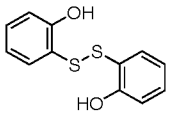
Experimental:

Reagents: All commercial materials were used as received unless otherwise noted. The following solvents were obtained from a dry solvent system and used without further purification: THF, diethyl ether, toluene, and DCM. Reagents were obtained from Aldrich or as noted, with the following exceptions: amino acids and resins for solid phase peptide synthesis were purchased from NovaBiochem; Biosynthesis grade DMF from EM Science; and all other solvents from Fisher Scientific (HPLC grade).

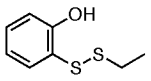
HPLC: All separations involved a mobile phase of 0.05% TFA (v/v) in water (solvent A)/0.0425% TFA in acetonitrile (solvent B). Preparative, semipreparative, and analytical HPLC separations were performed using a Rainin HXPL solvent delivery system equipped with a Rainin UV-1 detector and one of the following Dynamax-60Å C18 axial compression columns 250 mm in length equipped with a similarly packed guard column: 41.4 mm diameter (prep), 21.4 m diameter (semiprep), or 4.6 mm diameter (analytical). Separations were performed at flow rates of 48 mL/min (prep), 16 mL/min (semiprep), or 1 mL/min (analytical), and were monitored at a wavelength between 214 and 230 nm, depending on column loading. LCMS chromatographic separations were performed using a Waters 2695 Separations Module and a Waters 996 Photodiode Array Detector equipped with a Varian Microsorb C18 2 x 150 mm column at a flow rate of 0.2 mL/min.

ESMS and LCMS: Electrospray mass spectroscopy and LCMS analyses were obtained on a Waters Micromass ZQ mass spectrometer in conjunction with the Waters HPLC apparatus described above.

NMR: ^{1}H and ^{13}C NMR spectra were recorded on Bruker instruments in $CDCl_3$, CD_3OD or D_2O at 400 or 500 MHz for ^{1}H and 100 or 125 MHz for ^{13}C.

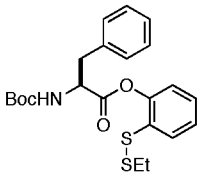


To a stirred, biphasic solution of 2-mercaptophenol (1.0 g, 7.9 mmol) in H_2O (5.1 mL) was added, drop wise, a solution of iodine (1.0 g 4.0 mmol) in methanol (3.5 mL). When the brown iodine color persisted the solution was diluted with ethyl acetate and water. The aqueous layer was removed and extracted with an additional portion of ethyl acetate. The combined organic layers were dried and washed with brine then dried (Na_2SO_4) and concentrated to give a brown oil which was used without purification (1.5 g). The product still contains iodine. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.33-7.37 (m, 2H), 7.22-7.24 (m, 2H), 6.99-7.01 (m, 2H), 6.82-6.85 (m, 2H), 6.22 (brs, 2H). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.35 (dt, J = 8.16, 1.65 Hz, 2H), 7.22 (dd, J = 7.52, 1.65 Hz, 2H), 7.00 (dd, J = 8.16, 1.15 Hz, 2H), 6.83 (dt, J = 7.52, 1.15 Hz, 2H), 6.22 (s, 2H) ^{13}C NMR ($CDCl_3$, 125 MHz): δ 157.3, 136.6, 133.6, 121.4, 120.3, 116.1. ESI-MS: Calcd. for $C_{12}H_{10}O_2S_2$ $[M+NH_4]^+$ 267.8 Found: 267.8.

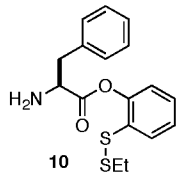


To a stirred solution of the disulfide (1.5 g crude, ~4 mmol) in CH_2Cl_2 (25 mL) was added ethyldisulfide (10.5 mL, 79.9 mmol) and then BF_3•OEt_2 (10.1 mL, 79.9 mmol). The reaction was stirred at room temperature for three hours and then carefully quenched by the addition of (aq.) $NaHCO_3$. The organic layer was drained and the aqueous layer was extracted with an additional portion of

CH_2Cl_2. The combined organic layers were dried ($MgSO_4$) and concentrated to give a yellow oil. Purification by silica gel chromatography (20% ethyl acetate in hexane) gave the desired product as a clear, slightly yellow oil (1.45 g, 99%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.48-7.51 (m, 1H), 7.28-7.32 (m, 1H), 6.99-7.01 (m, 1H), 6.86-6.9 (m, 1H), 6.34 (brs, 1H), 2.78 (q, J = 7.4 Hz, 2H), 1.35 (t, J = 7.4 Hz, 3 H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 156.9, 135.2, 132.2, 121.0, 116.2, 32.4, 14.1. ESI-MS: Calcd. for $C_8H_{10}OS_2$ $[M+Na]^+$ 208.8 Found: 208.8.

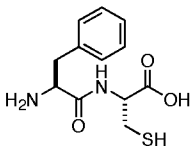


To a solution of the phenol (1.45 g, 8 mmol) and Boc-Phe-OH (2.65 g, 10 mmol) in CH_2Cl_2 (25 mL) and THF (5 mL) was added EDCI (1.92 g, 10 mmol) and DMAP (98 mg, 0.8 mmol). The resulting solution was stirred at room temperature for 18 hr at which point the volatile materials were removed *in vacuo*. The resulting oil was taken up in EtOAc and washed with 1N HCl, H_2O, and then brine. The organic layer was dried (Na_2SO_4) and concentrated to give a slightly yellow oil. Purification by silica gel chromatography (30% ethyl acetate in hexane) gave a clear, colorless oil (3.5 g, >99%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.78-7.81 (m 1H), 7.23-7.36 (m, 7H), 7.01-7.03 (m, 1H), 5.02 (d, J = 8.16 Hz, 1H), 4.86-4.91 (m, 1H), 3.31 (abx, J_{ab} = 13.98 Hz, J_{ax} = 5.41 Hz, J_{bx} = 7.22 Hz, 2H), 2.72 (q, J = 7.33 Hz, 2H), 1.42 (s, 9H), 1.29 (t, J = 7.33 Hz, 3H). ESI-MS: Calcd. for $C_{22}H_{27}NO_4S_2$ $[M+Na]^+$ 456.0 Found: 456.0.



10

To a 250 mL round-bottomed flask equipped with a magnetic stir bar was added the above phenylalanine derivative (3.0 g, 6.9 mmol) as well as a 4M solution of HCl in dioxane (86 mL, 345 mmol) and the reaction was stirred under argon at room temperature for 1.5 hr. At that point the reaction was concentrated *in vacuo*, leaving a white solid. This material was triturated with ether and subsequently concentrated. This process was repeated (3x) leaving the crude product as a white solid. The material was dissolved in 30% B, shell frozen and lyophilized to give a white powder (2.17 g, 94%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.93-7.95 (m, 1H), 7.52-7.42 (m, 7H), 7.23-7.21 (m, 1H), 4.81 (dd, J = 8.35, 5.73 Hz, 1H), 3.56 (abx, J_{ab} = 14.48 Hz, J_{ax} = 5.73 Hz, J_{bx} = 8.35 Hz, 2H), 2.85 (q, J = 7.34, 2H), 1.37 (t, J = 7.34, 3H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 169.0, 149.4, 135.6, 131.9, 131.3, 131.0, 130.7, 129.9, 129.6, 129.1, 123.9, 55.6, 38.0, 34.1, 14.9. ESI-MS: Calcd. for $C_{17}H_{19}NO_2S_2$ $[M+H]^+$ 334.1 Found: 334.2.

