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Synthesis and Evaluation of Fructose Analogues as Inhibitors of the D-Fructose Transporter GLUT5

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Abstract—We have examined the specificity and binding-site spatial requirements of the fructose transporter GLUT5. Interaction with a series of fructofuranosides and fructopyranosides suggests that both furanose and pyranose ring forms of D-fructose combine with GLUT5. The epimers of D-fructose all have low affinity for GLUT5 suggesting that the transporter requires all hydroxyls to be in the fructo-configuration. Similarly there is poor tolerance of all allyl derivatives of D-fructose except 6-*O*-allyl-D-fructofuranose. Therefore, the C-6 position offers the most suitable position for development of affinity probes and labels for exploring GLUT5 biochemistry.

Introduction

D-fructose is the second most abundant simple sugar in nature and so is a significant component of human dietary sugar intake. However, unlike D-glucose, which is absorbed across the intestine by the sodium dependent glucose transporter (SGLT1),[1] D-fructose uptake into mammals occurs via a specialized, sodium independent, facilitative transporter, GLUT5.[2,3] This protein is also present at high levels in the intestine, particularly the apical surface.[4] The GLUT5 protein shows sequence homology with a family of mammalian facilitative hexose transporters (GLUT isoforms 1–4) each of which shows a distinct pattern of subcellular distribution.[5,6] Isoforms 1-4 have high affinity for D-glucose but low affinity for D-fructose. By contrast, GLUT5 has low affinity for D-glucose and high affinity for D-fructose. The distribution of GLUT5 is quite widespread and also occurs at low levels in many tissues including the metabolically important peripheral tissues of fat and muscle.[6–8] The abundance and specificity of GLUT5 suggest that it performs a metabolically important role. However, to date the specificity and substrate structure requirements of GLUT5 have not been studied with isoform-specific chemical reagents and fructose analogues. We have therefore addressed this problem in the study described here.

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Despite its large-scale accessibility and the elucidation of its structure over a century ago,[9] the chemistry of fructose[10] makes the preparation of simple derivatives in tautomeric fixed structures, particularly in furanoid forms, difficult. Some simple derivatives of D-fructose exist with well defined ring shapes including in the pyranoid form: the tetraacetate,[11,12] the tetrabenzoate,[13] the 1,2:4,5 di-*O*-isopropylidene,[14] and the 2,3:4,5-di-*O*-isopropylidene[15] and in the furanoid form: the tetrabenzoate,[16] and in open chain: the tetracetates of 6-halo-6-deoxy-keto-D-fructoses.[17,18] The aim of our study was therefore to develop analogues of D-fructose in which each position was substituted and thereby determine the importance of each of the hydroxyl groups in the binding and interaction with GLUT5. In particular, we have studied the complete series of allyl substituted D-fructoses as these provide information on the steric constraints around the GLUT5 binding site. In addition, the allyl compounds described here can easily be further functionalized and will be useful for studying other proteins that specifically combine with D-fructose.

Results

Chemical synthesis

Several compounds used in the characterisation of the specificity of GLUT5 were synthesised by adaptation of previously described methods. 2,5-Anhydro-D-mannitol

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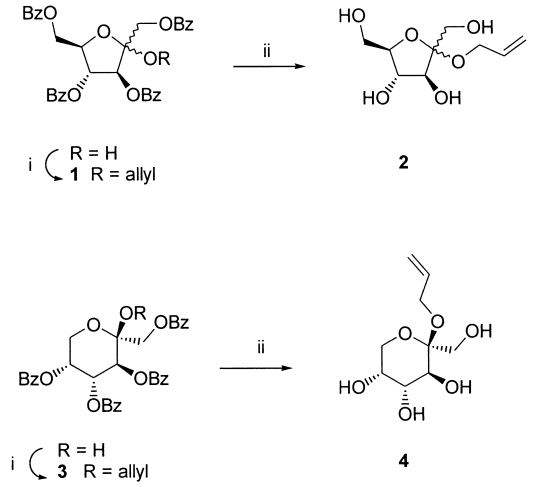
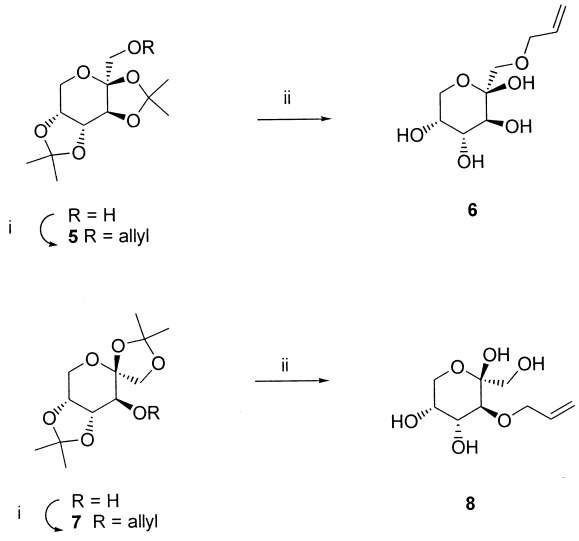


Scheme 1. Reagents and conditions: (i) allylmethylcarbonate, $Pd_2(dba)_3$, Ph_3P; (ii) MeONa, MeOH.

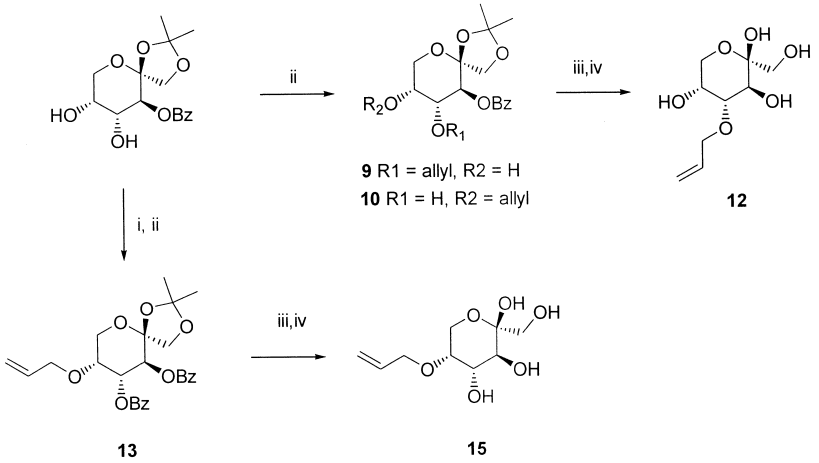


Scheme 2. Reagents and conditions: (i) allylbromide, NaH, DMF; (ii) TFA–H_2O.

was prepared as described.[19] 1,2-*O*-Isopropylidene-β-D-fructopyranose was prepared as described.[20,21] Methyl α,β-D-fructofuranosides[22] were prepared according to literature methods,[23] or from 1,3,4,6-tetra-*O*-benzoyl-D-fructofuranose which was methylated using methyl iodide and sodium hydride or silver oxide to give methyl 1,3,4,6-tetra-*O*-benzoyl-α,β-D-fructofuranosides which were deprotected using sodium methoxide. Methyl β-D-fructopyranoside was prepared by methylation of 1,3,4,5-tetra-*O*-benzoyl-D-fructopyranose using methyl iodide and sodium hydride or silver oxide which gave predominately (67% yield) methyl 1,3,4,5-tetra-O-benzoyl-β-D-fructopyranoside. Deprotection gave methyl β-D-fructopyranoside with data consistent with the literature.[22]

Allylation of the benzoylated fructofuranoses and fructopyranoses (Scheme 1) with sodium hydride and allyl bromide gave low yields. Better results were obtained for compounds **1** and **3** through a palladium (0) catalyzed allylation[24] with 93% and 88% yields, respectively. The anomeric ratio obtained for the allyl-tetrabenzoyl-fructofuranoside **1** was 7:3 in favor of the α anomer. Deprotections[25] of these benzoyl fructosides to give compounds **2** and **4** were performed with sodium methoxide in dry methanol with nearly 80% yields. The α and β alkyl fructofuranosides anomers were separated by chromatography.

