Novel carbohydrate derivatives as potential antitrypanosomal agents: chemical and parasitological studies

Grant no. OTKA-NKFIH NN-109671, 2013-2018.

FINAL REPORT

Our results are listed below with reference to the appropriate sections of the Research Proposal (RP).

Cf. Sections **2.2.1**, **2.2.3 & 3.6** of the Research Proposal (RP): Chemical syntheses, biochemical assays, molecular modelling

Initiated by Prof. H-J. Gabius (München) a collaboration was started to assay the biological activities of some sulfur- and selenium containing carbohydrates recently synthesized in our group. The inhibitory capacities of benzene-based mono- to trivalent dithiogalactosides increased with valency for the plant toxin VAA, potently blocking its binding to a lactose-presenting matrix and to human tumor cell lines. Human galectins were much less sensitive to the disulfides than the toxin. Selenodigalactoside and diselenodigalactoside were prepared and both compounds proved bioactive for the toxin. Remarkably, the Se-linked digalactoside proved active for human galectins as well [*BMCL 2015*].

We reported the synthesis of 12 new bivalent thio-, disulfido- and selenoglycosides attached to benzene/naphthalene cores. They present galactose, for blocking a plant toxin, or lactose, the canonical ligand of adhesion/growth-regulatory galectins. Molecular modelling revealed quasi unrestrained flexibility and interhead group distances between sugar units too small to bridge of two sites in the same lectin. In collaboration with the group of Prof. H-J. Gabius, inhibitory activities were detected by solid-phase assays using a surface-presented glycoprotein, with activity enhancements per sugar unit relative to free cognate sugar up to nearly 10-fold. Inhibitory activity was also seen on lectin binding to surfaces of human carcinoma cells using flow cytometry analysis. Monitoring of lectin binding in the presence of inhibitors was extended to sections of three types of murine organs as models. Naphthalene-based disulfide and selenide were found to present their sugar headgroup for productive lectin inhibition, documenting the perspective for broad applicability of the histochemical assay to measure inhibitions of lectin binding to natural glycans in tissues by glycoclusters [*BMC. 2017*].

Cf. Section **2.2.4** and **3.4** of the RP: Chemical syntheses, X-ray crystallography

Analogs of disulfide sugars wherein one or both sulfur atoms are replaced by selenium are of particular interest both chemically and for potential bioactivity. We have developed a convenient route to *Se*-*S*-glycoside derivatives using glycosyl isoselenuronium salts as glycosylselenenyl transfer reagents toward thiols. Aliphatic and aromatic thiols were found to react readily to furnish glycosylselenenylsulfide derivatives. *S*-glycosylselenenyl-cysteines were obtained similarly via reactions with *O,N*-protected cysteine. Reactions with monosaccharide thiols provided disaccharide mimics featuring *Se*-*S*- interglycosidic bonds. Further disaccharide mimics with *Se*-*Se* interglycosidic linkage were obtained from the starting isoselenuronium salts via reactions with protected monosaccharide derivatives bearing SeH groups in 6- or 4-position. The structures of the new derivatives were determined using NMR spectroscopy and X-ray diffraction methods. The novel glycomimetics are expected to open new perspectives in biological activities and/or mechanistic studies due, i.a., to the rather uncommon *Se*-*S*- or *Se*-*Se* bonds incorporated into a carbohydrate framework [*ChemSelect*, 2016].

Cf. Sections **2.2.3, 3.5 & 3.6** of the RP: Chemical syntheses, ITC, SPR, Molecular modelling

Aralkyl and aryl selenoglycosides as well as glycosyl selenocarboxylate derivatives were assayed on the activity of protein phosphatase-1 (PP1) and -2A (PP2A) catalytic subunits (PP1c and PP2Ac) in search of compounds for PP1c and PP2Ac effectors. The majority of tested selenoglycosides activated both PP1c and PP2Ac by ~2-4-fold in a phosphatase assay with phosphorylated myosin light chain substrate when the hydroxyl groups of the glycosyl moiety were acetylated, but they were without any effects in the non-acetylated forms. Possible molecular mechanisms were explored by surface plasmon resonance based binding experiments as well as by molecular docking calculations. Activator molecules caused a moderate increase in the phosphatase activity of HeLa cells and suppressed cell viability in 24 hour incubations [*BMC. 2018*].

Cf. Section **3.2** of the RP: Parasitology and biochemical assays

In collaboration with the group of M. Comini (Institut Pasteur, Montevideo) we have investigated the anti-trypanosomal activity and selectivity of a series of symmetric diglycosyl diselenides and disulfides. Of 18 compounds tested the fully acetylated forms of di-*β*-D-glucopyranosyl and di-*β*-D-galactopyranosyl diselenides displayed strong growth inhibition against the bloodstream stage of *Trypanosoma brucei*, the causative agent of the African sleeping disorder. Both compounds induced redox unbalance in the pathogen. Nonacetylated

versions of the same sugar diselenides proved to be, however, much less efficient or completely inactive to suppress trypanosome growth. *In vitro* NMR analysis indicated that diglycosyl diselenides react with glutathione, under physiological conditions, *via* formation of selenenylsulfide bonds. Our results suggest that non-specific cellular targets of the glucose and the redox metabolism of the parasite may be affected. These molecules are therefore promising leads for the development of novel multitarget antitrypanosomal agents [*IJPDD 2017*].

Cf. Section **3.3** of the RP: NMR spectroscopy

We have developed a broadband proton–proton-decoupled CPMG-HSQMBC method for the precise and direct measurement of long-range heteronuclear coupling constants. Our method, based on the Zangger–Sterk-based homodecoupling scheme efficiently removes unwanted proton–proton splittings from the heteronuclear multiplets, so that the desired heteronuclear couplings can be determined simply by measuring frequency differences between singlet maxima in the resulting spectra. The proposed pseudo-1D/2D pulse sequences were tested on nucleotides, a metal complex incorporating P heterocycles, diglycosyl (di)selenides and other carbohydrate derivatives, for the extraction of $^{n}J(^{1}H,^{77}Se)$, $^{n}J(^{1}H,^{13}C)$ and $^{n}J(^{1}H,^{31}P)$ values [*ChEurJ-a*].

We have reported another broadband homonuclear decoupled method, PSYCHE CPMG-HSQMBC, for precision measurement of multiple-bond heteronuclear couplings. The PSYCHE-scheme built in the pulse sequence efficiently eliminates unwanted proton-proton splittings from the heteronuclear multiplets to enable determination of long-range heteronuclear couplings simply by measuring frequency differences between peak maxima of pure antiphase doublets. This new measurement scheme can provide significant improvement in sensitivity as compared to the earlier Zangger-Sterk-based method. Applications of the proposed pulse sequence were demonstrated for the extraction of $^{n}J(^{1}H,^{77}Se)$ and $^{n}J(^{1}H,^{13}C)$ values, respectively, in carbohydrate molecules [*ChEurJ-b*].

References

[*BMCL 2015*] Sabine André, Katalin E. Kövér, Hans-Joachim Gabius, László Szilágyi: ***Thio- and selenoglycosides as ligands for biomedically relevant lectins: Valency–activity correlations for benzene-based dithiogalactoside clusters and first assessment***, Bioorg. Med. Chem. Lett., 25, 931–935, 2015.

[*BMC. 2017*] Herbert Kaltner, Tamás Szabó, Krisztina Fehér, Sabine André, Sára Balla, Joachim C. Manning, László Szilágyi, Hans-Joachim Gabius: ***Bivalent O-glycoside mimetics with S/disulfide/Se substitutions and aromatic core: Synthesis, molecular modeling and inhibitory activity on biomedically relevant lectins***, Bioorg. Med. Chem., 25, 3158-70, 2017.

[*ChemSelect, 2016*] Tünde-Zita Illyés, Sára Balla, Attila Bényei, Ambati Ashok Kumar, István Timári, Katalin E. Kövér, László Szilágyi: ***Exploring the Syntheses of Novel Glycomimetics. Carbohydrate Derivatives with Se-S- or Se-Se- Glycosidic Linkages***, ChemistrySelect 1, 2383–2388, 2016.

[*BMC. 2018*] Zoltán Kónya, Bálint Bécsi, Andrea Kiss, István Tamás, Beáta Lontay, László Szilágyi, Katalin E. Kövér, Ferenc Erdődi: ***Aralkyl selenoglycosides and related selenosugars in acetylated form activate protein phosphatase-1 and -2A***, Bioorg. Med. Chem. 26, 1875-1884, 2018.

[*IJPDD 2017*] Jaime Franco, Florencia Sardi, László Szilágyi , Katalin E. Kövér , Krisztina Fehér, Marcelo A. Comini: ***Diglycosyl diselenides alter redox homeostasis and glucose consumption of infective African trypanosomes***, Int. J. Parasitol.: Drugs & Drug Resist., 7, 303-313, 2017.

[*ChEurJ-a*] István Timári, Tünde Z. Illyés, Ralph W. Adams, Mathias Nilsson, László Szilágyi, Gareth A. Morris, Katalin E. Kövér: ***Precise Measurement of Long-Range Heteronuclear Coupling Constants by a Novel Broadband Proton–Proton-Decoupled CPMG-HSQMBC Method***, Chem. Eur. J. 21, 3472-3479, 2015.

[*ChEurJ-b*] István Timári, László Szilágyi, Katalin E. Kövér: ***PSYCHE CPMG–HSQMBC: An NMR Spectroscopic Method for Precise and Simple Measurement of Long-Range Heteronuclear Coupling Constants***, Chem. Eur. J. 21, 13939-13942, 2015.

[*TET 2018*] Tamás Szabó, László Szilágyi, Attila Bényei, Jaime Franco, Marcelo Comini: ***Bivalent glycoconjugates based on 1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione as a central scaffold. Chemical syntheses and anti-trypanosomal effects***, Tetrahedron, submitted, 2018.

Cf. Section **2.2.5** and **3.2** of the RP: Chemical syntheses, parasitology

Manuscript, submitted for publication to *Tetrahedron:*

Bivalent glycoconjugates based on 1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione as a central scaffold. Chemical syntheses and anti-trypanosomal effects.

Tamás Szabó[a], László Szilágyi[b*] and Attila Bényei[c], Jaime Franco[d], Marcelo Comini[d]

[a]Richter Gedeon Nyrt. Budapest, [b]Department of Organic Chemistry, [c]Department of Physical Chemistry, University of Debrecen, H-4032 Debrecen, Egyetem tér 1., Hungary, [d]Institut Pasteur, Montevideo, Uruguay.

Keywords:

heteroaromatics, fluorescence, thioglycoside, selenoglycoside, disulfide,

1,2,3-triazole

Declaration of interest: none.

Abstract

The heteroaromatic fused diazabicyclic "bimane" ring system, discovered four decades ago, is endowed with remarkable chemical and photophysical properties. Of the large number of derivatives obtained by chemical syntheses several low molecular-weight members are efficient fluorescent molecules ensuring widespread applications for them as biological labels. No carbohydrate derivatives of bimanes have, however, been described thus far. Here we report on the syntheses of a range of bimanes decorated with mono- or disaccharide residues for potential applications with carbohydrate-binding or -processing proteins or more complex biological systems. Mono- and disaccharide residues were attached to *syn*- or *anti*-bimane central cores via thio-, disulfido- or selenoglycosidic linkages to obtain novel fluorescent or nonfluorescent glycoconjugates. Cu(I)-catalyzed cycloaddition of glycosyl azides to a bimane diethynyl derivative furnished further bivalent glycoconjugates with sugar residues linked to the central bimane core via 1,2,3-triazole rings. We have determined the crystal and molecular structures of several glycosylated and non-glycosylated bimanes and report fluorescence data for the new compounds.

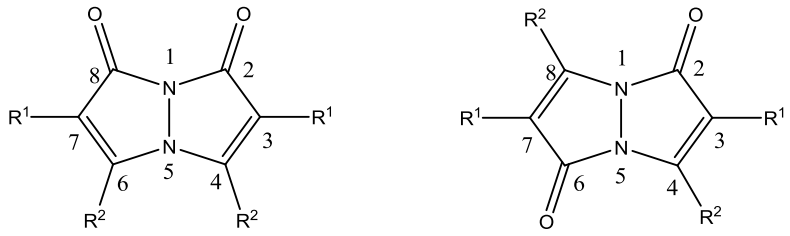
Introduction

Cell surface-presented glycans are targets of molecular recognition events in a wide array of physiological processes. Binding of proteins with carbohydrate-recognition domains, such as lectins, to glycan chains anchored to the cell wall play pivotal roles in intracellular signaling, cell-cell-, or cell-extracellular matrix interactions (for recent reviews see[1,2]) Small molecular weight oligo/multivalent glycoconjugates were often found instrumental to model the glycan partner in experimental settings under in vitro conditions.[3] Compounds displaying promising activity in these preliminary tests then qualify them to be carried over to further assays of increasing physiological relevance such as inhibition of lectin binding to cultured cells or histochemical assays in fixed animal tissue sections.[4] Although lectins are exempt of enzymic activity, as a rule (exceptions exist), *O*-glycosidic bonds in lectin-directed glycoconjugates are, however, frequently replaced by non-hydrolyzable (thio-, disulfido- or selenoglycosidic) linkages. This may be regarded as a prudential measure to avoid

inhibitor deactivation by glycanhydrolases or other carbohydrate processing enzymes that may be present in complex biological systems used for testing lectin inhibition activities.

