Chemistry
from Berry & Associates
TO ADVANCE THE LIFE SCIENCES
Issue 7 | January 2011

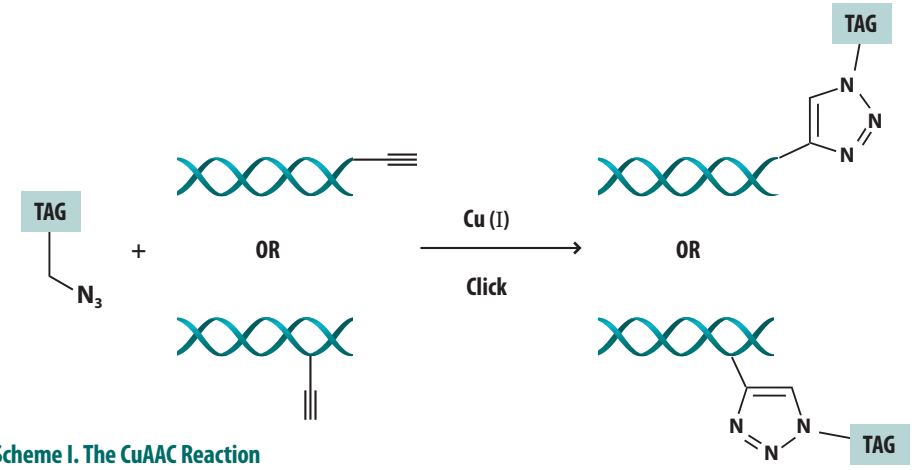
These Alkynes and Azides Make Click Reactions a Snap

The Cu(**I**) catalyzed [3+2] azide-alkyne cycloaddition (CuAAC) also known as the Click reaction, has become an invaluable tool in biochemical research. This cycloaddition reaction is remarkably efficient and reliable even in the presence of the diverse array of functional groups found in DNA and the variety of scientifically intriguing ligation partners. Development of the click methodology has led to the production and study of many modified DNA oligonucleotides (ODNs) for biological as well as nanotechnological and surface oriented applications. The beauty of the click cycloaddition lies in the efficient formation of a non-toxic triazole from biological building blocks that have been modified with non-perturbing azides and unactivated alkynes. For further reading on the evolution of the 1,3-dipolar cycloaddition for the labeling of DNA and beyond see references 1-6. We now offer a wide range of alkynes and azides suitable for click ligation.

Alkynes

It has been well documented that 5-ethynyl dU has many uses in biological research. Although studies have shown that it can undergo CuAAC,[6] its use for this purpose is not very efficient, especially if multiple copies of the cycloadduct are desired. We offer three additional alkyne tethered phorphoramidites that are superior choices as substrates for nucleic acid conjugation. Both 5-Octadiynyl-dU CEP (BA 0308)[6,7] and Alkynyl-modifier-C6-dT CEP (BA 0316) offer a longer alkyne appendage, thereby facilitating click chemistry especially for multiple ligations in the same ODN. 5-Propargyloxy-dU CEP (BA 0174) offers an intermediate alkyne

Continued on page 2



Scheme I. The CuAAC Reaction

In This Issue

Alkynes and Azides for Click Chemistry
Linear BBQ® Superquencher Application
Folate Labeled CEP and Azide
New Tools for Research: Thiol Sensors, Electrochemical Detection and Uridine Tagging

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.

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Alkynes and Azides

continued from page 1

tether length which also allows for efficient click ligation. In addition to the phosphoramidites, we offer the alkyne nucleoside precursors 5-(1,7-Octadiyn-1-yl)-2'-deoxyuridine (PY 7713), 5-(Propargyloxy)-2'-deoxyuridine (PY 7712) and 5'-(Dimethoxytrityl)-5-(propargyloxy)-2-deoxyuridine (PY 7714).

Azides

We now offer a variety of click ligation azides of many of the most popular labels including biotin (BT 1085), desthiobiotin (BT 1075), dansyl (FD 13005), and fluoroscein (FF 6110 and FF 6120). In addition, Aminooxy-TEG-azide (LK 4270) is a versatile new linker that can undergo a number of biologically significant coupling reactions and subsequent ligation *via* click methodology (see also page 7). Folate labeling is now simplified with our Folate-TEG azide (FC 8150).

Need and azide or an alkyne that you don't see? Feel free to contact us to discuss your research needs.

