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Hexose Keto-C-glycoside Conjugates: Design, Synthesis, Cytotoxicity, and Evaluation of Their Affinity for the Glucose Transporter Glut-1

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Abstract—The design, synthesis, cytotoxicity, and biological evaluation of carbohydrate/C-glycoside conjugates are described. The design concept is predicted on the idea that physiological barriers like the blood brain barrier could be crossed selectively by using glucose or glucose derivative/drug conjugates. The study demonstrates that, (1) carbohydrates and C-glycosides can be bonded at nonanomeric positions by the reaction of carbohydrate triflates with C-glycoside alkoxydes in the presence of DMPU; (2) there is a structure–activity relationship between the cytotoxicity of the conjugate and the nature of the carbohydrate residue; and (3) peracetylated hexose keto-C-glycoside conjugates are the most cytotoxic keto-C-glycosides. Copyright © 1996 Elsevier Science Ltd

Introduction

Although considerable effort has been invested in the development of new therapeutics with a higher spectrum of activity and potency, the effectiveness of a chemotherapeutic agent depends greatly on its bioavailability. To develop more effective cytotoxic C-glycosides, we have designed fatty acid keto-C-glycoside[1,2] and unsaturated epoxy-C-glycoside[3] conjugates, and have shown that coupling C-glycosides with arachidonic or docosahexaenoic acid led to cytotoxic agents with high potency. In addition, our results strongly suggest a correlation between the nature of the fatty acid and the supposed target of the drug.[3]

Some recent investigations have revealed that attachment of carbohydrate residues to peptides that are not glycosylated in nature can influence their biological functions.[4–8] However, little attention was paid to the role of glucose as conjugate for increasing the bioavailability of antineoplastic drugs, especially for the treatment of brain tumors. Indeed, glucose as a conjugate represents a useful drug targeting tool. First, glucose is the main nutrient of the brain and about 20% of the glucose in the bloodstream is metabolized in the brain. Thus, glucose is actively transported into the brain by the passive glucose transporter, GLUT-1.[9] This proteic transporter is located in the membrane of brain capillary endothelial cells composing the blood brain barrier (BBB), which excludes most hydrophilic molecules. Second, it has long be recognized that cancer cells have increased rates of glucose metabolism compared with healthy cells due to an increase expression of GLUT-1.[10–12]

Finding this rationale particularly engaging, we attempted to design and synthesize hexose keto-C-glycoside conjugates. This approach could offer several advantages: (1) the hexose moiety could be used as a vector to transport antineoplastic agents through the BBB; (2) the carbohydrate will decrease the lipoliphicity of the drugs avoiding secondary effects like myelotoxicity; (3) a considerable number of naturally occurring compounds with biological properties are glycosides, despite recent results,[13–15] the role of carbohydrate in the biological properties of glycosides is far from being fully established. A structure–activity relationship study of hexose keto-C-glycoside conjugates could lead to a better understanding of the role of carbohydrate moiety in glycosides and could allow the rational design of new antitumoral agents.

We have demonstrated that coupling an unsaturated-C-glycoside with a fatty acid increases dramatically the cytotoxicity of the drug toward tumoral cells.[1–3] It will be of a great interest to expand this work by investigating the properties of hexose, and peracetylated hexose, keto-C-glycoside conjugates. In human erythrocytes, a wide range of carbohydrates, bearing various groups at C-1 (OH, H, F...), inhibit the sugar-transport system.[16] However, inhibition by methyl glycopyranoside was not detectable. On the other hand, peptide-β-*O*-glucosides are transported through the BBB presumably by the GLUT-1 transporter.[8] With these structure–activity relationships in mind, it was therefore decided to explore the effects of connecting a keto-C-glycosides to the 1′ or the 6′ position of pyranosides. These compounds have been synthesized and evaluated for cytotoxicity against tumoral cells and

recognition of the free hexose conjugates by the GLUT-1 glucose transporter.

Chemistry

To prepare the conjugates, we have chosen the trichloroacetamidate procedure because previous studies have shown that the glucose transporters are specific for β-D-glucosides. This process is well known to lead mainly to the *trans* product when there is a participating group like acetate at C-2.[17] Thus, the glucose keto-C-glycoside conjugates were prepared as described in Scheme 1.

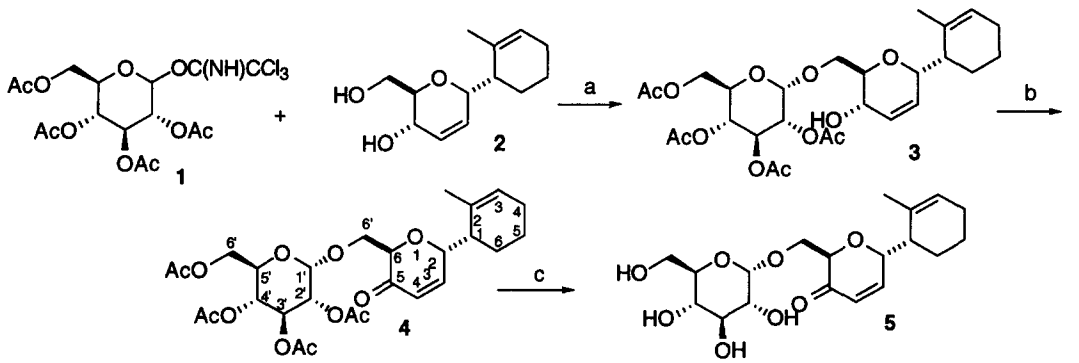
Condensation of C-glycoside **2** with the 1-*O*-trichloracetamidate **1** at −78 °C in the presence of trimethylsilyltrifluoromethanesulfonate afforded **3**, which was treated with pyridinium dichromate in the presence of 4 Å molecular sieves[18] to yield **4**. The structure of **4** was established using 1-D and 2-D NMR. The presence of the α,β-unsaturated ketone was dictated by the H-3 and H-4 pyran (py) signals [δ 6.1 (dd, 1 H, $J=2.3$ and 10.5 Hz, H-4 py), 6.98 (dd, 1 H, $J=1.7$ and 10.5 Hz, H-3 py)]. The presence of the enone demonstrated the chemoselectivity of the glycosidation. The formation of the glycosidic bond was deduced from the characteristic chemical shift of the C-1′ signal (d 101.2, d) on the ^{13}C spectra. The β-configuration of the glucose moiety was deduced from the large $J_{1',2'}=9.9$ Hz coupling constant. In addition, the value of the $J_{2',3'}=J_{3',4'}=J_{4',5'}=8.9$ Hz was consistent with a $^4C_1$ conformation. Deacetylation of conjugate **4** (K_2CO_3 aqueous methanol) gave the unprotected keto-C-glycoside conjugates **5** in 42% yield.

The C-glycoside **2** reacted chemoselectively with the 1-*O*-trichloroacetamidate of D-xylose, D-methyl-3-glucose and L-rhamnose to give the corresponding conjugates (yields 50%). After PDC oxidation the α,β-unsaturated keto-C-glycosides **6–8** were isolated in 63–67% yields (Fig. 1). Examination of the NMR data indicated that D-xylo and D-methyl-3-gluco conjugates **6** and **7** were in the β-configuration with the $^4C_1$ conformation. (Table 5 entries 3 and 5). For the L-rhamno-keto-C-glycoside **8**, the coupling constant values revealed *trans* diaxial relationships between H-3, H-4′ and H-5′. On the other hand, the $J_{1',2'}=1.7$ Hz and $J_{2',3'}=3.1$ Hz coupling constants indicated a *trans* diequatorial relationship between H-1′ and H-2′ and an axial-equatorial one between H-2′ and H-3′. These data supported for **8** an α-configuration in the $^1C_4$ conformation.