1-OH and 3-OH positions of D-fructose were *O*-allylated starting with and 2,3:4,5 di-*O*-isopropylidene-β-D- fructopyranose and 1,2:4,5-di-*O*-isopropylidene-β-D-fructopyranose[15] (Scheme 2). These hydroxyl groups were allylated with allyl bromide and sodium hydride[26] yielding **5** and **7**. Functionalization of 4-OH and 5-OH was realised through a multi-step process (Scheme 3). A previously described procedure for benzoylation of 1,2:4,5-di-O-isopropylidene-β-D-fructopyranose[27] was used followed by a selective hydrolysis of one *O*-isopropylidene group.[28] Mono-allylation conditions using palladium catalysis were then used to substitute both the 4-OH and 5-OH positions leading to **9** with 39%, and **10** with 32% yield. In addition, a selective additional benzoylation on the 4-OH position of 3-O-benzoyl-1,2-O-isopropylidene-β-D-fructopyranose was followed by the specific allylation



Scheme 3. Reagents and conditions: (i) BzCl, pyridine; (ii) allylmethylcarbonate, $Pd_2(dba)_3$, Ph_3P; (iii) MeONa, MeOH; (iv) TFA–H_2O (4:1).

of the 5-OH position to give **13**. Deprotection of all these protected fructose derivatives were realised through standard methods:benzoyl esters by transesterification with sodium methanolate and *O*-isopropylidene groups were removed with trifluoroacetic acid–water mixtures. At this stage, each position of D-fructose was functionalized with an allyl group with overall yields starting from D-fructose: 1-OH position **6** 30%, 2-OH position **2** and **4** 50%, 3-OH position **8** 30%, 4-OH position **12** 11%, and the 5-OH position **15** 17%.

Allylation of the 6-OH position took into account the preferential reactivity of the 6-hydroxyl in fructofuranosides[23] (Scheme 4). Reaction of methyl α-D-fructofuranose with monomethoxytrityl chloride afforded **16**; the free hydroxyl groups were then acetylated, which was followed by deprotection of the trityl ether to give **17**. To introduce an allyl group a palladium catalysed reaction[24,29] was used. De-acylation using sodium methoxide then gave **18**, which was followed by acidic cleavage of the glycosidic bond to afford 6-*O*-allyl-D-fructose, **19** (>20% overall yield from methyl α-D-fructofuranoside).

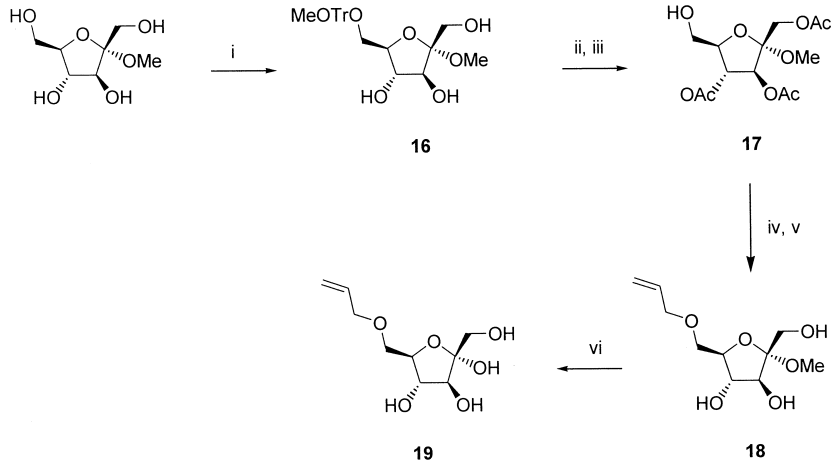
Biological evaluation

We have previously studied the interaction of D-glucose analogues with the binding site of the erythrocyte and adipocyte glucose transporters, GLUT1 and GLUT4, respectively.[30,31] For example, we have found that methyl and propyl glucosides are poorly tolerated at the exofacial binding site.[32,33] It was concluded that the D-glucose (in its pyranose ring form) enters the GLUT binding site in a specific orientation with C1 (the front of the molecule relative to the binding site) entering first. Conversely, substitutions into the C4 and C6 positions of D-glucose are well tolerated at the exofacial site.[32–34] Consideration of these factors led to the development of bis-hexose photoaffinity labels in which C4 positions are linked[35] and in which the linker is used as a platform for attachment of the bulky trifluoromethylazibenzoate moiety.[36] These compounds have been used extensively for studying GLUT1 biochemistry.[36] More importantly from the metabolic perspective, the photolabels have been used to study the effects of insulin on the subcellular localisation and trafficking of the insulin-responsive GLUT4 isoform.[37,38] In the present study we have probed the structure and steric factors that govern the interaction of D-fructose with its specific transporter GLUT5 so that similar photoaffinity probes can ultimately be developed for this system.

To evaluate the interaction of D-fructose analogues with GLUT5 we have chosen a CHO cell line in which the GLUT5 transporter is expressed at high levels.[39] The substrate for the assays is a tracer concentration (1 mM) of [^{14}C-U]-D-fructose. The ratio of GLUT5 mediated uptake to the endogenous uptake was <9:1. Preliminary studies established that the tracer uptake was linear for 10 min and that this radioactive substrate concentration was low compared with the half maximal affinity constant of GLUT5 for D-fructose. Therefore, the uptake in the presence of an analogue inhibitor is determined: $V_0/V = 1 + I/K_i$, where V_0 and V are the tracer uptake rates in the absence and presence of inhibitor, I is the inhibitor concentration and K_i is the half maximal inhibition constant or affinity constant.[31]

In Fig. 1A the interaction of GLUT5 with the furan compound 2,5-anhydro-D-mannitol is compared with the pyranose ring analogues methyl β-D-fructopyranoside and 1,2-*O*-isopropylidene β-D-fructopyranose. Each of these compounds inhibits the uptake of tracer D-fructose with similar potency (Table 1). Comparisons of the common structural features of 2,5-anhydro-D-mannitol with β-D-fructofuranose (Fig. 1B) indicate that the β-hydroxy is absent from the former compound but the molecules are elsewhere structurally identical. Additional data in Table 1 indicate that the methyl β-D-fructofuranoside compound also has high affinity (K_i = 15.5 mM) but that in comparison, the methyl α-D-fructofuranoside has reduced interaction (K_i = 32.4 mM). Therefore, the fructofuranose-C2 R configuration appears to be preferred.

The β-D-fructofuranose form is 20% of the total D-fructose added[40] and yet the affinity for the D-fructose mixture is similar to 2,5-anhydro-D-mannitol (Table 1).



Scheme 4. Reagents and conditions: (i) (MeOPh)Ph_2CCl, pyridine, 4 °C; (ii) Ac_2O, pyridine, 4 °C; (iii) AcOH; (iv) allylethylcarbonate, Ph_2P-C_4H_8-PPh_2; (v) MeONa, MeOH; (vi) dioxane, 0.5M HCl.

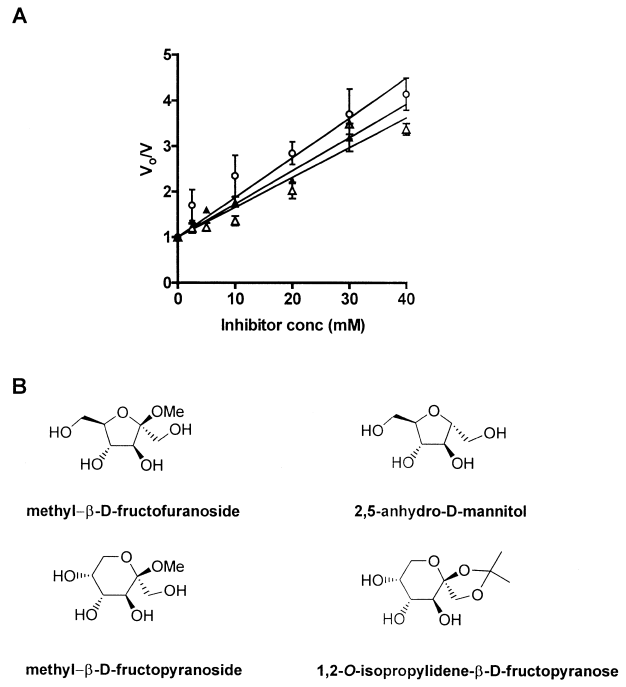


Figure 1. Comparison of the interaction of furanose and pyranose ring forms of fructose with GLUT5. In **A**, the uptake of 1 mM [^{14}C]-D-fructose into CHO cells expressing GLUT5 was measured in the presence of the indicated concentrations of 2,5-anhydro-D-mannitol (○), methyl-β-D-fructopyranoside (△) and 1,2-*O*-isopropylidene-β-D-fructopyranose (▲). K_i values were determined by linear regression fitting to Eqn. 1. The data points are mean±S.E.M. from 3 separate experiments. In **B**, the structures of furanose and pyranose derivatives of D-fructose are contrasted. The structures are similar around the ring oxygen and C1, C2, C3 and C4.