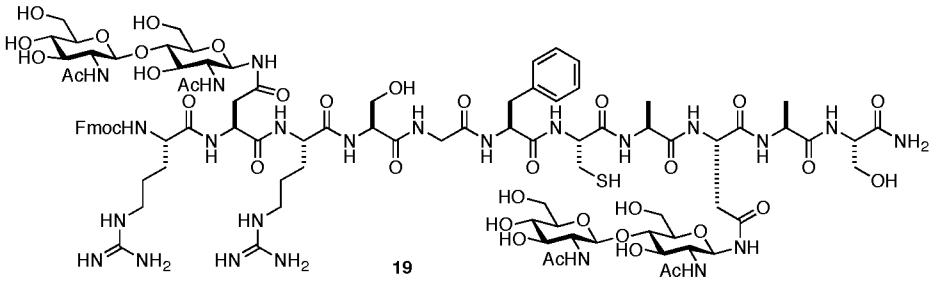


The phenylalanine derivative **10** (15 mg, 45 μmol) and L-cysteine (6 mg, 49 μmol) were placed into a LCMS vial along with a flea-sized stirbar. In a second vial were mixed MES-Na (25 mg, 150 μmol) and phosphate buffered saline (PBS) (0.2M NaCl, 0.2M phosphate, pH = 7.5, 2 mL). The MES-Na solution was then added directly to the amino acids, and the reaction was monitored by LCMS. After two hours the reaction appeared to be complete and TCEP (129 mg, 450 μmol) was added. This was stirred for 1 hour and then injected directly onto the HPLC for purification. The desired compound was obtained as a white powder after lyophilization (9.4 mg, 78%). ^{1}H NMR (D_2O): δ 7.36-7.45 (m, 3H), 7.31-7.33 (m, 2H), 4.63 (dd, J = 6.61, 4.83 Hz, 1H), 4.34 (dd, J = 7.24, 7.18 Hz, 1H), 3.25 (abx, J_{ab} = 14.1 Hz, J_{ax} = 7.18 Hz, J_{bx} = 7.24 Hz, 2H), 2.96 (abx,

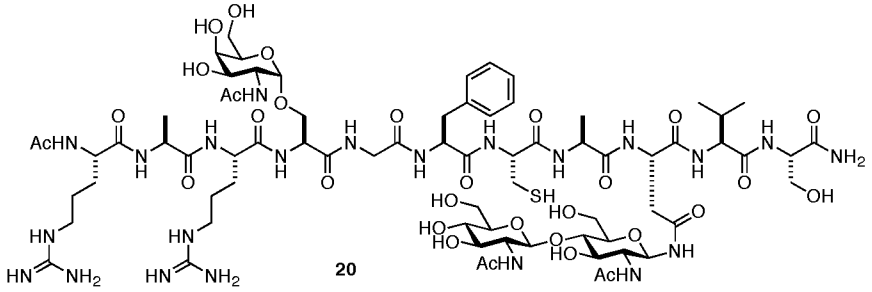
J_{ab} = 14.2 Hz, J_{ax} = 4.83 Hz, J_{bx} = 6.61 Hz, 2H). LCMS: 5-65%B over 20 min, rt = 8.53 min. HPLC: 5-95%B over 30 min, rt = 11.45 min. ESI-MS: Calcd. for $C_{12}H_{16}N_2O_3S$ $[M+H]^+$ 269.1, Found: 269.1

Typical ligation conditions:

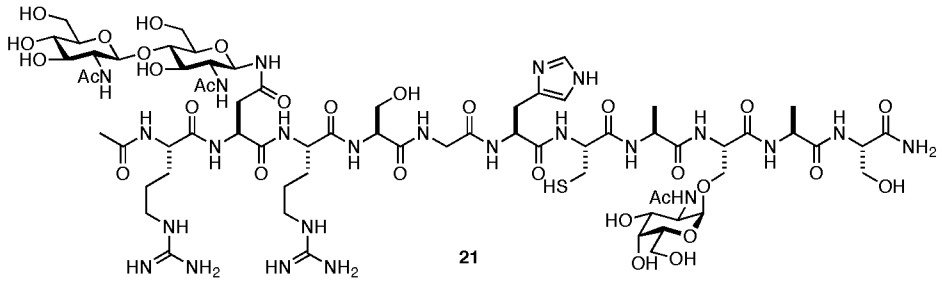
The two glycopeptide halves (**12**, 2.2 mg, 1.44 μmol) (**18**, 1.4 mg, 1.44 μmol) were placed in a LCMS vial along with a flea-sized stirbar. A stock solution of MESNa (18.3 mg, 111 mmol) in phosphate buffered saline (0.2M NaCl, 0.2M phosphate, pH = 7.4, 1 mL) was made and of this, 600 μL was added to the glycopeptides. The reaction was monitored by LCMS and, once finished, TCEP (25 mg, 0.087 mmol) was added and the solution stirred for 2 hr then injected directly onto the HPLC for purification.



LCMS: 5-65%B over 20 min, rt = 9.98 min. HPLC: 25-55%B over 30 min, rt = 7.65 min. ESI-MS: Calcd. for $C_{93}H_{138}N_{24}O_{37}S$ $[M+2H]^{2+}$ 1108.5, Found: 1108.6, $[M+3H]^{3+}$ 739.3, Found: 739.5.

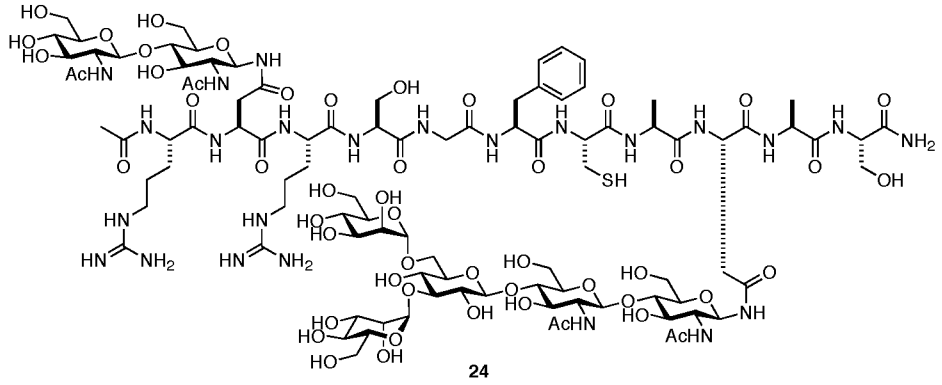


LCMS: 5-65%B over 20 min, rt = 11.40 min. HPLC: 5-65%B over 20 min, rt = 9.85 min. ESI-MS: Calcd. for $C_{73}H_{120}N_{22}O_{30}S$ $[M+2H]^{2+}$ 909.4, Found: 909.5, $[M+3H]^{3+}$ 606.6, Found: 606.8.



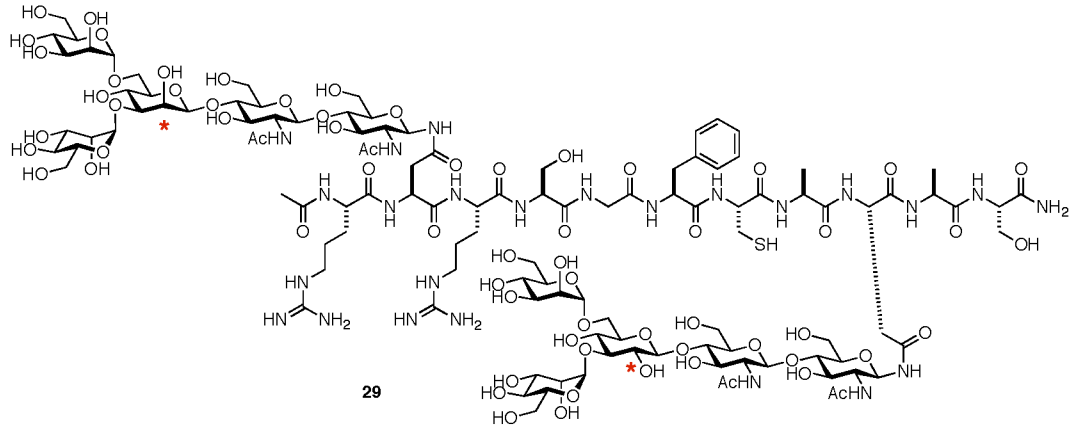
21

LCMS: 5-65%B over 20 min, rt = 4.02 min. HPLC: 5-65%B over 20 min, rt = 6.71 min. ESI-MS: Calcd. for $C_{73}H_{120}N_{22}O_{30}S$ $[M+2H]^{2+}$ 898.4, Found: 898.6.



