Samples were dissolved in methanol (1 mg/mL) for injection. Results are the average of three separate determinations.

Prodrug Disposition Studies. Two Long Evans male rats were dosed by gavage through an intragastric tube with 10 mg/kg of 6-methoxypurine arabinoside (1), or the molar equivalent of a prodrug, dissolved or uniformly suspended in water containing 0.1% Tween 80. The animals were then housed together in a metabolic cage for 48 h and given food and water ad libitum. Urine was collected for 0–24 and 24–48 h following administration of the test compound. Samples of the pooled urine were then filtered through 0.22-μm nylon filters. A WISP automatic injector was used to inject 100 μL of the filtered urine sample onto an Alltech/Applied Sciences C-18 column. The sample was eluted with a 45-min linear gradient of 0–20% CH_3CN in 25 mM phosphoric acid previously adjusted to pH 7.2 with ammonium hydroxide, followed by a 10-min purge of 80% CH_3CN in distilled water. Two variable-wavelength detectors set at 252 and 259 nm were used to monitor the eluate. Original conditions were regenerated with a 4-min linear gradient followed by a 15-min reequilibration phase. The standard error of these determinations was found to be less than 15% of the reported value as determined by multiple experiments (n = 3–7) with representative compounds.

Acknowledgment. We thank Mr. Aris Ragouzeos for assistance in obtaining the ^{1}H NMR spectra. Large quantities of compound 1 were prepared by Dr. B. Sickles of the Burroughs Wellcome Co. Chemical Development Labs. The assistance of Mr. Allen Jones and Ms. Laura Mansberg in proofreading this manuscript is gratefully acknowledged.

Registry No. 1, 91969-06-1; **2a**, 121032-46-0; **2b**, 137057-42-2; **2c**, 137057-43-3; **2d**, 137057-44-4; **2e**, 137057-45-5; **2f**, 137057-46-6; **2g**, 137057-47-7; **2h**, 137057-48-8; **2i**, 137057-49-9; **2j**, 137057-50-2; **2k**, 137057-51-3; **2l**, 137057-52-4; **2m**, 137057-53-5; **2n**, 137057-54-6; **3a**, 137057-55-7; **3b**, 137057-56-8; **3c**, 137091-64-6; **3d**, 137057-57-9; **3e**, 137057-58-0; **3f**, 137057-59-1; **3g**, 137057-60-4; **3h**, 137057-61-5; **3i**, 137057-62-6; **3j**, 137057-63-7; **3k**, 137057-64-8; **3l**, 137057-65-9; **3m**, 137057-66-0; **3n**, 137057-67-1; **4a**, 137057-68-2; **4b**, 137057-69-3; **4c**, 137057-70-6; **4d**, 137057-71-7; **4e**, 137057-72-8; **4f**, 137057-73-9; **5a**, 137057-74-0; **5b**, 137057-75-1; **5c**, 137091-65-7; **5d**, 137057-76-2; **5e**, 137057-77-3; **5f**, 137057-78-4; **5g**, 137057-79-5; **5h**, 137057-80-8; **5i**, 137057-81-9; **5j**, 137057-82-0; **6b**, 137057-83-1; **6c**, 137057-84-2; **6d**, 137057-85-3; **6e**, 137091-66-8; **6f**, 137057-86-4; **6g**, 137057-87-5; **6h**, 137057-88-6; **6i**, 137057-89-7; **6j**, 137057-90-0; **6k**, 137057-91-1; **6l**, 137057-92-2; **6m**, 137057-93-3; **6n**, 137057-94-4; **7**, 137057-95-5; **8**, 137057-96-6; **9**, 137057-97-7; **10**, 137058-03-8; **11b**, 137057-98-8; **11c**, 137057-99-9; **11d**, 137058-00-5; **11e**, 137058-01-6; **11f**, 137091-51-1; **12h**, 137058-02-7.

Supplementary Material Available: Analytical and spectral (^{1}H NMR assignments, multiplicities, and coupling constants, and mass spectral fragments) data for compounds **2a–n**, **3a–n**, **4a–f**, **5a–j**, **6b–n**, **7–10**, **11b–f**, **12e–h** (37 pages). Ordering information is given on any current masthead page.

