



Chemistry

from **Berry & Associates**

TO ADVANCE THE LIFE SCIENCES

Issue 3 | December 2007

CHEMISTRY from Berry & Associates | Issue 3 | December 2007 | Anthraquinone Phosphoramidites | Amine Modifiers | 6-Azapyrimidine Phosphoramidites & Nucleosides | Alkyne Modifiers | Fluorous Affinity Purification and Fluorous Tagging

Anthraquinone Phosphoramidites

In This Issue

Anthraquinone Phosphoramidites

New Amine Modifiers

6-Azapyrimidine Phosphoramidites & Nucleosides

Alkyne Modifiers for Click Conjugation

Fluorous Affinity Purification and Fluorous Tagging

Other New Phosphoramidites

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, nucleotides, 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.

Anthraquinones may be incorporated into oligonucleotides by a variety of methods using a host of different phosphoramidites. The anthraquinone moiety is useful for applications such as intercalation, duplex and triplex stabilization, photochemical immobilization, quenching of fluorescence, electrochemical detection, and charge transport through nucleic acids. Of the various anthraquinone phosphoramidites that have been explored, amides of anthraquinone-2-carboxylic acid are popular.¹⁻⁸ The linker between the anthraquinone amide and the phosphoramidite can be a simple aliphatic group with no DMT group (5'-incorporation only),^{1,4,5,8} an aliphatic linker with a DMT group,⁷ or a nucleoside with the anthraquinone tethered to the nucleobase or 2'-hydroxyl group.^{2,3,6} The latter two strategies allow internal incorporation into an oligonucleotide. Alternatively, the anthraquinone amide may be formed post-synthetically. For example, recent work by Barton and co-workers⁹ employs the acylation of an amine-modified oligonucleotide with anthraquinone-2-carbonyl chloride. Alternatively, an ethynyl-dU nucleotide was subjected to a

Given the large and growing body of work on anthraquinone-modified oligonucleotides, it is surprising that there are no commercially available phosphoramidites for their incorporation. We have chosen to offer a suite of new anthraquinone-bearing phosphoramidites.

palladium-catalyzed (Sonogashira) coupling with 2-iodoanthraquinone.⁹ Both processes were carried out while the oligonucleotide was still attached to the solid support. The Barton work also illustrates the importance of the tether in electronic coupling of the anthraquinone to the DNA π -stack.

Given the large and growing body of work on anthraquinone-modified oligonucleotides, it is surprising that there are no commercially available phosphoramidites for their incorporation. We have chosen to offer a suite of new anthraquinone-bearing phosphoramidites (Figure 1).¹⁰ The anthraquinone may be tethered to the oligonucleotide in a variety of ways in order to accommodate different applications. Anthraquinone-Pyrrolidine CEP (BA 0300) brings the anthraquinone very close to the DNA strand via an amide linkage to the amino group of 3-hydroxyprolinol, which serves as a substitute for the deoxyribose moiety.^{11,12} Anthraquinone-

Continued on page 2

ANTHRAQUINONE PHOSPHORAMIDITE COLLECTION

- Anthraquinone-Pyrrolidine CEP
- Anthraquinone-C2-dT CEP
- Anthraquinone-5-Ethynyl-dU CEP
- Anthraquinone-dU CEP

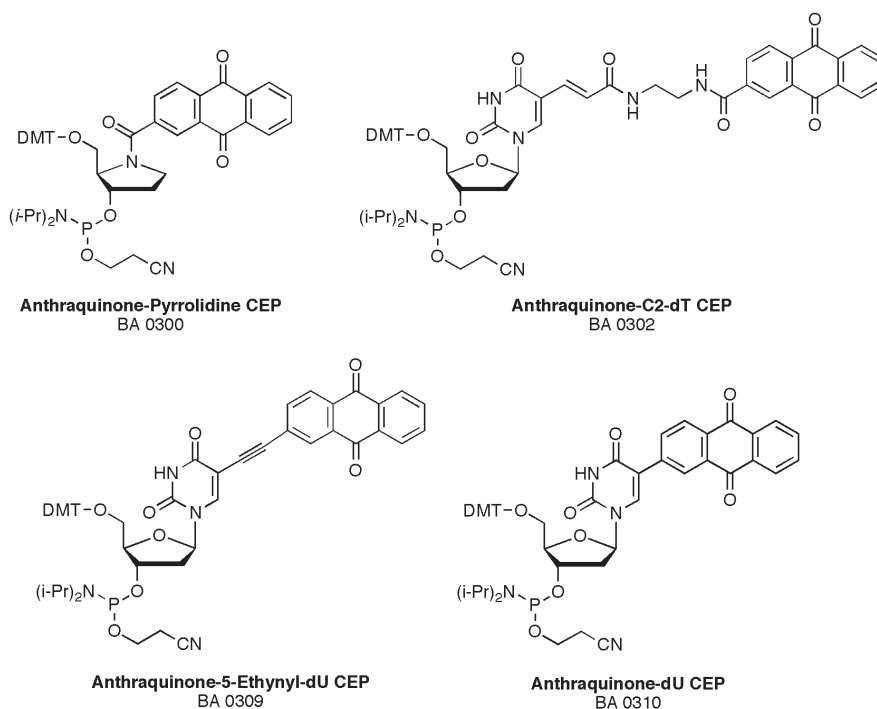


Figure 1. Phosphoramidites for the incorporation of anthraquinones into oligonucleotides.

Anthraquinone Phosphoramidites — Ordering Information			
Item	Catalog No.	Size/pack	Price (USD)
Anthraquinone-Pyrrolidine CEP	BA 0300	100 μ mol	\$395.00
		0.25 g	\$895.00
Anthraquinone-C2-dT CEP	BA 0302	100 μ mol	\$365.00
		0.25 g	\$650.00
Anthraquinone-5-Ethynyl-dU CEP	BA 0309	50 μ mol	\$275.00
		0.25 g	\$1095.00
Anthraquinone-dU CEP	BA 0310		Coming soon



Anthraquinone Phosphoramidites

Continued from front page

C2-dT CEP (BA 0302) features an electronically insulating tether that places the anthraquinone further from the oligonucleotide. Anthraquinone-5-Ethynyl-dU CEP (BA 0309) and Anthraquinone-dU CEP (BA 0310) provide an electronic coupling to the nucleobase π -stack, the latter involving direct coupling. Anthraquinone-5-Ethynyl-dU CEP allows a potential alternative to the use of 5-Ethynyl-dU CEP followed by a Sonogashira coupling with 2-iodoanthraquinone.⁹