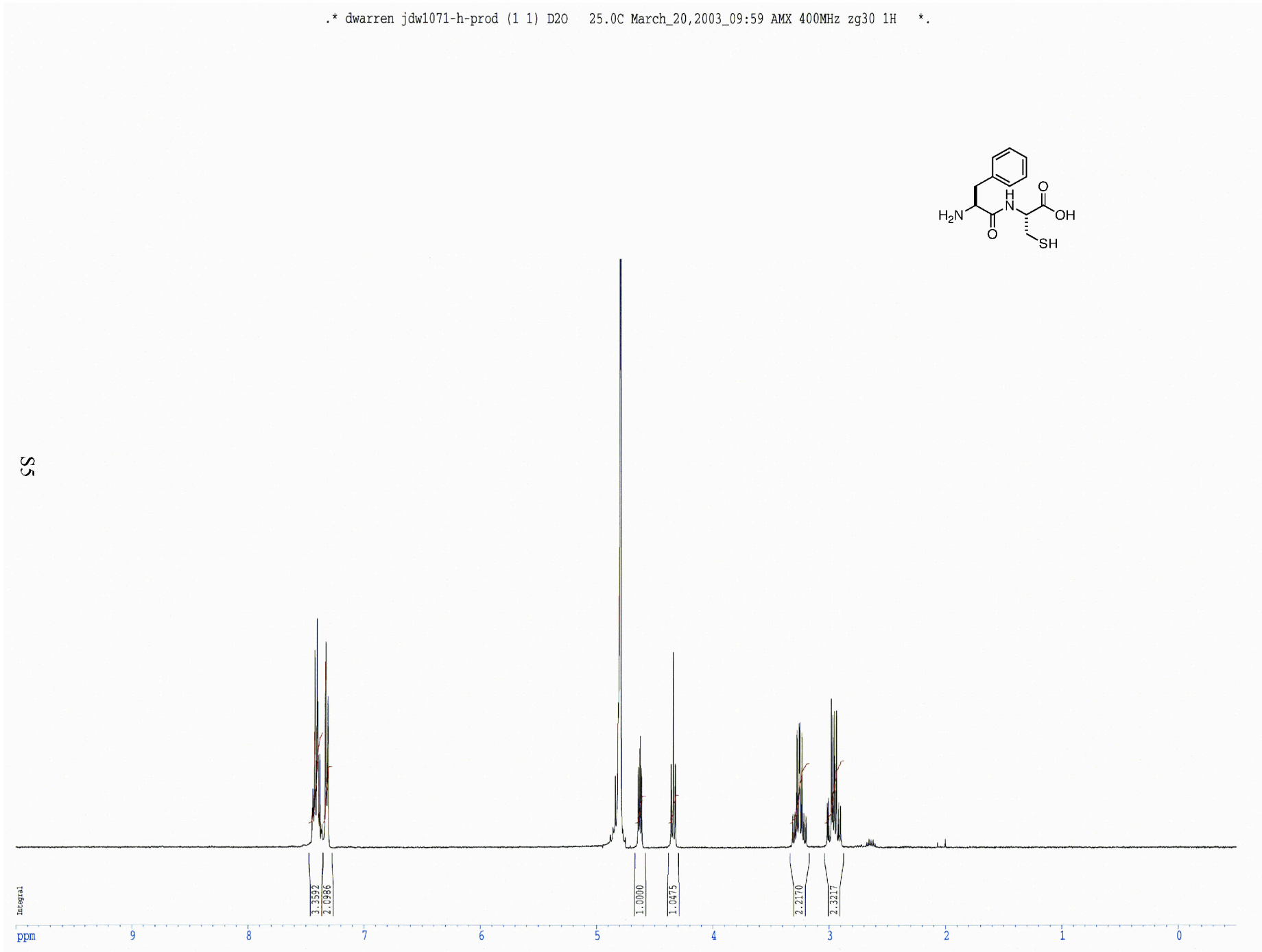
24

LCMS: 5-65%B over 20 min, rt = 7.81 min. HPLC: 5-65%B over 20 min, rt = 8.39 min. ESI-MS: Calcd. for $C_{98}H_{160}N_{24}O_{51}S$ $[M+2H]^{2+}$ 1261.5, Found: 1261.5, $[M+3H]^{3+}$ 841.4, Found: 841.5.



