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Very Important Publication

Vinyl Sulfonates: A Click Function for Coupling-and-Decoupling Chemistry and their Applications

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Abstract: The term coupling-and-decoupling (CAD) chemistry refers to applications in which efficient bond formation and subsequent cleavage between two moieties is required. Within this context, the scope of the vinyl sulfonate (VSO) group as an efficient tool for CAD chemistry is reported. The coupling step relies on the click features of the Michael-type addition of diverse nucleophiles to vinyl sulfonates as a valuable methodology. The feasibility of this strategy has been proved by the high yields obtained in mild conditions with model VSO derivatives. Cleavage of the resulting sulfonate adducts either through nucleophilic substitution with different nucleophiles (for alkyl VSO groups) or through hydrolysis (for both alkyl and aryl VSO) are successful strategies for the decoupling step, the former being the most promising, as the reaction proceeds under milder conditions with thiol nucleophiles. Moreover, the click VSO coupling chemistry proves to be orthogonal with the click CuAAC reaction, which enables the VSO-CAD methodology for the preparation of hetero-bifunctional clickable and cleavable linkers for double click modular strategies. The potential of the VSO-CAD chemistry is demonstrated in two biologically relevant examples: the decoupling of sulfonates with glutathione (GSH) under conditions compatible with those of living systems; and the synthesis of homo- and heterogeneous multivalent glycosylated systems from 1-thio and 1-azido or 1-azidoethyl sugar derivatives and bis-vinyl sulfonates (homo systems) or alkynyl-VSO bifunctional clickable-cleavable linkers (hetero systems). As proof of concept, the cleavable character of these multivalent systems was demonstrated by using one of them as a reversible linker for the non-covalent assembling and chemical decoupling of two model lectins.

Keywords: carbohydrates; click chemistry; coupling-and-decoupling chemistry; Michael addition; vinyl sulfonates

Introduction

The concept of "coupling-and-decoupling" (CAD) chemistry has been recently introduced by Bielski and Witczak as a strategy that aims at both the binding and subsequent disconnection of the target molecules, a desirable feature for a range of applications.[1] Among these applications some authors have highlighted the therapeutic delivery or targeted release, the decoupling of a molecule from a solid support or a surface of interest after performing chemical transformations on it (e.g., solid phase synthesis), the modification of the surface of a material or the quantification of the amount of compound bound to the surface or solid support.[1] Ideally, CAD chemistry requires that both the connection and disconnection steps proceed efficiently with high yields under mild conditions. Therefore, CAD chemistry benefits in the coupling step from the click chemistry concept,[2] whose development over the last decade represents a great advantage for chemists as it provides powerful tools to make new bonds and to easily functionalize a large variety of molecules and materials. However, CAD chemistry goes one step further as it demands reversibility in the click reaction or the presence of an additional cleavable motif in the linker.

Among the click reactions, the copper(I)-catalyzed azide-alkyne cycloaddition (CuAAC),[3] has been thoroughly exploited in research fields as diverse as polymer chemistry,[4] nanotechnology and materials science,[5] the synthesis of interlocked structures and molecular machines,[6] bioconjugation or other biolog-

ical applications,[7] taking advantage in the latter case of the copper-free version of the reaction.[8] Besides the CuAAC, other reactions,[9] such as the radical thiol-ene or thiol-yne reactions,[10] the inverse electron-demand Diels–Alder cycloaddition with tetrazines[11] or Michael-type addition reactions,[12] have been widely explored as alternative click reactions. Among the latter, the use of the vinyl sulfone (VS) group as Michael acceptor with different nucleophiles has been attracting much attention in the last few years due to its good stability in aqueous media, biocompatibility and excellent reactivity under mild conditions with thiols and amines. Hence, the Michael-type addition to VS, which is also orthogonal to the CuAAC, fulfils many of the criteria to be classified as a click reaction.[12,13] Thereby, VS derivatives have been employed for the bioconjugation of proteins,[14] in particular with polymers,[15] other biomolecules such as carbohydrates,[16] or fluorescent,[16a,17] electrochemically active[16a,18] or affinity-binding[16a,17] tags. The efficiency of Michael-type additions between VS and protein thiol and amine groups has been also exploited in their immobilization onto solid supports such as silica particles[16a,19] or in the development of glycoarrays to study glycoprotein-lectin interactions.[16c] Moreover, the VS group has been used in the development of non-viral gene delivery agents[20] and cyclodextrin-based carrier systems for targeted drug transport.[21]

The efficiency and versatility displayed by click Michael-type additions to VS prompted us to turn our attention to the vinyl sulfonate (VSO) group as a candidate for CAD chemistry. On one hand, the VSO displays a vinyl sulfonyl unit similar to the vinyl sulfone group that should enable them to act as suitable acceptors in click Michael-type additions (coupling). On the other hand, the VSO group incorporates a sulfonate group, liable to participate in substitution or hydrolysis reactions (decoupling).

Vinyl sulfonates have been previously employed as Michael acceptors[22] in the decoration of aminated polymers,[23] in the synthesis of dendrimers,[24] and as protein inhibitors whose mode of action is based on the thiol-Michael addition of cysteine residues present in the active site to the vinyl group.[25] Additionally, the VSO group has also been used in the synthesis of betylates, a type of charged compounds obtained by *N*-alkylation of the adducts obtained *via* aza-Michael addition of a secondary amine to a VSO group.[26] These studies are, however, restricted to scarce examples, and a more comprehensive and systematic research is needed in order to study the scope of the VSO group in both Michael-type additions (coupling) and the subsequent cleavage by nucleophilic substitution or hydrolysis (decoupling) to assess its suitability for CAD chemistry.

With this aim, herein we address the potential of the VSO function as a tool for CAD chemistry through the study of both coupling, based on Michael-type addition of different thiols, amines and alkoxides to model aliphatic and aromatic VSO derivatives, and nucleophilic or hydrolytic decoupling steps. Moreover, we exploit the features of the VSO group in two proof of concept biological applications: the decoupling of sulfonates with thiols, in particular glutathione (GSH), under conditions compatible with those of living systems, and the development of cleavable multivalent homo- and heterogeneous glycosylated systems.

Results and Discussion

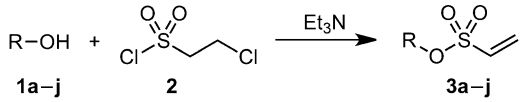
Vinyl Sulfonate-Based Click Coupling

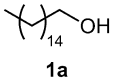
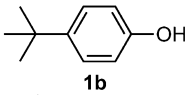
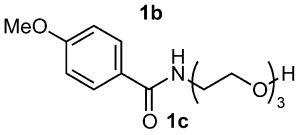
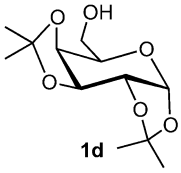
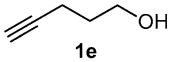
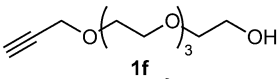
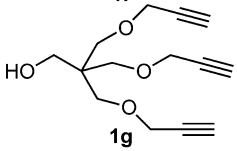
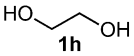
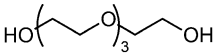
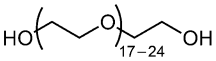
The VSO derivatives used in this study (**3a–j**) were easily obtained in good to excellent yields (71–99%) in most cases following a typical synthetic methodology based on the treatment of the corresponding alcohols **1a–j** with 2-chloroethanesulfonyl chloride (**2**) in the presence of Et_3N (Table 1). In this work we focused on the preparation of aliphatic and aromatic vinyl sulfonates, while their benzyl counterparts were not considered due to their higher reactivity that makes them more unstable and limits their application. In fact, benzyl VSO derivatives are scarce in the literature compared to the aromatic and aliphatic analogues.[25a]

To study the reactivity and scope of the VSO function as acceptor in Michael-type addition reactions, the aliphatic VSO derivative **3a** was first reacted with model nucleophiles (**4a–g**), among which are primary and secondary amines, thiols and alkoxides (Table 2). Typically, the reactions were performed with a twofold excess of the nucleophile using CH_2Cl_2/2-propanol (5:1) as solvent and mild conditions: room temperature and a catalytic amount of Et_3N. In the case of alkoxides, these nucleophiles were obtained *in situ* by treatment of the corresponding alcohol with a stronger base, i.e., *t*-BuOK. The corresponding Michael adducts **5a–e**, **g** were obtained with excellent yields (>90%) (Table 2, entries 1–5 and 7). High efficiency was also observed with aromatic VSO **3b** (Table 2, entries 8 and 9), as the corresponding Michael adducts **5h**, **i** were obtained in ≥85% isolated yield. In addition, a substrate bearing two VSO groups (**3i**) was also assayed leading to **5j** in 99% yield (Table 2, entry 10).

The high efficiency of the VSO group in Michael-type additions encouraged us to study the reaction with more valuable nucleophiles, specifically 1-thiosugar derivatives of per-*O*-acetylated glucose (**4i**),[27] and lactose (**4j**)[28] (Table 2, entries 11 and 12). Alkenyl

Table 1. Synthesis of vinyl sulfonate derivatives **3a–j**.[a]



Entry	Alcohol (**1**)	**3**	Yield [%]
1	 **1a**	**3a**	93
2	 **1b**	**3b**	71
3	 **1c**	**3c**	87
4	 **1d**	**3d**	91
5	 **1e**	**3e**	84
6	 **1f**	**3f**	89
7	 **1g**	**3g**	99
8	 **1h**	**3h**	48[b]
9	 **1i**	**3i**	55[b]
10	 **1j** (PEG1000)	**3j**	99[b]

[a] *Reaction conditions:* 2-chloroethanesulfonyl chloride (**2**, 1.5–2.0 equiv.), Et_3N (5.0 equiv.), CH_2Cl_2, 0–4 °C, 1 h.
[b] Reaction performed with **2** (3.0 equiv.) and Et_3N (10 equiv.).

VSO **3e** was chosen in this case and the reaction carried out with a catalytic amount of Et_3N in the presence of PPh_3 (0.3 equiv.). PPh_3 was used with a double purpose, to act as a catalyst in Michael-type reactions to vinyl groups,[29] and to prevent the oxidation of the thiols to disulfides due to its reductant character.[30] Hence, the corresponding products **5k**, **l** were obtained in 73% and 95% yield, respectively.

The assembly of results obtained demonstrates the good reactivity of the VSO function as Michael acceptor under mild conditions and supports its use as an efficient tool for a coupling strategy based on click Michael-type addition reactions.

Decoupling of Clicked Sulfonate Adducts

Having established the efficiency of the VSO function in click Michael-type addition coupling processes, we investigated the disconnection of the clicked adducts by two independent chemical strategies in which sulfonates are typically involved, namely, breaking of the C–O bond through nucleophilic substitution of aliphatic sulfonates and, alternatively, S–O bond cleavage, mainly *via* hydrolysis.[31]

To explore the nucleophilic displacement approach, compound **5g** was treated with a variety of organic and inorganic nucleophilic species, including amines (**4a**, **c**), thiols (**4g**, **i**) and azide (**4l**), halide (**4m**, **n**) and thiocyanate (**4o**) salts (Table 3). The reaction with amines and inorganic salts was performed by heating at 85 °C in DMF for 4 h, affording the expected substitution products (**6a–f**) in good yields (Table 3, entries 1–6). Interestingly, the reaction with methanol in the presence of an excess of LiOH also afforded the substitution product **6g** (Table 3, entry 7).

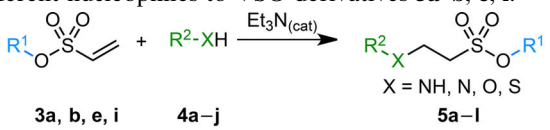
Although successful, the reaction with amines and inorganic nucleophiles requires relatively harsh conditions that might be still useful for important applications but may restrict the scope of this strategy, particularly when biologically relevant systems are involved. However, the increased nucleophilic character of thiols made it possible to overcome this limitation, as the reaction of **5g** with **4g**, **i** at room temperature in the presence of Cs_2CO_3, a relatively mild base, afforded the corresponding thioether products **6h**, **i** in good yields (82–89%) (Table 3, entries 8 and 9).

As an alternative disconnecting methodology, the hydrolysis of the clicked sulfonate adducts through cleavage of the S–O bond was explored (Table 4). Adducts **5g**, **i** were treated with $LiOH{\cdot}H_2O$ under different conditions. At room temperature in MeOH or an MeOH/H_2O (10:1) mixture the hydrolysis of **5g** did not take place and the starting material was recovered unaltered (Table 4, entries 1 and 2). When the temperature was raised to 65 °C, **5g** reacted to form **6g** (Table 4, entry 3), the product of the nucleophilic substitution instead of the hydrolysis. However, the hydrolysis reaction proceeded when heating at 85 °C using DMF as a solvent, affording cetyl alcohol (**1a**) and *tert*-butylphenol (**1b**) (Table 4, entries 4 and 5). These results confirm that hydrolysis is a valid decoupling methodology.

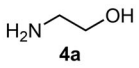
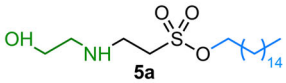
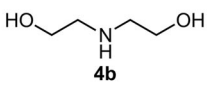
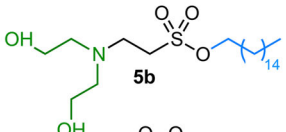
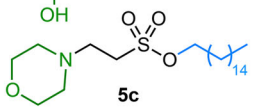
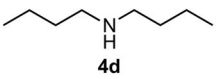
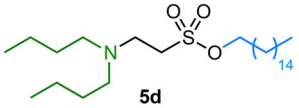
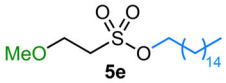
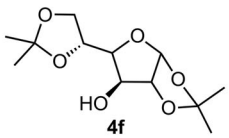
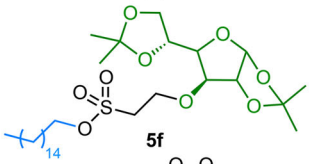
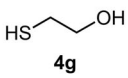
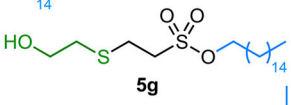
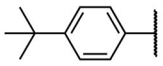
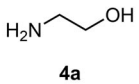
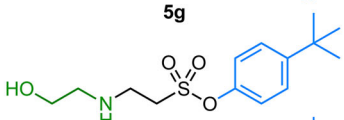
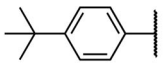
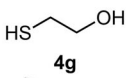
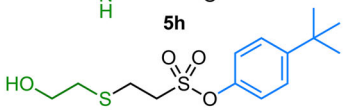
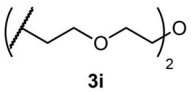
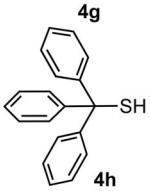
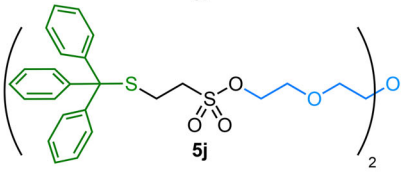
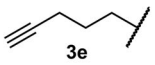
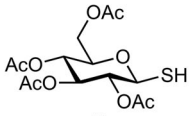
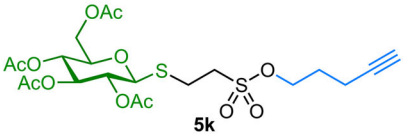
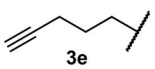
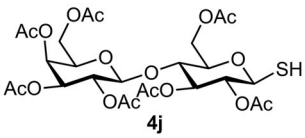
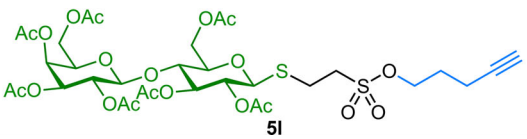
Orthogonality of VSO-Based Michael-Type Addition and CuAAC Reactions

A desirable additional feature of the coupling-and-decoupling chemistry based on the VSO group would be its orthogonality to other click reactions, as it would allow the development of modular double click strat-

Table 2. Michael-type addition of different nucleophiles to VSO derivatives **3a–b**, **e**, **i**.[a]

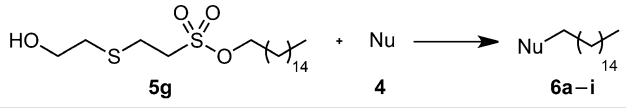


3a, b, e, i **4a–j** **5a–l**

Entry	R^1 (**3**)	Nucleophile (**4**)	Compound (**5**)	Yield [%] (Conditions)
1	 **3a**	 **4a**	 **5a**	92 (A)
2	 **3a**	 **4b**	 **5b**	98 (A)
3	 **3a**	 **4c**	 **5c**	99 (A)
4	 **3a**	 **4d**	 **5d**	99 (A)
5	 **3a**	NaOMe **4e**	 **5e**	100 (B)
6	 **3a**	 **4f**	 **5f**	67 (C)
7	 **3a**	 **4g**	 **5g**	98 (A)
8	 **3b**	 **4a**	 **5h**	92 (A)
9	 **3b**	 **4g**	 **5i**	85 (A)
10	 **3i**	 **4h**	 **5j**	99 (D)
11	 **3e**	 **4i**	 **5k**	73 (E)
12	 **3e**	 **4j**	 **5l**	95 (E)

[a] *Reaction conditions:* (A) **4a–d, g** (2.0 equiv.), $Et_3N_{(cat)}$, CH_2Cl_2/2-propanol (5:1), room temperature, 18–20 h. (B) **4e** (2.5 equiv.), THF or MeOH, room temperature, 1–2 h. (C) **4f** (2.0 equiv.), *t*-BuOK (0.2 equiv.), THF, room temperature, 18 h. (D) **4h** (4.0 equiv.), $Et_3N_{(cat)}$, CH_2Cl_2/2-propanol (5:1), room temperature, 18 h. (E) **4i**, **j** (1.5 equiv.), $Et_3N_{(cat)}$, PPh_3 (0.3 equiv.), CH_2Cl_2/2-propanol (5:1), room temperature, 18–48 h.

