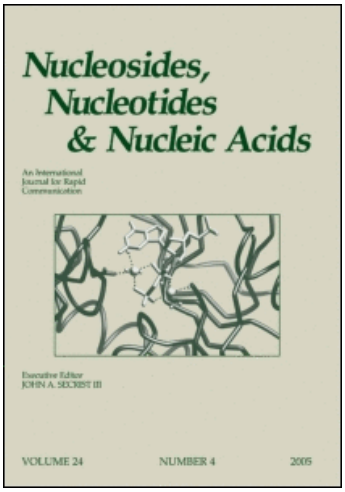

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SYNTHESIS AND ANTIVIRAL EVALUATION OF *N*-β-D-RIBOSIDES OF ERGOT ALKALOIDS

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ABSTRACT: *N*-β-D-Ribosides of agroclavine (**1**), elymoclavine (**2**), lysergene (**4**), lysergol (**3**), and 9,10-dihydrolysergol (**5**) were prepared by $SnCl_4$ catalyzed ribosylation of their TMS derivatives with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose. None of the new compounds exhibited activity against HIV or other viruses tested.

Ergot alkaloids (EA) exert a broad spectrum of pharmacological activities including central, neurohumoral and peripheral effects. Besides their activity mediated by neurotransmitter receptors,[1] clavine alkaloids possess also antibiotic and cytostatic activities. Agroclavine acts as an inhibitor of rhodomycin production by *Streptomyces purpurascens*;[2a] this antibiotic effect can be enhanced by reduction of the 8,9-double bond and aliphatic substitution at *N*-1 and *N*-6 atoms of ergoline.[2b] Some of these derivatives were found to be effective against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*.[2c] Antibiotic activity of EA was ascribed to inhibition of nucleic acid replicatory processes.[2c] Cytostatic activity of agroclavine and its derivatives were tested against mouse lymphoma L 5178y [3a] and the best activities were found with *N*-1 propylated clavine derivatives.[2c,3b,c] Such an antineoplastic activity is comparable with that of the clinically used cytostatics bleomycin, adriamycin and

daunomycin.[3b] 1-Propylagroclavine strongly inhibits incorporation of ^{3}H-thymidine into DNA but it does not influence activity of both α- and β-DNA-polymerase and RNA export from nucleus.[3b]

Antineoplastic and antiviral activity of various heterocycles could be augmented by their *N*-ribosylation. Preparation of *N*-ribosides of EA could create analogous compounds to nucleosides with the aglycon possessing both neurohumoral and cytostatic activity. *N*-Glycosides of indole compounds are rather scarce and *N*-glycosylation of EA has not been studied so far.

We report here the preparation of *N*-β-D-ribosides of ergot alkaloids and evaluation of their antiviral activity.

RESULTS AND DISCUSSION

The synthesis of these nucleosides was achieved using silylation procedure. Trimethylsilyl (TMS) derivatives of **1-5** were prepared by reaction with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide in MeCN (reflux, N_2).[4b] TMS derivatives were then treated with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**6**). From the different reaction conditions used, the best involved 1,2-dichloroethane as the solvent and $SnCl_4$ as the catalyst. After 30 minutes the reaction was quenched by sat. $NaHCO_3$. Evaporation and purification by flash chromatography afforded ribosides **7** - **11** in 20 - 40 % yields. Deprotection of these intermediates was carried out by treatment with a mixture of Et_3N : MeOH : H_2O 1/8/1 at room temperature overnight. Deprotected ribosides **12** - **16** were purified again by column chromatography.

Small amounts of α-anomer (6 - 19 %) were formed in all cases - *vide infra* - that were neither separable (even by HPLC) in protected nor in deprotected form from the major product. The use of TMS-OTf (2 eq) as a promoter gave similar results as $SnCl_4$, except that the yields were slightly lower.

