Synthesis and Biological Activity of Sinefungin Analogues

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A series of nucleosides (2–4) that derive from adenosine by chain extension at the 5′-end have been synthesized starting from the known phosphonate 7. The latter was first combined with 4-pentenal to give 8, which underwent chemical manipulations to provide triacetate 11, which was found suitable for the adenylation step. Further transformations, among them the Hofmann degradation of the amide group of compound 13, and final deprotection gave nucleosides 2–4. They were considered as analogues of sinefungin (1) and tested for their antileishmanial activity together with compounds 5 and 6, which were obtained independently. All the modifications with respect to sinefungin resulted in nearly complete loss of growth inhibitory activity. These results indicate that the 9′ terminal amino and carboxyl groups are necessary for the activity and that the presence of the amino group at C-6′ is not sufficient to maintain the antileishmanial effect. Some of the analogues however could antagonize or reverse the inhibitory activity of sinefungin (1).

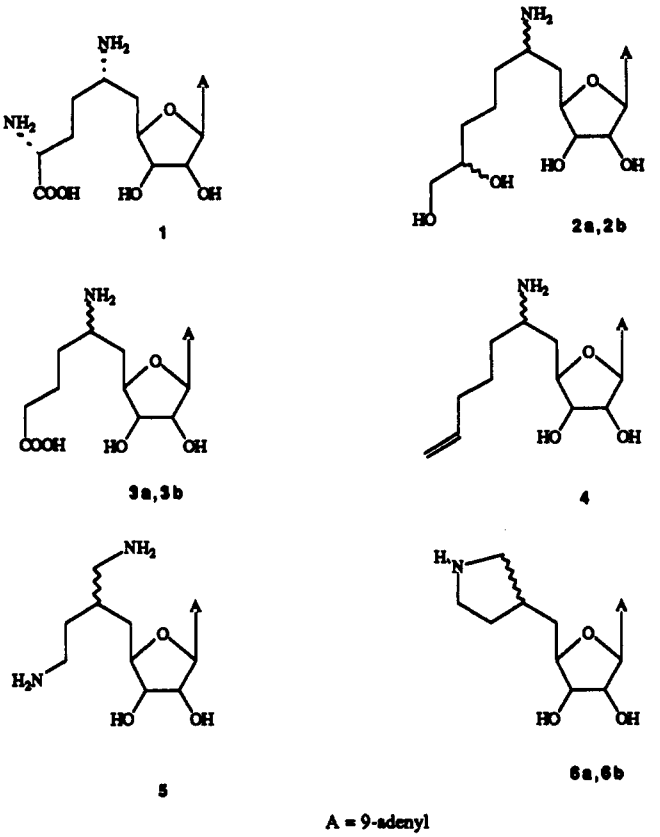
Sinefungin (1) is a nucleoside antibiotic isolated from the cultures of *Streptomyces griseolus* at Lilly Research Laboratories in the U.S.[1] and from cultures of *S. incarnatus* at Rhône Poulenc Industries in France.[2] The molecule is composed of an adenosine and an ornithine moiety linked together by a carbon–carbon bond at the 5′-end of the adenosine part.

After its discovery 1 was shown to be a potent inhibitor of the development of *Candida albicans* in vitro and in vivo.[3] Later the inhibitory effects of this antibiotic were demonstrated against various types of viruses such as Rous sarcoma, polyoma, vaccinia, and Epstein-Barr viruses in vitro.[4-6] These effects were observed at sinefungin concentrations ranging from 10 to 250 μM.

However, much lower sinefungin concentrations are needed to be effective against various parasites. Different species of *Trypanosoma*, *Leishmania*, and *Plasmodium falciparum* are inhibitied with 10 nM to 7 μM sinefungin in vitro.[7-9] In vivo low doses such as 0.05–5 mg/kg given i.p. 3–9 times to mice infected with *Trypanosoma b. brucei*, *T. congolense*, and *T. vivax* cured the animals,[10] furthermore one was found 73 times more active than sodium stibogluconate in *L. donovani* infected mice.[11] The compound is efficient also against cuteanous leishmaniasis in mice and hamsters without any toxic side effects.

Unfortunately however, 1 provokes severe nephrotoxicity in larger animals[12] that precludes its clinical use. These unfavorable toxic side effects prompted us to undertake a program to synthesize related molecules (Scheme I).

Scheme I



In the past few years a number of synthetic strategies have been elaborated to prepare 1[13] and various chain extended nucleosides at the 5′-end.[14] It is remarkable that the stereoselective methodology that was used in the most recent work served to establish the *S* configuration at C-6 for 1.[13a] In this paper we describe the synthesis and effects of some side-chain analogues of 1.

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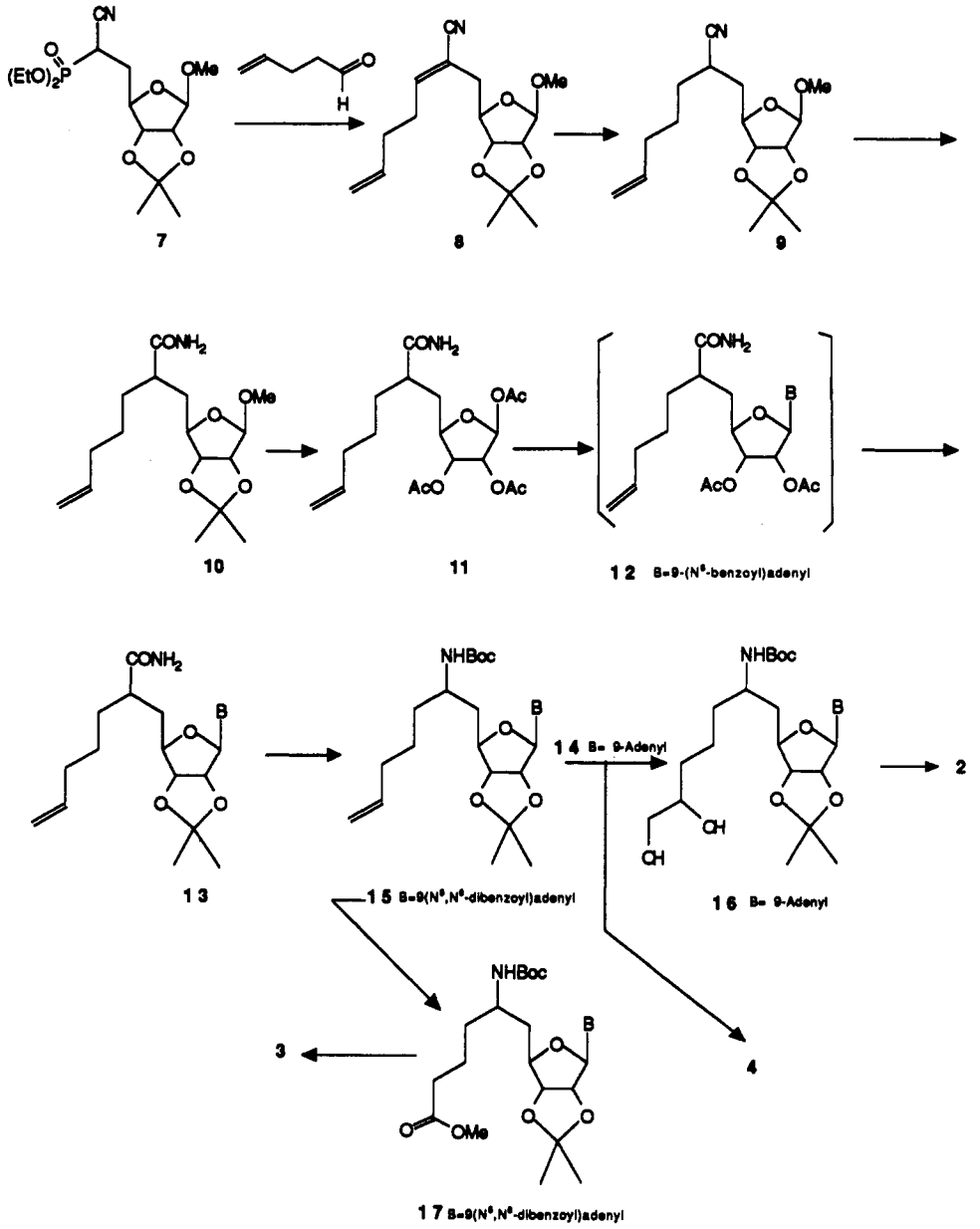
Scheme II