Therefore, either the β-hydroxyl of D-fructofuranose (which is absent in 2,5-anhydro-D-mannitol) is required for high affinity binding or alternatively β-D-fructopyranose also interacts with GLUT5. The latter possibility is consistent with the observed interaction of the β-pyranose ring analogues. Structural considerations of the common features of D-fructofuranose and D-fructopyranose suggest that the arrangement of hydroxyls at C1, C2, C3 and C4 and the ring oxygen are essentially the same in these two forms which only differ around the C5 and C6 positions. The specificity of GLUT5 therefore has evolved in such a way as to make use of D-fructose in all its ring forms.

We have examined the tolerance of D-fructose epimers by GLUT5 (Table 1). The C3, C4 and C5 epimers are all poorly tolerated. The C4 epimer, D-tagatose has slightly higher affinity than the C3 and C5 epimers D-psicose and L-sorbose, respectively. These data suggests that each of these hydroxyls in the fructo-configuration may be involved in H-bonding to GLUT5. However, it is also possible that the epimerized hydroxyls sterically reduce binding.

In order to explore the spatial requirements around the D-fructose binding site in GLUT5, we have chosen to substitute allyl groups at all the hydroxyl positions. In the case of the anomeric centre, we have compared methyl and allyl substitution. The allyl group was chosen as it is quite planar and therefore occupies a relatively fixed distance from the fructose ring. Second, it is a useful group for further modification. Comparison of

Table 1. Interaction of D-fructose analogues with the GLUT5 transporter[a]

Position substituted and derivative	Structure and compound number	Pyranose/furanose ratio (where known)		K_i±S.E.M. (mM)
		αP,βP	αF,βF	
D-fructose	Fig. 1	trace,75	4,21	15.5±2.9
C1,C2				
1,2-O-isopropylidene-β-D-fructopyranose	Fig. 1	100 βP		20.9±4.3
C1				
1-O-allyl-D-fructose	**6**	14,67	trace,19	105.6±19.0
C2				
Deoxy: 2,5-anhydro-D-mannitol	Fig. 1	100βF		12.6±1.8
methyl α-D-fructofuranoside	Fig. 1	100αF		32.4±4.1
allyl-α-D-fructofuranoside	**2**α	100αF		79.6±3.9
methyl-β-D-fructofuranoside	Fig. 1	100βF		15.5±1.8
methyl-β-D-fructopyranoside	Fig. 1	100βP		15.0±0.3
allyl-β-D-fructofuranoside	**2**β	100βF		65.1±5.8
allyl-β-D-fructopyranoside	**4**	100βP		28.5±3.1
C3				
Epimer: D-psicose		22,24	39,15	134.2±18.0
3-*O*-allyl-D-fructose	**8**	15,38	12,35	89.6±31.1
C4				
Epimer: D-tagatose		79,16	1,4	59.4±8.2
4-*O*-allyl-D-fructose	**12**	55,18	18,9	105.6±20.1
C5				
Epimer: L-sorbose		98αP		142.7±10.9
5-*O*-allyl-D-fructose	**15**	73,27	noF	153.0±36.6
C6				
6-*O*-allyl-D-fructose	**19**	100 α + βF, no open chain		20.3±0.8

[a]Uptake of 1 mM [^{14}C]-D-fructose into CHO cells expressing GLUT5 was measured at a range of concentrations of the D-fructose analogues (usually serial increases in concentrations up to 40 mM). The K_i values were calculated by least squares fitting to Eqn. 1. The tabulated values are the mean and SEM of 3–5 independent experiments. The pyranose/furanose ratios in solution are from literature values[40] or are estimated from ^{13}C NMR data for C2 of the compounds in D_2O. As discussed[40] values for the proportion of anomeric mixtures are approximations only because of inherent inaccuracies in peak intensities and peak area estimation by NMR.

the affinity (Table 1) for methyl β-D-fructofuranoside ($K_i = 15.5$ mM) and allyl β-D-fructofuranoside **2β** ($K_i = 65.1$ mM) suggests that the additional size of the allyl group reduces interaction with GLUT5 around the C2 position. Similarly, methyl β-D-fructopyranoside ($K_i = 15.0$ mM) has higher affinity than allyl β-D-fructopyranoside **4** ($K_i = 28.5$ mM). These data are consistent with the postulates that GLUT5 accepts both furanose and pyranose ring compounds, prefers the β configuration and has limited capacity to accept bulky groups at C2 (particularly in the α-configuration).

1-*O*-allyl-D-fructose **6** (a mixture of ≈20% furanose and 80% pyranose ring forms, Table 1) is poorly tolerated (Fig. 2). We have suggested above that the anomeric half of fructose is preferentially recognised as it is this half of the molecule that is similar in both furanose and pyranose forms of D-fructose. The anomeric center can therefore be considered as the "front" of the molecule relative to the binding site. The poor affinity of 1-*O*-allyl-D-fructose **6** may be due to a tight approach of the GLUT5 protein around C1.

3-*O*-, 4-*O*-, and 5-*O*-allyl-D-fructoses (compounds **8**, **12** and **15** all have low affinity (K_is≈80–100 mM; Table 1) for GLUT5. The GLUT5 protein must therefore quite tightly approach these positions when interacting with the associated hydroxyls. By contrast, the data on 6-*O*-allyl-D-fructose **19** ($K_i = 20$ mM, Fig. 2) suggest that this position occupies an open portion of the binding site. Data described here therefore suggests that the orientation of D-fructose as it enters GLUT5 is similar to the orientation of D-glucose in its interactions with GLUT1 and GLUT4. The "front" of D-glucose and D-fructose are most important for recognition. It is probably the accomodation, by GLUT5, of both the D-fructose hydroxyl and hydroxymethyl groups at the anomeric center that allows it to distinguish this substrate from D-glucose. 3-*O*-methyl-D-glucose has no detectable affinity for GLUT5 (unpublished data). Remarkably therefore, GLUT5 has evolved with the ability to exclude D-glucose while retaining recognition of both furanose and pyranose ring forms of D-fructose. This is a metabolically important property as D-fructose has low affinity for the glucose transporters (GLUTs 1–4). Therefore, if GLUT5 were absent, competition with circulating D-glucose would exclude D-fructose from significant transport via GLUTs 1–4 and consequently its access to intracellular metabolic processes.

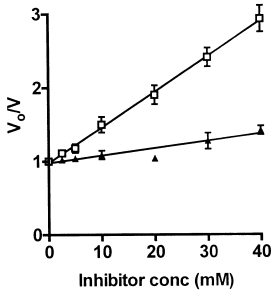


Figure 2. Interaction of GLUT5 with 1-*O*-allyl-D-fructose and 6-*O*-allyl-D-fructose. In **A**, the uptake of 1 mM [^{14}C]-D-fructose into CHO cells expressing GLUT5 was measured in the presence of the indicated concentrations of 1-*O*-allyl-D-fructose (▲) and 6-*O*-allyl-D-fructose (□). K_i values were determined by linear regression fitting to Eqn. 1. The data points are mean ±SEM from 3 separate experiments.

