Supplementary Materials For

Synthesis and Biological Assessment of a Triazine Dendrimer with 16 Paclitaxel Groups

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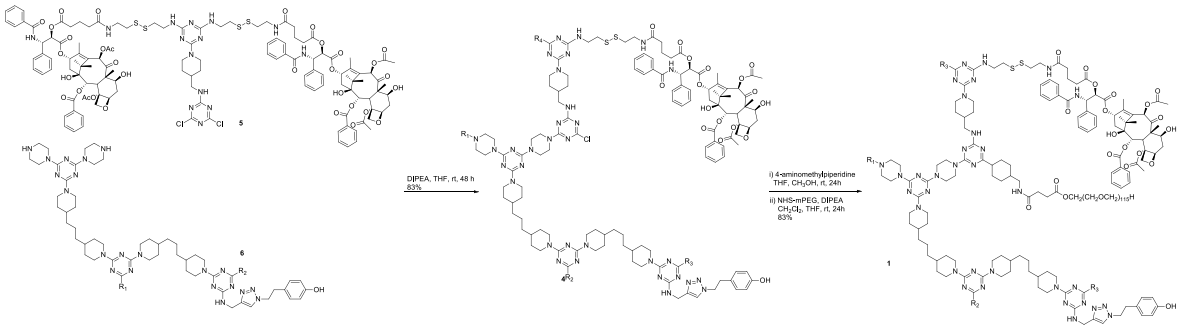
Table of Contents

Page

General Synthetic Procedures

All chemicals were purchased from Aldrich and Acros and used without further purification. All solvents were ACS grade and used without further purification. HPLC was carried out using an Agilent Technologies 1260 Infinity system and an Agilent Technologies 1260 Infinity DAD detector. NMR spectra were recorded on a Mercury 300 MHz spectrometer in $CDCl_3$. All ESI mass spectral analyses were carried out by an Agilent Technologies 6224 TOF LC/MS system. All MALDI mass spectral analyses were carried out by the Laboratory for Biological Mass Spectrometry at Texas A&M University.

Synthesis of prodrug 1



Compound **1**. To a solution of **4** (225 mg, 0.01 mmol) in chloroform (10 mL), THF (1.5 mL), and methanol (0.2 mL), a solution of 4-aminomethylpiperidine (0.12 mL, 1.58 mmol) in THF (4 mL) was added via dropping funnel over 20 min and the solution was stirred for 24 h at room temperature. The solution was washed two times with brine, dried over $MgSO_4$ and then the volume was reduced to 10 mL in vacuo. After addition of a solution of NHS-mPEG (CS type, MW 5 KDa, 555 mg, 0.11 mmol) and DIPEA (77 mg, 0.59 mmol) in dichloromethane (5 mL), the solution was stirred at room temperature for 24 h. After concentrated in vacuo, the residue was dissolved in deionized water and filtered. The resulting solution was diafilterated to remove low molecular weight impurities using Amicon stirring ultrafiltration cell equipment and a YM 10 membrane (MWXO: 10 KDa) in deionized water. The purified solution was concentrated to afford dendrimer **1** (521 mg, 83%). ^{1}H NMR ($CDCl_3$, 300 MHz): ^{1}H NMR (300 MHz, $CDCl_3$+MeOH-d_4): ^{1}H NMR (300 MHz, $CDCl_3$+MeOH-d_4): δ 8.04 (d, J = 6.3, 32H, i), 7.70 (d, J = 7.2, 32H, c), 7.64-7.22 (m, 176H, a, b, d, e, f, g, h), 6.80 (d, J = 7.5, 2H, v''), 6.71 (br s, 1H, q''), 6.61 (d, J = 6.0, 2H, u''), 6.30 (s, 16H, y), 6.06 (br, 16H, n), 5.84 (br, 16H, l), 5.60 (br, 16H, q), 5.35 (br, 16H, m), 4.91 (d, J = 8.7, 16H, u), 4.62 (br, 18H, h_e'', o''), 4.48-4.14 (m, 72H, w, t, b_e''), 3.82-3.64 (m, 82H, a'', r'', r, g'), 3.80-3.30 (mPEG), 3.34-3.24 (m, 48H, j', k''), 3.00 (br s, 18H, h_a'',s''), 2.76-2.58 (m, 96H, a''', h', i'), 2.50-2.34 (m, 72H, b_a'', d', f', v), 2.33 (s, 48H, j), 2.31-2.08 (m, 64H, d', f', o), 2.11 (s, 48H, k), 2.08-1.78 (m, 88H, c_e'', e', i_e'', v), 1.87 (s, 48H, z), 1.75-0.73 (m, 96H, d'', c_a'', g'', j'', i_a'', e'', f''), 1.64 (s, 48H, c'), 1.09 (s, 48H, b'), 1.07 (s, 48H, a'); MS (MALDI-TOF) calcd for complete PEGylation 63300, found 61100.

Figure S1. Mass spectrum of 1. Pegylation of **4** with 5kDa PEG broadens the spectrum of **1** in comparison to the starting material. The peak centered at 61kDa appears close to the theoretical expectation.

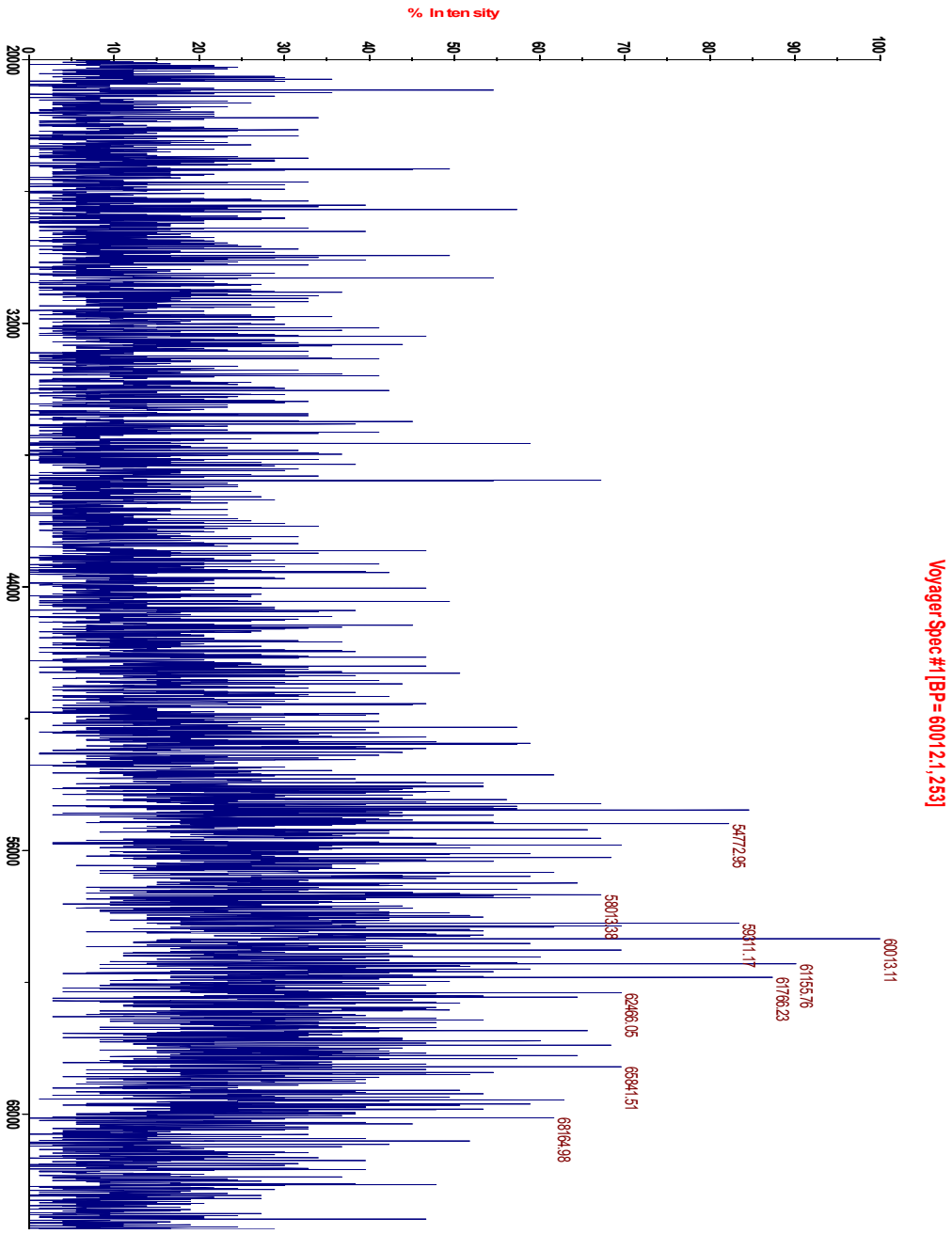


Figure S2. HPLC traces of **1**. For analytic HPLC of the dendrimers, a ZORBAX 300SB-C8 column (1.0 x 150 mm, 3.5m) was used with a gradient elution: 65% A to 20% A over 20 min and then keep 30% A (A = water with 0.1% TFA, B = acetonitrile with 0.1% TFA) with a flow rate of 50 μL/min. UV detection was performed at 227 nm. The two peaks are hypothesized to correspond to the target and one that is missing one PEG (perhaps due to incomplete reaction of monomer **4** and thus also missing 2 paclitaxels).

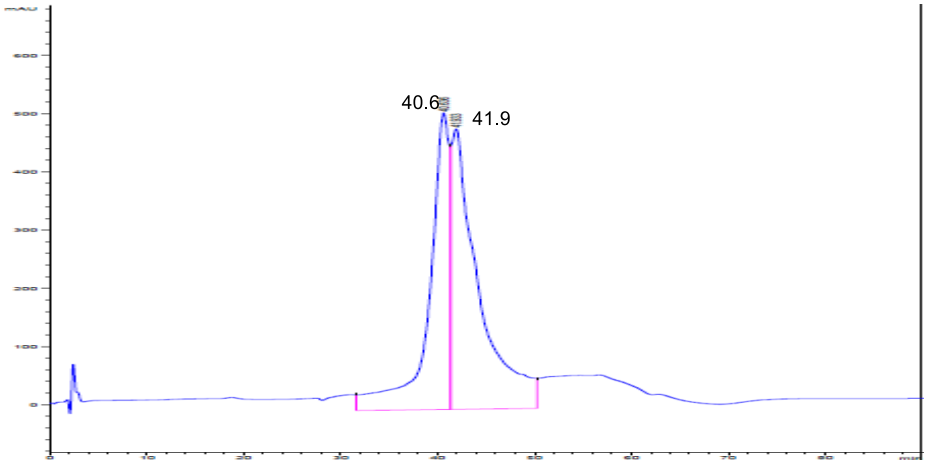


Figure S3. GPC traces of PEG 5K. The analytical GPC chromatogram obtained using 0.1 M $NaNO_3$ (aq) as an eluent with a RI detector. This trace is useful for assessing the amount of free PEG in **1**.

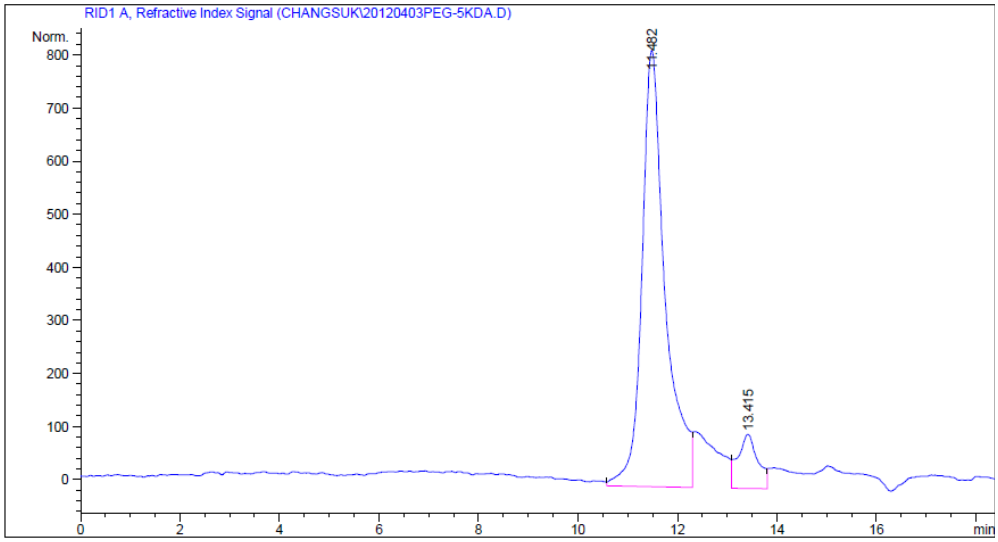


Figure S4. GPC traces of **1**. The analytical GPC chromatogram obtained using 0.1 M $NaNO_3$ (aq) as an eluent with a RI detector. Dialysis removes a majority of the free PEG.

