Chemistry
from Berry & Associates
TO ADVANCE THE LIFE SCIENCES
Volume 1, Issue 2 | Fall 2006

Ribonucleoside Phosphoramidites:
Focus on RNA synthesis

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 more than 130 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.

The importance of RNA oligonucleotides continues to increase due to their use in RNAi, antisense technology, and basic RNA-related research. We have recently focused on increasing our collection of RNA phosphoramidites for solid-phase oligoribonucleotide synthesis and submit the following products for your consideration.

PHOSPHORAMIDITE COLLECTION

- Convertible A, C, and G
- RNA Amino-modifiers
- Pseudouridine CEP
- 7-Deaza-A CEP
- 8-Aza-7-deaza-A CEP
- Pyridine-2-one CEP
- 2-Aminopurine Riboside CEP
- Nebularine (Purine Riboside) CEP
- Zebularine CEP

Convertible RNA Phosphoramidites (Convertible A, C, and G)

Through the efforts of Verdine, Xu, Harris, and others, convertible nucleoside phosphoramidites have been used for the site-specific incorporation of various amines and tethered functional groups into synthetic DNA.[1] The convertible nucleoside approach relies on the site-specific installation, through solid-phase synthesis, of a nucleoside derivative bearing a leaving group on its nucleobase. Treatment of the full-length chain with an amine nucleophile causes displacement of this leaving group and thus attachment of the amine (and any tethered groups or functionality) to the nucleobase. A key feature of this strategy is that such modifications do not disrupt Watson-Crick base-pairing. The applications of this approach are far-reaching and include isotopic labeling (e.g., with $^{15}NH_3$), the modification of helix stability, and the introduction of fluorescent markers.

Verdine, et al.,[1] have extended the convertible nucleoside strategy to the RNA arena. We now offer a suite of the requisite 2'-*O*-TBDMS phosphoramidites (Figure 1, next page). The inosine derivative O^6-Chlorophenyl-I CEP (Convertible A CEP) allows the formation of N^6-alkyladenosine residues. After incorporation into an oligoribonucleotide by standard phosphoramidite chemistry, treatment with ammonia, methylamine, or higher alkylamines, including those bearing tethered functional groups, leads to

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Berry In the News

Berry & Associates has been awarded a Phase II Small Business Innovation Research (SBIR) grant from the National Institutes of Health (NIH) to continue to develop fluorous affinity technology for the purification of oligonucleotides. To learn more about this method, see *Chemistry from Berry & Associates*, 2006, Vol. 1, Issue 1. Call to request a copy, or download it from our website.

Ribonucleoside Phosphoramidites

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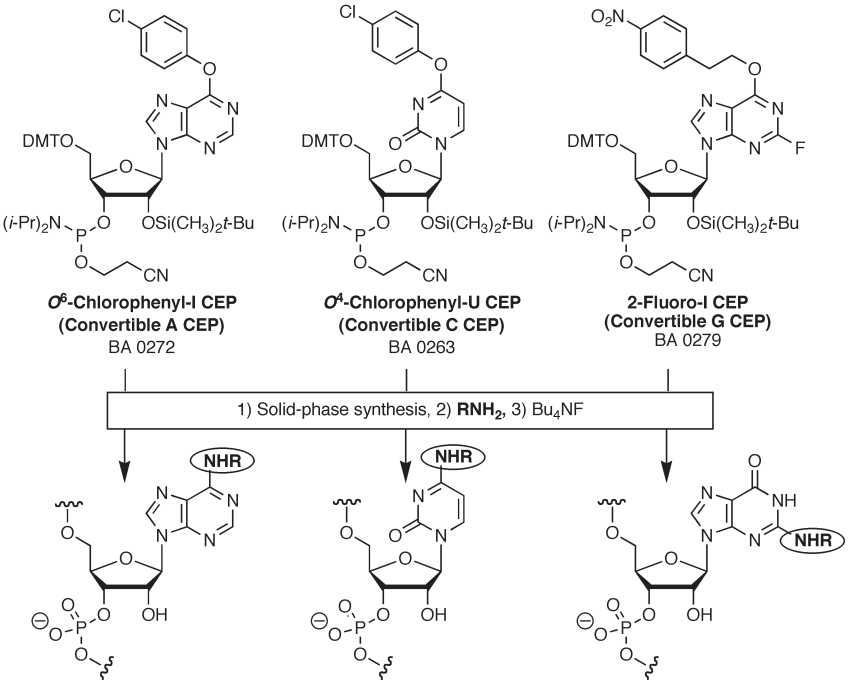


Figure 1. Convertible RNA phosphoramidites allow the formation of *N*-alkyl-adenosine, -cytidine, and -guanosine residues in synthetic oligoribonucleotides.

displacement of 4-chlorophenol with resultant installation of a 6-alkylamino group, i.e., producing N^6-alkyladenosine residues. Exemplary amines include ethylenediamine, 1,4-diaminobutane, ethanolamine, benzylamine, cystamine, and di(2-aminoethyl) disulfide.[1] In a similar fashion, O^4-Chlorophenyl-U CEP (Convertible C CEP) and 2-Fluoro-I CEP (Convertible G CEP) produce N^4-alkylcytidine and N^2-alkylguanosine residues, respectively. In the latter case, the leaving group is fluoride ion. The (4-nitrophenyl)ethyl (NPE) protecting group is removed from the O^6 position during the desilylation step.

RNA Amino-Modifiers

The popularity of Amino-modifier-C6-dT CEP for the introduction of an amine functional group into DNA prompts us to offer the RNA version, Amino-modifier-C6-U CEP (Figure 2).[2] If tethering a reporter group to the 2' oxygen via an amine is preferred, we now offer 2'-*O*-Aminolinker-U CEP,[2] an alternative to 2'-aminouridine and 2'-*O*-(2-aminoethoxy)uridine. Placing the amino group farther from the sugar ring may be advantageous in post-synthetic acylation reactions.