Synthesis of the C-glycoside hexose conjugates

We envisioned the synthesis of a 6→6 keto-C-glycoside conjugate as the reaction of a C-glycoside 6′-*O*-trifluoromethanesulfonate with the primary alkoxide of a carbohydrate as outlined in Scheme 2. The triflate **15** was prepared in 4 steps from the C-glycoside **2** (Scheme 3). The selective protection of **2** with triphenylmethyl chloride gave **12**. The 6′-*O*-triphenylmethyl ether **12** was treated with thexyldimethylsilyl chloride to give **13** quantitatively.

Removal of the triphenylmethyl group with formic acid[19] gave **14**. Finally, reaction of C-glycoside **14** with trifluoromethanesulfonic anhydride afforded the triflate **15**. With **15** in hand, we next turned to the condensation. Thus, 1,2,3,4-di-*O*-isopropylidene-D-galactose **16** was reacted with **15** (Scheme 4).



Scheme 1. Reagents: (a) TMSOTf −78 °C; (b) PDC 4 Å molecular sieves; (c) K_2CO_3, $MeOH/H_2O$.

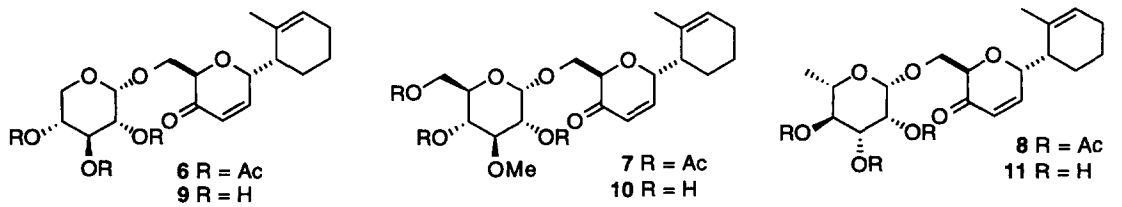
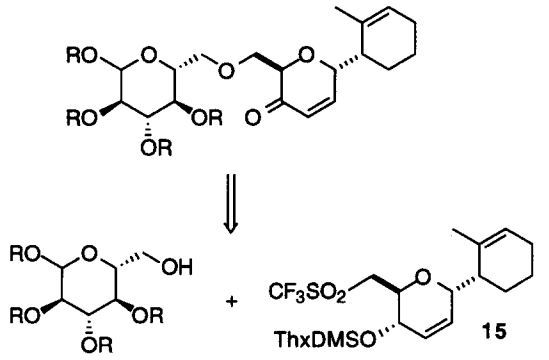


Figure 1.

Treatment of **16** with sodium hydride in THF followed by triflate **15** addition gave no condensation products. Several attemps were done with various additives like HMPA with no more success. However, when the reaction was performed in the presence of two equivalents DMPU, a new product was produced in a very clean fashion (yield 60%). The structure of **17** was deduced from the ^{1}H NMR 1-D and 2-D spectra. The signals at δ 5.71 and 5.77 were assigned to the 2H pyran double bond, whereas the doublet at δ 5.52 (1 H, $J=5$ Hz) was attributed to H-1. These data were consistent with the presence of a 6→6′ bond.

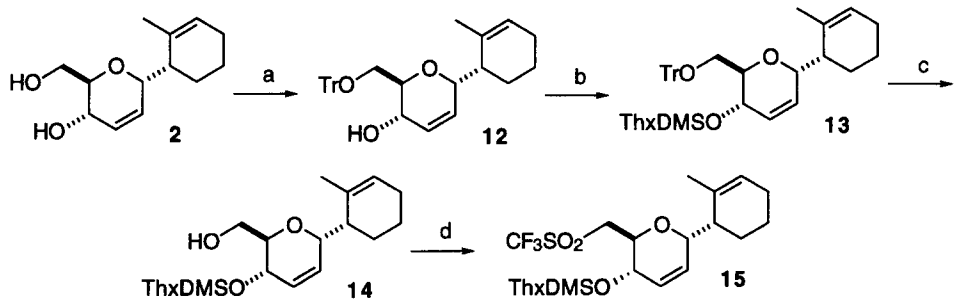
The thexyldimethylsilyl group was removed quantitatively (tBu$_4$NF, CH_3CN) then **18** was treated with pyridinium dichromate in the presence of 4 Å molecular sieves. Finally, removal of the acetals with 0.1 N hydrochloric acid afforded the unprotected conjugate **20**. The D-gluco 6→6 keto-C-glycoside conjugate **25** (Scheme 5) was prepared in the same fashion from 1,2,3,5 diisopropylidene-D-glucose (**21**). The formation of the 6→6′ linkage was deduced from the ^{1}H NMR spectrum and was ascertained by the upfield shift of the C-6 carbon (**22**, δ 71.76). After removal of the protecting group, the allylic alcohol was oxidized to give **24** in 72% yield. Treatment of the keto-C-glycoside **24** with trifluoroacetic acid afforded **25** as a mixture of α and β isomers.



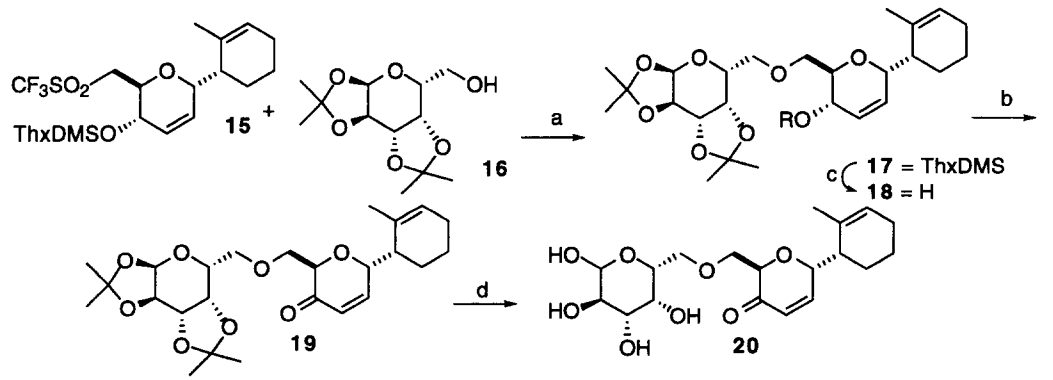
Scheme 2.

