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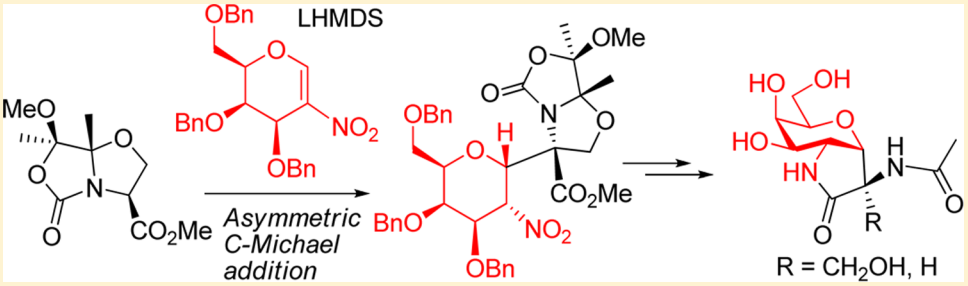
A Double Diastereoselective Michael-Type Addition as an Entry to Conformationally Restricted Tn Antigen Mimics

Carlos Aydillo, Claudio D. Navo, Jesús H. Busto, Francisco Corzana, María M. Zurbano, Alberto Avenoza,* and Jesús M. Peregrina*

Departamento de Química and Centro de Investigación en Síntesis Química, Universidad de La Rioja, 26006 Logroño, Spain

Supporting Information



ABSTRACT: A totally stereocontrolled *C*-Michael addition of serine-equivalent *C*-nucleophiles to tri-*O*-benzyl-2-nitro-D-galactal was used as the key step to synthesize several pyrano[3,2-*b*]pyrrole structures. These scaffolds could be regarded as conformationally restricted Tn antigen mimics, as we have demonstrated by biological assays. The pyranose rings retain their $^4C_1$ chair conformation, as shown by molecular modeling and NMR spectroscopy. The expected bioactivity was established by a competition-tailored enzyme-linked lectin assay using both soybean and *Vicia villosa* agglutinins as model lectins. The facile described synthetic route and the strategic combination of computational and experimental techniques to reveal conformational features and bioactivity demonstrate the prepared glycomimics to be promising candidates for further exploitation of this scaffold to give glycans for lectin blocking and vaccination.

INTRODUCTION

The α-D-*N*-acetylgalactosamine attached to the hydroxyl groups of serine/threonine side chains (the α-GalNAc−Ser/Thr substructure) is a specific human tumor-associated carbohydrate antigen (TACA) known as the Tn antigen[1] (see Figure 1) that is attracting a great deal of interest for the development of vaccines for cancer treatment.[2] This substructure appears in mucins, which are the most abundant form of *O*-linked glycoproteins.[3] Cells of the immune system are equipped with lectin-like receptors, and recent studies have shown that interactions between tumor-associated carbohydrate antigens and lectins play a crucial role in the metastatic cascade of some carcinoma cells.[4]

A precise understanding of the relationship between the carbohydrate moieties of glycopeptides and their biological activity remains limited by the presentation mode of the glycan moiety toward the corresponding biological targets when natural glycopeptides are used. In this sense, we have demonstrated that this presentation mode can be modulated by the nature of the underlying amino acid, involving different interactions, and that it is important for a positive biological response (molecular recognition).[5]

On the other hand, the syntheses of mimics of natural carbohydrates[6] and glycopeptides and glycoproteins[7] help us to complete our understanding of different biochemical processes and also offer a battery of new candidates for biological targets. In this way, recent developments in carbohydrate-based cancer vaccines have addressed the concept of introducing carbohydrate analogues into TACAs in order to improve their immunogenicity.[8] First, the strategic introduction of fluorine atoms into the glycan moiety of tumor-associated carbohydrate and glycopeptide antigens seems to be a particularly promising approach to improve this immunogenicity.[9] Second, in an attempt to circumvent hydrolytic degradation by endogenous glycosidases, a number of hydrolysis-resistant TACA mimics comprising *C*-glycosides[10] and *S*-glycosides[11] have been incorporated into carbohydrate-based vaccines. Finally, the use of homoserine and β^3-homothreonine conjugates instead of serine or threonine to construct mucin-like glycopeptide antigen analogues has also been proposed.[12]

Initially, the antigenicity of artificial TACA derivatives should be enhanced by minor structural modifications, assuming that the conformations remain similar to those of the natural antigens. In this work, we took one step forward in the process of designing new Tn antigens by exploring several structural modifications concomitantly to evaluate whether these modifications are able to induce biological activity similar to that observed in natural Tn antigens. In particular, these novel compounds are *C*-glycoside bicyclic systems in which the

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acetamido group of GalNAc does not show the methyl group and displays a *cis* disposition of the amide; there is either a free hydroxyl or no oxygen; and the sugar is connected to Cα of the amino acid, which is two atoms closer than in the real Tn antigen.

RESULTS AND DISCUSSION

On this basis, we report herein the synthesis and conformational analysis, in aqueous solution, of model neoglycoamino acids **1** and **2**, all in the form of diamides to simulate a peptide backbone. In all cases, the carbohydrate analogue of α-GalNAc is joined to the backbone by a cycle through the methyl group of the acetamide in an attempt to restrict the conformational flexibility of the most important dihedral angles. In one case (**2**), the α-GalNAc residue is *C*-linked to the backbone and a CH_2OH group is attached to Cα of the amino acid moiety. It is important to highlight that while in the natural derivative (Tn antigen) the amide of GalNAc adopts a typical *trans* disposition, in the mimics reported here this amide presents a *cis* disposition because the pyrrolidinone ring simulates the acetamido group of GalNAc (Figure 1).

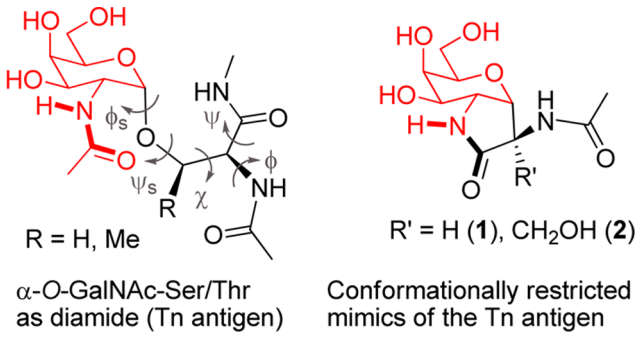
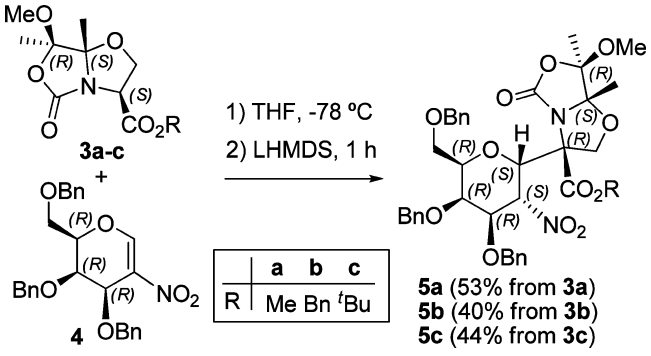


Figure 1. Tn antigen and conformationally restricted mimics.

To achieve the synthesis of these glycomimics and inspired by the well-known methodology described by Schmidt,[13] we envisioned the Michael reaction of a serine-equivalent *C*-nucleophile with a nitrogalactal derivative as a precursor of carbohydrate moiety (Scheme 1). In this context, we have

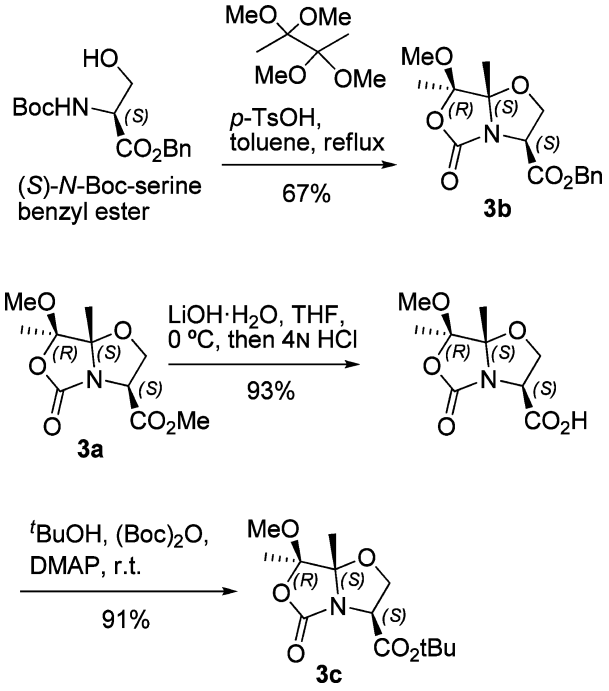
Scheme 1. Double Asymmetric *C*-Michael Reaction



reported that the serine equivalent **3a** behaves as an excellent chiral building block[14] in diastereoselective enolate alkylation reactions, allowing the synthesis of chiral quaternary α-alkyl-β-hydroxy-α-amino acids. More recently, we have demonstrated that this building block participates in a domino Michael/Dieckmann process[15] as an entry to α-(hydroxymethyl)-glutamic acid and other derivatives.