Elemental compositions of ribosides **12-16** were checked by high-resolution measurement of the $[M]^{+\cdot}$ ions in their EI mass spectra. **14**, **15** and **16** gave corresponding molecular ion as a base peak, the spectra of **12** and **13** were dominated by $[M-H]^+$ ion. In MS of **16** fragment ions originated from intrinsic cleavages of the sugar moiety have

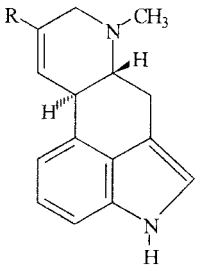
been found at *m/z* 284, 298 and 357, the rest of ions should be attributed to the aglycon. The ion m/z 256 characterizes the whole aglycon, ions *m/z* 223 and peaks at *m/z* 167 and 154 should stem from 9,10-saturated ABCD and ABC ring systems, respectively. Ion *m/z* 144 $[C_{10}H_{10}N]^+$ confirms the saturation of the D-ring.[5a] Fragment ion series *m/z* 282, 296 and 355 in MS of **15** is analogous to that observed for **16**. Ion m/z 254 characterizes the aglycon part, containing the double bond 9,10. In MS of **12** dominates $[M-H]^+$ analogously as in the MS of the parent aglycon.[5b] The ABC system lacking the sugar moiety is characterized by a doublet *m/z* 167 and 154,[5c] and the whole aglycon part by *m/z* 238 and 237.[5b] Aglycon part of **15** is documented by a doublet m/z 236/235. In **13** this part is indicated by ions *m/z* 254/253.

NOE's observed between H-2' and H-2 and between H-1' and H-4' mean that the involved protons are located on the same face of the furanose ring.[6a] Therefore, the anomeric configuration of compounds **12** - **16** is β-. This conclusion is further supported by observations on the mixtures of anomers: H-1β resonates upfield of H-1α,[6a,b] H-1β exhibits longer T_1 relaxation time than H-1α[6c] (studied on **16**); C-1'α resonates in all cases about 3 ppm upfield of C-1'β.[6b] Even though the use of ^{1}H NMR coupling constants for the determination of anomeric configuration of ribofuranoside nucleotides is unreliable, it should be noted that $J_{1',2'}$ was smaller for all α-ribosides (3.8 - 4.2 Hz) than for the β-ribosides (6.0 - 6.1).

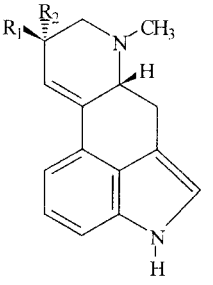
ANTIVIRAL ACTIVITY

The new ergot alkaloid ribosides **12, 14, 16** and all aglycons **1-5** were tested for their cytotoxicity, for their activity against the replication of HIV-1(III_B) and HIV-2(ROD) in acutely infected MT-4 cells and for their activity in persistently infected HUT-78/III_B cells. All compounds were inactive against the replication of HIV-1(III_B) and HIV-2(ROD) at subtoxic concentrations in acutely infected MT-4 cells. Of the established glycosylation inhibitors *N*-butyldeoxynojirimycin was also inactive, whereas 6-*O*-butanoylcastanospermine was active in the concentration range 10 - 20 μg/mL. The same holds true for the activity in persistently infected HUT-78/III_B cells while reference

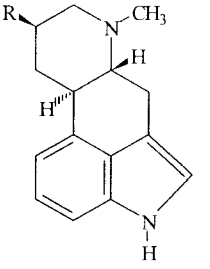
compound saquinavir (HIV protease inhibitor Ro31-5989) inhibited virus yield over a concentration range of 0.016 - 10 μg/mL.



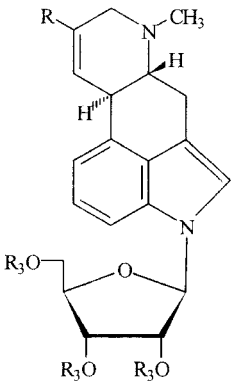
1 R = CH_3
2 R = CH_2OH
2a R = CH_2OAc



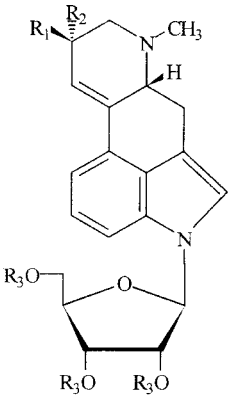
3 R_1 = CH_2OH, R_2 = H
3a R_1 = CH_2OAc, R_2 = H
4 R_1 , R_2 = CH_2



