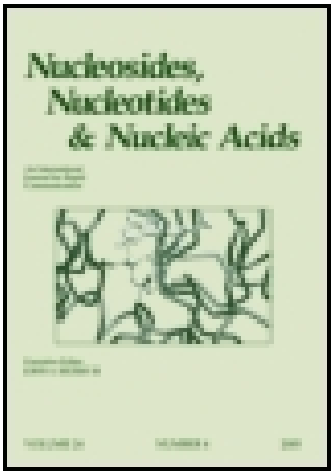

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Hexitol Nucleic Acids (HNA): Synthesis and Properties

B. De Bouvere [a] , L. Kerreinans [a] , C. Hendrix [a] , H. De Winter [a] , G. Schepers [a] , A. Van Aerschot [a] & P. Herdewijn [a]
[a] Rega Institute for Medical Research, Katholieke Universiteit Leuven , Minderbroedersstraat 10, 3000, Leuven, Belgium
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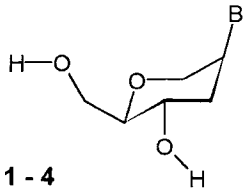
HEXITOL NUCLEIC ACIDS (HNA) : SYNTHESIS AND PROPERTIES

B. De Bouvere, L. Kerremans, C. Hendrix, H. De Winter, G. Schepers,
A. Van Aerschot* and P. Herdewijn
Rega Institute for Medical Research, Katholieke Universiteit Leuven,
Minderbroedersstraat 10, 3000 Leuven, Belgium.

ABSTRACT: While improved alkylation procedures have been worked out for the coupling of purine bases to the anhydrohexitol ring using sulphonate activating groups on the anhydrohexitol ring, the Mitsunobu reaction seems to be the method of choice for synthesis of the pyrimidine analogues. In a mixed sequence context, the anhydrohexitol oligonucleotides still display strong and very selective basepairing properties, with a strong preference for RNA as the complement.

Over the last years, very promising new constructs have been described hybridizing strongly and selectively with as well deoxy- as ribooligonucleotides. The peptide nucleic acids or PNA's are the most stable, but these polyamide linked acyclic nucleic acids suffer from low water solubility and limited uptake[1]. A more recent finding are the RNA selective N3'→P5' phosphoramidates, affording more stable duplexes with an average $\Delta Tm = 2°C$ per base pair versus RNA[2].

We recently described a new construct consisting of 1,5-anhydrohexitol nucleoside analogues **1-4** and discussed its structural resemblance with natural oligonucleotides[3]. While previously, synthesis of these analogues hA, hT, hC and hG (**1-4**) was time-consuming[4-6], new batches of the different monomers were obtained by alkylation of the respective nucleobases with the carbohydrate part. The latter was activated with either a triflate or tosylate as the leaving moiety analogously to Bisacchi et al.[7].



1 - 4

1 hA, B = adenin-9-yl
2 hT, B = thymin-1-yl
3 hC, B = cytosin-1-yl
4 hG, B = guanin-9-yl

