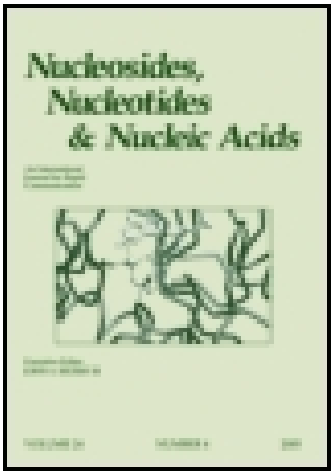


Nucleosides and Nucleotides
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lncn19

Oligodeoxynucleotides Embodying the Ambiguous Base Z, 5-Amino-imidazole-4-carboxamide

Sylvie Pochet [a] & Laurence Dugué [a]
[a] Unité de Chimie Organique URA CNRS 487, Département de BGM , Institut Pasteur 28, rue du Docteur Roux 75754, Paris Cedex, France
Published online: 24 Sep 2006.

To cite this article: Sylvie Pochet & Laurence Dugué (1995) Oligodeoxynucleotides Embodying the Ambiguous Base Z, 5-Amino-imidazole-4-carboxamide, Nucleosides and Nucleotides, 14:6, 1195-1210, DOI: 10.1080/15257779508010683

To link to this article: http://dx.doi.org/10.1080/15257779508010683

NUCLEOSIDES & NUCLEOTIDES, 14(6), 1195-1210 (1995)

OLIGODEOXYNUCLEOTIDES EMBODYING THE AMBIGUOUS BASE Z, 5-AMINO-IMIDAZOLE-4-CARBOXAMIDE

Sylvie Pochet* and Laurence Dugué

Unité de Chimie Organique, URA CNRS 487, Département de BGM, Institut Pasteur, 28, rue du Docteur Roux, 75754 Paris Cedex, France.

Abstract: Short oligomers containing 5-amino-1-(2-deoxy-β-D-ribofuranosyl)imidazole -4-carboxamide (dZ) were synthesized in solution using the phosphotriester methodology. Usual acyl groups were used for the canonical bases. For the exocyclic amino function of Z residue, the hydrogenolyzable benzyloxycarbonyl group was introduced.

INTRODUCTION

AICAR (5-amino-imidazole-4-carboxamide-riboside) is a central intermediate in the *de novo* purine biosynthetic pathway[1]. The base of this nucleotide is expected to pair with three of the four canonical bases of DNA, owing to the rotation of its carboxamide group (not shown). In a recent study[2], we suggested that this nucleotide, acting as a promutagen, could be converted into the corresponding 2'-deoxynucleoside 5'-triphosphate (dZTP), which in turn could be incorporated into DNA chains during replication and thereby induce mutations. We synthesized the triphosphate dZTP and showed that it served as a substrate for terminal deoxynucleotidyltransferase[2]. We also demonstrated that dZTP can be incorporated by DNA polymerase I (Klenow fragment) in place of dATP and dCTP (unpublished results). In order to further investigate the base-pairing properties of Z base in DNA duplexes, we needed sizeable amounts of oligonucleotides containing dZ at uniquely defined positions.

The chemical synthesis of an oligonucleotide containing 5-amino-1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (dZ) requires the preparation of a suitably protected dZ phosphotriester (or phosphoramidite). The sensitivity of the nucleoside dZ to ring closure into a purine and the possible dehydratation of the carboxamide group to form

a cyano group rendered the choice of a protecting group for the exocyclic amino function more delicate than for the standard bases.

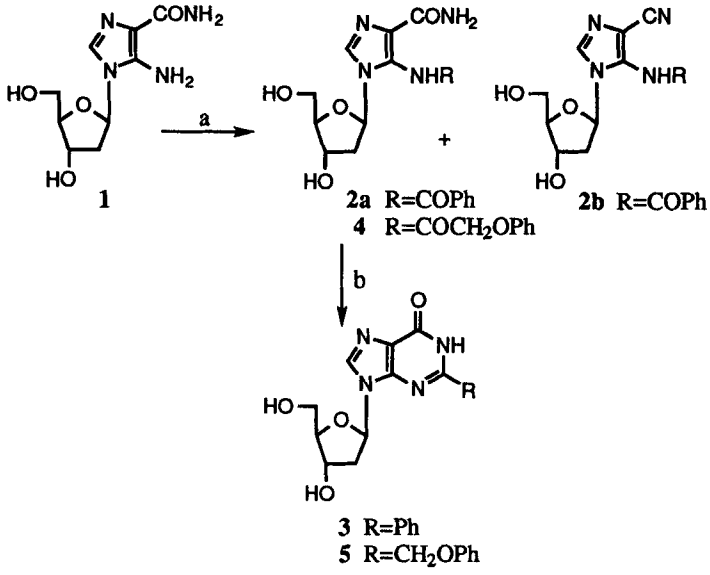
In this report, we describe the chemical synthesis of oligodeoxynucleotides containing the non-canonical base, 5-amino-imidazole-4-carboxamide. The hydrogenolyzable benzyloxycarbonyl (CBz) group was introduced for protecting the exocyclic amino function. Common acyl groups were found to lead to ring closure derivatives under alkaline conditions of deprotection. The short oligomers d(ZAC) and d(CACZAC) were synthesized following the phosphotriester method in solution. An oligodeoxynucleotide containing a dZ residue has been previously reported as a side-product in the incorporation of 2-azadeoxyinosine[3] following the phosphoramidite methodology.

RESULTS AND DISCUSSION

Perbenzoylation of 5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide under standard conditions led to the concomitant dehydration of the carboxamide function[4]. We have observed that the use of dichloromethane as solvent reduced this side reaction. Thus, peracylation of dZ (**1**) with a slight excess of benzoyl chloride in dichloromethane with pyridine, followed by treatment with 2N NaOH, afforded the N-benzoylated carboxamide **2a** (45% yield), along with the N-benzoylated carbonitrile **2b** (8% yield). Treatment of compound **2a** with 28% aqueous ammonia at 60°C afforded the inosine derivative **3** in quantitative yield (Scheme 1).

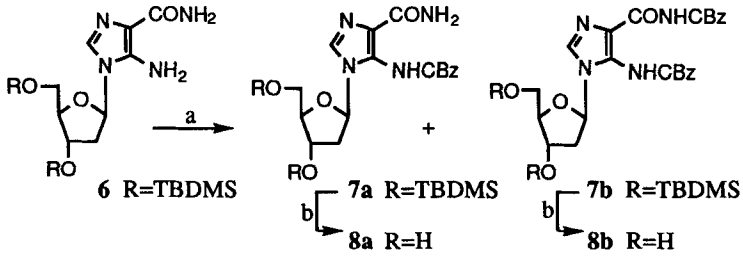
The phenoxyacetyl group (PhOAc) was introduced for protecting the exocyclic amino groups of 2'-deoxyadenosine and 2'-deoxyguanosine[5,6]. This group can be removed very quickly under mild alkaline condition at room temperature. Phenoxyacetylation of dZ was achieved via the transient procedure[7]. Thus, silylation with trimethylsilyl chloride in anhydrous dichloromethane in the presence of pyridine, followed by reaction of phenoxyacetyl chloride, gave the N-phenoxyacetyl derivative **4** in 30% yield (Scheme 1). In a preliminary attempt to cleave the PhOAc group, compound **4** was treated with aqueous ammonia. Unfortunately, after a few hours at room temperature compound **4** still remained, and only a trace of the nucleoside **1** could be detected. By raising the temperature, compound **1** was formed along with the product **5** from alkaline ring closure reaction.

We also investigated the protection of dZ using the 4-nitrophenylethyl (NPE) group, which can be cleaved by a mild β-elimination process. No reaction occurred between dZ (or 3',5'-O-diacetyl dZ) and 1-methyl-3-(*p*-nitrophenyl-ethoxycarbonyl)imidazolium chloride according to reported conditions for dA and dC[8,9], probably due to the low reactivity of the amino function of dZ.



