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Studies on the Uptake of Glucose Derivatives by Red Blood Cells

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Erythrocytes express the same glucose transporter (GLUT-1) as is present in the blood-brain barrier. With the aim of testing the viability of using this transport system to deliver glucosyl drug derivatives to the brain, the uptake of several dopamine–glucose conjugates and a few structurally related analogues by erythrocytes was studied with HPLC and ^{1}H MAS NMR spectroscopy. The results showed that slight structural changes determine the uptake of glycoconjugates by red blood cells. However, experiments in the presence of glucose transport inhibitors showed that none of the conjugates that efficiently crossed the cell membrane were transported by GLUT-1.

Introduction

Drug uptake in the brain is strictly regulated by the blood-brain barrier (BBB). This behavior relies on the structure of the brain vessels formed by endothelial cells joined by tight junctions and lined by astrocyte projections. Only a few substances needed for the proper function of the brain are able to cross this barrier.[1–3] This is the case of glucose, the main nutrient of the brain whose passage is facilitated by the glucose carrier GLUT-1.[4–6] The extraordinary efficiency of this transport system is demonstrated by the fact that more than 20% of the glucose in the bloodstream is metabolized in the brain. The high level of cerebral glucose uptake suggests GLUT-1 could be a useful carrier to deliver glucose-conjugated drugs efficiently and selectively to the brain. This approach has been used to facilitate the transport of different bioactive compounds across cell membranes that express the GLUT-1 protein.[7–14] However, no conclusive evidence showing that the resultant glycoconjugates cross the membranes by the glucose transport system has been reported.

In this context, studies to deliver dopamine to the central nervous system (CNS) using the glucose carrier GLUT-1 were initiated.[15–16] A variety of glycoconjugates with dopamine attached by ester, carbamate, and glycosidic bonds to different *O*-positions in the glucose were synthesized, and their affinity for the glucose carrier GLUT-1 using human erythrocytes was evaluated. Except for the glucose derivatives substituted at position C6, all other modifications of the sugar gave compounds with low or no binding to the carrier. Among the C6 substituted compounds, the carbamate **1** (Scheme 1) showed the highest affinity for the GLUT-1, its K_I being almost 15 times lower than the K_M of D-glucose. However, to exhibit affinity for GLUT-1 is not enough to penetrate the cell membrane by the glucose-transport system, as the subsequent translocation of bound substrate is the rate limiting step.[5] In fact, this compound was inactive when assayed in an experimental model of Parkinson's disease.[17] The lack of activity of **1** raised the question of whether this compound was able to cross the BBB using GLUT-1 or behaved just like a nontransported inhibitor.

To answer this question and get a better understanding of the structural requirements that glucose substituted at C6 must fulfill to be transported by GLUT-1, we have studied the uptake of carbamate **1** and a few structurally related analogues by erythrocytes using HPLC. These cells express the same GLUT-1 transporter present in the BBB. Thus, the crossing of the cell membrane was studied with analogue **2** (Scheme 1), in which the carbamate bond is replaced by an ether bond which is more stable chemically and enzymatically, and the phenylethylamine derivative **3**, which lacks the two phenolic hydroxyl groups. The γ-aminobutyric acid (GABA) derivative **4** was chosen as an analogue of **1** with a carboxylate ester instead of the aromatic ring. A group of dopamine derivatives that have the phenolic hydroxyl groups protected by methylene ketal (**5**), methyl (**6**), and acetyl (**7**) groups, was also included in the study. 3-*O*-methylglucose (**8**) known to be transported by GLUT-1 and not metabolized by cells, was used as a reference compound.

In addition, to study the transport of the glycoconjugates high-resolution proton magic angle spinning (MAS) NMR spectroscopy was used. This technique is increasingly used to investigate the metabolic profile of intact tissue and cell samples because it offers the advantage of not requiring sample preparation.[18–22] Moreover, it avoids the presence of artifacts or unwanted signals resulting from the processing of biological materials.

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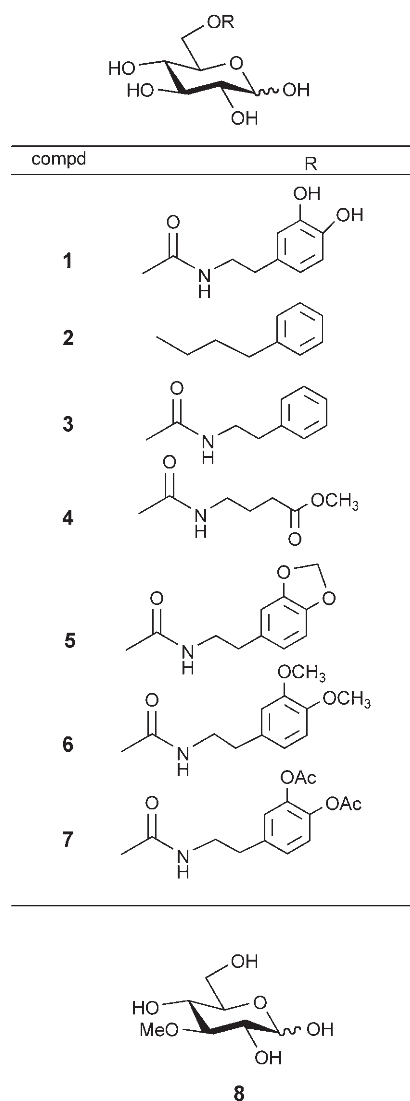

Scheme 1. Glycosyl derivatives.

Results and Discussion

Synthesis. Compound **2** was obtained by alkylation of partially benzylated glucose derivative **9**[16] with 3-phenylpropyl bromide to give **10**, which was submitted to debenzylation (Scheme 2). The synthesis of carbamates **3**–**7** was carried out by coupling the 6-*O*-imidazolylcarbonyl glucose derivative **11**[16] with the corresponding amine **12**–**16** to give conjugates **17**–**21**, followed by hydrogenolysis (Scheme 2). For **21**, the phenolic hydroxyl groups were acetylated (**22**) before removal of the benzyl groups.

Previously, amines **13**–**15** were synthesized in the following manner. Amine **13** was prepared by protection of the amino group of GABA with Cbz (**23**, Scheme 3), esterification with MeOH (**24**), and removal of Cbz group. Amines **14** and **15** were obtained from the *N*-Boc protected dopamine **25** by methylenation or methylation of phenolic hydroxyl groups to afford **26** and **27**, respectively, followed by removal of the Boc protecting group with trifluoroacetic acid.

Uptake experiments. Glucosyl derivatives **1**–**7** (15 mM) were incubated, in triplicate, in the presence of erythrocytes for 15 min and then blocking solution ($HgCl_2$, 2 mM) was added. Cells were washed with phosphate buffered solution (three times) and the contents of the isolated cells were released upon addition of aqueous 6% trichloroacetic acid (TCA) and analyzed by HPLC. The amount of glycoconjugate detected after cell lyses is shown in Table 1. Carbamate **1**, which in our previous study exhibited high affinity for GLUT-1, was not detected in the lysates. Similar results were obtained with the GABA derivative **4** and the diacetate **7**. On the other hand, compounds **2**, **3**, **5**, and **6** were able to cross the cell membrane, and the amount uptaken by cells varied from 0.8 to 4.9 mmol L^{-1}, as detected by HPLC.

It has been reported that certain catecholamines accumulate in the red blood cells because of their binding to hemoglobin.[23] Considering the possibility that the absence of peaks corresponding to compounds **1**, **4**, and **7** in the HPLC chromatograms of lysates could be due to their association with the proteins present in cells, we have also used high-resolution proton MAS NMR spectroscopy to study the transport of the glucosyl derivatives by recording the spectrum of whole red blood cells.

