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# α-D-Mannose derivatives as models designed for selective inhibition of Golgi α-mannosidase II

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ABSTRACT

Human Golgi α-mannosidase II (hGM) is a pharmaceutical target for the design of inhibitors with anti-tumor activity. Nanomolar inhibitors of hGM exhibit unwanted co-inhibition of the human lysosomal α-mannosidase (hLM). Hence, improving specificity of the inhibitors directed toward hGM is desired in order to use them in cancer chemotherapy. We report on the rapid synthesis of D-mannose derivatives having one of the RS-, R(SO)- or $R(SO_2)$- groups at the α-anomeric position. Inhibitory properties of thirteen synthesized α-D-mannopyranosides were tested against the recombinant enzyme *Drosophila melanogaster* homolog of hGM (dGMIIb) and hLM (dLM408). Derivatives with the sulfonyl [$R(SO_2)$-] group exhibited inhibitory activities at the mM level toward both dGMIIb ($IC_{50} = 1.5–2.5$ mM) and dLM408 ($IC_{50} = 1.0–2.0$ mM). Among synthesized, only the benzylsulfonyl derivative showed selectivity toward dGMIIb. Its inhibitory activity was explained based on structural analysis of the built 3-D complexes of the enzyme with the docked compounds.



## 1. Introduction

The natural indolizidine swainsonine and some related pyrrolidines (Scheme 1) are nanomolar inhibitors of retaining glycoside hydrolases belonging to the family 38, Golgi α-mannosidase II (GM) (E.C.3.2.1.114) and lysosomal α-mannosidase (LM) (E.C.3.2.1.24) [1]. It was previously shown that treatment of the animals with swainsonine induced a strong immunostimulatory activity that resulted in an anti-cancer effect [2] and significantly blocked growing tumor in the lung, liver, and spleen. It was demonstrated that its activity was associated with the modification of *N*-glycan biosynthesis in which GM is reversibly blocked. However, swainsonine causes a phenocopy of the lysosomal storage disease called α-mannosidosis. This phenomena is caused by the additional targeting of LM by swainsonine, and limits its use in the clinical cancer chemotherapy [3]. Therefore the finding of a selective GM inhibitor with minimized unwanted co-inhibition of LM has become challenge for a number of researchers [4–19].

Up to date, dozens of novel swainsonine analogs were proposed and tested [1]. However, most potent inhibitors of GM exhibit negligible or no selectivity. To design an effective selective inhibitor of GM is a major problem, mainly because of the structural similarities of the active sites in GM and LM. The both enzymes are from the same family with the almost identical structures in radius 10 Å around $Zn^{2+}$ ion co-factor, which resides at the bottom of the active site and is essential for catalytic activity of the enzymes as well as for strong binding of the inhibitors [20]. This is the main reason why binding affinities of small organic inhibitors such as swainsonine are similar for both enzymes with no selectivity observed. Rose and co-workers [21,22] proposed the design for a selective inhibitor which mimics a binding position of non-reducing-terminal *N*-acetylglucosamine (GlcNAc) residue of the GM substrate. Such type of inhibitor would be capable to attach to GM at its binding subsite which is missing in LM. Moreover, this residue is essential for substrate specificity since GM cleaves substrate without terminal GlcNAc 80-fold more slowly. On the other side,

*Abbreviations:* GM, Golgi α-mannosidase II; hGM, human Golgi α-mannosidase II; dGM, *Drosophila melanogaster* α-mannosidase II; dGMIIb, catalytic domain of *Drosophila melanogaster* Golgi α-mannosidase II, recombinant enzyme homolog of human Golgi α-mannosidase II; LM, lysosomal α-mannosidase; hLM, human lysosomal α-mannosidase; bLM, *Bos taurus* lysosomal α-mannosidase; dLM408, catalytic domain of *Drosophila melanogaster* lysosomal α-mannosidase, recombinant enzyme homolog of human lysosomal α-mannosidase.

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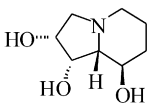
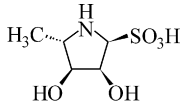


Swainsonine
$K_i$ = 3 nM
Golgi α-mannosidase II
(*Drosophila melanogaster*)



(2*R*,3*S*,4*S*,5*S*)-3,4-dihydroxy-5-methylpyrrolidine-2-sulfonic acid
$K_i$ = 50 nM
α-mannosidase (Jack bean)

**Scheme 1.** Nanomolar inhibitors of α-mannosidases belonging to the family 38.

LM is not able to cleave the GM substrate with terminal GlcNAc residue at the same concentration as GM does [21]. Recently, the GlcNAc binding subsite at the active site of *Drosophila melanogaster* GM (dGM) was explored by crystallographic approach [21,22]. The regions of the active sites, at which selective inhibitors as well as moiety of the substrate responsible for the substrate specificity, were characterized based on crystal structures of dGM in combination with native substrate [21,22,29], swainsonine [20] and its 5-substituted derivatives [4].

For purpose of finding a selective inhibitor of GM and better understanding the mechanism of action, some swainsonine derivates were synthesized and their biological activities were evaluated [1,4,13–15,18,19,23]. It was observed that 3- and 5-substituted swainsonine derivatives exhibited a weak selectivity for GM ($IC_{50}$ > 3–20-fold) at the nanomolar and micromolar levels, while 6- and 7-substituted derivatives exhibited even poorer activities (300–9000 less) at the micromolar and milimolar levels [14,15,18]. However, using of swainsonine and its derivatives as models of GM selective inhibitors or model compounds for the research of their action modes is complicated mainly because of the demands on their syntheses [18,19,24].

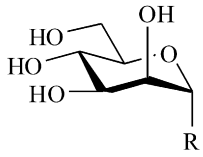
In this paper we describe the rapid synthesis of D-mannose derivatives substituted at the α-anomeric position as starting models for selective inhibitors of GM. Although it is known that six-member ring structures are not potent inhibitors of GM [1], the main goal of our study was to modify the aglycone unit of the mannosides to improve their selectivity directed toward GM. For their preparation we used the structural motif of an optimized synthesis described in our previous paper [25]. Moreover, α-D-mannose moiety is essential part of the native substrate of GM and its binding with the $Zn^{2+}$ ion co-factor at the active site of dGM has been previously characterized by crystallographic measurements [21,22]. A reference structure used for our inhibition assays, α-D-mannose, does not bind at the active site of the mannosidases (no inhibition activity up to 5 mM), thus an observed activity of the compounds can be easily assigned to a functional group attached at the α-anomeric position of the mannose unit.

## 2. Chemistry

A glycosylation of the corresponding alcohols and thioalcohols with mannose pentaacetate under a promotion of Lewis acid ($BF_3 \cdot OEt_2$) was used in this study for a coupling of the donor **1** with benzyl and 2-phenylethyl thioalcohols, yielding thioglycosides **2** and **3** [25]. The synthesis of other compounds (**14**–**20**) depicted in Fig. 1 with similar reaction conditions ($BF_3 \cdot OEt_2$ or $SnCl_4$ as Lewis acid) has been reported recently [26].

Thioglycosides are useful intermediates due to the presence of sulfur atom which can be oxidized to a various extent, in dependence of reaction conditions used. Both thioglycosides **2** and **3** were subjected to an oxidation which resulted in their conversion to sulfoxides **6** and **7**, or sulfones **10** [27] and **11** (Scheme 2).

