Chemistry from Berry & Associates

TO ADVANCE THE LIFE SCIENCES

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In This Issue

Founded in 1989 with roots in the nucleoside field, Berry & Associates soon moved into the chemistry of nucleic acids, resulting in a current portfolio of nearly 200 phosphoramidites and solid phase-linked monomers for oligonucleotide synthesis as well as hundreds of nucleosides, carbohydrates, spacers, fluorescent markers, quenchers, and heterocycles – all proudly made at our facility just outside of Ann Arbor, Michigan. Although our company is small, the credentials of our highly trained staff of chemists include over 400 publications and 80 patents in synthetic organic and medicinal chemistry. High quality chemicals, timeliness, and personalized service are the hallmarks of Berry & Associates.

Design. Develop. Deliver.

A Superior New Thiol-Modifier CPG

Incorporation of ether functionality redefines the state of the art

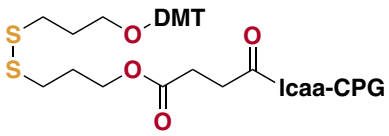
In synthesizing oligonucleotides, it can be useful to include a thiol or a disulfide as a modification to the oligonucleotide. These sulfur containing groups, not present in natural oligonucleotides, enable attachment of the oligonucleotide to surfaces or to other molecules of interest. When the modification is to occur at the 3'-terminus of the oligo, one common strategy is to link one end of a symmetrical alkyl disulfide to the solid support *via* an amide bond to the terminal amino group of the long chain amino alkyl moiety. The oligonucleotide chain is then extended from the other end of the disulfide.

For many years, C3-disulfide on lcaa-CPG (**BA 0067,** Figure 1) has been the principal 3'-thiol-modifier CPG option for oligonucleotide syntheses. The short alkyl chain of this product can pose limitations for certain applications but, unfortunately, lengthening the alkyl chain from three to six carbon atoms gives a support that is dysfunctional. Switching the support to aminopropyl-CPG gives better results. However, C6-disulfide on aminopropyl-CPG still does not measure up to the performance of C3-disulfide on lcaa-CPG.

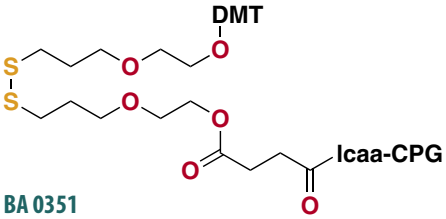
We have discovered that DNA synthesis is markedly enhanced by including ether functionality in the alkyl chain of the disulfide. ***Both superior oligo yield and greater maximum synthesis length are thereby achieved*** (Table 1, next page). Hence, we now offer Thiol-modifier-oxa-6-S-S CPG (**BA 0351**; patent pending), a new and

Continued on page 2

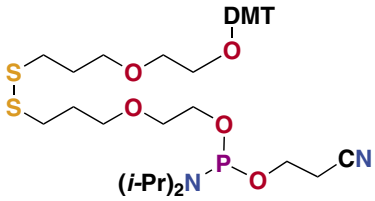
Figure 1. Selected thiol-modifiers



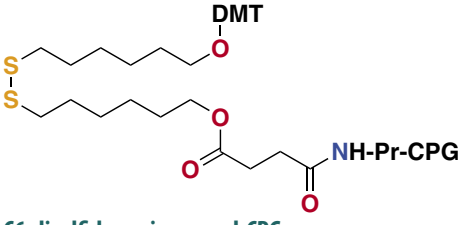
BA 0067



BA 0351



BA 0332



C6-disulfide-aminopropyl-CPG

Ether Enhanced Thiol Modifiers

Continued from page 1

improved 3'-thiol-modifier CPG which utilizes an ether-containing disulfide that has the same chain length as C6-disulfide. In order to demonstrate the yield advantage provided by BA 0351, crude oligos obtained from various thiol-modifier CPGs (200 nMol columns) were equally diluted and examined by HPLC (Figure 2). Needless to say, the oligo yield provided by BA 0351 is markedly superior to its predecessors.

