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Newsletter

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Deprotection — Volume 3 — Dye-Containing Oligonucleotides, an Update

While following our principle of "Deprotection to Completion", one should always adhere to our mandate of “Do No Harm”. This is especially true when dealing with expensive dyes since these modifications are often sensitive to basic deprotection conditions. Our dye deprotection table, first published in 2009[1] and subsequently updated in 2013[2], provides guidelines and recommendations for deprotection conditions of oligonucleotides containing a combination of dyes and/or quenchers. Over the years, this table has been a popular reference tool for our customers.

Since our last update, we have added a couple of rhodamine dyes to our catalog, AquaPhluor® 593 (AP593) and AquaPhluor® 639 (AP639). Both these dyes are part of the ELITechGroup AquaPhluor® family of fluorescent dyes. AP593 is a good substitute for Texas Red®[3] while AP639 is an alternative to Cyanine 5.[4] We have added these products to the deprotection table.

Identifying the optimum deprotection conditions requires connecting columns and rows of the interested dyes in the table. Referencing the table, the preferred deprotection methods for an oligonucleotide containing both Fluorescein and TAMRA are “E” and “F”. The choice of the “E” and “F” deprotection conditions, in this case, is mainly dictated by the TAMRA, which is more sensitive to basic deprotection conditions, relative to the Fluorescein. It is worth noting that the deprotection conditions apply to all product versions (5’-phosphoramidites, internal modifications such as dT-dye, and the supports).

As before, the methods for oligonucleotide deprotection (A-G) are as follows:

A. 30% NH_4OH 17 hours at 55 °C; sufficient to deprotect all standard bases, A/C/G/T.

B. 30% NH_4OH 17 hours at room temperature; sufficient to deprotect A, C, and dmf-dG.

C. 30% NH_4OH 2 hours at 65 °C; sufficient to deprotect A, C, and dmf-dG.

D. 30% NH_4OH 2 hours at room temperature; sufficient to deprotect only UltraMild monomers, Pac-dA, Ac-dC, iPr-Pac-dG when UltraMild Cap A is used.

E. 50 mM Potassium Carbonate in Methanol for 4 hours at room temperature; sufficient to deprotect only UltraMild monomers, Pac-dA, Ac-dC, iPr-Pac-dG when UltraMild Cap A is used.

F. Tert-Butylamine/water 1:3 (v/v) 6 hours at 60 °C; sufficient to deprotect A, C, and dmf-dG.

G. 30% Ammonium Hydroxide/40% Methylamine 1:1 (v/v) 10 minutes at 65 °C; sufficient to deprotect all standard bases, however, Ac-dC must be used.

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Table 1: Deprotection Conditions Suitable for Popular Dyes and Quenchers

	AP593	AP639	BHQ-1	BHQ-2 BBQ-650	BHQ-3	Cyanine 3	Cyanine 5	Fluorescein	HEX	JOE Dabcyl	TAMRA	TET Eclipse	Yakima Yellow
AP593	$A, B, C, G	$A, B, C, G	$A, B, C, G	$A, B, C	$B	B, C, G	⊗	$A, B, C, $G	B, G	$A, B, C, G	⊗	$A, B, C, G	$A, B, C
AP639	$A, B, C, G	A, B, C, F, G	$A, B, C, $F, G	A, B, C	$B	B, C, G	⊗	A, B, C, F, $G	B, G	A, B, C, G	F	A, B, C, F, G	A, B, C, F
BHQ-1	$A, B, C, G	$A, B, C, $F, G	$A, B..E, $F, G	$A, B..E	$B, D	B..E, G	D, E	$A, B, C, $F, $G	B, D, E, G	$A, B..E, G	E, $F	$A, B..E, $F, G	$A, B, C, D, $F
BHQ-2 BBQ-650	$A, B, C	A, B, C	$A, B..E	A..E	$B, D	B..E	D, E	A..E	B, D, E	A..E	E	A..E	A..D
BHQ-3	$B	$B	$B, D	$B, D	$B, D	$B, D	D	$B, D	$B, D	$B, D	⊗	$B, D	$B, D
Cyanine 3	B, C, G	B, C, G	B..E, G	B..E	$B, D	B..E, G	D, E	B..E, $G	B, D, E, G	B..E, G	E	B..E, G	B, C, D
Cyanine 5	⊗	⊗	D, E	D,E	D	D, E	D, E	D, E	D, E	D, E	E	D, E	D
Fluorescein	$A, B, C, $G	A, B, C, F, $G	$A, B..E, $F, $G	A..E	$B, D	B..E, $G	D, E	A..F, $G	B, D, E, $G	A..E, $G	E, F	A..F, $G	A..D, F
HEX	B, G	B, G	B, D, E, G	B, D, E	$B, D	B, D, E, G	D, E	B, D, E, $G	B, D, E, G	B, D, E, G	E	B, D, E, G	B, D
JOE Dabcyl	$A, B, C, G	A, B, C, G	$A, B..E, G	A..E	$B, D	B..E, G	D, E	A..E, $G	B, D, E, G	A..E, G	E	A..E, G	A..D
TAMRA	⊗	F	E, $F	E	⊗	E	E	E, F	E	E	E, F	E, F	F
TET Eclipse	$A, B, C, G	A, B, C, F, G	$A, B..E, $F, G	A..E	$B, D	B..E, G	D, E	A..F, $G	B, D, E, G	A..E, G	E, F	A..G	A..D, F
Yakima Yellow	$A, B, C	A, B, C, F	$A, B, C, D, $F	A..D	$B, D	B, C, D	D	A..D, F	B, D	A..D	F	A..D, F	A..D, F