The treatment of thioglycosides with equimolar amount of *m*CPBA at low temperature (−78 °C to −55 °C) afforded diastereomeric



**14** R = $OC_8H_{17}$
**15** R = $OC_{12}H_{25}$
**16** R = $OCH_2Cyh$
**17** R = $OCH_2CH_2Cyh$
**18** R = $SC_6H_{13}$
**19** R = $SO_2C_6H_{13}$
**20** R = $SO_2C_8H_{17}$
Cyh = 

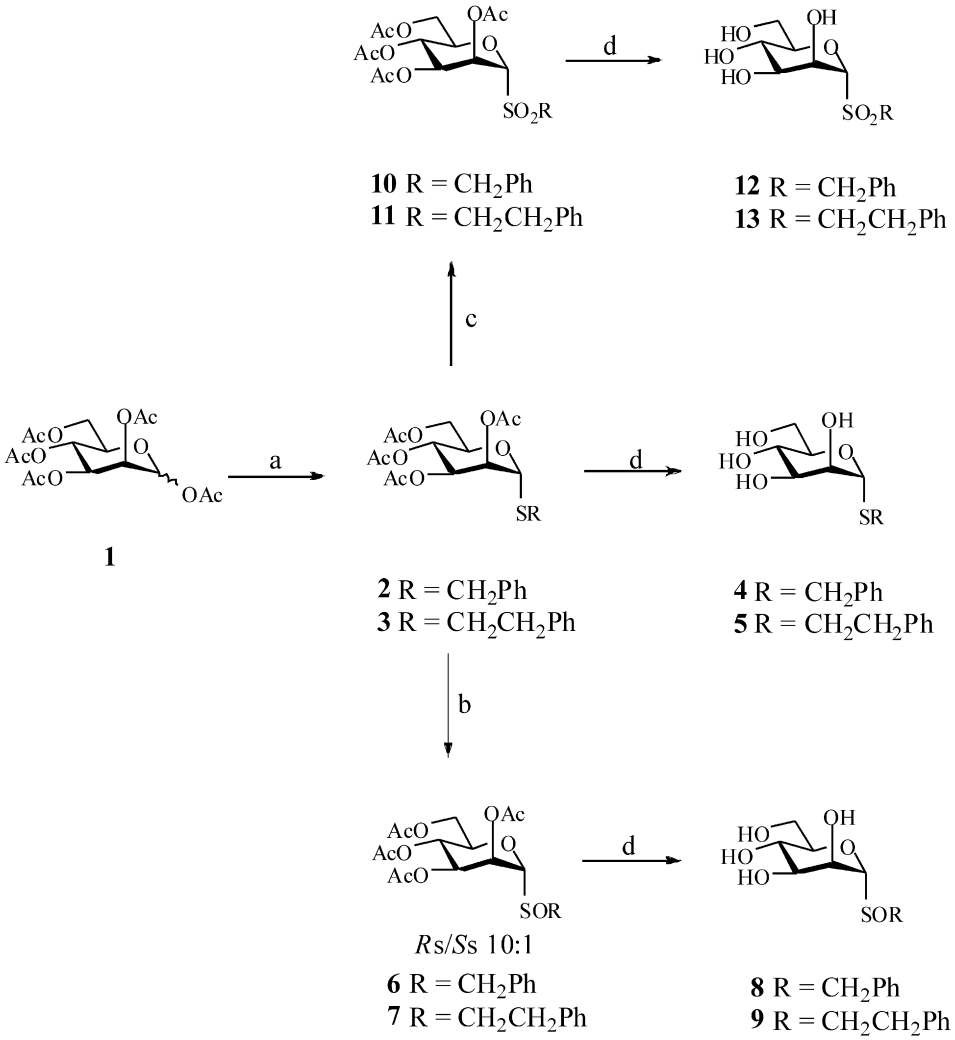
**Fig. 1.** The first set of the evaluated glycosides.

mixture of sulfoxides in moderate yield. Benzyl sulfoxide **6** was purified solely by a column chromatography while additional recrystallization was required to obtain pure major sulfur epimer of 2-phenylethyl sulfoxide **7**. Since under conditions used both sulfoxides were obtained as the mixtures of ($S_S$) and ($R_S$) epimers, it was necessary to distinguish their configuration which was established from $^1$H NMR spectra. In accordance with previous results [28], the diastereomeric ratio $R_S/S_S$ was found to be 10:1. In case of the sulfoxide **7**, the structure of major ($R_S$) epimer was also confirmed by X-ray crystallography (unpublished data). Taking into account similarity in the synthesis and chemical shifts in the $^1$H NMR spectra, the same configuration is proposed for the sulfoxide **6**.

Following our procedure described recently [26], oxidation of the thioglycosides carried out at room temperature with an excess of *m*CPBA smoothly provided sulfones **10** and **11**. The deacetylation under mild conditions using $K_2CO_3$ in MeOH with subsequent purification by column chromatography, afforded the target compounds **4** [25], **5** [25], **8**, **9**, **12** and **13**.

Transformation of the thioglycosides to the sulfoxides and the sulfones was clearly evident from their $^1$H and $^{13}$C NMR spectra. In $^1$H NMR spectra signals for H-1 of major acetylated sulfoxide and sulfone were upfield shifted in comparison with that of the starting thioglycoside; and the signal of the sulfoxide appeared at the lowest chemical shift from these sulfur-containing compounds. In $^{13}$C NMR spectra C-1 signal of the acetylated *S*-glycoside was the most upfield shifted, while C-1 signals of the oxidized compounds were observed at markedly higher values. The same trend in the chemical shifts for H-1 and C-1 was observed for series of deprotected compounds. All prepared derivatives discussed here were characterized by NMR and HRMS. Data are presented in the Experimental section.

The novel compounds of interest **8**, **9**, **12** and **13** were subjected to MS/MS analysis to verify their compositions. In tandem mass spectra all examined derivatives produced fragment ions corresponding to a number of cross-ring cleavages and total loss of monosaccharide (Y, Z - type ion cleavages) or phenylalkyl residue (B, C - type ion cleavages). However, their ratio significantly differed mainly in dependence on sulfur type linked on the aglycone of mannose. Generally, very similar patterns were observed in spectra recorded from sulfones **12** and **13** and differed from those obtained for sulfoxides **8** and **9**, suggesting different fragmentation pathways between mannose sulfoxides and sulfones with phenylalkyl function at anomeric position. Fig. 2 shows the representative tandem mass spectra acquired from **8** and **12**. The most abundant ions in spectra of deprotected sulfoxides were observed at *m*/*z* 234.02 ($C_1$) and corresponded to the loss of phenylalkyl residue from sulfur [Fig. 2, a)]. On the other side, the most




**Scheme 2.** *Reagents and conditions*. a) $BF_3 \cdot OEt_2$, alcohol, $CH_2Cl_2$, 24 h, rt; b) *m*CPBA, $CH_2Cl_2$, 45 min, −78 °C to −55 °C; c) *m*CPBA, $CH_2Cl_2$, 60 min, rt; d) $K_2CO_3$, MeOH, 10–30 min, rt.

