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New sialyl Lewis^x mimic containing an α-substituted β^3-amino acid spacer

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Abstract—A highly convergent and efficient synthesis of a new sialyl Lewis^x (sLe^x) mimic, which was predicted by computational studies to fulfil the spacial requirements for a selectin antagonist, has been developed. With a β^{2,3}-amino acid residue L-galactose (bioisostere of the L-fucose moiety present in the natural sLe^x) and succinate are linked, leading to a mimic of sLe^x that contains all the required pharmacophores, namely the 3- and 4-hydroxy group of L-fucose, the 4- and 6-hydroxy group of D-galactose and the carboxylic acid of *N*-acetylneuraminic acid. The key step of the synthesis involves a tandem reaction consisting of a N-deprotection and a suitable O→N intramolecular acyl migration reaction which is promoted by cerium ammonium nitrate (CAN). Finally, the new sialyl Lewis^x mimic was biologically evaluated in a competitive binding assay.

Keywords: sLe^x; Selectin antagonist; β^3-Amino acid; Glycomimetic

1. Introduction

Selectins[1] are involved in the orderly migration of leucocytes from blood vessels to the sites of inflammation.[2] Although extravasation of leucocytes represents an essential defense mechanism against inflammatory stimuli, excessive infiltration of leucocytes into the surrounding tissue can cause acute or chronic reactions as observed in reperfusion injuries, stroke, psoriasis, rheumatoid arthritis, or respiratory diseases.[2] An early step in this inflammatory cascade is mediated by selectin/carbohydrate interactions. Data from both selectin knock out mice[3a–c] and LAD type 2 patients,[3d] clearly demonstrate that the selectin–carbohydrate interaction is a prerequisite for the inflammatory cascade to take place. Since the tetrasaccharide sLe^x (**1**, Fig. 1) is the carbohydrate epitope recognized by E-selectin,[4] it became the lead structure for the design of selectin antagonists.[5]

The search for novel selectin antagonist with enhanced adhesion and improved pharmacokinetic properties[5] has led to numerous classes of antagonists. In the initial contributions,[6,7] the structure–activity relationship was elucidated, revealing the essential pharmacophores which are the carboxylic acid function of *N*-acetylneuraminic acid, the 3- and 4-hydroxyl group of L-fucose and the 4- and 6-hydroxyl group of D-galactose (highlighted in Fig. 1).[8] In addition, it has been shown that the D-GlcNAc moiety is not involved in binding. Its principal function is that of a rigid spacer to accommodate the appropriate spacial orientation of L-fucose and D-galactose.[5] For the design of simplified sLe^x antagonists[5] a dual strategy was pursued by (1) eliminating monosaccharide moieties by non-carbohydrate linkers, and (2) substituting metabolically labile O-glycosidic bonds.

Considering the glycoaminoacid mimics reported by Wong,[9] which exhibit remarkable pharmacological

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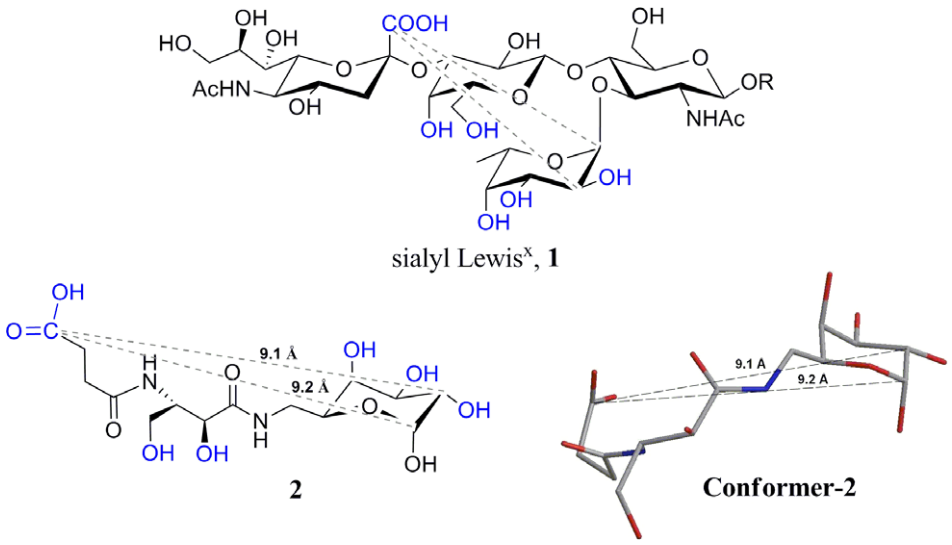


Figure 1. Identical spacial orientation of the pharmacophores in sLe^x (**1**) and the glycoaminoacid mimic **2**.

activity, we report herein the synthesis and the biological evaluation of the scaffold **2**, a member of a new family of antagonists based on a dihydroxylated β-amino acid as a substitute of galactose. This scaffold is decorated with a suitably modified L-fucose and a carboxylic acid moiety mimicking the native *N*-acetylneuraminic acid. The corresponding target molecule **2** (Fig. 1) therefore contains (2*S*,3*S*)-2-hydroxy-β^3-serine which is coupled with the free amino group of 6-amino-6-deoxy-L-galactose (L-Fuc replacement) and, at the other end, with a succinic acid (D-NeuNAc replacement). An important factor for affinity is the spacial orientation of the pharmacophores in the bioactive conformation.[5d,e] This is expected to be realized with the rigid diamide core.

