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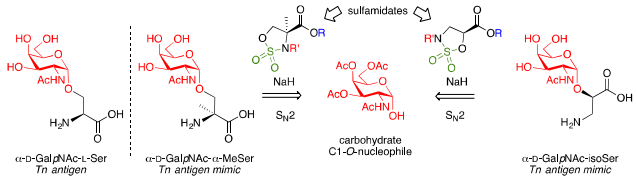
ABSTRACT

Starting from commercially available (*S*)-isoserine and effectively accessible (*S*)-α-methylserine, enantiopure cyclic sulfamidates have been prepared as chiral building blocks for the synthesis of various *S*- and *O*-glycosylated amino acid derivatives, including unnatural variants of the Tn antigen, through highly chemo-, regio- and stereoselective nucleophilic ring-opening reactions with carbohydrate C1-*S*- and C1-*O*-nucleophiles.

TOC



INTRODUCTION

MUC1 mucin is an *O*-glycoprotein that plays a pivotal role in the renewal and differentiation of the epithelium, cell adhesions, immune response and cell signalling.[1] In healthy cells, the mucin peptide is decorated with complex oligosaccharides; however, when overexpressed in tumour cancer cells, the backbone appears with simple, truncated carbohydrates. Incomplete glycosylation exposes glycopeptide epitopes, normally masked in healthy cells, to the immune system. Such tumour associated carbohydrate antigens [e.g., Tn antigen (α-D-Gal*p*NAc-L-Ser/Thr)] are thus attractive targets for the development of therapeutic cancer vaccines. However, to date, none of these vaccines based on this antigen have succeeded in clinical trials. The main drawback with therapeutic vaccines is that cancer cells can generate immune escape mechanisms,[2] which results in an increased tolerance of the antigens by the immune system.

An attractive approach to tackle this issue may be the use of unnatural derivatives that mimic the structure of the Tn antigen.[3] Because of this, nowadays, there is an active field of research focused on the design of the Tn antigen mimics acting as better candidates for anticancer vaccine generation.[4,5] Our group has contributed to this field by synthetizing and evaluating a new cancer vaccine incorporating α-D-Gal*p*NAc-α-MeSer (α-MeSer = α-methylserine).[6]

Linkage of α-GalNAc to Ser, Thr or, in general, hydroxyamino acids is a quite difficult synthetic operation due to the presence of the C2-acetamido group in 1,2-*cis* disposition. This group often directs glycosylation to form a β-linkage due to neighboring effects. These effects are particularly relevant when the carbohydrate acts as an electrophile, often through a planar, prochiral oxocarbenium intermediate. In our case, the unnatural glycosyl amino acid (α-D-Gal*p*NAc-α-MeSer) was prepared by two different methodologies, both based on

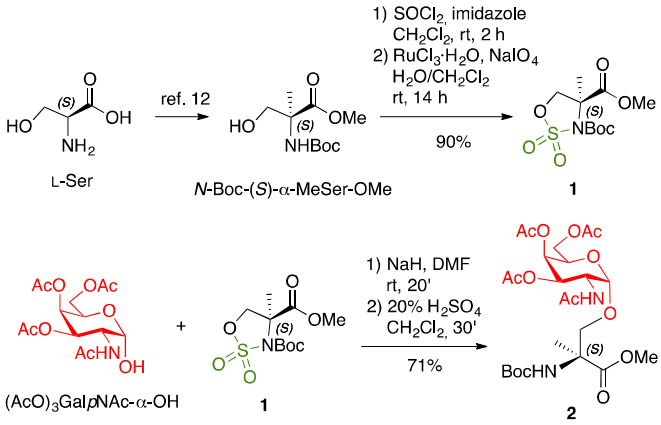
the nucleophilic attack of protected α-MeSer to carbohydrate electrophiles such as glycosyl halides (Koenigs-Knorr)[6] or 2-nitrogalactal (*O*-Michael addition).[5,7] These methodologies give mixtures of both α- and β-anomers, leading in the best conditions to ca. 25% of the undesired β-derivative, therefore they have the drawback of needing tedious chromatographic separation from the reaction mixture. One way of mitigating such neighboring effects is by using nucleophilic carbohydrates in which the anomeric carbon provides the atom defining the glycosydic bond (normally *O* or *S*), and maintains its configuration upon glycosylation.

Along these lines, several groups including ours have developed alternative glycosylation methods using the well-established sulfamidate chemistry.[8] Hence, we designed a versatile synthetic methodology based on the ring-opening of hindered cyclic sulfamidates derived from α-methylisoserine, with various 1-thiocarbohydrates as nucleophiles.[9] We demonstrated that this reaction proceeds with total inversion of the configuration at the quaternary electrophilic carbon, preserving the enantiomeric excess of the starting material. The synthesis and subsequent ring-opening of cyclic sulfamidates derived from L-serine and L-threonine with some 1-thiocarbohydrates have also been reported.[10] However, nucleophilic displacement reactions involving oxygenated nucleophiles derived from carbohydrates, especially pyranose C1-*O*-hemiacetals, are often problematic due to their poorer nucleophilicity and higher basicity compared to 1-thiocarbohydrates. To the best of our knowledge, this type of reaction has only been achieved using aziridine-2-carboxamides as electrophiles,[11] leading to α- and β-*O*-glycosyl serine conjugates in a highly stereoselective manner.

RESULTS AND DISCUSSION

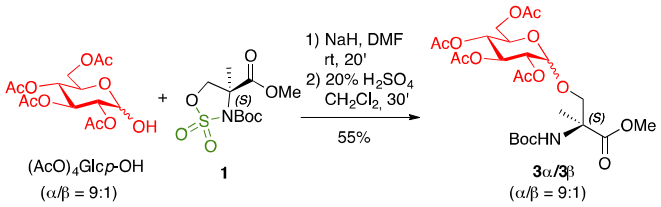
Inspired by the results reported with aziridine-2-carboxamides,[11] and encouraged by the good results obtained with 1-thiocarbohydrates using different sulfamidates derived from amino acids, we planned a new synthetic route to access mimics of the Tn antigen featuring α-methylserine (α-D-Gal*p*NAc-α-MeSer) and isoserine (α-D-Gal*p*NAc-α-*iso*Ser), by combining both synthetic strategies.

First, enantiopure sulfamidate **1** was synthesized from protected (*S*)-α-methylserine, which in turn can be accessed in a gram scale from L-serine following our published protocol.[12] Cyclic sulfamidate was formed by using the well-established two-step protocol consisting on treatment of protected amino acid with thionyl chloride in the presence of imidazole and subsequent oxidation of the intermediate sulfamidites with $RuCl_3/NaIO_4$. As a proof-of-concept, the subsequent nucleophilic ring-opening with $(AcO)_3$Gal*p*NAc-α-OH as a pyranose C1-*O*-nucleophile,[13] using sodium hydride as a base in DMF, led to the desired protected α-D-Gal*p*NAc-α-MeSer **2** in good yield and as a single α anomer without further chromatographic purification. Importantly, competitive elimination or deprotection reactions observed with natural analogues[14] were not observed. (Scheme 1).



Scheme 1. Synthesis and ring-opening reaction of α-MeSer-sulfamidate **1** with $(AcO)_3$Gal*p*NAc-α-OH to obtain the Tn antigen mimic **2**.

The scope of this new methodology was extended to other carbohydrates such as glucose. Hence, we carried out the ring-opening reaction of sulfamidate **1** with a mixture of α- and β-anomers (α/β = 9:1) of tetra-*O*-acetyl-D-glucopyranose hemiacetal, $(AcO)_4Glcp$-OH,[15] under the same conditions described above (Scheme 2). The corresponding protected α-*O*-glucosyl-α-methylserine **3** was efficiently obtained with the same high diastereomeric ratio of the starting carbohydrate (**3**α/**3**β = 9:1).

