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# Stereocontrolled 1-*S*-glycosylation and comparative binding studies of photoprobethiosaccharide conjugates with their *O*-linked analogs\*

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*Abstract*: The use of thioglycosides and other glycan derivatives with anomeric sulfur linkages is gaining increasing interest, both in synthesis and in various biological contexts. Herein, we demonstrate the occurrence and circumvention of anomerization during 1-S-glycosylation reactions, and present highly efficient and stereocontrolled syntheses of a series of photoprobe-thiosaccharide conjugates. Mutarotation of glycosyl thiols proved to be the origin of the anomeric mixtures formed, and kinetic effects could be used to circumvent anomerization. The synthesized carbohydrate conjugates were then evaluated by both solution- and solid-phase-based techniques. Both binding results showed that the S-linked glycosides interact with their cognate lectins comparably to the corresponding *O*-analogs in the present cases, thus demonstrating the reliability of the solid-support platform built upon our photo-initiated carbohydrate immobilization method for probing protein bindings, and showing the potential of combining these two means for studying carbohydrate–protein interactions.

*Keywords*: carbohydrates; photochemistry; protein interactions; organic materials; organic synthesis; stereocontrol.

## INTRODUCTION

Thiosaccharides and *S*-linked glycoconjugates are known as highly valuable substrate analogs of naturally occurring carbohydrates and carbohydrate-containing biomolecules for studies in glycobiology [1–6]. These molecules are favorable target molecules in synthesis, able to resist enzymatic or acidic hydrolysis [1,3], which significantly potentiates their use in biological studies, e.g., as inducers [7], enzyme inhibitors [8], therapeutics [9], or ligands for the purification of proteins by affinity chromatography [10]. Although the general use of thio-analogs as receptor-active carbohydrate ligands has occasionally been questioned, several studies have shown potent activities of these structures. For

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instance, *S*-linked carbohydrate residues in conjugate vaccines induce immune responses of comparable or higher magnitude, in terms of antibody titer, as their natural analogs [11], thus demonstrating the effectiveness and reliability of thiosaccharides and thioglycoconjugates as mimetics in glycobiological studies. Consequently, a considerable amount of effort has been made toward the synthesis and biological evaluations of such glycomimetics [12–16].

From the synthetic point of view, the importance of 1-thioaldoses and 1-thioglycosides has been recognized for a long time, and 1-*S*-alkylation routes to thioglycosides, which are easier to perform than the corresponding 1-*O*-alkylation methods, were documented by Horton already in the early 1960s [17]. Recently, these molecules are gaining renewed interest, and have found numerous novel applications in, for example, biohybrid polymer systems [18–20], dynamic combinatorial chemistry (DCC) [21–24], glycoclusters based on self-organizable scaffolds [25–27], glycoporphyrin-assisted photodynamic therapy (PDT) [25,28,29], bio-nanotechnology [30–32], and biologically important glycoconjugates [15,33]. Given the value of 1-thioaldoses and 1-thioglycosides, it is however surprising that anomerization during the 1-thioglycosylation processes, especially when using free 1-glycosyl thiols as donors, is rarely reported in the literature. In fact, it has been generally regarded that alkylation of glycosyl thiolates mainly occurs with retention of the anomeric configuration [34]. Anomerization of glycosyl thioethers, promoted by Lewis- or protic acids, has on the other hand been more widely realized and studied [35–38].

From the biological point of view, assessment of the general performances of *S*-linked carbohydrate entities, in comparison to the recognition properties of the *O*-linked analogs, both in solutionand solid-phase systems, are of considerable interest. Especially for carbohydrate–protein interactions, arguably the most important type of interaction, in-depth information of the binding properties is central to further developments. The conformational change of thiosaccharides, induced by the sulfur atom, and its correlation to affinity properties toward their cognate proteins have been well studied [39]. However, only limited reports can surprisingly be found where the binding properties between *S*- and *O*-linked glycan analogs have been compared [12,40–43].

Several methods to study carbohydrate-protein interactions can be employed to address this difference, e.g., isothermal titration calorimetry (ITC) [44], hemagglutination inhibition [40], precipitation inhibition [45], equilibrium dialysis [46], spectrophotometry [47], enzyme-linked immunosorbent assay (ELISA) [48], fluorescence assay [49,50], nuclear magnetic resonance (NMR) [51], surface plasmon resonance (SPR) [41,52], quartz-crystal microbalance (QCM) [53,54], carbohydrate microarrays [55], and scanning probe microscopy (SPM) [56]. Of these, two major methods are especially advantageous: ITC and solid-phase binding techniques, the combination of which results in elucidation of overall binding performances. ITC can directly provide thermodynamic data about molecular interactions, thus reliably conveying important binding information and supporting binding implications. On the other hand, techniques involving solid supports, such as microarrays, biosensors, and nanoplatform-based techniques, have more recently drawn increasing attention in glycobiology and glycomics [57]. This is in part due to the fact that solid surfaces are better mimics of cell surfaces than solution systems, and partly because of their ability to provide comprehensively hierarchical levels of molecular information [58-60]. Solid-supported techniques are thus playing crucial roles for studies of various carbohydrate-protein interactions, and as a result, methods for immobilization of carbohydrates on surfaces and resins have become an important research topic. In this context, the well-established perfluorophenylazide (PFPA) chemistry enables one-step, facile, and robust attachment of organic molecules on solid surfaces, by either thermal- or photo-activation, resulting in versatile surface modification for a wide range of applications [41,49,52–56,61–64].