Total Synthesis of Uracil Analogues of Sinefungin

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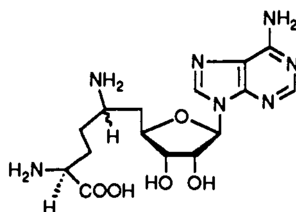
Analogues of sinefungin derivatives **18a** and **18b** have been prepared from uridine and L-aspartic acid. The key step in the synthesis was the coupling of the radical derived from 14 with the unsaturated amide 13. The latter was produced from the known *N*-hydroxy-2-thiopyridone ester of L-aspartic acid 12 with the olefin 11. Thus, the essential carbon skeleton was constructed by way of two radical coupling reactions. These analogues as well as 1a and 1b synthesized previously were tested for their antileishmanial effect in vivo and for their inhibitory activity of protein carboxymethylase (protein methylase II). The replacement of the adenine moiety by uracil or dihydrouracil considerably decreases the antiparasitic activity and the affinity for protein methylase II. The synthetic (*S*)-sinefungin was as active as the natural one. Interestingly, the C-6′ epimer **1b** was 50% less active in vitro than the natural sinefungin, but both had identical affinities for the target enzyme.

Natural sinefungin (**1a**) was isolated in 1973 from the cultures of *Streptomyces grisoleus*.[1] Its structure (**1a**) is composed of an adenosine unit to which has been attached at the C-5′ position an ornithine residue. The C-6′ chiral center has the *S* configuration, as has been confirmed by the recent synthesis of Rapoport[2] and in agreement with our own synthesis.[3]



1a: natural sinefungin (*S*)
b: episinefungin (*R*)

Natural sinefungin is a powerful antifungal agent particularly efficient against *Candida albicans*.[4] It possesses also a strong antiparasitic activity, especially against various species of *Leishmania*[5] and *Trypanosoma*.[6] However, sinefungin has serious side effects (nephrotoxicity in the dog and toxicity for bone marrow cells). In an effort to improve the therapeutic index of sinefungin, Secrist and co-workers have recently reported the synthesis of a series of analogues.[7] We ourselves disclosed a new

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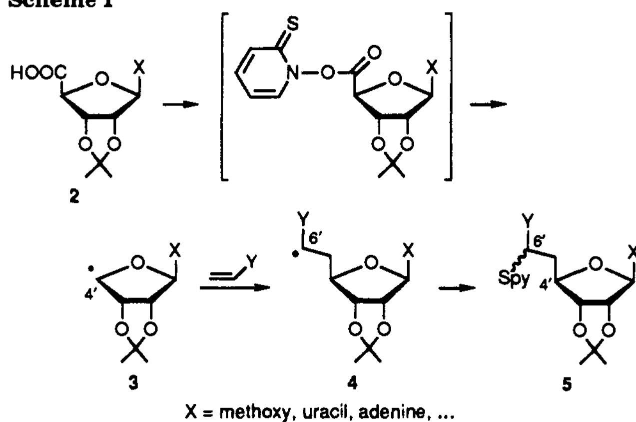
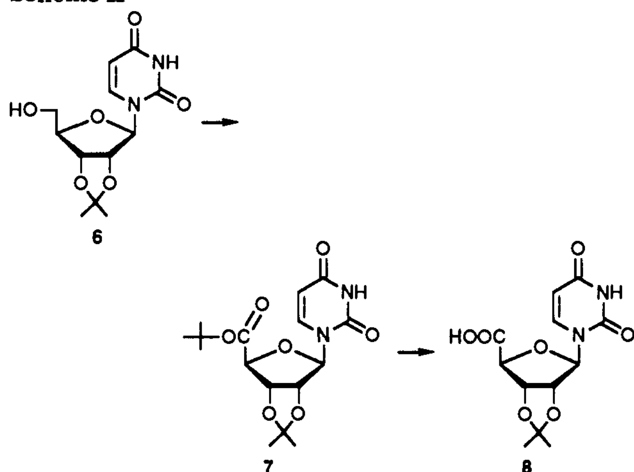
Scheme I



Scheme II



and simple synthesis[3] of sinefungin which affords the whole carbon skeleton of sinefungin. This approach allows modifications on the base portion of sinefungin. We present in this paper the synthesis of analogues **18a** and **18b** in which adenine is replaced by uracil and dihydrouracil, respectively. Our synthesis permits the construction of the carbon skeleton by two simple radical reactions. The activity of these analogues on the multiplication of *Leishmania donovani* promastigotes and their effect on the protein carboxylmethylase of these parasites is compared to the activity of the formerly described synthetic *S*- and *R*-sinefungin.

Chemistry

Our approach (Scheme I) is based on the photolysis of the *N*-hydroxy-2-thiopyridone derivative of the appropriate 5′-carboxylic acid. Thus, the acid **2** affords the radical **3** which adds to the appropriate electron-deficient olefin to give radical **4**. The latter then reacts[8] with the thiocarbonyl derivative of the precursor to give the 2-pyridylthio derivative **5** as a mixture of stereoisomers at the 6′-position. In contrast, the radical at the 4′-position reacts stereoselectively,[9,21] and only one configuration (less hindered side attack) is seen. The intermediate **5** is readily reduced by tin hydride reagents resulting in removal of the 2-pyridylthio residue.