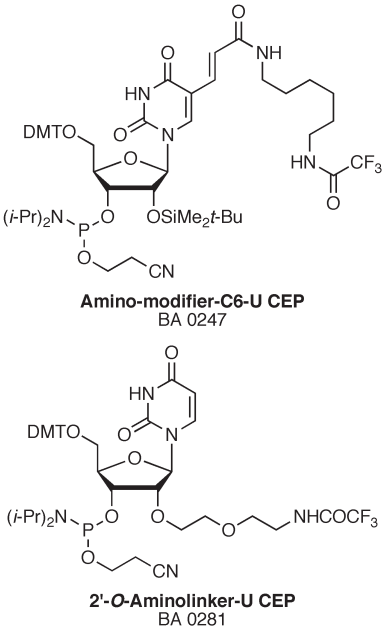


Figure 2. Amine-modifiers for RNA synthesis.

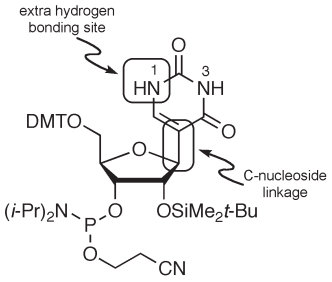
RNA Amidites for Alternate Base Pairing and Structural Studies

Pseudouridine CEP (Ψ CEP)

Pseudouridine (Ψ) is one of the most common modified nucleosides found in RNA, e.g., in tRNAs and snRNAs.[3] The uracil nucleobase is identical to that found in uridine except that it is attached to the ribose ring via C5 rather than N1, i.e., it is a C-nucleoside (Figure 3). Thus, in addition to the ability to form Watson-Crick base pairs with adenosine in the normal manner, Ψ has an additional hydrogen bond donor at N1. This difference can strongly influence the overall structure of an RNA oligonucleotide.[4] As an example of the consequences of the N1 hydrogen, the ability to coordinate a structural water molecule may result in rigidifying the nearby sugar-phosphate backbone and enhancing base stacking.[3]

The ability to install a Ψ residue site-specifically allows the systematic study of its effect on the structure, function and stability of RNA. Several strategies have been reported for the incorporation of Ψ during the chemical synthesis of RNA oligonucleotides.[5-9] We now offer Pseudouridine CEP (Ψ CEP, Figure 3) for this purpose. This particular version[5,8] of pseudouridine phosphoramidite relies on standard cyanoethyl phosphoramidite coupling chemistry, 2'-*O*-TBDMS protection, and no nucleobase protecting groups.[7]

The availability of totally synthetic Ψ-containing oligoribonucleotides has led to the synthesis of modified ribozymes and key portions of natural tRNAs and snRNAs and has generated numerous observations about the role of Ψ in RNA.[4-7,9,10,18] For example,[5] in double-stranded RNA, the N1 hydrogen projects into the deep and narrow major groove, and ^{1}H-NMR studies on synthetic duplex A-form RNA



Pseudouridine CEP (Ψ CEP)
BA 0280

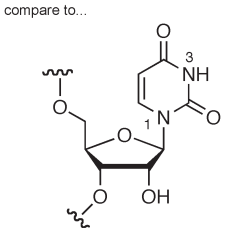
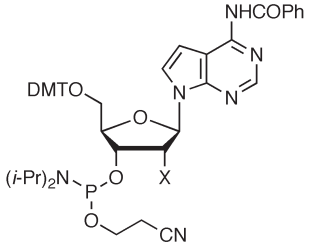


Figure 3. The phosphoramidite of pseudouridine (Ψ) and a comparison to uridine.

show that the uniquely visible[4,5,10] N1 hydrogen is normally non-bonded, but may be accessed with metal ions, spermidine, and charged peptide side chains. Replacement or addition of pseudouridine residues in synthetic anticodon domains of $tRNA^{Lys}$ (human and *E. coli*) had a dramatic effect on its structure.[10] In studies on synthetic fragments of 23S rRNA, altering of the number and position of Ψ residues showed a range of effects, both stabilizing and destabilizing.[9] It was proposed that Ψ may be stabilizing relative to U because of greater hydrophilicity, presumably due to additional hydrogen bonding via the N1 hydrogen.[9] It is hoped that the availability of Pseudouridine CEP may facilitate further research on the customization of the structure and function of RNA oligonucleotides.

7-Deaza-A CEP

The N7 imidazole nitrogen of purine nucleosides is known to take part in non-Watson-Crick hydrogen bonding and in metal chelation. "Deleting" the N7 nitrogen by replacing it with a CH group is a useful modification that has been accomplished in DNA and RNA oligonucleotides using the phosphoramidites of 7-deaza-dA (2'-deoxytubercidin) and 7-deaza-A (tubercidin), respectively. Early work by Seela[11] involved 7-Deaza-dA CEP (Figure 4), which was useful in showing that N7 of dA is an important hydrogen bond acceptor site for the endodeoxyribonuclease *Eco*RI. We now offer the ribonucleoside version, 7-Deaza-A CEP,[12] also known as Tubercidin CEP or C^7A CEP. This phosphoramidite has been used for studies of the role of the N7 adenosine nitrogen in the structure and function of tRNA and ribozymes.[12,13]



7-Deaza-dA CEP X=H (BA 0001)
7-Deaza-A CEP X=$OSi(CH_3)_2t$-Bu (BA 0268)

Figure 4. 7-Deaza-dA/A phosphoramidites, derivatives of 2'-deoxytubercidin and tubercidin .

