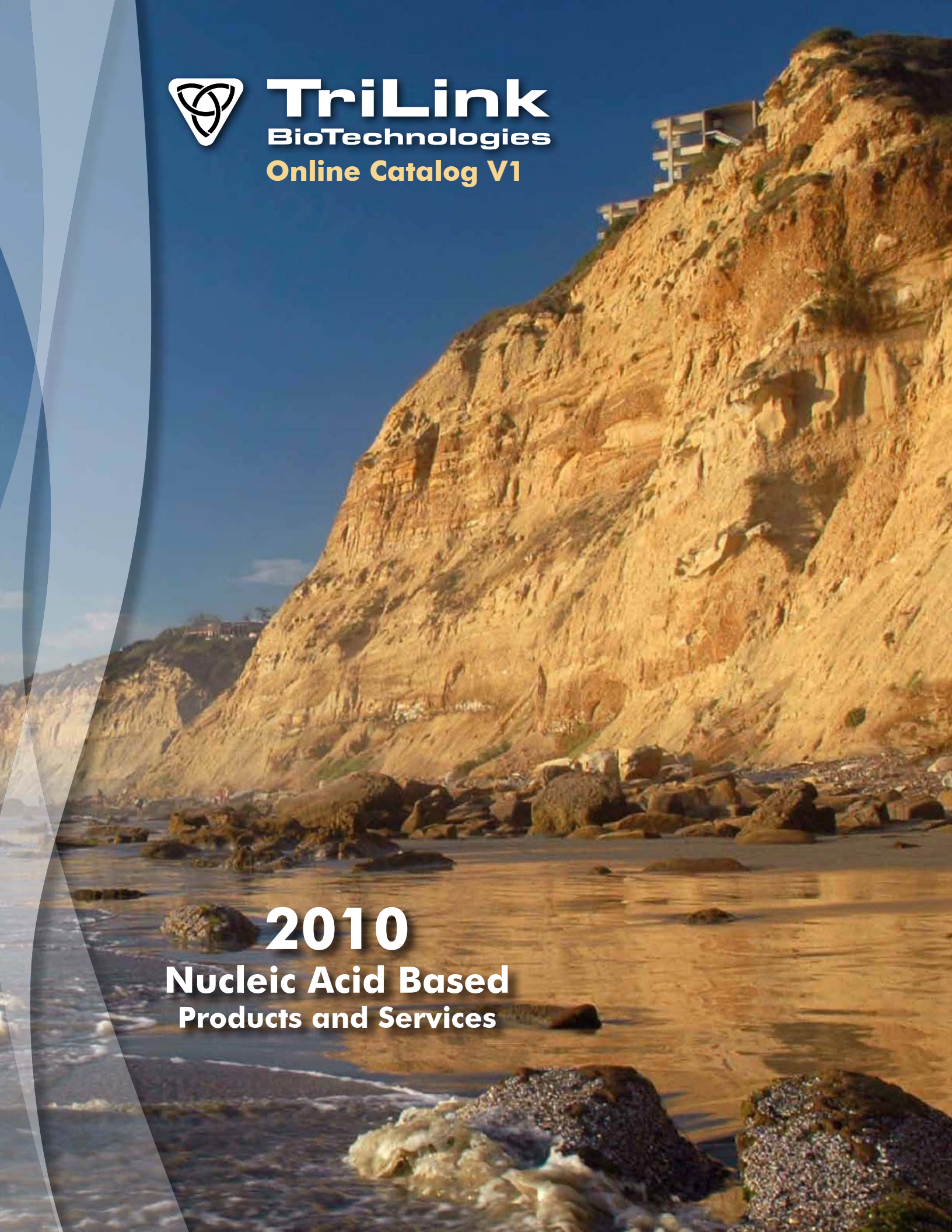

TriLink
BioTechnologies
Online Catalog V1
2010
Nucleic Acid Based
Products and Services

Ordering Information

800-863-6801
sales@trilinkbiotech.com
www.trilinkbiotech.com

All oligonucleotide sequences must be submitted electronically, either by email or online. Prices do not include shipping charges or applicable taxes. TriLink reserves the right to modify prices at any time or refuse to accept any order.

Payment

We accept payment by purchase order, wire transfer and credit card (MasterCard, Visa and American Express). Net payment is 30 days for open accounts in good standing. All first time customers are required to submit a credit application before receiving their first order.

Distributors

For a complete list of distributors, please see inside back cover.

Delivery

All products are shipped by overnight delivery to ensure quality. Within the US, shipments typically arrive by 10:30 am the following business day. Orders valued at $5000 or more are shipped with additional insurance. Products with stability concerns or in solution will be shipped on dry ice. F.O.B. San Diego.

Shipping Charges

Within the US standard shipping is approximately $15. International shipments range from $40 to $60. If your order requires dry ice or insurance, additional charges will apply. Please inquire for pricing specific to your location and order.

Quotations

Quotations for bulk syntheses, custom compounds and research services may be requested. To estimate oligonucleotide pricing see page 9. Direct inquires to sales@trilinkbiotech.com.

Technical Support

Our sales team is committed to fully supporting TriLink's products and services from initial customer inquiry to post delivery technical assistance. The answers to many common questions can be found online in our FAQ database. Our technical support team is available to speak with you Mon-Fri, 7am to 5pm PST.

800-863-6801
www.trilinkbiotech.com/faqs.asp

Signature TriLink Quality

TriLink has over 12 years of experience in making specialty modified oligonucleotides and nucleoside triphosphates. We believe it is our responsibility to work with our customers to overcome technical barriers. Consequently, we often prepare compounds that have not been made before or are not well established in the literature. Our extensive experience with unique modifications and our strong commitment to quality result in the best products available.

TriLink BioTechnologies

Nucleic Acid Based Products and Services

2010

Quick Guide

The TriLink Team

San Diego, California © Tim Banham

TriLink was founded in 1996 to meet a growing need in the marketplace for quality modified oligonucleotides. Still owned and managed by two of the original founders, TriLink now leads the industry in unique modified nucleic acids and mid-scale oligonucleotide synthesis.

Dear Colleagues,

We have grown so much over the past year! We have added nearly 40 new members to the TriLink family, almost doubling our size. It is your continued success that made this possible. We are very appreciative of the opportunities you gave us and, of course, the second chances when needed – this is a difficult science we practice. When we started TriLink, one of our stated objectives was to become our customers' *de facto* chemistry group. We wanted to be a member of your team. In many ways, we have succeeded in that goal for quite a number of you. I hope you have come to appreciate our commitment to overcoming your technical challenges, as much as we enjoy solving them.

Responding to our customer's needs, TriLink has vastly increased our synthesis capacity. We added a number of large-scale synthesizers, substantially increasing our 1-5 mmole scale capacity. We also added several 10-100 μmole scale mid-scale instruments and we even added a high throughput small-scale instrument. Naturally these were matched with the required number of HPLC purification instruments, lyophilization devices and QC capacity. We are grateful to be in a position where expansion is required.

We have also made significant advancements in product development. Our CleanAmp™ Primers, which were introduced in March of 2008, are now joined by our CleanAmp™ dNTPs. We are very proud of these two products. We believe they will have a very positive impact on the PCR field and become useful products in the years to come. We have several CleanAmp™ technology publications already out, and more that will be published soon. More CleanAmp™ Product information can be found in the orange tab section of this catalog.

Please take the time to peruse all our products and capabilities. We included all the technical information you have come to expect from a TriLink catalog, adding a number of articles about our new CleanAmp™ technologies. Even if you do not need one of our products today, you may find a useful piece of information inside that will help your research. We will be here when you need us, probably even bigger than we are today, and possibly even better able to serve you. See you then!

Best regards,

Richard Hogrefe, Ph.D.
CEO/President

La Jolla, California © istockphoto.com/Kazmaniac

Signature TriLink Quality and Service

TriLink is an industry leader in manufacturing high quality oligonucleotides and nucleoside triphosphates at small and mid-scales. We operate a GMP laboratory with a QSR environment. We believe it is our responsibility to work with our customers to push past technical barriers and create the programs they need. Our product management, manufacturing and quality assurance teams work together to understand the challenges of each synthesis and develop the best possible products.

Quality Assurance

All TriLink products undergo final analytical testing, typically a PAGE and MS analysis, to ensure the product meets our release specifications. The QC analyses are then incorporated in the certificate of analysis provided with each product. Every TriLink product and certificate of analysis is reviewed by a Quality Assurance Specialist and verified to be correct before shipment.

QSR and cGMP Manufacturing

TriLink will work with you to develop, document and validate processes and materials for your specific product needs. We will then manufacture your product under a quality system based on the FDA's Quality System Regulations (QSR) and the ISO 1345 standard.

Benefits of Manufacturing under QSR:

- Multi-gram batches
- Lot to lot consistency
- Optimized purification protocols
- Ultra-filtration desalting available

Program Guidelines Include:

- All personnel trained in QSR compliance
- SOPs & central records handling
- Supplier validation & traceable raw material
- Controlled access to dedicated laboratories

Please contact us to discuss your specific manufacturing needs.

Custom Chemistry and Contract Services

TriLink offers cutting edge services to researchers in the fields of gene therapy, nucleoside chemotherapy, oligonucleotide therapy and diagnostics. We offer custom small molecule synthesis including phosphoramidites and nucleoside mono-, di-, and triphosphates.

We provide a wide range of services including:

- Synthesis of specialty oligonucleotides
- Modification of nucleotides
- Bulk oligo and nucleoside synthesis
- QSR cGMP laboratory facilities available

Contract research and development project features:

- Developmental work on challenging compounds
- One on one interaction with a scientist
- Fixed weekly rates available
- Nucleoside Triphosphates, phosphoramidites and more

Collaborations

We are very interested in developing collaborative efforts with academic researchers and other companies. We have a research department and many years of experience helping our clients develop successful products. Please see page 153 for our ResearchRewards Grant Program, which is just one more way TriLink is giving support to the science that supports us.

About TriLink BioTechnologies

La Jolla, California © istockphoto.com/ddbell

Custom Oligonucleotide Synthesis

You Design. We Synthesize.

With our expertise and extensive selection of dyes, quenchers, modified bases, linkers, spacers and other conjugates, your creativity is the limit. TriLink specializes in challenging constructs such as oligonucleotides with numerous modifications, molecular beacons and probes with multiple modifications.

Consistently producing unique, superior grade oligonucleotides is an art form. Automated systems cannot recognize the nuances of distinct sequences and dye sets. Our skilled technicians isolate each compound using protocols designed to ensure we deliver the highest purity and yield for each oligonucleotide. By employing a hands-on approach, we offer a scalable process with unsurpassed quality and exceptional value.

All of these reasons make us a natural choice for oligonucleotide kit suppliers and testing facilities as well as pharmaceutical, biotech and academic researchers.

Simple Oligonucleotide Pricing

Estimating the price of your oligonucleotide is easy as 1, 2, 3.

1. Backbone
   Choose your backbone. See pages 16-19 for per base pricing at your desired scale.

2. Modifications
   Determine the modifications required. See pages 20-29 for fluorescent dye labeling, pages 38-46 for base modifications and pages 31-37 for other conjugates, linkers and spacers. Don't see the modification you need? Please inquire. TriLink also offers custom phosphoramidite synthesis, see page 51.

3. Purification
   Decide on a purification method. See pages 16-19 for pricing corresponding to the backbone of your oligonucleotide. Not sure which one to choose? Simply request a quote and indicate "Best Method" or ask our technical support team.

For a formal quotation, please email quotes@trilinkbiotech.com. Should your construct require special processing or an unusual chemistry, additional charges may apply. Mid-scale oligonucleotide and Molecular Beacon pricing can be found on pages 13 and 15, respectively. TriLink reserves the right to change pricing at any time.

Oligonucleotides for Diagnostics/OEM

Designing and developing specifications for your diagnostic kit components can be an impossible challenge without a nucleic acid chemist on your team. TriLink has over 13 years experience applying unique nucleic acid chemistry expertise to our customers' technical barriers.

Your Perfect Partner

We will work closely with you to tailor the manufacturing process to your specific program needs. Our extensive research in randomization optimization for the Roche NimbleGen platform is a great example of how we can make your product even better. Our process development and scale-up research in support of the ABI SOLiD™ System oligonucleotides is another example of what our technical expertise can do for you. To learn how our ingenuity can work for you, please contact our technical support team.

A Customized Program

Whether you are ordering your first oligonucleotide or are ready to set up a full supply program, TriLink is the right supplier for you. Material can be ordered in nmoles all the way up to multiple grams. We offer several levels of purification to meet your needs. Our crude material ranges from 60% - 80% pure. We will not ship any purified material unless it meets our 85% purity minimum without your request and approval. Greater purity can be achieved with additional purification steps at your request.

QC Analyses Available

- PAGE analysis & gel densitometry
- Mass spectroscopy
- RP/AX-HPLC analysis
- Synthesis report
- Other assays available

Additional Services Available

- Custom labeling & documentation
- Label control
- Research & development
- Manufacturing process optimization
- Custom phosphoramidite synthesis

QSR Manufacturing

Every product made at TriLink is manufactured under QSR, whether it is for diagnostics, therapeutics or research. At TriLink our top priority has always been manufacturing the highest quality products for our customers. We will work with you to develop, document and validate processes and materials for your specific program. We will then manufacture your product under a quality system based on QSR and the ISO 1345 standard.

Benefits of Manufacturing under QSR:

- Manufacturing process validation
- Optimized synthesis & purification protocols
- Locked process & specification documentation
- Separate batch record for each compound
- Dedicated program review

Program Guidelines Include:

- All personnel trained in QSR compliance
- SOPs & central records handling
- Supplier validation & traceable raw material
- Controlled access to dedicated laboratories

Roche NimbleGen Validated Random Nonamers

Randomers have become an essential tool in diagnostic research. They can help identify a gene that codes for a protein of interest when the specific sequence is unknown or amplify a genetic target to generate a pool of all available sequences. Commercial assays have been developed that use randomers to detect a single base mismatch polymorphism, as well as map chromosomal differences, most commonly deletions. These sensitive applications require oligos of random sequence, consistant base composition and minimal lot to lot variability.

TriLink has done extensive research and development to ensure our randomer oligos meet these crucial specifications. See our Randomer Oligonucleotide article in the Technical Information Section. We stock the more commonly requested randomers, as well as provide custom randomer synthesis. Please contact our sales team for more information.

Each and every lot of our validated products are tested by Roche NimbleGen prior to release. This is to ensure that they pass Roche NimbleGen's specifications for use in their array systems. TriLink is the only outside supplier of Roche NimbleGen validated oligonucleotides at this time. We are also the only supplier of pack sizes above 2 OD_{260} units, including Roche NimbleGen.

Roche NimbleGen Validated Random Nonamers

5' Cy Labeled Random Nonamers
Sold as a set: 1 vial of 5' Cy3 and 1 vial of 5' Cy5
Use: 2-color array systems

1 OD_{260} units of each	N46-0010-01	$100
2 OD_{260} units of each	N46-0010-02	$150
25 OD_{260} units of each	N46-0010-25	$800
50 OD_{260} units of each	N46-0010-50	$1500
100 OD_{260} units of each	N46-0010-100	$2600
200 OD_{260} units of each*	N46-0010-200	$4200

Roche NimbleGen Validated Unmatched 5' Cy3 Labeled Random Nonamers

5' Cy3 Labeled Random Nonamers
Use: 1-color array systems

1 OD_{260} units	N46-0007-01	$50
2 OD_{260} units	N46-0007-02	$75
25 OD_{260} units	N46-0007-25	$400
50 OD_{260} units	N46-0007-50	$750
100 OD_{260} units	N46-0007-100	$1300
200 OD_{260} units*	N46-0007-200	$2100

Roche NimbleGen Validated Random 7mers

5' Cy Labeled Random 7mers
Sold as a set: 1 vial of 5' Cy3 and 1 vial of 5' Cy5
Use: short labeling in CHiP-chip array systems

25 OD_{260} units of each	N46-0011-25	$800
50 OD_{260} units of each	N46-0011-50	$1500
100 OD_{260} units of each	N46-0011-100	$2600
200 OD_{260} units of each*	N46-0011-200	$4200

*Larger quantities available, please inquire.

Therapeutic Oligonucleotide Services

TriLink provides small-scale oligonucleotides for the discovery and pre-clinical stages of drug development to customers exploring a wide variety of DNA or RNA based therapies. Service options include synthesis in a QSR environment and full document control.

QSR & Document Control

We will work with you to develop, document and validate processes and materials for your specific product needs. Your product will then be manufactured in our laboratories, which are dedicated to production under a quality system based on QSR and the ISO 1345 standard.

Documentation under QSR includes:
- Standard operating procedures specific to your compound and specifications
- Central records handling and traceable batch records
- Supplier validation and traceable raw material

Conduct your pre-clinical studies with the confidence that your oligonucleotide will comply with FDA guidelines.

The TriLink-Avecia Alliance

From discovery to the clinic

The TriLink-Avecia Alliance allows easy transition from R&D and pre-clinical studies to clinical trials and commercial manufacturing of therapeutic oligonucleotides.

Working with the alliance minimizes your challenges and risks associated with transitioning from a research-grade vendor to an oligonucleotide development service and manufacturing company. We have harmonized our processes, so you can enjoy a smooth changeover when scaling up for clinical trials. For more information on the alliance go to our websites:

www.trilinkbiotech.com/alliance
www.aveciabiotech.com/oligomedicines

Mid-Scale Oligonucleotide Synthesis

TriLink specializes in mid-scale synthesis of both DNA and RNA oligonucleotides. Quantities range from 50 mg to 10 g. If you require more than 10 g, we recommend our alliance partner Avecia. All products are analyzed by PAGE, HPLC and MS. Turn around time varies based on sequence, purification and quantity requested. Additional services include modifications, process development and cGMP/QSR manufacturing.

DNA Pricing

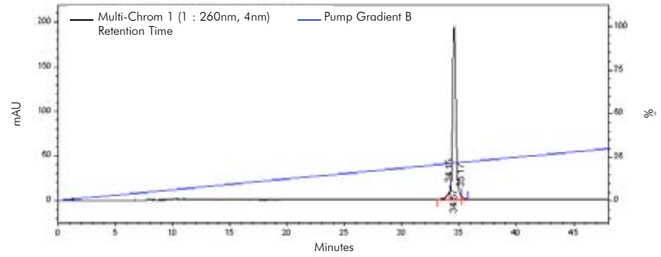
50 mg	$1,500	1.0 g	$5,500
100 mg	$1,825	1.25 g	$6,600
150 mg	$2,075	1.5 g	$7,350
200 mg	$2,350	2.0 g	$9,150
250 mg	$2,450	3.0 g	$12,375
300 mg	$2,700	5.0 g	$17,500
500 mg	$3,500	10.0 g	$30,000
750 mg	$4,450		

Prices are for unmodified oligonucleotides between 15 and 25 bases. It includes purification and diafiltration. Please request a quotation for other lengths or different quantities. The backbone may consist of phosphorothioate or phosphodiester linkages, or a combination of the two. Prices may vary due to sequence composition.

RNA Pricing

Set pricing for mid-scale RNA oligonucleotide synthesis is not available. The yield and quality of these syntheses are highly dependent on sequence. Please contact our technical support team to request a custom quotation.

The analysis below shows an AX-HPLC run on a mid-scale phosphodiester DNA oligonucleotide. The oligonucleotide is 34mer, HPLC purified.

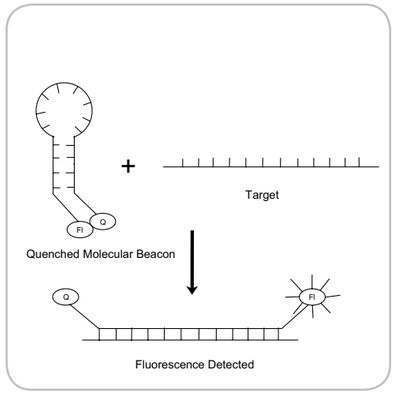


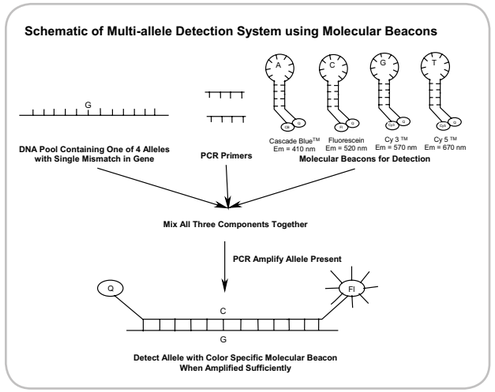
1 : 260 nm 4nm Results		
Retention Time	Area	Area Percent
34.15	158009	3.70
34.57	4072665	95.47
35.17	35063	0.82
Totals	4265737	100.00

Molecular Beacon Overview

In 1996 Tyagi and Kramer described a novel diagnostic assay utilizing oligonucleotides with a fluorescent dye at one terminus and a quenching dye at the other. These "molecular beacons" formed a hairpin when not hybridized to a target, thus placing the 5' and 3' termini, and their attached dyes, in close proximity. The fluorescent dye was quenched, yielding no signal. However, upon hybridization of the probe to the target, the dyes were separated and fluorescence was detected. (Tyagi, S., and Kramer, F.R. (1996) Nature Biotechnology, 14, 303-308.)

In order for the assay to work correctly the oligonucleotide conjugate must be of the highest quality as is expected from TriLink. We analyze the purity of every intermediate and pool only the purest fractions for your final product.

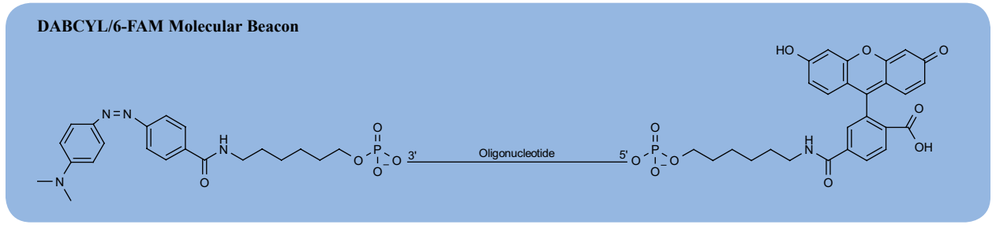




Quencher Selection

TriLink offers a wide range of quenchers including TAMRA, DABCYL, Black Hole Quenchers® and QSY®. Please refer to the Technical Information section of this catalog for more on quenchers. Additional information and structures can be found on page 30.

For licensing information and disclaimers, please see page 152.



Molecular Beacon Pricing

Targets for Molecular Beacons (0.2 umole scale, crude preparations) are available at no extra charge upon request. Limit one primer pair per Molecular Beacon. Prices are listed below for beacons up to 40 bases in length. Please inquire for (1) pricing regarding QSY quenchers (QSY-7®, QSY-9®, QSY-21®, QSY-35®), (2) larger scale syntheses or (3)other fluorophores and quenchers.

Pricing

5' Fluorophore	3' Quencher	0.2 umole scale			1.0 umole scale		
		without a licence*	with a license*	expected yield	without a licence*	with a license*	expected yield
Carboxy-X-Rhodamine™	Dabcyl or BHQ-2®	$600	$490	1-3 ODs	$700	$575	3-15 ODs
Coumarin™	Dabcyl or BHQ-1 or 2®	$600	$490	1-3 ODs	$700	$575	3-15 ODs
Cy3™	Dabcyl or BHQ-2®	$600	$490	1-3 ODs	$700	$575	3-15 ODs
Cy3.5™	Dabcyl or BHQ-2®	$750	$615	1-3 ODs	$850	$700	3-15 ODs
Cy5™	Dabcyl or BHQ-2®	$600	$490	1-3 ODs	$700	$575	3-15 ODs
Cy5.5™	Dabcyl or BHQ-2®	$750	$615	1-3 ODs	$850	$700	3-15 ODs
6-FAM	Dabcyl or BHQ-1®	$450	$370	3-5 ODs	$550	$450	10-20 ODs
HEX	Dabcyl or BHQ-1®	$450	$370	3-5 ODs	$550	$450	10-20 ODs
Rhodamine Green™	Dabcyl or BHQ-1®	$600	$490	1-3 ODs	$700	$575	3-15 ODs
Rhodamine Red™	Dabcyl or BHQ-2®	$600	$490	1-3 ODs	$700	$575	3-15 ODs
TAMRA	Dabcyl or BHQ-2®	$600	$490	1-3 ODs	$700	$575	3-15 ODs
TET	Dabcyl or BHQ-1®	$450	$370	3-5 ODs	$550	$450	10-20 ODs
Texas Red®	Dabcyl or BHQ-2®	$600	$490	1-3 ODs	$700	$575	3-15 ODs

*Molecular Beacons are sold under license from The Public Health Research Institute. The purchase of Molecular Beacons by end users is accompanied by limited rights under PHRI patents to use the probes for their research only. Commercial use or any other use of Molecular Beacons requires a license from PHRI, and no license rights for any such use are conveyed with the purchase of Molecular Beacons. No representation or warranty accompanies the sale of these products that their use, alone or in combination, or in any process will not infringe the claims of United States or foreign patents. TriLink will not be responsible for damages related to this product beyond replacement cost for any reason. |

See Licensing Information section for the disclaimer of license statement pertaining to Molecular Beacon products.

Primer Grade DNA Oligonucleotides

Our primer grade synthesis services include 40 nmole and 200 nmole scales. Per base price includes synthesis, deprotection, cartridge desalt, PAGE analysis and a certificate of analysis. Primers are phosphodiester DNA only and must be between 10 and 45 bases in length. If you require larger scale synthesis, modifications or additional purification, please see Standard Synthesis DNA Oligonucleotides on the opposite page.
Primer grade services include:

- No set up charge
- Free desalting
- PAGE analysis on every primer
- Certificate of analysis with every shipment

Synthesis Scale	Per Base Price	Minimum Desalt Yield (>20mer)	Desalt Purification	Cartridge Purification
40 nmole	$0.99	3 ODs	no charge	$15.00
200 nmole	$1.25	15 ODs	no charge	$15.00

Minimum charge per oligonucleotide: 40 nmole scale $18.00; 200 nmole scale $20.00.

Did You Know?

Every oligonucleotide sold by TriLink, even primer grade DNA, is analyzed by PAGE and usually MS as well. Many are also assayed by either AX-HPLC or RP-HPLC. Our strong quality commitment and our extensive nucleic acid chemistry experience results in the best oligonucleotides possible.

Standard DNA Oligonucleotides

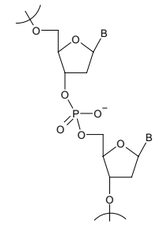
Our standard DNA synthesis ranges from 0.2 µmole to 15 µmole, in both phosphodiester and phosphorothioate backbones. We offer an extensive selection of oligo modifications, such as linkers, spacers, dyes and modified bases, please see pages 20-47.

For larger synthesis scales please refer to our mid-scale services on page 13 or request a custom quotation.

Per Base Pricing

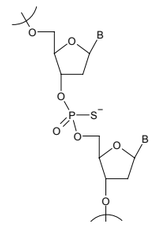
Phosphodiester

0.2 µmole $2.00
1.0 µmole $3.00
15 µmole $15.00
$40 minimum



Phosphorothioate

0.2 µmole $4.50
1.0 µmole $6.00
15 µmole $20.00
$50 minimum



Purification

Method*	0.2 - 1.0 µmole	15 µmole
Double RP-HPLC	$200	$375
Single RP-HPLC & RP-Cartridge	$150	$325
AX-HPLC	$225	$425
RP-Cartridge	$50	Not available
PAGE	$150	Inquire
Extra Conjugation Purification	$50	$100

*If you are not sure which purification is best for your oligonucleotide and application, see *When is my Oligonucleotide Pure Enough?* in the Technical Information section of this catalog, ask our technical support team or simply indicate "Best Method" when requesting your quotation.

Expected Yields

In general, yields of HPLC purified (~90%) unmodified oligonucleotides are as follows:

0.2 µmole scale: 5 - 15 OD_{260} units (~0.15 mg - 0.5 mg)
1.0 µmole scale: 20 - 60 OD_{260} units (~0.66 mg - 2 mg)
15 µmole scale: 300-750 OD_{260} units (~10 mg - 25 mg)

Yield and purity differences can be caused by many factors, such as sequence and length.
If you require a specific yield, please let us know when you place your order or request a quote.

Standard RNA Oligonucleotides

Our standard RNA synthesis services range from 0.2 μmole to 15 μmole scale, in both 2' OH RNA and 2' O-Methyl RNA backbones. Both 2' OH and 2' O-Methyl RNA oligonucleotides can be made with phosphorothioate linkages at no extra charge. We offer an extensive selection of oligo modifications, such as linkers, spacers, dyes and modified bases, please see pages 20-47.

For larger synthesis scales please request a custom quotation. Set pricing for mid-scale RNA oligonucleotide synthesis is not available, because the yield and quality of these syntheses is highly dependent on sequence.

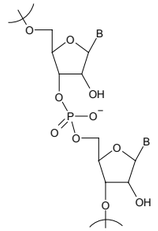
2' OH RNA

Per Base Pricing

0.2 μmole	$10.50
1.0 μmole	$14.50
15 μmole	$65.00

$100 minimum*

*This minimum refers only to the portion of the oligonucleotide with the 2' OH modification.



Purification

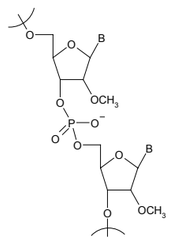
Method	0.2 - 1.0 μmole	15 μmole
PAGE	$200	Inquire
AX-HPLC	$250	$475

2' O-Methyl RNA

Per Base Pricing

0.2 μmole	$8.00
1.0 μmole	$12.00
15 μmole	$50.00

$50 minimum



Purification

Same as Standard DNA Synthesis.
See previous page.

Expected Yields

In general, yields of PAGE purified (~90%) unmodified 2' OH RNA oligonucleotides are as follows (2' O-Methyl yields are comparable to DNA yields as shown on the previous page):

0.2 μmole: 2 - 8 OD_{260} units (~0.07 mg - 0.27 mg)
1.0 μmole: 10 - 40 OD_{260} units (~0.33 mg - 1.33 mg)
15 μmole: 150-400 OD_{260} units (~4.45 mg - 13.33 mg)

Yield and purity differences can be caused by many factors, such as sequence and length. If you require a specific yield, please let us know when you place your order or request a quote.

Please see the Technical Information section for further information on oligonucleotide purification procedures.

Methylphosphonate and 2' Fluoro RNA Oligonucleotides

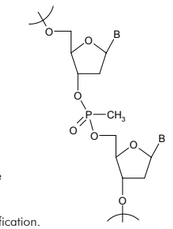
Methylphosphonate

Per Base Pricing

0.2 μmole	N/A
1.0 μmole	$35.00
15 μmole	$125.00

$150 minimum*

*This minimum refers only to the portion of the oligonucleotide with the methylphosphonate modification. This is to cover the additional deprotection processing required for this modification.

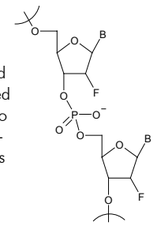


This molecule has a number of unusual properties. It is one of the few neutrally charged backbones that is readily available. This modification is most commonly used to protect the termini of oligonucleotides from enzymatic degradation. One disadvantage is that the racemic form of this modification reduces the ability of the oligonucleotide to hybridize to its target.

2' Fluoro RNA

Inquire for Pricing

Each 2' Fluoro RNA modified oligonucleotide must be quoted individually. The price is effected by the total number of 2' Flouro modifications and if the modifications are pyrimidines, purines or a combination of both.



The main applications of these 2' Fluoro modifications are ribozyme development (1,3), to increase T_m (2,6,7), aptamer selection (4,5) and nuclease resistance (7). Oligonucleotides containing 2' Fluoros have been reported to increase DNA-DNA T_m by 1.3°C per insertion (6).

1. Fell, et al. (1997) Antisense and Nucleic Acid Development 7, 319-326.
2. Pagratis, et al. (1997) Nature Biotechnology 15, 68-73.
3. Ito, et al. (1999) Nucl. Acids Symp. Ser 42, 277-278.
4. Pat. No. 5,475,096, Nexstar.
5. Kubik, et al. (1997) J. Immunol. 159, 259-267.
6. Sabahi, et al. (2001) Nucleic Acids Research 29, 2163-2170.
7. Kawasaki, et al. (1993) J. Medicinal Chemistry 36, 831-841.

Purification and Yields

Methylphosphonate and 2' Fluoro modified oligonucleotides can often be purified using the same methods listed for Standard DNA Synthesis. However, this depends on the design of the entire molecule. Yields are extremely variable, affected by modifications and sequence. Please contact us for information regarding purification methods and expected yields for your specific construct.

Oligonucleotide Conjugates

Our selection of conjugates includes over *sixty* fluorescent dyes. We also offer non-fluorescent conjugates, such as biotin, psoralen and cholesteryl. Most conjugates can be placed at the 5' terminus, and internally on any 2'-deoxy base. Many conjugates can also be placed at the 3' terminus. Separate pricing will be noted for these conjugates. Our standard protocol includes purification before and after conjugation.

Fluorescent Dye Labeling

Dyes with a range of wavelengths are available for oligonucleotide conjugation. Structures and list prices can be found on the following pages. Please refer to the selection chart in the Technical Information section for more information on available dyes and their spectra. For additional help please contact our technical support team.

Violet/Blue (Em_{max} 375-491 nm)	**Green/Yellow** (Em_{max} 492-585 nm)	**Green/Yellow** (Cont.) (Em_{max} 492-585 nm)	**Orange** (Em_{max} 586-647 nm)	**Red** (Em_{max} 647-700 nm)
P130	BODIPY 493/503	6-JOE	CAL Fluor® Red 590	Alexa Fluor® 633
7-Methoxycoumarin	DTAF	BODIPY 530/550	BODIPY 576/589	Alexa Fluor® 647
Cascade Blue	6-FAM (Fluorescein)	Alexa Fluor® 532	BODIPY 581/591	Quasar 670®
Alexa Fluor® 405	Dansyl-X	HEX	Alexa Fluor® 568	Cy5™
AMCA-X	Oregon Green 500	Carboxyrhodamine 6G	Texas Red-X	Naphthofluorescein
Alexa Fluor® 350	Alexa Fluor® 488	CAL Fluor® Orange 560	Cy3.5™	Alexa Fluor® 660
Pacific Blue	dT-FAM	Alexa Fluor® 555	ROX	Cy5.5™
Marina Blue	Oregon Green 488	BODIPY 558/568	CAL Fluor® Red 610	Alexa Fluor® 680
Dimethylaminocoumarin	Oregon Green 514	PyMPO	BODIPY-TR-X	Alexa Fluor® 700
	Rhodamine Green-X	Alexa Fluor® 546	Alexa Fluor® 594	Alexa Fluor® 750
	NBD-X	BODIPY 564/570	Alexa Fluor® 610	
	TET	Quasar 570®	CAL Fluor® Red 635	
	Alexa Fluor® 430	Cy3™		
	CAL Fluor® Gold 540	TAMRA-X		
	Alexa Fluor® 514	dT-TAMRA		
	2',4',5',7' Br_4-sulfoneflu.	Rhodamine Red-X		

Linkers

Prices for 0.2 and 1.0 μmole scale conjugations include the 5' or 3' C6-Amino linker if needed. Specialty linkers specified by the customer are not included. See page 34-36 for pricing. Linkers for larger scales are also not included in the conjugate price.

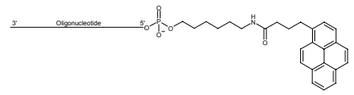
Purification

Some conjugates require only a single purification. See page 17-18 for pricing. The asterisked conjugates, however, require an extra purification step for an additional $50 (0.2 or 1.0 μmole scale) or $100 (15 μmole scale). Many of the dyes listed above are succinimidyl esters (SE), and require an amino labeled oligonucleotide unless specifically noted otherwise. Thiol reactive conjugates are also available for some of these dyes for an additional cost. Please inquire for availability and pricing.

Violet/Blue (Emission Max. 375-491 nm)

P130*

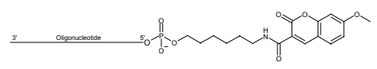
0.2 μmole	$125
1.0 μmole	$150
15 μmole	$500



Abs. max: 340 nm
Em. max: 376 nm
Ɛ at Abs. max: 43,000

7-Methoxycoumarin*
(7-methoxycoumarin-3-carboxy)

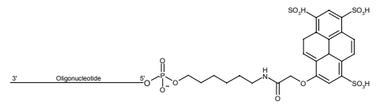
0.2 μmole	$175
1.0 μmole	$200
15 μmole	$600



Abs. max: 358 nm
Em. max: 410 nm
Ɛ at Abs. max: 26,000

Cascade Blue™*

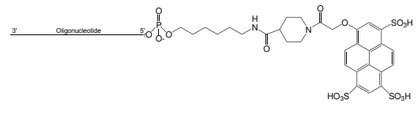
0.2 μmole	$225
1.0 μmole	$275
15 μmole	$700



Abs. max: 396 nm
Em. max: 410 nm
Ɛ at Abs. max: 29,000

Alexa Fluor® 405*

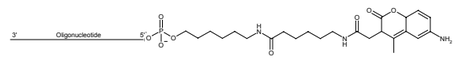
0.2 μmole	$395
1.0 μmole	$650
15 μmole	$2423



Abs. max: 400 nm
Em. max: 424 nm
Ɛ at Abs. max: 35,000

7-Aminocoumarin-X*
(AMCA-X)

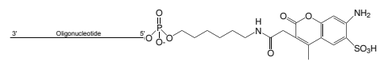
0.2 μmole	$175
1.0 μmole	$200
15 μmole	$600



Abs. max: 353 nm
Em. max: 442 nm
Ɛ at Abs. max: 19,000

Alexa Fluor® 350*

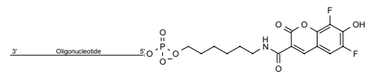
0.2 μmole	$300
1.0 μmole	$375
15 μmole	$800



Abs. max: 346nm
Em. max: 442nm
Ɛ at Abs. max: 19,000

Pacific Blue®*

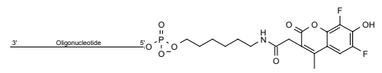
0.2 μmole	$175
1.0 μmole	$225
15 μmole	$650



Abs. max: 410 nm
Em. max: 455 nm
Ɛ at Abs. max: 46,000

Marina Blue®*

0.2 μmole	$175
1.0 μmole	$225
15 μmole	$650



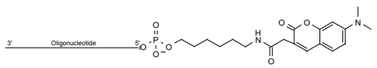
Abs. max: 365 nm
Em. max: 460 nm
Ɛ at Abs. max: 19,000

Ɛ is noted in L $mmole^{-1}$ cm^{-1}.
*These compounds require an extra purification step. See page 20 for details.

Violet/Blue (Emission Max. 375-491 nm) continued

Dimethylaminocoumarin®*

0.2 µmole	$175
1.0 µmole	$200
15 µmole	$600

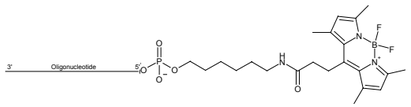


Abs. max: 376 nm
Em. max: 468 nm
Ɛ at Abs. max: 22,000

Green/Yellow (Emission Max. 492-585 nm)

BODIPY 493/503™*

0.2 µmole	$225
1.0 µmole	$275
15 µmole	$750

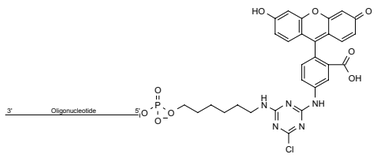


Abs. max: 500 nm
Em. max: 506 nm
Ɛ at Abs. max: 79,000

DTAF*

(4,6-Dichlorotriazinyl)aminofluorescein

0.2 µmole	$150
1.0 µmole	$175
15 µmole	$600

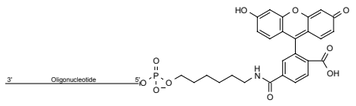


Abs. max: 492 nm
Em. max: 516 nm
Ɛ at Abs. max: 83,000

6-FAM (Fluorescein)

6-Carboxyfluorescein

	Amidite	SE*	3' Terminus
0.2 µmole	$100	$150	$100
1.0 µmole	$125	$175	$125
15 µmole	$500	$600	$700



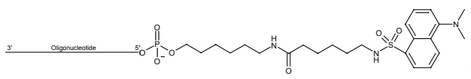
Amidite/SE version shown

Abs. max: 496 nm
Em. max: 516 nm
Ɛ at Abs. max: 83,000

Dansyl-X*

6-((5-Dimethylaminonaphthalene-1-sulfonyl)amino) hexanoate

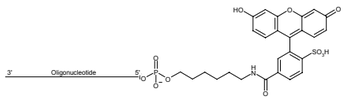
0.2 µmole	$125
1.0 µmole	$150
15 µmole	$500



Abs. max: 335 nm
Em. max: 518 nm
Ɛ at Abs. max: 4,200

Oregon Green 500™*

0.2 µmole	$175
1.0 µmole	$225
15 µmole	$650

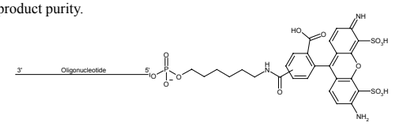


Abs. max: 499 nm
Em. max: 519 nm
Ɛ at Abs. max: 78,000

Alexa Fluor® 488* (mixed isomers)

Note: Impurities in this dye can affect the oligo conjugate product purity.

0.2 µmole	$375
1.0 µmole	$550
15 µmole	$1650



Abs. max: 495 nm
Em. max: 517 nm
Ɛ at Abs. max: 73,000

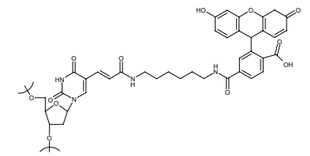
Ɛ is noted in L mmole⁻¹ cm⁻¹.
*These compounds require an extra purification step. See page 20 for details.

Green/Yellow (Emission Max. 492-585 nm) continued

dT-FAM

Fluorescein linked to 5 position of thymidine.

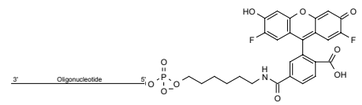
	5' or Internal	3' Terminus
0.2 µmole	$150	$100
1.0 µmole	$200	$125
15 µmole	$650	$700



Abs. max: 492 nm
Em. max: 520 nm
Ɛ at Abs. max: 74,850

Oregon Green 488™*

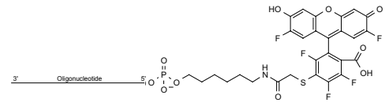
0.2 µmole	$175
1.0 µmole	$225
15 µmole	$650



Abs. max: 495 nm
Em. max: 521 nm
Ɛ at Abs. max: 76,000

Oregon Green 514™*

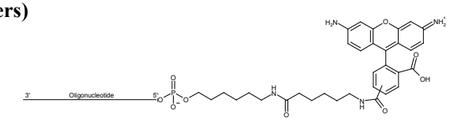
0.2 µmole	$175
1.0 µmole	$225
15 µmole	$650



Abs. max: 506 nm
Em. max: 526 nm
Ɛ at Abs. max: 85,000

Rhodamine Green-X™* (mixed isomers)

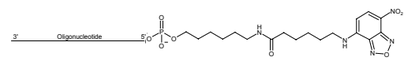
0.2 µmole	$225
1.0 µmole	$275
15 µmole	$750



Abs. max: 503 nm
Em. max: 528 nm
Ɛ at Abs. max: 74,000

NBD-X*

0.2 µmole	$125
1.0 µmole	$150
15 µmole	$500

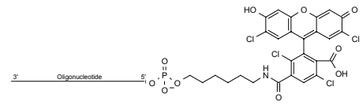


Abs. max: 466 nm
Em. max: 535 nm
Ɛ at Abs. max: 22,000

TET

Tetrachlorofluorescein

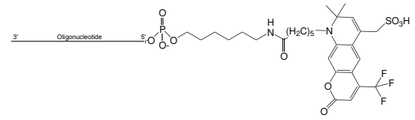
	Amidite	SE*
0.2 µmole	$100	$150
1.0 µmole	$125	$175
15 µmole	$500	$600



Abs. max: 521 nm
Em. max: 536 nm
Ɛ at Abs. max: 86,000

Alexa Fluor® 430*

0.2 µmole	$325
1.0 µmole	$400
15 µmole	$1000



Abs. max: 433 nm
Em. max: 539 nm
Ɛ at Abs. max: 15,000

CAL Fluor® Gold 540

	5' Terminus
0.2 µmole	$250
1.0 µmole	$275
15 µmole	$875

Structure not available from manufacturer.

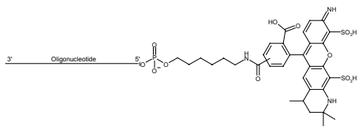
Abs. max: 522 nm
Em. max: 541 nm
Ɛ at Abs. max: 74,700

Ɛ is noted in L mmole⁻¹ cm⁻¹.
*These compounds require an extra purification step. See page 20 for details.

Green/Yellow (Emission Max. 492-585 nm) continued

Alexa Fluor® 514* (mixed isomers)

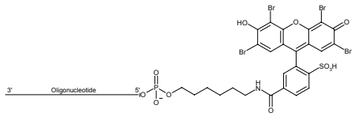
0.2 µmole	$385
1.0 µmole	$565
15 µmole	$1785



Abs. max: 518 nm
Em. max: 540 nm
Ε at Abs. max: 80,000

2',4',5',7'-Tetrabromosulfonefluorescein*

0.2 µmole	$175
1.0 µmole	$225
15 µmole	$650

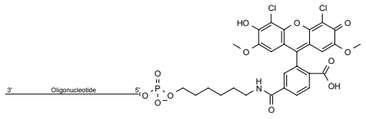


Abs. max: 529 nm
Em. max: 544 nm
Ε at Abs. max: 89,000

6-JOE*

6-Carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein

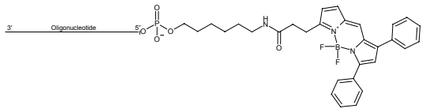
0.2 µmole	$200
1.0 µmole	$250
15 µmole	$700



Abs. max: 520 nm
Em. max: 548 nm
Ε at Abs. max: 75,000

BODIPY-530/550™*

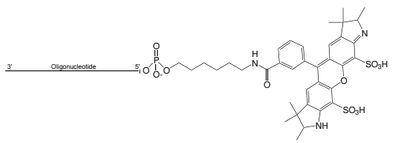
0.2 µmole	$225
1.0 µmole	$275
15 µmole	$750



Abs. max: 534 nm
Em. max: 554 nm
Ε at Abs. max: 77,000

Alexa Fluor® 532*

0.2 µmole	$385
1.0 µmole	$565
15 µmole	$1785

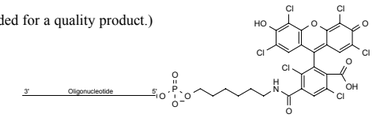


Abs. max: 530 nm
Em. max: 555 nm
Ε at Abs. max: 81,000

HEX

Hexachlorofluorescein (The SE dye is highly recommended for a quality product.)

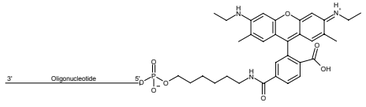
	Amidite	SE*
0.2 µmole	$100	$150
1.0 µmole	$125	$175
15 µmole	$500	$600



Abs. max: 535 nm
Em. max: 556 nm
Ε at Abs. max: 96,000

Carboxyrhodamine 6G™*

0.2 µmole	$175
1.0 µmole	$225
15 µmole	$650



Abs. max: 525 nm
Em. max: 555 nm
Ε at Abs. max:108,000

CAL Fluor® Orange 560

	5' Terminus
0.2 µmole	$250
1.0 µmole	$275
15 µmole	$875

Structure not available from manufacturer.

Abs. max: 540 nm
Em. max: 561 nm
Ε at Abs. max: 94,000

Ε is noted in L mmole^{-1} cm^{-1}.
*These compounds require an extra purification step. See page 20 for details.

Green/Yellow (Emission Max. 492-585 nm) continued

Alexa Fluor® 555*

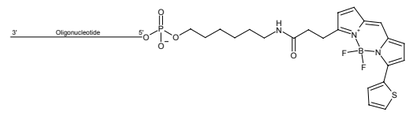
0.2 µmole	$405
1.0 µmole	$700
15 µmole	$2750

Structure not available from manufacturer.

Abs. max: 555 nm
Em. max: 572 nm
Ε at Abs. max:155,000

BODIPY 558/568™*

0.2 µmole	$225
1.0 µmole	$275
15 µmole	$750

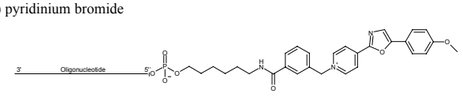


Abs. max: 558 nm
Em. max: 569 nm
Ε at Abs. max: 97,000

PyMPO*

1-(3-carboxybenzyl)-4-(5-(4-methoxyphenyl)oxazol-2-yl) pyridinium bromide

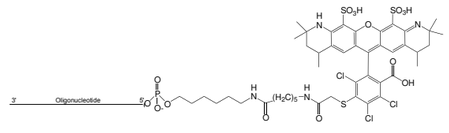
0.2 µmole	$150
1.0 µmole	$200
15 µmole	$600



Abs. max: 415 nm
Em. max: 570 nm
Ε at Abs. max: 26,000

Alexa Fluor® 546*

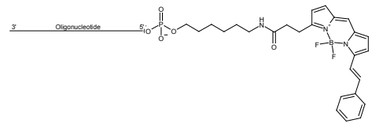
0.2 µmole	$395
1.0 µmole	$650
15 µmole	$2425



Abs. max: 554 nm
Em. max: 570 nm
Ε at Abs. max:112,000

BODIPY-564/570™*

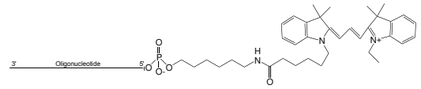
0.2 µmole	$225
1.0 µmole	$275
15 µmole	$750



Abs. max: 565 nm
Em. max: 571 nm
Ε at Abs. max:142,000

Quasar 570® *

0.2 µmole	$200
1.0 µmole	$250
15 µmole	$875

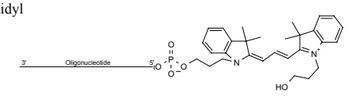


Abs. max: 547 nm
Em. max: 570 nm
Ε at Abs. max:115,000

Cy3™

Cy3 is available as both the amidite and as the succinimidyl ester. Our experience is that the SE is a better dye.

	Amidite	SE*
0.2 µmole	$125	$200
1.0 µmole	$150	$250
15 µmole	$500	$875



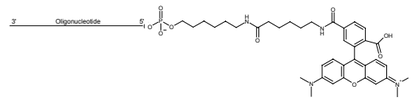
Amidite version shown

Abs. max: 550 nm
Em. max: 562 nm
Ε at Abs. max:150,000

TAMRA-X*

6-(Tetramethylrhodamine-5(6)-carboxamido) hexanoate

	Single Isomer	Mixed Isomers
0.2 µmole	$175	$175
1.0 µmole	$200	$200
15 µmole	$750	$600



Abs. max: 543 nm
Em. max: 571 nm
Ε at Abs. max: 92,000

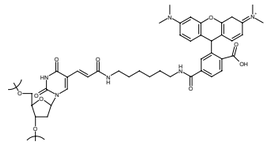
Ε is noted in L mmole^{-1} cm^{-1}.
*These compounds require an extra purification step. See page 20 for details.

Green/Yellow (Emission Max. 492-585 nm) continued

dT-TAMRA

6-Tetramethylrhodamine-5(6)-carboxamide attached to dT-C6 amine

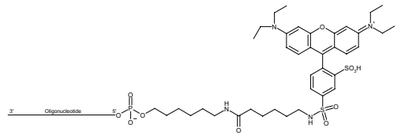
	5' or Internal
0.2 µmole	$175
1.0 µmole	$200
15 µmole	$750



Abs. max: 546 nm
Em. max: 576 nm
Ɛ at Abs. max: 95,000

Rhodamine Red-X™*

0.2 µmole	$175
1.0 µmole	$225
15 µmole	$650



Abs. max: 560 nm
Em. max: 580 nm
Ɛ at Abs. max:129,000

Orange (Emission Max. 586-647 nm)

CAL Fluor® Red 590

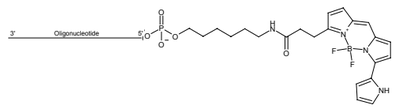
	5' Terminus
0.2 µmole	$250
1.0 µmole	$275
15 µmole	$875

Structure not available from manufacturer.

Abs. max: 566 nm
Em. max: 588 nm
Ɛ at Abs. max: 79,000

BODIPY-576/589™*

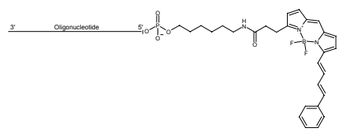
0.2 µmole	$225
1.0 µmole	$275
15 µmole	$750



Abs. max: 576 nm
Em. max: 590 nm
Ɛ at Abs. max: 83,000

BODIPY-581/591™*

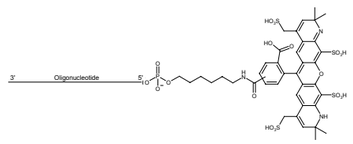
0.2 µmole	$225
1.0 µmole	$275
15 µmole	$750



Abs. max: 584 nm
Em. max: 592 nm
Ɛ at Abs. max:136,000

Alexa Fluor® 568* (mixed isomers)

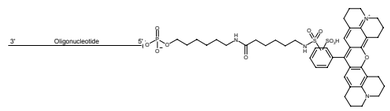
0.2 µmole	$385
1.0 µmole	$565
15 µmole	$1785



Abs. max: 578 nm
Em. max: 602 nm
Ɛ at Abs. max: 88,000

Texas-Red-X™* (mixed isomers)

0.2 µmole	$225
1.0 µmole	$250
15 µmole	$700



Abs. max: 583 nm
Em. max: 603 nm
Ɛ at Abs. max:112,000

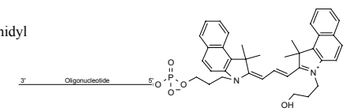
Ɛ is noted in L mmole^{-1} cm^{-1}.
*These compounds require an extra purification step. See page 20 for details.

Orange (Emission Max. 586-647 nm) continued

Cy3.5™

Cy3.5 is available as both the amidite and as the succinimidyl ester. Our experience is that the SE is a better dye.

	Amidite	SE*
0.2 µmole	$325	$300
1.0 µmole	$350	$550
15 µmole	$1375	$1950



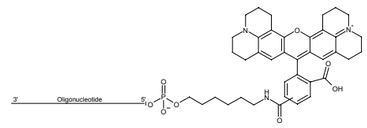
Amidite version shown

Abs. max: 588 nm
Em. max: 604 nm
Ɛ at Abs. max:150,000

Carboxy-X-Rhodamine™*

(X-Rhodamine, ROX)

	Single Isomer	Mixed Isomers
0.2 µmole	$200	$150
1.0 µmole	$225	$175
15 µmole	$650	$550



6-ROX structure shown

Abs. max: 580 nm
Em. max: 605 nm
Ɛ at Abs. max: 78,000

CAL Fluor® Red 610

	5' Terminus
0.2 µmole	$250
1.0 µmole	$275
15 µmole	$875

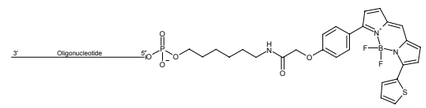
Structure not available from manufacturer.

Abs. max: 590 nm
Em. max: 610 nm
Ɛ at Abs. max:108,000

BODIPY-TR-X™*

Texas Red™ substitute

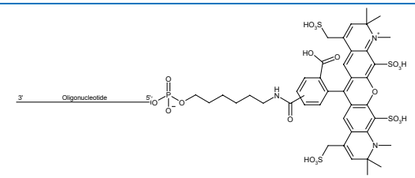
0.2 µmole	$250
1.0 µmole	$275
15 µmole	$750



Abs. max: 588 nm
Em. max: 616 nm
Ɛ at Abs. max: 68,000

Alexa Fluor® 594* (mixed isomers)

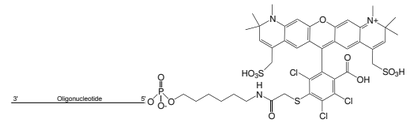
0.2 µmole	$390
1.0 µmole	$600
15 µmole	$1950



Abs. max: 590 nm
Em. max: 617 nm
Ɛ at Abs. max: 92,000

Alexa Fluor® 610*

0.2 µmole	$425
1.0 µmole	$725
15 µmole	$2850



Abs. max: 612 nm
Em. max: 628 nm
Ɛ at Abs. max:144,000

CAL Fluor® Red 635

	5' Terminus
0.2 µmole	$275
1.0 µmole	$300
15 µmole	$900

Structure not available from manufacturer.

Abs. max: 616 nm
Em. max: 636 nm
Ɛ at Abs. max:112,000

Red (Emission Max. 647-700 nm)

Alexa Fluor® 633*

0.2 µmole	$405
1.0 µmole	$725
15 µmole	$2850

Structure not available from manufacturer.

Abs. max: 621 nm
Em. max: 639 nm
Ɛ at Abs. max:159,000

Alexa Fluor® 647*

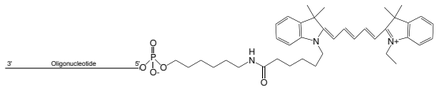
0.2 µmole	$425
1.0 µmole	$725
15 µmole	$2850

Structure not available from manufacturer.

Abs. max: 651 nm
Em. max: 672 nm
Ɛ at Abs. max:270,000

Quasar 670®*

0.2 µmole	$200
1.0 µmole	$250
15 µmole	$875

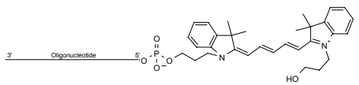


Abs. max: 644 nm
Em. max: 670 nm
Ɛ at Abs. max:187,000

Cy5™

Cy5 is available as both the amidite and as the succinimidyl ester. Our experience is that the SE is a better dye.

	Amidite	SE*
0.2 µmole	$125	$200
1.0 µmole	$150	$250
15 µmole	$500	$875



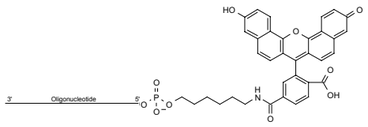
Amidite version shown

Abs. max: 649 nm
Em. max: 670 nm
Ɛ at Abs. max:250,000

Carboxynaphthofluorescein*

(5 and 6 mixed esters)

0.2 µmole	$125
1.0 µmole	$150
15 µmole	$500



Abs. max: 602 nm
Em. max: 672 nm
Ɛ at Abs. max: 42,000

Alexa Fluor® 660*

0.2 µmole	$395
1.0 µmole	$650
15 µmole	$2425

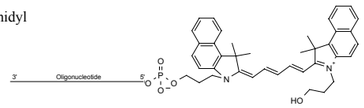
Structure not available from manufacturer.

Abs. max: 663 nm
Em. max: 690 nm
Ɛ at Abs. max:132,000

Cy5.5™

Cy5.5 is available as both the amidite and as the succinimidyl ester. Our experience is that the SE is a better dye.

	Amidite	SE*
0.2 µmole	$350	$395
1.0 µmole	$375	$650
15 µmole	$1500	$1950



Amidite version shown

Abs. max: 675 nm
Em. max: 694 nm
Ɛ at Abs. max:250,000

Alexa Fluor® 680*

0.2 µmole	$395
1.0 µmole	$650
15 µmole	$2425

Structure not available from manufacturer.

Abs. max: 684 nm
Em. max: 707 nm
Ɛ at Abs. max:183,000

Ɛ is noted in L mmole^{-1} cm^{-1}.
*These compounds require an extra purification step. See page 20 for details.

Red (Emission Max. 647-700 nm) continued

Alexa Fluor® 700*

0.2 µmole	$475
1.0 µmole	$750
15 µmole	$3000

Structure not available from manufacturer.

Abs. max: 702 nm
Em. max: 723 nm
Ɛ at Abs. max:196,000

Alexa Fluor® 750*

0.2 µmole	$425
1.0 µmole	$725
15 µmole	$2850

Structure not available from manufacturer.

Abs. max: 749 nm
Em. max: 782 nm
Ɛ at Abs. max:240,000

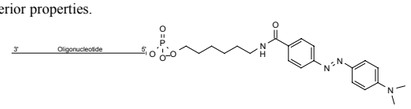
Ɛ is noted in L mmole^{-1} cm^{-1}.
*These compounds require an extra purification step. See page 20 for details.

Quenchers

DABCYL SE, (Carboxyamide linker)*

We use 3' amine labeled support or 5' C6 amino linker and DABCYL succinimidyl ester to prepare this labeled product with superior properties.

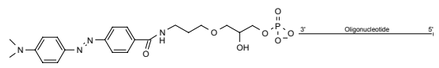
0.2 µmole	$125
1.0 µmole	$150
15 µmole	$500



Abs. max: 453 nm
ε at Abs. max: 32,000

3'-DABCYL CPG

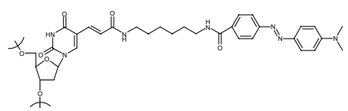
	500Å	1000Å
0.2 µmole	$40	$40
1.0 µmole	$50	$50
15 µmole	$400	$575



Abs. max: 453 nm
ε at Abs. max: 8,000

DABCYL-dT

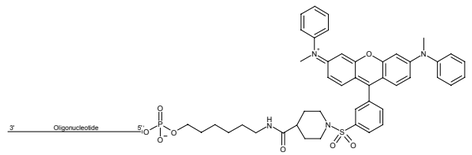
0.2 µmole	$140
1.0 µmole	$165
15 µmole	$600



Abs. max: 453 nm
ε at Abs. max: 22,700

QSY-7®*

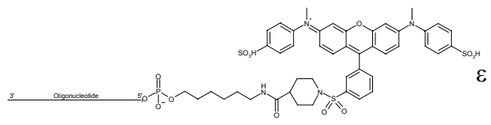
0.2 µmole	$175
1.0 µmole	$200
15 µmole	$700



Abs. max: 560 nm
ε at Abs. max: 90,000

QSY-9®*

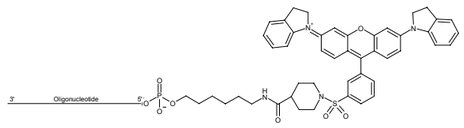
0.2 µmole	$175
1.0 µmole	$200
15 µmole	$700



Abs. max: 562 nm
ε at Abs. max: 88,000

QSY-21®*

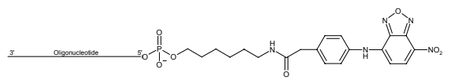
0.2 µmole	$175
1.0 µmole	$200
15 µmole	$700



Abs. max: 661 nm
ε at Abs. max: 90,000

QSY-35®*

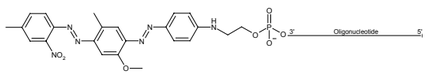
0.2 µmole	$175
1.0 µmole	$200
15 µmole	$700



Abs. max: 475 nm
ε at Abs. max: 23,000

Black Hole Quencher® 1 or 2

	5' or Internal	3' Terminus
0.2 µmole	$275	$75
1.0 µmole	$325	$150
15 µmole	$850	$1200



3' BHQ1 shown

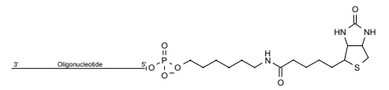
Abs. max: 480-580 nm
ε at Abs. max: 34,000

ε is noted in L mmole^{-1} cm^{-1}.
*These compounds require an extra purification step. See page 20 for details.

Non-Fluorescent Conjugates

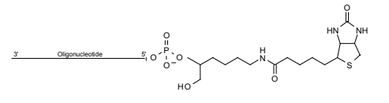
Biotin

	5' or Internal
0.2 µmole	$100
1.0 µmole	$125
15 µmole	$425



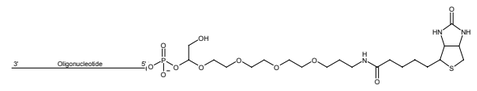
Biotin Diol Linker

	5' or Internal
0.2 µmole	$125
1.0 µmole	$150
15 µmole	$500



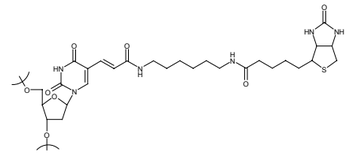
Biotin TEG

	5' Terminus	3' Terminus
0.2 µmole	$150	$75
1.0 µmole	$175	$125
15 µmole	$550	$700



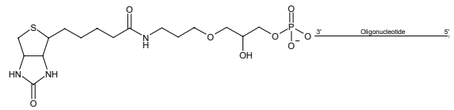
Biotin-dT

	5' or Internal
0.2 µmole	$175
1.0 µmole	$225
15 µmole	$900



Biotin BB

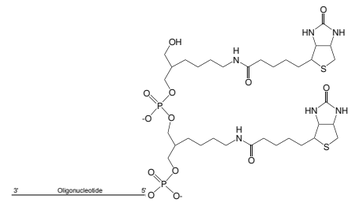
	3' Terminus	5' or Internal
0.2 µmole	$75	$100
1.0 µmole	$125	$125
15 µmole	$700	$425



3' Biotin BB shown

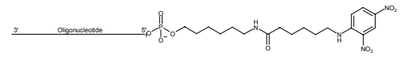
Dual Biotin

	5' Terminus
0.2 µmole	$250
1.0 µmole	$350
15 µmole	$900



DNP-X*

	5', 3' or Internal
0.2 µmole	$150
1.0 µmole	$175
15 µmole	$550

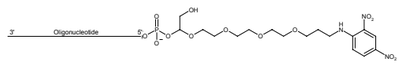


*These compounds require an extra purification step. See page 20 for details.

Non-Fluorescent Conjugates

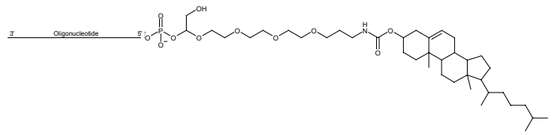
DNP TEG

	5' or Internal
0.2 µmole	$200
1.0 µmole	$225
15 µmole	$700



Cholesteryl TEG

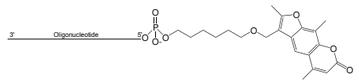
	5' Terminus	3' Terminus
0.2 µmole	$150	$75
1.0 µmole	$175	$125
15 µmole	$550	$700



Psoralen C6

Crosslinks to thymine or uracil when irradiated with longwave UV.

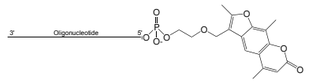
	5' or Internal
0.2 µmole	$125
1.0 µmole	$150
15 µmole	$475



Psoralen C2

Crosslinks to thymine or uracil when irradiated with longwave UV.

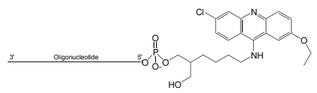
	5' or Internal
0.2 µmole	$125
1.0 µmole	$150
15 µmole	$475



Acridine

Used as an effective intercalating agent.

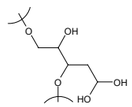
	3' Terminus	5' or Internal
0.2 µmole	$50	$225
1.0 µmole	$100	$250
15 µmole	$725	$750



Abasic

Used to create highly base-labile abasic site.

	5' or Internal
0.2 µmole	Inquire
1.0 µmole	Inquire
15 µmole	Inquire



Did You Know?

TriLink DNA Synthesis Reagents are now sold through Glen Research. To purchase any of the following products, please contact Glen Research via the phone, email or web address below.

- MMT- and TFA-C6-Amino Linker
- C6 and C3 Amino Modifier
- Spacer 18, Spacer 9, C-3 Spacer
- DADE, Carboxy Modifier
- Propyl- and Dabcyl-CPG
- CleanAmp™ Amidites

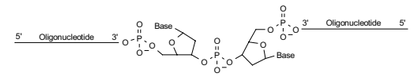
Phone: 703-437-6191 Email: orders@glenres.com Website: www.glenres.com

Inverted Oligonucleotides

There are many applications for reverse linked nucleotide modifications of oligonucleotides. They impart nuclease resistance and allow the study of strand orientation effects on molecular biological function. When placed internally the remaining portion of the oligo must be synthesized with reversed polarity. This and other such complicated constructs, must be quoted on a case-by-case basis. Please see Internal modification prices below to determine the cost of the reversed polarity portion of your oligonucleotide, if applicable.