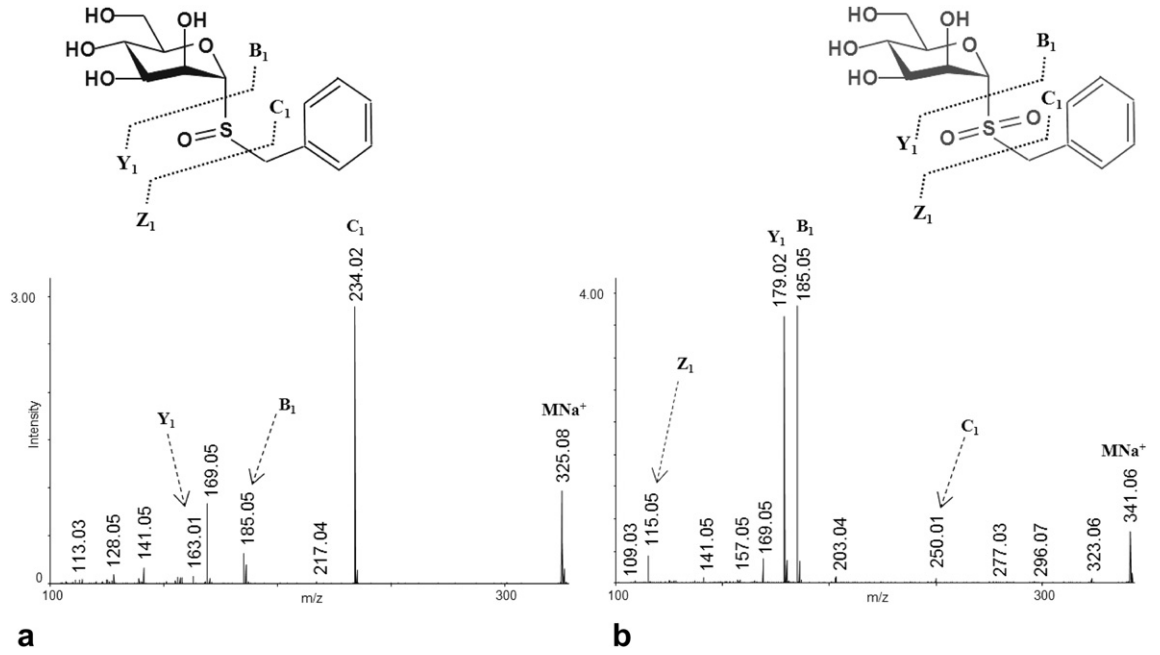



**Fig. 2.** MALDI-MS/MS spectra of novel mannose derivatives recorded for $MNa^+$ precursor ions at *m*/*z*: a) 325.07 (compound **8**); and b) 341.07 (compound **12**).

**Table 1**
Measured $IC_{50}$ values for both Golgi (dGMIIb) and lysosomal (dLM408) α-mannosidases.

| Compound | GM ($IC_{50}$/mM) | LM ($IC_{50}$/mM) |
|---|---|---|
| Swainsonine | $5.0 \times 10^{-6}$ | $11.0 \times 10^{-6}$ |
| Mannostatin A | $0.15 \times 10^{-3}$ | $3.0 \times 10^{-3}$ |
| α-D-mannose[a] | n.i | n.i |
| **4** | n.i. | n.i. |
| **5** | n.i. | n.i. |
| **8** | n.i. | n.i. |
| **9** | 2.5 | 1.5 |
| **12** | 2.0 | n.i. |
| **13** | 1.5 | 1.0 |
| **14** | n.i. | n.i. |
| **15** | n.i. | n.i. |
| **16** | n.i. | n.i. |
| **17** | n.i. | n.i. |
| **18** | n.i. | n.i. |
| **19** | 2.5 | 2.0 |
| **20** | 2.5 | 2.0 |

n.i. – No inhibition or inhibition <10% at 5 mM.
[a] A mixture of α/β ≈ 95:5.

prominent peaks in derivatives bearing sulfone group were consistent with a cleavage of the bond between a carbon of mannose and sulfur and these fragment ions appeared in **12** at *m*/*z* 179.02 ($Y_1$) and *m*/*z* 185.05 ($B_1$) [Fig. 2, b)].

## 3. Biological evaluation and docking

The $IC_{50}$ values (concentrations of inhibitor at which 50% of activity remains) were evaluated for two sets of the mannopyranosides (Table 1). The first set (Fig. 1) included 3 types of compounds (glycosides, thioglycosides and sulfones derived thereof) selected from recently prepared α-D-mannopyranosides: linear alkyl glycosides (compounds **14** and **15**), cyclohexylalkyl glycosides (compounds **16** and **17**), and linear alkyl thioglycosides and glycosyl alkyl sulfones (the compounds **18**, **19** and **20**) [26]. For the sulfones **19** and **20** inhibition activities toward both dGMIIb and dLM408 were found at the mM level ($IC_{50} = 2.0–2.5$ mM, Table 1) with no selectivity observed. The glycosides and thioglycosides did not show any activity in concentrations of the measurements (1–5 mM).

In general, α-D-mannopyranoside is a structural moiety of the α-mannosidase substrate and does not exhibit a significant inhibitory activity against the α-mannosidases. As the first step in their conversion to inhibitors of the retaining α-mannosidases, the glycosidic bond of a potential inhibitor should be converted to the structure not to be hydrolyzed by the enzyme. We suppose that the inactive glycosides and thioglycosides either weakly bind into the active site or after binding they can be immediately hydrolyzed by the enzyme. When the sulfanyl group was oxidized to a sulfoxide (compound **9**) or sulfone ones (compounds **19** and **20**), the inhibition of the enzyme became observable at the milimolar level. Another essential condition for proper binding of a structure into the active site of the enzyme is stabilization of a reactive conformation of the mannose ring upon binding. It was demonstrated by means of crystallographic measurements [21,22] that a conformational interconversion of the manose ring from the stable chair $^4C_1$ to the reactive skew boat $B_{2,5}$ conformation occurs upon binding substrate (or a mannoside inhibitor) to the active site of the enzyme. This reactive conformation of the mannosidase substrate [21,22] and substrate-enzyme covalent intermediate [29] are essential for the catalysis and a strong interaction with the $Zn^{2+}$ ion co-factor at the active site. Consequently, we can suppose that the oxidized forms of the thioglycosidic bond, mainly the sulfone one, in our active compounds prevent enzymatic hydrolysis; moreover it can preserve the mannose ring in a required reactive conformation upon binding to the active site.

To confirm this hypothesis we docked the sulfonyl derivatives as well as a nanomolar pyrrolidine inhibitor [(2*R*,3*S*,4*S*,5*S*)-3,4-dihydroxy-5-methylpyrrolidinium-2-sulfonate; Scheme 1] to the active site of dGM. As can be seen from Fig. 3, sulfonyl groups in both docked structures interact by hydrogen bonding with catalytic acid Asp341 and by dipole–ion interactions with Arg228 [$d(SO_2$-Asp341) = 1.80 Å and 2.02 Å; and $d(SO_2$-Arg228) = 3.22 Å and 2.67 Å for the pyrrolidine derivative and the mannoside, respectively]. It should be noted that Asp341 plays a role of the acid catalyst donating a proton onto the glycosidic oxygen of the substrate facilitating its hydrolysis. Thus, the sulfone group at the α-anomeric position can block its catalytic function and stabilize the mannose ring of the inhibitor in the reactive conformation necessary for an effective binding with the $Zn^{2+}$ ion co-factor. Indeed, after the post-docking minimization of the sulfonyl derivative in the active site, its mannose ring maintained the reactive skew boat conformation [Fig. 3, b)].

