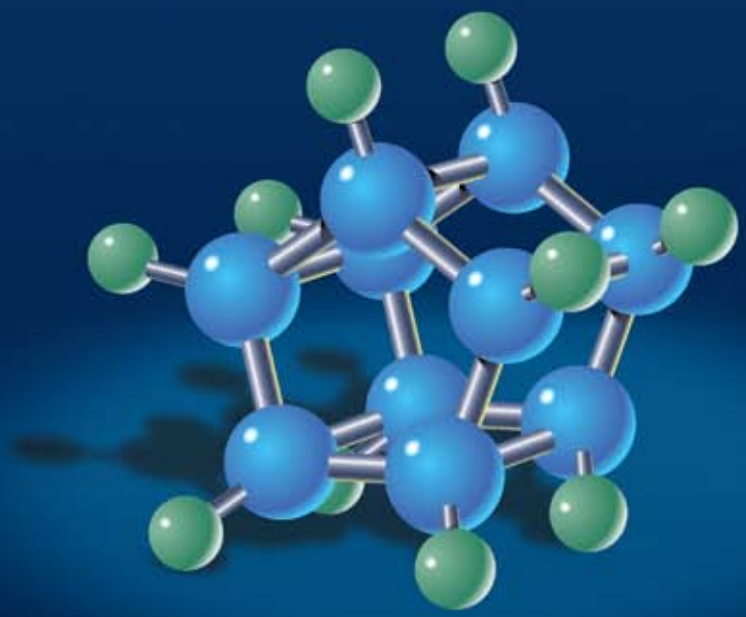
2008, Vol. 3.0

ChemGenes CORPORATION

Experience Nucleic Acid Expertise

New Featured Products

Sulfurizing Reagent
Reverse RNA Synthesis
TOM phosphoramidites
3′-tBDSilyl amidites
L-RNA amidites
7-Deaza ribo Products
8-Methyl ribo G
Universal Support



Introducing GMP-grade Manufacturing

ChemGenes has been in business for over 25 years and has recently moved into a state of the art facility in Wilmington, MA. ChemGenes has a full scale modernized lab with the facilities to manufacture in bulk while maintaining its high quality. We have added many new products to our original line to facilitate research in the area of biotechnology.

As the market for oligonucleotides continues to grow, ChemGenes remains committed to introducing novel products, while maintaining its existing product mix. We also have the capacity to custom synthesize products on request.

Our quality is guaranteed! We want to assure you that every product is of the highest purity and conforms to the certificate of analysis that accompanies it when shipped.

- ChemGenes takes pride in a long history of customer satisfaction in supplying the highest purity phosphoramidites & supports for oligonucleotide synthesis.
- Robust quality system to ensure full documentation of products for oligonucleotide Therapeutics.

- Each lot of phosphoramidite must pass an established testing criteria before it can be shipped to customers.

Required QC Tests for Most Phosphoramidites

Solubility test

- Amidites completely dissolve in Acetonitrile to make a 0.1M Solution (water<0.004-0.005gm/100ml). Leaves no visible particulate matter.

Coupling Efficiency

- The coupling efficiency of ChemGenes phosphoramidite products are functionally tested to 98% or better.

HPLC

- Greater than 98% purity by HPLC.

31P NMR

- Doublet peak or single peak.
- Position of each peak is known for each phosphoramidite.
- The value between the peaks is calculated and recorded.

UV – The UV test provides 4 values of data:

- The ratio between 250/260 nm.
- The ratio between 260/280 nm.
- Emax position.
- Extinction Coefficient.

MASS Spectrum

- ESI/MS performed on each product.

