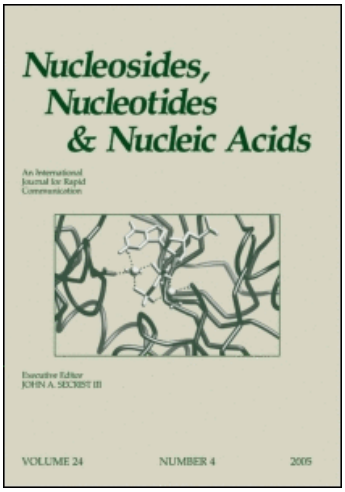

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Synthesis and Biological Activity of the Mono- and Diamino Analogues of 2'-Deoxyadenosine, Cordycepin, 9-(3-Deoxy-α-D-Threo-Pentofuranosyl)-Adenine (A Structural Component of Agrocin 84) and 9-(2-Deoxy-α-D-Threo-Pentofuranosyl)Adenine

Piet Herdewijn[a]; Jan Balzarini[a]; Rudi Pauwels[a]; Gerard Janssen[a]; Arthur Van Aerschot[a]; Erik De Clercq[a]
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SYNTHESIS AND BIOLOGICAL ACTIVITY OF THE MONO- AND DIAMINO ANALOGUES OF 2'-DEOXYADENOSINE, CORDYCEPIN, 9-(3-DEOXY-β-D-THREO-PENTOFURANOSYL)-ADENINE (A STRUCTURAL COMPONENT OF AGROCIN 84) AND 9-(2-DEOXY-β-D-THREO-PENTOFURANOSYL)ADENINE

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Abstract. The mono- and diamino analogues of 9-(2-deoxy-β-D-erythro-pentofuranosyl)adenine 1a, 9-(2-deoxy-β-D-threo-pentofuranosyl)adenine 4a, 9-(3-deoxy-β-D-erythro-pentofuranosyl)adenine 2a and 9-(3-deoxy-β-D-threo-pentofuranosyl)adenine 3a were synthesized by triphenylphosphine reduction of the corresponding azido compounds. The azido group was introduced by a substitution reaction with lithium azide on mesylates or, more directly, by reaction with lithium azide, triphenylphosphine and carbon tetrabromide. Of the newly synthesized compounds, only 3'-amino-2',3'-dideoxyadenosine proved, albeit slightly, inhibitory to murine leukemia L1210 and mammary carcinoma FM3A, and human B-lymphoblast Raji, T-lymphoblast Molt/4F and T-lymphocyte MT-4 cell proliferation in vitro (50 % inhibitory dose : 43.1-323 μM). None of the compounds inhibited human immunodeficiency virus-induced cytopathogenicity in MT-4 cells.

Nucleosides in which the carbohydrate part has been replaced by an aminosugar has been the subject of intensive research during the past two decades. Of special interest are the naturally occuring nucleoside antibiotics 2'-amino-2'-deoxyguanosine[1] and 3'-amino-3'-deoxyadenosine[2], both showing antitumor activity. The latter is also a structural part of puromycin[3], a strong inhibitor of protein biosynthesis, and of different other natural compounds such as antibiotic A201A, lysylaminoadenosine and homocitrullylaminoadenosine[4]. Nucleoside antibiotics with yet more complex formulae, containing aminosugar residues, are even more frequent[4].

With 5-iodo-2'-deoxyuridine as the starting material, a decrease in toxicity with only a slight loss of antiviral activity was noted upon

converting the 5'-hydroxyl group of 5-iodo-2'-deoxyuridine to an amino group[5]. The selectivity of these compounds against herpes simplex virus infection is based upon a selective phosphorylation by the virus-infected cells. Also, activity was seen against murine leukemia virus but not Rous sarcoma virus[5]. 5'-Amino-2',5'-dideoxythymidine by itself shows anti-herpes activity both *in vitro* and *in vivo*[6], whereas 3'-amino-2',3'-dideoxythymidine inhibits the replication of murine sarcoma 180 cells and murine L1210 cells[7]. Cytotoxic activity has also been described for 3'-amino-2',3'-dideoxycytidine[8]. Amino sugar modifications have been less frequently carried out with purine deoxynucleosides, partly because of synthetic problems. Of the four possible 2'- or 3'-deoxyadenosine analogues (Scheme 1), three of them, 1a, 2a and 3a, are natural products. 2'-Deoxyadenosine (1a) is a monomer building block for DNA synthesis, cordycepin (2a) was isolated from the culture filtrates of *Cordyceps militaris* and *Aspergillus nidulans*[9], whereas 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3a) is part of the antibiotic Agrocin 84[10].

We synthesized and evaluated all possible isomers of the four deoxyadenosines in which one or two hydroxyl groups are replaced by an amino-group. These compounds could possibly function as antimetabolites of the natural 2'-deoxyadenosine or adenosine, modulate the antineoplastic activity of cordycepin, or act as anomalous substrates or inhibitors of other enzymes. We were also interested in exploring the anti-HIV activity of these compounds since the number of nucleosides containing an amino sugar that have been tested against human immunodeficiency virus (HIV) is rather limited[11].

Only three aminosugar derivatives of deoxyadenosines have been described in the past : 3'-amino-2',3'-dideoxyadenosine[12,13], 2'-amino-2',3'-dideoxyadenosine[12] and 5'-amino-2',5'-dideoxyadenosine[14,15]. The syntheses, as described here, are based on either substitution of mesylates with the azido nucleophile or direct introduction of an azido group by the triphenylphosphine/carbon tetrabromide activation method[16]. All amino compounds could be obtained by triphenylphosphine reduction of the corresponding azido compound, following a procedure that was originally described by Staudinger[17].

CHEMISTRY

Three of the starting materials for the synthesis of the amino analogues : cordycepin[18] (2a), 9-(3-deoxy-β-D-*threo*-pentofuranosyl)adenine[19]

(3a) and 9-(2-deoxy-β-D-threo-pentofuranosyl)adenine[19] (4a) were described previously, while the fourth, 2'-deoxyadenosine (1a) is commercially available. For the synthesis of 9-(3-deoxy-β-D-threo-pentofuranosyl)adenine (3a), 3'-O-tosyladenosine (5) is required as starting material. 3'-O-p-Nitrobenzenesulfonyladenosine has been synthesized in 14 % yield from 5'-O-acetyladenosine[20]. 3'-O-Tosyladenosine can also be isolated as a minor compound during the 2'-O-tosylation of adenosine by the regioselective dibutyltin oxide activation method[21,22,23]. More recently, this compound has been synthesized by a phase-transfer catalysed reaction[24] but the yields were rather low (6 %) and the compound was isolated by HPLC. Our 4 step synthesis of 5 made use of the rather expensive t-butyldimethylsilyl protecting group, and resulted in a total yield of 36 %[25]. A transient protection of the 2'-OH with a benzoyl group, however, as depicted in Scheme 2, gave crystalline 3'-O-tosyladenosine in 3 steps in 43 % yield from adenosine with no chromatographic purification problems. This transient protection of the 2'-OH group through selective benzoylation is based on the steric hindrance of the 5'-O-protecting group together with the slight difference in acidity of both hydroxy groups. It was hoped that no acylmigration would occur during the tosylation step. This was indeed the case. The reaction mixture, after debenzoylation, contained mainly two compounds : the 3'-O-tosylated compound and the starting material.

Although selective mesylation of a carbohydrate hydroxyl group in the presence of the unprotected adenine base can be done with mesylchloride in pyridine, we prefer to protect the adenine base in order to prevent, as much as possible, nucleophilic attack of the base on the activated sugar during subsequent reactions[26]. Protection of the adenine base with a benzoyl group, to obtain 1b, 3b and 4b, was accomplished by the transient protection method[27]. The protected cordycepin 2b has been described previously[28].

Reaction of N-benzoyl-2'-deoxyadenosine[27] (1b) with 1.6 equivalents of methanesulfonyl chloride in pyridine proceeded smoothly at room temperature to give a 70 % yield of the 5'-O-mesylated compound 1c (Scheme 3). Reaction of 1c with lithium azide at 80°C gave a near quantitative yield of N-benzoyl-5'-azido-2',5'-dideoxyadenosine (7a) which could be further mesylated to 7b and converted into the diazido compound 8 without any problem.

Reaction of N-benzoyl-2'-deoxyadenosine (1b) with 2 equivalents of triphenylphosphine and carbon tetrabromide in the presence of 10 equivalents of lithium azide afforded directly 7a in 70 % yield together with 20 % of 8.

