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Synthesis of *O*-methylsulfonyl derivatives of D-glucose as potential alkylating agents for targeted drug delivery to the brain. Evaluation of their interaction with the human erythrocyte GLUT1 hexose transporter

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Abstract

In order to obtain hydrophilic analogues of 1,4-dimethylsulfonyloxybutane (busulfan) with enhanced selectivity and improved brain penetration, we have synthesized 6-*O*-methylsulfonyl-D-glucose, 3-*O*-methylsulfonyl-D-glucose, 3,6-di-*O*-methylsulfonyl-D-glucose, 4-*O*-methylsulfonyl-D-glucose, and 4,6-di-*O*-methylsulfonyl-D-glucose, and we have studied their interactions with the human erythrocyte GLUT1 hexose transport system. Mesylation of OH-4 and OH-6 of glucose resulted in a slightly diminished affinity for the GLUT1 glucose transporter, whereas mesylation of OH-3 led to complete loss of affinity.

Keywords: Drug-delivery; Blood–brain barrier; GLUT1 glucose transporter; Tumor cells; Targeting; Methylsulfonylglucose derivatives

1. Introduction

A limitation of the use of chemotherapeutic agents against cerebral tumors is their poor uptake into the central nervous system. This is principally due to the structure of cerebral vessels, which are composed of endothelial cells linked by tight junctions. This constitutes the blood–brain barrier (BBB), which provides an efficient protection to the brain against the penetration of exogenous hydrophilic substances [1]. Thus the chemotherapy of cerebral tumors relies principally on hydrophobic alkylating agents such as 1,4-dimethylsulfonyloxybutane (busulfan, **1**), tris(1-aziridinyl)phosphine sulfide (thiothepa), nitrosourea, or mustard derivatives [2]. However, because of their

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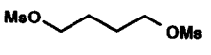
lipophilicity, these drugs are highly myelotoxic and are associated with several side effects. New strategies for cancer chemotherapy are hence necessary, and particularly the development of more hydrophilic, and thus less myelotoxic, antitumor agents.



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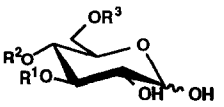
Because of the BBB, brain transport of essential hydrophilic nutrients and exit of metabolic wastes are mediated by a number of specific carriers. The large and uninterrupted energy demand of the brain is provided almost exclusively by D-glucose, which is transported through the BBB by one of the facilitative glucose transporters, the highly efficient erythrocyte/brain glucose transporter isoform GLUT1, which is present not only at the BBB, but also on erythrocytes and on several other cell types. The high level of cerebral glucose uptake [1] suggests GLUT1 to be a useful carrier to deliver glucose-conjugated drugs efficiently and selectively to the brain. Moreover, an accelerated rate of glucose transport associated with the expression of GLUT1 is one of the most characteristic biochemical events of neoplastic cells and transformed phenotypes [3–7]. In addition to increased brain penetration, this raises the possibility of specific targeting of glucose derived drugs to the tumor.

In a recent report [8], we described the synthesis of several derivatives of D-glucose, in which the dialkylating antitumor compound, chlorambucil, was linked to the C-6 position, and we have also studied the interaction of these compounds with the GLUT1 glucose transporter. This study was performed with human erythrocytes, since the availability of human erythrocytes allows structure-activity studies directly on the well documented human erythrocyte/brain GLUT1 transporter. All compounds were able to inhibit D-[^{14}C]glucose uptake in a concentration-dependent manner. One of them exhibited an approximately 160-fold higher ability to inhibit D-[^{14}C]glucose uptake mediated by the GLUT1 transporter than did glucose, and was also able to inhibit [^{3}H]-cytochalasin B binding to erythrocytes with approximately 1000-fold higher efficiency than did D-glucose, suggesting a specific interaction of the compound with the transporter protein. However, uptake studies with the [^{14}C]-labeled glucose-conjugate indicated that its interaction with GLUT1 is that of a non-penetrating inhibitor. This was attributed to the presence of the bulky, hydrophobic chlorambucil moiety.