Chemistry

The synthesis of the various sinefungin analogues 2–4 is outlined in Scheme II. The general strategy involved first the construction of the chain-extended ribose derivative 11. Then this compound 11 was adenylated, providing the protected nucleoside 13. In the last stages of the synthesis the appropriate functionalization manipulations to give the desired derivatives have been carried out.

The known ribose derived phosphonate 7[15] can be combined under various reaction conditions with 4-pentenal[16] to give a mixture of unsaturated nitriles 8. Our preference was to use methanol-containing magnesium methanolate in the Wittig–Horner reaction since without isolation of compound 8 the C-6, C-7 double bond of the latter could be reduced by simple addition of an excess of magnesium[17] to the reaction medium. This one-pot two-step sequence gave compound 9 in 90% overall yield as a mixture of epimers at C-6 in a ratio of approximately 1:1. To prepare the amide 10 starting from nitrile 9 the best results were obtained by refluxing 9 in *tert*-butyl alcohol containing 10 equiv of potassium hydroxide.[18] Interestingly, the two C-6′ epimers of amide 10 could be fully separated by column chromatography. However, for the sake of simplification, it was decided to carry out our program with compounds epimeric at C-6 with the expectation that the epimers could be separated at a later stage of the synthetic sequence.

Acidic hydrolysis of derivative 10 followed by acetylation of the resulting product gave the triacetate 11. Adenylation of this compound was best achieved by using the standard procedure.[19] Thus, when a 1,2-dichloroethane solution of N^6-benzoyl-N^6,N^9-bis(trimethylsilyl)adenine and 11 was refluxed overnight in the presence of trimethylsilyl triflate, the crude diacetylated derivative 12

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was obtained. This nucleoside 12 was not isolated since in order to have a compound appropriately protected to undergo the subsequent chemical transformations it was transformed into the 2',3'-O-isopropylidene derivative 13. We were also pleased to find that the two C-6 epimers of 13 were conveniently separable by column chromatography to give 13a and 13b. Also each C-6' epimers 13a and 13b was submitted to Hofmann degradation by using (bis(trifluoroacetoxy)iodo)benzene[20] in aqueous N,N-dimethylformamide to give the corresponding 6'-amino derivatives, which were isolated as their $N^{6'}$-tert-butyloxycarbonyl derivatives 14a and 14b in 83% yield.

These compounds 14 served as precursors of the various extended adenosine derivatives 2–4. Thus, the diol 2a (2b) was obtained by catalytic osmium tetraoxide/N-methylmorpholine oxide cis-hydroxylation[21] of the terminal double bond of 14a (14b) to give 16a (16b). Deprotection of the diol 16a (16b) was accomplished by acidic treatment to give nucleoside 2a (2b) as a pair of epimers at C-10', which could not be separated.

In another experiment the nucleoside derivatives 14 were N^6,N^6-dibenzoylated to give 15 and degraded to the nor-acids by mean of the Sharpless oxidation procedure.[22] The resulting acids were isolated and characterized as their methyl esters 17 after diazomethane treatment. Complete deprotection was achieved by saponification and acid hydrolysis to produce 3a and 3b.

Finally, another pair of adenosine derivatives 4 was obtained by simple acidic removal of the protecting groups of compound 14 (as a mixture of epimers).

Results and Discussion

Besides the three pairs of sinefungin analogues 2–4 that were synthesized as indicated above, we have also considered the related nucleosides 5–6 (Scheme I), which we have obtained by a another route.[23]

Accordingly, the eight compounds that have been examined for their eventual growth-inhibiting activity of parasites were the 9',10'-diol 2 (two C-6' epimers 2a and 2b) in which the terminal NH_2 and COOH groups at C-9' of 1 were substituted by OH and $CH(OH)CH_2OH$, respectively; the 9'-deaminosinefungin 3 (two epimers at C-6' 3a and 3b); the nucleoside 4 (considered as a mixture of C-6' epimers) where the therminal functions have been eliminated to be replaced by a 9',10'-double bond; the nucleoside 5 (mixture of C-6' epimers), which is deprived of carboxyl group and where the adenosyl moiety is attached at the C-2 position of putrescine and the pyrrolidine derivative 6 (two epimers 6a and 6b).