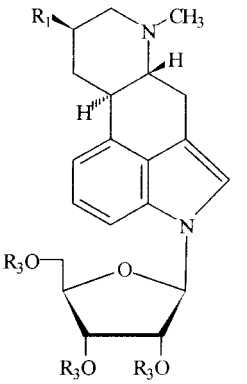
5 R = CH_2OH
5a R = CH_2OAc



7 R = CH_3 , R_3 = Bz
12 R = CH_3 , R_3 = H
8 R = CH_2OAc , R_3 = Bz
13 R = CH_2OH , R_3 = H



9 R_1 = CH_2OAc , R_2 = H, R_3 = Bz
14 R_1 = CH_2OH , R_2 = H, R_3 = H
10 R_1 , R_2 = CH_2 , R_3 = Bz
15 R_1 , R_2 = CH_2 , R_3 = H



11 R_1 = CH_2OAc, R_3 = Bz
16 R_1 = CH_2OH, R_3 = H

Compounds **12-16** and **1-5** were also tested for their broad spectrum antiviral activity in E_6SM cells cultures against *Herpes simplex* virus-1 (KOS), *Herpes simplex* virus-2 (G), *Vaccinia* virus, *Vesicular stomatitis* virus, thymidine kinase-deficient *Herpes simplex* virus-1 TK^- B2006 and *Herpes simplex* virus-1 TK^- VMW1837; in HeLa cell

cultures against *Vesicular stomatitis* virus, *Coxsackie* virus B4 and *Respiratory syncytial* virus and in Vero cell cultures against *Parainfluenza*-3 virus, *Reovirus*, *Sindbis* virus, *Coxsackie* virus B4 and *Punta Toro* virus. No specific antiviral activity was obtained with any of the compounds at concentrations (in average 50 to 200 μg/mL) that were below the cytotoxic threshold.

EXPERIMENTAL SECTION

All positive-ion electron impact mass spectra were recorded on a double-sector instrument (Finnigan MAT 90) of BE geometry (ionizing energy 70 eV, source temperature 200 °C, emission current 0.5 mA, accelerating voltage 5 kV; direct inlet, DIP temperature varied between 170 - 230 °C, samples dosed in microgram amounts for evaporation).

High-resolution measurements were carried out by the peak-matching method using Ultramark 1600F (PCR Inc., FL, U.S.A.) as a standard. The instrument was tuned to resolution of 8000 (10 % valley definition).

^{1}H and ^{13}C NMR spectra were measured on a Varian VXR-400 spectrometer (399.95 and 100.58 MHz, respectively) in CD_3OD at 25 °C. Residual solvent signal (δ_H 3.33, δ_C 49.3) served as an internal reference. Chemical shifts are given in the δ-scale; digital resolution was 0.0002 and 0.006 ppm, respectively. Carbon signal multiplicity was determined by an APT (Attached Proton Test) experiment. Manufacturer's software was used for 2D NMR (COSY, ROESY, HOM2DJ, HETCOR).

Clavines **1, 2, 3** and **5** were kindly donated by Galena Pharm. Co. Ltd. (Opava, Czech Republic), **4** was prepared from **2** according to previously published procedure.[4a] **2, 3** and **5** were acetylated by Ac_2O/Py (r.t., overnight) and purified by flash chromatography (SiO_2, CH_2Cl_2/MeOH 93 : 7) affording **2a**, **3a** or **5a** in approx. 90% yields. TMS derivatization of **1, 2a, 3a, 4** and **5a** was done as published previously.[4b]

General procedure for glycosylation - To a solution in 1,2-dichloroethane (6 mL) of the TMS derivative of the ergot alkaloids **1, 2a, 3a, 4** and **5a** prepared by refluxing (30 min) the aglycon (1 mmol) in acetonitrile (20 mL) with *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (280 μL, 1.5 mmol), **6** (550 mg, 1.1 mmol) was added and stirred

under nitrogen. $SnCl_4$ (0.6 mL) was slowly added under vigorous stirring. The resulting mixture was stirred at room temperature for 30 min. and was then poured into saturated $KHCO_3$ (100 mL) solution and extracted with $CHCl_3$ (3 × 50 mL). After drying over Na_2SO_4 and evaporation, the residue was chromatographed by flash chromatography using $CHCl_3$ as eluent to give peracylated glycoside. Further purification was done as indicated for each particular case.