For the synthesis of the desired analogues **18a** and **18b** we chose two intermediates, the acid **8** (Scheme II) and the olefin **13** (Scheme III). The known crystalline acid[10] **8** was prepared from the alcohol[11] **6** by way of the *tert*-butyl ester **7**.[12] Hydrolysis[21] of the latter yielded acid **8**.

We have recently described the synthesis of the olefin **13**,[3] which was prepared in the following way (Scheme III). The radical from the L-aspartic acid derivative[13] **12** was added to the olefin **11** to furnish the expected olefin **13** (62% isolated). The olefin **11** was in turn prepared from the bromacrylate[14] **9** by displacement with thiophenol (70%). The ester function was then converted to amide **11** (82%) using the amide of methylchloroaluminum, prepared in situ from trimethylaluminum and ammonium chloride in benzene at room temperature.[15]

Scheme III

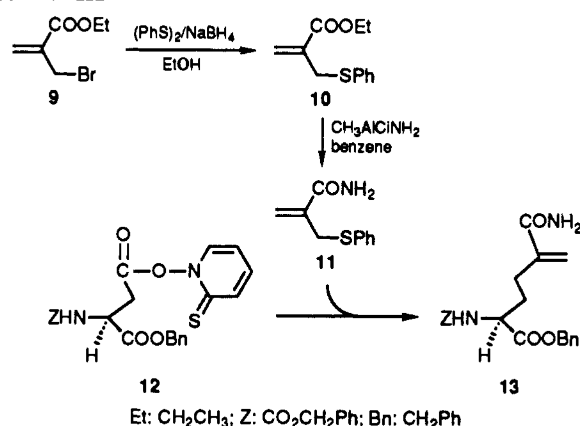


Table I. Growth Inhibition of *L. donovani* Promastigotes by Sinefungin Analogues

substrate	dose (μM)	% inhibn
natural sinefungin [(*S*)-**1a**]	0.26	100
synthetic sinefungin [(*S*)-**1a**]	0.26	88
epi-sinefungin [(*R*)-**1b**]	0.26	34
	1.30	91
compound **18a**	14	20
compound **18b**	14	19

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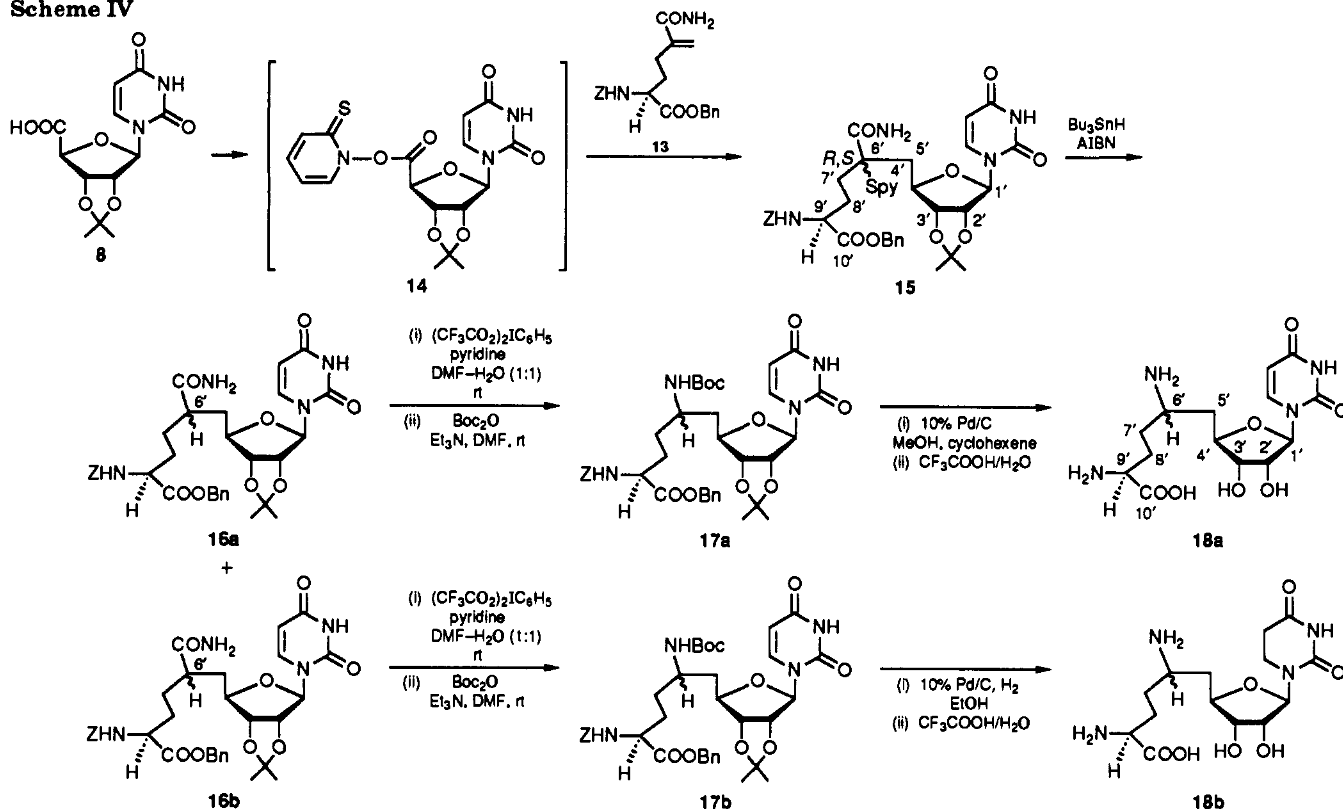
Scheme IV