8-Aza-7-deaza-A CEP

The phosphoramidite 8-aza-7-deaza-dA CEP, also known as PPA CEP, features a nucleobase that is isosteric with adenine but offers a different π-electron distribution and thus an altered dipole moment, resulting in stronger stacking interactions in oligonucleotides.[14] We now offer the ribonucleoside version, 8-Aza-7-deaza-A CEP (PPA Riboside CEP) for use in the synthesis of altered RNA oligonucleotides (Figure 5).[2]

2-Aminopurine Riboside CEP

Deletion of the O6 carbonyl oxygen of guanosine results in 2-aminopurine riboside (2-AP, Figure 6). The hydrogen bonding pattern of the 2-aminopurine nucleobase (N1 acceptor, H-N2 donor) is isomeric with that of adenosine (N1 acceptor, H-N6 donor). This nucleoside allows the study of the role of exocyclic functional groups, base stacking, and hydrogen bonding patterns in purine-containing nucleic acids. For example, replacement of guanosine residues with 2-AP in the core region of hammerhead ribozymes was useful in determining their role in stabilizing the transition state of ribozyme cleavage.[15] The nature of hydrogen-bonding between G-A mismatches in RNA internal loops was studied with 2-AP.[16] The role of hydrogen-bonding and stacking interactions in the stability of GNRA loops was probed using

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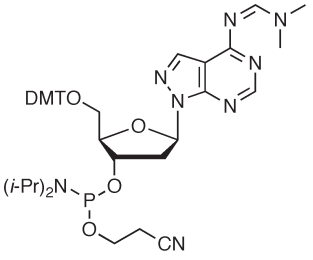
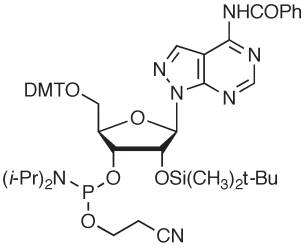
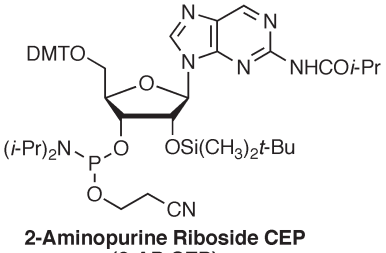


8-Aza-7-deaza-dA CEP
BA 0239



8-Aza-7-deaza-A CEP
BA 0267

Figure 5. 8-Aza-7-deaza-dA/A phosphoramidites.



2-Aminopurine Riboside CEP (2-AP CEP)
BA 0266

Figure 6. The phosphoramidite of 2-aminopurine riboside (2-AP).

Ribonucleoside Phosphoramidites

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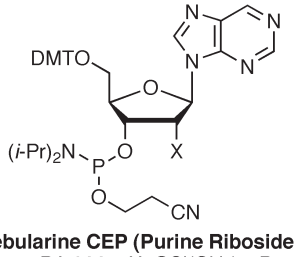
2-AP substitutions.[17] The thermodynamic parameters for RNA loops of the type $(A)_n$ were determined using time-resolved spectrofluorimetry on RNAs bearing 2-AP residues in place of A residues, since 2-AP is blue fluorescent and was found to have properties in the $(A)_n$ region that were otherwise very similar to adenosine.[18] In this sense, 2-AP can be used as a non-invasive conformational probe in RNA studies.

Of the different phosphoramidites that have been used for 2-aminopurine riboside incorporation into RNA oligonucleotides,[15-19] we have chosen to offer 2-Aminopurine Riboside CEP (Figure 6) in the particular form shown,[15,18] which appears to offer the best results in RNA synthesis yield and purity.

Nebularine CEP (Purine Riboside CEP)

Nebularine (purine riboside) (Figure 7) lacks exocyclic functional groups and offers an altered hydrogen bonding scheme while retaining base stacking ability. It can be viewed as an adenosine analog with the hydrogen bond donor deleted. Sequential replacement of conserved adenosine residues in hammerhead ribozymes by nebularine residues[20b,21] suggested the presence of interstrand non-Watson-Crick hydrogen bonding.[20b] Depending on the position of the nebularine



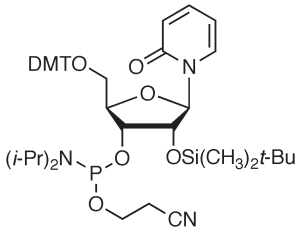
Nebularine CEP (Purine Riboside CEP)
BA 0265, X=OSi(CH$_3$)$_2$*t*-Bu
2'-Deoxynebularine CEP
BA 0016, X=H

Figure 7. The phosphoramidite of nebularine, also known as purine riboside, and its 2'-deoxy version.

residue, cleavage rates were either unchanged or diminished.[20b,21] Incorporation of nebularine into a GNRA tetraloop has also been useful for studying this type of RNA structural feature.[17] Nebularine has been installed into RNA using two different phosphoramidites, one with 2'-*O*-THP protection[16] and one with 2'-*O*-TBDMS protection.[17,20,21] We offer the latter, Nebularine CEP (Figure 7) as well as the 2-deoxy version, 2'-Deoxynebularine CEP (BA 0016).

Pyridin-2-one Riboside CEP

Beigelman and co-workers have carried out structure-activity studies on hammerhead ribozymes by substituting modified pyrimidines at various positions, where profound effects on ribozyme catalytic activity have been observed.[22] Pyridin-2-one Riboside CEP (Figure 8)[23] was found to be useful in these studies,



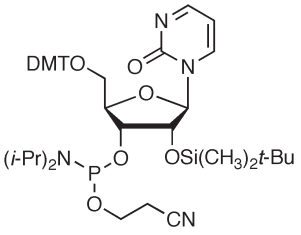
Pyridin-2-one Riboside CEP
BA 0264

Figure 8. The phosphoramidite of 1-ribofuranosyl-pyridin-2-one.

providing alterations in syn/anti nucleobase orientation, ribose puckering, and stacking ability due to dipole changes.[22-25] We now offer this intriguing 3-deazapyrimidine analog for use by the RNA community.

