Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 1999–2013

BIOORGANIC & MEDICINAL CHEMISTRY

Oligomers of Glycamino Acid

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Received 21 May 2001; accepted 5 December 2001

Abstract—Glycamino acids, a family of sugar amino acids, are derivatives of *C*-glycosides that possesses a carboxyl group at the *C*-1 position and an amino group replacing one of the hydroxyl groups at either the *C*-2, 3, 4, or 6 position. We have prepared a series of glucose-type glycamino acids as monomeric building blocks: these are derivatives of 2-NH_2-Glc-β-CO_2H **1**, 3-NH_2-Glc-β-CO_2H **2**, 4-NH_2-Glc-β-CO_2H **3**, and 6-NH_2-Glc-β-CO_2H **4** and constructed four types of homo-oligomers, β(1→2)-linked **I**, β(1→3)-linked **II**, β(1→4)-linked **III**, and β(1→6)-linked **IV**, employing the well-established *N*-Boc and BOP strategy. CD and NMR spectral studies of these oligomers suggested that only the β(1→2)-linked homo-oligomer possessed a helical structure that seems to be predetermined by the linkage position. Homo-oligomers with β(1→2)-linkages **I** and β(1→6)-linkages **IV** were also subjected to *O*-sulfation, and these *O*-sulfated oligomers were found to be able, in a linkage-specific manner, to effectively inhibit L-selectin-mediated cell adhesion, HIV infection, and heparanase activity without the anticoagulant activity associated with naturally occurring sulfated polysaccharides such as heparin.

Introduction

Considerable interest has been devoted to designing and constructing a new class of peptide molecules with the expectation that these molecules may provide stable alternatives to the biologically important natural α-peptides,[1] and a variety of oligomers have been prepared and studied with respect to their conformations.[2–8] Among them, β-peptides are composed of β-amino acids, in which an amino and carboxyl groups are separated by an additional methylene group, and their oligomers have been found to form stable helical conformations as comparable to those in naturally occurring peptides.[2] β-Amino acid derivatives that have been designed and prepared are counterparts of the natural α-amino acids, derivatives of 2-amino-carboxyl cyclo-hexane and -pentane, and 3-carboxy piperidine;[4,5,7] the biological activities of some of these oligomers have already been evaluated.[4j,5g,7]

Carbohydrate-derived amino acids, or sugar amino acids (also called glycamino acids)[9,10] have also been developed as non-natural amino acid analogues with additional functionality on the molecules, that is a hydroxyl group. They possess both a carboxylate group at the anomeric position and an amino group replacing one of the OH groups of the sugar. Several oligomers composed of glycamino acid analogues have been prepared and characterized their conformation, and some have been found to assume a rigid conformation by NMR and CD analysis.[11–20]

As a part of our ongoing program for developing a new class of molecules that possess the structural and functional features of both carbohydrates and peptides, we have reported the first homo-oligomers of glucose-based glycamino acids in which the glucose-based glycamino acid residues are connected via

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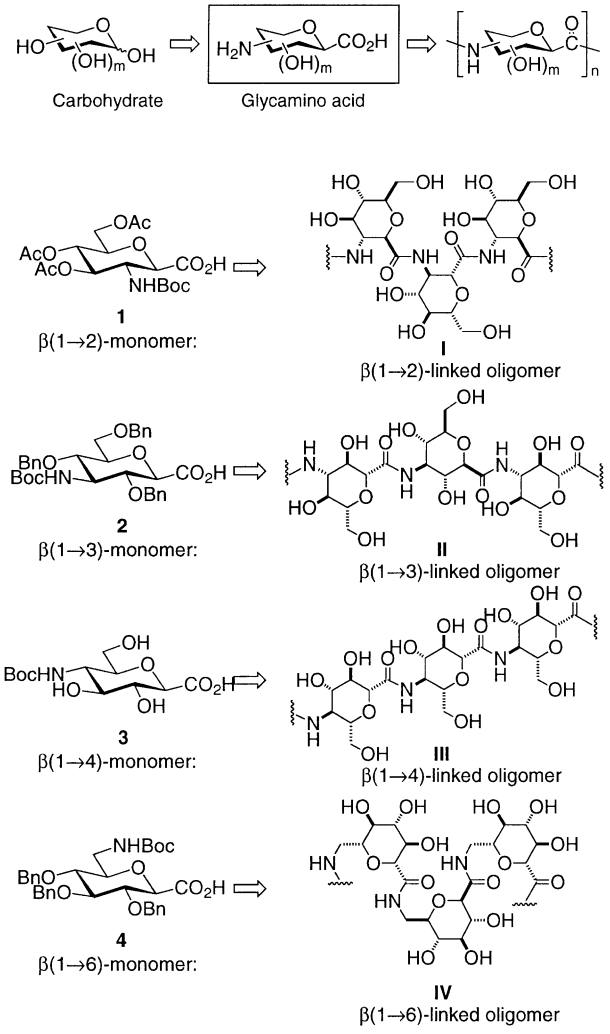
0968-0896/02/$ - see front matter
PII: S0968-0896(02)00020-2

β(1→2)-* (**I**)[21] and β(1→6)-linkages (**IV**).[22] We found that upon *O*-sulfation the amido-linked glycamino acid oligomers were able to inhibit the replication of HIV-1 and sialyl Lewis x-dependent cell adhesion. Because the β(1→2)-type glycamino acid can be recognized as a β-amino acid,[2a] we expected that the oligomer would show a rigid secondary conformation. We also wondered whether other type of linkages, such as β(1→3)- (**II**), β(1→4)- (**III**), and β(1→6)-linked (**IV**) oligomers might form a particular rigid conformation, and we therefore synthesized all the four types of oligomers of glucose-type glycamino acids (**I**–**IV**). We describe herein the detailed synthesis of monomers of the β(1→3)- and β(1→4)-glycamino acids and their oligomers and present the result of our biological evaluation and our conformational studies using CD and NMR spectroscopy.

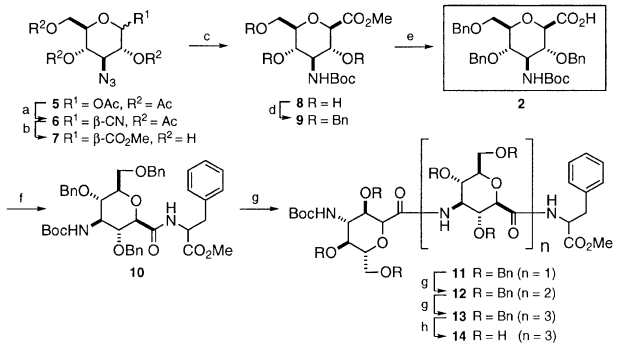


Results

Synthesis of β(1→3)-linked homooligomer 14 (Scheme 1)

The β(1→3)-monomer **2** was synthesized from a known 3-azido glucose derivative **5**.[23] Bromination[24] of **5** with $TiBr_4$ gave the glycosyl bromide, which was purified by silica gel chromatography (hexane–EtOAc 3:1) and immediately used for glycosylation with $Hg(CN)_2$[†] to afford the β-glycosyl cyanide **6** in 57% yield. Sequential treatment of **6** with: (i) NaOMe in MeOH and (ii) NaOH in water gave the *C*-1 carboxylate derivative,[25] which was purified with Dowex 1 [OH^-] resin and eluted with 2 N AcOH. Esterification of the free carboxylate was accomplished by dissolving the product in refluxing MeOH,[26] to afford the methyl ester **7** in 53% overall yield. The azido group of **7** was reduced with H_2S and the resulting amino group was protected with a Boc group (BocON[27] in MeOH) to give the *N*-Boc derivative **8**. The free OH groups of **8** were benzylated with NaH and BnBr, and the reaction products were treated with NaOMe (in order to convert the concomitant benzyl ester to the methyl ester) to give **9** in 49% overall yield. The methyl ester group of **9** was hydrolyzed with LiOH[28] to afford the β(1→3)-monomer **2** in 97% yield.

The oligomers were assembled in a manner similar to that used for the synthesis of the β(1→2)-linked oligomers:[21] The *C*-terminus of **2** was blocked with L-phenylalanine



Scheme 1. (a) (i) $TiBr_4$, CH_2Cl_2/EtOAc, 12 h, 0 °C to rt, 62%; (ii) $Hg(CN)_2$, $MeNO_2$, 1 h, 80 °C, 57%; (b) (i) 25% NaOMe, MeOH, 1 h, rt; (ii) NaOH, H_2O, 12 h, reflux; (iii) MeOH, 12 h, reflux, 53% overall; (c) (i) H_2S gas, pyridine/H_2O (1:1), 2 h, 0 °C then 12 h, rt; (ii) 5 equiv of Boc_2O, 5 equiv of Et_3N, MeOH, 12 h, rt, 77% overall yield; (d) (i) 6 equiv of BnBr, 4.5 equiv of 60% NaH, DMF, 16 h, rt; (ii) 25% NaOMe, MeOH, 30 min, rt, 49% overall; (e) 2 equiv of LiOH·H_2O, MeOH/THF/H_2O (3:3:1), 20 min, rt, 90%; (f) 1.2 equiv of L-phenylalanine methyl ester, 1.5 equiv of BOP, 3 equiv of DIEA, 16 h, 0 °C to rt, 55%; (g) (i) 2 N HCl in EtOAc, 3 h, 0 °C to rt; (ii) 1.2 equiv of **2**, 1.5 equiv of BOP, 3 equiv of BOP, 3 equiv of DIEA, 16 h, 0 °C to rt; (h) 20% $Pd(OH)_2$, H_2, MeOH, 16 h, rt, 85%.

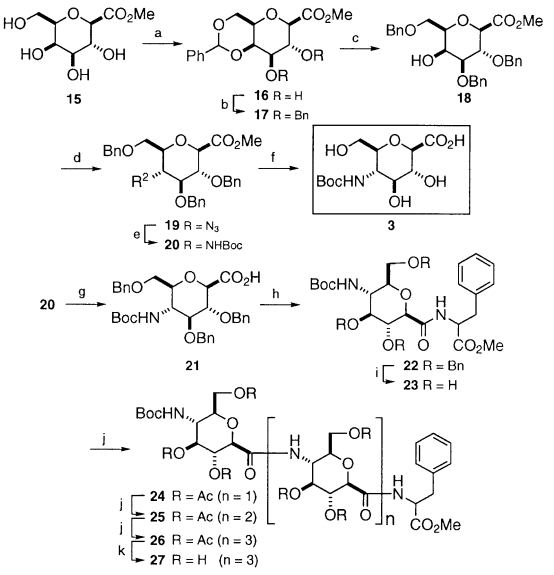
*In this text, we have used a nomenclature convention in which glycamino acids and their synthetic precursors are named as derivatives of the parent sugar [i.e., (2,4,6-tri-*O*-benzyl-3-*tert*-butoxycarbonylamido-3-deoxy-β-D-glucopyranosyl)-*C*-carboxylic acid for compound **2**), and accordingly the numbering of the skeletal carbons is based on that of the parent sugar. This convention is recommended for our present purposes because higher-carbon sugar names are not easily applied to describe the relationship between the structure of the compound and its IUPAC names provided in the Experimental. Compound **2** would be named, in another proper convention, as 2,6-anhydro-3,5,7-tri-*O*-benzyl-4-*tert*-butoxycarbonylamido-4-deoxy-D-glycero-D-gulo-hepturonic acid.

