

# ***NUCLEIC ACID FACILITY***

**CUSTOM OLIGONUCLEOTIDE SYNTHESIS**

**1999**



*Cancer Center*

*Department of Chemistry*

*University of Pennsylvania*



Welcome

## **Welcome to the NUCLEIC ACID FACILITY**

The Nucleic Acid Facility has provided high quality custom oligonucleotides to the university community for the past 15 years. Oligonucleotides from the facility are desalted and lyophilized, ready for use in PCR, sequencing, cloning, mutagenesis and many other experiments. Customer satisfaction is guaranteed.

In addition to the standard oligonucleotide synthesis, the facility offers ready made standard primers (HPLC purified) at low cost and the facility can furnish modified DNA or RNA for special needs. Among the special modifications provided routinely are: phosphorothioate oligonucleotide, methyl phosphonate DNA, 2'-OMe methyl phosphonate RNA, phosphorylation, cyclic oligonucleotides, oligonucleotide linkers, insertion of modified nucleosides into specific sequences, and labeling with digoxigenin, biotin, cholesterol, DNP, acridine, psoralen, dyes, and dual dyes.

We welcome inquiries about specialty synthesis items not covered in this catalog. Contact us for free consultation about your DNA application.

Custom oligonucleotides may be ordered online or by FAX and Email. Charges vary according to services. [Cancer Center members receive a 10% discount on all services.](#) Other non-profit and commercial organizations are invited to inquire about pricing. There is no charge for on-campus delivery. The charge for Federal Express shipment is \$10. Delivery times range from two days to two weeks, depending on the level of purification and modification required.

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**Web:** <http://www.med.upenn.edu/dna>

**Email:** [chemdna@mail.med.upenn.edu](mailto:chemdna@mail.med.upenn.edu)



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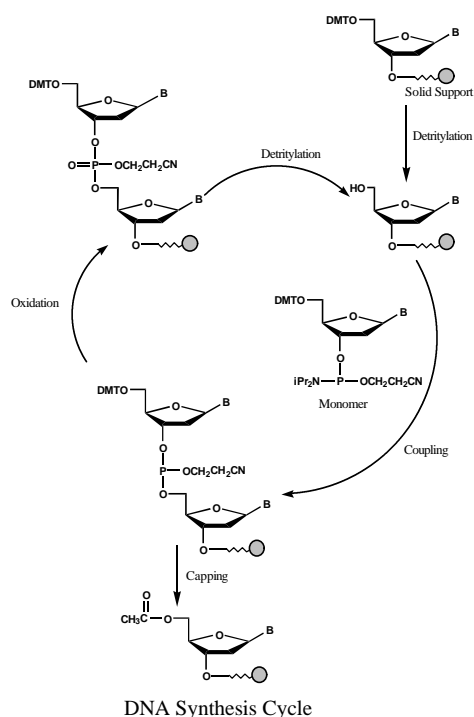
# Overview of DNA Synthesis

The field of DNA synthesis exploded with the pioneering work of Caruthers and his coworkers in the early 1980s. Caruthers' team is credited with the development of silica-based solid supports and the discovery of the highly efficient nucleoside phosphoramidite synthesis reagents. Phosphoramidites later led to the development of the first truly successful automated DNA synthesizers, having a major impact in the fields of molecular biology, biotechnology, and biological chemistry.

DNA synthesis is a cyclical process that assembles a chain of nucleotides from the 3'-end to the 5'-end. The 3'-nucleoside is covalently attached to a solid support and successive nucleotide monomers are added one by one through a cycle of four chemical reactions: detritylation, coupling, capping and oxidation. As the following illustration shows, the first step of the synthesis cycle is detritylation, where the dimethoxytrityl (DMT) group is removed with trichloroacetic acid to free the 5' hydroxyl for the coupling reaction. The next step is coupling, in which the 5'-OH of the oligonucleotide reacts with an activated monomer created by simultaneously adding the phosphoramidite nucleoside monomer and tetrazole. The tetrazole protonates the nitrogen of the phosphoramidite, making it susceptible to nucleophilic attack. The next step, capping, terminates any chains that did not undergo coupling. Since the unreacted chains have a free 5' OH, they are capped by acetylation with acetic anhydride and 1-methylimidazole. Capping minimizes the length of impurities, making it easy to do post-synthesis trityl-selective purification of the final product. Finally, the internucleotide linkage is converted from the phosphite to the more stable phosphotriester by oxidation with an iodine solution. For the synthesis of phosphorothioate oligonucleotides, the internucleotide phosphite is oxidized by a sulfuring reagent. After oxidation, the DMT group is removed with trichloroacetic acid and the cycle is repeated until chain elongation is complete. The amount of DMT released from each cycle is monitored to insure high coupling efficiency. The protected oligonucleotide with/without DMT is cleaved from the solid support with concentrated ammonium hydroxide. Ammonia treatment also removes the cyanoethyl phosphate protecting groups. The crude DNA in ammonium hydroxide solution is then heated to remove the protecting groups on the exocyclic amines of the bases.

Advances in nucleic acid chemistry have made possible the synthesis of oligonucleotides with modified backbones, non-standard bases, or with non-radioactive labels attached to the 3' or 5'-termini. RNA, OMe-RNA and other modified oligonucleotides can be synthesized with corresponding monomer phosphoramidites by using the same synthesis cycle as DNA.

All the oligonucleotides are synthesized on Expedite™ 8909 Nucleic Acid Synthesis Systems in this facility.



## References:

- Agrawal, S. (1993). *Protocols for Oligonucleotides and Analogs*, **20**.
- Agrawal S. (1994). *Protocols for Oligonucleotide Conjugates*, **26**.
- Beaucage, S. L. and Caruthers, M. H. (1981). *Tetrahedron Lett.*, **22**, 1859.
- Eckstein, F. (1991). *Oligonucleotides and Analogies*.
- Letsinger, R. L. finnan, J. L., Heavner, G. A., and Lunsford, W. B. (1975). *J. Am. Chem. Soc.*, **97**, 3278.
- McBride, L. J. and Caruthers, M. H. (1983). *Tetrahedron Lett.*, **24**, 245.
- Matteucci, M. D. and Caruthers, M. H. (1980). *Tetrahedron Lett.* **21**, 719.

# Overview of DNA Purification

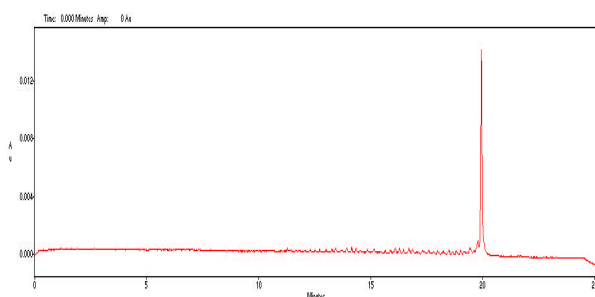
Our advanced DNA synthesizers and our care with chemical reagents result in high quality oligonucleotides. Desalted DNA is suitable for direct use in sequencing or PCR in many cases. However, we offer a range of DNA purification protocols using tC-18 Sep-Pak™ cartridge or reversed-phase HPLC. The recovery yield from oligo purification is 25-50% of the synthetic yield. DNA with modifications may give lower yields.

