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Synthesis and biological studies of glycosyl dopamine derivatives as potential antiparkinsonian agents

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Abstract

A new approach to deliver dopamine into the central nervous system, based on the use of D-glucose as transportable agent, has been studied. Glycosyl dopamine derivatives bearing the sugar moiety linked to either the amino group or the catechol ring of dopamine through amide, ester or glycosidic bonds were synthesised as potential antiparkinsonian agents. Studies on the binding to dopamine D_2 receptor, in vitro stability, and locomotive effect in mice of the synthetic glycoconjugates are reported.

Keywords: Glycosides; Neurologically active compounds; Neurotransmitter; Glucose carrier

1. Introduction

Parkinson's disease is a progressive disorder of the central nervous system (CNS) [1,2], whose major symptoms are stiffness of the muscles, difficulty in moving, and tremors. In biochemical terms, the disease is characterised by a reduced concentration of the neurotransmitter, dopamine in the brain. A possible therapy based on the use of dopamine itself is hampered by the fact that this substance is unable to cross the blood brain barrier (BBB). The usual treatment of this disorder is the use of levodopa, a dopamine precursor, which reverses symptoms. Levodopa enters the CNS trough active transport and is enzymatically cleaved in the brain to release dopamine. In fact, levodopa is actually a prodrug for dopamine. However, during chronic treatment with levodopa, a variety of problems may appear. These limitations have stimulated the search for new strategies [3,4]. One of these may rely on dopamine-derivatives able to penetrate the BBB, by making use of specific transport systems. It is known that glucose — the brain's source of energy — and other hexoses are transported across the BBB by the glucose carrier GLUT-1 [5,6]. This proteic transporter is located in the membrane of brain capillary endothelial cells composing the BBB. Thus, one could envisage a new approach to deliver dopamine into the CNS by linking the neurotransmitter to a sugar molecule so that the resulting glycoconjugate may cross the BBB using this carrier-mediated transport [7]. Upon reaching the CNS, the prodrug should be enzymatically cleaved to release the active compound (Fig. 1). Some authors have studied this approach in peptide drugs [8] and cytotoxic agents [9,10] delivery into the CNS.

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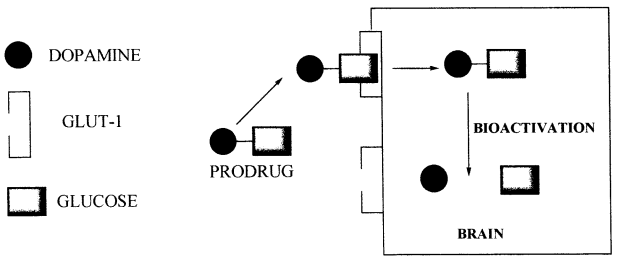
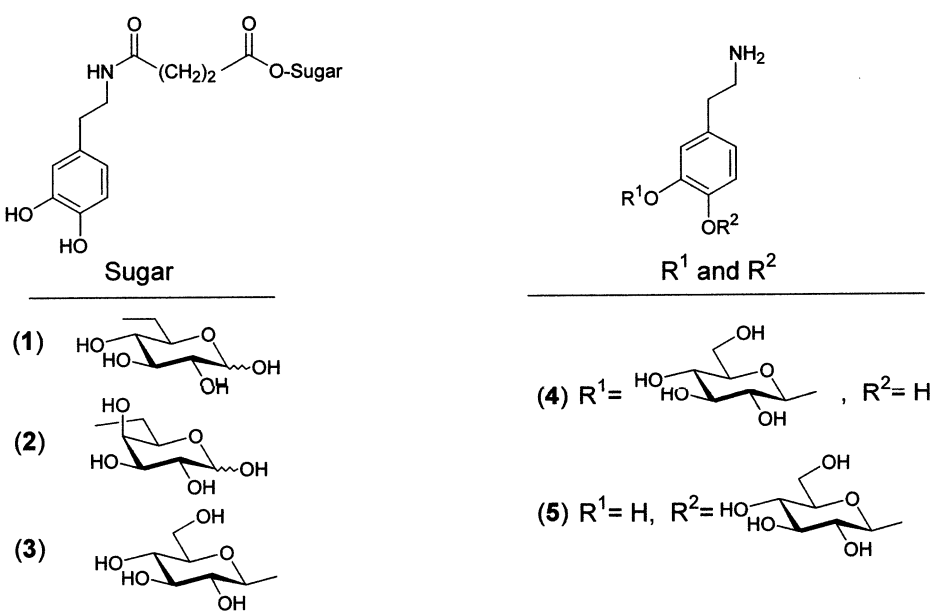


Fig. 1. Schematic representation of the new strategy to deliver dopamine into the CNS.

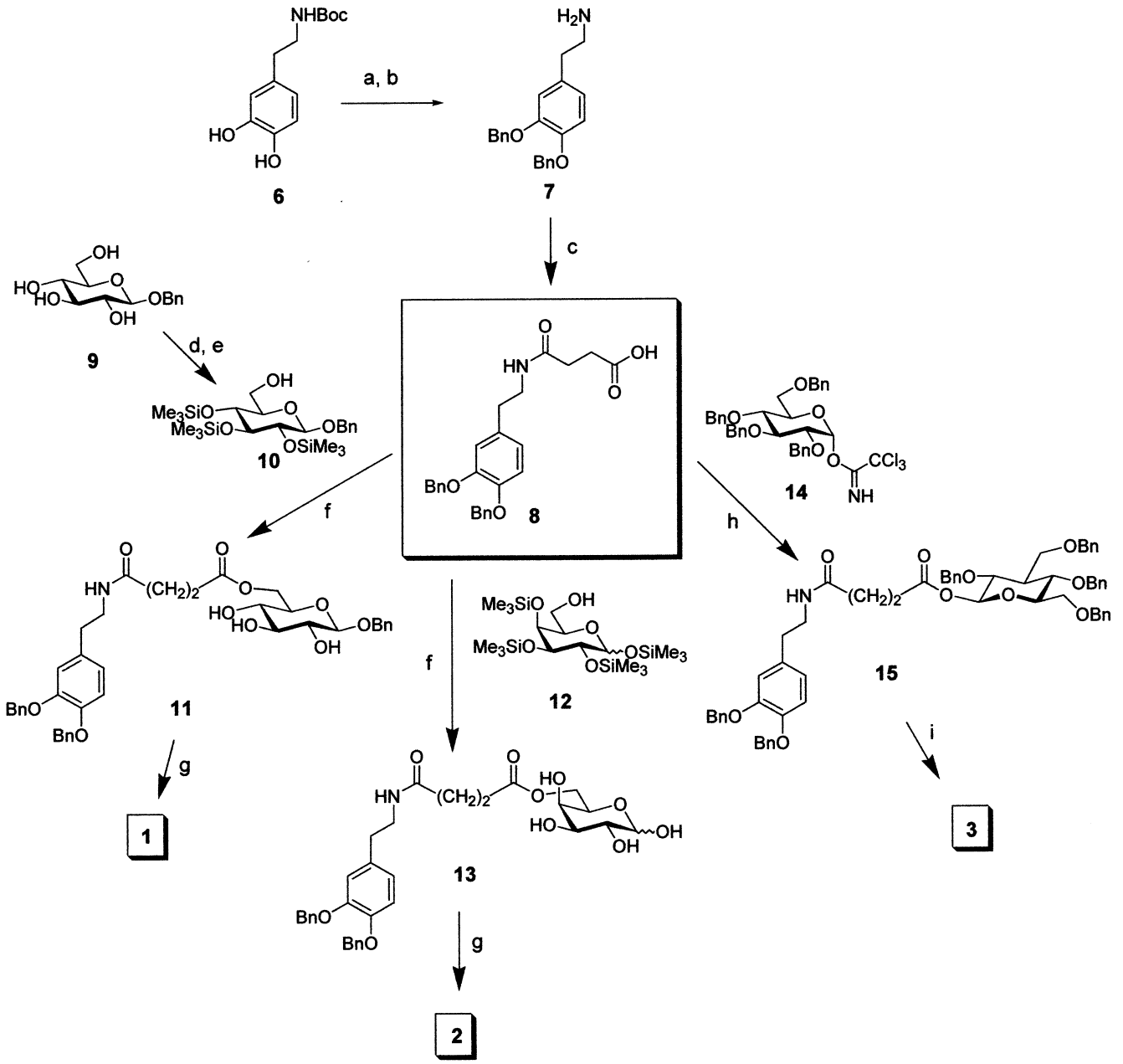
Following this strategy, we have synthesised several glycosyl derivatives of dopamine as potential antiparkinsonian agents. Sugar and neurotransmitter are linked through ester, amide and glycosidic bonds. These linkages can be cleaved enzymatically in the brain tissue to release dopamine. A series of compounds presents the amino group of dopamine linked to the C-6 (**1** and **2**, Scheme 1) or C-1(β) (**3**) position of the sugar through a succinyl linker. In another series, the sugar is attached to the phenolic groups of dopamine through a glycosidic bond (**4** and **5**). We report herein the synthesis, in vitro stability studies, binding to dopamine D_2 receptor, and behavioural activity, in an experimental model of Parkinson's disease, of compounds **1** to **5**.