Table 3. Nucleophilic displacement of sulfonate **5g**.[a]

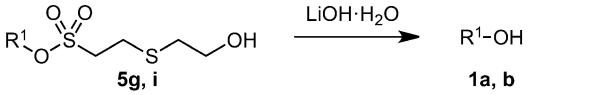


Entry	Nucleophile (**4**)	Compound (**6**)	Yield [%] (Conditions)
1	H_2N OH **4a**	HO N H 14 **6a**	95 (A)
2	HN O **4c**	N O 14 **6b**	79 (A)
3	NaN_3 **4l**	N_3 14 **6c**	86 (A)
4	KBr **4m**	Br 14 **6d**	90 (A)
5	KI **4n**	I 14 **6e**	82 (A)
6	KSCN **4o**	NCS 14 **6f**	99 (A)
7	MeOH	MeO 14 **6g**	99 (B)
8	HS OH **4g**	HO S 14 **6h**	82 (C)
9	AcO AcO OAc SH OAc **4i**	AcO AcO OAc O S 14 OAc **6i**	89[b] (C)

[a] *Reaction conditions:* (A) **4** (5.0 equiv.), DMF, 85 °C, 4 h; (B) LiOH·H_2O (5.0 equiv.), MeOH, 65 °C, 18 h; (C) **4g**, **i** (2.5 equiv.), Cs_2CO_3 (2.5 equiv.), DMF, room temperature, 24 h.

[b] Obtained as a ~8:92 mixture of the α and β anomeric isomers, respectively, which were separated by column chromatography to afford the β anomer in 82% yield and the α anomer in 7% yield.

Table 4. Hydrolytic decoupling of sulfonates **5g**, **i** with LiOH.[a]



Entry	R^1 (**5**)	Solvent	*T*	Yield [%] (Product)
1	14 **5g**	MeOH	r.t.	no reaction[b]
2	14 **5g**	MeOH/H_2O (10:1)	r.t.	no reaction[b]
3	14 **5g**	MeOH	65 °C	99 (**6g**)[c]
4	14 **5g**	DMF	85 °C	80 (**1a**)
5	**5i**	DMF	85 °C	63 (**1b**)

[a] Reaction time: 18–24 h.

[b] **5g** recovered.

[c] See Table 3, entry 7.

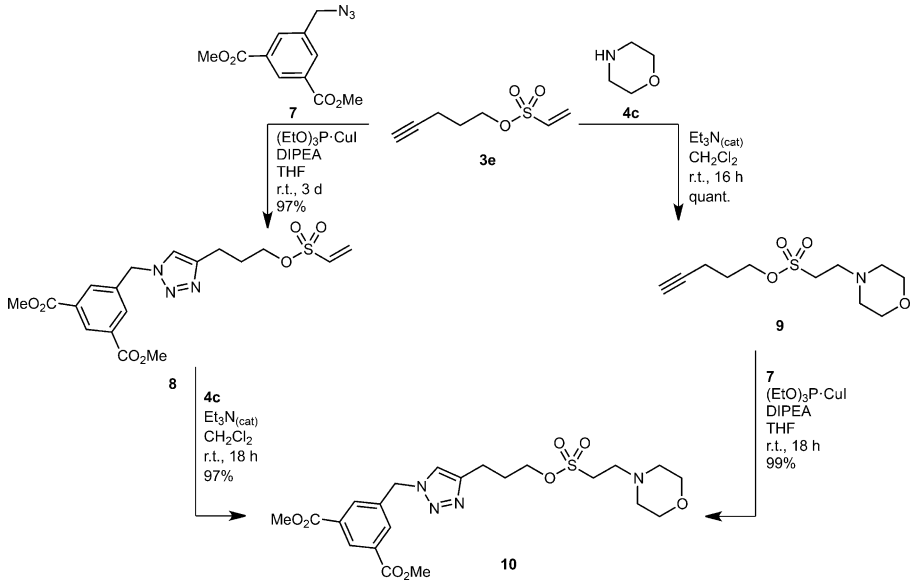
egies. For this reason, we decided to investigate the compatibility and orthogonality of the Michael-type addition to vinyl sulfonates with the CuAAC reaction.

For this purpose we selected compound **3e**, which displays a vinyl sulfonate and an alkyne function and we addressed the synthesis of model compound **10** by two alternative routes (Scheme 1). The first one involved initially the CuAAC reaction at room temperature between **3e** and azide **7** in the presence of DIPEA, using $(EtO)_3P{\cdot}CuI$[32] as catalyst, to yield **8**, followed by aza-Michael addition of morpholine (**4c**) to the VSO moiety of **8**, giving the difunctionalized product **10** (Scheme 1, *left*). In the second route the order of the click reactions was inverted, obtaining **9** by aza-Michael addition between **3e** and **4c**, followed by CuAAC reaction between **9** and **7** (Scheme 1, *right*). Both synthetic routes afforded **10** in excellent overall yield (94–99%) with no significant differences in the performance of the CuAAC (97–99% yield) or the aza-Michael reaction (97–100% yield) as a result of the changes in the reaction order.

These results clearly demonstrate the orthogonality under the conditions tested between the click Michael-type addition reactions to the VSO group and the CuAAC. In this sense, alkynyl-VSO derivatives like **3e–g** arise as hetero-bifunctional cleavable linkers that give access to cleavable click-click adducts. Such a CAD-click modular strategy would enable the design of complex systems in which different groups can be selectively coupled *via* orthogonal click chemistry reactions by choosing reagents with the appropriate functionalities and subsequently decoupled taking advantage of the VSO chemistry.

Proof of Concept Applications of VSO-CAD Chemistry

Having shown that the VSO group exhibits suitable reactivity features that could be exploited to make it an efficient click tool in CAD chemistry, we applied this methodology for two different proof of concept applications: (i) the coupling and decoupling with the biologically relevant tripeptide glutathione (GSH), and (ii) the synthesis of cleavable multivalent glycosylated systems.



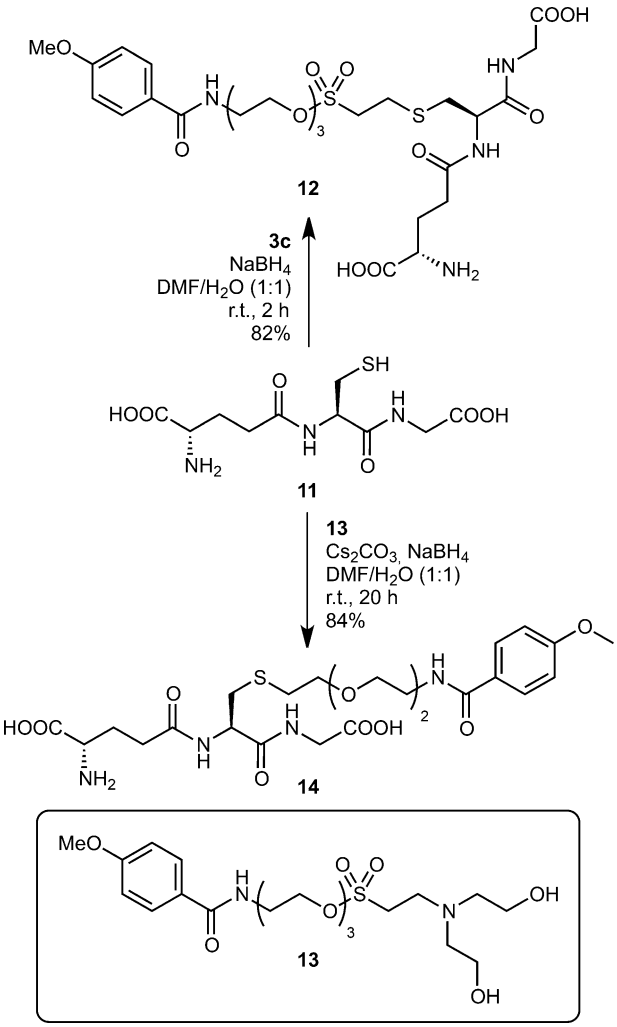
Scheme 1. Study on the orthogonality of VSO-based Michael addition and CuAAC click reactions.

Coupling and Decoupling with Glutathione

The non-protein peptide GSH (**11**) contains a cysteine residue that could act as a nucleophile in thiol-Michael reactions, making this a good model molecule to study the labelling of cysteine-containing proteins with VSO derivatives. Moreover, the thiol group present in this tripeptide might also participate in the decoupling of sulfonate adducts through nucleophilic substitution. The displacement of the sulfonate group by GSH is especially appealing from a biological perspective, since GSH is the most abundant small molecule thiol in the cell, reaching millimolar concentrations, and its homeostasis is important to prevent pathologies.[33]

Initially, we studied GSH (**11**) as a nucleophile in the thiol-Michael addition reaction to the model VSO **3c**, chosen for its higher solubility in polar solvents. The reaction was performed in a DMF/H_2O (1:1) mixture in the presence of $NaBH_4$ to prevent GSH oxidation, affording adduct **12** in 82% yield (Scheme 2, *top*). The reaction time was shortened to ensure that only the thiol and not the amino group participated in the Michael-type addition coupling, which was confirmed by 2D NMR spectroscopy of adduct **12** (see Figure S1 in the Supporting Information).

We then investigated the nucleophilic decoupling of sulfonate conjugates with GSH under non-biological conditions. Hence, adduct **13** (see the Supporting Information for synthetic details) was reacted with GSH under similar conditions to those of the coupling step, but in the presence of Cs_2CO_3, affording the nucleophilic displacement product **14** in 84% yield (Scheme 2, *bottom*). The chemoselectivity of the pro-



Scheme 2. VSO-CAD chemistry with GSH (**11**).

cess was confirmed again by 2D NMR experiments (Figure S2 in the Supporting Information).

Having demonstrated that GSH is a suitable nucleophile for the decoupling of sulfonate adducts, we tested this process under conditions compatible with those of biological systems. In order to estimate the concentration of thiol that can cleave the sulfonate group under biological conditions (i.e., aqueous medium, neutral pH and ionic strength), β-mercaptoethanol (**4g**) and the mannose-VSO derivative **15** (Figure 1a, see the Supporting Information for synthetic details) were selected as model system. Microtiter plates bearing amino groups were reacted with mannose-VSO derivative **15** and then incubated with concanavalin A conjugated with horseradish peroxidase (ConA-HRP) to afford the immobilization of ConA-HPR on the surface of the wells *via* ConA-mannose interaction (Figure 1b).[34] The wells were then incubated with different concentrations of β-mercaptoethanol **4g** up to 250.5 mM for 3 h, 8 h or 24 h at 37 °C in PBS buffer (pH ≈ 7.3) to cleave the sulfonate and disconnect the ConA-HRP from the well. Finally, the activity of the HRP that remains immobilized was determined by measuring the absorbance. Results demonstrate that both concentration and reaction time influence the rate of the decoupling reaction and support the feasibility of displacing the sulfonate group by intracellular thiols (Figure 1c, see also Figure S3 in the Supporting Information).

A similar experimental approach was assayed with GSH (**11**). As shown in Figure 1c, GSH (**11**) also displaces the sulfonate group. At concentrations of thiol ≥ 10 mM, β-mercaptoethanol (**4g**) shows better performance than GSH in the nucleophilic decoupling, as can be deducted from the lower peroxidase activity observed in the former which can be attributed to a higher degree of sulfonate cleavage in this case. However, in the 0.5–10 mM range, which corresponds to the intracellular concentrations of GSH,[33b] both **4g** and **11** display a similar activity.

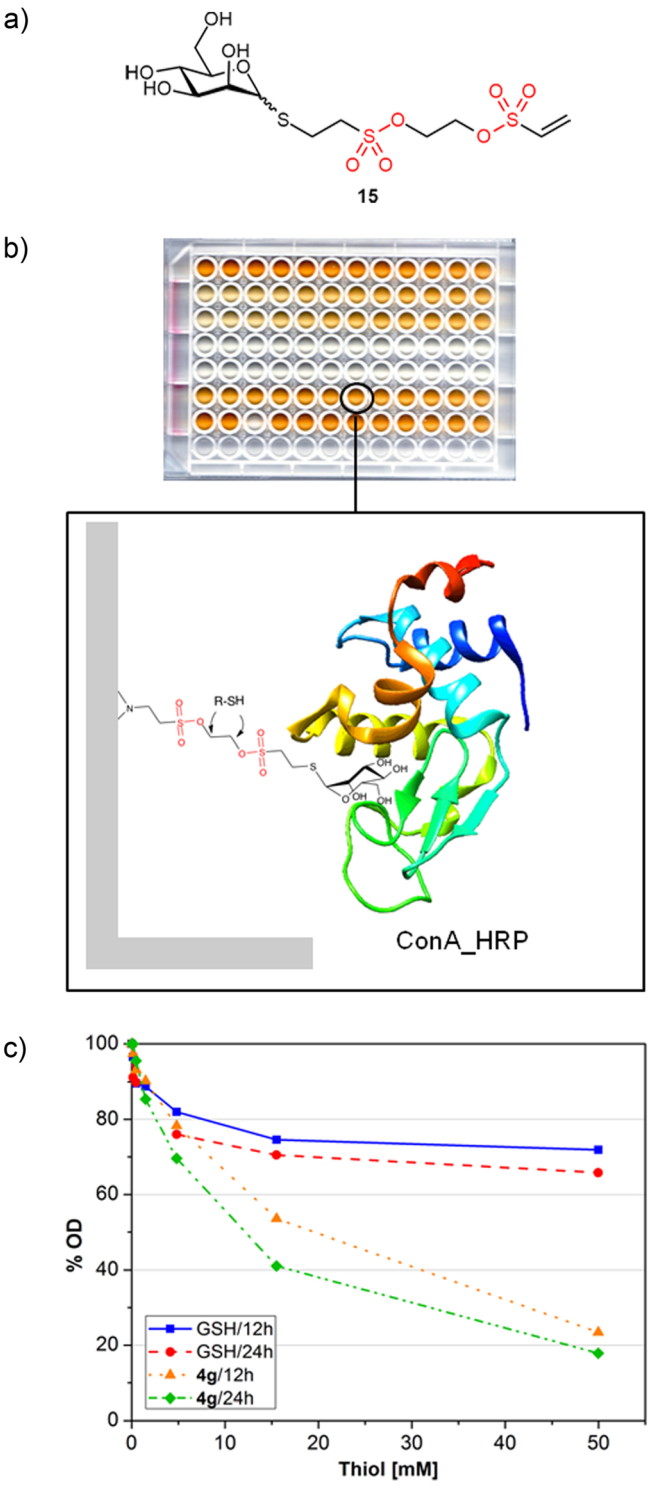
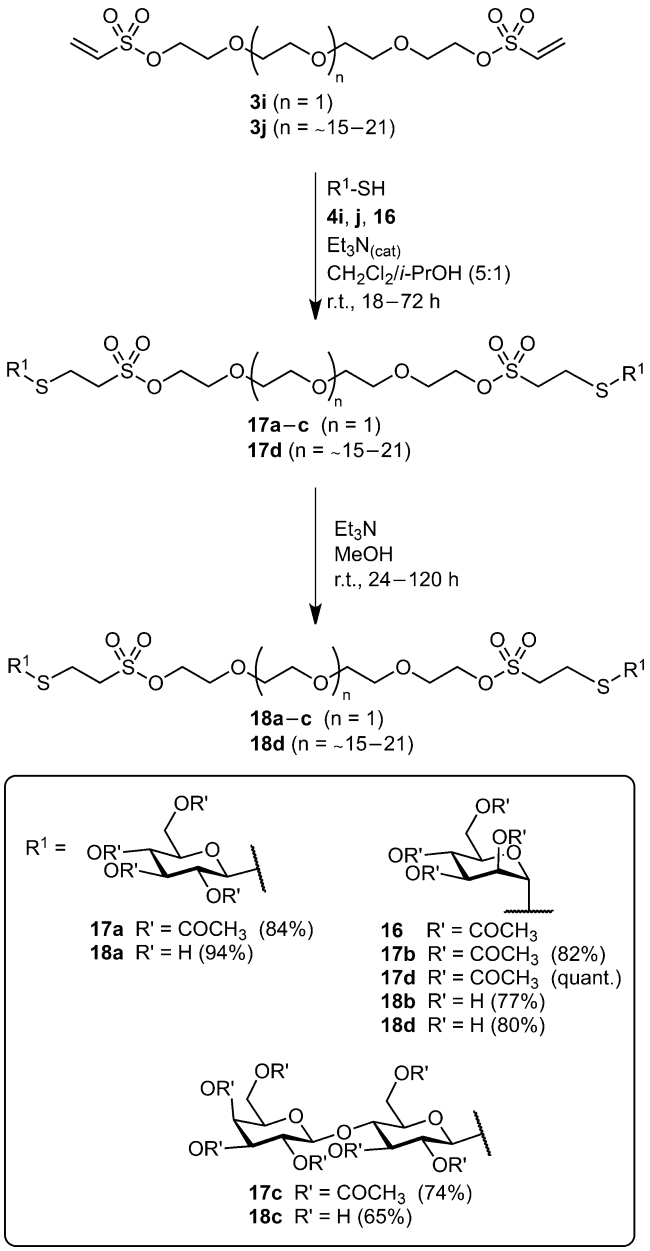


Figure 1. Displacement of the VSO group in conditions compatible with biological systems: **a**) Chemical structure of compound **15**; **b**) experimental set-up; **c**) comparison of the displacement by GSH or β-mercaptoethanol (**4g**).

