Chemistry
from Berry & Associates
TO ADVANCE THE LIFE SCIENCES
Issue 5 | November 2009

Staudinger Ligation

Effective Coupling of Amines and Azides

Bertozzi and co-workers have reported an ingenious extension of the Staudinger azide reduction that provides a simple way to covalently ligate an amine-containing component and an azide-containing component under extremely mild conditions.[1,2] The Staudinger ligation has been used for fluorescent labeling of DNA by site-specific ligation of a 5'-azido oligonucleotide with a preformed fluorescein-ligator conjugate.[3] The utility of this technology in living systems demonstrates the potential for re-engineering a variety of cell and tissue surfaces.[4] Additional applications include site selective post-translational modification of proteins with a biotin-phosphine ligator conjugate,[5] ligation of human recombinant thrombomodulin to liver islets through bifunctional poly(ethylene glycol) (PEG) linkers,[6] and liposome surface functionalization.[7]

Shown in Figure 1 (next page) are illustrative examples of a Staudinger ligation between an amine-bearing biological macromolecule and azido-TEG derivatives of biotin, dansyl, and fluorescein. The amine–containing component is first modified by acylating with the appropriately substituted triaryl phosphine. Subsequent treatment with the azide component results in a Staudinger reduction of the azide, followed by intramolecular trapping of the intermediate phosphorimidate by the *ortho*-methyl ester on the phospine, thereby ligating the label to the macromolecule. The Staudinger ligation proceeds at room temperature and physiological pH and a catalyst is not required. This ligation chemistry tolerates a wide variety of functional groups in the two components being linked.

We now offer AmAz Coupler (LK 4260) as a useful reagent for the installation of the phosphine moiety that is required for Staudinger ligations. AmAz Coupler II (LK 4265), which adds a 14-atom spacer to the phosphine moiety, is coming soon. Also now available is a selection of TEG-azide labels, including biotin (BT 1085), dansyl (FD 13005), and fluorescein (FF 6110). For additional details see the next page.

References

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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.

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Staudinger Ligation

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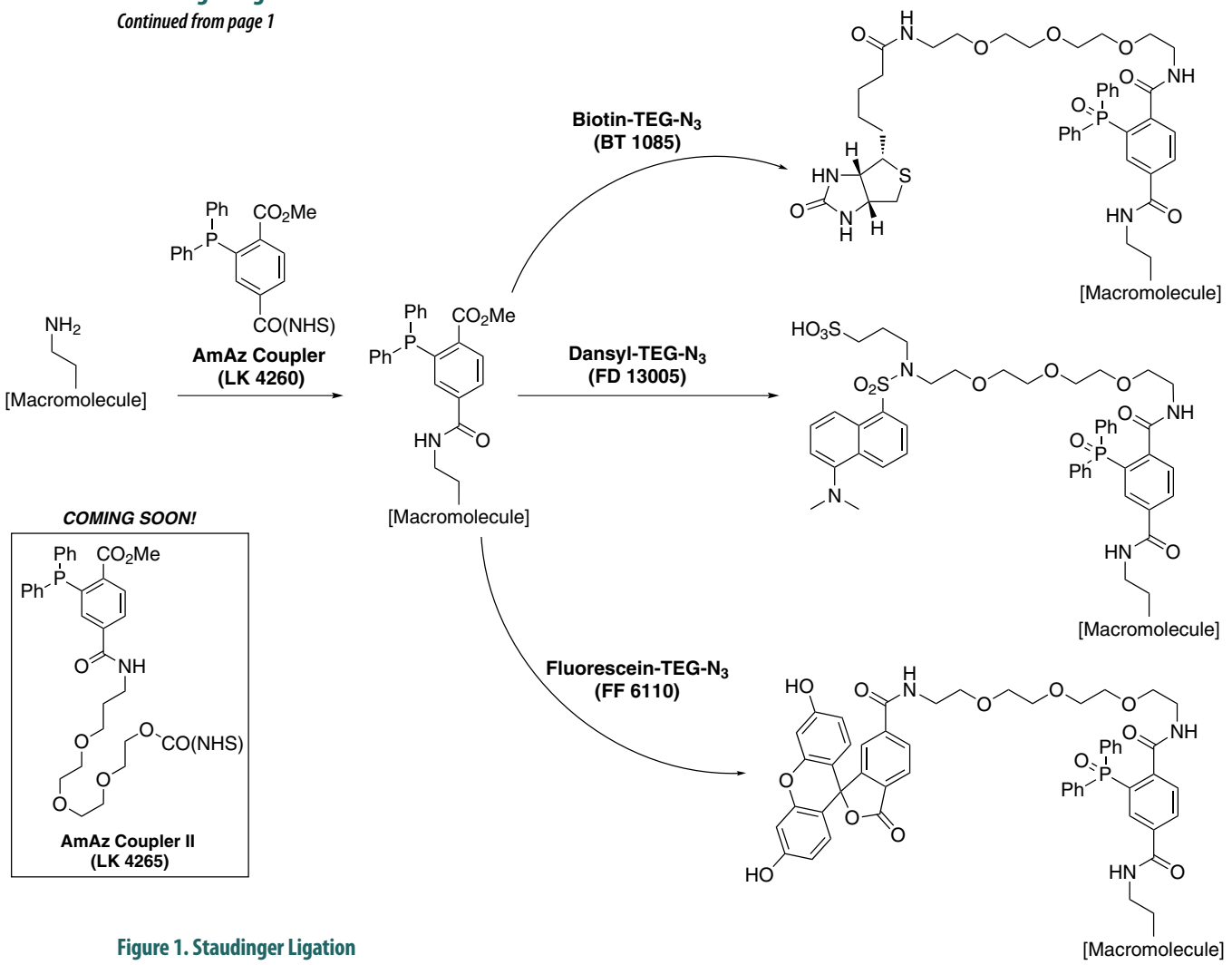