3'-3' Modifications

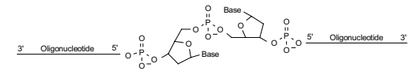
3' Terminus		Internal	
0.2 µmole	$30	0.2 µmole	$70
1.0 µmole	$50	1.0 µmole	$75
15 µmole	$400	15 µmole	$300



Internal 3'-3' linkage shown

5'-5' Modifications

5' Terminus		Internal	
0.2 µmole	$70	0.2 µmole	$70
1.0 µmole	$75	1.0 µmole	$75
15 µmole	$300	15 µmole	$300



Internal 5'-5' linkage shown

Terminal Phosphates

Phosphates can be placed on the 3' or 5' termini of oligonucleotides. A 3' terminal phosphate can be used to block elongation although other modifications, such as 3' Propyl or a 2',3'-Dideoxy modified base, are more effective terminators. A 5' terminal phosphate is needed for enzymatic ligation of two oligonucleotides to form longer oligonucleotides.

5' Phosphate

with PAGE purification		with HPLC purification	
0.2 µmole	$50	0.2 µmole	$100
1.0 µmole	$75	1.0 µmole	$100
15 µmole	$275	15 µmole	$375

5' Phosphorylation requires additional processing in order to ensure high quality and yield.

3' Phosphate

0.2 µmole	$30
1.0 µmole	$50
15 µmole	$400

Linkers

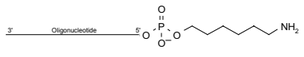
TriLink offers a wide range of linkers, allowing flexibility in oligonucleotide design. Linkers can be placed at the 3' terminus, 5' terminus or internally. The most common linkers are listed below. If TriLink is synthesizing a conjugated oligonucleotide for you, the 5' or 3' C6-Amino linker is included for no additional charge at 0.2 and 1.0 µmole scales.

5' and 3' Amino Linkers

These amino linkers are used to prepare oligonucleotides for most conjugations. The most common coupling partner is a succinimidyl ester (SE or NHS ester) to a primary amine, forming a covalent amide bond.

5' C6 Amino Linker

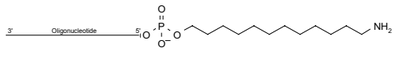
0.2 µmole	$45
1.0 µmole	$50
15 µmole	$250



5' C12 Amino Linker

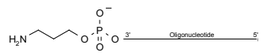
0.2 µmole	$125
1.0 µmole	$150
15 µmole	$400

Additional 0.2 or 1.0 µmole scale couplings are only $35.



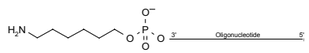
3' C3 Amino Linker

0.2 µmole	$30
1.0 µmole	$50
15 µmole	$400



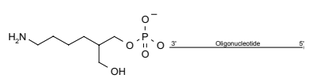
3' C6 Amino Linker

0.2 µmole	$20
1.0 µmole	$40
15 µmole	$325



3' C7 Amino Linker

0.2 µmole	$30
1.0 µmole	$50
15 µmole	$400



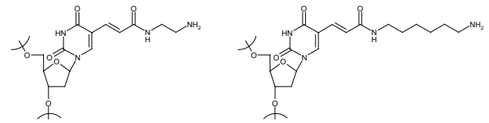
Linkers

Selective Placement Amino Linkers

The nucleoside linkers are attached to the pyrimidine base at the 5 position and the purine at the 8 position, which has a very moderate effect on hybridization with the corresponding target. Therefore you can place a modification such as a fluorescent dye where you want it.

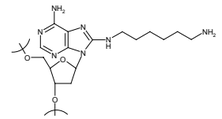
Thymidine-5-C2 and C6 Amino Linker

	5' or Internal	3' Terminus (C-6 only)
0.2 µmole	$125	$75
1.0 µmole	$150	$100
15 µmole	$525	$650



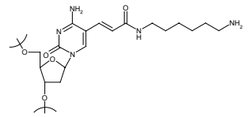
2'-Deoxyadenosine-8-C6 Amino Linker

	5' or Internal
0.2 µmole	$150
1.0 µmole	$175
15 µmole	$575



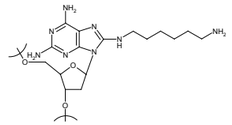
2'-Deoxycytidine-5-C6 Amino Linker

	5' or Internal	3' Terminus
0.2 µmole	$150	$100
1.0 µmole	$175	$125
15 µmole	$625	$700



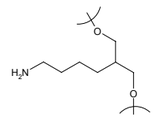
2'-Deoxyguanosine-8-C6 Amino Linker

	5' or Internal
0.2 µmole	$275
1.0 µmole	$300
15 µmole	$1150



C7 Internal Amino Linker

0.2 µmole	$125
1.0 µmole	$150
15 µmole	$400



Did You Know?

TriLink is the only licensee of the patented Phthalimidyl-3'-C6-Amino-CPG and Phthalimidyl-3'-C6-Amino-CPG reagents. We offer this product as an oligonucleotide modification; see product details on page 34. We also sell the support, exclusively through Glen Research.

The phthalimidyl protected 3' amino linkers are the best available on the market. Because the amine is buried within the linker, it is not subject to the loss of the protecting group and capping during the subsequent oligonucleotide synthesis cycles that occur with the alternatives. This capping side reaction can substantially lower the yield of amine labeled oligonucleotide available for conjugation. We use this reagent routinely for the manufacture of the more unusual oligonucleotides with multiple modifications ordered by our customers.

Linkers

5' and 3' Thiol Linkers

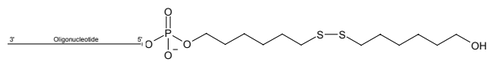
Thiol linkers can be used to form either reversible disulfide bonds or stable thiol ether linkages with maleimides. Care must be used when working with mercaptan labeled oligos. They will dimerize unless they are stored in a non-oxidizing environment. To avoid dimerization issues we recommend that you take delivery of the protected thiol (disulfide) form shown below. The reduction of the disulfide with TCEP can be done at the same time the maleimide is conjugated.

The purified yields of thiol modified oligonucleotides are often lower than the analogous amino modified oligonucleotide.

5' C6 Disulfide Linker

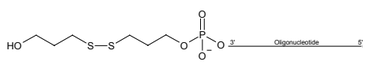
0.2 µmole	$180
1.0 µmole	$180
15 µmole	$500

Additional 0.2 or 1.0 µmole scale couplings only $35.



3' C3 Disulfide Linker

0.2 µmole	$75
1.0 µmole	$120
15 µmole	$500

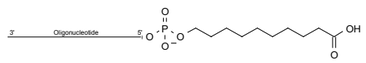


Carboxyl Linkers

Using our patented DADE Linker, we can readily prepare 5' carboxyl linkers. This linker can be used to conjugate to amines using conventional carbodiimide chemistry. Though less convenient than the solid phase method described in the Technical Information section, solution phase methods may be necessary at times. The DADE linker is also useful for conjugation to amine bearing supports for microchip assembly.

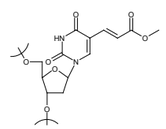
DADE Linker (5' Carboxyl Linker)

0.2 µmole	$125
1.0 µmole	$150
15 µmole	$400



Carboxy-dT Linker

0.2 µmole	$150
1.0 µmole	$175
15 µmole	$575



Did You Know?

TriLink's DADE (decanoic acid diester) linker is unique on the market. It offers a novel way of preparing conjugates more economically and with much more flexibility. It was originally designed to allow high-throughput screening of a large number of conjugates for therapeutic application. However, it has several other inherent advantages that make it a powerful tool for the scale up of oligonucleotide conjugates as well. We now sell our patented DADE Synthesis Reagent through Glen Research.

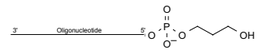
Spacers

Spacers impart a number of desirable characteristics, such as stability to enzymatic degradation. Multiple spacers can be placed end on end to create extremely long spacers. They can also be placed between other oligonucleotide modifications. Even though these spacers are shown on the 5' terminus of the oligonucleotide, they can be placed internally as well. The C3 (Propyl) Spacer can also be placed at the 3' terminus and is an effective terminator.

C3 (Propyl) Spacer

	3' Terminus	5' or Internal
0.2 µmole	$30	$75
1.0 µmole	$50	$100
15 µmole	$300	$250

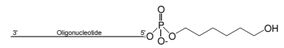
Additional 5' or internal 0.2 or 1.0 µmole scale couplings only $35.



5' version shown

C6 Spacer

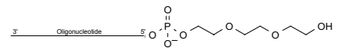
0.2 µmole	$150
1.0 µmole	$150
15 µmole	$400



Additional 0.2 or 1.0 µmole scale couplings only $50.

Spacer 9 (Triethylene glycol; PEG-150)

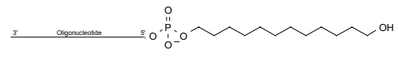
0.2 µmole	$125
1.0 µmole	$125
15 µmole	$300



Additional 0.2 or 1.0 µmole scale couplings only $35.

C12 Spacer

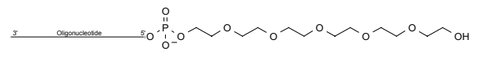
0.2 µmole	$140
1.0 µmole	$140
15 µmole	$350



Additional 0.2 or 1.0 µmole scale couplings only $35.

Spacer 18 (Hexaethylene glycol; PEG-282)

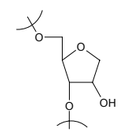
0.2 µmole	$140
1.0 µmole	$140
15 µmole	$350



Additional 0.2 or 1.0 µmole scale couplings only $35.

rSpacer

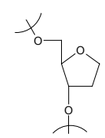
0.2 µmole	$225
1.0 µmole	$250
15 µmole	$650



Additional 0.2 or 1.0 µmole scale couplings only $100.

dSpacer

0.2 µmole	$135
1.0 µmole	$135
15 µmole	$350



Additional 0.2 or 1.0 µmole scale couplings only $35.

Modified Bases for Oligonucleotides

TriLink can incorporate many different modified bases into your oligonucleotide. Our "hands-on" approach to oligonucleotide synthesis is uniquely suited to this chemistry. The following pages contain structure and pricing information. Please inquire if the modification you desire is not listed. TriLink also offers custom phosphoramidite synthesis, see page 51.

Available Modified Bases

2' Deoxyribonucleoside (DNA)

Purines
- 2-Aminopurine-2'-deoxyriboside
- 8-Amino-2'-deoxyadenosine
- 8-Amino-2'-deoxyguanosine
- 8-Bromo-2'-deoxyadenosine
- 8-Bromo-2'-deoxyguanosine
- 7-Deaza-8-aza-2'-deoxyadenosine
- 7-Deaza-2'-deoxyadenosine
- 7-Deaza-2'-deoxyguanosine
- 7-Deaza-2'-deoxyxanthosine
- 2,6-Diaminopurine-2'-deoxyriboside
- Etheno-2'-deoxyadenosine
- N^6-Methyl-2'-deoxyadenosine
- O^6-Methyl-2'-deoxyguanosine
- O^6-Phenyl-2'-deoxyinosine
- 8-Oxo-2'-deoxyadenosine
- 8-Oxo-2'-deoxyguanosine
- 6-Thio-2'-deoxyguanosine

Pyrimidines
- 5'-Aminothymidine
- 5-Bromo-2'-deoxycytidine
- 5-Bromo-2'-deoxyuridine
- 5-(Carboxy)vinyl-2'-deoxyuridine
- 2'-Deoxypseudouridine
- 2'-Deoxyuridine
- 2,4-Difluorotoluyl
- 5,6-Dihydrothymidine
- 5,6-Dihydro-2'-deoxyuridine
- 5-(C2-EDTA)-2'-deoxyuridine
- N^4-Ethyl-2'-deoxycytidine
- 5-Fluoro-2'-deoxyuridine
- 5-Hydroxymethyl-2'-deoxyuridine
- 5-Hydroxy-2'-deoxycytidine
- 5-Hydroxy-2'-deoxyuridine
- 5-Iodo-2'-deoxycytidine
- 5-Iodo-2'-deoxyuridine
- 5-Methyl-2'-deoxycytidine
- O^4-Methylthymidine
- 5-Propynyl-2'-deoxycytidine
- 5-Propynyl-2'-deoxyuridine
- Pyrrolo-2'-deoxycytidine
- 4-Thio-2'-deoxyuridine
- 2-Thiothymidine
- 4-Thiothymidine
- Thymidine Glycol
- 6-O-TMP-5-F-2'-deoxyuridine
- C4-(1,2,4-Triazol-1-yl)-2'-deoxyuridine

Ribonucleoside (RNA)

- 2-Aminopurine
- 5-Bromouridine
- 2,6-Diaminopurine
- Inosine
- 5-Iodouridine
- 5-Methylcytidine
- 5-Methyluridine
- Puromycin
- Pyrrolocytidine
- 4-Thiouridine

2'-O-Methyl Ribonucleosides (2' OMe RNA)

- 2-Aminopurine-2'-O-methylriboside
- 5-Bromo-2'-O-methyluridine
- 3-Deaza-5-aza-2'-O-methylcytidine
- 2,6-Diaminopurine-2'-O-methylribose
- 5-Fluoro-2'-O-methyluridine
- 5-Fluoro-4-O-TMP-2'-O-methyluridine
- 2'-O-Methylinosine
- 5-Methyl-2'-O-methylcytidine
- 5-Methyl-2'-O-methylthymidine

Sugar Modified

- Aracytidine
- 3'-Deoxyadenosine
- 3'-Deoxycytidine
- 3'-Deoxyguanosine
- 3'-Deoxythymidine
- 2',3'-Dideoxyadenosine
- 2',3'-Dideoxycytidine
- 2',3'-Dideoxyguanosine
- 2',3'-Dideoxythymidine
- 5'-Iodothymidine
- 5'-O-Methylthymidine

Wobble and Universal Bases

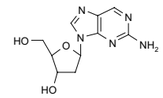
- 2'-Deoxyinosine
- 2'-Deoxyisoguanosine
- 2'-Deoxynebularine
- K-2'-deoxyribose
- 5-Methyl-2'-Deoxyisocytidine
- P-2'-deoxyribose

2' Deoxyribonucleoside Analogs - Purines

2-Aminopurine-2'-deoxyriboside

Use: Structure/activity studies

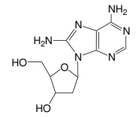
0.2 µmole	$125
1.0 µmole	$150
15 µmole	$375



8-Amino-2'-deoxyadenosine

Use: Triplex formation

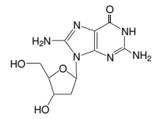
0.2 µmole	$200
1.0 µmole	$250
15 µmole	$700



8-Amino-2'-deoxyguanosine

Use: Triplex formation

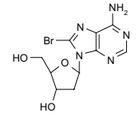
0.2 µmole	$200
1.0 µmole	$250
15 µmole	$700



8-Bromo-2'-deoxyadenosine

Use: Crystallography, protein cross-linking studies

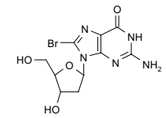
0.2 µmole	$110
1.0 µmole	$125
15 µmole	$325



8-Bromo-2'-deoxyguanosine

Use: Crystallography, protein cross-linking studies

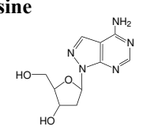
0.2 µmole	$100
1.0 µmole	$110
15 µmole	$300



7-Deaza-8-aza-2'-deoxyadenosine

Use: Duplex stabilization

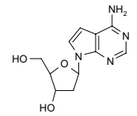
0.2 µmole	$200
1.0 µmole	$250
15 µmole	$325



7-Deaza-2'-deoxyadenosine

Use: Structure/activity studies

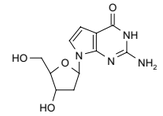
0.2 µmole	$200
1.0 µmole	$250
15 µmole	$700



7-Deaza-2'-deoxyguanosine

Use: Structure/activity studies

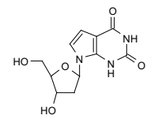
0.2 µmole	$200
1.0 µmole	$250
15 µmole	$700



7-Deaza-2'-deoxyxanthosine

Use: Structure/activity studies

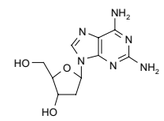
0.2 µmole	$200
1.0 µmole	$250
15 µmole	$700



2,6-Diaminopurine-2'-deoxyriboside

Use: Duplex stabilization

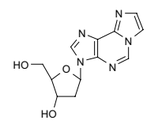
0.2 µmole	$100
1.0 µmole	$110
15 µmole	$325



Etheno-2'-deoxyadenosine

Use: Fluorescent nucleoside for structure/activity studies

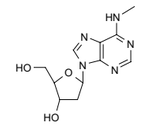
0.2 µmole	$100
1.0 µmole	$115
15 µmole	$325



N^6-Methyl-2'-deoxyadenosine

Use: Mutagenic effect studies

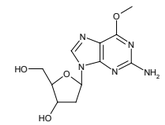
0.2 µmole	$130
1.0 µmole	$145
15 µmole	$425



O^6-Methyl-2'-deoxyguanosine

Use: Mutagenic effect studies

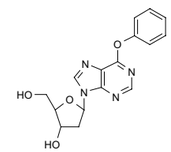
0.2 µmole	$100
1.0 µmole	$115
15 µmole	$325



O^6-Phenyl-2'-deoxyinosine

Use: Convertible nucleoside for structure/activity studies

0.2 µmole	$125
1.0 µmole	$150
15 µmole	$375

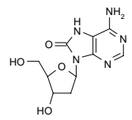


2' Deoxyribonucleoside Analogs - Purines continued

8-Oxo-2'-deoxyadenosine

Use: Structure/activity studies of damaged bases

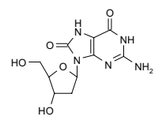
0.2 µmole	$125
1.0 µmole	$150
15 µmole	$375



8-Oxo-2'-deoxyguanosine

Use: Structure/activity studies of damaged bases

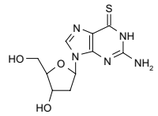
0.2 µmole	$200
1.0 µmole	$250
15 µmole	$700



6-Thio-2'-deoxyguanosine

Use: Photo-crosslinking and photoaffinity labeling studies

0.2 µmole	$225
1.0 µmole	$250
15 µmole	$775



Did You Know?

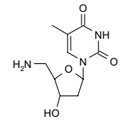
Prices listed are per coupling. You will receive a 25% discount on the second coupling and a 50% discount on all additional couplings of a particular base within a single oligonucleotide.

2' Deoxyribonucleoside Analogs - Pyrimidines

5'-Aminothymidine

Use: Ligation blocking, peptide fusion

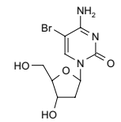
	5' Terminus
0.2 µmole	$120
1.0 µmole	$135
15 µmole	$400



5-Bromo-2'-deoxycytidine

Use: Crystallography, protein cross-linking studies

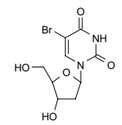
0.2 µmole	$90
1.0 µmole	$100
15 µmole	$250



5-Bromo-2'-deoxyuridine

Use: Crystallography, protein cross-linking studies

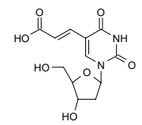
0.2 µmole	$90
1.0 µmole	$100
15 µmole	$250



5-(Carboxy)vinyl-2'-deoxyuridine (Carboxy Thymidine)

Use: Conjugation to amines

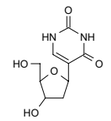
0.2 µmole	$130
1.0 µmole	$145
15 µmole	$450



2'-Deoxypseudouridine

Use: Structure/activity studies

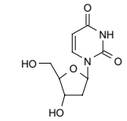
0.2 µmole	$180
1.0 µmole	$200
15 µmole	$600



2'-Deoxyuridine

Use: Duplex destabilization

0.2 µmole	$90
1.0 µmole	$100
15 µmole	$250



2,4-Difluorotoluyl

Use: Non-polar thymidine mimic

0.2 µmole	$250
1.0 µmole	$275
15 µmole	Inquire

5,6-Dihydrothymidine

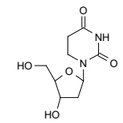
Use: Structure/activity studies of damaged bases

0.2 µmole	$160
1.0 µmole	$175
15 µmole	$450

5,6-Dihydro-2'-deoxyuridine

Use: Structure/activity studies of damaged bases

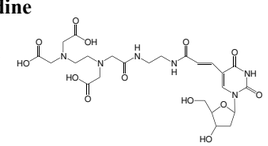
0.2 µmole	$160
1.0 µmole	$175
15 µmole	$450



5-(C2-EDTA)-2'-deoxyuridine

Use: Sequence specific cleavage of single & double-stranded DNA & RNA

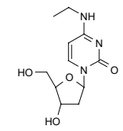
0.2 µmole	$350
1.0 µmole	$375
15 µmole	$1000



N⁴-Ethyl-2'-deoxycytidine

Use: Duplex destabilization

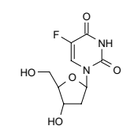
0.2 µmole	$165
1.0 µmole	$180
15 µmole	$475



5-Fluoro-2'-deoxyuridine

Use: Crystallography, protein cross-linking studies

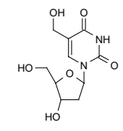
0.2 µmole	$115
1.0 µmole	$130
15 µmole	$350



5-Hydroxymethyl-2'-deoxyuridine

Use: Structure/activity studies of damaged bases

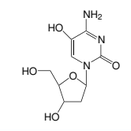
0.2 µmole	$165
1.0 µmole	$180
15 µmole	$475



5-Hydroxy-2'-deoxycytidine

Use: Structure/activity studies of damaged bases

0.2 µmole	$200
1.0 µmole	$225
15 µmole	$625

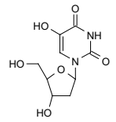


2' Deoxyribonucleoside Analogs - Pyrimidines continued

5-Hydroxy-2'-deoxyuridine

Use: Structure/activity studies of damaged bases

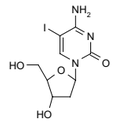
0.2 µmole	$165
1.0 µmole	$180
15 µmole	$475



5-Iodo-2'-deoxycytidine

Use: Crystallography, protein cross-linking studies

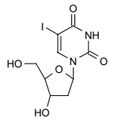
0.2 µmole	$115
1.0 µmole	$130
15 µmole	$350



5-Iodo-2'-deoxyuridine

Use: Crystallography, protein cross-linking studies

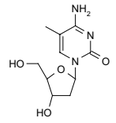
0.2 µmole	$90
1.0 µmole	$100
15 µmole	$250



5-Methyl-2'-deoxycytidine

Use: Duplex stabilization

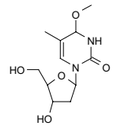
0.2 µmole	$90
1.0 µmole	$100
15 µmole	$300



O⁴-Methylthymidine

Use: Mutagenic effect studies

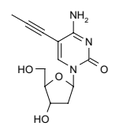
0.2 µmole	$115
1.0 µmole	$130
15 µmole	$350



5-Propynyl-2'-deoxycytidine

Use: Duplex stabilization

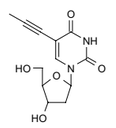
0.2 µmole	$100
1.0 µmole	$150
15 µmole	$300



5-Propynyl-2'-deoxyuridine

Use: Duplex stabilization

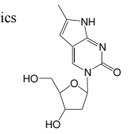
0.2 µmole	$100
1.0 µmole	$150
15 µmole	$300



Pyrrolo-2'-deoxycytidine

Use: Probing of DNA structure and dynamics

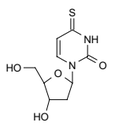
0.2 µmole	$170
1.0 µmole	$200
15 µmole	$600



4-Thio-2'-deoxyuridine

Use: Photocrosslinking and photoaffinity labeling experiments

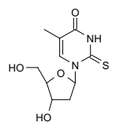
0.2 µmole	$200
1.0 µmole	$225
15 µmole	$650



2-Thiothymidine

Use: Protein-DNA interaction studies

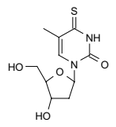
0.2 µmole	$200
1.0 µmole	$225
15 µmole	$650



4-Thiothymidine

Use: Photocrosslinking and photoaffinity labeling experiments

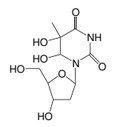
0.2 µmole	$200
1.0 µmole	$225
15 µmole	$650



Thymidine Glycol

Use: DNA repair enzyme studies

0.2 µmole	$250
1.0 µmole	$275
15 µmole	$850

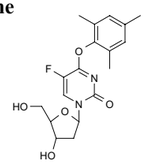


Deprotected Version Shown

6-O-(TMP)-5-F-2'-deoxyuridine

Use: Convertible nucleoside for structure/activity studies (converts to 5-F-dC)

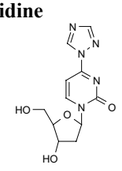
0.2 µmole	$160
1.0 µmole	$175
15 µmole	$450



C4-(1,2,4-Triazol-1-yl)-2'-deoxyuridine

(O4-Triazolyl-2'-deoxyuridine)
Use: Convertible nucleoside for structure/activity studies

0.2 µmole	$115
1.0 µmole	$130
15 µmole	$350

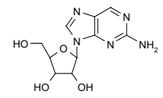


Ribonucleoside Analogs

2-Aminopurine

Use: Structure/activity studies

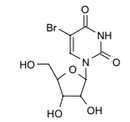
0.2 µmole	$225
1.0 µmole	$250
15 µmole	$800



5-Bromouridine

Use: Crystallography, protein cross-linking studies

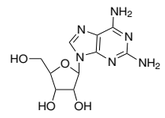
0.2 µmole	$150
1.0 µmole	$175
15 µmole	$450



2,6-Diaminopurine

Use: Duplex stabilization

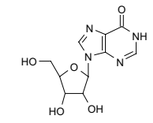
0.2 µmole	$225
1.0 µmole	$250
15 µmole	$800



Inosine

Use: Degenerate site

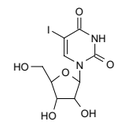
0.2 µmole	$150
1.0 µmole	$175
15 µmole	$450



5-Iodouridine

Use: Crystallography, protein cross-linking studies

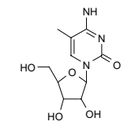
0.2 µmole	$150
1.0 µmole	$175
15 µmole	$450



5-Methylcytidine

Use: Duplex stabilization

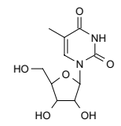
0.2 µmole	$150
1.0 µmole	$175
15 µmole	$450



5-Methyluridine

Use: Structure/activity studies

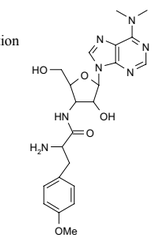
0.2 µmole	$150
1.0 µmole	$175
15 µmole	$450



Puromycin*

Use: Peptide-RNA fusion, blocks elongation

	3' Terminus
0.2 µmole	$75
1.0 µmole	$100
15 µmole	$575

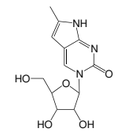


*Additional coupling discount does not apply.

Pyrrolocytidine

Use: Probing RNA structure, fluorescence is sensitive to environment

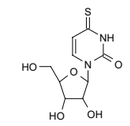
0.2 µmole	$250
1.0 µmole	$275
15 µmole	$850



4-Thiouridine

Use: Photocrosslinking and photoaffinity labeling experiments

0.2 µmole	$225
1.0 µmole	$250
15 µmole	$800

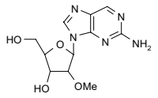


2'-O-Methyl Ribonucleoside Analogs

2-Aminopurine-2'-O-methylriboside

Use: Structure/activity studies

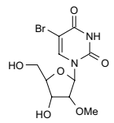
0.2 µmole	$200
1.0 µmole	$225
15 µmole	$650



5-Bromo-2'-O-methyluridine

Use: Crystallography, protein cross-linking studies

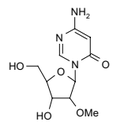
0.2 µmole	$165
1.0 µmole	$180
15 µmole	$475



3-Deaza-5-aza-2'-O-methylcytidine

Use: 5-azacytidine mimic

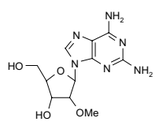
0.2 µmole	$200
1.0 µmole	$225
15 µmole	$650



2,6-Diaminopurine-2'-O-methylriboside

Use: Duplex stabilization

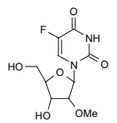
0.2 µmole	$200
1.0 µmole	$225
15 µmole	$650



5-Fluoro-2'-O-methyluridine

Use: Structure/activity studies

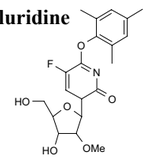
0.2 µmole	$200
1.0 µmole	$225
15 µmole	$650



5-Fluoro-4-O-TMP-2'-O-methyluridine

Use: Convertible nucleoside structure/ activity studies

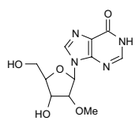
0.2 µmole	$200
1.0 µmole	$225
15 µmole	$650



2'-O-Methylinosine

Use: Degenerate site

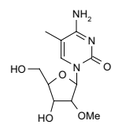
0.2 µmole	$120
1.0 µmole	$135
15 µmole	$400



5-Methyl-2'-O-methylcytidine

Use: Duplex stabilization

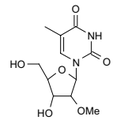
0.2 µmole	$120
1.0 µmole	$135
15 µmole	$400



5-Methyl-2'-O-methylthymidine

Use: Structure/activity studies

0.2 µmole	$120
1.0 µmole	$135
15 µmole	$400

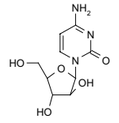


Sugar Modified Analogs

Aracytidine

Use: Structure/activity studies

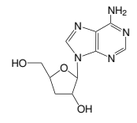
0.2 µmole	$115
1.0 µmole	$130
15 µmole	$350



3'-Deoxyadenosine

Use: Chain termination

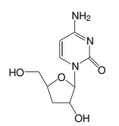
	3' Terminus
0.2 µmole	$75
1.0 µmole	$200
15 µmole	Inquire



3'-Deoxycytidine

Use: Chain termination

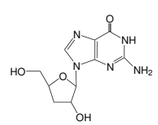
	3' Terminus
0.2 µmole	$75
1.0 µmole	$200
15 µmole	Inquire



3'-Deoxyguanosine

Use: Chain termination

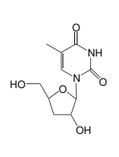
	3' Terminus
0.2 µmole	$75
1.0 µmole	$200
15 µmole	Inquire



3'-Deoxythymidine

Use: Chain termination

	3' Terminus
0.2 µmole	$75
1.0 µmole	$200
15 µmole	Inquire

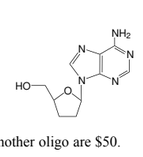


2',3'-Dideoxyadenosine

Use: Chain termination

0.2 µmole	$250
1.0 µmole	$250
15 µmole	Inquire

Additional 0.2 and 1.0 umol couplings on another oligo are $50. Requires PAGE purification.

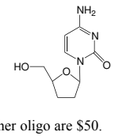


2',3'-Dideoxycytidine

Use: Chain termination

0.2 µmole	$250
1.0 µmole	$250
15 µmole	Inquire

Additional 0.2 and 1.0 umol couplings on another oligo are $50. Requires PAGE purification.

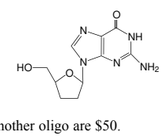


2',3'-Dideoxyguanosine

Use: Chain termination

0.2 µmole	$400
1.0 µmole	$400
15 µmole	Inquire

Additional 0.2 and 1.0 umol couplings on another oligo are $50. Requires PAGE purification.

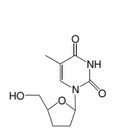


2',3'-Dideoxythymidine

Use: Chain termination

0.2 µmole	$250
1.0 µmole	$250
15 µmole	Inquire

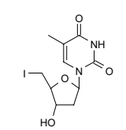
Additional 0.2 and 1.0 umol couplings on another oligo are $50. Requires PAGE purification.



5'-Iodothymidine

Use: Chain termination

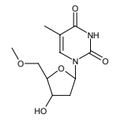
	5' Terminus
0.2 µmole	$115
1.0 µmole	$130
15 µmole	$350



5'-O-Methylthymidine

Use: Ligation blocking

	5' Terminus
0.2 µmole	$115
1.0 µmole	$130
15 µmole	$350

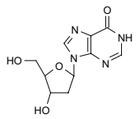


Wobble and Universal Bases

2'-Deoxyinosine

Use: Degenerate site

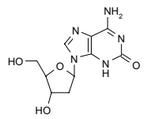
0.2 µmole	$90
1.0 µmole	$100
15 µmole	$250



2'-Deoxyisoguanosine

Use: Duplex stability studies

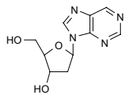
0.2 µmole	$130
1.0 µmole	$145
15 µmole	$400



2'-Deoxynebularine

Use: Degenerate site

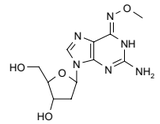
0.2 µmole	$90
1.0 µmole	$100
15 µmole	$300



K-2'-deoxyriboside

Use: Degenerate site

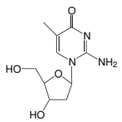
	3' Terminus	5' or Internal
0.2 µmole	$50	$160
1.0 µmole	$100	$175
15 µmole	Inquire	$450



5-Methyl-2'-Deoxyisocytidine

Use: Duplex stability studies

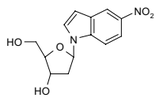
0.2 µmole	$115
1.0 µmole	$125
15 µmole	$275



5-Nitroindole-2'-deoxyriboside

Use: Universal base

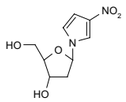
0.2 µmole	$110
1.0 µmole	$125
15 µmole	$300



3-Nitropyrrole-2'-deoxyriboside

Use: Universal base

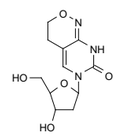
0.2 µmole	$110
1.0 µmole	$125
15 µmole	$300



P-2'-deoxyriboside

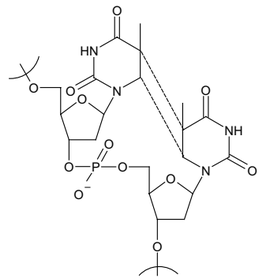
Use: Degenerate site

	3' Terminus	5' or Internal
0.2 µmole	$50	$160
1.0 µmole	$100	$175
15 µmole	Inquire	$450



Cis-Syn Thymidine Dimers

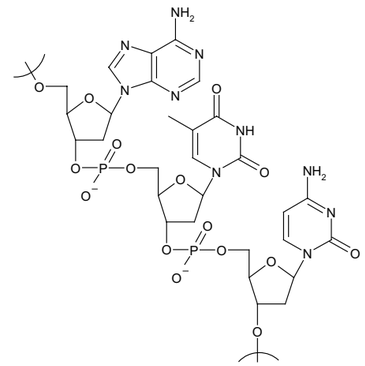
Cis-syn thymidine dimers are used in DNA damage and repair research. One of the major causes of DNA damage is UV from sunlight that results in dimerization of adjacent pyrimidine bases, forming cyclobutane dimers (CPDs). The majority of CPDs are cis-syn cyclobutane thymidine dimers. If you are interested in incorporating a cis-syn thymidine dimer in an oligonucleotide please inquire for a custom quotation.



Trimer Oligonucleotide Synthesis

TriLink now offers oligonucleotide synthesis using 2'-deoxynucleoside trimer phosphoramidites. This chemistry is the simplest approach to oligonucleotide-directed mutagenesis. Although there are 64 possible codon combinations, all 20 amino acids can be achieved with only 20 codons. Please inquire for a custom quotation. Learn more about trimer oligonucleotides at www.trilinkbiotech.com.

Trimer*	Amino Acid	Abbrev.	MW
d(AAA)	Lys	K	1911.5
d(AAC)	Asn	N	1887.5
d(ACT)	Thr	T	1774.5
d(ATC)	Ile	I	1774.5
d(ATG)	Met	M	1780.5
d(CAG)	Gln	Q	1869.5
d(CAT)	His	H	1774.5
d(CCG)	Pro	P	1845.5
d(CGT)	Arg	R	1756.5
d(CTG)	Leu	L	1756.5
d(GAA)	Glu	E	1893.5
d(GAC)	Asp	D	1869.5
d(GCT)	Ala	A	1756.5
d(GGT)	Gly	G	1762.5
d(GTT)	Val	V	1667.5
d(TAC)	Tyr	Y	1774.5
d(TCT)	Ser	S	1661.4
d(TGC)	Cys	C	1756.5
d(TGG)	Trp	W	1762.5
d(TTC)	Phe	F	1661.4



*Visit www.glenres.com for further information on trimer coding and physical parameters.

Stocked Randomer Oligonucleotide Products

Random Nonamers

5' Hydroxyl Random Nonamers (9mer "Wobble")

1 OD_{260} units	O-30103-01	$30
2 OD_{260} units	O-30103-02	$40

5' Amine Random Nonamers (9mer "Wobble")

1 OD_{260} units	O-30104-01	$30
2 OD_{260} units	O-30104-02	$40

6-FAM (Fluorescein) Antisense Oligo Controls

5' 6-FAM 20mer Phosphorothioate Oligonucleotides

2 OD_{260} units	O-30020-02	$35
5 OD_{260} units	O-30020-05	$50

S-18 Randomers

S-18 Randomer (Phosphorothioate 18mer Oligonucleotide)

50 nmoles	O-30030-50	$75

S-18 Randomer Control (Phosphodiester 18mer Oligonucleotide)

50 nmoles	O-30040-50	$75

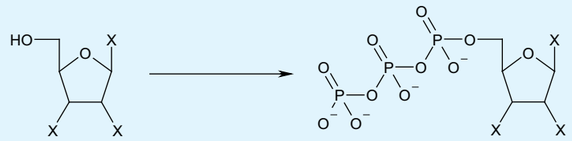
La Jolla, California © istockphoto.com/photo168

Nucleoside Triphosphates

It is likely that among TriLink's wide selection of modified nucleoside triphosphates you will find a compound to suit your application. Our nucleoside triphosphates are sold individually in 1, 5 and 10 µmole aliquots (approximately 0.5, 2.5 and 5 mg respectively) as 100 mM solutions in most cases. Please inquire for larger quantities or specific concentrations. All of our nucleotides are analyzed by HPLC, ^{31}P NMR, ^{1}H NMR, MS and UV Spectroscopy. Your order will be accompanied by a certificate of analysis and the HPLC analysis.

Custom Nucleoside Triphosphate Synthesis Service

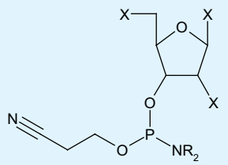
TriLink has developed detailed protocols to convert nucleosides into triphosphates. In this custom service the starting nucleoside can be made in house, customer supplied or purchased if it is commercially available. Each triphosphate is purified to greater than 90% by AX-HPLC and ^{31}P NMR and delivered as the lithium salt. Our service includes HPLC, ^{31}P NMR, ^{1}H NMR and MS analysis along with a certificate of analysis. Please email or call for a quote on your specific compound.



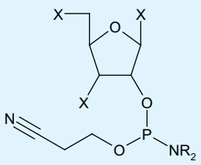
x = your imagination

Custom Phosphoramidite Synthesis Service

TriLink is unique in offering both custom phosphoramidite synthesis and incorporation into an oligonucleotide. As oligonucleotide chemists we understand how important it is to have high quality phosphoramidites going into each synthesis. In fact, we manufacture many of our own phosphoramidites including amino linkers, disulfides, spacers and our patented CleanAmp™ Amidites. If you would like a quotation for your custom phosphoramidite email quotes@trilinkbiotech.com. Please include the structure and the final yield required. All phosphoramidites are analyzed by RP-HPLC, ^{31}P NMR, ^{1}H NMR, MS and undergo a coupling test.



3' Phosphoramidite



2' Phosphoramidite

x = your imagination

Aminoallyl Nucleoside Triphosphates

Aminoallyl modified nucleoside triphosphates offer a more efficient method for high density labeling of DNA, than incorporation of pre-labeled NTPs. Following enzymatic incorporation of Aminoallyl NTPs, an amine reactive moiety, such as a fluorescent dye, biotin, hapten or an amine reactive protein, can be conjugated throughout the resultant DNA molecule. Aminoallyl NTPs are used for indirect DNA labeling in PCR, nick translation, primer extension reactions and cDNA synthesis.

Catalog #	Description	Page
N-2048	5-Aminoallyl-2'-deoxycytidine-5'-Triphosphate	56
N-2049	5-Aminoallyl-2'-deoxyuridine-5'-Triphosphate	56
N-1065	5-Aminoallylcytidine-5'-Triphosphate	62
N-1062	5-Aminoallyluridine-5'-Triphosphate	62

Biotin Nucleoside Triphosphates

Biotin labeled nucleic acids are frequently used in assays that require sensitive hybridization and strong detection, such as microarray target detection using labeled RNA. The quality of a labeled NTP is directly related to the level of incorporation; higher quality equals a higher level of incorporation. In a recent study, TriLink's Biotin-dUTP incorporated as much as 100%, as compared to the 30% mixture that limited the competitors products. TriLink offers both Biotin NTPs and Biotin-labeled custom oligonucleotides.

Catalog #	Description	Page
N-5001	Biotin-16-Aminoallyl-2'-deoxyuridine-5'-Triphosphate	57
N-5002	Biotin-16-Aminoallyl-2'-deoxycytidine-5'-Triphosphate	56
N-5003	Biotin-16-Aminoallylcytidine-5'-Triphosphate	63
N-5004	N^4-Biotin-OBEA-2'-deoxycytidine-5'-Triphosphate	63
N-5005	Biotin-16-Aminoallyluridine-5'-Triphosphate	63
N-5006	Biotin-16-7-Deaza-7-Aminoallyl-2'-deoxyguanosine-5'-Triphosphate	58
N-6003	5'-Biotin-G-Monophosphate	77
N-6004	5'-Biotin-A-Monophosphate	77
N-6005	5'-Biotin-dG-Monophosphate	77
N-6006	5'-Biotin-dA-Monophosphate	77

2' Fluoro Nucleoside Triphosphates

2' Fluoro nucleoside triphosphates are being utilized in an increasing number of applications in research and new drug development. Recently 2' Fluoro ATP was confirmed to be a universal tool for screening ATP-requiring enzymes in the investigation of pharamceutically applicable kinases. However, 2' Fluoro NTPs are most commonly enzymatically incorporated for improvement in (in vivo)italic stability in both DNA and RNA. They are used in the design and synthesis of aptamers, antagomirs and siRNA, because they impart increased target affinity and nuclease resistance while reducing immune response. 2' Fluoro NTPs are excellent substrates for both DNA and RNA polymerases, including the widely used T7 RNA polymerase.

Catalog #	Description	Page
N-1007	2'-Fluoro-2'-deoxyadenosine-5'-Triphosphate	71
N-1008	2'-Fluoro-2'-deoxycytidine-5'-Triphosphate	71
N-1009	2'-Fluoro-2'-deoxyguanosine-5'-Triphosphate	71
N-1010	2'-Fluoro-2'-deoxyuridine-5'-Triphosphate	72
N-1055	2'-Fluorothymidine-5'-Triphosphate	71

CAP and mCAP

CAP and mCAP are important reagents in the study of mRNA recognition and proper attachment of mRNA to the ribosome. CAP may also play a role in splicing and transport. In vivo(italics) addition of mCAP via a unique 5′-5′ linkage to the 5′ end of the immature mRNA is the process that makes mRNA ready for translation. CAP also provides significant resistance to 5′ exonucleases, allowing mRNA the time needed to get to the ribosome. TriLink is the least expensive source of CAP and mCAP in the market place.

Catalog #	Description	Page
N-7002	Guanosine-5'-Triphosphate-5'-Guanosine	74
N-7001	N^7-Me-Guanosine-5'-Triphosphate-5'-Guanosine	74

ε is noted in L $mmole^{-1}$ cm^{-1}.

NTP Quick Guide

Cat.#	Name	1 µmol	5 µmol	10 µmol	Pg
N-1001	2-Amino-ATP	$77.50	$365.00	$625.00	62
N-1002	2-Amino-6-Chloropurineriboside-TP	$97.50	$465.00	$850.00	62
N-1004	8-Aza-ATP	$250.00	$1,150.00	$2,250.00	62
N-1006	6-Chloropurineriboside-TP	$77.50	$365.00	$625.00	64
N-1007	2'-Fluoro-2'-dATP	$77.50	$365.00	$625.00	71
N-1008	2'-Fluoro-2'-dCTP	$52.50	$255.00	$415.00	71
N-1009	2'-Fluoro-2'-dGTP	$97.50	$465.00	$850.00	71
N-1010	2'-Fluoro-2'-dUTP	$52.50	$255.00	$415.00	72
N-1011	5-Iodo-CTP	$77.50	$365.00	$625.00	64
N-1012	5-Iodo-UTP	$77.50	$365.00	$625.00	64
N-1013	N^6-Methyl-ATP	$52.50	$255.00	$415.00	65
N-1014	5-Methyl-CTP	$77.50	$365.00	$625.00	64
N-1015	2'-O-Methyl-ATP	$27.50	$115.00	$205.00	72
N-1016	2'-O-Methyl-CTP	$27.50	$115.00	$205.00	72
N-1017	2'-O-Methyl-GTP	$27.50	$115.00	$205.00	72
N-1018	2'-O-Methyl-UTP	$27.50	$115.00	$205.00	73
N-1019	Pseudo-UTP	$77.50	$365.00	$625.00	65
N-1020	ITP	$52.50	$255.00	$415.00	64
N-1021	2'-O-Methyl-ITP	$77.50	$365.00	$625.00	72
N-1022	Puromycin-TP	$97.50	$465.00	$850.00	66
N-1023	Xanthosine-TP	$52.50	$255.00	$415.00	66
N-1024	5-Methyl-UTP	$77.50	$365.00	$625.00	65
N-1025	4-Thio-UTP	$97.50	$465.00	$850.00	66
N-1026	2'-Amino-2'-dCTP	$77.50	$365.00	$625.00	67
N-1027	2'-Amino-2'-dUTP	$77.50	$365.00	$625.00	67
N-1028	2'-Azido-2'-dCTP	$77.50	$365.00	$625.00	68
N-1029	2'-Azido-2'-dUTP	$77.50	$365.00	$625.00	68
N-1031	O^6-Methyl-GTP	$155.00	$695.00	$1,235.00	65
N-1032	2-Thio-UTP	$77.50	$365.00	$625.00	66
N-1033	Ara-CTP	$52.50	$255.00	$415.00	68
N-1034	Ara-UTP	$97.50	$465.00	$850.00	68
N-1035	5,6-Dihydro-UTP	$97.50	$465.00	$850.00	64
N-1036	2-Thio-CTP	$77.50	$365.00	$625.00	66
N-1037	6-Aza-CTP	$77.50	$365.00	$625.00	62
N-1038	6-Aza-UTP	$77.50	$365.00	$625.00	62
N-1039	N^1-Methyl-GTP	$77.50	$365.00	$625.00	65
N-1040	2'-O-Methyl-2-Amino-ATP	$77.50	$365.00	$625.00	72
N-1041	2'-O-Methylpseudo-UTP	$52.50	$255.00	$415.00	73
N-1042	N^1-Methyl-ATP	$77.50	$365.00	$625.00	65
N-1043	2'-O-Methyl-5-methyl-UTP	$52.50	$255.00	$415.00	72
N-1044	7-Deaza-GTP	$97.50	$465.00	$850.00	64

Cat.#	Name	1 µmol	5 µmol	10 µmol	Pg
N-1045	2'-Azido-2'-dATP	$77.50	$365.00	$625.00	68
N-1046	2'-Amino-2'-dATP	$52.50	$255.00	$415.00	67
N-1047	Benzimidazole-riboside-TP	$97.50	$465.00	$850.00	63
N-1048	Ara-ATP	$77.50	$365.00	$625.00	68
N-1052	8-Azido-ATP	$52.50	$255.00	$415.00	63
N-1053	5-Bromo-CTP	$155.00	$695.00	$1,235.00	63
N-1054	5-Bromo-UTP	$52.50	$255.00	$415.00	63
N-1055	2'-Fluoro-dTTP	$77.50	$365.00	$625.00	71
N-1056	3'-O-Methyl-ATP	$77.50	$365.00	$625.00	73
N-1057	3'-O-Methyl-CTP	$77.50	$365.00	$625.00	73
N-1058	3'-O-Methyl-GTP	$77.50	$365.00	$625.00	73
N-1059	3'-O-Methyl-UTP	$77.50	$365.00	$625.00	73
N-1061	7-Deaza-ATP	$77.50	$365.00	$625.00	64
N-1062	5-Aminoallyl-UTP	$52.50	$255.00	$415.00	62
N-1063	2'-Azido-2'-dGTP	$77.50	$365.00	$625.00	68
N-1064	2'-Amino-2'-dGTP	$77.50	$365.00	$625.00	67
N-1065	5-Aminoallyl-CTP	$77.50	$365.00	$625.00	62
N-1066	8-Oxo-GTP	$52.50	$255.00	$415.00	65
N-1067	2-Aminopurine-riboside-TP	$97.50	$465.00	$850.00	62
N-2002	2-Amino-6-chloropurine-2'-deoxyriboside-TP	$97.50	$465.00	$850.00	56
N-2003	2-Amino-2'-dATP	$77.50	$365.00	$625.00	56
N-2004	2-Aminopurine-2'-deoxyribose-TP	$97.50	$465.00	$850.00	56
N-2006	5-Bromo-2'-dCTP	$77.50	$365.00	$625.00	57
N-2008	5-Bromo-2'-dUTP	$27.50	$115.00	$205.00	57
N-2009	6-Chloropurine-2'-deoxyriboside-TP	$77.50	$365.00	$625.00	57
N-2010	7-Deaza-2'-dATP	$52.50	$255.00	$415.00	58
N-2011	7-Deaza-2'-dGTP	$155.00	$695.00	$1,235.00	58
N-2012	2'-dITP	$77.50	$365.00	$625.00	58
N-2016	5-Propynyl-2'-dCTP	$77.50	$365.00	$625.00	61
N-2017	5-Propynyl-2'-dUTP	$77.50	$365.00	$625.00	61
N-2019	Pseudo-2'-dUTP	$77.50	$365.00	$625.00	59
N-2020	2'-dUTP	$27.50	$115.00	$205.00	59
N-2022	5-Fluoro-2'-dUTP	$52.50	$255.00	$415.00	59
N-2023	5-Iodo-2'-dCTP	$97.50	$465.00	$850.00	59
N-2024	5-Iodo-2'-dUTP	$52.50	$255.00	$415.00	59
N-2025	N^6-Methyl-2'-dATP	$97.50	$465.00	$850.00	60
N-2026	5-Methyl-2'-dCTP	$52.50	$255.00	$415.00	60
N-2027	O^6-Methyl-2'-dGTP	$155.00	$695.00	$1,235.00	60
N-2028	N^2-Methyl-2'-dGTP	$250.00	$1,150.00	$2,250.00	60

NTP Quick Guide

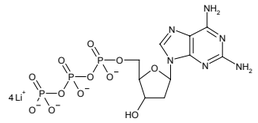
Cat.#	Name	1 µmol	5 µmol	10 µmol	Pg
N-2031	5-Nitro-1-Indolyl-2'-Deoxyribose-TP	$250.00	$1,150.00	$2,250.00	60
N-2033	8-Oxo-2'-dATP	$77.50	$365.00	$625.00	60
N-2034	8-Oxo-2'-dGTP	$77.50	$365.00	$625.00	60
N-2035	2-Thio-TTP	$97.50	$465.00	$850.00	61
N-2037	2'-dPTP	$97.50	$465.00	$850.00	58
N-2038	5-Hydroxy-2'-dCTP	$77.50	$365.00	$625.00	59
N-2039	5-Hydroxy-2'-dUTP	$97.50	$465.00	$850.00	59
N-2041	4-Thio-TTP	$77.50	$365.00	$625.00	61
N-2042	2-Thio-2'-dCTP	$97.50	$465.00	$850.00	61
N-2043	6-Aza-2'-dUTP	$77.50	$365.00	$625.00	56
N-2045	6-Thio-2'-dGTP	$250.00	$1,150.00	$2,250.00	61
N-2046	8-Chloro-2'-dATP	$77.50	$365.00	$625.00	57
N-2048	5-AA-2'-dCTP	$77.50	$365.00	$625.00	56
N-2049	5-AA-2'-dUTP	$52.50	$255.00	$415.00	56
N-2053	2'-Deoxy-L-ATP	$97.50	$465.00	$850.00	69
N-2054	2'-Deoxy-L-CTP	$97.50	$465.00	$850.00	69
N-2055	2'-Deoxy-L-GTP	$175.00	$775.00	$1,450.00	69
N-2056	2'-Deoxy-L-TTP	$97.50	$465.00	$850.00	70
N-2057	N^4-Methyl-2'-dCTP	$77.50	$365.00	$625.00	60
N-2058	2'-Deoxyzebularine-TP	$250.00	$1,150.00	$2,250.00	59
N-3001	3'-dATP	$77.50	$365.00	$625.00	70
N-3002	3'-dGTP	$77.50	$365.00	$625.00	70
N-3003	3'-dCTP	$77.50	$365.00	$625.00	70
N-3004	5-Methyl-3'-dUTP	$77.50	$365.00	$625.00	70
N-3005	3'-dUTP	$77.50	$365.00	$625.00	70
N-4001	2',3'-ddATP	$52.50	$255.00	$415.00	70
N-4002	2',3'-ddGTP	$52.50	$255.00	$415.00	71
N-4003	2',3'-ddUTP	$27.50	$115.00	$205.00	71
N-4004	2',3'-ddTTP	$27.50	$115.00	$205.00	71
N-4005	2',3'-ddCTP	$27.50	$115.00	$205.00	70
N-4007	3'-Azido-2',3'-ddATP	$97.50	$465.00	$850.00	68
N-4008	3'-Azido-2',3'-ddGTP	$97.50	$465.00	$850.00	69
N-4009	3'-Azido-3'-dTTP	$97.50	$465.00	$850.00	69
N-4010	3'-Amino-2',3'-ddATP	$175.00	$775.00	$1,450.00	67
N-4011	3'-Amino-2',3'-ddCTP	$175.00	$775.00	$1,450.00	67
N-4012	3'-Amino-2',3'-ddGTP	$175.00	$775.00	$1,450.00	67
N-4013	3'-Amino-3'-dTTP	$97.50	$465.00	$850.00	67
N-4014	3'-Azido-2',3'-ddCTP	$97.50	$465.00	$850.00	69

Cat.#	Name	1 µmol	5 µmol	10 µmol	Pg
N-4015	3'-Azido-2',3'-ddUTP	$97.50	$465.00	$850.00	69
N-4016	5-Bromo-2',3'-ddUTP	$97.50	$465.00	$850.00	69
N-4017	2',3'-ddITP	$155.00	$695.00	$1,235.00	71
N-5001	Biotin-16-AA-2'-dUTP	$525.00	N/A	N/A	57
N-5002	Biotin-16-AA-2'-dCTP	$525.00	N/A	N/A	56
N-5003	Biotin-16-AA-CTP	$525.00	N/A	N/A	63
N-5004	N^4Biotin-OBEA-2'-dCTP	$525.00	N/A	N/A	57
N-5005	Biotin-16-AA-UTP	$525.00	N/A	N/A	63
N-5006	7-Deaza-7-(Biotin-16)-AA-2'-dGTP	$725.00	N/A	N/A	58
N-5007	5-(Dabcyl-3-AA)-2'-dUTP	N/A	N/A	N/A	58
N-6001	ppGpp	$175.00	$775.00	$1,450.00	74
N-6002	pGp	$155.00	$695.00	$1,235.00	74
N-6003	5'-Biotin-GMP	$52.50	$255.00	$415.00	77
N-6004	5'-Biotin-AMP	$52.50	$255.00	$415.00	77
N-6005	5'-Biotin-dGMP	$52.50	$255.00	$415.00	77
N-6006	5'-Biotin-dAMP	$52.50	$255.00	$415.00	77
N-6009	5'-Amino-GMP	$52.50	$255.00	$415.00	77
N-6010	5'-Amino-AMP	$52.50	$255.00	$415.00	77
N-7001	mCAP	$77.50	$365.00	$625.00	74
N-7002	CAP	$77.50	$365.00	$625.00	74
N-8001	(1-Thio)-2'-dATP	$27.50	$115.00	$205.00	75
N-8002	(1-Thio)-2'-dCTP	$27.50	$115.00	$205.00	75
N-8003	(1-Thio)-2'-dGTP	$52.50	$255.00	$415.00	75
N-8004	(1-Thio)-2'-dTTP	$27.50	$115.00	$205.00	75
N-8005	(1-Thio)-ATP	$27.50	$115.00	$205.00	75
N-8006	(1-Thio)-CTP	$77.50	$365.00	$625.00	75
N-8007	(1-Thio)-GTP	$27.50	$115.00	$205.00	76
N-8008	(1-Thio)-UTP	$27.50	$115.00	$205.00	76
N-8009	(1-Thio)-2',3'-ddATP	$77.50	$365.00	$625.00	76
N-8010	(1-Thio)-2',3'-ddCTP	$77.50	$365.00	$625.00	76
N-8011	(1-Thio)-2',3'-ddGTP	$52.50	$255.00	$415.00	76
N-8012	(1-Thio)-2',3'-ddTTP	$52.50	$255.00	$415.00	76
N-8013	3'-Azido-(1-Thio)-2',3'-ddTTP	$97.50	$465.00	$850.00	75
N-8015	(1-Thio)-2',3'-ddUTP	$52.50	$255.00	$415.00	76
N-8051	(1-Borano)-2'-dCTP	$175.00	$775.00	$1,450.00	75
N-9001	Ganciclovir-TP	$77.50	$365.00	$625.00	72

Base Modified 2' Deoxynucleoside Triphosphates

2-Amino-2'-deoxyadenosine-5'-Triphosphate
(Diaminopurine deoxyribose)

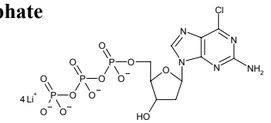
N-2003-1	1 µmole	$77.50
N-2003-5	5 µmoles	$365.00
N-2003-10	10 µmoles	$625.00



$C_{10}H_{17}N_6O_{12}P_3$
MW= 506.20 (free acid)
ε= 8743 @ 279 nm
Lithium salt
>90% purity

2-Amino-6-chloropurine-2'-deoxyriboside-5'-Triphosphate

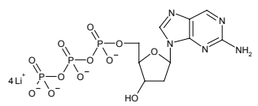
N-2002-1	1 µmole	$97.50
N-2002-5	5 µmoles	$465.00
N-2002-10	10 µmoles	$850.00



$C_{10}H_{15}N_5O_{12}P_3Cl$
MW= 525.63 (free acid)
ε= 6590 @ 306 nm
Lithium salt
>90% purity

2-Aminopurine-2'-deoxyriboside-5'-Triphosphate

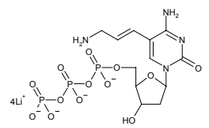
N-2004-1	1 µmole	$97.50
N-2004-5	5 µmoles	$465.00
N-2004-10	10 µmoles	$850.00



$C_{10}H_{16}N_5O_{12}P_3$
MW= 491.18 (free acid)
ε= 6422 @ 303 nm
Lithium salt
>90% purity

5-Aminoallyl-2'-deoxycytidine-5'-Triphosphate

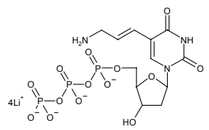
N-2048-1	1 µmole	$77.50
N-2048-5	5 µmoles	$365.00
N-2048-10	10 µmoles	$625.00



$C_{12}H_{21}N_4O_{13}P_3$
MW= 522.0 (free acid)
ε= 5041 @ 290 nm
Lithium salt
>90% purity

5-Aminoallyl-2'-deoxyuridine-5'-Triphosphate

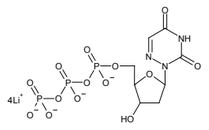
N-2049-1	1 µmole	$52.50
N-2049-5	5 µmoles	$255.00
N-2049-10	10 µmoles	$415.00



$C_{12}H_{20}N_3O_{14}P_3$
MW= 523.22 (free acid)
ε= 7100 @ 290 nm
Lithium salt
>90% purity

6-Aza-2'-deoxyuridine-5'-Triphosphate

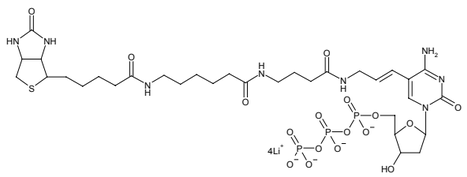
N-2043-1	1 µmole	$77.50
N-2043-5	5 µmoles	$365.00
N-2043-10	10 µmoles	$625.00



$C_8H_{14}N_3O_{14}P_3$
MW= 469.13 (free acid)
ε= 6254 @ 260 nm
Lithium salt
>90% purity

Biotin-16-Aminoallyl-2'-deoxycytidine-5'-Triphosphate

N-5002-050	50 nmoles	$197.50
N-5002-1	1 µmole	$525.00



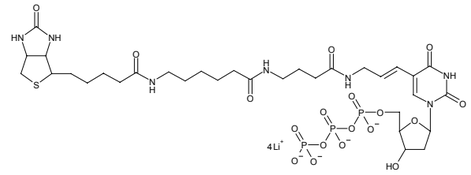
$C_{32}H_{53}N_8O_{17}P_3S$
MW= 947.80 (free acid)
ε= 7100 @ 290 nm
Lithium salt
>90% purity

ε is noted in L mmole^{-1} cm^{-1}.

Base Modified 2' Deoxynucleoside Triphosphates continued

Biotin-16-Aminoallyl-2'-deoxyuridine-5'-Triphosphate

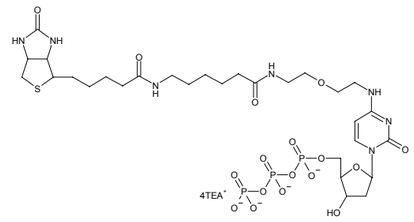
N-5001-050	50 nmoles	$197.50
N-5001-1	1 µmole	$525.00



$C_{32}H_{52}N_7O_{18}P_3S$
MW= 946.70 (free acid)
ε= 5040 @ 290 nm
Lithium salt
>90% purity

N⁴-Biotin-OBEA-2'-deoxycytidine-5'-Triphosphate

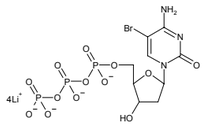
N-5004-050	50 nmoles	$197.50
N-5004-1	1 µmole	$525.00



$C_{29}H_{50}N_7O_{17}P_3S$
MW= 893.70 (free acid)
ε= 9100 @ 272 nm
Triethylammonium salt
>90% purity

5-Bromo-2'-deoxycytidine-5'-Triphosphate

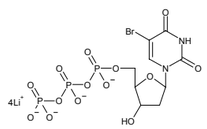
N-2006-1	1 µmole	$77.50
N-2006-5	5 µmoles	$365.00
N-2006-10	10 µmoles	$625.00



$C_9H_{15}N_3O_{13}P_3Br$
MW= 546.05 (free acid)
ε= 6825 @ 288 nm
Lithium salt
>90% purity

5-Bromo-2'-deoxyuridine-5'-Triphosphate

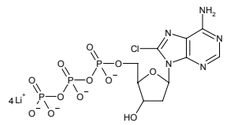
N-2008-1	1 µmole	$27.50
N-2008-5	5 µmoles	$115.00
N-2008-10	10 µmoles	$205.00



$C_9H_{14}N_2O_{14}P_3Br$
MW= 547.04 (free acid)
ε= 9220 @ 279 nm
Lithium salt
>90% purity

8-Chloro-2'-deoxyadenosine-5'-Triphosphate

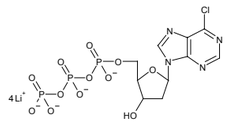
N-2046-1	1 µmole	$77.50
N-2046-5	5 µmoles	$365.00
N-2046-10	10 µmoles	$625.00



$C_{10}H_{15}N_5O_{12}P_3Cl$
MW= 525.63 (free acid)
ε= 14900 @ 262 nm
Lithium salt
>90% purity

6-Chloropurine-2'-deoxyriboside-5'-Triphosphate

N-2009-1	1 µmole	$77.50
N-2009-5	5 µmoles	$365.00
N-2009-10	10 µmoles	$625.00



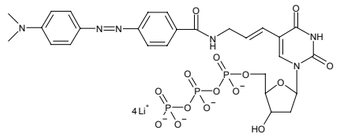
$C_{10}H_{14}N_4O_{12}P_3Cl$
MW= 510.61 (free acid)
ε= 6600 @ 264 nm
Lithium salt
>90% purity

ε is noted in L mmole^{-1} cm^{-1}.

Base Modified 2' Deoxynucleoside Triphosphates continued

5-(Dabcyl-3-Aminoallyl)-2'-deoxyuridine-5'-Triphosphate

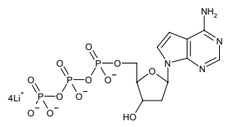
N-5007-0100	100 nmole	$197.50



$C_{27}H_{33}N_8O_{15}P_3$
MW= 774.51 (free acid)
ε= 32000 @ 467 nm
Lithium salt
>90% purity

7-Deaza-2'-deoxyadenosine-5'-Triphosphate

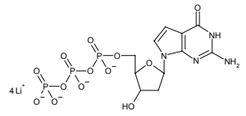
N-2010-1	1 µmole	$52.50
N-2010-5	5 µmoles	$255.00
N-2010-10	10 µmoles	$415.00



$C_{11}H_{17}N_4O_{12}P_3$
MW= 490.20 (free acid)
ε= 9500 @ 271 nm
Lithium salt
>90% purity

7-Deaza-2'-deoxyguanosine-5'-Triphosphate

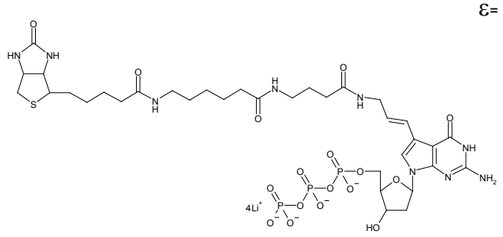
N-2011-1	1 µmole	$155.00
N-2011-5	5 µmoles	$695.00
N-2011-10	10 µmoles	$1235.00



$C_{11}H_{17}N_4O_{13}P_3$
MW= 506.00 (free acid)
ε= 10069 @ 259 nm
Lithium salt
>90% purity

7-Deaza-7-(Biotin-16)aminoallyl-2'-deoxyguanosine-5'-Triphosphate

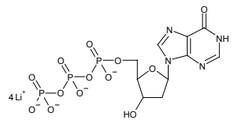
N-5006-050	50 nmoles	$215.00
N-5006-1	1 µmole	$725.00



$C_{34}H_{54}N_9O_{17}P_3S$
MW= 985.80 (free acid)
ε= 10100 @ 298 nm
Lithium salt
>90% purity

2'-Deoxyinosine-5'-Triphosphate

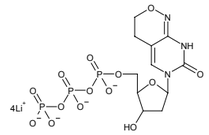
N-2012-1	1 µmole	$77.50
N-2012-5	5 µmoles	$365.00
N-2012-10	10 µmoles	$625.00



$C_{10}H_{15}N_4O_{13}P_3$
MW= 492.17 (free acid)
ε= 12300 @ 248.5 nm
Lithium salt
>90% purity

2'-Deoxy-P-nucleoside-5'-Triphosphate

N-2037-1	1 µmole	$97.50
N-2037-5	5 µmoles	$465.00
N-2037-10	10 µmoles	$850.00



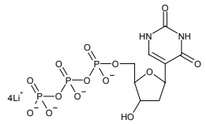
$C_{11}H_{18}N_3O_{14}P_3$
MW= 509.20 (free acid)
ε= 6473 @ 293 nm
Lithium salt
>90% purity

ε is noted in L mmole^{-1} cm^{-1}.