As the key step to synthesize the required glycomimics, we assayed the doubly diastereoselective Michael-type addition[16] of the above-mentioned chiral bicyclic enolate of **3a** on the nitrogalactal derivative **4**, which was obtained from tri-*O*-benzyl-D-galactal.[13] To this aim, compound **3a** was treated with compound **4** in the presence of lithium bis(trimethylsilyl)amide (LHMDS) as a base using tetrahydrofuran (THF) as the solvent at −78 °C. Surprisingly, after 1 h of reaction, a unique diastereoisomer **5a** from among the eight possible ones was obtained in 53% yield (Scheme 1). In order to study the effect of bulky esters in this serine-equivalent substrate, the same stereoselective Michael reaction was assayed with similar results from derivatives **3b** (R = Bn) and **3c** (R = ^{t}Bu).[17] The synthesis of bicyclic compound **3b** was achieved following the same procedure as described for compound **3a**[14] but starting from (*S*)-*N*-Boc-serine benzyl ester. However, derivative **3c** was obtained starting from bicyclic compound **3a**. The methyl ester group of **3a** was hydrolyzed in a basic medium, and after treatment with hydrochloric acid, the corresponding carboxylic acid group was esterified using a mixture of *tert*-butanol (^{t}BuOH), di-*tert*-butyl dicarbonate (Boc_2O), and 4-dimethylaminopyridine (DMAP) at room temperature (Scheme 2).

Scheme 2. Synthesis of Serine Equivalents 3b and 3c



Fortunately, we could obtain appropriate monocrystals of compound **5a** to be analyzed by X-ray diffraction (see the Supporting Information), and the absolute configurations obtained for the three stereogenic centers of this new compound are shown in Scheme 1. In this double diastereoselective Michael addition, three new stereocenters are created and controlled. Two of them come from the *O*-benzyl-protected 2-nitrogalactal **4** (the Michael acceptor). In this sense, Schmidt and co-workers previously explored Michael additions to 2-nitrogalactals and observed the same diastereoselectivity as described here.[13] However, the origin of this diastereoselectivity probably involves a complex mechanism,

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because, as described by Schmidt and co-workers, a change in the base and/or the type of nucleophile afforded different diastereoselectivities.[13c,d] On the other hand, the third stereocenter comes from the serine ester-derived bicyclic compound **3a** (the Michael donor), in which the precursor *C*-nucleophile is chiral. Once it is transformed into the corresponding enolate by the action of a base, the Cα carbon theoretically loses its chirality. Nevertheless, we observed total control of its configuration, as it retained the same configuration in the Michael adduct **5a** as in the starting material **3a**, although it is now designed as *R* because of a change in the priority of the groups attached to this stereocenter. This important feature was also observed for this serine analogue as a chiral building block in other asymmetric reactions previously published by us,[14,15] in which we proposed a highly pyramidalized ester enolate as the true source of this stereodifferentiation.

The next step was the transformation of the nitro group to an amine by treatment with platinized Raney Ni under an atmosphere of hydrogen.[13] Once the amino group was formed, it immediately reacted in the same medium with the methyl ester group to give the corresponding lactam **6** in 48% yield (Scheme 3). Hydrolysis of the acetal and cyclic carbamate groups of compound **6** by treatment with a 4 N aqueous solution of HCl at 40 °C followed by acetylation of the amino and hydroxyl groups with acetic anhydride (Ac_2O) in pyridine (Py) gave compound **7** in good yield (Scheme 3).

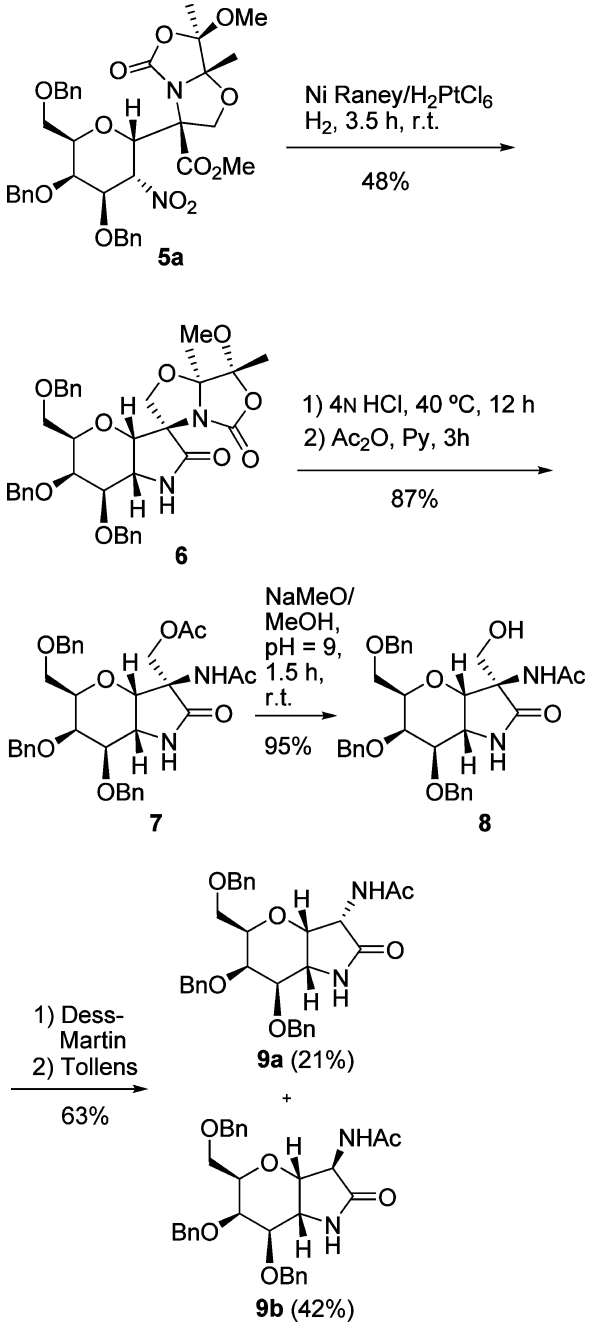
Treatment of compound **7** with sodium methoxide in methanol at pH 9 gave the corresponding free alcohol **8** in excellent yield (Scheme 3). Oxidation of this primary alcohol in two steps, first with Dess–Martin reagent and then with Tollens reagent, gave the corresponding carboxylic acid group, which in the acid workup used to precipitate the AgCl quickly suffered a decarboxylation reaction to give a mixture of compounds **9a** and **9b** (Scheme 3). The two compounds were separated by column chromatography, and their structures were elucidated by NMR experiments, in particular using 2D NOESY techniques. The H3–H3a and H3a–H7a cross-peaks in the NOESY spectrum of compound **9a** (Figure 2 top) reveal that **9a** shows an *S* configuration at C3. In contrast, there is no H3–H3a cross-peak in the NOESY spectrum of **9b** (Figure 2 bottom), and this fact along with the existence of the H3–H5 and H3–H7 cross-peaks confirms that **9b** shows an *R* configuration at C3.

Conformational Analysis in Solution by NMR Spectroscopy and Molecular Modeling. The three *O*-benzylated compounds **8**, **9a**, and **9b** can be regarded as conformationally restricted mimics of the Tn antigen. In order to demonstrate this feature, their benzyl groups were removed by hydrogenolysis using palladium carbon as a catalyst, giving compounds **2**, **epi-1**, and **1**, respectively (Figure 3), in good yields.

To understand the bioactivity of glycomimic motifs, is crucial to know their conformational preferences as well as their dynamics. The presentation mode of the carbohydrate moiety could also be of vital importance for establishing interactions with the corresponding molecular entities. On this basis, we report herein the conformational analysis of these Tn antigen mimics in aqueous solution by NMR spectroscopy and molecular modeling.