In order to restrict the bulkiness of the alkylating moiety, we sought to incorporate it into the glucose molecule, and decided the syntheses of some mesyl derivatives of D-glucose. Thus we could obtain analogues of busulfan which, because of their hydrophilic nature, would not concentrate in undesirable or non-relevant tissues such as bone marrow, but could be transported into the brain and into tumor cells by the GLUT1 transporter, owing to their structural resemblance to the natural substrate. In the present paper, we describe the syntheses of several mesylated derivatives of D-glucose together with preliminary results on their interaction with the human erythrocyte GLUT1 hexose transport system.

2. Results and discussion

In order to evaluate the positions of the sugar molecule which could be substituted with an *O*-methylsulfonyl group without altering the affinity to the GLUT1 transporter, a series of mono- and di-substituted methylsulfonyl derivatives of D-glucose (**2–6**) was synthesized.



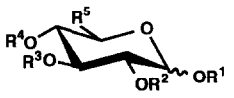
2 R^1 = H, R^2 = H, R^3 = Ms;
3 R^1 = Ms, R^2 = H, R^3 = H;
4 R^1 = Ms, R^2 = H, R^3 = Ms;
5 R^1 = H, R^2 = Ms, R^3 = H;
6 R^1 = H, R^2 = Ms, R^3 = Ms;

1,2,3,4-Tetra-*O*-trimethylsilyl-D-glucopyranose was prepared from 1,2,3,4,6-penta-*O*-trimethylsilyl-D-glucopyranose as previously described [9]. Conventional mesylation in the presence of pyridine gave the 6-*O*-mesyl compound **7** (74%) which was converted into the target compound, 6-*O*-methylsulfonyl-D-glucose (**2**, 98%) by acid catalyzed hydrolysis of the trimethylsilyl groups.

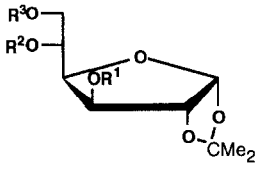
3-*O*-Methylsulfonyl-D-glucose (**3**) (80%) was obtained from 1,2,5,6-di-*O*-isopropylidene-3-*O*-methylsulfonyl-D-glucofuranose by a modification of a published method [10].

Selective hydrolysis of the 5,6-*O*-isopropylidene group of 1,2,5,6-di-*O*-isopropylidene-3-*O*-methyl-

sulfonyl-D-glucofuranose furnished **8** (62%) which was converted into the di-*O*-mesyl derivative **9** (69%) by selective mesylation of the primary hydroxyl group at −30 °C. Deisopropylidenation of **9** was effected in the presence of Amberlite IR-120 (H^+) ion exchange resin to afford 3,6-di-*O*-methylsulfonyl-D-glucose (**4**, 69%).



7 $R^1 = R^2 = R^3 = R^4 = Me_3Si$, $R^5 = CH_2OMs$
10 $R^1 = R^2 = R^3 = Ac$, $R^4 = Ms$, $R^5 = CH_2OTr$
11 $R^1 = R^2 = R^3 = H$, $R^4 = Ms$, $R^5 = CH_2OTr$
12 $R^1 = R^2 = R^3 = Ac$, $R^4 = Ms$, $R^5 = CH_2OMs$



8 $R^1 = Ms$, $R^2 = R^3 = H$
9 $R^1 = Ms$, $R^2 = H$, $R^3 = Ms$

Mesylation of 1,2,3-tri-*O*-acetyl-6-*O*-triphenylmethyl-D-glucopyranose [11] yielded the 4-*O*-mesyl derivative **10** (94%), which was deacetylated to give the 4-*O*-mesyl-6-*O*-trityl compound **11** (98%). Treatment of **11** with dilute hydrochloric acid afforded 4-*O*-methylsulfonyl-D-glucose **5** (96%).

The synthesis of **6** involved dimesylation of 1,2,3-tri-*O*-acetyl-β-D-glucopyranose [12] to give compound **12** (85%), which was deacetylated with dilute sodium hydroxide yielding 4,6-di-*O*-methylsulfonyl-D-glucose **6** (62%).

