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Synthesis of modified D-mannose core derivatives and their impact on GH38 α-mannosidases

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ABSTRACT

Nine new compounds having five- and modified six-member carbohydrate core derived from D-lyxose or D-mannose, and non-hydrolysable aglycones (benzylsulfonyl or aryl(alkyl)triazolyl) were synthesised to investigate their ability to inhibit the recombinant *Drosophila melanogaster* homologs of two human GH38 family enzymes: Golgi mannosidase II (dGMIIb) and lysosomal mannosidase (dLMII).

Two compounds were weak selective dGMIIb inhibitors showing IC_{50} at mM level. Moreover, it was found that another GH38 enzyme, commercial jack bean α-mannosidase, was inhibited by triazole conjugates regardless of the carbohydrate core while the corresponding sulfones were inactive.

1. Introduction

It is well known that carbohydrates play an essential role in many biological events. They are involved in fundamental physiological processes such as cell–cell communication, immune response, pathogen defence, cancerogenesis, cell adhesion and others.[1] Due to the important role of carbohydrates in medicine and biology the development and synthesis of novel functional structures with the desired properties is an expanding area of research that continuously provides a broad scale of new monovalent and multivalent compounds and glycoconjugates. These carbohydrate derivatives have been employed in many studies including assays of sugar processing enzymes with the aim to identify potent inhibitors of the biocatalysts. The enzymes include glycoside hydrolases which are well known to be affected by numerous natural and synthetic carbohydrate mimetics. For example, various glycosyl triazoles have been used to examine their ability to inhibit commercial glycosidases, such as *Escherichia coli* β-galactosidase, sweet almond β-glucosidase and bovine liver β-galactosidase.[2] The dependence of inhibition of two glucosidases, sweet almond β-glucosidase and yeast α-glucosidase,[3] as well as glycogen phosphorylase[4] on the glucosyl triazole anomeric configuration has been proven. On the other hand, only one of 4 tested glycosidases was affected by anomeric 1,5-anhydrosugars, which have been shown to be selective inhibitors of jack bean α-mannosidase.[5] Recently, endocyclic sulfur or selenium containing carbohydrate mimetics with the positively charged heteroatom have been reported to act as potent glycosidase inhibitors,[6] while glycosyl sulfoxides displayed only weak activity towards glycosidases.[7] Various *fuco*-configured pyrrolidotriazoles have been found to exhibit enhanced inhibition of α-fucosidase and their impact on several α-glucosidases was aglycone dependent.[8] Modulation of affinity and selectivity towards various glycosidases by ligand multivalency has been demonstrated for mono-, di- and tri-valent iminosugars[9] (Fig. 1).

In our ongoing research, synthetic mannose derivatives have been constructed as models for mycobacterial mannosyltansferase (ManT)[10–12] or eukaryotic α-mannosidases belonging to glycoside hydrolase families 38 and 47.[13,14] The model compounds failed to inhibit ManT, instead, they served as good acceptor substrates.[10–12] They were more potent inhibitors of GH47 than GH38 α-mannosidases.[13,14]

One of the GH38 enzymes, human Golgi α-mannosidase II (hGMII) (EC 3.2.1.114), is a very attractive target. It has been demonstrated that known potent hGMII inhibitors induce an immunostimulatory response that results in an anti-cancer effect.[15] However, they also showed an undesired co-inhibition of lysosomal α-mannosidase (LM) (EC 3.2.1.24). This limits their use in

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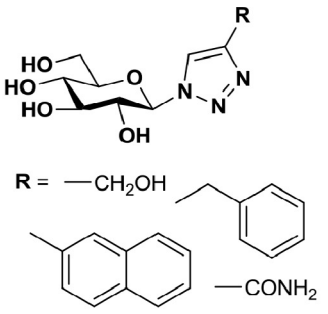
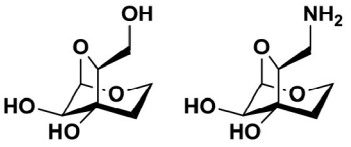
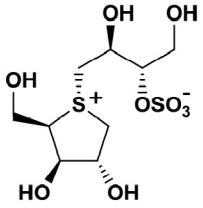


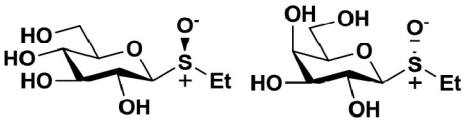
rabbit muscle glycogen phosphorylase *b*[4]



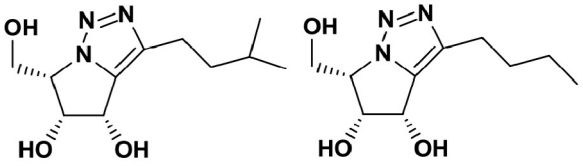
α-galactosidase, α-glucosidase
α-amylase, jack bean α-mannosidase[5]



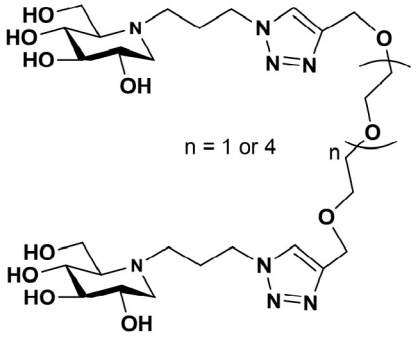
α-glucosidase, β-glucosidase
α-galactosidase, β-galactosidase
jack bean α-mannosidase[6]



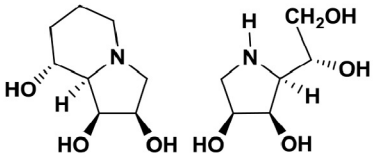
β-glucosidase
β-galactosidase
jack bean α-mannosidase[7]



α-glucosidase, β-glucosidase
α-galactosidase, β-galactosidase
jack bean α-mannosidase
α-L-fucosidase[8]



α-glucosidase, β-glucosidase
α-galactosidase, β-galactosidase
α-mannosidase, β-mannosidase
isomaltase, naringinase, amyloglucosidase[9]



human α-mannosidases
(lysosomal, neutral, Golgi II)[18]

Fig. 1. Examples of the compounds tested as inhibitors of various glycosidases.

clinical cancer chemotherapy[16] and justifies a continued search for a selective hGMII inhibitor.

All known inhibitors of hGMII and LM are characterised by cyclic structures and the most potent of them comprise endocyclic nitrogen.[17–19] On the other hand, to the best of our knowledge the potency and selectivity of five- and six-member ring compounds containing endocyclic oxygen, such as derivatives of D-lyxose and D-mannose, have not been investigated in detail. These

carbohydrate cores possess *cis*-configured OH groups at positions 2 and 3, which are necessary for binding to Zn^{2+} co-factor at the active site of dGMII.[20,21]

We have previously reported the synthesis of a series of 13 alkyl and phenylalkyl 1-thio-α-D-mannosides, sulfoxides and sulfones and the examination of their inhibitory effect towards two α-mannosidases, *Drosophila melanogaster* homologs of human Golgi mannosidase II (dGMIIb) and lysosomal mannosidase (dLMII). Some derivatives exhibited inhibitory activities at mM level towards both dGMIIb (IC_{50} = 1.5–2.5 mM) and dLMII (IC_{50} = 1.0–2.0 mM). However, only benzyl mannopyranosyl sulfone was selective towards dGMIIb.[13]

Subsequently, the phenylalkylsulfonyl group has been replaced with another non-hydrolysable phenyl- or phenylalkyl-triazolyl moiety. However, this alteration improved neither potency nor selectivity towards dGMII. In general, these triazole conjugates were better inhibitors of commercial *Canavalia enciformis* (jack bean) α-mannosidase (JBMan) (EC 3.2.1.24, GH38 family member) than dGMIIb and dLMII.[14]

With the aim to improve the selectivity towards dGMIIb, further modifications of the carbohydrate core of potential inhibitors of this enzyme were carried out, while keeping the same non-hydrolysable aglycones (benzylsulfonyl, phenyl- and benzyl-triazolyl). These modifications included elongation or deoxygenation at C-6 atom of D-mannose unit and alteration of D-mannose with D-lyxose. This paper deals with the synthesis of nine new derivatives and examination of their ability to inhibit the GH38 family enzymes: dGMIIb, dLMII and JBMan.

2. Results and discussion

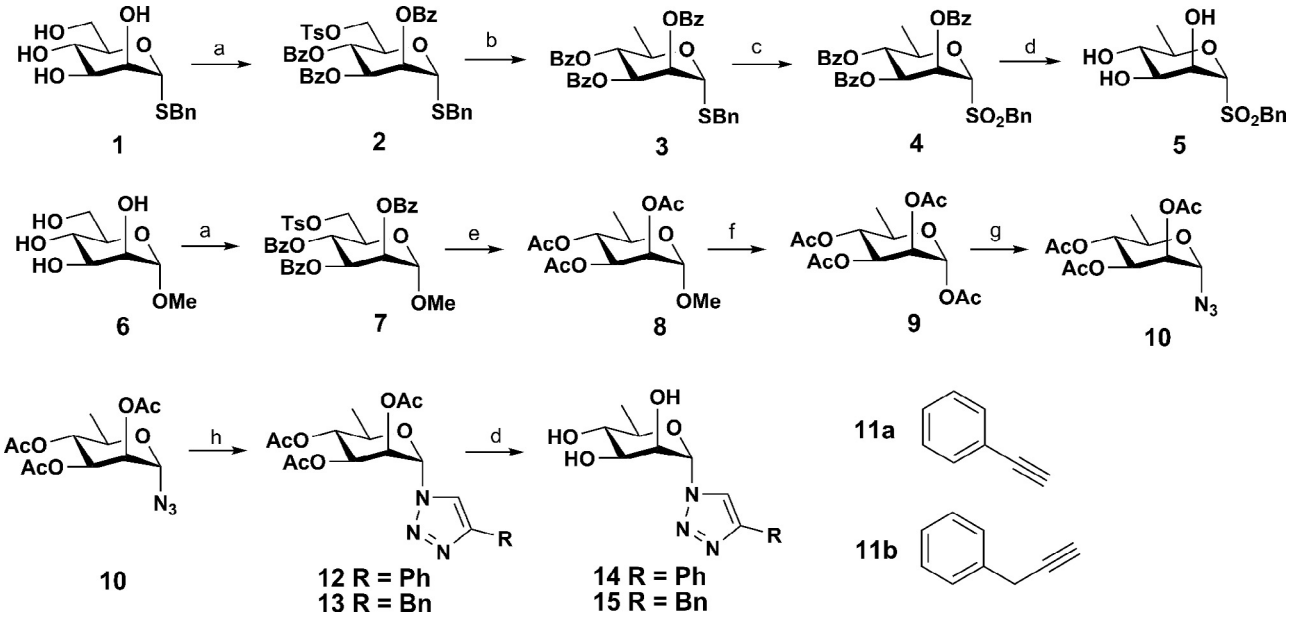
2.1. Synthesis

The first series of prepared compounds (Scheme 1) included D-rhamnosides, i.e. those having D-mannose unit deoxygenated at C-6 atom. Synthesis of benzyl α-D-rhamnosyl sulfone **5** commenced from benzyl 1-thio-α-D-mannopyranoside **1**.[13] One pot tosylation of primary hydroxyl group of **1** followed by benzoylation gave **2** in moderate yield. Subsequent tosyl group reduction with $NaBH_4$ provided fully protected benzyl 1-thio-α-D-rhamnoside **3**. Oxidation of rhamnoside **3** thio-functionality with *m*CPBA gave sulfone **4**. In final step, removal of benzoyl protective groups under mild conditions (K_2CO_3, MeOH) provided the desired benzyl α-D-rhamnosyl sulfone **5**.