In the present study, a series of *O*- and *S*-linked, photoactivatable PFPA-conjugated mono- and di-saccharides were synthesized. The thioglycosylation strategy utilizing unprotected glycosyl thiols was employed for the *S*-linked entities, able to substantially circumvent the labor-intensive and time-consuming nature of protection-glycosylation-deprotection routes generally required for synthesizing their *O*-linked analogs. Furthermore, a conspicuous mutarotation phenomenon during thioglycosylation

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was investigated, and the reaction conditions were adapted to achieve complete stereocontrol. Subsequently, the *O*- and *S*-linked carbohydrates were subjected to ITC analyses for comparison of their inherent protein binding properties. To the best of our knowledge, this is the first comparison of lectin binding affinities between thioglycosides and their oxygen analogs using the ITC technique. Moreover, the results well correlate with their surface binding responses on SPR sensors, demonstrating the capability and potential of combining both solution- and solid-phase-based biotechniques to study carbohydrate–protein interactions.

## **RESULTS AND DISCUSSION**

Six PFPA-conjugated carbohydrates were synthesized (Fig. 1). These include *S*- and *O*-linked mannosides **1** and **2**, glucosides **3** and **4**, and lactose derivatives **5** and **6**. The synthesis of the *O*-linked carbohydrate structures **2**, **4**, and **6** followed previously reported methods [55], modified for the present structures. Briefly, the compounds were synthesized by first allowing the peracetylated parent structures to



Fig. 1 Carbohydrate conjugates used in the study.

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react with an azide linker acceptor, catalyzed by Lewis acid. The resulting carbohydrate-tethered azides were then subjected to catalytic hydrogenation, after which subsequent amidation with NHS-activated PFPA-ester yielded the protected carbohydrate conjugates. The final unprotected PFPA-carbohydrates were obtained after Zemplén deacetylation. The stereochemistry during the glycosylation reactions was controlled using neighboring group participation by the 2-*O*-acetyl group.

A general route for synthesizing the target PFPA-thioglycosides **1**, **3**, and **5** was initially designed as shown in Scheme 1. The synthesis of compound **1** was first implemented, where unprotected mannose thiolate **7** [22] was directly alkylated by a tosylated oligoethylene linker **8** carrying an azide functionality. The reaction was carried out in methanol at ambient temperature, resulting in a mixture of  $\alpha$ -and  $\beta$ -thioglycosides after purification. Reducing (0 °C) or increasing (40–60 °C) the temperature did not prevent the mixture formation, and no reaction product was formed at any tested temperature. Thus, dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF) proved to be the best solvents for the reaction, whereas *N*,*N*'-dimethylpropyleneurea (DMPU) could work in special cases. DMSO turned out to be a remarkably good solvent to avoid 1-glycosyl thiol mutarotation and to enhance the coupling rate. However, DMF was chosen to further optimize the reaction conditions.



Scheme 1 Synthetic route to PFPA-thiomannoside 1 ( $\alpha/\beta = 5/1$  for compounds 9 and 1).

Free glycosyl 1-thiolates have generally been considered to be relatively configurationally stable compared to their oxygen analogs [65], in part explained by the poor orbital overlap between the anomeric carbon and the sulfur atom. Thus, ring opening and subsequent mutarotation of 1-thiosac-charides are hindered. Nevertheless, it has recently been demonstrated that anomerization readily occurs for a range of 1-thiosaccharides in aqueous solution in a pH-dependent process [66]. In the present case, the NMR analyses similarly revealed the formation of the  $\beta$ -anomer in the product mixture. This observation was corroborated by a value of the homonuclear three-bond coupling constant ( $J_{1,2}$ ) of 0.63 Hz [67], and the presence of a multiplet resonating at  $\delta$  3.41 ppm, which was assigned to mannose H-5 [68]. Thus, that anomerization was taking place under the present conditions could be confirmed.

Efforts were subsequently focused on controlling the stereochemistry in the synthesis. A number of factors, including reaction time, temperature, solvent, electrophile, and presence of base, were thus investigated. Firstly, the reaction time, temperature, and solvent were evaluated in the synthesis of mannose-azide **9** (Table 1). Four different polar aprotic solvents were tested: DMSO, DMF, DMPU, and acetonitrile. Using the most polar solvent, DMSO, the desired  $\alpha$ -product could be obtained at 20 °C in

8 h; and in a shorter time at higher temperatures from 50 to 100 °C. In all cases, no  $\beta$ -product was detected under these conditions. In the case of DMF at 20 °C, however, a mixture of  $\alpha$ - and  $\beta$ -anomers was produced after prolonged reaction time. Heating to 50–100 °C led to high conversion within 3 h, but the undesired  $\beta$ -product was again formed. When the reaction was carried out at 0 °C, no reaction could be observed after 24 h. DMPU is known to be a good substitute for the relatively toxic hexamethylphosphoramide (HMPA) as a (co)solvent in S<sub>N</sub>2 type reactions [69]. However, it proved unsuitable for this reaction under the tested conditions, where mainly disulfide and other unidentified side products were observed. In contrast, the desired  $\alpha$ -product was formed without any detectable  $\beta$ -anomer when the reaction was carried out in a microwave reactor at 60 °C for 30 min. Acetonitrile proved inefficient due to the low solubility of the starting thiosaccharide.

