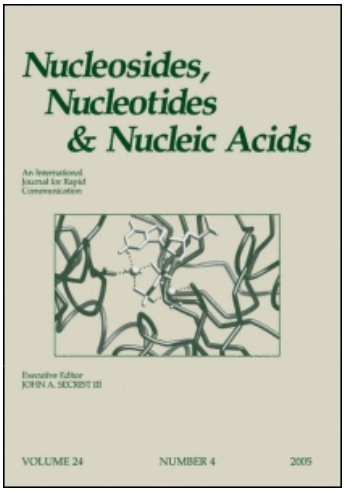

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Relationship Between Chemical Structure and Antileishmanial Effect of Sinefungine Analogues

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RELATIONSHIP BETWEEN CHEMICAL STRUCTURE AND ANTILEISHMANIAL EFFECT OF SINEFUNGINE ANALOGUES.

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Abstract The synthesis of various analogues of sinefungin (**1**), having structures **2-5**, has been developed by means of an original approach which uses radical chemistry. The study of their biological activities revealed that for the antileishmanial effect of sinefungin, the presence of the amino group at C-6' in the (*S*)-configuration and the presence of the carboxyl group at C-9' are necessary.

Introduction

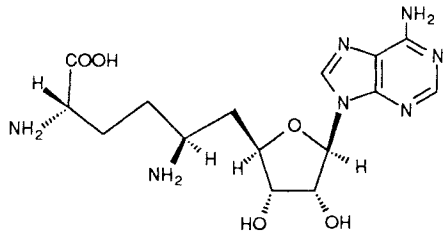
Leishmaniases are tropical diseases caused by protozoal parasites of the genus *Leishmania*. Pentavalent antimonials are among the first-line drugs against visceral leishmaniasis but resistance to these molecules occurs more and more frequently.[1] Thus, new drugs to treat these diseases are urgently needed. Sinefungin (SF) **1**, a natural nucleoside antibiotic[2] was shown to have potent antiparasitic, particularly antileishmanial activity *in vitro* [3] and in infected rodents *in vivo* [4] but has toxic side effects in dogs and goats[5] which precludes its clinical use in humans. In our laboratories, a synthetic program is underway with the aim to prepare active and less toxic SF analogues.[6-8] The present work was undertaken in order to understand the importance of the amino group at C-6', that of the carboxyl group at C-9' as well as of the side-chain length for the antileishmanial activity.

Chemistry

Recently, we have proposed new approaches for the formation of nucleophilic radicals and their eventual addition to olefins.[9-12] In this original procedure the required radicals are generated by simple treatment of an iodide with either the zinc/copper[9-11] or the zinc/iron couple.[12] Such a system avoids the formation of toxic by-products as observed when tin hydride derivatives are employed.[13] Moreover, the reaction conditions

are very mild and can be easily performed. We have used this newly developed chemistry to prepare sinefungin analogues **2-5** (Scheme 1) as illustrated below.

In a first series of experiments (Scheme 2) methyl 5-deoxy-5-iodo-2,3-*O*-isopropylidene-D-ribofuranoside (**6**) was combined as reported, in the presence of the bi-metallic couple, with either methyl acrylate (**7a**) or methyl 2-acetamidoacrylate (**7b**) to give, in acceptable yields, the octose derivatives **8** and **9**, respectively.[9,12] The next steps of the synthesis required adequate protecting group manipulation which permitted to obtain a 2-*O*-acetyl sugar derivative susceptible to undergo adenylation by the Vorbrüggen procedure.[14] To prepare such a derivative in a limited number of steps, conditions for the removal of the isopropylidene group avoiding the concomitant hydrolysis of the methyl ester had to be found. Accordingly, elimination of the isopropylidene group was performed in methanol containing ethylene glycol and two equivalents of p-toluenesulfonic acid. The resulting product was immediately acetylated using acetic anhydride in pyridine to give the methyl glycoside **10** (or **11**) (two epimers at C-1) in 82% yield (two steps). Adenylation of diacetate **10** (or **11**) was accomplished by overnight treatment with persilylated N^6-



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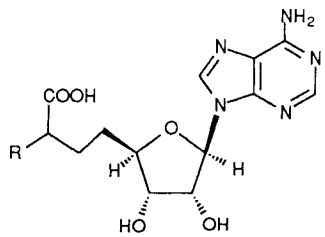
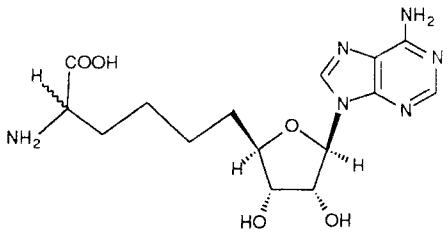


2 R= H
3 R= NH_2



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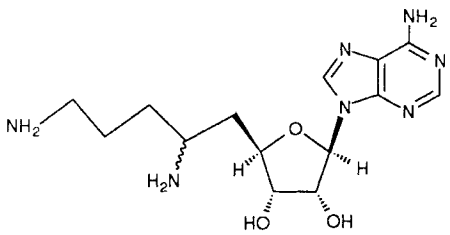

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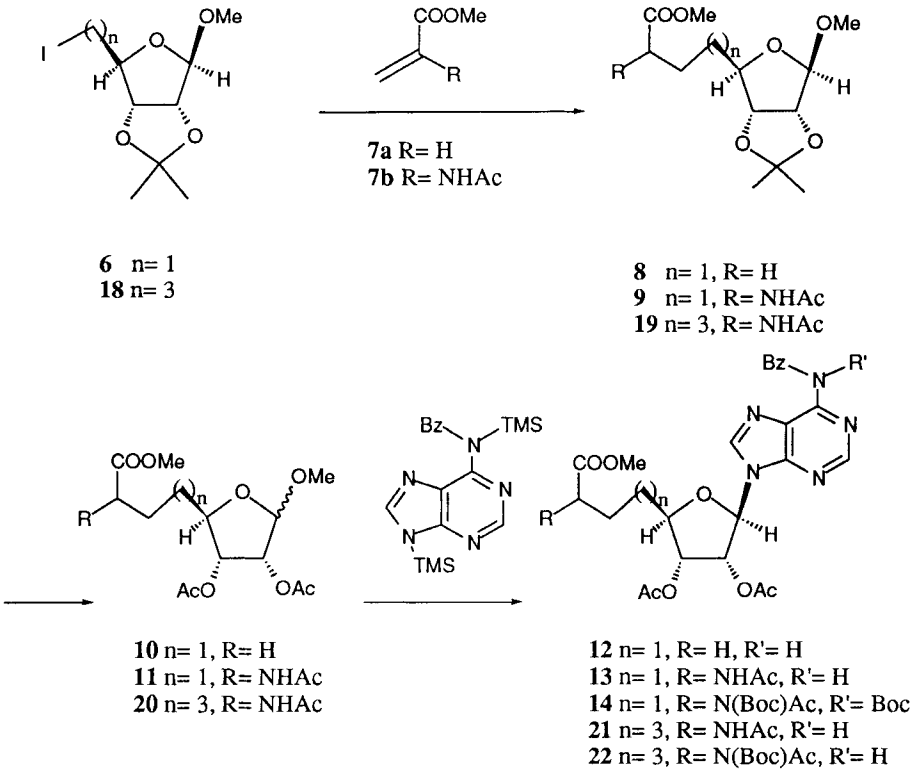
Scheme 1



Scheme 2

benzoyladenine in refluxing 1,2-dichloroethane in the presence of trimethylsilyl trifluoromethanesulfonate.[14] In this manner the fully protected nucleoside derivative **12** (or **13**) was obtained in 55% yield. Finally, when **12** was warmed in concentrated ammonia containing 20% methanol the corresponding nucleoside **2** was isolated in 85% yield after HPLC purification.

