

Chemistry

from **Berry & Associates**

TO ADVANCE THE LIFE SCIENCES

Issue 8 | December 2011

Introducing BCN: Our Newest Click-easy™ Alkynes for Cu-Free Click Conjugation

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Our Founder and Friend Remembered

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

Design. Develop. Deliver.

Since its introduction in 2002, the click reaction has become an immensely valuable tool spanning many facets of research from surface science to biomolecules. For seminal reading on the evolution of the use of this 1,3-dipolar cycloaddition in bioconjugation and bioorthogonal labeling, see references 1–5. Originally introduced as a copper(I)-catalyzed cycloaddition between an alkyne and azide, the click reaction is clean, efficient, and tolerant of a wide range of solvents and functional groups. However, the requisite copper can degrade oligonucleotides⁶ and can compromise cell function,⁷ thereby limiting the utility of the Cu-catalyzed click reaction for these purposes. Since Bertozzi's report in 2004,⁸ a large number of cyclooctynes have been developed to capitalize upon the unique nature of a ring-bound alkyne for strain-promoted alkyne-azide cycloaddition (SPAAC). And now, Berry & Associates is pleased to partner with SynAffix⁹ to bring you an exciting bicyclo[6.1.0]nonyne (BCN) alkyne scaffold for catalyst free clicking (Figure 1). This sub-

stituted cyclooctyne strikes the best balance between reactivity and lipophilicity of all the substituted cyclooctynes reported to date.¹⁰ Since a Cu(I) catalyst is not required for click reactions with azides, BCN modification provides a “no-muss, no fuss” solution to many oligo conjugation reactions.

The Click-easy™ BCNs (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.

In one illustrative example, 5'-Click-easy™ BCN CEP II (BA 0373)¹¹ is incorporated at the 5'-terminus of an oligo with >99% efficiency. Following a diethylamine wash, standard cleavage, and salt switch, a triethylamine salt of the T6 oligo was taken up in a buffer/acetonitrile solution and treated with PQQ-TEG azide (Figure 2). The reactivity of the quinone functionality in PQQ presents a formidable barrier to successful oligo conjugation via Cu-catalyzed click and Staudinger ligation strategies. The remarkably clean SPAAC conjugation of PQQ-TEG azide to the BCN-oligo, using approximately a three-fold molar excess of the azide, was a supremely gratifying conclusion to a nearly two-year search for successful PQQ ligation chemistry. Although the

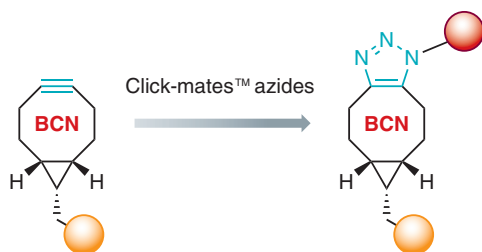


Figure 1. BCNs for copper-free clicking

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Copper-Free Click

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SPAAC ligation reaction shown in Figure 2 requires >26 hours, it is possible to increase the reaction rate by increasing the concentration of azide. For example, when the same BCN-oligo was treated with Biotin-TEG azide (BT 1085) in approximately 100-fold molar excess in the same reaction volume, the reaction was nearly complete in just 30 minutes (Figure 3).

Also in our Click-easy™ lineup for catalyst-free ligation are the MFCO or monofluorocyclooctynes reported by Pigge and co-workers.¹² We offer 5'-Click-easy™ MFCO CEP (BA 0368) for introduction of the cyclooctyne during oligo synthesis, and the analogous Click-easy™ MFCO-*N*-hydroxysuccinimide ester (LK 4300) for post synthetic incorporation.