Entry	Solvent	Temp. (°C)	Time (h) <sup>b</sup>	Product ratio, yield
1	DMSO	20	8	only $\alpha^{c}$
2	DMSO	50	0.5	only $\alpha^{c}$
3	DMSO	100	0.08	only $\alpha^{c}$
4	DMF	0	No reaction <sup>d</sup>	-
5	DMF	20	50	α/β: 7/1, 58 % <sup>e</sup>
6	DMF	50	3	α/β: 11/1, 64 % <sup>e</sup>
7	DMF	100	0.5	$\alpha/\beta$ : 4/1, 36 % <sup>e</sup>
8	DMPU	0	24	side products
9	DMPU	20	16	side products
10	DMPU	50	1.5	side products

Table 1 Time, temperature, and solvents evaluated<sup>a</sup>.

<sup>a</sup>In all entries, 1.2 equiv of glycosyl thiolate 7 ( $\alpha$ -anomer only) and 1 equiv of linker azide 8 were used.

<sup>b</sup>Time to reach full conversion.

<sup>c</sup>Determined by NMR, yields not determined.

<sup>d</sup>No reaction after 24 h, no mutarotation observed.

eIsolated yields, based on 50 mg of glycosyl thiolate scale reactions,  $\alpha/\beta$  ratio determined by NMR.

In principle, mutarotation of 1-thiosaccharides is likely the major pathway to account for the anomerization effect. To control the stereochemistry of the product, efforts can be directed to increase the rate of the desired  $S_N^2$  reaction. After the aforementioned evaluation of the effects from solvent and temperature, the properties of the electrophile were examined. Replacing the tosyl moiety for an iodo group was found to increase the reaction rate of the  $S_N^2$  displacement, so that the starting 1-thio- $\alpha$ -D-mannopyranose could be trapped to give the corresponding  $\alpha$ -glycoside product in high yield. When used with the thiolate at 50 °C in DMF, the reaction reached completion within 1 h, and only  $\alpha$ -product was produced with an isolated yield of 87 %. When the reaction could be detected and only  $\alpha$ -product was obtained. In contrast, when the iodo-linker was reacted with 1-thio- $\alpha$ -D-mannopyranose in MeOH at 50 °C, a mixture of anomeric products was again formed.

As reported, the mutarotation of 1-glycosyl thiols is pH-dependent [66], and the reaction is generally impaired under basic conditions. To test the effect from added base in the present study, the tosyl electrophile, which originally led to a mixture of anomeric products at 50 °C in DMF, was subjected to glycosylation using 1-thio- $\alpha$ -D-mannopyranose. The results were in this case very clear, and the addition of 50 equiv triethylamine (TEA) in the reaction mixture resulted in nearly pure  $\alpha$ -product, with only trace  $\beta$ -anomer formed. At higher temperature, 100 °C, the  $\beta$ -anomer formation was, however, only marginally suppressed, and could still be clearly detected. Varied ratios of TEA: from 5 equiv to neat TEA, did not improve the outcome. The coupling reaction rate was also considerably lower when

neat TEA was used. Thus, under these conditions, the basic environment was not able to efficiently block the mutarotation of the 1-thiomannose.

It has been proposed that anomerization of 1-thioglycosides can occur, albeit under Lewis acidic conditions [70]. However, experiments were carried out to exclude the possibility of product anomerization in the present case. Thus, leaving the glycoside product in the reaction vessel for prolonged times did not result in increased anomerization. Likewise, reactions using the purified  $\alpha$ -D-mannose thioglycoside **9**, in the presence of NaOTs ( $\geq$ 1 equiv) in DMF at 50 °C for up to one week, yielded no anomerization products. This supported the mechanistic origin to be mutarotation of the 1-glycosyl thiols, resulting in the formation of final anomeric product mixtures.

Glucose thiolates are generally regarded to be configurationally stable in glycosylation reactions. However, when 1-thio- $\beta$ -D-glucopyranose **11** was allowed to react with tosylated linker in MeOH/THF (v/v: 3/1) and heated up to 50 °C for 5 h, a mixture of anomeric 1-thioglucosides were formed with an  $\alpha/\beta$  ratio of 1/5 after column purification. Nevertheless, with the aforementioned reaction conditions, reactions of compound **11** with either linker **8** or **13** at 50 °C in DMF gave the pure  $\beta$ -product in very high yield (Scheme 2). For example, in the case of mixing compounds **11** and **8**, a yield of 98 % was obtained after column purification, and no  $\alpha$ -anomer product could be found within the NMR detection limit. It is noteworthy that in the case of mannose thiolate **7** as glycosyl donor with the tosylated linker **8**, a mixture of  $\alpha/\beta$  products was produced under similar reaction conditions. This may be attributed to, on the one hand, 1-thio- $\alpha$ -D-mannopyranose **7** being more vulnerable to mutarotation [66], and on the other hand, 1-thio- $\beta$ -D-glucopyranose **11** reacting faster in the S<sub>N</sub>2 displacement reactions for steric and stereoelectronic reasons [71]. Such reactivity is in accord with previous results showing that the anomeric *O*-1 in equatorial position is much more nucleophilic than that in the axial position [72,73]. This was also supported by the fact that the time for a full conversion of  $\beta$ -glucose thiolate in the current reaction was 1.5 h, in contrast to 3 h for the  $\alpha$ -mannose thiolate.

For *O*-glycosides, this anomeric reactivity difference has been explained by a kinetic anomeric effect, owing to the increased lone pair repulsion and orbital interaction for the equatorial anomer compared to the axial counterpart [71–73]. In principle, this could also be a possible factor for the different



Scheme 2 Synthetic routes to PFPA-thioglucoside 3 and PFPA-thiolactoside 5.

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thiosaccharide anomeric reactivities, although likely to a lower extent. Regardless, these results also correlate with literature reports, suggesting higher reactivity of  $\beta$ -thiolates than  $\alpha$ -thiolates [67].