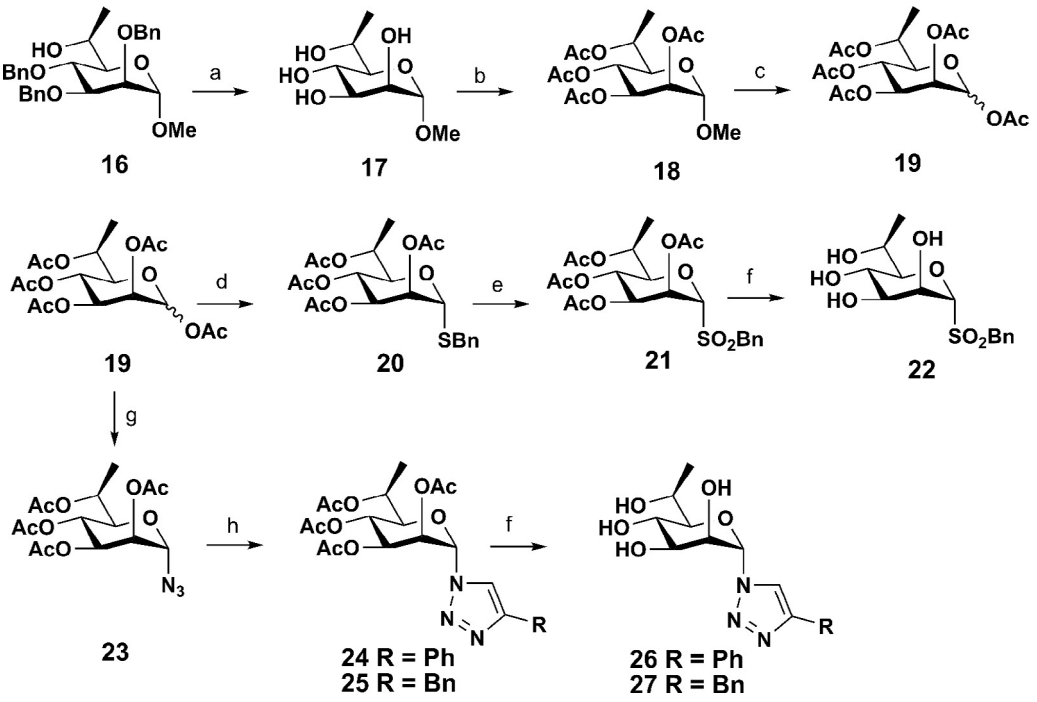
Other target derivatives, phenyl and benzyltriazolyl rhamnosides **14** and **15**, were synthesised by Cu(I) catalysed azide–alkyne cycloaddition (CuAAC) reaction of azide **10**[22] with the corresponding alkynes. Synthesis of **10** was accomplished from methyl α-D-mannopyranoside **6** by the same sequence as reported for **3** (tosylation, benzoylation, reduction), followed by debenzoylation and acetylation of secondary hydroxyls leading to peracetylated methyl α-D-rhamnoside **8**. Its acetolysis gave donor **9** for glycosylation of $TMSN_3$ catalysed by $SnCl_4$,[23] yielding **10**. The coupling of azide **10** with alkynes **11a** and **11b** carried out in DMF/H_2O (3:1) solvent mixture using copper(II) sulfate and sodium ascorbate[11,14,24] afforded **12** and **13** in moderate yields. Triazole conjugates **14** and **15** were obtained after saponification of acetyl protective groups.

The second group consisted of compounds having D-mannose unit elongated at C-6 (Scheme 2). Mannoside **6** was transformed over 5 steps to known intermediate **16** which was identified by NMR spectroscopy as the *S*-isomer at C-6.[25] Compound **16** was then converted to peracetylated donor **19**[26] in high overall yield. $SnCl_4$-catalysed glycosylation of benzylmercaptan with **19** afforded benzyl thioglycoside **20**, which was by the same reaction sequence as described for **3** converted to the required (6*S*) benzyl 6-*C*-methyl-α-D-mannopyranosyl sulfone **22**. The same approach reported for conjugates **14** and **15** was used also in the synthesis of triazoles **26** and **27**. Their synthesis commenced from azide **23** prepared from glycosyl donor **19** and $TMSN_3$.

The last group included derivatives comprising a five member D-lyxose ring (Scheme 3) possessing *cis*-OH group arrangement at positions 2 and 3. Sulfone **30** and triazoles **35** and **36** were obtained from the glycosyl acceptors ($TMSN_3$ and BnSH) and 1–acylated donors **28** or **31**, respectively, using analogous reaction sequences as depicted in Schemes 1 and 2.



Scheme 1. *Reagents and conditions:* (a) 1. TsCl, py/CH_2Cl_2, rt, 16 h; 2. BzCl, rt, 16h; **2** (50%), **7** (30%); (b) $NaBH_4$, DMF, 65 °C, 6 h; 34%; (c) *m*CPBA, CH_2Cl_2, rt, 3 h; 95%; (d) K_2CO_3, MeOH, rt; **5** (69%), **14** (83%), **15** (60%); (e) 1. $NaBH_4$, DMF, 65 °C, 4 h; 2. 0.02M MeONa, MeOH, 20 h, rt; 3. Ac_2O, py, 16 h, rt; 50% over 3 steps; (f) Ac_2O, AcOH, H_2SO_4(cat.), 1 h, 0 °C; 93%; (g) $TMSN_3$, $SnCl_4$, CH_2Cl_2, rt, 2 h; 85%; (h) **11a** or **11b**, $CuSO_4$, Na ascorbate, DMF/H_2O, 16 h, rt; **12** (90%), **13** (91%).



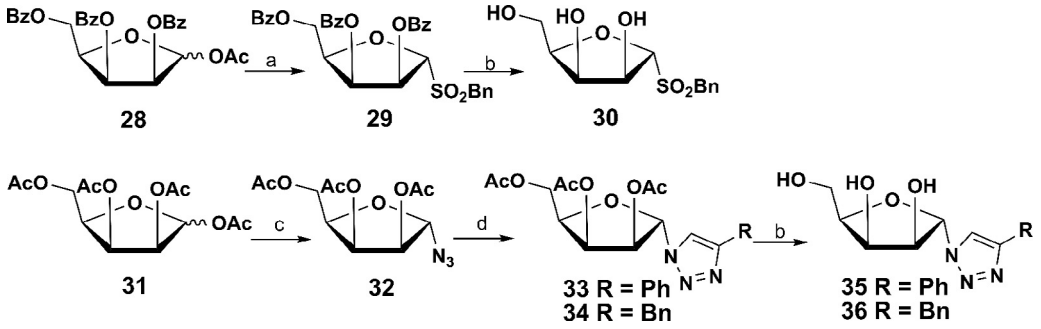
Scheme 2. *Reagents and conditions:* (a) H_2, 10% Pd/C, MeOH, rt, 4 h; 98%; (b) Ac_2O, py, 16 h, rt; 98%; (c) Ac_2O, AcOH, H_2SO_4(cat.), 1 h, 0 °C; 99%; (d) BnSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, 16 h; 70%; (e) *m*CPBA, CH_2Cl_2, rt, 3 h; 85%; (f) K_2CO_3, MeOH, rt; **22** (85%), **26** (80%), **27** (82%); (g) $TMSN_3$, $SnCl_4$, CH_2Cl_2, rt, 2 h; 88%; (h) **11a** or **11b**, $CuSO_4$, Na ascorbate, DMF/H_2O, 16 h, rt; **24** (91%), **25** (76%).

The structures of the targeted compounds and the intermediates were determined by NMR spectroscopy. The first series of compounds (structures **3**–**6**, **8**–**15**), featuring deoxygenation at C-6, were confirmed based on their characteristic shifts in the ^{13}C NMR spectra. For example, the C-6 signal of rhamnoside **3** appeared at δ = 17.0. The C-6 atom of mannoside **2** showed a significantly higher chemical shift (δ = 68.6). Moreover, the C-6 signals in all rhamnosides were easily identified in the ^1H NMR spectra (doublet at 1.2–1.4 ppm, *J* 6.2–6.5 Hz). Similarly, the doublet of C–7 (CH_3 function) of mannosides **16**–**27** (elongated at C-6) was observed in the same region and had a similar coupling constant. Oxidation of thioglycosides to the corresponding sulfones resulted in a lower chemical shift of the H-1 signal of the sulfones compared to the shifts of the thioglycosides. On the contrary, the C-1 signal of the sulfones had a significantly (by about 5–6 ppm) higher chemical shift than the same carbon of the thioglycosides. The opposite shift of anomeric signals was observed when glycosyl azides were coupled with alkynes providing glycosyl triazoles. The anomeric proton of the azide had a slightly lower chemical shift than that of the triazole derived thereof. Formation of glycosyl triazoles can also be identified in the ^1H NMR spectra by the presence of a singlet originating from hydrogen of triazolyl ring (NC═CH). This singlet appeared at 7.93–7.96 and 7.34–7.38 ppm for protected phenyl and benzyl triazoles, respectively. After deprotection of the saccharide core, the singlet resonance was shifted to higher values by about 0.5 ppm (8.50 and 7.85 ppm).

In summary, 9 new synthetic conjugates **5**, **14**, **15**, **22**, **26**, **27**, **30**, **35** and **36** were prepared by multistep synthesis in moderate to high yields.