Based on the above-mentioned results obtained from the evaluation of the alkyl sulfones **19** and **20** and crystallographic measurements of complexes of dGM with 5-substituted aryl derivatives of swainsonine selective inhibitors [4], the second set of the evaluated compounds (Scheme 2, Table 1) consisted of mannosides (thioglycosides **4** and **5**, sulfoxides **8** and **9**, sulfones **12** and **13**) having aromatic aglycone connected to the sulfur atom through a short alkyl

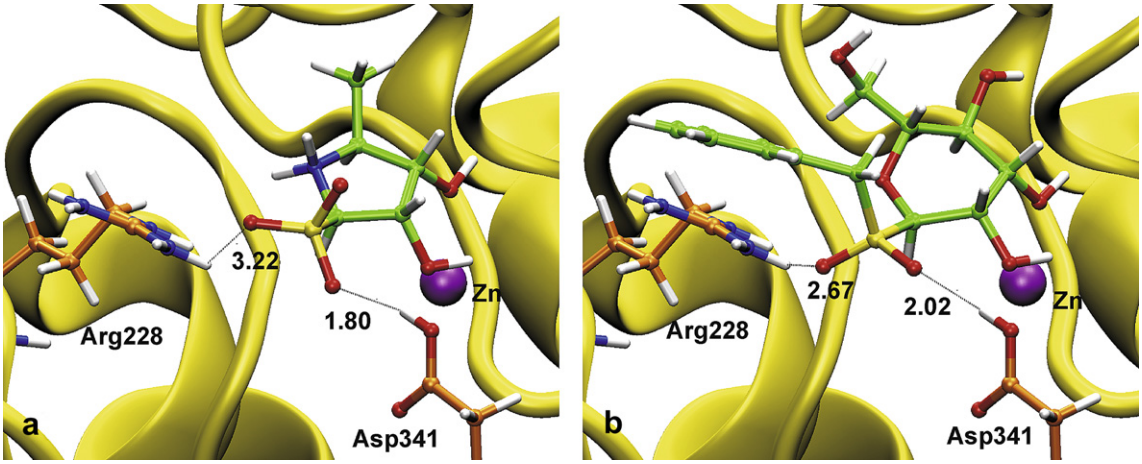



**Fig. 3.** a) A nanomolar inhibitor [(2*R*,3*S*,4*S*,5*S*)-3,4-dihydroxy-5-methylpyrrolidinium-2-sulfonate)] docked at the active site of dGM. The sulfonate group interacts with the catalytic acid (Asp341) and Arg228. b) Compound **12** docked at dGM. The same type of the interactions between the sulfonyl group and Asp341 and Arg228 is predicted by the modeling.

linker (one or two $CH_2$ groups). The main goal was to modify the aglycone unit of the glycosides to improve their selectivity toward GM. Therefore, we focused our attention on both groups of derivatives accessible from thioglycosides by oxidation because of inhibitory activity of sulfones found in the first set of the compounds.

The structures **9**, **12** and **13** exhibited inhibitory activities at the milimolar level ($IC_{50} = 1.0–2.5$ mM). The benzylsulfonyl derivative (**12**) selectively inhibited dGMIIb ($IC_{50} = 2$ mM), while no inhibition to dLM408 was observed in the measured range of 1–5 mM. The elongation of the aglycone linker by one $CH_2$ group from the benzylsulfone to the 2-phenylethylsulfone group (compound **13**) resulted in the loss of the selectivity. This derivative exhibited similar inhibiting properties toward both enzymes [$IC_{50}$(dGMIIb) = 1.5 mM and $IC_{50}$(dLM408) = 1.0 mM]. We docked **12** and **13** to the active sites of dGM and *Bos taurus* LM (bLM) to spot differences in binding posed and energies (Fig. 4 and Table S1 of Supplementary data). The derivative **12** prefers binding poses at which the aglycone chain interacts with a backbone and side chains of conserved Arg876 and Tyr267 in dGM [Fig. 4, a)] The identical binding pose of the aromatic chain was found for the docked 5-benzyl swainsonine at dGM (unpublished results). A similar binding position of an aromatic chain of nanomolar inhibitors close to Arg876 was recently described in the crystallographic measurements of 5-substituted aryl swainsonines with the dGM [4]. The interaction of **12** with tyrosine is missing in bLM because it is not conserved in known sequences of the lysosomal α-mannosidases [30]. Therefore, **12** prefers at bLM a pose in which the aglycone chain does not interact with the side chain of Arg823 (analogous to Arg876 in

