

Note

Analysis of glycosaminoglycan-derived oligosaccharides using fast-atom-bombardment mass-spectrometry

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Glycosaminoglycans (GAGs) are sulfated, linear polysaccharides found in mammalian tissues¹ that play an important role in cell–cell interaction² and demonstrate a wide variety of other important biological activities³. Polysaccharide lyases^{4,5} can break down these GAGs into smaller oligosaccharides that exist in an ionized form and are usually isolated as sodium salts for structure determination^{6–8}.

Carbohydrate structure determination is increasingly relying on spectroscopic methods, such as n.m.r. spectroscopy⁹ and fast-atom-bombardment mass-spectrometry (f.a.b.–m.s.)¹⁰. In the absence of large amounts of sample, negative-ion f.a.b.–m.s. analysis may represent one of the best methods for obtaining structural information on complex acidic oligosaccharides^{8,11,12}. Recently, we reported a method of performing f.a.b.–m.s. analysis on the underivatized sodium salts of sulfated oligosaccharides obtained from heparin using triethanolamine⁸, as the liquid f.a.b. matrix, in the negative-ion mode.

In the present work, we examined the ability to extend the mass range of this analytical method for the analysis of larger, heparin-derived oligosaccharides. In addition, a variety of disaccharides obtained from different GAGs were analyzed by f.a.b.–m.s., thus demonstrating the versatility of this technique.

Disaccharides derived from chondroitin or dermatan sulfate, and heparin or heparan sulfate were prepared or commercially obtained[†]. Each of the thirteen disaccharides analyzed by f.a.b.–m.s. gave appropriate molecule-ion peaks assignable to $[M + Na_x - H_{x+1}]^-$, $x = 0, 1, \dots, 4$ (Table I). Molecule ion peaks are easily observed with 20 nanomoles of sample. The most characteristic fragment ion observed^{8,12} in the spectra of sulfated oligosaccharides is associated with the loss of sulfation corresponding to $[M + Na_x - NaSO_3 - H_x]^-$. These disaccharides, prepared from chondroitin, dermatan

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† For structures 1–13, see Table I.

TABLE I

F.a.b.-m.s. data for oligosaccharides 1-13^a

Disaccharide	Molecule ions ^b (x)				Fragment ions
	0	1	2	3	4
(1) β -D-GlcAp-(1 \rightarrow 3)- α -D-GalpNH ₂	354				193 [M-HexN] ⁻ 175 [M-HexN-H ₂ O] ⁻ 175 [M-HexNAc] ⁻ 175 [M-HexNAc] ⁻ 378 [M+Na-NaSO ₃ -H] ⁻ 300 [M- Δ UA] ⁻ 378 [M+Na-NaSO ₃ -H] ⁻ 300 [M- Δ UA] ⁻
(2) Δ UAp-(1 \rightarrow 3)- α -D-GlcpNAc	378	400			378 [M+Na-NaSO ₃ -H] ⁻ 277 [M+Na-HexNAc4S-H] ⁻
(3) Δ UAp-(1 \rightarrow 3)- α -D-GalpNAc	378	400			277 [M+Na-HexNAc4S-H] ⁻ 458 [M+Na-NaSO ₃ -H] ⁻ 277 [M+Na-HexNAc6S] ⁻ 300 [M- Δ UA2S] ⁻ 458 [M+Na-NaSO ₃ -H] ⁻ 402 [M- Δ UA] ⁻
(4) Δ UAp-(1 \rightarrow 3)- α -D-GalpNAc4S	458	480	502		438 [M-2Na-NaSO ₃ -2H] ⁻ 416 [M+Na-NaSO ₃ -H] ⁻ 540 [M+3Na-NaSO ₃ -3H] ⁻ 360 [M+Na- Δ UA2S-H] ⁻ 582 [M+3Na-NaSO ₃ -3H] ⁻ 560 [M+2Na-NaSO ₃ -2H] ⁻
(5) Δ UAp-(1 \rightarrow 3)- α -D-GalpNAc6S	458	480	502		
(6) Δ UAp2S-(1 \rightarrow 3)- α -D-GalpNAc	458	480	502		
(7) Δ UAp2S-(1 \rightarrow 3)- α -D-GalpNAc4S		560	582	604	
(8) Δ UAp2S-(1 \rightarrow 3)- α -D-GalpNAc6S		560	582	604	
(9) Δ UAp-(1 \rightarrow 3)- α -D-GalpNAc4S6S		560	582	604	
(10) Δ UAp-(1 \rightarrow 4)- α -D-GlcpNS6S		518	540	562	
(11) Δ UAp2S-(1 \rightarrow 4)- α -D-GlcpNS		518	540		
(12) Δ UAp2S-(1 \rightarrow 4)- α -D-GlcpNS6S			620	642	664
(13) Δ UAp2S-(1 \rightarrow 3)- α -D-GalpNAc4S6S		640	662	684	