SCHEME 1: Alkaline rearrangement of 5-N acylated dZ a) BzCl in CH_2Cl_2/pyridine, then 2N NaOH or $PhOCH_2COCl$ via transient protection; b) 28% NH_4OH.

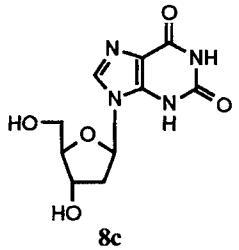
Finally, the exocyclic amino function of dZ was blocked as its 5-N-benzyloxycarbamate. The CBz group, which may be removed under neutral hydrogenolytic conditions, has been applied to nucleoside chemistry (phosphotriester methodology) by Rapoport[10,11]. These autors showed that the use of benzylchloroformate (CBzCl) is unsatifactory for the preparation of N-protected nucleosides, the method of choice involved 1-benzyloxycarbonyl-imidazolium or triazolium salts as effective acylation agent[10-12]. Attemps to acylate the 5-amino function of dZ with 1-(benzyloxycarbonyl)-3-methylimidazolium salt were not successful: acylation of compound **1** in water afforded the 5'-O-CBz derivative as the major product (46%) (data not shown). Prior to N-protection the hydroxy functions of dZ were silylated by reaction of *tert*-butyldimethylsilyl chloride (TBDMSCl) in DMF in the presence of imidazole to give the 3',5'-O-di-*tert*-butyldimethylsilyl derivative **6** in 68% yield. From compound **6**, a low yield of 5-N-protected nucleoside was obtained. The protection of the amino function was more conveniently effected by treatment of compound **6** with an excess of CBzCl in dichloromethane/pyridine. Two products were obtained, the 5-N-benzylcarbamate derivative **7a** (range of 39 to 46%) and the dibenzylcarbamate **7b** (17 to 27%), along with the starting material **6** (28 to 35%), in a ratio depending on the quantity of chloroformate and reaction time (Scheme 2). The mixture of nucleosides **7a** and **7b** could be separated



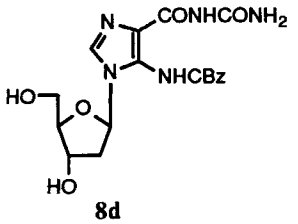
SCHEME 2: a) CBzCl in CH_2Cl_2/pyridine ; b) 1M TBAF in THF

more easily as their desilylated derivatives. Thus, treatment of **7a** and **7b** with tetra-*n*-butylammonium fluoride (TBAF) in THF yielded the corresponding nucleosides **8a** and **8b**. As described below, these two derivatives could be used as units for DNA synthesis.

The sensitivity of the monomers **8a** and **8b** to the reagents used in oligonucleotide synthesis was studied in order to assess its suitability as DNA building-block. It was equally important to ensure that the protecting group chosen would be removed quantitatively by hydrogenolysis at the end of oligonucleotide synthesis. Transfer hydrogenolysis of compounds **8a** and **8b** using 1,4-cyclohexadiene as hydrogen donor and 10% palladium on carbon as catalyst gave the expected nucleoside dZ in quantitative yield. Treatment of compounds **8a** and **8b** with 30% aqueous ammonia at 60°C afforded the derivatives **8c** (2'-deoxyxanthosine) and **8d**, respectively.



8c

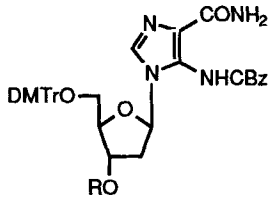


8d

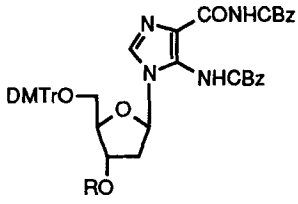
Thus, incomplete deprotection of CBz group in a synthetized oligomer should result in a mixture of two products, the correct sequence and a rearranged one. The nucleosides **8c** and **8d** could serve as standards in the base analysis by enzymatic digestion of synthetized oligomers.

The trimer d(ZAC) was chosen as a first synthetic target to demonstrate the suitability of the N-CBz blocking group for the Z residue following the phosphotriester approach. The preparation of the requisite monomer **10a** was undertaken according to classical methodology[13]. The 5'-hydroxyl function of **8a** was selectively protected as the

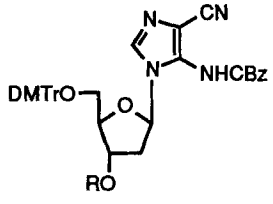
4,4'-dimethoxytrityl ether to give **9a** in 81% yield. The 3'-hydroxyl was then converted into its phosphotriester by reaction of 2-chlorophenylphosphorodichloridate and 2-cyanoethanol in pyridine. The desired product **10a** was isolated with 67% yield as a diastereomeric mixture (4:1) as evidenced by ^{1}H and ^{31}P-NMR spectroscopy. Concomitant dehydration of the carboxamide function was observed under the phosphorylation conditions used, and compound **10c** was formed in 20% yield. In the same manner, 5'-dimethoxytritylation followed by 3'-phosphorylation afforded compound **10b** from compound **8b**.



9a R=H
10a R=OP(O)OCH$_2$CH$_2$CN OPhCl



9b R=H
10b R=OP(O)OCH$_2$CH$_2$CN OPhCl

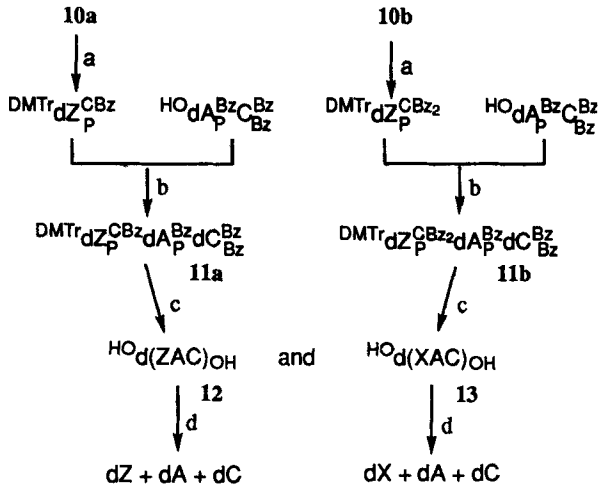


10c R=OP(O)OCH$_2$CH$_2$CN OPhCl

The trimer d(ZAC) was synthesized in solution from monomers **10a** and **10b** using the 5'-3' phosphotriester methodology illustrated in Scheme 3. After treatment of **10a** and **10b** with Et3N/pyridine/H2O (step a), the resulting decyanoethylated monomers were condensed with the dimer dA(Bz)C(Bz)Bz (step b) to give **11a** and **11b** in 86% and 60%, respectively. These fully protected trimers were characterized by ^{1}H- and ^{13}C-NMR spectroscopy. In particular, the integrity of the carboxamide function was confirmed. Deprotection of trimers **11a** and **11b** obtained from these two trials was accomplished according to the following conditions (step c): detritylation with a 2% benzene sulfonic acid (BSA) solution followed by chromatographic purification on silica gel of the resulting trimers, catalytic transfer hydrogenation on 10% Pd/C using 1,4-cyclohexadiene in ethyl acetate-ethanol (1/1), treatment of the filtrate with a 0.3M solution of tetramethylguanidinium-pyridine aldoximate (TMG-PAO) followed by 28% aqueous ammonia at 60°C.

We have observed that the time necessary for the CBz group to be completely removed is greater with trimers (six days) than with corresponding monomers (18h). Following gel filtration chromatography of the crude deprotected oligomers, analysis by reverse-phase HPLC showed in both cases the formation of two products (noted peaks A and B) (Figures 1 and 2).

After reverse-phase HPLC purification, an aliquot of each peak was submitted to enzymatic digestion with snake venom phosphodiesterase and bacterial alkaline phosphatase. HPLC analysis of the resulting monomers revealed for the major fraction



SCHEME 3: Block-synthesis of trimer in solution using phosphotriester methodology: a) Et_3N/pyridine/H_2O; b) TPSNT/pyridine; c) 2% BSA/CH_2Cl_2, 10% Pd/C with 1,4-cyclohexadiene, 0.3M TMG-PAO, then 28% NH_4OH at 60°C; d) snake venom phosphodiesterase and alkaline phosphatase.