2. Results and discussion

Chemical synthesis.—The glycosyl dopamine derivatives were obtained in short and effective syntheses involving 4–7 steps from dopamine. The synthesis of **1**–**3** (Scheme 2) was carried out from a common succinamic acid derivative **8**, which was coupled with the corresponding substituted monosaccharide. Previously, **8** was obtained from the *N*-Boc protected dopamine **6** [11] by benzylation of the phenolic groups, followed by removal of the Boc group, to give amine **7**, and subsequent treatment with succinic anhydride. The glucose and galactose derivatives with a free hydroxyl group at C-6 (intermediates **10** and **12**, respectively) were easily obtained by trimethylsilylation of benzyl β-D-glucopyranoside (**9**) and D-galactose, and selective methanolysis of the primary silyl group. The methanolysis was first tried under basic conditions (K_2CO_3–MeOH) [12] giving a mixture of partially silylated products. A cleaner reaction, however, was obtained when the persilylated compounds were treated with AcOH–MeOH [9]. Esterification of **10** with acid **8** in the presence of DCC and subsequent removal of the silyl groups gave **11**, which after hydrogenolysis led to target **1**. Similar transformations on **12** furnished the galactose-containing product **2**. The glycosyl dopamine derivative **3** was obtained by reaction of **8**



Scheme 1.

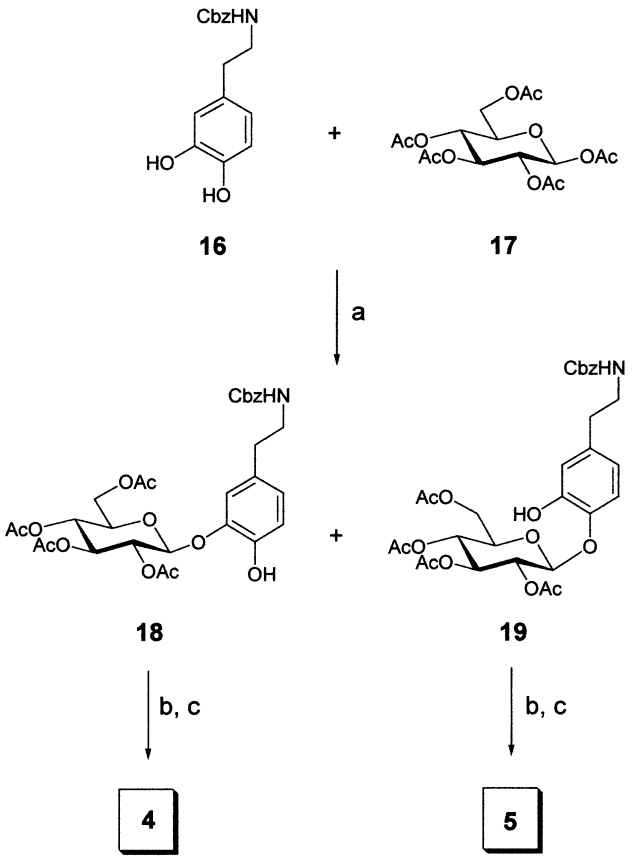


Scheme 2. Reagents and conditions. (a) BnBr, K_2CO_3, DMF, 60 °C, 24 h; (b) 5% CF_3COOH, CH_2Cl_2, rt, 5 h, 81% two steps; (c) succinic anhydride, Py, rt, 3 h, 94%; (d) $(Me)_3SiCl$, HMDS, rt, overnight, 74%; (e) 5% AcOH, MeOH–acetone, 0 °C, 1 h, 75%; (f) (1) **8**, DCC, DMAP, CH_2Cl_2, rt, 4 h; (2) 2% CF_3COOH, CH_3NO_2, 0 °C, 1 h, 69% for **11**, 78% for **13**; (g) H_2, MeOH, Pd–C, rt, 1 h, 99%; (h) **8**, CH_2Cl_2, 5% DMF, Ar, rt, 5 days, 40%; (i) H_2, MeOH–AcOEt, Pd–C, rt, 3 h, 99%.

with α-trichloroacetimidate **14** [13] giving the β-glucopyranosyl ester **15** in a stereospecific manner, followed by debenzylation.

Glycosides **4** and **5** were obtained from the N-protected dopamine **16** (Scheme 3). The reaction of **16** with glucose peracetate **17** promoted by $BF_3{\cdot}OEt_2$, gave glycosylation at HO-3 (**18**) and HO-4 (**19**) of the catechol ring in a 3:1 ratio. Deprotection steps on **18** and **19**, separately, afforded **4** and **5**, respectively as their trifluoroacetate salts.

Binding to dopamine D_2 receptor.—To test for the possibility that the intact glycoconjugates might be pharmacologically active, the affinity of compounds **1**–**5** for dopamine D_2 receptor was evaluated. None of the glycosyl dopamine derivatives inhibited the binding of ^{3}H-spiperone to D_2 receptor, showing pK_i val-

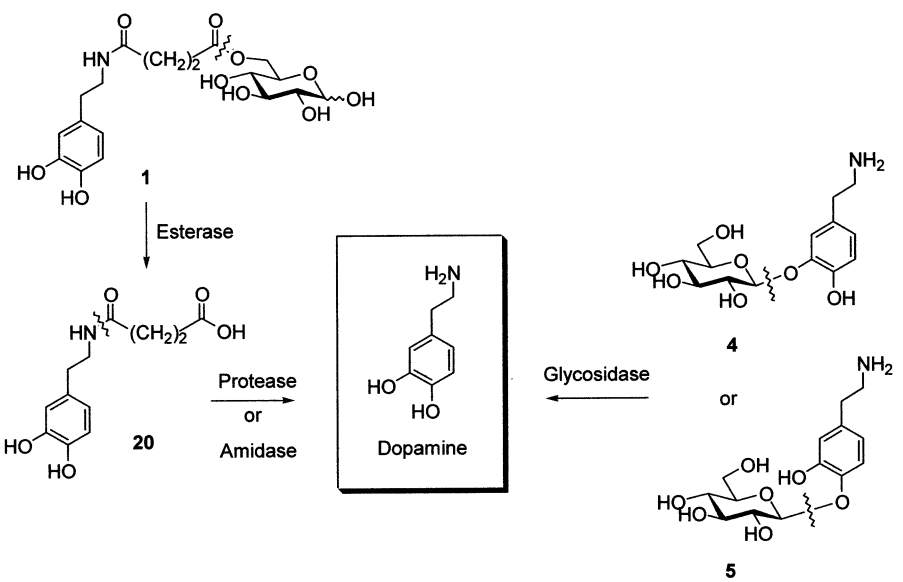


Scheme 3. Reagents and conditions. (a) $BF_3{\cdot}OEt_2$, CH_2Cl_2, Ar, rt, 4 h, 18% for **18** and 54% for **19**. (b) 0.1 M NaMeO–MeOH, rt, 1.5 h, 88% for **18**, 97% for **19**; (c) H_2, CF_3COOH (1.6 equiv), MeOH, Pd–C, rt, 1 h, 99%.

ues below 5. This result is in agreement with the generally accepted assumption that the amino and *m*-hydroxyl groups of the dopamine are essential for the interaction with the TM3 aspartic acid and TM5 serine, respectively, of the receptor [14].