Ordering information is on the following page

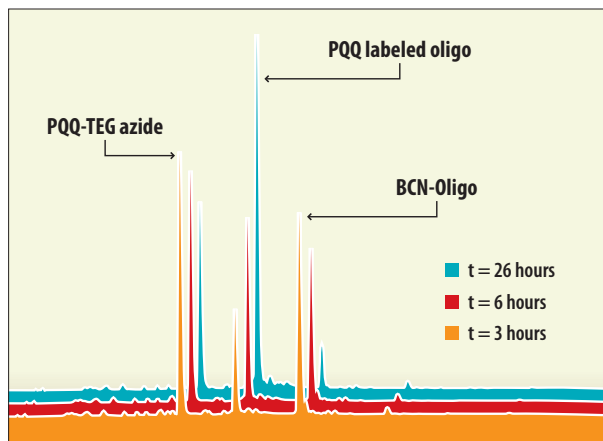


Figure 2 BCN-Oligo with PQQ-TEG azide (FC 8170)

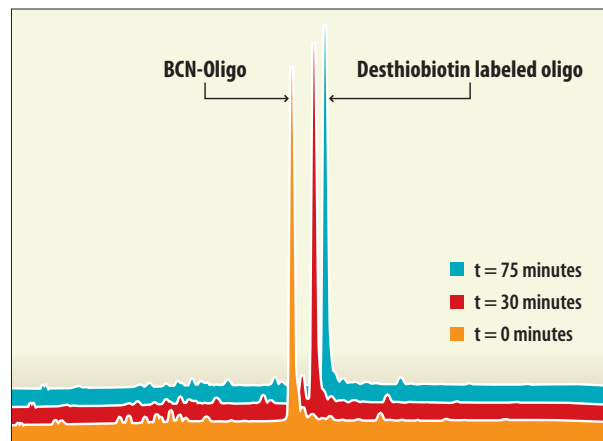
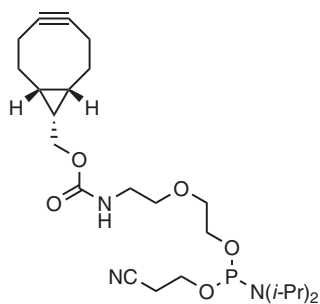
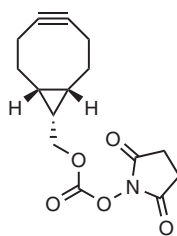


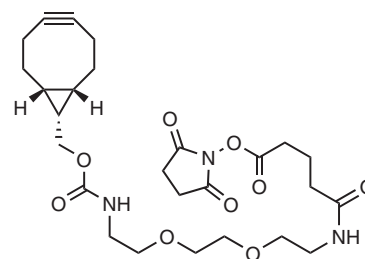
Figure 3 BCN-Oligo with excess Desthiobiotin-TEG azide (BT 1075)



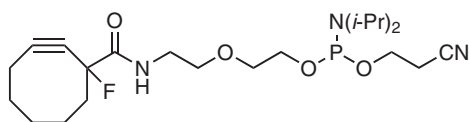
BA 0373



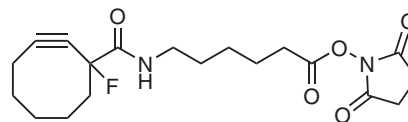
LK 4320



LK 4330



BA 0368



LK 4300

BA 0368 and LK 4300 are protected by US 7,807,619, the rights of which are assigned to The Regents of the University of California. Additional patents covering methods for their use in the modification of biomolecules are pending. They may be used for research purposes only. They are not licensed for resale and may only be used by the buyer. These products may not be used and are not licensed for clinical assays, where the results of such assays are provided as a diagnostic service. If a diagnostic or therapeutic use is anticipated, then a license must be requested from the University of California. The availability of such diagnostic and therapeutic use license(s) cannot be guaranteed from the University of California.

