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ORIGINAL ARTICLE

The versatility of *N*-alkyl-methoxyamine bi-functional linkers for the preparation of glycoconjugates

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Abstract The application of *N*-glycosyl-*N*-alkyl-methoxyamine bi-functional linkers for the synthesis of a variety of glycoconjugates is described. The linker contains a specific functional group, such as an amine, azide, thiol, or carboxylic acid, which can be used for conjugation methodologies that include amide ligation, sulfonylation, copper-mediated Huisgen cycloaddition or thiol-maleimide coupling. In this way, glycoconjugates equipped with biotin, a fluorescent reporter, or a protein were efficiently synthesised, thus demonstrating the versatility of this type of oxyamine linker for the construction of glycoconjugate probes.

Keywords Oxyamine · Glycoconjugate · Linker · Oligosaccharide · Probe

Introduction

A staggering number of proteins and lipids are glycosylated. [1, 2] This has spurred interest in studying the biological activities of glycoconjugates and the need to find efficient strategies for the conjugation of carbohydrates to molecules such as fluorescent reporters [3, 4], biotin [5], dendrimers [6], microarrays[1] and proteins [9, 10]. While many complex glycans have been prepared *de novo*, with the functionalised linkers being incorporated during the synthesis of a protected glycan,[2] the use of carbohydrates from natural sources requires conjugation strategies that are compatible with unprotected sugars. To this end, a linker is often attached to the free glycan and subsequently reacted, via the linker's handle, with the molecule of choice. Strategies that employ this concept include Kochetkov amination [14], to prepare glycosylamines that can be further functionalised, the use of oximes/hydrazides or oxyamines [15], and reductive amination,[3] although the last methodology affects the structural integrity of the reducing end carbohydrate.

Of these general ligation strategies, we were interested in exploring and extending the scope of oxyamine methodology. There are numerous elegant examples of the use of oxyamines in the synthesis of glycoconjugates, as reported by the groups of Peri [18], Boons [19], Blixt [20], Carrasco [21], Nitz [22] and Jensen [23], whereby *N*-methyloxyamine linkers of 'Type A' (Fig. 1) were described. In some instances however, the yield and efficiency of the linker preparation could be improved, and moreover, a general strategy that would allow for the installation of the functional group of choice on the linker terminus would be advantageous. Accordingly, we recently developed a highly efficient strategy for the synthesis of 'Type B' oxyamine linkers that contained azide (**1a**), amine (**1b** and **1c**), thiol (**1d** and **1e**) and carboxy (**1f** and **1 g**) bioorthogonal handles (Type B, Fig. 1) [24]. The linkers showed good hydrolytic stability, which was comparable to

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[1] For example: [7, 8]
[2] For example see: [11–13].
[3] For example, see: [16, 17]

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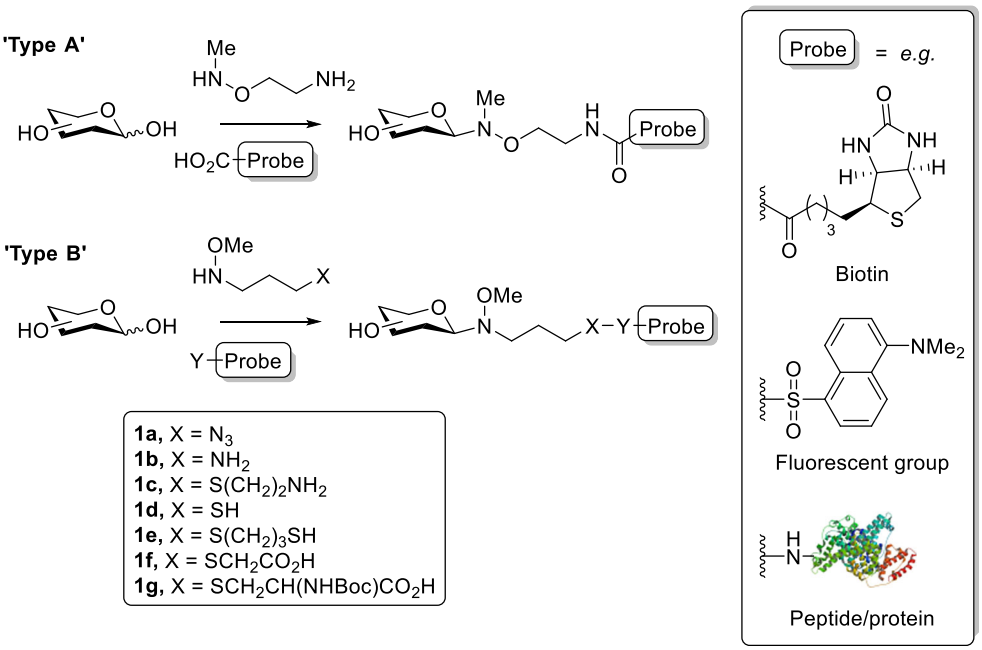


Fig. 1 'Type A' *N*-methyloxyamine and 'Type B' *O*-methyloxyamine linkers **1a–g**, and representative examples of probes that can be incorporated using these linkers

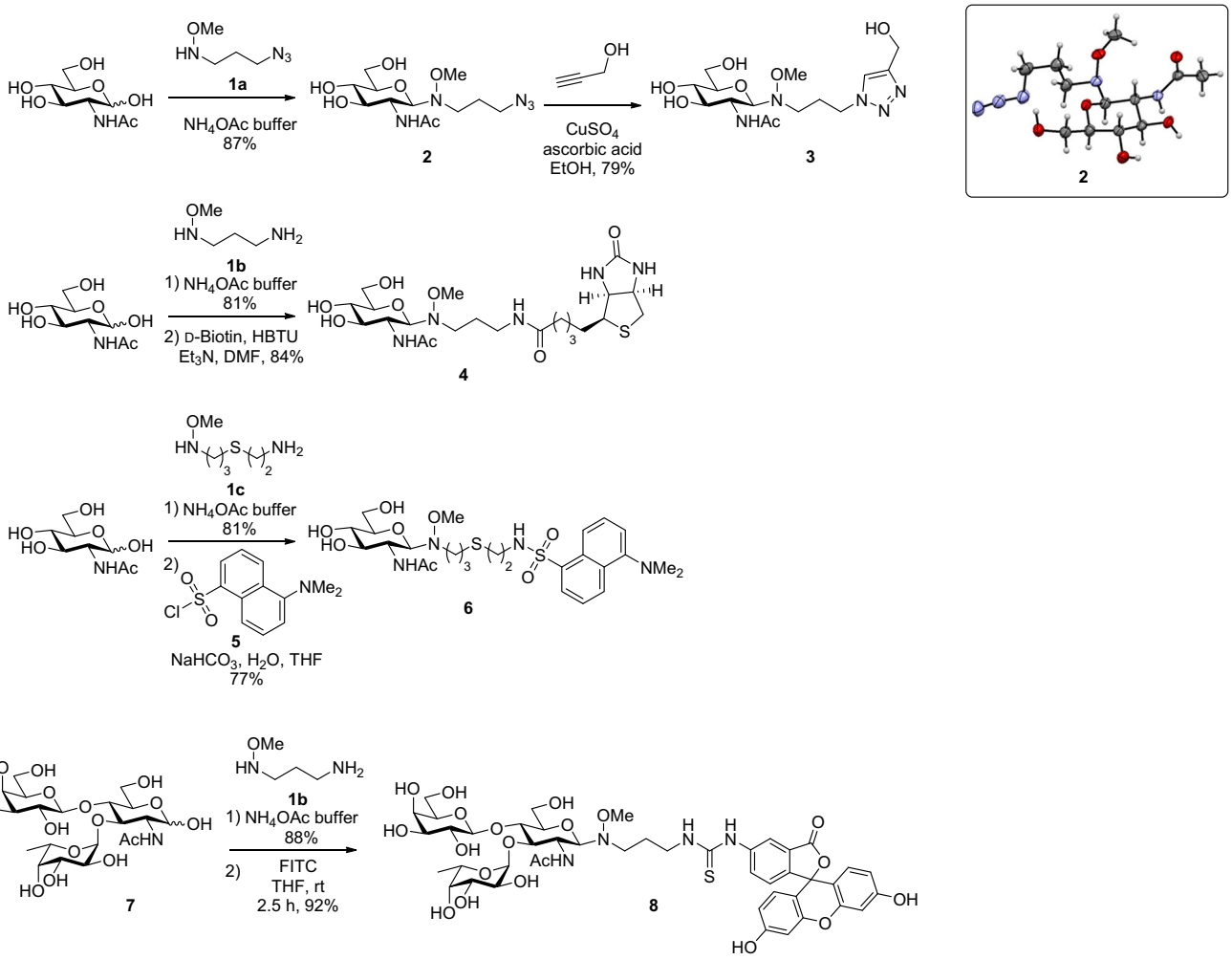
the 'Type A' glycoconjugates [25], however the scope and limitation of these 'Type B' oxyamine linkers for the preparation of glycoconjugates was not thoroughly explored. To this end, we herein provide insight into the application of the different *N*-oxyamine linkers, with a focus on the ability to conjugate the linkers to other molecules using chemoselective ligation methodologies that include amide formation [26, 27], sulfonylation [28], copper-mediated cycloaddition [29] and thiol-maleimide conjugation [30]. In this way, glycoconjugates equipped with biotin, a fluorescent reporter, or a protein were efficiently synthesised, thus demonstrating the versatility of the 'Type B' oxyamine linkers for the construction of glycoconjugate probes.