The conformational behavior of the three mimics **2**, **epi-1**, and **1** was examined by performing simulations using the molecular dynamics with time-averaged experimental restraints

Scheme 3. Transformation of Functional Groups in Compound 5a



(MD-tar) method with the restraints obtained from the 2D NOE experiments and following the protocol described by our group.[18a] The results obtained from the MD-tar simulations for all of the compounds showed a close agreement, in numerical terms, between the distances found in the refined models and the experimental NMR data (see the Supporting Information and Figure 3). As can be observed in Figure 3, both the natural and non-natural derivatives exhibit mainly an extended conformation for the backbone, and in general, the non-natural derivatives are more rigid than the natural Tn with serine. Another significant difference between the natural Tn and the novel derivatives is the relative orientation of the sugar moiety and the peptide backbone, which in the case of the natural Tn shows a clear parallel alignment. Additionally, the conforma-

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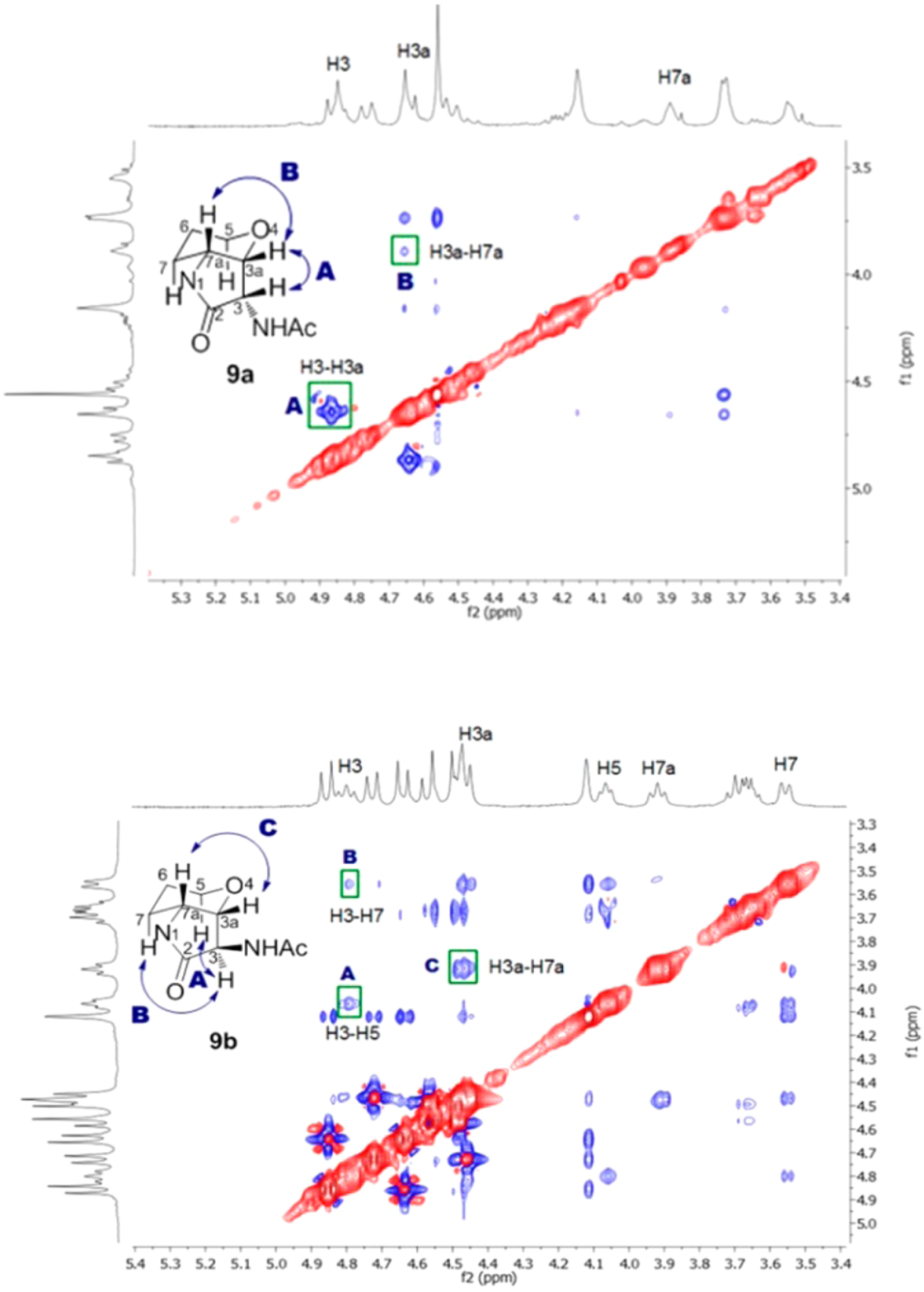

Figure 2. Elucidation of the structures of **9a** and **9b** from NOESY experiments (hydroxyl groups of the carbohydrate moieties have been omitted for clarity).

tions showed by these mimics are different from those observed in other published mucin-type glycopeptides.[18b,c] On the other hand, while for compound **1** only the $^4C_1$ conformation for the carbohydrate moiety was detected, derivatives **2** and **epi-1** displayed about a 20% population of a "twist-boat" conformation in solution. This conformation was also observed in the solid state of compound **5a** (see the Supporting Information).

Molecular Recognition. In the course of the rational design of glycomimics, it is mandatory for these neoglyco derivatives to retain bioactivity, that is, the capacity to serve as ligands for specific receptors. Thus, synthetic products need to

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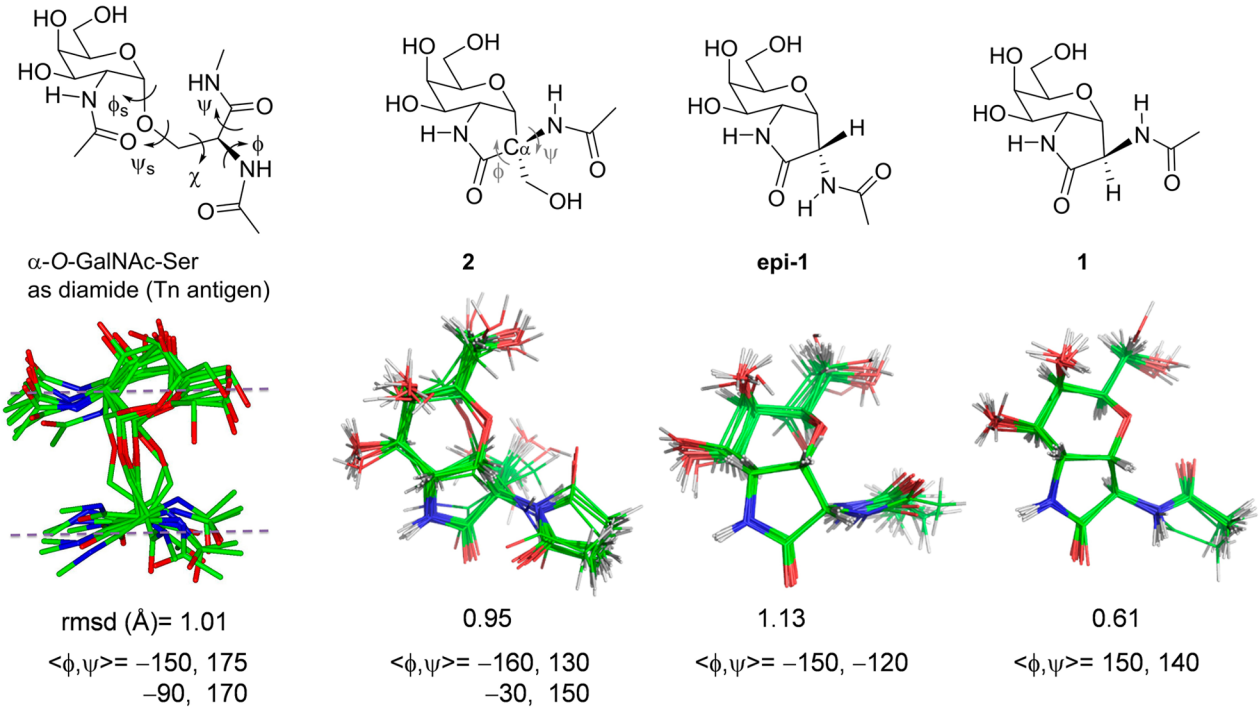

Figure 3. Ensembles for *α-O*-GalNAc-Ser (as a diamide) and compounds **2, epi-1**, and **1** as obtained from 80 ns MD-tar simulations. The rmsd values (in Å) for heavy-atom superimposition are also shown.

be rigorously controlled by respective assays. In our case, we studied the affinities of these non-natural compounds toward soybean agglutinin lectin (SBL) to compare them with that of Tn antigen. SBL was chosen as a biological target because it has been previously used to develop a model for lectin binding to MUC1 mucins, showing high affinity for GalNAc and also for Tn antigen.[19a] Moreover, SBL is a readily available and stable lectin, and its 3D structure bound to GalNAc is already known.[20] These affinities were estimated by a competition-tailored enzyme-linked lectin assay (ELLA) (see Figure 4, the Experimental Section, and the Supporting Information).