Compounds **2–6** were assayed for binding to the human erythrocyte GLUT1 transporter, and the results are summarized in Fig. 1 and Table 2. Data in Fig. 1 show that some of the assayed mesylated glucose derivatives were able to inhibit specific GLUT1-mediated, cytochalasin B-sensitive D-[^{14}C]-glucose uptake by human erythrocytes. The inhibition was dependent upon inhibitor concentration, and displayed inhibition curves similar to that of the inhibition by D-glucose itself. Table 1 indicates IC_{50} values and suggests that mesylation of OH-4 (**5**) or OH-6 (**2**) and, more importantly, simultaneous mesylation of OH-4 and OH-6 (**6**), is fairly well tolerated. In contrast, modification of OH-3 (**3** and **4**) results in a complete loss of affinity.

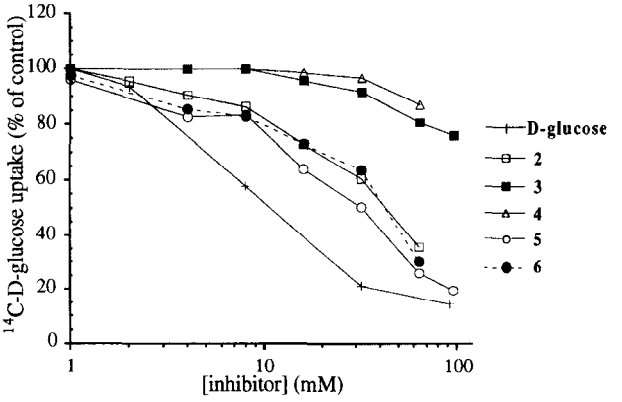


Fig. 1. Inhibition of D-[^{14}C]glucose uptake by human erythrocytes in the presence of compounds **2–6** or D-glucose. Uptake of D-[^{14}C]glucose (1 mM) by human erythrocytes was determined at room temperature for a 8 s incubation time in the presence of the indicated concentration of inhibitor. Glucose uptake represents specific GLUT1-mediated uptake deduced after substraction of non-specific association evaluated in the presence of 10 μM cytochalasin B. All D-[^{14}C]glucose uptake values were determined in quadruplicate. Relative standard deviations were always inferior to 4% of the values. For commodity, they are not shown.

Previous investigations of the structural requirements for binding to the GLUT1 transporter protein, based on inhibition studies of the glucose or sorbose uptake by various sugar derivatives, have suggested the existence of space on the GLUT1 D-glucose receptor site around C-3, C-4, and C-6, and the involvement of OH-1, OH-3, and OH-6 in binding to the transporter [13,14]. The sugar-carrier interaction is not simple, and small structural changes can result in a marked decrease or increase in affinity for the carrier which cannot be interpreted in terms of steric hindrance, hydrophobic binding or hydrogen bonding alone. For example, in spite of their structural resemblance, the affinity of the transported sugars, 3-*O*-allyl- and 3-*O*-(2′,3′-*exo*-epoxypropyl)-D-glucose (K_i 25 mM for both) was greater than that of 3-*O*-ethyl-(K_i 48 mM) or 3-*O*-propyl-D-glucose (K_i 59 mM). Our results show that unlike 3-*O*-alkyl substitution, 3-*O*-mesylation abolishes binding to the transporter. The loss of affinity seems to be due to steric hindrance, rather than to weaker hydrogen bonding, since the mesyl group is providing additional hydrogen-bond receptor sites.

