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OLIGODEOXYRIBONUCLEOTIDES CONTAINING N^7-(2-DEOXY-ß-D-*ERYTHRO*-PENTOFURANOSYL)ADENINE

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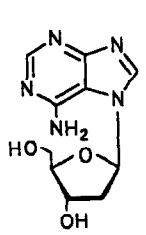
Abstract: The synthesis of N^7-(2-deoxy-ß-D-*erythro*-pentofuranosyl)adenine (^7A_d, **1**) is described. Compound **1** was protected at the 6-amino group with the dimethylaminomethylidene residue (**3a**), the DMT group was selected for OH-5' protection (**3b**) and the 3'-phosphonate **3c** was prepared. Solid-phase synthesis employing **3c** furnished oligonucleotides containing ^7A_d.

Watson-Crick base pairing between purine and pyrimidine bases is not restricted to DNA or RNA but can occur in oligomeric structures with a modified sugar phosphate backbone [1-3] or an altered anomeric centre [4]. From model building it is expected that oligonucleotide chains containing N^7-(2-deoxy-ß-D-*erythro*-pentofuranosyl)adenine (^7A_d, **1**) instead of dA (**2**) can form "reversed" Watson-Crick base pairs with complementary oligo(2'-deoxythymidylate). Here, the synthesis of compound **1** is described by two different routes: (i) glycosylation of 6-substituted purines and (ii) glycosylation of an imidazole precursor followed by anellation of the pyrimidine moiety. Furthermore, compound **1** is converted into the phosphonate **3c** which is employed in solid-phase synthesis of an oligonucleotide containing a N-7 linked adenine moiety.



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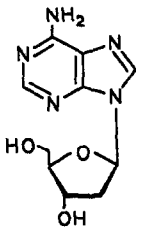

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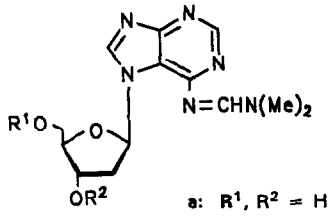
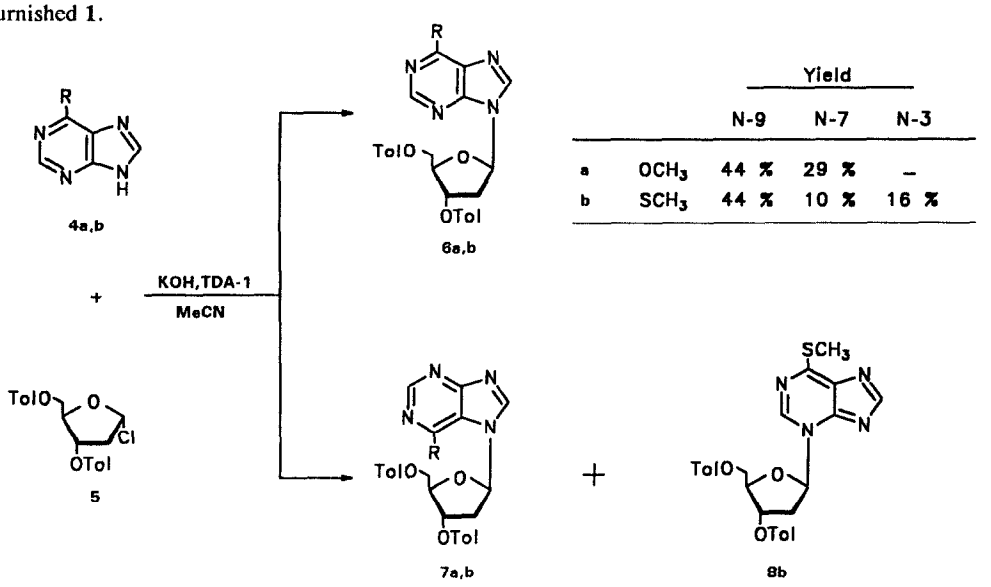