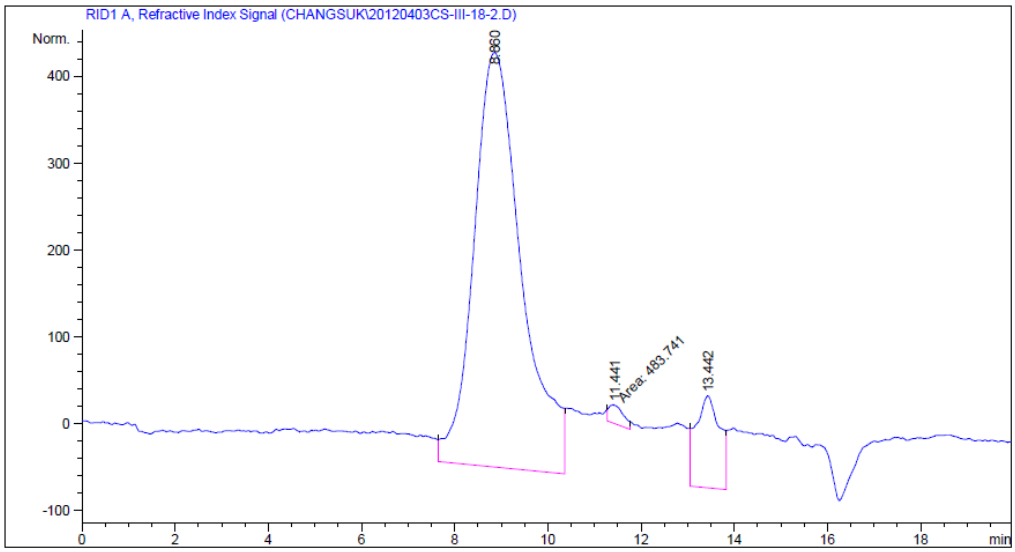


Figure S5. ^{1}**H NMR of 1.**

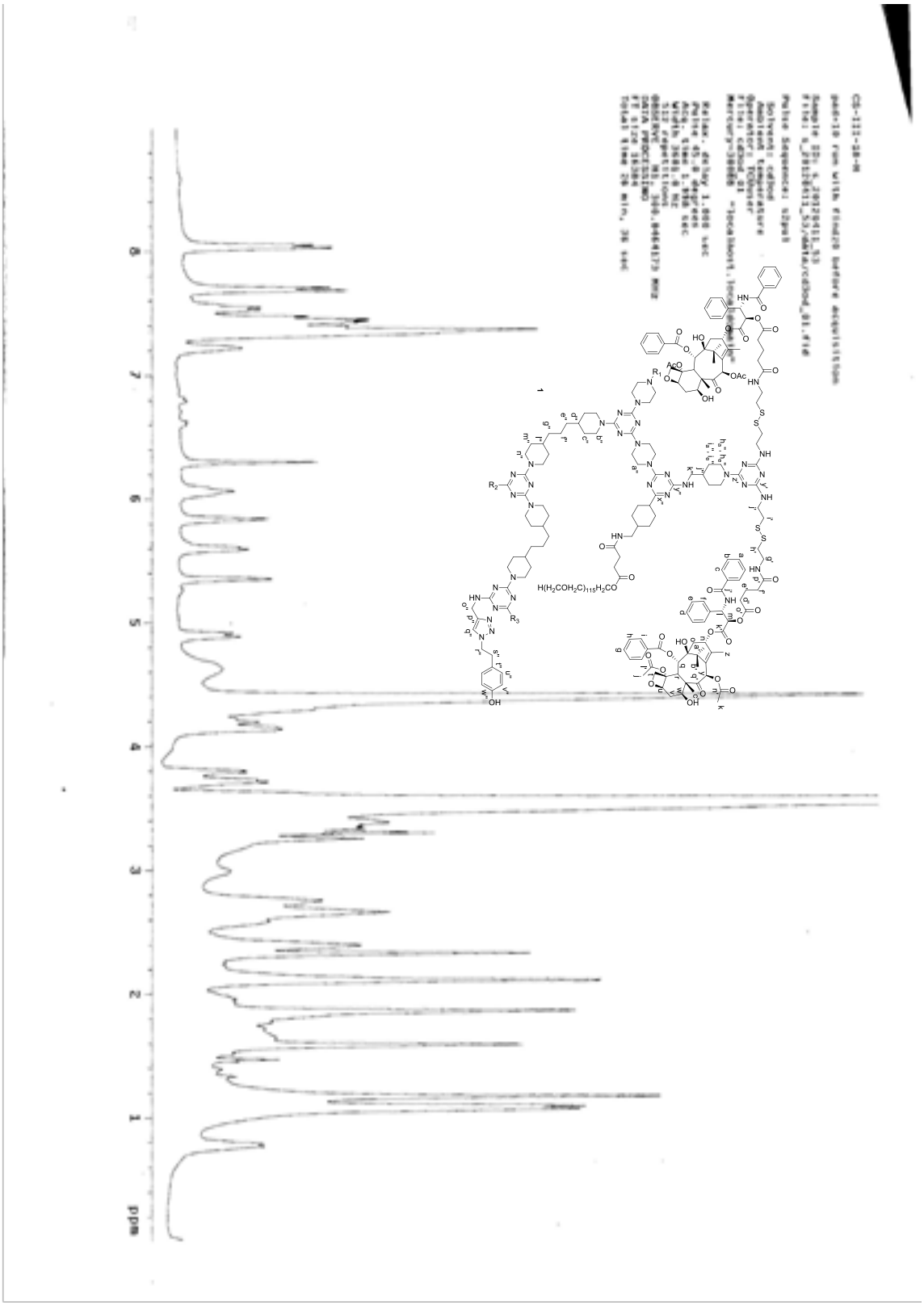
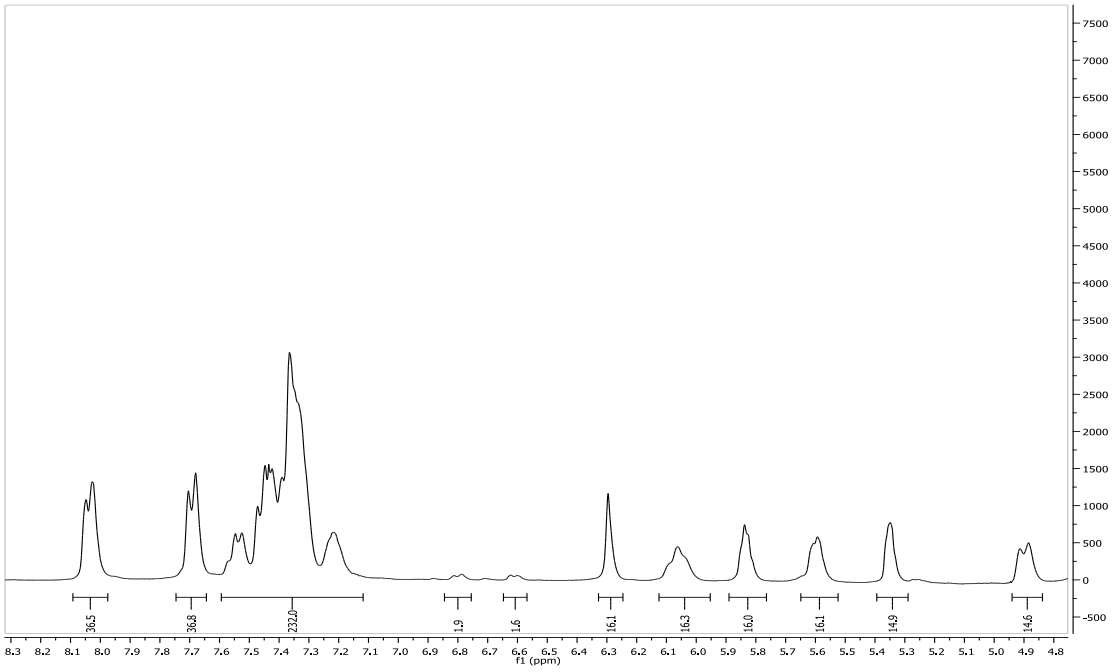
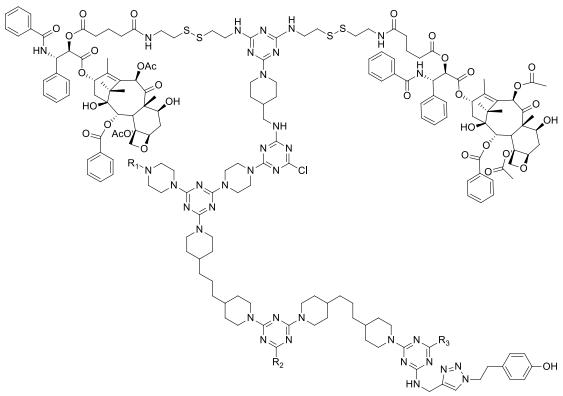


Figure S6. Expanded Region of 1. Comparison of the integration of the phenolic group (6.6 and 6.8 ppm) and that of 16 theoretical paclitaxel groups.



Synthesis of 4



Compound **4**. To a solution of **5** (599 mg, 0.236 mmol) in THF (4 mL), a solution of G2 dendron (**6**) (680 mg, 0.284 mmol) and DIPEA (0.15 mL, 0.89 mmol) in THF (3 mL) and water (1 mL) was added via dropping funnel over 20 min at 0 ^{o}C. The solution was stirred for 48h at room temperature and evaporated under vacuum. The residue was dissolved in dichloromethane (20 mL) and washed two times with brine, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (EtOAc:DCM:MeOH = 10:10:0.3 → DCM:MeOH = 10:1; TLC R_f = 0.23 with DCM:MeOH = 10:1) to give **4** as a solid (445 mg, 83 %). ^{1}H NMR (300 MHz, $CDCl_3$+MeOH-d_4): δ 8.14 (d, *J* = 6.9, 32H, i), 7.78 (d, *J* = 7.2, 32H, c), 7.64 (t, *J* = 7.2 Hz, 16H, g), 7.56-7.29 (m, 160H, a, b, d, e, f, h), 6.98(s, 1H, q''), 6.90 (d, *J* = 8.4, 2H, v''), 6.71 (d, *J* = 8.4, 2H, u''), 6.38 (s, 16H, y), 6.17 (t, *J* = 8.7, 16H, n), 5.95 (d, *J* = 3.9, 16H, l), 5.70 (d, *J* = 7.2, 16H, q), 5.46 (d, *J* = 4.2, 16H, m), 4.96 (d, *J* = 9.3, 16H, u), 4.72 (br s, 16H, h_e''), 4.60 (br s, 2H, o''), 4.48-4.23 (m, 72H, w, t, b_e''), 3.82 (br s, 50H, a'', r'', r), 3.64 (br s, 32H, g'), 3.57-3.31 (m, 48H, j', k''), 3.33 (br s, 16H, h_a''), 3.07 (t, *J* = 6.6, 2H, s''), 2.85 (br s, 32H, a'''), 2.77 (br s, 64H, h', i'), 2.56-2.36 (m, 72H, b_a'', d', f', v), 2.43 (s, 48H, j), 2.31-2.11 (m, 64H, d', f', o), 2.22 (s, 48H, k), 2.08-1.78 (m, 88H, c_e'', e', i_e'', v), 1.96 (s, 48H, z), 1.75-0.86 (m, 96H, d'', c_a'', g'', j'', i_a'', e'', f''), 1.69 (s, 48H, c'), 1.20 (s, 48H, b'), 1.17 (s, 48H, a'); ^{13}C NMR (75 MHz, $CDCl_3$+MeOH-d_4) δ = 205.7 (q'), 175.2(p'), 174.3(o'), 172.6(n'), 171.7(z'), 170.8(m'), 170.6(j'), 170.6(k'), 170.4(triazole-dendron), 168.3(l'), 167.1(z''), 166.9(triazole-dendron), 166.6(triazole-dendron), 165.5(y'), 143.4(r'), 138.5(g'), 135.6(g), 135.2(h'), 135.0(s'), 133.6(d), 131.8(i), 131.3(i'), 130.7(b), 130.3(a), 130.3(e), 130.3(h), 129.1(c), 128.6(f), 127.1(u''), 117.3(v''), 86.3(u), 82.8(s), 79.6(p), 78.2(t), 77.4(y), 76.7(q), 75.9(m), 73.7(n), 73.1(w),

59.9(x), 54.9(l), 47.7(r), 45.0(a''), 45.0(b''), 45.0(t'), 45.0 (k''), 41.2(x'), 39.9(u'), 39.5(v'), 39.0(w'), 38.6(e''), 38.1(d''), 37.7(o), 37.0(v), 36.4(f'), 34.5(d'), 34.0(c''), 31.8(i''), 31.3(j''), 31.3(s''), 28.1(b'), 25.5(f''), 24.2(j), 23.6(a'), 22.5(e'), 22.4(k), 16.1(z), 11.4(c'); MS (MALDI-TOF) calcd for $C_{1134}H_{1474}Cl_8N_{165}O_{257}S_{32}$ $(M+H)^+$ 22716.80895, found 22842.

Figure S7. Mass spectrum of 4. The expected ion is observed (22842) with a ionization-induced fragmentation (hypothesis) of a paclitaxel group (21972) and evidence of incomplete reaction of monomer (20408), a target corresponding to 7 additions instead of 8, ***or*** an ionization-induced fragmention (loss of **14**). The mass difference between these choices is 250 a.m.u. While the difference is closer to a fragmentation, it is not possible for definitive assessment.

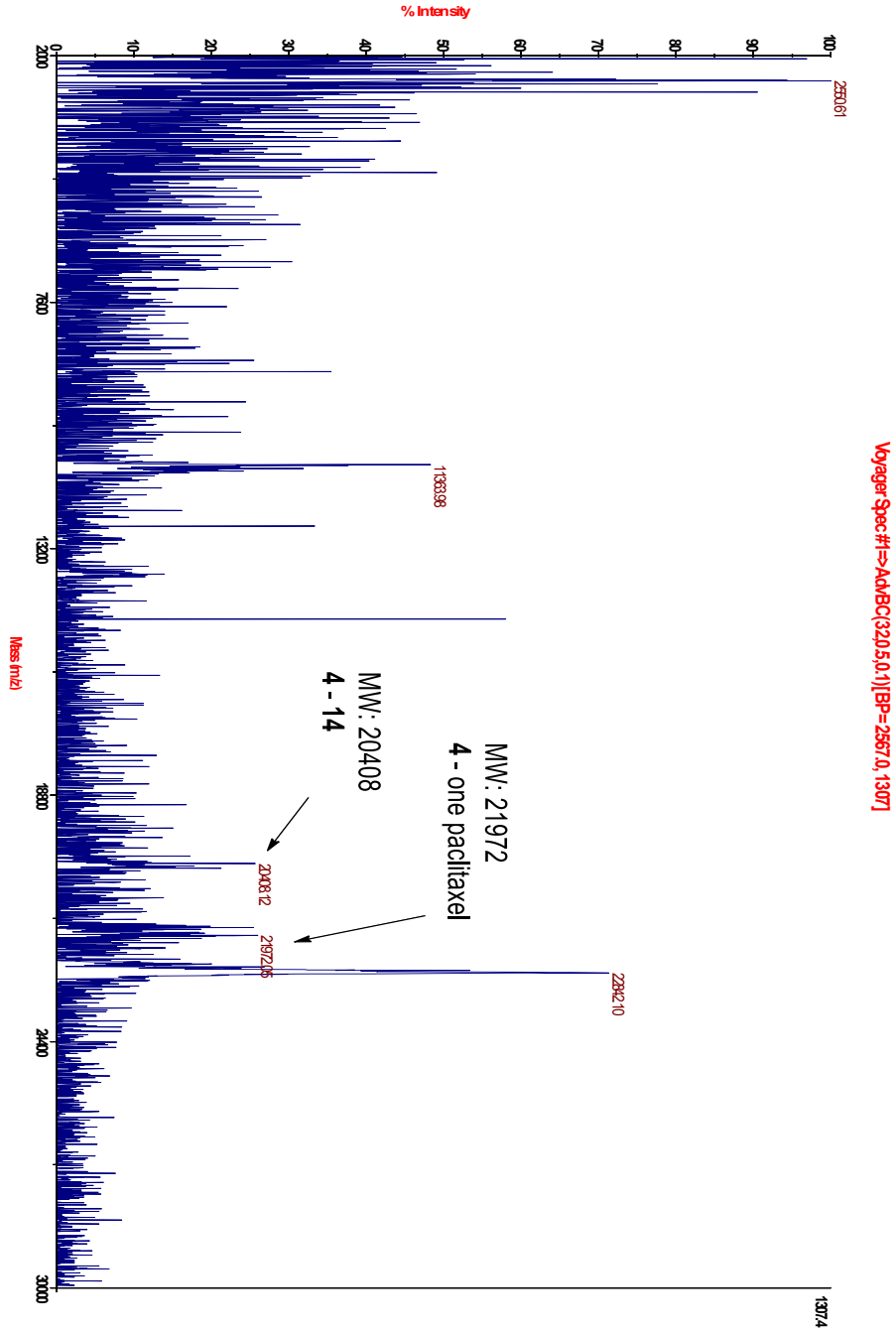


Figure S8. ^{1}**H NMR of 4.**

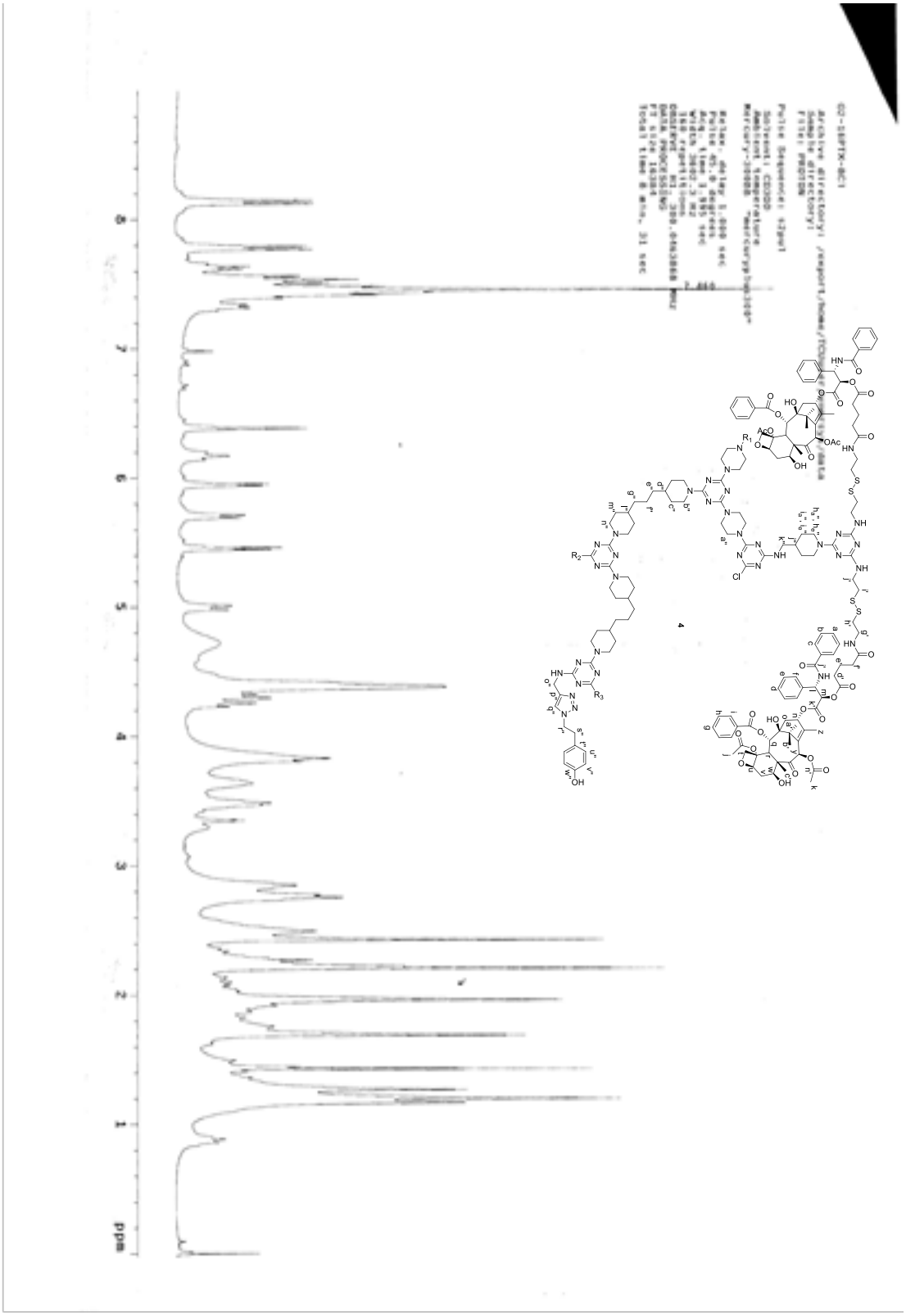


Figure S9 Expanded Region of the ^{1}H NMR of 4. This region shows good agreement between the signals for the phenolic group at the core of the dendrimer (6.6 and 6.8 ppm) and the paclitaxel groups. The expected ratio is 2:16 for u" and v" with l, m, n, and q.