The effect of modification at C-4 and/or C-6 is still more complex. For instance, 4-*O*-propyl-D-glu-

Table 1
Selected ^{1}H NMR data for compounds **2–12**

Compound	δ, p.p.m., multiplicity, J, Hz							
	H-1 ($J_{1,2}$)	H-2 ($J_{2,3}$)	H-3 ($J_{3,4}$)	H-4 ($J_{4,5}$)	H-5 ($J_{5,6}$), ($J_{5,6'}$)	H-6 ($J_{6,6'}$)	H-6′	Ms
2 [a] (α anomer)	5.17	3.43	3.6	3.37	3.99	← 4.53–4.35 →		3.11
	d, (3.8)	dd, (9.6)	t, (9)	dd, (10.1)	m, (2.5), (4.6)	m		s
(β anomer)	4.56	3.18	3.38	~ 3.1	3.59	← 4.53–4.35 →		3.13
	d, (7.9)	dd, (8.9)	t, (8.9)		m, (2.0), (5.5)	m		s
3 [a] (α anomer)	5.20	3.61	4.63	3.61	3.81	← 3.86–3.68 →		3.20
	d, (3.7)	dd, (9.7)	t, (9.5)	t, (9.4)	m, (2.77), (4.6)	m		s
(β anomer)	4.59	3.36	4.49	3.61	3.39	← 3.86–3.68 →		3.20
	3, (7.8)	dd, (9.5)	t, (9.1)	t (9.8)	m, (2.7), (5.0)	m		s
4 [a] (α anomer)	5.23	3.65	4.46	3.62	4.07	← 4.54–4.39 →		3.21, 3.13
	d, (3.7)	dd, (9.7)	t, (9.0)	dd, (10.1)	m	m		2s
(β anomer)	4.63	3.41	4.52	3.63	3.59–3.65	← 4.54–4.39 →		3.20, 3.12
	d, (7.8)	dd, (9.5)	t, (9.0)	t, (9.3)	m	m		2s
5 [a] (α anomer)	5.17	3.48	3.88	4.44	3.95	3.82	3.77	3.23
	d, (3.7)	dd, (9.6)	t, (9.2)	dd, (0.2)	m, (2.4), (4.3)	dd, (12.4)	dd	s
(β anomer)	4.56	3.24	3.69	4.43	3.55	3.70	3.67	3.23
	d, (7.9)	dd, (9.3)	t, (9.2)	t, (9.8)	m, (2.2), (5.1)	dd, (12.4)	dd	s
6 [a] (α anomer)	5.19	3.49	3.89	4.52–4.30	4.21	← 4.52–4.30 →		3.25, 3.13
	d, (3.7)	dd, (9.6)	t, (9.1)	(10.1)	m, (2.3), (4.6)	m		2s
(β anomer)	4.59	3.25	3.71	4.52–4.30	3.84	← 4.52–4.30 →		3.25, 3.11
	d, (7.9)	t, (9.3)	t, (9.2)	(9.2)	m, (2.3), (5.3)	m		2s
7 [b]	4.99	3.34	3.78	3.40	3.94	4.41	4.28	3.04
	d, (3.2)	dd, (9.1)	t, (8.5)	t, (9.7)	m, (2.3), (4.9)	dd, (11.1)	dd	s
8 [b]	5.91	4.69	5.07	4.16	3.6–3.8	← 3.6–3.8 →		3.04
	d, (3.6)	d, (0)	d, (2.6)	dd, (8.7)	m	m		s
9 [b]	5.94	4.77	5.11	4.23	4.09	4.52	4.34	3.15, 3.09
	d, (3.6)	d, (0)	d, (2.7)	dd, (9.4)	m, (2.3), (5.7)	dd, (11.1)	dd	2s
10 [b]	5.47	5.19	5.32	4.98	3.76	3.57	3.19	2.51, 2.46
	d, (8.1)	dd, (9.6)	t, (9.3)	t, (10.0)	m, (2.1), (4.0)	dd, (10.9)	dd	2s
11 [c] (α anomer)	5.22	3.51	3.84	4.48	4.12	3.45	3.15	3.02
	d, (3.8)	dd, (9.0)	t, (9.2)	t, (10.1)	m, (2.2), (6.0)	dd, (10.5)	dd	s
(β anomer)	4.57	3.26	3.63	4.46	3.69	3.48	3.14	3.01
	d, (7.9)	dd, (9.3)	t, (9.2)	t, (10.0)	m, (2.0), (6.0)	dd, (10.6)	dd	s
12 [b]	5.73	5.13	5.36	4.83	3.93	4.50	4.39	3.10, 3.09
	d, (8.3)	t, (9.5)	t, (9.2)	t, (10.0)	m, (2.2), (4.1)	dd, (11.5)	dd	2s

[a] Measured in 9:1 CD_3CN–CD_3CO_2D.
[b] Measured in $CDCl_3$.
[c] Measured in CD_3CN.