A novel class of carbohydrate structures designed along this principle, diglycosyl disulfides and disulfidoglycosides,(for a review see[5]), received attention recently for their various biological activities. Symmetric diglycosyl disulfides, as one of the first platforms in this line, were found to display lectin inhibition activities in solid phase tests and in tumor cell lines.[6,7]) Some oligovalent derivatives featuring mannosyl moieties attached to benzene central cores by disulfide linkages were shown to bind to concanavalin A with affinities surpassing that of the cognate sugar.[8] Remarkable inhibitory activities against *Trypanosoma cruzi*, the etiologic agent of Chagas's disease, were recorded in vitro for similar structures with galacto configurations.[9] Further glycoconjugates with galactose or lactose moieties attached to central naphthalene scaffolds proved to inhibit the binding of a plant agglutinin and different human galectins to tumor cell lines; this bioactivity being preserved when tested in animal tissue sections as well.[4] Exploring the scope for potential biorelevance of sugar mimetics with the glycosidic oxygen substituted with another VIth column heteroatom, selenium, di-*β*-D-galactopyranosyl selenide and -diselenide have been identified to display lectin inhibition efficiency comparable to that of their thio analogs.[10] On the other hand, we have recently observed significant growth inhibiton efficiency of symmetric diglycosyl diselenides against *T. brucei* species, the parasite causing African sleeping sickness.[11] As a further extension of the palette of three-bond interglyosidic connections with two non-oxygen heteroatoms (for a review see,[5]) we have recently introduced the disulfide analog selenosulfide linkage which is of rare occurrence in carbohydrate chemistry.[12]

Building upon various biological activities discussed above we have set out to explore novel glycoconjugates with a central heteroaromatic core called "bimane". Here we wish to report on the syntheses and properties of bivalent glycosyl derivatives based on this remarkable heterocyclic skeleton. The beautifully symmetric heterocyclic ring system, 1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (bimane) was discovered, after some preliminary attempts, by E.M. Kosower et al. in 1978;[13] an alternative synthesis being reported just recently.[14] Bimanes are fluorescent molecules; the *syn* isomers displaying much stronger effects than the *anti* forms (see Chart 1).[15] The latter show, on the other hand, low temperature phosphorescence.[16]



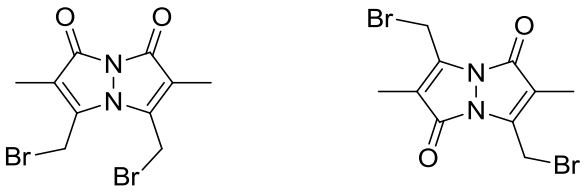
syn-(R_2,R_1)B *anti*-(R_2,R_1)B

Chart 1

Syn and *anti* forms of substituted bimanes labeled using the shorthand notation introduced by Kosower et al.[13]

Bimane fluorophores are extensively used in biophysical studies such as for monitoring conformational changes, motions and interactions in proteins.[17-20] We have envisioned that glycoconjugates endowed with fluorescent properties might be further exploited to explore potential interactions with cells or higher organisms. (see, e.g.,[21,22]). Another motivation for our studies was provided by the observation of antiparasitic activity of certain bimane derivatives.[23] Finally, while sugars are vital components of all biosystems and displaying a large variety of biological activities, to some surprise, no carbohydrate derivatives of bimanes have been described thus far to the best of our knowledge.

Haloalkyl-bimanes like the s*yn* and *anti* forms of dibromobimane (**DBB**) derivatives **1a** and **1b**[15] (Chart 2) are reactive species and reactions with thiols are of interest, in particular, for fluorescent labeling in biological systems.[17,18,20,19]



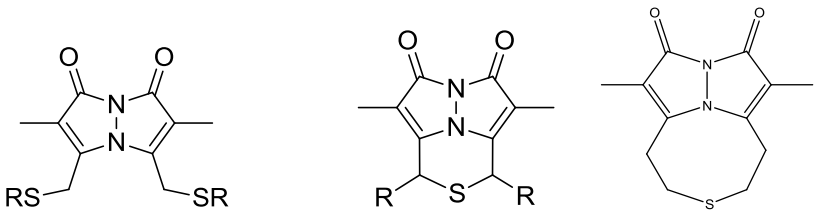
1a **1b**

Chart 2

Structures of *syn*-(CH_2Br, CH_3)B (**1a**) and *anti*-(CH_2Br, CH_3)B (**1b**)

Bis-thioether derivatives were obtained in smooth reactions of **DBB** with aliphatic/aromatic thiols, as well as with cysteine,[21] substituted cysteines[24] or glutathione.[25,21] Formation of thia-bridged

derivatives (Chart 3) as minor side products have also been observed in the reactions with thiols,[26,21] with sodium sulfide[26] or H_2S.[21]



syn-(RS,CH_3)B[21], *μ-(S)-syn*-(R,CH_3)B[26], *μ-(2-S-trimethylene)-syn*-(CH_2,CH_3)B[27]

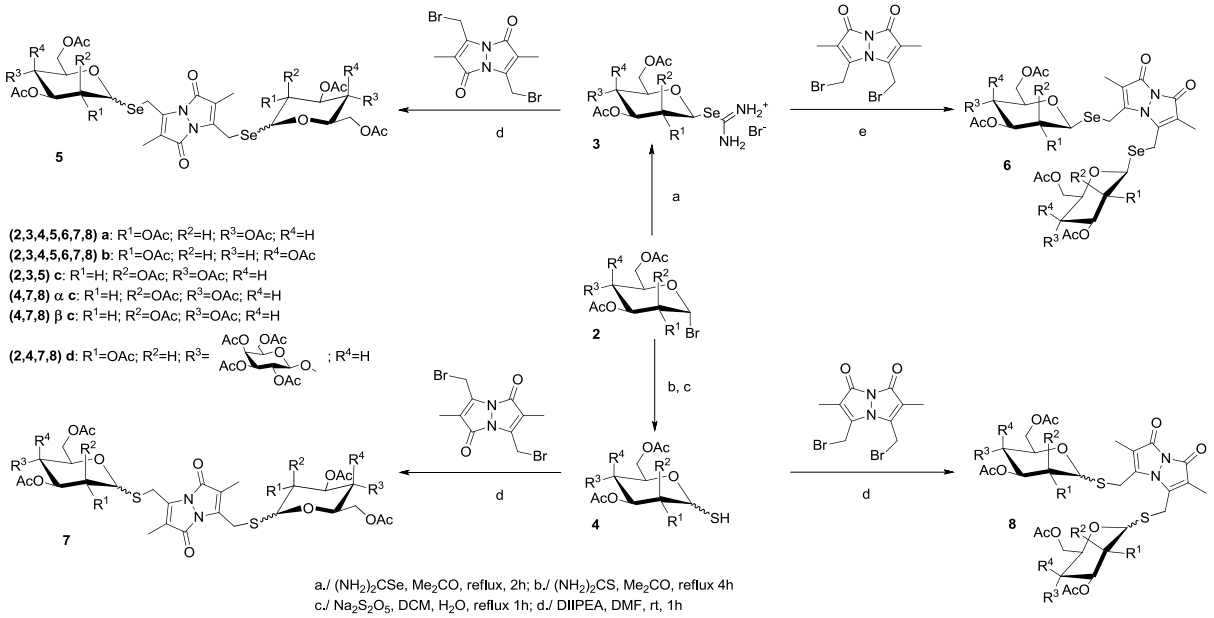
Chart 3. Bimane thioethers

These reactions have been proposed for the purpose of quantification of glutathione,[28] sulfide- or thiol levels or H_2S[21] in physiological concentrations. It is to be noticed that reaction of monochlorobimane (*syn*-(CH_2Cl,Me)B)) with selenocysteine did not result in the expected selenoether derivative; *syn*-(Me,Me)bimane was obtained instead via reduction of the CH_2Cl group by Se-cysteine.[29]

Results and Discussion

Dibromobimanes **1a** and **1b** (Chart 2) were found to be suitable starting compounds for the attachment of glycosyl residues to the bimane skeleton. First we have explored reactions of **1a** and **1b** with per-*O*-acetylated glycosyl thiols **4a,b,αc,βc,d** and observed the formation of bis-thioglycosylated bimanes in *syn* (**8a,b,αc,βc,d**) and *anti* forms (**7a,b,αc,βc,d**), respectively, under mild conditions and in good yields (Scheme 1 and Table 1). Reactions with *syn*- and *anti* **DBB (1a, 1b)** occurred with retention of the anomeric configurations of the starting thiols to provide β-glycosylated derivatives as ascertained trivially by the $J_{H1,H2}$ values for the gluco- (**7a, 8a**), galacto- (**7b, 8b**) or lacto (**7d, 8d**) configurations (see Experimental). Availability of mannosyl thiols in both anomeric configurations, α (**4αc**) and β (**4β c**),[30] enabled the preparation of both α and β thioglycosides (**7,8αc** and **7,8βc**, respectively). In these cases the anomeric configurations were deduced from NOESY spectra wherein the presence of crosspeaks between H1/H3 and H1/H5 of the mannosyl moiety clearly indicated β configurations for **7,8βc** whereas such crosspeaks were missing from the spectra of **7,8αc.** ^{1}H chemical shifts of H-5 were, furthermore, found highly diagnostic for the assignment of anomeric configurations of thiomannosides as values for α-anomers are ~ 0.5 ppm larger than those measured in the β-counterparts (see Experimental). Selenoglycosides cannot be prepared in analogy with glycosyl thiols because of the known instability of the corresponding

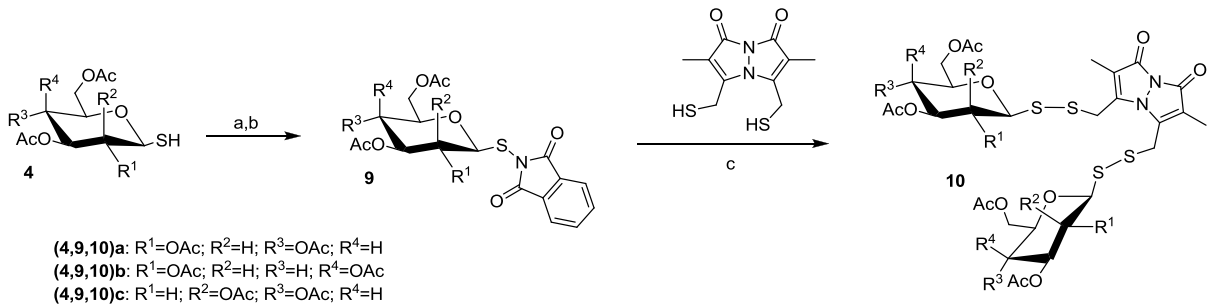
glycosyl selenols[31] On the other hand, selenoglycosides **6a,b** and **5a,b,c**, respectively, were readily obtained from **1a, 1b** using per-*O*-acetylated glycosyl *iso*selenuronium salts **3a,b,c** as glycosyl-selenenol equivalents following a procedure we have previously described[32] (Scheme 1 and Table 1).



Scheme 1

Syntheses of glycosylated bimanes with thio- or selenoglycoside linkages

In view of the versatile activities of carbohydrate molecules with disulfide glycosidic linkages observed in interactions with systems of increasing biorelevance[4] we have set out to prepare glycosylated bimanes featuring this type of glycosidic bond. Of the several methods/approaches available to synthesize unsymmetrical disulfides in general[33] and in sugar chemistry, in particular,[34,35-37] we have chosen to take advantage of the glycosylsulfenyl-transfer properties of glycosyl sulfenamides (**9**) in reactions with thiols.[36] Indeed, reactions of *syn*-(CH_2SH, CH_3)B (**1d**) with the appropriate *N*-phthalyl-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl)sulfenamides[36] furnished the desired bis(glycopyranosyl-dithio) bimane derivatives **10a-c** (Scheme 2, Table 1).

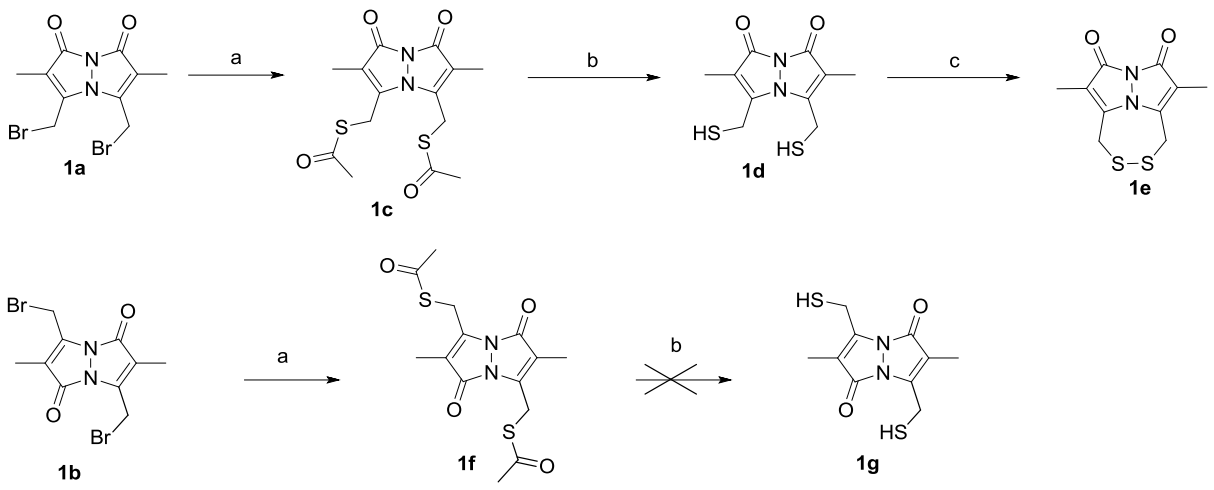


a./ AgOAc, EtOAc, 0-5 °C, 1h; b./ N-bromophthalimide, EtOAc, rt., 15 min; c./ DCM, rt., 15 min

Scheme 2

Syntheses of bis(glycopyranosyl-dithio)bimanes

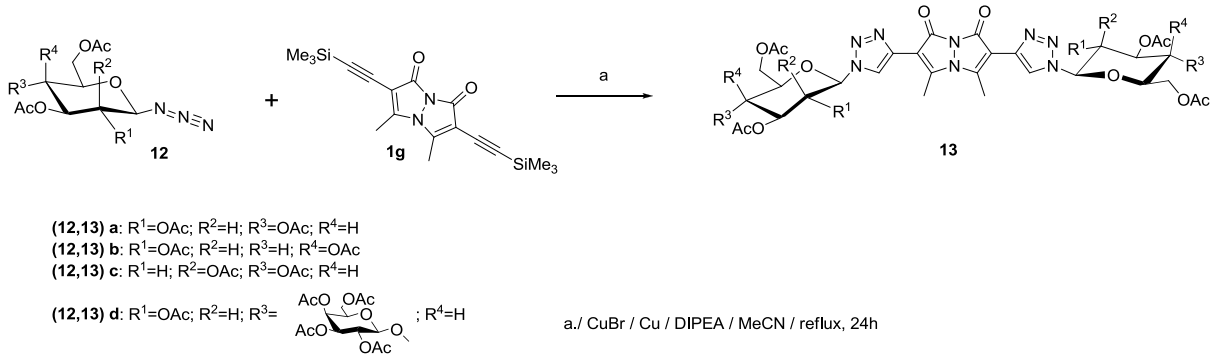
The required *syn*-(CH_2SH, CH_3)B (**1d**) was obtained from **1a** via rection with potassium thiolacetate followed by acidic deacetylation. No *anti*-thiol (**1g**) could, however, be obtained from **1b** as treatment of the thiolacetate **1f** with HCl/MeOH resulted in, surprisingly, total decomposition with the formation of polymeric products. Attempted deacetylation of **1f** under basic conditions (ammonia, LiOH) led to similar result. On the other hand, we have observed the formation of internal disulfide **1e** upon oxidation of **1d** with H_2O_2 (Scheme 3, Table 1).



a./ KSAc, DCM, rt., 2h; b./ 1N HCl, MeOH, rt., 16h; c./ 30 % H_2O_2, DCM, MeOH, rt., 2h

Scheme 3 Reactions to obtain bimane thiols from DBB

As a further option to attach glycosyl moieties to the bimane skeleton we have explored approaches by taking advantage of the Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC, "click") reaction (for a review of applications in carbohydrate chemistry, see[38]) for this purpose. We have selected the bimane diacetylene derivative **1g**[39] for reactions with glycosyl azides **12a,b,c,d** under "click" conditions and, to our satisfaction, 3,7-disubstituted bimane-triazole derivatives **13a,b,c,d** could be obtained in moderate to good yields (Scheme 4 and Table 1).