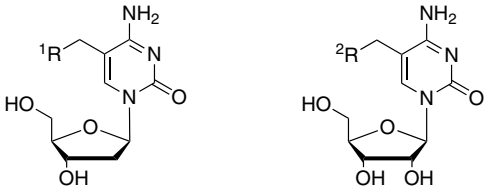
Figure 1. Staudinger Ligation

Staudinger Ligation Products—Ordering Information			
Item	**Catalog No.**	**Size/pack**	**Price (USD)**
AmAz Coupler	LK 4260		
NEW!		50 mg	$125.00
		100 mg	$200.00
		1 g	$1,500.00
Biotin-TEG-N_3	BT 1085		
NEW!		25 mg	$105.00
		100 mg	$365.00
Water Soluble Dansyl-TEG-N_3	FD 13005		
NEW!		25 mg	$95.00
		100 mg	$325.00
6-Carboxy-fluorescein-TEG-N_3	FF 6110		
NEW!		25 mg	$150.00
		100 mg	$495.00

Do you need a different azide? We would be happy to consider custom synthesis requests for your preferred azide coupling partner.

Oxidized Derivatives of 5-Methylcytidine

Emerging science implicates the oxidation of 5-methyl cytosine residues in genomic instability and the development of human diseases.[8-10] In response to customer requests, we now offer a family of four oxidized derivatives of 5-methylcytidine.



PY 7588 ^1R = OH
PY 7587 ^1R = OCH_2CH_2CN
PY 7596 ^2R = OH
PY 7586 ^2R = SO_3Na

Figure 2. Oxidized Derivatives of 5-Methylcytidine

References

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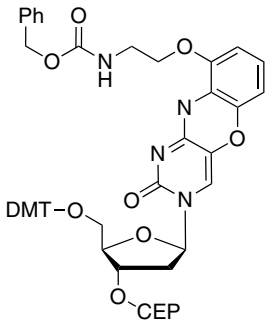
Oxidized Derivatives of 5-Methylcytidine—Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
5-Hydroxymethyl-2′-deoxycytidine	PY 7588		
NEW!		10 mg	$245.00
		50 mg	$875.00
5-[(2-Cyanoethoxy)methyl]-2′-deoxycytidine	PY 7587		
NEW!		10 mg	$210.00
		50 mg	$750.00
5-Hydroxymethylcytidine	PY 7596		
NEW!		50 mg	$450.00
		100 mg	$765.00
Cytidin-5-yl-methanesulfonate sodium salt hydrate	PY 7586		
NEW!		50 mg	$525.00
		100 mg	$879.00
5-Methylcytidine	PY 7637		
		1 g	$95.00
		5 g	$285.00

Do you need a related compound? Please let us know how we can help you with your 5-methylcytidine research efforts.