Cleavable Multivalent Glycosylated Systems

To further illustrate the potential of VSO-CAD chemistry, we employed this methodology in the synthesis of cleavable homogeneous as well as heterogeneous multivalent glycosylated systems.

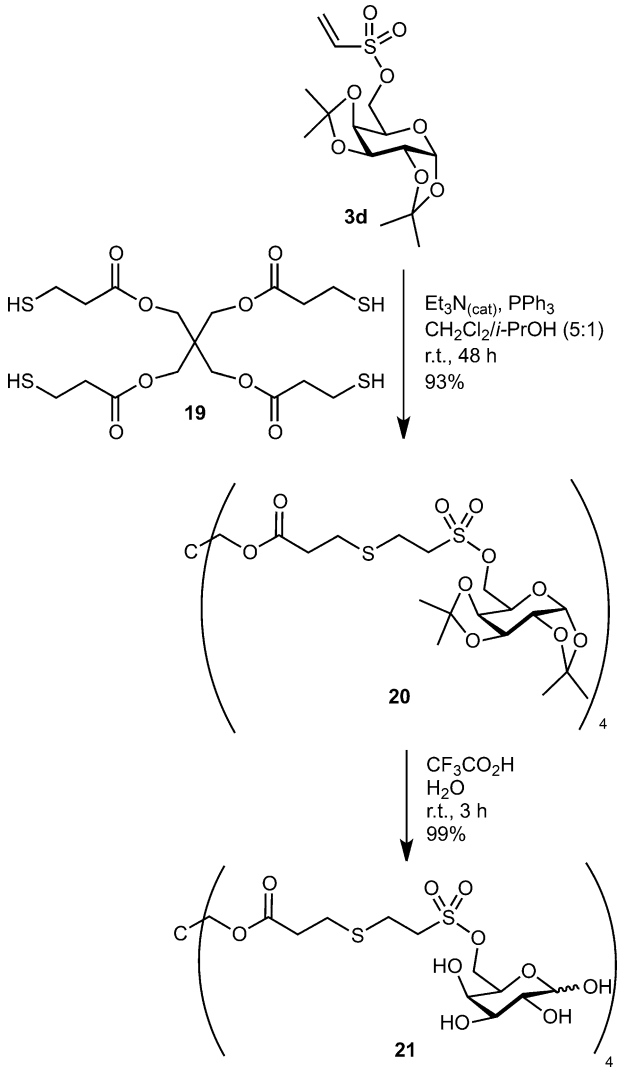
Initially, we prepared different homogeneous divalent systems bearing two sugar units of identical configuration: β-D-glucopyranose, α-D-mannopyranose and β-D-lactose (**18a–d**) (Scheme 3). These homodimers were obtained through thiol-Michael addition[35] of the corresponding per-*O*-acetylated 1-thiosugar derivatives **4i**, **j** and **16**[36] to bis-VSO linkers **3i** or **3j** under the optimized reaction conditions previously established, affording compounds **17a–d** in good yields (74–100%, Scheme 3), and subsequent removal of the *O*-acetyl protecting groups by treatment with Et_3N in MeOH at room temperature for 24–120 h with good yields (65–94%).



Scheme 3. Synthesis of homogeneous divalent glycosylated derivatives **18a–d**.

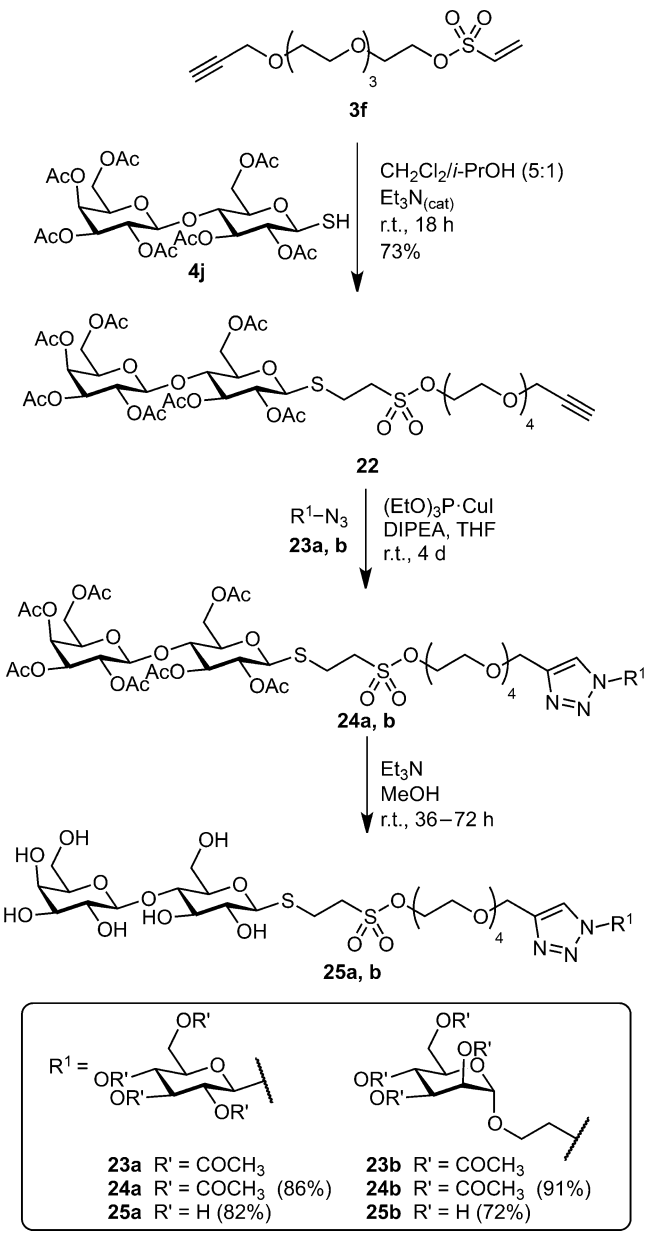
Moreover, we also synthesized the homogeneous tetravalent glycosylated system **21** incorporating four D-galactopyranose moieties (Scheme 4). Conversely to the precedent case, the strategy was based on the use of the tetrathiol scaffold **19**, derived from pentaerythritol, that was reacted with a VSO-sugar, the α-D-galactopyranose derivative **3d**, that bears a VSO group at the C-6 position. The target product **21** was easily obtained in 92% yield through a Michael-type addition reaction followed by cleavage of the *O*-acetal protecting groups with trifluoroacetic acid.

In a further step, we prepared heterogeneous glycosylated systems taking advantage of the orthogonality

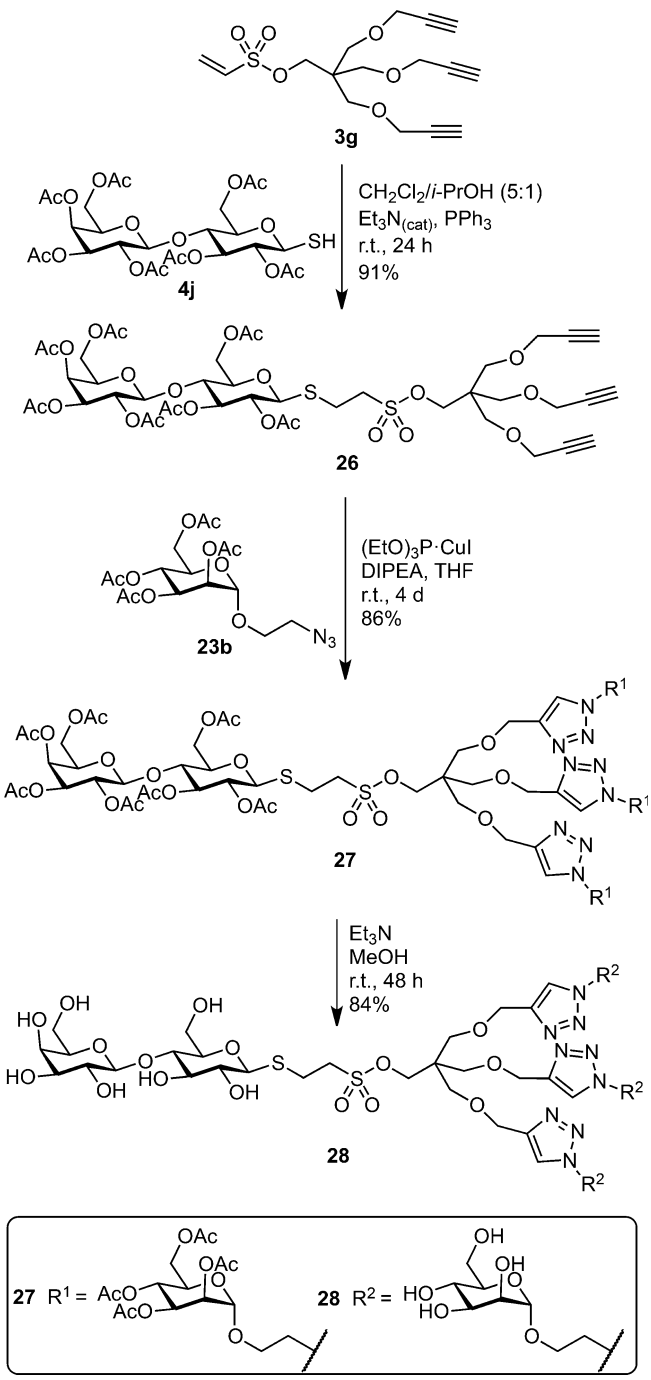


Scheme 4. Synthesis of the homogeneous tetravalent glycosylated system **21**.

between the CuAAC reaction and the Michael-type addition to VSO moieties. We envisaged the synthesis of heterodimeric glycosylated compounds starting from hetero-bifunctional alkynyl-VSO linkers such as **3f**[37] (Scheme 5). These linkers could be versatile reagents for conjugation chemistry as they provide access to cleavable double click adducts. Following the coupling strategy based on click thiol-Michael addition, adduct **22** was obtained in 73% yield from bis-VSO **3f** and per-*O*-acetylated 1-thio-lactose **4j**. Subsequent CuAAC reaction of the alkyne moiety in **22** with 2,3,4,6-tetra-*O*-acetyl-1-azido-β-D-glucopyranose[38] (**23a**) or 1-azidoethyl-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose[39] (**23b**) using $(EtO)_3P{\cdot}CuI$ as catalyst resulted in the connection of the second carbohydrate unit, affording compounds **24a**, **b** in good yields (86–91%), which were subsequently deprotected to yield the target compounds **25a**, **b** in 72–82%.



Scheme 5. Synthesis of heterogeneous divalent glycosylated systems **25a**, **b**.



Scheme 6. Synthesis of the heterogeneous tetravalent glycosylated system **28**.

Finally, following the same modular double click strategy, we synthesized a tetravalent glycosylated system exhibiting one lactose and three mannose units (Scheme 6), starting from a scaffold incorporating three alkyne and one VSO functions (**3g**).[40] Thiol-Michael addition of **4j** to the vinyl sulfonate and subsequent CuAAC with **23b** afforded compound **27** in 78% overall yield. De-*O*-acetylation of **27** gave the desired tetravalent glycosylated system **28**.

As proof of concept to verify the cleavable character of the multivalent systems developed and as additional evaluation of the potential interest of the VSO chemistry for reversible conjugation in the context of life sciences, compound **25b** was selected as a model to couple two lectins that can be decoupled by intracellular GSH (**11**). The affinity of ConA for mannose and that of the peanut agglutinin (PNA) for galactose makes them the lectins of choice.[34] For such an end ConA was adsorbed onto a microtiter well, incubated with compound **25b** and then with PNA conjugated with horseradish peroxidase (PNA-HRP) (Figure 2a). The peroxidase activity was assayed after incubation with different concentrations of GSH at 37 °C for

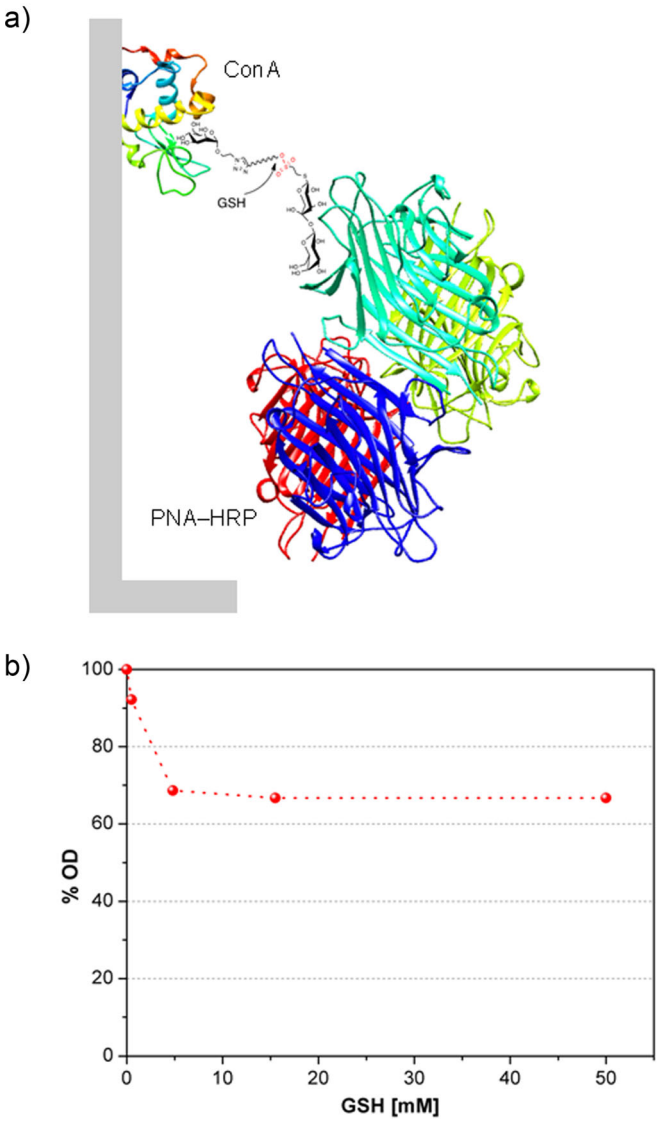


Figure 2. Evaluation of compound **25b** as a reversible linker to couple/decouple two different lectins (ConA and PNA): **a**) Experimental set-up; **b**) reduction of the peroxidase activity of PNA-HRP as a consequence of the decoupling by GSH at different concentrations.

20 h. As depicted in Figure 2b, increasing concentrations of GSH reduce the percentage of peroxidase activity down to 67%, a reduction similar to that achieved in the evaluation of **15**, (Figure 1c) where a physiological concentration of GSH (5 mM) yields a reduction of the activity down to 69%.