As a complementary reagent for 5'-thiol and disulfide modifications, we offer Thiol-modifier-oxa-6-S-S CEP (**BA 0332**). Again, this reagent has the same chain length as a C6-disulfide but one of the CH_2 units in the C6 chain is converted to an oxygen atom.

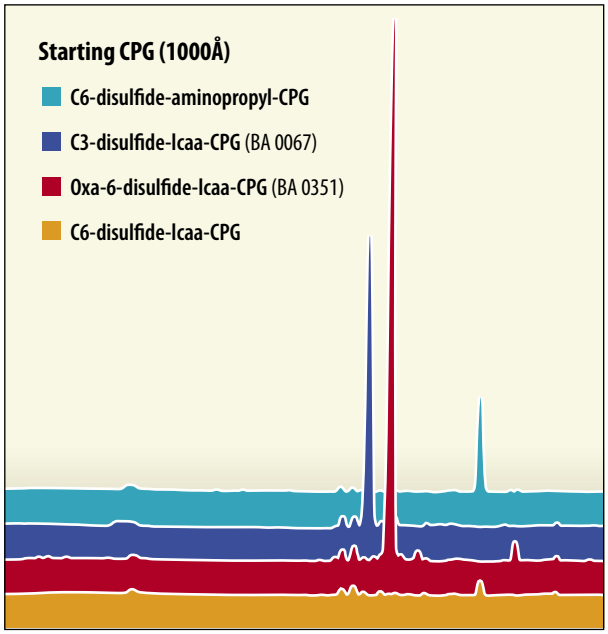


Figure 2. HPLC traces of various 5'-DMT-T6-(3'-disulfides) at equal dilution

Table 1. Synthesis Performance of Disulfide CPGs

Disulfide[a]	Support[a]	Maximum Length[b]	Relative Yield[c]	Catalog Number
DMT-O$(CH_2)_6$-S-S-$(CH_2)_6$O-	aminopropyl-CPG (1000 Å)	48	0.31	—
DMT-O$(CH_2)_3$-S-S-$(CH_2)_3$O-	lcaa-CPG (1000 Å)	100	1.00	BA 0067
DMT-O$(CH_2)_2$O$(CH_2)_3$-S-S-$(CH_2)_3$O$(CH_2)_2$O-	lcaa-CPG (1000 Å)	104	1.68	BA 0351
DMT-O$(CH_2)_6$-S-S-$(CH_2)_6$O-	lcaa-CPG (1000 Å)	9	0.05	—

(a) Standard succinate linker used between disulfide and support. (b) DMT histogram drops below 70% of initial maximum. (c) HPLC integration (AUC) for each 5'-DMT-T6-(3'-disulfide) peak at equivalent dilution.

Ordering Information—Ether Enhanced Thiol-Modifiers

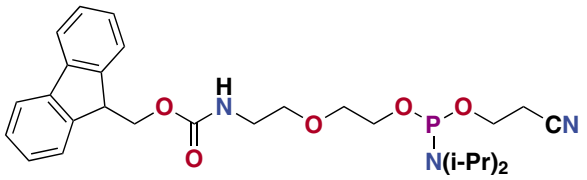
Item	Catalog No.	Size/Pack	Price (USD)
Thiol-modifier-oxa-6-S-S CPG	BA 0351		
NEW!		200 nMol columns/pack of 4	$69.00
		1 µMol columns/pack of 4	$115.00
		100 mg	$70.00
		1 g	$500.00
Thiol-modifier-oxa-6-S-S CEP	BA 0332		
NEW!		100 µMol	$145.00
		0.25 g	$360.00

Fmoc-Amino-Modifiers

The (fluorenylmethyloxy)carbonyl (Fmoc) group has been shown to be useful as an amine protecting group on oligonucleotide amino-modifiers.[1] It can be removed by standard cleave-deprotect protocols such as ammonium hydroxide. Alternatively, the Fmoc group can be selectively removed before cleavage of the oligonucleotide from the solid support,[2] thereby simplifying labeling of the resulting amino group by acylation. After the acylation is complete and excess reagents are washed away, the labeled oligonucleotide is cleaved from the support and further deprotected with ammonium hydroxide. We offer a variety of Fmoc-amino-modifier building blocks that are tailored to 3'-terminal, 5'-terminal, and internal incorporation in oligonucleotides.