8-oxoG-clamp

As the major oxidative damage metabolite of DNA, 8-oxo-dG serves as a marker for oxidative stress in cells. Many methods for detection of 8-oxo-dG exist, but until recently, a fluorescent probe for detection in DNA had not been developed. The Sasaki labs have identified a variation of Matteucci's "G-clamp"[11] that is specific for 8-oxo-dG. This fluorescent phenoxazine analog, **8-oxoG-clamp CEP** (Figure 3, BA 0339) appears to be highly specific for pairing with 8-oxo-dG.[12] As illustrated in Figure 4, the hydrogen bonding network imparts the high degree of selectivity of the 8-oxoG-clamp for 8-oxo-dG via the interaction with the Cbz group oxygen.[13] This interaction is attractive in the case of 8-oxo-dG through formation of a hydrogen bond, and repulsive in the case of dG. When incorporated into an oligonucleotide, 8-oxo-dG is selectively detected by fluorescence quenching of 8-oxoG-clamp. The pairing of 8-oxo-dG with 8-oxoG-clamp causes only a slight reduction in duplex stabilization.[13]



BA 0339

Figure 3. 8-oxoG-clamp CEP (BA 0339)

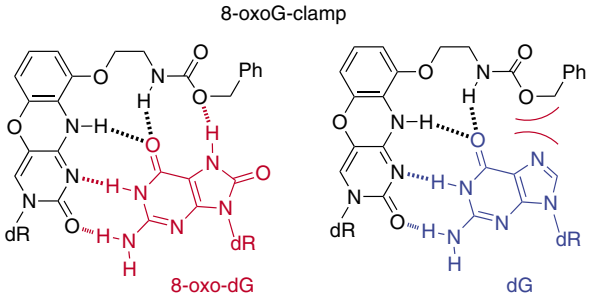


Figure 4. 8-oxoG-clamp H-Bonding

References

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8-oxo-dG-clamp CEP—Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
8-oxo-dG-clamp CEP	BA 0339		
NEW!		100 µmol	$505.00
		250 mg	$958.00

Expanded Genetic Alphabet

The flurry of recent publications by Romesberg and co-workers[14-20] describing the discovery, characterization and optimization of several novel base pairs which expand the genetic alphabet has caught our attention. Interestingly, their efforts to find novel base pairs have focused on hydrophobic interactions as the basis for pair recognition. The reliance on base pair hydrophobicity also disfavors pairing with the four natural nucleobases which, of course, depend upon the distinct H-bonding patterns of more polar heterobases to promote pair recognition. Shown in Figure 5 are **dNaM** and **d5SICS**, a matched pair of unnatural nucleosides which have pair recognition that rivals the A-T and G-C pairing in the natural genetic alphabet.

These unnatural bases have been shown to be well-replicated by DNA polymerases under steady-state conditions. They are also efficiently transcribed by T7 RNA polymerase in either direction. Finally, the Romesburg lab has also recently demonstrated that DNA containing the unnatural base pair is PCR amplified with efficiencies and fidelities approaching that of fully natural DNA, allowing for the sequence-specific amplification of unnatural DNA.