^a Abbreviations: Δ UAp, 4-deoxy- α -L-threo-hex-4-enopyranosyluronic acid; S, SO₃H⁻; NS, NHSO₃H⁻; [M + Na_x - H_{x+}]⁻.

sulfate, heparin, and heparan sulfate, were examined in an effort to understand whether sulfate groups were lost preferentially from specific positions. For example, a *O*-sulfate group is found at C-4 and C-6 in D-galactosamine units, and at C-2 of the unsaturated uronic acid unit in chondroitin and dermatan sulfate disaccharides. In heparin and heparan sulfate disaccharides, *N*-sulfate at C-2 and *O*-sulfate groups at C-3 and C-6 of the D-glucosamine unit, and at C-2 of the unsaturated uronic acid unit are commonly observed. The mass spectra of monosulfated disaccharides 4–6 demonstrated the presence of only weak fragment-ions corresponding to $[M + Na_x - NaSO_3 + H_x]^-$. The low intensity of these fragment ions can be contrasted to the intense $[M + Na_x - NaSO_3 - H_x]^-$ fragment ions observed in the disulfated (7–11), and trisulfated (12 and 13) disaccharides.

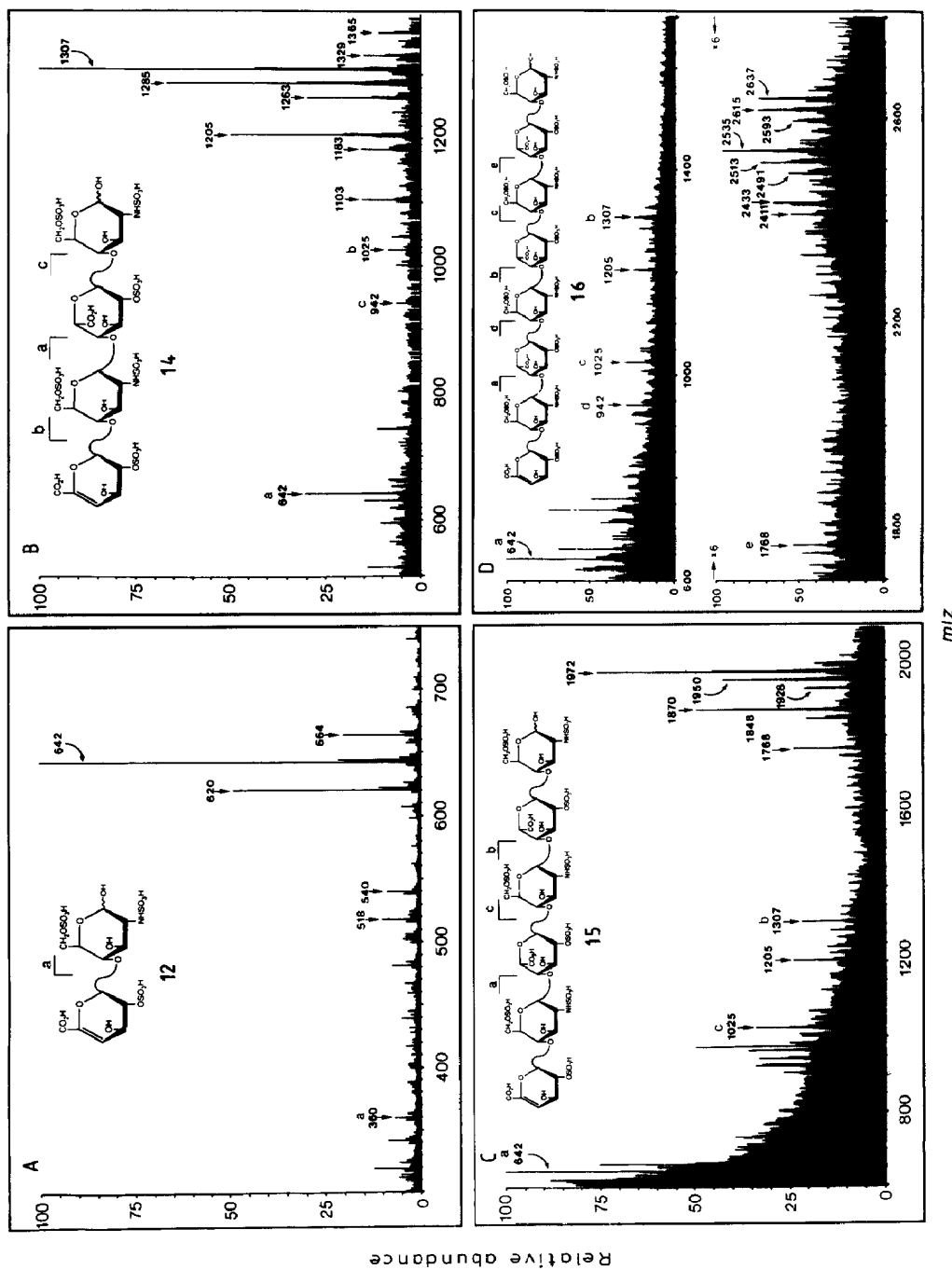
Based on these data, one might suggest that the weak $[M + Na_x - NaSO_3 - H_x]^-$ fragment-ion observed in the spectrum of the monosulfated disaccharides is due to reduced sensitivity of the negative-ion f.a.b.–m.s. analysis for ions having no sulfate groups. Unsulfated disaccharides 1, 2, and 3 did, however, give intense $[M - H]^-$ molecule ions. In addition, an equimolar mixture of unsulfated disaccharide 3 and monosulfated disaccharide 4 gave molecule ions of approximately equal intensity at m/z 378 and 480. This suggested that the reduced intensity of the $[M + Na_x - NaSO_3 - H]^-$ fragment ion observed in the f.a.b.–m.s. spectra of monosulfated disaccharides is not primarily the result of our ability to detect the unsulfated ion.