Synthesis Scale	Desalted Yield (20-mer)	HPLC Yield (20-mer)
25-nmole	5 OD	0.15 mg
50-nmole	10 OD	0.30 mg
0.2-μmole	40 OD	1.20 mg
1.0-μmole	160 OD	4.80 mg
10-μmole	1600 OD	48.00 mg

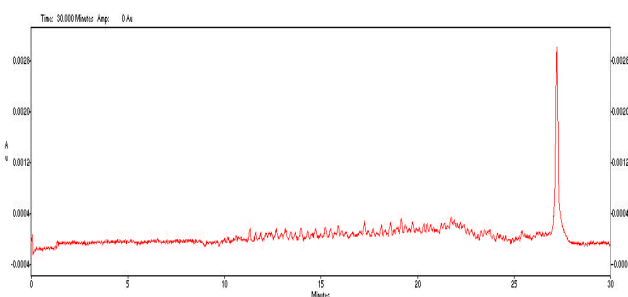
The step-wise coupling efficiency for DNA synthesis is about 99%. The final yield is dependent on the length and sequence of the oligo.

## DESALT PURIFICATION

After deprotection, the ammonia solution is removed from the oligonucleotide by vacuum. All the protected groups, small failure strands and ammonia salt are then removed by sodium acetate-ethanol precipitation. The resulting oligo is in sodium salt form. Desalted, quantified and lyophilized DNA, with synthesis report, is delivered in 2 business days.



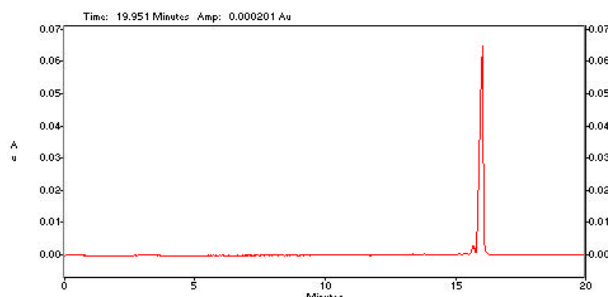
The electropherogram of a desalted 48-mer: 5'GCATGACTGGTGGTCAGCAATGGGCCCTGAGGAAACTCCAATCCC



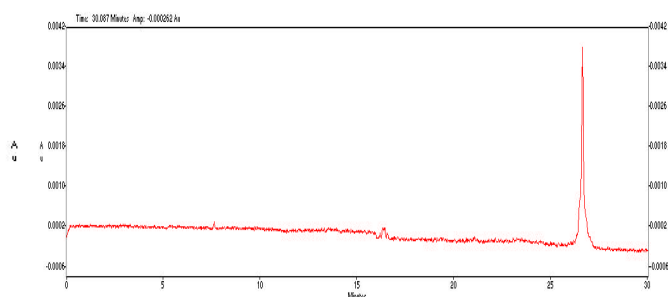
The electropherogram of desalted 89-mer: 5'GGAATTCCATATGAAAAGCCA TTTTGAACCACCTACCCTTCACGAACTGTATGATTAGACGTGACGGCC CCAAACCTTGTACCGGAGG

## HPLC Purification

The trityl-on full length DNA strands are separated from the trityl-on and trityl-off failure strands by HPLC on a reversed-phase PRP-1 Hamilton column (4.1 x 250 mm), and the trityl groups are removed on the column with trifluoroacetic acid. The full length oligos are collected, desalted with ethanol precipitation, quantified and lyophilized. HPLC-purified DNA, with HPLC and synthesis report, is delivered in 5 business days.



The electropherogram of an HPLC-purified 20-mer: 5'GTCATCATCATCCG GGTCTC

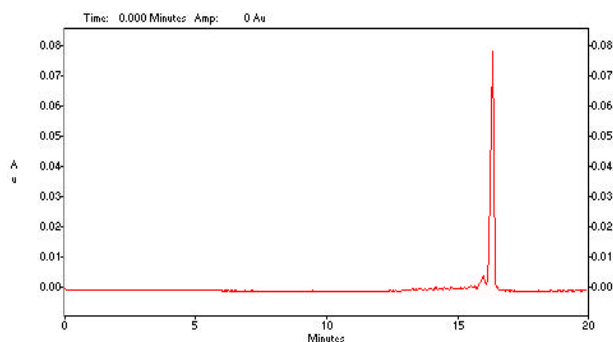


The electropherogram of an HPLC-purified 89-mer: 5'GGAATTCCATATG AAAAGCCATTTTGAACCACCTACCCTTCACGAACTGTATGATTAGACG TGACGGCCCCAAACCTTGTACCGGAGG

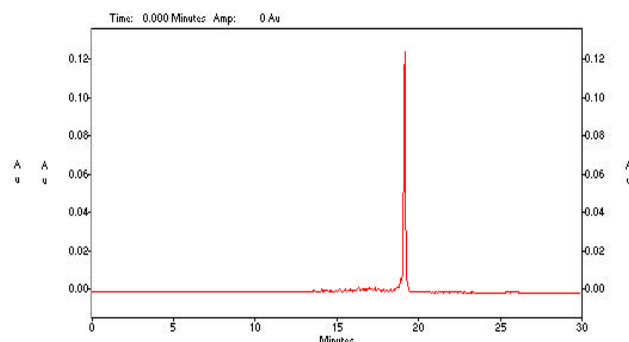
# Overview of DNA Purification

## Cartridge Purification

The trityl-on DNA strands are separated from the trityl-off failure strands on a tC-18 Sep-Pak™ cartridge. Detritylation is carried out on the cartridge with trifluoroacetic acid. Detrityled oligos are collected, desalted with ethanol precipitation, quantified and lyophilized. Cartridge purification is less effective than HPLC and recommended only for unmodified oligos less than 40 nucleotides in length. Cartridge purified DNA, with synthesis report, is delivered in 3 business days.



The electropherogram of a cartridge-purified 21-mer: 5'TGAAGGATGGACATGACGGAC



The electropherogram of a cartridge-purified 36-mer: 5'CAAACAGACACCATGGTTGTGCGGCGTATCTTCACC

## QUALITY CONTROL

To ensure the oligonucleotides are of high quality, all the oligonucleotides are synthesized by highly trained technicians using state of the art DNA synthesizers and high quality chemical reagents. The trityl color is monitored to ensure high coupling efficiency for each oligonucleotide synthesized in this facility. Any oligonucleotide that are not meet our standards will be remade. The quality of all the oligonucleotides with special modifications are checked by capillary electrophoresis(CE). At least 10% of the DNA oligonucleotides are analyzed by CE routinely.

## NOTES ABOUT DNA SAMPLES

All DNA samples are delivered as lyophilized powders and should be stored at -20°C. The DNA samples should be stored at -70°C if dissolved in water or buffer.