Scheme 4

Syntheses of glycosylated bimane-triazole derivatives

Table 1

List of the new compounds synthesized

Entry	Compound	Structure*		Yield (%)
		syn / anti	(R^2;R^1)B	
1.	**1c**	*syn*	(CH_2SAc;CH_3)B	62
2.	**1d**	*syn*	(CH_2SH;CH_3)B	87
3.	**1e**	*syn*	(CH_2S*;CH_3)B	63
4.	**1f**	*anti*	(CH_2SAc;CH_3)B	85
5.	**5a**	*anti*	(CH_2Se-β-GlcAc_4;CH_3)B	83
6.	**5b**	*anti*	(CH_2Se-β-GalAc_4;CH_3)B	59
7.	**5c**	*anti*	(CH_2Se-β-ManAc_4;CH_3)B	64

Entry	Compound	Structure*		Yield (%)
		syn / anti	(R^2;R^1)B	
8.	**6a**	*syn*	(CH_2Se-β-GlcAc_4;CH_3)B	62
9.	**6b**	*syn*	(CH_2Se-β-GalAc_4;CH_3)B	68
10.	**7a**	*anti*	(CH_2S-β-GlcAc_4;CH_3)B	80
11.	**7b**	*anti*	(CH_2S-β-GalAc_4;CH_3)B	84
12.	**7βc**	*anti*	(CH_2S-β-ManAc_4;CH_3)B	98
13.	**7αc**	*anti*	(CH_2S-α-ManAc_4;CH_3)B	77
14.	**7d**	*anti*	(CH_2S-β-LacAc_7;CH_3)B	95
15.	**8a**	*syn*	(CH_2S-β-GlcAc_4;CH_3)B	86
16.	**8b**	*syn*	(CH_2S-β-GalAc_4;CH_3)B	77
17.	**8βc**	*syn*	(CH_2S-β-ManAc_4;CH_3)B	73
18.	**8αc**	*syn*	(CH_2S-α-ManAc_4;CH_3)B	21
19.	**8d**	*syn*	(CH_2S-β-LacAc_7;CH_3)B	90
20.	**10a**	*syn*	(CH_2S_2-β-GlcAc_4;CH_3)B	81
21.	**10b**	*syn*	(CH_2S_2-β-GalAc_4;CH_3)B	71
22.	**10βc**	*syn*	(CH_2S_2-β-ManAc_4;CH_3)B	11
23.	**13a**	*syn*	(CH_3;TA-β-GlcAc_4)B	97
24.	**13b**	*syn*	(CH_3;TA-β-GalAc_4)B	57
25.	**13c**	*syn*	(CH_3;TA-β-ManAc_4)B	60
26.	**13d**	*syn*	(CH_3;TA-β-LacAc_7)B	57

* For the notation see Experimental

Of the new compounds the structures of glycosylated and non-glycosylated derivatives have been confirmed by crystallographic analyses. For ORTEP views of structures **1c** and **1d** (Fig. S1) as well as for details of structure determination and refinement see Supplementary Information (Table S1). **1e** (Fig. 1) represents the first example of a bimane structure with an intramolecular disulfide incorporated into a seven-membered ring anellated to the bimane skeleton.

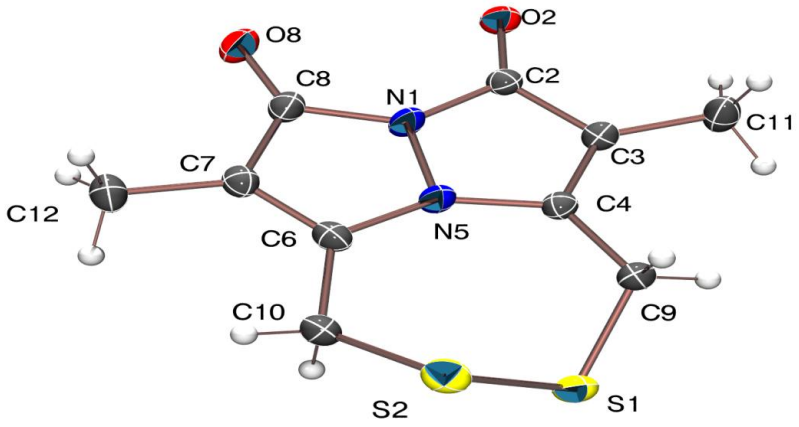


Figure 1

ORTEP View of **1e** at 50% probability level.

In case of **7a** (Fig. 2) one half of the molecule occupies the asymmetric unit and the other half is given by a twofold screw axis. For **1c**, **1e** and **8a** (Fig. 3) two independent molecules could be found in the asymmetric unit with small conformational differences (Fig. S2). Figure 4 shows the molecular structure of triazole derivative **13c**.

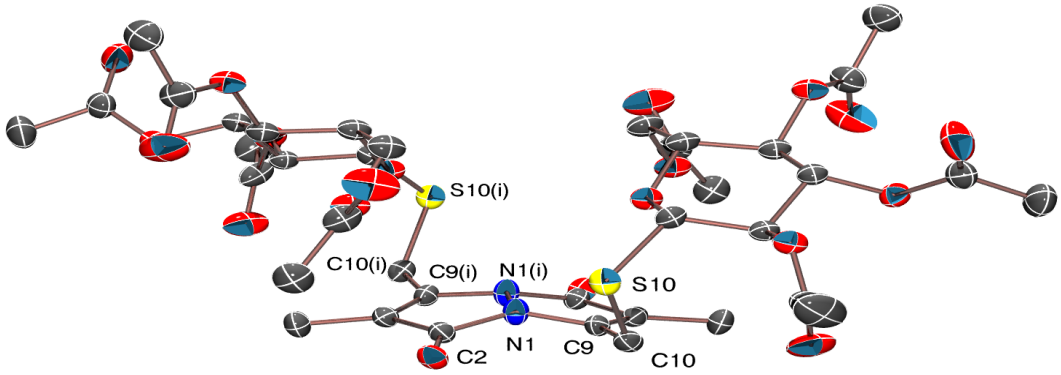


Figure 2

ORTEP view of **7a** at 30% probability level with partial numbering scheme. Hydrogen atoms are omitted for clarity. Symmetry code (i): -y+1, -x+1, -z+3/2.

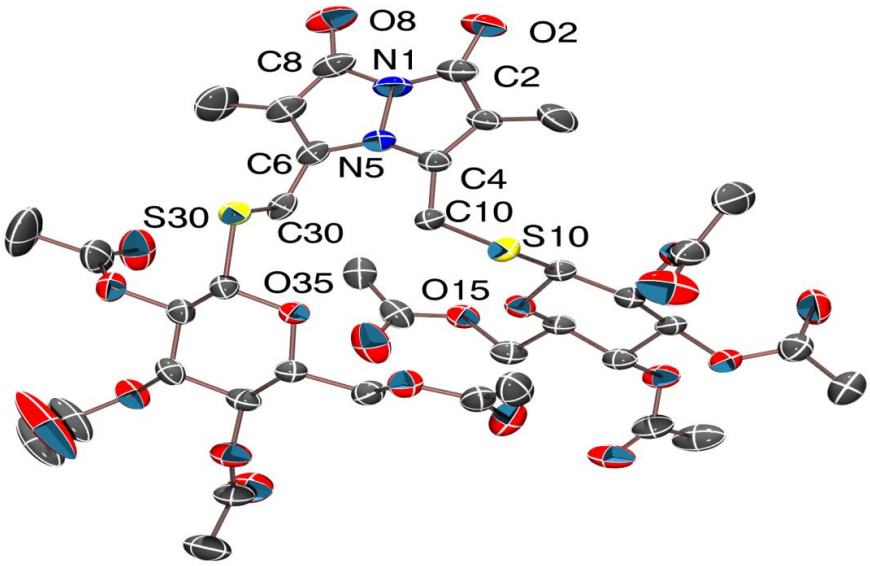


Figure 3

ORTEP view of **8a** with partial numbering scheme at 50 % probability level. Hydrogen atoms are omitted for clarity. Only one of the molecules in the asymmetric unit is shown.

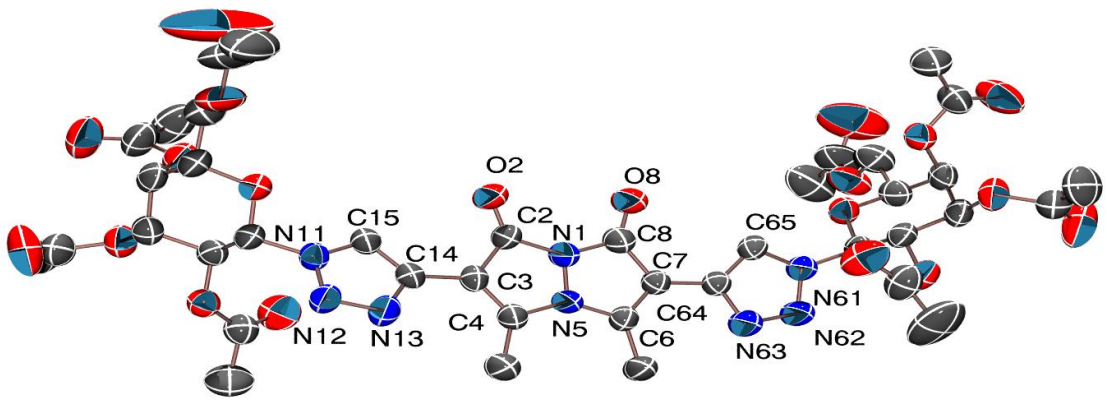


Figure 4

ORTEP view of **13c** at 50% probability level with partial numbering scheme. Hydrogen atoms are omitted for clarity.

Distances between glycosyl units in similar diglycosylated naphthalene derivatives have been shown to matter in sugar-lectin interactions.[4] Compounds **7a** and **13c** represent increasing interglycosidic separations as measured by the through-space distances between the anomeric C-atoms from the crystal structures (Figs. 5 & 6).

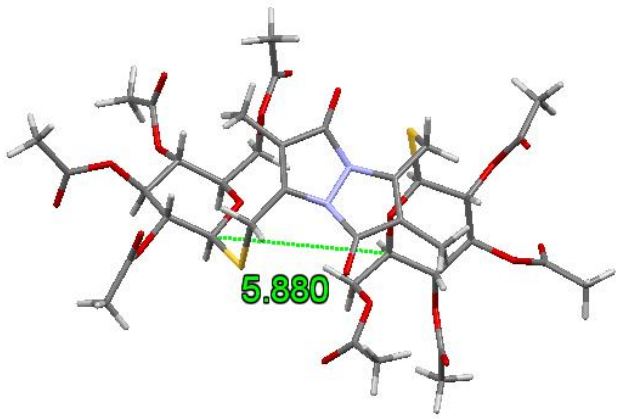


Figure 5

Interglycosidic separation distance (in Å) in **7a.**

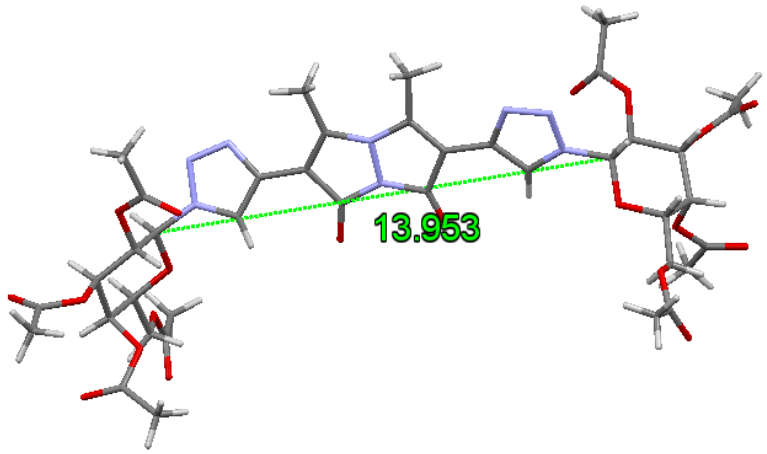


Figure 6

Interglycosidic separation distance (in Å) in **13c**.

Search of the Cambridge Structural Database[40] (Ver. 5.39 updates May, 2018) resulted 28 hits for *syn*- and 11 hits for *anti* bimane structures. Comparison of the bond distance and bond angle data found in the CSD reveals that the corresponding data in our compounds are in the expected range (Table S2). The bimane structures found in the CSD also revealed nonplanarity, i.e., > 0 deg., between the mean planes of the two anellated pyrazolone rings in several cases; values being varied between 0 and 50 deg. with well-defined maximum at <5 deg. It is gratifying, that for the cases investigated here we have often detected two independent molecules in the asymmetric unit of the crystal. It is of note that the extent of bending within these pairs varied significantly, for example, 13 / 22 deg. in **1c** or 5 / 19 deg. in **8a**. Even for **1e** where the two rings are bridged by a disulfide linkage the respective data are 4.4 / 18.8 deg. (Table S3). These results suggest that the planarity of the bimane backbone is not as prevalent, as it was suggested in early single crystal studies.[41,42] Further investigations of the same authors had shown the flexibility of the bimane system.[43,44] Our data suggest that lattice interactions can easily bend the ring system. Moreover, the bending occurs in both *syn*- (**8a**) and *anti* (**7a**) bimanes (Table S3). The relatively large bending (approx. 30 deg.) of the two pyrazolone rings when bridged by a short C-S-C spacer[25]can probably be attributed to steric constrains in part only.

Bimanes are fluorescent compounds with the *syn* isomers displaying distinctly stronger effect than their *anti* counterparts as noted above. As this photophysical property is influenced not only by *syn/anti* relationships but other structural subtleties as well,[45,46] it was of interest to investigate the effects, if any, of the pending glycosyl moieties on the fluorescent behavior of these molecules. First, we note that absorption and fluorescence spectra of compounds with the same glycosidic bond type but bearing different sugar moieties (eg. **8a,b,c,d**) are practically superimposable therefore data for glucosyl derivatives have only been listed in Table 2. Clearly, the sugar part has no effect on the optical absorption and fluorescence properties of these molecules with absorption/ emission maxima and extinction coefficients similar to those published for non-glycosylated bimane derivatives.[15,47] Data for a*nti*-bimane derivatives are not listed either because, in line with literature reports,[15,47] these isomers are non-fluorescent. It is of note, however, that the *syn* selenoglycoside **6a** was found nonfluorescent unlike its thio-analog **8a**. It is known, however, that when "Se is connected to the fluorophore (…) the efficient photoinduced eletron-transfer (PET) process between the Se and fluorophore quenches the fluorescence".[48] On the other hand, this behavior may also be related to small value of the extinction coefficient (800) for the band used for fluorescent excitation in **6a** (360 nm) in comparison with significantly higher values in other *syn*-bimanes (4 ~ 5000, Table 2).