2.2. Biological assay with the GH38 enzymes[13,14]

Compounds **5**, **14**, **15**, **22**, **26**, **27**, **30**, **35** and **36** were assayed towards dGMIIb, dLMII and JBMan (Table 1). None of the mannoside analogs served as a substrate of these enzymes or an inhibitor of dLMII. In contrast, the activity of dGMIIb, the most important enzyme, was negatively affected by benzyl α-D-rhamnosyl sulfone **5** (IC_{50} = 2.0 mM) and benzyl triazole **27** (IC_{50} = 3.0 mM) elongated at C-6 of the D-mannose unit, suggesting that these conjugates may work as selective dGMIIb inhibitors. Deoxygenation at C-6 of



Scheme 3. *Reagents and conditions:* (a) 1. BnSH, $BF_3{\cdot}OEt_2$, CH_2Cl_2, rt, 16 h, 2. *m*CPBA, CH_2Cl_2, rt, 3 h; 66%; (b) K_2CO_3, MeOH, rt; **30** (80%), **35** (88%), **36** (89%); (c) $TMSN_3$, $SnCl_4$, CH_2Cl_2, rt, 2 h; 78%; (d) **11a** or **11b**, $CuSO_4$, Na ascorbate, DMF/H_2O, 16 h, rt; **33** (91%), **34** (72%).

Table 1
Measured IC_{50} values for dGMIIb, dLMII and JBMan (in mM)

Compound	dGMIIb	dLManII	JBMan
5	2.0	n.i.	n.i.
14	n.i.	n.i.	2.3
15	n.i.	n.i.	2.5
22	n.i.	n.i.	n.i.
26	n.i.	n.i.	1.2
27	3.0	n.i.	1.2
30	n.i.	n.i.	n.i.
35	n.i.	n.i.	2.7
36	n.i.	n.i.	2.8
37	2.0[a]	n.i.[a]	2.0[b]
38[b]	5.0	0.5	1.1

n.i. - no inhibition or inhibiton < 10% at 5 mM.
[a] IC_{50} values taken from Poláková et al.[13]
[b] IC_{50} values taken from Poláková et al.[14]

D-mannose led to a replacement of hydroxymethyl with a CH_3-functionality in rhamnosyl sulfone **5**; however, both **5** and mannopyranosyl sulfone **37**[13] (IC_{50} = 2.0 mM, Fig. 2) exhibited the same selectivity and inhibitory activity towards dGMIIb. On the other hand, elongation at C-6 of D-mannose in sulfone **22** resulted in its inactivity towards dGMIIb. These observations indicate that the potency of benzyl sulfones is influenced by the nature and size of the C-6 substituent of the mannose core.

In general, the triazoles were less potent inhibitors of dGMIIb than sulfones[14] with the exception of C-6-chain elongated compounds. In this case, benzyl triazole **27** was better than the corresponding sulfone **22** and it was also a slightly better inhibitor of dGMIIb (IC_{50} = 3.0 mM) than benzyl mannosyl triazole **38**[14] (IC_{50} = 5.0 mM).

A more specific influence of the tested compounds on JBMan was observed. While all synthesised derivatives having a benzylsulfonyl aglycone were inactive, all glycosyl triazoles acted as JBMan inhibitors (IC_{50} = 1.2–2.8 mM). It is noteworthy that compounds **26** and **27** elongated at C-6 atom of D-mannose were approximately two times more efficient than triazoles **14**, **15** and **35**, **36**. These data are in line with our previous observation that triazoles with a non-modified mannose moiety are stronger JBMan inhibitors than the corresponding phenylalkyl mannosyl sulfones that also affect JBMan.[14] All the modification of D-mannose core performed in this study thus resulted in the inactivity of the benzylsulfonyl derivatives towards JBMan while inhibitory activity of the triazoles was not affected significantly.

In summary, the assays towards *Drosophila* homologs of the mammalian GH38 α-mannosidases (dGMIIb and dLMII) revealed that the synthesised compounds display poor inhibitory activity. Thus, modified D-mannose units as well as D-lyxose structural moieties are inappropriate carbohydrate cores for efficient and selective inhibitors of dGMIIb. The capability of all triazole conjugates to inhibit JBMan suggests that the plant GH38 enzyme is a member of another subfamily than dGMIIb and dLMII.

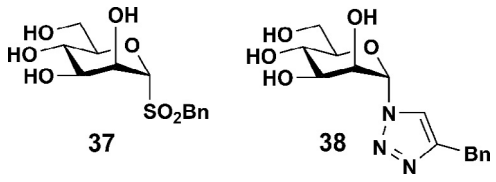


Fig. 2. Compounds previously tested as inhibitors of GH38 mannosidases.[13,14]

3. Conclusion

All phenyl- and benzyl-triazolyl conjugates act as selective JBMan inhibitors. Compounds with five- and modified six-member carbohydrate cores having endocyclic oxygen and either benzylsulfonyl or phenyl- and benzyl-triazolyl non-hydrolysable aglycones are inactive or poor inhibitors of dGMIIb. Alterations of D-mannose core, carried out with the intention to search for more efficient dGMIIb inhibitors, did not result in improved features of the tested compounds with respect to selectivity and potency towards this enzyme. Therefore, our current effort is focused on molecular modelling-assisted design of new carbohydrate core(s) of dGMIIb inhibitors that would better meet the requirements.

4. Experimental

4.1. General methods

TLC was performed on aluminium sheets precoated with silica gel 60 F_{254} (Merck). Flash column chromatography was carried out on silica gel 60 (0.040–0.060 mm, Merck) with distilled solvents (hexanes, ethylacetate, acetonitrile, methanol). Dichloromethane was dried (CaH_2) and distilled before use. All reactions containing sensitive reagents were carried out under an argon atmosphere. ^{1}H NMR and ^{13}C NMR spectra were recorded at 25 °C with a VNMRS 400 MHz Varian spectrometer, chemical shifts are referenced to either TMS (δ 0.00, $CDCl_3$ for ^{1}H) or HOD (δ 4.87, CD_3OD) for ^{1}H, and to internal $CDCl_3$ (δ 77.23) or CD_3OD (δ 49.15) for ^{13}C. Optical rotations were measured on a Jasco P2000 polarimeter at 20 °C. High resolution mass determination was performed by ESI–MS on a Thermo Scientific Orbitrap Exactive instrument operating in positive mode.

The conjugates **5**, **14**, **15**, **22**, **26**, **27**, **30**, **35** and **36** used in biological tests were lyophilised before the use. *p*-Nitrophenyl α-D-mannopyranoside (*p*NP-Man*p*), jack bean α-mannosidase and alkynes **11a** and **11b** were purchased from Sigma; and swainsonine was purchased from Calbiochem. Inhibition assays for GH family 38 enzymes were performed using a microplate reader (Epoch, BioTek and Gen^{5TM} data collection and analysis software).

4.2. Chemistry

4.2.1. Synthesis

4.2.1.1. General procedure for oxidation of thioglycoside (Method A). To a stirred and at 0 °C cooled solution of corresponding thioglycoside (1 eq) in CH_2Cl_2 (20 mL) *m*CPBA (2.5 eq, 77% peroxide content) was added. The reaction mixture was stirred at rt for 3 h, diluted with CH_2Cl_2 (20 mL), washed with satd $NaHCO_3$ (2 × 20 mL) and water (30 mL). The organic phase was dried (Na_2SO_4), filtered, concentrated and the crude product was purified by column chromatography (hexane:EtOAc).

4.2.1.2. General procedure for azide synthesis (Method B). A solution of donor (1eq) in CH_2Cl_2 (10 mL) was cooled at 0 °C and $TMSN_3$ followed 1M $SnCl_4$ in CH_2Cl_2 were added. After being stirred at rt for 2 h, the reaction was diluted with DCM (20 mL) and poured into cold satd. $NaHCO_3$ (20 mL). After 15 min stirring it was filtered through Celite, the organic phase was separated, dried (Na_2SO_4), filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc).

4.2.1.3. General procedure for CuAAC reaction (Method C). To a solution of azide (1 eq) in DMF: H_2O (1.6 mL, 3:1) alkyne **11a** or **11b** (1.1 eq) was added followed by sodium ascorbate (0.8 eq) and Cu(II) sulphate (0.4 eq). The reaction mixture was stirred at rt for 16 h. The reaction mixture was poured into satd. NH_4Cl (20 mL) and extracted with EtOAc (3 × 20 mL). The organic extracts were combined,

washed with water, dried and concentrated. The crude product was purified by column chromatography (hexane:EtOAc).

4.2.1.4. General procedure for deprotection (Method D). Protected compound (1eq) was dissolved in dry methanol (3–5 mL) and K_2CO_3 (0.5 eq) was added thereto. The reaction mixture was stirred at rt until disappearance of starting material, then filtered and concentrated. Purification by column chromatography (CH_3CN:MeOH or EtOAc:MeOH), evaporation and lyophilisation gave the target compound for biological evaluation.

4.2.1.5. Benzyl 2,3,4-tri-O-benzoyl-6-O-tosyl-1-thio-α-D-mannopyranoside (2).

To a stirred solution containing **1**[13] (0.56 g, 1.95 mmol) in CH_2Cl_2: pyridine (1:1, 15 mL) cooled down on an ice bath tosyl chloride (0.48 g, 2.53 mmol) was added. The resulting mixture was brought to rt and the stirring was continued for 16 h. The reaction mixture was diluted with CH_2Cl_2 (25 mL), washed with 1M HCl (5 × 10 mL), satd $NaHCO_3$ (3 × 10 mL), water (20 mL), dried with anhydrous Na_2SO_4, filtered and concentrated. The crude product was dissolved in pyridine (7 mL) and cooled down on an ice bath. Benzoyl chloride (0.64 g, 0.53 mL, 4.54 mmol) was added dropwise. The resulting mixture was brought to rt and the stirring was continued for 16 h. The reaction mixture was diluted with water (10 mL) and CH_2Cl_2 (30 mL). The organic phase was separated, washed with 1M HCl (3 × 15 mL), satd $NaHCO_3$ (3 × 10 mL), dried with anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexanes:EtOAc 10:1→3:1) to give **2** (0.73 g, 50% over 2 steps) as an oil. $[\alpha]_D$ +16 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03–7.17 (m, 24H, Ar), 5.80–5.75 (m, 2H, H-3, H-4), 5.69 (dd, 1H, $J_{2,3}$ 3.1 Hz, H-2), 5.28 (d, 1H, $J_{1,2}$ 1.4 Hz, H-1), 4.69 (m, 1H, H-5), 4.24 (dd, 1H, $J_{5,6b}$ 5.8 Hz, H-6b), 4.17 (dd, 1H, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 12.1 Hz, H-6a), 3.88 (d, 1H, *J* 13.5 Hz, SC*H*$_2$Ph), 3.80 (d, 1H, *J* 13.5 Hz, SC*H*$_2$Ph), 2.33 (s, 3H, CH_3(Ts)); ^{13}C NMR (100 Mz, $CDCl_3$): δ 165.8, 165.4, 165.3 (3 × Ph*C*O), 137.1–128.0 (Ar), 81.8 (C-1), 71.8 (C-2), 70.2 (C-3), 68.9 (C-5), 68.6 (C-6), 66.7 (C-4), 34.9 (S*C*H$_2$Ph), 20.9 (CH_3(Ts)). HRMS (MALDI): *m/z* calcd for $[C_{41}H_{36}O_{10}S_2]Na^+$: 775.1648. Found: 775.1661.

