Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

Potential use of synthetic alpha-galactosyl-containing glycotopes of the parasite *Trypanosoma cruzi* as diagnostic antigens for Chagas disease

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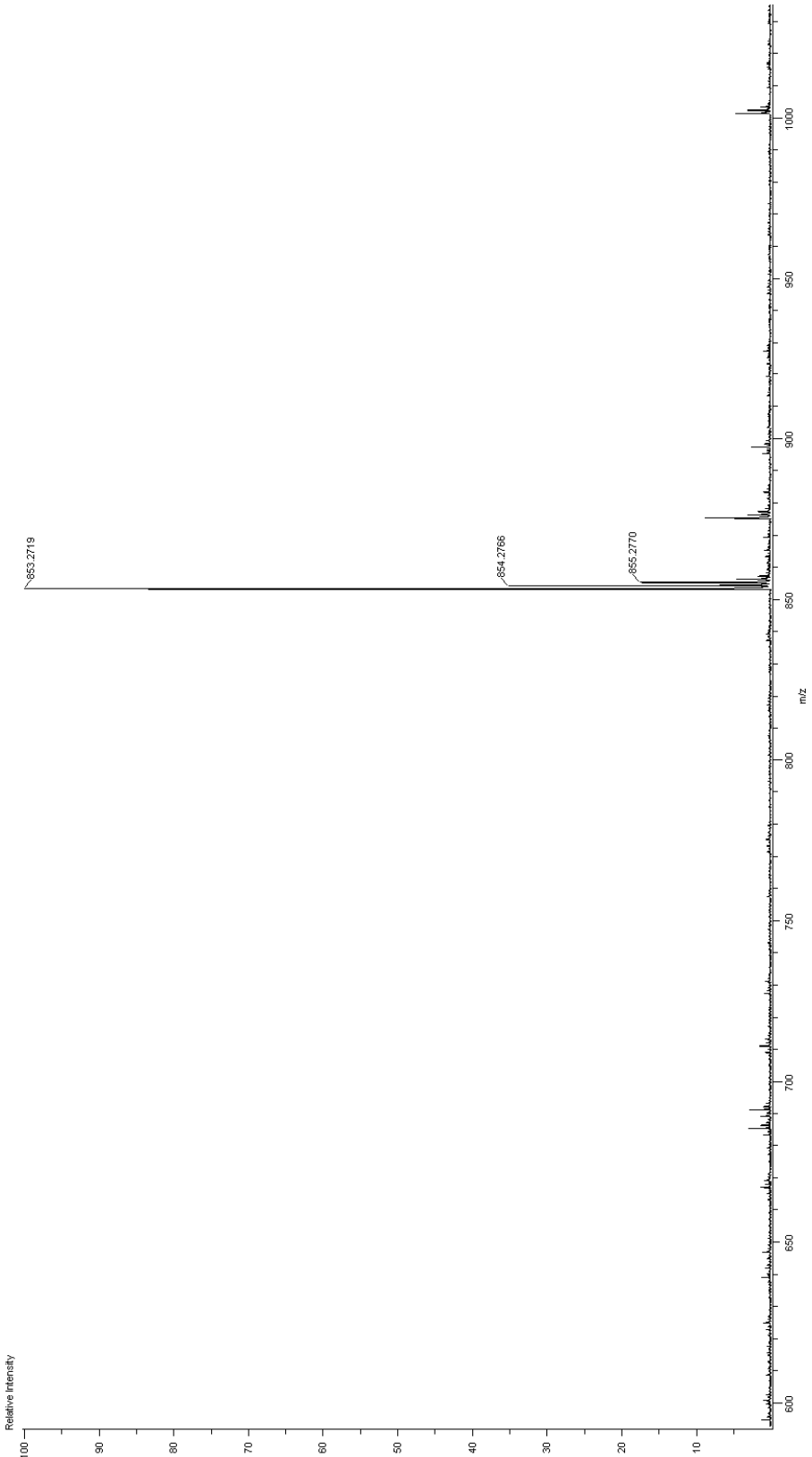
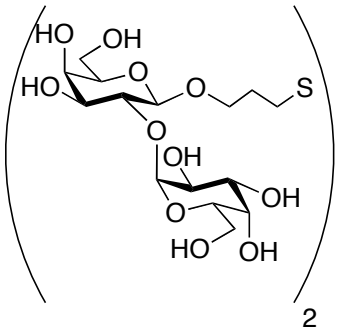
Abbreviations pertaining to Figures 2 and 3

ChSP	sera from chronic Chagasic patients, pooled
CL-ELISA	chemiluminescent-enzyme linked immunosorbent assay
NGP	neoglycoprotein
NHSP	normal human sera from healthy individuals, pooled
RLU	relative luminescence units

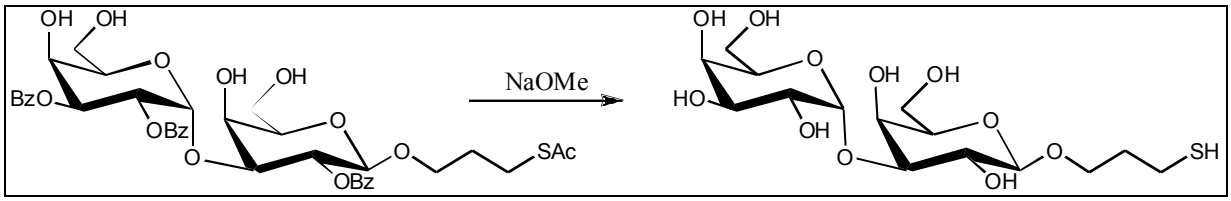
ESI-TOF HR mass spectrum of compound **2** (disulfide form)





ESI-TOF HR MS $[C_{30}H_{54}NaO_{22}S_2]^+$ calc. *m/z* = 853.2446, found 853.2719

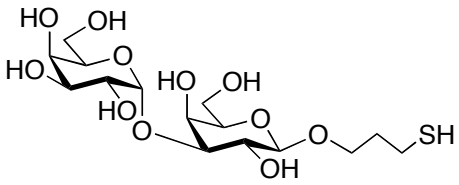
Synthesis of 3-mercaptopropyl α-D-galactosyl-(1→3)-β-D-galactoside (3)

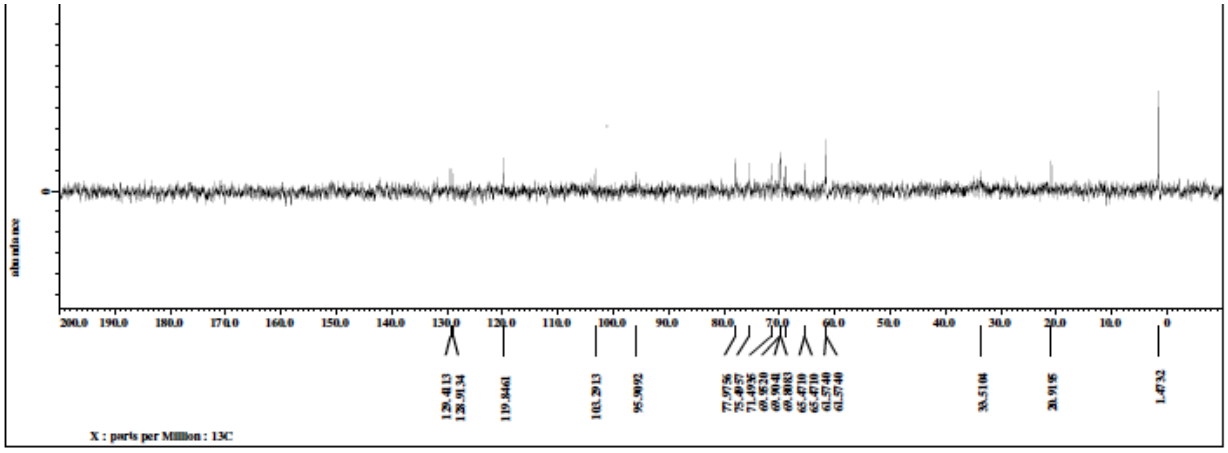


To a solution of **26** (136 mg, 0.176 mmol) in dry MeOH (3 mL), 1M NaOMe (1 mL) was added and the mixture was stirred under argon at room temp. for 45 min. Amberlite IRC-748 ion-exchange resin was added to neutralize NaOMe. Amberlite was filtered off and rinsed with MeOH. The resulting solution was then concentrated, dissolved in water (10 mL), and washed with $CHCl_3$ (3 × 5 mL). The aqueous layer was collected and lyophilized to afford **3** (72 mg, 99%) as an off-white solid. R_f 0.47 (5:1 iPrOH/H_2O w/ 3 drops AcOH); ^{1}H NMR (600 MHz, MeOD/D_2O) 5.03 (d, 1H, $J_{H\text{-}1'/H\text{-}2'}$ = 3.44 Hz, H-1'); 4.28 (d, 1H, $J_{H\text{-}1/H\text{-}2}$ = 7.56 Hz, H-1); 4.11 (dd, 1H); 3.89-3.49 (m, 11H); 2.70 (t, 2H, $OCH_2CH_2CH_2SH$, $OCH_2CH_2CH_2SH$); 1.90 (m, 2H, $OCH_2CH_2CH_2SH$, $OCH_2CH_2CH_2SH$); ^{13}C NMR (151 MHz; D_2O): δ 103.3, 95.9, 78.0, 75.5, 71.5, 70.0, 69.9. 69.8, 65.5, 61.6, 33.5, 20.9; ESI-TOF HR MS $[C_{30}H_{54}NaO_{22}S_2]^+$ calc. *m/z* = 853.2446, found 853.2406; ESI-TOF HR MS $[C_{15}H_{28}NaO_{11}S]^+$ calc. *m/z* = 439.1250, found 439.1006; ESI-TOF HR MS $[C_{15}H_{28}KO_{11}S]^+$ calc. *m/z* = 455.0989, found 455.0733.

A small sample of the disulfide form of compound **3** was reduced with Pierce* Immobilized TCEP Disulfide Reducing Gel. The supernatant was passed through a Sep-Pak C18 cartridge, and the major fraction was lyophilized and dissolved in degassed D_2O for ^{13}C NMR analysis (p. S5) and mass spectrometric analysis (p. S6)

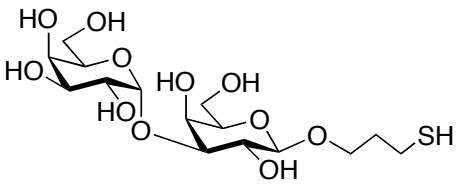
^{13}C NMR spectrum of compound **3**

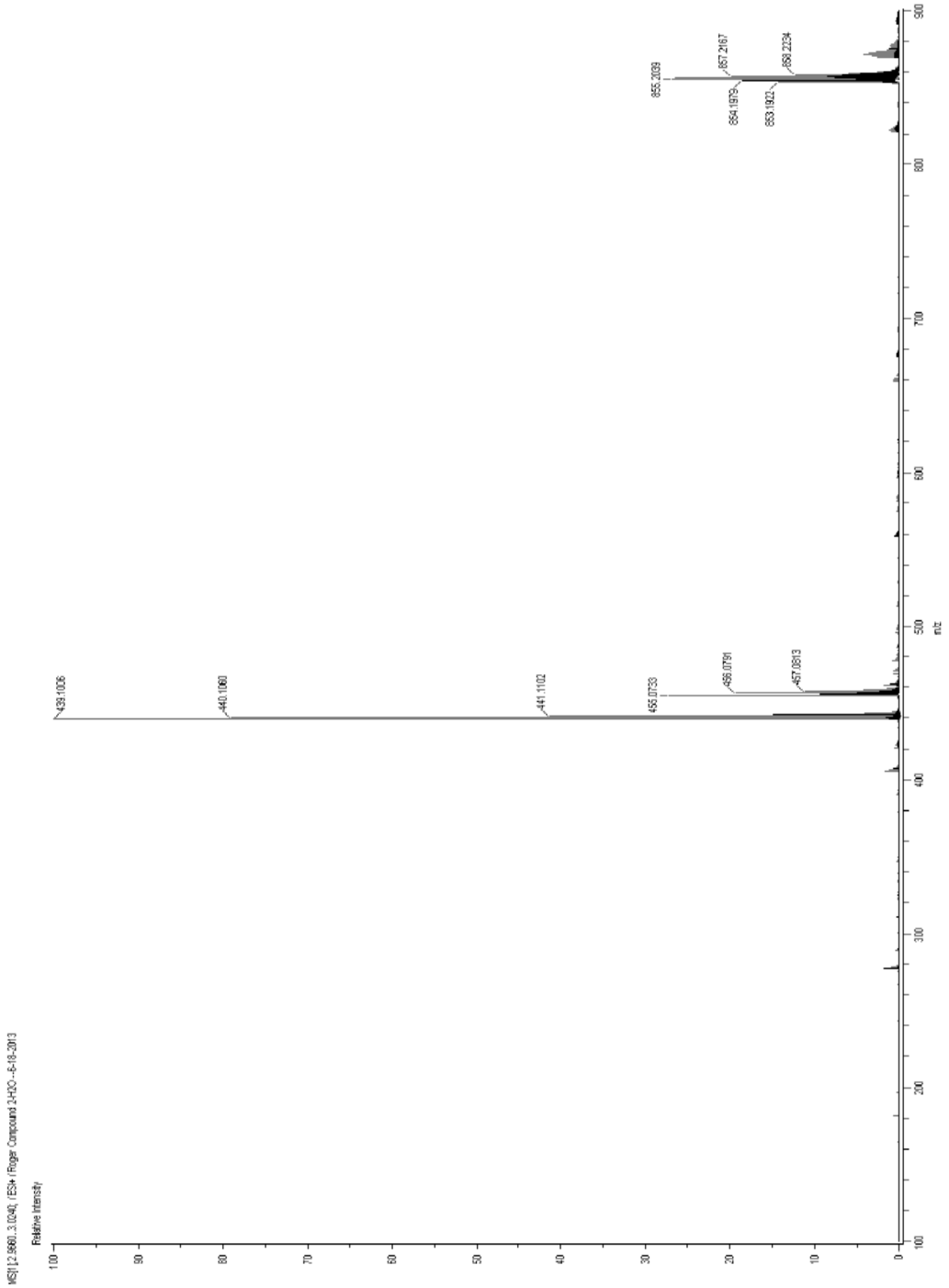




This ^{13}C NMR spectrum was measured under argon in degassed D_2O with a small quantity of CH_3CN for calibration purposes (119.68 and 1.47 ppm). The spectrum shows aromatic signals at 129.4 and 128.9 ppm, resulting from residual benzoic acid or benzoate, which could not be completely removed during the aqueous workup of the final deprotection step in the synthesis of compound **3**. Mass spectrometry of compound **3** (p. S6) confirms that all benzoate esters were successfully cleaved from the precursor **26**.