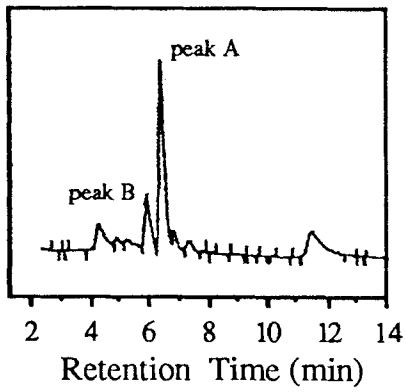


FIGURE 1: Reverse-phase HPLC profile of crude d(ZAC) from deprotection of **11a**.

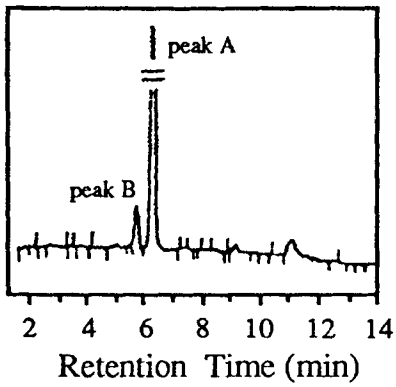


FIGURE 2: Reverse-phase HPLC profile of crude d(ZAC) from deprotection of **11b**.

(peak A) the expected ratio based on the extinction coefficients at 254 nm and the peak areas (Figure 3). The minor peak B corresponds to the alkaline rearrangement of the non-hydrogenolyzed Z^{CBz} residue to give the trimer d(XAC) (**13**), where dX corresponds to the nucleoside **8c** (Figure 4). Both starting synthons gave comparable yields. We thus isolated 11 A260 (4%) of trimer d(ZAC) (**12**) from 17 mg of detritylated **11a**, and 14 A260 (5%) of **12** from 20 mg of detritylated **11b**. A non-negligible adsorption of oligomers on the catalyst has been observed; thus, treatment of the catalyst with 0.3M TMG-PAO solution followed by ammonia resulted in another crop of recovered material.

We next carried out the synthesis of the hexamer d(CACZAC), using the block-strategy illustrated in Scheme 4. Deprotection of the fully protected hexamer **14** was realized according to the same protocol than for trimers **11** except that the hydrogenation step required a more important reaction time (three weeks as compared to six days) and led to the formation of numerous products. As control an aliquot of the hexamer **14** was treated according to the same deblocking conditions without the hydrogenation step. Comparative HPLC analysis of these two crude reaction mixtures allowed to select two major peaks. After reverse-phase HPLC purification, we isolated the hexamers **15** (14 A260) and **16** (15 A260) from 210 mg of fully protected hexamer **14**. The presence of numerous by-products and adsorption on the catalyst perhaps explain this poor yield of product. Hydrogenolysis using 1,4-cyclohexadiene as hydrogen donor and Pd black as catalyst (from commercial source or freshly prepared from $Pd(OAc)_2$), or using hydrogen with 10% Pd/C were inefficient in our hands for obtaining better yields.

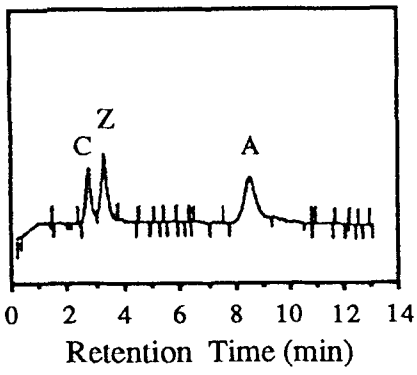


FIGURE 3: HPLC analysis of nucleotides from enzymatic digestion of **peak A.**

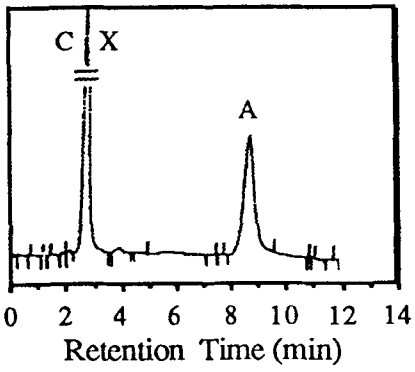
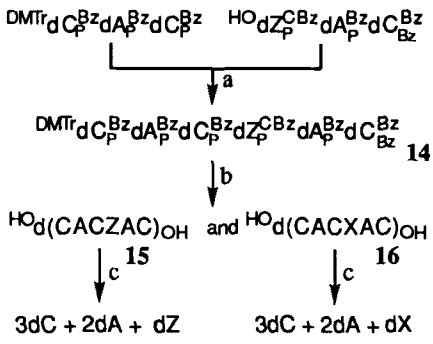


FIGURE 4: HPLC analysis of nucleotides from enzymatic digestion of **peak B.**



SCHEME 4: Block-synthesis of hexamer in solution using phosphotriester methodology: a) TPSNT/pyridine; b) 2% BSA/CH_2Cl_2, 10% Pd/C with 1,4-cyclohexadiene, 0.3M TMG-PAO, then 28% NH_4OH at 60°C; c) snake venom phosphodiesterase and alkaline phosphatase.

Enzymatic degradation and analysis of resulting nucleosides by reverse-phase HPLC confirmed the correct nucleoside ratio of 3:1:2 (dC:dZ:dA) for hexamer **15** (Figure 5) and the presence of the rearranged purine **8c** (dX) for hexamer **16**.

Although Z could be incorporated in DNA oligomers through the use of CBz protecting group, it afforded low yield. Considering that other acyl groups commonly used for protecting canonical bases resulted in the ring closure of the Z moiety, a suitable protecting group remains to be found for efficently using Z as a DNA building block.

The stability of oligomers containing the Z base together with those containing other bases able to pair ambiguously will be the subject of a separate publication.

EXPERIMENTAL SECTION

NMR spectra were recorded on a Bruker AC-300 spectrometer at 300 MHz for ^1H, 75 MHz for ^{13}C. Chemical shifts were reported in ppm (δ) relative to DMSO or TMS used as internal standard. Exchangeable protons were detected by addition of D_2O. ^{31}P-NMR spectra were recorded on a Bruker AC-300 spectrometer at 121 MHz. Chemical shifts were reported in ppm (δ) relative to H_3PO_4 used as external standard. Chemical ionisation mass spectra were measured on a NERMAG R10-10C apparatus (M for mass peak, B for base moiety). TLC was performed using Merck Kieselgel 60F254 precoated plates. Silica gel used for column chromatography was Kieselgel 9385A (230-400 mesh) or 7734 (70-230 mesh). Analysis and purification by HPLC were performed on a Perkin Elmer system using a reverse-phase column (5C18) with a linear gradient of acetonitrile (A) in 10mM triethylammonium acetate buffer (B) (pH 7.5) in 20 min at a flow rate of 1 ml/min (anal.) or 5.5 ml/min (prep.). Adenosine deaminase (type II), snake venom phosphodiesterase and bacterial alkaline phosphatase were purchased from the Sigma Chemical Company.

5-Amino-1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (1). Compound **1** was synthesized according to the previously reported procedure from 2'-deoxyinosine[2], itself obtained by large scale enzymatic deamination of 2'-deoxyadenosine with adenosine deaminase[14].

5-Benzamido-1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (2a) and 5-benzamido-1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carbonitrile (2b). Compound **1** (1.25 g, 5 mmol) was treated with a large excess of benzoyl chloride (3.48 ml, 30 mmol) according to a reported procedure[13] except that a mixture of CH_2Cl_2/pyridine (9 ml/3.5 ml) was used as solvent.