Table 2

Ultraviolet-visible absorption and emission properties of *syn*-bimanes [*syn*-(R^2;R^1)B]*

Compound	R^2	R^1	Absorption λ_{max}, nm (ε_{max})	Emission λ_{max}, nm (Φ_F)
1c	***CH_2SAc***	***CH_3***	375 (5600); 238 (21100)	445 (0.86)
1d	***CH_2SH***	***CH_3***	375 (5400); 237 (14300)	445 (0.70)
1e	***CH_2S****	***CH_3***	360 (4200); 232 (13100)	442 (0.74)
6a	***CH_2Se-β-$GlcAc_4$***	***CH_3***	360 (800); 237 (26100)	non-fluorescent
8a	***CH_2S-β-$GlcAc_4$***	***CH_3***	364 (5100); 255 (23100)	444 (0.89)
10a	***CH_2S_2-β-$GlcAc_4$***	***CH_3***	378 (4600); 245 (13200)	449 (0.57)
13a	***CH_3***	***TA-β-$GlcAc_4$***	370 (3500); 242 (4400)	475 (0.86)

* For the notation see Experimental

Biological evaluation

The anti-trypanosomal activities of the compounds listed in Table 3 were tested against the bloodstream stage of *Trypanosoma b. brucei*, the causative agent of the African sleeping sickness. Nifurtimox (Nfx), an established anti-trypanosomal drug was used as control. Added at a concentration of 5 µM, a subset of the tested bimane derivatives displayed weak to medium anti-proliferative activity (15-56 % growth inhibition). Remarkably, molecules with appreciable inhibitory activities are thio- disulfide derivatives; the most active bearing free thiol groups (10d); the activity being decreased when thiols are blocked such as in the disulfide 1e or thiomannoside 8βc.

Table 3. Anti-trypanosomal activities

Compound	**Cell viability (%)**	**2*SD**
1d	77.9	3.5
1e	43.2	14.1
8b	95.2	6.3
8a	91.2	5.2
8βc	86.5	4.1
8d	90.1	3.7
10b	95.3	0.2
13c	93.9	8.2
Control (Nfx 15 uM)	28.2	5.5

In summary we have described the syntheses and characterization of a set of novel derivatives, including those with appended mono- and disaccharide moieties, based on the 1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (bimane) heteroaromatic ring system. Mono- and disaccharide residues were attached to *syn*- or *anti*-bimane central cores via thio-, dithio- or selenoglycosidic linkages to obtain novel fluorescent or nonfluorescent glycoconjugates. Cu(I)-catalyzed cycloaddition of glycosyl azides to a bimane diethinyl derivative furnished further bivalent glycoconjugates with sugar residues linked to the central bimane core via 1,2,3-triazole rings. We have determined the crystal and molecular structures of several glycosylated and non-glycosylated bimanes and report fluorescence data for the new compounds. Based on previous experience these novel glycoconjugates may prove useful to probe interactions with proteins such as lectins[8,10] or systems with increasing biorelevance[4] or for testing their bioactivity such as antitrypanosomal properties.[9,11]

Experimental

General procedures

syn-($CH_2Br;CH_3$)B, *anti*-($CH_2Br;CH_3$)B,[15] *syn*-(CH_3;C≡CTMS)B,[39] *N*-phthalyl-*S*-(2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl)sulfenamides[36] per-*O*-acetylated-β-D-glycopyranosyl azides,[49] 2,3,4,6-Tetra-*O*-acetyl-β-D-glycopyranosyl *iso*selenuronium bromides[31,32], 1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranoses[50,51,30,52] and 1-thio-2,3,6-tri-*O*-acetyl-4-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-β-D-glucopyranose[53] were prepared according to literature procedures. TLC was performed on DC-Alurolle Kieselgel F254 (Merck), and the spots on the plates visualized under UV light and by heating. For column chromatography Kieselgel 60 (Merck, particle size 0.063-0.200) was used. NMR spectra were recorded on a Bruker Avance II 500 (500/125 MHz for $^1H/^{13}C$) spectrometer. Chemical shifts are referenced to internal TMS (^1H), or to the residual solvent signals (^{13}C). Mass spectra were measured using Bruker microOTOF-Q or Thermo LTQ FT Ultra spectrometers. UV and steady state fluorescence data were determined on a Perkin Elmer Lambda 11 and Jasco FP-8200 spectrometer using 1 µmol solutions in MeCN in a quartz cuvette of 10 mm length. The excitation and emission spectra were

recorded using 2.5 nm excitation, 5.0 nm emission bandwith and 200 nm/min scanning speed. Quantum yields (Φ) were calculated according to the equation:

$$\Phi F_s = \frac{F_s\,(\varepsilon_{ref}\,c_{ref}\,\Phi F_{ref})}{F_{ref}\,(\varepsilon_s\,c_s)}$$

using *syn*-(CH_3,CH_3)B as reference (ΦF_{ref} = 0.72)[15]. Symbols: F: integrated area under fluorescence curve; s: sample; ref: *syn*-(CH_3,CH_3)B; c: concentration; ε: absorption coefficient. X-ray diffraction data were collected at 293-298 K using a Bruker-D8 Venture diffractometer equipped with INCOATEC IμS 3.0 dual (Cu and Mo) sealed tube microsources and Photon 2 Charge-integrated Pixel Array detector. For compounds **1c, 1d, 1e** and **13c** Mo Kα (λ = 0.7107 Å) while for **7a** and **8a** Cu Kα (λ = 1.541 Å) radiation was applied. For the software used for data collection and processing see Supporting Information. The structures could be solved using direct methods and refined on F^2 using SHELXL program[54] incorporated into the APEX3 suite. Refinement was performed anisotropicaly for all non-hydrogen atoms. Hydrogen atoms atoms were placed into geometric positions except the SH protons in **1d** as these hydrogen atoms could be found at the difference electron density map. Tables were extracted from the edited CIF file using publCIF.[55] The PLATON program[56] was used for crystallographic calculations. Further information on the data collection and refinement for the respective compounds can be found in Table S1. CCDC numbers for compounds **1c, 1d, 1e, 7a, 8a, 13c** are 1847474-1847479, respectively. The absolute configurations for compounds **7a, 8a** and **13c** were determined on the basis of the configuration of stereogenic centers in the carbohydrate moiety.

***syn*-(CH_2SAc;CH_3)B**; **4,6-bis-[(acetylthio)methyl]-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (1c)**: 3.50 g (10.0 mmol) *syn*-(CH_2Br;CH_3)B **1a** was dissolved in 50 mL dichloro-methane, at room temperature in nitrogen atmosphere, then 3.43 g (30.0 mmol) potassium-thioacetate added. Stirring was continued at r.t. until TLC indicated complete conversion of the starting materials (1.5 hr)

to products, then 2.00 g silica gel added, the reaction mixture was filtered, and washed with 50 mL dichloro-methane. The filtrate was evaporated at reduced pressure and the residue crystallized from methanol, to yield 2.10 g (62 %) of **1c** as a yellow crystalline powder. Mp.: 131-135 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 4.25 (s, 4H, 2xCH_2); 2.43 (s, 6H, 2xCH_3); 1.79 (s, 6H, 2xCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 193.4 (S*C*O); 159.6 (C-2, C-8); 146.9 (C-4, C-6); 114.2 (C-3, C-7); 30.1 (CO*C*H_3); 22.4 (*C*H_2); 6.8 (*C*H_3). HRMS m/z Calcd for $C_{14}H_{16}N_2O_4S_2$ $[M+H]^+$: 341.062 Found: 341.062.

***syn*-(CH_2SH;CH_3)B**; **4,6-bis-(mercaptomethyl)-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (1d)**: 2.60 g (7.6 mmol) *syn*-(CH_2SAc;CH_3)B **1c** was added to a cold (0 °C) mixture of 65 mL methanol and 2.6 mL (36.4 mmol) acetylchloride. The reaction mixture was then warmed gradually to room temperature, and stirred overnight. When the reaction was complete (TLC) the mixture was cooled, the precipitated solid filtered and washed with cold methanol, to yield 1.70 g (87 %) of **1d** as a yellow powder. Mp.: 169-172 °C. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 3.88 (s, 4H, 2xC*H*$_2$); 3.75 (s, 2H, 2xS*H*); 1.79 (s, 6H, 2xC*H*$_3$); ^{13}C NMR (DMSO-d6, 125 MHz): δ 160.2 (C-2, C-8); 150.0 (C-4, C-6); 111.7 (C-3, C-7); 17.1 (*C*H_2); 6.4 (*C*H_3). HRMS m/z Calcd for $C_{10}H_{12}N_2O_2S_2$ $[M+H]^+$: 257.041 Found: 257.041.

***syn*-(CH_2S^*;CH_3)B**; **4,6-(dithiamethylene)-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (1e)**: 500 mg (0.51 mmol) *syn*-(CH_2SH;CH_3)B **1d** was dissolved in the mixture of 100 mL methanol and 50 mL dichloro-methane, then 3.6 mL 30 % hydrogen-peroxide added. Stirring was continued at r.t. until TLC indicated complete conversion of the starting materials (2 hr), then the reaction mixture was poured into 200 mL of 10 % sodium-bisulfite solution The methanol and dichloro-methane evaporated at reduced pressure, and the water phase extracted with 200 mL ethyl acetate. The organic phase was washed with water, dried on sodium-sulfate and evaporated. The crude product was purified by column chromatography (ethylacetate) to yield, 310 mg (63 %) of **1e** as a yellow powder. Mp.: 151-157 °C. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.12 (s, 4H, 2xC*H*$_2$); 1.81 (s, 6H,

2xCH_3); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 159.0 (C-2, C-8); 149.4 (C-4, C-6); 113.1 (C-3, C-7); 36.1 (CH_2); 6.5 (CH_3). HRMS m/z Calcd for $C_{10}H_{10}N_2O_2S_2$ $[M+Na]^+$: 277.008 Found: 277.007.

***anti*-(CH_2SAc;CH_3)B**; **4,8-bis-[(acetylthio)methyl]-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,7-diene-2,6-dione (1f)**: 800 mg (2.3 mmol) *anti*-(CH_2Br;CH_3)B **1b** was dissolved in 100 mL dichloro-methane, at room temperature in nitrogen atmosphere, then 784 mg (6.9 mmol) potassium-thioacetate added. Stirring was continued at r.t. until TLC indicated complete conversion of the starting materials (1.5 hr) to products, then 2.00 g silica gel added, and the reaction mixture was filtered, and washed with 50 mL dichloro-methane. The filtrate was evaporated, and the residue crystallized from methanol, to yield 660 mg (85 %) of **1f** as a white powder. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.19 (s, 4H, 2xCH_2); 2.35 (s, 6H, 2xCH_3); 1.94 (s, 6H, 2xCH_3); ^{13}C NMR (DMSO-d_6, 125 MHz): δ 194.0 (S*C*O); 160.3 (C-2, C-6); 143.9 (C-4, C-8); 114.3 (C-3, C-7); 30.0 (CO*C*H_3); 22.1 (CH_2); 6.7 (CH_3). HRMS m/z Calcd for $C_{14}H_{16}N_2O_4S_2$ $[M+Na]^+$: 363.045 Found: 363.044.

General procedure for the preparation of selenoglycosides

syn-(CH_2Br;CH_3)B **1a**, or *anti*-(CH_2Br;CH_3)B **1b** and 2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl isoselenuronium bromides **3a-c** were dissolved with stirring in DMF at r.t. under nitrogen and triethylamine added through a septum. Stirring was continued at r.t. until TLC indicated complete conversion of the starting materials (30 min), then the reaction mixture was poured into water. A solid deposited which was filtered, rinsed with 3x10 mL water and dried. The crude products were purified by column chromatography (toluene : isopropanol = 9.5 : 0.5) to yield the anti- (**5a-c)**, and syn- (**6a-b**) selenoglycosides.

***anti*-(CH_2Se-β-GlcAc$_4$;CH_3)B**; **4,8-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)seleno]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,7-diene-2,6-dione (5a)**: From 100 mg (0.29 mmol) *anti*-(CH_2Br;CH_3)B **1b**, 313 mg (0.58 mmol) 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isoselenuronium bromide **3a**, 0.2 mL (1.4 mmol) triethylamine, yield 239 mg (83 %) of **5a**, white powder. $[\alpha]_D^{27}$ – 5 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.23 (t, 2H, H-2,2'^{Glc}, $J_{1,2} = J_{2,3}$ 9.5 Hz); 5.10 (m, 6H, H-3,3'^{Glc}, H-4,4'^{Glc}, H-1,1'^{Glc}); 4.29 (dd, 2H, H-6a,6a'^{Glc}, $J_{5,6a}$ 4.5 Hz, $J_{a,b}$ 12.5 Hz); 4.14 (m, 4H, H-6b,6b'^{Glc}, C$H_{2a,a'}^{Bim}$, $J_{CH2a,b}$ 13.0 Hz); 3.93 (d, 2H, C$H_{2b,b'}^{Bim}$); 3.77 (m, 2H, H-5,5'^{Glc}); 2.09, 2.03, 2.02, 2.01 (s, 24H, 8xCOCH_3^{Glc}); 1.87 (s, 6H, 2xCH_3^{Bim}); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.6, 170.1, 169.5 (COCH$_3$)^{Glc}; 160.7 (C-2, C-6)^{Bim}; 147.2 (C-4, C-8)^{Bim}; 112.6 (C-3, C-7)^{Bim}; 77.3 (C-1,1')^{Glc}; 76.8 (C-5,5')^{Glc}; 73.5 (C-3,3')^{Glc}; 70.7 (C-2,2')^{Glc}; 68.0 (C-4,4')^{Glc}; 61.8 (C-6,6')^{Glc}; 20.7, 20.6 (COCH$_3$)^{Glc}; 12.6 (CH$_2$)^{Bim}; 6.7 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}Se_2$ $[M+Na]^+$: 1035.102 Found: 1035.102.

***anti*-(CH_2Se-β-GalAc$_4$;CH_3)B**; **4,8-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)seleno]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,7-diene-2,6-dione (5b)**: From 200 mg (0.57 mmol) *anti*-(CH_2Br;CH_3)B **1b**, 626 mg (1.2 mmol) 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl isoselenuronium bromide **3b**, 0.4 mL (2.9 mmol) triethyl-amine, yield 340 mg (59 %) of **5b**, white powder. $[\alpha]_D^{27}$ **+** 64 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.47 (d, 2H, H-4,4'^{Gal}, $J_{3,4}$ 2.5 Hz); 5.26 (t, 2H, H-2,2'^{Gal}, $J_{1,2} = J_{2,3}$ 10.0 Hz); 5.08 (m, 4H, H-3,3'^{Gal}, H-1,1'^{Gal}); 4.12 (m, 6H, H-6a,6a'^{Gal}, H-6b,6b'^{Gal}, $CH_{2a,a'}^{Bim}$); 3.99 (m, 2H, H-5,5'^{Gal}, $CH_{2b,b'}^{Bim}$); 2.16, 2.05, 2.04, 1.99 (s, 24H, 8xCOCH_3^{Gal}); 1.87 (s, 6H, 2xCH_3^{Bim}); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.3, 169.9, 169.8 (COCH$_3$)^{Gal}; 160.6 (C-2, C-6)^{Bim}; 147.1 (C-4, C-8)^{Bim}; 112.5 (C-3, C-7)^{Bim}; 78.2 (C-1,1')^{Gal}; 75.6 (C-5,5')^{Gal}; 71.4 (C-3,3')^{Gal}; 68.0 (C-4,4')^{Gal}; 67.3 (C-2,2')^{Gal}; 61.1 (C-6,6')^{Gal}; 20.7 (COCH$_3$)^{Gal}; 12.9 (CH$_2$)^{Bim}; 6.6 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}Se_2$ $[M+Na]^+$: 1035.102 Found: 1035.103.