The *N*-hydroxy-2-thiopyridone derivative 14 (Scheme IV) was prepared from 8 in THF by the usual mixed-anhydride method.[13,16] The irradiation of 14 in the presence of the olefin 13 gave the addition product 15 (50%) as a mixture of two stereoisomers at C-6′. The adduct 15 was reduced with tributyltin hydride in benzene under reflux in the presence of an initiator. The two reduction products **16a** and **16b**, epimeric at C-6′ were separated by medium-pressure chromatography over silica gel. The less polar compound was **16a** (40%) and the more polar one was **16b** (38%). These two amides were treated separately with [bis(trifluoroacetoxy)iodo]benzene in DMF–water in the presence of pyridine at room temperature.[17] The amines thus formed were at once converted to their *tert*-butoxycarbonyl derivatives **17a** (66%) and **17b** (68%), both isolated in crystalline form. Compound **17b** was deprotected by successive hydrogenolysis (H_2, 10% Pd/C) and acidic hydrolysis to furnish **18b** in which the double bond of the base was reduced. Compound **17a** was hydrogenolyzed using 10% Pd/C and cyclohexene as hydrogen donor to yield **18a**. The absolute configuration at C-6′ of **18a** and **18b** has not yet been determined.

Biological Activity

Biological testing of derivatives **18a** and **18b** shows that the replacement of the adenine portion of sinefungin by uracil or dihydrouracil considerably decreases the growth inhibitory activity against *L. donovani* (promastigotes) in comparison with natural sinefungin. The C-6′ epimer (*R*)-**1b** is active but 50% less than the natural one (Table I).

As sinefungin (**1a**) is an efficient inhibitor of transmethylases, the effect of the analogues was tested on protein carboxylmethyltransferase of leishmanial promastigotes. All the compounds assayed behaved as competitive inhibitors with respect to AdoMet. As shown in Table II, **1a** and **1b** have a similar affinity for this enzyme, with the same order of magnitude than the natural substrate, AdoMet. The replacement of the adenine portion of sinefungin by uracil decreased the affinity. The K_i/K_m ratio of the compound **18a** is 35 and 15 for S12 and P12 fractions, respectively, compared to 1 and 0.8 for sinefungin. These results indicate that the configuration of the C-6′ chiral center is not critical for the binding of the sinefungin to the enzyme, but seems to influence the uptake of the molecule. Furthermore, the adenine portion is important for the binding to this enzyme and for the growth-inhibitory effect.

Table II. Apparent Kinetic Constants of Sinefungin Analogues for PM II of *Leishmania donovani* Promastigotes

compounds	K_m and K_i (μM)[a] S12	P12
AdoMet	*48	*164
sinefungin [(*S*)-**1a**]	47	126
episinefungin [(*R*)-**1b**]	54	236
compound **18a**	1680	2500

[a] The apparent kinetic constants of PM II were determined on two subcellular fractions of *L. donovani* promastigotes: the supernatant (S12) and the proteins extracted from the 12000*g* pellet (P12) as described in the Experimental Section. All the compounds tested were competitive inhibitors with respect to AdoMet. The specific activities of these subcellular fractions, measured at their apparent V_m values, were 134 and 248 pmol of methyl incorporated mg^{-1} for S12 and P12, respectively.

Experimental Section

General. Column chromatography was carried out on silica gel 60 (0.040–0.063 mm). Thin-layer analytical plates were 60F254 (Merck). Unless stated otherwise, ^1H and ^{13}C NMR spectra were recorded on Bruker WP200 SY (200 MHz) or on Bruker AM (400 MHz) instruments. Chemical shifts (δ) are expressed in ppm from Me_4Si as internal standard. Coupling constants *J* are in hertz. Most spectra were taken in $CDCl_3$. In other cases, the solvent

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is specified. Melting points were taken on a Reicher apparatus and are not corrected. Infrared spectra were recorded on a Perkin-Elmer 297 instrument. Routine mass spectra were recorded on an AEI MS50, AEI MS9, and Kratos MS80 (for FAB spectra). High-resolution mass spectra (HRMS) were determined by the Service for Mass Spectra of the C.N.R.S. at Vernaison. Elemental analyses were carried out at the I.C.S.N.