†A commercially available $Hg(CN)_2$ was pretreated for the glycosyl cyanation by: (1) co-evaporation from a suspension of $Hg(CN)_2$ in toluene three times and (2) drying under vacuum for 2 h. Without this pretreatment, it was difficult to obtain the product.

methyl ester in the presence of benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent)[29] to give **10** in 55% yield. Removal of the Boc group and subsequent coupling with the monomeric **2** gave, sequentially, the protected forms of the dimer **11**, the trimer **12**, and the tetramer **13** in good yields. *O*-Benzyl groups of **13** were removed by hydrogenolysis to afford the OH-free tetramer **14** in 85% yield after purification by a Sephadex column chromatography, with a water elution.

Synthesis of β(1→4)-linked homooligomer 27[30] (Scheme 2)

The β(1→4)-monomer **3** was prepared from a known (β-D-galactopyranosyl)-*C*-carboxylic acid methyl ester (methyl 2,6-anhydro-D-glycero-L-manno-hepturonate) **15**.[31] Benzylidenation of **15** with benzaldehyde and formic acid[32] gave a benzylidene derivative **16**[33] (65% yield) which was then benzylated to give **17**. Reductive opening of the benzylidene group[34] with HCl–$Na(CN)BH_3$ gave the 2,3,6-tri-*O*-benzyl derivative **18** in 80% yield. Sequential treatment of **18** with: (i) trifluoromethanesulfonic anhydride (Tf_2O) and (ii) NaN_3 afforded the 4-azido derivative with a gluco-configuration **19**. The azido group of **19** was reduced with H_2S and protected with a Boc group to give **20** in 97% overall yield. Since we have experienced difficulty in coupling *O*-benzylated monomer **21**, we decided to perform the assembly of the oligomer using the monomeric derivative **3** with the non-protected OH groups, which was obtained from **20** by hydrogenolysis.



Scheme 2. (a) PhCHO, HCO_2H, 1 h, rt, 65%; (b) (i) 2.2 equiv of 60% NaH, 3 equiv of BnBr, DMF, 12 h, rt; (ii) 25% NaOMe, MeOH, 1 h, rt, 93% overall; (c) 6 equiv of $Na(CN)BH_3$, MS3A, HCl satd EtOAc, THF, 20 min, rt, 80%; (d) (i) Tf_2O, pyridine, CH_2Cl_2, 2 h, −5 °C; (ii) NaN_3, DMF, 12 h, rt, 60% overall; (e) (i) H_2S gas, pyridine/H_2O (1:1), 2 h, 0 °C then 12 h, rt; (ii) 10 equiv of Boc_2O, 9 equiv of Et_3N, MeOH, 12 h, rt, 97% overall; (f) (i) 20% $Pd(OH)_2$, H_2, THF/MeOH/H_2O (5:5:1), rt, 12 h; (ii) 2 equiv of LiOH, THF/MeOH/H_2O (3:3:1), 20 min, rt, 90% overall; (g) 2 equiv of LiOH, THF/MeOH/H_2O (3:3:1), 20 min, rt, 93%; (h) (i) 1.2 equiv of L-phenylalanine methyl ester, 2 equiv of BOP, 3 equiv of DIEA, DMF, 16 h, 0 °C to rt, 65%; (ii) 20% $Pd(OH_2)$, H_2, THF/MeOH/H_2O (5:5:1), rt, 12 h, 90%; (j) (i) 2 N HCl in EtOAc, 3 h, 0 °C to rt; (ii) 1.2 equiv of **3,** 2 equiv of BOP, 3 equiv of DIEA, DMF, 16 h, rt; (iii) Ac_2O, pyridine, 12 h, rt, 73% overall; (k) 25% NaOMe, MeOH (pH 11), 2 h, rt.

The first coupling with L-phenylalanine methyl ester was carried out with the benzylated monomer **21**, prepared by alkaline hydrolysis of the methyl ester of **20**, to give **22**, which was then hydrogenolyzed to give **23**. Removal of the Boc group and coupling with the OH free monomer **3** gave **24**, after purification, as the *O*-acetyl derivative. This sequence was repeated to give the *O*-acetylated derivatives of a trimer **25** and a tetramer **26** in good yields. *O*-Deacetylation of **26** with NaOMe in MeOH afforded the tetramer **27**.

Conformational analysis of the glycamino acid homooligomers

Circular dichroism (CD) techniques are extensively used to analyze the secondary structure of α-amino acid polypeptides.[35] In the case of non-α-peptides, efforts have been made to correlate CD spectra with types of secondary structure[36] using other structural data obtained by NMR or X-ray crystallography. We first examined CD spectra of all the oligomers with different linkages to see whether any of them had a rigid secondary structure and then used NMR measurement to further study their conformation.

Circular dichroism (CD). Figure 1a shows CD spectra for a series of β(1→2)-homo-oligomers. All of the β(1→2)-oligomers except the dimer displayed minima ($[\theta] = -8\times10^3 \sim -3\times10^3$) between 215 and 225 nm and maxima ($[\theta] = -2\times10^3 \sim 5\times10^3$) at 200 nm. For the β(1→2)-hexamer, molar ellipticity is 4×10^3 at 200 nm and -7×10^3 at 220 nm. The observed CD pattern of the β(1→2)-oligomers was quite similar to that of the 14-helical β-peptides reported by Seebach et al.,[5a,b,d] suggesting that the β(1→2)-oligomers do indeed adopt a 14-helical structure. However, the magnitudes of the molar ellipticity of the β(1→2)-oligomers were rather small when compared to the reported values; this finding suggests that β(1→2)-oligomers may equilibrate between 14-helix and unfolded conformation.

The β(1→3)-oligomers produced CD spectra with a negative band at about 205 nm (Fig. 1b). The CD spectra of the β(1→4)-oligomers had a positive band at about 218 nm and a negative band at 195 nm (Fig. 1c). The β(1→6)-oligomers had CD spectra with positive bands at about 198 and 218 nm and a negative band at 190 nm (Fig. 1d). From these CD studies, we concluded that among the glycamino acid oligomers, only the β(1→2)-oligomers likely possess a 14-helical structure.

Nuclear magnetic resonance (NMR). Further conformational studies by NMR were carried out for the β(1→2)-oligomers. The β(1→2)-oligomers were dissolved in pyridine-d_5 or H_2O/D_2O, with the former giving a better dispersion of the NMR signals. NMR signal assignments were established by DOF-COSY, TOCSY, ROESY, ^1H–^{13}C HSQC, and ^1H–^{13}C HSQC-TOCSY experiments.

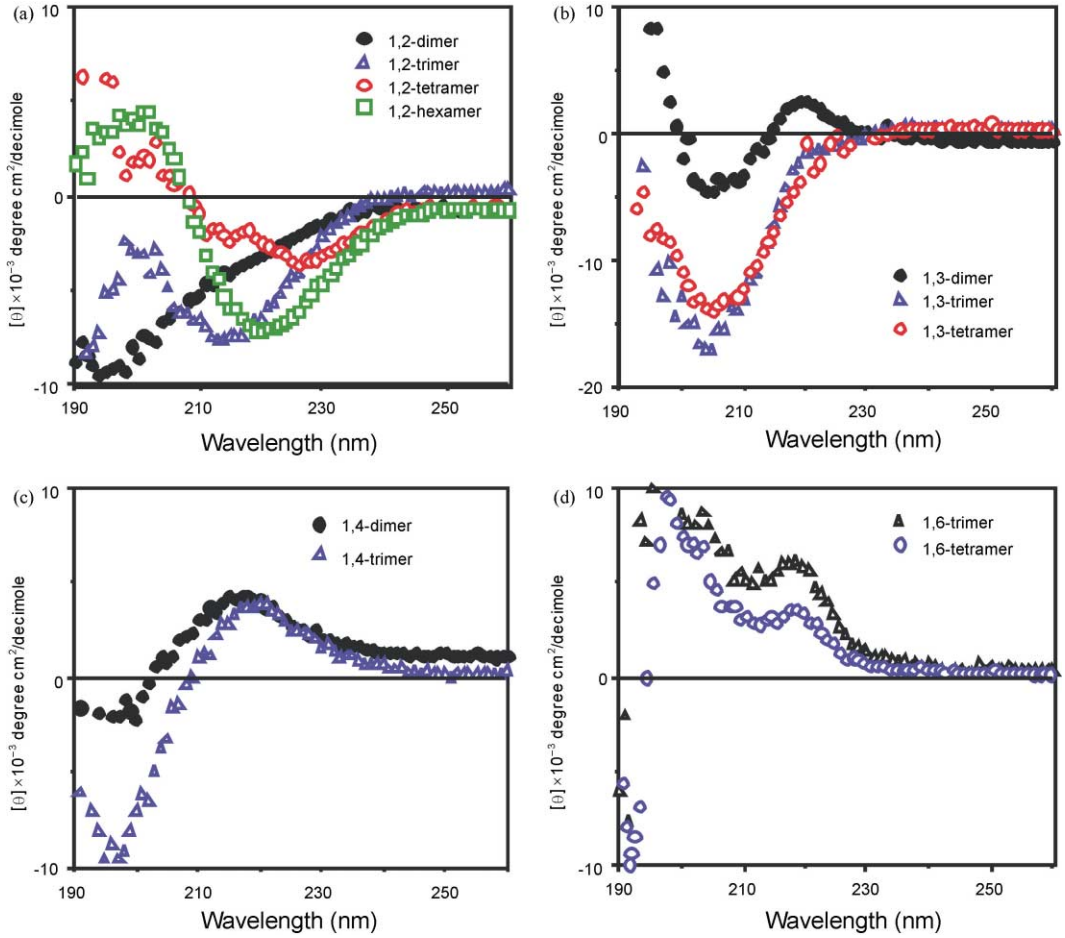


Figure 1. (a) CD spectra of β(1→2)-dimer, trimer, tetramer, and hexamer; (b) CD spectra of β(1→3)-dimer, trimer, and tetramer; (c) CD spectra of β(1→4)-dimer and trimer; (d) CD spectra of β (1→6)-trimer and tetramer.

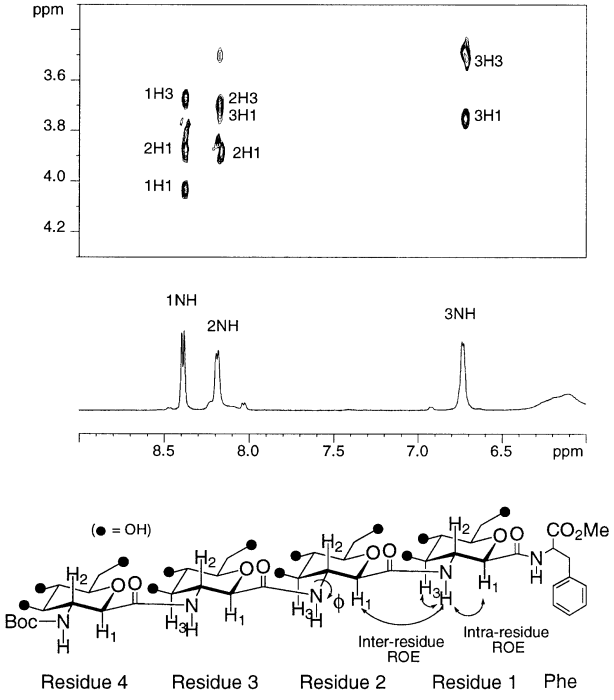


Figure 2. A part of ROESY spectrum of β(1→2) trimer in H_2O at 303 K.