Stability studies.—In order to determine the stability and ability to deliver dopamine after brain uptake, compounds **1**, **4**, and **5** were incubated in the presence of specific enzymes, rat plasma, and rat brain extracts. The presumed activation pathways for **1**, **4**, and **5** are outlined in Scheme 4. The degradation route for **1** involves the formation of the acid intermediate **20** through an esterase-catalysed hydrolysis, and subsequent cleavage of the amide bond, by amidase or protease activation, to give dopamine. On the other hand, glycosides **4** and **5** should give rise to the neurotransmitter through an activation mechanism catalysed by glucosidase.

The progress of the incubations were monitored by high-performance liquid chromatography (HPLC; UV detector, $\lambda = 280$ nm) using a reverse-phase column. Table 1 reports the half-life ($t_{1/2}$) of these compounds in the different incubation media, together with that of dopamine. Compound **1** was highly stable in water, where the half-life exceeded 15 days. In the presence of esterase (60 units/mL) and protease (30 units/mL) **1** underwent hydrolysis ($t_{1/2} = 7$ and 43 h, respectively). In plasma, **1** showed a moderate stability ($t_{1/2} = 3$ h); however, it slowly decomposed in brain extracts. In all the incubation media, the degradation of glycosyl–succinyl dopamine **1** led to the formation of the expected acid derivative **20**.



Scheme 4.

Table 1
Half-life of compounds **1**, **4**, **5**, and dopamine in different incubation media

Incubation mean	Half-lives (h)			
	1	**4**	**5**	Dopamine
Water	>144	>144	>144	27
Esterease [a]	7	–	–	–
Protease [b]	43	–	–	–
β-Glucosidase [c]	–	2	1.5	–
Plasma	3	>144	>144	2
Brain extract	21 [d]	132 [e]	67 [e]	3 [e]

[a] From bovine liver (60 units/mL).
[b] α-Chymotrypsin from bovine pancreas (30 units/mL).
[c] From almonds (20 units/mL).
[d] Rat brain extract soluble fractions.
[e] Rat brain homogenate, prepared following Ref. [18].

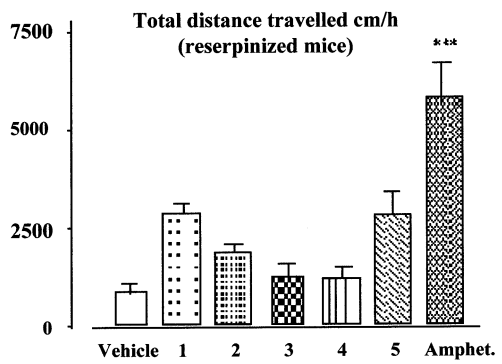


Fig. 2. Locomotor activity of compounds **1**–**5**.

This intermediate accumulated during the incubation but no appreciable release of dopamine was observed for the time that all **1** was consumed. The amide bond in **20** and in **1** was found to be highly resistant under the assayed conditions. Other prodrugs described in the literature met with highly stable amide bonds under in vitro conditions [15,16].

Glycosides **4** and **5** released dopamine in the presence of β-glucosidase from almonds (20 units/mL). However, they were very stable in plasma, as was expected, due to the low levels of β-glucosidase found in the blood [17]. In brain extract with glucosidase activity, as determined following a described procedure [18], glycosides **4** and **5** were slowly hydrolysed, showing differences in their rates of hydrolysis ($t_{1/2}$ for **4** and **5**, 5.6 and 2.8 days, respectively). In this medium, the amount of released dopamine during the incubation of **4** and **5** was low and constant, probably due to the slow activation of the prodrugs as compared to the degradation rate of dopamine ($t_{1/2}$ of dopamine in brain extract, 3 h).

In vivo activity.—Compounds **1**–**5** were tested for their ability to induce a recovery of motor activity in reserpinized mice (1 h). They were administered intraperitoneally at a dose of 10 and 100 mg/kg, and amphetamine (2.5 mg/kg, i.p.) was used as the reference drug. The results for locomotive activity (cm/h) for the 100 mg/kg dose are shown in Fig. 2. While amphetamine increased the locomotive activity in mice (5842.14 ± 949.77, $P < 0.01$), some glycosyl derivatives caused a slight modification during the hour following administration; however, this was not significant enough as to exhibit antiparkinsonian properties. Several factors can be responsible for the lack of in vivo activity of the glycosyl dopamine derivatives. One of them may be the inability of the compounds to cross the BBB. Also, their slow bioconversion into dopamine, as it has been observed during in vitro experiments, indicates poor properties of the compounds as prodrugs. Studies about the affinity of the glycosyl derivatives for the glucose carrier GLUT-1, and the synthesis of new prodrugs with modified linkages that may undergo faster enzyme catalysed hydrolysis, are currently in progress.

3. Experimental

General methods.—THF was distilled from Na/benzophenone and CH_2Cl_2 from CaH_2, immediately prior to use. *N*,*N*-Dimethylformamide was distilled over CaH_2 and was kept over molecular sieves (4 Å) under argon. Unless stated otherwise, materials were obtained from commercial sources (Aldrich, Sigma, Fluka) and used without further purification. All reactions were monitored by thin-layer chromatography (TLC), carried out on 0.20 mm E. Merck pre-coated Silica Gel 60 F-254 plates by using UV light (254/365 nm) and 10% H_2SO_4 in EtOH or a soln of ammonium molybdate and ceric ammonium sulfate in wa

ter–H_2SO_4 5% as developing agent. E. Merck Silica Gel 60 (230–400 mesh) was used for flash column chromatography. Analytical HPLC was carried out with a Water 600E multisolvent delivery system equipped with a reverse-phase (C18) column (Lichrospher 100RP-18, 14.5 cm, 5 μm, E. Merck), using as eluent, different mixtures of MeCN, MeOH and trifluoroacetic acid 5% in water, with a UV detector (Waters 484) at 280 nm. Melting points were measured with a Reicher Jung Thermovar microscoper and are uncorrected. Optical rotations were determined with a Perkin–Elmer 241-MC polarimeter. ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD on a Varian Gemini 200, a Bruker AMX-200, a Varian INOVA 300 or a Varian 400 spectrometer. Chemical shifts are given in ppm (δ). Microanalyses were performed with an Heraeus CHN-O analyzer.

N-tert-*Butoxycarbonyl-3,4-dihydroxiphenylethylamine* (**6**).—To a solution of dopamine hydrochloride (5 g, 26.4 mmol) in Et_3N and MeOH (1:9, 143 mL) was added di-*tert*-butyldicarbonate (6.331 g, 29 mmol) and the mixture was stirred at 40–50 °C for 30 min. After *tert*-butoxycarbonylation was complete (as evidenced by TLC using 3:1:0.5 hexane–EtOAc–MeOH), stirring was continued for 30 min more, the solvent was then evaporated under reduced pressure, and the residue treated with ice-cold dilute HCl (pH 2.15, 7×10^{-3} M) for 10 min and extracted immediately with EtOAc. The organic layer was dried ($MgSO_4$), then filtered, and concentrated. The residue was purified by flash chromatography (4:1:0.5 → 2:1:0.5 hexane–EtOAc–MeOH) to yield **6** as a solid (6.15 g, 92%); mp 136–138 °C; R_f 0.36 (2:1:0.15 hexane–EtOAc–AcOH); ^1H NMR (200 MHz, CD_3OD): δ 6.86 (d, *J* 8.0 Hz, 1 H, H-5 Ar), 6.82 (d, *J* 2.0 Hz, 1 H, H-2 Ar), 6.70 (dd, 1 H, *J* 1.9 and 8.0 Hz, H-6 Ar), 3.37 (t, 2 H, *J* 7.5 Hz, CH_2–NH), 2.77 (t, 2 H, *J* 7.5 Hz, CH_2–Ar), 1.61 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (50 MHz, CD_3OD): δ 158.4 (–CO–), 146.2 (C-3 Ar), 144.65 (C-4 Ar), 132.3 (C-1 Ar), 121.1 (C-6 Ar), 116.9 and 116.4 (C-2, C-5 Ar), 80.0 ($C(CH_3)_3$), 43.3 (CH_2–NH), 36.6 (CH_2–Ar), 28.8 ($C(CH_3)_3$); Anal. Calcd for $C_{13}H_{19}NO_4$: C, 61.63; H, 7.56; N, 5.53. Found: C, 61.20; H, 7.55; N, 5.39.