Zebularine CEP

Zebularine (a.k.a. 2-pyrimidinone riboside or ^{4H}C) is similar to cytidine except that it lacks a C4-amino group and thus makes one less hydrogen bond to G. Incorporation of zebularine into RNA strands may be accomplished with Zebularine CEP (Figure 9) using standard phosphoramidite chemistry.[26,27] Substitution of zebularine for cytidine amounts to "atomic mutagenesis",[27] allowing studies of the role of a particular hydrogen bond in RNA duplexes.[26-28] Replacing various cytidine residues in a hammerhead ribozyme with zebularine proved to be an effective probe of structure vs. catalytic activity, supporting the assignment of the proposed catalytically-active magnesium ion binding site.[26,28] Atomic mutagenesis



Zebularine CEP
BA 0254

Figure 9. The phosphoramidite of zebularine (2-pyrimidinone riboside).

at the G1:C72 base pair in class II *Escherichia coli* alanyl-tRNA synthetase using various G and C analogs (zebularine, 7-deaza-dG, inosine, 2-aminopurine) allowed dissection of the roles of various hydrogen bonds in the major and minor grooves, revealing the nature of the high degree of specificity afforded by major groove interactions at the end of the helix.[27] Finally, it should be noted that zebularine is fluorescent. Excitation of a zebularine-containing ribozyme at 298 nm caused emission at 367 nm. Upon annealing with a complementary strand, a small increase in fluorescence intensity (10%) was observed with no change in emission wavelength.[26]

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(2) This product is from our Experimental Grab Bag. The compounds in this unique collection have not been validated for any particular purpose and have not been proven in oligonucleotide synthesis. We hope that you may find them of interest, but please be aware that their purchase and use is at your own risk.

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RNA Phosphoramidites — Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
O^6-Chlorophenyl-I CEP	BA 0272		
(Convertible A CEP)		50 µmol	$275.00
		0.25 g	$975.00
O^4-Chlorophenyl-U CEP	BA 0263		
(Convertible C CEP)		100 µmol	$265.00
		0.25 g	$525.00
2-Fluoro-I CEP	BA 0279		
(Convertible G CEP)		50 µmol	$275.00
		0.25 g	$975.00
Amino-modifier-C6-U CEP	BA 0247		
		50 µmol	$275.00
		0.25 g	$975.00
2'-*O*-Aminolinker-U CEP	BA 0281		
		100 µmol	$460.00
		0.25 g	$975.00
Pseudouridine CEP	BA 0280		
(Ψ CEP)		100 µmol	$290.00
		0.25 g	$645.00
7-Deaza-A CEP	BA 0268		
		100 µmol	$300.00
		0.25 g	$725.00
8-Aza-7-deaza-A CEP	BA 0267		
(PPA Riboside CEP)		100 µmol	$495.00
		0.25 g	$1,195.00
2-Aminopurine Riboside CEP	BA 0266		
(2-AP CEP)		100 µmol	$305.00
		0.25 g	$775.00
Nebularine Riboside CEP	BA 0265		
(Purine Riboside CEP)		50 µmol	$245.00
		100 µmol	$405.00
		0.25 g	$895.00
Pyridin-2-one Riboside CEP	BA 0264		
		100 µmol	$305.00
		0.25 g	$775.00
Zebularine CEP	BA 0254		
		100 µmol	$217.50
		0.25 g	$495.00

Quenched Autoligation (QUAL) Probes

Improved methods for the detection of nucleic acids continue to be an active area of investigation. Non-enzymatic approaches involving fluorescence changes are attractive alternatives to enzyme-based methods such as PCR. Autoligation probes involve two short oligonucleotides, each of which bears a reactive functional group on one end. The two probes are designed such that they hybridize to the appropriate target sequence in an end-to-end fashion to place the two reactive functional groups in close proximity, thus promoting the formation of a covalent bond. Recent work by Kool and co-workers describes an imaginative autoligation strategy that results in the appearance of a fluorescence signal upon template-promoted ligation (Figure 10).[1] Two probes are used, one bearing a 3'-phosphorothioate (Probe 1) and the other a 5'-dabsylate and an internal fluorophore (Probe 2). The fluorophore of Probe 2 is quenched by the nearby dabsyl quencher and is thus dark. The two probes bear additional nucleotides and may bind to the correct sequence (if present) to place the sulfur nucleophile close to the 5'-*O*-dabsylate. A substitution reaction then occurs, displacing the dabsylate quencher and thus unquenching the fluorophore, resulting in a fluorescence signal. These "quenched autoligation probes" (QUAL probes) are more sensitive to single-nucleotide differences than most hybridization-based approaches. Further, the fluorescence change is permanent and is not subject to buffer or temperature. We now offer 5'-*O*-Dabsyl-T CEP (Figure 1) for the synthesis of 5'-*O*-dabsylate QUAL probes.

