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ARTICLE

Bifunctional mannoside-glucosinolate glycoconjugates as enzymatically triggered isothiocyanates and FimH ligands

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Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Glucosinolates are sulfur-containing secondary metabolites found in plants of the Brassicale order. They are precursors of isothiocyanate species, resulting from C-S hydrolysis catalysed by the thioglucohydrolase myrosinase. We describe the synthesis of bifunctional glucosinolate-mannoside glycoconjugates combining both the structural features of a substrate of myrosinase and a ligand of the lectin FimH. We show that these glycoconjugates serve as enzyme substrates and that myrosinase can indeed hydrolyze the glucosinolate moiety with affinities (K_M, V_{max}) comparable to the natural substrates glucomoringin and sinigrin. This enzymatic hydrolysis of the thioglycosidic bond led to the efficient formation of an isothiocyanate which was assessed by the formation of the corresponding dithiocarbamates derivatives. Finally, we show that our synthetic bifunctional glycoconjugates also serve as FimH ligands where the glucosinolate moiety does not hamper the interaction with the lectin. Our findings set the stage for an original bioconjugation tool, allowing for myrosinase-triggered specific labelling of lectins using glucosinolate glycoconjugates as non-toxic, water soluble isothiocyanate precursors.

Introduction

The myrosinase-glucosinolate (MG) reaction is a unique biochemical transformation in Nature, able to produce a reactive isothiocyanate, which is a well-known bioconjugation tool. Glucosinolates (Figure 2A, 1) as substrates of myrosinase have a long chemical and biochemical history. As early as the identification of the first glucosinolate sinalbine (Figure 2A, **1a**) in 1831 by P. J. Robiquet and F. Boutron-Charlard, the presence of a sulfur atom in the structure was assessed. The glucosinolate was identified as the preactive molecule, which could be further transformed into mustard oil.[1] This transformation, known as the "mustard oil bomb", is due to the activity of myrosinase.[2] The first synthesis of the glucosinolate glucotropaeolin (1b) by Ettlinger led to the structural assignment of these secondary metabolites.[3] They are specific biomarkers of the Brassicale order in which more than 140 glucosinolates were found associated with thioglucoside glucohydrolases named myrosinases (EC 3.2.1.147).[4] Glucosinolates and myrosinase root their relationship in a mechanism of defense of Brassicale plants against any aggression from bacteria to mammals.[5] The enzymatic action of myrosinase leads to the hydrolysis of the C-S anomeric bond of glucosinolates, forming D-glucose and a transient thiohydroxamic species which in turn undergoes a Lossen rearrangement liberating sulfate and an isothiocyanate (ITC) (**2**) (Figure 2A). This unique enzymatic reaction induced numerous studies on the enzymatic mechanism, as well as on the biological (antifungal, antibacterial, antiviral, insecticidal, repellent to animals) and even therapeutic activities (chemopreventive effects) of these secondary metabolites[6] which are mainly attributed to the isothiocyanate products. Thus glucosinolate extraction, analysis and synthesis have attracted numerous research efforts,[7] whereas little work has been devoted to evaluate myrosinase activity with various natural and artificial glucosinolates.[8] Indeed, as strong electrophiles, the ITCs are well known ligation agents, but are toxic, not easy to prepare and to store. Using the natural myrosinase-glucosinolate (MG) reaction would allow for the *in-situ* preparation of ITCs from a stable, non-toxic and water soluble GL precursor (Figure 1).

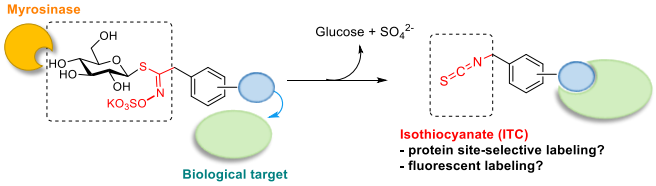

Figure 1 The Myrosinase-Glucosinolate (MG) tandem as a new tool to release reactive ITC?

Thus, the aim of this work is the study of the MG reaction as a potential new bioconjugational tool for specific labelling[9] through the use of synthetic glucosinolate glycoconjugates, designed to interact with a specific biological target. This initial study targets the bacterial lectin FimH, which has a well-defined specificity for α-D-manno pyranosides.[10]

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Electronic Supplementary Information (ESI) available: see DOI: 10.1039/x0xx00000x

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DOI: 10.1039/C8OB01128A

Results and Discussion

Our approach/strategy is to combine two structural features in one molecule in order to design/prepare glycoconjugates which can act at the same time as a substrate for the enzyme myrosinase and as a ligand for a specific biological target, such as lectin FimH in this present study. The design of the target glycoconjugates was based on our knowledge of myrosinase substrate specificity and the chemistry of glucosinolates on one hand and of synthetic ligands of the bacterial lectin FimH on the other hand. Indeed, it is known that an aromatic aglycone moiety considerably adds to the affinity of FimH mannoside ligands[10] due to π-π stacking interactions at the entrance of the lectin's carbohydrate recognition domain.[11] Recently, B. Ernst and co-workers[12a] and Janetka *et al.*[12b] have shown that biphenyl α-D-mannopyranosides show especially high affinity for FimH with K_d values in the nanomolar range in specific assays.[12c]

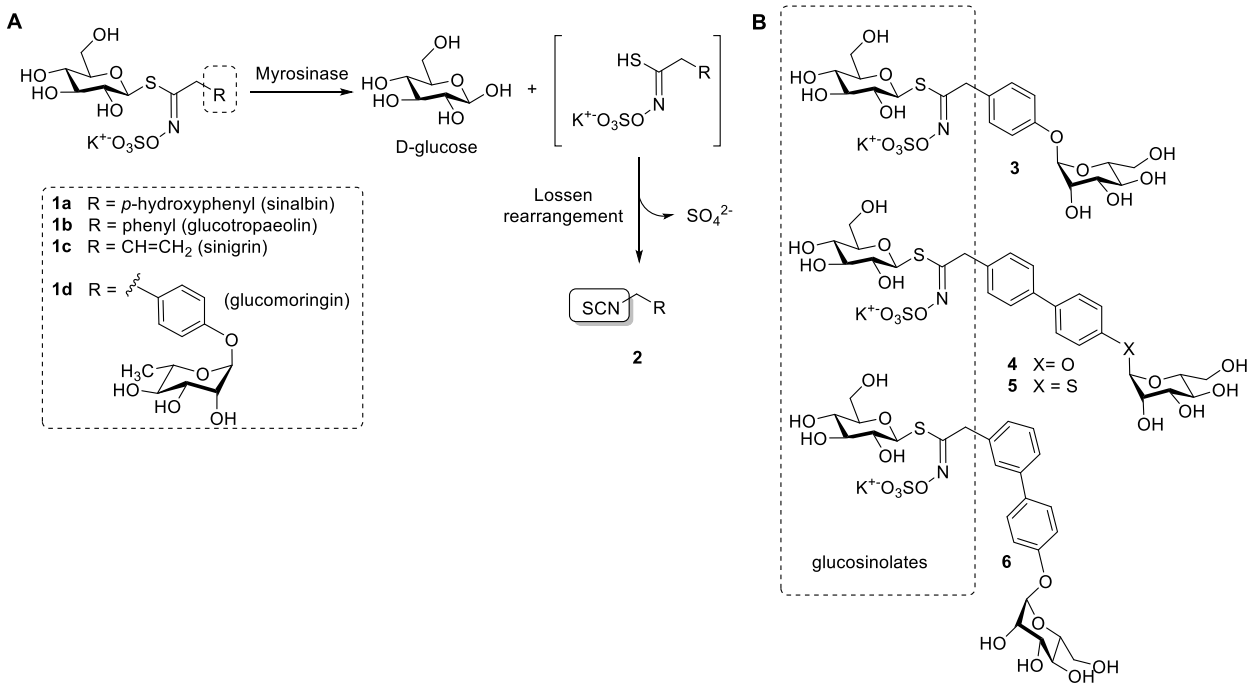

Figure 2 A: Generic glucosinolate structures and some classical examples (**1a**-**1d**). Myrosinase catalyzes the hydrolysis of glucosinolate thioglycosidic bond, generating a transient sulfated thiohydroxamic acid, which decomposes into sulfate and a reactive isothiocyanate (**2**). **B**: Targeted glucosinolate glycoconjugates (**3**-**6**), comprising α-D-mannopyranosyl moieties as FimH ligands.

Accordingly, glucosinolate glycoconjugates **3**-**6** (Figure 2B) comprising high-affinity binding motifs for FimH were first designed to explore the possibility of triggering isothiocyanate formation with myrosinase. The 4'-*O*-mannopyranosyl sinalbine analogue **3,** where the *O*-sulfated thiohydroximate part is separated from the α-D-mannopyranoside by a *p*-benzyl moiety constituted a first model substrate, and the biphenyl analogues **4**, **5**, and **6** were envisaged as advanced glucosinolate glycoconjugates. The *O*-glycosides **3** and **4** were directly inspired by literature[11] while the *S*-glycosidic mannoside **5** was considered to prevent hydrolysis of the *O*-glycosidic bond by an α-mannosidase.[12d] Hence, the replacement of the anomeric oxygen atom by a sulfur atom might lead to a more glycosidase-resistant ligand, without modifying much the ligand-protein interaction and could consequently lead to improved bioavailability. Glucosinolate **6** was designed in order to evaluate the influence of the spatial orientation of the α-D-mannopyranoside part in relation to the biphenyl aglycone moiety on FimH binding. The key step in glucosinolate synthesis is the formation of the thiohydroximate function. This can be achieved using various conditions starting from a nitroalkane, a nitrovinyl, an aldoxime or using the most recent approach starting from lactones.[7e,13] We chose to follow previous methods from our laboratory applying the Kulkarni conditions on nitrovinyl derivatives. First, the phenyl mannoside derivative **3** (Fig. 1) was prepared following the procedures previously reported by us.[14] To prepare the required biphenyl nitrovinyl compounds **14**-**16**, a three-step sequence was employed (Scheme 1). Formation of the *O*-glycoside intermediates bearing a biphenyl moiety was achieved according to methods developed in the literature.[12] Thus, glycosylation of the per-acetylated mannose with *p*-bromophenol gave the bromophenyl mannoside **9** in 68% yield. Then, a Suzuki-Miyaura reaction of the bromophenyl mannopyranoside **9** and *para*- or *meta*-formylphenylboronic acid, respectively, gave the biphenyl mannoside intermediates **11** and **13** in 84% and 80% respective yields. In the next step, Henry condensation afforded the nitrovinyl derivatives **14** and **16**, respectively, again in very good yields. Synthesis of the *S*-glycosidic biphenyl analogue was inspired by the efficient protocol developed by S. Messaoudi