Conclusions

We have demonstrated that the vinyl sulfonate group is an efficient tool for the connection and subsequent disconnection of molecular units in CAD chemistry. The connection step, based on click Michael-type addition reactions of different thiols, amines and alkoxides to the VSO group, afforded excellent results in model systems with reactions taking place under mild conditions with excellent yields. The disconnection is also easily attainable by means of nucleophilic (C–O bond cleavage in alkyl VSO) or hydrolytic (S–O bond cleavage in both alkyl and aryl VSO) displacement of the sulfonate adducts. In particular, the nucleophilic substitution with thiols is especially relevant as it proceeds under milder conditions, opening the possibility of implementing this strategy in biological applications. Moreover, the Michael-type addition-based coupling step is orthogonal with the CuAAC reaction enabling a synergism between both click reactions when using alkynyl-VSO bifunctional compounds that behave as cleavable linkers in more advanced double click strategies. These alkynyl-VSO systems thus are potential versatile reagents for reversible conjugation processes.

The potential of the VSO-CAD chemistry developed has been demonstrated in two relevant biological cases: the decoupling with GSH under conditions compatible to those in the living systems and the synthesis of a series of homo- and hetereogeneous multivalent glycosylated systems. In this latter case, bis-VSO and alkynyl-VSO bifunctional compounds are cornerstone elements that behave as clickable-cleavable linkers in a one-step (homo systems) or a modular approach (hetero systems), respectively, in conjunction with adequately derivatized thiol and azido sugars. As proof of concept, we have demonstrated the cleavable character of these systems by using one of them as a reversible linker for the non-covalent assembly of two different lectins that can be subsequently decoupled with intracellular concentrations of GSH in conditions compatible to those of the living systems.

Experimental Section

General Methods

Unless otherwise noted, commercially available reagents and solvents were used as purchased without further purification. 1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranose (**1d**),[41] 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (**4i**),[27] 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-(β-D-galactopyranosyl)-(1→4)-1-thio-β-D-glucopyranose (**4j**),[42] 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranose (**16**),[36] 2,3,4,6-tetra-*O*-acetyl-1-azido-β-D-glucopyranose (**23a**),[43] 1-azidoethyl-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranose (**23b**),[39] compounds **1f**,[44] **1g**,[40] **7**[45] and $(EtO)_3P{\cdot}CuI$[32a] were prepared according to literature procedures. TLC was performed on Merck Silica gel 60 F_{254} aluminum sheets. The TLC plates were stained with sulfuric acid (5% v/v in ethanol), potassium permanganate (1% w/v) in water or ninhydrin (0.3% w/v) in ethanol, or observed under UV light when applicable.

Flash column chromatography was performed with Silica gel 60 (Merck, 230–400 mesh ASTM). ^{1}H and ^{13}C NMR spectra were recorded at room temperature on a Varian Inova Unity (300 MHz) or Varian Direct Drive (400 MHz or 500 MHz) spectrometers. Chemical shifts are given in ppm and referenced to the signal of the residual protiated solvent (^{1}H: $\delta = 7.26$ for $CDCl_3$ and $\delta = 4.79$ for D_2O at room temperature, ^{13}C: $\delta = 77.16$ for $CDCl_3$). Electrospray ionization (ESI) or atmospheric-pressure chemical ionization (APCI) mass spectra were recorded with a Waters LCT Premier XE spectrometer (TOF). NALDI mass spectra were recorded on a Bruker Autoflex spectrometer. Melting points were measured with a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were recorded on a Perkin–Elmer 341 polarimeter at room temperature. IR spectra were recorded with a Perkin–Elmer Spectrum Two FTIR ATR spectrometer.

General Procedure for the Formation of VSO 3a–g

Under an inert atmosphere, a solution of alcohol **1a–g** and Et_3N (5.0 equiv.) in anhydrous CH_2Cl_2 (5–25 mL) was added to a solution of 2-chloroethanesulfonyl chloride (**2**, 1.5–2.0 equiv.) in anhydrous CH_2Cl_2 (5–20 mL), cooled in a water-ice bath. The mixture was stirred for 1 h at 0–4 °C under an inert atmosphere. Saturated $NaHCO_3$ was added and the phases separated. The organic layer was washed with water, dried with anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude residue was purified by column chromatography to afford the corresponding vinyl sulfonate derivatives **3a–g**.

Hexadecyl vinylsulfonate (3a): Obtained according to the general procedure from cetyl alcohol (**1a**, 400 mg, 1.65 mmol) as a white solid after column chromatography (SiO_2, CH_2Cl_2/hexane 1:1); yield: 514 mg (93%); mp 34–35 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 6.53$ (dd, $J = 16.6$, 9.6 Hz, 1H), 6.40 (d, $J = 16.7$ Hz, 1H), 6.11 (d, $J = 9.6$ Hz, 1H), 4.11 (t, $J = 6.6$ Hz, 2H), 1.71 (m, 2H), 1.40–1.25 (m, 26H), 0.86 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 132.7$, 130.0, 71.2, 32.1, 29.8, 29.8, 29.7, 29.7, 29.5, 29.5, 29.1, 29.1, 25.5, 22.8, 14.3; IR (neat): $\nu = 2917$, 2849, 1470, 1352, 1167 cm^{-1}.

4-(*tert*-Butyl)phenyl vinylsulfonate (3b): Obtained according to the general procedure from 4-(*tert*-butyl)phenol (**1b**, 600 mg, 1.65 mmol) as a colourless oil after column chromatography (SiO_2, hexane/Et_2O 4:1 to 2:1); yield: 683 mg (71%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.38$ (d, $J = 8.7$ Hz, 2H), 7.14 (d, $J = 8.8$ Hz, 2H), 6.67 (dd, $J = 16.6$, 10.0 Hz, 1H), 6.36 (d, $J = 16.7$ Hz, 1H), 6.15 (d, $J = 9.9$ Hz, 1H), 1.31 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 150.5$, 147.2, 132.3, 131.7, 126.9, 121.7, 34.7, 31.5; IR (neat): $\nu = 1503$, 1370, 1177, 1149 cm^{-1}.

Compound 3c: Obtained according to the general procedure from **1c** (see the Supporting Information for synthetic details) (150 mg, 0.53 mmol) as a colourless oil after column chromatography (SiO_2, CH_2Cl_2/MeOH 97:3); yield: 172 mg (87%); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.76$ (d, $J = 8.8$ Hz, 2H), 6.92 (d, $J = 8.8$ Hz, 2H), 6.63 (br, 1H), 6.56 (dd, $J = 16.6$, 9.9 Hz, 1H), 6.38 (d, $J = 16.7$ Hz, 1H), 6.08 (d, $J = 10.0$ Hz, 1H), 4.24 (m, 2H), 3.84 (s, 3H), 3.74 (m, 2H), 3.65 (m, 8H); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 167.1$, 162.2, 132.5, 130.1, 128.8, 126.6, 113.7, 70.7, 70.2, 69.9, 69.4, 68.8, 55.4, 39.7; HR-MS (ESI$^+$): $m/z = 374.1262$, calcd. for $C_{16}H_{24}NO_7S$ $[M+H]^+$: 374.1273; IR (neat): $\nu = 1605$, 1502, 1352, 1251, 1169 cm^{-1}.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-vinylsulfonyl-α-D-galactopyranose (3d): Obtained according to the general procedure from 1,2,3,4-di-*O*-isopropylidene-α-D-galactopyranose (**1d**, 450 mg, 1.73 mmol) as a yellowish solid after column chromatography (SiO_2, hexane/Et_2O 1:1); yield: 551 mg (91%); mp 131–132 °C; $[\alpha]_D^{22}$: −63.7° (c 1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.60$ (dd, $J = 16.7$, 9.9 Hz, 1H), 6.42 (d, $J = 16.7$ Hz, 1H), 6.11 (d, $J = 9.9$ Hz, 1H), 5.51 (d, $J = 4.9$ Hz, 1H), 4.62 (dd, $J = 7.9$, 2.5 Hz, 1H), 4.33 (dd, $J = 5.0$, 2.5 Hz, 1H), 4.29 (dd, $J = 10.8$, 5.1 Hz, 1H), 4.23 (m, 2H), 4.09 (ddd, $J = 7.1$, 5.1, 1.9 Hz, 1H), 1.53 (s, 3H), 1.42 (s, 3H), 1.33 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): $\delta = 132.7$, 130.2, 109.9, 109.1, 96.3, 70.8, 70.7, 70.5, 69.2, 66.2, 26.1, 26.0, 25.0, 24.6; HR-MS (ESI$^+$): $m/z = 351.1102$, calc. for $C_{14}H_{23}O_8S$ $[M+H]^+$: 351.1114; IR (neat): $\nu = 2921$, 1361, 1253, 1212, 1171 cm^{-1}.

4-Pentyn-1-yl vinylsulfonate (3e): Obtained according to the general procedure from 4-pentyn-1-ol (**1e**, 725 mg, 8.62 mmol) as a colourless oil after column chromatography (SiO_2, hexane/Et_2O 1:1); yield: 1.26 g (84%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 6.51$ (dd, $J = 16.6$, 9.7 Hz, 1H), 6.36 (d, $J = 16.6$ Hz, 1H), 6.11 (d, $J = 9.7$ Hz, 1H), 4.18 (t, $J = 6.1$ Hz, 2H), 2.28 (td, $J = 6.8$, 2.6 Hz, 2H), 1.96 (t, $J = 2.6$ Hz, 1H), 1.88 (quint, $J = 6.5$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 132.2$, 130.5, 82.1, 69.7, 69.1, 27.7, 14.6; IR (neat): $\nu = 3290$, 1388, 1356, 1168, 1009 cm^{-1}.

Propargyl vinylsulfonyl tetraethylene glycol (3f): Obtained according to the general procedure from **1f** (650 mg, 2.80 mmol) after column chromatography (SiO_2, EtOAc) as a brown oil; yield: 807 mg (89%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 6.59$ (dd, $J = 16.7$, 9.9 Hz, 1H), 6.36 (d, $J = 16.6$ Hz, 1H), 6.09 (d, $J = 9.9$ Hz, 1H), 4.22 (m, 2H), 4.15 (d, $J = 2.4$ Hz, 2H), 3.71 (m, 2H), 3.64 (m, 4H), 3.61 (m, 8H), 2.41 (t, $J = 2.4$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 132.7$, 130.1, 79.7, 74.6, 70.7, 70.6, 70.6, 70.4, 69.8, 69.1, 68.8, 58.4; HR-MS (ESI$^+$): $m/z = 323.1155$, calcd. for $C_{13}H_{23}O_7S$ $[M+H]^+$: 323.1164; IR (neat): $\nu = 3284$, 2870, 2119, 1745, 1456, 1351, 1169, 1093, 916 cm^{-1}.

Tripropargyl vinylsulfonyl pentaerythritol (3g): Obtained according to the general procedure from **1g** (300 mg, 1.20 mmol) as a brown syrup without column chromatography; yield: 408 mg (99%); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 6.53$ (dd, $J = 16.6$, 9.7 Hz, 1H), 6.37 (d, $J = 16.7$ Hz, 1H), 6.12 (d, $J = 9.7$ Hz, 1H), 4.08 (m, 8H), 3.49 (s, 6H), 2.42 (t, $J = 2.3$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 132.0$, 130.6, 79.6, 74.7, 69.5, 67.9, 58.7, 44.4; HR-MS (ESI$^+$): $m/z = 341.1058$, calc. for $C_{16}H_{21}O_6S$ $[M+H]^+$: 341.1059; IR (neat): $\nu = 3277$, 2119, 1745, 1479, 1339, 1169, 1085 cm^{-1}.

General Procedure for the Formation of Vinyl Sulfonates 3h–j

Under an inert atmosphere, a solution of alcohol **1h–j** and Et_3N (10.0 equiv.) in anhydrous CH_2Cl_2 (10–35 mL) was added to a solution of 2-chloroethanesulfonyl chloride (**2**, 3.0 equiv.) in anhydrous CH_2Cl_2 (5–30 mL) cooled in a water-ice bath. The mixture was stirred for 1 h at 0–4 °C under an inert atmosphere. Saturated $NaHCO_3$ was added and the phases separated. The organic layer was washed

with water, dried with anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude residue was purified by column chromatography to afford the corresponding vinyl sulfonate derivatives **3h–j**.

Bis(vinylsulfonyl) ethylene glycol (3h): Obtained according to the general procedure from ethylene glycol (**1h**, 500 mg, 8.06 mmol) as a brown syrup after column chromatography (SiO_2, CH_2Cl_2/EtOAc 9:1); yield: 945 mg (48%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.57 (dd, J = 16.6, 9.6 Hz, 2H), 6.44 (d, J = 16.7 Hz, 2H), 6.19 (d, J = 9.5 Hz, 2H), 4.34 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 132.1, 131.4, 67.3; HR-MS (ESI$^+$): m/z = 264.9819; calcd. for $C_6H_{10}O_6S_2Na$ [M+Na]$^+$: 264.9816; IR (neat): ν = 1354, 1167, 1040, 915, 778 cm^{-1}.

Bis(vinylsulfonyl) tetraethylene glycol (3i): Obtained according to the general procedure from tetraethylene glycol (**1i**, 1.50 g, 7.73 mmol) as a pale yellow syrup after column chromatography (SiO_2, EtOAc/CH_2Cl_2 4:1); yield: 1.58 g (55%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.62 (dd, J = 16.6, 9.9 Hz, 2H), 6.41 (d, J = 16.7 Hz, 2H), 6.12 (d, J = 9.9 Hz, 2H), 4.27 (m, 4H), 3.76 (m, 4H), 3.65 (s, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 132.7, 130.2, 70.8, 70.7, 69.8, 68.8; HR-MS (ESI$^+$): m/z = 375.0780; calcd. for $C_{12}H_{23}O_9S_2$ [M+H]$^+$: 375.0784; IR (neat): ν = 2869, 1460, 1350, 1167, 1132, 913, 784 cm^{-1}.

Bis(vinylsulfonyl) polyethylene glycol (3j): Obtained according to the general procedure from PEG1000 (**1j**, 1.08 g, 1.08 mmol) after column chromatography (SiO_2, CH_2Cl_2/MeOH 95:5) as a colourless syrup; yield: 1.26 g (99%); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.61 (dd, J = 16.6, 10.0 Hz, 2H), 6.39 (d, J = 16.7 Hz, 2H), 6.11 (d, J = 10.0 Hz, 2H), 4.25 (m, 4H), 3.74 (m, 4H), 3.62 (s, 88H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 132.8, 130.1, 70.9, 70.7, 70.7, 69.8, 68.9; HR-MS (ESI$^+$): m/z = 1096.5295 (n = 20) 1140.5558 (n = 21), 1184.5762 (n = 22), 1228.6028 (n = 23), 1272.6378 (n = 24); calcd. for $(C_2H_4O)_nC_4H_{10}NO_5S_2$ [M+NH$_4$]$^+$: 1096.5243 (n = 20) 1140.5505 (n = 21), 1184.5767 (n = 22), 1228.6030 (n = 23), 1272.6291 (n = 24); IR (neat): ν = 3507, 2868, 1722, 1644. 1454, 1350, 1170, 1091, 919 cm^{-1}.

General Procedure for the Michael-Type Addition of Nucleophiles to Vinyl Sulfonates (Conditions A)

Under an inert atmosphere, to a solution of vinyl sulfonate **3a** or **3b** (0.13–1.25 mmol) in CH_2Cl_2/2-propanol (5:1, 6–18 mL), previously degassed when thiols are used, the corresponding nucleophile (**4a–d**, **g**) (2.0 equiv.) and a catalytic amount of Et_3N were added. The mixture was stirred at room temperature for 18–20 h. The solvent was evaporated under reduced pressure and the residue was dissolved in CH_2Cl_2 and washed with H_2O. The organic layer was dried with Na_2SO_4, filtered and the residue purified by column chromatography to yield the Michael adducts **5a–d**, **g–i**.

Hexadecyl 2-(2-hydroxyethylamino)ethanesulfonate (5a): Obtained according to the general procedure from **3a** (125 mg, 0.38 mmol) and ethanolamine (**4a**) as a white solid after column chromatography (SiO_2, CH_2Cl_2/MeOH 92:8); yield: 136 mg (92%); mp 56–57 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.19 (t, J = 6.6 Hz, 2H), 3.61 (m, 2H), 3.27 (t, J = 6.3 Hz, 2H), 3.10 (t, J = 6.4 Hz, 2H), 2.75 (m, 2H), 2.54 (br, 2H), 1.69 (m, 2H), 1.22 (m, 26H), 0.83 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 70.3, 60.8, 50.9, 50.2, 43.5, 32.0, 29.7, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 25.5, 22.7, 14.2; HR-MS (ESI$^+$): m/z = 394.2973, calcd. for $C_{20}H_{44}NO_4S$ [M+H]$^+$: 394.2991; IR (neat): ν = 2917, 2849, 1469, 1346, 1165 cm^{-1}.