4.2.1.6. Benzyl 2,3,4-tri-O-benzoyl-6-deoxy-1-thio-α-D-mannopyranoside (3).

Compound **2** (0.65 g, 0.86 mmol) was dissolved in DMF (8 mL) and sodium borohydride (0.16 g, 4.23 mmol) was added slowly. The resulting mixture was stirred at 65 °C for 6 h. The reaction mixture was cooled to rt, neutralised with glacial AcOH and diluted with CH_2Cl_2 (30 mL). The organic phase was separated, washed with satd $NaHCO_3$ (3 × 20 mL), water (2 × 20 mL), dried with anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexanes:EtOAc 1:0 →12:1) to give **3** (0.17 g, 34%) as an oil. $[\alpha]_D$—36.7 (*c* 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07–7.22 (m, 20H, Ar), 5.77–5.74 (m, 2H, H-2, H-3), 5.68 (t, 1H, $J_{3,4}$ 6.4 Hz, $J_{4,5}$ 6.4 Hz, H-4), 5.35 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.51 (dq, 1H, H-5), 3.92 (d, 1H, *J* 9.0 Hz, SC*H*$_2$Ph), 3.85 (d, 1H, *J* 9.0 Hz, SC*H*$_2$Ph), 1.32 (d, 3H, $J_{5,CH3}$ 6.2 Hz, CH_3); ^{13}C NMR (100 Mz, $CDCl_3$): δ 165.8, 165.4, 165.3 (3 × Ph*C*O), 137.8–127.4 (Ar), 81.8 (C-1), 72.2 (2×) (C-2, C-4), 70.7 (C-3), 67.6 (C-5), 35.4 (S*C*H$_2$Ph), 17.0 (CH_3). HRMS (MALDI): *m/z* calcd for $[C_{34}H_{30}O_7S]Na^+$: 605.1610. Found: 605.1607.

4.2.1.7. Benzyl 2,3,4-tri-O-benzoyl-6-deoxy-α-D-mannopyranosyl sulfone (4).

Oxidation of compound **3** (0.09 g, 0.15 mmol) according to general procedure (Method A) and purification by column chromatography (hexanes:EtOAc 15:1→5:1) gave **4** (0.09 g, 95%) as an oil. $[\alpha]_D$—50.0 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.07–7.22 (m, 20H, Ar), 6.33 (dd, 1H, $J_{2,3}$ 3.8 Hz, H-2), 6.09 (dd, 1H, $J_{3,4}$ 10.0 Hz, H-3), 5.72 (t, 1H, $J_{4,5}$ 9.9 Hz, H-4), 4.97 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.88 (dq, 1H, H-5), 4.58 (d, 1H, *J* 14.4 Hz, SC*H*$_2$Ph), 4.37 (d, 1H, *J* 14.4 Hz, SC*H*$_2$Ph), 1.41 (d, 3H, $J_{5,CH3}$ 6.2 Hz, CH_3); ^{13}C NMR (100 Mz, $CDCl_3$): δ 165.8, 165.1, 165.0 (3 × Ph*C*O), 133.8–127.1 (Ar), 87.1 (C-1), 72.2 (C-5), 70.7 (C-4), 69.9 (C-3), 60.5 (SO_2CH_2Ph), 18.3 (CH_3). HRMS (MALDI): *m/z* calcd for $[C_{34}H_{40}O_9S]Na^+$: 637.1508. Found: 637.1515.

4.2.1.8. Benzyl 6-deoxy-α-D-mannopyranosyl sulfone (5).

Deprotection of compound **4** (0.065 g, 0.11 mmol) according to general procedure (Method D) and purification by column chromatography (CH_3CN:MeOH 1:0→15:1) gave **5** (0.022 g, 69%). White foam, $[\alpha]_D$ + 85 (*c* 0.6, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50–7.40 (m, 5H, Ar), 4.75 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1), 4.62 (d, 1H, *J* 14.1 Hz, SO_2CH_2Ph), 4.44 (dd, 1H, $J_{2,3}$ 3.6 Hz, H-2), 4.38 (d, 1H, *J* 14.1 Hz, SO_2CH_2Ph), 4.25 (m, 1H, H-5), 3.93 (dd, 1H, $J_{3,4}$ 9.4 Hz, H-3), 3.49 (t, 1H, $J_{4,5}$ 9.4 Hz H-4), 1.36 (d, 3H, $J_{5,CH3}$ 6.2 Hz, CH_3); ^{13}C NMR (100 Mz, $CDCl_3$): δ 137.1–131.4 (Ar), 89.9 (C-1), 73.7 (C-5), 71.4 (2×) (C-3, C-4), 65.5 (C-2), 56.4 (S*C*H$_2$Ph), 17.2 (CH_3). HRMS (MALDI): *m/z* calcd for $[C_{13}H_{18}O_6S]Na^+$: 325.0722. Found: 325.0745.

4.2.1.9. Methyl 2,3,4-tri-O-benzoyl-6-O-tosyl-α-D-mannopyranoside (7).

To a cooled (0 °C) solution of methyl-α-D-mannopyranoside **6** (1.18 g, 6.1 mmol) in dry pyridine (7 mL), TsCl (1.28 g, 6.7 mmol) in dry pyridine (5 mL) was added over 15 minutes. The reaction mixture was then stirred overnight at rt, the solvent was evaporated and the residue was purified by column chromatography (hexane:EtOAc 1:1→EtOAc) to afford methyl-6-O-tosyl-α-D-mannopyranoside (0.90 g, 2.6 mmol), which was dissolved in dry pyridine (12 mL). BzCl (1.5 mL, 12.9 mmol) was added dropwise over 20 minutes at 0 °C to precooled solution. After stirring at rt overnight, the reaction mixture was poured on ice cold water (100 mL) and extracted with DCM (2 × 20 mL). The combined organic layers were washed with aqueous 1M HCl (40 mL), water (40 mL), brine (40 mL), dried with Na_2SO_4, filtered and concentrated. The residue was washed with ethanol and the solvent was evaporated. Ethanol was added again and crystals were filtered, washed with small amount of cold EtOH and dried under reduced pressure to obtain **7** (1.2 g, 30% over 2 steps) as white crystals. $[\alpha]_D$—108 (c 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09–7.19 (m, 19H, Ar), 5.83 (dd, 1H, $J_{3,4}$ 9.8 Hz, H-3), 5.76 (t, 1H, $J_{4,5}$ 9.6 Hz, H-4), 5.62 (dd, 1H, $J_{2,3}$ 3.1 Hz, H-2), 4.91 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1), 4.28–4.24 (m, 3H, H-5, H-6a, H-6b), 3.49 (s, 3H, OCH_3), 2.35 (s, 3H, CH_3(Ts)); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.4(2×), 165.3 (3 × Ph*C*O), 144.8–128.0 (Ar), 98.5 (C-1), 70.3 (C-2), 69.7 (C-3), 68.7 (C-5), 68.4 (C-6), 66.8 (C-4), 55.6 (OMe), 21.6 (CH_3(Ts)). HRMS (MALDI): *m/z* calcd for $[C_{35}H_{32}O_{11}S]Na^+$: 683.1563. Found: 683.1553.

4.2.1.10. Methyl 2,3,4-tri-O-acetyl-α-D-rhamnopyranoside (8).

To a solution of **7** (0.87 g, 1.32 mmol) in DMF (15 mL) $NaBH_4$ (0.25 g, 6.60 mmol) was added and the suspension was stirred at 65 °C under inert atmosphere for 4 h. Then the reaction mixture was cooled to 0 °C, diluted with DCM (50 mL) and glacial acetic acid (1.8 mL) was added. It was stirred until evolution of hydrogen ceased. The solution was shaken with water (50 mL) and aqueous layer extracted with DCM (50 mL). The organic layers were combined, washed with water (3 × 50 mL), satd. aqueous $NaHCO_3$ (50 mL), brine (1 × 50 mL), dried with Na_2SO_4 and concentrated. The residue was purified by column chromatography (hexane:EtOAc 8:1→5:1) to afford methyl 2,3,4-tri-O-benzoyl-α-D-rhamnopyranoside (0.40 g, 62%).

Methyl 2,3,4-tri-O-benzoyl-α-D-rhamnopyranoside (0.40 g, 0.816 mmol) was dissolved in 0.02 M methanolic MeONa (10 mL) and stirred for 20 h at rt. The suspension was neutralised with AcOH, concentrated and the residue was purified by column chromatography (EtOAc:MeOH 10:1→5:1) yielding methyl α-D-rhamnopyranoside (0.13 g, 96%). Ac_2O (0.31 mL, 3.29 mmol) was added dropwise to the cooled (0 °C) solution of methyl

α-D-rhamnopyranoside (0.13 g, 0.73 mmol) in pyridine (2 mL). The solution was stirred at rt overnight and then poured on ice (25 mL), extracted with DCM (2×), the organic layers were combined and washed with 1 M HCl (2×), dried with Na_2SO_4 and concentrated to afford **8** (0.20 g, 90%) as a colourless oil. $[\alpha]_D$ + 49.7 (c 1.1, $CHCl_3$); lit[27] $[\alpha]_D$ + 53.7 (c 1.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.29 (dd, 1H, $J_{3,4}$ 10.2 Hz, H-3), 5.24 (dd, 1H, $J_{2,3}$ 3.5 Hz, H-2), 5.07 (t, 1H, $J_{4,5}$ 9.8 Hz, H-4), 4.63 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 3.86 (dq, 1H, $J_{5,CH3}$ 6.3 Hz, H-5), 3.39 (s, 1H, OCH_3), 2.15, 2.05, 1.99 (each s, each 3H, 3× CH_3CO), 1.24 (d, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 170.0(2×) (3 × CH_3CO), 98.5 (C-1), 71.1, 69.8, 69.1, 66.2 (C-2, C-3, C-4, C-5), 55.1(OMe), 20.9, 20.8, 20.7 (3 × CH_3CO), 17.4 (CH_3). HRMS (MALDI): *m/z* calcd for $[C_{13}H_{20}O_8]Na^+$: 327.1056. Found: 327.1059.

4.2.1.11. 1,2,3,4-Tetra-O-acetyl-α-D-rhamnopyranoside (9)[28].