⊗ = Incompatible $ = Denotes an acceptable, but not preferred method.

Notes:

1. JOE (5'-Dichloro-dimethoxy-Fluorescein Phosphoramidite) has not been tested with Condition F.

2. Fluorescein is compatible with deprotection in AMA without any degradation if the oligo is first treated with 30% NH_4OH to remove the pivaloyl protecting groups from the Fluorescein and then the 40% Methylamine is added to complete the deprotection of the bases.

References

1. *The Glen Report,* 2009, **21.2**, 11.
2. *The Glen Report*, 2013, **25.S**, 4.
3. *The Glen Report,* 2016, **28.2**, 8.
4. *The Glen Report*, 2020, **32.2**, 1-3.

New Products — UnySupport™ CPGs

Due to their versatility, it's no surprise that universal supports remain popular over requiring a support for each 3'-nucleoside or modification. Particularly when a special modification is only available as a phosphoramidite, a reliable universal support is essential. Our UnySupport, which is based on UnyLinker chemistry, was first introduced in 2008 (Figure 1).[1] Since then, we have included multiple versions: 500Å CPG, 1000Å CPG, high load CPG, and polystyrene.[2] We are pleased to introduce two new versions of UnySupport: 1400Å CPG and 2000Å CPG.

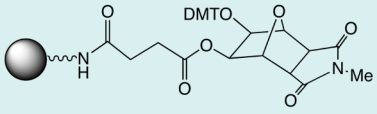

Figure 1. UnySupport Structure

Prior to now, our 2000Å CPG supports were limited to main DNA bases (A, C, G, and T). The introduction of UnySupport 2000Å CPG allows researchers to use all of our modifiers in a 2000Å environment. The 2000Å supports are best for very long (>150 mer) oligonucleotides. However, a caveat to using such a large pore size is the limited loading. For customers who do not wish to sacrifice this, we are also offering UnySupport 1400Å CPG, which is capable of synthesizing long oligonucleotides without compromising loading.

Oligonucleotide deprotection and cleavage from Glen UnySupport can be carried out with various methods: UltraFast, Standard, UltraMild, or gas phase. The cleavage mechanism is two steps and has been described in previous Glen Reports.[1] Deprotection with Ammonium Hydroxide:MethylAmine (AMA) 1:1 requires 1 hour at 65 °C or Ammonium Hydroxide for 8 hours at 55 °C. For sensitive minor bases or dyes, Glen UnySupport may be eliminated with 50 mM Potassium Carbonate in Methanol in 17 hours at room temperature or with Tert-Butylamine/water 1:3 (v/v) for 4 hours at 60 °C. Glen UnySupport is also compatible with Methylamine gas for 30 minutes at 65 °C at 30 psi.

References

1. *The Glen Report*, 2008, **20.2**, 10-11.
2. *The Glen Report*, 2009, **21.1**, 14.