Results and discussion

To synthesise the 'Type B' oxyamine linkers, acrolein was treated with either a functionalised thiol or with NaN_3, and the resulting Michael adduct was condensed with methoxyamine and then reduced with $NaCNBH_3$ to yield the oxyamines **1a**, **1c**, **1e–g** in a three-step one-pot synthesis, as previously reported [24]. The amine functionalised oxyamine **1b** containing the shorter linker was prepared via Staudinger reduction of azide **1a**, while the synthesis of oxyamine **1d** was achieved using thioacetate as the nucleophile and involved the deacetylation of the intermediate imine prior to the reduction step to avoid *S*-to-*N* acyl migration [24]. In this way, the oxyamine linkers were synthesised in 3–4 steps and in high yield (51–96%).

To explore the versatility of the oxyamine linkers, we first conjugated azide functionalised linker **1a** to GlcNAc to give neoglycoside **2** (Scheme 1). Crystallisation of **2** from MeOH/Et_2O then provided conclusive evidence for the formation of the β-pyranose configuration [31], which supported our earlier NMR analysis that revealed a $J_{1',2'}$ of 9.8 Hz and an HBMC between H-1′ and C-5′ [24]. The application of the azide functionalised oxyamine linker **1a** was then demonstrated by reacting neoglycoside **2** with propargyl alcohol under copper-mediated azide-alkyne cycloaddition conditions [29] to give triazole **3** in 79% yield. Here, propargyl alcohol was used as a model substrate to demonstrate proof-of-principle for the orthogonality of oxyamine linker **1a**. Given the ease of this copper-mediated 'click' reaction, it is envisioned that oxyamine linker **1a** will thus serve as a useful tool for the conjugation of glycans to other functionalised alkynes.

Next, we sought to prepare different classes of glycoconjugates using the amine-functionalised oxyamine linkers **1b** and **1c**. First, *N*-acetylglucosamine was coupled to the short-chain bi-functional amine linker **1b**, followed by conjugation of the resulting neoglycoside to D-biotin under standard peptide coupling conditions [26, 27]. The conjugation reaction with D-biotin proceeded smoothly under the mediation of *N,N,N′,N′*-tetramethyl-*O*-(*1H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) to give the biotinylated glycoconjugate **4** in 71% yield. The applications of such biotinylated glycans include streptavidin/biotin affinity chromatography and ELISA using streptavidin functionalised microarrays [32, 33]. In addition to the use of the amine-functionalised linkers for amide bond formation, these linkers can also be used for the synthesis of sulfonamides, as demonstrated by the synthesis of a dansylated fluorescent glycoconjugate. Here, GlcNAc was conjugated to oxyamine **1c** and the resulting glycoconjugate reacted with dansyl chloride (**5**) under basic conditions to give sulfonamide **6** in 77%

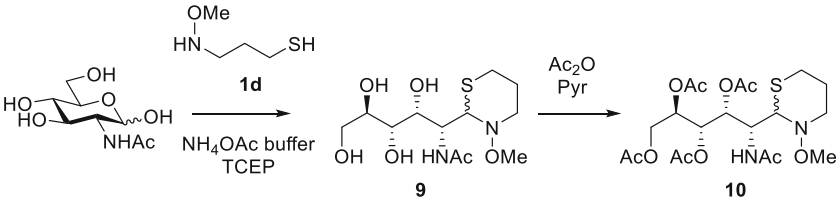


Scheme 1 Conjugation of azide- and amine-functionalised neoglycosides

yield. The synthesis of fluorescently labelled glycans was further demonstrated by the preparation of a fluorescein isothiocyanate (FITC) labelled Lewis^X glycan **8**. Lewis^X is a ligand for several immune receptors, including DC-SIGN [34], and is involved in numerous biological processes including cancer metathesis and viral infection.[4] To synthesise a Lewis^X-chemical probe, Lewis^X (**7**) [38] was coupled to bi-functional amine linker **1b** and treated with FITC under mild reaction conditions to give the fluorescein derivative **8** in excellent (92%) yield. The ease of this synthesis illustrates the power of the oxyamine-linker methodology for the preparation of more complex chemical probes. Fluorescent glycoconjugates have a number of applications, particularly in the study of glycan uptake and cellular trafficking by confocal microscopy or flow cytometry. As the physical-chemical properties of the glycoconjugates can affect cellular trafficking [3, 4], it is advantageous to have the choice of either oxyamine linker **1b** or **1c** for the attachment of the fluorophore.

Glycans containing thiol-functionalised linkers have a number of applications that include use in gold-plated microarrays [39] or as nucleophiles for Michael-addition [40]. Accordingly, we next attempted to conjugate the thiol-functionalised oxyamine **1d** to GlcNAc to afford the corresponding thiol-functionalised glycoconjugate. Unfortunately, this reaction did not yield the target glycoside but instead appeared to give a diastereomeric mixture of thiazinanes **9**, which presumably forms through a 6-endo-trig cyclization of an intermediate oxyimminium ion with the pendant thiol group (Scheme 2). Analysis by HRMS (*m/z* for $[C_{12}H_{25}N_2O_6S]^+$ calc. 325.1428, obs. 325.1445), as well as TLC [R_f = 0.32; 10:2:2:1, CH_2Cl_2:EtOH:MeOH:NH_3 (aq, 33%), v:v:v:v], clearly showed the formation of a new compound, however, as ^1H and ^{13}C NMR data for **9** suffered from significant peak broadening, even when using different solvents [D_2O, CD_3OD, pyridine-d5 or DMSO-d6] and varying temperatures (rt – 60 °C). Accordingly, the mixture of thiazinanes was acetylated (→**10**), and NMR data was obtained which allowed for the conclusive assignment of the structures of the two thiazine diastereoisomers (see SI for further details).

[4] For some representative papers see: [35–37]



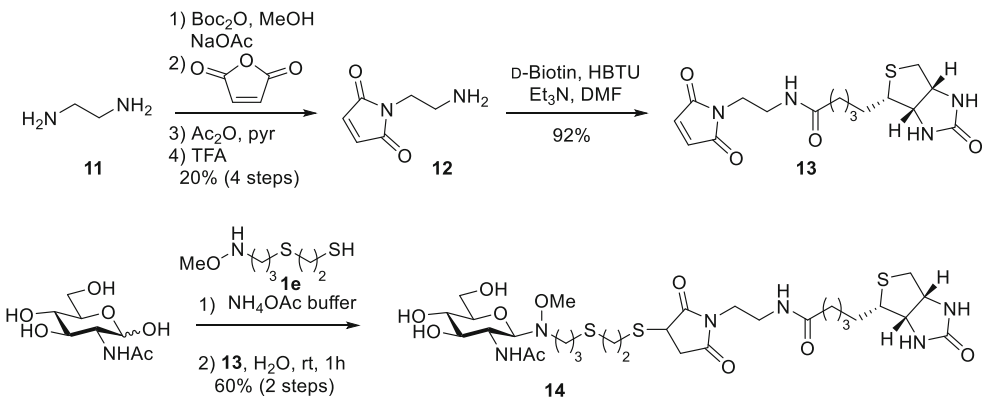
Scheme 2 The formation of *N*-methoxy-thiazinanes **7** which, after per-acetylation (→**10**), could be confirmed by NMR analysis

To prevent the formation of thiazinanes, the thiol functionalised linker **1e**, which contains an extended linker chain, was then used for the formation of glycoconjugates via maleimide-mediated conjugation (Scheme 3). To this end, the maleimide acceptor was prepared via Boc protection of ethylenediamine (**11**), condensation of the remaining amine with maleic anhydride to give a carboxy-amine that underwent ring-closure to form the maleimide, and Boc deprotection to yield functionalised amine **12** [41]. Conjugation of **12** to D-biotin via an HBTU-mediated peptide coupling then gave biotin-functionalised maleimide **13** in 92% yield. The conjugation of thiol-functionalised oxyamine **1e** with GlcNAc occurred smoothly and following coupling with biotin-functionalised maleimide **13** in water at room temperature gave the target glycoside **14** in good (60%) yield over the two steps after purification by size exclusion chromatography. An advantage of this approach for the formation of biotinylated glycoconjugates is the ease of the thiol-maleimide conjugation, as mild conditions are preferable when using small quantities of glycans. Accordingly, we envisioned this protocol to have wide application for the conjugation of glycans from both synthetic and biological sources.

