Synthesis and Anti-HIV Activity of Various 2′- and 3′-Substituted 2′,3′-Dideoxyadenosines: A Structure–Activity Analysis†

P. Herdewijn, R. Pauwels, M. Baba, J. Balzarini, and E. De Clercq*

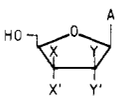
Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium. *Received May 11, 1987*

A systematic synthesis was undertaken of 2′,3′-dideoxyadenosine analogues with either an azido, fluorine, or hydroxyl group substituted in the "up" or "down" position of C-2 or C-3 of the sugar moiety. The compounds were evaluated against the cytopathogenicity of human immunodeficiency virus (HIV) for MT-4 cells. The four azido derivatives **6**, **7**, **8**, and **9** were synthesized by a nucleophilic displacement reaction with lithium azide on the mesylates **3**, **2**, **5**, and **4**. (Diethylamido)sulfur trifluoride was used for the synthesis of **10–12**. The compound **13** was obtained by 2′-deoxygenation of 9-(3-fluoro-3-deoxy-β-D-xylofuranosyl)adenine. Among the azido derivatives, compound **8** with the 3′-azido "down" was slightly more active than 2′,3′-dideoxyadenosine (**1**) but considerably more toxic, and, of the fluorine series, compound **11**, with the 2′-fluoro "up", was the most selective inhibitor of HIV, although it was less active than **1**. Hence, none of the newly synthesized compounds proved more selective in their anti-HIV activity than the parent compound, **1**.

The degree of inhibition of retroviruses by 2′,3′-dideoxythymidine (ddThd) strongly depends on the choice of the cell line used to monitor retrovirus replication.[1] These cell-dependent variations most probably reside in the differential abilities of the cells to phosphorylate the nucleosides to their 5′-triphosphates, a process that is usually very slow for 2′,3′-dideoxynucleosides.[2] Moreover, within the same cell line, the extent of phosphorylation may differ considerably from one 2′,3′-dideoxynucleoside to another, and this may explain at least in part the differences found for the inhibitory effects of 2′,3′-dideoxynucleosides on the replication of human immunodeficiency virus (HIV) in vitro.[3] The variability in phosphorylation capacity of different cells together with the differential sensitivity of the retrovirus-associated reverse transcriptase and host cell DNA polymerases toward the 2′,3′-dideoxynucleoside 5′-triphosphates should be taken into account in the design of selective antiviral agents against HIV.

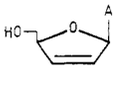
Since 3′-azido-2′,3′-dideoxythymidine (AZT), the drug that is currently advocated for the treatment of AIDS patients, suffers from a rather short half-life in the body[4] and furthermore suppresses bone marrow cell formation,[5] there is an urgent need for other compounds that are equally potent but more selective in their antiviral action. Starting from 2′,3′-dideoxyadenosine, we worked out two structure–function analyses, based on the positioning of the azido or fluorine substituent at the sugar moiety. The fluorine atom is the most electronegative atom that can be introduced in an organic compound and has a van der Waals radius (1.35 Å) comparable to that of hydrogen (1.17 Å). Substitution of fluorine for hydrogen has a strong effect on the electronic configuration of a molecule, often reflected by a dramatic change in biological activity [cf. the activity of 5-fluorouracil, 9-β-D-arabinofuranosyl-2-fluoroadenine, and 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)cytosine] and enzymatic stability. This susceptibility to enzymatic degradation can either increase or decrease following introduction of a fluorine atom: i.e., 3′-fluoro-2′,3′-dideoxythymidine is not degraded by pyrimidine nucleoside phosphorylase,[6] while both 9-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)adenine and 9-(3-fluoro-3-deoxy-β-D-xylofuranosyl)adenine are deaminated by adenosine deaminase at least as fast as, if not faster than, the unsubstituted nucleoside counterparts.[7] The susceptibility to adenosine deaminase[8] is essentially determined by the presence of a free hydroxyl at C-5′, and, hence, compounds like 2′,3′-dideoxyadenosine are readily deaminated.

Scheme I



(b)	X	X′	Y	Y′
1	H	H	H	H
2	H	H	H	OMs
3	H	H	OMs	H
4(a)	H	OMs	H	H
5	OMs	H	H	H
6	H	H	H	N_3
7	H	H	N_3	H
8	H	N_3	H	H
9	N_3	H	H	H
10	H	H	H	F
11	H	H	F	H
12	H	F	H	H
13	F	H	H	H
14	H	H	H	OH
15	H	H	OH	H
16(a)	H	OH	H	H
17	OH	H	H	H

(a) N-benzoyladenine instead of adenine (A)
(b) Numbers with an accent designate the presence of a 5′-monomethoxytrityl group



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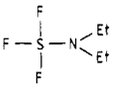

19

Herein we describe the synthesis and anti-HIV activity of a series of 2′,3′-dideoxyadenosine analogues substituted

† Dedicated to Prof. H. Vanderhaeghe, on the occasion of his 65th birthday.

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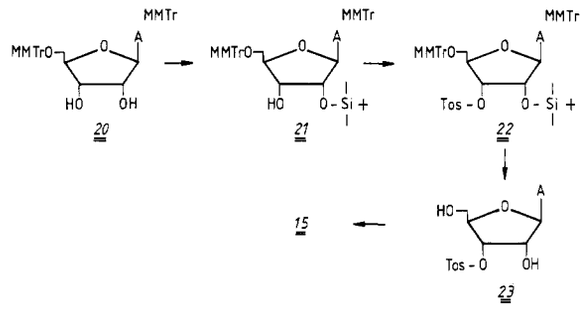
Scheme II



with an azido group or fluorine atom in all possible C-2′ and C-3′ positions (Scheme I). The substrate properties of the compounds for adenosine deaminase will be reported elsewhere.

Chemistry

Three of the four deoxyadenosine analogues (**14**, **16**, and **17**) that serve as starting materials for the synthesis of the azido analogues can be obtained by well-established procedures. Cordycepin (**14**) was synthesized according to Robins et al.,[9] and 6-*N*-benzoyl-5′-*O*-(monomethoxytrityl)-2′-deoxyadenosine (**16′**) has been previously described,[10] although the transient protection method[11] was used here for protection of the base. The 2-deoxy-β-D-*threo*-pentofuranosyl compound **17** was synthesized via stereospecific 2′-tosylation of adenosine, as described by Moffatt et al.,[12] followed by a hydride shift reaction with lithium triethylborohydride.[13] The latter can also be carried out with the 5′-O-protected derivative,[13,14] which gives **17′** directly. Under these conditions, trace amounts of 5′-*O*-(monomethoxytrityl)-2′-deoxyadenosine could be detected.