Item	Pack Size	Catalog No.
UnySupport™ 1400	0.1 g	20-5044-01
	0.25 g	20-5044-02
	1.0 g	20-5044-10
1 µmol columns	Pack of 4	20-5044-41
0.2 µmol columns	Pack of 4	20-5044-42
10 µmol columns (ABI)	Pack of 1	20-5044-13
UnySupport™ 2000	0.1 g	20-5042-01
	0.25 g	20-5042-02
	1.0 g	20-5042-10
0.2 µmol columns	Pack of 4	20-5042-42

New Products — LA CPGs

Locked Nucleic Acid (LNA) is a modified ribonucleic acid containing a methylene bridge connecting the 2'-oxygen and 4'-carbon atoms. LNA is a very popular backbone modification.[1, 2] The bicyclic structure introduces conformationally restrained units, locking the modified ribose into a C3'-endo conformation (Figure 1).[3] LNA-modified oligos are increasingly prominent for hybridization assays and probes due to enhanced thermal stability towards complementary oligos, without compromising base pairing specificity.[2] Each LNA modification can increase the melting temperature of a duplex by 2-8 °C.[4]

The improved binding of LNA oligos have been crucial for the detection of difficult samples, such as microRNA (miRNA).[5] DNA- or RNA-based technologies for miRNA analysis is complicated because the melting temperature is highly dependent on the GC content of the sequence. The T_m of a duplex can be designed by varying the LNA content, regardless of the presence of GC base pairs in a miRNA sequence. LNA probes can be used for detecting other challenging targets, including low-abundance, short, or highly similar sequences.[6]

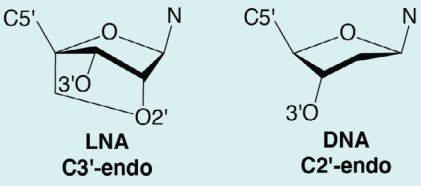

Figure 1. Sugar pucker conformations

Third generation antisense oligonucleotides (ASOs) containing chemical modifications, such as phosphorodiamidate morpholino oligomers (PMO), have gained FDA approval as early as 2016. LNA modifications have also found their place in these types of therapeutics.[7, 8] The enzymatic stability against RNase H1 of the constrained nucleotides provides more control when designing ASOs with LNAs. LNAs are commonly employed in ASOs as gapmers or mixers (Figure 2). Gapmers feature a block of DNA sandwiched between two terminal blocks made of a very stable backbone. LNA-DNA-LNA designs consist of at least four LNA nucleotides at the 5'- and 3'- ends.[6] In recent years, several LNA-based ASOs have been in or are currently in clinical trials (Table 1).[7,8]

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More research is coming out about the effects of using LNA-featured ASOs, especially when combined with other modifications such as phosphorothioate (PS) linkages. While gapmer ASOs offer enhanced nuclease resistance, they may come with the caveat of hepatotoxicity. It was very recently reported that gapmer ASO-mediated hepatotoxicity was reduced when combined with certain nucleobase modifications.[9] This was particularly true for 5-hydroxy-dC (10-1063), 8-bromo-dG (10-1027), 8-amino-dG (10-1079), and 2-thio-dT (10-1036), modifications that are also available at Glen Research.

LNA Supports

Our locked analog, or LA, phosphoramidite products have been discussed in previous Glen Reports (Figure 3).[1, 2] We often receive inquiries about LNA supports. Until now, a universal support was required to synthesize a 3'-LNA modified oligonucleotide. We are pleased to introduce LA CPGs (Figure 3). LA CPGs negate the need for Universal Supports, which typically require special cleavage conditions. As is the case for LA phosphoramidites, the pyrimidine bases are thymidine and 5-methylcytosine, rather than uracil and cytosine, respectively.

These supports are available as 1000Å CPG. LA CPGs are cleaved with standard protocols. It is advisable to avoid the use of methylamine when deprotecting oligos containing Bz-5-Me-C-LA, since this can result in introduction of an N4-methyl modification.

LNA oligonucleotides are water soluble, can be separated by gel electrophoresis and precipitated by ethanol. LA-containing oligonucleotides can be purified and analyzed using the same methods employed for standard DNA. LNA can be mixed with DNA and RNA, as well as other nucleic acid analogs, modifiers, and labels.