Finally, the versatility of the thiol-functionalised oxyamine linker was further demonstrated by the formation of a glycoprotein (Fig. 2a). For our study, the commercially available bifunctional cross-linker succinimidyl-4-[*N*-maleimidomethyl]cyclohexane-1-carboxylate (SMCC) **15** was used for the maleimide functionalisation of the lysine residues in Bovine Serum Albumin (BSA). The functionalised protein was then coupled to thiol-functionalised neoglycoside **16** to give glycoprotein **17**. Literature studies for the conjugation of glycans to proteins are often limited in experimental detail relating to glycan loading. Thus, we investigated a variety of reaction conditions and monitored the progress of the reactions using MALDI-TOF analysis to determine the average molecular weight of the protein after SMCC conjugation and subsequent glycosylation. From these studies it was determined that the reactions proceeded more efficiently when being performed in 10% MeCN in PBS, so as to prevent denaturisation of the protein while fully dissolving the SMCC cross-linker, and that optimal glycan coupling to the SMCC-BSA conjugate occurred after 60 min. Further time course and concentration studies then concluded that the most efficient glycoconjugation was achieved when BSA (10 mg/mL) was reacted with the SMCC cross linker **15** (180 equiv.) for 60 min before the addition of excess neoglycoside **16** (360 equiv.), and incubation of the glycoprotein at room temperature for a further 60 min (Fig. 2b). This led to an average of nine glycans per BSA.

Conclusions

In conclusion, we have demonstrated the versatility of the Type B oxyamine linkers by preparing several glycoconjugates. The formation of these glycoconjugates occurred in good yield through the use of chemical ligation reactions that include amide formation, sulfonylation, copper-mediated azide-alkyne cycloaddition and thiol-maleimide conjugations. In this way, representative biotinylated and fluorescent reporter groups were conjugated to GlcNAc, the more complex (FITC) labelled LewisX chemical probe was prepared, and a glycosylated protein was also synthesised using an optimised protein conjugation protocol. Given the ease and high-yielding synthesis of the 'Type B'



Scheme 3 Application of thiol oxyamine linker **1e** to form a biotinylated glycoconjugate

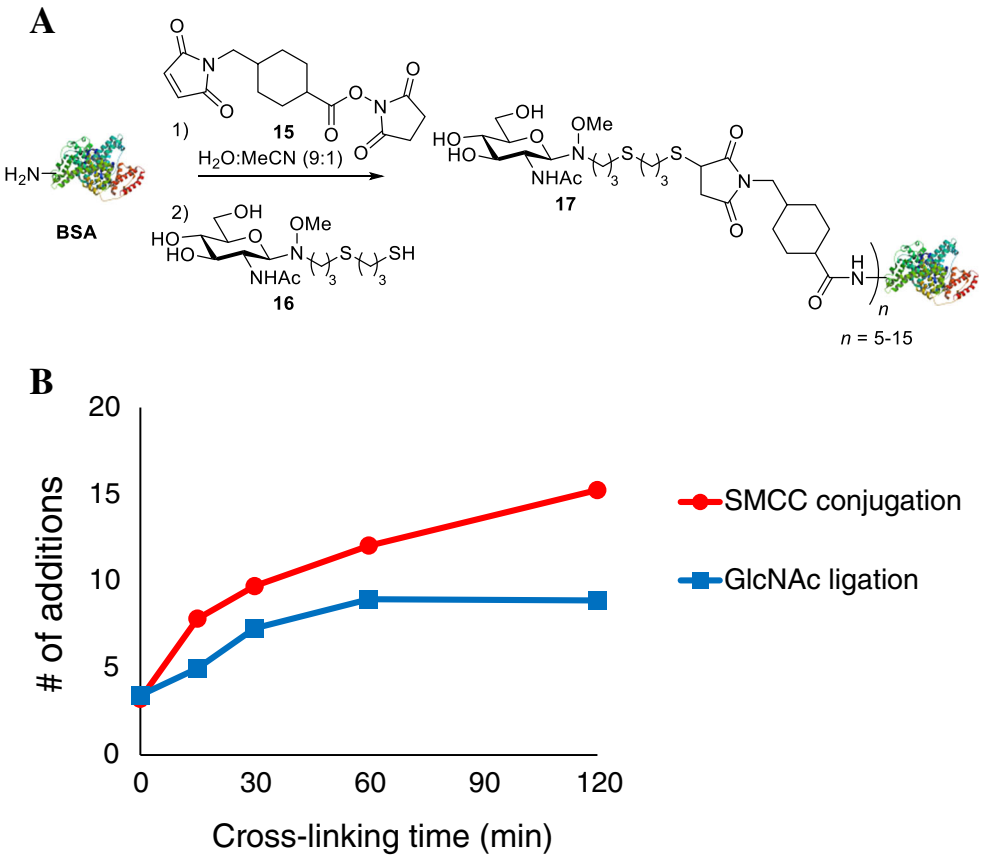


Fig. 2 **a** The synthesis of BSA-glycoprotein **17**. **b** SMCC conjugation and glycan ligation was monitored via MALDI-TOF analysis. Conjugation of cross-linker SMCC **15** was monitored at each time point. At each time point, an aliquot of the reaction mixture was taken, glycoside **16** (360 equiv.) added and incubated for 60 min, and then glycan loading determined

oxyamine linkers and our proof-of-concept studies for the formation of several classes of glycoconjugates, we envision that these 'Type B' linkers will thus find wide application in the synthesis of many other glycoconjugates using glycans from both synthetic and natural sources.

Experimental

General procedure for the chemical ligation of methoxyamine linkers with carbohydrates To a solution of carbohydrate (0.15 mmol) in an AcOH/NH_4OAc buffer (1.5 mL, 2.0 M, pH 4.5, freshly prepared), oxyamine linker (1.5 mmol, 10 equiv., HCl salt) [25] was added and the reaction mixture was stirred at rt. for 24 h. The crude mixture was then directly loaded onto a size exclusion column (Bio-Gel P-2, 1200 × 18 mm) and eluted with a 0.1 M aq. NH_4HCO_3 solution. Lyophilisation of the product fractions afforded the neoglycoside. Alternatively, the neoglycoside can be purified by directly loading the crude reaction mixture onto a reverse phase column (C_{18}) and/or by purification by silica gel column chromatography to afford the neoglycoside**.**

***N*-(2-Acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-(3-azidopropyl)-*O*-methylhydroxylamine (2)** *N*-Acetyl-glucosamine (34 mg, 0.15 mmol) and 3-azido-1-methoxyaminopropane hydrochloride (**1a**) (195 mg, 1.50 mmol) were reacted in an AcOH/NH_4OAc buffer (1.5 mL, 2.0 M, pH 4.5, freshly prepared) at rt. for 24 h. Purification by reverse phase chromatography (C_{18}, H_2O/MeOH, 100/0 → 60/40, *v*/v) afforded neoglycoside **2** (44.6 mg, 0.13 mmol, 87%) as a white solid. R_f = 0.64 (silica, CH_2Cl_2/EtOH/MeOH/NH_3 (aq. 35%), 10/2/2/1, *v*/v/*v*/v); $\alpha_D^{20.3}$ = −19 (c = 0.1, MeOH); IR (film) 3369, 3296, 3253, 3003, 2953, 2943, 2889, 2099, 2067, 1735, 1647, 1605, 1575, 1567, 1487, 1446, 1419, 1377, 1349, 1308, 1253, 1079, 1020, 828 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.33 (d,1H, $J_{1',2'}$ = 9.8 Hz, H-1′), 3.89 (dd, 1H, $J_{1',2'}$ = $J_{2',3'}$ = 9.6 Hz, H-2′), 3.88 (dd, 1H, $J_{5',6'}$ = 1.5 Hz, $J_{6a',6b'}$ = 12.3 Hz, H-6a′), 3.73 (dd, 1H, $J_{5',6'}$ = 5.3 Hz, $J_{6a',6b'}$ = 12.3 Hz, H-6b′), 3.51 (dd, 1H, $J_{2',3'}$ = $J_{3',4'}$ = 8.7 Hz, H-3′), 3.50 (s, 3H, OCH_3) 3.46–3.34 (m, 4H, H-4′, H-5′, CH_2–3), 3.09–2.98 (m, 2H, CH_2–1), 2.04 (s, 3H, CH_3 Ac), 1.88–1.74 (m, 2H, CH_2–2); ^{13}C NMR (125 MHz, D_2O) 173.9 (C = O), 90.0 (C-1′), 77.4 (C-5′), 75.5 (C-3′), 69.6 (C-4′), 61.0 (OCH_3), 60.8 (C-6′), 52.3 (C-2′), 49.0 (C-3), 48.7 (C-1), 25.7 (C-2), 22.1 (CH_3 Ac); HRMS(ESI) *m/z* for $[C_{12}H_{24}N_5O_6]^+$ calcd.: 334.1721, obsd.: 334.1719.

