Pergamon

Tetrahedron, Vol. 53, No. 12, pp. 4503-4510, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00123-3

N-Deoxyribosides of Ergot Alkaloids: Synthesis and Biological Activity

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Abstract: *N*-Deoxyribosides of agroclavine (**1**), lysergol (**2**), and 9,10-dihydrolysergol (**3**) were prepared by $SnCl_4$ -catalyzed N-glycosylation of their TMS derivatives with 1-chloro-3,5-di-*O*-toluoyl-2-deoxy-D-ribofuranose. None of the new compounds exhibited activity against HIV. © 1997 Elsevier Science Ltd.

Ergot alkaloids (EA) have a broad spectrum of pharmacological activities including central, neurohumoral, and peripheral effects. Besides their activity mediated by neurotransmitter receptors,[1] clavine alkaloids exhibit also antibiotic[2] and cytostatic activities. Some of these derivatives were found to be effective against *S. aureus, E. coli, Pseudomonas aeruginosa,* and *Candida albicans*.[4] Their antibiotic effect can be enhanced by reduction of the double bond in the ring D and by aliphatic substitution at the *N*-1 and *N*-6 atoms of ergoline.[3] Antibiotic activity of EA was ascribed to inhibition of nucleic acid replicatory processes.[4] Cytostatic activity of agroclavine and its derivatives were tested against mouse lymphoma L 5178y [5] and the best activities were found with *N*-1 propylated clavine derivatives that inhibit incorporation of ^{3}H-thymidine into DNA without influencing the activity of both α- and β-DNA-polymerase and RNA export from nucleus.[4,6,7] Such an antineoplastic activity is comparable with that of the clinically used cytostatics bleomycin, adriamycin, and daunomycin.[6]

Antineoplastic and antiviral activity of many heterocycles could be improved by their *N*-2-deoxy-ribosylation. Preparation of *N*-2-deoxyribosides of EA could create analogous compounds to nucleosides with the aglycon possessing both neurohumoral and cytostatic activity. *N*-2-deoxyglycosides of indole compounds are very scarce and this type of glycosylation of EA has not been studied so far.

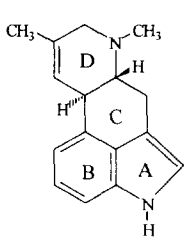
We report here the preparation of *N*-2-deoxy-D-ribosides of ergot alkaloids and evaluation of their antiviral activity.

Results and Discussion

All enzymatic methods tested[8] for the transfer of β-2-deoxyribosyl moiety to N-1 of agroclavine (**1**) using microbial deoxyribonucleotide transferases (e.g., from *E. coli*) failed presumably due to narrow specificity of the particular enzymes to the aglycone molecule. Therefore, a synthetic approach to the preparation of β-*N*-deoxyribosyl ergolines was attempted. As the representatives of different ergot alkaloid classes,

agroclavine (**1**) ($\Delta^{8,9}$-ergolines), lysergol (**2**) ($\Delta^{9,10}$-ergolines) and 9,10-dihydrolysergol (**3**) (9,10-dihydroergolines) were chosen to demonstrate versatility of the synthetic methods used.

2-Deoxy-3,5-di-*O*-*p*-toluoyl-α-D-ribofuranosylchloride[9] was used as a glycosyl donor. *N*-Glycosylation *via* respective sodium salt prepared with NaH, MeCN[10,11] or by powdered KOH in DMSO[12] followed by addition of sugar halogenide was attempted.[10,11] However, this method yielding preferentially β-deoxyribofuranosides, failed.



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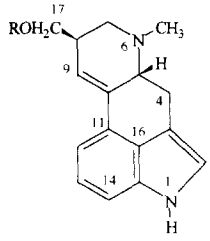

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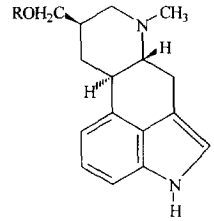
2a R = Ac