We first observed ^3J values for the coupling between NH and H2 protons in the β(1→2)-oligomers in H_2O because ^3J coupling constants between NH and H2 protons provide information about the dihedral angles (∅) (Fig. 2). NH protons of the β(1→2)-oligomers exhibited large J values ranging from 8.5 to 9.6 Hz, which corresponds to a nearly anti-periplanar arrangement of N–H and Cβ–H (H2). Large ^3J(NH,H2) values were also reported for the 14-helical β-peptide.[5b,d]

We next examined long-rang ROE cross-peaks to define a 14-helical structure. Figure 2 shows the ROESY spectrum observed for the β(1→2)-trimer. In this spectrum, the inter-residue [NH(i)–H1($i+1$)] and intra-residue [NH(i)-H3(i)/H1(i)] cross-peaks were observed. The intensities of the inter- and intra-residue ROE cross-peaks were almost same in both solvents (H_2O and pyridine-d_5), suggesting that the β(1→2)-trimer assumes a 14-helical conformation (see Fig. 2 and Table 1). However, no long-range ROE cross-peaks, which are used to identify the 14-helix in β-peptides,[4f,5a,b,d] could be observed, probably because an equilibrium between the 14-helix and unfolded conformations. For the β(1→2)-tetramer, similar ROE data were obtained (Table 1). Therefore, these ROE data revealed that the β(1→2)-trimer and tetramer in solution tends to assume a 14-helical conformation.[37]

Table 1. ROE cross-peak intensities of intra-residue ROE (NH(i) H1(i)H3(i) and inter-residue ROE (NH(i) ↔ H2(i+1↔) for β(1→2) trimer and tetramer[a]

	Trimer	Tetramer
(in pyridine)		
1NH ↔ 1H1	20	12
1NH ↔ 1H3	42	30
1NH ↔ 2H1	37	30
2NH ↔ 2H1	24	16
2NH ↔ 2H3	18	42
2NH ↔ 3H1	16	32
3NH ↔ 3H1		12
3NH ↔ 3H3		—
3NH ↔ 4H1		10
(in H_2O)		
1NH ↔ 1H1	18	—[b]
1NH ↔ 1H3	24	—
1NH ↔ 2H1	16	—
2NH ↔ 2H1	31	—
2NH ↔ 2H3	38	—
2NH ↔ 3H1	26	—
3NH ↔ 3H1		—
3NH ↔ 3H3		—
3NH ↔ 4H1		—

[a]Values are in arbitary unit.
[b]Analysis of β(1→2) tetramer in H_2O was hampered due to signal overlap in ROESY spectrum.

In order to gain insight into intramolecular hydrogen-bonding patterns, we examined the temperature dependence of amide proton chemical shifts. Data obtained for the β(1→2)-tetramer are shown in Table 2. These data suggest the existence of an intramolecular hydrogen bond for the NH of residues 2 and 3. In contrast, NH temperature coefficients for the other β(1→3)-oligomers ranged from 14.8 to 22.8, suggesting that the β(1→3)-oligomers did not form intramolecular hydrogen bonds (data not shown). Thus the β(1→3)-oligomers did not have well-defined conformation since the long range ROE was not found by NMR measurement, therefore, the conformation of β(1→3)-oligomers could be corresponding to the CD data. On the basis of the NMR and CD data, we concluded that β(1→2)-oligomers, except the dimer, assume a 14-helical conformation in solution.

Table 2. Temperature coefficient of amide protons of β(12) tetramer in pyridine-d_5[a]

NH (Phe)	12.0
1NH	12.8
2NH	7.1
3NH	6.3
4NH	14.3

[a]Values are in -ppb/K.

Biological evaluation of sulfated homo-oligomers as a stable analogue of the *O*-sulfated oligosaccharides in several assay systems. Sulfated oligosaccharides and polysaccharides are known to show a variety of biological activities in important biological systems.[38] However, these compounds are highly susceptible to glycosidase digestion and therefore have a very short half-lives. Since the glycamino acid-based oligomers are derivatives of *C*-glycosides and are not cleaved by glycosidases, we examined the effect of *O*-sulfation and evaluated the biological activities of glycamino acid-based oligomers in three biological assays. The β(1→2)- and β(1→6)-linked homo-oligomers were *O*-sulfated according to a published procedure,[39] and the degree of sulfation was estimated from the elemental analytical data.

Anti-HIV activity. It has been suggested that the anti-HIV activity of the *O*-sulfated oligosaccharides is highly dependent upon its degree of sulfation.[39] Sulfated polysaccharides such as heparin and dextran sulfate are known to inhibit HIV infection of T-cells,[40] but these compounds also show undesirable properties such as short half-life in circulation, poor bioavailability, and toxicity.[41] Therefore, a variety of *O*-sulfated oligosaccharides or anionic compounds have been prepared as a way of overcoming these drawbacks.[39–41]

Determination of the antiviral activity of the compounds was based on the inhibition of the expression of the HIV p24 protein in supernatant using a standard core ELISA assay, as described previously.[42] MT-2 cells are exposed to virus (HTLVRF) in the presence or absence of various concentrations of each of the synthesized analogues for 5 days at 37 °C. Viral production was then determined by ELISA. We observed that whereas the *O*-sulfated β(1→6)-linked tetramer was highly active in inhibiting HIV-1 infectivity, with an IC_{50} of 1 μM, the corresponding trimer showed no inhibitory activity up to 500 μM. In addition, the *O*-sulfated β(1→6)-linked tetramer was more potent than the β(1→2)-linked tetramer. It should be mentioned that because of their limited size, none of these compounds showed any heparin-like anticoagulant activity.

Inhibition of sialyl Lewis x selectin-mediated cell adhesion (Fig. 3). Because the interaction between sialyl Lewis x and selectins is now considered to be the first step in the intercellular adhesion leading to inflammation,[43] considerable effort has been devoted to developing an effective inhibitor of this process.

The inhibitory activity of these compounds against sialyl Lewis x-mediated cell adhesion was determined using an assay described previously:[44] COS cells expressing human E- or P-selectins were incubated with our synthesized compounds, and then incubated on surfaces bearing immobilized sialyl Lewis x-containing glycolipid. COS cells that bound to sialyl Lewis x were lysed and quantified spectrophotometrically. We observed that the *O*-sulfated β(1→2)- and β(1→6)-linked oligomers (up to tetramers) were weak inhibitors of E-selectin-dependent sialyl Lewis x adhesion; however, the *O*-sulfated β(1→2)-linked tetramer was an effective inhibitor of P-selectin-dependent adhesion, with an IC_{50} of 100 μM (Fig. 3). This observation can be explained by the fact that two binding sites have been suggested for P- and L-selectins: one site for sialyl Lewis x and the other for negatively charged carbohydrates.[45] In fact, sulfated oligosaccharides and tyrosine residues have been demonstrated to be ligands for P-selectin.[46]

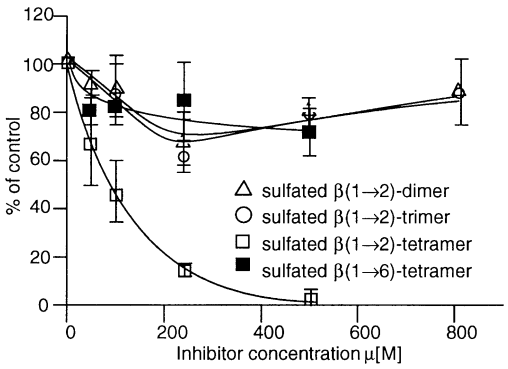


Figure 3. Inhibition of sialyl Lewis x and P-selectin-mediated cell adhesion with *O*-sulfated glycamino acid oligomers.

Inhibition of heparanase activity (Fig. 4). Since heparan sulfate proteoglycans are ubiquitous macromolecules and components of extracellular matrices and cell surfaces,[47] migration of T-cells and tumor cells is associated with the secretion of heparanase, which degrades heparan sulfate chains in the extracellular matrix.[48] Inhibition of heparanase activity by heparin fragments has been shown to decrease the heparanase-mediated infiltration of T-cells into inflammatory lesions[49] or of tumor cells into metastatic sites.[50] However, use of heparin fragments causes the unwanted side effect of blood anticoagulation.[41] It is therefore highly desirable to develop a compound that inhibits heparanase without producing such side effects.

Determination of the inhibitory activity of the compounds against heparanase was based on the inhibition of its hydrolase activity. $^{35}SO_4$-radiolabeled heparan sulfate proteoglycans[51] are incubated with a partially purified heparanase[52] and various concentrations of the *O*-sulfated amide-linked carbohydrate analogues or peptide–sugar hybrid analogues.[53] The incubation mixtures were then centrifuged through an Amicon® membrane filter (molecular weight cut-off 30,000), and the radioactivity in the filtrate (cleaved material) was determined to assess the inhibitory potency of the compounds. Among the *O*-sulfated carbohydrate analogues tested, the β(1→6)-linked tetramer was the most potent inhibitor of heparanase, with an IC_{50} of 0.6 μM; the β(1→6)-linked trimer (IC_{50}, 20 μM) and the β(1→2)-linked tetramer (IC_{50}, 10 μM) were less potent (Fig. 4).

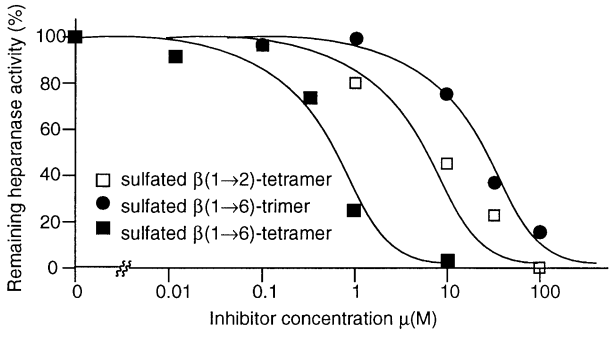


Figure. 4. Inhibition of heparanase activity with *O*-sulfated glycamino acid oligomers.

Our biological evaluation of oligomers of the glycamino acids as amide-linked oligosaccharide analogues has shown that the *O*-sulfated derivatives are equally or more potent anti-HIV agents than are the currently available anti-HIV agents that are sulfated derivatives of naturally occurring oligosaccharides. Furthermore, our compounds were found to be very potent inhibitors of heparanase, which is a key enzyme in cell migration, including tumor metastasis. In addition, we have shown that our designed β(1→2)-amide-linked analogues with *O*-sulfate groups are potent inhibitors of sialyl Lewis x-P-selectin-mediated cell adhesion. These biological activities were found to be dependent on the linkage position of the analogues. The *O*-sulfated derivatives of β(1→2)- and β(1→6)- linked oligomers that we have tested did not show any anticoagulant activity, which has been a major side effect of the *O*-sulfated natural-type oligosaccharide derivatives. Although we have not yet fully optimized the structure–activity relationships of these glycamino acid derivatives, our preliminary observations strengthen our hypothesis that these glycamino acid-based molecules have great potential for producing new and biologically effective compounds. These findings may also lead to the design of a new class of designed molecules: oligosaccharide and oligopeptide analogues with significant biological activities.

