Supporting information

Novel D-xylose derivatives stimulate muscle glucose uptake by activating AMP-activated protein kinase α

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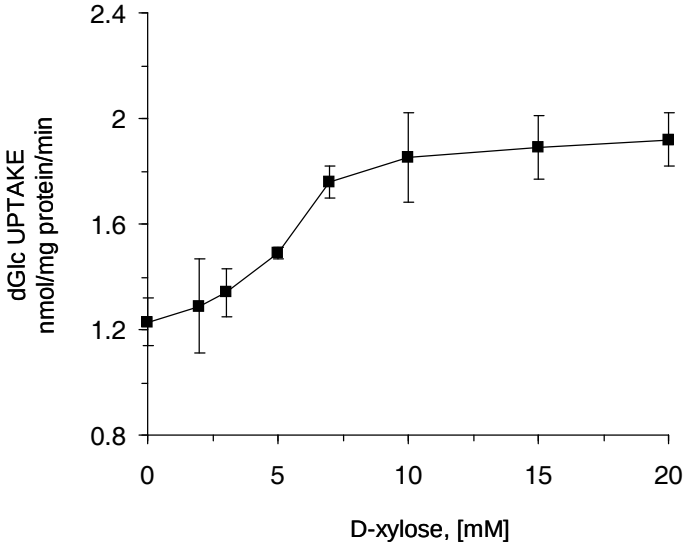
Supplemental Table 1: Elemental analyses and purity of main compounds.

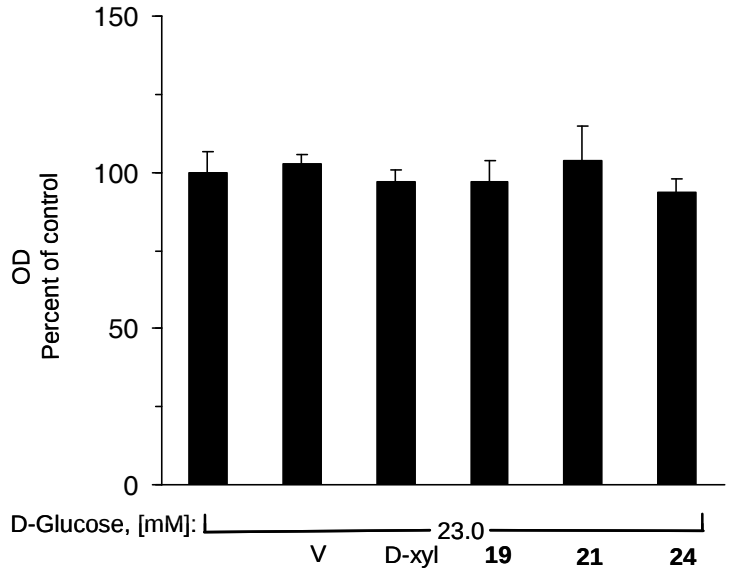
Comp.	Formula	MW	Calculated (%)			Found (%)			Purity (%)
			C	H	S	C	H	S	
1	$C_6H_{12}O_5$	164.07	43.90	7.37	-	42.59	6.92	-	95.46
2	$C_6H_{12}O_5$	164.07	43.90	7.37	-	42.89	6.97	-	96.14
3	$C_6H_{12}O_5$	164.07	43.90	7.37	-	43.75	7.17	-	98.47
4	$C_6H_{12}O_5$	164.07	43.90	7.37	-	43.59	7.87	-	96.96
7	$C_6H_{12}O_5$	164.07	43.90	7.37	-	43.38	7.21	=	98.33
16	$C_7H_{14}O_5$	178.18	43.18	7.92	-	46.99	7.77	-	96.54
17	$C_8H_{16}O_5$	192.21	49.19	8.72	-	49.31	8.31	-	97.77
18	$C_9H_{18}O_5$	206.12	51.02	7.92	-	52.11	8.20	-	97.17
19	$C_{23}H_{28}O_4S_2$	432.60	63.86	6.52	14.82	63.21	6.37	14.34	97.81
21	$C_{16}H_{24}O_4\ S_2$	344.49	55.62	7.28	18.52	52.21	7.37	18.14	97.68
24	$C_{17}H_{26}O_4\ S_2$	358.52	56.92	7.31	17.89	56.94	7.18	17.56	98.8
25	$C_{18}H_{28}O_4\ S_2$	372.54	58.03	7.58	17.21	58.14	7.08	17.36	98.15

Supplemental Figure 1. Dose response analysis of D-xylose-induced stimulation of hexose uptake in L6 myotubes.



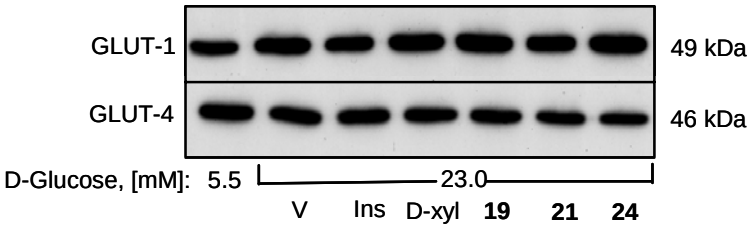
Myotube cultures, which were incubated with increasing concentrations of D-xylose for 6 h, were taken for the standard [^{3}H]dGlc uptake assay.

Supplemental Figure 2. Viability assay.



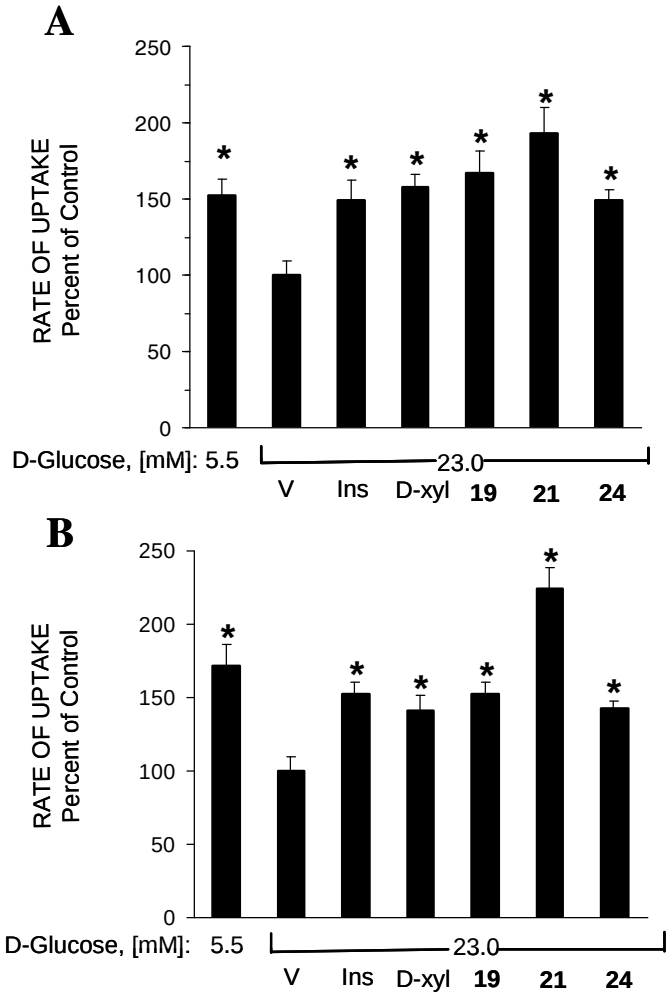
L6 myotubes were exposed to 20 mM D-xylose (7 h), 5 μM of **19** (18 h), 150 μM of **21** (7 h) or 50 μM of **24** (7 h). At the end of incubation the cultures were taken for the MTT viability assay, as described in the *Experimental section*..