To further demonstrate the generalizability of the method, disaccharide  $\beta$ -thiolactose **12** was as well used to make the corresponding PFPA-thiosaccharide conjugate **5** following the same optimized protocol (Scheme 2). The results were in this case similar to the corresponding mannose and glucose reactions. This protocol, in combination with a one-step method to convert free *O*-saccharides to their corresponding *S*-analogs [74], will thus provide an efficient and promising route to various *S*-linked glycoconjugates for glycobiological studies.

ITC analyses were subsequently carried out to provide thermodynamic information of the binding events for the different photoprobe-conjugated carbohydrate structures. Thus, the carbohydrate conjugates were titrated against buffer solutions containing Concanavalin A (Con A) or peanut agglutinin (PNA).

The resulting titration curves of *O*- and *S*-linked PFPA-mannose toward Con A in PBS buffer at pH 7.4, containing 1 mM of Mn<sup>2+</sup> and 1 mM of Ca<sup>2+</sup> are displayed in Fig. 2. The obtained thermodynamic data for both binding systems are presented in Table 2. Compared to the binding affinity of methyl  $\alpha$ -D-mannopyranoside toward Con A ( $K_a = 7.0 \times 10^3 \text{ M}^{-1}$ ) [75], the  $K_a$  values of both compounds 1 and 2 against Con A were comparable, with that of *O*-linked conjugate 2 being slightly higher than that of 1. In contrast, the affinities of lactose derivatives 5 and 6 against PNA were very similar, yet also in good agreement with the literature data, where a  $K_a$  value of 1.99 × 10<sup>3</sup> M<sup>-1</sup> was reported for the lactose-PNA binding [76].



**Fig. 2** Calorimetric titration of Con A (80  $\mu$ M) with carbohydrate derivatives **2** (a) and **1** (b) (5 mM) at 25 °C: top) raw data obtained from 20 automatic injections, of 2  $\mu$ L each; bottom) integrated curves showing experimental points (**■**) and best fits (-).

			$-\Delta G$	$-\Delta H$	$-T\Delta S$
Compound	Lectin	$K_{\rm a}({\rm M}^{-1}\times 10^{-3})$		(kcal/mol)	
1	Con A <sup>a</sup>	$4.06 \pm 0.65$	$4.9 \pm 0.4$	$12.8 \pm 1.1$	$7.9 \pm 0.9$
2	Con A <sup>a</sup>	$6.50 \pm 1.04$	$5.2 \pm 0.7$	$13.5 \pm 1.0$	$8.3 \pm 1.3$
5	PNA <sup>b</sup>	$1.82 \pm 0.17$	$4.3 \pm 0.8$	$27.2 \pm 2.1$	$22.8 \pm 2.1$
6	PNA <sup>b</sup>	$1.85 \pm 0.22$	$4.4 \pm 1.2$	$19.0 \pm 1.8$	$14.5 \pm 2.5$

**Table 2** Thermodynamic binding parameters of *S*- and *O*-linked PFPA-conjugated carbohydrates with lectins at 25  $^{\circ}$ C.

<sup>a</sup>Average of six experiments.

<sup>b</sup>Average of three experiments.

The SPR experiments were carried out following reported procedures [41]. The PFPA-carbohydrates were printed and subsequently immobilized on a SPR chip coated with a thin layer of poly(2ethyl-2-oxazoline) (PEOX) [77]. Each compound was spotted in six replicates using a robotic printer, and the resulting glycochip was subjected to three different concentrations of Con A solution, 100 nM, 1  $\mu$ M, and 10  $\mu$ M, for the lectin binding studies. The responses, in percent change in reflectivity ( $\Delta$ %R), were recorded simultaneously for all six spots by SPR imaging (Fig. 3). Upon Con A interrogation, good signal-to-noise and reproducible binding results were obtained, showing that the *S*-linked PFPA-carbohydrate 1 bound the lectin marginally weaker than its *O*-linked analog 2. This result agreed with the results obtained by the solution-based ITC technique, thus demonstrating the reliability of this solid-support-based platform in comparing lectin bindings between the *S*- and *O*-linked carbohydrate conjugates.



**Fig. 3** SPR responses of glycochips based on carbohydrates **1** and **2** toward Con A interrogation. Each data point (±SEM) represents the average response of six replicates for each compound.

#### CONCLUSIONS

In the present study, a series of photoprobe-conjugated carbohydrates were synthesized and their lectin binding properties evaluated. In the synthesis of the *S*-linked conjugates, anomerization was identified as an obstacle during glycosylation using unprotected 1-glycosyl thiols, and the origin of which proposed to be mutarotation of the reactant thiosaccharides. Design and optimization of the synthetic pro-

tocol, however, resulted in highly efficient syntheses of the target molecules, with complete control of the stereochemistry, and coupling yields up to 98 %. The method also proved applicable for synthesizing both mono- and di-saccharides. The synthesized conjugates were subsequently evaluated using solution-phase ITC and solid-phase SPR, respectively. Both methods reveal that the *S*- and *O*-linked glycosides show comparable lectin affinities, thus demonstrating the reliability of our solid-supported SPR platform, and the capability and potential of combining these two means for studying carbo-hydrate–protein interactions.