The effects of these molecules were tested on the growth of *Leishmania donovani* promastigotes in vitro. All the modifications resulted in nearly complete loss of growth inhibitory activity. (The IC50 of 1 is 0.025 μM; the analogues showed no effect even in 1000-fold higher concentration when tested individually; results not shown.) This suggests that the 9' terminal amino and carboxyl groups are necessary for the activity. Interestingly, 6a and 6b are devoid of activity, whereas the lactam derivatized sinefungin is a good growth inhibitor.[24]

Table I. Thymidine Uptake and Incorporation in *Leishmania donovani* Promastigotes Cultured in the Presence of the Analogues Alone or in Addition to Sinefungin[a]

compound	concn μM	sinefungin (μM)	cpm/mg protein TCA soluble	cpm/mg protein TCA insoluble
control	–	–	815 255 (100)	3 207 567 (100)
1	–	0.026	1 583 862 (194)	2 197 110 (68)
1	–	0.260	1 741 329 (213)	956 745 (30)
3a	1.25	0	1 499 100 (184)	1 772 064 (55)
3b	2.1	0	1 161 185 (142)	3 284 127 (102)
2a	11.6	0	823 954 (101)	3 148 603 (98)
2b	23.3	0	1 020 493 (125)	4 079 585 (127)
4	130	0	6 649 304 (815)	3 222 584 (100)
3a	1.25	0.026	1 556 483 (191)	1 459 000 (45)
3b	2.10	0.026	1 973 260 (242)	1 861 835 (58)
2a	11.60	0.026	919 098 (113)	3 391 425 (106)
2b	23.30	0.026	1 230 950 (151)	3 097 327 (96)
4	130.00	0.026	1 703 083 (209)	2 880 449 (90)

[a] Cells were cultured with the various compounds alone or in the presence of 0.026 μM sinefungin. After 4 h treatment, the cells were labelled with 2.5 μCi/mL of thymidine, recovered by centrifugation, and washed. Thymidine uptake and incorporation were measured respectively in the TCA-soluble and TCA-insoluble material (28). The values in parentheses represent the percentage of radioactivity with respect to the control.

Table II. Apparent Kinetic Values of Sinefungin Analogues for Protein Carboxyl Methyltransferase in Crude Extracts of *Leishmania donovani* Promastigotes

compound	K_m (μM)	K_i (μM)	K_i/K_m
AdoMet	55 (48)	–	–
AdoHcy		7.2	0.13
1	–	49	0.9
2	–	1697	30.9
2b	–	446	8.3
3a	–	165	3.0
3b	–	380	6.9
4	–	877	15.9
5	–	1119	23.3
6a	–	103	2.1
6b	–	140	2.9

One of the primary effect of 1 in *Leishmania* at the molecular level is the inhibition of thymidine incorporation into DNA.[25] As shown in Table I among the analogues tested only 3a inhibited thymidine incorporation after 4 h of treatment.

The lack of inhibitory effect of these analogues could be due to their poor penetration into the cells. As 1 is a very efficient transmethylase inhibitor,[4,24] the effect of some analogues was tested on protein carboxylmethyltransferases of leishmanial promastigotes. As shown in Table II all compounds tested behaved as weak competitive inhibitors with respect to AdoMet except the two cyclic derivatives 6a and 6b, and 3a, which correspond to the C-9' deamino sinefungin with the natural configuration at C-6' (this is tentatively suggested after pattern comparison of the ^1H NMR spectra of several derivatives of 1 and 3a when protected in the same manner). The K_i/K_m ratio of these molecules was 2, 2.9, and 3, respectively, whereas with 1 this ratio is 0.9. We have previously shown that AdoMet, the methyl group donor substrate in enzymatic transmethylations, antagonizes the effect of sine-

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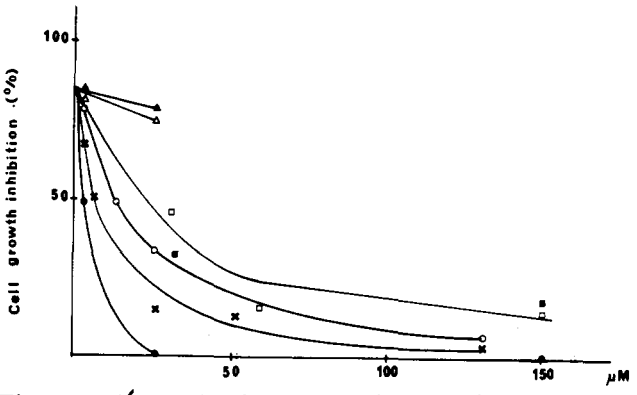

Figure 1. Antagonism between sinefungin and some of its derivatives on cell growth inhibition. (▲) **3a**; (△) **3b**; (●) **2a**; (○) **2b**; (×) **4**; (■) **6a**; (□) **6b**. Cells were cultured with 0.26 μM sinefungin alone or in addition to the various compounds for 4 days. Results are expressed as cell growth inhibition (%) in comparison to untreated cells.

Table III. Antagonism between Sinefungin and Some Analogues on Cell Growth of *L. donovani* Promastigotes[a]

hours of 0.26 μM sinefungin treatment before analogue addition	sinefungin (0.26 μM)	**2a** (260 μM)	**6a** (156 μM)	**5** (148 μM)	**4** (130 μM)
0	15	100	83	93	97
16		92			
24		59	74	80	65
48		41	37		41

[a] Results are expressed as percent cell growth after 4 days treatment.

fungin (1) when added together to the cultures.[26] Furthermore, AdoMet can reverse the inhibitory effect of 1 when added after it .

Sinefungin was shown to be nephrotoxic in goats;[12] thus, molecules that can modulate its activity are of potential interest. An eventual antagonistic effect of the analogues was tested in the presence of 0.26 μM 1, which alone provokes 85% growth inhibition in 3 days. Figure 1 shows that the strongest antagonists are **2a**, **2b**, **4**, **6a**, and **6b**. The analogues **3a** and **3b** had neither synergistic nor antagonist effect. Compounds **2a** and **4** antagonized also the inhibitory effect of 1 on thymidine incorporation (Table I); again **3a** and **3b** had no effect.

Sinefungin-induced growth inhibition could be antagonized by the analogues **2a**, **6a**, **4**, and **5** when added in 500–1000-fold excess with respect to the antibiotic (Table III). This effect decreased with the time lapse between sinefungin and analogue addition, and it was 40% after 48 h.

In conclusion, it seems that for the growth inhibitory activity the terminal NH_2 and COOH groups are necessary. When these functions are eliminated or replaced by some other groups the inhibitory effect of 1 disappears. These compounds, however, can antagonize the inhibitory activity of 1 when used in great excess. Further synthetic efforts are needed to clarify these preliminary observations.

Experimental Section

^{1}H NMR spectra were recorded on Brucker "WP 200" SY or "WP 400" spectrometers with Me_4Si as the internal standard. Chemical shifts are reported in ppm. ^{13}C NMR spectra were recorded on the same instruments operating at 50.31 and 100.62 MHz, respectively. Electron-impact (EI) and high-resolution (HR) mass spectra were measured on a AEI MS 50 mass spectrometer. For fast atom bombardment (FAB) mass spectra the instrument was a Kratos MS 80 using glycerol (or thioglycerol) as matrices. Microanalysis were performed by the Service de Microanalyses du C.N.R.S. Column chromatography was carried out on silica gel Kieselgel 60 type 7734 or 9385. Silica gel TLC was performed on Schleicher & Schuell plates with UV light for visualization.