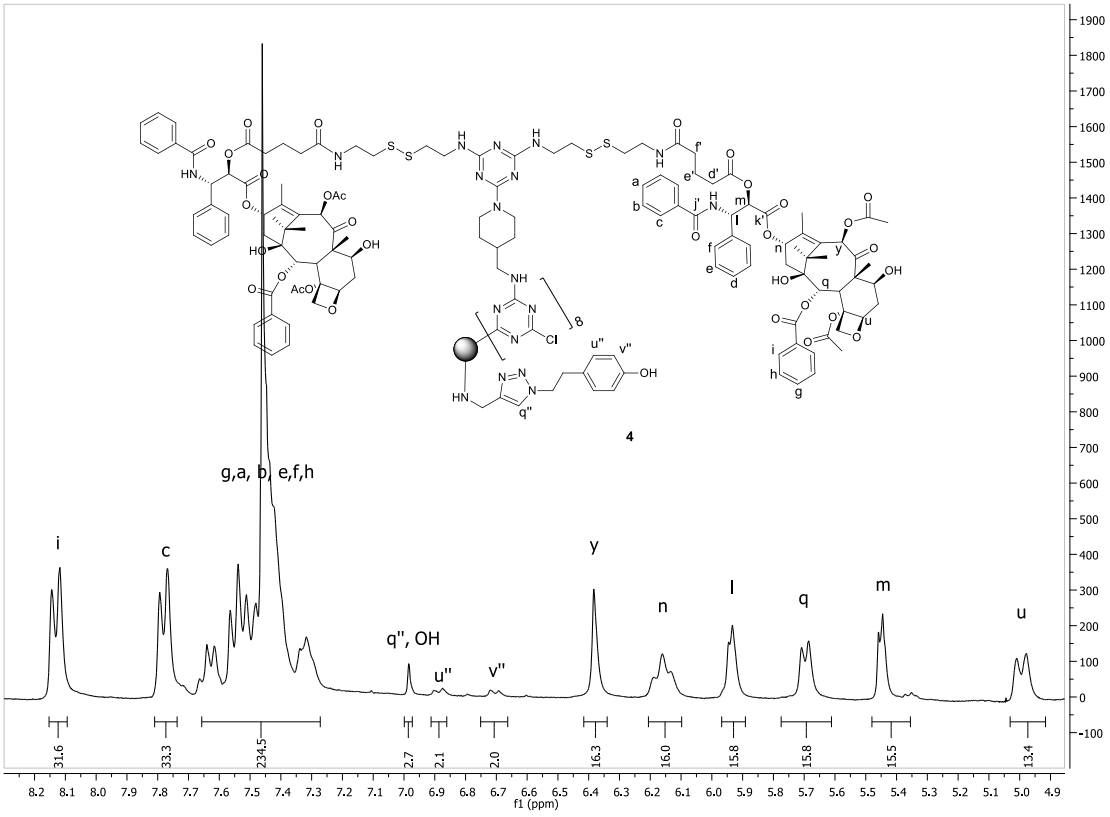
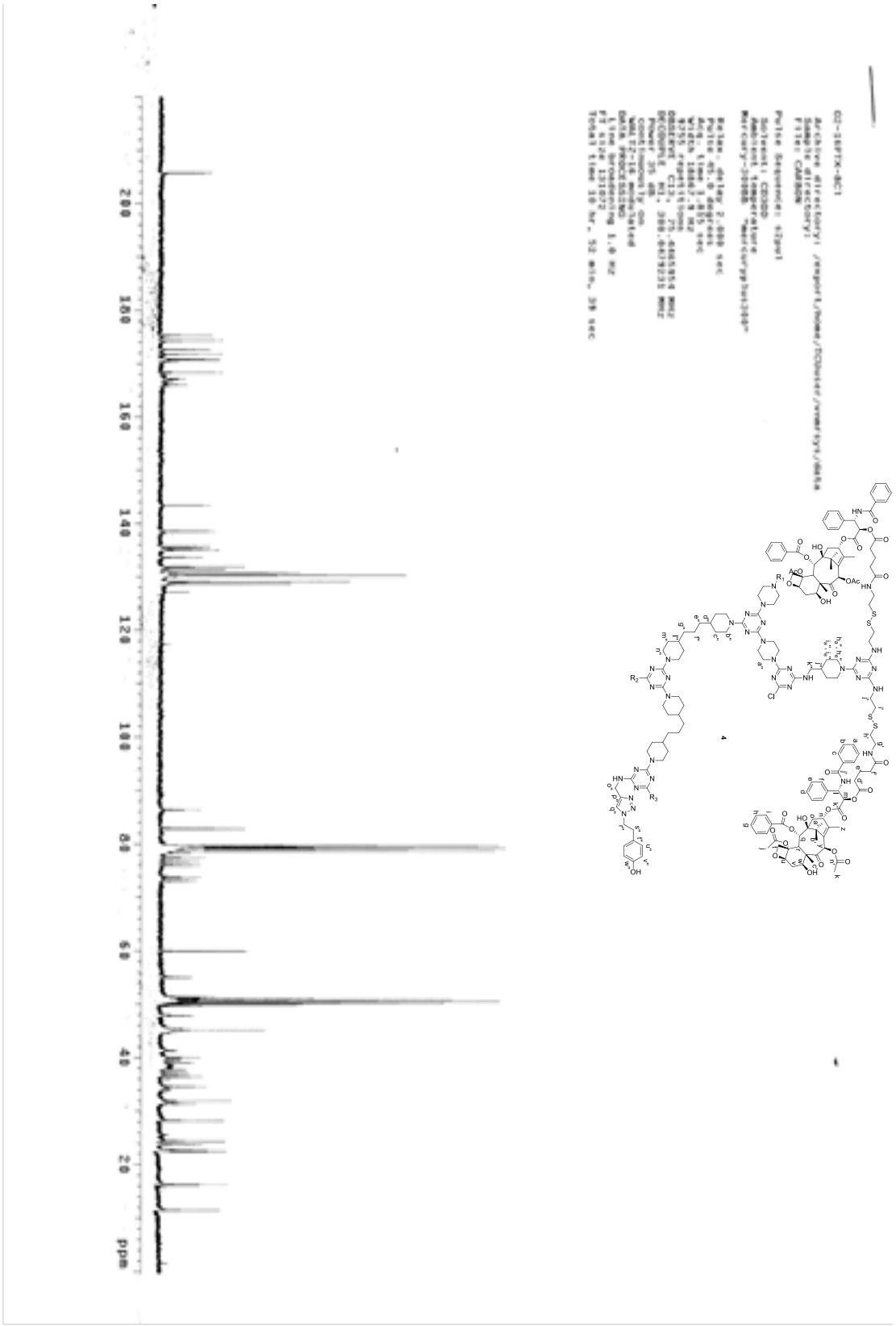
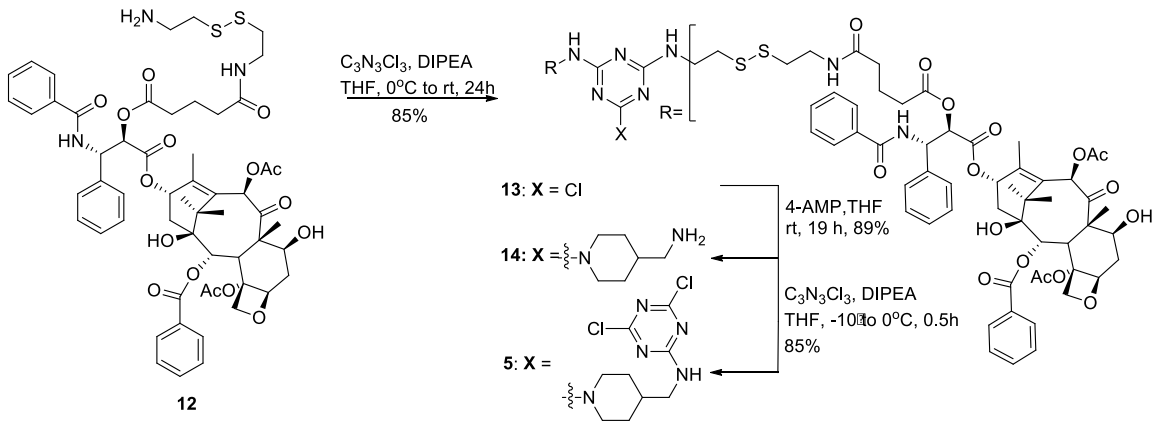


Figure S10. ^{13}C NMR of 4.



Synthesis of Dichlorotriazine 5



Dichlorotriazine **5**. To a solution of cyanuric chloride (79 mg, 0.426 mmol) in THF (5 mL), a solution of paclitaxel-AMP (**14**) (680 mg, 0.284 mmol) and DIPEA (0.15 mL, 0.852 mmol) in THF (15 mL) was added via drooping funnel over 20 min at -10 ^{o}C. The solution was stirred for 30 min at 0 ^{o}C and quenched with water. The solution was diluted with dichloromethane (30 mL) and washed three times with brine, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (DCM:MeOH = 19:1; TLC R_f = 0.29 with DCM:MeOH = 19:1) to give **5** as a foam (613 mg, 85 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.13 (d, *J* = 7.5, 4H, i), 7.77 (d, *J* = 7.5, 4H, c), 7.60 (t, *J* = 6.9 Hz, 2H, g), 7.52-7.27 (m, 20H, a, b, d, e, f, h), 6.31 (s, 2H, y), 6.28 (br s, 2H, n), 6.00 (d, *J* = 4.5, 2H, l), 5.68 (d, *J* = 6.6, 2H, q), 5.51 (br s, 2H, m), 4.96 (d, *J* = 9.3, 2H, u), 4.71 (br s, 2H, h_e''), 4.43 (t, *J* = 8.1, 2H, w), 4.29 (d, *J* = 8.1, 2H, t), 4.19 (d, *J* = 8.4, 2H, t), 3.81 (d, *J* = 6.3, 2H, r), 3.57-3.31 (m, 10H, g', j', k''), 2.96 (br s, 2H, h_a''), 2.89-2.74 (m, 8H, h', i'), 2.56-2.36 (m, 6H, d', f', v), 2.46 (s, 6H, j), 2.31-2.11 (m, 8H, d', f', o), 2.22 (s, 6H, k), 2.08-1.78 (m, 8H, e', i_e'', v), 1.96 (s, 6H, z), 1.75-1.61 (m, 1H, j''), 1.68 (s, 6H, c'), 1.25- 1.13 (m, 2H, i_a''), 1.22 (s, 6H, b'), 1.13 (s, 6H, a'); ^{13}C NMR (75 MHz, $CDCl_3$+MeOH-d_4) δ = 205.2 (q'), 174.8(p'), 173.8(o'), 172.0(n'), 171.3(z'), 170.4(m'), 170.3(j'), 170.3(k'), 167.8(l'), 167.1(z''), 165.5(y'), 142.8(r'), 138.0(g'), 135.1(g), 134.8(h'), 134.5(s'), 133.1(d), 131.3(i), 130.9(i'), 130.2(b), 129.8(a), 129.7(e), 129.7(h), 128.6(c), 128.2(f), 85.9(u), 82.3(s), 79.1(p), 77.7(t), 76.9(y), 76.2(q), 75.5(m), 73.2(n),

72.5(w), 59.4(x), 54.6(l), 47.7 (h''), 47.3(r), 44.5 (k''), 44.2(t'), 40.8(x'), 39.5(u'), 39.0(v'), 38.5(w'), 37.2(o), 36.5(v), 35.9(f'), 34.0(d'), 30.8(i''), 27.6(j''), 26.7(b'), 23.7(j), 23.0(a'), 22.0(e'), 21.9(k), 15.6(z), 10.9(c'); HRMS (ESI) calcd for $C_{124}H_{145}Cl_2N_{14}O_{32}S_4$ 2539.84092, found 2539.7546 $(M+H)^+$.

Figure S11. Mass spectrum of 5. The line at 1270 corresponds to the doubly-charged product. The line at 1721 is not identified and does not correspond to loss of paclitaxel (853 amu) or cleavage at the ester bond.