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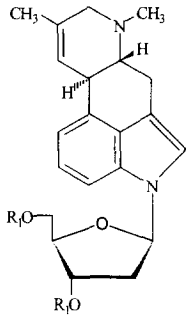
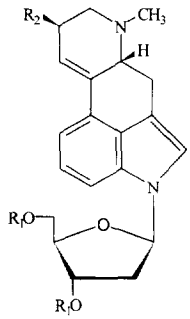
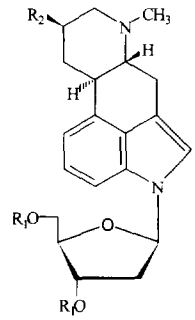
3a R = Ac



4a R_1 = Tol
4b R_1 = H



5a R_1 = Tol, R_2= CH_2OAc
5b R_1 = H, R_2 = CH_2OH



6 R_1 = Tol, R_2 = CH_2OAc
6a R_1 = H, R_2 = H

α-Anomers
7a R_1 = Tol
7b R_1 = H

α-Anomers
8a R_1 = Tol, R_2= CH_2OAc
8b R_1 = H, R_2 = CH_2OH

α-Anomers
9a R_1 = Tol, R_2 = CH_2OAc
9b R_1 = H, R_2 = H

Scheme 1

Glycosylation of *N*-1-trimethylsilyl (*N*-1-TMS) derivatives[13] of **1**, **2a** and **3a** under Friedel-Crafts catalysis using 1,2-dichloroethane as a solvent and $SnCl_4$ or TMS-*O*-Tf as catalysts failed presumably because of the fast decomposition of the glycosylation products. However, the reaction performed in MeCN catalysed by $SnCl_4$ was completed within 20 min. and the TMS derivatives of alkaloids reacted quantitatively. TLC revealed

two new similar products formed in approximately the same proportion. After their separation, ^{1}H NMR ($CDCl_3$) proved that both products were the protected *N*-2-deoxyribosides of the parent alkaloids. Substitution at N-1 of all **4 a,b**, **5 a,b**, and **6 a,b** was confirmed by the change of multiplicity of H-2 from dd into d. Nevertheless, the configuration at C-1' was not determined due to missing analogy in the literature. For biological studies only β-anomer was required.

It is known[14] that the above reaction preferentially provides the β-anomer and the α-anomer is formed consecutively by racemization of the product by the released HCl. Kinetic study revealed that in the first 1-2 minutes after addition of the catalyst ($SnCl_4$), mostly the product having lower R_f (product B) was formed, and within ca 20 min. an approximately equimolar mixture of both anomers was formed. Therefore, we have preliminarily assigned B-product to be β-anomer and the reaction conditions (concentrations, time, temperature) were optimized accordingly. This estimate was eventually confirmed by NOE experiments - *vide infra*. Optimum reaction conditions are given in the Experimental.

Table 1 ^{1}H NMR Data [ppm] of Ergot Alkaloid *N*-2-Deoxyribofuranosides (CD_3OD, 25 °C)

Protons	**4b**	**7b**	**5b**	**8b**	**6b**	**9b**
Aglycone moiety						
2	7.173	7.353	7.214	7.419	7.214	7.424
4*a*[b]	2.722	2.744	2.697	2.731	2.72-2.81[c]	2.78[a]
4*e*[b]	3.34[a]	3.368	3.602	3.629	3.544	3.587
5	2.517	2.535	3.33[a]	3.40[a]	2.566	2.80[a]
7*a*	3.012	3.030	2.487	2.543	2.419	2.664
7*e*	3.293	3.301	3.264	3.298	3.376	3.504
8	-	-	2.918	2.932	2.202	2.262
9*d*	6.228	6.238	6.463	6.479	2.72-2.81[c]	2.84[a]
9*u*	-	-	-	-	1.244	1.339
10	3.69[a]	3.697	-	-	3.056	3.172
12	6.993	6.996	7.205	7.213	6.958	6.980
13	7.148	7.146	7.170	7.172	7.168	7.181
14	7.284	7.321	7.347	7.384	7.323	7.376
17*d*[b]	1.816	1.820	3.664	3.677	3.646	3.681
17*u*[b]	-	-	3.580	3.594	3.528	3.557
NCH_3	2.517	2.533	2.707	2.747	2.733	2.880
Glycosidic moiety						
1'	6.399	6.347	6.396	6.365	6.406	6.367
2'*d*	2.613	2.822	2.624	2.838	2.614	2.836
2'*u*	2.318	2.413	2.322	2.410	2.324	2.396
3'	4.485	4.461	4.491	4.465	4.489	4.469
4'	3.948	4.094	3.955	4.104	3.953	4.106
5'*d*	3.730	3.738	3.735	3.735	3.732	3.736
5'*u*	3.689	3.654	3.693	3.653	3.692	3.658