Base Modified 2' Deoxynucleoside Triphosphates continued

2'-Deoxypseudouridine-5'-Triphosphate

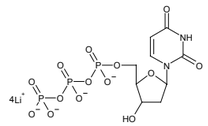
N-2019-1	1 µmole	$77.50
N-2019-5	5 µmoles	$365.00
N-2019-10	10 µmoles	$625.00



$C_9H_{15}N_2O_{14}P_3$
MW= 468.14 (free acid)
ε= 7385 @ 260 nm
Lithium salt
>90% purity

2'-Deoxyuridine-5'-Triphosphate

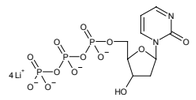
N-2020-1	1 µmole	$27.50
N-2020-5	5 µmoles	$115.00
N-2020-10	10 µmoles	$205.00



$C_9H_{15}N_2O_{14}P_3$
MW= 468.14 (free acid)
ε= 10100 @ 262 nm
Lithium salt
>90% purity

2'-Deoxyzebularine-5'-Triphosphate

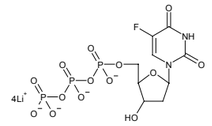
N-2058-1	1 µmole	$250.00
N-2058-5	5 µmoles	$1150.00
N-2058-10	10 µmoles	$2250.00



$C_9H_{12}N_2O_4$
MW= 458.19 (free acid)
ε= 5800 @ 302 nm
Lithium salt
>90% purity

5-Fluoro-2'-deoxyuridine-5'-Triphosphate

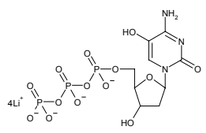
N-2022-1	1 µmole	$52.50
N-2022-5	5 µmoles	$255.00
N-2022-10	10 µmoles	$415.00



$C_9H_{14}N_2O_{14}P_3F$
MW= 486.13 (free acid)
ε= 7350 @ 268 nm
Lithium salt
>90% purity

5-Hydroxy-2'-deoxycytidine-5'-Triphosphate

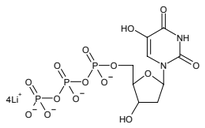
N-2038-1	1 µmole	$77.50
N-2038-5	5 µmoles	$365.00
N-2038-10	10 µmoles	$625.00



$C_9H_{16}N_3O_{14}P_3$
MW= 483.16 (free acid)
ε= 6500 @ 293 nm
Lithium salt
>90% purity

5-Hydroxy-2'-deoxyuridine-5'-Triphosphate

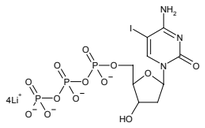
N-2039-1	1 µmole	$97.50
N-2039-5	5 µmoles	$465.00
N-2039-10	10 µmoles	$850.00



$C_9H_{15}N_2O_{15}P_3$
MW= 484.14 (free acid)
ε= 8200 @ 280 nm
Lithium salt
>90% purity

5-Iodo-2'-deoxycytidine-5'-Triphosphate

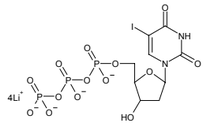
N-2023-1	1 µmole	$97.50
N-2023-5	5 µmoles	$465.00
N-2023-10	10 µmoles	$850.00



$C_9H_{15}N_3O_{13}P_3I$
MW= 593.05 (free acid)
ε= 5300 @ 294 nm
Lithium salt
>90% purity

5-Iodo-2'-deoxyuridine-5'-Triphosphate

N-2024-1	1 µmole	$52.50
N-2024-5	5 µmoles	$255.00
N-2024-10	10 µmoles	$415.00

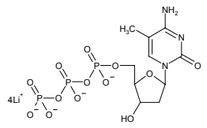


$C_9H_{14}N_2O_{14}P_3I$
MW= 594.04 (free acid)
ε= 8300 @ 287 nm
Lithium salt
>90% purity

ε is noted in L mmole^{-1} cm^{-1}.

Base Modified 2' Deoxynucleoside Triphosphates continued

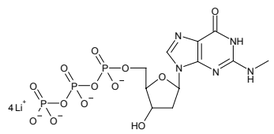
5-Methyl-2'-deoxycytidine-5'-Triphosphate



N-2026-1	1 µmole	$52.50
N-2026-5	5 µmoles	$255.00
N-2026-10	10 µmoles	$415.00

$C_{10}H_{18}N_3O_{13}P_3$
MW= 481.18 (free acid)
ε= 8900 @ 278 nm
Lithium salt
>90% purity

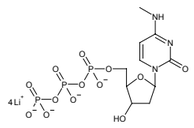
N^2-Methyl-2'-deoxyguanosine-5'-Triphosphate



N-2028-1	1 µmole	$250.00
N-2028-5	5 µmoles	$1150.00
N-2028-10	10 µmoles	$2250.00

$C_{11}H_{18}N_5O_{13}P_3$
MW= 521.21 (free acid)
ε= 14800 @ 254 nm
Lithium salt
>90% purity

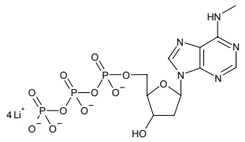
N^4-Methyl-2'-deoxycytidine-5'-Triphosphate



N-2057-1	1 µmole	$77.50
N-2057-5	5 µmoles	$365.00
N-2057-10	10 µmoles	$625.00

$C_{10}H_{18}N_3O_{13}P_3$
MW= 481.18 (free acid)
ε= 11700 @ 270 nm
Lithium salt
>90% purity

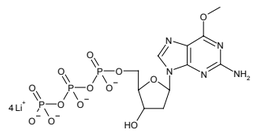
N^6-Methyl-2'-deoxyadenosine-5'-Triphosphate



N-2025-1	1 µmole	$97.50
N-2025-5	5 µmoles	$465.00
N-2025-10	10 µmoles	$850.00

$C_{11}H_{18}N_5O_{12}P_3$
MW= 505.21 (free acid)
ε= 15400 @ 269 nm
Lithium salt
>90% purity

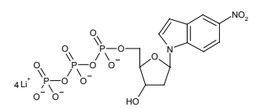
O^6-Methyl-2'-deoxyguanosine-5'-Triphosphate



N-2027-1	1 µmole	$155.00
N-2027-5	5 µmoles	$695.00
N-2027-10	10 µmoles	$1235.00

$C_{11}H_{18}N_5O_{13}P_3$
MW= 521.21 (free acid)
ε= 7100 @ 279 nm
Lithium salt
>90% purity

5-Nitro-1-indolyl-2'-deoxyribose-5'-Triphosphate

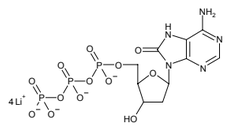


N-2031-1	1 µmole	$250.00
N-2031-5	5 µmoles	$1150.00
N-2031-10	10 µmoles	$2250.00

$C_{13}H_{17}N_2O_{14}P_3$
MW= 518.20 (free acid)
ε= 21735 @ 265 nm
Lithium salt
>90% purity

8-Oxo-2'-deoxyadenosine-5'-Triphosphate

(8-Hydroxy-2'-deoxyadenosine)

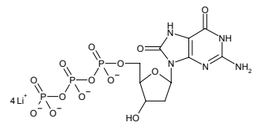


N-2033-1	1 µmole	$77.50
N-2033-5	5 µmoles	$365.00
N-2033-10	10 µmoles	$625.00

$C_{10}H_{16}N_5O_{13}P_3$
MW= 507.18 (free acid)
ε= 13600 @ 267 nm
Lithium salt
>90% purity

8-Oxo-2'-deoxyguanosine-5'-Triphosphate

(8-Hydroxy-2'-deoxyguanosine)



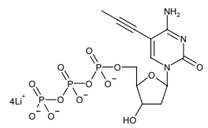
N-2034-1	1 µmole	$77.50
N-2034-5	5 µmoles	$365.00
N-2034-10	10 µmoles	$625.00

$C_{10}H_{16}N_5O_{14}P_3$
MW= 523.18 (free acid)
ε= 7868 @ 292 nm
Lithium salt
>90% purity

ε is noted in L mmole^{-1} cm^{-1}.

Base Modified 2' Deoxynucleoside Triphosphates continued

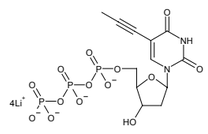
5-Propynyl-2'-deoxycytidine-5'-Triphosphate



N-2016-1	1 µmole	$77.50
N-2016-5	5 µmoles	$365.00
N-2016-10	10 µmoles	$625.00

$C_{12}H_{18}N_3O_{13}P_3$
MW= 505.21 (free acid)
ε= 9500 @ 295 nm
Lithium salt
>90% purity

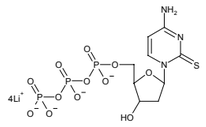
5-Propynyl-2'-deoxyuridine-5'-Triphosphate



N-2017-1	1 µmole	$77.50
N-2017-5	5 µmoles	$365.00
N-2017-10	10 µmoles	$625.00

$C_{12}H_{17}N_2O_{14}P_3$
MW= 506.19 (free acid)
ε= 11050 @ 291 nm
Lithium salt
>90% purity

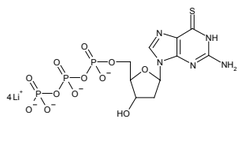
2-Thio-2'-deoxycytidine-5'-Triphosphate



N-2042-1	1 µmole	$97.50
N-2042-5	5 µmoles	$465.00
N-2042-10	10 µmoles	$850.00

$C_9H_{16}N_3O_{12}P_3S$
MW= 483.22 (free acid)
ε= 6500 @ 293 nm
Lithium salt
>90% purity

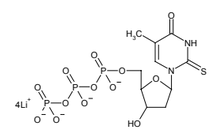
6-Thio-2'-deoxyguanosine-5'-Triphosphate



N-2045-1	1 µmole	$250.00
N-2045-5	5 µmoles	$1,150.00
N-2045-10	10 µmoles	$2,250.00

$C_{10}H_{16}N_5O_{12}P_3S$
MW= 523.24 (free acid)
ε= 24800 @ 341 nm
Lithium salt
>90% purity

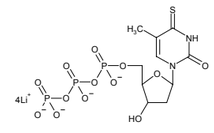
2-Thiothymidine-5'-Triphosphate



N-2035-1	1 µmole	$97.50
N-2035-5	5 µmoles	$465.00
N-2035-10	10 µmoles	$850.00

$C_{10}H_{17}N_2O_{13}P_3S$
MW= 498.23 (free acid)
ε= 13300 @ 277 nm
Lithium salt
>90% purity

4-Thiothymidine-5'-Triphosphate



N-2041-1	1 µmole	$77.50
N-2041-5	5 µmoles	$365.00
N-2041-10	10 µmoles	$625.00

$C_{10}H_{17}N_2O_{13}P_3S$
MW= 498.23 (free acid)
ε= 16300 @ 335 nm
Lithium salt
>90% purity

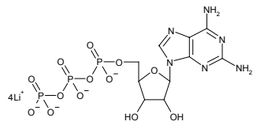
ε is noted in L mmole^{-1} cm^{-1}.

Nucleotides

Base Modified Ribonucleoside Triphosphates

2-Aminoadenosine-5'-Triphosphate
(Diaminopurine)

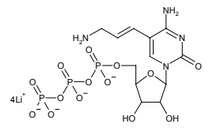
N-1001-1	1 µmole	$77.50
N-1001-5	5 µmoles	$365.00
N-1001-10	10 µmoles	$625.00



$C_{10}H_{17}N_6O_{13}P_3$
MW= 522.20 (free acid)
ε= 9894 @ 279 nm
Lithium salt
>90% purity

5-Aminoallylcytidine-5'-Triphosphate

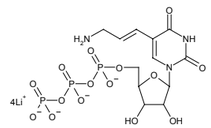
N-1065-1	1 µmole	$77.50
N-1065-5	5 µmoles	$365.00
N-1065-10	10 µmoles	$625.00



$C_{12}H_{21}N_4O_{14}P_3$
MW= 538.23 (free acid)
ε= 5041 @ 290 nm
Lithium salt
>90% purity

5-Aminoallyluridine-5'-Triphosphate

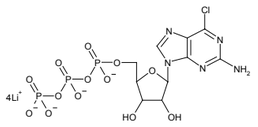
N-1062-1	1 µmole	$52.50
N-1062-5	5 µmoles	$255.00
N-1062-10	10 µmoles	$415.00



$C_{12}H_{20}N_3O_{15}P_3$
MW= 539.22 (free acid)
ε= 7100 @ 290 nm
Lithium salt
>90% purity

2-Amino-6-chloropurineriboside-5'-Triphosphate

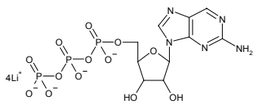
N-1002-1	1 µmole	$97.50
N-1002-5	5 µmoles	$465.00
N-1002-10	10 µmoles	$850.00



$C_{10}H_{15}N_5O_{13}P_3Cl$
MW= 541.63 (free acid)
ε= 6709 @ 307 nm
Lithium salt
>90% purity

2-Aminopurine-riboside-5'-Triphosphate

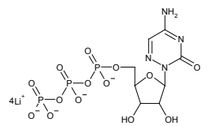
N-1067-1	1 µmole	$97.50
N-1067-5	5 µmoles	$465.00
N-1067-10	10 µmoles	$850.00



$C_{10}H_{16}N_5O_{13}P_3$
MW= 507.20 (free acid)
ε= 6422 @ 303 nm
Lithium salt
>90% purity

6-Azacytidine-5'-Triphosphate

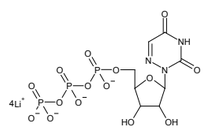
N-1037-1	1 µmole	$77.50
N-1037-5	5 µmoles	$365.00
N-1037-10	10 µmoles	$625.00



$C_8H_{15}N_4O_{14}P_3$
MW= 484.14 (free acid)
ε= 7473 @ 262 nm
Lithium salt
>90% purity

6-Azauridine-5'-Triphosphate

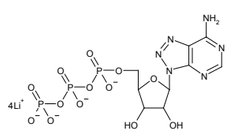
N-1038-1	1 µmole	$77.50
N-1038-5	5 µmoles	$365.00
N-1038-10	10 µmoles	$625.00



$C_8H_{14}N_3O_{15}P_3$
MW= 485.13 (free acid)
ε= 6254 @ 260 nm
Lithium salt
>90% purity

8-Azaadenosine-5'-Triphosphate

N-1004-1	1 µmole	$250.00
N-1004-5	5 µmoles	$1150.00
N-1004-10	10 µmoles	$2250.00



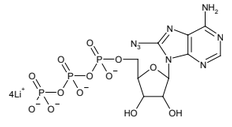
$C_9H_{15}N_6O_{13}P_3$
MW= 508.17 (free acid)
ε= 17830 @ 277 nm
Lithium salt
>90% purity

ε is noted in L mmole^{-1} cm^{-1}.

Base Modified Ribonucleoside Triphosphates continued

8-Azidoadenosine-5'-Triphosphate

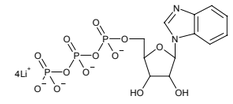
N-1052-1	1 µmole	$52.50
N-1052-5	5 µmoles	$255.00
N-1052-10	10 µmoles	$415.00



$C_{10}H_{15}N_8O_{13}P_3$
MW= 548.20 (free acid)
ε= 14500 @ 281 nm
Lithium salt
>90% purity

Benzimidazole-riboside-5'-Triphosphate

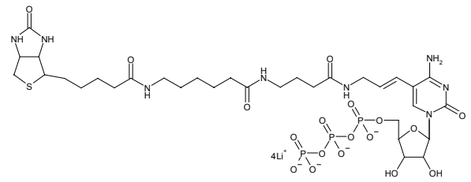
N-1047-1	1 µmole	$97.50
N-1047-5	5 µmoles	$465.00
N-1047-10	10 µmoles	$850.00



$C_{12}H_{17}N_2O_{13}P_3$
MW= 490.19 (free acid)
ε= 19800 @ 251 nm
Lithium salt
>90% purity

Biotin-16-Aminoallylcytidine-5'-Triphosphate

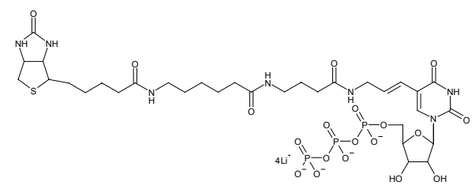
N-5003-0250	250 nmoles	$197.50
N-5003-1	1 µmole	$525.00
N-5003-2	2 µmoles	$915.00



$C_{32}H_{52}N_8O_{18}P_3S$
MW= 961.70 (free acid)
ε= 5040 @ 290 nm
Lithium salt
>90% purity

Biotin-16-Aminoallyluridine-5'-Triphosphate

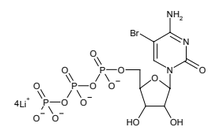
N-5005-0250	250 nmoles	$150.00
N-5005-1	1 µmole	$525.00
N-5005-2	2 µmoles	$750.00



$C_{32}H_{52}N_7O_{19}P_3S$
MW= 963.80 (free acid)
ε= 7100 @ 290 nm
Lithium salt
>90% purity

5-Bromocytidine-5'-Triphosphate

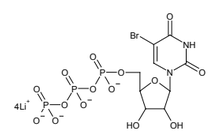
N-1053-1	1 µmole	$155.00
N-1053-5	5 µmoles	$695.00
N-1053-10	10 µmoles	$1235.00



$C_9H_{15}N_3O_{14}P_3Br$
MW= 562.05 (free acid)
ε= 6825 @ 288 nm
Lithium salt
>90% purity

5-Bromouridine-5'-Triphosphate

N-1054-1	1 µmole	$52.50
N-1054-5	5 µmoles	$255.00
N-1054-10	10 µmoles	$415.00



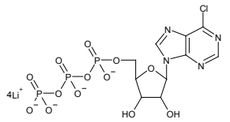
$C_9H_{14}N_2O_{15}P_3Br$
MW= 563.04 (free acid)
ε= 9220 @ 279 nm
Lithium salt
>90% purity

ε is noted in L mmole^{-1} cm^{-1}.

Base Modified Ribonucleoside Triphosphates continued

6-Chloropurineriboside-5'-Triphosphate

N-1006-1	1 µmole	$77.50
N-1006-5	5 µmoles	$365.00
N-1006-10	10 µmoles	$625.00

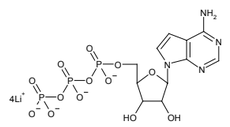


$C_{10}H_{14}N_4O_{13}P_3Cl$
MW= 526.61 (free acid)
ε= 9754 @ 262 nm
Lithium salt
>90% purity

7-Deazaadenosine-5'-Triphosphate

(Tubercidin Triphosphate)

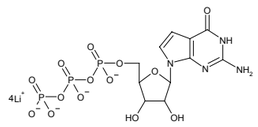
N-1061-1	1 µmole	$77.50
N-1061-5	5 µmoles	$365.00
N-1061-10	10 µmoles	$625.00



$C_{11}H_{17}N_4O_{13}P_3$
MW= 506.19 (free acid)
ε= 9500 @ 271 nm
Lithium salt
>90% purity

7-Deazaguanosine-5'-Triphosphate

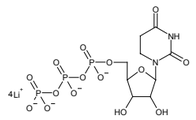
N-1044-1	1 µmole	$97.50
N-1044-5	5 µmoles	$465.00
N-1044-10	10 µmoles	$850.00



$C_{11}H_{17}N_4O_{14}P_3$
MW= 522.19 (free acid)
ε= 9145 @ 259 nm
Lithium salt
>90% purity

5,6-Dihydrouridine-5'-Triphosphate

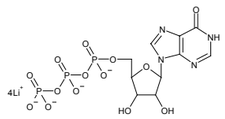
N-1035-1	1 µmole	$97.50
N-1035-5	5 µmoles	$465.00
N-1035-10	10 µmoles	$850.00



$C_9H_{17}N_2O_{15}P_3$
MW= 486.16 (free acid)
ε= N/A
Lithium salt
>90% purity

Inosine-5'-Triphosphate

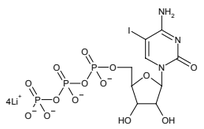
N-1020-1	1 µmole	$52.50
N-1020-5	5 µmoles	$255.00
N-1020-10	10 µmoles	$415.00



$C_{10}H_{15}N_4O_{14}P_3$
MW= 508.17 (free acid)
ε= 12700 @ 248 nm
Lithium salt
>90% purity

5-Iodocytidine-5'-Triphosphate

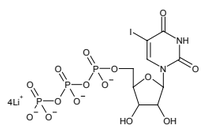
N-1011-1	1 µmole	$77.50
N-1011-5	5 µmoles	$365.00
N-1011-10	10 µmoles	$625.00



$C_9H_{15}N_3O_{14}P_3I$
MW= 609.05 (free acid)
ε= 8275 @ 294 nm
Lithium salt
>90% purity

5-Iodouridine-5'-Triphosphate

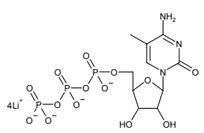
N-1012-1	1 µmole	$77.50
N-1012-5	5 µmoles	$365.00
N-1012-10	10 µmoles	$625.00



$C_9H_{14}N_2O_{15}P_3I$
MW= 610.04 (free acid)
ε= 6533 @ 287 nm
Lithium salt
>90% purity

5-Methylcytidine-5'-Triphosphate

N-1014-1	1 µmole	$77.50
N-1014-5	5 µmoles	$365.00
N-1014-10	10 µmoles	$625.00



$C_{10}H_{18}N_3O_{14}P_3$
MW= 497.18 (free acid)
ε= 7808 @ 279 nm
Lithium salt
>90% purity

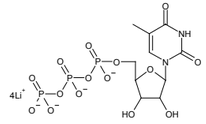
ε is noted in L mmole^{-1} cm^{-1}.

Base Modified Ribonucleoside Triphosphates continued

5-Methyluridine-5'-Triphosphate

(Thymidine RNA analog)

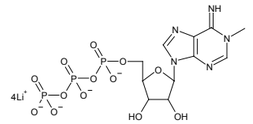
N-1024-1	1 µmole	$77.50
N-1024-5	5 µmoles	$365.00
N-1024-10	10 µmoles	$625.00



$C_{10}H_{17}N_2O_{15}P_3$
MW= 498.17 (free acid)
ε= 9650 @ 267 nm
Lithium salt
>90% purity

N¹-Methyladenosine-5'-Triphosphate

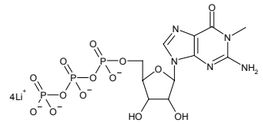
N-1042-1	1 µmole	$77.50
N-1042-5	5 µmoles	$365.00
N-1042-10	10 µmoles	$625.00



$C_{11}H_{20}N_5O_{13}P_3$
MW= 523.23 (free acid)
ε= 14600 @ 257 nm
Lithium salt
>90% purity

N¹-Methylguanosine-5'-Triphosphate

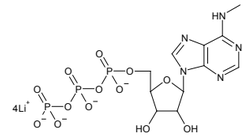
N-1039-1	1 µmole	$77.50
N-1039-5	5 µmoles	$365.00
N-1039-10	10 µmoles	$625.00



$C_{11}H_{18}N_5O_{14}P_3$
MW= 537.21 (free acid)
ε= 12950 @ 254 nm
Lithium salt
>90% purity

N⁶-Methyladenosine-5'-Triphosphate

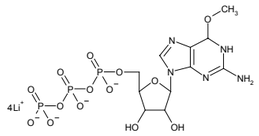
N-1013-1	1 µmole	$52.50
N-1013-5	5 µmoles	$255.00
N-1013-10	10 µmoles	$415.00



$C_{11}H_{18}N_5O_{13}P_3$
MW= 521.21 (free acid)
ε= 15567 @ 265 nm
Lithium salt
>90% purity

O⁶-Methylguanosine-5'-Triphosphate

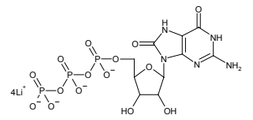
N-1031-1	1 µmole	$155.00
N-1031-5	5 µmoles	$695.00
N-1031-10	10 µmoles	$1235.00



$C_{11}H_{18}N_5O_{14}P_3$
MW= 537.21 (free acid)
ε= 5460 @ 280 nm
Lithium salt
>90% purity

8-Oxoguanosine-5'-Triphosphate

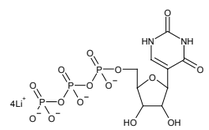
N-1066-1	1 µmole	$52.50
N-1066-5	5 µmoles	$255.00
N-1066-10	10 µmoles	$415.00



$C_{10}H_{16}N_5O_{15}P_3$
MW= 539.18 (free acid)
ε= 7868 @ 296 nm
Lithium salt
>90% purity

Pseudouridine-5'-Triphosphate

N-1019-1	1 µmole	$77.50
N-1019-5	5 µmoles	$365.00
N-1019-10	10 µmoles	$625.00



$C_9H_{15}N_2O_{15}P_3$
MW= 484.14 (free acid)
ε= 7546 @ 262 nm
Lithium salt
>90% purity

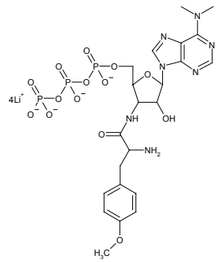
ε is noted in L mmole^{-1} cm^{-1}.

Nucleotides

Base Modified Ribonucleoside Triphosphates continued

Puromycin-5'-Triphosphate

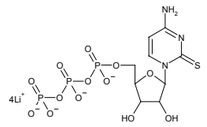
N-1022-1	1 µmole	$97.50
N-1022-5	5 µmoles	$465.00
N-1022-10	10 µmoles	$850.00



$C_{22}H_{32}N_7O_{14}P_3$
MW= 711.45 (free acid)
ε= 18800 @ 275 nm
Lithium salt
>90% purity

2-Thiocytidine-5'-Triphosphate

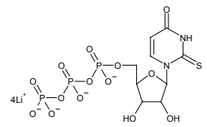
N-1036-1	1 µmole	$77.50
N-1036-5	5 µmoles	$365.00
N-1036-10	10 µmoles	$625.00



$C_9H_{16}N_3O_{13}P_3S$
MW= 499.22 (free acid)
ε= 11124 @ 247 nm
Lithium salt
>90% purity

2-Thiouridine-5'-Triphosphate

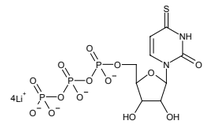
N-1032-1	1 µmole	$77.50
N-1032-5	5 µmoles	$365.00
N-1032-10	10 µmoles	$625.00



$C_9H_{15}N_2O_{14}P_3S$
MW= 500.20 (free acid)
ε= 13120 @ 274 nm
Lithium salt
>90% purity

4-Thiouridine-5'-Triphosphate

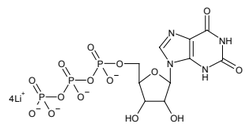
N-1025-1	1 µmole	$97.50
N-1025-5	5 µmoles	$465.00
N-1025-10	10 µmoles	$850.00



$C_9H_{15}N_2O_{14}P_3S$
MW= 500.20 (free acid)
ε= 16526 @ 331 nm
Lithium salt
>90% purity

Xanthosine-5'-Triphosphate

N-1023-1	1 µmole	$52.50
N-1023-5	5 µmoles	$255.00
N-1023-10	10 µmoles	$415.00



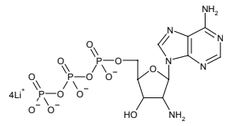
$C_{10}H_{15}N_4O_{15}P_3$
MW= 524.17 (free acid)
ε= 8900 @ 278 nm
Lithium salt
>90% purity

ε is noted in L mmole^{-1} cm^{-1}.

Sugar Modified Nucleoside Triphosphates continued

2'-Amino-2'-deoxyadenosine-5'-Triphosphate

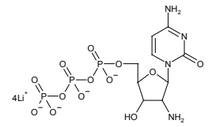
N-1046-1	1 µmole	$52.50
N-1046-5	5 µmoles	$255.00
N-1046-10	10 µmoles	$415.00



$C_{10}H_{17}N_6O_{12}P_3$
MW= 522.20 (free acid)
ε= 15400 @ 258 nm
Lithium salt
>90% purity

2'-Amino-2'-deoxycytidine-5'-Triphosphate

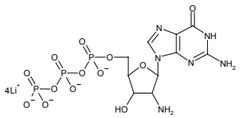
N-1026-1	1 µmole	$77.50
N-1026-5	5 µmoles	$365.00
N-1026-10	10 µmoles	$625.00



$C_9H_{17}N_4O_{13}P_3$
MW= 482.17 (free acid)
ε= 9100 @ 271 nm
Lithium salt
>90% purity

2'-Amino-2'-deoxyguanosine-5'-Triphosphate

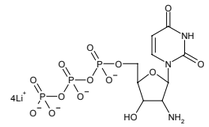
N-1064-1	1 µmole	$77.50
N-1064-5	5 µmoles	$365.00
N-1064-10	10 µmoles	$625.00



$C_{10}H_{17}N_6O_{13}P_3$
MW= 522.20 (free acid)
ε= 13600 @ 252 nm
Lithium salt
>90% purity

2'-Amino-2'-deoxyuridine-5'-Triphosphate

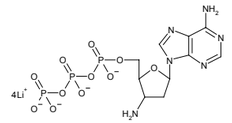
N-1027-1	1 µmole	$77.50
N-1027-5	5 µmoles	$365.00
N-1027-10	10 µmoles	$625.00



$C_9H_{16}N_3O_{14}P_3$
MW= 483.16 (free acid)
ε= 10100 @ 262 nm
Lithium salt
>90% purity

3'-Amino-2',3'-dideoxyadenosine-5'-Triphosphate

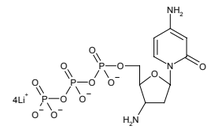
N-4010-1	1 µmole	$175.00
N-4010-5	5 µmoles	$775.00
N-4010-10	10 µmoles	$1450.00



$C_{10}H_{17}N_6O_{11}P_3$
MW= 490.20 (free acid)
ε= 15400 @ 258 nm
Lithium salt
>90% purity

3'-Amino-2',3'-dideoxycytidine-5'-Triphosphate

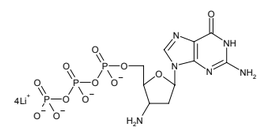
N-4011-1	1 µmole	$175.00
N-4011-5	5 µmoles	$775.00
N-4011-10	10 µmoles	$1450.00



$C_9H_{17}N_4O_{12}P_3$
MW= 466.17 (free acid)
ε= 9100 @ 271 nm
Lithium salt
>90% purity

3'-Amino-2',3'-dideoxyguanosine-5'-Triphosphate

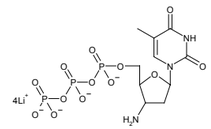
N-4012-1	1 µmole	$175.00
N-4012-5	5 µmoles	$775.00
N-4012-10	10 µmoles	$1450.00



$C_{10}H_{17}N_6O_{12}P_3$
MW= 506.20 (free acid)
ε= 13600 @ 252 nm
Lithium salt
>90% purity

3'-Amino-2',3'-dideoxythymidine-5'-Triphosphate

N-4013-1	1 µmole	$97.50
N-4013-5	5 µmoles	$465.00
N-4013-10	10 µmoles	$850.00



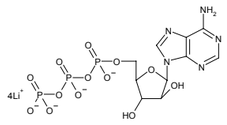
$C_{10}H_{18}N_3O_{13}P_3$
MW= 481.18 (free acid)
ε= 9650 @ 267 nm
Lithium salt
>90% purity

ε is noted in L mmole^{-1} cm^{-1}.

Sugar Modified Nucleoside Triphosphates continued

Araadenosine-5'-Triphosphate

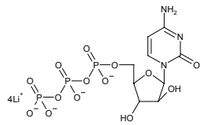
N-1048-1	1 µmole	$77.50
N-1048-5	5 µmoles	$365.00
N-1048-10	10 µmoles	$625.00



$C_{10}H_{16}N_5O_{13}P_3$
MW= 507.18 (free acid)
Ε= 15400 @ 258 nm
Lithium salt
>90% purity

Aracytidine-5'-Triphosphate

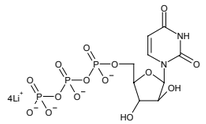
N-1033-1	1 µmole	$52.50
N-1033-5	5 µmoles	$255.00
N-1033-10	10 µmoles	$415.00



$C_9H_{16}N_3O_{14}P_3$
MW= 483.16 (free acid)
Ε= 9100 @ 271 nm
Lithium salt
>90% purity

Arauridine-5'-Triphosphate

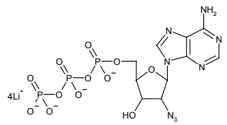
N-1034-1	1 µmole	$97.50
N-1034-5	5 µmoles	$465.00
N-1034-10	10 µmoles	$850.00



$C_9H_{15}N_2O_{15}P_3$
MW= 482.12 (free acid)
Ε= 10100 @ 262 nm
Lithium salt
>90% purity

2'-Azido-2'-deoxyadenosine-5'-Triphosphate

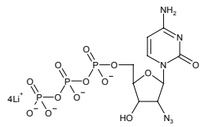
N-1045-1	1 µmole	$77.50
N-1045-5	5 µmoles	$365.00
N-1045-10	10 µmoles	$625.00



$C_{10}H_{15}N_8O_{12}P_3$
MW= 532.20 (free acid)
Ε= 15400 @ 258 nm
Lithium salt
>90% purity

2'-Azido-2'-deoxycytidine-5'-Triphosphate

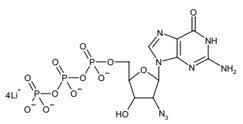
N-1028-1	1 µmole	$77.50
N-1028-5	5 µmoles	$365.00
N-1028-10	10 µmoles	$625.00



$C_9H_{15}N_8O_{13}P_3$
MW= 508.17 (free acid)
Ε= 9100 @ 271 nm
Lithium salt
>90% purity

2'-Azido-2'-deoxyguanosine-5'-Triphosphate

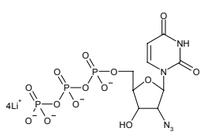
N-1063-1	1 µmole	$77.50
N-1063-5	5 µmoles	$365.00
N-1063-10	10 µmoles	$625.00



$C_{10}H_{15}N_8O_{13}P_3$
MW= 548.19 (free acid)
Ε= 13600 @ 252 nm
Lithium salt
>90% purity

2'-Azido-2'-deoxyuridine-5'-Triphosphate

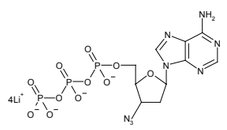
N-1029-1	1 µmole	$77.50
N-1029-5	5 µmoles	$365.00
N-1029-10	10 µmoles	$625.00



$C_9H_{14}N_5O_{14}P_3$
MW= 509.15 (free acid)
Ε= 10100 @ 262 nm
Lithium salt
>90% purity

3'-Azido-2',3'-dideoxyadenosine-5'-Triphosphate

N-4007-1	1 µmole	$97.50
N-4007-5	5 µmoles	$465.00
N-4007-10	10 µmoles	$850.00



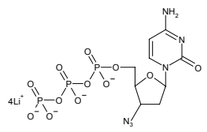
$C_{10}H_{15}N_8O_{11}P_3$
MW= 516.20 (free acid)
Ε= 15400 @ 258 nm
Lithium salt
>90% purity

Ε is noted in L mmole^{-1} cm^{-1}.

Sugar Modified Nucleoside Triphosphates continued

3'-Azido-2',3'-dideoxycytidine-5'-Triphosphate

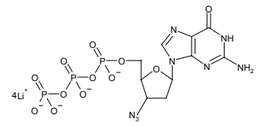
N-4014-1	1 µmole	$97.50
N-4014-5	5 µmoles	$465.00
N-4014-10	10 µmoles	$850.00



$C_9H_{15}N_6O_{12}P_3$
MW= 492.17 (free acid)
Ε= 9100 @ 271 nm
Lithium salt
>90% purity

3'-Azido-2',3'-dideoxyguanosine-5'-Triphosphate

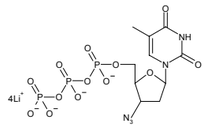
N-4008-1	1 µmole	$97.50
N-4008-5	5 µmoles	$465.00
N-4008-10	10 µmoles	$850.00



$C_{10}H_{15}N_8O_{12}P_3$
MW= 532.20 (free acid)
Ε= 13600 @ 252 nm
Lithium salt
>90% purity

3'-Azido-2',3'-dideoxythymidine-5'-Triphosphate

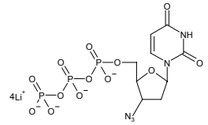
N-4009-1	1 µmole	$97.50
N-4009-5	5 µmoles	$465.00
N-4009-10	10 µmoles	$850.00



$C_{10}H_{16}N_5O_{13}P_3$
MW= 507.18 (free acid)
Ε= 9650 @ 267 nm
Lithium salt
>90% purity

3'-Azido-2',3'-dideoxyuridine-5'-Triphosphate

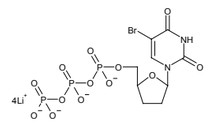
N-4015-1	1 µmole	$97.50
N-4015-5	5 µmoles	$465.00
N-4015-10	10 µmoles	$850.00



$C_9H_{14}N_5O_{13}P_3$
MW= 493.16 (free acid)
Ε= 10100 @ 262 nm
Lithium salt
>90% purity

5-Bromo-2',3'-dideoxyuridine-5'-Triphosphate

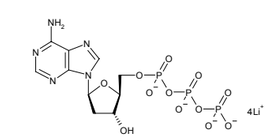
N-4016-1	1 µmole	$97.50
N-4016-5	5 µmoles	$465.00
N-4016-10	10 µmoles	$850.00



$C_9H_{14}N_2O_{13}P_3Br$
MW= 531.04 (free acid)
Ε= 9200 @ 279 nm
Lithium salt
>90% purity

2'-Deoxy-L-adenosine-5'-Triphosphate

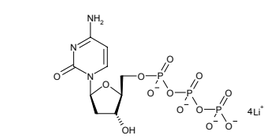
N-2053-1	1 µmole	$97.50
N-2053-5	5 µmoles	$465.00
N-2053-10	10 µmoles	$850.00



$C_{10}H_{16}N_5O_{12}P_3$
MW= 491.18 (free acid)
Ε= 15400 @ 258 nm
Lithium salt
>90% purity

2'-Deoxy-L-cytidine-5'-Triphosphate

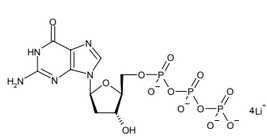
N-2054-1	1 µmole	$97.50
N-2054-5	5 µmoles	$465.00
N-2054-10	10 µmoles	$850.00



$C_9H_{16}N_3O_{13}P_3$
MW= 467.16 (free acid)
Ε= 9100 @ 271 nm
Lithium salt
>90% purity

2'-Deoxy-L-guanosine-5'-Triphosphate

N-2055-1	1 µmole	$175.00
N-2055-5	5 µmoles	$775.00
N-2055-10	10 µmoles	$1450.00



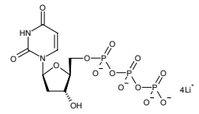
$C_{10}H_{16}N_5O_{13}P_3$
MW= 507.18 (free acid)
Ε= 13600 @ 252 nm
Lithium salt
>90% purity

Ε is noted in L mmole^{-1} cm^{-1}.

Nucleotides

Sugar Modified Nucleoside Triphosphates continued

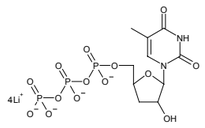
2'-Deoxy-L-thymidine-5'-Triphosphate



$C_{10}H_{17}N_2O_{14}P_3$
MW= 482.17 (free acid)
ε= 9650 @ 267 nm
Lithium salt
>90% purity

N-2056-1	1 µmole	$97.50
N-2056-5	5 µmoles	$465.00
N-2056-10	10 µmoles	$850.00

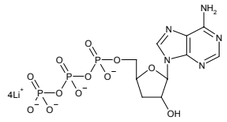
3'-Deoxy-5-methyluridine-5'-Triphosphate



$C_{10}H_{17}N_2O_{14}P_3$
MW= 482.17 (free acid)
ε= 8700 @ 260 nm
Lithium salt
>90% purity

N-3004-1	1 µmole	$77.50
N-3004-5	5 µmoles	$365.00
N-3004-10	10 µmoles	$625.00

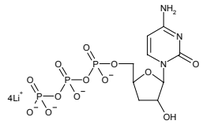
3'-Deoxyadenosine-5'-Triphosphate
(Cordycepin)



$C_{10}H_{16}N_5O_{12}P_3$
MW= 491.18 (free acid)
ε= 15400 @ 258 nm
Lithium salt
>90% purity

N-3001-1	1 µmole	$77.50
N-3001-5	5 µmoles	$365.00
N-3001-10	10 µmoles	$625.00

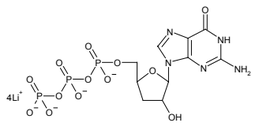
3'-Deoxycytidine-5'-Triphosphate



$C_9H_{16}N_3O_{13}P_3$
MW= 467.16 (free acid)
ε= 9100 @ 271 nm
Lithium salt
>90% purity

N-3003-1	1 µmole	$77.50
N-3003-5	5 µmoles	$365.00
N-3003-10	10 µmoles	$625.00

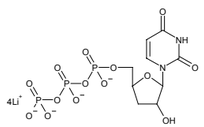
3'-Deoxyguanosine-5'-Triphosphate



$C_{10}H_{16}N_5O_{13}P_3$
MW= 507.18 (free acid)
ε= 13600 @ 252 nm
Lithium salt
>90% purity

N-3002-1	1 µmole	$77.50
N-3002-5	5 µmoles	$365.00
N-3002-10	10 µmoles	$625.00

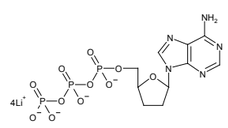
3'-Deoxyuridine-5'-Triphosphate



$C_9H_{15}N_2O_{14}P_3$
MW= 468.14 (free acid)
ε= 10100 @ 262 nm
Lithium salt
>90% purity

N-3005-1	1 µmole	$77.50
N-3005-5	5 µmoles	$365.00
N-3005-10	10 µmoles	$625.00

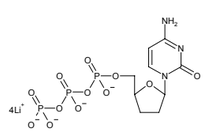
2',3'-Dideoxyadenosine-5'-Triphosphate



$C_{10}H_{16}N_5O_{11}P_3$
MW= 475.18 (free acid)
ε=15400 @ 258 nm
Lithium salt
>90% purity

N-4001-1	1 µmole	$52.50
N-4001-5	5 µmoles	$255.00
N-4001-10	10 µmoles	$415.00

2',3'-Dideoxycytidine-5'-Triphosphate



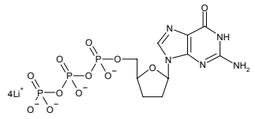
$C_9H_{16}N_3O_{12}P_3$
MW= 451.16 (free acid)
ε= 9100 @ 271 nm
Lithium salt
>90% purity

N-4005-1	1 µmole	$27.50
N-4005-5	5 µmoles	$115.00
N-4005-10	10 µmoles	$205.00

ε is noted in L mmole^{-1} cm^{-1}.

Sugar Modified Nucleoside Triphosphates continued

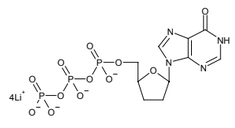
2',3'-Dideoxyguanosine-5'-Triphosphate



$C_{10}H_{16}N_5O_{12}P_3$
MW= 491.18 (free acid)
ε= 13600 @ 252 nm
Lithium salt
>90% purity

N-4002-1	1 µmole	$52.50
N-4002-5	5 µmoles	$255.00
N-4002-10	10 µmoles	$415.00

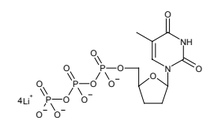
2',3'-Dideoxyinosine-5'-Triphosphate



$C_{10}H_{15}N_4O_{12}P_3$
MW= 476.17 (free acid)
ε= 12300 @ 248 nm
Lithium salt
>90% purity

N-4017-1	1 µmole	$155.00
N-4017-5	5 µmoles	$695.00
N-4017-10	10 µmoles	$1235.00

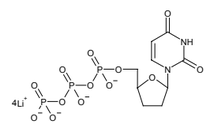
2',3'-Dideoxythymidine-5'-Triphosphate



$C_{10}H_{17}N_2O_{13}P_3$
MW= 466.17 (free acid)
ε= 9650 @ 267 nm
Lithium salt
>90% purity

N-4004-1	1 µmole	$27.50
N-4004-5	5 µmoles	$115.00
N-4004-10	10 µmoles	$205.00

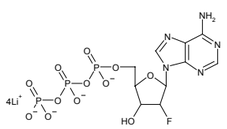
2',3'-Dideoxyuridine-5'-Triphosphate



$C_9H_{15}N_2O_{13}P_3$
MW= 452.14 (free acid)
ε= 10100 @ 262 nm
Lithium salt
>90% purity

N-4003-1	1 µmole	$27.50
N-4003-5	5 µmoles	$115.00
N-4003-10	10 µmoles	$205.00

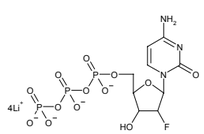
2'-Fluoro-2'-deoxyadenosine-5'-Triphosphate



$C_{10}H_{15}N_5O_{12}P_3F$
MW= 509.17 (free acid)
ε= 15400 @ 258 nm
Lithium salt
>90% purity

N-1007-1	1 µmole	$77.50
N-1007-5	5 µmoles	$365.00
N-1007-10	10 µmoles	$625.00

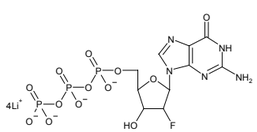
2'-Fluoro-2'-deoxycytidine-5'-Triphosphate



$C_9H_{15}N_3O_{13}P_3F$
MW= 485.15 (free acid)
ε= 9100 @ 271 nm
Lithium salt
>90% purity

N-1008-1	1 µmole	$52.50
N-1008-5	5 µmoles	$255.00
N-1008-10	10 µmoles	$415.00

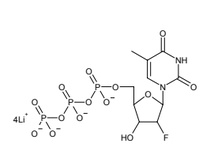
2'-Fluoro-2'-deoxyguanosine-5'-Triphosphate



$C_{10}H_{15}N_5O_{13}P_3$
MW= 525.17 (free acid)
ε= 13600 @ 252 nm
Lithium salt
>90% purity

N-1009-1	1 µmole	$97.50
N-1009-5	5 µmoles	$465.00
N-1009-10	10 µmoles	$850.00

2'-Fluoro-2'-deoxythymidine-5'-Triphosphate



$C_{10}H_{16}N_2O_{14}P_3F$
MW= 500.16 (free acid)
ε= 9650 @ 267 nm
Lithium salt
>90% purity

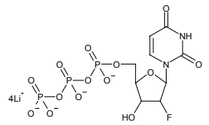
N-1055-1	1 µmole	$77.50
N-1055-5	5 µmoles	$365.00
N-1055-10	10 µmoles	$625.00

ε is noted in L mmole^{-1} cm^{-1}.

Sugar Modified Nucleoside Triphosphates continued

2'-Fluoro-2'-deoxyuridine-5'-Triphosphate

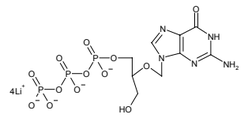
N-1010-1	1 µmole	$52.50
N-1010-5	5 µmoles	$255.00
N-1010-10	10 µmoles	$415.00



$C_9H_{14}N_2O_{14}P_3F$
MW= 486.13 (free acid)
ε= 10100 @ 262 nm
Lithium salt
>90% purity

Ganciclovir-5'-Triphosphate (S-isomer)

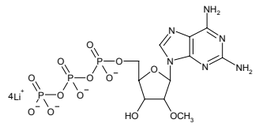
N-9001-1	1 µmole	$77.50
N-9001-5	5 µmoles	$365.00
N-9001-10	10 µmoles	$625.00



$C_9H_{16}N_5O_{13}P_3$
MW= 495.20 (free acid)
ε= 13600 @ 252 nm
Lithium salt
>90% purity

2'-O-Methyl-2-aminoadenosine-5'-Triphosphate
(Diaminopurine)

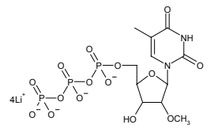
N-1040-1	1 µmole	$77.50
N-1040-5	5 µmoles	$365.00
N-1040-10	10 µmoles	$625.00



$C_{11}H_{19}N_6O_{13}P_3$
MW= 536.22 (free acid)
ε= 8675 @ 280 nm
Lithium salt
>90% purity

2'-O-Methyl-5-methyluridine-5'-Triphosphate

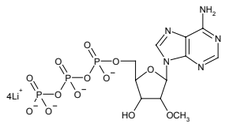
N-1043-1	1 µmole	$52.50
N-1043-5	5 µmoles	$255.00
N-1043-10	10 µmoles	$415.00



$C_{11}H_{19}N_2O_{15}P_3$
MW= 512.19 (free acid)
ε= 10360 @ 265 nm
Lithium salt
>90% purity

2'-O-Methyladenosine-5'-Triphosphate

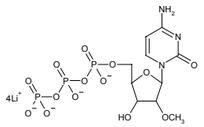
N-1015-1	1 µmole	$27.50
N-1015-5	5 µmoles	$115.00
N-1015-10	10 µmoles	$205.00



$C_{11}H_{18}N_5O_{13}P_3$
MW= 521.21 (free acid)
ε= 15400 @ 258 nm
Lithium salt
>90% purity

2'-O-Methylcytidine-5'-Triphosphate

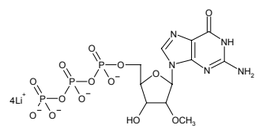
N-1016-1	1 µmole	$27.50
N-1016-5	5 µmoles	$115.00
N-1016-10	10 µmoles	$205.00



$C_{10}H_{18}N_3O_{14}P_3$
MW= 497.18 (free acid)
ε= 9100 @ 271 nm
Lithium salt
>90% purity

2'-O-Methylguanosine-5'-Triphosphate

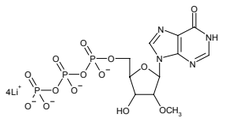
N-1017-1	1 µmole	$27.50
N-1017-5	5 µmoles	$115.00
N-1017-10	10 µmoles	$205.00



$C_{10}H_{17}N_2O_{15}P_3$
MW= 537.21 (free acid)
ε= 13600 @ 252 nm
Lithium salt
>90% purity

2'-O-Methylinosine-5'-Triphosphate

N-1021-1	1 µmole	$77.50
N-1021-5	5 µmoles	$365.00
N-1021-10	10 µmoles	$625.00



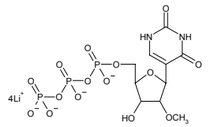
$C_{11}H_{13}N_4O_{13}P_3$
MW= 522.25 (free acid)
ε= 12200 @ 248 nm
Lithium salt
>90% purity

ε is noted in L mmole^{-1} cm^{-1}.

Sugar Modified Nucleoside Triphosphates continued

2'-O-Methylpseudouridine-5'-Triphosphate

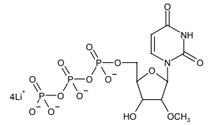
N-1041-1	1 µmole	$52.50
N-1041-5	5 µmoles	$255.00
N-1041-10	10 µmoles	$415.00



$C_{10}H_{17}N_2O_{15}P_3$
MW= 498.17 (free acid)
ε= 6475 @ 262 nm
Lithium salt
>90% purity

2'-O-Methyluridine-5'-Triphosphate

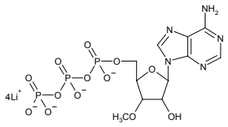
N-1018-1	1 µmole	$27.50
N-1018-5	5 µmoles	$115.00
N-1018-10	10 µmoles	$205.00



$C_{10}H_{17}N_2O_{15}P_3$
MW= 498.17 (free acid)
ε= 10100 @ 262 nm
Lithium salt
>90% purity

3'-O-Methyladenosine-5'-Triphosphate

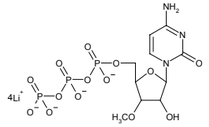
N-1056-1	1 µmole	$77.50
N-1056-5	5 µmoles	$365.00
N-1056-10	10 µmoles	$625.00



$C_{11}H_{18}N_5O_{13}P_3$
MW= 521.21 (free acid)
ε= 15400 @ 258 nm
Lithium salt
>90% purity

3'-O-Methylcytidine-5'-Triphosphate

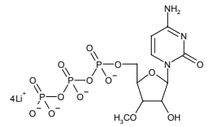
N-1057-1	1 µmole	$77.50
N-1057-5	5 µmoles	$365.00
N-1057-10	10 µmoles	$625.00



$C_{10}H_{18}N_3O_{14}P_3$
MW= 497.18 (free acid)
ε= 9100 @ 271 nm
Lithium salt
>90% purity

3'-O-Methylguanosine-5'-Triphosphate

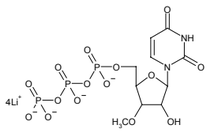
N-1058-1	1 µmole	$77.50
N-1058-5	5 µmoles	$365.00
N-1058-10	10 µmoles	$625.00



$C_{11}H_{18}N_5O_{14}P_3$
MW= 537.21 (free acid)
ε= 13600 @ 252 nm
Lithium salt
>90% purity

3'-O-Methyluridine-5'-Triphosphate

N-1059-1	1 µmole	$77.50
N-1059-5	5 µmoles	$365.00
N-1059-10	10 µmoles	$625.00



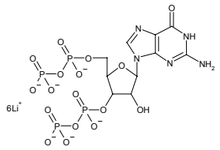
$C_{10}H_{17}N_2O_{15}P_3$
MW= 498.17 (free acid)
ε= 10100 @ 262 nm
Lithium salt
>90% purity

ε is noted in L mmole^{-1} cm^{-1}.

Bisphosphate Nucleosides

Guanosine-3',5'-Bisdiphosphate
(ppGpp)

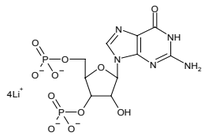
N-6001-1	1 µmole	$175.00
N-6001-5	5 µmoles	$775.00
N-6001-10	10 µmoles	$1450.00



$C_{10}H_{17}N_5O_{17}P_4$
MW= 603.16 (free acid)
Ɛ= 13600 @ 252 nm
Lithium salt

Guanosine-3',5'-Bisphosphate
(pGp)

N-6002-1	1 µmole	$155.00
N-6002-5	5 µmoles	$695.00
N-6002-10	10 µmoles	$1235.00

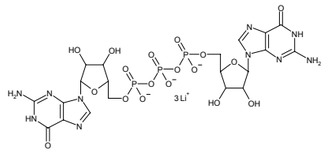


$C_{10}H_{15}N_5O_{11}P_2$
MW= 443.20 (free acid)
Ɛ= 13600 @ 252 nm
Lithium salt
>90% purity

CAP and mCAP

Guanosine-5'-Triphosphate-5'-Guanosine
(CAP)

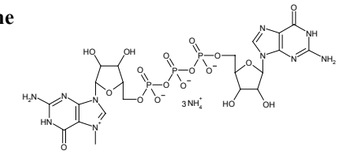
N-7002-1	1 µmole	$77.50
N-7002-5	5 µmoles	$365.00
N-7002-10	10 µmoles	$625.00



$C_{20}H_{27}N_{10}O_{18}P_3$
MW= 788.41 (free acid)
Ɛ= 21600 @ 260 nm
Lithium salt
>90% purity

N^7-Me-Guanosine-5'-Triphosphate-5'-Guanosine
(mCAP)

N-7001-1	1 µmole	$77.50
N-7001-5	5 µmoles	$365.00
N-7001-10	10 µmoles	$625.00

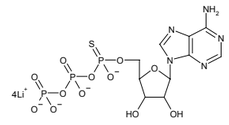


$C_{21}H_{30}N_{10}O_{18}P_3$
MW= 803.45 (free acid)
Ɛ= 21000 @ 254 nm
Ammonium salt
>90% purity

Alpha Phosphate Modified Nucleoside Triphosphates

Adenosine-5'-O-(1-Thiotriphosphate) (mixed isomers)

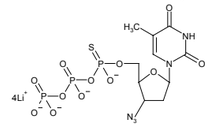
N-8005-1	1 µmole	$27.50
N-8005-5	5 µmoles	$115.00
N-8005-10	10 µmoles	$205.00



$C_{10}H_{16}N_5O_{12}P_3S$
MW= 523.24 (free acid)
Ɛ= 15400 @ 258 nm
Lithium salt
>90% purity

3'-Azido-2',3'-dideoxythymidine-5'-O-(1-Thiotriphosphate) (mixed isomers)

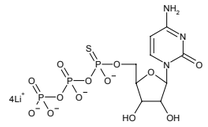
N-8013-1	1 µmole	$97.50
N-8013-5	5 µmoles	$465.00
N-8013-10	10 µmoles	$850.00



$C_{10}H_{16}N_5O_{12}P_3S$
MW= 523.24 (free acid)
Ɛ= 9650 @ 267 nm
Lithium salt
>90% purity

Cytidine-5'-O-(1-Thiotriphosphate) (mixed isomers)

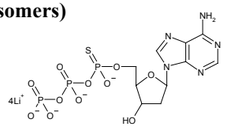
N-8006-1	1 µmole	$77.50
N-8006-5	5 µmoles	$365.00
N-8006-10	10 µmoles	$625.00



$C_9H_{16}N_3O_{13}P_3S$
MW= 499.22 (free acid)
Ɛ= 9100 @ 271 nm
Lithium salt
>90% purity

2'-Deoxyadenosine-5'-O-(1-Thiotriphosphate) (mixed isomers)

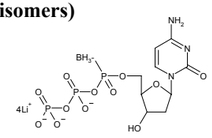
N-8001-1	1 µmole	$27.50
N-8001-5	5 µmoles	$115.00
N-8001-10	10 µmoles	$205.00



$C_{10}H_{16}N_5O_{11}P_3S$
MW= 507.24 (free acid)
Ɛ= 15400 @ 258 nm
Lithium salt
>90% purity

2'-Deoxycytidine-5'-O-(1-Boranotriphosphate) (mixed isomers)

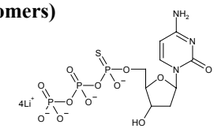
N-8051-1	1 µmole	$175.00
N-8051-5	5 µmoles	$775.00
N-8051-10	10 µmoles	$1450.00



$C_9H_{18}BN_3O_{12}P_3$
MW= 463.90 (free acid)
Ɛ= 9300 @ 271 nm
Lithium salt
>90% purity

2'-Deoxycytidine-5'-O-(1-Thiotriphosphate) (mixed isomers)

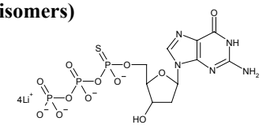
N-8002-1	1 µmole	$27.50
N-8002-5	5 µmoles	$115.00
N-8002-10	10 µmoles	$205.00



$C_9H_{16}N_3O_{12}P_3S$
MW= 483.22 (free acid)
Ɛ= 9100 @ 271 nm
Lithium salt
>90% purity

2'-Deoxyguanosine-5'-O-(1-Thiotriphosphate) (mixed isomers)

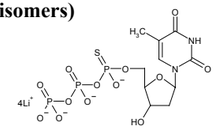
N-8003-1	1 µmole	$52.50
N-8003-5	5 µmoles	$255.00
N-8003-10	10 µmoles	$415.00



$C_{10}H_{16}N_5O_{12}P_3S$
MW= 523.24 (free acid)
Ɛ= 13600 @ 252 nm
Lithium salt
>90% purity

2'-Deoxythymidine-5'-O-(1-Thiotriphosphate) (mixed isomers)

N-8004-1	1 µmole	$27.50
N-8004-5	5 µmoles	$115.00
N-8004-10	10 µmoles	$205.00



$C_{10}H_{17}N_2O_{13}P_3S$
MW= 498.23 (free acid)
Ɛ= 9650 @ 267 nm
Lithium salt
>90% purity

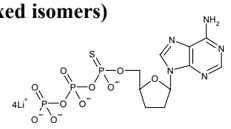
Ɛ is noted in L mmole^{-1} cm^{-1}.

Nucleotides

Alpha Phosphate Modified Nucleoside Triphosphates continued

2',3'-Dideoxyadenosine-5'-O-(1-Thiotriphosphate) (mixed isomers)

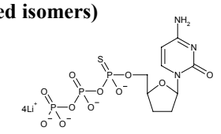
N-8009-1	1 µmole	$77.50
N-8009-5	5 µmoles	$365.00
N-8009-10	10 µmoles	$625.00



$C_{10}H_{16}N_5O_{10}P_3S$
MW= 491.25 (free acid)
ε= 15400 @ 258 nm
Lithium salt
>90% purity

2',3'-Dideoxycytidine-5'-O-(1-Thiotriphosphate) (mixed isomers)

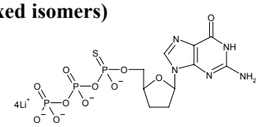
N-8010-1	1 µmole	$77.50
N-8010-5	5 µmoles	$365.00
N-8010-10	10 µmoles	$625.00



$C_9H_{16}N_3O_{11}P_3S$
MW= 467.22 (free acid)
ε= 9100 @ 271 nm
Lithium salt
>90% purity

2',3'-Dideoxyguanosine-5'-O-(1-Thiotriphosphate) (mixed isomers)

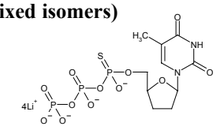
N-8011-1	1 µmole	$52.50
N-8011-5	5 µmoles	$255.00
N-8011-10	10 µmoles	$415.00



$C_{10}H_{16}N_5O_{11}P_3S$
MW= 507.24 (free acid)
ε= 13600 @ 252 nm
Lithium salt
>90% purity

2',3'-Dideoxythymidine-5'-O-(1-Thiotriphosphate) (mixed isomers)

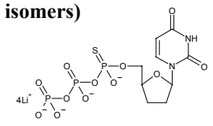
N-8012-1	1 µmole	$52.50
N-8012-5	5 µmoles	$255.00
N-8012-10	10 µmoles	$415.00



$C_{10}H_{17}N_2O_{12}P_3S$
MW= 482.23 (free acid)
ε= 9650 @ 267 nm
Lithium salt
>90% purity

2',3'-Dideoxyuridine-5'-O-(1-Thiotriphosphate) (mixed isomers)

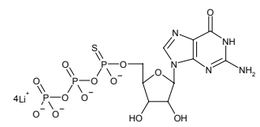
N-8015-1	1 µmole	$52.50
N-8015-5	5 µmoles	$255.00
N-8015-10	10 µmoles	$415.00



$C_9H_{15}N_2O_{12}P_3S$
MW= 468.2 (free acid)
ε= 10100 @ 262 nm
Lithium salt
>90% purity

Guanosine-5'-O-(1-Thiotriphosphate) (mixed isomers)

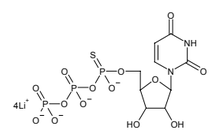
N-8007-1	1 µmole	$27.50
N-8007-5	5 µmoles	$115.00
N-8007-10	10 µmoles	$205.00



$C_{10}H_{16}N_5O_{13}P_3S$
MW= 539.24 (free acid)
ε= 13600 @ 252 nm
Lithium salt
>90% purity

Uridine-5'-O-(1-Thiotriphosphate) (mixed isomers)

N-8008-1	1 µmole	$27.50
N-8008-5	5 µmoles	$115.00
N-8008-10	10 µmoles	$205.00



$C_9H_{15}N_2O_{14}P_3S$
MW= 500.20 (free acid)
ε= 10100 @ 262 nm
Lithium salt
>90% purity

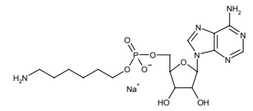
ε is noted in L mmole^{-1} cm^{-1}.

Labeled Nucleoside Monophosphates

5'-Amino-A-Monophosphate

Adenosine-5'-(6-Aminohexyl)-Monophosphate

N-6010-1	1 µmole	$52.50
N-6010-5	5 µmoles	$255.00
N-6010-10	10 µmoles	$415.00

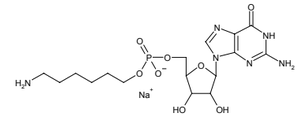


$C_{16}H_{27}N_6O_7P$
MW= 446.40 (free acid)
ε= 13700 @ 253 nm
Sodium salt
>90% purity

5'-Amino-G-Monophosphate

Guanosine-5'-(6-Aminohexyl)-Monophosphate

N-6009-1	1 µmole	$52.50
N-6009-5	5 µmoles	$255.00
N-6009-10	10 µmoles	$415.00

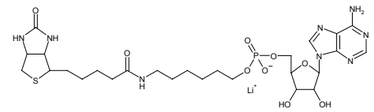


$C_{16}H_{27}N_6O_8P$
MW= 462.40 (free acid)
ε= 13700 @ 253 nm
Lithium salt
>90% purity

5'-Biotin-A-Monophosphate

Adenosine-5'-[N-(6-Biotinylamidohexyl)]-Monophosphate

N-6004-1	1 µmole	$52.50
N-6004-5	5 µmoles	$255.00
N-6004-10	10 µmoles	$415.00

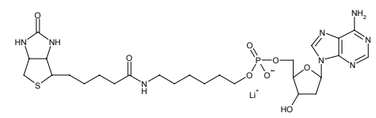


$C_{26}H_{41}N_8O_9PS$
MW= 672.70 (free acid)
ε= 15400 @ 259 nm
Lithium salt
>90% purity

5'-Biotin-dA-Monophosphate

2'-Deoxyadenosine-5'-[N-(6-Biotinylamidohexyl)]-Monophosphate

N-6006-1	1 µmole	$52.50
N-6006-5	5 µmoles	$255.00
N-6006-10	10 µmoles	$415.00

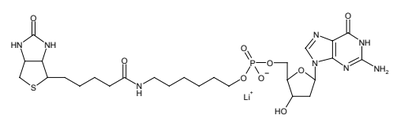


$C_{26}H_{34}N_8O_8PS$
MW= 656.75 (free acid)
ε= 15300 @ 258 nm
Lithium salt
>90% purity

5'-Biotin-dG-Monophosphate

2'-Deoxyguanosine-5'-[N-(6-Biotinylamidohexyl)]-Monophosphate

N-6005-1	1 µmole	$52.50
N-6005-5	5 µmoles	$255.00
N-6005-10	10 µmoles	$415.00

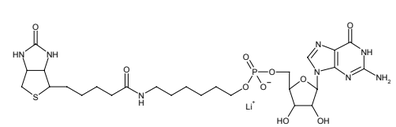


$C_{26}H_{39}N_8O_9PS$
MW= 672.69 (free acid)
ε= 13700 @ 252 nm
Lithium salt
>90% purity

5'-Biotin-G-Monophosphate

Guanosine-5'-[N-(6-Biotinylamidohexyl)]-Monophosphate

N-6003-1	1 µmole	$52.50
N-6003-5	5 µmoles	$255.00
N-6003-10	10 µmoles	$415.00



$C_{26}H_{41}N_8O_{10}PS$
MW= 688.70 (free acid)
ε= 13700 @ 253 nm
Lithium salt
>90% purity

ε is noted in L mmole^{-1} cm^{-1}.

Nucleotide Kits

For your convenience, we offer nucleotide kits that contain various combinations of our popular modified nucleoside triphosphates. These kits are perfect for all your assay development requirements and come with TriLink's high quality guarantee. Structures and physical data describing the individual products can be found in the preceding section on nucleoside triphosphates.

Alpha-Thiol Nucleotide Kits

These kits combine alpha-thiol nucleotides, useful for nick translation and other applications.

K-1001 **2'-Deoxynucleoside Alpha-Thiol Nucleotide Kit** **$135.00**

Contains 1.0 µmole each of 4 nucleotides:
N-8001 2'-Deoxyadenosine-5'-O-(1-Thiotriphosphate)
N-8002 2'-Deoxycytidine-5'-O-(1-Thiotriphosphate)
N-8003 2'-Deoxyguanosine-5'-O-(1-Thiotriphosphate)
N-8004 2'-Deoxythymidine-5'-O-(1-Thiotriphosphate)

K-1002 **Ribonucleoside Alpha-Thiol Nucleotide Kit** **$135.00**

Contains 1.0 µmole each of 4 nucleotides:
N-8005 Adenosine-5'-O-(1-Thiotriphosphate)
N-8006 Cytidine-5'-O-(1-Thiotriphosphate)
N-8007 Guanosine-5'-O-(1-Thiotriphosphate)
N-8008 Uridine-5'-O-(1-Thiotriphosphate)

K-1003 **2',3'-Dideoxynucleoside Alpha-Thiol Nucleotide Kit** **$260.00**

Contains 1.0 µmole each of 4 nucleotides:
N-8009 2',3'-Dideoxyadenosine-5'-O-(1-Thiotriphosphate)
N-8010 2',3'-Dideoxycytidine-5'-O-(1-Thiotriphosphate)
N-8011 2',3'-Dideoxyguanosine-5'-O-(1-Thiotriphosphate)
N-8012 3'-Deoxythymidine-5'-O-(1-Thiotriphosphate)

Chain Termination Kits

These kits contain nucleotides useful for chain termination protocols. We offer RNA as well as DNA terminators.

