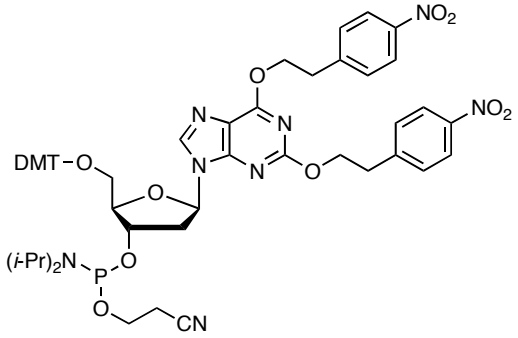
2'-Deoxyxanthosine CEP
(dX CEP)
Product No. BA 0313
Product Information

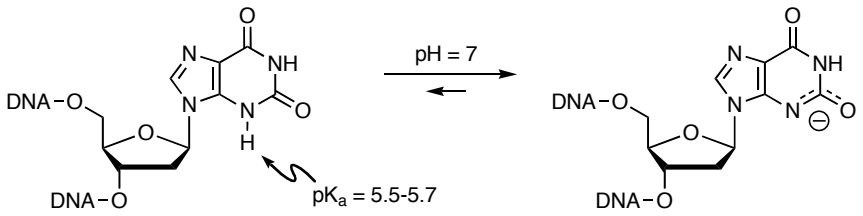


$C_{56}H_{61}N_8O_{12}P$
Mol. Wt.: 1069.10

2'-Deoxyxanthosine CEP (dX CEP) is useful for the installation of 2'-deoxyxanthosine (dX) nucleotides into oligonucleotides.[1,2]

Introduction: Xanthosine (X) and 2'-deoxyxanthosine (dX) have been incorporated into oligoribonucleotides[3] and oligodeoxyribonucleotides,[1,2,4-8] imparting a variety of properties to these nucleic acids. Examples include:

- The pKa of the proton at N^3 of dX is 5.5-5.7,[2,8] therefore dX is present as a mono-anion to the extent of about 95% at neutral pH.



2'-deoxyxanthosine (dX)

- dX residues have the same hydrogen-bond donor/acceptor pattern as thymidine, thus allowing base-pairing with dA.[8] This leads to a distortion of the helix, since two purines are paired. Stronger base stacking interactions are apparent. Duplex stability variations as a function of the opposing base pair have been studied.[4,8]

- dX residues are a product of deamination of dG.[2,8,9]

- dX is susceptible to depurination under acidic conditions, forming abasic sites.[2,8] At pH 4, dX depurinates more quickly than dG by a factor of about 1000. At neutral pH, dX has about the same stability as dG. The recognition and excision of dX by base excision repair enzymes has been studied.[2]

- dX has been considered to be a promiscuous or universal nucleoside.[1,8]

- dX may expand the genetic code when paired to a pyrimidine-2,4-diamine C-nucleoside.[6]

- Studies of polymerase-mediated incorporation of various nucleotides opposite dX have been carried out.[2,4]

- The presence of dX residues may lead to triplexes, quartets, and other G-stranded structures.[8]

For the incorporation of dX into oligonucleotides, we have chosen 2'-Deoxyxanthosine CEP (BA 0313), which bears 2-(4-nitrophenyl)ethyl (NPE) protecting groups on O^2 and O^6.[1,2] Other monomers for the incorporation of dX or X have been reported.[1,3,4-8] Those without O^2/O^6 protection appear to lead to by-products in their synthesis and use. A recent alternative to NPE protection involves allyl groups, which may be removed via Pd(0) catalysis.[8]

Coupling, deprotection, and purification:

Benner, Battersby, and co-workers[1] synthesized oligonucleotides using dX CEP and standard methods with the exception of an extended coupling time of 3 minutes, the use of 1*H*-imidazole-4,5-dicarbonitrile (0.25 M) as activator, and dichloroacetic acid (2.5% in dichloromethane) to detritylate. The alternate activator and deblock reagent were precautions to avoid depurination of dX. For cleavage and deprotection, the solid support bearing the trityl-off oligonucleotide was treated with concentrated aqueous ammonium hydroxide for 24-40 h at rt. Evaporation, suspension of the residue in water, filtration through a 0.2 μm filter, and evaporation was carried out. The residue was dissolved in and aqueous dioxane solution containing *N*,*N*,*N*',*N*'-tetramethylguanidinium 2-nitrobenzaldoximate and heated at 70 °C for 18 h, followed by quenching with sodium citrate and various extractions (see reference 1 for details). If the cleavage and deprotection was carried out with ammonium hydroxide only (48 h at 60 °C), about a third of the oligonucleotide had iso-dG residues, resulting from

displacement of the 2-(4-nitrophenyl)ethoxy group at position 6 of the purine nucleobase.

Wuenschell, O'Connor, and Termini[2] synthesized oligonucleotides using dX CEP via standard methods, except for the presence of 4 Å molecular sieves in the acetonitrile solution of the phosphoramidite. The oligonucleotide was synthesized trityl-off, then cleaved from the support with 30% aqueous ammonia at 50 °C for 16 h. The NPE protecting groups were subsequently removed with DBU in dry pyridine at rt for 18 h. Various additional manipulations (see reference 2 for details) gave the desired material. No mention of iso-dG formation was made.

In our hands, dissolving 2'-Deoxyxanthosine CEP in dry acetonitrile without sieves followed by standard protocols as recommended by the synthesizer manufacturer led to >95% yield of incorporation of dX nucleotides with no requirement for extended coupling times. The only modification was the use of 0.25 M 5-ethylthio-1*H*-tetrazole (ETT) rather than 1*H*-tetrazole, the use of which was not attempted. Cleavage from the controlled-pore glass solid support was carried out with concentrated aqueous ammonium hydroxide at rt as per normal protocols. The resultant ammonium hydroxide solution can be used to deprotect other (non-dX) nucleobase protecting groups (e.g., rt for 24 h or 55 °C for 16 h; see Benner, *et al*., for concerns about higher temperatures[1]). Under these conditions, we found that the NPE groups were not removed. Consult references 1 and 2 for protocols for NPE removal. Finally, note that the two NPE groups impart substantial lipophilicity to the oligonucleotide, resulting in a longer retention time on an RP-HPLC column than normal.

References:

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