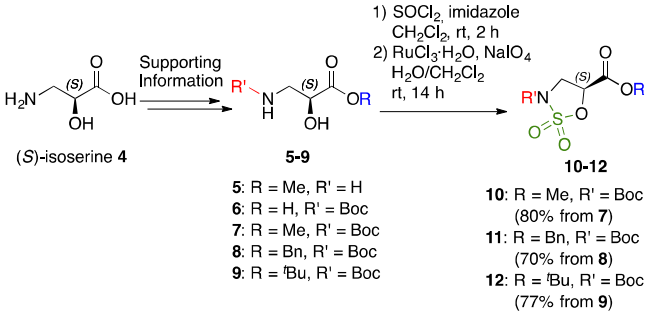


Scheme 2. Ring-opening reaction of α-MeSer sulfamidate **1** with $(AcO)_4Glcp$-OH.

We next extended this synthetic protocol to access new unnatural Tn antigen mimics, namely glycosyl β^2-amino acids. The proteolytic stability of glycosylated β-peptides toward glycosidases has been studied, showing that glyco-β-peptides are stable to degradation by proteolytic enzymes.[16] Additionally, the synthesis of new conjugates composed of β- or α,β-peptides functionalized with biologically active carbohydrate residue constitutes an important platform for dual structure–activity studies.[17] These results emphasize the potential use of β-peptides functionalized with carbohydrates for biological and biomedical investigations. Therefore, development of robust methodologies towards suitably protected, enantiopure α- or β-glycosyl-β-amino acids building blocks is synthetically valuable.

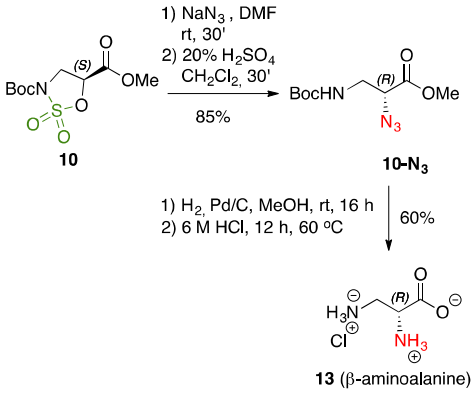
Starting from commercially available (*S*)-isoserine **4**, we first prepared several enantiopure isoserine derivatives **5**-**9**, which were used as starting material to synthesize enantiopure

cyclic sulfamidates **10-12** following standard methodologies (Scheme 3 and Supporting Information). Different protecting schemes were selected for these new sulfamidates in order to test the tolerance of our ring-opening methodology to different functional groups.

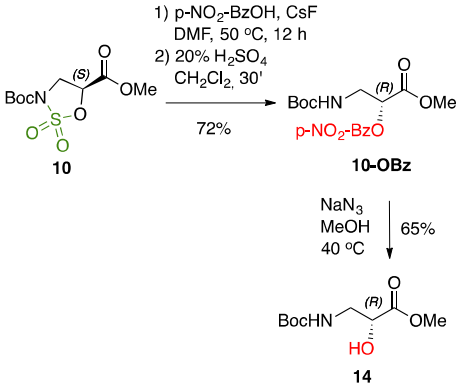


Scheme 3. Synthesis of cyclic sulfamidates derived from (*S*)-isoserine **4**.

Before synthesizing the required glycoconjugates, we examined the reactivity of this new isoserine-derived sulfamidates towards ring-opening reactions with sodium azide and cesium paranitrobenzoate as probe nucleophiles. The stereochemical outcome of the ring-opening reaction was confirmed to proceed with complete inversion of configuration at the Cα of isoserine sulfamidate, by derivatizing the corresponding adducts to known compounds 2,3-diaminopropionic acid **13** (β-aminoalanine, Scheme 4) and *N*-Boc-(*R*)-isoserine methyl ester **14** (Scheme 5) with matching optical properties.

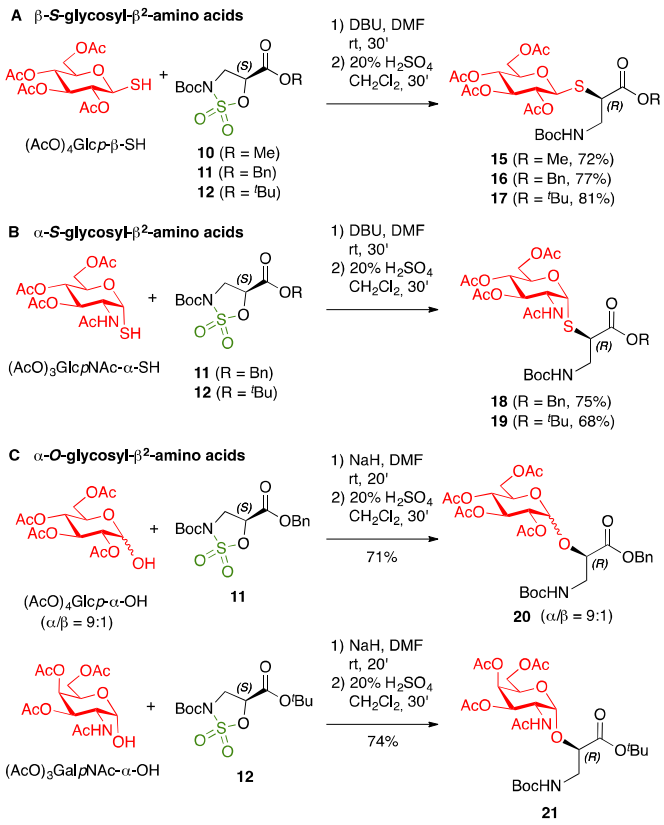


Scheme 4. Ring-opening reaction of isoserine sulfamidate **10** with sodium azide and synthesis of β-aminoalanine.



Scheme 5. Ring-opening reaction of isoserine sulfamidate **10** with a *O*-nucleophile and synthesis of a protected (*R*)-isoserine derivative.

The reactivity of these electrophiles towards glycosylation was first assayed with configurationally stable α- and β-1-thiocarbohydrates. Due to the growing importance of *S*-glycopeptides[18] as mimics of *O*-glycopeptides with singular properties, cysteine *S*-glycosylation, such as that found in glycopeptide bacteriocins,[19] has emerged as a powerful and synthetically accessible post-translational modification. Reaction of sulfamidates **10-12** with tetra-*O*-acetyl-β-1-thio-D-glucopyranose as a nucleophile using DBU as a base in DMF, led to protected β-*S*-glycosyl-β^2-amino acids **15-17** in good yields (panel A, Scheme 6). Analogous treatment of sulfamidates **11** and **12** with tri-*O*-acetyl-α-1-thio-*N*-acetylglucosamine produced α-*S*-glycosyl-β^2-amino acids **18** and **19**, respectively (panel B, Scheme 6). In both cases, the configuration of the glycosidic bond was fully maintained with respect to the anomeric configuration of the starting carbohydrates, as observed in the ^{1}H NMR spectra.

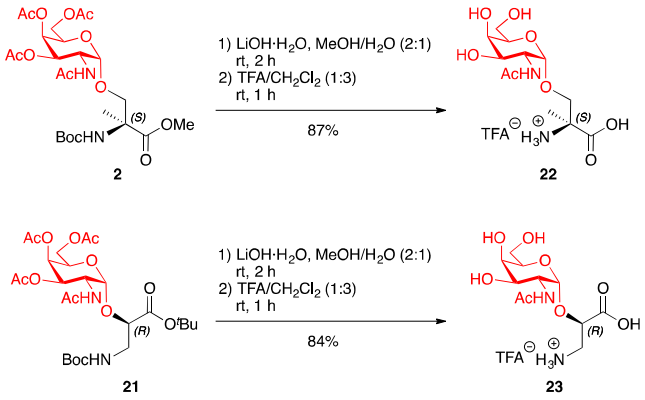


Scheme 6. Ring-opening reactions of isoserine sulfamidates with *S*- and *O*-carbohydrates as nucleophiles.

Finally, we assayed the ring-opening of sulfamidates **11** and **12** with tetra-*O*-acetyl-α-D-glucopyranose, (AcO)$_4$Glc*p*-α-OH,[15] and tri-*O*-acetyl-α-D-*N*-acetylgalactosamine, (AcO)$_3$Gal*p*NAc-α-OH,[13] respectively, as pyranose C1-*O*-nucleophiles using sodium hydride as a base in DMF (panel C, Scheme 6). As a result, α-*O*-glucosyl-β^2-amino acid **20** was obtained with high anomeric selectivity (**20**α/**20**β = 9:1, the same ratio as the starting material). Most importantly, α-*O*-Gal*p*NAc-β^2-amino acid **21** was obtained in good yield and as single α anomer. In both cases, nucleophilic attack occurred with complete inversion of configuration at the reacting center, and competitive elimination or deprotection reactions were not observed in any case.