One OD unit is the absorbency of a 1.0 mL oligonucleotide solution, measured at 260 nm in a 1.0 cm path-length cuvette. One OD unit is approximately equal to 33 micrograms of single-stranded DNA, or 5 nanomoles of a 20-mer.

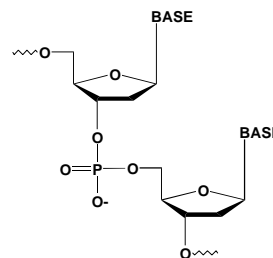
# Standard Oligonucleotide Synthesis

## DNA Synthesis

DNA (up to 200 nucleotides) is synthesized on Expedite™ 8909 Nucleic Acid Synthesis Systems using standard phosphoramidite chemistry. There are no set-up charges.

Synthesis Scale	Synthesis Charge/base		Purification Charge/oligo	
	CC*	PENN	Cartridge	HPLC
25-nmole	\$0.80	\$0.90	NA	NA
50-nmole	\$0.90	\$1.00	\$10	\$25
0.2- $\mu$ mole	\$1.10	\$1.20	\$10	\$25
1.0- $\mu$ mole	\$2.50	\$2.75	\$10	\$50
10- $\mu$ mole	\$14.00	\$15.50	NA	\$200

\* Cancer Center member rate.



DNA

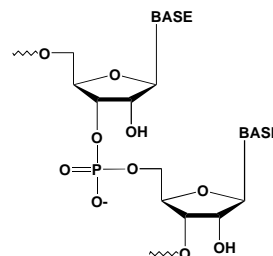
## RNA Synthesis

RNA (up to 40 nucleotides) is synthesized on Expedite™ 8909 Nucleic Acid Synthesis System with 2'-silyl protected RNA phosphoramidites. Deprotected and desalted RNA oligos are delivered in 10 business days. There are no set-up charges. No purification is available for RNA oligos.

Synthesis Scale	Yield(20-mer)	Charge/base
0.2- $\mu$ mole	15 OD	\$8.00
1.0- $\mu$ mole	70 OD	\$10.00

Yields of RNA oligo are greatly dependent on the sequence.

The coupling efficiency for RNA synthesis is about 95%.



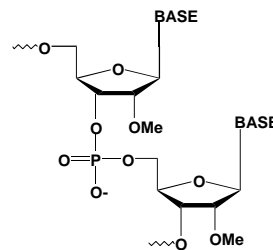
RNA

## 2'-OMe RNA Synthesis

2'-OMe RNA (up to 40 nucleotides) is synthesized with 2'-OMe RNA phosphoramidites. Deprotected and desalted oligos are delivered in 10 business days. There are no set-up charges. HPLC purification is available for 2'-OMe RNA oligos (charge is same as DNA purification).

Synthesis Scale	Yield(20-mer)	Charge/base	HPLC/oligo
0.2- $\mu$ mole	20 OD	\$8.00	\$25
1.0- $\mu$ mole	80 OD	\$10.00	\$50

The coupling efficiency for 2'-OMe RNA synthesis is about 95%.



2'-OMeRNA

## Chimeric Oligonucleotide Synthesis

Oligonucleotides containing a mixture of different sugars and/or backbone chemistries can be synthesized. Many possible chimeric oligonucleotides can be synthesized. Please call for more details.

# Standard Primers

All the primers listed below are HPLC purified and checked by CE to insure the high quality. For an updated list, please see our web site. If a common primer you require is not listed, it will be made available to you free of charge.

Primers are packaged in vials of 1 OD unit each; charge is \$10/ primer. Same day delivery.

Primers	Sequence	Length	T <sub>m</sub> (°C)	MW	ε *(OD/μmol)
T7 Promoter	5'-TAA TAC GAC TCA CTA TAG GG-3'	20-mer	51	6125	205
T3 Promoter	5'-CAA TTA ACC CTC ACT AAA GG-3'	20-mer	51	6054	203
M13 Forward (-20)	5'-GTA AAA CGA CGG CCA GTG-3'	18-mer	54	5558	188
M13 Forward (-41)	5'-GGT TTT CCC AGT CAC GAC-3'	18-mer	54	5451	163
M13 Reverse (-27)	5'-GGA AAC AGC TAT GAC CAT G-3'	19-mer	53	5846	200
M13 Reverse (-48)	5'-AGC GGA TAA CAA TTT CAC AC-3'	20-mer	51	6094	207
SP6 Promoter	5'-TAC GAT TTA GGT GAC ACT ATA G-3'	22-mer	53	6773	225
pBluescript SK	5'-CGC TCT AGA ACT AGT GGA TC-3'	20-mer	55	6117	194
pBluescript KS	5'-CTC GAG GTC GAC GGT ATC G-3'	19-mer	59	5854	180
Lambda gt11 (forward)	5'-GGT GGC GAC GAC TCC TGG AGC CCG-3'	24-mer	71	7396	223
Lambda gt11 (reverse)	5'-TTG ACA CCA GAC CAA CTG GTA ATG-3'	24-mer	59	7346	243
Lambda gt10 (forward)	5'-AGC AAG TTC AGC CTG GTT AAG-3'	21-mer	56	6470	212
Lambda gt10 (reverse)	5'-CTT ATG AGT ATT TCT TCC AGG GTA-3'	24-mer	55	7349	227
pBR322 Bam HI, CW	5'-CAC TAT CGA CTA CGC GAT CA-3'	20-mer	55	6046	192
pBR322 Bam HI, CCW	5'-ATG CGT CCG GCG TAG A-3'	16-mer	52	4922	155
pBR322 Eco RI, CW	5'-GTA TCA CGA GGC CCT T-3'	16-mer	50	4857	148
pBR322 Eco RI, CCW	5'-GAT AAG CTG TCA AAC-3'	15-mer	42	4584	158
pBR322 HIND III, CW	5'-GAC AGC TTA TCA TCG-3'	15-mer	44	4552	145
pBR322 HIND III, CCW	5'-GCA ATT TAA CTG TGA T-3'	16-mer	42	4895	162
pBR322 Pst I, CW	5'-GCT AGA GTA AGT AGT T-3'	16-mer	44	4960	168
pBR322 Pst I, CCW	5'-AAC GAC GAG CGT GAC-3'	15-mer	48	4611	156
pBR322 Sal I, CW	5'-ATG CAG GAG TCG CAT-3'	15-mer	46	4617	152
pBR322 Sal I, CCW	5'-AGT CAT GCC CCG CGC-3'	15-mer	52	4514	132
Random Hexamer	5'-NNNNNN-3'	6-mer	58	1792	12
PolydT20	5'-TTT TTT TTT TTT TTT TT-3'	20-mer	35	6022	158

\* Extinction Coefficient



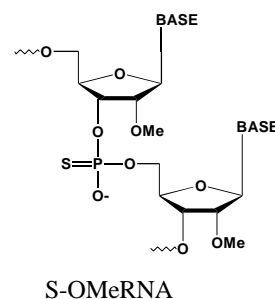
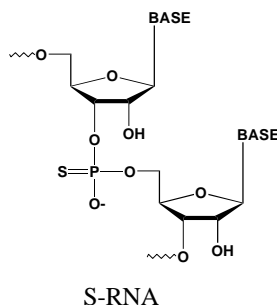
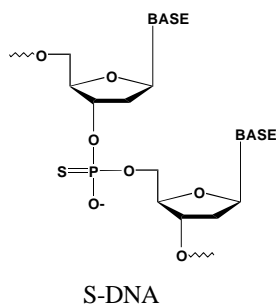
# Special Modifications

Special modifications of oligonucleotides with commercially available phosphoramidite reagents can be performed in our facility. For information about any oligonucleotide modification not listed, please call Dr. Xiaolin Zhang.