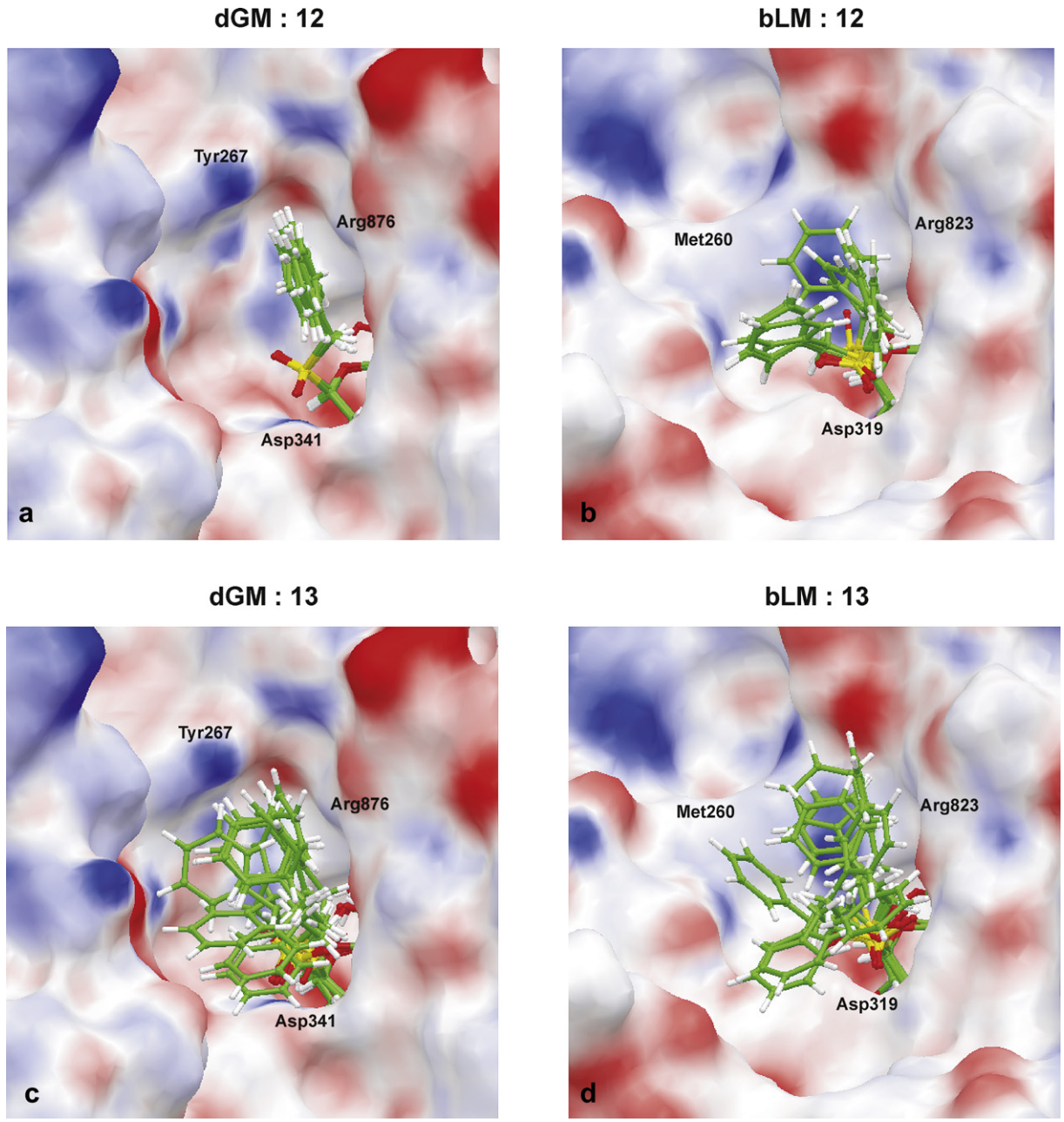



**Fig. 4.** Structures **12** and **13** docked into the active site of dGM and bLM (10 poses with the best docking Glide SP score, see also Table S1 in Supplementary data). a) **12** prefers at dGM specific positions at which a phenyl group interacts with the backbone and side chains of Arg876 and with the side chain of Tyr267. b) At hLM **12** prefers less ordered binding because of the missing Tyr residue and a different 3-D structure of the active site of bLM compared with dGM. c) and d) The elongation of an alkyl linker allowed a higher flexibility of the aglycone in **13** and caused less ordered binding at both dGM and bLM.

dGM), and various poses are predicted by the program (Fig. 4b). The binding energy for **12** is about 1.56 kcal mol$^{-1}$ (Glide SP docking score, Table S1, see Supplementary data) and 1.26 kcal mol$^{-1}$ (Glide XP docking score, Table S2, see Supplementary data) higher (more negative) in dGM *vs* bLM. This could explain the observed selectivity in the inhibition measurements for **12**. The elongation of an alkyl linker in **13** allowed a higher flexibility of the aglycone chain and its less ordered binding at both dGM and bLM [Figs. 4, c) and d)]. The binding energy for **13** is about 1.67 kcal mol$^{-1}$ (Glide SP docking score) and 1.12 kcal mol$^{-1}$ (Glide XP docking score) higher (more negative) in dGM than for bLM. The docking calculations predict the stronger binding of **12** and **13** toward dGM. The theoretical results predict both derivatives as selective inhibitors of GM, however according the experimental assay only **12** exhibited the selective binding against dGMIIb.

## 4. Conclusion

A rapid synthesis of a series of sulfur-containing α-D-mannopyranosides having the phenylalkyl functional group with one or two methylene group(s) long linker between the sulfur atom and the aromatic ring as the aglycone is described herein. Among the synthesized compounds, **6**, **7**, **8**, **9**, **11**, **12** and **13** are novel derivatives, first time described in this study. Five compounds showed the weak inhibitory activity directed toward Golgi and lysosomal α-mannosidases and benzylsulfonyl derivative in addition showed the selectivity against the former one. Because the reference α-D-mannose does not exhibit any binding affinity in the range of the measurement, the inhibition observed by the sulfonyl derivatives can mainly be assigned to the sulfonyl group attached at the α-anomeric position at the D-mannose moiety. Based on the data obtained from the theoretical modeling, the sulfonyl group at the α-anomeric position can interact with the catalytic acid of GM and block its catalytic function. It can stabilize the mannose ring in the reactive skew boat conformation which is necessary for an effective binding with the $Zn^{2+}$ ion co-factor. However, for better understanding the mechanism of selective binding of the benzylsulfonyl chain as aglycone in the mannoside, more precise calculations and crystallographic measurements are planned to be done.

## 5. Experimental

### 5.1. Chemistry

#### 5.1.1. General methods

Optical rotations were measured on a Perkin Elmer 241 polarimeter at 20 °C. Melting points were determined on a Kofler hot stage and are uncorrected. $^1$H NMR and $^{13}$C NMR spectra were recorded at 25 °C with VNMRS 400 MHz Varian or INOVA AV 600 MHz spectrometer. Chemical shifts are referenced to either TMS ($\delta$ 0.00, $CDCl_3$ for $^1$H) or HOD ($\delta$ 4.87, $CD_3OD$ for $^1$H), and to $CDCl_3$ ($\delta$ 77.23) or $CD_3OD$ ($\delta$ 49.15) for $^{13}$C, as internal standards. High resolution (HRMS) and tandem mass spectra (MS/MS) were recorded on the prototype quadrupole/TOF mass spectrometer. The instrument was calibrated externally using angiotensin I, bombesin, substance P and ACTH. The samples (~0.5 μL) were spotted onto a partially dried matrix (DHB), predeposited on the surface of a MALDI target. 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 (hexane, ethylacetate, acetonitrile, methanol). Reaction solvents were dried and distilled before use. All reactions containing sensitive reagents were carried out under argon atmosphere. p-Nitrophenyl α-D-mannopyranoside (PNP-Man*p*) was purchased from Sigma. Swainsonine and mannostatin A were purchased from Calbiochem.

#### 5.1.2. General procedure for glycosylation

To a stirred solution containing 1,2,3,4,6-penta-*O*-acetyl-D-mannopyranose (**1**) (2.5 g, 6.40 mmol, 1 eq) in $CH_2Cl_2$ (30 mL) the corresponding alcohol (1.25 eq) was added. The reaction mixture was stirred for 15 min, cooled down on an ice bath and $BF_3 \cdot OEt_2$ (3.18 g, 2.85 mL, 22.41 mmol, 3.5 eq) was added dropwise. The resulting mixture was stirred for 15 min, brought to rt and stirring was continued for 24 h. The reaction mixture was diluted with $CH_2Cl_2$ (150 mL) and poured into ice cold water (250 mL) under stirring. The organic phase was separated, washed with satd $NaHCO_3$ (3 × 100 mL), water (100 mL), dried with anhydrous $Na_2SO_4$, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc, 5:1 → 3:1).

*5.1.2.1. Benzyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside (2).* Yield 2.51 g; 86%; white crystals; mp 139–140 °C; lit [25] mp 137–138.5 °C; $[\alpha]_D = +158$ ($c = 0.5$, $CHCl_3$); lit [25] $[\alpha]_D^{27} = +154 \pm 0.9$ ($c = 1.0$, $CHCl_3$); HRMS: *m*/*z*: calcd for $C_{21}H_{26}O_9S$ $[M + Na]^+$: 477.1195; found: 477.1201.

*5.1.2.2. 2-Phenylethyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranoside (3).* Yield 2.25 g; 75%; yellowish solid; mp 82–83 °C; lit [25] mp 85.5–86.8 °C; $[\alpha]_D = +86$ ($c = 0.5$, $CHCl_3$); lit [25] $[\alpha]_D^{27} = +96 \pm 0.5$ ($c = 0.9$, $CHCl_3$); HRMS: *m*/*z*: calcd for $C_{22}H_{28}O_9S$ $[M + Na]^+$: 491.1352; found: 491.1365.