K-1004 **2',3'-Dideoxynucleotide Kit (DNA chain terminators)** **$160.00**

Contains 1.0 µmole each of 4 nucleotides:
N-4001 2',3'-Dideoxyadenosine-5'-Triphosphate
N-4005 2',3'-Dideoxycytidine-5'-Triphosphate
N-4002 2',3'-Dideoxyguanosine-5'-Triphosphate
N-4003 2'-3'-Dideoxyuridine-5'-Triphosphate

K-1005 **3'-Azido-2',3'-Dideoxynucleotide Kit (DNA chain terminators)** **$487.50**

Contains 1.0 µmole each of 5 nucleotides:
N-4007 3'-Azido-2',3'-dideoxyadenosine-5'-Triphosphate
N-4008 3'-Azido-2',3'-dideoxyguanosine-5'-Triphosphate
N-4009 3'-Azido-3'-deoxythymidine-5'-Triphosphate
N-4014 3'-Azido-2',3'-dideoxycytidine-5'-Triphosphate
N-4015 3'-Azido-2'-3'-dideoxyuridine-5'-Triphosphate

K-1006 **3'-Amino-2',3'-Dideoxynucleotide Kit (DNA chain terminators)** **$622.50**

Contains 1.0 µmole each of 4 nucleotides:
N-4010 3'-Amino-2',3'-dideoxyadenosine-5'-Triphosphate
N-4011 3'-Amino-2',3'-dideoxycytidine-5'-Triphosphate
N-4012 3'-Amino-2',3'-dideoxyguanosine-5'-Triphosphate
N-4013 3'-Amino-3'-deoxythymidine-5'-Triphosphate

K-1007 **3'-Deoxynucleotide Kit (RNA chain terminators)** **$387.50**

Contains 1.0 µmole each of 5 nucleotides:
N-3001 3'-Deoxyadenosine-5'-Triphosphate
N-3002 3'-Deoxyguanosine-5'-Triphosphate
N-3003 3'-Deoxycytidine-5'-Triphosphate
N-3004 3'-Deoxy-5-methyluridine-5'-Triphosphate
N-3005 3'-Deoxyuridine-5'-Triphosphate

2' Sugar Modified RNA Kits

These kits contain 2' modified ribonucleotides.

K-1008 **2'-Amino-2'-Deoxynucleotide Kit** **$207.50**

Contains 1.0 µmole each of 3 nucleotides:
N-1046 2'-Amino-2'-deoxyadenosine-5'-Triphosphate
N-1026 2'-Amino-2'-deoxycytidine-5'-Triphosphate
N-1027 2'-Amino-2'-deoxyuridine-5'-Triphosphate

K-1009 **Ara-Nucleotide Kit** **$227.50**

Contains 1.0 µmole each of 3 nucleotides:
N-1048 Araadenosine-5'-Triphosphate
N-1033 Aracytidine-5'-Triphosphate
N-1034 Arauridine-5'-Triphosphate

K-1010 **2'-Azido-2'-Deoxynucleotide Kit** **$232.50**

Contains 1.0 µmole each of 3 nucleotides:
N-1045 2'-Azido-2'-deoxyadenosine-5'-Triphosphate
N-1028 2'-Azido-2'-deoxycytidine-5'-Triphosphate
N-1029 2'-Azido-2'-deoxyuridine-5'-Triphosphate

K-1011 **2'-Fluoro-2'-Deoxynucleotide Kit** **$357.50**

Contains 1.0 µmole each of 5 nucleotides:
N-1007 2'-Fluoro-2'-deoxyadenosine-5'-Triphosphate
N-1008 2'-Fluoro-2'-deoxycytidine-5'-Triphosphate
N-1009 2'-Fluoro-2'-deoxyguanosine-5'-Triphosphate
N-1010 2'-Fluoro-2'-deoxyuridine-5'-Triphosphate
N-1055 2'-Fluorothymidine-5'-Triphosphate

K-1012 **2'-O-Methyl-Nucleotide Kit** **$110.00**

Contains 1.0 µmole each of 4 nucleotides:
N-1015 2'-O-Methyladenosine-5'-Triphosphate
N-1016 2'-O-Methylcytidine-5'-Triphosphate
N-1017 2'-O-Methylguanosine-5'-Triphosphate
N-1018 2'-O-Methyluridine-5'-Triphosphate

K-1013 **2' Sugar Super Modifier Kit** **$1135.00**

Contains 1 kit of each of the following:
K-1008 2'-Amino-2'-Deoxynucleotide Kit
K-1009 Ara-nucleotide Kit
K-1010 2'-Azido-2'-Deoxynucleotide Kit
K-1011 2'-Fluoro-2'-Deoxynucleotide Kit
K-1012 2'-O-Methyl-Nucleotide Kit

Post-Synthetic Modification Reagents

These kits contain nucleotides useful for post-synthetic labeling of DNA and RNA molecules.

K-1006 **3'-Amino-2',3'-Dideoxynucleotide Kit** **$622.50**

Contains 1.0 µmole each of 4 nucleotides:
N-4010 3'-Amino-2',3'-dideoxyadenosine-5'-Triphosphate
N-4011 3'-Amino-2',3'-dideoxycytidine-5'-Triphosphate
N-4012 3'-Amino-2',3'-dideoxyguanosine-5'-Triphosphate
N-4013 3'-Amino-3'-deoxythymidine-5'-Triphosphate

K-1008 **2'-Amino-2'-Deoxynucleotide Kit (For RNA)** **$207.50**

Contains 1.0 µmole each of 3 nucleotides:
N-1046 2'-Amino-2'-deoxyadenosine-5'-Triphosphate
N-1026 2'-Amino-2'-deoxycytidine-5'-Triphosphate
N-1027 2'-Amino-2'-deoxyuridine-5'-Triphosphate

Hybridization Modulation Kits

These kits contain nucleotides that have properties to increase the thermostability of duplexes, useful for studying hybridization properties of modified DNA and RNA or increasing thermostability for assay development.

K-1015 **RNA Thermostability Enhancer Kit** **$330.00**

Contains 1.0 µmole each of 4 nucleotides:

N-1001	2-Aminoadenosine-5'-Triphosphate
N-1002	2-Amino-6-chloropurineriboside-5'-Triphosphate
N-1014	5-Methylcytidine-5'-Triphosphate
N-1024	5-Methyluridine-5'-Triphosphate

K-1016 **DNA Thermostability Enhancer Kit** **$382.50**

Contains 1.0 µmole each of 5 nucleotides:

N-2003	2-Amino-2'-deoxyadenosine-5'-Triphosphate
N-2004	2-Aminopurine-2'-deoxyriboside-5'-Triphosphate
N-2016	5-Propynyl-2'-deoxycytidine-5'-Triphosphate
N-2017	5-Propynyl-2'-deoxyuridine-5'-Triphosphate
N-2026	5-Methyl-2'-deoxycytidine-5'-Triphosphate

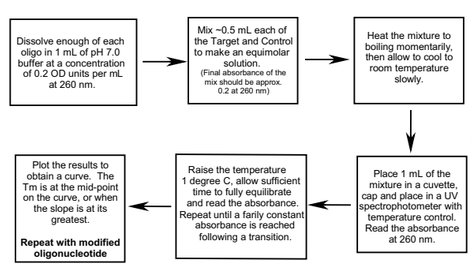
K-1017 **2'-Modified Thermostability Enhancer Kit** **$467.50**

Contains 1.0 µmole each of 9 nucleotides:

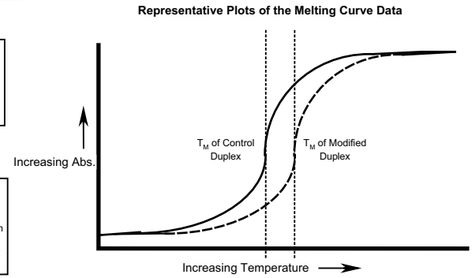
N-1007	2'-Fluoro-2'-deoxyadenosine-5'-Triphosphate
N-1008	2'-Fluoro-2'-deoxycytidine-5'-Triphosphate
N-1009	2'-Fluoro-2'-deoxyguanosine-5'-Triphosphate
N-1010	2'-Fluoro-2'-deoxyuridine-5'-Triphosphate
N-1055	2'-Fluorothymidine-5'-Triphosphate
N-1015	2'-O-Methyladenosine-5'-Triphosphate
N-1016	2'-O-Methylcytidine-5'-Triphosphate
N-1017	2'-O-Methylguanosine-5'-Triphosphate
N-1018	2'-O-Methyluridine-5'-Triphosphate

Determining Relative Thermostability by Melting Temperature

To determine how a proposed modification will effect the thermostability of a duplex, conduct the following simple melting temperature experiment using three oligonucleotides: a target sequence, a non-modified control probe, and the modified probe.



Representative Plots of the Melting Curve Data



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CleanAmp™

The Next Generation in Hot Start PCR

Solutions Start Here. TriLink's CleanAmp™ Product Line is an innovative new approach to Hot Start PCR. We have applied our expertise in modified nucleic acid chemistry to resolve common PCR problems. This advancement in Hot Start technology employs two widely ignored components of the PCR reagent mix: the primers and the dNTPs. Two distinct nucleic acid thermolabile protecting groups identified by TriLink's R&D team make the CleanAmp™ Primer and dNTP technology possible.

CleanAmp™ Products are activated during the initial heat cycle of Hot Start PCR. By remaining unreactive at lower temperatures, CleanAmp™ Products significantly reduce background amplification. A reduction or elimination of primer dimer and mis-priming can be achieved by adding CleanAmp™ Products into your PCR reaction.

Reasons to choose CleanAmp™ Products:

- Take advantage of a less expensive solution to high stringency PCR
- Achieve a significant reduction or elimination of off-target amplification
- Increase your amplicon yield and specificity in most cases
- Enjoy little change to current protocols when introducing CleanAmp™ Products
- Discover very reasonable licensing terms

Learn More About CleanAmp™ Products!

Visit www.trilinkbiotech.com/cleanamp

Check out all the great information in this catalog, including product details and benefits, application notes and an oligonucleotide synthesis protocol.

Read our recent publications:

- Lebedev AV, Paul N, Yee J, Kellum J, Timoshchuk VA, Hogrefe RI, Zon G. Chemically modified primers for improved hot start PCR. Collection Symposium Series. 2008;10:398–399.
- Lebedev AV, Paul N, Yee J, Timoshchuk VA, Shum J, Miyagi K, Kellum J, Hogrefe RI, Zon G. Hot start PCR with heat-activatable primers: a novel approach for improved PCR performance. Nucleic Acids Research. 2008 Nov; 36:e131.
- Shum J, Paul N. Chemically modified primers for improved multiplex polymerase chain reaction. Analytical Biochemistry. In Press.
- Koukhareva I, Haoqiang H, Yee J, Paul N, Hogrefe RI, Lebedev AV. A new approach to improved Hot Start PCR: Nucleoside 5'-triphosphates with thermolabile 3'-protecting group. Collection Symposium Series, 10, 259-263. 2008.
- Koukhareva I, Haoqiang H, Yee J, Shum J, Paul N, Hogrefe RI, Lebedev AV. Heat activatable 3'-modified dNTPs: Synthesis and application for Hot Start PCR. Nucleic Acids Symposium Series, 52, 259-260. 2008.

Ask our technical support team about your project today.

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CleanAmp™ dNTPs

CleanAmp™ dNTPs are the most versatile solution in TriLink's line of PCR enhancing products. CleanAmp™ dNTPs offer a universal approach to improved Hot Start PCR. Replacement of the standard dNTPs, the essential DNA polymerase substrate, with CleanAmp™ dNTPs, offers the same advantages as more costly Hot Start enzymes.

CleanAmp™ dNTPs offer precise control at the start of PCR thermal cycling by blocking DNA polymerase nucleotide incorporation until heat activated. The temperature-dependent control of DNA polymerase extension vastly reduces mis-priming, primer dimer formation and other common deleterious effects.

CleanAmp™ dNTPs:

- Offer significant cost savings over other Hot Start technologies
- Allow easy introduction into PCR reactions
- Reduce or eliminate primer dimer formation
- Reduce or eliminate mis-priming
- License the technology at reasonable rates

Improve Yield and Specificity

CleanAmp™ dNTPs vastly reduce mis-priming, primer dimer formation and other common deleterious effects (Figure 1). This improvement in the specificity of target amplification allows for enhanced detection of low input template concentrations (Figure 2).

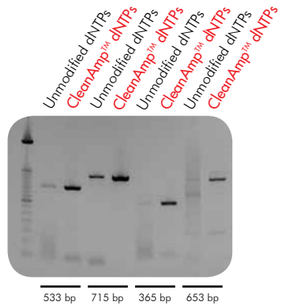

Figure 1: CleanAmp™ dNTPs improve amplification yield and specificity for a number of targets in PCR.

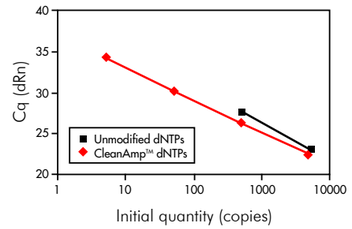

Figure 2: CleanAmp™ dNTPs improve the limit of detection in real-time PCR by two orders of magnitude.

A Flexible Solution

CleanAmp™ dNTPs are compatible with a number of thermostable DNA polymerases, allowing for greater flexibility in PCR design (Figure 3). The use of CleanAmp™ dNTPs in the experiment shown in Figure 3 eliminated or reduced primer dimer formations. Moreover, all of the DNA polymerases were able to produce the desired amplicon demonstrating the versatility of CleanAmp™ dNTPs.

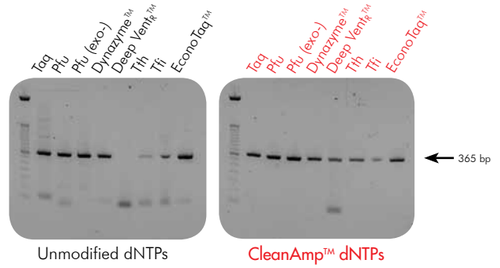

Figure 3: CleanAmp™ dNTPs are compatible with a number of commercially available DNA polymerases suited for PCR.

To learn more about CleanAmp™ dNTPs see pages 90-92 or go to www.trilinkbiotech.com/cleanamp.

Ordering:

CleanAmp™ dNTPs can be ordered online at www.trilinkbiotech.com/cleanamp.
They are sold as a mix of dATP, dCTP, dGTP and dTTP at 10 mM.

N-9501-2	2 µmole each (4 x 2)	$90
N-9501-10	10 µmole each (4 x 10)	$350

All CleanAmp™ dNTPs are analyzed by AX-HPLC, RP-HPLC, ^{31}P NMR, ^{1}H NMR, Mass Spec and are tested in a functional assay.

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CleanAmp™ Primers

CleanAmp™ Primers contain thermolabile chemical modifications that allow for primer-based Hot Start activation in PCR. These modifications prevent primer extension at the lower temperatures of PCR set-up and manipulation. A Hot Start thermal activation step removes the modification and generates the corresponding unmodified primer, which supports amplification of the desired target.

These novel primers allow you to:

- Save money by using a hot start solution that is only pennies per reaction.
- Introduce CleanAmp™ Primer modifications into any sequence.
- Depend on a guaranteed 10-15 OD yield from every synthesis.
- Receive primers in a high concentration stock solution.
- Scale-up to commercially viable amounts.
- License the technology at reasonable rates.

A Novel Technology

CleanAmp™ Primers specifically amplify your target by virtually eliminating extension off primer dimer (Figure 1A) and mis-priming (Figure 1B) events. Furthermore, CleanAmp™ Primers provide robust amplicon formation and are compatible with a number of standard DNA polymerases, such as *Taq*. (Figure 2)

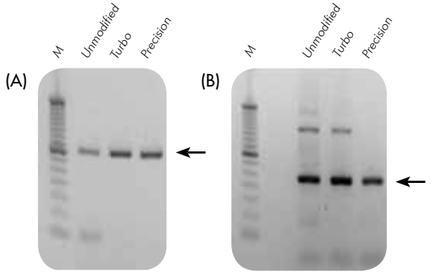
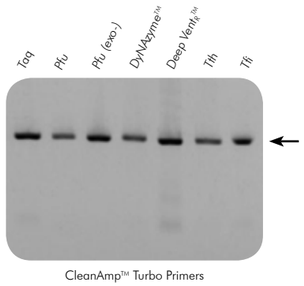


Figure 1: CleanAmp™ Primers improve PCR performance in systems prone to (A) primer dimer formation and (B) mis-priming. Both CleanAmp™ Turbo and Precision Primers improve the specificity of amplification, with Turbo Primers providing the greatest amplicon yield and Precision Primers providing the highest level of specificity.



CleanAmp™ Turbo Primers

Figure 2: CleanAmp™ Primers are beneficial when used with a number of thermostable DNA polymerases. The use of CleanAmp™ Turbo Primers provides robust amplification of the desired amplicon with primer dimer formation reduced or eliminated.

To learn more about CleanAmp™ Primers see pages 93-98 or go to www.trilinkbiotech.com/cleanamp.

Choose the Right Primer for You

CleanAmp™ Primers are available in two forms that differ in the rate of thermal activation into the corresponding unmodified primer. CleanAmp™ Turbo Primers activate more quickly than CleanAmp™ Precision Primers. The differential rate of activation of the two product types is beneficial for different applications and PCR needs:

CleanAmp™ Turbo Primers

- Fast cycling
- Multiplex PCR
- Improves amplicon yield
- Reduces mis-priming/primer dimer formation

CleanAmp™ Precision Primers

- Standard cycling
- One-step reverse-transcription PCR
- Improves specificity and limit of detection
- Greatest reduction in mis-priming/primer dimer formation

Ordering:

Order online at www.trilinkbiotech.com/cleanamp.
Primers are $250/pair, sold as phosphodiester DNA,
15-40 bases with a guaranteed minimum yield of 10 ODs.
Please inquire for special orders.

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CleanAmp™ Amidites

If you have oligonucleotide synthesis capabilities in-house, you may prefer making your own CleanAmp™ Primers. CleanAmp™ Amidites are available through our website, as well as through Glen Research.

Ordering:

CleanAmp™ Amidites can be ordered online, by phone or email.

CleanAmp™ - PAC-dA-CE	(100 µmoles)	X-9050-100	$100
CleanAmp™ - PAC-dG-CE	(100 µmoles)	X-9060-100	$100
CleanAmp™ - Ac-dC-CE	(100 µmoles)	X-9070-100	$100
CleanAmp™ - dT-CE	(100 µmoles)	X-9080-100	$100

Please inquire for bulk quantities. CleanAmp™ Amidites can also be purchased through Glen Research at www.glenres.com.

The Benefits of CleanAmp™ Technology for Less

CleanAmp™ Amidites allow you to manufacture CleanAmp™ Primers in-house using standard solid phase synthesis procedures. CleanAmp™ Amidites can be used on any DNA synthesizer. Make as much or as little material as you need, when you need it. Protocols for CleanAmp™ Primer synthesis can be found on pages 99-101. If followed precisely, it is very simple to prepare CleanAmp™ Primers.

Successful Syntheses with CleanAmp™ Amidites

Several properties of the CleanAmp™ Amidites and CleanAmp™ Primers are discussed below. Understanding these characteristics and carefully following the protocols provided will result in high quality CleanAmp™ Primers.

Thermolability of the Phosphorus Protecting Group
Devising a primer that would undergo Hot Start activation required the development of a phosphorus protecting group that would have the proper lability at 95°C. This had to be carefully balanced with the ability to prepare, deliver, store and handle the oligonucleotide. It is critical that the compound is handled appropriately and not subjected to elevated temperatures at any time.

Don't heat your CleanAmp™ Amidites or Primer above room temperature at any time during synthesis.
Don't use a centrifugal concentrator to dry your sample completely.

Base Lability of the Phosphorus Protecting Group
The CleanAmp™ Primer protecting group is a phosphotriester, like most other phosphorus protecting groups used for oligonucleotide synthesis. Phosphotriesters are labile to base, however the CleanAmp™ esters are much more stable than the ß-cyanoethyl group commonly used. Therefore we readily identified conditions that removed the protecting groups from the rest of the oligonucleotide, while leaving the CleanAmp™ modification in place.

Do use fast deprotecting phosphoramidites.
Do use methanolic potassium carbonate at room temperature for deprotection.
Don't use standard phosphoramidites or standard deprotection schemes.

Use of DMSO as the Storage Solution
Although reasonable stability of CleanAmp™ Primers can be acheived in aqueous buffers, we were very fortunate to discover that DMSO is an excellent stabilizing solution, allowing us to store these modified primers at room temperature for extended times.

Do use pure DMSO.
Don't use the standard mix of ACN/aq. TEA (1:1) to elute your CleanAmp™ Primer from a reverse phase cartridge.

Assaying the Final Product
Once the CleanAmp™ Primer is eluted, an important part of the protocol is the HPLC assay of the product. For the best success in a PCR reaction, the amount of unprotected primer should be less than 1%.

Don't allow more than 1% unmodified material in the sample mixture, and in the case of Precision Primers, no more than 20% of the singly modified species.
Do assay all of your CleanAmp™ Primers by RP-HPLC to ensure that specifications are met.

For full details on successful CleanAmp™ Primer synthesis visit: www.trilinkbiotech.com/cleanamp.

CleanAmp™ dNTP Application Note

By Tony Le, Jonathan Shum and Natasha Paul, Ph.D.; TriLink BioTechnologies

As a diagnostic tool in molecular biology, the Polymerase Chain Reaction (PCR) is one of the most powerful and commonly used techniques. However, inherent flaws can plague PCR, especially when nonspecific amplification such as primer dimer formation dominates the reaction. These off-target amplifications are generally due to the excess of primers in each experiment, where primer interaction and extension are almost inevitable (1). Competing off-target amplifications decrease the overall effectiveness of PCR by consuming key components within the reaction and inhibiting the amplification of the intended target (2).

Commonly used approaches to mitigate the formation of off-target amplification in PCR are termed Hot Start (3). In this process, the components of a PCR reaction are prevented from forming primer extension products until higher thermocycling temperatures are reached. Many developments have been made in this technology, including strategies that require the physical separation of reaction components, inhibition of DNA polymerase, the addition of accessory proteins and the use of chemically modified primers that are unextendable (2,3). While these modifications to PCR setup can be effective, they can also significantly increase the cost of reactions. One area that had not been explored is the concept of deoxynucleoside-5´-triphosphates (dNTPs) that are modified to allow for a Hot Start activation approach in PCR. Since dNTPs are ubiquitous in all PCR experiments, modified dNTPs can easily be incorporated in any reaction by simple substitution. CleanAmp™ dNTPs provide a novel, cost-effective and efficient route to Hot Start PCR that is amendable to use in standard PCR protocols, with a wide range of commonly-used DNA polymerases.

CleanAmp™ dNTPs contain thermolabile modification groups that allow for dNTP-mediated Hot Start activation in PCR. The introduction of temperature sensitive tetrahydrofuranyl protecting groups onto the 3'-hydroxyl of a dNTP blocks primer extension at the less stringent, lower temperatures of PCR reaction preparation (Figure 1). When the reaction is heated to the elevated temperatures of PCR, the protecting group is removed to form the corresponding standard dNTP, which is now a suitable DNA polymerase substrate. The loss of protecting group can be attributed to the acidification of Tris-based PCR buffers as temperature is increased (for example, an increase in temperature will cause a Tris-based reaction buffer at ~ pH 8 (25°C) to become ~ pH 6 at elevated temperatures (95°C)). This temperature-dependent control of dNTP activation has shown great promise for the use of CleanAmp™ dNTPs with DNA polymerases that utilize Tris-based PCR buffers, pH 8 to 9 at 25°C.

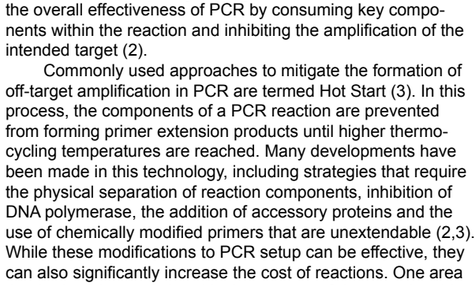


Figure 1: Hot Start Activation of CleanAmp™ dNTPs.

The efficacy of CleanAmp™ dNTPs is highlighted herein as they are able to decrease the formation of primer dimer products while increasing the specificity of the desired product. The following experiments explore the effectiveness of CleanAmp™ dNTPs in primer/template systems prone to primer dimer artifacts by comparing their performance to unmodified dNTPs and Hot Start DNA polymerases. Studies will also demonstrate the compatibility of CleanAmp™ dNTPs when used in combination with a variety of non-Hot Start and Hot Start DNA polymerases.

Eliminate Primer Dimer Formation, Increase Yield

CleanAmp™ dNTP modifications significantly lower or eliminate primer dimer formation while increasing target amplicon yield in endpoint PCR. A problematic 533 bp primer/template system from Lambda genomic DNA is prone to primer dimer formation in PCR, especially at low template concentrations (4). After 40 thermal cycles, reactions that employed natural dNTPs are compromised by competing amplification of non-target sequences (Figure 2). Under standard PCR conditions, the effect on specificity and sensitivity of detection is most significant at lower template concentrations as the target amplicon is completely absent and primer dimers predominate. When CleanAmp™ dNTPs are substituted for standard dNTPs, not only are primer dimers significantly reduced, the desired amplicon's yield is increased as well. By introducing CleanAmp™ dNTPs into a PCR setup, primer dimers and other off-target amplicons are significantly reduced, resulting in a cleaner PCR reaction. Although these results were obtained using 0.2 mM concentration, the most consistent results are seen with a dNTP concentration of 0.4 mM and a $MgCl_2$ concentration of 2.5 mM.

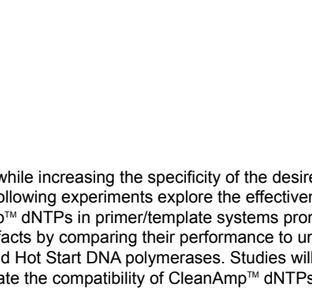


Figure 2: Endpoint PCR evaluation of CleanAmp™ dNTPs in a primer/template system prone to primer dimer. PCR Conditions[1] on page 92.

Improve Specificity Over a Greater Range of Template Concentrations

CleanAmp™ dNTPs provide amplification specificity over a greater range of template concentrations in real-time PCR. Real-time detection of the above-mentioned 533 bp target displays reduced sensitivity in real-time detection due to predominant primer dimer formation at lower template concentrations. The detection of the Lambda gDNA amplicon was tested over a variety of template concentrations ranging from 5-5,000 copies using *Taq*Man® probe detection in real-time PCR (Figure 3). When 5 to 50 copies of template were employed, the use of standard dNTPs failed to amplify the target band, while CleanAmp™ dNTPs successfully yielded the desired product at all template concentrations. When the resulting data was plotted in a standard curve, CleanAmp™ dNTPs afforded a 10-100 fold increase in sensitivity relative to standard dNTPs, thus allowing for detection over a wider range of template concentrations. By increasing the limit of detection, CleanAmp™ dNTPs give the user more options and fewer restrictions in experimental design for real-time target detection.

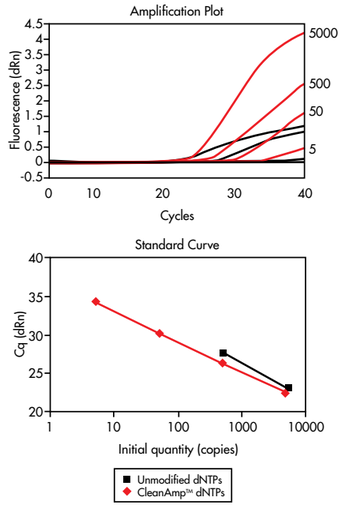
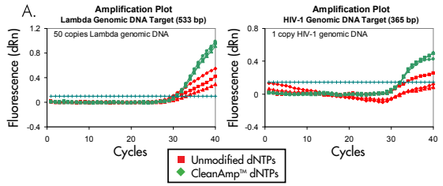


Figure 3: Real-time PCR detection of 5-5,000 copies of Lambda gDNA using CleanAmp™ dNTPs. PCR Conditions[1] on page 92.

Increase PCR Reproducibility

Real-time PCR results often vary and require multiple iterations to achieve reproducible data, especially when template concentrations are limiting (3,5). With CleanAmp™ dNTPs, PCR data fluctuates very little within an experimental run, allowing analysis with confidence. In the real-time experiment shown in Figure 4, CleanAmp™ dNTPs were tested against unmodified dNTPs at different template concentrations in two separate primer/template systems.



B.

Reproducibility of Real-Time Data
(Standard deviation of Ct Values)

	Copies	dNTP (std. dev.)	CleanAmp™ dNTP (std. dev.)
HIV-1 genomic DNA template	1	3.62	0.11
	5	0.24	0.08
	25	0.05	0.04
Lambda genomic DNA template	50	1.57	0.11
	500	0.32	0.09
	5000	0.22	0.07

Figure 4: SYBR Green real-time PCR assay where results of 1 copy of HIV-1 gDNA and 50 copies of Lambda gDNA were consistent when using CleanAmp™ dNTPs.
A. Amplification plots of the real-time data. B. Real-time data in chart form. PCR Conditions[1,2] on page 92.

For both the HIV-1 and Lambda DNA targets, CleanAmp™ dNTPs provide greater experimental precision, especially at lower template concentrations. The standard deviation at these low concentrations is high under standard conditions, yet decreases considerably when CleanAmp™ dNTPs are used in the reaction. Even at high template concentrations, where the standard deviation of reactions with standard dNTPs is low, CleanAmp™ dNTPs still produce a more consistent result. With CleanAmp™ dNTPs, more time is spent analyzing data instead of second-guessing PCR results.

Versatility of CleanAmp™ dNTPs

Though *Taq* is one of the most commonly used DNA polymerases in PCR, there are other thermostable DNA polymerases that may be amenable for use with CleanAmp™ dNTPs. To investigate further, seven non-Hot Start DNA polymerases were tested in PCR with endpoint agarose gel analysis to learn whether they could successfully amplify a 365 bp target band with either standard or CleanAmp™ dNTPs (Figure 5). When using standard dNTPs, Deep VentR™ amplified only primer dimer while *Tth* and *Tfi* DNA showed only slight amplification. Furthermore, off-target amplification is evident for most of the remaining DNA polymerases. In contrast, the introduction of CleanAmp™ dNTPs into these experiments eliminated or reduced primer dimer formations, and all of the DNA polymerases were able to produce the desired 365 bp amplicon. CleanAmp™ dNTPs work well together with a broad selection of DNA polymerases, in addition to *Taq* DNA polymerase, further demonstrating their versatility.

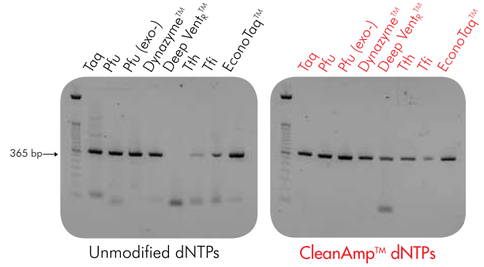


Figure 5: Evaluation of the performance of CleanAmp™ dNTPs in amplification reactions with a variety of thermostable DNA polymerases. PCR Conditions[2] on page 92.

Achieve Better Results Compared to Hot Start DNA Polymerases

In these experiments, reactions containing *Taq* DNA polymerase with CleanAmp™ dNTPs were compared to reactions with various Hot Start DNA polymerases and natural dNTPs for their ability to amplify a 365 bp target from HIV-1 (Figure 6) (3). When tested against other common Hot Start technologies, CleanAmp™ dNTPs provided a comparable reduction in primer dimer formation. Although all Hot Start approaches successfully suppressed off-target amplicon formation in this primer/template system, reactions containing *Taq* DNA polymerase and CleanAmp™ dNTPs outperformed the Hot Start technologies by providing a greater relative amplicon yield. Overall, when evaluated alongside other Hot Start technologies, CleanAmp™ dNTPs display comparable off-target results while enhancing target amplification. Other primer/template systems may require further optimization. See the CleanAmp™ dNTP Product Manual for specific details.

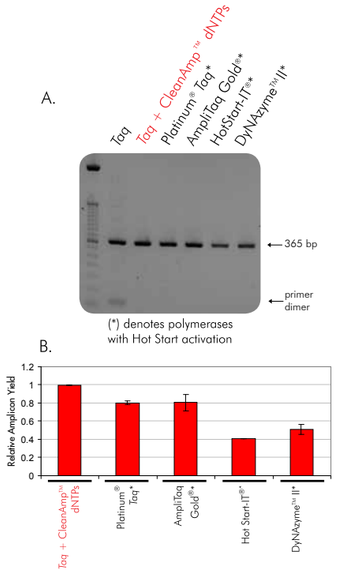
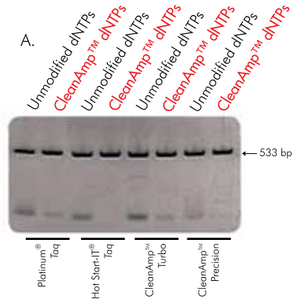


Figure 6: Comparison of CleanAmp™ dNTPs to other commercially available Hot Start DNA polymerases. A. Endpoint PCR analysis of amplification reactions containing 5 copies of HIV-1 genomic DNA. B. Graphical representation of the relative amplicon yield (normalized to *Taq* + CleanAmp™ dNTPs). PCR Conditions[2] on page 92.

Improve Performance with Other Hot Start Technologies

When used in combination with other Hot Start technologies, CleanAmp™ dNTPs allow for an even greater enhancement in PCR specificity. In Figure 7, experiments were conducted with a Lambda gDNA template system, known for its susceptibility to primer dimer formation. When Platinum® *Taq* and HotStart-IT® *Taq* were used with standard dNTPs, relative amplicon yield improved compared to *Taq* DNA polymerase, but primer dimer formation was still evident. With the addition of CleanAmp™ dNTPs, off-target amplification is significantly reduced or eliminated. Similarly, TriLink's CleanAmp™ Turbo and Precision Primers both increased relative amplicon yield, yet had some primer dimer byproducts. When used in conjunction with CleanAmp™ dNTPs, an improvement in PCR performance was seen, especially with CleanAmp™ Precision Primers, where off-target amplification was entirely eliminated. When used individually, each Hot Start approach improves the specificity and efficiency of PCR reactions, but this improvement is further enhanced when the Hot Start technologies are used in combination with CleanAmp™ dNTPs.



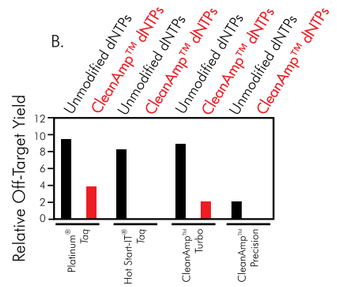


Figure 7: Comparison of commercially available Hot Start DNA polymerases used with and without CleanAmp™ dNTPs. A. Endpoint PCR analysis of amplification reactions containing 5 copies of HIV-1 genomic DNA. B. Graphical representation of the relative off-target amplification. PCR Conditions[1] on page 92.

Summary

CleanAmp™ dNTPs reduce or eliminate off-target amplification while enhancing target yield. Comparable or better performance than Hot Start DNA polymerases can be achieved at a fraction of the price.

PCR Conditions

1. PCR: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM MgCl2), Primers (0.2 µM), 0.2 mM dNTPs, 5,000 copies Lambda gDNA, 1.25 U *Taq* DNA polymerase, 50 µL. Thermal cycling: 95°C (10 min); [95°C (40 sec), 57°C (30 sec), 72°C (60 sec)] 40X.
2. PCR: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM MgCl2), Primers (0.4 µM) 0.2 mM dNTPs, 5 copies HIV-1 gDNA, DNA polymerase (var. U), 50 µL. Thermal cycling: 95°C (10 min); [95°C (40 sec), 56°C (30 sec), 72°C (2min)] 35X. 72°C (7min).

NOTE: TaqMan® probe (0.1 µM) and ROX were used for real-time experiments.

References

1. Brownie, J., Shawcross, S., Theaker, J., Whitcombe, D., Ferrie, R., Newton, C. and Little, S. (1997) The elimination of primer-dimer accumulation in PCR. Nucleic Acids Res, 25, 3235-3241.
2. Dang, C. and Jayasena, S.D. (1996) Oligonucleotide inhibitors of Taq DNA polymerase facilitate detection of low copy number targets by PCR. J Mol Biol, 264, 268-278.
3. Chou, Q., Russell, M., Birch, D.E., Raymond, J. and Bloch, W. (1992) Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. Nucleic Acids Res, 20, 1717-1723.
4. Lebedev, A.V., Paul, N., Yee, J., Timoshchuk, V.A., Shum, J., Miyagi, K., Kellum, J., Hogrefe, R.I. and Zon, G. (2008) Hot start PCR with heat-activatable primers: a novel approach for improved PCR performance. Nucleic Acids Res, 36, e131.
5. Svenstrup, H.F., Jensen, J.S., Bjornelius, E., Lidbrink, P., Birkelund, S. and Christiansen, G. (2005) Development of a quantitative real-time PCR assay for detection of Mycoplasma genitalium. J Clin Microbiol, 43, 3121-3128.
6. Koukhareva, I., Haoqiang, H., Yee, J., Paul, N., Hogrefe, R.I. and Lebedev, A.V. (2008) A New Approach to Improved Hot Start PCR: Nucleoside 5'-Triphosphates with Thermolabile 3'-Protecting Group. Collection Symposium Series, 10, 259-260.
7. Koukhareva, I., Haoqiang, H., Yee, J., Shum, J., Paul, N., Hogrefe, R.I. and Lebedev, A.V. (2008) Heat Activatable 3'-modified dNTPs: Synthesis and Application for Hot Start PCR. Nucleic Acids Symposium Series, 52, 259-260.

CleanAmp™ Primer Application Note

By Jonathan Shum, Joyclyn Yee, Elena Hidalgo Ashrafi, and Natasha Paul, Ph.D.; TriLink BioTechnologies

The Polymerase Chain Reaction (PCR) is a commonplace molecular biological method to amplify a DNA target of interest. Although PCR is a powerful technique, its sensitivity and reproducibility are often plagued by off-target amplifications. These include primer dimer and mis-priming products caused by the hybridization of primers to one another or to non-specific regions on the template. Such off-target amplifications lower the efficiency of PCR by effectively sequestering PCR substrates, such as the primers, the DNA polymerase, and the dNTPs, from amplifying the desired template. At lower template concentrations, the problem of primer dimer formation and mis-priming is exacerbated as less template is available causing an increase in undesired primer hybridization and extension. (1) Therefore, it is critical to substantially decrease or even eliminate off-target amplification, especially when available template is limited.

Attempts have been made to alleviate the problem of primer dimer formation by the use of Hot Start technologies, which include physical separation of reaction components, inhibition of the DNA polymerase, and the use of accessory proteins. Many of the specialized DNA polymerase compositions can add significant cost to the reaction by the need for extensive manipulations, such as in the preparation of the DNA polymerase. In contrast, primers, which have CleanAmp™ thermolabile protecting groups, represent a simple approach to Hot Start activation in PCR. These modifications can be easily introduced to any primer sequence using standard solid phase oligonucleotide synthesis protocols. In addition, CleanAmp™ modifications are compatible with many commonly used DNA polymerases.

The introduction of thermolabile CleanAmp™ modifications into PCR primers allows for greater control of primer hybridization and extension during PCR. The thermolabile primer modifications prevent DNA polymerase extension at the lower, less discriminating temperatures of reaction set-up and manipulation, but also display the flexibility to allow intended extension after activation at higher temperatures (Figure 1).

Furthermore, by using either the slow-releasing Precision primers or the faster-releasing Turbo Primers, the rate of formation of unmodified primer can be controlled to suit your reaction needs. Herein, we investigate the utility of the CleanAmp™ Primer modifications in standard PCR protocols and in more advanced techniques such as multiplex PCR, one-step RT-PCR and fast cycling PCR.

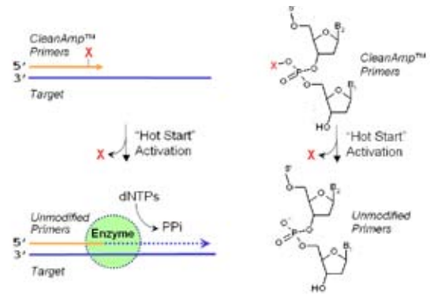
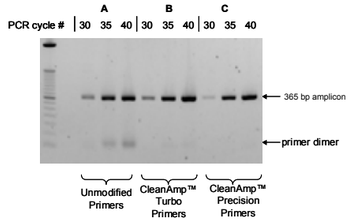


Figure 1: Hot Start Activation of CleanAmp™ Primers

General Applications

CleanAmp™ Turbo and Precision Primers significantly lower, if not eliminate off target amplification

Primer dimer formation has been found to be problematic in the amplification of a region of the HIV-1 tat genomic DNA. (1) In these studies, the reaction progression was monitored by removing aliquots after 30, 35 and 40 thermal cycles (Figure 2). Amplifications using unmodified PCR primers were found to be prone to robust primer dimer formation, which competes with the formation of the desired 365 bp amplicon (Figure 2A). By contrast, the introduction of Turbo Primers significantly reduced primer dimer formation and promoted an even greater target yield as compared to the unmodified primers (Figure 2B). For Turbo Primers, only a slight amount of primer dimer is seen after 40 cycles. In the same system, the use of Precision Primers yielded only the desired amplicon, with no detectable primer dimer formation. However, while the slower release of the Precision protecting groups significantly reduced primer dimer formation, robust target amplification was slightly delayed at 30 thermal cycles but fully recovered after 40 thermal cycles (Figure 2C). These studies demonstrated that Turbo Primers were able to efficiently form the desired amplicon, while significantly reducing primer dimer formation. Precision Primers were found to have the greatest utility when pure amplicon formation is required.



PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$), Primers (0.5 µM), dNTPs (0.2mM), 5 copies HIV-1 gDNA, *Taq* DNA polymerase (1.25U), 50 µL. Thermal cycling conditions: 95°C (10 min); [95°C (40 sec), 56°C (30 sec), 72°C (1 min)]40X, 72°C (7 min).

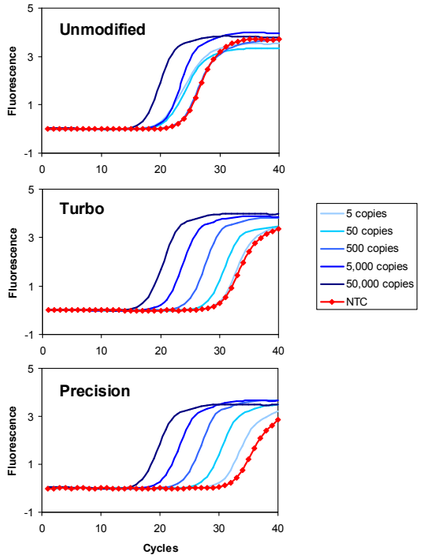
Figure 2: Endpoint PCR evaluation of CleanAmp™ Primers in a primer/template system prone to primer dimer. Aliquots of reaction were removed at 30, 35 and 40 cycles.

Mis-priming can also be a significant hindrance to the fidelity and efficiency of amplification of the desired target. In comparison to unmodified primers, Turbo Primers reduce much of the mis-priming products, with Precision Primers providing the greatest benefit. This reduction in off-target amplification is evident over a wide range of input template concentrations with improved amplicon yield relative to unmodified primers.

CleanAmp™ Primers provide amplification specificity over a large range of template concentrations.

Detection of a target at low concentrations is another difficulty encountered in PCR. Often, at low template concentrations, off-target amplifications compete with the desired amplification, complicating real-time PCR detection of the desired amplicon formation. CleanAmp™ Primers have been found to successfully amplify the correct amplicon at 10-100 fold lower template concentration as compared to unmodified primers. In Figure 3, the lower limit of detection of a 533 bp amplicon from Lambda genomic DNA was explored over a range of input template concentrations using SYBR Green® detection in real-time PCR. For the unmodified primers, the range of detection

started above 500 copies. Because the amplification curve coincided with the no template control (NTC) curve at 500 copies or less, it would be impossible to differentiate between desired amplicon formation and other off-target formation.



PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$), Precision CleanAmp™ Primers (0.5 µM), SYBR Green (.15X), ROX (30nM), dNTPs (0.2 mM), 0-50,000 copies Lambda gDNA, 1.25 U *Taq* DNA polymerase, 25 µL; reactions performed in duplicate. Thermal cycling conditions: 95°C (10 min); [95°C (40 sec), 57°C (30 sec), 72°C (1 min)] 40X.

Figure 3: SYBR Green® real-time PCR assay where 0-50,000 copies of Lambda gDNA were assayed using unmodified, Turbo and Precision Primers.

The use of Turbo Primers provides at least a ten-fold increase in detection, as the 50 copy concentration is distinguishable from the NTC curve. Precision Primers ultimately provide the greatest level of detection, detecting as low as five copies. This increased limit of detection using Precision Primers is indicative of their utility in a number of high-sensitivity downstream applications, such as single molecule detection.

CleanAmp™ Primers outperform other Hot Start Technologies

Since CleanAmp™ Primers provide significant benefit relative to reactions with unmodified primers, experiments were then carried out to compare the performance of CleanAmp™ Primers to that of other Hot Start technologies. In these studies, the performance of unmodified primers with one of a series of Hot Start DNA polymerases, such as a chemically modified version of *Taq* (2), was compared to the performance of CleanAmp™ Primers with unmodified *Taq* DNA polymerase. Amplicon was formed with equal or much lower yield than reactions that employed unmodified *Taq* DNA polymerase with Precision Primers (Figure 4A, B). Moreover, Turbo Primers and unmodified *Taq* DNA polymerase gave the greatest benefit, as the amplicon yield was much higher than each of the Hot Start polymerases examined. These findings are significant, as they indicate that CleanAmp™ Primers and unmodified *Taq* DNA polymerase can be employed without compromising amplicon yield, while efficiently reducing primer dimer formation and mis-priming.

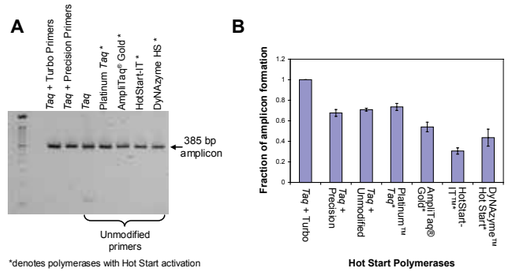


Figure 4: Comparison of CleanAmp™ Primers to other commercially available Hot Start DNA polymerases. A. Endpoint PCR analysis of amplification reactions containing five copies of HIV-1 genomic DNA. B. Graphical representation of the relative amplicon yield, normalized to reactions containing *Taq* DNA polymerase plus CleanAmp™ Turbo Primers.

CleanAmp™ Primers are compatible with other DNA polymerases

Taq DNA polymerase was used as a point of reference to determine whether Precision and Turbo CleanAmp™ Primers could be employed with other DNA polymerases in endpoint PCR experiments. In the endpoint reactions, seven DNA polymerases devoid of Hot Start activation were evaluated for their ability to robustly form the desired 365 bp amplicon (Figure 5). Each of the DNA polymerases examined was able to support efficient amplification of the DNA target. In all cases, with the exception of Deep Vent™ and *Tfi* polymerase, the units of DNA polymerase were kept constant. Overall, CleanAmp™ Primers are versatile as they can be used with other thermostable DNA polymerases in endpoint PCR. This compatibility gives great potential for the implementation of CleanAmp™ Primers in a number of applications.

PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$), Primers (0.4 µM), dNTPs (0.2mM), 5 copies HIV-1 gDNA, *Taq* DNA polymerase (1.25U), *Pfu* (2.5U), *Pfu*(exo-) (2.5U), Dynazyme™ (1.2U), Deep Vent™ (2.5U), *Tth* (2.5U), *Tfi*(20U), 50 µL. Thermal cycling conditions: 95°C (10 min); [95°C (40 sec), 56°C (30 sec), 72°C (1 min)] 35X, 72°C (7 min).

Figure 5: Evaluation of the performance of CleanAmp™ Primers in amplification reactions with a variety of thermostable DNA polymerases.

Multiplex PCR Applications

One promising application of PCR is the ability to amplify multiple targets in a single reaction. This approach, known as multiplex PCR, employs a distinct primer pair for each amplicon of interest. This application has been an essential tool for many different medical diagnostic and scientific applications, such as viral screens(3), where PCR based assays have proven to be more sensitive and less time consuming than traditional cell culture tests(4).

Although multiplex PCR has many advantages, there are inherent problems that inhibit robust amplification. One major factor is the increased propensity for primer dimer formation(5), which can result from the larger number of unique primer sequences in the reaction. Another challenge of multiplex PCR is the preferential amplification of certain targets(6). Therefore the design of multiple primer pairs that are both specific for a target of interest and exhibit a low level of off-target amplicon formation can be a challenge. Coupled with this decreased flexibility in primer design, individual primer pair concentrations must be optimized, such that amplification efficiencies of all targets are similar(7). This is a time consuming process, which has a low probability of success, should off-target amplicon formation dominate the reaction. Below, the ability of CleanAmp™ Primers to improve the specificity of amplicon formation for all targets in multiplex PCR is evaluated. The ability of CleanAmp™ Primers to reduce other competing off-target amplification, in single target reactions was applied to a multiplex PCR assay. A system where amplicon yield and PCR efficiency are extremely sensitive to primer dimer formation was used. Findings revealed minimal optimization of the design and concentration of the CleanAmp™ Turbo Primers, improving with reduced primer dimer formation.

Multiplexed target amplification at low template concentrations

When individual assays are combined into a single, multiplexed PCR assay, often template concentration must be increased to compensate for inefficient amplification. However, in clinical settings, where template sample is limited, increasing template concentration is not an option.

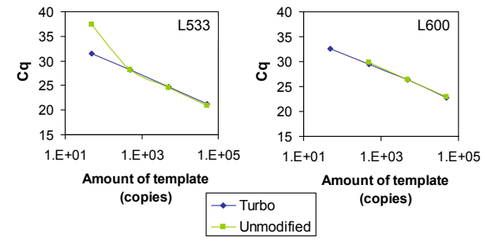
PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$), Primers (0.5 µM), 0.2 mM dNTPs, var. copies Lambda gDNA, 1.25 U *Taq* DNA polymerase, 50 µL.Thermal cycling conditions: 95°C (10 min); [95°C (40 sec), 56°C (30 sec), 72°C (2 min)] 35X, 72°C (7 min).

Figure 6: Multiplex reactions over a wide range of template amounts. A. Performance of Turbo and unmodified primers with a varying amount of template. B. Quantitative analysis of amplicon yield and primer dimer formation for experiment performed in Figure 6A. For each template concentration, amplicon yield was normalized to Turbo.

In these cases where greater sensitivity is necessary, Turbo Primers have demonstrated much promise. When compared to unmodified primers, amplicon formation in a triplet reaction was detected at a 100-fold lower input of template when using Turbo Primers (Figure 6A), with Turbo Primers efficiently detecting 50 copies of Lambda genomic DNA, where unmodified primers could only detect 5,000 copies. Furthermore, the longer targets appeared to amplify less efficiently than the shorter targets when using unmodified primers, with the longest 962 bp amplicon not forming until 50,000 copies of template were employed. On the other hand, at all template concentrations examined, Turbo Primers amplified all three targets with similar efficiency. Additionally the use of CleanAmp™ Primers improved PCR performance by reducing primer dimer formation (Figure 6B). In summary, efficient amplification by Turbo Primers is less restricted by target size limitations, all three amplicons being formed over a broad range of input template concentrations at increased sensitivity.

CleanAmp™ Turbo Primers outperform in real-time multiplex PCR

To confirm that the range of detection was also reproducible in quantitative real-time PCR, a duplex reaction using Taqman® probe detection was performed. Much like the endpoint experiments, at low template concentrations, the detection of amplification is much more sensitive using CleanAmp™ Turbo Primers. In this duplex reaction, the difference in Cq between unmodified and Turbo Primers increased as template concentration decreased (Figure 7). In some cases, such as in the L600 target, no Cq is observed at 50 copies for the unmodified primers. Turbo Primers provide earlier detection of successful amplification, whereas amplicon detection with unmodified primers is delayed or not present.



PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$), Primers (0.5 µM), 0.2 mM dNTPs, var. copies Lambda gDNA, TaqMan® probe (0.1 µM), ROX (30nM),1.25 U *Taq* DNA polymerase, 50 µL.Thermal cycling conditions: 95°C (10 min); [95°C (40 sec), 56°C (30 sec), 72°C (2 min)] 35X, 72°C (7 min).

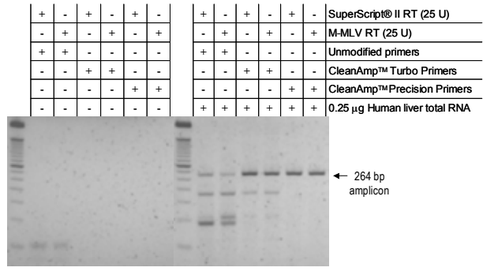
Figure 7: Real-time Multiplex PCR detection of a wide range of template amounts. Comparison of Turbo and unmodified primers varying concentration of template.

Overall, the use of CleanAmp™ Turbo Primers in multiplex PCR provides several advantages, which include greater amplicon yield and lower primer dimer formation. Turbo Primers provide great flexibility in assay design, as a wide range of primer concentrations produce robust, non-preferential amplification. Furthermore greater sensitivity is achieved for both endpoint and real-time assays, with a 100-fold increase in the limit of detection.

One-step RT-PCR Applications

With the advent of microarrays, the need to validate the massive amount of gene expression results has grown significantly. Reverse transcription PCR (RT-PCR) has become the gold standard for validation of microarray gene expression profiles(8,9). The typical RT-PCR reaction consists of a two-step protocol that involves a lower temperature reverse transcription step followed by an elevated temperature PCR step(10). The extra manipulation procedures inherent to a two-step protocol can introduce opportunities for contamination. A one-step RT-PCR protocol provides a streamlined, high-throughput technique that reduces the chances of contamination(11). Another advantage for a one-step protocol is that replicates will repeat both the reverse transcription and the PCR step. However, one-step RT-PCR is not without its own inherent problems.

In many cases one-step RT-PCR reactions are not as sensitive as two-step(12,13). The lack of sensitivity can be the result of reverse transcriptase(14) or DNA polymerase(15) mediated extension of primers to form primer-dimer and/or non-specific products at the less stringent temperatures of reverse transcription. To improve the sensitivity and specificity of RT-PCR, inhibition of such primer extension at lower temperatures is required.



Thermal cycling conditions: 42°C (30 min), 95°C (10 min), [95°C (30 sec), 60°C (30 sec), 72°C (30 sec)] 30X, 72°C (5 min). Reaction conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$), Primers (0.5 µM), polydT primer (1 µM), 0.16 mM dNTPs, 0-0.25 µg Human brain total RNA, 25 U reverse transcriptase, 0.3 U *Taq* DNA polymerase, 25 µL.

Figure 8: Evaluation of CleanAmp™ Primers in one-step reverse-transcription PCR using SuperScript® II Reverse Transcriptase (Invitrogen) and Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) (Invitrogen). For the gene of interest, the PCR primers were unmodified, contained CleanAmp™ Turbo modification or contained CleanAmp™ Precision modification. Reverse transcription utilized a polydT$_{18}$ primer. Reactions contained *Taq* DNA polymerase and SuperScript® II or M-MLV Reverse Transcriptase.

One approach to improving the specificity of one-step RT-PCR is to employ CleanAmp™ Primers. By introducing a CleanAmp™ Primer pair, only the RT primer can elongate during reverse transcription. This reduces lower-temperature, non-specific amplicon formation from extension of PCR primers. At higher temperature, the CleanAmp™ Primers are activated, allowing for greater specificity of primer extension during PCR. CleanAmp™ Primers provide a solution to non-specific amplifications and also enable other more universal RT priming methods for applications such as multiplex one-step RT-PCR.

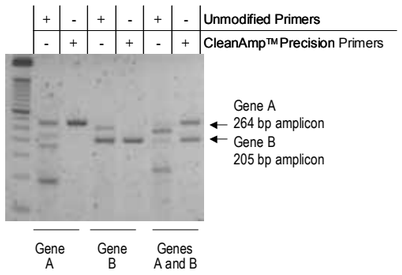
CleanAmp™ Primers improve sensitivity and specificity regardless of reverse transcriptase (RT) enzyme

CleanAmp™ Primers decrease competing PCR primer extension during the RT step with a number of commonly used RT enzymes. To illustrate this, PCR primer sequences were prepared as either a) standard, unmodified primers or b) one of two types of CleanAmp™ Primers (16,17). CleanAmp™ Turbo Primers and slower-releasing CleanAmp™ Precision Primers differ in the rate of temperature-induced formation of the corresponding unmodified primer. The following RT enzymes were tested: SuperScript® II Reverse Transcriptase (Invitrogen) and Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) (Invitrogen) (Figure 8), with similar results were found for both RT enzymes. The use of unmodified PCR primers resulted in formation of several non-specific amplicons, with the desired amplicon (264 bp) being formed at low relative abundance. Amplicon formation was enriched when CleanAmp™ Turbo Primers were used. In the case of CleanAmp™ Precision Primers, amplicon formation was also enriched to a slightly lesser degree. However, reactions containing Precision Primers displayed improved specificity.

CleanAmp™ Primers can also be utilized with reactions employing either total RNA or poly(A)+ RNA. Experiments have shown that with various RNA tissue sources, one-step RT-PCR displayed improved specificity when CleanAmp™ Primers were employed. Furthermore, one-step RT-PCR experiments have been evaluated in real-time qRT-PCR using CleanAmp™ Turbo and Precision Primers. The use of CleanAmp™ Primers enhanced the specificity of the reaction compared to the unmodified primers. CleanAmp™ Turbo Primers displayed the most significant increase in amplicon formation. Overall, CleanAmp™ Primers demonstrated marked improvement of sensitivity and specificity in one-step endpoint and real-time RT-PCR protocols.

CleanAmp™ Primers improve specificity of duplex one-step RT-PCR

CleanAmp™ Primers were examined for their ability to support multiplexed one-step RT-PCR. Gene A (264 bp) and Gene B (205 bp) were employed in singleplex and duplex reactions (Figure 9). In both singleplex and duplex reactions, unmodified primers produced off-target amplification products. CleanAmp™ Turbo and CleanAmp™ Precision Primers improved the specificity of singleplex reactions, CleanAmp™ Precision Primers providing the highest enrichment in multiplex amplification.



Reaction conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$), Primers (0.5 µM), polydT primer (1 µM), 0.16 mM dNTPs, 0-0.25 µg Human brain total RNA, 25 U reverse transcriptase, 0.3 U *Taq* DNA polymerase, 25 µL.

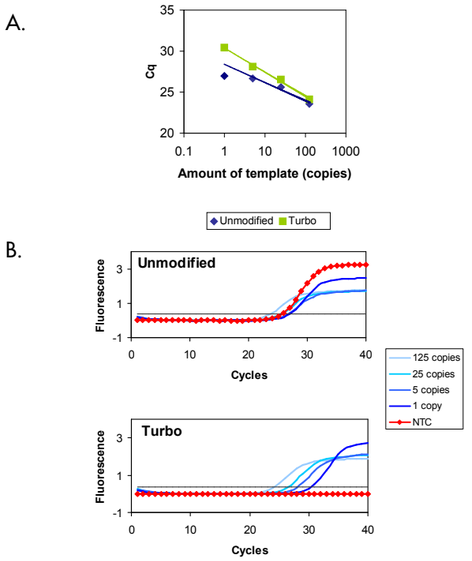
Figure 9: Evaluation of CleanAmp™ Primers in one-step reverse-transcription PCR in Human Liver Total RNA (Clontech) in singleplex and duplex. For the genes of interest (Gene A and Gene B), the PCR primers were unmodified or contained CleanAmp™ Precision modifications. Reverse transcription utilized a polydT$_{18}$ primer. Reactions contained *Taq* DNA polymerase and M-MLV Reverse Transcriptase.

This unique thermolabile modification protects PCR primers from extension during the RT step until thermally activated in the PCR step, thereby reducing non-specific product formation in one-step RT-PCR protocols. Non-Hot Start DNA polymerases, such as *Taq* DNA polymerase, and standard reverse transcriptases, such as M-MLV reverse transcriptase, are recommended. There is no need to use more expensive modified DNA polymerases or RT enzymes. Furthermore, poly dT$_{18}$ or random primers can be employed during the cDNA synthesis steps instead of gene-specific primer. This universal priming method allows for a more streamlined approach to multiplex one-step RT-PCR reactions. This is also a key advantage of CleanAmp™ Primers because other one-step RT-PCR kits do not recommend the use of poly dT$_{18}$ or random primers. In summary, CleanAmp™ Precision Primers are a significant improvement over unmodified primers when used in one-step RT-PCR.

Fast PCR Thermal Cycling Applications

In PCR applications such as diagnostics or medical testing, efforts have been directed at reducing the duration of the PCR amplification step, allowing for faster results and greater throughput. However, these faster cycling PCR protocols also have some drawbacks including a decrease in sensitivity and a loss in reproducibility (18). One suggested solution to decreased sensitivity is to increase primer concentration.
The main complication encountered with this solution is that an increase in primer concentration often leads to higher off-target amplification, which can increase the false positive rate in real-time experiments, especially with SYBR Green® detection (18). Therefore, prevention of off-target amplification at high primer concentration could lead ultimately to an increase in sensitivity when using fast cycling PCR. Herein, the utility of CleanAmp™ Turbo Primers in preventing off-target amplification at high primer concentration using fast cycling PCR protocols will be demonstrated.

CleanAmp™ Turbo Primers decrease off-target amplification in fast cycling PCR



PCR conditions: 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$), Primers (1.5 µM), 0.8 mM dNTPs, 0-125 copies HIV gDNA, 3.75 U *Taq* DNA polymerase, 25 µL 1:2000 SYBR Green™ I. Thermal cycling conditions: [95°C (10 sec), 66°C (30 sec)]40X.

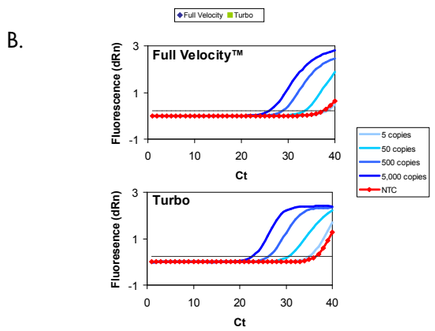
Figure 10: A. Amplification plots of CleanAmp™ Turbo Primers and unmodified primers in a primer/template system prone to primer dimer formation over a range of template concentrations. B. Corresponding standard curve comparing CleanAmp™ Turbo Primers and unmodified primers.

Turbo Primers were evaluated for their ability to reduce off-target amplification and to improve the sensitivity of real-time SYBR Green® assays using a typical fast cycling protocol (Figure 10). When unmodified primers were employed, interpretation of the amplification plots was complicated by strong amplification of the NTC sample. Although 125 copies of template DNA was accurately detected, the NTC sample amplified before each of the subsequent lower template concentrations. In comparison, the use of CleanAmp™ Turbo Primers allowed for detection down to a single copy. The NTC amplification curve did not cross the threshold line (Cq) during the 40 thermal cycles of the experiment. In addition, when the Cq values were plotted in standard curves (Figure 10B), CleanAmp™ Turbo Primers exhibited greater linearity compared to unmodified primers. This characteristic is most likely related to the suppression of primer dimer formation when CleanAmp™ Turbo Primers are used and demonstrates Turbo Primer's increased specificity over a large range of template concentrations.

CleanAmp™ Turbo Primers outperform other fast cycling technologies

Next, CleanAmp™ Turbo Primers were compared with Full Velocity™ SYBR Green® QPCR Mastermix (Stratagene/Agilent), a commercially available kit which is formulated specifically for fast cycling PCR. The experiment involved amplification of a 245 bp target from Lambda genomic DNA. To accurately compare the two products in the same experiment, a 5 minute initial denaturation time necessary for the Full Velocity™ Mastermix was used with these cycling conditions, we found that Turbo Primers had slightly greater sensitivity than the Full Velocity™ Master Mix.

As depicted in Figure 11A, the NTC curve overlapped with the 5 copy sample with Full Velocity™ Master Mix. However, when Turbo Primers were employed, 5 copies of template amplified before the NTC curve. These findings are likely due to a lower level of off-target amplification by Turbo Primers. When the experimental results were plotted in a standard curve, both approaches displayed good linearity over the range of concentrations evaluated. Although the efficiencies were similar for each technology, reactions that employed Turbo Primers had lower Cq's. This allowed for even greater speed of amplicon detection in a fast cycling protocol.



PCR conditions (Turbo Primers): 1X PCR buffer (20 mM Tris (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$), Primers (0.3 µM), 0.8 mM dNTPs, 0-10,000 copies Lambda gDNA, 3.75 U *Taq* DNA polymerase, 25 µL 1:2000 SYBR Green™ I. PCR conditions (Unmodified Primers): 1X Full Velocity™ MasterMix (Stratagene/Agilent), Primers (0.15 µM). Thermal cycling conditions: 95°C (5 min)[95°C (10 sec), 66°C (30 sec)] 40X.

Figure 11: A. Amplification plots of CleanAmp™ Turbo Primers and Full Velocity™ in a primer/template system prone to primer dimer formation over a range of template concentrations. B. Corresponding standard curve comparing CleanAmp™ Turbo Primers and Full Velocity™ SYBR Green® QPCR Master Mix.

Summary

In summation, CleanAmp™ Primers allow for greater control of primer hybridization and extension during PCR. Over a broad range of applications CleanAmp™ Primers reduce or eliminate off-target amplification. Greater amplicon yield is also achieved, due to improvement in specificity and sensitivity. It has been demonstrated that CleanAmp™ Primers outperform other technologies in multiple applications.

Which CleanAmp™ Primer is best for my application?

Turbo Primers	Precision Primers
Fast cycling	Standard cycling
Multiplex PCR	One-step reverse-transcription PCR
Improved amplicon yield	Improved specificity and limit of detection
Reduced mis-priming/primer dimer formation	Greatest reduction in mis-priming/primer dimer formation

For the most current CleanAmp™ Primer Application Note visit: www.trilinkbiotech.com/cleanamp.

References

1. Chou, Q., M. Russell, D.E. Birch, J. Raymond and W. Bloch. 1992. Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. Nucleic Acids Res 20:1717-1723.
2. Moretti, T., B. Koons and B. Budowle. 1998. Enhancement of PCR amplification yield and specificity using AmpliTaq Gold DNA polymerase. Biotechniques 25:716-722.
3. DeBiasi, R.L. and Kenneth L. Tyler, K.L. (2004) Molecular Methods for Diagnosis of Viral Encephalitis. Clin Microbiol Rev, 17, 903–925.
4. Gilbride, K.A., Lee, D.Y. and Beaudette, L.A. (2006) Molecular techniques in wastewater: Understanding microbial communities, detecting pathogens, and real-time process control. J Microbiol Methods, 66, 1-20.
5. Brownie, J., Shawcross, S., Theaker, J., Whitcombe, D., Ferrie, R., Newton, C. and Little, S. (1997) The elimination of primer-dimer accumulation in PCR. Nucl. Acids Res., 25, 3235-3241.
6. Henegariu, O., Heerema, N.A., Dlouhy, S.R., Vance, G.H. and Vogt, P.H. (1997) Multiplex PCR: Critical Parameters and Step-by-Step Protocol. BioTechniques, 23, 504-511.
7. Cinque, P., Bossolasco, S. and Lundkvist, A. (2003) Molecular analysis of cerebrospinal fluid in viral diseases of the central nervous system. J Clin Microbiol, 26, 1-28.
8. Canales, R.D., Y. Luo, J.C. Willey, B. Austermiller, C.C. Barbacioru, C. Boysen, K. Hunkapiller, R.V. Jensen, et al. 2006. Evaluation of DNA microarray results with quantitative gene expression platforms. Nature Biotechnology 24:1115-1122.
9. Qin, L., R.P. Beyer, F.N. Hudson, N.J. Linford, D.E. Morris and K.F. Kerr. 2006. Evaluation of methods for oligonucleotide array data via quantitative real-time PCR. BMC Bioinformatics 7:23.
10. Bustin, S.A. and T. Nolan. 2004. Pitfalls of Quantitative Real-Time Reverse-Transcription Polymerase Chain Reaction. Journal of biomolecular techniques 15:155-166.
11. Wacker, M.J. and M.P. Godard. 2005. Analysis of One-Step and Two-Step Real-Time RT-PCR Using SuperScript III. Journal of biomolecular techniques 16:266-271.
12. Lekanne Deprez, R.H., A.C. Fijnvandraat, J.M. Ruijter and A.F.M. Moorman. 2002. Sensitivity and accuracy of quantitative real-time polymerase chain reaction using SYBR green I depends on cDNA synthesis conditions. Analytical Biochemistry 307:63-69.
13. Suslov, O. and D.A. Steindler. 2005. PCR inhibition by reverse transcriptase leads to an overestimation of amplification efficiency. Nucleic Acids Research 33:e181.
14. Peters, I.R., C.R. Helps, E.J. Hall and M.J. Day. 2004. Real-time RT-PCR: considerations for efficient and sensitive assay design. Journal of Immunological Methods 286:203-217.
15. Chou, Q., M. Russell, D.E. Birch, J. Raymond and W. Bloch. 1992. Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. Nucleic Acids Research 20:1717-1723.
16. Kielar, D., D. W., T. Langmann, C. Aslanidis, M. Probst, M. Naruszewicz and G. Schmitz. 2001. Rapid quantification of human ABCA1 mRNA in various cell types and tissues by real-time reverse transcription-PCR. Clinical Chemistry 47:2089-2097.
17. Louwrier, A. and A. van der Valk. 2005. Thermally reversible inactivation of Taq polymerase in an organic solvent for application in hot start PCR. Enzyme and Microbial Technology 36:947-952.
18. Hilscher, C., Vahrson, W. and Dittmer, D.P. (2005) Faster quantitative real-time PCR protocols may lose sensitivity and show increased variability. Nucleic Acids Res, 33, e182.