Supplemental Figure 3. Lack of effect of **19**, **21** and **24** on total cell content of GLUT1 and GLUT4 in L6 myotubes.



L6 myotubes were treated as described in the legend to Figure 5. At the end of incubation the cultures were lysed and taken for the Western blots analysis of GLUT1 and GLUT4, as described in the *Experimental section.*

Supplemental Figure 4. Effects of D-xylose, **19**, **21** and **24** on the rate of hexose uptake in L6 myotubes under the conditions of the OPD assay



L6 myotubes expressing GLUT1myc (Panel A) or L6 GLUT4myc (Panel B) were preincubated in 5.5 mM or 23.0 mM glucose for 24 h. Three h before the assay the medium was changed to a serum free medium supplemented with 0.5% (w/v) BSA. The myotubes were then incubated 30 min with insulin [200 nmol/l] (Ins), 5 hours with 20 mM of D-xylose (D-xyl), 18 hours with 5 μM of **19**, 7 h with 150 μM of **21** and 50 μM of **24**. Control myotubes were incubated with vehicle DMSO (V) for 18 h. The cultures were then taken for the standard uptake assay. Results are expressed as percent of control: The 100% values for GLUT1myc- and GLUT4myc myotubes were 2.24 ± 0.44 and 1.19 ± 0.05 nmol dGlc/mg/protein/min *p<0.05 in comparison with V.

Non active derivatives: general comments and synthesis

The glycoside derivatives 1-O-tetrahydropyranyl-α-D-xylopyranoside (**28**) and isobutylcarbamate-O-α-D-xylopyranoside (**29**) (Supplemental Scheme 1), 1-benzyl-O-α-D-xylopyranoside (**30**) and 1-piperidine-N-β-D-xylopyranoside (**31**) (Supplemental Chart 1) were synthesized to determine whether a non methylether substitution in position 1 of D-xylose results in more potent derivates than **1**. Compound **1** was used as a starting material for the synthesis of **28** and **29** via a common synthetic intermediate 2,3,4-tri-O-benzyl-α-xylopyranose (**27**), which was synthesized according to a known procedure using KOH/bromotoluene treatment of methyl-O-D-glycosides, followed by demethylation by acid hydrolysis[1]. After recrystalisation from diethyl ether/isopropyl ether (3:7) the **27** was obtained. It was reacted with dihydropyran in dry dichloromethane and catalytic amount of PPTS[2]. The latter was prepared by reaction between *p*-toluenesulfonic acid monohydrate and pyridine. Compound **28** was obtained by debenzylation with H_2/Pd on active carbon treatment.

Compound **29** was synthesized by coupling isobutylchloroformate and **27** in pyridine and THF[3]. The resulting glycoside was debenzylated and **29** was obtained. A one-step synthesis to obtain benzyl-O-α-D-xylopyranoside (**30**) from D-xylose was performed by stirring D-xylose in benzylalcohol saturated with HCl gas, as described

by Deng et al.[4] (95% yield). The N-glycoside derivative of D-xylose (1-piperidine-N-β-D-xylopyranoside, **31**) was synthesized according to the general synthetic procedure of 1-piperidine-N-β-D-ribopyranoside, as described by Chavis et al.[5] These hydrophilic glycoside derivatives of D-xylose failed to increase glucose uptake in L6 myotubes (Supplemental table 2) probably due to poor cell permeability.

Therefore more lipophilic tri- and tetra esters derivatives of D-xylose were planned and synthesized. 1,2,3,4-O-tetra acetate-β-D-xylopyranoside (**32**), 1-O-methyl-2,3,4-O-tri-benzoate-β-D-xylopyranoside (**33**) and 1-O-methyl-2,3,4-O-pivaloyl-β-D-xylopyranoside (**34**) were synthesized according to published synthetic procedures (Supplemental Chart 1): **32** was obtained from the interaction between acetic anhydride/sodium acetate and D-xylose[6]; **33** and **34** were synthesized via coupling reaction between benzoyl or pivaloyl chloride, respectively, and the commercially available 1-O-β-methyl-D-xylopyranoside in dry pyridine, as described by Petrovic et al.[7] for **34** and in *Supplemental Synthetic Procedures* for **33**. Yet, these compounds also failed to stimulate hexose uptake in L6 myotubes (Supplementary table 2). We assumed that steric hindrances in the molecules prevent hydrolysis of the ester bonds to release D-xylose.

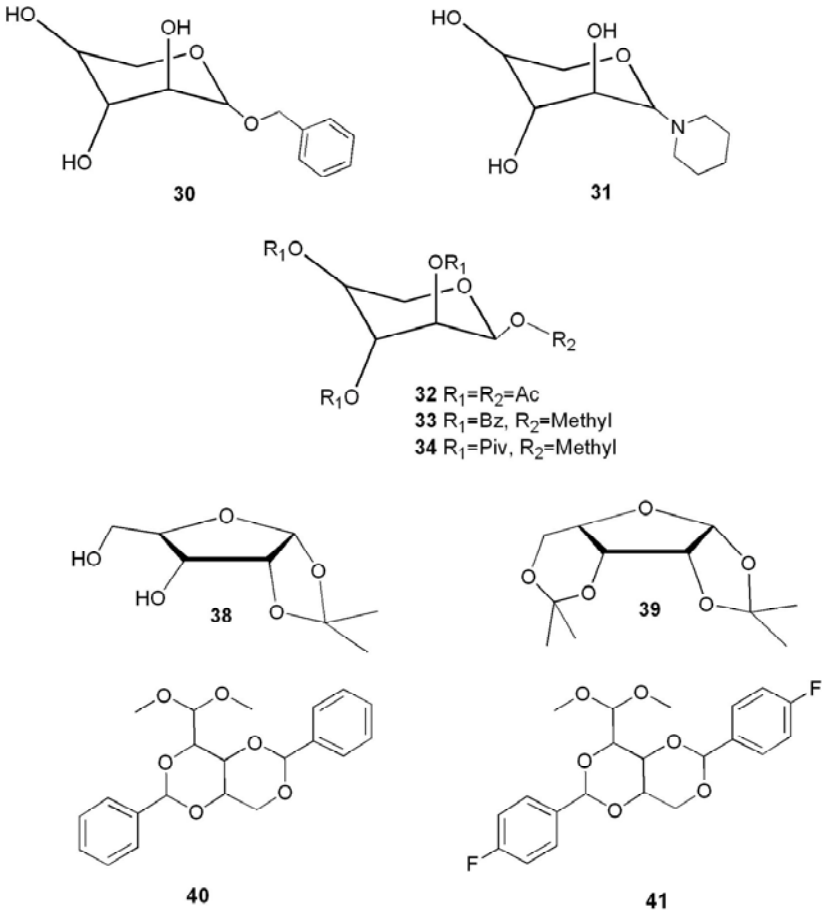
In addition, we asked whether a thioglycoside substitution would produced a more potent derivative that the methyl glycoside derivative (**1**). **32** was used as a starting material for the synthesis of ethyl-thio-β-D-xylopyranoside (**35**), propyl-thio-β-D-xylopyranoside (**36**) and dodecanoyl-thio-β-D-xylopyranoside (**37**) (Supplemental Scheme 2). The synthetic procedure for the preparation of acetylated glucose thioglycosides was adopted for the preparation of acetylated xylothioglycosides[8]. Briefly, **32** in dry dichloromethane was reacted with the appropriate thiol in the presence of $FeCl_3$ as a catalyst, followed by deacetylation with

sodium methoxide in methanol and purification by column chromatography. When tested in the hexose uptake-screening assay these derivatives were found inactive (Supplementary table 2).