References

- (1) (a) Gasper, S. M.; Schuster, G. B. *J. Am. Chem. Soc.* **1997**, *119*, 12762-12771. (b) Shao, F.; Augustyn, K.; Barton, J. K. *J. Am. Chem. Soc.* **2005**, *127*, 17445-17452.
- (2) Whittemore, N. A.; Mullenix, A. N.; Inamati, G. B.; Manoharan, M.; Cook, P. D.; Tuinman, A. A.; Baker, D. C.; Chambers, J. Q., *Bioconj. Chem.* **1999**, *10*, 261-270.
- (3) Tierney, M. T.; Grinstaff, M. W., *Org. Lett.* **2000**, *2*, 3413-3416.
- (4) Tierney, M. T.; Grinstaff, M. W., *J. Org. Chem.* **2000**, *65*, 5355-5359.
- (5) Koch, T.; Jacobsen, N.; Fensholdt, J.; Boas, U.; Fenger, M.; Jakobsen, M. H., *Bioconj. Chem.* **2000**, *11*, 474-483.
- (6) Al-Rawi, S.; Ahlborn, C.; Richert, C., *Org. Lett.* **2005**, *7*, 1569-1572.
- (7) Asanuma, H.; Hayashi, H.; Zhao, J.; Liang, X.; Yamazawa, A.; Kuramochi, T.; Matsunaga, D.; Aiba, Y.; Kashida, H.; Komiyama, M., *Chem. Commun.* **2006**, 5062-5064.
- (8) Shibata, A.; Ueno, Y.; Shinbo, K.; Nakanishi, M.; Matsuda, A.; Kitade, Y., *Bioorg Med. Chem. Lett.* **2006**, *16*, 1410-1413.
- (9) Gorodetsky, A. A.; Green, O.; Yavin, E.; Barton, J. K. *Bioconjugate Chem.* **2007**, *18*, 1434-1441.
- (10) These new compounds are from our Experimental Grab Bag. The compounds in this unique collection have not been validated for any particular oligonucleotide application. We hope that you may find them of interest, but please be aware that their purchase and use is at your own risk.
- (11) Ceulemans, G.; Van Aerschot, A.; Rozenski, J. Herdewijn, P. *Tetrahedron* **1997**, *53*, 14957-14974.
- (12) Schärer, O. D.; Ortholand, J.-Y.; Ganesan, A.; Ezaz-Nikpay, K.; Verdine, G. L. *J. Am. Chem. Soc.*, **1995**, *117*, 6623-6624.

New Amine Modifiers

Amine modifiers allow the installation of a primary amino group into an oligonucleotide for the purpose of post-synthetic modification with an acylating agent. There are a variety of factors that come into play in choosing an amine modifier, such as the choice of amine protecting group, what position the modifier will occupy in the oligonucleotide (5', 3', or internal), and how the amino group is attached to the backbone. Although we currently offer over a dozen of these important monomers, new variants are still of interest. We now introduce several new amine modifiers that span a range of options for the introduction of amino groups

NEW AMINE MODIFIERS

- **3'-Amino-Modifier CPG (500 Å)**
- **3'-Amino-Modifier CPG (1000 Å)**
- **Fmoc-Amino-Modifier-C6-dT CEP**
- **Amino-Modifier-15-dT CEP**
- **5-Aminoallyl-dU CEP**
- **Amino-Modifier-G CEP**

into oligonucleotides (Figure 2). For the installation of an amino group at the 3'-terminus of an oligonucleotide, a solid-support-linked monomer with a protected amine and DMT-protected alcohol is required. The (fluorenylmethyl) carbamoyl (Fmoc) group has been shown to be a useful amine protecting group for amine modification of oligonucleotides.¹ It is removed during cleavage/deprotection with ammonium hydroxide. Alternatively, the *Fmoc* group can be removed before cleavage of the oligonucleotide from the solid support,² e.g., with piperidine, simplifying the acylation process. After the acylation is complete, the labeled oligonucleotide can then be cleaved from the support and further deprotected

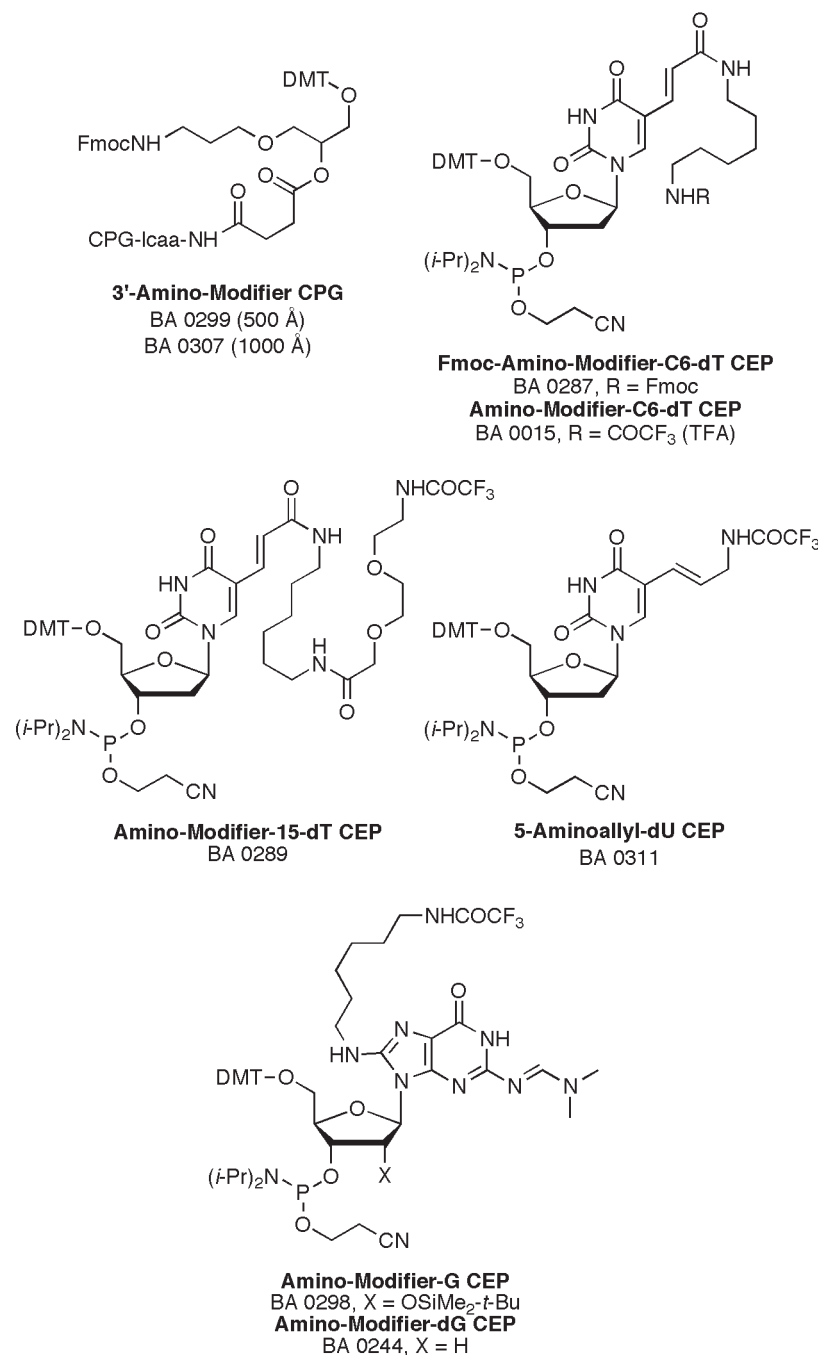


Figure 2. Phosphoramidites for the synthesis of amino-modified oligonucleotides.

with ammonium hydroxide. We offer a version of such an Fmoc-protected amino-modifier for installation of an amino group at the 3'-terminus, i.e., 3'-Fmoc-Amino-Modifier CPG, in both higher- and lower-loaded versions, namely BA 0299 (ca. 70-80 μmol/g

on 500 Å CPG) and BA 0307 (ca. 35-45 μmol/g on 1000 Å CPG). Both products feature a 7-atom spacer between the amino group and the O-DMT group.