α-Anomer **21** is particularly attractive, since it is a protected structural analogue of the Tn antigen,[20] in which the α-D-Gal*p*NAc is *O*-linked to isoserine (α-D-Gal*p*NAc-*iso*Ser).

Once the protected Tn antigen mimics **2** and **21** were obtained and considering the novelty of these structures, in order to demonstrate that the carbohydrate and amino acid moieties can be liberated without breaking the glycosidic bond, removal of the protecting groups were carried out in two steps to achieve the corresponding free Tn antigen mimics α-D-Gal*p*NAc-α-MeSer (**22**) and α-D-Gal*p*NAc-α-*iso*Ser (**23**), respectively. First, protected glycosyl amino acids were treated with lithium hydroxide monohydrate ($LiOH \cdot H_2O$) at room temperature, using a mixture of methanol/water (2:1) as a solvent. The basic hydrolysis reaction was followed by TLC until starting material disappeared and by ^1H NMR to test the hydrolysis of ester groups (AcO- of carbohydrate moiety and -$CO_2{}^t$Bu or -CO_2Me of amino acid moiety). The basic solution was neutralized with ion exchanger Dowex® 50W-X8, filtered, and evaporated to give the glycosyl amino acid whose amino group is already protected as Boc carbamate. Transformation of Boc to amino group was carried out by treatment with trifluoracetic acid (TFA) in dichloromethane at room temperature. Hence, the corresponding unprotected glycosyl amino acids **22** (87%) and **23** (84%) were obtained without further purification (Scheme 7).



Scheme 7. Deprotection of glycosyl amino acids **2** and **21** to obtain the free Tn antigen mimics α-D-Gal*p*NAc-α-MeSer (**22**) and α-D-Gal*p*NAc-α-*iso*Ser (**23**), respectively.

CONCLUSION

In summary, a new family of differently protected, enantiopure cyclic sulfamidates have been prepared from effectively accessible (*S*)-α-methylserine and commercially available (*S*)-isoserine. Such sulfamidates have been used as efficient electrophiles for glycosylation with C1-*S*- and C1-*O*-carbohydrates via highly chemo-, regio- and stereoselective ring-opening reaction in very mild conditions and with complete functional group tolerance. Of note, the special architecture of this unnatural amino acid derivatives precludes the undesired elimination reactions commonly observed on their natural analogues derived from L-Ser and L-Thr with basic nucleophiles. This methodology allows *S*- and *O*-glycosylation in an efficient and highly stereocontrolled manner, even with challenging carbohydrates such as α-Gal*p*NAc. As a result, analogues of the Tn antigen derived from α-methylated and β^2-amino acids are now accessible.

EXPERIMENTAL SECTION

General and Experimental Methods. Commercial reagents were used without further purification. Analytical thin layer chromatography (TLC) was performed on Macherey-Nagel precoated aluminium sheets with a 0.20 mm thickness of silica gel 60 with fluorescent indicator UV_{254}. TLC plates were visualized with UV light and by staining with phosphomolybdic acid (PMA) solution (5 g of PMA in 100 mL of absolute ethanol) or sulfuric acid-ethanol solution. Column chromatography was performed on silica gel (230−400 mesh). ^1H 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 experiments. 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. 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.

General Procedure for the Synthesis of Chiral Cyclic Sulfamidates. To a solution of imidazole (1.06 g, 15.7 mmol) in dichloromethane (15 mL), another solution of thionyl chloride (0.33 mL, 4.7 mmol) in dichloromethane (5 mL) was slowly added, at 0-5 °C for 15 min. The reaction mixture was then stirred at room temperature for 1 h and then cooled to -10 ^{o}C. To the resulting suspension, a solution of the corresponding *N*-Boc-protected hydroxyamino ester (2.6 mmol), in dichloromethane (8 mL) was added over 30 min at -10^{o} C and the mixture was then stirred at room temperature for 2 h. To the resulting suspension, water (30 mL) was added and the mixture was stirred at room temperature for 10 min. The organic phase was washed with 10% aqueous citric acid (25 mL) and brine (25 mL) and dried over sodium sulphate. The solids were removed by filtration and washed with dichloromethane. The combined filtrates were mixed with a 10% aqueous sodium periodate solution (25 mL) and cooled to 0 ^{o}C. To the well stirred mixture, ruthenium (III) chloride hydrate (6 mg, 0.26 mmol) was added and the reaction was vigorously stirred at 0 ^{o}C for 2 h and additional 2 h at room temperature. The organic phase was washed with 10% aqueous sodium ascorbate solution (8 mL) and filtered over silica gel. After evaporation of the volatiles, the product was chromatographed to give the corresponding sulfamidate.

*3-(tert-Butyl) 4-methyl (S)-4-methyl-1,2,3-oxathiazolidine-3,4-dicarboxylate 2,2-dioxide (**1**).* Following the general procedure described above for the synthesis of chiral cyclic sulfamidates and starting from (*S*)-*N*-Boc-α-MeSer-OMe (606 mg, 2.6 mmol), sulfamidate **1** was obtained as a white solid in a 90% yield (690 mg), after purification by a column chromatography with hexane/ethyl acetate (3:2). Physical data: mp 113-115 °C. $[\alpha]_D^{20}$ (*c* 1.00, $CHCl_3$): -10.7. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{10}H_{17}NO_7SNa^+$ 318.0618; Found 318.0627. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.56 (s, 9H, $C(CH_3)_3$), 1.81 (s, 3H, $C\alpha CH_3$), 3.85 (s, 3H, CO_2CH_3), 4.32 (d, 1H, J = 9.3 Hz $CH_2\beta$), 4.64 (d, 1H, J = 9.3 Hz, $CH_2\beta$).^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.6 ($C\alpha CH_3$), 27.9 ($C(CH_3)_3$), 53.7 (CO_2CH_3), 65.5 (CHα), 72.7 ($CH_2\beta$), 86.1 ($C(CH_3)_3$), 147.9, 169.2 (CO).

*(S)-3-tert-Butyl-5-methyl-1,2,3-oxathiazolidine-3,5-dicarboxylate 2,2-dioxide (**10**).* Following the general procedure described above for the synthesis of sulfamidates, and starting from *N*-Boc-(*S*)-isoserine methyl ester (**7**) (1.705 g, 7.8 mmol), sulfamidate **10** was obtained as a white solid in an 80% yield (1.80 g), after purification by a column chromatography with hexane/ethyl acetate (2:1). Physical data: mp 75-77 °C. $[\alpha]_D^{20}$ (*c* 1.00, $CHCl_3$): +0.9. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_9H_{15}NO_7SNa^+$ 304.0467; Found 304.0481. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.55 (s, 9H, $C(CH_3)_3$), 3.90 (s, 3H, CO_2CH_3), 4.17 (dd, 1H, J = 10.1, 7.4 Hz $CH_2\beta$), 4.26 (dd, 1H, J = 10.1, 7.4 Hz, $CH_2\beta$), 5.17 (t, 1H, J = 7.2 Hz, CHα). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.0 ($C(CH_3)_3$), 47.1 ($CH_2\beta$), 53.8 (CO_2CH_3), 72.5 (CHα), 86.3($C(CH_3)_3$), 148.3, 165.6 (CO).