1H NMR

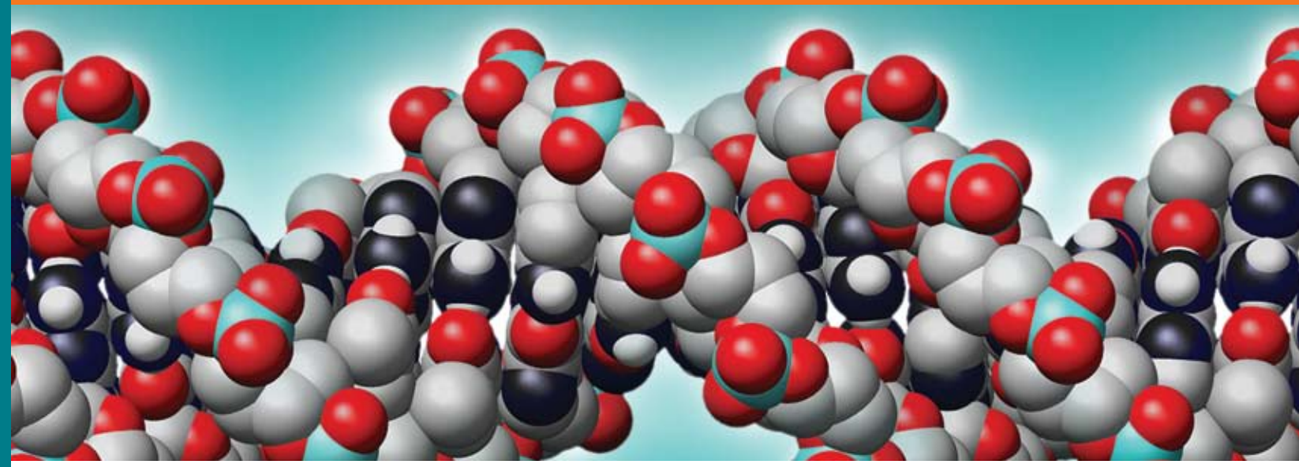
- Proton NMR analysis is performed for each product.

TLC

- Single or double spot (depends on the phosphoramidite) with no other visibly impurity on spotting, 0.2mg/spot.

ChemGenes CORPORATION

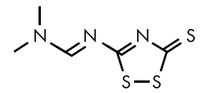
Sulfurizing Reagent



((Dimethylamino-methylidene)amino)-3H-1,2,4-dithiazoline-3-thione (DDTT)[3]

Available in bulk and formulated solution.
An efficient sulfurizing reagent with quantitative (greater than 99%) P/S conversion in oligonucleotide synthesis as compared to Beaucage reagent and has further advantage of greater stability in the formulated solutions.

Figure 1.



(DDTT)
Catalogue # RN-3155

A number of 1,2,4-dithiazolines are described in the literature[1,2] and demonstrate high sulfurizing efficiency in oligonucleotide synthesis.

Our data has also shown superior sulfurization by DDTT reagent in solution phase. Unlike Beaucage reagent, DDTT did not yield any detectable level of phosphodiester type by-product.

Recommended formulations are summarized in the table below (Table 1).

Table 1.

Recommended formulations for the synthesis of posphorothioate containing oligonucleotides and sulfurizing in solution phase.

DDTT Concentration	Solvent formulation v/v	Oxidation time	Comments
0.05 M	Py/ACN – 3:7 Py/THF – 2:8	**2.5 min**	Formulation used in oligonucleotide synthesis
0.1 M	Py Py/THF – 4:6	**2 min**	Formulation used in oligonucleotide synthesis
0.05 M	Acetonitrile	**10 min**	Formulation used in solution phase reaction

In solution phase we observed that DDTT (1-8 eq.) in Pyridine: acetonitrile ::60:40 converts amidite fully into phosphorothioate within 2 minutes of reaction time. The conversion was found to be almost quantitative >99% to P-S and negligible amount of P-O formation occurred, as analyzed by 31 P NMR.

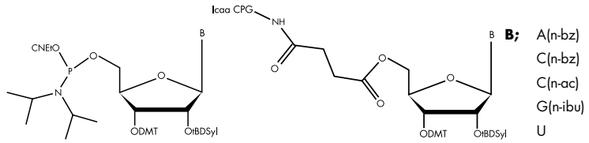
References:

1. Xu, Q., Musier-Forsyth, K., Hammer R. P. and Barany G. Nucleic Acid Research, 1996, 24, 1602-1607.
2. Xu, Q., Barany G., Hammer R. P. and Musier-Forsyth, K. Nucleic Acid Research, 1996, 24, 3643-3645.
3. Paten Pending, AM Chemicals, LLC

Reverse RNA

Phosphoramidites for Reverse RNA Synthesis:

The novel phosphoramidites, **A-n-bz, C-n-bz, C-n-ac, G-n-ibu and U** are produced with an HPLC purity of greater than 98% and 31P NMR purity greater than 99%. A novel process of reverse **5′→3′** directed synthesis of RNA oligomers has been developed. Using this method of synthesis, we demonstrated high quality RNA synthesis with **coupling efficiency approaching 99%.**