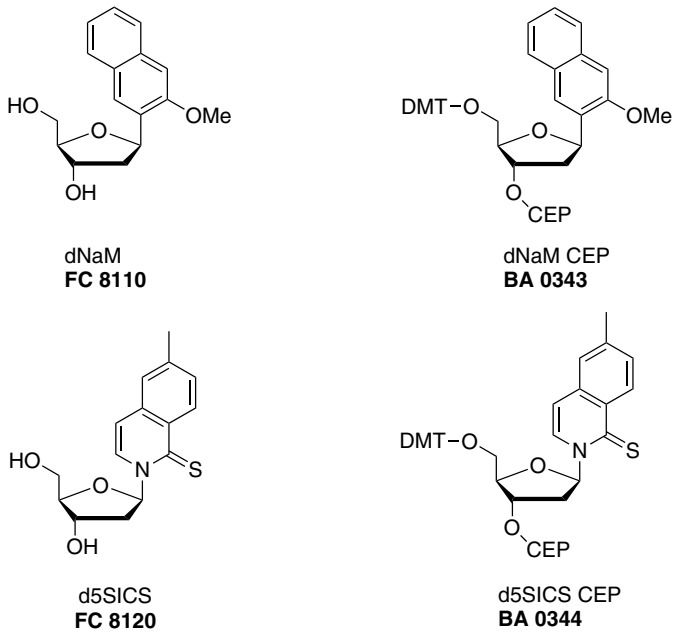

Figure 5. dNaM and d5SICS

As we went to press with this newsletter the chemistry effort for **dNaM** was complete and we were wrapping up **d5SICS**. Look for both of these additions to the genetic alphabet as the free nucleosides and their synthesizer-ready phosphoramidites in the new products section of our web site.

Expanded Genetic Alphabet—Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
dNaM	FC 8110		
NEW!		25 mg	$165.00
		50 mg	$495.00
dNaM CEP	BA 0343		
NEW!		100 µmol	$415.00
		250 mg	$995.00
d5SICS	FC 8120		
NEW!		25 mg	$390.00
		100 mg	$995.00
d5SICS CEP	BA 0344		
COMING SOON!		100 µmol	
		250 mg	

References

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New Quencher and Fluorophore Architectures

As a result of continued research we have been fine tuning the linker and framework architectures of some of our Blackberry Quencher and Fluorescein products in order to improve the cleavage rate and purity profile of tagged oligos. Thus we now provide three new quencher and fluorophore product options to suit specific needs.

3'-BBQ-650 CPG II

3'-BBQ-650 CPG II (BL 2020, Figure 6) is faster cleaving compared to its predecessor, 3'-BBQ-650 CPG (BL 2010). The best strategy for cleavage and deprotection of oligos constructed from BL 2020 is a two step procedure. Following synthesis, the resulting oligonucleotide should first be treated with 10% diethylamine in acetonitrile for 5–10 minutes in order to deprotect the backbone phosphate groups. Second, standard NH_4OH or AMA protocols are employed. The cleavage of poly-T oligos, which lack nucleobase protection, is complete within five minutes using NH_4OH. However, the resulting T_n- BBQ-650 oligos are stable to the more rigorous conditions required for nucleobase deprotection. The two step procedure minimizes side reactions that lead to quencher label loss and thus provides enhanced purity of the tagged oligo compared to a conventional one step protocol.

3'-BBQ-650 CPG III

Taking the architecture one step further, **3'-BBQ-650 CPG III** (BL 2030, Figure 6) utilizes a 1,3,5-triol framework, which is two methylene units longer than the 1,2,3-triol framework that is employed with 3'-BBQ-650 CPG (BL 2010) and 3'-BBQ-650 CPG II (BL 2020). The one-carbon extension between each of the oxygen atoms provides an architecture that allows a one step cleavage with AMA while also minimizing the occurrence of impurities that lack the quencher tag. The oligo purity observed with BL 2030 after a one step AMA cleave/deprotect rivals that seen with BL 2020 after the two step protocol. Like BL 2020, BL 2030 also has a fast cleaving linker but AMA appears to provide markedly superior oligo yield as compared to NH_4OH.

