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AN APPROACH TO NOVEL ELLIPTICINE GLYCOSIDES

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Abstract - Reactions of 6-methylellipticine (3a) with the aim of preparing various functionalized derivatives have been studied. A base-(LDA) mediated reaction of 3a with formaldehyde results in a C(11)-hydroxyethyl derivative (3d), which has been coupled with ribose-, glucose- and galactose derivatives to give, after deprotection, novel ellipticine glycosides 3e-g. The sequence of reactions constitute a new strategy for the synthesis of a wide range of ellipticine derivatives with improved solubility characteristics.

The alkaloid ellipticine (1a) and a variety of its derivatives have been isolated from several members of the Ochrosia species[1] and more recently from the stem bark of Strychnos dinklagei Gilg. (loganiceae)[2]. The parent alkaloid 1a and the oxygenated derivatives 1b and 1c exhibit broad-spectrum antitumour activity in experimental models. The synthetic ellipticinium salt 2 has been demonstrated to produce useful clinical responses in thyroid and renal carcinomas, soft tissue sarcomas and in particular in metastatic breast cancer where weekly doses of 80-100 mg/m^2 lead in 18-40% cases to complete and partial remissions. It is noteworthy that the commonly observed side-effects of cytostatics like cardio- and hepatotoxicity, as well as bone marrow depression and alopecia were not observed. Limiting side effects were nausea, hyposalivation, anorexia, periphlebitis and renal toxicity[3]. The extreme low water-solubility of many ellipticine derivatives constitutes a serious practical disadvantage and is a feature which resulted in the termination of a clinical study of 9-hydroxyellipticine. Side effects and solubility characteristics plead for the development of new, water-soluble ellipticine derivatives with improved chemotherapeutic index.

In the light of the aforementioned, a great deal of interest has recently focussed on the development of synthetic methodology for construction of the ellipticine system[4]. As a result of these studies a large variety of derivatives have been synthesized and screened for antitumour activity[5]. The finding that 9-hydroxyellipticine (1b) is the active metabolite of ellipticine (1a) (Chart I), together with recent suggestions concerning the mechanism of action of the alkaloid, constitute the background for the rational design of ellipticine derivatives which posses antitumour activity and improved water-solubility.

We have previously reported a facile approach to the construction of the ellipticine skeleton[6] and described its application in the preparation of novel C(11)-side chain substituted derivatives[7]. Of these (ellipticine analogues), the aza-mustard derivative 3b and the riboside 3c (Chart I) showed significant activity in in vivo screening in the P 388 murine leukemia (experimental tumour). The relatively low toxicity and improved solubility characteristics of 3c in particular have prompted us to investigate the chemistry of the ellipticine template with the aim of synthesizing a series of 9-hydroxyellipticine derivatives bearing a range of carbohydrate moieties.

This paper presents the method developed by us for the direct functionalization of the C(11)-side chain. The C(11)-hydroxyethyl derivative 3d has been converted into the corresponding glycosides