1-(β-D-Ribofuranosyl)-agroclavine 12. According to the general procedure **1** (236 mg, 1 mmol) was allowed to react with **6** (550 mg, 1.1 mmol). Flash chromatography of the residue on silica gel ($CHCl_3$) afforded **7** (238 mg, 35 %). This compound (**7**) (150 mg, 0.2 mmol) was further dissolved in a mixture of $MeOH/H_2O/Et_3N$ (8:1:1) (50 mL) and stirred 24 hours at room temperature. After evaporation the residue was chromatographed on silica gel with $CH_2Cl_2/MeOH/NH_4OH$ (90:10:0.2) to give **12** (58 mg, 79 %) as brownish amorphous solid. EI-MS [m/z (% rel. int.)] 371 (12), 370.1889 ($[M]^{+\cdot}$, calc. for $C_{21}H_{26}N_2O_4$ 370.1893, 71%), 369 (100), 355 (2), 280 (1), 279 (3), 238 (3), 237 (20), 235 (2), 223 (1), 221 (1), 207 (1), 180 (1), 167 (3), 154 (2), 108 (5). ^{1}H-NMR (CD_3OD) δ 1.788 (3H, mt, H-17), 2.438 (1H, ddd, *J* = 11.8, 4.0, 9.6 Hz, H-5), 2.458 (3H, s, N-Me), 2.673 (1H, ddd, *J* = 14.4, 11.8, 1.2 Hz, H-4*ax*), 2.935 (1H, dddq, *J* = 16.3, 3.7, 2.1, ~1 Hz, H-7*u*), 3.244 (1H, dmt, *J* = 16.3, H-7*d*), 3.294 (1H, dd, *J* = 14.4, 4.0 Hz, H-4*eq*), 3.614 (1H, dmt, *J* = 9.6 Hz, H-10), 3.756 (1H, dd, *J* = 12.0, 4.3 Hz, H-5'*u*), 3.820 (1H, dd, *J* = 12.0, 3.5 Hz, H-5'*d*), 4.041 (1H, ddd, *J* = 4.3, 3.8, 3.5 Hz, H-4'), 4.245 (1H, dd, *J* = 5.6, 3.8 Hz, H-3'), 4.410 (1H, dd, *J* = 6.0, 5.6 Hz, H-2'), 5.936 (1H, d, *J* = 6.0, H-1'), 6.183 (1H, mt, H-9), 6.960 (1H, dd, *J* = 7.3, 1.2 Hz, H-12), 7.125 (1H, dd, *J* = 8.2, 7.3 Hz, H-13), 7.178 (1H, d, *J* = 1.2 Hz, H-2), 7.300 (1H, dd, *J* = 8.2, 1.2 Hz, H-14). ^{13}C-NMR δ 21.14 (q, C-17), 27.47 (t, C-4), 41.09 (q, N-Me), 41.73 (d, C-10), 61.33 (t, C-7), 63.55 (t, C-5'), 65.39 (d, C-5), 72.30 (d, C-3'), 75.91 (d, C-2'), 86.05 (d, C-4'), 91.20 (d, C-1'), 109.24 (d, C-14), 113.70 (s, C-3), 114.64 (d, C-12), 120.28 (d, C-2), 120.94 (d, C-9), 124.27 (d, C-13), 128.61 (s, C-16), 133.24, 136.20 (2C, 2s, C-11, C-15).

1-(β-D-Ribofuranosyl)-elymoclavine 13. According to the general procedure **2a** (296 mg, 1 mmol) was allowed to react with **6** (550 mg, 1.1 mmol). Flash chromatography of the residue on silica gel ($CHCl_3$) afforded intermediate **8** (215 mg,