To the solution of **8** (0.20 g, 0.65 mmol) in AcOH (3.5 mL) and Ac_2O (1 mL) cooled to 0 °C was added conc. H_2SO_4 (0.15 mL). After 1 h of stirring at 0 °C, NaOAc·3H_2O (80 mg) was added and mixture was concentrated. The residue was purified by column chromatography (hexane:EtOAc 4:1→2:1) providing **9** (0.20 g, 93%) as a colourless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.02 (d, 1H, $J_{1,2}$ 2.0 Hz, H-1), 5.31 (dd, 1H, $J_{3,4}$ 10.2 Hz, H-3), 5.25 (dd, 1H, $J_{2,3}$ 3.5 Hz, H-2), 5.13 (t, 1H, $J_{4,5}$ 10.0 Hz, H-4), 3.94 (dq, 1H, $J_{5,CH3}$ 6.2 Hz, H-5), 2.17, 2.16, 2.07, 2.01 (each s, each 3H, 4× CH_3CO), 1.24 (d, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.1, 169.8(2×), 168.4 (4 × CH_3CO), 90.6 (C-1), 70.5 (C-4), 68.8 (C-3), 68.7 (C-5), 68.6 (C-2), 20.9, 20.8, 20.7(2×) (4 × CH_3CO), 17.4 (CH_3). HRMS (MALDI): *m/z* calcd for $[C_{14}H_{20}O_9]Na^+$: 355.1005. Found: 355.1011.

4.2.1.12. 2,3,5-Tri-O-acetyl-α-D-rhamnopyranosyl azide (10)[22].

Reaction of **9** (0.2g, 0.60 mmol, 1eq) and $TMSN_3$ (1 eq) catalysed by 1M $SnCl_4$ in CH_2Cl_2 (1 eq) according to general procedure (Method B) and purification by column chromatography (hexane:EtOAc 4:1→2:1) gave azide **10** (0.16 g, 85%) as a clear oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.32 (d, 1H, $J_{1,2}$ 2.0 Hz, H-1), 5.21 (dd, 1H, $J_{3,4}$ 10.2 Hz, H-3), 5.15 (dd, 1H, $J_{2,3}$ 3.5 Hz, H-2), 5.09 (t, 1H, $J_{4,5}$ 9.8 Hz, H-4), 4.04 (dq, 1H, $J_{5,CH3}$ 6.2 Hz, H-5), 2.17, 2.06, 1.99 (each s, each 3H, 3 × CH_3CO), 1.28 (d, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.9, 169.8(2×) (3 × CH_3CO), 87.5 (C-1), 70.5 (C-4), 69.5 (C-2), 68.6 (C-5), 68.3 (C-3), 20.8, 20.7, 20.6 (3 × CH_3CO), 17.4 (CH_3). HRMS (MALDI): *m/z* calcd for $[C_{12}H_{17}N_3O_7]Na^+$: 338.0964. Found: 338.0956.

4.2.1.13. 1-(2,3,4,-Tri-O-acetyl-α-D-rhamnopyranosyl)-4-phenyl-1,2,3-triazole (12).

Reaction of **10** (50 mg, 0.158 mmol) and alkyne **11a** according to general procedure (Method C) and purification by column chromatography (hexane:EtOAc 6:1) gave **12** as a colourless oil (59 mg, 90%). $[\alpha]_D$ + 16 (c 0.25, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.96 (s, 1H, C*H*N), 7.88–7.86 (m, 2H, Ar), 7.47–7.44 (m, 2H, Ar), 7.40–7.36 (m, 1H, Ar), 6.04 (dd, 1H, $J_{2,3}$ 3.7 Hz, H-2), 5.99 (d, 1H, $J_{1,2}$ 2.3 Hz, H-1), 5.91 (dd, 1H, $J_{3,4}$ 9.2 Hz, H-3) 5.20 (t, 1H, $J_{4,5}$ 9.0 Hz, H-4), 3.79 (dq, 1H, $J_{5,CH3}$ 6.5 Hz, H-5), 2.20, 2.07, 2.06 (each s, each 3H, 3 × CH_3CO), 1.27 (d, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.9, 169.8, 169.4 (3 × CH_3CO), 148.3 (NC═CH), 129.9, 128.9, 128.6, 125.9 (Ar), 119.4 (NC═*C*H), 83.8 (C-1), 70.8 (C-4), 70.1 (C-5), 68.9 (C-3), 68.6 (C-2), 20.8(2×), 20.6 (3 × *C*H_3CO), 17.3 (CH_3). HRMS (MALDI): *m/z* calcd for $[C_{20}H_{23}N_3O_7]Na^+$: 440.1434. Found: 440.1426.

4.2.1.14. 1-(2,3,4,-Tri-O-acetyl-α-D-rhamnopyranosyl)-4-benzyl-1,2,3-triazole (13).

Reaction of **10** (50 mg, 0.158 mmol) and alkyne **11b** according to general procedure (Method C) and purification by column chromatography (hexane:EtOAc 5:1→3:1) gave **13** as a colourless oil (62 mg, 91%). $[\alpha]_D$ + 48 (c 0.25, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35 (s, 1H, C*H*N), 7.33–7.25 (m, 5H, Ar), 5.93 (dd, 1H, $J_{2,3}$ 3.8 Hz, H-2), 5.89 (dd, 1H, $J_{3,4}$ 8.8 Hz, H-3), 5.84 (d, 1H, $J_{1,2}$ 2.4 Hz, H-1), 5.15 (t, 1H, $J_{4,5}$ 8.9 Hz, H-4), 4.12–4.08 (m, 2H, CH_2Ph), 3.75 (dq, 1H, $J_{5,CH3}$ 6.3 Hz, H-5), 2.17, 2.06, 2.04 (each s, each 3H, 3× CH_3CO), 1.23 (d, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.9, 169.7, 169.3 (3 × CH_3CO), 148.3 (NC═CH), 138.4, 128.7, 126.6 (Ar), 121.5 (NC═*C*H), 83.5 (C-1), 70.9 (C-4), 69.9 (C-5), 68.9 (C-3), 68.6 (C-2), 32.1 (*C*H_2Ph), 20.7(2×), 20.6 (3 × *C*H_3CO), 17.2 (CH_3). HRMS (MALDI): *m/z* calcd for $[C_{21}H_{25}N_3O_7]Na^+$: 454.1590. Found: 454.1580.

4.2.1.15. 1-(α-D-Rhamnopyranosyl)-4-phenyl-1,2,3-triazole (14).

Deprotection of **12** (59 mg, 0.141 mmol) according to general procedure (Method D) and purification by column chromatography (EtOAc) gave **14** as a colourless oil (34 mg, 83%). $[\alpha]_D$ + 49.4 (c 0.2, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.47 (s, 1H, C*H*N), 7.86–7.83 (m, 2H, Ar), 7.46–7.42 (m, 2H, Ar), 7.38–7.33 (m, 1H, Ar), 6.01 (d, 1H, $J_{1,2}$ 2.5 Hz, H-1), 4.72 (dd, 1H, $J_{2,3}$ 3.4 Hz, H-2), 4.13 (dd, 1H, $J_{3,4}$ 8.7 Hz, H-3), 3.59 (t, 1H, $J_{4,5}$ 8.7 Hz, H-4), 3.42 (dq, 1H, $J_{5,CH3}$ 6.3 Hz, H-5), 1.33 (d, 3H, CH_3); ^{13}C NMR (100 MHz, CD_3OD): δ 149.2 (NC═CH), 131.6, 130.1, 129.7, 126.9 (Ar), 122.2 (NC═*C*H), 88.7 (C-1), 73.8(2×) (C-4, C-5), 72.6 (C-3), 69.3 (C-2), 18.1 (CH_3). HRMS (MALDI): *m/z* calcd for $[C_{14}H_{17}N_3O_4]Na^+$: 314.1117. Found: 314.1119.

4.2.1.16. 1-(α-D-Rhamnopyranosyl)-4-benzyl-1,2,3-triazole (15).

Deprotection of **13** (57 mg, 0.132 mmol) according to general procedure (Method D) and purification by column chromatography (EtOAc:MeOH 10:1→5:1) gave **15** as a colourless oil (24 mg, 60%). $[\alpha]_D$−35.7 (c 0.1, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 7.84 (s, 1H, C*H*N), 7.31–7.18 (m, 5H, Ar), 5.90 (d, 1H, $J_{1,2}$ 2.5 Hz, H-1), 4.63 (dd, 1H, $J_{2,3}$ 3.5 Hz, H-2), 4.07 (s, 2H, CH_2Ph), 4.05 (dd, 1H, $J_{3,4}$ 8.8 Hz, H-3), 3.54 (t, 1H, $J_{4,5}$ 8.8 Hz, H-4), 3.31 (m, 1H, H-5), 1.27 (d, 3H, $J_{5,6}$ 6.3 Hz, CH_3); ^{13}C NMR (100 MHz, CD_3OD): δ 148.9 (NC═CH), 140.5, 129.8(2×), 127.7 (Ar), 123.7 (NC═*C*H), 88.5 (C-1), 73.7 (C-5) 73.6 (C-4), 72.6 (C-3), 70.4 (C-2), 32.7 (*C*H_2Ph), 18.1 (CH_3). HRMS (MALDI): *m/z* calcd for $[C_{15}H_{19}N_3O_4]Na^+$: 328.1273. Found: 328.1282.

4.2.1.17. (6S) Methyl 2,3,4-tri-O-benzyl-6-C-methyl-α-D-mannopyranoside (16).

Compound **16** was synthesised by procedure described previously.[25] $[\alpha]_D$—34.0 (*c* 0.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32–7.19 (m, 15H, Ar), 4.90–4.60 (m, 6H, 3× *C*H_2Ph), 4.67 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1), 4.04–4.00 (m, 2H, H-4, H-6), 3.82 (dd, 1H, $J_{3,4}$ 9.4 Hz, H-3), 3.71 (dd, 1H, $J_{2,3}$ 3.1 Hz, H-2), 3.32 (dd, 1H, $J_{4,5}$ 9.7 Hz, $J_{5,6}$ 1.8 Hz, H-5), 3.22 (s, 3H, OCH_3), 1.23 (d, 3H, *J* 6.5 Hz, C-7(CH_3)); ^{13}C NMR (100 Mz, $CDCl_3$): δ 138.5–138.3 (Ar), 128.3–127.5 (Ar), 99.5 (C-1), 80.4 (C-3), 75.4, 75.1, 74.9, 74.4 (C-2, C-4, C-5, *C*H_2Ph), 73.0, 72.3 (2 × *C*H_2Ph), 65.7 (C-6), 54.8 (OCH_3), 20.4 (C-7). HRMS (MALDI): *m/z* calcd for $[C_{29}H_{34}O_6]Na^+$: 501.2253. Found: 501.2274.

4.2.1.18. (6S) Methyl 6-C-methyl-α-D-mannopyranoside (17).