Methyl 6-Cyano-5,6,7,8,9,10,11-heptadeoxy-2,3-*O*-isopropylidene-β-D-*ribo*-6,10-dieneundecafuranoside (8). A magnesium methanolate solution was prepared by adding 7.93 g (33 mmol) of magnesium to 50 mL of absolute methanol. To the resulting solution, which was maintained at 18 °C under a N_2 atmosphere, was added 8 g (29 mmol) of diethyl cyanomethylphosphonate 7[15] over 30 min. After another 30 min 3.7 g of 4-pentenal[16] was added to the reaction mixture, which was kept under stirring for 3 h. At that time the reaction mixture was neutralized by addition of a 10% methanolic solution of oxalic acid. The solution was filtered and evaporated, and the residue was taken up in CH_2Cl_2. The organic phase gave, after washing with water, drying, and evaporation, a brown oil that was chromatographed over a silica gel column to give 5.07 g (yield 78%) of compound 8 as an oil: R_f (ethyl acetate/hexane, 1/9) 0.65; IR ($CHCl_3$) νmax 2215 cm^{-1};; ^{1}H NMR ($CDCl_3$) (mixture of 2 geometric isomers in 85/15 ratio) 6.50 and 6.26 (2 t, 1 H total, H-7), 5.82 (m, 1 H, H-10), 5.08 (m, 2 H, H-11), 5.02 and 5.00 (2 s, 1 H total, H-1), 4.65 (d, 1 H, H-2), 4.56 (d, 1 H, H-3), 4.40 (t, 1 H, H-4), 3.39 and 3.36 (2 s, 3 H total, OMe), 2.50 (m, 4 H, H-5 and H-8), 2.20 (t, 2 H, H-9), 1.48 (s, 3 H, C-Me), 1.32 (s, 3 H, C-Me); ^{13}C NMR ($CDCl_3$) 149.37 (C-7), 136.13 (C-10), 117.13 (CN), 116.09 (C-11), 112.76 ($C(Me)_2$), 111.91 (C-6), 110.08 (C-1), 85.51 (C-4), 85.14 (C-3), 83.57 (C-2), 55.33 (OMe), 39.52 (C-9), 30.88 (C-8), 26.58 and 25.19 (2 Me).

Methyl 6-Cyano-5,6,7,8,9,10,11-heptadeoxy-2,3-*O*-isopropylidene-β-D-*ribo*-10-eneundecafuranoside (9). (a) To a stirred and ice-cooled solution of 5 g (17 mmol) of 8 in 250 mL of absolute methanol was added 12.3 g (51 mmol) of magnesium turnings. After the complete dissolution of the metal the solution was neutralized by adding a 10% methanolic solution of oxalic acid. The resulting reaction product was isolated as above to give 4.7 g (yield 93%) of compound 9.

(b) Compound 9 can also be obtained in a one-pot reaction starting from 7. Thus, after completion of the Wittig–Horner reaction (see above), 200 mL of methanol and 15.8 g (660 mmol) of magnesium turnings were added, succesively. The final reaction product was isolated as in (a) and purified over a silica gel column (eluent: hexane/ethyl acetate/CH_2Cl_2, 90/3/7) to give 4.88 g (yield 75%) of compound 9 (2 epimers at C-6 in 6/4 ratio) as an oil: IR ($CHCl_3$) νmax 2240 cm^{-1}; ^{1}H NMR ($CDCl_3$) 5.81 (m, 1 H, H-10), 5.06 (m, 2 H, H-11), 5 (s, 1 H, H-1), 4.59 (m, 2 H, H-2 and H-3), 4.41 and 4.26 (t and dd, 1 H total, H-4), 3.38 and 3.33 (2 s, 3 H total, OMe), 2.81 (m, 1 H, H-6), 2.15 (m, 2 H, H-9), 2.00 and 1.8 (m, 2 H, H-5), 1.64 (m, 4 H, H-7 and H-8), 1.51 and 1.33 (2 s, 6 H, C-Me); ^{13}C NMR ($CDCl_3$) 137.68 (C-10), 121.47 (CN), 115.53 (C-11), 112.86 ($C(Me)_2$), 110.34 and 110.13 (C-1), 85.54 and 85.48 (C-4), 84.63 and 84.63 and 84.24 (C-3), 84.24 and 84.15 (C-2), 55.60 (OMe), 38.09 (C-9), 33.17 and 33.11 (C-5), 32.08 (C-6), 28.87 (C-7), 26.63 (Me), 25.23 (Me), 26.29 (C-8); MS (EI) *m/z* 280 (M − 15, 24). Anal. ($C_{16}H_{25}NO_4$) C, H, N.

Methyl 6-Carbamoyl-5,6,7,8,9,10,11-heptadeoxy-2,3-*O*-isopropylidene-β-D-*ribo*-10-eneundecafuranoside (10). A solution of 4 g (13 mmol) of nitrile 9 in *tert*-butyl alcohol containing 7.6 g (130 mmol) KOH was refluxed overnight under a nitrogen atmosphere. The mixture was diluted with CH_2Cl_2 and neutralized by saturation with CO_2. Filtration over Celite gave a solution, which was evaporated to give a residue that was soluble in CH_2Cl_2. The organic phase was washed with water, dried, and evaporated. The residue was chromatographed over a silica gel column (CH_2Cl_2/ethyl acetate gradient, 10–30% ethyl acetate) to give 3.37 g (yield 79%) of amide 10 (mixture of 2 epimers at C-6). Complete separation of these two epimers could be accomplished by medium-pressure chromatography using hexane/*tert*-amyl alcohol (gradient 10–40% *tert*-amyl alcohol) to give 2.1 g of the less polar amide **10a** and 1.05 g of the more polar amide **10b**.

10a (less polar amide): $[\alpha]_D = -12.8°$ ($c = 1$, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) 5.92 and 5.82 (2 bs, 2 H, $CONH_2$), 5.74 (m, 1 H, H-10), 4.97 (m, 2 H, H-11), 4.94 (s, 1 H, H-1), 4.54 (d, 1 H, H-2) 4.44 (d, 1 H, H-3), 4.14 (dd, 1 H, H-4), 3.28 (s, 3 H, OMe), 2.32 (m, 1 H,

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H-6), 1.96 (bt, 2 H, H-9), 1.72 and 1.56 (dd, 2 H, H-5), 1.56 and 1.31 (m, 2 H, H-7), 1.31 (m, 2 H, H-8), 1.19 and 1.34 (2 s, 6 H, C-Me); ^{13}C NMR ($CDCl_3$) 177.69 ($CONH_2$), 138.3 (C-10), 114.67 (C-11), 112.19 ($C(Me)_2$), 110.06 (C-1), 85.40 (C-4), 85.18 (C-3), 84.39 (C-2), 55.22 (OMe), 43.38 (C-6), 37.97 (C-5), 33.53 (C-9), 32.61 (C-7), 26.62 (C-8), 24.92 and 24.47 (2 Me); MS (CI, isobutane) m/z 314 (MH^+, 98). Anal. ($C_{16}H_{27}O_5N$) C, H, N.