2. Results and discussion

A conformational analysis of **2** by molecular dynamic studies indicated that the low energy **conformer-2** presented in Figure 1 exhibits distances between the carboxylate and C-1 and C-2 of L-galactose (9.2 and 9.1 Å, respectively), which are similar to the one found in the bioactive conformation[10] of sLe^x. **Conformer-2** is only less stable by 5.29 kJ/mol compared to its lowest energy conformation and is characterized by differing flexibilities of the dihedral angles θ, φ and ω (Fig. 2). Whereas θ and φ show considerable flexibility over the 10,000 ps period of the MD simulation, ω is rather stiff, leading to a partial preorganization of **2** in the bioactive conformation.

The synthesis started with the preparation of 6-amino-6-deoxy-L-galactose derivative **4** from commercially available L-galactose according to a known procedure[11] (Scheme 1). According to this procedure, the acetonation to achieve 1,2:3,4-di-*O*-isopropyliden-L-galactose (**3**), using $CuSO_4/H_2SO_4$ in acetone, gave only poor yields (55%).

Using an improved procedure[12] (polystyryl diphenyl phosphine–iodine complex, prepared in situ, in anhydrous acetone, at rt under nitrogen) **3** was obtained in 95% yield. Tosylation of the primary hydroxy group followed by substitution with sodium azide and catalytic hydrogenation (Pd/H_2) afforded the free amine **4** in 69% overall yield from L-galactose.

(2*S*,3*S*)-2-Hydroxy-β^3-serine methyl ester derivative **7**, was obtained diastereoselectively[13] from commercially available *O*-benzyl-L-serine via enolate formation (by KHMDS) of the corresponding fully protected β^3-serine **5** and the coupling of the enolate with racemic 2-[(4-methylphenyl)sulfonyl]-3-phenyloxaziridine (**6**) (Scheme 2).

The hydrolysis of methyl ester **7** afforded **8** in excellent yield without any traces of racemization.

Then, the 2-hydroxy-β^3-amino acid **8** was coupled with the previously prepared 6-amino-L-galactose derivative **4** by treatment with DCC and HOBT in CH_2Cl_2, yielding **9** in good yield (75%) (Scheme 3).

The last step of the synthesis was the introduction of a carboxylate side chain at the N-terminus of **9**. The first attempt to remove the 4-methoxybenzyl ether protection (PMB) with cerium ammonium nitrate (CAN)[14] was accompanied by the formation of several byproducts due to the cleavage of C-2–C-3 bond of the β-aminoacidic moiety. To avoid a possible interference of the free hydroxyl group with CAN, compound **9** was first acetylated and then treated with CAN. A single product was formed, which turned out not to be the desired N-deprotected derivative, but the product of a subsequent O→N intramolecular acetyl migration.[15]

When this O→N intramolecular acyl migration was applied to ester **10**, which was obtained by esterification

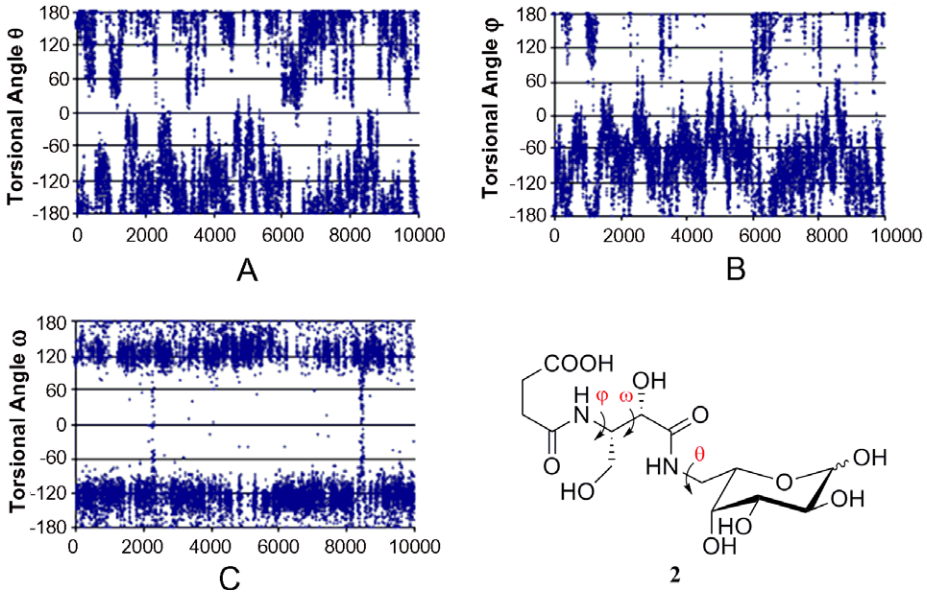
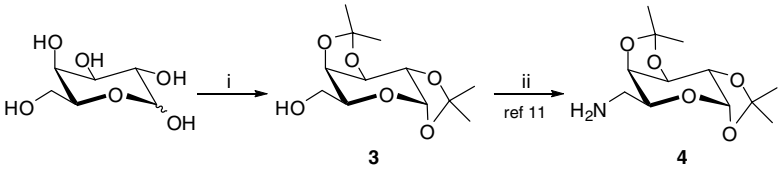
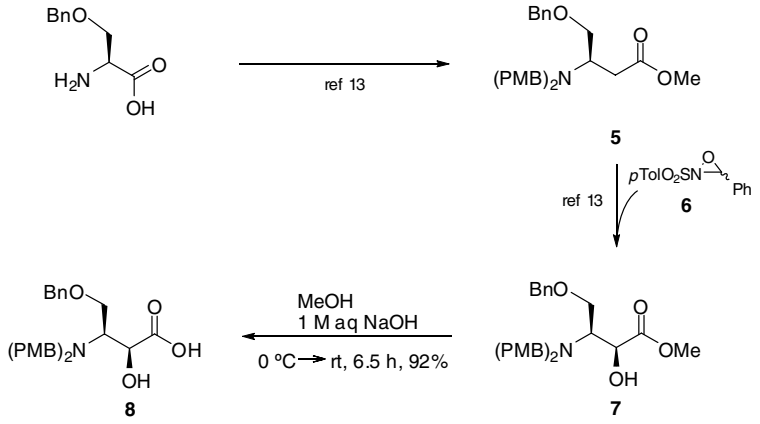