Many of the disaccharides analyzed also produced fragment ions under negative-ion f.a.b. conditions resulting from cleavage of glycosyl linkages, thus providing information on the saccharide sequence (Table I).

Examination of a homologous series of oligosaccharides based on the major repeating trisulfated disaccharide comprising heparin was undertaken to provide insight into common fragmentation pathways while extending the mass range of this mass spectral technique (Fig. 1). The f.a.b.–m.s. spectrum of heparin trisulfated disaccharide 12 showed molecule ions at m/z 664, 642, and 620, corresponding to $[M + 4 Na - 5 H]^-$, $[M + 3 Na - 4 H]^-$, and $[M + 2 Na - 3 H]^-$, respectively (Fig. 1A). In addition, fragment ions at m/z 540, 518, and 360 corresponding to $[M + 3 Na - NaSO_3 - 3 H]^-$, $[M + 2 Na - NaSO_3 - 2 H]^-$, and $[M + Na - \Delta UA2S - H]^{-*}$ are observed. To demonstrate the value of high resolution f.a.b.–m.s. (particularly useful for identifying unknown oligosaccharides), disaccharide 12 was analyzed. This analysis gave an accurate mass of 663.89260 (calc. 663.89130 for $C_{12}H_{14}NNa_4O_{19}S_3$ $[M + 4 Na - 5 H]^-$; $\Delta m = -1.3$ mmu).

Heparin-derived tetrasaccharide 14, hexasaccharide 15, and octasaccharide 16 were then analyzed (Figs. 1B–D) to examine the ability of f.a.b.–m.s. to analyze higher-molecular-weight oligosaccharides and to examine similarities in fragmentation patterns obtained in a homologous series of oligosaccharides. These are based on the repeating trisulfated disaccharide 12, representing the major structural unit in the heparin polysaccharide. Tetrasaccharide hexasulfate 14 (Fig. 1B) showed molecule ions

* Abbreviations: ΔUA , 4-deoxy- α -L-threo-hex-4-enopyranosyluronic acid; 2S, sulfonic acid group at C-2.