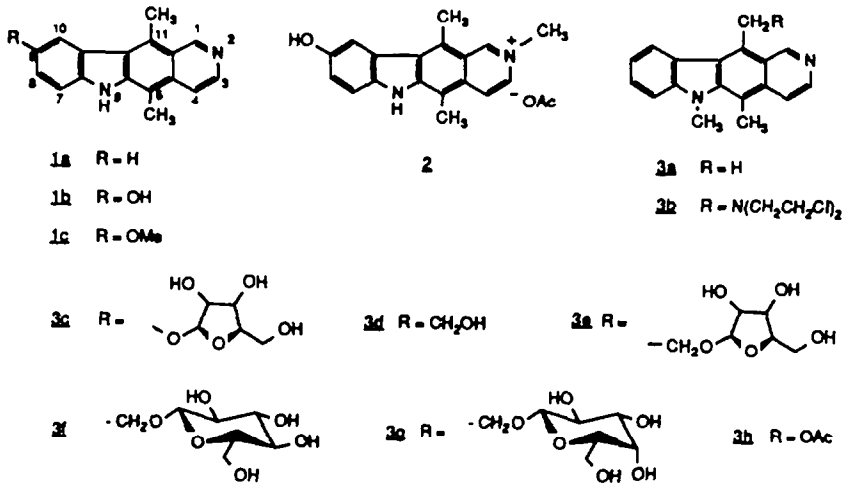
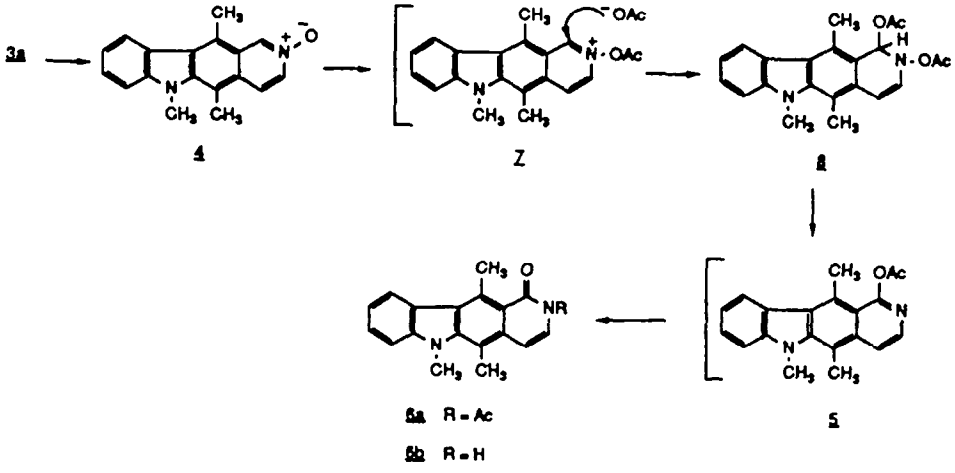
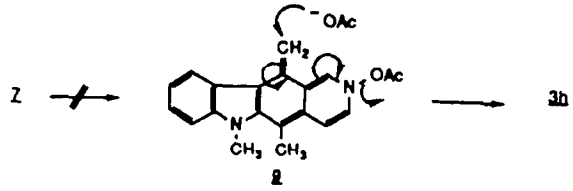


Chart I



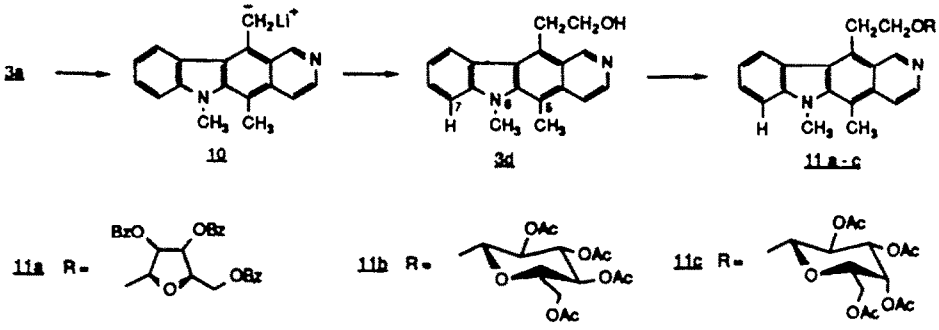


Scheme 1

(3e-g). Preliminary reports on these results have been communicated earlier[7].

Our initial approach to the functionalization of 3a considered the application of pyridine-N-oxide chemistry to the ellipticine template. Since the C(11)-methyl of 3a is conjugatively similar to the methyl group of picoline, it was projected that the N(2)-oxide (4, Scheme 1) could be converted into the acetoxymethyl derivative 3h, by reaction with acetic anhydride, following a mechanism analogous to the one proposed for the transformation of 2-methylpyridine-N-oxide to 2-acetoxymethylpyridine[8].

Starting out with ellipticine, the N(6)-methyl derivative (3a) was prepared in high yield and subsequently oxidized by m-chloroperbenzoic acid to the corresponding N(2)-oxide (4). When 4 was heated to reflux with acetic anhydride and sodium acetate, the reaction was found not to follow the expected and desired course, that is, 7 → 9 → 3h (Scheme 1). Instead, two products, namely 6a and 6b were isolated from the reaction mixture, the relative amounts of which varied with the conditions of workup of the mixture. The structures of both products could be elucidated from their spectral data[9] (vide experimental). It follows from these results that while the expected intermediate 7 is formed during the reaction, its further fate involves a collapse leading to 8, from which acetic acid is eliminated to give 1-acetoxy-6-methylellipticine (5). The formation of 6a presumably proceeds via N-acylation of 5 by acetic anhydride, followed by an attack of acetate ion on the acetoxy carbonyl moiety at C(1). Hydrolysis of 6a during workup leads to the pyridone derivative 6b. The fact that 4 is not converted to 3h implies that either deprotonation of 7 by acetate ion is not effectively competitive with its collapse (to 8) or that a nucleophilic attack on 9 is not a favoured process. Inspection of structure of 9 shows that the exocyclic methylene group constitutes the terminal end of two dienamine chromophores. In particular, conjugation with the indole nitrogen would confer a nucleophilic rather than an electrophilic character upon the exocyclic methylene system.