3,4-Dibenzyloxyphenylethylamoniun trifluoroacetate (**7**).—To a mixture of **6** (6.15 g, 24.2 mmol) in anhyd DMF (40 mL) containing K_2CO_3 (21.36 g, 217.8 mmol) was added benzyl bromide dropwise (7.20 mL, 60.53 mmol). The reaction was stirred at room temperature (rt), under argon for 24 h. After this time, the mixture was filtered through Celite and the solid washed with Et_2O (30 mL). The combined filtrate and washings were washed with ice-water (4 × 25 mL), an aq soln of 1 N HCl (3 × 15 mL) and brine. The organic layer was dried (Na_2SO_4) and concentrated to give a residue which was purified by flash chromatography. Elution with 7:1 → 3:1 hexane–EtOAc afforded *N-tert*-butoxycarbonyl - 3,4 - dibenzyloxyphenylethylamine (8.92 g, 85%) as a white solid; mp 106–108 °C; R_f 0.52 (3:1:0.05 hexane–EtOAc–AcOH); ^1H NMR (200 MHz, $CDCl_3$): δ 7.39 (m, 10 H, aromatics), 6.89 (d, 1 H, *J* 1.7 Hz, H-2 Ar), 6.71 (dd, *J* 8.0 and 1.7 Hz, H-6 Ar), 5.15 (s, 4 H, 2 CH_2–Ph), 4.45 (s, 1 H, NH), 3.32 (m, 2 H, CH_2–NH), 2.70 (t, 2 H, *J* 6.9 Hz, CH_2–Ar), 1.45 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR (50 MHz, $CDCl_3$): δ 155.8 (–CO–), 149.1 (C-3 Ar), 147.8 (C-4 Ar), 137.5 and 137.3 (C ipso Bn), 132.5 (C-1 Ar), 128.5–127.3 (10 C aromatics), 121.7 (C-6 Ar), 116.0 and 115.6 (C-5 and C-2 Ar), 79.2 ($C(CH_3)_3$), 71.6 and 71.5 (CH_2–Ph), 41.8 (CH_2–NH), 35.7 (CH_2–Ar), 28.4 ($C(CH_3)_3$); Anal. Calcd for $C_{27}H_{31}NO_4$: C, 74.80; H, 7.21; N, 3.14. Found C, 74.58; H, 7.25; N, 3.23.

This compound (7.34 g, 16.95 mmol) was treated with 5% TFA in CH_2Cl_2 (190 mL) at rt for 5 h. After this time, the solvent was removed under vacuum (co-evaporation with toluene) to obtain **7** as a white solid (8.48 g, 95%); mp 159–162 °C; R_f 0.32 (5:1 CH_2Cl_2–MeOH); ^1H NMR (300 MHz, CD_3OD): δ 7.65–7.47 (m, 10 H, aromatics), 7.20 (d, 1 H, *J* 8.2 Hz, H-5 Ar), 7.14 (d, 1 H, *J* 2.1 Hz, H-2 Ar), 7.00 (dd, 1 H, *J* 8.2 and 2.1 Hz, H-6 Ar), 5.32 and 5.30 (2s, 4 H, CH_2–Ph), 3.30 (t, 2 H, *J* 7.6 Hz, CH_2–NH), 3.04 (t, 2 H, *J* 7.6 Hz, CH_2–Ar); ^{13}C NMR (50 MHz, CD_3OD): δ 150.6 (C-3 Ar), 149.6 (C-4 Ar), 138.7 and 138.6 (C ipso Bn), 131.3 (C-1 Ar), 129.4–128.6 (10 C, aromatics), 122.9 (C-6 Ar), 117.1 and 117.0 (C-2 and C-5 Ar), 72.52 (CH_2–Ph),

41.95 (CH_2–NH), 34.04 (CH_2–Ar); Anal. Calcd for $C_{24}H_{24}F_3NO_4$: C, 64.42; H, 5.41; N, 3.13. Found: C, 64.21; H, 5.18; N, 3.24.

N-*(3,4-Dibenzyloxyphenylethyl)succinamic acid* (**8**).—A solution of **7** (1 g, 2.706 mmol) in pyridine (1.08 mL) was treated with succinic anhydride (542 mg, 5.412 mmol). The reaction mixture was stirred at rt for 3 h, then the solvent was co-evaporated with toluene, and the residue was purified by flash chromatography (15:1 → 10:1 → 5:1 CH_2Cl_2–MeOH) to give **8** as a white solid (1.103 g, 94%); mp 160–163 °C; R_f 0.40 (10:1 CH_2Cl_2–MeOH); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.39 (m, 10 H, aromatics), 6.89 (d, 1 H, *J* 8.1 Hz, H-5 Ar), 6.80 (d, 1 H, *J* 2.0 Hz, H-2 Ar), 6.71 (dd, 1 H, *J* 8.1 and 2.0 Hz, H-6 Ar), 5.55 (s, 1 H, NH), 5.17 and 5.15 (2s, 4 H, 2 CH_2–Ph), 3.45 (m, 2 H, *CH_2*–NH), 2.70 (t, 2 H, *J* 6.8 Hz, CH_2–Ar), 2.65 (t, 2 H, *J* 6.8 Hz, CH_2 succ.), 2.38 (t, 2 H, *J* 6.8 Hz, CH_2 succ.); Anal. Calcd for $C_{26}H_{27}NO_5$: C, 72.04; H, 6.26; N, 3.23. Found: C, 71.75; H, 6.19; N, 3.06.

Benzyl 2,3,4-tri-O-*trimethylsilyl-β*-D-*glucopyranoside* (**10**).—A mixture of chlorotrimethylsilane (1.410 mL, 11.1 mmol) and hexamethyl disilazane (0.78 mL, 3.70 mmol) was carefully added to a vigorously stirred solution of benzyl β-D-glucopyranoside (**9**, 500 mg, 1.85 mmol) in pyridine (8 mL) at 0 °C. The temperature was slowly raised to 20 °C and the solution was stirred overnight. Solvent and excess reagents were evaporated under reduced pressure. The resulting white solid was dissolved in pentane, washed with water, dried ($MgSO_4$) and concentrated to afford benzyl 2,3,4,6-tetra-*O*-trimethylsilyl-β-D-glucopyranoside (764 mg, 74%) as a syrup. To a solution of this compound (737 mg) in acetone (3 mL) were added MeOH (4.10 mL) and AcOH (0.15 mL), and the mixture was stirred for 2 h at 0 °C. After the addition of solid $NaHCO_3$ (242 mg), the mixture was concentrated and the residue was purified by flash chromatography (9:1 hexane–EtOAc) to yield **10** as a solid (481.5 mg, 75%); mp 53–55 °C; $[\alpha]_D^{20}$ −20° (*c* 1, $CHCl_3$); R_f 0.64 (3:1 hexane–EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$): 7.27–7.37 (m, 5 H, aromatics), 4.81 (d, 1 H, *J* 11.8 Hz, CH_2–Ph), 4.64 (d, 1 H, *J* 11.8 Hz, CH_2–Ph), 4.36 (d, 1 H, $J_{1,2}$ 7.32 Hz, H-1), 3.76 (m, 1 H, H-6′), 3.58 (m, 1 H, H-6), 3.44–3.32 (m, 3 H, H-2,-3,-4), 3.24 (m, 1 H, H-5), 0.18–0.10 (27 H, Si–*CH_3*); ^{13}C NMR (50 MHz, $CDCl_3$): δ 128.3 (2 C aromatics), 128.1 (2 C aromatics), 127.8 (C ipso Bn), 103.0 (C-1), 78.1–71.7 (C-2,-3,-4,-5), 71.7 (CH_2–Ph), 62.3 (C-6), 1.3, 1.1 and 0.9 (9 C, Si–CH_3).