***anti*-(CH_2Se-β-ManAc_4;CH_3)B**; **4,8-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)seleno]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,7-diene-2,6-dione (5c)**: From 50 mg (0.14 mmol) *anti*-(CH_2Br;CH_3)B **1b**, 157 mg (0.29 mmol) 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl isoselenuronium bromide **3c**, 0.2 mL (1.4 mmol) triethylamine, yield 92 mg (64 %) of **5c**, white powder. $[\alpha]_D^{27}$ – 18 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.72 (s, 2H, H-1,1'^{Man}); 5.42 (d, 2H, H-2,2'^{Man}, $J_{2,3}$ 3.5 Hz); 5.34 (t, 2H, H-4,4'^{Man}, $J_{3,4}$ = $J_{4,5}$ 10.0 Hz); 5.23 (dd, 2H, H-3,3'^{Man}); 4.31 (m, 4H, H-6a,6a'^{Man}, H-5,5'^{Man}, $J_{5,6a}$ 5.0 Hz); 4.16 (dd, 2H, H-6b,6b'^{Man}, $J_{5,6b}$ 1.9 Hz, $J_{6a,6b}$ 12.0 Hz); 3.96 (dd, 4H, 2xCH_2^{Bim}, $J_{CH2a,b}$ 13.0 Hz); 2.17, 2.10, 2.06, 1.98 (s, 24H, 8xCOCH_3^{Man}); 1.86 (s, 6H, 2xCH_3^{Bim}); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.5, 169.7 (COCH_3)^{Man}; 160.1 (C-2, C-6)^{Bim}; 145.4 (C-4, C-8)^{Bim}; 113.7 (C-3, C-7)^{Bim}; 77.9 (C-1,1')^{Man}; 71.1 (C-5,5')^{Man}; 70.6 (C-3,3')^{Man}; 69.8 (C-2,2')^{Man}; 66.1 (C-4,4')^{Man}; 62.3 (C-6,6')^{Man}; 20.9, 20.7 (COCH$_3$)^{Man}; 13.9 (CH$_2$)^{Bim}; 6.8 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}Se_2$ $[M+Na]^+$: 1035.102 Found: 1035.104.

***syn*-(CH_2Se-β-GlcAc_4;CH_3)B**; **4,6-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)seleno]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (6a)**: From 200 mg (0.57 mmol) *syn*-(CH_2Br;CH_3)B **1a**, 625 mg (1.2 mmol) 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isoselenuronium bromide **3a**, 0.2 mL (1.4 mmol) triethylamine, yield 358 mg (62 %) of **6a**, yellow powder. $[\alpha]_D^{27}$ – 5 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.22 (t, 2H, H-2,2'^{Glc}, $J_{2,3}$ = $J_{1,2}$ 9.5 Hz); 5.08 (t, 2H, H-3,3'^{Glc}, $J_{3,4}$ 9.5 Hz); 5.07 (t, 2H, H-4,4'^{Glc}); 4.81 (d, 2H, H-1,1'^{Glc}); 4.28 (dd, 2H, H-6a,6a'^{Glc}, $J_{5,6a}$ 5.0 Hz, $J_{6a,6b}$ 12.5 Hz); 4.14 (dd, 2H, H-6b,6b'^{Glc}, $J_{5,6b}$ 1.5 Hz); 4.08 (d, 2H, C$H_{2a,a'}^{Bim}$, $J_{CH2a,b}$ 13.0 Hz); 3.99 (d, 2H, C$H_{2b,b'}^{Bim}$); 3.73 (m, 2H, H-5,5'^{Glc}); 2.10, 2.06, 2.03, 2.02, (s, 24H, 8xCOCH_3^{Glc}); 1.90 (s, 6H, 2xCH_3^{Bim}); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.5, 169.9, 169.4 (COCH_3)^{Glc}; 160.0 (C-2, C-8)^{Bim}; 146.2 (C-4, C-6)^{Bim}; 114.2 (C-3, C-7)^{Bim}; 77.4 (C-1,1')^{Glc}; 76.8 (C-5,5')^{Glc}; 73.2 (C-3,3')^{Glc}; 70.3 (C-2,2')^{Glc}; 67.9 (C-4,4')^{Glc}; 61.9 (C-6,6')^{Glc}; 20.7, 20.5 (CH$_2$)^{Bim}; 13.6 (COCH$_3$)^{Glc}; 7.1 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}Se_2$ $[M+H]^+$: 1013.120 Found: 1013.123.

***syn*-(CH_2Se-β-GalAc$_4$;CH_3)B; 4,6-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)seleno]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (6b)**: From 100 mg (0.29 mmol) *syn*-(CH_2Br;CH_3)B **1a**, 313 mg (0.58 mmol) 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl isoselenuronium bromide **3b**, 0.2 mL (1.4 mmol) triethylamine, yield 176 mg (61 %) of **6b**, yellow powder. $[\alpha]_D^{27}$ – 75 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.45 (d, 2H, H-4,4'^{Gal}, $J_{3,4}$ 2.0 Hz); 5.24 (t, 2H, H-2,2'^{Gal}, $J_{1,2}$ = $J_{2,3}$ 10.0 Hz); 5.06 (dd, 2H, H-3,3'^{Gal}); 4.81 (d, 2H, H-1,1'^{Gal}); 4.11 (m, 8H, H-6a,6a'^{Gal}, H-6b,6b'^{Gal}, 2xCH_2^{Bim}); 3.96 (t, 2H, H-5,5'^{Gal}, $J_{5,6a}$=$J_{5,6b}$ 10.0 Hz); 2.19, 2.07, 2.06, 2.00 (s, 24H, 8xCOCH_3^{Gal}); 1.92 (s, 6H, 2xCH_3^{Bim}); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.3, 170.1, 169.8 (*C*OCH_3)^{Gal}; 160.0 (C-2, C-8)^{Bim}; 146.2 (C-4, C-6)^{Bim}; 114.0 (C-3, C-7)^{Bim}; 78.3 (C-1,1')^{Gal}; 76.1 (C-5,5')^{Gal}; 71.2 (C-3,3')^{Gal}; 67.5 (C-4,4')^{Gal}; 67.1 (C-2,2')^{Gal}; 61.3 (C-6,6')^{Gal}; 20.7 (CO*C*H_3)^{Gal}; 14.0 (*C*H_2)^{Bim}; 7.1 (*C*H_3)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}Se_2$ $[M+H]^+$: 1013.120 Found: 1013.123.

General procedure for the preparation of thioglycosides

syn-(CH_2Br;CH_3)B **1a**, or *anti*-(CH_2Br;CH_3)B **1b** was dissolved in DMF at r.t. under stirring in nitrogen atmosphere, and per-*O*-acetylated-1-thio-sugars **4a-d** added, followed by triethylamine. Stirring was continued at r.t. until TLC indicated complete conversion of the starting materials (30 min), then the reaction mixture was poured into water. A solid deposited which was filtered, rinsed with 3x10 mL water and dried. Purification by column chromatography (toluene : isopropanol = 9.5 : 0.5) yielded the anti- (**7a-d)** and syn- (**8a-d)** thioglycosides.

***anti*-(CH_2S-β-GlcAc$_4$;CH_3)B; 4,8-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thio]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,7-diene-2,6-dione (7a)**: From 100 mg (0.29 mmol) *anti*-(CH_2Br;CH_3)B **1b**, 219 mg (0.60 mmol) 1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose **4a**, 0.2 ml (1.4

mmol) triethyl-amine, yield 210 mg (80 %) of **7a**, white powder. $[\alpha]_D^{27}$ **+** 6 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.24 (t, 2H, H-2,2'^{Glc}, $J_{1,2} = J_{2,3}$ 9.5 Hz); 5.12 (t, 2H, H-4,4'^{Glc}, $J_{3,4} = J_{4,5}$ 9.5 Hz); 5.06 (t, 2H, H-3,3'^{Glc}); 4.76 (d, 2H, H-1,1'^{Glc}); 4.24 (dd, 2H, H-6a,6a'^{Glc}, $J_{5,6a}$ 4.5 Hz, $J_{6a,6b}$ 10.0 Hz); 4.16 (d, 2H, $CH_{2a,a'}{}^{Bim}$, $J_{CH2a,b}$ 14.5 Hz); 4.10 (dd, 2H, H-6b,6b'^{Glc}, $J_{5,6b}$ 1.5 Hz); 3.91 (d, 2H, $CH_{2b,b'}{}^{Bim}$); 3.75 (m, 2H, H-5,5'^{Glc}); 2.09, 2.04, 2.01, 2.00 (s, 24H, 8xCOC$H_3{}^{Glc}$); 1.89 (s, 6H, 2xC$H_3{}^{Bim}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.6, 170.1, 169.4 (COCH$_3$)^{Glc}; 160.5 (C-2, C-6)^{Bim}; 145.9 (C-4, C-8)^{Bim}; 113.8 (C-3, C-7)^{Bim}; 82.5 (C-1,1')^{Glc}; 76.0 (C-5,5')^{Glc}; 73.6 (C-3,3')^{Glc}; 69.8 (C-2,2')^{Glc}; 68.1 (C-4,4')^{Glc}; 61.8 (C-6,6')^{Glc}; 21.7 (CH$_2$)^{Bim}; 20.6 (COCH$_3$)^{Glc}; 6.6 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}S_2$ $[M+Na]^+$: 939.213 Found: 939.215.

***anti*-(CH$_2$S-β-GalAc$_4$;CH$_3$)B**; **4,8-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thio]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,7-diene-2,6-dione (7b)**: From 100 mg (0.29 mmol) *anti*-(CH$_2$Br;CH$_3$)B **1b**, 219 mg (0.60 mmol) 1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose **4b**, 0.2 ml (1.4 mmol) triethyl-amine, yield 220 mg (84 %) of **7b**, white powder. $[\alpha]_D^{27}$ – 40 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.44 (d, 2H, H-4,4'^{Gal}, $J_{3,4}$ 3.0 Hz); 5.20 (t, 2H, H-2,2'^{Gal}, $J_{1,2} = J_{2,3}$ 10.0 Hz); 5.07 (dd, 2H, H-3,3'^{Gal}); 4.78 (d, 2H, H-1,1'^{Gal}); 4.08 (m, 10H, H-6a,6a'^{Gal}, H-6b,6b'^{Gal}, 2xC$H_2{}^{Bim}$, H-5,5'^{Gal}); 2.16, 2.05, 2.04, 1.97 (s, 24H, 8xCOC$H_3{}^{Gal}$); 1.88 (s, 6H, 2xC$H_3{}^{Bim}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.3, 170.1, 169.6 (COCH$_3$)^{Gal}; 160.4 (C-2, C-6)^{Bim}; 145.7 (C-4, C-8)^{Bim}; 113.9 (C-3, C-7)^{Bim}; 83.4 (C-1,1')^{Gal}; 74.5 (C-5,5')^{Gal}; 71.6 (C-3,3')^{Gal}; 67.3 (C-4,4')^{Gal}, (C-2,2')^{Gal}; 61.0 (C-6,6')^{Gal}; 22.3 (CH$_2$)^{Bim}; 20.7, 20.5 (COCH$_3$)^{Gal}; 6.6 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}S_2$ $[M+Na]^+$: 939.213 Found: 939.210.

***anti*-(CH$_2$S-β-ManAc$_4$;CH$_3$)B**; **4,8-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)thio]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,7-diene-2,6-dione (7βc)**: From 100 mg (0.29 mmol) *anti*-(CH$_2$Br;CH$_3$)B **1b**, 219 mg (0.60 mmol) 1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose **4βc**, 0.2 ml (1.4 mmol) triethyl-amine, yield 260 mg (98 %) of **7βc**, white powder. $[\alpha]_D^{27}$ **+** 10 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.47 (d, 2H, H-2,2'^{Man}, $J_{2,3}$ 3.5 Hz); 5.29 (t, 2H, H-4,4'^{Man}, $J_{3,4} = J_{4,5}$ 10.0 Hz);

5.10 (dd, 2H, H-3,3′^{Man}); 5.04 (s, 2H, H-1,1′^{Man}); 4.24 (dd, 2H, H-6a,6a′^{Man}, $J_{5,6a}$ 5.0 Hz, $J_{6a,6b}$ 12.5 Hz); 4.15 (dd, 2H, H-6b,6b′^{Man}, $J_{5,6b}$ 2.0 Hz); 4.11 (d, 2H, $CH_{2a,a'}{}^{Bim}$, $J_{CH2a,b}$ 14.5 Hz); 3.95 (d, 2H, $CH_{2b,b'}{}^{Bim}$); 3.73 (m, 2H, H-5,5′^{Man}); 2.17, 2.09, 2.04, 1.96 (s, 24H, 8xCOC$H_3{}^{Man}$); 1.85 (s, 6H, 2xC$H_3{}^{Bim}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.7, 170.0, 169.5 (COCH$_3$)^{Man}; 160.2 (C-2, C-6)^{Bim}; 145.4 (C-4, C-8)^{Bim}; 114.0 (C-3, C-7)^{Bim}; 81.5 (C-1,1′)^{Man}; 76.5 (C-5,5′)^{Man}; 71.7 (C-3,3′)^{Man}; 69.9 (C-2,2′)^{Man}; 65.7 (C-4,4′)^{Man}; 62.6 (C-6,6′)^{Man}; 22.7 (CH$_2$)^{Bim}; 20.9, 20.7, 20.5 (COCH$_3$)^{Man}; 6.5 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}S_2$ $[M+Na]^+$: 939.213 Found: 939.214.

