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S-Michael Additions to Chiral Dehydroalanines as an Entry to Glycosylated Cysteines and a Sulfa-Tn Antigen Mimic

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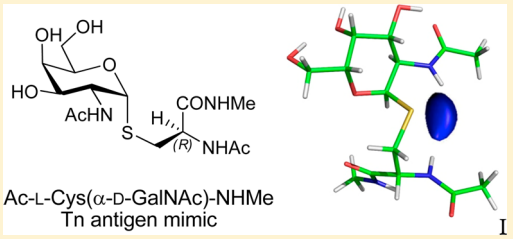
Supporting Information

ABSTRACT: Stereoselective sulfa-Michael addition of appropriately protected thiocarbohydrates to chiral dehydroalanines has been developed as a key step in the synthesis of biologically important cysteine derivatives, such as *S*-(β-D-glucopyranosyl)-D-cysteine, which has not been synthesized to date, and *S*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-cysteine, which could be considered as a mimic of Tn antigen. The corresponding diamide derivative was also synthesized and analyzed from a conformational viewpoint, and its bound state with a lectin was studied.



INTRODUCTION

Glycoproteins are ubiquitous components of cellular surfaces where their oligosaccharide moieties are involved in an extensive variety of molecular recognition events.[1] To study these binding interactions, the synthesis of structurally defined glycopeptides is of significant current interest.[2] As a result, chemical access to covalently modified proteins is a quickly expanding area in chemical biology.[3] In naturally occurring glycopeptides, the carbohydrate moiety and the peptide backbone are most commonly joined by an *O*-glycosidic linkage. However, these natural products present, in general, a low chemical stability, and, consequently, their use as therapeutics is hampered in many cases. Because of this, recent research has focused on the efficient synthesis of glycopeptide mimics with a stable glycosidic linkage as a fundamental tool for biological research.[4] In particular, *S*-linked glycopeptides may avoid this disadvantage due to their enhanced chemical stability and enzymatic resistance.[5]

Cysteine *S*-glycosylation[6] has recently emerged as a new post-translational modification found in glycopeptide bacteriocins glyocin F[7] and sublancin,[8] which contain a β-*N*-acetylglucosamine (β-GlcNAc) and a β-glucose (β-Glc), respectively. This glycosylation is essential for the antimicrobial activity.

Because of the emerging relevance of *S*-glycopeptides, a variety of glycosylation methods[6,2f] have been applied. These methods generally use protected carbohydrates as electrophiles and Cys derivatives as nucleophiles. Alternatively, nucleophilic thiocarbohydrate derivatives[9] have been used in the 1,4-conjugate addition to dehydroalanine (Dha) and dehydrobutyrine (Dhb) containing peptides with poor results in diastereoselectivity.[10] Particularly, Dha is an unsaturated amino acid residue of both biological and synthetic interest,[11] found in the initial stages of the biosynthesis of lanthionine-containing antibiotic peptides (lantibiotics).[12] Dha is also a useful chemical precursor to a range of post-translational modifications by the conjugate addition of thiols.[13] In this field, we have reported a biomimetic synthetic approach to lanthionine (Lan) by using an asymmetric sulfa-Michael addition of appropriately protected L- and D-Cys to a new chiral Dha derivative.[14]

RESULTS AND DISCUSSION

Herein, we report a new stereocontrolled entry to *S*-glycosyl cysteines, as building blocks for solid phase synthesis of glycopeptides, exploiting the high nucleophilicity of the sulfhydryl group of thiosugars in the diastereoselective Michael addition to chiral Dha **1** (see Table 1). The synthesis was previously communicated[15] and the absolute configuration of their stereogenic centers further established and corroborated by X-ray analysis.[14] Additionally, derivatives **1′** and **1″** and the enantiomers **ent-1** and **ent-1′** have been synthesized in a gram-scale (see the Supporting Information) and tested in the Michael reactions (see Table 1). Adequate monocrystals of compound **1′** were obtained and examined by X-ray diffraction analysis.

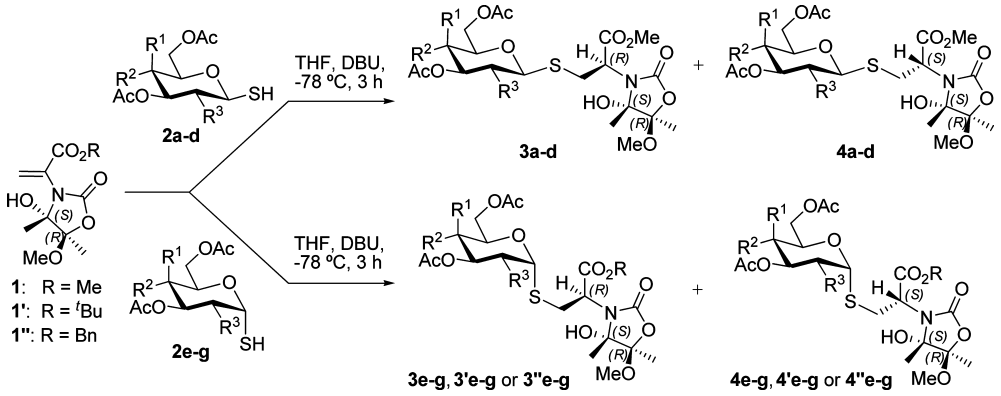
The asymmetric Michael reaction of Dha derivatives **1**, **1′**, and **1″** with different anomeric thioglycosylates in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a base and tetrahydrofuran (THF) as a solvent (Figure 1 and Table 1) was assayed. Under these conditions, mutarotation was not observed.[16]

This methodology was generalized to both α-thiosugars and β-thiosugars. The reaction with mono- and disaccharides, as well as with *N*-substituted 2-amino-2-deoxy-D-thiosugars, was also examined. Therefore, seven different thiosugars were used, tetra-*O*-acetyl-1-thio-β-D-glucose (**2a**), tetra-*O*-acetyl-1-thio-β-D-galactose (**2b**), tri-*O*-acetyl-2-benzyloxycarbonylamino-2-deoxy-1-thio-β-D-glucose (**2c**), tetra-*O*-acetyl-β-D-galactosyl-

Received: November 14, 2013
Published: December 27, 2013

   dx.doi.org/10.1021/ja411522f | J. Am. Chem. Soc. 2014, 136, 789−800

Table 1. Asymmetric Michael Reactions of Chiral Dehydroalanines 1, 1′, or 1″ and Thiosugars 2a−g



entry	dehydroalanine	R	thiosugar	R^1	R^2	R^3	yield (%)[a]	Michael adducts	diastereoselectivity (dr)[b]
1	**1**	Me	**2a**	H	OAc	OAc	84	**3a/4a**	>5:95
2	**1**	Me	**2b**	OAc	H	OAc	82	**3b/4b**	>5:95
3	**1**	Me	**2c**	H	OAc	NHCbz	56	**3c/4c**	>5:95
4	**1**	Me	**2d**	H	β-D-$(OAc)_4$Gal	OAc	78	**3d/4d**	>5:95
5	**1**	Me	**2e**	H	OAc	OAc	79	**3e/4e**	23/77
6	**1**	Me	**2f**	OAc	H	NHAc	67	**3f/4f**	>5:95
7	**1**	Me	**2g**	H	OAc	NHAc	80	**3g/4g**	>5:95
8	**1′**	tBu	**2f**	OAc	H	NHAc	86	**3′f/4′f**	>5:95
9	**1′**	tBu	**2g**	H	OAc	NHAc	74	**3′g/4′g**	>5:95
10	**1″**	Bn	**2g**	H	OAc	NHAc	71	**3″g/4″g**	15:85

[a]Yield of products after column chromatography. [b]See ref 18.

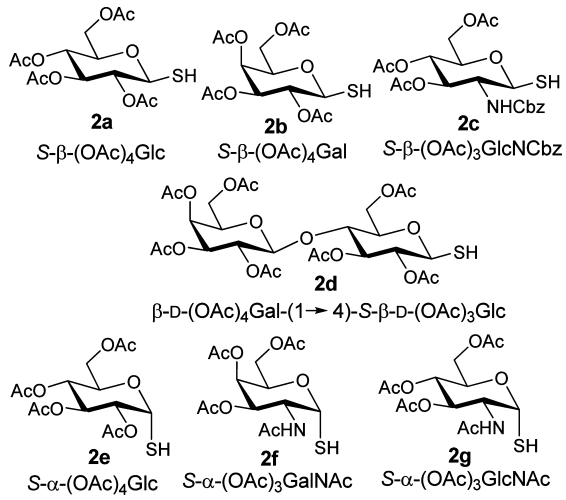


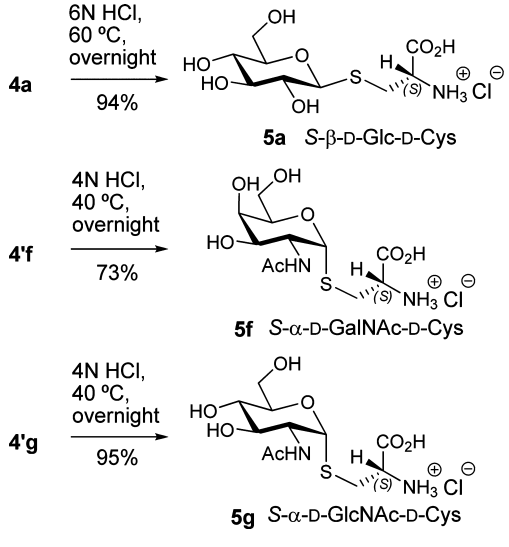
Figure 1. Thiosugars **2a−g** used as nucleophiles in the Michael additions to Dha derivatives.

(1→4)-tri-*O*-acetyl-1-thio-*β*-D-glucose or hepta-*O*-acetyl-1-thio-*β*-D-lactose (**2d**), tetra-*O*-acetyl-1-thio-*α*-D-glucose (**2e**), tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-*α*-D-galactose (**2f**), and tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-*α*-D-glucose (**2g**) (Figure 1). These protected thiosugars were prepared according to literature procedures.[17]

It is important to highlight that, in general, most of the tested thiosugars gave good yields and the diastereoselectivities obtained were excellent in all cases with a dr > 95:5,[18] except for the *α*-anomers **2e**[19a] (entry 5 in Table 1) and **2g** (entry 10 in Table 1) for which 23/77 and 15/85 lower diastereomeric ratios were obtained, respectively. For the rest of the compounds, purification of the crude reaction mixture by column chromatography gave exclusively one product, the corresponding protected *S*-glycosyl cysteine *β*-anomers (**4a−d**) or *S*-glycosyl cysteine *α*-anomers (**4f,g** and **4′f,g**) in good yields (56−86%) (Table 1).

In the case of product **4a**, representative of *β*-glycosidic derivatives, the absolute configuration of the new stereocenter was determined by transformation of this compound into the corresponding hydrochloride of the thioglycoamino acid *S*-*β*-D-Glc-D-Cys **5a**. This transformation was carried out in an aqueous 6 N HCl solution at 60 °C (Scheme 1). For this compound, we obtained a specific optical rotation value of +43. The reported value[19b,c] for *S*-*β*-D-Glc-L-Cys is −40, indicating

Scheme 1. Synthesis of *S*-Glycosyl Amino Acids 5a, 5f, and 5g

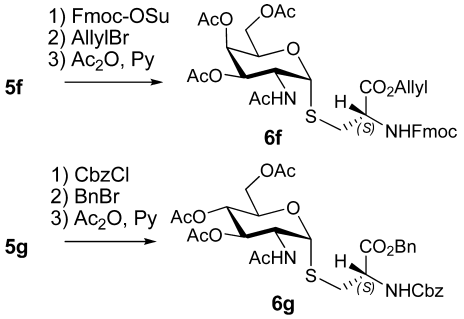


dx.doi.org/10.1021/ja411522f | J. Am. Chem. Soc. 2014, 136, 789−800

that the new stereogenic center created in the Michael reaction shows an *S*-configuration (Scheme 1).