Conclusion

We have described the synthesis of a new class of oligomers composed of glycamino acids, which bear a carboxylate at the anomeric position and an amino group replacing one of the hydroxyl groups of the monosaccharide. On the basis of the CD and NMR spectral analyses, they were found to form rigid secondary structures, in particular the β(1→2)-linked oligomers, which were very likely to form a 14-helical structure. In addition, since some divalent metal cations such as Ca^{2+} are known to form a complex with the OH groups of carbohydrates and such complexes play an important role in carbohydrate-receptor interactions, it would be of considerable interest to study the solution conformation of these glycamino acid-based oligomers in the presence of divalent metals. Though it may be too early to discuss the usefulness of these glycamino acid-based oligomers as alternate analogues of biologically active peptides or oligosaccharides, we have observed that, upon *O*-sulfation, these oligomers effectively inhibited HIV infection, sialyl Lewis x-mediated cell adhesion, and heparanase activity in a linkage-specific manner without evidence of the anticoagulant activity that is characteristic of the sulfated derivatives of naturally occurring oligosaccharides.

Experimental

Melting points were determined with a Fisher–Johns melting point apparatus and are uncorrected. Elemental analyses were performed by Galbraith Laboratories Inc. (Knoxville, TN, USA). Mass spectra were measured by Mass Spectrometry Laboratory, University of Illinois at

Urbana-Champaign (Urbana, IL, USA). Organic extracts were dried over anhydrous $MgSO_4$, and were concentrated under diminished pressure. Chromatography was performed on a column of silica gel (60–200 mesh, Fisher Scientific), and thin-layer chromatography was conducted on precoated plates silica gel 60 F_{254} (Merck). Gel filtration was performed on a column of Sephadex G-25 (Pharmacia). ^1H and ^{13}C NMR spectra were recorded with a Bruker AMX 300 or a JEOL GX-400 spectrometer.

1,2,4,6-Tetra-*O*-acetyl-3-azido-3-deoxy-D-glucopyranose (5). Compound **5** was prepared from 3-azido-3-deoxy-1,2:5,6-di-*O*-isopropylidene-β-D-glucofuranose[23] by acid hydrolysis and acetylation; ^1H NMR (300 MHz, $CDCl_3$) δ 6.25 (1H, d, $J=3.5$ Hz, H-1α), 5.68 (1H, d, $J=8.2$ Hz, H-1β), 5.07–4.93 (4H, m), 4.27–4.19 (2H, m), 4.11–3.93 (4H, m), 3.82–3.76 (1H, m, H-5), 3.70 (1H, t, $J=10.0$ Hz, H-4); mass spectrum (FAB) *m*/*e* 396 ((M + Na^+), calcd for $C_{14}H_{19}N_3O_9Na$ 396), 314 ((M−OAc), calcd for $C_{12}H_{16}N_3O_7$ 314).

2,6-Anhydro-3,5,7-tri-*O*-acetyl-2,6-anhydro-4-azido-4-deoxy-D-*glycero*-D-*gulo*-heptononitrile (6). $TiBr_4$ (22.6 g, 616 mmol) was added portionwise to a cooled solution of **5** (11.5 g, 30.8 mmol) in CH_2Cl_2 (150 mL)–EtOAc (50 mL) at 0 °C, and the mixture was stirred for 36 h at room temperature. The reaction mixture was cooled and NaOAc (22.6 g) was added to the mixture, and the resulting mixture was stirred for 1 h at room temperature. The mixture was diluted with CH_2Cl_2 and was successively washed with water, satd $NaHCO_3$, and brine, dried, and concentrated. The residue was roughly chromatographed on SiO_2, with hexanes–EtOAc 3:1, to give the glycosyl bromide (7.21 g, 62%) as a yellow oil, which was used for the next step without further purification.

The bromide (7.21 g, 190 mmol) and $Hg(CN)_2$ (9.60 g, 380 mmol) were suspended in CH_3NO_2 (10 mL), and the resulting reaction mixture was heated for 1 h at 80 °C. The reaction mixture was diluted with $CHCl_3$ and the precipitate was filtered off. The filtrate was washed with N KBr and brine, dried, and concentrated. The residue was chromatographed on SiO_2, with toluene–EtOAc 10:1, to give **6** (3.70 g, 57%) as a white powder, mp 120–124 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 5.26 (1H, t, $J=10.2$ Hz, H-4), 5.00 (1H, t, $J=10.0$ Hz, H-3), 4.30 (1H, d, $J=10.2$ Hz, H-1), 4.21 (1H, dd, $J=4.9$, 12.7 Hz, H-6a), 4.12 (1H, dd, $J=2.4$, 10.2 Hz, H-6b), 3.72–3.67 (1H, m, H-5), 3.66 (1H, t, $J=9.9$ Hz, H-2), 2.21, 2.13, 2.10 (3H each, 3s, 3×CH_3CO); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ 170.6, 169.1, 168.6 (3×CH_3CO), 114.2 (CN), 68.9, 67.5, 66.8, 64.7, 61.6, 20.8, 20.6, 20.5 (3×CH_3CO); mass spectrum (FAB) *m*/*e* 341 ((M + H^+), calcd for $C_{13}H_{17}N_4O_7$ 341).

Methyl 2,6-anhydro-4-azido-4-deoxy-D-*glycero*-D-*gulo*-hepturonate (7). The pH of a solution of **6** (3.40 g, 9.99 mmol) in MeOH (100 mL) was adjusted to 11 with 25% NaOMe–MeOH, and the mixture was stirred for 1 h at room temperature and concentrated. Solid NaOH (336 mg, 8.40 mmol) was added to a solution of the residue in water (100 mL), and the mixture was refluxed for 12 h. After cooled, the mixture was neutralized with Dowex 50W-X8 [H^+] resin, and the resin was filtered off. The filtrate was diluted with water (1 L) and was applied onto a column of Dowex 1 [OH-]-form resin. The column was washed with water and the product was eluted out with 2 N AcOH. The fractions containing the product were pooled and concentrated. A solution of the residue in MeOH (100 mL) was refluxed for 12 h and concentrated. The residue was chromatographed on SiO_2, with hexanes–EtOAc 1:2 to EtOAc only, to give **7** (1.30 g, 53%) as a colorless oil; ^1H NMR (300 MHz, CD_3OD) δ 4.10 (1H, q, $J=1.2$, 13.2 Hz, H-5), 3.88 (1H, d, $J=9.5$ Hz, H-2), 3.82 (1H, dd, $J=1.3$, 14.4 Hz, H-7a), 3.77 (3H, s, CO_2CH_3), 3.65 (1H, dd, $J=1.0$, 11.1 Hz, H-7b), 3.49 (1H, t, $J=9.6$ Hz, H-4), 3.61–3.33 (1H, m, H-3); ^{13}C NMR (72.5 MHz, CD_3OD) δ 171.6 (CO_2CH_3), 82.2, 81.1, 72.2, 72.0, 69.7, 62.3 (C-1,2,3,4,5,6), 52.8 (CO_2CH_3); mass spectrum (FAB) *m*/*e* 248 ((M + H^+), calcd for $C_8H_{14}N_3O_6$ 248).

Methyl 2,6-anhydro-4-*tert*-butoxycarbonylamido-4-deoxy-D-*glycero*-D-*gulo*-hepturonate (8). H_2S gas was bubbled through a solution of **7** (1.40 g, 5.66 mmol) in pyridine (50 mL)–water (50 mL) for 2 h at room temperature. The mixture was stirred for another 12 h at room temperature and concentrated. BOC-ON (2.82 g, 11.3 mmol) was added to a solution of the residue and Et_3N (1.58 mL, 11.3 mmol) in MeOH (100 mL), and the mixture was stirred for 12 h at room temperature and concentrated. The residue was chromatographed on SiO_2, with $CHCl_3$–MeOH, to give **8** (1.02 g, 77%) as a white powder, mp 148–151 °C; ^1H NMR (300 MHz, CD_3OD) δ 4.10 (1H, dd, $J=7.1$, 14.2 Hz, H-5), 3.88 (1H, d, $J=9.3$ Hz, H-2), 3.84 (1H, m, H-7a), 3.76 (3H, s, CO_2CH_3), 3.65 (1H, dd, $J=4.5$, 12.2 Hz, H-7b), 3.57–3.44 (2H, m, H-3,4); ^{13}C NMR (72.5 MHz, CD_3OD) δ 171.9 (C-1), 159.1 (NHO*C*O*t*Bu), 82.9, 81.1, 71.7, 69.5, 62.6, 61.5 (C-1,2,3,4,5,6), 52.8 (CO_2CH_3), 28.8 (*t*Bu); mass spectrum (FAB) *m*/*e* 322 ((M + H^+), calcd for $C_{13}H_{24}NO_8$ 322), 222 ((M–Boc + $2H^+$), calcd for $C_8H_{16}NO_6$ 222).

Methyl 2,6-anhydro-3,5,7-tri-*O*-benzyl-4-*tert*-butoxycarbonylamido-4-deoxy-D-*glycero*-D-*gulo*-hepturonate (9). NaH (60% mineral oil dispersion; 359 mg, 8.96 mmol) was added to a cooled solution of **8** (800 mg, 2.49 mmol) in DMF (30 mL), and the mixture was stirred for 10 min at 0 °C. BnBr (1.33 mL, 11.2 mmol) was added to the mixture, and the mixture was stirred for 12 h at room temperature. The reaction mixture was carefully poured into ice water, and extracted with EtOAc. The extracts were successively washed with water and brine, dried, and concentrated. The residue, a mixture of the methyl ester and the corresponding benzyl ester, was treated with 25% NaOMe–MeOH (200 μL) in MeOH (20 mL) for 2 h at room temperature. The reaction mixture was neutralized with Dowex 50W-X8 [H^+] resin, and the resin was filtered off and the filtrate was concentrated. The residue was chromatographed on SiO2, with hexanes only to hexanes–EtOAc 5:1, to give

9 (920 mg, 64%), mp 150–153 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35–7.18 (15H, m, Ph), 4.72–4.46 (6H, m, 3×C*H*$_2$Ph), 3.95–3.84 (2H, m), 3.72 (3H, s, CO_2CH_3), 3.77–3.59 (4H, m), 3.48–3.44 (1H, m); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ 169.5 (C-1), 155.3 (NHO*C*O*t*Bu), 137.9, 137.7 (*C*Ph), 128.30, 128.28, 128.11, 128.02, 127.91, 127.88, 127.77, 127.61 (Ph), 80.4, 79.6, 78.9, 73.4, 68.9, 73.97, 73.92, 73.89 (CH_2Ph), 52.3 (CO_2CH_3), 28.3 (*t*Bu); mass spectrum (FAB) *m*/*e* 592 ((M + H$^+$), calcd for $C_{34}H_{42}NO_8$ 592), 492 ((M–Boc + 2H$^+$), calcd for $C_{29}H_{35}NO_6$ 492).