***anti*-(CH_2S-α-ManAc$_4$;CH_3)B**; **4,8-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,7-diene-2,6-dione (7αc)**: From 100 mg (0.29 mmol) *anti*-(CH_2Br;CH_3)B **1b**, 219 mg (0.60 mmol) 1-thio-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose **4αc**, 0.2 ml (1.4 mmol) triethyl-amine, yield 201 mg (77 %) of **7αc**, white powder. $[\alpha]_D{}^{27}$ **+** 91 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.30 (m, 6H, H-2,2′^{Man}, H-1,1′^{Man}, H-4,4′^{Man}); 5.19 (dd, 2H, H-3,3′^{Man}, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 10.0 Hz); 4.36 (m, 2H, H-5,5′^{Man}); 4.26 (dd, 2H, H-6a,6a′^{Man}, $J_{5,6a}$ 5.5 Hz, $J_{6a,6b}$ 12.5 Hz); 4.12 (dd, 2H, H-6b,6b′^{Man}, $J_{5,6b}$ 2.0 Hz); 3.95 (d, 2H, $CH_{2a,a'}{}^{Bim}$, $J_{CH2a,b}$ 14.0 Hz); 3.88 (d, 2H, $CH_{2b,b'}{}^{Bim}$); 2.13, 2.06, 2.02, 1.93 (s, 24H, 8xCOC$H_3{}^{Man}$); 1.84 (s, 6H, 2xC$H_3{}^{Bim}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.4, 169.6 (COCH$_3$)^{Man}; 159.9 (C-2, C-6)^{Bim}; 143.8 (C-4, C-8)^{Bim}; 115.0 (C-3, C-7)^{Bim}; 81.5 (C-1,1′)^{Man}; 70.0 (C-5,5′)^{Man}; 69.4 (C-3,3′)^{Man}, 69.4 (C-2,2′)^{Man}; 66.1 (C-4,4′)^{Man}; 62.4 (C-6,6′)^{Man}; 22.4 (CH$_2$)^{Bim}; 20.8, 20.5 (COCH$_3$)^{Man}; 6.5 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}S_2$ $[M+Na]^+$: 939.213 Found: 939.213.

***anti*-(CH_2S-β-LacAc$_7$;CH_3)B**; **4,8-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucosyl)thio]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,7-diene-2,6-dione (7d)**: From 100 (0.29 mmol) mg *anti*-(CH_2Br;CH_3)B **1b**, 392 mg (0.60 mmol), 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-1-thio-2,3,6-tri-*O*-acetyl-β-D-glucopyranose **4d**, 0.1 ml (0.72 mmol) triethylamine, yield 407 mg (95 %) of **7d**, white powder. $[\alpha]_D{}^{27}$ **+** 6 (c 0.25 $CDCl_3$). ^{1}H NMR

($CDCl_3$, 500 MHz): δ 5.34 (d, 2H, H-4,4'^{Gal}, $J_{3,4}$ 3.5 Hz); 5.22 (t, 2H, H-3,3'^{Glc}, $J_{2,3} = J_{3,4}$ 9.5 Hz); 5.09 (t, 2H, H-2,2'^{Gal}, $J_{1,2} = J_{2,3}$ 8.0 Hz); 4.95 (dd, 2H, H-3,3'^{Gal}); 4.93 (t, 2H, H-2,2'^{Glc}, $J_{1,2}$ 9.5 Hz); 4.72 (d, 2H, H-1,1'^{Glc}); 4.48 (m, 4H, H-1,1'^{Gal}, H-6a,6a'^{Glc}, $J_{6a,6b}$ 12.5 Hz); 4.09 (m, 8H, $CH_{2a,a'}{}^{Bim}$, H-4,4'^{Glc}, H-6b,6b'^{Glc}, H-6b,6b'^{Gal}); 3.88 (m, 4H, $CH_{2b,b'}{}^{Bim}$, H-6a,6a'^{Gal}); 3.82 (t, 2H, H-5,5'^{Gal}, $J_{5,6a}$=$J_{5,6b}$ 10.0 Hz); 3.64 (m, 2H, H-5,5'^{Glc}); 2.14, 2.12, 2.06, 2.04, 2.03, 2.02, 1.96, 1.84 (s, 48H, 14xCOC$H_3{}^{Lac}$, 2xC$H_3{}^{Bim}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.3, 170.0, 169.6, 169.1 (COCH_3)^{Lac}; 160.5 (C-2, C-6)^{Bim}; 145.8 (C-4, C-8)^{Bim}; 113.8 (C-3, C-7)^{Bim}; 101.0 (C-1,1')^{Gal}; 82.4 (C-1,1')^{Glc}; 76.7 (C-5,5')^{Glc}; 75.9 (C-4,4')^{Glc}; 73.6 (C-3,3')^{Glc}; 71.0 (C-5,5')^{Gal}; 70.7 (C-3,3')^{Gal}; 70.3 (C-2,2')^{Gal}; 69.1 (C-2,2')^{Glc}; 66.6 (C-4,4')^{Gal}; 61.8 (C-6,6')^{Glc}; 60.8 (C-6,6')^{Gal}; 21.9 (CH$_2$)^{Bim}; 20.9, 20.8, 20.7, 20.6, 20.5 (COCH$_3$)^{Lac}; 6.6 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{62}H_{80}N_2O_{36}S_2$ $[M+Na]^+$: 1515.382 Found: 1515.390.

***syn*-(CH_2S-β-GlcAc$_4$;CH_3)B**; **4,6-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)thio]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (8a)**: From 100 mg (0.29 mmol) *syn*-(CH_2Br;CH_3)B **1a**, 219 mg (0.60 mmol) 1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose **4a**, 0.1 ml (0.72 mmol) triethylamine, yielding 225 mg (86 %) of **8a**, as a pale yellow powder. $[\alpha]_D{}^{27}$ – 97 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.24 (t, 2H, H-3,3'^{Glc}, $J_{2,3} = J_{3,4}$ 9.5 Hz); 5.08 (t, 2H, H-4,4'^{Glc}, $J_{4,5}$ 9.5 Hz); 5.03 (t, 2H, H-2,2'^{Glc}, $J_{1,2}$ 9.5 Hz); 4.53 (d, 2H, H-1,1'^{Glc}); 4.27 (dd, 2H, H-6a,6a'^{Glc}, $J_{5,6a}$ 5.0 Hz, $J_{6a,6b}$ 12.5 Hz); 4.13 (dd, 2H, H-6b,6b'^{Glc}, $J_{5,6b}$ 1.5 Hz); 4.11 (d, 2H, $CH_{2a,a'}{}^{Bim}$, $J_{CH2a,b}$ 14.0 Hz); 4.01 (d, 2H, $CH_{2b,b'}{}^{Bim}$); 3.73 (m, 2H, H-5,5'^{Glc}); 2.11, 2.06, 2.04, 2.02 (s, 24H, 8xCOC$H_3{}^{Glc}$); 1.94 (s, 6H, 2xC$H_3{}^{Bim}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.5, 170.0, 169.3 (COCH$_3$)^{Glc}; 159.9 (C-2, C-8)^{Bim}; 144.8 (C-4, C-6)^{Bim}; 115.0 (C-3, C-7)^{Bim}; 81.8 (C-1,1')^{Glc}; 76.5 (C-5,5')^{Glc}; 73.3 (C-3,3')^{Glc}; 69.5 (C-2,2')^{Glc}; 67.9 (C-4,4')^{Glc}; 61.8 (C-6,6')^{Glc}; 22.3 (CH$_2$)^{Bim}; 20.7, 20.6 (COCH$_3$)^{Glc}; 7.2 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}S_2$ $[M+H]^+$: 917.231 Found: 917.230.

***syn*-(CH_2S-β-GalAc_4;CH_3)B**; **4,6-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)thio]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (8b)**: From 200 mg (0.57 mmol) *syn*-(CH_2Br;CH_3)B **1a**, 438 mg (1.2 mmol) 1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose **4b,** 0.2 ml (1.4 mmol) triethyl amine, yield 401 mg (77 %) of **8b** as pale yellow powder. $[\alpha]_D^{27}$ – 86 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.45 (d, 2H, H-4,4'^{Gal}, $J_{3,4}$ 1.5 Hz); 5.20 (t, 2H, H-2,2'^{Gal}, $J_{1,2}$ = $J_{2,3}$ 10.0 Hz); 5.07 (dd, 2H, H-3,3'^{Gal}); 4.52 (d, 2H, H-1,1'^{Gal}); 4.10 (m, 8H, H-6a,6a'^{Gal}, H-6b,6b'^{Gal}, $CH_{2a,a'}{}^{Bim}$, $CH_{2b,b'}{}^{Bim}$); 3.96 (t, 2H, H-5,5'^{Gal}, $J_{5,6a}$=$J_{5,6b}$ 6.5 Hz); 2.20, 2.07, 2.06, 2.00 (s, 24H, 8xCOC$H_3{}^{Gal}$); 1.96 (s, 6H, 2xC$H_3{}^{Bim}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.2, 169.6 (COCH$_3$)^{Gal}; 159.8 (C-2, C-8)^{Bim}; 144.8 (C-4, C-6)^{Bim}; 114.6 (C-3, C-7)^{Bim}; 82.7 (C-1,1')^{Gal}; 75.0 (C-5,5')^{Gal}; 71.2 (C-3,3')^{Gal}; 67.0 (C-2,2')^{Gal}; 66.6 (C-4,4')^{Gal}; 61.3 (C-6,6')^{Gal}; 22.6 (CH$_2$)^{Bim}; 20.5 (COCH$_3$)^{Gal}; 6.9 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}O_{20}N_2S_2$ $[M+H]^+$: 917.231 Found: 917.232.

***syn*-(CH_2S-β-ManAc_4;CH_3)B**; **4,6-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)thio]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (8βc)**: From 200 mg (0.57 mmol) *syn*-(CH_2Br;CH_3)B **1a**, 438 mg (1.2 mmol) 1-thio-2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranose **4βc**, 0.2 ml (1.4 mmol) triethyl-amine, yield 382 mg (73 %) of **8βc** as pale yellow powder. $[\alpha]_D^{27}$ – 106 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.50 (d, 2H, H-2,2'^{Man}, $J_{2,3}$ 3.0 Hz); 5.26 (t, 2H, H-4,4'^{Man}, $J_{3,4}$ = $J_{4,5}$ 10.0 Hz); 5.11 (dd, 2H, H-3,3'^{Man}); 5.01 (s, 2H, H-1,1'^{Man}); 4.26 (dd, 2H, H-6a,6a'^{Man}, $J_{5,6a}$ 6.0 Hz, $J_{6a,6b}$ 12.5 Hz); 4.17 (m, 4H, H-6b,6b'^{Man}, $CH_{2a,a'}{}^{Bim}$); 3.93 (d, 2H, $CH_{2b,b'}{}^{Bim}$, $J_{CH2a,b}$ 15.0 Hz); 3.88 (m, 2H, H-5,5'^{Man}); 2.18, 2.09, 2.02, 1.92 (s, 24H, 8xCOC$H_3{}^{Man}$); 1.88 (s, 6H, 2xC$H_3{}^{Bim}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.0, 169.7, 169.3 (COCH$_3$)^{Man}; 158.7 (C-2, C-8)^{Bim}; 142.6 (C-4, C-6)^{Bim}; 114.9 (C-3, C-7)^{Bim}; 78.7 (C-1,1')^{Man}; 75.8 (C-5,5')^{Man}; 71.0 (C-3,3')^{Man}; 69.8 (C-2,2')^{Man}; 65.2 (C-4,4')^{Man}; 62.5 (C-6,6')^{Man}; 22.8 (CH$_2$)^{Bim}; 20.2 (COCH$_3$)^{Man}; 6.5 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}S_2$ $[M+H]^+$: 917.231 Found: 917.233.

***syn*-(CH_2S-α-ManAc$_4$;CH_3)B**; **4,6-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)thio]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (8αc)**: From 176 mg (0.50 mmol) *syn*-(CH_2Br;CH_3)B **1a**, 376 mg (1.0 mmol) 1-thio-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose **4αc**, 0.2 ml (1.4 mmol) triethyl-amine, yield 96.5 mg (21 %) of **8αc**, as pale yellow powder. $[\alpha]_D^{27}$ **+** 4 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.34 (dd, 2H, H-2,2'^{Man}, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 3.5 Hz); 5.30 (t, 2H, H-4,4'^{Man}, $J_{3,4} = J_{4,5}$ 10.0 Hz); 5.18 (dd, 2H, H-3,3'^{Man}); 5.12 (d, 2H, H-1,1'^{Man}); 4.34 (m, 2H, H-5,5'^{Man}); 4.27 (dd, 2H, H-6a,6a'^{Man}, $J_{5,6a}$ 6.0 Hz, $J_{6a,6b}$ 12.5 Hz); 4.21 (dd, 2H, H-6b,6b'^{Man} , $J_{5,6b}$ 2.0 Hz); 4.00 (d, 2H, $CH_{2a,a'}{}^{Bim}$, $J_{CH2a,b}$ 15.0 Hz); 3.91 (d, 2H, $CH_{2b,b'}{}^{Bim}$); 2.17, 2.13, 2.09, 1.99 (s, 24H, 8xCOC$H_3{}^{Man}$); 1.92 (s, 6H, 2xC$H_3{}^{Bim}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.9, 169.3, 169.0 (*C*OCH_3)^{Man}; 159.0 (C-2, C-8)^{Bim}; 142.9 (C-4, C-6)^{Bim}; 115.6 (C-3, C-7)^{Bim}; 80.0 (C-1,1')^{Man}; 69.6 (C-3,3')^{Man}; 69.1 (C-5,5')^{Man}; 69.0 (C-2,2')^{Man}; 65.6 (C-4,4')^{Man}; 62.1 (C-6,6')^{Man}; 22.6 (*C*H_2)^{Bim}; 20.4, 20.2 (CO*C*H_3)^{Man}; 6.6 (*C*H_3)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}S_2$ $[M+Na]^+$: 939.214 Found: 939.216.

***syn*-(CH_2S-β-LacAc$_7$;CH_3)B**; **4,6-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucosyl)thio]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (8d)**: From 100 mg (0.29 mmol) *syn*-(CH_2Br;CH_3)B **1a**, 392 mg (0.60 mmol) 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl-(1→4)-1-thio-2,3,6-tri-*O*-acetyl-β-D-glucopyranose **4e**, 0.1 ml (0.72 mmol) triethyl-amine, yield 383 mg (90 %) of **8d**, as pale yellow powder. $[\alpha]_D^{27}$ – 81 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.27 (d, 2H, H-4,4'^{Gal}, $J_{3,4}$ 5.0 Hz); 5.13 (t, 2H, H-3,3'^{Glc}, $J_{2,3} = J_{3,4}$ 10.0 Hz); 5.01 (t, 2H, H-2,2'^{Gal}, $J_{1,2} = J_{2,3}$ 10.0 Hz); 4.89 (dd, 2H, H-3,3'^{Gal}); 4.84 (t, 2H, H-2,2'^{Glc}); 4.43 (m, 4H, H-1,1'^{Gal}, H-1,1'^{Glc}); 4.38 (d, 2H, H-6a,6a'^{Glc}, $J_{6a,6b}$ 10.0 Hz); 3.98 (m, 10H, 2xC$H_2{}^{Bim}$, H-6b,6b'^{Glc}, H-6a,6a'^{Gal}, H-6b,6b'^{Gal}); 3.83 (t, 2H, H-4,4'^{Glc}, $J_{4,5}$ 10.0 Hz); 3.69 (t, 2H, H-5,5'^{Gal}, $J_{5,6a}$=$J_{5,6b}$ 9.5 Hz); 3.55 (m, 2H, H-5,5'^{Glc}); 2.07, 2.05, 1.98, 1.97, 1.96, 1.88, 1.83 (s, 48H, 14xCOC$H_3{}^{Lac}$, 2xC$H_3{}^{Bim}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.4, 169.9, 169.4, 169.0 (*C*OCH_3)^{Lac}; 159.7 (C-2, C-8)^{Bim}; 144.6 (C-4, C-6)^{Bim}; 114.7 (C-3, C-7)^{Bim}; 100.9 (C-1,1')^{Gal}; 81.7 (C-1,1')^{Glc}; 77.4 (C-5,5')^{Glc}; 75.6 (C-4,4')^{Glc}; 73.1 (C-3,3')^{Glc}; 70.8 (C-5,5')^{Gal};

70.6 (C-3,3')^{Gal}; 69.8 (C-2,2')^{Glc}; 69.0 (C-2,2')^{Gal}; 66.5 (C-4,4')^{Gal}; 61.9 (C-6,6')^{Glc}; 60.7 (C-6,6')^{Gal}; 22.4 (*C*H$_2$)^{Bim}; 20.5, 20.4 (CO*C*H$_3$)^{Lac}; 7.0 (*C*H$_3$)^{Bim}. HRMS m/z Calcd for $C_{62}H_{80}N_2O_{36}S_2$ $[M+H]^+$: 1493.400 Found: 1493.404.