Table 2
Inhibition of D-[^{14}C]glucose uptake into human erythrocytes by *O*-methylsulfonyl-D-glucose derivatives (**2–6**)

Compound	Substituted position of D-glucose		n [a]	IC_{50} (mM)
D-Glucose	–	–	1	10
2	–	O-6	2	≈ 52
3	O-3	–	1	> 100
4	O-3	O-6	3	> 100
5	O-4	–	1	≈ 32
6	O-4	O-6	2	≈ 42

IC_{50} values were deduced from experiments represented by Fig. 1. Concentration of D-[^{14}C]glucose was 1 μM.
[a] Number of separate experiments performed on different preparations of human erythrocytes from various blood donors. Within each separate experiment, each glucose uptake value was deduced from quadruplicate determinations.

cose (K_i 10 mM) displayed the same affinity as D-glucose (K_i 8–10 mM), whereas D-galactose (OH-4 inverted) shows markedly reduced affinity (K_i 85–90 mM). On the other hand, the high affinity of 6-deoxy-D-glucose (OH-6 absent, K_i 6.7 mM) was in contrast with the poor inhibitory activity of D-xylose (CH_2OH absent, K_i 69 mM) [13]. Moreover, analogues with bulky hydrophobic substituents (*O*-benzyl [14], chlorambucil [8]) at the C-6 position were also found to have high affinity for the transporter. Although binding efficiently to the GLUT1 exofacial site, C-6 substituted analogues were not transported into the cell, presumably because of strong hydrophobic interactions between the sugar derivative and some aromatic amino acids at or near the exofacial binding site of the transporter. In contrast, a glucose analogue with a bulky hydrophobic substituent at C-6, 6-deoxy-6-*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-amino-D-glucose, was demonstrated to be transported into human erythrocytes by the sugar transporter pathway, although at a very slow rate [15]. Our results show that mesylation of OH-4 of glucose affects to a smaller extent the affinity to GLUT1 than its inversion. Moreover, the slightly decreased affinity resulting from mesylation of OH-6 and dimesylation of OH-4 and OH-6 could be due to the combined effects of some steric hindrance and of the hydrogen bond acceptor properties of the mesyl group, leading to rather strong water-sugar interaction in the hydrophobic environment represented by the hydrophobic pocket of the transporter protein adjacent to C-4 and C-6 positions of bound D-glucose. Our findings suggest the potential value of D-glucose derivatives with electrophilic substituents at C-4 and C-6 as hydrophilic bi-alkylating agents which might replace advantageously busulfan and other bialkylating drugs, conferring them higher selectivity and bioavailability.

3. Experimental

General procedures.—All reactions were conducted under nitrogen. Solutions were concentrated to dryness under reduced pressure below 30 °C. Thin layer chromatography was performed on Silica Gel 60 F_{254} (E. Merck, Darmstadt, Germany) and Silica Gel 60 (230–400 mesh) was used for flash chromatography [16], with 9:1 (*A*), 7:3 (*B*), 1:1 (*C*), 3:7 (*D*) hexane–EtOAc, EtOAc (*E*), 8:2 EtOAc:MeOH (*F*), and 9.5:0.5 CH_2Cl_2–MeOH (*G*). Optical rotations were determined with a Roussel-Jouan Quick polarimeter. ^{1}H NMR spectra were recorded with a Brucker MSL 300 spectrometer. Chemical shifts are relative to tetramethylsilane as internal standard, or to the residual signal of the deuterated solvent used. Sample for elemental analysis was dried (13 Pa, 30 °C) in the presence of P_2O_5 for 8–10 h.