After mesylation in pyridine at room temperature, the protected cordycepin 2b[28] was treated with lithium azide in dimethylformamide at 80°C (Scheme 4). The yield of 6a was rather low probably due to partial debenzoylation. Therefore, the total reaction mixture, after the reaction with lithium azide, was debenzoylated directly with ammonia in methanol and purified by column chromatography. In this reaction, a second compound in 20 % yield was isolated and identified as 5-amino-1-(3,5-dideoxy-β-D-ribofuranosyl)imidazole-4-(N-benzoyl)carboxamidine-$N^5 \rightarrow$ 5'-cyclonucleoside. This compound was formed by attack of N^3 of the adenine base on the 5'-activated carbon atom, followed by opening of the pyrimidinium ring. 2'-O-mesylcordycepin 2d[25] was converted in a one pot reaction into N,N-dibenzoyl-2',5'-di-O- mesylcordycepin 2e by reaction with mesylchloride at 4°C followed by reaction with benzoylchloride at room temperature. Reaction of 2e with lithium azide in dimethylformamide at 100°C for 1.5 h gave N-benzoyl- 9-(5-azido-2-O-mesyl-3,5-dideoxy-β-D-erythro-pentofuranosyl)-adenine (6b) which could be converted into N-benzoyl-9-(2,5-diazido-2,3,5-trideoxy-β- D-threo-pentofuranosyl)adenine by further heating the reaction mixture at 100°C for 42 h. This compound was fully characterized after debenzoylation.

Mesylation of 3b was easier than the reaction with N-benzoyl-2'-deoxyadenosine (1b). After 1 h at room temperature with 1.5 equivalents of mesylchloride in pyridine, all the starting material was disappeared and already some dimesylated compound 3d was formed (Scheme 5). The latter could be easily obtained from 3b by reaction with 4 equivalents of mesylchloride at room temperature for 4 h. Reaction of 3c with lithium azide under the same reaction conditions as for the synthesis of 7a from 1c gave a much lower yield of 10. Conversion of 3d into 11 gave no problems.

4c was mesylated to 4d in the usual way and treated with lithium azide to obtain 12 (Scheme 6). This substitution reaction was also more difficult than the reaction with 1c. The same compound 4d, could be used directly in a one pot synthesis of 13. Therefore, N-benzoyl-9-(5-O-mesyl-

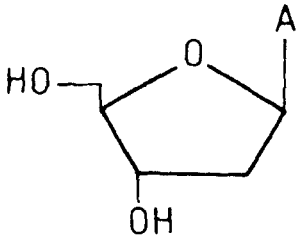
2-deoxy-ß-D-threo-pentofuranosyl)adenine (4d) was treated overnight with 1.25 eq of triphenylphosphine and carbon tetrabromide in dimethylformamide at room temperature in the presence of lithium azide, followed by heating the reaction mixture for 35 min at 100°C, to furnish 13 in 71 % yield.

The synthesis of the four different 2'- and 3'-azido-substituted 2',3'-dideoxyadenosine analogues (16, 17, 22, 23) has been described previously[25]. 3'-Azido-2',3'-dideoxyadenosine (22) can also be obtained in three steps from 4a by selective 5'-O-benzoylation to 4b, reaction with lithium azide in the presence of carbon tetrabromide and triphenylphosphine, and debenzoylation with ammonia in methanol[29].

All these compounds were debenzoylated with ammonia in methanol at room temperature overnight and the azido groups were reduced with triphenylphosphine[17] followed by hydrolysis of the phosphinimine with concentrated ammonia. These reactions were quantitative on T.L.C. and the yields obtained (Table 2) were dependent on the ease of crystallization (the yields mentioned are those of the first crystallization fraction). The complete reduction of the diazido compounds was confirmed by the absence of an azido absorption in the IR spectrum. The diamino compounds were isolated as monoacetates.

When comparing the chemical shift values of H-1' (ppm) for the different azido- (Table 1), hydroxy- (Scheme 1) and amino- (Table 2) 3'-deoxy-analogues, it is evident that H-1' of the compounds with the 2'-erythro-configuration always resonates at higher field than H-1' of the compounds with the 2'-threo-configuration. The opposite is true for the 2'-deoxynucleosides : H-1' of 3'-threo compounds resonates at a higher field than H-1' of 3'-erythro compounds, the values for 22 and 23 approaching one another.

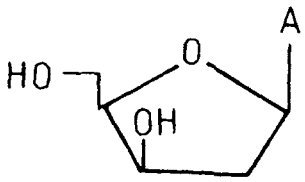
In the hydroxy- (Scheme 1) and azido- (Table 1) series, the coupling constants between H-1' and H-2' for the 3'-deoxy compounds with a 2'-erythro configuration varied between 2 and 3.2 Hz and for the 3'-deoxy compounds with a 2'-threo configuration they varied between 5 and 6.1 Hz. An analogous relationship was found for the 2'-deoxy compounds where the coupling constants between H-1',H-2' and H-1',H-2" were very similar in the 3'-erythro series but markedly different in the 3'-threo series. However, this relationship did not extend to the amino compounds (Table 2).



1a

H-1' : 6.39 ppm

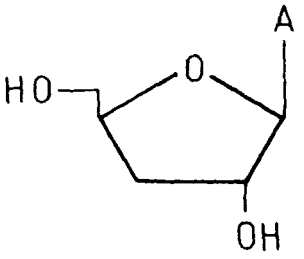
$J_{1',2'}$ and $J_{1',2''}$ = 6.7 and 7.3 Hz



4a

H-1' : 6.24 ppm

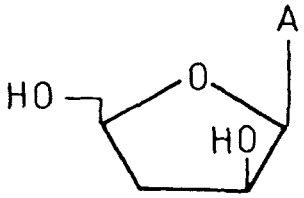
$J_{1',2'}$ and $J_{1',2''}$ = 2.6 and 8.3 Hz



2a

H-1' : 5.87 ppm

$J_{1',2'}$ = 2.1 Hz



3a

H-1' = 6.15 ppm

$J_{1',2'}$ = 5.1 Hz

Scheme 1

$J_{1',2'}$ values for the 3'-deoxy-ß-D-threo-pentofuranoses were higher than for 3'-deoxy-ß-D-erythro-pentofuranoses, although overlapping existed for the non-homologous molecules : for 9-(5-amino-3,5-dideoxy-ß-D-threo-pentofuranosyl)adenine (27) $J_{1',2'}$ is 4.2 Hz and for 9-(2-amino-2,3-dideoxy-ß-D-erythro-pentofuranosyl)adenine (28) it is 4.4 Hz. In the 2'-deoxy series, the values for 9-(3-amino-2,3-dideoxy-ß-D-threo-pentofuranosyl)adenine (35) did not correspond at all with those obtained for related

structures. It should be kept in mind, however, that all these compounds are not all in the same protonated form.

The electron impact mass spectra of some of the precursors and of the deoxyamino-adenosines show the expected fragmentation.[30] Major fragment ions in the mass spectra of the deoxyamino-adenosines are summarized in Table 3.

BIOLOGICAL ACTIVITY

The cytostatic effects of compounds 26 to 37 listed in Table 2 have been examined using several murine (leukemia L1210, mammary carcinoma FM3A) and human (B-lymphoblast Raji, T-lymphoblast Molt/4F, T-lymphocyte MT-4) cell lines. 3'-Amino-2',3'-dideoxyadenosine (compound 34) proved slightly inhibitory against the proliferation of L1210, FM3A, Raji, Molt/4F and MT-4 cells (50 %-inhibitory dose : 139, 54, 323, 43 and 104 μM, respectively). The other compounds were devoid of any significant cytostatic effect at 400 μM (data not shown).

When evaluated for their antiretroviral effects, none of the compounds proved inhibitory to human immunodeficiency virus (HIV)-induced cytopathogenicity in MT-4 cells at 500 μM (or, for compounds 28 and 34, 100 μM, the latter corresponding to the subtoxic concentration of these compounds) or Moloney murine sarcoma virus (MSV)-induced transformation of murine C3H embryo fibroblast cells at 400 μM (except for compounds 28 and 34, which showed a 50 % effective dose of 144 and 235 μM, respectively) (Table 2). In conclusion, none of the 2'-, 3'-, or 5'-monoamino-, or 2',5'- or 3',5'-diamino-substituted derivatives of adenosine had substantial antiretroviral and cytostatic properties *in vitro*.