## **EXPERIMENTAL SECTION**

**General.** Compounds **1** and **9** were prepared as previously reported [41]. Water used for analysis was obtained from a water purification system, and showed at least 18.2 M $\Omega$  resistivity. Con A from *Canavalia ensiformis*, and PNA from *Arachis hypogaea* were from commercial sources. The lectins were dialyzed in PBS buffer for 48 h before use. All other solvents and commercially available compounds were of HPLC, certified ACS, or reagent grade and used as received. PBS (pH 7.4) buffer was prepared by dissolving a phosphate-buffered saline tablet in 200 mL purified water to yield pH 7.4 phosphate buffer (PBS, 0.01 M) containing KCl (0.0027 M) and NaCl (0.137 M). <sup>1</sup>H and <sup>13</sup>C NMR data were recorded at 500 MHz (<sup>1</sup>H), or 125 MHz (<sup>13</sup>C). Chemical shifts are reported as  $\delta$  values (ppm) with D<sub>2</sub>O (<sup>1</sup>H:  $\delta$  = 4.79) as internal standard. *J* values are given in Hz. <sup>1</sup>H peak assignments were made by first-order analysis of the spectra supported by standard <sup>1</sup>H-<sup>1</sup>H correlation spectroscopy (COSY). Thin layer chromatography (TLC) was performed on precoated Silica gel 60 F<sub>254</sub> plates. Flash column chromatography was performed on silica gel 60, 0.040–0.063 mm. All experiments containing PFPA groups were conducted in the absence of light with all reaction flasks covered by aluminum foil to prevent decomposition.

**2-[2-(2-Azidoethoxy)ethoxy]ethyl 1-thio-β-D-glucopyranoside (14).** 1-thio-β-D-glucopyranose sodium salt **11** (55 mg, 0.25 mmol) and compound **8** (72 mg, 0.21 mmol) in dry DMF (4 mL) were stirred at 50 °C under nitrogen for 1.5 h. After completion of the reaction, the solvent was evaporated. Then the crude product was purified by flash column chromatography using solvent system DCM/MeOH (8:1 v/v), giving 73 mg of compound **14** (98 %). [ $\alpha$ ]<sub>D</sub> = -29 (c 1.5, MeOH); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  2.93 (m, 1 H, SCH<sub>2</sub>), 2.99 (m, 1 H, SCH<sub>2</sub>), 3.32 (at, 1 H, *J* = 9.8 and 9.2 Hz, H-2), 3.53–3.38 (m, 5 H, H-3, H-4, H-5, CH<sub>2</sub>N<sub>3</sub>), 3.84–3.67 (m, 9 H, 4 × CH<sub>2</sub>O, H-6a), 3.90 (dd, 1 H, *J* = 1.9 and 12.3 Hz, H-6b), 4.57 (d, 1 H, *J* = 9.8 Hz, H-1). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  29.10, 50.14, 60.87, 69.22, 69.38, 69.49, 70.14, 72.28, 77.15, 79.86, 85.41. HRMS: calcd. for C<sub>12</sub>H<sub>23</sub>N<sub>3</sub>O<sub>7</sub>S [M + Na]<sup>+</sup> 376.1149; observed 376.1142.

**2-{2-(2-(4-Azido-2,3,5,6-tetrafluorobenzamido)ethoxy]ethoxy}ethyl 1-thio-β-D-glucopyranoside (3).** Compound **14** (40 mg, 0.11 mmol) was dissolved in dry MeOH (2 mL), and Pd/C (15 mg) was added. The flask was purged with N<sub>2</sub> and exchanged with H<sub>2</sub>, after three times of which the solution was stirred vigorously at RT for 1.5 h. The reaction mixture was subsequently filtered through Celite and the solvent evaporated to give the amino product. To a solution of the crude amino product in dry DMF (2 mL) was added compound **10** (37 mg, 0.11 mmol). The reaction mixture was stirred in the dark at RT for 2 h under nitrogen atmosphere. Then the solvent was evaporated under reduced pressure, and the crude product was purified by flash column chromatography using solvent system DCM/EtOH (15:1 v/v), yielding 51 mg of compound **3** (86 %). [α]<sub>D</sub> = -24 (c 2.6, MeOH); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  2.91 (m, 1 H, SCH<sub>2</sub>), 2.96 (m, 1 H, SCH<sub>2</sub>), 3.29 (at, 1 H, *J* = 9.8 and 9.2 Hz, H-2), 3.50–3.36 (m, 3 H, H-3, H-4, H-5), 3.62 (t, 2 H, *J* = 5.1 Hz, CH<sub>2</sub>NHCO), 3.81–3.65 (m, 9 H, 4 × CH<sub>2</sub>O, H-6a), 3.89 (dd, 1 H, *J* = 1.9 and 12.3 Hz, H-6b), 4.54 (d, 1 H, *J* = 9.8 Hz, H-1). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  29.14, 39.69, 60.84, 68.47, 69.42, 69.43, 69.47, 70.05, 72.24, 77.13, 79.86, 85.42, 110.13, 122.43, 139.39, 141.38, 142.55, 144.46, 160.71. HRMS: calcd. for C<sub>19</sub>H<sub>24</sub>F<sub>4</sub>N<sub>4</sub>O<sub>8</sub>S [M + Na]<sup>+</sup> 567.1143; observed 567.1129.

