Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 1337–1346

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis of a 3-Deoxy-L-iduronic Acid Containing Heparin Pentasaccharide to Probe the Conformation of the Antithrombin III Binding Sequence†

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Received 17 February 1998; accepted 24 June 1998

Abstract—We report in this work the total synthesis of a close analogue of the pentasaccharide active site of heparin, in which the L-iduronic acid residue has been deoxygenated at position three. ^{1}H NMR studies demonstrated that, as anticipated, such a modification induces a shift of the conformational equilibrium toward $^1C_4$ (contribution to the conformational equilibrium rises from 37% to 65%) and a substantial decrease of the affinity for antithrombin III (K_d 0.154 μM versus 0.050 μM).

Introduction

Heparin, a complex anionic polysaccharide,[1] inhibits blood coagulation through activation of antithrombin III[2] (AT III), a physiological inhibitor of coagulation. Binding of heparin to AT III is mediated by the pentasaccharide sequence **1**.[3] Chemical synthesis of this pentasaccharide,[4] and its methyl glycoside **2**,[5] allowed careful analysis of the ^{1}H NMR coupling constants for the L-iduronic acid unit (unit G) which finally led us to conclude that the conformational equilibrium of this monosaccharide could not be explained by the presence of the two well known conformers $^4C_1$ and $^1C_4$ only, but that a third one, $^2S_0$, had also to be considered to account for the observed pattern of coupling constants.[6] The contribution of each of the conformers to the conformational equilibrium could be computed from ^{1}H NMR coupling constants[7] and it appeared that L-iduronic acid conformation is highly influenced by the substituents and the nature of the neighbouring units.[8] Thus, while $^1C_4$ was found to represent the predominant conformer in heparin, a significant shift of the conformational equilibrium toward $^2S_0$ was observed when L-iduronic acid was next to a 3-*O*-sulfated glucosamine unit (unit F).[9] Since this extra-sulfate, at position 3 of unit F, is the key structural element responsible for the binding to AT III[10] the question arises whether its function is to directly interact with AT III or to drive L-iduronic toward an 'active' $^2S_0$ conformation, or both. The same issue is raised by the extra sulfate group introduced on the H unit in various oligosaccharides,[11] which confers to these compounds a higher affinity for AT III[12] and strongly favours the $^2S_0$ conformation. To assess the role of the conformation itself in the interaction, it is necessary to compare compounds that contain the same potential interaction sites (same pattern of sulfates and carboxylates) yet differ in their conformation. We reasoned that deoxygenation at position 3 of unit G in **2** should shift the conformational equilibrium toward $^1C_4$, by decreasing the non bonding interactions occurring between O-3 and the other axial substituents in this conformer. In the present article,[13] we report the synthesis of **30** (deoxygenated **2**) and the comparison of the biophysical and biochemical properties of **2** and **30**.

Key words: L-Iduronic acid; pentasaccharide; heparin; antithrombin III.
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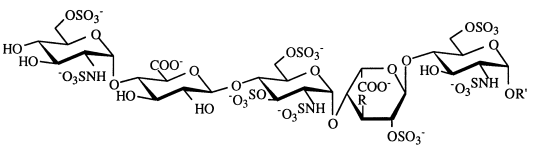
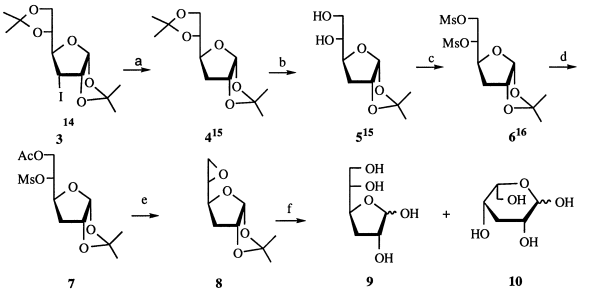
0968-0896/98/$—see front matter
PII: S0968-0896(98)00127-8



D **E** **F** **G** **H**

1: R = OH, R′ = H
2: R = OH, R′= Me
30: R = H, R′ = Me



Scheme 2. Reagents and conditions: (a) $NiCl_2$, $NaBH_4$, DMF, 96%; (b) 60%, AcOH, rt, 72 h, 78%; (c) MsCl, pyr, rt, 16 h, 99%; (d) AcOK, DMF, 80 °C, 48 h, 63%; (e) *t*BuOH, CH_2Cl_2, 100%; (f) 0.1 N H_2SO_4, 100%; (f) 0.1 N H_2SO_4, 50 °C, 2 h, 100%.

Results and Discussion

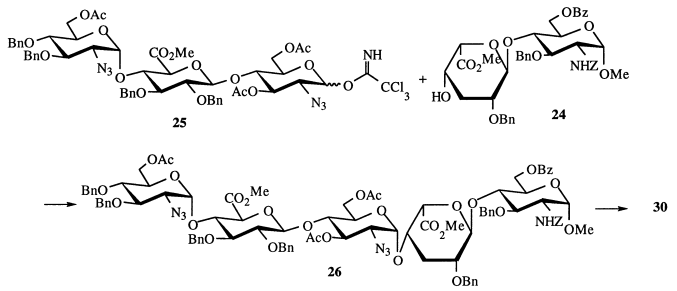
General strategy. The general selected synthetic strategy is outlined in Scheme 1. The known[11b] trisaccharide imidate **25** had already been successfully used as a glycosyl donor for the synthesis of heparin pentasaccharidic fragments.[11b,12] The major task of the present work was thus the selective synthesis of the disaccharidic building block **24**, the glycosylation of which by **25** should then provide the protected pentasaccharide **26**, that would be easily converted into the final product **30** following a well established methodology.[4,5]

Chemical synthesis of the disaccharidic building block 24. We initially decided to prepare the disaccharide **24** starting from 3-deoxy-L-idose (3-deoxy-L-*lyxo*-hexose) eventually obtained from diacetone glucose via the known[14] **3** as shown in the self explanatory Scheme 2.

Anomeric protons corresponding to the α and β anomers of **9** and **10** were identified in the ^1H NMR spectrum in deuterium oxide solution of the mixture, and the proportion of the various constituents at equilibrium could be easily determined. As shown in Table 1 the furanoid forms are predominant in the case of 3-deoxy-L-*lyxo*-hexose (3-deoxy-L-idose).

This feature can be easily explained. The noteworthy known[17] proportion (see Table 1) of furanoid forms in the aqueous solution of 3-deoxy-D-*ribo* hexose as compared to D-glucose (which does not give rise to furanoid forms at equilibrium in aqueous solution) arises from the lack, in the 3-deoxy compound, of the most unfavourable interaction between the side chain at C-4 and the oxygen atom at C-3 which are cis to each other in glucofuranose. This trend is even more pronounced in the case of 3-deoxy-L-*lyxo*-hexose because of the lower stability of the pyranoid forms of L-idose itself. The previously reported[18] strategy for the synthesis of pyranoid L-iduronic acid derivatives is thus not applicable for the preparation of 3-deoxy analogues. Acetylation of 3-deoxy-L-idose would mainly provide an unwanted furanoid derivative.