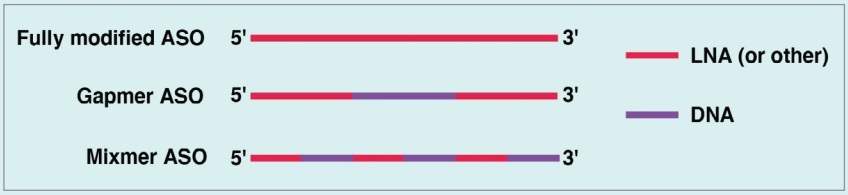

Figure 2. ASO design

Table 1. Clinical trials on LNA-based ASOs

Drug Candidate	Chemistry	Target	Trial Stage	Disease(s)
ISTH0036	LNA	TGF beta 2	Phase 1 (complete, 2018)	Primary open angle glaucoma
OGX-427	LNA	Hsp27 mRNA	Phase 2 (complete, 2022)	Metastatic bladder cancer
Cobomarsen	LNA	miRNA-155	Phase 2 (term., 2022)	Cutaneous T-cell lymphoma
Miravirsen	LNA/PS	miRNA-122	Phase 2 (complete, 2018)	Hepatitis C
BP1001	LNA	Grb2	Phase 2 (recruiting)	Acute Myeloid Leukemia

PS = phosphorothioate linkages

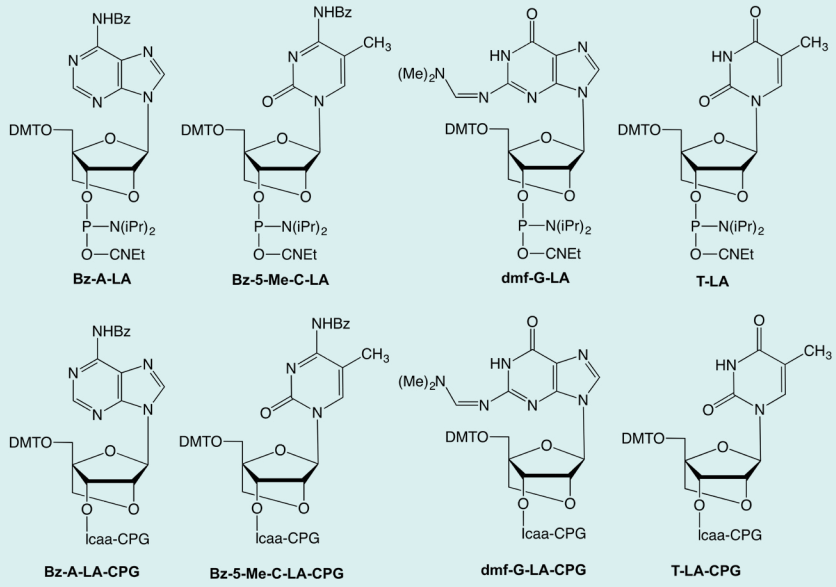

Figure 3. LA Products

Item	Pack Size	Catalog No.
Bz-A-LA-CPG	0.1 g	20-2501-01
	0.25 g	20-2501-02
	1.0 g	20-2501-10
1 µmol columns	Pack of 4	20-2501-41
0.2 µmol columns	Pack of 4	20-2501-42
10 µmol columns (ABI)	Pack of 1	20-2501-13
15 µmol columns (Expedite)	Pack of 1	20-2501-14
Bz-5-Me-C-LA-CPG	0.1 g	20-2511-01
	0.25 g	20-2511-02
	1.0 g	20-2511-10
1 µmol columns	Pack of 4	20-2511-41
0.2 µmol columns	Pack of 4	20-2511-42
10 µmol columns (ABI)	Pack of 1	20-2511-13
15 µmol columns (Expedite)	Pack of 1	20-2511-14
dmf-G-LA-CPG	0.1 g	20-2529-01
	0.25 g	20-2529-02
	1.0 g	20-2529-10
1 µmol columns	Pack of 4	20-2529-41
0.2 µmol columns	Pack of 4	20-2529-42
10 µmol columns (ABI)	Pack of 1	20-2529-13
15 µmol columns (Expedite)	Pack of 1	20-2529-14
T-LA-CPG	0.1 g	20-2531-01
	0.25 g	20-2531-02
	1.0 g	20-2531-10
1 µmol columns	Pack of 4	20-2531-41
0.2 µmol columns	Pack of 4	20-2531-42
10 µmol columns (ABI)	Pack of 1	20-2531-13
15 µmol columns (Expedite)	Pack of 1	20-2531-14

References

1. *The Glen Report*, 2003, **16.2**, 5.
2. *The Glen Report*, 2018, **30.2**, 8-9.
3. M. Petersen, *et al.*, *J Mol Recognit*, 2000, **13**, 44-53.
4. D.A. Braasch; D.R. Corey, *Chem Biol*, 2001, **8**, 1-7.
5. T. Ouyang; Z. Liu; Z. Han; Q. Ge, *Anal Chem*, 2019, **91**, 3179-3186.
6. S. Mishra, *et al.*, *Nano Lett*, 2021, **21**, 9061-9068.
7. K. Dhuri, *et al.*, *J Clin Med*, 2020, **9**, 2004-2028.
8. A.M. Quemener; M.L. Centomo; S.L. Sax; R. Panella, *Molecules*, 2022, **27**, 536-562.
9. T. Yoshida, *et al.*, *Nucleic Acids Res*, 2022, **50**, 7224-7234.