10b (more polar amide): $[\alpha]_D$ = −38.9° (c = 1, CH_2Cl_2); ^{1}H RMN ($CDCl_3$) 6.04 and 5.69 (2 s, 2 H, $CONH_2$), 5.77 (m, 1 H, H-10), 4.97 (m, 2 H, H-11), 4.92 (s, 1 H, H-1), 4.58 (d, 1 H, H-2), 4.49 (d, 1 H, H-3), 4.17 (t, 1 H, H-4), 3.34 (s, 3 H, OMe), 2.30 (m, 1 H, H-6), 2.03 (bt, 2 H, H-9), 1.91 and 1.6 (2 bd, 2 H, H-5), 1.60 and 1.45 (2 m, 2 H, H-7), 1.35 (m, 2 H, H-8), 1.45 and 1.28 (2 s, 6 H, Me); ^{13}C NMR ($CDCl_3$) 177.94 ($CONH_2$), 138.29 (C-10), 114.79 (C-11), 112.47 ($C(Me)_2$), 109.64 (C-1), 85.5 (C-4), 84.97 (C-3), 84.17 (C-2), 55.10 (OMe), 43.30 (C-6), 37.85 (C-5), 33.63 (C-9), 31.51 (C-7), 26.58 (C-8), 26.57 and 25.07 (2 Me); MS (CI, isobutane) m/z 314 (MH^+, 59). Anal. ($C_{16}H_{27}O_5N$) C, H, N.

Methyl 6-Carbamoyl-5,6,7,8,9,10,11-heptadeoxy-1,2,3-tri-*O*-acetyl-β-D-*ribo*-10-eneundecafuranoside (11). To a solution of 1.6 g (5 mmol) of the epimeric amides **10** in water/dioxane (1/1) was added 1 g of Dowex 50W-X8 (H^+). The reaction was stirred at room temperature for 2 days. The solution was filtered and washed with CH_2Cl_2. The aqueous phase was evaporated to give 1.2 g of crude product, which was taken up in a mixture of acetic anhydride/pyridine (2/3). After overnight reaction the solution was evaporated and the residue disolved in CH_2Cl_2. The usual treatment of the organic phase and chromatography over silica gel (elution with ethyl acetate/CH_2Cl_2, 2/8) provided 1.2 g (yield 61%) of triacetate **11** as an oil; ^{1}H NMR ($CDCl_3$) 6.10 (s, 1 H, H-1), 5.95 and 5.83 (2 s, 2 H, $CONH_2$), 5.83 (m, 1 H, H-10), 5.33 (d, 1 H, H-2), 5.16 (m, 1 H, H-3), 5.00 (m, 2 H, H-11), 4.23 (m, 1 H, H-4), 2.46 (m, 1 H, H-6), 2.08 (s, 9 H, $MeCO_2$), 2.08 (m, 2 H, H-9), 1.62 (m, 2 H, H-5), 1.50 (m, 2 H, H-7), 1.40 (m, 2 H, H-8); MS (EI) m/z 385 (M^+, 8); (CI, isobutane) m/z 386 (MH^+, 3), 326 ($MH^+ - MeCO_2H$, 100).

9-(6′-Carbamoyl-10′-ene-5′,6′,7′,8′,9′,10′,11′-heptadeoxy-2′,3′-*O*-isopropylidene-β-D-*ribo*-1′,4′-undecafuranosyl)-adenine (13). To 1.8 g (4.7 mmol) of N^6-benzoyl-N^6,N^9-bis-(trimethylsilyl)adenine was added a solution of 1 g (2.6 mmol) of **11** in 10 mL of 1,2-dichloroethane (DCE) and 0.32 mL of a 25% solution of trimethylsilyl triflate in DCE, successively. The mixture was refluxed for 16 h under a N_2 atmosphere. The reaction mixture was diluted with CH_2Cl_2 and washed with a saturated aqueous solution of $NaHCO_3$ and with an aqueous saturated solution of NaCl. The organic phase was evaporated, and the residue was taken up in ethyl acetate. This solution was kept in the refrigerator overnight to allow the precipitation of N^6-benzoyladenine. The filtrate was evaporated to give 1.7 g of a residue, which was dissolved in 10 mL of a solution of methanol/concentrated aqueous ammonia (7/3), which was maintained overnight at 60 °C. Evaporation gave a residue, which was suspended in 20 mL of acetone. Then 4 mL of a 25% trimethylsilyl triflate in DCE was added slowly. After 1 h the clear solution was neutralized by addition of concentrated aqueous ammonia and evaporated. The residue was dissolved in CH_2Cl_2, and the solution washed with water. The organic residue was chromatographed over silica gel using a 0–10% gradient of ethanol in acetone to give a 63% overall yield of amide epimers (6/4 ratio).

13a: R_f (acetone/EtOH, 8/2) 0.67; $[\alpha]_D$ + 15° (c = 1, MeOH); ^{1}H NMR ($CDCl_3$) 8.35 (s, 1 H, H-2), 7.87 (s, 1 H, H-8), 6.01 (d, 1 H, H-1′), 5.85 and 5.63 (2 s, 2 H, $CONH_2$), 5.70 (m, 1 H, H-10′), 5.56 (dd, 1 H, H-2′), 4.93 (m, 2 H, H-11′), 4.85 (dd, 1 H, H-3′), 4.27 (bt, 1 H, H-4′), 2.15 (m, 1 H, H-6′), 1.99 and 1.88 (2 m, 2 H, H-5′), 1.91 (bt, 2 H, H-9′), 1.59 and 1.37 (2 m, 2 H, H-7′), 1.59 and 1.37 (2 s, 6 H, Me), 1.24 (m, 2 H, H-8′); ^{13}C NMR (CD_3OD) 180.37 ($CONH_2$), 157.36 (C-6), 153.97 (C-2), 150.34 (C-4), 141.77 (C-8), 139.32 (C-10′), 120.60 (C-5), 115.32 (C(Me)2), 115.06 (C-11′), 91.46 (C-1′), 86.31 (C-4′), 85.95 (C-3′), 84.82 (C-2′), 43.68 (C-6′), 37.18 (C-5′), 34.32 (C-9′), 33.51 (C–7′), 27.36 (C-8′), 27.37 and 25.53 (2 Me); MS (EI) m/z 416 (M^+, 14). Anal. ($C_{20}H_{28}O_4N_6$) C, H, N.