2,6-Anhydro-3,5,7-tri-*O*-benzyl-4-*tert*-butoxycarbonylamido-4-deoxy-D-*glycero*-D-*gulo*-hepturonic acid (2). A mixture of **9** (900 mg, 1.52 mmol) and LiOH·H_2O (128 mg, 3.05 mmol) in THF (15 mL)–MeOH (15 mL)–water (5 mL) was stirred for 20 min at room temperature. The reaction mixture was neutralized with Dowex 50W-X8 [H$^+$] resin, and the resin was filtered off. The filtrate was concentrated, and the residue was chromatographed on SiO_2, with $CHCl_3$–MeOH (10:1 to 5:1), to give **2** as a colorless amorphous (880 mg, 97%), mp 118–120 °C; ^{1}H NMR (300 MHz, CD_3Cl/CD_3OD 3:1) δ 7.33–7.10 (15H, m, Ph), 4.78–4.42 (6H, m, CH_2Ph), 4.12–3.43 (7H, m), 1.42 (9H, s, NHCO_2*tBu*); ^{13}C NMR (72.5 MHz, CD_3OD/$CDCl_3$ 3:1) δ 155.6 (NHO*C*O*t*Bu), 137.8 (*C*Ph), 129.9, 128.5, 128.4, 128.2, 127.9, 127.7, 127.0, 126.6 (Ph), 80.2, 79.4, 79.0, 78.7, 73.9, 73.5, 73.3 (CH_2Ph), 50.8 (CO_2CH_3), 28.4 (*t*Bu); mass spectrum (FAB) *m*/*e* 600 ((M + Na$^+$), calcd for $C_{33}H_{39}NO_8Na$ 600).

(2,4,6-Tri-*O*-benzyl-3-*tert*-butoxycarbonylamido-3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-*N*-L-phenylalanine methyl ester (10). Method A. DEPC (67.4 μL, 413 μmol) was added to a cooled solution of **2** (159 mg, 275 μmol), L-phenylalanine methyl ester (71.2 mg, 330 μmol), and Et_3N (115 μL, 826 μmol) in DMF (10 mL) at 0 °C, and the mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with EtOAc, and was successively washed with water, sat. $NaHCO_3$, and brine, dried, and concentrated. The residue was chromatographed on SiO_2, with toluene–EtOAc 5:1, to give **10** (75.2 mg, 37%) as a white powder.

Method B. To a cooled solution of **2** (166 mg, 287 μmol) in DMF (20 mL) were added BOP reagent (74.4 mg, 345 μmol) and DIEA (150 μL, 863 μmol), and the mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with EtOAc, and was successively washed with water, satd $NaHCO_3$, and brine, dried, and concentrated. The residue was chromatographed on SiO_2, with toluene–EtOAc 5:1, to give **10** (116 mg, 55%) as a white powder, mp 183–186 °C; ^{1}H NMR 300 MHz, $CDCl_3$) δ 7.36–7.05 (20H, m, Ph), 5.05 (1H, br s, NH), 4.80 (1H, dd, *J* = 6.3, 13.4 Hz, C*H*CO_2CH_3), 4.71–4.46 (6H, m, 3×C*H*$_2$Ph), 4.02 (1H, d, *J* = 5.9 Hz, H-2), 3.91–3.83 (3H, m), 3.69–3.64 (3H, m), 3.68 (3H, s, CO_2CH_3), 3.03 (1H, dd, *J* = 5.5, 13.9 Hz, C*H*$_2$Ph of L-Phe), 2.84 (1H, dd, *J* = 7.0, 13.6 Hz, C*H*$_2$Ph of L-Phe), 1.44 (9H, s, NHCO_2*tBu*); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ 171.9, 169.3, 155.2 (C-1, CO), 137.80, 137.75, 135.7 (*C*Ph), 129.0, 128.5, 128.3, 128.23, 128.17, 128.14, 128.0, 127.8, 127.7, 127.5, 127.0 (Ph), 79.3, 78.0, 77.1, 73.43, 73.36, 52.8 ($CHCO_2CH_3$ of L-Phe), 52.2 (CO_2CH_3 of L-Phe), 37.3 (*C*H2Ph of L-Phe), 28.4 (*t*Bu); mass spectrum (FAB) *m*/*e* 739 ((M + H$^+$), calcd for $C_{43}H_{51}N_2O9$ 739), 639 ((M–Boc + 2H$^+$), calcd for $C_{38}H_{43}N_2O_7$ 639).

(2,4,6-Tri-*O*-benzyl-3-*tert*-butoxycarbonylamido-3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-3-*N*-(2,4,6-tri-*O*-benzyl-3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-*N*-L-phenylalanine methyl ester (11). To a cooled solution of **10** (147 mg, 199 μmol) in EtOAc (**10** mL) was added 4 N HCl/EtOAc (10 mL), and the mixture was stirred for 1 h at 0 °C. The reaction mixture was concentrated and the residue was washed with Et_2O to give the amino derivative, which was used for the next step without further purification. To a solution **2** (172 mg, 298 μmol) in DMF (20 mL) were added the amino derivative, BOP reagent (176 mg, 398 μmol) and DIEA (104 μL, 597 μmol), and the mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with EtOAc and was successively washed with water, satd $NaHCO_3$, and brine, dried, and concentrated. The residue was chromatographed on SiO_2, with toluene–EtOAc 5:1 to 1:1, to give **11** (138 mg, 58%) as a white powder, mp 219–221 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30–7.07 (35H, m, Ph), 5.22 (1H, d, *J* = 8.2 Hz, NH), 4.78 (1H, t, *J* = 6.5 Hz, C*H*CO_2CH_3), 4.65–4.35 (12H, m, 6×C*H*$_2$Ph), 4.24 (1H, t, *J* = 7.9 Hz), 3.96 (1H, d, *J* = 7.8 Hz), 3.86–3.73 (4H, m), 3.69–3.54 (7H, m), 3.63 (3H, s, CO_2CH_3), 3.46–3.38 (1H, m), 3.05 (1H, dd, *J* = 6.0, 13.9 Hz, C*H*$_2$Ph of L-Phe), 2.94 (1H, dd, *J* = 7.0, 13.8 Hz, C*H*$_2$Ph of L-Phe), 1.42 (9H, s, NHCO_2*t*Bu); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ 171.8, 169.1, 155.5, 153.1 (CO), 137.94, 137.87, 137.7, 137.4, 135.8 (*C*Ph), 129.0, 128.4, 128.2, 128.0, 127.94, 127.89, 127.81, 127.7 (Ph), 73.5, 28.2 (*t*Bu); mass spectrum (FAB) *m*/*e* 1099 ((M–Boc + 2H$^+$), calcd for $C_{66}H_{72}N_3O_{12}$ 1099).

(2,4,6-Tri-*O*-benzyl-3-*tert*-butoxycarbonylamido-3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-3-*N*-(2,4,6-tri-*O*-benzyl-3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-3-*N*-(2,4,6-tri-*O*-benzyl-3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-*N*-L-phenylalanine methyl ester (12). The dimeric derivative **11** (113 mg, 94.2 μmol) in EtOAc (10 mL) was treated with 4 N HCl/EtOAc (10 mL) for 1 h at 0 °C, and the mixture was concentrated. The residue was washed with Et_2O to give the amino derivative, which was used for the next step without further purification. To a solution of **2** (81.7 mg, 114 μmol) in DMF (20 mL) were added the amino derivative, BOP reagent (83.4 mg, 189 μmol), and DIEA (49.3 μL, 283 μmol), and the mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with EtOAc and was successively washed with water, satd $NaHCO_3$, and brine, dried, and concentrated. The residue was chromatographed on SiO_2, with toluene–EtOAc 1:1, to give **12** (63 mg, 40%) as a white powder, mp 177–178 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31–7.04 (50H, m, Ph), 6.86 (1H, d, *J* = 7.9 Hz, NH), 6.76 (1H, d, *J* = 8.7 Hz, NH), 4.80 (1H, dd, *J* = 6.9, 13.2 Hz, C*H*CO_2CH_3), 4.67–4.38 (18H, m, 9×C*H*$_2$Ph), 4.33–4.23 (2H, m), 4.15 (1H, m), 4.05 (1H, d, *J* = 6.4 Hz), 3.89 (1H, t, *J* = 6.3 Hz), 3.83–3.52

(12H, m), 3.65 (3H, s, CO_2CH_3), 3.40 (1H, m), 3.03 (1H, dd, $J=5.7$, 13.8 Hz, CH_2Ph of L-Phe), 2.82 (1H, dd, $J=6.7$, 13.6 Hz, CH_2Ph of L-Phe), 1.41 (9H, s, $NHCO_2tBu$); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ171.8, 168.2 (CO), 137.9, 137.8, 135.8 (*C*Ph), 129.0, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7 (Ph), 73.5, 73.1, 72.8, 28.4 (*t*Bu); mass spectrum (FAB) *m*/*e* 1557 ((M−Boc+2H$^+$), calcd for $C_{94}H_{101}N_4O_{17}$ 1557).

(2,4,6-Tri-*O*-benzyl-3-*tert*-butoxycarbonylamido-3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-3-*N*-(2,4,6-tri-*O*-benzyl-3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-3-*N*-(2,4,6-tri-*O*-benzyl-3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-3-*N*-(2,4,6-tri-*O*-benzyl-3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-*N*-D-phenylalanine methyl ester (13). The trimetic derivative **12** (45 mg, 27.1 μmol) in EtOAc (10 mL) was treated with 4 N HCl/EtOAc (10 mL) for 1 h at 0 °C, and the mixture was concentrated. The residue was washed with Et_2O to give an amino derivative which was used for the next step without further purification. To a solution of **2** (23.5 mg, 40.7 μmol) in DMF (10 mL) were added the amino derivative, BOP reagent (24.0 mg, 54.3 μmol) and DIEA (10.4 μL, 59.7 μmol), and the mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with EtOAc and was successively washed with water, satd $NaHCO_3$, and brine, dried, and concentrated. The residue was chromatographed on SiO_2, with toluene–EtOAc 1:1, to give **13** (27.1 mg, 47%) as a white powder, mp 162–165 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.28–7.06 (65H, m, Ph), 6.87 (1H, d, $J=8.7$ Hz, NH), 6.84 (1H, d, $J=8.8$ Hz, NH), 6.61 (1H, d, $J=8.7$ Hz, NH), 4.80 (1H, dd, $J=7.0$, 13.1 Hz, $CHCO_2CH_3$), 4.68–4.38 (24H, m, 9×CH_2Ph), 4.33–4.16 (2H, m), 4.15–4.05 (3H, m), 3.91 (1H, t, $J=6.2$ Hz), 3.81–3.50 (21H, m), 3.65 (3H, s, CO_2CH_3), 3.40 (1H, m), 3.04 (1H, dd, $J=5.6$, 13.6 Hz, CH_2Ph of L-Phe), 2.82 (1H, dd, $J=6.6$, 13.6 Hz, CH_2Ph of L-Phe), 1.42 (9H, s, $NHCO_2tBu$); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ 171.4, 168.8 (CO), 138.8, 138.3 (*C*Ph), 128.48, 128.38, 128.33, 128.1, 127.9 (Ph), 77.8, 28.2 (*t*Bu); mass spectrum (FAB) *m*/*e* 2018 ((M−Boc+2H$^+$), calcd for $C_{122}H_{131}N_5O_{22}$ 2018).

(3-*tert*-Butoxycarbonylamido-3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-3-*N*-(3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-3-*N*-(3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-3-*N*-(3-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-*N*-L-phenylalanine methyl ester (14). The tetrametic derivative **13** (27.1 mg, 12.8 μmol) was hydrogenated over 20% $Pd(OH)_2$ (20 mg) in THF (10 mL)–MeOH (10 mL)–water (2 mL) for 12 h at room temperature. The reaction mixture was filtered through a Celite pad and the filtrate was concentrated. The residue was purified on Sephadex G-25, with water, and the fractions containing the product were pooled and concentrated. The product was lyophilized from water to give **14** (12 mg, 90%); ^1H NMR (300 MHz, D_2O) δ 7.28–7.12 (5H, m, Ph), 3.87–3.73 (15H, m), 3.65–3.48 (4H, m), 3.62 (3H, s, CO_2CH_3), 3.45–3.34 (10H, m), 3.10–2.98 (1H, m, CH_2Ph of L-Phe), 1.31 (9H, s, $NHCO_2tBu$); mass spectrum (FAB) *m*/*e* 1037 ((M+2H$^+$), calcd for $C_{43}H_{67}N_5O_{24}$ 1037.

