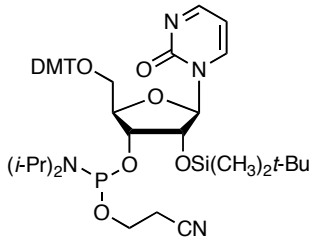
Zebularine CEP (BA 0254)

Product Information



Zebularine (a.k.a. 2-pyrimidinone-1-β-D-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 using standard phosphoramidite chemistry.[1,2] Substitution of zebularine for cytidine amounts to "atomic mutagenesis",[2] allowing studies of the role of a particular hydrogen bond in RNA duplexes.[1-3] 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.[1,3] Atomic mutagenesis 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.[2] 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.[1]

Coupling: Stockley and co-workers[3] reported the use of an ABI 391 synthesizer and standard 1 μmol DNA protocols, except the coupling times were extended to 15 min with an additional 1 s acetonitrile delivery at 7.5 min. They used ^{Bz}A, ^{Bz}C, and ^{iBu}G amidites.

In our hands, the standard 1 μmol RNA protocol on an Expedite 8909 synthesizer (12 min coupling times and standard dilutions) led to incorporation of zebularine with ca. 98% efficiency.

Cleavage and nucleobase deprotection: Literature reports recommend the use of methanol saturated with ammonia for cleavage and base deprotection.[4]

Caution: Carry out the cleavage/deprotection for 12-14 h.[3] Longer contact with ammonia leads to undesired side reactions.[1,3]

Desilylation and purification: "Standard methods" were employed in literature reports, e.g., with NEt_3•3HF at 30 °C for 30 h.[1-4]

References:

1. Adams, C. J.; Murray, J. B.; Arnold, J. R. P.; Stockley, P. G. *Tetrahedron Lett* **1994**, *35*, 1597-1600.

2. Beuning, P. J.; Gulotta, M.; Musier-Forsyth, K. *J. Am. Chem. Soc*. **1997**, *119*, 8397-8402.

3. Murray, J. B.; Adams, C. J.; Arnold, J. R. P.; Stockley, P. G. *Biochem. J*. **1995**, *311*, 487-494.

4. Murray, J. B.; Collier, A. K.; Arnold, J. R. P. *Anal. Biochem*. **1994**, *218*, 177-184.