1-DEAZA-2'-DEOXYADENOSINE: PHOSPHONATE AND PHOSPHORAMIDITE BUILDING BLOCKS FOR SOLID-PHASE OLIGONUCLEOTIDE SYNTHESIS

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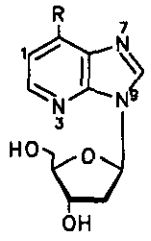
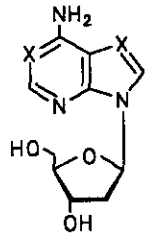
Abstract—— The synthesis of the 3'-[(2-cyanoethyl)diisopropylphosphoramidite] (**3b**) and the 3'-phosphonate (**3a**) of 1-deaza-2'-deoxyadenosine (**1b**) is described. For this purpose compound **1b** was protected at the 6-amino group with a benzoyl residue. Ensuing 4,4'-dimethoxytritylation of **1b** and phosphitylation afforded the P(III) derivatives(**3a**)and(**3b**). They were successfully employed in solid-phase oligodeoxyribonucleotide synthesis of d(c^1A-c^1A-c^1A-A-A-A) (**6**). ^{13}C-Nmr and ^{15}N-nmr spectra of the compounds(**1-5**)are discussed.

INTRODUCTION

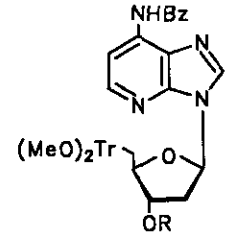
Oligonucleotides containing imidazo[4,5-*b*]pyridine (1-deazapurine) nucleosides (e.g. **1b**) cannot form regular Watson-Crick base pairs with complementary dT-residues. This has already been demonstrated in the case of oligomers containing 1,7-dideazaadenosine (**2b**).[1] However, in the case of **1b**, Hoogsteen base pairing is still possible allowing the formation of duplexes of an unusual structure. Furthermore, differences in protonation, glycosylic bond stability, and interaction with nucleoside-metabolizing enzymes are expected for such oligonucleotides compared to those containing 2'-deoxyadenosine (**2a**). In the following we describe the synthesis of suitably protected oligonucleotide building blocks (**3a,b**) of 1-deaza-2'-deoxyadenosine (c^1A_d, **1b**) and their use in solid-phase oligodeoxyribonucleotide synthesis.



1a: R = NO_2
1b: R = NH_2



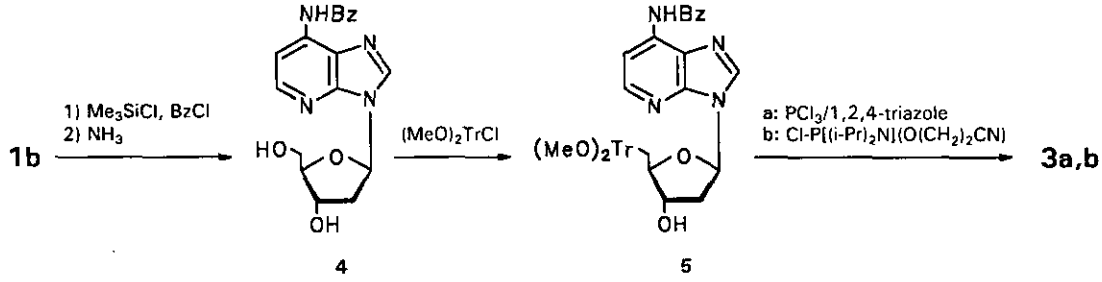
2a X = N
2b X = CH



3a R = -PH(O)O$^-$$Et_3NH^+$
3b R = -P[(i-Pr)$_2$N](O(CH_2)$_2$CN)

RESULTS AND DISCUSSION

The nitronucleoside (**1a**) has already been synthesized[2] and the amino compound (**1b**) was obtained by deoxygenation[3] from the ribonucleoside or by enzymatic transglycosylation.[4] We have prepared **1b** from **1a** by reduction with Raney nickel-catalyst/hydrazine in 90% yield.



As the assignment of the anomeric configuration of **1a** was tentative we have used ^{1}H-nmr NOE difference spectroscopy.[5] According to Table 1 the configuration at the glycosylic bond was ß-D and the position of glycosylation was N-9.

Table 1. NOE Data of **1a** upon Irradiation of H-1' and H-8.[a,b]

Irradiation	NOE (%)				
	H-8	*H*-1'	H_α-2'	H_β-2'	*H*-4'
H-1'	4.6	-	6.9	-	1.8
H-8	-	4.4	-	3.4	-

a) DMSO-d_6 at 23°C. b) Purine numbering.