For the complete deprotection of compound **13**, having an *N*-acetylamino substituent at position C-7', a supplementary step was necessary. Thus, to render the *N*-acetyl group[15] more labile to ammonia treatment, **13** was treated with di-*tert*-butyldicarbonate in the presence of an 0.1 equivalent of *N,N*-dimethylaminopyridine in refluxing acetonitrile to give **14**. Ammonia treatment of this compound provided a N^6,$N^{7'}$-di-*tert*-butyloxycarbonyl derivative whose complete deprotection was immediately accomplished by a short treatment with trifluoroacetic acid. In this manner the resulting nucleoside **3** was obtained in 85% yield after HPLC purification.

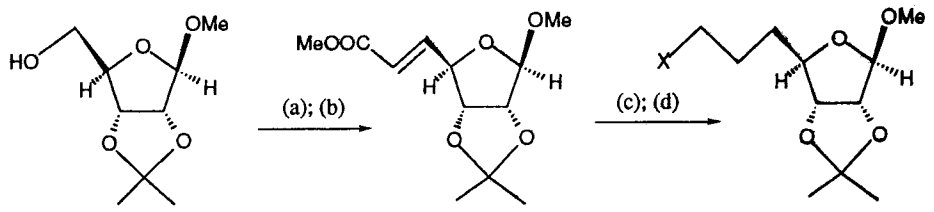
The same synthetic strategy was applied for the preparation of the 6'-deaminosinefungin **4**.[16] Thus, methyl 7-iodo-2,3-*O*-isopropylidene-5,6,7-trideoxy-β-D-riboheptafuranoside (**18**) was prepared in four steps from methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (**15**) as outlined in Scheme 3. As for the synthesis of **9**, iodide **18** was combined with methyl 2-acetamidoacrylate (**7b**) to provide the expected protected amino acid **19** having the sugar moiety of 6'-deaminosinefungin (**4**). Subsequent transformation of this compound by methanolysis and acetylation of the 2,3-diol produced **20**. Adenylation of the latter provided the protected nucleoside **21** which was treated with di-*tert*-butyldicarbonate using the same conditions as in the case of **13**. However, surprisingly, the N^6-position of the adenine moiety was not protected in this reaction and compound **22** was isolated. Successive treatments of **22** with ammonia and trifluoroacetic acid led to 6'-desaminosinefungin (**4**) in 75% yield after HPLC purification.

The route which was followed for the preparation of decarboxy sinefungin (**5**) is outlined in Scheme 4. *N*-(2-iodoethyl)trifluoroacetamide was treated by the zinc-iron couple/sodium iodide/pyridine system in the presence of the known unsaturated nitrile **23**[6] to give methyl 6-cyano-2,3-*O*-isopropylidene-5,6,7,8,9-pentadeoxy-9-trifluoroacetamido-β-D-*ribo*-nonafuranoside (**24**) (two epimers at C-6) in 85% yield.[12] The next step of this sequence was the hydratation of nitrile **24** to give the corresponding amide **25**. In our hand the best reaction conditions were those which make use of potassium superoxide in dimethylsulfoxide.[17] At the end of the reaction the excess of base was neutralized by addition of an ethyl acetate/ ethyl trifluroroacetate mixture to insure the eventual reprotection of the amine at C-9. Under these conditions the amide **25** was obtained in 80% yield. Reaction of this amide with iodobenzene bis(trifluoroacetate)[18] led to the Hofmann degradation product which was isolated after *N*-trifluoroacetylation to give **26** in 82% yield. Methanolysis of this compound followed by acetylation provided the required diacetate **27** to be adenylated. Indeed, by using the same conditions as those which served for the other nucleosides, the protected decarboxy sinefungin **28** was obtained in 53% yield. A simple treatment of derivative **28** with ammonia followed by HPLC purification furnished 9'-decarboxysinefungin (**5**)[19] (two epimers at C-6) in 82% yield.

Biological activity

As mentionned above, the four sinefungin (**1**) analogues **2-5** were synthesized to permit the study of the importance of the C-6' NH_2 and C-9' carboxyl groups as well as the effect of side-chain shortening for the antileishmanial effect.

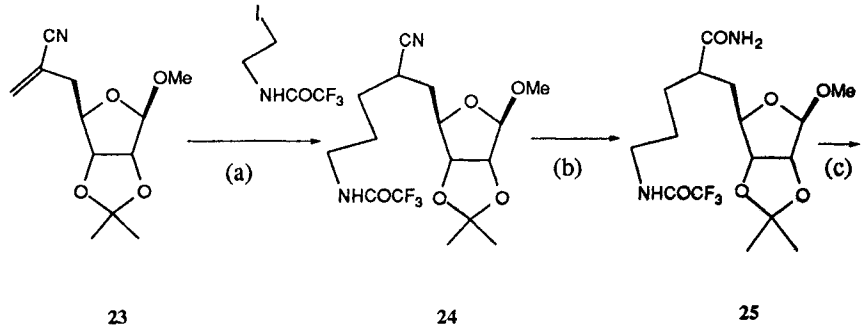
In Table I the activities of the new compounds : **2**, **3**, **4** and **5** are compared with those of **1**. The results clearly indicate that removal of the C-6' amino group, side-chain



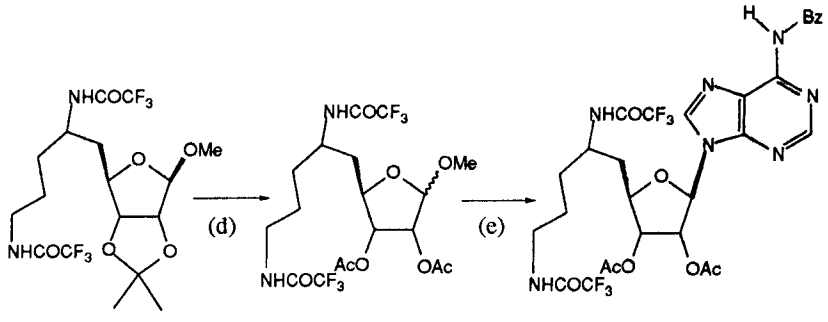
15 16 17 X= OH
18 X= I

a Reagents: (a) DCA, DCC, DMSO; (b) methyl (triphenylphosphoranylidene)acetate; (c) $NaBH_4$, LiCl, ethanol, THF; (d) methyltriphenylphosphonium iodide, DMF.

Scheme 3 [a]



23 24 25



26 27 28

a Reagents: (a) Zn/ $FeCl_3$, pyridine; (b) KO_2, DMSO; (c) [bis(trifluoroacetoxy)iodo]benzene, DMF/ H_2O then ethyl trifluoroacetate; (d) MeOH, pTSA then Ac_2O, pyridine; (e) N^6-benzoyl-N^6,N^9-bis-(trimethylsilyl)adenine, trimethylsilyl trifluoromethanesulfonate, DCE.

Scheme 4 [a]

Table I. Effect of the newly synthesized sinefungin analogues on the growth of *L. donovani* promastogotes

Analogue	IC50 (μM)	ICMax
2	880	2 mM → 65%
3	960	2 mM → 69%
4	1180	2 mM → 61%
5	> 3000	3 mM → 37%
SF **1**	0.009	0,03 μM → 100%

shortening or removal of the terminal carboxyl group leads to a very severe decrease in the effect on parasite multiplication. The least active molecule is 9'-decarboxysinefungin **5** indicating that the terminal carboxyl group is crucial for interference with cell growth.