Similarly, the representative α-glycosyl compounds **4f** and **4g** were transformed into the hydrochlorides *S*-α-D-GalNAc-D-Cys **5f** and *S*-α-D-GlcNAc-D-Cys **5g**, respectively, using the conditions described above. Unfortunately, these *S*-glycoamino acids were formed together with the corresponding hydrolysis products of the acetamido group, precluding the purification of the target compounds. This issue was overcome by using softer acid hydrolysis conditions and the *tert*-butyl derivatives **4′f** and **4′g** as starting materials, diminishing considerably the hydrolysis of acetamido group. Surprisingly, compounds **5a**, **5f**, and **5g**[19c] have not been synthesized to date (Scheme 1). In fact, it is important to highlight that, to the best of our knowledge, this is the first time that this type of glycoconjugates, in which both the carbohydrate and the cysteine belong to D-series, is described. Taking into account that neither *S*-α-D-GalNAc-L-Cys nor *S*-α-D-GlcNAc-L-Cys are described, and to determine the absolute configuration of the Michael adducts, compounds **5f** and **5g** were converted into protected derivatives **6f** and **6g**, respectively (Scheme 2). These

Scheme 2. Determination of the Absolute Configuration of Michael Adducts

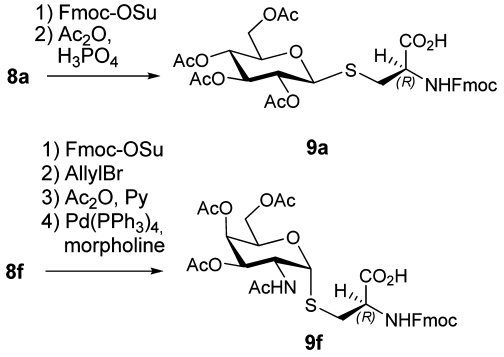


conversions involved three steps: (a) protection of the amino group as Cbz (**6g**) or Fmoc (**6f**) carbamate derivatives, (b) acid group conversion to formation of allyl (**6f**) or benzyl (**6g**) ester derivatives, and (c) acetylation of hydroxyl groups (experimental details in the Supporting Information). The comparison of the spectroscopic data of these compounds with those described[5b,c,20a] for the well-kown derivatives Fmoc-L-Cys(α-D-(OAc)$_3$GalNAc)-OAllyl and Cbz-L-Cys(α-D-(OAc)$_3$GlcNAc)-OBn confirmed that the new stereocenter created in the Michael reaction shows also an *S*-configuration.

To access the L-Cys-containing glycopeptides, we assayed the asymmetric Michael reaction of thiosugars **2a** and **2f** with the acceptors **ent-1** and **ent-1′**, enantiomers of chiral Dha derivatives **1** and **1′**, respectively, using the aforementioned conditions (Scheme 3). The best results provided **7a** and **7′f** with complete diastereoselectivity and in 79% and 76% yield, respectively. After chromatographic purification, compounds **7a** and **7′f** were hydrolyzed to the corresponding hydrochlorides *S*-β-D-Glc-L-Cys **8a**[19c] and *S*-α-D-GalNAc-L-Cys **8f**, respectively (Scheme 3).

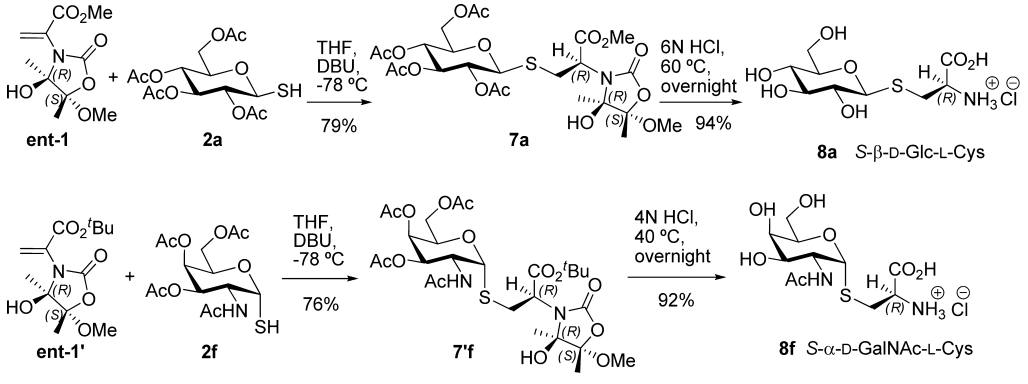
Glycoamino acids **8a** and **8f** were transformed into the corresponding building blocks **9a** and **9f**, which are ready to use in solid-phase synthesis. First, the free amine in compound **8a** was protected with Fmoc employing Fmoc−OSu in a basic medium. This compound then was treated with acetic anhydride (Ac_2O) in the presence of phosphoric acid to acetylate the hydroxyl groups of the carbohydrate moiety, giving compound **9a**.[8c,20b] Compound **8f** was converted into the building block **9f** following four steps: (a) Fmoc protection of the amino group with Fmoc−OSu, (b) transformation of the acid group into the corresponding allyl ester, (c) acetylation[20a] of the carbohydrate hydroxyls with Ac_2O and pyridine (Py), and (d) conversion of the allyl ester group into the corresponding carboxylic acid by treatment with $Pd(PPh_3)_4$ in morpholine (Scheme 4).

Scheme 4. Synthesis of Building Blocks 9a and 9f

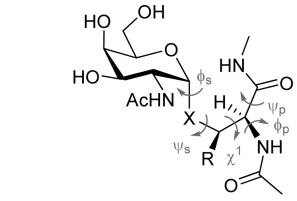


Starting from building block **9f**, we synthesized the corresponding diamide **10f**, which can be regarded as the shortest *S*-glycopeptide analogue of the *O*-glycopeptide (Figure 2). We previously synthesized and analyzed the conformational properties in water of Ac-L-Ser(α-D-GalNAc)-NHMe and Ac-L-Thr(α-D-GalNAc)-NHMe as model glycopeptides.[21] Notably,

Scheme 3. Asymmetric Michael Reactions of Chiral Dehydroalanines and Thiosugars 2a and 2f



dx.doi.org/10.1021/ja411522f | J. Am. Chem. Soc. 2014, 136, 789−800



X = O, R = Me: Ac-L-Thr(α-D-GalNAc)-NHMe
(*Tn antigen* derived from *Thr*)
X = O, R = H: Ac-L-Ser(α-D-GalNAc)-NHMe
(*Tn antigen* derived from *Ser*)
X = S, R = H: Ac-L-Cys(α-D-GalNAc)-NHMe
(compound **10f**)

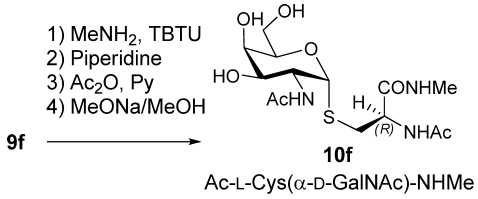
Figure 2. Tn antigen derivatives and compound **10f** conformationally studied in water.

we found that water molecules between the carbohydrate and the underlying amino acid contribute to stabilize defined conformations, which may be relevant for important biological features. Moreover, Ac-L-Thr/Ser(α-D-GalNAc)-NHMe are particularly important because they include the substructure of Tn antigen, which can be found in cancer cells (Figure 2) and, consequently, attract attention in developing Tn-based vaccines and other therapeutic approaches based on Tn expression.[22]

To understand the bioactivity of glycomimic motifs, it is crucial to determine their conformational preferences and dynamics. The presentation mode of the carbohydrate moiety is important for the recognition by the corresponding molecular entities. On this basis, we compared the conformational features in water of Ac-L-Ser(α-D-GalNAc)-NHMe and Ac-L-Thr(α-D-GalNAc)-NHMe with the sulfa-mimic Ac-L-Cys(α-D-GalNAc)-NHMe (**10f**) to understand the differences induced by the sulfur atom (Figure 2).

Target compound **10f** was synthesized stating from building block **9f** (Scheme 5). Initially, the acid group was transformed

Scheme 5. Synthesis of Ac-L-Cys(α-D-GalNAc)-NHMe 10f



into the corresponding methyl amide using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) as a coupling agent. Deprotection of Fmoc with piperidine, followed by acetylation of the amino group with Ac_2O in pyridine, gave the corresponding diamide derivative, which was treated with sodium metoxide (MeONa) to deacetylate the hydroxyl groups of the carbohydrate moiety (Scheme 5).

We performed then the conformational analysis in aqueous solution on compound **10f** through NMR experiments and molecular dynamics (MD) simulations. The relevant proton−proton distances obtained from 2D-NOESY experiments (Figure 3) were then used as restraints in MD simulations with time-averaged restraints (MD-tar)[23] to obtain a distribution of low-energy conformers able to quantitatively reproduce the NMR data (see the Experimental Section).

Figure 4 shows the conformer distribution obtained from these simulations for the backbone of compound **10f**, as well as

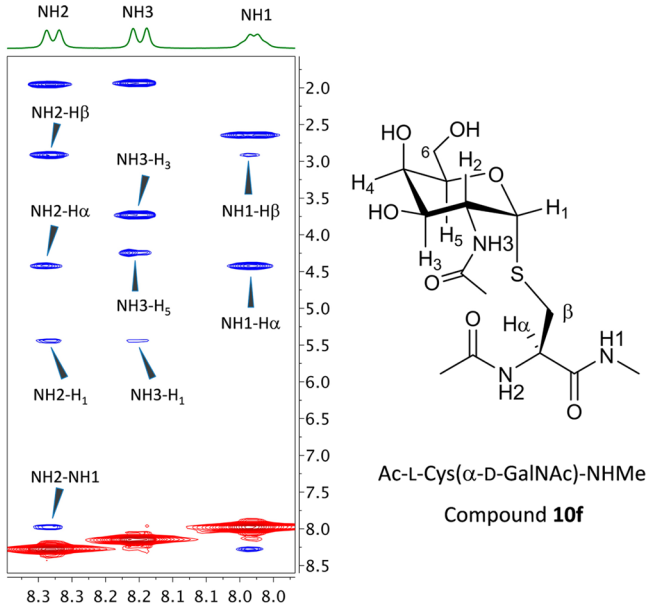


Figure 3. 2D-NOESY for Ac-L-Cys(α-D-GalNAc)-NHMe (**10f**) in H_2O/D_2O (9:1) at 400 MHz (pH 5.5, 298 K).

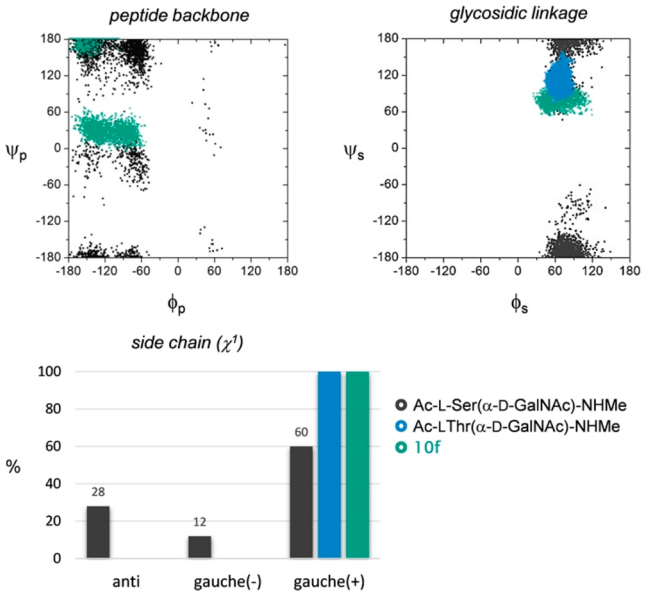


Figure 4. Distributions for the peptide backbone, glycosidic linkage, and side-chain obtained from the MD-tar simulations for Ac-L-Ser(α-D-GalNAc)-NHMe, Ac-L-Thr(α-D-GalNAc)-NHMe, and Ac-L-Cys(α-D-GalNAc)-NHMe (**10f**).

for the glycosidic linkage and for the side-chain. A comparison to the natural Tn antigens is also shown.[21]

For compound **10f**, the peptide backbone exhibited mainly folded (helix-like) conformations, corroborated by the NOE intensities observed for the crosspeaks NH2−NH1, NH1−Hα, and NH2−Hα (Figure 3). A small population of extended conformations (about 10% of the total trajectory time) was also observed. Conversely, and as described previously by us, both natural Tn antigens prefer extended conformations for the underlying amino acid. Concerning the glycosidic linkage, the ϕ_s (O5−C1−S−Cβ) dihedral angle is quite rigid, in accordance with the exoanomeric effect. However, significant differences

dx.doi.org/10.1021/ja411522f | J. Am. Chem. Soc. 2014, 136, 789−800

are observed in the ψ_s (C1−S−Cβ−Cα) torsional angle when compared to the natural derivatives (Figure 4).