For applications requiring a nucleobase-tethered amine, we offer

Continued on page 4

New Amine Modifiers

Continued from page 3

several new options, all allowing internal or 5' installation. The new Fmoc-protected compound Fmoc-Amino-Modifier-C6-dT CEP (BA 0287) offers 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. The TFA group cannot be removed without cleavage of the oligonucleotide from the resin. Also new is Amino-Modifier-15-dT CEP (BA 0289), which is similar to Amino-Modifier-C6-dT CEP except that it offers a longer tether that includes an amphipathic glycol ether region. For those requiring a short tether between the amine and the nucleobase, 5-Aminoallyl-dU CEP (BA 0311) is in development. Finally, we now introduce Amino-Modifier-G CEP (BA 0298), the ribose version of our existing product Amino-Modifier-dG CEP (BA 0244).

References

- (1) Nelson, P. S.; Kent, M.; Muthini, S. *Nucl. Acids Res.* **1992**, *20*, 6253-6259.
- (2) Gartner, Z. J.; Kanan, M. W.; Liu, D. R. *J. Am. Chem. Soc.* **2002**, *124*, 10304-10306; see Supporting Information, p. 3. (b) Gartner, Z. J.; Tse, B. N.; Grubina, R.; Doyon, J. B.; Snyder, T. M.; Liu, D. R. *Science* **2004**, *305*, 1601-1605; see Supporting Online Material, p. 2.

New Amine Modifiers — Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
3'-Amino-Modifier CPG (500 Å)	BA 0299	100 mg	\$90.00
		1 g	\$595.00
3'-Amino-Modifier CPG (1000 Å)	BA 0307	100 mg	\$85.00
		1 g	\$525.00
Fmoc-Amino-Modifier-C6-dT CEP	BA 0287	100 µmol	\$180.00
		0.25 g	\$325.00
Amino-Modifier-15-dT CEP	BA 0289	100 µmol	\$470.00
		0.25 g	\$795.00
5-Aminoallyl-dU CEP	BA 0311		Coming soon
Amino-Modifier-G CEP	BA 0298	50 µmol	\$275.00
		0.25 g	\$975.00

6-Azapyrimidine Phosphoramidites and Nucleosides

The pK_a values of the N-3 hydrogen atoms of uridine and thymidine are 9.3 and 10.0, respectively, which is high enough that these positions are fully protonated at physiological pH and thus capable of hybridization in nucleic acids (Figure 3). Replacement of the methine groups at position 6 of uridine and thymidine with nitrogen atoms affords 6-Aza-2'-deoxyuridine¹ (PYA 11057) and 6-Azathymidine² (PYA 11058), modifications that strongly affect the acidity of the N-3 hydrogen atoms ($pK_a = 6.8$ and 7.0 , respectively³). Thus, at physiological pH, these residues will be significantly deprotonated, bestowing the uracil bases with a negative charge and thus greatly affecting their properties.

Further, there are significant conformational changes imparted by the presence of the 6-aza substitution. For example, 6-Aza-2'-deoxyuridine has a high-*anti* conformation and is present in solution with *N*-type sugar pucker.³

We now offer the nucleosides 6-Aza-2'-deoxyuridine and 6-Azathymidine (Figure 3) as well as their phosphoramidites for nucleic acid synthesis, i.e., 6-Aza-dU CEP and 6-Azathymidine CEP (Figure 4).

Seela and Chittepudi reported that 6-aza-dU nucleotides may be incorporated into oligodeoxyribonucleotides using 6-Aza-dU CEP (BA 0303).^{3,4} The *N*³-*o*-anisoyl protecting group⁵ is necessary to afford stability. DNA duplexes containing 6-aza-dU•dA base pairs were studied³ and

are less stable than T•dA base pairs at neutral pH due to deprotonation of the N-3 hydrogen on 6-aza-dU. However, at lower pH, duplex stability increases as N-3 becomes protonated and therefore able to hydrogen bond to dA. The fact that 6-aza-dU residues are deprotonated at neutral pH results in easy metal-DNA (M-DNA) formation.³ Canonical DNA forms M-DNA only at high pH, whereas 6-aza-dU-containing DNA can form M-DNA at neutral or high pH. Finally, oligonucleotides containing 6-aza-dU show enhanced resistance to 3'-exonucleases (e.g., snake venom phosphodiesterase).

The phosphoramidite 6-Azathymidine CEP (BA 0306) has been incorporated into oligo-

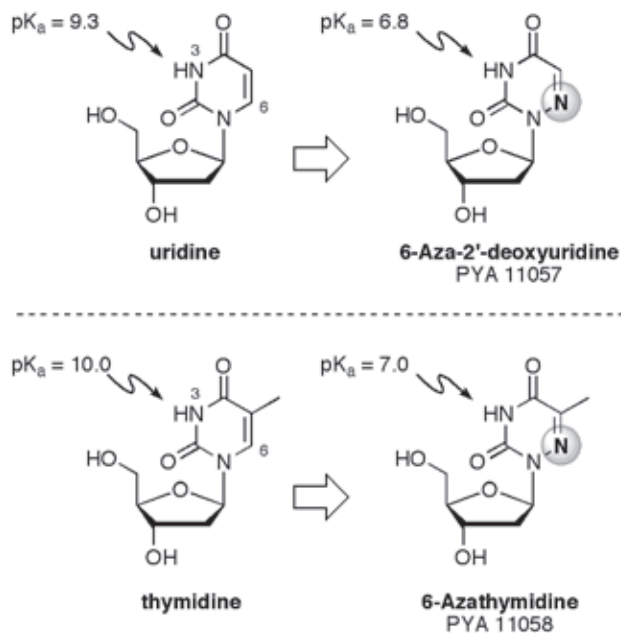


Figure 3. 6-Aza modifications of uridine and thymidine lead to higher acidity of the N-3 hydrogen atoms.

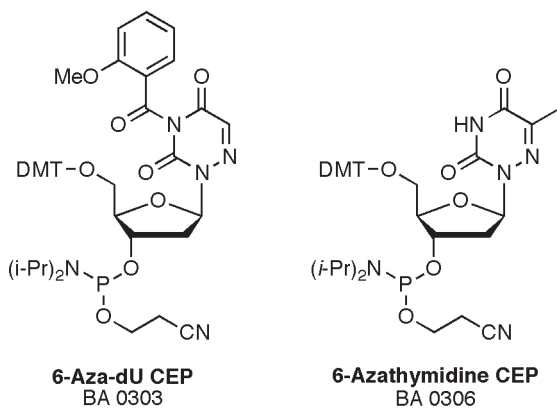


Figure 4. New phosphoramidites for the incorporation of 6-azapyrimidines into nucleic acids.

nucleotides,² where it imparts nuclease resistance when installed at the 5'-position. Duplexes with DNA or RNA are only slightly destabilized, and heteroduplexes with RNA support RNase-H cleavage.