EXPERIMENTAL SECTION

Melting points were determined in capillary tubes with a Büchi-Tottoli apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer 257 spectrophotometer on samples in potassium bromide disks at 1.5 %. Ultraviolet spectra were recorded with a Beckman UV 5230 spectrophotometer. Electron impact mass spectra were determined with an AEI MS-12 apparatus, at 70 eV electron energy and 170-220°C ion source temperature. Samples were introduced by direct insertion (M = molecular ion, B = base fragment, S = sugar fragment). The ^{1}H NMR and ^{13}C NMR spectra

Table 1. ^{1}H NMR data of the azido-substituted compounds

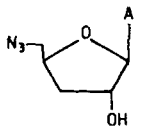
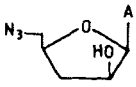
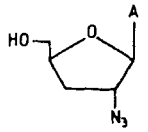
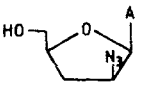
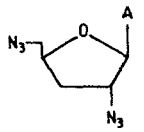
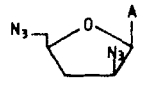
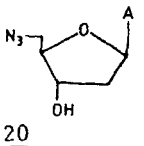
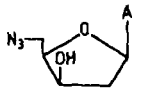
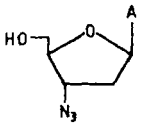
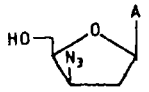
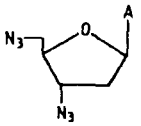
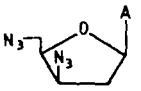
	H-1'	$J_{1',2'}$	H-2'	H-3'	H-4'	H-5'	H-2 H-8	NH_2 OH[2]
	5.94	2.4	4.76	2.04 2.30	4.49	3.58	8.18 8.29	7.27 5.74
14								
	6.19	5.1	4.49	1.88- 2.40	4.24	3.60	8.14 8.17	7.20 5.52
15								
	6.00	3.1	4.82	1.90- 2.60	4.30	3.60	8.16 8.38	7.29 5.18
16								
	6.36	6.1	4.79	1.95- 2.58	4.17	3.69	8.16 8.40	7.27 5.19
17								
	6.03	3.2	5.07	2.10- 2.78	4.44	3.60	8.17 8.31	7.30
18								
	6.41	5.9	4.85	2.20 2.64	4.33	3.68	8.18 8.23	7.32
19								
	6.40	6.8 6.8	2.36 2.95	4.43	3.99	3.57	8.17 8.34	7.26 5.47
20								

Table 1 (continued)

	6.30	2.6 8.3	2.38 2.84	4.38	4.09	3.60	8.19 8.38	7.38 6.30
21								
	6.32	6.6 6.6	2.44 2.96	4.61	3.94	3.61	8.15 8.34	7.30 5.34
22								
	6.30	3.8 7.3	2.50- 3.08	4.59	4.16	3.73	8.17 8.25	7.30 5.07
23								
	6.38	6.6 6.6	2.60 3.13	4.70	4.09	3.66	8.19 8.37	7.31
24								
	6.30	4.6 7.2	2.65- 3.08	4.66	4.29	3.67	8.16 8.27	7.27
25								

All spectra were taken in DMSO-d_6 with tetramethylsilane as internal standard.

Table 2. ^{1}H NMR[a] data and biological activities of the amino-substituted compounds

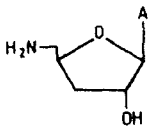
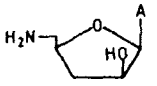
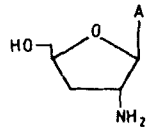
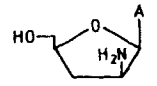
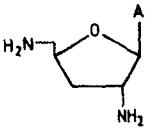
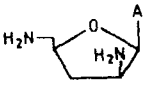
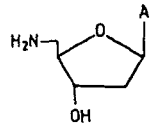
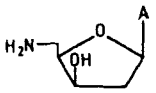
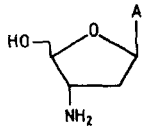
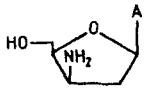
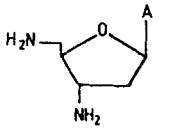
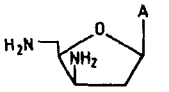
	H-1'	$J_{1',2'}$	H-2 H-8	Inhibition of HIV-induced cytopathogenicity in MT-4 cells ED_{50}[b] (μg/ml)	Inhibition of MT-4 cell growth ID_{50}[c] (μM)	Inhibition of MSV-induced transformation of C3H cells ED_{50}[d] (μM)
 26	5.87	2.4	8.14 8.31	> 500	> 500	> 400
 27	6.09	4.2	8.12 8.30	> 500	> 500	> 400
 28	5.73	4.4	8.13 8.30	> 100	220 ± 30	144 ± 65
 29	6.11	6.4	8.12 8.38	> 500	> 500	> 400
 30	5.70	4.4	8.14 8.29	> 500	> 500	> 400
 31	6.08	6.2	8.32 8.12	> 500	> 500	> 400

Table 2 (continued)

 32	6.33	7.0 7.0	8.14 8.34	> 500	> 500	> 400
 33	6.26	2.2 8.3	8.14 8.36	> 500	> 500	> 400
 34	6.31	5.5 6.2	8.13 8.33	> 100	104 ± 1	235 ± 12
 35	6.19	6.2 6.8	8.14 8.50	> 500	> 500	> 400
 36	6.28	5.3 7.0	8.13 8.31	> 500	> 500	> 400
 37	6.17	4.6 7.4	8.13 8.54	> 500	> 500	> 400

[a]All spectra were taken in DMSO-d_6 with tetramethylsilane as internal standard; chemical shift in δ values (ppm); coupling constants in hertz.

[b]50 %-effective dose, required to inhibit HIV-induced cytopathogenicity in human MT-4 cells by 50 %.

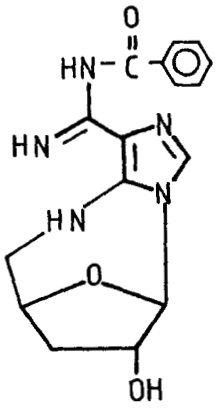
[c]50 %-cytotoxic dose, required to inhibit MT-4 cell growth by 50 %.

[d]50 %-effective dose, required to inhibit MSV-induced transformation of murine C3H fibroblast cells by 50 %.

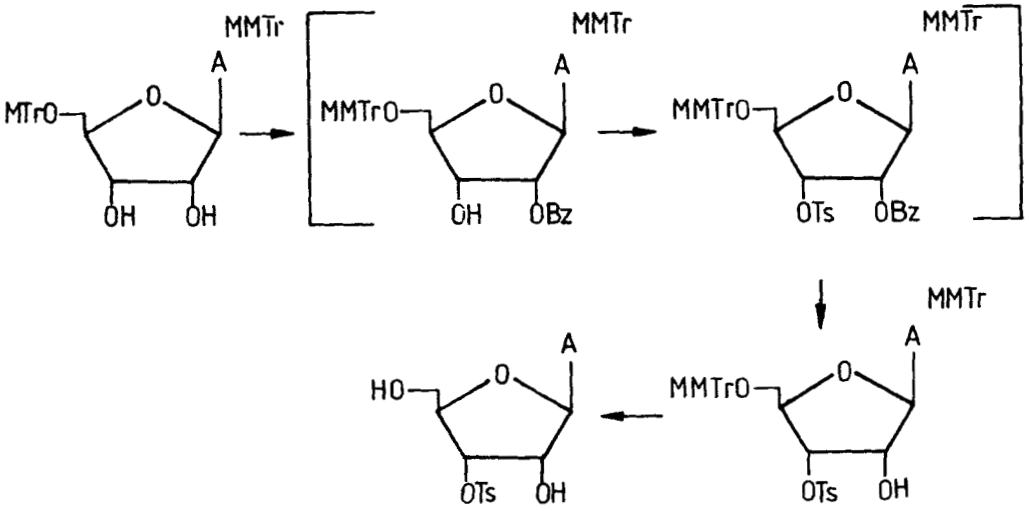
Table 3. Major diagnostic fragment ions in the mass spectra of the deoxy-amino-adenosines[a]

