Chemistry
from Berry & Associates
TO ADVANCE THE LIFE SCIENCES
Issue 9 | May 2013

In This Issue

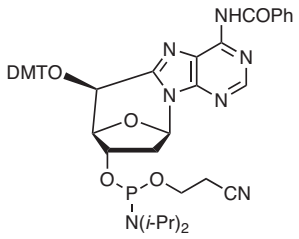
Founded in 1989 with roots in the nucleoside field, Berry & Associates soon moved into the chemistry of nucleic acids, resulting in a current portfolio of nearly 200 phosphoramidites and solid phase-linked monomers for oligonucleotide synthesis as well as hundreds of nucleosides, carbohydrates, spacers, 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.

8,5'-Cyclodeoxypurines

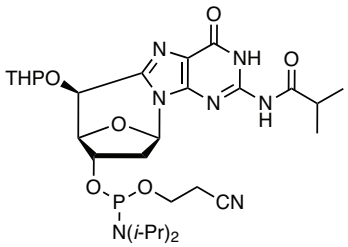
Tools for Investigating DNA Damage and Repair

The naturally occurring nucleosides, cyclo-dG and cyclo-dA are bridged cyclonucleosides that result from oxidative free radical damage to DNA. This specialized "tandem lesion" encompasses damage to both the sugar and the base moiety of the same purine nucleoside. This oxidative damage plays a significant role in mutagenesis, carcinogenesis and aging.[1] Both the bulky 8,5'-Cyclodeoxyadenosine (cyclo-dA) and the 8,5'-Cyclodeoxyguanosine (cyclo-dG) lesions have been shown to be present in human cells.[1,2] Cyclo-dA and Cyclo-dG are both strong blockers of gene expression in CHO and human cells. These lesions can be repaired *via* nucleoside excision repair mechanisms, but not by base excision repair mechanisms.[3] Structural investigations have shown that cyclo-dG stacks in the DNA duplex, retains Watson-Crick bonding with dC, but perturbs the helix structure near the lesion.[4]

Both cyclo-dA and cyclo-dG are valuable tools for investigations into DNA damage and repair. We offer the traditionally DMT protected cyclo-dA CEP (BA 0329), but the difficulties encountered when developing a practical synthesis for a cyclo-dG phosphoramidite led us away from the conventional 5'-DMT protection. A better alternative, which dramatically increases the overall yield of the synthesis, is to utilize a 5'-tetrahydropyranyl (THP) protecting group. We have found that BA 0382 couples



BA 0329 (5'-S)-8,5'-Cyclodeoxyadenosine CEP



BA 0382 (5'-S)-8,5'-Cyclodeoxyguanosine (THP) CEP

efficiently in automated oligonucleotide synthesis. Furthermore, the THP protection is cleanly removed by treatment with 3% TCA in CH_2Cl_2. This is readily accomplished by adding two or three 15-minute deprotect steps at the end of the cyclo-dG cycle. Note that THP removal will not give the characteristic color associated with DMT removal.

Continued on page 3

Copper-Free Click with BCN—new lower prices!

During the last year, many reports have appeared in the literature describing the utility of the bicyclo[6.1.0]nonyne (BCN) scaffold for copper free click reactions in a variety of biologically significant applications (Figure 1). Quantum dots have been modified *via* copper free click ligation to a BCN tethered protein;[1] the efficacy of next-generation viral vectors was reportedly increased *via* strain promoted azide alkyne cyclization with a BCN moiety;[2] and increased signal to noise ratios in ELISA assays can be achieved using BCN and copper free click.[3] In addition, selective enrichment of glycoproteins *via* copper free click reaction with BCN modified agarose beads has been reported,[4] and BCN has been genetically encoded into proteins for efficient fluorescent tagging *via* the click reaction.[5]

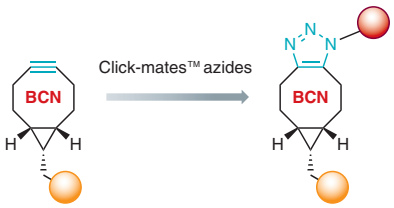
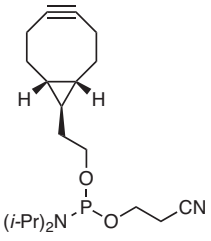


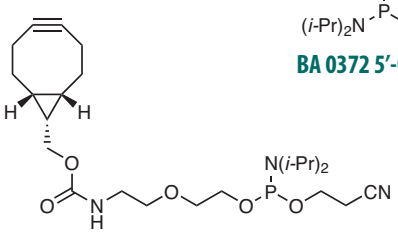
Figure 1. BCNs for copper-free clicking.