The synthesis of 3′-*O*-tosyladenosine (**23**), which serves as starting material for the preparation of **15** (Scheme II), is not so straightforward.[15] Since a tosylation of 5′-O-monomethoxytritylated adenosine gives predominantly the 2′-*O*-tosyl derivative, together with the 2′,3′-ditosylated analogue, we used a step by step procedure starting from bis(monomethoxytrityl)adenosine **20**. Monomethoxytritylation of adenosine with 3 equiv of monomethoxytrityl chloride (MMTrCl) yielded **20** in 76% yield. Bistritylation was preferred over 5′-O-monotritylation because of the ready separation from the side products and because of the advantage of an N-protecting group during the tosylation step (**21** to **22**). The position of the second MMTr group at N-6 follows from the bathochromic shift of the UV maximum (from 259 to 274 nm). Protection of the 2′-OH group with *tert*-butyldimethylsilyl chloride–silver nitrate–pyridine in tetrahydrofuran[16] gave **21** in a 60% yield. When the reaction was carried out in a mixture of tetrahydrofuran–dimethylformamide (3:1), the selectivity changed and greater amounts of the 3′-*O*-silyl derivative were obtained, which could be due to silyl migration during the reaction. As mentioned by Ogilvie et al.,[17] this isomerization could be accelerated in dimethylformamide, where product distribution changes with time. Compound **21** could be easily separated from the 3′-silylated isomer by column chromatography. When the reaction is carried out on a larger scale (20 g), the usual laboratory equipment does not allow a complete separation. Then, the 2′-silylated compound **21** is often contaminated with some 3′-isomer. However, tosylation of **21** to **22** gives a compound that is crystalline and thus more readily separable. Desilylation followed by demonomethoxytritylation gives 3′-*O*-tosyladenosine (**23**). A distinction between 2′-*O*-tosyladenosine and 3′-*O*-tosyladenosine can be made by comparison of the shift values for H-2′ and H-3′ in the ^{1}H NMR spectrum. In the case of 3′-*O*-tosyladenosine, the 2′-proton and 3′-proton appear as overlapping double doublets at δ 5.10, while both protons are separated by approximately 1.1 ppm in 2′-*O*-tosyladenosine (H-2′ δ 5.48, H-3′ δ 4.35). In this reaction sequence, the positional isomers are best separated at the stage of 6-*N*,5′-*O*-bis(monomethoxytrityl)-3′-*O*-(*tert*-butyldimethylsilyl)adenosine [TLC: hexane–EtOAc (3:2), R_f 0.42] and 6-*N*,5′-*O*-bis(monomethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)adenosine (R_f 0.58). The separation of tetrasubstituted adenosines like **22** and of mixtures of 2′-*O*-tosyladenosine (TLC: EtOAc, R_f 0.33) and 3′-*O*-tosyladenosine (R_f 0.30) is a more difficult undertaking. Rearrangement of 3′-*O*-tosyladenosine by a procedure described previously[13] yielded **15** at 90%. In contrast with our experience with the 2′-*O*-tosyl analogue,[14] prior protection of the 5′-OH of 3′-*O*-tosyladenosine with a monomethoxytrityl group gave a lower yield following this rearrangement reaction.

The synthesis of 3′-azido-2′,3′-dideoxyadenosine has been described in our previous publication.[14] However, the low electronegativity of the azido group as compared with a mesyloxy group is responsible for the low yield of the detritylation reaction leading to **8**. The glycosidic bond of the 3′-azido derivative is more susceptible to acid cleavage. Therefore, 9-[5-*O*-(monomethoxytrityl)-3-*O*-mesyl-2-deoxy-β-D-*threo*-pentofuranosyl]adenine, obtained by mesylation of **17′**, was first detritylated with *p*-toluenesulfonic acid and subsequently converted into **8** with lithium azide in DMF. This reaction sequence, which gives a more satisfactory yield than the previous method,[14] was also used for the synthesis of the other azido compounds. Thus, this three-step procedure was used to convert 5′-(monomethoxytrityl)cordycepin **14′** into **7** and 5′-(monomethoxytrityl)-3′-deoxy-*ara*-A **15′** into **6**. In the case of **16′**, it is best to reduce the nucleophilic character

Table I. Reaction Conditions for the Nucleophilic Substitution Reaction at the C-2′ and C-3′ Positions

compd	starting	reactn time, h	yield, %
6	**3**	24[a]	93
7	**2**	40[b]	89
8	**5**	4[a]	90
9	**4**	6[a]	65[d]
10	**15′**	14[c]	76[e]
12	**17′**	1[c]	52

[a] Reaction in DMF at 80 °C with 10 equiv of LiN_3. [b] Reaction in DMF at 100 °C with 10 equiv of LiN_3. [c] Reaction in CH_2Cl_2 at room temperature with DAST. [d] Total yield after debenzoylation. [e] Total yield after detritylation.

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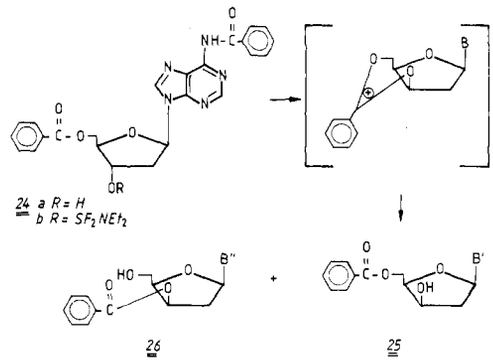
Scheme III

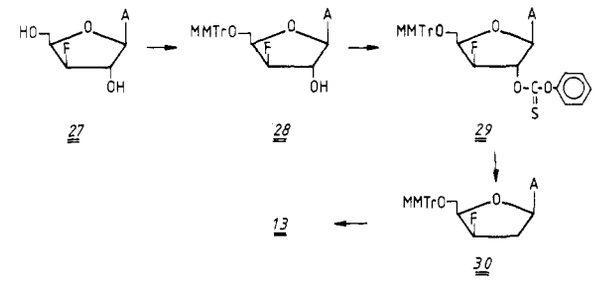


of the N-3 of adenine by protecting the base with a benzoyl group to avoid a nucleophilic attack of the nitrogen at the C-3′. The reaction sequence leading to **9** also needed an additional step to remove the benzoyl group.

The reaction conditions needed for the converson of **2**, **3**, **4**, and **5** to **7**, **6**, **9**, and **8**, respectively, are summarized in Table I. These conditions clearly reflect the well-known influence of steric factors (adenine moiety and sugar conformation) and electronic factors (due to the proximity of the anomeric center) on substitution reactions at C-2′ and C-3′. It reflects the unfavorable dipolar interactions between the approaching nucleophile or the leaving group and the anomeric substituents in the S_N2 transition state. The same effects were observed in the reaction of **15′** with (diethylamido)sulfur trifluoride (**19**) (DAST).[18] This S_N2 displacement with inversion of the stereochemistry requires 14 h at room temperature before it is completed (76% yield after detritylation) while the analogous reaction[14] with **17′** is completed within 1 h.

When 5′-*O*-(monomethoxytrityl)-2′-deoxyadenosine was treated with DAST, nearly all the starting material was converted into the N^3,3′-cyclonucleoside. Protection of the amino group of the base with a benzoyl group, in order to lower the nucleophilic character of the N-3 atom, did not prove feasible because of the incompatibility of DAST with this protecting group. When 6-*N*,5′-*O*-dibenzoyl-2′-deoxyadenosine (**24a**) was reacted with DAST, at least five compounds were formed, which showed totally different UV maxima (259, 261, 273, 296, and 300 nm). As follows from the ^{1}H NMR spectra of these compounds, four of the five isolated compounds have 5-*O*-benzoyl-2-deoxy-β-D-xylofuranose (**25**) or 3-*O*-benzoyl-2-deoxy-β-D-xylofuranose (**26**) as the sugar moiety. The principal product formed in this reaction is 9-(5-*O*-benzoyl-2-deoxy-β-D-*threo*-pentofuranosyl)adenine (**25**) (B′ = adenine), an N-debenzoylated product the structure of which was proven by 5′-O-debenzoylation and comparison with an authentic sample prepared by the method of Hansske and Robins.[13] It can be concluded from these data and products that the inversion of configuration at the 3′-position is due to a neighboring group participation reaction of the 5′-*O*-benzoyl group with the 3′-activated intermediate **24b**[18] according to Scheme III. An analogous 5′→3′ neighboring group participation reaction via an acyloxonium ion has been previously described for 6-*N*,5′-*O*-dipivaloyl-2′,3′-anhydroadenosine.[19] Instead of further exploring these reactions with other protecting groups, we switched to Barton's deoxygenation method.[20] Protection of the 5′-hydroxyl group of 9-(3-fluoro-3-deoxy-β-D-xylo-

Scheme IV



furanosyl)adenine[21] (**27**) with monomethoxytrityl chloride gave **28**, which was reacted with *O*-phenyl chlorothioformate in the presence of 4-(dimethylamino)pyridine, as described by Robins et al.[20] (Scheme IV). A standard reduction procedure of **29** with tri-*n*-butyltin hydride and 2,2′-azobis(2-methylpropionitrile) gave **30**. The 5′-monomethoxytrityl group was removed with *p*-toluenesulfonic acid (2%) in chloroform–methanol (8:2). The total yield for the conversion of **28** into **13**, after crystallization, was 56.5%.