2a: 0.90 g (51%). Rf (CH_2Cl_2/MeOH: 80/20) = 0.37. ^1H NMR (DMSO-d_6) δ: 2.24 (m, 1H, H2'); 2.36 (m, 1H, H2''); 3.54 (m, 2H, H5' and H5''); 3.78 (m, 1H, H4'); 4.28 (m, 1H, H3'); 5.00 (brs, 1H, OH5'); 5.30 (brs, 1H, OH3'); 5.79 (t, 1H, H1', J = 6.7 Hz); 7.08 (brs, 1H, $CONH_2$); 7.30 (brs, 1H, $CONH_2$); 7.54 (t, 2H, H Arom. of Bz, J = 7.6 Hz); 7.63 (t, 1H, H Arom. of Bz, J = 7.2 Hz); 8.00 (s, 1H, H2); 8.02 (d, 2H, H Arom. of Bz, J = 7.7 Hz); 10.25 (brs, 1H, NHCOPh). ^{13}C NMR (DMSO-d_6) δ: 41.18 (C2'); 61.54 (C5'); 70.54 (C3'); 84.07 and 87.85 (C1' and C4'); 127.71 (C of Im.); 128.10 (C2 and C6 of Bz and C of Im.); 128.63 (C3 and C5 of Bz); 132.33 (C4 of Bz); 132.60 (C2 of Im.); 133.19 (C1 of Bz); 163.99 (4-$CONH_2$); 166.84 (5-NHCOPh). MS (CI, NH_3) m/z 347 (M+H); 231 (B+H). **2b**: 0.12 g (7%). Rf (CH_2Cl_2/MeOH: 80/20) = 0.53. ^1H NMR (DMSO-d_6) δ: 2.34 (m, 1H, H2'); 2.72 (m, 1H, H2''); 3.57 (m, 2H, H5' and H5''); 3.89 (m, 1H, H4'); 4.44 (m, 1H, H3'); 5.00 (brs, 1H, OH5'); 5.30 (brs, 1H, OH3'); 5.41 (t, 1H, H1', J = 6.8 Hz); 7.58 (m, 3H, H Arom. of Bz); 8.13 (dd, 2H, H Arom. of Bz, J = 7.6 Hz); 8.36 (s, 1H, H2).

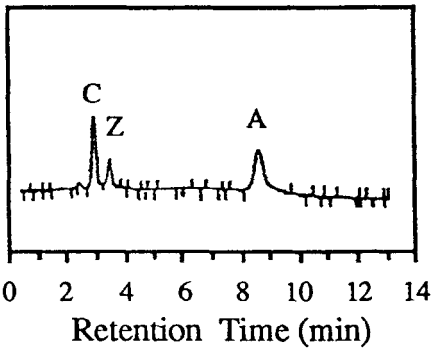


FIGURE 5: HPLC analysis of nucleotides from enzymatic digestion of **hexamer 15.**

2-Phenyl-2'-deoxyinosine (3). Compound **2a** (30 mg) was treated with 28% aqueous ammonia (3 ml) at 60°C until TLC showed that no starting material remained. After evaporation, the product was crystallized from EtOH/H_2O to give pure **3**. Rf (CH_2Cl_2/MeOH: 80/20) = 0.60. ^1H NMR (DMSO-d_6) δ: 2.33 (m, 1H, H2'); 2.70 (m, 1H, H2"); 3.56 (m, 2H, H5' and H5"); 3.87 (m, 1H, H4'); 4.43 (m, 1H, H3'); 4.96 (t, 1H, OH5', J = 5.3 Hz); 5.40 (d, 1H, OH3', J = 4 Hz); 6.40 (t, 1H, H1', J = 6.8 Hz); 7.58 (m, 4H, H Arom. of Bz and NH); 8.12 (dd, 2H, H Arom. of Bz, J = 8.0 Hz, J = 1.6 Hz); 8.34 (s, 1H, H8). ^{13}C NMR (DMSO-d_6) δ: 40.20 (C2'); 61.56 (C5'); 70.66 (C3'); 83.31 and 87.84 (C1' and C4'); 122.89 (C5), 127.76 (C2 and C6 of Bz); 128.58 (C3 and C5 of Bz); 131.26 (C4 of Bz): 132.11 (C1 of Bz); 139.05 (C8); 148.19 (C4); 153.26 (C2); 157.26 (C6). MS (CI, NH_3) m/z 329 (M+H); 213 (B+H). Anal. Calcd for $C_{16}H_{16}N_4O_4$: C, 58.50; H, 4.80; N, 17.08. Found: C, 58.52; H, 4.85; N, 17.20.

5-Phenoxyacetylamino-1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (4). To a solution of compound **1** (120 mg, 0.50 mmol) in dry pyridine (5 ml) was added trimethylsilyl chloride (0.50 ml, 4 mmol). After stirring for 1 hour, phenoxyacetyl chloride (0.09 ml, 0.62 mmol) was added and the reaction was allowed to stir at room temperature for 4 h. The reaction was quenched by addition of water (1 ml) to the cooled reaction mixture, followed after 15 min by 25% aqueous ammonia (1 ml). After an additional 15 min the reaction was concentrated *in vacuo*. The product was then purified by column chromatography on silica gel using a 0 to 10% gradient of MeOH in CH_2Cl_2 to give compound **4** (55 mg, 30%). Rf (CH_2Cl_2/MeOH: 80/20) = 0.48. ^1H NMR (DMSO-d_6) δ: 2.20 (m, 1H, H2'); 2.30 (m, 1H, H2"); 3.52 (m, 2H, H5' and H5"); 3.78 (m, 1H, H4'); 4.30 (m, 1H, H3'); 4.70 (s, 2H, CH_2 of PhOAc); 5.00 (brs, 1H, OH5'); 5.30 (brs, 1H, OH3'); 5.75 (t, 1H, H1', J = 6.7 Hz); 6.80 - 7.30 (m, 7H, H Arom. and $CONH_2$); 7.97 (s, 1H, H2); 10.40 (brs, 1H, NHCO). ^{13}C NMR (MeOH-d_4) δ: 42.42 (C2'); 62.77 (C5'); 68.37 (CH_2 of PhOAc); 71.98 (C3'); 86.19 and 89.13 (C1' and C4'); 115.99 (C2 and C6 of Ph); 122.98 (C4 of Ph); 128.24 and 128.94 (C4 and C5 of Im.); 130.74 (C3 and C5 of Ph); 134.77 (C2 of Im.); 159.14 (C1 of Ph); 166.78 ($CONH_2$); 171.95 ($NHCOCH_2OPh$). MS (CI, NH_3) m/z 377 (M+H); 359 (M+H-18); 261 (B+H); 243 (dZ+H).

2-Phenoxymethyl-2'-deoxyinosine (5). Compound **4** (30 mg) was treated with 28% aqueous ammonia at 60°C for 10 h to give after purification on silica gel plate compound **5**. Rf (CH_2Cl_2/MeOH: 80/20) = 0.56. ^1H NMR (DMSO-d_6) δ: 2.30 (m, 1H,

H2'); 2.60 (m, 1H, H2"); 3.52 (m, 2H, H5' and H5"); 3.84 (m, 1H, H4'); 4.37 (m, 1H, H3'); 5.00 (s, 2H, CH_2 of PhOAc); 5.00 (brs, 1H, OH5'); 5.30 (brs, 1H, OH3'); 6.30 (t, 1H, H1', J = 6.7 Hz); 7.00 (m, 3H, H Arom.); 7.30 (m, 2H, H Arom.); 8.32 (s, 1H, H8). MS (CI,NH_3) m/z 359 (M+H); 260 (B+18); 243 (B+H).