Fmoc-5'-amino-modifier-5 CEP

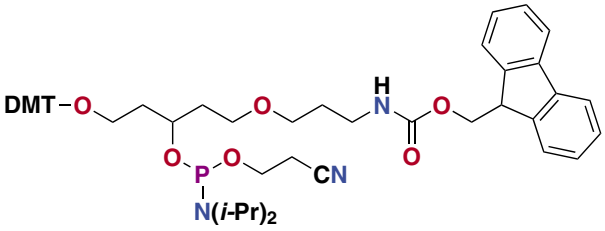
A new addition to our catalog, Fmoc-5'-amino-modifier-5-CEP (**BA 0354**) is designed to be an alternative to the old standby, 5'-Amino-modifier-5-CEP (BA 0030), which has an MMT protecting group.



BA 0354

Fmoc-amino-modifier III CEP

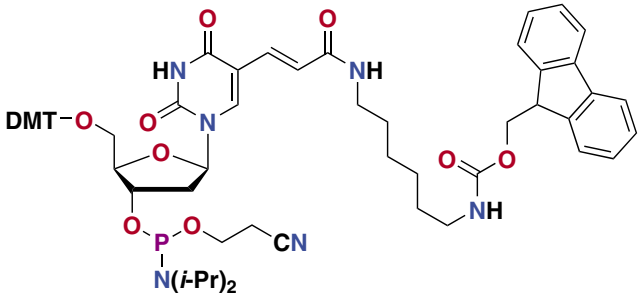
Also a new addition to our catalog, Fmoc-amino-modifier III CEP **(BA 0335)** is suited either to 5'-terminal or internal incorporation in oligonucleotides. It contains a *1,3,5-triol framework* which serves two functions. First, this framework disfavors cyclic phosphate formation when the DMT group is removed, thereby minimizing loss of the amine and any tag that is acylated thereto. Second, the *1,3,5-triol framework* maintains the natural 3-carbon atom internucleotide phosphate distance that is provided by ribose or 2-deoxyribose.



BA 0335

Fmoc-Amino-Modifier-C6-dT CEP

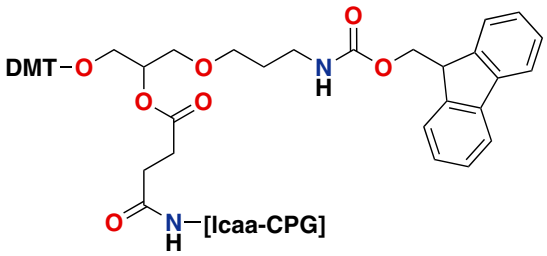
For applications requiring a nucleobase-tethered amine at internal or 5'-positions, we offer Fmoc-Amino-Modifier-C6-dT CEP (**BA 0287**), which provides the possibility of on-bead acylation as discussed above. It is an alternative to the venerable Amino-modifier-C6-dT CEP (**BA 0015**), which bears a trifluoroacetyl (TFA) protecting group. On-bead acylation is not feasible for BA 0015 since TFA group cannot be removed without cleavage of the oligonucleotide from the support.



BA 0287

3'-Fmoc-amino-modifier CPG

For the installation of an amino group at the 3'-terminus of an oligonucleotide, we offer an Fmoc-protected amino-modifier that incorporates a *1,2,3-triol framework* that is attached *via* a succinate linker to lcaa-CPG. Two versions are available, namely **BA 0299** (ca. 70–80 µMol/g on 500 Å CPG) and **BA 0307** (ca. 35–45 µMol/g on 1000 Å CPG).