3a-c

a: R^1, R^2 = H
b: R^1 = $(Me)_2Tr$, R^2 = H
c: R^1 = $(Me)_2Tr$, R^2 = $HPO^-Et_3NH^+$ (O)

Previous investigations on the glycosylation of 6-chloropurine or 2,6-dichloropurine anions with the halogenose **5** showed, that the usual site of glycosylation is N-9. The N-7 regioisomer is formed only as minor product [5]. In order to increase the N-7 regioselectivity 6-substituted purine derivates such as 6-methoxypurine (**4a**) or the 6-methylthiopurine (**4b**) are used during nucleobase anion glycosylation [6,7]. Reaction of the **4a** or **4b** anions with the halogenose **5** [8] furnished the N-7 isomers **7a** (29%) or **7b** (10%) together with the N-9 compounds **6a** (44%) and **6b** (44%), stereoselectively. As it can be seen the N-7/N-9 ratio was significantly shifted towards N-7 in case of the 6-methoxypurine (**4a**). The N-3 compound **8b**

(16%) was isolated as third glycosylation product from **4b**. Compounds **9** and **10** were isolated after detoluoylation with methanolic ammonia at room temperature. Treatment of **9** at elevated temperature furnished **1**.



		Yield		
		N-9	N-7	N-3
a	OCH_3	44 %	29 %	–
b	SCH_3	44 %	10 %	16 %

The position of glycosylation was determined by ^1H NOE difference spectroscopy [9]. Upon irradiation of H-1' compounds **1** and **9** exhibited NOEs at the amino or the methoxy group together with those of H-8 confirming N-7 as glycosylation sites. In the case of **8b** an NOE was detected at H-2 but not at H-8 (Table 1).

Table 1. NOE Data (%) of **1**, **8b**, **9** and **10** upon Irradiation of H-1'.[a]

Compound	H-2'	H-4'	H-2	H-8	NH_2	OCH_3
1	7.5	3.2	-	8.2	2.0	-
8b	6.2	1.4	9.3	-	-	-
9	6.6	2.0	-	3.1	-	1.4
10	6.8	1.9	-	1.3	-	-

a) DMSO-d_6 at 23°C.

Further evidence for N-7 as glycosylation site came from the ^{13}C NMR spectra. Chemical shifts were assigned on the basis of J(H,C) coupling constants. The N-7 isomers exhibit downfield shifts of C-4 being approximately equivalent to the upfield shift of C-5. In the case of the N-3 compound **8b**, the C-8 signal is shifted downfield and C-2 is shifted upfield.

Table 2. ^{13}C NMR Chemical Shifts of Purine 2-Deoxyribonucleosides.[a,b]

	C-2	C-4	C-5	C-6	C-8	SCH_3	OCH_3
1	152.6	159.4	110.4	151.6	143.6	-	-
2	152.5	148.9	119.4	156.2	139.7		
3a	152.0	160.9	116.2	154.5	143.6	-	-
3b	152.1	160.9	116.3	154.6	143.3	-	-
6a	151.8	151.6	121.4	160.5	142.8	-	54.1
6b	151.6	147.8	131.5	160.6	143.5	11.3	-
7a	151.8	161.9	111.7	156.5	145.1	-	54.4
7b[c]	152.7	158.6	122.3	153.2	143.3	12.3	-
8b	139.0	147.8	136.0	160.1	158.0	12.0	-
9	151.6	161.5	111.8	156.6	144.6	-	54.3
10	151.5	157.9	122.1	152.9	145.4	12.0	-

	C-1'	C-2'	C-3'	C-4'	C-5'	CN
1	85.4	40.9	69.3	87.7	60.4	
2	84.1	DMSO	71.1	88.1	62.0	
3a	86.5	40.7	69.9	88.0	61.2	156.6
3b	85.8	40.5	70.0	85.7	63.9	156.6
6a	84.3	35.6	74.9	81.9	64.0	
6b	84.4	35.6	74.9	81.9	64.0	
7a	86.4	37.6	74.6	81.8	64.2	
7b[c]	87.0	41.0	74.5	83.3	61.8	
8b	90.7	37.1	74.6	83.2	63.9	
9	86.4	41.0	70.2	88.1	61.7	
10	86.2	41.0	69.9	88.1	60.9	

[a] Spectra measured in DMSO-d_6 rel. to TMS. [b] From $^1H,^{13}C$ gated-decoupled spectra. [c] In $CDCl_3$.