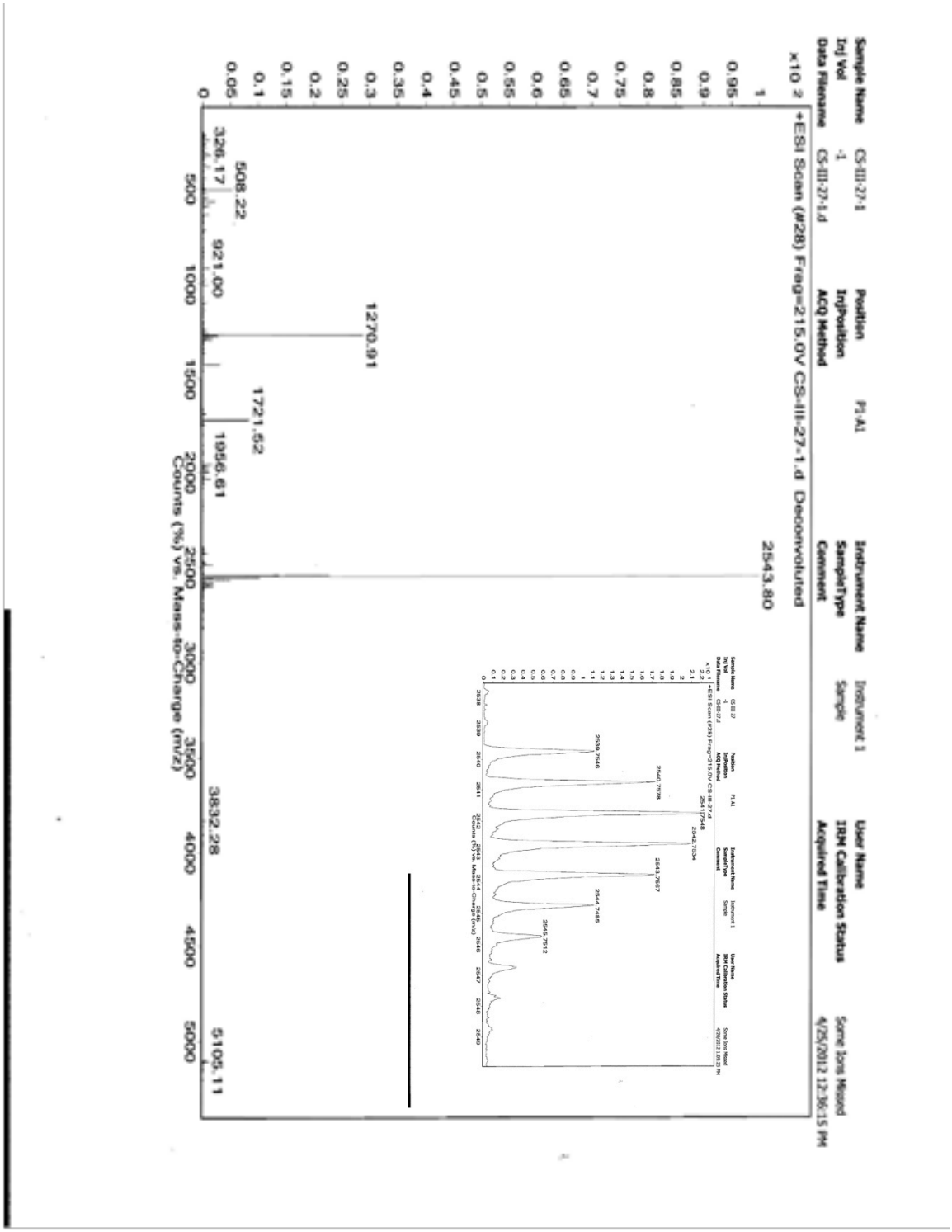


Figure S12. ^{1}**H NMR of 5.**

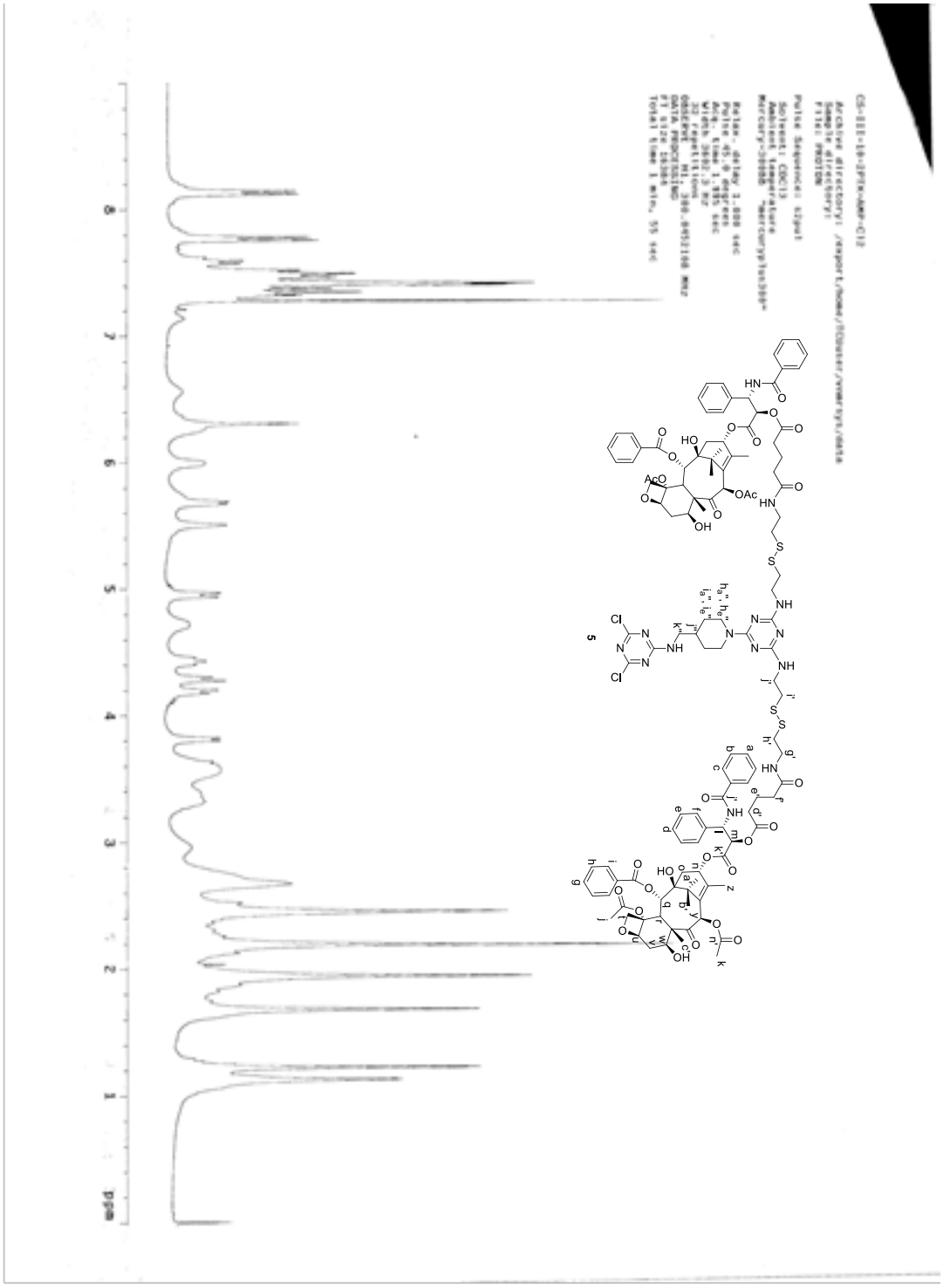
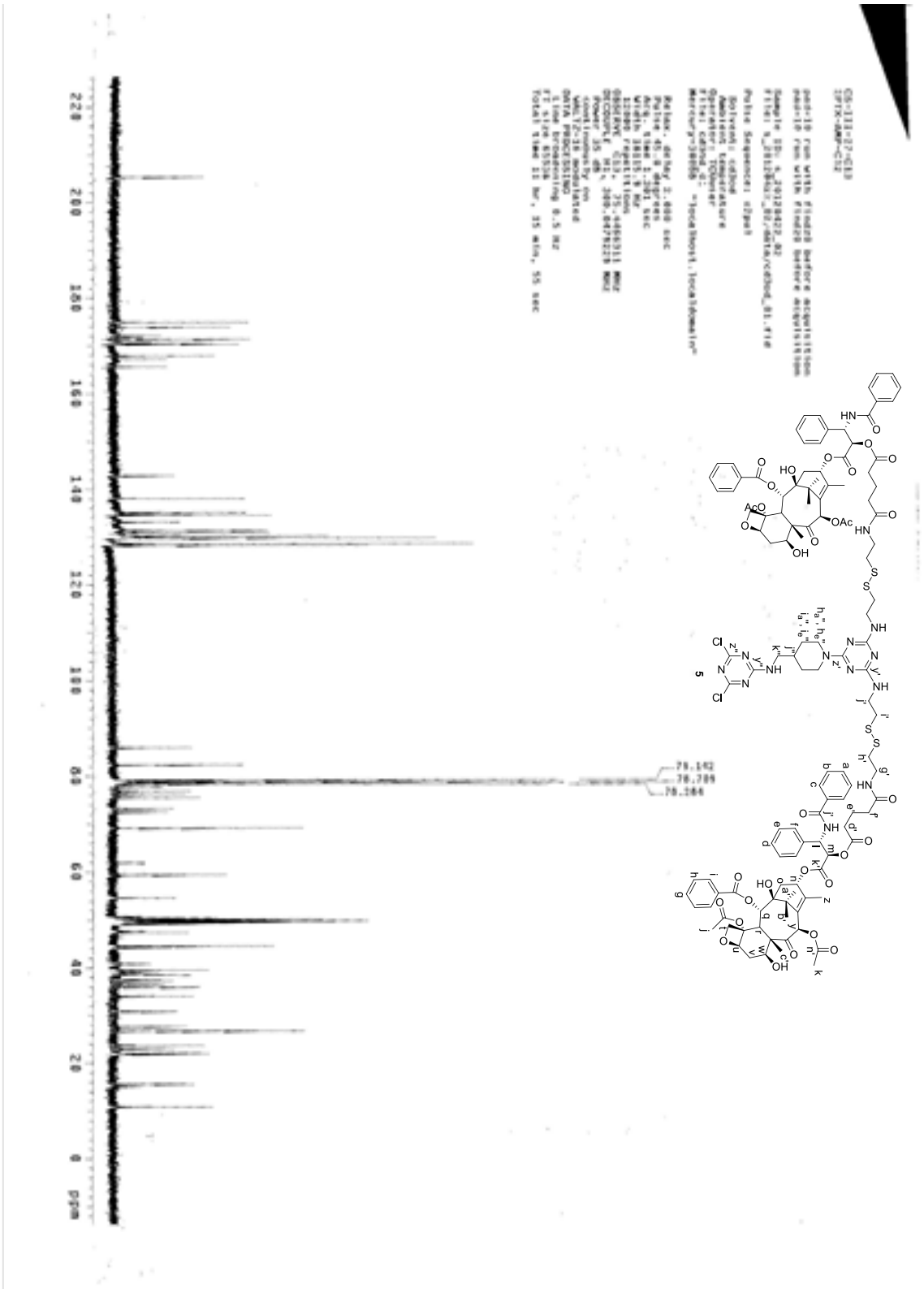
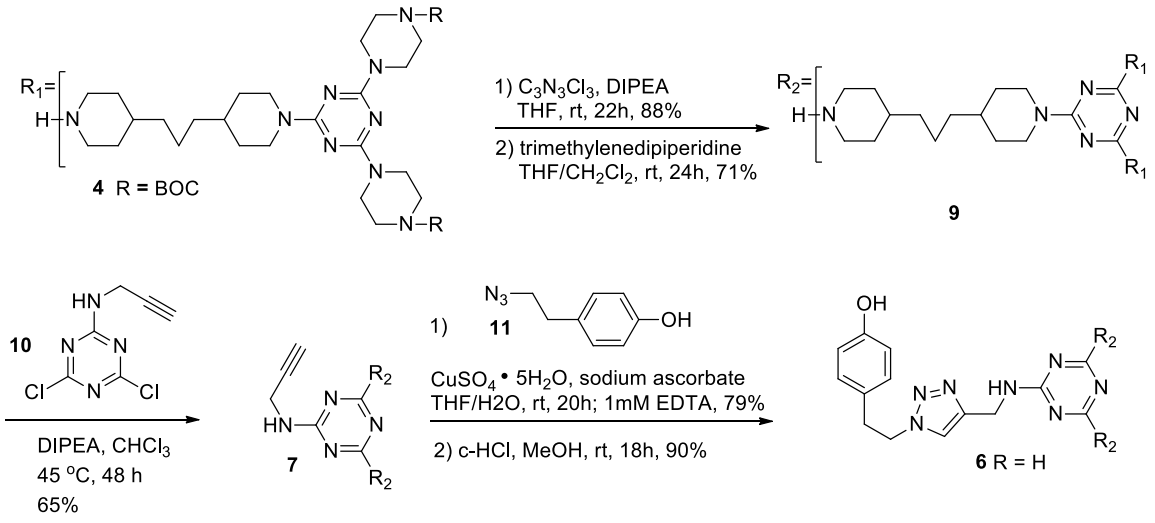


Figure S13. ^{13}C NMR of 5.



Synthesis of 6



Dendrimer **6**. To a mixture of **S1** (171 mg, 0.049 mmol) methanol (4 mL), conc-HCl (12N, 2.5 mL) was slowly added at room temperature and stirred for 18h. The solution was diluted with dichloromethane (20 mL) and basified (pH = 14) with 5 M NaOH (aq) solution, and the resulting milky suspension was extracted with dichloromethane (5 x 20 mL). The organic extractions were combined, and then the solvent was removed *in vacuo* to afford the product **6** as a white solid (119 mg, 90%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.34 (br s, 1H, q''), 6.89 (d, *J* = 8.1, 2H, v''), 6.70 (d, *J* = 8.1, 2H, u''), 4.68 (t, *J* = 12.0, 24H, b_e''), 4.47 (t, *J* = 7.2, 2H, o''),4.19 (br s, NH), 3.74 (br s, 32H, a''), 3.35 (br s, 2H, r''), 3.06 (t, *J* = 7.2, 2H, s''), 2.85 (br s, 32H, a'''), 2.73 (t, *J* = 12.3, 24H, b_a''), 1.71 (d, *J* = 11.4, 24H, c_e''), 1.79-0.94 (m, 72H, c_a'',d'', e'', f'', g'') ^{13}C NMR (75 MHz, $CDCl_3$+MeOH-d_4) δ 167.7, 167.0, 166.9, 166.6, 166.3, 157.6(w''), 148.1(p''), 131.3(u''), 129.3(t''), 124.2(q''), 117.3(v''), 53.7(r''), 47.1(a''), 45.5(b''), 45.2(a'''), 38.5(e''), 38.0(d''), 37.6(s''), 33.9(c''), 23.5(f''); LRMS (ESI) calcd for $C_{142}H_{230}N_{53}O$ 2693.9576, found 2693.96 $(M+H)^+$.

Figure S14. Mass spectrum of 6. The line at 1347 corresponds to doubly-charged **6.**

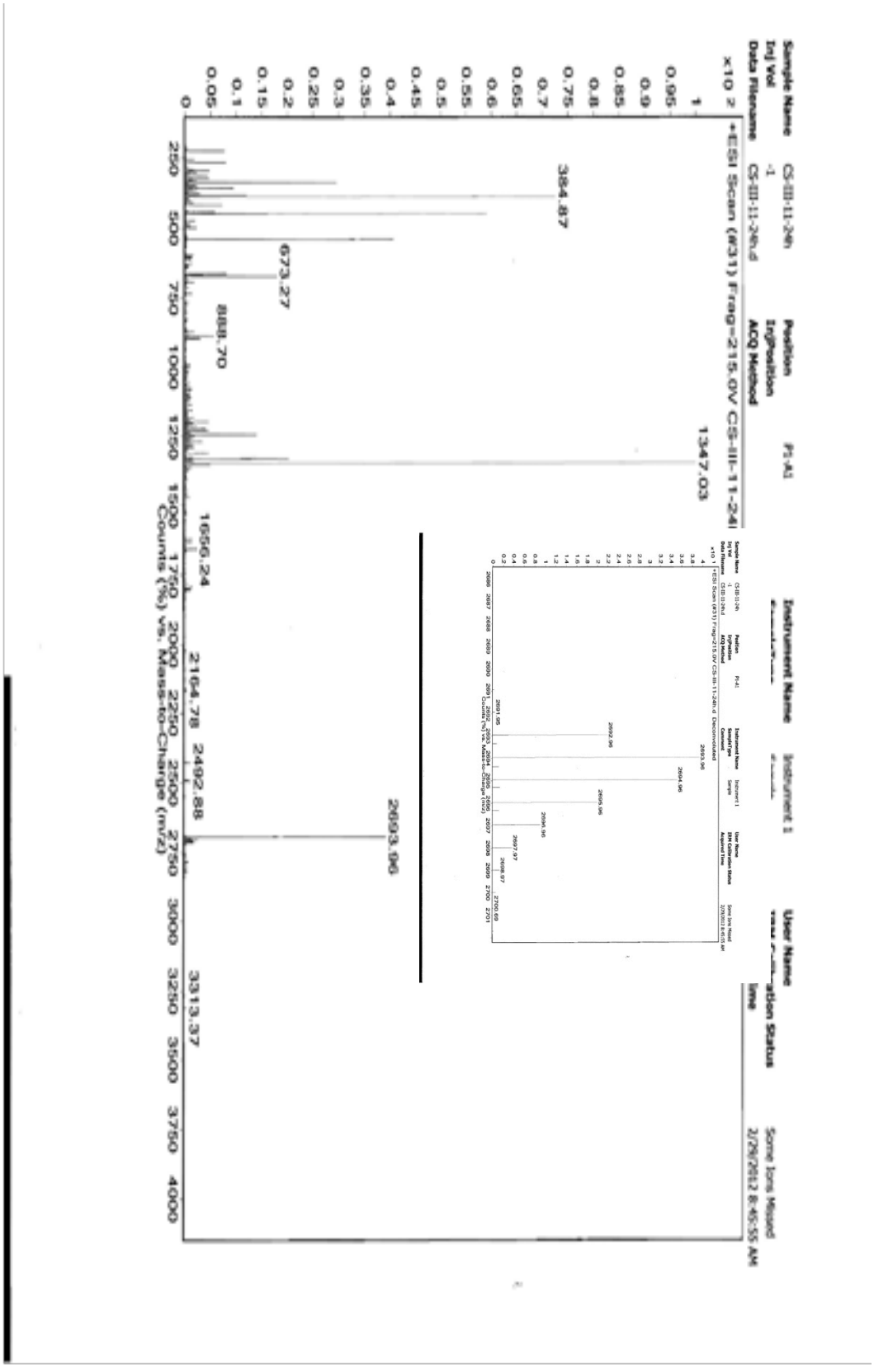


Figure S15. ^{1}H NMR of 6.