Modified oligonucleotides are delivered in 10 business days. HPLC purification is recommended to ensure quality.

## Phosphorothioate Oligonucleotide

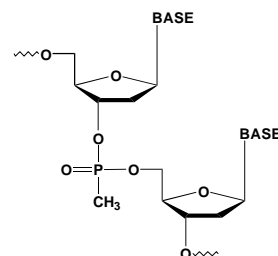
Phosphorothioate analogues of DNA, RNA and OMe-RNA have sulphur in place of oxygen as one of the non-bridging ligands bonded to phosphorus. Thiolation of the oligo phosphates at any or all positions are possible at no extra charge. Purification is available using HPLC only.



## Methyl Phosphonate DNA

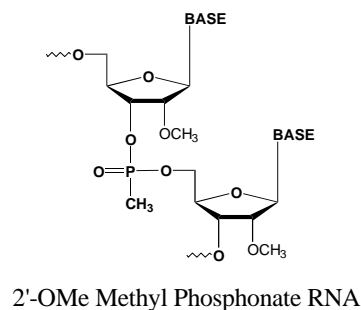
DNA oligo containing one or more methyl phosphonate linkages can be synthesized. HPLC purification is available.

Synthesis Scale	Charge/base
0.2-μmole	\$8.00
1.0-μmole	\$10.00



## 2'-OMe Methyl Phosphonate RNA

The 2'-OMe methyl phosphonate RNA has uncharged backbone linkages. Please call for more details.

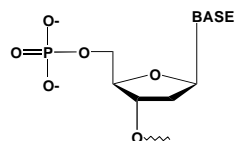


# Special Modifications

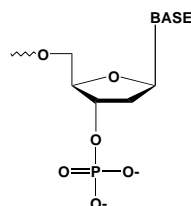
## Phosphorylation

The standard synthetic oligonucleotide has an OH group on both 5' and 3'-end. Phosphorylation at 5' and/or 3' end of the oligo is available. Price includes HPLC purification.

Phosphorylation	0.2- $\mu$ mole/oligo	1.0- $\mu$ mole/oligo
5'-Phosphorylation	\$35	\$65
3'-Phosphorylation	\$35	\$70



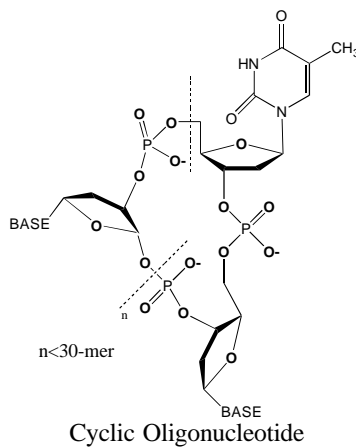
5'-Phosphorylated oligo



3'-Phosphorylated oligo

## Cyclic Oligonucleotides

Cyclic oligonucleotides of length within the range of 2 to 20 nucleotides can be prepared. Please call for more details.

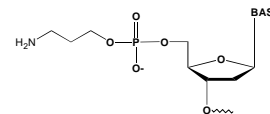


# Special Modifications

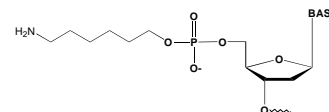
## Oligonucleotide Linkers

Oligonucleotide can be linked with different functional groups through a variety of hydrocarbon chains to fit the desired applications. Price includes HPLC purification.

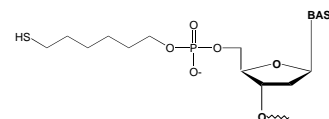
Oligonucleotide Linkers	200nmole/oligo	1.0-μmole/oligo
5'-C3-Amino-oligo	\$35	\$65
5'-C6-Amino-oligo	\$35	\$65
5'-C12-Amino-oligo	\$45	\$75
3'-C3-Amino-oligo	\$30	\$60
3'-C6-Amino-oligo	\$30	\$60
3'-C7-Amino-oligo	\$30	\$60
Amino-C2-dT-oligo	inquire	inquire
Amino-C6-dT-oligo	inquire	inquire
Carboxy-dT-oligo	inquire	inquire
5'-HS-C6-oligo	\$40	\$70
3'-HS-C3-oligo	\$35	\$65
Spacer 9-oligo	\$40	\$70
Spacer C3-oligo	\$40	\$70
Spacer 18-oligo	\$45	\$75
dSpacer-oligo	\$45	\$75
3'-Spacer-C3-oligo	\$35	\$70