The decreased activity of these analogs can be due either to no or bad uptake by the cells or to a low affinity for the cellular target. We have previously published that SF is taken up by an AdoMet permease[20] and that its main cellular target in these promastigotes is protein carboxyl-methylase or protein methylase II[21,22]. As shown in Table II analogues **2**, **3** and **4** have very low affinity for the AdoMet transporter whereas 9'-decarboxysinefungin (**5**) seems to be recognized by this permease. However, none of the four analogues (including **5**) are recognized by the leishmanial PM II which may explain that they are devoid of activity.

The comparison of the activity of the newly synthesized SF analogs with those prepared before is presented in Table III (chemical structures are shown in scheme 5). These data indicate that even the smallest structural modification involves a 50% decrease in the *in vitro* activity. The three analogues whose activity is the closest to SF are **33a** where the adenosine moiety is replaced by uridine[8], 4',5'-dehydrosinefungin (**31**), a natural nucleoside carrying a double bond in position C-4' and 9'-deaminosinefungin (**30**).[7] However as **33** is not recognized by the AdoMet permease[20] and by PMII[8], its antileishmanial effect is probably due to its interaction with other cellular targets.

In conclusion, data presented in this article show that for the antileishmanial activity the presence of the amino group at C-6' in the natural *S*-configuration and of the 9'-carboxyl group are essential. The fact that the 9'-deamino derivatives **30** and **35** are active suggest that some modifications at the side chain remain possible without excessive loss of biological effects.

Table II. Effect of the molecules on the uptake of AdoMet by the promastigotes of *L. donovani* and on the activity of leishmanial PMII

Substrat	AdoMet uptake IC50 (μM)	Ki PMII (μM)
AdoMet	0,6 ± 0.10	Km : 142
AdoHcy	17 ± 3.00	-
1 (SF)	0.2 ± 0.02	62
2	500 ± 100	no activity at 2 mM
3	227 ± 50	no activity at 2 mM
4	185 ± 60	2299
5	1.0 ± 0.30	2940

Experimental section

^1H and ^{13}C NMR spectra were recorded on Brucker WP 200 SY and AC 300 spectrometers. Chemical shifts are reported in ppm. Electron impact (EI) and chemical ionization (CI, isobutane) mass spectra were measured on AEI MS 50 and AEI MS 9 mass spectrometers, respectively. High resolution (HR) mass spectra and fast atom bombardment (FAB) mass spectra, using glycerol (or thioglycerol) matrices, were obtained with a Kratos MS 80 instrument. Microanalyses were performed by the Service de Microanalyse du CNRS. Column chromatography was carried out on Silica gel Kieselgel 60. Silicagel TLC was performed on Schleicher and Schuell plates with UV light for visualisation. Analytical and preparative HPLC of the nucleoside derivatives were accomplished on RP-18 columns using the 0.1% aqueous trifluoroacetic acid/ acetonitrile (95/ 5) and acetonitrile solvent system.

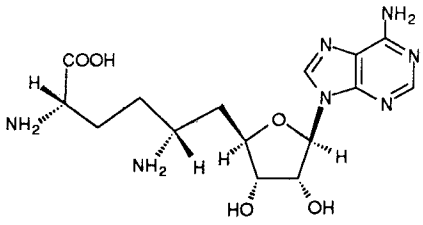
Methyl 2,3-di-O-acetyl-7-methoxycarbonyl-5,6,7-trideoxy-D-ribo-heptafuranoside (**10**). To a solution of **8**[9,12] (115 mg, 0.4 mmol) in methanol (4 ml), p-toluenesulfonic acid (150 mg, 0.8 mmol) was added. The mixture was stirred at room temperature for 4 h. Then 1 ml of pyridine was added and the solution evaporated. The residue was taken up overnight in pyridine (3 ml) containing acetic anhydride (2 ml). Work up of the reaction and silica gel column chromatography of the resulting oil gave methyl 2,3-di-O-acetyl-7-methoxycarbonyl-5,6,7-trideoxy-D-*ribo*-heptafuranoside **10** (110 mg) (2 epimers at C-1); ^1H NMR ($CDCl_3$) 5.20 - 4.85 (m, 3H, H-1, H-2, H-3), 4.06 (m, 1H, H-4), 3.67 (s, 3H, $MeCO_2$), 3.41 and 3.37 (2s, 3H, MeO_a and MeO_b), 2.38 (m, 2H, H-7), 2.10 (1s, 3H, MeCO), 2.05 (1s, 3H, MeCO), 1.69 (m, 4H, H-5, H-6); ^{13}C NMR ($CDCl_3$) 173.7, 169.8, 169.7, 106.0 and 101.1, 80.4 and 80.3, 75.1 and 74.7, 72.5 and 70.6, 55.3 and 55.1, 51.5, 34.1, 33.7, 21.3, 20.9, 20.6; Calcd for $C_{14}H_{22}O_8$: C, 52.82; H, 6.97. Found: C, 53.08; H, 6.86.

N^9-[2',3'-di-O-acetyl-7'-methoxycarbonyl-5',6',7'-trideoxy-β-D-ribo-1',4'-heptafuranosyl]-N^6-benzoyladenine (**12**). To N^6-benzoyl-N^6,N^9-bis-(trimethylsilyl)adenine (340 mg, 0.85 mmol) was added succesively, a solution of diacetate **10** (200 mg , 0.5 mmol) in 1,2-dichloromethane (8 ml) and trimethylsilyl trifluoromethanesulfonate (30 μl, 0.15 mmol). The mixture was refluxed for 48 h under N_2 atmosphere.

Table III. Relationship between the chemical structure and the effect of the analogs on the growth of *L. donovani* promastigotes.

Analogue	IC50 (μM)
1 [SF]	0.009
(*S*) **33a** [(*S*)-6'-SFU][8]	0.014
31 [Dehydro-SF]	0.020
30 [9'-deamino-SF][7]	0.040
32[6]	0.200
29 [6'-episinefungin][8]	0.270
(*R*) **33a** [(*R*)-6'-SFU][8]	0.280
34 [Cyclo-SF][28]	0.370
35[7]	0.400
33b [SF-T][8]	27.00
2	880
3	960
4	1150
5	>2960

The reaction mixture was diluted with CH_2Cl_2 and washed with a saturated aqueous solution of $NaHCO_3$ and with brine. The organic phase was evaporated and the residue taken up in ethyl acetate. The resulting solution was kept in the refrigerator overnight to precipitate the excess of N^6-benzoyladenine. The filtrate was evaporated and the residue chromatographed over silica gel using an acetone/heptane gradient (10 to 100%) to give N^9-[2',3'-di-*O*-acetyl-7'-methoxycarbonyl-5',6',7'-trideoxy-β-D-*ribo*-1',4'-heptafuranosyl]-N^6-benzoyladenine **12** as a foam (150 mg) in 56 % yield; ^{1}H NMR ($CDCl_3$) 9.14 (s, 1H, NH), 8.79 (s,1H, H-2), 8.14 (s, 1H, H-8), 8.02 (d, 1H, Ar), 7.83 (d, 1H, Ar), 7.50 (m, 3H, Ar), 6.16 (d, 1H, H-1'), 5.96 (m, 1H, H-2'), 5.47 (m, 1H, H-3'), 4.22 (m, 1H, H-4'), 3.65 (s, 3H, $MeCO_2$), 2.36 (m, 2H, H-7'), 2.14 (s, 3H, MeCO), 2.06 (s, 3H, MeCO), 1.93 (m, 4H, H-5', H-6'); ^{13}C NMR ($CDCl_3$) 173.4, 169.6, 169.4, 152.6, 151.6, 142.9, 141.8, 133.5, 128.6, 128.4; 128.0; 127.3; 123.9; 186.5; 82.2; 73.3; 73.1; 55.5; 33.3; 32.3; 20.8, 20.5, 20.3; MS (CI) *m/z* 526 $(M+H)^+$; HRMS (CI) Calcd. for $C_{25}H_{28}O_8N_5$: 526.1937. Found: 526.1895.