Compd	Ion structure													
	M	M-CH_2X	M-CH_2X-HX	BCHOHCHXH	HBCH=CHCHXH	HBCH=CHCHX	HBCH=CHXH	BCHOH	$HBCH=CH_2$	$B=CH_2$	H_2B	HB	S	S-H
26	250(1)	221(25)	203(5)	194(11)	-	-	178(41)	164(20)	162(4)	148(10)	136(100)	135(41)	116(5)	115(2)
27	250(1)	221(40)	203(7)	194(15)	-	-	178(56)	164(41)	162(4)	148(14)	136(100)	135(38)	116(8)	115(2)
28	250(5)	220(11)	203(8)	-	-	-	177(7)	164(100)	162(2)	148(5)	136(54)	135(32)	-	115(55)
29	250(2)	220(4)	203(3)	-	-	-	177(2)	164(100)	-	148(2)	136(29)	135(16)	116(3)	115(12)
30	-	220(29)	203(17)	193(3)	-	-	177(16)	164(58)	162(2)	148(5)	136(100)	135(39)	-	114(41)
31	249(2)	220(5)	203(7)	193(2)	-	-	177(9)	164(100)	162(3)	148(6)	136(87)	135(46)	115(5)	114(6)
32	250(8)	221(18)	-	-	191(1)	190(1)	-	-	162(72)	148(2)	136(100)	135(58)	116(7)	115(3)
33	250(7)	221(20)	203(24)	-	191(13)	190(11)	-	-	162(100)	148(2)	136(100)	135(93)	116(5)	115(3)
34	250(0.5)	220(5)	-	-	190(16)	189(2)	-	164(11)	162(44)	148(2)	136(100)	135(54)	116(7)	115(23)
35	250(1)	220(20)	203(18)	-	190(58)	189(6)	-	164(11)	162(36)	148(4)	136(67)	135(100)	116(4)	115(4)
36	249(0.5)	220(10)	-	-	190(4)	189(3)	-	-	162(61)	148(2)	136(100)	135(34)	-	114(30)
37	249(3)	220(3)	203(7)	-	190(12)	189(7)	-	164(7)	162(31)	148(2)	136(100)	135(62)	-	114(18)

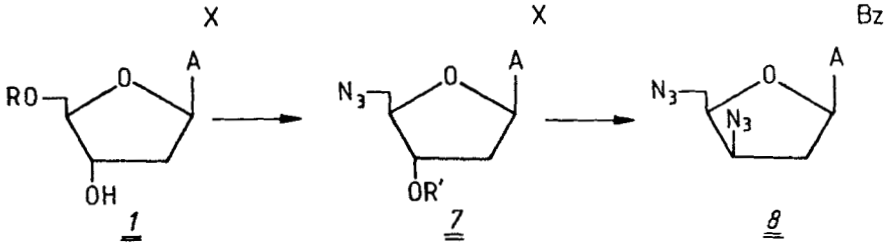
[a]Relative intensities are indicated in parentheses. X = O or NH; B = adenine fragment; S = sugar fragment.



9



Scheme 2

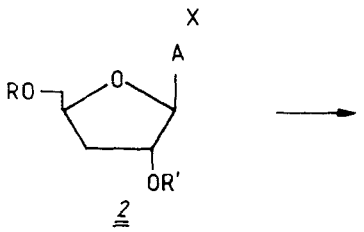


1 7 8

a) R = H ; X = H
b) R = H ; X = Bz
c) R = Ms ; X = Bz

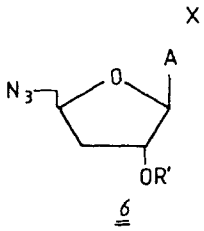
a) R' = H ; X = Bz
b) R' = Ms ; X = Bz

Scheme 3



2

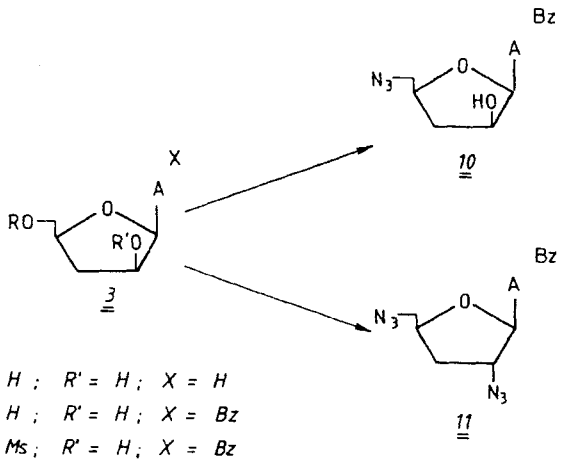
a) R = H ; R' = H ; X = H
b) R = H ; R' = Bz ; X = Bz_2
c) R = Ms ; R' = Bz ; X = Bz_2
d) R = H ; R' = Ms ; X = H
e) R = Ms ; R' = Ms ; X = Bz_2



6

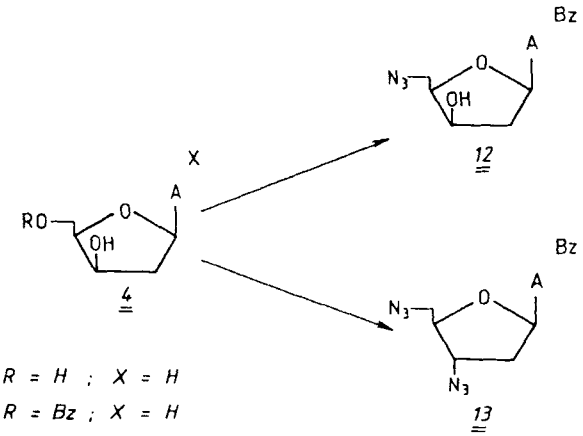
a) R' = Bz ; X = Bz_2
b) R' = Ms ; X = Bz

S c h e m e 4



a) R = H ; R' = H ; X = H
b) R = H ; R' = H ; X = Bz
c) R = Ms ; R' = H ; X = Bz
d) R = Ms ; R' = Ms ; X = Bz

S c h e m e 5



a) R = H ; X = H
b) R = Bz ; X = H
c) R = H ; X = Bz
d) R = Ms ; X = Bz

S c h e m e 6

were determined with a JEOL FX 90Q spectrometer with tetramethylsilane as internal standard (s = singlet, d = doublet, t = triplet, br = broad signal, q = quadruplet, = multiplet) unless stated otherwise. Precoated Merck silica gel F254 plates were used for TLC, and the spots were examined with UV light and sulfuric acid-anisaldehyde spray. Column chromatography was performed on Merck silica gel (0.063-0.200 mm). Anhydrous solvents were obtained as follows : pyridine was refluxed overnight with potassium hydroxide, and distilled; dichloromethane was stored for 1 week over anhydrous calcium chloride, filtered, and distilled; methanol was dried by distillation after it had been refluxed overnight with magnesium-iodine; water was removed from N,N-dimethylformamide by distillation with benzene followed by distillation in vacuo; acetonitrile was first refluxed over phosphorus pentoxide and distilled.

3'-O-Tosyladenosine (5). To a solution of 5.7 g (7 mmol) of N^6,5'-O-di(monomethoxytrityl)adenosine[25] in 100 mL of anhydrous pyridine, cooled to -15°C, was added dropwise, over a period of 30 min, a solution of 0.88 mL (7.5 mmol) of benzoyl chloride in 10 mL of anhydrous pyridine. The reaction mixture was further stirred for 2 h in an ice/NaCl bath and allowed to warm up to 0°C. To this mixture was added 13.3 g (70 mmol) of p-toluenesulfonyl chloride and the mixture was stirred for 6 days at room temperature. After addition of 20 mL of methanol, the reaction mixture was poured into water (500 mL) and extracted twice with Et_2O (2 x 300 mL). The combined organic layer was dried, evaporated, coevaporated with toluene and treated overnight with a saturated solution of ammonia in methanol (300 mL). Column chromatographic purification ($CHCl_3$-MeOH 99.5-0.5) (T.L.C. $CHCl_3$-MeOH 98-2) gave 4.54 g (4.7 mmol, 67 % yield) of N^6,5'-O-di(monomethoxytrityl)-3'-O-tosyladenosine. UV (MeOH) λ_{max} 275 nm (ε 25,400). ^{1}H NMR ($CDCl_3$) δ : 1.29 (s, CH_3); 3.09 (dd, H-5'); 3.43 (dd, H-5", $J_{5',5"}$ = 10.7 Hz); 3.78 (s, CH_3O); 3.81 (s, CH_3O); 4.38 (m, H-4'); 4.98-5.34 (m, H-2', H-3'); 5.86 (d, J = 5.05 Hz, H-1'); 6.74-8.00 (aromatic H) ppm.