[a] Strongly coupled, center of multiplet;
[b] Symbols used in all ^{1}H NMR data tables: *e* - equatorial, *a* - axial, *d* - downfield, *u* - upfield
[c] Obscured by the solvent signal

The reaction was quenched by sat. $NaHCO_3$ and the products extracted by chloroform. Purification by flash chromatography afforded 2-deoxy-ribosides **4a**, **5a** and **6a** in 20 - 40 % yields. The respective α-anomers (10 - 20 % yields) were also isolated for the spectral studies. These intermediates were deprotected by a mixture of Et_3N : MeOH : H_2O 1/8/1 (40 °C, 3 days). 2-Deoxy-ribosides **4b**, **5b**, **6b** and their α-anomers **7b**, **8b** and **9b** were purified by column chromatography.

Compounds **4b**, **5b**, **7b**, **8b** gave molecular ions in the EI MS, **6b** and **9b** displayed $[M+H]^+$ ions under electrospray ionization conditions. All observed fragments correspond to the aglycon fragmentation.[12-18] Mass spectra of anomeric pairs showed small differences in intensity.

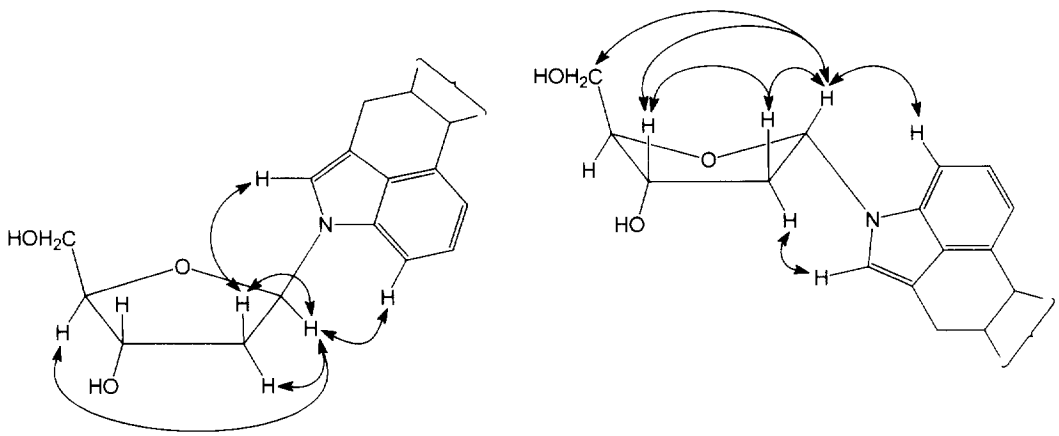
Table 2. ^{13}C NMR Data [ppm] of Ergoline *N*-2-Deoxyribofuranosides (CD_3OD, 25 °C)

Carbons	**4b**	**7b**	**5b**	**8b**	**6b**	**9b**
Aglycone moiety						
2	120.01	121.06	120.76	121.85	120.19	121.51
3	113.75	113.33	111.43	110.86	112.29	111.15
4	27.55	27.57	27.37	27.28	27.13	27.75
5	65.52	65.61	64.73	64.77	68.96	69.01
N-Me	41.15	41.16	43.71	43.62	43.01	42.51
7	61.42	61.45	57.88	57.82	61.32	60.99
8	133.27	133.24	39.45	39.33	39.10	38.74
9	120.98	121.08	124.72	124.59	31.50	32.03
10	41.84	41.86	136.36	136.29	40.95	40.66
11	133.10	133.07	128.80	128.61	133.19	132.62
12	114.62	114.50	114.30	114.16	115.18	115.18
13	124.31	124.17	123.04	122.93	124.55	124.50
14	109.06	109.23	110.39	110.51	109.37	109.78
15	136.06	135.93	135.39	135.23	135.81	135.73
16	128.59	128.67	128.26	128.23	128.20	128.19
17	21.13	21.13	65.22	65.15	65.94	65.55
Glycosidic moiety						
1′	88.26	87.95	88.34	88.20	88.31	88.23
2′	41.35	41.52	41.26	41.64	41.36	41.64
3′	73.10	72.57	73.09	72.62	73.09	72.65
4′	86.87	87.00	86.98	87.10	86.93	87.15
5′	63.94	63.61	63.93	63.56	63.93	63.59