We thus explored a second route starting from the known[19] methyl 2-*O*-benzyl-3-deoxy-α-D-ribo-hexopyranoside **11** wherein, in contrast to the first approach, the pyranoid form is locked throughout the synthesis (Scheme 3). The conversion of the iodo derivative **12** into the hex-5-enopyranoside **13** was first attempted using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) either in tetrahydrofuran,[20] acetonitrile,[21] or dimethylsulfoxide;[22] in all cases **13** was only isolated in poor yields. Finally, a satisfactory reaction (79%) was observed upon treatment of **12** with a refluxed solution of sodium methoxide in methanol although some substituted product **14** was also isolated (17%).



Scheme 1. Synthetic route to **30**.

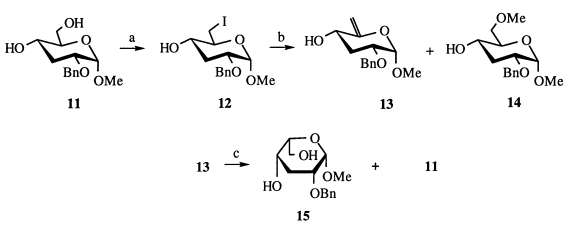
Table 1. Proportion(%) of pyranoid and furanoid forms at equilibrium in deuterium oxide solution

	Pyranoid forms			Furanoid forms		
	α	β	total	α	β	total
D-Glucose (at 31 °C)[17]	38	62	100	–	–	–
3-Deoxy-D-*ribo*-hexose (at 31 °C)[17] (3-deoxy-D-glucose)	24.5	55	79.5	5	15.5	20.5
D-Idose (at 31 °C)[17]	38.5	36	74.5	11.5	14	25.5
3-Deoxy-L-*lyxo*-hexose (3-deoxy-L-idose) (at 27 °C)	12	12	24	57	19	76

A detrimental extensive migration of the 4-*O*-acetyl group to the primary position was observed during the removal of the trityl group of **17** under various classical conditions[23] (Scheme 4). We found that a 2 min treatment at 0 °C of a dichloromethane solution of **17** with 60% aqueous perchloric acid limited the migration (10%) and provided the expected primary alcohol **18** in satisfactory yield (70%). The primary alcohol function and the benzyl ether group were simultaneously oxidised according to Sharpless[24] to give, after treatment with diazomethane, the L-iduronic acid derivative **20** as a crystalline product. ^{1}H NMR data at 250 MHz of **20** ($CDCl_3$ solution) are in agreement with a $^1C_4$ conformation. Formation of the glycosyl chloride **21** was finally achieved using dichloromethyl methyl ether in the presence of zinc chloride[25] at 60 °C. When this reaction was conducted at room temperature, anomerization (88%) to the α-methyl glycoside was observed.

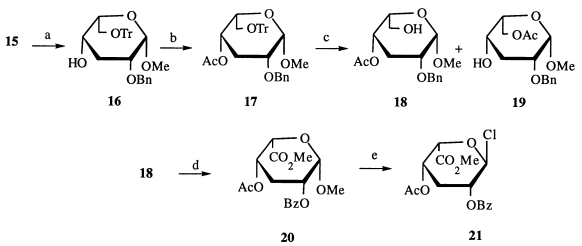
The glycosyl donor **21** was now reacted at −15 °C in dichloromethane with the known[26] alcohol **22** in the presence of silver triflate to give selectively, albeit in limited yield (36%), the crystalline disaccharide **23** (Scheme 5). The expected alcohol **24** was finally obtained by selective de-*O*-acetylation of **23**.

Preparation of the pentasaccharide 30. The alcohol **24** was condensed with the previously reported[11b] imidate **25** to give the protected pentasaccharide **26** (Scheme 6). The final deprotecting steps were achieved as extensively developed by us in this field.[4,5]

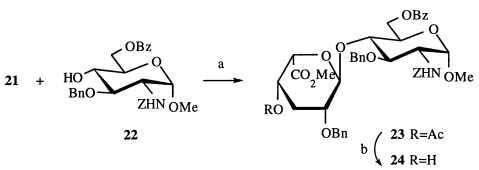


Scheme 3. Reagents and conditions: (a) Ph_3P, I_2, imidazole, toluene, 70 °C, 3 h, 83%; (b) MeONa/MeOH, reflux, 16 h, **13**: 79%, **14**: 18%; (c) BH_3/THF then H_2O_2/NaOH, 78%, ido/gluco (5.5/1).

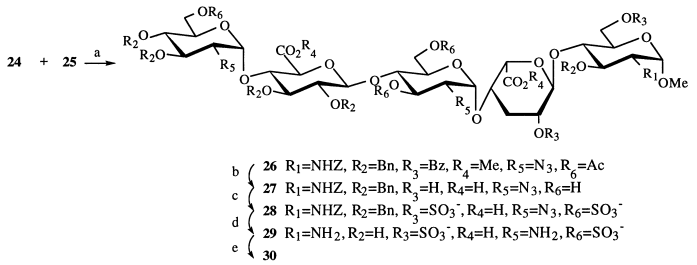
^{1}H NMR analysis of pentasaccharide 30. ^{1}H NMR data for **30** (Table 2) were obtained at 500 MHz.[27] Signals were assigned through a phase sensitive COSY experiment.[28] The spectrum was acquired as 4 K×512 (sine bell weighting was applied in each dimension), zero-filled to 4 K×2 K prior to double Fourier Transform, not symmetrised. Signal assignments are in total agreement with the structure. The ^3J coupling constants were obtained from the 1-D spectrum. The coupling constants observed for the anomeric protons confirmed the expected configurations at these carbons. Concerning the L-iduronic acid unit G, most noticeable is the expected upfield shift of the two protons G-3ax and G-3eq of the deoxy position observed at 2.29 and 2.24 ppm versus 4.17 for G-3 in **2**. The observation of a ^4J long-range coupling constant between the anomeric proton of L-iduronic acid residue G (observed as a broad singlet) and one of the two protons at position 3 of the same unit is an indication for predominance of $^1C_4$ conformation for the G unit. The equatorial position was



Scheme 4. Reagents and conditions: (a) TrCl, pyridine, 80 °C, 16 h; (b) Ac_2O, Pyr, rt, 80%; (c) 60% aq $HClO_4$, 0 °C, 5 min, **18**: 70% **19**: 10%; (d) $RuCl_3$/$NaIO_4$, H_2O/CH_3CN/CCl_4, rt, 24 h then CH_2N_2/ether, 60%; (e) $CHCl_2OCH_3$, $ZnCl_2$, 60 °C, 5 h, 70%.



Scheme 5. Reagents and conditions: (a) AgOTf, CH_2Cl_2, −15 °C, 3 h, 36%; (b) HCl/MeOH, CH_2Cl_2, 3 days, 33%.