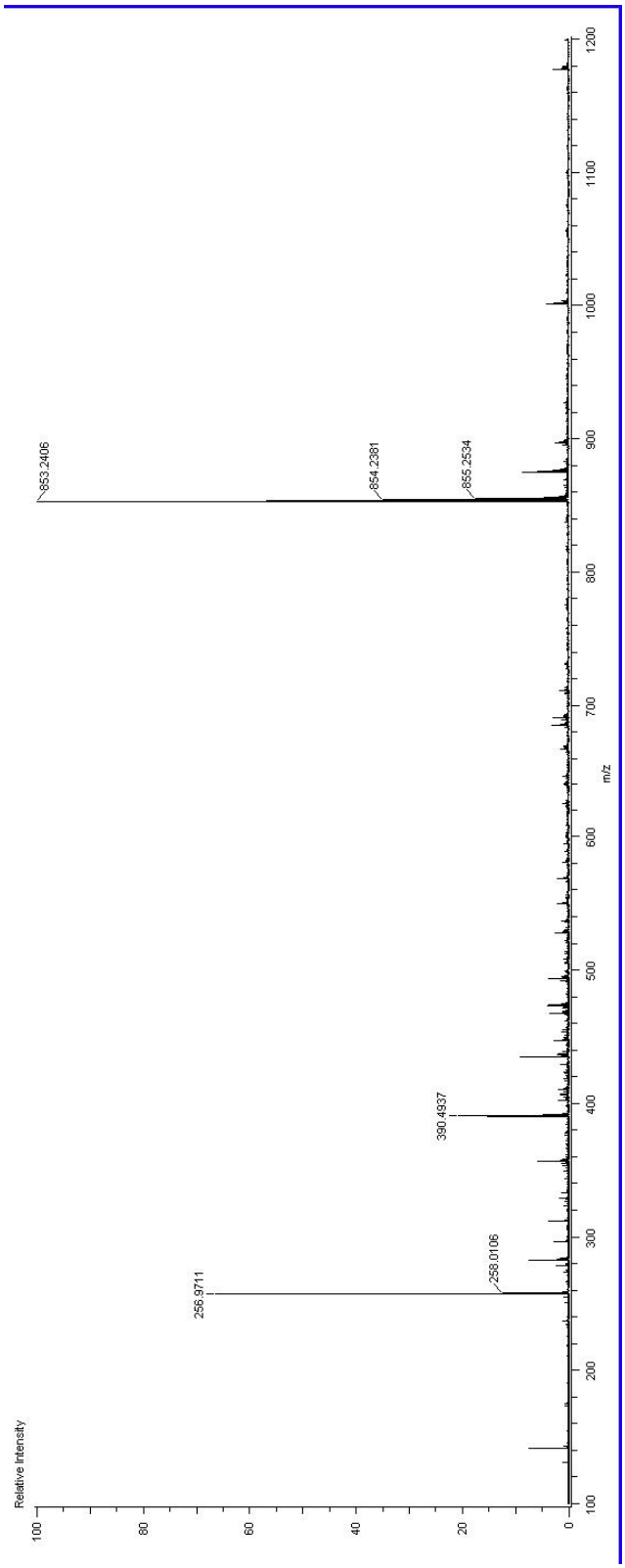
ESI-TOF HR mass spectrum of compound **3**

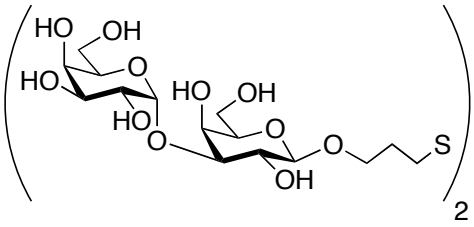




ESI-TOF HR MS $[C_{15}H_{28}NaO_{11}S]^+$ calc. *m/z* = 439.1250, found 439.1006
ESI-TOF HR MS $[C_{15}H_{28}KO_{11}S]^+$ calc. *m/z* = 455.0989, found 455.0733
Note that a) compound **3** was dissolved in D_2O, followed by the addition of H_2O to accomplish deuterium to hydrogen exchange; b) some oxidation to the disulfide occurred.

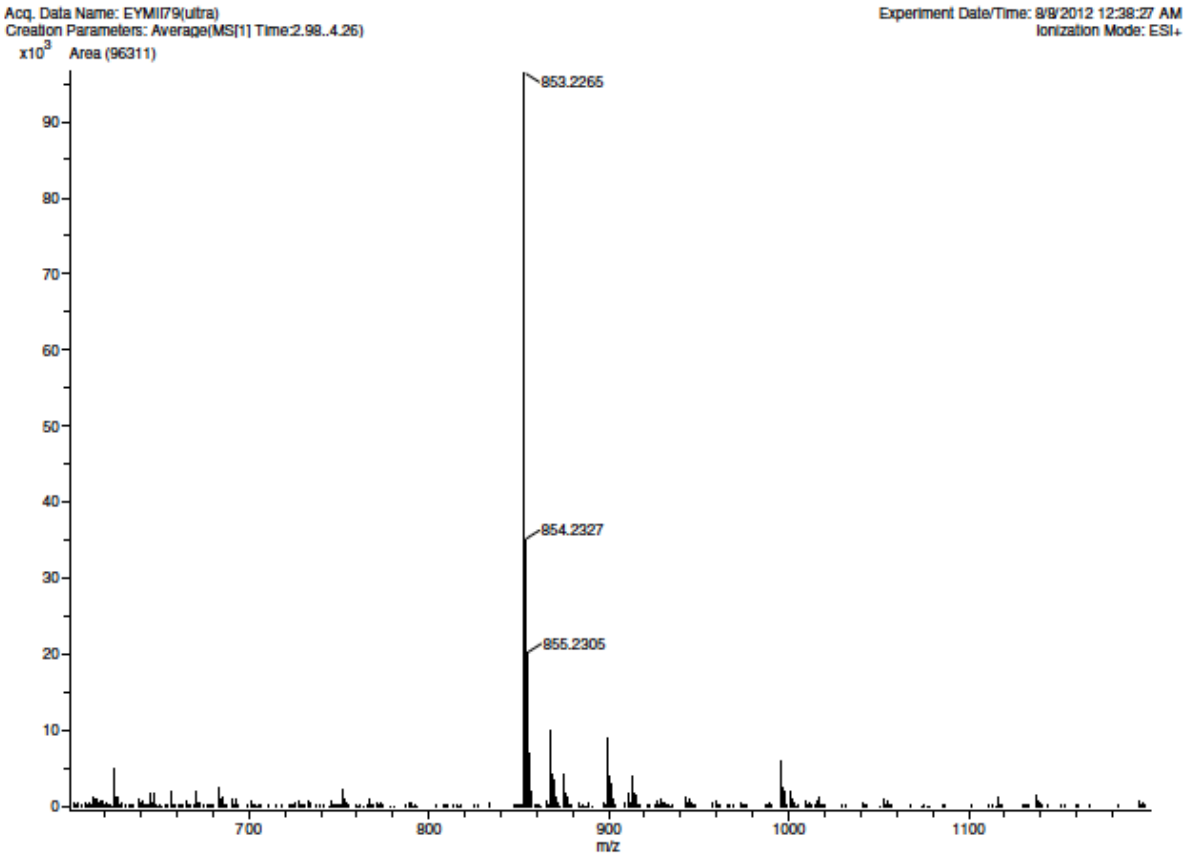
ESI-TOF HR mass spectrum of compound **3** (disulfide form)

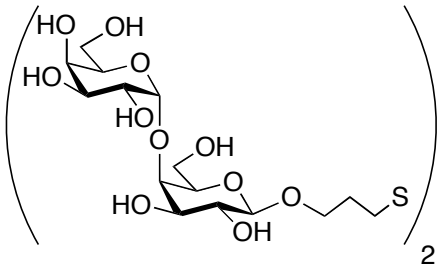




ESI-TOF HR MS $[C_{30}H_{54}NaO_{22}S_2]^+$ calc. *m/z* = 853.2446, found 853.2406

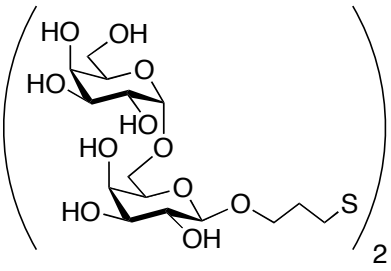
ESI-TOF HR mass spectrum of compound **4** (disulfide form)

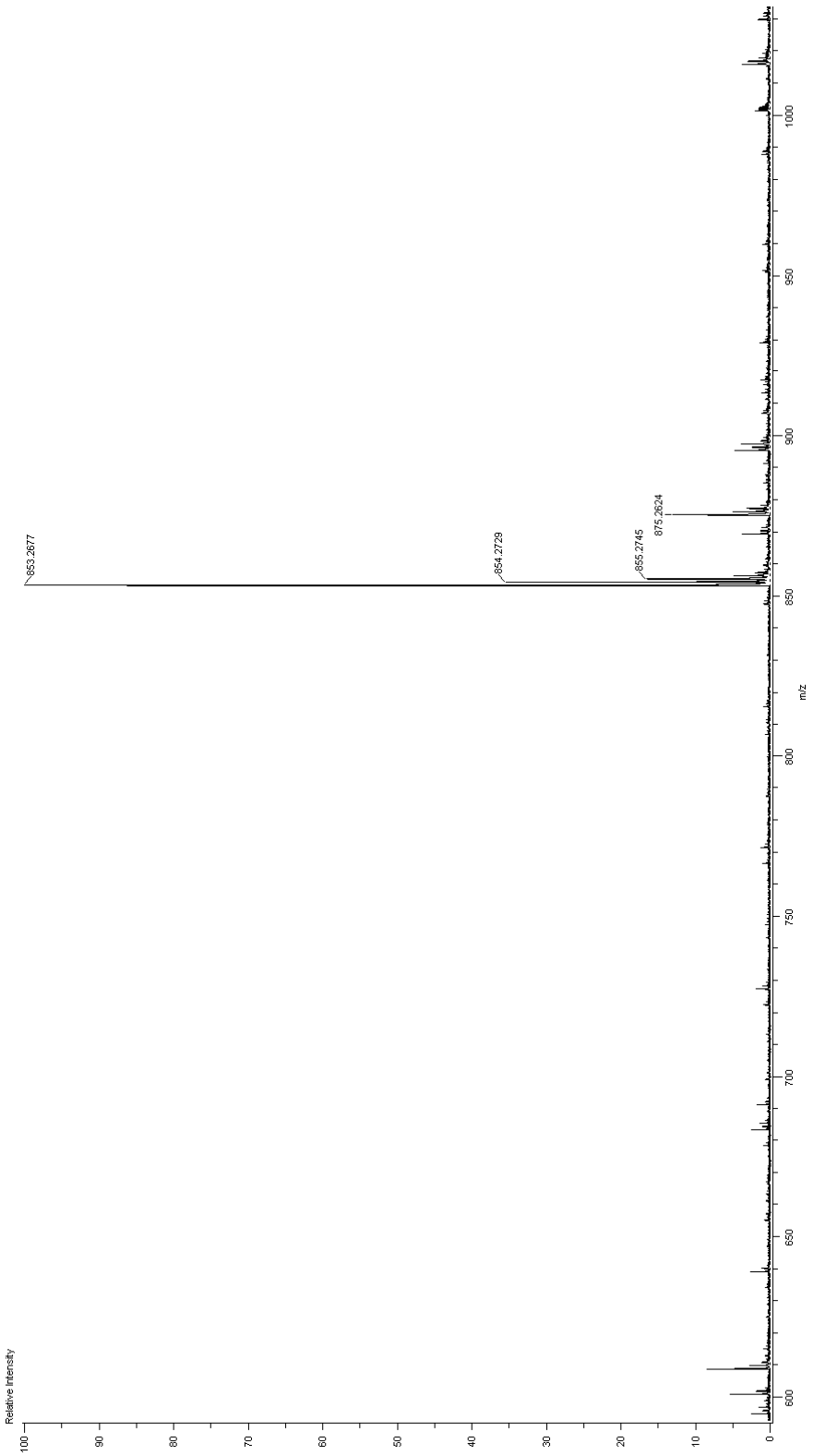




ESI-TOF HR MS $[C_{30}H_{54}NaO_{22}S_2]^+$ calc. *m/z* = 853.2446, found 853.2265

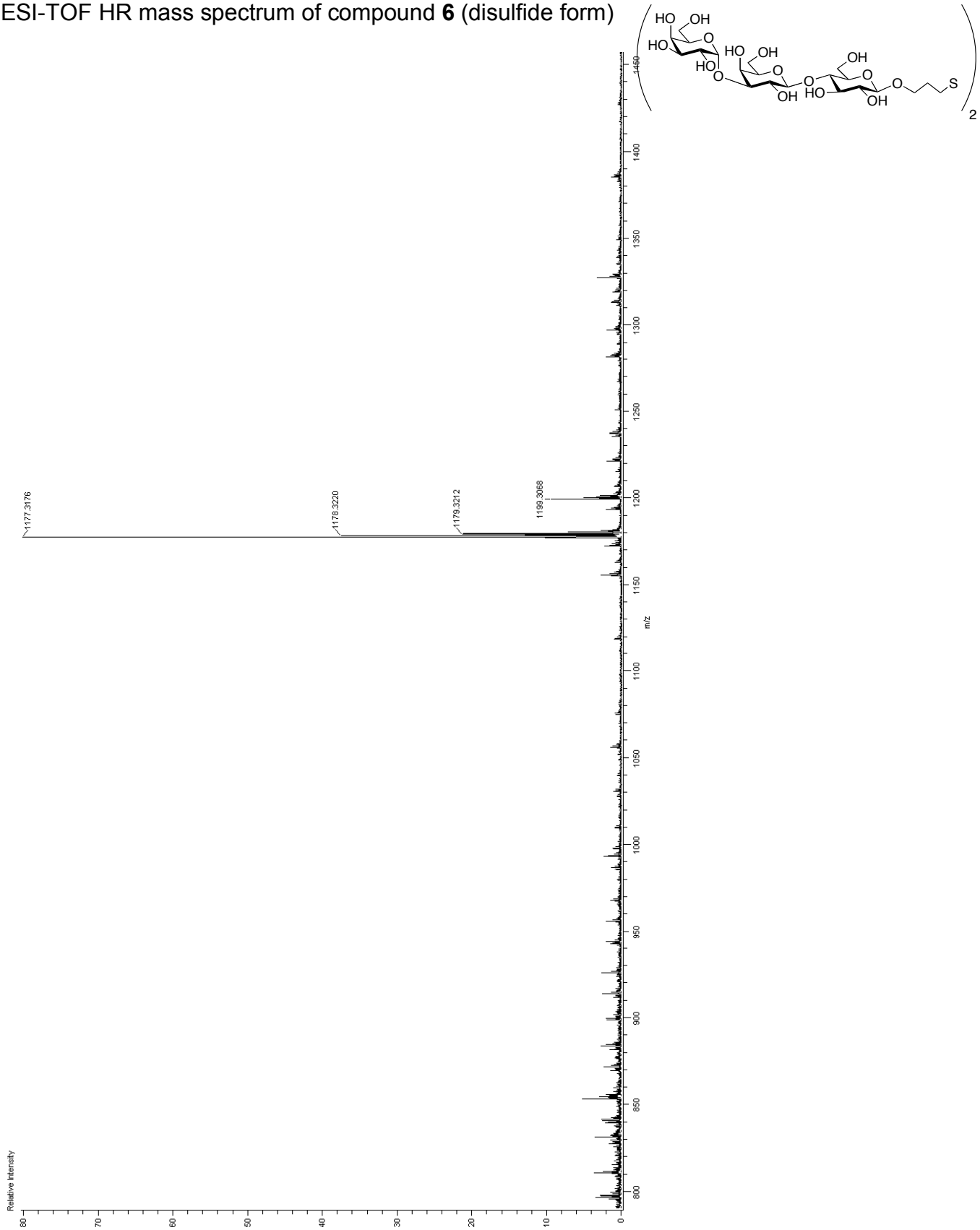
ESI-TOF HR mass spectrum of compound **5** (disulfide form)