This compound (3.86 g, 4 mmol) was stirred overnight in 80 % acetic acid at room temprature. The solvent was evaporated, the residue was diluted with Et_2O, filtered and the precipitate was recrystallized from MeOH furnishing 1.35 g (3.2 mmol, 80 % yield) of 3'-O-tosyladenosine (mp 161-163°C)[25].

N-Benzoyl-9-(3-deoxy-β-D-threo-pentofuranosyl)adenine (3b). 3a[19] was protected with a benzoyl group according to the method of Ti et al.[27] Instead of crystallization, however, the total reaction mixture was, after evaporation of the solvent, applied onto a silica column and eluted with 1) $CHCl_3$-MeOH 95:5, 2) $CHCl_3$-MeOH 90:10. Starting with 2.67 g (10.6 mmol) of 3a, 3.21 g (9.05 mmol, 85 %) of 3b was obtained. mp (MeOH) : 178-179°C. MS (m/e) : 355 (M^+). UV (MeOH) λ_{max} : 280 nm (ε22,200). ^1H NMR (DMSO-d_6) δ : 1.82-2.42 (m, H-3', H-3"); 3.64 (m, H-5', H-5"); 4.12 (m, H-4'); 4.60 (m, H-2'); 5.13 (t, 5'-OH); 5.47 (d, 2'-OH); 6.31 (d, J = 5.5 Hz, H-1'); 7.50-7.68 (m) and 7.97-8.14 (m) (benzoyl); 8.64 and 8.72 (2xs, H-8 and H-2); 11.13 (s, NH) ppm.

N-Benzoyl-9-(2-deoxy-β-D-threo-pentofuranosyl)adenine (4c). The transient protection method was followed[27]. The H_2O layer was evaporated and the title compound was purified by column chromatography ($CHCl_3$-MeOH 96:4) and crystallized from MeOH in 84 % yield (2.98 g, 8.4 mmol) starting from 2.51 g (10 mmol) of 4a. mp : 176-178°C; UV (MeOH) λ_{max} 280 nm (ε22,000); MS (m/e) 355 (M^+). ^1H NMR (DMSO-d_6) δ : 2.21-2.55 (m, H-2'); 2.60-3.03 (m, H-2"); 3.72 (m, H-5', H-5"); 3.97 (m, H-4'); 4.41 (m, H-3'); 4.69 (t, 5'-OH); 5.52 (d, 3'-OH); 6.45 (dd, J = 2.0 and 8.0 Hz, H-1'); 7.51-7.70 (m) and 7.98-8.15 (m) (benzoyl); 8.68 and 8.75 (2xs) (H-8 and H-2) ppm.

N-Benzoyl-5'-O-mesyl-2'-deoxyadenosine (1c). A solution of 1.4 mL (18.15 mmol, 1.65 equiv.) of methanesulfonyl chloride in 10 mL of pyridine was added dropwise over a period of 30 min to a solution of 3.55 g (10 mmol) of N-benzoyl-2'-deoxyadenosine[27] in 100 mL of anhydrous pyridine and the mixture was stirred for 6 h at room temperature. After addition of MeOH (1 mL), the mixture was concentrated to about 15 mL, diluted with H_2O (100 mL) and extracted with EtOAc (100 mL). The organic layer was dried, evaporated and purified by column chromatography ($CHCl_3$-MeOH 95:5). The title compound was crystallized from MeOH : 3.03 g (7 mmol, 70 % yield). mp 140-141°C; UV (MeOH) λ_{max} 280 nm (ε20,900); MS (m/e) no M^+; 319 (M-MsOH-H_2O); 239 (B+H). ^1H NMR (DMSO-d_6) δ: 2.88 (m, H-2', H-2"); 3.11 (s, CH_3); 4.17 (m, H-4'); 4.46 (m, H-5', H-5"); 4.58 (m, H-3'); 5.56 (3'-OH); 6.56 (t, J = 6.8 Hz, H-1'); 7.46-7.65 (m) and 7.98-8.21 (m) (benzoyl); 8.60 and 8.73 (2xs, H-8 and H-2); 11.12 (s, NH) ppm.

N-Benzoyl-5'-azido-2',5'-dideoxyadenosine (7a). A mixture of 1.8 g (4.15 mmol) of 1c and 740 mg (15 mmol) of lithium azide in 10 mL of dime-

thylformamide was heated for 30 min at 80°C. The mixture was concentrated to about 5 mL, diluted with EtOAc (100 mL) and washed with H_2O (50 mL). The H_2O layer was extracted with EtOAc (50 mL) and the combined organic layer was dried and evaporated. The title compound was purified by column chromatography ($CHCl_3$-MeOH 96:4) followed by crystallization from CH_2Cl_2. mp 121-123°C [lit.[16] 102°C (dec)]. IR (KBr) 2100 cm^{-1} (N_3). MS (m/e) 380 (M^+). UV (MeOH) λ_{max} 280 nm (ε 21,000). ^1H NMR ($CDCl_3$) δ : 2.36-3.08 (m, H-2', H-2"); 3.61 (d, H-5', H-5"); 4.16 (q, H-4'); 4.35 (3'-OH); 4.70 (m, H-3'); 6.47 (t, J = 6.4 Hz, H-1'); 7.45-7.63 (m) and 7.93-8.10 (m) (benzoyl); 8.25 and 8.75 (2xs, H-8 and H-2); 9.39 (s, NH) ppm.

N-Benzoyl-3'-O-mesyl-5'-azido-2',5'-dideoxyadenosine (7b). A solution of 380 mg (1 mmol) of 7a and 0.2 ml (2.5 mmol) of methanesulfonyl chloride in 10 mL of anhydrous pyridine was kept at room temperature for 4 h. MeOH (1 mL) was added, the solvent was evaporated and the residue was dissolved in $CHCl_3$ (50 mL) and washed with H_2O (50 mL). Column chromatographic purification ($CHCl_3$-MeOH 98:2) yielded 455 mg (99 %) of 7b as a foam. UV (MeOH) λ_{max} 279 nm (ε 20,400); IR (KBr) 2110 cm^{-1} (N_3). ^1H NMR ($CDCl_3$) δ : 2.65-2.96 (m, H-2'); 3.06-3.41 (m, H-2"); 3.71 (d, H-5', H-5"); 4.43 (m, H-4'); 5.47 (m, H-3'); 6.48 (dd, J = 6.6 and 7.3 Hz, H-1'); 7.53 (m) and 7.92-8.07 (m) (benzoyl); 8.24 and 8.72 (2xs, H-8 and H-2); 9.46 (s, NH) ppm.

N-Benzoyl-9-(3,5-diazido-2,3,5-trideoxy-β-D-threo-pentofuranosyl)-adenine (8). After the mixture of 760 mg (1.66 mmol) of 7b and 830 mg (17 mmol) of lithium azide in 15 mL of dimethylformamide was heated for 6 h 30 min at 80°C, it was concentrated to 5 mL, diluted with $CHCl_3$ (100 mL) and washed with H_2O (50 mL). The organic layer was dried, evaporated and purified by column chromatography ($CHCl_3$-MeOH 99:1) yielding 500 mg (1.23 mmol, 74 %) of the title compound. MS (m/e) 405 (M^+). IR (KBr) 2100 cm^{-1} (N_3). UV (MeOH) λ_{max} 280 nm (ε20,900). ^1H NMR ($CDCl_3$) δ : 2.58-3.17 (m, H-2', H-2"); 3.72 (m, H-5', H-5"); 4.14-4.52 (m, H-3', H-4'); 6.49 (dd, J = 3.3 and 7.3 Hz, H-1'); 7.54 (m) and 7.93-8.09 (m) (benzoyl); 8.37 and 8.74 (2xs, H-8 and H-2); 9.31 (s, NH) ppm.

Alternative synthesis of 7a and 8. A solution of 710 mg (2 mmol) of N-benzoyl-2'-deoxyadenosine, 1.05 g (4 mmol) of triphenylphosphine, 1.33 g (4 mmol) of carbon tetrabromide and 1 g (20 mmol) of lithium azide was kept overnight at room temperature. The mixture was concentrated to 5 mL, diluted with EtOAc (100 mL) and washed with H_2O (100 mL). The organic

layer was dried, evaporated and purified by column chromatography ($CHCl_3$-MeOH 98:2) which yielded 530 mg (1.4 mmol, 70 %) of 7a and 160 mg (0.4 mmol, 20 %) of 8.