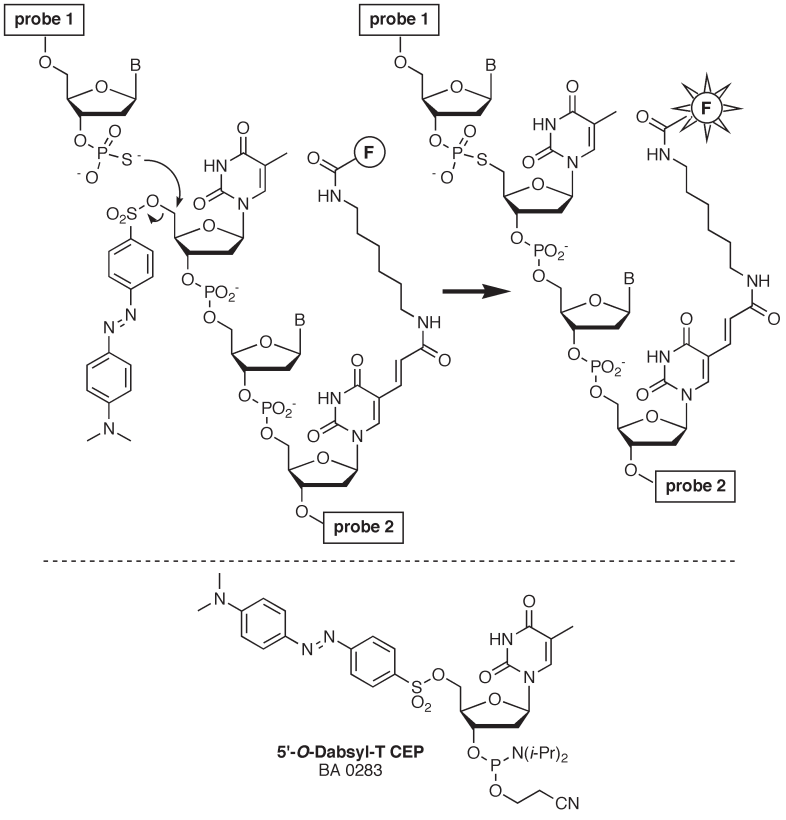


Figure 10. Quenched autoligation probes form a covalent bond in the presence of an appropriate target sequence, displacing dabsylate and thus unquenching Probe 2, resulting in a fluoresence signal. Probe 2 is made with 5'-*O*-Dabsyl-T CEP.

References

(1) (a) Sando, S.; Kool, E. T. *J. Am. Chem. Soc.* **2002**, *124*, 2096-2097. (b) Review: Silverman, A. P.; Kool, E. T. *Trends in Biochem.* **2005**, *23*, 225-230. (c) Review: Silverman, A. P.; Kool, E. T. *Chem. Rev.* **2006**, *106*, 3775-3789.

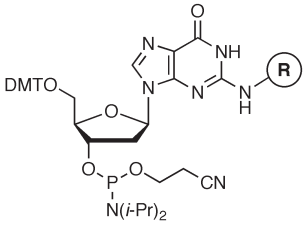
Quenched Autoligation (QUAL) Probes — Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
5'-*O*-Dabsyl-T CEP	BA 0283		
		100 µmol	$225.00
		0.25 g	$575.00

Other New Phosphoramidites

N^2-Alkyl-2'-deoxyguanosine phosphoramidites —A steric tool box

Guanine bases in DNA are susceptible to *N*-alkylation by various carcinogens, leading to miscoding and mutagenicity. Choi and Guengerich have prepared a series of N^2-alkyl-2'-deoxyguanosine phosphoramidites where the alkyl group ranges in size from methyl to anthracenylmethyl for studies on the effect of the size of these groups on the catalytic efficiency and fidelity of various DNA polymerases.[1] We now offer the N^2-methyl-, N^2-ethyl-, and N^2-isobutyl-dG phosphoramidites[1] as well an additional bulkier choice, the N^2-neopentyl version[2] (Figure 11). Researchers may find this "steric tool box" useful for probing the steric requirements at N^2 of dG in various applications.



R = CH_3 **N^2-Methyl-dG CEP** (BA 0249)
R = CH_2CH_3 **N^2-Ethyl-dG CEP** (BA 0076)
R = $CH_2CH(CH_3)_2$ **N^2-Isobutyl-dG CEP** (BA 0250)
R = $CH_2C(CH_3)_3$ **N^2-Neopentyl-dG CEP** (BA 0200)

increasing steric bulk

Figure 11. N^2-Alkyl-2'-deoxyguanosine phosphoramidites of increasing steric bulk.

Phosphoramidites with methylated nucleobases

The methylation and demethylation of DNA continues to be an actively studied area. We offer several phosphoramidites that may be useful for making methylated oligonucleotides in a site-specific manner (Figure 12).[2] The site of methylation may be an exocyclic amino group, a ring nitrogen, or in the case of 3-Deaza-3-methyl-dA CEP, at the ring carbon of a deaza analog. The latter compound may be interesting due to the role of the 3-methyl group in locking the nucleobase in the anti conformation.[3]

8-Vinyl-dA CEP—An alternative to 2-aminopurine

The phosphoramidite 8-Vinyl-dA CEP (Figure 13) has been used to incorporate fluorescent 8-vinyl-deoxyadenosine (8vdA) residues into oligo-nucleotides and has been proposed as an alternative to 2'-deoxyribofuranosyl-2-aminopurine (2AP).[4] The 8vdA-labeled oligonucleotides form more stable duplexes than 2AP-labeled versions when flanked by dA or T residues. The fluorescence quantum yield of 8vdA-labeled oligonucleotides is significantly higher than that of the 2AP versions.

Continued on page 8

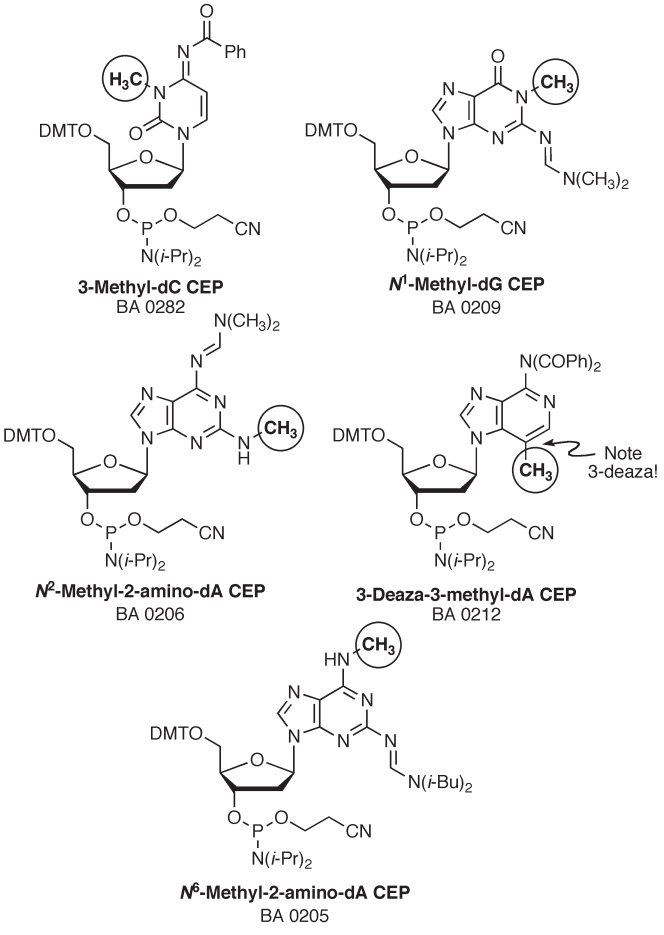


Figure 12. Phosphoramidites for making base-methylated oligonucleotides.