**2-[2-(2-Azidoethoxy)ethoxy]ethyl 4-***O*-**β**-**D**-galactopyranosyl-1-thio-**β**-**D**-glucopyranoside (**15**). β-thiolactose sodium salt **12** (92 mg, 0.24 mmol) and compound **13** (57 mg, 0.20 mmol) in dry DMF (4 mL) were stirred at 50 °C under nitrogen for 1 h. After completion of the reaction, the solvent was evaporated. Then the crude product was purified by flash column chromatography using solvent system DCM/MeOH (7:1 v/v), giving 84 mg of compound **15** (82 %). [ $\alpha$ ]<sub>D</sub> = -19 (c 1.4, MeOH); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>): δ 2.93 (m, 1 H, SCH<sub>2</sub>), 2.99 (m, 1 H, SCH<sub>2</sub>), 3.38 (at, 1 H, *J* = 9.8 and 9.2 Hz, H-2), 3.85–3.47 (m, 19 H, 4 × CH<sub>2</sub>O, CH<sub>2</sub>N<sub>3</sub>, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6a, H-6a'), 4.01–3.90 (m, 2 H, H-6b, H-6b'), 4.46 (d, 1 H, *J* = 7.9 Hz, H-1'), 4.60 (d, 1 H, *J* = 9.8 Hz, H-1). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>): δ 29.10, 50.15, 60.21, 61.03, 68.56, 69.23, 69.40, 69.50, 70.15, 72.02, 72.54, 75.36, 75.73, 78.15, 78.67, 85.28, 102.88. HRMS: calcd. for C<sub>18</sub>H<sub>33</sub>N<sub>3</sub>O<sub>12</sub>S [M + Na]<sup>+</sup> 538.1677; observed 538.1661.

**2-(2-(4-Azido-2,3,5,6-tetrafluorobenzamido)ethoxy)ethoxy)ethyl 4-***O*-β-D-galactopyranosyl-1-thio-β-D-glucopyranoside (5). Compound 15 (67 mg, 0.13 mmol) was dissolved in dry MeOH (2 mL), and Pd/C (20 mg) was added. The flask was purged with N<sub>2</sub> and exchanged with H<sub>2</sub>, after three times of which the solution was stirred vigorously at RT for 2 h. The reaction mixture was subsequently filtered through Celite and the solvent evaporated to give the amino product. To a solution of the crude amino product in dry DMF (2 mL) was added compound 10 (43 mg, 0.13 mmol). The reaction mixture was stirred in the dark at RT for 2 h under nitrogen atmosphere. Then the solvent was evaporated under reduced pressure, and the crude product was purified by flash column chromatography using solvent system DCM/EtOH (12:1 v/v), yielding 71 mg of compound 5 (77 %). [α]<sub>D</sub> = -22 (c 1.0, MeOH); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  2.92 (m, 1 H, SCH<sub>2</sub>), 2.97 (m, 1 H, SCH<sub>2</sub>), 3.35 (at, 1 H, *J* = 9.8 and 9.2 Hz, H-2), 3.84–3.52 (m, 19 H, 4 × CH<sub>2</sub>O, CH<sub>2</sub>NHCO, H-2', H-3, H-3', H-4, H-4', H-5, H-5', H-6a, H-6a'), 3.99-3.91 (m, 2 H, H-6b, H-6b'), 4.45 (d, 1 H, *J* = 7.9 Hz, H-1'), 4.57 (d, 1 H, *J* = 9.8 Hz, H-1). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  29.16, 39.73, 60.20, 61.02, 68.50, 68.56, 68.99, 69.46, 70.06, 70.95, 72.00, 72.55, 75.37, 75.74, 78.20, 78.68, 85.31, 102.91, 110.18, 122.43, 139.42, 141.40, 142,60, 144.56, 160.68. HRMS: calcd. for C<sub>25</sub>H<sub>34</sub>F<sub>4</sub>N<sub>4</sub>O<sub>13</sub>S [M + Na]<sup>+</sup> 729.1671; observed 729.1654.

Isothermal titration calorimetry (ITC). Isothermal titration microcalorimetry was performed in PBS buffer (pH 7.4), containing MnCl<sub>2</sub> (1 mM) and CaCl<sub>2</sub> (1 mM). The concentration of Con A was 80  $\mu$ M, and that of PFPA-mannose was 5 mM. The concentration of PNA was 10.6  $\mu$ M, and that of PFPA-lactose was 1 mM. In each individual experiment, the carbohydrate solution, ~40  $\mu$ L total, was injected through the computer-controlled 40- $\mu$ L micro-syringe at an interval of 4 min into the lectin solution (cell volume = 200  $\mu$ L), which is prepared in the same PBS buffer as for the PFPA-carbohydrates, while stirring at 350 rpm. A total of 20 injections were performed. Control experiments were carried out by making identical injections of carbohydrates into a cell containing the buffer without the protein. This nonspecific heat was then subtracted from the total heat generated from the specific carbohydrate–protein interactions. The experimental data were processed and fitted to a theoretical titration curve, using a standard one-site binding model with  $K_a$  (the association constant in M<sup>-1</sup>) and  $\Delta H$ (the enthalpy change in kcal/mol) as adjustable parameters.

Surface plasmon resonance (SPR). Previously reported procedures of glycochip fabrication were followed in the study [41]. Briefly, gold-coated glass substrates were initially functionalized with a PFPA-disulfide derivative, after which PEOX was attached to the substrates through UV-irradiation. Solutions of PFPA-derivatized carbohydrates were subsequently printed onto the PEOX-coated gold substrates, and attached by UV-irradiation. SPR measurements were performed using an SPR imager equipment, operating at ambient temperature. The flow rate of the solutions throughout the experiments was maintained at 100  $\mu$ L/min. The glycochips were first equilibrated with PBS containing 0.1 % (w/v) Tween 20 (running buffer), then treated with 0.2 % (w/v) BSA in PBS containing 0.1 % (w/v) Tween 20 in order to prevent nonspecific binding, and subsequently re-equilibrated with running buffer. All protein binding cycles were initiated by equilibrating the glycochips with running buffer, followed by the specific protein solution, and finally re-equilibration with running buffer. After protein binding, the glycochips were regenerated with urea (8 M), and then re-equilibrated with running buffer.