Procedure for Synthesis of CleanAmp™ Primers

Reagents	Vendor
CleanAmp™ DNA phosphoramidites	TriLink BioTechnologies, Inc.
Fast deprotecting DNA phosphoramidites	Glen Research
Synthesis Column containing UnyLinker™	Glen Research
Acetonitrile, dried to ≤ 20 ppm water (ACN)	Fisher Scientific
Dichloromethane	Fisher Scientific
Activated Molecular Sieves	TriLink BioTechnologies, Inc.
Cap A reagent with Phenoxyacetic anhydride	Glen Research
Cap B reagent with N-Methyl-Imidazole	Glen Research
HPLC Grade Water	Fisher Scientific
50 mM Potassium carbonate in Methanol	Glen Research
1M TEAA, pH 7.2 (Triethylammonium Acetate)	TriLink BioTechnologies, Inc.
2% TFA in water (Trifluoroacetic Acid)	TriLink BioTechnologies, Inc.
DMSO anhydrous	Fisher Scientific

Procedure:

A. Synthesis of CleanAmp™ oligonucleotides on solid support at 1 µmole scale :

1. Prepare solutions of each fast deprotecting phosphoramidite monomer in anhydrous acetonitrile (ACN) using manufacturer's instructions. We recommend using a concentration of 0.067 M. **Higher concentrations may lead to precipitation of the amidite.**
2. Prepare 0.067 M solutions of each CleanAmp™ phosphoramidite (except for CleanAmp™ -dG) in dry acetonitrile in a standard DNA synthesizer bottle. Prepare 0.067 M solution of CleanAmp™-dG phosphoramidite monomer in mixture of dry dichloromethane (DCM):ACN (1:1) in a standard DNA synthesizer bottle.

   To properly dissolve a 100 umole bottle of a CleanAmp™ monomer to obtain a 0.067 M solution, use 1.50 mL of the appropriate solvent.

3. Add activated molecular sieves (20 beads/mL) to each amidite bottle, flush with dry argon gas, recap the bottle, seal using parafilm and keep it overnight at room temperature before use.
4. Purchase, or manually fill, a 1 µmole synthesis column with UnyLinker™ support.
5. Load the DNA synthesizer with the reagents listed in the Reagents table in the appropriate well according to manufacturer's instructions.
   a. Load the amidites. Use a spare port for the CleanAmp™ monomer(s).
   b. Load the ancillary reagents. It is critical that the appropriate capping reagents are used for fast deprotecting monomers. "Cap A" must be the phenoxyacetyl anhydride version of the reagent. DO NOT USE ACETIC ANHYDRIDE.
6. Follow an automated synthesis protocol for fast deprotecting phosphoramidite monomers as recommended by the instrument manufacturer with the following exception: increase the coupling time for CleanAmp™ phosphoramidites to ten minutes.
7. At the last coupling cycle, leave the DMT group "On".
8. When synthesis is completed, dry the column using argon flow.
9. It is recommended to proceed with the cleavage/deprotection of CleanAmp™ oligonucleotide immediately after completion of the synthesis, otherwise keep the dry column at –20°C.

B. Cleavage/Deprotection of CleanAmp™ oligonucleotides:

1. Transfer support (with oligonucleotide) from the column to a screw cap 8 mL glass vial.
2. Add 6 mL of freshly prepared 50 mM potassium carbonate solution in MeOH.
3. Place the vial on a rotary mixer for 20 hrs at room temperature.
4. Let support settle and transfer supernatant solution into a 50 mL conical tube.
5. Wash support with 2 mL of 1 M Triethyl Ammonium AAcetatel (TEAA) and add to the deprotection solution.
6. Measure the total crude yield using UV spectrophotometer.
7. Process the solution immediately using SepPak isolation/purification, otherwise keep the solution at –80°C to –20°C.

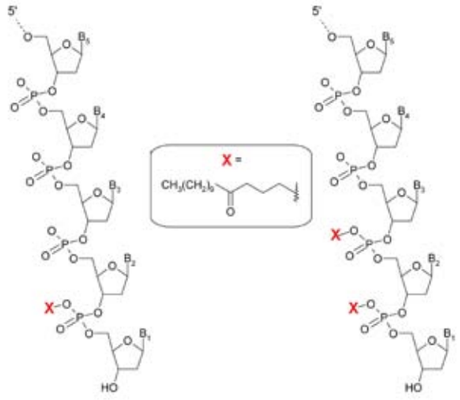
C. SepPak isolation and purification procedure:

1. Fit a 60 mL syringe to SepPak cartridge; part # 20515.
2. Equilibrate cartridge with 10 mL of acetonitrile, followed by 10 mL of 50% acetonitrile in 100 mM TEAA and finally by 15 mL of 25 mM TEAA.

3. Prepare sample for loading:
   a. Add 2 mL of 1 M TEAA to the sample and pipette the solution into 5 mL syringe fitted with a luer lock and a 0.44 micron filter disc. Filter the solution into a 50 mL conical tube.
   b. Rinse the deprotection vessel with another 2 mL of water, passing that through the filter and into the tube with the sample.
   c. Dilute the solution to 50 mL with 1 M TEAA.
4. Load the solution to cartridge with a flow rate of 1-2 mL/min and pour into the 60 mL loading syringe on cartridge. Collect and read the absorbance of the flow-through to ensure the oligo is bound to the cartridge.
5. Rinse the cartridge with 20 mL of water.
6. Pass 10 mL of 2% TFA through the cartridge over 3 minutes. Observe appearance of orange colored band.
7. Immediately rinse SepPak with 20 mL HPLC water.
8. Pass 10 mL of 1 M TEAA through the cartridge over 5 minutes to neutralize acid.
9. Wash SepPak with a purification buffer:
   a. For CleanAmp™ Turbo Oligos, apply 15 mL of 15% ACN, 100 mM TEAA to cartridge. Collect rinse.
   b. For CleanAmp™ Precision Oligos, apply 15 mL of 25% ACN, 100 mM TEAA to cartridge. Collect rinse.
10. Rinse cartridge with 20 mL of water. Collect rinse.
11. Elute samples using DMSO.
   a. Attach a 3mL slip tip syringe to the cartridge.
   b. Add 200 µL DMSO to syringe and elute into a microtube, labeled fraction 1.
   c. Remove syringe from cartridge, pull out plunger, reattach the syringe to the cartridge, and push air into same graduated tube to completely remove all the DMSO.
   d. Add 200 µL DMSO to syringe and elute into a fresh microtube (fraction 2) using plunger. Add another 200 µL to DMSO to syringe and elute into the same graduated tube for a total of 400 µL DMSO in fraction 2.
   e. Add 200 µL DMSO to syringe and elute into a fresh microtube (fraction 3).
12. Cap, vortex and spin down fractions. Read fraction 2 only. If yield is less than 10 OD_{260} units, read the absorbance of fraction 3. Add just enough of the material in fraction 3 to the material in fraction 2 to achieve 10 OD_{260} units.
13. Determine molar concentration of purified oligonucleotide in DMSO solution using the absorbance reading and the calculated extinction coefficient. If necessary, dilute with DMSO to obtain a 0.200 mM solution.
14. Analyze purified CleanAmp™ oligonucleotides by RP-HPLC to ensure that no more than 1-2% of unmodified primer is in the purified sample, and no more than 20% of the singly modified species if a Precision Primer was prepared.
15. Store the oligonucleotide at 4°C or less. Although it is very stable at room temperature in the DMSO solution, long term storage is improved at lower temperatures.

HPLC Analysis of CleanAmp™

1. Analyze a 0.2 OD_{260} unit sample of the isolated material by reverse phase HPLC to determine the level of contamination by oligonucleotides not protected by the CleanAmp™ moiety.
2. Method:
   a. Column: Waters µBondapak 8 mm RP cartridge (WAT 027324) or comparable.
   b. Buffer A: 100 mM TEAA; Buffer B: ACN
   c. Gradient: 0-100% B over 40 minutes
   d. Flow Rate: 1 mL/minute
   e. Observe at 260 nm.
3. Determine the mobility of the unprotected oligonucleotide by heating another 0.2 OD_{260} unit sample for 40 minutes at 95°C, which will completely remove the CleanAmp™ modification from the oligonucleotide.
4. Turbo should have less than 1% of the fully deprotected oligonucleotide while Precision should contain less than 2% of that contaminant. Precision should have less than 20% of the singly modified material, which elutes between the unmodified product and the doubly modified Precision oligonucleotide.
5. If specifications are not met, the benefits of the CleanAmp™ technology may still be experienced. The system is very robust and some PCR applications are less demanding than others.



CleanAmp™ Turbo Primers
Single 4-oxo-tetradecyl modification

CleanAmp™ Precision Primers
Double 4-oxo-tetradecyl modification

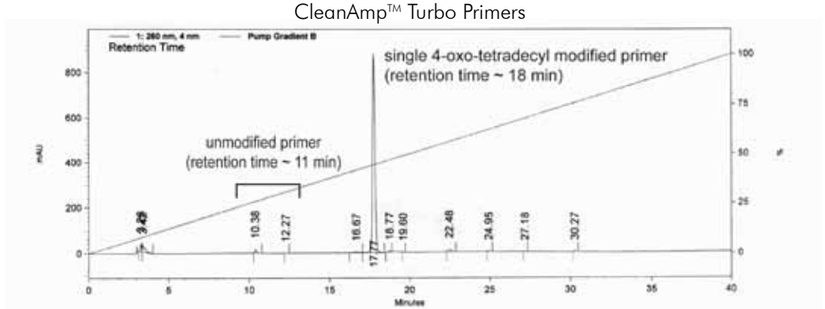
For the most current CleanAmp™ Primer protocol visit:
www.trilinkbiotech.com/cleanamp.

*See next page for representative RP-HPLC analysis of CleanAmp™ Turbo and Precision Primers.

Reverse-phase HPLC analysis:
CleanAmp™ Primers

RP3-HPLC : Waters uBondapak C18, 10 µm, 125 A, 300x3.9 mm
Buffer A : 100 mM TEAA pH = 7-7.5 Buffer B : Acetonitrile
Gradient : 0-100% B over 40 min Flow Rate : 1 mL/min Temperature : RT
Injection : 30 µL

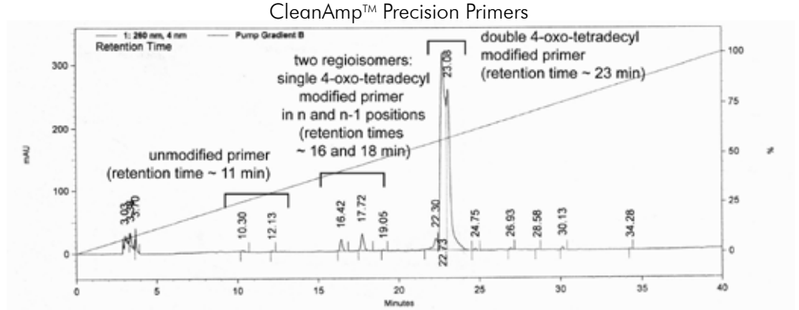
CleanAmp™ Turbo Primers



1: 260 nm, 4 nm Results

Retention Time	Area	Area Percent
3.28	222971	2.15
3.42	344063	3.32
10.38	102868	0.99
12.27	6196	0.06
16.67	57024	0.55
17.77	9367907	90.43
18.77	55087	0.53
19.60	10305	0.10
22.48	134106	1.29
24.95	23730	0.23
27.18	7994	0.08
30.27	26505	0.26
Totals	10358756	100.00

CleanAmp™ Precision Primers



1: 260 nm, 4 nm Results

Retention Time	Area	Area Percent
3.03	66785	0.55
3.38	149969	1.24
3.70	115176	0.96
10.30	20631	0.17
12.13	8460	0.07
16.42	189062	1.57
17.72	332858	2.76
19.05	11175	0.09
22.30	281620	2.34
22.73	6632543	55.04
23.08	4124618	34.23
24.75	31559	0.26
26.93	29226	0.24
28.58	11771	0.10
30.13	40921	0.34
34.28	4071	0.03
Totals	12050445	100.00

La Jolla, California © istockphoto.com/aimintang

A Short History of Oligonucleotide Synthesis

By Richard Hogrefe, Ph.D.; TriLink BioTechnologies

In order to offer an exhaustive report on the history of oligonucleotide synthesis, it would be necessary to examine the history of modern biotechnology, for the two are tightly interwoven. For example, early biotech innovations such as fermentation, pasteurization, and vaccine development, were key stepping stones to the birth of oligonucleotide synthesis. Another event crucial to establishing this novel chemistry was the passage of the Bayh-Dole act. This legislation allowed academic institutions to own and patent federally funded research, which in turn made it possible to secure the huge levels of capital investment necessary to finance the development of groundbreaking diagnostics and therapeutics. In an attempt to present a condensed chronicle of recent scientific contributions that led specifically to the launch of oligonucleotide synthesis, this article will discuss certain biotech milestones, beginning in the 1950's.

First, for the uninitiated, an oligonucleotide, in the strictest sense, is a short piece of nucleic acid less than 50 nucleotides in length. In the past 20 years, the meaning has broadened to include all chemically synthesized nucleic acids, regardless of the length.

Next, it is necessary to have some understanding of why researchers initially became interested in synthesizing these molecules. Although the general make-up of nucleic acids and their biological function as carriers of the genetic code was known by the mid-1940's, it was the landmark paper published by Watson and Crick in 1953 describing DNA's double helix structure (Watson and Crick, 1953) that revealed the link between the chemistry of genetics and the biological result. Thus was born molecular biology, the science of investigating the interface between biology and chemistry. It was only natural that chemists would soon have an interest in trying to synthetically prepare some of the newly elucidated bio-macromolecules: proteins and nucleic acids. Although the biotech industry came into existence through the growing knowledge about biological systems at the molecular level, it was ultimately fueled by the development of precise tools. It was the ability to simulate and modify biological systems, through tools such as recombinant protein synthesis and cloning techniques that allowed science to create new biomolecules. Because the chemistry was somewhat simpler, the synthesis of peptides (or small proteins) developed faster than that of oligonucleotides. Consequently, peptide synthesizers were the first automated systems available. Vega Biotechnologies pioneered this field and also introduced the first DNA synthesizer, which can be viewed in the Smithsonian. Although it used chemistry that is now outdated, it marked the first attempt to make oligonucleotide chemistry easily available to research laboratories.

However, general accessibility to the chemistry would require many advancements before reliable, automated oligonucleotide synthesis was a reality. The beginning of the era of investigating the human genome can be dated to the collaboration between Professor Marvin Caruthers of the University of Colorado, and Professor Leroy Hood of CalTech, when they set out to automate Caruthers' new phosphoramidite oligonucleotide synthesis chemistry. This collaboration, which formed Applied Biosystems, Incorporated (ABI), commercialized the first phosphoramidite DNA synthesizer in the early 1980's. Many labs now had routine access to oligonucleotides, which was critical in advancing the overall understanding of biological systems.

The invention of Polymerase Chain Reaction (PCR) by Kary Mullis in the 1980s proved to be the catalyst for the rapid development of a myriad of applications, including the more sophisticated sequencing methods, which made it possible to sequence the human genome. The following sections of this article will examine in greater detail the contributions of some of the influential researchers who were the direct parents of oligonucleotide synthesis.

First Dinucleotide

The first published account of the directed chemical synthesis of an oligonucleotide occurred in 1955 when Michelson and Todd reported the preparation of a dithymidinyl nucleotide (Michelson and Todd, 1955).

In their report, the phosphate link between two thymidine nucleosides was made by first preparing the 3' phosphoryl chloride of a 5' benzoyl protected thymidine, using phenylphosphoryl dichloride. This was then reacted with the 5' hydroxyl of a 3' protected thymidine. The chemistry worked

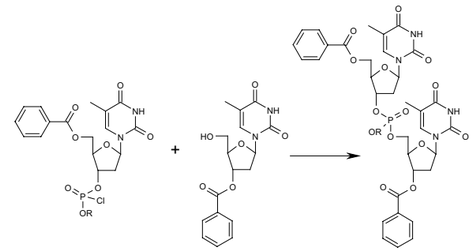


Figure 1: Phosphoryl chloridate method described by Michelson and Todd.

reasonably well, albeit slowly. Additionally, the phosphoryl chloride intermediate was not stable, being susceptible to hydrolysis. Figure 1 shows the basic scheme.

The Khorana Contribution

In the late 1950's a creative and forward-thinking researcher at the University of Chicago by the name of H. Gobind Khorana became interested in the synthesis of oligonucleotides. He introduced two concepts to the field that made possible the convenient synthesis of oligonucleotides more than just a few bases long. One concept, the on-off protection scheme necessary for sequential oligonucleotide synthesis, is still widely used today by oligonucleotide chemists, virtually unmodified from Khorana's initial publications (Schaller, e.t al., 1963; Smith, et. al., 1961). The other was the first use of a stable phosphorylated nucleoside that coupled to the desired nucleoside when activated (Khorana, et. al., 1956).

This protocol, called the phosphodiester method of oligonucleotide synthesis (Figure 2), is the same cyclic scheme used today with the exception of the addition of one step, oxidation. In place of the hydrolysable phosphoryl chloride, he prepared 3' phosphates of the 5' protected nucleoside using phosphorochloridates that then hydrolyzed to the phosphomonoester. These 5' protected nucleoside 3' phosphates were subsequently activated using a condensation reagent, such as dicyclohexyl carbodiimide (DCC), to couple to the 5' hydroxyl of another 3'-protected

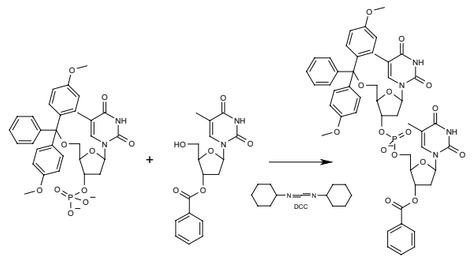


Figure 2: Khorana's Phosphodiester Coupling Method

nucleoside. This method was revolutionary at the time and produced a truly remarkable feat: the synthesis of an active 72-mer tRNA molecule, which was published in Nature (Khorana, 1970).

Like most archetypes, the method did have shortcomings. Because the phosphate itself was not protected, branching at the internucleotide phosphate linkages of the previous couplings was a major problem. As a result, it was necessary to follow a very arduous multi-step purification process in which the branched contaminants were removed. However, as the oligonucleotide length increased, the percentage of branching also increased, making purification even more challenging. The solution phase chemistry made the process very slow, because the oligonucleotide had to be purified or precipitated between steps to remove excess reagents. When one considers the magnitude of the task, the accomplishment of preparing an active tRNA molecule becomes even more remarkable.

Khorana's most lasting contribution, however, was in the area of nucleoside protecting groups. The key to developing an efficient, cyclic, step-wise synthesis is a good protecting group scheme that allows the selective removal of a specific protecting group at the desired time. To make matters more challenging, the protecting groups must be removable almost quantitatively. Otherwise, the yield of desired product will be low and the product itself may be irresolvable from contaminants. To raise the bar even further, purines are susceptible to depurination under mildly to moderately acidic conditions (pH 4-5 for extended periods, pH 1-3 for fairly short periods), so strong acids should be avoided.

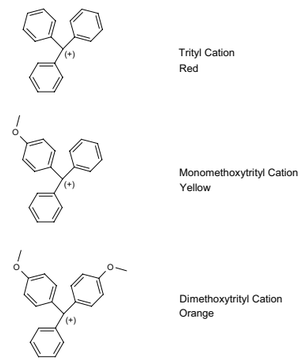


Figure 3: Different Trityls have a Range of Absorbances

The solution Khorana offered for 5' hydroxyl protection, the dimethoxytrityl (DMT) protecting group (Smith, et. al., 1961), is ubiquitous in oligonucleotide chemistry today. The combination of good general stability and easy removal with mild acid has been unbeatable. Several options are available, such as leuvenyl and FMOC, but none are as popular as the unique trityl family.

The reason this triphenyl methyl ether cleaves so readily under acidic conditions lies in the fact it is one of the few molecules that actually likes to form a carbocation. The back bonding of the pi electron cloud system formed by the three phenyl groups is sufficient to allow the methyl carbon to remain stable as a positively charged species under very mildly acidic conditions. Like many carbocations, the trityls have a distinctive color when ionized, which has turned into an extremely useful diagnostic tool. The dimethoxytrityl (DMT) carbocation has a very strong orange color in mild acid that has a high extinction coefficient, which means that even at very low concentrations it can still be accurately measured optically. The monomethoxytrityl (MMT) carbocation has a yellow color, while the parent trityl (Tr) itself is deep red (Figure 3). The efficiency of each cycle of nucleoside addition can be followed by measuring the absorbance of the released DMT and comparing it to the previous step.

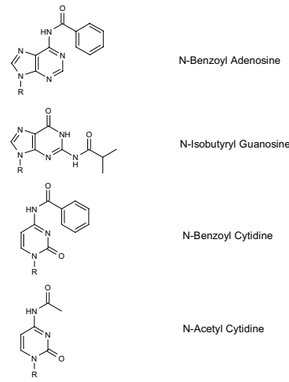


Figure 4: Exocyclic Amine Protecting Groups

Khorana also introduced the protecting groups for the nucleosidyl exocyclic amines that are today known as the standard protecting groups; isobutyryl for guanosine and benzoyl for adenosine and cytidine (Schaller, et. al., 1963; Brown, et. al., 1979) (Figure 4). Although others exist, these are the most commonly used groups today, with the possible exception of acetyl-protected cytidine, which is more readily removed compared to benzoyl.

Professor Khorana influenced many with his work, both through his publications and through his labs, where many of the great names in oligonucleotide chemistry passed as graduate students, post-docs, or visiting scholars. Those names include Marvin Caruthers, who will be discussed later in this article, and Robert Letsinger, who worked nearby at Northwestern University and developed two important steps in the field: solid phase synthesis and phosphite-triester chemistry.

The Letsinger Epoch

Professor Letsinger began his career at Northwestern University in the late 1940's as a boron chemist. He was a significant player in that field, but in the early 1960's he turned his sights onto a newly emerging field, biomacromolecule

synthesis. At that time, the target was peptide synthesis. However, a twist of fate moved Letsinger from peptide to oligonucleotide chemistry in the mid-1960's.

Dr. Letsinger was developing a peptide synthesis scheme using solid phase chemistry that had originated mainly for the support of catalysts. Letsinger utilized flow-through technology with a cyclic chemistry scheme of adding units sequentially. When applied to peptide synthesis, it added an internal filtering system that proved to be an incredibly important step forward. However, he wasn't the only researcher following this lead. Another scientist, Bob Merrifield, was also investigating the synthesis of peptides using solid phase technology. At the time they were neck and neck in the process of discovery and struggling to publish their findings as soon as possible. Bob Merrifield submitted his seminal paper describing the solid phase synthesis of peptides first and eventually won the Nobel Prize for his work. This unexpected scoop prompted Letsinger to regroup and focus his attention on another nascent chemistry: oligonucleotide synthesis. He rapidly converted his method using solid phase synthesis for peptides to the improvement of the oligonucleotide synthesis procedures taught by Khorana, thus converting a stroke of hard luck into scientific advancements that benefited an entire industry.

Letsinger made three major contributions to the field. First, he introduced solid phase chemistry as stated above. Secondly, he introduced the phosphotriester method of synthesis, an important improvement on Khorana's phosphodiester method. Finally, he introduced a radical departure, the P(III) based phosphite-triester method, which is the root of Marvin Caruthers' phosphoramidite method.

Solid Phase Synthesis

Letsinger's first support for peptide synthesis was described in papers published in 1963 and 1964 (Letsinger and Kornet, 1963; Letsinger, et. al., 1964). The support consisted of what was called a "popcorn" polymer, a styrene-divinylbenzene polymer that had the unfortunate property of swelling in some solvents. In 1965 he published the first paper describing the solid-phase synthesis of dimer and trimer oligonucleotides using the same support (Letsinger

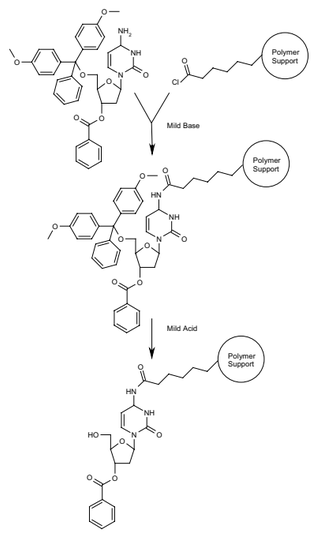


Figure 5: Coupling of dC to Polymer Support

and Mahadevan, 1965). In the initial report, 2' deoxycytidine (dC) was attached through the amine at the 4 position of the base itself to acid chloride modified support and forming an amide bond that was cleaved with ammonium hydroxide. The 3' hydroxyl of the dC was protected with a benzoyl group and the 5' position with a DMT group. The DMT group was then removed with mild acid to prepare the support bound nucleoside for oligonucleotide synthesis. The attachment was made to the support, which was activated to an acid chloride, thus forming an amide bond that was cleavable with base (Figure 5).

Through the 1960's he continued to explore the solid phase synthesis technique. He quickly determined that the best approach to solid phase synthesis was to attach the 3' hydroxyl to the support, as is done today. In fact, the graduate student instrumental in that work was Marvin Caruthers. Letsinger explored a number of polymer formulations, but never found a solution to the problem of swelling that was so detrimental to the chemistry. That role fell to his former student, Caruthers, who will shortly have his own section in this history.

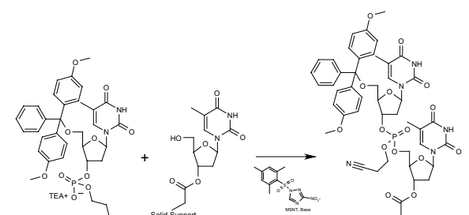


Figure 6: Phosphotriester Approach

Phosphotriester Chemistry

In the late 1960's, Letsinger published the first paper on the phosphotriester method of oligonucleotide synthesis (Letsinger, et. al., 1969) (Figure 6). The key advance of this method was the protection of the phosphate group to prevent the branching that plagued the phosphodiester approach. The protecting group most commonly used was the ß-cyanoethyl group that is easily removed with ammonium hydroxide (Letsinger and Ogilvie, 1969). An o-chlorophenyl was also used but it required a more complicated deprotection mixture. It turned out, however, that the key to pushing the efficiency of the reaction, which reached levels in excess of 95% per step, was the selection of a proper activator. Mesityl sulfonyl chloride (MSCl) and mesityl sulfonyl nitrotriazole (MSNT) were by far the most popular (Devine and Reese, 1986; Letsinger and Ogilvie, 1969).

This was the first chemistry that was simple enough to reproduce successfully in many labs. The combination of a chemistry that worked relatively easily with solid phase methodology led to the creation of the first viable automated and semi-automated DNA synthesizers, exemplified by the early instruments developed by Vega Biotechnologies. Another early entrant was Ron Cook and his company Biosearch. He introduced the SAM I in the late 1970's, which was based on phosphotriester chemistry and was the most popular instrument of its era. These instruments allowed non-chemists to prepare simple oligonucleotides, and created the ability to probe genes with radio-labeled oligonucleotides prepared with the exact sequence desired. Thus equipped, the industry was primed for the emerging techniques of gene mapping, PCR, and target validation.

However, the phosphotriester chemistry still suffered from critical drawbacks. Among them was the fact that despite the

years of work by a number of research groups, the average step-wise efficiency could never reproducibly be raised above 97%, and often failed to reach 95%. This limited the method to the routine synthesis of oligonucleotides less than 20 bases in length. Another problem was the extensive coupling time, which resulted in cycle times that commonly ran longer than an hour and a half.

Phosphite-Triester Chemistry

In the mid-1970's, Letsinger published the first papers describing the phosphite-triester method of oligonucleotide chemistry (Letsinger, et. al., 1975; Letsinger and Lunsford, 1976) (Figure 7). This chemistry is based on the use of reactive phosphorus in the P(III) state, instead of the classic P(V) phosphoryl chemistry. The scheme required an additional step in the synthesis cycle, oxidation, in order to prepare the natural P(V) backbone. The major advantage of this chemistry was the significant reduction in time required for coupling due to the highly reactive nature of the nucleoside phosphomonochloridite intermediate.

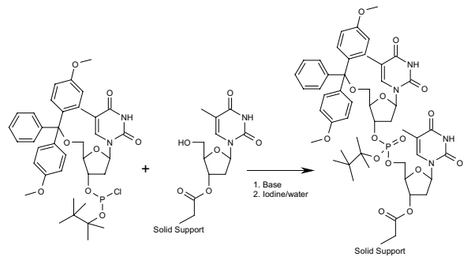


Figure 7: Phosphite-Triester Method

The fact that the P(III) intermediate is more reactive than the P(V) species is not intuitive. One would suspect, in the absence of data, that because of the doubly bonded oxygen, the P(V) would be more reactive to attack by a nucleophile based on it's enhanced electronegativity. However, the determining factor of the reaction rate turns out to be the difference in the energy of formations for the transitional intermediates of the P(III) species versus P(V). As shown in Figure 8, a trigonal bipyrimidal intermediate is formed. The doubly bonded oxygen hinders the transition from the tetrahedron configuration into the planar much more than the lone pair of electrons.

Oxidation of the phosphite-triester intermediate

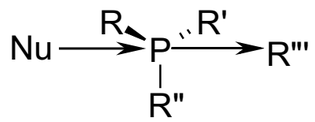


Figure 8: Trigonal Bipyrimidal Intermediate

into a phosphotriester was needed in order to stabilize the backbone. This oxidation was required at each step of the cycle because of the instability of the phosphite-triester intermediate to the acid required to remove the DMT group. Fortunately, a very simple mixture of iodine, water and some base very efficiently and quantitatively oxidizes phosphorus within seconds.

The research community was quick to accept this new chemistry as a significant step forward. Not only could standard DNA be prepared faster, but the door was opened for the investigation into a variety of backbone modified oligonucleotides. Biologics, a company partially comprised of former Letsinger students, marketed an automated synthesizer based on this chemistry and another was in development by Vega Biotechnologies. However, the early form of the phosphite-triester chemistry did indeed have major drawbacks.

The most significant problem was the highly reactive nature of the nucleoside phosphomonochloridite intermediate. It was very susceptible to hydrolysis. The intermediate was not easy to store and therefore was best made just prior to each coupling. Another issue was that the formation of active intermediate was very tricky. The phosphodichloridite activating reagent had to be added to the 5' protected nucleoside in such a manner as to maximize the formation of desired intermediate while reducing the formation of 3'-3' dimer (Figure 9). The formation of this side-product did double damage in that it reduced the amount of desired material and increased the amount of unused phosphodichloridite that remained in solution. This unused reagent would very efficiently cap off the growing chain before the desired intermediate had time to couple. That was the reason that an excess of the reagent could not be used to reduce formation of the 3'-3' adduct. Using too few equivalents of the phosphodichloridite had a like-wise harmful effect in that too much 3'-3' adduct would be formed, reducing the concentration of active nucleoside reagent below a critical threshold. Increasing the concentration of the reagents to combat that only led to the opposite effect and an even less controllable reaction.

The protocols designed to optimize this reaction

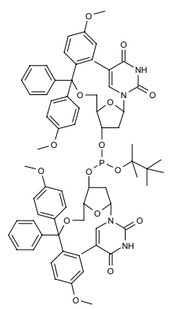


Figure 9: 3'-3' Adduct

called for the slow addition of a very slight excess of solubilized 5' protected nucleoside to a solution of RO-PCl_2 at extremely cold temperatures (-78° C). As it turned out, the combination of the requirement for preparing the active reagent just prior to each coupling, and the need for arduous conditions during this activation, removed nearly all of the advantages brought about by the faster coupling time.

This problem was not solved until the early 1980's. A serendipitous discovery was made by a graduate student that showed if there was a rapid introduction of the phosphodichloridite reagent to the nucleoside at room temperature, it formed a useful active reagent without making too much of the 3'-3' adduct or leaving too much phosphodichloridite in the mix (Hogrefe, 1987). This improved method was later coupled with a scavenger system involving trityl alcohol that selectively removed any excess RO-PCl_2 from the reaction mixture. It was this new protocol, which finally allowed the development of a practical automated DNA synthesizer with coupling times of 15 minutes or less. This instrument was also developed by Vega Biotechnologies in collaboration with Letsinger. Although this particular instrument was a significant improvement over the phosphotriester instruments described earlier in this section, it was never sold. The phosphodichloridite method was soon eclipsed by a new chemistry discovered by Marvin Caruthers, the phosphoramidite method. It solved many of the problems that clouded the entry of the phosphodichloridite method into the market.

Caruthers: Right Chemistry, Right Time

After Caruthers left Letsinger's lab he did a post-doc with Khorana, who was now at MIT. In 1973, he joined the faculty at the University of Colorado, where he continued his research in the synthesis of oligonucleotides. Over the next decade Caruthers worked out the solution to two major problems, the swelling of the organic polymer supports and the instability of the phosphitylated active nucleoside intermediate. In 1981, he published the use of inorganic matrices as supports for oligonucleotide synthesis (Matteucci and Caruthers, 1981). Originally, chromatography grade silica was used. It was much later supplanted by controlled pore glass (CPG). This solved the swelling problem experienced with the polymer supports being used at the time, thus increasing efficiency by allowing freer flow of reagents over the support. It was easier to rinse the support of old reagents prior to the next step and easier to access all portions of the support. This reduced washing time and improved coupling efficiency, as any contamination left on the support invariably interfered with the desired reaction through hydrolysis, premature oxidation, or undesired capping.

The Phosphoramidite Approach

As far as modifying Letsinger's overall phosphite-triester method, Caruthers' contribution appears almost trivial: simply, the exchange of one leaving group, a chloride, for another, an amine (Beaucage and Caruthers, 1981; Mc Bride and Caruthers, 1983). However, this subtle change was pivotal to the development of routine oligonucleotide synthesis because it resulted in very significant changes to the properties of the molecule. Now the phosphitylated nucleoside intermediate or phosphoramidite could be made in advance, isolated as a stable solid and stored until needed. The phosphoramidite was then activated just prior to coupling by simply adding a weak acid, tetrazole. This allowed the commercial scale manufacture and distribution of DNA synthesis reagents.

As shown in Figure 10, the only difference between the phosphoramidite synthesis cycle and the existing method offered by Letsinger was the activiation step. The cycle starts with the deprotection of the 5' hydroxyl of the nucleoside bound to the support. The removal of the DMT group is normally done with a mild solution (2-3%) of either dicholoracetic acid or trichloroacetic acid in dichloromethane. Next, the support is washed well with acetonitrile to remove all traces of acid and reduce adventitious water. Now come

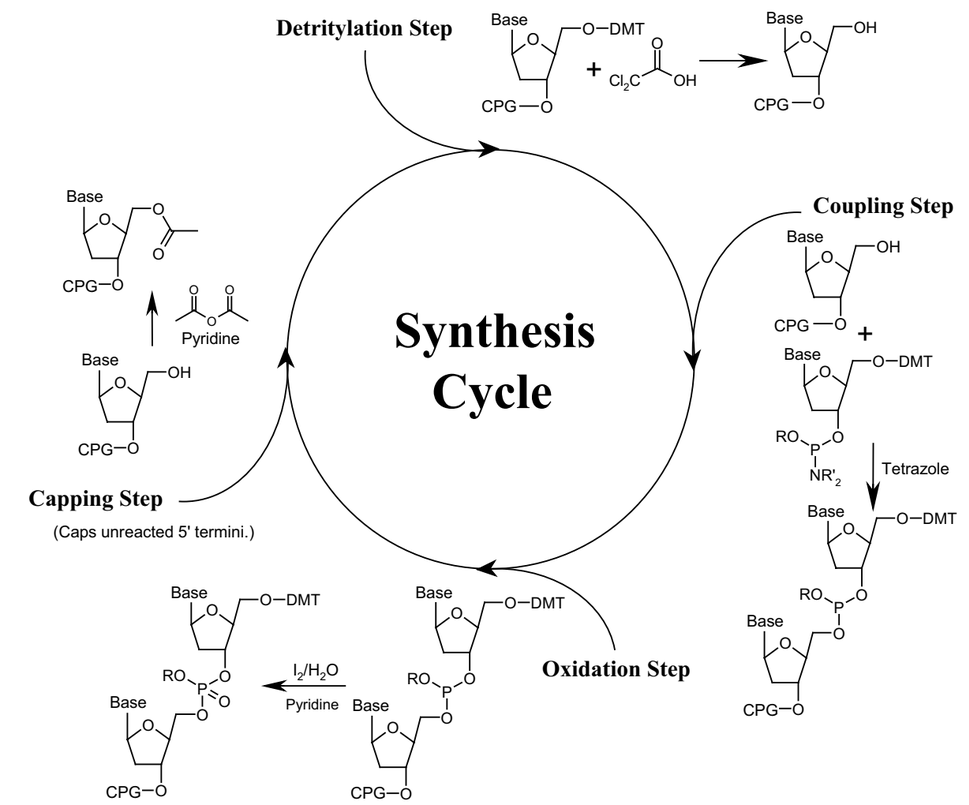


Figure 10: Synthesis Cycle

the most important steps, activation of the phosphoramidite and coupling to the 5' hydroxyl of the bound nucleoside. The activation is done with tetrazole, an unusual secondary amine that actually acts as a mild acid. The donation of the proton allows the formation of a thermodynamically favored anionic aromatic ring. The pKa of this acid is high enough that it does not remove the DMT group from the reagent, yet it is still acidic enough to activate the phosphoramidite.

Besides the delicate balance in activator pKa needed to achieve efficient activation without loss of the 5' hydroxyl protecting group (Figure 11), the actual mechanism is

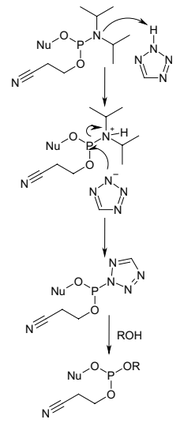


Figure 11: Mechanism of Activation of Phosphoramidite by Tetrazole

interesting, and controversial. The currently accepted mechanism is that the amine becomes protonated (slow step), followed by rapid replacement of the protonated amine with the nearby recently generated tetrazolide. It is actually this intermediate that reacts with the 5' hydroxyl. The evidence for this was found through ^{31}P NMR experiments (Berner et al, 1989). The chemical shift of the phosphorus after activation suggests that an aromatic amine is bound to it, similar to what would be obtained if excess tetrazole was added to a nucleoside phosphochloridite.

After coupling, the support is oxidized using essentially the same iodine/water/base reagent originally described by Letsinger. Then the few remaining unreacted strands ending with free hydroxyls are capped using a mixture of acetic anhydride and N-methylimidazole.

This cycle, elegant in its simplicity, has remained virtually unchanged for almost two decades.

With a viable chemistry in hand, Caruthers was introduced to Hood: an instrument was designed and ABI was born. Prior to the introduction of the phosphoramidite method, the synthesis of oligonucleotides was largely in the hands of experienced chemists. The instruments were temperamental and when combined with the chemistries of the time, nearly impossible to run successfully. At its most advanced state in the mid-1980's, the phosphodichloridite method was tricky and required a high degree of experience to reproduce successfully. Coupled with the phosphoramidite method, the automated synthesizer became useful to those who could best make use of it, the biologists.

Conclusion

Without a doubt, the oligonucleotide synthesis methods used today are the fruits of decades of research by progressive scientists such as Khorana, Letsinger, and Caruthers. As the field advances, TriLink follows the tradition of seeking better ways of making oligonucleotides. Today the challenges lie in the need for increasingly larger quantities of modified oligonucleotides for therapeutic applications; better high-throughput methods for the screening and PCR markets; and improved synthesis quality of dye modified oligonucleotides for the diagnostic industry. Chemists are indeed still needed for this work, but to use an old cliché, we've come a long way.

References

1. Beaucage SL, Caruthers MH. (1981) Tetrahedron Lett. 22, 1859-62.
2. Berner S, Muehlegger L, Seliger H. (1989) Nucleic Acids Research 17(3), 853-64.
3. Brown EL, Belagaje R, Ryan MJ, Khorana HG. (1979) Methods Enzymol. 68, 109.
4. Devine KG, Reese CB. (1986) Tetrahedron Lett. 27(45), 5529-32.
5. Hogrefe RI. (1987) Ph.D. Dissertation. Northwestern University, Evanston, IL.
6. Khorana HG, Tener GM, Moffatt JG, Pol EH. (1956) Chem. & Ind. London, 1523.
7. Khorana HG. (1970) Nature 227, 27-34.
8. Köster H, Sinha ND. (1984) US Patent No. 4725677.
9. Letsinger RL, Kornet MJ. (1963) J. Am. Chem. Soc. 85(19), 3045-6.
10. Letsinger RL, Kornet MJ, Mahadevan V, Jerina DM. (1964) J. Am. Chem. Soc. 86(23), 5163-5.
11. Letsinger RL, Mahadevan V. (1965) J. Am. Chem. Soc. 87(15), 3526-7.
12. Letsinger RL, Ogilivie KK. (1969) J. Am. Chem. Soc. 91(12), 3350-5.
13. Letsinger RL, Ogilivie KK, Miller PS. (1969) J. Am. Chem. Soc. 91(12), 3360-5.
14. Letsinger RL, Finnan JL, Heavner GA, Lunsfold WB. (1975) J. Am. Chem. Soc. 97, 3278-9.
15. Letsinger RL, Lunsford WB. (1976) J. Am. Chem. Soc. 98, 3655-61.
16. Matteucci MD, Caruthers MH. (1981) J. Am. Chem. Soc. 103, 3185-91.
17. McBride LJ, Caruthers MH. (1983) Tetrahedron Lett. 24, 245-8.
18. Michelson AM, Todd AR. (1955) J. Chem. Soc., 2632.
19. Schaller H, Weimann G, Lerch B, Khorana HG. (1963) J. Am. Chem. Soc. 85, 3821.
20. Smith M, Rammler DH, Goldberg IH, Khorana HG. (1961) J. Am. Chem. Soc. 84, 430-440.
21. Watson JD, Crick FH. (1953) Nature 171, 73.

Troubleshooting the Synthesis of Modified Oligonucleotides

By Richard Hogrefe, Ph.D.; TriLink BioTechnologies

Over the past decade, the level of sophistication regarding oligonucleotide synthesis has grown tremendously. Because the applicability of unmodified oligonucleotides is fairly limited, a great deal of time and money is spent on developing modified oligonucleotides. Most researchers find that some degree of oligonucleotide "tweeking" is necessary to get the desired properties for diagnostic and therapeutic applications.

Although the diagnostic and therapeutic industries have different needs, there is considerable overlap in the chemistries being developed. The diagnostic industry is confronted with two primary challenges: stringency and sensitivity (and consequently background noise). Unfortunately, these two requirements are almost mutually exclusive. In order to have high sensitivity, one must have a robust system with probes that will bind available targets with high affinity. However, in driving the equilibrium towards duplex formation, there is an increased binding to non-targets, leading to false positives. In order to solve these problems, researchers have turned to a number of oligonucleotide modifications, including base, sugar and backbone modifications to control hybridization properties. Conjugating reporter groups, such as fluorophores, chemiluminescent reagents and microchips can enhance sensitivity.

The therapeutic industry has a slightly broader range of problems to solve. Although there is disagreement about the severity of the various issues, and the solutions, most would agree that the following are the primary considerations:

1. *In vivo* stability
2. Strong hybridization properties to target while maintaining high stringency - *in vivo*
3. Circulatory lifetime
4. Tissue and cellular targeting
5. Metabolic properties (toxicity)

It was obvious from the outset that unmodified oligonucleotides would not become a viable drug candidate. A series of modifications enhancing the ability of the oligonucleotide to overcome these obstacles was introduced; phosphorothioates being the most popular of the second generation drugs. These modifications led to the discovery of new modes of action, such as the aptamers of Gilead and ISIS.

As our understanding of biological processes becomes more sophisticated, the demands on chemists increase to produce more radical departures from nature in order to control them. Now we are entering the third generation of drugs. Most of the major players have introduced oligonucleotides with more sophisticated chemistries: Gilead's propynyls, Lynx's phosphoramidates, ISIS's MMIs and MOEs, and Genta's chiral methylphosphonates.

With each new twist, the problems multiply for the synthetic chemists responsible for the preparation of these compounds. Compatibility issues between the new generations of oligonucleotides and the classic methods of synthesis abound. In some cases, such as with peptide nucleic acids, the entire synthesis scheme had to be redeveloped.

A brief review of several case histories demonstrating how to solve some synthesis problems are presented.

Case History 1: Synthesis

Problem

In developing the use of chirally pure methylphosphonate dimer synthons to prepare oligonucleotides, it was found that the phosphoramidite synthons rapidly lost their ability to couple, even while stored dry under anhydrous conditions.

Observations

1. Amidite was pure by ^{1}H and ^{31}P NMR, HPLC, MS.
2. Synthons coupled well immediately after purification (>95%), only to rapidly lose efficiency over the next couple of days, in some cases to 20% or less.
3. Extensive co-evaporations and dry down times were not successful in recovering efficiency.
4. Using analytical techniques described above, no change in synthon was observed, despite loss of coupling efficiencies.

Experiments

1. Repurify on a silica dimer that coupled at 25% efficiency (95% originally).
Purpose: Try to remove undetectable impurity (salts, etc.)
Result: No improvement.
Conclusion: Contaminant co-migrates with product. Not from original synthesis of synthon, or would have been present from beginning.
2. Activate synthon with tetrazole in NMR tube. Compare to DMT-T amidite standard.
Purpose: Determine if synthon converts cleanly to tetrazolide intermediate.
Result: Very little active material observed with dimer synthon (>10%). Most of the amidite converted to hydrolyzed side-product.
Conclusion: It's water.
3. Treat dimer synthon with high quality molecular sieves. Test by NMR and coupling.
Purpose: Try to remove water, and thereby increase coupling efficiency.
Result: After 2 days over sieves, NMR showed over 50% active reagent. Coupling efficiency >95%.
Conclusion: Molecular sieves are a viable solution.

Follow Up

All the dimer synthons are now treated with 3 Å molecular sieves for two days prior to use. In fact, this is done with any new or modified reagent. It is better to be safe, than to lose the results of many hard weeks in the lab. We now prepare our own molecular sieves.

Case History 2: Deprotection

Problem

2'-O-silyl RNA monomers have become the most popular reagents for the synthesis of RNA, mainly because of the "first in" principle and cost savings. However, there are a number of complaints about biologically inactive products being produced with these synthons. At the time of this work, tetrabutylammonium fluoride (TBAF) was the reagent of choice for removal of the silyl group.

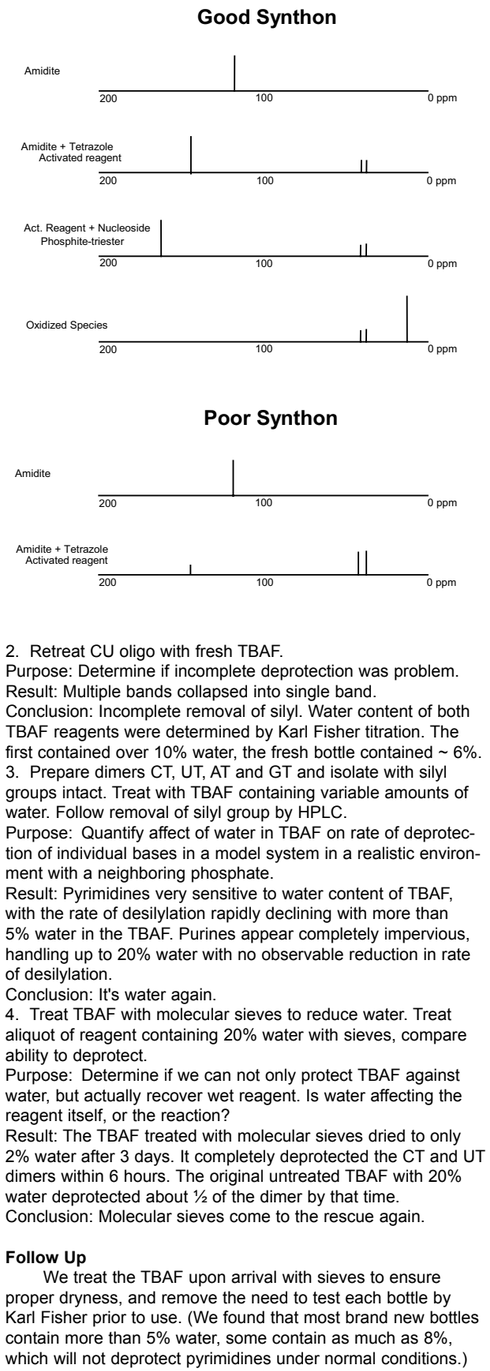
Observations

1. Variable quality of RNA.
2. Quality of pyrimidine rich sequences were not as high as purine rich.
3. Biological quality of longer strands (40mer +) often poor.
4. Oligos that appeared pure on 20 cm gels showed extra banding when run on 40cm gels. (Prof. Paul Miller, Johns Hopkins University, originally made this observation.)

Experiments

1. Synthesize alternating AG and CU RNA oligos, deprotect as usual, analyze on gel.
Purpose: Repeat Miller's observation, and to begin exploring purine vs. pyrimidine issue.
Result: Significant banding of CU oligo with no major band, AG oligo was a clean single band
Conclusion: Definitely a difference between AG and CU oligos.

31P NMR Experiment to Test for Water in Amidite



2. Retreat CU oligo with fresh TBAF.
Purpose: Determine if incomplete deprotection was problem.
Result: Multiple bands collapsed into single band.
Conclusion: Incomplete removal of silyl. Water content of both TBAF reagents were determined by Karl Fisher titration. The first contained over 10% water, the fresh bottle contained ~ 6%.
3. Prepare dimers CT, UT, AT and GT and isolate with silyl groups intact. Treat with TBAF containing variable amounts of water. Follow removal of silyl group by HPLC.
Purpose: Quantify affect of water in TBAF on rate of deprotection of individual bases in a model system in a realistic environment with a neighboring phosphate.
Result: Pyrimidines very sensitive to water content of TBAF, with the rate of desilylation rapidly declining with more than 5% water in the TBAF. Purines appear completely impervious, handling up to 20% water with no observable reduction in rate of desilylation.
Conclusion: It's water again.
4. Treat TBAF with molecular sieves to reduce water. Treat aliquot of reagent containing 20% water with sieves, compare ability to deprotect.
Purpose: Determine if we can not only protect TBAF against water, but actually recover wet reagent. Is water affecting the reagent itself, or the reaction?
Result: The TBAF treated with molecular sieves dried to only 2% water after 3 days. It completely deprotected the CT and UT dimers within 6 hours. The original untreated TBAF with 20% water deprotected about ½ of the dimer by that time.
Conclusion: Molecular sieves come to the rescue again.

Follow Up

We treat the TBAF upon arrival with sieves to ensure proper dryness, and remove the need to test each bottle by Karl Fisher prior to use. (We found that most brand new bottles contain more than 5% water, some contain as much as 8%, which will not deprotect pyrimidines under normal conditions.) We use the small TBAF (5 mL) bottles from Aldrich so that they are not in use for a long period of time. The combination of fresh reagent well dried with sieves has led to a track record of no failed deprotections over several hundred syntheses.

Needless to say, we now treat every water sensitive reagent with high quality molecular sieves prior to use. This work was published by Hogrefe et al. in the paper: *The Effect of Excess Water on the Rate of Desilylation of Tetrabutylammonium Fluoride*.

Case History 3: Deprotection

Problem

The synthesis of methylphosphonate oligonucleotides is no trivial matter. One problem is that the backbone is very sensitive to base and will completely degrade under normal deprotection conditions using ammonium hydroxide. The use of ethylenediamine (EDA) as an alternative was established by Paul Miller. The problem was that EDA transaminated N^4-benzoyl cytidine, leading to undesired EDA adducts. We could see these compound as later eluting species on gel, but not on RP-HPLC. This problem was exacerbated during scale up. Miller used a primary treatment with hydrazine to remove the benzoyl group prior to treatment with EDA. This method was not amenable to scale up. Use of a breif treatment with ammonium hydroxide was not very effective at scale and led to loss of product due to insolubility of the oligo in aq. ammonium hydroxide. Somehow the NH_4OH permanently bound the oligo to the support. Not only that, but degradation of MP oligos to single nucleosides showed other modifications as well.

We had two problems here. One was to fix a known problem, EDA adduct formation with benzoyl-C, the second was to first determine what caused the unknown modification, then fix it. To top it off, we also had a solubility problem that limited our options.

Observations

1. During scale up transamination of benzoyl-C with EDA was found to occur with a rate of up to 15% per C.
2. Other modifications were > 10%, but still significant.
3. EDA is the best solvent for MP oligos.
4. Other pre-treatments, such as NH_3 saturated isopropanol, etc., for the removal of bz-C prior to EDA failed.

Experiments

1. Synthesize 9mers consisting of dA, dC, dG or T surrounded by T's using C-bz, C-ibu, G-ibu, and A-bz. Deprotect using:
    a. first hyrdrazine, then EDA/ethanol (Miller)
    b. first NH_4OH, then EDA/ethanol (Sarin)
    c. EDA/ethanol alone

Purpose: Determine extent of problem, compare methods.
Result: dA, dC-ibu and T oligomers deprotect cleanly in all systems. dC-Bz yielded EDA adduct in every case. dG yielded unidentified modification in methods a and c, but not with b. Unfortunately, b yielded ½ the product the other methods did.
Conclusion: The first problem was neatly solved with a simple change, ibu instead of bz protection on dC. However, dG is also modified, something not reported in the literature. We tried O_6 protected dG-ibu (diphenylcarbamoyl, DPC), but that exacerbated the problem.
2. Treat dG-ibu and dG-ibu-DPC with methods a, b and c.
Purpose: Look at modification of dG in isolated environment.
Result: No modification.
Conclusion: dG-ibu must be modified during synthesis, making it susceptible to transamination during deprotection. We suspected dimethylamino pyridine (DMAP), which we were required to use because MP oligomers are base labile enough to be cleaved by N-methylimidazole (NMI).
3. Cycle 20 artificial couplings over support bound dG-ibu (the other bases were done as well). By deblocking, then capping the support prior to running coupling cycles, the monomer is not elongated into an oligo. Each of the reagents, dichloroacetic acid, oxidizer, caps, amidites, etc., were replaced with acetonitrile (ACN) one at a time and the process repeated. The process was then repeated with the variation of replacing all but one of the reagents with ACN. The supports were deprotected by methods a, b, and c above.
Purpose: To study the effect of each reagent on ibu-dG under simulated oligo synthesis conditions, both independently and in conjunction with other reagents.
Result: DMAP formed adducts with ibu-dG and DPC-ibu-dG in methods a and c, but not in b.
Conclusion: It is not surprising that ibu-dG formed EDA adducts. This was reported by Eadie. What was surprising was that the putative solution, O_6 DPC protected dG, was significantly more modified. The NH_4OH pre-treatment served to revert the modification back to desired product.

Follow Up

Now that we knew the culprit, and how to fix it, we merely had to apply our knowledge. We couldn't use method b, which called for a pre-treatment of NH_4OH before EDA, because it yielded very low yields on scale-up. EDA is the best solvent for that step, but to use it as the principle deprotecting reagent a new capping routine would have to be discovered to avoid NMI or DMAP. Fortunately, one last experiment gave us the needed clue. It was found that a very short treatment of 10 min with 30% NH_4OH, or a 30 min treatment with 2% NH_4OH in ACN/EtOH was sufficient to revert the DMAP-dG adduct. Furthermore, it was found that the 2% NH_4OH solution degraded the MP backbone at a very acceptable low rate of 1%/hour.

We solved the problem by first treating the support bound oligo with the mild NH_4OH solution to revert dG adducts, followed with one volume of EDA after 30 min to complete the deprotection. Our deprotections went cleanly and our yields were acceptable. And we had a one pot method that was faster, cleaner and more simple than before. Also, the use of dC^{ibu} instead of dC^{bz} and this deprotection method eliminated the transamination side product. This work was also published by Hogrefe et al. in the paper: *Deprotection of Methylphosphonate Oligonucleotides Using a Novel One-Pot Procedure*.

Case History 4: Purification

Problem

In the search for an uptake enhancer, a certain peptide with biological activity of its own was deemed a viable candidate for conjugation to the terminus of an oligo. It was synthesized and purified using RP-HPLC with 0.1% Trifluoro acetic acid (TFA)/ACN as the eluent. The oligo was found to be too active. It had the same activity profile as the free peptide.

Observations

1. Conjugated oligo was clean on HPLC. Free peptide and oligonucleotide separated very well independently.
2. Conjugated oligo clean on mass spec, until we requested closer examination of lower mass region. Sure enough, there was the peptide. To quantitate the peptide, two different solvents had to be used, one to determine the oligo, the other to determine the peptide. The intensity of the peptide peak was compared to an internal standard, gramicidin. This took several months to complete.

Experiments

1. Mix peptide and oligo together (10/1 peptide/oligo). Analyze by Reverse Phase-HPLC and quantitate area of peptide and oligo.
Purpose: Try to determine if peptide sticks to oligo, and in what ratio.
Result: 3 molecules of peptide bound to every oligo.
Conclusion: The peptide sticks to the oligo.
2. Attempt same experiment with normal phase and AX columns.
Purpose: Try other HPLC approaches.
Result: Same results as with RP.
Conclusion: This complex is really tight!
3. Try every molecular weight cut off filter, solid phase extraction, and separation tool known to man, short of a Waring blender.
Purpose: Try to rip the complex apart.
Result: Nothing worked.
Conclusion: This complex is really, really tight!
4. Try electrophoresis.
Purpose: Try to rip the complex apart using fact that peptide is positively charged.
Result: Removed peptide.
Conclusion: Electrophoresis is the route of choice.

Follow Up

After successfully removing the free peptide, the compound was found to be completely inactive. Although not happy about the result, we were not entirely displeased. Have you ever contemplated pilot plant scale electrophoresis gels? Not a thought for the timid.

Case History 5: Purification

Problem

The lesson from the last case was that analytical methods are needed along with purification methods. The lesson here is to believe what you see. Prior to the development of mass spectroscopy (MS) for the analysis of Methylphosphonate (MP) oligonucleotides, you based purity on the integrated area of the putative product peak as determined by HPLC (MP oligos, having no charge, do not run on gel or CE well). What more can you do? First off, you keep looking for something new, and secondly, you believe what you see.

After much trial and tribulation, Genta developed a new purification scheme using normal phase chromatography. It worked very, very well for a number of compounds early on and was used exclusive of other techniques. However, one particular compound was going to be conjugated and therefore subjected to RP-HPLC analysis.

Observations

1. A new peak corresponding to 30% of the total area was seen in front of the main product peak. This information was passed on, but discounted as an artifact of the system, even though it repeated.
2. At this time, ESI-MS of MP oligos became available to us. Low and behold, the compound contained 30% n-1 product.

Experiments

1. Full length oligo and n-1, n-2, n-3 oligos were prepared and subjected to analysis.
Purpose: Determine if failure products resolve from product.
Result: n-1 did not resolve. n-2, etc. did resolve.
Conclusion: There was no separation of n and n-1.
2. Prepare a number of compounds containing different deletions.
Purpose: Explore dependence of chromatographic properties on sequence.
Result: T's do not effect retention time of oligos using normal phase HPLC.
Conclusion: AMP oligo containing a 5′ terminal T will not resolve from an n-1 product, if it terminates in A, C, or G; n-2 if the n-1 terminates in T, etc.

Follow Up

The chromatographic properties of MP oligos on normal phase HPLC was so well defined by base composition that our consultant, LCResources, was able to develop a formula predicting the retention times of various oligomers. However, that did not solve our problem. The simple solution to this problem was to design our oligomers such that they don't end with a T.

DNA Linker and Spacer Reagents and Their Utility

By David Combs, Ph.D.; TriLink BioTechnologies

5'-Amino Linkers (TFA/MMT)

As DNA-based microarrays and multi-label diagnostic systems increase in their importance, the need for methods to readily prepare functionalized oligonucleotides also grows. Currently the most common modification is a primary amine at the 5'-terminus of the oligonucleotide, which can be introduced using amino-phosphoramidites protected with base labile trifluoroacetate (1) or acid labile monomethoxytrityl (2). Both the TFA protected and MMT protected 5'-amino-modifiers can be used in an automated synthesizer or manually coupled (Figure 1). The base labile TFA moiety is employed in cases where the amino-modified oligo is directly isolated from the cleaved and deprotected oligonucleotide. The acid labile MMT protecting group is stable to the basic cleavage and deprotection conditions and can be used as a 'handle' in RP-high performance liquid chromatography to readily isolate the oligonucleotide from failure sequences. The MMT group is subsequently removed using acidic conditions (2). A variety of moieties can be attached to the 5'-primary amine including fluorescent dyes (3,4) biotin (5) and EDTA (6).

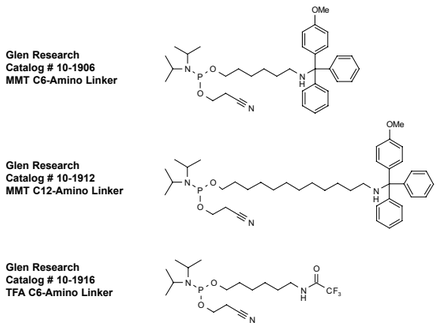


Figure 1: 5' Amino Linker Structures manufactured by TriLink and sold through Glen Research

C3, C6 vs C12 Amino Linkers

A wide selection of linker lengths for incorporation between the reactive or diagnostic moiety and the oligonucleotide have been prepared. The shorter carbon chain linkers (C3: $(CH_2)_3$) can be used in instances where the proximity of the oligonucleotide poses no problems. The longer chain linkers (C6 and C12) are typically used when the oligonucleotide must be spaced far enough from the corresponding undesired interactions (7).

3'-Amino-CPG

Introduction of modifications at the 3'-end of an oligonucleotide in concert with various 5'-modifications has become a very powerful tool. One of the more useful modifications at the 3'-end is the introduction of a 3'-amino group which can be used to post-synthetically introduce a variety of products like fluorescent chromophores, biotin, enzymes and polypeptides. In addition to this, some 3'-amino modified oligonucleotides have been found to be more resistant to endonucleases making them candidates for antisense research (8).

Incorporation of a 3'-amino function requires a protecting group that is stable throughout the multi-step oligonucleotide solid phase synthesis. It is also mandatory that the moiety be readily cleaved from the solid support using standard deprotection conditions. A number of linkers have been developed which introduce the 3'-amino modification.

One of the supports used to introduce the 3'-amino modification is the branched FMOC-protected amino C3 and C7 CPG (Figure 2). The linker is bound to the CPG via a succinate bond through the secondary hydroxyl group while the oligonucleotide is elaborated at the DMT protected hydroxy group upon removal. A drawback to using this reagent is that the FMOC protecting group is labile to the synthesis conditions of the oligonucleotide, which leads to capping of the amine and reduced yields (9).

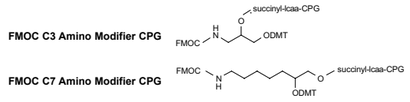


Figure 2: 3' Amino Linker Structures, FMOC protected

To overcome this inherent problem of the FMOC protection a number of other protecting groups have been developed, with the phthalimidyl derivative giving superior results (8) (Figure 3). However this reagent requires a costly multiple step synthesis.

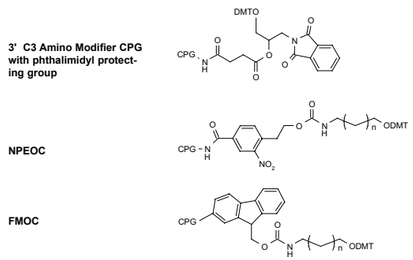


Figure 3: Other 3' Amino Linkers

A number of other interesting reagents have been developed using carbamate moieties to attach the linker to the solid supports while acting as a protecting group for the amino functionality (10). The advantage of such a protecting group is that if the protecting group is removed during oligonucleotide synthesis the impurities will be washed away and final purification made considerably easier.

We currently sell a C6 variant of 3'-amino CPG support through Glen Research which was first published several years ago (Figure 4) (11,12) along with a novel C3 analog. These new supports take advantage of the stability of the phthalimidyl group as an amino protecting group with the additional advantage as the bridging unit between the linker and CPG support.

A comparison of the commonly used 3'-amino modifier C7 (purchased from Glen Research, Sterling, VA.) with the phthalimidyl-3'-C6-Amino-CPG was done. It was found that the phthalimidyl-3'-C6-Amino-CPG was a more reliable reagent giving oligo product with a higher conjugation efficiency.

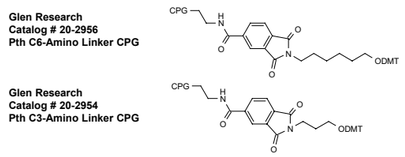


Figure 4: 3' Amino Linker Structures manufactured by TriLink and sold through Glen Research

5'-Aldehyde Modification

Another approach for introducing reactive functionality at the 5'-end that can be further modified post synthetically is the 5'-aldehylde. In contrast to the 5'-amino and 5'-thiol modifications, which are nucleophilic substitutions, the aldehyde modifier is an electrophilic substitution. The aldehyde moiety can be reacted with a variety of substituted hydrozinos and semicarbazides to form stable hyrozones and semicarbazides, respectively (13). The 5'-Aldehydes can also react with amines to form Schiff's base, but the Schiff's base must be reduced to form a stable linkage.

Two phosphoramidites that incorporate an aldehyde moiety on the 5'–terminus are available. The amidites introduce the aldehyde via a benzaldehyde functional group but vary in the linker length (C6 vs C2). Both aldehyde modifiers are incorporated using standard solid phase synthesis methodologies. However, the 5'-aldehyde modifier C2 is protected and the aldehyde must be freed using detritylation conditions with 80% acetic acid or 2% aqueous trifluoroacetic acid after purification.

5'-Spacers

A number of different spacer phosphoramidites are available (Figure 5). These spacer amidites differ in the number of atoms and are typically used to bridge sections of oligonucleotides. In addition spacers can be used in conjunction with 3' and 5'-amino-modifiers and/or additional spacers to place tags at greater distances from the oligonucleotide and reduces interaction between the oligonucleotide and the fluorescent dye (14). They can also increase hybridization to a support bound oligonucleotide by reducing steric interaction between support and bound oligo (15). The C3 spacer can be used to mimic the three carbon spacing between the 3'- and 5'-hydroxyls of the oligonucleotide (16) or replace a base within a sequence when the base is unknown. The dSpacer can be used to mimic abasic sites within an oligonucleotide and is known to undergo β-elimination reactions and lead to single strand scission of DNA (17,18).

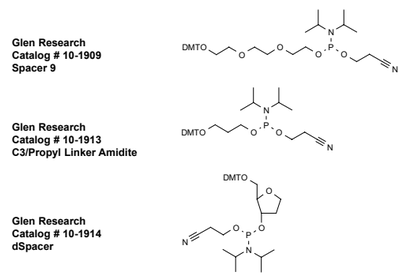


Figure 5: Spacers

5'-Thiol-Modifiers

As the number of thiol specific dyes continue to increase so does the interest in 3'- and 5'-thiol modified oligonucleotides. Like the 3'- and 5'- amino modifiers the thiol modification can be used to introduce biotin (19). In addition to this powerful tool, thiols can be conjugated with a variety of fluorescent probes (20) via reactions of the thiol with iodoacetate and maleimide derivatives of the dye to form thioether linkages, enzymes such as horseradish peroxidase, and peptides via a disulfide linkage (21), and bound to metal surfaces (22).

Thiol modification at the 5'-end of the oligonucleotide is typically done using the 5'-thiol-modifier C6 or the 5'-thiol modifier C6 S-S (Figure 6). Like the MMT protected amino linker the trityl protecting group on the 5'-thiol-modifier C6 is usually kept on after cleavage of the oligonucleotide to aid in purification. However, because the trityl group is not as acid labile, unlike MMT or DMT, it must be removed by oxidation with silver nitrate. Although successfully used, oxidative detritylation can be a problem.

An alternative to this reagent is the 5'-thiol-modifier C6 S-S. As with other reagents the trityl protecting group can be kept to aid in purification of the oligonucleotide. The thiol is then freed by treatment with dithiothreitol (DTT).