Scheme 6. Reagents and conditions: (a) TMSOTf, CH_2Cl_2, −20 °C, 64%; (b) LiOOH, THF; (c) $Et_3N{:}SO_3$, DMF; (d) H_2, Pd/C; (e) $Pyr{:}SO_3$, water (35% from **24**).

assigned to the proton at 2.24 ppm on the basis of this small long-range coupling. It is noteworthy that such a long-range coupling is not observed in the proton ^1H NMR spectrum of **1** and **2** where the anomeric proton of the L-iduronic acid residue appears as a doublet. No such coupling was detected for the other proton at G-3. The ^3J coupling constants for all ring protons are shown in Table 2.

Conformation of L-iduronic acid and biological activity. It is now well documented[6–9] that in heparin and heparin related oligosaccharides, L-iduronic acid stands in equilibrium between the three different conformers $^1C_4$, $^4C_1$ and $^2S_0$ depicted in Scheme 7. The predominance of the $^2S_0$ conformer is easily revealed by comparison of the $J_{2,3}$ and $J_{3,4}$ set of coupling constants. Thus while $J_{2,3}$ and $J_{3,4}$ have similar values in the presence of the chair forms only, $J_{2,3}$ becomes much larger than $J_{3,4}$ (and can reach 9 Hz) when the $^2S_0$ conformer contributes significantly to the conformational equilibrium. Thus, from a qualitative standpoint, the weak difference between $J_{2,3}$ and $J_{3,4}$ (Table 2) indicates that the $^2S_0$ conformer is present in **30** although to a rather low extent.

Table 2. Chemical shifts (ppm) and coupling constants (Hz) observed for **30**

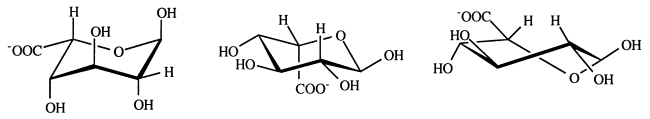
δ	D	E	F	G		H
H-1	5.60	4.59	5.17	5.30		4.99
H-2	3.22	3.37	3.41	4.35		3.24
H-3	3.58	3.82[a]	4.38	2.29 ax	2.24 eq	3.63
H-4	3.54	3.81[a]	3.92	4.20		3.72
H-5	3.86	3.73	4.05	4.65		3.96
H-6	4.34		4.47			4.34
H-6′	4.12		4.18			4.24
$^3J_{1,2}$	3.8	7.9	3.5	2.6		3.6
$^4J_{1,3eq}$					∼1	
$^3J_{2,3}$	10.0	9.4	10.6	4.6	5.5	10.3
$^3J_{3,4}$	9.2	9.2	9.1	4.6	4.0	8.7
$^2J_{3,3'}$				−15.1		
$^3J_{4,5}$	9.6	9.6	10.0	2.7		9.9
$^3J_{5,6}$	2.2		1.7			2.1
$^3J_{5,6'}$	2.0		1.6		∼1	5.6
$^2J_{6,6'}$	−11.1		−11.2			−11.7

[a]Assignments of these two protons can be reversed.

To obtain a more precise evaluation we used the method reported by Ferro et al.,[7] where the participation of each conformer to the conformational equilibrium is computed from the interproton coupling constants of the iduronate ring. The values obtained in the present case for **30** are depicted in Table 3. As expected, the predominant conformer is $^1C_4$, the participation of which amounts to 65%. The $^2S_0$ conformer, which participates to the extent of 64 % in **2**[7], only represent 24% in **30** (Table 3).

The presence of the $^2S_0$ conformer can also be probed through NOE experiments.[8] Thus, whereas the H-2/H-5 distance in the chair conformers is too large to give rise to an observable NOE, these two protons are much closer (2.3 Å) in the case of the skewed boat (Scheme 7), and a NOE is observed. Since the $^2S_0$ conformer participates to a greater extent to the conformational equilibrium in **2**, a stronger NOE should be observed in this compound, compared to **30**. NOE build-up experiments on these two pentasaccharides were thus designed, and four similar phase sensitive[30] NOESY spectra were acquired at 283 K (2 K×400 pts) with mixing periods of 100, 150, 200, and 250 ms. NOESY crosspeaks intensities were evaluated using the UXNMR program. Some pairs of protons give rise to NOE that can be easily observed and quantified, the results being reported in Table 4. It clearly appears that, while similar values are obtained for various pairs of proton in **2** and **30**, an obvious difference is noted concerning the H-2G/H-5G NOE and a much stronger effect is observed in **2** compared to **30**.

In conclusion the present ^1H NMR data indicate that, as expected, the removal of the oxygen at C-3 of the iduronate ring induces a shift of the conformational equilibrium of this unit at the expense of the $^2S_0$ conformer. In order to evaluate the influence of this conformational change on the affinity for AT III of such



$^1C_4$ $^4C_1$ $^2S_0$

Scheme 7.

pentasaccharides, we finally compared the binding constants of **2** and **30**. These constants were measured by fluorescence spectroscopy using a technique already reported.[31]

As shown in Table 5, the pentasaccharide **2** has a much higher affinity for antithrombin. Since **2** and **30** have the same sulfation pattern, and considering that the interaction with the protein mainly involves the sulfate and the carboxylate groups,[12,32] we conclude that the affinity for the protein is highly influenced by the conformation of G. This affinity is higher when the $^2S_0$ conformation at L-iduronic acid predominates. This conclusion was recently strengthened by the finding that a conformationally constrained heparin-like pentasaccharide where the iduronic acid unit is fixed in the $^1C_4$ conformation displays a very low activity in an AT III mediated anti-factor Xa assay.[33] However since the compounds used in the present study can reach several conformations, one cannot tell whether the predominance of the $^2S_0$ state of L-iduronate is important for initial recognition by the protein or if it favours the subsequent induced fit known to occur during the assembling of the antithrombin–pentasaccharide complex.

Experimental

General. All solvents and reagents were of the best commercially available grade or were purified and dried according to standard procedures. Reactions were monitored by TLC on silica gel 60 F_{254} (Merck) with detection by charring with H_2SO_4. Unless otherwise stated, column chromatography was performed on silica gel 60 (E. Merck 63-200 μm). ^{1}H NMR spectra were recorded with Bruker AM100, AC 250, AM400 or AM 500 instruments for solution in $CDCl_3$ (internal Me_4Si) unless otherwise stated. MS analyses were performed on Nermag R10-10 instrument using chemical ionisation (NH_3) and detection of positive ions. Melting points were determined in capillary tubes with a Büchi 510 apparatus, and are uncorrected. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter at 23 ± 3 °C. Elemental analyses were carried out at the Service Central d'Analyses (C.N.R.S., Vernaison, France)

3-Deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranose (4). To a solution of 3-deoxy-3-iodo-1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (2 g, 5.5 mmol), and $NaBH_4$ (0.82 g, 21.5 mmol) in anhydrous DMF (40 mL), wad added $NiCl_2$ (1.7 g, 13.2 mmol) at 0 °C. After 30 min at room temperature, the solution was concentrated. A solution of the residue in chloroform was washed with water, dried ($MgSO_4$) and concentrated to yield **4** (1.26 g, 96%), which was directly engaged in the next step.