Benzyl 1'-Uracil-1-yl-9'-[(benzyloxycarbonyl)amino]-6'-(*S*,*R*)-carbamoyl-1',5',6',7',8',9'-hexadeoxy-2',3'-*O*-isopropylidene-6'-(pyridylthio)-β-D-*ribo*-1',4'-decafuranuronate (15). To the acid 8 (1 mmol) in anhydrous tetrahydrofuran (10 mL) was added *N*-methylmorpholine (1 mmol) and isobutyl chloroformate (1 mmol). After the mixture was stirred for 15 min at 0 °C under argon, the sodium salt of *N*-hydroxy-2-thiopyridone (0.178 g, 1.2 mmol) was added. The reaction mixture was stirred under argon at 0 °C for 1 h with exclusion of light (aluminum foil) to form 14. The mixture was then transferred dropwise to a solution of olefin 13 (2.37 g, 6 mmol), and the yellow solution was irradiated with a tungsten lamp (250 watts) at 0 °C for 15 min. The reaction mixture was diluted with $CHCl_3$ (100 mL) and washed with saturated sodium hydrogen carbonate solution (50 mL) and with water (50 mL). The organic phase was dried over $MgSO_4$, and after filtration it was evaporated under reduced pressure. The residue thus obtained was chromatographed on silica gel using gradient elution. Elution with ethyl acetate–methanol 9.5:0.5 gave the coupled derivative 15 (50%), which was a mixture of two isomers. It had mp (CH_2Cl_2–hexane) 100–102 °C; MS *m/z* (FAB) 760 (MH^+). Anal. ($C_{38}H_{41}N_5O_{10}S\cdot{}^1/_2H_2O$) C, H, N, S.

Benzyl 1'-Uracil-1-yl-9'-[(benzyloxycarbonyl)amino]-6'(*R* or *S*)-carbamoyl-1',5',6',7',8',9'-hexadeoxy-2',3'-*O*-isopropylidene-β-D-*ribo*-1',4'-decafuranuronate (16a and 16b). The mixture of isomers 15 (1.139 g, 1.5 mmol) in anhydrous benzene (10 mL) was treated under reflux with tributyltin hydride (1.21 mL, 4.5 mmol) and α,α'-azoisobutyronitrile (0.024 g, 0.15 mmol) for 3 h under argon. Thin-layer chromatography (ethyl acetate–*tert*-butyl alcohol 9:1) showed the presence of two isomers at position 6'. The solvent was removed under reduced pressure. The residue was chromatographed on a column of silica gel (ethyl acetate–*tert*-butyl alcohol 9.5:0.5) permitting separation of 16a (40%) and 16b (38%).

Isomer 16a: mp (from CH_2Cl_2–hexane) 94–95 °C; $[\alpha]^{20}{}_D$ +25° (*c* 1.0, $CHCl_3$); MS *m/z* (FAB) 651 (MH^+); ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (m, 10 H, Ph), 7.23 (d, 1 H, H-6, $J_{6,5}$ = 8 Hz), 6.15 (br s, 1 H, $CONH_2$), 6.05 (br s, 1 H, $CONH_2$), 5.79 (d, 1 H, N*H*Z), 5.76 (d, 1 H, H-5, $J_{5,6}$ = 8 Hz), 5.60 (s, 1 H, H-1'), 5.2 (s, 2 H, CH_2Ph), 5.16 (br s, 3 H, CH_2Ph, NH), 5.01 (d, 1 H, H-2'), 4.65 (t, 1 H, H-3'), 4.26 (m, 1 H, H-4'), 4.10 (m, 1 H, H-9'), 2.40 (m, 1 H, H-6'), 2.03, 1.76, 1.51 (m, 6 H, 2 H-5', 2 H-7', 2 H-8'), 1.55, 1.33 (2 s, 6 H, CMe_2); ^{13}C NMR ($CDCl_3$) δ 177.2 ($CONH_2$), 172.2 ($NHCO_2Bn$), 163.6 (C-4), 156.2 (CO_2Bn), 150.3 (C-2), 142.4 (C-6), 114.7 ($C(CH_3)_3$), 102.7 (C-5), 93.8 (C-1'), 84.7, 84.0, 83.7 (C-4', C-3', C-2'), 67.1, 66.3 (CH_2Ph), 53.8 (C-9'), 42.2 (C-6'), 35.9 (C-5'), 29.8, 28.6 (C-7', C-8'), 27.2, 25.3 (CMe_2). Anal. ($C_{33}H_{38}N_4O_{10}$) C, H, N.