Results and Discussion

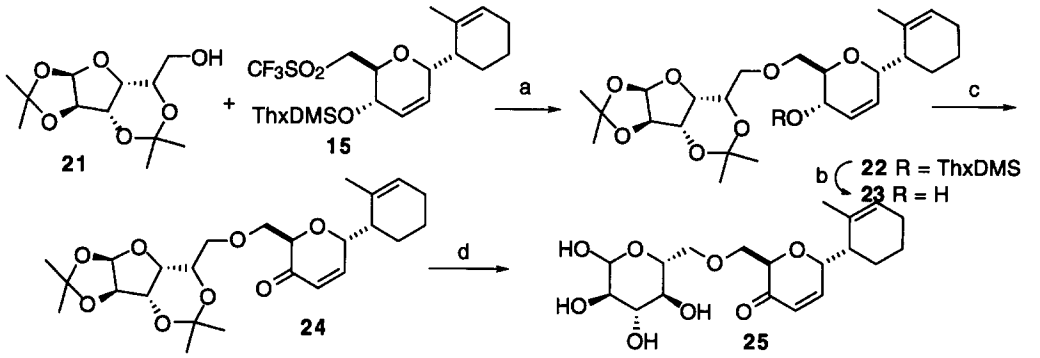
The in vitro cytotoxic activities of the compounds described in this paper were tested against RAJI cells. This cell line was selected to compare the cytotoxicity of fatty acid[3] and monosaccharide keto-C-glycoside conjugates. Raji cells are cultured malignant cell lines derived from a human lymphoma. The cytotoxicity results are summarized in Table 1. We first turned our attention to the peracetylated derivatives **4**, **6**, and **8**. These compounds showed cytotoxic activities ranging from 25.4 to 52.9 μM. Comparison with **A**, **B**, and **C** indicated that the peracetylated keto-C-glycoside conjugates are more potent agents than the keto-C-glycosides **B** and the fatty acid conjugate **C**.



Scheme 3. Reagents: (a) TrCl pyridine; (b) ThxDMSCl imidazole; (c) HCO_2H, d tfO_2, pyridine.



Scheme 4. Reagents: (a) NaH; (b) tBu$_4$NF; (c) PDC 4 Å molecular sieves; (d) 1 N HCl.

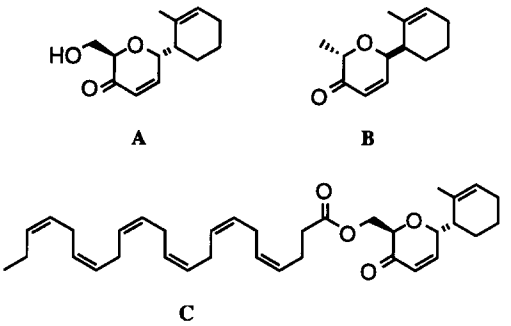


Scheme 5. Reagents: (a) NaH; (b) tBu$_4$NF; (c) PDC 4 Å molecular sieves; (d) CF_3CO_2H:MeOH, 95:5.

In addition, these data suggested the possibility of a relationship between the structure of the carbohydrate moiety and the cytotoxic activity. Examination of the IC_{50} for the unprotected derivatives **5**, **9**, and **11** revealed an activity drop that could be related to the diminution of the lipophilicity of the products. However, **5**, **9**, and **11** were more cytotoxic than chlorambucil and 5-fluorodeoxy-uridine. Finally, the 6→6′ conjugates **20** and **25** were found to be completely inactive.

Next, the free hexose keto-C-glycoside conjugates **5**, **9**, **10**, and **11** were evaluated for their interaction with the glucose transporter GLUT-1 of human erythrocytes. These cells express the same transporter GLUT-1 as that of the BBB. The interaction of the drug conjugates with GLUT-1 was determined by the efficiency of these compounds to prevent the uptake of [^{14}C]glucose in human erythrocytes. The nonspecific uptake of [^{14}C]glucose by human erythrocytes was ascertained by a preliminary treatment with cytocholasine B, a GLUT-1 inhibitor.

Table 1. Cytotoxic activity of keto-C-glycoside conjugates



Product	IC_{50} (μM) Raji
5-Fluorodeoxy-uridine[a]	NT[b]
Chlorambucil[a]	298
A[a]	280
B[a]	82.3
C[a]	62
4	31.5
6	52.9
8	25.4
5	111.1
9	127.3
11	125
20	NT
25	NT

[a]See ref. 3.
[b]Nontoxic.

Table 2 summarizes the effect of the conjugates on [^{14}C]glucose uptake. Our preliminary results showed that the conjugates inhibited the glucose uptake. The IC_{50} ranged from 1.5 to 4.5 mM. Conjugates **9** and **11** were poorly active. Examination of the structures suggested that the presence of a free hydroxyl or an hydroxymethyl connected to C-1′ or C-5′ is required to inhibit the glucose uptake (entries 3 and 5). The presence of a methoxy group at C-3′ led to the conjugate with the higher inhibitory properties. This effect could be related to an increase in the lipophilicity.

In addition, our results were compared with those recorded for chlorambucil glucose conjugates (Fig. 2). Most of these molecules, recently studied in our group,[20] inhibited the glucose uptake with similar IC_{50} values. Finally, Table 2 suggested that the inhibition of the glucose uptake by **5**, **9**, **10**, and **11** is neither related to the nature of the carbohydrate moiety nor to the type of the conjugate linkage.

In summary, the synthesis of monosaccharide keto-C-glycoside conjugates has been accomplished. The cytotoxicity of these compounds was optimum for the peracetylated derivatives. Moreover, the presence of an O-glycosidic linkage is critical for the cytotoxic activity.

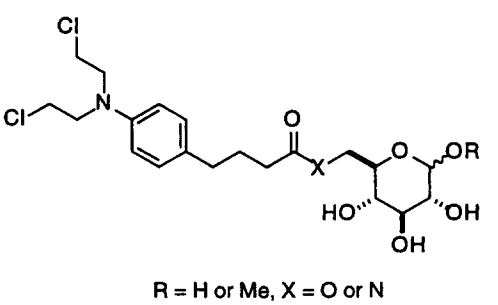


Figure 2. Chlorambucil glucose conjugates.

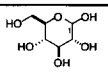
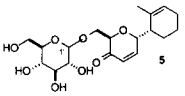
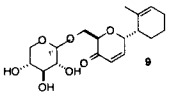
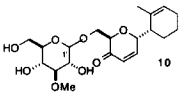
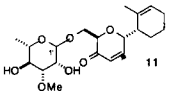
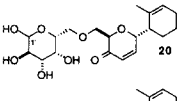
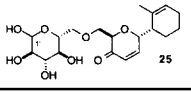
Keto-C-glycoside conjugates prevent the uptake of [^{14}C] glucose by human GLUT-1 glucose transporter. In addition, the results suggested that the presence of an hydroxyl or an hydroxymethyl group at C-1′ or C-5′ is necessary.

Experimental

General techniques

NMR spectra were recorded unless noted in $CDCl_3$ soln. ^{1}H and ^{13}C resonance spectra were recorded at 300.13 and 75.47 MHz, respectively, using tetramethylsilane as internal standard. THF and toluene were distilled from sodium benzophenone ketyl. Dichloromethane and acetonitrile were distilled from P_2O_5 and stored over 4 Å molecular sieves. All reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plate by using UV light or an ethanolic anisaldehyde acid-heat as developing agent. E. Merck silica gel 60 (particle 0.04–0.063 mm) was used for flash column chromatography. Mass spectra were measured on a Perkin–Elmer SCIEX API III equiped with a thermospray ion source. Microanalyses were performed by the Laboratoire central de Microanalyse du CNRS, Vernaison France.