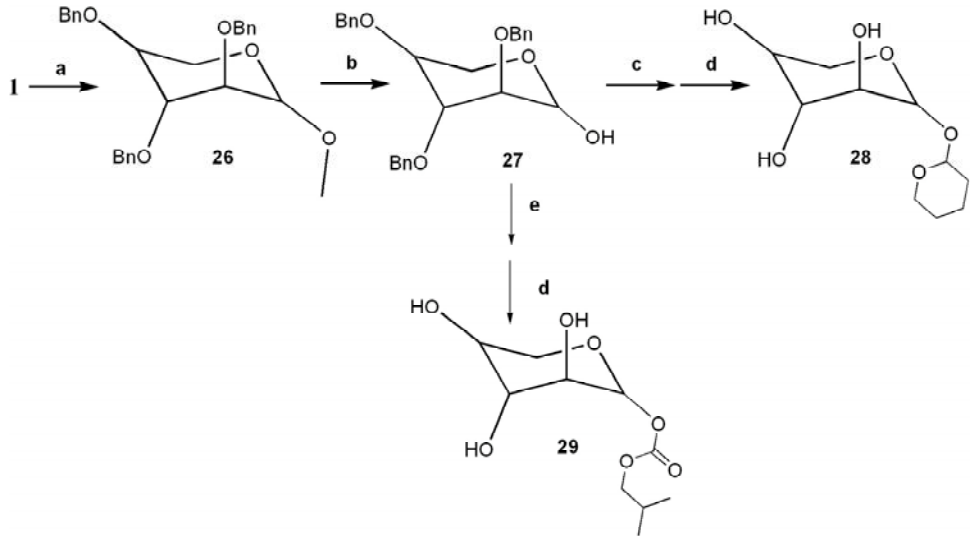
Another class of D-xylose derivatives was prepared by introducing acetal or ketal moieties into the pentose structure as described by others (Supplementary Chart 1): 1,2-O-isopropyledene-α-D-xylofuranose (**38**)[9], 1,2:3,5-O-diisopropyledene-α-D-xylofuranose (**39**)[10], 2,4:3:5-Di-O-benzyledene-α-D-xylose-dimethylacetal (**40**)[11] and 2,4:3:5-Di-O-*p*-fluorobenzyledene-α-D-xylose-dimethylacetal (**41**)[11]. Theoretically, these derivatives might loose (spontaneously or emzimaticly) the acetal/ketal moiety to release D-xylose or D-xylose dimethyl ether introcelulary. However, none of these compounds was found active in the screening assay. (Supplementary table 2).

Finally, to improve the cell permeability of **1** coupled it to methyl PEG to obtain Di-4-O-(1-O-methyl-α-D-xylopyranoside)-phosphoryl-O-methylpolyethelene glycol (**42**). Methyl PEG and imidazole (1:2 molar reatio) reacted with phosphorus trichloride. The imidazole moiety was replaced by tetrazol followed by treatment of the intermediate complex with a large excess of **5.** This was followed with oxidazion of the phosphite to phosphate[12] and deprotection with iodine[13] to give **42** (Supplementary Scheme 3). This PEG derivative of D-xylose also failed to stimulate hexose uptake in L6 myotubes (Supplementary table 2).

Supplemental Chart 1. Structures of non-active D-xylose derivatives

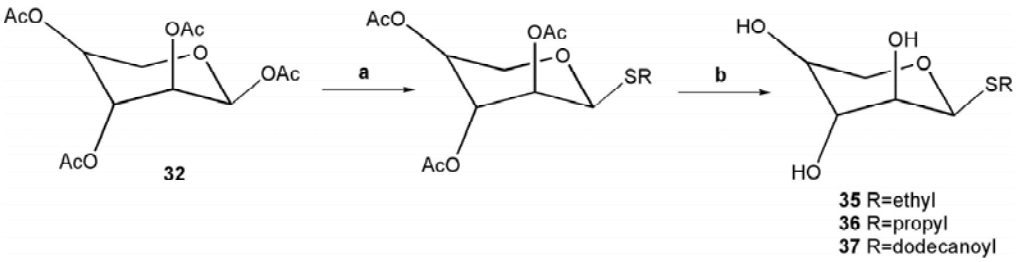


Supplemental Scheme 1. Synthesis of **28** and **29**



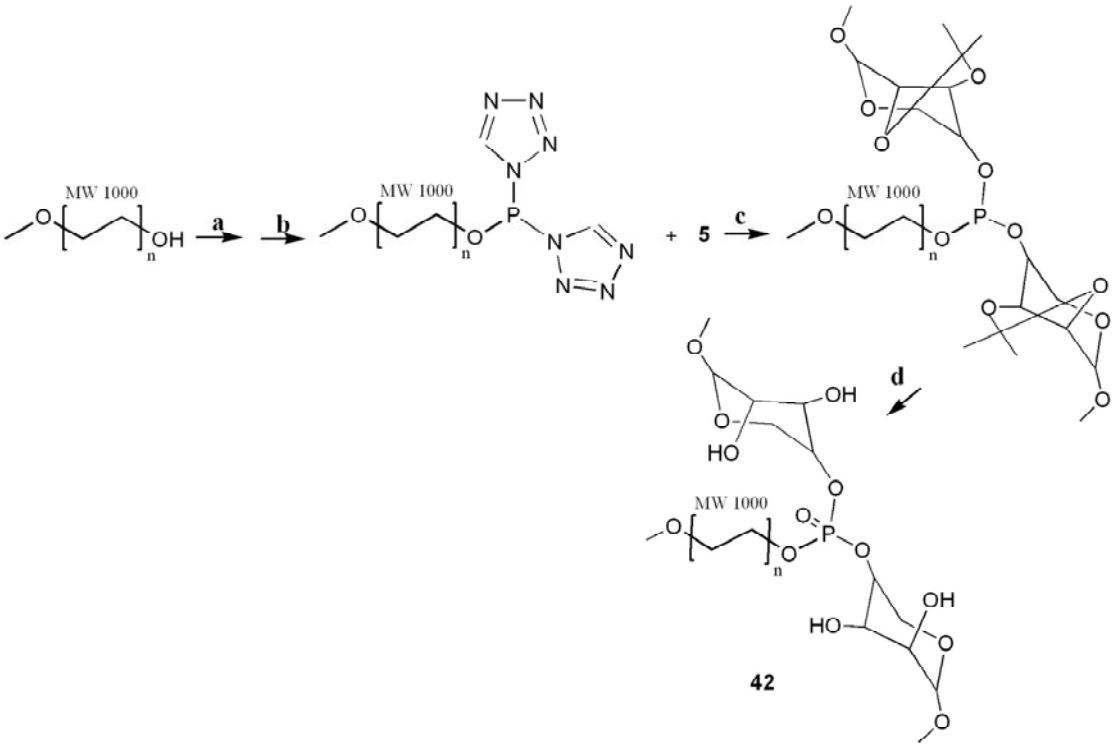
Reagents and conditions: (a) Benzyl Bromide, KOH, reflux, THF, 15 h. (b) Acetic acid, 2M HCl, 15 h, 80° C. (c) PPTS, dihydropyrane, dry chloroform, 6 h, room temperature. (d) H2/10% Pd on activated carbon, 60 atm., ethanol, 16 h. (e) isobutylchloroformate in dry THF, pyridine, 4° C, 3 h.

Supplemental Scheme 2. Synthesis of thioxyloglycosides



Reagents and conditions: (a) RSH, $FeCl_3$, molecular seave 3 Å, dichloromethane, 3 h, (b) Sodium methoxide, methanol, room temperature, 40 min.