In solid or semisolid samples molecular motion is restricted or anisotropic and, consequently, dipolar coupling and chemical shift anisotropy contribute to broaden the resonance lines. However, if the sample is mechanically spun at an angle θ, an angle relative to the static magnetic field B_o, such that $3\cos^2\theta-1=0$, a high resolution spectra could be obtained. The value of the angle that fulfills the condition is 54° 44′, the so-called "magic angle." In addition, spinning the sample at the magic angle reduces the broadening due to magnetic susceptibility effects. In fact, all interactions proportional to $3\cos^2\theta'-1$, where θ' is the angle between B_o and the internuclear vector are averaged to zero.

Herein, we used proton T_2-filtered MAS NMR spectroscopy to investigate the uptake of glycoconjugates by erythrocytes. The T_2-filter consisted of a CPMG pulse sequence with a train of thirty 180° rf pulses spaced 2 ms apart. The effective echo time was 60 ms. Thus, the combination of both methods effectively reduces the broadening of the spectral lines due to restricted molecular mobility and local field inhomogeneity. In addition, to suppress the water protons signal low power continuous rf irradiation during the waiting period was applied.

The experimental protocol was similar to that employed in the analysis by HPLC. Glucosyl derivatives (15 mM) were incubated in the presence of erythrocytes for 15 min and then blocking solution ($HgCl_2$, 2 mM) was added. Cells were washed with deuterated phosphate buffered solution (three times) and analyzed by MAS NMR spectroscopy. For comparative purposes, we have carried out the uptake experiment with 3-*O*-methylglucose (**8**), considering that its transport across the cell membrane is mediated by the GLUT-1 protein. In Figure 1, the proton MAS spectra of a) erythrocytes, and erythrocytes incubated with b) **2**, c) **3**, d) **5**, e) **6**, and f) **8** are shown. Compounds **1**, **4**, and **7** gave a NMR spectrum essentially identical to that

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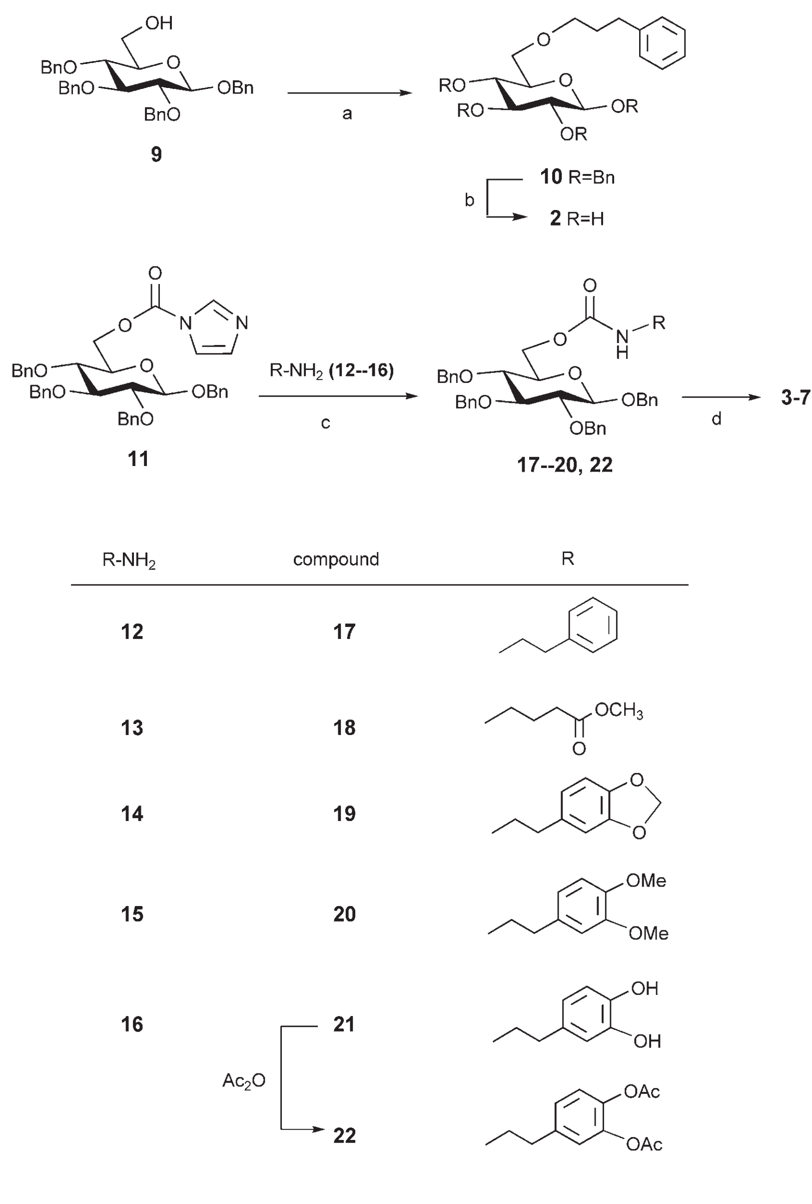

Scheme 2. Reagents and conditions: a) $Ph(CH_2)_3Br$ (2.5 equiv), NaH (3 equiv), THF, 60 °C, 12 h, 90 %; b) Pd/C, H_2, 1:1 AcOEt-MeOH, RT, 4 h, quant.; c) **12** (1.2 equiv), Et_3N, THF, 80 °C, 8 h, 92 %; **13** (1.1 equiv), Et_3N, THF, 40 °C, 6 h, 48 %; **14** (1 equiv), Et_3N, THF, 60 °C, 20 h, 81 %; **15** (0.9 equiv), Et_3N, THF, 60 °C, 14 h, 72 %; **16** (1.1 equiv), Py, RT, 72 h, then, Ac_2O, RT, 1.5 h, 39 % two steps; d) Pd/C, H_2, THF, RT, 25 h, quant.

of erythrocytes (Figure 1 a), indicating that these compounds do not cross the cell membrane.

The spectrum of erythrocytes incubated with **2**, **3**, **5**, and **6** showed the resonances from the phenyl ring of the substituent bound to glucose (δ = 6.83–7.35 ppm), which appeared in a region of the spectrum clearly differentiated from the signals of cell metabolites. In addition, the spectrum of the sample with **5** contained the signal for the methylene ketal protons at δ = 5.94 ppm, and samples with **6** contained the peaks assigned to the *O*-methyl groups (δ = 3.84 ppm). The spectrum of the sample incubated with 3-*O*-methylglucose (**8**) showed the signals associated with the carbohydrate derivative, although overlapping with those from metabolites in the erythrocytes, and the peak assigned to the *O*-methyl group (δ = 3.62 ppm).

Therefore, NMR spectroscopy experiments confirmed in a qualitative manner the results obtained by HPLC. These results indicate that little structural changes in the substituent of the glucose determine the uptake of the glycoconjugates by red blood cells. It seems that the presence of polar groups, such as phenol, is detrimental for the uptake as shown by the different results obtained with dopamine derivative **1** as opposed to the phenylethyl derivative **3** or the dopamine derivatives with protected phenolic hydroxyl groups **5** and **6**. Moreover, the polar effect seems to alter uptake more adversely than steric hindrance. When the phenyl ring in **3** was replaced by the smaller but more polar methyl carboxylate, the resulting compound **4** did not cross the membrane. In the case of the diacetate **7**, the hydrolysis of labile phenol ester bonds, to produce the nontransported dopamine derivative **1**, cannot be excluded.

To know if the uptake of **2**, **3**, **5**, and **6** was mediated by the GLUT-1 transporter, the incubations were carried out in the presence of maltosyl isothiocyanate (MITC),[24] a potent irreversible inhibitor of glucose transport in human erythrocytes, and of the GLUT-1 reversible inhibitor cytochalasin B (CCB, 50 μM). As shown in Table 2, similar values of compound **2**, **3**, **5**, or **6** were found in the lysates in the presence or absence of GLUT-1 inhibitors (entries 1–4). Neither the incubation at lower concentration of one of the glycoconjugates, **5** (5 mM), in the presence of saturating concentrations of CCB (50 and 200 μM, entries 5 and 6) nor glucose substrates (50 mM, data not shown) produce an effect on uptake. The proton MAS NMR spectrum of erythrocytes incubated with **5** (15 mM) in the presence of CCB (50 μM) was similar to that obtained in the absence of CCB (Figure 2).