Benzyl 6-O-*[*N-*(3,4-dibenzyloxyphenylethyl)-succinamyl]-β*-D-*glucopyranoside* (**11**).—A stirred solution of acid **8** (53 mg, 0.122 mmol) in dry CH_2Cl_2 (1.52 mL) was treated with compound **10** (54 mg, 0.111 mmol), DCC (26.3 mg, 0.128 mmol) and DMAP (1 mg, 0.008 mmol). The mixture was kept at rt for 3 h and then filtered through Celite. The filtrate was diluted with CH_2Cl_2 and washed with an aq soln of 5% AcOH and water, dried (Na_2SO_4) and concentrated. The residue was treated with a 2% soln of TFA in nitromethane (5.24 mL) for 1 h at 0 °C and then $NaHCO_3$ (178 mg) was added. The reaction mixture was filtered and concentrated. The residue was purified by flash chromatography (50:1 → 30:1 CH_2Cl_2–MeOH) to give **11** as a solid (76 mg, 69%); mp 46–49 °C; $[\alpha]_D^{20}$ −24.4° (*c* 1, $CHCl_3$); R_f 0.52 (9:1 CH_2Cl_2–MeOH); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.25–7.45 (m, 15 H, aromatics), 6.87 (d, 1 H, *J* 8.2 Hz, H-5 Ar), 6.77 (d, 1 H, *J* 1.9 Hz, H-2 Ar), 6.67 (dd, 1 H, *J* 8.2 and 1.9 Hz, H-6 Ar), 5.67 (m, 1 H, NH), 4.89 (d, 1 H, *J* 11.7 Hz, CH_2–Ph), 4.59 (d, 1 H, *J* 5.6 Hz, CH_2–Ph), 4.36 (d, 1 H, *J* 7.3 Hz, H-1), 4.53 (dd, 1 H, $J_{6,6'}$ 12.0 Hz, $J_{5,6}$ 4.1 Hz, H-6), 4.24 (dd, 1 H, $J_{6,6'}$ 12.0 Hz, $J_{5,6'}$ 1.7 Hz, H-6′), 3.36–3.54 (m, 6 H, H-2,-3,-4,-5, *CH_2*–NH), 2.58–2.67 (m, 4 H, CH_2 succ.), 2.35 (m, 2 H, CH_2–Ar); ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.2 (–CONH–), 171.7 (–COO–), 137.3, 137.2 and 137.0 (3 C ipso Bn), 149.0 and 147.7 (C-3,-4 Ar), 132.1 (C-2 Ar), 127.3–128.5 (15 C aromatics), 121.63 (C-6 Ar), 115.8 and 115.5 (C-2,-5 Ar), 101.8 (C-1), 76.1, 73.8, 73.7, 69.6 (C-2,-3,-4,-5), 71.5, 71.3 and 71.1 (CH_2–Ph), 63.4 (C-6), 40.7 (CH_2–NH), 34.9 (CH_2–Ar), 30.8 and 29.4 (CH_2 succ.). Anal. Calcd for $C_{39}H_{43}NO_{10}$: C, 68.31; H, 6.33; N, 2.04. Found: C, 68.05; H, 6.20; N, 1.92.

6-O-*[*N-*(3,4-Dihydroxyphenylethyl)-succinamyl]-α,β*-D-*glucopyranose* (**1**).—A mixture of **11** (24 mg, 0.035 mmol) Pd/C (40 mg) and MeOH (3 mL) was stirred under hydrogen at rt for 1 h. The catalyst was removed by filtration and the solvent evaporated to give a syrup, which was dissolved in 95:5 water–Me_3CN and freeze-dried to afford **1** as a white powder (15 mg, 99%); mp 80–85 °C, R_f 0.34 (4:1:1 EtOAc–AcOH–water); $[\alpha]_D^{20}$ +28.9° (*c* 0.7, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 6.87 (d, 1 H, *J* 8.0 Hz, H-5 Ar), 6.83 (d, 1 H, *J* 2.0 Hz, H-2 Ar), 6.72 (dd, 1 H, *J* 8.0 and 2.0 Hz, H-2 Ar), 5.27 and 4.67 (2d, 1 H, *J* 3.6 and 7.8 Hz, H-1), 4.41 and 4.39 (2dd, 1 H, H-6), 4.53 and 4.58 (2dd, 1 H, H-6′), 2.65 (t, 2 H, *J* 6.7 Hz, CH_2CON); ^{13}C NMR (50 MHz, CD_3OD): δ 174.3 and 174.2 (2 C, CO), 146.2 and 144.7 (C-3 and C-4 Ar), 132.1 (C-1 Ar), 121.0 (C-6 Ar), 116.8 and 116.3 (C-2 and C-5 Ar), 98.3 (C-1β), 94.0 (C-1α), 77.9–70.6 (4 C, C-2,-3,-4,-5), 65.1 and 65.0 (C-6), 42.3 (CH_2NH), 35.8 (CH_2Ar), 31.4 and 30.4 (2 C, CH_2 succ.); Anal. Calcd for $C_{18}H_{25}NO_{10}$: C, 52.05; H, 6.07; N, 3.37. Found: C, 52.25; H, 5.95; N, 3.22.

1,2,3,4-Tetra-O-*trimethylsilyl*-α-D-*galactopyranose* (**12**).—Compound **12** was prepared according to the procedure described for the synthesis of **10**. After column chromatography (10:1 → 8:1 hexane–EtOAc), compound **12** was obtained as a solid, in 75% yield; mp 54–55 °C; R_f 0.60 (3:1 hexane–EtOAc); $[\alpha]_D^{20}$ +92° (*c* 1, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.11 (d, 1 H, *J* 1.8 Hz, H-1), 3.87–3.85 (m, 3 H, H-2,-3,-4), 3.96 (m, 1 H, H-5), 3.80 (m, 1 H, H-6), 3.61 (m, 1 H, H-6′), 0.16–0.12 (36 H, m, $(CH_3)_3Si$; ^{13}C NMR (50 MHz, $CDCl_3$): δ 94.5 (C-1), 73.9–69.7 (C-2,-3,-4,-5), 63.2 (C-6), 0.1–0.7 (12 C, $(CH_3)_3Si$).

6-O-*[*N-*(3,4-Dibenzyloxyphenylethyl)succinamyl]-β*-D-*galactopyranose* (**13**).—Compound **13** was prepared following a similar procedure as described for **11**. After purification by flash chromatography (15:1 CH_2Cl_2–MeOH), **13** was obtained as a solid (99 mg, 78%); mp 160–163 °C; R_f 0.35 (7:1 CH_2Cl_2–MeOH); $[\alpha]_D^{20}$ +26.6° (*c* 0.8, 1:1 $CHCl_3$–MeOH); ^{1}H NMR (400 MHz, 1:1 $CDCl_3$–CD_3OD): δ 7.38–7.23 (m, 10 H, aromatics), 6.81 (d, 1 H, *J* 8.2 Hz, H-5 Ar), 6.76 (d, 1 H, *J* 1.9 Hz, H-2 Ar), 6.64 (dd, 1 H, *J* 1.8 and 8.2 Hz, H-6 Ar), 5.24 and 4.35 (2d, 1 H, *J* 3.1 and 7.0 Hz H-1), 5.06 and 5.04 (2s, 4 H, CH_2–Ph), 2.61 (2 H, t, *J* 7.3 Hz, CH_2CON); ^{13}C NMR (50 MHz, 1:1 $CDCl_3$–CD_3OD): δ 172.6 and 172.0 (2 C, CO), 148.5 and 147.0 (2 C, C-3 and C-4 Ar), 136.8 and 136.7 (2 C, C ipso Bn), 132.3 (C-1 Ar), 121.3 (C-6 Ar), 115.5 and 115.2 (C-2 and C-5 Ar), 71.2 and 71.0 (2 C, CH_2–Ph), 72.9–67.2 (C-2,-3,-4,-5), 63.5 and 63.1 (C-6), 40.4 (CH_2–Ar), 34.9 (CH_2–NH), 29.9 and 28.8 (2 CH_2 succ.); Anal. Calcd for $C_{32}H_{37}NO_{10}$: C, 64.53; H, 6.26; N, 2.35. Found: C, 64.20; H, 6.12; N, 2.20.