*(S)-5-Benzyl-3-tert-Butyl 1,2,3-oxathiazolidine-3,5-dicarboxylate 2,2-dioxide (**11**).* Following the general procedure described above for the synthesis of sulfamidates, and starting from hydroxyamino ester **8** (767 mg, 2.6 mmol), sulfamidate **11** (650 mg, 70%) was obtained as a white solid, after column chromatography in hexane/ethyl acetate (1:1). Physical data: mp 65-67 °C. $[\alpha]_D^{20}$ (*c* 1.00, $CHCl_3$): +3.1. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{15}H_{19}NO_7SNa^+$ 380.0780; Found 380.0801. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.54 (s, 9H, $C(CH_3)_3$), 4.14 (dd, 1H, *J* = 10.2, 7.4 Hz, $CH_2\beta$), 4.24 (dd, 1H, *J* = 10.2, 7.3 Hz, $CH_2\beta$), 5.16 (t, 1H, *J* = 7.2 Hz, CHα), 5.30 (s, 2H, CH_2Ph), 7.38 (s, 5H, arom). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.0 ($C(CH_3)_3$), 47.1 ($CH_2\beta$), 68.9 (CH_2Ph), 72.5 (CHα), 86.3 ($C(CH_3)_3$), 128.8, 129.0, 129.2, 134.1 (arom), 148.3, 164.9 (CO).

*(S)-di-tert-Butyl 1,2,3-oxathiazolidine-3,5-dicarboxylate 2,2-dioxide (**12**).* Following the general procedure described above for the synthesis of sulfamidates, and starting from hydroxyamino ester **9** (679 mg, 2.6 mmol), sulfamidate **12** (647 mg, 77%) was obtained as a white solid, after column chromatography in hexane/ethyl acetate (4:1). Physical data: mp 93-95 °C. $[\alpha]_D^{20}$ (*c* 1.00, $CHCl_3$): +2.1. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{12}H_{21}NO_7SNa^+$ 346.0936; Found 346.0899. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.53 (s, 9H, $C(CH_3)_3$), 1.55 (s, 9H, $C(CH_3)_3$), 4.10 (dd, 1H, *J* = 10.2, 7.6 Hz $CH_2\beta$), 4.21 (dd, 1H, *J* = 10.2, 7.2 Hz, $CH_2\beta$), 5.04 (t, 1H, *J* = 7.3 Hz, CHα). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.0 ($2C(CH_3)_3$), 47.4 ($CH_2\beta$), 72.9 (CHα), 85.4 ($C(CH_3)_3$), 86.0 ($C(CH_3)_3$), 148.4, 163.8 (CO).

General Procedure for the Ring-opening Reactions of Sulfamidates with Carbohydrate C1-*O*-Nucleophiles. Sodium hydride 60% dispersion in mineral oil (12 mg, 0.30 mmol) was added to a solution of the corresponding protected carbohydrate C1-*O*-nucleophile (0.30 mmol) in dry DMF (2 mL) in a nitrogen atmosphere using standard schlenk techniques. The resulting mixture was stirred at room temperature for 5 min and it was then added by cannula to a solution of the corresponding sulfamidate (0.30 mmol) in dry DMF (1 mL). The reaction mixture was kept at room temperature and stirred for 20 min. After that time, the volatiles were removed, and the residue dissolved in a mixture of 20% aqueous H_2SO_4/dichloromethane (1:1), which was stirred for 30 min at room temperature to hydrolyze the sulfamic acid intermediate. The reaction crude was isolated after extraction with dichloromethane (2 x 5 mL), dried over anhydrous Na_2SO_4, and concentrated. That crude was purified by silica gel column chromatography and the corresponding protected glycosyl amino acid was obtained.

*(2S)-O-((3',4',6')-Tri-O-acetyl-2'-acetamido-2'deoxy-α-D-galactopyranosyl)-N-(tert-butoxycarbonyl)-2-methylserine methyl ester (**2**).* Following the general procedure described above for the ring-opening reactions of sulfamidates with carbohydrate C1-*O*-nucleophiles, and starting from 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranose (105 mg, 0.30 mmol) and sulfamidate **1** (88 mg, 0.30 mmol), protected glycosyl amino acid **2** (119 mg, 71%) was obtained as an oil, after column chromatography in hexane/ethyl acetate (1:1). Physical data: $[\alpha]_D^{20}$ (*c* 1.00, $CHCl_3$): +61.3. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{24}H_{38}N_2O_{13}Na^+$ 585.2272; Found 585.2270. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.47 (s, 9H, $C(CH_3)_3$), 1.49 (s, 3H, $C\alpha CH_3$), 1.99 (s, 3H,

NHCOCH_3), 2.00 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.17 (s, 3H, CH_3CO), 3.77 (s, 3H, CO_2CH_3), 3.79-3.84 (d, 1H , J = 10.4 Hz, $CH_2\beta$), 4.01-4.19 (m, 4H, 1$CH_2\beta$, 2H_{6s}, H_{5s}), 4.57 (ddd, 1H, J = 11.3, 9.6, 3.7 Hz, H_{2s}), 4.93 (d, J = 3.7 Hz, H_{1s}), 5.08 (dd, J = 11.3, 3.3 Hz, H_{3s}), 5.32 (s, 1H, N*H*Boc), 5.38 (d, J = 3.2 Hz, H_{4s}), 6.15 (d, 1H, J = 9.6 Hz, N*H*Ac). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.7, 20.8, 20.9 (3*C*H_3CO), 23.3 (NHCO*C*H_3), 28.1 ($NCO_2C(CH_3)_3$), 28.5 (Cα*C*H_3), 42.9 ($CH_2\beta$), 51.6 (C_{2s}), 62.0 (C_{6s}), 68.3 (C_{4s}), 68.8 (C_{5s}), 71.5 (C_{3s}), 76.4 (CHα), 80.2 ($C(CH_3)_3$), 83.5 ($CO_2C(CH_3)_3$), 98.5 (C_{1s}), 155.7, 169.4, 170.8, 171.2 (CO).

*(2S)-O-((2',3',4',6')-Tetra-O-acetyl-D-glucopyranosyl)-N-(-tert-butoxycarbonyl)-2-methylserine methyl ester (**3**).* Following the general procedure described above for the ring-opening reactions of sulfamidates with carbohydrate C1-*O*-nucleophiles, and starting from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (100 mg, 0.29 mmol) and sulfamidate **1** (50 mg, 0.17 mmol), protected glycosyl amino acid **3** (53 mg, 55%) was obtained as an oil in a 9:1 ratio in favor of the α-anomeric isomer, after column chromatography in hexane/ethyl acetate (1:1). Physical data: $[\alpha]_D^{20}$ (*c* 1.00, $CHCl_3$): +40.5. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{24}H_{37}NO_{14}Na^+$ 586.2106; Found 586.2113. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.44 (s, 9H $NCO_2C(CH_3)_3$), 1.52 (s, 3H, CαCH_3), 2.01 (s, 3H, COCH_3), 2.02 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.10 (s, 3H, $COCH_3$), 3.70-3.75 (m, 1H, $CH_2\beta$), 3.75 (s, 3H, CO_2CH_3), 3.95-4.02 (m, 1H, H_{5s}), 4.06-4.13 (m, 2H, 1 $CH_2\beta$, 1 H_{6s}), 4.23-4.30 (m, 1H, 1 H_{6s}), 4.83 (dd, 1H, J = 10.2, 3.7 Hz, H_{2s}), 5.01-5.07 (m, 2H, H_{1s}, H_{4s}), 5.37-5.45 (m, 2H, H_{3s}, N*H*Boc). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 20.7 (4*C*H_3CO), 20.9 (Cα*C*H_3), 28.4 ($NCO_2C(CH_3)_3$), 52.9 (CO_2CH_3), 59.8 (Cα), 61.8 (C_{6s}), 67.6 (C_{5s}), 68.4 (C_{4s}), 70.3 (C_{3s}),

70.3 (C_{2s}), 70.9 (C_{β}), 96.4 (C_{1s}), 154.5 ($NCO_2C(CH_3)_3$), 169.7, 170.2, 170.3, 170.3, 170.8, 173.0 (CO).