Therefore, results using GLUT-1 inhibitors indicate that this carrier protein is not responsible for the cellular uptake of glucose conjugates. Although compounds **2**, **3**, **5**, or **6** must bind

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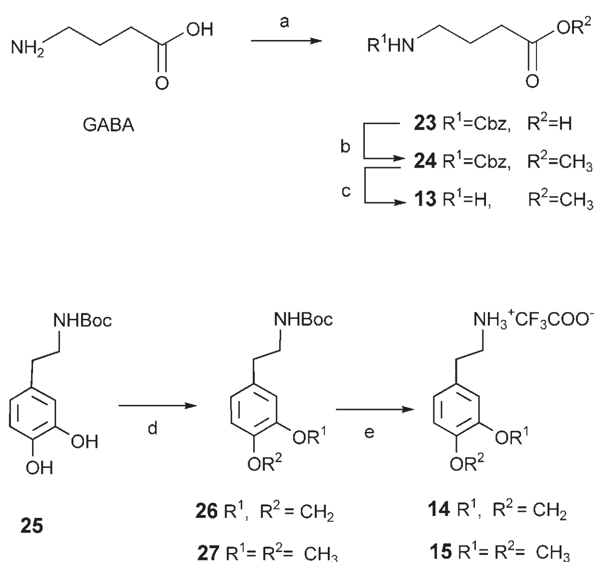

Scheme 3. Reagents and conditions: a) Benzyl chloroformate (1.5 equiv), $NaOH_{aq}$, RT, 1 h, 54%; b) MeOH, 4-dimethylaminopyridine, DCC, CH_2Cl_2, RT, 1 h, 96%; c) Pd/C, H_2, TFA, MeOH, RT, 2 h, quant.; d) CH_2Br_2 (10 equiv), K_2CO_3 (4 equiv), DMF, 65 °C, 5 h, 63% of **26**; $Me_2(SO_4)$ (2.2 equiv), K_2CO_3 (6 equiv), acetone, RT, 72 h, 85% of **27**; e) TFA, CH_2Cl_2, quant.

Table 1.
Uptake of glycoconjugates by red blood cells after 15 min incubation.[a]

Compound[b]	Uptake [mmol L^{-1} red cells]
1	0
2	4.9 ± 0.4
3	2.5 ± 0.9
4	0
5	1.8 ± 0.3
6	0.80 ± 0.01
7	0

[a] Determined by HPLC (see text). [b] Final concentration: 15 mM.

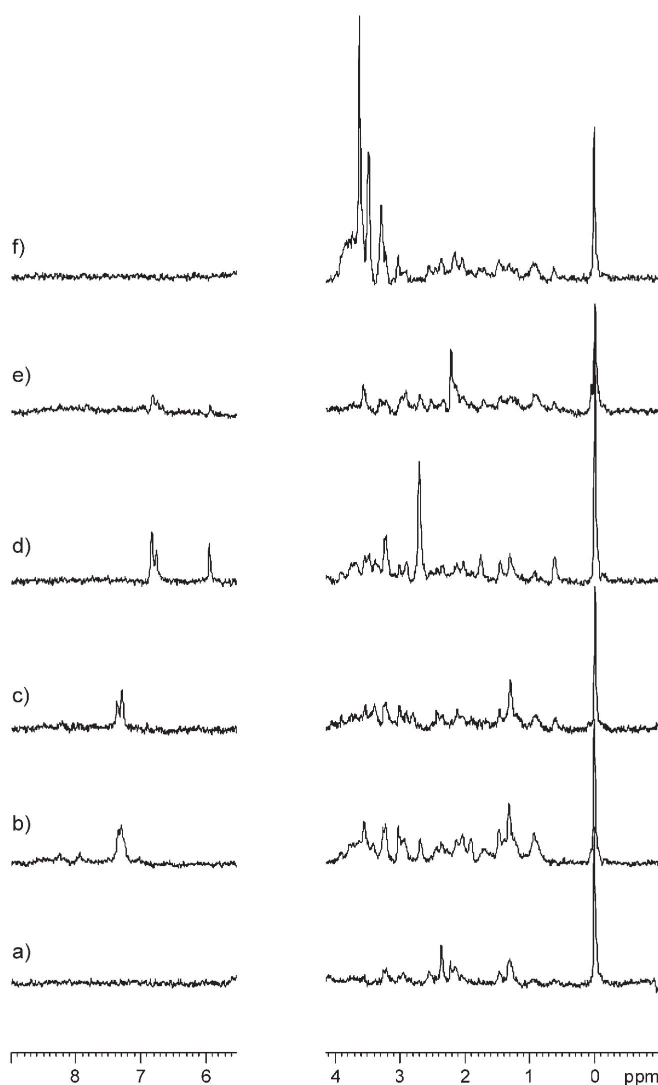

Figure 1. ^{1}H MAS NMR spectra corresponding to samples of a) erythrocytes, and erythrocytes incubated with b) **2**, c) **3**, d) **5**, e) **6**, and f) **8**. The spectrum of erythrocytes incubated with **2**, **3**, **5** and **6** showed the resonances from the phenyl ring of the substituent at C-6 appearing in a region of the spectrum clearly differentiated from signals of cell metabolites. The spectrum of sample incubated with **8** showed also the signals for the carbohydrate derivative, although overlapping with those from metabolites in the erythrocytes, and the peak assigned to the *O*-methyl group ($\delta = 3.62$ ppm).

to the glucose carrier, being analogous in structure to other C6 modified glucoses that showed high affinity for GLUT-1,[16] it seems that the subsequent translocation from membrane bound to cytoplasm, which is the rate-limiting step, should be precluded from occurring.

Additional evidence suggesting that GLUT-1 is not involved in the passage of the glycoconjugates was the uptake of the carbamate **30**, obtained as depicted in Scheme 4, which presents the dopamine moiety at the anomeric position. After incubation of **30** (15 mM) with erythrocytes under similar conditions as above, the glucose conjugate was detected in lysates in an amount similar to that obtained with the C6 substituted regioisomer **6** (0.5 mmol mL^{-1} for **30** versus 0.8 mmol mL^{-1} for **6**). In our previous work,[16] we showed that the substitution of glucose at the anomeric position leads to compounds with low or no binding to GLUT-1; therefore, the uptake of **30** seems to support a mechanism of penetration different from the use of the GLUT-1 transporter.

Table 2.
Uptake of glycoconjugates by red blood cells after incubation (15 min) in presence or absence of GLUT-1 inhibitors.[a]

entry	compd	conct [mM]	uptake [mmol L^{-1} red cells] no inhibitor	MITC[b]	CCB[c]
1	**2**	15	4.9 ± 0.4	5.3 ± 0.5	5.6 ± 0.7
2	**3**	15	2.5 ± 0.9	2.0 ± 0.4	1.8 ± 0.9
3	**5**	15	1.8 ± 0.3	2.1 ± 0.4	1.6 ± 0.2
4	**6**	15	0.80 ± 0.01	0.7 ± 0.05	0.7 ± 0.05
5	**5**	5	0.46 ± 0.06	–	0.56[d] ± 0.08
6	**5**	5	0.46 ± 0.06	–	0.60[e] ± 0.09

[a] Determined by HPLC (see text). [b] MITC: maltosyl isothiocyanate. [c] CCB: cytochalasin B (50 μM). [d] CCB, 50 μM. [e] CCB, 200 μM.