Figure 2. The plots A, B, and C show the fluctuations of the θ, φ, and ω torsional angles in compound **2** monitored during the 10,000 ps MD simulation in a box of TIP3P water molecules; A, B: the θ and φ dihedral angles are highly variable within the MD simulation time frame; C: the ω torsion angle remains practically unchanged during the MD simulation.



Scheme 1. Synthesis of 6-amino-6-deoxy-L-galactose derivative **4**. Reagents and conditions: (i) PDP/I_2, anhyd $(CH_3)_2CO$, rt, 1 h, 95%; (ii) (a) TsCl, anhyd py, 0 °C→rt, 45 min, 86%; (b) NaN_3, anhyd DMF, 115 °C, 15 h, 94%; (c) H_2, 5% Pd/C, MeOH, ultrasound, 90%.



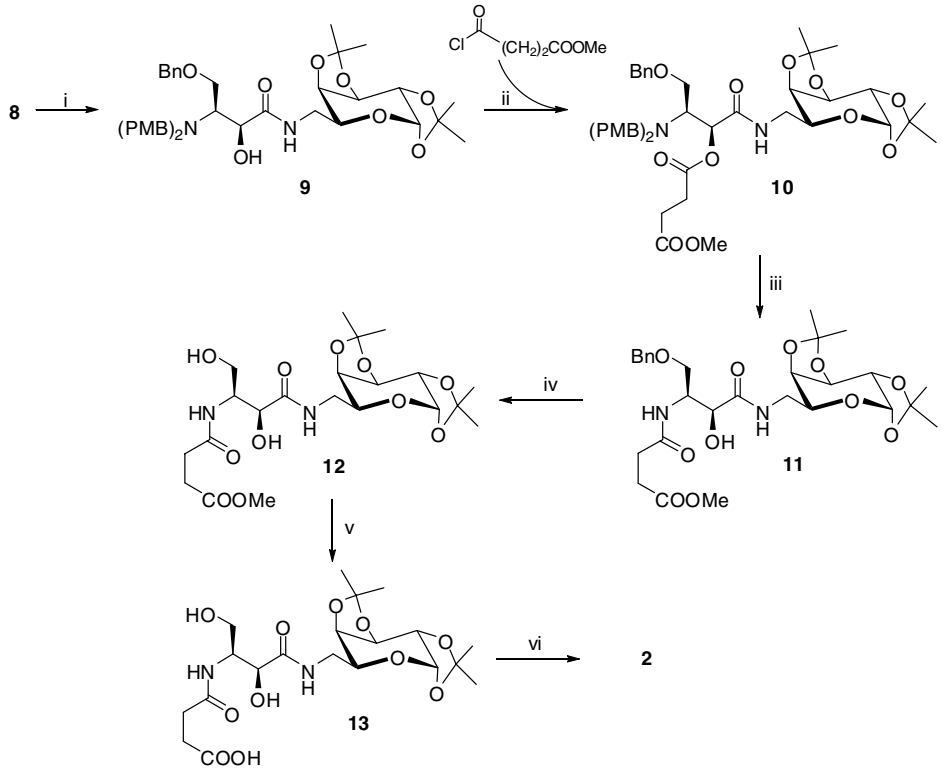
Scheme 2. Synthesis of protected (2*S*,3*S*)-2-hydroxy-β^3-serine **8**.

of **9** with 3-carbomethoxypropionyl chloride, product **11** could be isolated in excellent 90% yield (Scheme 3).

Finally, the removal of the protecting groups by hydrogenolysis on Pd/C in an ultrasound bath (→**12**), followed by alkaline hydrolysis (→**13**), and acidic hydrolysis (TFA/H_2O) of the isopropylidene groups (Scheme 3) yielded **2** as an anomeric mixture ($\alpha/\beta = 1{:}2$).