All 2′-fluoro nucleosides of the arabinofuranosyl conformation have so far been synthesized by a condensation reaction of protected 2-deoxy-2-fluoro-D-arabinose with the appropriate base.[22a-d] Because DAST causes little of the competing elimination reaction,[14] this reagent was used for the synthesis of **11** starting from cordycepin. When 5′-*O*-tritylcordycepin was treated with DAST in dichloromethane at reflux, followed by a detritylation procedure with 80% aqueous acetic acid, a 10% yield of 9-(2-fluoro-2,3-dideoxy-β-D-*threo*-pentofuranosyl)adenine (**11**) was isolated. The isolation of pure **11** from the reaction mixture was rather difficult because of the presence of a side compound with similar polarity. This reaction demonstrates again that it is very difficult, although not impossible, to introduce directly a fluorine atom in the 2′-"up" position by an S_N2 displacement. This S_N2 reaction is facilitated by the absence of an oxygen function (or another electronegative substituent) on the adjacent (2′ or 3′) carbon atom. The ^{1}H NMR and ^{13}C NMR spectra of the synthesized compounds are summarized in Table II. From literature data[21,22b,22d,23a-c] it is clear that, only in the case when the fluorine atom is situated in the 2′-"up" position, the signal assigned to H-8 in the ^{1}H NMR spectrum is split into a doublet with a coupling constant between 2 Hz and 3 Hz. This is confirmed for the dideoxy analogues. Here, a coupling constant of 2.6 Hz was found for **11**. The ^{1}H NMR spectrum of **10** also differs from that of **11**, as far as concerns the values for $J_{1',2'}$, which are 0 and 3.6 Hz, respectively. The ^{13}C NMR spectra show a long-range coupling between C-8 and fluorine for **11** ($J_{8,F}$ = 4.9 Hz) and for **13** ($J_{8,F}$ = 8.5 Hz).

Anti-HIV Activity

The procedure to measure anti-HIV activity in MT-4 cells has been described previously.[24] Five days after

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Table II. ^1H and ^{13}C NMR Spectral Data[a]

Azido-Substituted 2′,3′-Dideoxyadenosines

	^1H NMR		^{13}C NMR				
compd	H-1′	$^3J_{1',2'}$ and $^3J_{1',2''}$	C-1′	C-2′	C-3′	C-4′	C-5′
6	6.00	3.1	88.2	64.8	31.1	80.8	62.2
7	6.36	6.1	83.6	62.0	30.1	79.2	61.7
8	6.32	2 × 6.6	83.4	36.2	61.2	84.8	61.5
9	6.30	3.8 and 7.3	82.3	37.5	61.1	82.6	59.9

Fluoro-Substituted 2′,3′-Dideoxyadenosines

	^1H NMR							
compd	H-1′	$J_{1',F}$	H-2′ (H-2″)[c]	$J_{2',F}$	H-3′ (H-3″)[c]	$J_{3',F}$	H-4′	$J_{4',F}$
10	6.62	18	5.59	52.7	2.40	*b*	4.37	*b*
11	6.28	16.7	5.41	54.7	2.46	*b*	4.23	*b*
12	6.39	0	2.90	*b*	5.44	53.4	4.25	26.8
13	6.42	0	2.96	*b*	5.43	54.5	4.14	29.5

	^{13}C NMR									
compd	C-1′	$J_{C1',F}$	C-2′	$J_{C2',F}$	C-3′	$J_{C3',F}$	C-4′	$J_{C4',F}$	C-5′	$J_{C5',F}$
10	88.5	36.6	96.8	178.2	32.1	20.8	81.3	0	61.7	0
11	83.7	17.1	91.4	186.7	32.5	19.5	77.7	0	62.9	0
12	84.2	0	36.9	20.8	95.1	173.3	85.6	22.6	61.2	11.0
13	82.2	0	38.7	20.8	92.4	179.4	84.3	19.5	58.6	9.8

[a] All spectra were run in DMSO-d_6, which was used as internal standard (39.6 ppm) in the ^{13}C NMR spectra; TMS was used as standard for the ^1H NMR spectra; chemical shifts in δ values (ppm); coupling constants in Hz. [b] Values not determined. [c] Values correspond to the center of the multiplet.

Table III. Anti-HIV Activity and Cytotoxicity of 2′,3′-Dideoxyadenosine Analogues[24] in MT-4 Cells

compd	CD_{50},[a] μM	ED_{50},[b] μM	selectivity index[c]
1	889	6.2	148
6	>625	215	>2.9
7	625	55	11.4
8	10	5	2
9	551	>625	<0.9
10	>625	>625	
11	>625	35	>18
12	557	50	11.1
13	>625	221	>2.8
18	34	>25	<1.2
AZT	4	0.006	666

[a] Fifty percent cytotoxic dose, or dose required to reduce the viability of uninfected MT-4 cells by 50% following a 5-day incubation period in the presence of the compound. [b] Fifty percent effective dose, or dose required to protect 50% of the HIV-infected MT-4 cells against destruction following a 5-day incubation period in the presence of the compound. [c] Ratio of CD_{50} to ED_{50}.

either mock-infected MT-4 cells or MT-4 cells infected with HIV [at 10 $CCID_{50}$ (50% cell culture infective dose) per well] had been incubated in the presence of appropriate concentrations of the test compounds, the number of viable cells was determined in a blood cell counting chamber after trypan blue staining. The ED_{50} was defined as the concentration of compound required to confer 50% protection of HIV-infected cells in terms of cell viability.

The anti-HIV activity and cytotoxicity of the 2′,3′-dideoxyadenosine analogues is shown in Table III. 2′,3′-Dideoxyadenosine (1) and 2′,3′-didehydro-2′,3′-dideoxyadenosine (18) were included in the assays as reference materials. Among the azido derivatives, compound 8 turned out to be the most potent anti-HIV agent. It was also the most cytotoxic. Its selectivity index was 2, as compared to 148 for dideoxyadenosine (1). The azido compound with the best selectivity index (compound 7) was 10-fold less active than 1. The two other compounds, 6 and 9, were virtually inactive against HIV and noncytotoxic. Of the fluoro compounds, compound 11 with fluorine in the "up" position at C-2′ was the most potent inhibitor of HIV. Yet, it was about 6 times less active than dideoxyadenosine (1). An important difference between the azido and fluoro compounds was the very low toxicity of 12 compared to that of 8. The toxicity of 8 was comparable to that of AZT. However, the anti-HIV activity of 8 in MT-4 cells was about 800-fold lower than that of AZT. From these data it is clear that introduction of a fluorine atom or azido group in the sugar part of dideoxyadenosine (1) leads to a marked reduction of the anti-HIV activity in all but one case, namely, 3′-azido-2′,3′-dideoxyadenosine (8). However, the toxicity of 8 is such that it does not seem to be a candidate worthy of further exploration for its potential in the treatment of AIDS.