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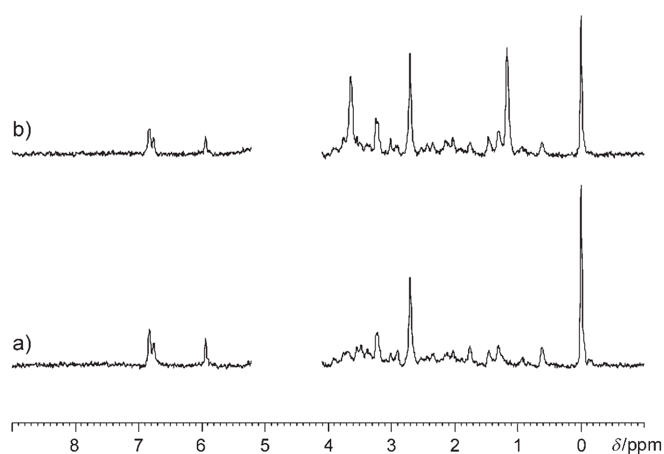
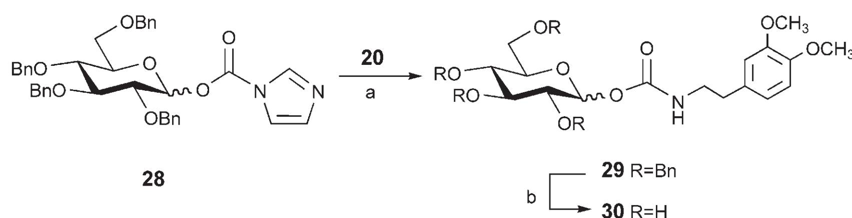


Figure 2. ^{1}H MAS NMR spectra corresponding to samples of erythrocytes incubated with **5** in the a) absence and b) presence of CCB (50 μM).



Scheme 4. Reagents and conditions: a) **20** (1.1 equiv), Et_3N, THF, 80 °C, 18 h, 37%; b) Pd/C, H_2, THF, RT, 4 h, quant.

Conclusions

The high expression of GLUT-1 in the BBB has stimulated the development of strategies to use this transport system to facilitate the entry of drugs into the brain.[25] Within this framework, we initiated studies on a new approach to deliver dopamine to the central nervous system by evaluating the interaction of a variety of glucosyl dopamine derivatives with GLUT-1.[16] The results showed that only compounds with the dopamine residue linked at position C6 of glucose showed high affinity for GLUT-1. In this work, we have studied the uptake by erythrocytes of **1**, a compound with demonstrated high affinity for GLUT-1, and some structurally related analogues. We have found that whereas **1** does not penetrate the cell, other derivatives with the phenolic hydroxyl groups protected or without these groups are able to cross the membrane. However, experiments in the presence of GLUT-1 inhibitors have shown that the passage is not mediated by GLUT-1. Although the mechanism of penetration is unknown, these results draw attention to the possibility of associating GLUT-1 to the transport of other bioactive glucosyl derivatives across cell membranes designed by a similar approach.

On the other hand, the observation that slight structural changes in the substituent linked to glucose can alter the ability of the sugar derivatives to cross the erythrocyte membrane, may lead to practical applications in the field of medicinal chemistry. For example, in the design of drugs for the treatment of *Plasmodium falciparum*-infected erythrocytes, the parasite that causes malaria requires a continuous supply of glucose to survive and proliferate. Recent studies[26,27] that show mechanistic differences between the parasite glucose transporter PfHT and mammalian GLUT-1, stimulate investigations on new drugs based on glucose derivatives with selective inhibition on the parasite transporter. However, to reach their target, they must first cross the membrane of the infected erythrocytes.

Experimental Section

General Methods: Chemicals were purchased puriss p.a. from commercial suppliers or purified by standard techniques. Solvents were distilled over drying agents: dimethylformamide, BaO; dichloromethane, CaH_2; tetrahydrofuran, sodium/benzophenone ketyl; acetonitrile, CaH_2; and pyridine, BaO. Thin-layer chromatography (TLC) was performed on aluminum sheets 60 F_{254} Merck silica gel and compounds were visualized by irradiation with UV light and/or by treatment with a solution of Ce_2MoO_4 or 5% H_2SO_4 in EtOH, followed by heating. Flash column chromatography was performed using thick walled columns, employing silica gel (Merck 60: 0.040–0.063 mm). The eluent used is indicated, and solvent ratios refer to volume. Melting points are not corrected. Optical rotations were recorded on a Perkin–Elmer 241 Polarimeter (λ = 589 nm, 1dm cell). ^{1}H NMR spectra were registered at 400, 300, or 200 MHz, and ^{13}C NMR were obtained at 100, 75, or 50 MHz using $CDCl_3$, CD_3OD, D_2O, or DMSO as solvent at room temperature. Chemical shift values are reported in parts per million (δ). Values of coupling constant (J) are reported in hertz (Hz), and spin multiplicities are indicated by the following symbol: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Mass spectroscopy spectra were registered on a HP series 1100 MSD spectrometer.

Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-(3-phenylpropyl)-β-D-glucopyranoside (10): Sodium hydride (26.6 mg, 1.1 mmol) was added to a solution of **9** (200 mg, 0.37 mmol) in dry THF (3.7 mL) under Ar, and the mixture was stirred at RT for 10 min. Then, tetrabutylammonium bromide (59.6 mg, 0.19 mmol) was added and the mixture was stirred for a further 10 min. After this time elapsed, 3-phenylpropyl bromide (0.15 mL, 1.0 mmol) was added dropwise, and the reaction mixture was heated at 60 °C for 12 h. After cooling, the reaction was quenched with methanol (1 mL) and the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl ether (8 mL) and washed with cool water (4×4 mL), dried over Na_2SO_4, and concentrated. The residue was purified by column chromatography (hexane-AcOEt, 6:1) to give **10** (219 mg, 90%). R_f: 0.42 (hexane-AcOEt, 4:1); $[\alpha]_D = -9.8°$ ($c = 1.6$, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.4–7.2 (m, 25 H), 5.0–4.6 (m, 8 H), 4.27 (d, 1 H, J = 7.7 Hz), 3.7–3.4 (m, 8 H), 2.7–2.6 (m, 2 H), 1.91 ppm (q, 2 H, J = 6.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 141.9, 138.6, 138.5, 138.2, 137.5, 128.5–125.7, 102.7, 84.8, 82.3, 78.0, 77.7, 75.6, 75.1, 74.0, 69.7, 71.1, 70.2, 32.4, 31.3 ppm. MS (ES) m/z (calcd 658.3): 681.3 $[M+Na]^+$.

6-*O*-phenylpropyl-α,β-D-glucopyranose (2): 10% Pd/C (217 mg) was added to a solution of **10** (130 mg, 0.20 mmol) in 1:1 AcOEt-MeOH (20 mL), and the reaction mixture was stirred under H_2 for

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4 h. Then, the mixture was filtered through Celite and the solvent evaporated to dryness obtaining **2** (59 mg, quantitative). R_f: 0.4 (CH_2Cl_2-MeOH, 5:1); $[\alpha]_D=+24.0°$ (c=1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ=7.5–7.3 (m, 5H), 5.29 (d, <1H, J=3.4 Hz), 4.65 (d, <1H, J=7.8 Hz), 4.1–4.0 (m, 1H), 3.93 (dd, <1H, J=2.0, J=10.7 Hz), 3.9–3.3 (m, 7H), 2.87 ppm (t, 2H, J=7.8 Hz), 2.06 (m, 2H); ^{13}C NMR (125 MHz, CD_3OD): δ=143.3, 129.5, 129.3, 126.8, 98.2, 94.0, 78.2–73.8, 72.1–71.4, 33.3, 32.7 ppm. MS (ES) m/z (calcd 298.1): 321.0, $[M+Na]^+$. Anal. Calcd for $C_{15}H_{22}O_6$: C, 60.39; H, 7.43. Found: C, 59.93; H, 7.21.