(1-(3-(Methoxy(2-acetamido-2-deoxy-β-D-glucosyl)amino) propyl)-1*H*-1,2,3-triazol-4-yl)methanol (3) To a solution of

azide **2** (11.8 mg, 35 μmol) and propargyl alcohol (0.1 mL) in ethanol (1 mL), a solution of L-ascorbic acid (6.2 mg, 35 μmol) and $CuSO_4$ (1 mg) were added and the reaction mixture was stirred for 3 d at rt. The crude mixture was filtered, the residue washed with H_2O (2 mL), and the combined solvents concentrated in vacuo. Purification by reverse phase chromatography (C_{18}, H_2O/MeOH, 100/0 → 90/10, *v*/v) yielded triazole **3** (10.7 mg, 79%) as a colourless oil. R_f = 0.53 (silica, CH_2Cl_2/EtOH/MeOH/NH_3 (aq. 33%), 5/2/2/1, *v*/v/*v*/v); α_D^{19} = −0.82 (c = 0.1, MeOH); IR (film) 3350, 2934, 2874, 1643, 1555, 1442, 1378, 1319, 1227, 1104, 1054, 1033, 1021 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 7.99 (s, 1H, triazole), 4.73 (s, 2H, C = C-CH_2), 4.58–4.45 (m, 2H, CH_2 H-3), 4.33 (d, 1H, $J_{1',2'}$ = 9.8 Hz, H-1′), 3.91–3.85 (m, 2H, H-2′, H-6a′), 3.73 (dd, 1H, $J_{5',6b'}$ = 5.4 Hz, $J_{6a',6b'}$ = 12.4 Hz, H-6b′), 3.53 (dd, 1H, $J_{2',3'}$ = 8.7 Hz, $J_{3',4'}$ = 9.7 Hz, H-3′), 3.50 (s, 3H, OCH_3), 3.42 (t, 1H, $J_{2',3'} = J_{4',5'}$ = 8.7 Hz, H-4′), 3.40–3.35 (m, 1H, H-5′), 3.03–2.89 (m, 2H, H-1), 2.24–2.10 (m, 2H, H-2), 2.06 (s, 3H, CH_3 Ac); ^{13}C NMR (125 MHz, CD_3OD) δ 173.9 (C = O Ac), 146.7 (Cq C = C), 124.0 (CH = C), 90.1 (C-1″), 77.4 (C-5′′), 75.4 (C-3″), 69.6 (C-4″), 61.2 (OCH_3), 60.8 (C-6″), 54.5 (CH_2 allylic), 52.3 (C-2″), 48.4 (C-1′), 48.2 (C-3′), 27.0 (C-2′), 22.1 (CH_3 Ac); HRMS(ESI) *m/z* calcd. For $[C_{15}H_{28}N_5O_7]^+$: 390.1983, obsd.: 390.1986.

***N*-(3-(methoxy[2-acetamido-2-deoxy-β-D-glucopyranosyl]-amino)propyl)-D-biotinamide (4)** *N*-Acetyl-glucosamine (34.0 mg, 0.15 mmol) and 3-(methoxyamino)propan-1-amine hydrochloride **1b** (211 mg, 1.50 mmol) were reacted in an AcOH/NH_4OAc buffer (1.5 mL, 2.0 M, pH 4.5, freshly prepared) at rt. for 24 h. Purification by size exclusion chromatography (Bio-Gel P-2) afforded *N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-(3-aminopropyl)-*O*-methylhydroxylamine (28.3 mg, 0.092 mmol, 81%). R_f = 0.16 (silica, CH_2Cl_2/EtOH/MeOH/NH_3 (aq. 35%), 5/2/2/1, *v*/v/*v*/v); $\alpha_D^{20.3}$ = −21 (c = 0.1, MeOH); IR (film) 3401, 3375, 3322, 3311, 3291, 3278, 2938, 2885, 2727, 2058, 1651, 1567, 1490, 1459, 1437, 1376, 1315, 1257, 1108, 1023, 633 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.41 (d, 1H, $J_{1',2'}$ = 9.9 Hz, H-1′), 3.92 (dd, 1H, $J_{5',6'}$ = 0.9 Hz, $J_{6a',6b'}$ = 12.5 Hz, H-6a′), 3.85 (dd, 1H, $J_{1',2'} = J_{2',3'}$ = 9.8 Hz, H -2′), 3.75 (dd, 1H, $J_{5',6'}$ = 4.9 Hz, $J_{6a',6b'}$ = 12.5 Hz, H-6b′), 3.55 (dd, 1H, $J_{2',3'} = J_{3',4'}$ = 9.1 Hz, H-3′), 3.52 (s, 3H, OCH_3), 3.45–3.40 (m, 2H, H-4′, H-5′), 3.12–3.01 (m, 4H, CH_2–1, CH_2–3), 2.06 (s, 3H, CH_3 Ac), 1.93 (p, 2H, $J_{1,2} = J_{2,3}$ = 7.1 Hz, CH_2–2); ^{13}C NMR (125 MHz, D_2O) δ 174.1 (C = O Ac), 90.5 (C-1′), 77.4 (C-5′), 75.2 (C-3′), 69.6 (C-4′), 61.1 (OCH_3), 60.8 (C-6′), 52.3 (C-2′), 47.9 (C-1), 37.6 (C-3), 24.5 (C-2), 22.1 (CH_3 Ac); HRMS(ESI) *m/z* calcd. For $[C_{12}H_{26}N_3O_6]^+$: 308.1816, obsd.: 308.1820. To a solution of *N*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-*N*-(3-aminopropyl)-*O*-methylhydroxylamine (12.0 mg, 39.1 μmol) in DMF (0.2 mL), D-biotin (13.0 mg, 53.7 μmol), Et_3N (12 μL) and HBTU (30.0 mg, 79 μmol) were added and the reaction mixture was stirred for 2 h at rt. To the reaction mixture, H_2O (2 mL) was added and the white precipitate was filtered and washed with water (2 × 1 mL). The residue was then purified by C-18 reverse phase chromatography (eluted with H_2O), concentrated in vacuo, followed by silica gel flash column chromatography (CH_2Cl_2/EtOH/MeOH/NH_3(aq. 33%), 15/2/2/1 → 10/2/2/1, *v*/v/*v*/v) to afford glycoconjugate **4** (17.6 mg, 84%) as a colourless oil. R_f = 0.58 (CH_2Cl_2/EtOH/MeOH/NH_3 (aq. 33%), 5/2/2/1, *v*/v/*v*/v); $\alpha_D^{20.0}$ = +3.1 (c = 0.2, MeOH); IR (film) 3343, 2927, 2857, 1671, 1652, 1637, 1559, 1542, 1457, 1437, 1395, 1375, 1317, 1266, 1110, 1078, 1053, 1033 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.58 (dd, 1H, $J_{7,8}$ = 4.7 Hz, $J_{6,7}$ = 7.9 Hz, H-7), 4.39 (dd, 1H, $J_{5,6}$ = 4.5 Hz, $J_{6,7}$ = 7.9 Hz, H-7), 4.30 (d, 1H, $J_{1'',2''}$ = 9.8 Hz, H-1″), 3.90–3.84 (m, 2H, H-6a″, H-2″), 3.71 (dd, 1H, $J_{5'',6''}$ = 5.4 Hz, $J_{6a'',6b''}$ = 12.5 Hz, H-6b″), 3.49 (t, 1H, $J_{3'',4''} = J_{4'',5''}$ = 8.9 Hz, H-3″), 3.47 (s, 3H, OCH_3), 3.42–3.34 (m, 2H, H-4″, H-5″), 3.34–3.28 (m, 1H, H-5), 3.28–3.22 (m, 1H, H-3a′), 3.22–3.15 (m, 1H, H-3b′), 3.03 (m, 3H, CH_2–1′, H-8a), 2.75 (d, 1H, $J_{8a,8b}$ = 13.1 Hz, H-8b), 2.23 (t, 2H, $J_{1,2}$ = 7.2 Hz, CH_2–1), 2.02 (s, 3H, CH_3*NAc*), 1.78–1.50 (m, 6H, CH_2–2′, CH_2–2, CH_2–4), 1.44–1.34 (m, 2H, CH_2–3); ^{13}C NMR (125 MHz, D_2O) δ 176.6 (C = O amide), 174.0 (C = O acetamide), 165.3 (C = O carbamide), 90.0 (C-1″), 77.3 (C-5″), 75.5 (C-3″), 69.6 (C-4″), 62.0 (C-6), 61.0 (OCH_3), 60.8 (C-6″), 60.1 (C-7), 55.3 (C-5), 52.2 (C-2″), 48.7 (C-1′), 39.6 (C-8), 37.0 (C-3′), 35.4 (C-1), 27.7 (C-3), 27.6 (C-4), 25.9 (C-2′), 25.0 (C-2), 22.1 (CH_3 Ac); HRMS(ESI) *m/z* calcd. For $[C_{22}H_{39}N_5O_8SNa]^+$: 556.2414, obsd.: 556.2416.