General procedure for the preparation of *bis*(glycopyranosyl-dithio)bimanes

syn-(CH$_2$SH;CH$_3$)B **1d** was dissolved in DMF at r.t. with stirring in nitrogen atmosphere, then N-phthalyl-S-(2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl)sulfenamides **9a-c** added. Stirring was continued at r.t. until TLC indicated complete conversion of the starting materials (30 min), then the reaction mixture was poured into water. A solid deposited which was filtered, rinsed with 3x10 mL water and dried. The crude peracetylated products were purified by column chromatography (toluene : isopropanol = 9.5 : 0.5) to yield the disulfido glycosides **10a-c**.

***syn*-(CH$_2$S$_2$-β-GlcAc$_4$;CH$_3$)B**; **4,6-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)dithio]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (10a)**: From 130 mg (0.51 mmol) *syn*-(CH$_2$SH;CH$_3$)B **1d**, 650 mg (1.3 mmol) N-phthalyl-S-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)sulfenamide **9a**, yield 406 mg (81 %) of **10a**, pale yellow powder. $[\alpha]_D^{27}$ – 161 (c 0.25 CDCl$_3$). ^{1}H NMR (CDCl$_3$, 500 MHz): δ 5.38 (t, 2H, H-2,2'^{Glc}, $J_{1,2} = J_{2,3}$ 9.5 Hz); 5.29 (t, 2H, H-3,3'^{Glc}); 5.15 (t, 2H, H-4,4'^{Glc}, $J_{3,4} = J_{4,5}$ 9.5 Hz); 4.59 (d, 2H, H-1,1'^{Glc}); 4.27 (m, 6H, 2xC*H*$_2^{Bim}$, H-6a,6a'^{Glc}, $J_{6a,6b}$ 12.5 Hz, $J_{5,6a}$ 5.0 Hz); 3.96 (d, 2H, H-6b,6b'^{Glc}); 3.85 (m, 2H, H-5,5'^{Glc}); 2.10, 2.06, 2.03 (s, 24H, 8xCOC*H*$_3^{Glc}$); 1.96 (s, 6H, 2xC*H*$_3^{Bim}$); ^{13}C NMR (CDCl$_3$, 125 MHz): δ 170.5, 169.9, 169.3, 169.0 (*C*OCH$_3$)^{Glc}; 159.9 (C-2, C-8)^{Bim}; 144.5 (C-4, C-6)^{Bim}; 116.1 (C-3, C-7)^{Bim}; 86.1 (C-1,1')^{Glc}; 77.3 (C-5,5')^{Glc}; 73.4 (C-3,3')^{Glc}; 68.6 (C-2,2')^{Glc}; 67.8 (C-4,4')^{Glc}; 61.8 (C-6,6')^{Glc}; 32.7 (*C*H$_2$)^{Bim}; 20.6, 20.5 (CO*C*H$_3$)^{Glc}; 7.4 (*C*H$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}S_4$ $[M+Na]^+$: 1003.158 Found: 1003.153.

***syn*-(CH_2S_2-β-$GalAc_4$;CH_3)B**; **4,6-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)dithio]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (10b)**: From 68 mg (0.27 mmol) *syn*-(CH_2SH;CH_3)B **1d**, 340 mg (0.67 mmol) N-phthalyl-S-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)sulfenamide **9b**, yield 185 mg (71 %) of **10b**, pale yellow powder. $[\alpha]_D^{27}$ – 108 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.55 (t, 2H, H-2,2'^{Gal}, $J_{1,2}$ = $J_{2,3}$ 10.0 Hz); 5.49 (dd, 2H, H-4,4'^{Gal}, $J_{3,4}$ 3.0 Hz, $J_{4,5}$ 0.5 Hz); 5.12 (dd, 2H, H-3,3'^{Gal}); 5.11 (d, 2H, H-1,1'^{Gal}); 4.19 (m, 6H, H-6a,6a'^{Gal}, H-6b,6b'^{Gal}, $CH_{2a,a'}{}^{Bim}$); 4.06 (td, 2H, H-5,5'^{Gal}, $J_{5,6a}$=$J_{5,6b}$ 6.5 Hz, $J_{4,5}$ 0.5 Hz); 4.01 (d, 2H, $CH_{2a,a'}{}^{Bim}$, $J_{CH2a,b}$ 14.5 Hz); 2.22, 2.07, 2.05, 2.01 (s, 24H, 8xCOC$H_3{}^{Gal}$); 1.97 (s, 6H, 2xC$H_3{}^{Bim}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.4, 170.0, 169.4 (COCH$_3$)^{Gal}; 160.0 (C-2, C-8)^{Bim}; 144.3 (C-4, C-6)^{Bim}; 116.2 (C-3, C-7)^{Bim}; 87.0 (C-1,1')^{Gal}; 75.5 (C-5,5')^{Gal}; 71.5 (C-3,3')^{Gal}; 67.2 (C-2,2')^{Gal}; 66.1 (C-4,4')^{Gal}; 61.9 (C-6,6')^{Gal}; 32.7 (CH$_2$)^{Bim}; 20.7, 20.6 (COCH$_3$)^{Gal}; 7.5 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}S_4$ $[M+Na]^+$: 1003.158 Found: 1003.152.

***syn*-(CH_2S_2-β-$ManAc_4$;CH_3)B**; **4,6-bis-{1-[(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)dithio]methyl}-3,7-dimethyl-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (10c)**: From 130 mg (0.51 mmol) *syn*-(CH_2SH;CH_3)B **(1d)**, 650 mg (1.3 mmol) N-phthalyl-S-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)sulfenamide **9c**, yield 54 mg (11 %) of **10c**, pale yellow powder. $[\alpha]_D^{27}$ – 18 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 5.53 (d, 2H, H-2,2'^{Man}, $J_{2,3}$ 3.0 Hz); 5.27 (t, 2H, H-4,4'^{Man}, $J_{3,4}$ = $J_{4,5}$ 10.0 Hz); 5.04 (dd, 2H, H-3,3'^{Man}); 4.97 (s, 2H, H-1,1'^{Man}); 4.29 (m, 6H, H-6a,6a'^{Man}, H-6b,6b'^{Man}, $CH_{2a,a'}{}^{Bim}$); 3.89 (d, 2H, $CH_{2b,b'}{}^{Bim}$, $J_{CH2a,b}$ 14.5 Hz); 3.79 (m, 2H, H-5,5'^{Man}); 2.17, 2.10, 2.07, 1.99 (s, 24H, 8xCOC$H_3{}^{Man}$); 1.98 (s, 6H, 2xC$H_3{}^{Bim}$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.0, 169.5, 169.1 (COCH$_3$)^{Man}; 159.6 (C-2, C-8)^{Bim}; 143.7 (C-4, C-6)^{Bim}; 116.4 (C-3, C-7)^{Bim}; 88.9 (C-1,1')^{Man}; 76.7 (C-5,5')^{Man}; 71.0 (C-3,3')^{Man}; 68.8 (C-2,2')^{Man}; 64.8 (C-4,4')^{Man}; 61.9 (C-6,6')^{Man}; 32.2 (CH$_2$)^{Bim}; 20.2, 20.1 (COCH$_3$)^{Man}; 7.1 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{38}H_{48}N_2O_{20}S_4$ $[M+Na]^+$: 1003.158 Found: 1003.150.

General procedure for the preparation of triazole glycosides

syn-(CH_3;C≡CTMS)B **1g** was dissolved in MeCN at r.t. with stirring in nitrogen atmosphere, then per-*O*-acetylated 1-azido-sugars **12a-d**, copper(I)-bromide, copper dust added, followed by N,N-diisopropylethylamine. Stirring was continued at reflux until TLC indicated complete conversion of the starting materials (6 hr), then silica gel and celite was added, and the reaction mixture evaporated to dryness. Purification by column chromatography (dichloromethane, then ethylacetate) yielded the triazole glycosides **13a-d**.

***syn*-(CH_3;TA-β-GlcAc$_4$)B**; **4,6-dimethyl-3,7-bis[(1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-1H-1,2,3-triazol-4-yl)]-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (13a)**: From 100 mg (0.28 mmol) *syn*-(CH_3;C≡CTMS)B **1g**, 261 mg (0.70 mmol) 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide **12a**, 12 mg (0.05 mmol) copper(I)-bromide, 16 mg (0.25 mmol) copper dust, 0.1 mL (0.57 mmol) N,N-diisopropylethylamine yield 175 mg (97 %) of **13a**, yellow powder. $[\alpha]_D^{27}$ – 125 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.92 (s, 2H, H-5,5'^{Taz}); 6.25 (d, 2H, H-1,1'^{Glc}, $J_{1,2}$ 9.5 Hz); 5.97 (t, 2H, H-3,3'^{Glc}, $J_{2,3}$ = $J_{3,4}$ 9.5 Hz); 5.73 (t, 2H, H-4,4'^{Glc}, $J_{4,5}$ 9.5 Hz); 5.27 (t, 2H, H-2,2'^{Glc}); 4.22 (dd, 2H, H-6a,6a'^{Glc}, $J_{5,6a}$ 5.5 Hz, $J_{6a,b}$ 13.0 Hz); 4.13 (m, 4H, H-6b,6b'^{Glc}, H-5,5'^{Glc}); 3.06 (s, 6H, 2xCH_3^{Bim}); 2.07, 2.00, 1.85 (s, 24H, 4xCOCH_3^{Glc}); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.6, 169.4, 168.7 (*C*OCH$_3$)^{Glc}; 157.1 (C-2, C-8)^{Bim}; 145.5 (C-4, C-6)^{Bim}; 138.0 (C-4,4')^{Taz}; 122.6 (C-3, C-7)^{Bim}; 107.7 (C-5,5')^{Taz}; 85.2 (C-1,1')^{Glc}; 74.5 (C-5,5')^{Glc}; 73.4 (C-3,3')^{Glc}; 69.8 (C-2,2')^{Glc}; 68.2 (C-4,4')^{Glc}; 61.8 (C-6,6')^{Glc}; 20.7, 20.3 (CO*C*H$_3$)^{Glc}; 13.4 (*C*H$_3$)^{Bim}. HRMS m/z Calcd for $C_{40}H_{46}N_8O_{20}$ $[M+H]^+$: 959.290 Found: 959.292.

***syn*-(CH_3;TA-β-GalAc$_4$)B**; **4,6-dimethyl-3,7-bis[(1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1H-1,2,3-triazol-4-yl)]-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (13b)**: From 100 mg (0.28 mmol) *syn*-(CH_3;C≡CTMS)B **1g**, 261 mg (0.70 mmol) 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl azide **12b**, 12 mg (0.05 mmol) copper(I)-bromide, 16 mg (0.25 mmol) copper dust, 0.1 mL (0.57 mmol) N,N-diisopropylethylamine, yield 150 mg (57 %) of **13b**, orange-yellow powder. $[\alpha]_D^{27}$ – 123 (c 0.25 $CDCl_3$).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.70 (s, 2H, H-5,5'^{Taz}); 6.03 (d, 2H, H-1,1'^{Gal}, $J_{1,2}$ 9.0 Hz); 5.83 (t, 2H, H-2,2'^{Gal}, $J_{2,3}$ 9.0 Hz); 5.55 (d, 2H, H-4,4'^{Gal}, $J_{3,4}$ 2.0 Hz); 5.39 (dd, 2H, H-3,3'^{Gal}); 4.32 (t, 2H, H-5,5'^{Gal}, $J_{5,6a}$=$J_{5,6b}$ 5.0 Hz); 4.20 (dd, 2H, H-6a,6a'^{Gal}, $J_{6a,6b}$ 10.0 Hz); 4.12 (dd, 2H, H-6b,6b'^{Gal}); 3.03 (s, 6H, 2xCH_3^{Bim}); 2.23, 2.00, 1.86 (s, 24H, 4xCOCH_3^{Gal}); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.3, 170.0, 168.7 (*C*OCH$_3$)^{Gal}; 157.1 (C-2, C-8)^{Bim}; 145.5 (C-4, C-6)^{Bim}; 138.0 (C-4,4')^{Taz}; 121.9 (C-3, C-7)^{Bim}; 107.7 (C-5,5')^{Taz}; 86.0 (C-1,1')^{Gal}; 73.8 (C-5,5')^{Gal}; 71.2 (C-3,3')^{Gal}; 67.8 (C-2,2')^{Gal}; 67.0 (C-4,4')^{Gal}; 61.4 (C-6,6')^{Gal}; 20.7, 20.6 (CO*C*H$_3$)^{Gal}; 13.4 (*C*H$_3$)^{Bim}. HRMS m/z Calcd for $C_{40}H_{46}N_8O_{20}$ $[M+H]^+$: 959.290 Found: 959.290.