13b: R_f (acetone/EtOH, 8/2) 0.57; $[\alpha]_D$ −0.5° (c = 1, MeOH); ^{1}H NMR ($CDCl_3$) 8.31 (s, 1 H, H-2), 7.88 (s, 1 H, H-8), 6.24 and 5.79 (2 s, 2 H, $CONH_2$), 5.95 (d, 1 H, H-1′), 5.94 (2 s, 2 H, NH_2), 5.76 (m, 1 H, H-10′), 5.47 (dd, 1 H, H-2′), 4.96 (m, 2 H, H-11′), 4.92 (dd, 1 H, H-3′), 4.25 (dd, 1 H, H-4′), 2.38 (m, 1 H, H-6′), 2.17 and 2.05 (2 m, 2 H, H-5′), 2.05 (bd, 2 H, H-9′), 1.80 and 1.65 (2 m, 2 H, H-7′), 1.60 and 1.37 (2 s, 6 H, Me), 1.43 and 1.35 (2 m, 2 H, H-8′); ^{13}C NMR (CD_3OD) 180.58 ($CONH_2$), 157.38 (C-6), 153.96 (C-2), 150.32 (C-4), 141.75 (C-8), 139.50 (C-10′), 120.65 (C-5), 115.82 ($C(Me)_2$), 115.10 (C-11′), 90.95 (C-1′), 85.98 (C-4′), 85.49 (C-3′), 85.29 (C-2′), 43.80 (C-6′), 37.14 (C-5′), 34.53 (C-9′), 33.90 (C-7′), 27.53 (C-8′), 27.70 and 25.54 (2 Me); MS (EI) m/z 416 (M^+, 14). Anal. ($C_{20}H_{28}O_4N_6$) C, H, N.

***N*⁹-(6′-(*N*-(*tert*-Butyloxycarbonyl)amino)-10′-ene-5′,6′,7′,8′,9′,10′,11′-heptadeoxy-2′,3′-*O*-isopropylidene-β-D-*ribo*-1′,4′-undecafuranosyl)adenine (14).** To a solution of 100 mg (0.24 mmol) of compound **13** (**13a** or **13b**) in 2 mL of a 1/1 mixture of *N*,*N*-dimethylformamide (DMF)/water was added 155 mg (0.36 mmol) of (bis(trifluoroacetoxy)iodo)benzene. Then, after 15 min, pyridine (0.038 mL) was added, and the reaction mixture was stirred for 4 h. The solution was evaporated to dryness, and the residue was dissolved in 4 mL of a mixture dioxane/water/triethylamine (2/1/1). This solution was treated with 63 mg (29 mmol) of di-*tert*-butyl dicarbonate. After 4 h the solvents were removed by evaporation, CH_2Cl_2 was added, and the solution after washing with water was dried and evaporated. The residue was purified by silica gel column chromatography to give 98 mg (yield 84%) of compound **14**.

14a: ^{1}H NMR ($CDCl_3$) 8.32 (s, 1 H, H-2), 7.88 (s, 1 H, H-8), 6.38 (s, 2 H, NH_2), 6.03 (d, 1 H, H-1′), 5.66 (m, 1 H, H-10′), 5.50 (dd, 1 H, H-2′), 4.88 (m, 2 H, H-11′), 4.86 (dd, 1 H, H-3′), 4.65 (d, 1 H, NH-t-Boc), 4.30 (m, 1 H, H-4′), 3.60 (m, 1 H, H-6′), 1.88 (m, 4 H, H-9′ and H-5′), 1.68 (m, 2 H, H-7′), 1.31 (m, 2 H, H-8′), 1.52 and 1.31 (2 s, 6 H, $C(Me)_2$), 1.32 (s, 9 H, $C(Me)_3$); ^{13}C NMR ($CDCl_3$) 155.95 (C-6), 155.66 (C=O), 153.17 (C-2), 149.47 (C-4), 140.12 (C-8), 138.43 (C-10′), 120.51 (C-5), 114.71 (C-11′), 114.57 ($C(Me)_2$), 90.70 (C-1′), 84.82 (C-4′), 84.54 (C-3′), 84.00 (C-2′), 79.18 ($C(Me)_3$), 48.30 (C-6′), 38.90 (C-5′), 34.82 (C-9′), 33.39 (C-7′), 28.48 ($C(Me)_3$), 27.30 and 25.57 ($C(Me)_2$), 25.16 (C-8′); MS (EI) m/z 488 (M^+, 59). Anal. ($C_{24}H_{36}N_6O_5$) C, H, N.

14b: ^{1}H NMR ($CDCl_3$) 8.35 (s, 1 H, H-2), 7.95 (s, 1 H, H-8), 6.12 (s, 2 H, NH_2), 6.02 (d, 1 H, H-1′), 5.73 (m, 1 H, H-10′), 5.45 (dd, 1 H, H-2′), 4.93 (m, 2 H, H-11′), 4.88 (dd, 1 H, H-3′), 4.56 (d, 1 H, NH-t-Boc), 4.26 (m, 1 H, H-4′), 3.65 (m, 1 H, H-6′), 1.95 (bt, 2 H, H-9′), 1.82 (m, 2 H, H-5′), 1.65 and 1.50 (2 m, 2 H, H-7′), 1.50 and 1.31 (2 s, 6 H, $C(Me)_2$), 1.30 (m, 2 H, H-8′), 1.30 (s, 9 H, $C(Me)_3$); ^{13}C NMR ($CDCl_3$) 155.88 (C-6), 155.61 (C=O), 153.26 (C-2), 149.67 (C-4), 140.11 (C-8), 138.51 (C-10′), 120.61 (C-5), 114.81 (C-11′), 114.80 ($C(Me)_2$), 90.49 (C-1′), 84.64 (C-4′), 84.64 (C-3′), 84.08 (C-2′), 79.26 ($C(Me)_3$), 48.55 (C-6′), 38.68 (C-5′), 34.99 (C-9′), 33.51 (C-7′), 28.55 ($C(Me)_3$), 27.39 and 25.61 ($C(Me)_2$), 25.23 (C-8′); MS (EI) m/z 488 (M^+, 59). Anal. ($C_{24}H_{36}N_6O_5$) C, H, N.

