The **Glen Report**

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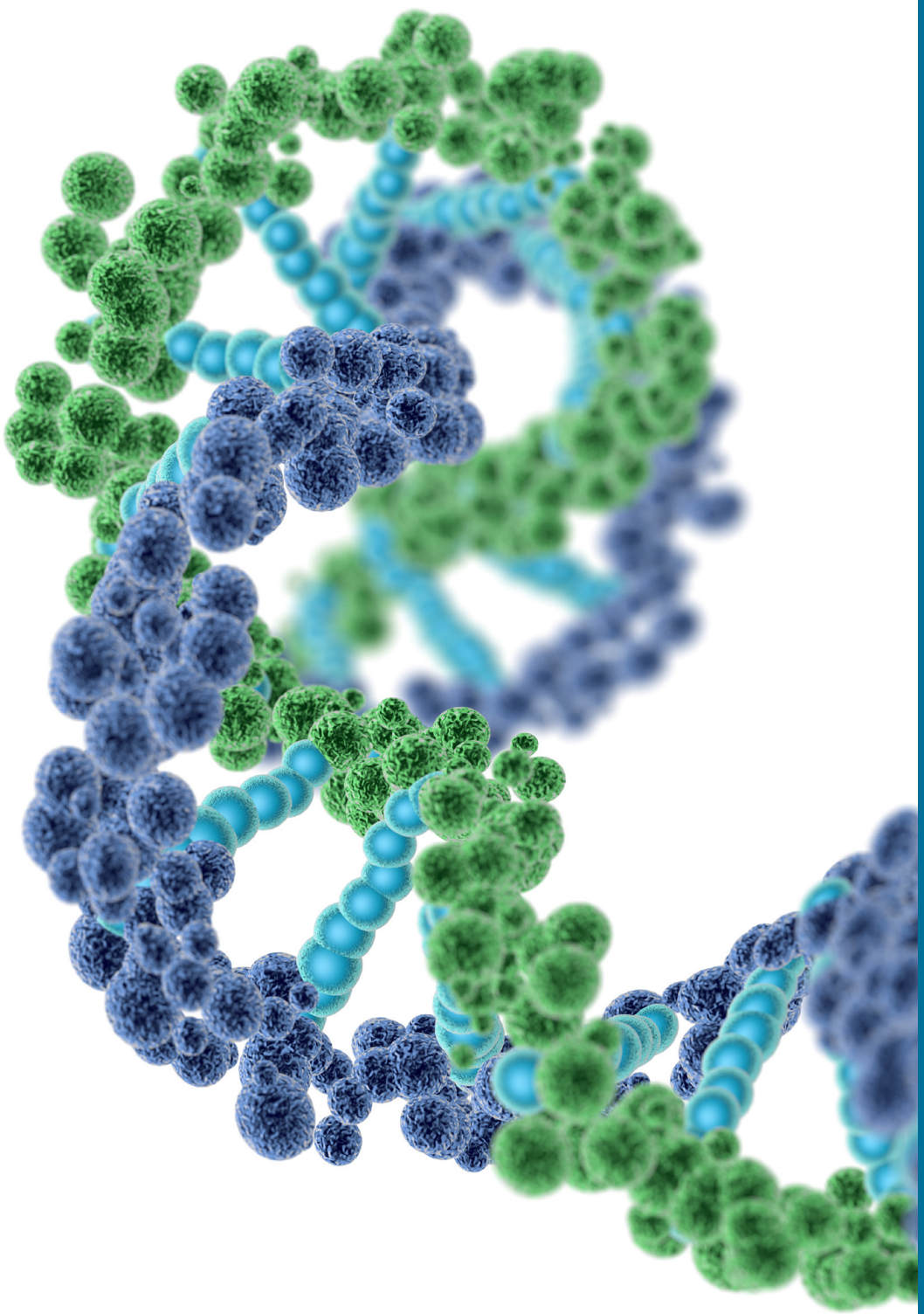

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GLEN RESEARCH
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NEW PRODUCT

Serinol Nucleic Acids

Serinol nucleic acid (SNA) is an acyclic phosphodiester backbone based on serinol (2-amino-1,3-propanediol). It shares the same three carbon skeleton as ribose and has nucleobases that are one atom further away from the skeleton than standard nucleic acids (Figure 1). SNA was first described almost 30 years ago, and in the years since, many investigations have been published on their properties and usage.

Early studies focused on the properties of oligonucleotides containing a small number of SNA substitutions. In these studies, it was found that SNA was destabilizing in terms of duplex stability. One single substitution reduced melting temperatures by 0.5-12 °C depending on the sequence and substitution location.[1, 2] Later studies also looked at oligonucleotides composed entirely of SNA. SNA oligonucleotides will hybridize with SNA, RNA as well as DNA in an antiparallel fashion to form right-handed helices, and the resulting structures are relatively stable.[3] In fact, SNA binds RNA more strongly than DNA does. As one would expect, SNA is very stable to nucleases. Even in the presence of snake venom phosphodiesterase, a standard exonuclease for base composition analysis of oligonucleotides, SNA is relatively stable.[4]

One property of SNA that sets it apart from most other modified nucleic acid backbones is its chirality. At the monomer level, serinol building blocks are achiral. It is only when the monomers are assembled in a linear chain that there can be chirality, and even then, it is sequence dependent. To differentiate one terminus from the other, it is convenient to label them as the *S* and *R* termini based on the stereochemistry of the terminal monomer (Figure 1). Instead of sequences expressed as 5' to 3', they are written as *S* to *R* instead. Palindromic sequences will be achiral while all others will be chiral. For example, ATT is chiral while TAT is not. Along these same lines, the enantiomer of an SNA oligonucleotide is the same sequence in reverse. For ATT, that would be TTA. Furthermore, an SNA oligonucleotide can hybridize with both DNA and L-DNA (Figure 2). Using the ATT example again, SNA ATT can hybridize with both DNA AAT (right hand helix) and L-DNA TAA (left hand helix).

In recent years, SNA has been used in several applications. SNA has been used in RNA interference.[5] When SNA was substituted into the 5' and 3' termini of the passenger strand and the 3' terminus of the guide strand, nuclease resistance, guide strand selectivity and RNA interference activity were all improved. SNA has also been used in molecular beacons to detect RNA in cells.[6] Sequences constructed of entirely SNA backbones, including the fluorophores and quenchers, successfully detected target RNA with signal to noise ratios as high as 930. Furthermore, an all SNA sequence was evaluated for its ability to facilitate splice switching.[4] In an *in vitro* cellular model of Duchene muscular dystrophy, 41-52% exon skipping was observed. Finally, the use of SNA as the foundation of a helical amplification system that is triggered by hybridization was demonstrated.[7]

To give researchers access to more tools for therapeutic oligonucleotides as well as other unique applications, Glen Research is introducing the SNA phosphoramidites of A, C, G and T (Figure 3). These reagents are chiral and stereopure. They should be used in the same way as 3' phosphoramidites to give oligonucleotides with an *S* terminus that can be treated as the 5' and an *R*