4-(Benzyloxycarbonylamino)butanoic acid (23): Benzylchloroformate (21.3 mL, 149 mmol) and 4 N NaOH (60 mL) were added to a solution of 4-aminobutyric acid (10 g, 97 mmol) in 2 N NaOH (130 mL) cooled at 0 °C. The reaction mixture was stirred for 1 h, then treated with concentrated HCl solution to pH 4, and a precipitate was formed. The solid was extracted with diethyl ether (3× 150 mL) and washed with 1N $NaHCO_3$ (3×100 mL). The aqueous phase was cooled at 0 °C, acidified with 1N HCl to pH 4, and the white precipitate formed was filtered and crystallized from water to give **23** (12.4 g, 54%). R_f: 0.58 (AcOEt); ^{1}H NMR (300 MHz, $CDCl_3$): δ=10.7 (s, 1H), 7.4–7.2 (m, 5H), 6.2 (s, 1H), 5.2 (d, 2H, J=8.3 Hz), 3.2 (t, 2H, J=6.6 Hz), 2.40 (t, 2H, J=7.1 Hz), 1.8 ppm (q, 2H, J=6.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ=178.8, 156.9, 136.7, 128.8, 128.4, 128.2, 67.1, 40.5, 31.4, 25.1 ppm.

Methyl 4-(benzyloxycarbonylamino)butanoate (24): 23 (474 mg, 2.0 mmol) and 4-dimethylaminopyridine were added to a solution of methanol (126 μL, 5 mmol) in CH_2Cl_2 (4 mL). The mixture was cooled at 0 °C and dicyclohexylcarbodiimide (412 mg, 2 mmol) dissolved in CH_2Cl_2 (4 mL) was added dropwise over 10 min. The reaction was stirred at RT for 1 h. Then, the mixture was filtered through Celite and concentrated. The residue was purified by column chromatography (hexane-AcOEt, 2:1) to give **24** (480 mg, 96%). R_f: 0.53 (hexane-AcOEt, 1:1); ^{1}H NMR (200 MHz, $CDCl_3$): δ= 7.4–7.3 (m, 5H), 5.13 (s, 2H), 4.98 (s, 1H), 3.69 (t, 3H), 3.27 (td, 2H, J=13.0 Hz, J=6.8 Hz), 2.40 (t, 2H, J=7.3 Hz), 1.87 ppm (q, 2H, J= 7.1 Hz).

3-(Methoxycarbonyl)propylammonium trifluoroacetate (13): Trifluoroacetic acid (0.23 mL, 3.0 mmol) and 10% Pd/C (440 mg) were added to a solution of **24** (480 mg, 1.9 mmol) in methanol (27 mL), stirring at RT under H_2 for 2 h. Then, the mixture was filtered through Celite and the solvent evaporated to dryness obtaining **13** (442 mg, quantitative). ^{1}H NMR (300 MHz, $CDCl_3$): δ=3.87 (s, 3H), 3.17 (t, 2H, J=7.7 Hz), 2.69 (t, 2H, J=7.2 Hz), 2.13 ppm (q, 2H, J= 7.2 Hz). MS (ES) m/z (calcd 217.1): 104.1 $[M-CF_3COO]^+$.

***N*-(*tert*-butylcarbonyl)-3,4-dihydroxyphenylethylamine (25):** Ditertbutyldicarbonate (500 mg, 2.3 mmol) was added to a solution of dopamine hydrochloride (400 mg, 2.11 mmol) in 9:1 methanol-triethylamine (13 mL). The reaction mixture was stirred at 50 °C for 30 min. After cooling, the mixture was concentrated and the residue was treated with diluted HCl (7 mM, pH 2) for 120 min, then, extracted with AcOEt. The organic phase was dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (hexane-AcOEt-methanol, 4:1:0.5→2:1:0.5) to give **25** (490 mg, 92%). R_f: 0.36 (hexane-AcOEt-AcOH, 2:1:0.15); Mp: 136–138 °C; ^{1}H NMR (200 MHz, CD_3OD): δ=6.86 (d, J=8.0 Hz), 6.82 (d, 1H, J=2.0 Hz), 6.70 (dd, 1H, J=1.9 Hz, J=8.0 Hz), 3.37 (t, 2H, J= 7.5 Hz), 2.77 (t, 2H, J=7.5 Hz), 1.61 ppm (s, 9H); ^{13}C NMR (50 MHz, CD_3OD): δ=158.4, 146.2, 144.7, 131.4, 121.1, 116.9, 116.4, 80.0, 43.3, 36.6, 28.8 ppm. 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.

***N*-(*tert*-butylcarbonyl)-3,4-methylenedioxyphenylethylamine (26):** Dibromomethane (1.65 mL, 24 mmol) and potassium carbonate (1.33 g, 9.6 mmol) were added to a solution of **25** (600 mg, 2.4 mmol) in dry dimethylformamide (6 mL) under Ar. The reaction mixture was stirred at 65 °C for 5 h. After cooling, the mixture was filtered through Celite and concentrated. The residue was purified by column chromatography (hexane-AcOEt, 5:1) to give **26** (400 mg, 63%). R_f: 0.78 (hexane-AcOEt, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.73 (d, 1H, J=7.9 Hz), 6.66 (d, 1H, J=1.3 Hz), 6.62 (dd, 1H, J=7.8 Hz, J=1.5 Hz), 5.91 (s, 2H), 4.59 (s, 1H), 3.32 (t, 2H, J= 6.5 Hz), 2.69 (t, 2H, J=6.5 Hz), 1.42 ppm (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=155.8, 147.7, 146.0, 132.7, 121.6, 109.1, 108.25, 100.8, 79.1, 41.9, 35.9, 28.4 ppm. MS (ES) m/z (calcd 265.1): 288.1 $[M+Na]^+$.

***N*-(*tert*-butylcarbonyl)-3,4-dimethoxyphenylethylamine (27):** Potassium carbonate (3.27 g, 23.7 mmol) and dimethylsulphate (0.82 mL, 8.69 mmol) were added to a solution of **25** (1 g, 3.95 mmol) in acetone (20 mL). The reaction mixture was stirred at RT during 72 h. Then, the mixture was filtered through Celite and concentrated. The residue was purified by column chromatography (hexane-AcOEt, 10:1→5:1) to give **27** (940 mg, 85%). R_f: 0.66 (hexane-AcOEt, 1:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.78 (d, J= 8.2 Hz), 6.72 (d, 1H, J=2.0 Hz), 6.69 (m, 1H), 4.59 (s, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.34 (t, 2H, J=6.5 Hz), 2.72 ppm (t, 2H, J= 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ=155.8, 148.8, 147.5, 131.4, 120.6, 111.8, 111.2, 79.1, 55.8, 55.7, 41.8, 35.7, 28.3 ppm. MS (ES) m/z (calcd 281.1): 304.1 $[M+Na]^+$.

3,4-Methylenedioxyphenylethylammonium trifluoroacetate (14): 26 (400 mg, 1.5 mmol) was treated with a solution of CF_3COOH-CH_2Cl_2 (5:95, 15 mL) stirring at RT for 20 h, then evaporated to dryness to obtain **14** (520 mg, quantitative). ^{1}H NMR (300 MHz, CD_3OD): δ=6.8–6.7 (m, 3H), 5.93 (s, 2H), 3.12 (t, 2H, J=7.3 Hz), 2.86 ppm (t, 2H, J=7.2 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ=149.6, 148.3, 131.4, 123.0, 109.9, 109.5, 102.5, 42.1, 34.3 ppm. MS (ES) m/z (calcd 279.1): 166.1 $[M-CF_3COO]^+$.

3,4-Dimethoxyphenylethylammonium trifluoroacetate (15): 27 (620 mg, 2.2 mmol) was treated with a solution of CF_3COOH-CH_2Cl_2 (5:95, 12 mL) stirring at RT for 8 h. Then, the mixture was concentrated to dryness to obtain **15** (650 mg, quantitative). ^{1}H NMR (300 MHz, CD_3OD): δ=6.90 (d, 1H, J=8.5 Hz), 6.87 (d, 1H, J= 1.8 Hz), 6.80 (dd, 1H, J=8.4 Hz, J=1.8 Hz), 3.82 (s, 3H), 3.80 (s, 3H), 3.16 (t, 2H, J=7.5 Hz), 2.89 ppm (t, 2H, J=7.3 Hz); ^{13}C NMR (75 MHz, CD_3OD): δ=150.7, 149.6, 130.6, 122.2, 113.5, 113.3, 56.5, 56.4, 42.1, 34.1 ppm. MS (ES) m/z (calcd 295.1): 182.1 $[M-CF_3COO]^+$.

Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-[*N*-(phenylethyl)aminocarbonyl]-β-D-glucopyranoside (17): Triethylamine (1.3 mL) and **11** (940 mg, 1.48 mmol) were added to a solution of **12** (0.154 mL, 1.23 mmol) in dry THF (4 mL), stirring at 80 °C under Ar for 8 h. After cooling at RT, the mixture was concentrated and the residue was purified by column chromatography (hexane-AcOEt, 3:1→2:1) to give **17** (780 mg, 92%). R_f: 0.53 (hexane-AcOEt, 1:1); $[\alpha]_D=-6.8°$ (c=1.4, CH_2Cl_2); ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.4–7.2 (m, 25H), 5.0–4.3 (m, 12H), 3.66 (t, 2H, J=8.4 Hz), 3.6–3.4 (m, 3H), 2.82 ppm (t, 2H, J= 7.5 Hz). MS (ES) m/z (calcd 687.3): 710.3 $[M+Na]^+$.

Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-[3-(methoxycarbonyl)propylaminocarbonyl]-β-D-glucopyranoside (18): 13 (290 mg, 1.25 mmol) was reacted under similar conditions as described for **12**, and the residue was purified by column chromatography (toluene-AcOEt, 6:1) to give **18** (303 mg, 48%). R_f: 0.13 (hexane-AcOEt, 3:1); $[\alpha]_D=-7.6°$ (c=1.4, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ=7.4–7.3 (m, 20H), 5.0–4.6 (m, 10H), 4.51 (d, 1H, J=7.6 Hz), 3.66 (s, 3H), 3.7–3.5 (m, 4H), 3.22 (c, 2H, J=6.6 Hz), 2.36 (t, 2H, J=7.3 Hz), 1.83 ppm (q, 2H, J=7.1 Hz); ^{13}C NMR (50 MHz, $CDCl_3$): δ=173.7, 156.3, 138.6, 138.4, 137.9, 137.4, 129.0–127.8, 102.7, 84.8, 2.3, 77.9, 77.8–71.3, 63.5, 51.8, 40.5, 31.3, 25.2 ppm. MS (ES) m/z (calcd 683.3): 706.3 $[M+Na]^+$.

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Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-[*N*-(3,4-methylenedioxyphenylethyl) aminocarbonyl]-β-D-glucopyranoside (19): 14 (279 mg, 1.0 mmol) was reacted under similar conditions as described for **12**, and the residue was purified by column chromatography (hexane-AcOEt, 5:1) to give **19** (590 mg, 81%). R_f: 0.53 (toluene-AcOEt, 5:1); $[\alpha]_D = -6.7^\circ$ ($c = 1.2$, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.3$–7.2 (m, 20H), 6.64 (d, 1H, $J = 8.3$ Hz), 6.59 (d, 1H, $J = 1.1$ Hz), 6.54 (dd, 1H, $J = 8.3$ Hz, $J = 1.1$ Hz), 5.80 (s, 2H), 4.9–4.3 (m, 12H), 3.58 (t, 2H, $J = 9.3$ Hz), 3.5–3.3 (m, 3H), 2.64 ppm (t, 2H, $J = 6.8$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 156.0$, 147.8, 146.2, 138.4, 138.3, 137.7, 137.2, 132.3, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.8, 127.8, 127.7, 121.6, 109.0, 108.4, 102.4, 100.9, 84.6, 82.2, 77.3, 75.7, 75.0, 74.9, 73.2, 71.16, 63.2, 42.3, 35.7 ppm. MS (ES) *m/z* (calcd 731.3): 754.3 $[M+Na]^+$.

Benzyl 2,3,4-tri-*O*-benzyl-6-*O*-[*N*-(3,4-dimethoxyphenylethyl)aminocarbonyl]-β-D-glucopyranoside (20): 15 (350 mg, 1.19 mmol) was reacted under similar conditions as described for **12**, and the residue was purified by column chromatography (toluene-AcOEt, 20:1→10:1→5:1) to give **20** (640 mg, 72%). R_f: 0.57 (toluene-AcOEt, 5:1); $[\alpha]_D = +5.5^\circ$ ($c = 0.5$, CH_2Cl_2); ^1H NMR (200 MHz, CD_3OD): $\delta = 7.4$–7.2 (m, 20H), 6.8–6.7 (m, 3H), 5.0–4.5 (m, 12H), 3.85 (s, 3H), 3.83 (s, 3H), 3.9–3.8 (m, 5H), 2.80 ppm (t, 2H, $J = 7.0$ Hz); ^{13}C NMR (50 MHz, CD_3OD): $\delta = 156.0$, 149.0, 147.6, 138.4, 138.2, 137.7, 137.1, 131.0, 128.6, 128.4, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.9, 127.8, 127.6, 120.6, 111.8, 111.3, 102.4, 63.2, 55.8, 43.0, 35.6 ppm. MS (ES) *m/z* (calcd 747.3): 770.3 $[M+Na]^+$.

Benzyl 6-*O*-[*N*-(3,4-diacetoxyphenylethyl)aminocarbonyl]-2,3,4-tri-*O*-benzyl-β-D-glucopyranoside (22): 11 (1.2 g, 1.9 mmol) was dissolved in dry pyridine (6 mL) and a suspension of dopamine (405.3 mg, 2.1 mmol) in pyridine (3.5 mL) was added dropwise under Ar. The reaction mixture was stirred at RT for 72 h. After TLC indicated completion of the reaction, acetic anhydride (4 mL) was added and the reaction was continued at RT for 1.5 h. Then, the mixture was concentrated and the residue was purified by column chromatography (hexane-AcOEt, 5:1→2:1→0:1) to give **22** (590 mg, 39%, two steps). R_f: 0.45 (hexane-AcOEt, 1:1); $[\alpha]_D = -4.6^\circ$ ($c = 1.1$, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.3$–7.1 (m, 20H), 7.0–6.9 (m, 3H), 5.58 (s, <1H), 5.1–5.0 (m, 1H), 4.8–4.7 (m, 3H), 4.7–4.4 (m, 8H), 4.3–4.2 (m, 1H), 3.5–3.4 (m, 2H), 3.20 (t, 2H, $J = 7.2$ Hz), 2.62 (t, 2H, $J = 7.0$ Hz), 2.08 (s, 3H), 2.06 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 168.7$, 168.5, 156.5, 142.3, 140.9, 138.8, 138.6, 138.2, 138.1, 137.6, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.2, 124.0, 123.7, 102.8, 84.9, 82.5, 78.1, 77.7, 77.3, 75.9, 75.3, 73.5, 71.4, 60.7, 42.3, 35.7, 20.9, 20.8 ppm.

6-*O*-[*N*-(phenylethyl)aminocarbonyl]-α,β-D-glucopyranose (3): To a solution of **17** (680 mg, 1 mmol) in AcOEt-MeOH (1:1, 20 mL), 10% Pd/C (1.1 g) was added and the reaction mixture was stirred under H_2 for 5 h. Then, the mixture was filtered through Celite and concentrated. The residue was purified by column chromatography (CH_2Cl_2-MeOH, 30:1→4:1) to give **3** (235 mg, 70%). R_f: 0.2 (CH_2Cl_2-MeOH, 5:1); $[\alpha]_D = +38^\circ$ ($c = 1.0$, MeOH); ^1H NMR (300 MHz, CD_3OD): $\delta = 7.3$–7.2 (m, 5H), 5.07 (d, <1H, $J = 3.6$ Hz), 4.46 (d, <1H, $J = 7.8$ Hz), 4.4–4.1 (m, 3H), 3.9–3.8 (m, 2H), 3.7–3.6 (m, 1H) 3.12 (t, 2H, $J = 8.2$ Hz), 2.77 ppm (t, 2H, $J = 7.5$ Hz). MS (ES) *m/z* (calcd 327.1): 328.1 $[M+H]^+$. Anal. Calcd for $C_{15}H_{21}NO_7$: C, 55.04; H, 6.47; N, 4.28. Found: C, 54.63; H, 6.27; N, 3.91.