*(2R)-O-((2',3',4',6')-Tetra-O-acetyl-D-glucopyranosyl)-N-(tert-butoxycarbonyl)isoserine benzyl ester (**20**).* Following the general procedure described above for the ring-opening reactions of sulfamidates with carbohydrate C1-*O*-nucleophiles, and starting from 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (70 mg, 0.20 mmol) and sulfamidate **11** (72 mg, 0.20 mmol), protected glycosyl amino acid **20** (89 mg, 71%) was isolated as a colorless oil in a 9:1 ratio in favor of α-anomeric isomer, after column chromatography in hexane/ethyl acetate (5.5:4.5). Physical data: $[\alpha]_D^{20}$ (*c* 1.00, $CHCl_3$): +54.3. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{29}H_{39}NO_{14}Na^+$ 648.2268; Found 648.2271. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44 (s, 9H, $C(CH_3)_3$), 2.00-2.06 (m, 9H, Ac), 2.09 (s, 3H, Ac), 3.54-3.62 (m, 2H, $CH_2\beta$), 4.03-4.15 (m, 2H, H_{6s}, H_{5s}), 4.27 (dd, 1H, *J* = 12.3, 4.0 Hz, H_{6s}), 4.38 (t, 1H, *J* =5.0 Hz, CHα), 4.87 (dd, 1H, *J*= 12.4, 4.3 Hz, H_{3s}), 4.91-4.99 (m, 1H, N*H*Boc), 5.06 (t, 1H, *J* = 9.9 Hz, H_{4s}), 5.12-5.18 (m, 2H, CH_2Ph), 5.25 (d, 1H, *J* = 3.3 Hz, H_{1s}), 5.51 (t, 1H, *J* = 9.8 Hz, H_{3s}), 7.29-7.41 (m, 5H, arom). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.8 (CH_3CO), 28.5 ($C(CH_3)_3$), 42.7 ($CH_2\beta$), 61.9 (C_{6s}), 67.5 (CH_2Ph), 68.2 (C_{5s}), 68.7 (C_{4s}), 70.0 (C_{3s}), 70.5 (C_{2s}), 74.2 (CHα), 80.1 ($C(CH_3)_3$), 95.0 (C_{1s}), 128.2, 128.8, 128.9, 135.3 (arom), 155.4, 169.3, 169.7, 170.6, 170.9, 171.1, 171.6 (CO).

*(2R)-O-((3',4',6')-Tri-O-acetyl-2'-acetamido-2'-deoxy-α-D-galactopyranosyl)-N-(tert-butoxycarbonyl)isoserine tert-butyl ester (**21**).* Following the general procedure described above for the ring-opening reactions of sulfamidates with carbohydrate C1-*O*-nucleophiles,

and starting from 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-galactopyranose (70 mg, 0.20 mmol) and sulfamidate **12** (69 mg, 0.20 mmol), protected glycosyl amino acid **21** (42 mg, 68%) was obtained as an oil, after column chromatography in hexane/ethyl acetate (1:1). Physical data: $[\alpha]_D^{20}$ (*c* 1.00, $CHCl_3$): +41.5. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{26}H_{42}N_2O_{13}Na^+$ 613.2585; Found 613.2557. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.41-1.50 (m, 18H $NCO_2C(CH_3)_3$, $C(CH_3)_3$), 2.01 (s, 3H, NHCOC*H*$_3$), 2.01-2.05 (m, 6H, 2Ac), 2.10 (s, 3H, Ac), 3.54 (t, 2H, *J* = 4.8, $CH_2\beta$), 4.03-4.16 (m, 2H, H_{5s}, H_{6s}), 4.21-4.28 (m, 2H, CHα, H_{6s}), 4.37 (ddd, 1H, *J* = 11.0, 8.4, 5.4 Hz, H_{2s}), 4.82 (d, 1H, *J* = 3.6 Hz, H_{1s}), 4.91 (br s, 1H, N*H*Boc), 5.15 (t, 1H, *J* = 9.8 Hz, H_{4s}), 5.26 (dd, 1H, *J* = 20.1, 10.4, H_{3s}) 6.60 (d, 1H, *J* = 8.9 Hz, N*H*Ac). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.7, 20.8, 20.9 (*C*H_3CO), 23.3 (NHCOC*H*$_3$), 28.1 ($NCO_2C(CH_3)_3$), 28.5 ($C(CH_3)_3$), 42.9 ($CH_2\beta$), 51.6 (C_{2s}), 62.0 (C_{6s}), 68.3 (C_{4s}), 68.8 (C_{5s}), 71.5 (C_{3s}), 76.4 (CHα), 80.2 ($C(CH_3)_3$), 83.5 ($CO_2C(CH_3)_3$), 98.5 (C_{1s}), 155.7, 169.4, 170.8, 171.2 (CO).

General Procedure for the Ring-opening Reactions of Sulfamidates with Carbohydrate C1-*S*-Nucleophiles. DBU (19 μL, 0.13 mmol) was added to a solution of the corresponding thiocarbohydrate (0.12 mmol) in dry DMF (1 mL) in a nitrogen atmosphere using standard schlenk techniques. The resulting mixture was stirred at room temperature for 5 min and it was then added by cannula to a solution of the corresponding sulfamidate (0.12 mmol) in dry DMF (1 mL). The reaction mixture was kept at room temperature and stirred for 30 min. After that time, the volatiles were removed, and the residue dissolved in a mixture of 20% aqueous H_2SO_4/dichloromethane (1:1), which was stirred for 30 min at room temperature to hydrolyze the sulfamic acid intermediate. The

reaction crude was isolated after extraction with dichloromethane (2 x 5 mL), dried over anhydrous Na_2SO_4, and concentrated. That crude was purified by silica gel column chromatography to give the corresponding protected *S*-glycosyl amino acid.

*(2R)-2-((2',3',4',6')-Tetra-O-acetyl-β-D-glucopyranosylthio)-3-tert-butoxycarbonylamino propionic acid methyl ester (**15**)*. Following the general procedure described above for the ring-opening reactions of sulfamidates with carbohydrate C1-*S*-nucleophiles, and starting from 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (44 mg, 0.12 mmol) and sulfamidate **10** (34 mg, 0.12 mmol), protected *S*-glycosyl amino acid **15** (49 mg, 72%) was obtained as a colorless oil, after column chromatography in hexane/ethyl acetate (6.5:3.5). Physical data: $[\alpha]_D^{20}$ (*c* 1.00, $CHCl_3$): +196.3. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{23}H_{35}NO_{13}SNa^+$ 588.1727; Found 588.1707. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44 (s, 9H, $C(CH_3)_3$), 2.00 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.09 (s, 3H, Ac), 3.30-3.45 (m, 1H, $CH_2\beta$), 3.58-3.69 (m, 1H, $CH_2\beta$), 3.71-3.80 (m, 5H, CHα, H_{5s}, CO_2CH_3), 4.11-4.22 (m, 2H, H_{6s}), 4.82 (d, 1H, J = 10.2, H_{1s}), 4.97 (t, 1H, J = 9.7, H_{2s}), 5.04 (t, 1H, J = 9.7, H_{4s}), 5.14 (t, 1H, J = 5.6 Hz, N*H*Boc), 5.23 (t, 1H, J = 9.3, H_{3s}). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.7 ($4CH_3CO$), 28.5 ($C(CH_3)_3$), 41.7 ($CH_2\beta$), 45.8 (CHα), 52.8 (CO_2CH_3), 62.3 (C_{6s}), 68.4 (C_{4s}), 69.9 (C_{2s}), 73.8 (C_{3s}), 76.0 (C_{5s}), 80.0 ($C(CH_3)_3$), 82.2 (C_{1s}), 155,9, 169.4, 169.6, 170.2, 170.7, 171.2 (CO).