5-(dimethylamino)-*N*-(2-((3-(methoxy(2-acetamido-2-deoxy-β-D-glucopyranosyl)amino)-propyl)-thio)ethyl)naphthalene-1-sulfonamide (6) *N*-Acetylglucosamine (12.0 mg, 54.2 μmol) and oxyamine **8** (89.1 mg, 0.54 mmol) were reacted in an AcOH/NH_4OAc buffer (1.5 mL, 2 M, freshly prepared, pH 4.5) at rt. for 72 h. Purification by reverse phase chromatography (C_{18}, H_2O/MeOH, 100/0 → 90/10, *v*/v), followed by silica gel flash column chromatography (CH_2Cl_2/EtOH/MeOH/NH_3 (aq. 33%), 10/2/2/1 → 5/2/2/1, *v*/v/*v*/v) afforded 2-(3-[methoxy-(2-acetamido-2-deoxy-β-D-glucopyranosyl)amino]propylthio)ethan-1-amine as a colourless oil (16.1 mg, 81%). R_f = 0.53 (CH_2Cl_2/EtOH/MeOH/NH_3 (aq. 33%), 5/2/2/1, *v*/v/*v*/v); $\alpha_D^{20.3}$ = −25 (c = 0.1, MeOH); IR (film) 3287, 2938, 2056, 1690, 1650, 1559, 1434, 1375, 1316, 1131, 1080, 1034, 947, 632 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.34 (d, 1H, $J_{1',2'}$ = 9.7 Hz, H-1′), 3.93–3.85 (m, 2H, H-2′, H-6a′), 3.89 (t, 1H, $J_{1',2'} = J_{2',3'}$ = 9.7 Hz, H-2′), 3.73 (dd, 1H, $J_{5',6'}$ = 5.3 Hz, $J_{6a',6b'}$ = 12.4 Hz, H-6b′), 3.56–3.47 (m, 4H, H-2′, OCH_3), 3.46–3.37 (m, 2H, H-4′, H-5′), 3.07–3.00 (m, 2H, CH_2–1),

2.85 (t, 2H, $J_{4,5}$ = 6.6 Hz, CH_2–5), 2.71–2.54 (m, 4H, CH_2–3, CH_2–4), 2.04 (s, 3H, CH_3 Ac), 1.81 (m, 2H, CH_2–2). ^{13}C NMR (125 MHz, D_2O) δ 173.9 (C = O), 90.1 (C-1′), 77.5 (C-5′), 75.5 (C-3′), 69.7 (C-4′), 61.1 (OCH_3), 60.9 (C-6′), 52.3 (C-2′), 50.3 (C-1), 39.4 (C-5), 32.9 (C-3), 28.4 (C-4), 26.4 (C-2), 22.2 (CH_3 Ac); HRMS(ESI) *m/z* calcd. For $[C_{14}H_{30}N_3O_6S]^+$: 368.1850, obsd.: 368.1864. To a solution of 2-(3-[methoxy-(2-acetamido-2-deoxy-β-D-glucopyranosyl)amino]propylthio)ethan-1-amine (10.9 mg, 29.7 μmol) in H_2O (2 mL), $NaHCO_3$ (15 mg, 179 μmol) was added. Dansylchloride (12.0 mg, 44.5 μmol) was dissolved in distilled THF (1 mL) and added drop wise to the reaction mixture. After 2 h the THF was concentrated in vacuo and the remaining aqueous reaction mixture was purified by reverse phase chromatography (C_{18}, H_2O/MeOH, 100/0 → 25/75, *v*/v) to give fluorescent glycoside **6** (13.8 mg, 77%); R_f = 0.50 (CH_2Cl_2/MeOH, 8/1, *v*/v); $\alpha_D^{17.8}$ = −14.4 (c = 0.5, MeOH); IR (film) 3304, 3283, 3088, 2939, 2795, 1650, 1572, 1503, 1456, 1409, 1374, 1356, 1316, 1232, 1201, 1142, 1076, 1037, 945, 793, 686, 625, 573, 554, 536 cm^{-1}; ^{1}H NMR (500 MHz, CD_3OD) δ 8.57 (d, 1H, $J_{2,3}$ = 8.6 Hz, H-2), 8.34 (d, 1H, $J_{3,4}$ = 8.7 Hz, H-4), 8.21 (d, 1H, $J_{7,8}$ = 7.3 Hz, H-8), 7.62–7.56 (m, 2H, H-3, H-7), 7.28 (d, 1H, $J_{6,7}$ = 7.6 Hz, H-6), 4.22 (d, 1H, $J_{1'',2''}$ = 9.8 Hz, H-1″), 3.85 (dd, 1H, $J_{5'',6a'}$ = 1.8 Hz, $J_{6a'',6b''}$ = 12.0 Hz, H-6a″), 3.78 (dd, 1H, $J_{1'',2'}$ = $J_{2'',3''}$ = 9.9 Hz, H-2″), 3.68 (dd, 1H, $J_{5'',6b''}$ = 5.6 Hz, $J_{6a',6b''}$ = 12.0 Hz, H-6b″), 3.47 (s, 3H, OCH_3), 3.41 (dd, 1H, $J_{2'',3'}$ = $J_{3'',4''}$ = 9.0 Hz, H-3″), 3.36–3.27 (m, 1H, H-4″), 3.21 (ddd, 1H, $J_{5'',6a''}$ = 1.9 Hz, $J_{5'',6b''}$ = 5.4 Hz, $J_{4'',5''}$ = 9.6 Hz, H-5″), 3.02 (t, 1H, $J_{4',5'}$ = 7.2 Hz, H-5′), 2.98–2.85 (m, 8H, H-1′, 2 × *N*-CH_3), 2.49–2.32 (m, 4H, H-3′, H-4′), 1.95 (s, 3H, CH_3 Ac), 1.69–1.59 (m, 1H, H-2′); ^{13}C NMR (125 MHz, CD_3OD) δ 173.3 (C = O Ac), 153.2, 137.1, 131.32, 131.30 (C-1/4a/5/8a), 130.9 (C-2), 130.1 (C-8), 129.2, 124.3 (C-3/C-7), 120.6 (C-4), 116.5 (C-6), 92.4 (C-1″), 79.8 (C-5″), 77.5 (C-3″), 71.8 (C-4″), 62.9 (C-6″), 62.2 (OCH_3), 54.1 (C-2″), 51.4 (C-1′), 45.8 ($N(CH_3)_2$), 43.9 (C-5′), 32.4 (C-4′), 30.2 (C-3′), 28.5 (C-2′), 23.0 (CH_3 Ac); HRMS(ESI) *m/z* calcd. For $[C_{26}H_{41}N_4O_8S_2]^+$: 601.2360, obsd.: 601.2362.