6-O-Methylsulfonyl-1,2,3,4-tetra-O-trimethylsilyl-D-glucopyranose (**7**).—To a soln of 1,2,3,4-tetra-*O*-trimethylsilyl-D-glucopyranose [8] (486 mg, 1 mmol) in anhyd pyridine (5 mL) at 0 °C was added methylsulfonyl chloride (0.0967 mL, 1.25 mmol) and the soln was stirred at room temperature. After 4 h, the mixture was concentrated and the residue was purified by flash chromatography (hexane followed by solvent *A*) to yield syrupy **7** (405 mg, 74%): $[\alpha]_D^{20}$ +78° (*c* 0.3, $CHCl_3$); R_f 0.19 (solvent *A*). Anal. Calcd for $C_{19}H_{46}O_8SSi_4$: C, 41.72; H, 8.48; S, 5.86. Found: C, 41.86; H, 8.38; S, 5.91

6-O-Methylsulfonyl-D-glucose (**2**).—A soln of **7** (169 mg, 0.223 mmol) in Me_2CO (1.12 mL) and MeOH (1.12 mL) was treated with 0.001 N HCl for 25 min at 60 °C, then neutralized with Amberlite IR-45 (OH^-) ion exchange resin, filtered, and concd. Chromatography of the residue (solvent *A*, followed by solvent *E*) gave amorphous **2** (90 mg, 87%) as an anomeric mixture ($\alpha/\beta \sim 1.2$): $[\alpha]_D^{20}$ +52° (equilibrium; *c* 0.15, EtOH); R_f 0.24 (solvent *F*). Anal. Calcd. for $C_7H_{14}O_8S$: C, 32.56; H, 5.46; S, 12.42. Found: C, 32.43; H, 5.85; S, 12.45.

3-O-Methylsulfonyl-D-glucose (**3**).—Acid hydrolysis of 1,2,5,6-di-*O*-isopropylidene-3-*O*-methylsulfonyl-α-D-glucofuranose [10] (677 mg, 2 mmol) with Amberlite IR 120 (H^+) ion exchange resin (4 mL) in water (7 mL) at 60 °C for 8 h gave crystalline **3** (415 mg, 80%): mp 130 °C, lit. 133–134 °C [10]; $[\alpha]_D^{20}$ +67° (*c* 0.5, water), changing to +59° in 2 d, lit. +77° (water), changing to +56.9° in 2 d [10]; R_f 0.437 (solvent *F*). Anal. Calcd for $C_7H_{14}O_8S$: C, 32.56; H, 5.46; S, 12.42. Found: C, 32.86; H, 5.81; S, 12.30.

1,2-O-Isopropylidene-3-O-methylsulfonyl-α-D-glucofuranose (**8**).—To a solution of 1,2,5,6-di-*O*-isopropylidene-3-*O*-methylsulfonyl-D-glucofuranose (8.45 g, 25 mmol) in AcOH (22.5 mL), water (6.75 mL) was added slowly at 50 °C. The mixture was heated for 7 h, then concd, and the residue purified by flash chromatography (hexane, followed by solvents *B* and *D*) to yield syrupy **8** (4.62 g, 62%): R_f 0.39 (solvent *E*). Anal. Calcd for $C_{10}H_{18}O_8S$: C, 40.26; H, 6.08; S, 10.75. Found: C, 40.86; H, 5.81; S, 10.39.

1,2-O-Isopropylidene-3,6-di-O-methylsulfonyl-α-D-glucofuranose (**9**).—To a solution of **8** (4.85 g, 16.25

mmol) and pyridine (1.97 mL, 24.375 mmol) in dry CH_2Cl_2 (32.5 mL) was added methylsulfonyl chloride (1.38 mL, 17.87 mmol) at −30 °C. The mixture was stirred for 2 h at −30 °C, stored at −20 °C for 18 h and, after treatment with an excess of water at 0 °C, the mixture was diluted with CH_2Cl_2, washed with water, $NaHCO_3$, and then evaporated. The residue was crystallized from diisopropyl ether to give **9** (4.18 g, 69%). The mother liquor furnished another crop of product (0.59 g): m.p. 95 °C; $[\alpha]_D^{20}$ −20° (*c* 0.5, $CHCl_3$); R_f 0.423 (solvent *G*). Anal. Calcd for $C_{11}H_{20}O_{10}S_2$: C, 35.10; H, 5.36; S, 17.04. Found: C, 35.21; H, 5.37; S, 16.96.