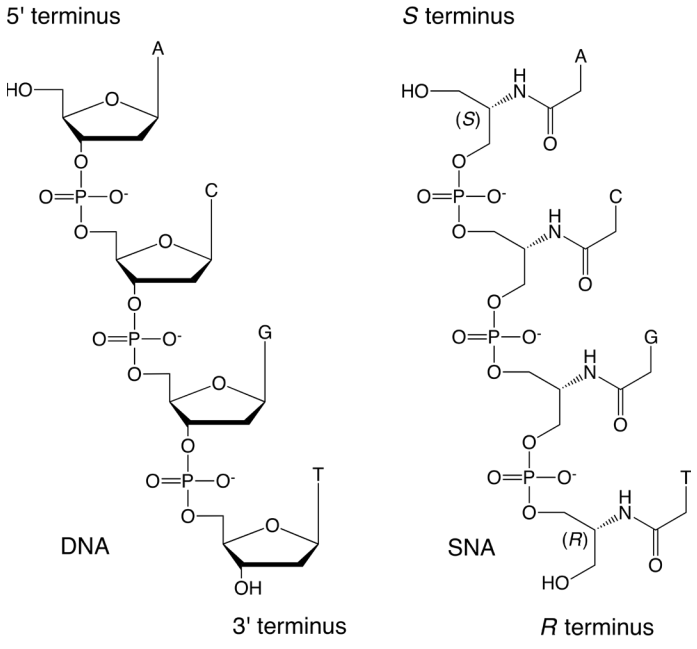


Figure 1. Serinol Nucleic Acid (SNA). SNA and DNA both share a 3-carbon phosphodiester backbone.

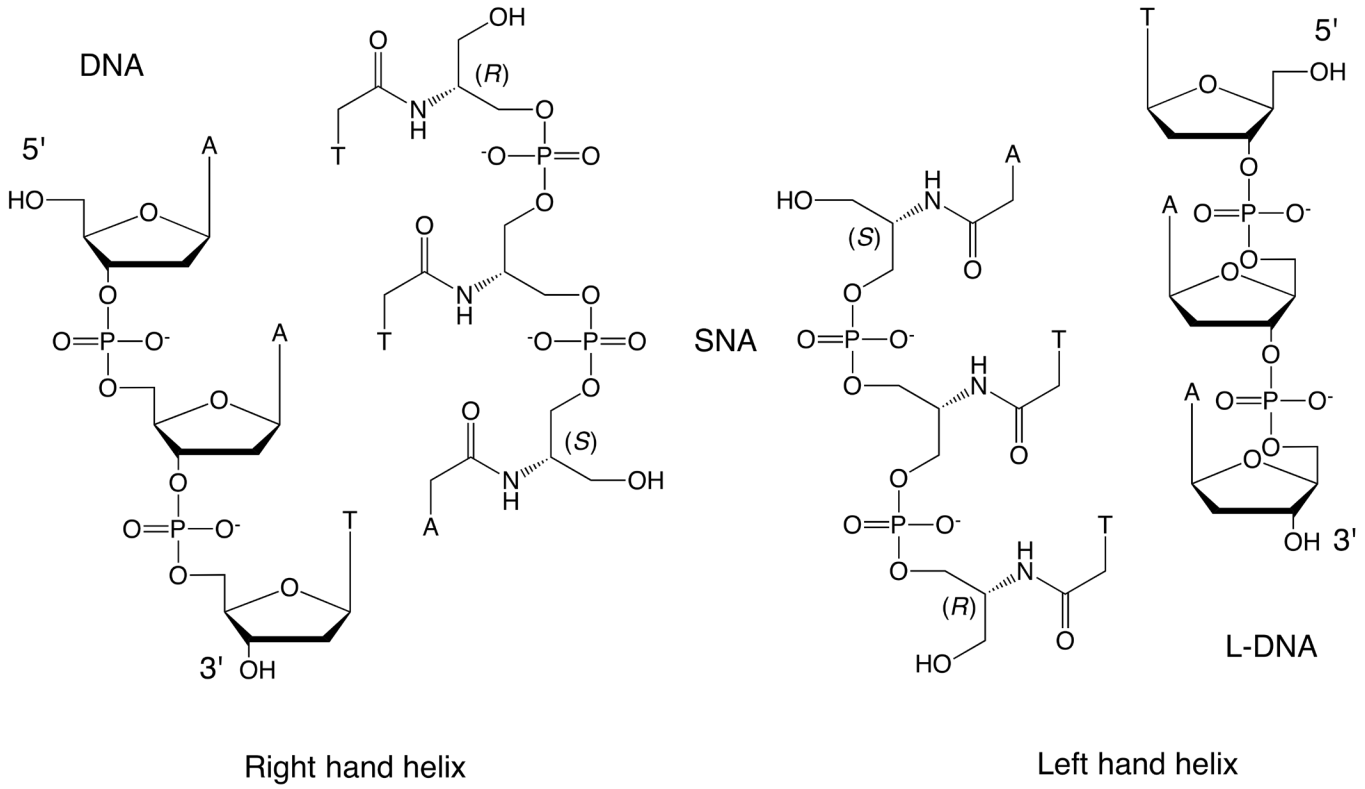


Figure 2. Unique base pairing properties of SNA. A single SNA sequence can hybridize to a complementary strand of DNA or a different complementary strand of L-DNA to form right- and left-handed helices, respectively.

terminus that can be treated as the 3'. To synthesize the mirror image of an SNA sequence, one only needs to reverse the order of addition of the phosphoramidites. These SNA phosphoramidites are somewhat different from our Serinol line of modifiers.[8, 9] While both share the same achiral serinol backbone, the modifiers are not stereopure (Figure 4).

In our hands, the SNA phosphoramidites can be attached with standard coupling times. Deprotection is carried out as dictated by the nucleobase protecting groups and will typically be for 4-8 h at 55 °C with ammonium hydroxide (more on that below). The use of methylamine-containing solutions should be avoided to prevent any N4-methylation of C. SNA can be processed and purified using the same techniques as standard oligonucleotides.

During our initial literature review of SNA, we found one report that showed SNA with diaminopurine was susceptible to loss of the nucleobase via cleavage of the amide bond to give a pseudo abasic site.[10]

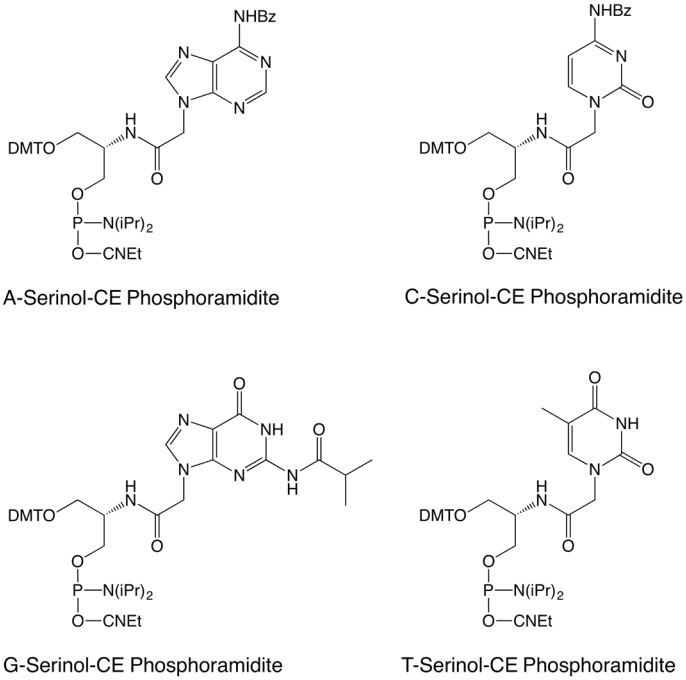
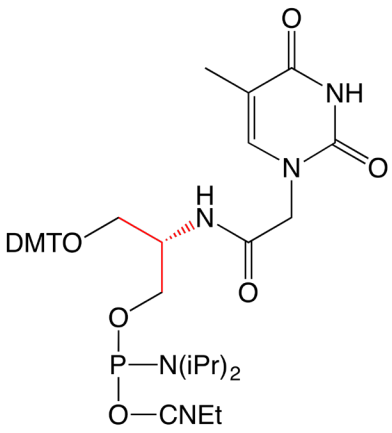
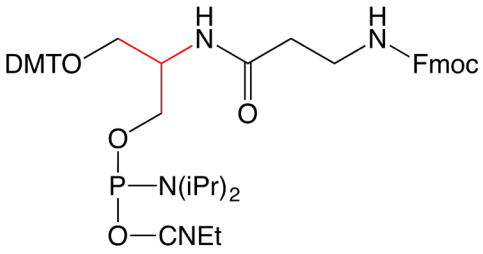