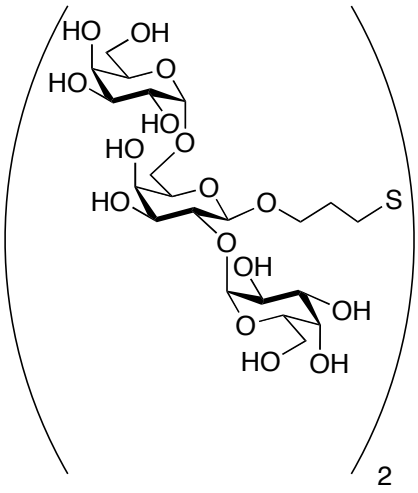
ESI-TOF HR MS $[C_{30}H_{54}NaO_{22}S_2]^+$ calc. *m/z* = 853.2446, found 853.2677

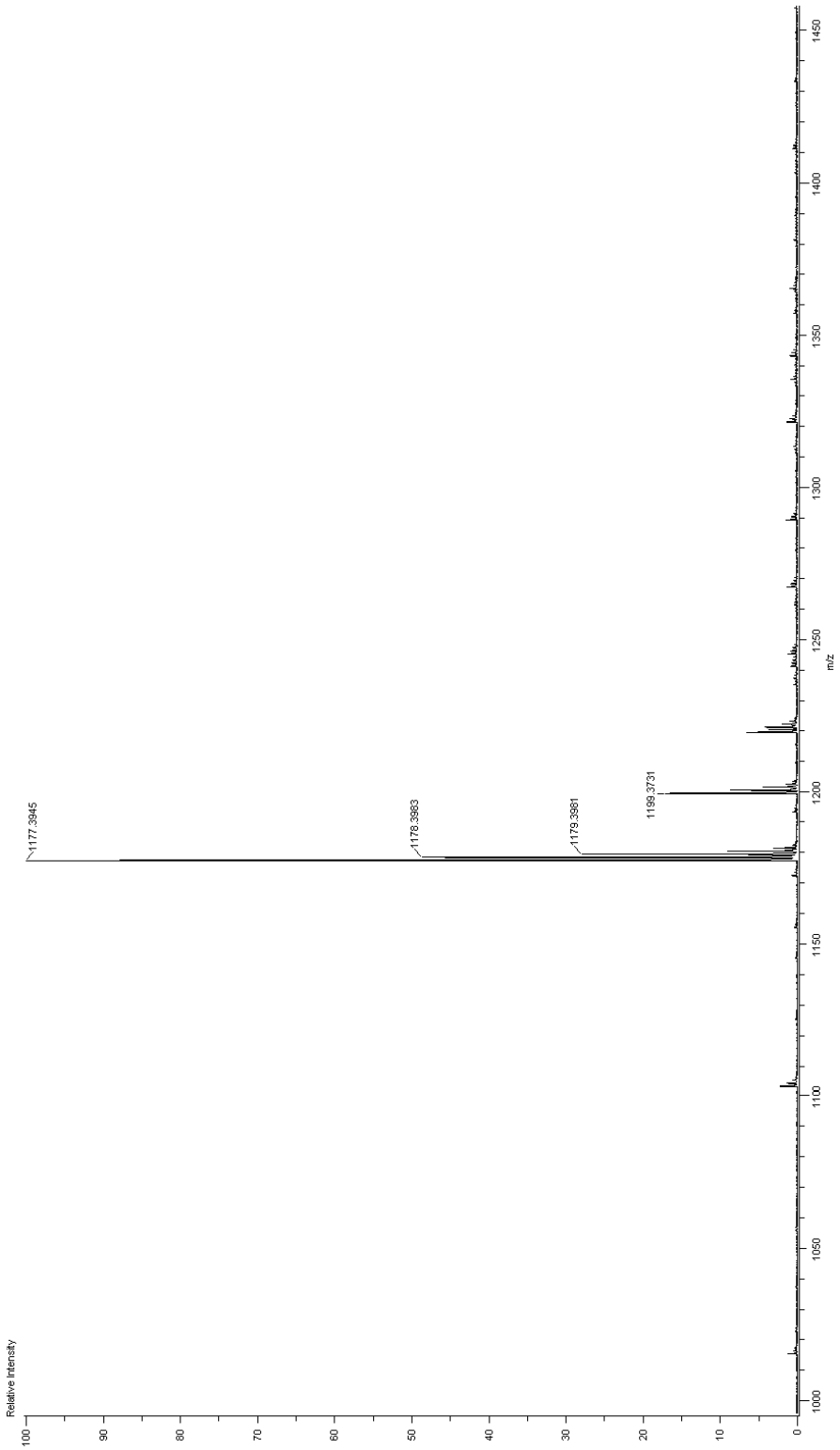
ESI-TOF HR mass spectrum of compound **6** (disulfide form)



ESI-TOF HR MS $[C_{42}H_{74}NaO_{32}S_2]^+$ calc. *m/z* = 1177.3502, found 1177.3176

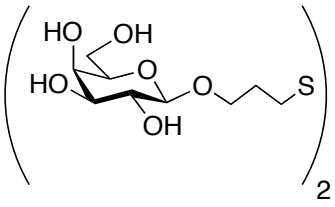
ESI-TOF HR mass spectrum of compound **7** (disulfide form)

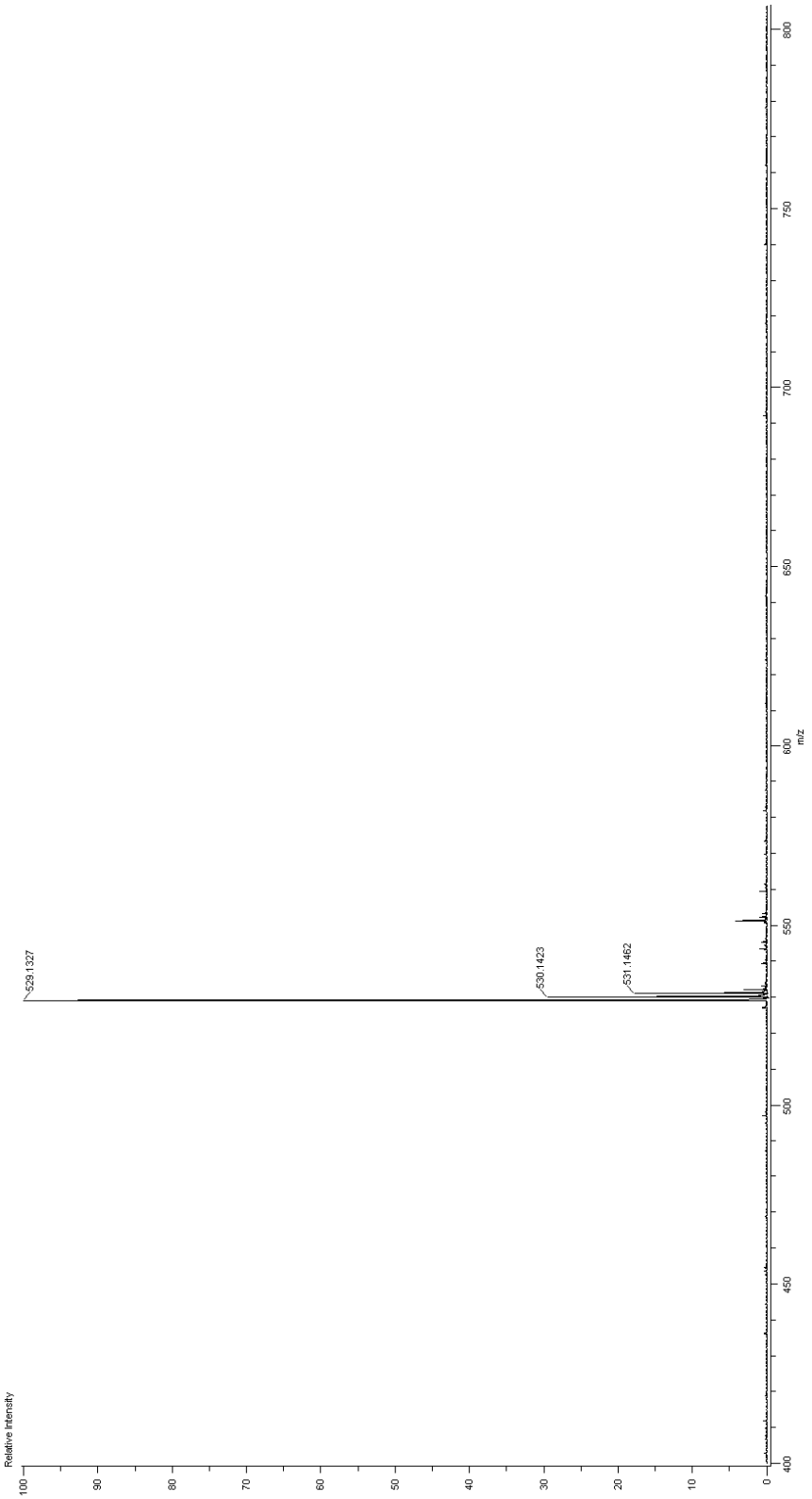




ESI-TOF HR MS $[C_{42}H_{74}NaO_{32}S_2]^+$ calc. *m/z* = 1177.3502, found 1177.3945

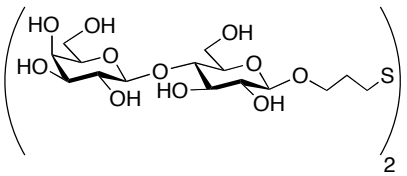
ESI-TOF HR mass spectrum of compound **8** (disulfide form)

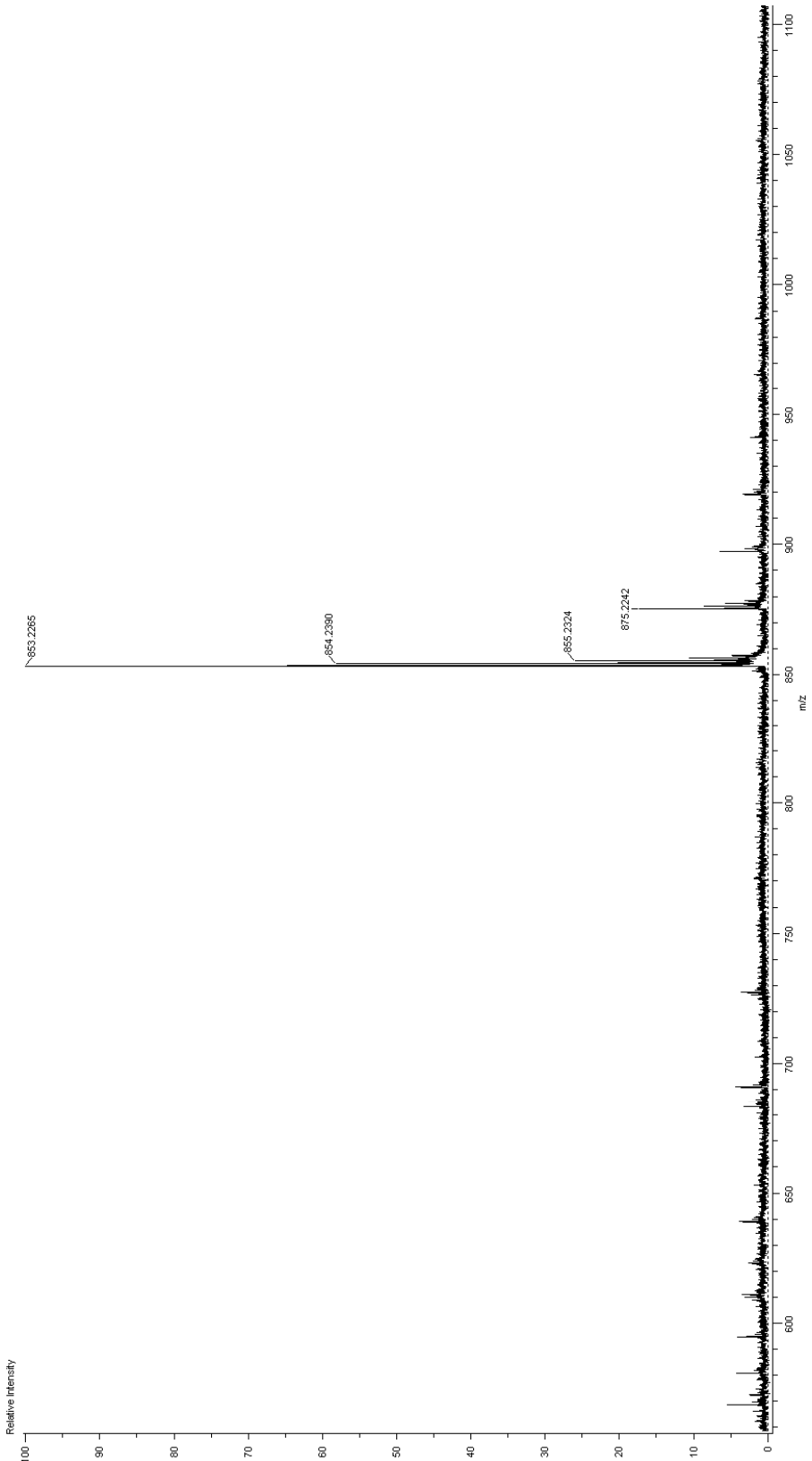




ESI-TOF HR MS $[C_{18}H_{34}NaO_{12}S_2]^+$ calc. *m/z* = 529.1389, found 529.1327

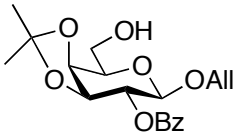
ESI-TOF HR mass spectrum of compound **9** (disulfide form)

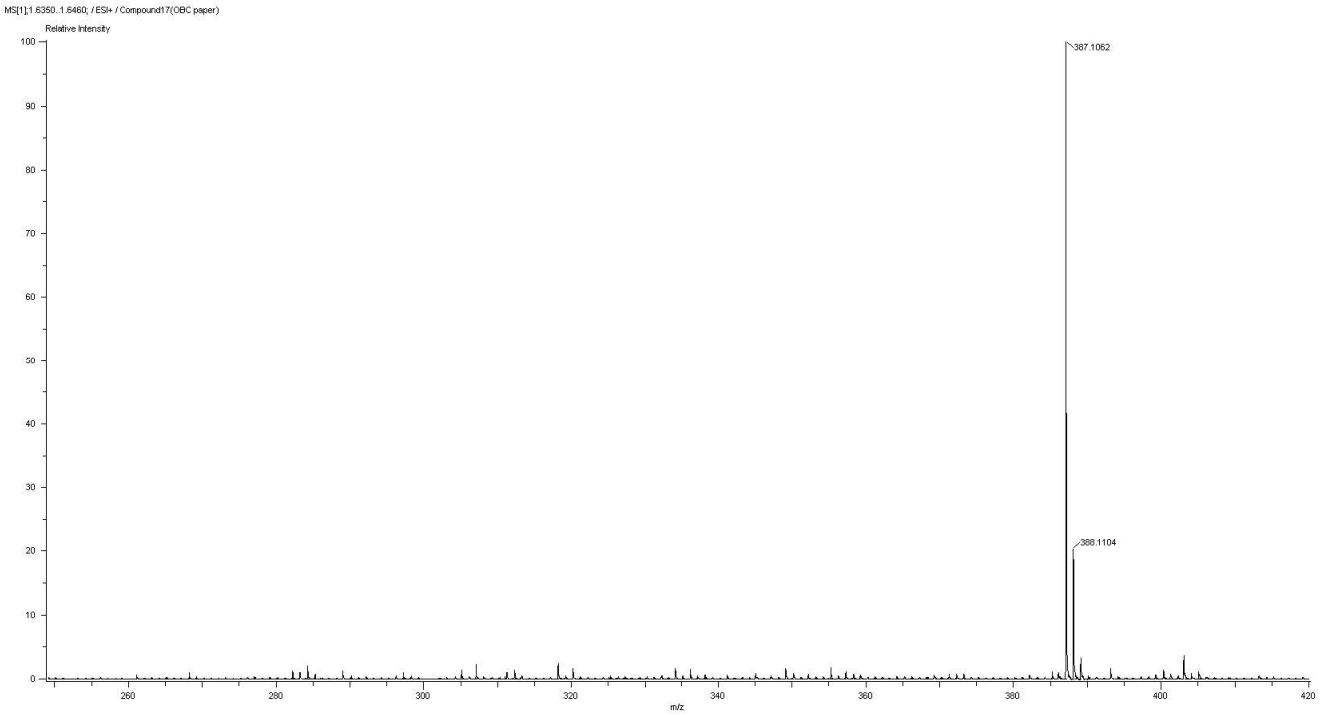




ESI-TOF HR MS $[C_{30}H_{54}NaO_{22}S_2]^+$ calc. *m/z* = 853.2446, found 853.2265