Hexadecyl 2-[bis(2-hydroxyethyl)amino]ethanesulfonate (5b): Obtained according to the general procedure from **3a** (150 mg, 0.45 mmol) and diethanolamine (**4b**) as a white solid; yield: 193 mg (98%); mp 55–57 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.20 (t, J = 6.6 Hz, 2H), 3.59 (t, J = 5.0 Hz, 4H), 3.27 (m, 4H), 3.05 (t, J = 6.4 Hz, 2H), 2.64 (t, J = 5.0 Hz, 4H), 1.70 (m, 2H), 1.22 (m, 26H), 0.83 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 70.3, 59.5, 56.6, 48.7, 48.6, 32.0, 29.7, 29.7, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 25.5, 22.7, 14.2; HR-MS (ESI$^+$): m/z = 438.3256, calcd. for $C_{22}H_{48}NO_5S$ [M+H]$^+$: 438.3253; IR (neat): ν = 2917, 2850, 1361, 1341, 1158 cm^{-1}.

Hexadecyl 2-morpholinoethanesulfonate (5c): Obtained according to the general procedure from **3a** (75.0 mg, 0.23 mmol) and morpholine (**4c**) as a yellow oil; yield: 94.8 mg (99%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.21 (t, J = 6.6 Hz, 2H), 3.68 (m, 4H), 3.26 (m, 2H), 2.84 (m, 2H), 2.47 (t, J = 4.6 Hz, 4H), 1.72 (m, 2H), 1.24 (m, 26H), 0.86 (t, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 70.3, 66.9, 53.4, 52.3, 47.9, 32.0, 29.8, 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, 29.1, 25.6, 22.8, 14.2; HR-MS (ESI$^+$): m/z = 420.3157, calcd. for $C_{22}H_{46}NO_4S$ [M+H]$^+$: 420.3148; IR (neat): ν = 2917, 2848, 1351, 1160, 1118 cm^{-1}.

Hexadecyl 2-(dibutylamino)ethanesulfonate (5d): Synthesized following the general procedure from **3a** (125.0 mg, 0.38 mmol) and dibutylamine (**4d**) as a yellow oil after column chromatography (SiO_2, hexane/Et_2O 2:1); yield: 173 mg (99%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.19 (t, J = 6.6 Hz, 2H), 3.19 (m, 2H), 2.96 (m, 2H), 2.40 (t, J = 7.3 Hz, 4H), 1.71 (quint, J = 6.8 Hz, 2H), 1.47–1.24 (m, 34H), 0.87 (m, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 69.9, 53.8, 47.8, 47.7, 32.0, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 29.3, 29.1, 25.6, 22.8, 20.6, 14.2, 14.1; HR-MS (ESI$^+$): m/z = 462.3972, calcd. for $C_{26}H_{56}NO_3S$ [M+H]$^+$: 462.3981; IR (neat): ν = 2915, 1472, 1348, 1157 cm^{-1}.

Hexadecyl 2-methoxyethanesulfonate (5e) (Conditions B): To a solution of **3a** (50.0 mg, 0.15 mmol) in anhydrous THF or MeOH (5 mL) a solution of NaOMe (**4e**) in MeOH (1 M, 376 µL, 0.376 mmol) was added. The mixture was stirred at room temperature for 1–2 h under an argon atmosphere. The excess of NaOMe was neutralized with Amberlite® IRA-120H. The resin was removed by filtration and the solvent evaporated under reduced pressure to yield **5e** as a brown syrup; yield: 55 mg (quant.); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.21 (t, J = 6.6 Hz, 2H), 3.80 (t, J = 6.3 Hz, 2H), 3.37 (s, 3H), 3.34 (t, J = 6.1 Hz, 2H), 1.72 (quint, J = 6.7 Hz, 2H), 1.25 (m, 26H), 0.88 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 70.7, 66.1, 59.0, 50.4, 32.0, 29.8, 29.8, 29.7, 29.6, 29.6, 29.5, 29.3, 29.2, 25.5, 22.8, 14.2; HR-MS (ESI$^+$): m/z = 387.2527, calcd. for $C_{19}H_{40}O_4SNa$ [M+Na]$^+$: 387.2545; IR (neat): ν = 2916, 2850, 1473, 1403, 1347 cm^{-1}.

Compound 5f (Conditions C): To a solution of **3a** (50 mg, 0.15 mmol) and 1,2,5,6-di-*O*-isopropylidene-α-D-glucofuranose (**4f**) (78 mg, 0.30 mmol) in anhydrous THF (5 mL), *t*-BuOK (3.0 mg, 0.03 mmol) was added. The mixture was stirred for 18 h at room temperature under an argon atmosphere. The solvent was removed under vacuum and the resi-

due purified by column chromatography (SiO_2, Et_2O/hexane 1:1) to afford **5f** as a colourless syrup; yield: 60 mg (67%); $[\alpha]_D^{22}$: −14.2° (c 1, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ = 5.83 (d, J = 3.6 Hz, 1H), 4.57 (d, J = 3.7 Hz, 1H), 4.22 (m, 3H), 4.10–3.96 (m, 5H), 3.90 (d, J = 2.9 Hz, 1H), 3.37 (m, 2H), 1.72 (m, 2H), 1.48 (s, 3H), 1.41(s, 3H), 1.34 (s, 3H), 1.30 (s, 3H), 1.25 (m, 26H), 0.87 (t, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 112.1, 109.3, 105.4, 83.0, 82.6, 81.1, 72.4, 70.5, 67.6, 64.4, 50.5, 32.0, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 29.3, 29.2, 27.0, 26.9, 26.3, 25.6, 25.5, 22.8, 14.2; HR-MS (ESI$^+$): m/z = 593.3727, calcd. for $C_{30}H_{57}O_9S$ $[M+H]^+$: 593.3723; IR (neat): ν = 2917, 2850, 1472, 1380, 1355 cm^{-1}.

Hexadecyl 2-(2-hydroxyethylthio)ethanesulfonate (5g): Obtained according to the general procedure from **3a** (350 mg, 1.05 mmol) and 2-mercaptoethanol (**4g**) as a white solid after column chromatography (SiO_2, Et_2O/hexane 1:1); yield: 424 mg (98%); mp 69–70 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 4.19 (t, J = 6.6 Hz, 2H), 3.74 (q, J = 5.3 Hz, 2H), 3.34 (m, 2H), 2.93 (m, 2H), 2.72 (t, J = 5.9 Hz, 2H), 2.58 (br, 1H), 1.70 (quint, J = 6.8 Hz, 2H), 1.22 (m, 26H), 0.84 (t, J = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 70.5, 61.1, 50.6, 35.2, 31.9, 29.7, 29.7, 29.6, 29.5, 29.4, 29.4, 29.2, 29.0, 25.4, 25.3, 22.7, 14.1; HR-MS (ESI$^+$): m/z = 433.2415, calcd. for $C_{20}H_{42}NO_4S_2Na$ $[M+Na]^+$: 433.2422; IR (neat): ν = 2917, 2849, 1471, 1340, 1159, 1037 cm^{-1}.

4-(*tert*-Butyl)phenyl 2-(2-hydroxyethylamino)ethanesulfonate (5h): Prepared following the general method from **3b** (120 mg, 0.50 mmol) and ethanolamine (**4a**) as a yellowish solid after column chromatography (SiO_2, CH_2Cl_2/MeOH 95:5); yield: 138 mg (92%); mp 83–84 °C; ^1H NMR (300 MHz, $CDCl_3$): δ = 7.37 (d, J = 8.6 Hz, 2H), 7.16 (d, J = 8.7 Hz, 2H), 3.61 (m, 2H), 3.43 (t, J = 6.4 Hz, 2H), 3.19 (t, J = 6.4 Hz, 2H), 2.75 (m, 3H), 1.27 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 150.5, 146.7, 126.9, 121.4, 60.8, 50.9, 50.1, 43.3, 34.6, 31.3; HR-MS (ESI$^+$): m/z = 302.1411, calcd. for $C_{14}H_{24}NO_4S$ $[M+H]^+$: 302.1426; IR (neat): ν = 1371, 1150, 1114, 1107, 1058, 1012 cm^{-1}.

4-(*tert*-Butyl)phenyl 2-(2-hydroxyethylthio)ethanesulfonate (5i): Obtained according to the general method from **3b** (300 mg, 1.25 mmol) and 2-mercaptoethanol (**4g**). The resulting mixture was extracted with saturated NaCl (2× 10 mL) and the organic layer dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (SiO_2, Et_2O/hexane 1:1) to afford **5i** as a colourless syrup; yield: 338 mg (85%). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.39 (d, J = 8.7 Hz, 2H), 7.17 (d, J = 8.8 Hz, 2H), 3.75 (t, J = 6.0 Hz, 2H), 3.51 (m, 2H), 3.06 (m, 2H), 2.73 (t, J = 6.0 Hz, 2H), 2.66 (br, 1H), 1.29 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 150.5, 146.6, 126.9, 121.3, 61.1, 50.5, 35.1, 34.6, 31.3, 25.2; HR-MS (ESI$^+$): m/z = 319.1042, calcd. for $C_{14}H_{23}O_4S_2$ $[M+H]^+$: 319.1038; IR (neat): ν = 1503, 1364, 1174, 1147 cm^{-1}.

Bis(tritylthioethanesulfonate) tetraethylene glycol (5j) (Conditions D): A solution of **3i** (50 mg, 0.134 mmol) and trityl thiol (**4h**) (147 mg, 0.534 mmol) and a catalytic amount of Et_3N in CH_2Cl_2/2-propanol (5:1, 6 mL) was stirred at room temperature for 18 h under an inert atmosphere. The solvent was removed under reduced pressure and the residue purified by column chromatography (SiO_2, CH_2Cl_2/EtOAc 1:0 to 4:1) to afford **5j** as a pale yellow syrup; yield: 123 mg (99%). ^1H NMR (300 MHz, $CDCl_3$): δ = 7.34 (d, J = 7.4 Hz, 12H), 7.17 (m, 18H), 4.05 (m, 4H), 3.52 (m, 12H), 2.62 (m, 8H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 144.1, 129.5, 128.2, 127.0, 70.7, 70.6, 69.3, 69.0, 67.6, 49.7, 25.2; HR-MS (ESI$^+$): m/z = 949.2543, calcd. for $C_{50}H_{54}O_9S_4Na$ $[M+Na]^+$: 949.2548; IR (neat): ν = 1596, 1493, 1444, 1353, 1165 cm^{-1}.

Compound 5k (Conditions E): Under argon, to a solution of **3e** (55.4 mg, 0.30 mmol) and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (**4i**) (174 mg, 0.48 mmol) in degassed CH_2Cl_2/2-propanol (5:1, 6 mL), PPh_3 (25.0 mg, 0.10 mmol) and a catalytic amount of Et_3N were added. The resulting mixture was stirred at room temperature for 18 h under an inert atmosphere. The solvent was removed under vacuum and the crude purified by column chromatography (SiO_2, EtOAc/hexane 6:4) as a white solid; yield: 125 mg (73%); $[\alpha]_D^{22}$: −16.5° (c 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 5.23 (t, J = 9.4 Hz, 1H), 5.04 (m, 2H), 4.56 (d, J = 10.1 Hz, 1H), 4.37 (t, J = 6.1 Hz, 2H), 4.18 (m, 2H), 3.74 (ddd, J = 10.1, 4.6, 3.0 Hz, 1H), 3.47 (m, 2H), 3.16 (ddd, J = 14.0, 10.9, 5.2 Hz, 1H), 2.99 (ddd, J = 14.1, 10.9, 5.4 Hz, 1H); 2.37 (td, J = 6.8, 2.7 Hz, 2H), 2.10 (s, 3H), 2.05 (s, 3H), 2.03 (m, 4H), 2.01 (s, 3H), 1.97 (quint, J = 6.4 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.8, 170.2, 169.5, 84.1, 82.2, 76.3, 73.6, 70.1, 69.6, 68.6, 68.3, 62.1, 51.5, 28.0, 24.2, 20.8, 20.8, 20.7, 20.7, 14.9; HR-MS (ESI$^+$): m/z = 556.1539, calcd. for $C_{21}H_{34}O_{12}S_2N$ $[M+NH_4]^+$: 556.1522; m/z = 561.1094, calcd. for $C_{21}H_{30}O_{12}S_2Na$ $[M+Na]^+$: 561.1076; IR (neat): ν = 3362, 2504, 1644, 1430, 1349, 1055 cm^{-1}.

Compound 5l: Prepared from **3e** (32.0 mg, 0.18 mmol) and (2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-1-thio-β-D-glucopyranose (**4j**) stirring for 48 h under conditions E as a colourless syrup after column chromatography (SiO_2, CH_2Cl_2/EtOAc 9:1 to 7:3); yield: 142 mg (95%); $[\alpha]_D^{22}$: −10.8° (c 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 5.30 (d, J = 3.2 Hz, 1H), 5.17 (dd, J = 11.7, 6.7 Hz, 1H), 5.05 (dd, J = 10.4, 7.9 Hz, 1H), 4.91 (m, 2H), 4.50 (m, 2H), 4.46 (d, J = 7.8 Hz, 1H), 4.32 (t, J = 6.1 Hz, 2H), 4.04 (m, 3H), 3.85 (t, J = 6.8 Hz, 1H), 3.73 (t, J = 9.5 Hz, 1H), 3.61 (ddd, J = 9.9, 5.6, 1.8 Hz, 1H), 3.47 (ddd, J = 14.4, 11.3, 5.1 Hz, 1H), 3.39 (ddd, J = 14.4, 11.2, 4.9 Hz, 1H), 3.09 (ddd, J = 14.3, 11.4, 4.9 Hz, 1H), 2.92 (ddd, J = 14.2, 11.3, 5.1 Hz, 1H), 2.32 (td, J = 6.8, 2.6 Hz, 2H), 2.10 (s, 3H), 2.10 (s, 3H), 2.02 (s, 3H), 2.00 (m, 10H), 1.95–1.90 (m, 5H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.4, 170.3, 170.1, 170.0, 169.6, 169.6, 169.1, 101.1, 83.8, 82.2, 77.1, 76.0, 73.4, 71.0, 70.8, 70.0, 69.8, 69.1, 68.5, 66.7, 61.9, 60.9, 51.4, 27.9, 24.1, 20.8, 20.7, 20.7, 20.7, 20.6, 20.5, 14.8; HR-MS (ESI$^+$): m/z = 827.2104, calcd. for $C_{33}H_{47}O_{20}S_2$ $[M+H]^+$: 827.2102; IR (neat): ν = 1741, 1366, 1212, 1040, 912 cm^{-1}.

General Procedure for Nucleophilic Decoupling of 5g (Conditions A)

A solution of sulfonate **5g** (0.14–0.5 mmol) and the corresponding nucleophile (**4a**, **c**, **l–o**) (5.0 equiv.) in DMF (6–20 mL) was stirred at 85 °C for 4 h. The solvent was evaporated under reduced pressure and the residue was dissolved in CH_2Cl_2, washed with H_2O and saturated NaCl. The organic layer was dried with anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. Compounds **6c–f** are described in the Supporting Information.

2-Hexadecylaminoethanol (6a): Obtained according to the general method from **5g** (100 mg, 0.24 mmol) and ethanola-

mine (**4a**) as a pale yellow solid; yield: 64 mg (95%); mp 50–51 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.64 (m, 2H), 2.76 (m, 2H), 2.61 (t, J = 7.2 Hz, 2H), 2.52 (br, 2H), 1.46 (m, 2H), 1.24 (m, 26H), 0.87 (t, J = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 60.8, 51.3, 49.7, 32.1, 30.0, 29.8, 29.8, 29.8, 29.8, 29.7, 29.5, 27.4, 22.8, 14.2; HR-MS (ESI$^+$): m/z = 272.2941, calcd. for $C_{17}H_{38}NO$ $[M+H]^+$: 272.2953; IR (neat): ν = 2914, 2846, 1467, 1442, 1065 cm^{-1}.

4-Hexadecylmorpholine (6b): Synthesized following the general procedure from **5g** (100 mg, 0.24 mmol) and morpholine (**4c**) in the presence of Et_3N (0.17 mL, 1.22 mmol) as a colourless oil; yield: 68 mg (79%); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.74 (m, 4H), 2.48 (m, 4H), 2.35 (m, 2H), 1.52 (m, 2H), 1.24 (m, 26H), 0.86 (t, J = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 66.8, 59.3, 53.7, 32.0, 29.8, 29.8, 29.8, 29.7, 29.7, 29.6, 29.5, 27.6, 26.4, 22.8, 14.2; HR-MS (ESI$^+$): m/z = 312.3260, calcd. for $C_{20}H_{42}NO$ $[M+H]^+$: 312.3266; IR (neat): ν = 2923, 2853, 1738, 1456, 1118 cm^{-1}.