1-Deaza-2'-deoxyadenosine (**1b**) exhibits a pK_a of 4.6 determined at 280 nm and 265 nm.[6] Compared to 1,7-dideaza-2'-deoxyadenosine (**2b**: pK_a 6.1)[1] the nucleoside is less basic but easier to protonate than 2'-deoxyadenosine (**2a**: pK_a 3.8). The ^{15}N-nmr data of **1b** are shown in Table 2.

Table 2. ^{15}N-Nmr Chemical Shifts and [^{15}N,^{1}H] Coupling Constants[a)] of Compounds (**1b**) and (**2a**)[7] measured in DMSO-d_6.[b)]

Compound	N-1	N-3	N-7	N-9	NH_2
1b	-	-150.1	-142.4	-210.5	-313.7
2a	-145.1	-157.8	-140.4	-207.6	-299.1

Coupling Constants [Hz]

Compound	N-1 H-2	N-3 H-2	N-7 H-8	N-9 H-1' and H-8	NH_2
1b	-	d, 10	d, 15	dd, 4 and 9	t, 90
2a	d, 17	d, 15	d, 12	d, 10	t, 88

a) Relative to nitromethane. b) Purine numbering.

The imidazole nitrogens of **1b** (Table 2) show very similar chemical shifts to those of **2a**. Nitrogen-3 can be assigned according to the coupling with H-2. As it can be seen from the Table 2 the exocyclic amino group of **1b** is upfield shifted compared to the parent nucleoside (**2a**). This is in line with an increase of the electron density within the π-system of the 1-deazapurine over the purine moiety.

Next, the exocyclic amino group of **1b** was benzoylated *via* the transient protection of the sugar hydroxyls[8] and compound(**4**)was isolated crystalline (mp 127°C, acetone). In order to ensure the compatibility of the benzoyl group in **4** with that of bzA_d, hydrolysis was carried out in conc. aqueous NH_4OH at 50°C. Tlc monitoring confirmed that deprotection gave back nucleoside(**1b**)as the only reaction product. Quantitative kinetic data were obtained uv spectrophotometrically.[9] The half-life of **4** was 115 min, which was increased

compared to bz^6A_d (71 min^1) but was still in a range suitable for oligonucleotide synthesis. The 4,4'-dimethoxytrityl residue was introduced under standard conditions affording **5** (mp 129°C, hexane-ether).
To our knowledge ^{13}C-nmr chemical shifts have not been unambiguously assigned in the case of 1-deazapurine nucleosides. We have made a complete assignment in the case of the compounds of Table 3 on the basis of J(C,H) coupling constants. The chemical shifts of C-1, C-2, C-8, and those of the sugar moiety were assigned by 2D ^{1}H,^{13}C-correlation spectra. Using the gate decoupled mode carbon-5 exhibits a dd multiplicity due to couplings to H-1 and H-8, whereas C-4 shows a td multiplicity resulting from couplings to H-2, H-8, and H-1'.

Table 3. ^{13}C-Nmr Chemical Shifts of Imidazo[4,5-*b*]pyridine Nucleosides.[a,b]

Compd	C-8[c]	C-5[d]	C-6[d]	C-1[c]	C-2[c]	C-4[d]	CO[d]	C-1'[c]	C-2'[c]
1a	147.9	127.2	143.7	112.1	144.8	150.0	-	84.1	39.4
1b	139.4	123.6	147.2	102.3	144.2	146.4	-	84.5	39.3
3a	142.0	126.6	137.1	109.1	144.8	147.0	166.1	83.8	37.8
4	142.2	126.8	137.2	109.1	144.8	147.0	166.2	84.0	39.5
5	142.2	126.7	137.1	109.0	144.9	147.1	166.2	83.6	39.2

	C-3'[c]	C-4'[c]	C-5'[c]	$\underline{C}H_3CH_2N$[c]	$CH_3\underline{C}H_2N$[c]	OCH_3[c]	C-DMTr[d]
1a	70.5	88.1	61.5	-	-	-	-
1b	71.4	88.1	62.2	-	-	-	-
3a	72.9	84.9	63.8	8.5	45.2	54.9	85.5
4	70.9	88.0	61.9	-	-	-	-
5	70.8	85.8	64.1	-	-	54.9/55.0	85.5

[a] Spectra were measured in DMSO-d_6 rel. to TMS. [b] Purine numbering. [c] From [^{1}H,^{13}C] correlation spectra. [d] From ^{1}H,^{13}C gated-decoupled spectra.