$N^6,N^6,2'$-O-Tri-benzoyl-5'-O-mesylcordycepin (2c). A mixture of 850 mg (1.5 mmol) of $N^6,N^6,2'$- O-tri-benzoylcordycepin[28] and 0.23 ml (3 mmol) of mesyl chloride in 10 mL of anhydrous pyridine was kept overnight at room temperature. MeOH (1 mL) was added, the solvent was evaporated and the residue was dissolved in CH_2Cl_2 (100 mL). The solution was washed with H_2O (100 mL), dried, evaporated and filtered trough a short silica column ($CHCl_3$-MeOH 98:2). The conversion to the 5'-O-mesylated compound 2c was quantitative (960 mg, 1.5 mmol). MS (m/e) : no M^+, 342 (B). UV (MeOH) λ_{max} 273 nm (ε 21,500). ^1H NMR ($CDCl_3$) δ : 2.24-3.01 (m, H-3' H-3"); 2.93 (s, CH_3); 4.50 (m, H-5', H-5"); 4.78 (m, H-4'); 6.02 (m, H-2'); 6.29 (d, J = 0.9 Hz, H-1'); 7.20-7.66 (m) and 7.75-8.15 (m) (benzoyl); 8.26 and 8.64 (2xs, H-8 and H-2) ppm.

5'-Azido-5'-deoxycordycepin (14). A solution of 960 mg (1.5 mmol) of 2c in 15 mL of dimethylformamide was heated at 80°C for 2 h in the presence of 250 mg (5 mmol) of lithium azide. The reaction mixture was evaporated, diluted with H_2O (50 mL) and extracted with CH_2Cl_2 (100 mL). The organic layer was purified by column chromatography ($CHCl_3$-MeOH 99:1) yielding 580 mg of 6a [IR (KBr) 2110 cm^{-1} (N_3); UV (272 nm)]. ^1H NMR spectrum showed that the compound was not pure. The reaction mixture itself was rather complex because of N-debenzoylation and N-3' attack on the 5'-O-position as a side reaction. The impure compound (580 mg) was directly debenzoylated with ammonia in methanol (50 mL) overnight. After evaporation of the solvent, a crystalline precipitate was formed which was recrystallized from MeOH : 210 mg (0.76 mmol, 50 % yield) of 14. mp : 196-197°C. IR (KBr) 2110 cm^{-1} (N_3). UV (MeOH) $_{max}$ 258 nm (15,600). Anal. ($C_{10}H_{12}N_8O_2$) C, H, N. When the total reaction mixture was directly debenzoylated and purified by column chromatography, 100 mg (0.3 mmol, 20 %) of 9 was isolated. MS (m/e) 327 (M^+). IR (KBr) no azide band. UV (MeOH) λ_{max} : 343 nm (ε 16,300); 267 nm (ε 17,600); 248 nm (ε 17,500). ^1H NMR (DMSO-d_6) δ 2.16 (m, H-3', H-3"); 3.35 (m, H-5', H-5"); 4.18 (m, H-4'); 4.96 (m,H-2'); 5.64 (d, 2'-OH); 5.92 (s, H-1'); 7.64 (m) and 8.08 (m) (benzoyl); 7.90 (brs), 8.30 (brs), 10.06 (brs) (3 x NH) ppm.

N,N-Dibenzoyl-2',5'-di-O-mesylcordycepin (2e). A solution of 990 mg (3 mmol) of 2'-O-mesylcordycepin[25] (2d) and 0.75 mL (10 mmol) of methane-

sulfonyl chloride in 30 mL of anhydrous pyridine was kept overnight at 4°C. To this mixture was added 1.8 mL (15 mmol) of benzoyl chloride and the mixture was stirred for 5 h at 15°C and concentrated. The residue was dissolved in $CHCl_3$ (100 mL), washed with H_2O (100 mL), dried and purified by column chromatography ($CHCl_3$-MeOH 99:1). UV absorbing fractions were collected, washed with 5 % of sodium bicarbonate (3 x 100 mL), dried and evaporated yielding 1.59 g (2.58 mmol, 86 %) of 2e. MS (m/e) no M^+, 342 (B); UV (MeOH) λ_{max} 274 nm (ε 21,600). ^1H NMR ($CDCl_3$) δ : 2.61 (m, H-3', H-3"); 2.95 (s, CH_3); 3.19 (s, CH_3); 4.47 (m, H-5', H-5"); 4.73 (m, H-4'); 5.82 (m, H-2'); 6.28 (brs, H-1'); 7.42 (m) and 7.83 (m) (benzoyl); 8.25 and 8.63 (2 x s, H-8 and H-2) ppm.

9-(2,5-Diazido-2,3,5-trideoxy-β-D-threo-pentofuranosyl)adenine (19). A solution of 1.85 g (3 mmol) of 2e and 1.5 g (30 mmol) of lithium azide in 30 mL of dimethylformamide was heated at 100°C for 1.5 h. After addition of $CHCl_3$ (150 mL) and washing with H_2O (100 mL), the organic layer was dried, evaporated and purified by column chromatography ($CHCl_3$-MeOH 99:1). This gave 1.15 g (2.51 mmol, 84 % yield) of 6b. [UV (MeOH) λ_{max} : 280 nm; IR (KBr) 2110 cm^{-1} (N_3); ^1H NMR ($CDCl_3$) δ : 2.25-2.73 (m, H-3', H-3"); 3.23 (s, CH_3); 3.67 (2 x dd, H-5', H-5"); 4.63 (m, H-4'); 5.74 (m, H-2'); 6.27 (brs, H-1'); 7.30-7.62 (m) and 7.92-8.12 (m) (benzoyl); 8.29 and 8.71 (2 x s, H-8 and H-2); 9.31 (s, NH) ppm]. This compound was further treated with lithium azide (1 g) in dimethylformamide (20 mL) at 100°C for 42 h. The reaction mixture was concentrated to 5 mL, diluted with EtOAc (100 mL), washed with H_2O (100 mL), dried and evaporated. The residue was diluted with a saturated solution of ammonia in methanol (50 mL), kept overnight at room temperature and evaporated. The title compound 19 was purified by column chromatography ($CHCl_3$-MeOH 98:2) and crystallized from MeOH : 320 mg (1.06 mmol, 35 % yield). mp : 210-211°C; UV (MeOH) λ_{max} 260 nm (ε15,800); IR (KBr) 2110 cm^{-1} (N_3); MS (m/e) 301 (M^+). Anal. ($C_{10}H_{11}N_{11}O$) C, H, N.

N-Benzoyl-9-(5-O-mesyl-3-deoxy-β-D-threo-pentofuranosyl)adenine (3c). 1.3 g (3.66 mmol) Of 3b and 0.43 mL (5.5 mmol) (1.5 equiv.) of methanesulfonyl chloride in 20 mL of anhydrous pyridine was stirred for 1 h at room temperature. MeOH was added, the mixture was evaporated and divided between 200 mL of EtOAc-H_2O (1:1). The H_2O layer was extracted with EtOAc (100 mL) and the combined organic layer was dried, evaporated and purified by column chromatography ($CHCl_3$-MeOH 97:3) yielding 1.37 g

(3.16 mmol, 86 %) of 3c as a foam. UV (MeOH) λ_{max} : 280 nm (ε 21,000); MS (m/e) : no M^+; 337 (M-MsOH); 239 (B+H). ^1H NMR ($CDCl_3$) δ : 2.31 (m, H-3', H-3"); 3.03 (s, CH_3); 4.18-4.83 (m, H-2', H-4', H-5', H-5"); 5.36 (2'-OH); 6.24 (d, J = 3.6 Hz, H-1'); 7.33-7.56 (m) and 7.80-7.99 (m) (benzoyl); 8.29 (s, 2 H, H-8 and H-2); 9.37 (s, NH) ppm. When the original reaction mixture was stirred for 3 h, a 30 % yield of the dimesyl derivative 3d was obtained.