***N*⁹-(6′-(*N*-(*tert*-Butyloxycarbonyl)amino)-10′-ene-5′,6′,7′,8′,9′,10′,11′-heptadeoxy-2′,3′-*O*-isopropylidene-β-D-*ribo*-1′,4′-undecafuranosyl)-*N*⁶,*N*⁶-dibenzoyladenine (15).** A solution of 90 mg (0.18 mmol) of compound **14** in 10 mL of pyridine containing 0.055 mL of benzoyl chloride was kept overnight at 0 °C. The solution was concentrated under reduced pressure and diluted with CH_2Cl_2. The organic phase was washed and dried in the usual manner. The solvent was evaporated to give a residue, which was purified by column chromatography, yielding 100 mg (78%) of compound **15**: ^{1}H NMR ($CDCl_3$) 8.72 (s, 1 H, H-2), 8.05 (s, 1 H, H-8), 7.95 and 7.50 (m, 10 H, arom.), 6.03 (d, 1 H, H-1′), 5.75 (m, 1 H, H-10′), 5.55 (dd, 1 H, H-2′), 4.95 (m, 2 H, H-11′), 4.85 (dd, 1 H, H-3′), 4.42 (m, 1 H, H-4′), 4.40 (m, 1 H, NH-t-Boc), 3.60 (m, 1 H, H-6′), 2.40–2.10 (m, 4 H, H-5′ and H-9′), 1.60 and 1.33 (2 s, 6 H, Me), 1.60 (m, 4 H, H-7′ and H-8′), 1.40 (bs, 9 H, $C(Me)_3$).

***N*⁹-(6′-(*N*-(*tert*-Butyloxycarbonyl)amino)-2′,3′-*O*-isopropylidene-5′,6′,7′,8′,9′-pentadeoxy-β-D-*ribo*-1′,4′-undecafuranosyl)adenine (16).** To a solution of 40 mg (0.8 mmol) of **14** (**14a** or **14b**) in 1 mL of acetonitrile were added 33 mg of *N*-methylmorpholine oxide hydrate and 0.6 mL of a 4-mmol aqueous solution of osmium tetraoxide. The reaction was left overnight. The reagent was decomposed by passing H_2S gas through the mixture, and then the solution was filtered and evaporated. The residue was dissolved in CH_2Cl_2, and the organic phase was washed with a brine solution, dried, and evaporated. The reaction product was purified by column chromatography to give 30 mg (yield 70%) of compound **16**.

16a: ^{1}H NMR (CD_5N) 8.72 (s, 1 H, H-2), 8.52 (s, 1 H, H-8), 8.48 (bs, 2 H, NH_2), 7.46 (d, 1 H, NH-t-Boc), 6.47 (d, 1 H, H-1'), 5.80 (dd, 1 H, H-2'), 5.60 (m, 2 H, OH), 5.18 (dd, 1 H, H-3'), 4.70 (m, 1 H, H-4'), 4.08 (m, 1 H, H-10'), 3.97 (m, 1 H, H-6'), 3.86 (m, 2 H, H-11'), 2.12 (m, 3 H, H-9' and H-5'), 1.65 (bs, 6 H, $C(Me)_2$), 1.44 (bs, 9 H, $C(Me)_3$), 1.44 (m, 2 H, H-8'); ^{13}C NMR (CD_5N) 157.59 (C-6), 156.58 (CO_2-t-Bu), 153.88 (C-2), 153.87 (C-4), 140.68 (C-8), 123.91 (C-5), 114.40 ($C(Me)_2$), 90.92 (C-1'), 85.58 (C-4'), 85.05 (C-3'), 84.66 (C-2'), 78.53 ($C(Me)_3$), 72.70 (C-10'), 67.49 (C-11'), 49.19 (C-6'), 39.82 (C-5'), 36.41 (C-9'), 34.38 (C-7'), 28.87 ($C(Me)_3$), 27.61 and 25.88 ($C(Me)_2$), 22.76 (C-8'); MS (EI) m/z 523 ($(M + H)^+$, 3); HRMS found 523.2893, calculated for $C_{24}H_{39}N_6O_7$ 423.2880.

16b: ^{1}H NMR (CD_5N) 8.60 (s, 1 H, H-2), 8.47 (s, 1 H, H-8), 8.47 (bs, 2 H, NH_2), 7.57 (d, 1 H, NH-t-boc), 6.55 (d, 1 H, H-1'), 5.78 (dd, 1 H, H-2'), 5.50 (m, 2 H, OH), 5.26 (dd, 1 H, H-3'), 4.75 (m, 1 H, H-4'), 4.22 (m, 1 H, H-10'), 4.07 (m, 1 H, H-6'), 3.92 (m, 2 H, H-11'), 2.20 (m, 1 H, 2.12 (m, 1 H, H-5') 2.00 (m, 2 H, H-9'), 1.7 (m, 2 H, H-7'), 1.52 (m, 2 H, H-8'), 1.62 and 1.40 (2 s, 6 H, $C(Me)_2$), 1.52 (s, 9 H, $C(Me)_3$); ^{13}C NMR (CD_5N) 157.78 (C-6), 156.42 (CO_2-t-Bu), 154.05 (C-2), 151.31 (C-4), 140.62 (C-8), 123.19 (C-5), 114.71 ($C(Me)_2$), 90.88 (C-1'), 85.50 (C-4'), 85.28 (C-3'), 84.94 (C-2'), 78.40 ($C(Me)_3$), 72.82 (C-10'), 67.68 (C-11'), 49.31 (C-6'), 39.80 (C-5'), 36.54 (C-9'), 34.63 (C-7'), 29.00 ($C(Me)_3$), 27.79 and 26.03 ($C(Me)_2$), 22.93 (C-8'); MS (EI) m/z 523 ($(M^+, H)^+$, 3), HRMS found 523.2872, calculated for $C_{24}H_{39}N_6O_7$ 523.2880.

***N*^{9}-(6'-(*N*-(*tert*-Butyloxycarbonyl)amino)-2',3'-*O*-isopropylidene-9'-(methoxycarbonyl)-5',6',7',8',9'-pentadeoxy-β-D-*ribo*-1',4'-nonafuranosyl)adenine (17).** To a solution of 50 mg (0.7 mmol) of compound **15** (**15a** or **15b**) in 2 mL of a mixture of water/acetonitrile/CCl_4 (3/2/2) were added 63 mg (29 mmol) of sodium periodate and 0.37 mg of ruthenium chloride trihydrate. The reaction was maintained at room temperature during 4 h under vigorous stirring. Then ether was added and the organic phase was washed and dried. The residue that was obtained after evaporation of the solvent was dissolved in methanol, and the resulting solution was treated with a solution of diazomethane in ether. The solvents were removed, and column chromatography of the residue gave 36 mg (yield 69%) of compound 17.