Supplemental Scheme 3. Synthesis of PEG derivative of **1**



Reagents and conditions: (a) PCl_3, Imidazole 2 eq., dry dichloromethane, inert atmosphere, room temperature, 1 h. (b) Tetrazole 2 eq., 5° C, 2 h, inert atmosphere, (c) dry pyridine/dichloromethane, 7 days. (d) I_2, methanol/H_2O, 16 h.

Supplemental synthetic procedures

1-O-Tetrahydropyranyl-α-D-xylopyranoside (**28**)

One gram of **27**[1], 1.5 mL of dihydropyran and 0.4 g of pyridinium-*p*-toluenesulfanate[2] were dissolved in 25 mL of dry chloroform and placed at room temperature for 6 h. Excess of dihydropyran and chloroform were evaporated under vacuum and the residue was filtered through a silica column (petroleum ether:ethyl acetate (40:60). The organic solvents were evaporated and remaining yellow syrup was dissolved in 25 ml of ethanol followed by the addition of 1 g of 10% Pd/activated carbon. The mixture was placed in a hydrogenation apparatus for 16 h (pressure of H_2 was 60 atm). Then solution was then filtered and the ethanol evaporated under

vacuum. A preparative HPLC acetonitrile:water (increasing linear acetonitrile gradient) gave pure **28** was obtained as a colorless syrup. Yield=9.0%, Anal. $C_{10}H_{18}O_6$: C, 51.28; H, 7.74. Found: C, 51.02; H, 7.63, ^{1}H NMR (DMSO) δ, ppm, 1.54 (m, 4H, [C-H$_2$ 3,4 tetrahydropyran]), 1.78 (m, 2H, [C-H$_2$ 2 tetrahydropyran]), 3.29 (d, 1H, [C-*e*H5]), 3.49 (q, 1H, [C-H3]), 3.68 (m, 2H, [C-H$_2$ 5 tetrahydropyran]), 3.73 (d, 1H, [C-*a*H5]), 3.87 (m, 1H, [C-H4]), 3.97 (m, 1H, [C-H2]), 4.9 (t, 1H, [C-H1 tetrahydropyran]), (α) 5.6 (d, 1H, [C-H1])

Isobutylcarbamate-O-α-xylopyranoside (**29**)

Isobutyl chloroformate (0.62 mL) in dry THF (2 mL) was added to an ice-cold stirred solution of 1.0 g of **27** in 15 mL of dry THF and 0.4 ml of dry pyridine[3]. The reaction mixture was slowly warmed to room temperature, and incubated for 3 hours. Following filtration, the solvent was removed under reduced pressure. Dichloromethane (50 mL) was then added and the organic phase was washed 3 times with water. The organic layer was separated and dried over anhydrous magnesium sulfate, filtered and distilled to give yellow syrup. It was dissolve in 25 mL of ethanol, followed by the addition of 1 g of 10% Pd/activated carbon. The mixture was then placed in a hydrogenation apparatus for 16 h (60 atm), followed by filtration. The ethanol was evaporated under vacuum, and silica column chromatography (diethyl ether/methanol, 40:60) produced pure **29** as colorless syrup. Yield 11%. Anal. $C_{10}H_{18}O_7$: C, 47.99; H, 7.25. Found: C, 45.91; H, 7.27, ^{1}H NMR (DMSO) δ, ppm, 0.86 (d, 6H, [C-H$_3$]), 1.03 (m, 1H, [CH$_3$-C-H-CH$_3$]), 3.21 (q, 1H, [C-H3]), 3.52 (d, 2H, [C-H5]), 3.94 (t, 1 H, [C-H4]), 4.19 (d, 2H, [O-CH$_2$-]), 5.01 (q, 1H, [C-H2]), (α), 5.31 (d, 1H, [C-H1]).

1-Benzyl-O-α-D-xylopyranoside (**30**)

This compound was synthesized according to Deng et al.[4] with minor modifications. D-xylose (10 g) was gradually added to benzyl alcohol (30 mL) that had been saturated with HCl gas. Following mixing at room temperature for 16 h. Cold saturated water solution of bicarbonate was used carefully for neutralization of HCl, then the solution was filtered and water with benzyl alcohol were distillated out by high vacuum pressure. Resulting syrup was resolved in ethanol, filtered and ethanol was evaporated. Despite many attempts to crystallize **30** the compound was obtained as colorless syrup, which was purified by silica gel chromatography (ethanol: cyclohexane, 50:50). Yield=45%, Anal. $C_{12}H_{16}O_5$: C, 59.99; H, 6.71. Found: C, 59.81; H, 6.73, ^{1}H NMR (DMSO) δ, ppm, 3.21 (d, 2H, [C-*e*H5]), 3.42 (q, 1H, [C-H3]), 3.9 (d, 2H, [C-*a*H5]), 4.45 (s, 2H, [-C-H_2-]), 4.52 (m, 1H, [C-H4]), 4.57 (m, 1H, [C-H2]), (α), 4.97 (d, 1H, [C-H1]), 7.1-7.3 (m, 5 H, [Bz]).

Piperidine-N-β-D-xylopyranoside (**31**)

D-xylose (5 g) was dried following its suspended in toluene, which was then evaporated to dryness under vacuum. This procedure was repeated three times. The dried D-xylose was added to 5 mL of dry methanol, stoichiometric volume of piperidine (3.3 mL) in the presence of freshly dried 3Å molecular sieve[5]. The reaction was left under nitrogen atmosphere for three days at room temperature. The resulting syrup was purified by silica chromatography using methanol as an eluent. Recrystallization attempts of the glycoside from cold methanol failed due to the instability of the compound. Yield (syrup)=4.0%. Anal. $C_{10}H_{19}$ NO_4: C, 55.29; H, 8.81; N, 6.46. Found: C, 55.81; H, 8.79, N, 5.87. ^{1}H NMR (DMSO) δ, ppm, 1.51 (m,

6H, [-C-$\underline{H}_2$-]), 2.27 (t, 4H, [N-C-$\underline{H}_2$-]), 3.21 (q, 1H, [C-$\underline{H}$3]), 3.52 (d, 2$\underline{H}$, [H5]), 3.94 (t, 1H, [$\underline{H}$4]), 5.01 (q, 1H, [$\underline{H}$2]), (β), 4.81 (d, 1H, [$\underline{H}$1]).

1,2,3,4-O-tetraacetyl-β-D-xylopyranoside (**32**)

The compound was produced according to general procedure described by Wolfrom and Thompson[6]. D-xylose (9 g) was added gradually over 30 min to a solution of 5 g sodium acetate (5 g) in 70 mL of boiling acetic anhydride. The solution was cooled down to 30° C and 200 mL of water were carefully added. The solution was then left stirring at room temperature for 3 hours. The resulting white crystalloid mass was filtered, washed in cold water and petroleum ether and recrystallized from ethanol. The crystals were washed with cold pentane and traces of organic solvents were evaporated in a vacuum desiccator for 16 h and **32** was obtained. Yield=81%, m.p.=124°-125° C. Anal. $C_{13}H_{18}O_9$: C, 49.09; H, 5.69. Found: C, 50.81; H, 5.86, ^{1}H NMR ($CDCl_3$) δ, ppm, 1.98 (s, 9H [C-$\underline{H}_3$]), 2.04 (s, 3H [glycoside position C-$\underline{H}_3$]), 3.29 (d, 1H, [C-*a*$\underline{H}$5]), 3.69 (m, 1H, [C-$\underline{H}$4]), 3.95 (d, 1H, [C-e$\underline{H}$5]), 4.55 (q, 1H, [C-$\underline{H}$3]), 4.85 (q, 1H, [C-$\underline{H}$2]), (β), 5.18 (d, 1H, [C-$\underline{H}$1])