Empirical rules for the determination of anomeric configuration based on the differences in proton or carbon chemical shifts[19] worked well with *N*-ribofuranosides of ergot alkaloids[16]. However, they completely failed in this series because of small difference (0.03 - 0.05 ppm and 0.08 - 0.31 ppm for ^1H and ^{13}C, respectively). Equally useless were the coupling constants $J_{1',2'}$ (Table 3), again due to a small difference (about 0.9 Hz) between both pairs. An attempt to make use of longer longitudinal relaxation times of H-1′ reported for β-anomers[16,20] was unsuccessful owing to nearly equal T_1 values. The unambiguous answer was provided by NOE measurements that established which deoxyribose protons are located on the same side of the five-membered ring, regardless its conformation. The compounds in which H-1′ had a contact with H-4′ were

assigned to the β-series, those exhibiting crosspeaks H-1′*vs.* H-3′ and H-1′ *vs.* H-5′ were placed into the α-series (Fig. 1).

We have observed that in all studied compounds, H-2 is the most downfield resonating aromatic proton in the α-series whereas in the β- one the order of chemical shifts of H-2 and H-14 is reversed. This empirical rule might be useful in configuration assignment of the analogous compounds (2-deoxyribosides of indolic compounds) (Table 1). Smaller, however, consistent differences can be observed in chemical shifts of the protons at C-1'.



β-deoxyriboside α-deoxyriboside

Figure 1 Diagnostic Nuclear Overhauser Effects in both α- and β- Deoxyribosides

Table 3 Proton - Proton Coupling Constants (*J* [Hz]) of the Glycosidic Moiety of the Ergot Alkaloid 2-Deoxyribofuranosides.

Protons	**4b**	**7b**	**5b**	**8b**	**6b**	**9b**
1`, 2*u*`	6.0	5.1	5.9	5.0	5.9	4.9
1`, 2*d*`	8.2	7.4	8.1	7.5	8.1	7.5
2*u*`, 3`	3.0	5.0	3.1	4.9	3.0	4.8
2*d*`, 3`	6.3	7.5	6.3	7.5	6.4	7.5
3`, 4`	3.1	4.9	3.1	4.7	3.1	4.7
4`, 5*u*`	4.8	4.7	5.0	4.7	4.8	4.7
4`, 5*d*`	4.4	3.5	4.6	3.6	4.4	3.6
2*u*`, 2*d*`	-13.5	-13.9	-13.5	-13.9	-13.5	-13.9
5*u*`, 5*d*`	-11.8	-11.9	-11.8	-11.9	-11.8	-11.9

Antiviral activity

New ergot alkaloid β-2-deoxyribosides **4b**, **5b** and **6b** were tested for their cytotoxicity, and for activity against the replication of HIV-1(III_B) and HIV-2(ROD) in acutely infected MT-4 cells, and 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, where the reference compound saquinavir (HIV protease inhibitor Ro31-5989) inhibited virus yield in the concentration range of 0.016 - 10 μg/ml.

The compounds will be further evaluated for their potential cytostatic activity.

EXPERIMENTAL

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). For the measurement of the positive-ion electrospray ionisation spectra (ESI) the sample was dissolved in methanol : water (1/1, v/v, 10 pmol/μl) and it was continuously infused through a stainless capillary held at 3.0 kV into the electrospray ion source (Finnigan MAT) via linear syringe pump at a rate of 30 μl/min. A mixture of polypropylene glycols M_r = 425 was used as an internal calibrant for high resolution mass measurements.