at m/z 1329, 1307, 1285, and 1263, corresponding to $[M + Na_x - H_{x+1}]^-$, where $x = 8-5$. Fragment ions at m/z 1205 and 1103 result from the loss of sulfate groups and correspond to $[M + 7 Na - NaSO_3 - 7 H]^-$ and $[M + 7 Na - 2 NaSO_3 - 6 H]^-$, respectively. A sodium chloride cluster with the molecule ion is observed at m/z 1365 corresponding to $[M + 7 Na - 8 H + NaCl]^-$. Fragment ions associated with the scission of glycosyl linkages were at m/z 1025, 942, and 642, corresponding to $[M + 5 Na - \Delta UA2S - 5 H]^-$, $[M + 5 Na - HexNS6S - 5 H]^{-*}$, and $[M + 3 Na - \{UA2S-(1 \rightarrow 4)-HexNS6S\} - 3 H]^-$.

Hexasaccharide nonasulfate **15** (Fig. 1 C) showed molecule ions at m/z 1972, 1950, and 1928 corresponding to $[M + 11 Na - 12 H]^-$, $[M + 10 Na - 11 H]^-$, and $[M + 9 Na - 10 H]^-$. Fragment ions at m/z 1870, 1848, and 1768 correspond to $[M + 11 Na - NaSO_3 - 11 H]^-$, $[M + 10 Na - NaSO_3 - 10 H]^-$, and $[M + 11 Na - 2 NaSO_3 - 10 H]^-$, respectively. Loss of a disaccharide unit is observed as fragment ions at m/z 1307 and 1205 assigned to $[M + 7 Na - \{\Delta UA-(1 \rightarrow 4)-HexNS6S\} - 8 H]^-$ and $[M + 7 Na - \{\Delta UA(1 \rightarrow 4)-HexNS6S\} - NaSO_3 - 7 H]^-$, respectively. Loss of three sugar residues was observed as a fragment ion at m/z 1025, assigned to $[M + 5 Na - \{\Delta UA2S-(1 \rightarrow 4)-HexNS6S-(1 \rightarrow 4)-UA2S\} - 5 H]^-$. Loss of a tetrasaccharide is seen as a fragment ion at m/z 642 assigned as $[M + 3 Na - 3 H - \{UA2S-(1 \rightarrow 4)-HexNS6S-(1 \rightarrow 4)-UA2S-(1 \rightarrow 4)-HexNS6S\}]^-$. This also corresponds to a disaccharide **12** subunit.

Octasaccharide dodecasulfate **16** (Fig. 1D) showed molecule ions at m/z 2637, 2615, and 2593, corresponding to $[M + 15 Na - 16 H]^-$, $[M + 14 Na - 15 H]^-$, and $[M + 13 Na - 14 H]^-$, respectively. Fragment ions at m/z 2535 and 2513 correspond to $[M + 15 Na - NaSO_3 - 15 H]^-$ and $[M + 14 Na - NaSO_3 - 14 H]^-$, respectively. Loss of a tetrasaccharide gave fragment ions at m/z 1307 and 1205, assigned to $[M + 7 Na - 7 H - \{\Delta UA2S-(1 \rightarrow 4)-HexNS6S-(1 \rightarrow 4)-UA2S-(1 \rightarrow 4)-HexNS6S\}]^-$ and $[M + 7 Na - 7 H - \{UA2S-(1 \rightarrow 4)-HexNS6S-(1 \rightarrow 4)-UA2S-(1 \rightarrow 4)-HexNS6S\} - NaSO_3]^-$. Loss of five sugar residues could be seen as a fragment ion at m/z 1025 and 942, corresponding to $[M + 5 Na - 5 H - \{HexNS6S-(1 \rightarrow 4)-UA2S-(1 \rightarrow 4)-HexNS6S-(1 \rightarrow 4)-UA2S-(1 \rightarrow 4)-HexNS6S\}]^-$ and $[M + 5 Na - 5 H - \{UA2S-(1 \rightarrow 4)-HexNS6S-(1 \rightarrow 4)-UA2S-(1 \rightarrow 4)-HexNS6S-(1 \rightarrow 4)-UA2S\}]^-$. Finally, the fragment ion at m/z 642 resulted from the loss of a hexasaccharide and was assigned to $[M + 3 Na - \{UA2S-(1 \rightarrow 4)-HexNS6S-(1 \rightarrow 4)-UA2S-(1 \rightarrow 4)-HexNS6S-(1 \rightarrow 4)-UA2S-(1 \rightarrow 4)-HexNS6S\} - 3 H]^-$.