References

- (1) (a) Kara, J.; Sorm, F. *Coll. Czech. Chem. Commun.* **1963**, *28*, 1441-1448. (b) Holy, A.; Cech, D. *Coll. Czech. Chem. Commun.* **1974**, *39*, 3157-3167. (c) Drasar, P.; Hein, L.; Beranek, J. *Coll. Czech. Chem. Commun.* **1976**, *41*, 2110-2123. (d) Freskos, J. N. *Nucleosides Nucleotides* **1989**, *8*, 549-555.
- (2) Sanghvi, Y.; Hoke, G. D.; Freier, S. M.; Zounes, M. C.; Gonzalez, C.; Cummins, L.; Sasmor, H.; Cook, P. D. *Nucleic Acids Res.* **1993**, *21*, 3197-3203.
- (3) Seela, F.; Chittepu, P. *J. Org. Chem.* **2007**, *72*, 4358-4366.
- (4) For the incorporation of 6-azauridine into oligoribonucleotides, see: (a) Beigelman, L.; Karpeisky, A.; Usman, N. *Nucleosides Nucleotides* **1995**, *14*, 895-899 and (b) Oyelere, A. K.; Strobel, S. A. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1851-1858.
- (5) Welch, C. J.; Chattopadhyaya, J. *Acta Chem. Scand. Ser. B* **1983**, *37*, 147-150.

6-Azapyrimidine — Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
6-Aza-2'-deoxyuridine	PYA 11057	25 mg	\$55.00
		100 mg	\$175.00
6-Azathymidine	PYA 11058	25 mg	\$105.00
		100 mg	\$295.00
6-Aza-dU CEP	BA 0303	100 μ mol	\$395.00
		0.25 g	\$895.00
6-Azathymidine CEP	BA 0306	100 μ mol	\$345.00
		0.25 g	\$895.00



Alkyne Modifiers for Click Conjugation

Alkyne-bearing oligonucleotides are useful for a variety of applications. For example, 5-ethynyl-dU, 5-propynyl-dC, and 5-propynyl-dU nucleotides lead to duplex stabilization. If the alkyne is terminal, transition-metal couplings may be performed on the alkyne-modified oligonucleotide.¹ We have several alkyne-bearing phosphoramidites in our product line, e.g., the terminal alkynes 5-Ethynyl-dU CEP and 5-Propargyloxy-dU CEP² (Figure 5). We now introduce 5-Octadiynyl-dU CEP (Figure 5) for alkyne modification and describe an additional use for alkyne-modified oligonucleotides, namely the click reaction of alkynes with azides.

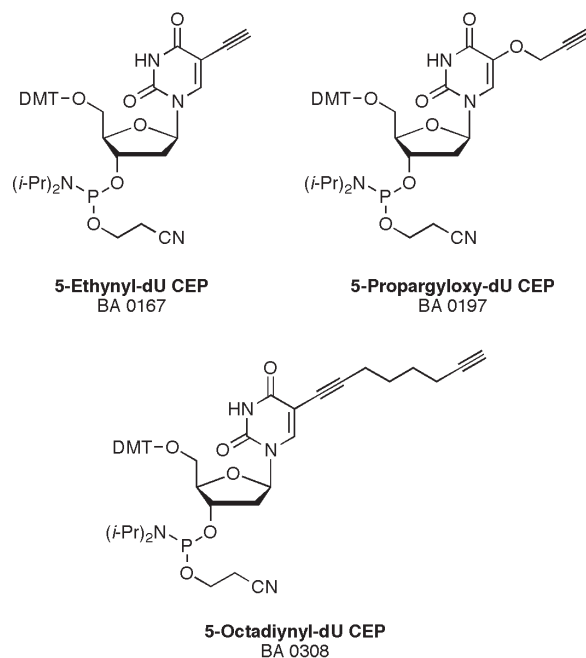
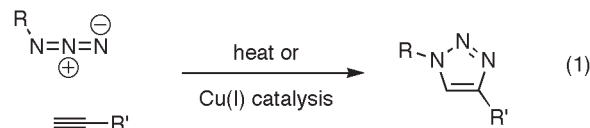


Figure 5. Phosphoramidites for the incorporation of terminal alkynes into oligonucleotides.

Huisgen³ pioneered the field of 1,3-dipolar cycloaddition chemistry, wherein five-membered heterocycles are formed by the combination of two molecules that bear certain unsaturated groups. A powerful example is the 1,3-dipolar cycloaddition of alkynes with azides (RN₃) to form triazoles, a reaction that occurs thermally with no additional reagents (eq. 1).⁴ A major advance was made independently by the groups of Sharpless⁵ and Meldal,⁶ who discovered that Cu(I) catalysis allows the cycloaddition to be performed at room temperature. Sharpless and co-workers coined the term “click reaction” to refer to simple reactions that require only benign conditions, occur in high yield, and are easy to perform. The copper-catalyzed azide/alkyne cycloaddition reaction⁷ is the most popular example of a

click reaction and is now essentially implied when one encounters the term “click”.



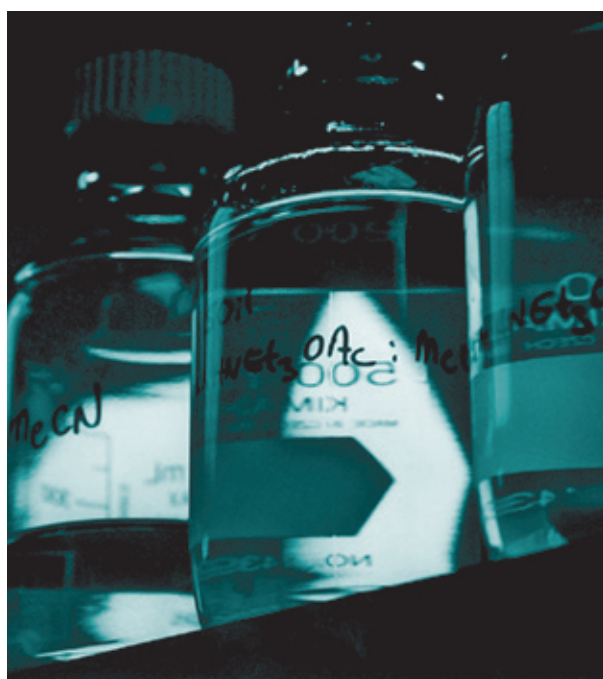
In the oligonucleotide field, the click reaction of azides with alkynes is of increasing importance, and provides an alternative to polar (nucleophile-electrophile) chemistry for the conjugation of various species to nucleic acids. The click reaction of azides with alkynes is bioorthogonal to conjugation techniques such as the acylation of amines or the alkylation or conjugate addition reactions of thiols. We wish to provide researchers with a “click toolbox” for carrying out nucleic acid conjugation using various alkynes and azides. Herein we describe three initial offerings in this vein.