Ordering Information—BCN Alkynes for Copper-Free Clicking

Catalog Number	Name	Size	Price
BA 0373	5'-Click-easy™ BCN CEP II	100 μmol	\$499.00
		250 μmol	\$1100.00
LK 4320	Click-easy™ BCN N-hydroxysuccinimide ester I	25 mg	\$125.00
		50 mg	\$185.00
LK 4330	Click-easy™ BCN N-hydroxysuccinimide ester II	10 mg	\$150.00
		25 mg	\$220.00

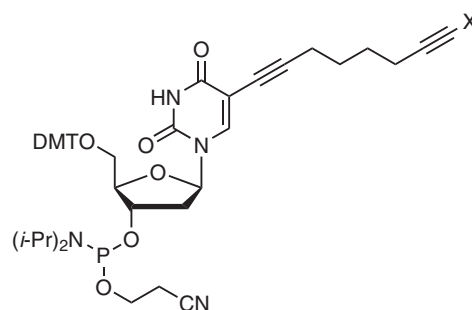
Catalog Number	Name	Size	Price
BA 0368	5'-Click-easy™ MFCO CEP	50 μmol	\$325.00
		100 μmol	\$590.00
LK 4300	Click-easy™ MFCO-N-hydroxysuccinimide ester	25 mg	\$255.00
		50 mg	\$470.00

Protected Alkynes for Double and Triple Clicking

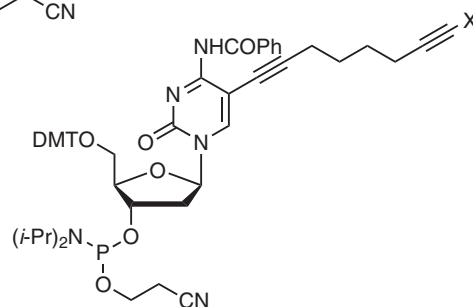
Although the click reaction with standard alkynes and cyclooctynes is straightforward and efficient, it does not lend itself well to the sequential labeling of oligonucleotides. Fortunately, the base-click platform provides tools for introduction of up to three different labels using variably protected diynes as shown by Carell and co-workers.¹³ To affect the triple click, the three orthogonally protected diyne substituted bases could be incorporated into an oligo, followed by on column click reaction of the free alkyne, deprotection of the TMS alkyne during cleavage of the oligo from the solid support with ammonia, a second click, TBAF deprotection of the TIPS and then the final click.

We now offer the three requisite alkynes in the dU family: 5-Octadiynyl-dU CEP (BA 0308), 5-Octadiynyl-TMS-dU CEP (BA 0364), and 5-Octadiynyl-TIPS-dU CEP (BA 0369). For oligos requiring dC, the unprotected (BA 0366, 5-Octadiynyl-dC CEP) and the TMS protected (BA 0365, 5-Octadiynyl-TMS-dC CEP) alkynes are available as well.

BA 0308, BA 0369, BA 0364, BA 0366, BA 0365: These compounds are sold under license from baseclick GmbH, and 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.



BA 0308 X = H
BA 0364 X = TMS
BA 0369 X = TIPS



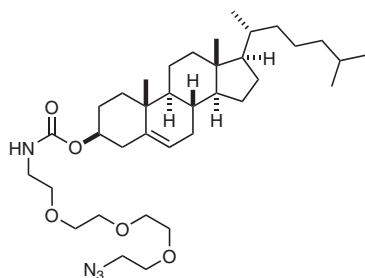
BA 0366 X = H
BA 0365 X = TMS

Ordering Information—Protected Alkynes

Catalog Number	Name	Size	Price
BA 0308	5-Octadiynyl-dU CEP	100 μmol	\$255.00
		0.25 g	\$550.00
BA 0364	5-Octadiynyl-TMS-dU CEP	2 g minimum order	Call for pricing
BA 0369	5-Octadiynyl-TIPS-dU CEP	2 g minimum order	Call for pricing
BA 0365	5-Octadiynyl-TMS-dC CEP	2 g minimum order	Call for pricing
BA 0366	5-Octadiynyl-dC CEP	2 g minimum order	Call for pricing

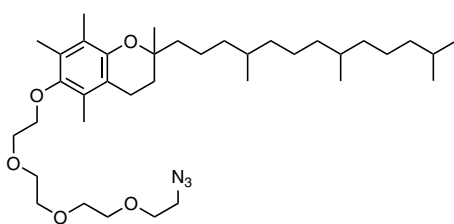
More New Click-mates™ azides

Due to the efficiency and simplicity of the click reaction, biologically significant azides are in high demand. For those who prefer the reliability of cholesterol for its lipophilicity and ability to improve efficiency of delivery of oligonucleotides to targeted cells, we now offer FC 8180 Cholesteryl-TEG azide.