For Tn serine derivative, this angle is close to 180°, while the threonine derivative shows an "eclipsed" conformation ($\psi_s \approx$ 120°). However, for the *S*-glycosyl derivative **10f**, a major conformation with ψ_s around 60° was observed. It is important to note that this conformer lies at one of the local minima calculated for methyl 4-thio-α-maltoside.[24] Consequently, using different underlying amino acids (serine, threonine, or cysteine), it is possible to modulate the values of ψ_s, ranging from 60° to 180°. With respect to the side chain, defined by the χ^1 (X−Cβ−Cα−N) torsion angle, the simulations indicated that the side chain of compound **10f** is rather rigid, exhibiting only the *gauche*(+) conformer, similarly to Ac-L-Thr(α-D-GalNAc)-NHMe. Through all of these observations, it can be concluded that the conformational behavior of compound **10f** is closely related to Tn antigen containing threonine as the underlying amino acid. In fact, as can be observed in Figure 5,

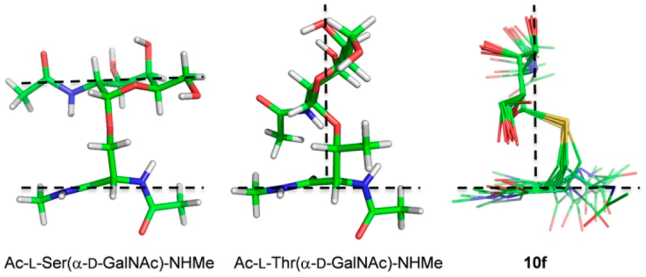


Figure 5. Representative conformations for natural Tn antigens, along with calculated ensemble obtained for compound **10f** from MD-tar simulations.

while in serine derivative the carbohydrate moiety is almost parallel to the peptide backbone, in both Tn bearing threonine and **10f** compounds, the GalNAc unit adopts a perpendicular disposition.

The 3D-disposition of compound **10f** allows the formation of a water pocket between the NHAc group of GalNAc (N3) and the NH of the backbone (N2). This is similar to that previously reported[21b] for compound Ac-L-Thr(α-D-GalNAc)-NHMe, and it is characterized by a density of 3.7 times the bulk density (Figure 6). This spatial disposition of **10f** is corroborated by a medium NOE peak between the NH2 proton of the acetyl group of the amino acid and the H_1 proton of the carbohydrate (NH2−H_1, Figures 3 and 6). The presence of these structured water molecules may contribute to rigidify the molecule.

Recent developments in carbohydrate-based cancer vaccines have addressed the concept of introducing analogues into tumor-associated carbohydrate antigens to improve their immunogenicity.[25] Different strategies, including incorporation of fluorine atoms,[26] synthesis of *C*-glycoside[27] and *S*-glycoside derivatives,[28] and the use of homoserine and β^3-homothreonine conjugates,[29] have been reported to prepare mucin-like glycopeptides antigen analogues. Recently, we contributed to this field by synthesizing a conformationally restricted Tn antigen mimic.[27h] However, *S*-glycoside analogues of Tn antigen have been scarcely used to develop anticancer vaccines.[28b,30] Because the *O*-glycosidic linkage of the Tn antigen can be chemically or enzymatically cleaved, the higher stability of the sulfa-Tn vaccine has been attributed to be the source of its superior activity at lower doses when compared to the natural Tn.

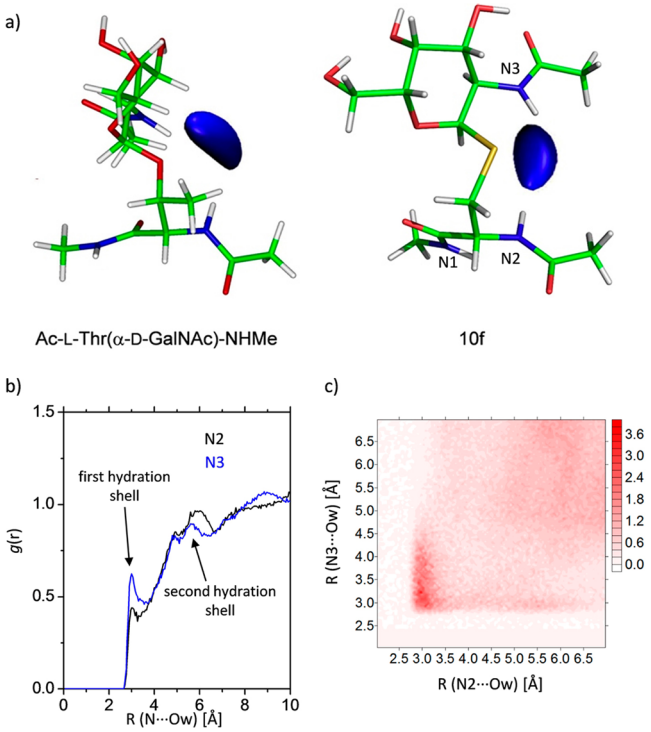


Figure 6. (a) Comparison between the inter-residue water pockets deduced from the MD-tar simulations of Ac-L-Thr(α-D-GalNAc)-NHMe and Ac-L-Cys(α-D-GalNAc)-NHMe (**10f**). (b and c) Radial pair distribution function (RDF) and two-dimensional RDF for N2 and N3 found in the 16 ns MD_{H2O}-tar simulations of **10f**.

The binding affinities of the sulfa-mimic **10f** and the natural Tn antigen serine derivative to *Soybean agglutinin* (SBA) lectin were studied by an enzyme-linked lectin assay (ELLA). We selected SBA as the binding target because it is a well-known, readily available, and stable lectin with high affinity toward GalNAc, Tn antigen, and other mimics.[27h,31] The X-ray structure of this protein bound to galactose has been described.[32] To compare the relative affinities of **10f** and Ac-L-Ser(α-D-GalNAc)-NHMe to SBA lectin, we used a competitive ELLA (Table 2 and Supporting Information). There-

Table 2. Inhibition Ratio (%) of Compounds Ac-L-Ser(α-D-GalNAc)-NHMe and Ac-L-Cys(α-D-GalNAc)-NHMe (10f) at Different Concentrations Employing 100 nmol of Ala-Pro-Asp-Thr(α-D-GalNAc)-Arg Bound to SBA Lectin

nmol	Ac-L-Ser(α-D-GalNAc)-NHMe	**10f**
50	72.2	54.4
100	91.5	83.9
150	95.3	91.3

fore, Ala-Pro-Asp-Thr(α-D-GalNAc)-Arg glycopeptide (100 nmol per well) was covalently linked to a 96-well plate, and a fixed amount of SBA lectin was then added. The competitive assay was carried out at three different concentrations of **10f** and the natural Tn serine derivative, as summarized in Table 2. The results obtained from these assays are represented as the inhibition ratio (%). This ratio is defined as the optical density (OD) observed at 450 nm in the presence of each Tn derivative (**10f** or the natural Tn antigen) divided by the OD in the absence of the corresponding Tn derivative. From these experiments, it can be inferred that both compounds present

dx.doi.org/10.1021/ja411522f | J. Am. Chem. Soc. 2014, 136, 789−800

similar inhibition ratios, although slightly higher values were observed for the natural Tn serine-derivative antigen. Of note, these results agree with those of a recently reported comparative binding study of *S*- and *O*-glycoconjugates to different lectins, which showed that both types of derivatives exhibited similar affinities.[33]

To explain these experimental results, a putative 3D model of the complex of **10f** with SBA was established using MD simulations (Figure 7a). As expected, these calculations indicate

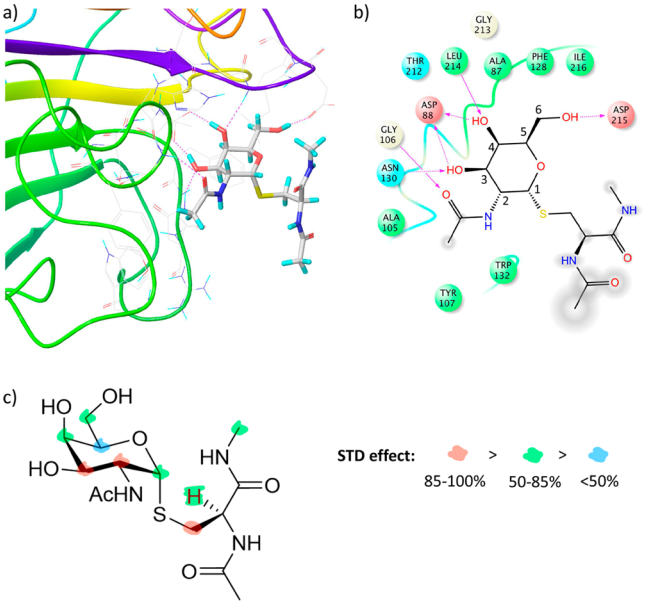


Figure 7. (a) Snapshot taken from the 25 ns MD simulations of compound **1of** bound to SBA lectin. (b) Ligand interaction diagram for SBA:**10f** complex obtained using Maestro 9.3.5 software. (c) Intensity diagram for protons of compound **10f** obtained from the saturation-transfer difference (STD) NMR experiments of a sample containing **10f** and SBA lectin.

that compound **10f** interacts with the lectin mainly through the carbohydrate moiety. Hydrogen bonds between the sugar and the lectin are observed (Figure 7b). For instance, OH-3 and OH-4 interact with Asp88, OH-6 participates in a hydrogen bond with Asp215, and OH-3 interacts with Asn130. These interactions are similar to those found in the crystal structure of SBA bound to galactose.[32] This model was experimentally corroborated by studying the interactions of compound **10f** with SBA lectin through saturation transfer difference (STD)-NMR experiments[34] (Experimental Section). Unequivocal clear STD signals were detected for the carbohydrate moiety (with STD effects >85%) of compound **10f** (Figure 7c), which prove the predicted specific interactions with SBA lectin. Therefore, these experiments corroborated the binding epitope previously proposed by the MD simulations.

CONCLUSION

We have described a novel and straightforward methodology for the stereoselective synthesis of different *S*-glycosyl cysteine derivatives. This strategy is based on the double asymmetric sulfa-Michael addition reaction of adequately protected thiosugars to chiral dehydroalanines. These compounds were easily transformed into the corresponding glycosyl amino acid building blocks, which are ready to use in phase solid synthesis. The conformational analysis of a sulfa-Tn antigen mimic has been studied through NMR experiments and MD simulations. The conformational properties and the first hydration shell of this compound were analyzed and compared to those of the natural *O*-glycosyl analogues. The 3D spatial disposition of the sulfa-Tn mimic is closely related to the natural Tn carrying threonine as the underlying residue. In fact, both molecules expose the GalNAc moiety perpendicularly to the amino acid backbone and accommodate a similar water pocket between the peptide and the sugar moieties. Finally, the binding affinity of the sulfa-mimic Tn antigen toward *Soybean agglutinin* (SBA) lectin was studied through enzyme-linked lectin assays and compared to that showed by the natural Tn serine-derivative, demonstrating that both molecules have similar preferences for this lectin. This methodology is currently being expanded in our lab to obtain longer glycopeptides of biological relevance.

EXPERIMENTAL SECTION

Reagents and General Procedures. Commercial reagents were used without further purification. Solvents were dried and redistilled prior to use in the usual way. All reactions were performed in oven-dried glassware with magnetic stirring under an inert atmosphere unless noted otherwise. Analytical thin layer chromatography (TLC) was performed on glass plates precoated with a 0.25 mm thickness of silica gel. The TLC plates were visualized with UV light and by staining with Hanessian solution (ceric sulfate and ammonium molybdate in aqueous sulfuric acid) or sulfuric acid−ethanol solution. Column chromatography was performed on silicagel (230−400 mesh). Optical rotations (OR) were measured with a polarimeter at a concentration (*c*) expressed in g/100 mL. ^{1}H and ^{13}C NMR spectra were measured with a 400 MHz spectrometer with TMS as the internal standard. Multiplicities are quoted as singlet (s), broad singlet (br s), doublet (d), doublet of doublets (dd), triplet (t), or multiplet (m). Spectra were assigned using COSY and HSQC. All NMR chemical shifts (δ) were recorded in ppm, and coupling constants (J) were reported in Hz. The results of these experiments were processed with MestreNova software. Melting points were determined on a Büchi melting-point apparatus and are uncorrected. Optical rotations were measured on a polarimeter from solutions in 1.0 dm cells of capacity 1.0 or 0.3 mL. High-resolution electrospray mass (ESI) spectra were recorded on a microTOF spectrometer; accurate mass measurements were achieved by using sodium formate as an external reference.

NMR Experiments. NMR experiments were performed on a 400 spectrometer at 298 K. Magnitude-mode ge-2D COSY spectra were acquired with gradients by using the cosygpqf pulse program with a pulse width of 90°. Phase-sensitive ge-2D HSQC spectra were acquired by using *z*-filter and selection before t1 removing the decoupling during acquisition by use of the invigpndph pulse program with CNST2 (J^{HC}) = 145. Phase-sensitive ge-2D NOESY experiments were performed. NOE intensities were normalized with respect to the diagonal peak at zero mixing time. The saturation-transfer difference (STD) NMR experiments[34] (Supporting Information) were achieved using standard conditions on a 55 μM/2.75 mM lectin/ligand sample (molar ratio 1:50) in D_2O at 298 K at 400 MHz. The saturation time was 2s, at an on-resonance frequency of δ 0.370 ppm. No spin-lock filter and no water suppression schemes were employed.

X-ray Diffraction Analysis. ORTEP diagrams are presented in the Supporting Information. The SHELXL97 program[35] was used for the refinement of crystal structures, and hydrogen atoms were fitted at theoretical positions. CIF files in the Supporting Information contain the supplementary crystallographic data for this Article.