Table 2. Inhibition of glucose uptake by keto-C-glycosides

Entries	Products	IC_{50} (mM)
1		10
2	 5	4.5
3	 9	>4
4	 10	1.5
5	 11	>>4
6	 20	4
7	 25	3

General procedure for the preparation of the keto-C-glycoside 1→6 peracetylated conjugates (4, 6, 7, and 8)

[2*S*,5α,6β]-1-(5,6-Dihydro-6-(glucopyranosyl)oxymethyl]-5-hydroxy-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (3). In a flame dried round botton flask was placed successively tetra-*O*-acetyl-1-trichloroacetamidyl-D-glucose (**1**) (0.666 g, 3.00 mmol), [2*S*,5α,6β]-1-(5,6-dihydro-6-hydroxymethyl]-5-hydroxy-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (**2**) (0.8 g, 3.2 mmol), CH_2Cl_2 (15 mL), and 4 Å MS (0.3 g). The soln was cooled to −78 °C, then trimethylsilyltrifluoromethanesulfonate (0.115 mL, 0.60 mmol) was added. After 5 min, satd $NaHCO_3$ (3 mL) was added and the solution allowed to reach 0 °C. The soln was diluted with (15 mL), filtrated on celite, dried ($MgSO_4$), then the solvent was evapd, and the product was purified (flash chromatogaphy hexane:EtOAc, 1:1) to yield 0.831 g (50%) of **3**.

Condensation between **1** and the peracetylated 1-*O*-trichloroacetamidate of D-xylose, 3-*O*-methyl-D-glucose and L-rhamnose was performed in the same fashion. In each case, the corresponding conjugates were isolated in 50% yield.

[2*S*,5α,6β]-1-(5,6-dihydro-6-(glucopyranosyl)oxymethyl]-5-oxo-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (4). To a soln of **3** (1.1 g, 2 mmol) in CH_2Cl_2 (10 mL) was added 4 Å molecular sieves (2 g) and PDC (0.9 g, 2.4 mmol). After 1.5 h, celite (2 g) and EtOAc (20 mL) were added and the resulting slurry was filtered

Table 3. Physical properties of carbohydrate keto-C-glycoside conjugates

Products	Yields (%)	$[\alpha]_D^{20}$ [a]	Formula	Calcd (%)		Found (%)	
				C	H	C	H
5	42	−25	$C_{19}H_{28}O_8$, $1.5H_2O$	55.47	7.54	55.05	7.15
6	61	−85	$C_{24}H_{32}O_{10}$, $1.5H_2O$	56.80	6.90	56.97	6.62
9	40	−50	$C_{18}H_{26}O_7$, $1.5H_2O$	56.99	7.61	56.52	7.37
7	68	−45	$C_{26}H_{36}O_{11}$, $1.5H_2O$	56.62	7.07	56.74	6.77
10	52	−50	$C_{20}H_{30}O_8$, H_2O	57.69	7.64	57.7	7.16
8	67	−55	$C_{25}H_{34}O_{10}$, $1.5H_2O$	57.58	7.10	57.08	6.72
11	43	−55	$C_{19}H_{28}O_7$, $2H_2O$	56.43	7.92	56.00	7.99
19	72	−85	$C_{25}H_{36}O_8$, $1.5H_2O$	62.24	7.88	62.68	7.75
20	35	−13	$C_{19}H_{28}O_8$, $1.5H_2O$	55.47	7.54	55.13	7.37
24	68	−35	$C_{25}H_{36}O_8$, $0.5H_2O$	63.42	7.82	63.73	7.91
25	43	−40	$C_{19}H_{28}O_8$, $1.5H_2O$	55.47	7.54	55.10	7.25

[a] *c* 0.1 (MeOH).

Table 4. ^{1}H NMR data for carbohydrate keto-C-glycoside conjugates

Products	Pyran						Cyclohexene						Monosaccharide										
	2	3	4	6	6′	6′	1	3	4	5	o	Me	1	2	3	4	5	6	6				
4	5.08	6.98	6.1	4.25	4.4	4.62	2.35	5.6	1.77	1.63	1.47	1.77	4.38	5.07	5.56	5.38	3.68	4.11	4.32	2.11[a]	2.10[a]	2.02[a]	1.99[a]
							2.51		1.98	1.98	1.77												
5[c]	5.02	7.01	6.08	4.4	3.87	4.05	2.52	5.56	1.77	1.63	1.47	1.77	4.32	3.15	3.36	3.2	3.29	3.57	3.83				
		7.03		4.43				5.61	1.98	1.98	1.77		4.34					3.63					
6	5.03	7.01	6.11	4.38	3.82	4.22	2.35	5.7	1.77	1.6	1.47	1.75	4.32	4.87	5.15	4.95	4.04			2.05[a]	2.01[a]	1.99[a]	
		7.02	6.18				2.52		1.98	1.98	1.80		4.38				4.12						
9	5.05	6.98	6.18	4.42	3.88	4.03	2.35	5.64	1.77	1.6	1.47	1.77	4.23	3.28	3.43	3.68	3.9						
							2.55		1.98	1.98	1.68						3.92						
7	5.05	6.99	6.11	4.33	3.86	4.26	2.35	5.65	1.77	1.63	1.47	1.77	4.27	4.89	3.4	5.02	3.55	4.08	4.24	2.10[a]	2.09[a]	2.04[a]	
							2.56		1.98	1.98	1.77		4.38										
10	5.03	6.99	6.11	4.36	3.88	4.23	2.35	5.63	1.77	1.63	1.47	1.77	4.34	4.82	3.38	3.62	3.37	3.83	3.92				
							2.54		1.98	1.98	1.77												
8	5.01	7.03	6.21	4.42	3.66	4.2	2.35	5.64	1.77	1.63	1.47	1.77	4.68	5.18	5.2	5.04	3.85	1.21		2.11[a]	2.06[a]	2.02[a]	
							2.56		1.98	1.98	1.77			5.25	5.23			1.24					
11[c]	4.92	7.02	6.17	4.41	3.86	3.96	2.42	5.65	1.72	1.63	1.49	1.77	4.68	3.81	3.61	3.42	3.54	1.25					
			6.19	4.44			2.5		1.92	1.98	1.81												
19	5.05	6.97	6.13	4.35	3.82	3.93	2.38	5.5	1.88	1.5	1.81	1.75	5.5	4.3	4.6	4.2	3.88	3.66		1.48[b]	1.43[b]	1.31[b]	
	5.09		6.16	4.4			2.57		1.98											1.49[b]	1.44[b]	1.32[b]	
20[c]	5	7.2	6.4	4.31	3.76	3.96	2.35	5.65	1.88	1.63	1.47	1.76	4.57	3.7		4.12		3.6					
				4.36			2.51		1.98	1.98	1.81												
24	5.04	6.95	6.12	4.33	3.78	3.97	2.35	5.65	1.77	1.6	1.47	1.75	5.99	4.58	4.17	4.22	3.6	3.6	3.55	1.31[b]	1.32[b]	1.35[b]	1.49[b]
	5.14	6.97	6.17	4.39			2.54		1.98	1.98	1.8	1.8											
25[c]	4.91	6.97	6	4.11	3.83	3.59	2.42	5.62	1.77	1.63	1.47	1.77	4.35	3.19	3.25	3.01	3.59	3.51	3.53				
	5.01	7.08		4.19	3.86	3.61	2.58		1.98	1.98	1.47		5.52	3.21	3.43	3.18							

[a]MeCO.
[b]Me isopropylidene.
[c]Acetone-d_6.

through a celite pad that was washed with Et_2O (2 × 10 mL). The solvent was removed under vacuum then the resulting black residue was purified by flash chromatography (20% EtOAc:hexane CH_2Cl_2, 1:1) to yield 0.695 g (63%) of [2*S*,5α,6β]-1-(5,6-dihydro-6-(glucopyranosyl)oxymethyl]-5-oxo-2*H*-pyran-2-yl)-methylcyclohex-1-ene (**4**). $[\alpha]_D^{20}$ (*c* 0.1 MeOH) −5; MS *m/z* $C_{27}H_{36}O_{12}$: $[M+K]^+$ 592.1.