Methyl 2,6-anhydro-5,7-*O*-benzylidene-D-*glycero*-L-*manno*-hepturonate (16). A mixture of (β-D-galactopyranosyl)-*C*-carboxylic acid methyl ester **15** (7.0 g, 31.5 mmol) and HCO_2H (50 mL) in benzaldehyde (50 mL) was stirred for 1 h at room temperature. The reaction mixture was diluted with EtOAc and was carefully neutralized with solid anhydrous $NaHCO_3$ (30 g). The suspension was filtered through a Celite pad, and the filtrate was successively washed with satd $NaHCO_3$, water, and brine, dried, and concentrated. The residue was chromatographed on SiO_2, with hexanes–EtOAc 1:1, to give **16** (6.1 g, 61%), mp 54–57 °C; ^1H NMR (300 MHz, CD_3OD) δ 7.32–7.30 (2H, m, Ph), 7.14–7.11 (3H, m, Ph), 5.35 (1H, s, C*H*Ph), 4.00–3.49 (6H, m), 3.55 (3H, s, CO_2CH_3), 3.39 (1H, dd, $J=3.5$, 9.5 Hz); ^{13}C NMR (72.5 MHz, CD_3OD) δ 171.3 (CO_2CH_3), 139.7 (*C*Ph), 130.0, 129.0, 127.5 (Ph), 102.3 (*C*HPh), 80.7, 77.5, 74.5, 71.6, 70.1, 69.6 (C-1,2,3,4,5,6), 52.7 (CO_2CH_3); mass spectrum (FAB) *m*/*e* 311 ((M+H$^+$), calcd for $C_{15}H_{19}O_8$ 311).

Methyl 2,6-anhydro-3,4-di-*O*-benzyl-5,7-*O*-benzylidene-D-*glycero*-L-*manno*-hepturonate (17). NaH (60% mineral oil dispersion; 1.09 g, 27.2 mmol) was added portionwise to a cooled solution of **16** (3.80 g, 11.4 mmol) and benzyl bromide (6.08 mL, 51.1 mmol) in DMF (50 mL) at 0 °C, and the mixture was stirred for 12 h at room temperature. The excess NaH was destroyed by the addition of MeOH with cooling, and the mixture was poured into ice-water and extracted with EtOAc. The extracts were washed with water and brine, dried, and concentrated. The residue was treated with NaOMe in MeOH (100 mL) at pH ∼11 for 2 h at room temperature. The reaction mixture was neutralized with Dowex 50W-X8 [H$^+$] resin, and the resin was filtered off and the filtrate was concentrated. The residue was chromatographed on SiO_2, with hexanes only to hexanes–EtOAc 3:1, to give **17** (5.20 g, 93%) as a colorless oil; ^1H NMR (300 MHz, $CDCl_3$) δ 7.56–7.54 (2H, m, Ph), 7.36–7.25 (13H, m, Ph), 5.44 (1H, s, C*H*Ph), 4.90 (1H, d, $J=10.9$ Hz, CH_2Ph), 4.72–4.53 (3H, m, CH_2Ph), 4.28–4.20 (2H, m), 4.12 (1H, d, $J=3.1$ Hz), 3.91–3.84 (2H, m), 3.73–3.67 (4H, m, H-7a and CO_2CH_3), 3.61 (1H, dd, $J=3.4$, 9.4 Hz, H-7b); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ 168.7 (CO_2CH_3), 138.3, 138.1, 137.8 (*C*Ph), 128.9, 128.3, 128.2, 128.1, 127.9, 127.7, 127.6, 126.4 (Ph), 101.3 (*C*HPh), 80.7, 78.4, 73.4, 71.2, 70.2, 69.0 (C-1,2,3,4,5,6), 75.3, 75.2 (CH_2Ph), 52.3 (CO_2CH_3); mass spectrum (FAB) *m*/*e* 491 ((M+H$^+$), calcd for $C_{29}H_{31}O_7$ 491).

Methyl 2,6-anhydro-3,4,7-tri-*O*-benzyl-D-*glycero*-L-*manno*-hepturonate (18). Saturated HCl/EtOAc was added dropwise to a suspension of **17** (1.80 g, 3.67 mmol), $NaBH_3CN$ (1.38 g, 22.0 mmol), and activated molecular sieves 3A (3 g) in THF (100 mL) until the pH of the mixture became under 1.0, and the resulting mixture was stirred for 20 min at room temperature. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated. The residue was chromatographed on SiO_2, with toluene only to toluene–EtOAc 5:1, to give **18** (1.71 g, 94%); ^1H NMR (300 MHz, $CDCl_3$) δ 7.34–7.25 (15H, m, Ph), 4.80–4.53 (6H, m, 3×CH_2Ph), 4.14–4.03 (2H, m), 3.83 (1H, d, $J=9.8$ Hz, H-1), 3.78–3.71 (2H, m), 3.67 (3H, s, CO_2CH_3), 3.61–3.55

(2H, m, H-7a, 7b); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ 168.1 (CO_2CH_3), 137.7, 137.5, 137.4 (*C*Ph), 128.2, 128.1, 128.0, 127.7, 127.6, 127.53, 127.45 (Ph), 81.9, 78.2, 77.3, 73.3, 71.2, 68.9 (C-1,2,3,4,5,6), 75.5, 75.4, 74.9 (CH_2Ph), 51.4 (CO_2CH_3);. mass spectrum (FAB) *m*/*e* 493 ((M + H$^+$), calcd for $C_{29}H_{33}O_7$ 493).

Methyl 2,6-anhydro-5-azido-3,4,7-tri-*O*-benzyl-5-deoxy-D-*glycero*-D-*gulo*-hepturonate (19). Trifluoromethanesulfonic anhydride (973 μL, 5.76 mmol) was added dropwise to a cooled solution of **18** (1.90 g, 3.86 mmol) and pyridine (2.15 mL, 15.4 mmol) in CH_2Cl_2 at −15 °C, and the mixture was stirred for 2 h at −15 °C. The reaction mixture was diluted with CH_2Cl_2, and was successively washed with dil HCl, water, satd $NaHCO_3$, and water, dried, and concentrated to give the triflate derivative, which was used for the next step without further purification. The triflate derivative was treated with NaN_3 (1.27 g, 19.3 mmol) in DMF (50 mL) for 12 h at room temperature. The reaction mixture was diluted with EtOAc, and washed with water and brine, dried, and concentrated. The residue was chromatographed on SiO_2, with toluene–EtOAc 20:1, to give **19** (1.20 g, 60%) as a colorless oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.24 (15H, m, Ph), 4.91–4.51 (6H, m, 3×CH_2Ph), 3.87–3.53 (6H, m), 3.71 (3H, s, CO_2CH_3), 3.34–3.29 (1H, m, H-6); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ 168.1 (CO_2CH_3), 162.0 (CO), 137.6, 137.1 (*C*Ph), 128.4, 128.2, 127.9, 127.8, 127.7, 127.6, 77.2, 75.1, 74.6, 73.4, 68.9, 52.3 (CO_2CH_3); mass spectrum (FAB) *m*/*e* 518 ((M + H$^+$), calcd for $C_{29}H_{32}N_3O_6$ 518).

Methyl 2,6-anhydro-3,4,7-tri-*O*-benzyl-5-*tert*-butoxycarbonylamido-5-deoxy-D-*glycero*-*gulo*-hepturonate (20). H_2S gas was bubbled through a solution of **19** (1.20 g, 2.31 mmol) in pyridine (50 mL)–water (50 mL) for 1 h at room temperature. The mixture was concentrated to give the crude amino derivative which was dried azeotropically by toluene twice. BOC-ON (5.77 g, 23.2 mmol) was added portionwise to a solution of the residue and Et_3N (3.23 g, 23.2 mmol) in MeOH (50 mL), and the resulting mixture was stirred for 12 h at room temperature. The reaction mixture was concentrated and the residue was chromatographed on SiO_2, with hexanes–EtOAc 5:1, to give **20** (1.31 g, 95%), mp 137–139 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33–7.25 (15H, m, Ph), 4.85–4.50 (6H, m, 3×CH_2Ph), 3.92 (1H, d, *J* = 9.2 Hz), 3.82–3.58 (4H, m), 3.72 (3H, s, CO_2CH_3), 3.48 (1H, t, *J* = 9.4 Hz), 1.40 (9H, s, *t*Bu); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ 169.4 (CO), 128.44, 128.40, 128.3, 128.1, 127.92, 127.86, 127.6 (Ph), 80.3, 78.1, 74.8, 73.5, 69.8, 52.3 (CO_2CH_3), 28.3 (*t*Bu); mass spectrum (FAB) *m*/*e* 592 ((M + H$^+$), calcd for $C_{34}H_{42}NO_8$ 592), 492 ((M–Boc + 2H$^+$), calcd for $C_{29}H_{34}NO_6$ 492).

2,6-Anhydro-5-*tert*-butoxycarbonylamido-5-deoxy-D-*glycero*-D-*gulo*-hepturonic acid (3). Compound **20** (500 mg, 845 μmol) was hydrogenated over 20% $Pd(OH)_2$ (400 mg) in MeOH (20 mL) for 16 h at room temperature. The reaction mixture was filtered through a Celite pad, and the filtered was concentrated. LiOH·H_2O (70.9 mg, 1.69 mmol) was added portionwise to a cooled solution of the residue in THF (20 mL)–MeOH (20 mL)–water (5 mL) at 0 °C, and the mixture was stirred for 20 min at 0 °C. The reaction mixture was neutralized with Dowex 50W-X8 [H$^+$] resin, and the resin was filtered off. The filtrate was concentrated and the residue was purified on Sephadex G-25, with water. The fractions containing the product were pooled and concentrated, and the residue was lyophilized from water to give **3** (212 mg, 82% overall), mp 204–208 °C. ^{1}H NMR (300 MHz, D_2O) δ 3.72–3.49 (3H, m), 3.43–3.25 (4H, m), 1.33 (9H, s, *t*Bu); ^{13}C NMR (72.5 MHz, D_2O) δ 159.0 (CO), 81.9, 79.5, 79.1, 75.6, 72.7, 61.9 (C-1,2,3,4,5,6), 28.2 (*t*Bu); mass spectrum (FAB) *m*/*e* 307 (M +, calcd for $C_{12}H_{21}NO_8$ 307).