3. Biological evaluation

Various formats of cell-free competitive binding assays under static conditions have been reported.[16–18] We used microtiter plates coated with human recombinant E-selectin/IgG or P-selectin/IgG, which were incubated with **2** and commercially available sLe^x/biotin-polyacry-



Scheme 3. Synthesis of mimic **2**. Reagents and conditions: (i) DCC, HOBT, **4**, anhyd CH_2Cl_2, rt, 15 h, 75%; (ii) anhyd py, CH_2Cl_2, rt, 4 h, 90%; (iii) CAN, MeCN/H_2O, 0 °C→rt, 19 h, 90%; (iv) H_2, Pd/C 5%, MeOH, ultrasound, 40 °C, 22 h, 93%; (v) MeOH, 1 M aq NaOH, 0 °C→rt, 3 h, 91%; (vi) TFA/H_2O, rt, 3 h, 80%.

late. After unbound ligand and polymer were washed from the plate, streptavidin/horseradish peroxidase conjugate was added to enable colorimetric determination of binding.[18b] As a reference compound, sialyl Lewis^x with an IC_{50} value of 0.9 mM for E-selectin and $\geqslant 3$ mM for P-selectin was used. With **2**, a moderate inhibition of the interaction of sLe^x/E-selectin ($IC_{50} \geqslant 6$ mM) could be detected. However, the interaction of P-selectin with the sLe^x-polyacrylate polymer could not be inhibited with **2** (see Table 1).

4. Conclusion

The new sLe^x mimic **2** was designed and synthesized starting from an L-galactose derivative **4** and orthogonally protected (2*S*,3*S*)-2-hydroxy-β^3-serine **8** in six steps. Our synthesis features the use of cerium ammonium nitrate (CAN) to perform a useful O→N intramolecular acyl migration reaction on the succinoyl ester **10**. However, biological testing revealed that this sLe^x mimic shows only low affinity of E-selectin ($IC_{50} = 6$ mM) and no activity (IC_{50} >10 mM) for P-selectin, respectively.

Since the spatially correct arrangement of the pharmacophores can be realized in **2**, the low affinity can also be due to the considerable conformational flexibility of the dihedral angles θ and φ (Fig. 2A and B) leading to substantial cost of entropy upon binding.

Table 1. Biological evaluation data

Test compound	E-Selectin (mM)	P-Selectin (mM)
Sialyl Lewis^x (**1**)	0.9	$\geqslant 3$
Glycoaminoacid (**2**)	$\geqslant 6$	>10

5. Experimental

5.1. General methods

Triphenyl phosphine polymer-bound was purchased from Fluka Chemical Co. All moisture-sensitive reactions were performed under a nitrogen atmosphere using oven-dried glassware. Solvents were dried over standard drying agents and freshly distilled prior to use. Reactions were monitored by TLC (precoated silica gel plate F254, Merck). Column chromatography: Merck Kieselgel 60 (70–230 mesh); flash chromatography: Merck Kieselgel 60 (230–400 mesh). Optical rotations were measured at 25 ± 2 °C in the stated solvent. ^1H and ^{13}C NMR spectra were recorded on NMR spectrometers operating at 400 or 500 MHz and 50, 100 or 125 MHz, respectively. Wherever necessary, two-dimen-

sional ^1H–^1H COSY experiments were carried out for complete signal assignments. Combustion analyses were performed using CHNS analyzer.

5.2. 1,2:3,4-Di-*O*-isopropylidene-α-L-galactopyranose (3)

To a magnetically stirred suspension of dry polystyryl diphenyl phosphine (1.12 g, ≈3.34 phosphine units) in anhydrous acetone (10 mL) at rt, a solution of I_2 (0.85 g, 3.34 mmol) in the same solvent (30 mL) was added dropwise in the dark and under dry nitrogen atmosphere. After 15 min, solid L-galactopyranose (0.33 g, 1.67 mmol) was added in one portion to the suspension. TLC monitoring ($CHCl_3$/CH_3OH, 9:1) showed that the starting sugar was completely consumed within 30 min. The reaction mixture was then filtered through a glass sinter funnel and washed with acetone. The solvent was removed under reduced pressure and the solid residue recrystallized from $CHCl_3$/hexane (1:2) to give the final product **3** (0.41 g, 95% yield); $[\alpha]_D^{25}$ −59.5 (*c* 1.5, $CHCl_3$), lit.[19] $[\alpha]_D^{25}$ −55.0 (*c* 3.6, $CHCl_3$). ^1H and ^{13}C NMR spectral data matched that reported.[12]

5.3. 6-Amino-6-deoxy-1,2:3,4-di-*O*-isopropylidene-α-L-galactopyranose (4)

The title compound was prepared following May's procedure[11] starting from **3**.

5.4. (2*S*,3*S*)-4-(Benzyloxy)-3-[di(4-methoxybenzyl)-amino]-2-hydroxybutanoic acid (8)