Scheme 2

In the search of other approaches for side chain functionalization of 3a, we have examined the base-mediated reactions of the ellipticine template. The C(11)-methyl, in view of its conjugation with C(2)-nitrogen, can be easily deprotonated by lithium diisopropylamide. When the resulting anion (10, Scheme 2) was quenched with formaldehyde, the hydroxymethylation product 3d (Chart I) was formed in practically useful yields. The fact that it was indeed the C(11)-methyl group which had undergone the hydroxymethylation process, was established by Differential Nuclear Overhauser Experiments. In support of the structure, it was observed that the irradiation of the C(6)-methyl group led to enhancement of the signals of the C(5)-methyl and the C(7)-proton.

The conversion of 3d to the corresponding glycosides was achieved in two steps. Glycosidation of 3d was carried out by $SnCl_4$ catalyzed condensation with the appropriate acetate or benzoate esters of ribose, glucose and galactose. The stereochemistry of the glycosidic linkage in the products 11a-c was shown to be β by NMR spectroscopic analysis. For the coupling constants between H(2') and the anomeric proton values of 7.5 to 8 Hz were observed[10].

The protective groups on the carbohydrate moieties were removed to yield the ellipticine derivatives 3e-g.

Cytostatic activities of the compounds obtained and further derivatization of the C(11)-methyl group in ellipticine will be reported elsewhere.

EXPERIMENTAL

General
All melting points are uncorrected. IR spectra were recorded on a Perkin Elmer 1310 spectrophotometer. The absorptions are given in cm^{-1}. PMR spectra were run on Bruker WM 250 and AC 200 instruments. Unless stated otherwise, IR and NMR spectra were taken in $CHCl_3$ and $CDCl_3$ respectively. Mass spectra were obtained with a Varian Matt 711 spectrometer. Thin-layer chromatography was carried out with silica gel F 254 plates.

6-Methylellipticine (3a)
To a suspension of 1.28 g NaH (53 mmol) in 120 ml dry DMF in a nitrogen atmosphere was added 10 g (41 mmol) ellipticine in portions. After the evolution of hydrogen had ceased, 6 g (42 mmol) methyl iodide in 65 ml of DMF was added dropwise. After stirring overnight at room temperature, 500 ml water was added and the mixture was extracted with chloroform. The combined chloroform layers were dried (sodium sulphate) and filtered over basic alumina. Evaporation and recrystallisation of the residue from ethanol/water (260/160 ml) yielded 8.58 g of 3a (81%) as yellow needles; m.p. 207-208°C; IR: 1595, 1470; PMR: δ 3.00 (s, 3H, C(5)-CH_3), 3.14 (s, 3H. C(11)-CH_3), 4.08 (s, 3H, N-CH_3), 7.30, 7.58 (2t, 2H, J = 8, H-8 + H-9), 7.38 (d, J = 8, H-7), 7.86 (d, 1H, J = 7, H-4), 8.32 (d, 1H, J = 8, H-10), 8.46 (d, 1H, J = 7, H-3), 9.64 (s, 1H, H-1); MS: m/z = 260.1307 (calc. for $C_{18}H_6N_2$: 260.1301. Found, C, 82.9; H, 6.3; N, 10.7; $C_{18}H_6N_2$ requires C, 83.04; H, 6.19; N, 10.76.

6-Methylellipticinium-2-oxide (4).
To an ice-cold solution of 1.04 g (4 mmol) 3a in 60 ml methylene chloride was added 1.05 g m-chloroperbenzoic acid (5.2 mmol), followed, after stirring for 2 h, by a second portion (0.5 g) of the acid. The solution was stirred overnight, diluted with chloroform and washed (3x) with aqueous $NaHCO_3$ and once with sat. NaCl. After drying (Na_2SO_4) the solvent was evaporated and the residue dissolved in 5 ml DMF. From the solution yellow-brownish crystals were obtained. Yield: 75%; m.p. 185-192°C; IR (KBr): 1585, 1470, 1385, 1185; PMR: 3.03, 3.06 (2s, 6H, C(5)-CH_3, C(11)-CH_3), 4.12 (s, 3H, N-CH_3, J = 7.7), 7.31, 7.59 (2t, 2H, H-8 H-9), 7.40 (d, 1H, H-7, J = 7.9), 7.90 (d, 1H, H-4, J = 7.2), 8.12 (d, 1H, H-3, J = 7.4), 8.30 (d, 1H, H-10, J = 7.9), 9.18 (s, 1H, H-1); MS: m/z = 276.1258 (calc. for $C_{18}H_{16}N_2O$: 276.1262).