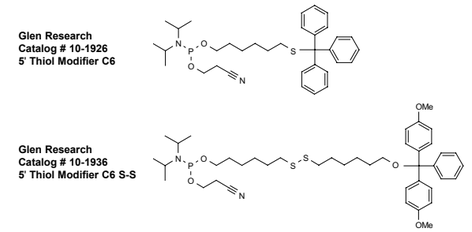


Figure 6: 5' Thiol Linkers

References

1. Greene, T. W.: Wuts, G. M. Protective Groups in Organic Synthesis 3rd Ed. John Wiley & Sons, Inc. New York
2. Connolly, B. A. (1987) Nucleic Acids Research, 15, 3131.
3. Connolly, B. A.; Rider, P. (1985) Nucleic Acids Research, 13, 4485.
4. Zuckerman, R.; Corey, D.; Shultz, P. (1987) Nucleic Acids Research, 15, 5305.
5. Kempe, T. et al. (1985) Nucleic Acids Research. 13, 45.
6. Dreyer, G. B.; Dervan, P. B. (1985) Proc. Natl. Acad. Sci. USA, 82, 968.
7. Hogrefe, R. I.; Mackie, H.; Powell, M. L. (1988) Amer. Biotechnologies News Ed. 6, 47.
8. Vu, H.; Joyce, N.; Rieger, M.; Walker, D.; Goldknopt, I.; Hill, T. S.; Jayaraman, K.; Mulvey, D. (1995) Bioconjugate Chem. 6, 599.
9. Trilink catalog, p. 94.
10. Aviño, A.; Garcia, R. G.; Albericio, F.; Mann, M.; Wilm, M.; Nebauer, G.; Eritja, R. (1996) Bioorganic & Medicinal Chemistry 4, 1649.
11. Petrie, C. R.; Reed, M. W., Adam, D. A.; Meyer, R. B. Jr. (1992) Bioconjugate Chem., 3, 85.
12. Reed, M. W.; Meyer, R. B. Jr., Petrie, C. R.; Tobone, J. C. U.S. Patent 5,419,966 (1995).
13. Shchepino, M. S.; Korshun, V. A. (2001) Nucleosides, Nucleotides & Nucleic Acids 20, 369.
14. Trawick, B. N.; Osiek, T. A.; Bashkin, J. K. (2001) Bioconjugate Chem. 12, 900.
15. Takeshita, M.; Chang, C. N.; Johnson, F.; Will, S.; Grollman, A.P. (1987) J. Biol. Chem. 262, 10171.
16. Kalnik, M.W.; Chang, C.N.; Grollman, A. P.; Patel, D.J. (1988), Biochemistry 27, 924.
17. Sproat, B. S.; Beijer, B. S.; Rider, P.; Neuner, P. (1987) Nucleic Acids Research, 15, 4837.
18. Hermanson, G. T. Bioconjugate Techniques, Acedemic Press, San Diego.
19. Antopolsky, M.; Azhayev, A. (2001) Nucleosides, Nucleotides & Nucleic Acids 20, 539.
20. Doronina, S. O.; Guzaev, A. P.; Monoharan, M. (2001) Nucleosides, Nucleotides & Nucleic Acids 20, 1007.

Phthalimidyl Protected 3' Amine Modifying Reagent Enhances Yield of Conjugated Product

By Richard Hogrefe, Ph.D, Paul Imperial, Kelly Christianson and Terry Beck; TriLink BioTechnologies

The reagent most commonly used to modify the 3' terminus of an oligonucleotide is the 3' Amino Modifier C-7 available through several distributors (Figure 1). It is based on a branched alkyl amine with two hydroxyl groups, one for linkage to a solid support and the other for coupling with a nucleoside phosphoramidite. Unfortunately, it suffers one serious drawback; the flourenylmethoxycarbonyl (FMOC) group protecting the primary amine is unstable making the amine susceptible to irreversible acetylation during the capping step of the DNA synthesis cycle.

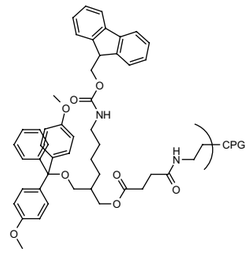


Figure 1: Structure of 3'-Amino Modifier C-7 CPG

An interesting alternative, which placed the amine within the actual link to the solid support using a base labile phthalimide (Figure 2), was published several years ago (Petrie, *et al*. 1992). However, after the initial report, this innovative reagent was not seen in the literature again.

Always seeking new ways to make higher quality modified oligonucleotides, we synthesized the phthalimide protected amino support and compared it to the 3' Amino Modifier C-7 support. The Phthalimidyl-3'-Amino-CPG was synthesized by TriLink's organic chemistry group according to the reported procedure with slight modification. The 3' Amino Modifier C-7 CPG and the Phthalimidyl-3'-Amino-CPG are available through Glen Research, Sterling, VA.

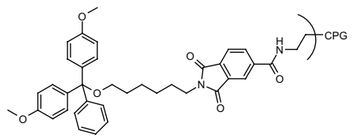


Figure 2: Structure of Phthalimide 3'-Amino Modifier C-6 CPG

The two types of 3' amino labeling support (1 µmole each) were loaded into synthesis columns and a 20mer phosphodiester oligonucleotide, 5'-GTC-ATC-TGA-TAG-CAC-GTC-GA-(Linker)-NH_2 -3', was prepared using an Expedite 8909 and standard conditions. Three columns of each support were synthesized. The oligonucleotides were deprotected by overnight treatment at 55°C with 1.5 ml of fresh concentrated aqueous ammonium hydroxide in 4 ml capped vials. After deprotection the oligonucleotides were decanted, the beads rinsed, and the combined solutions dried. The samples were ethanol precipitated and aliquots containing 30 OD_{260} units of each of the six samples were placed into separate 1.5 ml microtubes and dried in preparation for conjugation.

Each of the samples was conjugated to DABCYL succinimidyl ester for 6 hours under identical conditions. The mixtures were then run through a 10 ml G-25 column using dH_2O as the eluent to remove excess dye. The conjugated oligonucleotides were analyzed by reverse-phase (RP) HPLC.

The results of the conjugations are shown in Table 1 below. The HPLC chromatograms shown in Figures 3 and 4 are typical of the set and clearly illustrate the difference in product quality between the two supports.

In order to substantiate the hypothesis that acetylation of the amine functionality is the root cause of the lower conjugation efficiency, an additional 1µmole scale column of the same sequence as above was synthesized with each support, with the dimethoxytrityls left on. These oligonucleotides were purified by RP-HPLC on a Waters µBondapak C-18 column (8x100 mm) using 0.05 M triethylammonium acetate (pH 7.2) and a gradient of acetonitrile. After pooling and drying the fractions containing the entire DMT-on product, the trityl protecting groups were removed and the oligonucleotide dried, and then repurified using the same RP-HPLC conditions. The oligonucleotides were then analyzed by mass spectroscopy (see Figures 5 and 6). The product isolated from the Amino Modifier C-7 support contains an additional major peak (~20%) at +42 au (6368). This corresponds to the mass of an acetyl capping group.

	Synthesis	Crude Yield (in OD_{260} units)	Percent Conjugated
Phthalimidyl-CPG	1a	90	83%
	1b	91	82%
	1c	93	82%
C-7 3'-Amino-Modifier	2a	103	65%
	2b	109	68%
	2c	111	67%

Table 1: DABCYL conjugation results

In summation, the Phthalimide-3'Amino-CPG is superior to the 3' Amino Modifier C-7 for most applications. By its very nature, the conjugation efficiency is not susceptible to lot-to-lot variations or to synthesis issues. It is therefore a more reliable reagent for use in high throughput and microspotting applications where reproducibility and high conjugation efficiencies are very desirable. As a less expensive reagent with superior properties, it is the obvious choice for 3' amino labeling of oligonucleotides.

References

1. Petrie, Charles R., Reed, Michael W., Adams, David A., Meyer, Rich B. Jr., An Improved CPG Support for the Synthesis of 3' -Amine-Tailed Oligonucleotides, 1992, Bioconjugate Chem., Volume 3, 85-87.
2. Reed, Michael W., Meyer, Jr., Rich B., Petrie, Charles R., Tabone, John C. U.S. Patent 5,419,966. 1995.

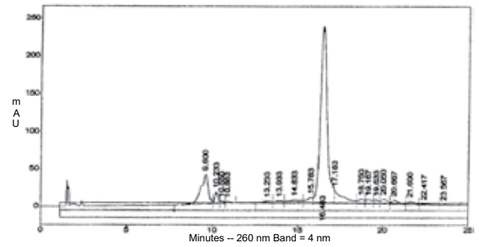


Figure 3: RP-HPLC chromatogram of crude conjugation mixture with Phthalimidyl 3'-Amino support. Conjugation product run at ~16.5 mins.

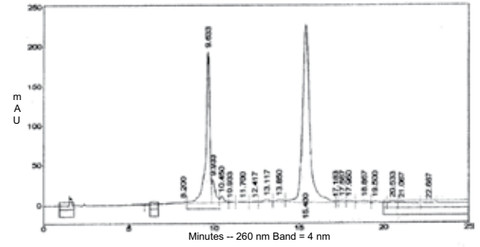


Figure 4: RP-HPLC chromatogram of crude conjugation mixture with 3'-Amino Modifier C7 support. Conjugation product run at ~15.4 mins.

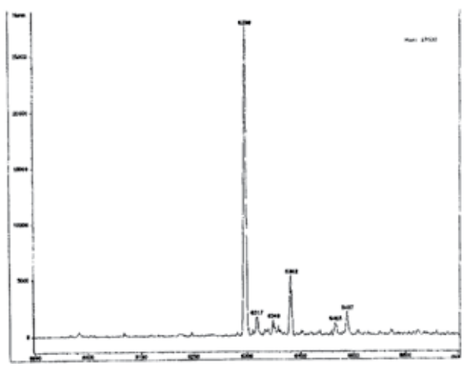


Figure 5: Electrospray mass spectroscopy analysis of HPLC purified oligonucleotide synthesized off of phthalimide-3' CPG.

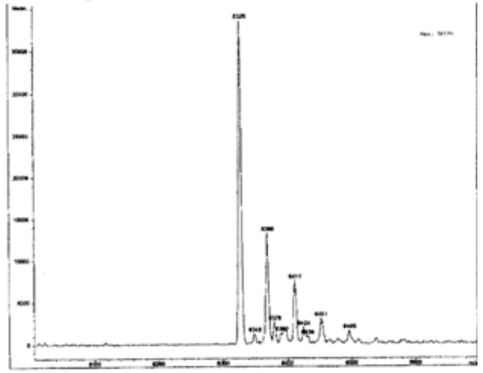


Figure 6: Electrospray mass spectroscopy analysis of HPLC purified oligonucleotide synthesized off of 3'-Amino Modifier C-7 CPG.

Comparison of Deprotection Methods for the Phthalimidyl Amino CPGs

By Richard Hogrefe, Ph.D., Paul Imperial, Alexandre Lebedev, Ph.D. and Kristin Rossum; TriLink BioTechnologies

One of the concerns regarding the use of the phthalimidyl 3'-amino CPG is the efficiency of the cleavage reaction from support by ammonium hydroxide. Although the accompanying article on these molecules offer data using deprotection conditions of conc. ammonium hydroxide at 55° C for 15 hours, we do not feel that we adequately addressed the question regarding efficiency of cleavage. Also, we wanted to compare the cleavage efficiencies of the phthalimidyl amino supports under a variety of other, commonly used oligonucleotide deprotection conditions. Many conditions have been developed to deprotect oligonucleotides that contain modifications.

We also wanted to explore the AMA (ammonium hydroxide/methylamine) system, which is a newer fast deprotection method. It is becoming the reagent of choice for many high throughput synthesis laboratories with its fast 10-minute deprotection rate.

As a control we compared our results to the deprotection of a thymidyl 20mer prepared from a standard, ester linked, thymidine CPG.

Results

The experiment was carried out by synthesizing a thymidyl 20mer on a 15 μmole scale on each of the following supports; phthalimidyl-C-3 amino-CPG, phthalimidyl-C-6 amino-CPG, and T-CPG. The initial dimethoxytrityls were collected so that the exact starting scale could be determined, as well as the coupling efficiencies.

Support	Avg. Coupling Efficiency
Thymidine (1)	98.8%
Thymidine (2)	98.7%
Phthalimidyl-C-3-amino (1)	97.4%
Phthalimidyl-C-3-amino (2)	97.8%
Phthalimidyl-C-6-amino (1)	96.9%
Phthalimidyl-C-6-amino (2)	97.8%

Table 1: Coupling Efficiencies

After synthesis, the supports were dried and carefully weighed out in 1 μmole scale aliquots into 4 mL screw top vials. Aliquots were then subjected, in duplicates, to the deprotection conditions shown in Table 2. The product was carefully isolated and the yield determined by absorbance at 260 nm. Table 2 shows the results of these deprotections, averaged and normalized to account for differences in the actual amount deprotected and for differences in coupling efficiencies. The results are given as ratios to the highest yielding deprotection off the thymidine support, which was potassium carbonate in methanol.

The products were analyzed by PAGE for purity. The products all looked exceptional with little n-1 evident in any of the syntheses. No evidence of any partially deprotected species as shown in the Figure 1 was observed by mass spectral analyses.

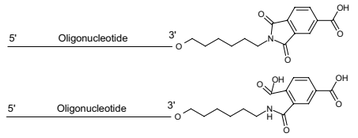


Figure 1: Partially protected species

Method	Temp °C	Time	C-3 Pth	C-6 Pth	T-20 Std
NH_4OH	RT	24 hrs	0.92	0.92	0.99
NH_4OH	RT	48 hrs	0.80	0.87	1.00
NH_4OH	55°	15 hrs	0.83	0.82	0.97
NH_4OH	65°	4 hrs	0.72	0.79	0.96
3/1 NH_4OH/EtOH	RT	48 hrs	0.87	0.91	0.95
1/1 $MeNH_2$/NH_4OH [ref 1]	55°	10 min.	1.04	1.11	0.90
1/1 $MeNH_2$/NH_4OH	55°	15 hrs	1.13	1.18	0.98
0.4 M NaOH in 4/1 MeOH/ H_2O [ref 2]	RT	15 hrs	0.97	1.00	0.90
0.05 M K_2CO_3 in MeOH [ref 3]	RT	15 hrs	0.15	0.13	1.00

Table 2: Deprotection Conditions and Yield Determination (norm.) NH_4OH- 30% aq. sol.; $MeNH_2$- 40% aq. soln.

Discussion

The results confirm the anecdotal knowledge that the phthalimidyl amino supports do not fully cleave using ammonium hydroxide, although the differences of 10% to 20% seem hardly worth consideration given the benefits shown in the last article. In general, the various ammonium hydroxide conditions all yielded equal amounts of amino labeled product, which was 80-90% of the yield from the thymidine support, after taking into account the slightly lower coupling efficiencies.

The actual yields were about 70% of the yield recovered from the thymidine support. An additional 10% or more was lost due to the lower coupling efficiencies of about 1% per cycle as shown in Table 1. We also see similar lowered efficiencies with almost all of the non-nucleosidyl modified supports. We have no proven explanation for the phenomenon, although one could suggest that the non-nucleosidyl derivatives do not extend away from the support properly.

Since we see no partially protected species as shown in Figure 1, we can only assume that the two phthalimidyl amide bonds cleave well before the linkage to the support. The aromatic amide link apparently does not cleave under these conditions, or we would see some of those side products in the crude product mix.We were pleased to find that the AMA system resulted in the highest yield with our linker. We believe that the extra absorbance is due to released aromatic linker side product. Although the longer deprotection with AMA gave slightly better results, the 10-minute deprotection with AMA is an excellent choice for this support. It is convenient to prepare and well suited for today's high throughput requirements. We are exploring this reagent more fully.

One disappointment was that the potassium carbonate deprotection conditions do yield considerably less product and should not be considered for use with the phthalimidyl protected amino supports. This deprotection condition is useful for phosphoramidite Cy5 dye. In conclusion, we found that with one exception, K_2CO_3, the phthalimidyl-3'-amino supports can be deprotected with any of the common deprotection conditions with good results.

References

1. Reddy, M. P.; Farooqui, F.; Hanna, N. B. (1995) Tetrahedron Letters 36 (49): 8929-32.
2. Kim, M.; Guengerich, F. P. (1997) Chemical Research in Toxicology 10: 1133-1143.
3. Schnetz-Boutaud, N. C.; Mao, H.; Stone, M. P.; Marnett, L. J. (2000) Chemical Research in Toxicology 13 (2): 90-95.

DADE: A Pre-Activated Carboxyl Linker, *Applications and Methods*

By Richard Hogrefe, Ph.D.; TriLink BioTechnologies

TriLink's DADE (decanoic acid diester) linker (Figure 1) is unique on the market. It offers a novel way of preparing conjugates more economically and with much more flexibility. It was originally designed to allow high throughput screening of a large number of conjugates for therapeutic application. However, it has several other inherent advantages that make it a powerful tool for the scale up of oligonucleotide conjugates as well.

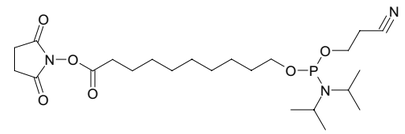


Figure 1: Structure of DADE linker

The linker is used as shown in Figure 2. After coupling to the 5' terminus of an oligonucleotide, it is now ready to be coupled to any primary amine. This greatly increases the number of conjugates that can be readily prepared. Previously, either an activated carboxylic acid or maleimidyl derivative had to be laboriously prepared in order to prepare a conjugate to an oligonucleotide with a primary amine or thiol. The only alternative was to react the oligonucleotide with either a heterobifunctional or homobifunctional linker, which added costly steps to the process and was often difficult to reproduce. Now, for the same price as a 5' amino linker, researchers can quickly screen through a number of interesting conjugates that were chemically difficult if not impossible to synthesize and do it in a controlled, single step fashion that enhances the probability of success.

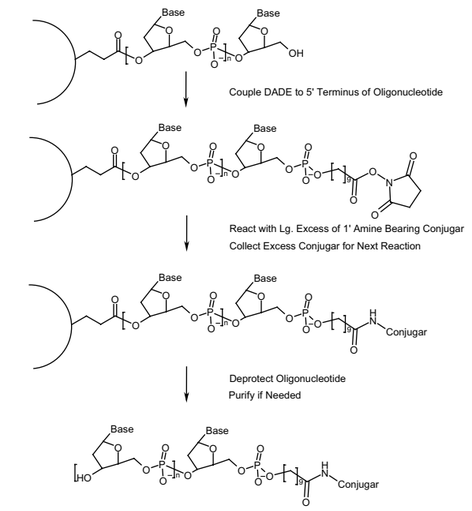


Figure 2: A typical application of the DADE linker

The fact that the conjugation chemistry is accomplished while the oligonucleotide is still support bound adds several advantages of its own. The choice of conjugar is often limited by the requirement that some water (20-50%) is needed to dissolve the oligonucleotide in the conjugation mixture. Solid phase conjugations allow the use of completely organic systems, even dichloromethane. This allows the ready conjugation of very lipophilic compounds to oligonucleotides. These fatty conjugars can then act as purification handles for RP-purification of the conjugate.

Another advantage of this system is that the reagent used in excess, the amine, is not affected by the reaction and is therefore recoverable. In fact, the reagent can be used exactly as is for the next reaction, especially if the excess is in the range of 100 fold or more. The use of 1% of the conjugar will not be noticeable kinetically for at least many reactions. At that time the reaction mix can be replenished. Besides the obvious cost savings, this will also improve overall efficiency in that much larger excesses are now feasible even in large-scale conjugations. By driving the reaction further to completion, the need for downstream operations, such as purifications, are reduced or even eliminated.

All in all, DADE offers enough advantages that it should be worthy of consideration for your high throughput or scale-up conjugation operation.

Sample Applications of DADE

1. Conjugation of the Lipid Octadecylamine to a Phosphorothioate

Octadecylamine was conjugated to a phosphorothioate oligonucleotide.

a. The DADE linker was coupled to the support bound oligonucleotide (5 μmole) using 50 μmoles as per the synthesis method supplied with the linker. The sulfurization was carried out using 10 equivalents of Beaucage reagent.

b. After the linker modified support-bound oligonucleotide was washed and dried, the beads were added to a vial containing 10 equivalents of 1-octadecylamine (stearylamine) dissolved in 2 mL of dichloromethane with 10% triethylamine. The reaction was allowed 4 hours at room temperature with continuous mixing. The beads were then washed well with dichloromethane and dried.

c. The oligonucleotide conjugate was then deprotected and cleaved from support using 2 mL of conc. ammonium hydroxide for 5 hours at 65° C, after which time the reagent was decanted and the beads rinsed with 4 mL of 25% acetonitrile in water. The combined solutions were dried *in vacuo*. The resulting residue was reconstituted in 1 ml of 25% acetonitrile in water and the yield determined (965 OD_{260} units).

d. The crude product was analyzed and purified on a Waters (Milford, MA) μBondapak C-18 RP-column (4.6 x 305 mm) using a gradient of acetonitrile (5% to 70% over 70 minutes) in 100 mM triethylammonium acetate, pH 7, at a flow rate of 1 ml/min. The conjugation occurred with 80% efficiency. The product was isolated using the same method with injection of 200 OD_{260} units of the crude sample per run. The isolated oligonucleotide conjugate was analyzed by electrospray mass

spectroscopy and found to have the correct mass (6585 au). The final yield was 435 OD_{260} units; 2.25 µmoles; 45% of overall theoretical yield from the starting scale of 5 µmoles.

2. High Throughput Screening of Conjugates
A number of amine bearing conjugates can be rapidly prepared using a variation of the following concept. This method could also be used to screen a number of different conditions to optimize the formation of a conjugate if you already have chosen one.

a. Prepare a 15 µmole scale synthesis of the oligonucleotide sequence of interest. Remove a very small amount of support from the synthesis column and reseal the column. This sample will be deprotected and serve as the unmodified standard.

b. Couple DADE to the oligonucleotide using the procedures supplied with the reagent. This scale of synthesis will require 100 µmoles of reagent.

c. Dry the support and divide into 15 equal aliquots, approximately 1 µmole each. For most syntheses, this will give the equivalent of 0.3-0.5 µmole of isolatable full-length product.

d. Place the support in 15 separate 2 mL glass screw-cap vials in which the conjugations will be carried out.

e. Dissolve the amines to be conjugated separately in the solvents of choice to a concentration of approximately 0.05 M. The conjugar will not be affected by the reaction and is therefore recoverable, allowing you to use large excesses of the reagent. We commonly will use 100 fold excesses (200µL of solution). As to preferred solvents, we have successfully carried out conjugations in acetonitrile, dichloromethane (methylene chloride), DMSO, high quality DMF (free of amine contaminants), and water. We add 10% volume of triethylamine to organic reactions, although this may not be necessary when large excesses of conjugar are used, and 10% volume of freshly prepared 0.5 M sodium bicarbonate to aqueous reactions.

f. Add the dissolved conjugars to the vials containing the support. Up to 14 reactions can be done simultaneously from this scale. The 15th will be kept as a retainer. A small aliquot of this material should be deprotected to use as a control during analysis of the oligonucleotide conjugates. Analysis of this material by PAGE or HPLC will also establish the efficiency of the DADE coupling.

g. Place the conjugation reactions on a rotary mixer and allow the reactions at least 4 hours. We recommend overnight reactions to ensure high coupling yields.

h. Decant the reagents from the beads and store for later use. Rinse the beads with fresh solvent, the same as used for the conjugation, followed by a rinse with acetone. Allow the beads to dry, then deprotect with conc. aqueous ammonium hydroxide as usual.

i. The oligonucleotide conjugates are now ready for use if crude samples are sufficient. Of course, they can be purified by HPLC or PAGE at this time. We recommend that the products are all analyzed by PAGE or HPLC and compared to the controls.

3. Scale up of Oligonucleotide Conjugates
This process is essentially the same as that described for the high throughput application above. The only difference is scale.

a. Synthesize the sequence of interest on solid support. For this example we will work with a 15 µmole scale synthesis. Remove a very small amount of support from the synthesis column and reseal the column. This sample will be deprotected and serve as the unmodified control.

b. Couple DADE to the oligonucleotide using the procedures supplied with the reagent. This scale of synthesis will require 100 µmoles of reagent.

c. Dissolve 100 fold excess of the amine (1500 µmoles for this example) to be conjugated in the solvent of choice. See Example 1 for ideas regarding solvent choice. Add 10% volume of triethylamine to organic reactions, although this may not be necessary when large excesses of conjugar are used, and 10% volume of freshly prepared 0.5 M sodium bicarbonate to aqueous reactions. Attempt to make your conjugation solution at approximately 0.1 M. A 0.1 M solution will result in 1.5 mL of solution (0.15 mL of which is triethylamine.)

d. Place the support bound oligonucleotide in a vial that can be sealed and is big enough to contain the support and the reaction mix. A small aliquot of this material should be deprotected to use as a control during analysis of the oligonucleotide conjugates. Analysis of this material by PAGE or HPLC will also establish the efficiency of the DADE coupling.

e. Follow steps g, h and i from section 2.

4. **Preparation of Oligonucleotides with 5' Carboxylic Acid Linkers**
Merely using the following deprotection scheme allows the use of DADE to directly prepare 5' COOH linkers on oligonucleotides.

a. Prepare the sequence of interest on solid support in the scale desired. Remove a very small amount of support from the synthesis column and reseal the column. This sample will be deprotected and serve as the unmodified control.

b. Couple the appropriate amount of DADE linker to the oligonucleotide using the procedures supplied with the reagent. DADE can be added to the modified reagent port of any DNA synthesizer like 5' amino linkers or 5' biotin phosphoramidites.

c. Deprotect the oligonucleotide at room temperature for 48 hours with the following reagent:
0.4 M NaOH in Methanol/H_2O (4:1)
(The time can be reduced if fast deprotecting monomers are used. Refer to the manufacturer of your phosphoramidites for specific deprotection requirements using the 0.4 M NaOH solution if you are uncertain.)

d. Isolate the oligonucleotide by first passing the reaction through the desalting process of your choice, and then purify as usual. The product can also be used crude after desalting.

DADE offers many opportunities to discover new solutions to old problems. TriLink now sells the DADE DNA Synthesis Reagent through Glen Research.

Use of Custom Synthesized Phosphoramidite Reagents

Before you grab a bottle of acetonitrile to dissolve your custom synthesized phosphoramidite, we suggest you take a few minutes to read these words of advice.

1. Pushing the envelope of scientific knowledge: Most likely very little is known about your custom compound. We do have some suggestions on how your amidite may behave based on our experience with similar compounds. And we can recommend procedures that should work in most cases.

Further, since this particular amidite was synthesized by us only once (and perhaps for the first time by anyone), some of the contaminants that are removed from standard amidites using established protocols may still remain in your compound. At some point it becomes more cost effective to accept the compound as it stands, than to continue to pay for methods of development. This is particularly true of compounds that are very experimental or are merely a means to answer a research question. Purification is usually the most costly step in an amidite synthesis. Multiple purifications can lead to very low yields and high costs. The methods suggested below can help overcome some minor contamination issues by optimizing other parameters.

2. Solvent selection and dryness: Most nucleoside phosphoramidites are soluble in acetonitrile, which is the common solvent for DNA synthesis. However, more lipophilic amidites, such as fatty acid amidites, require dichloromethane. Although dichloromethane can be used in most synthesizers, there are problems with flow rate changes and volatility. Ask your manufacturer if there are any compatibility problems if you decide to use your compound on a synthesizer.

It is also important to use solvent that contains less than 30 ppm water, preferably 10 ppm or less. This can be prepared by using high quality solvent, such as DNA synthesis grade acetonitrile, adding molecular sieves and allowing it to stand at least 24 hours before use. (We found that the Trap-paks from Perseptive Biosystems are the best quality sieves to use for this purpose.) We strongly recommend you use a titration device to test the water content of your solvents. TriLink can supply highly dried solvents over sieves for your amidite if you are unsure of the quality of your supply.

3. More about water: There are two common contaminates arising from the amidite itself that can greatly affect the quality of your synthesis; excess base and water. Neither contaminant is detectable in the phosphorus NMR or HPLC analysis of your compound. The excess base comes from the purification process and is removed using extensive drying at the end of the synthesis. If this is done carefully then excess base will not be a problem. We co-evaporate your compound extensively to remove the base.

Water is less easily eliminated. Some custom amidites tend to cling to water, or are hygroscopic enough to rapidly draw water out of the atmosphere even during fast transfers. The solution to this is to dry the dissolved amidite with high quality molecular sieves (3 Å) just prior to use. The Trap-paks work well here also. The large packs can be cut open and used for several bottles.

Our procedure with any expensive amidite, whether commercially purchased or manufactured by us, is to dissolve the amidite in the appropriate solvent and then add a single layer of sieves to the bottom of the vial. The vial is sealed and allowed to stand overnight. This will drop the water content to acceptable limits.

4. Concentration: Most instruments use between 0.05 M (approx. 50 mg/mL) and 0.1 M solutions of the amidites. In our estimation, the higher the concentration the better. We recommend using a concentration of 0.1 M for both automated and manual syntheses using modified reagents.

5. Coupling time: The steric hindrance of the amidite dictates the required coupling time. Sometimes only trial and error will provide an answer. We generally use coupling times of 5 minutes for a modified 2' deoxynucleoside and 15 minutes for everything else, including non-nucleosidyl amidites.

If there is a reported reagent that is similar to yours, use that coupling time. Actually, in most cases it is best to double the coupling time. It is very rare to have an amidite that couples worse with extended coupling times, although it is known to occur (methylphosphonates, for example). Another recommendation is to double or even triple couple an important nucleoside. If you are concerned at all about coupling efficiency, this is a good way to boost an 80% coupling to 95% or better. We will describe this process below.

6. Stability of your reagent to the rest of the DNA synthesis process: Another major consideration, and one that should be addressed before a custom synthesis is ordered, is the stability of the reagent to the rest of the coupling cycle chemistries. Is your compound stable to acid, oxidizer or capping reagents? If the answer is no to any one of these reagents, then changes must be made to either the compound or to the synthesis scheme. TriLink can help you develop preliminary stability experiments that will answer some of the questions. However, in the end, an oligonucleotide must be made and tested. A mass spec analysis of your first oligonucleotide is recommended.

7. Deprotection method: This is another stability issue. Many compounds, such as dyes, are not stable to the standard deprotection conditions using conc. NH_4OH at 55º C for 15 hours. There are many alternative methods available. Once again, trial and error may be necessary to optimize this step.

8. Coupling method - automated vs manual: We have learned the most efficient way to use precious amidites is to do the couplings by hand. This is how we couple most of our modified nucleosides. It us gives precise control over the quantity of reagents used and coupling time. An instrument uses more reagents because of priming needs, etc. A manual coupling can be done with just the right amount of material and little to no wastage. This can be critical if you have limited amounts of material.

9. Single coupling vs double or triple coupling: If you really want to make sure your amidite couples well, then it may be best to do multiple couplings. This is done by simply repeating the coupling step one or two times in the cycle before oxidation as illustrated below.

1. Deblock
2. Couple #1 (add amidite and activator to column)
3. Repeat step #2 as many times as needed
4. Oxidize
5. Cap

If your reagent is coupling at 80% efficiency, then the second coupling will get you to 96% (80% of the remaining 20%), etc. Theoretically, although we will never get to 100% as Aristotle explained, we should get extremely close.

10. If all else fails… TriLink can conduct all of the experiments described above and prepare your oligonucleotide for you. Many clients contract to have this done from the beginning of the program. Our goal is to help you succeed with your research.

An Antisense Oligonucleotide Primer

By Richard Hogrefe, Ph.D.; TriLink BioTechnologies
(an adaptation of this article was published in *Antisense and Nucleic Acid Drug Development*, 9, 351-357 1999. Table 1 updated 2007.)

Antisense oligonucleotides are short, synthetic strands of DNA (or analogs) that are complimentary, or antisense, to a target sequence (DNA or RNA) designed to halt a biological event, such as transcription, translation or splicing. After a period of doubt (Gura, 1995; Stein, 1995), antisense has been resurrected as a powerful tool for the molecular biologist and the first antisense drug (Isis's Fomivirsen) recently received FDA approval. The antisense field is experiencing an explosion of interest now that the phenomenon of the inhibition of gene expression by antisense oligonucleotides is more or less universally acknowledged. This essay will hopefully help the novice understand a few key principles regarding the use of antisense technology, as well as learn ways to avoid costly errors that nearly caused the premature death of the field.

Paul Zamecnik is generally attributed with publishing the first paper describing the use of antisense oligonucleotides (Stephenson, 1978). The early theory held by many was that if a synthetic oligonucleotide was annealed to a single stranded mRNA (or genetic DNA), the ribosome (or polymerase) would not continue reading the code and fall off, thus effecting 'hybrid arrest'. In fact, it was later learned that ribosomes and polymerases are indeed more like trains and will quickly read through the hybridized region. The true mechanism of action, in fact, is enzymatic cleavage of the RNA strand by RNase H (Minshull and Hunt, 1986; Dash, *et al.* 1987; Walder and Walder, 1988).

Even before the role of RNase H was discovered, it became apparent that, in order to develop an antisense drug, one of the hurdles that needed to be overcome was the rapid degradation of the oligonucleotide in the blood and in cells by both exonucleases and endonucleases. To remedy this, modified backbones were introduced that resisted nuclease degradation (Blake, *et al*., 1985; Agrawal and Goodchild, 1987). These modifications included subtle as well as not too subtle changes to either the phosphate or the sugar portion of the oligonucleotide. As it turned out, phosphorothioates, one of the easiest modifications to synthesize, have been the most successful to date (Matsukura, *et al*., 1987; Stein, *et al*., 1988).

One of the most simple and straightforward modifications that can be made to an oligonucleotide is to replace a non-bridging oxygen on the phosphate backbone with sulfur, producing a phosphorothioate linkage. The ability of this modification to retard nuclease degradation of oligonucleotides was long known (Matzura and Eckstein, 1968). It was later learned that this modification is also a substrate for RNase H (Stein, *et al*., 1988; Furdon, *et al*., 1989). These properties, combined with the relative ease of synthesis, have led to the ascendancy of this compound as an antisense drug. However, the road has not been easy. It was rapidly discovered that these compounds exhibited several unexpected properties *in vivo* (Srinivasan and Iversen, 1995). Despite the issues, most of the compounds progressing through clinical trials at this time are phosphorothioates (Table 1).

Target Validation versus Antisense Drug Development

The design of your experiments is predicated on where your interest in antisense lies. Of the many potential applications of antisense, two are most prominent: target validation and the use of an oligonucleotide as an actual therapeutic agent. Target validation refers to the use of antisense oligonucleotides in cell culture to determine if down regulating a certain gene target will give desired biological results (i.e. tumor cell line reduction, etc.). This information is often used to help develop more classic small molecule drugs. Fortunately, much of the early phase work needed to develop a therapeutic agent is similar to what is needed to conduct target validation experiments, which is why so many antisense drug companies are now antisense target validation companies as well. However, several significant differences do exist. Those interested in using antisense as a tool to study genes will most likely work exclusively with cells, which requires less material, but more attention to the issue of cellular uptake. Those interested in developing an antisense drug must concern themselves with scale up, toxicity, delivery, pharmacokinetics, and the FDA, of course.

One very important difference in developing an *in vitro* assay or a therapeutic drug is choice of oligonucleotide construct. When designing an antisense drug, one of the overriding concerns needs to be the ability to scale up the synthesis of the construct for a reasonable cost. This is the reason that most of the constructs in clinical trials are phosphorothioate oligonucleotides with no other modification. They are the least expensive of the constructs that have the correct properties for an antisense drug - nuclease resistance and retention of RNase H activity. However, there are problems with phosphorothioates that will be discussed in more detail below. When designing an antisense *in vitro* assay for target validation you have more flexibility and can choose from the newer "second generation" constructs that will also be discussed below.

The other major difference between *in vivo* and *in vitro* work is cellular uptake and delivery. Perhaps one of the most surprising findings to come out in the last 15 years in this field is that while delivery into cells *in vitro* appears to be difficult, *in vivo* delivery does not appear to be a problem. That statement is very bold and simplistic - and even controversial - but is well supported since all of the oligonucleotides in clinical trials are administered as saline solutions without delivery vehicles. The whole field of antisense drug development almost collapsed when it was determined that a delivery system was needed in order to be successful *in vitro*. The potential cost of developing such a vehicle for systemic delivery of oligonucleotides *in vivo* frightened an investment community that already spent a great deal of money and was losing patience. Fortunately, a brave few went ahead and injected animals anyway only to discover that in some instances results were obtained that were convincingly due to an antisense mechanism (Dean and McKay 1994; Desjardins and Iverson, 1995). The occasional positive therapeutic effects that cannot be proved to be antisense should be considered an added bonus (Stein, 1995; Ramasamy, *et al.*, 1996, Boiziau, *et al*., 1997). In any case, regardless of whether you are developing an antisense drug or an *in vitro* assay, your initial studies will most likely be with cells in culture and therefore you will have to be concerned with uptake.

Choosing an Oligonucleotide Construct

As stated earlier, the choice of backbone construct depends on your final goal. If you are designing an antisense drug, you must consider the cost to synthesize the final product, and if it is feasible in large scale. The chemistry must precede the biology to a large extent. This should be a primary concern. If you have novel chemistry, or very complicated chemistry, seek the counsel of someone experienced with oligonucleotide synthesis from a commercial and scale up perspective. Make sure that others can reproduce your work.

The most popular modification for antisense oligonucleotides continues to be phosphorothioates. These oligonucleotides can be obtained for fairly reasonable prices and in kilogram scales. Antisense firms still commit a great deal of their development budget to improving the synthesis of these compounds. They obviously expect a continual stream of phosphorothioate oligonucleotides to enter the clinic, as well as obtain FDA approval. Phosphorothioate oligonucleotides are probably a good choice if you wish to rapidly develop a program. Besides price, phosphorothioate oligonucleotides have demonstrated success in a science where success has often been difficult to achieve.

However, there are some problems with phosphorothioate oligonucleotides. The backbone is chiral, resulting in a racemic mixture of 2^n oligonucleotide species (where n = number of phosphorothioate internucleotide linkages) instead of a single compound. The overall mixture has a lower T_m than its corresponding phosphodiester oligonucleotide (LaPlanche, *et al.*, 1986). Phosphorothioate oligonucleotides have been known to exhibit unusual properties *in vivo*, both desirable and undesirable. Some of those effects are due to the affinity phosphorothioates show for proteins (Brown, *et al*., 1994). Phosphorothioates also have a reputation for being toxic (Srinivasan and Iversen, 1995), although that may be a sequence specific phenomenon or due to contamination in early oligonucleotide preparations. Another problem for some is that the NIH patented phosphorothioate oligonucleotides for antisense applications. Be prepared to pay Uncle Sam for the right to use this compound as a therapeutic agent once you succeed.

For those who want to have alternatives, or wish to develop an antisense *in vitro* assay as an endpoint, other possibilities do exist. These "second generation" oligonucleotide constructs are available commercially and the less complex ones are not much more expensive than phosphorothioate oligonucleotides at the smaller scales. In fact, most of them include some phosphorothioate linkages, and many are still completely modified with phosphorothioates. A common design is to have nuclease resistant arms (such as 2'-O-methyl (OMe) nucleosides) that surround a phosphorothioate modified deoxyribose core that retains the RNase H activity of the oligonucleotide (Agrawal and Goodchild, 1987, Giles and Tidds, 1992). Oligonucleotides that contain mixtures of chemistry are called chimeric oligonucleotides. Chimeric oligonucleotides containing 2'-OMe arms were used to help understand the underlying principles of the RNase H mechanism (Hogrefe, *et al*., 1990). The most significant enhancements offered by this class of compound are a general reduction in toxicity, increased hybrid stability, and increased nuclease stability (Peng Ho, *et al*., 1998; Zhou and Agrawal, 1998). These all combine to yield a compound more reproducibly active when used in an *in vitro* assay and are your best choice for such work.

Since 1987, various groups have staked out several specific constructs as proprietary. In the end you may have to seek counsel of a good patent attorney and obtain a license for a particular construct, but for research purposes a number of choices are available commercially. A good starting point is to use an oligonucleotide eighteen nucleotides in length that has six 2'-OMe nucleotides at both the 5' and 3' ends, leaving a core of six 2'-deoxyribose nucleosides with phosphorothioate internucleotide linkages (Monia, *et al.*, 1993; Metelev, *et al.*, 1994). The arms may or may not contain phosphorothioate linkages for best results. Removing phosphorothioate linkages may reduce toxicity, however it also reduces nuclease resistance. You have to see what works best with your system.

"Third generation" compounds are also in development. These constructs are a return to the original concept of hybrid arrest and depend on extreme hybridization enhancement using highly modified oligonucleotides. These modifications include 2'-MOEs (Monia, 1997), N3'-P5' phosphoramidates (Gryaznov and Chen, 1994; Mignet and Gryaznov, 1998), PNA's (Hanvey, *et al*., 1992), chirally pure methylphosphonates (Reynolds *et al*., 1996), MMIs (Morvan, *et al*., 1996; Swayze, 1997), and others. While most of these constructs work to some extent, all have at least one significant problem, such as solubility, delivery, or cost of synthesis. At this time, it is probably best not to explore these types of compounds unless you have extensive in-house experience.

As a final word regarding the oligonucleotide itself, whatever the construct you choose, be certain of the integrity of the compound. Many of the failed experiments and false conclusions of the past were due to contamination. Although there has been significant improvement over the years, there is still a need to be attentive to purity, particularly as the modification requirements increase.

Choice of Sequence

There is no sure way to determine *a priori* where on a particular gene is the most active site for an antisense oligonucleotide, although advice does exist (Cohen, 1989; Woolf, *et al*., 1992; Brysch and Schlingensiepen, 1994). The region surrounding the start codon (AUG) site is probably the most popular, followed by site mutations. Recently, targeting splicing sites has become increasingly popular in order to inhibit the mRNA processing mechanism as opposed to the message (Sierakowaka, *et al*., 1996).

For every site of interest, design up to ten different sequences along the region, trying to maximize hybridization while avoiding sequences with regions of polyguanosine or G-C arms that will form strong hairpins. There is a good chance that one of those ten sequences will be active. Some of the chimeric oligonucleotides have even better success rates.

You do have to be concerned with what is commonly referred to as the CpG effect. Some oligonucleotide sequences that contain the dinucleotide CpG cause a fairly profound stimulation of the immune system (Krieg, 1998). The explanation is that in mammalian cells, most of the exposed cytosine is methylated at the 5 position. Bacterial cytosine is not methylated. Apparently, mammals have developed an immune response to non-methylated genomic material as a defense against bacteria. Several groups are actually exploiting this effect for its therapeutic value (Klinman, 1998; Millan, *et al*., 1998). If you are concerned about the effect a CpG may have on your system, a simple experiment is to replace all the cytidines 5' to guanosines with 5-methylcytidine which will inhibit the effect (Boggs, *et al*., 1997).

Developing a Successful *In Vitro* Experiment

Whether you are developing an antisense drug or an *in vitro* assay, the initial experiments will most likely be with cells in culture. This, of course, is your endpoint when developing an *in vitro* assay, but it is also highly recommended when developing an antisense drug. In general, it is easier to look for true antisense indicators such as reduction of target mRNA or protein *in vitro* than *in vivo*. The screening process is also far more economical. What must be remembered is that to be successful *in vitro*, you must use a delivery system. Conversely, you can go forward with your *in vivo* experiments *sans* carrier once you've discovered a good target. There does not appear to be a satisfactory explanation for this phenomenon.

Fortunately, we do seem to have a reasonable solution to the problem of *in vitro* cellular uptake. The most effective delivery system has turned out to be cationic lipids (Capaccioli, *et al*., 1993; Lappalainen, *et al*., 1994; Quattrone, *et al*., 1995), which have become the standard for *in vitro* work. The one caveat is that there is no universal cationic

Table 1: Sampling of Oligonucleotides in Clinical Trials

Company	Compound	Phase	Disease	Target	Mode of Action	Chemistry	Trial Number	Outcome
A.C. James Canc. Hosp.	GTI 2040	I	Myeloid Leukemia	Unknown	Antisense	Phosphorothioate	NCT00070551	Ongoing
Advanced Viral Research	AVR118	II	Cachexia	Cytoprotective	Immune-Active	PNA	NCT00127517	Ongoing
Aegera	AEG35156	I	Advanced cancers	XIAP (caspase inhibitor)	Antisense	Phosphodiester		Terminated
Aegera	AEG35156	I/II	Acute myeloid leukemia	XIAP (caspase inhibitor)	Antisense	Phosphodiester	NCT00363974	Ongoing
Aegera	AEG35156	I	Advanced tumors	XIAP (caspase inhibitor)	Antisense	Phosphodiester	NCT00372736	Ongoing
Aegera	AEG35156	I	Solid tumors	XIAP (caspase inhibitor)	Antisense	Phosphodiester	NCT00357747	Ongoing
Aegera	AEG35156	I/II	Non-small cell lung cancer	XIAP (caspase inhibitor)	Antisense	Phosphodiester	NCT00558922	Ongoing
Aegera	AEG35156	I/II	Human Mammary Carcinoma	XIAP (caspase inhibitor)	Antisense	Phosphodiester	NCT00558545	Ongoing
Aegera	AEG35156	I/II	Pancreatic Cancer	XIAP (caspase inhibitor)	Antisense	Phosphodiester	NCT00557596	Ongoing
Alnylam	ALN-RSV01	II	Resp. syncytial virus	viral gene	siRNA	RNA	NCT00658086	Ongoing
Amarin (Ester Neurosciences)	EN101	II	Myasthenia gravis	acetylcholine esterase	Antisense	Mixed Chemistry		Ongoing
Antisense Pharma	AP 12009	II	Glioblastoma	TGF-beta2 (tumor factor)	Antisense	Phosphorothioate	NCT00431561	Ongoing
Antisense Pharma	AP 12009	III	Anaplastic Astrocytoma	TGF-beta2 (tumor factor)	Antisense	Phosphorothioate	NCT00761280	Ongoing
Antisense Thera./Teva	ATL1102	IIa	Multiple sclerosis	VLA-4	Antisense	Phosphorothioate		Ongoing
Antisense Therapeutics	ATL1102	I	Asthma	VLA-4	Antisense	Phosphorothioate		Ongoing
Antisense/Isis	ATL1102	II	Multiple sclerosis	VLA-4	Antisense	Mixed Chemistry		Ongoing
Archemix	ARC1779	II	Von Willebrand Disease	Platelets	Aptamer	Unknown	NCT00694785	Ongoing
AVI Biopharma	Resten-NG	II/III	Restenosis	c-myc	Antisense	Nugene		Completed
AVI Biopharma	Resten-MP	II	Restenosis	c-myc	Antisense	Nugene		Completed
AVI Biopharma	AVI-5126	I	CABG	c-myc	Antisense	Nugene		Terminated
AVI Biopharma	AVI-4065	II	Hepatitis C	NS3 (HCV protease)	Antisense	Nugene		Completed
AVI Biopharma	AVI-4557	I/II	Drug metabolism	CYP3A4	Antisense	Nugene		Completed
AVI Biopharma	AVI-4658	I/Ib	Muscular dystrophy	dystrophin	Antisense	Phosphorothioate		Ongoing
California Cancer Cons.	GTI 2040	II	Breast cancer	Ribonucleotide Reductase	Antisense	Phosphorothioate	NCT00068588	Ongoing
Coley Pharmaceuticals	ProMune	II	Cancer	TLR9	Immune-Active	Phosphorothioate		Completed
Dynavax	1018 ISS(Tolamba)	III	Ragweed allergy	TLR9	Immune-Active	Phosphorothioate		Terminated
Dynavax	1018 ISS(Heplisav)	III	Hepatitis B	TLR9	Immune-Active	Phosphorothioate	NCT00435812	Ongoing
Dynavax	1018 ISS	II	Non-Hodgkin's Lymphoma	TLR9	Immune-Active	Phosphorothioate	NCT00251394	Ongoing
Dynavax Technologies Corp	1018 ISS	I	Hepatitis B	TLR9	Immune-Active	Phosphorothioate	NCT00511095	Ongoing
Dynavax Technologies Corp	1018 ISS	I	Colorectal Neoplasms	TLR9	Unknown	Phosphorothioate	NCT00403052	Ongoing
Enzon Therapeutic	HGTV-43	I/II	HIV	viral replication genes	Antisense	DNA		Ongoing
Enzon Pharmaceuticals, Inc.	EZN-2968	I	Lymphoma	HIF-1α	Immune-Active	LNA	NCT00466583	Ongoing
EORTC	Genasense	II	Prostate cancer	Bcl-2	Antisense	Phosphorothioate	NCT00085228	Ongoing
Genta	Genasense	III	Acute myeloid leukemia	Bcl-2	Antisense	Phosphorothioate	NCT00024440	Ongoing
Genta	Genasense	II	Prostate cancer	Bcl-2	Antisense	Phosphorothioate		Completed
Genta	Genasense	II/III	Non-small cell lung cancer	Bcl-2	Antisense	Phosphorothioate	NCT00030641	Ongoing
Genta Incorporated	Genasense	III	Melanoma (Skin)	Bcl-2	Antisense	Phosphorothioate	NCT00016263	Ongoing
Genta Incorporated	Genasense	II/III	Melanoma	Bcl-2	Antisense	Phosphorothioate	NCT00543205	Ongoing
Genta Incorporated	Genasense	I/II	Chronic Lymphocytic Leukemia	Bcl-2	Antisense	Phosphorothioate	NCT00078234	Ongoing
Genta Incorporated	Genasense	III	Multiple Myeloma and Plasma Cell Neoplasm	Bcl-2	Antisense	Phosphorothioate	NCT00017602	Ongoing
Genta Incorporated	Genasense	I	Solid Tumors	Bcl-2	Antisense	Phosphorothioate	NCT00636545	Ongoing
Gentium/Dana-Farber	Defibrotide	III	VOD	Unknown	Unknown	Random mixture	NCT00358501	Ongoing
Geron	GRN163L	I/II	Chronic lymphocyte leukemia	telomerase	Antisense	Thiophosphoramidate	NCT00124189	Ongoing
Geron	GRN163L	I	Solid tumor malignancies	telomerase	Antisense	Thiophosphoramidate	NCT00310895	Ongoing
Idera	IMO-2055	II	Renal cell carcinoma	TLR9	Immune-Active	Phosphodiester	NCT00729053	Ongoing
Idera	IMO-2125	I	Hepatitis C	TLR9	Unknown	DNA	NCT00728936	Ongoing
Idera	IMO-2055	I	Non-small cell lung cancer	TLR9	Immune-Active	Phosphodiester	NCT00633529	Ongoing
Immune Response	Amplivax	II	HIV	TLR9	Immune-Active	Phosphodiester		Completed
Imperial College London	AVI-4658 (PMO)	I/II	Duchenne muscular dystrophy	dystrophin	Antisense	Phosphorothioate	NCT00159250	Ongoing
Inst. for Drug Dev.	GTI 2040	I	Solid Tumors		Antisense	Phosphorothioate		Completed
Isis	Vitravene	N/A	CMV retinitis	IE2	Antisense	Phosphorothioate		Completed
Isis	Alicaforsen	II	Ulcerative colitis	ICAM1	Antisense	Phosphorothioate		Completed
Isis	ISIS 113715	II	Diabetes	PTP-1B	Antisense	Mixed Chemistry	NCT00455598	Ongoing
Isis	ISIS 301012	II	High Cholesterol	apoB-100	Antisense	Mixed Chemistry	NCT00362180	Ongoing
Isis	ISIS 104838	II	Rheumatoid Arthritis	TNF-alpha	Antisense	Mixed Chemistry		Completed
Isis Pharmaceuticals	ISIS 2302	III	Crohn's Disease	ICAM-1	Antisense	Phosphorothioate	NCT00048295	Ongoing
Isis/Lilly	LY2181308	II	Cancer	survivin	Antisense	Mixed Chemistry	NCT00642018	Ongoing
Lorus	GTI-2040	II	Renal cell carcinoma	R2 comp of RNR	Antisense	Phosphorothioate		Completed
Ludwig Inst for Cancer Res	CpG7909	I	Melanoma	Melan-A	Immune-Active	Unknown	NCT00112229	Ongoing
MethylGene	MGCD0103	I/II	Solid tumors	HCAC	Antisense	Mixed Chemistry	NCT00372437	Ongoing
MethylGene	MGCD0103	I	Hematological cancer	HCAC	Antisense	Mixed Chemistry	NCT00511576	Ongoing
National Cancer Institute	VEGF-AS	I/II	Kaposi's sarcoma	vascular endothelial GF	Antisense	Phosphorothioate		Completed
National Cancer Institute	OGX-011	II	Breast Cancer	secretory protein clusterin	Antisense	Mixed Chemistry	NCT00258375	Ongoing
NeoPharm	LErafAON	I	Cancer	c-raf	Antisense	Unknown	NCT00100672	Ongoing
Norris Comprehensive Cancer Center	Veglin™ (VEGF-AS)	I/II	Mesothelioma	angiogenesis	Antisense	Phosphorothioate	NCT00668499	Ongoing
Oncogenex	OGX-011	I/II	Cancer	secretory protein clusterin	Antisense	Mixed Chemistry	NCT00327340	Ongoing
OncoGenex Technologies	OGX-427	I	Tumors	Hsp27	Antisense	Unknown	NCT00487786	Ongoing
Opko Health (formerly Acuity)	Bevasiranib (Cand5)	III	Neovascular AMD	VEGF	siRNA	RNA	NCT00499590	Ongoing
Opko Health (formerly Acuity)	Bevasiranib (Cand5)	II	Neovascular AMD	VEGF	siRNA	RNA		Completed
OSI Pharm (Eyetech)	Macugen	N/A	Neovascular AMD	VEGF	Aptamer	Phosphorothioate	NCT00354445	Ongoing
OSI Pharm (Eyetech)	Macugen	II/III	Diabetic Macular Endema	VEGF	Aptamer	Phosphorothioate		Completed
OSI Pharm (Eyetech)	Macugen	II	Retinal Vein Occlusion	VEGF	Aptamer	Phosphorothioate		Completed
Princess Margaret Hospital	GTI 2040	I	Prostate cancer	Ribonucleotide Reductase	Antisense	Phosphorothioate		Completed
Santaris	SPC2996	I/II	Chronic lymphocyte leukemia	Bcl-2	Antisense	Phosphorothioate	NCT00285103	Ongoing
Sirna (formerly Ribozyme)	SIRNA-027	I	Macular degeneration	VEGFR-1	siRNA	RNA		Completed
Southwest Oncology Group	EGFR AS	III	Cancer	EGFR	Antisense	Phosphorothioate	NCT00049543	Ongoing
Southwest Oncology Group	Genasense	II	Lymphoma	Bcl-2	Antisense	Phosphorothioate	NCT00080847	Ongoing
Topigen	TPI-ASM8	II	Allergic asthma	CCR3, ILreceptor3 & 5, GM-CSF	Antisense	Phosphorothioate	NCT00550797	Ongoing
University of British Col.	OGX-011	II	Prostate cancer	Unknown	Antisense	Mixed Chemistry	NCT00138918	Ongoing
University of Chicago	Genasense	I/II	Lung Cancer	bcl-2	Anitsense	Phosphorothioate	NCT00005032	Ongoing
Univ. of Pennsylvania	c-myb AS	II	Cancer	c-myb	Antisense	Phosphorothioate	NCT00002592	Ongoing
University of Pennsylvania	busulfan	II	Leukemia	c-myb	Antisense	Unknown	NCT00002592	Ongoing
University of Pennsylvania	c-myb AS ODN	I	Hematologic Malignancies	c-myb	Antisense	Unknown	NCT00780052	Ongoing
University of Pittsburgh	Diabetes-suppressive dendritic cell vaccine	I	Type 1 Diabetes	CD40, CD80 and CD86	Antisense	Phosphorothioate	NCT00445913	Ongoing
UPMC Cancer Centers	CpG 7909	I	Melanoma	CD8+ T-cell	Immune-Active	Unknown	NCT00471471	Ongoing
VasGene	Veglin	II	Cancer	VEGF isoforms	Antisense	Phosphorothioate		Completed

lipid that works for all cell lines and with all constructs. Worse yet, sometimes a mixture of different cationic lipids at specific concentrations is required to optimize delivery into your particular cell line. The fact is that you have to hand tailor the cationic lipid mixture to fit your cell line and oligonucleotide construct.

Many commercially available cationic lipids exist. It is highly likely that amongst them at least one uptake system can be found that works. However, if you are exploring your own cell line or oligonucleotide construct, then you are going to have to do some experimentation to find the right delivery system. To make it easier, kits are available that contain various lipid mixtures for this purpose.

The best way to begin developing a successful *in vitro* assay is to determine conclusively if good cellular uptake is occurring with your delivery system by using a fluorescently labeled oligonucleotide and fluorescence microscopy to observe uptake (Noonberg, *et al*., 1992; Sasaski, *et al*., 1995). The sequence is relatively unimportant here. All that matters is that the construction is the same as what you intend to use. Although a fluorescent molecule must be added, this does not appear to affect uptake. Since most fluorescent microscopes come equipped with filters for fluorescein, that fluorophore will serve well. Please note that the fluorescent molecule must be introduced during the synthesis of the oligonucleotide. Therefore a separate preparation of your compound is required. As an alternative, inexpensive fluorescently labeled oligonucleotides with mixed base compositions are available for this purpose from commercial sources.

The experiment to study the uptake of your fluorescently labeled oligonucleotide is fairly straightforward (Shoji, *et al*., 1991; Sasaki, *et al*., 1995). The fluorescently labeled oligonucleotide is mixed with the cationic lipid mixture(s) according to the manufacturer's instructions. These are applied to the cells as 1 to 3 micromolar solutions. The cells are harvested and fixed, then viewed under the microscope. The desired effect is uptake into the nucleus of the cells. A fair proportion of the cells should have fluorescence in the nuclei. This is indicative of proper delivery. If a punctate pattern (isolated spots of fluorescence) is visible in the cytoplasm instead, then you are merely observing endoplasmic sequestering, which was the fate of most of the oligonucleotides used in the early experiments without delivery systems (Shoji, *et al*., 1991).

Once you have found a satisfactory lipid mixture, you can test your antisense sequences with the confidence that you are truly looking at activity. It is actually a good idea to periodically test your delivery system using a fluorescent oligonucleotide to make sure that the cell line hasn't transformed in some manner that changes uptake properties.

One last question to answer in regard to *in vitro* experimentation is how much oligonucleotide is required. Usually 5 to 10 ODs (~150 - 300 μg) which is readily obtained from a 200 nmole scale synthesis is more than enough. For more extensive experimentation, a 1 μmole scale synthesis will generally yield 1 to 2 mg of purified oligonucleotide, depending on the construct. The extra material will also allow you to retain sample to compare with new batches of oligonucleotide in case activity changes, which is not uncommon.

Moving to *In Vivo* Experiments

When you start your *in vivo* studies you have a whole new set of concerns. First, you must be assured of obtaining a reasonable quantity of your oligonucleotide. Initially, your requirements will not be extensive. A fairly comprehensive rodent study can be conducted with 50 mg of oligonucleotide. At a common dose of 5 mg/kg, 500 inoculations can be made to mice that normally weigh 0.02 kg. However, a rat study will obviously go through 50 mg much more rapidly. Later experiments will require grams of material. If you are using standard phosphorothioate oligonucleotides, or one of the more common chimeric oligonucleotides, supply will not be a significant concern. However, if your construct is fairly complicated, be sure to investigate scale up issues early in the program, rather than later. Nothing is worse than spending millions developing a drug only to find out it is next to impossible to manufacture for a reasonable fee.

Along with quantity goes quality. It is even more important to be sure of the quality of your material. Toxicity due to contaminants is very easy to avoid if proper precautions are taken. Be picky and willing to pay good money for good material, whether it is from an in-house source or from an external vendor.

A very significant concern is how to interpret the results of your experiments and prove you have an antisense drug. This has been a topic of controversy from the beginning. It is difficult to locate and quantitate the reduction of both target mRNA and protein product. Still, it has been done (Monia, 1997) and is the best way to be confident in an antisense mechanism. One strong argument that is very persuasive is championed by adherents to the "So what?" school of thought. If the compound does what it was meant to do, why argue? The practical course is to accept positive results and continue towards a drug product. You can always continue the search for the mechanism of action later with the hope of discovering the Holy Grail of drug development - true rational drug design.

Another concern, or perhaps a relief, is that you can throw out the delivery system you so arduously developed for your cell work. Most *in vivo* oligonucleotide solutions are merely saline. The solutions are injected in various ways, including ocular and other locations even more difficult to imagine enduring. Oral delivery has also been examined (Agrawal, *et al*., 1995). Despite the apparent success of some ongoing trials using no delivery system, there is still a fairly universal belief that a good method of systemic delivery to specified tissues can only be advantageous and lead to a higher success rate. The merging needs, in fact, have led to merging companies, such as the purchase of Lynx's therapeutic oligonucleotide technology by Inex of Canada, a delivery company. Until such a delivery system emerges, your best course is to just use a saline solution.

Another set of experiments unique to the development of an antisense therapeutic is the need for pharmacokinetic studies. These animal experiments require injection of the test subject with an oligonucleotide labeled with a radioactive isotope (Agrawal, *et al*., 1991; Cossum, *et al*., 1993; Iverson, *et al*., 1994). The most common isotopes are sulfur-35 and tritium (hydrogen-3). These can be made in-house, although they can also be obtained commercially. For a very simple study regarding circulatory lifetime and urine and fecal elimination, as little as 1 to 5 μCi per mouse will suffice, depending on the sensitivity of your equipment. More significant quantities will be needed to follow the degradation rate *in vivo* in the next stage of investigations.

The road to FDA approval from here is still long and difficult. However, you should now be well on your way and thinking about the second and third drugs in your pipeline.

Final Words

Regardless of your intended use for antisense oligonucleotides, if you pay attention to those who have gone before, you stand a good chance of succeeding. This is especially true if you are developing an *in vitro* assay. If all else fails, there are companies in existence that are in the business of helping you develop your antisense assays. It may cost you more at first, but the expertise you purchase is usually well worth the price if your goals are commercial in nature. If you prefer to do it yourself, then go forward with the confidence of knowing your chances will be good in the long run and with the knowledge that there are plenty of people out there willing to answer your questions.

References

1. Agrawal, K., and Riftina, F. (1979) Nucleic Acids Research., 6, 3009-3023.
2. Agrawal, S., and Goodchild, J. (1987) Tetrahedron Letters., 28, 3539-3542.
3. Agrawal, S., Temsamani, J. & Tang, J.Y.(1991) Proc. National Acad. Sci., 88, 7595-7599
4. Agrawal, S., Zhang, X., Lu, Z., Zhao, H., Tamburin, J.M., Yan, J., Cai, H., Diasio, R.B., Habus, I., Jiang, Z., Iyer, R.P., Yu, D. and Zhang, R. (1995) Biochemical Pharmacology, 50, 571-576.
5. Blake, K., Murakami, A., Spitz, S.A., Glave, S.A., Reddy, M.P., Ts'o, P.O.P., & Miller, P.S. (1985) Biochemistry, 24, 6139-6145.
6. Boggs, R.T., McGraw, K., Condon, T., Flournoy, S., Villiet, P., Bennett, C. F., & Monia, B. P. (1997) Antisense Nucleic Acid Drug Dev., 7, 461-471.
7. Boiziau, C., Dausse, E., Mishra, R., Duconge, F., & Toulme, J.-J. (1997) Antisense Nucleic Acid Drug Dev., 7, 369-380
8. Brown, D.A., Kang, S.H., Gryaznov, S.M., DeDionisio, L., Heidenreich, O., Sullivan, S., Xu, X., Nerenberg, M. I. (1994) J. Biol. Chem., 269, 26801-5.
9. Brysch, W., and Schlingensiepen, K. (1994) Cellular and Molecular Neurobiology, 14, 557-568.
10. Capaccioli, S., Pasquale, G.D., Mini, E., Mazzei, T, and Quattrone, A. (1993) Biochem. And Biophys. Res. Comm., 197, 818-825.
11. Cohen, J.S. (1989) TIBS, 10, 435-437.
12. Cossum, P.A., Sasmor, H., Dellinger, D., Truong, L., Cummins, L., Owens, S.R., Markham, P.M., Shea, J.P. and Crooke, S. (1993) Journal of Pharm. and Exper. Therapeutics, 267, 1181-1190.
13. Dash, P., Lotan, I., Knapp, M., Kandel E.R. and Goelet, P. (1987) Proc. Natl Acad. Sci. USA, 84, 7896-7900.
14. Dean, N.M., and McKay, R (1994) Proc. Natl Acad. Sci. USA, 91, 11762-11766.
15. Desjardins, J.P., and Iverson, P.L. (1995) J. of Pharmacology and Experimental Therapeutics, 275, 1608-1613.
16. Furdon, P.J., Domininski, Z., and Kole, R. (1989) Nucleic Acids Research., 17, 9193-9204.
17. Giles, R.V., & Tidd, D.M.(1992) Nucleic Acids Research, 20, 763-770.
18. Gryaznov, S., and Chen, J.K. (1994) Journal of American Chemical Society, 116, 3143-3144.
19. Gura, T. (1995) Science, 70, 575-577.
20. Hanvey, J.C., Peffer, N.J., Bisis, J.E., Thomson, S.A., Cadilla, R., Josey. J.A., Ricca, D.J., Hassman, C.F., Bonham, M.A., Au, K.G., Carter, S.G., Bruckenstein, D.A., Boyd, A.L., Noble, S.A., and Babis, L.E. (1992) Science, 258, 1481-1485.
21. Hogrefe, H.H., Hogrefe, R.I., Walder, R.Y., Walder, J.A. (1990) Journal of Biological Chemistry, 265, 5561-5566.
22. Iversen, P.L., Mata, J., Tracewell, W.G., & Zon, G. (1994) Antisense Research and Development, 4, 43-52
23. Klinman, D.M. (1998) Antisense Nucleic Acid Drug Dev., 8, 181-184.
24. Krieg, A. M. (1998) Handb. Exp. Pharmacol, 131(Antisense Research and Application), 243-262.
25. LaPlanche, L.A., James, T.L., Powell, C., Wilson, W.D., Uznanski, B., Stec, W.J., Summers, M.F., and Zon, G. (1986) Nucleic Acids Research, 14, 9081-9093.
26. Lappalainen, K., Urtti, A., Soderling, E., Jaaskelainene, I., Syrjanen, K., and Syrjanen, S. (1994) Biochemica and Biophysica Acta, 201-208.
27. Matsukura, M., Shinozuka, K., Zon, G., Mitsuya, H., Reitz, M., Cohen, J.S., Broder, S. (1987) Phosphorothioate analogs of oligodeoxynucleotides: inhibitors of replication and cytopathic effects of human immunodeficiency virus, Proc. Natl Acad. Sci. USA, 84, 7706–7710.
28. Matzura, H., and Eckstein, F. (1968) Eur. J. Biochem., 3, 448-452.
29. Metelev, V., Lisziewicz, J., and Agrawal, S. (1994) Bioorg. Med. Chem. Lett., 4, 2929-34.
30. Mignet, N., and Gryaznov,S.M. (1998) Nucleic Acids Research, 26, 431-438.
31. Minshull, J., and Hunt, T. (1986) Nucleic Acids Research, 14, 6433-6451.
32. Millan, C.L.B., Weeratna, R., Krieg, A. M., Siegrist, C., Davis, H.L. (1998)Proc. Natl. Acad. Sci. U. S. A., 95, 15553-15558
33. Miller, P.S. (1991) Bio/Technology, 9, 358-362.
34. Monia, B.P., Lesnik, E. A., Gonzalez, C., Lima, W.F., McGee, D., Guinosso, C. J., Kawasaki, A. M., Cook, P. D., & Freier, S. M. (1993) J. Biol. Chem., 268, 14514-22.
35. Monia, B.P. (1997) Ciba Found. Symp., 209 (Oligonucleotides as Therapeutic Agents), 107-123.
36. Morvan, F., Sanghvi, Y., Perbost, M., Vasseur, J.-J., Bellon, L., (1996) Journal of American Chemical Society, 118, 255-256.
37. Noonberg, Sarah B., Weiss, Tania L., Garovoy, Marvin R., Hunt, C. Anthony (1992) Antisense Res. Dev., 2, 303-13.
38. Quattrone, A., Pasquale, G.D., and Capaccioli, S. (1995) Biochemica, 1, 25-29.
39. Peng Ho, S., Livanov, V., Zhang, W., Li, J., Lesher, T. (1998) Mol. Brain Res., 62, 1-11.
40. Ramasamy, R., Kanagaratnam, R., Misiura, K., Rebowski, G., Amerakoon, R., and Stec, W.J. (1996) Biochem. and Biophys. Res. Comm., 218, 930-933.
41. Reynolds, M.A., Hogrefe, R.I., Jaeger, J.A., Schwartz, D.A., Riley, T.A., Marvin, W.B., Daily, W.J., Vaghefi, M.M., Beck, T.A., Knowles, S.K., Klem, R.E. & Arnold, L.J.(1996) Nucleic Acids Research., 24, 4584-4591.
42. Sasaki, M., Watanabe, M., Kawakubo, Y., Komiyama, M., Kobayashi, M., & Fujita, H. (1995) Photomed., Photobiol., 17, 103-4
43. Shoji, Y., Akhtar, S., Periasamy, A., Herman, B., & Juliano, R. L (1991) Nucleic Acids Research, 19, 5543-5550
44. Sierakowaka, H., Sambade, M. J., Agrawal, S., & Kole, R. (1996) Proc. Natl. Acad. Sci. U. S. A., 93, 12840-12844.
45. Srinivasan, S. K., & Iversen, P. L. (1995) Journal of Clinical Laboratory Analysis, 9, 129-137.
46. Stein, C.A., Subasingfe, C., Shinozuka, K., and Cohen, J.S. (1988) Nucleic Acids Research., 16, 3209-3221.
47. Stein, C.A., (1995) Nature Medicine, 1, 1119-1120.
48. Stephenson, M.L., and Zamecnik, P.C. (1978) Proc. Natl Acad. Sci. USA, 75, 1302-1306.
49. Swayze, E.E., Bhat, B., Peoc'h, D., Sanghvi, Y. S. (1997) Nucleosides Nucleotides, 16, 971-972.
50. Walder, R.Y., and Walder, J.A. (1988) Proc. Natl Acad. Sci. USA, 85, 5011-5015.
51. Woolf, T.M., Melton, D.A., and Jennings, C.G.B. (1992) Proc. Natl Acad. Sci. USA, 89, 7305-7309.
52. Zhou, W., and Agrawal, S. (1998) Bioorg. Med. Chem. Lett., 8, 3269-3274.