References

1. V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, **2002**, *41*, 2596–2599.

2. C.W. Tornoe, C. Christensen and M. Meldal, *J. Org. Chem.*, **2002**, *67*, 3057–3064.

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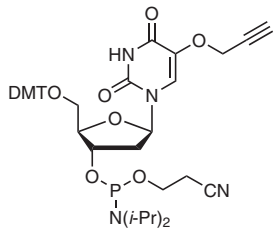
4. P. M. E. Gramlich, C. T. Wirges, A. Manetto and T. Carell, *Angew. Chem., Int. Ed.*, **2008**, *47*, 8350–8358.

5. (a) R. Huisgen, *Proc. Chem. Soc., London*, **1961**, 357–369. (b) R. Huisgen, *Angew. Chem., Int. Ed. Engl.*, **1963**, *2*, 565–632. (c) R. Huisgen, 1,3-Dipolar Cycloadditional Chemistry, Wiley,New York, 1984.

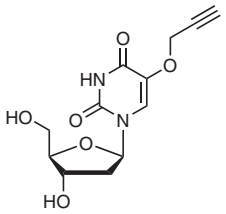
6. Gierlich, J.; Burley, G. A.; Gramlich, P. M. E.; Hammond, D. M.; Carell, T. *Org. Lett.* **2006**, *8*, 3639-3642.

7. (a) Seela, F.; Sirivolu, V. R. *Chem. Biodiversity* **2006**, *3*, 509-514; (b) Seela, F.; Sirivolu, V. R. *Helv. Chim. Acta* **2007**, *90*, 535-552; (c) 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.

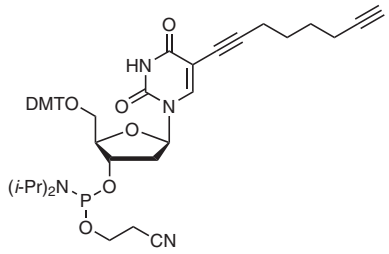
Alkynes



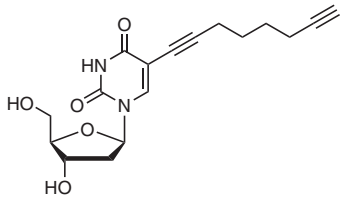
5-Propargyloxy-dU CEP (BA 0174)



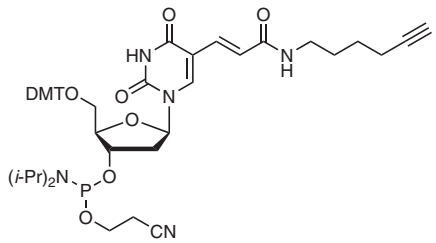
5-(Propargyloxy)-2′-deoxyuridine (PY 7712)



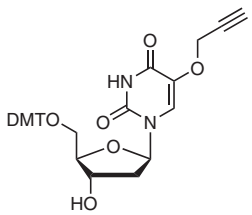
5-Octadiynyl-dU CEP (BA 0308)



5-(1,7-Octadiyn-1-yl)-2′-deoxyuridine (PY 7713)



Alkynyl-modifier-C6-dT (CEP BA 0316)



5′-(Dimethoxytrityl)- 5-(propargyloxy)-2′-deoxyuridine (PY 7714)

Ordering Information—Alkynes and Azides

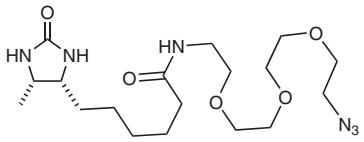
Catalog Number	Name	Size	Price
BA 0174	5-Propargyloxy-dU CEP		
		100 µmol	$355.00
		0.25 g	$875.00
BA 0308	5-Octadiynyl-dU CEP		
		100 µmol	$255.00
		0.25 g	$595.00
BA 0316	Alkynyl-modifier-C6-dT CEP		
		100 µmol	$360.00
		0.25 g	$795.00
BT 1075	Desthiobiotin-TEG azide		
		25 mg	$185.00
		100 mg	$595.00
BT 1085	Biotin-TEG azide		
		25 mg	$225.00
		100 mg	$595.00
FD 13005	Water soluble dansyl-TEG azide		
		25 mg	$95.00
		100 mg	$325.00
FF 6110	6-Carboxyfluorescein-TEG azide		
		25 mg	$175.00
		100 mg	$595.00