From these experiments, it can be inferred that lectin shows a preference for the Tn antigen, with derivative **2** proving to be the best non-natural candidate. In fact, derivatives **1** and **2** inhibit at a concentration over 500 μM, showing adequate values of inhibition at a concentration of 1000 μM (see Figure 4 and the Supporting Information). However, it is important to highlight that although compounds **1** and **2** are not structurally close analogues of the Tn antigen, they are recognized by the SBL lectin and thus can be regarded as mimics of the Tn antigen. This is an example in which it may be the case that close structural similarity between the original and the mimic is not needed or desirable. The configuration of Cα appears to affect greatly the ligand−mucin interactions, which is in agreement with the similar affinity observed for substrates **2** and **1** in comparison to **epi-1**. To explain these experimental results, a putative 3D model of the three complexes with SBL was deduced using MD simulations (see Figure 5 and Supporting Information). The difference between the SBL affinities observed for derivatives **1** and **epi-1** could be attributed to the fact that Hα of compound **1** is involved in a hydrophobic patch formed with the aromatic ring of Phe128 of SBL. This stabilizing interaction is not possible in the case of compound **epi-1** (see the Supporting Information). The protein−glycopeptide interactions are similar to those found in the modeled 3D structure of the SBL:GalNAc complex.[20]

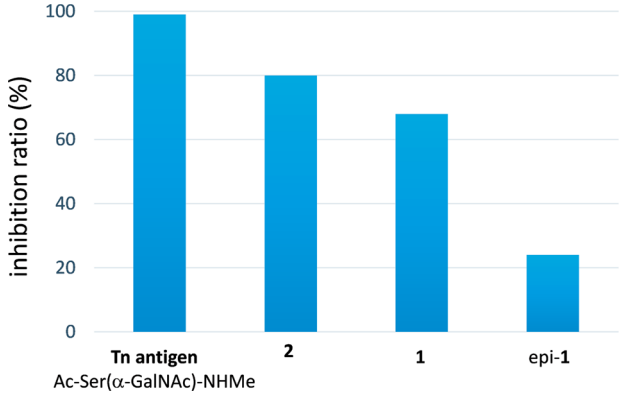

Figure 4. Inhibition ratio (%) for Tn antigen and mimics **1**, **2**, and **epi-1** at a concentration per well of 1000 μM in the competition ELLA assay (see the Supporting Information).

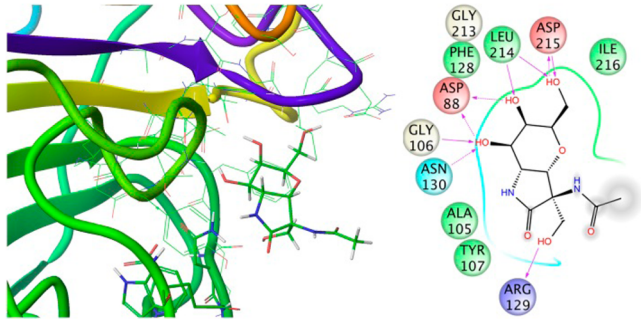

Figure 5. Snapshot taken from the unrestrained 20 ns MD simulation for the SBL:**2** complex, together with the ligand interaction diagram obtained using Maestro 9.3.5 software.

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The higher affinity of compound **2** may be explained by the extra hydrogen bond present in the bound state for this compound. In fact, the CH_2OH group participates in a highly populated hydrogen bond with arginine-129.

To generalize this result, we evaluated an additional lectin, *Vicia villosa*-B-4 agglutinin (VVL), which also shows specificity for the epitope α-*N*-acetylgalactosamine,[19b,c] the glycan moiety of the Tn antigen. The affinity results from the competition ELLA assay are depicted in the Supporting Information, and the behavior of tested compound **2** with VVL was similar to that found for SBL lectin.

CONCLUSION

Although some examples of the synthesis of *C*-glycosides through an asymmetric Michael addition have been published, it is important to note that to the best of our knowledge this is the first time that *C*-glycosides have been obtained using a double asymmetric Michael addition on the well-known 2-nitroglycal derivatives. Indeed, the double asymmetric *C*-Michael additions of three serine-equivalent *C*-nucleophiles to tri-*O*-benzyl-2-nitro-D-galactal have been described. The reactions took place with complete stereoselectivity, allowing for the preparation of three pyrano[3,2-*b*]pyrrole structures. The presented facile synthetic route afforded not only stereoselectivity but also products that maintained the biomimetic conformational properties of the resulting scaffold, as documented by combined molecular modeling and NMR spectroscopic analyses. The sugar moiety retained bioactivity for the tested model lectin, and therefore, these synthetic scaffolds can be regarded as conformationally restricted Tn antigen mimics. This fact was experimentally demonstrated by competition-tailored enzyme-linked lectin assays. In a general context, we have used a strategy that combines stereoselective synthesis of glycomimics with affinity testing, which has not only allowed us to explore these scaffolds as a potential source for new candidates for lectin blocking but also enabled us to gain insights into structural aspects of the recognition process that have relevance for understanding and exploiting lectin−carbohydrate interactions.

EXPERIMENTAL SECTION

General Procedures. Solvents were purified according to standard procedures. Column chromatography was performed using silica gel 60 (230−400 mesh). ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz spectrometer using $CDCl_3$, CD_3OD, or D_2O as the solvent; chemical shifts are reported in parts per million on the δ scale, and coupling constants are reported in hertz. All of the resolved signals in the ^{1}H NMR spectra were assigned on the basis of coupling constants and ge-COSY and ge-HSQC experiments performed on the 400 MHz spectrometer. The results of these experiments were processed with MestReC and MestreNova software. Melting points were determined on a 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. Electrospray mass spectra were recorded on a micrOTOF spectrometer; accurate mass measurements were achieved by using sodium formate as an external reference. Copies of NMR spectra for all new compounds are provided in the Supporting Information.

NMR Experiments. NMR experiments were performed on a 400 MHz 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 908. Phase-sensitive ge-2D HSQC spectra were acquired by using *z*-filter and selection before t_1, removing the decoupling during acquisition by use of the invigpndph pulse program with CNST2 (J_{HC}) = 145. 2D NOESY experiments were conducted by using phase-sensitive ge-2D NOESY with WATERGATE for H_2O/D_2O (9:1) spectra. NOE intensities were normalized with respect to the diagonal peak at zero mixing time. Distances involving NH protons were semiquantitatively determined by integrating the volume of the corresponding cross-peaks.

X-ray Diffraction Analysis:[21] A summary of crystal data for compound **5a** is presented in the Supporting Information. The SHELXL97 program[22] was used for the refinement of crystal structures, and hydrogen atoms were fitted at theoretical positions.

Benzyl (3*S*,7*R*,7a*S*)-7-Methoxy-7,7a-dimethyl-5-oxotetrahydro-5*H*-oxazolo[4,3-*b*]oxazole-3-carboxylate (3b). A round-bottom flask was charged with (*S*)-*N*-Boc-serine benzyl ester (2.10 g, 7.12 mmol), 2,2,3,3-tetramethoxybutane (2.54 g, 14.24 mmol), toluene (50 mL), and $TsOH·H_2O$ (203 mg, 1.07 mmol). The solution was heated under reflux and stirred overnight. The reaction mixture was cooled to room temperature and diluted with diethyl ether (50 mL), and the reaction was quenched with saturated $NaHCO_3$ (50 mL). The aqueous phase was extracted with diethyl ether (2 × 25 mL). The organic layers were combined, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated, and the crude product was purified by column chromatography (hexane/ethyl acetate, 8:2) to give benzyl ester bicyclic compound **3b** as a yellow oil (1.53 g, 4.77 mmol, 67%). $[\alpha]_D^{25} = -85.2$ (*c* 1.02, $CHCl_3$). HRMS ESI+ (*m*/*z*): 344.1119 [M + Na^+]; calcd for $C_{16}H_{19}NO_6Na^+$, 344.1105. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.29 (s, 3H, CH_3), 1.55 (s, 3H, CH_3), 3.44 (s, 3H, OCH_3), 4.10 (dd, *J* = 5.9, 8.8 Hz, 1H, CH_2), 4.27 (t, *J* = 9.0 Hz, 1H, CH_2), 4.79 (dd, *J* = 5.9, 8.9 Hz, 1H, CH), 5.22 (q, *J* = 12.1 Hz, 2H, C*H*$_2$Ph), 7.35 (m, 5H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 15.5 (CH_3), 16.3 (CH_3), 51.0 (OCH_3), 60.1 (CH), 66.7 (CH_2), 67.7 (CO_2 CH_2), 101.5 ($CNCH_3OCH_2$), 107.2 (CCH_3OCH_3), 128.5 (Ph), 128.7 (Ph), 128.7 (Ph), 135.0 (Ph), 160.6 (NCO_2), 169.9 (CO_2CH_2).