1-O-methyl-2,3,4-tribenzoyl-β-D-xylopyranoside (**33**)

The compound was synthesized according to procedure of Hewitt and Fletcher anomeric D-arabinopyranose tetrabenzoates[14] with some modifications. Briefly, to an ice-cold mixture of 5 g commercially available 1-O-methyl-β-D-xylopyranoside in 38 mL dry pyridine/dichloromethane (1:1) 15 ml of benzoyl chloride were gradually added over 30 min. The solution was maintained stirring in an ice bath for 1 h and then at room temperature for 24 h. The reaction mixture was then cooled to 4ºC and quenched by careful addition of 100 mL of cold water followed by addition of 100

mL of chloroform. Following phase separation, the organic phase was washed three times with water and dried with anhydrous magnesium sulfate and filtered out. Traces of chloroform and pyridine were evaporated and the remaining amorphic solid residue was crystallized from hot ethanol to obtain white crystals. Yield=43%, m.p; 93-96° C. Anal. $C_{27}H_{24}O_8$: C, 68.06.29; H, 5.08. Found: C, 65.31; H, 5.32, ^{1}H NMR ($CDCl_3$) δ, ppm, 3.3 (q, 1H, [C-H3]), 3.56 (s, 3H, [C-H_3]), 4.1 (d, 2H, [C-H5]), 4.4 (t, 1H, [C-H4]), 4.7 (q, 1 H, [C-H2]), (β), 5.2 (d, 1 H, [C-H1]), 7.2-7.4 (m, 15 H, [Bz]).

1-S-ethyl-β-D-xylopyranoside (**35**), **1-S-propyl-β-D-xylopyranoside** (**36**) and **1-S-dodecanoyl-β-D-xylopyranoside** (**37**). All three compounds were synthesized according to the method of Disgupta and Garegg[8,] using **32** rather than β-D-glucopyranose pentaacetate as a starting material. Briefly, a solution of 1 g of **32** in 5 mL of dichloromethane was stirred for 90 min at room temperature with 1 g of freshly dried molecular sieves 4Å. The solution was then cooled to 4ºC and anhydrous $FeCl_3$ (1.2 mol. equiv.) was added. When the $FeCl_3$ crystals were dissolved, the appropriate thiol (1.0 mol. equiv.) was quickly added and the reaction mixture was kept stirring at room temperature for 4 h and then filtered. Ice-cold saturated solution of sodium bicarbonate was added carefully to the filtrate and the organic layer was separated, washed by water, dried with anhydrous magnesium sulfate, filtered and the dichloromethane was evaporated. Crude syrups of the respective thyoglycosides were obtained and passed through a silica gel column (eluate: methanol/chloroform ,40:60). For deacetylation[15] the obtained crude material was dissolved in 10 mL of dry methanol, to which 3 mL of freshly prepared solution of sodium methoxide (0.5 g in 100 mL of methanol) was added and stirred at room temperature for 20 min. The resulting amorphous precipitate was filtered, dissolved in methanol. The resulting

sodium salts was removed by stirring the solution with Amberlite IR-120 (cation exchange) resin. The solution was then filtered, the methanol was evaporated and the resulting material was purified by silica gel chromatography (methanol:petrolium ether (70:30). All three thioglycosides were obtained as syrups, despite many crystallization attempts. Yield = 32, 39 and 12%., for **35**, **36** and **37**, respectively: **35:** Anal. $C_7H_{14}O_4S$: C, 43.29; H, 7.26; S, 16.5. Found: C, 43.17; H, 7.18; S, 16.38. ^{1}H NMR (D_2O) δ, ppm, 1.27 (t, 3H, [C-H$_3$]), 2.57 (m, 2H [S-C-H$_2$-]), 3.35 (d, 1H, [C-*e*H5]), 3.41 (m, 1H, [C-H4]), 3.65 (d, 1H, [C-*a*H5]), 3.92 (q, 1H, [C-H3]) 4.52 (q, 1H, [C-H2]) (β), 5.17 (d, 1H, [C-H1]). **36:** Anal. $C_8H_{16}O_4S$: C, 46.13; H, 7.74; S, 15.38. Found: C, 46.17; H, 7.28; S, 15.08. ^{1}H NMR (D_2O) δ, ppm, 0.96 (t, 3H, [C-H$_3$]), 1.52 (m, 2H, [S-C-H$_2$-C-H$_2$-]), 2.59 (t, 2H, [S-CH$_2$-]), 3.59 (d, 1H, [C-*e*H5]), 4.19 (m, 1H, [C-H4]), 4.85 (d, 1H, [C-*a*H5]), 5.18 (q, 1H, [C-H3]), 5.22 (q, 1H, [C-H2]), (β) 5.32 (d, 1H, [C-H1]). **37:** Anal. $C_{17}H_{34}O_4S$: C, 61.04; H, 10.24; S, 9.58. Found: C, 61.07; H, 10.12; S, 8.87. ^{1}H NMR (D_2O) δ, ppm, 0.91 (t, 3H, [C-H$_3$]), 1.1-1.5 (m, 18H, [-C-H$_2$-]), 1.61 (m, 2H, [S-C-H$_2$-C-H$_2$-]), 2.65 (t, 2H, [S-C-H$_2$]), 3.55 (d, 1H, [C-*e*H5]), 3.61 (m, 1H, [C-H4]), 4.05 (d, 1H, [C-*a*H5]), 4.19 (q, 1H, [C-H3]), 5.12 (q, 1H, [C-H2]), (β), 5.20 (d, 1H, [C-H1]).

2,4:3,5-Di-O-*p*-fluorobenzylidene-D-xylose-dimethyl acetal (**41**)

The synthesis was carrying out according to procedure of Ferrier and Hatton[43] with minor modifications: 5 g of D-xylose, 4 mL of 32% HCl and 20 mL of *p*-fluorobenzaldehyde were added to 100 mL of methanol at stirred at -20ºC for 30 min. It was then left stirring at 4ºC for three days. The resulting white crystalline mass was filtered out, washed in cold water and cold methanol and recrystallized from ether.

Yield=27%, m.p.=223º-225º C. Anal. $C_{21}H_{22}F_2O_4$: C, 61.76; F, 9.3; H, 5.43. Found: C, 61.75; F, 8.99 H, 5.39, ^{1}H NMR ($CDCl_3$) δ, ppm, 3.37 (s, 6H, [C-H$_3$]), 3.93 (m, 1H, [C-H4]), 4.01 (q, 1H, [C-H2]), 4.11 (q, 1H, [C-H3]) 4.15 (d, 2H, [C-H5]), 4.54 (d, 1H, [C-H1]), 5.69 (s, 2H, [H-C(O)2Bz]), 7.1 (d, 4H, [Bz]), 7.55 (d, 4H, [Bz]).

Di-4-O-(1-O-methyl-α-D-xylopyranoside)-phosphoryl-O-methyl-polyethelene glycol (**42**)

Five g of methyl-O-PEG (MW=1000) were dissolved in dry toluene (100 mL) and dried in a Dean-Stark trap connected to a reflex apparatus. The toluene was distilled out and dry dichloromethane (10 mL) was under nitrogen atmosphere and the mixture was stirred. When methyl-O-PEG completely dissolved, PCl_3 (0.54 mL) and imidazole (1.02 g) in 10 mL dry dichloromethane were added slowly. The solution was stirred at room temperature for 1 h and then cooled in an ice bath. Tetrazole (0.35 g) in dry dichloromethane (10 mL) was then gradually added over 30 min and the reaction mixture was kept stirring at room temperature for 2 h. Compound **5** (1.43 g) in 10 ml of dry pyridine was then added, and the solution was kept stirring at room temperature for 7 days. It was then filtered, the organic solvents were evaporated and the remaining solid crude was dissolved in minimal amount of methanol. The solution was passed throw silica gel column (chloroform: petroleum ether 40:60 was used as a eluent). Organic solvents were evaporated and white crude mass was dissolved in 30 ml of methanol with 1 mL of water. 0.15 g of iodine was added and then solution was steering 24 hours. Mixture was filtered, methanol was evaporated and 20 ml of water was added, then solution was filtered again. The water was removed by lyophilization and the remaining white solid powder was dissolved in small amount of acetonitrile and purified by preparative HPLC (eluent: 100% acetonitrile, wave length=220 nM).