Ordering Information—Alkynes and Azides

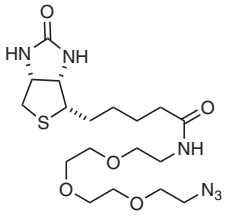
Catalog Number	Name	Size	Price
FF 6120	6-Carboxyfluorescein-dipivalate-TEG azide		
		25 mg	$175.00
		100 mg	$595.00
LK 4270	Aminooxy-TEG azide		
		100 mg	$325.00
PY 7713	5-(1,7-Octadiyn-1-yl)-2'-deoxyuridine		
		50 mg	$68.00
		250 mg	$225.00
PY 7714	5'-O-(Dimethoxytrityl)-5-(propargyloxy)-2'-deoxyuridine		
		100 mg	$275.00
		500 mg	$575.00
		1 g	$1,195.00
FC 8150	Folate TEG azide		
		5 mg	Inquire
		10 mg	Inquire

The purchase of these products for use in applications relating to copper catalyzed azide-alkyne cycloaddition chemistry ("Click Chemistry") includes a limited, nontransferable license to intellectual property owned by TSRI to use this product solely for internal non-commercial research activities and specifically excludes clinical, therapeutic, or diagnostic use in humans or animals. Information regarding a license for commercial use in Click Chemistry may be obtained directly from The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA 92037, or by contacting 858-784-8140 or click@scripps.edu.

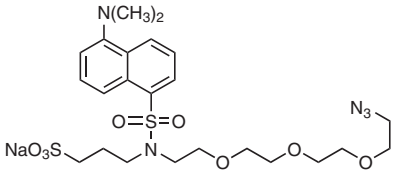
Azides



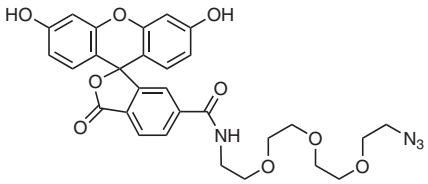
Desthiobiotin-TEG azide (BT 1075)



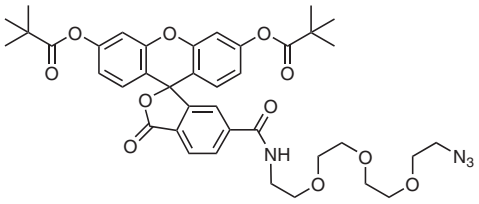
Biotin-TEG azide (BT 1085)



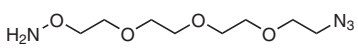
Water soluble dansyl-TEG azide (FD 13005)



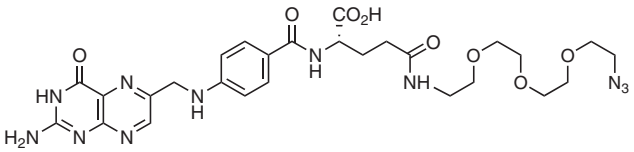
6-Carboxyfluorescein-TEG azide (FF 6110)



6-Carboxyfluorescein-TEG azide dipivalate (FF 6120)



Aminooxy-TEG azide (LK 4270)

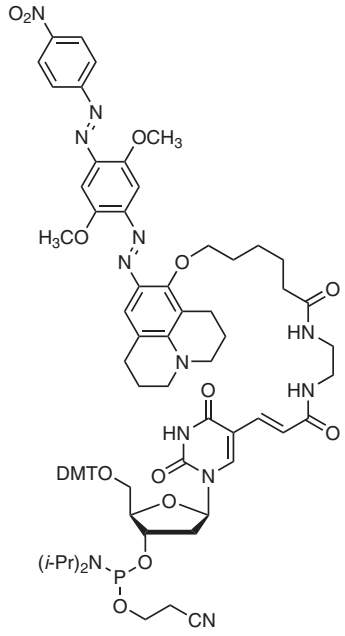


Folate-TEG azide (FC 8150)

Exciting new applications of BlackBerry® Quenchers:

Linear Superquenchers

Recent reports from Zheng and co-workers at the University of Toronto have illustrated the value of our BlackBerry® quencher BBQ-650™-dT CEP (BL 1010) in photodynamic molecular beacons (PMBs).[1] PMBs exhibit single base mismatch sensitivity and increase singlet oxygen production when hybridized with a specific target sequence. Zheng and co-workers have developed a fully automated synthesis of PMBs that utilize a linear architecture consisting of three consecutive BL 1010 nucleosides to create a *superquencher*. These PMBs are labeled with the photosensitizer pyropheophorbide which is stable to multiple DNA synthesis cycles as well as the methylamine/ammonium hydroxide deprotection and cleavage conditions. The linear approach simplifies PMB production as only one specialty quencher phosphoramidite is required whereas branched superquenchers require a trebler phosphoramidite in addition to the quencher CEP. The addition of multiple quencher units increases the hydrophobicity thereby facilitating purification. The ease of access to linear superquenchers has allowed the researchers to investigate the insertion of these molecular beacons into nanoparticles.[2] These studies have also uncovered a promising new technique for DNA-nanoparticle manipulation. The insertion of the linear superquenchers into lipid nanoparticles and subsequent opening of the molecular beacons resulted in irreversible aggregation of nanoparticles. This aggregation was selectively induced through target nucleic acid recognition.



BL 1010

References:

1. Lovell, J. F.; Chen, J.; Huynh, E.; Jarvi, M. T.; Wilson, B. C.; Zheng, G. *Bioconjugate Chem.* **2010**, *21*, 1023-1025.

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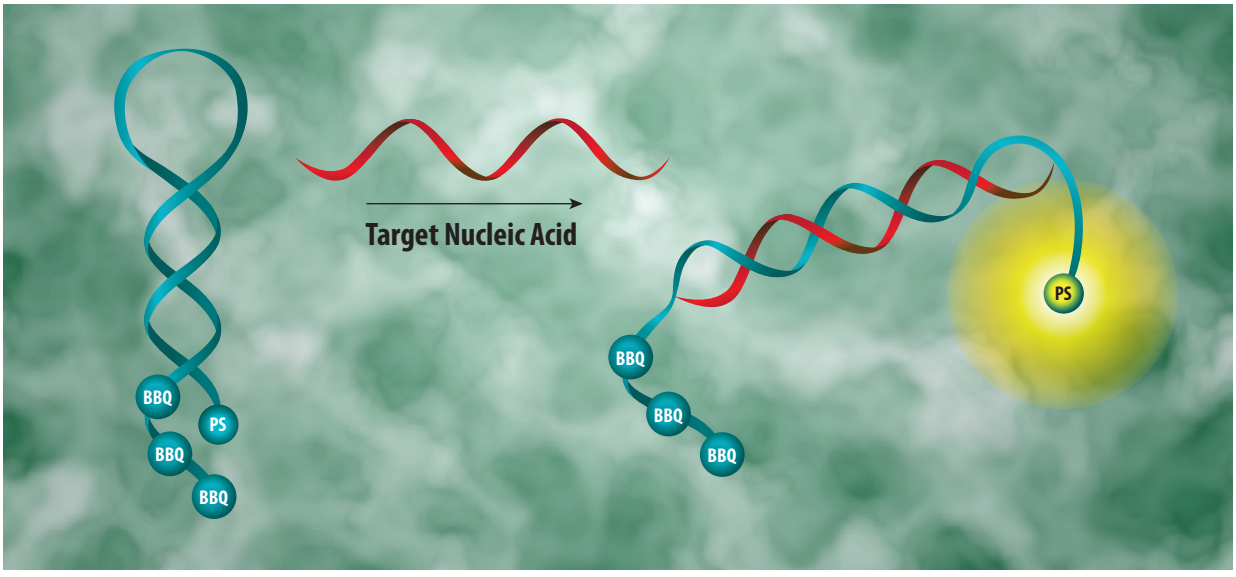
Ordering Information—Linear Superquenchers

Catalog Number	Name	Size	Price
BL 1010	BBQ-650™-dT CEP		
		50 umol	$150.00
		100 umol	$275.00
		0.25 g	$650.00