***tert*-Butyl (3*S*,7*R*,7a*S*)-7-Methoxy-7,7a-dimethyl-5-oxotetrahydro-5*H*-oxazolo[4,3-*b*]oxazole-3-carboxylate (3c).** Methyl ester bicyclic compound **3a** (4.00 g, 16.31 mmol) was dissolved in THF (25 mL), and the solution was stirred at 0 °C. $LiOH·H_2O$ (3.42 g, 81.57 mmol) was then added as a solution in water (25 mL), and the mixture was stirred for 5 min. Next, the reaction was quenched with 4 N HCl (20 mL) to reach pH 1. Ethyl acetate (25 mL) was added, and the aqueous layer was extracted with more ethyl acetate (4 × 25 mL). The combined organic layers were dried over anhydrous Na_2SO_4, and the solvent was evaporated to give the bicyclic acid compound as a white crystalline solid (3.50 g, 15.17 mmol, 93%) that could be used without further purification. This compound was dissolved in ^{t}BuOH (25 mL), and Boc_2O (6.62 g, 30.34 mmol) and DMAP (556.0 mg, 4.55 mmol) were added. The solution was stirred at room temperature until completion of the reaction (3 h) as monitored by TLC. The solvent was evaporated, and the crude material was purified by column chromatography (hexane/ethyl acetate, 1:1) to give bicyclic *tert*-butyl ester compound **3c** (3.97 g, 13.80 mmol, 91%) as a white solid. Mp: 35−37 °C. $[\alpha]_D^{25} = -112.1$ (*c* 1.07, $CHCl_3$). HRMS ESI+ (*m*/*z*): 310.1276 [M + Na^+]; calcd for $C_{13}H_{21}NO_6Na^+$, 310.1261. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.34 (s, 3H, CH_3), 1.48 (s, 9H, ^{t}Bu), 1.55 (s, 3H, CH_3), 3.45 (s, 3H, OCH_3), 4.05 (dd, *J* = 5.8, 8.8 Hz, 1H, CHC*H*$_2$), 4.23 (t, *J* = 8.9 Hz, 1H, CHC*H*$_2$), 4.64 (dd, *J* = 5.8, 9.0 Hz, 1H, C*H*CH$_2$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.6 (CH_3), 16.4 (CH_3), 27.9 ($C(CH_3)_3$), 51.0 (OCH_3), 60.7 ($CHCH_2$), 66.9 ($CHCH_2$), 82.8 ($C(CH_3)_3$), 101.5 ($CNCH_3OCH_2$), 107.0 (CCH_3OCH_3), 160.7 (NCO_2), 169.0 (CO_2^{t}Bu).

Double Diastereoselective Michael-Type Addition. Compounds **5a**, **5b**, and **5c** were synthesized using tri-*O*-benzyl-2-nitro-D-galactal[13c] (**4**) as the Michael acceptor and the corresponding chiral bicyclic enolates of **3a−c** as serine-equivalent *C*-nucleophiles. The experimental details of these double diastereoselective Michael-type additions are described in the following three paragraphs.

Methyl (3R,7R,7aS)-3-((2′S,3′S,4′R,5′R,6′R)-4′,5′-Bis(benzyloxy)-6′-(benzyloxymethyl)-3′-nitrotetrahydro-2H-pyran-2′-yl)-7-methoxy-7,7a-dimethyl-5-oxotetrahydro-2H-oxazolo[3,2-c]oxazole-3-carboxylate (5a). Tri-*O*-benzyl-2-nitro-D-galactal (**4**) (848 mg, 1.84

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mmol) was added to a dry THF solution (48 mL) of compound **3a** (450 mg, 1.84 mmol) under an Ar atmosphere at −78 °C. A 1 M THF solution of LHMDS (4.8 mL, 4.8 mmol) was added dropwise under vigorous stirring. After 1 h of reaction, a saturated solution of NH_4Cl (50 mL) was added, and the mixture was warmed to room temperature. The crude mixture was diluted with diethyl ether, and the water phase was extracted with more diethyl ether. The organic phases were collected, washed with brine, and dried with anhydrous Na_2SO_4. Concentration and purification of the crude product by silica gel column chromatography (hexane/ethyl acetate, 9:1) afforded compound **5a** (686 mg, 0.97 mmol, 53%) as a white solid. Mp: 106−108 °C. $[\alpha]_D^{25}$ = −5.1 (*c* 1.03, $CHCl_3$). HRMS ESI+ (*m*/*z*): 729.2632 $[M + Na]^+$; calcd for $C_{37}H_{42}N_2O_{12}Na^+$, 729.2630. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (s, 3H, Me), 1.43 (s, 3H, Me), 3.35 (s, 3H, OMe), 3.70−3.74 (m, 1H, BnOCH^aH^b), 3.78−3.82 (m, 1H, BnOCH^aH^b), 3.88 (s, 3H, CO_2Me), 4.18−4.23 (m, 1H, $H^{5'}$) 4.23−4.31 (m, 2H, $H^{6'}$, H^{2a}), 4.39−4.45 (m, 2H, PhCH^cH^dO, $H^{4'}$), 4.51 (d, 1H, *J* = 11.6 Hz, PhCH^cH^dO), 4.55−4.62 (m, 3H, H^{2b}, PhCH_2O), 4.66 (d, 1H, *J* = 12.0 Hz, PhCH^eH^fO), 4.79 (d, 1H, *J* = 11.5 Hz, PhCH^eH^fO), 5.08 (t, 1H, *J* = 6.0 Hz, $H^{3'}$), 6.13 (d, 1H, *J* = 4.8 Hz, $H^{2'}$) 7.19−7.40 (m, 15H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.4 (Me), 19.2 (Me), 51.9 (OMe), 53.7 (CO_2Me), 66.0 (BnOCH_2), 67.4 (C^3), 71.3 (C^2), 71.7 ($C^{5'}$), 71.9 ($C^{2'}$), 72.8 (PhCH^cH^dO), 73.3 (PhCH^eH^fO), 73.4 ($C^{4'}$), 73.9 (PhCH^gH^hO), 77.3 ($C^{3'}$), 77.4 ($C^{6'}$), 101.9 (C^{7a}), 109.6 (C^7), 127.7, 128.1, 128.3, 128.3, 128.4, 128.5, 128.7, 136.9, 137.9, 138.5 (Arom.), 152.5 (C^5), 168.5 (CO_2Me).

*Benzyl (3R,7R,7aS)-3-((2′S,3′S,4′R,5′R,6′R)-4′,5′-Bis(benzyloxy)-6′-(benzyloxymethyl)-3′-nitrotetrahydro-2H-pyran-2′-yl)-7-methoxy-7,7a-dimethyl-5-oxotetrahydro-2H-oxazolo[3,2-c]oxazole-3-carboxylate (**5b**).* Tri-*O*-benzyl-2-nitro-D-galactal (600 mg, 1.3 mmol) was added to a dry THF solution (35 mL) of compound **3b** (418 mg, 1.3 mmol) under an Ar atmosphere at −78 °C. A 1 M THF solution of LHMDS (3.5 mL) was added dropwise under vigorous stirring. After 1 h of reaction, a saturated solution of NH_4Cl (40 mL) was added, and the mixture was warmed to room temperature. The crude mixture was diluted with diethyl ether, and the water phase was extracted with more diethyl ether. The organic phases were collected, washed with brine, and dried with anhydrous Na_2SO_4. Concentration and purification of the crude product by silica gel column chromatography (hexane/ethyl acetate, 9:1) afforded compound **5b** (410 mg, 0.52 mmol, 40%) as a colorless oil. $[\alpha]_D^{25}$ = −18.6 (*c* 1.00, $CHCl_3$). HRMS (ESI) *m*/*z*: 783.3138 $(M + H)^+$; calcd for $C_{43}H_{47}N_2O_{12}^+$, 783.3124. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36 (s, 3H, Me), 1.37 (s, 3H, Me), 3.34 (s, 3H, OMe), 3.73−3.85 (m, 2H, BnOCH_2), 4.21−4.23 (m, 1H, $H^{5'}$), 4.25−4.31 (m, 2H, $H^{6'}$, H^{2a}), 4.35−4.41 (m, 1H, $H^{4'}$), 4.42−4.45 (m, 1H, PhCH^aH^bO), 4.46 (s, 2H, PhCH_2O), 4.59−4.62 (m, 3H, H^{2b}, PhCH^aH^bO, PhCH^cH^dO), 4.69−4.74 (m, 1H, PhCH^cH^dO), 5.01 (t, 1H, *J* = 5.2 Hz, $H^{3'}$), 5.28 (d, 1H, *J* = 12.3 Hz, $CO_2CH^eH^f$Ph), 5.38 (d, 1H, *J* = 12.3 Hz, $CO_2CH^eH^f$Ph), 6.19 (d, 1H, *J* = 3.0 Hz, $H^{2'}$), 7.20−7.39 (m, 20H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 17.4 (CH_3), 19.2 (CH_3), 52.0 (OCH_3), 66.8 (BnOCH_2), 67.5 ($CO_2CH^eH^f$Ph), 68.3 (C^3), 71.3 (C^2), 71.9 ($C^{2'}$), 72.0 ($C^{5'}$), 72.6 (PhCH_2O), 73.3 (PhCH^aH^bO), 73.4 ($C^{4'}$), 73.7 (PhCH^cH^dO), 77.2 ($C^{3'}$), 77.4 ($C^{6'}$), 101.8 (C^{7a}), 109.7 (C^7), 127.5, 127.9, 128.3, 128.3, 128.6, 128.7, 128.8, 134.7, 137.0, 137.9, 138.6 (Arom.), 152.3 (C^5), 167.7 (CO_2Bn).