***N*-(2-Acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl)-*N*-(2-((3-(3-(3′,6′-dihydroxy-3-oxo-3*H*-spiro[isobenzofuran-1,9′-xanthen]-5-yl)thioureido)propyl)-*O*-methylhydroxylamine (8)** To a solution of Lewis^X (5.6 mg, 10.6 μmol) in a AcOH/NH_4OAc buffer (0.5 mL, 2 M, freshly prepared, pH 4.5), 3-(methoxyamino)propan-1-amine hydrochloride (**1b**) (15.9 mg, 113.2 μmol) was added and the reaction mixture was stirred at 40 °C for 35 h. The crude mixture was directly loaded on a size exclusion column (Bio-Gel P-2, 1200 × 18 mm) and eluted with 0.1 M NH_4HCO_3 (aq). Lyophilization of the product fractions afforded the neoglycoside (5.7 mg, 88%). R_f = 0.10 (CH_2Cl_2:EtOH:MeOH:NH_3(35% aq), 5:2:2:1, *v*/v); $\alpha_D^{19.5}$ = −9.3 (c = 0.2, MeOH); IR (film) 3341, 2925, 2852, 17,117, 1647, 1586, 1466, 1451, 1415, 1380, 1350, 1302, 1233, 1193, 1085, 1026, 968, 917 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 5.12 (d, 1H, $J_{1''',2'''}$ = 3.9 Hz, H-1‴), 4.84 (q, 1H, $J_{5''',6'''}$ = 6.7 Hz, H-5‴), 4.49–4.42 (m, 1H, H-1′, H-1″), 4.05 (m, 2H, H-2′, H-6a'), 3.93–3.81 (m, 5H, H-3‴, H-4″, H-4′, H-3′, H-6b'), 3.80 (d, 1H, $J_{3''',4'''}$ = $J_{4''',5'''}$ = 2.9 Hz, H-4‴), 3.76 (m, 3H, H-6a", H-6b", H-2‴), 3.64 (dd, 1H, $J_{3'',4''}$ = 3.2 Hz, $J_{2'',3''}$ = 9.9 Hz, H-3″), 3.60–3.55 (m, 1H, H-5″), 3.54–3.46 (m, 1H, H-5″), 3.54–3.46 (m, 5H, OCH_3, H-5′, H-2″), 3.11–2.92 (m, 4H, CH_2–1, CH_2–3), 2.02 (s, 3H, CH_3 NAc), 1.95 (m, 2H, CH_2–2), 1.17 (d, 3H, $J_{5''',6'''}$ = 6.7 Hz, H-6‴); ^{13}C NMR (125 MHz, D_2O) 170.5 (C = O), 101.8 (C-1″), 98.7 (C-1‴), 90.5 (C-1′), 76.9 (C-5′), 76.1 (C-3′), 74.8 (C-5″), 73.2 (C-4′), 72.4 (C-3″), 71.8 (C-4‴), 70.9 (C-2″), 69.1 (C-3‴), 68.3 (C-4″), 67.6 (C-2‴), 66.7 (C-5‴), 61.4 (C-6″), 60.9 (OCH_3), 59.7 (C-6′), 52.5 (C-2′), 47.2 (C-1), 37.5 (C-3), 24.5 (C-2), 22.1 (CH_3 NAc), 15.2 (C-6‴); HRMS(ESI) m/z calcd. For $[C_{24}H_{46}N_3O_{15}]^+$: 616.2923, obsd.: 616.2938. To a solution of *N*-(2-acetamido-2-deoxy-3-*O*-(α-L-fucopyranosyl)-4-*O*-(β-D-galactopyranosyl)-β-D-glucopyranosyl)-*N*-(3-aminopropyl)-*O*-methylhydroxylamine (12.0 mg, 0.0195 mmol) in H_2O (0.5 mL), was added $NaHCO_3$ (9 mg, 0.107 mmol) followed by fluorescein isothiocyanate isomer I (12.5 mg, 0.032 mmol) in THF (0.25 mL). The reaction was stirred at room temperature for 2.5 h, then concentrated in vacuo and purified by reverse phase chromatography (C_{18}, H_2O/MeOH, 100/0 → 10/90, *v*/v) to give fluorescent glycoside **8** (18 mg, 92%); R_f = 0.08 (silica, CH_2Cl_2/MeOH/AcOH, 70/29/1, *v*/v/v); $\alpha_D^{21.1}$ = − 0.17 (c = 0.7, H_2O); IR (film) 3356, 2929, 1656, 1576, 1505, 1464, 1391, 1329, 1212, 1170, 1111, 1081, 1039, 1021, 915, 852, 809, 770, 670 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 7.58–7.38 (m, 2H, H-aromatic), 7.23–7.07 (m, 3H, H-aromatic), 6.53–6.48 (m, 4H, H-aromatic), 5.10 (d, 1H, $J_{1'''',2''''}$ = 3.9 Hz, H-1⁗), 4.82 (q, 1H, $J_{5'''',6''''}$ = 6.6 Hz, H-5⁗), 4.41 (d, 1H, $J_{1'',2''}$ = 7.0 Hz, H-1″), 4.37 (d, 1H, $J_{1''',2''}$ = 7.2 Hz, H-1‴), 4.08 (s, 1H, H-2″), 3.94 (d, $J_{6a'',6b'}$ = 11.7 Hz, H-6a″), 3.90–3.75 (m, 6H, H-3⁗, H4‴, H-4″, H-3″, H6b″, and H-4⁗), 3.74–3.57 (m, 6H, H-3a′, H-3b′, H-6a‴, H-6b‴, H-3‴, H-2⁗), 3.55–3.44 (m, 6H, OCH_3, H-5″, H-5‴, H-2‴), 3.02 (br s, 2H, H-1′), 2.04 (s, 3H, CH_3 Ac), 1.91–1.81 (m, 2H, H-2′), 1.15 (d, 3H, $J_{5'''',6''''}$ = 6.6 Hz, H-6⁗); ^{13}C NMR (150 MHz, D_2O) δ 180.5 (C = S), 174.0 (C = O), 173.8 (C = O NAc), 158.7 (C-quart), 158.6 (C-quart), 141.1 (C-quart), 138.1 (C-quart), 131.5 (C-aromatic), 131.3 (C-aromatic), 125.8 (C-aromatic), 124.6 (C-aromatic), 122.8 (C-aromatic), 112.4 (C-quart), 112.3 (C-quart), 103.6 (C-quart), 103.5 (C-aromatic), 101.9 (c-1‴), 98.7 (C-1⁗), 90.0 (C-1″), 77.0 (C-5″), 76.3 (C-3″), 74.8 (C-5‴), 73.5 (C-4″), 72.3 (C-3‴), 71.8 (C-4⁗), 70.9 (C-2‴), 69.1 (C-3⁗), 68.2 (C-4‴), 67.6 (C-2⁗), 66.6 (C-5⁗), 61.4 (C-6‴), 59.8 (C-6′

′), 52.4 (C-2″), 48.7 (C-1′), 42.9 (C-3′), 25.9 (C-2′), 22.3 (CH_3 Ac), 15.2 (C-6‴′); HRMS(ESI) *m/z* calcd. For $[C_{45}H_{57}N_4O_{20}S]^+$ 1005.3281, obsd.: 1005.3278.

D-*gluco*-2-(1-Acetamido-2,3,4,5-tetrahydroxy-pentyl)-3-methoxy-1,3-thiazinane (9) *N*-Acetylglucosamine (34 mg, 0.15 mmol) and 3-(methoxyamino)propane-1-thiol (**1d**) (182 mg, 1.50 mmol) were reacted in an AcOH/NH_4OAc buffer (1.5 mL, 2.0 M, pH 4.5, freshly prepared) containing 5% TCEP at rt. for 72 h. Purification by reverse phase chromatography (C_{18}, H_2O/MeOH, 100/0 → 60/40, *v*/v) afforded thiazinane **9** (44 mg, 89%) as an colourless oil. R_f = 0.32 (silica, CH_2Cl_2/EtOH/MeOH/NH_3 (aq. 33%), 10/2/2/1, *v*/v/*v*/v); IR (film) 3298, 2940, 1646, 1545, 1428, 1374, 1318, 1284, 1190, 1079, 1037, 951, 867, 844, 694, 632 cm^{-1}; ^1H NMR (300 MHz, D_2O, 50 °C) δ 4.85–4.62 (m, 2H), 4.63–4.38 (m, 2H), 4.12–3.72 (m, 10H), 3.35–2.91 (m, 4H), 2.40–2.13 (m, 5H), 2.11–1.77 (m, 1H); ^{13}C NMR (150 MHz, D_2O) δ 174.1, 173.8, 72.2, 71.3, 69.3, 68.4, 68.0, 62.6, 62.3, 58.6, 58.0 53.5, 50.6, 27.2, 22.1, 22.0, 17.8; HRMS(ESI) *m/z* calcd. For $[C_{12}H_{25}N_2O_6S]^+$: 325.1428, obsd.: 325.1426.