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 267 spectrophotometer on samples in potassium bromide disks at 1.5%. Ultraviolet spectra were recorded with a Beckman UV 5230 spectrophotometer. The ^1H NMR and ^{13}C NMR spectra were determined with a JEOL FX 90Q spectrometer with tetramethylsilane as internal standard for the ^1H NMR spectra and DMSO-d_6 (39.6 ppm) for the ^{13}C NMR spectra (s = singlet, d = doublet, t = triplet, br s = broad signal, m = multiplet). Mass spectra were determined with an AEI MS-12 apparatus. 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 nm). Anhydrous solvents were obtained as follows: tetrahydrofuran was obtained by distillation after reflux overnight with lithium aluminum hydride; pyridine was refluxed overnight in *p*-toluenesulfonyl chloride, distilled, refluxed overnight in potassium hydroxide, and distilled again; dichloromethane was stored for 1 week in anhydrous calcium chloride, filtered, and distilled; water was removed from *N,N*-dimethylformamide by distillation with benzene followed by distillation in vacuo.

6-*N*,5′-*O*-Bis(monomethoxytrityl)adenosine (20). A mixture of 5.34 g (20 mmol) of adenosine and 18.3 g (60 mmol) of 4-anisylchlorodiphenylmethane in pyridine (200 mL) was stirred overnight at 50 °C. After addition of 10 mL of MeOH and stirring for another 2 h, the solvent was evaporated and the residual oil was divided between H_2O (250 mL) and $CHCl_3$ (250 mL). The

(24) Pauwels, R.; De Clercq, E.; Desmyter, J.; Balzarini, J.; Goubau, P.; Herdewijn, P.; Vanderhaeghe, H.; Vandeputte, M. *J. Virol. Methods* **1978**, *16*, 171.

organic layer was dried, evaporated, and coevaporated with toluene. Column chromatographic separation [(1) $CHCl_3$; (2) $CHCl_3$–MeOH, 99:1] of the tritylated adenosine derivatives yielded 12.4 g (15.3 mmol, 76%) of **20**: UV (MeOH) λ_{max} 274 nm (ϵ 19100); ^1H NMR ($CDCl_3$) δ 3.20 (m, 1 H, H-5′), 3.46 (m, 1 H, H-5″), 3.77 (s, 6 H, 2 CH_3), 4.35 (m, 2 H, H-3′ and H-4′), 4.70 (dd, 1 H, H-2′), 5.89 (d, 1 H, J = 5.5 Hz, H-1′), 6.77 (2 d, 4 H) and 7.26 (m, 24 H) (phenyl), 7.99 (s, 1 H) and 8.06 (s, 1 H) (H-2 and H-8).

6-*N*,5′-*O*-Bis(monomethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)adenosine (21). To a mixture of 12.2 g (15 mmol) of **20**, 5.46 mL (67.5 mmol) of pyridine, and 3.06 g (18 mmol) of silver nitrate in anhydrous THF (500 mL) was added 2.94 g (19.5 mmol) of *tert*-butyldimethylsilyl chloride. The course of the reaction was followed by TLC:hexane–EtOAc (3:2). The reaction mixture was stirred for 7 h at room temperature, filtered, and evaporated. The residue was dissolved in CH_2Cl_2 (300 mL) and washed twice with sodium bicarbonate (5%, 2 × 200 mL). The organic layer was dried and evaporated, leaving an oil, which was purified by column chromatography with hexane–EtOAc (4:1) as eluent: 8.33 g (9 mmol, 60%) yield. This compound still contained the 3′-O-silylated derivative as a minor impurity. The characterization was carried out after tosylation when the product could be crystallized more easily.

6-*N*,5′-*O*-Bis(monomethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-3′-*O*-tosyladenosine (22). A solution of 7.86 g (8.5 mmol) of **21** and 16.2 g (85 mmol) of tosyl chloride in 250 mL of pyridine was stirred for 3 days at room temperature. The reaction mixture was cooled in an ice bath, MeOH (20 mL) was added, and the mixture was stirred overnight. After evaporation of the solvent, the residue was taken up in Et_2O (500 mL) and washed with H_2O (3 × 500 mL). The organic layer was dried, evaporated, and coevaporated with toluene. The title compound was crystallized from Et_2O–hexane (85:15): 7.75 g (7.18 mmol, 84.5%) yield; mp 134–137 °C; UV (MeOH) λ_{max} 273 nm (ϵ 27200); ^1H NMR ($CDCl_3$) δ −0.29 and −0.08 (2 s, 2 CH_3), 0.72 (s, *tert*-butyl), 2.36 (s, CH_3 phenyl), 3.06 (m, H-5′), 3.42 (m, H-5″), 3.75 (s, 2 CH_3O), 4.18 (m, H-4′), 5.10 (m, H-2′ and H-3′), 5.85 (d, J = 5.2 Hz, H-1′), 6.74, 7.25 and 7.64 (m, aromatic H), 7.83 (s) and 7.89 (s) (H-2 and H-8).

3′-*O*-Tosyladenosine (23). A solution of 7.55 g (7 mmol) of **22** in 100 mL of 1 M tetrabutylammonium fluoride in THF was stirred for 1 h at room temperature and evaporated. The residue was dissolved in $CHCl_3$ (200 mL), washed with H_2O (3 × 200 mL), dried, and evaporated. Detritylation was carried out by stirring the residue in 200 mL of HOAc (80%) overnight at room temperature. Evaporation, coevaporation with toluene, and chromatographic purification ($CHCl_3$–MeOH, 90:10) yielded 2.71 g (6.44 mmol, 92%) of **23**, which was crystallized from MeOH: mp 162–165 °C; UV (MeOH) λ_{max} 258 nm (ϵ 16000); ^1H NMR (DMSO-d_6) δ 2.43 (s, 3 H, CH_3), 3.10–3.60 (m, H-5′ and H-5″, partially hidden by HOD), 4.08 (m, 1 H, H-4′), 4.76–5.08 (m, 2 H, H-2′ and H-3′), 5.60 (dd, 1 H, 5′-OH), 5.88 (d, 2′-OH), 5.97 (d, J = 5.7 Hz, H-1′), 7.38 (br s, 2 H, NH_2), 5.49 (d, 2 H) and 5.88 (d, 2 H) (phenyl), 8.13 (s, 1 H, H-2), 8.31 (s, 1 H, H-8). Anal. ($C_{17}H_{19}N_5O_6S$) C, H, N.

9-[5-*O*-(Monomethoxytrityl)-3-deoxy-β-D-*threo*-pentofuranosyl]adenine (15′). A solution of 1.88 g (7.5 mmol) of **15** and 2.78 g (9 mmol) of 4-anisylchlorodiphenylmethane in anhydrous pyridine (50 mL) was stirred for 2 days at room temperature. The reaction mixture was poured into H_2O (200 mL) and extracted with EtOAc (2 × 100 mL). The organic layer was dried, evaporated, and coevaporated with toluene. The residue was applied on a silica column and eluted first with CH_2Cl_2 followed by CH_2Cl_2–MeOH (95:5). The title compound was isolated as a foam in 76% (2.98 g, 5.7 mmol) yield (some 10% of the ditritylated compound was isolated): UV (MeOH) λ_{max} 259 (ϵ 15200), 233 nm (16800); ^1H NMR ($CDCl_3$) δ 1.84–2.40 (m, 2 H, H-3′ and H-3″), 3.40 (m, 2 H, H-5′ and H-5″), 3.70 (s, 3 H, CH_3), 4.22 (m, 1 H, H-4′), 4.52 (m, 1 H, H-2′), 5.84 (br s, 1 H, OH), 6.15 (d, 1 H, J = 4.2 Hz, H-1′), 6.64 (br s, 2 H, NH_2), 6.79 (d, 2 H) and 7.26 (m, 12 H) (phenyl), 8.03 (s, 1 H, H-2), 8.16 (s, 1 H, H-8).