Application Note — Spacers for Aptamer and DNAzyme/ ribozyme Study

Glen Research offers many different spacer products that serve a variety of functions. Most commonly, spacer modifiers are used to increase the distance between two portions of an oligonucleotide. A dendrimer may require additional room to minimize crowding between branches while additional spacing between a label (fluorophore, biotin, etc.) and the oligonucleotide can be desirable in certain applications. Our spacer products can also be used as polymerase blockers, cleavable linkages and stable abasic site substitutes. What we would like to highlight in this article is the use of spacers to probe and optimize the structure of functional oligonucleotides.

Functional oligonucleotides include aptamers, ribozymes and DNAzymes. Aptamers are affinity agents that are similar to antibodies, in many ways, while DNAzymes and ribozymes are catalytic DNA and RNA, respectively. All these entities function based on the three-dimensional structures that they fold up into, similar to how proteins work. Due to practical limitations in the development of these functional oligonucleotides, their sequences are far from optimal. They may be longer than necessary, and even if the minimal functional sequence is obtained, minor substitutions of the sequence can dramatically change the folded structure and improve functionality. One method of optimizing such sequences is to use spacers in place of nucleosides to determine the relative importance of individual residues. To keep the overall length essentially the same, 3-carbon spacers such as Spacer Phosphoramidite C3, dSpacer CE Phosphoramidite and rSpacer TBDMS CE Phosphoramidite are typically used (Figure 1). The last two retain a sugar-like ring and as such, should be less disruptive substitutions relative to Spacer C3.

Earlier, Wang *et al.* probed the sequence of the well-studied DNAzyme, 10-23.[1] 10-23 consists of a 15 nt catalytic motif that catalyzes the cleavage of RNA.[2] Like many other DNAzymes, 10-23 requires substrate binding regions to guide the catalytic motif (Figure 2) to a desired RNA target, and by customizing the substate binding regions, 10-23 can cleave almost any RNA sequence in a sequence-specific manner with multiple turnover. The researchers synthesized 15 analogs of 10-23, each with one nucleotide replaced with dSpacer, and evaluated RNA cleavage relative to the native DNAzyme. To no surprise, most of these analogs exhibited slower catalysis (Table 1). In some cases, there was no cleavage at all. Only one position, T_8, was amenable to this substitution, resulting in somewhat improved catalysis. Subsequently, 11 analogs with Spacer C3 in place of dSpacer were synthesized and studied.

Table 1. Relative catalytic rates of DNAzyme 10-23 spacer analogs.

Nucleotide	Relative Catalytic Rates (WT = 100)		
	dSpacer	Spacer C3	Deletion
G_1	65	43	
G_2	15	19	
C_3	54	40	
T_4	7	6	
A_5	2		
G_6	0		
C_7	7	9	
T_8	124	129	46
A_9	6	5	
C_{10}	4	4	
A_{11}	9	10	
A_{12}	12	11	
C_{13}	0		
G_{14}	0		
A_{15}	9	11	

Their catalytic activity largely mirrored those of the dSpacer series. Finally, since the thymine nucleobase was clearly not essential, a T_8 deletion analog was evaluated, and catalytic rates were reduced by roughly two-fold.