29

LCMS: 5-45%B over 20 min, rt = 10.82 min. HPLC: 5-45%B over 20 min, rt = 9.31 min. ESI-MS: Calcd. for $C_{98}H_{160}N_{24}O_{51}S$ $[M+2H]^{2+}$ 1504.6, Found: 1504.9, $[M+3H]^{3+}$ 1003.4, Found: 1003.7.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N
O
OH
SH
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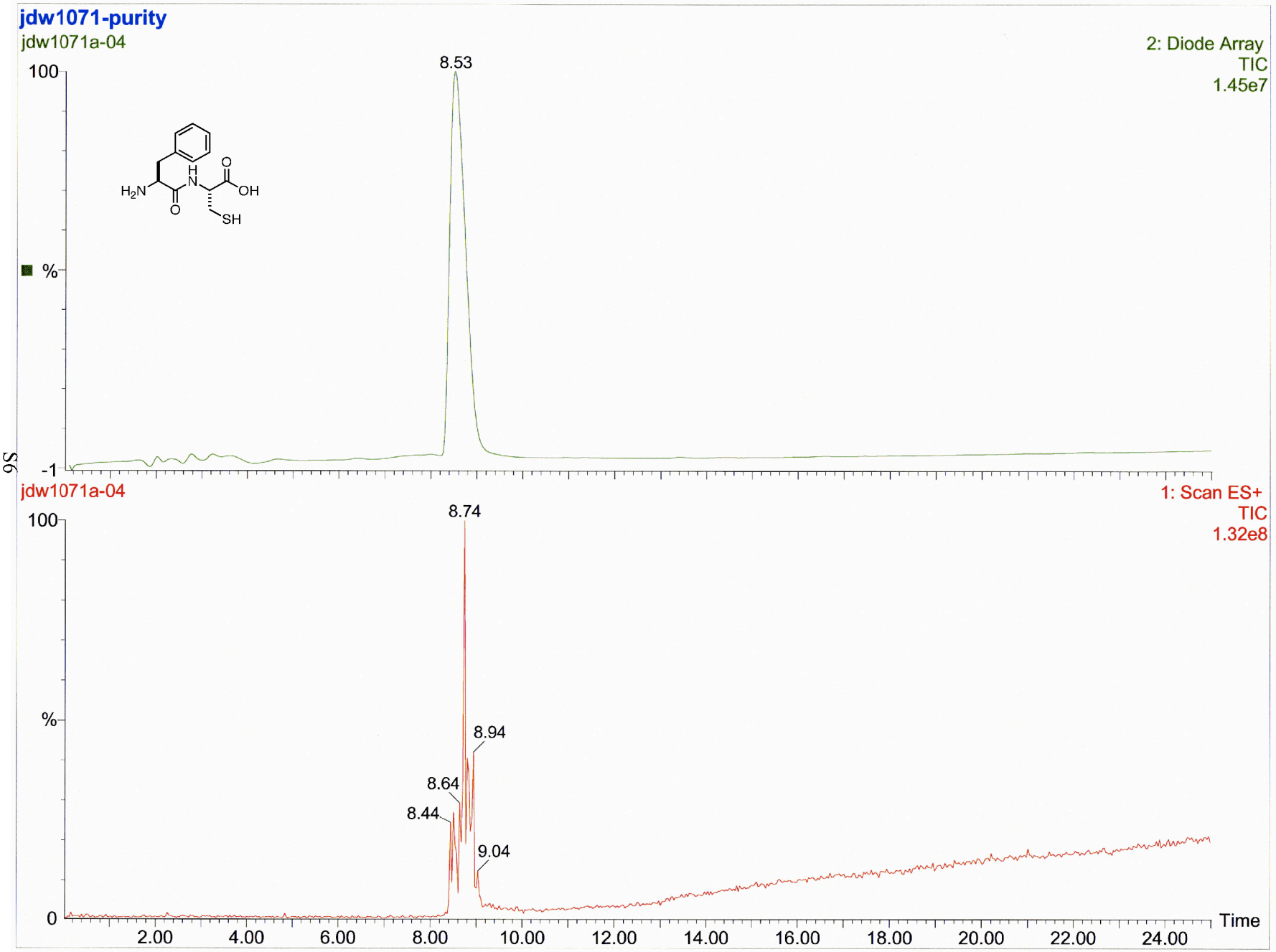
1.0000
1.0475
2.2170
2.3217
ppm
9
8
7
6
5
4
3
2
1