3′-DMT-2′-tBDSilyl-5′-Amidites 3′-DMT-2′-tBDSilyl-5′-CPG

B	Protection	Catalog #
A	n-bz	**ANP-3401 (Amidite) N-6201 (CPG)**
C	n-bz	**ANP-3402 (amidite) N-6202 (CPG)**
G	n-ibu	**ANP-3403 (amidite) N-6203 (CPG)**
U	n/a	**ANP-3404 (amidite) N-6204 (CPG)**
C	n-ac	**ANP-3405 (amidite) N-6204 (CPG)**

Synthesis of varieties of therapeutic grade RNA and siRNA etc. requires a modification or labeling of 3′- end of an oligonucleotide. The synthesis of 3′- end modified RNA requiring lipophilic, long chain ligands or chromophores, using **3′→5′** synthesis methodology is challenging, requires corresponding solid support and generally results in low coupling efficiency and lower purity of the final oligonucleotide in general because of a large amount of truncated sequences containing the desired hydrophobic modification. We have approached this problem by developing reverse RNA monomer phosphoramidites for RNA synthesis in **5′→3′**- direction. They lead to very clean oligonucleotide synthesis allowing for introduction of various modifications at the 3′- end, cleanly and efficiently.

Applications:

A vast number of applications are possible for easy attachment at 3′- End of an oligonucleotide.

1. For attachment of bulky molecules at the 3′- end of the RNA, such as cholesterol, long chain aliphatic chains such as C-18, triethylene glycols, hexaethylene glycols: Direct coupling with these amidites can be achieved easily.
2. Attachment of Polyethylene Glycols such as PEG 2000 and PEG 4500 amidites at the 3′- end of the RNA molecule.
3. For easy attachment of 3′- thiol modification. 3′- Disulfides from readily available amidites, via C-3 disulfide, C-6 disulfide.
4. 3′- Biotin attachment via biotin amidite in a single step and avoiding biotin CPG.
5. Modification of 3′- end of the sense strand of siRNA. The modification of the overhang of the sense strand (3′- End) of siRNA is not expected to affect targeted mRNA recognition, as the antisense siRNA strand guides target recognition. Useful modification for improvement of delivery of siRNA can be easily designed.

High Purity Synthetic RNA

RNA Synthesis Approach in the Reverse Direction **leads to smooth 3′-conjugation of macromolecules to Synthetic RNA. Reverse RNA Synthesis Results in Complete Absence of M+1 Species.**

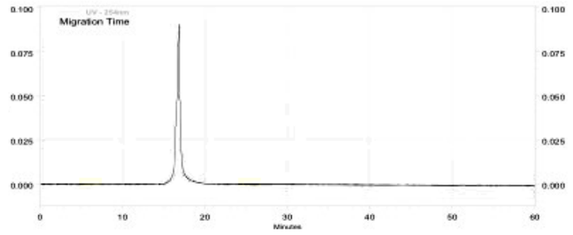


Figure 1. RNA with 3′-Cholesterol

Capillary Gel Electrophoresis (CE) and HPLC purification. Reverse direction (5′→3′) synthesis and Expedite model 8909- 1 umole scale. Purity; 99.9%.

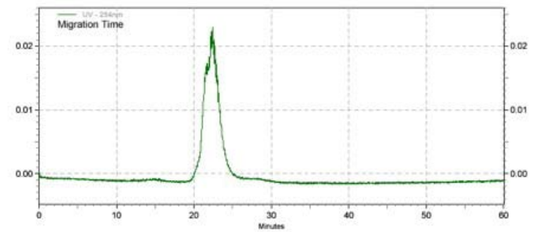


Figure 2a. RNA with 3′-PEG attachment

Capillary Gel Electrophoresis (CE) of the HPLC purified 21- mer RNA with 3′- PEG(Poly ethyleneglycol; MW; 2000). Made by Reverse RNA synthesis method (**5′→3′**-direction). Expedite model 8909- 1 umole scale. Purity; 100%

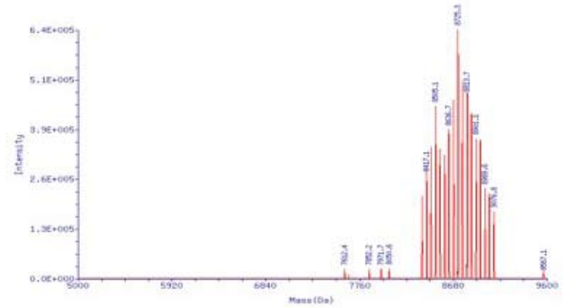


Figure 2b. RNA with 3′-PEG attachment

ESI Mass Spectral analysis of 21- mer RNA with 3′- PEG -2000 attachment, purified RNA as shown in Fig. 2a. The synthesis was carried out in reverse direction (**5′→3′** direction). The PEG-2000 was attached as last step via the corresponding phosphoramidite, ChemGenes catalog; CLP-3119.