Hexadecyl methyl ether (6g) (Conditions B): To a solution of **5g** (50.0 mg, 0.12 mmol) in anhydrous MeOH (5 mL) was added a solution of $LiOH{\cdot}H_2O$ (25.0 mg, 0.60 mmol) in anhydrous MeOH (1 mL). The mixture was stirred at 65 °C for 18 h. The solvent was removed under vacuum and the residue was redissolved in CH_2Cl_2 (15 mL) and washed with H_2O (15 mL). The organic layer was dried with anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude was purified by column chromatography (SiO_2, hexane/Et_2O 2:1) to afford **6g** as a colourless syrup; yield: 31 mg (99%). The spectroscopic data match those reported in the literature.[46] ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.36 (t, J = 6.7 Hz, 2H), 3.32 (s, 3H), 1.56 (quint, J = 6.7 Hz, 2H), 1.25 (m, 26H), 0.86 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 73.1, 58.7, 32.1, 29.9, 29.8, 29.8, 29.7, 29.5, 26.3, 22.9, 14.3.

2-Hexadecylthioetanol (6h) (Conditions C): To a solution of **5g** (85 mg, 0.21 mmol) in degassed anhydrous DMF (6 mL), 2-mercaptoethanol (**4g**) (41.0 mg, 0.52 mmol) and Cs_2CO_3 (171 mg, 0.53 mmol) were added. The mixture was stirred at room temperature for 24 h under argon. The solvent was removed under reduced pressure and the residue was dissolved in CH_2Cl_2 (25 mL) and washed with H_2O (2× 25 mL). The organic layer was dried with Na_2SO_4 and concentrated to afford **6h** as a syrup; yield: 52 mg (82%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.71 (t, J = 5.9 Hz, 2H), 2.72 (t, J = 5.9 Hz, 2H), 2.51 (t, J = 7.4 Hz, 2H), 2.25 (br, 1H), 1.57 (quint, J = 7.1 Hz, 3H), 1.25 (m, 26H), 0.87 (t, J = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 60.3, 45.9, 35.5, 32.1, 31.8, 29.9, 29.8, 29.8, 29.8, 29.7, 29.7, 29.5, 29.4, 29.0, 22.8, 14.3; HR-MS (ESI$^+$): m/z = 303.2719, calcd. for $C_{18}H_{39}OS$ $[M+H]^+$: 303.2722; IR (neat): ν = 3299, 2917, 2849, 1472, 1462, 1047 cm^{-1}.

2,3,4,6-Tetra-*O*-acetyl-1-hexadecylthio-β-D-glucopyranose (6i): Synthesized following conditions C from 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (**4i**) (188 mg, 0.52 mmol) as a white solid after column chromatography (SiO_2, Et_2O/hexane 1:1); yield: 100 mg (82%); mp 88–89 °C; $[\alpha]_D^{22}$: −25.0° (c 1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.20 (t, J = 9.3 Hz, 1H), 5.06 (t, J = 9.7 Hz, 1H), 5.02 (t, J = 9.9 Hz, 1H), 4.46 (d, J = 10.0 Hz, 1H), 4.23 (dd, J = 12.3, 4.9 Hz, 1H), 4.11 (dd, J = 12.3, 2.3 Hz, 1H), 3.68 (ddd, J = 9.9, 4.8, 2.4 Hz, 1H), 2.63 (m, 2H), 2.05 (s, 3H), 2.03 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.55 (m, 2H), 1.23 (m, 26H), 0.84 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.7, 170.3, 169.5, 169.5, 83.7, 76.0, 74.0, 70.0, 68.4, 62.3, 32.0, 30.1, 29.8, 29.8, 29.7, 29.6, 29.5, 29.3, 28.9, 22.8, 20.8, 20.7, 20.7, 14.2; HR-MS (ESI$^+$): m/z = 611.3228, calcd. for $C_{30}H_{52}O_9SNa$ $[M+Na]^+$: 611.3230; IR (neat): ν = 2916, 2849, 1740, 1366, 1225 cm^{-1}.

Procedure for Hydrolytic Decoupling Experiments of 5g, i

A solution of sulfonate **5g**, **i** (0.12–0.46 mmol) and $LiOH{\cdot}H_2O$ (5.0 equiv.) in MeOH (6 mL), MeOH/H_2O (11 mL, 10:1) or anhydrous DMF (5 mL) was stirred at the corresponding temperature (see Table 4) for 18–24 h. The reactions in MeOH or MeOH/H_2O were diluted with CH_2Cl_2 (15 mL) and H_2O (15 mL). The layers were separated and the aqueous phase was further extracted with CH_2Cl_2 (15 mL). The combined organic layers were dried with Na_2SO_4, filtered and concentrated under reduced pressure. In the DMF experiments the solvent was removed under vacuum. See the Supporting Information for full details.

Orthogonality of VSO-Based Michael-Type Addition and CuAAC Reactions

Compound 8: Under argon, to a solution of **3e** (52.0 mg, 0.30 mmol) and **7** (50.0 mg, 0.20 mmol) in degassed anhydrous THF (5 mL), $(OEt)_3P{\cdot}CuI$ (21.0 mg, 0.06 mmol) and DIPEA (145 μL, 0.60 mmol) were added. The mixture was stirred at room temperature under an inert atmosphere for 3 d. The solvent was removed under reduced pressure and the residue purified by column chromatography (SiO_2, CH_2Cl_2/hexane 70:30 to CH_2Cl_2/AcOEt 70:30) to afford **8** as a colourless syrup; yield: 82.5 mg (97%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.59 (s, 1H), 8.09 (s, 2H), 7.36 (s, 1H), 6.50 (dd, J = 16.6, 9.8 Hz, 1H), 6.33 (d, J = 16.6 Hz, 1H), 6.08 (d, J = 9.8 Hz, 1H), 5.56 (s, 2H), 4.12 (t, J = 6.1 Hz, 2H), 3.89 (s, 6H), 2.79 (t, J = 7.3 Hz, 2H), 2.08 (quint, J = 6.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.6, 136.0, 133.2, 132.3, 131.6, 130.8, 130.4, 69.8, 53.2, 52.6, 28.5, 21.5; HR-MS (ESI$^+$): m/z = 424.1170, calcd. for $C_{18}H_{22}N_3O_7S$ $[M+H]^+$: 424.1178; IR (neat): ν = 3445, 1724, 1438, 1355, 1249 cm^{-1}.

4-Pentyn-1-yl 2-morpholinoethanesulfonate (9): A solution of **3e** (100 mg, 0.57 mmol), morpholine (100 μL, 1,15 mmol) and a catalytic amount of Et_3N in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature for 16 h under argon. The resulting mixture was diluted with CH_2Cl_2 (20 mL) and washed with H_2O (25 mL). The organic layer was dried with anhydrous Na_2SO_4 and the solvent removed under vacuum to yield **9** as a colourless syrup; yield: 150 mg (quant.). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.30 (t, J = 6.1 Hz, 2H), 3.63 (t, J = 4.7 Hz, 4H), 3.25 (m, 2H), 2.80 (m, 2H), 2.43 (t, J = 4.6 Hz, 4H), 2.30 (td, J = 6.8, 2.5 Hz, 2H), 1.98 (t, J = 2.4 Hz, 1H), 1.90 (quint, J = 6.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 82.2, 69.9, 68.3, 66.7, 53.2, 52.1, 47.7, 27.9, 14.7; HR-MS (ESI$^+$): m/z = 262.1110, calcd. for $C_{11}H_{20}NO_4S$ $[M+H]^+$: 262.1113. IR (neat): ν = 3286, 2962, 2930, 2858, 2808, 1348 cm^{-1}.

Compound 10 (from 8): A solution of **8** (75 mg, 0.18 mmol), morpholine (**4c**) (30 μL, 0.35 mmol) and a cata-

lytic amount of Et_3N in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature for 18 h under argon. The resulting mixture was diluted with CH_2Cl_2 (15 mL) and washed with H_2O (20 mL). The organic layer was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to yield **10** as a syrup; yield: 88 mg (97%).

From 9: Under argon, to a solution of **9** (90 mg, 0.34 mmol) and **7** (107 mg, 0.43 mmol) in degassed anhydrous THF (5 mL), $(EtO)_3P{\cdot}CuI$ (37.0 mg, 0.10 mmol) and DIPEA (250 μL, 1.42 mmol) were added. The mixture was stirred at room temperature under an inert atmosphere for 18 h. The solvent was removed under reduced pressure and the residue purified by column chromatography (SiO_2, CH_2Cl_2/EtOAc 80:20 to CH_2Cl_2/MeOH 96:4) to afford **10** as a colourless syrup; yield: 175 mg (99%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.53 (s, 1H), 8.04 (s, 2H), 7.35 (s, 1H), 5.53 (s, 2H), 4.20 (t, J = 6.1 Hz, 2H), 3.85 (s, 6H), 3.59 (t, J = 4.6 Hz, 4H), 3.23 (t, J = 7.4 Hz, 2H), 2.76 (t, J = 7.5 Hz, 4H), 2.39 (t, J = 4.6 Hz, 4H), 2.04 (quint, J = 9.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 165.4, 135.9, 133.0, 131.4, 130.7, 121.4, 69.0, 66.6, 53.2, 53.0, 52.5, 52.0, 47.7, 28.6, 21.4; HR-MS (ESI$^+$): m/z = 511.1847, calcd. for $C_{22}H_{31}N_4O_8S$ $[M+H]^+$: 511.1863; IR (neat): ν = 2961, 1728, 1437, 1346, 1248, 1168, 1013, 998 cm^{-1}.

Coupling-and-Decoupling with GSH

Compound 12: Under an inert atmosphere, to a solution of reduced glutathione (GSH, **11**) (63.3 mg, 0.21 mmol) and $NaBH_4$ (7.0 mg, 0.187 mmol) in degassed DMF/H_2O (10 mL, 1:1) was added **3c** (70.0 mg, 0.187 mmol). The resulting mixture was stirred at room temperature for 2 h. H_2O (50 mL) was added and the solution was freeze-dried. The residue was purified by column chromatography (SiO_2, CH_3CN/H_2O 4:1). The product-containing fractions were combined, the volatiles removed under reduced pressure and the remaining solution freeze-dried to afford **12** as a white solid; yield: 104 mg (82%); $[\alpha]_D^{22}$: −13.4° (c 0.5, DMF/H_2O 1:1); mp 204–205 °C. ^{1}H NMR (500 MHz, D_2O): δ = 7.79 (d, J = 8.9 Hz, 2H), 7.08 (d, J = 8.9 Hz, 2H), 4.60 (dd, J = 8.8, 5.0 Hz, 1H), 4.39 (m, 2H), 3.90 (s, 3H), 3.86–3.71 (m, 11H), 3.59 (m, 4H), 3.10 (dd, J = 14.2, 4.9 Hz, 1H), 2.93 (m, 3H), 2.55 (td, J = 7.5, 2.2 Hz, 1H), 2.18 (q, J = 7.3 Hz, 2H); ^{13}C NMR (126 MHz, D_2O): δ = 176.0, 174.8, 173.8, 171.5, 170.0, 162.0, 129.1, 125.9, 114.0, 70.3, 69.7, 69.5, 68.9, 68.4, 55.5, 54.1, 52.8, 49.5, 43.3, 39.5, 33.0, 31.4, 26.2, 24.6; HR-MS (ESI$^+$): m/z = 681.2117, calcd. for $C_{26}H_{41}N_4O_{13}S_2$ $[M+H]^+$: 681.2112; IR (neat): ν = 2970, 1738, 1365, 1216 cm^{-1}.

Compound 14: A mixture of **13** (67.0 mg, 0.14 mmol) (see the Supporting Information for synthetic details), GSH (**11**) (65.0 mg, 0.21 mmol), Cs_2CO_3 (70 mg, 0.21 mmol) and $NaBH_4$ (8.0 mg, 0.21 mmol) in degassed DMF/H_2O (4 mL, 1:1) was stirred at room temperature under an argon atmosphere for 20 h. The resulting mixture was diluted with H_2O (20 mL) and freeze-dried. The residue was purified by column chromatography (SiO_2, CH_3CN/H_2O 4:1). The product-containing fractions were combined and the volatiles removed under vacuum. The resulting solution was freeze-dried to afford **14** as a white solid; yield: 67 mg (84%); $[\alpha]_D^{22}$: −19.0° (c 0.5, DMF/H_2O 1:1). ^{1}H NMR (500 MHz, D_2O): δ = 7.78 (d, J = 8.9 Hz, 2H), 7.08 (d, J = 8.9 Hz, 2H), 4.53 (dd, J = 8.9, 4.8 Hz, 1H), 3.90 (s, 3H), 3.81–3.67 (m, 11H), 3.60 (t, J = 5.2 Hz, 2H), 3.02 (dd, J = 14.1, 4.9 Hz, 1H), 2.82 (dd, J = 13.9, 8.9 Hz, 1H), 2.71 (t, J = 6.5 Hz, 2H), 2.54 (t, J = 7.5 Hz, 2H), 2.16 (q, J = 7.3 Hz, 2H); ^{13}C NMR (126 MHz, D_2O): δ = 176.0, 174.8, 173.9, 171.7, 170.2, 161.9, 129.1, 125.9, 114.0, 69.5, 69.4, 69.3, 68.8, 55.5, 54.1, 53.1, 43.3, 39.5, 33.1, 31.4, 31.0, 26.2; HR-MS (ESI$^+$): m/z = 573.2224, calcd. for $C_{24}H_{37}N_4O_{10}S$ $[M+H]^+$: 573.2230; IR (neat): ν = 3284, 1604, 1504, 1303, 1088 cm^{-1}.

General Procedure for the Synthesis of Protected Divalent Homogeneous Glycosylated Systems 17a–d

A solution of **3i** or **3j** (0.13–0.27 mmol), the corresponding acetylated-1-thiosugar (**4i**, **j**, **16**) (3.0–4.0 equiv.) and a catalytic amount of Et_3N in degassed CH_2Cl_2/2-propanol (5:1, 6–12 mL) was stirred at room temperature under an argon atmosphere for 18–72 h. The resulting mixture was concentrated under reduced pressure and the residue was purified by column chromatography

Compound 17a: Obtained from **3i** (50 mg, 0.13 mmol) and 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (**4i**) following the general procedure as a brown syrup after column chromatography (SiO_2, Et_2O/EtOAc 70:30); yield: 114 mg (84%); $[\alpha]_D^{22}$: −17.0° (c 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.21 (t, J = 9.4 Hz, 2H), 5.02 (t, J = 9.7, 2H), 4.99 (t, J = 9.7, 2H), 4.58 (d, J = 10.1 Hz, 2H), 4.35 (m, 4H), 4.15 (m, 4H), 3.74 (m, 6H), 3.64 (m, 8H), 3.58–3.44 (m, 4H), 3.15 (ddd, J = 14.1, 11.1, 5.1 Hz, 2H), 2.99 (ddd, J = 14.1, 11.0, 5.2 Hz, 2H), 2.08 (s, 6H), 2.02 (s, 6H), 2.01 (s, 6H), 1.98 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.7, 170.1, 169.4, 169.4, 84.2, 76.1, 73.6, 70.8, 70.6, 69.7, 69.6, 69.0, 68.3, 62.1, 51.6, 24.3, 20.7, 20.7, 20.6; HR-MS (ESI$^+$): m/z = 1103.2437, calcd. for $C_{40}H_{63}O_{27}S_4$ $[M+H]^+$: 1103.2440; IR (neat): ν = 1737, 1354, 1217, 1091, 1034 cm^{-1}.

Compound 17b: Obtained according to the general procedure from **3i** (100 mg, 0.27 mmol) and 2,3,4,6-tetra-*O*-acetyl-1-thio-α-D-mannopyranose (**16**) after column chromatography (SiO_2, CH_2Cl_2/EtOAc 1:1 to 1:4) as a colourless oil; yield: 240 mg (82%); $[\alpha]_D^{22}$: +74.0° (c 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.30 (s, 2H), 5.29 (d, J = 3.5 Hz, 2H), 5.22 (t, J = 9.8 Hz, 2H), 5.15 (dd, J = 10.0, 3.3 Hz, 2H), 4.33 (m, 6H), 4.22 (dd, J = 12.2, 6.8 Hz, 2H), 4.09 (d, J = 12.0 Hz, 2H), 3.73 (t, J = 4.4 Hz, 4H), 3.61 (m, 8H), 3.54 (dd, J = 10.2, 5.6 Hz, 2H), 3.43 (ddd, J = 14.5, 10.8, 5.2 Hz, 2H), 3.06 (m, 4H), 2.13 (s, 6H), 2.08 (s, 6H), 2.03 (s, 6H), 1.95 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.7, 169.9, 169.8, 169.7, 83.0, 70.7, 70.6, 70.6, 69.7, 69.5, 69.3, 69.0, 66.3, 62.7, 50.4, 25.2, 20.9, 20.7, 20.6; HR-MS (ESI$^+$): m/z = 1103.2406, calcd. for $C_{40}H_{63}O_{27}S_4$ $[M+H]^+$: 1103.2440; IR (neat): ν = 1742, 1368, 1219, 1167 cm^{-1}.