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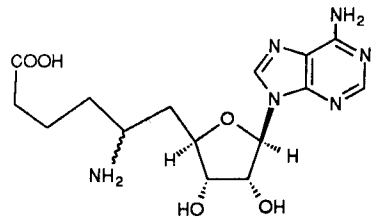

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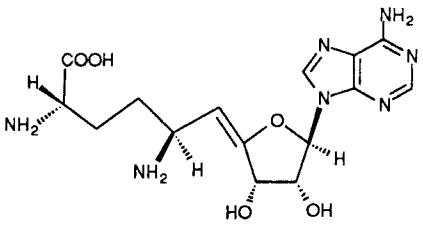

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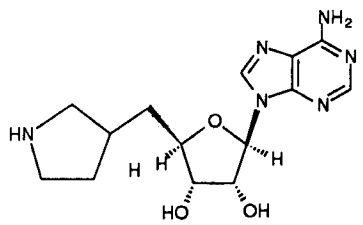

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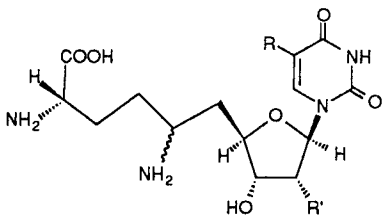
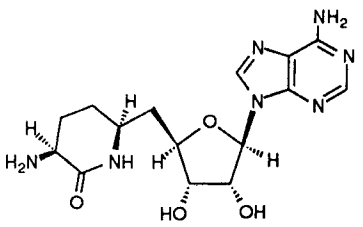


33a R= H, R'= OH
33b R= Me, R'= H



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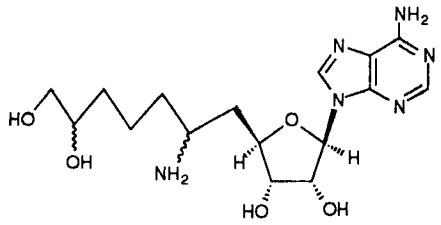

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Scheme 5

N^9-[7'-carboxy-5',6',7'-trideoxy-β-D-ribo-1',4'-heptafuranosyl]-adenine (**2**). A 10 ml solution of 38 mg of compound **12** in methanol/32% ammonia (1/4) was maintained at 60°C during 24 h. After evaporation of the solvents the residue was taken up in 0.1 N aqueous HCl and the solution washed with ether and neutralized with NH_4OH. Purification by C18 reverse phase HPLC gave 20 mg of nucleoside **2**. ^1H NMR (CD_3OD) 8.43 (s,1H, H-2), 8.36 (s, 1H, H-8), 6.01 (d, 1H, H-1'), 4.69 (t, 1H, H-2'), 4.14 (t, 1H, H-3'), 4.04 (m, 1H, H-4'), 2.29 (m, 2H, 2H-7'), 1.79 (m, 4H, H-5', H-6'); MS (FAB, glycerol) *m/z* 324 $(M+H)^+$.

Methyl 7-acetamido-2,3-O-isopropylidene-7-methoxycarbonyl-5,6,7-trideoxy-β-D-ribo-1',4'-heptafuranoside (**9**). To methyl 5-deoxy-5-iodo-2,3-*O*-isopropylidene-β-D-riboside (**6**) (1g, 3mmol) in ether (4ml) was added gradually a solid mixture (2 g) of zinc powder/CuI (1/1, weight to weight) and 5 ml of a solution of methyl 2-acetamidoacrylate (1.57 g, 10 mmol) in THF/H_2O (8/2). The reaction mixture was stirred with a vibromixer until the disappearance of the iodide. Then the reaction mixture was diluted with ether and filtered over Celite. Usual treatment of the organic phase and chromatography (heptane/acetone gradient) of the residue gave the extended carbohydrate derivative **9** (633 mg) in 63% yield as an oil. ^1H NMR ($CDCl_3$) 6.80 (s, 1H, NH), 4.92 (s, 1H, H-1), 4.58 (m, 3H, H-2, H-3, H-7), 4.12 (m, 1H, H-4), 3.76 (s, 3H, COOMe), 3.33 (s, 3H, OMe), 2.16 (s, 3H, COMe), 2.14-1.30 (m, 4H, H-5, H-6), 1.30 (s, 3H, Me); 1.26 (s, 3H, Me); ^{13}C NMR ($CDCl_3$) 172.5, 171.3, 112,5, 110, 86,4, 85,5, 84,2, 55,3, 52,6, 52.4 and 52.1, 31.0, 29.4, 26.6, 25.1, 23.3; MS (EI) *m/z* 330 $(M^{\cdot+}-1)$, 316 $(M^{\cdot+}-15)$, 300 $(M^{\cdot+}-31)$; Calcd for $C_{15}H_{25}NO_7$: C, 54.37; H, 7.60; N: 4.23. Found: C, 54.35; H, 7.61; N, 4.23.

Methyl 7-acetamido-2,3-di-O-acetyl-7-methoxycarbonyl-5,6,7-trideoxy-β-D-ribo-heptafuranoside (**11**). This compound was obtained in 83% yield using the conditions which served for the preparation of derivative **10** starting from **8**. ^1H NMR ($CDCl_3$) 6.30 (d, 1H, NH), 5.14 (m, 2H, H-2, H-3), 4.85 (s, 1H, H-1), 4.64 (m, 1H, H-7), 4.07 (m, 1H, H-4), 3.75 (s, 3H, COOMe), 3.36 (s, 3H, OMe), 2.10 (s, 3H, COMe), 2.05 (s, 3H, COMe), 2.03 (s, 3H, COMe), 2.0 - 1.5 (m, 4H, H-5, H-6); ^{13}C NMR ($CDCl_3$) 172.8, 169.9, 169.7, 106,1, 80.1, 75.1, 74.2, 55.3, 52.4 and 51.8, 51.7, 30.1, 28.5, 23.1, 20.6; MS (CI) *m/z* 376 $(M+H)^+$, 344 $(M+H)^+$-32; HRMS (CI): Calcd for $C_{16}H_{26}NO_9$: 376.1607. Found: 376.1579.

N^9-[7'-acetamido-2',3'-di-O-acetyl-7'-methoxycarbonyl-5',6',7'-trideoxy-β-D-ribo-heptafuranosyl]-N^6-benzoyladenine (**13**). This compound was obtained in 48% yield from **11** using identical conditions as in the case of the preparation of derivative **12**. ^1H NMR ($CDCl_3$) 9.50 (s, 1H, NH), 8.81 (s, 1H, H-2), 8.39 (s, 1H, H-8), 8.29 (d, 2H, Ar), 8.05 (m, 3H, Ar), 6.58 (m, 1H, NHAc), 6.1 (d, 1H, H-1'), 6.00 (t, 1H, H-2'), 5.47 (m, 1H, H-3'), 4.60 (m, 1H, H-7'), 4.20 (m, 1H, H-4'), 3.37 and 3.62 (2s, 3H, $COOMe_a$ and $COOMe_b$), 2.2-1.5 (m, 13H, COMe, NHCOMe, H-5', H-6'); ^{13}C NMR ($CDCl_3$) 132.9, 128.9, 128.0, 86.8, 81.9, 73.2, 52.6, 51.8 and 51.6, 29.0, 28.5, 23.2, 20.7, 20.5; MS (FAB, glycerol) *m/z* 605 $(M+Na)^+$, 583 $(M+H)^+$; Calcd for $C_{27}H_{30}N_6O_7$: C, 55.66; H, 5.19; N, 14.43. Found: C, 55.48; H, 5.26; N, 14.15.