Compound **16** (2.2 g, 4.60 mmol) was dissolved in MeOH (50 mL) and 10% Pd/C was added. The solution was stirred under a H_2 atmosphere for 4 h, the catalyst was then filtered off and washed with MeOH (30 mL). The solvent was evaporated and purification of the crude product by column chromatography ($CHCl_3$:MeOH 10:1→5:1) yielded **17** (0.94 g, 98%) as an oil. $[\alpha]_D$ + 30.0 (*c* 0.2, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 4.71 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1), 4.14 (dq, 1H, J_d 1.9 Hz, J_q 6.5 Hz, H-6), 3.82–3.79 (m, 2H, H-2, H-4), 3.68 (dd, 1H, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 9.5 Hz, H-3), 3.38 (s, 3H, OCH_3), 3.29 (dd, 1H, $J_{4,5}$ 9.7 Hz, H-5), 1.32 (d, 3H, *J* 6.6 Hz, C-7(CH_3)); ^{13}C NMR (100 Mz, CD_3OD): δ 102.8 (C-1), 75.9 (C-5), 72.9 (C-3), 72.1, 68.3 (C-2, C-4), 65.9 (C-6), 55.2 (OCH_3), 20.2 (C-7). HRMS (MALDI): *m/z* calcd for $[C_8H_{16}O_6]Na^+$: 231.0845. Found: 231.0901.

4.2.1.19. (6S) Methyl 2,3,4-tri-O-acetyl-6-C-methyl-α-D-mannopyranoside (18).

A solution of compound **17** (0.9 g, 4.32 mmol) in pyridine (15 mL) was cooled at 0 °C and acetic anhydride (30 mL) was added. The

reaction mixture was stirred at rt for 16 h, then diluted with CH_2Cl_2 (50 mL) and washed with satd. $NaHCO_3$ (3 × 30 mL) and 1M HCl (3 × 20 mL). The organic phase was separated, dried with anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (hexanes:EtOAc 10:1→2:1) to give **18** (1.60 g, 98%) as an oil. $[\alpha]_D$ + 45.2 (*c* 0.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.31–5.25 (m, 3H, H-2, H-3, H-4), 5.07 (dq, 1H, J_d 1.9 Hz, J_q 6.6 Hz, H-6), 4.78 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 3.76 (dd, 1H, $J_{4,5}$ 9.4 Hz, $J_{5,6}$ 2.1 Hz, H-5), 3.40 (s, 3H, OCH_3), 2.16, 2.10, 2.01, 1.97 (each s, each 3H, 4 × CH_3CO), 1.35 (d, 3H, *J* 6.6 Hz, C-7(CH_3)); ^{13}C NMR (100 Mz, $CDCl_3$): δ 170.7, 170.2, 170.0, 169.7 (4× CH_3CO), 99.1 (C-1), 71.5 (C-5), 69.7, 69.5 (C-2, C-3), 66.5 (C-6), 65.8 (C-4), 55.4 (OCH_3), 21.2, 21.1, 20.8, 20.7 (4 × CH_3CO), 15.9 (C-7). HRMS (MALDI): *m/z* calcd for $[C_{16}H_{24}O_{10}]Na^+$: 399.1267. Found: 399.1279.

4.2.1.20. (6S) Benzyl 2,3,4-tri-O-acetyl-6-C-methyl-1-thio-α-D-mannopyranoside (20).

To a solution of **19**[26] (prepared in 96% yield from **18** by acetolysis as described for compound **8**) (56 mg, 0.14 mmol) in CH_2Cl_2 (3 mL) benzyl mercaptan (32.5 µL, 0.28 mmol) and BF_3OEt_2 (52.6 µL, 0.42 mmol) were added dropwise at 0 °C. After being stirred at rt for 16 h, the reaction was diluted with DCM (15 mL) and poured into cold satd. $NaHCO_3$ (10 mL). The organic phase was separated, washed with water, dried (Na_2SO_4), filtered and concentrated. The residue was purified by column chromatography hexane:EtOAc 7:1→3:1) to give **20** (45 mg, 70%) as a clear oil. $[\alpha]_D$ + 132 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.24–7.19 (m, 5H, Ar), 5.32–5.26 (m, 2H, H-2, H-4), 5.18 (dd, 1H, $J_{2,3}$ 3.4 Hz, $J_{3,4}$ 10.0 Hz, H-3), 5.09 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 5.06 (dq, 1H, J_d 2.0 Hz, J_q 6.7 Hz, H-6), 4.19 (dd, 1H, $J_{4,5}$ 9.8 Hz, $J_{5,6}$ 2.0 Hz, H-5), 3.68 (d, 1H, *J* 11.9 Hz, CH_2Ph), 3.66 (d, 1H, *J* 11.9 Hz, CH_2Ph), 2.06, 2.05, 1.96, 1.89 (each s, each 3H, 4 × CH_3CO), 1.26 (d, 3H, *J* 6.6 Hz, C-7(CH_3)); ^{13}C NMR (100 Mz, $CDCl_3$): δ 170.7, 170.0, 169.9, 169.7 (4× CH_3CO), 136.5, 129.1, 128.9, 127.7 (Ar), 81.6 (C-1), 72.2 (C-5), 70.9 (C-2), 70.1 (C-3), 66.5 (C-6), 66.0 (C-4), 34.6 (CH_2Ph), 21.2, 21.1, 20.8, 20.7 (4 × CH_3CO), 16.1 (C-7). HRMS (MALDI): *m/z* calcd for $[C_{22}H_{28}O_9S]Na^+$: 491.1352. Found: 491.1371.

4.2.1.21. (6S) Benzyl 2,3,4,6-tetra-O-acetyl-6-C-methyl-α-D-mannopyranosyl sulfone (21).

Oxidation of **20** (40 mg, 0.08 mmol) according to general procedure (Method A) and purification by column chromatography (hexanes:EtOAc 3:1→1.5:1) provided **21** (36 mg, 85%) as a clear oil. $[\alpha]_D$ + 54 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45–7.40 (m, 5H, Ar), 5.90 (dd, 1H, $J_{2,3}$ 3.7 Hz, H-2), 5.55 (dd, 1H, $J_{3,4}$ 9.8 Hz, H-3), 5.36 (t, 1H, $J_{4,5}$ 9.8 Hz, H-4), 5.11 (dq, 1H, J_d 2.3 Hz, J_q 6.6 Hz, H-6), 4.78 (d, 1H, $J_{1,2}$ 1.7 Hz, H-1), 4.60 (dd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6}$ 2.3 Hz, H-5), 4.48 (d, 1H, *J* 14.2 Hz, CH_2Ph), 4.28 (d, 1H, *J* 14.2 Hz, CH_2Ph), 2.12, 2.11, 2.03, 1.98 (each s, each 3H, CH_3CO), 1.44 (d, 3H, *J* 6.6 Hz, C-7(CH_3)); ^{13}C NMR (100 Mz, $CDCl_3$): δ 170.4, 4 169.6, 169.4, 169.3 (4× CH_3CO), 130.9, 129.6, 129.5, 126.7 (Ar), 86.2 (C-1), 76.6 (C-5), 69.3 (C-3), 66.3 (C-6), 64.8(2×) (C-2, C-4), 57.3 (CH_2Ph), 21.1, 20.8, 20.7, 20.6 (4 × CH_3CO), 16.2 (C-7). HRMS (MALDI): *m/z* calcd for $[C_{22}H_{28}O_{11}S]Na^+$: 523.1250. Found: 523.1257.

4.2.1.22. (6S) Benzyl 6-C-methyl-α-D-mannopyranosyl sulfone (22).

Deprotection of compound **21** (30 mg, 0.06 mmol) according to general procedure (Method D) and purification by column chromatography (CH_3CN:MeOH 9:1) afforded **22** (17 mg, 85%) as a white foam. $[\alpha]_D$ + 98 (*c* 0.4, MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ 7.51–7.40 (m, 5H, Ar), 4.84 (d, 1H, $J_{1,2}$ 1.6 Hz, H-1), 4.60 (d, 1H, *J* 14.0 Hz, CH_2Ph), 4.41 (dd, 1H, $J_{2,3}$ 3.1 Hz, H-2), 4.36 (d, 1H, *J* 14.0 Hz, CH_2Ph), 4.17 (dq, 1H, J_d 1.8 Hz, J_q 6.6 Hz, H-6), 4.05–3.96 (m, 3H, H-3, H-4, H-5), 1.43 (d, 3H, *J* 6.7 Hz, C-7(CH_3)); ^{13}C NMR (100 Mz, CD_3OD): δ 132.4, 129.9, 129.8, 126.37 (Ar), 90.6 (C-1), 81.5 (C-5), 73.2, 67.3 (C-3, C-4), 66.6 (C-2), 65.9 (C-6), 57.3 (CH_2Ph), 20.4 (C-7). HRMS (MALDI): *m/z* calcd for $[C_{14}H_{20}O_7S]Na^+$: 355.0827. Found: 355.0841.

4.2.1.23. (6S) 2,3,4,6-Tetra-O-acetyl-6-C-methyl-α-D-mannopyranosyl azide (23).

Reaction of **19** (0.2g, 0.50 mmol, 1eq) and $TMSN_3$ (2.5eq) catalysed with 1M $SnCl_4$ in CH_2Cl_2 (0.9 eq) according to general procedure (Method B) and purification by column chromatography (hexane:EtOAc 3:1→1.5:1) provided **23** (0.17 g, 88%) as a clear oil; $[\alpha]_D$ + 30.0 (*c* 0.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.46 (d, 1H, $J_{1,2}$ 1.8 Hz, H-1), 5.33 (t, 1H, $J_{3,4}$ 10.0 Hz, H-4), 5.23 (dd, 1H, $J_{2,3}$ 3.4 Hz, H-3), 5.16 (dd, 1H, 1H, H-2), 5.11 (dq, 1H, J_d 2.1 Hz, J_q 6.6 Hz, H-6), 3.94 (dd, 1H, H-5), 2.18, 2.11, 2.02, 1.98 (each s, each 3H, 4 × CH_3CO), 1.36 (d, 3H, *J* 6.6 Hz, C-7(CH_3)); ^{13}C NMR (100 Mz, $CDCl_3$): δ 170.5, 170.0, 169.9, 169.6 (4× CH_3CO), 87.9 (C-1), 73.8 (C-5), 69.3 (C-2), 68.7 (C-3), 66.2 (C-6), 65.3 (C-4), 21.1, 21.0, 20.7(2×) (4 × CH_3CO), 15.9 (C-7). HRMS (MALDI): *m/z* calcd for $[C_{15}H_{21}O_9N_3]Na^+$: 410.1176. Found: 410.1181.

4.2.1.24. (6S) 1-(2,3,4,6-Tetra-O-acetyl-6-C-methyl-α-D-mannopyranosyl)-4-phenyl-1,2,3-triazole (24).