Through our partnership with SynAffix,[6] we offer several BCN alkyne tools for catalyst free click conjugation, and we have recently reduced our prices for the BCN phosphoramidites. The Click-easy™ BCNs (BA 0372, BA 0373, LK 4320, and LK 4330) can be used to efficiently prepare oligonucleotides and other biomolecules labeled with the BCN motif. In our hands, 5'-BCN-oligonucleotides react cleanly with a variety of azide reagents. Even PQQ-TEG azide (FC 8170), which contains a highly reactive quinone functionality, is smoothly ligated to a BCN-oligo. This substituted cyclooctyne strikes the best balance between reactivity and lipophilicity of all the substituted cyclooctynes reported to date.[7] Since a Cu(I) catalyst is not required for click reactions with azides, BCN modification provides "no-muss, no fuss" access to many oligo conjugation products.

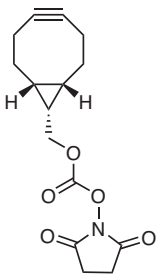
New Lower Prices on BA 0372 and BA 0373!



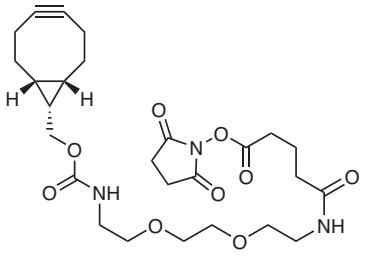
BA 0372 5'-Click-easy™ BCN CEP



BA 0373 5'-Click-easy™ BCN CEP II



LK 4320 Click-easy™ BCN N-hydroxysuccinimide ester I



LK 4330 Click-easy™ BCN N-hydroxysuccinimide ester II

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6. For more information regarding SynAffix, see www.synaffix.com.

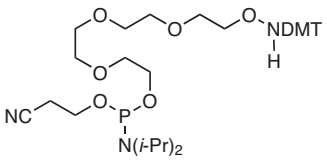
7. Debets, M.F.; van Berkel, S.S.; Dommerholt, J.; Dirks, A.J.; Rutjesvan, F.P.J.T.; van Delft, F.L. *Accounts of Chem. Res.* **2011**, *44*, 805-815.

Ordering Information—Copper-Free Clicking with BCN

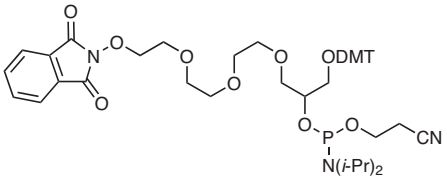
Catalog Number	Name	Size	Price
BA 0372	5'-Click-easy™ BCN CEP I		
		100 µmol	$370.00
		250 µmol	$875.00
BA 0373	5'-Click-easy™ BCN CEP II		
		100 µmol	$299.00
		250 µmol	$710.00
LK 4320	Click-easy™ BCN N-hydroxysuccinimide ester I		
		10 mg	$80
		50 mg	$185.00
		100 mg	$345.00
LK 4330	Click-easy™ BCN N-hydroxysuccinimide ester II		
		10 mg	$150.00
		25 mg	$220.00

The Aminooxy Toolbox

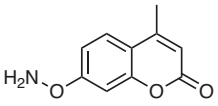
The use of oxime formation in ligation reactions of oligonucleotides has been widely established in the literature for over a decade.[1] 5'-Aminooxy-modifiers have been used in oxime ligation for peptide-oligonucleotide conjugates,[2] for attachment of nucleosides to solid supports,[3] and for head to tail cyclization of oligonucleotides.[4] We offer a variety of aminooxy moieties to facilitate your research. For example, the use of BA 0350 at the 5'-terminus produces an oxyamine upon cleavage of the N-DMT protecting group. Alternatively, Aminooxy-modifier CEP (BA 0374) allows for the internal incorporation of the aminooxy moiety upon phthalimide removal. LK 4270 facilitates orthogonal conjugation *via* oxime-conjugation at one end and triazole-click at the other. Coumarin derivatives such as 7-O-Amino-4- methylumbelliferone (HC 9070) have been shown to be useful in a simple spectroscopic assay for aldehydes in biologically relevant media.[5]