N^9-[7'-N-acetyl-(N-tert-butyloxycarbonyl)amino-2',3'-di-O-acetyl-7'-methoxycarbonyl-5',6',7'-trideoxy-β-D-ribo-heptafuranosyl]-N^6-benzoyladenine (**14**). A solution of compound **13** (40 mg, 0.07 mmol) in acetonitrile (2 ml) was warmed during 40 min in the presence of di-*tert*-butyl dicarbonate (65 µl, 0.29 mmol) and DMAP (1 mg). The reaction product was purified by silica gel chromatography to give the protected nucleoside **14** (50 mg) in 93% yield as a foam. ^1H NMR ($CDCl_3$) 8.87 (s,1H, H-2), 8.17 (s, 1H, H-8), 7.89 (d, 2H, Ar), 7.54 (m, 1H, Ar), 7.46 (m, 2H, Ar), 6.13 (d, 1H, H-1'), 6.00 (s, 1H, H-2'), 5.48 (m, 1H, H-3'), 4.64 (m, 1H, H-7'), 4.22 (m, 1H, H-4'), 3.72 and 3.68 (2s, 3H, $COOMe_a$ and $COOMe_b$), 2.14 (s, 3H, COMe), 2.07 (s, 3H, COMe), 2,00 and 1,98 (2s, 3H, $NCOMe_a$ and $NCOMe_b$), 1.92 (m, 4H, H-5,' H-6'), 1.47 (s, 9H, Me-Boc), 1.30 (s, 9H, Me-Boc); ^{13}C NMR ($CDCl_3$) 171.1, 171.9, 170.6, 170.5, 169.8, 160.0, 153.0, 152.0, 151.5, 144.2, 136.0, 133.1, 129.3, 129.0, 124.0, 87.2, 82.4, 73.6, 73.3, 52.1 and 51.9, 29.5, 29.0, 28.3, 27.9, 23.6, 21.0, 20.8; MS (FAB, glycerol) *m/z* 701 $(M+Na)^+$-104; Calcd for $C_{37}H_{46}N_6O_{13}$,1/2 H_2O: C, 56.13; H, 5.98; N, 10.61. Found: C, 56.05; H, 5.96; N, 10.48.

Deprotection. A solution of 30 mg of nucleoside derivative **14** in 10 ml of methanol/ammonia 32% (4/1) was maintained at 60°C during 24 h. Then the solvent was evaporated and the residue taken up in a solution of 5% aqueous trifluoroacetic acid (1 ml). Final purification was accomplished by reverse phase HPLC[7] to

give 11 mg of nucleoside **3** (85%). ^{1}H NMR (CD_3OD) 8.40 (s,1H, H-2), 8.39 (s, 1H, H-8), 6.02 and 6.00 (2s, 1H, H-1'$_a$ and H-1'$_b$), 4.73 (m, 1H, H-2'), 4.21 (m, 1H, H-3'), 4.03 (m, 1H, H-4'), 3.91 (m, 1H, H-7'), 2.1-1.8 (m, 4H, H-5', H-6'); MS (FAB, glycerol) *m/z* 338 $(M+H)^+$.

Methyl 5,6-dehydro-5,6-dideoxy-6-methoxycarbonyl-2,3-O-isopropylidene-β-D-ribo-hexafuranoside (**16**). To a solution of methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (**15**) (2 g, 9.8 mmol) in DMSO (50 ml) containing dicyclohexyl carbodiimide (6.13 g, 30 mmol) was added 0.41 ml of dichloroacetic acid.[23] The reaction mixture was stirred for 3 h; at that time pyridine (0.8 ml) and methyl (triphenylphosphoranylidene)acetate (4 g) were added. After overnight stirring the solution was cooled in an ice bath and 200 ml of ethyl acetate containing acetic acid (2.3 ml) was added. The organic phase was washed with an aqueous sodium bicarbonate solution and water, then dried and evaporated. The oil was chromatographed to give 2.51 g of the unsaturated ester **16** (yield 98%). ^{1}H NMR ($CDCl_3$) 6.92 (dd, 1H, H-5), 6.05 (dd, 1H, H-6), 5.05 (s, 1H, H-1), 4.80 (d, 1H, H-4), 4.65 (q, 2H, H-2, H-3), 3.75 (s, 3H, COOMe), 3.40 (s, 3H, OMe), 1.50 (1s, 3H, Me), 1.30 (1s, 3H, Me); ^{13}C NMR ($CDCl_3$) 165.8, 146.6, 122.4, 112.9, 109.9, 86.2, 85.5, 84.2, 65.6, 55.1, 26.6, 25.2; MS (CI) *m/z* 259 $(M+H)^+$, 227 $(M+H)^+$-32; Calcd for $C_{12}H_{18}O_6$: C, 55.80; H, 7.03. Found: C, 55.76; H, 7.12.

Methyl 5,6-dideoxy-2,3-O-isopropylidene-β-D-ribo-heptafuranoside (**17**). To a stirred solution of unsaturated ester **16** (2.5 g, 9.6 mmol) in THF (40 ml) were added at room temperarure LiCl (0.82 g), $NaBH_4$ (0.73 g) and ethanol (60 ml). Then, after 30 h, a 10% citric acid aqueous solution was added at 0°C (pH 4). Usual work up and silica gel chromatography (ethyl acetate/heptane (4/6) eluant) provided alcohol **17** (1.01 g) in 45% yield. ^{1}H NMR ($CDCl_3$) 4.96 (s, 1H, H-1), 4.60 (d, 1H, H-2), 4.53 (d, 1H, H-3), 4.16 (m, 1H, H-4), 3.66 (t, 2H, H-7), 3.33 (s, 3H, OMe), 2.43 (s, 1H, OH), 1.66 (m, 4H, H-5, H-6), 1.50 (s, 3H, Me), 1.33 (s, 3H, Me); ^{13}C NMR ($CDCl_3$) 112.2, 109.5, 87.0, 85.4, 84.1, 61.9, 54.8, 31.4, 29.4, 26.4, 24.9; MS (IC) *m/z* 233 $(M+H)^+$, 215 $(M+H)^+$-18, 201 $(M+H)^+$-32; Calcd for $C_{11}H_{20}O_5$: C, 56.88; H, 8.68. Found: C, 56.72; H, 8.76.