6-*O*-[3-(methoxycarbonyl)propylaminocarbonyl]-α,β-D-glucopyranose (4): 10% Pd/C (56 mg) was added to a solution of **18** (141 mg, 0.21 mmol) in 1:1 AcOEt-MeOH (5 mL) and the reaction mixture was stirred under H_2 for 4 h. Then, the mixture was filtered through Celite and the solvent evaporated to dryness to obtain **4** (67 mg, quantitative). R_f: 0.3 (CH_2Cl_2-MeOH, 5:1); $[\alpha]_D = +53^\circ$ ($c = 1.0$, MeOH); ^1H NMR (300 MHz, CD_3OD): $\delta = 5.01$ (d, <1H, $J = 3.7$ Hz), 4.46 (d, <1H, $J = 7.8$ Hz), 4.35–4.13 (m, 2H), 3.9–3.8 (m, <1H), 3.66 (s, 3H), 3.5–3.3 (m, >3H), 3.13 (t, 2H, $J = 6.8$ Hz), 2.36 (t, 2H, $J = 7.5$ Hz), 1.78 ppm (q, 2H, $J = 7.8$ Hz). Anal. Calcd for $C_{12}H_{21}NO_9$: C, 44.58; H, 6.55; N, 4.33. Found: C, 44.12; H, 6.05; N, 4.43.

6-*O*-[*N*-(3,4-methylenedioxy-phenylethyl)aminocarbonyl]-α,β-D-glucopyranose (5): 10% Pd/C (693 mg) was added to a solution of **19** (460 mg, 0.63 mmol) in THF (63 mL) and the reaction mixture was stirred under H_2 for 4 h. Then, the mixture was filtered through Celite and the solvent evaporated to dryness to obtain **5** (234 mg, quantitative). R_f: 0.2 (CH_2Cl_2-MeOH, 5:1); $[\alpha]_D = +18^\circ$ ($c = 1.0$, MeOH); ^1H NMR (300 MHz, DMSO): $\delta = 6.8$–6.7 (m, 2H), 6.63 (d, 1H, $J = 7.7$ Hz), 5.94 (s, 2H), 4.88 (d, <1H, $J = 2.3$ Hz), 4.3–4.1 (m, >2H), 4.0–3.9 (m, 1H), 3.7–3.6 (m, <1H), 3.5–3.2 (m, 4H), 3.1–2.9 (m, 5H), 2.60 ppm (t, 2H, $J = 7.6$ Hz); ^{13}C NMR (75 MHz, DMSO): $\delta = 162.2$, 156,3, 147.2, 145.5, 133.2, 121.5, 109.0, 108.1, 100.6, 96.9, 92.3, 76.5, 74.7, 73.9, 72.9, 72.2, 70.6, 70.2, 69.5, 60.8, 57.1, 42.1, 35.0 ppm. MS (ES) *m/z* (calcd 371.1): 394.1 $[M+Na]^+$. Anal. Calcd for $C_{16}H_{21}NO_9$: C, 51.75; H, 5.70; N, 3.77. Found: C, 51.30; H, 5.31; N, 3.62.

6-*O*-[*N*-(3,4-dimethoxyphenylethyl)aminocarbonyl]-α,β-D-glucopyranose (6): 20 (550 mg, 0.74 mmol) was reacted under similar conditions as described for **19**. The residue was purified by column chromatography (AcOEt-MeOH, 5:1) to give **6** (200 mg, 70%). R_f: 0.2 (AcOEt-MeOH, 5:1); $[\alpha]_D = +29^\circ$ ($c = 2.3$, MeOH); ^1H NMR (300 MHz, CD_3OD): $\delta = 6.9$–6.7 (m, 3H), 5.04 (d, <1H, $J = 3.6$ Hz), 4.43 (d, <1H, $J = 7.9$ Hz), 4.3–4.0 (m, 2H), 3.9–3.6 (m, 7H), 3.4–3.1 (m, 5H), 2.67 ppm (t, 2H, $J = 7.3$ Hz); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 159.1$, 159.1, 150.4, 149.0, 133.4, 122.1, 113.7, 113.1, 98.2, 94.0, 77.9, 76.2, 75.7, 74.7, 73.8, 71.8, 71.5, 71.1, 65.2, 61.6, 56.5, 56.5, 43.6, 36.6 ppm. MS (ES) *m/z* (calcd 387.1): 410.1 $[M+Na]$. Anal. Calcd for $C_{17}H_{25}NO_9$: C, 52.71; H, 6.50; N, 3.62. Found: C, 52.34; H, 6.78; N, 3.45.

6-*O*-[*N*-(3,4-diacetoxyphenylethyl)aminocarbonyl]-α,β-D-glucopyranose (7): 22 (300 mg, 0.37 mmol) was reacted under similar conditions as described for **19** to give **7** (160 mg, quantitative). R_f: 0.4 (CH_2Cl_2-MeOH, 6:1); $[\alpha]_D = +18^\circ$ ($c = 1.0$, MeOH); ^1H NMR (300 MHz, CD_3OD): $\delta = 7.3$–7.2 (m, 3H), 5.24 (d, <1H, $J = 3.1$ Hz), 4.64 (d, <1H, $J = 7.9$ Hz), 4.5–4.3 (m, 2H), 4.1–4.0 (m, 1H), 3.84 (t, 1H, $J = 9.4$ Hz), 3.6–3.4 (m, 5H), 3.30 (t, 1H, $J = 8.6$ Hz), 2.93 ppm (t, 2H, $J = 7.9$ Hz); ^{13}C NMR (75 MHz, CD_3OD): $\delta = 170.2$, 159.0, 158.8, 143.4, 142.0, 139.6, 128.1, 124.8, 124.4, 98.2, 93.9, 77.8, 76.1, 75.6, 74.6, 73.7, 71.7, 71.5, 71.0, 65.1 (−), 43.1 (−), 36.2 (−), 20.6, 20.5 ppm. MS (ES) *m/z* (calcd 443.1): 466.1 $[M+Na]^+$. Anal. Calcd for $C_{19}H_{25}NO_{11}$: C, 51.47; H, 5.68; N, 3.16. Found: C, 51.05; H, 5.35; N, 2.86.

2,3,4,6-Tetra-*O*-benzyl-1-*O*-[*N*-(3,4-dimethoxyphenylethyl)aminocarbonyl]-α,β-D-glucopyranoside (29): 15 (207 mg, 0.70 mmol) was added to a solution of **28** (490 mg, 0.77 mmol) in THF-triethylamine (3:1, 12 mL), stirring at 80 °C under Ar for 18 h. After cooling at RT, the mixture was concentrated and the residue was purified by column chromatography (hexane-AcOEt, 2:1) to give **29** (193 mg, 37%). R_f: 0.33 (hexane-AcOEt, 2:1); $[\alpha]_D = +42.8^\circ$ ($c = 1.1$, CH_2Cl_2); ^1H NMR (300 MHz, $CDCl_3$): $\delta = 7.3$–7.1 (m, 20H), 6.8–6.7 (m, 3H), 6.27 (d, <1H, $J = 3.3$ Hz), 5.55 (d, <1H, $J = 8.9$ Hz), 4.9–4.4 (m, 10H), 4.1–3.5 (m, 15H), 3.41 (t, 2H, $J = 6.7$ Hz), 2.74 ppm (t, 2H, $J = 6.5$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 154.4$, 154.2, 148.8, 147.5, 138.5, 138.3, 138.1, 137.9, 137.7, 137.4, 130.8, 128.4, 128.3, 128.3, 128.2, 128.2, 128.1, 127.9, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5, 120.5, 111.7, 111.6, 111.2, 111.2, 95.0, 90.6, 84.6, 81.7, 80.9, 78.6, 73.4, 73.3, 72.9, 72.3, 67.9, 55.7, 55.7, 42.2, 35.2 ppm. MS (ES) *m/z* (calcd 747.3): 770.2 $[M+Na]^+$.