5-Amino-1-(3,5-di-O-*tert*-butyldimethylsilyl-2-deoxy-β-D-ribofuranosyl) imidazole-4-carboxamide (6). To a solution of **1** (2.2 g, 9.1 mmol) in anhydrous DMF (70 ml) containing imidazole (5.4 g, 80 mmol) was added TBDMSCl (6.0 g, 40 mmol). The mixture was stirred at room temperature overnight. The solvent was removed *in vacuo* and the residue dissolved in CH_2Cl_2. The solution was washed with 10% $NaHCO_3$, then water, and was dried over sodium sulfate. After evaporation of the solvent, the crude product was purified by column chromatography on silica gel using a 0 to 3% gradient of MeOH in CH_2Cl_2 to give compound **6** as a white foam (2.9 g, 68%). Rf (CH_2Cl_2/MeOH: 95/5) = 0.31. ^1H NMR ($CDCl_3$) δ: 0.08, 0.09, 0.10 and 0.11 (each s, each 3H, CH_3Si); 0.90 and 0.91 (each s, each 9H, CH_3 of t-BuSi); 2.12 (m, 1H, H2'); 2.62 (m, 1H, H2"); 3.77 (dd, 1H, H5', J = 2.3 Hz, J = 11.4 Hz); 3.85 (dd, 1H, H5", J = 2.8 Hz, J = 11.2 Hz); 3.93 (m, 1H, H4'); 4.53 (dt, 1H, H3', J = 2.6 Hz, J = 6.3 Hz); 5.57 (s, 2H, NH_2); 5.88 (dd, 1H, H1', J = 5.8 Hz, J = 8.4 Hz); 6.49 (brs, 2H, $CONH_2$); 6.98 (s, 1H, H2). ^{13}C NMR ($CDCl_3$) δ: -5.50, -5.43, -4.88 and -4.70 (CH_3Si); 17.82 and 18.10 (C of t-Bu); 25.76 and 25.85 (CH_3 of t-BuSi); 39.50 (C2'); 62.72 (C5'); 71.98 (C3'); 82.85 and 86.97 (C1' and C4'); 112.91 (C4 of Im.); 127.35 (C2 of Im.); 142.73 (C5 of Im.); 166.77 (4-$CONH_2$). Anal. Calcd for $C_{21}H_{42}N_4O_4$: C, 53.60 ; H, 8.96 ; N; 11.86. Found: C, 53.74; H, 8.96; N, 11.86.

Benzyloxycarbonylation of Compound 6. To a cooled solution of **6** (1.45 g, 3.08 mmol) in a mixture of anhydrous CH_2Cl_2/pyridine (20 ml/10 ml) was added dropwise benzyloxycarbonyl chloride (0.7 ml, 1.6 mmol) in CH_2Cl_2 (2 ml). The solution was left stirring at room temperature. After 2 h, TLC (CH_2Cl_2/MeOH, 95/5) showed the partial conversion of starting material into two new compounds with higher Rfs. Extra benzyloxycarbonyl chloride (1.4 ml in two portions at 3h intervals) was added and the reaction mixture was left overnight at room temperature. Reaction was quenched by addition of MeOH, and the solvent was removed *in vacuo*. The residual syrup was dissolved in CH_2Cl_2, washed with water, and dried. After removal of the solvent, the crude product was purified by column chromatography on silica gel using a gradient of MeOH in CH_2Cl_2. Compound **7b** was eluted first (0.1 g, 4%), followed by a mixture of **7b** and **7a** (0.8 g, 1:2), and then pure **7a** (0.32 g, 17%). The starting material was recovered from the latter eluted fractions (0.41 g, 28%). The mixture of compounds **7a** and **7b** was used without further purification in the next step.

7a: Rf (CH_2Cl_2/MeOH: 95/5) = 0.35. ^1H NMR ($CDCl_3$) δ: 0.01-0.03 (each s, each 3H, CH_3Si); 0.90 and 0.91 (each s, each 9H, CH_3 of t-Bu); 2.24 (m, 1H, H2'); 2.44 (m, 1H, H2"); 3.70 (m, 2H, H5' and H5"); 3.84 (m, 1H, H4'); 4.44 (m, 1H, H3'); 5.10 (m, 2H, CH_2 of CBz); 5.96 (t, 1H, H1'); 6.80 (brs, 2H, $CONH_2$); 7.30 (m, 5H, H Arom. of CBz); 7.72 (s, 1H, H2). ^{13}C NMR ($CDCl_3$) δ: -4.70 and -4.65 (CH_3Si); 17.97 and 18.40 (C of t-Bu); 25.73 and 25.98 (CH_3 of t-Bu); 42.65 (C2'); 62.66 (C5'); 68.02 (CH_2 of CBz); 71.55 (C3'); 85.64 and 87.72 (C1' and C4'); 127.78-128.70 (C Arom.of CBz, C4 and C5 of Im.); 131.95 (C2 of Im.); 135.35 (C1 of CBz); 154.48 (5-NHCOOBn); 165.63 (4-$CONH_2$). **7b**: Rf (CH_2Cl_2/MeOH: 95/5) = 0.39. ^1H NMR ($CDCl_3$) δ: -0.07, -0.04, 0.01 and 0.03 (each s, each 3H, CH_3Si); 0.90 and 0.95 (each s, each 9H, CH_3 of t-Bu); 1.95 (m, 2H, H2' and H2"); 3.64 (m, 2H, H5' and H5"); 3.73 (m, 1H, H4'); 4.31 (m, 1H, H3'); 5.15 (m, 4H, CH_2 of CBz); 5.58 (t, 1H, H1'); 7.20 (m, 10H, H Arom. of CBz); 7.64 (s, 1H, H2); 8.02 (brs, 1H, NHCO). ^{13}C NMR ($CDCl_3$) δ: -4.85 and -4.70 (CH_3Si); 17.97 and 18.40 (C of t-Bu); 25.73 and 25.96 (CH_3 of t-Bu); 42.52 (C2'); 62.53 (C5'); 69.17 and 69.28 (CH_2 of CBz); 71.47 (C3'); 84.38 and 87.86 (C1' and C4'); 127.79-128.63 (s, C Arom.of CBz, C4 and C5 of Im.); 132.65 (C2 of Im.); 134.68 and 134.80 (C1 of CBz); 150.83 (4-CONHCOOBn); 151.29 (5-NHCOOBn); 163.37 (4-CONHCOOBn).

General procedure for desilylation of compounds 7a and 7b. Silylated compounds (1 mmol) were dissolved in dry THF (2.5 ml), and 1M TBAF in THF (2.5 ml) was added. After 20 min stirring at room temperature, TLC showed complete reaction. The mixture was evaporated to dryness, and the crude product was purified by column chromatography on silica gel eluting with THF/MeOH (4/1). Typically, 0.62 g (1.02 mmol) of **7a** treated as above gave 0.30 g (78%) of **8a** as a foam.

5-(Benzyloxycarbonyl)amino-1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (8a). Rf (CH_2Cl_2/MeOH: 80/20) = 0.30. ^1H NMR (DMSO-d_6) δ: 2.20 (m, 1H, H2'); 2.32 (m, 1H, H2"); 3.53 (m, 2H, H5' and H5"); 3.81 (m, 1H, H4'); 4.30 (m, 1H, H3'); 4.98 (t, 1H, OH5', J = 5.3 Hz); 5.12 (brs, 2H, CH_2 of CBz); 5.31 (d, 1H, OH3', J = 4.2 Hz); 5.81 (t, 1H, H1', J = 6.7 Hz); 7.08 and 7.27 (each s, 2H, $CONH_2$); 7.37 (m, 5H, H Arom. of CBz); 7.93 (s, 1H, H2); 9.26 (brs, 1H, NHCO). ^{13}C NMR (DMSO-d_6) δ: 40.73 (C2'); 61.29 (C5'); 66.10 (CH_2 of CBz); 70.31 (C3'); 83.26 and 87.59 (C1' and C4'); 127.52-128.27 (4s, C Arom. of CBz, C4 and C5 of Im.); 132.08 (C2 of Im.); 136.36 (C1 of CBz); 154.64 (5-NHCOOBn); 163.57 (4-$CONH_2$). MS (CI, NH_3) m/z 377 (M+1); 261 (B+1). Anal. Calcd for $C_{17}H_{20}N_4O_6 + H_2O$: C, 51.77; H, 5.62; N, 14.20. Found: C, 52.01; H, 5.35; N, 14.41.