D-*gluco*-2-(1-Acetamido-2,3,4,5-tetra-acetoxy-pentyl)-3-methoxy-1,3-thiazinane (10) The mixture of thiazinanes **9** (12.0 mg, 37.0 μmol) was co-evaporated with pyridine:acetic anhydride (3 mL, 2/1, *v*/v) and dissolved in acetic anhydride (2 mL) and pyridine (1 mL) and stirred at rt. overnight. The crude reaction mixture was concentrated and purified by silica gel flash column chromatography (PE/EtOAc, 95/5 → 80/20, *v*/v) to give diastereomeric thioaminals **10** (17.8 mg, 98%) as a colorless oil. R_f = 0.38 (EtOAc); IR (film) 2951, 2847, 2816, 1742, 1668, 1514, 1429, 1370, 1313, 1211, 1033, 953, 916, 859, 845, 795, 729, 646, 633 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$, 50 °C) δ 6.06 (d, 1H, $J_{NH,2'}$ = 9.8 Hz, NH *isomer A*), 5.64 (d, 1H, $J_{NH,2'}$ = 10.1 Hz, NH *isomer B*), 5.50 (dd, 1H, $J_{3',4'}$ = 2.0 Hz, $J_{2',3'}$ = 9.4 Hz, H-3′ *isomer A*), 5.43 (dd, 1H, $J_{3',4'}$ = 2.0 Hz, $J_{2',3'}$ = 8.6 Hz, H-3′ *isomer B*), 5.43–5.37 (m, 2H, H-4′ *isomer A + B*), 5.23–5.13 (m, 2H, H-5′ *isomer A + B*), 4.84 (dt, 1H, $J_{1',2'} = J_{2',3'}$ = 5.5 Hz, $J_{2',NH}$ = 10.0 Hz, H-2′ *isomer B*), 4.53 (bd, 1H, $J_{1',2'}$ = 4.5 Hz, $^1J_{CH}$ = *147 Hz,* H-1′ *isomer A*), 4.40 (dt, 1H, $J_{1',2'} = J_{2',3'}$ = 4.9 Hz, $J_{2',NH}$ = 9.7 Hz, H-2′ *isomer A*), 4.28 (dd, 1H, $J_{5',6a'}$ = 3.2 Hz, $J_{6a',6b'}$ = 12.4 Hz, H-6a′ *isomer B*), 4.22 (dd, 1H, $J_{5',6a'}$ = 2.9 Hz, $J_{6a',6b'}$ = 12.5 Hz, H-6b′ *isomer A*), 4.15–4.07 (m, 2H, H-6b′ *isomer A + B*), 3.90 (bd, 1H, $J_{1',2'}$ = 5.2 Hz, $^1J_{CH}$ = *149 Hz,* H-1′ *isomer B*), 3.68 (s, 3H, OCH_3*isomer A*), 3.63–3.52 (m, 1H, H-1_{eq}*isomer A*), 3.49 (s, 3H, OCH_3*isomer B*), 3.39 (dt, $J_{1eq,2eq} = J_{1eq,2ax}$ = 4.3 Hz, $J_{1eq,1ax}$ = 13.3 Hz, H-1_{eq}*isomer B*), 2.95 (dt, 1H, $J_{2eq,3ax}$ = 1.7 Hz, $J_{2ax,3ax} = J_{3eq,3ax}$ = 13.0 Hz, H-3_{ax}*isomer B*), 2.83 (ddd, 1H, $J_{1ax,2eq}$ = 2.9 Hz, $J_{1ax,2ax}$ = 13.0 Hz, $J_{1ax,-1eq}$ = 15.5 Hz, H-1_{ax}*isomer B*), 2.75–2.57 (m, 3H, H-3_{eq}*isomer B*, H-1_{ax}*isomer A*, H-3_{ax}*isomer A*, H-3_{eq}*isomer A),* 2.18 (s, 3H, CH_3-Ac), 2.10 (s, 3H, CH_3 -Ac), 2.04 (s, 3H, CH_3 -Ac), 2.03 (s, 3H, CH_3 -Ac), 2.02 (s, 3H, CH_3 -Ac), 2.00 (s, 3H, CH_3 -Ac), 1.97–1.84 (m, 5H, CH_3 -Ac, H-2_{eq}, H-2_{ax}*isomer A*), 1.49 (bd, 1H, H-2_{eq}*isomer B*); ^{13}C NMR (150 MHz, $CDCl_3$, 50 °C) δ 171.2 (C = O), 170.8 (C = O), 170.53 (C = O), 170.46 (C = O), 170.3 (C = O), 170.2 (C = O), 169.9 (C = O), 169.8 (2 × C = O), 169.6 (C = O), 70.9, 70.5, 69.9, 69.4 (C-3′, C-4′ *isomer A + B*) 69.0 (C-5′ *isomer A + B*), 68.6 (C-1′ *isomer B*), 67.6 (C-1′ *isomer A*), 62.3 (C-6′ *isomer A/B*), 62.1 (C-6′ *isomer A/B*), 59.8 (OCH_3*isomer B*), 58.2 (OCH_3*isomer A*), 52.9 (CH_2NO *isomer B*), 51.6 (CH_2NO *isomer A*), 51.5 (C-2′ *isomer A*), 49.5 (C-2′ *isomer B*), 28.1 (CH_2S *isomer B*), 26.8 (CH_2S *isomer A*), 23.4 (C-CH_2-C *isomer A/b)*, 23.2 (C-CH_2-C *isomer A/b*), 20.9, 20.8, 20.7 (CH_3 Ac); TOCSY NMR (300 MHz, D_2O, 50 °C) *Isomer a* δ 3.59 (excited, app. bd, $J_{1ax,1eq}$ = 14.6 Hz, H-1 eq), 2.84 (app. bt, $J_{1ax,1eq} = J_{1ax,2ax}$ = 13.1 Hz, H-1ax), 2.13 (app. bq, $J_{1ax,2ax} = J_{2ax,2eq} = J_{2ax-3ax}$ = 13.0 Hz, H-2 ax), 1.50 (app. bd, $J_{2eq,2ax}$ = 14.3 Hz, H-2 eq), 2.97 (app. bt, $J_{2ax,3ax} = J_{3ax,3eq}$ = 13.0 Hz, H-3ax), 2.64 (app. bd, $J_{3eq,3ax}$ = 13.3 Hz, H-3 eq); HRMS(ESI) *m/z* calcd. For $[C_{20}H_{33}N_2O_{10}S]^+$: 493.1850, obsd.: 493.1856.

1-(2-D-biotinamidoethyl)-1*H*–pyrrole-2,5-dione (13) To a solution of 2-aminoethyl maleimide **12**[30] (159 mg, 1.13 mmol) and D-biotin (363.4 mg, 1.31 mmol) in DMF (10 mL), HBTU (516 mg, 1.36 mmol) and triethylamine (0.1 mL) were added and the reaction mixture was stirred at rt. for 2 h. The crude reaction mixture was concentrated and purified by reverse phase column chromatography (C_{18}, H_2O/MeOH, 100/0 → 70/30, *v*/v) and size exclusion chromatography (Sephadex LH-20, CH_2Cl_2/MeOH, 50/50, *v*/v) to give biotin functionalised maleimide **13** as a white solid (380 mg, 1.04 mmol, 92%). R_f = 0.59 (silica, CH_2Cl_2/MeOH, 80/20, *v*/v); $\alpha_D^{20.6}$ = + 26.5 (c = 0.2, MeOH); IR (film) 3283, 3087, 2936, 2864, 1701, 1552, 1461, 1439, 1408, 1360, 1332, 1266, 1169, 1101, 1055, 1033, 828, 763, 696 cm^{-1}; ^1H NMR (500 MHz, CD_3OD) δ 6.82 (s, 2H, HC = CH), 4.60 (s, 1H, NH), 4.49 (dd, $J_{6,7}$ = 4.9 Hz, $J_{7,8}$ = 7.7 Hz, 1H, H-7), 4.67 (dd, 1H, $J_{6,7}$ = 4.9 Hz, $J_{5,6}$ = 7.8, Hz H-6), 3.61 (dd, 2H, $J_{1',2'}$ = 5.5 Hz, CH_2–1′), 3.36 (dd, 2H, $J_{1',2'}$ = 5.5 Hz, CH_2–2′), 3.24–3.17 (m, 1H, H-5), 2.93 (dd, 1H, $J_{7\text{-}8a}$ = 5.0 Hz, $J_{8a\text{-}8b}$ = 12.8 Hz, H-8a), 2.70 (d, 1H, $J_{8a\text{-}8b}$ = 12.8 Hz, H-8b), 2.12 (dt, 2H, $J_{1a,2}$ = 3.4 Hz, $J_{1b,2}$ = 7.4 Hz, CH_2–1), 1.79–1.66 (m, 1H, CH_2–4a), 1.66–1.52 (m, 3H, CH_2–2, CH_2–4b), 1.50–1.35 (m, 2H, CH_2–3); ^{13}C NMR (125 MHz, CD_3OD) δ 176.3 (C = O amide), 172.6 (2 × C = O *maleimide*), 166.1 (C = O *carbamate*), 135.5 (C = C), 63.3 (C-6), 61.6 (C-7), 56.9 (C-5), 41.0 (C-8), 38.8 (C-2′), 38.4 (C-1′), 36.7 (C-1), 29.7 (C-3), 29.4 (C-4), 26.6 (C-2); HRMS(ESI) *m/z* calcd. For $[C_{16}H_{23}N_4O_4S]^+$: 367.1435, obsd.: 367.1429.