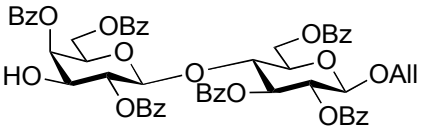
ESI-TOF HR mass spectrum of compound **17**

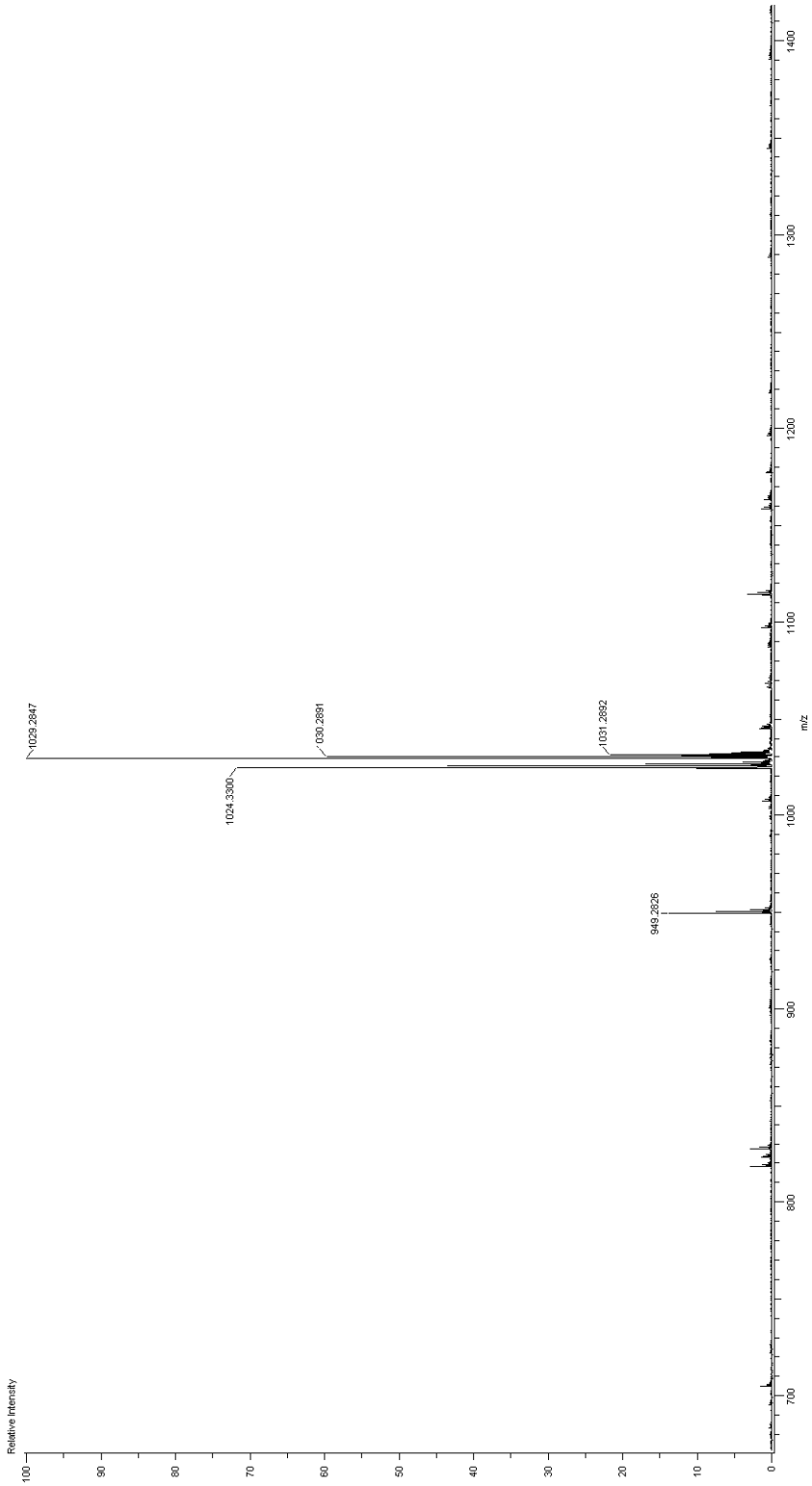




ESI-TOF HR MS $[C_{19}H_{24}NaO_7]^+$ calc. *m/z* = 387.1420, found 387.1062

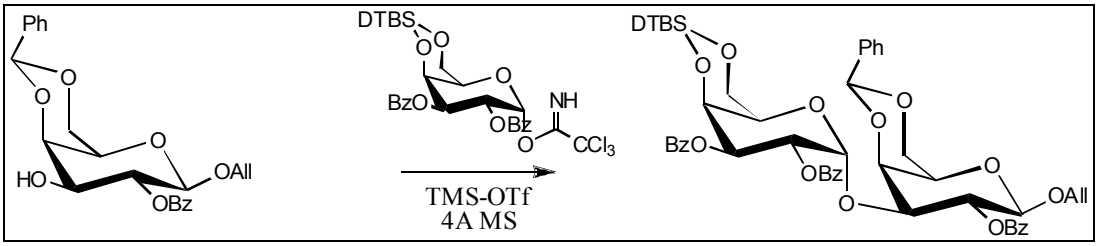
ESI-TOF HR mass spectrum of compound **21**





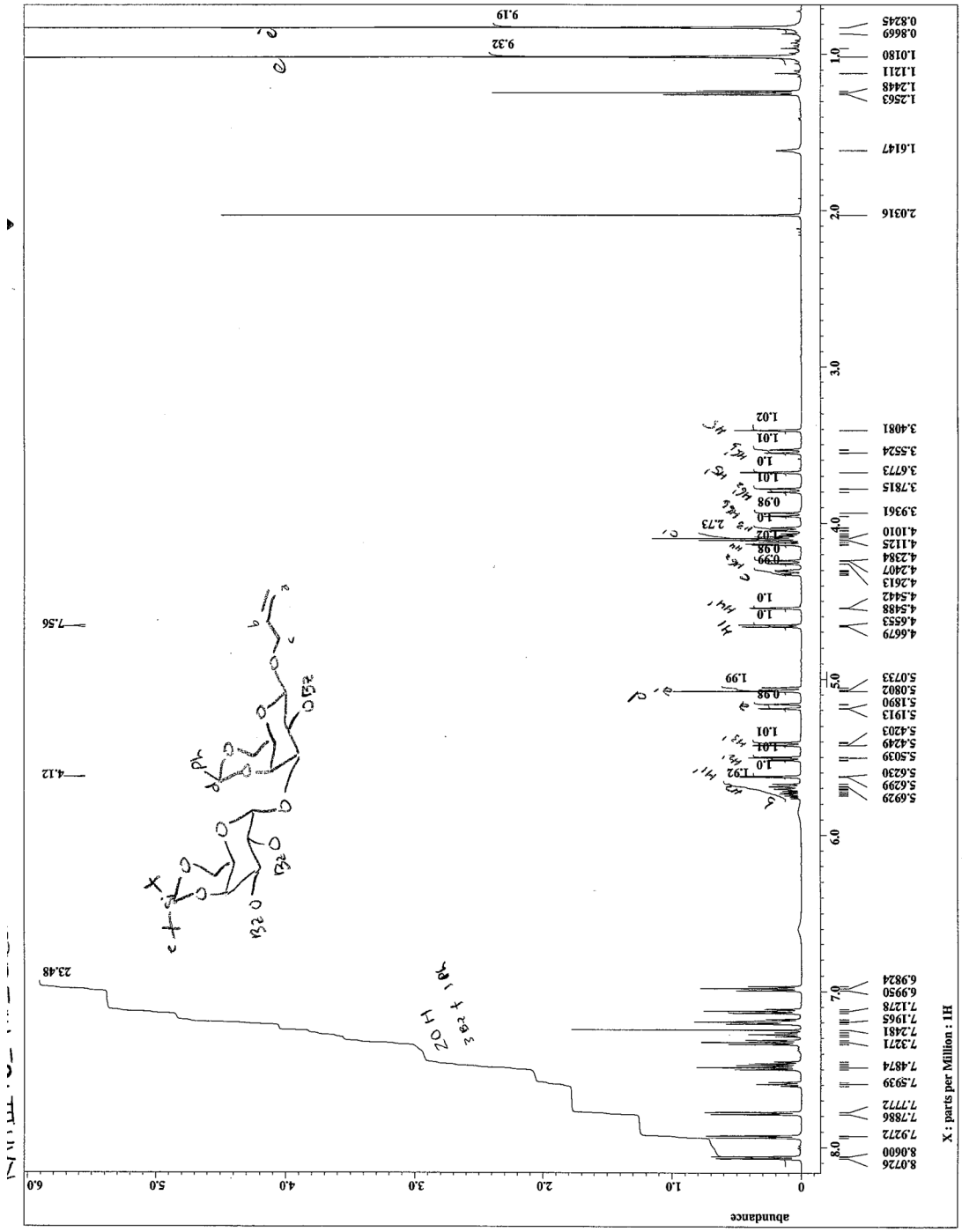
ESI-TOF HR MS $[C_{57}H_{50}NaO_{17}]^+$ calc. *m/z* = 1029.2946, found 1029.2847
ESI-TOF HR MS $[C_{57}H_{54}NO_{17}]^+$ (ammonium adduct) calc. *m/z* = 1024.3392, found 1024.3300

Synthesis of allyl 2,3-di-*O*-benzoyl-4,6-*O*-di-*tert*-butylsilyl-α-D-galactosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactoside (23)

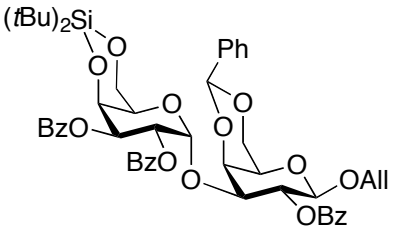


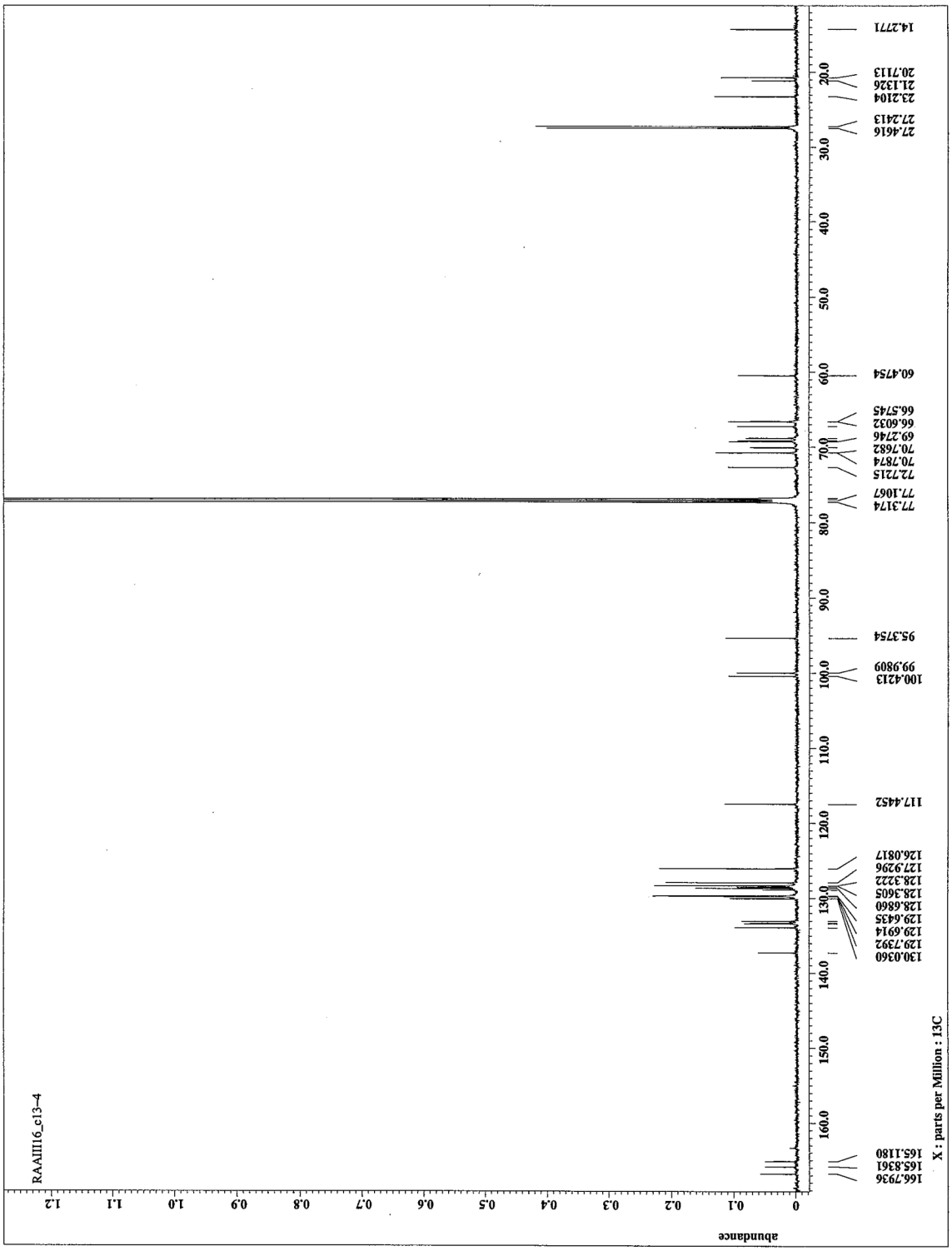
To a solution of acceptor **18** (196 mg, 0.475 mmol), donor **22** (408 mg, 0.606 mmol), and freshly activated crushed 4 Å MS in dry DCM (11 mL) cooled to 0 ^{o}C, TMS-OTf (6 µL) was added and the mixture was stirred under argon at 0 ^{o}C for 1.25 h. After disappearance of starting material **18**, TEA (100 µL, 0.717 mmol) was added, and the mixture was stirred at room temp. for 20 min. $CHCl_3$ (60 mL) was added and molecular sieves were filtered off. The organic layer was washed with water (4 × 25 mL), brine solution (1 × 25 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography (2:1 Hex/EtOAc) to afford **23** (404 mg, 92%) as a white solid. R_f 0.40 (1:1 EtOAc/Hex); $[\alpha]_D$ +140.14 (*c*, 1.0, Acetone); ^{1}H NMR (600 MHz, $CDCl_3$) 8.07 (d, 2H, Ar); 7.93 (d, 2H, Ar); 7.78 (d, 2H, Ar); 7.59 (t, 1H, Ar); 7.49 (m, 3H, Ar); 7.33 (t, 2H, Ar); 7.28 (t, 1H, Ar); 7.20 (t, 3H, Ar); 7.13 (t, 2H, Ar); 6.98 (t, 2H, Ar); 5.72 (m, 1H, $OCH_2CH{=}CH_2$); 5.69 (dd, 1H, $J_{H\text{-}1/H\text{-}2}$ = 7.56 Hz, $J_{H\text{-}2/H\text{-}3}$ = 10.31 Hz, H-2); 5.63 (d, 1H, $J_{H\text{-}1'/H\text{-}2'}$ = 4.12 Hz, H-1'); 5.51 (dd, 1H, $J_{H\text{-}1'/H\text{-}2'}$ = 4.12 Hz, $J_{H\text{-}2'/H\text{-}3'}$ = 10.31 Hz, H-2'); 5.41 (dd, 1H, $J_{H\text{-}2'/H\text{-}3'}$ = 10.31 Hz, $J_{H\text{-}3'/H\text{-}4'}$ = 2.75 Hz, H-3'); 5.18 (dd, 1H, *J* = 17.18 Hz, $OCH_2CH{=}CH_2$); 5.08 (s, 1H, $PhCHO_2$); 5.06 (dd, 1H, *J* = 10.31 Hz, $OCH_2CH{=}CH_2$); 4.66 (d, 1H, $J_{H\text{-}1/H\text{-}2}$ = 7.56 Hz, H-1); 4.55 (d, 1H, $J_{H\text{-}3'/H\text{-}4'}$ = 2.75 Hz, H-4'); 4.32 (m, 1H, $OCH_2CH{=}CH_2$); 4.25 (m, 1H, H-6_a); 4.14 (d, 1H, $J_{H\text{-}3/H\text{-}4}$ = 3.44 Hz, H-4); 4.09 (m, 1H, $OCH_2CH{=}CH_2$); 4.04 (dd, 1H, $J_{H\text{-}2/H\text{-}3}$ = 10.31 Hz, $J_{H\text{-}3/H\text{-}4}$ = 3.44 Hz, H-3); 3.95 (dd, 1H, *J* = 12.37 Hz, H-6_b); 3.79 (dd, 1H, *J* = 13.06 Hz, H-$6'_a$); 3.68 (s, 1H, H-5'); 3.54 (dd, 1H, *J* = 13.06 Hz, H-$6'_b$); 3.41 (s, 1H, H-5); 1.02 (s, 9H, tBu); 0.82 (s, 9H, tBu); ^{13}C NMR (151 MHz; $CDCl_3$): δ 171.2, 166.8, 165.8, 165.1, 137.3, 133.9, 133.38, 133.26, 133.0, 130.0, 129.74, 129.69, 129.64, 128.9, 128.68, 128.52, 128.36, 128.32, 127.9,