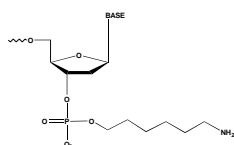
5'-C3-Amino-oligo



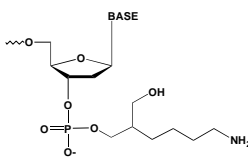
5'-C6-Amino-oligo



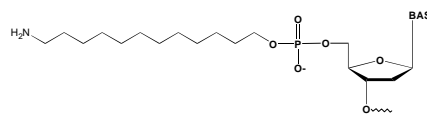
5'-HS-C6-oligo



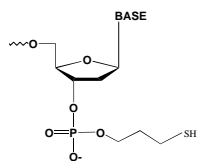
3'-C6-Amino-oligo



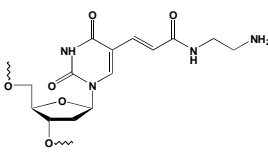
3'-C7-Amino-oligo



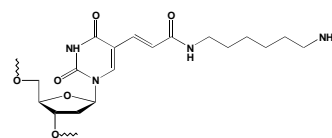
5'-C12-Amino-oligo



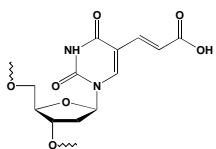
3'-HS-C3-oligo



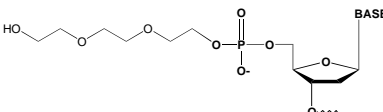
Amino Modifier-C2-dT-oligo



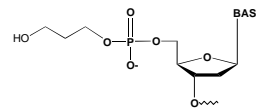
Amino Modifier-C6-dT-oligo



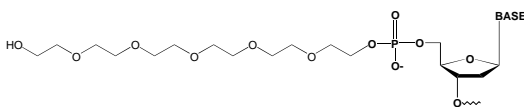
Carboxy-dT oligo



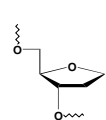
Spacer 9-oligo



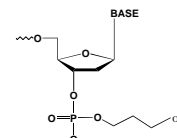
Spacer C3-oligo



Spacer 18-oligo



dSpacer-oligo



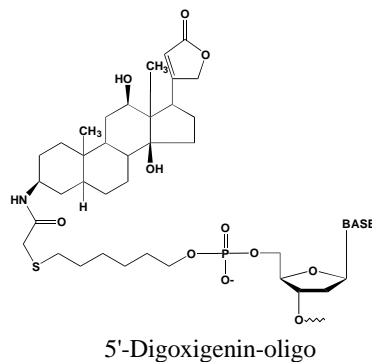
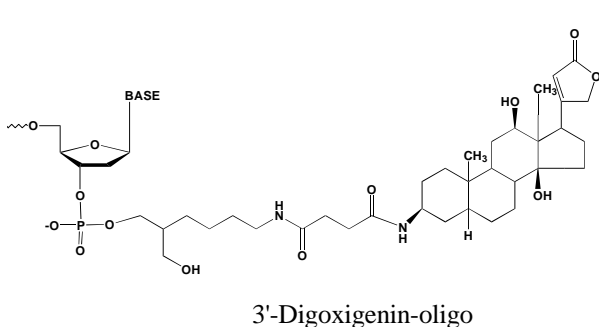
3'-Spacer-C3-oligo

# Special Modifications

## Digoxigenin labeling

Price includes HPLC purification.

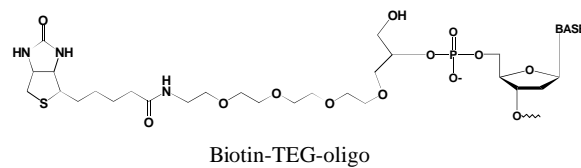
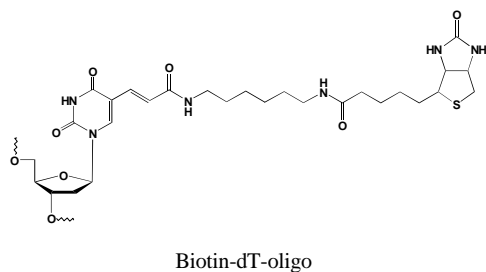
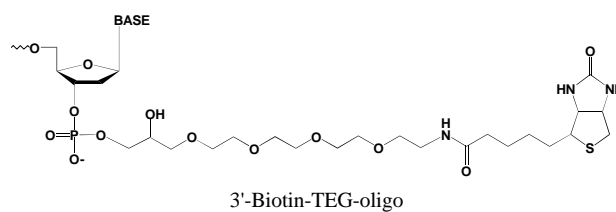
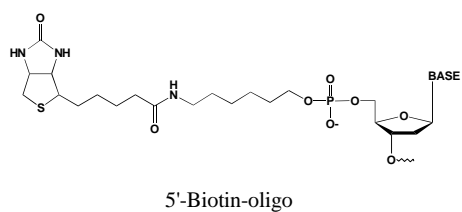
Digoxigenin labeling	200nmole/oligo	1.0-μmole/oligo
5'-Digoxigenin-oligo	\$100	\$150
3'-Digoxigenin-oligo	\$80	\$120



## Biotin Labeling

Price includes HPLC purification. For multiple labeling, please call.

Biotinylation	200nmole/oligo	1.0-μmole/oligo
5'-Biotin-oligo	\$40	\$70
3'-Biotin-TEG-oligo	\$35	\$70
Biotin-dT-oligo	Internal labeling. Inquire	
Biotin-TEG-oligo	Internal labeling. Inquire	

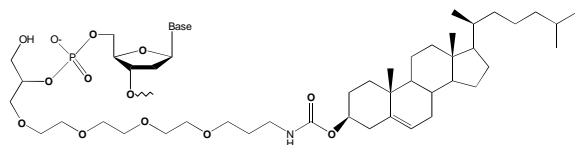


# Special Modifications

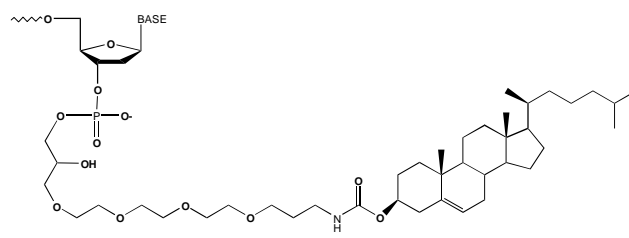
## Cholesterol Labeling

Potential therapeutic oligonucleotides must permeate the cell membrane for optimal activity. The addition of lipophilic groups to an oligonucleotide is expected to enhance activity. Price includes HPLC purification. For multiple labeling, please call.

Cholesterol Labeling	200nmole/oligo	1.0-μmole/oligo
5'-Cholesteryl-TEG-oligo	\$80	\$120
3'-Cholesteryl-TEG-oligo	\$35	\$70



5'-Cholesteryl-TEG-oligo

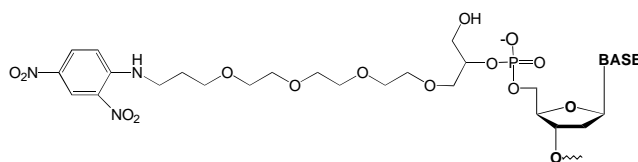


3'-Cholesteryl-TEG-oligo

## DNP Labeling

Oligonucleotide labeled with 2,4-dinitrophenyl (DNP) can be detected with anti-DNP antibodies. Price includes HPLC purification. For multiple labeling, please call.

DNP Labeling	200nmole/oligo	1.0-μmole/oligo
5'-DNP-TEG-oligo	\$80	\$120



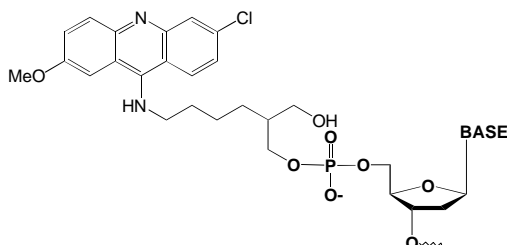
5'-DNP-TEG-oligo

# Special Modifications

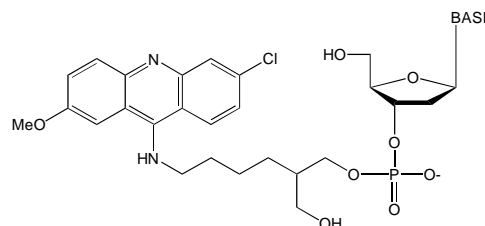
## Acridine Labeling

Acridine is an effective intercalating agent. Price includes HPLC purification. For multiple labeling, please call.