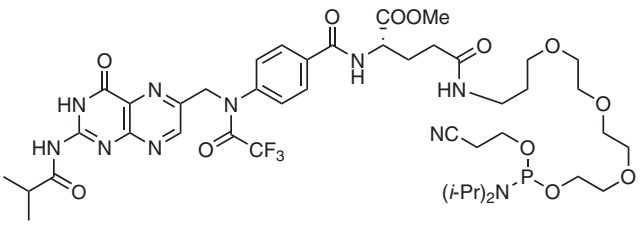


Folate Labeled CEP: siRNAs and beyond

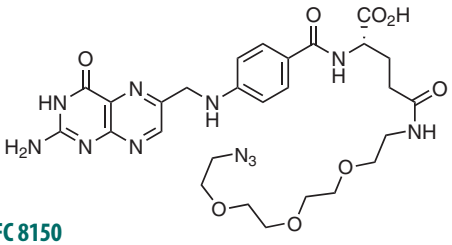
Recent years have seen an intensification of research in the area of folate-targeted therapeutics specifically designed as cancer treatments. Folic acid is an essential vitamin for cell maintenance and proliferation. In typical mammalian cells, a low affinity reduced folate carrier[1] or a protein coupled folate transporter[2] deliver the folate moiety. An orthogonal biological pathway has evolved in cancer cells where folate receptors (FR) are over expressed and bind folate conjugates with high affinity. Receptor mediated endocytosis is then the relevant mechanism for the delivery of the folate conjugate into the cancer cells.[3] The prominence of FRs in ovarian and lung among other cancers has catalyzed the exploration of folate conjugates of siRNAs, proteins and nanoparticles as potential chemotherapeutic agents.

Berry & Associates is proud to introduce our Folate CEP (BA 0349) for use in appending the folate moiety to oligonucleotides.[4]

For post-synthetic folate labeling *via* Click chemistry, we also offer Folate-TEG azide (FC 8150).



BA 0349



FC 8150

References

1. Matherly, L. H.; Hou, Z.; Deng, Y. *Cancer Metastasis Rev.* **2007**, *26*, 111–128.

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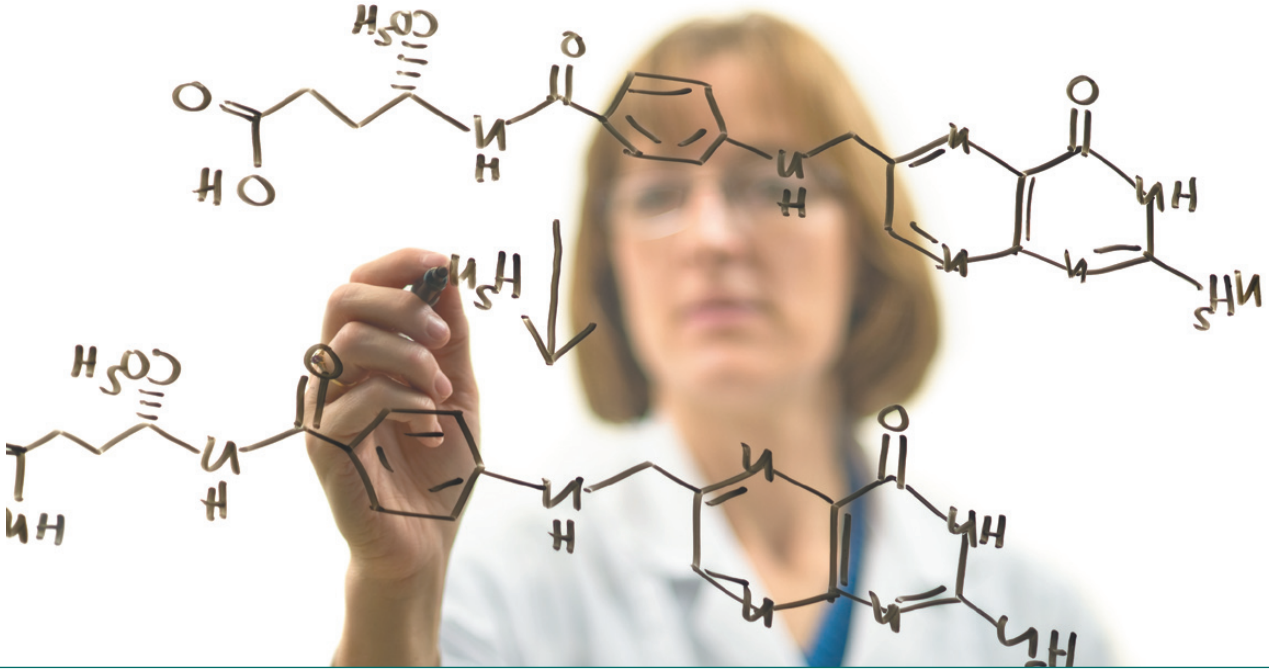
4. Patent pending.