Calculated Molecular Weight: 8684.1

Observed Molecular Weight: 8681.1

Note: There is a distribution of at least 14 PEG species of the RNA on both sides of the Calculated molecular weight with PEG-2000. Thus species from 8417.1 to 8945.3 are present with a molecular weight difference of a glycol unit (+/- 44).

Salient Features:

A vast number of applications are possible for easy attachment at 3′- End of an oligonucleotide.

1. The crude RNA's have much closer impurities (M-1) in the conventional method (**3′→5′**-direction), as compared to reverse RNA synthesis (**5′→3′** direction). Therefore, after purification, RNA synthesized by reverse RNA synthesis are purer.
2. The feature mentioned above is much more visible in the synthesis of cholesterol attached to 3′- end of RNA. Therefore it is easier to purify RNA with cholesterol at 3′- end synthesized by reverse RNA synthesis.
3. M+1 impurities are essentially absent in the RNAs synthesized by reverse RNA synthesis. It is postulated that in the molecule, ribonucleoside- 3′- DMT-2′- tBDsilyl-5′- phosphoramidites, the 3′- DMT is not cleaved by 5-ethylthiotetrazole during the coupling time of oligonucleotide chain extension.
4. RNA containing macromolecules at the 3′- end which are generally inaccessible by conventional methods (**3′→5′**) are easily synthesized by reverse RNA synthesis (**5′→3′** direction). These RNAs can be produced in high purity.
5. 3′- PEG RNA (21- mer) was synthesized, and after purification, purity was essentially 100% (see figs. 2a & 2b).

TOM RNA[1,2,3] (2′-Triisopropylsilyl-oxy methyl amidites)

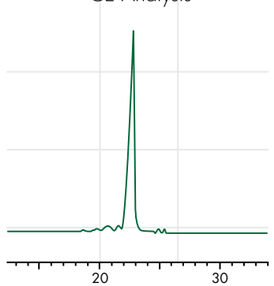
Highly suitable for Large Scale Therapeutic Grade RNA Synthesis & Long RNA sequences.

Perfected Technology at ChemGenes makes Available Bulk quantities (multi-kilo gram scale batches) & Affordable Prices.
Produced Under GMP Guidelines

The following superior qualities makes TOM amidites most desirable for Therapeutic oligonucleotide production.

- Highest coupling kinetics and efficiency per step, due to lower steric hindrance compared to conventional 2′-TBDMS RNA monomers. A very fast coupling time of TOM amidites (2-4 minutes for different scale of synthesis), and complete absence of 3′ impurities, due to optimized manufacturing conditons for TOM amidites. No possibility of 2′ to 3′ migration during oligo synthesis or during work up. Results in highest purity oligonucleotides. *The synthesis resembles DNA Synthesis like behaviour.*
- Coupling Efficiency consistently greater than 98% per step.
- Short deprotection time for n- protecting group with a time of 4 hrs -6 hours depending on chain length, and with liberal amount of water. *{Labile Base Protecting groups used are (n-acetyl-A ; n-acetyl-C and n-acetyl –G) Easy removal of TOM protecting group is achieved under mild conditions and in significantly excess amount of water without any side effects}*
- No formaldehyde adduct formation is detected in the oligonucleotides made by using TOM amidite chemistry, after desilylation step using 1 M TBAF. Single Ion –Exchange purification and ESI/Mass analysis showed no peak corresponding to hydroxymethyl group (CH_2 –OH) either as single unit or multiple units, with mass corresponding to 30 Daltons or its multiples (Fig.1a and Fig.1b).
- A comparative study of the quality of oligonucleotides synthesized using TOM amidites and tBDSilyl amidites showed that the RNA oligos synthesized using TOM amidites were found to result in far superior quality after single Ion-Exchange purification (Capillary Gel analysis and ESI MS Data).
- There is an overall reduced preparation time involved using TOM amidite chemistry, starting with base deprotection, TOM deprotection and overall ease of purification resulting in very high purity oligonucleotides.