Unrestrained Molecular Dynamics Simulations. All of the molecular dynamics simulations were carried out on the Finis-Terrae cluster belonging to the Centro de Supercomputación de Galicia (CESGA), Spain. The starting geometries for the complexes were generated from the available data deposited in the Protein Data Bank (pdb code 1SBF) and modified accordingly. Each model complex was immersed in a 10 Å-sided cube with pre-equilibrated TIP3P water

dx.doi.org/10.1021/ja411522f | J. Am. Chem. Soc. 2014, 136, 789−800

molecules. To equilibrate the system, we followed a protocol consisting of 10 steps. First, only the water molecules are minimized, and then heated to 300 K. The water box, together with Na^+ ions, was then minimized, followed by a short MD simulation. At this point, the system was minimized in the four following steps with positional restraints imposed on the solute, decreasing the force constant step by step from 20 to 5 kcal mol^{-1}. Finally, a nonrestraint minimization was performed. The production dynamics simulations were accomplished at a constant temperature of 300 K (by applying the Berendsen coupling algorithm for the temperature scaling) and constant pressure (1 bar). The Particle Mesh Ewald Method, to introduce long-range electrostatic effects, and periodic boundary conditions were also used. The SHAKE algorithm for hydrogen atoms, which allows using a 2 fs time step, was also employed. Finally, a 9 Å cutoff was applied for the Lennard-Jones interactions. MD simulations were performed with the sander module of AMBER 11.0 (parm99 force field),[36] which was implemented with GAFF parameters[37] to accurately simulate compound **10f**. A simulation length of 25 ns and the trajectory coordinates were saved each 0.5 ps. The data processing of the generated trajectories was done with the ptraj module of Amber 11.0 and with the Carnal module of Amber 6.0.

MD Simulations with Time-Averaged Restraints (MD-tar). MD-tar simulations were performed with AMBER 11 (parm99 force field), which was implemented with GAFF parameters. Distances derived from NOE interactions were included as time-averaged distance restraints. A $\langle r^{-6}\rangle^{-1/6}$ average was used for the distances. Final trajectories were run using an exponential decay constant of 2000 ps and a simulation length of 16 ns in explicit TIP3P water molecules.

General Procedure for the Sulfa-Michael Additions. The corresponding α,β-dehydroamino acid (1 equiv) was dissolved in dry THF (concentration of 10 mg/mL), and the resulting solution was introduced in a Schlenk tube under an argon atmosphere. This solution was stirred and cooled to −78 °C. The corresponding thiocarbohydrate (**2a**−**g**, 1.2 equiv) dissolved in THF was introduced into the Schlenk tube by a syringe, and DBU (1.2 equiv) was then added slowly by another syringe. After the mixture was stirred at this temperature for 3 h, a saturated NH_4Cl solution (the same volume as THF) was added. This mixture was stirred vigorously while it was allowed to warm to room temperature. After that, the reaction crude was diluted with ethyl ether, and the aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with brine and dried with anhydrous Na_2SO_4. The solvent was filtered and evaporated, and the residue was purified by silica gel column chromatography to give the corresponding sulfa-Michael adduct.

*Methyl (2S)-2-((4S,5R)-4,5-Dimethyl-4-hydroxy-5-methoxy-2-oxooxazolidin-3-yl)-3-(tetra-O-acetyl-β-D-glucosylthio)propanoate (**4a**).* Compound **4a** was obtained from α,β-dehydroamino acid **1** (550 mg, 2.24 mmol) and tetra-*O*-acetyl-1-thio-β-D-glucose **2a** (980 mg, 2.69 mmol) following the procedure described for sulfa-Michael additions, using DBU (403 μL, 2.69 mmol) as a base. After column chromatography (ethyl acetate/hexane, 1:1), compound **4a** was obtained as a white foam (1.147 g, 1.88 mmol, 84%). $[\alpha]_D^{25} = -34.5$ (*c* 1.03, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.40 (s, 3H; CH_3), 1.57 (s, 3H; CH_3), 1.99 (s, 3H; Ac), 2.02 (s, 3H; Ac), 2.04 (s, 3H; Ac), 2.05 (s, 3H; Ac), 3.31 (dd, *J* = 5.3, 14.3, Hz, 1H; $CHCH_2$), 3.43 (s, 3H; OCH_3), 3.49−3.62 (m, 1H; $CHCH_2$), 3.76 (s, 3H; CO_2CH_3), 3.70−3.78 (m, 1H; H-5), 4.08−4.19 (m, 2H; H-6a; H-6b), 4.19−4.29 (m, 1H; $CHCH_2$), 4.64 (d, *J* = 10.1 Hz, 1H; H-1), 4.95−5.09 (m, 2H; H-2; H-4), 5.21 (t, *J* = 9.3 Hz, 1H; H-3). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 15.6 (CH_3), 19.8 (CH_3), 20.4, 20.5, 20.6, 20.7 (Ac), 30.7 ($CHCH_2$), 50.8 (OCH_3), 52.9 (CO_2CH_3), 55.8 ($CHCH_2$), 62.0 (C-6), 68.0 (C-4), 70.0 (C-2), 73.4 (C-3), 76.2 (C-5), 84.9 (C-1), 90.0 ($CNCH_3OH$), 108.4 (CCH_3OCH_3), 154.5 (NCO_2), 169.3, 169.4, 169.5, 170.0, 170.4 (4×Ac; CO_2CH_3). HRMS ESI+ (*m*/*z*): calcd for $C_{24}H_{35}NO_{15}SNa^+$ $[M + Na]^+$ 632.1620, found 632.1626.

*Methyl (2S)-2-((4S,5R)-4,5-Dimethyl-4-hydroxy-5-methoxy-2-oxooxazolidin-3-yl)-3-(tetra-O-acetyl-β-D-galactosylthio)propanoate (**4b**).* Compound **4b** was obtained from α,β-dehydroamino acid **1** (200 mg, 0.82 mmol) and tetra-*O*-acetyl-1-thio-β-D-galactose **2b** (327 mg, 0.82 mmol) following the procedure for sulfa-Michael additions, using DBU (136 μL, 0.89 mmol) as a base. After column chromatography (ethyl acetate/hexane, 1:1), **4b** was obtained as a white amorphous solid (406 mg, 0.67 mmol, 82%). $[\alpha]_D^{25} = -24.6$ (*c* 1.00, $CHCl_3$). Mp: 64−66 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.43 (s, 3H; CH_3), 1.60 (s, 3H; CH_3), 1.99 (s, 3H; Ac), 2.04 (s, 3H; Ac), 2.08 (s, 3H; Ac), 2.19 (s, 3H; Ac), 3.36 (dd, *J* = 5.0, 14.8 Hz, 1H; $CHCH_2$), 3.44 (s, 3H; OCH_3), 3.53−3.59 (m, 2H; $CHCH_2$; OH), 3.77 (s, 3H; CO_2CH_3), 3.98 (t, *J* = 6.31 Hz, 1H; H-5), 4.07−4.17 (m, 2H; H-6a; H-6b), 4.21−4.25 (m, 1H; $CHCH_2$), 4.59 (d, *J* = 9.9 Hz, 1H; H-4), 5.03−5.06 (m, 1H; H-2), 5.24 (t, *J* = 9.9 Hz, 1H; H-3), 5.43 (d, *J* = 3.2 Hz, 1H; H-1). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 15.5 (CH_3), 19.8 (CH_3), 20.6, 20.7, 20.8, 20.9 (Ac), 30.2 ($CHCH_2$), 50.8 (OCH_3), 53.0 (CO_2CH_3), 55.9 ($CHCH_2$), 61.8 (C-6), 67.0 (C-3), 67.2 (C-1), 71.6 (C-2), 75.1 (C-5), 85.0 (C-4), 90.1 ($CNCH_3OH$), 108.5 (CCH_3OCH_3), 154.5 (NCO_2), 169.4, 169.7, 170.0, 170.3, 170.4 (4×Ac; CO_2CH_3). HRMS ESI+ (*m*/*z*): calcd for $C_{24}H_{35}NO_{15}SNa^+$ $[M + Na]^+$ 632.1620, found 632.1606.

*Methyl (2S)-2-((4S,5R)-4,5-Dimethyl-4-hydroxy-5-methoxy-2-oxooxazolidin-3-yl)-3-(tri-O-acetyl-2-benzyloxycarbonylamino-2-deoxy-β-D-glucosylthio)propanoate (**4c**).* Compound **4c** was obtained from α,β-dehydroamino acid **1** (22 mg, 0.09 mmol) and tri-*O*-acetyl-2-benzyloxycarbonyl-2-deoxy-1-thio-β-D-glucose **2c** (45 mg, 0.10 mmol) following the procedure for sulfa-Michael additions, using DBU (15 μL, 0.10 mmol) as a base. After column chromatography (ethyl acetate/hexane, 1:1), **4c** was obtained as a white amorphous solid (36 mg, 0.05 mmol, 56%). $[\alpha]_D^{25} = -27.4$ (*c* 1.00, $CHCl_3$). Mp: 68−70 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.40 (s, 3H; CH_3), 1.59 (s, 3H; CH_3), 1.93 (s, 3H; Ac), 2.02 (s, 3H; Ac), 2.06 (s, 3H; Ac), 3.31 (dd, *J* = 4.5, 14.9 Hz, 1H; $CHCH_2$), 3.44 (s, 3H; OCH_3), 3.63−3.72 (m, 3H; $CHCH_2$; H-5; H-4), 3.76 (s, 3H; CO_2CH_3), 4.10−4.23 (m, 2H; H-6a; H-6b), 4.28 (d, *J* = 6.1 Hz, 1H; $CHCH_2$), 4.84 (d, *J* = 10.0 Hz, 1H; H-1), 4.95 (d, *J* = 8.8 Hz, 1H; NHCbz), 5.01−5.13 (m, 3H; H-2; OCH_2Ph), 5.20 (d, *J* = 9.2 Hz, 1H; H-3), 7.29−7.43 (m, 5H; Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 15.7 (CH_3), 19.9 (CH_3), 20.6, 20.7, 20.7 (Ac), 30.8 ($CHCH_2$), 51.0 (OCH_3), 53.1 (CO_2CH_3), 55.6 ($CHCH_2$), 56.0 (C-4), 62.3 (C-6), 67.2 (CH_2Ph), 68.5 (C-2), 73.2 (C-3), 76.3 (C-5), 86.0 (C-1), 90.3 ($CNCH_3OH$), 108.6 (CCH_3OCH_3), 128.2, 128.4, 128.7 (Ph), 154.8 (NCO_2), 155.9 (NCO_2Bn), 169.6, 169.6, 170.7, 170.8 (3×Ac; CO_2CH_3). HRMS ESI+ (*m*/*z*): calcd for $C_{30}H_{40}N_2O_{15}SNa^+$ $[M + Na]^+$ 723.2042, found 723.2037.