*(2R)-2-((2',3',4',6')-Tetra-O-acetyl-β-D-glucopyranosylthio)-3-tert-butoxycarbonylamino propionic acid benzyl ester (**16**)*. Following the general procedure described above for the ring-opening reactions of sulfamidates with carbohydrate C1-*S*-nucleophiles, and starting

from 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (66 mg, 0.18 mmol) and sulfamidate **11** (64 mg, 0.18 mmol), protected *S*-glycosyl amino acid **16** (89 mg, 77%) was obtained as an oil, after column chromatography in hexane/ethyl acetate (6:4). Physical data: $[\alpha]_D^{20}$ (*c* 1.00, $CHCl_3$): +8.3. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{29}H_{39}NO_{13}SNa^+$ 664.2040; Found 664.2151. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.43 (s, 9H, $C(CH_3)_3$), 1.95 (s, 3H, Ac), 2.00 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.07 (s, 3H, Ac), 3.39 (ddd, 1H, *J* = 13.9, 7.6, 5.9 Hz, $CH_2\beta$), 3.58-3.72 (m, 2H, $CH_2\beta$, H_{5s}), 3.77 (t, 1H, *J* = 7.2, CHα), 4.06-4.21 (m, 2H, H_{6s}), 4.74 (d, 1H, *J* = 10.3, H_{1s}), 4.88-5.06 (m, 2H, H_{2s}, H_{4s}), 5.07-5.26 (m, 4H, N*H*Boc, H_{3s}, CH_2Ph), 7.33-7.41 (m, 5H, arom). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.7 (*C*H_3CO), 28.5 ($C(CH_3)_3$), 41.8 ($CH_2\beta$), 45.9 (CHα), 62.3 (C_{6s}), 67.5 (*C*H_2Ph) 68.4 (C_{4s}), 69.8 (C_{2s}), 73.8 (C_{3s}), 75.9 (C_{5s}), 80.0 ($C(CH_3)_3$), 82.2 (C_{1s}), 128.4, 128.8, 128.9, 135.4 (arom), 155,9, 169.4, 169.5, 170.2, 170.4, 170.7 (CO).

*(2R)-2-((2',3',4',6')-Tetra-O-acetyl-β-D-glucopyranosylthio)-3-tert-butoxycarbonylamino propionic acid tert-butyl ester (**17**).* Following the general procedure described above for the ring-opening reactions of sulfamidates with carbohydrate C1-*S*-nucleophiles, and starting from 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranose (36 mg, 0.10 mmol) and sulfamidate **12** (32 mg, 0.10 mmol), protected *S*-glycosyl amino acid **17** (49 mg, 81%) was obtained as a colorless oil, after column chromatography in hexane/ethyl acetate (6:4). Physical data: $[\alpha]_D^{20}$ (*c* 1.00, $CHCl_3$): +30.7. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{26}H_{41}NO_{13}SNa^+$ 630.2196; Found 630.2203. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44 (s, 9H, $NCO_2C(CH_3)_3$), 1.47 (s, 9H, $C(CH_3)_3$), 2.00 (s, 3H, Ac), 2.03 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.08 (s, 3H, Ac), 3.13-3.39 (m, 1H, $CH_2\beta$), 3.56 (t, 1H, *J* = 11.2, CHα), 3.63-3.83

(m, 2H, $CH_2\beta$, H_{5s}), 4.07-4.23 (m, 2H, H_{6s}), 4.86-5.10 (m, 3H, H_{1s}, H_{2s}, H_{4s}), 5.13-5.28 (m, 2H, N*H*Boc, H_{3s}). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.7 (*C*H_3CO), 28.0 (C(*C*H_3)$_3$), 28.5 (NCO_2C(*C*H_3)$_3$), 41.3 ($CH_2\beta$), 46.7 (CHα), 62.4 (C_{6s}), 68.5 (C_{4s}), 69.7 (C_{2s}), 73.9 (C_{3s}), 75.8 (C_{5s}), 79.8 (NCO_2C(*C*H_3)$_3$), 81.8 (C_{1s}), 82.4 (*C*(*C*H_3)$_3$), 155,9, 169.6, 169.7, 170.2, 170.2, 170.7 (CO).

*(2R)-2-((3',4',6')-Tri-O-acetyl-2'-acetamido-2'-deoxy-α-D-glucopyranosylthio)-3-tert-butoxycarbonylamino propionic acid benzyl ester (**18**)*. Following the general procedure described above for the ring-opening reactions of sulfamidates with carbohydrate C1-*S*-nucleophiles, and starting from 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-α-D-glucopyranose (36 mg, 0.10 mmol) and sulfamidate **11** (32 mg, 0.10 mmol), protected *S*-glycosyl amino acid **18** (48 mg, 75%) was obtained as a colorless oil, after column chromatography in hexane/ethyl acetate (1:1). Physical data: $[\alpha]_D^{20}$ (*c* 0.50, $CHCl_3$): +42.8. HRMS (ESI-TOF) m/z: $[M + Na]^+$; Calcd for $C_{29}H_{40}N_2O_{12}SNa^+$ 663.2200; Found 663.2194. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.42 (s, 9H, C(CH_3)$_3$), 1.90 (s, 3H, NHCOC*H*$_3$), 2.03 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.08 (s, 3H, Ac), 3.43-3.60 (m, 2H, $CH_2\beta$), 3.72 (t, 1H, *J* = 8.4 Hz, CHα), 4.03-4.12 (m, 1H, H_{6s}), 4.24-4.37 (m, 2H, H_{6s}, H_{5s}), 4.51 (ddd, 1H, *J* = 11.0, 8.5, 5.4 Hz, H_{2s}), 4.94-5.08 (m, 2H, N*H*Boc, H_{3s}), 5.12 (d, 1H, *J* = 9.6 Hz, H_{4s}), 5.16-5.20 (m, 2H, C*H*$_2$Ph), 5.66-5.75 (m, 2H, H_{1s}, N*H*Ac), 7.28-7.44 (m, 5H, arom). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.8 (*C*H_3CO), 23.3 (NHCO*C*H_3), 28.5 (C(*C*H_3)$_3$), 41.9 ($CH_2\beta$), 45.4 (CHα), 52.5 (C_{2s}), 62.0 (C_{6s}), 67.8 (*C*H_2Ph), 68.0 (C_{4s}), 69.2 (C_{5s}), 71.3 (C_{3s}), 80.1 (*C*(*C*H_3)$_3$), 83.9 (C_{1s}), 128.4, 128.7, 128.9, 135.2 (arom), 155.7, 169.4, 170.3, 170.9, 171.0, 171.8 (CO).

*(2R)-2-((3',4',6')-Tri-O-acetyl-2'-acetamido-2'-deoxy-α-D-glucopyranosylthio)-3-tert-butoxycarbonylamino propionic acid tert-butyl ester (**19**).* Following the general procedure described above for the ring-opening reactions of sulfamidates with carbohydrate C1-*S*-nucleophiles, and starting from 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-1-thio-α-D-glucopyranose (36 mg, 0.10 mmol) and sulfamidate **12** (32 mg, 0.10 mmol), protected *S*-glycosyl amino acid **19** (42 mg, 68%) was obtained as a colorless oil, after column chromatography in hexane/ethyl acetate (1:1). Physical data: $[\alpha]_D^{20}$ (*c* 0.50, $CHCl_3$): +29.3. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{26}H_{42}N_2O_{12}SNa^+$ 629.2356; Found 629.2303. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.44 (s, 9H $NCO_2C(CH_3)_3$), 1.47 (s, 9H, $C(CH_3)_3$), 1.95 (s, 3H, NHCOCH_3), 2.04 (s, 3H, Ac), 2.06 (s, 3H, Ac), 2.11 (s, 3H, Ac), 3.42-3.58 (m, 3H, $CH_2\beta$, CHα), 4.04-4.17 (m, 1H, H_{6s}), 4.26-4.42 (m, 2H, H_{6s}, H_{5s}), 4.52 (ddd, 1H, J = 11.0, 8.4, 5.4 Hz, H_{2s}), 4.99-5.10 (m, 2H, N*H*Boc, H_{3s}), 5.16 (t, 1H, J = 12.6 Hz, H_{4s}), 5.70-5.82 (m, 2H, H_{1s}, N*H*Ac). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 20.8 (CH_3CO), 23.3 ($NHCOCH_3$), 28.0 ($C(CH_3)_3$), 28.5 ($NCO_2C(CH_3)_3$), 41.7 ($CH_2\beta$), 45.9 (CHα), 52.7 (C_{2s}), 62.0 (C_{6s}), 68.0 (C_{4s}), 69.1 (C_{5s}), 71.2 (C_{3s}), 82.9 ($C(CH_3)_3$), 84.1 (C_{1s}), 155,8, 169.4, 170.0, 170.5, 170.9, 171.9 (CO).