Fragmentation patterns of the oligosaccharide homologs were similar and easily interpretable, and generally resulted in sufficient information to definitively assign the sequence. The heparin-derived octasaccharide having twelve sulfate groups and sixteen negative charges represents the largest oligosaccharide yet analyzed in our laboratory. A molecular ion of m/z 2637 and its characteristic fragmentation pattern confirmed the

* Abbreviations: Hex, hexose; HexN, hexosamine; NS, 2-deoxy-2-sulfamido.

Fig. 1. Negative-ion f.a.b. spectra of sulfated oligosaccharides from heparin **12** (A), **14** (B), **15** (C), and **16** (D). The molecule ions are at m/z 642 (A), 1307 (B), 1972 (C), and 2637 (D). A sixfold scaling of the y-axis is used $> m/z$ 1700 in spectrum D. The fragment ions carry the glycosyl oxygen atom.

structure assigned to it by use of both enzymic methods and high-field ^1H -n.m.r. spectroscopy. It may be possible to analyze even larger oligosaccharide sulfates by f.a.b.-m.s. if the sample is available in sufficient amount and its purity (lack of both saccharidic and salt contaminants) can be assured.

EXPERIMENTAL

Materials. — Heparin sodium salt, from bovine lung (160 units/mg), was obtained from Sigma Chemical Co., St. Louis, MO. Heparin lyase (EC 4.2.2.7) was purified¹³ from *Flavobacterium heparinum* (5 munits/ μg ; 1 munit = 1 nmol of product formed/min). Chondroitin sulfate, dermatan sulfate, and hyaluronic acid disaccharides were purchased from Seikagaku America. Triethanolamine, used as the f.a.b.-m.s. matrix, and chondrosine were obtained from Sigma Chemical Co. Deuterium oxide and 4,4-dimethyl-4-sila-(2,3- $^2\text{H}_4$)pentanoate [$(^2\text{H}_4)$ -TSP] were from Aldrich Chemical Co. (Milwaukee, WI). All reagents used in electrophoresis were from Fisher Chemical Co. (Fairlawn, NJ).

General methods. — U.v. spectra were recorded with a Shimadzu Model UV-160 spectrophotometer, ^1H -n.m.r. spectra at 360 MHz with a Bruker WM360 and at 600 MHz with a Bruker AMX-600 spectrometer, and mass spectra with a VG Analytical Ltd. ZAB-HF spectrometer. Strong-anion exchange, high-pressure liquid chromatography (SAX-h.p.l.c.) used dual, face programmable, Shimadzu (Kyoto, Japan) LC-7A titanium-based pumps. The system was equipped with a Rheodyne (Cotati, CA) #7125 titanium injector and a Pharmacia LKB (Piscataway, NJ) 2141 variable-wavelength, u.v. detector with a Shimadzu Chromatopac C-R2A integrating recorder. Preparative separations relied on a 2×25 cm SAX-h.p.l.c. column containing 5- μm particle size Spherisorb, and analytical separations on a 4.6 mm \times 25 cm column of identical packing, both from Phase Separations, Norwalk, CT. Electrophoresis was done on a Hoefer (San Francisco, CA) SE600 vertical-slab-gel unit equipped with a Bio-Rad (Richmond, CA) Model 1420B power source.

Depolymerization of heparin. — Heparin sodium salt (8 mg/mL) was depolymerized at 30° with the appropriate heparin lyase (120 munits/mL) in a solution of 0.2M NaCl and 5mM sodium phosphate at pH 7.0. The reaction was monitored by removing aliquots and measuring A_{232} after a 1:100 dilution into 0.03M HCl. A constant value of A_{232} in the presence of active enzyme indicated that the reaction was complete. The reaction was terminated, either before completion (in the preparation of higher oligosaccharides) or at completion (for the preparation of disaccharides) by thermally inactivating the enzyme (100° for 1 min).