Reaction of **23** (40 mg, 0.1 mmol) and alkyne **11a** according to general procedure (Method C) and purification by column chromatography (hexane:EtOAc 4:1→1:1) gave **24** as a colourless oil (46 mg, 91%). $[\alpha]_D$ + 51.6 (*c* 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.98 (s, 1H, C*H*N), 7.87–7.83 (m, 2H, Ar), 7.48–7.38 (m, 3H, Ar), 6.13 (d, 1H, $J_{1,2}$ 2.4 Hz, H-1), 6.09 (dd, 1H, $J_{2,3}$ 3.8 Hz, H-2), 5.96 (dd, 1H, $J_{3,4}$ 9.3 Hz, H-3), 5.43 (t, 1H, $J_{4,5}$ 9.3 Hz, H-4), 5.11 (dq, 1H, J_d 2.8 Hz, J_q 6.6 Hz, H-6), 3.64 (dd, 1H, H-5), 2.12, 2.05, 2.04, 2.03 (each s, each 3H, 4 × CH_3CO), 1.22 (d, 3H, *J* 6.6 Hz, C-7(CH_3)); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 169.9, 169.7, 169.4 (4 × CH_3CO), 148.6 (N*C*═CH), 129.8, 129.1, 128.8, 126.0, 119.7 (Ar, NC═*C*H), 84.2 (C-1), 75.2 (C-5), 69.3 (C-3), 68.4 (C-2), 65.8(2×) (C-4, C-6), 21.0, 20.7, 20.6 (2×) (4 × CH_3CO), 14.3 (C-7). HRMS (MALDI): *m/z*: calcd for $[C_{23}H_{27}N_3O_9]Na^+$: 512.1645. Found: 512.1649.

4.2.1.25. (6S) 1-(2,3,4,6-Tetra-O-acetyl-6-C-methyl-α-D-mannopyranosyl)-4-benzyl-1,2,3-triazole (25).

Reaction of **23** (51 mg, 0.13 mmol) and alkyne **11b** according to general procedure (Method C) and purification by column chromatography (hexane:EtOAc 4:1→1:1) gave **25** as a colourless oil (50 mg, 76%). $[\alpha]_D$—20.0 (*c* 0.1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33–7.22 (m, 6H, C*H*N, Ar), 6.00 (dd, 1H, $J_{2,3}$ 3.9 Hz, H-2), 5.97 (d, 1H, $J_{1,2}$ 2.2 Hz, H-1), 5.91 (dd, 1H, $J_{3,4}$ 9.3 Hz, H-3), 5.38 (t, 1H, $J_{4,5}$ 9.4 Hz, H-4), 5.05 (dq, 1H, J_d 2.7 Hz, J_q 6.6 Hz, H-6), 4.12 (m, 2H, PhCH_2), 3.51 (dd, 1H, H-5), 2.18, 2.08, 2.02, 2.01 (each s, each 3H, 4 × CH_3CO), 1.13 (d, 3H, *J* 6.6 Hz, C-7(CH_3)); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.0, 169.7, 169.4 (4 × CH_3CO), 148.8 (N*C*═CH), 138.6, 129.8, 128.9, 128.8, 126.7, 121.8 (Ar, NC═*C*H), 84.1 (C-1), 74.9 (C-5), 69.3 (C-3), 68.5 (C-2), 65.8(2×) (C-4, C-6), 32.2 (CH_2Ph), 21.1, 20.9, 20.8, 20.7 (4 × CH_3CO), 15.9 (C-7). HRMS (MALDI): *m/z*: calcd for $[C_{24}H_{29}N_3O_9]Na^+$: 526.1802. Found: 526.1809.

4.2.1.26. (6S) 6-C-Methyl-1-α-D-mannopyranosyl-4-phenyl-1,2,3-triazole (26).

Deprotection of **24** (45 mg, 0.09 mmol) according to general procedure (Method D) and purification by column chromatography (CH_3CN:MeOH 10:1→5:1) gave **26** as white powder (23.6 mg, 80%). $[\alpha]_D$ + 48 (*c* 0.2, MeOH). ^{1}H NMR (400 MHz, CD_3OD): δ 8.49 (s, 1H, C*H*N), 7.86–7.84 (m, 2H, Ar), 7.47–7.36 (m, 3H, Ar), 6.13 (d, 1H, $J_{1,2}$ 3.0 Hz, H-1), 4.72 (t, 1H, $J_{2,3}$ 3.3 Hz, H-2), 4.18 (dd, 1H, $J_{3,4}$ 8.0 Hz, H-3), 4.13 (dq, 1H, J_d 2.8 Hz, J_q 6.5 Hz, H-6), 4.01 (t, 1H, $J_{4,5}$ 8.4 Hz, H-4), 3.14 (dd, 1H, $J_{5,6}$ 2.6 Hz, H-5), 1.22 (d, 3H, *J* 6.5 Hz, C-7(CH_3)); ^{13}C NMR (100 MHz, CD_3OD): δ 149.1 (N*C*═CH), 131.4, 130.0, 129.6, 127.8, 121.9 (Ar, NC═*C*H), 88.5 (C-1), 80.4 (C-5), 73.1 (C-3), 70.0 (C-2), 68.7

(C-4), 65.9 (C-6), 20.0 (C-7). HRMS (MALDI): *m/z*: calcd for $[C_{15}H_{19}N_3O_5]Na^+$: 344.1222. Found: 344.1230.

4.2.1.27. (6S) 4-Benzyl-6-C-methyl-1-α-D-mannopyranosyl-1,2,3-triazole (27).

Deprotection of **25** (37 mg, 0.073 mmol) according to general procedure (Method D) and purification by column chromatography (CH_3CN:MeOH 10:1→5:1) gave **27** as white powder (20 mg, 82%). $[\alpha]_D$—27.3 (*c* 0.1, MeOH). ^1H NMR (400 MHz, CD_3OD): δ 7.81 (s, 1H, C*H*N), 7.29–7.18 (m, 5H, Ar), 6.00 (d, 1H, $J_{1,2}$ 2.8 Hz, H-1), 4.66 (t, 1H, $J_{2,3}$ 3.2 Hz, H-2), 4.09–4.05 (m, 4H, H-3, H-6, CH_2Ph), 3.94 (t,1H, $J_{3,4}$ 8.6 Hz, H-4), 2.95 (dd, 1H, $J_{5,6}$ 2.6 Hz, H-5), 1.12 (d, 3H, *J* 6.5 Hz, C-7(CH_3)); ^{13}C NMR (100 MHz, CD_3OD): δ 148.9 (N*C*═CH), 140.2, 129.7, 129.6, 127.5, 123.5 (Ar, NC═*C*H), 88.4 (C-1), 80.1 (C-5), 73.0 (C-3), 69.9 (C-2), 68.5 (C-4), 65.6 (C-6), 32.5 (Ph*C*H_2), 19.9 (C-7). HRMS (MALDI): *m/z*: calcd for $[C_{16}H_{21}N_3O_5]Na^+$: 358.1379. Found: 358.1387.

4.2.1.28. Benzyl 2,3,5-tri-O-benzoyl-α-D-lyxofuranosyl sulfone (29).

To a precooled at 0 °C solution of **28**[29] (0.22 g, 0.44 mmol) in CH_2Cl_2 (5 mL) benzyl mercaptan (68 mg, 64 μL, 0.55 mmol) and BF_3OEt_2 (0.22g, 0.19 mL, 1.53 mmol) were added dropwise. After being stirred at rt for 16 h, the reaction was diluted with DCM (15 mL) and poured into cold satd. $NaHCO_3$ (10 mL). The organic phase was separated, washed with water, dried (Na_2SO_4), filtered and concentrated. The residue was dissolved in CH_2Cl_2 (10 mL), cooled at 0 °C and treated with *m*CPBA (2.5eq) according to general procedure (Method A). The crude product was purified by column chromatography (hexane:EtOAc 6:1→4:1) to afford **29** (0.15 g, 66% over 2 steps, purity > 95%) as a clear oil; $[\alpha]_D$ + 55 (*c* 0.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.02–7.28 (m, 20H, Ar), 6.40 (dd, 1H, $J_{2,3}$ 5.4 Hz, H-2), 6.15 (dd, 1H, $J_{3,4}$ 5.1 Hz, H-3), 5.14 (m, 1H, H-4), 5.05 (d, 1H, $J_{1,2}$ 4.1 Hz, H-1), 4.79 (dd, 1H, $J_{4,5b}$ 6.9 Hz, $J_{5a,5b}$ 11.8 Hz, H-5b), 4.62 (dd, 1H, $J_{4,5a}$ 5.1 Hz, H-5a), 4.58 (d, 1H, *J* 14.0 Hz, CH_2Ph), 4.30 (d, 1H, *J* 14.0 Hz, CH_2Ph); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.2, 165.1, 164.6 (3 × Ph*C*O), 133.6–128.4 (*Ph*CO, CH_2*Ph*), 90.7 (C-1), 80.2, 72.2, 71.1, 60.6, 56.7 (C-2, C-3, C-4, C-5, *C*H_2Ph). HRMS (MALDI): *m/z*: calcd for $[C_{33}H_{28}O_9S]Na^+$: 623.1352. Found: 623.1361.

4.2.1.29. Benzyl α-D-lyxofuranosyl sulfone (30).

Deprotection of compound **29** (0.12 g, 0.20 mmol) according to general procedure (Method D) and purification by column chromatography (CH_3CN:MeOH 10:1→5:1) gave **30** (46 mg, 80%). Yellow oil, $[\alpha]_D$ + 32 (*c* 0.3, MeOH); ^1H NMR (400 MHz, CD_3OD): δ 7.76–7.41 (m, 5H, Ar), 4.78 (t, 1H, $J_{3,4}$ 5.1 Hz, H-3), 4.70 (d, 1H, $J_{1,2}$ 5.2 Hz, H-1), 4.61 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.41 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.34–4.32 (m, 1H, H-4), 4.28 (dd, 1H, $J_{2,3}$ 4.8 Hz, H-2), 3.88 (dd, 1H, $J_{4,5a}$ 4.4 Hz, H-5a), 3.84 (dd, 1H, $J_{4,5b}$ 6.3 Hz, $J_{5a,5b}$ 12.0 Hz, H-5b); ^{13}C NMR (100 MHz, CD_3OD): δ 132.3, 129.8 (CH_2*Ph*), 94.3 (C-1), 85.7, 73.1, 72.6 (C-2, C-3, C-4), 61.4 (C-5), 56.8 (*C*H_2Ph). HRMS (MALDI): *m/z*: calcd for $[C_{12}H_{16}O_6S]Na^+$: 311.0565. Found: 311.0571.

4.2.1.30. 2,3,5-Tri-O-acetyl-α-D-lyxofuranosyl azide (32).

