

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

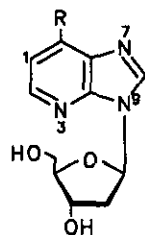
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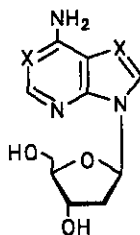
Abstract— The synthesis of the 3'-[(2-cyanoethyl)diisopropylphosphoramidite] (3b) and the 3'-phosphonate (3a) of 1-deaza-2'-deoxyadenosine (1b) is described. For this purpose compound 1b was protected at the 6-amino group with a benzoyl residue. Ensuing 4,4'-dimethoxytritylation of 1b and phosphitylation afforded the P(III) derivatives (3a) and (3b). They were successfully employed in solid-phase oligodeoxyribonucleotide synthesis of d(c¹A-c¹A-c¹A-A-A-A) (6). ¹³C-Nmr and ¹⁵N-nmr spectra of the compounds (1-5) are discussed.

INTRODUCTION

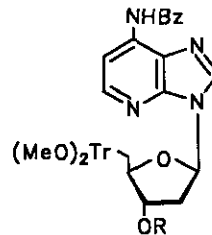
Oligonucleotides containing imidazo[4,5-*b*]pyridine (1-deazapurine) nucleosides (e.g. 1b) cannot form regular Watson-Crick base pairs with complementary dT-residues. This has already been demonstrated in the case of oligomers containing 1,7-dideazaadenosine (2b).¹ However, in the case of 1b, Hoogsteen 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 (c¹A_d, 1b) and their use in solid-phase oligodeoxyribonucleotide synthesis.



1a: R = NO₂
1b: R = NH₂



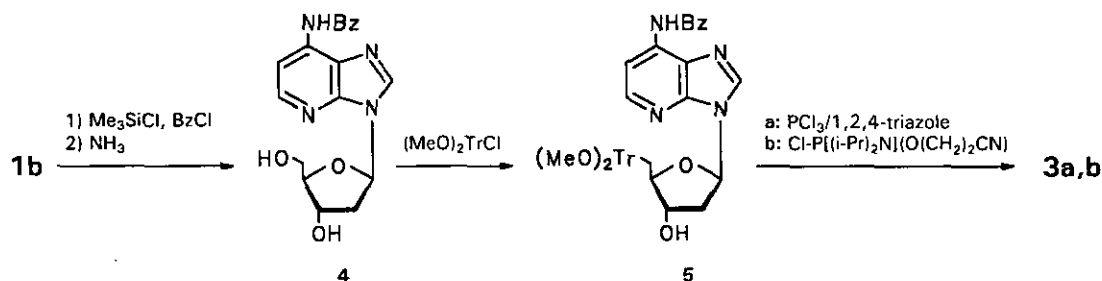
2a X = N
2b X = CH



3a R = -PH(O)O⁻Et₃NH⁺
3b R = -P[(i-Pr)₂N](O(CH₂)₂CN)

RESULTS AND DISCUSSION

The nitronucleoside (**1a**) has already been synthesized² and the amino compound (**1b**) was obtained by deoxygenation³ 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 ¹H-nmr NOE difference spectroscopy.⁵ According to Table 1 the configuration at the glycosylic bond was β-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-d₆ 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 , ^1H] Coupling Constants^{a)} of Compounds (**1b**) and (**2a**)⁷ measured in $\text{DMSO}-d_6$.^{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 hydroxyls⁸ 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 bz⁶A_d (71 min¹) 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 ¹³C-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 ¹H, ¹³C-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. ¹³C-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)	<u>CH</u> 3CH2N ^c)	CH3 <u>CH</u> 2N ^c)	OCH3 ^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 [¹H, ¹³C] correlation spectra. ^d) From ¹H, ¹³C gated-decoupled spectra.

Compound (**5**) was converted into the phosphonate¹⁰ [**3a** (³¹P-nmr (DMSO-*d*₆): δ = 2.56 ppm; $J(\text{P}, \text{C}-3') = 5.0$ Hz and $J(\text{P}, \text{C}-4') = 5.0$ Hz, $^1J(\text{P}, \text{H}) = 587$ Hz)] as well as into the β -cyanoethylphosphoramidite¹¹ [**3b** (³¹P-nmr (DMSO-*d*₆): δ = 148.2 ppm (dd, $J(\text{P}-\text{H}) = 5.7$ Hz, 11.0 Hz) and δ = 148.2 ppm (dd, $J(\text{P}-\text{H}) = 6.2$ Hz, 11.3 Hz)].

The phosphonate (**3a**) was then used in solid-phase oligonucleotide synthesis¹¹ 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/H₂O/THF was carried out on the oligomeric level. The (MeO)₂Tr-protected oligonucleotide was split off from the polymer support by conc. aq. NH₄OH and purified by RP-18 hplc. Detritylation was accomplished with 80% HOAc/H₂O within 30 min and was followed by neutralization. The oligomer (**6**) d(c¹A-c¹A-c¹A-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 phosphatase¹⁰ and analysis by RP-18 hplc (Figure).

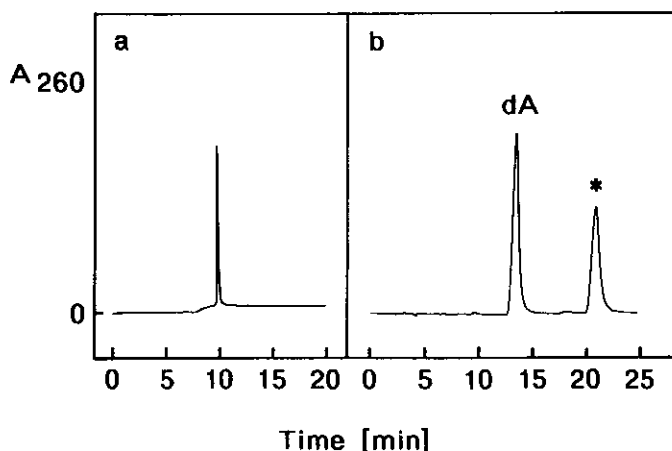


Figure. Hplc profiles of **6**; a) and its enzymatic digest b) after tandem hydrolysis with snake-venom phosphodiesterase and alkaline phosphatase in 0.1 M Tris-HCl (pH 8.3). Gradient a) 0-20% MeCN in 0.1 M (Et₃NH)OAc (pH 7.0)/MeCN, 95:5; b) in the absence of MeCN.

The incorporation of c¹A_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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