Three fractions with very close retention time were obtained: the first was the desired compound (**42**), the second was the mono phosphate ester and the third was the unsubstituted O-methyl-PEG ether. **42** was dissolved in a small volume of ethanol and recrystallized from cold diethyl ether: n-hexane (40:60). Yield=8%. ^{1}H NMR (DMSO) δ, ppm, 3.31 (s, 3H, [O-C-$\underline{H}_3$]), 3.47 (s, 3H, [PEG-O-C-$\underline{H}_3$]), 3.51 (m, ≈90H, [-O-C-$\underline{H}_2$-]), 3.67 (q, 2H, [P-O-C$\underline{H}_2$]), 3.75 (m, 1H, [C-$\underline{H}$4]), (α), 5.09 (d, 1H, [$\underline{H}$1]). ^{31}P NMR (DMSO) δ, ppm, 0.97 [s, $\underline{P}$O(O-R)$_3$].

Supplemental Table 2: Non-active D-xylose derivatives: summary of maximal tested concentrations, effects on dGlc uptake in L6 myotubes, minimal cytotoxic concentrations and the synthetic procedures of.

Compound	Concentration	Effect on dGlc uptake (fold of control)	Minimal cytotoxic concentration	Synthetic procedure
2	20 mM	1.03 ± 0.06	50 mM	Ref. 16
4	20 mM	0.99 ± 0.07	50 mM	Refs. 9, 17
7	20 mM	1.01 ± 0.09	50 mM	Scheme 1
8	20 mM	0.94 ± 0.07	50 mM	Ref. 18
18	20 mM	1.05 ± 0.11	50 mM	Scheme 2
19	200 µM	1.06 ± 0.03	1 mM	Scheme 1S
25	250 µM	1.01 ± 0.11	600 µM	Scheme 2
27	100 µM	0.94 ± 0.04	200 µM	Scheme 1S
28	1 mM	0.99 ± 0.04	5 mM	Scheme 1S
30	100 µM	0.90 ± 0.19	500 µM	Ref. 4
31	400 µM	0.94 ± 0.08	1 mM	Ref. 5
32	200 µM	0.99 ± 0.05	500 µM	Ref. 6
33	150 µM	1.01 ± 0.16	250 µM	Ref. 14
34	100 µM	1.03 ± 0.05	150 µM	Ref. 7
35	500 µM	0.99 ± 0.08	1 mM	Scheme 2S
36	100 µM	0.92 ± 0.08	200 µM	Scheme 2S
37	150 µM	0.98 ± 0.08	400 µM	Scheme 2S
38	50 µM	0.94 ± 0.09	150 µM	Ref. 9
39	50 µM	1.08 ± 0.09	100 µM	Ref. 10
40	150 µM	1.06 ± 0.09	300 µM	Ref. 11
41	150 µM	0.93 ± 0.01	250 µM	Ref. 11
42	600 µM	1.01± 0.12	1 mM	Scheme 3S

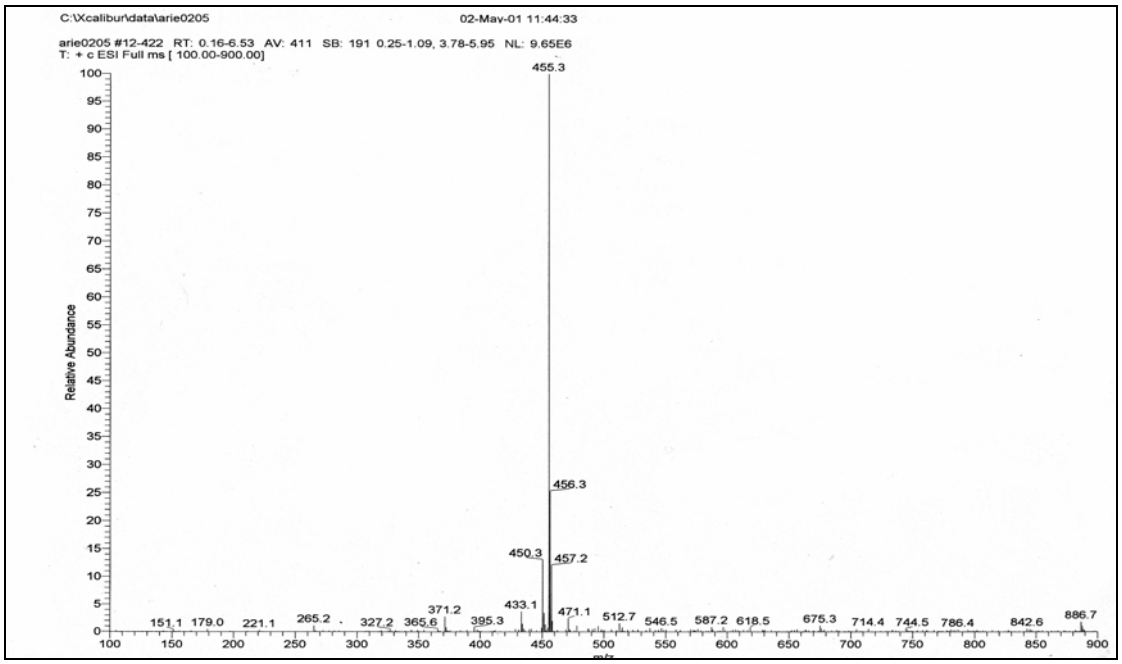
Supplemental Table 3: Western blot protocols.

Primary antibody against	Blocking buffer	Wash buffer	Antibody dilution & buffer	Wash buffer	Secondary Antibody	Wash
GLUT1	TBST1 + 5% BSA	_	1:1000 TBST1 + 5% BSA	TBST1, 0.75% BSA, 0.5% Igepal	1:10000 TBST1, RT, 45 min.	TBST2 + 0.1% Triton X-100
GLUT4	TBST1 + 5% BSA	_	1:1000 TBST1 + 5% BSA	TBST1, 0.75% BSA, 0.5% Igepal	1:10000 TBST1, RT, 45 min.	TBST2 + 0.1% Triton X-100
AMPKα	TBST2 + 5% NFDM	TBST2	1:2000 TBST2 + 5% BSA	TBST2	1:3000 TBST2 + 5% NFDM, RT, 1h	TBST2
pThr^{172}-AMPKα	TBST2 + 5% NFDM	TBST2	1:1000 TBST2 + 5% BSA	TBST2	1:3000 TBST2 + 5% NFDM, RT, 1h	TBST2
Akt/PKBα	TBST2 + 5% NFDM	_	1:1000 TBST2 + 5% NFDM	TBST2	1:3000 TBST2 + 5% NFDM, RT, 1h	TBST2
pSer^{473}-Akt/PKBα	TBST2 + 5% NFDM	TBST2	1:2500 TBST2 + 5% BSA	TBST2	1:3000 TBST2 + 5% NFDM, RT, 1h.	TBST2
pThr^{308}-Akt/PKBα	TBST2 + 5% NFDM	_	1:500 TBST2 + 5% NFDM	TBST2	1:3000 TBST2 + 5% NFDM, RT, 1h.	TBST2
AS160	TBS + 3% NFDM	_	1:1000 TBS + 5% NFDM	Water	1:3000 TBS + 3% NFDM, RT, 1h	TBST2
pThr^{642}-AS160	TBST2 + 5% BSA	_	1:1000 TBST2 + 3% BSA	TBS	1:3000 TBST2 + 5% BSA, RT, 1h	TBST2