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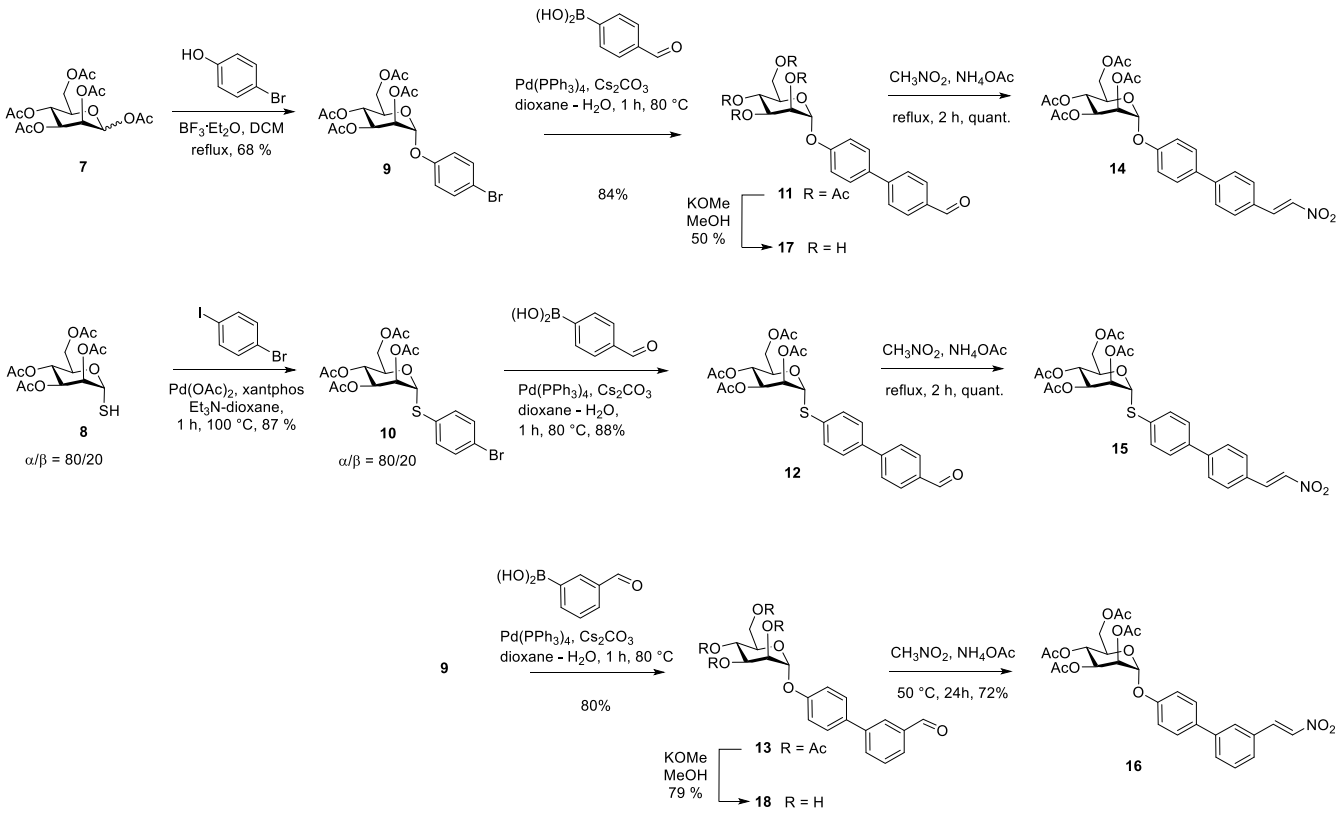
DOI: 10.1039/C8OB01081A

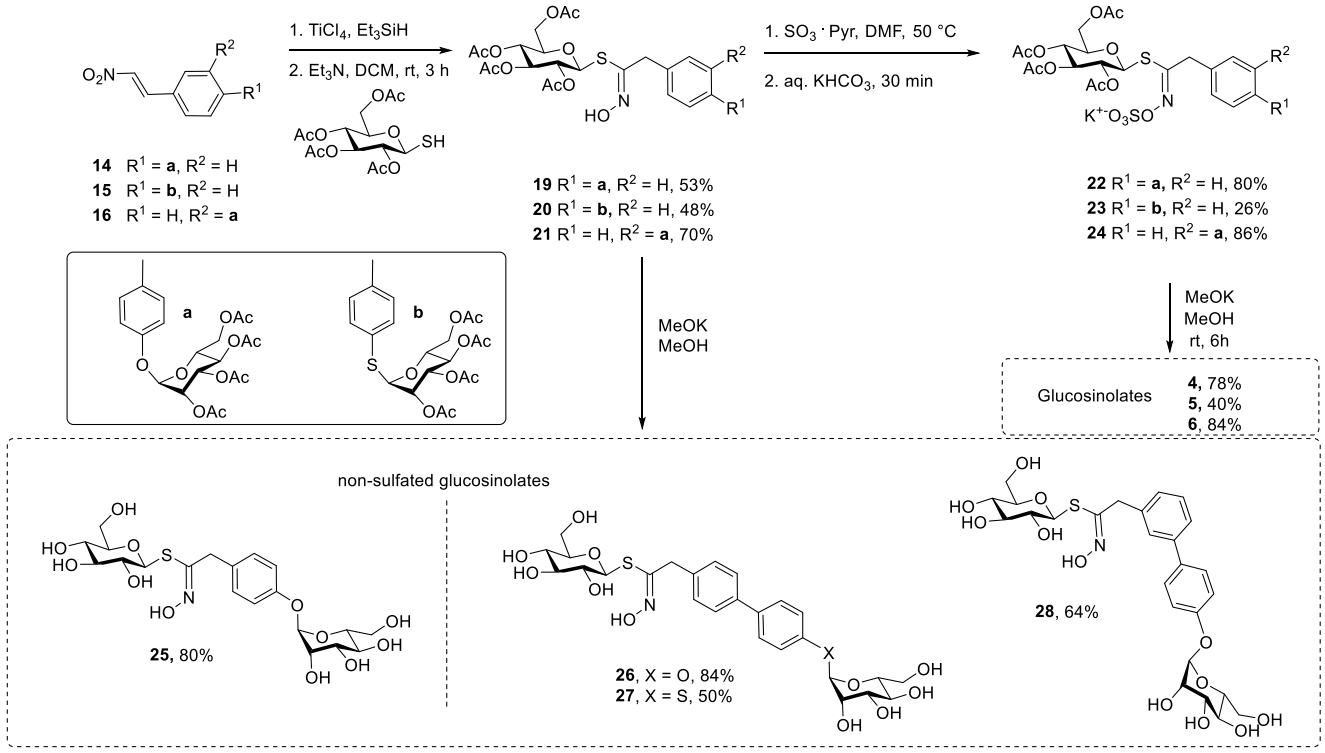
and coworkers applying palladium catalysis. [15] Tetra-*O*-acetyl-1-thio-β-D-mannopyranoside (**8**) was used as a 80/20 α/β-anomeric mixture to furnish the *p*-phenyl-thiomannopyranoside **10** after a short optimisation process in 87% as a 80/20 α/β-anomeric mixture. This ratio suggests that no anomerisation occurs during the coupling process. In the next step, the Suzuki-Myaura cross-coupling reaction with an excess of 4-formylphenylboronic acid afforded the biphenylaldehyde **12** in 88% yield. Then again Henry condensation was applied to obtain the nitrovinyl derivative **15** in quantitative yield. In order to evaluate the influence of the glucosinolate moiety on FimH binding, the unprotected intermediate aldehydes **17** and **18** were also prepared. Thus, aldehydes **11** and **13** were deacetylated under Zemplén conditions using potassium methoxide in methanol to lead to the desired compounds in reasonable yields after purification by reverse phase chromatography (50% and 79% respectively).



Scheme 1 Synthesis of the nitrovinyl precursors **14** to **16**.

The three biphenyl nitrovinyl mannoside precursors **14-16** were then used in the following key step towards the target glucosinolates. For the formation of the thiohydroximate function the Kulkarni protocol was applied to prepare the hydroximoyl chloride with titanium chloride and triethylsilane (Scheme 2).[16] Then, under basic conditions a transient nitrile oxide was formed and trapped with β-thio-D-glucopyranose to form the thiohydroximate function in acceptable yields in most cases. A modest yield of 53% was observed with the *para*-substituted biphenyl derivative **14** while with the *meta*-substituted biphenyl derivative **16** the yield was better (70%). The preparation of the *S*-mannopyranoside **20** turned out to be more difficult. The yields were much lower when conditions developed for the *O*-mannopyranoside were applied (26% yield). Decreasing the temperature to -10°C during the Kulkarni reaction allowed some improvement and isolation of the thiohydroximate **20** in 48% yield. The different yields observed between *m*- and *p*-substitution on one hand and *O*- an *S*-mannopyranosides on the other hand might be the result of the Kulkarni conditions. Indeed, the Lewis acid activation of the nitrovinyl moiety with titanium chloride could induce, through the conjugated system, a glycosidic bond cleavage. This could explain the lower yields observed with the *p*-substituted *S*- and *O*-glycosidic bond for the thiohydroximate **19** and **20**. The *m*-nitrovinyl derivative **16** resulted in the thiohydroximate **21** in a better 70% yield. Sulfation of the thiohydroximate moiety was next achieved using pyridine sulfur trioxide complex in DMF. Again, with the *O*-glycosidic bond the sulfation was very efficient, producing the sulfated glucosinolates **22** and **24** in very good yield (80% and 86%) while a lower yield of 26% was observed for **23**. The synthesis of the glucosinolates **4**, **5**, and **6** was finalized by transesterification using a catalytic amount of potassium methoxide and purification through C18 reverse phase chromatography to remove traces of sulfate salts. There again the *O*-glycosidic bonds were more stable under these conditions and afforded **4** and **6** efficiently with yields close to

80%. While with the *S*-mannoside **23**, some degradation occurred during the deacetylation reaction and hence the final glucosinolate **5** was obtained in a much lower 40% yield. The non-sulfated thiohydroximates **25-28** were also prepared in good yields (80%, 84%, 50% and 64% respectively) following the same deprotection process as previously described for aldehydes **17** and **18**.



Scheme 2 Synthesis of glucosinolates from the corresponding nitrovinyl derivatives.

Myrosinase activity

Once the synthesis of the glucosinolate analogues completed, the next step was to determine whether myrosinase could still hydrolyze those artificial GLs or not. To evaluate the enzyme activity, we followed a procedure previously developed in our laboratory.[8]

The myrosinase hydrolysis of both natural and synthetic glucosinolates could be followed by CE/C4D (capillary electrophoresis/contactless capacitively coupled conductivity detector) in less than 10 min using 7 µL reaction mixture. This approach to enzyme analysis by CE is very simple, economic, fast and robust. Two steps are required: 1) incubation of the reaction mixture in a micro vial; 2) electrophoretic analysis in the CE capillary to detect the released sulfate product (SO_4^{2-}). Using this technique, the K_M for the enzymatic reaction with sinigrin **1c** was determined as 0.63 mM, (Table 1, entry 1). This value is in the same range as the one we previously reported, as well as the value obtained using the conventional photometric approach to enzyme activity determination. This K_M value served as a reference for comparison with the different glucosinolate derivatives **3**, **4**, **5**, and **6**. V_{max} are also reported (myrosinase was used at 0.05 U. mL^{-1} for all enzymatic assays).

Table 1. Screening of myrosinase* substrates using capillary electrophoresis (for structures of **1a**-**1d** see Figure 2).

Entry	Glucosinolates	K_M (mM) [a]	V_{max} (mM.min^{-1})	V_{max}/K_M (min^{-1})
1	**1c** (sinigrin)	0.63±0.10 0.11±0.01[b]	0.31±0.01 -	0.49 -
2	**1b** (glucotropaeolin)	0.12±0.01[b]	-	-
3	**1a** (sinalbin)	0.07±0.01[b]	-	-
4	**1d** (glucomoringin)	2.60±0.19[b]	-	-
5	**3**	1.31±0.26	0.12±0.01	0.09
6	**4**	3.33±0.79	0.59±0.04	0.18
7	**5**	1.19±0.28	0.42±0.02	0.35
8	**6**	1.78±0.23	0.36±0.01	0.20

*myrosinase : thioglucosidase from *Sinapis alba* (white mustard) seed.