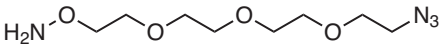
BA 0350 5'-Aminooxy-modifier-11 CEP



BA 0374 Aminooxy-modifier CEP



HC 9070 7-O-Amino-4-methylumbelliferone



LK 4270 Aminooxy-TEG azide

Ordering Information—Aminooxy Toolbox

Catalog Number	Name	Size	Price
BBA 0350	5'-Aminooxy-modifier-11 CEP		
		100 µmol	$235.00
		0.25 g	$785.00
		1 g	$2500.00
BA 0374	Aminooxy-modifier CEP		
		100 µmol	$260.00
		0.25 g	$865.00
LK 4270	Aminooxy-TEG azide		
		100 mg	$325.00
		1 g	$2600.00
HC 9070	7-O-Amino-4-methylumbelliferone		
		5 mg	$95.00
		25 mg	$245.00

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8,5'-Cyclodeoxypurines

Continued from page 1

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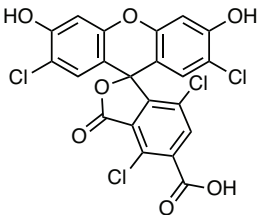
Ordering Information—8,5'-Cyclodeoxypurines

Catalog Number	Name	Size	Price
BA 0382	BA 0379 (5'-S)-8,5'-Cyclodeoxyguanosine (THP) CEP		
		50 umol	$1250.00
BA 0329	BA 0329 (5'-S)-8,5'-Cyclodeoxyadenosine CEP		
		50 umol	$557.50
		0.25 g	$2,495.00

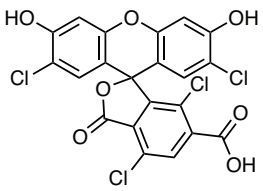
Tetrachlorofluorescein: New Tools and New Applications

Tetrachlorofluorescein (TET) has an absorbance maximum of 519 nm and an emission maximum of 539 nm.[1] As with fluorescein, TET can be used to label DNA oligos for a variety of investigative applications. We have recently introduced several new TET products into our lineup. These products range from the basic fluorophores such as 5-Carboxy TET (FF 6150) and 6-Carboxy TET (FF 6160) to exotic probes such as TET TInsP$_5$ (PH 1200). Our TET lineup also contains chemically reactive products, such as 5-Carboxy-TET dipivalate NHS ester (FF 6155) for incorporation *via* nucleophilic displacement, 6-Carboxy-TET-TEG azide (FF 6130) for click chemistry applications and 5'-Tetrachlorofluorescein CEP (BA 0377) for automated incorporation into oligonucleotides.

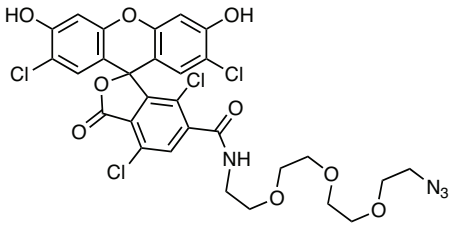
The environmental utility of TET TInsP$_5$ (PH 1200) was recently highlighted in the literature.[2,3] This fluoroescent substrate analog of phytic acid was shown to allow direct measurement of the phosphate ester bond-cleavage reaction for determiniation of phytase activity. The TET TInsP$_5$ molecular probe should prove useful for assessing the nutritive status of P in surface water ecosystems. The advent of a fluorescent substrate analog of phytic acid affords environmental scientists the means to unambiguously quantify an extremely small amount of phytase-generated product(s), enabling the measurement of phytase activity, over a reasonably short time duration, in an environmental sample containing low concentrations of enzyme. We also continue to offer TInsP$_5$ (PH 1000), an earlier prototype of a chromophoric substrate analog of phytic acid that also allows the measurement of phytase activity.[3,4]