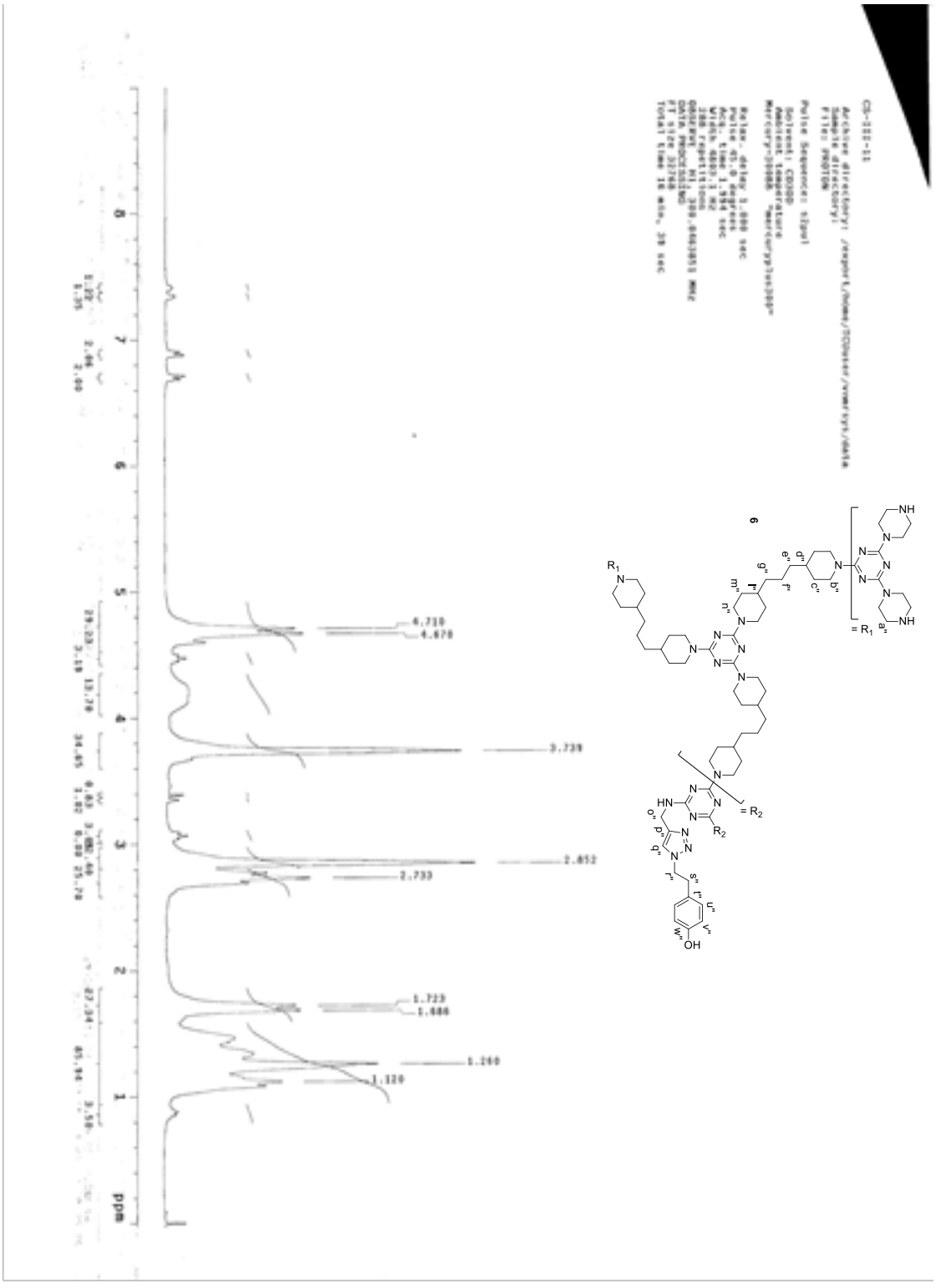
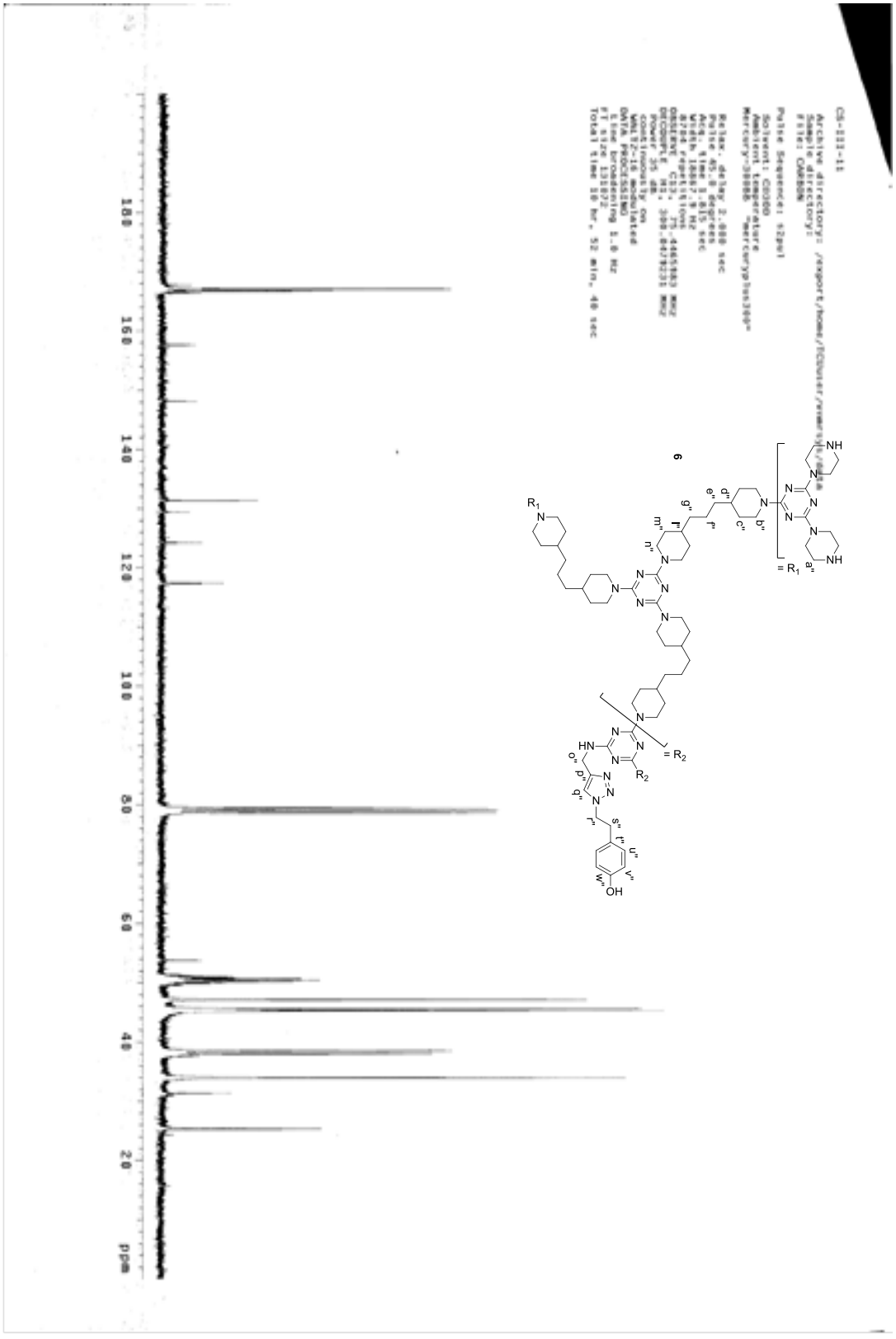
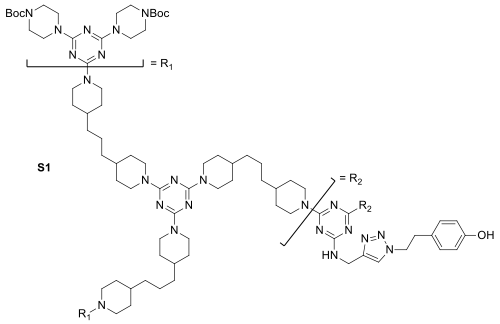


Figure S16. ^{13}C NMR of 6.



Synthesis of S1



Intermediate S1. To a solution of **7** (149 mg, 0.045 mmol) and 4-(2-azidoethyl)phenol **11** (11 mg, 0.067 mmol) in THF (1 mL), a solution of $CuSO_4 \cdot 5H_2O$ (2.2 mg, 0.01 mmol) in water (0.2 mL) followed by sodium-L-ascorbate (18 mg, 0.09) was added at room temperature and stirred for 20h. The solution was diluted with dichloromethane (20 mL) and washed three times with 1mM EDTA (20 mL), brine, dried over $MgSO_4$ and concentrated. The crude product was purified by column chromatography (DCM:MeOH = 97:3 → DCM:MeOH = 92:8; TLC R_f = 0.33 with DCM:MeOH = 92:8) to give **S1** as a solid (124 mg, 79 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.06 (s, 1H, q''), 6.78 (d, *J* = 8.4, 2H, v''), 6.63 (d, *J* = 7.5, 2H, u''), 4.68 (br s, 24H, b_e''), 4.43 (t, *J* = 6.9, 2H, o''), 3.72 (br s, 32H, a''), 3.34-3.18 (m, 2H, r''), 3.02 (t, *J* = 6.9, 2H, s''), 2.85 (br s, 32H, a'''), 2.73 (t, *J* = 11.7, 24H, b_a''), 1.71 (d, *J* = 11.1, 24H, c_e''), 1.79-0.94 (m, 72H, c_a'',d'', e'', f'', g''), 1.46 (s, 72H, Boc) ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 165.3, 164.9, 154.8(Boc), 129.6(u''), 122.7(q''), 116.3(v''), 79.8(Boc), 43.5(a''), 42.9(b''), 36.8(e''), 36.3(d''), 36.0(s''), 32.2(c''), 28.4(Boc), 23.5(f''); LRMS (ESI) calcd for $C_{182}H_{294}N_{53}O_{17}$ 3494.377, found 3494.35 $(M+H)^+$.

Figure S17. Mass Spectrum of S1.

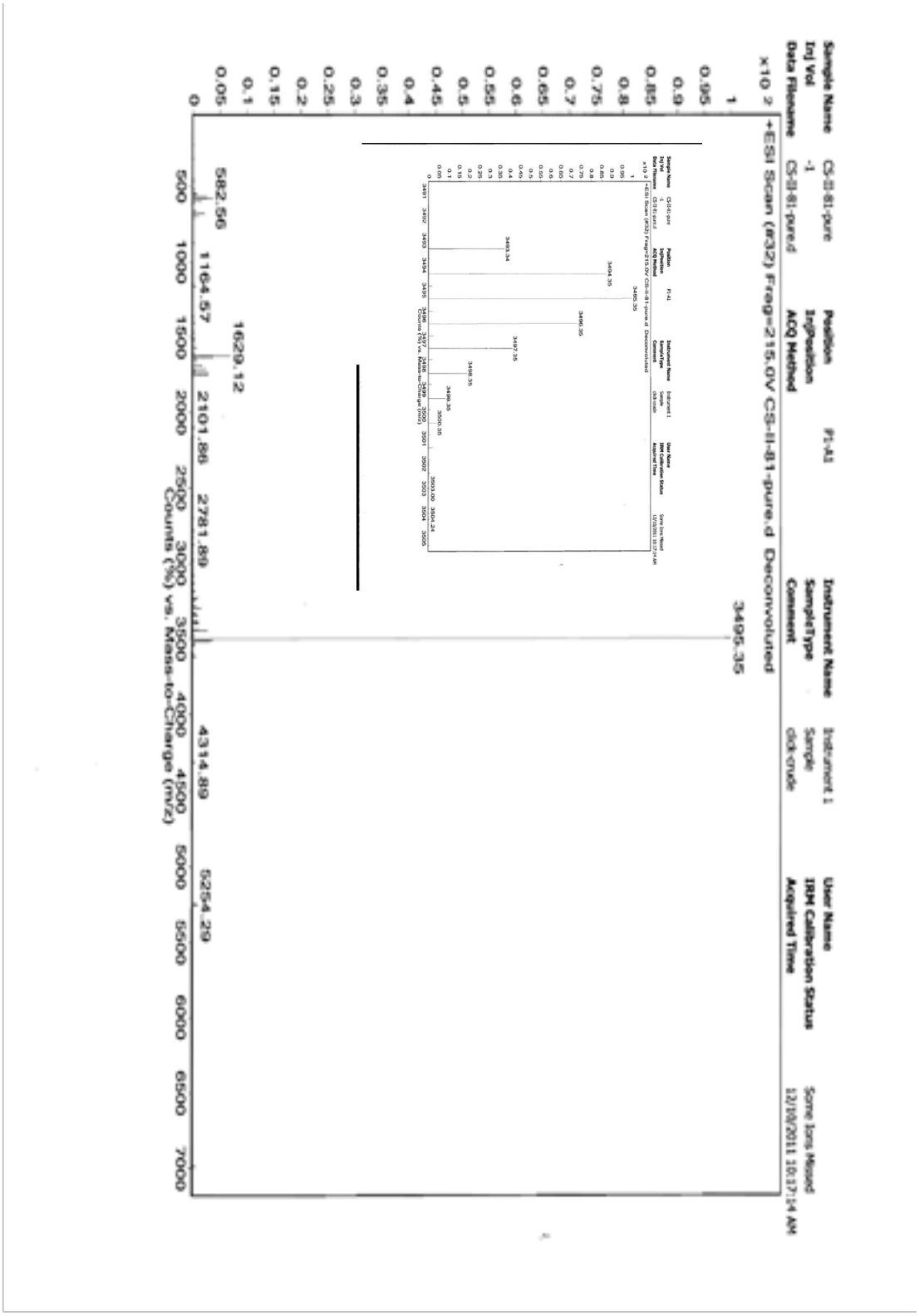


Figure S18. ^{1}H NMR Spectrum of S1.