Figure 3. SNA Phosphoramidites



T-Serinol-CE Phosphoramidite



Amino-Modifier Serinol Phosphoramidite

Figure 4. SNA phosphoramidites vs Serinol modifiers. SNA phosphoramidites are stereopure while Serinol modifiers are not.

To evaluate how susceptible this was in the standard bases, oligonucleotides were synthesized with multiple insertions of either SNA A, C, G or T. The oligonucleotides were deprotected at 55 °C and aliquots were removed after about 4, 7 and 22 h. In each case, we were able to find loss of the nucleobase-acetic acid by ESI-MS, and the relative amounts are summarized (Table 1). In the case of A and G, the cleavage was high enough that a nucleobase-acetic acid was identified by RP-HPLC. After 22 h of deprotection, the average loss was just over 1%. For a 4 h deprotection, that would be about 0.2%, which is relatively manageable. Deprotections at 55 °C should not exceed 8 h, and oligonucleotides with many purine insertions will likely be more challenging to purify.

Table 1. Nucleobase loss. Oligonucleotides were deprotected at 55 °C for 22 h, and nucleobase loss was quantified by ESI-MS.

Nucleobase	Nucleobase loss (%)
A	2.0
C	0.7
G	1.3
T	0.3

Item	Pack Size	Catalog No.
A-Serinol-CE Phosphoramidite	0.25 g	10-2390-02
	0.5 g	10-2390-05
C-Serinol-CE Phosphoramidite	0.25 g	10-2391-02
	0.5 g	10-2391-05
G-Serinol-CE Phosphoramidite	0.25 g	10-2392-02
	0.5 g	10-2392-05
T-Serinol-CE Phosphoramidite	0.25 g	10-2393-02
	0.5 g	10-2393-05

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TECHNICAL BRIEF

Backbone Modifications

Previously, we discussed nucleobase and sugar conformations and modifications.[1,2] In this issue, we are highlighting the third component of nucleic acids: its backbone. The natural nucleic acid backbone is comprised of 3'-5'-phosphodiester linkages. As is the case of nucleobase and sugar components, researchers have studied the effects of non-natural modifications of this phosphate backbone. There are numerous ways to modify the natural phosphate backbone, including the overall stereochemistry via L-DNA monomers, 2'-5'-linkages, methyl phosphonate linkages, and replacement of non-bridged oxygen atom(s) with sulfur (Figure 1). This, of course, is not a comprehensive list of all modifications studied, but for the purposes of this article, we will be focusing on backbone modifications offered by Glen Research.

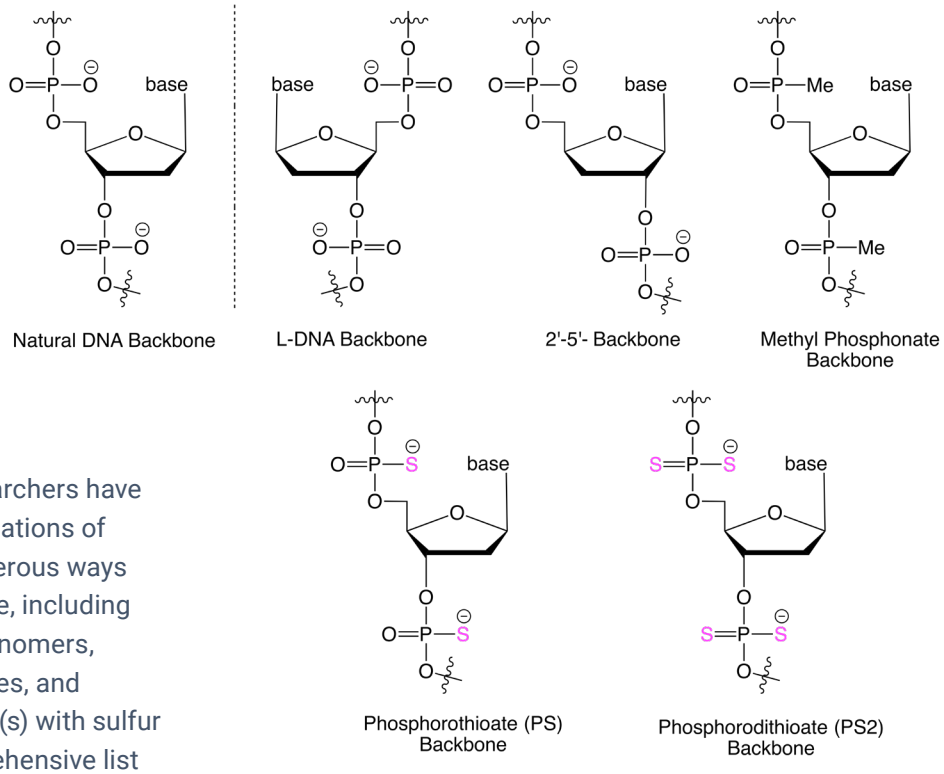

Figure 1. Backbone modifications

Out of the five modifications, L-DNA stands apart from the rest as it does not involve a chemical substitution. Instead, an oligonucleotide made up of L-DNA monomers is the mirror image of naturally occurring D-DNA. These L-DNA oligonucleotides have been discussed in previous Glen Reports.[3] Due to its structure, L-DNA is not recognized by naturally occurring DNA-binding proteins. This allows L-DNA oligonucleotides to evade degradation by nucleases. L-DNA oligonucleotides have been used in various applications, including aptamers, molecular beacons, and drug delivery nanostructures.[3]

Oligonucleotides containing 2'-5'-phosphate linkages selectively bind to complementary, single stranded RNA sequences. These duplexes do not activate RNase H and also exhibit less nonspecific binding to cellular proteins. It has been observed that 2'-5'-linkages reduce the duplex melting temperature by about 0.5 °C per insertion.[4]

These 2'-5'-linkages have been utilized in various applications. Despite poor binding with complementary DNA, 2'-5'-linkages have a stabilizing effect for triplex forming oligonucleotides (TFO). Addition of this modified backbone into a homopyrimidine oligonucleotide enhanced triplex stability under physiological conditions.[5] A combination of 3'-deoxy-2'-phosphoramidites and 2'-deoxy-3'-phosphoramidites have been used to produce a gapmer with 2'-5'-linked ends and 3'-5'-linked central regions. A 3'-deoxynucleoside at the 3'-terminus of an otherwise normal oligonucleotide effectively blocks polymerase extension.