Acridine Labeling	200nmole/oligo	1.0-μmole/oligo
5'-Acridine-oligo	\$80	\$120
3'-Acridine-oligo	\$35	\$70



5'-Acridine-oligo

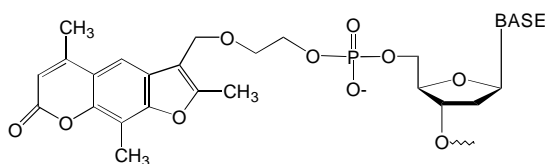


3'-Acridine-oligo

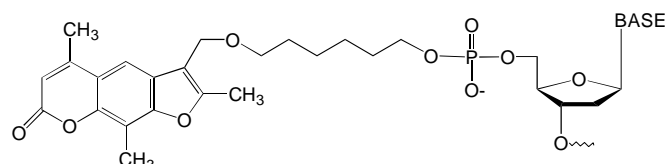
## Psoralen Labeling

Psoralen C2 placed at the 5' terminus of an oligonucleotide serves effectively as a cross-linking reagent in double-stranded oligonucleotides. Price includes HPLC purification. For multiple labeling, please call.

Psoralen Labeling	200nmole/oligo	1.0-μmole/oligo
5'-Psoralen-C2-oligo	\$70	\$100
5'-Psoralen-C6-oligo	\$70	\$100



5'-Psoralen-C2-oligo



5'-Psoralen-C6-oligo

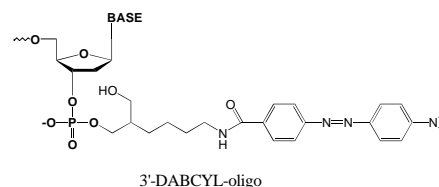
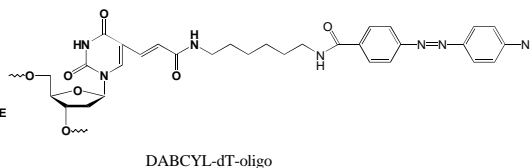
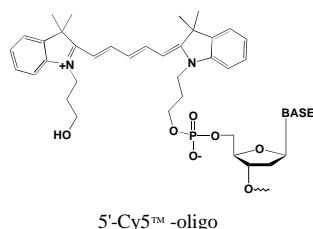
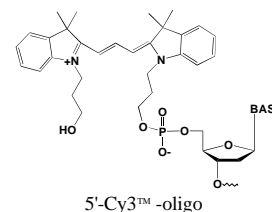
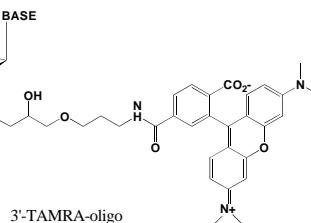
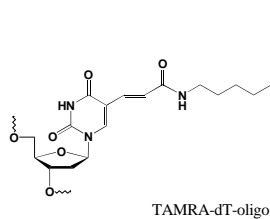
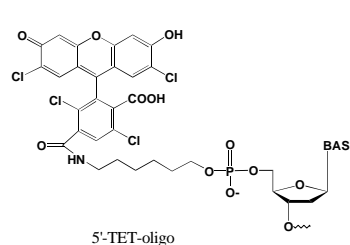
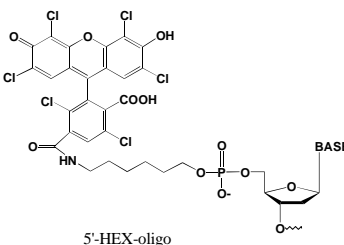
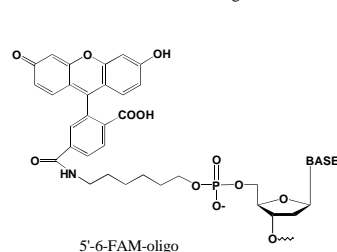
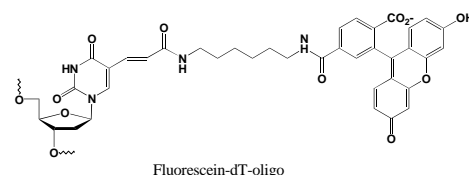
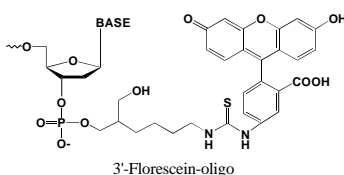
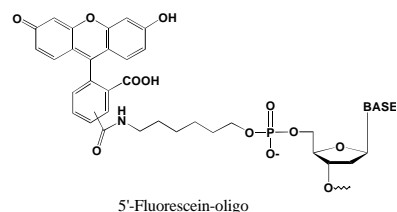
# Special Modifications

## Dye Labeling

Oligonucleotide can be labeled with different dyes at either end and/or internally. The area of fluorescence detection of DNA hybrids is developing rapidly, particularly in the areas of fluorescence in situ hybridization and in hybrid detection on DNA chips. Fluorescent labels provide for very sensitive detection. Under normal laboratory conditions the fluorescein-labeled oligo can detect  $5 \times 10^{-17}$  moles of target molecules. For any dye labeling not listed, please call the facility. Price includes HPLC purification.

Dye labeling	200nmole	1.0μmole	Color	Absorbance (max)	Emission (max)	$\epsilon$ (cm <sup>-1</sup> M <sup>-1</sup> )*
5'-Fluorescein-oligo	\$50	\$80	Green	494nm	525nm	73,000
3'-Fluorescein-oligo	\$40	\$70	Green	494nm	525nm	73,000
5'-6-FAM-oligo	\$80	\$120	Green	494nm	525nm	73,000
5'-HEX-oligo	\$80	\$120	Pink	535nm	556nm	73,000
5'-TET-oligo	\$80	\$120	Orange	521nm	536nm	73,000
Fluorescein-dT-oligo	inquire	inquire	Green	494nm	525nm	73,000
TAMRA-dT-oligo	\$130	\$180	Rose	565nm	580nm	89,000
3'-TAMRA-oligo	\$40	\$70	Rose	565nm	580nm	89,000
5'-Cy3 <sup>TM</sup> -oligo	\$80	\$120	Orange	550nm	570nm	150,000
5'-Cy5 <sup>TM</sup> -oligo	\$80	\$120	Far red	649nm	670nm	250,000
DABCYL-dT-oligo	\$130	\$180	--	453nm	none	32,000
3'-DABCYL-oligo	\$50	\$80	--	453nm	none	32,000

\* Extinction Coefficient



# Special Modifications

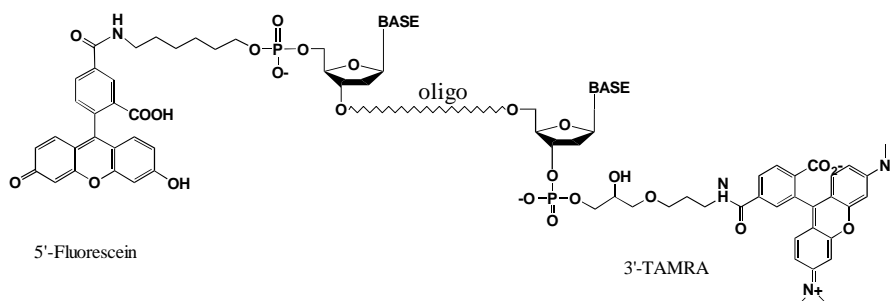
## Dual Dye Labeling

In many cases, it is desirable to label an oligo with more than one dye. Several current diagnostic assays utilizing oligo with two dyes-labeled that carries both a reporting and a quenching dye on opposite termini, such as TaqMan probe for the Perkin-Elmer TaqMan system and molecular beacon. We can finish those probes at low cost. Before ordering, please call for more details. Price includes HPLC purification.