N-Benzyloxycarbonyl-5-(benzyloxycarbonyl)amino-1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (8b). Rf (CH_2Cl_2/MeOH: 80/20) = 0.60. ^1H NMR (DMSO-d_6) δ: 2.26 (m, 1H, H2'); 2.33 (m, 1H, H2"); 3.54 (m, 2H, H5' and H5"); 3.83 (m, 1H, H4'); 4.31 (m, 1H, H3'); 5.02 (t, 1H, OH5', J = 5.2 Hz); 5.13 (s, 2H, CH_2 of 5-CBz); 5.19 (s, 2H, CH_2 of 4-CBz); 5.86 (t, 1H, H1', J = 6.6 Hz); 7.40 (m, 10H, H Arom. of CBz); 8.05 (s, 1H, H2); 9.58 (brs, 1H, 5-NHCO); 9.84 (s, 1H, 4-CONH). ^{13}C NMR (DMSO-d_6) δ: 40.76 (C2'); 61.18 (C5'); 66.26 and 66.46 (CH_2 of CBz); 70.24 (C3'); 83.58 and 87.82 (C1' and C4'); 126.02 (C4 of Im.); 127.67-128.32 (6s, C Arom.of CBz); 130.00 (C5 of Im.); 132.76 (C2 of Im.); 135.82 and 136.10 (C1 of CBz); 150.35 (4-CONHCOOBn); 154.12 (5-NHCOOBn); 158.57 (4-CONHCOOBn). MS (CI, NH_3) m/z 511 (M+H); 395 (B+H).

2'-deoxyxanthosine (8c). Compound **8a** (20 mg) was treated by 28% aqueous ammonia (3 ml) at 60°C for 10h. After evaporation of solvent, the product was purified by HPLC on a reverse-phase column using a 5-25% gradient of A in buffer B in 20 min (Rt = 2.70 min). ^1H NMR (DMSO-d_6) δ: 2.25 (m, 1H, H2'); 2.40 (m, 1H, H2"); 3.60 (m, 2H, H5' and H5"); 3.88 (m, 1H, H4'); 4.33 (m, 1H, H3'); 5.02 (bs, 1.2H, OH5' and OH3'); 6.15 (t, 1H, H1', J = 6.9 Hz); 8.10 (s, 1H, H2); 8.40 (very bs, 1H, NH); 10.50 (brs, 0.5H, NH). ^{13}C NMR (DMSO-d_6) δ: 40.08 (C2'); 61.60 (C5'); 71.02 (C3'); 85.16 and 87.97 (C1' and C4'); 116.03 (C5); 134.99 (C8); 141.72 (C4); 151.86 (C2); 158.26 (C6). MS (CI, NH_3) m/z 269 (M+H); 250 (M-18); 178 (B+18); 153 (B+H). UV (H_2O, pH 5.5) l 238 (10500), 262sh (12270); (H_2O, pH 11.4) l 249 (12800), 278 (10770).

5-(Benzyloxycarbonyl)amino-1-(2-deoxy-β-D-ribofuranosyl)imidazole-4-carbonylurea (8d). Compound **8b** (20 mg) was treated by 28% aqueous ammonia (3 ml) at 60°C for 18h. Rf (CH_2Cl_2/MeOH: 80/20) = 0.37. ^1H NMR (DMSO-d_6) δ: 2.26 (m, 1H, H2'); 2.33 (m, 1H, H2"); 3.54 (m, 2H, H5' and H5"); 3.83 (m, 1H, H4'); 4.31 (m, 1H, H3'); 5.02 (t, 1H, OH5', J = 5.2 Hz); 5.13 (s, 2H, CH_2 of 5-CBz); 5.86 (t, 1H, H1', J = 6.6 Hz); 7.20 (m, 6H, H Arom. of CBz and $CONH_2$); 7.75 (brs, 1H, $CONH_2$); 8.10 (s, 1H, H2); 8.92 (brs, 1H, 4-CONH); 9.58 (brs, 1H, 5-NHCO). ^{13}C NMR (DMSO-d_6) δ: 40.75 (C2'); 61.18 (C5'); 66.44 (CH_2 of CBz); 70.25 (C3'); 83.60 and 87.84 (C1' and C4'); 125.41 (C4 of Im.); 127.66, 127.90, 128.28 (C Arom. of CBz); 130.04 (C5 of Im.); 133.12 (C2 of Im.); 136.14 (C1 of CBz); 152.82 (4-CONHCONH$_2$); 154.16 (5-NHCOOBn); 161.24 (4-CONHCONH$_2$).

5-(Benzyloxycarbonyl)amino-1-(5-O-dimethoxytrityl-2-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (9a). Compound **8a** (0.65 g, 1.73 mmol) was dried by coevaporation with pyridine (2 x 2 ml) and then dissolved in pyridine (15 ml).

Dimethoxytrityl chloride (0.64 g, 1.90 mmol) was added to the cooled solution. The reaction was allowed to stir for 2 h at room temperature. MeOH (5 ml) was then added to the mixture and the solvent was removed *in vacuo*. The residue was dissolved in CH_2Cl_2, washed in turn with $NaHCO_3$, water and dried (Na_2SO_4). The product was then purified by column chromatography on silica gel using a 0 to 5% gradient of MeOH in $CH_2Cl_2$to yield compound **9a**. To the resulting foam dissolved in $CH_2Cl_2$was added vigorously petroleum ether. The precipitated product was filtered off and dried *in vacuo* to give **9a** as a white powder (0.95 g, 81%). Rf (CH_2Cl_2/MeOH: 90/10) = 0.50. ^{1}H NMR (DMSO-d_6) δ: 2.28 (m, 1H, H2'); 2.39 (m, 1H, H2''); 3.16 (m, 2H, H5' and H5''); 3.73 (s, 6H, OCH_3); 3.93 (m, 1H, H4'); 4.29 (m, 1H, H3'); 5.11 (brs, 2H, CH_2 of CBz); 5.38 (d, 1H, OH3', J = 4.4 Hz); 5.85 (t, 1H, H1', J = 6.5 Hz); 7.09 (s, 1H, $CONH_2$); 6.87 (d, 4H, H Arom. of DMT); 7.30 (m, 15H, H Arom. of CBz, DMT and $CONH_2$); 7.78 (s, 1H, H2); 9.32 (brs, 1H, NHCO). ^{13}C NMR (DMSO-d_6) δ: 40.25 (C2'); 54.92 (OCH_3 of DMT); 63.81 (C5'); 66.11 (CH_2 of CBz); 70.35 (C3'); 82.50 (C1'); 85.51 (Cq of DMT); 85.71 (C4'); 113.10 (C3 and C5 of DMT); 126.60 (C4' of DMT); 127.47-128.30 (5s, C Arom.of CBz and DMT); 129.79 (C2 and C6 of DMT); 131.60 (C2 of Im.); 135.30 and 135.40 (C1 of DMT); 136.36 (C1 of CBz); 144.64 (C1' of DMT); 154.10 (5-NHCOOBn); 157.96 (C4 of DMT); 163.50 (4-$CONH_2$).

N-Benzyloxycarbonyl-5-(benzyloxycarbonyl)amino-1-(5-O-dimethoxytrityl-2-deoxy-β-D-ribofuranosyl)imidazole-4-carboxamide (9b). Compound **8b** (0.66 g, 1.30 mmol) was tritylated according to the procedure given for **8a**. The precipitation procedure afforded compound **9b** as a white powder (0.80 g, 75%). ^{1}H NMR (DMSO-d_6) δ: 2.30 (m, 1H, H2'); 2.40 (m, 1H, H2''); 3.16 (m, 2H, H5' and H5''); 3.73 (s, 6H, OCH_3); 3.95 (m, 1H, H4'); 4.30 (m, 1H, H3'); 5.12 (brs, 2H, CH_2 of 5-CBz); 5.20 (s, 2H, CH_2 of 4-CBz); 5.40 (d, 1H, OH3'); 5.91 (t, 1H, H1', J = 6.6 Hz); 6.87 (d, 4H, H Arom. of DMT); 7.32 (m, 19H, H Arom. of CBz and DMT); 7.84 (s, 1H, H2); 9.65 (brs, 1H, 5-NHCO); 9.85 (s, 1H, 4-CONH). ^{13}C NMR (DMSO-d_6) δ: 40.49 (C2'); 54.89 (OCH_3 of DMT); 63.78 (C5'); 66.24 and 66.46 (CH_2 of CBz); 70.30 (C3'); 83.26 and 85.52 (C1' and C4'); 113.11 (C3 and C5 of DMT); 126.08 (C4 of Im.); 126.61 (C4' of DMT); 127.58-128.31 (6s, C Arom.of CBz and DMT); 129.59 (C2 and C6 of DMT); 130.14 (C5 of Im.); 132.32 (C2 of Im.); 135.27 and 135.37 (C1 of DMT); 135.82 and 136.08 (C1 of CBz); 144.61 (C1' of DMT); 150.35 (4-CONHCOOBn); 154.14 (5-NHCOCBz); 157.96 (C4 of DMT); 158.60 (4-CONHCOOBn).