3,6-Di-O-methylsulfonyl-D-glucose (**4**).—A mixture of **9** (376 mg, 1 mmol), water (2 mL), and Amberlite IR-120 (H^+) ion exchange resin (2 mL) was stirred at 60 °C overnight, then filtered and evaporated. Chromatography (solvent *G*) of the residue gave the anomeric mixture of **4** as a white powder: $[\alpha]_D^{20}$ +28° (equilibrium; *c* 0.5, water); R_f 0.355 (8.5:1.5 CH_2Cl_2–MeOH). Anal. Calcd for $C_8H_{16}O_{10}S_2 \cdot CH_3OH$: C, 29.34; H, 5.47; S, 17.41. Found: C, 29.30; H, 5.35; S, 17.28.

1,2,3-Tri-O-acetyl-4-O-methylsulfonyl-6-O-triphenylmethyl-D-glucopyranose (**10**).—To a solution of 1,2,3-tri-*O*-acetyl-6-*O*-triphenylmethyl-D-glucopyranose [11] (1.096 g, 2 mmol) and triethylamine (0.502 mL, 3.6 mmol) in dry CH_2Cl_2 (8 mL), methylsulfonyl chloride (0.186 mL) was added. The mixture was stirred for 7 h at room temperature, then diluted with CH_2Cl_2, and washed with water, dil HCl, and $NaHCO_3$, dried, and concentrated. Flash chromatography (continuous gradient from hexane to solvent *B*) of the residue gave **10** (1.19 g, 94%) as a white powder: $[\alpha]_D^{20}$ +47° (*c* 0.2, $CHCl_3$); R_f 0.600 (solvent *D*). Anal. Calcd for $C_{32}H_{34}O_{11}S$: C, 61.33; H, 5.47; S, 5.12. Found: C, 61.33; H, 5.69; S, 4.98.

4-O-Methylsulfonyl-6-O-triphenylmethyl-D-glucopyranose (**11**).—A mixture of **10** (0.944 g, 1.5 mmol), CH_2Cl_2 (3 mL), MeOH (9 mL), and aq 2N NaOH (0.294 mL) was stirred at room temperature for 7 min, then neutralized with Amberlite IRC-50 (H^+) ion exchange resin, filtered, and concentrated. Flash chromatography (solvent *G*, followed by solvent *B*) of the residue gave **11** (0.74 g, 98%) as a white powder: $[\alpha]_D^{20}$ +50° (equilibrium; *c* 0.2, $CHCl_3$); R_f 0.342 (solvent *D*). Anal. Calcd for $C_{26}H_{28}O_8S \cdot EtOAc$: C, 61.22; H, 6.16; S, 5.45. Found: C, 61.27; H, 5.89; S, 5.55.

4-O-Methylsulfonyl-D-glucose (**5**).—A solution of **11** (0.5 g, 1 mmol) in EtOH (5 mL) was treated with 0.02 N HCl (5 mL) at 60 °C for 2 h, then crystals of triphenylmethanol were filtered off, the soln was neutralized with Amberlite IR-45 (OH^-) ion exchange resin, filtered, and concd. Chromatography (solvent *B*, followed by solvent *C*) of the residue yielded **5** (249 mg, 96%) as a hygroscopic powder: $[\alpha]_D^{20}$ +57° (equilibrium; *c* 0.2, H_2O, 2 d); R_f 0.420 (solvent *F*). Anal. Calcd for $C_7H_{14}O_8S$: C, 32.56; H, 5.46; S, 12.42. Found: C, 32.94; H, 5.51; S, 11.99.