0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jdw1071a-04
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%
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8.64
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9.04
100
%
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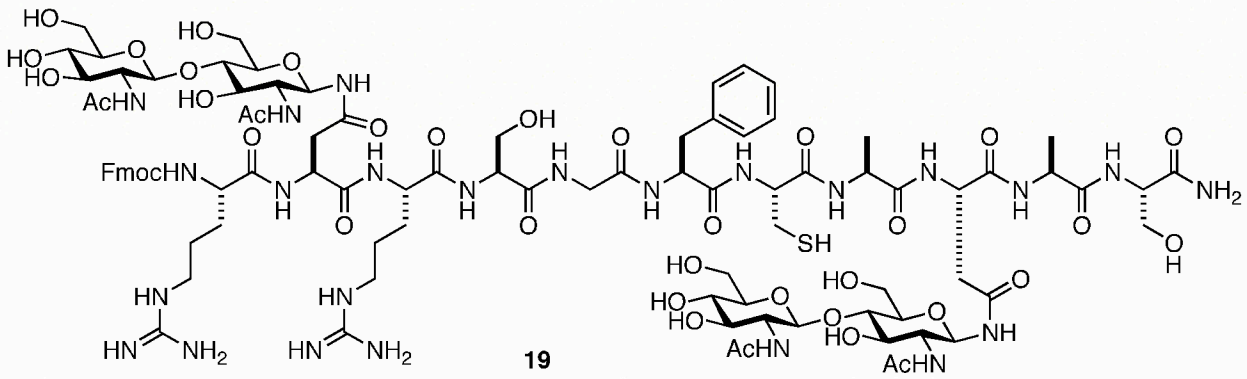
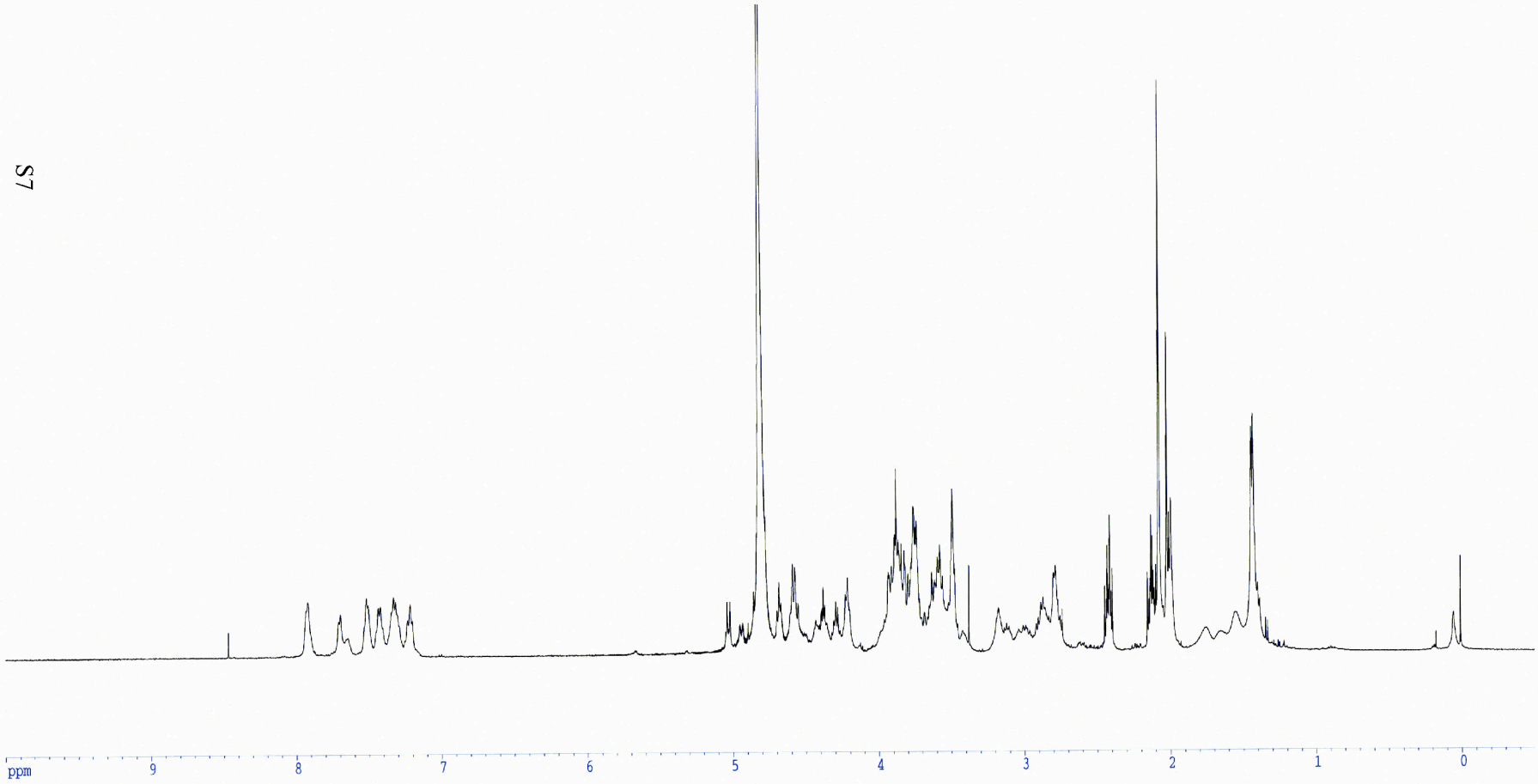
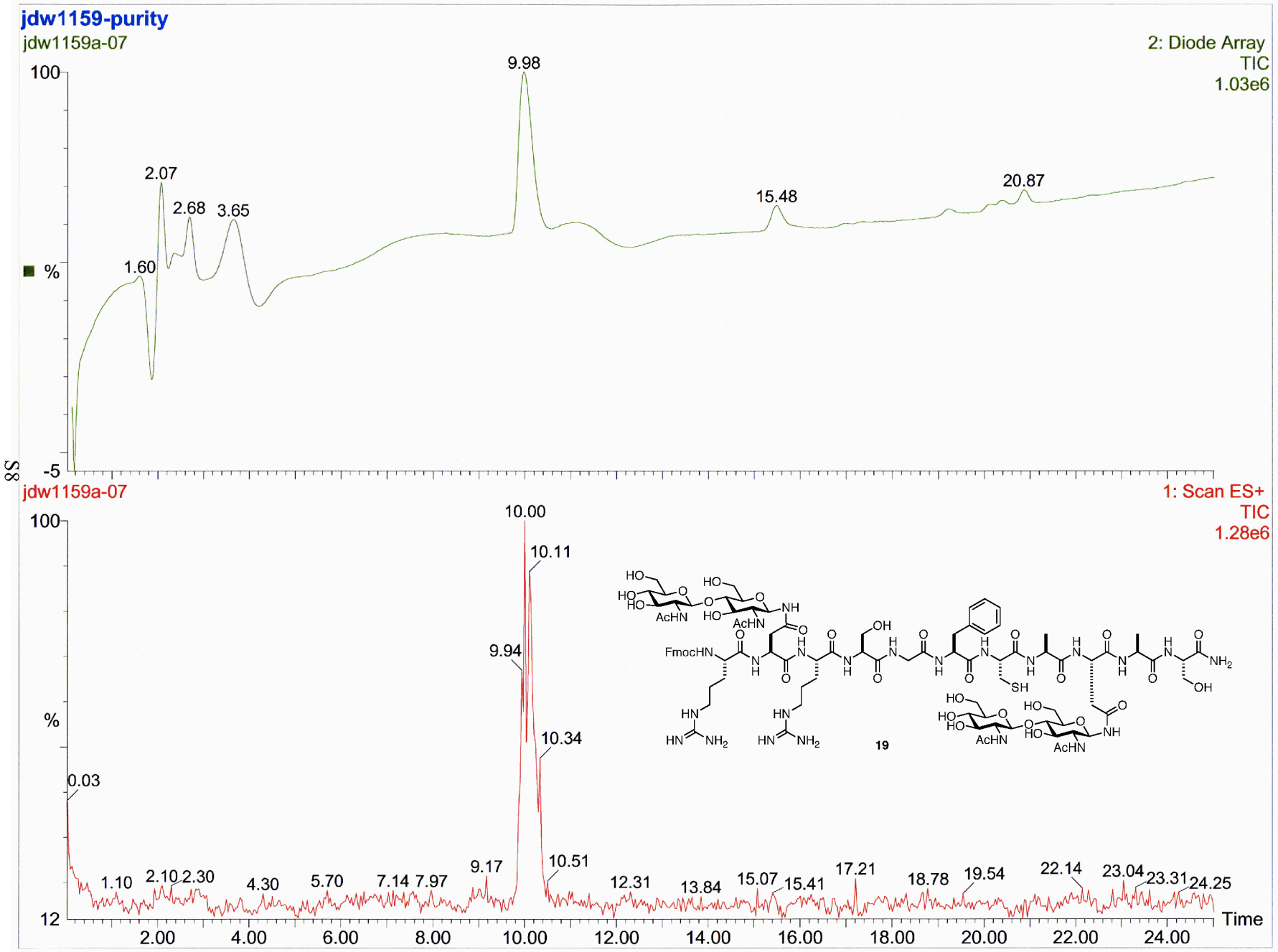