N-Benzoyl-9-(5-azido-3-deoxy-β-D-threo-pentofuranosyl)adenine (10). A mixture of 1.3 g (3 mmol) of 3c and 490 mg (10 mmol) of lithium azide in 10 mL of dimethylformamide was heated at 80°C for 1 h. The reaction mixture was evaporated, diluted with H_2O (30 mL) and extracted with $CHCl_3$ (50 mL). The organic layer was dried and evaporated. Column chromatographic purification ($CHCl_3$-MeOH 98:2) yielded 460 mg (1.2 mmol, 40 %) of the title compound as a foam while 300 mg (0.7 mmol, 23 %) of the starting material was recuperated. MS (m/e) 380 (M^+). UV (MeOH) λ_{max} 280 nm (ε 20,600); IR (KBr) 2110 cm^{-1} (N_3). ^1H NMR ($CDCl_3$) δ : 1.93-2.70 (m, H-3', H-3"); 3.56 (d, H-5', H-5"); 4.27 (m, H-4'); 4.65 (m, H-2'); 5.34 (2'-OH); 6.19 (d, J = 4.2 Hz, H-1'); 7.35-7.58 (m) and 7.85-8.03 (m) (benzoyl); 8.25 and 8.41 (2 x s, H-8 and H-2); 9.28 (NH) ppm.

N-Benzoyl-9-(2,5-di-O-mesyl-β-D-threo-pentofuranosyl)adenine (3d). A mixture of 500 mg (1.41 mmol) of 3b and 0.44 mL (5.64 mmol) of methanesulfonyl chloride in 15 mL of pyridine was stirred for 4 h at room temperature. MeOH (1 mL) was added, the reaction mixture was concentrated, diluted with CH_2Cl_2 (100 mL) and washed with H_2O (100 mL). The organic layer was dried, evaporated, coevaporated with toluene and purified by column chromatography ($CHCl_3$-MeOH 97:3). This yielded 700 mg (1.37 mmol, 97 %) of the di-O-mesylated derivative 3d as a foam. UV (MeOH) λ_{max} 280 nm (ε 20,800); MS (m/e) : no M^+; 319 (M-2MsOH); 239 (B + H). ^1H NMR ($CDCl_3$) δ : 2.09-2.30 (m, H-3'); H-3" partially hidden by the mesyl groups; 2.74 (s, CH_3); 3.07 (s, CH_3); 4.53 (m, H-4', H-5', H-5"); 5.47 (m, H-2'); 6.50 (d, J = 4.4 Hz, H-1'); 7.56 (m) and 8.03 (m) (benzoyl); 8.34 and 8.73 (2 x s, H-8 and H-2); 9.24 (s, NH) ppm.

N-Benzoyl-9-(2,5-diazido-2,3,5-trideoxy-β-D-erythro-pentofuranosyl)-adenine (11). A solution of 510 mg (1 mmol) of 3d in 10 mL of dimethylformamide was heated at 80°C for 24 h in the presence of 490 mg (10 mmol) of lithium azide. The reaction mixture was concentrated and purified by column chromatography ($CHCl_3$-MeOH 99:1) yielding 300 mg (0.74 mmol, 74 %)

of 11. UV (MeOH) λ_{max} : 280 nm (ε20,800); IR ($CHCl_3$) 2110 cm^{-1} (N_3); MS (m/e) 405 (M^+). ^1H NMR ($CDCl_3$) δ : 2.04-2.76 (m, H-3', H-3"); 3.66 (2 x dd, H-5', H-5"); 4.55 (m, H-4'); 4.90 (m, $J_{2',3'}$ = 2.6 Hz, $J_{2',3"}$ = 6.4 Hz, H-2'); 6.06 (d, J = 2.2 Hz, H-1'); 7.55 (m) and 7.93-8.10 (m) (benzoyl); 8.30 and 8.76 (2 x s, H-8 and H-2); 9.24 (NH) ppm.

N-Benzoyl-9-(5-O-mesyl-2-deoxy-β-D-threo-pentofuranosyl)adenine (4d). To a solution of 1.07 g (3 mmol) of 4c in 30 mL of pyridine, cooled in an icebath, was added dropwise 0.26 mL (3.3 mmol) of mesyl chloride in 10 mL of pyridine over a period of 30 min. After stirring overnight at 4°C, TLC ($CHCl_3$-MeOH 85-15) showed that the reaction mixture still contained starting material, and another 0.2 mL of methanesulfonyl chloride was added. After stirring for 2 h at 4°C, MeOH (1 mL) was added and the reaction mixture was evaporated. The residue was diluted with H_2O (50 mL), extracted twice with EtOAc (2 x 100 mL), dried, evaporated and coevaporated with toluene. The title compound (4d) crystallized after addition of CH_2Cl_2 : 1.23 g (2.84 mmo, 95 %). mp : 131-133°C. UV (MeOH) λ_{max} : 280 nm (ε 20,600); MS (m/e) : no M^+; 319 (M-MsOH-H_2O); 239 (B+H). ^1H NMR ($CDCl_3$) δ : 2.50-3.10 (m, H-2', H-2"); 3.02 (s, CH_3); 4.13-4.86 (m, H-3', H-4', H-5', H-5"); 6.22 (dd, J = 3.0 and 8.7 Hz, H-1'); 6.95 (d, 3'-OH); 7.41-7.66 (m) and 7.93-8.08 (m) (benzoyl); 8.16 and 8.76 (2 x s, H-8 and H-2); 9.08 (s, NH) ppm.

N-Benzoyl-9-(5-azido-2,5-dideoxy-β-D-threo-pentofuranosyl)adenine (12). A solution of 433 mg of 4d and 490 mg (10 mmol) of lithium azide in 10 mL of dimethylformamide was heated at 80°C for 7 h. The mixture was concentrated to 5 mL and divided between EtOAc (50 mL) and H_2O (50 mL). The EtOAc layer was dried, evaporated and purified by column chromatography ($CHCl_3$-MeOH 97:3). The title compound was crystallized from MeOH yielding 273 mg (0.63 mmol, 63 %). mp : 107°C (softens). MS (m/e) : 380 (M^+); UV (MeOH) λ_{max} 280 nm ($_\varepsilon$ 21,000); IR (KBr) : 2110 cm^{-1} (N_3). ^1H NMR ($CDCl_3$) δ : 2.42-3.15 (m, H-2', H-2"); 3.66 (m, H-5', H-5"); 4.02 (m, H-4'); 4.46 (m, H-3'); 6.20 (dd, J = 2.8 and 9.0 Hz, H-1'); 6.77 (3'-OH); 7.50 (m) and 7.91-8.06 (m) (benzoyl); 8.21 and 8.70 (2 x s, H-8 and H-2); 9.39 (NH) ppm.

N-Benzoyl-9-(3,5-diazido-2,3,5-trideoxy-β-D-erythro-pentofuranosyl)-adenine (13). A solution of 433 mg (1 mmol) of 4d, 330 mg (1.25 mmol) of triphenylphospine, 415 mg (1.25 mmol) of carbon tetrabromide and 490 mg (10 mmol) of lithium azide was kept at room temprature overnight and hea-

ted for 35 min at 100°C. The reaction mixture was concentrated to 5 mL, diluted with $CHCl_3$ (50 mL), washed with H_2O (50 mL), dried and evaporated. Column chromatographic purification ($CHCl_3$-MeOH 99.5:0.5) yielded 288 mg (0.71 mmol, 71 %) of the title compound 13. mp (MeOH) : 146-147°C; UV (MeOH) λ_{max} 279 nm (ε 20,700); IR (KBr) 2,100 cm^{-1} (N_3); MS (m/e) : 405 (M^+). ^1H NMR ($CDCl_3$) δ : 2.42-2.78 (m, H-2'); 2.97-3.32 (m, H-2"); 3.66 (m, H-5', H-5"); 4.09 (m, H-4'); 4.61 (m, H-3'); 6.38 (dd, J = 5.5 and 6.8 Hz, H-1'); 7.50 (m) and 7.94-8.09 (m) (benzoyl); 8.21 and 8.78 (2 x s, H-8 and H-2); 9.23 (NH) ppm.

Debenzoylation of 7a, 8, 10, 11, 12 and 13. Removal of the benzoyl group from the adenine base was carried out with ammonia in methanol at room temperature overnight. After evaporation of the solvent, the different compounds were crystallized from MeOH (15, 18, 21 24, 25) or from EtOH-Et_2O (20).

9-(5-Azido-3,5-dideoxy-β-D-threo-pentofuranosyl)adenine (15). Starting material 7a : 1 mmol. Yield : 200 mg (0.72 mmol, 72 %). mp = 202-203°C. UV (MeOH) λ_{max} : 258 nm (ε 15,600). Anal. ($C_{10}H_{12}N_8O_2$) C, H, N.

9-(2,5-Diazido-2,3,5-trideoxy-β-D-erythro-pentofuranosyl)adenine (18). Starting material 8 : 0.74 mmol. Yield : 135 mg (0.45 mmol, 61 %). mp 155-156°C. UV (MeOH) λ_{max} : 259 nm (ε 14,900). Anal. ($C_{10}H_{11}N_{11}O$) C, H, N.

