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New Chirally Modified Bisphosphonates for Synthesis of Individual Beta,Gamma-CHX-Deoxynucleotide Diastereomers

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Abstract

Individual diastereomers of CXY bisphosphonate analogues of dNTPs or NTPs are useful chemical stereoprobes to investigate interactions within the chiral active site environment of enzymes such as polymerases and kinases. We previously reported synthetic access to β,γ -CHX-dGTPs (X = F or Cl) via a bisphosphonate synthon with an (R)-methyl mandelate auxiliary and have extended this approach to dTTP and dATP analogues. As removal of the chiral auxiliary by (Pd/C) hydrogenolysis is incompatible with the cytosine heterocycle and also with X = Br, we have now designed bisphosphonate synthons using (R)-(+)- α -ethylbenzylamine or methyl (R)-(-)-phenylglycine auxiliaries and equipped with an o-nitrobenzyl ester protecting group allowing photochemical deprotection. These new synthons have made possible the first syntheses of individual dCTP and monobromo-substituted dNTP β,γ -CHX diastereomers.

Keywords

chiral bisphosphonates; dNTP probes; polymerase mechanism

RESULTS AND DISCUSSION

β,γ -CXY-Modified deoxynucleoside 5'-triphosphate analogues are useful chemical probes to investigate DNA polymerase structure and function. Conventional synthetic methods generate a mixture of diastereomers in the case of nonequivalent CXY groups (X = Y). We have addressed the problem of preparing individual diastereomers of β,γ -CXY-dNTPs by conversion of corresponding prochiral bisphosphonate precursors to chiral synthons. We previously reported synthesis of individual stereoisomers of β,γ -CHX-dGTP (X = F, Cl)^{1,2} and β,γ -CHF-ATP³ using stereoselective Mitsunobu condensation to connect an (R)-methyl mandelate auxiliary to the corresponding prochiral bisphosphonic acids, resulting in diastereomers susceptible to separation by standard preparative reverse phase HPLC (Scheme 1). A cognate approach using methyl (R)-(-)-phenylglycine as the auxiliary has been used to obtain α,β -CHX-ATP stereoisomers.⁴

The (Pd/C) hydrogenolysis deprotection step in this procedure² is incompatible with both the cytosine heterocycle and a bromo-substituted methylenebisphosphonate. We have now devised chiral CHX bisphosphonate (*R*)-(+)- α -ethylbenzylamide synthons equipped with an *o*-nitrobenzyl ester protecting group, resulting in isomer pairs that are chromatographically separable (Scheme 2). Selective acidic hydrolysis of the amide provides a phosphonate site for coupling to an activated dCMP, followed by photochemical deprotection to give the desired individual β,γ -CHX-dCTP diastereomer (X = F, Cl, Br). Product nucleotide configurations were assigned by x-ray crystallography of the ternary DNA-pol β complexes.⁵ These probes are providing insights into the base-pairing dependent mechanism of pol β DNA gap repair.^{5,6}

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Scheme 1. Synthesis of chiral bisphosphonates using *(R)*- or *(S)*-methylmandelate esters

Scheme 2.

o-Nitrobenzyl ester (*R*)-(+)- α -ethylbenzylamide route to individual diastereomers of β,γ -CHX-dNTPs