[a] K_M = Michaelis Menten constant (mM); [b] data from our previous work.[8]

Natural glucosinolates with small aromatic side chains such as benzyl (glucotropaeolin) **1b** or *p*-hydroxybenzyl (sinalbin) **1a** (Table 1, entries 2 and 3) showed higher affinities than the bulkier natural glucomoringin **1d** which incorporate in its structure a L-rhamnopyranoside moiety.[14] The results obtained with the small library of glucosinolates **3**-**6** (Table 1) were

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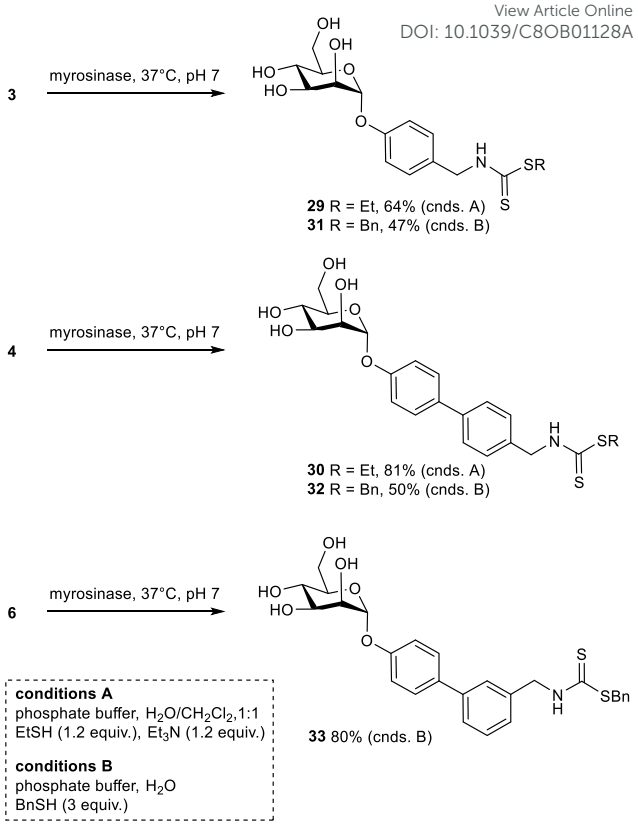
consistent with our previous data.[8] The K_M value determined for these four glucosinolates were 1.31, 3.33, 1.19, and 1.78 mM, respectively (Table 1, entries 5-8) which show that these artificial glucosinolates are still good substrates of myrosinase. The analogue **3** (entry 5) showed a similar K_M value as glucomoringin **1d**. In terms of catalytic efficiency, glucosinolates **3**, **4** and **6** seemed to be hydrolysed with similar rates although with lower overall efficiency than the natural substrate sinigrin **1c**. The thiomannopyranoside **5** (entry 7) turned out to be slightly better substrate of myrosinase than the three *O*-glycosides, having a global efficiency closer to sinigrin. Finally, it can be concluded from these experiments that the biphenyl moiety do not dramatically influence the myrosinase activity when compared to the natural glucosinolate glucomoringin **1d** and the *S*-glycoside analogue **5** is also well recognized by myrosinase. Our project relies on the myrosinase ability to convert the glucosinolate moiety into a reactive isothiocyanate function. We have thus optimized a process to prove the formation of the isothiocyanate product by trapping it with a thiol to form a dithiocarbamate derivative, which is easier to detect analytically, especially by NMR. The reactivity of myrosinase on glucosinolates could be assessed using a simple biphasic system or direct reaction in water.

Myrosinase was diluted in a phosphate buffer at pH 7, and the same volume of dichloromethane containing an excess of ethanethiol and triethylamine was added. The glucosinolate analogues were then added and reacted with myrosinase at 37°C until hydrolysis was completed. The formed isothiocyanate was subsequently trapped in the dichloromethane phase by ethanethiol. This protocol was applied to glucosinolates **3** and **4**, the resulting dithiocarbamates **29** and **30** were isolated in 64% and 81% yields respectively (conditions A, Scheme 3).

To evaluate the reactivity of the isothiocyanate in the buffer solution only, we tested the MG reaction without CH_2Cl_2 and Et_3N. Surprisingly, the condensation with the isothiocyanate was not observed. The glucosinolate analogues **3**, **4** and **6** were also tested with benzylmercaptan and the corresponding dithiocarbamates **31**, **32**, and **33** were isolated in reasonable to good yields (47%, 50%, and 80%, respectively). These complementary results confirm that the synthetic glucosinolates prepared here, are indeed substrates of myrosinase from *Sinapis alba*. The observed affinities as well as reactivities are comparable to the hydrolysis of the natural substrates of myrosinase. Moreover, the MG reaction produced the corresponding isothiocyanates, as proven by trapping with benzylmercaptan at neutral pH. Further work is ongoing in the lab on the study of the kinetics of the hydrolysis and the reactivity of the ITC towards nucleophiles.



Scheme 3: Hydrolysis of the glucosinolates **3**, **4**, and **6** by myrosinase and subsequent trapping reaction with mercaptans.

Impact of glucosinolate moieties on the interaction with lectins

Having the glucosinolate mannosides **3**-**6** in hands, we decided to explore their ability to inhibit the interaction with various lectins using the "glycoprofile" technique.[17] Four glucosinolates **3**, **4** and **6** and natural glucomoringin **1d** were explored for their interaction with 19 lectins using GLYcoDIAG company technology.[18]

The interaction with ten mannose selective lectins were explored: six lectins from vegetal (ConA, LcH, PSA, VFA, GNA, HHA), two bacterial lectins (BC2L-A, FimH) and two recombinant human lectins (Langerin and DC SIGN) and a further nine other lectins chosen for their various specificity toward carbohydrate structures.[18a] The three artificial glucosinolates **3**, **4**, **6** tested, showed good inhibition levels of the interaction with mannose selective lectins with a stronger effect on ConA, PSA, BC2LA and FimH, while no or very little inhibition effect was detected with the other set of lectins. On the contrary, glucomoringin **1d** showed no or little inhibition of the interaction with various carbohydrate selective lectins except for the specific L-rhamnose lectin CorM. Finally, this glycoprofile allowed us to assess that these novel glycoconjugates retain good affinities with lectins, the glucosinolate moiety having a small impact on the recognition pattern, and that the side chain mainly drives the interaction with lectins.

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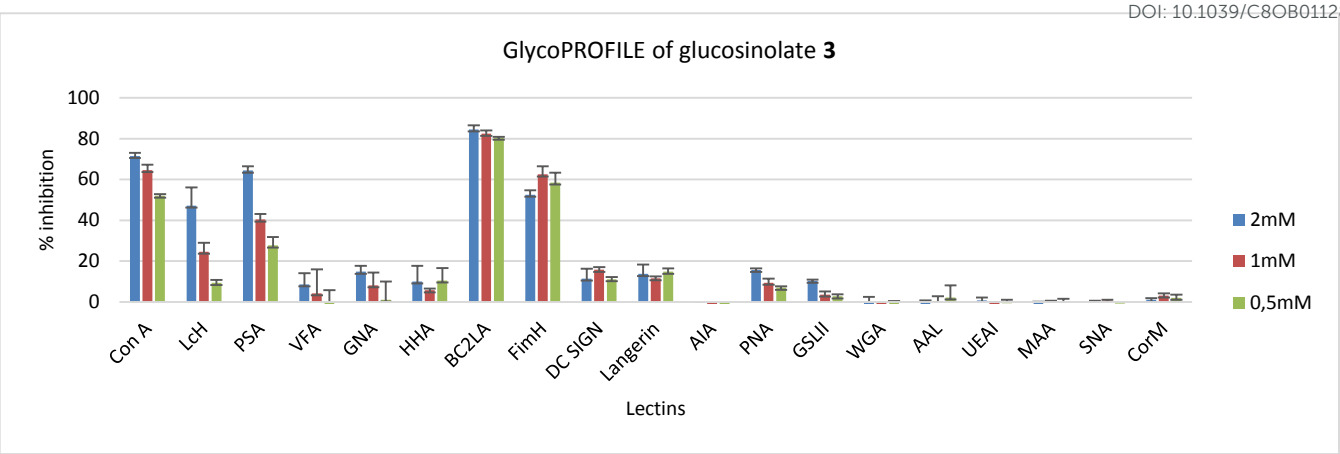

Figure 3 : Example of a glycoprofile for Glucosinolate 3 : inhibition of the interaction using three different concentrations of glucosinolate (2mM, 1mM and 0.5 mM) with 19 lectins using Concanavalin A (ConA), Lens Culinaris Agglutinin (LcH), Pisum Sativum Agglutinin (PSA), Vicia Faba Agglutinin (VFA) ; Galanthus Nivalis Agglutinin (GLN/GNA), Hippeastrum Hybrid Agglutinin (HHA), Burkholderia cenocepacia lectin A (BC2LA), E. coli Type I fimbrial lectin (FimH), DC-SIGN extracellular domain (DC-SIGN), Langerin extracellular domain (Langerin), Artocarpus intergrifolia Agglutinin (AIA), Peanut Agglutinin (PNA), Griffonia Simplicifolia Lectin II (GSLII), Wheat Germ Agglutinin (WGA), Aleuria Aurantia Lectin (AAL), Ulex Europeus Agglutinin (UEA-I), Maackia amurensis Agglutinin (MAA), Elderberry Lectin (SNA), Coregonus lavaretus marenae (CorM). Each experiment has been set in triplicate.

Furthermore, we studied glucosinolates **3**-**6,** for their potential to inhibit the adhesion of live type 1 fimbriated *E. coli* bacteria to mannan, which is mediated by the lectin FimH. To gain a more detailed understanding of the interaction of particular moieties of our synthetic ligands with FimH, the deacetylated desulfoglucosinolate **19** and **21** were also tested. Hence, the myrosinase substrates **3**-**6**, the desulfoglucosinolate analogues **25**-**28** (Scheme 2) and **17** and **18** (Scheme 1) were compared in microplate-based adhesion inhibition assays, where methyl α-D-mannopyranoside (MeMan) and *p*-nitrophenyl α-D-mannopyranoside (*p*NPMan) were tested on the same microtiter plate. We used fluorescent *E. coli* bacteria according to a published assay[19] where fluorescent read-out can be correlated with bacterial adhesion. In the employed adhesion inhibition assay, the mannan-coated microplate surface competes with the tested inhibitors for binding to the bacteria. Serial dilutions of inhibitors were applied to obtain sigmoidal inhibition curves from which IC_{50} values can be deduced, reflecting the inhibitor concentration, which causes 50% inhibition of bacterial binding to the polysaccharide mannan. However, as the absolute IC_{50} values obtained in independent assays are typically found to differ significantly, the inhibitory potency of synthetic inhibitors is related to standard inhibitors (MeMan, *p*NPMan), which are tested in parallel in the same experiment on the same plate. Thus relative inhibitory potencies (RIP values) are obtained, which are suited to compare individual compounds even when they were not assayed in the same experiment. The determined IC_{50} and RIP values of the tested compounds are collected in Table 2. All tested inhibitors exceeded the inhibitory potency of MeMan (Table 2) and were thus more potent inhibitors of type 1 fimbriae-mediated bacterial adhesion to the mannan-coated surface employed in our assay.