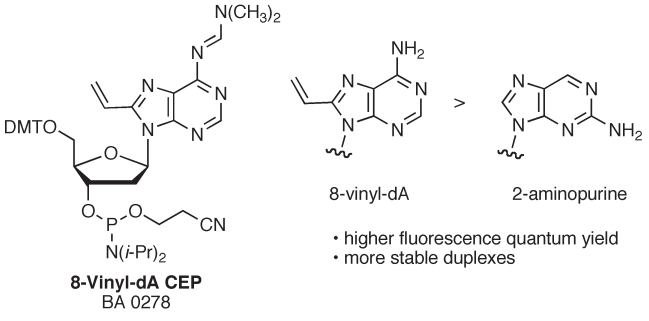


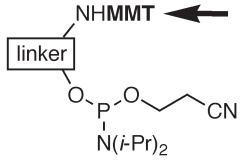
Figure 13. 8-Vinyl-dA is an alternative to 2-aminopurine.

Other New Phosphoramidites

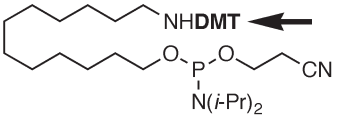
Continued from page 7

5'-Amino-modifier-C_{12}-DMT CEP—A complement to MMT amino-modifiers

As a complement to amine modifiers that bear monomethoxytrityl (MMT) groups, we offer a dimethoxytrityl (DMT) version for applications that require milder detritylation conditions (Figure 14). The installation of an amino-modifier at the 5'-terminus of an oligonucleotide provides, via amide bond formation, a handle for the attachment of a variety of chemical species. If purification of the amine-modified oligonucleotide is



5'-Amino-modifiers with an MMT group



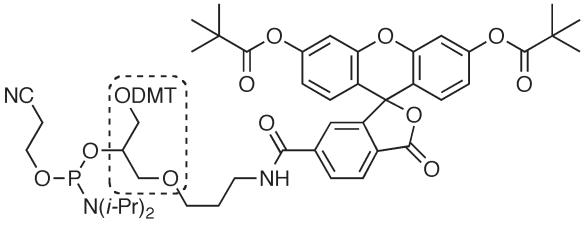
5'-Amino-modifier-C_{12}-DMT CEP
BA 0262

Figure 14. A new 5'-Amino-modifier with a more acid-labile amine protecting group.

desired, it is common to employ an MMT group on the amine. The lipophilicity of the MMT group aids in reversed-phase purification techniques and may also be useful for assaying the coupling yield. For applications where acid sensitivity may be an issue, we offer 5'-Amino-modifier-C_{12}-DMT CEP,[2] which uses the more acid-labile DMT protecting group. Amine-modified oligonucleotides have been synthesized using closely related DMT-bearing amino-modifier phosphoramidites.[5]

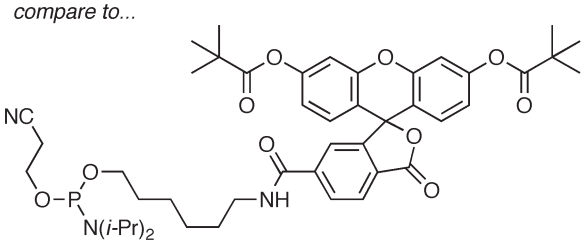
5'-Fluorescein II CEP (6-FAM II CEP)—A DMT-bearing 6-FAM analog

For the installation of fluorescein at the 5'-terminus of an oligonucleotide, the phosphoramidite 6-FAM, which does not bear a DMT group, is a popular choice (Figure 15). However, the lack of a trityl group precludes multiple additions or assaying the coupling step. We now introduce 5'-Fluorescein II CEP (6-FAM II), which features the same tether length as 6-FAM but includes a DMT group.



5'-Fluorescein II CEP (6-FAM II)
BA 0253

compare to...



5'-Fluorescein CEP (6-FAM)
BA 0054

Figure 15. A new version of 6-FAM that bears a DMT group.

References

(1) Choi, J.-Y.; Guengerich, F. P. *J. Biol. Chem.* **2004**, *279*, 19217-19229.

(2) This product is from our Experimental Grab Bag. The compounds in this unique collection have not been validated for any particular purpose and have not been proven in oligonucleotide synthesis. We hope that you may find them of interest, but please be aware that their purchase and use is at your own risk.

(3) Irani, R. J.; SantaLucia, J., Jr., *Nucleosides, Nucleotides, and Nucleic Acids*, **2002**, *21*, 737-751.

(4) Ben Gaied, N.; Glasser, N.; Ramalanjaona, N.; Beltz, H.; Wolff, P.; Marquet, R.; Burger, A.; Mely, Y. *Nucl. Acids Res.* **2005**, *33*, 1031-1039.

(5) (a) Sinha, N. D.; Cook, R. M. *Nucleic Acids Res.* **1988**, *16*, 2659-2669. (b) Guar, R. K. *Nucleosides & Nucleotides* **1991**, *10*, 895-909.