Keto-C-glycoside conjugates **6–8** were prepared in the same fashion and were isolated in 61, 68 and 67% yields, respectively.

General procedure for the preparation of C-glycoside conjugates (5, 9–11)

[2*S*,5α,6β]-1-(5,6-dihydro-6-(glucopyranosyl)oxymeth-

Table 5. Coupling constants for carbohydrate keto-C-glycoside conjugates

Entry	C-Glycosides	Pyran						cy[a]	Monosaccharide						
		1,3	1,4	3,4	6,6′	6,6′	6′,6′	1,1	1′,2′	2′,3′	3′,4′	4′,5′	5′,6′	5′,6′	6′,6′
1	**4**	1.7	2.3	10.5	2.7	4.1	12.1	5.3	9.9	8.9	8.9	8.9	2.3	4.6	12.1
2	**5**	1.9	2.8	10.8	2.9	7.7	12.4	5.25	7.5	8.8	8.6	9.8	3.7	5.2	12.4
3	**6**	1.8	2.6	10.8	2.5	4.1	10.7	5.3	7.4	9.2	9.2	5.3	11.6	7.5?	
4	**9**	2	2.5	10.7	4	6.4	11	5.5	6.9	8.9	7.9	4.8	8.9	11.7	
5	**7**	1.7	2.4	10.6	2.7	3.7	10.7	5.3	8.1	9.6	9.3	9.4	3.4	4.7	11.9
6	**10**	1.9	2.5	10.6	2.4	4.2	10.5	4.7	7.9	9.6	9.3	9.3	3.5	7	10.3
7	**8**	1.7	2.4	10.7	2.6	5	10.3	5	1.7	3.1	9.2	9.1	5.8		
8	**11**[b]	1.8	2.5	10.7	3.2	6	11.6	5.5	4	0	9.1	9.1	6.4		
9	**19**	1.8	2.4	11	2.8	5.1	10.5	—	5	2.4	1.8	4.7	7.1	10.2	
10	**20**[b]	1.9	2.6	10.5	3	6.1	10.4	—	7.5	8	2.5	4.3	6	6.6	10
11	**24**	1.8	2.6	10.8	2.8	5.2	10.8	5.1	4	0.8	3.9		3.8	7.2	10.8
12	**25**[b]	1.8	2.4	10.8	3	6	11	5.25	8.1	9.6	8.4	9.75	2.8	7	11
						6.3			3.75				2.2	5.6	11

[a]Cyclohexene.
[b]Acetone-d_6.

Table 6. ^{13}C NMR data for carbohydrate keto-C-glycoside conjugates

Product	Pyran						Cyclohexene							Monosaccharide								
	2	3	4	5	6	6′	1	2	3	4	5	6	Me	1′	2′	3′	4′	5′	6′			
4	70.30	151.86	126.70	194.75	78.33	70.90	42.83 42.85	131.45	126.54	25.24	22.88	18.16	24.1	101.2	70.42	75.35	72.46	66.42	61.25 61.82	169.18[b] 169.42[b]	170.15[b] 20.55[b]	170.94[b]
5	70.71	150.74	126.49	193.94	79.12	67.65	42.41	132.36	126.02	25.26	22.47	20.59	24.11	103.22	74.04	76.50	76.83	70.28	61.75			
6	70.76	151.70	126.50 126.73	191.91	78.46	68.94	42.88 43.27	131.8	126.12	25.24	22.34	20.63	24.17	101.1	68.94	72.47	71.35	62.41		169.83[b]	169.87[b]	20.63[b]
9	71.17 71.5	151.04	126.95	195.05	78.33	67.25 67.46	42.70 43.07	131.83 132.45	126.70	25.18	22.50	18.16 20.52	23.85 24.25	103 103.37	72.92	75.30	69.49	65.17				
7	69	151.84	126.73	195.05	78.41	72.06	42.87	132.27	126.59	25.26	22.26	20.74	24.18	101.49	71.79	81.11	72.66	72.06	62.16	168.95[b]	169.33[b]	170.68[b] 20.74[b]
10	72.53	152.04	126.61	195.06	78.42	71.23	42.80	132.14	126.30	25.18	22.86	20.92	24.07	101.38	71.53	83.70	69.68	75.24	59.91			
8	72.45	151.07	127.6.	195.05	78.92	68.60	42.59	131.83	127.43	25.21	22.44	20.80	24.22	98.23	70.18	66.36	69.41	68.60	20.80	169.92[b]	20.61[b]	
11[a]	71.66	150.97 152.73	126.98	195.88	78.36	66.98	42.77 43.20	132.86	126.98	25.19	22.60	20.61	24.32	100.30	70.45	71.42	72.39	68.58	17.96			
19	71.94	151.06	126.79	195	78.44	72.48	42.87 43.33	132.31 133.00	125.97	25.27	22.36	21.70	24.33	98.24	70.51	71.16	71.31	67.02	70.51	20.71[d] 24.33[d]	21.40[d] 24.96[d]	
20[a]	71.55	151.88 154.01	127.13	195.75 195.86	79.29 79.67	71.92	43.7 43.43	133.51 134.31	125.86 126.74	25.15	22.19	21.35	24.38	98.4 103.84	74.31	70.52	70.71	71.17	71.73			
24	71.45	150.78 152.65	126.86 127.01	195.04	78.40 78.86	71.99	42.84 43.38	132.08 132.94	126.71 126.88	25.22	22.51	21.35	23.90 24.17	106.29	83.89	74.83	79.33	74.87	71.83	20.61[d] 23.86[d]	21.72[d] 26.45[d]	23.67[d] 27.06[d]
25[a]	71.53	151.07	127.4	195.04	79.53	72.02	43.51 43.75	133.1	125.74 126.85	25.10	22.29 22.94	21.40	24.40	98.06	76.52	77.92	76.14	74.88	71.81			

[a]Acetone-d_6.
[b]MeCO.
[c]MeCO.
[d]Me isop.

yl]-5-oxo-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (4). To a soln of **4** (0.230 g, 0.44 mmol) in MeOH (1.58 mL) and H_2O (0.176 mL) was added K_2CO_3 (0.030 g, 0.22 mmol). After 1 h the solution was filtered. Concentration in vacuo, then flash chromatography (10% MeOH in EtOAc) yielded 0.071 g (42%) of [2*S*,5α,6β]-1-(5,6-dihydro-6-(glucopyranosyl)oxymethyl]-5-oxo-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (**5**).

Desacetylation of **6**–**8** led to the conjugate **9**–**11** in 40, 52, and 43% yields, respectively.