1-*O*-[*N*-(3,4-dimethoxyphenylethyl)aminocarbonyl]-α,β-D-glucopyranose (30): 10% Pd/C (165 mg) was added to a solution of **29**

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(162 mg, 0.22 mmol) in THF (12 mL) and the reaction mixture was stirred under H_2 for 4 h. Then, the mixture was filtered through Celite and the solvent evaporated to dryness to obtain **30** (84 mg, quantitative). $[\alpha]_D = +24^\circ$ ($c=1.0$, MeOH); ^{1}H NMR (300 MHz, DMSO): $\delta=7.35$ (t, <1 H, $J=5.9$ Hz), 7.21 (t, <1 H, $J=6.2$ Hz), 6.85 (dd, 1 H, $J=8.2$ Hz, $J=1.5$ Hz), 6.79 (d, 1 H, $J=1.3$ Hz), 6.71 (dd, 1 H, $J=8.2$ Hz, $J=2.0$ Hz), 5.81 (d, <1 H, $J=3.9$ Hz), 5.24 (d, <1 H, $J=8.7$ Hz), 5.1–4.8 (m, 3 H), 4.5–4.4 (m, 1 H), 4.26 (t, <1 H, $J=7.0$ Hz), 4.16 (t, <1 H, $J=6.7$ Hz), 3.74 (s, 3 H), 3.71 (s, 3 H), 3.6–3.5 (m, 1 H), 3.5–3.4 (m, 3 H), 3.29 (s, 8 H), 3.2–3.1 (m, 4 H), 2.66 ppm (t, 2 H, $J=8.3$ Hz); ^{13}C NMR (75 MHz, DMSO): $\delta=155.0$, 154.7, 148.5, 147.1, 131.7, 120.3, 120.3, 112.3, 111.8, 94.8, 92.2, 77.4, 76.8, 74.4, 72.9, 70.6, 69.5, 69.4, 68.2, 60.7, 60.5, 57.0, 55.4, 55.3, 42.0, 41.9, 34.8, 34.8 ppm. MS (ES) m/z (calcd 387.1): 410.1 $[M+Na]^+$. Anal. Calcd for $C_{17}H_{25}NO_9$: C, 52.71; H, 6.50; N, 3.62. Found: C, 52.27; H, 6.28; N, 3.38.

Biological methods. *Chemicals:* Cytochalasin B (CCB) and 3-*O*-methylglucose (3-OMG) were purchased from Sigma–Aldrich. All other chemicals used were of the highest purity commercially available.

Solutions: Phosphate-buffered saline (PBS) 25 mM, 1% NaCl (pH 7.4). Stopping solution consisting of 2 mM $HgCl_2$, 1.25 mM KI, and 1% NaCl aqueous solution. DDS 10 mM in deuterated PBS.

Preparation of intact erythrocytes: Fresh erythrocytes drawn from healthy blood donors, collected on EDTA and remaining after removal of platelets and leucocytes, were washed at RT in ten volumes of isotonic phosphate buffered saline solution (PBS), centrifuged (15 min, 2500 g), resuspended for 20 min at 37 °C with fresh PBS to deplete intracellular sugar levels, and centrifuged and washed three more times in the same way at RT. The obtained erythrocytes were resuspended in PBS to a hematocrit of 40% (controlled by microhematocrit centrifugation) and kept at 4 °C.

Glycoconjugates uptake measurements: The erythrocytes (40% hematocrit) were suspended in 75 mM glucose derivatives (in PBS) (final concentration 15 mM, final hematocrit 32%) and placed in a shaker at 37 °C (150 rpm). At various times (5, 15, 30, 50, and 150 min), 160 µL aliquots of red blood cell suspensions were added to 1 mL of transport stopping solution at 4 °C. The red blood cells were pelleted by centrifugation (3 min, 1000 g, 4 °C), the upper solution was collected by aspiration and the pellet washed twice with fresh PBS. The contents of isolated cells were released upon addition of 1 mL of aqueous 6% trichloroacetic acid (TCA), and clarified by centrifugation (10 min, 1000 g, 4 °C). The supernatant was lyophilized and dissolved in H_2O for measurements of glucose derivative uptake by HPLC. The rate of entry of glycoconjugates detected after cell lyses was linear for at least 15 min and, hence, this time was chosen for further experiments. The experimental protocol used in proton MAS NMR spectroscopy studies was similar to that employed for the analysis by HPLC. Glucosyl derivatives (15 mM) were incubated in the presence of erythrocytes for 15 min and then stopping solution ($HgCl_2$, 2 mM) was added. Cells were washed with deuterated phosphate buffered solution (three times) and analyzed by NMR.

Glycoconjugates uptake measurements in presence of irreverversible GLUT-1 inhibitor(MITC): The erythrocytes (40% hematocrit) were treated with a fresh solution 200 mM of MITC in PBS, (final concentration 40 mM, final hematocrit 32%) and placed in a shaker at 37 °C (150 rpm) during 30 min. Then, the suspension was diluted 50-fold by the addition of a solution containing 100 mM glycine, 150 mM NaCl and 5 mM sodium phosphate, pH 8. The red blood cells were pelleted by centrifugation (3 min, 1000 g, 4 °C) and washed once with glycine-PBS, followed by three washes with fresh PBS. The isolated red cells were diluted with PBS to the initial hematocrit (40%) and incubated with glycoconjugates as described above.

Glycoconjugates uptake measurements in presence of GLUT-1 inhibitor: The erythrocytes (40% hematocrit) were suspended in solutions of glucose derivatives (in PBS, final hematocrit 32%) and the GLUT-1 inhibitor (Cytochalasin B, 50 and 200 µM) was added. The cells were incubated as described above.

HPLC assays: All analyses were performed on a Vertex P680 HPLC pump equipped with a PDA-100 photodiode array detector and a 20 µL Rheodyne inyector. Data acquisition and processing were accomplished with the CHROMELEON 6.60 software. The guardcolumn was a Pathfinder RP_{18} (4.0×20 mm) and the column was a Licrosorb RP_{18} (5 µm, 4.6×250 mm), both kept at 25 °C in a Thermostatted Column Compartment TCC-100. The mobile phase was a mixture of H_2O-CH_3CN. The flow rate was 1.0 mL min^{-1}. For analysis conditions, see Table 3.

Table 3. HPLC conditions for analysis of compounds **2**–**7** and **30**.

Compound[a]	H_2O:CH_3CN	Retention time [min]	λ [nm]
1	95:5	14.3, 16.9	280
2	80:20	15.4, 18.0	254
3	80:20	8.8, 9.6	254
4	86:14→70:30	10.0	245
5	80:20	9.0, 10.0	284
6	85:15	12.7, 13.9	280
7	80:20	9.5, 10.2	280
30	85:15	10.0, 11.0	280

[a] Labeled with phenylhydrazine[28] for HPLC analysis.

NMR spectroscopy: High-resolution proton MAS NMR measurements were performed on a Bruker Avance 400 wide bore (89 mm Ø) spectrometer (Bruker Instruments, Karlsruhe, Germany) operating at 9.4 T (proton Larmor frequency at 400.14 MHz). All spectra were acquired at 20 °C (293 K) using a Bruker double tuned broadband solid state CP/MAS probe head. After incubating the cells in the medium with the corresponding compound, they were washed with deuterated phosphate buffered solution and centrifuged to form pellets. Typically, for each sample approximately 60 µL of cell pellets were placed into a 4 mm Ø zirconia rotor. The spectra were acquired using a 90° pulse of 3.35 µs followed by a T_2-filter (60 ms). The spinning rate was 2.2 or 2.4 kHz. The spectral width was 8.3 kHz, 16 k data points and the number of scans varied between 1000 and 2000 with a repetition rate of 5 s. The residual water signal was suppressed by presaturation on the water resonance. All FIDs were processed with a 2 Hz line-broadening and the chemical shifts referenced to internal DDS (0.00 ppm).

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