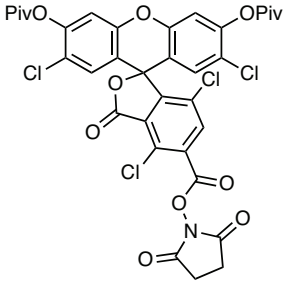
FF 6150 5-Carboxy-TET



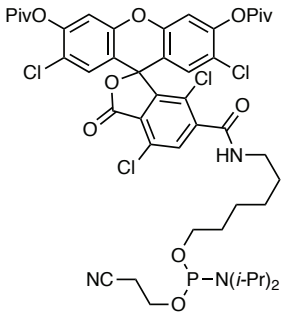
FF 6160 6-Carboxy-TET



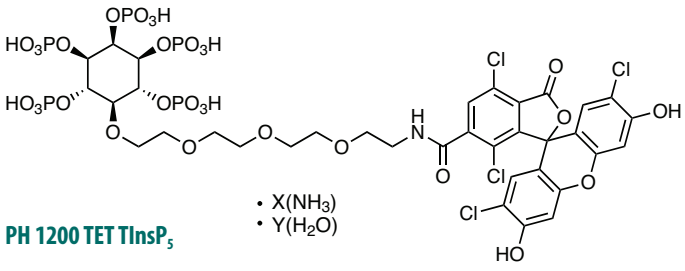
FF 6130 6-Carboxy-TET-TEG azide



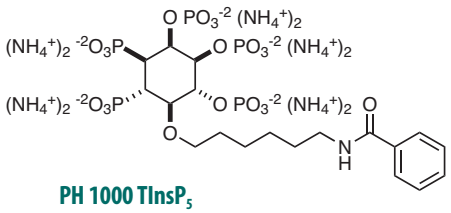
FF 6155 5-Carboxy-TET dipivalate N-hydroxysuccinimide ester



BA 0377 5'-Tetrachlorofluorescein CEP



PH 1200 TET TInsP$_5$



PH 1000 TInsP$_5$

References

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See ordering information on page 5

Azidocoumarins

These new 3'-Azidocoumarins are profluorophores that become highly fluorescent following [3+2] cycloaddition with alkynes (Figure 2).[1] As a result, this class of compounds provides a variety of useful tools for both copper catalyzed and copper free click bioconjugation. The profluorescent nature of the 3-azidocoumarins provides a highly sensitive signal upon ligation *via* click chemistry without adding background fluorescence from unreacted excess reagent. We now offer FC 8200, FC 8205 and FC 8210,[2] which have reactive linkers that are widely applicable to profluorescent tagging of biomolecules *via* amide bond chemistry. We are continuing to develop new azidocoumarin products, and are happy to entertain linker design suggestions from interested customers.

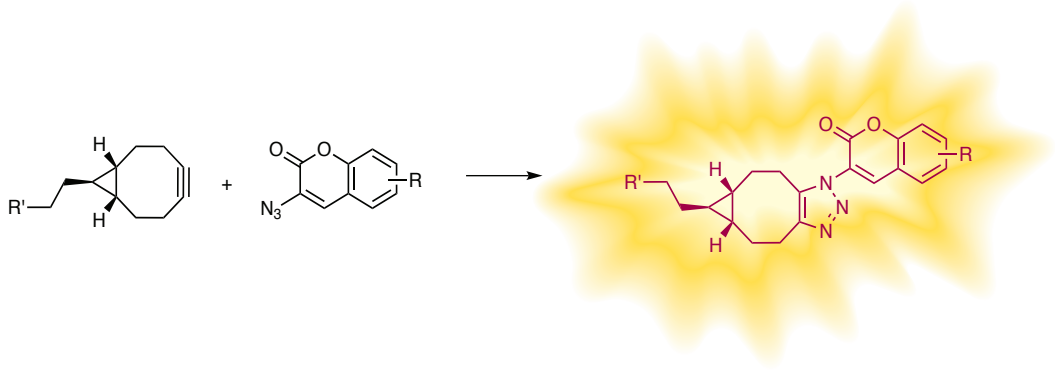
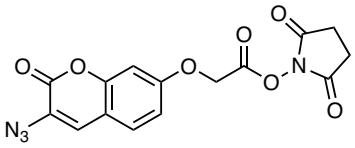
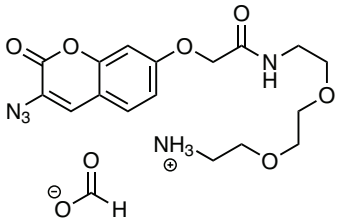


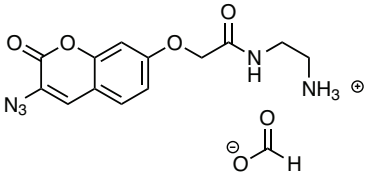
Figure 2. Azidocoumarin fluorescence after copper-free click reaction..