126.1, 117.4, 100.4, 100.0, 95.4, 72.7, 70.78, 70.77, 70.1, 69.27, 69.15, 68.8, 67.2, 66.60, 66.57, 60.5, 27.5, 27.2, 23.2, 21.1, 20.7, 14.3.

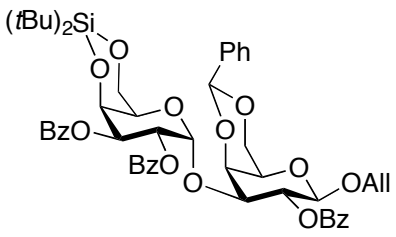


^{1}H NMR spectrum of compound **23**

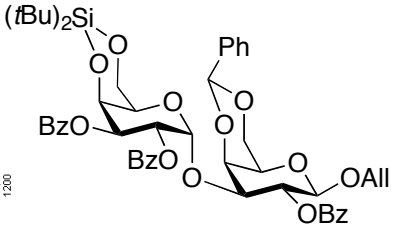


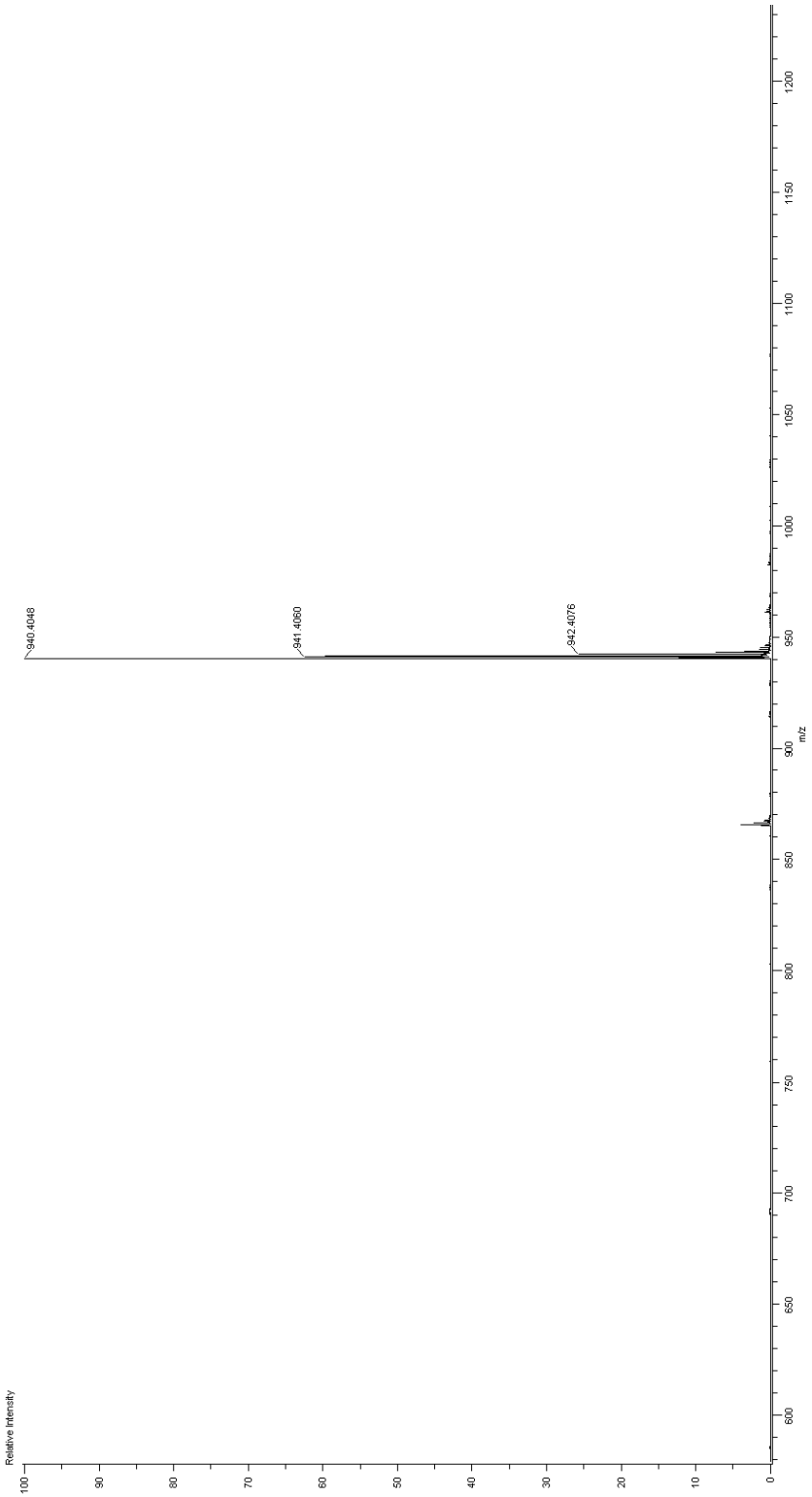


^{13}C NMR spectrum of compound **23**



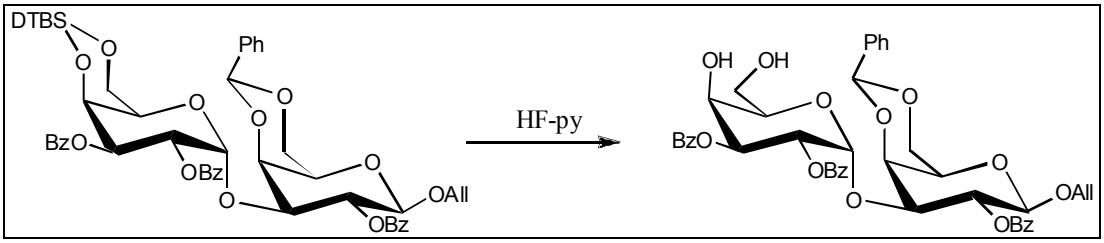
ESI-TOF HR mass spectrum of compound **23**



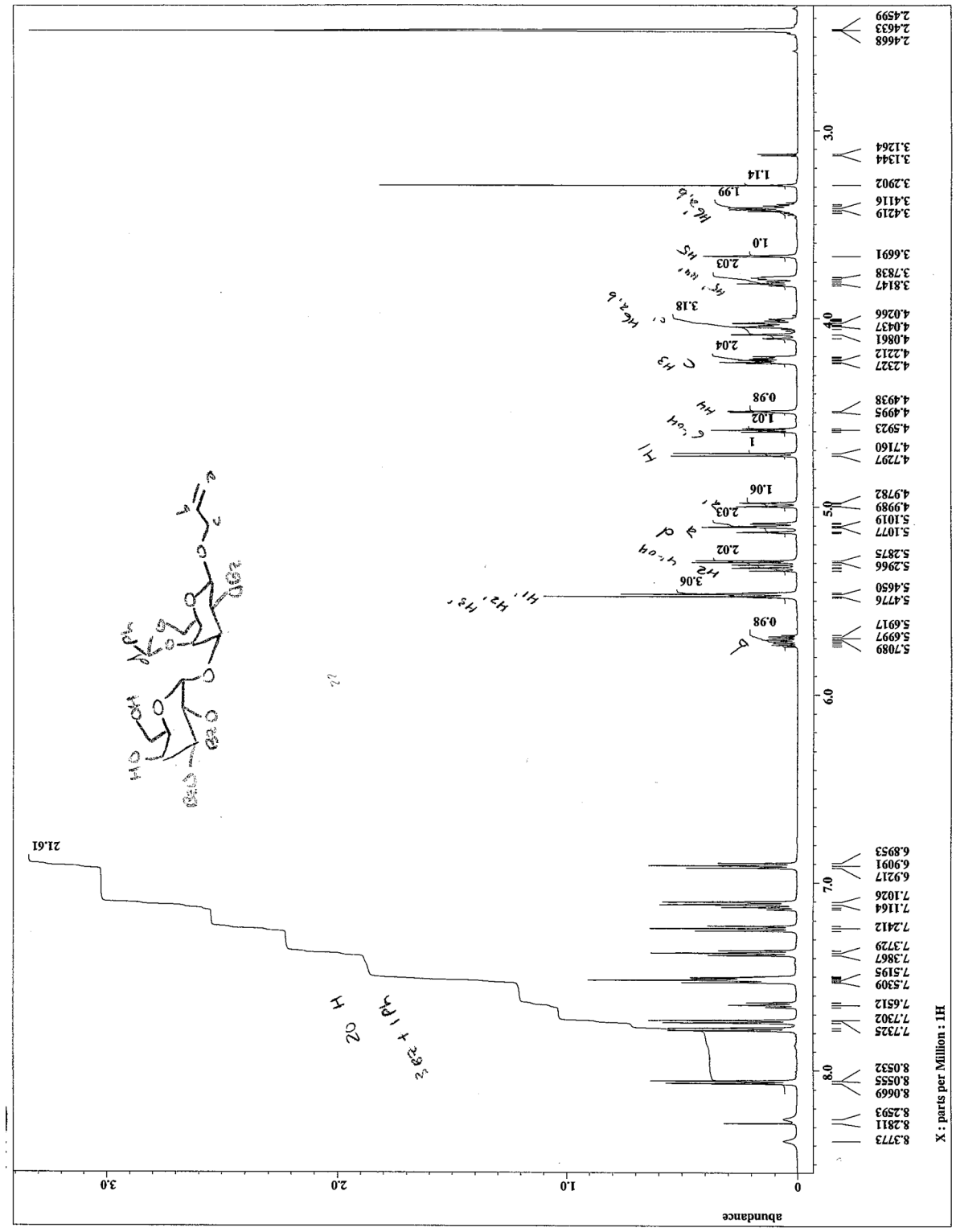


ESI-TOF HR MS $[C_{51}H_{62}NO_{14}Si]^+$ (ammonium adduct) calc. *m/z* = 940.3940, found 940.4048

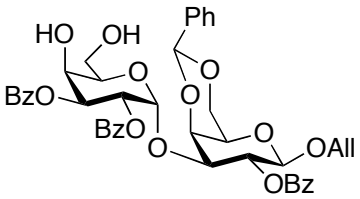
Synthesis of allyl 2,3-di-*O*-benzoyl-α-D-galactosyl-(1→3)-2-*O*-benzoyl-4,6-*O*-benzylidene-β-D-galactoside (24)