Fluorescein III CEP

The 1,3,5-triol framework has similarly been employed as an architecture for **Fluorescein III CEP** (BA 0334, Figure 7). This latest addition is two methylene units longer than the 1,2,3-triol framework that is employed with 5'-Fluorescein II CEP (BA 0253) and FDMT-5'-Fluorescein CEP (FL 1700). In this case the extended framework serves two functions. First, the one-carbon

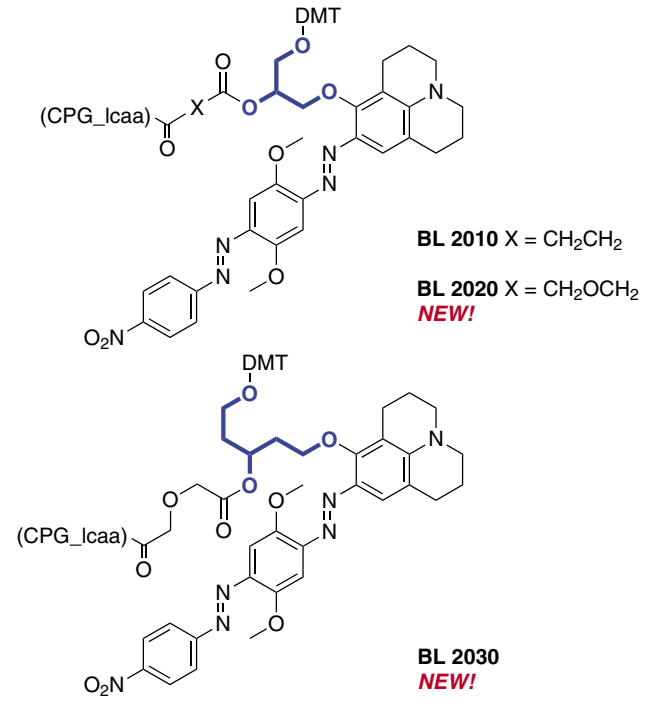

Figure 6. Blackberry Quencher-650 CPGs

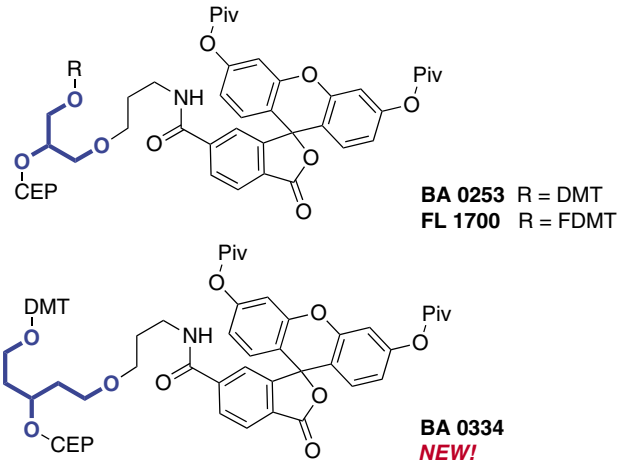

Figure 7. Fluorescein Phosphoramidites

extension between oxygen atoms minimizes the potential for fluorescein label loss and allows one step cleavage with standard NH_4OH or AMA protocols. Second, the distance between phosphate groups maintains the natural three carbon atom internucleotide phosphate distance, thereby diminishing duplex destabilization when the fluorescein label is incorporated at internal sequence locations.[21]

Continued on back cover

By Popular Demand

Since our last newsletter, a variety of individual products have been added to our catalog (Figure 9). These compounds are the result of continued in-house research efforts and customer inquiries. We are always looking to serve our customers' needs and are happy to consider catalog additions based upon popular demand.

N^2-Benzyl-dG CEP

N^2-modified guanosine residues have been observed in DNA following exposure to aldehyde and epoxide carcinogens, and it has been shown that synthetic incorporation of various N^2-alkyl-deoxyguanosines causes preferential misincorporation and strong blockage of replicative polymerases.[22] **N^2-Benzyl-dG CEP** (BA 0337, Figure 8) is one more useful tool for scientists studying the effects of DNA damage and repair. It joins our family of N^2-alykyl-dG CEP products: N^2-Methyl-dG CEP (BA 0249); N^2-Ethyl-dG CEP (BA 0076); N^2-Isobutyl-dG (BA 0250); and N^2-Neopentyl-dG CEP (BA 0200).