*Methyl (S)-3-amino-2-hydroxypropanoate hydrochloride (**5**).* Acetyl chloride (2 mL) was added dropwise at 0 °C in about 20 min to absolute methanol (12 mL) through a dropping funnel. After complete addition, the ice bath was removed and (*S*)-isoserine **4** (1.0 g, 9.52 mmol) was added in one portion and the solution was heated to reflux for 2 h. After that, the reaction mixture was allowed to cool to room temperature and the volatiles were

removed under reduced pressure to give the methyl ester hydrochloride **5** (1.11 g, 98%) as a white solid, which was used without further purification in the next step. The spectroscopic data are consistent with those described in the literature.[21]

*(S)-3-((tert-Butoxycarbonyl)amino)-2-hydroxypropanoic acid (**6**).* (*S*)-Isoserine **4** (1.04 g, 9.8 mmol) was dissolved in 1 M aqueous NaOH (20 mL) and dioxane (10 mL) at 0° C and treated with di-*tert*-butyl dicarbonate (2.57 g, 11.8 mmol) and the mixture was allowed to warm to room temperature and stirred for 24 h. The dioxane was then evaporated and the aqueous layer was washed with diethyl ether (30 mL) to remove di-*tert*-butyl dicarbonate. Ethyl acetate (50 mL) was then added to the aqueous layer and the mixture was stirred while a 20% aqueous H_2SO_4 solution was added to give pH 2-3. The organic layer was separated. The aqueous layer was saturated with NaCl and extracted with ethyl acetate (4 x 50 mL). The combined organic layers were dried and filtered and the volatiles were removed to give compound **6** (1.83 g, 91%), as a white solid. Physical data: mp 85-88 °C. $[\alpha]_D^{20}$ (*c* 1.00, MeOH): +6.7. HRMS (ESI-TOF) m/z: $[M + H]^+$; Calcd for $C_8H_{15}NO_5H^+$ 206.1028; Found 206.1019. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.38 (s, 9H, $C(CH_3)_3$), 3.24 (dd, 1H, J = 13.9, 6.6 Hz, $CH_2\beta$), 3.39 (dd, 1H, J = 13.8, 4.0 Hz, $CH_2\beta$), 4.07-4.18 (m, 1H, CHα). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.7 ($C(CH_3)_3$), 45.1 ($CH_2\beta$), 71.2 (CHα), 80.3($C(CH_3)_3$), 158.5, 175.9 (CO).

*Methyl (S)-3-((tert-butoxycarbonyl)amino)-2-hydroxypropanoate (**7**).* Compound **5** (1.11 g, 9.32 mmol) was suspended in THF (24 mL) and triethylamine (2.84 mL, 20.5 mmol) was added and the mixture cooled down to 0 °C. Di-tert-butyl dicarbonate (2.06 g, 9.44 mmol),

dissolved in THF (8 mL), was added slowly under nitrogen atmosphere over 1 h using a dropping funnel. The resulting mixture was left stirring for 14 h at room temperature and afterward was stirred at 50 °C for additional 3 h. The volatiles were then removed and the crude residue was partitioned between ethyl ether (400 mL) and saturated $NaHCO_3$ solution (20 mL). The aqueous phase was extracted with ethyl ether three times. The combined organic layers were dried over Na_2SO_4, filtered and concentrated in vacuo to give **7** (1.705 g, 82%) as a colorless oil, which was used as such for the next step. $[\alpha]_D^{20}$ (*c* 1.00, MeOH): +38.2. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_9H_{18}NO_5^+$ 220.1185; Found 220.1177. The spectroscopic data are consistent with those described in the literature.[22]

*Benzyl (S)-3-((tert-butoxycarbonyl)amino)-2-hydroxypropanoate (**8**).* To a stirring solution of *N*-Boc-(*S*)-isoserine (**6**) (517 mg, 2.52 mmol) in DMF (100 mL) was added Cs_2CO_3 (822 mg, 2.52 mmol) and the stirring was continued 30 min. Benzyl bromide (300 μL, 2.52 mmol) was then added and the resulting solution was stirred for 18 h. The reaction mixture was then diluted with ethyl acetate (25 mL), washed with lithium bromide (3 x 15 mL), $NaHCO_3$ (2 x 15 mL), and brine (2 x 15 mL). The organic layer was dried over sodium sulfate. The volatiles were then removed under reduced pressure and the resulting tan oil was purified by flash chromatography, using a mixture of hexane/ethyl acetate (7:3), to afford the product **8** (45%) as a colorless oil. Physical data: $[\alpha]_D^{20}$ (*c* 1.00, $CHCl_3$): -3.7. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{15}H_{21}NO_5Na^+$ 318.1317; Found 318.1327. ^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.43 (s, 9H, $C(CH_3)_3$), 3.34-3.56 (m, 2H, $CH_2\beta$), 4.30 (dd, 1H, J = 9.3, 4.9 Hz, CHα), 4.93 (s, 1H, N*H*Boc), 5.15-5.26 (m, 2H, CH_2Ph), 7.29-7.42 (m, 5H, arom). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.3 ($C(CH_3)_3$), 44.0 ($CH_2\beta$), 67.7

(*C*H_2Ph), 70.4 (CHα), 79.9(*C*(CH_3)$_3$), 128.5, 128.6, 128.7, 135.0 (arom), 156.2, 173.0 (CO).

*tert-Butyl (S)-3-((tert-butoxycarbonyl)amino)-2-hydroxypropanoate (**9**).* *tert*-Butanol (1.60 g, 21.6 mmol), DCC (3.50 g, 10.5 mmol) and CuCl (38 mg, 0.38 mmol) were stirred under exclusion of light for 3 days. The mixture was diluted with dry dichloromethane (10 mL) and a solution of the compound **6** (1.07 g, 5.2 mmol) in dry dichloromethane (15 mL) was added at room temperature. After stirring for 3 h, *N*,*N'*-dicyclohexylurea was filtered off and, then, the solvent removed in vacuo. The remaining powder was subjected to flash chromatography (hexane/ethyl acetate, 7:3) to give protected amino acid **9** (1.11 g, 4.26 mmol, 82%) as a white solid. Physical data: mp 83-86 °C. $[\alpha]_D^{20}$ (*c* 1.00, $CHCl_3$): +11.8. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{12}H_{23}NO_5H^+$ 262.1654; Found 262.1642. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.37 (s, 9H, C(CH_3)$_3$), 1.42 (s, 9H, $NCO_2C(CH_3)_3$), 3.42 (d, 2H, *J* = 4.5 Hz, CH_2β), 4.09 (t, 1H, *J* = 4.3 Hz, CHα). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.3 (C(*C*H_3)$_3$), 28.7 (C(*C*H_3)$_3$), 44.1 (CH_2β), 70.7 (CHα), 79.8, 83.4 (*C*(CH_3)$_3$), 156.3, 172.7 (CO).

*Methyl (R)-2-azido-3-((tert-butoxycarbonyl)amino)propanoate (**10-N_3**).* To a well stirred solution of sulfamidate **10** (33 mg, 0.12 mmol) in DMF (1 mL), at room temperature, sodium azide (31 mg, 0.47 mmol) was added in one portion. The reaction was stirred for 30 min and the remaining DMF was evaporated under vacuum conditions. The crude was dissolved in dichloromethane (5 mL) and an aqueous solution 20% H_2SO_4 (5 mL) was added. The reaction mixture was stirred at room temperature for 30 min and the solvent

mixture was extracted with dichloromethane. The combined organic phase was dried with Na_2SO_4, concentred under vacuum and purified by column chromatography, using hexane/ethyl acetate (6.5:3.5) as an eluent, to give the required product **10-N$_3$** (25 mg, 85%) as a colorless oil. Physical data: $[\alpha]_D^{20}$ (*c* 1.01, $CHCl_3$): +100.5. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_9H_{16}N_4O_4Na^+$ 267.1069; Found 267.1097. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.45 (s, 9H, $C(CH_3)_3$), 3.35-3.48 (m, 1H, $CH_2\beta$), 3.51-3.67 (m, 1H, $CH_2\beta$), 3.82 (s, 3H, CO_2CH_3), 4.15 (t, 1H, *J* = 5.7 Hz, CHα), 4.92 (br s, 1H, N*H*Boc). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.4 ($C(CH_3)_3$), 41.8 ($CH_2\beta$), 53.0 (CO_2CH_3), 61.7 (CHα), 80.2 ($C(CH_3)_3$), 155.7, 169.4 (CO).