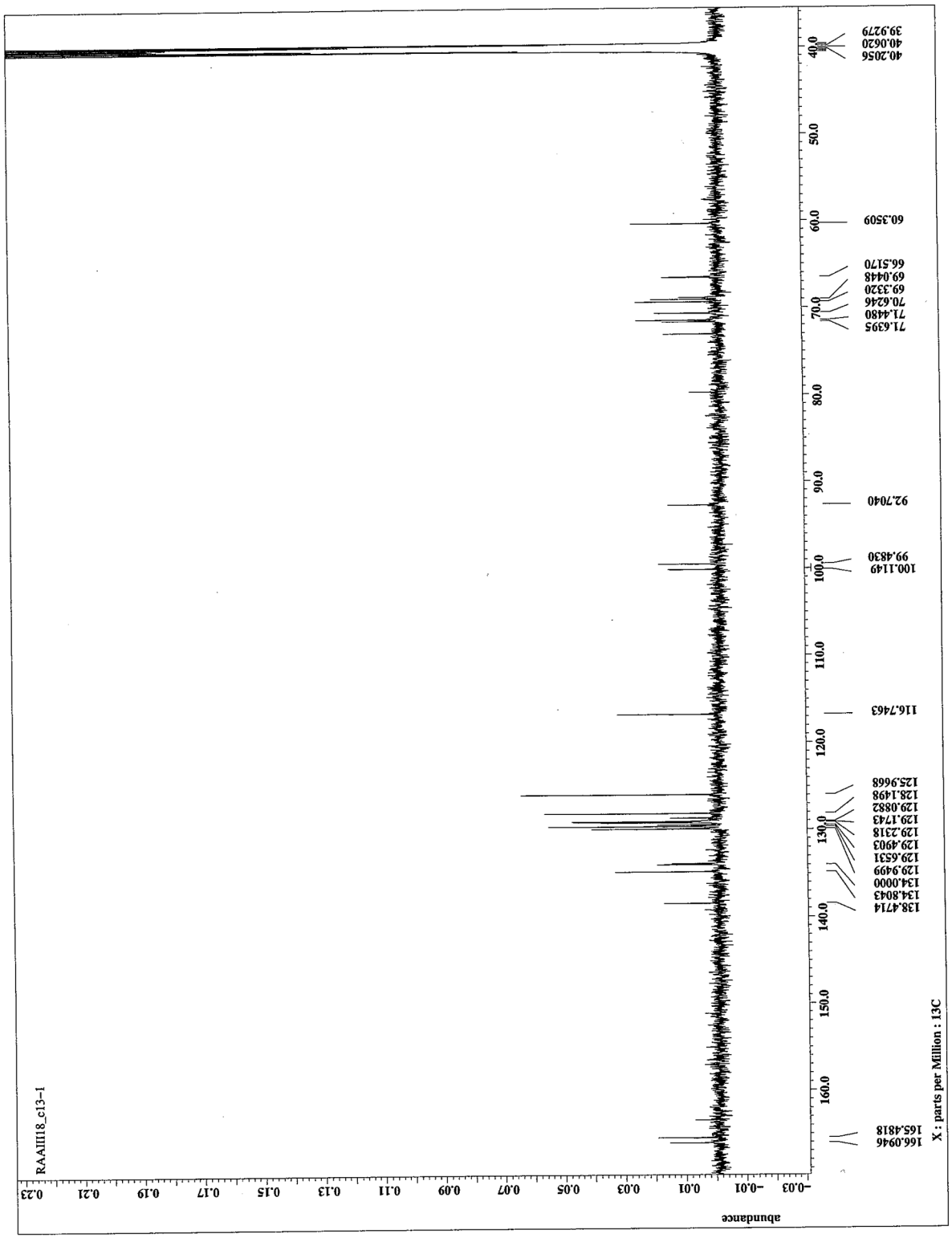


To a solution of **23** (394 mg, 0.427 mmol) in dry THF (6 mL) cooled to 0 °C, HF-pyr (214 µL, 8.3537 mmol, volume accounted for HF and by percentage of HF in HF-pyr solution) was added and the mixture was stirred under argon to room temp. for 1 h. The reaction mixture was cooled to 0 °C and saturated $NaHCO_3$ solution was added and stirred for 15 min. The reaction mixture was extracted with EtOAc (60 mL). The organic layer was washed with water (4 × 30 mL). The aqueous layer was re-extracted with EtOAc (2 × 20 mL). The organic layers were combined, washed with brine solution (1 × 25 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography (10:1 $CHCl_3$/MeOH) to afford **24** (300 mg, 90%) as a white solid. R_f 0.30 (9:1 $CHCl_3$/MeOH); $[\alpha]_D$ +180.18 (*c*, 1.0, MeOH); ^{1}H NMR (600 MHz, CD_3OD) 8.08 (d, 2H, Ar); 7.79 (d, 2H, Ar); 7.74 (d, 2H, Ar); 7.65 (t, 1H, Ar); 7.51 (m, 4H, Ar); 7.37 (t, 2H, Ar); 7.24 (t, 2H, Ar); 7.21 (t, 3H, Ar); 6.91 (t, 2H, Ar); 5.71 (m, 1H, OCH_2CH=CH_2); 5.47 (m, 3H, H-1', H-2', H-3'); 5.33 (dd, 1H, $J_{H\text{-}1/H\text{-}2}$ = 8.25 Hz, $J_{H\text{-}2/H\text{-}3}$ = 10.31 Hz, H-2); 5.29 (d, 1H, *J* = 5.50 Hz, 4'-OH); 5.14-5.08 (m, 2H, $PhCHO_2$, OCH_2CH=CH_2); 4.99 (dd, 1H, *J* = 10.31 Hz, OCH_2CH=CH_2); 4.72 (d, 1H, $J_{H\text{-}1/H\text{-}2}$ = 8.25 Hz, H-1); 4.59 (dd, 1H, *J* = 6.19 Hz, 6'-O*H*); 4.50 (d, 1H, $J_{H\text{-}3/H\text{-}4}$ = 3.44 Hz, H-4); 4.24-4.20 (m, 2H, H-3, OCH_2CH=CH_2); 4.08-4.00 (m, 3H, H-$6_{a,b}$, OCH_2CH=CH_2); 3.82 (dd, 1H, $J_{H\text{-}5'/H\text{-}6'a}$ = 6.19 Hz, $J_{H\text{-}5'/H\text{-}6'b}$ = 6.87 Hz, H-5'); 3.79 (m, 1H, H-4'); 3.67 (s, 1H, H-5); 3.42 (m, 2H, H-6'$_{a,b}$); ^{13}C NMR (151 MHz; CD_3OD): δ 171.2, 166.8, 165.8, 165.1, 137.3, 133.9, 133.4, 133.3, 133.0, 130.0, 129.7, 129.7, 129.6, 128.9, 128.7, 128.5, 128.4, 128.3, 127.9, 126.1, 117.4, 100.4, 100.0, 95.4, 72.7, 70.8 (2), 70.1, 69.3, 69.2, 68.8, 67.2, 66.6 (2), 60.5, 27.5, 27.2, 23.2, 21.1, 20.7, 14.3.

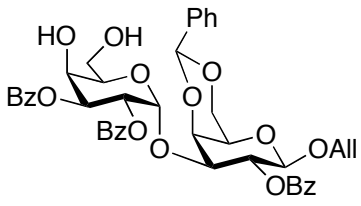


^{1}H NMR spectrum of compound **24**

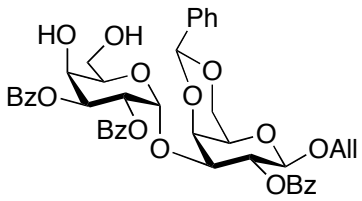


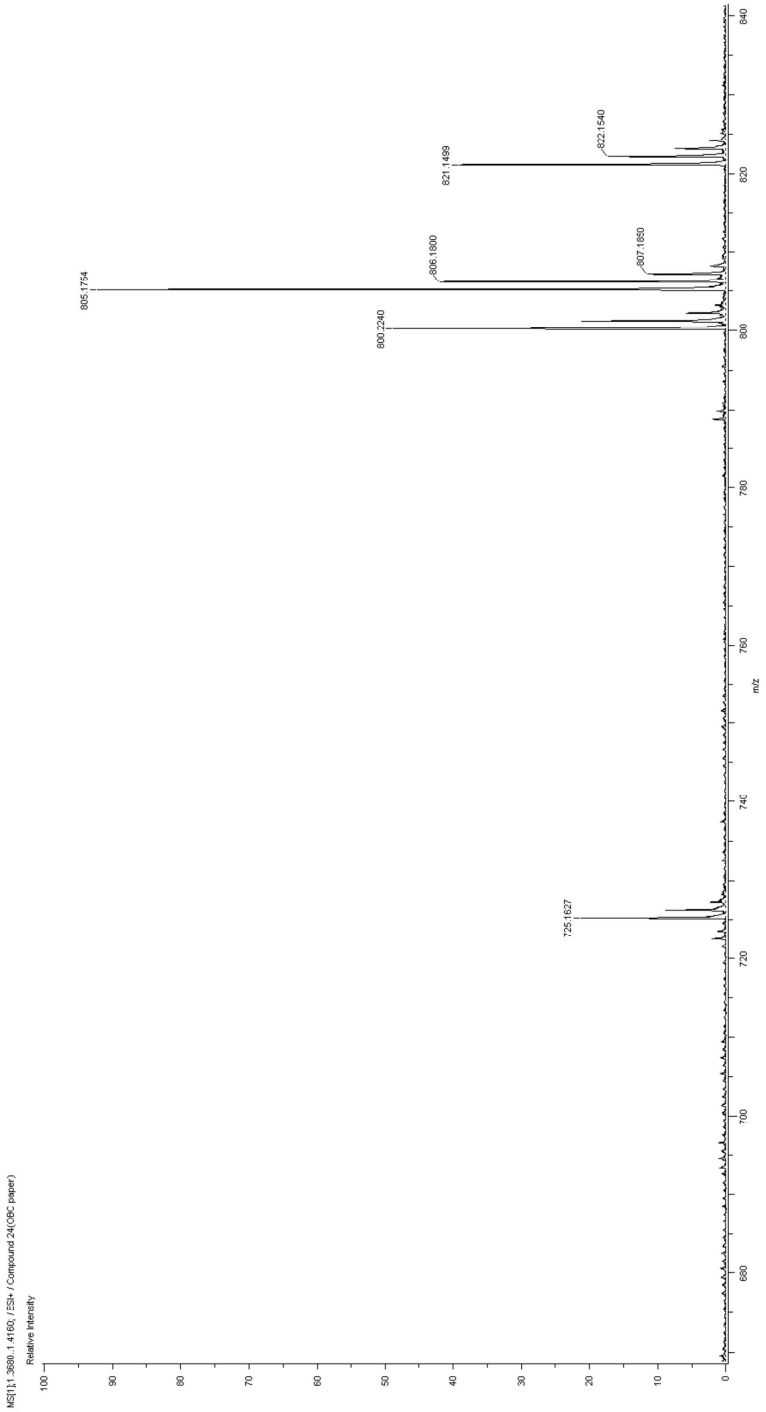


^{13}C NMR spectrum of compound **24**



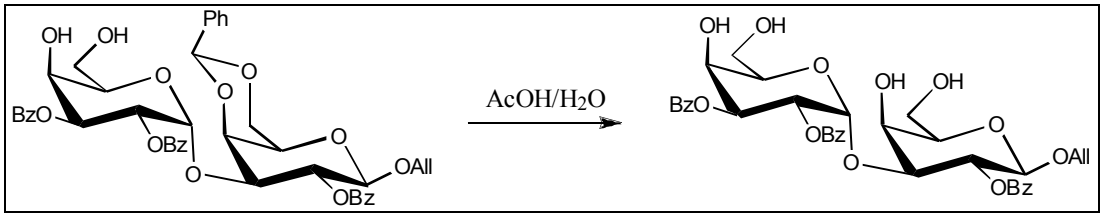
ESI-TOF HR mass spectrum of compound **24**



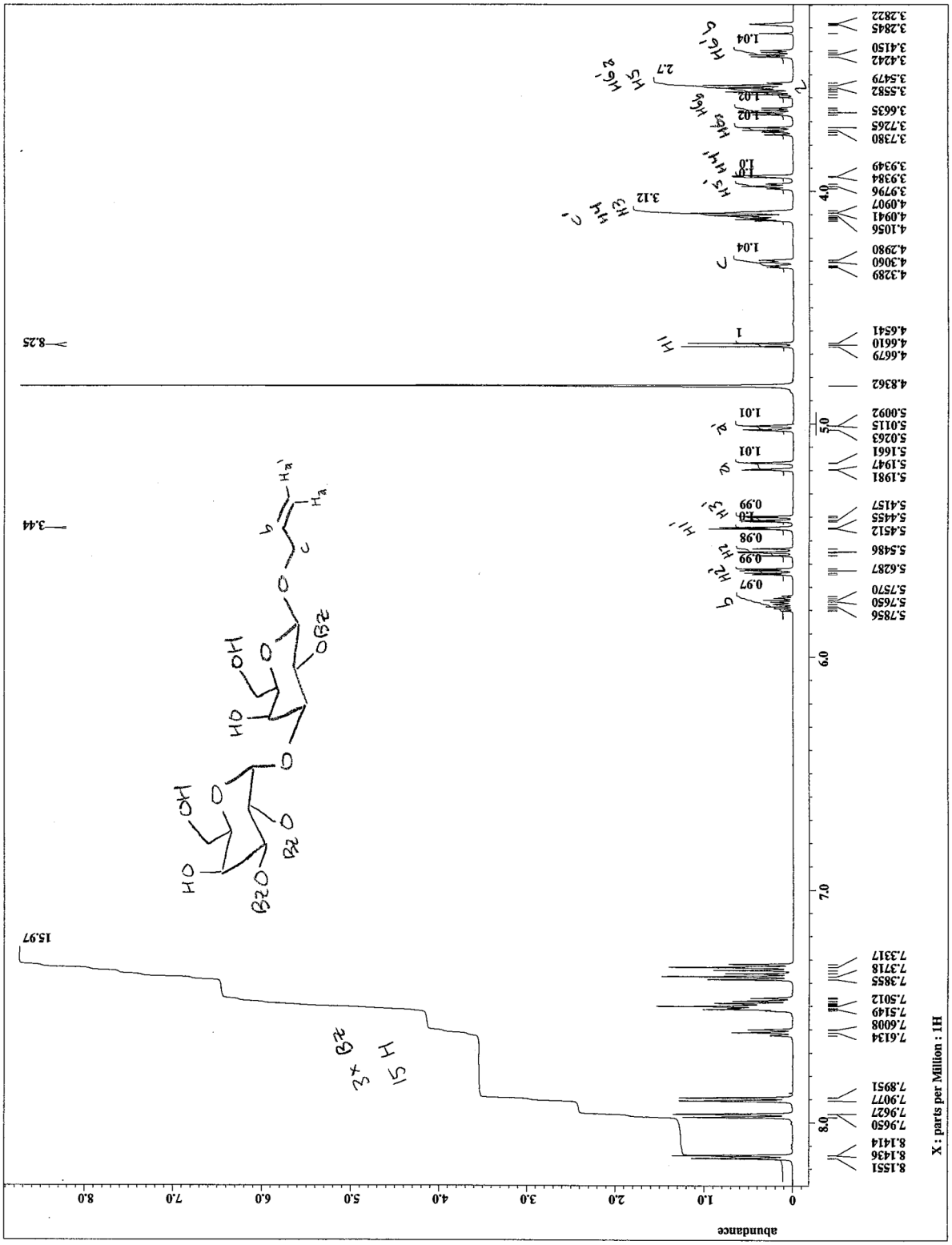


ESI-TOF HR MS $[C_{43}H_{46}NO_{14}]^+$ (ammonium adduct) calc. *m/z* = 800.2918, found 800.2240
ESI-TOF HR MS $[C_{43}H_{42}NaO_{14}]^+$ calc. *m/z* = 805.2472, found 805.1754
ESI-TOF HR MS $[C_{43}H_{42}KO_{14}]^+$ calc. *m/z* = 821.2212, found 821.1499

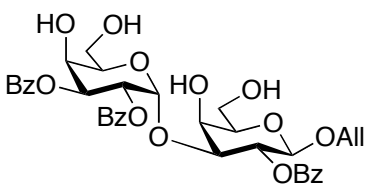
Synthesis of allyl 2,3-di-*O*-benzoyl-α-D-galactosyl-(1→3)-2-*O*-benzoyl-β-D-galactoside (25)