Table 2. Inhibition of type 1 fimbriae-mediated *E. coli* adhesion to mannan-coated microtiter plates under static conditions.[a]

Entry	compounds		IC_{50} (SD)[b] (μmol/L)	RIP (SD)[c]
	p-NPMan		187 (10.3)	81.4 (15.3)
1	glucosino-lates	**3**	144.9 (5.9)	34.75 (1.05)
2		**4**	20.55 (3.15)	267.0 (11.0)
3		**5**	36.0 (19.0)	169.7 (38.4)
4		**6**	41.4 (12.0)	260.0 (2.75)
5	desulfo-glucosino-sinolates	**25**	82.8 (0.1)	60.8 (0.7)
6		**26**	31.85 (3.85)	218.5 (10.5)
7		**27**	23.0 (3.0)	174.8 (5.4)
8		**28**	33.0 (15.0)	511.0 (22.0)
9	aldehydes	**17**	23.4 (16.0)	441.0 (20.8)
10		**18**	6.5 (1.0)	776.0 (1.14)

[a] IC_{50} and RIP (relative inhibitory potency, based on methyl α-D-mannopyranoside (MeMan) with IP = 1) values are averaged from mean values from three independent tests; [b] Note that IC_{50} values can vary significantly in independent experiments as live bacteria are investigated. Therefore, IC_{50} values cannot be correlated with RIP values throughout the table. However, all RIP values can be compared to one another as they are all referenced to the same standard compound (MeMan) tested in the same experiment; [c] RIP = IC_{50} (MeMan)/IC_{50} (compound) as tested in the same individual experiment. SD: standard deviation; *p*NPMan: *p*-nitrophenyl-α-D-mannopyranoside.

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As expected, the biphenyl mannoside structures (**4**-**6**, **26**-**28**, **17**-**18**, Table 2) were more potent ligands than the phenylglucosinolate **3** and the corresponding desulfo-glucosinolate **25** with RIP values at least 4 times higher than **3** or **25**. Interestingly, the aldehyde derivatives **17** and **18** showed especially high RIP values, **18** surpassing the inhibitory potency of MeMan by almost 780 times. This corresponded to an IC_{50} value of 6.5 µmol in our experiment. In addition, all tested biphenyl mannosides showed better inhibition than *p*NPMan, which equally provides an aromatic aglycon to interact with the bacterial lectin's binding site and thus can be regarded as a suitable reference compound for comparison. Overall, analysis of the results obtained with the desulfoglucosinolates **25**-**28** and the glucosinolates **3**-**6** revealed some reduction of the corresponding RIP values in comparison to the tested aldehydes.

Molecular modeling/docking

In order to rationalize the differences observed for the RIP values of the synthetic myrosinase substrates and to obtain some insight in the molecular details of binding, we performed docking studies with the program Glide[20] implemented in the Schrödinger software package. For that purpose, glucosinolates **4** and **6**, desulfoglucosinolates **26** and **28**, and the aldehydes **17** and **18** were chosen. As the FimH protein occurs with the tyrosine gate flanking the entrance of the carbohydrate binding domain (Tyr 48 and Tyr 137) in a "closed" conformation as well as in a more "open" form, we used both the open[21] and the closed gate crystal structures[22] of FimH for docking. Docking was carried out in extra precision mode and keeping the receptor in a fixed conformation. Moreover, flexibility was allowed for the input ligands, meaning that several conformers per ligand (five at most) were generated.

Results, showing the highest docking score for each ligand, are listed in Table 3 (for comprehensive results, see Supporting Information). More negative docking scores correlate with higher affinity.

Table 3. Docking scores[a] and binding energies[b] in open and closed gate conformation of FimH for selected glycoside ligands.

Entry	Ligand	Docking score Open gate	Docking score Closed gate	Binding energy (kJ.mol^{-1}) Open gate	Binding energy (kJ.mol^{-1}) Closed gate
1	**MeMan**	-8.514	-8.212	-53.44	-53.634
2	**4**	-10.082	-9.81	-87.681	-85.935
3	**6**	-11.677	-10.971	-86.179	-91.79
4	**26**	-9.85	-9.067	-64.511	-72.885
5	**28**	-11.101	-8.88	-89.092	-92.167
6	**17**	-8.781	-8.557	-70.932	-74.287
7	**18**	-8.847	-9.785	-73.13	-77.781

[a] Calculated with the Glide program; [b] calculated via MM-GBSA method from the docking output

For a better correlation with the experimental results, docking outputs were re-scored through a MM-GBSA[23] calculation in which the two important tyrosine residues (Tyr 48 and 137) were set flexible. The binding energies corresponding to the score values are listed in Table 3. Hence, the expected affinity ranking is found for the glucosinolates (**4**, **6**) and their desulfo counterparts (**26**, **28**) with FimH in the open gate conformation. This is slightly different in the closed gate conformation, where **6** and **28** bind with very similar affinity. However, the calculated binding energy values for the aldehyde derivatives **17** and **18** do not correlate with the respective RIPs in both open and closed gate conformation of FimH. This finding might be rationalized with the possibility of a strong non-specific interaction of the aromatic aldehyde moiety with a peripheral area of the carbohydrate recognition domain.[24] The structures of the ligand-FimH complexes resulting from the docking confirm the expected recognition mode of FimH: a tight interaction with the biphenylmannoside residue while the side chain of the glucosinolate protrudes on the outside of the lectin (Fig. 4). Additionally, our docking poses hint on the influence of the position of the glucosinolate substituent on the biphenyl moiety. In the open gate conformation, the *m*-substitution results in the flanking of the biphenyl linker by the tyrosine residues 48 and 137 (Tyr gate) while the glucoside portion binds via an H-bond network to a lectin subsite located above the Tyr gate. A comparable situation is observed for the *p*-substituted derivative **4** but with no binding of the glucoside residue into the aforementioned subsite. However, a π-π stacking of the aromatic linker with the tyrosine gate may favor FimH binding of **4** over **6**. As shown by the values of binding energies (Table 3), the mode of interaction varies when FimH is used in the closed gate conformation (Fig. 5). Here, the benefit of the *m*-substitution can be seen by first comparing the complexes with the glucosinolates. Ligand **6** binds stronger than **4** because its sulfate group is positioned so as to allow an ionic interaction with the side chain of Arg 98. As observed in the open gate conformation, the binding of the desulfoglucosinolate derivatives with the closed gate FimH is clearly influenced by the substitution pattern on the biphenyl linker. While the two compounds **26** and **28** exhibit the same number of H-bonds with the protein residues, the orientation of their respective aglycone part is quite different. The biphenyl moiety of **26**, which is lifted to the right, lays above a hydrophilic domain

whereas the same aromatic part on **28** is set in a much favorable situation, close to the phenyl ring of tyrosine 48.

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DOI: 10.1039/C8OB01128A

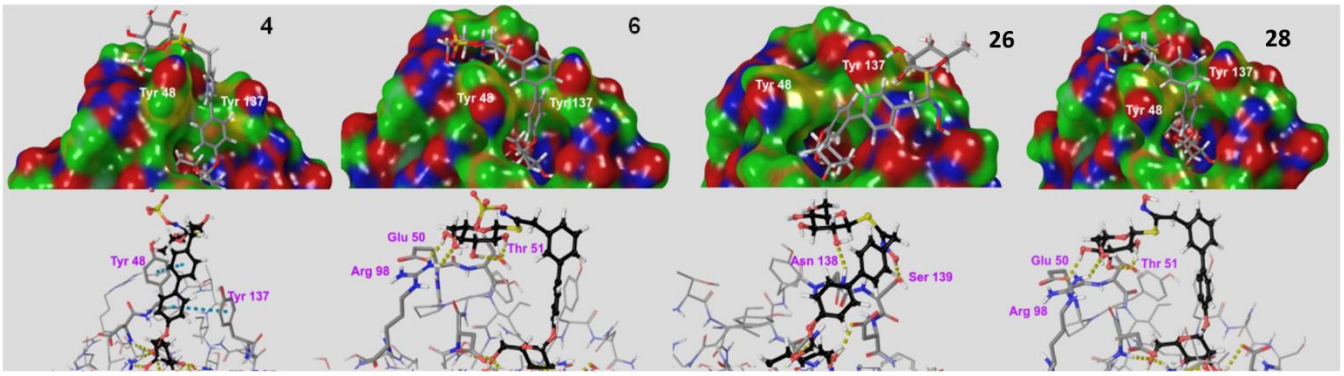

Figure 4 : Complexes of the open gate crystal structure of FimH with ligands **4**, **6**, **26** and **28**, respectively; the docking results from the MM-GBSA calculation. Top row: partial charge coloured Connolly descriptions [25] (negative partial charges coloured in red, positive in blue). Bottom row: highlights of the interactions of the ligands with some residues of the lectin; the dotted yellow lines represent the H-bonds, the dotted blue lines represent the π-π stacking.

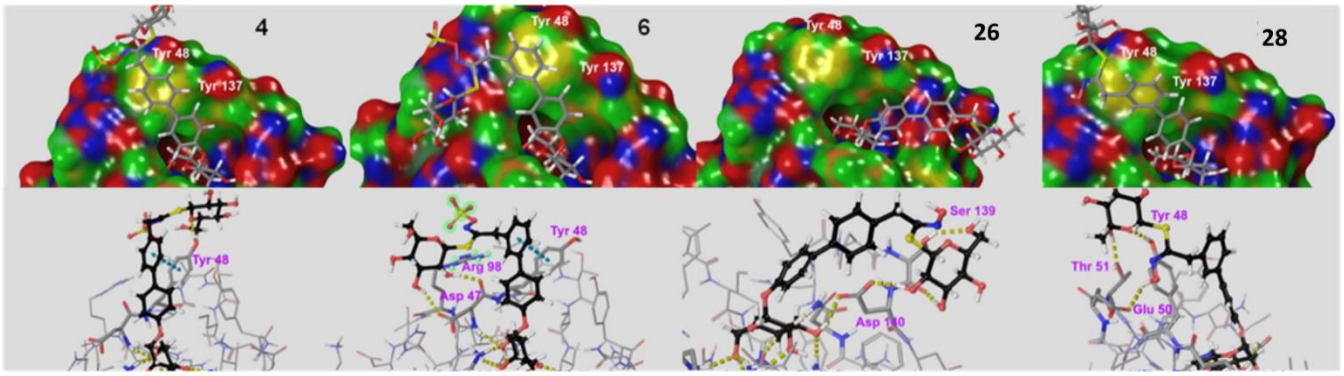

Figure 5. Complexes of the closed gate crystal structure of FimH with ligands **4**, **6**, **26** and **28**, respectively; the docking results from the MM-GBSA calculation. Top row: partial charge coloured Connolly descriptions. Bottom row: highlights of the interactions of the ligands with some residues of the lectin; the dotted yellow lines represent the H-bonds, the dotted blue lines represent the π-π stacking; in the case of ligand **6**, the green highlight represents the ionic interaction between the sulfate and the arginine 98.