29 %) as brown amorphous solid. Compound **8** (140 mg, 0.19 mmol) was dissolved in a mixture of $MeOH/H_2O/Et_3N$ (8:1:1) and stirred 24 hours at room temperature. Reaction was completed by a short heating to 50 °C. After evaporation the residue was chromatographed on silica gel with $CH_2Cl_2/MeOH/NH_4OH$ (90:22:0.3) to give **13** (53 mg, 73 %) as brownish amorphous solid. EI-MS [m/z (% rel. int.)] 386.1839 ($[M]^{+\cdot}$, calc. for $C_{21}H_{26}N_2O_5$ 386.1842, 83 %), 385 (100), 254 (25), 253 (64), 249 (28), 235 (25), 233 (22), 223 (22), 167 (19), 149 (39), 133 (42), 122 (53), 105 (69), 77 (42), 57 (67), 55 (42). ^{1}H-NMR (CD_3OD) δ 2.622 (3H, s, N-Me), 2.707 (1H, ddd, *J* = 11.7, 9.4, 3.7 Hz, H-5), 2.807 (1H, ddd, *J* = 14.1, 11.7, 1.7 Hz, H-4*ax*), 3.215 (1H, ddd, *J* = 16.1, 3.7, 2.2, H-7*eq*), 3.395 (1H, dd, *J* = 14.1, 3.7 Hz, H-4*eq*), 3.541 (1H, ddd, *J* = 16.1, 2.0, 0.5 Hz, H-7*ax*), 3.759 (1H, dd, *J* = 12.1, 4.2 Hz, H-5'*u*), 3.791 (1H, m, H-10), 3.821 (1H, dd, *J* = 12.1, 3.5 Hz, H-5'*d*), 4.042 (1H, ddd, *J* = 4.2, 3.8, 3.5 Hz, H-4'), 4.126 (2H, m, H-17), 4.250 (1H, dd, *J* = 5.6, 3.8 Hz, H-3'), 4.353 (1H, dd, *J* = 6.1, 5.6 Hz, H-2'), 5.946 (1H, d, *J* = 6.1 Hz, H-1'), 6.514 (1H, m, H-9), 7.034 (1H, dd, *J* = 7.2, 1.3 Hz, H-12), 7.159 (1H, dd, *J* = 8.3, 7.2 Hz, H-13), 7.234 (1H, d, *J* = 1.7 Hz, H-2), 7.337 (1H, dd, *J* = 8.3, 1.3 Hz, H-14). ^{13}C-NMR (CD_3OD) δ 27.37 (t, C-4), 40.92 (d, C-10), 41.23 (q, N-Me), 57.71 (t, C-7), 63.55 (t, C-5'), 65.26 (t, C-17), 65.68 (d, C-5), 72.33 (d, C-3'), 75.98 (d, C-2'), 86.16 (d, C-4'), 91.20 (d, C-1'), 109.51 (d, C-14), 113.23 (s, C-3), 114.74 (d, C-12), 120.61 (d, C-2), 121.82 (d, C-13), 124.33 (d, C-9), 128.57 (s, C-16), 132.41 (s, C-8), 136.28 (s, C-11), 136.72 (s, C-15).

1-(β-D-Ribofuranosyl)-lysergol 14. According to the general procedure **3a** (296 mg, 1 mmol) was allowed to react with **6** (550 mg, 1.1 mmol). Flash chromatography of the residue on silica gel ($CHCl_3$) afforded compound **9** (230 mg, 29 %) as colourless amorphous solid. Compound **9** (139 mg, 0.19 mmol) was dissolved in a mixture of $MeOH/H_2O/Et_3N$ (8:1:1) and stirred 24 hours at room temperature. Reaction was completed by a short heating upto 50 °C. After evaporation the residue was chromatographed on silica gel with $CH_2Cl_2/MeOH/NH_4OH$ (90:22:0.3) to give **14** (58 mg, 79 %) as colourless amorphous solid. EI-MS [m/z (% rel. int.)] 388 (5), 387 (22), 386.1838 ($[M]^{+\cdot}$, calc. for $C_{21}H_{26}N_2O_5$ 386.1842, 100 %), 355 (5), 296 (15), 282 (2), 268 (4), 267 (18), 255 (8), 254 (48), 253 (10), 235 (5), 233 (5), 223 (9), 222 (5), 221 (14), 220 (4), 219 (4), 207 (5), 194 (4), 193 (11), 192 (8), 180 (5), 167 (5), 154 (5), 74 (4), 57 (4).