Reaction of **31**[30] (0.53g, 1.66 mmol, 1eq) and $TMSN_3$ (1.3eq) catalysed by 1 M $SnCl_4$ in CH_2Cl_2 (1.05eq) according to general procedure (Method B) and purification by column chromatography (hexane:EtOAc 3:1→1.5:1) gave azide **32** (0.39 g, 78%) as a clear oil; $[\alpha]_D$ + 82 (*c* 0.8, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 5.51 (t, 1H, $J_{3,4}$ 5.2 Hz, H-3), 5.43 (d, 1H, $J_{1,2}$ 3.0 Hz, H-1), 5.08 (dd, 1H, $J_{2,3}$ 5.0 Hz, H-2), 4.55 (dt, 1H, H-4), 4.22 (dd, 1H, $J_{4,5a}$ 4.8 Hz, H-5a), 4.17 (dd, 1H, $J_{4,5b}$ 7.4 Hz, $J_{5a,5b}$ 11.8 Hz, H-5b), 2.05, 2.04, 2.03 (each s, each 3H, 3 × CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 169.5, 169.3 (3 × CH_3CO), 93.0 (C-1), 76.8 (C-4), 75.4 (C-2), 70.7 (C-3), 62.4 (C-5), 20.8, 20.5, 20.4 (3 × *C*H_3CO). HRMS (MALDI): *m/z*: calcd for $[C_{11}H_{15}N_3O_7]Na^+$: 324.0808. Found: 324.0814.

4.2.1.31. 1-(2,3,5-Tri-O-acetyl-α-D-lyxofuranosyl)-4-phenyl-1,2,3-triazole (33).

Reaction of **32** (0.13 g, 0.43 mmol) and alkyne **11a** according to general procedure (Method C) gave **33** after purification by column chromatography (hexane:EtOAc 4:1→2.5:1). White powder (0.16 g, 91%). $[\alpha]_D$ + 25 (*c* 0.2, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.93 (s, 1H, C*H*N), 7.84–7.81 (m, 2H, Ar), 7.47–7.33 (m, 3H, Ar), 6.21 (d, 1H, $J_{1,2}$ 4.6 Hz, H-1), 6.10 (t, 1H, $J_{2,3}$ 4.8 Hz, H-2), 5.87 (t, 1H, $J_{3,4}$ 4.8 Hz, H-3), 4.83 (dt, 1H, H-4), 4.35 (dd, 1H, $J_{4,5a}$ 4.8 Hz, $J_{5a,5b}$ 11.9 Hz, H-5a), 4.29 (dd, 1H, $J_{4,5b}$ 7.1 Hz, H-5b), 2.09, 2.07, 2.04 (each s, each 3H, 3 × CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 169.3, 169.1 (3 × CH_3CO), 148.2 (N*C*═CH), 129.9, 128.8, 128.5, 125.9, 119.2 (Ar, NC═*C*H), 89.7 (C-1), 78.4 (C-4), 75.2 (C-2), 71.0 (C-3), 61.9 (C-5), 20.7, 20.4, 20.3 (3 × *C*H_3CO). HRMS (ESI-MS): *m/z*: calcd for $[C_{19}H_{21}N_3O_7]Na^+$: 426.1277. Found: 426.1280.

4.2.1.32. 1-(2,3,5-Tri-O-acetyl-α-D-lyxofuranosyl)-4-benzyl-1,2,3-triazole (34).

Reaction of **32** (0.13 g, 0.43 mmol) and alkyne **11b** according to general procedure (Method C) gave **34** after purification by column chromatography (hexane:EtOAc 4:1→2.5:1). White powder (0.13 g, 72%). $[\alpha]_D$—36 (*c* 0.25, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 7.34–7.22 (m, 6H, C*H*N, Ar), 6.07 (d, 1H, $J_{1,2}$ 4.5 Hz, H-1), 6.01 (t, 1H, $J_{2,3}$ 4.7 Hz, H-2), 5.83 (t, 1H, $J_{3,4}$ 4.8 Hz, H-3), 4.76 (dt, 1H, H-4), 4.29 (dd, 1H, $J_{4,5a}$ 4.9 Hz, $J_{5a,5b}$ 11.8 Hz, H-5a), 4.24 (dd, 1H, $J_{4,5b}$ 7.2 Hz, H-5b), 4.11 (m, 2H, CH_2Ph), 2.14, 2.07, 2.06 (each s, each 3H, 3 × CH_3CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 169.5, 169.2 (3 × CH_3*C*O), 148.4 (N*C*═CH), 138.6, 128.9, 128.8, 126.8, 121.3 (Ar, NC═*C*H), 89.6 (C-1), 78.4 (C-4), 75.3 (C-2), 71.2 (C-3), 62.1 (C-5), 32.3 (*C*H_2Ph), 20.9, 20.6, 20.5 (3 × CH_3CO). HRMS (MALDI): *m/z*: calcd for $[C_{20}H_{23}N_3O_7]Na^+$: 440.1434. Found: 440.1439.

4.2.1.33. 1-α-D-Lyxofuranosyl-4-phenyl-1,2,3-triazole (35).

Deprotection of **33** (0.12 g, 0.30 mmol) according to general procedure (Method D) and purification by column chromatography (CH_3CN:MeOH 1:0→6.5:1) gave **35**. White powder (72 mg, 88%). $[\alpha]_D$ + 23.3 (*c* 0.2, MeOH); ^1H NMR (400 MHz, CD_3OD): δ 8.53 (s, 1H, C*H*N), 7.86–7.80 (m, 2H, Ar), 7.45–7.32 (m, 3H, Ar), 6.09 (d, 1H, $J_{1,2}$ 5.8 Hz, H-1), 4.86 (dd, 1H, $J_{2,3}$ 4.4 Hz, H-2), 4.50 (m, 1H, H-4), 4.43 (t, 1H, $J_{3,4}$ 4.1 Hz, H-3), 3.89 (dd, 1H, $J_{4,5a}$ 4.6 Hz, $J_{5a,5b}$ 11.8 Hz, H-5a), 3.82 (dd, 1H, $J_{4,5b}$ 6.2 Hz, H-5b); ^{13}C NMR (100 MHz, CD_3OD): δ 149.1 (N*C*═CH), 131.5, 130.0, 129.4, 126.8, 121.2 (Ar, NC═*C*H), 94.1 (C-1), 84.3 (C-4), 78.2 (C-2), 72.7 (C-3), 61.7 (C-5). HRMS (MALDI): *m/z*: calcd for $[C_{13}H_{15}N_3O_4]Na^+$: 300.0960. Found: 300.0969.

4.2.1.34. 4-Benzyl-(1-α-D-lyxofuranosyl)-1,2,3-triazole (36).

Deprotection of compound **34** (0.10 g, 0.24 mmol) according to general procedure (Method D) and purification by column chromatography (CH_3CN:MeOH 1:0→6.5:1) gave **36**. White powder (62 mg, 89%). $[\alpha]_D$ + 68.6 (*c* 0.2, MeOH); ^1H NMR (400 MHz, CD_3OD): δ 7.90 (s, 1H, C*H*N), 7.31–7.18 (m, 5H, Ar), 6.00 (d, 1H, $J_{1,2}$ 5.8 Hz, H-1), 4.79 (dd, 1H, $J_{2,3}$ 4.5 Hz, H-2), 4.43 (m, 1H, H-4), 4.38 (t, 1H, $J_{3,4}$ 4.1 Hz, H-3), 4.07 (s, 2H, CH_2Ph), 3.85 (dd, 1H, $J_{4,5a}$ 4.6 Hz, $J_{5a,5b}$ 11.8 Hz, H-5a), 3.79 (dd, 1H, $J_{4,5b}$ 6.2 Hz, H-5b); ^{13}C NMR (100 MHz, CD_3OD): δ 148.7 (N*C*═CH), 140.3, 129.6, 127.5, 122.8 (Ar, NC═*C*H), 94.1 (C-1), 84.2 (C-4), 78.1 (C-2), 72.7 (C-3), 61.7 (C-5), 32.6 (*C*H_2Ph). HRMS (MALDI): *m/z*: calcd for $[C_{14}H_{17}N_3O_4]Na^+$: 314.1117. Found: 314.1124.

4.3. Biochemistry

4.3.1. Enzyme preparation

The purification and characterisation of recombinant *Drosophila melanogaster* Golgi (dGMIIb) and lysosomal (dLMII) mannosidases was carried out as we described recently.[31]

4.3.2. Class II α-mannosidase assay[13]

The supernatants of yeast expressing soluble forms of the α-mannosidase were incubated with the substrate *p*NP-Man*p* at 37 °C for 2–3 h. The standard assay mixture consisted of 50 mM sodium acetate buffer (pH 4.5 for JBMan, pH 5.2 for dLManII or pH 5.8 for dGMIIb), 2 mM *p*NP-Man*p* (from 100 mM stock solution in DMSO), 1–5 μL enzyme (supernatant of the culture medium) and in case of dGMIIb final 0.2 mM $CoCl_2$ in a total reaction volume of 50 μL. A blank sample contained no enzyme. The samples were prepared in triplicates. The reactions were terminated by the addition of 0.5 mL of 100 mM Na_2CO_3 and the absorbance was recorded at 410 nm (spectrophotometer).

4.3.2.1. Inhibition assays[13]. Inhibition assays of derivatives **5**, **14**, **15**, **22**, **26**, **27**, **30**, **35** and **36** with dLMII and dGMIIb were carried out in the conditions outlined above and the IC_{50} values were determined. Stock concentrations of inhibitors were made up in DMSO to a concentration of 100 mM and stored at –20 °C. The inhibitory effect of DMSO was tested for both enzymes. The 5% DMSO was selected as a maximal final concentration in the assay. This concentration causes 10% or 15% of inhibition of dLMII or dGMIIb, eventually. For this reason maximal concentration of the tested compounds in the reaction was 5 mM and data were obtained in triplicate. The inhibitory effect of the compounds was calculated by percentage of inhibition towards a control sample containing the same concentration of DMSO. Swainsonine was used as standard mannosidase inhibitor.

Inhibition of jack bean α-mannosidase activity was performed in microtitre plates in a final volume of 50 μL according to a published protocol.[31,32] The tested compounds **5**, **14**, **15**, **22**, **26**, **27**, **30**, **35** and **36** were prepared as stock solutions in DMSO (100 mM) and data were obtained in triplicate over a concentration range of 1–5 mM. Jack bean α-mannosidase (5μL) was pre-incubated for 5 min at 20 °C with the inhibitor under evaluation. The reaction mixture contained 80 mM sodium acetate buffer (pH 4.5) and 5 mM *p*NP-Man*p*. The absorbance of the reaction was measured at 405 nm using microplate reader.

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Supplementary material

^1H and ^{13}C NMR spectra of new compounds. Supplementary data to this article can be found online at doi:10.1016/j.carres.2016.04.004.

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