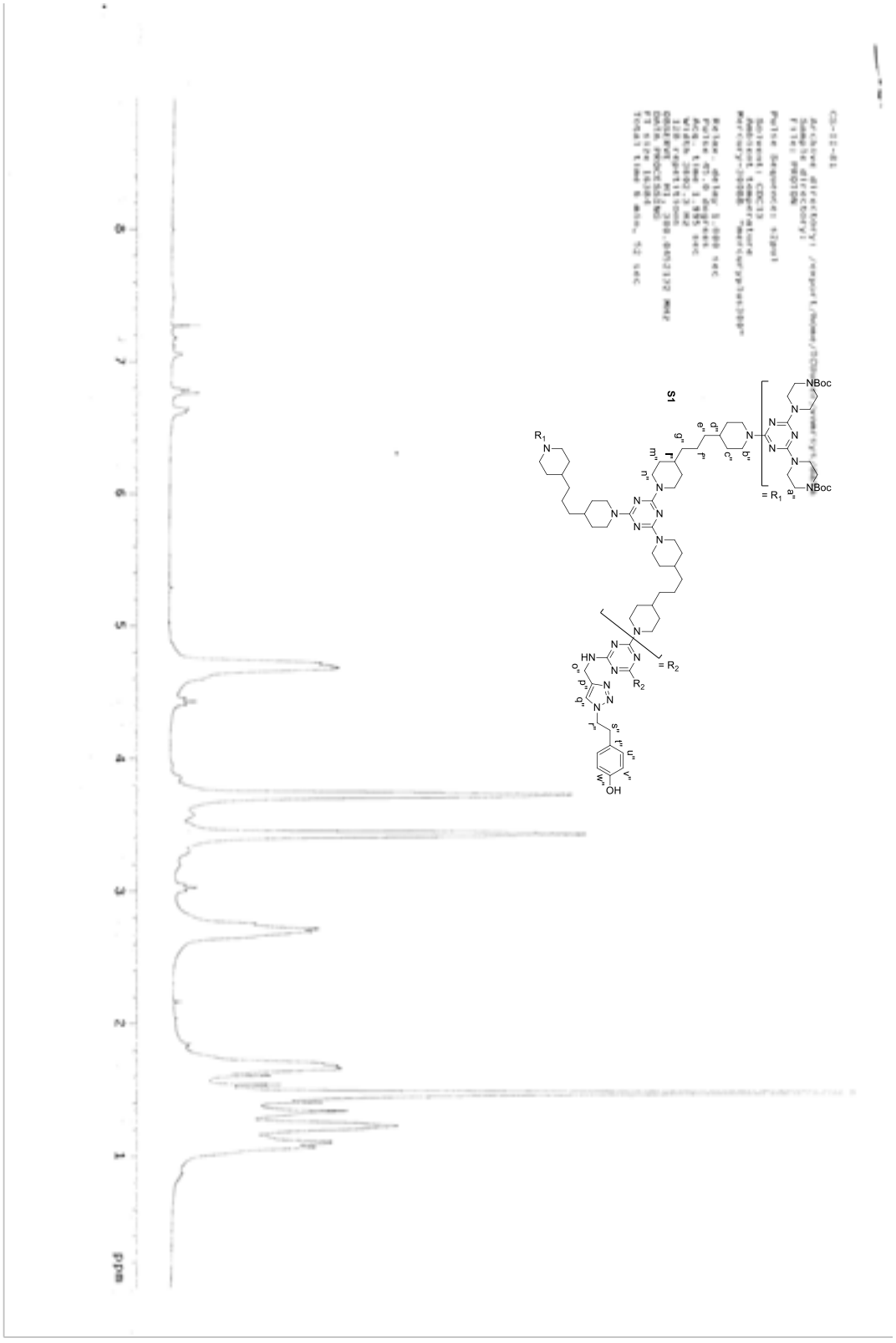
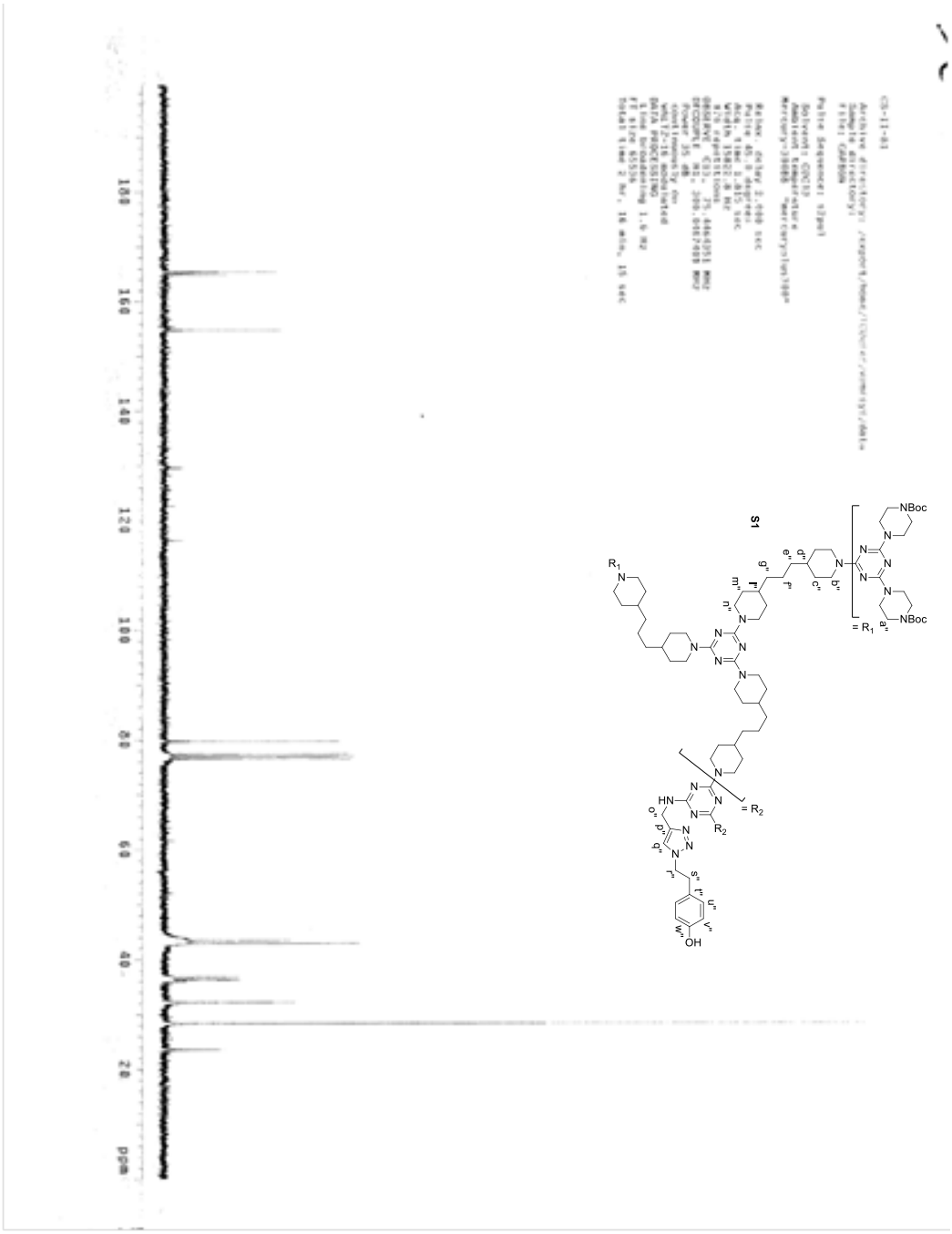
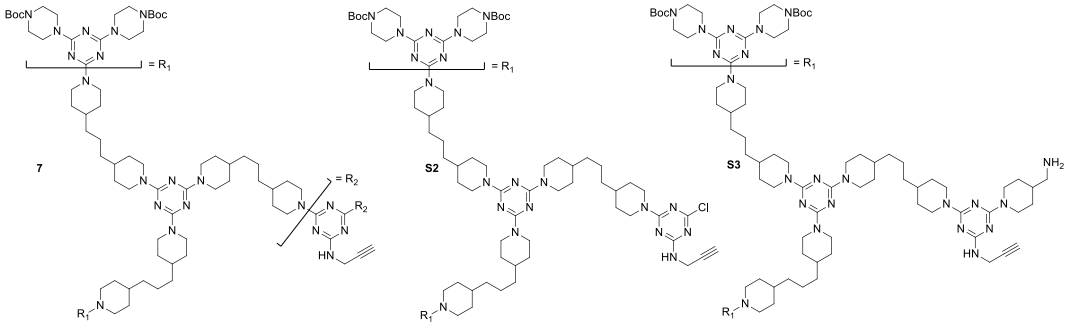


Figure S19. ^{13}C **NMR Spectrum of S1.**



Synthesis of 7 with a Strategy to Remove Sideproduct S2 by Conversion to S3



Intermediate 7. Compound **9** (716 mg, 0.447 mmol), dichlorotriazine **10** (22 mg, 0.112 mmol) and DIPEA (160 μL, 0.89 mmol) were dissolved in $CHCl_3$ (3 mL) at room temperature then warmed up 45 ^oC and stirred for 48h. The reaction mixture was dried under vacuo and an excess amount of compound **9** was recovered through column chromatography (DCM:MeOH = 19:1→DCM:MeOH = 22:3). Semi-pure compound of **11** with a little amount of **S2** was further purified after addition of excess amount of aminomethylpiperidine (~100 equiv.) in THF (10 mL) at room temperature for 12h. Dendron **11** was isolated from **S3** by silica gel chromatography (DCM:MeOH = 19:1; TLC R_f = 0.31 with DCM:MeOH = 19:1) to give a white solid **7** (242 mg, 65%) in THF (10 mL) at room temperature for 12h. ^1H NMR (300 MHz, $CDCl_3$): δ 4.71 (d, *J* = 10.5, 24H, b_e''), 4.18 (d, *J* = 10.5, 2H, o'') 3.73 (s, 32H, a'''), 3.43 (s, 32H, a''), 2.71 (q, *J* = 11.4, 24H, b_a''), 2.18 (br s, 1H, q''), 1.68 (br s, 24H, C_e''), 1.48 (s, 64H, Boc), 1.39-0.84 (m, 72H, C_a'',d'', e'', f''); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4(z''), 165.3(z'), 164.9(y'), 154.8(Boc), 79.7(Boc), 43.5 (b''), 42.9(a''), 36.8(e''), 36.3 (d''), 32.2(c''), 28.4(Boc), 23.6(f''); LRMS (ESI) calcd for $C_{174}H_{285}N_{50}O_{16}$ 3331.30247, found 3331.30 $(M+H)^+$

Figure S20. Mass Spectrum of 7. The trace shows loss of BOC and isobutylene as commonly observed and attributed to ionization-induced fragmentation.

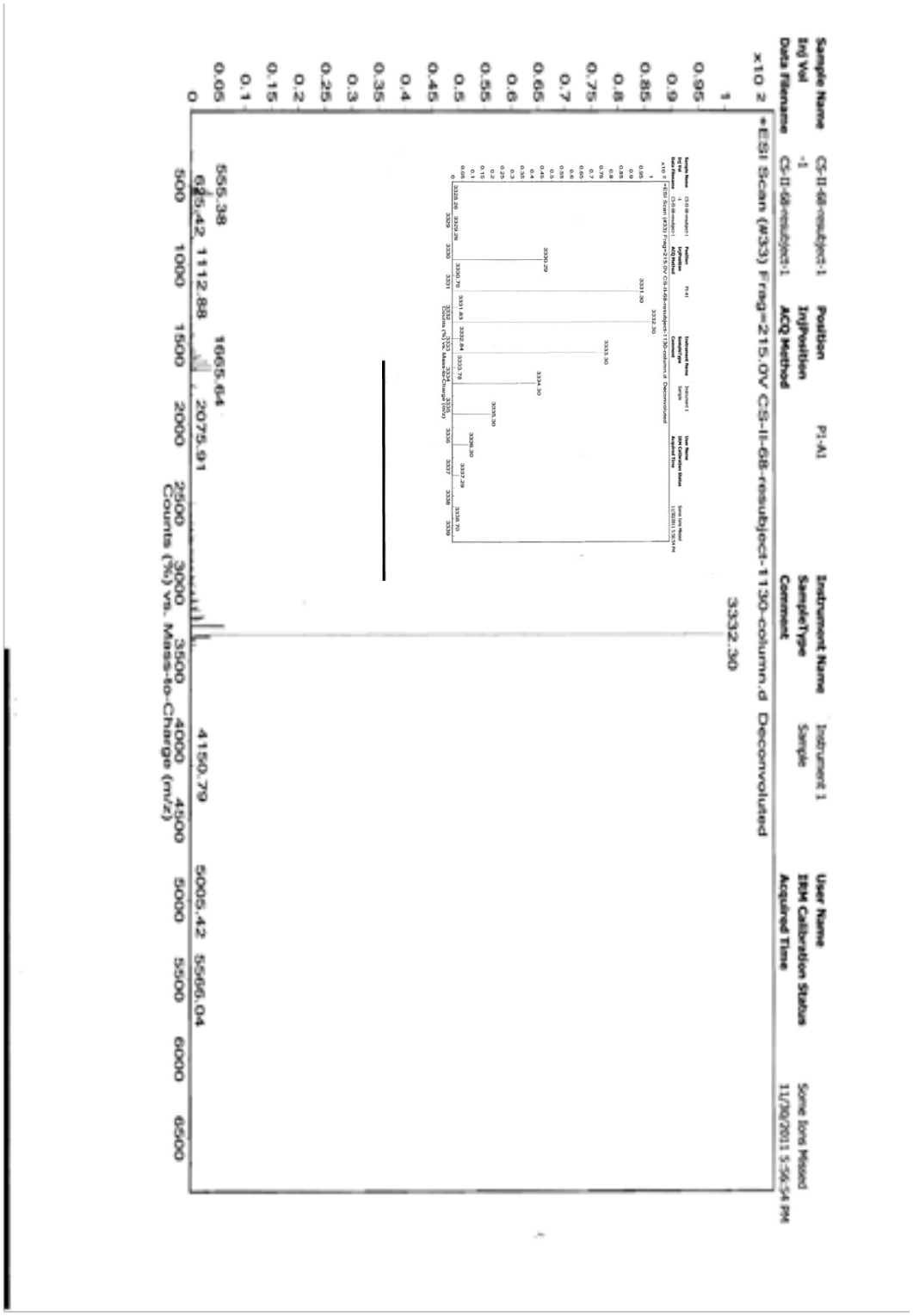


Figure S22. ^{1}H NMR Spectrum of 7.