^{1}H-NMR (CD_3OD) δ 2.606 (3H, s, N-Me), 2.326 (1H, dd, *J* = 11.4, 10.8 Hz, H-7ax), 2.650 (1H, ddd, *J* = 14.5, 11.4, 1.7 Hz, C-4*ax*), 2.87 (1H, mt, H-8), 3.153 (1H, ddmt, *J* = 11.4, ~2 Hz, H-7*eq*), 3.160 (1H, ddd, *J* = 11.4, 5.3, 1.5 Hz, H-5), 3.555 (1H, dd, *J* = 10.8, 7.2 Hz, H-17*u*), 3.566 (1H, dd, *J* = 14.5, 5.3 Hz, H-4*eq*), 3.642 (1H, dd, *J* = 10.8, 6.1, H-17*d*), 3.756 (1H, dd, *J* = 12.0, 4.2 Hz, H-5'*u*), 3.818 (1H, dd, *J* = 12.0, 3.6 Hz, H-5'*d*), 4.042 (1H, ddd, *J* = 4.2, 3.7, 3.6 Hz, H-4'), 4.247 (1H, dd, *J* = 5.6, 3.7 Hz, H-3'), 4.426 (1H, dd, *J* = 6.0, 5.6 Hz, H-2'), 5.944 (1H, d, *J* = 6.0 Hz, H-1'), 6.438 (1H, mt, H-9), 7.191 (1H, dd, *J* = 7.4, 1.2 Hz, H-12), 7.157 (1H, dd, *J* = 7.5, 7.4, H-13), 7.217 (1H, d, *J* = 1.7 Hz, H-2), 7.358 (1H, dd, *J* = 7.5, 1.2 Hz, H-14). ^{13}C-NMR δ 27.94 (t, C-4), 40.07 (d, C-8), 44.28 (q, N-Me), 58.32 (t, C-7), 63.57 (t, C-5'), 64.93 (d, C-5), 65.67 (t, C-17), 72.33 (d, C-3'), 75.80 (d, C-2'), 86.12 (d, C-4'), 91.26 (d, C-1'), 110.20 (d, C-14), 112.36 (s, C-3), 114.09 (d, C-12), 120.75 (d, C-2), 123.05 (d, C-9), 124.57 (d, C-13), 128.48, 129.50 (2C, 2s, C-16, C-11), 136.48, 136.61 (2C, 2s, C-10, C-15).

1-(β-D-Ribofuranosyl)-lysergene 15. According to the general procedure **4** (236 mg, 1 mmol) was allowed to react with **6** (550 mg, 1.1 mmol). Flash chromatography of the residue on silica gel ($CHCl_3$) afforded compound **10** (183 mg, 27 %) as brownish amorphous solid. Compound **10** (136 mg, 0.2 mmol) was dissolved in a mixture of MeOH/H_2O/Et_3N (8:1:1) and stirred 24 hours at room temperature. After evaporation the residue was chromatographed on silica gel with CH_2Cl_2/MeOH/NH_4OH (90:9:0.2) to give **15** (46 mg, 63 %) as brown amorphous solid. EI-MS [m/z (% rel. int.)] 369 (6), 368.1733 ($[M]^{+\cdot}$, calc. for $C_{21}H_{24}N_2O_4$ 368.1736, 100 %), 367 (17), 278 (7), 277 (5), 263 (4), 249 (5), 247 (4), 238 (4), 237 (13), 236 (58), 235 (62), 234 (7), 233 (10), 221 (10), 220 (9), 219 (10), 205 (6), 192 (7), 154 (5), 122 (8), 105 (12), 77 (11), 57 (5), 55 (5). ^{1}H-NMR (CD_3OD) δ 2.542 (3H, s, N-Me), 2.651 (1H, ddd, *J* = 14.6, 11.6, 1.9 Hz, H-4*ax*), 3.210 (1H, ddd, *J* = 12.4, 1.9, 1.9 Hz, H-7*eq*), 3.236 (1H, ddd, *J* = 11.6, 5.9, 2.0 Hz, H-5), 3.524 (1H, d, *J* = 12.4 Hz, H-7*ax*), 3.519 (1H, dd, *J* = 14.6, 5.9 Hz, H-4*eq*), 3.756 (1H, dd, *J* = 12.0, 4.2 Hz, H-5'*u*), 3.819 (1H, dd, *J* = 12.0, 3.5 Hz, H-5'*d*), 4.046 (1H, ddd, *J* = 4.2, 3.8, 3.5 Hz, H-4'), 4.246 (1H, dd, *J* = 5.6, 3.8 Hz, H-3'), 4.420 (1H, dd, *J* = 6.0, 5.6 Hz, H-2'), 5.008 (1H, m, H-17*u*), 5.104 (1H, m, H-17*d*), 5.948 (1H, d, *J* = 6.0 Hz, H-1'), 7.001 (1H, d, *J* = 2.0 Hz, H-9), 7.184 (1H, dd, *J* = 8.0, 7.4 Hz, H-13), 7.241 (1H, d, *J* = 7.9 Hz, H-2), 7.261 (1H, ddd, *J* = 7.4, 0.7, 0.6 Hz, H-12), 7.394 (1H, dd,

J = 8.0, 0.7 Hz, H-17). ^{13}C-NMR (CD_3OD) δ 27.65 (t, C-4), 43.22 (q, N-Me), 59.86 (t, C-7), 63.54 (d, C-5'), 63.86 (d, C-5), 72.32 (d, C-3'), 75.85 (d, C-2'), 86.14 (d, C-4'), 91.25 (d, C-1'), 110.82 (d, C-14), 112.19 (t, C-17), 112.27 (s, C-3), 114.48 (d, C-12), 121.14 (d, C-2), 122.96 (d, C-9), 124.65 (d, C-13), 128.79 (s, C-16), 136.58 (s, C-15), 137.29 (s, C-10), 141.82 (s, C-8).