3-Deoxy-1,2-*O*-isopropylidene-α-D-*ribo*-hexofuranose (5). A solution of 3-deoxy-1,2:5,6-di-*O*-isopropylidene-α-D-*ribo*-hexofuranose (**4**) (107 mg, 0.44 mmol) in aq acetic

Table 3. Participation of the three conformers to the conformational equilibrium of **2**[8] and **30**

	$^1C_4$	$^4C_1$	$^2S_0$
30	65	11	24
2	36	0	64

Table 5. Dissociation constants of antithrombin III and compounds **2** and **30**

	K_d (μM)
30	0.154
2	0.050

Table 4. Relative intensities of NOEs (%) observed for some pairs of protons in **2** and **30**

	H-2G/H-5G	H-5G/H-4G	H-1E/H-6F	H-1E/H-5E	H-5F/H-6F	H-1E/H-4F
30	8.0	33.4	30.5	49	25	23.2
2	13.6	34.6	32.5	52.5	26.4	23.2
30/2	0.59	0.97	0.94	0.93	0.95	1.00

acid (60%, 1 mL) was stirred for 60 h at room temperature, and concentrated. The residue was taken up in a chloroform/hexane mixture and **5** (70 mg, 78%) was filtered off. Mp 82–83 °C (chloroform/hexane). Lit.[15a,b] mp 84 °C.

3-Deoxy-1,2-*O*-isopropylidene-5,6-di-*O*-methanesulfonyl-α-D-*ribo*-hexofuranose (6). Methanesulfonyl chloride (0.48 mL, 6.2 mmol) was added at 0 °C to a solution of **5** (500 mg, 2.4 mmol) in dry pyridine (5 mL). The resulting solution was stirred for 24 h at room temperature, and concentrated. A solution of the residue in dichloromethane was washed with water (3×30 mL), dried ($MgSO_4$) and concentrated. Column chromatography (chloroform/ethyl acetate, 3/1) gave **6** (874 mg, 99%), mp 90–91 °C (acetone/water). $[\alpha]_D$ −1 (*c* 1.1, chloroform). Lit.[16] mp 84.5–85.5 °C (ether/pentane) $[\alpha]^{26}_D$ −1° (*c* 2.46, chloroform). ^{1}H NMR: δ 5.88 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.98 (m, 1H, $J_{4,5}$ = 6 Hz, $J_{5,6a}$ = 3 Hz, $J_{5,6b}$ = 5 Hz, H-5), 4.83 (dd, 1H, $J_{2,3a}$ = 4.5 Hz, H-2), 4.58 (dd, 1H, $J_{6a,6b}$ = 12 Hz, H-6a), 4.42 (dd, 1H, H-6b), 4.40 (dt, 1H, $J_{3e,4}$ = 4.5 Hz, $J_{3a,4}$ = 12 Hz, H-4), 3.17 and 3.12 (2s, 6H, 2 CH_3S), 2.30 (dd, 1H, $J_{3a,3e}$ = 14 Hz, H-3e), 2.00–1.84 (ddd, 1H, H-3a), 1.50 and 1.30 (2s, 6H, $2CH_3$). MS: 378 (M + 18), 361 (M + 1), 345, 320, 284.

6-*O*-Acetyl-3-deoxy-1,2-*O*-isopropylidene-5-*O*-methanesulfonyl-α-D-ribo-hexofuranose (7). A mixture of **6** (120 mg, 0.33 mmol) and potassium acetate (74 mg, 0.83 mmol) in dry DMF (1 mL) was stirred for 60 h at 80 °C and concentrated. A solution of the residue in dichloromethane, was washed with water, dried ($MgSO_4$), and concentrated. Column chromatography (toluene/ethyl acetate, 4/1) gave **7** (68 mg, 63%). $[\alpha]_D$ +4 (*c* 0.8, chloroform). ^{1}H NMR: δ 5.82 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.98 (m, 1H, $J_{4,5}$ = 4 Hz, $J_{5,6a}$ = 3.0 Hz, $J_{5,6b}$ = 7 Hz, H-5), 4.79 (dd, 1H, $J_{2,3a}$ = 4.5 Hz, H-2), 4.44 (dd, 1H, $J_{6a,6b}$ = 12 Hz, H-6a), 4.40–4.36 (dt, 1H, $J_{3e,4}$ = 4.5 Hz, $J_{3a,4}$ = 12 Hz, H-4), 4.14 (dd, 1H, H-6b), 3.10 (s, 3H, CH_3S), 2.08 (dd, 1H, $J_{3a,3e}$ = 14 Hz, H-3e), 2.01 (s, 3H, OAc), 1.96 (ddd, 1H, H-3a), 1.50 and 1.30 (2s, 6H, $2CH_3$). MS: 342 (M + 18), 325 (M + 1), 309, 284. Anal. calcd for $C_{12}H_{20}O_8S$ (324.352): C, 44.44; H, 6.21. Found: C, 44.80; H, 6.28.

5,6-Anhydro-3-deoxy-1,2-*O*-isopropylidene-β-L-lyxo-hexofuranose (8). A solution of potassium *tert*-butoxide (179 mg, 1.6 mmol, 2 equiv) in *tert*-butanol (3 mL) was added, at 0 °C, to a solution of **7** (254 mg, 0.8 mmol) in anhydrous dichloromethane (5 mL). The reaction mixture was stirred for 6 h at room temperature, filtered, and concentrated. Column chromatography (chloroform/ethyl acetate, 10/1) gave **8** (345 mg, 95%). $[\alpha]_D$ −5 (*c* 2.7, chloroform). ^{1}H NMR: δ 5.80 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.74 (dd, 1H, $J_{2,3a}$ = 4 Hz, H-2), 4.15 (dt, 1H, $J_{3a,4}$ = 11 Hz, $J_{3e,4}$ = 4.5 Hz, $J_{4,5}$ = 4 Hz, H-4), 3.03 (m, 1H, H-5), 2.80 (m, 2H, H-6a,6b), 2.16 (dd, 1H, $J_{3a,3e}$ = 14 Hz, H-3e), 1.85 (ddd, 1H, H-3a), 1.49 and 1.31 (2s, 6H, $2CH_3$). MS: 204 (M + 18), 187 (M + 1), 171, 146. Anal. calcd for $C_9H_{14}O_4$ (186.209): C, 58.05; H, 7.58. Found: C, 58.03; H, 7.65.