*tert-Butyl (3R,7R,7aS)-3-((2′S,3′S,4′R,5′R,6′R)-4′,5′-Bis(benzyloxy)-6′-(benzyloxymethyl)-3′-nitrotetrahydro-2H-pyran-2′-yl)-7-methoxy-7,7a-dimethyl-5-oxotetrahydro-2H-oxazolo[3,2-c]oxazole-3-carboxylate (**5c**).* Tri-*O*-benzyl-2-nitro-D-galactal (77 mg, 0.27 mmol) was added to a dry THF solution (7 mL) of compound **3c** (90 mg, 0.20 mmol) under an Ar atmosphere at −78 °C. A 1 M THF solution of LHMDS (0.7 mL) was added dropwise under vigorous stirring. After 1 h of reaction, a saturated solution of NH_4Cl (10 mL) was added, and the mixture was warmed to room temperature. The crude mixture was diluted with diethyl ether, and the water phase was extracted with more diethyl ether. The organic phases were collected, washed with brine, and dried with anhydrous Na_2SO_4. Concentration and purification of the crude product by silica gel column chromatography (hexane/ethyl acetate, 9:1) afforded compound **5c** (66 mg, 0.088 mmol, 44%) as a colorless oil. $[\alpha]_D^{25}$ = −13.7 (*c* 1.00, $CHCl_3$). HRMS (ESI) *m*/*z*: 749.3264 $(M + H)^+$; calcd for $C_{40}H_{49}N_2O_{12}^+$, 749.3280. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36 (s, 3H, Me), 1.43 (s, 3H, Me), 1.50 (s, 9H, ^{t}Bu), 3.35 (s, 3H, OMe), 3.75−3.79 (m, 2H, BnOCH_2), 4.21−4.28 (m, 3H, $H^{5'}$, $H^{6'}$, H^{2a}), 4.41−4.44 (m, 2H, PhCH^aH^bO, $H^{4'}$), 4.51−4.66 (m, 5H, PhCH_2O, H^{2b}, PhCH^aH^bO, PhCH^cH^dO), 4.77 (d, 1H, *J* = 6.0 Hz, PhCH^cH^dO), 5.10 (t, 1H, *J* = 3.0 Hz, $H^{3'}$), 6.08 (d, 1H, *J* = 3.0 Hz, $H^{2'}$), 7.26−7.34 (m, 15H, Ph). ^{13}C NMR (400 MHz, $CDCl_3$): δ 17.2 (CH_3), 19.2 (CH_3), 27.6 ($C(CH_3)_3$), 51.8 (OCH_3), 66.8 (BnOCH_2), 68.1 (C^3), 70.9 (C^2), 72.0 ($C^{5'}$), 72.3 ($C^{2'}$), 72.7 (PhCH^aH^bO), 73.1 (PhCH_2O), 73.3 ($C^{4'}$), 73.8 (PhCH^cH^dO), 77.1 ($C^{3'}$), 77.4 ($C^{6'}$), 83.8 ($C(CH_3)_3$), 101.4 (C^{7a}), 109.5 (C^7), 127.4, 127.9, 128.2, 128.4, 128.6, 136.9, 137.9, 138.5 (Arom.), 152.0 (C^5), 166.4 ($CO_2{}^t$Bu).

(3*R*,3a*S*,5*R*,6*R*,7*R*,7a*S*,7′*R*,7′a*S*)-6,7-Dibenzyloxy-5-(benzyloxymethyl)-7′-methoxy-7′,7′a-dimethylspiro[1,3a,5,6,7,7a-hexahydropyran[3,2-*b*]pyrrol-3,3′-2*H*-oxazolo[4,3-*b*]oxazol]-2,5′-dione (6). Raney Ni (2.00 g) was suspended in H_2O (12 mL), and hexachloroplatinic acid (50 mg) and sodium hydroxide 20% (400 μL) were added under stirring. The mixture was heated at 50 °C. After the mixture was stirred for 2.5 h, sodium hydroxide 40% (6 mL) was added, keeping the stirring and the heating. After 1.5 h of stirring, a white cloud in the top of the flask appeared, which was removed by decantation; the resulting solution was then washed with warm water (3 × 15 mL) and ethanol (3 × 15 mL). The catalyst obtained was suspended in ethanol (10 mL) and prehydrogenated for 10 min. An ethanol solution (7 mL) of compound **5a** (160 mg, 0.23 mmol) was added, and the mixture was stirred under molecular hydrogen at room temperature and atmospheric pressure for 3.5 h. The crude product was filtered, and the liquid phase was concentrated and purified by silica gel column chromatography (dichloromethane/methanol, 99:1), to afford compound **6** (70 mg, 0.11 mmol, 48%) as a colorless oil. $[\alpha]_D^{25}$ = +4.2 (*c* 1.00, $CHCl_3$). HRMS (ESI) *m*/*z*: 645.2812 $(M + H)^+$; calcd for $C_{36}H_{41}N_2O_9^+$, 645.2807. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.59 (s, 3H, CH_3), 1.62 (s, 3H, CH_3), 3.49 (s, 3H, OCH_3), 3.57 (d, 1H, *J* = 5.7 Hz, H^7), 3.72−3.76 (m, 2H, CH_2OBn), 4.12−4.19 (m, 2H, H^5, H^6), 4.37 (d, 1H, *J* = 9.5 Hz, OCH^aH^bPh), 4.55 (s, 2H, $H^{2'}$), 4.61−4.73 (m, 5H, H^{7a}, OCH_2Ph, OCH^aH^bPh,OCH^cH^dPh), 4.92−4.97 (m, 2H, OCH^cH^dPh, H^{3a}), 7.29−7.40 (m, 15H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 16.9 (CH_3CN), 18.0 (CH_3COMe), 51.6 (CH_3O), 57.8 (C^{7a}), 67.5 (CH_2OBn), 68.69 (C^3), 69.1 (OCH_2Ph), 71.0 (C^{3a}), 71.9 (OCH^aH^bPh), 72.3 (C^5), 73.5 ($C^{2'}$), 73.9 (OCH^cH^dPh), 76.8 (C^6), 80.6 (C^7), 103.0 ($C^{7'a}$), 108.1 ($C^{7'}$), 127.7, 127.8, 127.8, 127.8, 128.1, 128.4, 128.5, 128.7, 137.7, 138.1, 138.2 (Ph), 154.8 ($C^{5'}$), 173.6 (C^2).

(3*R*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamide-3-acetoxymethyl-6,7-dibenzyloxy-5-(benzyloxymethyl)-2-oxo-1,3a,5,6,7,7a-hexahydropyran[3,2-*b*]pyrrole (7). An aqueous 4 N HCl solution (2.5 mL) was added to a THF solution of compound **6** (150 mg, 0.23 mmol), and the mixture was heated at 40 °C under stirring. After 12 h of stirring, the crude product was concentrated and redissolved in a mixture of pyridine and acetic anhydride (2:1, 6 mL) under stirring. The mixture was stirred for 3 h and then concentrated, and the crude product was purified by silica gel column chromatography (dichloromethane/methanol, 15:1), affording compound **7** (122 mg, 0.20 mmol, 87%) as a colorless oil. $[\alpha]_D^{25}$ = +56.2 (*c* 1.00, $CHCl_3$). HRMS (ESI) *m*/*z*: 603.2701 $(M + H)^+$; calcd for $C_{34}H_{39}N_2O_8^+$, 603.2701. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.96 (s, 3H, CH_3COO), 1.98 (s, 3H, CH_3CON), 3.55 (d, 1H, *J* = 7.6 Hz, H^7), 3.61−3.65 (m, 2H, CH_2OBn), 4.05−4.09 (m, 1H, H^5), 4.13 (d, 1H, *J* = 4.4 Hz, H^6), 4.27 (d, 1H, *J* = 11.6 Hz, PhCH^aH^bO), 4.31 (t, 1H, *J* = 7.6 Hz, H^{7a}), 4.46 (d, 1H, *J* = 11.7 Hz, PhCH^cH^dO), 4.51 (s, 2H, AcOCH_2), 4.52 (d, 1H, *J* = 11.6 Hz, PhCH^aH^bO), 4.58 (d, 1H, *J* = 11.7 Hz, PhCH^cH^dO), 4.66−4.72 (m, 2H, PhCH^eH^fO, H^{3a}), 4.88 (d, 1H, *J* = 11.6 Hz, PhCH^eH^fO), 6.18 (s, 1H, CONH), 6.42 (s, 1H, CONH), 7.25−7.40 (m, 15H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 20.9 ($OCOCH_3$), 23.2 ($NCOCH_3$), 56.0 (C^{7a}), 63.2 (C^5), 63.3 (C^3), 67.8 (CH_2OBn), 71.5 ($AcOCH_2$), 71.7 ($PhCH_cH_dO$), 73.5 ($PhCH_aH_bO$), 74.4 ($PhCH_eH_fO$), 75.7 (C^{3a}), 76.5 (C^6), 81.2 (C^7), 127.8, 127.8, 127.9,

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128.0, 128.3, 128.5, 128.6, 128.8, 137.6, 138.1, 138.3 (Ph), 170.1 ($OCOCH_3$), 170.7 ($NCOCH_3$), 172.4 (C^2).