FC 8200 Azidocoumarin N-hydroxysuccinimide ester



FC 8205 Azidocoumarin-spacer-12-amine formate



FC 8210 Azidocoumarin-spacer-6-amine formate

References

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2. These iON Linkers™ are manufactured and sold under a license agreement with A&Q Nano (www.aqnano.com).

Ordering Information—Azidocoumarins

Catalog Number	Name	Size	Price
FC 8200	Azidocoumarin N-hydroxysuccinimide ester		
		10 mg	$100
		50 mg	$405
FC8205	Azidocoumarin-spacer-12-amine formate		
		10 mg	$100
		50 mg	$405
FC 8210	Azidocoumarin-spacer-6-amine formate		
		10 mg	$100
		50 mg	$405

Tetrachlorofluorescein

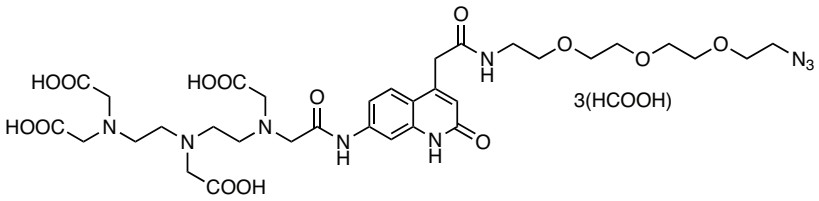
Continued from page 4

Ordering Information—Tetrachlorofluorescein

Catalog Number	Name	Size	Price
BA 0377	5′-Tetrachlorofluorescein CEP		
		100 µmol	$345.00
		0.25 g	$860.00
FF 6130	6-Carboxy-TET-TEG azide		
		1 mg	$295.00
FF 6150	5-Carboxy-TET	50 mg	$200.00
		250 mg	$850.00
FF 6155	5-Carboxy-TET dipivalate N-hydroxysuccinimide ester		
		50 mg	$450.00
		250 mg	$1950.00

Catalog Number	Name	Size	Price
FF 6160	6-Carboxy-TET	50 mg	$200.00
		250 mg	$850.00
PH 1000	$TInsP_5$		
		10 mg	$195.00
		25 mg	$345.00
		100 mg	$875.00
PH 1200	TET $TInsP_5$		
		1 mg	$495.00

DTPA-Quinolone-TEG azide



FC 8190 DTPA-Quinolone-TEG azide

A number of luminescent probes based on the chelation of lanthanide ions with organic chromophores have been reported.[1-6] FC 8190 is our first product for this approach to luminescence.[7] We now offer FC 8190 as a novel tool which allows the user to add the lanthanide of choice to create a probe that suits their needs.[7]

The design of FC 8190 incorporates three elements in a single organic molecule that sequesters lanthanide ions (Figure 3): (1) a diethylenetriaminepentaacetic acid (DTPA) fragment, which serves to chelate Lanthanides such as Eu^{+3} or Tb^{+3}; (2) a quinolone chromophore, which serves as an antenna for ultraviolet light; and (3) a hydrophilic tether with a distal azide, which provides a site for conjugation to biomolecules *via* the click reaction.

The luminescence exhibited by such organic lanthanide chelates makes them useful probes for investigating biological processes. A model for the photochemical function of FC 8190, when coordinated to Eu^{+3} is shown in Figure 3. The quinolone chromophore absorbs UV light and then transmits energy to the chelated europium ion, which in turn emits discrete bands of visible light. The large Stokes shift, long luminescence lifetime and narrow emission bands that are observed provide the capability for highly sensitive detection.