Isomer 16b: mp (from CH_2Cl_2–pentane) 92–93 °C; $[\alpha]^{20}{}_D$ +25° (*c* 1.0, $CHCl_3$); MS *m/z* (FAB) 651 (MH^+); ^1H NMR (400 MHz, $CDCl_3$) δ 7.28 (m, 10 H, Ph), 7.12 (d, 1 H, H-6), 6.26 (br s, 1 H, $CONH_2$), 6.12 (br s, 1 H, $CONH_2$), 5.76 (d, 1 H, N*H*Z), 5.67 (d, 1 H, H-5, $J_{5,6}$ = 8 Hz), 5.46 (s, 1 H, H-1'), 5.14 (s, 2 H, CH_2Ph), 5.07 (s, 2 H, CH_2Ph), 5.0 (d, 1 H, H-2', $J_{2',3'}$ = 6 Hz), 4.60 (t, 1 H, H-3', $J_{3',4'}$ = 6 Hz), 4.40 (m, 1 H, H-4'), 4.04 (m, 1 H, H-9'), 2.33 (m, 1 H, H-6'), 1.86, 1.70, 1.47 (m, 6 H, 2 H-5', 2 H-7', 2 H-8'), 1.53, 1.33 (2s, 6 H, CMe_2); ^{13}C NMR ($CDCl_3$) δ 177.7 ($CONH_2$), 172.2 ($NHCO_2Bn$), 163.9 (C-4), 156.1 (CO_2Bn), 150.3 (C-2), 142.6 (C-6), 114.6 ($C(CH_3)_3$), 102.5 (C-5), 94.0 (C-1'), 85.1, 84.3, 83.9 (C-4', C-3', C-2'), 67.1, 66.9 (CH_2Ph), 53.7 (C-9'), 42.2 (C-6'), 35.6 (C-5'), 29.9, 28.4 (C-7', C-8'), 27.2, 25.4 (CMe_2). Anal. ($C_{33}H_{38}N_4O_{10}$) C, H, N.

Benzyl 1'-Uracil-1-yl-9'-[(benzyloxycarbonyl)amino]-6'(*R* or *S*)-[(*tert*-butoxycarbonyl)amino]-1',5',6',7',8',9'-hexadeoxy-2',3'-*O*-isopropylidene-β-D-*ribo*-1',4'-decafuranuronate (17a and 17b). To the amide 16a (0.228 g, 0.35 mmol) in a mixture of dimethylformamide (5.0 mL) and water (5.0 mL) was added iodosobenzene bis(trifluoroacetate) (0.226 g, 0.525 mmol). After the mixture was stirred at room temperature for 30 min, pyridine (57 μL, 2 equiv) was added. Stirring was continued for 2 h at room temperature. The solvent was removed under reduced pressure, and the residue was then treated with toluene which was again removed under reduced pressure to eliminate the last traces of pyridine. The amine obtained was treated at 0 °C with di-*tert*-butyl dicarbonate (0.092 g, 1.2 equiv) in dimethylformamide (2 mL) with addition of triethylamine (49 μL, 1 equiv). After stirring for 2 h at room temperature, the solution was evaporated under reduced pressure. The residue was purified on silica gel (ethyl acetate–hexane 8:2), affording the crystalline sinefungin derivative 17a (66%): mp (from CH_2Cl_2–pentane) 85–86 °C; $[\alpha]^{20}{}_D$ 24° (*c* 0.5, $CHCl_3$); IR ν_{max} ($CHCl_3$) 1720, 1695, 1580 cm^{-1}; MS *m/z* (FAB) 623 (MH^+ – Boc); ^1H NMR (400 MHz, $CDCl_3$) δ 9.23 (s, 1 H, NH), 7.35 (s, 10 H, Ph), 7.13 (d, 1 H, H-6, $J_{6,5}$ = 8 Hz), 5.7 (d, 1 H, H-5), 5.56 (d, 1 H, N*H*Z), 5.50 (br s, 1 H, H-1'), 5.18 (q, 2 H, CH_2Ph), 5.10 (q, 2 H, CH_2Ph), 4.96 (d, 1 H, H-2', $J_{2',3'}$ = 6 Hz), 4.63 (dd, 1 H, H-3', $J_{3',4'}$ = 4.5 Hz), 4.41 (m, 2 H, N*H*Boc, H-9'), 4.08 (m, 1 H, H-4', $J_{4',5'}$ = 9 Hz), 3.7 (m, 1 H, H-6'), 1.86, 1.76, 1.70, 1.56 (m, 6 H, 2H-5', 2H-7', 2H-8'), 1.53 (s, 3 H, CH_3 of CMe_2), 1.41 (s, 9 H, CH_3 of Boc), 1.31 (s, 3 H, CH_3 of CMe_2). Anal. ($C_{37}H_{46}N_4O_{11}\cdot{}^1/_2H_2O$) C, H, N.