9-(2-*O*-Mesyl-3-deoxy-β-D-*threo*-pentofuranosyl)adenine (3). A mixture of 994 mg (1.9 mmol) of **15′** and 0.5 mL of mesyl chloride in anhydrous pyridine (25 mL) was stirred overnight at 4 °C. After addition of 1 mL of H_2O, the mixture was stirred for another 30 min, poured into H_2O (100 mL), and extracted twice with $CHCl_3$ (2 × 100 mL). The organic layer was dried, evaporated, and coevaporated with toluene. The residue was dissolved in 30 mL of 80% HOAc. The detritylation was finished after 4.5 h. Evaporation, coevaporation with toluene, and washing of the residue with Et_2O (2 × 100 mL) gave a crystalline residue, which was recrystallized from MeOH: yield 403 mg (1.22 mmol, 64%); mp 243 °C dec; UV (MeOH) λ_{max} 258 nm (ϵ 15100); ^1H NMR (DMSO-d_6) δ 2.52 (m, 2 H, H-3′ and H-3″), 3.10 (s, 3 H, CH_3), 3.67 (m, 2 H, H-5′ and H-5″), 4.15 (m, 1 H, H-4′), 5.12 (br s, 1 H, OH), 5.52 (m, 1 H, H-2′), 6.40 (d, 1 H, J = 5.5 Hz, H-1′), 7.28 (br s, 2 H, NH_2), 8.16 (s, 1 H, H-2), 8.35 (s, 1 H, H-8).

9-(2-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)adenine (6). A solution of 380 mg (1.15 mmol) of **3** in DMF (10 mL) containing 560 mg of LiN_3 (10 equiv) was heated for 24 h at 80 °C. After evaporation of the solvent, the resulting syrup was purified by column chromatography ($CHCl_3$–MeOH, 95:5): yield 290 mg (1.05 mmol, 93%); mp (MeOH) 204–205 °C; UV (EtOH) λ_{max} 260 nm (ϵ 15800); IR (KBr) 2100 cm^{-1} (N_3); ^1H NMR (DMSO-d_6) δ 1.90–2.60 (m, 2 H, H-3′ and H-3″), 3.60 (m, 2 H, H-5′ and H-5″), 4.30 (m, 1 H, H-4′), 4.82 (m, 1 H, H-2′), 5.18 (t, 1 H, J = 5.5 Hz, OH), 6.00 (d, 1 H, J = 3.1 Hz, H-1′), 7.29 (br s, 2 H, NH_2), 8.16 (s, 1 H, H-2), 8.38 (s, 1 H, H-8); ^{13}C NMR (DMSO-d_6) 119.1 (C-5), 138.9 (C-8), 148.8 (C-4), 152.6 (C-2), 156.1 (C-6) ppm. Anal. ($C_{10}H_{12}N_8O_2$) C, H, N.

9-[5-*O*-(Monomethoxytrityl)-3-deoxy-β-D-*erythro*-pentofuranosyl]adenine (14′). A mixture of 2.51 g (10 mmol) of cordycepin[9] and 3.70 g (12 mmol) of 4-anisylchlorodiphenylmethane in anhydrous pyridine (150 mL) was stirred for 5 h at 50 °C. The reaction mixture was concentrated to 50 mL, poured into H_2O (200 mL), and extracted twice with EtOAc (200 mL). The combined organic layer was dried, evaporated, and coevaporated with toluene. The residual oil was purified by column chromatography on silica (eluent $CHCl_3$–MeOH, 97:3): yield 3.87 g (7.4 mmol, 74%); UV (MeOH) λ_{max} 258 nm (ϵ 14900); ^1H NMR ($CDCl_3$) δ 1.90–2.40 (m, 2 H, H-3′ and H-3″), 3.18–3.57 (m, 2 H, H-5′ and H-5″), 3.78 (s, 3 H, CH_3), 4.53–4.91 (m, 2 H, H-2′ and H-4′), 6.02 (d, 1 H, J = 1.1 Hz, H-1′), 6.72 (br s, 2 H, NH_2), 6.79 (d, 2 H) and 7.25 (m, 12 H) (phenyl), 8.12 (s, 1 H, H-2), 8.29 (s, 1 H, H-8).

9-(2-*O*-Mesyl-3-deoxy-β-D-*erythro*-pentofuranosyl)adenine (2). A mixture of 680 mg (1.3 mmol) of **14′** and 0.3 mL of mesyl chloride in anhydrous pyridine (20 mL) was stirred at 0 °C overnight. After addition of 1 mL of H_2O and further stirring for 15 min, the reaction mixture was poured into H_2O (100 mL) and extracted twice with $CHCl_3$ (2 × 100 mL). The organic layer was dried, evaporated, and coevaporated with toluene. The residue was dissolved in 50 mL of HOAc (80%). The mixture was stirred for 5 h, evaporated, and coevaporated with toluene, and the residue was triturated twice with 100-mL portions of Et_2O. The title compound was crystallized from MeOH: 300 mg (0.91 mmol, 70%) yield; mp 212 °C dec; UV (MeOH) λ_{max} 259 nm (ϵ 15400); ^1H NMR (DMSO-d_6) δ 2.12–2.70 (m, H-3′ and H-3″), 3.36 (s, 3 H, CH_3), 3.64 (m, 2 H, H-5′ and H-5″), 4.36 (m, 1 H, H-4′), 5.18 (t, 1 H, J = 5.3 Hz, OH), 5.64 (m, 1 H, H-2′), 6.25 (d, 1 H, J = 1.8 Hz, H-1′), 7.31 (br s, 2 H, NH_2), 8.16 (s, 1 H, H-2), 8.36 (s, 1 H, H-8).

9-(2-Azido-2,3-dideoxy-β-D-*threo*-pentofuranosyl)adenine (7). A mixture of 296 mg (0.9 mmol) of **2** and 440 mg of LiN_3 (10 equiv) in DMF (10 mL) was heated for 40 h at 100 °C. The reaction mixture was evaporated and purified by column chromatography ($CHCl_3$–MeOH, 90:10), yielding 220 mg (0.8 mmol, 89%) of **7**: mp (MeOH) 234 °C dec; UV (MeOH) λ_{max} 259 nm (ϵ 15700); IR (KBr) 2100 cm^{-1} (N_3); ^1H NMR (DMSO-d_6) δ 1.95–2.58 (m, 2 H, H-3′ and H-3″), 3.69 (m, 2 H, H-5′ and H-5″), 4.17 (m, 1 H, H-4′), 4.79 (m, 1 H, H-2′), 5.19 (t, 1 H, J = 5.3 Hz, OH), 6.36 (d, 1 H, J = 6.2 Hz, H-1′), 7.27 (br s, 2 H, NH_2), 8.16 (s, 1 H, H-2), 8.40 (s, 1 H, H-8); ^{13}C NMR (DMSO-d_6) δ 118.55 (C-5), 139.08 (C-8), 149.37 (C-4), 152.63 (C-2), 156.04 (C-6) ppm. Anal. ($C_{10}H_{12}N_8O_2$) C, H, N.