(3*R*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamido-6,7-dibenzyloxy-5-(benzyloxymethyl)-3-(hydroxymethyl)-2-oxo-1,3a,5,6,7,7a-hexahydropyrane[3,2-*b*]pyrrole (8). A methanol solution of NaOMe (2 mL, 0.5M) was added to a methanol solution (4 mL) of compound **7** (122 mg, 0.20 mmol). The mixture was stirred for 1.5 h, and Dowex sulfonic acid resin was then added. The liquid phase was filtered and concentrated, and the residue was purified by silica column chromatography (dichloromethane/methanol, 15:1) to afford compound **8** (107 mg, 0.19 mmol, 95%) as a colorless oil. $[\alpha]_D^{25}$ = +89.8 (*c* 1.00, $CHCl_3$). HRMS (ESI) *m*/*z*: 561.2603 (M + H)$^+$; calcd for $C_{32}H_{36}N_2O_7^+$, 561.2595. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.03 (s, 3H, CH_3), 3.50−3.54 (m, 1H, C*H*^{a}H^{b}OBn), 3.62 (d, 1H, *J* = 8.0 Hz, H^7), 3.72−3.81 (m, 2H, CH^a*H*^{b}OBn, OC*H*^{c}H^{d}Ph), 4.03 (d, 1H, *J* = 12.0 Hz, OCH^c*H*^{d}Ph), 4.13 (d, 1H, *J* = 4.5 Hz, H^6), 4.15−4.24 (m, 1H, H^5), 4.39 (t, 1H, *J* = 7.2 Hz, H^{7a}), 4.53 (d, 1H, *J* = 11.6 Hz, OC*H*^{e}H^{f}Ph), 4.54 (s, 2H, C*H*$_2$OH), 4.60 (d, 1H, *J* = 11.9 Hz, OC*H*^{g}H^{h}Ph), 4.68 (d, 1H, *J* = 6.3 Hz, H^{3a}), 4.72 (d, 1H, *J* = 11.6 Hz, OCH^e*H*^{f}Ph), 4.90 (d, 1H, *J* = 11.9 Hz, OC*H*^{g}H^{h}Ph), 6.60 (s, 1H, NH), 6.86 (s, 1H, NH), 7.19−7.52 (m, 15H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.8 (CH_3), 56.4 (C^{7a}), 63.9 (OCH^{c}H^{d}Ph, C^3), 67.4 (CH_2OBn), 71.6 (C^6), 71.7 (OCH^{e}H^{f}Ph), 73.5 (*C*H$_2$OH), 74.3 (OCH^{g}H^{h}Ph), 76.6 (C^{3a}), 76.7 (C^5), 81.3 (C^7), 127.7, 127.9, 127.9, 128.0, 128.2, 128.5, 128.6, 128.8, 137.6, 137.8, 138.0 (Ph), 170.6 ($NCOCH_3$), 174.1 (C^2).

(3*S*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamido-6,7-dibenzyloxy-5-(benzyloxymethyl)-2-oxo-1,3a,5,6,7,7a-hexahydropyran[3,2-*b*]-pyrrole (9a) and (3*R*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamido-6,7-dibenzyloxy-5-(benzyloxymethyl)-2-oxo-1,3a,5,6,7,7a-hexahydropyran[3,2-*b*]pyrrole (9b). A dichloromethane solution of Dess−Martin periodinane (2 mL) was added to a dichloromethane solution (2 mL) of compound **8** (100 mg, 0.18 mmol). The mixture was stirred at room temperature for 1 h. A saturated water solution of NaS_2O_3 and $NaHCO_3$ (10 mL, 1:1) was added, and the resulting mixture was diluted with dichloromethane (10 mL). The phases were separated, and the aqueous one was washed with dichloromethane (3 × 10 mL). The combined organic phases were washed with brine, dried with Na_2SO_4, and concentrated. Meanwhile, $AgNO_3$ (365 mg, 2.1 mmol) was dissolved in the minimum volume of water, and an aqueous 10% NaOH solution was added dropwise. A dark-brown precipitate appeared, and NH_4OH was then added dropwise until it was totally redissolved. This solution was added to a dichloromethane solution (5 mL) of the previously obtained crude product, and the mixture was heated at 50 °C under stirring for 2 h. A black precipitate (Ag^0) was observed as it reacted. The pH was adjusted to 7 using 2 N HNO_3 solution, and then 2 N HCl and saturated NH_4Cl solutions were added in order to precipitate AgCl, which was filtered off. The filtrate was concentrated and extracted with ethyl acetate and H_2O. The combined organic phases were dried (Na_2SO_4), concentrated, and purified by silica gel column chromatography (chloroform/methanol, 95:5) to afford compounds **9a** (20 mg, 0.038 mmol, 21%) and **9b** (40 mg, 0.076 mmol, 42%) as yellow oils.

Data for (3*S*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-**9a**: $[\alpha]_D^{25}$ = +31.6 (*c* 1.00, $CHCl_3$). HRMS (ESI) *m*/*z*: 531.2471 (M + H)$^+$; calcd for $C_{31}H_{35}N_2O_6^+$, 531.2490. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.06 (s, 3H, CH_3), 3.55 (d, 1H, *J* = 5.0 Hz, H^7), 3.70−3.79 (m, 2H, BnOC*H*$_2$), 3.84−3.91 (m, 1H, H^{7a}), 4.16 (s, 1H, H^6), 4.16−4.19 (m, 1H, H^5), 4.52 (d, 1H, *J* = 12.0 Hz, PhC*H*^{a}H^{b}O), 4.56 (s, 2H, PhC*H*$_2$O), 4.62−4.69 (m, 2H, PhC*H*^{c}H^{d}O, H^{3a}), 4.77 (d, 1H, *J* = 12.0 Hz, PhCH^a*H*^{b}O), 4.81−4.90 (m, 2H, PhCH^c*H*^{d}O, H^3), 5.86 (s, 1H, HN), 6.03 (d, 1H, *J* = 8.0 Hz, AcN*H*), 7.26−7.47 (m, 15H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 22.8 (CH_3), 52.7 (C^3), 56.8 (C^{7a}), 67.3 (BnO*C*H$_2$), 69.1 (C^{3a}), 72.0 (PhCH^{a}H^{b}O), 72.8 (C^6), 73.7 (PhCH$_2$O), 74.4 (PhCH^{c}H^{d}O), 76.5 (C^5), 80.6 (C^7), 127.7, 127.8, 127.9, 128.0, 128.0, 128.5, 128.6, 128.9, 137.5, 137.6, 138.1 (Ph), 170.7, 173.6 (NCO, C^2).

Data for (3*R*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-**9b**: $[\alpha]_D^{25}$ = +76.7 (*c* 1.00, $CHCl_3$). HRMS (ESI) *m*/*z*: 531.2471 (M + H)$^+$; calcd for $C_{31}H_{35}N_2O_6^+$, 531.2490. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.08 (s, 3H, CH_3), 3.56 (d, 1H, *J* = 8.2 Hz, H^7), 3.65−3.69 (m, 2H, BnOC*H*$_2$), 3.92 (t, 1H, *J* = 8.2 Hz, H^{7a}), 4.07 (t, 1H, *J* = 6.4 Hz, H^5), 4.12 (s, 1H, H^6), 4.20−4.53 (m, 3H, PhC*H*^{a}H^{b}O, PhC*H*^{c}H^{d}O, H^{3a}), 4.57 (d, 1H, *J* = 11.7 Hz, PhCH^c*H*^{d}O), 4.64 (d, 1H, *J* = 11.5 Hz, PhC*H*^{e}H^{f}O), 4.73 (d, 1H, *J* = 11.4 Hz, PhCH^a*H*^{b}O), 4.77−4.83 (m, 1H, H^3), 4.86 (d, 1H, *J* = 11.5 Hz, PhCH^e*H*^{f}O), 6.13 (d, 1H, *J* = 7.5 Hz, AcN*H*), 6.31 (s, 1H, NH), 7.26−7.45 (m, 15H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$): δ 23.2 (CH_3), 50.4 (C^3), 51.7 (C^{7a}), 68.3 (BnO*C*H$_2$), 70.8 (C^6), 71.6 (PhCH^{a}H^{b}O), 72.4 (C^5), 73.6 (PhCH^{c}H^{d}O), 74.7 (PhCH^{e}H^{f}O), 78.0 (C^{3a}), 82.0 (C^7), 127.9, 128.0, 128.2, 128.4, 128.5, 128.6, 128.8, 137.4, 137.9, 138.3 (Ph), 171.2, 171.9 (NCO, C^2).