Compound (**5**) was converted into the phosphonate[10] [**3a** (^{31}P-nmr (DMSO-d_6): δ = 2.56 ppm; J(P,C-3') = 5.0 Hz and J(P,C-4') = 5.0 Hz, ^{1}J(P,H) = 587 Hz] as well as into the ß-cyanoethylphosphoramidite[11] [**3b** (^{31}P-nmr (DMSO-d_6): δ = 148.2 ppm (dd, J(P-H) = 5.7 Hz, 11.0 Hz) and δ = 148.2 ppm (dd, J(P-H)= 6.2 Hz, 11.3 Hz)].

The phosphonate (**3a**) was then used in solid-phase oligonucleotide synthesis[11] using an ABI-380B synthesizer. The protocol of detritylation, activation (pivaloyl chloride), coupling, and capping followed the ABI user bulletin.[12] Oxidation with iodine in pyridine/H_2O/THF was carried out on the oligomeric level. The $(MeO)_2$Tr-protected oligonucleotide was split off from the polymer support by conc. aq. NH_4OH and purified by RP-18 hplc. Detritylation was accomplished with 80% HOAc/H_2O within 30 min and was followed by neutralization. The oligomer (**6**) d(c^1A-c^1A-c^1A-A-A-A) was again submitted to RP-18 hplc, desalted, and lyophilized. The composition of **6** was derived from enzymatic digestion with snake-venom phosphodiesterase followed by alkaline phosphatase[10] and analysis by RP-18 hplc (Figure).

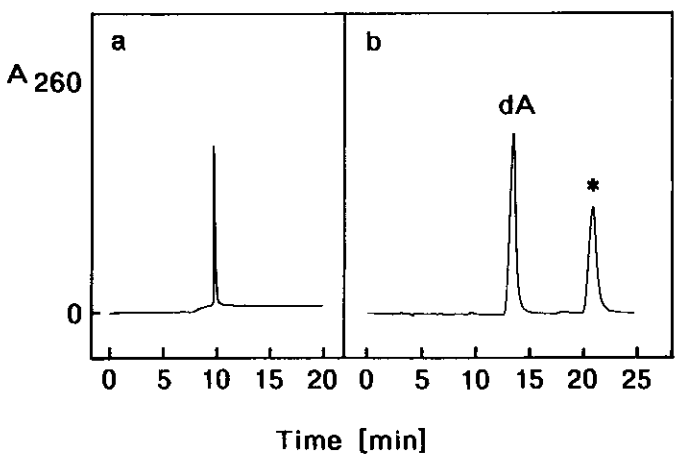


Figure. Hplc profiles of **6**; a) and its enzymatic digest b) after tandem hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase in 0.1 M Tris-HCl (pH 8.3). Gradient a) 0-20% MeCN in 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN, 95:5; b) in the absence of MeCN.

The incorporation of c^1A_d (**1b**) into an oligonucleotide opens the possibility to synthesize oligonucleotides forming duplexes by exclusive Hoogsteen base pairing. This work is under current investigation.

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REFERENCES

1. F. Seela and T. Wenzel, Helv. Chim. Acta, 1992, 75, 1111.
2. G. Cristalli, S. Vittori, A. Eleuteri, M. Grifantini, R. Volpini, G. Lupidi, L. Capolongo, and E. Pesenti, J. Med. Chem., 1991, 34, 2226.
3. T. Sugawara, T. Ishikura, T. Itoh, and Y. Mizuno, Nucleosides, Nucleotides, 1982, 1, 239.
4. I.A. Mikhailopulo, A.I. Zinchenko, S.B. Bokut, N.V. Dudchik, V.N. Baraj, E.N. Kalinichenko, H. Rosemeyer, and F. Seela, 1992, in preparation.
5. H. Rosemeyer, G. Tóth, and F. Seela, Nucleosides, Nucleotides, 1989, 8, 587.
6. A. Albert and E. P. Serjant, 'The Determination of Ionization Constants', Chapman & Hall Ltd., London, 1971.
7. H. Rosemeyer and F. Seela, Helv. Chim. Acta, 1988, 71, 1573.
8. G.S. Ti, B.L. Gaffney, and R.A. Jones, J. Am. Chem. Soc., 1982, 104, 1316.
9. F. Seela and K. Kaiser, Helv. Chim. Acta, 1988, 71, 1813.
10. B. C. Froehler, P. G. Ng, and M. D. Matteucci, Nucleic Acids Res., 1986, 14, 5399.
11. N. D. Sinha, J. Biernat, J. McManus, and H. Köster, Nucleic Acids Res., 1984, 12, 4539.
12. Applied Biosystems, 'User Bulletin', 1990, pp. 6-15.

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