*Methyl (2S)-2-((4S,5R)-4,5-Dimethyl-4-hydroxy-5-methoxy-2-oxooxazolidin-3-yl)-3-(hepta-O-acetyl-β-D-lactosylthio)propanoate (**4d**).* Compound **4d** was obtained from α,β-dehydroamino acid 1 (200 mg, 0.82 mmol) and hepta-*O*-acetyl-1-thio-β-D-lactose **2d** (535 mg, 0.82 mmol) following the procedure for sulfa-Michael additions, using DBU (136 μL, 0.89 mmol) as a base. After column chromatography (ethyl acetate/hexane, 1:1), **4d** was obtained as a white amorphous solid (574 mg, 0.64 mmol, 78%). $[\alpha]_D^{25} = -21.0$ (*c* 1.00, $CHCl_3$). Mp: 97−99 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.36 (s, 3H; CH_3), 1.53 (s, 3H; CH_3), 1.92 (s, 3H; Ac), 2.00 (s, 3H; Ac), 2.01 (s, 3H; Ac), 2.02 (s, 3H; Ac), 2.03 (s, 3H; Ac), 2.05 (s, 3H; Ac), 2.11 (s, 3H; Ac), 3.24 (dd, *J* = 4.6, 14.9 Hz, 1H; $CHCH_2$), 3.40 (s, 3H; OCH_3), 3.55 (dd, *J* = 10.3, 14.9 Hz, 1H; $CHCH_2$), 3.60−3.68 (m, 2H; H_{Glc}-5; OH), 3.69−3.78 (m, 4H; CO_2CH_3; H_{Glc}-4), 3.85 (t, *J* = 6.8 Hz, 1H; H_{Gal}-5), 3.98 (dd, *J* = 5.9, 12.1 Hz, 1H; H_{Glc}-6a), 4.04−4.11 (m, 2H; H_{Gal}-6a; H_{Gal}-6b), 4.19 (dd, *J* = 4.5, 10.3 Hz, 1H; $CHCH_2$), 4.44 (d, *J* = 7.9 Hz, 1H; H_{Gal}-1), 4.51 (dd, *J* = 1.5, 12.0 Hz, 1H; H_{Glc}-6b), 4.59 (d, *J* = 10.0 Hz, 1H; H_{Glc}-1), 4.85−4.95 (m, 2H; H_{Glc}-2; H_{Gal}-3), 5.05 (dd, *J* = 7.9, 10.4 Hz, 1H; H_{Gal}-2), 5.16 (t, *J* = 9.1 Hz, 1H; H_{Glc}-3), 5.30 (d, *J* = 3.0 Hz, 1H; H_{Gal}-4). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 14.2 (CH_3), 15.6 (CH_3), 19.8, 20.5, 20.6, 20.6, 20.7, 20.7, 21.0 (Ac), 30.6 ($CHCH_2$), 50.9 (OCH_3), 52.9 (CO_2CH_3), 55.8 ($CHCH_2$), 60.9 (C_{Gal}-6), 62.1 (C_{Glc}-6), 66.7 (C_{Gal}-4), 69.1 (C_{Gal}-2), 70.3 (C_{Glc}-2), 70.8 (C_{Gal}-5), 71.0 (C_{Gal}-3), 73.3 (C_{Glc}-3), 75.9 (C_{Glc}-4), 77.1 (C_{Glc}-5), 84.8 (C_{Glc}-1), 90.0 ($CNCH_3OH$), 101.0 (C_{Gal}-1), 108.5 (CCH_3OCH_3), 154.5 (NCO_2), 169.1, 169.3, 169.6, 169.6, 170.1, 170.1, 170.3, 170.4 (7×Ac; CO_2CH_3). HRMS ESI+ (*m*/*z*): calcd for $C_{36}H_{51}NO_{23}SNa^+$ $[M + Na]^+$ 920.2465, found 920.2470.

dx.doi.org/10.1021/ja411522f | J. Am. Chem. Soc. 2014, 136, 789−800

*Methyl 2-((4S,5R)-4,5-Dimethyl-4-hydroxy-5-methoxy-2-oxooxazolidin-3-yl)-3-(tetra-O-acetyl-α-D-glucosylthio)propanoate, Mixture (**3e/4e**).* Diastereomeric mixture **3e**/**4e** was obtained from α,β-dehydroamino acid **1** (101 mg, 0.41 mmol) and tetra-*O*-acetyl-1-thio-α-D-glucose **2e** (151 mg, 0.41 mmol) following the procedure for sulfa-Michael additions, using DBU (67 μL, 0.45 mmol) as a base. After column chromatography (ethyl acetate/hexane, 1:1), diastereomeric mixture **3e**/**4e** was obtained as a colorless syrup (197 mg, 0.32 mmol, 79%) in a diastereomeric ratio 23/77, as determined by ^{1}H NMR. ^{1}H NMR data of major compound (400 MHz, $CDCl_3$): δ (ppm) 1.46 (s, 3H), 1.59 (s, 3H), 2.00 (s, 3H), 2.06 (s, 3H), 2.12 (s, 3H), 2.16 (s, 3H), 3.27−3.41 (m, 3H), 3.44 (s 3H), 3.78 (s, 3H), 4.08−4.24 (m, 2H), 4.26−4.37 (m, 2H), 5.19−5.36 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 15.7, 19.8, 20.6, 20.7, 20.9, 29.9, 50.9, 53.1, 53.4, 62.2, 66.1, 69.2, 69.6, 70.8, 81.3, 90.6, 108.3, 154.4, 169.5, 169.7, 169.8, 170.1, 170.7. HRMS ESI+ (*m*/*z*): calcd for $C_{24}H_{35}NO_{15}SNa^+$ [M + Na]$^+$ 632.1620, found 632.1617.

*Methyl (2S)-2-((4S,5R)-4,5-Dimethyl-4-hydroxy-5-methoxy-2-oxooxazolidin-3-yl)-3-(tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactosylthio)propanoate (**4f**).* Compound **4f** was obtained from α,β-dehydroamino acid **1** (424 mg, 1.73 mmol) and tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-α-D-galactose **2f** (690 mg, 1.90 mmol) following the procedure for sulfa-Michael additions, using DBU (310 μL, 2.07 mmol) as a base. After column chromatography ($CHCl_3$/MeOH, 95:5), **4f** was obtained as a white amorphous solid (704 mg, 0.64 mmol, 67%). $[\alpha]_D^{25}$ = +76.6 (*c* 1.10, $CHCl_3$). Mp: 179−181 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.41 (s, 3H; CH_3), 1.57 (s, 3H; CH_3), 1.97 (s, 3H; Ac), 2.00 (s, 3H; Ac), 2.07 (s, 3H; Ac), 2.14 (s, 3H; Ac), 3.31−3.35 (m, 2H; $CHCH_2$), 3.43 (s, 3H; OCH_3), 3.77 (s, 3H; CO_2CH_3), 4.01−4.22 (m, 4H; $CHCH_2$; H-6a; H-6b; OH), 4.52 (t, *J* = 6.3 Hz, 1H; H-5), 4.61−4.71 (m, 1H; H-2), 5.06 (dd, *J* = 3.2, 11.7 Hz, 1H; H-3), 5.40 (d, *J* = 2.8 Hz, 1H; H-4), 5.78 (d, *J* = 5.4 Hz, 1H; H-1), 6.16 (d, *J* = 7.2 Hz, 1H; NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 15.8 (CH_3), 19.9 (CH_3), 20.7, 20.7, 20.8, 23.1 (Ac), 28.8 ($CHCH_2$), 48.7 (C-2), 50.9 (OCH_3), 53.0 (CO_2CH_3), 53.1 ($CHCH_2$), 62.0 (C-6), 67.2 (C-4), 67.6 (C-5), 68.4 (C-3), 82.5 (C-1), 90.6 ($CNCH_3OH$), 108.3 (CCH_3OCH_3), 154.6 (NCO_2), 169.6, 170.2, 170.6, 171.1, 171.3 (4×Ac; CO_2CH_3). HRMS ESI+ (*m*/*z*): calcd for $C_{24}H_{36}N_2O_{14}SNa^+$ [M + Na]$^+$ 631.1785, found 631.1788.

*Methyl (2S)-2-((4S,5R)-4,5-Dimethyl-4-hydroxy-5-methoxy-2-oxooxazolidin-3-yl)-3-(tri-O-acetyl-2-acetamido-2-deoxy-α-D-glucosylthio)propanoate (**4g**).* Compound **4g** was obtained from α,β-dehydroamino acid **1** (143 mg, 0.58 mmol) and tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-α-D-glucose **2g** (234 mg, 0.64 mmol) following the procedure for sulfa-Michael additions, using DBU (105 μL, 0.70 mmol) as a base. After column chromatography ($CHCl_3$/MeOH, 95:5), **4g** was obtained as a white foam (284 mg, 0.46 mmol, 80%). $[\alpha]_D^{25}$ = +54.7 (*c* 1.14, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.38 (s, 3H; CH_3), 1.53 (s, 3H; CH_3), 1.91 (s, 3H; Ac), 1.98 (s, 3H; Ac), 1.99 (s, 3H; Ac), 2.06 (s, 3H; Ac), 3.23−3.35 (m, 2H; $CHCH_2$), 3.39 (s, 3H; OCH_3), 3.72 (s, 3H; CO_2CH_3), 4.05−4.28 (m, 3H; H-6a; H6-b; $CHCH_2$), 4.28−4.38 (m, 1H; H-5), 4.38−4.47 (m, 1H; H-2); 4.98−5.09 (m, 2H; H-3; H-4), 5.57 (d, *J* = 5.4 Hz, 1H; H-1), 6.19 (d, *J* = 8.0 Hz, 1H; NHAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 15.6 (CH_3), 19.8 (CH_3), 20.6, 20.7, 20.7, 23.0 (Ac), 29.8 ($CHCH_2$), 50.9 (OCH_3), 52.5 (C-2), 53.0 (CO_2CH_3), 53.3 ($CHCH_2$), 62.0 (C-6), 68.3 (C-4), 68.7 (C-5), 71.1 (C-3), 83.2 (C-1), 90.7 ($CNCH_3OH$), 108.3 (CCH_3OCH_3), 154.7 (NCO_2), 169.4, 169.5, 170.7, 171.0, 171.6 (4xAc; CO_2CH_3). HRMS ESI+ (*m*/*z*): calcd for $C_{24}H_{36}N_2O_{14}SNa^+$ [M + Na]$^+$ 631.1785, found 631.1780.

*tert-Butyl (2S)-2-((4S,5R)-4,5-Dimethyl-4-hydroxy-5-methoxy-2-oxooxazolidin-3-yl)-3-(tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactosylthio)propanoate (**4′f**).* Compound **4′f** was obtained from α,β-dehydroamino acid **1′** (720 mg, 2.51 mmol) and tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-α-D-galactose **2f** (1.00 g, 2.73 mmol) following the procedure for sulfa-Michael additions, using DBU (310 μL, 2.07 mmol) as a base. After column chromatography ($CHCl_3$/MeOH, 95:5), **4′f** was obtained as a white amorphous solid (1.40 g, 2.15 mmol, 86%). $[\alpha]_D^{25}$ = +89.3 (*c* 1.00, $CHCl_3$). Mp: 184−186 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.41 (s, 3H; CH_3), 1.44 (s, 9H; ^{t}Bu), 1.56 (s, 3H; CH_3), 1.96 (s, 3H; Ac), 1.99 (s, 3H; Ac), 2.07 (s, 3H; Ac), 2.14 (s, 3H; Ac), 3.29 (d, *J* = 7.6 Hz, 2H; $CHCH_2$), 3.41 (s, 3H; OCH_3), 4.00 (t, *J* = 7.6 Hz, 1H; $CHCH_2$), 4.06−4.19 (m, 3H; H-6a; H6-b; OH), 4.53 (t, *J* = 6.3 Hz, 1H; H-5), 4.62−4.72 (m, 1H; H-2), 5.06 (dd, *J* = 3.2, 11.7 Hz, 1H; H-3), 5.39 (d, *J* = 2.6 Hz, 1H; H-4), 5.72 (d, *J* = 5.4 Hz, 1H; H-1), 6.14 (d, *J* = 7.5 Hz, 1H; NH). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 15.4 (CH_3), 19.8 (CH_3), 20.6, 20.7, 20.7, 23.1 (Ac), 27.8 ($C(CH_3)_3$), 28.7 ($CHCH_2$), 48.6 (C-2), 50.7 (OCH_3), 54.1 ($CHCH_2$), 61.9 (C-6), 67.2 (C-4), 67.7 (C-5), 68.4 (C-3), 82.6 ($C(CH_3)_3$), 83.1 (C-1), 90.3 ($CNCH_3OH$), 108.2 (CCH_3OCH_3), 154.6 (NCO_2), 168.0, 170.2, 170.7, 171.1, 171.2 (4xAc; $CO_2{}^t$Bu). HRMS ESI+ (*m*/*z*): calcd for $C_{27}H_{42}N_2O_{14}SNa^+$ [M + Na]$^+$ 673.2249, found 673.2243.

*tert-Butyl (2S)-2-((4S,5R)-4,5-Dimethyl-4-hydroxy-5-methoxy-2-oxooxazolidin-3-yl)3-(tri-O-acetyl-2-acetamido-2-deoxy-α-D-glucosylthio)propanoate Ester (**4′g**).* Compound **4′g** was obtained from α,β-dehydroamino acid **1′** (504 mg, 1.75 mmol) and tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-α-D-glucose **2g** (765 mg, 2.10 mmol) following the procedure for sulfa-Michael additions, using DBU (288 μL, 1.92 mmol) as a base. After column chromatography ($CHCl_3$/MeOH, 19:1), **4′g** was obtained as a white amorphous solid (845 mg, 1.30 mmol, 74%). $[\alpha]_D^{25}$ = +59.5 (*c* 0.99, $CHCl_3$). Mp: 71−73 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.39 (s, 3H; CH_3), 1.42 (s, 9H; ^{t}Bu), 1.53 (s, 3H; CH_3), 1.92 (s, 3H; Ac), 1.99 (s, 3H; Ac), 2.00 (s, 3H; Ac), 2.07 (s, 3H; Ac), 3.19−3.33 (m, 2H; $CHCH_2$), 3.39 (s, 3H; OCH_3), 4.00 (dd, 1H; *J* = 5.9, 9.0 Hz, $CHCH_2$), 4.08 (d, 1H; *J* = 12.2 Hz; H-6a), 4.20−4.30 (m, 2H; H-6b; OH), 4.30−4.37 (m, 1H; H-5), 4.44 (dd, 1H; *J* = 2.6, 5.3 Hz; H-2), 5.13−4.97 (m, 2H; H-3; H-4), 5.56 (d, *J* = 5.4 Hz, 1H; H-1), 6.15 (d, *J* = 7.8 Hz, 1H; NHAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 15.4 (CH_3), 19.7 (CH_3), 20.6, 20.7, 20.7, 23.1 (Ac), 27.8 ($C(CH_3)_3$), 29.4 ($CHCH_2$), 50.7 (OCH_3), 52.6 (C-2), 54.2 ($CHCH_2$), 61.9 (C-6), 68.2 (C-4), 68.6 (C-5), 71.2 (C-3), 82.8 ($C(CH_3)_3$), 83.1 (C-1), 90.4 ($CNCH_3OH$), 108.2 (CCH_3OCH_3), 154.7 (NCO_2), 168.0, 169.3, 170.8, 170.9, 171.7 (4×Ac; $CO_2{}^t$Bu). HRMS ESI+ (*m*/*z*): calcd for $C_{27}H_{42}N_2O_{14}SNa^+$ [M + Na]$^+$ 673.2249, found 673.2258.