CE Analysis



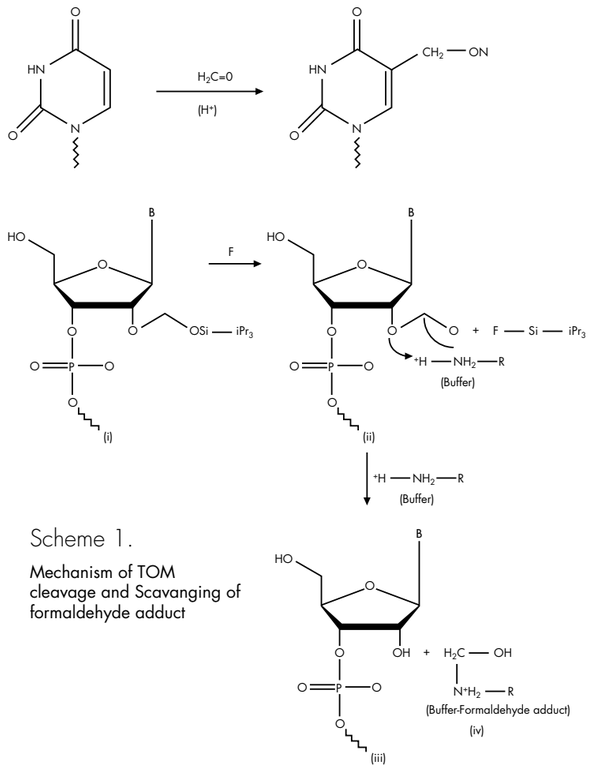
12 Mer RNA @ 15 Micromole

Studies to Confirm Absence of Formaldehyde Adduct formation :

During the loss of silyl and generation of formaldehyde and C-5 formylation or n-formylation of nucleoside bases does not occur due to presence of aq TRIS buffer,Ph 7.4, which captures all the formaldehyde formed and prevents any base modification as analyzed by ESI mass spectral data. None of the Mass spectral data showed any peak corresponding to hydroxymethyl group (CH_2 –OH) either as single unit or multiple units, with mass corresponding to 30 or its multiples (Fig. 1a and Fig. 1b) adduct is seen (see the ESI MS data).

ESI/ MS data is presented here to substantiate clean RNA synthesis. No peak corresponding to the formaldehyde adduct (molecular ion + 30) has been detected.

It is likely that formaldehyde formed after the cleavage is captured by buffer, which is mildly basic, pH 7.4. Because of acidic nature of formaldehyde addition to pyrimidines (see Scheme below) pH 7.4 is the recommended during desilylation step.



Scheme 1.

Mechanism of TOM cleavage and Scavanging of formaldehyde adduct

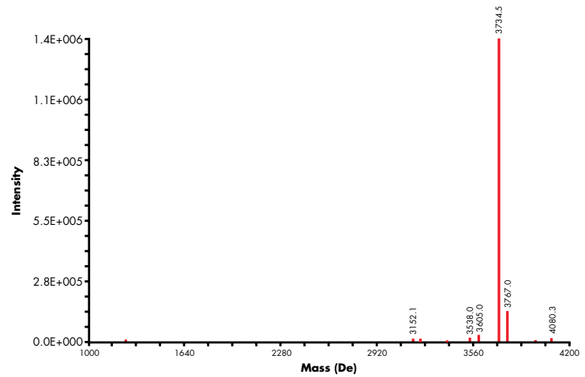


Figure 1a. 15umole scale synthesis of a 12-mer

Shows correct and clean Mass. No M+30 or multiples of M+30 are seen.

Observed: MW: 3715.3
Target: MW: 3715.3

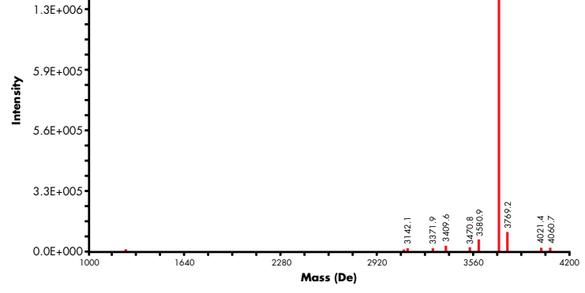


Figure 1b. 15umole scale synthesis of a another 12-mer

Shows correct and clean Mass. No M+30 or multiples of M+30 are seen.