2',3'-Dideoxyinosine

2',3'-Dideoxyinosine (PR 3727, Figure 8) combines the utility of a universal base with the chain termination of a dideoxyriboside. It joins our existing series of 2',3'-dideoxy-nucleoside products: 2',3'-ddA (PR 3495); 2',3'-ddG (PR 3505); 2',3'-ddC (PY 7325); 2',3'-ddU (PY 7278); and 3'-dT (PY 7277).

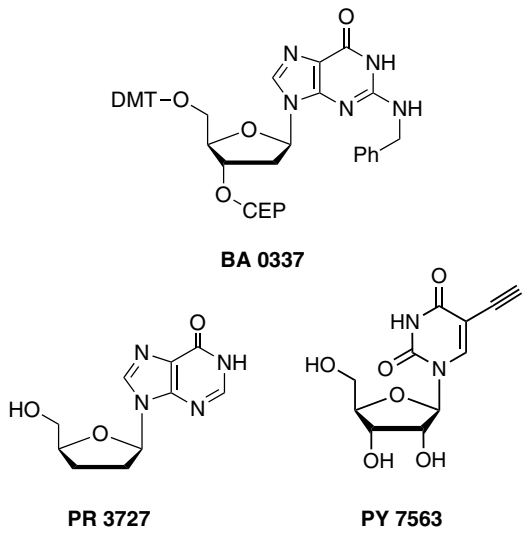

Figure 8. New Nucleoside Products

5-Ethynyluridine

5-Ethynyluridine (PY 7563, Figure 8) now joins 5-Ethynyl-2'-deoxyuridine (PY 7562) in the nucleosides section of our catalog.

Pyrroloquinoline Quinone

Pyrroloquinoline Quinone (HC 9090, Figure 9) is also known in the literature as PQQ and Methoxatin. This functional group-packed heterocycle is a coenzyme for various oxidoreductases, including alcohol dehydrogenase, aldehyde dehydrogenase and D-glucose dehydrogenase.[23]

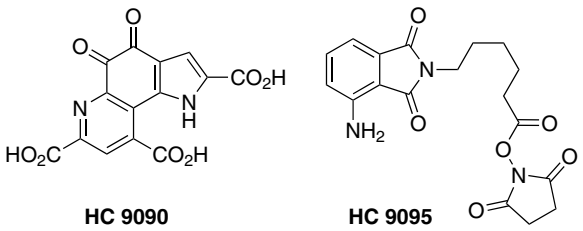

Figure 9. New Heterocycles

Pro-luminol tag

Pro-luminol tag (HC 9095, Figure 9) is a reagent which can be used to install a latent chemiluminescent tag on macromolecules. The tag can be chemically triggered to liberate luminol upon demand. Luminol is one of the most widely used chemiluminescent substrates and luminol based indirect bioassays are linear and efficient. HC 9095 is designed for post synthetic modification of biological macromolecules bearing free amino groups (Figure 1o, next page). Such 3-aminophthalimide tagged macromolecules liberate luminol when treated with hydrazine.[24] The tagged macromolecules are also highly fluorescent, thereby providing an exceptionally elegant utility in dual fluorescence and chemiluminescence studies.

References

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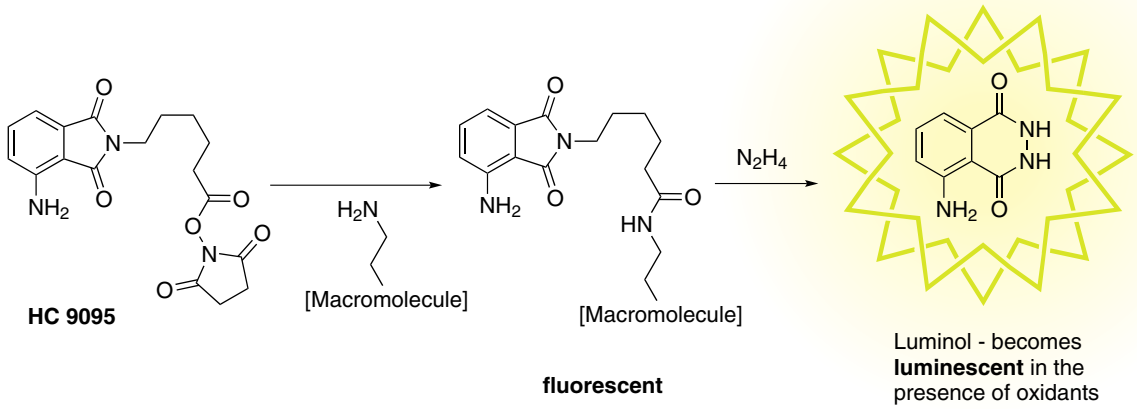