An attractive click strategy for nucleic acid conjugation involves the reaction of alkyne-bearing oligonucleotides with azide-bearing species (fluorophores, biotins, proteins, sugars, etc.). Ideally, the alkyne component would be built into the oligonucleotide during automated synthesis.^{8,9} For the installation of an alkyne-bearing nucleoside into an oligonucleotide, we offer 5-Ethynyl-dU CEP (BA 0167), 5-Propargyloxy-dU CEP (BA 0174),² and 5-Octadiynyl-dU CEP (BA 0308), Figure 5. 5-Ethynyl-dU CEP allows the synthesis of modified oligonucleotides that have been shown by Carell and co-workers¹¹ to be capable of undergoing click reactions with azides bearing a variety of groups (sugar, coumarin, fluorescein). However, the short, rigid nature of the alkyne group in 5-ethynyl-dU limits the utility of the click reaction if multiple incorporations are desired. The longer, more flexible tether in 5-Octadiynyl-dU CEP allows efficient click chemistry, as shown by the groups of Seela^{10,12} and Carell,^{11,13,14} even with multiple incorporations. Click reactions with the above azides¹¹ as well as AZT,^{10,12} a pinacyanol dye,¹³ and azide-terminated glass slides¹⁴ were successful. Further, the 5-octadiynyl-dU modification is similar to the well-known propynyl-dU modification, resulting in a slight stabilization of DNA duplexes.¹²

Regarding the click reaction of oligonucleotides, copper(I) salts can cause strand breaks, perhaps by hydroxyl radical production.¹¹ To avoid this problem, modified conditions have been studied that protect the integrity of biomolecules.¹⁵ Instead of using air-sensitive Cu(I) salts, copper sulfate pentahydrate may be reduced *in situ* to the Cu(I) oxidation state with water-

soluble reducing agents such as sodium ascorbate or tris(carboxyethyl)phosphine hydrochloride (TCEP).¹⁶ Further, the Cu(I)-stabilizing ligand tris(benzyltriazolylmethyl)amine (TBTA)¹⁶ may be added, which accelerates the rate of the reaction and protects the Cu(I) center from oxidation under aerobic conditions.^{15,17} Finally, the conjugation of 5-octadiynyl-dU-modified oligonucleotides may be performed in solution or while still bound to the CPG solid support.¹⁰⁻¹³

Other click conjugation products, both alkynes and azides, are in development; please check our web site for the latest developments.



Alkyne Modifiers — Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
5-Ethynyl-dU CEP	BA 0167	100 μmol	\$315.00
		0.25 g	\$775.00
5-Propargyloxy-dU CEP	BA 0174	100 μmol	\$355.00
		0.25 g	\$875.00
5-Octadiynyl-dU CEP	BA 0308	100 μmol	\$255.00
		0.25 g	\$595.00

References

- (1) (a) Graham, D.; Parkinson, J. A.; Brown, T. *J. Chem. Soc., Perkin Trans. 1* **1998**, 1131-1138. (b) Gorodetsky, A. A.; Green, O.; Yavin, E.; Barton, J. K. *Bioconjugate Chem.* **2007**, *18*, 1434-1441.
- (2) This compound is from our Experimental Grab Bag. The compounds in this unique collection have not been validated for any particular oligonucleotide application. We hope that you may find them of interest, but please be aware that their purchase and use is at your own risk.
- (3) Huisgen, R. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley-Interscience: New York, 1984; Vol. 1, Ch. 1.
- (4) Huisgen, R.; Szeimies, G.; Moebius, L. *Chem. Ber.* **1967**, *100*, 2494-2507.
- (5) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596-2599.
- (6) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057-3064.
- (7) Reviews: (a) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004-2021. (b) Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.* **2006**, 51-68.
- (8) The alternate strategy, wherein an azide is incorporated by automated synthesis, is problematic due to the known propensity of azides to react with P(III) species such as phosphoramidites, though azides may be installed post-synthetically. Alkynes can also be installed post-synthetically.
- (9) For other examples of oligonucleotide conjugation using the click reaction of azides with alkynes, see: (a) Seo, T. S.; Li, Z.; Ruparel, H.; Ju, J. *J. Org. Chem.*, **2003**, *68*, 609-612. (b) Seo, T. S.; Bai, X.; Ruparel, H.; Li, Z.; Turro, N. J.; Ju, J. *Proc. Nat. Acad. Sci. USA*, **2004**, *101*, 5488-5493. (c) Devaraj, N. K.; Miller, G. P.; Ebina, W.; Kakaradov, B.; Collman, J. P.; Kool, E. T.; Chidsey, C. E. D. *J. Am. Chem. Soc.* **2005**, *127*, 8600-8601. (d) Chevolut, Y.; Bouillon, C.; Vidal, S.; Morvan, F.; Meyer, A.; Cloarec, J.-P.; Jochum, A.; Praly, J.-P.; Vasseur, J.-J.; Souteyrand, E. *Angew. Chem. Int. Ed.* **2007**, *46*, 2398-2402. (e) Kumar, R.; El-Sagheer, A.; Tumpance, J.; Lincoln, P.; Wilhelmsson, L. M.; Brown, T. *J. Am. Chem. Soc.* **2007**, *129*, 6859-6864.
- (10) Seela, F.; Sirivolu, V. R. *Chem. Biodiversity* **2006**, *3*, 509-514.
- (11) Gierlich, J.; Burley, G. A.; Gramlich, P. M. E.; Hammond, D. M.; Carell, T. *Org. Lett.* **2006**, *8*, 3638-3642.
- (12) Seela, F.; Sirivolu, V. R. *Helv. Chim. Acta* **2007**, *90*, 535-552.
- (13) Hammond, D. M.; Manetto, A.; Gierlich, J.; Azov, V. A.; Gramlich, P. M. E.; Burley, G. A.; Maul, M.; Carell, T. *Angew. Chem. Int. Ed.* **2007**, *46*, 4184-4187.
- (14) Rozkiewicz, D. I.; Gierlich, J.; Burley, G. A.; Gutmiedl, K.; Carell, T.; Ravoo, B. J.; Reinhoudt, D. N. *ChemBioChem* **2007**, *8*, 1997-2002.
- (15) Wang, Q.; Chan, T. R.; Hilgraf, R.; Fokin, V. V.; Sharpless, K. B.; Finn, M. G. *J. Am. Chem. Soc.* **2003**, *125*, 3192-3193.
- (16) Commercially available from Aldrich and other chemical suppliers.
- (17) Chan, T.; Hilgraf, R.; Sharpless, K. B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 2853-2855.

Fluorous Affinity Purification and Fluorous Tagging

Highly fluorinated organic compounds are both hydrophobic and lipophobic, preferring instead to associate with other fluorinated substances. Organic molecules that have both an organic domain and a perfluoroalkyl domain (e.g., a linear perfluoroalkyl “ponytail”) are known as *fluorous molecules*,¹ and may be separated from non-fluorous molecules by interaction with fluorinated separation media such as Fluoro-Pak columns.^{2,3} Fluorous-fluorous interactions are strong and selective (“like dissolves like”). The Fall 2006 edition of *Chemistry from Berry & Associates*⁴ introduced Fluorous Affinity Purification of Oligonucleotides, a higher affinity alternative to DMT-on reversed-phase cartridge purification.² It relies on the strong interaction of fluorous-tagged oligonucleotides with the fluorous adsorbent in Fluoro-Pak columns. The fluorous tag took the form of a fluorous dimethoxytrityl (FDMT) group,

which was installed using the appropriate FDMT-bearing nucleoside phosphoramidite (Figure 6). After fluorous purification on Fluoro-Pak columns with on-column detritylation, high recoveries of oligonucleotides were obtained, free from failure sequences, even with 100-mers.² The FDMT group also facilitates RP-HPLC purification.⁵ We now describe several new fluorous products for use in oligonucleotide chemistry. Figure 6 shows one of these new products, FMMT-*N*²-iBu-dG CEP (FL 1220), a fluorous monomethoxytrityl (FMMT) version of our existing product FDMT-*N*²-iBu-dG CEP (FL 1200). The more stable FMMT group may be of interest in applications where there are several purine nucleotides near the 5'-terminus, a scenario that can sometimes lead to premature detritylation during RP-HPLC purification.