6-O-*[*N-*(3,4-Dihydroxyphenylethyl)-succinamyl]-α,β*-D-*galactopyranose* (**2**).—Compound **2** was obtained by catalytic hydrogenation of **13** (94 mg, 0.16 mmol), as described for **1**, using 10% Pd/C (183 mg) and MeOH (10 mL). Work-up and final lyophilization analogously to **1**, afforded **2** as a white solid (65.7 mg, 99%), mp 97–100 °C; R_f 0.30 (4:1:1 EtOAc–AcOH–water); $[\alpha]_D^{20}$ +25.8° (*c* 0.4, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 6.90 (d, 1 H, *J* 8.0 Hz, H-5 Ar), 6.83 (d, 1 H, *J* 2.0 Hz, H-2 Ar), 6.72 (dd, 1 H, *J* 2.0 and 8.0 Hz, H-6 Ar), 5.33 and 4.62 (2d, 1 H, *J* 3.3 Hz and 7.6 Hz, H-1), 3.54 (m, 2 H, CH_2–NH), 2.65–2.84 (m, 6 H, CH_2–Ar); ^{13}C NMR (50 MHz, CD_3OD): δ 174.7 and 174.5 (2 C, CO), 145.8 and 144.3 (C-3 and C-4 Ar), 132.3 (C-1 Ar), 121.3 (C-6 Ar), 116.6 and 117.0 (C-2 and C-5), 76.0–69.1 (4 C, C-2,-3,-4,-5), 65.2 and 64.9 (C-6), 42.2 (CH_2–NH), 35.6 (CH_2–Ar), 31.4 and 30.4 (2 C, CH_2 succ.); Anal. Calcd for $C_{18}H_{25}NO_{10}$: C, 52.05; H, 6.07; N, 3.37. Found: C, 52.31; H, 5.93; N, 3.19.

1-O-*[*N-*(3,4-Dibenzyloxyphenylethyl)-succinamyl]-2,3,4,6-tetra*-O-*benzyl*-β-D-*glucopyranose* (**15**).—A solution of **14** (Ref. [13], 394 mg, 0.577 mmol) in CH_2Cl_2 and DMF (95:5, 13.1 mL) was treated with acid **8** (227 mg, 0.524 mmol) at rt and under argon for 5 days. Then, CH_2Cl_2 was added (10 mL) and the mixture was washed with ice-water (4 × 5 mL), dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (5:1 → 4:1 → 3:2 hexane–EtOAc) to give **15** as a white solid (220 mg, 40%); mp 75–78 °C; R_f 0.395 (1:1 hexane–EtOAc); $[\alpha]_D^{20}$ +10.4° (*c* 1, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ

7.04–7.38 (m, 30 H, aromatics), 6.81 (d, 1 H, *J* 8.2 Hz, H-5 Ar), 6.76 (d, 1 H, *J* 2.1 Hz, H-2 Ar), 6.61 (dd, 1 H, *J* 2.1 Hz and 8.2 Hz, H-6 Ar), 5.53 (d, 1 H, *J* 7.9 Hz, H-1), 5.38 (m, 1 H, NH), 5.07 and 5.06 (2s, 4 H, 2 CH_2–Ph), 4.83–4.36 (m, 8 H, 4 CH_2–Ph), 3.69–3.45 (m, 6 H, H-2,-3,-4,-6,-6′), 3.34 (m, 2 H, C*H*$_2$NH), 2.62–2.55 (m, 4 H, CH_2 succ. and CH_2–Ar), 2.24 (m, 2 H, CH_2 succ.); ^{13}C NMR (50 MHz, $CDCl_3$): δ 171.4 (*C*OO), 170.7 (*C*ONH), 147.8 and 149.1 (C-3 and C-4 Ar), 137.3–138.4 (C ipso Bn), 132.3 (C-1 Ar), 121.6 (C-6 Ar), 115.6, 115.9 (C-5, C-2 Ar), 94.3 (C-1), 84.8, 80.9, 77.2, 75.6 (C-2,-3,-4,-5), 75.6–71.3 (CH_2Ph); 68.1 (C-6), 40.7 (CH_2NH), 35.1 (CH_2Ar), 30.8 and 29.7 (2 CH_2 succ.); Anal. Calcd for $C_{60}H_{58}NO_{10}$: C, 75.61; H, 6.13; N, 1.47. Found: C, 75.31; H, 6.09; N, 1.52.

1-O-*[*N-*(3,4-Dihydroxyphenylethyl)-succinamyl]-β*-D-*glucopyranose* (**3**).—A solution of **15** (146 mg, 0.153 mmol) was hydrogenolyzed in 3:2 MeOH–EtOAc, in the presence of Pd/C (175 mg). After 3 h, work-up was carried out as described for **1**, giving **3** as a solid (63 mg, 99%); R_f 0.32 (4:1:1 EtOAc–AcOH–water); $[\alpha]_D^{20}$ −4° (*c* 1, MeOH); ^{1}H NMR (300 MHz, CD_3OD): δ 6.87 (d, 1 H, *J* 8.1 Hz, H-5 Ar), 6.83 (d, 1 H, *J* 2.0 Hz, H-2 Ar), 6.72 (dd, *J* 8.1 and 2.0 Hz, H-6 Ar), 5.67 (d, 1 H, *J* 8.1 Hz, H-1), 2.68 (t, 2 H, *J* 6.7 Hz, CH_2CON); ^{13}C NMR (50 MHz, CD_3OD): δ 174.0 and 173.1 (2 CO), 146.2 and 144.7 (C-3 and C-4 Ar), 132.0 (C-1 Ar), 121.3 (C-6 Ar), 116.8 and 116.3 (C-2 and C-5 Ar), 95.8 (C-1), 78.8–71.0 (4 C, C-2,-3,-4,-5), 62.3 (C-6), 42.4 (CH_2NH), 35.9 (CH_2–Ar), 31.2 and 30.4 (2 CH_2 succ.); Anal. Calcd for $C_{18}H_{25}NO_{10}$: C, 52.04; H, 6.07; N, 3.37. Found: C, 52.27; H, 5.93; N, 3.15.

N-*Benzyloxycarbonyl-3,4-dihydroxyphenylethylamine* (**16**).—To a solution of dopamine hydrochloride (2 g, 10.5 mmol) in MeOH (33 mL) and Et_3N (4.4 mL, 31.6 mmol), was added benzylchloroformate dropwise (1.9 mL) at 0 °C. Then, the stirred solution was allowed to attain rt and the stirring was continued for 2 h. The reaction mixture was neutralised with amberlite IR 150 (H^+) filtered, and concentrated to give a residue, which was purified by flash chromatography (2:1 → 1:1 hexane–EtOAc), affording compound **16** as a solid (2.11 g, 70%); mp 125–127 °C; R_f 0.35 (2:1:0.15 hexane–EtOAc–AcOH); ^{1}H NMR (200 MHz, $CHCl_3$): δ 6.67 (d, 1 H, *J* 8.1 Hz, H-5 Ar), 6.65 (d, 1 H, *J* 3.0 Hz, H-2 Ar), 6.53 (m, 1 H, H-6 Ar), 5.06 (s, 2 H, CH_2Ph), 3.27 (t, 2 H, *J* 7.5 Hz, CH_2NH), 2.62 (t, 2 H, *J* 7.7 Hz, CH_2Ar); ^{13}C NMR (50 MHz, CD_3OD): 159.1 (CO), 146.5 (C-3 Ar), 145.0 (C-4 Ar), 138.7 (C ipso), 132.3 (C-1 Ar), 129.7–129.0 (4 C, aromatics), 121.4 (C-6 Ar), 117.2 and 116.6 (C-2 and C-5 Ar), 67.5 (CH_2–Ph), 44.0 (CH_2–NH), 36.8 (CH_2–Ar); Anal. Calcd for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32; N, 5.16. Found: C, 70.71; H, 6.39; N, 5.02.