*(R)-2,3-Diaminopropanoic acid hydrochloride (**13**).* The catalyst (Pd/C, 2.5 mg, 10% mass) was suspended in methanol (3 mL) into a schlenk reactor and prehydrogenated for 10 min. Then, we added, in one portion, compound **10-N$_3$** (25 mg, 0.1 mmol) dissolved in methanol (3 mL) and the reaction mixture was vigorously stirred at room temperature for 16 h. The reaction mixture was filtered through diatomaceous earth and concentrated in vacuo. Subsequently, the residue was dissolved in an aqueous solution of 6 M HCl (6 mL) and kept stirring at 60 °C for 12 h. The aqueous phase was evaporated and the residue dissolved in H_2O (2 mL) and eluted through a reverse-phase Sep-pak C18 cartridge to obtain, after evaporation, the corresponding compound **13** (9 mg, 60%) as a colorless oil. Physical data: $[\alpha]_D^{20}$ (*c* 0.50, 1 M HCl): -24.2. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_3H_8N_2O_2H^+$ 105.0664; Found 105.0672. The spectroscopic data are consistent with those described in the literature.[23]

*(R)-3-((tert-Butoxycarbonyl)amino)-1-methoxy-1-oxopropan-2-yl 4-nitrobenzoate (**10-OBz**).* Sulfamidate **10** (225 mg, 0.80 mmol), CsF (134 mg, 0.88 mmol), and *p*-nitrobenzoic acid (*p*-NO_2-BzOH) (147 mg, 0.88 mmol) were dissolved in DMF (5 mL) and the mixture was heated at 50° C for 12 h, until the total disappearance of starting material monitored by TLC. After the volatiles were evaporated, the residue was dissolved in a mixture of aqueous 20% H_2SO_4/CH_2Cl_2 (1:1, 10 mL) and it was stirred at room temperature for 30 min. The aqueous phase was extracted with dichloromethane (3 x 15 mL), the combined organic phases were dried (Na_2SO_4) and evaporated to give a residue, which was purified by silica gel column chromatography (hexane/ethyl acetate, 6.5:3.5). In this way, compound **10-OBz** (212 mg, 72%) was isolated as an oil. Physical data: $[\alpha]_D^{20}$ (*c* 0.50, $CHCl_3$): +0.8. HRMS (ESI-TOF) m/z: $[M + Na]^+$ Calcd for $C_{16}H_{20}N_2O_8Na^+$ 391.1117; Found 391.1249. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.43 (s, 9H, $C(CH_3)_3$), 3.75-3.80 (m, 2H, $CH_2\beta$), 3.81 (s, 3H, CO_2CH_3), 4.86 (br s, 1H, NH), 5.38 (t, 1H, J = 4.6 Hz, CHα), 8.20-8.34 (m, 4H, arom). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 28.4 ($C(CH_3)_3$), 41.4 ($CH_2\beta$), 53.0 (CO_2CH_3), 73.0 (CHα), 80.3 ($C(CH_3)_3$) 123.8, 131.3, 134.7, 151.0 (arom), 155.8, 164.1, 168.4 (CO).

*Methyl (R)-3-((tert-butoxycarbonyl)amino)-2-hydroxypropanoate (**14**).* A solution of *p*-nitrobenzoate protected compound **10-OBz** (151 mg, 0.41 mmol) and sodium azide (80 mg, 1.22 mmol) in dry MeOH (10 mL) was warmed at 40 ^{o}C for 14 h under nitrogen. The solvent was removed on a rotary evaporator, and the hydroxyl free compound was purified by column chromatography using hexane/ethyl acetate (7:3) to give **14** (59 mg, 65%) as an oil. Physical data: $[\alpha]_D^{20}$ (*c* 1.00, MeOH): -39.1. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd

for $C_9H_{18}NO_5^+$ 220.1185; Found 220.1174. The spectroscopic data are consistent with those described in the literature for its enantiomer.[22]

General Procedure for the Synthesis of Free Tn Antigen Mimics. Deprotection of hydroxyl, carboxylic acid and amino groups of protected glycosyl amino acids were carried out in two steps following this protocol. Protected glycosyl amino acid (0.1 mmol) was dissolved in a mixture of methanol/water (2:1, 3 mL) and after the addition of lithium hydroxide monohydrate ($LiOH \cdot H_2O$, 1.0 mmol), the mixture was stirred at room temperature until starting material was consumed by TLC monitoring (2 h). Additionally, the reaction was monitored by ^1H NMR to test the hydrolysis of all ester groups. The basic solution was neutralized with ion exchanger Dowex® 50W-X8, filtered and evaporated to give an oil that was treated with trifluoracetic acid (TFA, 1 mL) in dichloromethane (3 mL) at room temperature. After stirring for 1 h, the volatiles were removed and the corresponding unprotected glycosyl amino acid was obtained as a white solid without further purification.

*(2S)-O-(2'-Acetamido-2'-deoxy-α-D-galactopyranosyl)-2-methylserine (**22**).* Following the general procedure and starting from compound **2** (50 mg, 0.09 mmol), free glycosyl amino acid **23** was obtained without further purification (25 mg, 87%). Physical data: $[\alpha]_D^{20}$ (*c* 1.00, H_2O): +57.1. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{12}H_{23}N_2O_8^+$ 323.1449; Found 323.1459. ^1H NMR (300 MHz, D_2O) δ (ppm): 1.53 (s, 3H, CCH_3), 1.99 (s, 3H, $NHCOCH_3$), 3.69-3.76 (m, 2H, $2H_{6s}$), 3.84-3.89 (m, 2H, $H_{5s,}$ H_{3s}), 3.90-3.93 (m, 2H, $CH_2\beta$), 3.94-3.96 (m, 1H, H_{4s}), 4.10 (dd, 1H, J = 11.80, 3.80 Hz, H_{2s}), 4.92 (d, 1H, J = 3.80

Hz, H_{1s}). ^{13}C NMR (75 MHz, D_2O) δ (ppm): 18.0 (CCH_3), 21.8 ($NHCOCH_3$), 49.6 (C_{2s}), 60.2 (C_α), 61.2 (C_{6s}), 67.2 (C_{5s}), 68.2 (C_{4s}), 70.2 (C_β), 71.5 (C_{3s}), 98.1 (C_{1s}), 172.3 ($NHCOCH_3$), 174.5 (CO_2H).

(2R)-O-(2'-Acetamido-2'-deoxy-α-D-galactopyranosyl)isoserine ***(23)***. Following the general procedure and starting from compound **21** (50 mg, 0.08 mmol), free glycosyl amino acid **23** was obtained without further purification (22 mg, 84%). Physical data: $[\alpha]_D^{20}$ (*c* 1.00, H_2O): +51.3. HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{11}H_{21}N_2O_8^+$ 309.1292; Found 309.1290. ^1H NMR (300 MHz, D_2O) δ (ppm): 1.93 (s, 3H, $NHCOCH_3$), 3.35 (dd, 2H, *J* = 5.02, 2.79 Hz, $CH_2\beta$), 3.61-3.69 (m, 2H, $2H_{6s}$), 3.83-3.90 (m, 3H, H_{3s}, H_{4s}, H_{5s}), 4.10 (dd, 1H, *J* = 10.91, 3.88 Hz, H_{2s}), 4.54 (t, 1H, *J* = 5.01, Hα), 5.04 (d, 1H, *J* = 3.89 Hz, H_{1s}). ^{13}C NMR (75 MHz, D_2O) δ (ppm): 21.9 ($NHCOCH_3$), 40.6 (C_β), 49.4 (C_{2s}), 61.3 (C_{6s}), 67.1 (C_{5s}), 68.3 (C_{4s}), 71.2 (C_α), 72.1 (C_{3s}), 96.9 (C_{1s}), 171.8 ($NHCOCH_3$), 174.8 (CO_2H).

ASSOCIATED CONTENT

Supporting Information. Additional experimental section and copies of NMR spectra for all new compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: xxxx

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Notes

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