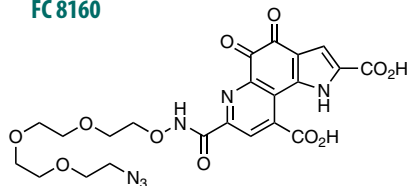


FC 8180

Since cholesteryl labeling does have some limitations, we have added another alternative to our vitamin lineup. We have previously reported on our folate TEG azide (FC 8150), and now we also offer a vitamin E analog, FC 8160 Tocopherol-TEG azide. Our new offerings also include the cofactor PQQ-TEG azide (FC 8170) for use as a colorimetric probe for biomolecules.¹⁴

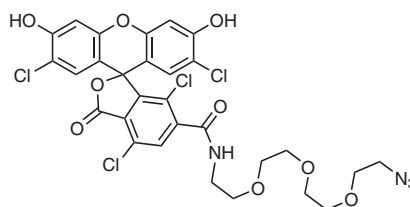


FC 8160



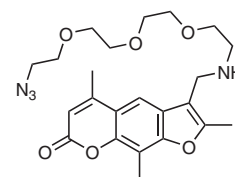
FC 8170

For the convenient ligation of 6-tetrachloro fluorescein either via click chemistry or Staudinger ligation, give our 6-TET-TEG azide



FF 6130

(FF 6130) a try. Tetrachloro fluorescein has been widely used for labeling a variety of biomolecules and has the advantage of being fluorescent at physiological pH. Lastly,



PS 5030

Psoralean TEG azide (PS 5030) is now available.

If you are in need of an azide but don't see it on our website, just give us a call. We will be happy to help!

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Ordering Information—Click-Mates Azides

Catalog Number	Name	Size	Price
FC 8170	PQQ-TEG azide	1 mg	\$295.00
FF 6130	6-TET-TEG azide	1 mg	\$295.00
FC 8160	Tocopherol-TEG azide	5 mg	\$725.00
		10 mg	\$975.00
FC 8180	Cholesteryl-TEG azide	25 mg	\$175.00
		100 mg	\$595.00
PS 5030	Psoralean TEG azide	1 mg	\$210.00
		5 mg	\$450.00

5-Hydroxymethyl-2'-deoxycytidine tools for epigenetic research

The 5-methylation of 2'-deoxycytidine is an important and well studied modification of DNA. This methylation does not impact base pairing but it alters DNA in ways that affect the binding of transcription factors and subsequent gene expression. Therefore, cytosine methylation is an important epigenetic marker. The widespread 5-hydroxymethylation of cytosine in DNA of brain tissue has also been documented.¹ The exact function of this new "sixth base" is the subject of much current research. As shown in Figure 4, DNA methyltransferases (DNMT) catalyze the *in vivo* conversion of dC to mC and subsequent oxidation by ten-to-eleven translocation oxidases (TET) affords hmC. It has been further postulated that a complete cycle involving further oxidation to fC and then to caC followed by decarboxylation to dC could exist *in vivo*.² Recently, fC has been discovered in embryonic stem cells with concentrations decreasing as cell differentiation proceeds.³

To facilitate research in this area, we offer a collection of 5hmC derivatives including the nucleoside standards 5-Hydroxymethylcytidine (PY 7596) and 5-Hydroxymethyl-2'-deoxycytidine (PY 7588), and the phosphoramidite *N*-Benzoyl-5-((2-cyanoethoxy)methyl)-2'-