N-*Benzyloxycarbonyl-3,4-dihydroxy-3*-O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl)-phenylethylamine* (**18**) *and* N-*benzyloxycarbonyl-3,4-dihydroxy-4*-O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*glucopyranosyl)phenylethylamine* (**19**).—To a solution of **16** (1.5 g, 5.22 mmol) and β-D-glucose pentaacetate (**17**, 3.06 g, 7.83 mmol) in dry CH_2Cl_2 (53 mL) was added $BF_3{\cdot}OEt_2$ dropwise (3.2 mL, 26.10 mmol), stirring under argon for 4 h. Then, the mixture was diluted with CH_2Cl_2 and washed with satd $NaHCO_3$, water and dried ($NaSO_4$). Evaporation of the solvent, followed by purification by silica gel chromatography (2:1 → 1:1 hexane–EtOAc) afforded compounds **18** (0.58 g, 18%) and **19** (1.74 g, 54%) as solids; **18**: mp 42–45 °C; R_f 0.40, (1:1 hexane–EtOAc); $[\alpha]_D^{20}$ −13.2° (*c* 1, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32 (m, 5 H, aromatics), 6.85 (m, 2 H, H-5 and H-6 Ar), 6.80 (s, 1 H, H-2 Ar), 5.94 (s, 1 H, OH), 4.93 (d, 1 H, *J* 7.3 Hz, H-1), 4.81 (s, 1 H, NH), 4.26 (dd, 1 H, $J_{5,6}$ 5.1 Hz, $J_{6,6'}$ 12.4 Hz, H-6), 4.18 (dd, 1 H, $J_{5,6'}$ 2.4 Hz, $J_{6,6'}$ 12.4 Hz, H-6′), 3.82 (m, 1 H, H-5), 3.41 (m, 2 H, *CH*$_2$–NH), 2.72 (t, 2 H, *J* 6.59 Hz, *CH*$_2$–Ar), 2.05 (m, 12 H, 4 CH_3, Ac); ^{13}C NMR (50 MHz, $CDCl_3$). δ 177.1 (CO), 170.6–169.3 (4 C, CO, Ac), 156.2 (C-3 Ar), 145.9 (C-4 Ar), 144.0 (C ipso Bn), 136.4 (C-1 Ar), 130.7–128.5 (5 C, aromatics), 125.4 (C-6 Ar), 118.0 (C-2 Ar), 116.3 (C-5 Ar), 101.4

(C-1), 72.2–68.0 (C-2,-3,-4,-5), 66.6 (CH_2Ph), 61.6 (C-6), 42.0 (CH_2–NH), 35.3 (CH_2–Ar); Anal. Calcd for $C_{30}H_{35}NO_{13}$: C, 58.34; H, 5.71; N, 2.27. Found: C, 58.20; H, 5.90; N, 2.10. **19**: mp 87–89 °C, R_f 0.30 (1:1 hexane–EtOAc), $[\alpha]_D^{20}$ −65.7° (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 7.36 (m, 5 H, aromatics), 6.88 (d, 1 H, *J* 8.1 Hz, H-5 Ar), 6.79 (d, 1 H, *J* 2.09 Hz, H-2 Ar), 6.63 (dd, 1 H, *J* 8.2 and 2.1 Hz, H-6 Ar), 6.02 (s, 1 H, OH), 5.11 (s, 2 H, CH_2Ph), 4.91 (m, 1 H, H-1), 4.31 (dd, 1 H, $J_{6,6'}$ 12.4 Hz, $J_{6,5}$ 5.3 Hz, H-6), 4.19 (dd, 1 H, $J_{6,6'}$ 12.4 Hz, $J_{5,6'}$ 2.63 Hz, H-6′), 3.83 (m, 1 H, H-5), 3.43 (m, 2 H, CH_2–NH), 2.74 (m, 2 H, CH_2Ar), 2.11 (m, 12 H, 4 CH_3, Ac); ^{13}C NMR (50 MHz, $CDCl_3$): 177.1 (NH–CO–), 170.5–169.3 (–CO–, Ac), 156.2 (C-3 Ar), 147.4 (C-4 Ar), 142.8 (C ipso Bn), 136.4 (C-1 Ar), 128.0–128.4 (5 C, aromatics), 120.41 (C-6 Ar), 118.0 (C-2 Ar), 101.6 (C-1), 72.1, 71.2 and 68.0 (C-2,-3,-4,-5), 66.5 (CH_2Ph), 61.6 (C-6), 42.0 (CH_2NH), 35.4 (CH_2Ar), 20.6–20.5 (4 C, CH_3, Ac); Anal. Calcd for $C_{30}H_{35}NO_{13}$: C, 58.34; H, 5.71; N, 2.27. Found: C, 58.12; H, 6.00; N, 2.33.

3,4-Dihydroxy-3-O-(β-D-glucopyranosyl)-phenylethylamonium trifluoroacetate (**4**).—Compound **18** (490 mg, 0.793 mmol) was dissolved in NaMeO (0.1 M, 15 mL) and the mixture was stirred for 1.5 h at rt. Then the reaction mixture was neutralized with amberlite IR-120 (H^+), filtered and concentrated. The residue was purified by silica gel chromatography (7:1 EtOAc–MeOH) to yield the deacetylated glycoside (314 mg, 88%); R_f 0.67 (4:1 EtOAc–MeOH) which was dissolved (180 mg, 0.40 mmol) in MeOH (6 mL), and treated with TFA (26 μL, 1.6 equiv) and Pd/C (90 mg) and stirred under hydrogen at rt for 1 h. The catalyst was removed by filtration and the solvent and excess of TFA were evaporated giving **4** as a syrup (170 mg, 99%); R_f 0.28 (4:2:1 EtOAc–AcOH–water); $[\alpha]_D^{20}$ −35.7° (*c* 1, MeOH); ^1H NMR (300 MHz, CD_3OD): δ 7.30 (m, 1 H, H-2 Ar), 7.02 (m, 2 H, H-5 and H-6 Ar), 4.96 (d, 1 H, *J* 7.4 Hz, H-1), 4.13 (dd, 1 H, $J_{6,6'}$ 12.0 Hz, $J_{6,5}$ 2.1 Hz, H-6), 3.88 (dd, 1 H, $J_{6,6'}$ 12.0 Hz, $J_{6',5}$ 6.2 Hz, H-6′), 3.33 (2 H, t, *J* 8.0 Hz, CH_2NH), 3.04 (2 H, t, *J* 8 Hz, CH_2Ar); ^{13}C NMR (50 MHz, CD_3OD): δ 147.9 (C-3 Ar), 147.2 (C-4 Ar), 129.6 (C-1 Ar), 125.3 (C-6 Ar), 119.5 (C-2 Ar), 117.8 (C-5 Ar), 104.4 (C-1), 78.7, 77.9, 75.1, 71.8 (C-2,-3,-4,-5), 62.9 (C-6), 42.3 (CH_2–NH), 34.2 (CH_2–Ar); Anal. Calcd for $C_{16}H_{22}F_3NO_9$: C, 44.76; H, 5.16; N, 3.26. Found: C, 44.62; H, 5.20; N, 3.14. ESMS: $[M - CF_3COO^-]$ 316.3.

3,4-Dihydroxy-4-O-(β-D-glucopyranosyl)-phenylethylamonium trifluoroacetate (**5**).—Compound **5** was prepared from **19** as described for **4**. Thus, **5** was obtained as a syrup (1.2 g, 99%); R_f 0.25 (4:2:1 EtOAc–AcOH–water); $[\alpha]_D^{20}$ −39.7° (*c* 1, MeOH); ^1H NMR (300 MHz, CD_3OD): δ 7.36 (d, 1 H, *J* 8.3 Hz, H-5 Ar), 6.97 (d, 1 H, *J* 2.2 Hz, H-2 Ar), 6.88 (dd, 1 H, *J* 2.2 and 8.3 Hz, H-6 Ar), 4.91 (d, 1 H, *J* 7.6 Hz, H-1), 3.32 (t, 2 H, *J* 7.6 Hz, CH_2NH), 3.03 (t, 2 H, *J* 7.6 Hz, CH_2Ar); ^{13}C NMR (50 MHz, CD_3OD): δ 148.7 (C-3 Ar), 145.9 (C-4 Ar), 133.4 (C-1 Ar), 121.1 (C-6 Ar), 119.3 (C-2 Ar), 117.5 (C-5 Ar), 104.3 (C-1), 78.2, 77.5, 74.8 and 71.2 (C-2,-3,-4,-5), 62.3 (C-1), 41.9 (CH_2–NH), 33.9 (CH_2–Ar); Anal. Calcd for $C_{16}H_{22}F_3NO_9$: C, 44.76; H, 5.16; N, 3.26. Found: C, 44.62; H, 5.22; N, 3.31. ESMS: $[M - CF_3COO^-]$ 316.3.

D_2 receptor binding assays.—[^3H]spiperone (104 Ci/mmol) were obtained from Amersham International (England), unlabelled R(+)-SCH23390·HCl from Research Biochemicals Inc. (RBI, USA), and unlabelled spiperone, sulpiride·HCl and haloperidol from Sigma (USA). The reference drugs were stored in 1 mM soln at −20 °C. The new and reference drugs were diluted to the required concentration on ice, immediately before binding assays. Male Sprague–Dawley rats were killed by decapitation and their brains were rapidly removed and dissected on an ice-cold plate. Striatal membrane preparations were obtained by homogenization (Polytron homogenizer, setting 6.10 s) in 50 mM Tris–HCl (pH 7.7 at 25 °C; about 100 μL per mg of tissue) containing 5 mM EDTA; the homogenates were centrifuged (49,000*g* for 15 min at 4 °C, Sorvall RC-26 plus), resuspended in 50 mM Tris–HCl buffer (pH 7.4 at 25 °C), and centrifuged again (same conditions), and the final pellets were stored at −80 °C pending use. Just before binding assays, the pellets were resuspended (1.25 mg original wet weight per 750 μL) in 50 mM Tris–HCl buffer (pH 7.4 at

25 °C) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ and 1 mM $MgCl_2$.