3-Deoxy-L-*lyxo*-hexose (9) and (10). A solution of **7** (100 mg, 0.5 mmol) in aq sulfuric acid (0.1 N, 2 mL) was stirred at 50 °C for 2 h, neutralised by 1 N aq sodium hydroxide, and concentrated. Column chromatography (chloroform/methanol, 10/1) gave 3-deoxy-L-*lyxo*-hexose (70 mg, 80%). ^{1}H NMR (D_2O): δ 5.31 (d, 1H, $J_{1,2}$ = 4 Hz, H-1 β-fur), 5.24 (s, H-1 α-fur), 5.11 (s, H-1 β-fur), 4.5–3.5 (m, 5H, H-2, H-4, H-5, H-6a,6b), 2.19–1.89 (m, 2H, H-3a,3b); ^{13}C NMR (D_2O): δ 102.85 (C-1, α-fur), 98.04 (C-1, β-pyr) 35.51 et 31.43 (C-3, α- and β-pyr), 33.87 (C3, α-fur), 33.83 (C-3, β-fur).

Methyl 2-*O*-benzyl-3,6-dideoxy-6-iodo-α-D-*ribo*-hexopyranoside (12). A solution of **11** (64 mg, 0.24 mmol), triphenylphosphine (173 mg, 0.66 mmol, 3 equiv), imidazole (45 mg, 0.66 mmol, 3 equiv), and iodine (74 mg, 0.29 mmol, 1.3 equiv) in anhydrous toluene (2 mL) was stirred at 70 °C for 3 h. The reaction mixture was washed with aq $Na_2S_2O_3$ (10%, 2 mL), aq saturated $NaHCO_3$, water, dried ($MgSO_4$), and concentrated. Column chromatography (chloroform/acetone, 5/1) gave **12** (75 mg, 83%). $[\alpha]_D$ +59 (*c* 1.4, chloroform). ^{1}H NMR: δ 7.40 (m, 5H, arom), 4.86 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.67 (AB, 2H, CH_2Ph), 3.67–3.30 (m, 5H, H-2,4,5,6a,6b), 3.56 (s, 3H, OCH_3), 2.30 (m, 1H, H-3e), 1.98 (q, 1H, H-3a). MS: 396 (M + 18), 379 (M + 1), 364 (-CH_3), 347 (-OH). Anal. calcd for $C_{14}H_{19}O_4I$ (378.208): C, 44.46; H, 5.06. Found: C, 44.26; H, 5.11.

Methyl 2-*O*-benzyl-3,6-dideoxy-α-D-*ribo*-hex-5-enopyranoside (13). Sodium methoxide (1 M) in methanol (10 mL) was added to a solution of **12** (720 mg, 1.9 mmol) in methanol (10 mL). The mixture was stirred at 80 °C for 12 h, cooled, and diluted with water (25 mL) and dichloromethane. The organic layer was washed with cold aq diluted HCl, water, dried ($MgSO_4$), and concentrated. Column chromatography (chloroform/acetone, 5/1) gave first **13** (375 mg, 79%), mp 59–60 °C (ethyl acetate/hexane). $[\alpha]_D$ +52 (*c* 2, chloroform). IR (chloroform) V_{max} (cm^{-1}): 1665 (C=C) 2940, 3020 (benzyl). ^{1}H NMR: δ 7.45–7.35 (m, 5H, arom), 4.80–4.60 (m, 5H, H-1,6a,6b, CH_2Ph), 4.10 (m, 1H, $J_{3e,4}$ = 5.5 Hz, $J_{3a,4}$ = 11 Hz, $J_{4,OH}$ = 8 Hz, H-4), 3.75–3.45 (ddd, $J_{2,3e}$ = 4.5 Hz, $J_{1,2}$ = 3 Hz, $J_{2,3a}$ = 11 Hz, H-2), 3.50 (s, 3H, OCH_3), 2.30–2.15 (m, 1H, $J_{3a,3e}$ = 11 Hz, H-3e), 2.05–1.90 (q, 1H, H-3a). MS: 268 (M + 18), 234, 219. Anal. calcd for $C_{14}H_{18}O_4$ (250.296): C, 67.18; H, 7.15. Found: C, 66.84; H, 7.44.

Then **14** was eluted (4 mg, 17.5%).

Methyl 2-*O*-benzyl-3-deoxy-6-*O*-methyl-α-D-*ribo*-hexopyranoside (14). $[\alpha]_D$ +35° (*c* 0.7, chloroform). ^{1}H NMR: δ 7.40 (m, 5H, arom), 4.67 (d, 1H, $J_{1,2}$ = 4 Hz, H-1), 4.55 (AB, 2H, CH_2Ph), 3.70–3.45 (m, 5H, H-2,4,5,6a,6b), 3.40 (2s, 6H, $2CH_3$), 2.20 (m, 1H, H-3e), 1.80 (m, 1H, H-3a). Anal. calcd for $C_{15}H_{22}O_5$ (282.338): C, 63.81; H, 7.85. Found: C, 63.74; H, 7.92.

Methyl 2-*O*-benzyl-3-deoxy-β-L-*lyxo*-hexopyranoside (15). A 1 M solution of BH_3 in THF (4.8 mL) was added to a solution of **13** (300 mg, 1.2 mmol) in dry THF (10 mL). The reaction mixture was stirred for 1 h then diluted with ethanol. Aqueous NaOH, (3 M, 3 mL) and H_2O_2 (3 mL) were added, and after 2 h stirring at 50 °C, the solution was diluted with chloroform, washed with water, dried ($MgSO_4$), and concentrated. Column chromatography (toluene/methanol, 8/1) gave **15** (212 mg, 66%) then **11** (38 mg, 12%). **15**: mp 63 °C (ethyl acetate/hexane). $[\alpha]_D$ +94 (*c* 2.4, chloroform). ^{1}H NMR: δ 7.45–7.30 (m, 5H, arom), 4.80 (AB, 2H, CH_2Ph), 4.42 (d, 1H, $J_{1,2}$ = 0.5 Hz, H-1), 4.03 (dd, 1H, $J_{6a,6b}$ = 9 Hz, $J_{5,6a}$ = 4.5 Hz, H-6a), 3.85 (dd, 1H, $J_{5,6b}$ = 3 Hz, H-6b), 3.75 (m, 2H, H-2,4), 3.60 (2s, 4H, H-5 and OCH_3), 2.20 (dt, 1H, $J_{3a,3e}$ = 10 Hz, $J_{2,3e}$ = 3 Hz, $J_{3e,4}$ = 3 Hz, H-3e), 1.70 (dt, 1H, $J_{2,3a}$ = 3 Hz, $J_{3a,4}$ = 3 Hz, H-3a). MS: 286 (M + 18), 269 (M + 1), 254 (-OCH_3), 237, 108. Anal. calcd for $C_{14}H_{20}O_5$ (268.311): C, 62.67; H, 7.51. Found: C, 62.76; H, 7.62.