Methyl Phosphonamidites are used to produce oligonucleotides containing methyl phosphonates. These backbones are neutral (not charged) and decrease the overall polarity compared to a negatively charged phosphodiester backbone. While oligonucleotides bearing methyl phosphonate linkages are still taken up into cells, they do so through a different mechanism and to a lesser extent relative to other backbone modifications.[6, 7] Methyl phosphonates confer resistance to nucleases. Each methyl phosphonate linkage introduces a chiral center into the oligonucleotide. Methyl phosphonolated oligonucleotides have been evaluated in antisense therapeutics.[8]

Introducing phosphorothioate (PS) linkages are unique from the others in this list because no special phosphoramidite is required. A standard phosphoramidite is used on a synthesizer and the oxidizer is replaced with sulfurizing reagent. While several sulfurizing reagents have been used and evaluated in the literature, such as the Beaucage Reagent, we recommend DDTT ((dimethylamino-methylidene)amino)-3H-1,2,4-dithiazole-3-thione) (Figure 2) for sulfurization. Sulfur atoms are larger than oxygen and better at dispersing the negative charge in a PS linkage. This provides resistance to nuclease degradation. Phosphorothioates are often used in antisense oligonucleotides (ASOs). In fact, PS linkages have been utilized in 10 of the 18 FDA-approved therapeutic oligonucleotides.[9] Similar to methyl phosphonates, phosphorothioates are chiral and will produce two diastereomers per insertion.

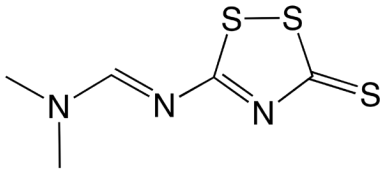

Figure 2. Sulfurizing Reagent II (DDTT)

Phosphorodithioate linkages are prepared by combining thiophosphoramidites with the above mentioned, sulfurization reagent. Phosphorodithioates reduce oligonucleotide complexity because each insertion is achiral. Phosphorodithoates and their use in oligonucleotide therapeutics have recently been discussed.[10] Glen Research also offers 2'-OMe thiophosphoramidites.

Modifications of the phosphate backbone lead to differences in nucleic acid activity. With various substitutions, nuclease resistance and thermal stability can be altered to best fit one's needs (Table 1).

Table 1. Overview of standard and modified backbones available at Glen Research

Modification	Nuclease Resistance	Chirality	Thermal Stability
D-DNA		Achiral	
L-DNA	✓	Achiral	
2'-5'-Linkages	✓	Achiral	∇
Methyl Phosphonates	✓	Chiral	∇
Phosphorothioates	✓	Chiral	*
Phosphorodithioates	✓	Achiral	∇

∇ = decreased, * = depends on stereopurity[11, 12]

Item	Product (Phosphoramidite only)	Catalog No.
L-DNA	beta-L-Pac-dA-CE	10-2101
	beta-L-Ac-dC-CE	10-2115
	beta-L-iPr-Pac-dG-CE	10-2121
	beta-L-dT-CE	10-2130
2'-5'- Phosphoramidites	3'-dA-CE	10-1004
	3'-dC-CE	10-1064
	3'-dG-CE	10-1074
	3'-dT-CE	10-1084
Methyl Phosphonamidites	dA-Me	10-1100
	Ac-dC-Me	10-1115
	dG-Me	10-1120
	dT-Me	10-1130
Phosphorothioates	Sulfurizing Reagent II	40-4037
Phosphorodithioates	dA-Thiophosphoramidite	10-1700
	dC-Thiophosphoramidite	10-1710
	dG-Thiophosphoramidite	10-1720
	dT-Thiophosphoramidite	10-1730

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NEW PRODUCT

5X Tris-Borate-EDTA (TBE) Buffer

We offer a few buffers already that are used for oligonucleotide processing, analysis, and purification. A couple of these are ammonium acetate buffers, triethylammonium acetate (TEAA) and hexylammonium acetate (HAA), commonly used as mobile phase buffers for reverse phase high-performance liquid chromatography (RP-HPLC). We also have a quenching buffer that is used to stop the 2'-desilylation reaction for RNAs. We are happy to expand our buffer offerings by introducing 5X Tris-Borate-EDTA (TBE) Buffer.

When diluted to 1X working concentration, TBE is a popular running buffer for various applications:

- Electrophoresis of nucleic acids in agarose and polyacrylamide gels analyzing RNA and DNA fragments smaller than 1,500 bp[1]
- Plasmid characterization and extraction
- DNA automated sequencing gels
- DNA fingerprinting
- Detection of DNA polymorphisms
- Restriction enzyme mapping[2]

This electrophoresis buffer is essential for the success of agarose gels and polyacrylamide gel electrophoresis (PAGE) because it allows current to flow through the gel while keeping oligonucleotide samples at biologically appropriate pH to maintain their charge and structure.[3]

It is compatible with both non-denaturing and denaturing gels and yields higher resolution of smaller fragments. TBE has a higher buffer capacity, compared to other running buffers, meaning it performs better over a longer period of time.

Our TBE is made with high quality reagents and is tested to ensure the buffer is free of RNase contamination. Once diluted 5-fold, TBE buffer contains 89 mM Tris Borate and 2 mM EDTA at pH 8.3 and is ready for use in gel electrophoresis.

For successful gel electrophoresis experiments when using our 1X TBE buffer, we generally recommend following some tips:

- Load equal volumes of DNA and ladder DNA
- Stick to using one loading dye per gel
- Avoid high salt concentrations in your samples

Item	Pack Size	Catalog No.
5X Tris-Borate-EDTA (TBE) Buffer	1 L	60-4130-80

References

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APPLICATION NOTE

RNA Synthesis

Long RNA synthesis has become increasingly popular for various applications. Many researchers find themselves needing very long (100mer+) RNA oligonucleotides for real-time PCR, sgRNA for CRISPR technology[1-3], or short mRNA sequences.[4] Long RNAs are also important for studying genomic pathways and drug discovery. Finally, the regulatory role of long RNA in biology requires the synthesis and analysis of long RNAs.[5]

Traditionally, RNA synthesis is less efficient than DNA synthesis because of the 2'-O-protecting group required for RNA. The most common protecting group at this position is a silyl group that tends to be bulky and can impede coupling through steric hindrance. TBDMS is the standard 2'-silyl group while TOM is popular as well. TOM is often recommended for long RNA synthesis because the oxymethyl spacer extends the bulky silyl group further away from the active phosphoramidite center (Figure 1). In addition, the impact of secondary structure in regard to RNA complicates the isolation of long, single-stranded RNAs.