6-*N*-Benzoyl-3′-*O*-mesyl-2′-deoxyadenosine (4). 6-*N*-Benzoyl-5′-*O*-(monomethoxytrityl)-2′-deoxyadenosine[10] (1.88 g, 3 mmol) was treated with 0.75 mL of mesyl chloride in pyridine (50 mL) at room temperature for 5 h. After addition of 1 mL of H_2O and further stirring for 10 min, the reaction mixture was poured into H_2O (200 mL) and extracted twice with $CHCl_3$ (2 × 150 mL). The combined organic layer was dried, evaporated, and coevaporated with toluene. The resulting oil was stirred for 20

min in 50 mL of $CHCl_3$–MeOH (8:2) containing 2% *p*-toluenesulfonic acid. After neutralization with NaOH (1 *N*) and addition of 50 mL of H_2O, the organic layer was separated. The H_2O layer was extracted again with $CHCl_3$ (100 mL), and the combined extracts were washed with H_2O. After drying and evaporation, the title compound was crystallized from MeOH: 950 mg (2.19 mmol, 73%); mp 153 °C; UV (MeOH) λ_{max} 280 nm (ϵ 21 300); ^1H NMR ($CDCl_3$) δ 2.66 (m, 1 H, H-2'), 3.34 (m, 1 H, H-2''), 3.12 (s, 3 H, CH_3), 3.89 (m, 2 H, H-5' and H-5''), 4.46 (m, 1 H, H-4'), 5.54 (d, J = 5.3 Hz, H-3'), 6.40 (dd, J = 5.3 Hz and 9.0 Hz, H-1'), 7.53 (m) and 7.94 (m) (5 H, phenyl), 8.13 (s, 1 H, H-2), 8.68 (s, 1 H, H-8).

9-(3-Azido-2,3-dideoxy-β-D-*threo*-pentofuranosyl)adenine (9). A solution of 866 mg (2 mmol) of **4** in DMF (20 mL) containing 980 mg of LiN_3 (10 equiv) was heated for 6 h at 80 °C. The reaction mixture was evaporated and purified by column chromatography ($CHCl_3$–MeOH, 95:5). 9-(3-Azido-2,3-dideoxy-β-D-*threo*-pentofuranosyl)-*N*-benzoyladenine: IR (KBr) 2100 cm^{-1} (N_3); ^1H NMR (DMSO-d_6) δ 2.45–3.20 (m, 2 H, H-2' and H-2''), 3.76 (m, 2 H, H-5' and H-5''), 4.19 (m, 1 H, H-4'), 4.60 (m, 1 H, H-3'), 5.00 (br s, 1 H, OH), 6.44 (dd, 1 H, J = 3.1 Hz and 7.7 Hz, H-1'), 7.62 and 8.00 (2 m, 5 H, phenyl), 8.57 (s, 1 H, H-2), 8.75 (s, 1 H, H-8). The benzoyl group was removed with methanol saturated with ammonia at room temperature overnight, and **9** was crystallized from MeOH: total yield 359 mg (1.3 mmol, 65%); mp 195–196 °C; IR (KBr) 2100 cm^{-1} (N_3); UV (MeOH) λ_{max} 259 nm (ϵ 15 600); ^1H NMR (DMSO-d_6) δ 2.50–3.08 (m, 2 H, H-2' and H-2''), 3.73 (t, 2 H, H-5' and H-5''), 4.16 (m, 1 H, H-4'), 4.59 (m, 1 H, H-3'), 5.07 (t, 1 H, J = 5.5 Hz, OH), 6.30 (dd, 1 H, J = 3.8 Hz and 7.3 Hz, H-1'), 7.3 (br s, 2 H, NH_2), 8.17 (s, 1 H, H-2), 8.25 (s, 1 H, H-8); ^{13}C NMR (DMSO-d_6) 118.9 (C-5), 138.6 (C-8), 149.1 (C-4), 152.7 (C-2), 156.0 (C-6) ppm. Anal. ($C_{10}H_{12}N_8O_2$) C, H, N.

9-(3-*O*-Mesyl-2-deoxy-β-D-*threo*-pentofuranosyl)adenine (5). A solution of 1.05 g (2 mmol) of **17**[14] in pyridine (20 mL) was mesylated overnight with 0.5 mL of mesyl chloride at 0 °C. After addition of 1 mL of H_2O and a further stirring for 15 min, the mixture was poured into H_2O (100 mL) and extracted twice with $CHCl_3$ (100 mL). The organic layer was dried, evaporated, and coevaporated with toluene, leaving an oil, which was dissolved in 30 mL of CH_2Cl_2–MeOH (8:2) containing 1% of *p*-toluenesulfonic acid. The reaction mixture was stirred for 35 min at room temperature, 3.2 mL of 1 N NaOH was added, and the solvent was evaporated. Column chromatography on silica ($CHCl_3$–MeOH, 90:10) and crystallization from MeOH yielded 480 mg (1.46 mmol, 73%) of **5**: mp 199–200 °C; UV (MeOH) λ_{max} 259 nm (ϵ 15 800); ^1H NMR (DMSO-d_6) δ 2.45–3.25 (m, 2 H, H-2' and H-2''), 3.28 (s, 3 H, CH_3), 3.68 (t, 2 H, H-5' and H-5''), 4.15 (m, 1 H, H-4'), 4.98 (t, 1 H, J = 5.3 Hz, OH), 5.39 (m, 1 H, H-3'), 6.37 (dd, 1 H, J = 3.5 Hz and 7.5 Hz, H-1'), 7.28 (br s, 2 H, NH_2), 8.15 (s) and 8.17 (s) (2 × 1 H, H-2 and H-8).

9-(3-Azido-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)adenine (8). A mixture of 500 mg (1.5 mmol) of **5** and 750 mg of LiN_3 (10 equiv) in 15 mL of DMF was heated for 4 h at 80 °C. The reaction mixture was evaporated and purified by column chromatography, leaving 373 mg (1.35 mmol, 90%) of **7**, which was crystallized from MeOH: melting point and physical data are in agreement with previous reports;[14,25] ^1H NMR (DMSO-d_6) δ 2.25–2.64 (m, 1 H, H-2'), 2.80–3.12 (m, 1 H, H-2''), 3.61 (m, 2 H, H-5' and H-5''), 3.94 (m, 1 H, H-4'), 4.61 (m, 1 H, H-3'), 5.34 (t, J = 5.7 Hz, OH), 6.32 (t, J = 6.59 Hz, H-1'), 7.3 (br s, 2 H, NH_2), 8.15 (s, 1 H, H-2), 8.34 (s, 1 H, H-8); ^{13}C NMR (DMSO-d_6) 36.25 (C-2'), 61.2 (C-3'), 61.5 (C-5'), 83.4 (C-1'), 84.8 (C-4'), 119.2 (C-5), 139.6 (C-8), 148.9 (C-4), 152.5 (C-2), 156.1 (C-6) ppm.