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## REFERENCES

- 1. H. Driguez. Top. Curr. Chem. 187, 85 (1997).
- 2. D. Horton, J. D. Wander. In *The Carbohydrates: Chemistry and Biochemistry*, pp. 799–842, Academic Press, New York (1980).
- 3. L. A. Marcaurelle, C. R. Bertozzi. Chem.-Eur. J. 5, 1384 (1999).
- 4. K. Pachamuthu, R. R. Schmidt. Chem. Rev. 106, 160 (2005).
- 5. Z. Witczak, J. Culhane. Appl. Microbiol. Biotechnol. 69, 237 (2005).
- 6. X. Qian, K. Sujino, M. M. Palcic, R. M. Ratcliffe. J. Carbohydr. Chem. 21, 911 (2002).
- 7. J. Pazur, B. Liu, N. Li, Y. Lee. J. Protein Chem. 9, 143 (1990).
- M. Singer, M. Lopez, L. F. Bornaghi, A. Innocenti, D. Vullo, C. T. Supuran, S.-A. Poulsen. Bioorg. Med. Chem. Lett. 19, 2273 (2009).
- J. L. C. Wright. In *Biochemistry and Genetic Regulation of Commercially Important Antibiotics*, L. C. Vining (Ed.), Addison-Wesley, London (1983).
- 10. R. T. Lee, S. Cascio, Y. C. Lee. Anal. Biochem. 95, 260 (1979).
- 11. D. R. Bundle, J. R. Rich, S. Jacques, H. N. Yu, M. Nitz, C.-C. Ling. Angew. Chem., Int. Ed. 44, 7725 (2005).
- 12. U. Nilsson, R. Johansson, G. Magnusson. Chem.-Eur. J. 2, 295 (1996).
- 13. D. A. Thayer, H. N. Yu, M. C. Galan, C.-H. Wong. Angew. Chem., Int. Ed. 44, 4596 (2005).
- 14. H. Driguez. ChemBioChem 2, 311 (2001).
- 15. D. P. Gamblin, E. M. Scanlan, B. G. Davis. Chem. Rev. 109, 131 (2009).
- 16. S. Knapp, E. Darout, B. Amorelli. J. Org. Chem. 71, 1380 (2006).
- 17. D. Horton, D. H. Hutson. In *Advances in Carbohydrate Chemistry*, D. Horton (Ed.), pp. 123–199, Academic Press (1963).
- 18. C. Xue, S. P. Jog, P. Murthy, H. Liu. Biomacromolecules 7, 2470 (2006).
- 19. S. F. M. van Dongen, H.-P. M. de Hoog, R. J. R. W. Peters, M. Nallani, R. J. M. Nolte, J. C. M. van Hest. *Chem. Rev.* **109**, 6212 (2009).
- 20. Y. Chen, G. Chen, M. H. Stenzel. Macromolecules 43, 8109 (2010).
- R. Caraballo, H. Dong, J. P. Ribeiro, J. Jiménez-Barbero, O. Ramström. Angew. Chem., Int. Ed. 49, 589 (2010).
- 22. Z. Pei, R. Larsson, T. Aastrup, H. Anderson, J.-M. Lehn, O. Ramström. *Biosens. Bioelectron.* 22, 42 (2006).
- 23. R. Caraballo, M. Sakulsombat, O. Ramström. Chem. Commun. 46, 8469 (2010).
- 24. S. André, Z. Pei, H.-C. Siebert, O. Ramström, H.-J. Gabius. Bioorg. Med. Chem. 14, 6314 (2006).
- 25. Y. M. Chabre, R. Roy. In *Advances in Carbohydrate Chemistry and Biochemistry*, D. Horton (Ed.), pp. 165–393, Elsevier (2010).
- 26. A. Dondoni, A. Marra. Chem. Rev. 110, 4949 (2010).
- A. Nelson, J. M. Belitsky, S. Vidal, C. S. Joiner, L. G. Baum, J. F. Stoddart. J. Am. Chem. Soc. 126, 11914 (2004).
- 28. A. A. Aksenova, Y. L. Sebyakin, A. F. Mironov. Russ. J. Bioorg. Chem. 27, 124 (2001).
- 29. S. Hirohara, M. Obata, H. Alitomo, K. Sharyo, T. Ando, S. Yano, M. Tanihara. *Bioconjugate Chem.* 20, 944 (2009).
- 30. J. Fernandez, C. Mellet, J. Defaye. J. Inclusion Phenom. Macrocyclic Chem. 56, 149 (2006).