6-Methyl-1,2-dihydroellipticin-1-one (5) and 2-acetyl-6-methyl-1,2-dihydroellipticin-1-one (6).
A mixture of 55 mg (0.2 mmol) N-oxide 4 and 4 mg sodium acetate (0.05 mmol) was refluxed in 2 ml acetic anhydride. After 1.5 h the starting material had disappeared and the acetic anhydride was removed by coevaporation with toluene. The residue was extracted with chloroform and the combined chloroform extracts washed with saturated $NaHCO_3$ and saturated NaCl. After drying and evaporating the solvent, the residue was applied to a silica gel column and eluted with dichloromethane/methanol gradient. Two products were obtained; 6a (20%) and 6b (20%).
6a: IR: 1710, 1672, 1570, 1235; PMR: 2.82 (s, 3H, (C=O)-CH_3), 2.85 (s, 3H, C(5)-CH_3), 3.36 (s, 3H, C(11)-CH_3), 4.09 (s, 3H, N-CH_3), 6.72 (d, 1H, J = 8.5, H-1), 7.29, 7.52 (2t, 2H, J = 8.0, H-8+H-9), 7.39 (d, 1H, J = 8.1, H-7), 7.86 (d, 1H. J = 8.4, H-3), 8.35 (d, 1H, J = 8.0, H-10).
6b: IR: 1640, 1600, 1575, 1370; PMR ($CDCl_3/CD_3OD$): 2.85 (s, 3H, C(5)-CH_3), 3.47 (s, 3H, C(11)-CH_3), 4.05 (s, 3H, N-CH_3), 6.74 (d, 1H, J = 7.6, H-4), 6.97 (d, 1H, J = 7.7, H-3), 7.24, 7.47 (2t, 2H, J = 7.7, H-8 + H-9), 7.34 (d, 1H, J = 8.0, H-7), 8.31 (d, 1H, J = 7.4, H-10).

11-(2-Hydroxyethyl)-5,6-dimethylpyrido[4,3-b]carbazole (3d)
To a mixture of 300 ml dry THF and 8.6 ml di-isopropyl amine in a nitrogen atmosphere was added 37.5 ml of a 1.6 M solution of n-butyllithium (60 mmol) in hexane. The mixture was stirred at room temperature during 15 min, coooled to -78°C and 5.2 g (20 mmol) 6-methyl ellipticine (3a) was added in portions. After 5 h stirring at -78°C, 1.2 g (40 mmol) paraformaldehyde was added and the suspension was stirred for 2 h at 0°C and overnight at room temperature. After addition of 20 ml saturated $NaHCO_3$, followed by 500 ml water, the mixture was extracted with chloroform (500 ml). After washing the chloroform layer (sat. NaCl), drying (Na_2SO_4), the solvent was evaporated and the residue was treated with 50 ml chloroform giving 2.7 g of 3d. Concentration of the chloroform layer produced an addition crop of 3d, (0.5 g). Total yield: 56%; m.p. 232-240°C; IR (KBr): 3200, 2960, 2920, 2850, 1590, 1475, 1440, 1390, 1240; PMR (DMSO-d_6): 3.07 (s, 3H, C(5)-CH_3), 3.90 (dt, 4H, C(11)-CH_2-CH_2), 4.17 (s, 3H, N-CH_3), 5.08 (t, 1H, J = 5.2, OH), 7.34, 7.66 (2t, 2H, J = 8.0), H-8 + H-9), 7.65 (d, 1H, J = 8.0, H-7), 8.02 (d, 1H, J = 6.0, H-4), 8.37 (d, 1H, J = 7.9, H-10), 8.46 (d, 1H, J = 5.8, H-3), 9.71 (s, 1H, H-1); MS: m/z = 290.1402 (calc. for $C_{19}H_{16}N_2O$: 290.1419.