Methyl 2-*O*-benzyl-3-deoxy-6-*O*-trityl-β-L-*lyxo*-hexopyranoside (16). A solution of **15** (340 mg, 1.26 mmol) and trityl chloride (432 mg, 1.55 mmol, 1.2 equiv) in dry pyridine (8 mL) was stirred at 80 °C until TLC (chloroform/ethyl acetate, 10/1) showed complete conversion of **15**. After concentration, column chromatography (chloroform/acetone, 10/1) gave **16**. ^{1}H NMR: δ 7.60–7.20 (m, 20H, arom), 4.80 (AB, 2H, CH_2Ph), 4.40 (d, 1H, $J_{1,2}$ = 1 Hz, H-1), 3.80–3.40 (m, 5H, H-2,4,5,6a,6b), 3.65 (s, 3H, OCH_3), 2.30 (dt, $J_{3a,3e}$15 Hz, $J_{2,3e}$ = 3 Hz, $J_{3e,4}$ = 3 Hz, H-3e), 1.65 (dt, 1H, $J_{2,3a}$ = 3 Hz, $J_{3a,4}$ = 3 Hz, H-3a).

Methyl 4-*O*-acetyl-2-*O*-benzyl-3-deoxy-6-*O*-trityl-β-L-*lyxo*-hexopyranoside (17). Acetic anhydride (5.2 mmol, 4 equiv) was added to a solution of **16** (corresponding to 1.26 mmol of diol **15**) in dry pyridine (8 mL) at 0 °C. The reaction mixture was stirred for 3 h at rt then concentrated. A solution of the residue in chloroform was washed with water, dried ($MgSO_4$), and concentrated. Column chromatography (chloroform/acetone, 10/1) gave **17** (696 mg, 100%), mp 108–109 °C (ethyl acetate/hexane). $[\alpha]_D$ +80 (*c* 0.7, chloroform). ^{1}H NMR: δ 7.50–7.25 (m, 20H, arom), 5.25 (m, 1H, $J_{3a,4}$ = 4 Hz, $J_{3e,4}$ = 4 Hz, $J_{4,5}$ = 2 Hz, H-4), 4.65 (AB, 2H, CH_2Ph), 4.45 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 3.95 (m, 1H, $J_{5,6a}$ = 9 Hz, $J_{5,6b}$ = 5 Hz, H-5), 3.55 (m, 1H, $J_{2,3e}$ = 4 Hz, $J_{2,3a}$ = 4 Hz, H-2), 3.50 (s, 3H, OCH_3), 3.45–3.30 (m, 2H, $J_{6a,6b}$ = 15 Hz, H-6a,6b), 2.40 (dt, 1H, $J_{3a,3e}$ = 15 Hz, H-3e), 1.75 (dt, 1H, H-3a). Anal. calcd for $C_{35}H_{36}O_6$ (552.669): C, 76.06; H, 6.57. Found: C, 75.58; H, 6.97.

Methyl 4-*O*-acetyl-2-*O*-benzyl-3-deoxy-β-L-*lyxo*-hexopyranoside (18). Sixty percent aq perchloric acid (6 mmol, 10 equiv) was added to a solution of **17** (330 mg, 0.6 mmol) in dichloromethane (12 mL), at 0 °C. The reaction mixture was stirred for 2 min then poured into a dropping funnel containing cold water and dichloromethane. The organic layer was washed with water, dried ($MgSO_4$), and concentrated. Column chromatography (chloroform/acetone, 10/1) gave first **18** (130 mg, 70%). $[\alpha]_D$ +58 (*c* 0.6. chloroform). ^{1}H NMR: δ 7.45–7.35 (m, 5H, arom), 4.95 (m, 1H, $J_{3e,4}$ = 4.5 Hz, $J_{3a,4}$ = 4 Hz, $J_{4,5}$ = 2.5 Hz, H-4), 4.72 (AB, 2H, CH_2Ph), 4.50 (d, 1H, $J_{1,2}$ = 2 Hz, H-1), 3.90 (m, 1H, $J_{5,6a}$ = 5 Hz, $J_{6a,6b}$ = 12 Hz, H-6a), 3.83 (1H, $J_{5,6b}$ = 6 Hz, H-5), 3.70 (1H, H-6b), 3.65 (1H, $J_{2,3e}$ = 4.5 Hz, $J_{2,3a}$ = 4 Hz, H-2), 3.62 (s, 3H, OCH_3), 2.52 (s, 1H, OH), 2.40 (dt, $J_{3a,3e}$ 15 Hz, H-3e), 2.10 (s, 3H, OAc), 1.70 (dt, H-3a). MS: 328 (M + 18), 311 (M + 1), 300, 296, 279. Anal. calcd for $C_{16}H_{22}O_6$ (310.348): C, 61.92; H, 7.15. Found: C, 61.93; H, 7.20.

Then **19** was eluted (18 mg, 10%).

Methyl 6-*O*-acetyl-2-*O*-benzyl-3-deoxy-β-L-*lyxo*-hexopyranoside (19). Melting point 50–51 °C (methanol). $[\alpha]_D$ +72 (*c* 0.7, chloroform). ^{1}H NMR: δ 7.45–7.30 (m, 5H, arom), 4.80 (AB, 2H, CH_2Ph), 4.45–4.35 (m, 3H, H-1,6a,6b), 3.80–3.70 (m, 3H, H-2,4,5), 3.60 (s, 3H, OCH_3), 2.35 (dt, 1H, $J_{3a,3e}$ = 15 Hz, $J_{2,3e}$ = 2.5 Hz, $J_{3e,4}$ = 2.5 Hz, H-3e), 2.10 (s, 3H, OAc), 1.70 (dt, 1H, $J_{2,3a}$ = 2.7 Hz, $J_{3a,4}$ = 2.7 Hz, H-3a). Anal. calcd for $C_{16}H_{22}O_6$ (310.348): C, 61.92; H, 7.15. Found: C, 62.04; H, 7.28.

Methyl (methyl 4-*O*-acetyl-2-*O*-benzoyl-3-deoxy-β-L-*lyxo*-hexopyranosyl)uronate (20). A mixture of **18** (220 mg, 0.7 mmol), ruthenium (III) chloride hydrate (3 mg, 0.015 mmol, 2.2% equiv), sodium periodate (600 mg, 2.8 mmol, 4 equiv), water (9 mL), acetonitrile (6 mL) and tetrachloromethane (6 mL) was vigorously stirred for 30 h at room temperature then diluted with chloroform. The organic layer was separated, the aq layer was treated twice with chloroform. The organic solutions were pooled and concentrated. A solution of the residue in ether (5 mL) was treated with an excess of diazomethane in ether, at 0 °C, and concentrated. Column chromatography (ethyl acetate/hexane, 1/1) gave **20** (135 mg, 54%), mp 175 °C (ethyl acetate). $[\alpha]_D$ +122 (*c* 0.5, chloroform). ^{1}H NMR: 8.20 and 7.60–7.50 (m, 5H, arom), 5.35–5.30 (m, 2H, H-2,4), 4.67 (d, 1H, $J_{1,2}$ = 1.5 Hz, H-1), 4.43 (d, 1H, $J_{4,5}$ = 2 Hz, H-5), 3.85 (s, 3H, CO_2CH_3), 3.65 (s, 3H, OCH_3), 2.60 (dt, 1H,

$J_{3a,3e}$ = 15 Hz, $J_{2,3e}$ = 3.5 Hz, $J_{3e,4}$ = 3.5 Hz, H-3e), 2.13 (dt, 1H, $J_{2,3a}$ = 4 Hz, $J_{3a,4}$ = 4 Hz, H-3a), 1.90 (s, 3H, OAc). Anal. calcd for $C_{17}H_{20}O_8$ (352,342): C, 57.95; H, 5.72. Found: C, 58.03; H, 5.68.