Two additional fluorous-trityl-bearing phosphoramidites are shown in Figure 7. The fluorous affinity purification of oligonucleotides is a powerful technique, but if oligonucleotides with different 5'-nucleobases are being made, it is necessary to have bottles of the four FDMT-tagged nucleoside phosphoramidites installed. However, if a short hydroxyalkyl group is tolerated at the 5'-terminus, a single phosphoramidite, FDMT-On Purification Modifier (FL 1400), is an effective way to enable fluorous purification in a variety of applications. This phosphoramidite installs a fluorous dimethoxytrityl group via a short tether. After fluorous affinity purification and detritylation, a 3-hydroxy-1-methylpropyl group remains.⁶ For applications requiring the purification of 5'-amine-modified oligonucleotides, trityl-on

purification is an option, typically involving a monomethoxytrityl (MMT) group. In cases where the separation of the trityl-on oligonucleotides from failure sequences and other non-trityl-bearing materials is more difficult, FMMT-5'-Amino-modifier-C6 CEP (FL 1500) is a useful alternative (Figure 7). It employs a fluorous version of the MMT group, i.e., a fluorous monomethoxytrityl (FMMT) group. This phosphoramidite behaves similarly to the MMT version during synthesis, but the final FMMT-bearing oligonucleotide is more strongly retained on fluorous or reversed-phase adsorbents, providing greater selectivity than that observed for MMT-bearing oligonucleotides.

While many of our fluorous products focus on the purification of oligonucleotides, fluorous tags have other potential applications in nucleic acid chemistry. We now introduce two new fluorous monomers for the permanent fluorous-tagging of oligonucleotides (Figure 8). Fluorous Modifier CEP (FL 1600) is useful for placing a fluorous tag internally or at the 5'-terminus of an oligonucleotide, whereas 3'-Fluorous Modifier CPG (FL 1610) is effective for attaching a 3'-fluorous tag. In addition to providing a purification handle, fluorous modifications enable applications where fluorophilicity or high hydrophobicity is desired. For example, the presence of a fluorous tag in an oligonucleotide may allow its immobilization onto fluorous-coated glass slides.^{7,8} Alternatively, placing fluorous monomers at strategic sites in an oligonucleotide may allow intra- or intermolecular fluorous-fluorous interactions, enhancing the attraction between various regions of an oligonucleotide.⁹

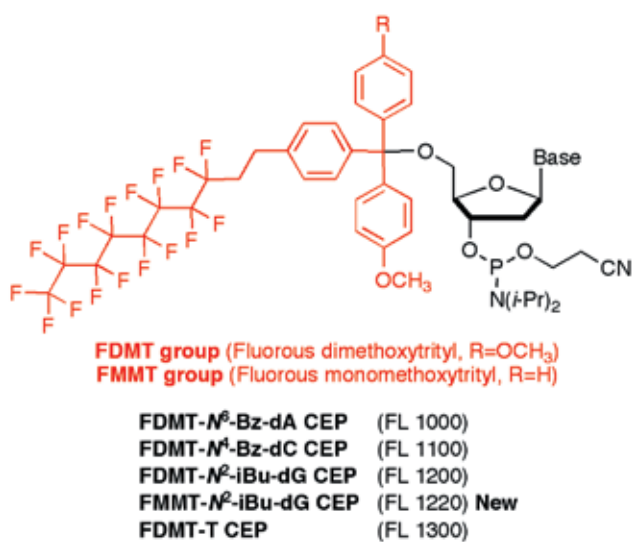


Figure 6. Fluorous trityl phosphoramidites for fluorous affinity purification of oligonucleotides.

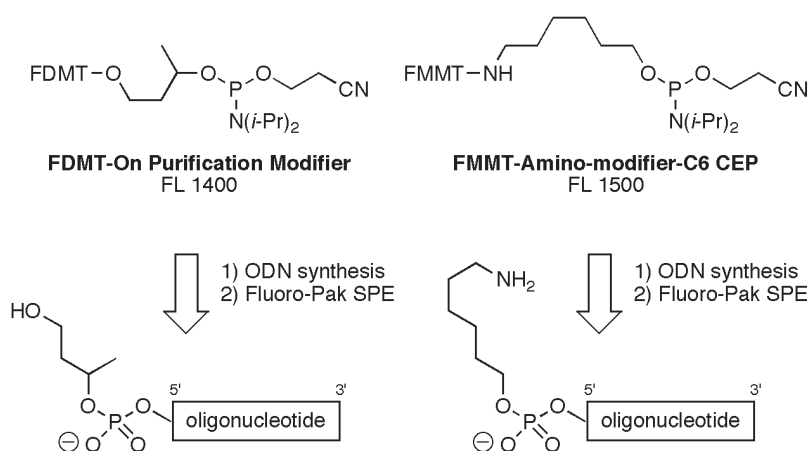


Figure 7. New fluoros phosphoramidites for fluoros affinity purification of oligonucleotides.

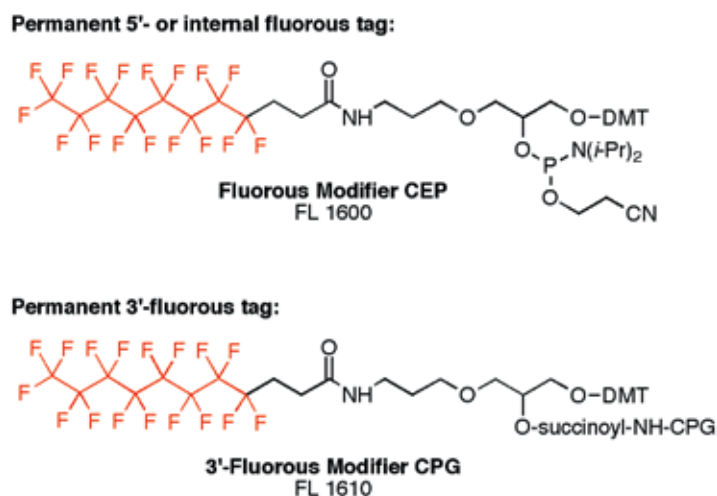


Figure 8. New fluoros phosphoramidites for permanent fluoros tagging of oligonucleotides.

We continue to offer new products for the introduction of fluoros tags into oligonucleotides. Coming soon are fluoros-tagged optical reagents such as dabcylys and fluoresceins; see our web site.