Observed: MW: 3734.5
Target: MW: 3734.5

References :

1. Tom Amidites are intellectual property of Qiagen Inc., Germantown , MD and licensed to ChemGenes Corp. for sales in Therapeutics RNA market.
2. Stefan Pitsch, Patrick A. Weiss, Luzi Jenny, Alfred Stutz, and Xiaolin Wu, Helv. Chem.Acta- 3773-95, Vol. 84 (2001).
3. TOM Protecting group chemistry is covered by US PAtent No. 5,986,084. ChemGenes Corp. holds license agreement with Qiagen Inc. for worldwide supply for Therapeutics market.

ChemGenes CORPORATION

L-DNA & L-RNA

Ultra Pure L-DNA and L-RNA amidites and Supports

Applications in Modified Molecular Beacons (MMB's) and Microarray platform with recognition tags (MPRT)
Recently several applications of L- DNA have been described. One significant research led to development of modified molecular beacons (MMB) consisting of L-DNA as the stem part of a Molecular Beacon (MB) probe, which prevent non specific folding of the MB. These modified MBs, have been reported to provide excellent probes for many biological roles which MBs play (Youngmi Kim, Chaoyong James Yang and Weihong Tan, Nucl. Acids Res., 35, 21, 7279-7287, 2007).

Another significant application involves design of probes with a L- DNA sequence stem as part of probes having labels for recognition. This technique needs only one design and was applied on a microarray platform. This is a very useful technique and has tremendous potential in wide applications using a single microarray platform (Hauser, N.C., Martinez, R., Jacob, A., Rupp, S., Hoheisel, J.D., and Matysiak, S., Nucl. Acids Res., 34, 5101-5011, 2006).

ChemGenes has been instrumental in providing key L- DNA amidite ingredients, which involve synthesis of molecular beacons and microarrays with recognition tags. In order to further advance this field, we are providing special pricing.

Special Small Quantity Pricings:

Catalog #	Product Name	Quantity & Price
L- DNA Amidites:		
ANP-8031	B-L- deoxy adenosine (n-bz)-OP	$22/100umol ; $46/250 mg
ANP-8035	B-L- deoxy cytidine (n-ac)-OP	$22/100umol; $46/250mg
ANP-8033	B-L- deoxy guanosine (n-ibu)-OP	$22/100umol; $46/250 mg
ANP-8032	B-L- deoxy thymidine-OP	$22/100umol; $46/250 mg
L- DNA Supports:		
N-3521-05 & -10	B-L- deoxy adenosine (n-bz)-3'-lcaa **CPG**	
N-3525-05 & -10	B-L- deoxy cytidine (n-ac)- 3'-lcaa-**CPG**	
N-3523-05 & -10	B-L- deoxy guanosine (n-ibu)-3'-lcaa -**CPG**	
N-3524-0-5 & -10	B-L- deoxy thymidine-3'- Lcaa –lcaa-**CPG**	

L-DNA Bulk CPG			Pack of 4 columns		Pack of 10 columns	
100 mg	500mg	1g	0.2 imol	1.0umol	0.2 umol	1.0umol
$18.00	$42.00	$58.00	$16.00	$32.00	$32.00	$64.00

ChemGenes CORPORATION

Spiegelmers

We anticipate same technology will be used in RNA beacons as well. We are tyerefore offering special pricing for small quantity L-RNA amidites and supports.

Catalog #	Product Name	Quantity & Price
L-RNA Amidites:		
ANP-4841	B-L- ribo adenosine-(n-bz)-2'-tBDSilyl-3'-OP	$26/100umol ; $48/250 mg
ANP-4846	B-L- ribo cytidine(n-ac)-2'-tBDsilyl-3'-OP	$26/100umol; $48/250mg
ANP-4843	B-L- ribo guanosine (n-ibu)-2'-tBDSilyl-3'-OP	$26/100umol; $48/250 mg
ANP-4844	B-L- ribo uridine-2'-tBDsilyl-3'-3'-OP	$26/100umol; $48/250 mg
L-RNA Supports:		
N-4691-05 & -10	B-L- ribo adenosine (n-bz)-2'-tBDsilyl-3'-lcaa- **CPG**	
N-4696-05 & -10	B-L- ribo cytidine (n-ac)- 2'-tBDsilyl-3'-lcaa- **CPG**	
N-4693-05 & -10	B-L- ribo guanosine (n-ibu)-2'-tBDSilyl-3'-lcaa- **CPG**	
N-4694-0-5 & -10	B-L- ribo uridine-2'-tBDsilyl-3'- lcaa- **CPG**	