Other Phosphoramidites — Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
N^2-Methyl-dG CEP	BA 0249		
		100 µmol	$355.00
		0.25 g	$875.00
N^2-Ethyl-dG CEP	BA 0076		
		100 µmol	$355.00
		0.25 g	$875.00
N^2-Isobutyl-dG CEP	BA 0250		
		100 µmol	$355.00
		0.25 g	$875.00
N^2-Neopentyl-dG CEP	BA 0200		
		100 µmol	$355.00
		0.25 g	$875.00
3-Methyl-dC CEP	BA 0282		
		100 µmol	$195.00
		0.25 g	$445.00
N^1-Methyl-dG CEP	BA 0209		
		100 µmol	$355.00
		0.25 g	$875.00
N^2-Methyl-2-amino-dA CEP	BA 0206		
		100 µmol	$355.00
		0.25 g	$875.00
3-Deaza-3-methyl-dA CEP	BA 0212		
		50 µmol	$375.00
		0.25 g	$1495.00
N^6-Methyl-2-amino-dA CEP	BA 0205		
		100 µmol	$355.00
		0.25 g	$875.00
8-Vinyl-dA CEP	BA 0278		
		100 µmol	$275.00
		0.25 g	$775.00
5'-Amino-modifier-C12-DMT CEP	BA 0262		
		100 µmol	$73.50
		0.25 g	$200.00
5'-Fluorescein II CEP	BA 0253		
(6-FAM II)		100 µmol	$255.00
		0.25 g	$495.00
5'-Fluorescein CEP	BA 0054		
(6-FAM)		25 g min. order	Call for pricing

Diazaindacene NHS Ester—A new fluorophore

Lightner and co-workers[1] reported the synthesis and spectroscopic properties of fluorophores based on the 3a,4a-diazaindacene ring system ("xanthglows"). Key properties include a high quantum yield (>0.9) and a large Stokes shift (>100 nm). The relatively small size of this type of fluorophore may also be an advantage. We now introduce an amine-reactive version, Diazaindacene NHS Ester (Figure 16), for incorporation of the diazaindacene into biomolecules. The absorption maximum of the corresponding carboxylic acid is reported to be 425 nm in methanol, with an extinction coefficient of 15,300. The emission maximum of the carboxylic acid is 535 nm in methanol with a quantum yield of 0.91.[1a] When Diazaindacene NHS Ester was used to install this fluorophore onto a 5'-amino-modified oligonucleotide,[2] the absorption and emission maxima appear at 430 nm and 530 nm, respectively, in aqueous

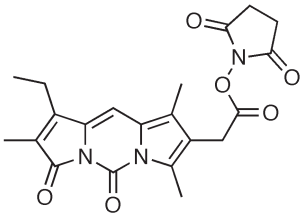


Figure 16. Diazaindacene, shown as its NHS ester, is a compact new fluorophore exhibiting an emission maximum at 530-535 nm, a large Stokes shift, and a high quantum yield.

$MgCl_2$/KCl/Tris-HCl (pH 8.0). For comparison, an analogous TET-labeled oligonucleotide showed absorption and emission maxima at 522 nm and 538 nm, respectively. A comparison of equimolar amounts of these two oligonucleotides indicated that the diazaindacene-labeled material exhibited a fluorescence intensity of 6% of the TET-labeled material, consistent with TET's larger extinction coefficient for absorption. When used in nucleic acid hybridization probes that rely on contact quenching, diazaindacene was efficiently quenched by dabcyl, BHQ-1, and BHQ-2; in FRET quenching mode, BHQ-2 was a good choice. While our initial studies have focused on nucleic acid labeling, where we believe diazaindacene is of interest because of its small size and large Stokes shift, this new fluorophore may also be useful for protein labeling.

References

(1) (a) Brower, J. O.; Lightner, D. A. J. *Org. Chem.* **2002**, *67*, 2713-2716. (b) Boiadjiev, S. E.; Lightner, D. A. *J. Phys. Org. Chem.* **2004**, *17*, 675-679. (c) Woydziak, Z. R.; Boiadjiev, S. E.; Norona, W. S.; McDohagh, A. F.; Lightner, D. A. *J. Org. Chem.* **2005**, *70*, 8417-8423.

(2) We thank Professor Salvatore A. E. Marras for these data.

Diae NHS Ester — Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
Diazaindacene NHS Ester	FD 13001		
		10 mg	$72.50
		50 mg	$269.50

A Selection of Nucleosides

While this issue of *Chemistry from Berry & Associates* focuses largely on monomers for nucleic acid synthesis, our company was founded on the chemistry of nucleosides. We continue to expand what is perhaps the world's largest collection of unusual nucleosides. Continuing the RNA theme of this issue, Figure 17 shows some rare ribonucleosides from our collection, many of which are new products. They are sold in small quantities for your convenience, but we are able to supply gram quantities if you require it—just call for pricing. Showdomycin, pseudouridine, pyrazofurin, formycin A, and formycin B are all C-ribonucleosides. Tubercidin, 5-iodotubercidin, toyocamycin, and triciribine are 7-deazaadenosines. 5-Iodotubercidin is a competitive inhibitor of MAP kinase ERK2. It also inhibits insulin receptor kinase, adenosine kinase, and various serine and threonine kinases. Triciribine (TCN) inhibits Akt (protein kinase B) in a very selective fashion, resulting in apoptosis in human cancer cells.[1] It also inhibits DNA synthesis,[2] and as its monophosphate, is active against HIV type 1.[3] Allopurinol riboside is the 8-aza-7-deaza analog of inosine. TOG (7-thia-8-oxoguanosine or immunosine) is an antiviral and immunostimulatory agent that activates, among other things, Toll-like receptor 7 (TLR7).[4]

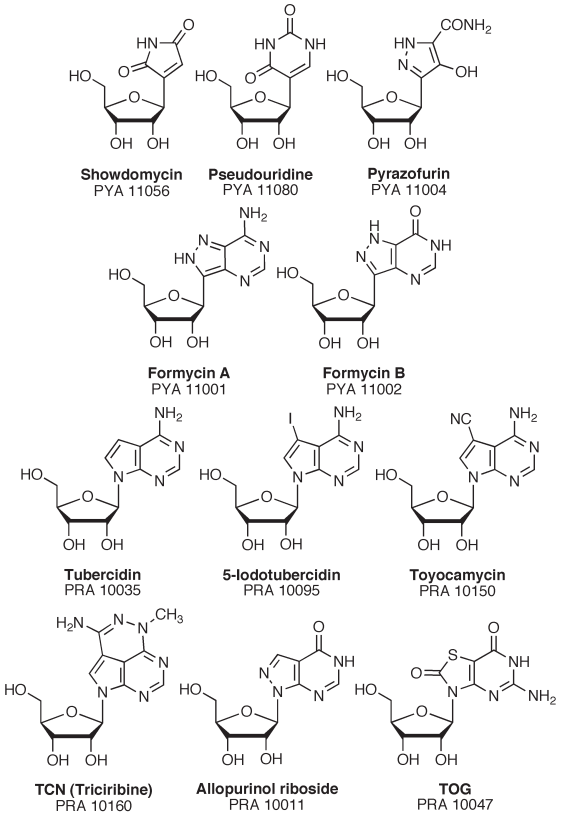


Figure 17. A selection of rare ribonucleosides from our collection.