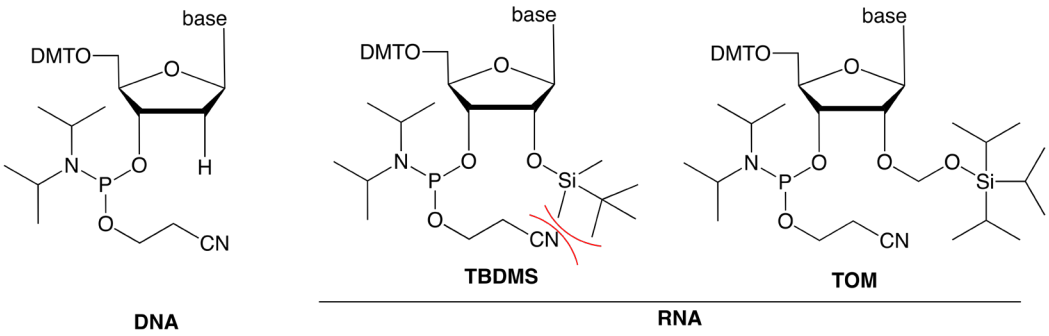
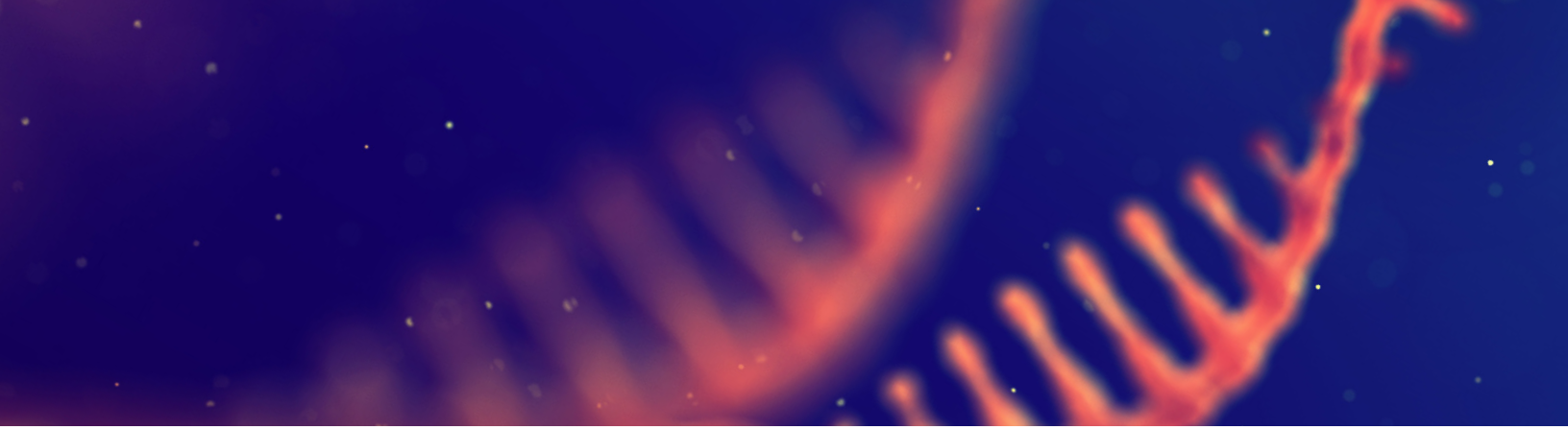


Figure 1. DNA and standard RNA phosphoramidites

To better support this research, we sought out to provide a quantitative assessment of various factors impacting the quality of RNA synthesis, such as RNA monomers and universal supports.

To do this, we synthesized 20mer oligonucleotides under various conditions and extrapolated the data to determine efficiency for longer syntheses. DCA deblock was chosen for these experiments to mimic recommended conditions for long oligonucleotide synthesis.[6] A 6 min coupling time was used with 0.25M ETT as the activator for all RNA monomers.[7] We compared DNA, TBDMS, and TOM monomers as well as the impact of our two universal supports: Universal Support (US) III PS and Glen UnySupport™ CPG.



Our methods are as follows:

- **Synthesis.** On an ABI 394 synthesizer, a 20mer oligonucleotide was synthesized on a 1.0 umol support, with the following sequence using DNA, TBDMS, and TOM phosphoramidites:

  5'-UUG UUC UUA UUG UUC UUA UU*-3'
  *For DNA control, T was used in place of U.

- **Cleavage & Deprotection.** Each oligonucleotide was deprotected according to support recommendations.
  - For oligonucleotides synthesized using US III PS, oligonucleotides were incubated in 2M ammonia in methanol for 60 min at RT. Without drying down, an equal volume of AMA was added to the solution, and deprotection was continued for 10 min in a 65 °C water bath. The solution was filtered and dried in a sterile microcentrifuge tube using a steady flow of argon to evaporate the liquid.
  - For oligonucleotides synthesized using Glen UnySupport CPG, oligonucleotides were incubated in AMA for 60 min in a 65 °C water bath. The solution was filtered and dried in a sterile microcentrifuge tube using a steady flow of argon to evaporate the liquid.

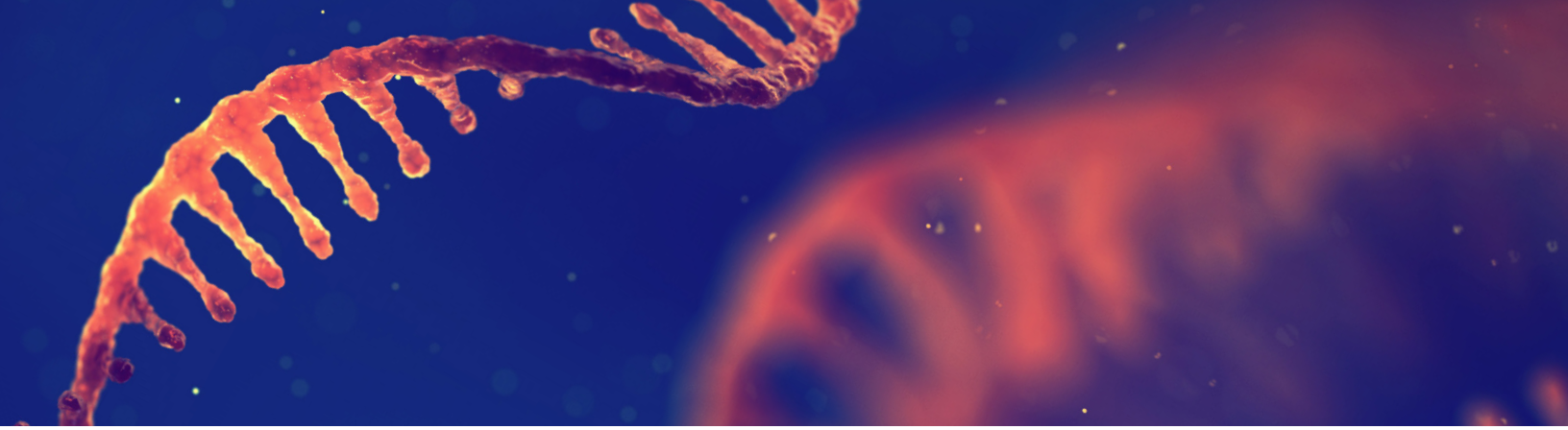
- **2'-Desilylation.** The oligonucleotides were redissolved in DMSO (115 µL) and warmed in a 65 °C water bath until fully dissolved. TEA (60 µL) and TEA•3HF (75 µL) were added to the reaction. The mixture was heated in a 65 °C water bath for 2.5 h. The reaction was quenched by adding 750 µL RNA quenching buffer.

- **Desalting.** To remove reaction conditions from the previous step, the oligonucleotide was desalted using a Glen Gel-Pak™ 1.0 desalting column. The recommended conditions were followed, and crude oligonucleotides were eluted in 0.1M RNase-free TEAA and analyzed by reverse-phase high-performance liquid chromatography (RP-HPLC).

Coupling efficiency was calculated based on crude oligonucleotide purity. As expected, DNA phosphoramidite coupling performance was the best of the three followed by TOM phosphoramidites and then TBDMS phosphoramidites (Table 1). Crude purity of TOM- and TBDMS-prepared oligonucleotides synthesized on US III PS were 80.1 and 77.6%, respectively (Figure 2). Based on MS and RP-HPLC analysis of our crude RNA oligonucleotides, we detected a small degree of DMT loss. The full-length DMT-OFF oligonucleotide co-eluted with failure sequences. The loss of the trityl group likely occurred during the drying step necessary for cleavage and deprotection and the 2'-desilylation reaction. This was unavoidable and has implications when it comes to choosing a purification method for your long RNA oligonucleotides. This was a contributing factor to the coupling efficiency differences between DNA and RNA in Table 1.

