Synthesis of Tumor-Associated Le^aLe^x hexasaccharides: Instability of a Thiol-containing Oligosaccharide in Mass Spectrometry and Hyper-metalation detected by ESI FAIMS.

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Table 1: listing of ions detected by ESI FAIMS HRMS for hexasaccharide 2

Ion	Molecular Formula	Calculated <i>m/z</i>	Measured Accurate ^a <i>m/z</i>	mass error (ppm)
[M + 2H] ²⁺	C ₄₆ H ₈₂ N ₂ O ₂₉	563.2496	563.2512	3
[M + H + NH ₄] ²⁺	C ₄₆ H ₈₅ N ₃ O ₂₉	571.7629	571.7635	1
[M + 2NH ₄] ²⁺	C ₄₆ H ₈₉ N ₄ O ₂₉	580.2762	580.2776	2
[M + H + Na] ²⁺	C ₄₆ H ₈₁ N ₂ O ₂₉ Na	574.2406	574.2431	4
[M + 2Na] ²⁺	C ₄₆ H ₈₀ N ₂ O ₂₉ Na ₂	585.2316	585.2345	5

Table 2: listing of ions detected by ESI FAIMS HRMS for hexasaccharide 3

Ion	Molecular Formula	Calculated <i>m/z</i>	Measured Accurate ^a <i>m/z</i>	mass error (ppm)
[M+2H] ²⁺	C ₄₆ H ₈₃ N ₃ O ₂₉	570.7551	570.7554	1
[M+H+NH ₄] ²⁺	C ₄₆ H ₈₆ N ₄ O ₂₉	579.2683	579.2693	2
[M+H+Na] ²⁺	C ₄₆ H ₈₂ N ₃ O ₂₉ Na	581.7460	581.7467	1

Table 3: listing of ions detected by ESI FAIMS HRMS for hexasaccharide 4

Ion	Molecular Formula	Calculated m/z	Measured Accurate ^a m/z	mass error (ppm)
$[M-H_2O-H+3Na]^{2+}$	$C_{46}H_{77}N_2O_{28}SNa_3$	603.2033	603.2016	-3
$[M-H_2O-2H+4Na]^{2+}$	$C_{46}H_{76}N_2O_{28}SNa_4$	614.1943	614.1937	-1
$[M-H_2O-3H+5Na]^{2+}$	$C_{46}H_{75}N_2O_{28}SNa_5$	625.1852	625.1863	2
$[M-H_2O-4H+6Na]^{2+}$	$C_{46}H_{74}N_2O_{28}SNa_6$	636.1762	636.1795	5
$[M-H_2O-2H+3Na+NH_4]^{2+}$	$C_{46}H_{80}N_3O_{28}SNa_3$	611.7171	611.7159	-2
$[M-H_2O-3H+4Na+NH_4]^{2+}$	$C_{46}H_{79}N_3O_{28}SNa_4$	622.7075	622.7076	0
$[M-H_2O-3H+3Na+2NH_4]^{2+}$	$C_{46}H_{83}N_4O_{28}SNa_3$	620.2298	620.2292	-1

Table 4: listing of ions detected by ESI FAIMS HRMS for LeX disulfide 23

Ion	Molecular Formula	Calculated <i>m/z</i>	Measured Accurate ^a <i>m/z</i>	mass error (ppm)
[M + 2H] ²⁺	C ₅₂ H ₉₄ N ₂ O ₃₀ S ₂	645.2661	645.2682	3
[M + H + NH ₄] ²⁺	C ₅₂ H ₉₇ N ₃ O ₃₀ S ₂	653.7794	653.7820	4
[M+ 2NH ₄] ²⁺	C ₅₂ H ₁₀₀ N ₄ O ₃₀ S ₂	662.2927	662.2942	2
[M + H + Na] ²⁺	C ₅₂ H ₉₃ N ₂ O ₃₀ S ₂ Na	656.2571	656.2581	2
[M + 2Na] ²⁺	C ₅₂ H ₉₂ N ₂ O ₃₀ S ₂ Na ₂	667.2481	667.2508	4

ESI FAIMS HRMS Compound **2** (see text for expansion)

ESI FAIMS HRMS Compound **3** (see text for expansion)

FIA 2015-JULY-10 MG193A 48 (9.699) Sm (SG, 2x3.00); Cm (45:49)

10-Jul-2015

TOF MS ES+
5.56e3

ESI FAIMS HRMS Compound **4** (see text for expansion)

ESI FAIMS HRMS Le^x-Hexyl disulfide **23** (see text for expansion)

ESI HRMS Compound 2

MG1.5.76.1/190A

FIAQ15410 377 (19.570) Sb (0,1.00); Sm (Mn, 2x0.50); Cm (376:382-162:168)

Scan ES+
9.20e5

ESI HRMS Compound 3

MG1.5.91.1/193A

FIAQ15409 45 (2.343) Sb (0,1.00); Sm (Mn, 2x0.50); Cm (44:48-24:28)

Scan ES+
7e6

ESI HRMS Compound **4** (see text for expansion)