19

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%
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10.11
9.94
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1.10
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2.30
4.30
5.70
7.14
7.97
9.17
12.31
13.84
15.07
15.41
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23.04
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Time
19

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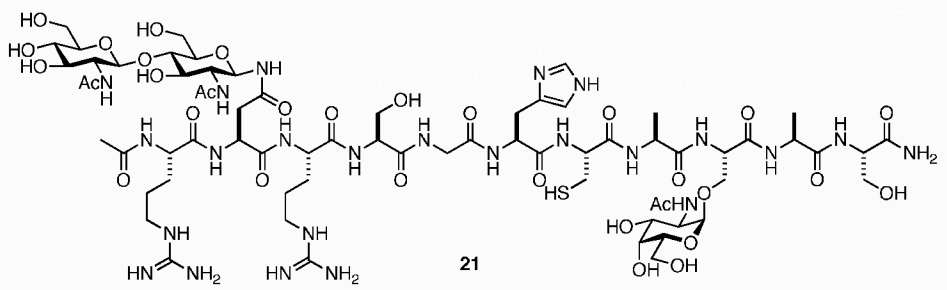
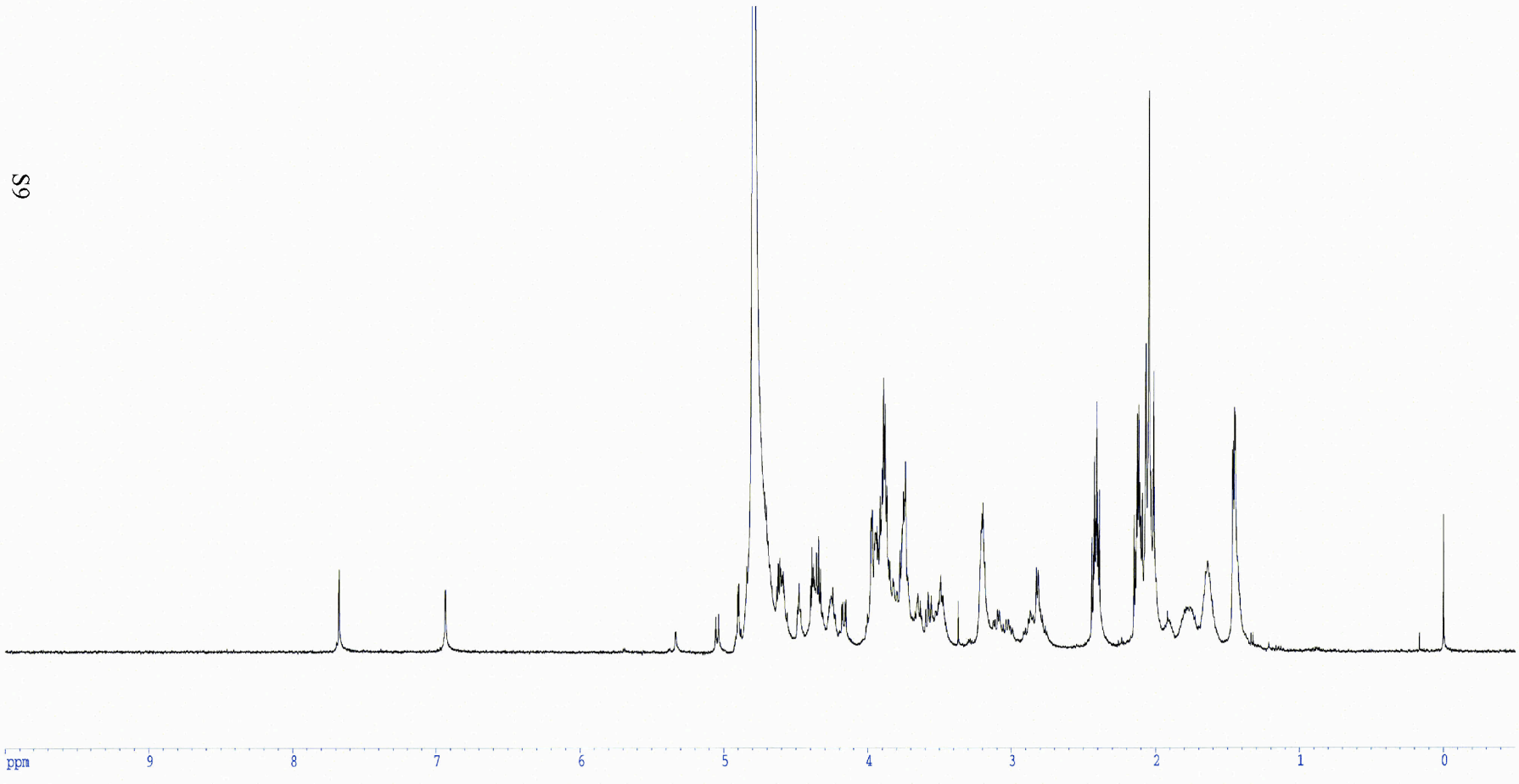
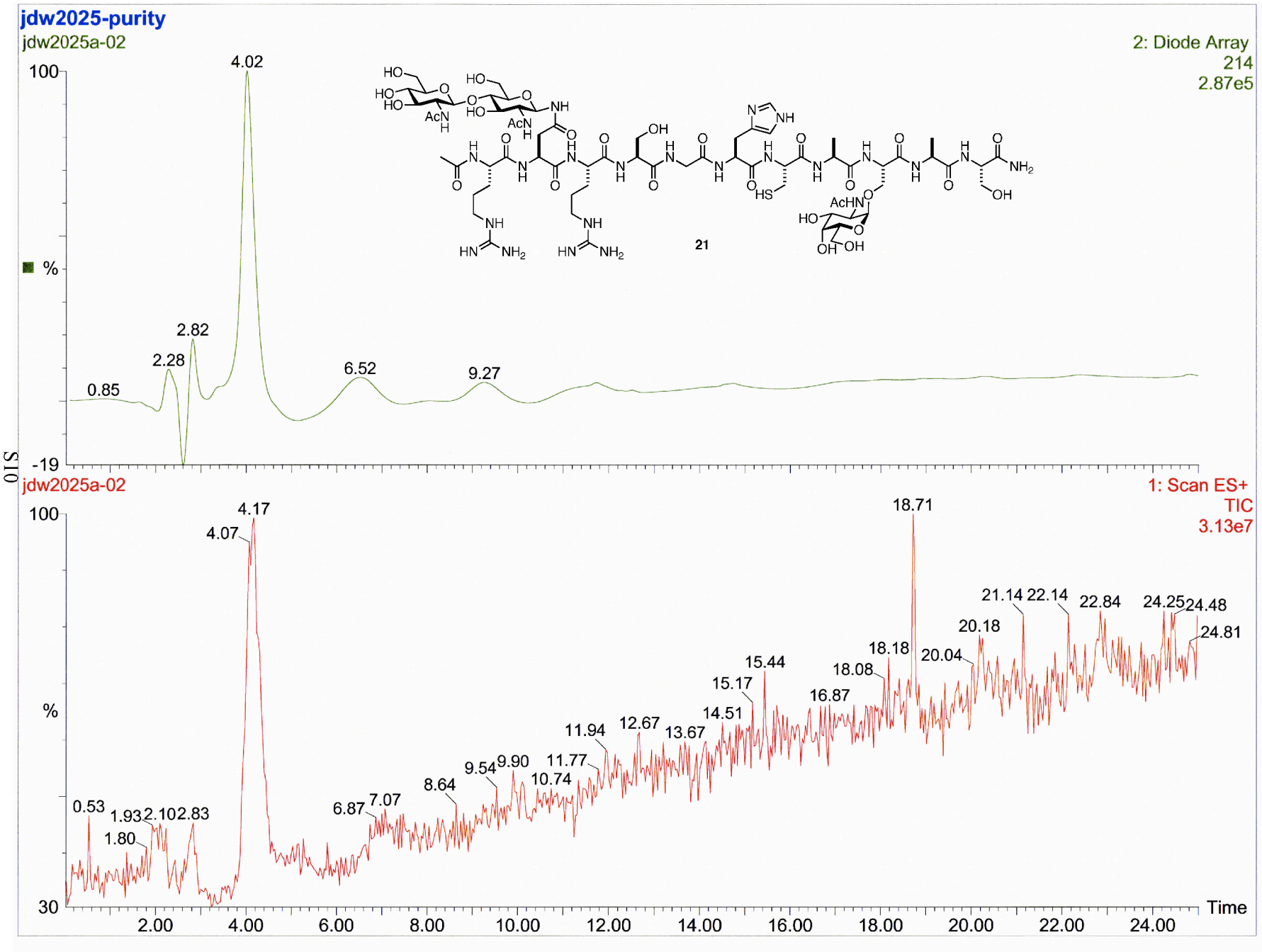