*Benzyl (2S)-2-((4S,5R)-4,5-Dimethyl-4-hydroxy-5-methoxy-2-oxooxazolidin-3-yl)-3-(tri-O-acetyl-2-acetamido-2-deoxy-α-D-glucosylthio)propanoate (**4″g**).* Compound **4″g** was obtained from α,β-dehydroamino acid **1″** (271 mg, 0.85 mmol) and tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-α-D-glucose **2g** (337 mg, 0.92 mmol) following the procedure for sulfa-Michael additions, using DBU (161 μL, 1.00 mmol) as a base. After column chromatography ($CHCl_3$/MeOH, 19:1), **4″g** was obtained as a white amorphous solid (411 mg, 0.60 mmol, 71%) with the presence of minor compound **3″g** in a ratio **3″g**/**4″g** of 15:85. $[\alpha]_D^{25}$ = +60.4 (*c* 1.03, $CHCl_3$). Mp: 46−48 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.36 (s, 3H; CH_3), 1.52 (s, 3H; CH_3), 1.91 (s, 3H; Ac), 1.99 (s, 3H; Ac), 2.00 (s, 3H; Ac), 2.00 (s, 3H; Ac), 3.26−3.48 (m, 5H; OCH_3; $CHCH_2$), 4.01−4.12 (m, 1H; H-6a), 4.14−4.23 (m, 2H; H-6b; $CHCH_2$), 4.28−4.36 (m, 1H; H-5), 4.38−4.47 (m, 1H; H-2), 5.01−5.09 (m, 2H; H-3; H-4), 5.15 (s, 2H; CH_2Ph), 5.59 (d, *J* = 5.4 Hz, 1H; H-1), 6.23 (d, *J* = 7.8 Hz, 1H; NHAc), 7.30 (m, 5H; Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 15.5 (CH_3), 19.7 (CH_3), 20.6, 20.6, 20.7, 23.0 (Ac), 29.7 ($CHCH_2$), 50.7 (OCH_3), 52.6 (C-2), 54.5 ($CHCH_2$), 62.0 (C-6), 67.8 (CH_2Ph), 68.3 (C-4), 68.7 (C-5), 71.2 (C-3), 83.2 (C-1), 90.6 ($CNCH_3OH$), 108.4 (CCH_3OCH_3), 128.1, 128.4, 128.6, 135.0 (Ph), 154.7 (NCO_2), 168.9, 169.3, 170.7, 171.0, 171.6 (4×Ac; CO_2Bn). HRMS ESI+ (*m*/*z*): calcd for $C_{30}H_{40}N_2O_{14}SNa^+$ [M + Na]$^+$ 707.2092, found 707.2090.

*S-(β-D-Glucosyl)-D-cysteine Hydrochloride (**5a**).* Compound **4a** (220 mg, 0.36 mmol) was introduced in a flask with 6 N HCl (5 mL). The mixture was stirred overnight at 60 °C. The solvent was removed in vacuo, and the crude was dissolved in water (3 mL) and extracted with ethyl acetate (3 mL). Aqueous layer was evaporated to give **5a** (*S*-β-D-Glc-D-Cys·HCl) as a colorless syrup (108 mg, 0.34 mmol, 94%). $[\alpha]_D^{25}$ = +43.0 (*c* 0.56, H_2O). ^{1}H NMR (400 MHz, D_2O): δ (ppm) 3.21−3.55 (m, 6H; $CHCH_2$; H-2; H-4; H-3; H-5), 3.71 (dd, *J* = 6.3, 12.2 Hz, 1H; H-6a), 3.80−3.97 (m, 1H; H-6b), 4.31−4.40 (m, 1H; $CHCH_2$), 4.62 (d, *J* = 9.7 Hz, 1H; H-1). ^{13}C NMR (100 MHz, D_2O): δ (ppm) 31.1 ($CHCH_2$), 53.2 ($CHCH_2$), 60.9 (C-6), 69.4 (C-4), 72.0

dx.doi.org/10.1021/ja411522f | J. Am. Chem. Soc. 2014, 136, 789−800

(C-2), 76.9 (C-3), 79.9 (C-5), 85.8 (C-1), 174.0 (CO_2H). HRMS ESI + (m/z): calcd for $C_9H_{18}NO_7S^+$ $[M]^+$ 284.0798, found 284.0799.

*S-(2-Acetamido-2-deoxy-α-D-galactosyl)-D-cysteine Hydrochloride (**5f**).* Compound **4′f** (1.40 g, 2.15 mmol) was introduced in a flask with 4 N HCl (25 mL). The mixture was stirred overnight at 40 °C. The solvent was removed in vacuo, and the crude was dissolved in water (20 mL) and extracted with ethyl acetate (20 mL). Aqueous layer was evaporated, and the crude was purified with a LC-18 SPE tube to give **5f** (*S*-α-D-GalNAc-D-Cys·HCl) as a colorless syrup (567 mg, 1.57 mmol, 73%). $[\alpha]_D^{25}$ = +141.6 (*c* 1.00, H_2O). ^{1}H NMR (400 MHz, D_2O): δ (ppm) 2.02 (s, 3H; Ac), 3.10 (dd, *J* = 7.8, 14.9 Hz, 1H; $CHCH_2$), 3.34 (dd, *J* = 4.6, 14.9 Hz, 1H; $CHCH_2$), 3.69−3.81 (m, 2H; H-6a; H-6b), 3.84 (dd, *J* = 3.1, 11.4 Hz, 1H; H-3), 3.99 (d, *J* = 3.1 Hz, 1H; H-4), 4.23 (dd, *J* = 4.8, 7.2 Hz, 1H; H-5), 4.29 (dd, *J* = 4.6, 7.6 Hz, 1H; $CHCH_2$), 4.36 (dd, *J* = 5.5, 11.4 Hz, 1H; H-2), 5.59 (d, *J* = 5.5 Hz, 1H; H-1). ^{13}C NMR (100 MHz, D_2O): δ (ppm) 21.9 (Ac), 30.5 ($CHCH_2$), 50.0 (C-2), 52.9 ($CHCH_2$), 61.1 (C-6), 67.4 (C-3), 68.3 (C-4), 72.1 (C-5), 84.3 (C-1), 170.2 (Ac), 174.8 (CO_2H). HRMS ESI+ (m/z): calcd for $C_{11}H_{21}N_2O_7S^+$ $[M]^+$ 325.1064, found 325.1056.

*S-(2-Acetamido-2-deoxy-α-D-glucosyl)-D-cysteine Hydrochloride (**5g**).* Compound **4′g** (845 mg, 1.30 mmol) was introduced in a flask with 4 N HCl (20 mL). The mixture was stirred overnight at 40 °C. The solvent was removed in vacuo, and the crude was purified by a LC-18 SPE tube to give **5g** (*S*-α-D-GlcNAc-D-Cys·HCl) as a colorless syrup (447 mg, 95%). This compound was obtained along with a small impurity (83/17 ratio by NMR) corresponding to the hydrolysis of the *N*-acetyl group of carbohydrate moiety, which was not possible to separate. ^{1}H NMR (400 MHz, D_2O): δ (ppm) 1.96 (s, 3H; Ac), 3.09 (dd, *J* = 6.9, 14.9 Hz, 1H; $CHCH_2$), 3.27 (dd, *J* = 4.7, 14.9 Hz, 1H; $CHCH_2$), 3.42 (t, *J* = 9.4 Hz, 1H; H-4), 3.60 (dd, *J* = 8.8, 10.9 Hz H-3), 3.68−3.82 (m, 2H; H-6a; H-6b), 3.91 (ddd, *J* = 2.3, 5.1, 10.1 Hz 1H; H-5), 4.04 (dd, *J* = 5.3, 11.0 Hz, 1H; H-2), 4.28 (dd, *J* = 4.8, 6.9 Hz, 1H; $CHCH_2$), 5.45 (d, *J* = 5.3 Hz, 1H; H-1). ^{13}C NMR (100 MHz, D_2O): δ (ppm) 21.9 (Ac), 30.5 ($CHCH_2$), 52.6 ($CHCH_2$), 53.7 (C-2), 60.3 (C-6), 70.0 (C-4), 70.6 (C-3), 72.9 (C-5), 84.2 (C-1), 169.9 (Ac), 174.5 (CO_2H). HRMS ESI+ (m/z): calcd for $C_{11}H_{21}N_2O_7S^+$ $[M]^+$ 325.1064, found 325.1059.

*S-(Tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactosyl)-N-Fmoc-D-cysteine Allyl Ester (**6f**).* (1) Compound **5f** (567 mg, 1.57 mmol) was dissolved in water (10 mL), and $NaHCO_3$ (263 mg, 3.14 mmol) was added with stirring. Fmoc-OSu (793 mg, 2.35 mmol) was then added as a solution in acetonitrile (20 mL). The resulting mixture was stirred overnight at room temperature. Water was then added, and the mixture was acidified to a pH of 1 with HCl 2 N. Next, acetonitrile was evaporated, and the mixture was filtered. Fmoc-protected derivative was collected and dried as a white solid (608 mg, 1.11 mmol, 70%). (2) Fmoc-D-Cys-(α-D-GalNAc)-OH (518 mg, 0.94 mmol) was introduced as a solution in dry DMF (10 mL), under argon atmosphere, in a Schlenk previously charged with 3 Å molecular sieves and Cs_2CO_3 (370 mg, 1.13 mmol). Allyl bromide (131 μL, 1.51 mmol) was then added by a syringe, and the mixture was stirred overnight at room temperature. After that, the solvent was evaporated, and the crude was dissolved in AcOEt and filtered. Evaporation of the solvent yielded the compound Fmoc-D-Cys(α-D-GalNAc)-OAllyl (217 mg, 0.37 mmol, 37%). (3) Treatment with Ac_2O in pyridine (1:2, 5 mL) at room temperature for 1 h produced compound **6f** (249 mg, 0.35 mmol, 94%), which was purified by a silica gel column chromatography ($CHCl_3$/AcOEt 1:1) affording **6f** as a colorless syrup. Its diastereoisomer Fmoc-L-Cys(α-D-$(OAc)_3$GalNAc)-OAllyl was synthesized for comparative purposes by the method reported in the literature,[20a] and their ^{1}H NMR spectra were compared, finding that these spectroscopic data are different (see the Supporting Information). $[\alpha]_D^{25}$ = +70.8 (*c* 1.00, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.94 (s, 3H; Ac), 1.99 (s, 3H; Ac), 2.05 (s, 3H; Ac), 2.18 (s, 3H; Ac), 3.07 (dd, *J* = 5.8, 14.0 Hz, 1H; $CHCH_2$), 3.27 (dd, *J* = 4.5, 13.8 Hz, 1H; $CHCH_2$), 3.95−4.17 (m, 2H; H-6a; H-6b), 4.27 (t, *J* = 6.2 Hz, 1H; CH_{Fmoc}), 4.36 (t, *J* = 6.1 Hz, 1H; H-5), 4.40−4.56 (m, 1H; CH_{2Fmoc}), 4.56−4.62 (m, 1H; CH_{2Fmoc}), 4.63−4.72 (m, 3H; $CHCH_2$; CH_{2Allyl}), 4.78 (ddd, *J* = 5.4, 8.5, 11.9 Hz, 1H; H-2), 5.00 (dd, *J* = 3.1, 11.7 Hz, 1H; H-3), 5.26−5.46 (m, 3H; H-4; CH═CH_2), 5.58 (d, *J* = 5.2 Hz, 1H; H-1), 5.66 (d, *J* = 8.6 Hz, 1H; NHAc), 5.80 (d, *J* = 7.8 Hz, 1H; NHFmoc), 5.92 (ddd, *J* = 6.0, 11.2, 16.5 Hz, 1H; CH═CH_2), 7.37 (dd, *J* = 6.5, 13.5 Hz, 2H; Fmoc), 7.40−7.50 (m, 2H; Fmoc), 7.60−7.64 (m, 2H; Fmoc), 7.80 (d, *J* = 7.4 Hz, 2H; Fmoc). ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 20.4, 20.7, 20.7, 23.2 (4xAc), 33.5 ($CHCH_2$), 47.0 (CH_{Fmoc}), 48.1 (C-2), 53.7 ($CHCH_2$), 62.0 (C-6), 66.6 (CH_2Allyl), 66.6 (CH_{2Fmoc}), 67.1 (C-4), 67.5 (C-5), 68.2 (C-3), 85.6 (C-1), 119.6 (CH═CH_2), 120.0, 124.8, 124.8, 127.1, 127.1, 127.7 (Fmoc), 131.1 (CH═CH_2), 141.3, 141.3, 143.6, 143.7 (Fmoc), 155.7 (NCO_2), 169.7, 170.2, 170.3, 170.5, 170.9 (4×Ac; CO_2allyl). HRMS ESI+ (m/z): calcd for $C_{35}H_{40}N_2O_{12}SNa^+$ $[M + Na]^+$ 735.2194, found 735.2202.