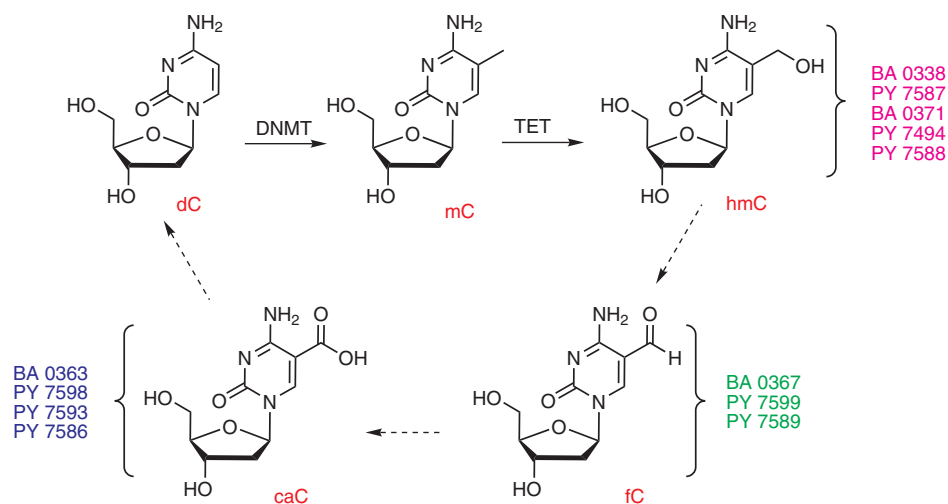


Figure 4. Cytosine methylation cycle and related Berry & Associates Products

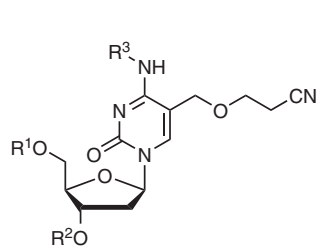
deoxycytidine CEP (BA 0338) which was described by Sowers and co-workers in 1997.⁴ We also carry the corresponding nucleoside 5-[(2-Cyanoethoxy)methyl]-2'-deoxycytidine (PY 7587).

As an alternative protection scheme, we now provide BA 0371, the cyclic carbamate phosphoramidite described by Carell and co-workers.⁵ This phosphoramidite can be efficiently incorporated into oligonucleotides and the carbamate can be removed with base during the cleavage process. This enables a straight-forward, gentle method for incorporation of the nucleobase

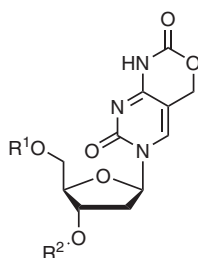
of 5-hydroxymethyl cytosine into oligonucleotides.

For the human p53 gene, the predominant mutation is a C to T transition, and Matsuda and co-workers have reported findings that indicate this transition can be caused by the formation of 5-formyl-2'-deoxycytidine.⁶ Based on their findings, we have introduced BA 0367 into our product line as a useful tool for further studies. Both 5-Formylcytidine (PY 7599) and 5-Formyl-2'-deoxycytidine (PY 7589) nucleosides are also available.

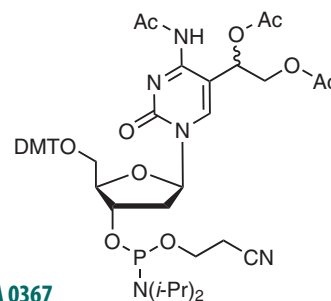
Continued on page 6



BA 0338 $R^1 = \text{DMT}, R^2 = \text{CEP}, R^3 = \text{Bz}$
PY 7587 $R^1 = R^2 = \text{H}$



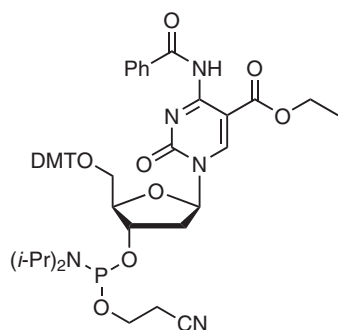
BA 0371 $R^1 = \text{DMT}, R^2 = \text{CEP}$
PY 7594 $R^1 = R^2 = \text{H}$