Methyl 7-iodo-2,3-O-isopropylidene-5,6,7-trideoxy-β-D-ribo-heptafuranoside (**18**). To a solution of alcohol **17** (0.8 g, 3.4 mmol) in DMF was added methyltriphenoxyphosphonium iodide (2.4 g, 5.3 mmol). After 3 h the solution was evaporated and the residue taken up in ethyl acetate. The precipitate was discarded and the filtrate washed with a solution of sodium thiosulfate. The organic phase was evaporated to give after silica gel chromatography purification the iodo derivative **18** (994 mg) in 85% yield. ^{1}H NMR ($CDCl_3$) 4.95 (s, 1H, H-1), 4.60 (d, 1H, H-2), 4.56 (d, 1H, H-3), 4.17 (dd, 1H, H-4), 3.36 (s, 3H, OMe), 3.23 (t, 2H, H-7), 1.99 (m, 2H, H-6); 1.68 (m, 2H, H-5); 1.49 (s, 3H, Me); 1.32 (s, 3H, Me); ^{13}C NMR ($CDCl_3$) 112.5, 109.8, 86.2, 85.6, 84.3, 55.3, 36.1, 30.4, 26.6, 25.2, 6.1; MS (EI) *m/z* 327 $(M^{\cdot+}-15)$, 311 $(M^{\cdot+}-31)$, 267 $(M^{\cdot+}-75)$, 214 $(M^{\cdot+}-128)$.

Methyl 9-acetamido-2,3-O-isopropylidene-9-methoxycarbonyl-5,6,7,8,9-pentadeoxy-β-D-ribo-nonafuranoside (**19**). Application to compound **18** (1 g, 2.9 mmol) of the reaction conditions, which were used for the preparation of derivative **9**, led to the protected amino acid **19** (652 mg) after silica gel chromatography (elution with ethyl acetate/heptane gradient, 20 to 80%) in 62% yield. ^{1}H NMR ($CDCl_3$) 6.75 (d, 1H, NH), 4.92 (s, 1H, H-1), 4.70 - 4.40 (m, 3H, H-2, H-3, H-9), 4.13 (m, 1H, H-4), 3.77 (s, 3H, COOMe), 3.33 (s, 3H, OMe), 2.30 (s, 3H, COMe), 2.00-1.30 (m, 8H, H-5, H-6, H-7, H-8), 1.46 (s, 3H, Me), 1.30 (s, 3H, Me); ^{13}C NMR ($CDCl_3$) 173.2, 169.7, 112.3, 109.6, 87.0, 85.6, 84.2, 54.9, 52.3, 52.1, 34.8, 32.6, 26.6, 25.9, 25.1, 23.2; MS (EI) *m/z* 358 $(M^{\cdot+}-1)$, 344 $(M^{\cdot+}-15)$, 328 $(M^{\cdot+}-31)$; Calcd for $C_{17}H_{29}NO_7$: C, 56.81; H, 8.13; N, 3.90. Found: C, 57.04; H, 7.96; N, 4.05.

Methyl 9-acetamido-2,3-di-O-acetyl-5,6,7,8,9-pentadeoxy-9-methoxycarbonyl-β-D-ribo-nonafuranoside (**20**). Application to **19** of the reaction conditions, which served for the preparation of diacetate **10**, provided compound **20** in 85% yield. ^{1}H NMR ($CDCl_3$) 6.20 (d, 1H, NH), 5.19 (m, 2H, H-2, H-3), 4.86 (s, 1H, H-1), 4.61 (m, 1H, H-9), 4.05 (m, 1H, H-4), 3.75 (s, 3H, COOMe), 3.37 (s, 3H, OMe), 2.10 (1s, 3H, COMe), 2.05 (1s, 3H, COMe), 2.03 (1s, 3H, COMe), 2.12-1,20 (m, 8H, H-5, H-6, H-7, H-8); ^{13}C NMR ($CDCl_3$) 173.1, 169.9, 169.8, 106.0, 80.6, 75.2, 74.6, 55.2, 52.3, 52.1, 34.5, 32.4, 25.3, 25.1, 23.1, 20.6; MS (CI) *m/z* 404 $(M+H)^+$, 372 $(M+H)^+$-32; HRMS (CI) Calcd for $C_{18}H_{30}NO_9$: 404.1920. Found: 404.1928.

N^9-(9'-acetamido-2',3'-di-O-acetyl-5',6',7',8',9'-pentadeoxy-9'-methoxycarbonyl-β-D-ribo-1',4'-nonafuranoside)-N^6-benzoyladenine (**21**). Adenylation of diacetate **20** (380 mg, 0.93 mmol) was accomplished as in the case of nucleoside derivatives **12** and **13**. Silica gel chromatography purification (elution with acetone/heptane gradient, 10 to 100%) gave nucleoside derivative **20** (190 mg) in 57% yield as a mixture of epimers at C-9. ^{1}H NMR ($CDCl_3$) 9.08 (s, 1H, NH), 8.79 and 8.77 (2s,1H, H-2_a and H-2_b), 8.14 and 8.12 (2s, 1H, H-8_a and H-8_b), 8.01 (d, 2H, Ar), 7.55 (m, 3H, Ar), 6.20-5.90 (m, 3H, NH, H-1', H-2'), 5.46 (m, 1H, H-3'), 4.60 (m, 1H, H-9'), 4.18 (m, 1H, H-4'), 3.72 (s, 3H, COOMe), 2.15 (s, 3H, COMe), 2.07 (s, 3H, COMe), 2.01 (s, 3H, COMe), 1.90-1.20 (m, 8H, H-5', H-6', H-7', H-8'); ^{13}C NMR ($CDCl_3$) 173.6, 170.6, 170.5, 170.3, 152.6, 150.8, 143.5, 133.2, 129.3, 128.8, 87.2, 82.8, 73.8, 73.5, 52.9, 52.3, 33.1, 31.8, 25.8, 25.3, 22.4, 20.5, 20.4; MS (CI) *m/z* 611 $(M+H)^+$, 551 $(M+H)^+$-60; Calcd for $C_{29}H_{34}N_6O_9$: C, 57.03; H, 5.61; N, 13.76. Found: C, 56.69; H, 5.46; N, 14.31.

N^9-[7'-(N-tert-butyloxycarbonyl)acetamido-2',3'-di-O-acetyl-5',6',7',8',9'-pentadeoxy-7'-methoxycarbonyl-β-D-ribo-1',4'-nonafuranoside]-N^6-benzoyladenine (**22**). As in the case of its lower homolog **13**, compound **21** was treated under reflux in acetonitrile in the presence di-*tert*-butyl dicarbonate (70 µl) and DMAP. Silica gel chromatography gave nucleoside **22** (50 mg) in 90% yield. ^{1}H NMR (CD_3CN) 8.81 and 8.79 (2s,1H, H-2_a and H-2_b), 8.38 and 8.36 (2s, 1H, H-8_a and H-8_b), 7.80 (d, 2H, Ar.), 7.62 (m, 1H, Ar.), 7.51 (m, 2H, Ar.), 6.18 (d, 1H, H-1'), 5.98 (m, 1H, H-2'), 5.48 (m, 1H, H-3'), 4.29 (m, 1H, H-9'), 4.19 (m, 1H, H-4'), 3.65 and 3.63 (2s, 3H, $COOMe_a$ and $COOMe_b$), 2.30-2.15 (m, 17H, COMe, NCOMe, H-5', H-6', H-7', H-8'), 1.31 (s, 9H, Boc); MS (FAB, glycerol) *m/z* 733 $(M+Na)^+$, 711 $(M+H)^+$.