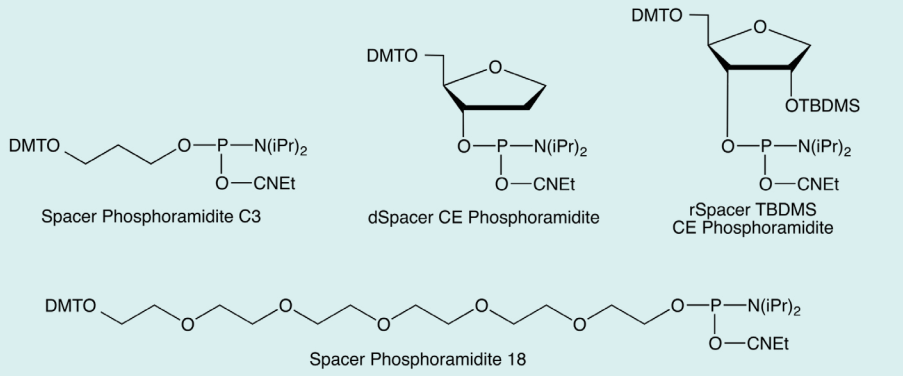

Figure 1. Highlighted Spacer Phosphoramidites

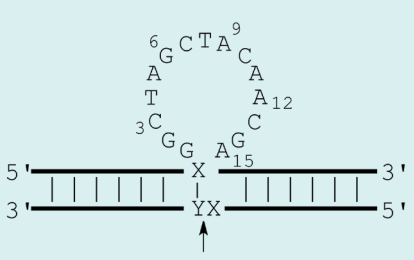

Figure 2. DNAzyme 10-23. Secondary structure of DNAzyme (top strand) shown bound to substrate (bottom strand). The arrow indicates the position of RNA cleavage. Y = U; X = A/G.

Spacer C3 substitution affinity ratio for SL1*	32	5700	1200	96	0.8	0.8	0.9	0.8	1.1	0.9	390	10000	13000	30000	280	2.2	3.1	610	4300	30000	30000	9200	30000	34	7600	30000	4600	190	1.5
Position, 5'-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
SL1**	U	U	A	C	G	A	C	U	A	C	G	U	U	A	C	A	C	G	C	G	U	U	U	A	U	A	G	C	G
SL2**	U	U	A	C	Spacer 18						G	U	U	A	C	A	C	G	C	G	U	U	U	A	U	A	G	C	G

Figure 3. Plate-derived growth factor B aptamer sequence optimization with spacers. *Affinity ratios, more than 1 is reduced affinity and less than 1 is enhanced affinity. Red highlights reduction of affinity by >10 fold; blue highlights affinity that is relatively unchanged. **U residues have a linker attached hydrophobic modification at the 5-position.

In a different study, Davies *et al.* performed a DNA aptamer selection for platelet-derived growth factor B (PDGF-BB).[3] Using a library of 40 randomized positions, they isolated a high affinity aptamer after eight selection rounds. The resulting 40 nt aptamer was systematically truncated to a 29 nt sequence (Figure 3, SL1) with no loss of activity, and from there, further optimization was achieved with spacers. 29 sequences, each with one nucleotide substituted with Spacer C3, were synthesized and tested for binding. As was the case with DNAzyme 10-23, the majority of these sequences had much less affinity for PDGF-BB relative to the control; however, unlike 10-23, there were many more where activity stayed relatively consistent, suggesting that the nucleobases at those positions were not critical for activity. Notably, there were six consecutive positions (5-10) where this was the case. A shorter sequence, where all six of these nucleotides were replaced with one Spacer 18 (Figure 1), was synthesized (Figure 3, SL2). This sequence was 16 atoms shorter with five fewer negative charges yet also retained binding affinity. Subsequently, the authors performed further series of systematic substitutions to tune both binding affinity and nuclease resistance. The final optimized sequence retained the internal Spacer 18 modification and was a potent inhibitor of PDGF-BB.

The above are just two of many examples in the literature where spacers played key roles in structure and function investigations. Spacers continue to be a popular product line for Glen Research, and customers who are not very familiar with our spacer offerings might want to take a closer look at them.

References

1. B. Wang, L. Cao, W. Chiuman, Y. Li, and Z. Xi, *Biochemistry*, 2010, **49**, 7553-62.
2. S.W. Santoro, and G.F. Joyce, *Proc Natl Acad Sci U S A*, 1997, **94**, 4262-6.
3. D.R. Davies, *et al.*, *Proc Natl Acad Sci U S A*, 2012, **109**, 19971-6.

Item	Pack Size	Catalog No.
Spacer Phosphoramidite C3	100 µmol	10-1913-90
	0.25 g	10-1913-02
dSpacer CE Phosphoramidite	100 µmol	10-1914-90
	0.25 g	10-1914-02
rSpacer TBDMS CE Phosphoramidite	50 µmol	10-3915-95
	100 µmol	10-3915-90
	0.25 g	10-3915-02
Spacer Phosphoramidite 18	100 µmol	10-1918-90
	0.25 g	10-1918-02

Product Review — Fluorescein Dyes

Fluorescein is a bright green dye first synthesized by Adolf von Baeyer in 1871. Since then, it has been used in a wide range of applications including leveling equipment, optometry and water tracing. It was even used to turn the Chicago River green for St. Patrick's Day celebrations for several years.