Conclusions

Our study showed that combining within the same molecule a FimH ligand and a substrate of myrosinase preserves the recognition of the two moieties by both proteins, without significant loss of activity of any of the two. Myrosinase was still able to hydrolyze the glucosinolate moiety with fairly good and comparable activities (K_M, V_{max}) to the natural substrate glucomoringin. The feasibility of the hydrolysis and the Lossen rearrangement leading to an isothiocyanate, which could be trapped with simple thiols, was also verified. The RIP values determined in adhesion inhibition studies with *E. coli* proved that the glucosinolate moiety of our synthetic mannosides is not detrimental to the interaction with FimH. Moreover, molecular modelling gave us some hint on the possible mode of interaction with FimH, in favour of *m*-substitution for the biphenylmannoside while the docking results for the aldehyde derivatives do not fit with the experimental data. In this regard, further practical and theoretical investigations will be carried out in order to understand the binding details of these ligands.

Thus, myrosinase-glucosinolate reaction proved to be a valuable biochemical system able to produce isothiocyanates *in vitro* which can be further reacted with nucleophiles. The next step is to use it as a labelling tool that could be extended to other biologically relevant ligand/drug. In doing so, the MG system could be used as a new bioconjugation device for *in vitro* chemical ligation. Further work is currently ongoing in our group in this perspective, in terms of both synthetic design and biological application.

Experimental

General methods: flash silica column chromatography was performed on silica gel 60N (spherical, neutral, 40-63µm, Merck) or using a Reveleris® flash chromatography system. The reactions were monitored by thin layer chromatography (TLC) on silica gel 60F254 precoated aluminium plates. Compounds were visualised under UV light and by charring with a 10% H_2SO_4 ethanolic solution, a solution of potassium permanganate. Solvents were dried by standard methods: THF was purified with a dry station GT S100 immediately prior use, dichloromethane was distilled over P_2O_5; dried methanol from

ACROS ORGANICS, *N*,*N*-dimethylformamide and dioxane were dried over molecular sieves; pyridine and triethylamine were dried over potassium hydroxide. Molecular sieves were activated prior to use by heating for 4h at 500°C. All other commercial solvents and reagents were used without further purification. All reactions were carried out under dry argon atmosphere. Melting points were determined in open capillary tubes using a Büchi 510 apparatus and are uncorrected. Optical rotation were measured at 20°C using a Perkin Elmer 341 polarimeter with a path length of 1 dm, values are given in deg dm^{-1} g^{-1} mL^{-1} with concentrations reported in g.100 mL^{-1}. ^1H NMR and ^{13}C NMR were recorded with Bruker Avance II 400 or Bruker DPX 250 spectrometers. Assignments were based on DEPT 135 sequence, homo- and heteronuclear correlations. Chemical shifts were reported in parts per million (ppm) using tetramethylsilane as the internal standard. For the ^{13}C NMR in deuterated water, acetone was used as internal standard. Coupling constants (*J*) are reported and expressed in Hertz (Hz), splitting patterns are designated as b (broad), s (singlet), d (doublet), dd (doublet of doublet), q (quartet), dt (doublet of triplet), ddd (doublet of doublet of doublet), m (multiplet). High-resolution mass spectra (HRMS) were performed on a Maxis Bruker 4G by the "Federation de Recherche" ICOA/CBM (FR2708) platform in the electrospray ionisation (ESI) mode. The infrared spectra of compounds were recorded on a Thermo Scientific Nicolet iS10. The following solvents have been abbreviated: ethyl acetate (EA), petroleum ether (PE), tetrahydrofuran (THF), diethyl ether (Et_2O) and *N*,*N*-dimethylformamide (DMF), methanol (MeOH). Thioglucosidase from *Sinapis alba* (white mustard) seed (myrosinase, EC 3.2.1.147, 25U, ≥100 units. g^{-1}) was purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France). Experimental procedures and analytical data for compounds **6**, **13**, **16**, **21**, **24**, **26**-**28** and **17**-**18** are given in the supporting information.

General procedure 1: tetraacetylated (4-bromophenyl)-α-D-mannopyranoside **9-10** (1 equiv.), 4- or 3-formylphenyl boronic acid (3 equiv.), caesium carbonate (3 equiv.), and tetrakis(triphenylphosphine)palladium (10 mol%) were heated at 80°C in dioxane/water (84/16, 0.055 M) for 1 h, under argon atmosphere. The solvent was removed under reduced pressure and the crude was purified by silica gel column chromatography (PE/EA: 100/0 to 40/60).

[4'-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosylsulfanyl) biphenyl-4-yl]carboxaldehyde 12: general procedure 1 was followed with tetraacetylated (4-bromophenyl)-1-thio-α-D-mannopyranoside **10** (3 g, 5.79 mmol), 4-formylphenyl boronic acid (2.6 g), caesium carbonate (3.11 g), and tetrakis(triphenylphosphine)palladium (650 mg) in dioxane/water 84/16 (105 mL). The desired product **12** was obtained as a mixture of α/β anomers (80/20) as a yellow foam (2.7 g, 88%).

R_f = 0.45 (PE/EA : 5/5); δ_H (400 MHz, $CDCl_3$) 10.05 (1.2H, s, CHO), 7.95 (2.4H, d, ^3J 7.9 Hz, CH_{Ar}), 7.71 (2.4H, bd, ^3J 7.9 Hz, CH_{Ar}), 7.61 - 7.53 (4.8H, m, CH_{Ar}), 5.69 (0.30H, d, $^3J_{2,3}$ 3.4 Hz, $H_{2\beta}$), 5.56 (1H, bs, $H_{1\alpha}$), 5.51 (1H, s, $H_{2\alpha}$), 5.45 - 5.26 (2.4H, m, $H_{3\alpha}$, $H_{4\alpha}$, $H_{4\beta}$), 5.08 (0.30H, dd, $^3J_{3\text{-}4}$ 10.0 Hz, $^3J_{3\text{-}2}$ 3.4 Hz, $H_{3\beta}$), 4.98 (0.25H, bs, $H_{1\beta}$), 4.59 - 4.50 (1H, m, $H_{5\alpha}$), 4.32 (1.4H, dd, $^2J_{6a\text{-}6b}$ 12.2 Hz, $^3J_{6a\text{-}5}$ 5.6 Hz, $H_{6a\alpha}$, $H_{6a\beta}$), 4.21 (0.30H, dd, $^2J_{6a\text{-}6b}$ 12.2 Hz, $^3J_{6a\text{-}5}$ 2.6 Hz, $H_{6b\beta}$), 4.15 (1H, dd, $^2J_{6a\text{-}6b}$ 12.2 Hz, $^3J_{6a\text{-}5}$ 2.4 Hz, $H_{6b\alpha}$), 3.78 - 3.71 (0.30H, m, $H_{5\beta}$), 2.22 (0.8H, s, $CH_{3\ Ac\ \beta}$), 2.16 (3H, s, $CH_{3\ Ac\ \alpha}$), 2.10 (0.91H, s, $CH_{3\ Ac\ \beta}$), 2.08 (3H, s, $CH_{3\ Ac\ \alpha}$), 2.04, 2.03, (7.1H, s x 3, $CH_{3\ Ac\ \alpha,\ \beta}$), 1.99 (0.83H, s, $CH_{3\ Ac\ \beta}$); δ_C (100 MHz, $CDCl_3$) 191.8 (HC=O), 170.6 (C=O $_{Ac\ \beta}$), 170.5 (C=O $_{Ac\ \alpha}$), 170.1 (C=O $_{Ac\ \beta}$), 170 (C=O $_{Ac\ \beta}$), 169.9 (C=O $_{Ac\ \alpha}$), 169.8 (C=O $_{Ac\ \alpha}$), 169.7 (C=O $_{Ac\ \alpha}$), 169.6 (C=O $_{Ac\ \beta}$), 145.98 (Cq $_{Ar\ \beta}$), 145.95 (Cq $_{Ar\ \alpha}$), 139.4 (Cq $_{Ar\ \beta}$), 139.37 (Cq $_{Ar\ \alpha}$), 135.5 (Cq $_{Ar\ \alpha}$), 133.9 (Cq $_{Ar\ \beta}$), 133.3 (Cq $_{Ar\ \alpha}$), 132.2 (CH $_{Ar\ \beta}$), 132.1 (CH $_{Ar\ \alpha}$), 130.7 (CH $_{Ar\ \beta}$), 130.3 (CH $_{Ar\ \alpha}$), 128.0 (CH $_{Ar\ \alpha}$), 127.9 (CH $_{Ar\ \beta}$), 127.54 (CH $_{Ar\ \alpha}$), 126.9 (CH $_{Ar\ \beta}$), 85.5 (C-1 $_{\alpha}$), 85.4 (C-1 $_{\beta}$), 76.6 (C-5 $_{\beta}$), 71.8 (C-3 $_{\beta}$), 70.9 (C-2 $_{\alpha}$), 70.6 (C-2 $_{\beta}$), 69.7 (C-5 $_{\alpha}$), 69.3 (C-4 $_{\alpha}$ or C-3 $_{\alpha}$), 66.3 (C-3 $_{\alpha}$ or C-4 $_{\alpha}$),65.8 (C-4 $_{\beta}$), 62.8 (C-6 $_{\beta}$), 62.4 (C-6 $_{\alpha}$), 20.9 ($CH_{3Ac\ \alpha}$), 20.8 ($CH_{3Ac\ \beta}$), 20.7, 20.63 (2 x $CH_{3Ac\ \alpha}$), 20.6, 20.5 ($CH_{3Ac\ \alpha}$); IR (neat) ν (cm^{-1}) = 1736 (C=O), 1698 (HC=O), 1604, 1515, 1483 (C=C $_{Ar}$), 1212 (C-O), 812,751 (C_{sp2}-H $_{Ar}$); ESI$^+$ HRMS $[M+Na]^+$ *m/z* calcd. 567.1295 for $C_{27}H_{29}NaO_{10}S$, found 567.1298.

[4'-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)biphenyl-4-yl]carboxaldehyde 11: general procedure 1 was followed with tetraacetylated 4-bromophenyl-α-D-mannoside **9** (1.6 g),[14] 4-formylphenylboronic acid (1.43 g), caesium carbonate (3.11 g), and tetrakis(triphenylphosphine)palladium (356 mg) in dioxane/water (84/16) (58 mL). The desired product **11** was obtained as a yellow foam (1.42 g, 84%).

R_f = 0.46 (PE/EA : 5/5); $[\alpha]_D^{20}$ +79.6 (*c* 0.69 in MeOH); δ_H (400 MHz, $CDCl_3$) 10.05 (1H, s, HC=O), 7.94 (2H, d, ^3J 7.9 Hz, H_{Ar}), 7.71 (2H, d, ^3J 7.9 Hz, H_{Ar}), 7.6 (2H, d, ^3J 8.4 Hz, H_{Ar}), 7.20 (2H, d, ^3J 8.4 Hz, H_{Ar}), 5.61 - 5.57 (2H, m, H_1, H_3), 5.49 - 5.46 (1H, m, H_2), 5.40 (1H, t, $^3J_{4\text{-}3}$ $^3J_{4\text{-}5}$ 10.2 Hz, H_4), 4.30 (1H, dd, $^3J_{6a\text{-}6b}$ 12.2 Hz, $^3J_{6a\text{-}5}$ 5.2 Hz, H_{6a}), 4.15 - 4.07 (2H, m, H_{6b}, H_5), 2.22, 2.07, 2.05, 2.04 (12H, s x 4, $CH_{3\ Ac}$); δ_C (100 MHz, $CDCl_3$) 192 (HC=O), 170.6, 170.1, 170.07, 169.8 (4 x C=O $_{Ac}$), 156.1 (Cq $_{Ar}$) , 146.4 (Cq $_{Ar}$), 135.2 (Cq $_{Ar}$), 134.7 (Cq $_{Ar}$), 130.5 (CH $_{Ar}$), 128.8 (CH $_{Ar}$), 127.4 (CH $_{Ar}$), 117.1 (CH $_{Ar}$), 95.9 (C-1), 69.5 (C-2 or C-5), 69.49 (C-5 or C-2), 69.0 (C-3), 66.1 (C-4), 62.2 (C-6), 21.0, 20.85, 20.83, 20.82 (4 x $CH_{3\ Ac}$); IR (neat) ν (cm^{-1}) = 1742 (C=O $_{Ac}$), 1698 (C=O ald), 1602, 1523, 1495 (C=C $_{Ar}$), 1212 (C-O), 847, 818 (C_{sp2}-H $_{Ar}$); ESI$^+$ HRMS $[M+H]^+$ *m/z* calcd. 529.1704 for $C_{27}H_{29}O_{11}$, found 529.1708.