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- 31. M. Marradi, M. Martín-Lomas, S. Penadés. In Advances in Carbohydrate Chemistry and Biochemistry, D. Horton (Ed.), pp. 211–290, Elsevier (2010).
- 32. M. Yu, Y. Yang, R. Han, Q. Zheng, L. Wang, Y. Hong, Z. Li, Y. Sha. Langmuir 26, 8534 (2010).
- F. Nicotra, L. Cipolla, F. Peri, B. L. Ferla, C. Redaelli. In Advances in Carbohydrate Chemistry and Biochemistry, D. Horton (Ed.), pp. 353–398, Elsevier (2007).
- 34. S. Josse, J. Le Gal, M. Pipelier, J. Cléophax, A. Olesker, J.-P. Pradère, D. Dubreuil. *Tetrahedron Lett.* **43**, 237 (2002).
- 35. G.-J. Boons, T. Stauch. Synlett 906 (1996).
- 36. D. T. Connolly, S. Roseman, Y. C. Lee. Carbohydr. Res. 87, 227 (1980).
- 37. M.-O. Contour, J. Defaye, M. Little, E. Wong. Carbohydr. Res. 193, 283 (1989).
- 38. K. P. R. Kartha, P. Cura, M. Aloui, S. K. Readman, T. J. Rutherford, R. A. Field. *Tetrahedron: Asymmetry* **11**, 581 (2000).
- 39. A. Aguirre-Valderrama, J. A. Dobado. J. Carbohydr. Chem. 25, 557 (2006).
- 40. L. Bhattacharyya, C. F. Brewer. Eur. J. Biochem. 176, 207 (1988).
- 41. L. Deng, O. Norberg, S. Uppalapati, M. Yan, O. Ramström. Org. Biomol. Chem. 9, 3188 (2011).
- 42. M. Köhn, J. M. Benito, C. Ortiz Mellet, T. K. Lindhorst, J. M. García Fernández. *ChemBioChem* 5, 771 (2004).
- 43. A. García-Herrero, E. Montero, J. L. Muñoz, J. F. Espinosa, A. Vián, J. L. García, J. L. Asensio, F. J. Cañada, J. Jiménez-Barbero. *J. Am. Chem. Soc.* **124**, 4804 (2002).
- 44. T. K. Dam, C. F. Brewer. Chem. Rev. 102, 387 (2002).
- 45. R. N. Knibbs, N. Agrwal, J. L. Wang, I. J. Goldstein. J. Biol. Chem. 268, 14940 (1993).
- 46. M. Cho, R. D. Cummings. Biochemistry 35, 13081 (1996).
- 47. B. D. Polizzotti, R. Maheshwari, J. Vinkenborg, K. L. Kiick. Macromolecules 40, 7103 (2007).
- 48. J. Gervay, K. D. McReynolds. Curr. Med. Chem. 6, 129 (1999).
- 49. X. Wang, O. Ramström, M. Yan. Anal. Chem. 82, 9082 (2010).
- 50. J. Tejler, H. Leffler, U. J. Nilsson. Bioorg. Med. Chem. Lett. 15, 2343 (2005).
- 51. M. A. Johnson, B. M. Pinto. Carbohydr. Res. 339, 907 (2004).
- 52. A. Tyagi, X. Wang, L. Deng, O. Ramström, M. Yan. Biosens. Bioelectron. 26, 344 (2010).
- 53. O. Norberg, L. Deng, T. Aastrup, M. Yan, O. Ramström. Anal. Chem. 83, 1000 (2011).
- 54. O. Norberg, L. Deng, M. Yan, O. Ramström. Bioconjugate Chem. 20, 2364 (2009).
- 55. Z. Pei, H. Yu, M. Theurer, A. Waldén, P. Nilsson, M. Yan, O. Ramström. *ChemBioChem* 8, 166 (2007).
- C. Madwar, W. Chu Kwan, L. Deng, O. Ramström, R. Schmidt, S. Zou, L. A. Cuccia. *Langmuir* 26, 16677 (2010).
- 57. K. Larsen, M. B. Thygesen, F. Guillaumie, W. G. T. Willats, K. J. Jensen. *Carbohydr. Res.* 341, 1209 (2006).
- 58. Y. C. Lee, R. T. Lee. Acc. Chem. Res. 28, 321 (1995).
- 59. J. J. Lundquist, E. J. Toone. Chem. Rev. 102, 555 (2002).
- 60. M. Cohen, A. Varki. OMICS 14, 455 (2010).
- 61. L. Liu, M. Yan. Angew. Chem., Int. Ed. 45, 6207 (2006).
- 62. L.-H. Liu, M. M. Lerner, M. Yan. Nano Lett. 10, 3754 (2010).
- 63. L.-H. Liu, M. Yan. Acc. Chem. Res. 43, 1434 (2010).
- X. Wang, E. Matei, L. Deng, O. Ramström, A. M. Gronenborn, M. Yan. Chem. Commun. 47, 8620 (2011).
- 65. H. S. Isbell, W. Pigman. In *Advances in Carbohydrate Chemistry*, D. Horton (Ed.), pp. 13–65, Elsevier (1970).
- 66. R. Caraballo, L. Deng, L. Amorim, T. Brinck, O. Ramström. J. Org. Chem. 75, 6115 (2010).
- 67. H. N. Yu, C.-C. Ling, D. R. Bundle. J. Chem. Soc., Perkin Trans. 1 832 (2001).
- 68. D. Crich, H. Li. J. Org. Chem. 65, 801 (2000).
- 69. T. Mukhopadhyay, D. Seebach. Helv. Chim. Acta 65, 385 (1982).

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- 70. L. Encinas, J. L. Chiara. Eur. J. Org. Chem. 2163 (2009).
- 71. V. G. S. Box. Heterocycles 31, 1157 (1990).
- 72. V. G. S. Box. Heterocycles 19, 1939 (1982).
- 73. R. R. Schmidt, J. Michel. Tetrahedron Lett. 25, 821 (1984).
- 74. G. J. L. Bernardes, E. J. Grayson, S. Thompson, J. M. Chalker, J. C. Errey, F. El Oualid, T. D. W. Claridge, B. G. Davis. *Angew. Chem., Int. Ed.* 47, 2244 (2008).
- 75. F. P. Schwarz, K. D. Puri, R. G. Bhat, A. Surolia. J. Biol. Chem. 268, 7668 (1993).
- 76. G. B. Reddy, V. R. Srinivas, N. Ahmad, A. Surolia. J. Biol. Chem. 274, 4500 (1999).
- 77. H. Wang, L. Li, Q. Tong, M. Yan. ACS Appl. Mater. Interfaces 3, 3463 (2011).