Methyl 4-*O*-acetyl-2-*O*-benzoyl-1-chloro-1,3-di-deoxy-α-L-*lyxo*-hexopyranosyl uronate (21). A solution of **20** (100 mg, 0.28 mmol) and a catalytic amount of anhydrous zinc chloride in dichloromethyl methyl ether (5 mL) was heated at 60 °C for 5 h, diluted with toluene, filtered and concentrated. The residue was chromatographed to give **21** (68 mg, 67%). ^{1}H NMR: δ 8.15–7.53 (m, 5H, arom), 6.40 (s, 1H, H-1), 5.47 (m, 1H, H-4), 5.25 (m, 1H, H-2), 4.92 (d, $J_{4,5}$ = 2 Hz, H-5), 3.85 (s, 3H, CO_2CH_3), 2.65 (dt, 1H, J_{gem} = 16 Hz, $J_{2,3a}$ = 3.5 Hz, H-3a), 2.46 (dm, 1H, J_{gem} = 16 Hz, H-3e), 1.97 (s, 3H, OAc).

Methyl 6-*O*-benzoyl-3-*O*-benzyl-2-(benzyloxycarbonyl)-amino-2-deoxy-4-*O*-(methyl-4-*O*-acetyl-2-*O*-benzoyl-3-deoxy-α-L-*lyxo*-hexopyranosyluronate)-α-D-glucopyranoside (23). A mixture of **21** (prepared and directly engaged, from 480 mg, 1.4 mmol of **20**), methyl 6-*O*-benzoyl-3-*O*-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside **22** (1.54 mmol, 1.1 equiv), and 4 Å molecular sieves, in anhydrous dichloromethane (5 mL) was stirred for 40 min at room temperature under argon then cooled to −15 °C. Silver triflate (400 mg, 1.54 mmol) was added, and the reaction mixture was stirred in the dark for 3 h, diluted with CH_2Cl_2, filtered, and concentrated. Column chromatography (chloroform/acetone, 50/1) and crystallisation from ethyl acetate gave **23**, (413 mg, 35% from **20**), mp 136 °C (ethyl acetate). $[\alpha]_D$ +52 (*c* 0.4, chloroform). ^{1}H NMR: δ 8.08, 7.98, 7.53–7.28 (m, 20H, arom), 5.33 (s, 1H, H-1), 5.03 (m, 4H, CO_2CH_3, NH, H-4′), 4.95 (m, 1H, H-2′), 4.90 (d, 1H, $J_{4',5'}$ = 2.2 Hz, H-5′), 4.86 (dd, 1H, J_{gem} = 12 Hz, $J_{5,6a}$ = 2.2 Hz, H-6a), 4.73 (AB, CH_2Ph), 4.69 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.44 (dd, 1H, J_{gem} = 12 Hz, $J_{5,6b}$ = 4.5 Hz, H-6b), 4.15 (dt, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10 Hz, $J_{2,NH}$ = 10 Hz, H-2), 4.10 (t, 1H, $J_{3,4}$ = 10 Hz, $J_{4,5}$ = 10 Hz, H-4), 3.97 (ddd, 1H, $J_{4,5}$ = 10 Hz, $J_{5,6a}$ = 2.2 Hz, $J_{5,6b}$ = 4.5 Hz, H-5), 3.77 (t, 1H, $J_{2,3}$ = 10 Hz, $J_{3,4}$ = 10 Hz, H-3), 3.45 (s, 3H, CO_2CH_3), 3.40 (s, 3H, OCH_3), 2.32 (dm, 1H, J_{gem} = 16 Hz, H-3′e), 2.21 (dt, 1H, J_{gem} = 16 Hz, $J_{2,3a}$ = 4 Hz, $J_{3a,4}$ = 4 Hz, H-3′a), 1.91 (s, 3H) OAc). MS: 859 (M + 17), 842 (M), 828, 752, 391. Anal. calcd for $C_{45}H_{47}O_{15}N$ (841.869): C, 64.20 H, 5.63. Found: C, 64.33 H, 5.66.

Methyl 6-*O*-benzoyl-3-*O*-benzyl-2-(benzyloxycarbonyl)-amino-2-deoxy-4-*O*-(methyl-2-*O*-benzoyl-3-deoxy-α-L-*lyxo*-hexopyranosyluronate)-α-D-glucopyranoside (24). A solution of hydrogen chloride in methanol (prepared from 1.5 mL of acetyl chloride and 2.2 mL of methanol) was added to a solution of **23** (700 mg, 0.83 mmol) in anhydrous dichloromethane (12 mL). The reaction mixture was stirred for 3 days at room temperature, washed with aq saturated $NaHCO_3$, water, dried ($MgSO_4$), and concentrated. Column chromatography on silica gel (ethyl acetate/hexane, 1/1) gave **24**, (220 mg, 33%). $[\alpha]_D$ +47 (*c* 0.6, chloroform). ^{1}H NMR: δ 8.08, 7.88, 7.55–7.25 (m, 20H, arom), 5.34 (s, 1H, H-1′), 5.03–5.01 (m, 3H, CO_2CH_2Ph, NH,), 5.01 (m, 1H, H-2′), 4.90 (dd, 1H, J_{gem} = 12 Hz, $J_{5,6a}$ = 2.0 Hz, H-6a), 4.83 (d, 1H, $J_{4',5'}$ = 1 Hz, H-5′), 4.78–4.60 (AB, CH_2Ph), 4.69 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.45 (dd, 1H, J_{gem} = 12 Hz, $J_{5,6b}$ = 4.5 Hz, H-6b), 4.16 (dt, 1H, $J_{1,2}$ = 3.5 Hz, $J_{2,3}$ = 10 Hz, $J_{2,NH}$ = 10 Hz, H-2), 4.10 (t, 1H, $J_{3,4}$ = 10 Hz, $J_{4,5}$ = 10 Hz, H-4), 3.98 (ddd, 1H, $J_{4,5}$ = 10 Hz, $J_{5,6a}$ = 2.0 Hz, $J_{5,6b}$ = 4.5 Hz, H-5), 3.94 (m, 1H, H-4′), 3.78 (t, 1H, $J_{2,3}$ = 10 Hz, $J_{3,4}$ = 10 Hz, H-3), 3.53 (s, 3H, CO_2CH_3), 3.38 (s, 3H, OCH_3), 2.23 (m, 2H, H-3′e, H-3′a). MS: 817 (M + 17), 801 (M + 1), 769, 522. Anal. calcd for $C_{43}H_{45}O_{14}N$ (799.831): C, 64.57; H, 5.67; N, 1.75. Found: C, 64.54; H, 5.62; N, 1.70.