Dual Dye Labeling	200nmole	1.0-μmole
TaqMan Probe	\$1.20/base of DNA +\$150	\$2.75/base of DNA +\$300
Molecular Beacon	\$1.20/base of DNA +\$150	\$2.75/base of DNA +\$300

## TaqMan™ Probe

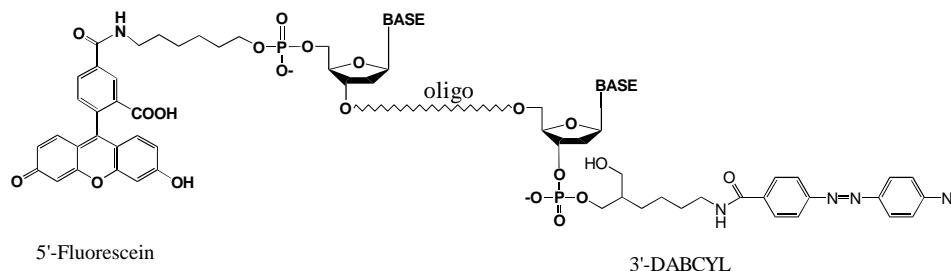
The assay exploits the 5' nuclease activity of *Taq* DNA Polymerase to allow direct detection of the PCR product by the release of the fluorescent reporter during PCR amplification. The TaqMan probe consists of an oligonucleotide with a 5'-reporter dye (fluorescein, FAM or TET) and a 3'-quencher dye, TAMRA. The fluorescence of the reporter is quenched by the TAMRA dye. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence. If hybridization occurs, probe cleavage by *Taq* polymerase takes place during polymerization of the targeted amplicon, resulting in separation of the reporter and quencher, and causing the reporter dye fluorescence to increase.



TaqMan probe with fluorescein as fluorophore and TAMRA as quencher

## Molecular Beacon

A molecular beacon probe has its natural fluorescence quenched in solution unless it is hybridized to the target sequence. A molecular beacon contains a fluorophore at one terminus (such as fluorescein, FAM, HEX, TET, Edeans, Texas or others) and DABCYL as universal quencher at the other. The probe sequence is in the center of the molecule and the bases towards both termini are self-complementary and about 5-8 nucleotides in length depending on the size of the probe sequence. The lengths of the probe sequences are chosen to maximize the separation of the fluorophore and quencher molecules when the probe is hybridized to the target.



Molecular Beacon with fluorescein as fluorophore and DABCYL as quencher.



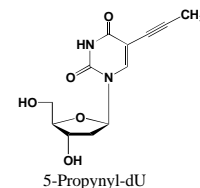
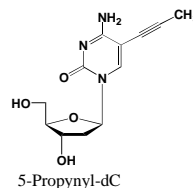
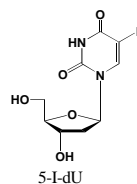
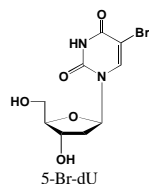
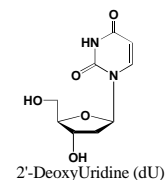
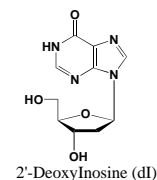
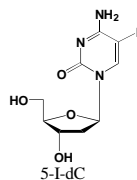
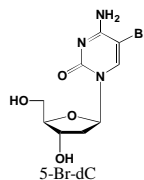
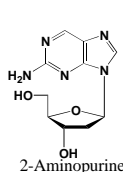
# Special Modifications

## Modified Nucleosides

Modified nucleosides can be incorporated into the oligonucleotide sequence at any sites.

### Common Modified Nucleosides

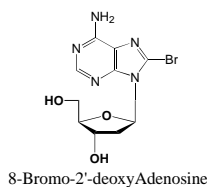
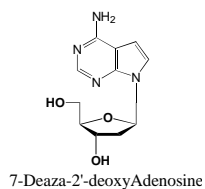
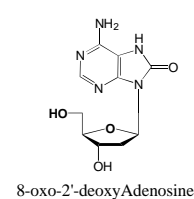
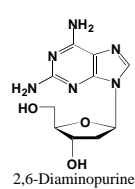
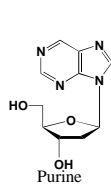
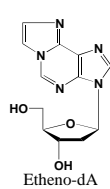
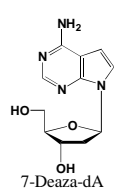
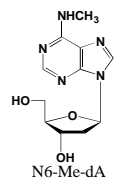
Modified Nucleosides	200nmole/site	1.0-μmole/site
2'-Deoxyinosine (dI)	\$8	\$12
2'-DeoxyUridine (dU)	\$8	\$12
2-Aminopurine	\$25	\$35
5-Br-dC	\$15	\$25
5-I-dC	\$15	\$25
5-Br-dU	\$15	\$25
5-I-dU	\$15	\$25
5-Propynyl-dC	\$20	\$30
5-Propynyl-dU	\$15	\$25



### Other Modified Nucleosides

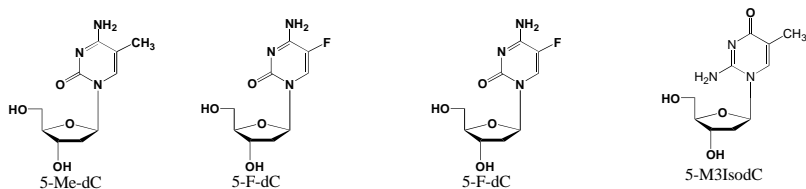
Please call for more details.