In exactly the same way the amide 16b (0.195 g) gave the 6'-epi derivative 17b (68%): mp 85–86 °C (from CH_2Cl_2–pentane); $[\alpha]^{20}{}_D$ +16° (*c* 1, $CHCl_3$); IR ν_{max} 1720, 1695, 1045 cm^{-1}; MS *m/z* (FAB) 623 (MH^+ – Boc); ^1H NMR (400 MHz, $CDCl_3$) δ 9.31 (s, 1 H, NH), 7.35 (s, 10 H, 2 × Ph), 7.26 (d, 1 H, H-6, $J_{6,5}$ = 8 Hz), 5.71 (d, 1 H, H-5, $J_{5,6}$ = 8 Hz), 5.65 (d, 1 H, N*H*Z), 5.56 (br s, 1 H, H-1'), 5.16 (q, 2 H, OCH_2Ph), 5.10 (s, 2 H, OCH_2Ph), 4.90 (d, 1 H, H-2', $J_{2,3'}$ = 5 Hz), 4.60 (dd, 1 H, H-3', $J_{3',4'}$ = 4.5 Hz), 4.55 (d, 1 H, N*H*Boc), 4.38 (m, 1 H, H-9'), 4.08 (m, 1 H, H-4'), 3.71 (m, 1 H, H-6'), 1.93, 1.81, 1.48 (m, 6 H, 2H-5', 2H-7', 2H-8'), 1.53 (s, 3 H, CH_3 of CMe_2), 1.4 (s, 9 H, CH_3 of Boc), 1.31 (s, 3 H, CH_3 of CMe_2). Anal. ($C_{37}H_{46}N_4O_{11}$) C, H, N.

1'-Uracil-1-yl-6'(*R* or *S*),9'-diamino-1',5',6',7',8',9'-hexadeoxy-β-D-*ribo*-1',4'-decafuranuronic Acid (18a). Derivative 17a (109 mg, 0.15 mmol) was hydrogenolyzed in methanol (4 mL) and cyclohexene (1 mL) using 10% palladium on charcoal (430 mg) at reflux during 30 min. After filtration through Celite, the solvent was removed. The residue was washed with ether. The Boc and ketal protecting groups were removed with 80% trifluoroacetic acid at 0 °C overnight. After evaporation in vacuo, the residue was chromatographed on a RP8 column eluting with $MeOH–CH_2Cl_2–NH_4OH$ 5:4:1. Removal of solvents yielded 18a (90%): mp 155 °C (dec, MeOH–ether); $[\alpha]^{20}{}_D$ +32° (*c* 1.1, H_2O); MS *m/z* (FAB) 359 (MH^+), ^1H NMR (400 MHz, D_2O) δ 7.70 (d, 1 H, H-6, $J_{6,5}$ = 8 Hz), 5.95 (d, 1 H, H-5), 5.80 (d, 1 H, H-1', $J_{1',2'}$ = 2 Hz), 4.50 (br s, 1 H, H-2'), 4.2 (br s, 2 H, H-3', H-4'), 3.9 (m, 1 H, H-9'), 3.65 (m, 1 H, H-6'), 2.25 (m, 2 H, 2H-5'), 2.05–1.9 (m, 4 H, 2H-7', 2H-8'); HRMS found 359.1566 (M), calcd for $C_{14}H_{23}N_4O_7$ 359.1557 (M).

1'-(Dihydrouracil-1-yl)-6'(*R* or *S*),9'-diamino-1',5',6',7',8',9'-hexadeoxy-β-D-*ribo*-1',4'-decafuranuronic Acid (18b). Derivative 17b (145 mg, 0.2 mmol) was hydrogenolyzed in ethanol using 10% palladium on charcoal (145 mg) at 2 atm pressure overnight. After filtration through Celite, the solvent was removed. The residue was washed with ether. The Boc and ketal protecting groups were then removed with 80% trifluoroacetic acid at 0 °C overnight. After evaporation in vacuo, the residue was chromatographed on a RP8 column eluting with $MeOH–CH_2Cl_2–NH_4OH$ 5:4:1. After removal of solvents, 18b was obtained (85%): $[\alpha]^{20}{}_D$ +0° (c 1, H_2O); MS *m/z* (FAB) 361 (MH^+); ^1H NMR (400 MHz, D_2O) δ 5.60 (d, 1 H, H-1', $J_{1',2'}$ = 4 Hz), 4.30 (br s, 1 H, H-2'), 4.0 (br s, 2 H, H-3', H-4'), 3.8 (t, 1 H, H-9', *J* = 6 Hz), 3.55 (m, 3 H, H-6', 2H-6), 2.80 (br s, 2 H, 2H-5'), 2.4–1.75 (m, 6 H, 2H-5, 2H-7', 2H-8'); HRMS found 361.1723 (M), calcd for $C_{14}H_{25}N_4O_7$ 361.1713 (M).

