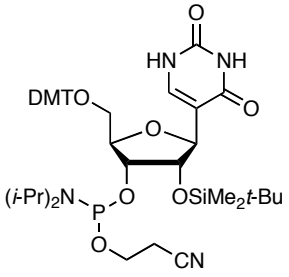
Pseudouridine CEP (BA 0280)

Product Information



Pseudouridine (Ψ) is one of the most common modified nucleosides found in RNA, e.g., in tRNAs and snRNAs.[1] 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. 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.[2] 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.[3-7] We now offer Pseudouridine CEP (Ψ CEP, BA 0280) for this purpose. This particular version[3,6] of pseudouridine phosphoramidite relies on standard cyanoethyl phosphoramidite coupling chemistry, 2'-*O*-TBDMS protection, and no nucleobase protecting groups.[5]

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.[2-5,7,8,9] For example,[1] 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 show that the uniquely visible[2,3,8] 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.[8] 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.[7] 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.[7] It is hoped that the availability of Pseudouridine CEP may facilitate further research on the customization of the structure and function of RNA oligonucleotides.

Coupling, cleavage, and nucleobase deprotection: In our hands, standard RNA coupling protocols using a 12 minute coupling time were successful in achieving >95%

coupling yields. Cleavage and nucleobase protection was accomplished by first passing 3:1 concentrated ammonium hydroxide:ethanol through the column over 1-1.5 h, then heating the eluate overnight at 55 °C. Please consult references 3 and 6 for further information on the use of this phosphoramidite in the synthesis and purification of pseudouridine-containing oligoribonucleotides, as well as references 4,5, and 7 for studies on related versions of pseudouridine phosphoramidite. Briefly, Agris and co-workers[6] used standard coupling methods. Cleavage from the CPG was accomplished with ethanol saturated with anhydrous ammonia for 12-17 h at 55 °C. Desilylation was carried out using Bu_4NF in THF as usual. Hall and McLaughlin[3] used concentrated aqueous ammonia/ethanol (3/1) for 6 h for their cleavage/deprotection. The silyl group was removed with 1 M Bu_4NF in THF for 16 h at rt.

References:

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(3) Hall, K. B.; McLaughlin, L. W. *Nucleic Acids Res.* **1992**, *20*, 1883-1889. See also reference 4 for a closely related phosphoramidite (NEtMe rather than N(*i*-Pr)$_2$).
(4) Gasparutto, D.; Livache, T.; Bazin, H.; Duplaa, A.-M.; Guy, A.; Khorlin, A.; Molko, D.; Roget, A.; Teoule, R. *Nucleic Acids Res.* **1992**, *20*, 5159-5166. This work describes a phosphoramidite similar to Pseudouridine CEP, but with an *N*-ethyl-*N*-methyl phosphoramidite.
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