BA 0367

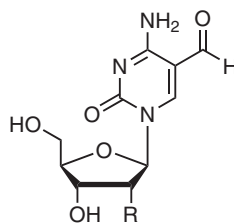
5-Hydroxymethyl-2'-deoxycytidine tools

Continued from page 5



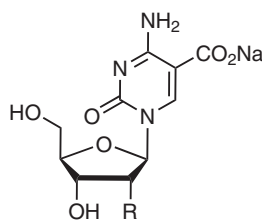
BA 0363

Lastly, we have added several 5-carboxy cytidines to our collection of useful tools. These include 5-Carboethoxy-dC CEP (BA 0363), Cytidine-5-carboxylic acid, sodium salt (PY 7598), 2'-Deoxycytidine-5-carboxylic acid, sodium salt (PY 7593)⁷ and the related Cytidin-5-yl-methanesulfonate sodium salt hydrate (PY 7586).



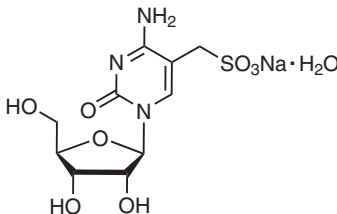
PY 7599 R=OH

PY 7589 R=H



PY 7598 R=OH

PY 7593 R=H



PY 7586

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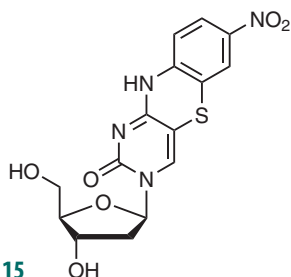
Ordering Information—5-Hydroxy

Catalog Number	Name	Size	Price
PY 7596	5-Hydroxymethylcytidine	50 mg	\$295.00
		100 mg	\$495.00
PY 7588	5-Hydroxymethyl-2'-deoxycytidine	10 mg	\$245.00
		50 mg	\$875.00
BA 0338	N-Benzoyl-5-((2-cyanoethoxy)methyl)-2'-deoxycytidine CEP	15 g minimum order	Call for pricing
PY 7587	5-[(2-Cyanoethoxy)methyl]-2'-deoxycytidine	10 mg	\$210.00
		50 mg	\$750.00
BA 0371	5-Hydroxymethyl-dC cyclic carbamate CEP	50 μmol	\$540.00
		100 μmol	\$825.00
PY 7594	5-Hydroxymethyl-2'-deoxycytidine cyclic carbamate	10 mg	\$150.00
		50 mg	\$525.00
BA 0367	Masked 5-formyl-dC CEP	50 μmol	\$660.00
		100 μmol	\$1200.00

Catalog Number	Name	Size	Price
PY 7599	5-Formylcytidine	5 mg	\$191.50
		10 mg	\$325.00
PY 7589	5-Formyl-2'-deoxycytidine	5 mg	\$185.00
		10 mg	\$315.00
BA 0363	5-Carboethoxy dC CEP	5 g minimum order	Call for pricing
PY 7598	Cytidine-5-carboxylic acid, sodium salt	10 mg	\$225.00
		50 mg	\$690.00
PY 7593	2'-Deoxycytidine-5-carboxylic acid, sodium salt	25 mg	\$147.00
		100mg	\$355.00
PY 7586	Cytidin-5-yl-methanesulfonate sodium salt hydrate	50 mg	\$525.00
		100mg	\$879.00

Assorted tools of interest

The arena of fluorescent base analogs has expanded in recent years due to the preparation and characterization of the tC family of tricyclic cytosine probes. Unlike other fluorescent base analogs, the tC compounds do not exhibit extensive quenching in the base-stacking environment of double stranded DNA. These nucleobase analogs are thus, uniquely suited for investigation of the dynamics of nucleic acid



PYA 11115

structure.¹ In 2009, tC_{nitro} was introduced² and further characterized³ by Wilhelmsson and co-workers as the energy acceptor in the first all nucleobase FRET pair.² To facilitate your research, we now offer PYA 11115 tC_{nitro} nucleoside, and the corresponding phosphoramidite is available from our colleagues at Glen Research (10-1518).