MG.1.5.98.1/195A

FIAB15594 503 (9.588) AM (Cen,6, 80.00, Ht,10000.0,622.03,1.00); Sb (99,10.00); Sm (Mn, 3x3.00); Cm (502:508)

General Experimental Procedures

^1H NMR (600.13 or 400.13 MHz) and ^{13}C NMR (150.9 or 100.6 MHz) spectra were recorded for compounds solubilized in CDCl_3 (internal standard, for ^1H residual CHCl_3 δ_{H} 7.24; for ^{13}C CDCl_3 δ_{C} 77.0), CD_3OD (internal standard, for ^1H residual CD_2HOD δ_{H} 3.30; for ^{13}C CD_3OD δ_{C} 49.0) or D_2O [external standard, for ^1H (sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) δ_{H} 0.00; for ^{13}C δ_{H} 0.0)]. ^1H NMR and ^{13}C NMR chemical shifts are reported in part per million (ppm). Coupling constants (J) were obtained from a first-order analysis of one-dimensional spectra and are reported in hertz (Hz). Assignments of proton and carbon resonances were based on two dimensional ^1H – ^1H COSY and ^{13}C – ^1H HSQC correlation experiments as well as 1D TOCSY experiments. ^1H NMR data are reported using standard abbreviations: singlet (s), doublet (d), triplet (t), doublet of doublet (dd), doublet of doublet of doublet (ddd), triplet of doublet (td), broad signal (bs) and multiplet (m). Analytical thin-layer chromatography (TLC) was performed on aluminum plates precoated with Silica Gel 60 F_{254} (250 μm) containing a fluorescent indicator. The plates were visualized under UV and/or charred with a 20% solution of H_2SO_4 in EtOH. Compounds were purified by flash chromatography with Silica Gel 60 (230–400 mesh) unless otherwise stated. Solvents were distilled and dried according to standard procedures and organic solutions were dried over Na_2SO_4 and concentrated under reduced pressure. Molecular sieves were activated by heating at 300 $^\circ\text{C}$ overnight under reduced pressure over P_2O_5 . Zinc powder (2 g) was activated by successive washings with HCl 2 N (25 mL), H_2O (50 mL), MeOH (50 mL) and Et_2O (50 mL) on a fritted glass. The sonication reactions were carried out in a thermostated (50 ± 4 $^\circ\text{C}$) ultrasonic cleaning bath at 40 kHz and involved the partial immersion of the reaction vessel (10 mL round-bottomed flask equipped with a sealed glass cap) into the water tank (dimensions were $292 \times 152 \times 152$ mm with a liquid holding capacity of 5.71 L). Reverse-phase HPLC purifications were carried out on Prep Nova Pak[®] HR C18, 6 μm 60 $\text{\AA}$ columns using mixtures of HPLC grade acetonitrile and milli-Q water. Purifications by gel permeation chromatography were carried out on a Biogel P2 column (100×1 cm) eluted with water or 0.05 M aq AcONH_4 . Optical rotations were measured at 22 $^\circ\text{C}$ and are reported as follows: $[\alpha]_{\text{D}}$ (c in grams per 100 mL, solvent). Mass spectra were obtained under electrospray ionization (ESI) on a high resolution mass spectrometer equipped with a TOF analyzer.

Chemical structure of compound 10 is shown above the spectrum. The structure is a dimeric molecule consisting of two pyranose rings linked by an oxygen atom. The left ring has an AcO group at C2, a ClAcO group at C3, and an OAc group at C4. The right ring has an OBn group at C2, an NHAc group at C3, and an OAc group at C4. The two rings are connected via an oxygen atom at C1 of the left ring and C1 of the right ring.

¹H NMR spectrum (CDCl₃) of compound 10. The x-axis represents chemical shift in ppm, ranging from 0 to 10. The spectrum shows several peaks, with integration values provided below the baseline: 5.37, 0.89, 2.01, 1.00, 1.95, 0.95, 1.02, 1.05, 5.01, 5.28, 2.09, 5.66, 3.12, 6.10, 2.39, 2.35, 4.95, 9.34. The peaks are assigned to various protons in the molecule, including aromatic protons (7.0-7.5 ppm), anomeric protons (4.5-5.5 ppm), and aliphatic protons (1.0-4.0 ppm).

Compound **10**, 100 MHz, 297 K, CDCl₃

Compound 11, 400 MHz, 296 K, CDCl₃

Compound **11**, 100 MHz, 296 K, CDCl₃

Compound **12**, 600 MHz, 295 K, CDCl₃

Compound **12**, 600 MHz, 295 K, CDCl₃

Compound **12**, 125 MHz, 295 K, CDCl₃

Compound **12**, 125 MHz, 295 K, CDCl₃

Compound **13**, 600 MHz, 296 K, CDCl₃

Compound **13**, 600 MHz, 296 K, CDCl₃

Compound **13**, 125 MHz, 296 K, CDCl₃

Compound **13**, 125 MHz, 296 K, CDCl₃

Compound **14**, 600 MHz, 296 K, CDCl₃

Compound **14**, 600 MHz, 296 K, CDCl₃

Compound **14**, 125 MHz, 296 K, CDCl₃

Compound **14**, 125 MHz, 296 K, CDCl₃

Compound **15**, 600 MHz, 295 K, CDCl₃

Compound **15**, 600 MHz, 295 K, CDCl₃

Compound **15**, 125 MHz, 295 K, CDCl₃

Compound **15**, 125 MHz, 295 K, CDCl₃

Chemical structure of the compound is shown above the spectrum. The structure is a complex oligosaccharide derivative with various substituents including a phenyl group, a TCAHN group, and a repeating unit with a chlorine atom.