Synthesis of keto-C-glycoside 6→6 conjugates

[2*S*,5α,6β]-1-(5,6-dihydro-5-hydroxy-6-[(triphenylmethyl)oxymethyl]-2*H*-pyran-2yl)-methyl-cyclohex-1-ene (12). To a soln of 6-(6-hydroxymethyl 4-hydroxy-2*H*-pyran-2-yl)-1-methyl-cyclohex-1-ene (**2**) (8.42 g, 37.54 mmol) in CH_2Cl_2 (37.54 mL) was added triphenylmethyl chloride (14.65 g, 52.56 mmol), pyridine (15.18 mL, 187.70 mmol) and a catalytic amount of DMAP. After 2.5 h, the soln was diluted with 5 vol. CH_2Cl_2, washed twice with brine, and dried ($MgSO_4$). The solvent was removed under vacuum, and the resulting oil was purified by flash chromatography (CH_2Cl_2) to give 13.54 g of **12** (75%). ^1H NMR ($CDCl_3$, 300.13 MHz): δ 1.47 (m, 1H, H-6), 1.63 (m, 1H, H-5), 1.77 (m, 1H, H-6), 1.77 (s, 3H, Me C-2), 1.77 (m, 1H, H-4), 1.98 (m, 1H, H-4), 1.98 (m, 1H, H-5), 2.12 (m, 0.33H, H-1), 2.43 (m, 0.66H, H-1), 3.4 (dd, 0.33H, J=6.5 and 9.7 Hz, H-6′ py), 3.42 (dd, 0.66H, J=6.5 and 9.7 Hz, H-6′ py), 3.52 (dd, 0.66H, J=6.5 and 9.7 Hz, H-6′ py), 3.62 (dd, 0.33H, J=6.5 and 9.7 Hz, H-6′ py), 3.9–4.05 (m, 2H, H-5 py and H-6 py), 4.44 (m, 0.33H, H-2 py), 4.48 (m, 0.66H, H-2 py), 5.52 (m, 0.33H, H-3), 5.58 (m, 0.66H, H-3), 5.72 (ddd, 0.66H, J=2.1, 2.15 and 10.6 Hz, H-3 py), 5.87 (ddd, 0.33H, J=2.7, 3.35 and 10.6 Hz, H-4 py), 5.90 (ddd, 0.66H, J=2.7, 3.35 and 10.6 Hz, H-4 py), 7.3–7.5 (m, 15H, triphenylmethyl); ^{13}C NMR ($CDCl_3$, 75.44 MHz): δ 22.58 (t, C-5), 22.58 (d, C-6), 22.58 (d, C-5), 24.12 (d, Me C-3), 25.37 (t, C-4), 22.58 (t, C-5), 22.58 (t, C-6), 24.12 (q, Me C-3), 22.58 (t, C-6), 24.12 (q, Me C-3), 25.37 (t, C-4), 42.87 (d, C-1), 42.62 (d, C-1), 63.91 (t, C-6′ py), 64.35 (t, C-6′ py), 64.94 (d, C-6 py), 70.80 (d, C-2 py), 72.62 (d, C-2 py), 75.01 (d, C-5 py), 75.29 (d, C-5 py), 125.53 (d, 3), 127.18, 127.18, 127.18, 127.94, 128.66, 128.66 (C-3 py, C-4 py and C_6H_6 triphenylmethyl), 143.74 (s, triphenylmethyl).

[2*S*,5α,6β]-1-(5,6-Dihydro-5-[(thexyldimethylsilyl)oxy]-6-[(triphenylmethyl)oxymethyl]-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (13). In a flame-dried round-bottom flask was placed successively [2*S*,5α,6β]-1-(5,6-dihydro-5-hydroxy-6-[(triphenylmethyl)oxymethyl]-2*H*-pyran-2-yl)-methyl-cyclohex-1-en (**12**) (2.9 g, 5.00 mmol), imidazole (2.723 g, 40.00 mmol), dimethylformamide (7 mL), thexyldimethylsilyl chloride (2.156 mL, 11.00 mmol), and a catalytic amount of dimethylaminopyridine. The solution was stirred overnight, diluted with hexane (25 mL), then washed with water (2 × 1 mL). The organic soln was dried ($MgSO_4$), then the solvent was removed to give **13** as an oil that was used without further purification.

[2*S*,5α,6β]-1-(5,6-Dihydro-5-[(thexyldimethylsilyl)oxy]-6-hydroxymethyl-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (14). A soln of **13** (1.72 g, 3.13 mmol) in Et_2O (6.26 mL) was treated with HCO_2H (6.26 mL) and water (0.313 mL). After 30 min the solvent was removed under vacuum and then purified by flash chromatography (CH_2Cl_2:MeOH, 98:2) to yield 0.91 g (80%) of [2*S*,5α,6β]-1-(5,6-dihydro-5-[(thexyldimethylsilyl)-oxy]-6-hydroxymethyl-2*H*-pyran-2-yl)-methyl-cyclohex-

1-ene (**14**). ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 0.16 (s, 3H, H-Me Si), 0.26 (m, 3H, H-Me Si), 0.88 (s, 3H, H-Me thex), 0.88 (s, 3H, H-Me thexyl), 1.47 (m, 1H, H-6), 1.63 (m, 1H, H-5), 1.68 (m, 1H, H-6), 1.69 (d, 1H, J=7.5 Hz, H-thexyl), 1.77 (s, 3H, Me C-2), 1.77 (m, 1H, H-4), 1.98 (m, 1H, H-4), 1.98 (m, 1H, H-5), 2.47 (m, 1H, H-1), 3.56 (m, 1H, H-6 py), 3.68 (dd, 1H, J=5.3 and 10.4 Hz, H-6′ py), 3.8 (dd, 1H, J=3.1 and 10.4 Hz, H-6′ py), 4.06 (m, 1H, H-5 py), 4.44 (m, 1H, H-2 py), 5.54 (m, 1H, H-3), 5.75 (ddd, 1H, J=1.6, 2.6 and 10.6 Hz, H-4), 5.8 (ddd, 1H, J=2.08, 2.08 and 10.6 Hz, H-3 py); ^{13}C NMR ($CDCl_3$, 75.44 MHz): δ −3.16 (s, C-Me Si), −2.32 (s, Me Si), 18.41 (s, Me thex), 20.08 (s, C-Me thex), 20.14 (t, C-6), 23.27 (t, C-5), 24,1 (q, Me C-2), 25.30 (t, C-4), 34.11 (d, C-H thex), 43.23 (d, C-1), 62.09 (t, C-6′), 63.29 (d, C-5 py), 74.42 (d, C-2 py), 75.15 (d, C-6 py), 125.10 (d, C-3), 125.60 (d, C-3), 127.64 (d, C-4 py), 130.13 (d, C-4 py).

[2*S*,5α,6β]-1-(5,6-Dihydro-5-[(thexyldimethylsilyl)oxy]-6-[(trifluoromethanesulphonyl]oxymethyl]-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (15). A soln of trifluoromethanesulfonic anhydride (0.277 mL, 1.60 mmol) in CH_2Cl_2 (3 mL) was added to a soln of pyridine (0.145 mL, 1.80 mmol) in CH_2Cl_2 (15 mL) at 15 °C. To this soln was added **14** (0.363 g, 1.00 mmol) in CH_2Cl_2 (7.5 mL) and the reaction was stirred for 1.5 h at −15 °C before being poured into a satd soln of $NaHCO_3$ (15 mL). The organic layer was decanted, dried ($MgSO_4$), then evapd in vacuo to give an oil that was used without further purification.