1,2,3-Tri-O-acetyl-4,6-di-O-methylsulfonyl-D-glucopyranose (**12**).—To a soln of 1,2,3-tri-*O*-acetyl-D-glucopyranose [12] (321 mg, 1.05 mmol) in anhyd pyridine (2.1 mL) at 0 °C was added methylsulfonyl chloride (0.203 mL, 2.62 mmol) and the soln was stirred at room temperature. After 3 h the mixture was concd, the residue in CH_2Cl_2 was washed with dil HCl, water, and $NaHCO_3$, then crystallized from MeOH to give compound **12**. Recrystallization from MeOH furnished the pure β-anomer (414 mg, 85%): mp 168–169 °C; $[\alpha]_D^{20}$ 0° (*c* 0.2, $CHCl_3$); R_f 0.458 (8:2 EtOAc–hexane). Anal. Calcd for $C_{14}H_{22}O_{13}S_2$: C, 36.36; H, 4.80; S, 13.87. Found: C, 36.16; H, 4.67; S, 13.55.

4,6-Di-O-methylsulfonyl-D-glucose (**6**).—To a solution of **12** (287 mg, 0.62 mmol) in dry CH_2Cl_2 (4.3 mL), MeOH satd with ammonia (4.3 mL) was added. The mixture was stirred for 6 h at room temperature, then concd. Flash chromatography (solvent *E* followed by solvent *F*) of the residue gave **6** (129 mg, 62%), as a white powder: $[\alpha]_D^{20}$ +60° (equilibrium; *c* 0.2, H_2O); R_f 0.513 (solvent *F*). Anal. Calcd for $C_8H_{16}O_{10}S_2 \cdot CH_3OH$: C, 29.34; H, 5.47; S, 17.41. Found: C, 29.56; H, 5.15; S, 17.08.

Preparation of erythrocytes.—Fresh erythrocytes drawn from healthy blood donors, collected on citrate and remaining after removal of platelets and leucocytes, were washed at room temp in ten vol of isotonic phosphate buffered saline soln (PBS), centrifuged (15 min, 2500 *g*), resuspended for 20 min at 37 °C with fresh PBS, and centrifuged and washed three more times in the same way at room temp. The erythrocytes thus obtained were resuspended in PBS to a hematocrit of 30% (controlled by microhematocrit centrifugation) and kept at 4 °C.

Inhibition of D-[^{14}C]glucose uptake by human erythrocytes with methylsulfonyl glucose derivatives.—Compounds were solubilized in PBS or Me_2SO (usually 240 mM stock soln). Final Me_2SO concn did not exceed 4% in order to prevent hemolysis of erythrocytes. D-[^{14}C]Glucose uptake was determined at room temp on 40 μL of erythrocyte-PBS suspension. D-[^{14}C]Glucose uptake was started by the addition of 10 μL of D-[^{14}C]glucose (final concn 1 mM;

0.33 μCi/mL, final hematocrit 20%). Preliminary experiments showed that D-[^{14}C]glucose uptake was linear with time up to 8 s, in agreement with previously published results [17], and was proportional to the erythrocyte concn up to a final hematocrit of 25%. In further experiments, glucose uptake was stopped after 8 s by adding 750 μL of an ice-cold blocking soln, modified from Jarvis [18], containing phloretin (0.1 mM), $HgCl_2$ (2 μM), and cytochalasin B (16 μM) in an isotonic aq soln of NaCl (140 mM) and KI (2 mM). The resulting suspension was transferred to an Eppendorf microtube containing 200 μL of dibutylphtalate and immediately centrifuged (1 min, 2500 *g*). The cell pellet was thus rapidly separated from the reacting soln by the dibutylphtalate layer. The upper solution was removed by aspiration, the tube was gently rinsed with ice-cold saline buffer, and the dibutylphtalate layer was then discarded. The erythrocyte pellet was treated with 1 mL of aq 6% trichloroacetic acid, centrifuged (1 min, 2500 *g*), and the deproteinized supernatant was counted by liquid scintillation.

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