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

General procedure for glycosylation - To a solution in MeCN (6 ml) of the TMS derivative (1 mmol) of the respective ergot alkaloid 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-ribofuranosylchloride (320 mg, 0.8 mmol) was added and the mixture was stirred under nitrogen at 10 °C. $SnCl_4$ (0.6 ml dissolved in 2.5 ml MeCN) was slowly added under vigorous stirring. The resulting mixture was stirred at room temperature for 10 min. and 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 glycosides (α and β). On the TLC (silica gel 60, F_{254}, Merck; mobile phase - chloroform with

4% MeOH) all α-anomers studied (in protected form) migrate slightly faster than the β-anomers. In deprotected form the α- and β- anomers are inseparable. The respective products were deacylated with a mixture of $MeOH/H_2O/Et_3N$ = 8:1:2 (50 ml, 72 hours, 40 °C) under stirring. After evaporation, the residue was chromatographed on silica gel with $CH_2Cl_2/MeOH/NH_4OH$ (90:10:0.2).

1-(β-D-2'-Deoxyribofuranosyl)-agroclavine 4b. According to the general procedure *N*-TMS derivative of **1** (236 mg, 1 mmol) was allowed to react with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-ribofuranosylchloride (320 mg, 0.8 mmol). Flash chromatography of the residue on silica gel (toluene/$CHCl_3$ = 15 : 85) afforded **4a** (204 mg, 35 %) and α-anomer **7a** (94 mg, 16 %). These compounds yielded after deprotection and further chromatography **4b** (95 mg, 75 %) or **7b** (38 mg, 68 %) as brownish amorphous solids. EI-MS of **4b** [m/z (% rel. int.)] 355 (20), 354.1931 (calc. 354.1943 for $C_{21}H_{26}N_2O_3$, 86%), 353 (100), 339 (4), 323 (5), 294 (3), 293 (4), 264 (4), 263 (9), 238 (12), 237 (41), 221 (3), 207 (3), 167 (4), 154 (3), 149 (9), 132 (3), 108 (6), 57 (3), 43 (3).

1-(β-D-2'-Deoxyribofuranosyl)-lysergol 5b. According to the general procedure *N*-TMS derivative of **2a** (296 mg, 1 mmol) was allowed to react with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-ribofuranosylchloride (320 mg, 0.8 mmol). Flash chromatography of the residue on silica gel (toluene/$CHCl_3$ = 15 : 85) afforded **5a** (214 mg, 33 %) and α-anomer **8a** (90 mg, 14 %). These compounds yielded after deprotection and further chromatography **5b** (84 mg, 69 %) or **8b** (34 mg, 67 %) as white amorphous solids. EI-MS of **5b** [m/z (rel. int. %)]: 371 (25), 370.1902 (calc. 370.1893 for $C_{21}H_{26}N_2O_4$, 100%), 339 (6), 307 (4), 280 (10), 255 (15), 254 (82), 253 (11), 223 (15), 221 (15), 193 (15), 192 (11), 180 (9), 167 (5), 154 (6), 149 (5), 111 (6), 74 (8), 57 (8), 43 (13), 41 (7).

1-(β-D-2'-Deoxyribofuranosyl)-9,10-dihydrolysergol 6b. According to the general procedure *N*-TMS derivative of **2a** (298 mg, 1 mmol) was allowed to react with 2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-ribofuranosylchloride (320 mg, 0.8 mmol). Flash chromatography of the residue on silica gel (toluene/$CHCl_3$ = 15 : 85) afforded **6a** (246 mg, 38 %) and α-anomer **9a** (117 mg, 18 %). These compounds yielded after deprotection and further chromatography **6b** (105 mg, 75 %) or **9b** (45 mg, 68 %) as white microcrystalline solids. ESI-MS of **6b** [m/z (rel. int. %): 374 (25), 373.2128 (calc. 373.2127 for $C_{21}H_{29}N_2O_4$, 100%), 257 (38), 208 (53), 193 (15), 192 (10), 182 (28), 180 (16), 168 (23), 167 (28), 155 (18), 154 (33), 144 (4).

The methods used for measuring anti-HIV activity (in MT-4 cells) have been described elsewhere.[21,22]

ACKNOWLEDGEMENT. This work was supported by EU grant PECO ERBCIPDCT 930194 and by the grant No. 203/96/1267 from the Grant Agency of the Czech Republic. Thanks are due to Dr. A. Píškala, Inst. of Organic Chemistry and Biochemistry, CAS, Prague for valuable advice and moral support throughout the work.

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(*Received in UK* 23 *December* 1996; *revised* 27 *January* 1997; *accepted* 30 *January* 1997)