#### 5.1.3. General procedure for oxidation of phenylalkyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranosides to sulfoxides

A solution of compound (**2**) (0.2 g, 0.44 mmol, 1 eq) or (**3**) (0.4 g, 0.85 mmol, 1 eq) in $CH_2Cl_2$ (10 mL) was cooled at −78 °C and treated with *m*CPBA (1 eq based on 77% peroxide content). The reaction mixture was stirred and allowed gradually to warm to −55 °C over 45 min period, then poured into ice cold satd. $NaHCO_3$ (20 mL), diluted with $CH_2Cl_2$ (20 mL). The organic phase was separated, washed with satd $NaHCO_3$ (2 × 15 mL) and water (20 mL), dried with anhydrous $Na_2SO_4$, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc, 3:1 → 1:1.5).

*5.1.3.1. ($R_S$)-benzyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl sulfoxide (6).* Yield 0.14 g, 66%; white solid; mp 162–65 °C; $[\alpha]_D = +34$ ($c = 0.5$, $CHCl_3$); HRMS: *m*/*z*: calcd for $C_{21}H_{26}O_{10}S$ $[M + Na]^+$: 493.1144; found: 493.1191.

*5.1.3.2. ($R_S$)-2-phenylethyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl sulfoxide (7).* The title product (**7**) was obtained by crystalisation (EtOH) of the *S*-epimeric mixture of the sulfoxides obtained by column chromatography. Yield 0.31 g; 74%; white solid; mp 150–151 °C; $[\alpha]_D = +56$ ($c = 0.5$, $CHCl_3$); HRMS: *m*/*z*: calcd for $C_{22}H_{28}O_{10}S$ $[M + Na]^+$: 507.1301; found: 507.1295.

#### 5.1.4. General procedure for oxidation of phenylalkyl 2,3,4,6-tetra-O-acetyl-1-thio-α-D-mannopyranosides to sulfones

To a stirred and at 0 °C precooled solution containing compound (**2**) (0.2 g, 0.44 mmol, 1 eq) or (**3**) (0.2 g, 0.43 mmol, 1 eq) in $CH_2Cl_2$ (10 mL) *m*CPBA (2.5 eq based on 77% peroxide content) was added. The reaction mixture was stirred at rt for 1 h, diluted with $CH_2Cl_2$ (20 mL), washed with satd $NaHCO_3$ (2 × 15 mL) and water (20 mL). The organic phase was dried with anhydrous $Na_2SO_4$, filtered and concentrated. The crude product was purified by column chromatography (hexane:EtOAc, 3:1 → 1.5:1).

*5.1.4.1. Benzyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl sulfone (10).* Yield 0.20 g; 96%; white solid; mp 169–172 °C; lit [27] mp 174–176 °C; $[\alpha]_D = +86$ ($c = 0.5$, $CHCl_3$); lit [27] $[\alpha]_D^{20} = +83.5 \pm 1$ ($c = 0.46$, $CHCl_3$); HRMS: *m*/*z*: calcd for $C_{21}H_{26}O_{11}S$ $[M + Na]^+$: 509.1094; found: 509.1105.

**Table 2**
$^1$H NMR chemical shifts of **2**, **3**, **6**, **7**, **10** and **11**.

| | H-1 ($J_{1,2}$) | H-2 ($J_{2,3}$) | H-3 ($J_{3,4}$) | H-4 ($J_{4,5}$) | H-5 ($J_{5,6}$) | H-6 ($J_{6,6'}$) | H-6′ ($J_{5,6'}$) | Aglycone ($J_{H,H}$) | Acetyl groups |
|---|---|---|---|---|---|---|---|---|---|
| **2**[a] | 5.11 s | 5.33–5.26 m | 5.33–5.26 m | 5.33–5.26 m | 4.37 m (2.2) | 3.95 dd (−12.1) | 4.29 dd (5.3) | 7.32–7.26 m, 5H, Ar, 3.81 d (13.5), 1H, $CH_2$, 3.74 d (13.5), 1H, $CH_2$ | 2.12, 2.11, 2.04, 1.97 each s |
| **3**[b] | 5.27 s | 5.34 dd (3.2) | 5.32–5.25 m | 5.32–5.25 m (9.5) | 4.35 ddd (2.2) | 4.07 dd (−12.2) | 4.30 dd (5.5) | 7.31–7.19 m, 5H, Ar, 2.95–2.89 m, 3H and 2.86–2.80 m, 1H, $CH_2CH_2$ | 2.17, 2.05 (2x), 1.99 each s |
| **6**[a] | 4.54 d (1.5) | 5.83 dd (3.7) | 5.58 dd (9.8) | 5.31 t (9.6) | 4.11 ddd (2.2) | 4.04 dd (−12.1) | 4.22 dd (5.6) | 7.37–7.30 m, 5H, Ar, 4.27 d (13.4), 1H, $CH_2$, 4.05 d (13.4), 1H, $CH_2$ | 2.13, 2.08, 2.03, 2.00 each s |
| **7**[b] | 4.67 d (1.8) | 5.86 dd (3.7) | 5.57 dd (9.7) | 5.32 t (9.7) | 4.10–4.04 m | 4.10–4.04 m (−12.4) | 4.24 dd (5.9) | 7.35–7.24 m, 5H, Ar, 3.19–3.12 m, 4H, $CH_2CH_2$ | 2.17, 2.05, 2.04, 2.02 each s |
| **10**[a] | 4.68 d (1.9) | 5.89 dd (3.7) | 5.59 dd (9.3) | 5.28 t (9.6) | 4.72 ddd (2.3) | 4.15 dd (−12.5) | 4.28 dd (5.8) | 7.43–7.40 m, 5H, Ar, 4.53 d (14.2), 1H, $CH_2$, 4.26 d (14.2), 1H, $CH_2$ | 2.14, 2.11, 2.07, 1.99 each s |
| **11**[b] | 4.79 d (2.2) | 5.96 dd (3.6) | 5.59 dd (9.1) | 5.28 t (9.4) | 4.67 ddd (2.3) | 4.15 dd (−12.5) | 4.26 dd (6.1) | 7.34–7.22 m, 5H, Ar, 3.46–3.36 m, 2H, $CH_2$, 3.23–3.18 m, 1H, $CH_2$, 3.14–3.09 m, 1H, $CH_2$ | 2.16, 2.07, 2.03, 2.01 each s |

[a] 400 MHz in chloroform-*d*.
[b] 600 MHz in chloroform-*d*.

*5.1.4.2. 2-Phenylethyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl sulfone (11).* Yield 0.20 g; 97%; white solid; mp 99–102 °C; $[\alpha]_D = +50$ ($c = 0.5$, $CHCl_3$); HRMS: *m*/*z*: calcd for $C_{22}H_{28}O_{11}S$ $[M + Na]^+$: 523.1250; found: 523.1254.

$^1$H and $^{13}$C NMR data for compounds **2**, **3**, **6**, **7**, **10** and **11** are listed in Tables 2 and 3.