Conclusion

The GLUT5 protein is a versatile transporter in that it has evolved to accept D-fructose in both furanose and pyranose ring shapes with high affinity and yet can exclude D-glucopyranoses. The 2,5-anhydro-D-mannitol, methyl β-D-fructopyranoside and 1,2-*O*-isopropylidene-β-D-fructopyranose are tolerated while 3-*O*-methyl-D-glucose is totally excluded from interaction. Structural considerations of the common features of D-fructofuranose and D-fructopyranose suggest that the arrangement of hydroxyls at C1, C2, C3 and C4 and the ring oxygen are essentially the same in these two forms. They only differ around the C5 and C6 positions. Data presented here suggest that the GLUT5 transporter is highly specific for the fructo-hydroxyls and will not accept D-fructose epimers. It is concluded that there is a close approach of the GLUT5 binding site to these hydroxyl positions. Consistent with this, we find that allyl substitutions are not well tolerated at positions C1, C2, C3, C4 and C5. However, the bulky allyl group is tolerated at the C6 position of D-fructofuranose. C6 substituted fructofuranoses will therefore be useful as lead compounds for generation of new affinity probes to specifically tag the GLUT5 protein. Such reagents can be used to study its expression in cells and tissues and to investigate the regulation of its subcellular localization.

Experimental

General. Methanol was distilled over magnesium, pyridine was dried over KOH, dioxane and tetrahydrofuran were distilled over $LiAlH_4$, anhydrous DMF was purchased from Aldrich in Sure-Seal bottles. Column chromatography was performed as described.[41,42] NMR spectra were recorded for solutions in D_2O (internal 3-(trimethylsilyl)-propionic acid) in CD_3OD (residual absorption of solvent) and in $CDCl_3$ (internal tetramethyl silane). FAB-mass spectra were measured in the positive or negative mode with a VG Autospec Fisons instrument (the samples were dissolved in *m*-nitrobenzyl alcohol). An Optical Activity Ltd polarimeter was used for the determination of optical rotations at ≈20 °C. Elemental analysis was performed by the micro-analysis service at the University of Bath. Attributions of anomeric carbons of fructose derivatives were based on ^{13}C NMR of fructose in D_2O and CD_3OD[40] (F: Furanose ring, P: Pyranose ring). All evaporations of volatiles were performed at t < 30 °C under reduced pressure.

Allyl 1,3,4,6-tetra-*O*-benzoyl-α,β-D-fructofuranoside (1). 1,3,4,6-tetra-*O*-benzoyl-β-D-fructofuranose (1 g, 1.678 mmol), $Pd_2(dba)_3$ [trisdibenzylideneacetone palladium (0)] (38.6 mg, 0.042 mmol) and Ph_3P [triphenylphosphine] (44 mg, 0.167 mmol) was dissolved in freshly distilled dioxane (10 mL). Then allyl methyl carbonate (572 μL, 5.03 mmol) was added. The solution was stirred at 50 °C

for 1 h. The solvent was evaporated and the syrup purified on silica gel by flash chromatography with 4:1 petroleum ether–EtOAc. Compound **1** (0.997 g, 1.567 mmol) was isolated with 93% yield: $[\alpha]_D^{20}$ +5 (*c* 1.15, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 8.11–7.93 (m, 8H, Ph-H), 7.62–7.21 (m, 12H, Ph-H), 6.12 (d, 0.3H, $J_{3,4}$ 6.71 Hz, H-3β), 6.13–5.82 (m, 2H, $J_{3,4}$ 1.23 Hz, H-3α, H-4β, H-2′), 5.58 (dd, 0.7 Hz, $J_{4,5}$ 4.58 Hz, H-4α), 5.41–5.35 (m, 0.7 H, H-3′aα), 5.30–5.25 (m, 0.3H, H-3′aβ), 5.15–5.11 (m, 0.7H, H-3′bα), 5.10–5.07 (m, 0.3H, H-3′bβ), 4.95 (d, 0.7H, $J_{1a,1b}$ 12.51 Hz, H-1aα), 4.85 (dd, 0.7H, $J_{6a,6b}$ 11.9 Hz and $J_{5,6a}$ 3.36 Hz, H-6aα), 4.78 (dd, 0.3H, $J_{6a,6b}$ 11.0 Hz and $J_{5,6a}$ 3.66 Hz, H-6aβ), 4.71 (dd, 0.7H, $J_{5,6b}$ 3.89 Hz, H-6bα), 4.67–4.55 (m, 1.6H, H-1b, H-6bβ, H-5), 4.45 (d, 0.7H, H-1bα). ^{13}C NMR ($CDCl_3$; 75 MHz): δ 166.2–164.7 (CO), 134.1–128.3 (Ph-H, allyl C-Hα,β), 116.4 (allyl C-2′α), 116.3 (allylC-2′β), 107.4 (C-2α), 103.5 (C-2β), 81.9, 81.0, 78.9, 78.8, 78.0, 77.3, 63.7 (C-1β), 63.6 (C-1α), 63.2 (C-6β), 62.3 (C-6α), 59.7 (allyl C-1′α), 58.3 (allyl C-1′β). Anal. calcd for $(C_{37}H_{32}O_{10}\cdot CH_3CH_2OH)$: C, 68.61; H, 5.61. Found: C, 68.60: H, 5.58.

Allyl α,β-D-fructofuranosides (2α, 2β). Allyl-1,3,4,6-tetra-*O*-benzoyl-D-fructofuranoside **1** (2.65 g, 4.17 mmol) was suspended in dry MeOH (10 mL) and NaOMe (0.4 g, 7.4 mmol) was added. The solution was stirred at room temperature for 4 h, then neutralized with Dowex 50W-8X, evaporated and purified on silica gel by flash chromatography with 9:1 EtOAc–(MeOH-H_2O 2:1). Allyl-α-D-fructofuranoside **2α** (512 mg, 2.33 mmol) and allyl-β-D-fructofuranoside **2β** (235 mg, 1.07 mmol) were isolated with 56% and 25% yields, respectively: **2α** $[\alpha]_D^{20}$ +20.3 (c 0.985, CH_3OH). ^1H NMR ($CDCl_3$, 400 MHz): δ 5.99–5.89 (m, 1H, H-2′), 5.31–5.26 (m, 1H, H-3′a), 5.11–5.08 (m, 1H, H-3′b), 4.19–4.13 (m, 1H, H-1′a), 4.19–4.13 (m, 1H, H-1′a), 4.10–4.04 (m, 2H, H-1′b and H-3), 3.90 (dd, 1H, $J_{3,4}$ 4.88 Hz and $J_{4,5}$ 7.02 Hz, H-4), 3.85 (m, 1H, H-5), 3.76 (dd, 1H, $J_{5,6a}$ 2.74 Hz and $J_{6a,6b}$ 11.9 Hz, H-6a), 3.71 (d, 1H, $J_{1a,1b}$ 11.9 Hz, H-1a), 3.64 (d, 1H, H-6b), 3.62 (d, 1H, H-1b), ^{13}C NMR ($CDCl_3$, 100 MHz): δ 136.7 (allyl CH), 116.2 (allyl CH_2), 109.0 (C-2), 84.0 (C-5), 83.3 (C-3), 78.5 (C-4), 63.5 (C-6), 62.6 (C-1), 61.6 (allyl CH_2). MS (FAB(+), NBA): $m/z = 243$ ($M^+ + Na$). MS (FAB (−),NBA): m/z 219 ($M^+ - H$). Anal. calcd ($C_9H_{16}O_6\cdot H_2O$) C, 48.10 H, 7.40. Found: C, 48.10; H, 7.39. **2β** $[\alpha]_D^{20}$ −27.1 (c 1.66, CH_3OH). ^1H NMR ($CDCl_3$, 400 MHz): δ 5.90–5.84 (m, 1H, H-2′), 5.25–5.21 (m, 1H, H-3′a), 5.06–5.03 (m, 1H, H-3′b), 4.21–3.90 (m, 4H), 3.74–3.48 (m, 5H). ^{13}C NMR ($CDCl_3$; 100 MHz): δ 137.1 (allyl CH), 116.2 (allyl CH_2), 105.7 (C-2), 83.7 (C-5), 78.6 (C-3), 77.3 (C-4), 65.1 (C-6), 63.7 (C-1), 62.0 (allyl CH_2)). MS (FAB(+), NBA): $m/z = 243$ ($M^+ + Na$). MS (FAB (−), NBA): m/z 219 ($M^+ - H$). Anal. calcd ($C_9H_{16}O_6.0.5\ H_2O$): C, 47.16; H, 7.48, Found: C, 47.10; H, 7.39.