9-(2-Fluoro-2,3-dideoxy-β-D-*erythro*-pentofuranosyl)adenine (10). A solution of 732 mg (1.4 mmol) of **15'** and 0.75 mL of (diethylamido)sulfur trifluoride in anhydrous CH_2Cl_2 (30 mL) was stirred at room temperature for 14 h. The reaction mixture was poured into 30 mL of sodium bicarbonate (10%), and the organic layer was separated, dried, and evaporated. The resulting yellow foam was diluted with 20 mL of $CHCl_3$–MeOH (8:2), containing 2% *p*-toluenesulfonic acid, and stirred for 15 min at room temperature. After neutralization with NaOH (2 N, 1.05 mL), the mixture was evaporated and purified by column chromatography ($CHCl_3$–MeOH, 97:3). The title compound was crystallized from MeOH: 269 mg (1.06 mmol, 76%), yield; mp 238 °C; UV (MeOH) λ_{max} 258 nm (ϵ 15 900); ^1H NMR (DMSO-d_6) δ 2.00–2.80 (m, 2 H, H-3' and H-3''), 3.63 (m, 2 H, H-5' and H-5''), 4.37 (m, 1 H, H-4'), 5.11 (t, 1 H, J = 5.5 Hz, OH), 5.59 (m, 1 H, $J_{2',F}$ = 52.7 Hz, H-2'), 6.62 (d, 1 H, $J_{1',F}$ = 18 Hz, H-1'), 7.66 (br s, 2 H, NH_2), 8.52 (s, 1 H, H-2), 8.72 (s, 1 H, H-8); ^{13}C NMR (DMSO-d_6) 119.0 (C-5), 138.8 (C-8)8 148.7 (C-4), 152.6 (C-2), 156.0 (C-6) ppm. Anal. ($C_{10}H_{12}N_5O_2F$) C, H, N.

9-[5-*O*-(Monomethoxytrityl)-3-fluoro-3-deoxy-β-D-*xylo*-pentofuranosyl]adenine (28). A mixture of 538 mg (2 mmol) of 9-(3-fluoro-3-deoxy-β-D-*xylo*-pentofuranosyl)adenine[21] and 772 mg (2.5 mmol) of 4-anisylchlorodiphenylmethane in 50 mL of anhydrous pyridine was stirred overnight at room temperature. The reaction mixture was concentrated, diluted with H_2O (100 mL), and extracted with $CHCl_3$ (2 × 100 mL). The organic layer was dried and evaporated, and the residual oil was purified by column chromatography ($CHCl_3$–MeOH, 99:1 followed by 95:5), leaving the title compound as a colorless foam: 736 mg (1.36 mmol, 68%) yield; UV (MeOH) λ_{max} 260 nm (ϵ 15 100); ^1H NMR ($CDCl_3$) δ 3.56 (m, H-5', H-5''), 3.76 (s, CH_3O), 4.20 (dm, $J_{4',F}$ = 28 Hz, H-4'), 4.66 (dm, $J_{2',F}$ = 16 Hz, H-2'), 5.16 (dm $J_{3',F}$ = 54 Hz, H-3'), 6.18 ("s", H-1'), 6.60 (br s, NH_2), 6.82 (d) and 7.10–7.58 (m) (trityl), 7.80 (s, H-2), 8.23 (s, H-8).

9-[5-*O*-(Monomethoxytrityl)-3-fluoro-3-deoxy-2-*O*-(phenoxythiocarbonyl)-β-D-*xylo*-pentofuranosyl]adenine (29). A mixture of 541 mg (1 mmol) of **28**, 250 mg of 4-(dimethylamino)pyridine, and 200 μL of *O*-phenyl chlorothioformate in 15 mL of CH_3CN was stirred overnight at room temperature. The reaction mixture was evaporated, diluted with H_2O (50 mL), and extracted with $CHCl_3$ (2 × 50 mL). TLC ($CHCl_3$–MeOH, 95:5) showed only one compound, which was identified after flash chromatography ($CHCl_3$–MeOH, 98:2): yield 623 mg (0.92 mmol, 92%) as a foam; UV (MeOH) λ_{max} 259 (ϵ 15 300), 233 nm (16 700); ^1H NMR ($CDCl_3$) δ 3.59 (br t, H-5', H-5''), 3.78 (s, CH_3O), 4.53 (dm, $J_{4',F}$ = 28 Hz, H-4'), 5.40 (dm, $J_{3',F}$ = 50 Hz, H-3'), 6.02 (dm, $J_{2',F}$ = 13 Hz, H-2'), 6.11 (br s, NH_2), 6.52 (d, J = 1.3 Hz, H-1'), 6.70–6.94 and 7.00–7.55 (m, trityl and phenyl), 7.95 (s, H-2), 8.36 (s, H-8).

9-(3-Fluoro-2,3-dideoxy-β-D-*threo*-pentofuranosyl)adenine (13). A stream of N_2 was bubbled into a solution of 541 mg (0.8 mmol) of **29** in 40 mL of toluene for 10 min. Then, 400 μL of tri-*n*-butyltin hydride and 40 mg of 2,2'-azobis(2-methylpropionitrile) were added, and the reaction mixture was heated for 45 min at 80 °C. The mixture was evaporated and applied onto a silica gel column, which was eluted with $CHCl_3$–MeOH, 97:3. This afforded 353 mg (0.67 mmol, 84% yield) of **30**. As shown by ^1H NMR, this compound was still contaminated with a trace of tri-*n*-butyltin hydride, and a complete identification was carried out after detritylation. ^1H NMR ($CDCl_3$): δ 2.35– = 27 Hz, H-4'), 5.25 (m, $J_{3',F}$ = 53 Hz, H-3'), 6.53 (dd, partially hidden by the NH_2 protons, H-1'), 6.65 (br s, NH_2), 6.8 (d) and 7.15–7.58 (m) (trityl), 7.95 (s, H-2), 8.3 (s, H-8). Therefore, the 5'-monomethoxytrityl compound **30** was dissolved in 10 mL of a solution of *p*-toluenesulfonic acid (2%) in $CHCl_3$–MeOH (8:2). After 20 min, 5.25 mL of NaOH (0.2 N) was added, and the mixture was evaporated and purified by column chromatography ($CHCl_3$–MeOH, 90:10), yielding 124 mg (0.49 mmol, 73%) of **13**, which was crystallized from MeOH–Et_2O: mp 188–190 °C; UV (EtOH) λ_{max} 259 nm (ϵ 15 700); ^1H NMR (DMSO-d_6) δ 2.96 (m, H-2', H-2''), 3.74 (m, H-5', H-5''), 4.14 (m, $J_{4',F}$ = 29.5 Hz, H-4'), 5.43 (m, $J_{3',F}$ = 54.5 Hz, H-3'), 6.42 (dd, J = 3.1 Hz and 7.03 Hz, H-1'), 8.11 (s, H-2), 8.20 (s, H-8); ^{13}C NMR (DMSO-d_6) 118.7 (C-5), 138.2 (d, J = 8.5 Hz, C-8), 149.3 (C-4), 152.8 (C-2), 156.0 (C-6) ppm. Anal. ($C_{10}N_{12}N_5O_2F$) C, H, N.

5'-*O*-Tritylcordycepin. A solution of 500 mg (2 mmol) of cordycepin and 1.11 g (4 mmol) of triphenylmethyl chloride in anhydrous pyridine (30 mL) was kept at 50 °C overnight. The reaction mixture was concentrated, diluted with $CHCl_3$ (100 mL), washed with H_2O (2 × 100 mL), dried, and evaporated. Column chromatographic purification on silica ($CHCl_3$–MeOH, 95:5) yielded 867 mg (1.76 mmol, 88%) of 5'-*O*-tritylcordycepin, which could be crystallized from CH_2Cl_2–toluene: mp 210–211 °C; UV (MeOH) λ_{max} 260 nm (ϵ 15 600); ^1H NMR ($CDCl_3$) δ 1.98–2.40 (m, 2 H, H-3' and H-3''), 3.39 (m, 2 H, H-5' and H-5''), 4.77 (m, 2 H,

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H-2′ and H-4′), 6.04 (d, J = 1.1 Hz, H-1′), 6.83 (br s, 2 H, NH_2), 7.23 (m, trityl), 8.13 (s, 1 H, H-2), 8.27 (s, 1 H, H-8).