11-[2(2,3,5-Tribenzoyl-β-D-ribofuranos-1-yl)oxyethyl]-5.6-dimethyl-pyrido[4,3-b]-carbazole (11a)
To a solution of 1.8 g (3.75 mmol) 1-O-acetyl-2,3,5-tri-O-benzoyl- -D-ribofuranose in 15 ml acetonitrile was added 0.67 ml SnCl . After stirring for 15 min, 0.43 g 3d was added and the suspension was stirred at room temperature untill a brownish-green solution was obtained (2.5 h). The reaction mixture was poured into 100 ml of chloroform, washed with sat. $NaHCO_3$ and sat. NaCl and dried (Na_2SO_4). The product was purified by column chromatography (silica gel, ethyl acetate/hexanes 1:1); yellow foam. IR: 2970, 1725, 1600, 1470, 1450, 1280, 1110; PMR: 3.02 (s, 3H, C(5)-CH_3), 3.95-4.52 (m, 4H, C(11)-CH_2CH_2), 4.07 (s, 3H, N-CH_3), 4.18 (dd, 1H, J = 5.8, J = 11.6, H-5A'), 4.55 (dd, 1H, J = 4.4, J = 11.5, H-5B'), 4.63 (m, 1H, H-4'), 5.30 (s, 1H, H-1'), 5.65 (d, 1H, J = 5.0, H-2'), 5.64 (dd, 1H, J = 5.0, J = 6.2, H-3'), 7.25-7.57 (m, 12H), 7.87-7.99 (m, 7H), 8.30 (d, 1H, J = 7.8, H-10), 8.48 (d, 1H, J = 6.2, H-3), 9.68 (s, 1H, H-1).

5,6-Dimethyl-11-[2-(β-D-ribofuranos-1-yl)oxyethyl]-pyrido[4,3-b]-carbazole (3e)
To a solution of 11a (0.61 g, 0.83 mmol) in a mixture of 5 ml THF and 5 ml of methanol was added a catalytic amount of sodium methoxide in methanol. After one night at room temperature, the solvents were evaporated and the residue crystallised from methanol/ethyl acetate. Yield: 0.22 g (63%); m.p. 119-124°C; IR (KBr): 3300, 2910, 1585, 1470, 1440, 1240, 1100, 1030; PMR (DMSO-d_6): 3.08 (s, 3H, C(5)-CH_3), 4.05 (s, 3H, N-CH_3), 4.81 (s, 1H, H-1'), 7.34 (t, 1H, J = 6.4, H-8 or H-9), 7.64 (m, 2H, H-7+H-8 or H-9), 8.05 (d, 1H, J = 6.2, H-4), 8.33 (d, 1H, J = 8.0, H-10), 8.46 (d, 1H, J = 6.2, H-3), 9.70 (s, 1H, H-1).

11-[2-(2,3,4,6-Tetraacetyl-β-D-glucopyranos-1-yl)oxyethyl]-5,6-dimethyl-pyrido[4,3-b]-carbazole (11b)
The same procedure was followed as for the preparation of 11a, starting from 3d and 1,2,3,4,6-penta-O-acetyl-β-D-glucopyranose. Yield: 35%; yellow foam; IR: 2950, 1740, 1590, 1460, 1230, 1030; PMR: 2.00-2.07 (4s, 12H, 4x$COCH_3$), 3.04 (s, 3H, C(5)-CH_3), 3.65 (..,1H,H-5'), 3.89-4.34 (m, 6H, H-6A', H-6B', CH_2-CH_2),4.54(d, 1H, J = 7.8, H-1'), 4.98-5.18 (m, 3H, H-2', H-3', H-4'), 7.28 (t, 1H, J = 7.9, H-8 or H-9), 7.40 (d, 1H, J = 8.1, H-7), 7.57 (t, 1H, J = 7.4, H-8 or H-9), 7.88 (d, 1H, J = 5.5, H-4), 8.24 (d, 1H, J = 7.9, H-10), 8.47 (d, 1H, H-4), 9.65 (s, 1H, H-1); ^{13}C-NMR ($CDCl_3$, 50 MHz): 13.7 (C(5)-Me), 20.2 (4x (C=O)-CH_3), 28.1 (C(11)-CH_2), 33.7 (N-CH_3), 61.8 (C-6'), 67.8 (C-4'), 6.87 (C(11)-CH_2CH_2O), 69.9 (C-2'), 71.6 (C-3'), 72.6 (C-5'), 100.7 (C-1'), 102.5 (C-7), 109.5 (C-5), 115.3 (C-4), 119.8 (C-9), 122.0 (C-11a), 123.0 (C-10), 124.7 (C-10a,b), 127.3 (C-8), 127.9 (C-11), 134.0 (C-4a), 140.2 (C-3), 141.7 (C-5a), 144.9 (C-6a), 149.0 (C-1), 169.5 (4x $COCH_3$).