Fluorous Affinity Purification and Tagging — Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
FMMT- <i>N</i> ² - <i>i</i> Bu- <i>d</i> G CEP	FL 1220		Inquire for pricing
FDMT-On Purification Modifier	FL 1400	100 μmol	\$75.00
		250 μmol	\$175.00
FMMT-Amino-modifier-C6 CEP	FL 1500		Inquire for pricing
Fluorous Modifier CEP	FL 1600	100 μmol	\$75.00
		250 μmol	\$175.00
3'-Fluorous Modifier CPG	FL 1610	100 mg	\$85.00
		1 g	\$475.00

References

- (1) *Handbook of Fluorous Chemistry*; Gladysz, J. A.; Curran, D. P.; Horváth, I. T., Eds.; Wiley-VCH: Weinheim, 2004.
- (2) Pearson, W. H.; Berry, D. A.; Stoy, P.; Jung, K.-Y.; Sercel, A. D. *J. Org. Chem.* **2005**, *70*, 7114-7122.
- (3) Fluoro-Pak is a trademark of Berry & Associates, Inc. Products for fluoros affinity purification and fluoros tagging of oligonucleotides are subject to patent applications filed by Berry & Associates, Inc. Further, the use of these products is licensed under U.S. Patents 6,673,539, 6,156,896; 5,859,247; and 5,777,121 and one or more pending patents owned or controlled by Fluorous Technologies, Inc.
- (4) Download at <http://www.berryassoc.com/literature/BerryNews1-1.pdf>.
- (5) Fluorous-tagged oligonucleotides are highly retained on both fluoros and traditional reversed-phase adsorbents, allowing easy separation from non-fluorous-tagged oligos and by-products.
- (6) Alternatively, Fluorous Chemical Phosphorylation Reagent II (F-CPR-II CEP, see www.berryassoc.com, Product Number FL 1360) allows fluoros affinity purification with concomitant 5'-phosphorylation, which is especially attractive when synthesizing longmers that will be used in ligase reactions. Thus, if a 5'-phosphate is tolerated in a particular application, F-CPR-II may be used as a common handle for fluoros purification, obviating the need to choose a different fluoros phosphoramidite for each different 5'-terminal nucleotide.
- (7) Fluorous Technologies, Inc., offers Fluorous Modified Glass Slides for the immobilization of fluoros-tagged molecules for microarray formation; see <http://www.fluorous.com>. The slides feature excellent spot morphology, high signal-to-noise ratios, low and uniform background fluorescence levels, and low non-specific binding, since the fluoros surface around the spot does not interact well with non-fluorous molecules. The ability to use the fluoros tag as both a purification handle and an immobilization handle is also an advantage. Further, the fluoros immobilization is potentially reversible.
- (8) (a) Pohl and co-workers detected carbohydrate-lectin interactions using fluoros modified slides bearing fluoros-tagged carbohydrates; see: Ko, K.-S.; Jaipuri, F. A.; Pohl, N. L. *J. Am. Chem. Soc.* **2005**, *127*, 13162-13163. (b) Spring and co-workers showed that fluoros-tagged small molecules could be immobilized on fluoros modified a glass surface and used to facilitate detection of protein-ligand binding interactions; see: Nicholson, R. L.; Ladlow, M. L.; Spring, D. R. *Chem. Commun.* **2007**, 3906-3908. (c) Schreiber and co-workers employed fluoros-immobilized small-molecule arrays to screen for histone deacetylase inhibitors; see: Vegas, A. J.; Bradner, J. E.; Tang, W.; McPherson, O. M.; Greenberg, E. F.; Koehler, A. N.; Schreiber, S. L. *Angew. Chem. Int. Ed.* **2007**, *46*, 7960-7964.
- (9) Examples: Placing fluoros tags in the stem region of molecular beacons or next to optical tags to enhance fluorescence quenching.

Other Phosphoramidites

Fmoc-Pyrrolidine CEP — Installation of 3-hydroxyprolinol nucleotides

Verdine and co-workers¹ have described the use of Fmoc-Pyrrolidine CEP (Figure 9) to install (2*R*,3*S*)-2-hydroxymethyl-3-hydroxypyrrolidine (3-hydroxyprolinol) residues into DNA (see 1). Such oligonucleotides were found to be potent and selective inhibitors of *E. coli* 3-methyladenine DNA glycosylase II (AlkA). The pyrrolidine ring, which is protonated under experimental conditions, is proposed to mimic the charged transition state 2 encountered during nucleoside hydrolysis. Related 3-hydroxyprolinol-bearing oligonucleotides have also been made and subjected to hybridization studies.²

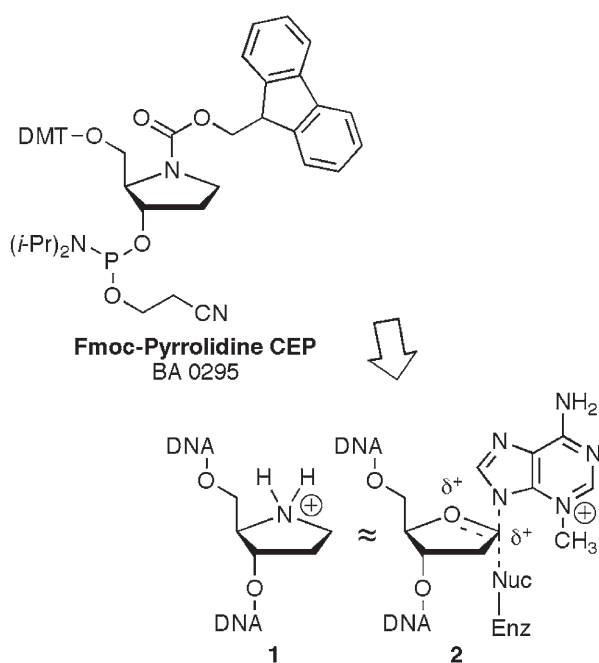


Figure 9. 3-Hydroxyprolinol nucleotides mimic the transition state for enzymatic nucleoside hydrolysis.

Alternate Base Pairing — Pseudo-dG

Isoguanosine 3 and its 2'-deoxy variant 4 are important in the field of non-natural base pairing.³ Berry, Townsend, and co-workers⁴ introduced 5, an analog of 3, differing by the transposition of the atoms/groups at positions 2 and 3 of the nucleobase (Figure 10). Hosmane and co-workers⁵ have recently synthesized the nucleosides 6 and its isomer 7 for the purpose of studying their base pairing characteristics. We offer the names pseudo-dG for 7 and pseudo-iso-G/dG for 5 and 6.

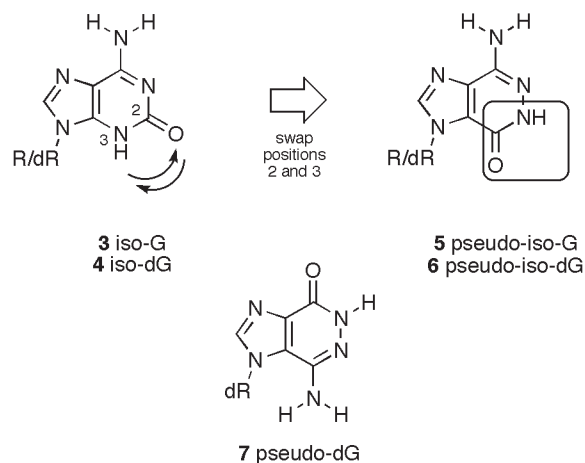


Figure 10. Purine base analogs for the study of non-natural base pairing.