Deprotection. Nucleoside derivative **22** (44 mg) was disolved in a solution of MeOH/ammonia 32% (4/1) (10 ml) which was maintained 24 h at 55°C. After a trifluoacetic acid (5% in H_2O) treatment (as in the case of the preparation of the lower homolog **3**) the deprotected nucleoside was purified by C_{18} reverse phase chromatography[7] to give *N^9*-[9'-amino-9'-carboxy-5',6',7',8',9'-pentadeoxy-β-D-*ribo*-1',4'-nonafuranoside]-adenine (**4**) (17 mg) as a mixture of two epimers at C-9' in 75% yield as an amorphous solid. ^{1}H NMR (CD_3OD) 8.42 and 8.38 (2s,1H, H-2a and H-2b), 8.37 and 8.33 (2s, 1H, H-8a and H-8b), 6.01 and 5.99 (2s, 1H, H-1'$_a$ and H-1'$_b$), 4.69 (t, 1H, H-2'$_a$ and H-2'$_b$), 4.33 (m, 1H, H-3'), 4.13 (t, 1H, H-9'), 4.02 (m, 1H, H-4'), 2,00-1,20 (m, 8H, H-5', H-6', H-7', H-8'); MS (FAB, glycerol) *m/z* 367 $(M+H)^+$.

Methyl 6-cyano-2,3-O-isopropylidene-5,6,7,8,9-pentadeoxy-9-trifluoroacetamido-β-D-ribo-nonafuranoside (**24**). To $FeCl_3$, $6H_2O$ (40 mg, 0.25 mmol) was added a solution of NaI (75 mg, 0.5 mmol) in pyridine (0.5 ml). The resulting suspension was stirred vigourously with a vibromixer before adding, under a nitrogen atmosphere, zinc powder (200 mg, 3 mmol). After 5 min a pyridine solution (0.5 ml) of the unsaturated nitrile **23**[6] (120 mg, 0.5 mmol) and *N*-(2-iodoethyl)trifluoroacetamide (140 mg, 1 mmol)were added. The reaction product was purified by silica gel chromatography to give methyl 6-cyano-2,3-isopropylidene-5,6,7,8,9-pentadeoxy-9-trifluoroacetamido-β-D-*ribo*-nonafuranoside **24** (162 mg) in 85% yield. IR 3327 cm^{-1} NHCO, 2242 cm^{-1} CN, 1730 cm^{-1} $COCF_3$; ^{1}H NMR ($CDCl_3$) 6.65 (s, 1H, NH), 4.96 (s, 1H, H-1), 4.56 (m, 2H, H-2, H-3), 4.38 and 4.24 (2m, 1H, H-4_a and H-4_b), 3.42 (m, 2H, H-9), 3.38 and 3.33 (2s, 3H, OMe_a and OMe_b), 2.80 (m, 1H, H-6), 1.70 (m, 6H, H-5, H-7, H-8), 1.47 (s, 3H, Me), 1.31 (s, 3H, Me); ^{13}C NMR ($CDCl_3$) 121.4 and 120.9, 122.7; 110.0, 85.2, 84.3, 83.8, 55.5, 39.0, 37.7 and 37.1, 29.6 and 28.6, 28.2, 27.9, 26.4, 25.0; MS (EI) *m/z* 365 ($M^{\cdot+}$-15), 349 ($M^{\cdot+}$-31), 305 ($M^{\cdot+}$-75); Calcd for $C_{16}H_{23}N_2O_5F_3$: C, 50.52; H, 6.09, N: 7.36. Found: C, 50.57; H, 6.02; N, 7.23.

Methyl 6-carboxamido-2,3-O-isopropylidene-5,6,7,8,9-pentadeoxy-9-trifluoroacetamido-β-D-ribo-nonafuranoside (**25**). To a solution of nitrile **24** (200 mg, 0.5 mmol) in DMSO (2 ml) was added slowly at 0°C under a nitrogen atmosphere a solution of KO_2 (130 mg, 1.8 mmol) in DMSO. The reaction mixture was stirred during 24 h. Although the reaction was not complete it was worked up by adding a (6/4) mixture of ethyl acetate and ethyl trifluoroacetate. The organic phase residue was chromatographed (using acetone/heptane (1/1) as eluant) to recover unreacted starting material **24** (62 mg) and amide **25** (116 mg) as an epimeric mixture in 80% yield (calculated on reacted starting material). ^{1}H NMR (CD_3OD) 4.88 (s, 1H, H-1), 4.59 (m, 2H, H-2, H-3),4.12 (m, 1H, H-4), 3.35 and 3.33 (2s, 3H, OMe_a and OMe_b), 3.30 (m, 2H, H-9), 2.56 and 2.42 (2m, 1H, H-6_a and H-6_b), 2.00-1.50 (m, 6H, H-5, H-7, H-8), 1.40 (s, 3H, Me), 1.28 (s, 3H, Me); ^{13}C NMR (CD_3OD) 179.9, 118.2, 113.2, 111.1 and 110.8, 86.6 and 86.5, 86.3

and 86.1, 85.5 and 85.1, 55.5, 43.9 and 43.8, 40.5, 38.8 and 38.6, 31.3, 27.5, 26.7, 25.0; MS (EI) *m/z* 383 ($M^{\cdot+}$-15), 367 ($M^{\cdot+}$-31), 338 ($M^{\cdot+}$-60); MS (CI) *m/z* 399 $(M+H)^+$, 367 $(M+H)^+$-32; HRMS (CI) Calcd for $C_{16}H_{26}F_3N_2O_6$: 399.1743. Found: 399.1755.

Methyl 6,9-di(trifluoroacetamido)-2,3-O-isopropylidene-5,6,7,8,9-pentadeoxy-β-D-ribo-nonafuranoside **(26).** To a solution of amide **25** (110 mg, 0.27 mmol) in a DMF/H_2O (1/1) mixture were added [bis(trifluoroacetoxy)iodo]benzene (185 mg, 0.54 mmol) and, after 20 min, pyridine (46 µl, 0.54 mmol). The mixture was stirred during 3 h at room temperature. The solvents were evaporated and the residue treated for 2.5 h with a 2/1 mixture of ethyl trifluoroacetate and triethylamine in CH_2Cl_2 solution (10 ml). The reaction mixture was worked up in ethyl acetate and silica gel chromatography (ethyl acetate/heptane gradient) of the crude reaction product provided the title compound **26** (105 mg) as a mixture of two epimers at C-6 in 82% yield. ^1H NMR ($CDCl_3$) 7.50 (d, 1H, NH), 7.10 (m, 1H, NH), 4,98 and 4.95 (2s, 1H, H-1_a et H-1_b), 4.61 (d, 1H, H-2), 4.52 (d, 1H, H-3), 4,40-4,00 (m, 2H, H-4, H-6), 3.39 (s, 3H, OMe), 3.34 (m, 2H, H-9), 1,90-1,40 (m, 6H, H-5, H-7, H-8), 1.47 (s, 3H, Me), 1.31 (s, 3H, Me); ^{13}C NMR ($CDCl_3$) 157.7 and 157.4, 118.3 and 113.7, 112.9, 110.6 and 110.4, 85.0 and 84.9, 84.8 and 84.5, 84.4 and 83.7, 55.5, 48.8 and 47.9, 39.6, 39.1 and 37.8, 31.2 and 31.0, 26.5, 25.4, 25.0; MS (EI) *m/z* 451 ($M^{\cdot+}$-15), 435 ($M^{\cdot+}$-31); MS (CI) *m/z* 467 $(M+H)^+$, 435 $(M+H)^+$-32; Calcd for $C_{17}H_{24}F_6N_2O_6$: C,43.78; H, 5.19; N, 6.01. Found: C, 43.59; H, 5.32; N, 6.23.