To a stirring solution of **7**, prepared as already reported,[13] (1.04 g, 2.00 mmol) in MeOH (13 mL) was added dropwise aq NaOH (1.0 M solution in H_2O, 4.0 mL) at 0 °C. The reaction, allowed to warm to rt, was stirred for 6.5 h and then it was diluted with EtOAc (100 mL). The organic phase was treated with a solution of HCl (3 M, 2 × 100 mL), washed with brine (50 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue was purified by flash chromatography ($CHCl_3$/MeOH, 9:1) to afford **8** (0.90 g, 92%) as a pale yellow oil. $[\alpha]_D^{25}$ +44.0 (*c* 0.9, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 3.45 (ddd, 1H, $J_{3,2}$ 11.0, $J_{3,4a}$ 9.6, $J_{3,4b}$ 3.4 Hz, H-3), 3.82 (s, 6H, 2 × OCH_3), 3.84 (d, 1H, $J_{2,3}$ 11.0 Hz, H-2), 3.89 (d, 2H, $J_{a,b}$ 12.0 Hz, 2 × CH_aPMB), 3.98 (dd, 1H, $J_{4a,4b}$ 11.3, $J_{4a,3}$ 9.6 Hz, H_a-4), 4.10 (dd, 1H, $J_{4b,4a}$ 11.3, $J_{4b,3}$ 3.4 Hz, H_b-4), 4.22 (d, 2H, $J_{b,a}$ 12.0 Hz, 2 × CH_bPMB), 4.59 (d, 1H, $J_{a,b}$ 11.7 Hz, CH_aPh), 4.73 (d, 1H, $J_{b,a}$ 11.7 Hz, CH_bPh), 6.89 (d, 4H, J_{ortho} 8.8 Hz, ArH), 7.25 (d, 4H, J_{ortho} 8.8 Hz, ArH), 7.38–7.48 (m, 5H, PhH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 54.2, 55.1, 60.1, 64.1, 66.2, 73.9, 114.6, 128.1, 128.6, 131.6, 136.9, 160.2, 175.0. Anal. Calcd for $C_{27}H_{31}NO_6$: C, 69.66; H, 6.71; N, 3.01. Found: C, 69.42; H, 6.69; N, 3.03.

5.5. *N*1-(1,2:3,4-Di-*O*-isopropylidene-6-deoxy-α-L-galactopyranos-6-yl)-(2*S*,3*S*)-4-(benzyloxy)-3-[di(4-methoxybenzyl)amino]-2-hydroxybutanamide (9)

HOBT (0.35 g, 2.58 mmol) was added to a stirred solution of $\beta^{2,3}$-amino acid **8** (0.60 g, 1.29 mmol) in anhydrous CH_2Cl_2 (10 mL) at rt. After 30 min, to the resulting mixture a solution of compound **4** (0.30 g, 1.17 mmol) and DCC (0.40 g, 1.42 mmol) in the same solvent (10 mL) was added dropwise over 5 min at 0 °C. The solution was allowed to warm slowly to rt and, after 15 h, most of the solvent was evaporated under reduced pressure and replaced by EtOAc. The precipitate was filtered and the solution was washed with saturated $NaHCO_3$ solution, brine until neutral, then dried (Na_2SO_4), and concentrated under reduced pressure. Chromatography of the crude residue on silica gel (hexane/EtOAc, 1:1) afforded the oily coupling product **9** (0.62 g, 75%). $[\alpha]_D^{25}$ +8.5 (*c* 0.9, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 1.31, 1.32, 1.43, 1.45 (4s, 12H, 4 × CH_3), 3.24 (ddd, 1H, $J_{6'a,6'b}$ 13.9, $J_{6'a,5'}$ 8.1, $J_{6'a,NH}$ 4.4Hz, H_a-6′), 3.36–3.43 (m, 1H, H-3), 3.51 (ddd, 1H, $J_{6'b,6'a}$ 13.9, $J_{6'b,5'}$ 6.8, $J_{6'b,NH}$ 4.9 Hz, H_b-6′), 3.67 (d, 2H, $J_{a,b}$ 13.2 Hz, 2 × CH_aPMB), 3.73 (d, 2H, $J_{b,a}$ 13.2 Hz, 2 × CH_bPMB), 3.79 (s, 6H, 2 × OCH_3), 3.80–3.85 (m, 1H, H-5′), 3.92 (dd, 1H, $J_{4a,4b}$ 10.7, $J_{4a,3}$ 4.4 Hz, H_a-4), 3.95–4.08 (m, 3H, H-4′, H-2, H_b-4), 4.27 (dd, 1H, $J_{2',1'}$ 4.9, $J_{2',3'}$ 1.9 Hz, H-2′), 4.50 (dd, 1H, $J_{3',4'}$ 7.8, $J_{3',2'}$ 1.9 Hz, H-3′), 4.55 (d, 1H, $J_{a,b}$ 11.7 Hz, CH_aPh), 4.58 (d, 1H, $J_{b,a}$ 11.7 Hz, CH_bPh), 5.48 (d, 1H, $J_{1',2'}$ 4.9 Hz, H-1′), 6.84 (d, 4H, J_{ortho} 8.3 Hz, ArH), 7.18 (d, 4H, J_{ortho} 8.3 Hz, ArH), 7.28–7.40 (m, 5H, PhH), 7.50–7.58 (m, 1H, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.9, 24.7, 25.2, 26.2, 39.7, 54.7, 55.5, 59.5, 66.3, 68.4, 69.6, 70.7, 71.0, 71.7, 73.7, 96.5, 108.9, 109.6, 114.1, 127.9, 128.7, 130.6, 131.1, 138.1, 159.0, 173.8. Anal. Calcd for $C_{39}H_{50}N_2O_{10}$: C, 66.27; H, 7.13; N, 3.96. Found: C, 66.50; H, 7.11; N, 3.93.