### 2'-DeoxyAdenosine Analogs

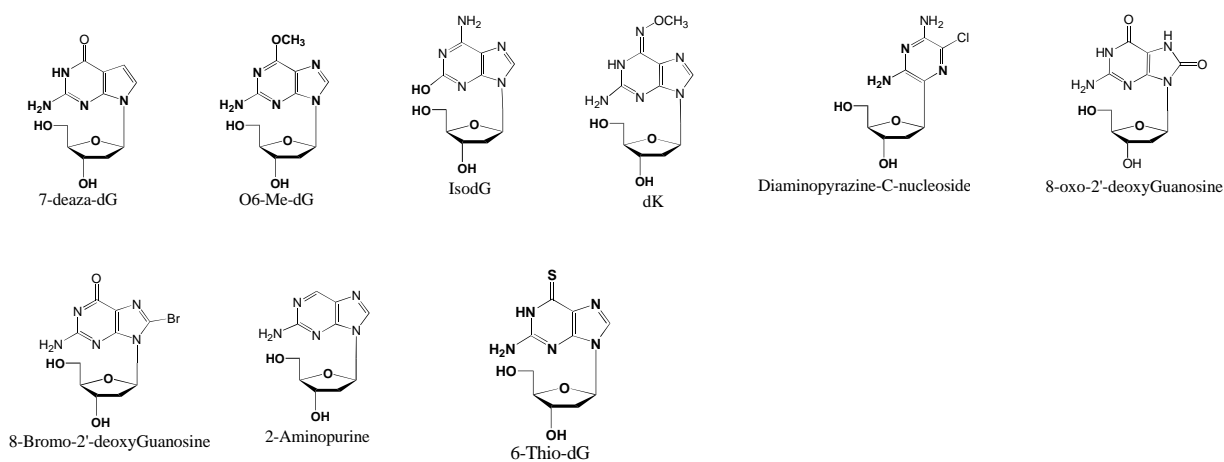


# Special Modifications

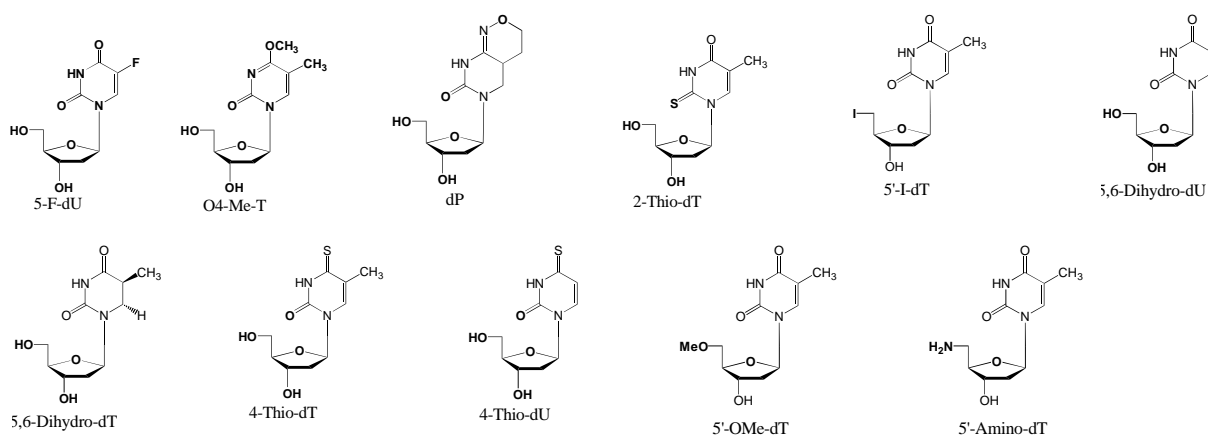
## 2'-Deoxycytidine Analogs



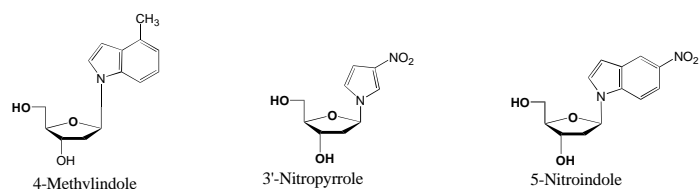
## 2'-DeoxyGuanosine Analogs



## Thymidine Analogs

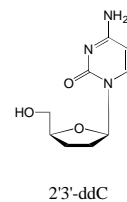
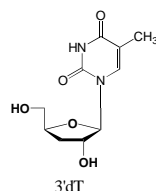
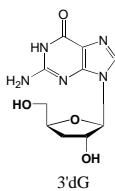
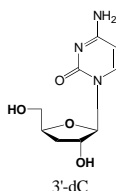
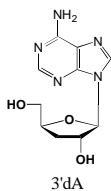


## Other 2'-Deoxyriboside Analogs

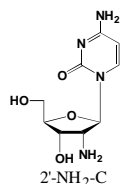
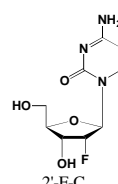
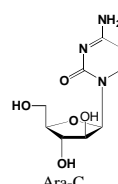
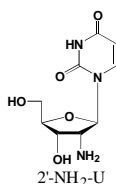
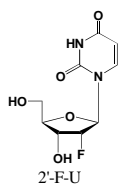
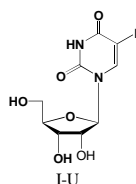
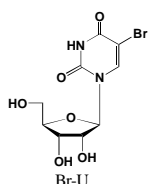


# Special Modifications

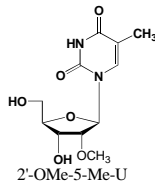
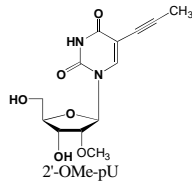
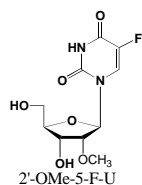
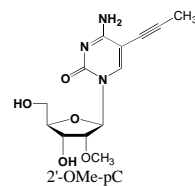
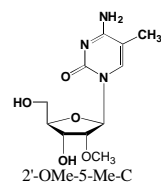
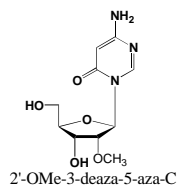
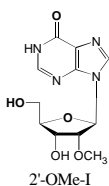
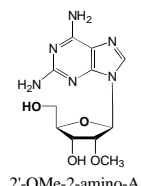
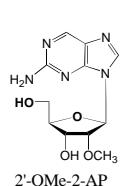
## 3'-Deoxyribose Analogs



## RNA Analogs



## 2'OMe-RNA Analogs





## NUCLEIC ACID FACILITY

University of Pennsylvania Cancer Center  
School of Arts and Sciences, Department of Chemistry

337 Cret Chem Bldg., 231 S. 34th St., Philadelphia, PA 19104-5323  
Phone: (215) 898-1584 Fax: (215) 572-2159  
Order online at [www.med.upenn.edu/naf](http://www.med.upenn.edu/naf)



Serial No. \_\_\_\_\_

Principal Investigator: \_\_\_\_\_

Date: \_\_\_\_/\_\_\_\_/\_\_\_\_

User: \_\_\_\_\_

Phone: \_\_\_\_\_

Account No.: \_\_\_\_\_

Delivering Address (include department or school):  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Sequence Name: \_\_\_\_\_

Synthesis Scale:

☐ 25-nomle(no purification option)

☐ 50-nomle ☐ 0.2- $\mu$ mole

☐ 1.0- $\mu$ mole ☐ 10- $\mu$ mole

Purification: ☐ None

☐ Cartridge ☐ HPLC

Special Requirement: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

Sequence(5' to 3'):

--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--

16 17 18 19 20 21 22 23 24 25 26 27 28 29 30

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31 32 33 34 35 36 37 38 39 40 41 42 43 44 45

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46 47 48 49 50 51 52 53 54 55 56 57 58 59 60

### IUB Mixed Base Symbols:

B=(C,G,T/U)

D=(A,G,T/U)

H=(A,C,T/U)

K=(G,T/U)

M=(A,C)

N=(A,C,G,T/U)

R=(A,G)

S=(C,G)

V=(A,C,G)

Y=(C,T/U)

W=(A,T/U)

X=Other