General procedure 2: ammonium acetate (1.1 equiv.) was added to a solution of mannoside **11**-**13** (1 equiv.) in nitromethane (0.08 M). The reaction mixture was heated for 2 to 24h. The solvent was removed under reduced pressure and the crude was purified by silica gel column chromatography (PE/EA : 100/0 to 40/60) (compound **16**). For compounds **14** and **15**, the residue obtained after evaporation was taken up with ethyl acetate and washed 2 times with saturated aqueous NH_4Cl, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure to give the desired product which was used in the next step without any further purification.

General procedure 3, thiohydroximate formation: titanium tetrachloride (2.2 equiv.) was added dropwise to a stirred

solution of triethylsilane (2.1 equiv.) and biphenylnitrovinyl derivative **14-16** (1 equiv.) in anhydrous dichloromethane (0.06 M) under argon atmosphere. The mixture was stirred at room temperature overnight. The reaction mixture was quenched by addition of water, then the aqueous phase was extracted 2 times with dichloromethane. The combined organic phases were dried over $MgSO_4$ and the solvent evaporated under reduced pressure. The residue was taken up with anhydrous dichloromethane (0.06 M) and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (1.2 equiv.) and triethylamine (3 equiv.) were sequentially added and the reaction mixture was stirred for 3h at rt. The solvent was evaporated under reduced pressure and the crude residue was purified by silica gel column chromatography (PE/EA: 100/0 to 50/50) to give the desired product.

***(Z)*-*S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl) [4'-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)biphenyl-4-yl]aceto thiohydroximate 19**: general procedure 2 was followed from mannoside **11** (1.75 g), ammonium acetate (280 mg) in nitromethane (47 mL) for 2h at reflux. The desired product **14** was obtained after work up as a yellowish foam (1.86 g, 99%). General procedure 3 was then followed from biphenyl nitrovinyl derivative **14** (300 mg), titanium tetrachloride (130 µL.) and triethylsilane (180 µL) in anhydrous dichloromethane (9 mL). For the second step, were used 9 mL of anhydrous dichloromethane, 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-gluco pyranose (232 mg) and triethylamine (220 µL). The desired product **19** was obtained after purification as a yellowish foam (260 mg, 53%).

R_f = 0.60 (PE/EA : 4/6); $[\alpha]_D^{20}$ +36.5 (*c* 0.94 in MeOH); δ_H (400 MHz, $CDCl_3$) 8.67 (s, 1H, NOH), 7.58 - 7.50 (4H, m, H_{Ar}), 7.33 (2H, d, ^3J 7.6 Hz, H_{Ar}), 7.17 (2H, d, ^3J 8.0 Hz, H_{Ar}), 5.64 - 5.55 (2H, m, H_{3M}, H_{1M}), 5.49-5.46 (1H, m, H_{2M}), 5.40 (1H, t, $^3J_{4-5}$ $^3J_{4-3}$ 10.0 Hz, H_{4M}), 5.13-4.95 (m, 3H, H_{2G}, H_{3G}, H_{4G}), 4.88 (1H, d, $^3J_{1-2}$ 10.0 Hz, H_{1G}), 4.30 (1H, dd, $^3J_{6a-6b}$ 12.2 Hz, $^3J_{6a-5}$ 4.6 Hz, H_{6aM}), 4.17-3.97 (6H, m, H_{6bM}, H_{5M}, H_{6aG}, H_{6bG}, $CH_2C{=}N$), 3.61-3.52 (1H, m, H_{5G}), 2.22, 2.08, 2.07, 2.05, 2.01, 1.97 (s x 6 , 24H, $CH_{3\ Ac}$); δ_C (100 MHz, $CDCl_3$) 170.7, 170.6, 170.3, 170.2, 170.1, 169.9, 169.4, 169.2 (C=O), 155.3 (Cq $_{Ar}$ or C=N), 151.1 (C=N or Cq $_{Ar}$), 139.6 (Cq $_{Ar}$), 135.5 (Cq $_{Ar}$), 134.7 (Cq $_{Ar}$), 128.6 (CH $_{Ar}$), 128.3 (CH $_{Ar}$), 127.4 (CH $_{Ar}$), 117.0 (CH $_{Ar}$), 96.0 (C-1_M), 79.6 (C-1_G), 75.8 (C-5_G), 73.8, 70.1 (C-2_G or C-3_G or C-4_G), 69.5 (C-2_M), 69.3 (C-5_M), 69.0 (C-3_M), 68.1 (C-2_G or C-3_G or C-4_G), 66.1 (C-4_M), 62.2, 62.3 (C-6_M, C-6_G), 38.5 ($CH_2C{=}N$), 20.9, 20.8, 20.79, 20.78, 20.66, 20.62 (6 x CH_3); IR (neat) ν (cm^{-1}) = 1742 (C=O), 1632 (C=C), 1602, 1496 (C=C $_{Ar}$), 1220, 1186, 1127 (C-O), 847, 818 (C_{sp2}-H $_{Ar}$); ESI^+ HRMS $[M+H]^+$ *m/z* calcd. 920.2641 for $C_{42}H_{50}NO_{20}S$, found 920.2636.

(*Z*)-*S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl) [4'-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosylsulfanyl)biphenyl-4-yl]acetothiohydroximate 20 : general procedure 2 was followed from mannoside **12** (2.6 g), ammonium acetate (420 mg) in nitromethane (70 mL) for 2h at reflux. The desired product **15** was obtained after work up as a yellowish foam (2.9 g, 100%). General procedure 3 was then followed from biphenyl nitrovinyl derivative 15 (2.3 g), titanium tetrachloride (946 µL.) and triethylsilane (1.31 mL) in anhydrous dichloromethane (65 mL). For the second step, were used 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (818 mg, 0.9 equiv.) and triethylamine (1.64 mL) and 65 mL of anhydrous dichloromethane. The desired product **20** was obtained after purification (PE/EA: 100/0 to 20/80) as a mixture of α/β anomers (75/25) as a yellow foam (1 g, 48%).

R_f =0.25 (PE/EA : 5/5); δ_H (400 MHz, $CDCl_3$) 8.25-8.20 (m, 1H, NOH), 7.60-7.52 (7.2H, m, CH $_{Ar\ \alpha+\beta}$), 7.34 (2.4 H, d, ^3J 7.8 Hz, CH $_{Ar\ \alpha+\beta}$), 5.68 (0.2H, d, $^3J_{2-3}$ 3.2 Hz, $H_{2\beta}$), 5.55-5.50 (2H, m, $H_{1M\ \alpha}$, $H_{2M\ \alpha}$), 5.39-5.26 (2.3H, m, $H_{3M\ \alpha}$, $H_{4M\ \alpha}$, $H_{4\beta}$), 5.12-4.95 (3.7H, m, H_{2G}, H_{3G}, H_{4G}, $H_{3\beta}$, $H_{1\beta}$), 4.87 (1H, d, $^3J_{1-2}$ 10.0 Hz, H_{1G}), 4.59-4.53 (1H, m, $H_{5M\ \alpha}$), 4.32 (1.2H, dd, $^2J_{6a-6b}$ 12.2 Hz, $^3J_{6a-5}$ 5.5 Hz, $H_{6aM\alpha}$, $H_{6aM\ \beta}$), 4.21-4.01 (3.3H, m, $H_{6bM\ \alpha}$, H_{6aG}), 3.98 (2H, bs, $CH_2C{=}N$), 3.76-3.70 (0.2H, m, $H_{5M\ \beta}$), 3.60-3.53 (1H, m, H_{5G}), 2.22 (0.6H, s, $CH_{3\ M\ \beta}$), 2.16 (3H, s, CH_3), 2.10 (0.6H, s, $CH_{3\ M\ \beta}$), 2.08, 2.07, 2.05 (9H, s x 3, CH_3), 2.04 (0.6H, s, $CH_{3\ M\ \beta}$), 2.02, 2.00 (6H, 2s, CH_3), 1.99 (0.6H, s, $CH_{3\ M\ \beta}$), 1.97, 1.96 (6H, 2s, CH_3); δ_C (100 MHz, $CDCl_3$) 170.7 (C=O$_\beta$), 170.6, 170.5, 170.2 (C=O$_\beta$), 170.2, 170.1 (C=O$_\beta$), 170.0, 169.9, 169.8, 169.6 (C=O$_\beta$), 169.2, 169.1 (C=O), 151.0 (C=N), 140.4 (Cq $_{Ar\ \beta}$), 140.3 (Cq $_{Ar}$), 139.3 (Cq $_{Ar}$), 135.4 (Cq $_{Ar}$), 132.5 (CH $_{Ar\ \beta}$), 132.4 (CH $_{Ar}$), 131.9 (Cq $_{Ar}$), 128.8, 127.8, 127.6 (CH $_{Ar\ \beta}$), 127.5 (CH $_{Ar}$), 85.8 (C-1_M), 85.7 (C-1_β), 79.6 (C-1_G), 76.5 (C-5_β), 75.8 (C-5_G), 73.8 (C-2_G or C-3_G or C-4_G), 71.9 (C-3_β), 71.0 (C-1 or C-2_M), 70.7 (C-2 $_\beta$), 70.1 (C-2_G or C-3_G or C-4_G), 69.7 (C-5_M), 69.5 (C-3_M or C-4_M), 68.1 (C-2_G or C-3_G or C-4_G), 65.8 (C-4 $_\beta$), 66.4 (C-3_M or C-4_M), 62.8 (C-6 $_\beta$), 62.5 (C-6_G or C-6_M), 62.3 (C-6_M or C-6_G), 38.5 ($CH_2C{=}N$), 21.0, 20.8, 20.7, 20.65, 20.62 ($CH_{3\ Ac}$); IR (neat) ν (cm^{-1}) = 1743 (C=O), 1662 (C=O), 1484 (C=C $_{Ar}$), 1217 (C-O), 912 (C_{sp2}-H $_{Ar}$); ESI^+ HRMS $[M+H]^+$ *m/z* calcd. 936.2413 for $C_{42}H_{50}NO_{20}S$, found 936.2414.

General Procedure 4, sulfation: sulfur trioxide-pyridine complex (5 equiv.) was added to a solution of thiohydroximate (1 equiv.) in anhydrous DMF (0.4 M). The suspension was heated at 50°C overnight. It was then cooled at 0°C, quenched by addition of a 0.5M aqueous $KHCO_3$ solution (10 equiv.) and then stirred for 30 minutes at room temperature. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (EA/MeOH 9/1).