5.6. 1-{[(1*S*,2*S*)-3-(Benzyloxy)-2-[di(4-methoxybenzyl)-amino]-1-[(1,2:3,4-di-*O*-isopropylidene-6-deoxy-α-L-galactopyranos-6-yl)carbamoyl]propyl} 4-methyl succinate (10)

3-Carbomethoxy propionyl chloride (0.17 g, 1.16 mmol) and anhydrous pyridine (94 μL, 1.16 mmol) were added to a solution of compound **9** in anhydrous CH_2Cl_2 (11 mL) at 0 °C. The stirring solution was allowed to warm slowly to rt and after 4 h most of the solvent was evaporated under reduced pressure and replaced by EtOAc. The organic phase was washed with brine (2 × 30 mL), dried (Na_2SO_4), and concentrated under reduced pressure. The residue oil was purified by silica gel chromatography (hexane/EtOAc, 8:2→6:4) to give the oily product **10** (0.56 g, 90%). $[\alpha]_D^{25}$ +18.0 (*c* 0.8, $CHCl_3$);

^{1}H NMR (500 MHz, $CDCl_3$) δ 1.29, 1.32, 1.43, 1.45 (4s, 12H, 4 × CH_3), 2.58–2.68 (m, 4H, CH_2–CH_2), 3.05 (ddd, 1H, $J_{6'a,6'b}$ 13.9, $J_{6'a,5'}$ 8.8, $J_{6'a,NH}$ 3.5 Hz, H_a-6′), 3.51–3.56 (m, 1H, H-2), 3.60 (d, 2H, $J_{a,b}$ 13.1 Hz, 2 × CH_aPMB), 3.65 (d, 2H, $J_{b,a}$ 13.1 Hz, 2 × CH_bPMB), 3.66 (s, 3H, CH_3OCO), 3.67–3.80 (m, 10H, H_b-6′, H-5′, 2 × OCH_3, 2 × H-3), 4.13 (dd, 1H, $J_{4',3'}$ 7.8, $J_{4',5'}$ 1.6 Hz, H-4′), 4.27 (dd, 1H, $J_{2',1'}$ 4.9, $J_{2',3'}$ 2.5 Hz, H-2′), 4.44 (s, 2H, CH_2Ph), 4.56 (dd, 1H, $J_{3',4'}$ 7.8, $J_{3',2'}$ 2.5 Hz, H-3′), 5.46 (d, 1H, $J_{1',2'}$ 4.9 Hz, H-1′), 5.51 (d, 1H, $J_{1,2}$ 4.3 Hz, H-1), 6.54 (dd, 1H, $J_{NH,6'b}$ 8.0, $J_{NH,6'a}$ 3.5 Hz, NH), 6.82 (d, 4H, J_{ortho} 8.7 Hz, ArH), 7.27 (d, 4H, J_{ortho} 8.7 Hz, ArH), 7.30–7.39 (m, 5H, PhH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.8, 25.4, 26.1, 26.4, 29.2, 29.6, 39.6, 52.2, 54.4, 55.6, 58.3, 67.2, 67.8, 71.0, 71.2, 71.9, 72.9, 73.3, 96.6, 109.2, 109.8, 114.0, 127.9, 128.1, 128.7, 130.5, 132.1, 138.7, 159.0, 169.4, 171.1, 173.1. Anal. Calcd for $C_{44}H_{56}N_2O_{13}$: C, 64.38; H, 6.88; N, 3.41. Found: C, 64.19; H, 6.85; N, 3.43.

5.7. Methyl 3-{[(1*S*,2*S*)-1-[(benzyloxy)methyl]-2-hydroxy-2-([1,2:3,4-di-*O*-isopropylidene-6-deoxy-α-L-galactopyranos-6-yl]carbamoyl)ethyl]carbamoyl}propanoate (11)

A solution of CAN (4.9 mL, 2.9 mmol) in H_2O was added to a stirring solution of compound **10** (0.48 g, 0.58 mmol) in MeCN at 0 °C. The reaction was kept to 0 °C for 1 h and then was allowed to warm to rt. After 18 h, the reaction mixture was quenched by the addition of a saturated $NaHCO_3$ solution (50 mL) and extracted with $CHCl_3$. The organic layer was washed with brine until neutral, dried (Na_2SO_4), and concentrated under reduced pressure. The residue, after chromatography on silica gel ($CHCl_3$/MeOH, 95:5), gave the oily product **11** (0.20 g, 90%). $[\alpha]_D^{25}$ −11.0 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.31, 1.33, 1.45, 1.47 (4s, 12H, 4 × CH_3), 2.50 (t, 2H, $J_{1,2}$ 6.8 Hz, CH_2–CH_2), 2.65 (t, 2H, $J_{2,1}$ 6.8 Hz, CH_2–CH_2), 3.28 (ddd, 1H, $J_{6'a,6'b}$ 13.9, $J_{6'a,5'}$ 8.8, $J_{6'a,NH}$ 4.0 Hz, H_a-6′), 3.61–3.68 (m, 5H, H_b-6′, CH_aPMB, CH_3OCO), 3.84–3.90 (m, 2H, H-5′, CH_bPMB), 4.16 (dd, 1H, $J_{4',3'}$ 7.9, $J_{4',5'}$ 1.8 Hz, H-4′), 4.23 (br s, 1H, H-1), 4.30 (dd, 1H, $J_{2',1'}$ 4.9, $J_{2',3'}$ 2.4 Hz, H-2′), 4.35–4.40 (m, 1H, H-2), 4.48 (d, 1H, $J_{a,b}$ 11.7 Hz, CH_aPh), 4.52 (d, 1H, $J_{b,a}$ 11.7 Hz, CH_bPh), 4.59 (dd, 1H, $J_{3',4'}$ 7.9, $J_{3',2'}$ 2.4 Hz, H-3′), 5.51 (d, 1H, $J_{1',2'}$ 4.9 Hz, H-1′), 6.31 (br d, 1H, $J_{NH,1}$ 6.8 Hz, NH_{serine}), 7.22–7.26 (m, 1H, $NH_{galactosamine}$), 7.30–7.40 (m, 5H, PhH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.8, 25.3, 26.3, 26.4, 29.6, 31.2, 40.0, 52.2, 53.7, 66.6, 69.7, 70.9, 71.2, 72.1, 73.5, 96.7, 109.1, 109.9, 128.2, 128.4, 128.9, 137.8, 171.9, 173.3, 173.6. Anal. Calcd for $C_{28}H_{40}N_2O_{11}$: C, 57.92; H, 6.94; N, 4.82. Found: C, 58.14; H, 6.91; N, 4.84.