21

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6.52
9.27
21
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TIC
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%
30
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4.07
18.71
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2.10
2.83
1.80
6.87
7.07
8.64
9.54
9.90
10.74
11.77
11.94
12.67
13.67
14.51
15.17
15.44
16.87
18.08
18.18
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21.14
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22.84
24.25
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Time

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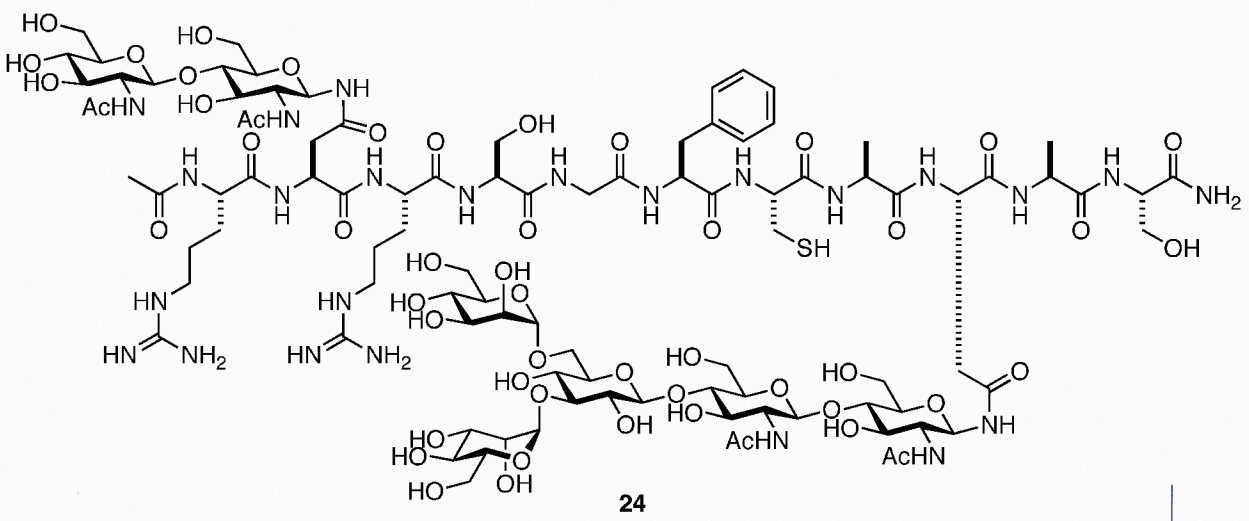
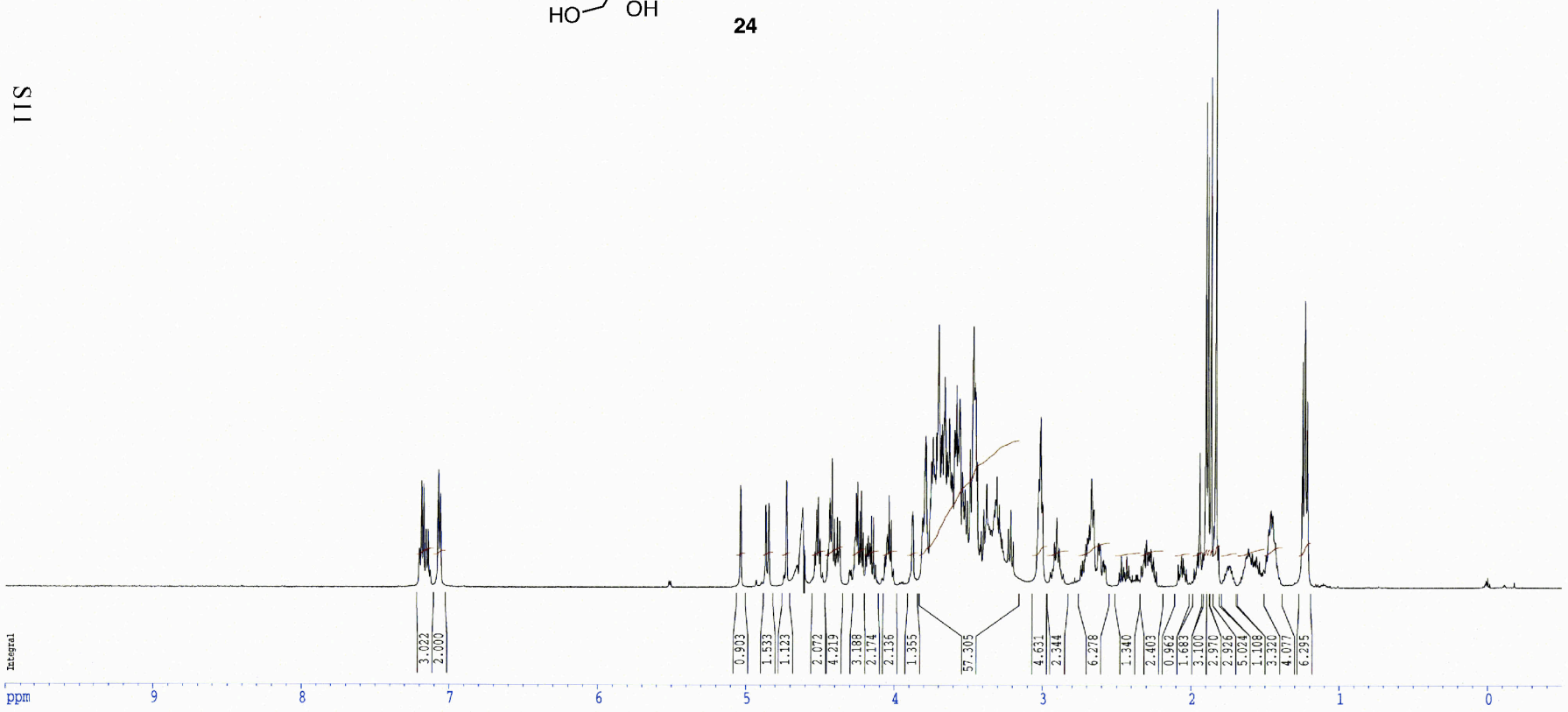
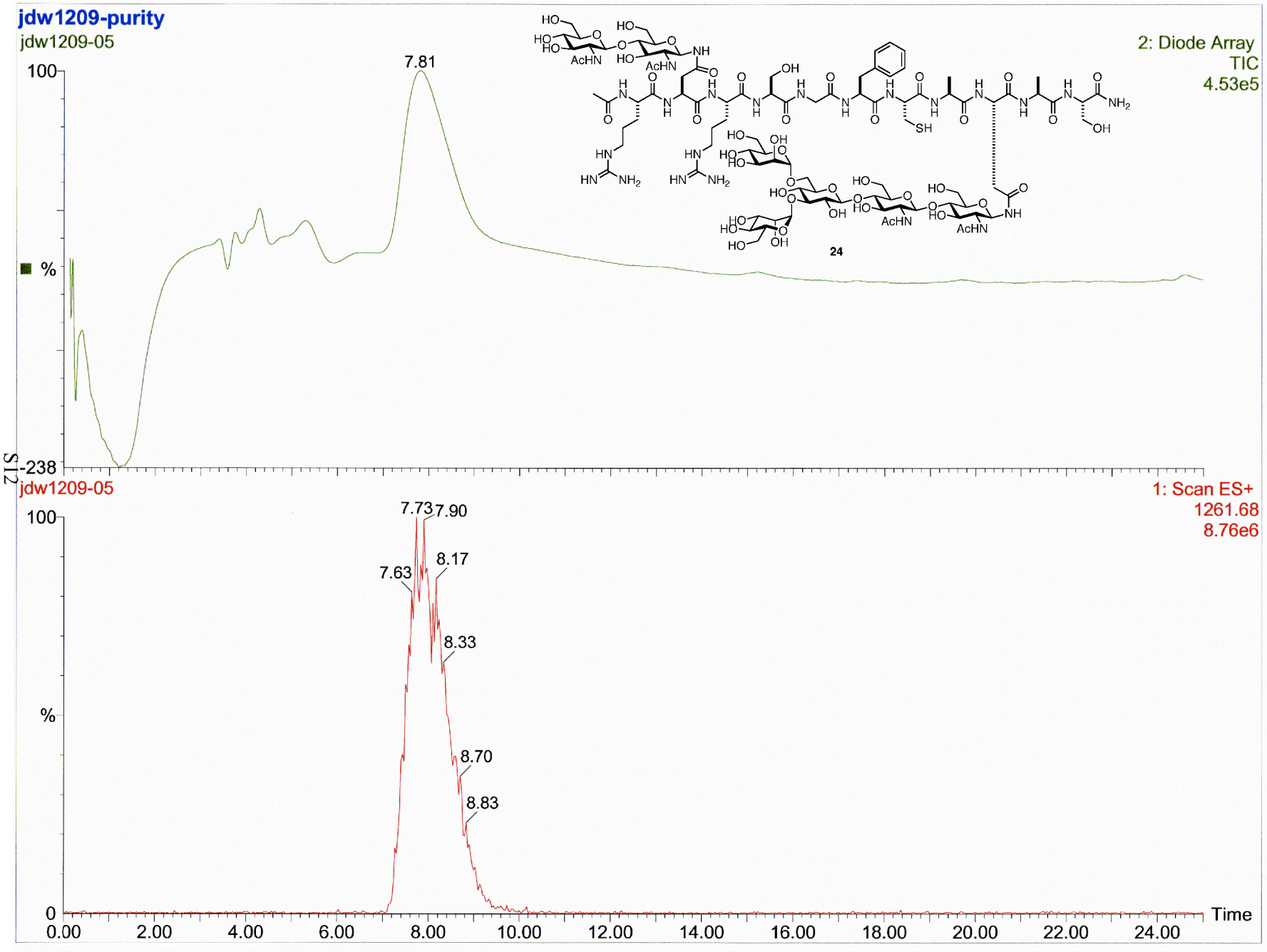


24

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%
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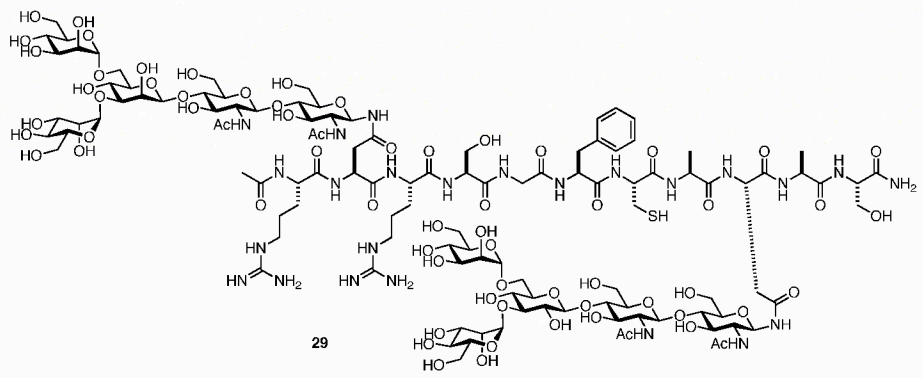