BA 0299 and BA 0307

See back page for ordering information

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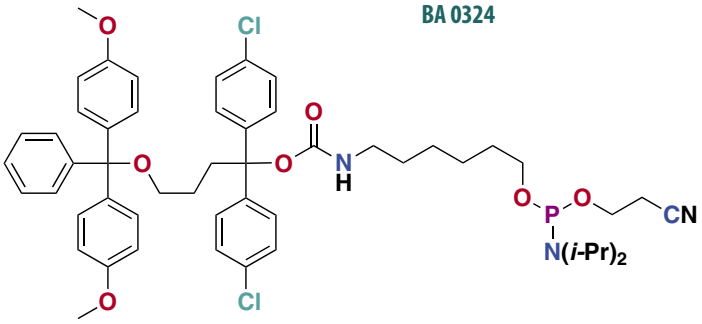
Cmoc-5'-amino-modifier-C6 CEP

We hear from our customers that N-protecting groups for 5'-amino-modifiers can pose a bit of a challenge to automated synthesis. DMT protection is frequently too labile for the amino group. MMT is usually preferred for its greater stability as an N-protecting group, however, it has two practical disadvantages: (1) It requires a custom deprotection protocol since NH-MMT is slightly more robust than O-DMT. (2) Automated synthesizers are designed to quantify DMT cleavage, not MMT cleavage. While the aforementioned Fmoc protected 5'-amino-modifiers offer one potential solution to these disadvantages, sometimes it is preferable to have acid-labile N-protection.

Considering this feedback, our chemists have designed a novel N-protecting group that is based upon the colorimetric-oxycarbonyl (Cmoc) concept shown in Figure 3 below. In essence, the Cmoc group is an O-DMT that is tethered to an N-bis(4-chlorophenyl)methoxy-carbonyl protecting group. Both



protecting groups are acid labile but only the DMT cation generates a colorimetric signal because the tether quenches the bis(4-chlorophenyl)-methyl-carbocation in an intra-molecular fashion, affording a neutral tetrahydrofuran derivative.

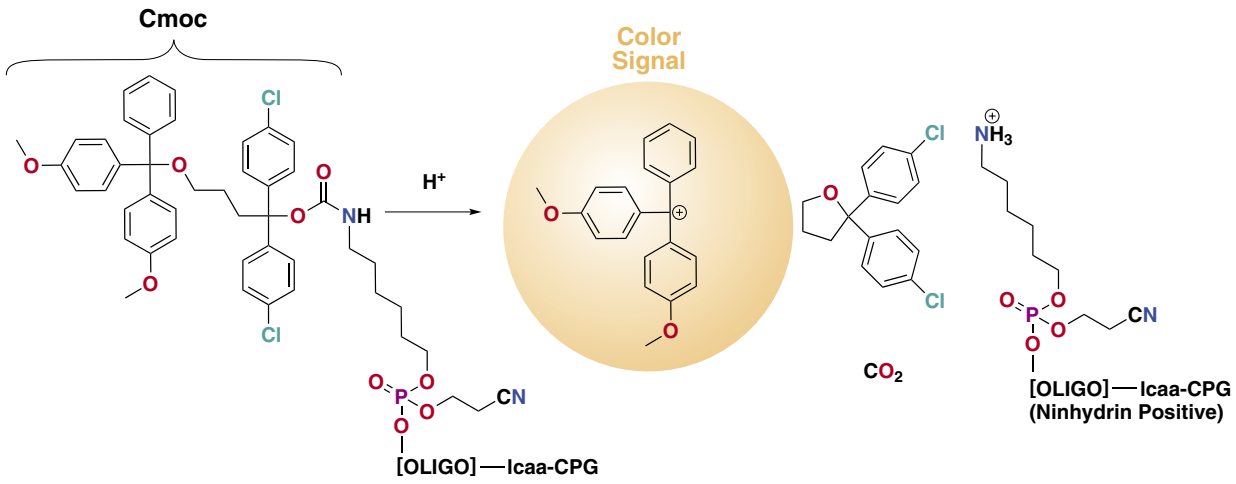
The acid lability of the Cmoc group allows the choice between on-column deprotection, and off column deprotection. Being more lipophilic than a traditional DMT group, the Cmoc protecting group does a superb job in facilitating SPE purification of full length oligos. If you like the DMT-on purification strategy, you will similarly like the Cmoc-on purification strategy.

