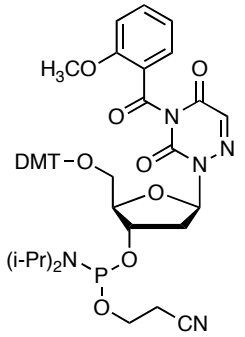
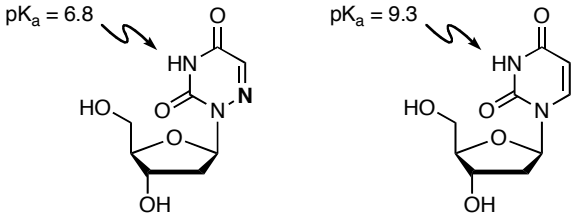
6-Aza-dU CEP
Product No. BA 0303
Product Information



$C_{46}H_{52}N_5O_{10}P$
Mol. Wt.: 865.91

As compared to dU residues, 6-aza-dU nucleotides are deprotonated at neutral pH, have enhanced 3'-exonuclease stability, and exhibit a high-anti glycosidic bond conformation. Metal-DNA (M-DNA) complexes are formed at neutral pH.

6-Aza-2'-deoxyuridine (6-aza-dU)[1] has a nitrogen atom in place of the methine group at position 6 of the uracil ring. This results in a significant lowering of the pKa of the N^3 hydrogen.[2] Unlike dU, 6-aza-dU is deprotonated at neutral pH, thus bestowing the uracil base with a negative charge. Further, there are significant conformational changes imparted by the presence of the 6-aza substitution: 6-Aza-dU has a high-anti conformation and is present in solution with *N*-type sugar pucker.



Seela and Chittepu have reported that 6-aza-dU nucleotides may be incorporated into oligodeoxyribonucleotides using phosphoramidite chemistry.[2,3] The N^3-*o*-anisoyl protecting group[4] is necessary to afford stability. DNA duplexes containing 6-aza-dU•dA base pairs have been studied[2] and are less stable than T•dA base pairs at neutral pH due to deprotonation of the N^3 hydrogen on 6-aza-dU. However, at lower pH, duplex stability increases as N^3 becomes protonated and therefore able to hydrogen bond to dA. The fact that 6-aza-dU residues are deprotonated at neutral pH results in easy metal-DNA (M-DNA) formation.[2] Canonical DNA forms M-DNA only at high pH, whereas 6-aza-dU-

containing DNA can form M-DNA at neutral or high pH. Finally, oligonucleotides containing 6-aza-dU show enhanced resistance to 3'-exonucleases (e.g., snake venom phosphodiesterase).

Coupling, deprotection, and purification: 6-Aza-dU CEP couples with greater than ≥95% efficiency using the standard protocols recommended for popular synthesizers. Seela[2] recommends deprotection of oligomers with 25% aqueous ammonia at 60 °C for 16 h.[5] Purification of the trityl-on oligonucleotide was accomplished by RP-HPLC (RP-18 column), after which the trityl group was removed with 2.5 % dichloroacetic acid in dichloromethane for 5 min. The detritylated oligomers were further purified via RP-HPLC and desalted on a short RP-18 silica column.

References:

1. (a) Kara, J.; Sorm, F. *Coll. Czech. Chem. Commun.* **1963**, *28*, 1441-1448. (b) Holy, A.; Cech, D. *Coll. Czech. Chem. Commun.* **1974**, *39*, 3157-3167. (c) Drasar, P.; Hein, L.; Beranek, J. *Coll. Czech. Chem. Commun.* **1976**, *41*, 2110-2123. (d) Freskos, J. N. *Nucleosides Nucleotides* **1989**, *8*, 549-555.

2. Seela, F.; Chittepu, P. *J. Org. Chem.* **2007**, *72*, 4358-4366.

3. For the incorporation of 6-azauridine into oligoribonucleotides, see: (a) Beigelman, L.; Karpeisky, A.; Usman, N. *Nucleosides Nucleotides* **1995**, *14*, 895-899 and (b) Oyelere, A. K.; Strobel, S. A. *Nucleosides Nucleotides Nucleic Acids* **2001**, *20*, 1851-1858.

4. Welch, C. J.; Chattopadhyaya, J. *Acta Chem. Scand. Ser. B* **1983**, *37*, 147-150.

5. In our hands, the N^3-*o*-anisoyl protecting group was removed in a few minutes with concentrated ammonium hydroxide. Presumably, other standard cleavage/deprotection protocols are viable.