9-(5-Azido-2,5-dideoxy-β-D-erythro-pentofuranosyl)adenine (20). Starting material 10 : 2 mmol. Yield : 415 mg (1.5 mmol, 75 %). mp = 60°C (soften), as ethanol solvate mp 78-79°C (dec)[15]. UV (MeOH) λ_{max} 258 nm (ε15,100). Anal. ($C_{10}H_{12}N_8O_2$.EtOH) C, H, N.

9-(5-Azido-2,5-dideoxy-β-D-threo-pentofuranosyl)adenine (21). Starting material 11 : 1.66 mmol. Yield : 1370 mg (1.34 mmol, 81 %). mp 164-165°C. UV (MeOH) λ_{max} : 260 nm (ε 15,200). Anal. ($C_{10}H_{12}N_8O_2$) C, H, N.

9-(3,5-Diazido-2,3,5-trideoxy-β-D-erythro-pentofuranosyl)adenine (24). Starting material 12 : 1.2 mmol. Yield : 260 mg (0.86 mmol, 72 %). mp 132-133°C. UV (MeOH) λ_{max} : 259 nm (ε15,700). Anal. ($C_{10}H_{11}N_{11}O$) C, H, N.

9-(3,5-Diazido-2,3,5-trideoxy-β-D-threo-pentofuranosyl)adenine (25). Starting material 13 : 1.23 mmol. Yield : 350 mg (1.16 mmol, 95 %). mp : decomposition starts at 220°C. UV (MeOH) λ_{max} : 260 nm (ε 14,900). Anal. ($C_{10}H_{11}N_{11}O$) C, H, N.

Reduction of the azido group. Reductions were carried out on a 0.5 to 1 mmol scale in pyridine (3 mL/mmol) with 3 equivalents of triphenylphosphine for the monoazido compounds and with 5 equivalents of triphenylphosphine for the diazido compounds. After stirring for 3 h at room temperature, 3 mL of concentrated ammonia (32 %) was added, and the reaction mixture was stirred further for 10 h. Although some of the hydrolyses were completed after 2 h, an incubation time up to 10 h was needed for the slowest reactions to reach completion. The reaction mixtures were evaporated and purified by column chromatography (EtOAc-MeOH-NH_3 32 % 70:25:5). The compounds were precipitated from CH_3CN. The reactions were quantitative on TLC, the isolated yields being those obtained after the first precipitation. Some of the compounds were isolated as acetates which is clear from their elemental analysis and ^1H NMR spectrum. For the ^1H NMR spectra, only values for H-1' and H-8/H-2 are mentioned. Values for the other protons are not always easy to determine with a 90 MHz apparatus and these frequently overlap with each other and with the HOD and DMSO peaks.

9-(5-Amino-2,5-dideoxy-β-D-erythro-pentofuranosyl)adenine (32). 89 % Yield. mp 184-186°C (lit. 185-187°C[14]; 181-183°C[15]). UV (H_2O) λ_{max}: 260 nm (ε 15,200).

9-(5-Amino-2,5-dideoxy-β-D-threo-pentofuranosyl)adenine (33). 88 % Yield. mp 213-215°C (dec). UV (H_2O) λ_{max} 260 nm (ε 14,800). Anal. ($C_{10}H_{14}N_6O_2$) C, H, N.

9-(3-Amino-2,3-dideoxy-β-D-erythro-pentofuranosyl)adenine (34). 79 % Yield. UV (H_2O)λ_{max} : 260 nm (ε 15,100). Anal. ($C_{10}H_{14}N_6O_2$.1/2 CH_3COOH) C, H, N.

9-(3-Amino-2,3-dideoxy-β-D-threo-pentofuranosyl)adenine (35). 92 % Yield. mp 199-200°C (dec). UV (H_2O)λ_{max} : 260 nm (ε 15,200). Anal. ($C_{10}H_{14}N_6O_2.H_2O$) C, H, N.

9-(3,5-Diamino-2,3,5-trideoxy-β-D-erythro-pentofuranosyl)adenine (36). 86 % Yield. UV (H_2O) λ_{max} 260 nm (ε 15,100). Anal. ($C_{10}H_{15}N_7O$. $CH_3COOH.H_2O$) C, H, N.

9-(3,5-Diamino-2,3,5-trideoxy-β-D-threo-pentofuranosyl)adenine (37). 73 % Yield. UV (H_2O)λ_{max} 260 nm (ε 15,200). Anal. ($C_{10}H_{15}N_7O$. $CH_3COOH.H_2O$) C, H, N.

9-(5-Amino-3,5-dideoxy-β-D-erythro-pentofuranosyl)adenine (26). 90 % Yield. mp 222°C (fast dec.). UV (H_2O)λ_{max} : 260 nm (ε 14,900). Anal. ($C_{10}H_{14}N_6O_2$.1/2 CH_3COOH) C, H, N.

9-(5-Amino-3,5-dideoxy-β-D-threo-pentofuranosyl)adenine (27). 75 % Yield. mp 148°C. UV (H_2O) λ_{max} 260 nm (ε 15,100). Anal. ($C_{10}H_{14}N_6O_2$. 1/2 CH_3COOH) C, H, N.

9-(2-Amino-2,3-dideoxy-β-D-erythro-pentofuranosyl)adenine (28). 58 % Yield. mp 183-185°C (lit. 183.5-185.5[12]). UV (H_2O) λ_{max} : 260 nm (ε 14,900).

9-(2-Amino-2,3-dideoxy-β-D-threo-pentofuranosyl)adenine (29). 56 % Yield. mp 208-210°C (dec). UV (H_2O) λ_{max} : 261 nm (ε15,100). Anal. ($C_{10}H_{14}N_6O_2$) C, H, N.

9-(2,5-Diamino-2,3,5-trideoxy-β-D-erythro-pentofuranosyl)adenine (30). 65 % Yield. UV (H_2O) λ_{max} : 260 nm (ε 15,200). Anal. ($C_{10}H_{15}N_7O$. $CH_3COOH.H_2O$) C, H, N.

9-(2,5-Diamino-2,3,5-trideoxy-β-D-threo-pentofuranosyl)adenine (31). 51 % Yield. UV (H_2O)λ_{max} : 260 nm (ε 15,400). Anal. ($C_{10}H_{15}N_7O$. $CH_3COOH.H_2O$) C, H, N.

ELEMENTAL ANALYSIS

Compound	Found			Calculated		
	C	H	N	C	H	N
14	43.57	4.46	40.52	43.48	4.38	40.56
15	43.75	4.50	40.18	43.48	4.38	40.56
18	40.06	3.84	51.12	39.87	3.68	51.14
19	40.00	3.83	51.05	39.87	3.68	51.14
20	44.87	5.44	34.78	44.72	5.63	34.76
21	43.76	4.43	40.45	43.48	4.38	40.56
24	39.55	3.69	51.44	39.87	3.68	51.14
25	40.05	3.80	50.98	39.87	3.68	51.14
26	46.93	5.67	29.79	47.14	5.75	29.98
27	47.04	5.76	29.80	47.14	5.75	29.98
29	47.60	5.67	33.71	47.99	5.64	33.58
30	43.91	6.34	29.85	44.03	6.47	29.95
31	44.05	6.40	29.91	44.03	6.47	29.95
33	48.16	5.74	33.57	47.99	5.64	33.58
34	47.17	5.67	31.11	47.14	5.75	29.98
35	44.52	5.90	31.32	44.77	6.01	31.33
36	44.01	6.38	29.94	44.03	6.47	29.95
37	44.28	6.49	29.79	44.03	6.47	29.95

Cytostatic and antiretroviral test procedures. Cytostatic assays were performed according to previously established procedures.[31,32] The tumor cell lines used in this study were murine leukemia L1210, murine mammary carcinoma FM3A, human B-lymphoblast Raji, human T-lymphoblast Molt/4F and human T-lymphocyte MT-4 cells.

Human immunodeficiency virus (HIV) infection was carried out with the HTLV-III_B strain. The virus was prepared from the supernatant of HTLV-III_B-infected HUT-78 cell cultures. The antiviral assays were based upon the inhibition of HIV-induced cytopathogenicity in human MT-4 cells, as described previously.[33]

The inhibitory effects of the compounds on the transformation of C3H mouse embryo cells by Moloney murine sarcoma virus (MSV) were determined by microscopic examination of the cell cultures 6 days after infection, as previously reported.[34]

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