References

(1) Yang, L.; Dan, H. C.; Sun, M.; Liu, Q.; Sun, X.-m.; Feldman, R. I.; Hamilton, A. D.; Polokoff, M.; Nicosia, S. V.; Herlyn, M.; Sebti, S. M.; Cheng, J. Q. *Cancer Res.* **2004**, *64*, 4394-4399.

(2) Wotring, L. L.; Townsend, L. B., Jones, L. M.; Borysko, K. Z.; Gildersleeve, D. L.; Parker, W. B. *Cancer Res.* **1990**, *50*, 4891-4899.

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(4) Lee, J.; Chuang, T.-H.; Redecke, V.; She, L.; Pitha, P. M.; Carson, D. A.; Raz, E.; Cottam, H. B. *Proc. Natl Acad. Sci. USA* **2003**, *100*, 6646-6651 and references therein.

Ribonucleosides — Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
Showdomycin	PYA 11056	10 mg	$295.00
		≥1 g	call
Pseudouridine	PYA 11080		
(From fermentation* or synthetic**)		10 mg*	$95.00*
		50 mg*	$170.00*
		1 g*	$995.00*
		1 g**	$1550.00**
Pyrazofurin	PYA 11004	10 mg	$245.00
		≥1 g	call
Formycin A	PYA 11001	10 mg	$245.00
		≥1 g	call
Formycin B	PYA 11002	10 mg	$195.00
		≥1 g	call
Tubercidin	PRA 10035		
(From fermentation* or synthetic**)		10 mg*	$75.00*
		25 mg**	$325.00**
		100 mg*	$245.00*
		100 mg**	$995.00**
		1 g*	$2395.00*
5-Iodotubercidin	PRA 10095		
		5 mg	$97.50
		25 mg	$395.00
Toyocamycin	PRA 10150		
(From fermentation* or synthetic**)		10 mg*	$75.00*
		25 mg**	$325.00**
		100 mg*	$245.00*
		100 mg**	$995.00**
		1 g*	$2395.00*
TCN (Triciribine)	PRA 10160	10 mg	$237.50
Allopurinol Riboside	PRA 10011		
		25 mg	$97.50
		1 g	$1450.00
TOG	PRA 10047		
(7-Thia-8-oxoguanosine)		10 mg	$395.00
		100 mg	$1200.00
		≥1 g	call

Something for the Environmental Chemist...

T-IP$_5$ — A probe for measuring phytase activity

The chemistry at Berry & Associates often involves nucleosides and nucleotides, but we are fundamentally synthetic organic chemists with broad interests. A recent collaboration with Professor Duane F. Berry, an expert in soil biochemistry and environmental chemistry at Virginia Tech, serves to illustrate the point.

Phytases catalyze the hydrolysis of phytic acid (*myo*-inositol hexakisphosphate, Figure 18), producing orthophosphate. Phytic acid is the major phosphorus-containing component of animal feed grain and is excreted in large quantities by swine and poultry, which lack phytase. In order to understand the fate of phytic acid in complex samples (e.g., soil, water-sediment environments, rumen, manure), it is important to develop a specific and sensitive quantitative enzyme assay for measuring phytase activity. We have synthesized T-IP$_5$ (tethered inositol pentakisphosphate) as a novel chromophoric substrate analog of phytic acid that allows the direct measurement of phytase activity.[1] Cleavage of phosphate ester bonds by phytase leads to dephosphorylated phosphatidylinositol intermediates that bear a tethered chromophore and may thus be quantified using reversed phase chromatography with UV detection. These intermediates have been identified and the HPLC assay of phytase activity has been optimized.[1b] We now offer T-IP$_5$ for those interested in the environmental issues surrounding phosphorus cycling.

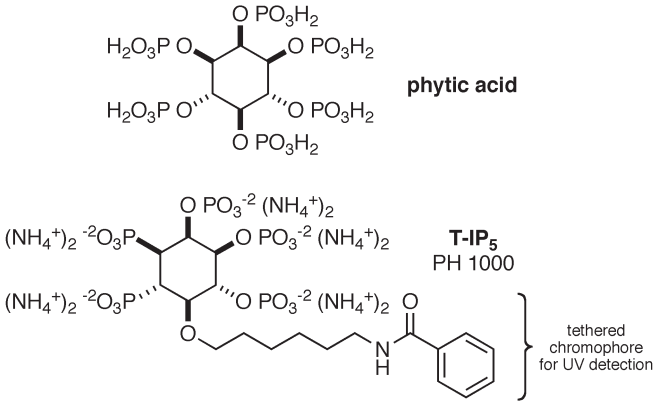

Figure 18. T-IP$_5$ is an analog of phytic acid that bears a tethered chromophore, making it useful in the analysis of phytase enzyme activity.

References

(1) (a) Berry, D. F.; Berry, D. A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3157-3161. (b) Berry, D. F.; Shang, C.; Waltham Sajdak, C. A.; Zelazny, L. W. *Soil Biol. & Biochem.* **2006**, in press.

T-IP$_5$ — Ordering Information

Item	Catalog No.	Size/pack	Price (USD)
T-IP$_5$	PH 1000		
		10 mg	$195.00
		25 mg	$345.00
		100 mg	$875.00

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