*S-((Tri-O-acetyl)-2-acetamido-2-deoxy-α-D-glucosyl)-N-Cbz-D-cysteine Benzyl Ester (**6g**).* (1) Compound **5g** (244 mg, 0.68 mmol) was dissolved in water (2.5 mL), and $NaHCO_3$ (114 mg, 1.36 mmol) was added with stirring. The resulting solution was cooled at 0 °C, and Cbz-Cl (145 μL, 1.02 mmol) was added as a solution in 1,4-dioxane (2.5 mL). The resulting mixture was stirred at 0 °C for 1 h and allowed to warm to room temperature overnight. Water was then added, and the mixture was acidified to a pH of 1 with HCl 2 N. The aqueous layer was extracted with AcOEt (3 × 10 mL) and $CHCl_3$/^{i}PrOH (3:1) (4 × 10 mL). The organic layers were combined and evaporated, and the crude was purified by LC-18 SPE tube to give the *N*-Cbz-protected derivative (91 mg, 0.20 mmol, 29%). (2) Cbz-D-Cys(α-D-GlcNAc)-OH was introduced as a solution in dry DMF (2 mL), under argon atmosphere, in a Schlenk tube previously charged with 3 Å molecular sieves and Cs_2CO_3 (77 mg, 0.24 mmol). Benzyl bromide (38 μL, 0.32 mmol) was then added by a syringe, and the mixture was stirred overnight at room temperature. Next, the solvent was evaporated, and the crude was dissolved in AcOEt and filtered. Evaporation of the solvent gave the benzyl ester derivative Cbz-D-Cys(α-D-GlcNAc)-OBn (60 mg, 0.11 mmol, 55%). (3) Treatment with Ac_2O and pyridine (1:2, 1.5 mL) at room temperature for 1 h yielded compound **6g** (42 mg, 0.063 mmol, 57%), which was purified by a silica gel column chromatography (hexane/AcOEt 3:7) affording **6g** as a colorless syrup. The spectroscopic data of this compound were found different from those of its diastereoisomer Cbz-L-Cys(α-D-$(OAc)_3$GlcNAc)-OBn reported in the literature.[5c] $[\alpha]_D^{25}$ = +71.0 (*c* 1.01, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.94 (s, 3H; Ac), 2.03 (s, 3H; Ac), 2.03 (s, 3H; Ac), 2.05 (s, 3H; Ac), 3.01 (dd, *J* = 5.6, 13.9 Hz, 1H; $CHCH_2$), 3.21 (dd, *J* = 4.5, 13.9 Hz, 1H; $CHCH_2$), 4.05 (d, *J* = 10.5 Hz, 1H; H-6a), 4.15−4.28 (m, 2H; H-6b; H-5), 4.47 (ddd, *J* = 11.0, 8.7, 5.4 Hz, 1H; H-2), 4.61−4.71 (m, 1H; $CHCH_2$), 4.98 (dd, *J* = 11.1, 9.4 Hz, 1H; H-3), 5.03−5.21 (m, 5H; H-4; 2×CH_2Ph), 5.44 (d, *J* = 5.3 Hz, 1H; H-1), 5.64 (d, *J* = 7.7 Hz, 1H; NHCbz), 5.76 (d, *J* = 8.4 Hz, 1H; NHAc), 7.36 (m, 10H; 2xPh). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 20.6, 20.7, 20.7, 23.2 (4×Ac), 34.6 ($CHCH_2$), 52.5 (C-2), 54.0 ($CHCH_2$), 61.8 (C-6), 67.3 (CH_2Ph), 67.9 (CH_2Ph), 68.0 (C-4), 68.9 (C-5), 71.2 (C-3), 85.8 (C-1), 128.1, 128.3, 128.6, 128.6, 128.8, 128.8, 134.8, 136.0 (2×Ph), 155.7 (NCO_2), 169.2, 169.9, 170.1, 170.7, 171.6 (4×Ac; CO_2Bn). HRMS ESI+ (m/z): calcd for $C_{32}H_{38}N_2O_{12}SNa^+$ $[M + Na]^+$ 697.2038, found 697.2035.

*Methyl (2R)-2-((4R,5S)-4,5-Dimethyl-4-hydroxy-5-methoxy-2-oxooxazolidin-3-yl)-3-(tetra-O-acetyl-β-D-glucosylthio)propanoate (**7a**).* Compound **7a** was obtained from α,β-dehydroamino acid **ent-1** (2.20 g, 8.97 mmol) and tetra-*O*-acetyl-1-thio-β-D-glucose **2a** (3.57 g, 9.85 mmol) following the procedure for sulfa-Michael additions, using DBU (1.60 mL, 10.50 mmol) as a base. After column chromatography (ethyl acetate/hexane, 1:1), **7a** was obtained as a white amorphous solid (4.32 g, 7.09 mmol, 79%). $[\alpha]_D^{25}$ = +11.3 (*c* 1.00, $CHCl_3$). Mp: 54−56 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.42 (s, 3H; CH_3), 1.57 (s, 3H; CH_3), 2.02 (s, 3H; Ac), 2.03 (s, 3H; Ac), 2.07 (s, 3H; Ac), 2.09 (s, 3H; Ac), 3.37 (dd, *J* = 10.0, 14.9, Hz, 1H; $CHCH_2$), 3.44 (s, 3H; OCH_3), 3.54−3.65 (m, 1H; $CHCH_2$), 3.71−3.83 (m, 1H; H-5), 3.79 (s, 3H; CO_2CH_3), 4.09−4.26 (m, 2H; H-6a; $CHCH_2$), 4.26−4.35 (m, 1H; H-6b), 4.56 (d, *J* = 10.0 Hz, 1H; H-1), 5.03−5.15 (m, 2H; H-2; H-4), 5.22 (t, *J* = 9.5 Hz, 1H; H-3). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 15.4 (CH_3), 19.6 (CH_3), 20.6, 20.6, 20.7, 20.7 (Ac), 30.1 ($CHCH_2$), 50.8 (OCH_3), 53.0 (CO_2CH_3), 55.7 ($CHCH_2$), 61.4 (C-6), 67.7 (C-4), 69.3 (C-2), 73.6 (C-3), 76.3 (C-5), 84.7 (C-1), 90.1

dx.doi.org/10.1021/ja411522f | J. Am. Chem. Soc. 2014, 136, 789−800

($CNCH_3OH$), 108.4 (CCH_3OCH_3), 154.7 (NCO_2), 169.2, 169.4, 169.4, 170.0, 170.6 (4×Ac; CO_2CH_3). HRMS ESI+ (m/z): calcd for $C_{24}H_{35}NO_{15}SNa^+$ $[M + Na]^+$ 632.1620, found 632.1630.

tert-Butyl (2R)-2-((4R,5S)-4,5-Dimethyl-4-hydroxy-5-methoxy-2-oxooxazolidin-3-yl)-3-(tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactosylthio)propanoate (7′f). Compound 7′**f** was obtained from α,β-dehydroamino acid **ent-1**′ (764 mg, 2.66 mmol) and tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-α-D-galactose **2f** (1.06 g, 2.93 mmol) following the procedure for sulfa-Michael additions, using DBU (529 μL, 3.19 mmol) as a base. After column chromatography ($CHCl_3$/MeOH, 19:1), 7′**f** was obtained as a white amorphous solid (1.31 g, 2.02 mmol, 76%). $[\alpha]_D^{25}$ = +141.8 (*c* 1.00, $CHCl_3$). Mp: 140−142 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 1.45 (s, 12H; $C(CH_3)_3$; CH_3), 1.57 (s, 3H; CH_3), 1.96 (s, 3H; Ac), 1.99 (s, 3H; Ac), 2.03 (s, 3H; Ac), 2.14 (s, 3H; Ac), 3.33 (dd, *J* = 7.3, 10.5 Hz, 2H; $CHCH_2$), 3.41 (s, 3H; OCH_3), 4.05−4.18 (m, 3H; H-6a; H-6b; $CHCH_2$), 4.52 (t, *J* = 6.4 Hz, 1H; H-5), 4.60−4.77 (m, 1H; H-2), 5.02 (dd, *J* = 3.0, 11.6 Hz, 1H; H-3), 5.37 (d, *J* = 2.4 Hz, 1H; H-4), 5.64 (d, *J* = 5.3 Hz, 1H; H-1), 5.95 (d, *J* = 8.1 Hz, 1H; NHAc). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 15.3 (CH_3), 19.9 (CH_3), 20.7, 20.7, 20.8, 23.2 (Ac), 27.9 ($C(CH_3)_3$), 30.8 ($CHCH_2$), 48.6 (C-2), 50.7 (OCH_3), 56.4 ($CHCH_2$), 61.8 (C-6), 67.2 (C-4), 67.7 (C-5), 68.4 (C-3), 83.3 ($C(CH_3)_3$), 85.5 (C-1), 90.5 ($CNCH_3OH$), 108.1 (CCH_3OCH_3), 154.8 (NCO_2), 168.3, 170.2, 170.7, 170.7, 171.1 (4×Ac; $CO_2{}^t$Bu). HRMS ESI+ (m/z): calcd for $C_{27}H_{42}N_2O_{14}SNa^+$ $[M + Na]^+$ 673.2249, found 673.2257.

S-(β-D-Glucosyl)-L-cysteine Hydrochloride (8a). Compound **7a** (4.32 g, 7.09 mmol) was introduced in a flask with 6 N HCl (100 mL). The mixture was stirred overnight at 60 °C. The solvent was removed in vacuo, and the crude was dissolved in water (25 mL) and extracted with ethyl acetate (25 mL). Aqueous layer was evaporated to give **8a** (*S*-β-D-Glc-L-Cys·HCl) as a colorless syrup (2.13 g, 6.66 mmol, 94%). $[\alpha]_D^{25}$ = −37.4 (*c* 0.63, H_2O). ^{1}H NMR (400 MHz, D_2O): δ (ppm) 3.14 (dd, *J* = 8.0, 15.3 Hz, 1H; $CHCH_2$), 3.36−3.47 (m, 3H; $CHCH_2$; H-2; H-4), 3.47−3.56 (m, 2H; H-3; H-5), 3.72 (dd, *J* = 5.8, 12.4 Hz, 1H; H-6a), 3.88−3.99 (m, 1H; H-6b), 4.02 (dd, *J* = 3.7, 7.8 Hz, 1H; $CHCH_2$), 4.56 (d, *J* = 9.7 Hz, 1H; H-1). ^{13}C NMR (100 MHz, D_2O): δ (ppm) 30.4 ($CHCH_2$), 54.5 ($CHCH_2$), 60.9 (C-6), 69.4 (C-4), 71.7 (C-2), 77.0 (C-3), 80.1 (C-5), 84.7 (C-1), 172.2 (CO_2H). HRMS ESI+ (m/z): calcd for $C_9H_{18}NO_7S^+$ $[M + H]^+$ 284.0798, found 284.0787. A small percentage of mutarotation was observed in the ^{1}H NMR spectrum.[16]

S-(2-Acetamido-2-deoxy-α-D-galactosyl)-L-cysteine Hydrochloride (8f). Compound 7′**f** (1.31 g, 2.02 mmol) was introduced in a flask with 4 N HCl (25 mL). The mixture was stirred overnight at 40 °C. The solvent was removed in vacuo, and the crude was dissolved in water (10 mL) and extracted with ethyl acetate (10 mL). Aqueous layer was evaporated to give **8f** (*S*-α-D-GalNAc-L-Cys·HCl) as a colorless syrup (670 mg, 1.85 mmol, 92%). $[\alpha]_D^{25}$ = +144.9 (*c* 1.00, H_2O). ^{1}H NMR (400 MHz, D_2O): δ (ppm) 2.05 (s, 3H; Ac), 3.09−3.19 (m, 1H; $CHCH_2$), 3.34 (dd, *J* = 6.2, 15.0 Hz, 1H; $CHCH_2$), 3.74−3.87 (m, 3H; H-6a; H-6b; H-3), 4.00 (s, 1H; H-3), 4.04−4.11 (m, 1H; $CHCH_2$), 4.26−4.36 (m, 1H; H-5), 4.40 (dd, *J* = 5.4, 11.4 Hz, 1H; H-2), 5.54 (d, *J* = 5.3 Hz, 1H; H-1). ^{13}C NMR (100 MHz, D_2O): δ (ppm) 21.9 (Ac), 33.1 ($CHCH_2$), 49.9 (C-2), 54.4 ($CHCH_2$), 61.2 (C-6), 67.4 (C-3), 68.4 (C-4), 72.4 (C-5), 86.2 (C-1), 172.3 (CO_2H), 174.6 (Ac). HRMS ESI+ (m/z): calcd for $C_{11}H_{21}N_2O_7S^+$ $[M]^+$ 325.1064, found 325.1061.