17a: ^{1}H NMR ($CDCl_3$) 8.68 (s, 1 H, H-2), 8.15 (s, 1 H, H-8), 7.85–7.46 and 7.36 (m, 10 H, arom.), 6.08 (d, 1 H, H-1'), 5.40 (dd, 1 H, H-2'), 4.83 (dd, 1 H, H-3'), 4.46 (m, 1 H, NH-t-Boc), 4.26 (m, 1 H, H-4'), 3.66 (m, 1 H, H-6'), 3.60 (s, 3 H, OMe), 2.23 (m, 3 H, H-9' and H-5'), 1.80 (m, 2 H, H-5' and H-7'), 1.62 (m, 1 H, H-7'), 1.62 and 1.38 (2 s, 6 H, $C(Me)_2$), 1.45 (bs, 9 H, $C(Me)_3$), 1.35 (m, 2 H, H-8'); ^{13}C NMR ($CDCl_3$) 173.76 (CO_2Me), 172.26 (2 COAr), 155.63 ($NHCO_2$-t-Bu), 152.39 (C-6), 152.00 (C-2), 148.00 (C-4), 144.00 (C-8), 134.36–128.78 (12 Ar), 128.70 (C-5), 115.06 ($C(Me)_2$), 90.60 (C-1'), 84.43 (C-4'), 84.24 (C-3'), 84.06 (C-2'), 79.46 ($C(Me)_3$), 51.40 (CO_2Me), 48.05 (C-6'), 39.24 (C-9'), 34.82 (C-5'), 33.63 (C-7'), 28.49 ($C(Me)_3$), 27.33 and 25.56 ($C(Me)_2$), 22.37 (C-8'); MS (EI) m/z 728 (M^+, 3); HRMS found 728.3183, calculated for $C_{38}H_{44}N_6O_9$ 728.3169.

17b: ^{1}H NMR ($CDCl_3$) 8.72 (s, 1 H, H-2), 8.28 (s, 1 H, H-8), 7.90–7.50 and 7.40 (m, 10 H, arom.), 6.10 (d, 1 H, H-1'), 5.36 (dd, 1 H, H-2'), 4.85 (dd, 1 H, H-3'), 4.46 (m, 1 H, NH-t-Boc), 4.26 (m, 1 H, H-4'), 3.70 (m, 1 H, H-6'), 3.63 (s, 3 H, OMe), 2.26 (t, 2 H, H-9'), 1.77 (m, 1 H, H-5'), 1.58 (m, 3 H, H-5' and H-7'), 1.56 and 1.30 (2 s, 6 H, $C(Me)_2$), 1.35 (bs, 9 H, $C(Me)_3$), 1.35 and 1.28 (m, 2 H, H-8'); ^{13}C NMR ($CDCl_3$) 173.70 (CO_2Me), 172.15 (2 COAr), 155.38 ($NHCO_2$-t-Bu), 154.76 (C-6), 152.48 (C-2), 147.93 (C-4), 144.06 (C-8), 134.41–128.83 (12 Ar), 129.62 (C-5), 115.26 ($C(Me)_2$), 90.26 (C-1'), 84.32 (C-4'), 84.14 (C-3'), 84.14 (C-2'), 79.17 ($C(Me)_3$), 51.52 (CO_2Me), 48.11 (C-6'), 38.63 (C-9'), 34.96 (C-5'), 33.71 (C-7'), 28.53 ($C(Me)_3$), 27.38 and 25.57 ($C(Me)_2$), 22.43 (C-8'); MS (EI) m/z 728 (M^+, 3); HRMS found 728.3159, calculated for $C_{38}H_{44}N_6O_9$ 728.3169.

Deprotection. The various adenosine derivatives **14**, **16**, and **17** were deprotected to the corresponding nucleosides in the following manner: Compounds **16** and **14**, which have acid labile protection only, were first dissolved in trifluoroacetic acid for immediate removal of the *tert*-butyloxycarbonyl group. After 1 min the solution was diluted with an equal volume of water. After standing overnight the solvents were evaporated to give the expected nucleoside **2** (FABMS glycerol, m/z 383 $(M + H)^+$) and **4** (FABMS glycerol m/z 349 $(M + H)^+$) as their trifluoroacetate salts. In the case of compound **3** its protected derivative **17** was first dissolved in dioxane/water (3/1) and treated during 2 h with 5 equiv of NaOH. The solution was dry evaporated, and the residue was taken into pyridine/NH_4OH (9/1). The resulting solution was maintained at 50 °C overnight. The residue that was obtained after evaporation of the solvents was treated as above to give **3** (FABMS, glycerol, m/z 367 $(M + H)^+$) after purification by C_{18} reverse-phase HPLC using $MeOH/H_2O/NH_4OH$ (70/29/1).

Biological Methods. Cells. *Leishmania donovani* promastigotes (strain LRC L-52) originated from the strain collection of the World Health Organization's International Reference Center for leishmaniasis (WHO-LRC) were kindly provided by Dr. L. F. Schnur (Hebrew University, Hadassah Medical School, Jerusalem, Israel).

Culture Medium. This was composed of 45% Dulbecco modified Eagle medium, 45% RPMI 1640 medium containing 25 mM HEPES (*N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid (pH 7.4)), and 10% heat-inactivated fetal calf serum. Streptomycin at 5 μg/mL, penicillin at 5 units/mL, and kanamycin at 5 μg/mL were also added. Incubation was performed at 26 °C.

Effect of the Compounds on Cell Multiplication and Viability. Promastigotes at 0.5×10^6 cells in 500 μL were seeded in 24-well Nunclon multiwell plates. Compounds to be tested were added a few hours later at various concentrations in 25 μL of H_2O, alone or with 0.26 μM sinefungin. Each test was performed in duplicate, and untreated cultures were run in parallel. After 3 or 4 days of culture, promastigotes were counted in a hemacytometer. Cells in control wells grew to a final density of 3.4×10^7 organisms/mL. Viability of the promastigotes was measured by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay method.[27]

Effect of the Compounds on Thymidine Incorporation. Promastigotes (5×10^6 cells/mL) were inoculated into 25-mL Nunclon flasks containing 5 mL of the above described medium. The cultures were left to equilibrate in the incubator for 3–4 h, and then the compound to be tested was added at the desired concentration for 4 h in the absence or in the presence of 0.026 μM sinefungin (**1**). Labeling was performed with 2.5 μCi/mL of [*methyl*-^{3}H]thymidine (52 Ci/mmol, Amersham France) for 1 h. After labeling, the cells were centrifuged, rinsed twice in cold phosphate-buffered saline (PBS). The uptake into the soluble pool and the incorporation into nucleic acid were obtained from the cold TCA-soluble (trichloroacetic acid) and insoluble materials, respectively.[28] Protein concentration was determined by the Lowry procedure[29] with bovine serum albumin as the standard.

Protein Methyltransferase Activity. *S*-Adenosylmethionine:protein–carboxyl methyltransferase activity which catalyzes the posttranslational methylation of carboxyl residues of aspartic and glutamic acids in proteins was measured in cell-free extracts using a published procedure[30] with α-globulin as substrate. The kinetic constants were calculated from Lineweaver and Burk plots.[31] Protein concentration was measured by the dye adsorption method of Bradford[32] using bovine serum albumin as the standard.

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