Purification of heparin-derived oligosaccharides. — Disaccharides **11** and **12**, and tetrasaccharide **14** were prepared from the mixture of heparin-derived oligosaccharides by preparative SAX-h.p.l.c. as previously described⁵. Disaccharide **10** was obtained as the content of a very minor peak (1.5% of the final product mixture) which was eluted on analytical SAX-h.p.l.c. just prior to disaccharide **11**. Heparin-derived hexasaccharide **15** and octasaccharide **16** were obtained by partial depolymerization of heparin.

When the reaction was completed at ~ 80%, oligosaccharides **15** and **16** could be obtained in reasonable yields (~ 3.5 and 5.0% of the product mixture, respectively), being eluted late from the SAX-h.p.l.c. column⁵.

Oligosaccharide purity. — Oligosaccharides **2–16** contain an unsaturated uronic acid group (Δ UAp, 4-deoxy- α -threo-hex-4-enopyranosyluronic acid or Δ UAp2S, 4-deoxy- α -L-threo-hex-4-enopyranosyluronic acid 2-sulfate) at their nonreducing end terminus as a result of being prepared with a polysaccharide lyase⁴. The presence of this group permitted an assessment of oligosaccharide purity (*i.e.*, by detecting a single peak at A_{232} on analytical SAX-h.p.l.c.). Heparin-derived oligosaccharides **10–12** and **14–16** were analyzed by SAX-h.p.l.c. using a gradient of concentration Y [Y in molarity] at any time (X in s) = $0.0002 X + 0.2$] of NaCl, pH 3.5 at 1.5 mL/min⁵. Hyaluronic acid, chondroitin sulfate- and dermatan sulfate-derived disaccharides had a lower proportion of sulfate groups and, thus, were analyzed by use of a gradient of concentration Y [Y in molarity] at any time (X in sec) = $0.0002 X$] of NaCl, pH 3.5 at 1.5 mL/min^{14,15}. In addition, these commercial disaccharide standards were shown to be pure by the manufacturer using a Sphodex RS, type DC-613, 10 μ m, 150 \times 6 mm h.p.l.c. column and an isocratic elution at 1 mL/min with 13:3:4 (v/v) acetonitrile-methanol-0.5M ammonium formate, pH 4.5, and a RiChrosorb-NH₂, 250 \times 2.5 mm h.p.l.c. column, eluted at 1.5 mL/min with 16–400mM sodium phosphate and u.v. detection at A_{232} ¹⁶. Disaccharides **3**, **4**, **5**, **10**, and **12** also showed a single peak on reversed-phase, ion-pairing h.p.l.c. using suppressed conductivity detection¹⁴. Sulfated oligosaccharides **4–16** showed a single band on gradient poly(acrylamide) gel electrophoresis (PAGE) with Alcian Blue staining^{17,18}. Chondrosine (**1**) was obtained and used without any assessment of its purity.

Characterization. — Full ¹H-n.m.r. spectral data have already been published for heparin-derived oligosaccharides **12** and **14**, thus establishing their structure^{5,19,20}. ¹H-N.m.r. spectra were recorded for solutions in ²H₂O (99.996 atom %) with (²H₄)-TSP (99 + atom %) as internal standard. The 360-MHz ¹H-n.m.r. spectra of the standard disaccharides derived from hyaluronic acid, chondroitin sulfate, and dermatan sulfate (**1–9** and **13**) were consistent with the structures listed by the manufacturer.

Δ UAp-(1 \rightarrow 4)- α -D-GlcpNS6S (**10**). ¹H-N.m.r. (360 MHz, ²H₂O): δ 5.86 (d, 1 H, $J_{3,4}$ 3.72 Hz, H-4'), 5.48 (d, 1 H, $J_{1,2}$ 3.55 Hz, H-1), 5.22 (d, 1 H, J 5.45 Hz, H-1'), 4.39 (m, 1 H, J 11.1, 3.6 Hz, H-6b), 4.27 (d, 1 H, J 2.19 Hz, H-3'), 4.24 (m, 1 H, J 11.2, 3.6 Hz, H-6a), 4.16 (m, 1 H, J 9.89 Hz, H-5), 3.85 (m, 1 H, H-2'), 3.85 (dd, 1 H, J 5.44, 10.01 Hz, H-4), 3.74 (dd, 1 H, J 8.91, 10.30 Hz, H-3), and 3.30 (dd, 1 H, J 3.56, 10.40 Hz, H-2).