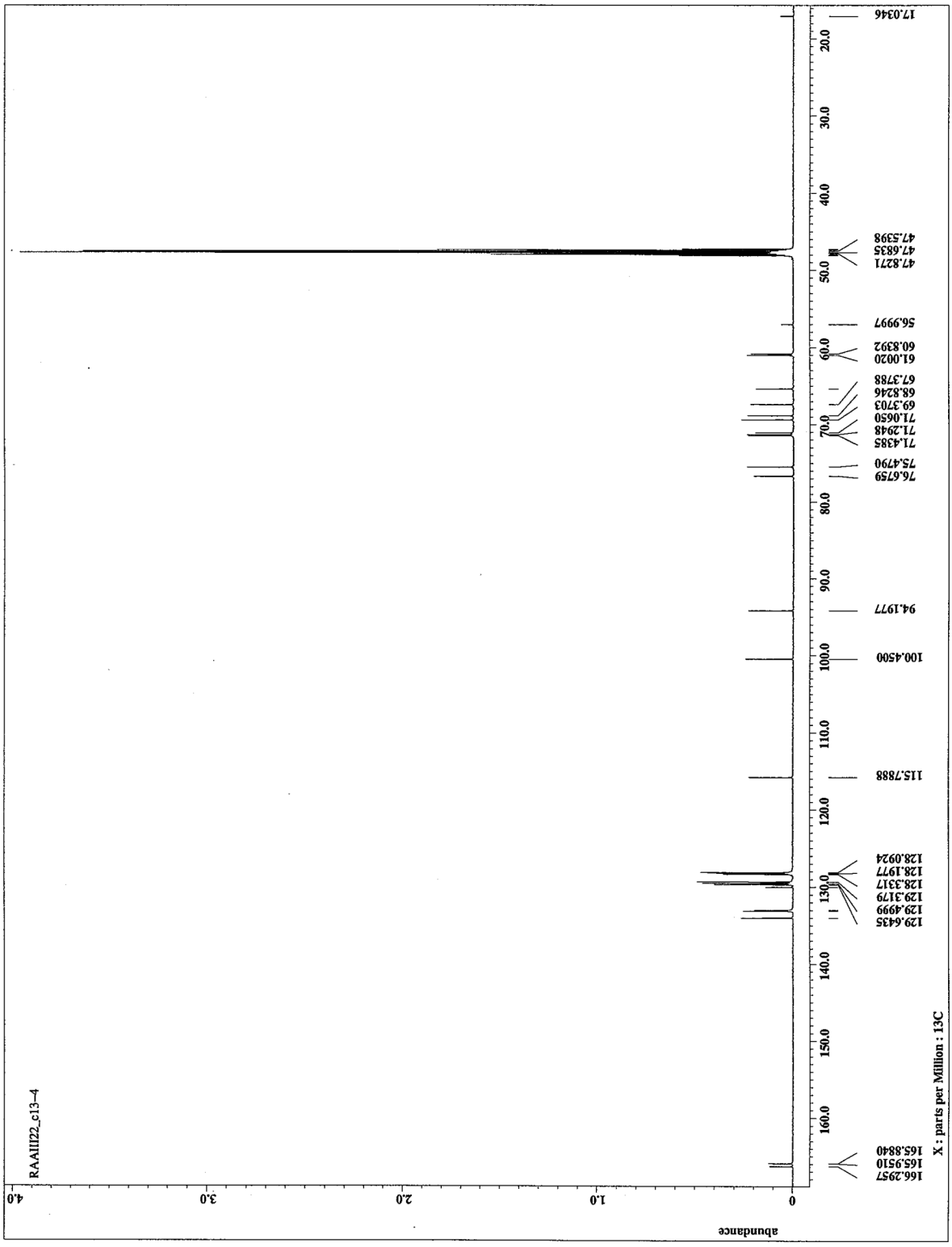


To **24** (248 mg, 0.317 mmol) was added AcOH (16 mL) and water (4 mL). The mixture was stirred at 80 ^{o}C for 5 h. The mixture was then allowed to cool to room temp. and was then co-evaporated with EtOH. The crude product was purified by flash chromatography (20:1 → 15:1 $CHCl_3$/MeOH) to afford **25** (187 mg, 85%) as clear oil. R_f 0.45 (9:1 $CHCl_3$/MeOH); $[\alpha]_D$ +100.10 (*c*, 1.0, MeOH); ^{1}H NMR (600 MHz, CD_3OD) 8.15 (d, 2H, Ar); 7.96 (d, 2H, Ar); 7.90 (d, 2H, Ar); 7.61 (t, 1H, Ar); 7.52-7.46 (m, 4H, Ar); 7.39-7.31 (m, 4H, Ar); 5.81-5.74 (m, 1H, OCH_2CH=CH_2); 5.64 (dd, 1H, $J_{H\text{-}1'/H\text{-}2'}$ = 3.44 Hz, $J_{H\text{-}2'/J\text{-}3'}$ = 11.00 Hz, H-2'); 5.55 (dd, 1H, $J_{H\text{-}1/H\text{-}2}$ = 7.56 Hz, $J_{H\text{-}2/H\text{-}3}$ = 9.62 Hz, H-2); 5.45 (d, 1H, $J_{H\text{-}1'/H\text{-}2'}$ = 3.44 Hz, H-1'); 5.41 (dd, 1H, $J_{H\text{-}2'/H\text{-}3'}$ = 11.00 Hz, $J_{H\text{-}3'/H\text{-}4'}$ = 2.75 Hz, H-3'); 5.18 (dd, 1H, *J* = 17.18 Hz, OCH_2CH=CH_2), 5.02 (dd, 1H, *J* = 10.31 Hz, OCH_2CH=CH_2); 4.66 (d, 1H, $J_{H\text{-}1/H\text{-}2}$ = 8.25 Hz, H-1); 4.31 (m, 1H, OCH_2CH=CH_2); 4.13-4.08 (m, 3H, H-3, H-4, OCH_2CH=CH_2); 3.98 (dd, 1H, $J_{H\text{-}5'/H\text{-}6'a}$ = 5.50 Hz, $J_{H\text{-}5'/H\text{-}6'b}$ = 6.19 Hz, H-5'); 3.94 (d, 1H, $J_{H\text{-}3'/H\text{-}4'}$ = 2.06 Hz, H-4'); 3.74 (m, 1H, H-6_a); 3.66 (m, 1H, H-6_b); 3.6-3.54 (m, 2H, H-5, H-$6'_a$); 3.41 (m, 1H, H-$6'_b$); ^{13}C NMR (151 MHz; CD_3OD): δ 171.2, 166.8, 165.8, 165.1, 137.3, 133.9, 133.4, 133.3, 133.0, 130.0, 129.7, 129.7, 129.6, 128.9, 128.7, 128.5, 128.4, 128.3, 127.9, 126.1, 117.4, 100.4, 100.0, 95.4, 72.7, 70.8 (2), 70.1, 69.3, 69.2, 68.8, 67.2, 66.6 (2), 60.5, 27.5, 27.2, 23.2, 21.1, 20.7, 14.3.

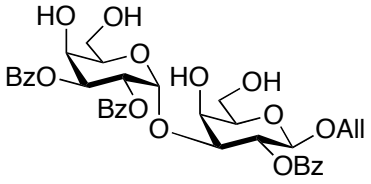


^{1}H NMR spectrum of compound **25**

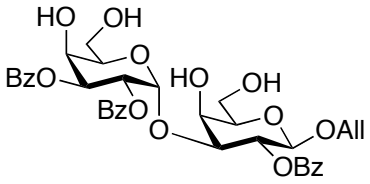


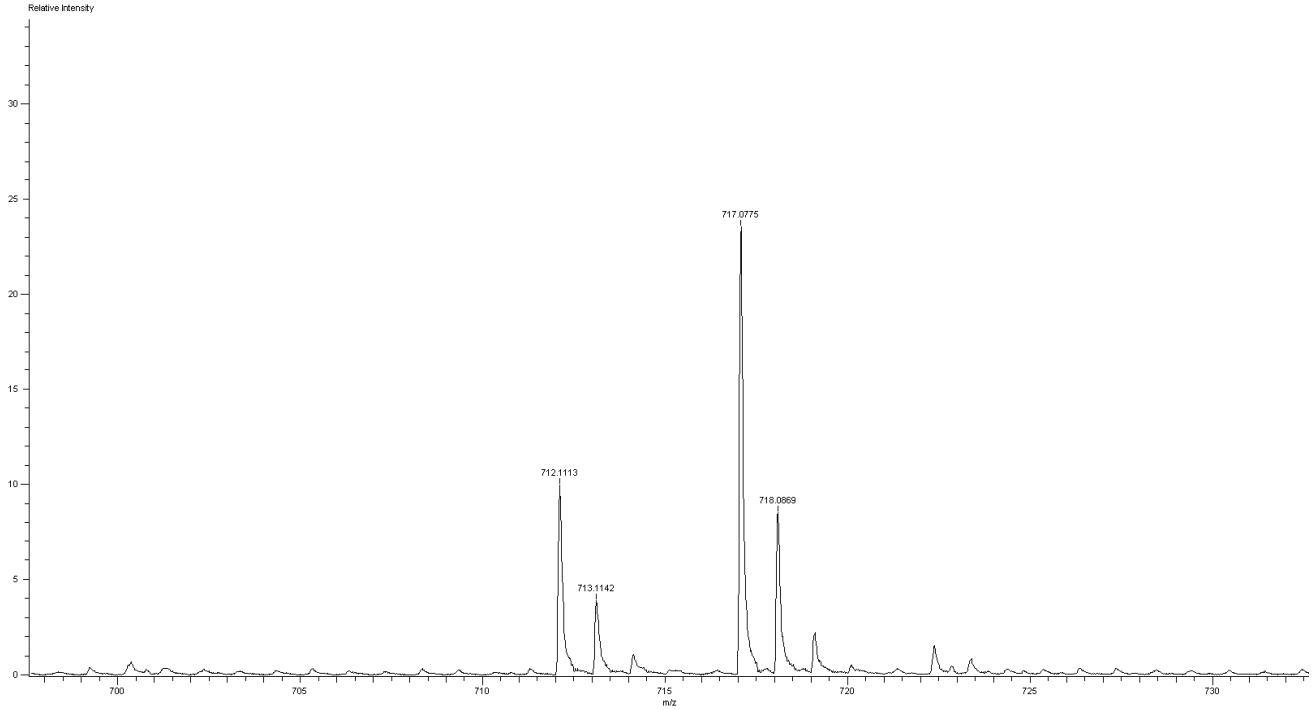


^{13}C NMR spectrum of compound **25**



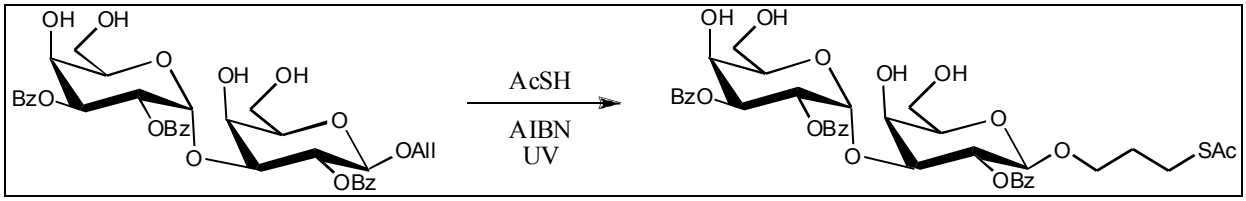
ESI-TOF HR mass spectrum of compound **25**



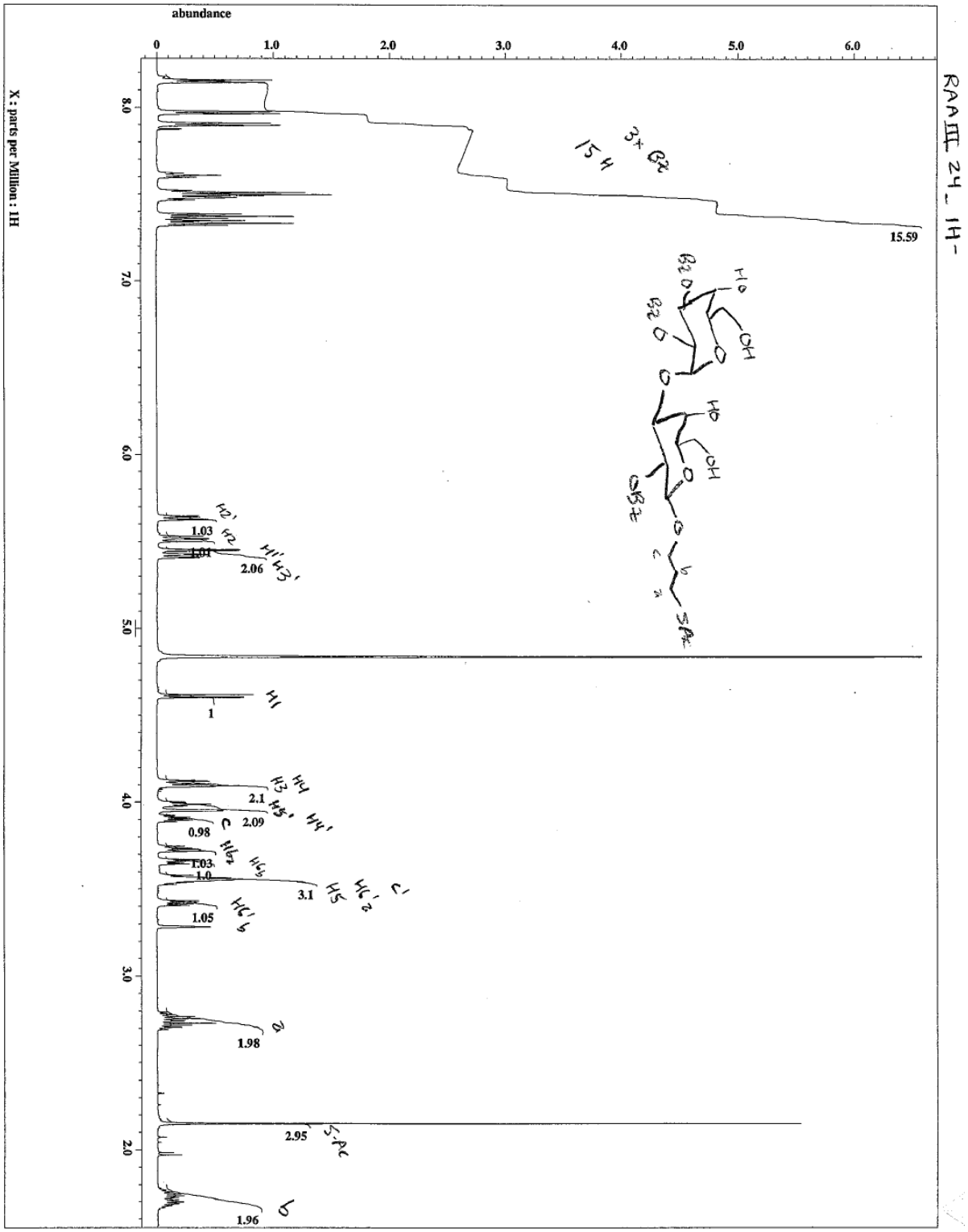


ESI-TOF HR MS $[C_{36}H_{42}NO_{14}]^+$ (ammonium adduct) calc. *m/z* = 712.2605, found 712.1113
ESI-TOF HR MS $[C_{36}H_{38}NaO_{14}]^+$ calc. *m/z* = 717.2159, found 717.0853

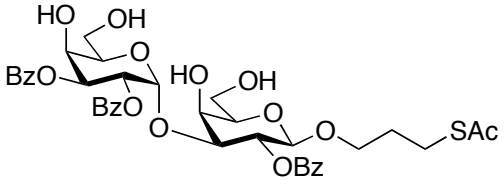
Synthesis of 3-(acetylthio)propyl 2,3-di-*O*-benzoyl-α-D-galactosyl-(1→6)-2-*O*-benzoyl-β-D-galactoside (26)