1-(β-D-Ribofuranosyl)-9,10-dihydrolysergol 16. According to the general procedure **5a** (298 mg, 1 mmol) was allowed to react with **6** (550 mg, 1.1 mmol). Flash chromatography of the residue on silica gel ($CHCl_3$) afforded compound **11** (237 mg, 32 %) as colourless amorphous solid. Compound **11** (149 mg, 0.2 mmol) was dissolved in a mixture of MeOH/H_2O/Et_3N (8:1:1) and stirred 24 hours at room temperature. Reaction was finished by a short heating upto 50 °C. After evaporation the residue was chromatographed on silica gel with CH_2Cl_2/MeOH/NH_4OH (90:22:0.3) to give **14** (58 mg, 79 %) as white crystals from MeOH. EI-MS [m/z (% rel. int.)] 389 (24), 388.1993 ($[M]^{+\cdot}$, calc. for $C_{21}H_{28}N_2O_5$ 388.1998, 100 %), 387 (2), 298 (4), 257 (4), 256 (23), 255 (5), 223 (4), 197 (2), 168 (2), 167 (4), 154 (5), 144 (5), 98 (2). ^{1}H-NMR (CD_3OD) δ 1.090 (1H, ddd, J = 13.4, 1.9, 1.3 Hz, H-9*ax*), 2.09 (1H, mt, H-8), 2.133 (1H, ddd, J = 11.2, 10.0, 4.1 Hz, H-5), 2.494 (3H, s, N-Me), 2.618 (1H, ddd, J = 14.5, 11.2, 1.6 Hz, H-4*ax*), 2.674 (1H, dmt, J = ~12 Hz, H-9*eq*), 2.866 (1H, ddmt, J = ~12, ~10 Hz, H-10), 3.161 (1H, dmt, J = 11.2 Hz, H-7*eq*), 3.262 (1H, ddd, J = 13.4, 1.9, 1.3 Hz, H-7*ax*), 3.405 (1H, dd, J = 14.5, 4.1 Hz, H-4*eq*), 3.474 (1H, dd, J = 10.9, 6.8 Hz, H-17*u*), 3.583 (1H, dd, J = 10.9, 5.4 Hz, H-17*d*), 3.752 (1H, dd, J = 12.0, 4.3 Hz, H-5'*u*), 3.814 (1H, dd, J = 12.0, 3.5 Hz, H-5'*d*), 4.035 (1H, ddd, J = 4.3, 3.8, 3.5 Hz, H-4'), 4.241 (1H, dd, J = 5.6, 3.8 Hz, H-3'), 4.410 (1H, dd, J = 6.0, 5.6 Hz, H-2'), 5.942 (1H, d, J = 6.0 Hz, H-1'), 6.907 (1H, dd, J = 7.3, 1.0 Hz, H-12), 7.131 (1H, dd, J = 8.2, 7.3 Hz, H-13), 7.175 (1H, d, J = 1.6 Hz, H-2), 7.308 (1H, dd, J = 8.2, 1.0 Hz, H-14). ^{13}C-NMR δ 27.75 (t, C-4), 32.10 (t, C-9), 39.61 (d, C-8), 41.41 (d, C-10), 43.69 (q, N-Me), 59.76 (t, C-7), 63.57 (t, C-5'), 66.55 (t, C-17), 69.05 (d, C-5), 72.33 (d, C-3'), 75.91 (d, C-2'), 86.05 (d, C-4'), 91.20 (d, C-1'), 109.17 (d, C-14), 113.36 (s, C-3), 115.02 (d, C-12), 120.12 (d, C-2), 124.39 (d, C-13), 128.50 (s, C-16), 134.27, 136.03 (2C, 2s, C-11, C-15).

The methods used for measuring anti-HIV activity (in MT-4 cells) and broad-spectrum antiviral activity (in E_6SM, HeLa and Vero cells) have been described elsewhere.[7a,b]

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