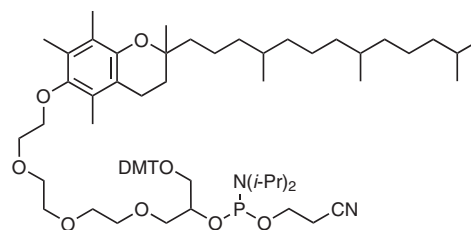
In the search for effective *in vivo* carriers for therapeutic applications of siRNAs, Nishina, Unno and coworkers utilized alpha-tocopherol (vitamin E) as a carrier molecule.⁴ They hypothesized that a molecule that had its own transport pathway, was essential for target tissue cells, yet was not synthesized within the cells would be an ideal *in vivo* carrier conjugate. Their results indicate that alpha-tocopherol is a safe and effective carrier for delivery

of siRNA into the liver. Following their lead, we have modified alpha-tocopherol with the mixed polarity TEG linker, and produced the corresponding phosphoramidite (BA 0357) which is useful for modification of oligonucleotides either internally or at the 5'-terminus. The tocopherol tag is also available as the TEG azide (FC 8160) for ligating via click chemistry (*vide infra*).

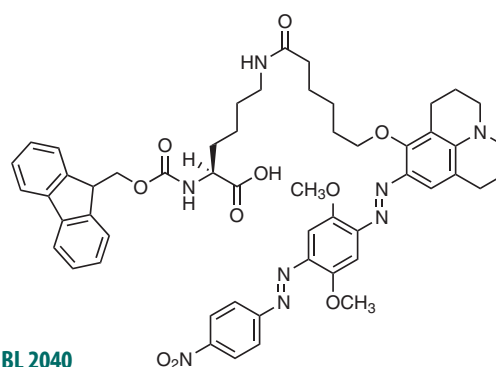
We are happy to introduce Fmoc-Lysine(BBQ-650TM)-OH (BL 2040), our first quencher for peptide applications. This protected lysine derivative allows for incorporation of our Blackberry[®] Quencher which is ideal for the quenching of long-wavelength reporter fluorophores in a variety of probes for use with contact or FRET quenching. Please consult our website or give us a call if you are interested in more information about our Blackberry[®] Quenchers.

References

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BA 0357



BL 2040

Ordering Information—Assorted

Catalog Number	Name	Size	Price
BA 0357	Tocopherol-TEG CEP	10 g minimum order	
		Call for pricing	
PYA 11115	tC _{nitro} Nucleoside	5 mg	\$138.00
		25 mg	\$575.00
BL 2040	Fmoc-Lysine(BBQ-650 TM)-OH	50 mg	\$465.00
		100 mg	\$710.00

Our Founder and Friend Remembered



As a result of a tragic accident this summer, we sadly had to face the untimely passing of Dave Berry, the founder of Berry & Associates, Inc.

Dave founded Berry & Associates in 1988 after having received his Ph.D. in Medicinal Chemistry under Dr. Leroy Townsend at the University of Michigan. Prior to receiving his Ph.D., Dave had spent 7 years in the Anti-Cancer group at Warner Lambert/Parke-Davis. It was during those 7 years that Dr. Berry learned a valuable lesson: pretty isn't always the way to go, and sometimes it is just necessary to roll up one's sleeves to move the chemistry forward. To independently put his philosophy into practice, Dave "rolled up his sleeves" and with his own hands built the building for the Berry & Associates laboratories.

Dave's business model was simple: provide high quality materials at a fair price and in a timely manner. As a result of his perseverance and stellar work ethic, Dave nurtured Berry & Associates from a one-man business into a successful and experienced Research, Development and Production company.

A firm believer in the power of education, Dave was a highly regarded mentor among his Berry & Associates colleagues and customers. Dave worked to encourage chemistry students by generously sharing his knowledge and time with those who sought advice, as well as by financially supporting the Eastern Michigan University Chemistry program.

For over two decades, Dave surrounded himself with talented associates who shared his vision for the company. We are proud of what Dave accomplished over the years, and will be honored to continue his mission. To our many customers, associates and vendors who have expressed sympathy, concern, and support, we extend our heartfelt thanks and our guarantee to continue to provide quality reagents worthy of the Berry & Associates name.

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