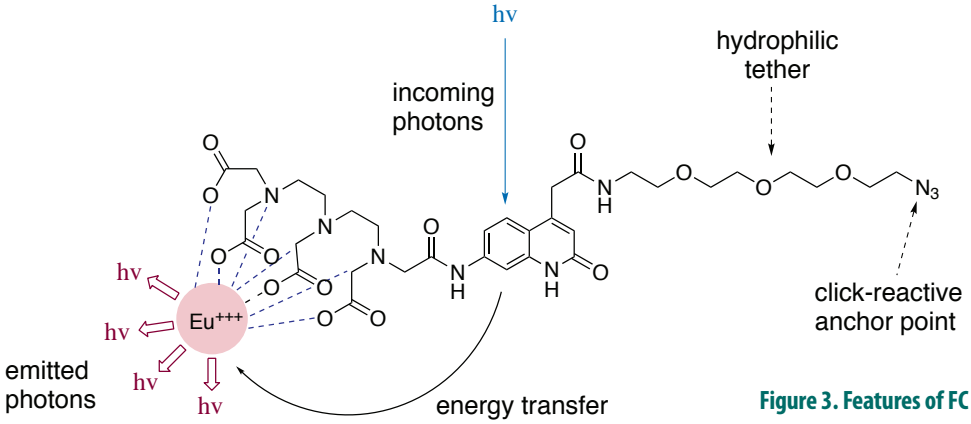


Figure 3. Features of FC 8190.

Ordering Information—DTPA-Quinolone-TEG-Azide

Catalog Number	Name	Size	Price
FC 8190	DTPA-Quinolone-TEG azide		
		1 mg	$120.00
		5 mg	$540.00
		10 mg	$975.00

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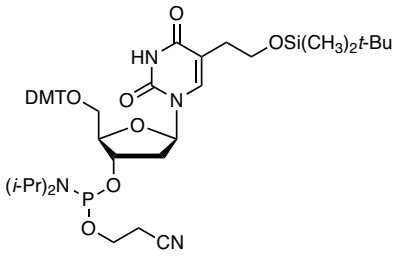
7. Manufactured and sold under a license agreement with the University of Medicine and Dentistry of New Jersey.

Branching Out Beyond the Single Strand

Chain assembly on solid support traditionally provides linear oligonucleotides. However, branched oligonucleotides have shown promise in areas such as gene conversion,[1] and nanotechnology.[2] Several tools are known for use in the preparation of branched oligos, including the commercially available doubler and trebler CEPs from Glen Research,[3] and the 2',3'-O-bisphosphormidites reported by Damha[4].

We are pleased to now offer a deoxyuridine branching phosphoramidite 5-(2-Hydroxyethyl) dU CEP (BA 0378) for use in the solid supported synthesis of branched oligonucleotides. An important feature of this new product is the *t*-butyldimethylsilyl (TBS) group, which protects the hydroxyethyl branching point. Unlike the TBS-protected secondary alcohols that are common in RNA synthesis, the TBS-protected primary alcohol of BA 0378 is labile to the solution of 3% trichloroacetic acid (TCA) in dichloromethane that is normally used for DMT deprotection in oligo synthesis. The TBS group on BA 0378 does, however, remain intact when 2.5% dichloroacetic acid (DCA) in dichloromethane is employed as the DMT deprotect reagent. Thus, the choice of aggressive deprotection with TCA or mild deprotection with DCA affords significant synthetic flexibility. If two identical branches are desired, the 5'-DMT group and the TBS group may both be removed with TCA, followed by concurrent synthesis of both branches (**1** → **2,** Figure 4.). Alternatively, if different branches are desired, it is possible to selectively remove the 5'-DMT with DCA and synthesize the first branch (**1** → **3**), then subsequently remove the TBS with TCA and synthesize the second branch (**3** → **4**).

Another use for BA 0378 is as a "hydroxyl modifier." The liberated hydroxyl group serves as a convenient attachment point for phosphoramidite coupling, which may be accomplished on the solid support. This allows the incorporation of a variety of reagents that are typically used to install 5'-labels with concomitant chain termination. For example, thiol modifiers such as BA 0332 and BA 0037 may be connected in the last automated synthesis cycle yet the disulfide resides at an internal sequence position of the oligo (**3** → **5**).



BA 0378 5-(2-Hydroxyethyl)dU CEP

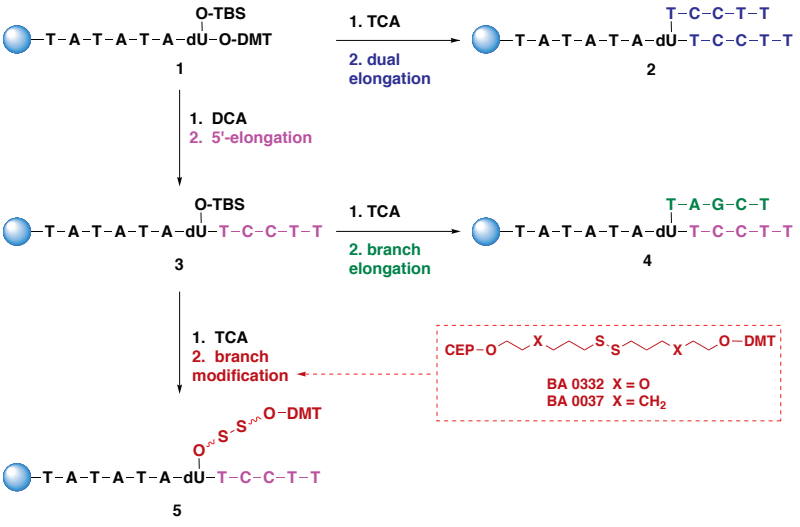


Figure 4. Branching Flexibility with BA 0378.