Randomer Oligonucleotides

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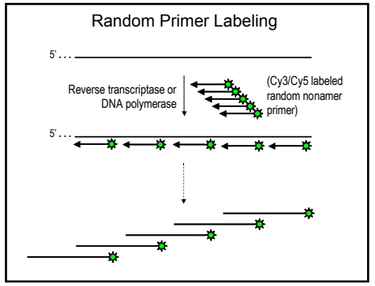
Introduction

There has been a recent resurgence in the interest in oligonucleotides containing sites with more than one base. These sites, known as either “random”, “wobble” or “degenerate” sites, have long been a synthetic target for oligonucleotide chemists. The need originated from early probe studies using synthetic oligonucleotides to hunt for the gene of a particular protein. Each amino acid is encoded by a trinucleotide codon, the third and sometimes the second base of which can often consist of several different nucleosides and still correctly encode for the amino acid desired. This is called a degenerate site in the genetic code. If the specific sequence for the gene is not known, then it is necessary to probe for all potential codons that result in the correct amino acid sequence. Therefore the probe must also have degenerate sites, or mixtures of all possible bases, in order to identify a gene that has the code for the protein of interest.

Another application for oligonucleotides with degenerate sites is the random amplification of a genetic target to generate a pool of all the available sequences. This random amplification is done with oligonucleotides containing a number of degenerate sites containing all four nucleosides. A number of commercial diagnostic assays were developed using oligonucleotides containing degenerate sites, or “randomers” for short. One such assay was designed by Incyte. They developed an assay that could detect a single base mismatch polymorphism using a two dye nonamer randomer probe system coupled with their microarray detection system. The sample and a WT control were both amplified using the randomers, but with different dyes. The labeled amplicons were analyzed with a microarray, yielding a plot of data. The polymorphism was detected if the fluorescence intensity from the two plots deviated from each other (3,4,5,6).



Other companies have also employed dye labeled randomers in their microarray assay systems (1). These assays may, for example, use dye labeled random primers to map differences in chromosomes. Such differences in the chromosomes can be detected, the results of which have diagnostic implications.

In another application of randomers the sequence is only random in select sites, reducing the number of compounds needed to determine the sequence. A common requirement of all these applications is that the oligonucleotides are as random as possible, and are as reproducible as possible from lot to lot.

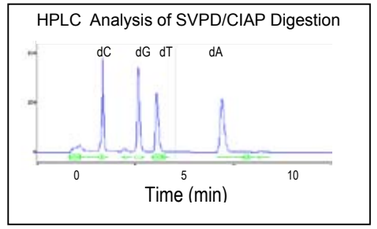
A Synthetic Challenge

The synthetic problems stem mainly from the fact that there are B^n sequences in the mixture, where B = the number of bases (4 if totally degenerate) and n = the number of sites that are random. Thus a nonamer randomer is actually 4^9 different sequences; 262,144 different individual oligonucleotides, nine bases in length. Many of the sequences will have similar properties during manufacturing. Some, however, like the homopolymers, will have vastly different characteristics.

The challenge lies in manufacturing a dye modified randomer oligonucleotide that is highly purified of shorter fragments, chemical modifications, and extra dye, while still maintaining a reproducible random mixture of sequences throughout the process which includes the step-wise chemical synthesis of the oligonucleotide, preliminary purification, followed by conjugation to the dye, repurification and then final processing. The randomness of the sequence is potentially changed at each step, requiring extreme care in order to manufacture a reproducible, high quality product.

Determining Base Composition

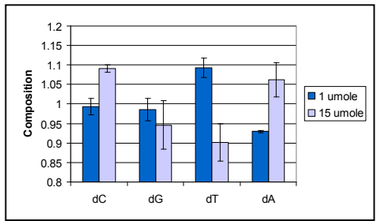
Determining base composition is accomplished by digesting the oligonucleotide using a combination of snake venom phosphodiesterase (SVPD) and alkaline phosphatase, yielding the free nucleosides which are quantified using RP-HPLC to yield the ratio of each base in the oligonucleotide (2).



The Synthesis Step

Although there is a subroutine on most commercial DNA synthesizers that will allow for the synthesis of degenerate sites consisting of mixes of 2, 3 or all 4 bases, you would be ill-advised to use it. Most machines apply pulses of each of the required nucleoside phosphoramidites to the column sequentially. Any deviation in flow rate between reagent bottles caused by restrictions can cause major changes in the resulting ratio of bases. It is much better to pre-mix the bases in the ratio you desire in a single bottle. Be sure to take into account the different molecular weights of each base.

Scale plays a bigger role in determining the ratio in instruments that use a single, gas driven flow through the column, like the Expedite 8909. In the case shown below, the same mix of reagents in the same machine gave considerably different ratios of each base at 1 and 15 µmole scales. (The ratios of the bases were not optimized to synthesize a 1:1:1:1 mixture in this experiment.)



Another consideration is the starting ratio of bases on the solid support. It is important that the mixture is as close to 1:1:1:1 as possible, and that the support itself is well mixed to ensure uniformity throughout the batch. The proper mixture of supports needed to prepare a perfect 1:1:1:1 ratio of bases is best determined using empirical data.

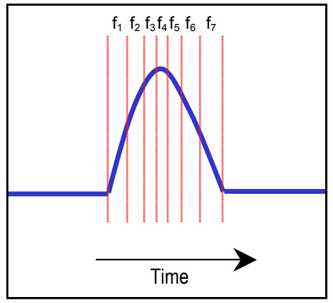
The Purification Step

A much larger potential impact on lot to lot variability can come from the purification step. Each oligonucleotide that is purified undergoes selection in order to remove impurities to the degree required to achieve the desired quality. The most common contaminants include shorter fragments from incomplete synthesis, oligonucleotides with some protecting groups still intact, and inadvertently modified oligonucleotides. These contaminants often elute close to the desired product, necessitating tight cuts in order to obtain high quality material. Unfortunately, in the case of randomers, which contain many different sequences, the elution profile of the desired product is much broader than a discreet oligonucleotide product. In fact, often the elution profile of the product overlaps that of the n-1 side-product elution profile making absolute removal of that contaminant impossible if complete integrity of the randomness of the desired product is to be retained.

To determine if the randomness of the mixture changes across an HPLC peak a nonamer randomer that originally yielded an overall base mixture close to 1:1:1:1 was fractionated by RP-HPLC as shown in the figure below.

The results show that in the beginning of the peak the guanosine nucleoside was enriched, while the back of the peak showed a lack of the sequences bearing guanosine. The adenosine, cytosine and thymidine ratios were essentially even throughout the fractions, equally sharing the change in the guanosine ratio through the elution profile.

It is very important to include the entire peak when purifying by HPLC. To do so, the synthesis must be very good to minimize side products and allow maximal recovery of product.



The Conjugation Step

The next step in which the ratio of bases can be inadvertently changed is during the conjugation step where a succinimidyl activated dye is attached to an amino labeled nonamer. We were concerned that the conjugation would skew the ratio of bases, especially if one or more bases are more reactive than the others.

We tested that concept by removing aliquots of oligonucleotide at various points of the conjugation reaction to see if the base composition of the resulting dye labeled product changed. We found very little difference in the base composition of the oligonucleotide as the efficiency of the reaction went from 20% to 100% completion, suggesting that there is no preference. However, the step immediately after conjugation is purification to remove the excess dye, which is another fractional purification as described above.

Summary

In order to prepare a successful randomer oligonucleotide, it is necessary to take the following steps:

1. Bring an assay in-house such as the described enzymatic digestion assay to test ratios.
2. Prepare your support by mixing it very well, and test random aliquots to assess both mixing and the base ratio. Adjust accordingly.
3. Prepare your amidite mixtures in one bottle and test the coupling of the prepared mixtures to ensure less than 10% overall variation in base ratio.
4. Ensure your DNA synthesizer is running very well to ensure high coupling efficiency.
5. Purify all of the full length material without cutting out the side fractions, hence the requirement for a near perfect synthesis.
6. Ensure a clean conjugation, purification, desalting process to remove excess dye and maximize yield.

A more simple solution would be to buy your randomer oligonucleotides from TriLink, of course. That way you can work on your next experiment, instead of your next oligonucleotide synthesis.

References

1. Proceedings of the National Academy of Science. (2006) 103: 4534.
2. Wiley, John and Sons. Current Protocols. Nucleic Acid Chemistry. (2000) 10.6.1-10.6.6.
3. The Journal of Neuroscience. (2004) 24: 2866.
4. Genome Biology. (2004) 5: R19.
5. Journal of Biology. (2006) 5: 3.
6. Nucleic Acids Research. (2001) 29: e41.

Quenchers

Great advances have been made in the area of quenchers over the last decade. The limitations of DABCYL and TAMRA for the development of multiplex systems quickly became apparent. These quenchers were not very efficient with the red-shifted dyes. Two different quenching systems were developed to overcome those limitations, the QSY® series and the Black Hole Quenchers (BHQ)®. Both offer specific quenchers for different emission ranges. The QSY quenchers are analogs of fluorescein.

There is now a specific quencher for everything from blue dyes such as Pacific Blue to the extremely red-shifted dyes such as Cy5.5. Although DABCYL has nearly become synonymous with the term “quencher” because of the early Molecular Beacon publications, one can now choose from more specialized quenchers - at little or no added cost. Table 1 will help you determine the appropriate quencher for your application. The more popular dye/quencher combinations are listed in Table 2.

The difference between QSY-7 and QSY-9 is water solubility of the conjugate and is of more concern to the chemist than to the end user. We do allow you to specify, however, if your experiments require one or the other.

Not only does TriLink offer each of these quenchers, we also offer a wide selection for placement on the oligonucleotide. These quenchers can be placed at the 3′ or 5′ terminus or internally at any deoxy base, which can be modified at the 5 position. There are some limitations based on the companion fluorescent dye chosen.

Figure 1: Quencher Structures

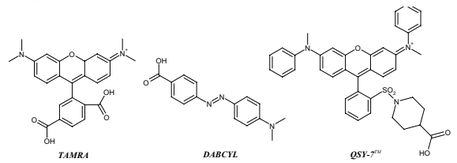


Table 1: Spectral Data of Quenchers

	Abs max	Ext. Coef.	Quenching Range
DABCYL	453 nm	32,000	380-530 nm
QSY-35	472 nm	23,500	410-500 nm
BHQ-1	534 nm	n/a	480-580 nm
QSY-7	560 nm	92,000	500-600 nm
QSY-9	562 nm	85,000	500-600 nm
BHQ-2	579 nm	n/a	550-650 nm
QSY-21	660 nm	89,000	590-720 nm
BHQ-3	672 nm	n/a	620-730 nm

Table 2: This dye selection chart provides a quick reference to the dyes we provide, spectral ranges and offers a starting point for quencher-dye pairing.

Dyes	Em_{max}	Abs_{max}	Quencher Guidelines*		
P130	376nm	340nm	BHQ-1 Abs_{max}: 534nm	QSY-35 Abs_{max}: 475nm	DABCYL Abs_{max}: 479nm
7-Methoxycoumarin	410nm	358nm			
Cascade Blue	410nm	396nm			
Alexa Fluor® 350	442nm	346nm			
7-Aminocoumarin-X	442nm	353nm			
Pacific Blue	451nm	416nm			
Marina Blue	459nm	362nm			
Dimethylaminocoumarin	468nm	376nm			
BODIPY 493/503	509nm	500nm			
DTAF	516nm	492nm			
6-FAM (Fluorescein)	516nm	496nm			
Dansyl-X	518nm	335nm			
Oregon Green 500	519nm	499nm			
Alexa Fluor® 488	519nm	495nm			
dT-FAM	520nm	492nm			
Oregon Green 488	521nm	495nm			
Rhodol Green	523nm	496nm			
Oregon Green 514	526nm	506nm			
Rhodamine Green-X	528nm	503nm			
NBD-X	535nm	466nm			
TET	536nm	521nm			
Alexa Fluor® 430	541nm	434nm		QSY-7 & QSY-9 Abs_{max}: 560nm	
2', 4', 5', 7'-Tetrabromosulfonefluorescein	544nm	529nm			
6-JOE	548nm	520nm			
BODIPY-530/550	551nm	534nm			
Alexa Fluor® 532	554nm	532nm			
HEX	556nm	535nm			
Carboxyrhodamine 6G	557nm	524nm	BHQ-2 Abs_{max}: 579nm		
Alexa Fluor® 555	565nm	555nm			
BODIPY 558/568	568nm	559nm			
BODIPY-564/570	570nm	564nm			
Cy3	570nm	550nm			
PyMPO	570nm	415nm			
Alexa Fluor® 546	573nm	556nm			
TAMRA-X/dT-TAMRA	576nm	546nm			
Rhodamine Red-X	580nm	560nm			
BODIPY-576/589	589nm	576nm			
BODIPY-581/591	591nm	581nm			
Alexa Fluor® 568	603nm	578nm			
Texas-Red-X	603nm	583nm			
Cy3.5	604nm	588nm			
Carboxy-X-Rhodamine (ROX)	605nm	580nm			
BODIPY-TR-X	616nm	588nm			
Alexa Fluor® 594	617nm	590nm			
Alexa Fluor® 633	647nm	632nm		QSY-21 Abs_{max}: 661nm	
Alexa Fluor® 647	665nm	650nm			
Cy5	670nm	649nm			
Carboxynaphthofluorescein	672nm	602nm			
Alexa Fluor® 660	690nm	663nm			
Cy5.5	694nm	675nm			
Alexa Fluor® 680	702nm	679nm			
Alexa Fluor® 700	723nm	702nm			
Alexa Fluor® 750	775nm	749nm			

***There is no guarantee a quencher will be effective in your construct. Quencher-dye efficiencies must be empirically determined in your experimental conditions.**

Pseudo-complementary Nucleic Acids

By Natasha Paul, Ph.D.; TriLink BioTechnologies

Pseudo-complementary (pc) nucleic acids contain base analogs that form weak base pairs with one another but form strong base pairs with standard bases(1,2). Consequently, pc-nucleic acids have diminished intramolecular and intermolecular secondary structures and can readily hybridize to unmodified nucleic-acids. The differential hybridization property of pc-nucleic acids is an attractive feature which can be utilized in microarray studies and in the targeting of duplex DNA.

One example of a pseudo-complementary base pair is the one between 2-aminoadenine (nA) and 2-thiothymine (sT) (Figure 1). This family of base analogs has been studied extensively in a number of polymer systems, which include DNA, RNA, and PNA(1,3,4,5). In comparison to the Watson-Crick base pair between adenine (A) and thymine (T), the pair between nA and sT is unstable because of the steric clash between the exocyclic amine of nA and the large size of the sulfur atom of sT. While the nA:sT base pair is unstable, the base pairing strength of the A:sT pair is similar to that of an A:T base pair, and the nA:T pair is more stable than A:T, presumably because three hydrogen bonds can now be formed between nA and T(6,7,8,9,10,11). This dynamic hybridization property of pc-nucleic acids is a desirable characteristic, which has found utility in a number of applications.

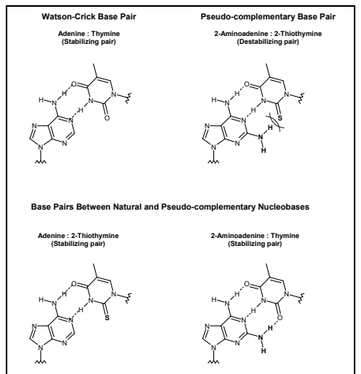


Figure 1: Proposed Base Pairs Between Natural and Pseudo-complementary Nucleobases. Differences between the pseudo-complementary and the corresponding natural nucleobases are represented in bold. Steric clashes within a base pair are represented by the dashed arcs.

Literature precedence has described how hybridization of a nucleic acid sequence to its corresponding complement can be hindered by significant secondary structure(12,13,14,15). Figure 2 depicts the advantage of utilizing pc-nucleic acids in hybridization studies, such as those which employ microarrays. As the degree of secondary structure should be significantly diminished for sequences composed of pc-nucleic acids, more representative hybridization to tiling microarrays is predicted. Advancements towards this ultimate goal have focused on incorporation of the corresponding pseudo-complementary nucleoside triphosphates during polymerase-mediated replication of the corresponding template. To accomplish this goal, the corresponding pseudo-complementary nucleoside triphosphate should be readily incorporated opposite its natural nucleobase with high fidelity. For example, in DNA polymerase replication, nA is selectively incorporated opposite thymine and sT is selectively incorporated opposite adenine, establishing the fidelity of recognition in DNA polymerase replication(16,17,18). Work with pc-nucleic acids has been recorded in a number of published manuscripts(3,16,17,18,19) as well as within a recent patent application filed by Sampson et al. from Agilent(20). Overall, encouraging results have been obtained, allowing for further advances towards more uniform nucleic acid hybridization.

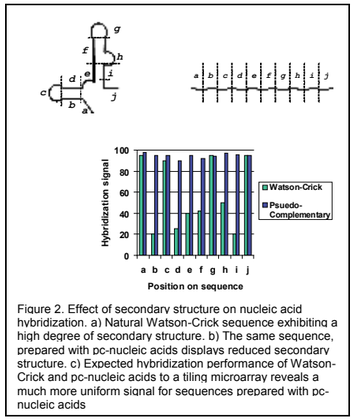
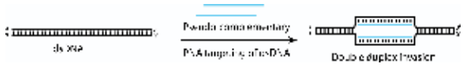


Figure 2. Effect of secondary structure on nucleic acid hybridization. a) Natural Watson-Crick sequence exhibiting a high degree of secondary structure. b) The same sequence, prepared with pc-nucleic acids displays reduced secondary structure. c) Expected hybridization performance of Watson-Crick and pc-nucleic acids to a tiling microarray reveals a much more uniform signal for sequences prepared with pc-nucleic acids

Peptide Nucleic Acids (PNAs) are noncharged polyamide sequences that form very stable duplexes with a complementary DNA strand(21). One interesting application of PNAs involves the use of pseudo-complementary PNAs to target duplex DNA (Figure 3)(4). The resultant complex is a stable double duplex invasion structure. If PNAs with the natural nucleobases attached were used, the duplex invasion would not be very efficient, as the dsDNA would remain intact and the two PNA strands would bind to one another with high affinity. The ability of pc-PNA to perform double duplex invasion has been utilized in a number of applications, which include inhibition of transcription initiation(4), modification of restriction endonuclease activity(22,23), and site-specific hydrolysis of duplex DNA(24).



Overall, the significant reduction of secondary structure in pc-nucleic acids is an attractive characteristic with a large number of potential applications ranging from nucleic acid detection and non-standard nucleic acid-based structure formation. As pseudo-complementary sequences form unstable sequences with one another and stronger complexes with their natural complement, there is a great potential for the development of novel applications that rely on the differential hybridization properties of nucleic acids.

TriLink is currently collaborating with Professor Howard Gamper of Thomas Jefferson University and was funded by a STTR grant to study pc-nucleic acids(25,26). Please contact us if you are interested in learning more about this exciting technology.

References

1. Biochemistry (1996) 35, 11170-11176.
2. Nucleic Acids Research (1996) 15, 2470-2475.
3. Biochemistry (2004) 43, 10224-10236.
4. Proceedings of the National Academy of Sciences (1999) 96 11804-11808.
5. RNA (2005) 11, 1441-1447.
6. FEBS Letters (1990) 274, 103-106.
7. Biochemistry (1983) 22, 597-600.
8. Biochemistry (1984) 23, 6723-6732 .
9. Nucleic Acids Research (1988) 16, 305-317.
10. Biochemical Biophysical Research Communications (1984) 118, 848-853.
11. Biochemistry (1996) 35, 11170-11176.
12. Journal of Molecular Biology (1987) 197, 349-362.
13. Journal of the American Chemical Society (2001) 123, 10805-10813.
14. Nucleic Acids Research (1998) 26, 4975-4982.
15. Biochemistry (1991) 30, 10606-10613.
16. Nucleic Acids Research (1995) 23, 885-892.
17. Nucleic Acids Research (2005) 33, 5640 - 5646.
18. Journal of the American Chemical Society (2006) 128, 396-397.
19. RNA (2005) 11, 1441-1447.
20. US Patent Application Number US2004/0086880 A1 (May 6, 2004).
21. Science (1991) 254, 1497-1500.
22. EMBO Reports (2002) 3, 956-961.
23. Nucleic Acids Research (2003) 31, 3929-3935.
24. Nucleic Acids Research (2004) 32, e153.
25. Nucleic Acids Research (2008) 36, 3409-3419.
26. Nucleic Acids Research (2008) 36, 6999-7008.

Understanding Oligonucleotide Synthetic Yields

By Richard Hogrefe, Ph.D.; TriLink BioTechnologies

Most oligonucleotides are ordered by the scale instead of the quantity, particularly the smaller scales of 15 µmole and lower, with no guarantee of final yield. This is because every oligonucleotide is a unique molecule, with unique synthetic properties based on sequence and modifications. The quality of a synthesis can be affected greatly by the sequence, such as G rich sequences. It is not easy to predict how much material will result from any particular synthesis. The price of the oligonucleotide is based on the amount of material and labor needed to prepare that synthesis based on scale. We can either guarantee only a small amount from any synthesis to ensure full delivery, or send the customer the entire resulting yield (which is what TriLink and most of our competitors do).

There are many factors other than sequence and the attached modifications that can affect yield as well, such as reagent quality and even weather. These topics will be discussed shortly, but first it may help to have a brief overview of how yield is measured and reported and how even that process can affect the final yield of a product.

Measuring Up

The yield on your certificate of analysis (COA) is reported in one of two ways. It will be reported either in units of mass or in optical density units measured at 260 nm (OD_{260}). The yields from all larger scale syntheses (100 µmole and above) are reported in units of mass, milligrams or grams. The yields of the small scale syntheses are conventionally reported in OD_{260} units.

All oligonucleotides, even those made at the multi-gram scale, are measured for yield using absorbance. Since most orders are placed at scales yielding 0.05-10 milligram of product, obtaining an accurate weight on routine syntheses is not possible. UV spectroscopy is a much more accurate way to measure the yield of oligonucleotides since it ignores salt, water and other residuals of the synthesis that would affect the weight. The oligonucleotide is dissolved in water or buffer and the absorbance at 260 nm determined. This absorbance number is reported as the yield on the COA as it is the most accurate representation of the yield. The conversion to mass uses an approximation, as will be discussed in the next section, and therefore is not an accurate representation.

Larger scales are generally ordered by mass, not yield, simply because it is the convention. The yields on the COA are likewise reported in units of mass for similar reasons.

Conversions

Although the absorbance is the most accurate representation of the yield, it is by itself not very useful. Almost all reactions are expressed in terms of moles of reagents required. The yield reported as OD_{260} units on the COA can be converted to µmoles using Beer's Law that relates absorbance to concentration using another figure found on TriLink's COA, the extinction coefficient (ε), which is a constant unique to every substance.

Beer's Law: Absorbance = [concentration] ε
which can be derived to: [concentration] = Absorbance / ε

The units of ε are (OD_{260} units)(mL) (µmole)$^{-1}$, the units of concentration are (µmole)(mL)$^{-1}$, and absorbance is expressed in OD_{260} units. Since the absorbance readings we report are already normalized to 1 mL, the final calculation yields the number of µmoles of product directly, with no further math required.

For example, a reported yield of 100 OD_{260} units for a product with an ε of 175 (OD_{260} units)(mL) (µmole)$^{-1}$ calculates to a yield of 0.571 µmole (100/175).

Unfortunately, this very convenient calculation is premised on a number that is itself obtained by calculation, and sometimes with some guesswork added in. The ε is calculated for each and every oligonucleotide sold by TriLink. Different formulations exist for this calculation, the accuracy of each increases as more of the complexities of the system are taken into account.

The most accurate method is to generate the ε experimentally, but this is a very long and difficult process. TriLink does the next best thing in using what is called the nearest neighbor model. We take into account the affect on the absorbance of each nucleoside by the neighboring nucleoside, which can be considerable. However, even this fairly complicated calculation is only accurate to within 10%.

The guesswork occurs when some modified nucleosides and other compounds are added to an oligonucleotide. We can only use an educated guess as to what will be the affect of the modification on absorbance. We have determined the ε of some dyes we sell experimentally, but those were on unique oligonucleotides that may or may not extrapolate well across the spectrum of sequences.

Therefore, despite using a more complicated formula than many vendors, the ε offered on our COA is still only a reasonable approximation of the true figure. Be prepared to vary the concentration of oligonucleotide reagents by as much as 10% in order to obtain perfect results.

If you wish to determine the mass of the oligonucleotide, multiply the number of moles by the molecular weight (found on the COA in grams per mole, or perhaps more usefully, µg per µmole). This number is calculated for the free acid. Be sure to account for the salt form if you intend to actually weigh the samples. This is not reasonable for scales of 1 µmole and less, as the amount delivered makes it impractical.

What affects the yield of your oligonucleotide?
Synthesis

The amount of product theoretically possible from any particular synthesis is determined by the quality of the synthesis itself, generally done on an automated synthesizer. The coupling efficiency of the synthesis is very important. At TriLink we do our best to optimize this efficiency using modified programming and paying special attention to reagent preparation. The results of a drop in coupling efficiency of just a few percentage points on the yield can be devastating. This is easily demonstrated by calculating the theoretical yield with the following formula:

$$x^y$$

where x is the average coupling efficiency and y the number of couplings.

For example, a synthesis of a 30mer (which requires 29 couplings) with an average coupling efficiency of 99% theoretically yields 75% of product (0.99^{29}). That same synthesis at 98% efficiency will have a maximum yield of only 55%. That one percent costs nearly half the material. Consider the difficulty of making a 70mer. Even at 99%, the best one could hope for is 50% yield. At 98% it becomes an abysmal 25% yield.

It is very difficult to maintain every machine every day at an operational efficiency of 99% or greater, despite claims to the contrary. Not only does the instrument have to be finely tuned to operate at 99%+, but other factors have to be perfect, such as the moisture content in the acetonitrile and phosphoramidite quality. The weather plays a role, as well. Extremely humid days will adversely affect the quality of synthesis by making near complete water removal almost impossible, despite using rigorous anhydrous chemistry techniques.

Modified reagents often have poor coupling efficiencies for a variety of reasons. It is not unusual for a reagent to have a coupling efficiency as low as 90%.

Some modified oligonucleotides are prepared by conjugation to an amine, thiol or carboxyl functionality. This reaction is affected by many factors as well, including the quality and age of reagent, the sequence of oligonucleotide and the quality of the buffers. Reactions can range from 10% to nearly quantitative. A mediocre conjugation can have a considerable affect on final product yield. At TriLink we do our best to control the quality of all reagents through our ISO/GMP program, as well as operate with well established SOPs and well trained personnel. Despite our best efforts, some reactions will still go poorly time to time due to sequence effects.

Deprotection

After synthesis, the oligonucleotide is deprotected with base. With simple oligonucleotides this presents little difficulty, as long as the deprotecting reagents are fresh. However particular modifications require deprotecting conditions so mild that the bases are not fully deprotected. In most cases, some damage occurs to the bases themselves, but particularly to dye labeled compounds. These side reactions all result in loss of yield.

Purification

Depending on the quality of the synthesis, purification can be the step where the most yield is lost. A high quality synthesis will have only a moderate amount of impurities to remove, allowing a larger cut of the product peak. Moderate and poor syntheses will have more contaminating fragments that will crowd into the product peak, requiring a tighter cut to obtain an acceptable purity. Regardless of the quality of the synthesis, the overall process of purification is costly to yield. Upwards of 50% of the theoretical yield will be consumed in many preps for a wide variety of reasons.

Oligonucleotides that are partially deprotected and those containing degraded or damaged dyes often share enough properties with the product to make purification difficult. In some cases, the modifications on an oligonucleotide will cause the product to co-elute with shorter fragments, which is the case with unprotected primary amine modified oligonucleotides.

The requested purity makes a considerable impact on yield. The difference in delivered product between a final purity of 90% and 95% can be several fold.

What should I expect from my 1 µmole scale modified oligonucleotide order?

Here is a hypothetical synthesis to illustrate how yield is affected. We will return to the example of the 30mer described above, but add the following: it has a 5' dye and a 3' quencher. This hypothetical dye is only available as a succinimidyl ester, therefore requiring post-synthesis conjugation to a 5' amino labeled oligonucleotide. The 3' quencher in this case is a support bound reagent from which the synthesis begins, requiring no further chemistry. The oligonucleotide is further modified with three modified bases, each of which couple at 93% in this example. The amino linker couples with an efficiency of 95%.

The theoretical maximum synthetic yield based on an average of 99% for the other 26 couplings is calculated thusly:

$$(0.99^{26})(0.93^{3})(0.95) = 0.59$$

The deprotection of the oligonucleotide always results in a small amount of undeprotected bases, undesired modifications, and in this case, some degradation of the quencher. It also involves transfers and filtrations that invariably result in most of the loss. Overall a loss of another 25% of potential product due to chemical modifications, incomplete deprotections and manual manipulation is not unusual. Our yield is now at 44%, or 0.44 µmole.

The protecting group on the amino group is left on to improve the purification of the amino labeled intermediate prior to conjugation. Without it, the amino labeled oligonucleotide will elute with the failure sequences on HPLC. It is common to lose 10% of this group prior to purification during deprotection and work-up, lowering our yield to 0.40 µmole.

This product is purified, resulting in another loss of at least 25% of the product due to contamination with the aforementioned undesired modified materials. We are now at 0.30 µmole of intermediate.

The conjugation of a dye generally goes at 70-90% unless there is a sequence or reagent issue. Let us assume an efficiency of 80%. The yield is now at 0.24 µmole.

This material is now re-purified to remove unconjugated material, free dye, and any side products due to damaged dyes, resulting in a further loss of 20% of the product, reducing the product to 0.19 µmole.

This is followed by a series of manipulations to desalt the sample, convert it to the proper salt and precipitate it, and then remove aliquots for final analysis prior to delivery. Unfortunately, this series of steps is more costly than appreciated, resulting in an overall loss of another 20% of the product, reducing the final yield to 0.15 µmoles.

Given a 30mer with an ε of 345 (OD_{260} units)(mL) (µmole)$^{-1}$ and a molecular weight of 10,500 g/m, or 10.5 mg/µmole, this final yield would correspond to a delivery of 52 OD_{260} units, 1.6 mg, of product.

We would consider this an excellent yield for a highly modified compound like this. Yields of 20-40 OD_{260} units for compounds of this sort are much more common. The losses described can easily be compounded by many different factors, some completely out of our control (mostly construct/sequence issues) and some within our control (level of training, routine maintenance). A compound with high guanosine content can cause the loss of at least half of the material if it has secondary structure that makes purification difficult. Also, recall that a day with higher than usual humidity can result in the loss of a third of the product right from the onset of the synthesis by dropping coupling efficiency just one percentage point.

Why is my yield different this time although the order is exactly the same?

We understand how annoying it must be for a customer to obtain different quantities of material for subsequent batches of the exact same oligonucleotide. Much of the answer is in the preceding paragraphs. Subtle changes in one aspect of the synthesis can have grave consequences on the yield.Also, many of our reagents have a single source, upon whom we are dependent for high quality material. If that falters we must make do with what we can obtain.

When should I request a set quantity?

Almost all mid-scale syntheses (50 mg and larger) are ordered by set quantity, usually in units of mass. Some customers order 10 mg samples, depending on us to decide on the proper starting scale.

There are cases when ordering a set quantity for smaller amounts may make sense. Many diagnostic firms require guaranteed amounts of material delivered so that they can produce the allotted number of kits, even at small quantities. This will increase your cost considerably in some cases since we may have to prepare a larger scale to ensure the yield you requested. It is best to order by the scale and trust us to do the best possible under the current circumstances.

A Table of General Expectations

For a general guideline, see tables of expected yields on pages 17 and 18.

Thermostability of Modified Oligonucleotide Duplexes

By Richard Hogrefe, Ph.D. and Alexandre Lebedev, Ph.D.; TriLink BioTechnologies

We are often asked about the relative thermostabilities of the duplexes of DNA and RNA with various backbone and sugar modified oligonucleotides. The simple answer is shown in the following set of duplexes listed by increasing thermostability (for oligonucleotides with the same sequence). The thermostability of the duplexes is characterized by melting temperature (Tm) at which half of the DNA or RNA molecules are in a double stranded form, while the other half of the molecules are represented by single strand form.

Least Stable >> Increase of Tm >> Most Stable
DNA:DNA < DNA:RNA < RNA:RNA < RNA:2'OMe RNA

The A-form of a duplex is characterized by 3'-endo conformation of the sugar (1). It is normally formed by two RNA molecules and is more stable than the B-form of DNA:DNA duplex which is characterized by 2'-endo conformation of sugar (1). The DNA:RNA hybrid duplexes have a conformation with both A-form and B-form characteristics with a predominance of A-form features (2). For the duplexes of DNA with DNA-RNA-DNA chimeras, the extent of A-form characteristics depends on the composition and ratio of deoxyribo- and ribonucleotides in the duplex. (A RNA stretch as few as three ribonucleotides possesses A-form conformational features sufficient for recognition and cleavage by RNase H of a DNA/RNA/DNA chimera hybridized to a DNA target (3).)

The commonly available sugar modified oligonucleotides are 2'-OMe, 2'-fluoro and 2'-amino derivatives. The presence of 2'-OMe nucleosides in DNA or RNA strands enhances thermostability of the duplexes. Modification of the 2'-deoxyribose with 2'-fluoro enhances the thermostability of the DNA-DNA duplexes by 1.3° C per insertion (4), while the 2'-amino modification destabilizes duplexes (5). A combination of 2'- fluoro and 2' OMe nucleosides will enhance the thermostability of a duplex considerably, without resorting to more expensive nucleosides with modified bases.

Phosphorothioate oligonucleotides are commonly used in target validation or antisense experiments (6). Due to the chirality of the internucleotide phosphorothioate phosphorus (Sp and Rp), standard phosphorothioate oligonucleotides are chemically synthesized as a mixture of 2^n diastereomers (where n is the number of internucleotide phosphorothioate linkages). Overall, the combination of the Sp and Rp chiral forms in the phosphorothioate oligonucleotide destabilize DNA:RNA and DNA:DNA duplexes. For example, a complex of a 15mer phosphorothioate oligonucleotide and a control phosphodiester of the same sequence with a complementary 15mer oligonucleotide RNA had Tm's of 33.9° C and 45.1° C respectively, at described conditions. Due to the stabilization effect of the 2'-OMe group, the difference between Tm is reduced considerably for 2' OMe-RNA phosphorothioate oligonucleotides. For the duplex with DNA the control 2'-OMe-RNA strand gave a Tm of 62.8° C, whereas the 2'-OMe phosphorothioate analog gave a Tm of 57.7° C (4).

In several publications individual stereo defined diastereomers of phosphorothioate oligonucleotides were synthesized and investigated (6). Chirally pure Sp-phosphorothioate oligonucleotides have been shown to enhance the thermal stability of the duplexes with DNA and RNA compared to Rp diastereomers or mixed Sp and Rp phosphorothioates (6). However, the chirally pure phosphorothioate oligonucleotides are very expensive to make. So far mixed Sp/Rp phosphorothioate oligonucleotides, especially 2' OMe analogs, are adequate for most applications and do not affect the thermostability adversely as to warrant the added expense required for the synthesis of chirally pure phosphorothioate oligonucleotides.

Another common backbone modification offered by TriLink is oligonucleotides containing neutral methylphosphonate linkages. These oligonucleotide analogs are also prepared as a mixture of Sp and Rp diastereomers (2^n diastereomers, where n is the number of methylphosphonate internucleotide linkages). The Sp/Rp mixed version of methylphosphonate oligonucleotides, which is all that is readily available, produce complexes with complementary DNAs and RNAs. However, the thermostability of these complexes is significantly lower than similar phosphodester DNA-DNA and DNA-RNA duplexes. The destabilizing effect of methylphosphonate oligonucleotides on the complexes with DNA or RNA is stronger than phosphorothioate oligonucleotides.

A chirally pure Rp methylphosphonate oligonucleotide analog, on the other hand only slightly destabilizes the complex with an RNA target; while the stability of the complex with a DNA target is greatly enhanced (8) compared to phosphodiester oligonucleotide. For instance, with RNA target, an Rp/Sp-mixed all-methylphosphonate oligonucleotide 15mer had a Tm of 34.3° C, whereas the phosphodiester control had a Tm of 60.8° C. An oligonucleotide with alternating Sp/Rp-mixed methylphosphonate and phosphodiester linkages had a Tm of 40.6° C, while the Rp chirally pure version of the same oligonucleotide had a Tm of 55.1° C, just slightly less than the control hybrid (7). Unfortunately, the chirally pure methylphosphonates are not available commercially, and can only be obtained through Genta, Inc. TriLink is capable of synthesizing these molecules with Genta's explicit written permission on a case by case basis.

References

1. W. Saenger, Principles of Nucleic Acid Structure, C.R. Cantor, Ed., Springer Advanced Texts in Chemistry (Springer-Verlag, New York, 1984).
2. Lesnik, et al. (1993) Biochemistry 32, 7832-7838.
3. Hogrefe, et al. (1990) J. Biol. Chem. 265, 5561-5566.
4. Kawasaki, et al. (1993) J. Med Chem. 36, 831-841.
5. Aurup, et al. (1994) Nucleic Acids Res. 22, 20-24.
6. Stec, et al. (1994) Angew. Chem. Int. Ed. Engl. 33, 709-722.
7. Reynolds, et al. (1996) Nucl. Acids Res. 24, 4584-4591.
8. Vyazovkina, E.V., Savchenko, E.V., Lokhov, S.G., Engels, J.W., Wickstrom, E., and Lebedev, A.V. (1994) Nucl.Acids Res. 22, 2401-2405.

When is my Oligonucleotide Pure Enough?

By Richard Hogrefe, Ph.D.; TriLink BioTechnologies

This is a very common question we receive from our clients. How do you know what purity is needed and what to ask for? The answer is predicated on many factors and can be complicated. The decision will vary depending on whether it is a research scale or a commercial scale synthesis; if it is a novel compound or one we made several times already; how highly modified it is; what is its application; and so on. In the end, your TriLink Product Specialist is your best way to get the answers you need for your specific problem. However, there are several guidelines we can offer that will help you make some general decisions before you place a call for advice.

How is Purity Defined?

Usually, the percent purity seen on most Certificate of Analyses (COA) for an oligonucleotide is the result of the HPLC analysis of the final product. This number can be erroneous for many reasons. For example, contaminants probably exist that do not separate from the product peak. Reverse phase (RP-) HPLC can often be very poor at separating n-1, n-2 and even shorter fragments from the main product peak. Also, there are many other contaminants that can be in a product sample that do not absorb light at 260 nm, such as excess salts and purification matrix (especially in PAGE purified samples). It is very hard to determine the amount of salt in sub-milligram samples.

A sample that is 90% pure by RP-HPLC analysis observed at a wavelength of 260 nm may only be 80% pure by anion exchange (AX-) HPLC at the same wavelength. Furthermore, the same compound could be only 70% pure if free dye is measured at an alternative wavelength, or only 60% pure by mass if excess salt is taken into account. Generally, the analytical method chosen gives the purity based on the factors important to the application. Therefore, purity is defined by the method(s) of analysis, which is determined by the application.

Common Oligonucleotide Analytical Methods

Polyacrylamide Gel Electrophoresis (PAGE)

PAGE separates oligonucleotides on the basis of mass/charge ratios by passing an electrical charge across a gel matrix. The negatively charged oligonucleotides are drawn through the matrix. A common method of detection is UV backshadowing, where a UV lamp is shined on the gel which is on a fluorescent background. The oligonucleotides absorb the UV light, leaving a shadow on the background. Denaturing PAGE (the gel has a high urea content to force any double strands to separate into single strands during the analysis) is the most common analytical tool for the analysis of oligonucleotides.

PAGE is good for qualitatively examining a product. It is very effective for separating oligonucleotides based on length. However, it is a very poor quantitative tool and has a fairly narrow linear range. Better quantitation can be obtained if the oligonucleotides are radiolabeled prior to analysis, but this is costly and time consuming for commercial production.

PAGE analysis is inexpensive and very adequate for showing the basic quality of an oligonucleotide. In the case of basic primers and research compounds it is more than adequate as the sole final QC analysis for all but the most demanding applications. Every oligonucleotide sold at TriLink is analyzed by PAGE at no additional charge.

Spectral Analysis

Every oligonucleotide is quantitated by measuring the absorbance of a solution of the sample at 260 nm. Spectral analysis of oligonucleotides in both the UV and visible ranges can also be very useful. We use the ratio of the absorbance of the oligonucleotide at 260 nm and the absorbance of a dye at its absorbance maximum to determine if any free dye remains in the sample. This is a very important assay and part of the final analysis performed by TriLink on every dye-labeled oligonucleotide as a free service.

High Performance Liquid Chromatography (HPLC)

Due to its versatility, HPLC has long been the most commonly used analytical tool for determining the purity of many classes of compounds. There are also many different classes of HPLC. The two most commonly used in oligonucleotide chemistry are reverse phase (RP), which separates on the basis of mass/lipophilicity ratio, and anion exchange (AX), which separates on the basis of anionic mass/charge ratio.

A RP matrix generally consists of a silica, glass or polymer particle coated covalently with a long chain hydrocarbon, the most common being octadecyl, or C-18. An AX matrix has a coating of cationic molecules, most commonly a form of diethylaminoethyl (DEAE) or a quarternary amine.

The most widely used and least expensive matrix available to oligonucleotide chemists historically was RP, and still is. AX chromatography was very difficult to perform in the early days because of manufacturing problems and reproducibility issues. On the other hand, RP matrices have over 50 years of commercial development behind them.

However, RP-HPLC has many problems, the most glaring being its inability to separate oligonucleotides of similar length, longer than approximately a 12mer (Figure 1). Sequence alone can greatly affect the retention of an oligonucleotide, to the extent that in a few cases a shorter fragment will retain longer than the full length product. For example, a product that contains a primary amino functionality for conjugation purposes will often elute along with the failure sequences because the amine is polar enough to change the lipophilicity of the oligonucleotide and overcome the mass difference.

AX-HPLC is very good at separating shorter fragments from the full length product (Figure 2, as compared to Figure 1). In more recent years it has become more reproducible.

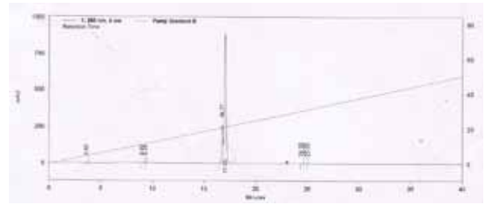


Figure 1: RP-HPLC analysis of a mixture of 6 polythymidine oligonucleotides: T12mer, T18mer - T22mer.

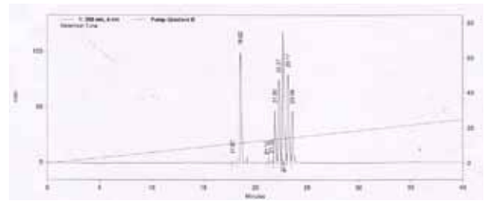


Figure 2: AX-HPLC analysis of a mixture of 6 polythymidine oligonucleotides: T12mer, T18mer - T22mer.

Analytical columns are much more expensive and do not separate some chemical modifications, such as dyes, as well as RP.

It should be kept in mind that it is difficult to predict with certainty the ability of any method to separate the contaminants of interest without synthesizing the suspected contaminants as analytical references. This is commonly done for the development of HPLC analytical methods for the analysis of pharmaceutical grade oligonucleotides. Therefore it must be understood that the purity number obtained from HPLC, especially when it is the only analytical method used, is only an approximation of the purity.

Which method is best, AX or RP-HPLC? That is difficult to answer definitively. It was already pointed out that the two methods are better at separating different types of contaminants. Having an analysis of your compound by both AX and RP-HPLC is better than either one alone; however, in general, analysis by AX is preferable. We often see more contaminants using this method. All syntheses of 50 mgs or greater are analyzed by AX-HPLC free of charge, as are many of our small scale modified compounds.

One significant problem that plagues HPLC is that it is not effective at separating fragments longer than 50-60 bases in length, and that is in the absolute best cases with AX. RP is much less effective for the separation of standard unmodified oligonucleotides, and without careful methods development must be considered unusable for anything over ten to twelve bases in length.

Mass Spectroscopy (MS)

Recently, this assay has become routinely available from oligonucleotide firms, including TriLink. MS analysis has become a great tool for the analysis of products and side-products. Determining the mass of a compound is an excellent way to help verify its identity and to determine unknown contaminants. However, this method also has limitations, some of them rather significant.

For instance, having a found mass identical to that calculated does not confirm a structure. For example, an oligonucleotide with the sequence ACTCGCTTGACAGAGT is a very innocuous looking 16mer that has the same mass as the self-annealing sequence CCCCAAAATTTTGGGG, which has vastly different properties. In this case PAGE will easily differentiate between the two.

There are two MS methods typically used for the analysis of oligonucleotides, electrospray (ES) and MALDI. ES is very accurate, usually giving masses within 1 or 2 amu of the calculated figure. However, it is easily affected by the quality of the sample, particularly excess salts, necessitating stringent desalting and handling of the samples. MALDI is much more tolerant of sample quality, particularly salt content, but is easily thrown out of calibration. Further, the level of error can be large, hiding many modifications. In longer oligonucleotides the error can be as much as a whole nucleotide.

We use ES unless MALDI is requested, or in special circumstances where ES will not work.

Special Assays

There are many assays available to the oligonucleotide analytical chemist. In most cases their value is nowhere near their cost in time, money and material. Most are only needed for clinical samples or for oligonucleotides being used in special applications. In fact, the basic assays listed in the above sections are all that are needed for all GMP diagnostic and pre-clinical therapeutic syntheses, with perhaps the addition of an endotoxin assay in the latter case. Specifically, these assays include the aforementioned endotoxin assay, bioburden assay, capillary electrophoresis, base composition sequence analysis, hybrid melting temperature, water content, elemental analysis, NMR and metal content by atomic absorption. The costs of some of these assays are very high for what they return.

The endotoxin and bioburden assays are useful for late stage pre-clinical samples, particularly for material going into expensive mammals such as pigs, dogs or primates. The rest should only be considered if your regulatory department or the FDA requests them or if needed in your unique situation due to the composition of the compound, in which case most likely TriLink will suggest it in the course of developing specifications for the product.

An Application Driven Decision

Although it may be obvious that purity is determined by the application, the purity required by each application is not obvious. For instance, you may expect that a compound being injected into humans has a much higher purity requirement than a primer sold in a kit. That is not true in all cases. For instance, some assays require absolutely no contamination with particular sequences, especially target sequences, while a therapeutic drug can tolerate a small percentage of other sequences, as long as the individual components are positively identified and are reproducible from lot to lot.

Purity requirements also normally change as the product progresses toward commercialization. On the other hand, some assays require high quality material at the beginning just to allow development, with the introduction of robustness to the assay coming later in the process. This is often true in the development of very exacting microarray assays using dye labeled oligonucleotides. Since every situation is unique, the best we can do is offer basic guidelines for the following general classes of oligonucleotides ordered from TriLink.

1. General biological research grade, non-diagnostic primers:

Many of the compounds ordered world wide on a daily basis fall into this category. These consist of the oligonucleotides used for basic biological research, such as primers used for simple amplification or extension experiments. These compounds are often pure enough as crude desalts. In most cases a purity of 70% is more than sufficient. The impurities should be spread out fairly evenly among the possible shorter fragments. All oligonucleotides should be desalted to less than 20% salt by weight by either cartridge or precipitation at the least to remove excess salts that may interfere with enzymes.

Assays to request and results to expect:

PAGE is more than sufficient for these compounds. Make sure that the product bands are strong and that the contaminants are faint if observable at all. There should not be a large n-1 band; this is often indicative of other problems.

2. General biological research grade probes and modified oligonucleotides:

These compounds are generally only prepared once or twice at most with any particular sequence. Purity is often limited by the number and type of modifications. We attempt to achieve as close to 90% as possible, although sometimes we cannot achieve greater than 70% -80%.

For most applications in general research that use modified oligonucleotides, such as studying the physical properties of modified oligonucleotides or how they affect a biological system at a cellular level, 80% will often be more than adequate. Once again, we attempt to achieve at least 90% purity on every compound. However, there are some applications where higher purity may be necessary, usually in respect to a specific contaminant. For example, any free dye present in a labeled oligonucleotide sample can wreak havoc with most assays. Occasionally, there are some combinations of sequence, modifications and dye that do not allow for good purification.

Assays to request and results to expect:

In many cases PAGE is sufficient. If the material is purified, the band should be sharp and the contamination very minimal. With some modifications and some sequences it is very hard to obtain products that are single bands on PAGE, however it should be very close to a single band, or an explanation should be accompanying the COA detailing why the data appears as it does.

For highly modified compounds, or for orders of 50 mg or more, a greater number of assays are used to ascertain the quality of the final compound. The suite still includes PAGE, since it does quickly show us problems that other methods do not. We also analyze the compound by AX-HPLC. We will include an analysis by RP-HPLC if needed. Both PAGE and HPLC analyses should show strong product bands or peaks with the contaminations each no more than a few percent of the overall mixture.

All dye labeled compounds are analyzed by UV/Visible spectroscopy to ensure that the dye still has the desired spectral qualities and did not undergo chemical degradation during synthesis. We also check that the oligonucleotide/dye ratio is correct for the number of dyes attached to the oligonucleotide. This is an approximation based on calculated extinction coefficients; however, repeat syntheses are compared to previous lots to ensure reproducibility. This data is hard to interpret and should be discussed with a TriLink representative if you intend to use this as a specification for future releases of a product.

Mass spectroscopy is also run on many small scale oligonucleotides and routinely on all 50 mg or larger orders free of charge. It is considered more of a qualitative, as opposed to a quantitative, method. It is also extremely valuable for process control, giving us an excellent tool to identify the appropriate product in a complex mixture.

3. Commercial diagnostic grade primers and probes:

The specifications for these products should be set no higher than what is needed for the assay to work routinely. The reason is that you want the assay to be robust, but at the lowest price possible. In this case, 80-90% is also usually good enough with the same careful attention given to the removal of free dyes or other interfering contaminants. Some specific applications may require higher purity, 95% or greater. This is actually quite rare. It must be understood that yield will be greatly affected as higher purity is desired. Some contaminants, such as free dye, must be removed as completely as possible.

Some sequences and/or modifications do not allow high purity. We are often limited by impure dyes in the manufacturing of fluorescent oligonucleotides.

Assays to request and results to expect:

All the assays discussed in the preceding section on general biological research grade products apply for this category of products. The only consideration here is that assays that may have been optional previously are now mandatory. Each relevant assay must be run. The desired results are set by the product specifications, which were in turn set by the validation runs, which establish the capabilities and reproducibility of the synthetic protocol. Of course, all specifications are subordinated to the product's required performance in the assay.

There are times when additional analysis is required. We do have the capabilities of testing the randomness of oligonucleotides bearing degenerate positions using a base composition assay. This special service requires enzymatic degradation of the oligonucleotide. Most of the other special assays are unnecessary for diagnostic products.

4. Therapeutic oligonucleotides:

The purity requirements for a therapeutic candidate are quite unique in many ways. This is one case where it is definitely not in your best interest to obtain as high a quality as possible at all times. In fact, we recommend a sort of concave profile for your product's purity as it runs its course through research and into the clinic.

At the very early stages of research you want to be sure to be working with as high a quality of material as possible. Much time has been wasted as a result of poor quality syntheses leading to cytotoxicity. Therefore, you want a much higher purity than is normally available from most small scale providers. At TriLink we specialize in the synthesis of these types of compounds and understand how to reduce unwanted cytotoxicity.

Once a target molecule is prepared and is going to be scaled up for animal studies, a more complicated strategy must evolve regarding the purity of the product. The main consideration is that it is critical that the drug stays as pure, or better yet, gets increasingly purer during each stage of the development of the commercial manufacturing protocol. It is important to not set extremely high purity specifications when the drug is being prepared at a relatively small scale for use in pre-clinical animal studies and even early phase clinical trials. If that purity is not met when the compound is scaled up for later clinical phases, you may have to repeat all your earlier work with the newer material – a very costly mistake.

A good rule of thumb is to use 80-85% pure material for pre-clinical animal studies, 85-90% for Phase I toxicity studies and 90-95% for Phase II/III studies.

It is actually more critical to remove small molecule contaminants from the beginning of the program, such as organics and salts, than it is to have an extremely clean 260 nm profile on HPLC. We always use care to remove salts and other contaminants from oligonucleotides that are to be used in cellular or animal experiments. It is not possible to assay for some of the small molecule contaminants using standard assays, and the ones required are usually impractical during early stage research, but our expertise in this area is sufficient for you to trust our products for all early stage pharmaceutical research.

Assays to request and results to expect:

Again, all of the assays applicable described for use with research grade oligonucleotides will be used for these oligonucleotides. In your early research the normal high quality you have come to expect from TriLink will suffice for your research compounds. As your program progresses, the number and complexity of the assays automatically ratchet up as well. As you get closer to a Phase I clinical study your regulatory department will be exploring the specifications of the oligonucleotide and the assays required to file an IND. Assays that test the bioburden and endotoxin contamination become necessary for the batches made for the final pre-clinical higher order animal toxicity studies. Sequence analysis becomes critical, using either classical or newer MS methodology.

The more esoteric assays, such as metal content, etc., may or may not be required, depending on the viewpoint of your regulatory staff and their interactions with the FDA. In our experience, the FDA will not require much beyond that described above if the manufacturing protocols being followed are considered standard.

TriLink's alliance with Avecia, the leading manufacturer of oligonucleotide drugs for the clinic, is a wonderful resource for you at this stage. Avecia will take an active role in helping with assay development as soon as you are ready to discuss taking your program to the next level. Since our methods are harmonized, it will be easy for you to transition to clinical scale manufacturing.

Summary

Hopefully you found some useful hints in this article that will help you make decisions regarding the purity of your oligonucleotide. Please ask your TriLink representative for more guidance if needed. We are happy to help.

An Introduction to Extinction Coefficients and Molecular Weights of Oligonucleotides

By Judy Ngo and Shawna Oliva; TriLink BioTechnologies

Every TriLink Certificate of Analysis lists two numbers crucial to the use of an oligonucleotide: the extinction coefficient and the molecular weight. These numbers are needed to calculate important data, such as determining molar concentrations and preparing stock solutions. This paper will offer an explanation of the extinction coefficients and molecular weights of oligonucleotides, discuss the means by which the numbers are derived, and give practical examples on how to use the data provided by TriLink.

The optical density unit, or more commonly the OD_{260} unit, is a spectrophotometric measurement of an oligonucleotide. Each of the bases in a nucleic acid strand has an absorbance at or near 260 nanometers, due to their conjugated double bond systems. Because the exact base sequence and composition is known, the OD_{260} unit is a very accurate and convenient method to quantify an oligonucleotide. The OD_{260} unit is a normalized unit of measurement that is defined as the amount of oligonucleotide required to give an absorbance reading of 1.0 at 260 nanometers in 1.0 milliliter of solution using a 1.0 centimeter light path. Utilizing absorbance measurements is the recommended method for quantitating or aliquoting an oligonucleotide. Often, the total mass of oligonucleotide is too low to accurately weigh on a balance. In addition, spectrophotometric measurements will be accurate regardless of any excess salt present in the sample. An absorbance reading will also ensure that a compound is fully solubilized prior to use.

Associating the OD_{260} unit with the amount of oligo present is done mathematically using a physical constant known as the extinction coefficient. It is a component of Beer's Law: $A = \varepsilon Cl$; where A is absorbance, C is concentration, l is the path length, and ε is the extinction coefficient, a constant for the material being analyzed. The extinction coefficient takes into account the effects of the neighboring bases as well as the absorbance of each of the individual bases. Because it is dependent on the exact nucleotide composition and sequence, the extinction coefficient is unique to every oligonucleotide. There are several ways to determine the extinction coefficient of an oligonucleotide. The "nearest neighbor" method gives greater accuracy than the more common method of merely adding the individual bases and multiplying by the individual extinction coefficients. TriLink uses this "nearest neighbor" model to calculate the extinction coefficient of each oligonucleotide. The next page shows the formula for calculating the extinction coefficient as well as the established constants for both the DNA and RNA nucleotides and dimer pairs. TriLink's website conveniently offers a spreadsheet that automatically calculates the extinction coefficient of an oligonucleotide.

The extinction coefficient is listed on TriLink's Certificate of Analysis and is expressed in ODs/µmole. These units are mathematically derived and equivalent to the standard equation units, L $mmole^{-1}$ cm^{-1}. Please note that although TriLink uses the relatively accurate "nearest neighbor" model to calculate the extinction coefficient, it is still just an approximation and may be off by as much as 10%.

Another important piece of data found on TriLink's Certificate of Analysis is the molecular weight. The molecular weight of an oligonucleotide is simply the mass of the compound in grams per mole (6.02×10^{23} molecules). It is the sum of each of the component molecular weights of all the atoms the oligonucleotide may contain. This value is needed for converting OD_{260} units into units of mass. TriLink gives the molecular weight of the free acid form of the oligonucleotide. The next page shows the information used to calculate the molecular weight of an unmodified oligonucleotide.

Since TriLink provides the final yield of the product in OD_{260} units, it is important to understand how to use this data to convert from OD_{260} units into other desired units. Below are examples of conversions to some of the more commonly used units. If you have any further questions, please contact our Client Relations Department. We will be happy to assist you.

Oligo Unit Conversion

Sample sequence:

5' AGC TAA GTC ACT GCC ATT GA 3'

Extinction Coefficient (ε)*: 195.5 OD units/µmole

Molecular Weight (MW): 6101.0 g/mole

OD (A_{260}): 32.4

Example 1: Converting to µmoles

µmoles of oligo = OD (A_{260}) ÷ ε
= 32.4 ÷ 195.5 OD units/µmole
= 0.166 µmoles

Example 2: Converting to µgrams

(Use the µmoles calculated in Example 1)

µgrams of oligo = µmoles x MW
= 0.166 µmoles x 6101.0 g/mole
= 1012.8 µgrams

*If you are working in L $mmole^{-1}cm^{-1}$ units, you will first need to convert the ε to OD units/µmole.

ε L $mmole^{-1}cm^{-1}$ ÷ 1000 = ε OD units/µmole

195,500 L $mmole^{-1}cm^{-1}$ ÷ 1000 = 195.5 OD units/µmole

Oligonucleotide Extinction Coefficient Formula

	ε 260		ε 260
pdA	15.4	dCpdG	9.0
pdC	7.4	dCpT	7.6
pdG	11.5	dGpdA	12.6
pT	8.7	dGpdC	8.8
dApdA	13.7	dGpdG	10.8
dApdC	10.6	dGpT	10.0
dApdG	12.5	TpdA	11.7
dApT	11.4	TpdC	8.1
dCpdA	10.6	TpdG	9.5
dCpdC	7.3	TpT	8.4

To calculate the number of OD_{260} units per µmole of oligonucleotide with the sequence 5' DpEp…KpL, use the following formula with the table above:

ε 260 DpEpFpGp…KpL = [2(ε DpE + ε EpF + ε FpG + .. + ε KpL) - ε E - ε F - ε G - .. - ε K]

Note that you do not include the 3' and 5' terminal bases in the second part (monomers) of the equation, whereas you use every dimer pair in the first part.

Molecular Weight Calculations

To calculate the molecular weight of an oligonucleotide, use the formula below. (Or, go to our website to download the latest version of our molecular weight calculator as an Excel spreadsheet.)

(# of dA in sequence)	x	249.24
+ (# of dC in sequence)	x	225.23
+ (# of dG in sequence)	x	265.2
+ (# of T in sequence)	x	240.23
+ (# of phosphodiester linkages in sequence)	x	63.97
+ (# of phosphorothioate linkages in sequence)	x	80.03
+ 2 for 3' and 5' terminal hydrogens		

= Molecular weight of oligonucleotide

Modified Nucleoside Triphosphate Applications
An Overview of the SELEX Process

By Stephanie M. Schuitt, TriLink BioTechnologies

TriLink's wide selection of modified nucleoside triphosphates offers researchers many novel ways to explore existing technologies typically used in drug discovery. One example is the SELEX (Systematic Evolution of Ligands by Exponential Enrichment) process of developing oligonucleotide aptamers. The SELEX process allows for the simultaneous screening of 1 x 10^{15} different oligonucleotides against a target of interest, such as a protein. The main goal of SELEX is to identify a small subset of aptamers from the original library that bind to the target (1). Three separate laboratories were simultaneously working on the development of this technology: Larry Gold and Craig Tuerk at the University of Colorado Boulder, Jack Szostak and Andy Ellington at Massachusetts General Hospital, and Gerald Joyce at the Scripps Institute in La Jolla, CA (2). The Colorado group was granted a patent in 1993.

The word aptamer comes from the Greek word "aptus", meaning "to fit" (3). An aptamer is an oligonucleotide ligand, generally 15-60 bases in length. It binds to the target of interest through conformational recognition, as opposed to the standard recognition mechanism for nucleic acids: hydrogen bonding of the bases to another nucleic acid strand. Oligonucleotides are often represented as linear sequences, but in reality they fold into specific conformations. It is these shapes that allow the lock-and-key fit between an aptamer and its target (4). As mentioned above, the ultimate goal of SELEX is to find an aptamer that binds to the active site of the target molecule.

Advantages of Aptamers

- Straightforward synthesis
- Easily modified to increase resistance to endonucleases
- Favorable toxicity profiles
- Greater stability than monoclonal antibodies
- Useful as therapeutic agents
- Highly specific; can discriminate between closely related proteins (5)

The SELEX Process

1. Define target molecule. (The target molecule can be a protein, small molecule, or a supramolecular structure.)
2. Create a "library" of random oligonucleotides (~1 x 10^{15} oligonucleotides) The random pool of DNA generally has primer binding sites at the end of each oligonucleotide and wobble bases in between (6). This provides an efficient way to find and PCR amplify oligonucleotides that bind to the target molecule.
3. Expose the oligonucleotide "library" to the target molecule. A few of these oligonucleotides in the library will bind to the target and are then considered aptamers.
4. Non-binding oligonucleotides are separated from the binding oligonucleotides. Those that bind are amplified and then put through several additional selection cycles.
5. The number of high affinity binding molecules is reduced from trillions, to a small number through this process.
6. Individual aptamers are isolated, sequenced and refined using modified nucleoside triphosphates. For example, 2'-Fluoro-dCTP (TriLink Catalog # N-1008) and 2'-Fluoro-dUTP (TriLink Catalog # N-1010) are commonly used to modify aptamers. These types of modifications increase the stability of the aptamer and make it more resistant to endonuclease degradation (7).

Gilead currently holds the patent rights to the SELEX technology and has licensed it to Archemix, who is using the technology to develop pharmaceutical applications for aptamers. Archemix has sublicensed this technology to NOXXON, who is using it for the development of their Spiegelmer® technology (http://www.noxxon.com).

SomaLogic (http://www.somalogic.com), a company founded by one of the original inventors, Larry Gold, has developed their own technology, called PhotoSELEX. This technology involves photoaptamers, which use a 5-Bromo-2'-deoxyuridine-5'-Triphosphate (Br-dU) (TriLink Catalog # N-2008) residue instead of a standard Thymidine. In addition to the conformational binding, there is also covalent bonding between the Br-dU residue of the aptamer and a tyrosine amino acid on the target. The Br-dU oligonucleotides are exposed to radiation and those that are in the correct conformation will covalently crosslink to specific sites on the target molecule. Those that do not crosslink are washed away, while those that remain are amplified by PCR. These photoaptamers are put into arrays so that large numbers of proteins can be tested at the same time (8).

SELEX is just one of many applications that modified nucleoside triphosphates can be used in. TriLink offers a wide variety of modified nucleoside triphosphates that can be used for research purposes.

References

1. Archemix http://www.archemix.com (accessed December 2002)
2. Burke, Donald, SELEX tutorial, In vitro selections: Evolution in a tube http://bl-chem-ernie.chem.indiana.edu/~dhburke/SELEX.htm
3. Jayasena, S.D. Aptamers: An Emerging Class of Molecules that Rival Antibodies in Diagnostics (1999) Clinical Chemistry 45:9 1628-1650.
4. Somalogic http://www.somalogic.com (accessed December 2002)
5. Archemix http://www.archemix.com (accessed December 2002)
6. Burke, Donald, In vitro selections: Evolution In a Tube http://bl-chem ernie.chem.indiana.edu/~dhburke/SELEX.htm
7. Archemix http://www.archemix.com (accessed December 2002)
8. Somalogic http://www.somalogic.com (accessed December 2002)

Enzymatic Activity of Selected Nucleoside 5'-Triphosphates and Their Analogs

By Alexandre Lebedev, Ph.D.; TriLink BioTechnologies

Nucleoside 5'-triphosphate derivatives and their analogs (NTPDA) have been extremely helpful in explaining many important details of the mechanism of reactions catalyzed by a family of NTP dependent enzymes. Different versions of NTPDAs represent various modifications of the bases, sugars, triphosphate chain and the combinations of those. Many of the modifications result in changes in the essential properties of the nucleoside 5'-triphosphates, and most importantly their behavior in the enzymatic reactions.

1. Substrate activity of NTPDAs often changes compared to that of the parent NTPs (usually reduced).

2. NTPDAs may become inhibitors of the enzymatic reactions through various mechanisms. For example:

   a. NTPDAs (depending on the type of modification) may physically block the binding, catalytic or allosteric sites of the enzyme thus competing with the substrate, the reaction product or an allosteric regulator(s).
   b. NTPDAs may cause a termination of the enzymatic chain reaction by producing a modified product lacking the chemical function required for continuation of the enzymatic process.
   c. NTPDAs possessing a reactive group(s) may also cause a chemical modification of the crucial enzyme functions responsible for substrate recognition, catalytic action, or conformation stability of the peptide structure (or disrupt the association forces keeping the functional multiunit enzymes).

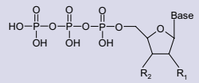
The tables on the following pages are examples of different enzymatic activities of the selected nucleoside 5'-triphosphate derivatives and their analogs. Where it is known, the mode of NTPDA action and the research or practical application(s) is briefly indicated with a reference to a primary source of information.

The data presented in the tables should not be considered as a complete compilation by any means. Rather, it is an overview of the main classes of NTPDAs and illustration of their ability to serve as tools for the different biochemical applications.