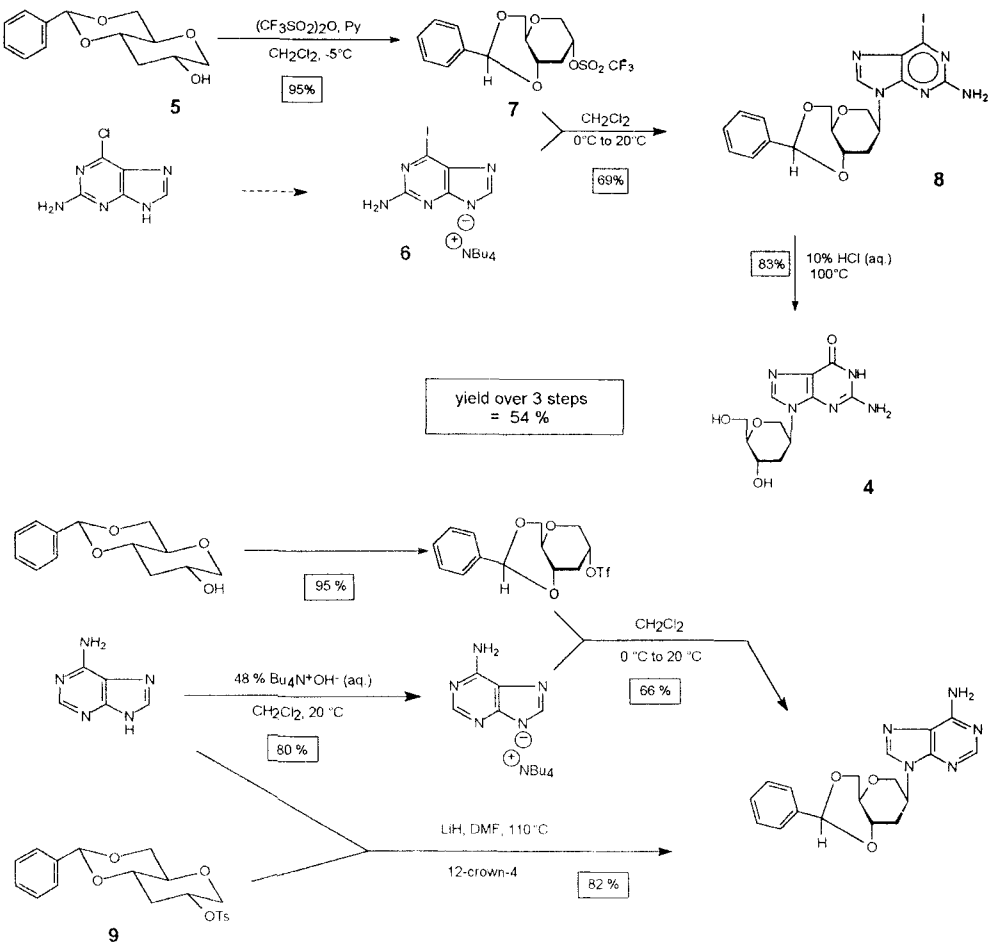


FIG. 1. Different alkylation strategies for synthesis of the purine anhydrohexitol derivatives **1** (hA) and **4** (hG).

Using the readily soluble tetrabutylammonium salt **7** of 2-amino-6-iodopurine[7], alkylation of the triflate activated anhydrohexitol **6** was straightforward affording **8** in 69% isolated yield. Deprotection of the benzylidene moiety was accomplished concomitantly with hydrolysis of the iodo group upon treatment with a 10% aqueous HCl solution at reflux yielding 83% of the deoxyguanosine analogue **4**. Applying the same methodology for synthesis of the adenosine congener **1**, alkylation of the triflate **6** afforded 66% of the benzylidene protected hA. However, making use of the tosylate **9**,

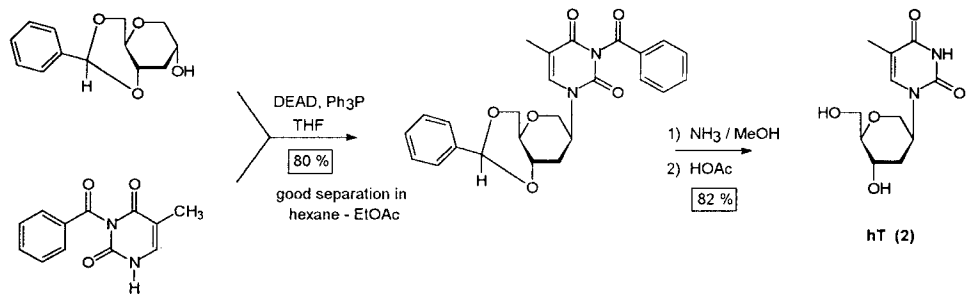


FIG. 2. Mitsunobu reaction as applied for the synthesis of the anhydrohexitol thymine congener **2**.

TABLE 1 : Melting temperatures for mixed sequences highlighting the selectivity of HNA for RNA versus DNA. Tm's were determined at 4 μm in a phosphate buffer pH 7.5 containing 0.1 M NaCl.

Sequence	RNA	DNA
h(CGACGGCG)-propanediol	65.3	46.3
d(CGACGGCG)-propanediol	45.7	51.5
h(CACCGACGGCGC)-propanediol	79.8	59.2
d(CACCGACGGCGC)-propanediol	63.1	69.4
h(AGG GAG AGG AGA)-propanediol	84.0	64.8
d(AGG GAG AGG AGA)-propanediol	47.6	49.0

optimisation of the old protocol using LiH in DMF at 110°C in the presence of 12-crown-4 gave an excellent 82% yield for the alkylation in a more convenient way.

Alkylation of pyrimidines on many occasions has been much more problematic, which proved to be the case here as well. Evaluating different sulphonates as leaving moieties in the presence of either LiH, NaH or KH always gave a mixture of the N^1- and O^2-alkylated species in more or less the same ratio. Best results were obtained with the Li salt of thymine but scaling-up gave solubility problems. In view of the low yields for alkylation in the pyrimidine series, we turned our attention back to the Mitsunobu reaction[4,6], which afforded excellent results for the thymine derivative (80% for the alkylation) when the reaction was performed in THF. These reactions are currently being further optimized and applied to other pyrimidines as well.

As shown before[3], a HNA-hexamer and a HNA-dodecamer mixed purine sequence hybridized strongly with as well DNA as RNA, providing a more stable duplex than the one made up of either the respective DNA-DNA or DNA-RNA counterparts. Especially the HNA-RNA interaction is very strong, indicated by an increase of the Tm versus the DNA-RNA control duplex with 36°C for the purine dodecamer (Table 1) and 34°C for the hexamer[3]. Melting studies of duplexes with mixed sequences confirm the strong and very selective base-pairing properties of HNA with a complementary RNA sequence as exemplified by the data of Table 1.

Modelling studies as well indicate the potential of HNA to hybridize straightforwardly with its complementary ribooligonucleotide. A 1100 psec thermodynamics simulation of a HNA-RNA octamer duplex in water did not change the overall picture which resembles very much a classical A-RNA double helix.

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