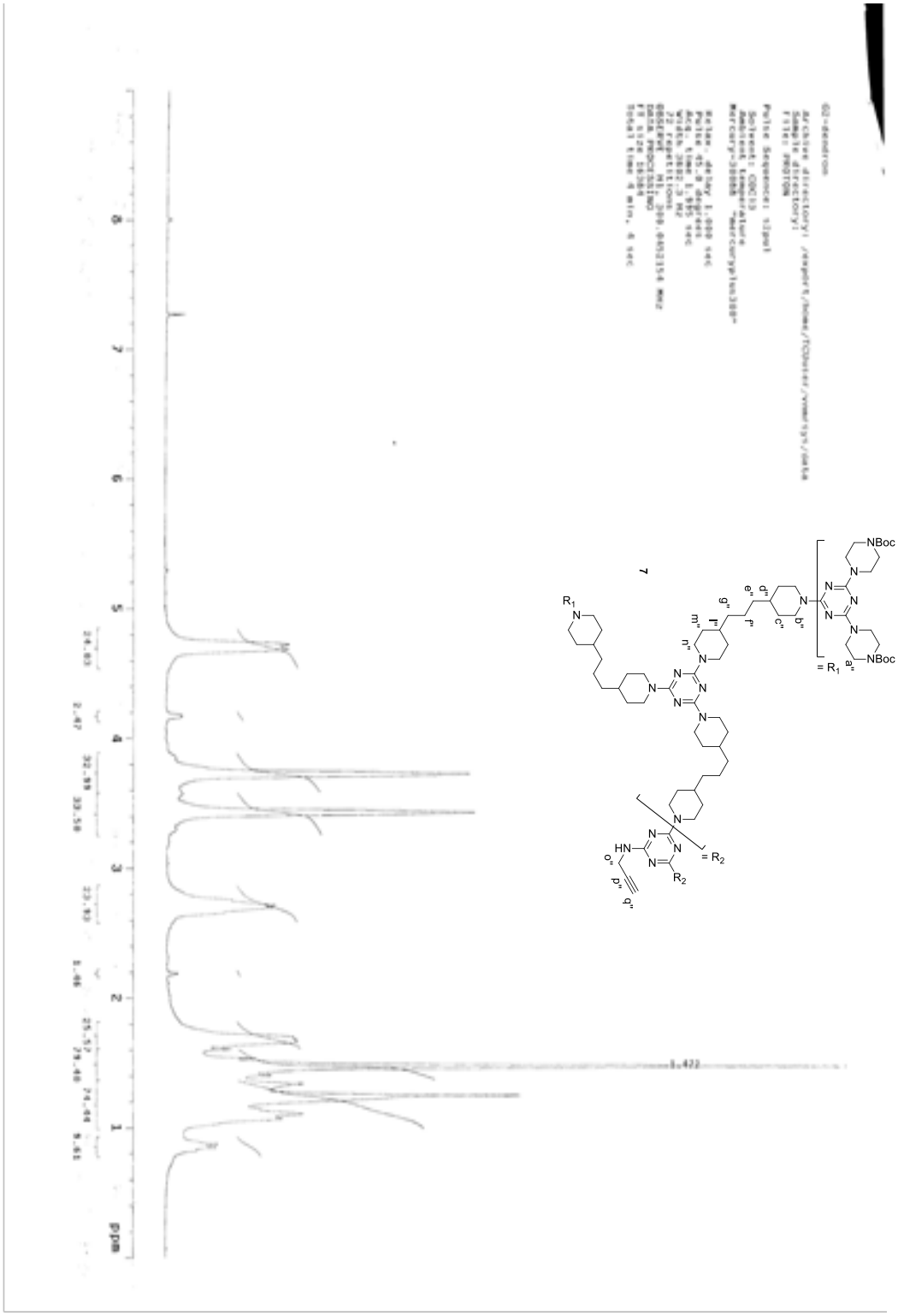
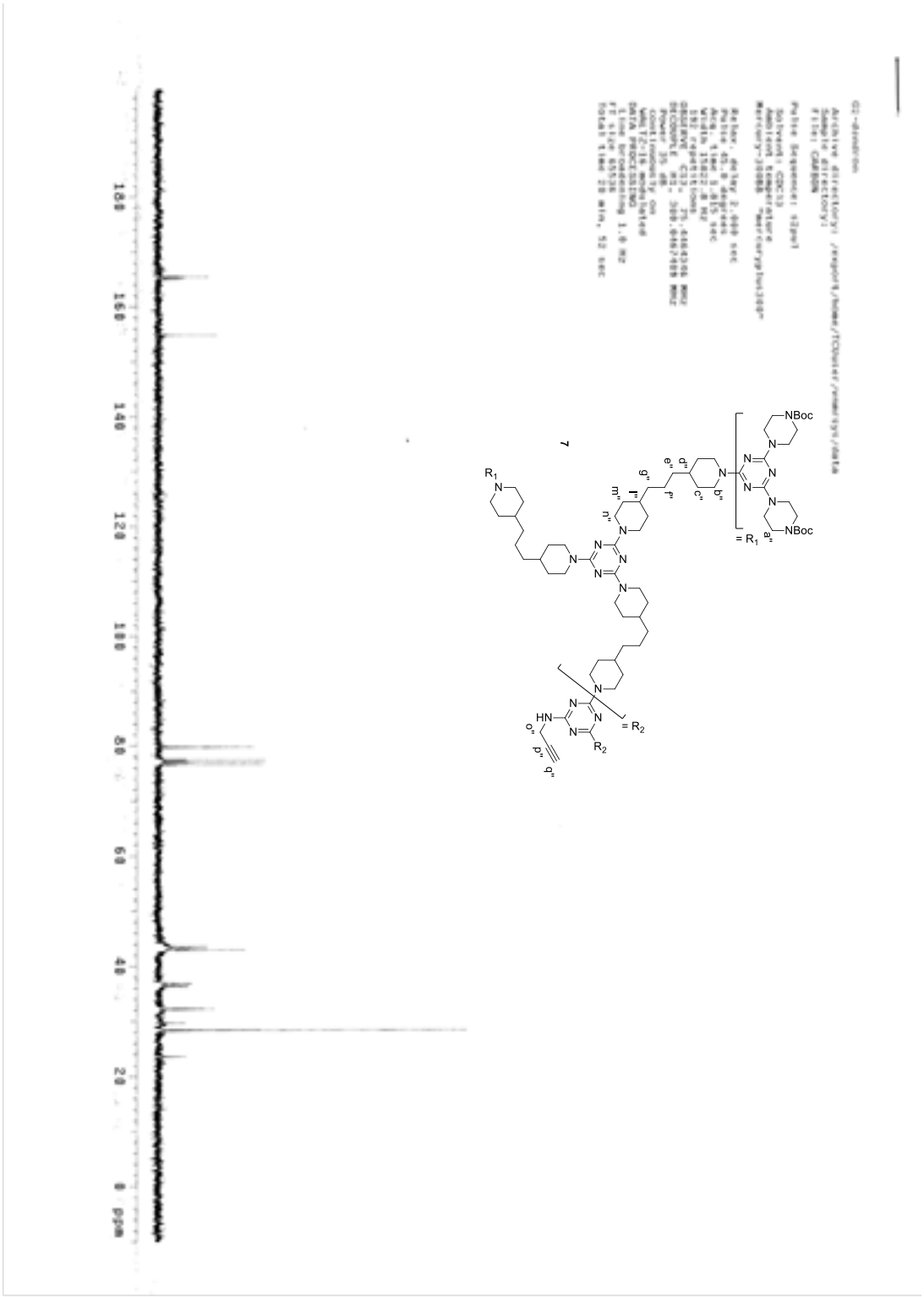
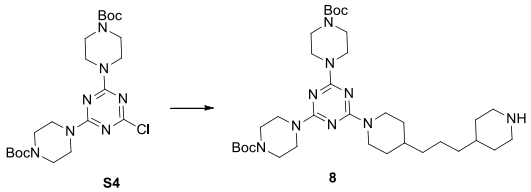


Figure S23. ^{13}C **NMR Spectrum of 7.**



Synthesis of 8



Compound 8. To a solution of monochlorotriazine **S4** (4.3g, 8.88 mmol) in dichloromethane (80 mL) and methanol (8 mL), a solution of trimethylene dipiperidine (11.2 g, 53.3 mmol) in dichloromethane (20 mL) and methanol (2 mL) was added at rt then stirred for 16 h. The reaction was washed brine, dried over $MgSO_4$, and then concentrated. The crude product was purified by column chromatography (DCM:MeOH = 97:3→DCM:MeOH = 17:3; TLC R_f = 0.3 with DCM:MeOH = 17:3) to give **8** as a foam (5.16 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.67 (d, *J* = 12.9, 2H, b_e''), 3.70 (s, 8H, a'''), 3.40 (s, 8H, a''), 3.06 (d, *J* = 12.0, 2H, n_e''), 2.91 (br s, 1H, NH), 2.69 (t, *J* = 11.4, 2H, b_a''), 2.58 (t, *J* = 12.0, 2H, n_a''), 1.68 (d, *J* = 12.3, 4H, C_e'', m_e''), 1.46 (s, 18H, Boc), 1.39-1.00 (m, 12H, C_a'',d'', e'', f'', g''); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 165.4(z'), 164.9(y'), 154.8(Boc), 79.7(Boc), 46.5(n''), 43.4(b''), 42.9(a''), 37.2(g''), 36.7(e''), 36.3(i''), 35.9(d''), 33.1(m''), 32.2(c''),28.4(Boc), 23.4(f''); HRMS (ESI) calcd for $C_{34}H_{60}N_9O_4$ 658.4768, found 658.5027 $(M+H)^+$.

Figure S24. Mass Spectrum of 8. The trace shows loss of BOC and isobutylene as commonly observed and attributed to ionization-induced fragmentation.

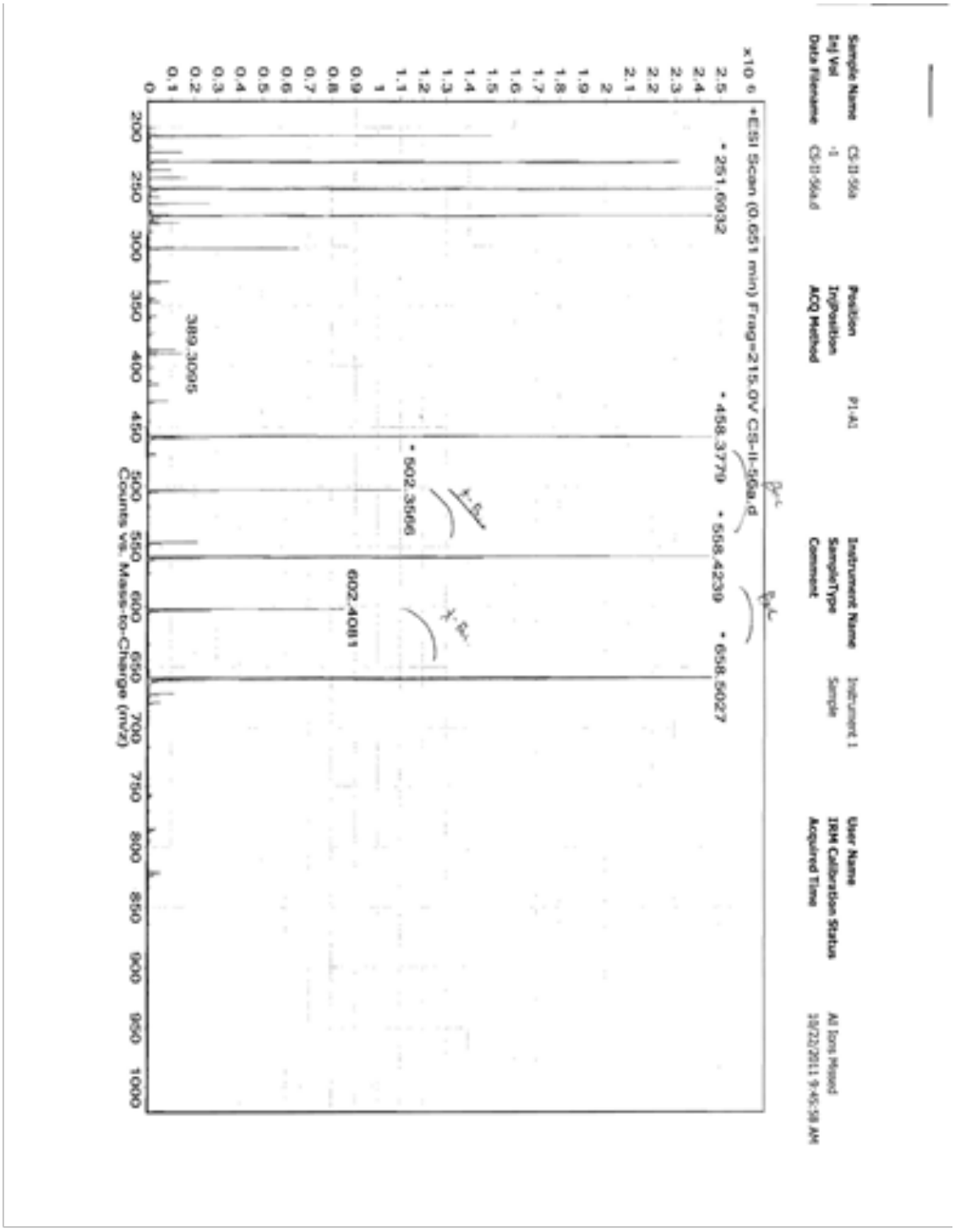


Figure S25. ^{1}H NMR Spectrum of 8.

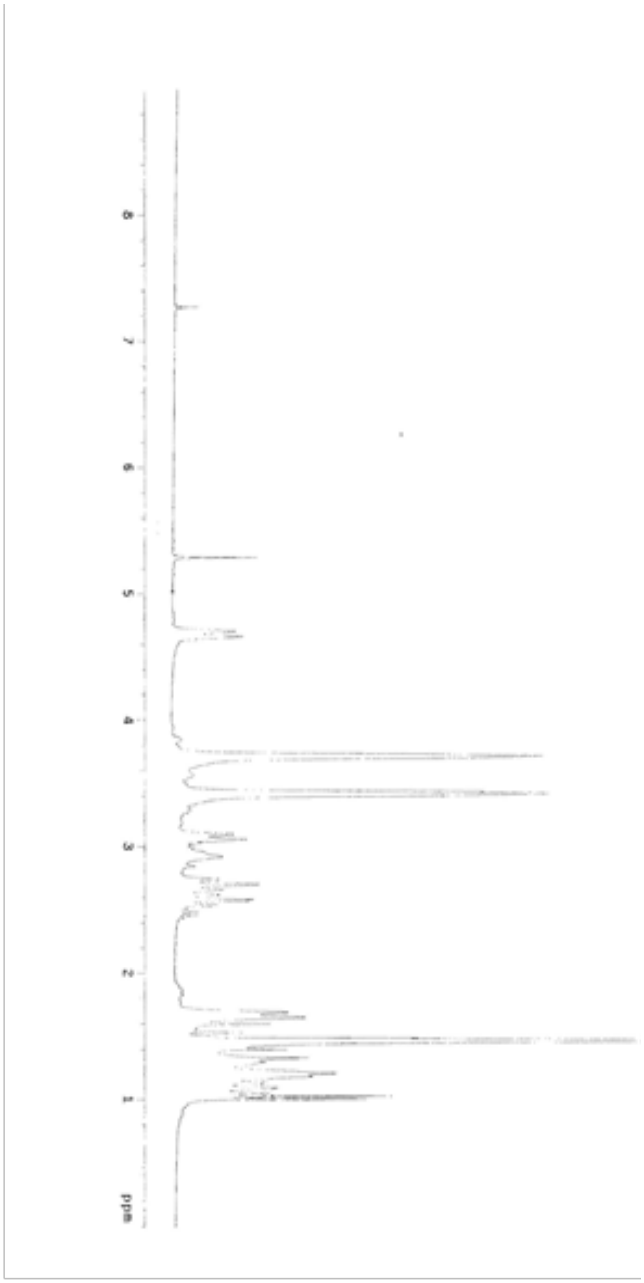
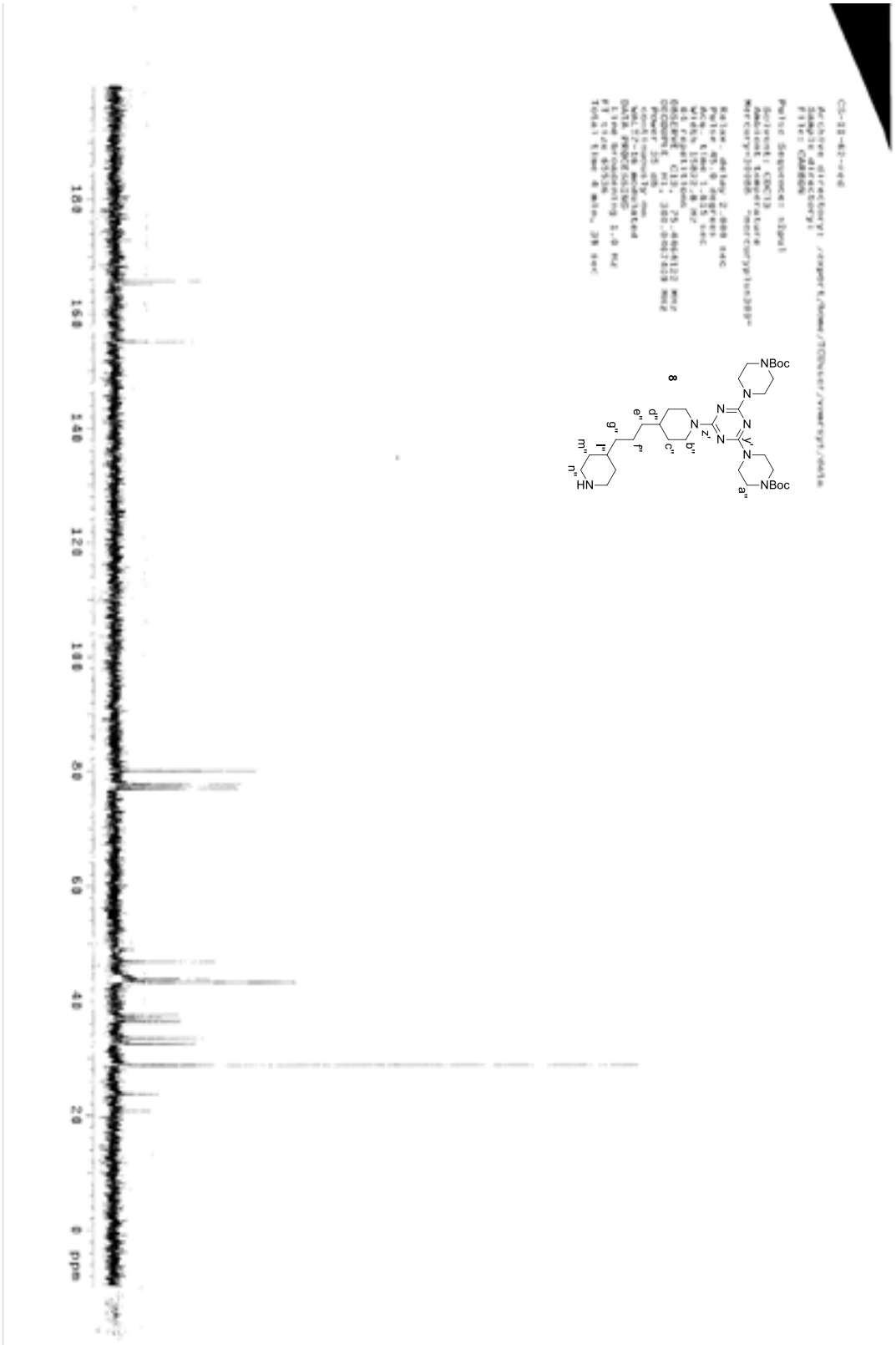
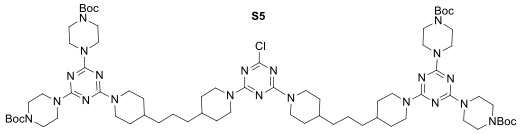