For our customers, fluorescein is more commonly known as the most popular fluorophore for oligonucleotide labeling as well as biomolecular labeling in general. The molecule has excitation and emission wavelengths of 495 and 521 nm, respectively, and is conveniently excited by the 488 nm spectral line of an argon-ion laser. With a very high quantum yield, fluorescein is one of the brighter small molecule dyes available. In the context of oligonucleotide synthesis, fluorescein has the added advantage of being very stable to the basic environments that are necessary during oligonucleotide deprotection, which is not always the case for fluorophores.

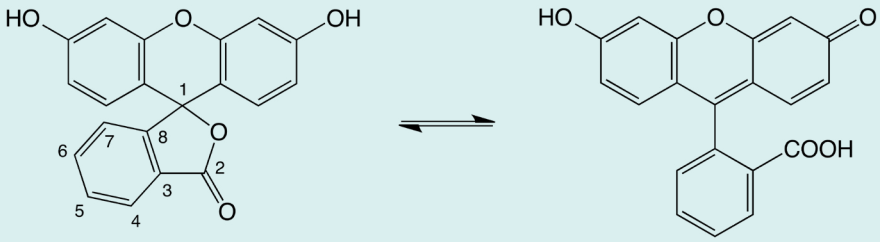

Figure 1. Fluorescein structure

Table 1. Spectral properties of fluorescein and its derivatives

	Absorbance Maximum (nm)	Emission Maximum (nm)	Color
Fluorescein	494	525	Green
Tetrachloro-Fluorescein	521	536	Orange
Hexachloro-Fluorescein	535	556	Pink
Dichloro-dimethoxy-Fluorescein	525	548	Orange/Pink
SIMA (HEX)	538	551	Pink
Yakima Yellow®	530	549	Yellow

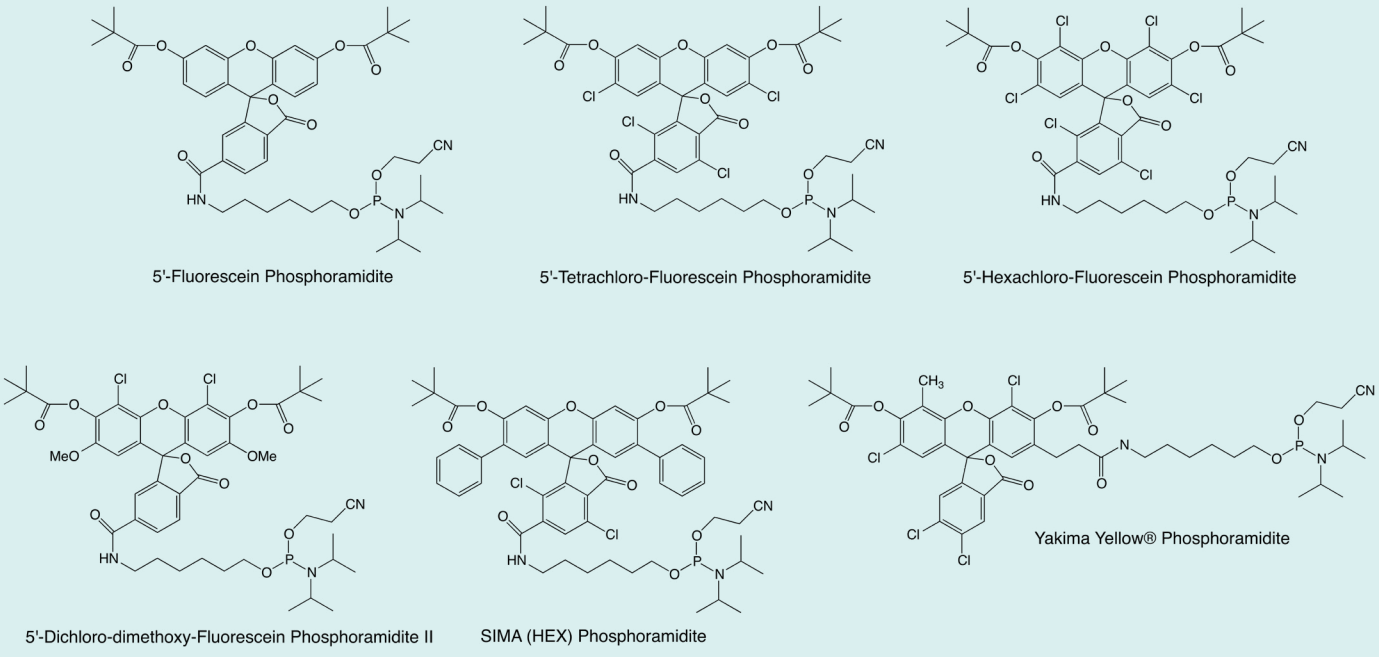

Figure 2. 5'-Fluorescein phosphoramidite and its derivatives

Deprotection of such oligonucleotides is covered in more depth in a separate article in this issue.[1]