Methyl *O*-(6-*O*-acetyl-2-azido-3,4-di-*O*-benzyl-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl-2,3-di-*O*-benzyl-β-D-glucopyranosyluronate)-(1→4)-*O*-(3,6-di-*O*-acetyl-2-azido-2-deoxy-α-D-glucopyranosyl)-(1→4)-*O*-(methyl 2-*O*-benzoyl-3-deoxy-α-L-*lyxo*-hexopyranosyluronate)-(1→4)-6-*O*-benzoyl-3-*O*-benzyl-2-(benzyloxycarbonyl)amino-2-deoxy-α-D-glucopyranoside (26). A solution of **25** (340 mg, 0.28 mmol) and **24** (225 mg, 0.28 mmol), in dry CH_2Cl_2 (7 mL) containing 4 Å molecular sieves (500 mg), was stirred 30 min at room temperature under argon then cooled to −20 °C. A 0.04 M solution of trimethylsilyl trifluoromethanesulfonate (490 μL, 0.019 mmol) in CH_2Cl_2 was added. After 30 min the mixture was neutralised with solid $NaHCO_3$, filtered, and concentrated. The residue was chromatographied on Sephadex LH 20 ($CHCl_3$/methanol, 1/1). Further purification was achieved on silica gel ($CHCl_3$/EtOAc, 7/1) yielding **26** (334 mg, 64%) as a white foam. $[\alpha]_D$ + 81 (*c* 1.2, $CHCl_3$). ^{1}H NMR: δ 7.42–7.28 (m, H, arom.), 5.53 (d, 1 H, $J_{1,2}$ = 3.8 Hz, H-1D), 5.46 (d, 1 H, $J_{1,2}$ = 2.1 Hz, H-1G), 5.33 (dd, 1 H, $J_{2,3}$ = 10.8 Hz, $J_{3\text{-}4}$ = 9.1 Hz, H-3F), 5.03 (2d, 2 H, $J_{1,2}$ = 3.5 Hz, H-1F and H-1H), 4.36 (d, 1 H, $J_{1,2}$ = 7.8 Hz, H-1E), 3.86 (d, 1 H, $J_{4\text{-}5}$ = 9.7 Hz, H-5E), 3.76 (s, 3 H, COOMe), 3.49 (dd, 1 H, $J_{2,3}$ = 9.1 Hz, H-2E), 3.45 (s, 6 H, OMe and COOMe), 3.34 (dd, 1 H, $J_{2,3}$ = 10.4 Hz, H-2D), 3.21 (dd, 1 H, $J_{2,3}$ = 10.8 Hz, H-2F), 2.50–2.42 (m, 1 H, H-3′G); 2.14–2.10 (m, 1 H, H-3G), 2.10, 2.06, 2.05 (3 s, 9 H, Ac). MS-ESI, positive mode: $(M + Na)^+$ *m*/*z* 1873.9, $(M + 2Na)^{2+}$ *m*/*z* 948.6. Anal. calcd. for $C_{96}H_{103}N_7O_{31}$: C, 62.30; H, 5.61; N, 5.30. Found: C, 62.03; H, 5.62; N, 5.33.

Methyl *O*-(2-deoxy-2-*N*-sulfonato-6-*O*-sulfonato-α-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyluronate)-(1→4)-*O*-(2-deoxy-2-*N*-sulfonato-3,6-di-*O*-sulfonato-α-D-glucopyranosyl)-(1→4)-*O*-(3-deoxy-2-*O*-sulfonato-α-L-

***lyxo*-hexopyranosyluronate)-(1→4)-*O*-2-*N*-sulfonato-6-*O*-sulfonato-α-D-glucopyranoside, decasodium salt (30).** (i) Saponification. An aq solution of H_2O_2 (30%, 8.5 mL) was added to a cooled (−5 °C) solution of **26** (0.387 g, 0.21 mmol) in THF (21 mL), and aq LiOH (0.7 M, 5 mL) was introduced dropwise. After 16 h at room temperature methanol (20 mL) and aq NaOH (4 M, 5.5 mL) were added. Twenty-four hours later the mixture was acidified (6 M aq HCl) and diluted with H_2O. The compound was extracted with CH_2Cl_2, washed with 10% aq Na_2SO_3, H_2O, dried, and concentrated to give white a powder (0.282 g,90%). Tlc: $R_f = 0.55$, EtOAc/pyridine/acetic acid/water, 12/2/0.6/1. (ii) *O*-Sulfonation. A solution of the above compound in dry DMF (8.0 mL) and $Et_3N{:}SO_3$ complex (0.860 g, 4.75 mmol) was heated at 50 °C for 20 h. After cooling aq $NaHCO_3$ (1.6 g in 19 mL of H_2O) was added. After 16 h stirring, evaporation to dryness gave a white residue that was treated with CH_2Cl_2/methanol, 1/1 (40 mL). After filtration and concentration, the solid residue was treated with CH_2Cl_2/methanol, 8/2 (25 mL), and after filtration the solution was concentrated. (iii) Hydrogenation. A solution of the *O*-sulfonated compound in *tert*-butyl alcohol/water, 13/20 (33 mL) was stirred with 10% Pd/C (300 mg), under hydrogen, for 48 h then filtered and concentrated. The treatment was repeated (at which point no signals of benzyl group were detected by ^{1}H NMR). (iv) *N*-sulfonation. The hydrogenated compound was dissolved in water (10 mL) and the pH was adjusted to 9.5 with 2 M NaOH. Sulfur trioxide–pyridine complex (89.0 mg, 0.57 mmol) was added, and the pH was maintained to 9.5 by addition of 2 M NaOH. After 1 h at room temperature more complex (89.0 mg, 0.57 mmol) was added. The mixture was chromatographed on Sephadex G-25 (500 mL) equilibrated with 0.2 N NaCl. The fractions containing the expected product were pooled and purified on Q Sepharose fast-flow (100 mL) using a NaCl gradient (0.75–1.2 M). Pooled fractions were desalted on Sephadex G-25 (500 mL) and lyophilised to give **30** (113 mg, 35% over four steps). $[\alpha]_D$ +63 (*c* 0.2, H_2O). ^{1}H NMR: see Table 2. MS-ESI, negative mode: monoisotopic mass: 1710.7; chemical mass: 1712,09; experimental mass: 1711.6.

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