As a new product we now offer Cmoc-5'-amino-modifier-C6 CEP (**BA 0324**; patent pending) as an orthogonal alternative to Fmoc-5'-amino-modifier-5-CEP (**BA 0354**). This is one of our grab bag products. It meets the BerrySelect™ purity and performance standards, but has not previously appeared in the literature. We hope that you find it interesting and useful for your research.

If you have other amino-modifiers that might benefit from Cmoc protection, give us a call and we would be happy to discuss making them for you.

See back page for ordering information

Figure 3. Cmoc deprotection reaction



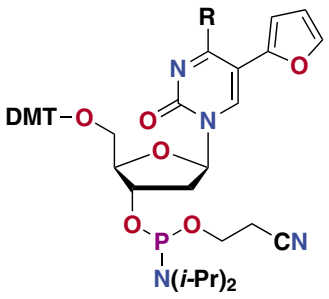
New BerrySelect™ Specialty Phosphoramidites

5-(Furan-2-yl)-dU CEP and 5-(Furan-2-yl)-dC CEP

Tor and co-workers have reported the preparation and photophysical characteristics of a number of small, fluorescent isosteric nucleosides that are capable of normal Watson-Crick base paring in unaltered duplexes.[3-8] These probes are useful tools for studying nucleic acid sequence, structure, dynamics and recognition.

BA 0346 and **BA 0347** are phosphoramidites of pyrimidine nucleosides wherein a furan ring is placed at the 5-position.[3,4] Interesting emission wavelength and/or intensity variations have been observed when atypical pairing changes the micro-environment around a furanyl-pyrimidine. For example, when paired to 8-oxo-dG in the complimentary strand, significant emission quenching of 5-(furan-2-yl)-dC is observed relative to dG pairing, while emission enhancement is observed when paired with T.[8] This emission sensitivity makes BA 0347 useful in the preparation of probes for the detection of oxidative DNA damage. Alternatively, DNA probes that contain a furanyl-pyrimidine at a selected sequence position show a significant emission enhancement when hybridization results in pairing to an abasic residue as compared to purine pairing.[3] This property makes BA 0346 and BA 0347 useful in the construction of probes for detecting sequence-specific depurination.

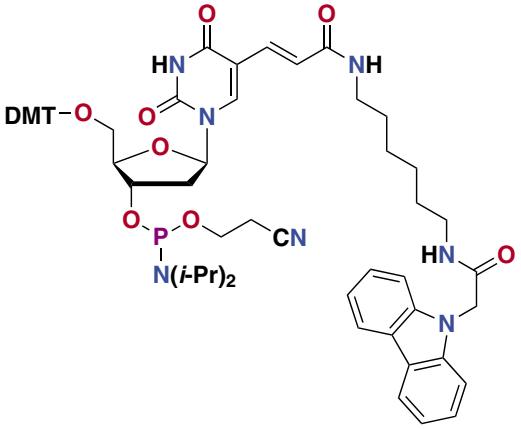
Also offered as new products are the parent nucleosides, 5-(Furan-2-yl)-dU and 5-(Furan-2-yl)-dC (catalog numbers **PY 7053** and **PY 7054**).