Compound 17c: Obtained following the general procedure from **3i** (152 mg, 0.41 mmol) and **4j** as a colourless syrup after column chromatography (SiO_2, CH_2Cl_2/MeOH 98:2); yield: 500 mg (74%); $[\alpha]_D^{22}$: −11.5° (c 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.30 (d, J = 3.4 Hz, 2H), 5.16 (t, J = 9.2 Hz, 2H), 5.05 (dd, J = 10.4, 7.8 Hz, 2H), 4.91 (m, 4H), 4.52 (d, J = 9.9 Hz, 2H), 4.47 (m, 2H), 4.32 (t, J = 4.3 Hz, 4H), 4.04 (m, 6H), 3.85 (t, J = 6.8 Hz, 2H), 3.72 (m, 6H), 3.61 (m, 10H), 3.47 (m, 2H), 3.09 (ddd, J = 14.0, 11.4, 4.9 Hz, 2H), 2.94 (ddd, J = 14.1, 11.3, 5.1 Hz, 2H), 2.10 (s, 6H), 2.09 (s, 6H), 2.02 (s, 6H), 2.00 (s, 18H), 1.92 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.4, 170.2, 170.0, 169.9,

169.5, 169.0, 101.0, 83.8, 77.0, 76.0, 73.4, 70.9, 70.7, 70.6, 70.5, 69.9, 69.5, 69.0, 68.9, 66.6, 61.9, 60.7, 51.5, 24.1, 20.7, 20.6, 20.6, 20.6, 20.4; HR-MS (ESI$^+$): m/z = 1679.4128, calcd. for $C_{64}H_{95}O_{43}S_4$ $[M+H]^+$: 1679.4130; IR (neat): ν = 1745, 1368, 1217, 1167, 1045 cm^{-1}.

Compound 17d: Obtained following the general method from **3j** (200 mg, 0.17 mmol) and **16** as a colourless syrup after purification by column chromatography (SiO_2, CH_2Cl_2/MeOH 96:4); yield: 324 mg (quant.); $[\alpha]_D^{22}$: +19.3° (c 1, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.28 (m, 4H), 5.21 (t, J = 9.9 Hz, 2H), 5.15 (dd, J = 9.9, 3.1 Hz, 2H), 4.32 (m, 6H), 4.21 (dd, J = 12.2, 6.8 Hz, 2H), 4.07 (dd, J = 12.2, 2.2 Hz, 2H), 3.72 (t, J = 4.4 Hz, 4H), 3.59 (s, 81H), 3.53 (m, 2H), 3.42 (ddd, J = 14.5, 10.9, 5.2 Hz, 2H), 3.04 (m, 4H), 2.12 (s, 6H), 2.07 (s, 6H), 2.01 (s, 6H), 1.94 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.7, 169.8, 169.7, 169.7, 83.0, 70.6, 70.6, 69.7, 69.4, 69.2, 68.9, 66.3, 62.6, 50.4, 25.2, 20.8, 20.7, 20.6; HR-MS (ESI$^+$): m/z = 921.3684 (n = 20) 943.3814 (n = 21), 965.3962 (n = 22), 987.4042 (n = 23), 1009.4241 (n = 24), calcd. for $(C_2H_4O)_nC_{32}H_{54}N_2O_{23}S_4$ $[M+2NH_4]^{2+}$: 921.3621 (n = 20), 943.3753 (n = 21), 965.3884 (n = 22), 987.4015 (n = 23), 1009.4146 (n = 24); IR (neat): ν = 2868, 1746, 1367, 1222, 1102 cm^{-1}.

Synthesis of Tetravalent Glycosylated System 21

Compound 20: Under an argon atmosphere, to a solution of **3d** (400 mg, 1.14 mmol), thiol **19** (111 mg, 0.23 mmol) and PPh_3 (36.0 mg, 0.14 mmol) in degassed CH_2Cl_2/2-propanol (18 mL, 5:1). a catalytic amount of Et_3N was added and the mixture was stirred at room temperature for 48 h. The solvent was removed under vacuum and the residue purified by column chromatography (SiO_2, CH_2Cl_2/EtOAc 9:1 to 0:1) to give **20** as a colourless oil; yield: 405 mg (93%); $[\alpha]_D^{22}$: +39.8° (c 1, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.52 (d, J = 4.9 Hz, 4H), 4.62 (dd, J = 7.9, 2.5 Hz, 4H), 4.41–4.30 (m, 12H), 4.21 (dd, J = 7.9, 2.0 Hz, 4H), 4.15 (s, 8H), 4.08 (ddd, J = 7.0, 4.6, 1.9 Hz, 4H), 3.42 (m, 8H), 2.97 (m, 8H), 2.82 (t, J = 7.1 Hz, 8H), 2.65 (t, J = 6.9 Hz, 8H), 1.52 (s, 12H), 1.43 (s, 12H), 1.32 (s, 24H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 171.1, 110.0, 109.1, 96.3, 70.8, 70.7, 70.4, 69.6, 66.4, 62.3, 50.7, 42.4, 34.5, 26.9, 26.1, 26.1, 25.4, 25.0, 24.5; HR-MS (APCI$^+$): m/z = 1911.4725, calcd. for $C_{73}H_{116}O_{40}S_8Na$ $[M+Na]^+$: 1911.4706; IR (neat): ν = 2990, 2983, 1742, 1356, 1210, 1164 cm^{-1}.

Compound 21: Compound **20** (101 mg, 0.053 mmol) was dissolved in a CF_3CO_2H/H_2O mixture (1 mL, 9:1) and the mixture was stirred at room temperature for 3 h. Toluene (5 mL) was added and the solvents removed under reduced pressure. H_2O (20 mL) was added to the crude and the resulting mixture was freeze-dried to afford **21** as a white solid; yield: 83.0 mg (99%); $[\alpha]_D^{22}$: +33.6° (c 0.5, H_2O); ^{1}H NMR (400 MHz, D_2O): δ = 5.32 (d, J = 3.6 Hz, 0.4H, α-anomer), 4.64 (d, J = 7.9 Hz, 0.6H, β-anomer), 4.50 (m, 2H), 4.38 (dd, J = 8.0, 4.0 Hz, 0.4H, α-anomer), 4.32–4.26 (s, 2H), 4.07–4.01 (m, 1.6H), 3.91 (dd, J = 10.4, 3.0 Hz, 0.4H, α-anomer), 3.85 (dd, J = 10.5, 3.5 Hz, 0.4H, α-anomer), 3.70 (m, 2.6H), 3.54 (dd, J = 10.0, 7.8 Hz, 0.6H, β-anomer), 3.06 (t, J = 7.7 Hz, 2H), 2.95 (t, J = 6.5 Hz, 2H), 2.83 (t, J = 6.1 Hz, 2H); ^{13}C NMR (101 MHz, D_2O): δ = 174.4, 173.9, 97.1, 93.0, 73.1, 72.3, 71.0, 70.6, 69.6, 69.5, 69.0, 68.8, 68.8, 63.5, 63.3, 50.4, 43.7, 42.6, 34.8, 34.7, 27.2, 27.1, 25.1, 25.0; HR-MS (ESI$^+$): m/z = 1586.2686, calcd. for $C_{49}H_{88}NO_{40}S_8$ $[M+NH_4]^+$: 1586.2648. IR (neat): ν = 3365, 1730, 1351, 1161, 973 cm^{-1}.

Synthesis of Protected Heterogeneous Glycosylated Systems 24a, 24b and 27

Compound 22: Under argon, to a solution of **3f** (50.0 mg, 0.16 mmol), **4j** (125 mg, 0.19 mmol) in degassed CH_2Cl_2/2-propanol (6 mL, 5:1), a catalytic amount of Et_3N was added and the mixture was stirred at room temperature for 18 h under an inert atmosphere. The solvent was removed under reduced pressure and the residue purified by column chromatography (SiO_2, CH_2Cl_2/EtOAc 2:1 to 1:2) to give **22** as a syrup; yield: 108 mg (73%); $[\alpha]_D^{22}$: −11.7° (c 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.27 (d, J = 3.3 Hz, 1H), 5.13 (t, J = 9.2 Hz, 1H), 5.01 (dd, J = 10.4, 7.8 Hz, 1H), 4.88 (m, 2H), 4.49 (m, 2H), 4.43 (d, J = 7.9 Hz, 1H), 4.29 (m, 2H), 4.12 (d, J = 2.3 Hz, 2H), 4.08–3.95 (m, 3H), 3.83 (t, J = 6.8 Hz, 1H), 3.68 (m, 3H), 3.58 (m, 13H), 3.45 (m, 2H), 3.06 (ddd, J = 14.2, 11.3, 4.9 Hz, 1H), 2.91 (ddd, J = 14.1, 11.2, 5.1 Hz, 1H), 2.40 (t, J = 2.4 Hz, 1H), 2.07 (s, 3H), 2.06 (s, 3H), 1.98 (s, 3H), 1.96 (s, 9H), 1.88 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.4, 170.2, 170.0, 169.9, 169.5, 169.5, 169.0, 101.0, 83.8, 79.7, 76.9, 76.0, 74.6, 73.4, 70.9, 70.7, 70.6, 70.5, 70.5, 70.3, 69.9, 69.5, 69.0, 68.9, 66.6, 62.0, 60.8, 58.3, 51.4, 24.1, 20.7, 20.7, 20.6, 20.6, 20.5, 20.5, 20.4; HR-MS (ESI$^+$): m/z = 975.2811, calcd. for $C_{39}H_{59}O_{24}S_2$ $[M+H]^+$: 975.2838; IR (neat): ν = 2923, 1745, 1434, 1368, 1217 cm^{-1}.

Compound 24a: A solution of **22** (233 mg, 0.24 mmol) and **23a**[38,43] (153 mg, 0.41 mmol) in anhydrous THF (12 mL) was degassed by bubbling argon. $(EtO)_3P{\cdot}CuI$ (25.3 mg, 0.071 mmol) and DIPEA (165 µL, 0.95 mmol) were added and the mixture was stirred for 4 d under an argon atmosphere. The solvent was removed under reduced pressure and the crude purified by column chromatography (SiO_2, CH_2Cl_2/MeOH 96:4) to give **24a** as a cream-coloured foam; yield: 289 mg, (86%); $[\alpha]_D^{22}$: −14.6° (c 1, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.77 (s, 1H), 5.85 (m, 1H), 5.38 (m, 2H), 5.29 (dd, J = 3.5, 1.2 Hz, 1H), 5.17 (m, 2H), 5.04 (dd, J = 10.4, 7.8 Hz, 1H), 4.90 (m, 2H), 4.63 (s, 2H), 4.53 (d, J = 10.1 Hz, 1H), 4.47 (m, 2H), 4.30 (m, 2H), 4.24 (dd, J = 12.6, 5.0 Hz, 1H), 4.03 (m, 5H), 3.86 (t, J = 6.8 Hz,1H), 3.70 (m, 3H), 3.60 (m, 13H), 3.48 (m, 2H), 3.09 (ddd, J = 14.1, 11.3, 4.9 Hz, 1H), 2.94 (ddd, J = 14.2, 11.3, 5.1 Hz, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (m, 6H), 1.98 (s, 3H), 1.97 (s, 3H), 1.90 (s, 3H), 1.81 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ = 170.5, 170.5, 170.3, 170.1, 170.0, 169.9, 169.6, 169.6, 169.4, 169.1, 168.9, 145.9, 121.1, 101.1, 85.6, 83.8, 77.0, 76.1, 75.0, 73.5, 72.7, 71.0, 70.7, 70.6, 70.6, 70.5, 70.5, 70.4, 69.9, 69.8, 69.5, 69.1, 68.9, 67.7, 66.7, 64.4, 62.0, 61.6, 60.8, 51.5, 24.2, 20.8, 20.7, 20.7, 20.7, 20.6, 20.6, 20.6, 20.5, 20.5, 20.5, 20.2; HR-MS (ESI$^+$): m/z = 1348.3972, calcd. for $C_{53}H_{78}N_3O_{33}S_2$ $[M+H]^+$: 1348.3959; IR (neat): ν = 1743, 1433, 1367, 1218, 1036 cm^{-1}.

Compound 24b: A solution of **22** (108 mg, 0.11 mmol) and **23b**[39] (64 mg, 0.15 mmol) in anhydrous THF (12 mL) was degassed with Ar. $(EtO)_3P{\cdot}CuI$ (10.9 mg, 0.031 mmol) and DIPEA (71 µL, 0.41 mmol) were added and the mixture was stirred for 3 d under an argon atmosphere. The solvent was removed under reduced pressure and the crude purified by column chromatography (SiO_2, CH_2Cl_2/MeOH 96:4) to give

24b as a white foam; yield: 140 mg (91%); $[\alpha]_D^{22}$: +5.5° (c 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.64 (s, 1H), 5.27 (dd, J = 3.5, 1.2 Hz, 1H), 5.13 (m, 4H), 5.02 (dd, J = 10.4, 7.9 Hz, 1H), 4.90 (dd, J = 10.4, 3.5 Hz, 1H), 4.85 (t, J = 9.7 Hz, 1H), 4.72 (s, 1H), 4.61 (s, 2H), 4.53 (m, 3H), 4.45 (m, 2H), 4.28 (m, 2H), 4.12 (dd, J = 12.3, 5.2 Hz, 1H), 4.04 (m, 3H), 3.96 (m, 2H), 3.83 (m, 2H), 3.68 (m, 3H), 3.62–3.56 (m, 13H), 3.49 (m, 2H), 3.41 (ddd, J = 14.4, 11.4, 4.8 Hz, 1H), 3.06 (ddd, J = 14.2, 11.5, 4.8 Hz, 1H), 2.91 (m, 1H), 2.07 (s, 3H), 2.06 (s, 3H), 2.06 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.96 (m, 12H), 1.91 (s, 3H), 1.88 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.4, 170.4, 170.2, 170.0, 169.9, 169.8, 169.8, 169.5, 169.5, 169.0, 145.1, 123.9, 101.0, 97.4, 83.7, 76.9, 76.1, 73.4, 70.9, 70.7, 70.6, 70.5, 70.4, 70.4, 69.9, 69.6, 69.5, 69.1, 69.0, 68.9, 68.9, 68.8, 66.6, 66.2, 65.6, 64.3, 62.1, 61.9, 60.8, 51.4, 49.6, 24.1, 20.7, 20.7, 20.6, 20.6, 20.5, 20.4; HR-MS (ESI$^+$): m/z = 1392.4211, calcd. for $C_{55}H_{82}N_3O_{34}S_2$ $[M+H]^+$: 1392.4221; IR (neat): ν = 1744, 1433, 1368, 1218 cm^{-1}.

Compound 26: Under argon, to a solution of **3g** (70.0 mg, 0.20 mmol), **4j** (168 mg, 0.26 mmol) in degassed CH_2Cl_2/2-propanol (12 mL, 5:1), PPh_3 (16.3 mg, 0.06 mmol) and a catalytic amount of Et_3N were added and the mixture was stirred at room temperature for 24 h under an inert atmosphere. The solvent was removed under reduced pressure and the residue purified by column chromatography (SiO_2, CH_2Cl_2/EtOAc 9:1 to 7:3) to give **26** as a syrup; yield: 187 mg (91%); $[\alpha]_D^{22}$: −2.5° (c 1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 5.33 (d, J = 3.4 Hz, 1H), 5.20 (t, J = 9.1 Hz, 1H), 5.09 (dd, J = 10.5, 7.8 Hz, 1H), 4.94 (m, 2H), 4.50 (m, 3H), 4.21 (m, 2H), 4.12–4.01 (m, 9H), 3.87 (t, J = 6.8 Hz, 1H), 3.74 (t, J = 9.4 Hz, 1H), 3.64 (m, 1H), 3.53–3.48 (m, 7H), 3.40 (m, 1H), 3.11 (ddd, J = 16.2, 11.5, 4.8 Hz, 1H), 2.96 (ddd, J = 14.1, 11.5, 4.9 Hz, 1H), 2.45 (s, 3H), 2.13 (s, 6H), 2.05 (s, 3H), 2.03 (m, 9H), 1.94 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.6, 170.4, 170.2, 170.1, 169.7, 169.6, 169.2, 101.2, 84.2, 79.6, 76.2, 74.9, 74.9, 73.5, 71.1, 70.9, 70.0, 69.5, 69.2, 68.2, 66.7, 62.1, 60.9, 58.8, 51.0, 44.5, 24.4, 20.9, 20.8, 20.8, 20.7, 20.6. HR-MS (ESI$^+$): m/z = 1015.2520, calcd. for $C_{42}H_{56}O_{23}S_2Na$ $[M+Na]^+$: 1015.2551; IR (neat): ν = 1742, 1366, 1213, 1166, 1083, 1042 cm^{-1}.