2,6-Anhydro-3,4,7-tri-*O*-benzyl-5-*tert*-butoxycarbonylamido-5-deoxy-D-*glycero*-D-*gulo*-hepturonic acid lithium salt (21). LiOH·H_2O (213 mg, 5.07 mmol) was added portionwise to a cooled solution of **20** (1.50 g, 2.53 mmol) in THF (30 mL)−MeOH (30 mL)−water (10 mL) at 0 °C, and the mixture was stirred for 20 min at 0 °C. The reaction mixture was neutralized with Dowex 50W-X8 [H$^=$] resin, and the resin was filtered off. The filtrate was concentrated and the residue was chromatographed on SiO_2, with $CHCl_3$–MeOH 10:1 to 5:1, to give 21 (1.31 g, 90%), mp 215–217 °C. ^{1}H NMR (300 MHz, CD_3OD) δ 7.34–7.24 (15H, m, Ph), 4.82–4.56 (6H, m, 3×CH_2Ph), 3.79–3.51 (7H, m), 3.37 (3H, s, CO_2CH_3), 1.41 (9H, s, *t*Bu); ^{13}C NMR (72.5 MHz, CD_3OD) δ 159.1 (CO), 140.8, 139.5, 138.5 (*C*Ph), 129.1, 128.8, 128.5, 128.4, 128.2, 128.1, 127.9, 127.8, 127.5 (Ph), 84.7, 82.1, 79.9, 79.1, 78.6, 28.8 (*t*Bu); mass spectrum (FAB) *m*/*e* 585 (M$^+$, calcd for $C_{33}H_{38}NO_8Li$ 585).

(2,3,6-Tri-*O*-benzyl-4-*tert*-butoxycarbonylamido-4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-*N*-L-phenylalanine methyl ester (22). BOP reagent (919 mg, 2.08 mmol) was added to a cooled solution of **21** (600 mg, 1.03 mmol), D-phenylalanine methyl ester (269 mg, 1.25 mmol), and DIEA (543 μL, 3.15 mmol) in DMF (30 mL) at 0 °C, and the mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with EtOAc, and was successively washed with water, satd $NaHCO_3$, and brine, dried, and concentrated. The residue was chromatographed on SiO_2, with toluene–EtOAc 5:1, to give **22** (631 mg, 82%), mp 184–186 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32–7.19 (18H, m, Ph), 7.11–7.07 (2H, m, Ph), 6.90 (1H, d, *J* = 7.7 Hz, NH), 4.81 (1H, dd, *J* = 6.1, 13.7 Hz, C*H*CO_2CH_3), 4.73–4.52 (6H, m, 3×CH_2Ph), 4.00 (1H, d, *J* = 9.2 Hz), 3.79 (1H, t, *J* = 7.0 Hz), 3.69–3.52 (4H, m), 3.61 (3H, s, CO_2CH_3), 3.11 (1H, q, *J* = 6.0, 14.6 Hz, CH_2Ph of D-Phe), 3.07 (1H, q, *J* = 6.0, 14.1 Hz, CH_2Ph of L-Phe), 1.40 (9H, s, *t*Bu); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ 171.3, 168.7 (CO), 138.0, 135.7 (*C*Ph), 129.2, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6, 127.0 (Ph), 80.4, 79.6, 78.2, 77.9, 74.0, 73.6, 70.5, 52.8 (*C*HCO_3CH_3 of Phe), 52.1 (CO_2CH_3 of Phe), 37.7 ($C(CH_3)_3$) 28.3 (*t*Bu); mass spectrum (FAB) *m*/*e* 739 ((M + H$^+$), calcd for $C_{43}H_{51}N_2O_9$ 739), 638 ((M−Boc + 2H$^+$), calcd for $C_{38}H_{42}N_2O7$ 638).

(4-*tert*-Butoxycarbonylamido-4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-*N*-L-phenylalanine methyl ester (23). Compound **22** (200 mg, 271 μmol) was hydrogenated

over 20% $Pd(OH)_2$ (150 mg) in THF (20 mL)–MeOH (20 mL)–water (4 mL) for 12 h at room temperature. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated to give **23** (120 mg, 95%) as a colorless oil; ^{1}H NMR (300 MHz, CD_3OD) δ 7.21–7.09 (5H, m, Ph), 4.62 (1H, dd, J = 5.9, 8.2 Hz, C*H*CO_2CH_3), 3.62–3.45 (3H, m), 3.59 (3H, s, CO_2CH_3), 3.35–3.21 (4H, m), 3.08 (1H, dd, J = 5.8, 13.8 Hz, CH_2Ph of L-Phe), 2.96 (1H, dd, J = 8.3, 13.7 Hz, CH_2Ph of L-Phe), 1.35 (9H, s, *t*Bu); ^{13}C NMR (72.5 MHz, CD_3OD) δ 173.1, 172.3, 158.7 (CO), 138.0, (*C*Ph), 130.2, 129.5, 127.9 (Ph), 81.3, 80.5, 78.8, 76.3, 74.0, 63.0, 54.9, 53.8, 52.8 (CO_2CH_3 of Phe), 38.1 ($C(CH_3)_3$), 28.7 (*t*Bu); mass spectrum (FAB) *m*/*e* 469 ((M + H$^+$), calcd for $C_{22}H_{33}N_2O_9$ 469).

(2,3,6-Tri-*O*-acetyl-4-*tert*-butoxycarbonylamido-4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-4-*N*-(2,3,6-tri-*O*-acetyl-4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-*N*-L-phenylalanine methyl ester (24). To a cooled solution of **23** (150 mg, 320 μmol) in EtOAc (30 mL) was added 4 N HCl/EtOAc (30 mL) at 0 °C, and the mixture was stirred for 1 h at room temperature. The reaction mixture was concentrated and the residue was washed with Et_2O to give the amino derivative, which was used for the next step without further purification. To a solution of **3** (118 mg, 384 μmol) in DMF (20 mL) were added the amino derivative, BOP reagent (283 mg, 640 μmol), and DIEA (167 μL, 960 μmol), and the mixture was stirred for 16 h at room temperature. Pyridine (30 mL) and acetic anhydride (15 mL) were then added to the mixture, and the resulting mixture was stirred for 16 h at room temperature. The reaction mixture was diluted with EtOAc, and was successively washed with dil HCl, water, satd $NaHCO_3$, and brine, dried, and concentrated. The residue was chromatographed on SiO_2, with toluene–EtOAc 5:1 to 1:1, to give **24** (160 mg, 58%), mp 254–257 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31–7.23 (3H, m, Ph), 7.13–7.10 (2H, m, Ph), 6.89 (1H, d, J = 8.1 Hz, NH), 6.69 (1H, d, J = 9.0 Hz, NH), 5.15–5.03 (4H, m), 4.78 (1H, dd, J = 6.5, 14.4 Hz, C*H*CO_2CH_3), 4.69 (1H, d, J = 9.3 Hz, NH), 4.38–4.00 (5H, m), 3.88 (1H, d, J = 9.5 Hz, H-1a), 3.80 (1H, d, J = 9.7 Hz, H-1b), 3.73–3.63 (6H, m), 3.72 (3H, s, CO_2CH_3), 3.14 (1H, dd, J = 1.4, 14.1 Hz, CH_2Ph of L-Phe), 3.08 (1H, dd, J = 2.1, 16.3 Hz, CH_2Ph of L-Phe), 2.14, 2.06, 2.042, 2.040, 2.03, 1.98 (3H each, s, 6CH_3CO), 1.41 (9H, s, *t*Bu); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ 171.1, 171.0, 170.7, 170.5, 169.7, 169.3, 167.6, 166.5, 155.0 (CO), 135.6 (*C*Ph), 129.1, 128.5, 127.0 (Ph), 80.4, 77.4, 75.7, 75.6, 72.9, 69.3, 69.1, 63.0, 52.7, 52.3, 49.7 (CO_2CH_3 of Phe), 37.6 ($C(CH_3)_3$), 28.1 (*t*Bu), 20.9, 20.8, 20.6, 20.5, 20.4 (CH_3CO) ; mass spectrum (FAB) *m*/*e* 910 ((M + H$^+$), calcd for $C_{41}H_{56}N_3O_{20}$ 910).

(2,3,6-Tri-*O*-acetyl-4-*tert*-butoxycarbonylamido-4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-4-*N*-(2,3,6-tri-*O*-acetyl-4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-4-*N*-(2,3,6-tri-*O*-acetyl-4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-*N*-L-phenylalanine methyl ester (25). The pH of a solution of the dimeric derivative (**24**) (160 mg, 176 μmol) in MeOH (30 mL) was adjusted to 10∼11 by the addition of 25% NaOMe–MeOH, and the mixture was stirred for 1 h at room temperature. The reaction mixture was neutralized with Dowex 50W-X8 [H$^+$], and the resin was filtered off. The filtrate was concentrated to give the *O*-deacetylated derivative, which, in EtOAc (30 mL), was then treated with 4 N HCl/EtOAc (30 mL) for 1 h at 0 °C, and the mixture was concentrated to afford the amino derivative, which was used for the next step without further purification. To a solution of 3 (65 mg, 211 μmol) in DMF (20 mL) were added the amino derivative, BOP reagent (156 mg, 352 μmol), and DIEA (92 μL, 528 μmol), and the mixture was stirred for 12 h at room temperature. Pyridine (20 mL) and acetic anhydride (10 mL) were added to the reaction mixture at 0 °C, and the mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with EtOAc, and was successively washed with dil. HCl, water, satd $NaHCO_3$, and brine, dried, and concentrated. The residue was chromatographed on SiO_2, with toluene–EtOAc 5:1 to 1:1, to give 25 (152 mg, 71% overall), mp 234 °C–dec. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31–7.23 (3H, m, Ph), 7.18–7.10 (2H, m, Ph), 6.90 (1H, d, J = 8.0 Hz, NH), 6.74 (1H, d, J = 8.1 Hz, NH), 5.16–5.03 (6H, m), 4.76 (1H, dd, J = 7.4, 13.7 Hz, C*H*CO_2CH_3), 4.65 (1H, br s, NH), 4.33–4.04 (8H, m), 3.91 (1H, d, J = 9.2 Hz, H-1), 3.81 (1H, d, J = 8.5 Hz, H-1), 3.75–3.69 (8H, m), 3.71 (3H, s, CO_2CH_3), 3.13 (2H, m, CH_2Ph of L-Phe), 2.14, 2.04, 1.99 (3H each, s, 9×CH_3CO), 1.41 (9H, s, *t*Bu); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ 171.2, 171.0, 170.6, 170.48, 170.45, 169.7, 169.6, 169.4, 167.7, 167.6, 166.5 (CO), 135.6 (*C*Ph), 129.1, 128.5 (Ph), 80.5, 80.4, 75.63, 75.59, 72.8, 69.74, 69.70, 68.7, 63.3, 63.0, 53.1, 52.2 (CO_2CH_3 of Phe), 28.1 (*t*Bu), 21.0, 20.9, 20.7, 20.6, 20.5, 20.4, 20.2 (CH_3CO); mass spectrum (FAB) *m*/*e* 1225 ((M + H$^+$), calcd for $C_{54}H_{73}N_4O_{28}$ 1225).