Figure 10. Chemistry of Pro-luminol tag (HC 9095)

By Popular Demand—Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
N^2 Alkyl Deoxyguanosine Phosphoramidites			
N^2-Benzyl-dG CEP	BA 0337		
NEW!		100 µmol	$425.00
		250 mg	$975.00
N^2-Methyl-dG CEP	BA 0249		
		100 µmol	$355.00
		250 mg	$875.00
N^2-Ethyl-dG CEP	BA 0076		
		100 µmol	$355.00
		250 mg	$875.00
N^2-Isobutyl-dG CEP	BA 0250		
		100 µmol	$355.00
		250 mg	$875.00
N^2-Neopentyl-dG CEP	BA 0200		
		100 µmol	$355.00
		250 mg	$875.00
2'3'-Dideoxy-Nucleosides			
2',3'-Dideoxyinosine	PR 3727		
NEW!		100 mg	$75.00
		1 g	$225.00
		5 g	$890.00
2',3'-Dideoxyadenosine	PR 3495		
		25 mg	$55.00
		100 mg	$117.50
		1 g	$595.00
		10 g	$4,250.00
2',3'-Dideoxyguanosine	PR 3505		
		25 mg	$105.00
		100 mg	$240.00
		1 g	$1,495.00

Item	Catalog No.	Size/pack	Price (USD)
2'3'-Dideoxy-Nucleosides *(continued)*			
2',3'-Dideoxyuridine	PY 7278		
		100 mg	$95.00
		500 mg	$395.00
2',3'-Dideoxycytidine	PY 7325		
		25 mg	$55.00
		100 mg	$95.00
		1 g	$495.00
		10 g	$3,750.00
3'-Deoxythymidine	PY 7277		
		25 mg	$55.00
		100 mg	$117.50
		1 g	$495.00
		10 g	$3,000.00
Ethynyluridines			
5-Ethynyluridine	PY 7563		
NEW!		10 mg	$151.00
		100 mg	$1,220.00
5-Ethynyl-2'-deoxyuridine	PY 7562		
		10 mg	$90.00
		100 mg	$690.00
New Heterocyclic Compounds			
Pyrroloquinoline quinone (PQQ)	HC 9090		
NEW!		10 mg	$120.00
		50 mg	$400.00
Pro-luminol tag	HC 9095		
NEW!		10 mg	$75.00
		50 mg	$210.00

New Quencher and Fluorophore Architectures

(continued from page 5)

References

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Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
3′-BBQ-650 CPG III	BL 2030		
NEW!		200 nmol column/pk of 4	$80.00
		1 µmol column/pk of 4	$230.00
		100 mg	$147.00
		1 g	$1,140.00
3′-BBQ-650 CPG II	BL 2020		
NEW!		200 nmol column/pk of 4	$75.00
		1 µmol column/pk of 4	$210.00
		100 mg	$135.00
		1 g	$1,050.00
3′-BBQ-650 CPG	BL 2010		
		200 nmol column/pk of 4	$75.00
		1 µmol column/pk of 4	$210.00
		100 mg	$135.00
		1 g	$1,050.00
5′-Fluorescein III CEP	BA 0334		
NEW!		100 µmol	$380.00
		250 mg	$605.00
5′-Fluorescein II CEP	BA 0253		
		100 µmol	$255.00
		250 mg	$495.00
FDMT-5′-Fluorescein CEP		FL 1700	
		100 µmol	$385.00

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