(*Z*)-*S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl) [4'-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)biphenyl-4-yl]aceto thiohydroximate *N*,*O*-sulfate potassium salt 22 : general procedure 4 was followed from thiohydroximate **17** (440 mg) and sulfur trioxide-pyridine complex (380 mg) in anhydrous DMF (6 mL). The desired compound **22** was obtained as a yellowish oil (400 mg, 80%).

R_f = 0.37 (EA/MeOH : 9/1); $[\alpha]_D^{20}$ +34.6 (*c* 1.53 in MeOH); δ_H (400 MHz, $CDCl_3$) 7.65-7.58 (4H, m, CH_{Ar}), 7.47 (2H, d, ^3J 7.9 Hz, CH_{Ar}), 7.22 (2H, d, ^3J 8.4 Hz, CH_{Ar}), 5.67 (s, 1H, H_{1M}), 5.53-5.46 (2H, m, H_{3M}, H_{2M}), 5.33 (1H, t, $^3J_{4-3}$ $^3J_{4-5}$ 9.8 Hz, H_{4M}), 5.20-5.09 (2H, m, H_{1G}, H_{2G}), 4.99 (1H, t, $^3J_{4-3}$ $^3J_{4-5}$ 9.7 Hz, H_{4G}), 4.88 (1H, t, $^3J_{3-2}$ $^3J_{3-4}$ 9.6 Hz, H_{3G}), 4.24 (1H, dd, $^2J_{6a-6b}$ 11.9 Hz, $^3J_{6a-5}$ 5.3 Hz, H_{6aM}), 4.17-4.05 (5H, m, H_{5M}, H_{6aG}, H_{6bM}, $CH_2C{=}N$), 3.91 (1H, d, $^2J_{6b-6a}$ 10.8 Hz, H_{6bG}), 3.77-3.71 (1H, m, H_{5G}), 2.19, 2.06, 2.03, 2.01, 1.98, 1.96, 1.93, 1.91 (24H, 8 s, $CH_{3\ Ac}$); δ_C (100 MHz, $CDCl_3$) 172.22,

172.2, 171.6, 171.5, 171.48, 171.4, 171.1, 170.8 (C=O $_{Ac}$), 157.8 (C=N or Cq $_{Ar}$), 156.5 (Cq $_{Ar}$ or C=N), 140.7 (Cq $_{Ar}$), 136.8 (Cq $_{Ar}$), 135.9 (Cq $_{Ar}$), 130.0 (CH $_{Ar}$), 129.2 (CH $_{Ar}$), 128.2 (CH $_{Ar}$), 118.2 (CH $_{Ar}$), 97.1 (C-1_M), 80.8 (C-1_G), 76.7 (C-5_G), 75.0 (C-3_G), 71.3 (C-2_G), 70.63, 70.59, 70.5 (C-5_M, C-3_M, C-2_M), 69.3 (C-4_G), 67.1 (C-4_M), 63.4 (C-6_M or C-6_G), 63.1 (C-6_G or C-6_M), 39.2 (CH_2 CN), 20.64, 20.62, 20.60, 20.53, 20.49 (CH_3 $_{Ac}$); IR (neat) ν (cm^{-1}) = 1742 (C=O), 1666 (C=C), 1603, 1497 (C=C $_{Ar}$), 1230, 1127 (C-O), 847, 818 (C_{sp2}-H Ar); ESI$^-$ HRMS [M-K]$^-$ *m/z* calcd. 998.2064 for $C_{42}H_{48}NO_{23}S_2$, found 998.2069.

***(Z)-S*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl) [4'-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosylsulfanyl)biphenyl-4-yl] acetothiohydroximate *N*,*O*-sulfate potassium salt 23**: general procedure 4 was followed from thiohydroximate **20** (950 mg) and sulfur trioxide-pyridine complex (811 mg) in anhydrous DMF (15 mL). After 24h at 50°C, sulfur trioxide-pyridine complex (811 mg, 5.1 mmol, 5 equiv.) was added again, and the reaction mixture was heated for an additional 24h. The desired compound **23** was finally obtained as a yellow foam (290 mg, 26%).

R_f = 0.35 (EA/MeOH : 9/1); $[\alpha]_D^{20}$ +122.1 (*c* 0.13 in MeOH); δ_H (250 MHz, CD_3OD) 7.70-7.57 (6H, m, CH_{Ar}), 7.49 (2H, d, ^3J 8.2 Hz, CH_{Ar}), 5.70-5.57 (1H, m, H_{1M}), 5.54-5.48 (1H, m, H_{2M}), 5.32-5.27 (1H, m, H_{3M}), 5.20-5.09 (2H, m, H_{1G}, H_{2G}), 5.05-4.83 (3H, m, H_{3G}, H_{4G}, H_{5M}), 4.62-4.49 (1H, m, H_{4M}), 4.37-4.20 (1H, m, H_{6aM}), 4.18-4.04 (4H, m, $H_{6'M}$, H_{6aG}, CH_2CN), 3.91 (1H, dd, $^2J_{6a-6b}$ 12.3 Hz, $^3J_{6-5}$ 2.4 Hz, H_{6aG}), 3.83-3.66 (1H, m, H_{5G}), 2.15, 2.08, 2.02, 2.00, 1.99, 1.98, 1.93, 1.91 (24H, 8s, CH_3 $_{Ac}$); δ_C (62.5 MHz, CD_3OD) 172.3, 172.2, 171.5, 171.47, 171.1, 170.8 (C=O), 157.6 (C=N), 141.8 (Cq $_{Ar}$), 140.3 (Cq $_{Ar}$), 136.7 (Cq $_{Ar}$), 133.9 (CH $_{Ar}$), 132.7 (Cq $_{Ar}$), 130.1 (CH $_{Ar}$), 128.8 (CH $_{Ar}$), 128.4 (CH $_{Ar}$), 86.6 (C-1_M), 80.9 (C-1_G), 76.7, 75.0, 71.9, 71.3, 71.0, 71.8, 69.3, 67.5, 63.6, 63.1, 39.2 (CH_2CN), 20.67, 20.61, 20.5, 20.45 (CH_3); IR (neat) ν (cm^{-1}) = 1742 (C=O), 1666, 1603 (C=O), 1497, 1434, 1367 (C=C $_{Ar}$), 1230 (C-O), 1035, 1001, 979 (C_{sp2}-H $_{Ar}$); ESI$^-$ HRMS [M-K]$^-$ *m/z* calcd. 1014.1836 for $C_{42}H_{48}NO_{22}S_3$, found 1014.1858.

General procedure 5: potassium methoxide (0.4 equiv.) was added to a solution of acetylated compound (1 equiv.) in anhydrous methanol (0.15 M). The reaction mixture was stirred at room temperature during 6h. The solvent was then evaporated under reduced pressure and the crude product was purified using Reveleris® column chromatography on C-18 reverse phase (H_2O/MeOH : 100/0 to 0/100).

***(Z)-S*-(β-D-Glucopyranosyl) [4'-(α-D-mannopyranosyloxy) biphenyl-4-yl]acetothiohydroximate *N*,*O*-sulfate potassium salt 4:** Ggeneral procedure 5 was followed with acetylated compound **22** (400 mg, 0.38 mmol, 1 equiv.) to give product **4** as a white resin (207 mg, 78 %).

$[\alpha]_D^{20}$ +55.7 (*c* 0.98 in MeOH); δ_H (400 MHz, D_2O) 7.43 (4H, d, ^3J 8.3 Hz, CH_{Ar}), 7.36 (2H, d, ^3J 8.0 Hz, CH_{Ar}), 7.10 (2H, d, ^3J 8.5 Hz, CH_{Ar}), 5.59 (1H, s, H_{1M}), 4.77-4.70 (1H, m, H_{1G}), 4.18 (1H, bs, H_{2M}), 4.13-4.04 (3H, m, H_{6M}, H_{3M}), 3.82 (1H, t, $^3J_{4-3}$ $^3J_{4-5}$ 9.8 Hz, H_{4M}), 3.78-3.65 (3H, m, CH_2C=N, H_{5M}), 3.65-3.54 (2H, m, H_{6G}), 3.48-3.41 (1H, m, H_{4G}), 3.41-3.32 (2H, m, H_{2G}, H_{3G}), 3.24-3.18 (1H, m, H_{5G}); δ_C (100 MHz, D_2O, internal acetone) 163.0 (Cq $_{Ar}$), 155.7 (C=N), 139.4 (Cq $_{Ar}$), 135.0 (Cq $_{Ar}$), 134.5 (Cq $_{Ar}$), 129.2 (CH $_{Ar}$), 128.6 (CH $_{Ar}$), 127.7 (CH $_{Ar}$), 117.9 (CH $_{Ar}$), 98.7 (C-1_M), 82.1 (C-1_G), 80.4 (C-5_G), 77.6 (C-2_G or C-3_G), 73.9 (C-5_M), 72.5 (C-3_G or C-2_G), 71.1 (C-3_M), 70.6 (C-2_M), 69.3 (C-4_G), 67.1 (C-4_M), 61.2 (CH_2C=N), 60.9 (C-6_G), 38.6 (C-6_M); IR (neat) ν (cm^{-1}) = 3384 (O-H), 1607, 1497 (C=C), 1231, 1056 (C-OH); ESI$^-$ HRMS [M-K]$^-$ *m/z* calcd. 662.1219 for $C_{26}H_{32}NO_{15}S_2$, found 662.1224.

***(Z)-S*-(β-D-Glucopyranosyl) [4'-(α-D-mannopyranosylsulfanyl) biphenyl-4-yl]acetothiohydroximate *N*,*O*-sulfate potassium salt 5**: general procedure 5 was followed from acetylated compound **23** (280 mg, 0.27 mmol, 1 equiv.) to give product 5 as a white resin (76 mg, 40%).

$[\alpha]_D^{20}$ +126.6 (*c* 1.23 in MeOH); δ_H (400 MHz, CD_3OD) 7.66-7.55 (6H, m, CH $_{Ar}$), 7.50 (2H, d, ^3J 8.2 Hz, CH $_{Ar}$), 5.48 (1H, d, $^3J_{1-2}$ 1.5 Hz, H_{1M}), 4.56 (1H, d, $^3J_{1-2}$ 9.5 Hz, H_{1G}), 4.30 (1H, d, ^2J 16.1 Hz, CH_2C=N), 4.13-4.03 (3H, m, H_{2M} , H_{4M}, CH_2C=N), 3.89-3.68 (5H, m, H_{3M}, H_{5M}, H_{6aG}, H_{6aM}, H_{6bM}), 3.66-3.58 (1H, m, H_{6bG}), 3.27-3.21 (2H, m, H_{3G}, H_{5G}), 3.19-3.13 (2H, m, H_{2G},H_{4G}); δ_C (100 MHz, CD_3OD) 159.1 (C=N), 139.8, 139.0, 135.5, 133.5 (Cq $_{Ar}$), 131.9, 128.6, 127.0, 126.9 (CH $_{Ar}$), 89.0 (C-1_M), 81.5 (C-1_G), 80.8 (C-5_G or C-3_G), 78.0 (C-2_G or C-4_G), 74.3 (C-4_M), 72.8 (C-4_G or C-2_G), 72.3 (C-2_M), 71.8 (C-5_M or C-3_M), 69.8 (C-3_G or C-5_G), 67.3 (C-3_M or C-5_M), 61.4 (C-6_G or C-6_M), 61.2 (C-6_M or C-6_G), 37.9 (CH_2C=N); IR (neat) ν (cm^{-1}) = 3380 (O-H), 1484 (C=C $_{Ar}$), 1276 (C-O), 1057 (C_{sp2}-H $_{Ar}$); ESI$^-$ HRMS [M-K]$^-$ *m/z* calcd. 678.0990 for $C_{26}H_{32}NO_{14}S_3$, found 678.0996.