5.8. Methyl 3-{[(1*S*,2*S*)-2-hydroxy-1-(hydroxymethyl)-2-([1,2:3,4-di-*O*-isopropylidene-6-deoxy-α-L-galactopyranos-6-yl]carbamoyl)ethyl]carbamoyl}propanoate (12)

A solution of compound **11** (0.20 g, 0.35 mmol) in MeOH (4 mL) was added to a stirring suspension of 5% palladium on carbon (0.07 g) in the same solvent (5 mL) and then was hydrogenated (1 atm) at 40 °C. The flask was immersed in an ultrasound cleaning bath filled with water and sonicated for 22 h. Then the suspension was filtered through Celite® and the solid washed twice with MeOH (2 × 5 mL). The organic phase was evaporated down under reduced pressure to afford the oily product **12** (0.16 g, 93%). $[\alpha]_D^{25}$ +0.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.33, 1.36, 1.47, 1.49 (4s, 12H, 4 × CH_3), 2.51–2.57 (m, 2H, CH_2–CH_2), 2.63–2.69 (m, 2H, CH_2–CH_2), 3.37 (ddd, 1H, $J_{6'a,6'b}$ 13.4, $J_{6'a,5'}$ 8.3, $J_{6'a,NH}$ 4.4 Hz, H_a-6′), 3.65–3.72 (m, 4H, H_b-6′, CH_3OCO), 3.74 (dd, 1H, $J_{Ha,Hb}$ 11.9, $J_{Ha,1}$ 4.9 Hz, CH_aOH), 3.87 (ddd, 1H, $J_{5',6'a}$ 8.3, $J_{5',6'b}$ 3.9, $J_{5',4'}$ 1.9 Hz, H-5′), 4.05 (dd, 1H, $J_{Hb,Ha}$ 11.9, $J_{Hb,1}$ 1.5 Hz, CH_bOH), 4.15–4.18 (m, 1H, H-1), 4.19 (dd, 1H, $J_{4',3'}$ 7.8, $J_{4',5'}$ 1.9 Hz, H-4′), 4.27–4.30 (m, 1H, H-2), 4.31 (dd, 1H, $J_{2',1'}$ 4.9, $J_{2',3'}$ 2.4 Hz, H-2′), 4.61 (dd, 1H, $J_{3',4'}$ 7.8, $J_{3',2'}$ 2.4 Hz, H-3′), 5.20 (br s, 1H, OH), 5.50 (d, 1H, $J_{1',2'}$ 4.9 Hz, H-1′), 5.90 (br s, 1H, OH), 6.77 (d, 1H, $J_{NH,1}$ 6.3 Hz, NH_{serine}), 7.54–7.64 (m, 1H, $NH_{galactosamine}$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 22.7, 23.2, 24.3, 27.4, 28.0, 28.7, 38.1, 50.2, 54.0, 60.5, 64.4, 68.8, 69.1, 70.1, 75.5, 94.6, 107.1, 107.9, 171.6, 173.2, 173.4. Anal. Calcd for $C_{21}H_{34}N_2O_{11}$: C, 51.42; H, 6.99; N, 5.71. Found: C, 51.28; H, 6.96; N, 5.73.

5.9. 3-{[(1*S*,2*S*)-2-Hydroxy-1-(hydroxymethyl)-2-([1,2:3,4-di-*O*-isopropylidene-6-deoxy-α-L-galactopyranos-6-yl]carbamoyl)ethyl]carbamoyl}propanoic acid (13)