Allyl 1,3,4,5-tetra-*O*-benzoyl-β-D-fructopyranoside (3). Same conditions as **2**. The product **3** (0.937 g, 1.473 mmol) was isolated after crystallization in absolute ethanol with 88% yield: data in accordance with the literature.[43]

Allyl β-D-fructopyranoside (4). Allyl-1,3,4,5-tetra-*O*-benzoyl-β-D-fructopyranoside **3** (0.7 g, 1.1 mmol) was suspended in freshly distilled MeOH (10 mL) with NaOMe (0.1 g, 1.85 mmol) and stirred at room temperature for 14 h. The reaction was neutralized with Dowex 50W-8X. The resin was removed by filtration and washed with MeOH. The filtrate was evaporated and the syrup obtained, purified on silica gel by flash chromatography with 4:1 EtOAc–(MeOH–H_2O 2:1). Allyl β-D-fructopyranoside **4** (0.182 g, 0.245 mmol) was isolated after crystallization in water with 75% yield: $[\alpha]_D^{20}$ −137 (c 0.71, CH_3OH). ^1H NMR (CD_3OD, 400 MHz): δ 5.98–5.89 (m, 1H, H-2′), 5.33–5.27 (m, 1H, H-3′a), 5.13–5.09 (m, 1H, H-3′b), 4.13–4.02 (m, 1H, H-1′), 3.93 (d, 1H, $J_{3,4}$ 9.77 Hz, H-3), 3.85–3.83 (m, 1H, H-5), 3.81 (dd, 1H, $J_{4,5}$ 3.36 Hz, H-4), 3.76 (d, 1H, $J_{1a,1b}$ 11.29 Hz, H-1a), 3.75 (dd, 1H, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.69 (d, 1H, H-1b), 3.66 (dd, 1H, $J_{5,6b}$ 1.83 Hz, H-6b). ^{13}C NMR (CD_3OD, 100 MHz): δ 136.4 (allyl CH), 116.0 (allyl CH_2), 102.0 (C-2), 71.5 (C-4), 71.0 (C-5), 70.4 (C-3), 65.3 (C-6), 63.4 (C-1), 62.8 (allyl CH_2). Anal. calcd ($C_9H_{16}O_6$): C, 49.09; H, 7.32. Found: C, 49.2; H, 7.33.

1-*O*-Allyl-D-fructose (6). 5[26] (780 mg, 2.6 mmol) was dissolved in 4:1 TFA–H_2O and stirred at room temperature for 4 h. TFA was removed by co-evaporation with MeOH until no smell was noticeable. The pale yellow syrup obtained was purified on silica gel by flash chromatography with EtOAc then 4:1 EtOAc–(MeOH–H_2O 2:1). Compound **6** (435 mg, 1.97 mmol) was isolated with 76% yield: $[\alpha]_D^{20}$ −29 (c 1.865, CH_3OH). ^1H NMR (CD_3OD, 400 MHz): δ 5.98–5.87 (m, 1H, H-2′), 5.33–5.26 (m, 1H, H-3′a), 5.18–5.15 (m, 1H, H-3′b), 4.12–3.39 (m, 9H). ^{13}C NMR (CD_3OD, 100 MHz): δ 136 (allyl-CH), 135.9 (allyl-CH), 135.8 (allyl-CH), 102.6 (C-2β-F), 101.5 (C-2α-P), 99.0 (C-2β-P), 83.4 (CH), 83.1 (CH), 78.4 (CH), 78.0 (CH), 76.9 (CH), 76.5 (CH), 73.7 (CH_2), 73.6 (CH_2), 73.5 (CH_2), 73.4 (CH_2), 72.8 (CH_2), 72.3 (CH_2), 71.6 (CH), 71.3 (CH), 71.0 (CH), 70.8 (CH), 70.6 (CH_2), 70.5 (CH), 70.0 (CH), 69.7 (CH), 65.0 (CH_2), 64.8 (CH_2), 64.6 (CH_2), 64.4 (CH_2), 63.9 (CH_2), 63.2 (CH_2). MS (FAB(+), NBA): m/z 243 ($M^+ + Na$). Anal. calcd ($C_9H_{16}O_6$) C, 49.09; H, 7.32. Found: C, 49.5; H, 7.34.

3-*O*-Allyl-1,2,4,5-di-*O*-isopropylidene-β-D-fructopyranose (7). 1,2,4,5-di-*O*-isopropylidene fructopyranose[14] (1.5 g, 5.77 mmol) was dissolved in 10 mL of dry THF and then chilled to −20 °C in an ethanol–dry ice bath. NaH (0.353 g, 9.3 mmol) and allylbromide (1.8 mL, 3.6 eq) were added. The solution was allowed to return to room temperature and stirred for 21 h. H_2O (50 mL) was added, and the solution was extracted with EtOAc (3×25 mL), The organic layers were collected, washed with H_2O (2×50 mL), dried over Na_2SO_4 and evaporated. The pale yellow liquid was subjected to flash chromatography with 3:1 petroleum ether–ethyl acetate. The product **7** (1.36 g, 4.53 mmol) was isolated as a pale yellow liquid with 78.5%. $[\alpha]_D^{20}$ −100 (c 1.5, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 5.95–5.85 (m, 1H, H′-2), 5.32-5.15 (m, 2H, H′-3), 4.44–4.39 (m, 1H, H′-1a), 4.30 (dd, 1H, $J_{4,3}$ 7.33 Hz and $J_{4,5}$ 5.5 Hz H-4), 4.21–4.19 (m, 1H, H-5), 4.15–3.92 (m, 5H, H′-1b, H-6, H-1), 3.44 (dd, 1H, $J_{3,5}$ 2.14 Hz and $J_{3,4}$, H-3), 1.54 (s, 3H, CH_3), 1.49 (s, 3H, CH_3), 1.41 (s, 3H, CH_3), 1.36 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 134.5 (C′-2), 116.7 (C′-3), 112 ($C(Me)_2$); 108.7 ($C(Me)_2$), 104.3 (C-2), 77.5 (CH), 75.8 (CH), 73.7 (CH),

72.1 (CH_2), 71.6 (CH_2), 59.9 (CH_2), 28.0 (CH_3), 26.7 (CH_3), 26.1 (CH_3), 25.8 (CH_3). MS (FAB(+), NBA): *m/z* 301 ($M^+ + H$). Anal. calcd ($C_{15}H_{24}O_6$): C, 59.98; H, 8.05. Found: C, 60.2; H, 8.15.

3-*O*-Allyl-D-fructose (8). Compound **7** (810 mg, 2.7 mmol) was dissolved in 4:1 TFA–H_2O (2 mL) and stirred at room temperature for 4 h. TFA was removed by co-evaporation with MeOH. The syrup was purified on silica gel by flash chromatography with EtOAc then 4:1 EtOAc–(MeOH–H_2O 2:1) giving **8** (497 mg, 2.26 mmol) as an oil with 83% yield. $[\alpha]_D^{20}$ +7.7 (*c* 1, CH_3OH). ^1H NMR (D_2O, 400 MHz): δ 6.00–5.70 (m, 1H, H-2′), 5.20–5.10 (m, 2H, H-3′), 4.12–3.27 (m, 9H). ^{13}C NMR (D_2O, 100 MHz): δ 134.7–134.1 (allyl CH), 120.1–119.5 (allyl CH_2), 103.0 (C-2α-F), 102.8 (C-2β-F), 99.9 (C-2α-P), 96.8 (C-2β-P), 90.0, 89.9, 84.3, 84.2, 82.5, 77.4, 76.1, 75.7, 75.4, 73.1, 72.1, 72.0, 70.2, 70.1, 64.5, 63.8, 63.4, 62.7, 62.5 (2C), 62.2, 61.9, 61.8. MS (FAB(+), NBA): *m/z* 243 ($M^+ + Na$). Anal. calcd ($C_9H_{16}O_6$): C, 49.09; H, 7.32. Found: C, 49.0; H, 6.95.