*5.1.5. General procedure for removal of acetyl groups*

Acetylated compound was dissolved in MeOH (5 mL) and $K_2CO_3$ (0.5 eq) was added. The reaction mixture was stirred for at rt (10–30 min), filtered and concentrated. The crude product was purified by column chromatography ($CH_3CN$:MeOH, 20:1 → 9:1).

*5.1.5.1. Benzyl 1-thio-α-D-mannopyranoside (4).* Yield 89.8 mg; 95%; pale yellow oil; $[\alpha]_D = +344$ ($c = 0.5$, MeOH); lit [25] $[\alpha]_D^{27} = +342 \pm 1.8$ ($c = 0.55$, MeOH); HRMS: *m*/*z*: calcd for $C_{13}H_{18}O_5S$ $[M + Na]^+$: 309.0773; found: 309.0801.

*5.1.5.2. 2-Phenylethyl 1-thio-α-D-mannopyranoside (5).* Yield 0.12 g; 94%; pale yellow oil; $[\alpha]_D = +168$ ($c = 0.5$, MeOH); lit [25] $[\alpha]_D^{27} = +198 \pm 0.5$ ($c = 0.9$, MeOH); HRMS: *m*/*z*: calcd for $C_{14}H_{20}O_5S$ $[M + Na]^+$: 323.0929; found: 323.0951.

*5.1.5.3. ($R_S$)-benzyl α-D-mannopyranosyl sulfoxide (8).* Yield 60.1 mg; 94%; oil; $[\alpha]_D = +61$ ($c = 0.5$, MeOH); HRMS: *m*/*z*: calcd for $C_{13}H_{18}O_6S$ $[M + Na]^+$: 325.0722; found: 325.0740.

*5.1.5.4. ($R_S$)-2-phenylethyl α-D-mannopyranosyl sulfoxide (9).* Yield 80.1 mg; 97%; oil; $[\alpha]_D = +110$ ($c = 0.5$, MeOH); HRMS: *m*/*z*: calcd for $C_{14}H_{20}O_6S$ $[M + Na]^+$: 339.0878; found: 339.0910.

*5.1.5.5. Benzyl α-D-mannopyranosyl sulfone (12).* Yield 48.3 mg; 87%, yellowish oil; $[\alpha]_D = +105$ ($c = 0.5$, MeOH); HRMS: *m*/*z*: calcd for $C_{13}H_{18}O_7S$ $[M + Na]^+$: 341.0671; found: 341.0689.

*5.1.5.6. 2-Phenylethyl α-D-mannopyranosyl sulfone (13).* Yield 58.5 mg; 88%; white solid; $[\alpha]_D = +86$ ($c = 0.5$, MeOH); HRMS: *m*/*z*: calcd for $C_{14}H_{20}O_7S$ $[M + Na]^+$: 355.0827; found: 355.0854.

$^1$H and $^{13}$C NMR data for compounds **4**, **5**, **8**, **9**, **12** and **13** are listed in Tables 4 and 5.

*5.2. Biology*

*5.2.1. Enzyme preparation*

The purification and characterization of recombinant *D. melanogaster* Golgi (dGMIIb) and lysosomal (dLM408) mannosidases were done with procedures used for relative α-mannosidases [31]. PCR fragments encoding soluble forms of dGMIIb and dLM408 (i.e., lacking the sequences encoding the putative *N*-terminal cytoplasmic and transmembrane domains) were cloned to *Pichia pastoris* GS115 cells using modified plasmid pICZαC (Invitrogen). The α-mannosidases were expressed in a secreted form to condition medium. The supernatant of condition medium was used as a crude enzyme in α-mannosidase assay or for further purification of His-tagged enzymes.

**Table 3**
$^{13}$C NMR chemical shifts of **2**, **3**, **6**, **7**, **10** and **11**.

| | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 | Aglycone | Acetyl groups |
|---|---|---|---|---|---|---|---|---|
| **2**[a] | 81.6 | 70.8 | 69.9 | 66.5 | 69.2 | 62.5 | 137.0, 129.2, 128.9, 127.7 Ar, 34.9 $CH_2$ | 170.8, 170.0 (2x), 169.9 $CH_3CO$, 21.1, 21.0, 20.9, 20.8 $CH_3CO$ |
| **3**[b] | 82.8 | 71.3 | 69.6 | 66.5 | 69.2 | 62.6 | 140.0, 128.8, 128.7, 126.8 Ar, 36.2 $CH_2$, 32.9 $CH_2$ | 170.8, 170.1, 170.0, 169.9 $CH_3CO$, 21.1, 20.9 (2x), 20.8 $CH_3CO$ |
| **6**[a] | 89.9 | 67.0 | 69.2 | 65.9 | 74.8 | 62.5 | 130.6, 129.3, 129.0, 128.7 Ar, 56.2 $CH_2$ | 170.6, 169.8, 169.7, 169.6 $CH_3CO$, 21.0, 20.9, 20.8, 20.7 $CH_3CO$ |
| **7**[b] | 91.3 | 67.2 | 69.0 | 65.9 | 74.7 | 62.5 | 138.5, 129.1, 128.8, 127.2 Ar, 52.2 $CH_2$, 28.3 $CH_2$ | 170.6, 169.8 (2x), 169.6 $CH_3CO$, 21.1, 20.9, 20.8 (2x) $CH_3CO$ |
| **10**[a] | 86.3 | 64.7 | 69.1 | 65.5 | 73.6 | 62.8 | 131.1, 129.6, 129.4, 127.1 Ar, 57.3 $CH_2$ | 170.6, 169.9, 169.4, 169.3 $CH_3CO$, 21.0, 20.9 (2x), 20.7 $CH_3CO$ |
| **11**[b] | 88.2 | 64.9 | 69.0 | 65.6 | 73.8 | 62.7 | 137.3, 129.2, 128.6, 127.5 Ar, 52.5 $CH_2$, 28.0 $CH_2$ | 170.6, 169.8, 169.4 (2x) $CH_3CO$, 20.8 (2x), 20.7, 20.6 $CH_3CO$ |

[a] 100 MHz in chloroform-*d*.
[b] 150 MHz in chloroform-*d*.