S-(Tetra-O-acetyl-β-D-glucosyl)-N-Fmoc-L-cysteine (9a). Compound **8a** (975 mg, 3.05 mmol) was dissolved in water (25 mL), and $NaHCO_3$ (511 mg, 6.08 mmol) was added with stirring. Fmoc-OSu (1.54 g, 4.57 mmol) was then added as a solution in acetonitrile (50 mL). The resulting mixture was stirred at room temperature overnight. Water was then added, and the mixture was acidified to pH = 1 with 2 N HCl. Next, acetonitrile was evaporated, and the mixture was filtered. Fmoc-protected derivative, Fmoc-L-Cys(β-D-Glc)-OH, was collected and dried as a white amorphous solid (902 mg, 1.79 mmol, 59%). This compound was suspended in Ac_2O (10 mL), and a few drops of H_3PO_4 (85 wt % in water) were added. The reaction was stirred for 2 h at room temperature and then quenched with saturated solution of $NaHCO_3$ (30 mL). The solution was acidified to pH = 1 with 2 N HCl and then extracted with ethyl acetate (3 × 15 mL). This crude compound was purified by flash silica gel chromatography (CH_2Cl_2/MeOH, 95:5) to give building block **9a** (566 mg, 0.84 mmol, 47%) as a colorless syrup. $[\alpha]_D^{25}$ = −6.2 (*c* 1.00, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.01 (s, 3H; Ac), 2.04 (s, 3H; Ac), 2.06 (s, 3H; Ac), 2.10 (s, 3H; Ac), 3.10 (dd, *J* = 4.3, 14.2 Hz, 1H; $CHCH_2$), 3.30 (d, *J* = 10.7 Hz, 1H; $CHCH_2$), 3.62−3.69 (m, 1H; H-5), 4.07−4.30 (m, 3H; $CHCH_{2Fmoc}$, H-6a; H-6b), 4.35−4.58 (m, 3H; $CHCH_{2Fmoc}$, H-1), 4.59−4.67 (m, 1H; $CHCH_2$), 4.99 (t, *J* = 9.7 Hz, 1H; H-2), 5.05 (m, 1H; H-4), 5.22 (t, *J* = 9.3 Hz, 1H; H-3), 5.94 (d, *J* = 7.1 Hz, 1H; NH_{Fmoc}), 7.32 (t, *J* = 7.4 Hz, 2H; Fmoc), 7.40 (t, *J* = 7.4 Hz, 2H; Fmoc), 7.55−7.64 (m, 2H; Fmoc), 7.77 (d, *J* = 7.5 Hz, 2H; Fmoc). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 20.6, 20.6, 20.7, 20.7 (Ac), 33.0 ($CHCH_2$), 47.2 ($CHCH_{2Fmoc}$), 53.8 ($CHCH_2$), 62.4 (C-6), 67.2 ($CHCH_{2Fmoc}$), 68.6 (C-4), 69.7 (C-2), 73.5 (C-3), 75.9 (C-5), 84.2 (C-1), 120.0, 125.1, 125.3, 127.2, 127.8 (Fmoc), 141.3, 143.7, 143.7 (Fmoc), 156.1 (NCO_2), 169.4, 169.6, 170.2, 171.1, 176.7 (4×Ac; CO_2H). HRMS ESI− (m/z): calcd for $C_{32}H_{34}NO_{13}S^-$ $[M - H]^-$ 672.1756, found 672.1778.

S-(Tri-O-acetyl-2-acetamido-2-deoxy-α-D-galactosyl)-N-Fmoc-L-cysteine (9f). Compound **8f** (670 mg, 1.85 mmol) was treated following the same methodology for the synthesis of Fmoc-D-Cys(α-D-$(OAc)_3$GalNAc)-OAllyl (**6f**). (1) Fmoc-OSu, $NaHCO_3$, H_2O/acetonitrile (1:2), room temperature, overnight (738 mg, 1.35 mmol, 73%). (2) AllylBr, Cs_2CO_3, DMF, 3 Å molecular sieves, overnight (277 mg, 0.47 mmol, 35%). (3) Ac_2O, pyridine, 1 h (298 mg, 0.42 mmol, 89%). All physical and spectroscopic properties of Fmoc-L-Cys(α-D-$(OAc)_3$GalNAc)-OAllyl were identical to those reported in the literature.[20a] (4) Fmoc-L-Cys(α-D-$(OAc)_3$GalNAc)-OAllyl (298 mg, 0.42 mmol) was dissolved in dry THF (10 mL) under argon atmosphere. $Pd(PPh_3)_4$ (5.2 mg, 4.5 × 10^{-3} mmol) and morpholine (182 μL, 2.1 mmol) were added, and the resulting solution was stirred at room temperature for 1 h. Next, the solvent was removed, and the crude was dissolved in $CHCl_3$/^{i}PrOH (3:1, 10 mL). The solution was washed with 1 N HCl (2 × 5 mL), and the combined aqueous phases were extracted with more $CHCl_3$/^{i}PrOH (2 × 5 mL). The organic layers were combined, dried with anhydrous Na_2SO_4, and solvent was removed. The crude product was purified by column chromatography (ethyl acetate/hexane 7:3 to CH_2Cl_2/MeOH 9:1) to give building block **9f** (268 mg, 0.40 mmol, 95%) as a yellow amorphous solid. $[\alpha]_D^{25}$ = +120.3 (*c* 1.00, $CHCl_3$). Mp: 102−104 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 2.01 (s, 3H; Ac), 2.02 (s, 3H; Ac), 2.04 (s, 3H; Ac), 2.20 (s, 3H; Ac), 3.08 (d, *J* = 13.8 Hz, 1H; $CHCH_2$), 3.36 (d, *J* = 12.4 Hz, 1H; $CHCH_2$), 4.04−4.13 (m, 1H; H-6a), 4.20−4.30 (m, 2H; H-6b; $CHCH_{2Fmoc}$), 4.33−4.44 (m, 1H; $CHCH_{2Fmoc}$), 4.45−4.56 (m, 2H; $CHCH_{2Fmoc}$; H-5), 4.73−4.89 (m, 2H; H-2; $CHCH_2$), 4.94−5.03 (m, 1H; H-3), 5.40 (s, 1H; H-4), 5.58 (s, 1H; H-1), 6.42 (s, 1H; NHFmoc), 6.64 (s, 1H; NHAc), 7.31−7.46 (m, 4H; Fmoc), 7.60−7.68 (m, 2H; Fmoc), 7.79 (d, *J* = 7.4 Hz 2H; Fmoc). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 20.6, 20.7, 20.7, 23.1 (Ac), 36.4 ($CHCH_2$), 47.1 ($CHCH_{2Fmoc}$), 48.4 (C-2), 54.6 ($CHCH_2$), 61.6 (C-6), 67.0 ($CHCH_{2Fmoc}$), 67.2 (C-4), 68.0 (C-5), 68.3 (C-3), 87.4 (C-1), 120.0, 125.1, 125.1, 127.1, 127.8, 128.6, 128.7 (Fmoc), 141.3, 141.4, 143.7, 143.9 (Fmoc), 155.9 (NCO_2), 170.4, 170.6, 171.2, 171.4, 174.7 (4×Ac; CO_2H). HRMS ESI− (m/z): calcd for $C_{32}H_{35}N_2O_{12}S^-$ $[M - H]^-$ 671.1916, found 671.1904.

S-(2-Acetamido-2-deoxy-α-D-galactosyl)-N-(acetyl)-L-cysteine Methylamide (10f). Fmoc-L-Cys(α-$(OAc)_3$-D-GalNAc)-OH (**9f**, 268 mg, 0.40 mmol) was dissolved in dry acetonitrile (10 mL) in the presence of 3 Å molecular sieves. TBTU (350 mg, 1.09 mmol) was added in one portion, followed by DIEA (732 μL, 4.2 mmol). After the mixture was stirred for 5 min, $MeNH_2$·HCl (170 mg, 2.52 mmol) was added under argon atmosphere. The reaction was stirred overnight, the mixture was then filtered off over a Celite pad, and brine was added to the remaining solution. Aqueous layer was extracted with CH_2Cl_2 (2 × 5 mL), and organic layers were combined and washed with 1 N HCl (2 × 5 mL) and $NaHCO_3$ (2 × 5 mL) saturated solution. After evaporation of the solvents, the reaction crude was purified by column chromatography (CH_2Cl_2/MeOH, 95:5) yielding

dx.doi.org/10.1021/ja411522f | J. Am. Chem. Soc. 2014, 136, 789−800

Fmoc-L-Cys(α-D-(OAc)$_3$GalNAc)-NHMe (158 mg, 0.23 mmol, 57%). This Fmoc-derivative was treated with piperidine (20% in acetonitrile, 10 mL) for 30 min at room temperature. The solvent was evaporated and the crude purified by flash column chromatography (CH_2Cl_2/MeOH, 90:10) yielding L-Cys(α-D-(OAc)$_3$GalNAc)-NHMe (102 mg, 0.22 mmol, 98%). This amine was reacted with Ac_2O in the presence of pyridine (1:2, 5 mL) for 1 h at room temperature. The solvent was evaporated, and the reaction crude was purified by column chromatography (CH_2Cl_2/MeOH, 95:5) yielding Ac-L-Cys(α-D-(OAc)$_3$GalNAc)-NHMe (96 mg, 0.19 mmol, 87%). This peracetylated carbohydrate was treated with 0.5 M NaOMe solution in MeOH (2 mL) for 1.5 h. After neutralization with Dowex 50WX8, the solution was evaporated, and the crude was purified by a LC-18 SPE tube to give Ac-L-Cys(α-D-GalNAc)-NHMe **10f** as a white foam (58 mg, 0.15 mmol, 78% and 38% overall yield from **9f**). $[\alpha]_D^{25}$ = +73.4 (*c* 0.50, H_2O). ^{1}H NMR (400 MHz, D_2O): δ (ppm) 2.04 (s, 3H; AcN2), 2.06 (s, 3H; AcN3), 2.75 (s, 3H; NHMe), 2.97−3.10 (m, 2H; CHCH_2), 3.79 (d, *J* = 6.0 Hz, 2H; H-6a; H-6b), 3.84 (dd, *J* = 2.4, 11.2 Hz, 1H; H-3), 4.00 (s, 1H; H-4), 4.26 (t, *J* = 6.0 Hz, 1H; H-5), 4.36 (dd, *J* = 5.5, 11.4 Hz, 1H; H-2), 4.53 (t, *J* = 6.4 Hz, 1H; C*H*CH$_2$), 5.55 (d, *J* = 5.5 Hz, 1H; H-1). ^{1}H NMR (400 MHz, D_2O/H_2O, 9:1): δ (ppm) 7.96−8.02 (m, 1H; NH1), 8.15 (d, *J* = 8.0 Hz, 1H; NH3), 8.28 (d, *J* = 7.5 Hz, 1H; NH3). ^{13}C NMR (100 MHz, D_2O): δ (ppm) 21.8 (Ac), 21.8 (Ac), 25.8 (NHMe), 31.7 (CHCH$_2$), 50.0 (C-2), 54.0 (CHCH$_2$), 61.0 (C-6), 67.4 (C-3), 68.4 (C-4), 71.6 (C-5), 84.6 (C-1), 172.5 (CONHMe), 174.3 (Ac), 174.5 (Ac). HRMS ESI+ (*m*/*z*): calcd for $C_{14}H_{26}N_3O_7S^+$ $[M + H]^+$ 380.1486, found 380.1471.

■ ASSOCIATED CONTENT

Supporting Information

Experimental and computational details, X-ray data, and spectroscopic characterization of all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank the Ministerio de Economía y Competitividad (project CTQ2012-36365/FEDER). We also thank CESGA for computer support. We appreciate the helpful comments of Dr. Gonzalo Jiménez-Osés.

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