4-*O*-Allyl-3-*O*-benzoyl-1,2-*O*-isopropylidene-β-D-fructopyranose (9) and 5-*O*-allyl-3-*O*-benzoyl-1,2-*O*-isopropylidene-β-D-fructopyranose (10). Same conditions as **1** but using 3-*O*-benzoyl-1,2-*O*-isopropylidene-fructopyranose[28] (1.625 g, 5 mmol) and allylmethylcarbonate (0.81 mL, 7.13 mmol). Compounds **9** (580 mg, 1.59 mmol) and **10** (710 mg, 1.95 mmol) were isolated as oils with 39% and 32% yields respectively. **9** $[\alpha]_D^{20}$ −113.5 (c 1.26, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 8.11–8.09 (2H, m, Ph-H), 7.61–7.57 (m, 1H, Ph-H), 7.49–7.45 (m, 2H, Ph-H), 5.79–5.69 (m, 1H, H-2′), 5.58 (d, 1H, $J_{3,4}$ 9.77 Hz, H-3), 5.21–5.13 (m, 1H, H-3′a), 5.12–5.04 (m, 1H, H-3′b), 4.14–4.09 (m, 2H, H-1′), 4.05–3.99 (m, 3H), 3.95–3.91 (m, 3H), 2.77 (s, 1H, OH), 1.51 (s, 3H, CH_3), 1.41 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$; 100 MHz): δ 165.9 (CO), 134.1–128.5 (PhCH, allyl CH), 117.7 (allyl CH_2), 111.9 (CMe_2), 104.7 (C-2), 76.5 (CH), 71.9 (CH_2), 71.2 (CH_2), 68.6 (CH), 67.3 (CH), 63.3 (CH_2), 26.6 (CH_3), 26.3 (CH_3). MS (FAB(+), NBA): *m/z* 365 ($M^+ + H$). Anal. calcd ($C_{19}H_{24}O_7$): C, 62.63; H, 6.64. Found: C, 62.70; H, 6.83. **10** $[\alpha]_D^{20}$ −141 (c 1.68, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 8.12–8.09 (m, 2H, Ph-H), 7.61–7.55 (m, 1H, Ph-H), 7.47–7.41 (m, 2H, Ph-H), 6.00–5.90 (m, 1H, H-2′), 5.43 (d, 1H, $J_{3,4}$ 10.07 Hz, H-3), 5.35–5.30 (m, 1H, H-3′a), 5.24–5.21 (m, 1H, H-3′b), 4.26–4.22 (m, 1H, H-1′a), 4.14–4.00 (m, 3H, H-1′b, H-4, H-1a), 3.96–3.88 (m, 3H, H-6, H-1b), 3.77 (d, 1H, $J_{5,6}$ 1.83 Hz, H-5), 2.56 (d, 1H, $J_{4,OH}$ 11.29 Hz, OH), 1.51 (s, 3H, CH_3), 1.45 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.7 (CO), 134.2–128.4 (Ph-CH), 117.9 (CH_2), 112.0 (CMe_2), 104.6 (C-2), 76.6 (CH), 71.9 (CH_2), 70.7 (CH_2), 70.6 (CH), 69.6 (CH), 60.7 (CH_2), 26.6 (CH_3), 26.3 (CH_3). MS (FAB(+), NBA): *m/z* 365 ($M^+ + H$). Anal. calcd ($C_{19}H_{24}O_7$): C, 62.63; H, 6.64. Found: C, 62.40; H, 6.65.

4-*O*-Allyl-1,2-*O*-isopropylidene-β-D-fructopyranose (11). Same conditions as **4**. Compound **9** (287 mg, 1.11 mmol) was isolated as an oil with 78% yield. $[\alpha]_D^{20}$ −137.5 (c 0.96, CH_3OH). ^1H NMR (CD_3OD, 400 MHz): δ 6.05–5.95 (m, 1H, H-2′), 5.35–5.29 (m, 1H, H-3′a), 5.17–5.13 (m, 1H, H-3′b), 4.24–4.19 (m, 1H, H-1′a), 4.15–4.09 (m, 2H, H-1′β, H-1a), 4.03–4.02 (m, 1H, H-5), 3.90–3.87 (m, 2H, H-4 and H-1b), 3.76 (d, 1H, $J_{6a,6b}$ 10.07, H-6a), 3.64 (dd, 1H, $J_{3,5}$ 2.14 Hz and $J_{3,4}$ 12.51 Hz, H-3), 3.52 (dd, 1H, $J_{5,6b}$ 3.35 Hz, H-6b), 1.45 (s, 3H, CH_3), 1.40 (s, 3H, CH_3). ^{13}C NMR (CD_3OD, 100 MHz): δ 136.6 (allyl CH), 117.3 (allyl CH_2), 113.1 (CMe_2), 107.7 (C-2), 79.8 (CH), 72.8 (CH_2), 71.7 (CH_2), 68.2 (CH), 67.9 (CH), 65.3 (CH_2), 27.3 (CH_3), 26.4 (CH_3). MS (FAB(+), NBA): *m/z* 261 ($M^+ + H$). Anal. calcd ($C_{12}H_{20}O_6$): C, 55.37; H, 7.74. Found: C, 55.25; H, 7.95.

4-*O*-Allyl-D-fructose (12). Same conditions as **6**. Compound **12** (206 mg, 0.936 mmol) was isolated as an oil with 90% yield. $[\alpha]_D^{20}$ −21.3 (c 0.61, CH_3OH). ^1H NMR (CD_3OD, 400 MHz): δ 6.04–5.85 (m, 1H, H-2′), 5.35–5.25 (m, 1H, H-3′a), 5.16–5.13 (m, 1H, H-3′b), 4.23–3.45 (m, 9H). ^{13}C NMR (CD_3OD, 100 MHz): δ 136.7, 136.2, 136.1, 117.4, 117.3, 117.0, 106.3 (C-2α-F), 103.5 (C-2β-F), 99.3 (C-2β-P), 84.8, 82.5, 79.3, 77.3, 72.2, 72.1, 71.8, 68.5, 68.2, 65.9, 64.6, 64.4, 64.3, 63.1, 62.9. MS (FAB(+), NBA): *m/z* 243 ($M^+ + Na$). Anal. calcd ($C_9H_{16}O_6$): C, 49.09; H, 7.32 > Found: C, 49.40; H, 7.21.

5-*O*-Allyl-3,4-di-*O*-benzoyl-1,2-*O*-isopropylidene-β-D-fructopyranose (13). Same conditions as **1**. Compound **13** (0.478 mg, 1.021 mmol) was isolated with 87% yield. $[\alpha]_D^{20}$ −177 (c 1.815, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz): δ 8.12–8.10 (m, 2H, Ph-H), 8.02–7.99 (m, 1H, Ph-H), 7.59–7.55 (m, 1H, Ph-H), 7.51–7.44 (m, 2H, Ph-H), 7.37–7.32 (m, 3H, Ph-H), 5.95 (d, 0.64H, $J_{3,4}$ 10.7 Hz, H-3), 5.87–5.77 (m, 0.64H, allyl H-2′), 5.73–5.58 (m, 1.72H, H-3, allyl H-2′ and H-4), 5.23–5.18 (m, 0.64H, H-3′a), 5.17–5.11 (m, 0.36H, H-3′a), 5.10–5.06 (m, 0.64H, H-3′), 5.03–5.00 (m, 0.36H, H-3′b), 4.19–3.90 (m, 7H, H-1, H-5, H-6 and H-1′), 1.53 (s, 3H, CH_3), 1.44 (s, 1.2H, CH_3), 1.43 (s, 1.8H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.1–165.9 (CO), 134.4–128.1 (PH-H), 117.5 (C-3′), 117.3 (C-3′), 112.1 (C-2′), 104.9 (C-2), 104.8 (C-2), 74.5 (CH), 74.3 (CH), 72.3 (CH), 72 (CH_2), 71.7 (CH_2), 70.8 (CH_2), 69.1 (CH), 68.9 (CH), 67.5 (CH), 67.1 (CH_2), 65.8 (CH_2), 62.5 (CH_2), 26.6 (CH_3), 26.5 (CH_3), 26.3 (CH_3), 26.1 (CH_3). MS (FAB(+), NBA): *m/z* 491($M^+ + Na$), 469 ($M^+ + H$). Anal. calcd ($C_{26}H_{27}O_8$): C, 66.66; H, 6.02. Found: C, 66.70; H, 6.15.