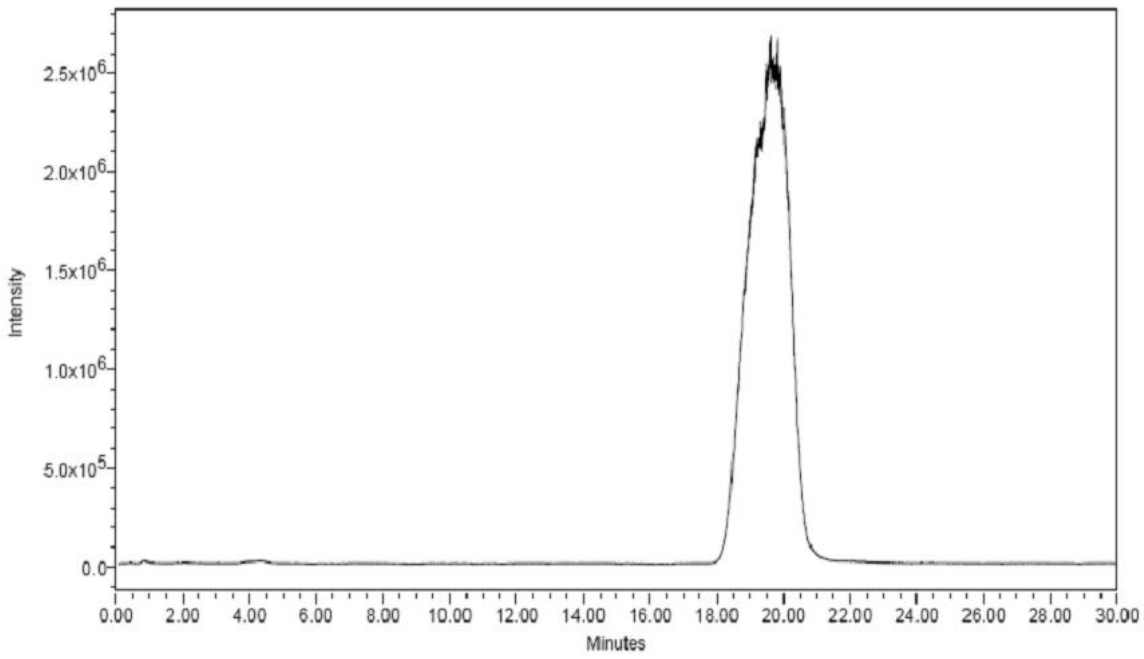
Buffers: TBS, 15 mM Tris-HCl, pH 7.6, and 15 mM NaCl; TBST1, TBS supplemented with 0.5% (v/v) Tween 20; TBST2, TBS with 0.1% Tween 20. Concentrations of BSA and non-fat dry milk (NFDM) are given in w/v units. Concentrations of Tween 20, Triton X-100 and Igepal are given in v/v units.

Tracings for lead compounds: 19, 21 and 24

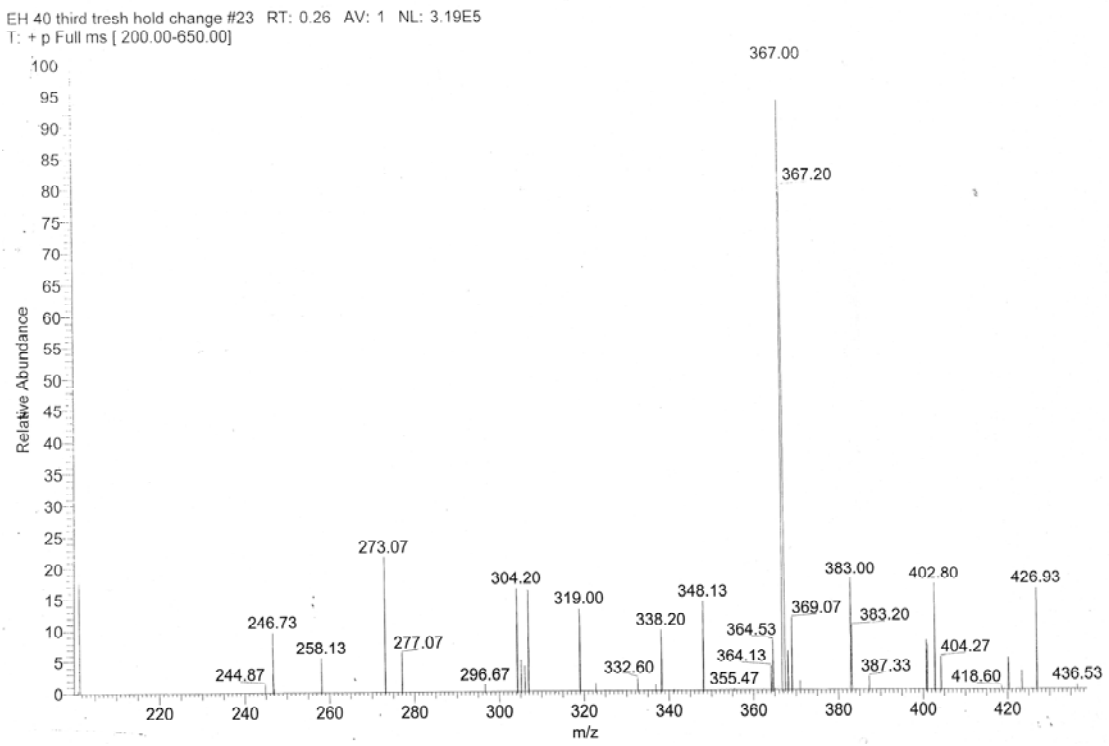
ESMS spectrum of **19**: [MW+Na^+]=455.3, calcd: 455.6