Alternatively, compound **1** was synthesized via glycosylation of 5-amino-4-imidazolecarbonitrile with the halogenose **5** [8], which gave a much better N-7/N-9 ratio (2:1) [10]. Anellation of the pyrimidine ring was achieved by condensation of the imidazole intermediate **11** with diethoxymethyl acetate followed by treatment with methanolic ammonia. Crystalline **1** (m.p. 178°C, MeOH) was obtained in 75% yield.

Acid-catalyzed depurination of dA is the most critical step during oligonucleotide synthesis. As it has been reported that adenine N^7-ribofuranoside is hydrolytically more labile than the N-9 compound [10] the stability of the N-glycosylic bond of compound **1** was determined. Hydrolysis of **1** was followed UV-spectrophotometrically at 273 nm resulting in a half life of 4.7 min (0.1 N HCl) compared to 95 min for dA [12]. The reduced stability impairs the choice of the amino protecting group of **1**. Because of the stabilizing effect of the dimethylaminomethylene residue [12-14] the amidine **3a** (m.p. 183-184°C, acetone/MeOH) was

prepared. The 3'-phosphonate **3c** (^{31}P NMR (DMSO)d_6: δ = 0.95 ppm, ^1J (PH) = 582 Hz) was then isolated after 4,4'-dimethoxytritylation (**3b**) [15] followed by reaction with PCl_3/1,2,4-triazole.

Next, compound **3c** was employed in solid-phase oligonucleotide synthesis [16] using the standard protocol of phosphonate chemistry [17]. After coupling and oxidation the DMT-derivative of **12** and **13** were removed from the solid support and purified on RP-18 HPLC. Upon detritylation (aq. 50% AcOH), purification on RP-18 HPLC, and desalting the oligomers were lyophilized.

Figure 1a shows the HPLC profile of d(T-T-A-^{7}A-T-T) (**12**). The composition of the oligomer was determined after tandem hydrolysis with snake venom phosphodiesterase followed by alkaline phosphatase (Fig. 1c). The nucleoside **1** (Figure 1b) showed an almost identical retention time as dT but a higher mobility as dA (Fig. 1c). Oligonucleotides containing **1** are expected to form different base pairing pattern as those containing dA. Experiments regarding this behaviour are under current investigation.

d(T-T-A-^{7}A-T-T)
12
d(A-A-A-A-^{7}A-A)
13

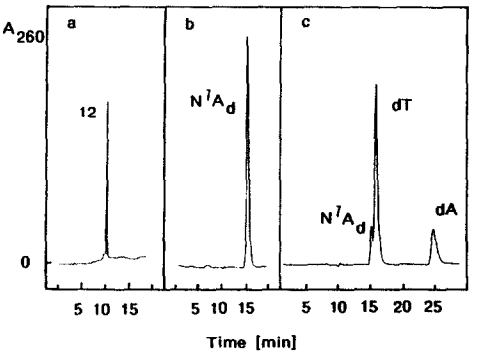


Figure 1. HPLC profiles of a) oligomer **12**; b) ^7A_d (**1**); c) digest of **12** after snake-venom phosphodiesterase and alkaline phosphatase treatment in 0.1 M Tris-HCl (pH 8.3). Gradient: a) 0-20% MeCN in 0.1 M (Et_3NH)OAc (pH 7.0)/MeCN, 95:5; b) and c) in the absence of MeCN.

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