5-*O*-Allyl-1,2-*O*-isopropylidene-D-fructopyranose (14). Same conditions as **4**. Compound **14** (0.15 g, 0.577 mmol) was isolated as a white solid with 68% yield. $[\alpha]_D^{20}$ −150 (c 0.4, CH_3OH). ^1H NMR ($CDCl_3$, 400 MHz): δ 5.99–5.88 (m, 1H, H-2′), 5.35–5.30 (m, 1H, H-3a′), 5.23–5.19 (m, 1H, H-3b′), 4.34–4.29 (m, 1H, H-1a′), 4.21–4.17 (m, 1H, H-1b′), 4.1–3.94 (m, 4H); 3.78–3.74 (m, 1H), 3.59 (d, 1H), 2.89 (s, 2H, OH), 1.49 (s, 3H, CH_3), 1.42 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 134.3 (C-2′), 117.5 (C-3′), 111.9 (C-1), 105.5 (C-2), 76.3 (CH), 74.2 (CH_2), 71.6 (CH_2), 71.2 (CH), 69.7 (CH), 63.5 (CH_2), 26.9 (CH_3), 26.0 (CH_3). MS (FAB(+), NBA): *m/z* 259 ($M^+ - H$). Anal. calcd ($C_{12}H_{20}O_6$): C, 55.37; H, 7.74. Found: C, 55.50; H, 7.80.

5-*O*-Allyl-D-fructopyranose (15). Same conditions as **6**. Compound **15** (0.152 g, 0.69 mmol) was isolated as an oil with 93% yield. $[\alpha]_D^{20}$ −89 (c 1.28, CH_3OH). ^1H NMR (CD_3OD, 400 MHz): δ 6.02–5.91 (m, 1H, H-2′), 5.34–

5.28 (m, 1H, H-3′a), 5.17–5.13 (m, 1H, H-3′b), 4.19–4.08 (m, 2H, H-1′), 3.95–3.5 (m, 7H, H-1, H-3, H-4, H-5, H-6). ^{13}C NMR (CD_3OD, 100 MHz): δ 136.5 (allyl CH), 136.4 (allyl CH), 117.2 (allyl CH_2), 101.3 (C-2α), 99.0 (C-2β), 78.5 (CH), 78.3 (CH), 72.1 (CH_2), 72.0 (CH), 71.6 (CH), 71.4 (CH), 71.3 (CH), 70.2 (CH_2), 66.1 (CH_2), 63.0 (CH_2), 62.0 (CH_2), 61.4 (CH_2). MS (FAB(+), NBA): *m/z* 243 ($M^+ + Na$). Anal. calcd ($C_9H_{16}O_6$): C, 49.09; H, 7.32. Found: C, 48.7; H, 7.70.

Methyl 6-*O*-(diphenyl-(4-methoxyphenyl)-methyl)-α-D-fructofuranoside (16). To a cooled (4 °C) solution of methyl α-D-fructofuranoside[23] (1.0 g, 5.15 mmol) in 10 mL pyridine, 4-methoxyphenyldiphenylmethyl chloride (1.7 g, 5.5 mmol) was added and the solution was left overnight at 4 °C. After addition of water, extraction was performed (CH_2Cl_2), the organic layer washed with water, dried and concentrated with toluene. The residue was purified by column chromatography on silica gel (CH_2Cl_2–CH_3OH (95-5)) affording **16** (1.35 g, 56%) as a colorless glass. $[\alpha]_D^{20} + 51°$ (c 0.73, CH_2Cl_2). ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.0–7.2 (m, 12 H, C_6H_5 and H-Ar meta to OCH_3), 6.9 (d, *J* 8 Hz, 2 H, H-Ar ortho to OCH_3), 4.6–3.2 (m, 14 H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 158.2 (C *ipso* of -Ar-OCH_3), 143.7 (C *ipso* of -C_6H_5), 134.8 (C *para* of -Ar-OCH_3, 130.1 (C *para* of -C_6H_5), 128.1, 127.5 (C *ortho* and *meta* of -C_6H_5), 126.7 (C *ortho* of-Ar-OCH_3), 112.8 (C *ortho* of -Ar-OCH_3), 108.2 (C-2), 86.6 (C(Ar)3), 82.6, 80.6, 78.2 (C-3, -4, -5), 63.5, 58.5 (C-1, -6), 54,7 ($ArOCH_3$), 48.2 (OCH_3). Anal. calcd ($C_{27}H_{30}O_7$): C, 69.51; H, 6.48. Found: C, 69.53; H, 6.50.

Methyl 1,3,4-tri-*O*-acetyl-α-D-fructofuranoside (17). To a stirred solution of **16** (1.05 g, 2.25 mmol) in pyridine (10 mL) at 4 °C, acetic anhydride (4 mL) was added in portions and the reaction mixture stirred at room temperature for 24 h. Ice was then added and the reaction mixture was extracted with CH_2Cl_2. The organic layer was washed with water, dried and concentrated with toluene in vacuo. The residue was added to acetic acid (5 mL). Water was added in portions until turbidity and then the solution was stirred at room temperature for 6 h. Solvent was removed with toluene before being purified by column chromatography on silica gel (CH_2Cl_2/CH_3OH (95:5)) affording **17** (695 mg, 96%) as a colorless oil. $[\alpha]_D^{20}$ +81 (c 1.2, AcOEt). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.3 (d, 1 H, *J* 2 Hz, H-3), 4.95 (dd, 1 H, *J* 2 Hz and *J* 5 Hz, H-4), 4.1 and 4.1 (AB system, 2 H, *J* 12 Hz, H-1), 4.0 (m, 1 H, H-5), 3.8 and 3.7 (AB part of an ABMX system *J* 12 Hz, 2 H, H-6), 3.28 (s, 3 H, OCH_3), 2.06, 2.04 and 2.01 (3 s, 9 H, $COCH_3$).^{13}C NMR (75 MHz, $CDCl_3$): δ 170.2, 169.9, 168.9 (CO), 108.2 (C-2), 82.6, 79.7, 78.0 (C-3, -4, -5), 61.5, 58.2 (C-1, -6), 48.3 (OCH_3), 20.3 ($COCH_3$). Anal. calcd ($C_{13}H_{22}O_9$): C, 48.44; H, 6.88. Found: C, 48.42; H, 6.89.

Methyl 6-*O*-allyl-α-D-fructofuranoside (18). To a stirred solution of **17** (580 mg, 1.81 mmol) under argon in dry THF (12 mL) was added a solution of tris-(dibenzylideneacetone)-dipalladium(0) (30 mg) and 1,4-bis-(diphenylphosphino)butane (105 mg) in dry THF (10 mL). Allyl ethyl carbonate (1.1 g, 8.46 mmol) was then added and the solution was stirred at 50 °C overnight. After cooling and evaporation of the solvent, column chromatography of the residue (toluene:AcOEt (4:1)), afforded methyl 6-*O*-allyl-1,3,4-tri-*O*-acetyl-α-D-fructofuranoside (390 mg) and unreacted **17** (155 mg). Methyl 6-O-allyl-1,3,4-tri-*O*-acetyl-α-D-fructofuranoside was then dissolved in dry methanol (10 mL) and sodium methoxide (250 μL of a 1.5 M in methanol) was then added. After stirring overnight, water (10 mL) was added and the solution concentrated to one third under reduced pressure. The pH was adjusted to 6–7 (0.1 N HCl) and the solution washed with ethyl acetate. The organic layer was extracted with water and the combined aqueous layers evaporated to dryness. The residue was taken up in acetone and after filtration, evaporation yielded 209 mg (50%) of **18** as a colorless oil. $[\alpha]_D^{20}$ +80 (c 0.48, CH_3OH). ^{1}H NMR (300 MHz, $(CD_3)_2CO$); δ 5.9 (m, 1 H, H-2′), 5.1 and 5.25 (m, 2 H, H-3′), 3.5–4.2 (m, 12 H), 3.27 (s, 3 H, OCH_3).^{13}C NMR (75 MHz, $(CD_3)_2CO$); δ 135.7 (C-2′), 116.6 (C-3′), 108.8 (C-2), 82.2, 81.5, 79.0 (C-3, -4, -5), 58.2, 72.5, 70.9 (C-1′, C-1, -6), 48.7 (OCH_3). HRFABMS (NBA) calcd for $C_{10}H_{18}O_6Na$: 257.1001 ($M^+ + Na$). Found: 257.1000.