(2,3,6-Tri-*O*-acetyl-4-*tert*-butoxycarbonylamido-4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-4-*N*-(2,3,6-tri-*O*-acetyl-4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-4-*N*-(2,3,6-tri-*O*-acetyl-4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-4-*N*-(2,3,6-tri-*O*-acetyl-4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-*N*-L-phenylalanine methyl ester (26). The pH of a solution of the trimeric derivative (**25**) (57 mg, 46.6 μmol) in MeOH (10 mL) was adjusted to 10∼11 by the addition of 25% NaOMe–MeOH, and the mixture was stirred for 1 h at room temperature. The reaction mixture was neutralized with Dowex 50W-X8 [H$^+$], and the resin was filtered off. The filtrate was concentrated to give the *O*-deacetylated derivative, which, in EtOAc (10 mL), was then treated with 4 N HCl/EtOAc (10 mL) for 1 h at 0 °C, and the mixture was concentrated to afford the amino derivative, which was used for the next step without further purification. To a solution of **3** (17 mg, 56 μmol) in DMF (10 mL) were added the amino derivative, BOP reagent (41 mg, 93 μmol), and DIEA (73 μL, 140 μmol), and the mixture was stirred for 12 h at room temperature. Pyridine (10 mL) and acetic anhydride (5 mL) were added to the reaction mixture at 0 °C, and the mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with EtOAc, and was successively washed with dil HCl, water, sat. $NaHCO_3$, and brine, dried, and concentrated. The residue was chromatographed on

SiO_2, with toluene–EtOAc 5:1 to 1:1, to give **26** (31 mg, 43% overall), mp 225 °C–dec. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31–7.25 (3H, m, Ph), 7.17–7.10 (2H, m, Ph), 6.90 (1H, s, NH), 6.78 (2H, br s, NH), 5.23–5.01 (8H, m), 4.76 (1H, m, C*H*CO_2CH_3), 4.65 (1H, br s, NH), 4.26–4.01 (10H, m), 3.93–3.62 (10H, m), 3.73 (3H, s, CO_2CH_3), 3.10 (2H, m, CH_2Ph of L-Phe), 2.13, 2.07, 2.02 (3H each, s, 9×CH_3CO), 1.42 (9H, s, *t*Bu); ^{13}C NMR (72.5 MHz, $CDCl_3$) δ 171.2, 170.5, 169.6, 169.4, 167.7, 166.5 (CO), 135.6 (*C*Ph), 129.1, 128.5, 127.0 (Ph), 80.5, 75.6, 72.9, 69.0, 63.0, 53.1, 52.8 (CO_2CH_3 of Phe), 28.1 (*t*Bu), 20.9, 20.8, 20.6, 20.5, 20.4 (CH_3CO); mass spectrum (FAB) *m*/*e* 1540 ((M + H$^+$), calcd for $C_{67}H_{90}N_5O_{36}$ 1540), 1440 ((M−Boc + 2H$^+$), calcd for $C_{62}H_{82}N_5O_{34}$ 1440).

(4-*tert*-Butoxycarbonylamido-4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-4-*N*-(4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-4-*N*-(4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-4-*N*-(4-deoxy-β-D-glucopyranosyl)-*C*-carboxamido-*N*-L-phenylalanine methyl ester (27). Tetrameric derivative (**26**) (31 mg, 20.2 μmol) was *O*-deacetylated with NaOMe−MeOH for 1 h at room temperature. The reaction mixture was neutralized with Dowex 50W-X8 [H$^+$], and the resin was filtreated off. The filtrate was concentrated and the residue was purified on Sephadex G-25, with water, to give **27** (14 mg, 67%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.21–7.10 (5H, m, Ph), 4.61 (1H, m, C*H*CO_2CH_3), 3.63–3.52 (19H, m), 3.34–3.25 (12H, m), 3.02 (2H, m, CH_2Ph of L-Phe), 1.31 (9H, s, *t*Bu); ^{13}C NMR (72.5 MHz, DMSO-d_6) δ 173.1, 166.0 (CO), 138.0 (*C*Ph), 129.5, 128.6 (Ph), 28.1 (*t*Bu); mass spectrum (FAB) *m*/*e* 1036 ((M + H$^+$), calcd for $C_{43}H_{66}N_5O_{24}$ 1036), 936 ((M–Boc + 2H$^+$), calcd for $C_{38}H_{58}N_5O_{22}$ 936).

General experimental procedure for *O*-sulfation of oligomers

O-Sulfation of the oligomers was performed based on the published procedure by Uryu et al.[38c] A mixture of tetramer (such as 25 or 59) (45 mg, 0.043 mmom) and sulfur trioxide pyridine complex (2 equiv per free OH groups; 178 mg, 1.12 mmol) in pyridine (8 mL) was stirred for 2 h at 80 °C, and cooled. Saturated aqueous $Ba(OH)_2$ solution was added to the reaction mixture until it became slightly alkaline (pH 8.0 judged by a pH paper). The formed white precipitate was removed by centrifugation with a microcentrifuge (Eppendorf Centrifuge 5415C) at 13×1000 cpm for 10 min. The clear supernatant was collected and was passed through a column of Dowex 50W-X8[Na]$^+$ (1×5 cm), eluted with water. The eluant was collected and concentrated in vacuo. The residue was chromatographed, for desalting, on a column of Sephadex G-15 (1×65 cm), eluted with water. Each fraction was examined with an Azure assay[54] and the positive fractions were pooled and concentrated in vacuo. The residue was lyophilyzed from water (10 mL) to give the *O*-sulfated oligomers.

The number of sulfate groups was determined by combution analysis (performed by Galbraith Laboratories, Knoxville, TN, USA) based on the sulfur content versus those for C, H, and N. Under these conditions, the average number of the sulfate groups was estimated to be two per each glycamino acid residue.

Inhibition of HIV infection to MT-2 cells

Determination of the antiviral activity of the compounds against HIV infection was based on the inhibition of the expression of p24 in supernatant using the virus capture assay method as described previously.[42] Briefly, MT-2 cells are exposed to virus (HTLVRF) in the presence or absence of various concentrations of each of the synthesized analogues for 5 days at 37 °C in c-RPMI [RPMI-1640 (Bio-Whittaker, Walkersville, MD, USA)] supplemented with 10% fetal bovine serum (HyClone, Logan, UT, USA), 2 mM L-glutamine (GIBCO, Grand Island, NY, USA), and 10 mM HEPES. The number of MT-2 cells was adjusted to 40,000 cells/mL. Supernatants were collected and lysed with 1% Triton X-100. The wells of an enzyme-linked immunosorbent assay (ELISA) place (Costar, Cambridge, MA, USA) were coated overnight with purified anti-p24 monoclonal antibody, 1 μg/well in 50 mM Tris, pH 9.5, and blocked with 3% bovine serum albumin (BSA) in phosphate-buffered saline (PBA) for 1 h at 37 °C. The HIV-containing lysed culture supernatants were added to the wells followed by incubation for 1 h at 37 °C. After washing the wells six times with 200 μL of PBS containing 0.05% Tween 20, biotinylated polyclonal HIV IgG was added to the wells, and incubated for 1 h at 37 °C. The wells were again washed six times with 200 μL of PBS containing 0.05% Tween 20 and horse radish peroxidase (HRP)–streptavidin conjugate was added to the wells and incubated for 20 min at room temperature. Substrate (TMB) was added in acetate buffer and the reaction stopped after 15 min by adding 0.5 M H_2SO_4. The plates were read at 450 nm with a spectrophotometric plate reader.

Inhibition of sialyl Lewis x-L-selectin mediated cell adhesion. Determination of the inhibitory activity of the compounds against sialyl Lewis x-mediated cell adhesion was based on the inhibition of the selectin expressing COS cell adhesion to immobilized sialyl Lewis x-containing glycolipid derivative as described previously.[44] Briefly, aliquots (50 μL) of ethanol/water (1:1) containing phosphatidylcholine (0.5 μM), cholesterol (1.0 μM) and sialyl Lewis x-containing glycolipid derivative (0.1 or 0.8 μM) were added to microwells, and incubated uncovered at ambient temperature for 90 min to allow lipid absorption. Wells were preblocked with HEPES-buffered DMEM supplemented with 25 mg/mL BSA for 10 min at 37 °C. COS-1 cells which had been transfected 48 h earlier with plasmids encoding E- or P-selectin were harvested with hypertonic PBS containing 1 mM EDTA, collected by centrifugation and resuspended in the BSA/DMEM at 1.7×10^5 cells/mL. An aliquot (300 μL) of the cell suspension was preincubated with the compounds at the indicated concentrations for 30 min at 37 °C with end-over-end rotation, and then added to the preblocked wells. Adhesion was allowed to proceed for 45 min at 37 °C. To remove nonadherent cells, the plates were immersed

in PBS, inverted, and placed in a Plexiglas box with was sealed to exclude air. The box was centrifuged at 110*g*, and then resubmerged in PBS. The plate was then removed, righted (while still immersed); removed from the PBS, and excess surface buffer was removed by aspiration, leaving 300 μL/well. Adherent cells were lysed by addition of 20 μL of 10% Triton X-100 to each well, and 80 μL were removed to a fresh 96-well plate for quantitation. Cell adhesion was quantitated by measuring lactate dehydrogenase activity in the cell lysate after addition of 120 μL of 0.1 M potassium phosphate pH 7.0 containing 0.7 mM NADH and 4.7 mM pyruvate. The decrease in absorbance at 340 nm as a function of time was measured simultaneously in each well using a Molecular Devices UV multiwell kinetics plate reader.

Inhibition of heparanase activity

A typical assay is as follows: Radiolabelled heparan sulfate solution (typically 1–5 μL; containing approximately 2500 cpm of 35S-labeled HS)[51] were mixed in with a buffer into a final reaction mixture volume of 100 μL, consisting of 25 mM KCl, 50 mM NaCl, 5 mM $MgCl_2$, 50 mM Tris–HCl buffered at pH 6.8, and 0.1% Triton X-100 (TKM/Triton buffer) and an aliquot of heparanase (typically 1 μL) obtained by the published procedure,[52] were incubated for 1 h at 37 °C in the presence of the compounds. The reaction was stopped by the addition of 100 μL of 2 M guanidinium chloride, vortexed and the total solution was applied to either a Centricon 30 (commercially available from Amicon, Inc., Beverly, MA, USA). Samples were then centrifuged at 3000*g* for 20 min in a Beckman AccuspinFR in a swing bucket rotor at room temperature. Another 100 μL aliquot of 2 M guanidinium chloride buffer was added and centrifuged additional 15 min to maximize the recovery of the filtrate. Filtrate was then counted for radioactivity with 1.5 mL of HighSafe 3 (Pharmacia) using a Beckman LS-5801, typically until the levels of counting error becomes less than 5%.

Circular dichroism (CD) spectra

Samples (2 mg) were dissolved in 500 μL of H_2O and data were collected on a Jasco J-600 spectropolarimeter at 20 °C in a CD cell with a 1.0 mm optical path length.

NMR spectroscopy

NMR spectra were measures on a Bruker DRX-600 and DRX-400 spectrometers. Samples (5–6 mg) were dissolved in H_2O/D_2O or pyridine-d_5. Probe temperature was set between 5 and 30 °C. DQF-COSY, TOCSY, ROESY, ^1H–^{13}C HSQC, and ^1H–^{13}C HSQC-TOCSY were recorded using standard pulse sequences. ^3J(NH,H2) coupling constants were obtained from 1D spectra. ROESY data for integration of peak volumes were collected with mixing times of 300 ms at 303 K. The temperature coefficients of the amide protons were studied by collecting 1D spectra at six different temperatures between 5 and 30 °C in 5 ° increments and are reported in-ppb/K.

Acknowledgements

We are grateful to Dr. K. Ikeda (University of Shizuoka, Japan) for performing the NMR experiments. Some of the NMR studies were performed in the Biochemistry NMR Facility at Johns Hopkins University, which was established by a grant from the National Institutes of Health (GM 27512) and a Biomedical Shared Instrumentation Grant (1S10-RR06262–0). A fellowship from Uehara Memorial Foundation (to Y.S.) is gratefully acknowledged. This research was partly supported by NIH grant GM52324 (to Y.I.).

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