Biological Methods. Cells. *L. 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).

Growth of Organisms. The promastigotes were grown at 26 °C, in a semi-defined medium composed of 45% Dulbecco's modified Eagle's medium, 45% RPMI 1640 medium containing 25 mM Hepes [*N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (pH 7.4)], 10% heat-inactivated fetal calf serum, streptomycin

5 μg/mL, penicillin 5 units/mL and kanamycin at 5 μg/mL.

Effect of the Compounds on Cell Multiplication. Promastigotes at 0.5×10^6 cells in 500 μL of medium 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. Each test was performed in duplicate, and untreated cultures were run in parallel. After 3 days of culture, promastigotes were counted in a hemacytometer. Cells in control wells grew to a final density of 3.5×10^{-7} organisms mL^{-1}.

Cell Fractionation. Cells were disrupted by five cycles of freezing in dry ice–ethanol and quick thawing at 37 °C in Tris-HCl 10 mM at pH 7.0 containing 2-mercaptoethanol 10 mM and PMSF 0.1 mM. Centrifugation of the homogenate was performed in a Beckman J 2/21 ME centrifuge equipped with a J 20 rotor at 4 °C for 20 min. The supernatant was taken up whereas the 12000g pellet was resuspended in 1 mL of the same buffer. The proteins were extracted with 0.2% Triton X 100 in 1.7 M NaCl. The DNA was then precipitated at 4 °C for 1 h upon addition of 10% PEG (w/w) in 1.3 M NaCl (final concentrations). The suspension was centrifuged at 12000g for 15 min, and the supernatant was dialyzed and assayed for enzyme activity.

Protein Methylase Activity. Protein methylase II (EC 2.1.1.77) activity was assayed at 37 °C by measuring the incorporation of radiolabeled methyl groups into α-globulin after separation from unreacted AdoMet by precipitation of the methylated protein with TCA, as described by Kim and Paik.[18]

The apparent kinetic constants were calculated from Lineweaver–Burk plots.[19] Protein concentration was measured by the dye adsorption method of Bradford,[20] using bovine serum albumin as the standard.

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(±)-3-Allyl-7-halo-8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepines as Selective High Affinity D1 Dopamine Receptor Antagonists: Synthesis and Structure–Activity Relationship

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Substituted 1-phenyl-3-benzazepines form a class of compounds possessing potent and selective affinity for the D1 DA receptor. 7,8-Dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine (SKF 38393) and its 6-halo analogues are potent and selective D1 receptor agonists. Recently, the 3-allyl derivatives of SKF 38393 and its analogues were described as selective D1 agonists with higher D1 efficacy and CNS potency. In order to extend these results to compounds in the 7-halo-8-hydroxy-substituted antagonist series, we have synthesized and pharmacologically characterized 3-allyl analogues of 7-substituted (Cl, Br, H) 8-hydroxy-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepines. These 3-allyl derivatives were compared with their 3-methyl and 3-unsubstituted analogues in terms of their D1 receptor affinity and selectivity. The results have been used to generate structure–affinity relationships. The D1 receptor affinity, for 3-substitution, is found to be in the order: methyl > allyl > H. For 7-substitution, the affinity is in the order: Cl = Br > H. The 3-allyl compounds show affinity close to that of the parent (3-methyl) compounds while exhibiting a slightly diminished D1 selectivity. However, the greater lipophilicity of the 3-allyl compounds may enable them to cross the blood–brain barrier more readily and thereby exhibit higher in vivo CNS potency. Thus 3-allylbenzazepines have potential as high affinity selective D1 antagonists.

Dopamine (DA) receptors have been implicated in neuropsychiatric diseases, and consequently there is considerable continuing interest in the search for suitable selective and potent DA receptor agonists and antagonists which can be useful both as agents for therapeutic purposes and as tools for pharmacological and biochemical research.[1,2] Until recently, the DA receptors were classified into D1 and D2 subtypes on the basis of available biochemical and pharmacological evidence.[3-5] The recent discovery of the existence of new DA receptor subtypes (D3, D4, and D5), has stimulated renewed interest in the chemistry and pharmacology of agents acting selectively at these receptor subtypes.[6-8] While the physiological and pathophysiological roles of these new receptor subtypes are as yet unknown, it is known that D1 and D2 receptor antagonists have clinical applications as potential antipsychotics in the treatment and management of schizo-

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