The nucleosides 6 and 7 were studied in solution and by computer modeling.⁵ Pseudo-dG 7 was found to form homo-pairs (7 • 7, see Figure 11), corresponding to what would be an anti-parallel arrangement in a nucleic acid. Evidence for three of the four possible intramolecular hydrogen bonds was obtained. Pseudo-dG 7 also hetero-pairs with pseudo-iso-dG 6 as shown, corresponding to a parallel arrangement. Although the nucleosides 6 and 7 were not studied in oligonucleotides, they would be attractive nucleic acid modifications in homo- and hetero-pairing modes. It would also be interesting to study them in pairs with canonical nucleotides. For example, replacing dG with pseudo-dG 7 opposite a dC nucleotide (see Figure 11) may lead to G•C base-pair leveling.

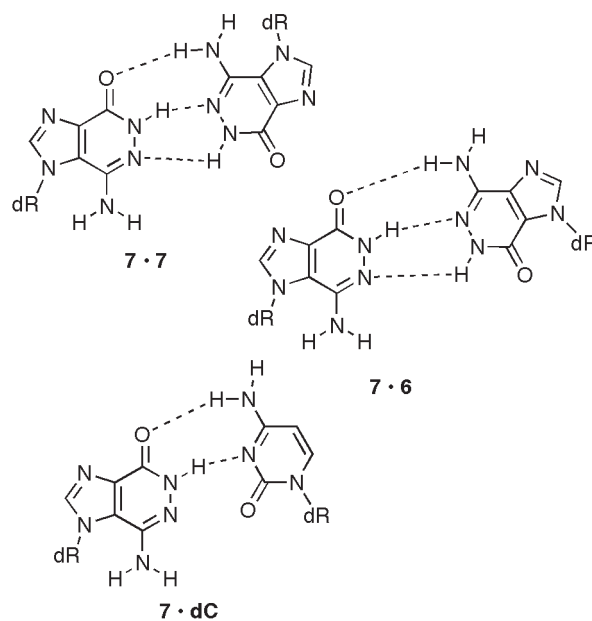


Figure 11. Base-pairing schemes for purine analogs 6 and 7.

We now offer the phosphoramidite of **7**, i.e., Pseudo-dG CEP (BA 0312, Figure 12). Although this compound has not been proven in the oligonucleotide arena and thus belongs in our Experimental Grab Bag,⁶ we hope that it may find interest in the scientific community.

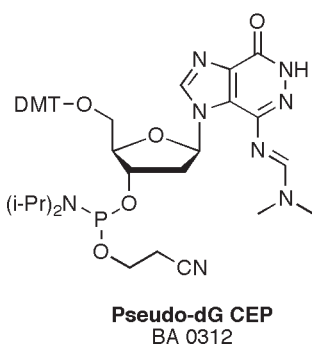


Figure 12. A phosphoramidite for the incorporation of pseudo-dG **7**.

Formylindole-dT CEP — An Aldehyde Modifier for Conjugation

Aldehydes are attractive electrophiles for bioconjugation, since they react with nucleophiles such as amines and hydrazines to form imines and hydrazones, respectively. For the incorporation of an aldehyde functional group into an oligonucleotide, its reactivity often necessitates carrying it through solid-phase synthesis in protected or otherwise masked form, thus requiring one or more post-synthetic unmasking transformations. Saito and co-workers⁷ reported that an aldehyde may be incorporated directly using phosphoramidite **8** via solid-phase nucleic acid synthesis (Figure 13).

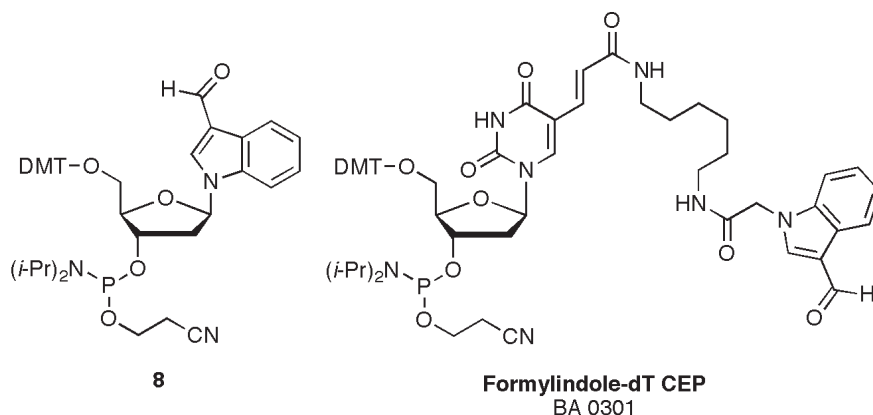


Figure 13. Aldehyde modifiers.

The electron-donating indole ring provides some stabilization of the aldehyde while still retaining enough electrophilic character to allow conjugation with hydrazines and hydrazones. We now offer Formylindole-dT CEP (Figure 13),⁶ which features a tether between the formylindole nucleus and the oligonucleotide strand. Internal and 5'-incorporation are possible.

References

- (1) Schärer, O. D.; Ortholand, J.-Y.; Ganesan, A.; Ezaz-Nikpay, K.; Verdine, G. L. *J. Am. Chem. Soc.*, **1995**, *117*, 6623-6624.
- (2) Ceulemans, G.; Van Aerschot, A.; Rozenski, J. Herdewijn, P. *Tetrahedron* **1997**, *53*, 14957-14974.
- (3) Switzer, C. Y.; Moroney, S. E.; Benner, S. A. *J. Am. Chem. Soc.* **1989**, *111*, 8322-8323.
- (4) Berry, D. A.; Wotring, L. L.; Drach, J. C.; Townsend, L. B. *Nucleosides Nucleotides* **1994**, *13*, 2001-2011.
- (5) Ujjinamatada, R. K.; Paulman, R. L.; Ptak, R. G.; Hosmane, R. S. *Bioorg. Med. Chem.* **2006**, *14*, 6359-6367.
- (6) These new compounds are from our Experimental Grab Bag. The compounds in this unique collection have not been validated for any particular oligonucleotide application. We hope that you may find them of interest, but please be aware that their purchase and use is at your own risk.
- (7) Okamoto, A.; Tainaka, K.; Saito, I. *Tetrahedron Lett.* **2002**, *43*, 4581-4583.

Other Phosphoramidites — Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
Fmoc-Pyrrolidine CEP	BA 0295	5 g min. order	Call for pricing
Pseudo-dG CEP	BA 0312		
		100 μ mol	\$345.00
		0.25 g	\$795.00
Formylindole-dT CEP	BA 0301		
		100 μ mol	\$395.00
		0.25 g	\$645.00

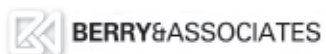


Berry & Associates, Inc.

2434 Bishop Circle East, Dexter, Michigan 48130 USA

Phone 734-426-3787 • Toll Free 800-357-1145 • Fax 734-426-9077

www.berryassoc.com | orders@berryassoc.com | techhelp@berryassoc.com



2434 Bishop Circle East
Dexter, Michigan 48130 USA

In This Issue

Anthraquinone Phosphoramidites

New Amine Modifiers

6-Azapyrimidine Phosphoramidites
& Nucleosides

Alkyne Modifiers for Click Conjugation

Fluorous Affinity Purification and
Fluorous Tagging

Other New Phosphoramidites