1-(2-D-biotinamidoethyl)-3-(3-(3-(methoxy[2-acetamido-2-deoxy-β-D-glucopyranosyl]-amino) propylthio)propylthio)pyrrolidine-2,5-dione (14) *N*-

Acetylglucosamine (7.8 mg, 35.3 μmol) and 3-(3-(methoxyamino)propylthio)propane-1-thiol (**1e**) (69.0 mg, 0.35 mmol) were reacted in a mixture of AcOH/NH_4OAc buffer (0.2 mL, 2 M, freshly prepared, pH 4.5), ethanol (0.2 mL) and CH_2Cl_2 (0.1 mL) at 40 °C for 72 h. The crude reaction mixture was directly loaded on a size exclusion column (Bio-Gel P-2, 600 × 10 mm) and eluted with 0.1 M aq. NH_4HCO_3. Lyophilisation of the product fractions afforded 1,2-bis(3-[3-(methoxy-[2-acetamido-2-deoxy-β-D-glucopyranosyl]amino)propylthio]propyl)-disulfane (9.0 mg, 64%) as a colorless oil. R_f (Sulfhydryl) = 0.34 (10/2/2/1, CH_2Cl_2/EtOH/MeOH/NH_3(aq, 33%)), R_f = (disulfide) = 0.17 (10/2/2/1, CH_2Cl_2/EtOH/MeOH/NH_3(aq, 33%)); $\alpha_D^{19.8}$ = +3.5 (c = 0.1, MeOH); IR (film) 3313, 3273, 3094, 3070, 2656, 2909, 1632, 1557, 1487, 1439, 1355, 1215, 1195, 1111, 1038, 1000, 986, 903, 755, 721, 691, 620, 604 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.34 (d, 2H, $J_{1',2'}$ = 9.8 Hz, H-1′), 3.92–3.86 (m, 4H, H-6a′, H-2′), 3.74 (dd, 2H, $J_{5',6b'}$ = 5.4 Hz, $J_{6a',6b'}$ = 12.4 Hz, H-6b′), 3.55–3.46 (m, 8H, OCH_3, H-3′), 3.44–3.36 (m, 4H, H-4′, H-5′), 3.10–3.00 (m, 4H, CH_2–1), 2.85 (t, 4H, $J_{5,6}$ = 7.1 Hz, CH_2–6), 2.70 (t, 4H, $J_{4,5}$ = 7.1 Hz, CH_2–4), 2.68–2.57 (m, 4H, CH_2–3), 2.04 (s, 6H, CH_3*N*Ac), 2.00 (p, 4H, $J_{4,5} = J_{5,6}$ = 7.0 Hz, CH_2–5), 1.86–1.78 (m, 4H, CH_2–2); ^{13}C NMR (125 MHz, D_2O) δ 173.8 (C = O), 90.0 (C-1′), 77.4 (C-5′), 75.5 (C-3′), 69.6 (C-4′), 61.1 (OCH_3), 60.8 (C-6′), 52.3 (C-2′), 50.3 (C-1), 36.5 (C-6), 29.4 (C-4), 28.6 (C-3), 27.9 (C-5), 26.3 (C-2), 22.1 (CH_3*N*Ac); HRMS(ESI) *m/z* calcd. For $[C_{30}H_{59}N_4O_{12}S_4]^+$: 795.3007, obsd.: 795.3018; HRMS(ESI) *m/z* calcd. For $[C_{15}H_{31}N_2O_6S_2]^+$: 399.1618, obsd.: 399.1609. To a solution of 1,2-bis(3-[3-(methoxy-[2-acetamido-2-deoxy-β-D-glucopyranosyl]amino)propylthio]propyl)-disulfane (7.0 mg, 17.6 μmol) in H_2O (1 mL), maleimide **13** (9.0 mg, 24.6 mmol) was added, and the reaction mixture was stirred at rt. for 1 h. The crude reaction mixture was purified using size exclusion chromatography (Bio Gel P-2) to yield glycoconjugate **14** (12.6 mg, 16.5 μmol, 94%) as a colourless oil. R_f = 0.54 (CH_2Cl_2/EtOH/MeOH/NH_3 (aq. 33%), 5/2/2/1, *v*/v/*v*/v); IR (film) 3338, 3286, 2933, 2871, 2859, 1700, 1648, 1559, 1452, 1439, 1332, 1265, 1188, 1107, 1080, 1026, 969, 901, 847, 760,699 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 4.60 (dd, 1H, $J_{7,8}$ = 5.0 Hz, $J_{6,7}$ = 7.8 Hz, H-7), 4.42 (dd, 1H, J_{5-6} = 4.5 Hz, J_{6-7} = 7.8 Hz, H-6), 4.32 (d, 1H, $J_{1'''',2''''}$ = 9.8 Hz, H-1′′′′), 4.02–3.98 (m, 1H, H-1′′), 3.91–3.85 (m, 2H, H-2′′′′, H-6a′′′′), 3.72 (dd, 1H, $J_{5'''',6''''}$ = 5.2 Hz, $J_{6a'''',6b''''}$ = 12.3 Hz, H-6b′′′′), 3.71–3.58 (m, 2H, CH_2–1′), 3.53–3.46 (m, 4H, H-3′′′′, OCH_3), 3.45–3.35 (m, 4H, CH_2–2′, H-5′′′′, H-4′′′′), 3.35–3.24 (m, 2H, CH_2–5, H-2a′′), 3.06–2.95 (m, 3H, CH_2–1′′′, H-8a), 2.93–2.79 (m, 2H, CH_2–6′′′), 2.77 (d, 1H, J_{8a-8b} = 13.0 Hz, H-8b), 2.72 (m, 5H, H-2b′′, CH_2–4′′′, CH_2–3′′′), 2.19 (t, 1H, $J_{1,2}$ = 7.3 Hz, CH_2–1), 2.03 (s, 3H, CH_3 Ac), 1.96–1.87 (m, 2H, CH_2–5′′′), 1.84–1.76 (m, 2H, CH_2–2′′′), 1.76–1.66 (m, 1H, H-4a), 1.62–1.50 (m, 3H, H-4b, CH_2–2), 1.42–1.33 (m, 2H, CH_2–3); ^{13}C NMR (125 MHz, D_2O) δ 182.0, 180.8 (2 × C = O *pyrrolidine*), 179.5 (C = O *biotin*), 176.3 (C = O *NAc*), 167.8 (*C* = O *carbamate-biotin*), 92.6 (C-1′′′′), 80.0 (C-4′′′′), 78.1 (C-3′′′′), 72.2 (C-5′′′′), 64.6 (C-6), 63.7 (OCH_3), 63.4 (C-6′′′′), 62.8 (C-7), 57.8 (C-5), 54.9 (C-2′′′′), 52.9 (C-1′′′), 42.5 (C-1′′), 42.3 (C-8), 41.2 (C-1′), 39.1 (C-2′), 38.5 (C-2′′), 38.0 (C-1), 32.4 (C-6′′′), 32.3 (C-4′′′), 31.2 (C-3′′′), 30.6, 30.5 (C-5′′′, C-3), 30.2 (C-4), 28.9 (C-2′′′), 27.5 (C-2), 24.8 (CH_3 Ac); HRMS(ESI) *m/z* calcd. For $[C_{31}H_{53}N_6O_{10}S_3]^+$: 765.2980, obsd.: 765.2975.

Glycoprotein 17 To a solution of Bovine Serum Albumin (1 mg, 0.015 μmol) in PBS (1 mL), SMCC cross-linker **15** (0.9 mg, 2.73 μmol) in MeCN (0.1 mL) was added and the reaction mixture incubated at rt. To 10 μL of the crude reaction mixture, neoglycoside **16** (2.2 mg, 5.45 μmol) in water (10 μL) was added and the reaction mixture was allowed to stir at rt. to obtain glycoprotein **17**.

MALDI-TOF To a solution of cinnapinic acid (15 mg) in MeCN:H_2O (1 mL, 1/1, *v*/v), TFA (1 μL) was added and the mixture was vortexed for 3 min and centrifuged for 10 min, to obtain the matrix solution. A 1–2 μL aliquot of the glycoprotein (~1.0 mg/mL) was added to 20 μL of the matrix solution and a pipette was used to mix the sample. Next, 2 μL of the sample/matrix was spotted on a MALDI-plate, dried for 1 h, and measured using MALDI-TOF.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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