References

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Ordering Information—5-(2-Hydroxyethyl)-dU CEP

Catalog Number	Name	Size	Price
BA 0378	5-(2-Hydroxyethyl)-dU CEP		
		100 µmol	$315.00
		0.25 g	$685.00
BA 0037	Thiol-modifier-C6-S-S CEP		
		20 g minimum	Inquire
BA 0332	Thiol-modifier-oxa-C6-S-S CEP		
		100 uMol	$145.00
		0.25 g	$360.00

Photolabile Strand Breaker Revisited

The introduction of nicks into DNA using photolabile nucleotides or nucleotide surrogates (so-called "caged strand-breaks") has been addressed using several strategies.[1] Nucleosidic caged strand-breaking monomers have been reported by the groups of Giese, Marx, Sheppard, Kotera, and Pirrung; see the review by Mayer and Heckel.[1a] Several non-nucleosidic caged strand-breaking monomers have also been reported.[2-7] Taylor, *et al.*[3-6] and Raynor, *et al.*[7] have reported several non-nucleosidic monomers that allow cleavage of oligonucleotides to liberate both daughter strands. Zhang and Taylor[6] developed what we call the Caged Strand Breaker CEP (BA 0315), which produces the phosphorylated daughter strands **1** and **2** and the by-product **3** upon irradiation (Figure 5). This monomer is the most advanced iteration of theUrdea/Taylor/Raynor strategy, and allows complete removal of the photolabile protecting group from both daughter strands, the phosphorylation of both daughter strands, and the avoidance of a second β-elimination step. Greenberg *et al*. have recently illustrated the utility of this Caged Strand Breaker CEP in his examination of the effect of strand breaks on the reactivity of apyrimidinic/apurinic sites in nucleosome core particles.[7]

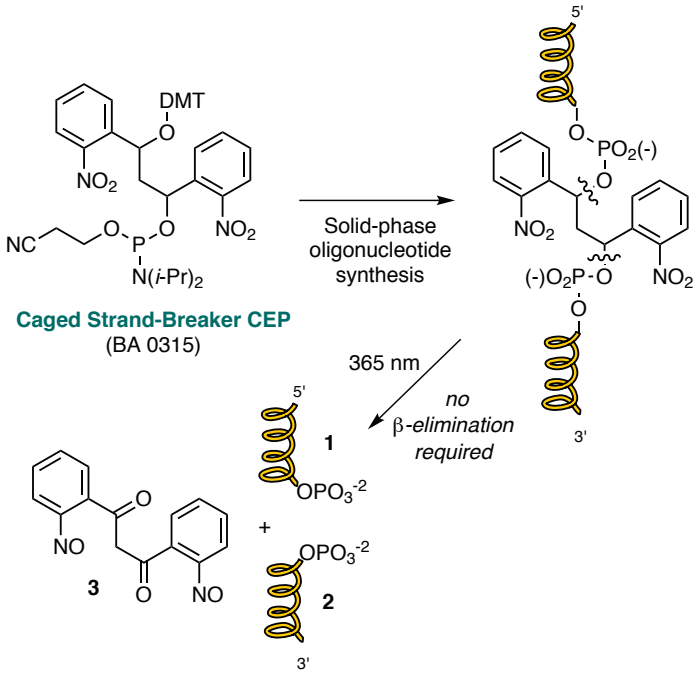

Figure 5. Illumination of an oligonucleotide bearing a Caged Strand-Breaker unit at 365 nm leads to strand cleavage, leaving phosphates on both daughter strands (6 and 7) with no ß-elimination required.[6]

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Ordering Information—Caged Strand Breaker CEP

Catalog Number	Name	Size	Price
BA 0315	Caged strand breaker CEP		
		100 umol	$340.00
		0.25 g	$795.00

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