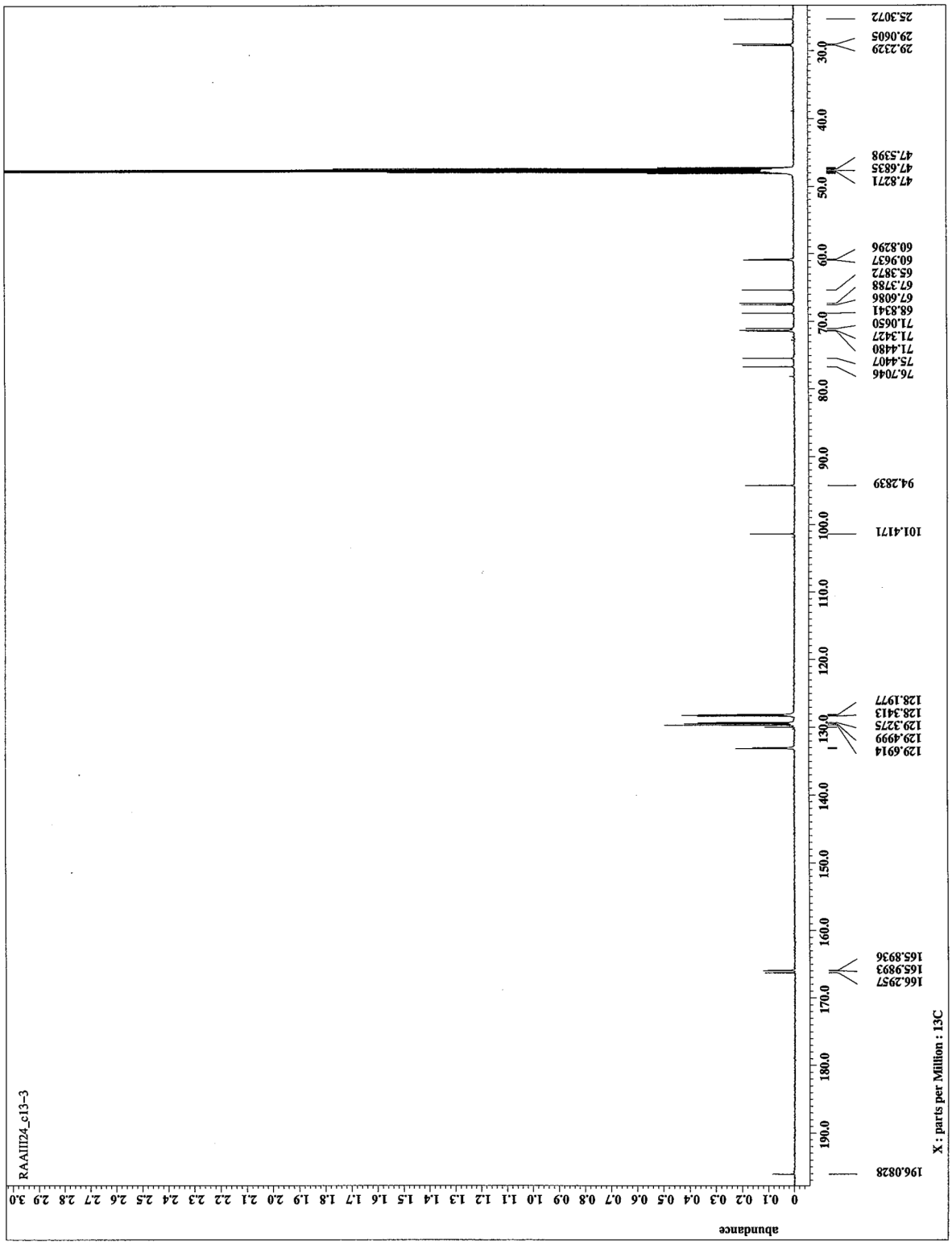


To a solution of **25** (157 mg, 0.226 mmol) and AIBN (19 mg, 0.113 mmol) in dry THF (3 mL), AcSH (129 µL, 1.808 mmol) was added and the mixture was irradiated (350 nm) and stirred under argon for 10 h. The reaction mixture was co-evaporated with toluene and concentrated. The crude product was purified by flash chromatography (20:1 → 15:1 $CHCl_3$/MeOH) to afford **26** (157 mg, 90%) as a clear oil. R_f 0.30 (15:1 $CHCl_3$/MeOH); $[\alpha]_D$ +190.19 (*c*, 1.0, MeOH); ^{1}H NMR (600 MHz, MeOD) 8.16 (d, 2H, Ar); 7.96 (d, 2H, Ar); 7.90 (d, 2H, Ar); 7.61 (t, 1H, Ar); 7.52-7.46 (m, 4H, Ar); 7.39-7.31 (m, 4H, Ar); 5.64 (dd, 1H, $J_{H\text{-}1'/H\text{-}2'}$ = 3.44 Hz, $J_{H\text{-}2'/J\text{-}3'}$ = 10.31 Hz, H-2'); 5.52 (dd, 1H, $J_{H\text{-}1/H\text{-}2}$ = 8.25 Hz, $J_{H\text{-}2/H\text{-}3}$ = 9.62 Hz, H-2); 5.45 (d, 1H, $J_{H\text{-}1'/H\text{-}2'}$ = 3.44 Hz, H-1'); 5.42 (dd, 1H, $J_{H\text{-}2'/H\text{-}3'}$ = 10.31 Hz, $J_{H\text{-}3'/H\text{-}4'}$ = 3.44 Hz, H-3'); 4.61 (d, 1H, $J_{H\text{-}1/H\text{-}2}$ = 8.25 Hz, H-1); 4.13-4.09 (m, 2H, H-3, H-4); 3.99 (dd, 1H, $J_{H\text{-}5'/H\text{-}6'a}$ = 5.50 Hz, $J_{H\text{-}5'/H\text{-}6'b}$ = 6.19 Hz, H-5'); 3.95 (d, 1H, $J_{H\text{-}3'/H\text{-}4'}$ = 2.06 Hz, H-4'); 3.91 (m, 1H, OC*H*$_2$CH$_2$CH$_2$SAc); 3.73 (m, 1H, H-6$_a$); 3.66 (m, 1H, H-6$_b$); 3.58-3.53 (m, 3H, H-5, H-6'$_a$, OC*H*$_2$CH$_2$CH$_2$SAc); 3.42 (m, 1H, H-6'$_b$); 2.80-2.70 (m, 2H, OCH$_2$CH$_2$C*H*$_2$SAc, OCH$_2$CH$_2$C*H*$_2$SAc); 2.15 (s, 3H, SAc); 1.78-1.66 (m, 2H, OCH$_2$C*H*$_2$CH$_2$SAc, OCH$_2$C*H*$_2$CH$_2$SAc); ^{13}C NMR (151 MHz; CD_3OD): δ 171.2, 166.8, 165.8, 165.1, 137.3, 133.9, 133.4, 133.3, 133.0, 130.0, 129.7 (2), 129.6, 128.9, 128.7, 128.5, 128.4, 128.3, 127.9, 126.1, 117.4, 100.4, 100.0, 95.4, 72.7, 70.8 (2), 70.1, 69.3, 69.2, 68.8, 67.2, 66.6, 66.6, 60.5, 27.5, 27.2, 23.2, 21.1, 20.7, 14.3.

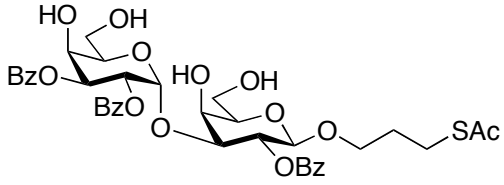


^{1}H NMR spectrum of compound **26**

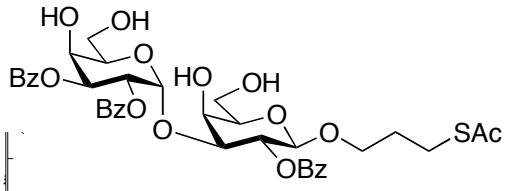


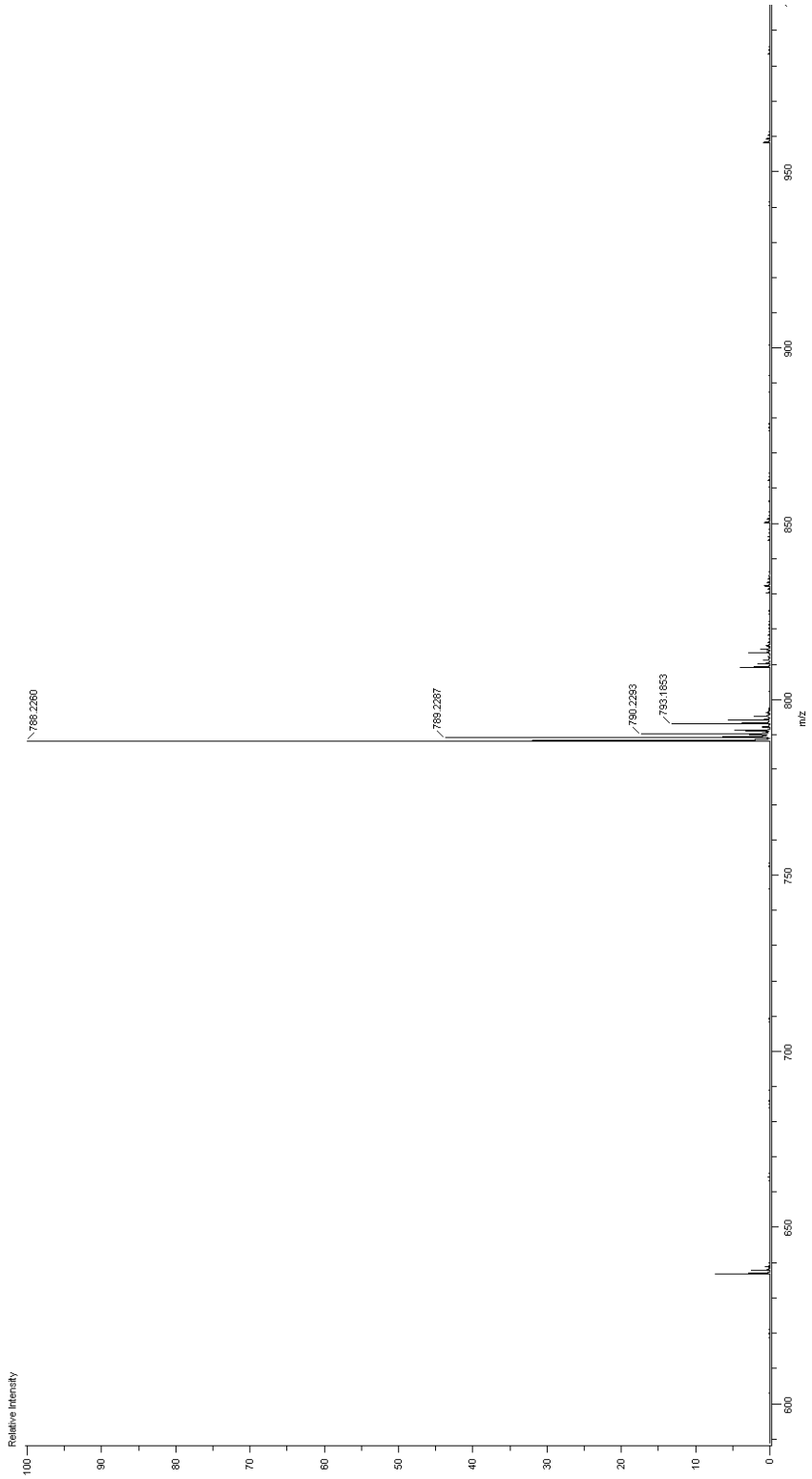


^{13}C NMR spectrum of compound **26**



ESI-TOF HR mass spectrum of compound **26**





ESI-TOF HR MS $[C_{38}H_{46}NO_{15}S]^+$ (ammonium adduct) calc. *m/z* = 788.2588, found 788.2260

ESI-TOF HR MS $[C_{38}H_{42}NaO_{15}S]^+$ calc. *m/z* = 793.2142, found 793.1853

Protocol for the conjugation of thiols to BSA

For the conjugation of mercaptopropyl glycosides to maleimide-activated BSA a conjugation kit "Imject Maleimide Activated Carrier Protein Spin Kit" from Thermo Scientific, product #77667, was used, and the protocol provided by the manufacturer was followed.

Tris(2-carboxyethyl)phosphine (TCEP, 0.8 mg, 2.79 μmol) was dissolved in 250 μL of Imject Maleimide Conjugation Buffer (83 mM sodium phosphate buffer, 0.1 M EDTA, 0.9 M sodium chloride, 0.02% sodium azide, pH 7.2) and added to microcentrifuge tubes containing sugar-disulfide (2.40 μmol), and stirred. After 1 hour, 10 μL was removed to determine the initial thiol concentration. Vials of maleimide-activated BSA (2 mg, 15-25 moles of maleimide/mole of BSA) were reconstituted by adding 200 μL of ultrapure water. The remaining 240 μL of sugar-conjugation buffer solution was added to each vial. Vials were flushed with argon, sealed with parafilm, and mixed for 3 hours on a shaker. Reaction Buffer was prepared (0.1 M sodium phosphate, pH 8.0, containing 1 mM EDTA) and a solution of Ellman's Reagent [5,5'-dithiobis-(2-nitrobenzoic acid) = DTNB] (4 mg DTNB in 1mL of Reaction Buffer). After 3 hours, 18.3 μL was removed from each conjugation solution to determine the thiol concentration after the conjugation. Each sample to be tested was diluted to 250 μL with Reaction Buffer and added to a test tube containing 50 μL of Ellman's Reagent Solution and 2.5 mL of Reaction Buffer, and mixed at room temperature for 15 minutes. With a spectrophotometer set to 412 nm, the absorbance of each sample was measured. Using the molar extinction coefficient of 2-nitro-5-thiobenzoic acid (TNB, ε = 14,150 $M^{-1}cm^{-1}$), the concentration of sulfhydryls in each sample and the amount of sugar loaded (average: 2.0μmol) was determined.

	Before conjugation		After conjugation		
	Absorbance	Concentration of thiol (M)	Absorbance	Concentration of thiol (M)	Amt. conjugated thiol (moles)
1-BSA	0.8693	4.30E-06	0.5189	2.47E-06	1.83E-06
2-BSA	0.8758	4.33E-06	0.5201	2.47E-06	1.86E-06
3-BSA	0.885	4.38E-06	0.5481	2.61E-06	1.77E-06
4-BSA	0.743	3.68E-06	0.3604	1.71E-06	1.96E-06
5-BSA	0.9797	4.85E-06	0.4673	2.22E-06	2.62E-06
6-BSA	0.954	4.72E-06	0.3802	1.81E-06	2.91E-06
7-BSA	0.8624	4.27E-06	0.4701	2.24E-06	2.03E-06
8-BSA	0.9886	4.89E-06	0.5986	2.85E-06	2.04E-06
9-BSA	0.8123	4.02E-06	0.4122	1.96E-06	2.06E-06
Cys-BSA	1.6741	8.28E-06	1.2943	6.16E-06	2.12E-06

Conjugates were then diluted to 1mL and added to Amicon Ultra 3K Centrifugal Filter Devices for desalting. Filters were centrifuged for 20 minutes at 4000xg, then 1mL of ultrapure water was added to the filter, and centrifuging was continied for 20 minutes at

4000xg. The filtrate tube was then removed, filters were inverted and centrifuged at 1000xg for 2 minutes to collect in the concentrate tube. The collected material was lyophilized, and stock solutions of the protein were prepared. The protein concentrations were determined using a Pierce BCA (bicinchoninic acid) Protein Assay Reagent kit using a spectrophotometer at a detection wave length of 562nm.

CL-ELISA protocol (pertaining to Figure 2)

NGPs (125 ng each), diluted in 0.1 M carbonate-bicarbonate buffer, pH 9.6, were immobilized overnight at 4°C on a 96-well Maxisorp microplate (NUNC, Thermo Fisher Scientific). The microplate was then washed three times with 200 µL phosphate-saline buffer (PBS, pH 7.4) containing 0.05% Tween 20 (PBS-T). Free binding sites were blocked by incubation with 5% serum bovine albumin (BSA) in PBS. 50 µL of ChSP or NHSP (diluted at 1/100 or 1/300 in 5% BSA-PBS) were added to each well. Finally, the microplate was incubated with biotinylated anti-human IgG (GE Healthcare) (1/5000 dilution, 50 µL/well) followed by streptavidin-horseradish peroxidase (HRP) (Thermo Fisher Scientific) (1/5000 dilution, 50 µL/well). All incubations were carried out at 37°C for 1h. Between incubation steps the microplate was washed 3x with 200 µL PBS-T, except for the last step with streptavidin-HRP, in which the plate was washed 4x with 200 µL PBS-T. After addition of 50 µL SuperSignal ELISA Pico Chemiluminescent Substrate (Thermo Fisher Scientific), diluted 1/8 in 0.1 M carbonate-bicarbonate buffer, pH 9.6, antibody-antigen complexes were immediately detected using a Luminoskan luminometer (Labsystems, Thermo). The assay readouts were expressed as relative luminescence units (RLU).