Table 1. Summary of coupling efficiencies

Universal Support	Monomer	Coupling Efficiency
US III PS	DNA	99.7%
	TOM	98.9%
	TBDMS	98.7%
UnySupport CPG	TBDMS	97.7%



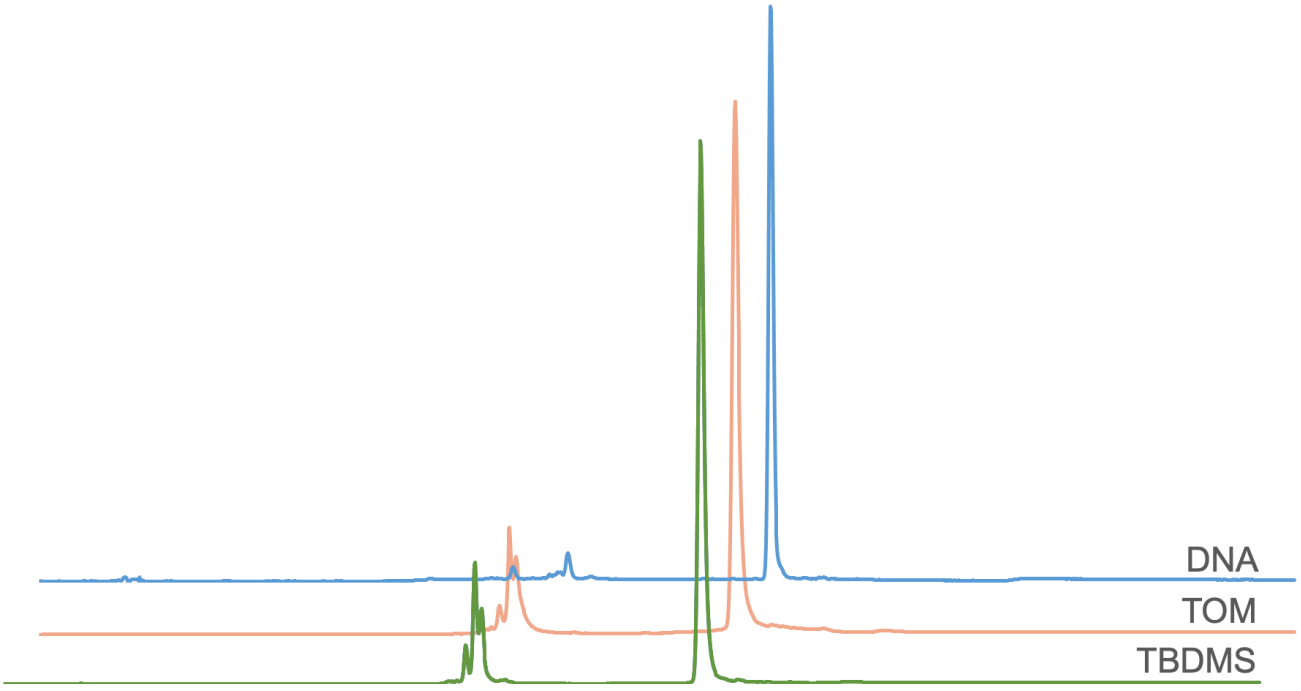


Figure 2. Crude RP-HPLC chromatograms of 20mer oligonucleotides synthesized on US III PS with DNA (blue), TOM (orange), and TBDMS (green) monomers.

Although only 20mer oligonucleotides were synthesized in this exercise, we can use these values to extrapolate the expected overall purity of a 100mer. We recognize a few caveats with this, namely the fact that failure sequences absorb less at 254nm than the full-length oligonucleotide. It is worth remembering that this extrapolation will predict what the chromatogram looks like, rather than the ultimate yield. Using the following equation, a 100mer made of TOM affords a 33% crude oligonucleotide purity while TBDMS provides a 27% crude oligonucleotide purity.

$$CE^{L}$$

where CE = coupling efficiency (written as a decimal) and
L = length

Unsurprisingly, US III PS offered better results than UnySupport CPG (Table 1, Figure 3). UnySupport requires very harsh conditions to cleave the oligonucleotide from the support. For example, standard nucleobase deprotection using AMA requires 65 °C for 10 min but UnySupport requires AMA at 65 °C for 1 hr for complete cleavage. Using this method, we observed some cleavage that yielded shorter DMT-ON fragments in our crude HPLC (highlighted with the red box). This is particularly concerning considering reverse phase cartridge purification would not be able to distinguish between the full-length DMT-ON sequence and the DMT-ON cleavage fragments.

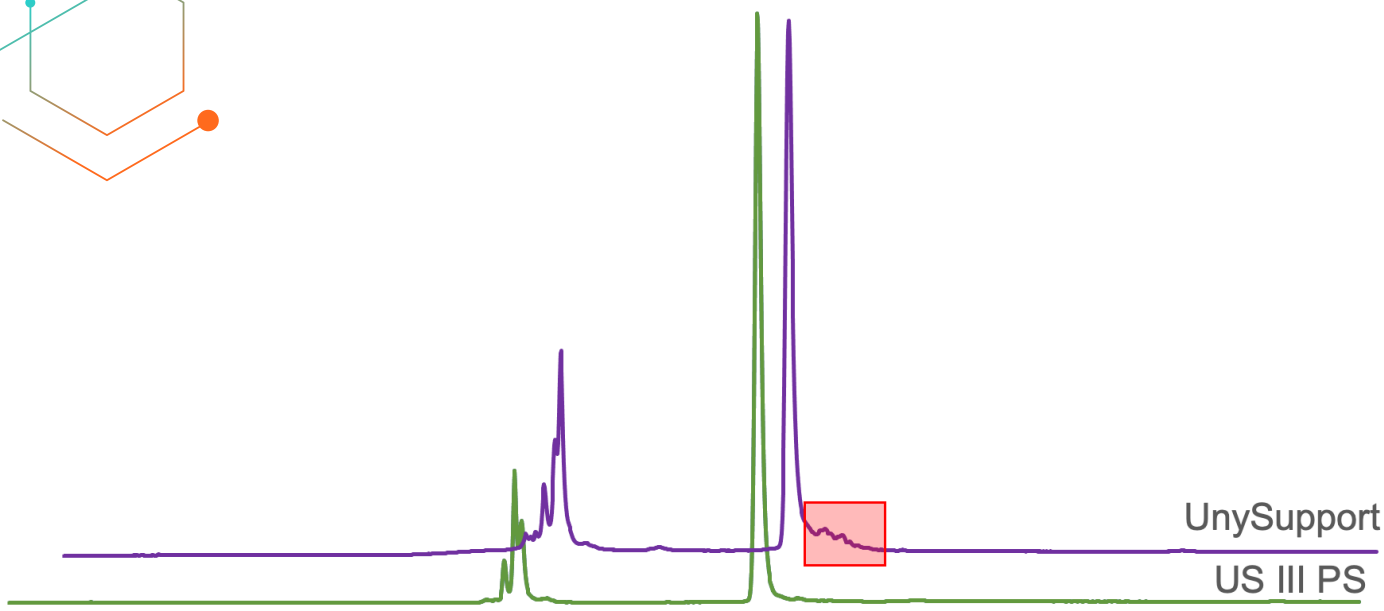


Figure 3. Crude RP-HPLC chromatograms of 20mer RNA oligonucleotide synthesized with TBDMS monomers on UnySupport (purple) and US III PS (green).