9-(2-Fluoro-2,3-dideoxy-β-D-*threo*-pentofuranosyl)adenine (11). A mixture of 493 mg (1 mmol) of 5′-*O*-tritylcordycepin and 0.5 mL of DAST in anhydrous CH_2Cl_2 (10 mL) was refluxed for 3 h, cooled to room temperature, and poured into a $NaHCO_3$ solution (10%). The organic layer was separated, dried, and evaporated. The resulting oil was dissolved in 80% acetic acid, heated for 20 min at 100 °C, and evaporated. The reaction mixture was purified by preparative TLC ($CHCl_3$–MeOH, 85:15, two developments), and trace amounts of an unidentified side compound were removed by chromatography on a XAD column (100–200 μm) with EtOH–H_2O (1:9) as eluent. The UV-absorbing fractions were collected, and the solvent was removed by lyophilization, giving 25 mg (0.1 mmol, 10%) of **11**: UV (MeOH) λ_{max} 258 nm (ε 15 100); MS, m/e (relative intensity) 253 (19.3, M^+), 223 (0.9, M – CH_2O), 222 (4.9, M – CH_2OH), 202 (9.8, M – CH_2OH – HF), 164 (66.4, BCHO + H), 135 (100, B + H), 108 (32.8, B + H – HCN); ^1H NMR (DMSO-d_6) δ 2.00–2.92 (m, H-3′ and H-3″), 3.57 (m, H-5′ and H-5″), 4.23 (m, H-4′), 5.41 (m, $J_{2',F}$ = 54.7 Hz, H-2′), 6.28 (dd, $J_{1',2'}$ = 3.6 Hz, $J_{1',F}$ = 16.7 Hz, H-1′), 7.27 (br s, NH_2), 8.16 (s, H-2), 8.23 (d, J = 2.4 Hz, H-8); ^{13}C NMR (DMSO-d_6) 139.5 (d, J = 4.9 Hz, C-8), 152.7 (C-2) ppm. Anal. ($C_{10}H_{12}N_5O_2F$) C, H, N.

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Registry No. 1, 4097-22-7; **2**, 110143-00-5; **3**, 110142-98-8; **4**, 110143-01-6; **5**, 110143-04-9; **6**, 110142-99-9; **7**, 79872-72-3; **8**, 66323-44-2; **9**, 110143-03-8; **9** (6-*N*-benzoyl), 110143-02-7; **10**, 110143-05-0; **11**, 110143-10-7; **12**, 87418-35-7; **13**, 110143-09-4; **14′**, 51763-58-7; **15**, 6998-75-0; **15′**, 110142-97-7; **17′**, 108895-39-2; **18**, 7057-48-9; **20**, 110142-94-4; **21**, 110142-95-5; **22**, 110142-96-6; **23**, 10992-57-1; **27**, 20535-16-4; **28**, 110143-06-1; **29**, 110143-07-2; **30**, 110143-08-3; adenosine, 58-61-7; cordycepin, 73-03-0; 6-*N*-benzoyl-5′-*O*-(monomethoxytrityl)-2′-deoxyadenosine, 24816-13-5; tritylcordycepin, 90813-62-0.

Optimization and in Vivo Evaluations of a Series of Small, Potent, and Specific Renin Inhibitors Containing a Novel Leu-Val Replacement[1,2]

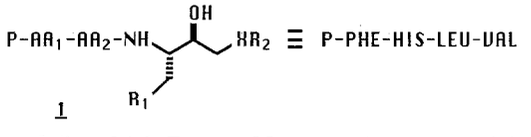
Joseph F. Dellaria,* Robert G. Maki, Barbara A. Bopp, Jerome Cohen, Hollis D. Kleinert, Jay R. Luly, Ilmar Merits, Jacob J. Plattner, and Herman H. Stein

Abbott Laboratories, Abbott Park, Illinois 60064. *Received April 24, 1987*

Further structure–activity relationships (SAR) for a novel dipeptide series of renin inhibitors are reported. These inhibitors retain the Phe^8-His^9 portion of angiotensinogen and employ a unique Leu^{10}-Val^{11} replacement [(LVR), ref 2]. SAR at the Leu^{10} side chain revealed that the LVR derived from cyclohexylalanine provided a nearly 10-fold boost in potency for the final inhibitor. In addition SAR work was carried out to delineate the relationships between binding potency and (1) the size, shape, and charge of the side chain at the His^9 position; (2) the size and topology of the side chain at the Phe^8 site; and (3) the size of the Phe^8 N-protecting group. One of the more potent inhibitors, **12**, was shown to provide a substantial antihypertensive effect in a sodium depleted monkey model when administered intravenously. Metabolism work, in Sprague–Dawley rats, provided insights into the susceptibility of **12** to significant hepatic clearance and provided encouraging evidence for intestinal absorption.

A major regulatory mechanism for the maintenance of blood pressure in mammals is the renin–angiotensin system (RAS).[3,4,5a] The RAS is a multiregulated cascade of enzyme mediated proteolytic events that converts angiotensinogen to angiotensin II (AII) and angiotensin III (AIII), the principle pressor agents of the system (Scheme I). The major pharmacological effects of AII and AIII are vasoconstriction and stimulation of the adrenal cortex to release aldosterone, which in turn induces sodium retention. Inhibition of angiotensin converting enzyme (ACE),[6] which catalyzes the second step in the RAS, has established that obstruction of this system prior to formation of AII or AIII can effectively reduce blood pressure in hypertensive patients.

The success of ACE inhibitors as antihypertensive agents has encouraged many to seek an inhibitor of the first step of the RAS, namely, the renin catalyzed cleavage of angiotensinogen to angiotensin I (AI). In previous work from our laboratories,[2] a novel series of small, potent, and selective renin inhibitors of the general structure **1** was



1

disclosed (in which P = an N-protecting group; AA_1 and AA_2 = amino acid residues; X = NH, O, CH_2, or SO_n (n = 0 or 2); R_1 = isopropyl; and R_2 = a lipophilic group). These inhibitors were designed as "transition-state

(1) Abbreviations follow IUPAC-IUB Commission on Biochemical Nomenclature for amino acids and peptides: *Eur. J. Biochem.* **1984**, *158*, 9–31. Additional abbreviations used are as follows: Ac, acetyl; Bn, benzyl; BSA, bovine serum albumin; Cbz, benzyloxycarbonyl; DMF, dimethylformamide; DMSO, dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; Etoc, (ethyloxy)carbonyl; Ibu, isobutyryl; LVR, Leu-Val replacement; MCPBA, *m*-chloroperoxybenzoic acid; PMSF, phenylmethanesulfonyl fluoride; Poa, phenoxyacetyl; sg, silica gel; Tba, *tert*-butylacetyl.

(2) For the previous paper in this series, see: Luly, J. R.; Yi, N.; Soderquist, J.; Stein, H.; Cohen, J.; Perun, T. J.; Plattner, J. J. *J. Med. Chem.* **1987**, *30*, 1609.

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(5) For other relevant literature, refer to the preceding paper in this series, ref 2 this paper: (a) ref 1–3; (b) ref 13–15 provided historical background concerning the previous use of transition-state analogues in renin inhibitors.

(6) For a recent review, refer to: Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev* **1985**, *5*, 483.

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