Striatal membrane preparation (750 μL aliquots) were added to ice-cold tubes containing: (a) 100 μL of [^{3}H]spiperone, (b) 50 μL of ketanserin (final concentration 50 nM) to block 5-HT_{2A} receptors; and either (c) 100 μL of buffer (for total binding assay) or (d) 100 μL of sulpiride (final concentration 10 μM) to allow quantification of unspecific binding by [^{3}H]spiperone, or (e) 100 μL of the compounds to be tested (final assay vol was 1 mL). All assays were performed in duplicate. Incubations (15 min at 37 °C) were stopped by rapid vacuum filtration through GF-52 glass fibre filters (Schleicher and Schuell) in a Brandel M-30 cell harvester. The filters were rinsed three times with 3 mL of ice-cold 50 mM Tris–HCl buffer (pH 7.4), and radioactivity was determined by liquid scintillation counting in a Beckman LS-6000LL apparatus.

Statistical analysis.—Competition analyses in binding assays were carried out with the aid of the Graphpad Prism software (v 2.1) (GraphPad, San Diego, CA), and K_i values were calculated as $K_i = IC_{50}/(1 + L/K_d)$, where L is the concentration and K_d the apparent dissociation constant of the ligand.

Stability studies.—All incubations were carried at 37 °C in a shaker (Adolf Kühner AG CH-4127) at 200 rpm. At various time intervals, aliquots were removed and immediately analysed by HPLC. The mobile phase was 95:5 water 0.1% TFA–MeCN for compound **1** and dopamine and 97.5:2.5 water 0.1% TFA–MeOH for compounds **4** and **5**. The flow rate was fixed at 0.5 mL/min for all the stability experiments. Bovine liver esterase (EC 3.1.1.1), α-chymotrypsin from bovine pancreas (EC 3.4.21.1), and β-glucosidase from almonds (EC 3.2.1.21) were purchased from Sigma.

Enzyme stability studies

Esterase. To 500 μL of a stock solution of **1** (1.0 mM) in phosphate buffer (0.1 M, pH 7.2) preincubated at 37 °C, 380 μL of buffer and 120 μL of an esterase solution (0.5 units/μL) in buffer, were added. Aliquots (40 μL) were removed and immediately analysed by HPLC as described above.

Protease. To 500 μL of a solution of **1** (1.0 mM) in phosphate buffer (0.1 M, pH 8.0), were added 190 μL of buffer, 60 μL of an enzyme solution (0.5 units/μL) in phosphate buffer and 250 μL of a solution of $CaCl_2$ (40 mM) in water. Aliquots (40 μL) were removed and analysed by HPLC.

Glucosidase. Compound **4** or **5** was dissolved in phosphate buffer (0.1 M, pH 5.0) at a concentration of 10 mM. To 50 μL of this solution, 750 μL of buffer preincubated at 37 °C and 200 μL of a glucosidase solution (0.1 units/μL) in buffer (pH 5), were added. The samples were incubated and analysed by HPLC.

Stability in plasma.—To 10 mL of rat blood was added 90 μL of EDTA and the suspension was centrifuged at 10,000*g* for 15 min. Plasma was kept in eppendorf tubes at 4 °C and it was used immediately for the stability studies. Bioactivation experiments were initiated by adding a solution (50 μL, 10 mM in phosphate buffer 0.1 M, pH 7.01) of compounds **1**, **4**, **5** or dopamine, to 950 μL of plasma preincubated at 37 °C (final compounds concentration 0.5 mM). Aliquots (50 μL) were removed and 0.8 N $HClO_4$ (50 μL) was added. The resulting mixtures were centrifuged (15 000 *g*, 15 min, 4 °C) to remove precipitated proteins. A 50 μL aliquot of the supernatant layer was subjected to HPLC analyses (mobile phase for compound **1**: 95:5 → 93:7 → 70:30 → 95:5 water 0.1% TFA–Me_3CN; for compounds **4** and **5**: 97.5:2.5 water 0.1% TFA–MeOH; for dopamine: 95:5 water 0.1% TFA–Me_3CN.

Stability in rat brain extract

Compound **1** *and dopamine*. The entire rat brain was removed after incision of the skull, rinsed with phosphate buffer, dried, weighed (6.78 g) and then homogenized in phosphate buffer (0.1 M, pH 7.3) 1:4 w/v brain–phosphate buffer, using a mechanical tissue homogeniser for 5 min, at 4 °C. The homogenate was centrifuged at 60 000 *g* for 35 min at 4 °C, and the supernatant was aliquoted and stored at −80 °C until it was to be used for the experimental studies. Compound **1** or dopamine (50 μL of stock solution 10.0 mM) was incubated at 37 °C with brain preparation (950 μL) (final concentration 0.5 mM). At

various time points, 50 μL were removed and treated as described for plasma experiments.

Compounds **4** *and* **5**. For these compounds a brain homogenate with β-glycosidase activity was obtained as described by Withers and co-workers [18]. A rat brain was rinsed in distilled water, blotted and weighed (1.8 g); then 4.5 mL of 25 mM citrate–50 mM phosphate buffer, pH 5.0, containing EDTA (1 mM), dithiothreitol (4 mM) and Triton X-100 (0.1% v/v) was added and the tissues were treated for 1 min with a mechanical tissue homogeneizer. β-Glucosidase activity of the homogenate was measured and expressed as nmol of *p*-nitrophenyl β-D-glucopyranoside hydrolysed per h and per mg of tissue; the resulting value, 4.45 nmol/h mg, is in good agreement with published data. Tissue homogenates were frozen and stored at −80 °C. For the stability experiments 300 μL of a stock solution of **4** or **5** (1.0 mM) in buffer (pH 5) was added to 300 μL of the brain homogenate and incubated at 37 °C. At various time intervals, aliquots (50 μL) were removed and treated as described for plasma experiments.

Behaviour experiments.—Mice (weighing 25 ± 5 g) were housed in groups of 12 (mice) under regulated conditions (light/dark cycle between 8.00 and 20.00 h at 21 ± 1 °C) in standard Makrolon cages ($215 \times 465 \times 145$ mm). The animals received standard laboratory chow and tap water ad lib until the beginning of the experiments. Experimental animals were pretreated with 5 mg/kg of reserpine intraperitoneally, 18 h prior to experiments. Locomotive activity, rearing and velocity, were measured always at the same time of day (9–17 h), to avoid variation due to circadian rhythms, with a video computerised animal observation system (EthoVision V. 1.90, Noldus Information Technology, Wageningen, The Netherlands). Activity (total distance travelled in cm), rearing (changes upper 15% in corporal surface) and velocity, were recorded in foursquare test arenas ($50 \times 50 \times 30$ cm) via a video camera fixed on the ceiling above the arenas. Animals were tested for 1 h and the recorded information was relayed to a monitor and a video tracking motion analysis.

Drugs and chemicals.—All compounds were administered in 0.01 mL/g injections intraperitoneally. Reserpine (Sigma, St Louis, MO) was dissolved in 1% AcOH in water and new drugs were prepared in saline, immediately prior to the experiments. Methanol, citric acid monohydrate, perchloric acid, and NaCl were all of analytical grade (E. Merck, Darmstadt, Germany). Dopamine hydrochloride, 3,4-dihydroxyphenylacetic acid (DOPAC), 3-methoxytyramine HCl (3-MT), homovanillic acid (HVA) and 1-octanesulphonic acid (sodium salt) were purchased from Sigma.

Statistical analysis and graphics.—Pharmacological calculations were obtained by using the PCS program (Pharmacologic Calculation System; Tallarida and Murray, 1987) or PRISM program. The statistical significance of differences between means were determined by the Newman–Keuls test, and the differences with probabilities lower than 0.05 were considered statistically significant.

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