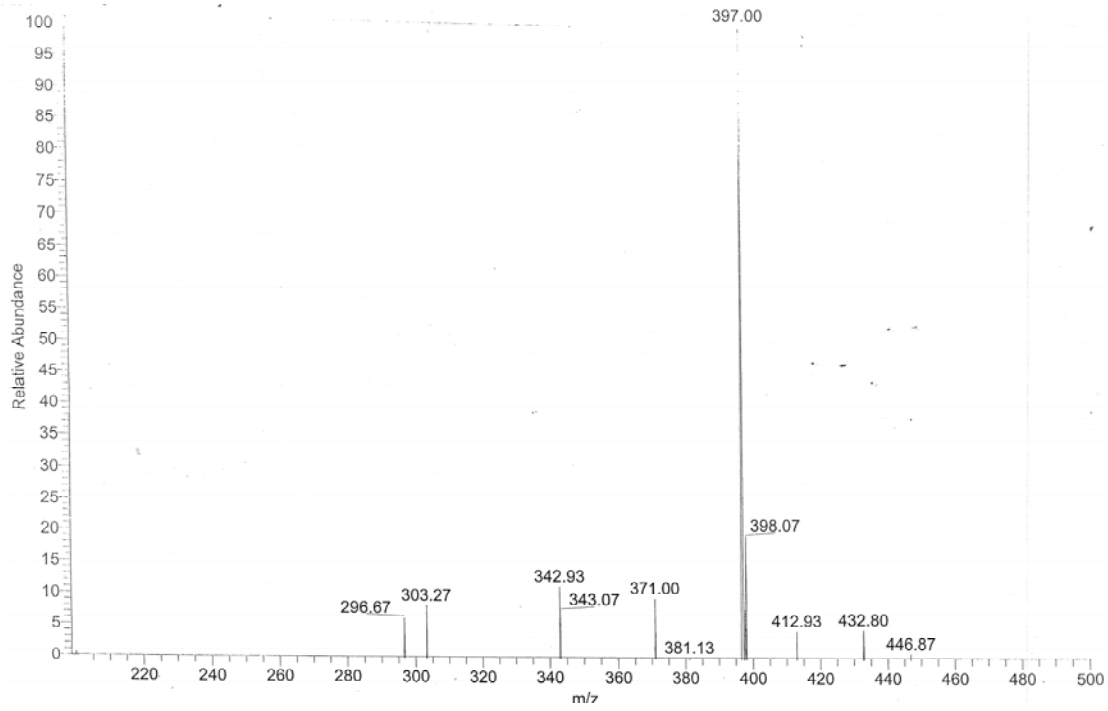
HPLC profile of **19**



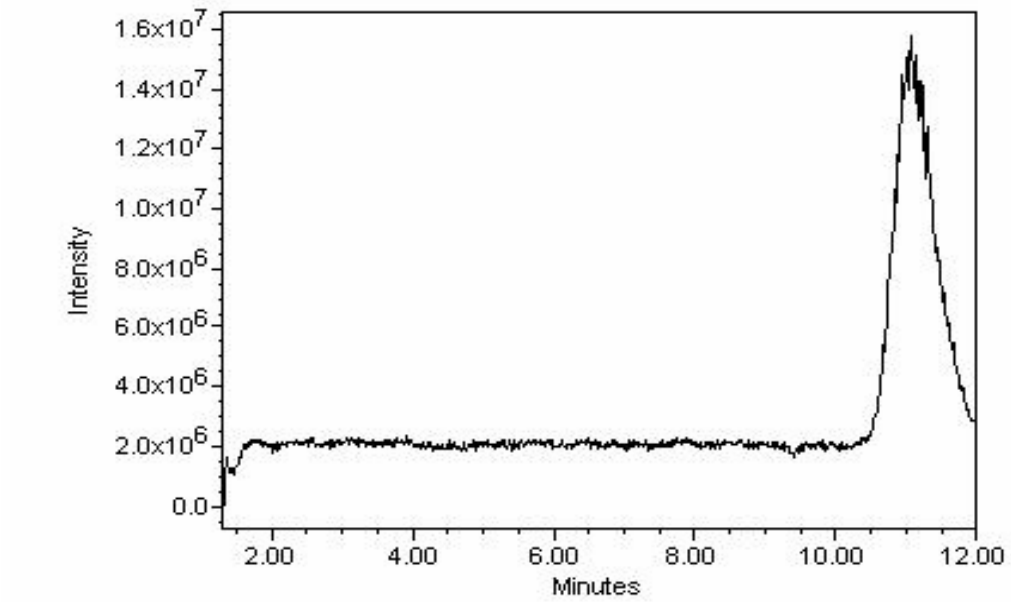
ESMS spectrum of **21**: [MW+Na^+]=367.0, calcd: 367.49



ESMS spectrum of **24**: [MW+K^+]=397.0, calcd: 397.52



HPLC profile of **24**



Supplemental References:

1. Tejima, S.; Ness, R. K.; Kaufman, R. L.; Fletcher, H. G. Some partially benzylated pentose derivatives. *Carbohydr Res* **1968,** 7, 485-490.
2. Miyashita, N.; Toshikoshi, A.; Grieco, P. Pyridinium p-toluenesulfonate. A mild and efficient catalyst for the tetrahydropyranylation of alcohols. *J Org Chem* **1977**, 42, 3772-3774.
3. Corey, E. J.; Suggs, J. W. Cleavage of allyloxycarbonyl protecting group from oxygen and nitrogen under mild coditions by nickel carbonyl. *J Org Chem* **1973,** 38, 3223-3224.
4. Deng, S.; Yu, B.; Lou, Y.; Hui, Y. First Total Synthesis of an Exceptionally Potent Antitumor Saponin, OSW-1. *J Org Chem* **1999,** 64, 202-208.
5. Chavis, C.; De Gourcy, C.; Imbach, J.-L. N.M.R. studies of D-ribosylamines in solution: derivatives of hydroxylamine, hydrazine, thiosemicarbazide, and secondary amines. *Carbohydrate Res* **1984,** 135, 13-27.
6. Wolform, M.; Thompson, A. Acetylation. In *Methods in Carbohydrate Chemistry*, Whistler, R.; Wolfrom, M.; BeMiller, J., Eds. Academic Press: New York, 1963; Vol. 2, pp 212-213.
7. Petrovic, V.; Tomic, S.; Ljevakovic, D.; Tomasic, J. Synthesis of acylated methyl beta-D-xylopyranosides and their enzymic deacylations by rabbit serum esterases. *Carbohydr Res* **1997,** 302, 13-18.
8. Disgupta, F.; Garegg, P. J. Synthesis of ethyl and phenyl 1-thio-1,2-*trans*-D-glycopyranosides from the corresponding per-*O*-acetylated glycopyrnoses having a 1,2-*trans*-configuration using anhydrous ferric chloride as a promoter. *Acta Chem Scand* **1989,** 43, 471-475.
9. Moravcova, J.; Capkova, J.; Stanek, J. One-pot synthesis of 1,2-O-isopropylidene-alfa-D- xylopyranose. *Carbohydrate Research* **1994,** 263, 61-66.
10. Baker, B. R.; Schaub, R. E. Puromycin. Synthetic studies. XIII. Syntheis of 6-dimethylamino-9-(3'-amino-3'-deoxy-beta-D-arabinofuranosyl)-purine. *J Am Chem Soc* **1955,** 77, 5900-5905.
11. Ferrier, R.; Hatton, L. The acid-catalyzed condensation of D-xylose with benzaldehyde in the presence of alcohols. Two diastereoisomeric 1,2:3,5-Di -O-benzylidene-alfa-D-xylofuranoses. *Carbohydrate Res* **1967,** 5, 132-139.
12. Parang, K. Polymer-supported reagents for methylphosphorylation and phosphorylation of carbohydrates. *Bioorg Med Chem Lett* **2002,** 12, 1863-1866.
13. Szarek, W. A.; Zamojski, A.; Tiwari, K. N.; Ison, E. R. A new, facile method for cleavage of acetals and dithioacetals carbohydrate derivatives. *Tetrahedron Lett* **1986,** 27, 3827-3830.
14. Fletcher, H. The anomeric D-arabinopyranose tetrabenzoates. In *Methods in Carbohydrate Chemistry*, Wilfrom, M.; BeMiller, J., Eds. Academic Press: New York, 1963; Vol. 2, pp 234-336.
15. Thompson, A.; Wolform, M.; Pascu, E. Deacetylation. In *Methods in Carbohydrate Chemistry*, Whistler, R. L.; Wolfrom, M.; BeMiller, J., Eds. Academic Press: New York, 1963; Vol. 2, pp 215-218.
16. Bowering, W. An alternative synthesis of 2-O-methyl-D-xylose. *Can J Chem* **1958,** 36, 283-284.
17. Levine, P.; Raymond, A. 3-methyl-xylose and 5-methyl-xylose. *Journal Biol Chem* **1933,** 102, 317-324.

18. de Bruyne, C. K.; Loontiens, F. G. Synthesis of alkyl beta-xylopyranoside in the presence of mercuric salts. *Nature* **1966,** 209, 396-397.