11-[2-(β-D-Glucopyranos-1-yl)oxyethyl]-5,6-dimethylpyrido[4,3-b]-carbazole (3f)
Deprotonation of 11b was carried out as described for 3e. Yield: 25%; yellow crystals; m.p. 161-164°C (methanol); IR (KBr): 3300, 1590, 1475, 1445, 1390, 1245; NMR (DMSO-d_6): 3.09 (s, 3H, C(5)-CH_3), 3.19 (m, 3H, H-2', H-3', H-5'), 3.51 (m, 2H, H-6', H-4'), 3.70 (d, 1H, J = 10.5, H-6A'), 4.04 (m, 4H, C(11)-CH_2-CH_2), 4.19 (s, 3H, N-CH_3), 4.56 (d, 1H, J = 7.6, H-1'), 4.52 (t, 1H, OH), 4.95 (m, 2H, 2x OH), 5.09 (d, 1H, J = 4.3, OH), 7.35 (t, 1H, H-8 or H-9), 7.67 (m, 2H, H-10 + H-8 or H-9), 8.04 (d, 1H, J = 6.2, H-4), 8.38 (d, 1H, J = 8.0, H-10), 8.47 (d, 1H, J = 6.2, H-3), 9.75 (s, 1H, H-1).

11-[2-(2,3,4,6-Tetraacetyl-β-D-galactopyranos-1-yl)oxyethyl]-5,6-dimethyl-pyrido[4,3-b]-carbazole
The same procedure was followed as for the preparation of 11a, starting from 3d and 1,2,3,4,6-penta-O-acetyl-β-D-galactopyranose. 50% (66% based on recovered starting material) of 11c was obtained as a yellow foam; IR ($CHCl_3$): 2985, 1745, 1595, 1465, 1370, 1240; ^{1}H-NMR ($CDCl_3$, 250 MHz): 2.02-2.12 (4s, 12H, (C=O)-CH_3), 2.99 (s, C(5)-CH_3), 3.88 (dd, 1H, J = 6.4, J = 6.7, H-5'), 4.06 (s, 3H, N-CH_3), 4.00-4.31 (m, 6H, H-6A', H-6B' + CH_2CH_2), 4.56 (d, 1H, J = 8.0, H-1'), 4.96 (dd, 1H, J = 10.4, J = 3.4, H-3'), 5.22 (dd, 1H, J = 10.4, J = 8.0, H-2'), 5.34 (d, 1H, J = 3.5, H-4'), 7.29 (t, 1H, J = 7.6, H-8 or H-9), 7.36 (d, 1H, J = 8.1, H-7), 7.57 (t, 1H, J = 7.6, H-8 or H-9), 7.87 (d, 1H, J = 6.2, H-4), 8.22 (d, 1H, J = 7.8, H-10), 8.46 (d, 1H, J = 6.0, H-3), 9.64 (s, 1H, H-1); ^{13}C-NMR ($CDCl_3$, 50 MHz): 13.8 (C(5)-CH_3), 20.4 (4x (C=O)-CH_3), 28.3 (C(11)-CH_3), 33.8 (N-CH_3), 61.2 (C-6'), 66.9 (C-4'), 68.6 (C-2'), 68.7 (C(11)-CH_2CH_2O), 70.6 (C-3'), 70.8 (C-5'), 101.2 (C-1'), 108.7 (C-7), 109.7 (C-5), 115.4 (C-4), 120.0 (C-9), 122.0 (C-11a), 123.2 (C-10), 124.9 (C-10a,b), 127.5 (C-8), 128.4 (C-11), 134.3 (C-4a), 139.6 (C-3), 142.0 (C-5a), 145.0 (C-6a), 148.5 (C-1), 169.5 (4x COCH).

11-[2-(β-D-Galactopyranos-1-yl)oxyethyl]-5,6-diethyl-pyrido[4,3-b]-carbazole (3g)
Deprotection of 11c was carried out as described for 3e. Yield: 49%; m.p. 245-252°C; IR (KBr): 3370, 1590, 1465, 1440, 1390, 1245; NMR (DMSO-d_6): 7.35 (t, 1H, H-8 or H-9), 7.66 (m, 2H, H-7 + H-8 or H-9), 8.03 (d, 1H, J = 6.2, H-4), 8.36 (d, 1H, J = 7.8, H-10), 8.46 (d, 1H, J = 6.2, H-3), 9.72 (s, 1H, H-1).

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