6-*O*-Allyl-D-fructose (19). To a solution of **18** (105 mg, 0.45 mmol) in 1,4-dioxane (1 mL) was added 0.5 M hydrochloric acid (1 mL) and the solution was stirred overnight at 60 °C. The solvent was removed and the residue co-evaporated twice with water before adjusting the pH to 7 with Amberlite IRA-93 (OH^-). After filtration, removal of water (evaporation or lyophilisation) oily **19** was isolated (79 mg, 80%). $[\alpha]_D^{20}$ +13° (10 min)→ +18° (15 h) (c 0.28, CH_3OH), ^{1}H NMR (300 MHz, (D_2O), δ 6.1 (m, 1 H, H-2′), 5.6–5.4 (m, 2 H, H-3′), 3.6–4.4 (m, 9 H). ^{13}C NMR (75 MHz, D_2O): δ 136.9 (C-2′), 121.1 (C-3′), 107.3 (C-2β), 104.3 (C-2α), 84.4, 82.7, 79.4 (C-3β, -4β, -5β), 81.7, 78.0, 77.6 (C-3α,-4α,-5α), 74.7, 73.7, 72.3, 65.5, 65.4 (C-6, C-1 and C-1′). HRFABMS (NBA) calcd for $C_9H_{16}O_6Na$: 243.0845 ($M^+ + Na$). Found: 243.0847.

Fructose transport assays. CHO cells expressing GLUT5[39] were grown to confluence in 19 mm culture dishes. Growth medium was removed by washing 3 times with KRPB (Krebs-Ringer Phosphate buffer; 130 mM NaCl, 5 mM KCl, 1.3 mM $CaCl_2$, 1.3 mM $MgSO_4$ and 10 mM Na_2HPO_4, pH 7.4). Cells were then maintained in this buffer at 37 °C for 10 min. Uptake was initiated by adding D-[^{14}C-U]-fructose to give a final concentration of 1 mM, 0.3 μCi in 0.25 mL. After 10 min at 37 °C, 1 mL of ice cold buffer containing 0.3 mM phloretin was added to terminate the reaction. The dishes were washed rapidly 3 times and then cell radioactivity was extracted into 0.5 mL of 0.1 M NaOH and estimated by scintillation counting.

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References

1. Wright, E. M. (special issue). *Am. J. Physiol.* **1998**, *38,* G879-G882.
2. Kayano, T.; Burant, C. F.; Fukumoto, H.; Gould, G. W.; Fan, Y. S.; Eddy, R. L.; Byers, M. G.; Shows, T. B.; Seino, S.; Bell, G. I. *J. Biol. Chem.* **1990**, *265*, 13276.
3. Rand, E. B.; Depaoli, A. M.; Davidson, N. O.; Bell, G. I.; Burant, C. F. *Am. J. Physiol.* **1993**, *264,* G1169-G1176.
4. Hundal, H. S.; Ahmed, A.; Guma, A.; Mitsumoto, Y.; Marette, A.; Rennie, M. J.; Klip, A. *Biochem. J.* **1992**, *286*, 339.
5. Mueckler, M. *Eur. J. Biochem.* **1994**, *219*, 713.
6. Gould, G. W.; Holman, G. D. *Biochem. J.* **1993**, *295*, 329.
7. Hajduch, E.; Darakhshan, F.; Hundal, H. S. *Diabetologia* **1998**, *41*, 821.
8. Zierath, J. R.; Nolte, L. A.; Wahlstrom, E.; Galuska, D.; Shepherd, P. R.; Kahn, B. B.; Wallberg-Henriksson, H. *Biochem. J.* **1995**, 517.
9. Fischer, E. *Ber. Dtsch. Chem. Ges* **1884**, *17*, 574.
10. Lichtenthaler, F. W. *Carbohydr. Res.* **1998**, *313*, 69.
11. Hudson, C. S.; Brauns, D. H. *J. Am. Chem. Soc.* **1915**, *37*, 2736.
12. Pascu, E.; Rich, F. V. *J. Am. Chem. Soc.* **1933**, *55*, 3018.
13. Lichtenthaler, F. W.; Klotz, J.; Flath, F. J. *Liebigs Annalen* **1995**, 2069.
14. Mio, S.; Ueda, M.; Hamura, M.; Kitagawa, J.; Sugai, S. *Tetrahedron* **1991**, *47*, 2145.
15. Brady, R. F. J. *Carbohydr. Res.* **1970**, *15*, 35.
16. Van Cleve, J. W. *Methods Carbohydr. Chem.* **1963**, *2*, 237.
17. Brauns, D. H. *J. Am. Chem. Soc* **1920**, 1850.
18. Fellahi, M.; Morin, C. *Carbohydr. Res.* **1999**, *322*, 142.
19. Horton, D.; Philips, K. *Carbohydr. Res.* **1973**, *30*, 367.
20. Szarek, W. A.; Zamojski, A.; Tiwari, K. N.; Ison, E. R. *Tetrahedron Lett.* **1986**, *27*, 3827.
21. Lee, C. K. *Carbohydr. Res.* **1987**, *170*, 255.
22. Johnson, L.; Verraest, D. L.; Vanhaveren, J.; Hakala, K.; Peters, J. A.; Vanbekkum, H. *Tetrahedron:Asymmetry* **1994**, *5*, 2475.
23. Furneaux, R. H.; Tyler, P. C.; Whitehouse, L. A. *Tetrahedron Lett.* **1993**, *34*, 3613.
24. Lakhmiri, R.; Lhoste, P.; Sinou, D. *Tetrahedron Lett.* **1989**, *30*, 4669.
25. Binder, T. P.; Robyt, J. F. *Carbohydr. Res.* **1984**, *132*, 173.
26. Diaz, R. R.; Melgarejo, C. R.; Lopezespinosa, M. T. P.; Cubero, I. I. *J. Org. Chem.* **1994**, *59*, 7928.
27. Szeja, W. *Pol. J.Chem.* **1980**, *54*, 1301.
28. Fischer, E.; Noth, H. *Ber. Dtsch. Chem. Ges.* **1918**, *51*, 321.
29. Lakhmiri, R.; Lhoste, P.; Sinou, D. *Synth. Commun.* **1990**, *20*, 1551.
30. Barnett, J. E. G.; Holman, G. D.; Munday, K. A. *Biochem. J.* **1973**, *131*, 211.
31. Rees, W. D.; Holman, G. D. *Biochim. Biophys. Acta.* **1981**, *642*, 251.
32. Barnett, J. E. G.; Holman, G. D.; Chalkley, R. A.; Munday, K. A. *Biochem. J.* **1975**, *145*, 417.
33. Holman, G. D.; Rees, W. D. *Biochim. Biophys. Acta.* **1982**, *685*, 78.
34. Abbadi, M.; Holman, G. D.; Morin, C.; Rees, W. D.; Yang, J. *Tetrahedron Lett.* **1999**, *40*, 5861.
35. Holman, G. D.; Midgley, P. J. W. *Carbohydr. Res.* **1985**, *135*, 337.
36. Clark, A. E.; Holman, G. D. *Biochem. J.* **1990**, *269*, 615.
37. Yang, J.; Holman, G. D. *J. Biol. Chem.* **1993**, *268*, 4600.
38. Koumanov, F.; Yang, J.; Jones, A. E.; Hatanaka, Y.; Holman, G. D. *Biochem. J.* **1998**, *330*, 1209.
39. Inukai, K.; Katagiri, H.; Takata, K.; Asano, T.; Anai, M.; Ishihara, H.; Nakazaki, M.; Kikuchi, M.; Yazaki, Y.; Oka, Y. *Endocrinology* **1995**, *136*, 4850.
40. Angyal, S. J.; Bethell, G. S. *Aust. J. Chem.* **1976**, *29*, 1249.
41. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
42. Morin, C. *J. Chem. Educ.* **1988**, *65*, 903.
43. Raaijmakers, H. W. C.; Arnouts, E. G.; Zwanenburg, B.; Chittenden, G. J. F. *Carbohydr. Res.* **1994**, *257*, 293.