Compound 27: A solution of **26** (103 mg, 0.10 mmol) and **23b** (174 mg, 0.42 mmol) in anhydrous THF (6 mL) was degassed by flowing argon. $(EtO)_3P{\cdot}CuI$ (37.0 mg, 0.10 mmol) and DIPEA (220 µL, 1.25 mmol) were added and the mixture was stirred for 4 d under an argon atmosphere. The solvent was removed under reduced pressure and the crude purified by column chromatography (SiO_2, CH_2Cl_2/MeOH 98:2 to 95:5) to give **27** as a colourless syrup; yield: 200 mg (86%); $[\alpha]_D^{22}$: +8.8° (c 1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ = 7.71 (s, 3H), 5.33 (d, J = 3.4 Hz, 1H), 5.24–5.18 (m, 10H), 5.06 (dd, J = 10.4, 7.9 Hz, 1H), 4.96 (dd, J = 10.4, 3.4 Hz, 1H), 4.89 (t, J = 9.7 Hz, 1H), 4.79 (d, J = 1.4 Hz, 3H), 4.67–4.56 (m, 13H), 4.51 (m, 2H), 4.18 (m, 5H), 4.10 (m, 5H), 4.02 (m, 4H), 3.89 (ddd, J = 10.8, 6.3, 4.2 Hz, 4H), 3.75 (t, J = 9.4 Hz, 1H), 3.68 (ddd, J = 10.2, 6.0, 2.1 Hz, 1H), 3.62 (m, 3H), 3.46–3.40 (m, 8H), 3.05 (ddd, J = 15.7, 10.6, 5.4 Hz, 1H), 2.89 (ddd, J = 14.0, 10.7, 5.6 Hz, 1H), 2.12 (s, 3H), 2.11 (m, 9H), 2.08 (s, 3H), 2.07 (m, 9H), 2.03 (s, 3H), 2.01 (m, 18H), 1.96 (s, 9H), 1.93 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 170.6, 170.5, 170.4, 170.2, 170.1, 170.0, 169.7, 169.7, 169.2, 145.1, 124.0, 101.2, 97.6, 83.7, 76.9, 76.3, 73.5, 71.0, 70.8, 70.1, 69.2, 69.2, 69.0, 69.0, 67.9, 66.8, 66.3, 65.8, 64.7, 62.3, 62.1, 60.8, 50.8, 49.7, 44.9, 24.3, 20.9, 20.9, 20.8, 20.8, 20.8, 20.8, 20.7, 20.7, 20.6; HR-MS (ESI$^+$): m/z = 2245.6907, calcd. for $C_{90}H_{125}N_9O_{53}S_2$ $[M+H]^+$: 2245.6876; IR (neat): ν = 1747, 1369, 1224, 1139, 1047 cm^{-1}.

General Procedure for *O*-Acetyl Deprotection

A solution of **17a–d**, **24a**, **b** or **27** (0.06–0.08 mmol) and Et_3N (0.8 mL) in anhydrous MeOH (8 mL) was stirred for 24–120 h at room temperature. The mixture was concentrated under reduced pressure and the residue purified by column chromatography (SiO_2, CH_3CN/H_2O 4:1 to 3:1). The product-containing fractions were combined and the volatiles removed under reduced pressure. The resulting solution was freeze-dried to yield the corresponding deprotected homo- and heterogeneous divalent glycosylated systems **18a–d**, **25a**, **b** or **28**.

Compound 18a: Obtained from **17a** (86.0 mg, 0.078 mmol) as a white foam after stirring for 24 h; yield: 56.0 mg (94%); $[\alpha]_D^{22}$: −22.4° (c 1, MeOH). ^1H NMR (500 MHz, D_2O): δ = 4.65 (d, J = 9.9 Hz, 2H), 4.52 (m, 4H), 3.93 (d, J = 12.4 Hz, 2H), 3.89 (m, 4H), 3.82–3.71 (m, 14H), 3.52 (m, 4H), 3.44 (t, J = 9.3 Hz, 2H), 3.37 (t, J = 9.4 Hz, 2H), 3.26 (ddd, J = 15.3, 9.1, 6.1 Hz, 2H), 3.17 (ddd, J = 14.7, 9.1, 6.4 Hz, 2H); ^{13}C NMR (126 MHz, D_2O): δ = 85.6, 79.9, 77.1, 72.1, 70.4, 69.8, 69.6, 69.4, 68.4, 60.8, 50.5, 23.4; HR-MS (ESI$^+$): m/z = 767.1573, calcd. for $C_{24}H_{47}O_{19}S_4$ $[M+H]^+$: 767.1594; 784.1843, calc. for $C_{24}H_{50}NO_{19}S_4$ $[M+NH_4]^+$: 748.1860; IR (neat): ν = 3380, 2880, 1349, 1166, 1041 cm^{-1}.

Compound 18b: Synthesized from **17b** (90.0 mg, 0.082 mmol) as a white solid after stirring for 24 h; yield: 48.0 mg (77%); $[\alpha]_D^{22}$: +132° (c 1, MeOH). ^1H NMR (400 MHz, D_2O): δ = 5.44 (d, J = 1.5 Hz, 2H), 4.53 (m, 4H), 4.11 (dd, J = 3.3, 1.5 Hz, 2H), 4.03 (ddd, J = 9.2, 6.4, 2.2 Hz, 2H), 3.94 (dd, J = 12.3, 2.3 Hz, 2H), 3.90 (m, 4H), 3.85–3.75 (m, 16H), 3.71 (t, J = 9.7 Hz, 2H), 3.17 (m, 4H); ^{13}C NMR (101 MHz, D_2O): δ = 85.2, 73.3, 71.4, 71.0, 70.4, 69.8, 69.6, 68.4, 67.0, 60.8, 49.7, 24.2; HR-MS (ESI$^+$): m/z = 767.1607, calcd. for $C_{24}H_{47}O_{19}S_4$ $[M+H]^+$: 767.1594; 784.1879, calc. for $C_{24}H_{50}NO_{19}S_4$ $[M+NH_4]^+$: 748.1860; IR (neat): ν = 3366, 2928, 1350, 1166, 1072 cm^{-1}.

Compound 18c: Obtained from **17c** (98.0 mg, 0.058 mmol) as a white solid after stirring for 5 d; yield: 41.0 mg (65%); $[\alpha]_D^{22}$: −6.0° (c 1, H_2O). ^1H NMR (400 MHz, D_2O): δ = 4.70 (d, J = 9.9 Hz, 2H), 4.54 (m, 4H), 4.50 (d, J = 7.8 Hz, 2H), 4.02 (dd, J = 12.4, 2.1 Hz, 2H), 3.97 (d, J = 3.4 Hz, 2H), 3.91 (m, 4H), 3.86–3.76 (m, 20H), 3.73–3.65 (m, 8H), 3.59 (dd, J = 10.0, 7.8 Hz, 2H), 3.45 (m, 2H), 3.24 (m, 4H); ^{13}C NMR (101 MHz, D_2O): δ = 102.8, 85.4, 78.7, 78.1, 75.7, 75.3, 72.5, 71.8, 70.9, 70.4, 69.8, 69.6, 68.5, 68.4, 61.0, 60.1, 50.4, 23.4; HR-MS (ESI$^+$): m/z = 1091.2650, calcd. for $C_{36}H_{67}O_{29}S_4$ $[M+H]^+$: 1091.2651; 1108.2909, calc. for $C_{36}H_{70}NO_{29}S_4$ $[M+NH_4]^+$: 1108.2916; IR (neat): ν = 3356, 2881, 1639, 1347, 1072 cm^{-1}.

Compound 18d: Obtained from **17d** (146 mg, 0.077 mmol) as a colourless syrup after stirring for 24 h; yield: 96 mg (80%); $[\alpha]_D^{22}$: +54.7° (c 1, H_2O); ^1H NMR (400 MHz, D_2O): δ = 5.43 (s, 2H), 4.53 (t, J = 4.1 Hz, 4H), 4.10 (m, 2H), 4.02 (t, J = 7.8, 2H), 3.94 (d, J = 12.5 Hz, 2H), 3.89 (m, 4H), 3.74 (m, 114H), 3.15 (m, 4H); ^{13}C NMR (101 MHz, D_2O): δ = 85.3, 73.3, 71.4, 71.0, 70.3, 69.7, 69.5, 68.4, 67.0, 60.8, 49.7,

24.2; HR-MS (ESI$^+$): m/z = 753.3242 (n = 20), 797.3489 (n = 22), 819.3611 (n = 23); calcd. for $(C_2H_4O)_nC_{16}H_{32}O_{15}S_4$ $[M+2NH_4]^{2+}$: 753.3199 (n = 20), 797.3461 (n = 22), 819.3592 (n = 23); IR (neat): ν = 3378, 2870, 1454, 1350, 1092 cm^{-1}.

Compound 25a: Obtained from **24a** (99 mg, 0.073 mmol) as a white foam after stirring for 72 h; yield: 53.0 mg (82%); $[\alpha]_D^{22}$: −9.2° (c 0.5, H_2O); ^{1}H NMR (500 MHz, D_2O): δ = 8.31 (s, 1H), 5.79 (d, J = 9.2 Hz, 1H), 4.77 (s, 2H), 4.67 (d, J = 9.9 Hz, 1H), 4.49 (m, 3H), 4.04 (t, J = 9.2 Hz, 1H), 4.00 (d, J = 10.6 Hz, 1H), 3.94 (m, 2H), 3.87 (dd, J = 10.2, 6.1 Hz, 3H), 3.83–3.64 (m, 27H), 3.57 (dd, J = 9.8, 7.9 Hz, 1H), 3.42 (dd, J = 9.9, 8.5 Hz, 1H), 3.26 (ddd, J = 15.0, 9.2, 6.1 Hz, 1H), 3.17 (ddd, J = 14.6, 9.1, 6.3 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ = 144.2, 124.3, 102.8, 87.4, 85.4, 78.8, 78.7, 78.0, 75.9, 75.7, 75.3, 72.5, 72.2, 71.8, 70.9, 70.3, 69.7, 69.6, 69.5, 69.5, 69.5, 69.0, 68.9, 68.5, 68.3, 63.0, 61.0, 60.4, 60.1, 50.4, 23.4; HR-MS (ESI$^-$): m/z = 884.2647, calcd. for $C_{31}H_{54}N_3O_{22}S_2$ $[M-H]^-$: 884.2640; IR (neat): ν = 2874, 1743, 1367, 1217, 1036, 913 cm^{-1}.

Compound 25b: Obtained from **24b** (100 mg, 0.071 mmol) as a white foam after stirring for 36 h; yield: 48.0 mg (72%); $[\alpha]_D^{22}$: +5.8° (c 1, H_2O). ^{1}H NMR (500 MHz, D_2O): δ = 8.14 (s, 1H), 4.82 (d, J = 1.7 Hz, 1H), 4.73 (s, 2H), 4.71 (m, 2H), 4.67 (d, J = 9.9 Hz, 1H), 4.49 (m, 3H), 4.13 (ddd, J = 11.0, 7.0, 4.0 Hz, 1H), 3.99 (m, 2H), 3.95 (d, J = 3.4 Hz, 1H), 3.87 (m, 3H), 3.83–3.55 (m, 27H), 3.42 (dd, J = 9.9, 8.5 Hz, 1H), 3.25 (ddd, J = 15.0, 9.2, 6.0 Hz, 1H), 3.16 (ddd, J = 14.6, 9.1, 6.3 Hz, 1H), 3.08 (ddd, J = 9.8, 5.7, 2.3 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ = 143.9, 125.6, 102.8, 99.5, 85.4, 78.7, 78.1, 75.7, 75.3, 72.8, 72.5, 71.8, 70.9, 70.4, 70.3, 69.8, 69.7, 69.6, 69.6, 69.5, 68.9, 68.5, 68.4, 66.3, 65.5, 63.0, 61.0, 60.6, 60.1, 50.4, 50.1, 23.4; HR-MS (ESI$^+$): m/z = 930.3068, calcd. for $C_{33}H_{60}N_3O_{23}S_2$ $[M+H]^+$: 930.3059; IR (neat): ν = 3358, 1738, 1368, 1352, 1217 cm^{-1}.

Compound 28: Prepared from **27** (103 mg, 0.071 mmol) as a white foam after stirring for 48 h; yield: 56.0 mg (84%); $[\alpha]_D^{22}$: +27.0° (c 1, H_2O); ^{1}H NMR (500 MHz, D_2O): δ = 8.09 (s, 3H), 4.82 (d, J = 1.7 Hz, 3H), 4.69 (m, 6H), 4.65 (d, J = 7.6 Hz, 1H), 4.61 (s, 6H), 4.48 (d, J = 7.8 Hz, 1H), 4.17 (s, 2H), 4.12 (m, 3H), 4.00–3.91 (m, 5H), 3.88 (dd, J = 3.3, 1.7 Hz, 3H), 3.84–3.72 (m, 7H), 3.71–3.56 (m, 16H), 3.49 (s, 6H), 3.41 (dd, J = 9.9, 8.6 Hz, 1H), 3.12–3.07 (m, 4H), 3.00 (ddd, J = 14.6, 9.2, 6.1 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ = 144.0, 125.5, 102.9, 99.5, 85.4, 78.7, 78.1, 75.7, 75.3, 72.7, 72.5, 71.9, 70.9, 70.4, 69.9, 68.8, 68.5, 67.1, 66.3, 65.4, 63.4, 61.0, 60.6, 60.1, 50.1, 44.3, 23.4; HR-MS (ESI$^+$): m/z = 1446.4847, calcd. for $C_{52}H_{88}N_9O_{34}S_2$ $[M+H]^+$: 1146.4875; IR (neat): ν = 3362, 2928, 2504, 1644, 1349, 1055 cm^{-1}.

Displacement of the Sulfonate Group of 15 by β-Mercaptoethanol (4g) and GSH (11)

Commercial amino-functionalized plates (CovaLink, NH plates, Nunc) were reacted with compound **15** (see the Supporting Information for synthetic details) (200 µL/well, 1 mg mL^{-1} in 50 mM HEPES pH 8) at 37 °C for 8 h. The unreacted **15** was removed by washing with PBST (300 µL/well, 3×3 min). Wells were incubated with serial dilutions of the nucleophile (200 µL/well of either GSH (**11**) or β-mercaptoethanol (**4g**) in PBS) at 37 °C for different times. Wells were washed with PBST (300 µL/well, 3×3 min) and then incubated with a solution of ConA-HRP (200 µL/well, 2 µg mL^{-1} in PBS with 1 mM $CaCl_2$ and 1 mM $MnCl_2$) at 37 °C for 1 h. The unbounded lectin was washed with PBST (300 µL/well, 3×3 min) and the presence of HRP-coupled lectin was revealed by incubation with a solution of *o*-phenylenediamine [200 µL/well, 0.04% (w/v) in 100 mM Na_2HPO_4, 50 mM citrate pH 5, 0.05% (v/v) H_2O_2] at 37 °C for 45 min. Values of absorbance were normalized to that of the blank (in absence of nucleophile).

Coupling-and-Decoupling of Two Model Lectins by Compound 25b

ConA (200 µL/well, 210 µg mL^{-1} in PBS with 1 mM $CaCl_2$ and 1 mM $MnCl_2$) was adsorbed on the surface of ELISA plate wells by incubation at 37 °C for 3.5 h. Wells were washed with PBST (300 µL/well, 3×3 min) and then incubated with a solution of compound **25b** (200 µL/well, 210 µg mL^{-1} in PBS with 1 mM $CaCl_2$ and 1 mM $MnCl_2$) at 37 °C for 1 h. Wells were washed with PBST (300 µL/well, 3×3 min) and then incubated with a solution of ConA-HRP (200 µL/well, 2 µg mL^{-1} in PBS with 1 mM $CaCl_2$ and 1 mM $MnCl_2$) at 37 °C for 1 h. After washing (300 µL PBST/well, 3×3 min), wells were incubated with a solution of PNA-HRP (200 µL/well, 1 µg mL^{-1} in PBS) at 37 °C for 1 h. Wells were washed (300 µL PBST/well, 3×3 min) and then incubated with serial dilutions of GSH (**11**) in PBS at 37 °C for 20 h. The unbounded PNA-HRP was washed with PBST (300 µL/well, 3×3 min) and the presence of HRP-coupled lectin was revealed by incubation with a solution of *o*-phenylenediamine [200 µL/well, 0.04% (w/v) in 100 mM Na_2HPO_4, 50 mM citrate pH 5, 0.05% (v/v) H_2O_2] at 37 °C for 45 min. Values of absorbance were normalized to that of the blank (in absence of GSH).

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