Integration values for the peaks are provided below the x-axis:

- 2.03
- 34.03
- 0.97
- 0.87
- 1.00
- 1.15
- 1.05
- 0.93
- 2.03
- 2.85
- 4.08
- 4.00
- 0.95
- 1.88
- 1.12
- 4.13
- 2.02
- 2.85
- 1.03
- 7.19
- 1.13
- 1.04
- 0.90
- 2.83
- 2.76
- 2.75
- 2.16
- 2.29
- 8.67
- 9.50
- 3.12

Compound **16**, 600 MHz, 295 K, CDCl₃

Chemical structure of the compound is shown above the spectrum. The structure is a complex glycoside derivative, featuring a central sugar core (likely a hexose) with various protecting groups and substituents. The substituents include a phenyl group (Ph), a hydroxyl group (HO), a TCAHN group, an acetoxy group (AcO), a pivaloyloxy group (OPiv), a benzoyloxy group (OBn), an acetamido group (NHAc), and a poly(6-chlorohexyl) chain (indicated by the subscript 6).

The ^{13}C NMR spectrum displays the following chemical shifts (ppm):

- 177.68
- 170.14
- 169.45
- 169.40
- 162.21
- 138.98
- 138.91
- 138.59
- 138.06
- 136.72
- 129.44
- 128.49
- 128.42
- 128.38
- 128.32
- 128.21
- 127.87
- 127.85
- 127.60
- 127.56
- 127.49
- 127.36
- 127.05
- 126.21
- 101.90
- 99.53
- 99.45
- 99.28
- 97.02
- 92.22
- 81.27
- 79.87
- 76.61
- 75.99
- 74.40
- 74.18
- 73.80
- 73.45
- 73.34
- 73.05
- 72.60
- 70.85
- 70.63
- 69.34
- 69.02
- 68.65
- 68.55
- 68.36
- 66.48
- 66.04
- 61.03
- 59.92
- 45.02
- 38.72
- 32.49
- 29.69
- 29.21
- 27.11
- 26.57
- 25.18
- 23.15
- 21.21
- 20.79
- 16.71

Compound **16**, 125 MHz, 295 K, CDCl₃

Compound 17, 600 MHz, 295 K, CDCl₃

Compound **17**, 600 MHz, 295 K, CDCl₃

Compound **17**, 125 MHz, 295 K, CDCl₃

Compound **17**, 125 MHz, 295 K, CDCl₃

Compound **18**, 600 MHz, 295 K, CDCl₃

Compound **18**, 600 MHz, 295 K, CDCl₃

Compound **18**, 125 MHz, 295 K, CDCl₃

Compound **18**, 125 MHz, 295 K, CDCl₃

Compound **19**, 600 MHz, 295 K, CDCl₃

Compound **19**, 600 MHz, 295 K, CDCl₃

Compound **19**, 125 MHz, 295 K, CDCl₃

Compound **19**, 125 MHz, 295 K, CDCl₃

Compound **20**, 600 MHz, 295 K, CDCl₃

Compound **20**, 600 MHz, 295 K, CDCl₃

Compound **20**, 125 MHz, 295 K, CDCl₃

Compound **20**, 125 MHz, 295 K, CDCl₃

Compound **21**, 600 MHz, 295 K, CDCl₃

Compound **21**, 600 MHz, 295 K, CDCl₃

Compound **21**, 125 MHz, 295 K, CDCl₃

Compound **21**, 125 MHz, 295 K, CDCl₃

Compound **22**, 600 MHz, 295 K, CDCl₃

Compound **22**, 600 MHz, 295 K, CDCl₃

Compound **22**, 125 MHz, 295 K, CDCl₃

Compound **22**, 125 MHz, 295 K, CDCl₃

Compound **2**, 600 MHz, 295 K, D₂O

Compound **2**, 600 MHz, 295 K, D₂O

Compound 2, 125 MHz, 295 K, D₂O

Compound **2**, 125 MHz, 295 K, D₂O

Compound 3, 600 MHz, 295 K, D₂O

Compound **3**, 600 MHz, 295 K, D₂O

Compound 3, 125 MHz, 295 K, D₂O

Compound **3**, 125 MHz, 295 K, D₂O

Compound 4, 600 MHz, 295 K, D₂O

Compound **4**, 600 MHz, 295 K, D₂O

Compound 4, 125 MHz, 295 K, D₂O

Compound **4**, 125 MHz, 295 K, D₂O