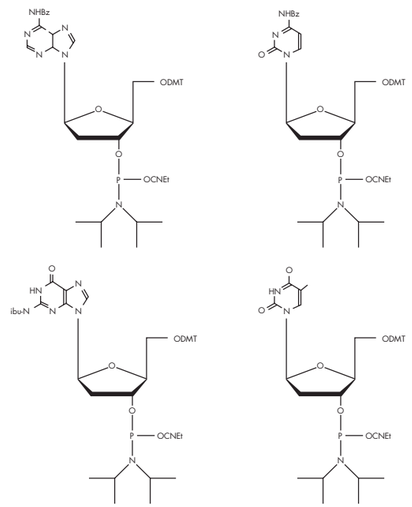
L-RNA Bulk CPG			Pack of 4 columns		Pack of 10 columns	
100 mg	500mg	1g	0.2 imol	1.0umol	0.2 umol	1.0umol
$18.00	$42.00	$58.00	$16.00	$32.00	$32.00	$64.00

***full Quality Control including Optical Rotation validation

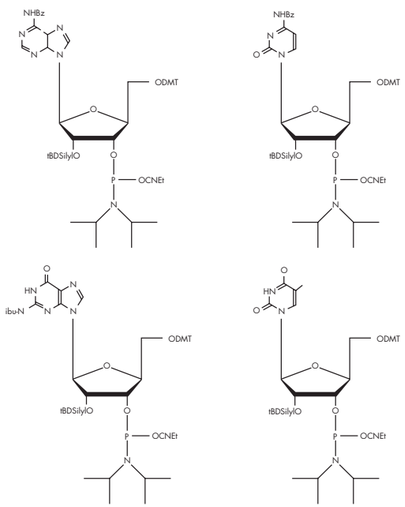
Key Features:

- L-RNA shows extraordinary stability in intracellular environments. Selective binding of long chain L-RNA to specific biological macromolecules has been searched via combinatorial libraries (Klussmann S, Nolte A, Bald R, Erdmann VA, Furste JP, Nature Biotechnology, 14 (9): 1112-1115 SEP 1996).

L-deoxy nucleoside phosphoramidites



L-2' -tBDSilyl ribo nucleoside phosphoramidites



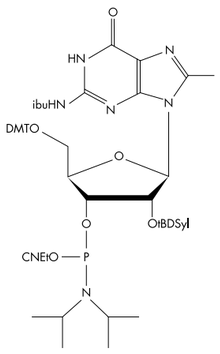
8-Methyl dG & rG Phosphoramidites

8-Methyl-rGuanosine:

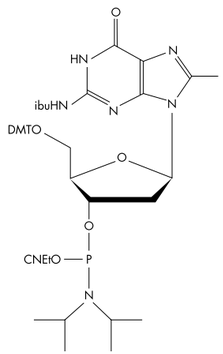
- Powerful Z–DNA stabilizer
- Reading effects B & Z transformation in oligonucleotides
- Can be incorporated into appropriate positions of synthetic DNA

Applications:

- For study of functional role in Gene Expression, transcription control etc.
- Selectivity of Z DNA in protein interactions
- DNA supercoiling modulation
- Selective Targeting of proteins or enzymes
- Aptamer Design and Therapeutic development



8-Methyl ribo Guanosine
CED OP
Catalog #:
ANP-6274



8-Methyl deoxy Guanosine
CED OP
Catalog #:
ANP-9274

7-Deaza Products

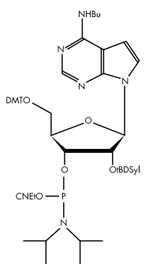
7- Deaza- Purine-Phosphoramidites:

7- Deaza-modification finds extensive application in molecular biology & design of oligos with 7- deaza-substitution, in place of multiple G's in the sequence.

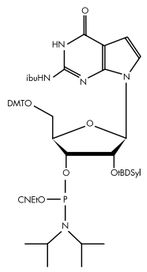
Key Features:

- To avoid extensive secondary structure formation in oligos and thereby improve targeted hybridization more effectively.
- Antiparallel triple helix formation with double stranded DNA is favored with this modification.