General procedure for phosphorylation of nucleosides 9a and 9b. Tritylated nucleosides and 1,2,4-triazole (3.35 eq) was dried by coevaporations with anhydrous pyridine, and then dissolved in pyridine (10 ml/mmol). To the cooled solution was added 2-chlorophenylphosphorodichloridate (1.66 eq). After stirring 15 min at room temperature, 2-cyanoethanol (8 eq) was added. TLC showed that the reaction was complete after about 1 h 30. The reaction mixture was dissolved in CH_2Cl_2 and washed in turn with 5% sodium hydrogenphosphate, 5% $NaHCO_3$, water, then dried and concentrated. The crude product was purified by column chromatography on silica gel eluting with CH_2Cl_2/MeOH.

Phosphorylation of compound **9a** (0.95 g, 1.4 mmol) according to the general procedure gave compound **10a** (0.87 g, 67%) and compound **10c** (0.25 g, 20%). **10a**: Rf (CH_2Cl_2/MeOH: 90/10) = 0.16. ^{1}H NMR (DMSO-d_6) δ: 2.64 (m, 1H, H2'); 2.74 (m, 1H, H2''); 2.95 (t, 2H, CH_2CN, J = 5.6 Hz); 3.23 (m, 2H, H5' and H5''); 3.72 (s, 6H, OCH_3); 4.22 (m, 0.8H, H4'); 4.30 (m, 0.2H, H4'); 4.34 (dt, 1H, OCH_2, J = 5.7 Hz, J = 1.8 Hz); 4.36 (dt, 1H, OCH_2); 5.10 (brs, 2H, CH_2 of CBz); 5.17 (m, 0.2H, H3'); 5.24 (m, 0.8H, H3'); 5.87 and 5.90 (each t, 1H, H1', J = 6.1 Hz); 6.84 (d, 4H, H Arom. of DMT, J = 8.7 Hz); 7.12 (s, 1H, $CONH_2$); 7.20-7.40 (m, 18H, H Arom. and $CONH_2$); 7.58 (d, 1H, H Arom. of ClPh); 7.79 and 7.80 (each s, 1H, H2); 9.36 (brs, 1H, NHCO). ^{13}C NMR (DMSO-d_6) δ: 19.12 and 19.23 (CH_2CN); 38.80 (C2'); 55.12

(OCH_3 of DMT); 63.21 (C5'); 63.87(t, OCH_2); 66.41 (CH_2 of CBz); 79.38 (t, C3'); 82.91 (C1'); 83.80 (m, C4'); 86.07 (Cq of DMT); 113.32 (C3 and C5 of DMT); 118.06 (CN); 121.67 (C4 of ClPh); 124.48 and 124.54 (C2 of ClPh); 126.89-129.77 (9s, C Arom.of CBz, ClPh, DMT, C4 and C5 of Im.); 130.83 (C2 and C6 of DMT); 131.89 (C2 of Im.); 135.28 and 135.33 (C1 of DMT); 136.49 (C1 of CBz); 144.58 (C1' of DMT); 145.75 (m, C1 of ClPh); 154.83 (5-NHCOOBn); 158.22 (C4 of DMT); 163.61 (4-$CONH_2$). ^{31}P NMR (DMSO-d_6) δ: -5.46 ppm (75%) and -5.42 ppm (25%). Anal. Calcd for $C_{47}H_{45}N_5O_{11}PCl + H_2O$: C, 60.03; H, 5.04; N, 7.45. Found: C, 60.07; H, 4.75; N, 7.56. **10c**: Rf (CH_2Cl_2/MeOH: 90/10) = 0.34. ^1H NMR (DMSO-d_6) δ: 2.77 (m, 2H, H2' and H2"); 2.96 (t, 2H, CH_2CN, J = 5.7 Hz); 3.20 (m, 2H, H5' and H5"); 3.72 (s, 6H, OCH_3); 4.23 (m, 1H, H4'); 4.34 (t, 1H, OCH_2, J = 5.9 Hz); 4.37 (t, 1H, OCH_2, J = 5.8 Hz); 5.17 (s, 2H, CH_2 of CBz); 5.23 (m, 1H, H3'); 6.00 (t, 1H, H1', J = 6.4 Hz); 6.84 (d, 4H, H Arom. of DMT, J = 7.5 Hz); 7.30 (m, 17H, H Arom.); 7.58 (d, 1H, H Arom. of ClPh); 7.96 (s, 1H, H2); 10.15 (brs, 1H, NHCO). ^{13}C NMR (DMSO-d_6) δ: 19.12 and 19.23 (CH_2CN); 38.44 (C2'); 55.10 (OCH_3 of DMT); 63.19 (C5'); 63.89 and 63.96 (OCH_2); 67.21 (CH_2 of CBz); 79.05 and 79.12 (C3'); 83.55 (C1'); 84.19 and 84.25 (C4'); 86.03 (Cq of DMT); 113.29 (C3 and C5 of DMT); 114.38 (4-CN); 118.07 (CN); 121.65 (C4 of ClPh); 124.47 and 124.56 (C2 of ClPh); 126.88-129.75 (9s, C Arom.of CBz, ClPh, DMT, C4 and C5 of Im.); 130.81 (C2 and C6 of DMT); 134.72 (C2 of Im.); 135.22 and 135.25 (C1 of DMT); 135.92 (C1 of CBz); 144.55 (C1' of DMT); 145.71 and 145.73 (C1 of ClPh); 153.75 (5-NHCOCBz); 158.21 (C4 of DMT). ^{31}P NMR (DMSO-d_6) δ: -5.59 ppm and -5.41 ppm.

Phosphorylation of compound **9b** (0.78 g, 0.96 mmol) according to the general procedure gave compound **10b** (0.68 g, 68%). ^1H NMR (DMSO-d_6) δδ: 2.75 (m, 2H, H2' and H2"); 2.95 (dt, 2H, CH_2CN, J = 5.5 Hz, J < 1 Hz); 3.23 (m, 2H, H5' and H5"); 3.72 (s, 6H, OCH_3); 4.26 (m, 1H, H4'); 4.36 (m, 2H, OCH_2); 5.10 (brs, 2H, CH_2 of 5-CBz); 5.20 (s, 2H, CH_2 of 4-CBz); 5.25 (m, 1H, H3'); 5.95 (m, 1H, H1'); 6.84 (d, 4H, H Arom. of DMT); 7.30 (m, 22H, H Arom.); 7.60 (2d, 1H, H Arom. of ClPh); 7.90 and 7.91 (each s, 1H, H2); 9.69 (brs, 1H, 5-NHCOOBn); 9.91 (s, 1H, 4-CONHCOOBn). ^{31}P NMR (DMSO-d_6) δ: -5.49 ppm (60%) and -5.43 ppm (40%).