BA 0346 — R = OH
BA 0347 — R = NHBz

Carbazole dT CEP

Template-directed DNA ligation has many potential applications including photo-regulated diagnostic and therapeutic agents,[9] nano-architecture construction,[10] DNA computing,[11] and DNA-based memory.[12] The research of Fujimoto, Saito and co-workers illustrates the utility of 5-vinyl-dU[13] and a carbazole-tethered 5-carboxyvinyl-dU[14] for photo-induced DNA ligation. The carbazole-tethered version gives a photo-triggered system where ligation and splitting can be repeated by irradiating at 366 nm. Use of this wavelength minimizes pyrimidine dimer formation, which is a problem when irradiating the simple vinyl analog at 302 nm. When photo-ligation takes place in the presence of template DNA, the data suggest that the carbazole group intercalates into the duplex that is formed. This intercalation prevents photo induced splitting, allowing control of the ligation/splitting based on the presence or absence of the template DNA strand. Carbazole dT CEP (**BA 0345**) enables the automated synthesis of oligonucleotides that contain carbazole-tethered vinyl dU.



BA 0345

8-Styryl-dG CEP

Ogasawara and co-workers have reported the use of 8-substituted dG derivatives that provide reversible duplex regulation *via* a light induced *trans-cis* isomerization.[15,16] The *trans* isomer of 8-styryl-2'-deoxyguanosine (^{8ST}G) is one such photochromic nucleoside (PCN). When a 12-bp duplex containing ^{8ST}G is irradiated for 5 minutes at 370 nm, the double bond isomerizes to the *cis* geometry with 86% conversion. Subsequent irradiation for 2 minutes at 254 nm returns the double bond to the *trans* geometry with 94% conversion. Both *trans* and *cis* isomers are thermally stable but readily interconvert at room temperature upon irradiation with light of the appropriate wavelength. The *T*m value of the duplex containing a *trans*-PCN is 7.9 °C higher than the *T*m value of the same duplex containing a *cis*-PCN. When three ^{8ST}G insertions are inserted into a 20-bp duplex, the *trans*-PCNs permit duplex formation whereas the *cis*-PCNs cause denaturation of the duplex. This phe-

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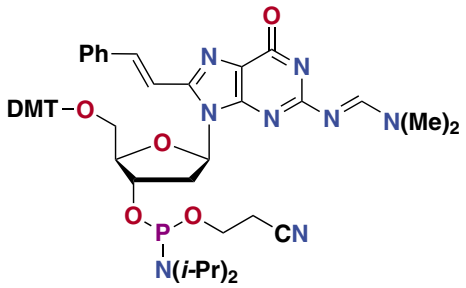
New BerrySelect™ Specialty Phosphoramidites

Continued from page 5

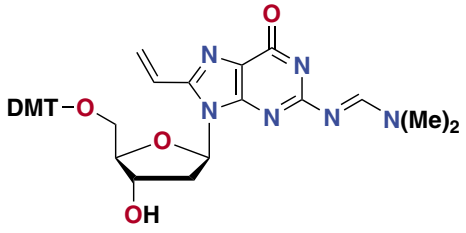
nomenon is evidenced by changes in the circular dichroism spectra before and after irradiation of the duplex containing *trans*-PCNs at 370 nm. Conversely, when the single strands containg *cis*-PCNs are irradiated at 254 nm hybridization occurs as the *trans* isomer is formed.

Berry & Associates now offers 8-Styryl-dG CEP (**BA 0352**) for the in automated synthesis of PCN-containing oligos.



BA 0352

Substitution of other aromatic moieties for the phenyl ring provides PCNs that operate at different wavelengths. For example with 8-(2-napthalen-2-yl)vinyl-2'-deoxyguanosine (^{8NV}G) the *trans* to *cis* conversion occurs with 410 nm irradiation and reverts at 290 nm and with 8-(2-fluoren-2-yl)vinyl-2'-deoxyguanosine (^{8FV}G)) the *trans* to *cis* conversion occurs with 420 nm irradiation and reverts at 310 nm. ^{8ST}G, ^{8NV}G, and ^{8FV}G all have the common synthetic precursor 8-vinyl-dG. Thus we now also offer **PR 3335** as a versatile synthetic intermediate for the preparation of these and other PCNs.