[2*S*,5α,6β]-1-(5,6-Dihydro-5-[(thexyldimethylsilyl)oxy]-6-(1,2,3,4-di-*O*-isopropylidene-D-galactopyranosyl)oxymethyl]-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (17). NaH (50% in paraffin, 0.144 g, 3.00 mmol) was washed three times with pentane under nitrogen then diisopropylidene galactopyranose **16** (0.093 g, 0.36 mmol) in THF (30 mL) and DMPU (4.5 mL) was added at 0 °C. After 30 min, a soln of **15** (0.463 g, 1.00 mmol) in THF (15 mL) was added slowly. After 3 h stirring, the solution was diluted with Et_2O then poured in satd Na_2HPO_4 (15 mL). The organic layer was decanted, then the water layer was extracted twice with Et_2O. The combined organic layers were washed with brine, dried ($MgSO_4$), then evapd in vacuo. Purification by flash chromatography (CH_2Cl_2) yielded 0.13 g (60%) of [2*S*,5α,6β]-1-(5,6-dihydro-5-[(thexyldimethylsilyl)oxy]-6-(1,2,3,4-di-*O*-isopropylidene-galactopyranosyl)oxymethyl]-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (**17**). $[\alpha]_D^{20}$ (*c* 0.1 MeOH) +5; ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 0.13 (s, 3H, Me Si), 0.16 (s, 3H, Me Si), 0.83 (s, 6H, Me thexyl), 0.84 (s, 6H, Me thexyl), 1.33 (s, 3H, isopropylidene), 1.34 (s, 3H, isopropylidene), 1.45 (s, 3H, isopropylidene), 1.55 (s, 3H, isopropylidene), 1.47 (m, 1H, H-6), 1.63 (m, 2H, H-5 and CH thexyl), 1.68 (m, 1H, H-6), 1.69 (d, 1H, J=7.5 Hz, H-thexyl), 1.77 (s, 3H, Me C-2), 1.77 (m, 1H, H-4), 1.98 (m, 1H, H-4), 1.98 (m, 1H, H-5), 2.20 (m, 0.33H, H-1), 2.41 (m, 0.66H, H-1), 3.57 (dd, 1H, J=6.3 and 10.2 Hz, H-6 py), 3.58 (dd, 1H, J=6.3 and 10.2 Hz, H-6 py), 3.6 (dd, 1H, J=6.3 and 10.6 Hz, H-6), 3.7 (dd, 1H, J=4.2 and 10.4 Hz, H-6), 3.72 (dd, 1H, J=4.54 and 10.15 Hz, H-6′ py), 4.03 (m, 3H, H-5, H-5 py, H-6 py), 4.29 (m, 0.33H, H-2 py), 4.29 (dd, 1H, J=2.4 and 5 Hz, H-2), 4.29 (dd, 1H, J=2 and 8 Hz, H-4), 4.4 (m, 0.33H, H-2 py), 4.58 (dd, 1H, J=2.4 and 8 Hz, H-3), 5.52 (d, 1H, J=5 Hz,), 5.6 (m, 1H, H-3), 5.71 (ddd, 1H, J=1.4, 2 and 10.5 Hz, H-4 py), 5.77 (ddd, 1H, J=2.2, 2.36 and 10.6 Hz, H-3 py). Anal. calcd for $C_{33}H_{56}SiO_8$: C, 65.13; H, 9.21 found: C, 65.36; H, 9.79.

[2*S*,5α,6β]-1-(5,6-Dihydro-6-(1,2,3,4-di-*O*-isopropylidene-galactopyranosyl)oxy-methyl]-5-hydroxy-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (18). [2*S*,5α,6β]-1-(5,6-Dihydro-5-[(thexyldimethylsilyl)oxy]-6-(1,2,3,4-di-*O*-isopropylidene-galactopyranosyl)oxymethyl]-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (**18**) (0.608 g, 1 mmol) was added to a soln of tetrabutylammonium fluoride (3.3 mmol) in THF (25 mL). After 1 h, the solvent was removed and **18** was isolated by flash chromatography (30% EtOAc in hexane) (yield 0.318 g, 68%).

[2*S*,5α,6β]-1-(5,6-Dihydro-6-(1,2,3,4-di-*O*-isopropylidene-galactopyranosyl)oxymethyl]-5-oxo-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (19). To a soln of **18** (0.43 g, 1 mmol) in CH_2Cl_2 (5 mL) was added 4 Å molecular sieves (1 g) and PDC (0.75 g, 1 mmol). After 1 h, EtOAc (15 mL) was added and the resulting slurry was filtered through a celite pad that was washed with Et_2O (2 × 10 mL). The solvent was removed under vacuum then the resulting black residue was purified by chromatography (flash 10% EtOAc in hexane) to yield 0.31 g (72%) of [2*S*,5α,6β]-1-(5,6-dihydro-6-(1,2,3,4-di-*O*-isopropylidene-galactopyranosyl)oxymethyl]-5-oxo-2*H*-pyran-2-yl)methyl-cyclohex-1-ene (**19**).

[2*S*,5α,6β]-1-[5,6-dihydro-6-(galactopyranosyl)oxy-methyl]-5-oxo-2*H*-pyran-2-yl)methyl-cyclohex-1-ene (20). A soln of [2*S*,5α,6β]-1-[5,6-dihydro-6-(1,2,3,4-di-*O*-isopropylidene-galactopyranosyl)oxy methyl]-5-oxo-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (0.464 g, 1 mmol) in a mixture of MeOH (5 mL) and HCl (2 N, 2 mL) was stirred overnight at 40 °C. The solution was neutralized with IR 45 (OH-) then evapd under vacuum. Keto-C-glycoside **20** was isolated by flash chromatography (5% MeOH in EtOAc) in 40% yield (0.153 g).

[2*S*,5α,6β]-1-(5,6-dihydro-5-[(thexyldimethylsilyl)oxy]-6-(1,2,3,5-di-*O*-isopropylidene-glucofuranosyl)oxymethyl]-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (22). NaH (50% in parrafin, 0.096 g, 2 mmol) was washed three times with pentane under nitrogen then 1,2,3,5-di-*O*-isopropylidene-D-glucose **21** (0.13 g, 0.5 mmol) in THF (30 mL) and DMPU (0.3 mL) was added at 0 °C. After 30 min, a soln of **15** (0.463 g, 1.00 mmol) in THF (2.5 mL) was added slowly. After 3 h stirring, the solution was diluted with EtOAc then poured in satd soln of Na_2HPO_4 (15 mL). The organic layer was decanted, then the water layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried ($MgSO_4$), then evaporated in