General Procedure 6: 0.67 U of myrosinase from *Sinapis alba* (white mustard) seed (10 U/mL) solution was added to a solution of glucosinolate (0.1 mmol, 1 equiv.) and benzylmercaptan (0.3 mmol, 3 equiv.), in a mixture of water/phosphate buffer pH = 7 (2/1) (0.029 M). The mixture was stirred at 37°C for 24h, then purified using Reveleris® column chromatography on C-18 reverse phase (H_2O/MeOH : 100/0 to 0/100).

***S*-Benzyl-*N*-(4-(α-D-mannopyranosyloxy)benzyl)dithiocarbamate 31:** general procedure 6 was followed from glucosinolate **3** (30 mg, 0.0480 mmol, 1 equiv.) to give product **31** as a solid (10.2 mg, 47%).

$[\alpha]_D^{20}$ +72.6 (*c* 0.74 in MeOH); δ_H (400 MHz, CD_3OD) 7.39-7.04 (9H, m, CH $_{Ar}$), 5.46 (1H, s, H_1), 4.84 (2H, s, CH_2N) 4.54 (2H, s, CH_2S), 3.99 (1H, s, H_2), 3.89 (1H, dd, $^3J_{3-4}$ 9.4 Hz, $^3J_{3-2}$ 3.4 Hz, H_3), 3.78-3.68 (3H, m, H_{6a}, H_{6b}, H_4), 3.61-3.55 (1H, m, H_5); δ_H (100 MHz, CD_3OD) 197.6 (C=S), 155.9 (Cq $_{Ar}$), 137.3 (Cq $_{Ar}$), 131.1 (Cq $_{Ar}$), 129.0 (CH $_{Ar}$), 128.7 (CH $_{Ar}$), 128.1 (CH $_{Ar}$), 126.8 (CH $_{Ar}$), 116.4 (CH $_{Ar}$), 98.8 (C-1), 73.9 (C-5), 71.0 (C-3), 70.6 (C-2), 66.9 (C-4), 61.3 (C-6), 49.4 (CH_2N), 38.81 (CH_2S); ESI$^+$ HRMS [M+H]$^+$ *m/z* calcd. 452.1196 for $C_{21}H_{25}NO_6S_2$, found 452.1195.

***S*-Benzyl-*N*-(4-(4-(α-D-mannopyranosyloxyphenyl)benzyl) dithiocarbamate 32:** general procedure 6 was followed from glucosinolate **4** (16 mg, 0.0228 mmol, 1 equiv.) to give product **32** as a solid (6 mg, 50 %).

$[\alpha]_D^{20}$ +66.9 (*c* 0.35 in MeOH); δ_H (400 MHz, CD_3OD) 7.63-7.16 (13H, m, CH_{Ar}), 5.52 (1H, s, H_1), 4.94 (2H, s, CH_2N), 4.56 (2H, s, CH_2S), 4.03 (1H, s, H_2), 3.92 (1H, dd, $^3J_{3-4}$ 9.4 Hz, $^3J_{3-2}$ 3.4 Hz, H_3), 3.80-3.70 (3H, m, H_6, H_4), 3.66-3.60 (1H, m, H_5); δ_C (100 MHz, CD_3OD) 199.2 (C=S), 157.5 (Cq $_{Ar}$), 141.1 (Cq $_{Ar}$), 138.7 (Cq $_{Ar}$), 137.3 (Cq $_{Ar}$), 136.2 (Cq $_{Ar}$), 130.1 (CH $_{Ar}$), 129.5 (CH $_{Ar}$), 129.0 (CH $_{Ar}$), 128.3 (CH $_{Ar}$), 127.7 (CH $_{Ar}$), 118.1 (CH $_{Ar}$), 100.2 (C-1), 75.4 (C-5), 72.4 (C-3), 72.0 (C-2), 68.4 (C-4), 62.7 (C-6), 51.0 (CH_2N), 40.3 (CH_2S); ESI$^+$ HRMS $[M+H]^+$ *m/z* calcd. 528.1509 for $C_{27}H_{29}NO_6S_2$, found 528.1508.

***S*-Benzyl-*N*-(3-(4-(α-D-mannopyranosyloxyphenyl)benzyl) dithiocarbamate 33:** general procedure 6 was followed from glucosinolate **6** (30 mg, 0.0427 mmol, 1 equiv.) to give the desired product as a solid **33** (18 mg, 80%).

$[\alpha]_D^{20}$ +75.3 (*c* 1.5 in MeOH); δ_H (400 MHz, CD_3OD) 7.58-7.15 (13H, m, CH $_{Ar}$), 5.53 (1H, s, H_1), 4.97 (2H, s, CH_2N), 4.56 (2H, s, CH_2S), 4.03 (1H, s, H_2), 3.93 (1H, dd, $^3J_{3-4}$ 9.4 Hz, $^3J_{3-2}$ 3.3 Hz, H_3), 3.81-3.70 (3H, m, H_6, H_4), 3.66-3.60 (1H, m, H_5); δ_C (100 MHz, CD_3OD) 199.3 (C=S), 157.5 (Cq $_{Ar}$), 142.2 (Cq $_{Ar}$), 139.2 (Cq $_{Ar}$), 138.7(Cq $_{Ar}$), 136.4 (Cq $_{Ar}$), 130.5 (CH $_{Ar}$), 130.1 (CH $_{Ar}$), 129.5 (CH $_{Ar}$), 129.1 (CH $_{Ar}$), 128.2 (CH $_{Ar}$), 127.5 (CH $_{Ar}$), 127.2 (CH $_{Ar}$), 126.7 (CH $_{Ar}$), 118.1 (CH $_{Ar}$), 100.2 (C-1), 75.4 (C-5), 72.4 (C-3), 72.0 (C-2), 68.4 (C-4), 62.7 (C-6), 51.3 (CH_2N), 40.2 (CH_2S); ESI$^+$ HRMS $[M+H]^+$ *m/z* calcd. 528.1509 for $C_{27}H_{29}NO_6S_2$, found 528.1510

Capillary Electrophoresis procedure followed protocols earlier developed in our laboratory.[8] Substrates are used at large excess relative to the enzyme (about 100 times). For this reason, Myrosinase was used at 0.05 U.mL^{-1} for all assays. The myrosinase activity was determined by following the hydrolysis of the glucosinolate substrate, the SO_4^{2-} produced was detected by the C^4D and quantified. The volume of the reaction mixture was set down to 7 µL, instead of the 100 µL used previously, and was done in a mico-vial of the CE instrument autosampler.

The nonlinear curve fitting program PRISM® 5.04 (GraphPad, San Diego, California, USA) was used to determine K_M and V_{max} according to the following equation:

$$V_i = \frac{V_{max} \times [S]}{K_m + [S]}$$

where V_i is the reaction rate, K_M is the Michaelis Menten constant, V_{max} is the maximum reaction velocity and [S] is the substrate (glucosinolate) concentration.

Adhesion inhibition tests:

Bacterial inhibition studies: buffers: PBS buffer: PBS tablets were obtained from GIBCO containing phosphate (as sodium phosphates), 10 mM, potassium chloride (KCl), 2.68 mM, sodium chloride (NaCl), 140 mM, pH = 7.45; PBST buffer: PBS buffer + 0.05% v/v Tween®20; carbonate buffer solution (pH 9.6): sodium carbonate (10.6 g) and sodium hydrogen carbonate (8.40 g) were dissolved in bidest. Water (1.0 L), pH values were adjusted by using 0.1 M HCl or 0.1 M NaOH.

Bacterial culture: The bacterial *E. coli* strain PKL1162[26] was cultured from a frozen stock in LB media (+ampicillin 100 mg/mL and chloramphenicol 50 mg/mL) overnight at 37°C. The bacterial pellet resulting after centrifugation and decantation of media was washed twice with PBS (2 mL) and suspended in PBS buffer afterwards. The bacterial suspension was adjusted to OD_{600} = 0.4 with PBS.

The inhibition assay was performed according to the literature.[19] Black microtiter plates (Nunc, MaxiSorp) were incubated overnight with mannan from Saccharomyces cerevisiae (1.2 mg/mL carbonate buffer, 120 µL/well) at 37°C at 100 rpm. After washing three times with PBST microtiter plates have been blocked with PVA (poly vinyl alcohol) by adding a solution of 1 % PVA in PBS (120 µL/well) and incubation at room temperature, 3 h, 100 rpm. Afterwards plates were washed with PBST twice and PBS once. Finally, a serial dilution of the particular inhibitor was prepared (50 µL/well) and the bacterial suspension was added (50 µL/well). After incubation for one hour at 37°C and 100 rpm, microtiter plates were washed three times with PBS and filled with PBS (100 µL/well) for terminal fluorescence intensity read out (excitation wavelength 485 nm, emission wavelength 535 nm).

Molecular modeling

Molecular modeling was performed using the Schrödinger software package implementing the Maestro interface.[27] The ligands were built using Maestro then minimized using MacroModel,[28] with the OPLS3 force field in implicit water (GB/SA continuum solvation model). The minimized structures were prepared for docking using LigPrep.[29] The docking studies were performed on the open gate (PDB code: 1klf),[21] or on the closed gate (PDB code: 1uwf) crystal structure of FimH.[22] Receptor grids suitable for docking were built with the Glide docking software,[20] by defining a outer box of 20 Å centered on the ligand from the crystal structure. Each grid was generated using the OPLS3 force field and including aromatic protons as H-bond donors. Extra precision (XP) docking was carried out with Glide, setting the ligand flexible and including aromatic protons as H-bond donors. At most five poses per ligand were written out, discarding poses as duplicate if both RMS deviation was less than 0.5 Å and the maximum atomic displacement was less than 1.3 Å. For re-scoring, the docking outputs were processed in a MM-GBSA (molecular mechanics-generalized born surface area) calculation,[23] giving the free energy of binding in kJ.mol^{-1}. The MM-GBSA calculation was performed using the VGSB solvation model and the OPLS3 force field. Hierarchical sampling was carried out, allowing flexibility of the two important tyrosine residues (Tyr 48 and 137) of the receptor. The extensive docking and MM-GBSA results are listed in Tables S2-S5 (see Supporting Information).

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work has been partly supported by the Université Orléans, the Centre National de la Recherche Scientifique (CNRS), the

Labex SynOrg (ANR-11-LABX-0029). G. C. thanks the Labex SynOrg (ANR-11-LABX-0029) for the doctoral fellowship. T.K. L. thanks the *International* Center at Christiana Albertina University of Kiel for funding this French-German collaboration.

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View Article Online
DOI: 10.1039/C8OB01128A

Bifunctional mannoside-glucosinolate glycoconjugates as enzymatically triggered isothiocyanates and FimH ligands.

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The synthesis of glucosinolate-mannoside glycoconjugates combining both the structural features of a myrosinase substrate and a FimH ligand are described.