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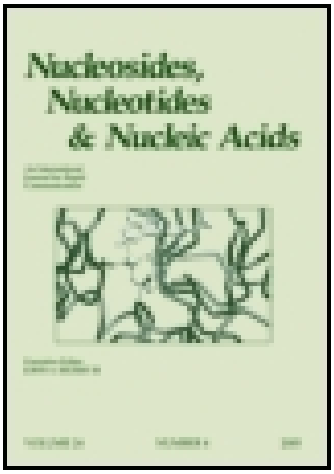

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Chemical and Enzymatic Synthesis of 2′-Deoxy-iso-inosine and Its Incorporation into DNA

Jean-Jacques Beaussire [a] & Sylvie Pochet [a]
[a] Unité de Chimie Organique, URA-CNRS 487, Département de BGM , Institut Pasteur, 28, rue du Dr Roux , 75724, Paris Cedex
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NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 805-808 (1995)

CHEMICAL AND ENZYMATIC SYNTHESIS OF 2'-DEOXY-*ISO*-INOSINE AND ITS INCORPORATION INTO DNA

Jean-Jacques Beaussire and Sylvie Pochet*
Unité de Chimie Organique, URA-CNRS 487, Département de BGM,
Institut Pasteur, 28, rue du Dr Roux, 75724 Paris Cedex.

Abstract: Two procedures for the preparation of 2'-deoxy-*iso*-inosine (**1**) are presented. Synthesis of 3'-phosphoramidite (**7**) and 3'-phosphonate (**8**) derivatives are described, as well as an oligodeoxynucleotide containing *iso*-I.

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In 1962, Rich[1] proposed the *iso*-C/*iso*-G base pairing system as potentially suitable for incorporation into nucleic acids because it forms a hydrogen-bonding pattern distinct from the natural A/T(U) or G/C base pairs. Benner et al.[2] demonstrated that *iso*-G can be incorporated into RNA using d-*iso*-C containing templates and T7 RNA polymerase. However, the fidelity of incorporation of *iso*-C opposite *iso*-G with DNA polymerase was low due to partial deamination of *iso*-C to produce dU, and to tautomeric equilibria of *iso*-G, the minor imino-enol form being complementary to T(U).

Our research group is concerned with diversifying the set of building blocks that can be incorporated in nucleic acids *in vitro* and *in vivo*. We are currently improving the *iso*-C/*iso*-G scheme for both criteria of stability and replicative fidelity of the purine and pyrimidine deoxynucleoside partners. The pairing ambiguity of *iso*-G with U depends on the presence of the 6-amino group as in A. Removing this amino group leads to the base 2-hydroxypurine (*iso*-I) able to form two hydrogen-bonds with *iso*-C as A/T, but which should not pair with any of the four canonical bases.

In this paper we report the synthesis of 2'-deoxy-*iso*-inosine (**1**) by two different routes : chemical deamination of the easily accessible 2-aminopurine-2'-deoxyriboside (**2**) and enzymatic transglycosylation of 2-hydroxypurine using a crude preparation of nucleoside deoxyribosyltransferases.

The nitrous acid deamination of 2-aminopurine riboside for the synthesis of *iso*-I riboside used by Holy[3] has been applied to the deoxy series.

Preparation of nucleoside analogs by biotransformation is a promising alternative to chemical synthesis. Nucleoside deoxyribosyltransferases are known to catalyze the exchange of the deoxyribosyl moiety between a 2'-deoxyribonucleoside (donor) and a

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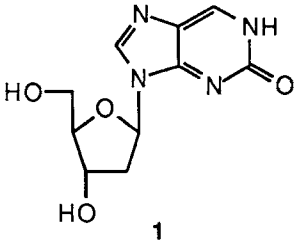
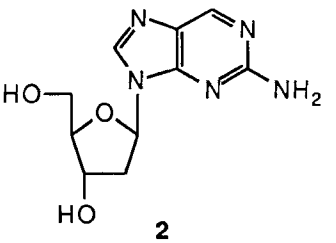
purine or pyrimidine base (acceptor). However, the reaction proceeds with a low yield when cell-free extracts of microbial cells are used. Enzyme purification and limited stability restrain their use in large-scale procedures. Recently, Hutchinson et al.[4] have demonstrated the beneficial effect of organic solvents on the enzymatic synthesis of nucleoside analogs using a crude preparation of N-deoxyribosyltransferases from Lactobacillus *leichmannii*. Following this procedure, 2'-deoxy and 2',3'-dideoxy-ribonucleosides of 2-aminopurine have been synthesized in 57% and 80% yield, respectively.[5]



1



2

First, we synthesized 2-amino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (**2**) on a mmol scale by incubating 2-aminopurine (1 eq.) and 2'-deoxycytidine (3 eq.) in 10 mM citrate buffer (pH 6) with a crude extract of N-deoxyribosyltransferases from L. *leichmannii* in the presence of ethanol (10% v/v) at 40°C for 2 days. Purification by chromatography on silica gel afforded compound **2** in 86% yield. Reaction of **2** with $NaNO_2$ in acetic acid at 4°C yield 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purin-2(3H)-one (**1**) in 70% yield. The structure of compound **1** was established by ^1H and ^{13}C NMR spectroscopy[6].