Synthesis of fully protected trinucleotides. They were synthesized in solution using the phosphotriester methodology. As an example, the trimer **11a** was prepared by condensation of detritylated dimer HOA(Bz)pC(Bz)Bz (0.67 g, 0.70 mmol) and 1.3 eq of decyanoethylated monomer DMTZ(CBz)p (0.84 g, 0.9 mmol) (from **10a** treated with triethylamine/pyridine/water: 1/3/1 for 20 min) using 1.2 eq of TPSNT (0.32 g, 0.84 mmol) in anhydrous pyridine (7 ml) at room temperature. The reaction was monitored by TLC (CH_2Cl_2/MeOH: 90/10). The reaction was quenched with saturated $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was washed with water, dried and concentrated to dryness. The crude product was purified by column chromatography on silica gel eluting with a 0 to 5% gradient of MeOH in CH_2Cl_2. Fractions containing the trimer were rechromatographed on Sephadex LH-20 eluting with 90/10 THF/MeOH. After precipitation from CH_2Cl_2/petroleum ether, oligomers were obtained in 86% for trimer DMTZ(CBz)pA(Bz)pC(Bz)Bz (**11a**) and 60% for trimer DMTZ(CBz)^{2}pA(Bz)pC(Bz)Bz (**11b**).

Synthesis of fully protected hexanucleotide. The fully protected hexamer **14** was obtained by condensation of the decyanoethylated trimer DMTC(Bz)pA(Bz)pC(Bz)p with the detritylated trimer HOZ(CBz)pA(Bz)pC(Bz)Bz (Rf = 0.32 in CH_2Cl_2/MeOH: 90/10). After classical work-up, the hexamer **14** was obtained with 76%.

General procedure for deprotection and purification of synthesized oligomers. The protected oligonucleotides were first detritylated with a cold 2% BSA solution in CH_2Cl_2/MeOH (70/30) for 20 min. Classical work-up and purification by column chromatography on silica gel afforded the 5' free oligonucleotides with yields ranging from 50 to 80%. In the second stage, the N-CBz protecting group was removed

by catalytic transfer hydrogenation using 1,4-cyclohexadiene in the presence of 10% palladium on charcoal or palladium black. As an example, the protected trimer HOZ(CBz)pA(Bz)pA(Bz)Bz (20 mg, 11 mmol) dissolved in 1 ml of EtOH/EtOAc (1/1) was treated with 100 mg of 10% Pd/C and 0.5 ml of 1,4-cyclohexadiene. The suspension was stirred under a nitrogen atmosphere until TLC showed no starting material remained. The catalyst was decanted, rinsed with hot EtOH/EtOAc and the filtrates were concentrated. TLC of the reaction mixture showed partial adsorption of oligonucleotides on the catalyst, which was treated separately for the next step. In the last step, the N-benzoyl and 2-chlorophenyl protecting groups were removed according to standard conditions. The oligomer (filtrates and catalyst separately) was treated overnight with a 0.3M TMG-PAO solution (10 eq per phosphate) in dioxan/water (1/1) at room temperature. The solvent was removed *in vacuo*, and the residue heated with excess 28% aqueous ammonia at 60°C for 10 h. After evaporation, the crude product was applied to a gel filtration column (Sephadex G-10) eluted with a 50mM triethylammonium bicarbonate buffer (pH 7.5). The crude deprotected oligomer was then purified by HPLC on a reverse-phase column (C-18 5 μ, 250 mm) eluting with a 5 to 25% gradient of A in B in 20 min at a flow rate of 5.5 ml/min. Differents peaks (monitored at 254 nm) were collected, lyophilized, and a small amount of each (1 A260) subjected to enzymatic digestion and base analysis.

Trimer d(ZAC). From **11a** : 27 mg (14 mmol) of fully protected trimer **11a** afforded after detritylation 17 mg (10 mmol, 72%) of 5'OH protected trimer. Catalytic transfer hydrogenolysis on 10% Pd/C with 1,4-cyclohexadiene gave a compound with lower Rf (0.14 in 90/10 CH_2Cl_2/MeOH) than the starting material (0.58 and 0.55). After a week, the supernatant was concentrated and subjected to 0.3M TMG-PAO followed by aqueous ammonia treatment. Analysis by reverse-phase HPLC showed two major peaks with retention times of 6.00 min (18%) and 6.40 min (82%). Enzymatic digestion of each fraction determined that the second peak had the correct sequence, the first one corresponded to the sequence with the rearranged CBz nucleoside **8c**. The catalyst treated as above gave also the two products in the same ratio. After HPLC purification, the trimer d(ZAC) **12** was isolated in a 4% overall yield (11 A260, 360 mg) along with trimer **13** (2 A260). From **11b** : 20 mg (11 mmol) of detritylated trimer **11b** was treated as above to give 14 A260 (460 mg, 5% yield) of pure trimer d(ZAC) **12**.

Hexamer d(CACZAC). 210 mg of fully protected hexamer **14** was deprotected following the general protocol, the hydrogenation step requiring three weeks. Ater HPLC purification, 14 A260 of the correct hexamer **15** (<1% yield) and 15 A260 of the rearranged hexamer **16** were isolated.

Base analysis. 1 A260 unit of an oligonucleotide was dissolved in 200 ml of 100mM TEAA (pH 7.5). Snake venom phosphodiesterase (10 ml, 5 mg) and alkaline phosphatase (2 ml, 6 mg) were added and the mixture incubated at 37°C for 1 h. The reaction was stopped by heating at 80°C for 3 min and dessalted on a Sep-Pak C18. The resulting nucleosides were analyzed by HPLC on a reverse-phase column using a 5 to 25% gradient of A in B in 20 min at a flow rate of 1 ml/min The eluate was monitored at 254 nm and the following elution times were observed : dC 2.69 min; dZ 3.29 min; dA 8.63 min. The areas under each peak were determined by integration and the amounts of each base present determined using the following extinction coefficients (M^{-1} cm^{-1}): dC (6×10^3), dZ (9.4×10^3), dA (14.3×10^3), all at 254 nm. The rearranged nucleoside **8c** gave a peak with a retention time close to dC (2.70 min) using the above elution conditions.

ACKNOWLEDGEMENT. We thank Professor Jean Igolen for his constant interest in this work.

REFERENCES

1 In " DNA replication", Kornberg, A and Baker, T.A. Ed., Freeman, W.H. and Company, NY, 1992.
2. Pochet, S.; D'Ari, R. *Nucleic Acids Res.* **1990**, 18, 7127-7131.
In this paper, the base is abbreviated Z, the corresponding 2'-deoxynucleoside as dZ.
3. Fernandez-Forner, D.; Eritja, R.; Bardella, F.; Ruiz-Perez, C.; Solans, X.; Giralt, E.; Pedroso, E. *Tetrahedron* **1991**, 47, 8917-8930.
4. Chambers, R. D. ; Bryce, M. R.; Mullins, S. T. ; Parkin, A. *Nucleosides and Nucleotides* **1988**, 7(3), 339-346.
5. Schulhof, J. C.; Molko, D.; Téoule, R. *Nucleic Acids Res.* **1987**, 15, 397-416.
6. Wu, T.; Ogilvie, K.K.; Pon, R.T. *Nucleic Acids Res.* **1989**, 17, 3501-3517.
7. Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, 104, 1316-1319.
8. Himmelsbach, F.; Pfleiderer, W. *Tetrahedron Letters* **1983**, 24, 3583-3586.
9. Himmelsbach, F.; Schulz, B.S.; Trichtinger, T.; Charabula, R.; Pfleiderer, W. *Tetrahedron* **1984**, 40, 59-72.
10. Watkins, B. E.; Rapoport, H. *J. Org. Chem.* **1982**, 47, 4471-4477.
11. Watkins, B. E.; Kiely, J. S. ; Rapoport, H. *J. Am. Chem. Soc.* **1982**, 104, 5702-5708.
12. Guibé-Jampel, E.; Bram, G.; Vilkas, M. *Tetrahedron Letters* **1969**, 40, 3541-3544.
13. Jones, R.A. In *"Oligonucleotide synthesis: a practical approach"*, Gait, M.J. Ed., IRL Press: Oxford, 1984; pp.23-34.
14. Robins, M.J. ; Basom, G. L. *Can. J. Chem.* **1973**, 51, 3161-3169.

Received July 4, 1994
Accepted November 28, 1994