vacuo. Purification by flash chromatography (CH_2Cl_2) yielded 0.22 g (50%) of [2*S*,5α,6β]-1-(5,6-dihydro-5-[(thexyldimethylsilyl)oxy]-6-(1,2,3,5-di-*O*-isopropylidene-glucofuranosyl)oxymethyl]-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (**22**). $[\alpha]_D^{20}$ (*c* 0.1 MeOH) +30; ^{1}H NMR ($CDCl_3$, 300.13 MHz): δ 0.14 (s, 3H, Me Si), 0.16 (s, 3H, Me Si), 0.87 (s, 6H, Me thexyl), 0.88 (s, 6H, Me thexyl), 1.34 (s, 3H, isopropylidene), 1.38 (s, 3H, isopropylidene), 1.44 (s, 3H, isopropylidene), 1.47 (m, 1H, H-6), 1.63 (m, 2H, H-5 and CH thexyl), 1.68 (m, 1H, H-6), 1.69 (d, 1H, *J*=7.5 Hz, H-thexyl), 1.77 (s, 3H, Me C-2), 1.77 (m, 1H, H-4), 1.98 (m, 1H, H-4), 1.98 (m, 1H, H-5), 2.47 (m, 1H, H-1), 3.56 (m, 1H, H-6 py), 3.6 (m, 1H, H-5′), 3.68 (m, 1H, H-6′ py), 3.7 (m, 1H, H-6′), 3.8 (m, 1H, H-6′ py), 3.8 (m, 1H, H-6′), 4..59 (d, 1H, *J*=3.9 Hz, H-2′), 14.06 (m, 1H, H-5 py), 4.22 (d, 1H, *J*=Hz, H-3′), 4.31 (m, 0.33H, H-2 py), 4.35 (dd, 1H, *J*=3.8 and 7.1 Hz, H-4′), 4.36 (m, 0.66H, H-2 py), 5.55 (m, 0.33H, H-3), 5.6 (m, 0.66H, H-3), 5.78 (ddd, 1H, *J*=1.7, 2.2 and 10.6 Hz, H-4 py), 5.8 (ddd, 1H, *J*=2, 2.4 and 10.6 Hz, H-3 py), 6 (d, 1H, *J*=3.9 Hz, H-1′); ^{13}C NMR ($CDCl_3$, 75.44 MHz): δ −3.96 (q, Me Si), −2.91 (q, Me Si), 18.54 (q, Me thexyl), 19.88 (t, C-6), 20.09 (q, Me thexyl), 22.92 (t, C-5), 23.9 (q, Me isopropylidene), 24.03 (q, Me isopropylidene), 25.21 (t, C-4), 26.47 (q, Me isopropylidene), 27.1 (q, Me isopropylidene), 34.09 (d, C-H thexyl), 42.79 (d, C-1), 63.47 (d, C-5 py), 71.42 (d, C-6′ py), 71.76 (d, C-6′), 72.49 (t, C-5′), 73.29 (d, C-2 py), 74.93 (d, C-3′), 75.07 (d, C-6 py), 79.36 (d, C-4′), 83.98 (d, C-2′), 105.19 (s, isopropylidene), 106.29 (d, C-1′), 112.04 (s, isopropylidene), 125.36 (d, C-3), 127.66 (d, C-4 py), 130.89 (d, C-3 py), 131.0 (s, C-2).

[2*S*,5α,6β]-1-(5,6-Dihydro-6-(1,2,3,5-di-*O*-isopropylidene-glucofuranosyl)oxymethyl]-5-hydroxy-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (23). [2*S*,5α,6β]-1-(5,6-Dihydro-5-[(thexyldimethylsilyl)oxy]-6-(1,2,3,5-di-*O*-isopropylidene-glucofuranosyl)oxymethyl]-2*H*-pyran-2-yl)-methy l-cyclohex-1-ene (**22**) (0.608 g, 1 mmol) was added to a solution of tetrabutylammonium fluoride (1.037 g, 3.3 mmol) in THF (25 mL). After 20 min, the solvent was removed then the resulting oil was dissolved in CH_2Cl_2. The soln was washed with water to yield **23** that was used without further purification.

[2*S*,5α,6β]-1-(5,6-dihydro-6-(1,2,3,5-di-*O*-isopropylidene-D-glucofuranosyl)oxymethyl]-5-oxo-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (24). To a soln of **23** (0.466 g, 1 mmol) in CH_2Cl_2 (5 mL) was added 4 Å molecular sieves (1 g) and PDC (0.752 g, 1 mmol). After 1 h, Et_2O (15 mL) was added and the resulting slurry was filtered through a celite pad that was washed with Et_2O (2 × 10 mL). The solvent was removed under vacuum then the resulting black residue was purified by flash chromatography (10% EtOAc in hexane) to yield 0.315 g (68%) of [2*S*,5α,6β]-1-(5,6-dihydro-6-(1,2,3,5-di-*O*-isopropylidene-glucofuranosyl)oxymethyl]-5-oxo-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (**24**).

[2*S*,5α,6β]-1-(5,6-Dihydro-6-(D-glucopyranosyl)oxymethyl]-5-oxo-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (25). [2*S*,5α,6β]-1-(5,6-Dihydro-6-(1,2,3,5-di-*O*-isopropylidene-glucofuranosyl)oxymethyl]-5-oxo-2*H*-pyran-2-yl)-methyl-cyclohex-1-ene (**24**) (0.464, 1 mmol) was disolved in 5 mL of a mixture of trifluoroacetic acid and MeOH (95:5). After 15 min, the soln was evapd. The residue was distilled several times with MeOH under vacuum. Keto-C-glycoside **25** was isolated by flash chromatography (EtOAc) in 43% yield (0.165 g).

Biological methods

Cytotoxicity. The human lymphoma RAJI[21] (B-cell derived) were grown in RPMI 1640 (Gibco BRL) supplemented with 10% fetal calf serum (FCS) 1% glutamine and without antibiotics. They were usually seeded at 250.000 cells/mL medium, incubated at 37 °C in a humidified atmosphere of 5% CO_2 and subcultured twice a week. Drugs were kept frozen at −20 °C and freshly dissolved in dimethyl sulphoxide (DMSO) at a final concentration of 0.2% in culture medium. Cells, in exponentional growth, were incubated with the drugs over a range of concentrations for 48 h at 37 °C. The cells were then washed twice with isotonic phosphate buffer (PBS) and pelleted by centrifugation for 10 min at 120 *g*. They were resuspended at a density of 106 cells/mL in PBS. Propidium iodide (PI) was added during 5 min after which the cells were examined by flow cytometry in an Epics Profile Analyzer II (Coulter).

GLUT-1. Preparation of biological materials: fresh erythrocytes drawn from healthy blood donors, collected on citrate and remaining after removal of platelets and leucocytes, were washed at room temperature in 10 vols of isotonic phosphate buffer (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 temperature. The erythrocytes thus obtained were resuspended in PBS to a haematocrit of 30% (controlled by microhaematocrit centrifugation) and kept at 4 °C.

Glucose uptake. Compounds were solublized in PBS or DMSO. Glucose uptake was determimed at room temperature on 40 μL of erythrocyte–PBS suspension. Glucose uptake was started by addition of 10 μL of D[I-^{14}C]glucose (final concentration 1 mM; 0.33 μCi/mL, final haematocrit 20%). Preliminary experiments showed that the uptake was linear with time up to 8 s (Fig. 1), and proportional to the erythrocyte concentration up to a final hematocrit of 25%. Glucose uptake was stopped after 8 s by adding 750 μL of an ice-cold blocking soln, modified from Jarvis,[22] containing phloretin (0.1 mM in ethanol), $HgCl_2$ (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 dibutylphthalate and immediately centrifuged (1 min, 2500 *g*). The cell pellet was thus rapidly separated from the reacting solution by the dibutylphthalate layer. The upper solution was

removed by aspiration, the tube was gently rinsed with ice-cold saline, and the dibutylphthalate layer was then discarded; the pellet was treated with 1 mL of aq 6% trichloroacetic acid, centrifuged (1 min, 2500 *g*), and the deproteinized supernatent was counted by liquid scintillation. Following this standard procedure, the values of V_{max} and K_m for glucose alone, respectively 40.5 mmol/l/min, and 4 mM, are in good agreement with published data.[23-25]

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