Ordering Information—Folate Labeled CEP

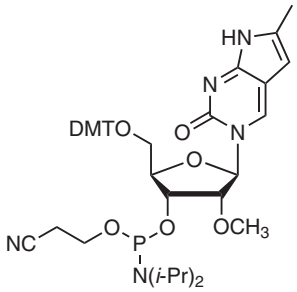
Catalog Number	Name	Size	Price
BA 0349	Folate-12 CEP		
		50 µmol	$548.00
		100 µmol	$843.00
FC 8150	Folate TEG Azide		
		5 mg	Inquire
		10 mg	Inquire



New Tools for Advancing Your Research

Fluroescent Nucleoside Phosphoramidites:

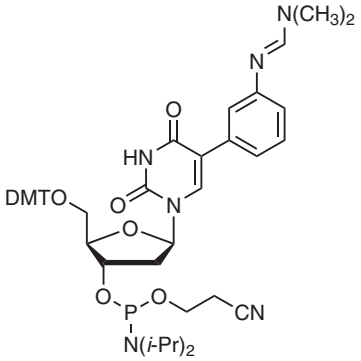
Pyrrolo-C (PC) is a fluorescent analog of cytidine.[1] It is highly fluorescent, the 2'-deoxy version exhibiting an emission maximum at 473 nm when incorporated into a 19-mer oligodeoxyribonucleotide, where it base-pairs normally with dG. Pyrrolo-C has proven to be useful for monitoring RNA secondary structure formation, where its fluorescence is reversibly quenched upon base-pairing.[2] PC has been used to follow the kinetics of formation and dissociation of an RNA/DNA complex and has been used to monitor the thermal denaturation of the central segment of an RNA duplex.[2] Most recently, PC has been incorporated into native and minimal hammerhead ribozymes at cleavage site position C17, where it was found to be capable of efficient photocrosslinking to G12, resulting in catalytically active RNA that was useful in structural studies.[3] Our new 2'-*O*-Me-Pyrrolo-C CEP (BA 0356) should behave in oligonucleotide synthesis in a manner similar to the other modified 2'-*O*-Me nucleoside CEPs.



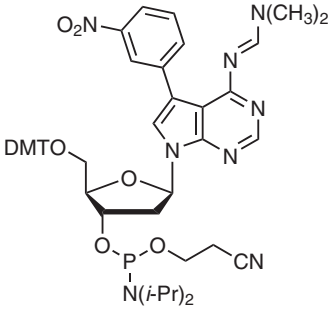
BA 0356

We also offer the 2'-OTBS phosphoramidite of pyrrolo-C (BA 0245) as well as the 2'-deoxyribo version, pyrrolo-dC CEP (BA 0170). An analogous product (R = O-TOM) is also available from Glen Research, our development partner for Pyrrolo-C phosphoramidites.

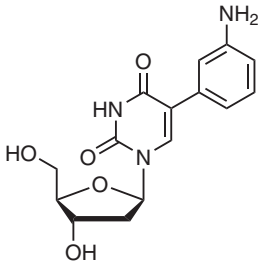
We offer the two nucleosides (PYA 11090 and PYA 11092) as well as the simple fluorescent pyrrolocytosine heterocycle (i.e., pyrrolo-C aglycone (HC 9060). [4]