7- Deaza-ribo A & G Phosphoramidites:



7-Deaza Adenosine
CED OP
Catalog #: **ANP-7101**



7-Deaza Guanosine
CED OP
Catalog #: **ANP-7301**

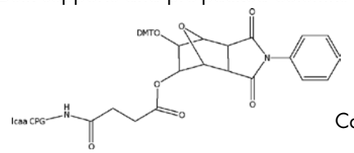
7- Deaza-7-iodo-dA and dG Nucleosides: Key Modifications:

- For conversion of 7-deaza-7-iodo to C-7-Modified-7-deaza-dA and dG-Modified nucleoside Triphosphates
- Extensive application in molecular biology for diagnostics and sequencing.
- 7- position modifications do not interfere in either PCR or oligo hybridizations.
- The 7- deaza-nucleoside phosphates and triphosphates are currently used in DNA sequencing.

Universal and Non-Cleavable Supports

UnyLinker Universal Support for Synthesis of Oligonucleotides:

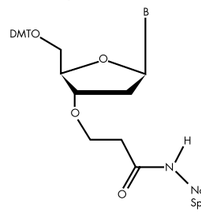
- Technology Licensed from Isis Pharmaceuticles
- CPG and Polystyrene supports
- Bulk supports and pre-packed columns Available



Catalog # **N-4000**

Non Cleavable Supports & Columns:

- Non- Cleavable inert Supports & Columns
- Uniform Particle Size
- Long Chain Spacer on Rigid non-swellable Support
- Two particle sizes are available; 15-20 um & 60-70 um



B	Protection	Catalog #
A	n-bz	**N-7521**
C	n-bz	**N-7522**
G	n-ibu	**N-7523**
T	n/a	**N-7524**

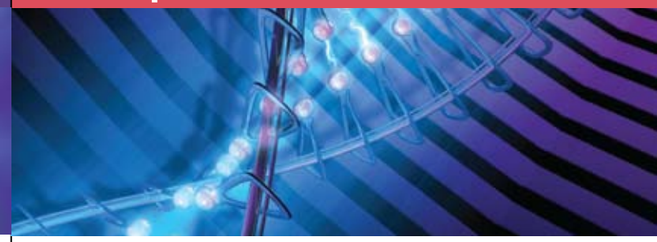
Key Features:

- Fully compatible with standard phosphoramidite reagents and synthesis conditions
- Has standard DMT group and requires standard deblock solutions for oligonucleotides synthesis
- Coupling efficiency greater than or equal to 99%
- Results in clean oligonucleotides
- Clean and standard succinate linkage and quantitative cleavage from support with ammonium incubation.

Applications:

- High Coupling efficiency leads to pure oligos
- Combinatorial library screening
- High purity Long chain oligos (up to 55 –mer synthesized)
- PCR amplication of bead bound oligo is done efficiently

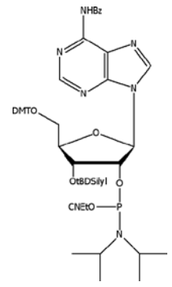
3′-tBDSilyl RNA Phosphoramidites



- Allows the synthesis of 2′-5′-linked oligos.
- RNA 2′,5′-duplexes are not substrates of the enzyme RNAse. However, they can inhibit the RNaseH mediated cleavage of a natural DNA: RNA substrate.

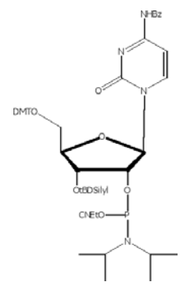
Useful Applications

- Determine their exact biological role.
- Extend their biological half life.
- Alter the biological activity of the core structure.



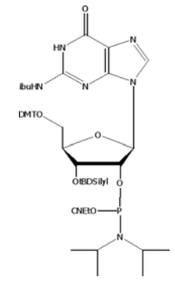
Adenosine (n-bz) 3′-tBDSilyl
CED OP

Catalog #
ANP-5681



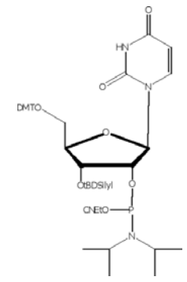
Cytidine (n-bz) 3′-tBDSilyl
CED OP

Catalog #
ANP-5682



Guanosine (n-ibu) 3′-tBDSilyl
CED OP

Catalog #
ANP-5683



Uridine 3′-tBDSilyl
CED OP

Catalog #
ANP-5684

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