**Table 4**
$^1$H NMR chemical shifts of **4**, **5**, **8**, **9**, **12** and **13**.

| | H-1 ($J_{1,2}$) | H-2 ($J_{2,3}$) | H-3 ($J_{3,4}$) | H-4 ($J_{4,5}$) | H-5 ($J_{5,6}$) | H-6 ($J_{6,6'}$) | H-6′ ($J_{5,6'}$) | Aglycone ($J_{H,H}$) |
|---|---|---|---|---|---|---|---|---|
| **4**[a] | 5.08 d (1.0) | 3.96–3.74 m | 3.96–3.74 m | 3.69–3.65 m | 3.69–3.65 m | 3.96–3.74 m | 3.96–3.74 m | 7.39–7.23 m, 5H, Ar, 3.96–3.74 m, 2H, $CH_2$ |
| **5**[b] | 5.26 d (1.0) | 3.93–3.90 m | 3.93–3.90 m | 3.69–3.65 m | 3.69–3.65 m (2.3) | 3.85 dd (−11.9) | 3.76 dd (5.9) | 7.29–7.18 m, 5H, Ar, 2.96–2.92 m, 3H and 2.87–2.84 m, 1H, $CH_2CH_2$ |
| **8**[b] | 4.56 d (1.6) | 4.33 dd (3.4) | 3.86 dd (9.3) | 3.71 t (9.5) | 3.60–3.57 m (2.0) | 3.91 dd (−12.1) | 3.68 dd (6.7) | 7.42–7.38 m, 5H, Ar, 4.45 d (13.6), 1H, $CH_2$, 4.05 d (13.7), 1H, $CH_2$ |
| **9**[a] | 4.74 d (1.3) | 4.43 dd (3.2) | 3.87–3.84 m | 3.72 t (9.5) | 3.45–3.41 m | 3.87–3.84 m (−12.2) | 3.65 dd (6.6) | 7.36–7.32 m, 5H, Ar, 3.45–3.41 m, 1H and 3.22–3.10 m, 3H, $CH_2CH_2$ |
| **12**[a] | 4.79 d (1.2) | 4.42 dd (3.7) | 3.97 dd (9.3) | 3.69 t (9.3) | 4.26–4.23 m (2.1) | 3.93 dd (−12.2) | 3.72 dd (6.7) | 7.50–7.38 m, 5H, Ar, 4.65 d (14.0), 1H and 4.40 d (14.0), 1H, $CH_2$ |
| **13**[b] | 5.00 d (1.3) | 4.56 dd (3.7) | 4.03 dd (9.2) | 3.73 t (9.6) | 4.23–4.20 m (2.1) | 3.92 dd (−12.1) | 3.71 dd (6.6) | 7.35–7.23 m, 5H, Ar, 3.58–3.48 m, 2H and 3.20–3.11 m, 2H, $CH_2CH_2$ |

[a] 400 MHz in chloroform-*d*.
[b] 600 MHz in chloroform-*d*.

### 5.2.2. α-mannosidase assay

The supernatants of yeast expressing soluble forms of the α-mannosidase were incubated with the substrate PNP-Man*p* at 37 °C for 2–3 h. The standard assay mixture consisted of 50 mM sodium acetate buffer pH 5.2 for LM or pH 5.8 for GMIIb, 2 mM PNP-Man*p* (from 100 mM stock solution in DMSO), 1–5 μL enzyme (supernatant of the culture medium) and in case of GMIIb final 0.2 mM $CoCl_2$ in the total reaction volume 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 absorbances were recorded at 410 nm (spectrophotometer).

### 5.2.3. Inhibition assays

Inhibition assays of new mannose analogs with LM 408 and GMIIb were carried out at the conditions outlined above and the $IC_{50}$ values were determined. Stock concentrations of inhibitors were made up in DMSO in the 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 LM or GMIIb, eventually. For this reason maximal concentration of the tested compounds in the reaction was 5 mM. The inhibitory effect of the compounds was calculated in percentage of inhibition toward control sample containing the same concentration of DMSO. The swainsonine and mannostatin A were used as standard mannose inhibitors.

**Table 5**
$^{13}$C NMR chemical shifts of **4**, **5**, **8**, **9**, **12** and **13**.

| | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 | Aglycone |
|---|---|---|---|---|---|---|---|
| **4** | 85.1 | 73.6/75.2 | | 69.0 | 73.6 | 62.9 | 139.7, 130.4, 129.6, 128.2 Ar, 35.2 $CH_2$ |
| **5**[b] | 86.6 | 73.8/75.1 | | 69.0 | 73.3 | 62.9 | 142.0, 129.7, 129.5, 127.4 Ar, 37.3 $CH_2$, 33.5 $CH_2$ |
| **8**[b] | 94.2 | 68.2 | 72.9 | 67.9 | 81.6 | 63.2 | 132.1, 130.9, 129.9, 128.6 Ar, 55.5 $CH_2$ |
| **9**[a] | 95.9 | 68.3 | 72.7 | 67.8 | 81.4 | 63.1 | 140.3, 129.9, 129.8, 127.9 Ar, 52.5 $CH_2$, 29.1 $CH_2$ |
| **12**[a] | 89.7 | 65.3 | 71.3 | 66.1 | 78.5 | 61.7 | 131.0, 128.4, 128.3, 127.8 Ar, 55.7 $CH_2$ |
| **13**[b] | 93.2 | 66.9 | 72.9 | 67.8 | 80.0 | 63.2 | 139.6, 129.9, 129.7, 127.9 Ar, 52.7 $CH_2$, 28.7 $CH_2$ |

[a] 100 MHz in chloroform-*d*.
[b] 150 MHz in chloroform-*d*.

### 5.3. Molecular modeling

The crystal structures of dGM with nanomolar inhibitor swainsonine (PDB ID: 3BLB) [4] and bLM (PDB ID: 1OD7) [30] were used as 3-D enzyme models of hGM and hLM for docking of synthesized mannosides by the GLIDE program [32,33] of the Schrödinger package [34].

For docking all molecules of water at the active site of dGM were deleted except one (WAT1820, numbering according to PDB ID: 3BLB). This water has shown to be conserved in crystal structures of dGM either with intact substrates and inhibitors [4,5,21,22]. In all docking calculations the catalytic acid (Asp341 in dGM and Asp319 in bLM) was modeled in the protonated form in accordance with its catalytic role as a general acid. The pyrrolidine derivative from Scheme 1 was docked with the protonated ring nitrogen and ionized sulfonate group as predicted for pH = 6.5 by the Epik program [35] of the Schrödinger package. The mannoside ligands were docked into the active site in the reactive skew boat $B_{2,5}$ conformation of the mannose ring. For the docking calculations default values of parameters were used with selected constraints that scored only poses at which the interactions between the $Zn^{2+}$ ion and 2-OH and 3-OH groups of the mannosides were included. The receptor box for the docking conformational search was centered at the $Zn^{2+}$ ion co-factor bound at the bottom of the active site with a size of 39 × 39 × 39 Å using partial atomic charges for the receptor from the OPLS2005 force field except for the $Zn^{2+}$ and side chains of His90, Asp92, Asp204, Arg228, Tyr269, Asp341 and His471 (analogous residues were selected for bLM). For these structural fragments the charges were calculated at the quantum mechanics level with the DFT (Density Functional Theory) method (M05-2X) [36,37] using hybrid quantum mechanics/molecular mechanics (QM/MM) model (M05-2X/LACVP*:OPLS2005) with the Qsite program [38] of the Schrödinger package. The grid maps were created with no Van der Waals radius and charge scaling for the atoms of the receptor. Flexible docking in standard (SP) and extra (XP) precision was used. The potential for nonpolar parts of the ligands was softened by scaling the Van der Waals radii by a factor of 1.0 for atoms of the ligands with partial atomic charges less than specified cutoff of 0.15. The 5000 poses were kept per ligand for the initial docking stage with scoring window of 100 kcal $mol^{-1}$ for keeping initial poses; the best 400 poses were kept per ligand for energy minimization. The ligand poses with RMS deviations less than 0.5 Å and maximum atomic displacement less than 1.3 Å were discarded as duplicates.

The post-docking minimization for 10 ligand poses with the best docking score was performed and optimized structures were saved for subsequent analyses using the MAESTRO [39] viewer of the Schrödinger package.

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## Appendix. Supplementary data

$^{1}H$ and $^{13}C$ NMR spectra of the compounds **2**–**13**. Tables with docking scores for complexes dGM:**12**, dGM:**13**, bLM:**12** and bLM:**13**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2011.01.012.

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