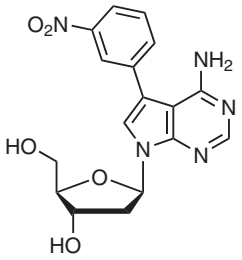
BA 0342



BA 0355



PY 7717

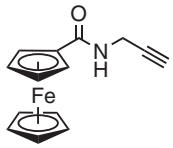


PRA 10032

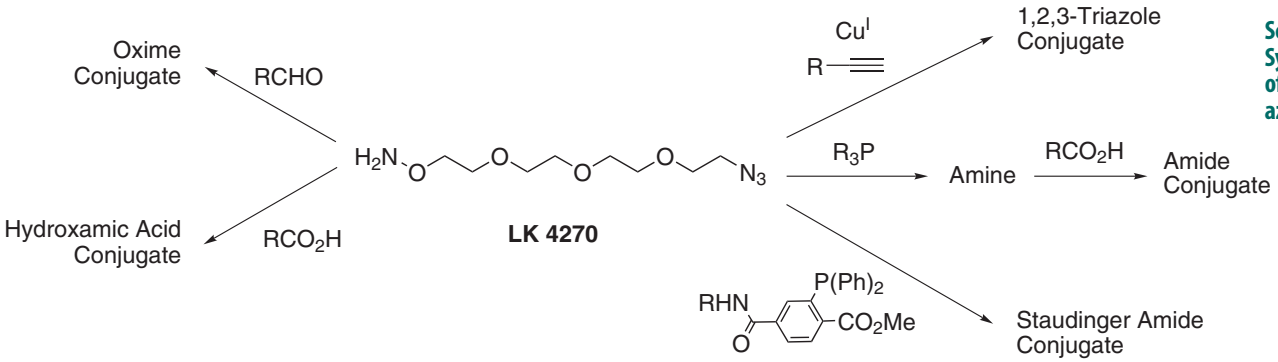
Electrochemical Detection:

Electrochemical detection is a less expensive alternative to common optical methods in DNA biosensors and chips. Hocek and coworkers[5] have shown that when aminophenyl and nitrophenyl substituted 2'-deoxyribonucleosides are incorporated into oligonucleotides, they exhibit excellent electrochemical label properties. Both types of markers in the same oligonucleotide can be easily detected and differentiated since the aminophenyl tag is irreversibly oxidized, and the nitophenyl tag is irreversibly reduced. Our diverse phosphoramidite collection now includes 5-(3-Aminophenyl)-2'-dU CEP (BA 0342) and **5**-(3-Nitrophenyl)-2'-dA CEP (BA 0355). We also offer the corresponding nucleosides 5-(3-Aminophenyl)-2'-deoxyuridine (PY 7717) and 5-(3-Nitrophenyl)-2'-deoxyadenosine (PRA 10032).

Redox labeled nucleotides, amino acids and peptides are essential tools in the development of biosensors. In particular, ferrocene conjugates of nucleotides aid in electrochemical detection of oligonucleotides on electrode surfaces.[6a] Ferrocene is a stable moiety that has highly desirable reversible and tunable electrochemical and spectroscopic properties. Ferrocenoyl propargylamide (FPA, FC 8100) is a simple molecule that can be used for on-column derivatization of oligonucleotides.[6b]



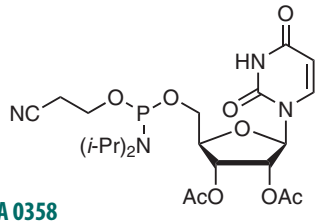
FC 8100



Scheme II: Synthetic Utility of Aminooxy-TEG-azide (LK 4270)

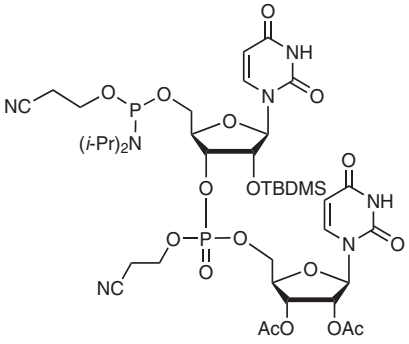
Uridine Tagging:

2',3'-Di-*O*-acetyl-U-5'-CEP (BA 0358) has been reported in the literature as a useful tool for the preparation of uridylated amino acid. Such modified amino acids have then been incorporated into nucleopeptides such as the uridylated viral genome-linked peptide found in the terminal protein of then poliovirus.[7] In addition, this phosphoramidite can be used in the formation of nucleoside



BA 0358

diphosphate sugars which are valuable tools in glycobiology.[8] Nucleopeptides from poliovirus and coxsackie virus contain



BX 00004

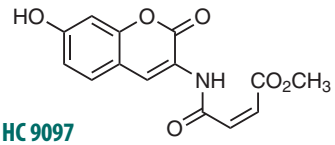
tyrosine residues modified with two uridine nucleotide units (Org. Biomol. Chem., 2006, **4**, 3576-86).[9] At Berry & Associates, we have the expertise to make U-p-U-5'-CEP (BX 00004) and other dinucleotide phosphoramidites according to your specific needs. Due to their unknown stability and sparse demand to date, these items are not routinely kept in stock. They require a 6–10 week lead time.