One molar solution of aq NaOH (0.78 mL, 0.78 mmol) was added to a stirring solution of compound **12** (0.13 g, 0.26 mmol) in MeOH (13 mL) at 0 °C. The reaction mixture was allowed to warm slowly to rt and after 3 h (TLC monitoring; $CHCl_3$/CH_3OH, 8:2) was quenched with some drops of acetic acid until neutral. The precipitate was filtered and the organic phase was evaporated under reduced pressure to afford the oily product **13** (0.11 g, 91%). $[\alpha]_D^{25}$ +9.0 (*c* 0.8, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD) δ 1.33, 1.36, 1.43, 1.47 (4s, 12H, 4 × CH_3), 2.40–2.52 (m, 4H, CH_2–CH_2), 3.35 (dd, 1H, $J_{6'a,6'b}$ 14.0, $J_{6'a,5'}$ 8.1 Hz, H_a-6′), 3.48 (dd, 1H, $J_{6'b,6'a}$ 14.0, $J_{6'b,5'}$ 4.4 Hz, H_b-6′), 3.61–3.66 (m, 2H, CH_2OH), 3.94–3.99 (m, 1H, H-5′), 4.20 (br d, 1H, $J_{2,1}$ 3.7 Hz, H-2), 4.24–4.31 (m, 2H, H-4′, H-1), 4.34 (dd, 1H, $J_{2',1'}$ 5.0, $J_{2',3'}$ 2.5 Hz, H-2′), 4.63 (dd, 1H, $J_{3',4'}$ 8.0, $J_{3',2'}$ 2.5 Hz, H-3′), 5.48 (d, 1H, $J_{1',2'}$ 5.0 Hz, H-1′); ^{13}C NMR (125 MHz, CD_3OD)

δ 23.2, 24.5, 25.1, 26.3, 34.0, 34.6, 40.7, 55.3, 61.1, 67.4, 71.9, 72.2, 72.9, 73.2, 97.8, 109.9, 110.5, 174.7, 176.3, 181.0. Anal. Calcd for $C_{20}H_{32}N_2O_{11}$: C, 50.42; H, 6.77; N, 5.88. Found: C, 50.28; H, 6.79; N, 5.89.

5.10. 3-{[(1*S*,2*S*)-2-Hydroxy-1-(hydroxymethyl)-2-([6-deoxy-L-galactopyranos-6-yl]carbamoyl)ethyl]carbamoyl}propanoic acid (2)

A solution of glycoaminoacid **13** (0.11 g, 0.23 mmol) in TFA/H_2O (9:1, 5 mL) was stirred at rt. After 3 h, the reaction solvent was evaporated under reduced pressure to afford a crude that was purified by Sephadex G-10 column leading to the mimic **2** as a mixture of β and α anomers in 2:1 ratio (0.067 g, 80%). ^{1}H NMR (500 MHz, D_2O) δ 2.33–2.41 (m, 4H, CH_2–CH_2), 3.30–3.38 (m, 2.66H, H-2′β, 2 × H-6′β, 2 × H-6′α), 3.52 (dd, 0.66H, $J_{3',2'}$ 10.0, $J_{3',4'}$ 3.3 Hz, H-3′β), 3.53–3.60 (m, 2H, CH_2OH), 3.63 (br dd, 0.66H, $J_{5',6'a}$ 7.4, $J_{5',6'b}$ 5.0 Hz, H-5′β), 3.68 (dd, 0.34H, $J_{2',3'}$ 10.3, $J_{2',1'}$ 3.6 Hz, H-2′α), 3.72 (br dd, 0.34H, $J_{3',2'}$ 10.3, $J_{3',4'}$ 3.1 Hz, H-3′α), 3.76 (br d, 0.66H, $J_{4',3'}$ 3.3 Hz, H-4′β), 3.82 (br d, 0.34H, $J_{4',3'}$ 3.1 Hz, H-4′α), 3.96–4.02 (m, 0.34H, H-5′α), 4.11–4.23 (m, 2H, H-1, H-2), 4.43 (d, 0.66H, $J_{1',2'}$ 7.8 Hz, H-1′β), 5.12 (d, 0.34H, $J_{1',2'}$ 3.6 Hz, H-1′α); ^{13}C NMR (125 MHz, D_2O) δ 31.5, 32.5, 41.7 (C-6′β), 41.8 (C-6′α), 55.7 (C-2), 61.8 (CH_2OH), 70.5 (C-2′α, C-5′α), 71.3 (C-3′α), 71.4 (C-4′β), 71.8 (C-4′α), 73.6 (C-1), 74.1 (C-2′β), 74.9 (C-3′β, C-5′β), 94.6 (C-1′α), 98.8 (C-1′β). Anal. Calcd for $C_{14}H_{24}N_2O_{11}$: C, 42.43; H, 6.10; N, 7.07. Found: C, 42.30; H, 6.09; N, 7.09.

5.11. Computational methods

Molecular dynamics simulations were performed using the AMBER 7.0 suite programs[20] using the SANDER module. The simulated structure **2** was built up by module XLEAP of AMBER using the AMBER force field PARM99.[21] The GLYCAM 2000 parameters[22,23] were implemented for the oligosaccharide simulations. The charges were taken from ab initio calculations performed by GAUSSIAN 98 molecular modelling package,[24] using the Hartree–Fock method with 6-31G* basic set. The MD simulation of **2** were performed with a time step of 1 fs in a cubic box of water 8.0 Å to the side containing 1102 TIP3P water molecules.[25] After heating and equilibration for 50 ps the simulations were performed for 10 ns, under periodic boundary conditions, at constant pressure (1 atm), and constant temperature (300 K). Energy minimizations and MD simulations were performed with a dielectric constant of unity, and a cut-off value for non-bonded interactions of 8 Å. The 1–4 electrostatic and van der Waals interactions were scaled by the standard values (SCEE = 1.2, SCNB = 2.0).

Post-processing of the trajectories (torsional, distance and energy analysis) was performed using the CARNAL module of AMBER 7.0 package.

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