Methyl 2,3-di-O-acetyl-6,9-di(trifluoroacetamido)-5,6,7,8,9-pentadeoxy-D-ribo-nonafuranoside **(27).** This compound was prepared by using the conditions which were applied for the preparation of derivative **10.** Thus, starting from **26** (100 mg, 0.21 mmol) the desired compound **27** (93 mg) was obtained as a mixture of two epimers at C-9' in 86% yield as an oil. ^1H NMR (CD_3CN) 7.65 (m, H, NH), 7.35 (m, H, NH), 5.15-4.95 (m, 2H, H-2, H-3), 4.97 (s, 1H, H-1), 4.07 (m, 2H, H-4, H-6), 3.34 and 3.33 (2s, 3H, OMe_a and OMe_b), 3.30 (m, 2H, H-9), 2.04 (2s, 3H, OMe), 2.00 and 1.98 (2s, 3H, $COMe_a$ and $COMe_b$), 2.1-1.8 (m, 2H, H-5), 1.55(m, 4H, H-7, H-8); ^{13}C NMR (CD_3CN) 170.7 and 170.5, 107.0 and 106.9, 79.0 and 78.4, 75.6 and 75.4, 75.3 and 75.0, 55.4 and 55.3, 48.8 and 48.5, 40.2, 39.8, 32.2 and 31.9, 25.6 and 25.5, 20.5; MS (CI) *m/z* 479 $(M+H)^+$-32, 419 $(M+H)^+$-32 -60.

N^9-[2',3'-di-O-acetyl-6',9'-di(trifluoroacetamido)-5',6',7',8',9'-pentadeoxy-β-D-ribo-nonafuranosyl]-N^6-benzoyladenine **(28).** This compound was prepared under the conditions which were used in the case of nucleoside drivative **12.** Thus, diacetate **27** (110 mg, 0.21 mmol) was combined with *N^6*-benzoyl-*N^6,N^9*-bis-(trimethylsilyl)adenine (130 mg, 0.21 mmol) in dichloroethane (8 ml) in the presence of trimethylsilyl trifluoromethanesulfonate (65 µl, 0.33 mmol). The reaction product was purified by silica gel chromatography (acetone/heptane gradient 10 to 100%) to give the title product **28** (80 mg) as an epimeric mixture at C-6' in 53% yield. ^1H NMR (CD_3CN) 9.56 (s, 1H, NH), 8.67 (s, 1H, H-2), 8.29 (s, 1H, H-8), 8.00 (d, 2H, Ar), 7.63 (m, 2H, NH), 7.52 (m, 3H, Ar), 6.16 (d, 1H, H-1'), 6.07 and 5.98 (2t, 1H, H-$2'_a$ and H-$2'_b$), 5.48 (m, 1H, H-3'), 4.25 (m, 1H, H-4'), 4.04 (m, 1H, H-6'), 3.22 (m, 2H, H-9'), 2.10 and 2.09 (2s, 3H, $COMe_a$ and $COMe_b$), 2.00 and 1.94 (2s, 3H, $COMe_a$ and $COMe_b$), 1.94 (m, 2H, H-5'), 1.53 (m, 4H, H-7', H-8'); ^{13}C NMR (CD_3CN) 181.2, 170.5, 170.3, 166.3, 164.2, 152.7, 152.6, 150.8, 143.8 and 143.6, 134.5, 133.3, 129.4, 128.9, 125.4, 87.7, 81.0 and 80.2, 74.2 and 74.0, 73.3 and 73.1, 48.6 and 48.0, 39.7, 38.0 and 37.7, 32.2 and 31.7, 25.5 and 25.4, 20.5 and 20.3; MS (CI) *m/z* 718 $(M+H)^+$, 614 $(M+H)^+$-106; Calcd for $C_{29}H_{29}F_6N_7O_8$: C,48.54; H, 4.07; N, 13.66. Found: C, 48.74; H, 3.98; N, 13.39.

Deprotection. The above nucleoside derivative **28** (33 mg) was disolved in a solution of MeOH/ammonia 32% (4/1) (10 ml) which was maintained 24 h at 55°C. The deprotected nucleoside was purified by C_{18} reverse phase chromatography[7] to give *N^9*-(6',9'-diamino-5',6',7',8',9'-pentadeoxy-β-D-*ribo*-nonafuranosyl)-adenine **5** (12 mg) as a mixture of two C-6' epimers in 77% yield. ^1H NMR (CD_3OD) 8.43 (s, 1H, H-2), 8.42 (s, 1H, H-8), 6.22 (d, 1H, H-1'), 4.97 and 4.83 (2t, 1H, H-$2'_a$ and H-$2'_b$), 4.47 (m, 2H, H-3', H-4'), 3.64 (m, 1H, H-6'), 3.20 (m, 2H, H-9'), 2.5-2.1 (m, 2H, H-5'), 1.94 (m, 4H, H-7', H-8'); ^{13}C NMR (CD_3OD) 157.4, 141.7 and 141.5, 91.1, 83.0 and 81.7, 75.2, 74.6, 51.1 and 49.7, 40.3, 38.1 and 37.3, 31.9, 24.9; MS (FAB, glycerol) *m/z* 360 $(M+Na)^+$, 338 $(M+H)^+$.

Biological Methods.

Parasites. For this study a strain of *Leishmania donovani* particularly sensitive to SF (**1**)was used. The promastigotes of this strain (MHOM/1N/80/DD8) originating from the culture collection of the WHO reference center in London School of Hygiene and Tropical Medicine were kindly provided by Dr. D. Evans.

Growth culture. Cells were grown in a semi-defined RPMI-1640 medium (pH 7.4) containing 2 mM glutamine, 25 mM Hepes [N-2(hydroxyethyl)piperazine-N'-2-ethanesulfonic acid)] 10% heat-inactivated fetal calf serum, streptomycin 5 µg/ml and penicillin 5 U/ml. Medium components were from Gibco (France) and the serum from Flobio (France).

Effect of the compounds on parasite-viability. Parasites were seeded at 1 x 10^6 cells/ml in 24-well microplates (Nunclon) with 0.5 ml medium per well and incubated at 26°C. Compounds to be tested were added 3-4 hours later at various concentrations in 25 µl H_2O. Each test was performed in duplicate and untreated cultures were run in parallel. After 3 days of incubation the viability of the cells was estimated by a colorimetric method using 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl-tetrazolium bromide (MTT). The dye reduction mainly reflects mitochondrial dehydrogenase activity.[24] The percentage of inhibition was calculated with respect to untreated control cultures run in parallel. The IC50 value represents the concentration provoking 50% of growth inhibition.

Protein methylase activity. Protein methylase II (EC 2.1.1.77) activity was determined at 37°C by measuring the incorporation of radiolabelled methyl groups into α-globulin after separation from unreacted AdoMet by precipitation of the methylated protein with trichloroacetic acid as described by Kim and Paik.[25] The apparent kinetic constants were calculated from Lineweaver-Buck plots.[26] Protein concentration was measured by the dye adsorption method of Bradford[27] using bovine serum albumin as the standard.

AdoMet uptake. Parasites preincubated with various concentrations of labelled AdoMet (S-Adenosyl-L-[methyl]-^{3}H]methionine (2.5 µLi/ml, 550 mLi/mmol) purchased from Amersham, France, in the presence or absence of the compound to be tested were incubated for 30 min at 20°C. Then the cultures were layered, in Eppendorf tubes, on top of a 9:1 mixture of dibutyl phthalate (d = 1.045) and liquid parafin (d = 0.88) and centrifuged for 1 min in an Eppendorf microfuge.[20] The supernatant was removed by careful aspiration, and the pellet resuspended in 1 M NaOH, hydrolysed for 30 min at 80°C, mixed with liquid scintillation fluid and the radioactivity counted (total uptake). The apparent affinities of the transport elements were calculated using the Lineweaver-Burk plot.[26]

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