Linkers and Spacers:

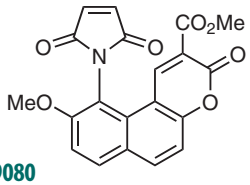
Aminooxy-TEG-azide (LK 4270) is a dual purpose linker that has numerous potential applications. Acylation of the oxyamine end affords a hydroxamic acid that bears a TEG-linked azide group. Hydroxamic acids have long been known to be useful as carboxylic acid mimics. Thus, an advantage of LK 4270 is that it allows the introduction of the TEG-azide functionality while retaining comparable acidity to the original carboxylic acid. Alternatively, it is possible for the oxyamine end to condense with an aldehyde, affording an oxime that bears a TEG-linked azide group. Subesquently, the azide group is available for use in a variety of well known ligation paradigms.[9]

Thiol Sensors:

Thiols are significant factors in many biological systems, and variations in cellular thiol levels have been associated with a number of disease states including psoriasis, cancer, and AIDS. For this reason, thiol probes that are fast, selective and sensitive are critical in the development of assays that hinge on thiol detection. Xi and co-workers[13] have reported on the properties and utility of the coumarin-maleimide as a thiol probe. They have shown that this probe is water soluble, has efficient PET quenching, and offers



HC 9097



HC 9080

significant turn-on fluorescence in the presence of GSH or cys. They show the Glutathione reductase probe to be highly selective for thiols over competing amino acids, and demonstrated the high sensitivity for low levels of thiols. We now offer Glutathion reductase probe (HC 9097) in addition to MMBC (HC 9080) to help met your thiol detection needs.

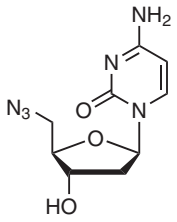
Continued on back cover

New Tools for Research

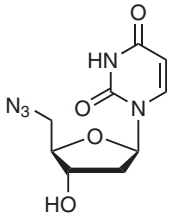
Continued from page 7

5'-Azide Building Blocks:

Chemically modified nucleobases have a variety of uses in biological research. Such nucleobases have been utilized in nucleic acid sequencing, analysis of protein-nucleic acid intermediates, and genotype determination. In addition, aminoglycoside-nucleobase conjugates are of current interest in HIV research where the modified nucleobase is coupled to a small molecule to leverage the natural and selective H-bonding to enhance interaction with the targeted RNA.[14] The 5'-azides 5'-Azido-2',5'-dideoxyuridine (PY 7276) and 5'-Azido-2',5'-dideoxycytidine (PY 7219) are valuable building blocks for the prepareation of modified nucleobase conjugates as well as 5'-amino triphosphate nucleotides.[15]



PY 7219



PY 7276

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Ordering Information—New Tools for Research

Catalog Number	Name	Size	Price
PY 7276	5'-Azido-2',5'-dideoxyuridine		
		25 mg	$98.00
		100 mg	$345.00
PY 7219	5'-Azido-2',5'-dideoxycytidine		
		25 mg	$98.00
		100 mg	$345.00
BA 0358	2',3'-Di-O-acetyl-U-5'-CEP		
		100 µmol	$155.00
		0.25 g	$465.00
BA 0342	5-(3-Aminophenyl)-2'-dU CEP		
		100 µmol	$375.00
BA 0355	5-(3-Nitrophenyl)-2'-dA CEP		
		100 µmol	$315.00
		0.25 g	$595.00
BA 0356	2'-O-Methyl-pyrrolo-C CEP		
		100 µmol	$395.00
		0.25 g	$875.00
PY 7717	5-(3-Aminophenyl)-2'-deoxyuridine		
		25 mg	$235.00
		100 mg	$795.00
PRA 10032	5-(3-Nitrophenyl)-2'-deoxyadenosine		
		10 mg	$119.00
		50 mg	$495.00
LK 4270	Aminooxy-TEG-azide		
		100 mg	$325.00
BA 0354	Fmoc-5'-amino-modifier-5 CEP		
	100 µmol	95.00	
		0.25 g	$190.00
HC 9097	7-O-Amino-4-methylumbelliferone		
		10 mg	$125.00
		50 mg	$365.00
HC 9080	MMBC		
		10 mg	$249.00
FC 8100	Ferrocenoyl propargylamide		
		10 mg	$98.00
		100 mg	$175.00
		250 mg	$275.00

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