We next investigated the possible enzymatic trans-glycosylation of 2-hydroxypurine. In a study of the specificity of L. *helveticus* N-deoxyribosyltransferase, Cardinaud[7] observed the transfer of 2-hydroxypurine. Identification was performed by chromatography of the radioactive deoxynucleoside produced, but neither the yield nor the stereochemistry of the transfer reaction were given. Employing encapsulated bacterial cells, Holy and Vortruba[8] have shown that 2-aminopurine was converted in 73% yield, while 2-hydroxypurine was not a substrate.

We have performed the synthesis of **1** on a large-scale using a crude extract of N-deoxyribosyltransferases from L. *leichmannii*. Thymidine was used as the donor to permit more efficient chromatographic separation since the alternative donor, 2'-deoxycytidine, migrated close to the resulting 2-hydroxypurine nucleoside. Thus, 2-hydroxypurine (1 eq.) and thymidine (3 eq.) were incubated in 10 mM citrate buffer (pH 6) at 40°C with a crude extract of L. *leichmannii* in presence of ethanol. The reaction was monitored by HPLC. After 5 days, equilibrium was reached and only one nucleoside

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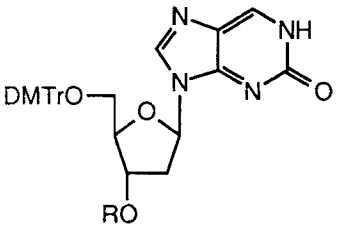
was formed. The mixture was concentrated to dryness and purified by chromatography on silica gel to yield 86% of d-*iso*-I (**1**). Spectral characteristics of **1** were identical to those of the nucleoside obtained by deamination of 2-aminopurine-2'-deoxyriboside[6].

The use of a crude extract of L. *leichmannii* made the transfer reaction a convenient route to produce d-*iso*-I on a larger scale as compared to the alternative chemical route. Further advantages of this route are that 2-hydroxypurine is commercially available, d-*iso*-I can be conveniently isolated by chromatography and excess thymidine can be recycled.

The nucleoside **1** was converted into the 5'-DMTr derivative **6** in 65% yield. The glycosidic bond was found to be stable towards detritylation conditions (2% trichloroacetic acid, 2% benzensulfonic acid, 80% AcOH). Phosphitylation of **6** with 2-cyanoethyl N,N,N',N'-tetraisopropylphosphoramidite and diisopropylammonium tetrazolide in CH_2Cl_2 afforded the 3'-phosphoramidite **7**[9] in 63% yield. The 3'-phosphonate **8**[10] was also synthesized by reaction of **6** with tris-(1,2,4-triazolyl)-phosphite in dry CH_3CN followed by hydrolysis with aqueous $(Et_3NH)HCO_3$ solution in 57% yield.



6 R = H
7 R = $P(OCH_2CH_2CN)(NiPr_2)$
8 R = H-$P(O)O^-$ Et_3NH^+

Incorporation of phosphoramidite **7** into oligodeoxynucleotide was accomplished using an automated DNA synthesizer (Applied Biosystems model 380A). As an example, a 21-mer (5'-CCGGAACGCGCC***iso*-I**CCACTGCA-3') containing one d-*iso*-I residue was synthesized on a 1 μmol scale using a 0.17M concentration of **7** in CH_3CN. A normal synthesis cycle was used except the coupling time for **7** was 15 min. Coupling yield measured by the amount of released DMTr cation was satisfactory (30% overall yield). The crude oligomer was purified by reverse-phase HPLC. Enzymatic degradation of an aliquot (snake venom phosphodiesterase and alkaline phosphatase) followed by HPLC analysis of the resulting monomers indicated the expected ratios. The spectral absorption at 315 nm of d-*iso*-I allowed the correct sequence of synthesized oligomer to be confirmed; access to such oligomers was thereby demonstrated.

During the progress of this work, Seela[11] reported a chemical route to d-*iso*-I starting from 2'-deoxyguanosine in 35% yield.

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Elongation reactions by DNA polymerases using oligomers containing *iso*-I residue as template and natural triphosphates are under way. The 5'-triphosphate derivative of d-*iso*-I has been prepared. Experiments aimed at incorporating this new base into DNA by enzyme action will be reported elsewhere.

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6 **1** : UV (H_2O) λmax = 314 nm (3700). MS (CI, NH_3) m/z 253 (M+H). HPLC (C18, 0 to 15% CH_3CN in 10mM TEAA over 20 min. at a flow rate of 5.5 min.) Rt = 10.3 min. ^1H NMR (D_2O) δ : 2.20 (m, 1H, H2'); 2.72 (m, 1H, H2"); 3.74 (m, 2H, H5' and H5"); 4.06 (m, 1H, H4'); 4.56 (m, 1H, H3'); 6.26 (t, 1H, H1', $J_{1,2'} = J_{1,2"} = 6.8$ Hz); 8.32 (s, 1H, H8); 8.38 (s, 1H, H6). ^{13}C NMR (D_2O) δ : 39.16 (C2'); 62.18 (C5'); 71.73 (C3'); 84.62 (C1'); 87.98 (C4'); 124.96 (C5); 139.58 (C6); 147.67 (C8); 158.41 (C2); 159.28 (C4). Anal. Calcd for $C_{10}H_{12}N_4O_4$ (252.2 +1.5 H_2O): C, 43.00; H, 5.41; N, 20.06. Found: C, 43.15; H, 5.12; N, 20.45.
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9 **7** : ^{31}P NMR ($CDCl_3$): 147.0 and 146.8 ppm.
10 **8** : ^{31}P NMR ($CDCl_3$): 1.1 ppm ($^1J_{PH} = 621$ Hz, $^3J_{PH} = 8.9$ Hz).
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