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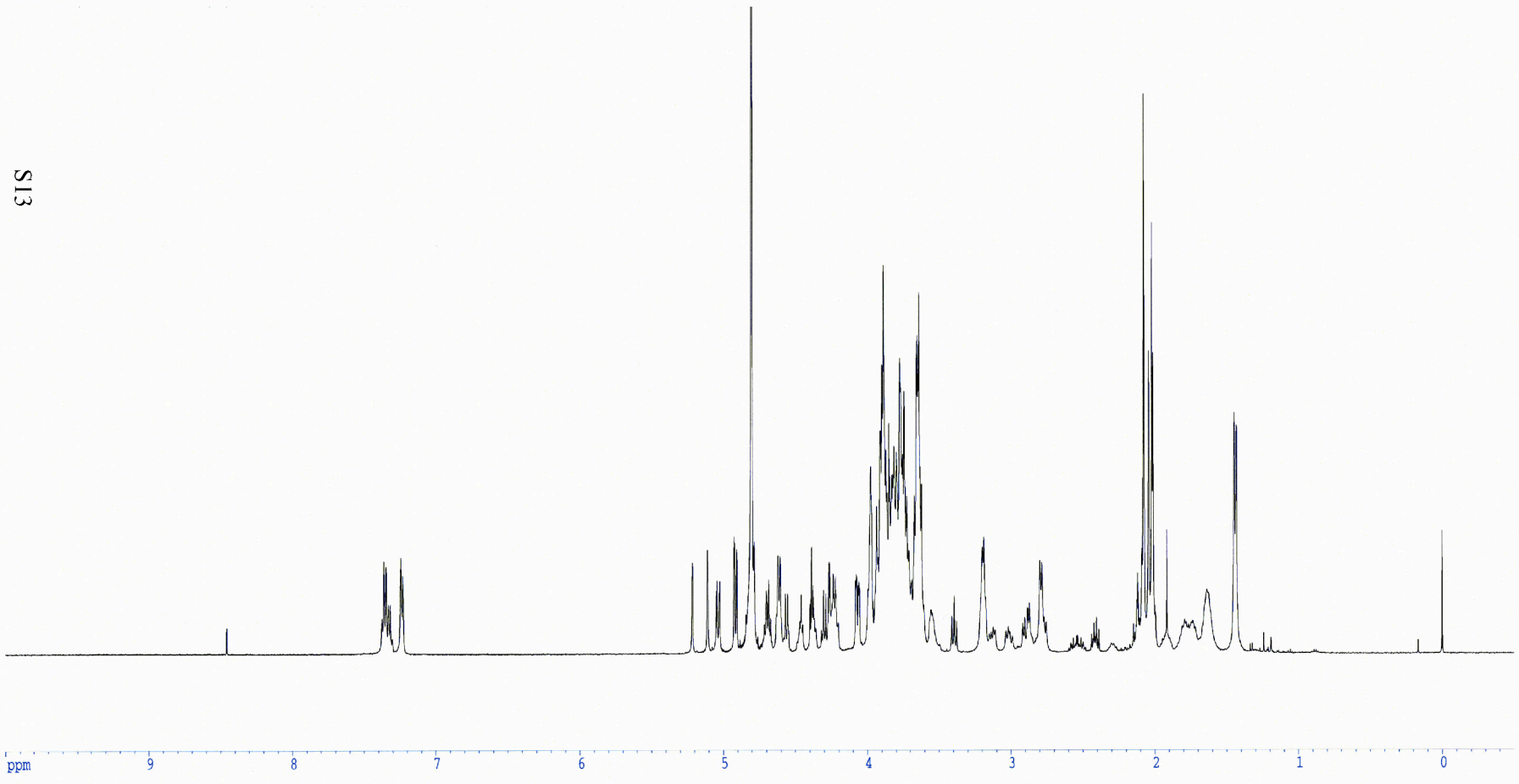

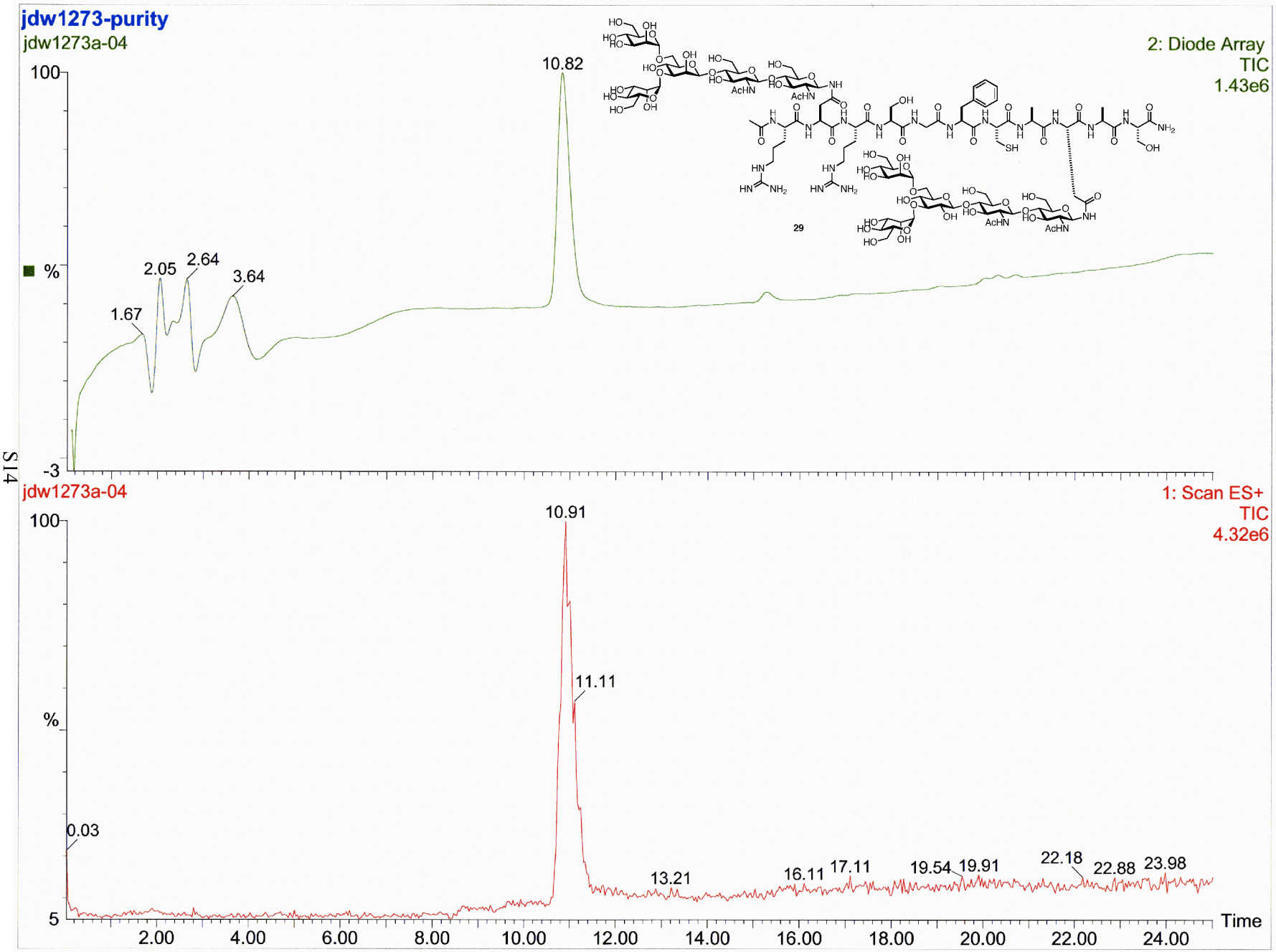
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%
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10.91
11.11
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13.21
16.11
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19.54
19.91
22.18
22.88
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Time
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