Δ UAp2S-(1 \rightarrow 4)- α -D-GlcpNS (11). ¹H-N.m.r. (360 MHz, ²H₂O): δ 5.99 (d, 1 H, J 3.79 Hz, H-4'), 5.53 (d, 1 H, J 2.43 Hz, H-1'), 5.46 (d, 1 H, J 3.52 Hz, H-1), 4.57 (m, 1 H, J 2.63 Hz, H-2'), 4.3 (m, 1 H, J 4.15 Hz, H-3'), 3.97 (m, 1 H, J 9.77 Hz, H-5), 3.84 (m, 2 H, J < 1.0 Hz, H-6), 3.80 (m, 1 H, J 9.31 Hz, H-4), 3.72 (m, 1 H, J 9.53 Hz, H-3), and 3.27 (dd, 1 H, J 3.54, 10.14 Hz, H-2).

Δ UAp2S-(1 \rightarrow 4)- α -D-GlcpNS6S-(1 \rightarrow 4)- α -L-IdoAp2S-(1 \rightarrow 4)- α -D-GlcpNS6S-(1 \rightarrow 4)- α -L-IdoAp2S-(1 \rightarrow 4)- α -D-GlcpNS6S (**15**). ¹H-N.m.r. (600 MHz, ²H₂O): δ 5.97 (d, J 4.2 Hz, H-4 of Δ UAp2S residue), 5.50 (d, J 2.1 Hz, H-1 of Δ UAp2S residue),

5.44–5.37 (m, 3 H, H-1 of GlcpNS6S residues), 5.24 (br. s, 2 H, H-1 of IdoAp2S residues), 4.61 (br. s, 1 H, H-2 of Δ UAp2S residue), and 3.31–3.23 (m, 3 H, H-2 of GlcpNS6S residues). Gradient PAGE analysis gave a single band¹⁸ corresponding to an oligosaccharide having an estimated¹⁷ mol. wt. of 2000. Exhaustive treatment of **15** with heparin lyase afforded 3 equivalents of disaccharide **12** by analytical SAX-h.p.l.c., further confirming its structure.

Δ UAp2S-(1 \rightarrow 4)- α -D-GlcpNS6S-(1 \rightarrow 4)- α -L-IdoAp2S-(1 \rightarrow 4)- α -D-GlcpNS6S-(1 \rightarrow 4)- α -L-IdoAp2S-(1 \rightarrow 4)- α -D-GlcpNS6S-(1 \rightarrow 4)- α -L-IdoAp2S-(1 \rightarrow 4)- α -D-GlcpNS6S (**16**). ¹H-N.m.r. (600 MHz, ²H₂O): δ 6.00 (br. d, 1 H, H-4 of Δ UAp2S residue), 5.51 (br. s, 1 H, H-1 of Δ UAp2S residue), 5.48–5.37 (m, 4 H, H-1 of GlcpNS6S residues), 5.24 (br. s, 3 H, H-1 of IdoAp2S residue), 4.62 (br. s, 1 H, H-2 of Δ UAp2S residue), and 3.32–3.25 (m, 4 H, H-2 of GlcpNS6S residues). Gradient PAGE analysis gave a single band¹⁸ corresponding to an oligosaccharide having an estimated mol. wt. of 2600. Exhaustive treatment of **16** with heparin lyase afforded 4 equivalents of disaccharide **12** by analytical SAX-h.p.l.c., further confirming its structure.

Fast-atom-bombardment mass-spectrometry. — Mass spectra were obtained with a VG ZAB-HF instrument in the fast-atom-bombardment (f.a.b.) ionization mode. The f.a.b. ion source used was a standard VG Analytical, Inc. system, equipped with a saddle-field atom gun. Xe was used for the bombarding fast-atom beam. Typical operating conditions were: Beam energies of 8 keV and neutral beam currents equivalent to 1.5 mA, supplied by an ION TECH (Model B 50) current and voltage regulator/meter. Negative-ion f.a.b. spectra were obtained by signal adding four or eight scans with the use of the multichannel analysis (MCA) software of the VG 11-250J data system.

The solutions of oligosaccharides were prepared for negative-ion f.a.b.-m.s. by dissolving the solid sample in doubly-distilled, de-ionized water to a concentration of 10–20 μ g/ μ L. Typically triethanolamine (2 μ L) was placed on a standard VG stainless-steel probe tip to which the oligosaccharide solution (1.5 μ L) was added.

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