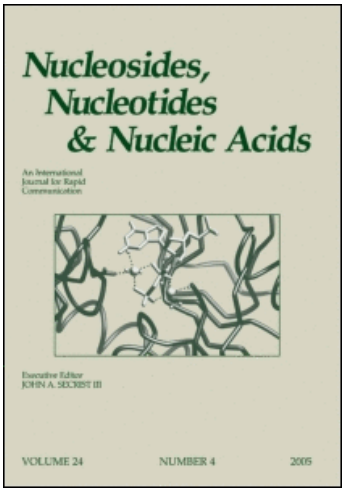

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Replicative Capability of Anhydrohexitol Analogues of Nucleotides

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REPLICATIVE CAPABILITY OF ANHYDROHEXITOL ANALOGUES OF NUCLEOTIDES

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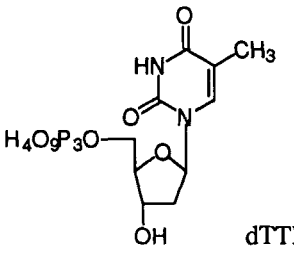
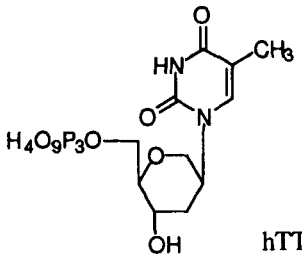
ABSTRACT : The reactivity of hTTP, the anhydrohexitol triphosphate analogue of dTTP, was investigated in enzymatic replication assays using deoxyadenosine (dA) or the anhydrohexitol analogue of dA (hA) as templates.

Molecules able to replicate and to propagate *in vivo* and *ex vivo* sequences of the four canonical bases grafted to backbones differing from the ribose and deoxyribose phosphodiester motifs of RNA and DNA would enable numerous scientific and industrial advances. 1,5-Anhydrohexitol nucleic acids (HNA) are the informational polymers with a six-membered carbohydrate moiety showing the closest chemical and structural resemblance to DNA. They represent a stable and selective pairing system which make them potential useful as antisense polymers.



dTTP



hTTP

We examined the ability of hTTP to substitute for dTTP in primer extension reactions catalyzed by several DNA polymerases. For this purpose, oligonucleotides listed above were synthesized.

Primer	5'-d(CAGGAAACAGCTATGAC)-3'
Template 1	3'-d(GTCCTTTGTCGATACTGACCCC)-5'
Template 2	3'-d(GTCCTTTGTCGATACTGhACCCC)-5'

As shown in Figure 1, the Klenow fragment of *Escherichia coli* DNA polymerase I deficient in 3'→5' exonuclease activity (KFexo⁻) catalyzes the specific incorporation of hTTP in response to dA in the template. When KFexo⁻ (0.06 U/μL) was incubated at 37 °C for 3 min in the presence of hTTP (10 μM) with the labelled primer/template **1** duplex (200 nM), primer +1 was produced with an efficiency comparable to the incorporation of dTTP (10 μM). In the elongation conditions used, neither hTTP nor dTTP was incorporated opposite C, G or T in template, indicating that hTTP requires correct Watson-Crick pairing to react (not shown). In the presence of a mixture of hTTP and dGTP, the full length product was formed with a little pausing at the position +2. Synthesis of the full length product could be enhanced by using a higher concentration of KFexo⁻ (0.07U/μL).

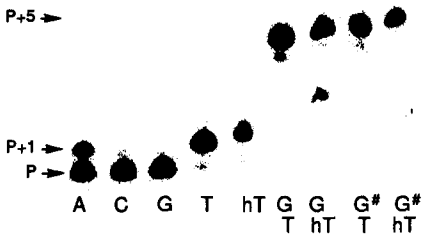


Figure 1 : Primer extension using template **1** catalyzed by KFexo⁻ (0.06 U/μL, or 0.07 U/μL when indicated by #).

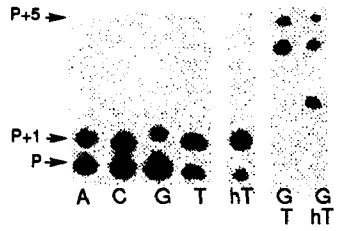


Figure 2 : Primer extension using template **2** catalyzed by KFexo⁻ (0.05 U/μL).

In addition, the analogue hA was found to command the incorporation of both dTTP or and hTTP but not of non-complementary dNTPs. As illustrated in Figure 2, when KFexo⁻ (0.05 U/μL) was incubated at 37 °C for 3 min with the labelled primer/template **2** duplex (80 nM) in the presence of hTTP (10 μM), primer +1 was produced with an efficiency comparable to the incorporation of dTTP (10 μM) (Figure 2). In the presence of a mixture of hTTP and dGTP (10 μM each), primer +4 and +5 were formed with a little pausing at the position +2.

Taq polymerase and Sequenase were also found to catalyze the specific incorporation of hTTP in both these copying assays. Altogether, these results exemplify the biosynthetic potential that can be explored by subjecting artificial monomers to natural enzymes, and augur favourably for the proliferation of new types of nucleic acids.

REFERENCE

Hendrix, C.; Rosemeyer, H.; de Bouvere, B.; Van Aerschot, A.; Seela, F. and Herdewijn, P. *Chem. Eur. J.*, **1997**, 3(9), 1513-1520.