Considering we synthesized oligonucleotides that were 20 nucleotides long, one thing we did not evaluate in this study is the impact of pore sizes on the length of oligonucleotides that can be prepared on certain solid supports. Our general rule of thumb for synthesis length is shown below, and the Glen UnySupport CPG used in this experiment was 1000 Å.

- 500 Å CPG is up to 50mer
- 1000 Å CPG is compatible with 75-100mer
- 2000 Å CPG is for >100mer
- PS is comparable to 1000 Å CPG

Based on our data, we can conclude some key takeaways when it comes to long RNA synthesis and RNA synthesis in general:

TOM offers a slightly higher coupling efficiency than TBDMS, which makes a bigger impact the longer the oligonucleotide length is.

Some loss of the DMT group is unavoidable under all conditions evaluated. This most likely occurs during drying the oligonucleotide down and the 2'-desilylation reaction. If pursuing reverse phase purification techniques, this will negatively impact the final yield of recovery as the DMT-OFF full length is washed away with any failure sequences. Another consideration to take is the finite hydrophobicity of the trityl group becomes less effective at DMT-ON purification as the very polar phosphate backbone increases in length. To circumvent these concerns, ion exchange chromatography or PAGE purification may be better options.

US III PS provides cleaner crude results than that of UnySupport. Some strand cleavage occurs during the harsh conditions required for UnySupport cleavage, which yields DMT-ON fragment sequences. The presence of these fragments may interfere with purification using reverse phase techniques.

Relevant Products

Product Line	Product Name	Catalog No.
TBDMS	Bz-A-CE	10-3003
	Ac-C-CE	10-3015
	Ac-G-CE	10-3025
	U-CE	10-3030
TOM	A-TOM-CE	10-3004
	C-TOM-CE	10-3014
	G-TOM-CE	10-3024
	U-TOM-CE	10-3034
Universal Supports	Universal Support III PS	26-5010
	Glen UnySupport™ 1000	20-5041
Deblock	3% Dichloroacetic acid in Dichloromethane	40-4040
Activator	0.25M 5-Ethylthio-1H-Tetrazole (ETT) in Anhydrous Acetonitrile	30-3140
RNA Quenching Buffer	Quenching buffer for Glen-Pak™ purification	60-4120
Desalting Columns	Glen Gel-Pak™ 1.0 Desalting Column	61-5010

References

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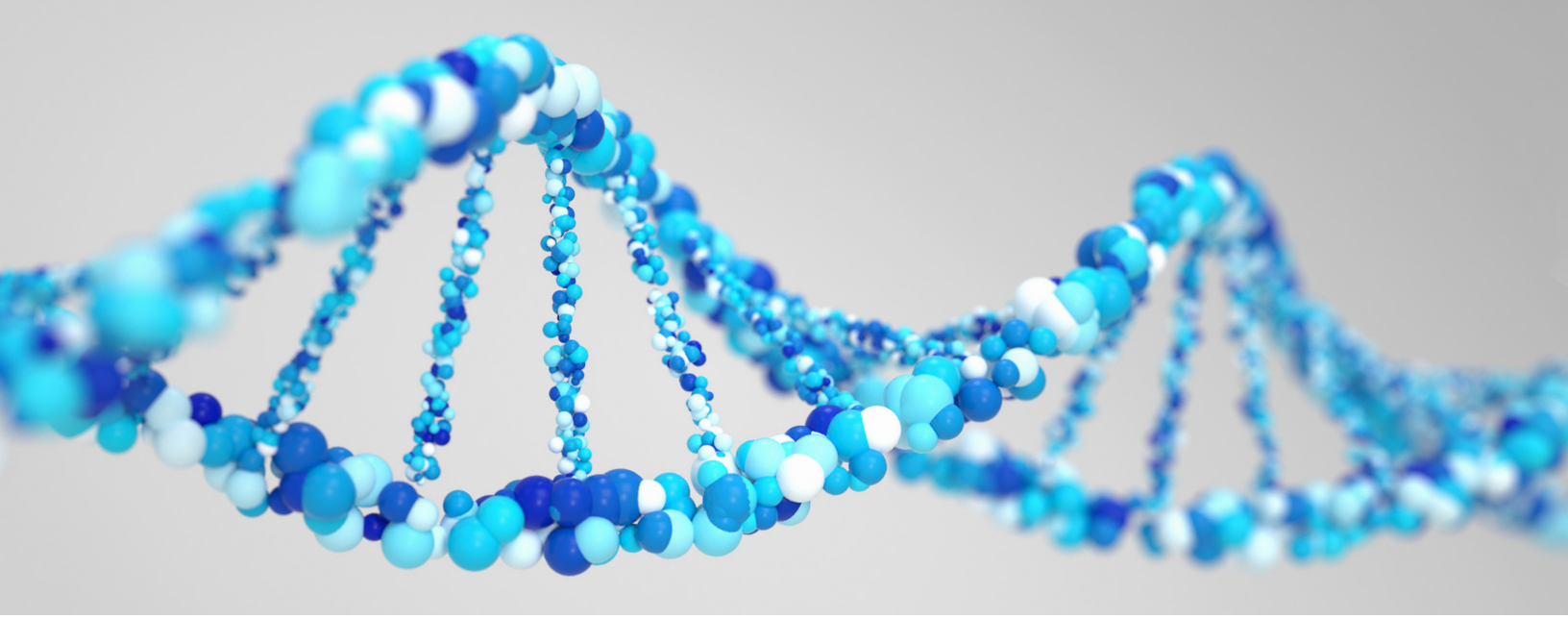
APPLICATION NOTE

Thermal-Aqueous Deprotection of 5'-Trityl Amino Modifiers

Trityl-protected amino modifiers offer an effective strategy for synthesizing amino-labeled oligonucleotides, owing to the versatility of the protecting group.[1] This trityl moiety can either be cleaved by the synthesizer to enable on-column conjugation or be retained as a purification aid for subsequent reverse-phase high-performance liquid chromatography (RP-HPLC) and Glen-Pak™ cartridges. Given these advantages, our assortment of trityl-protected amino modifiers enjoy widespread popularity among our customers.

Over the years, we have published several articles focusing on the utilization of trityl-protected amino modifiers,[1-5] and one of these articles addressed proper retention and removal of the trityl group as needed.[4] Specifically, MonoMethoxyTrityl (MMT) amino modifiers traditionally necessitated treatment with 20% aqueous acetic acid for an hour at room temperature post-purification to eliminate the MMT moiety. However, recent advancements have introduced an acid-free method, detailed in a recent journal publication, which enables quantitative removal of MMT under neutral aqueous conditions at 60 °C for 60 minutes.[6] This process involves heating the oligonucleotide to hydrolytically cleave the MMT-amine bond, resulting in



the formation of MMT-OH. Although MMT removal can be reversible, the precipitation of MMT-OH, which is insoluble in aqueous conditions, drives the MMT removal to completion (refer to Figure 1). In contrast, the analogous MMT removal conducted in a 1:1 mixture of acetonitrile and water achieved only 55% completion. Furthermore, the authors demonstrated that phosphate buffer saline (PBS) (pH 7.4) and Tris-EDTA (TE) buffer (pH 8.0) did not hinder MMT removal, whereas concentrated ammonium hydroxide did. Notably, this methodology extends to DiMethoxyTrityl (DMT) groups attached to hydroxyl groups, albeit requiring a more rigorous condition of 95 °C for 90 minutes due to the increased difficulty of DMT removal.