Abbreviations used in Tables:

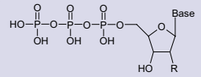
AMV RT	Avian Myeloblastosis Virus Reverse Transcriptase
ATPase	Adenosine 5'-triphosphatase
ASS	Adenylylsuccinate Synthesase
ATP/CTP tRNA NT	ATP/CTP Dependent tRNA Nucleotidyl Transferase
DD DP	DNA Dependant DNA Polymerase
DD RP	DNA Dependant RNA Polymerase
RD DP	RNA Dependant DNA Polymerase
RD RP	RNA Dependant DNA Polymerase
RNR	Ribonucleotide Reductase
PNP	Polynucleotide Phosphorylase
Poly(A) Pol	Polyadenylate Polymerase
PRPPS	Phosphoribosylpyrophosphate Synthesase
TNDT	Terminal Nucleotidyl Transferase
tRNA NT	tRNA Nucleotidyl Transferase
aa tRNA S	Aminoacyl tRNA Synthesase
2',5'-OAS	2',3'-Oligoadenylate Synthesase
HIV RT	Human Immunodeficiency Virus Reverse Transcriptase
AC	Adenylate Cyclase
GC	Guanylate Cyclase
CK	Creatine Kinase
HK	Hexokinase
PK	Pyruvate Kinase
PFK	Phosphofructose Kinase

Table 1: Sugar modified nucleoside 5'-triphosphate analogs



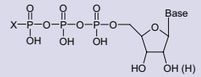
Application	Mode of Action	R_2	R_1	Base	Enzyme class	References
DNA Sequencing	Chain Termination	H	H	A,C,G,T,I	DD DP	1,2
		H	OH	A,C,G,U	DD RP	3-6
		F	OH	A,C,G,U	DD RP	14
		NH_2	OH	A,C,G,U	DD RP	15-18
		Me	OH	A,C,G,U	DD RP	15, 25-27
	Substrate	OH	F	A,C,G,U	DD DP	13
RNA Sequencing	Chain Terminator	H	OH	A,C,G,U	RD RP	3,9
		OMe	OH	A,C,G,U	DD RP	25
		Me	OH	A,C,G,U	DD RP	15, 25-27
		N_3	OH	A,C,G,U	DD RP	15
Synthesis Of Modified DNA	Substrate	OH	F	A,C,G,U	DD DP	13
Synthesis Of Modified RNA	Substrate	OH	OMe	A	DD RP	11
		NH_2	OH	A,C,G,U	DD RP	15-18
		NH_2	OH	A	tRNA-NT	19-21
Ribosomal Research	Substrate	NH_2	OH	A	tRNA-NT	19-21
Enzymatic Research	Inhibition	H	OH	A,C,G,U	DD RP	7
		H	OH	A,C,G,U	aa tRNA S	8
		OH	OMe	A	aa tRNA S	8
		OH	Ara-OH	A,C	RNR	10
		NH_2	OH	A,G	RD RP, DD RP	22,23
		H	OH	A	Poly(A) Pol	24
		NH_2	Ara-OH	G	RD RP DD RP	22
		N_3	OH	A,G	RD RP DD RP	22,23
		OMe	OH	A	aa tRNA S	8
		CHO	CHO	A,C,G,U	DD RP	30,31
		H	OH	U,T	DD RP	64
		Xylo-OH	OH	U,T	DD RP	67
	Activation	OH	OMe	A	RNR	10
	Chain Termination	NCS	H	A	DD RP	17
		$NHC(O)CH_2Br$	OH	A	DD RP	17
		2',3'-Epoxide	2',3'-Epoxide	A	DD DP	28
	Modification of Enzyme	OH	Arylazido	A	ATP-ase	12
		NCS	H	A	DD RP	17
		$NHC(O)CH_2Br$	OH	A	DD RP	17
		CHO	CHO	A	ATP-ase	29
		CHO	CHO	A	aa tRNA S	32-34

Table 2: Base modified nucleoside 5'-triphosphate analogs



Application	Mode of Action	R	Base	Enzyme class	References
DNA Sequencing	Substrate	H	7-Deaza (A,G,I)	DD DP	38-41
		H	N^4-Me-C	DD DP	46
Synthesis Of Modified DNA	Substrate	H	Benzimidazole	DD DP	35
		H	5-Me-C	DD DP	42-44
		H	7-Deaza (A,G)	Hum. telomerase	45
		H	N^6,N^4-Etheno-2,6-DAP	DD DP	47
		H	Dihydrothymidine	DD DP	48
Synthesis Of Modified RNA	Substrate	OH	5-Br-U	DD RP	49
		OH	5-I-U	DD RP	49
		OH	5-I-C	tRNA NT	50
		OH	5-Formyl U (β anomer)	DD RP	53-56
		OH	2-Thio U	DD RP	59
		OH	5-Br-C	DD DP	59
		OH	Formycin A	DD RP	60
PCR	Substrate	H	N^4-Me-C	DD DP	46
Enzymatic Research	Substrate	H	Benzimidazole	RNR	10
		H	5-Br-U	DD RP	36,37
		OH	8-Azido-A	DD RP	51
		OH	5-Formyl U (α anomer)	DD RP	53-56
		OH	4-Thio-U	DD RP	57-59
		OH	Xanthine	ASS	61
	Inhibition	H	7-Deaza (A,G)	Hum. telomerase	45
		OH	8-Azido-A	aa tRNA S	8
		OH	8-Br-A	aa tRNA S	8
		OH	8-Cl-A	aa tRNA S	8
		OH	9-Purinyl	aa tRNA S	8
	Modification of Enzyme	H	5-I-U	DD RP	49
Ribosomal Research	Photomodification of Ribosomal protiens	OH	3-(Azidooxymethylphenyl)-G	DD DP	52

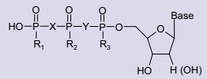
Table 3: Nucleoside 5'-triphosphate analogs with modified γ-phosphate



Application	Mode of Action	X	Nucleoside	Enzyme class	References
Enzymatic Research	Substrate	Anilide	rA	DD RP	141
		4-Azidoanilide	r(A, C, G)	DD RP	142 - 144
		4-Azidoanilide	rA	aa tRNA S	145-150
		1-(5-sulfonatenapthyl)-amide	rA	DD RP	160, 161
		1-(5-sulfonatenapthyl)-amide	r(A,C,G,U)	SVPD	162
		1-(5-sulfonatenapthyl)-amide	d(C, T)	SVPD	162
	Inhibition	Anilide	rA	aa tRNA S	138-140
		4-Azidoanilide	rA	aa tRNA S	145-150
		4-Azidoanilide	rA, Etheno-rA	CK	151-153
		$2,4,6\text{-}(Me)_3\text{-}C_6H_4\text{-}C(O)O\text{-}$	rA	aa tRNA S	155, 156
		$BrCH_2C(O)NH\text{-}C_6H_4O\text{-}$	rG	Ribosome	159
		4-(N-2-Chloroethyl, N-methylamino)-benzylamide	dA, dT	RD DP, DD DP, HK	163-165
		N-Methyl,N-(4-azidobenzyl)-amide	rA	aa tRNA S	124, 148
	Enzyme Modification	N_3	rG	DD RP	154
		$2,4,6\text{-}(Me)_3\text{-}C_6H_4\text{-}C(O)O\text{-}$	rA	ATP-ase	157
		$FSO_2\text{-}C_6H_4\text{-}C(O)O\text{-}$	rA	PK	158
		4-(N-2-Chloroethyl, N-methylamino)-benzylamide	dA, dT	RD DP, DD DP, HK	163-165
		N-Methyl,N-(4-azidobenzyl)-amide	rA	aa tRNA S	124, 148
		Cyclic trimetaphosphate*	rA, Etheno-rA	aa tRNA S	166
		Cyclic trimetaphosphate*	rA	DD RP	167, 168
	Enzyme Photo- Modification	4-Azidoaniline	r(A, C, G)	DD RP	142 - 144
		4-Azidoaniline	rA	aa tRNA S	145-150
		4-Azidoaniline	rA, Etheno-rA	CK	151-153
		4-Azidobenzylamide	rG	DD RP	154

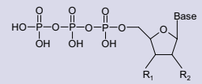
* Nucleoside 5'-Trimetaphosphate

Table 4: Nucleoside 5'-triphosphate analogs with modified triphosphate chain



Application	Mode of Action	X	Y	R_1	R_2	R_3	Nucleoside	Enzyme class	References
DNA Sequencing	Substrate	O	O	O^-	O^-	S^-	dA, dT	DD DP	110-117
		O	O	O^-	O^-	S^-	d(A,C,G,T)	DD DP	118
		O	O	O^-	O^-	BH_3	5-(Me,Et,Br,or I) C	DD DP	137
Synthesis Of Modified DNA	Substrate	O	O	O^-	O^-	S^-	dA, dT	DD DP	110-117
		O	O	O^-	O^-	Me	dT	DD DP, TDNT, HIV-RT, AMV-RT	133,134
Enzymatic Research	Inhibition	CF_2	O	O^-	O^-	O^-	rG	DD DR	66
		CH_2	O	O^-	O^-	O^-	rA	Various enzymes	67-70
		CH_2	O	O^-	O^-	O^-	rA	PFK	72
		O	CH_2	O^-	O^-	O^-	rA	AC	73
		NH	O	O^-	O^-	O^-	rA, rG	Various enzymes	74-82
		O	NH	O^-	O^-	O^-	rA	Various enzymes	67-69
	Substrate	CH_2	O	O^-	O^-	O^-	rA	CK	71
		CH_2	O	O^-	O^-	O^-	rA	DD DP	66
		O	CH_2	O^-	O^-	O^-	rA	CK	71
		O	NH	O^-	O^-	O^-	rA	DD RP, CK	83
		O	O	O^-	O^-	S^-	r(A,C,G,U)	Myosine	84, 85
		O	O	O^-	O^-	S^-	rA	Kinases	86-92
		O	O	O^-	O^-	S^-	rA	PRPPS	93
		O	O	O^-	O^-	S^-	rA, rG	DD RP	94-99
		O	O	O^-	O^-	S^-	rA	2',5' OAS	100, 101
		O	O	O^-	O^-	S^-	rA	PNP	102, 103
		O	O	O^-	O^-	S^-	rA	tRNA NT	104
		O	O	O^-	O^-	S^-	rA	RNA ligase	105
		O	O	O^-	O^-	S^-	rA	GS	106
		O	O	O^-	O^-	S^-	rA	AS	107, 108
		O	O	O^-	O^-	S^-	rA	UDP GPP, GP UT	109
		O	O	O^-	O^-	S^-	dA, dT	DD DP	110-117
		O	O	O^-	O^-	S^-	dA	AMV RT	119
		O	O	O^-	S^-	O^-	rA	Kinases	86-92, 120-122
		O	O	O^-	S^-	O^-	rA	aa tRNA S	123, 124
		O	O	O^-	S^-	O^-	rA	ATP-ase	125
		O	O	O^-	S^-	O^-	dA	DD DP	110-111
		O	O	S^-	O^-	O^-	rA	Kinases	126-129
		O	O	S^-	O^-	O^-	rA, rG	ATP and GTP-ases	125, 130-132
		O	O	O^-	O^-	Me	dT	DD DP, TDNT, HIV-RT, AMV-RT	133,134
		O	O	O^-	O^-	BH_3	d(A,C,G,T)	DD DP	135, 136

Table 5: Sugar and Base modified nucleoside 5'-triphosphate analogs



Application	Mode of Action	R_1	R_2	Base	Enzyme class	References
DNA Sequencing	Chain Terminator	NH_2	H	5-(2-Br-vinyl)-U	DD DP	35
		NH_2	H	5-(2-Br-vinyl)-U	AMV RT	35
		N_3	H	5-(2-Br-vinyl)-U	DD DP	35
		N_3	H	5-(2-Br-vinyl)-U	AMV RT	35
RNA Sequencing	Inhibition of DNA synthesis	H	OH	5-F-C	DNA Primase	7
	Inhibition of DNA synthesis	H	OH	5-F-U	DNA Primase	7
Enzymatic Research	Inhibition	N_3	H	Ribavirin	RD RP	22
		Xylo-OH	OH	5-F-U	DD RP	64
		Xylo-OH	OH	5-Cl-U	DD RP	64
		Xylo-OH	OH	5-Br-U	DD RP	64
		Xylo-OH	OH	5-I-U	DD RP	64
		Xylo-OH	OH	5-Ethyl-U	DD RP	64
		Xylo-OH	OH	5-Propyl-U	DD RP	64
		Xylo-OH	OH	5-Butyl-U	DD RP	64
	Modification of Enzyme	CHO	CHO	Etheno-A	Nitrogenase	65
	Photo-Modification of Enzyme	OH	OH	8-Azido-A	DD RP	62
		Arylazido	OH	8-Azido-A	ATP-ase	63

References

1. Sanger, F., Nicklen, S., and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. U.S.A., vol.74, pp. 5463-5467
2. Tabor, S., and Richardson, C. C. (1989) Proc. Natl. Acad. Sci. U.S.A., vol.86, pp.4076-4080
3. Axelrod, V.D., and Kramer, F.R. (1985) Biochemistry, vol.24, pp.5716-5723.
4. Saneyoshi, M., Tohyama, J., Nakayama, C., Takiya, S., and Iwabuchi, M. (1981) Nucleic Acids Res., vol.9, pp.3129-3138
5. Suchodolnik, R. J. (1979) Progr. Nucleic Acid Res. Mol. Biol., vol.22, pp.193-292
6. Parvin, J. D., Smith, F. I., and Palese, P. (1986) DNA, vol.5, pp.167-171
7. Izuta, S., Koshaka-Ichikawa, M., Yamaguchi, T., and Saneyoshi, M. (1996) J.Biochem (Tokyo), vol.119, pp.1038-1044
8. Freist, W., and Cramer, F. (1980) Eur. J. Biochem., vol.107, pp.47-50
9. Kramer, F.R., and Mills, D.R. (1978) Proc. Natl. Acad. Sci. U.S.A., vol.75, pp. 5334-5338
10. Follmann, H., and Hogenkamp, H. P. C. (1971) Biochemistry vol.10, pp. 186-192
11. Gerard, G. F., Rottman, F., and Boezi, J. A. (1971) Biochemistry, vol.10, pp.1974-1980
12. Russel J., Stella J., Guillroy J. J., and Guillroy R. J. (1976) Biochem. Biophys. Res. Comm., vol.70, pp.1225-1234
13. Ono, T., Scalf, M., and Smith, L.M. (1997) Nucleic Acids Res., vol.25, pp. 4581-4588
14. Mikhailopulo, I.A., Prikota, T.I., Poopeiko, N.E., Sivets, G.G., Kvasyuk, E.I., Sviryaeva, T.V., Savochkina, L.P., and Bibilashvilli, R.S. (1989) FEBS Lett., vol.250, pp.139-141
15. Kutateladze, T., Beabealasvilli, R., Azhaev, A., and Kraevsky A. (1983) FEBS Lett., vol.153, pp.420-426
16. Trauman, J.T., and Klenow H. (1968) Mol. Pharmacol., vol.4, pp.77-86
17. Armstrong, V.W., and Eckstein, F. (1979) Biochemistry, vol.18, pp.5117-5122
18. Armstrong, V.W., and Eckstein, F. (1976) Eur. J. Biochem., vol.70, pp.33-38
19. Fraser, T.H., and Rich, A. (1974) Proc. Natl. Acad. Sci. U.S.A., vol.72, pp.3044-3047
20. Sprinzl, M., and Cramer F. (1979) Prog. Nucleic Acid Res. Mol. Biol., vol.22, pp. 2-70
21. Kukhanova, M., Victorova, L., Bourd, S., Gottikh, B., Krayevsky, A., and Sprinzl, M. (1981) FEBS Lett., vol.118, pp.176-179
22. Pravdina, N. F., Papchikhin, A. V., Purygin, P. P., and Galegov, G. A. (1989) Mol. Genet., Mikrobiol.Virusol. pp.29-33 (in Russian)
23. Pravdina, N. F., Kraevskii, A. A., Skaptsova, N. V., Florent'ev, V. L., and Galegov, G.A. (1987) Mol. Genet., Mikrobiol.Virusol. pp.39-42 (in Russian)
24. Rose, K. M., Bell, L. E., and Jacob, S. T. (1977) Nature, vol.267, pp.178-180
25. Axelrod, V. D., Vartikyan, R. M., Aivazashvilli, V. A., and Bebelasvilly R. S. (1978) Nucleic Acids Res., vol.5, pp.3549-3563
26. Aivazashvili, V. A., Mickailov, S. N., Padyukova, N. S., Karpeisky, M. Y., and Bibilashvili, R. S. (1986) Bioorgan. Khim., vol.12, pp.708-710 (in Russian)
27. Aivasashvili, V. A., Beabealashvili, R. Sh., and Florentiev, V. L. (1977) Molekul. Biol., vol.11, pp.854-863 (in Russian)
28. Abboud M. M., Sim W. J., Loeb L. A., and Mildvan A. S. (1978) J. Biol. Chem., vol.253, pp.3415-3421
29. Kumar, G., Karla, V. K., and Brodie, A. F. (1979) J. Biol. Chem., vol.254, pp.1964-1971
30. Malcolm A. D., Moffatt J. R. (1978) Biochem. J., vol.175, pp.189-192
31. Slepneva I. A. (1980) Molec. Biol. Rep., vol. 6, p.31-34
32. Fayat G., Hountondji C., and Blanquet S. (1979) Eur. J. Biochem., vol.96, pp.87-92
33. Gorshkova I. I., *et al.* (1981) Biokhimia, vol.46, pp.699-707 (In Russian)
34. Baltzinger M., Fasiolo F., and Remy P. (1979) Eur. J. Biochem., vol.97, pp.481-494
35. Atrazhev, A. M., Dyatkina, N. B., Rozovskaya, T. A., Aleksandrova, L. A., Chidzhavadze, Z. G., and Von Janta-Lipinski, M. (1987) Bioorgan. Khim., vol.13, pp.1375-1381 (in Russian)
36. Ovchinnikov, Yu. A., Efimov, V. A., Chekhmakhcheva O. G., et. al. (1981) Biorgan. Khimia, vol.7, pp.485-493
37. Ovchinnikov, Yu. A., Efimov, V. A., and Chekhmakhcheva O. G. (1979) Biorgan. Khimia, vol.5, pp.138-143
38. Barr *et al.* (1986) Bio Techniques, vol.4, pp.428-432
39. Mizusawa, S., Nishimura, S., and Seela, F. (1986) Nucleic Acids Res., vol.14, pp.1319-1324
40. Seela F., and Roling A. (1991) Nucleic Acids Res., vol.20, pp.55-61
41. Dierick H., Stul M., De Kelver W., Marynen P., and Cassiman J.-J. (1993) Nucleic Acids Res., vol.21, pp.4427-4428
42. Ono, T., Uehara, Y., Kurishita, A., Tawa, R., and Sakurai, H. (1993) Age Ageing (Eng.), vol.22, (Supplement. No.1) pp.S34-S43
43. Munzel, P.A., Pfohl-Leszkowicz, A., Röhrdanz, E., Keith, G., Dirheimer, G., and Bock, K. W. (1991) Biochem. Biopharmacol., vol.42, pp.365-371
44. Hernandez, L., Petropoulos, C. J., Hughes, S. H., and Lijinsky, W. (1991) Mol. Carcinog., vol.4, pp.203-209
45. Fletcher, T. M., Salazar, M., and Chen, S.-F. (1996) Biochemistry, vol.35, pp.15611-15617
46. Li S., Haces A., Stupar L., Gebeyehu G., and Pless R. C. (1993) Nucleic Acids Res., vol.21, pp.2709-2714
47. Cowart, M., and Benkovic, S. J. (1991) Biochemistry, vol.30, pp.788-796
48. Ide, H., and Wallace, S. S. (1988) Nucleic Acids Res., vol.16, pp.11339-11354
49. Sverdlov, E. D., Tsarev, S. A., and Begar, V. A. (1980) FEBS Lett., vol.114, pp.111-114
50. Sprinzl, M., van der Haar, F., Schlimme, H.S., and Cramer F. (1972) Eur. J. Biochem., vol.25, pp.262-266
51. Cartwright, I. L., and Hutchinson, D. W. (1980) Nucleic Acids Res., vol.8, pp.1675-1691
52. Maasen, J. A., and Moller, W. (1978) J. Biol. Chem., vol.253, pp.2777-2783
53. Armstrong V.W., Sternbach H., and Eckstein F. (1976) Biochemistry, vol. 15, pp.2086-2091
54. Armstrong V.W., Sternbach H., and Eckstein F. (1977) Methods Enzymol., vol. 46, pp.346-352
55. Armstrong V.W., Sternbach H., and Eckstein F. (1975) Z. Physiol. Chem., vol.356, pp.218-219
56. Armstrong V.W., Sternbach H., and Eckstein F. (1974) FEBS Lett., vol.44, pp.157-159
57. Frischauf A. M., and Scheit K. H. (1973) Biochem Biophys. Res. Communs., vol.53, pp.1227-1223
58. Scheit K. H., and Frischauf A. M. (1974) Z. Physiol. Chem., vol.335, pp.759-763
59. Nawrot, B., Hottmuller, P., and Sprinzl, M. (1996) Chemical Papers-Chemicke Zvesti, vol.50, pp.151-155
60. Piccirilli, J. A., Moroney, S. E., and Benner, S. A. (1991) Biochemistry, vol.30, pp.10350-10356
61. Kang C., Sun N., Honzatko R. B., and Fromm H.J. (1994) J. Biol. Chem., vol.269, pp.24046-24049
62. Woody, A.-Y. M., Vader, C. R., Woody, R. W., and Haley, B. E. (1984) Biochemistry, vol.23, pp.2843-2848
63. Schafer, H.-J., Scheurich, P., Rathgeber, G., *et al.* (1980) Biochem., Biophys. Res. Commun., vol.95, pp.562-568
64. Nakayama C., and Saneyoshi M. (1985) J. Biochem., vol. 98, pp.417-425
65. Gvozdev, R. I., Kotelnikov, A. I., Pivovarov, A. D., et. al. (1975) Bioorgan. Khimia, vol.1, pp.1207-1214
66. Arabshahi, L., Khan, N. N., Butler, M., Noonan, T., Brown, N. C., and Wright, G. E. (1990) Biochemistry, vol.29, pp.6820-6826
67. Picher, M., Sevigny, J., D'Orleans-Juste, P., and Beaudoin, A. R. (1996) Biochem. Pharmacol., vol.51, pp.1453-1460
68. Aksoy, M. O., and Kelsen, S. G. (1994) Am. J. Respir. Cell Mol. Biol., vol.10, pp.230-236
69. Thévenod, F., Anderie, I., and Schulz, I. (1994) J. Biol. Chem., vol.269, pp.24410-24417
70. Tobin, T., Akera, T., Lee, C. Y., and Brody, T. M. (1974) Biochem. Biophys. Acta, vol.345, pp.102-117
71. Milner-White, E. J., and Rycroft, D. S.(1983) Eur. J. Biochem., vol.133, pp.169-172
72. Barzu O., Tilinca R., Porutui D. *et al.*(1977) Arch. Biochem. Biophys., vol.182, pp.42-51
73. Krug, F., Parikh, I., Illiano, G., and Cuatrecasas, P. (1973) J. Biol. Chem., vol.248, pp.1203-1206
74. Wang, Z. W., and Kotlikoff, M. I. (1996) Am. J. Physiol., vol.271, pp.L100-L105
75. Hehl, S., and Neumcke, B. (1994) Eur. Biophys. J., vol.23, p.231.
76. Yeung, S.-M. H., and Green, R. D. (1983) J. Biol. Chem., vol. 258, pp.2334-2339
77. Krall, F. J., Leshon, S. C., and Korenman, S. G. (1982) Biochem. J., vol.205, pp.249-255
78. Miller, L. D. P., Petrozzino, J. J., and Connor, J. A. (1995) J. Neurosci., vol.15, pp.8320-8330
79. Paz, M. M., Ramos, M., Ramirez, G., and Souza, D. (1994) FEBS Lett., vol.355, pp.205-208
80. Kopf, G. S., and Woolkalis, M. J. (1991) Methods Enzymol., vol.195, p.257-266
81. Yount, R. G., Babkock, D., Ballantyne, W., Ojala, D.(1971) Biochemistry, vol.10, pp.2484-2489
82. Gresser, M. J., Beharry, S., and Moennich, D. M. C. (1984) Curr. Topics Cell Regul., vol.24, p.365-378
83. Ma Q-F., Babbitt P. C., and Kenyon G. L. (1988) J. Am. Chem. Soc., vol. 10, pp. 4060-4061
84. Connolly, B. A., and Eckstein, F. (1981) J. Biol. Chem., vol. 256, pp.9450-9456
85. Eckstein, F., and Goody, R. S. (1976) Biochemistry, vol.15, pp.1685-1691
86. Jaffe, E. K., and Cohn, M. (1979) J. Biol. Chem., vol.254, pp.10839-10845
87. Jaffe, E. K., Nick, J., and Cohn, M. (1982) J. Biol. Chem., vol.256, pp.7650-7656
88. Burgers, P. M. J., and Eckstein (1980) J. Biol. Chem, vol.255, pp.8229-8233
89. Cohn, M., Shin, H., and Nick, J. (1982) J. Biol. Chem., vol.257, pp.7646-7649
90. Romaniuk, P. J., and Eckstein, F. (1981) J. Biol. Chem., vol.256, pp.7322-7328
91. Tomasselli, A. G., and Noda, I. H. (1983) Eur. J. Biochem., vol.132, pp.109-115
92. Kalbitzer, H. R., Marquetant, R., Connolly, B. A., and Goody, R. S. (1983) Eur. J. Biochem., vol.133, pp.221-227
93. Gibson, K. J., and Switzer, R. L. (1980) J. Biol. Chem., vol.255, pp.694-696
94. Armstrong, V. W., Yee, D., and Eckstein, F. (1979) Biochemistry, vol.18, pp.4120-4123
95. Matzura, H., and Eckstein, F. (1968) Eur. J. Biochem., vol.3, pp.448-452
96. Eckstein, F., and Gindl, H. (1970) Eur. J. Biochem., vol.13, pp.558-564
97. Eckstein, F., Armstrong, V. W., and Sternbach, H. (1976) Proc. Natl. Acad. Sci. U.S.A., vol.73, pp.2987-2990
98. Burgers, P. M. J., and Eckstein (1978) Proc. Natl. Acad. Sci. U.S.A., vol.75, pp.4798-4800
99. Yee, D., Armstrong, V. W., and Eckstein, F. (1979) Biochemistry, vol.18, pp.1245-1252
100. Suhadolnik, R. J., and Choongeun, L. (1985) Biochemistry, vol.24, pp.551-555
101. Karilo, K., Sobol, R. W., Jr., Suhadolnik, L., Li, S.-W., Reichenbach, N. L., Suhadolnik, R. J., Charubala, R., and Pfleiderer, W. (1987) Biochemistry, vol.26, pp.7127-7135
102. Burgers, P. M. J., and Eckstein F. (1979) Biochemistry, vol.18, pp.450-454
103. Marlier, J. F., Bryant, F. R., and Benkovic, S. J. (1981) Biochemistry, vol.20, pp.2212-2219

104. Eckstein, F., Sternbach, H., and von der Haar, F. (1977) Biochemistry, vol.16, pp.3429-3432
105. Bryant, F. R., and Benkovic, S. J. (1982) Biochemistry, vol.21, pp.5877-5885
106. Senter, P. D., Eckstein, F., Mülsch, A., and Böhme, E. (1983) J. Biol. Chem., vol.258, pp.6741-6745
107. Gerlt, J. A., Coderre, J. A., and Wolin, M. S. (1980) J. Biol. Chem., vol.255, pp.331-334
108. Eckstein, F., Romaniuk, P. J., Heideman, W., and Storm, D. R. (1981) J. Biol. Chem., vol.256, pp.9118-9120
109. Sheu, K.-F. R., Richard, J. P., and Frey, P. A. (1979) Biochemistry, vol.18, pp.5548-5556
110. Eckstein, F., and Thomson, B. J. (1995) Methods in Enzymol., vol. 262, pp. 189-202
111. Burgers, P. M. J., and Eckstein (1979) J. Biol. Chem, vol.254, pp.6889-6893
112. Brody, R.S., and Frey, P.A. (1981) Biochemistry, vol.20, pp.1245-1252
113. Romaniuk, P. J., and Eckstein, F. (1982) J. Biol. Chem., vol.257, pp.7684-7688
114. Gupta, A., DeBrosse, C., and Benkovic, S. J. (1982) J. Biol. Chem., vol.257, pp.7689-7692
115. Brody, R. S., Adler, S., Modrich, P., Stec, W. J., Lesnikowski, Z. J., and Frey, P. A. (1982) Biochemistry, vol.21, pp.2570-2572
116. Eckstein, F., and Jovin, T. M., (1983) Biochemistry, vol.22, pp.4546-4550
117. Gerlt, J. A., Demon, P. C., and Mehdi, S. (1982) J. Amer. Chem. Soc., vol.104, pp.2848-2856
118. Nakamaye K. L., Gish G., Eckstein F., and Vosberg H.-P. (1988) Nucleic Acids Res., vol.16, pp.9947-9959
119. Barlett, P. A., and Eckstein, F. (1982) J. Biol. Chem., vol.257, pp.8879-8884
120. Darby, M. K., and Trayer, I. P. (1982) Eur. J. Biochem., vol.129, pp.555-560
121. Lee, C. S., and O'Sullivan, W. J. (1984) Biochim. Biophys. Acta, vol.787, pp.131-137
122. Bolen, D. W., Stingelin, J., Bramson, H. N., and Kaizer, E. T. (1980) Biochemistry, vol.19, pp.1176-1182
123. Connolly, B. A., von der Haar F., and Eckstein, F. (1980) J. Biol. Chem., vol.255, pp.11301-11307
124. Smith, L. T., and Cohn, M. (1982) Biochemistry, vol.21, pp.1530-1534
125. Senter, P. D., Eckstein, F., and Kagawa, Y. (1983) Biochemistry, vol.22, pp.5514-5518
126. Orr., G. A., Simons, J., Jones, S. R., Chin, G. J., and Knowels, J. R. (1978) Proc. Natl. Acad. Sci. U.S.A, vol.75, pp.2230-2233
127. Richard, J. P., and Frey, P. A. (1978) J. Amer. Chem. Soc., vol.100, pp.7757-7758.
128. Richard, J. P., and Frey, P. A. (1982) J. Amer. Chem. Soc., vol.104, pp.3476-3481
129. Pliura, D. H., Schomburg, D., Richard, J. P., Frey, P. A., and Knowels, J. R. (1980) Biochemistry, vol.19, pp.325-329
130. Tsai, M. D., and Chang, T. T. (1980) J. Amer. Chem. Soc., vol.102, pp.5416-5418
131. Webb, M. R., Trentham, D. R. (1980) J. Biol. Chem., vol.255, pp.1775-1779
132. Webb, M. R. (1982) Methods Enzymol., vol.87, pp.301-316
133. Higuchi, H., Endo, T., and Kaji, A. (1990) Biochemistry vol.29, pp.8747-9753
134. Victorova, L. S., Dyatkina, N. B., Mozzherin, D., Atrazhev, A. M., Krayevsky, A. A., and Kukhanova, M. K. (1992) Nucleic Acids Res., vol.20, pp.783-789
135. Li, H., Porter, K., Huang, F., and Shaw, B. R. (1995) Nucleic Acids Res., vol.23, pp.4495-4501
136. Tomasz, J., Shaw, B. R., Porter, K., Spielvogel, B.F., and Sood, A. (1992) Angew. Chem, vol.104, p.1404
137. He K., Porter K. W., Hasan A, Briley J. D., and Shaw B. R. (1999) Nucleic Acids Res., vol.27, pp.1788-1794
138. Babkina, G. T., Knorre, V. L., Knorre, D. G., and Lavrik, O. I. (1974) Dokl. Akad. Nauk SSSR, vol.216, pp.1165-1167 (in Russian)
139. Ankilova, V. N., Knorre, D. G., Kravchenko, V. V., Lavrik, O. I., and Nevinsky, G. A. (1975) FEBS Lett., vol.60, pp.172-175
140. Akhverdyan, V. Z., Kisselev, L. L., Knorre, D. G., Lavrik, O. I., and Nevinsky, G. A. (1976) Dokl. Akad. Nauk SSSR, vol.226, pp.698-701 (Russian)
141. Grachev M. A., and Zaychikov E. F. (1974) FEBS Lett., vol.49, pp.163-167
142. Sverdlov E. D., Tsarev S. A., and Levitan, T. L. (1979) in "Macromolecules in functioning cell" / Salvatore F., and Marino, G., ed., N.Y., Plenum Press, pp.149-158
143. Sverdlov E. D., Tsarev S. A., Modyanov, N. N., et. al. (1978) Bioorgan Khimia, vol.4, pp.1278-1280
144. Grachev, M. A., and Zaychikov, E. F. (1981) FEBS Lett., vol.137, pp.23-26
145. Akhverdyan V. Z., Kisselev L. L., Knorre D. G. *et al*. (1977) J. Mol. Biol., vol.113, pp.475-501
146. Budker V. G., Knorre D. G., Kravchenko V. V. *et al*. (1974) FEBS Lett., vol.49, pp.159-162
147. Bulychev, N. V., Lavrik, O. I., and Nevinsky, G. A. (1980) Molekul. Biol., vol.14, pp.558-567 (Russian)
148. Lavrik, O. I., Nevinsky, G. A., and Ryazankin, I. A. (1979) Molekul. Biol., vol.13, pp.1001-1011 (Russian)
149. Nevinsky, G. A., Lavrik, O. I., Favorova, O. O., Kisselev, L. L. (1979) Biorg. Khimia, vol.5, pp.352-364 (Russian)
150. Krauspe, R. and Lavrik, O. I. (1980) In Proc. Conf. "Biol. Implications of Nucleic Acid - Protein Interactions", Augustinjak J. Ed., N. Y. - Oxford, Elsevier / North - Holland Biomedical Press, pp.452-454
151. Nevinsky, G. A., and Denisov, A.Yu. (1981) Bioorgan. Khimia, vol.7, pp.1693-1700 (Russian)
152. Denisov A. Yu., Nevinskii G.A., and Lavrik O.I. (1982) Biokhimiya (Moscow), vol.47, pp.184-190. (Russian)
153. Denisov A. U., Nevinsky G.A., and Lavrik O.I. (1982) Bioorgan. Khimia, vol.8, pp.72-79 (Russian)
154. Sverdlov E. D., Tsarev S. A., and Kuznetsova H. F. (1980) FEBS Lett., vol.112, pp.296-298
155. Kovaleva G. K., *et al*. (1978) Biokhimia, Vol. 43, pp.525-534 (Russian)
156. Krauspe, R., Kovaleva, G. K., Gulyaev, N. N., et. al. (1978) Biokhimia, vol.43, pp.656-661 (Russian)
157. Kozlov, I. A., Shalamberidze, M. V., Novikova, I. Yu., et. al. (1977) Biokhimia, vol.42, pp.1704-1710 (Russian)
158. Likos, J. J., and Colman, R. F. (1981) Biochemistry, vol.20, pp.491-499
159. Eckstein, F., Bruns, W., and Parmeggiani, A. (1975) Biochemistry, vol.14, pp.5225-5232
160. Yarbrough, L. B. (1978) Biochem. Biophys. Res. Commun., vol.81, pp.35-41
161. Yarbrough, L. B., Schlageck, J. G., and Baughman, M. (1979) J. Biol. Chem., vol.254, pp.12069-12073
162. Pollack, S., and Auld, D.S. (1982) Analytical Biochemistry, vol.127, pp.81-88.
163. Buneva V. N., Kudryashova N.V., Kurbatov V.A., and Romashenko, A.G. (1978) Biokhimia, vol. 43, pp.2261-2263 (Russian)
164. Buneva V. N., Demidova T., Knorre D.G., *et al*. (1980) Molek. Biologia, vol.14, pp.1080-1087 (Russian)
165. Buneva, V. N., Dobrikova, E. J., and Knorre, D. G. (1981) FEBS Lett., vol.135, pp.159-163
166. Vlassov, V. V., Grachev, M. A., Lavrik, O. I. (1983) "Affinity Modification of Biopolymers", p.173, Knorre, D. G. ed., Novosibirsk, Nauka (Russian)
167. Smirnov, Yu. B., Lipkin, V. M., Ovchinnikov, Yu. A., et. al. (1981) Bioorgan. Khimia, vol.7, pp.1113-1116
168. Grachev, M. A., and Mustayev, A. A. (1982) FEBS Lett., vol.137, pp.89-94

^{31}P Chemical Shifts in NMR Spectra of Nucleotide Derivatives and Some Related Organophosphorus Compounds*

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Phosphorus Nuclear Magnetic Resonance (^{31}P NMR) spectroscopy is widely used in the investigation of structure and chemical reactions of mono-, oligo and polynucleotides, their analogs, and derivatives. Many of the aspects of ^{31}P NMR spectroscopy were the subject of reviews [1], and systematic compilation of ^{31}P NMR data is presented in the literature [2].

The theory of ^{31}P NMR chemical shifts is very complex and is not very well developed compared to ^{1}H NMR. In this short introduction we cannot go into any details of ^{31}P NMR spectroscopy. Some facts below about ^{31}P NMR of nucleotide derivatives, including data presented in the tables, are brought here for informational purposes and are intended to help our customers in their routine work.

The Typical range of the ^{31}P chemical shifts of most known nucleotide derivatives covers the region from 200 ppm to -30 ppm. Many of P(III) derivatives resonate in a lower magnetic field (200 ppm to 50 ppm) compared to P(V) derivatives (70 ppm to -30 ppm). As one can deduce from the tables below, there is no unique correlation between ^{31}P NMR chemical shifts and the nature of the substituents on the phosphorus atom. However, a huge volume of the experimental data on nucleotides and their derivatives accumulated in ^{31}P NMR spectroscopy over 50 years, has helped introduce a simple set of rules that are useful in the interpretation of ^{31}P chemical shift changes [2].

In our work on the synthesis of different nucleotide derivatives (as well as other phosphorus containing compounds), ^{31}P NMR serves as one of the most important tools for evaluation of the structure, assurance of quality, and integrity of the products. When you are ordering our products you may request ^{31}P NMR spectra of the nucleotide derivatives to be recorded and sent to you with the compounds. ^{31}P NMR spectra are usually easy to interpret and some reference data presented in the Tables should help you in most cases. However, we are glad to assist you in the assignment of all the signals and in the complete interpretation of the ^{31}P NMR spectra.

I. Pentavalent Phosphorus

A. Phosphate mono-, di-, triesters and their mono- and diamides

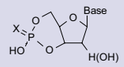
X	R^1	R^2	R^3	δ, ppm	Solvent	Reference
O	-OH	-OH	5'-O-Nucleoside	1.2; 2.8-3.6; 0.4-1.2	H_2O, pH 7.0; H_2O; Pyridine	3 2 1
O	-OH	-OH	3'-O-Nucleoside	1.0	H_2O, pH 7.0	3
O	3'-O-Nucleoside	-OH	5'-O-Nucleoside	-2.0-1.0	Pyridine	1
O	-OAryl	-OH	5'- or 3'-O-Nucleoside	-(5.0-6.0)	1,4-dioxane; Pyridine	1
O	-OAlkyl	-OAlkyl	5'- or 3'-O-Nucleoside	-(0.7-3.0)	Pyridine	1
O	-OAryl	-OAlkyl	5'- or 3'-O-Nucleoside (or -OAlkyl)	-7.7	Pyridine	1
O	-OAryl	-OAryl	5'- or 3'-O-Nucleoside	-(12.0-13.0)	Pyridine	1
O	$-NH_2$	-OH	5'- or 3'-O-Nucleoside	8.8-9.3	D_2O	1
O	-NH Alkyl	-OH	5'- or 3'-O-Nucleoside	5.0-6.5	Pyridine	1
O	-NH Aryl	-OH	5'- or 3'-O-Nucleoside	-1.0	Pyridine	1
O	5'-or 3'-O-Nucleoside	$-NH_2$	5'-or 3'-O-Nucleoside	10.8-12.1	D_2O, Pyridine	1
O	5'-or 3'-O-Nucleoside	-NHAlkyl	5'-or 3'-O-Nucleoside	8.4-9.2	Pyridine	1
O	5'-or 3'-O-Nucleoside	-NHAryl	5'-or 3'-O-Nucleoside	2.6-2.8	Pyridine	1
O	5'-or 3'-O-Nucleoside	-NHAryl	-OAryl	-(2.2-2.8)	Pyridine	1
O	5'-or 3'-O-Nucleoside	-NHAryl	-NHAryl	2.9	Pyridine	1
S	-OH	-OH	5'-or 3'-O-Nucleoside	43.5**	H_2O	1
S	5'- O-Nucleoside	-OH	5'-or 3'-O-Nucleoside	55.0-57.0**	H_2O	1, 3
S	5'- O-Nucleoside	-OAlkyl (-CNEt)	3'- O-Nucleoside	69.0**	H_2O	3

**Denotes range of ^{31}P chemical shifts for two diastereomers

* The ^{31}P chemical shifts are referenced to 85% H_3PO_4 and positive values mean downfield shifts of ^{31}P signals. Only ^{31}P NMR spectra recorded with spin-spin decoupling {^{1}H}-^{31}P have been considered. The chemical shifts and coupling constants are reported as a range or as an average number from multiple data points.

I. Pentavalent Phosphorus (continued)

B. Nucleoside 3',5'-cyclophosphate



X	δ, ppm	Solvent	Reference
O	-(1.5-2.0)	D_2O, pH7	1
S	52.0-55.0**	Pyridine	1

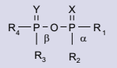
**Denotes range of ^{31}P chemical shifts for two diastereomers

C. Nucleoside 2',3'-cyclophosphate

X	δ, ppm	Solvent	Reference
O	17.6-18.5	DMF; Pyridine;MeOH	1
S	75.0-76.0**	H_2O	1

**Denotes range of ^{31}P chemical shifts for two diastereomers

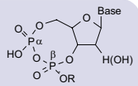
D. Pyrophosphate derivatives of nucleosides



X	Y	R_1	R_2	R_3	R_4	δα, ppm	δβ, ppm	Jaß, Hz	Solvent	Reference
O	O	5'-O-Nucleoside	-OH	-OH	-OH	-10.0	-6.0	20.7	H_2O	3
O	O	3'-O-Nucleoside	-OH	-OH	-OH	-(10.0-11.0)	-(5.0-6.0)	21.4	H_2O	3
O	O	5'-O-Nucleoside	-OH	-OH	5'-or 3'-O-Nucleoside	-(10.0-11.0)	-10.0	n/a	Pyridine	1
O	O	5'-O-Nucleoside	-OH	-OAryl	-OH	-11.2	-16.6	17.0	Pyridine	1
O	O	5'-O-Nucleoside	-OAryl	-OAryl	5'-O-Nucleoside	-19.0	-19.0	n/a	Pyridine	1
O	O	5'-O-Nucleoside	-OH	-OH	$-NH_2$	-11.3	-0.8	20.0	D_2O, pH10	1
O	O	5'-O-Nucleoside	-OH	-NHAryl	5'-O-Nucleoside	-11.2	-6.8	17.5	Pyridine	1
O	O	5'-O-Nucleoside	-NHAryl	-NHAryl	5'-O-Nucleoside	-(7.0-7.4)	-(7.0-7.4)	n/a	Pyridine	1
S	O	5'-O-Nucleoside	-OH	-OH	-OH	42.1**	-6.3**	30.8	H_2O	1
O	S	5'-O-Nucleoside	-OH	-OH	-OH	-11.7**	34.0**	31.2	H_2O	1

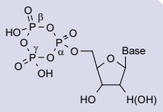
**Denotes range of ^{31}P chemical shifts for two diastereomers

E. Nucleoside 3',5'-cyclopyrophosphate



R	δα, ppm	δβ, ppm	Jαβ, Hz	Solvent	Reference
H	-13.7	-14.4	27.6	DMF	1
3'-O-Nucleoside	-13.0	-15.0	22.0	H_2O, pH 9	1

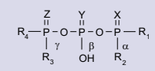
F. Nucleoside 5'-O-trimetaphosphate



δα	δβ	δγ	Jαβ, Hz	Jβγ, Hz	Jαγ, Hz	Solvent	Reference
-23.0	-24.0	-24.6	23.5	23.0	23.7	Pyridine	1

I. Pentavalent Phosphorus (continued)

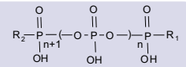
G. Triphosphate derivatives of nucleosides



X	Y	Z	R_1	R_2	R_3	R_4	δα, ppm	δβ, ppm	δγ, ppm	Jαβ, Hz	Jβγ, Hz	Solvent	Reference
O	O	O	-OH	-OH	-OH	-OH	-5.3(d)	-18.6(t)	-5.3(d)			H_2O	3
O	O	O	5'-O-Nuc.	-OH	-OH	-OH	-(10.0-11.0)(d)	-(20.0-23.0)(t)	-(5.0-7.7)(d)	20.0	20.0	H_2O,pH 7.0	1, 2, 3
O	O	O	5'-O-Nuc.	-OH	-OH	5'-O-Nuc.	-11.0(d)	-22.6(t)	-11.0(d)	n/a	n/a	H_2O, pH7.0	3
O	O	O	5'-O-Nuc.	-OH	-OH	-OAryl	-14.0	-24.5	-19.7	n/a	n/a	DMF:Pyridine=1:1	1
O	O	O	5'-O-Nuc.	-Alkyl	-OH	-OH	25.0(d)**	-(23.5-24.0)**	-9.7(d)**	21.8	19.5	D_2O	3, 5, 6
O	O	O	5'-O-Nuc.	-OH	-OH	-NHAlkyl	-11.8	-22.0	-3.6	22.5	22.5	DMSO: Pyridine=1:1	1
O	O	O	5'-O-Nuc.	-OH	-OH	-NHAryl	-11.4	-23.2	-10.2	n/a	n/a	H_2O, pH11	1
S	O	O	5'-O-Nuc.	-OH	-OH	-OH	42.0-44.0**	-(21.1-22.0)**	-(4.7-9.6)**	23.0-31.8	19.3-20.5	H_2O, pH 7.0	3, 4
O	S	O	5'-O-Nuc.	-OH	-OH	-OH	-11.4**	30.0**	-6.0**	6.5	27.3	H_2O, pH 7.0	1
O	O	S	5'-O-Nuc.	-OH	-OH	-OH	-10.6	-22.0	35.0	19.6	29.0	H_2O, pH 7.0	1

**Denotes range of ^{31}P chemical shifts for two diastereomers

H. Polyphosphate derivatives of Nucleosides



n=2,3,...

N	R^1	R^2	δp1, ppm	δpn, ppm	δn+1, ppm	$J_{1,2}$, Hz	J(n+1)n, Hz	Solvent	Reference
2	5'-O-Nuc.	-OH	-11.0 (d)	-22.7(d)	-10.3	19.0-20.0	19.0-20.0	H_2O	3
2, 3	5'-O-Nuc.	5'-O-Nuc.	-(10.8-11.2)(d)	-22.7(d)	-(10.8-11.2)	19.0-20.0	19.0-20.0	H_2O, pH 7.0	1, 3

II. Trivalent Phosphorus Compounds

R_1	R_2	R_3	δ, ppm	Solvent	Reference
3'-O-Nucleoside	-Cl	-OAlkyl	165.0-170.0	***	2
3'-or 5'-O-Nucleoside	$-N(Alkyl)_2$	-OAlkyl	149.0-150.0**	CD_3CN; $CDCl_3$	3
-OAlkyl	$-N(Alkyl)_2$	$-N(Alkyl)_2$	131.0-138.0	***	2
-Cl	-Cl	-Cl	215.0-220.0	***	2
-OAlkyl	-Cl	-Cl	177.0-180.0	***	2
-OAryl	-OAryl	-Cl	153.0-157.0	***	2
-OAryl	-Cl	-Cl	176.0-184.0	***	2
-OAryl	$-N(Alkyl)_2$	$-N(Alkyl)_2$	131.0	***	2
-OAryl	-OAryl	$-N(Alkyl)_2$	141.0	***	2
3'-or 5'-O-Nucleoside	3'-or 5'-O-Nucleoside	-OAlkyl	140.0-141.0**	$CDCl_3$	1

**Denotes range of ^{31}P chemical shifts for two diastereomers
***See ref.2 for solvent information

References

1. A.V. Lebedev, A.I. Rezvukhin, Nucl. Acids Res., 1984, V.12, N14 5547-5566.
2. M.M. Crutchfield, *et. al*, ^{31}P Nuclear Magnetic Resonance in Topics in Phosphorus Chemistry. New York, Intescience Publishers, 1967.
3. TriLink Biotechnologies, Inc. data.
4. J. Ludwig, F. Eckstein, J. Org. Chem., 1989, V.54, N3, 631-635.
5. Higuchi H., Endo T., Kaji A., 1990, Biochemistry, 29, 8747-8753.
6. Victorova L.S., Dyatkina N.B., Mozzherin D.F., Atkazhev A.M., Crayevsky A.A., Kukhanova M.K., Nucl. Acids Res., 1992, V.20, N4, 783-789.

Nucleoside Triphosphate Bibliography

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Product	Catalog #	Application	Title	Author	Journal	Year
2'-Fluoro-2'-dCTP, 2'-Fluoro-2'-dUTP	N-1008, N-1010	SELEX	Water soluble RNA based antagonist of AMPA receptors	Du M, et al	Neuropharmacology	2008
2'-Fluoro-2'-dCTP, 2'-Fluoro-2'-dUTP	N-1008, N-1010	Therapeutic	Multivalent 4-1BB binding aptamers costimulate CD8+ T cells and inhibit tumor growth in mice	McNamara J, et al	The Journal of Clinical Investigation	2008
N6-Methyl-ATP	N-1013	ATP hydrolysis	Asymmetric nucleotide transactions of the HslUV protease	Yakamavich J, Baker T, Sauer R	Journal of Molecular Biology	2008
3'-dGTP, 2'-O-Me-GTP	N-1017, N-3002	Therapeutic	The Imidazopyrrolopyridine Analogue AG110 Is a Novel, Highly Selective Inhibitor of Pestiviruses That Targets the Viral RNA-Dependent RNA Polymerase at a Hot Spot for Inhibition of Viral Replication	Paeshuyse J, et al	Journal of Virology	2007
3'-dGTP, 2'-O-Me-GTP, 2'-O-Me-ITP, ITP, 2',3'-ddGTP	N-1017, N-1020, N-1021, N-3002, N-4002	Therapeutic	Effects of Mutagenic and Chain-Terminating Nucleotide Analogs on Enzymes Isolated from Hepatitis C Virus Strains of Various Genotypes	Heck J, et al	Antimicrobial Agents and Chemotherapy	2008
4-Thio-UTP	N-1025	PCR, cross-linking	Eukaryotic ribosomal protein RPS25 interacts with the conserved loop region in a dicistroviral intergenic internal ribosome entry site	Nishiyama T, et al	Nucleic Acid Research	2007
4-Thio-UTP	N-1025	Microarray	Microarray analysis of newly synthesized RNA in cells and animals	Kenzelmann M, et al	Proceedings of the National Academy of Sciences of the United States of America	2007
8-Oxo-GTP, 8-Oxo-2'-dGTP	N-1066, N-2034	Incorporation	Assessing biomarkers of oxidative stress: Analysis of guanosine and oxidized guanosine nucleotide triphosphates by high performance liquid chromatography with electrochemical detection	Bolin C, Cardozo-Pelaez F	Journal of Chromatography B: Biomedical and Science Applications	2007
2'-deoxydiaminopurine-5'-TP	N-2003	Enzymatic synthesis, Glycerol nucleic acid	Enzymatic synthesis of DNA on glycerol nucleic acid templates without stable duplex formation between product and template	Ching-Hsuan Tsai, Jingyang Chen, and Jack W. Szostak	Proceedings of the National Academy of Sciences of the United States of America	2007
2'-deoxydiaminopurine-5'-TP, 2'-dITP, 2'-dUTP, 5-Methyl-2'-dCTP	N-2003, N-2012, N-2020, N-2026	PCR	Inversing the natural hydrogen bonding rule to selectively amplify GC-rich ADAR-edited RNAs	Suspène R, et al	Nucleic Acid Research	2008
5-Bromo-2'-dUTP	N-2008	Reverse Transcription, therapeutic	Interactions between HIV-1 Reverse Transcriptase and the Downstream Template Strand in Stable Complexes with Primer-Template	Rutvisuttinunt W, Meyer P, Scott W	PLoS ONE	2008

Product	Catalog #	Application	Title	Author	Journal	Year
5-AA-2'-dCTP, 5-AA-2'-dUTP, 5-Propynyl-2'-dCTP, 5-Propynyl-2'-dUTP	N-2016, N-2017, N-2048, N-2049	PCR	Systematic characterization of 2'-deoxynucleoside- 5'-triphosphate analogs as substrates for DNA polymerases by PCR and kinetic studies on enzymatic production of modified DNA	Kuwahara M, et al	Nucleic Acid Research	2006
8-Oxo-2'-dGTP	N-2034	Therapeutic	Selection of Anthrax Toxin Protective Antigen Variants that Discriminate Between the Cellular Receptors TEM8 and CMG2 and Achieve Targeting of Tumor Cells	Chen K, et al	Journal of Biological Chemistry	2008
8-Oxo-2'-dGTP	N-2034	Substrate specificity	Structural Basis for Different Substrate Specificities of Two ADP-Ribose Pyrophosphatases from Thermus thermophilus HB8	Wakamatsu T, et al	Journal of Bacteriology	2008
8-Oxo-2'-dGTP	N-2034	Therapeutic	Trace amounts of 8-oxo-dGTP in mitochondrial dNTP pools reduce DNA polymerase ? replication fidelity	Pursell Z, et al	Nucleic Acid Research	2008
3'-dGTP	N-3002	Therapeutic	P-TEFb is Critical for the Maturation of RNA Polymerase II into Productive Elongation In Vivo	Ni Z, et al	Molecular and Cellular Biology	2008
3'-dCTP	N-3003	Therapeutic	Mechanism of Activation of ß-d-2'-Deoxy-2'-Fluoro-2'-C-Methylcytidine and Inhibition of Hepatitis C Virus NS5B RNA Polymerase	Murakami E, et al	Antimicrobial Agents and Chemotherapy	2007
3'-dUTP	N-3005	Transcription	Transcription coupled nucleotide excision repair in Escherichia coli can be affected by changing the arginine at position 529 of the β subunit of RNA polymerase	Ganesan A, et al	DNA Repair (Amst)	2007
3'-Azido-2',3'-ddATP, 3'-Azido-2',3'-ddGTP	N-4002, N-4007	Reverse Transcription, therapeutic	3'-Azido-2',3'-dideoxynucleoside 5'-triphosphates inhibit telomerase activity in vitro, and the corresponding nucleosides cause telomere shortening in human HL60 cells	Liu X, et al	Nucleic Acid Research	2007
3'-Azido-2',3'-ddATP, 3'-Azido-2',3'-ddCTP, 3'-Azido-2',3'-ddGTP	N-4007, N-4008, N-4014	Therapeutic	A novel mechanism of selectivity against AZT by the human mitochondrial DNA polymerase	Hanes J, Johnson K	Nucleic Acid Research	2007
3'-Azido-2',3'-ddTTP	N-4009	Reverse Transcription, therapeutic	Molecular Mechanism by Which the K70E Mutation in Human Immunodeficiency Virus Type 1 Reverse Transcriptase Confers Resistance to Nucleoside Reverse Transcriptase Inhibitors	Sluis-Cremer N, et al	Antimicrobial Agents and Chemotherapy	2007
3'-Azido-2',3'-ddTTP	N-4009	Therapeutic	Mechanisms by Which the G333D Mutation in Human Immunodeficiency Virus Type 1 Reverse Transcriptase Facilitates Dual Resistance to Zidovudine and Lamivudine	Zelina S, et al	Antimicrobial Agents and Chemotherapy	2008
ppGpp	N-6001	Replication	Nutritional Control of Elongation of DNA Replication by (p)ppGpp	Wang J, Sanders G, Grossman A	Cell	2007
ppGpp	N-6001	Therapeutic	Interaction of Bacillus subtilis CodY with GTP	Handke L, Shivers R, Sonenshein A	Journal of Bacteriology	2008

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TriLink's Publications, Patents and Presentations

Publications

Koukhareva, I., Lebedev, A. 3'-Protected 2'-Deoxynucleoside 5'-Triphosphates as a Novel Tool for Heat-Triggered Activation of PCR. *Analytical Chemistry* 2009, 81 (in press).

Lebedev, A. Heat Activatable Primers for Hot Start PCR: Oligonucleotide Synthesis and Basic PCR Set-up in *Current Protocols in Nucleic Acid Chemistry*, Wiley Interscience 2009 (in press).

Ashrafi, E., Paul, N. Heat Activatable Primers for Hot Start Primers for Hot Start PCR and Hot Start One-step RT-PCR - End Point and Real-Time Experiments in *Current Protocols in Molecular Biology*, Wiley Interscience 2009 (in press).

Timoshchuk, V., Hogrefe, R. The "Corey Reagent", 3,5-Di-tert-butyl-1,2-benzoquinone, as a Modifying Agent in the Synthesis of Fluorescent and Double-Headed Nucleotides. *Nucleosides, Nucleotides and Nucleic Acids* 2009 (in press).

Shum, J., Paul, N. Chemically modified primers for improved multiplex polymerase chain reaction. *Analytical Chemistry* 2009, 388: 266-272.

Koukhareva I., Huang, H., Yee, J., Shum, J., Paul, N., Hogrefe, R., Lebedev, A. Heat Activatable 3'-Modified dNTPs: Synthesis and Application for Hot Start PCR. *Nucleic Acids Symposium Series* 2008, Oxford University Press, No. 52: 259-260.

Koukhareva I., Huang, H., Yee, J., Paul, N., Hogrefe, R., Lebedev, A. A new Approach to Improved Hot Start PCR: Nucleoside 5'-Triphosphates with Thermolabile 3'-Protecting Group. *Collection Symposium Series* 2008, Academy of Sciences of the Czech Republic, 10: 259-263.

Lahoud, G., Timoshchuk, V., Lebedev, A., De Vega, M., Salas, M., Arar, K., Hou, Y., Gamper, H., Jr. Enzymatic Synthesis of Structure-Free DNA with Pseudo-Complementary Properties. *Nucleic Acids Research* 2008, 36: 3409-3419.

Lahoud, G., Timoshchuk, V., Lebedev, A., Arar, K., Hou, Y., Gamper, H., Jr. Properties of pseudo-complementary DNA substituted with weakly pairing analogs of guanine or cytosine. *Nucleic Acids Research* 2008, 36: 6999-7008.

Nawrot, B., Paul, N., Rebowska, B., Stec, J. Significance of stereochemistry of 3'-terminal phosphorothioate-modified primer in DNA polymerasemediated chain extension. *Molecular Biotechnology* 2008, 40(2): 119-126.

Lebedev, A., Combs, D., Hogrefe, R. Preactivated Carboxyl Linker for the Rapid Conjugation of Alkylamines to Oligonucleotides on Solid Support. *Bioconjugate Chemistry* 2007, 18: 1530-1536.

Pirollo, K., Rait, A., Zhou, Q., Hwang, S., Dagata, J., Zon, G., Hogrefe, R., Palchik, G., Chang, E. Materializing the Potential of Small Interfering RNA via a Tumor-Targeting Nanodelivery System. *Cancer Research* 2007, 67(7): 2938-2943.

Hogrefe, R., Lebedev, A., Zon, G. Kathleen F. Pirollo K., Rait A., Zhou O., Yu, W., Chang E. Chemically modified short interfering hybrids (siHYBRIDS): Nanoimmunoliposome delivery in vitro and in vivo for RNAi of HER-2. *Nucleosides, Nucleotides & Nucleic Acids* 2006, 25: 889-907.

Hussein, Y., Anderson, N., Lian, T., Abdou, I., Strekowski, L., Victor A. Timoshchuk, V., Vaghefi, M., Netzel, T. Solvent and Linker Influences on AQ•-/dA•+ Charge-Transfer State Energetics and Dynamics in Anthraquinonyl-Linker-Deoxyadenosine Conjugates. *Journal of Physical Chemistry A.* 2006, 110: 4320-4328.

Pirollo, K., Zon, G., Rait, A., Zhou, Q., Yu, W., Hogrefe, R., Chang, E. Tumor-Targeting Nanoimmunoliposome Complex for Short Interfering RNA Delivery. *Human Gene Therapy* 2006, 17: 117-124.

Timoshchuk, V. Functionalization of Pyrimidine and Purine Nucleosides at C4 and C6: C-Nucleophilic Substitution of Their C4- and C6-(1,2,4-Triazol-1-yl) Derivatives. Nucleosides, *Nucleotides and Nucleic Acids* 2005, 24: 1043-1046.

Koukhareva I., Vaghefe M., and Lebedev A. Synthesis and properties of NTP analogs with modified triphosphate chains in Nucleoside triphosphates: *Chemistry, Biotechnology and Biological Applications*, Taylor and Francis 2005: 39-104.

Koukhareva, I., Lebedev, A. Chemical route to the capped RNAs. *Nucleosides, Nucleotides & Nucleic Acids* 2004, 23(10): 1667-1680.

Timoshchuk, V., Hogrefe, R., Vaghefi, M. Improved and Reliable Synthesis of 3' - Azido-2',3'-dideoxyguanosine Derivatives. *Nucleosides, Nucleotides & Nucleic Acids* 2004, 23[1-2]: 171-181.

Wang, K., Li, J., Fang, X., Schuster, S., Vicens, M., Kelley, S., Lou, H., Li, J., Beck, T., Hogrefe, R., Tan, W. Novel Fluorescent Molecular Beacon DNA Probes for Biomolecular Recognition. *Biomedical Photonics Handbook* 2003, 57: 1-23.

Fang, X., Cao, Z., Beck, T., Tan, W. Molecular Aptamer for Real-time Oncoprotein latelet-derived Growth Factor Monitoring by Fluorescence Anisotropy. *American Chemical Society* 2001, 73(23): 5752-5757.

Lebedev, A., Koukhareva, I., Beck, T., Vaghefi, M. Preparation of oligodeoxynucleotide 5'-triphosphates using solid support approach. *Nucleosides, Nucleotides & Nucleic Acids* 2001, 4-7[20]: 1403-1409.

Zhang, P., Beck, T., Tan, W. Design of a Molecular Beacon DNA Probe with Two Fluorophores. *Wiley-VCH Verlag GmbH* 2001, 40(2): 402-405.

Hogrefe, R. An Antisense Oligonucleotide Primer. *Antisense and Nucleic Acid Drug Development* 1999, 9: 351-357.

Patents and Pending Patents

Lebedev, A., Koukhareva I. Chemically Modified Nucleoside 5'-Triphosphates for Thermally Initiated Amplification of Nucleic Acid. Provisional Patent Application 61/056,324, May 27, 2008.

Zon, G., Lebedev, A. Chemically Modified Oligonucleotide Primers for Nucleic Acid Amplification. Patent Applications AU2007268075, CA2653841, EP2032714, US2007281308, WO2007139723, December 6, 2007.

Hogrefe, R., Vaghefi, M. Pre-activated carbonyl linkers for modification of oligonucleotides. Patent 6,320,041, November 20, 2001.

Presentations

Modified dNTPs for Improved PCR Performance – An Innovative use of Nucleoside Protecting Groups. Paul, N. TIDES: Oligonucleotide and Peptide Technology and Product Development, Las Vegas, NV, May 17-20, 2009.

The Next Generation in Hot Start PCR - CleanAmp™ dNTPs. Paul, N. Quantitative PCR. San Diego, CA, March 16-18, 2009.

The Next Generation in Hot Start PCR - CleanAmp™ Primers and dNTPs. Paul, N. qPCR Event 2009, Munich, Germany, March 9-11, 2009.

Modified dNTPs for Improved PCR Performance – An Innovative use of Nucleoside Protecting Groups. Paul, N. qPCR Symposium USA, Millbrae, CA, November 10-13, 2008.

Chemically Modified dNTPs for Improved Hot Start PCR. Paul, N. 16th Nucleic Acid-Based Technologies, Baltimore, MD, June 18-20, 2008.

A New General Approach to Hot Start PCR: Nucleoside 5'-Triphosphates with Thermolabile 3'-Protecting Group. Lebedev, A. XIVth Symposium on Chemistry of Nucleic Acid Components Conference, Czech Republic, June 8, 2008.

Unique Approaches to Hot Start PCR. Paul, N. Quantitative PCR Meeting, San Diego, CA, April 22, 2008.

Chemically Modified Primers for Improved Hot Start PCR. Paul, N. Cambridge Healthtech Institute's 14th Annual Nucleic Acid-Based Technologies Meeting, Arlington, VA, June 26-28, 2006.

The Challenge of Synthesizing Randomer Oligonucleotides for the Diagnostic Industry. Beck, T. TIDES: Oligonucleotide and Peptide Technology and Product Development, La Costa, CA, May 1-4, 2006.

Posters

Chemically Modified dNTPs for Improved Hot Start PCR.
Shum, J., Le, T., Yee, J., Huang, H., Timoshchuk V., Paul, N., Hogrefe, R., Koukhareva, I., Lebedev, A.
Genomic Tools, San Francisco, CA, June 8-10, 2009.
Bio-IT World, Boston, MA, April 27-29, 2009.
Quantitative PCR, San Diego, CA, March 16-18, 2009.
Molecular Medicine Tri-Conference, San Francisco, CA, February 24-27, 2009.
qPCR Symposium, San Francisco, CA, November 10-12, 2008.
Nucleic Acid Based Technologies, Baltimore, MD, June 18-20, 2008.

Chemically Modified Primers for Improved Multiplexed PCR.
Yee, J., Hildalgo-Mendez, E., Lebedev, A., Hogrefe, R., Zon, G., Le, T., Paul, N., Shum, J.
Genomic Tools, San Francisco, CA, June 8-10, 2009.
Bio-IT World, Boston, MA, April 27-29, 2009.
Quantitative PCR, San Diego, CA, March 16-18, 2009.
Molecular Medicine Tri-Conference, San Francisco, CA, February 24-27, 2009.
qPCR Symposium, San Francisco, CA, November 10-12, 2008.
Discovery2Diagnostics, San Diego, CA, October 20-22, 2008.
Data-Driven Discovery, Providence, RI, September 22-25, 2008.

A New General Approach to Hot Start PCR: Nucleoside 5'-Triphosphates with Thermolabile 3'-Protecting Group.
Koukhareva, I., Huang, H., Yee, J., Paul, N., Hogrefe, R., Lebedev, A.
XIVth Symposium on Chemistry of Nucleic Acid Components, Czech Republic, June 8, 2008.

Chemically Modified Primers for Hot Start PCR.
Lebedev, A., Paul, N., Yee, J., Kellum, J., Timoshchuk, V., Shum, J., Hogrefe, R., Zon, G.
Beyond Genome, San Francisco, CA, June 8-11, 2008.
XIVth Symposium on Chemistry of Nucleic Acid Components, Czech Republic, June 8, 2008.
Oligonucleotide and Peptide Technology and Product Development, Las Vegas, CA, May 19-21, 2008.
Quantitative PCR, San Diego, CA, April 21-23, 2008.

Chemically Modified Primers for Improved Hot Start PCR.
Lebedev, A., Hogrefe, R., Paul, N., Timoshchuk, V., Yee, J., Zon, G.
Gordon Research Conference (Nucleosides, Nucleotides, & Oligonucleotides), Newport, RI, July 1, 2007.
Gordon Research Conference (Nucleic Acids), Newport, RI, June 3, 2007.
Oligonucleotide and Peptide Technology and Product Development, Las Vegas, CA, May 21-23, 2007.
Quantitative PCR, San Diego, CA, March 19-21, 2007.
Discovery 2 Diagnostics Conference, Boston, MA, September 25-27, 2006.
XVII International Roundtable on Nucleosides, Nucleotides and Nucleic Acids, Bern, Switzerland, September 3-7, 2006.
Nucleic Acid Based Technologies, Arlington, VA, June 26, 2006.

Chemical Route to the Capped RNAs.
Koukhareva, I., Lebedev, A.
XVI International Roundtable on Nucleosides, Nucleotides and Nucleic Acids, Minneapolis, MN, September 12-16, 2004.

Functionalization of Pyrimidine and Purine Nucleosides at C4 and C6: C-Nucleophilic Substitution of Their C4- and C6-(1,2,4-Triazol-1-yl) Derivatives.
Timoshchuk, V.
XVII International Roundtable on Nucleosides, Nucleotides and Nucleic Acids, Minneapolis, MN, September 12-16, 2004.

Improved and Reliable Synthesis of 3'-Azido-2',3'-dideoxyguanosine Derivatives.
Timoshchuk, V., Vaghefi. M.
Gordon Research Conference, Salve Regina University, Newport, RI, July 20-25, 2003.

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