Glen Research carries many versions of fluorescein, currently five phosphoramidites, five supports, one NHS ester and one azide. A couple of these are based on 5-fluorescein isothiocyanate (5-FITC) while the rest are derived from 6-carboxyfluorescein (6-FAM). The number denotes the attachment point of the linker based on the interesting numbering system of fluorescein (Figure 1). For those interested in how one decides which phosphoramidite or synthesis support to use, this topic was reviewed earlier.[2]

Glen Research also carries derivatives of fluorescein. These contain substitutions on the aromatic rings that red-shift the spectral properties of the fluorophore for researchers that require multiple wavelength/dye applications (Table 1). They include 6-carboxy versions of tetrachloro-fluorescein (TET), hexachloro-fluorescein (HEX), dichloro-dimethoxy-fluorescein (JOE) and dichloro-diphenyl-fluorescein (SIMA). It should be noted that SIMA is a much more deprotection stable substitute for HEX. We also have Yakima Yellow®, a non-symmetrical tetrachloro-bisalkylated-fluorescein. A comparison of their structures based on the 5'-phosphoramidites reinforces how similar they are (Figure 2). In case one was wondering, each of the fluoresceins and its derivatives are protected by 2 pivaloyl groups on the aromatic hydroxyls that render the molecule non-fluorescent. It is only when these groups are released during deprotection that the expected color and emission of the dyes are realized.

Fluorescein and its related derivatives continue to be very popular fluorophore labels for oligonucleotides and represent a significant portion of our fluorophore portfolio. In future articles, we will review other major families of fluorophores that we also carry.

References

1. *The Glen Report*, 2022, **34.2**, 1.
2. *The Glen Report*, 2016, **28.2**, 5.

Item	Pack Size	Catalog No.
5'-Fluorescein Phosphoramidite	50 µmol	10-5901-95
	100 µmol	10-5901-90
	0.25 g	10-5901-02
5'-Tetrachloro-Fluorescein Phosphoramidite	50 µmol	10-5902-95
	100 µmol	10-5902-90
	0.25 g	10-5902-02
5'-Hexachloro-Fluorescein Phosphoramidite	50 µmol	10-5903-95
	100 µmol	10-5903-90
	0.25 g	10-5903-02
5'-Dichloro-dimethoxy-Fluorescein Phosphoramidite II (Joe II)	50 µmol	10-5906-95
	100 µmol	10-5906-90
	0.25 g	10-5906-02
SIMA (HEX) Phosphoramidite	50 µmol	10-5905-95
	100 µmol	10-5905-90
	0.25 g	10-5905-02
Yakima Yellow® Phosphoramidite	50 µmol	10-5921-95
	100 µmol	10-5921-90
	0.25 g	10-5921-02

Notes:

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Technical Snippets

Are BHQ's compatible with CSO oxidizer?

Black Hole Quenchers™ (BHQ) are some of our most popular quenchers. BHQ's are robust and quench a variety of popular fluorophores. Due to their popularity, they are frequently used in probes containing other modifications. These other modifications may require special ancillary reagents, which opens the question: Is BHQ compatible with alternative reagents, such as CSO oxidizer?

Yes! We investigated the compatibility between CSO and BHQ-1. In our hands, we found CSO oxidation (3 min) was comparable to the standard I_2 oxidation in a short strand containing BHQ-1 dT. While we only tested compatibility with BHQ-1, there is no reason to believe that other BHQ's or any of our other quencher offerings would have any issues. CSO is an excellent alternative to iodine, and we have yet to encounter a phosphoramidite that is incompatible with CSO.

Products:

0.5M CSO in Anhydrous Acetonitrile (40-4632)

Does Glen Research sell molecular trap packs?

The answer is no. While water is the enemy in oligonucleotide synthesis coupling and anything that can be done to reduce the water content present can only be beneficial, we have found that molecular trap packs or sieves are not necessary, at least for our reagents. Our labs do not use molecular trap packs, and as such, we do not sell them.