***syn*-(CH$_3$;TA-β-ManAc$_4$)B**; **4,6-dimethyl-3,7-bis[(1-(2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl)-1H-1,2,3-triazol-4-yl)]-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (13c)**: From 100 mg (0.28 mmol) *syn*-(CH$_3$;C≡CTMS)B **1g**, 261 mg (0.70 mmol) 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranosyl azide **12c**, 12 mg (0.05 mmol) copper(I)-bromide, 16 mg (0.25 mmol) copper dust, 0.1 mL (0.57 mmol) N,N-diisopropylethylamine, yield 156 mg (60 %) of **13c**, orange-yellow powder. $[\alpha]_D^{27}$ – 127 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.43 (s, 2H, H-5,5'^{Taz}); 6.24 (s, 2H, H-1,1'^{Man}, $J_{1,2}$ 0.5 Hz); 5.67 (dd, 2H, H-2,2'^{Man}, $J_{2,3}$ 3.5 Hz); 5.38 (t, 2H, H-4,4'^{Man}, $J_{3,4}$ = $J_{4,5}$ 10.0 Hz); 5.31 (dd, 2H, H-3,3'^{Man}); 4.28 (dd, 2H, H-6a,6a'^{Man}, $J_{5,6a}$ 5.5 Hz, $J_{6a,6b}$ 13.5 Hz); 4.23 (dd, 2H, H-6b,6b'^{Man}, $J_{5,6b}$ 2.5 Hz); 3.98 (m, 2H, H-5,5'^{Man}); 2.95 (s, 6H, 2xCH_3^{Bim}); 2.18, 2.08, 2.07, 1.98 (s, 24H, 4xCOCH_3^{Man}); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 170.6, 169.8, 169.4 (*C*OCH$_3$)^{Man}; 157.0 (C-2, C-8)^{Bim}; 145.4 (C-4, C-6)^{Bim}; 137.6 (C-4,4')^{Taz}; 121.3 (C-3, C-7)^{Bim}; 107.4 (C-5,5')^{Taz}; 84.5 (C-1,1')^{Man}; 76.0 (C-5,5')^{Man}; 70.7 (C-3,3')^{Man}; 68.6 (C-2,2')^{Man}; 64.9 (C-4,4')^{Man}; 61.9 (C-6,6')^{Man}; 20.6, 20.4 (CO*C*H$_3$)^{Man}; 13.1 (*C*H$_3$)^{Bim}. HRMS m/z Calcd for $C_{40}H_{46}N_8O_{20}$ $[M+H]^+$: 959.290 Found: 959.290.

***syn*-(CH$_3$;TA-β-LacAc$_7$)B**; **4,6-dimethyl-3,7-bis[(1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactosyl-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucosyl)-1H-1,2,3-triazol-4-yl)]-1,5-diazabicyclo[3.3.0]octa-3,6-diene-2,8-dione (13d)**: From 100 mg (0.28 mmol) *syn*-(CH$_3$;C≡CTMS)B **1g**, 464 mg (0.70 mmol) 2,3,4,6-tetra-*O*-

acetyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl azide **12d**, 12 mg (0.05 mmol) copper(I)-bromide, 16 mg (0.25 mmol) copper dust, 0.1 mL (0.57 mmol) N,N-diisopropylethylamine, yield 247 mg (57 %) of **13d**, yellow powder. $[\alpha]_D^{27}$ – 117 (c 0.25 $CDCl_3$). ^{1}H NMR ($CDCl_3$, 500 MHz): *δ* 8.49 (s, 2H, C-5,5'^{Taz}); 5.91 (d, 2H, H-1,1'^{Glc}, $J_{1,2}$ 10.0 Hz); 5.57 (t, 2H, H-2,2'^{Glc}, $J_{2,3}$ 10.0 Hz); 5.44 (t, 2H, H-3,3'^{Glc}, $J_{3,4}$ 10.0 Hz); 5.36 (d, 2H, H-4,4'^{Gal}, $J_{3,4}$ 5.0 Hz); 5.11 (t, 2H, H-2,2'^{Gal}, $J_{1,2}$ = $J_{2,3}$ 10.0 Hz); 5.00 (dd, 2H, H-3,3'^{Gal}); 4.57 (d, 2H, H-1,1'^{Gal}); 4.50 (d, 2H, H-6a,6a'^{Gal}, $J_{6a,6b}$ 15.0 Hz); 4.03 (m, 12H, H-4,4'^{Glc}, H-5,5'^{Glc}, H-6a,6a'^{Glc}, H-6b,6b'^{Glc}, H-5,5'^{Gal}, H-6b,b'^{Gal}); 3.00 (s, 6H, 2xCH_3^{Bim}); 2.14, 2.08, 2.05, 2.03, 1.95, 1.84 (s, 48H, 14xCOCH_3^{Lac}); ^{13}C NMR ($CDCl_3$, 125 MHz): *δ* 170.4, 170.3, 170.0, 169.7, 168.9 (COCH$_3$)^{Lac}; 157.1 (C-2, C-8)^{Bim}; 145.6 (C-4, C-6)^{Bim}; 138.1 (C-4,4')^{Taz}; 121.4 (C-3, C-7)^{Bim}; 107.6 (C-5,5')^{Taz}; 101.1 (C-1,1')^{Gal}; 85.5 (C-1,1')^{Glc}; 75.6 (C-5,5')^{Gal}; 75.6 (C-5,5')^{Glc}; 72.7 (C-3,3')^{Glc}; 71.0 (C-3,3')^{Gal}; 70.8 (C-4,4')^{Glc}; 70.6 (C-2,2')^{Glc}; 69.1 (C-2,2')^{Gal}; 66.7 (C-4,4')^{Gal}; 61.8 (C-6,6')^{Gal}; 60.9 (C-6,6')^{Glc}; 20.7, 20.5 (COCH$_3$)^{Lac}; 13.3 (CH$_3$)^{Bim}. HRMS m/z Calcd for $C_{64}H_{78}N_8O_{36}$ $[M+H]^+$: 1535.459 Found: 1535.461.

Acknowledgement

Financial support from the National Research Development and Innovation Office of Hungary for NKFIH-OTKA NN-109671 and from Richter Gedeon Zrt., Hungary (to T.Sz.), is gratefully acknowledged. The research was also supported by the EU and co-financed by the European Regional Development Fund under the projects GINOP-2.3.2-15-2016-00008 and GINOP-2.3.3-15-2016-00004. Our thanks go also to the Department of Applied Chemistry, University of Debrecen, for the HRMS measurements, as well as to Miklós Nagy for useful discussion of fluorescence data.

References

1. Gabius, H. J.; Manning, J. C.; Kopitz, J.; André, S.; Kaltner, H. *Cell.Molec.Life Sci.* **2016,** *73*, 1989-2016.

2. Varki, A. *Glycobiology* **2017,** *27*, 3-49.

3. Roy, R.; Murphy, P. V.; Gabius, H. J. *Molecules* **2016,** *21*, 629, and references therein.

4. Kaltner, H.; Szabó, T.; Fehér, K.; André, S.; Balla, S.; Manning, J. C.; Szilágyi, L.; Gabius, H. J. *Bioorg.Med.Chem.* **2017,** *25*, 3158-3170.

5. Szilágyi, L.; Varela, O. *Curr.Org.Chem.* **2006,** *10*, 1745-1770.

6. André, S.; Pei, Z. C.; Siebert, H. C.; Ramström, O.; Gabius, H. J. *Bioorg.Med.Chem.* **2006,** *14*, 6314-6326.

7. Martin-Santamaria, S.; André, S.; Buzamet, E.; Caraballo, R.; Fernandez-Cureses, G.; Morando, M.; Ribeiro, J. P.; Ramirez-Gualito, K.; de Pascual-Teresa, B.; Canada, F. J.; Menendez, M.; Ramström, O.; Jiménez-Barbero, J.; Solis, D.; Gabius, H. J. *Org. Biomol.Chem.* **2011,** *9*, 5445-5455.

8. Murthy, B. N.; Sinha, S.; Surolia, A.; Jayaraman, N.; Szilágyi, L.; Szabó, I.; Kövér, K. E. *Carbohydr.Res.* **2009,** *344*, 1758-1763.

9. Gutierrez, B.; Munoz, C.; Osorio, L.; Fehér, K.; Illyés, T. Z.; Papp, Z.; Kumar, A. A.; Kövér, K. E.; Sagua, H.; Araya, J. E.; Morales, P.; Szilágyi, L.; González, J. *Bioorg.Med.Chem.Lett.* **2013,** *23*, 3576-3579.

10. André, S.; Kövér, K. E.; Gabius, H. J.; Szilágyi, L. *Bioorg.Med.Chem.Lett.* **2015,** *25*, 931-935.

11. Franco, J.; Sardi, F.; Szilágyi, L.; Kövér, K. E.; Fehér, K.; Comini, M. A. *Int.J. Parasitol.Drugs Drug Resist.* **2017,** *7*, 303-313.

12. Illyés, T. Z.; Balla, S.; Bényei, A.; Kumar, A. A.; Timári, I.; Kövér, K. E.; Szilágyi, L. *ChemistrySelect* **2016,** *1*, 2383-2388.

13. Kosower, E. M.; Pazhenchevsky, B.; Hershkowitz, E. *J.Amer. Chem.Soc.* **1978,** *100*, 6516-6518.

14. Neogi, I.; Das, P. J.; Grynszpan, F. *Synlett* **2017,** *29*, 1043-1046.

15. Kosower, E. M.; Pazhenchevsky, B. *J.Amer. Chem.Soc.* **1980,** *102*, 4983-4993.

16. Chwee, T. S.; Lim, G. S.; Wong, Z. C.; Sullivan, M. B.; Fan, W. Y. *Phys.Chem. Chem.Phys.* **2016,** *18*, 7404-7413.

17. Kosower, N. S.; Kosower, E. M.; Newton, G. L.; Ranney, H. M. *Proc.Nat.Acad. Sci.USA* **1979,** *76*, 3382-3386.

18. Mansoor, S. E.; Farrens, D. L. *Biochemistry* **2004,** *43*, 9426-9438.

19. Mansoor, S. E.; DeWitt, M. A.; Farrens, D. L. *Biochemistry* **2010,** *49*, 9722-9731.

20. Taraska, J. W.; Puljung, M. C.; Zagotta, W. N. *Proc.Nat.Acad. Sci.USA* **2009,** *106*, 16227-16232.

21. Montoya, L. A.; Shen, X. G.; McDermott, J. J.; Kevil, C. G.; Pluth, M. D. *Chem. Sci.* **2015,** *6*, 294-300.

22. Newton, G. L.; Fahey, R. C.; Rawat, M. *Microbiology-Sgm* **2012,** *158*, 1117-1126. Erratum: **2012**, *158*, 2450.

23. Kosower, E. M.; Radkowsky, A. E.; Fairlamb, A. H.; Croft, S. L.; Neal, R. A. *Eur.J. Med.Chem.* **1995,** *30*, 659-671. Erratum: **1996**, *31*, 172.

24. Krishnan, B.; Szymanska, A.; Gierasch, L. M. *Chem.Biol.& Drug Design* **2007,** *69*, 31-40.

25. Radkowsky, A. E.; Kosower, E. M.; Eisenberg, D.; Goldberg, I. *J.Amer.Chem. Soc.* **1986,** *108*, 4532-4541.

26. Radkowsky, A. E.; Kosower, E. M. *J.Amer. Chem.Soc.* **1986,** *108*, 4527-4531.

27. Kosower, E. M.; Zbaida, D.; Baudhuin, M.; Marciano, D.; Goldberg, I. *J.Amer. Chem.Soc.* **1990,** *112*, 7305-7314.

28. Čapek, J.; Hauschke, M.; Bruckova, L.; Rousar, T. *J.Pharmacol.Toxicol.Meth.* **2017,** *88*, 40-45.

29. Imai, T.; Kurihara, T.; Esaki, N.; Mihara, H. *Anal.Biochem.* **2017,** *532*, 1-8.

30. Haque, M. B.; Roberts, B. P.; Tocher, D. A. *J.Chem.Soc.Perkin Trans.1* **1998**, 2881-2889.

31. Wagner, G.; Nuhn, P. *Arch. Pharm.* **1964,** *297*, 462-473.

32. Kumar, A. A.; Illyés, T. Z.; Kövér, K. E.; Szilágyi, L. *Carbohydr.Res.* **2012,** *360*, 8-18.

33. Musiejuk, M.; Witt, D. *Org.Prep.Proc.Int.* **2015,** *47*, 95-131.

34. Szilágyi, L.; Illyés, T. Z.; Herczegh, P. *Tetrahedron Lett.* **2001,** *42*, 3901-3903.

35. Stellenboom, N.; Hunter, R.; Caira, M. R.; Szilágyi, L. *Tetrahedron Lett.* **2010,** *51*, 5309-5312.

36. Illyés, T. Z.; Szabó, T.; Szilágyi, L. *Carbohydr.Res.* **2011,** *346*, 1622-1627.

37. Morais, G. R.; Springett, B. R.; Pauze, M.; Schroder, L.; Northrop, M.; Falconer, R. A. *Org.Biomol.Chem.* **2016,** *14*, 2749-2754.

38. He, X. P.; Zeng, Y. L.; Zang, Y.; Li, J.; Field, R. A.; Chen, G. R. *Carbohydr. Res.* **2016,** *429*, 1-22.

39. Kosower, E. M.; BenShoshan, M. *J.Org.Chem.* **1996,** *61*, 5871-5884.

40. Groom, C. R.; Bruno, I. J.; Lightfoot, M. P.; Ward, S. C. *Acta Cryst.B-Struct.Sci. Cryst.Eng.Mater.* **2016,** *72*, 171-179.

41. Kosower, E. M.; Bernstein, J.; Goldberg, I.; Pazhenchevsky, B.; Goldstein, E. *J.Amer. Chem.Soc.* **1979,** *101*, 1620-1621.

42. Goldberg, I.; Bernstein, J.; Kosower, E. M. *Acta Cryst.B-Struct.Sci.* **1982,** *38*, 1990-2000.

43. Goldberg, I.; Kosower, E. M. *J.Phys.Chem.* **1982,** *86*, 332-335.

44. Goldberg, I.; Bernstein, J.; Kosower, E. M.; Goldstein, E.; Pazhenchevsky, B. *J. Heterocycl.Chem.* **1983,** *20*, 903-912.

45. Huppert, D.; Kanety, H.; Pines, E.; Kosower, E. M. *J.Phys.Chem.* **1981,** *85*, 3387-3390.

46. Kosower, E. M.; Kanety, H.; Dodiuk, H.; Hermolin, J. *J.Phys.Chem.* **1982,** *86*, 1270-1277.

47. Kosower, E. M.; Kanety, H.; Dodiuk, H. *J.Photochem.* **1983,** *21*, 171-182.

48. He, J.; Jia, L.; Li, Y.; Liu, S.; Chen, R.; Jin, L.; Jin, J.; Zheng, C.; Fan, Q.; Huang, W. *Chem. Commun.* **2018**, DOI: 10.1039/C8CC01267A

49. Györgydeák, Z.; Szilágyi, L.; Paulsen, H. *J.Carbohydr.Chem.* **1993,** *12*, 139-163.

50. Černy, M.; Štanek, J.; Pacák, J. *Monatsh. Chem.* **1963,** *94*, 291-294.

51. Durette, P. L.; Shen, T. Y. *Carbohydr. Res.* **1980,** *81*, 261-274.

52. Gamblin, D. P.; Garnier, P.; van Kasteren, S.; Oldham, N. J.; Fairbanks, A. J.; Davis, B. G. *Angew.Chem.Int.Ed.* **2004,** *43*, 828-833.

53. Štanek, J.; Šindlerová, M.; Černy, M. *Collect. Czech. Chem. Commun.* **1965,** *34*, 297-303.

54. Sheldrick, G. M. *Acta Cryst.A* **2008,** *64*, 112-122.

55. Westrip, S. P. *J.App.Cryst.* **2010,** *43*, 920-925.

56. Spek, A. L. *Acta Cryst.D-Biol.Cryst.* **2009,** *65*, 148-155.