(3*R*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamido-6,7-dihydroxy-5-hydroxymethyl-2-oxo-1,3a,5,6,7,7a-hexahydropyrano[3,2-*b*]-pyrrole (1). Hydrogenolysis of a methanol/ethyl acetate solution (4:1, 5 mL) of compound **9b** (40 mg, 0.075 mmol) was performed at ambient pressure and temperature using Pd−C (30 mg) and 3 drops of concentrated HCl as the catalyst. The reaction mixture was stirred for 36 h, after which the catalyst was filtered over diatomaceous earth. The filtrate was concentrated and purified by extractions with water and ethyl acetate to afford compound **1** (18 mg, 0.07 mmol, 93%) in the aqueous phase as a yellow oil. $[\alpha]_D^{25}$ = +80.2 (*c* 0.74, H_2O). HRMS (ESI) *m*/*z*: 283.0899 (M + Na)$^+$; calcd for $C_{10}H_{16}N_2NaO_6^+$, 283.0901. ^{1}H NMR (400 MHz, D_2O): δ 2.08 (s, 3H, CH_3), 3.66−3.75 (m, 3H, H^{7a}, HOC*H*$_2$), 3.79 (dd, 1H, *J* = 9.3, 2.6 Hz, H^7), 3.89 (t, 1H, *J* = 5.9 Hz, H^5), 3.93 (d, 1H, *J* = 2.6 Hz, H^6), 4.70 (dd, 1H, *J* = 10.3, 7.9 Hz, H^{3a}), 4.87 (d, 1H, *J* = 10.4 Hz, H^3). ^{13}C NMR (100 MHz, D_2O): δ 21.7 (CH_3), 49.9 (C^3), 51.8 (C^{7a}), 61.2 (CH_2OH), 67.1 (C^6), 72.7 (C^7), 73.4 (C^5), 76.4 (C^{3a}), 173.5 (C^2), 174.8 ($NHCOCH_3$).

(3*S*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamido-6,7-dihydroxy-5-hydroxymethyl-2-oxo-1,3a,5,6,7,7a-hexahydropyrano[3,2-*b*]pyrrole (epi-1). Hydrogenolysis of a methanol/ethyl acetate (4:1, 5 mL) and acetic acid (0.2 mL) solution of compound **9a** (30 mg, 0.056 mmol) was performed at atmospheric pressure and room temperature using Pd−C (20 mg) as a catalyst. The reaction mixture was stirred for 36 h, after which the catalyst was filtered over diatomaceous earth. The filtrate was concentrated and purified by extractions with water and ethyl acetate to afford compound **epi-1** (14 mg, 0.053 mmol, 95%) in the aqueous phase as a colorless oil. $[\alpha]_D^{25}$ = +41.4 (*c* 0.72, H_2O). HRMS (ESI) *m*/*z*: 283.0914 (M + H)$^+$; calcd for $C_{10}H_{16}N_2NaO_6^+$, 283.0901. ^{1}H NMR (400 MHz, D_2O): δ 2.11 (s, 3H, CH_3), 3.71 (d, 1H, *J* = 3 Hz, HOC*H*^{a}H^b), 3.86 (d, 1H, *J* = 4 Hz, H^{7a}), 3.89 (d, 1H, *J* = 2.6 Hz, H^7), 3.95 (m, 1H, HOCH^a*H*^{b}), 4.00−4.04 (m, 1H, H^5), 4.16 (dd, 1H, *J* = 2.8, 4.7 Hz, H^6), 4.65 (m, 1H, H^{3a}), 4.92 (d, 1H, *J* = 5.4 Hz, H^3). ^{13}C NMR (100 MHz, D_2O): δ 21.7 (CH_3), 53.4 (C^3), 57.2 (C^{7a}), 58.7 (CH^{a}H^b), 67.3 (C^6), 67.7 (C^{3a}), 70.1 (C^7), 77.0 (C^5), 174.8 ($NHCOCH_3$), 175.5 (C^2).

(3*R*,3a*S*,5*R*,6*R*,7*R*,7a*S*)-3-Acetamido-6,7-dihydroxy-3,5-bis-(hydroxymethyl)-2-oxo-1,3a,5,6,7,7a-hexahydropyrano[3,2-*b*]-pyrrole (2). Hydrogenolysis of a methanol/dichloromethane solution (3:1, 8 mL) of compound **8** (72 mg, 0.13 mmol) was performed at atmospheric pressure and room temperature using Pd−C (46 mg) as a catalyst. The reaction mixture was stirred for 12 h, after which the catalyst was filtered over diatomaceous earth. The filtrate was concentrated and purified by extractions with water and ethyl acetate to afford compound **2** (20 mg, 0,069 mmol, 54%) in the aqueous phase as a colorless oil. $[\alpha]_D^{25}$ = +64.3 (*c* 0.75, H_2O). HRMS (ESI) *m*/*z*: 291.1180 (M + H)$^+$; calcd for $C_{11}H_{19}N_2O_7^+$, 291.1187. ^{1}H NMR (400 MHz, CD_3OD): δ 2.02 (s, 3H, CH_3), 3.71−3.75 (m, 1H, HOC*H*^{a}H^{b}CH), 3.78−3.83 (m, 2H, HOCH^a*H*^{b}CH, HOC*H*^{c}H^d), 3.92−3.95 (m, 3H, HOCH^c*H*^{d}, H^{7a}, H^6), 4.00−4.04 (m, 1H, H^7), 4.05−4.10 (m, 1H, H^5), 4.99 (d, 1H, *J* = 7.8 Hz, H^{3a}). ^{13}C NMR (400 MHz, CD_3OD): δ 21.8 (CH_3), 53.7 (C^{7a}), 60.3 (HOCH^{c}H^d), 61.1 (HOCH^{a}H^b), 64.9 (C^3), 67.9 (C^7), 71.8 (C^6), 75.2 (C^{3a}), 76.9 (C^5), 173.5 ($NHCOCH_3$), 174.9 (C^2).

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

molecules. We equilibrated the system as follows. First, only the water molecules were minimized. The water box, together with Na^+, was then minimized, and this was followed by a short MD simulation at 300 K. 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, an unrestrained 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 atm). The particle-mesh Ewald method and periodic boundary conditions were also used. The SHAKE algorithm for hydrogen atoms, which allows the use of 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), which was implemented with GAFF parameters to accurately simulate the Tn mimics. A simulation length of 20 ns for each complex was obtained, 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). Once the assignment of the NMR spectra was completed (see the Experimental Section), 2D NOESY experiments in H_2O/D_2O (9:1) at 25 °C and pH 5.2 were then carried out for glycopeptides **1**, **2**, and **epi-1**. Distances involving the key NH protons were semiquantitatively determined from the integrated volumes of the corresponding cross-peaks. MD-tar simulations were performed with AMBER 11 (parm99 force field), which was implemented with GAFF parameters. Distances derived from the NOE interactions were included as time-averaged distance restraints. An $\langle r^{-6}\rangle^{-1/6}$ average was used for the distances. Final trajectories were run using an exponential decay constant of 8000 ps and a simulation length of 80 ns with a dielectric constant $\varepsilon = 80$.

Enzyme-Linked Lectin Assay (ELLA). The plate (Pierce amine-binding, maleic anhydride 96-well plate) was coated with 100 μL/well of a solution of 70 nmol of glycopeptide APDT*R (where * = α-*O*-GalNAc) in carbonate/bicarbonate buffer (0.2 M, pH 9.4) and incubated overnight at 25 °C. The unbound sites were then blocked by adding 200 μL/well of blocking buffer (Thermo Scientific SuperBlock Blocking Buffer, product no. 37515). After 1 h at 25 °C, the blocking buffer was removed, and the plate wells were washed (3 × 200 μL/well) with PBST [phosphate-buffered saline (0.1 M sodium phosphate, 0.15 M sodium chloride, pH 7.2) containing 0.05% Tween-20 detergent; product no. 28320]. As the next step, the wells were incubated with biotin-conjugated soybean lectin or *V. villosa*-B-4 agglutinin from EY Laboratories (100 μL, diluted 1/150 in PBST buffer) and increasing amounts of the different Tn mimics for 2 h. The wells were then washed with PBST (3 × 200 μL/well, 2 min/well) and treated with horseradish peroxidase (HRP)-conjugated streptavidin from Rockland (100 μL, diluted 1/4000 in PBST buffer) for 1 h at 25 °C. The wells were again washed first with PBST (3 × 200 μL/well, 2 min/well) and then with 350 μL of water. TMB was added (90 μL/well), and after incubation for 10 min, the reaction was terminated by the addition of 50 mL/well of stop solution (1 M H_2SO_4). Absorbance detection of the wells was immediately performed at 450 nm using an ELISA plate reader. The average absorbance intensities of three replicates were plotted against the Tn mimic concentration.

■ ASSOCIATED CONTENT

Supporting Information

Named and labeled organic structures, 1D and 2D NMR spectra for all new compounds, X-ray data for compound **5a** (CIF), ELLA experiments, and data corresponding to molecular dynamics simulations. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*Fax: +34 941 299621. E-mail: alberto.avenoza@unirioja.es.

*E-mail: jesusmanuel.peregrina@unirioja.es.

Notes

The authors declare no competing financial interest.

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■ DEDICATION

Dedicated to Professor Carmen Nájera on the occasion of her 60th birthday.

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