We successfully validated this new protocol through the following experimental procedures:

- Synthesized 5'-Amino-Modifier C6-T_6.
- Deprotected the synthesized compound using ammonium hydroxide.
- Introduced 1x PBS.
- Evaporated the resulting solution to dryness.
- Resuspended the solid residue in water.
- Subjected the solution to heating at 60 °C for 60 minutes.

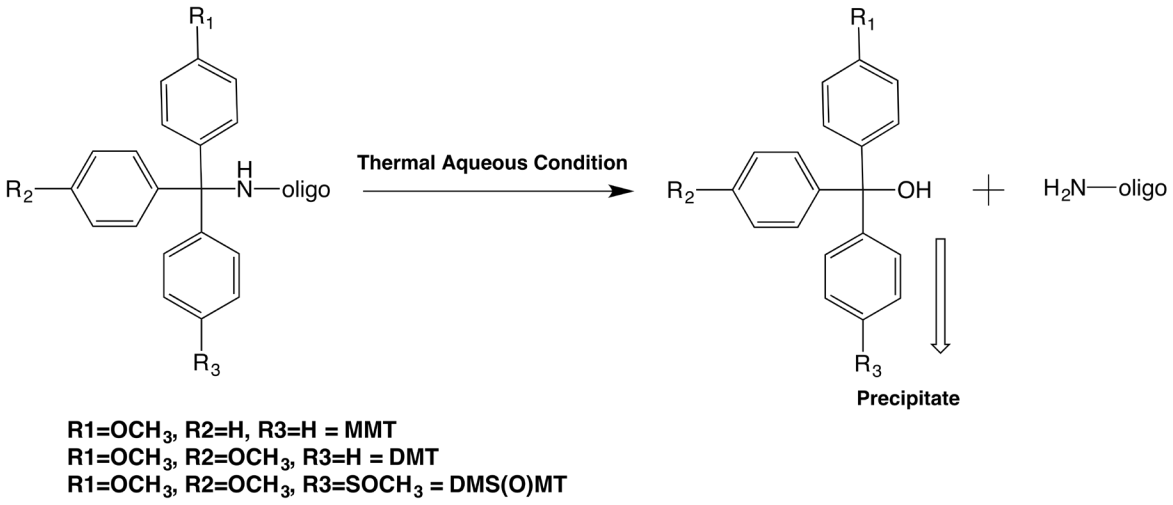


Figure 1. Thermal aqueous deprotection

The oligonucleotide was analyzed by RP-HPLC post-deprotection, post-resuspension, and post-heating (Table 1). As anticipated, the trityl groups remained intact during the deprotection process. However, during evaporation and subsequent resuspension, approximately 14% of the MMT groups were removed. This result is consistent with the use of our evaporation apparatus, which uses heat to aid in the drying of the deprotected oligonucleotide. When the gaseous ammonia is depleted while water is still present, the trityl removal process gets a head start. Consequently, we recommend the addition of 45 mg of Tris base per mL of deprotection solution during the drying phase for such oligonucleotides to retain the trityl-protecting group.[4] This quantity corresponds to an initial concentration of 0.37 M, a significant amount of Tris base. At this concentration, Tris base not only ensures that ammonium counterions do not trigger premature detritylation but also serves as a sufficiently basic agent to prevent thermal aqueous cleavage of the MMT groups.

Table 1. 5'-Trityl removal during deprotection and aqueous heating. Oligonucleotide quantities were determined by RP-HPLC.

	After deprotection (NH_4OH)	Evaporation & redissolve (H_2O)	After heating
MMT-NH-T_6	0%	14%	100%
DMS(O)MT-NH-T_6	0%	16%	100%
DMT-T_6	0%	0%	100%

We extended the same experimental procedure to DMS(O)MT-NH-T_6 and DMT-T_6, yielding results consistent with those reported in the literature (refer to Table 1). Notably, DMS(O)MT exhibits even greater lability than MMT, suggesting the potential for lower temperatures or shorter incubation times during deprotection. For DMT-ON oligonucleotides, prolonged heating at 95 °C may not always be optimal depending on the oligonucleotide composition; however, it presents another viable option for purification and workup. While we didn't explore the application of our other MMT-protected amino modifiers, such as C12 and C5, we anticipate their behavior to mirror that of the C6 version.

As our experienced customers are aware, oligonucleotides are prone to depurination when exposed to acids, particularly in the absence of protective groups. Hence, the opportunity to circumvent acid treatment entirely in the final step is noteworthy, and we encourage users to explore this alternative approach.

Item	Pack Size	Catalog No.
5'-Amino-Modifier C6	100 µmol	10-1906-90
	0.25 g	10-1906-02
5'-DMS(O)MT-Amino-Modifier C6	100 µmol	10-1907-90
	0.25 g	10-1907-02

References

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Technical Snippets

Why are certain modifications only available as NHS esters?

Some modifications (such as azidobutyrate or certain rhodamine dyes) are not available or preferred as phosphoramidites. This is either due to chemical instability, incompatibility with standard phosphoramidite coupling conditions, or the modification may not survive even mild oligonucleotide deprotection conditions.

For example, azide phosphoramidites are unstable because the azide group can be reduced by the P(III) center of the phosphoramidite via the Staudinger reaction. On the other hand, rhodamines (TAMRA and ROX) are sensitive to base and experience degradation during oligonucleotide deprotection and cleavage. These dyes are comprised of two structural elements: (1) the modified rhodamine dye and (2) a 6-carboxyl aryl linker. The extent of degradation depends on the fluorophore and may be managed. TAMRA has been prepared as a phosphoramidite and solid support but UltraMild conditions are strongly advised. A phosphoramidite version of ROX is not available. Post-synthesis labeling techniques are often preferred for installing these dyes into an oligonucleotide.

In other cases, there may be a phosphoramidite version available but the NHS ester is preferred. This is particularly true for modifications that are very sensitive to coupling and/or deprotection conditions, such as sulfoCyanine 5 NHS Ester and Methylene Blue NHS Ester. Post-synthetic labelling with the NHS ester precludes the need for using UltraMild conditions.

Relevant products:

Azidobutyrate NHS Ester (50-1904)

ROX NHS Ester (50-5911)

TAMRA NHS Ester (50-5910)

sulfoCyanine 5 NHS Ester (50-5915)

Methylene Blue NHS Ester (50-1960)

Can one use the Glen Gel-Pak™ columns for counterion exchanges?

Glen Gel-Pak columns are not designed to exchange counterions during the purification. These columns are mainly designed for the desalting of oligonucleotides, clean-up of conjugation reactions, and the removal of deprotection solution including the released protecting groups.

The Glen Gel-Pak desalting protocol calls for a buffer during the elution step of the desired product. Based on the nature of the buffer, some counterion exchange can occur, however, this is not sufficient to exchange all of the counterions. Therefore, we recommend using other more efficient methods such as ethanol precipitation to exchange the counterions.

Relevant products:

Glen Gel-Pak™ 1.0 Desalting Column (61-5010)

Glen Gel-Pak™ 2.5 Desalting Column (61-5025)

Glen Gel-Pak™ 0.2 Desalting Column (61-5002)

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