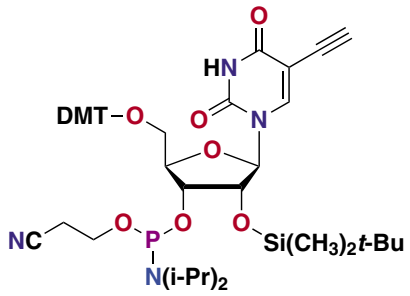


PR 3335

5-Ethynyl-U CEP

One of our customers noticed all the alkyne-modified DNA building blocks in a prior newsletter[17] and inquired about 2'-TBDMS protected 5-ethynyluridine. Hence, we now offer 5-Ethynyluridine CEP (**BA 0353**) as a new product.

Give us a call if you need other alkyne-modified RNA building blocks.

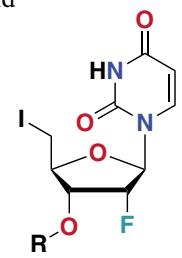


BA 0353

2'-Fluoro-5'-iodo deoxyuridine CEP

Another customer-requested product that is new to our catalog is 2'-Fluoro-5'-iodo deoxyuridine CEP (**BA 0348**). We also offer its parent nucleoside, 2'-Fluoro- 5'-iodo deoxyuridine (**PY 7612**).

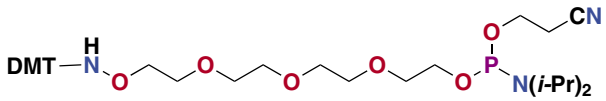
As Grab Bag products, these compounds meet BerrySelect™ purity and performance standards, but have not previously appeared in the literature. We hope that you may find them interesting and useful for your research.



PY 7612 — R = H
BA 0348 — R = CEP

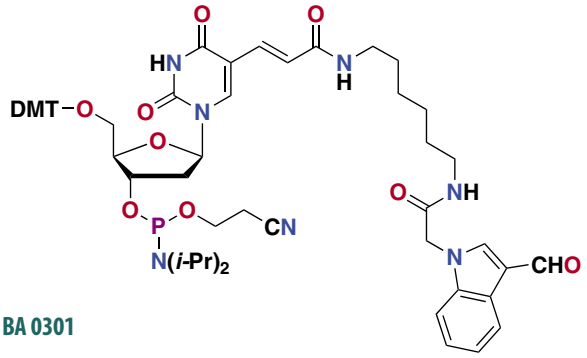
5'-Aminooxy-modifier-11 CEP

The use of oxime formation in ligation reactions of oligonucleotides has been widely established in the literature for over a decade.[18] As examples, 5'-aminooxy-modifiers have been used in oxime ligation for peptide-oligonucleotide conjugates,[19] attachment of nucleosides to solid supports,[20] and head to tail cyclization of oligonucleotides.[21] For the synthesis of an oligo bearing a 5'-aminooxy group we now offer 5'-Aminooxy-modifier-11 CEP (**BA 0350**).



BA 0350

In an earlier newsletter we described the utility of Formylindole-dT CEP (**BA 0301**).[22] The electron-donating indole ring provides sufficient aldehyde stabilization to accommodate DNA synthesis yet it retains enough electrophilic character to allow conjugation with hydrazines. By analogy it also can react with oxyamines to generate an oxime.



BA 0301

We postulate that the concurrent use of BA 0350 at the 5'-terminus and BA 0301 elsewhere in an oligonucleotide should permit the head to tail cyclization of oligos *via* oxime ligation. Routine cleavage of the 5'-DMT group should trigger the cyclization reaction. In principle, carrying out this cyclization on-support should reduce inter-strand ligation and promote intra-strand ligation.

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