Figure S26. ^{13}**C NMR Spectrum of 8.**



Synthesis of S5



Monochlorotriazine S5. To a solution of compound **8** (3.99 g, 6.08 mmol) and DIPEA (1.6 mL, 9.12 mmol) in THF (40 mL) a solution of cyanuric chloride (561 mg, 3.04 mmol) was added at 0^{o}C. After 30 min, the reaction warmed to room temperature then stirred for 22 h. The reaction was quenched with water (20 mL) removed under vacuo and all of volatile solvent was removed under vacuo. The residue was dissolved in dichloromethane and organic layer was washed with brine, dried over $MgSO_4$, and then concentrated. The crude product was purified by column chromatography (EtOAc:hexanes = 3:7; TLC R_f = 0.22 with EtOAc:hexanes = 3:7) to give **S5** as a foam (3.84 g, 88%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.69 (d, *J* = 10.8, 8H, b_e''), 3.73 (s, 16H, a'''), 3.43 (s, 16H, a''), 2.75 (q, *J* = 13.8, 8H, b_a''), 1.71 (t, *J* = 9.3, 8H, C_e''), 1.47 (s, 32H, Boc), 1.39-1.00 (m, 24H, C_a'',d'', e'', f''); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.0(y'), 154.8(Boc), 79.8(Boc), 43.8(n''), 43.5(b''), 43.0(a''), 36.7(g''), 36.6(e''), 36.2(i''), 36.0(d''), 32.2(m''), 32.2(c''),28.4(Boc), 23.5(f''); HRMS (ESI) calcd for $C_{71}H_{117}ClN_{21}O_8$ 1426.90825, found 1426.9116 $(M+H)^{+}$.

Figure S27. Mass Spectrum of S5. The line at 713 corresponds to doubly-charged **S5**. The other lines are not assigned.

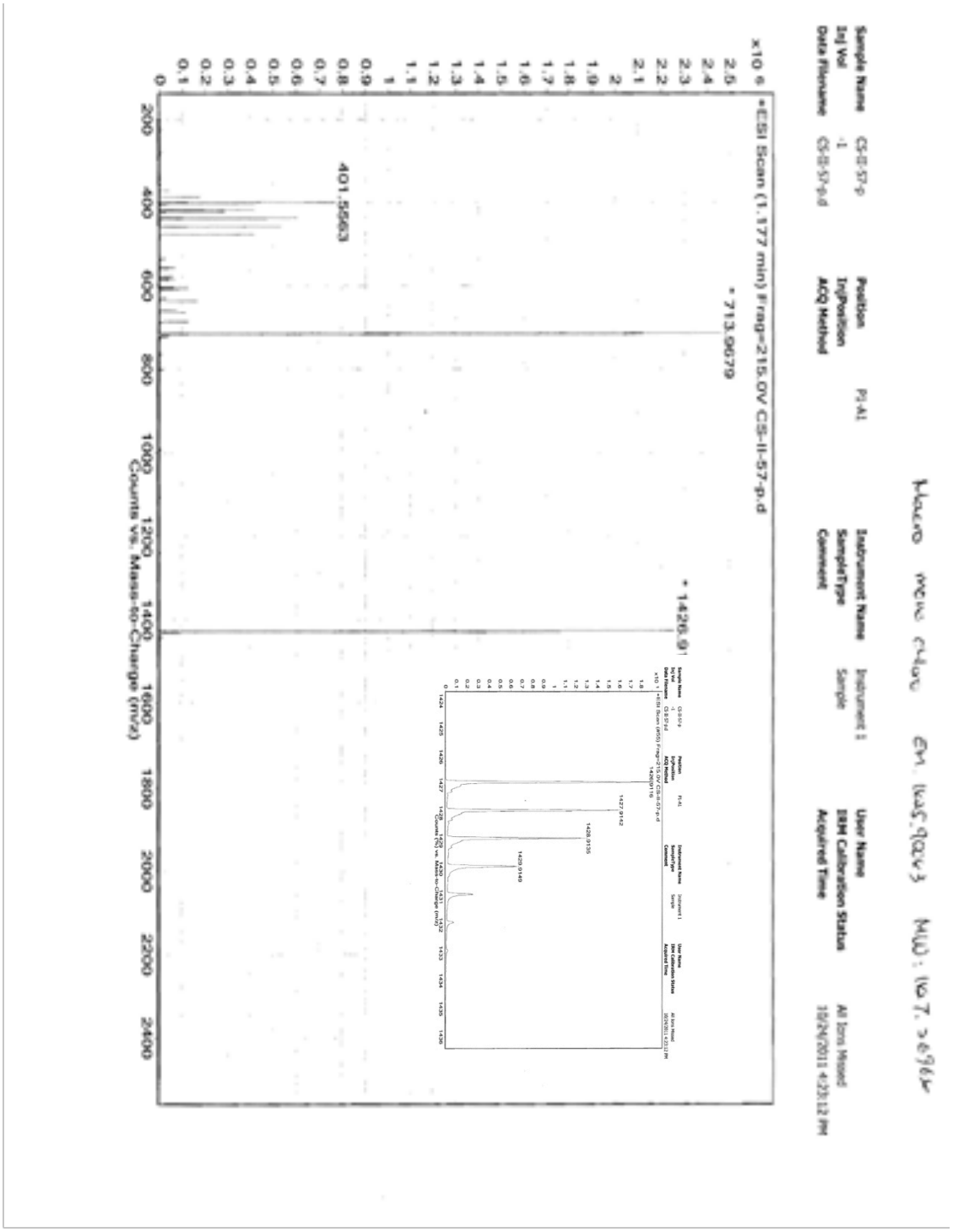


Figure S28. ^{1}**H NMR of S5.**

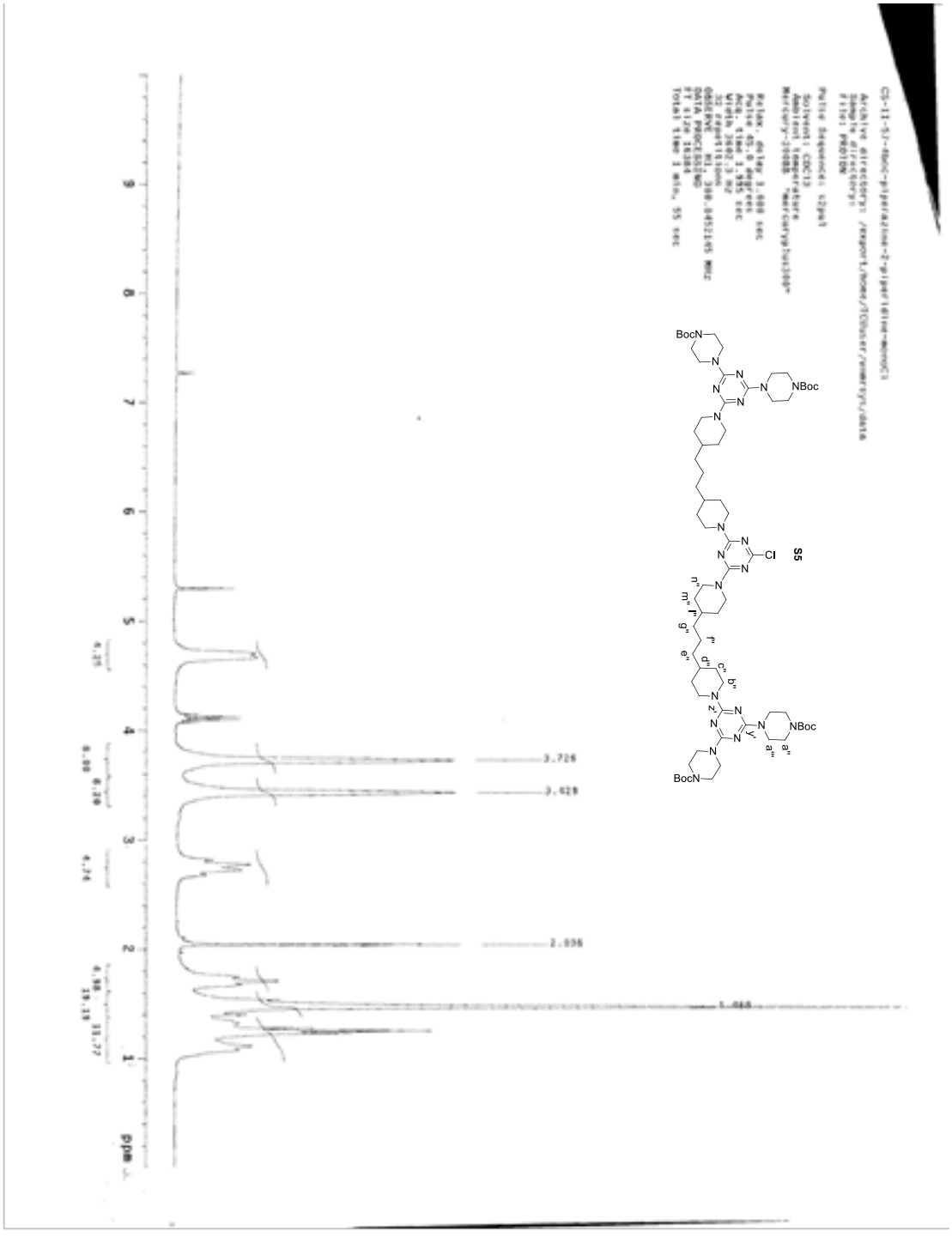
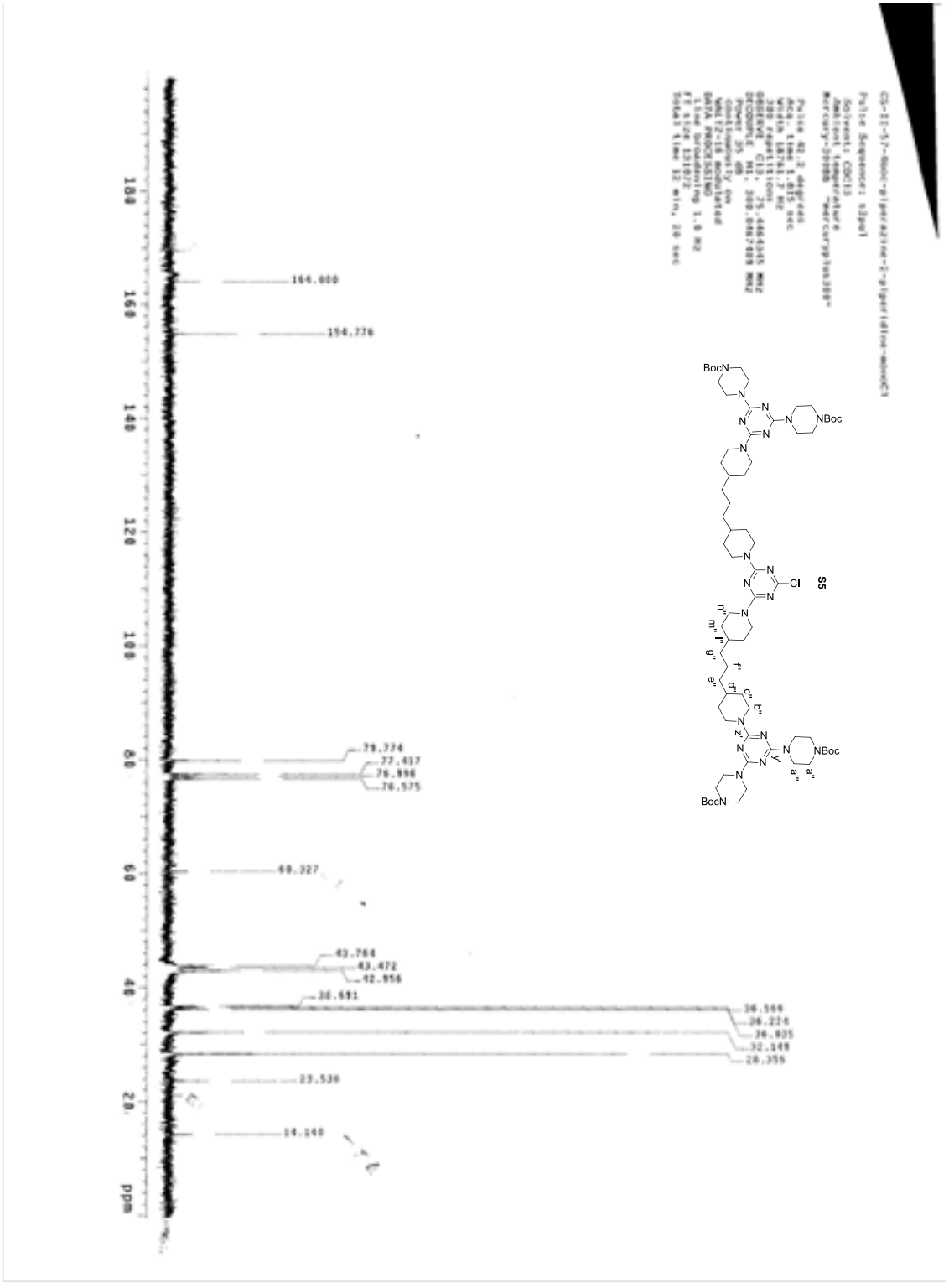
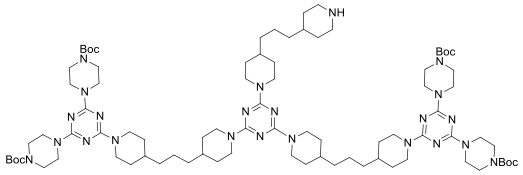


Figure S29. ^{13}C NMR of S5.



Synthesis of 9



9

Compound 9. To a solution of monochlorotriazine **S5** (3 g, 2.1 mmol) in dichloromethane (20 mL) and THF (20 mL), trimethylene dipiperidine (2.2 g, 10.5 mmol) was added at rt then stirred for 24 h. The reaction was washed brine, dried over $MgSO_4$, and then concentrated. The crude product was purified by column chromatography (DCM:MeOH = 19:1→DCM:MeOH = 22:3; TLC R_f = 0.28 with DCM:MeOH = 22:3) to give **9** as a solid (2.39 g, 71%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.72 (d, *J* = 11.4, 12H, b_e''), 3.73 (s, 16H, a'''), 3.43 (s, 16H, a''), 2.71 (q, *J* = 11.1, 12H, b_a''), 1.68 (br s, 12H, C_e''), 1.48 (s, 32H, Boc), 1.39-1.00 (m, 36H, C_a'',d'', e'', f''); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.3(z''), 165.3(z'), 164.9(y'), 154.8(Boc), 79.8(Boc), 43.5 (b''), 42.9(a''), 36.9(e''), 36.3 (d''), 32.2(c''), 28.4(Boc), 23.6(f''); HRMS (ESI) calcd for $C_{84}H_{142}N_{23}O_8$ 1601.1412, found 1601.1502 $(M+H)^+$.

Figure S30. Mass Spectrum of 9. The line at 801 corresponds to doubly-charged **9**. The lines at lower m/z are not assigned.

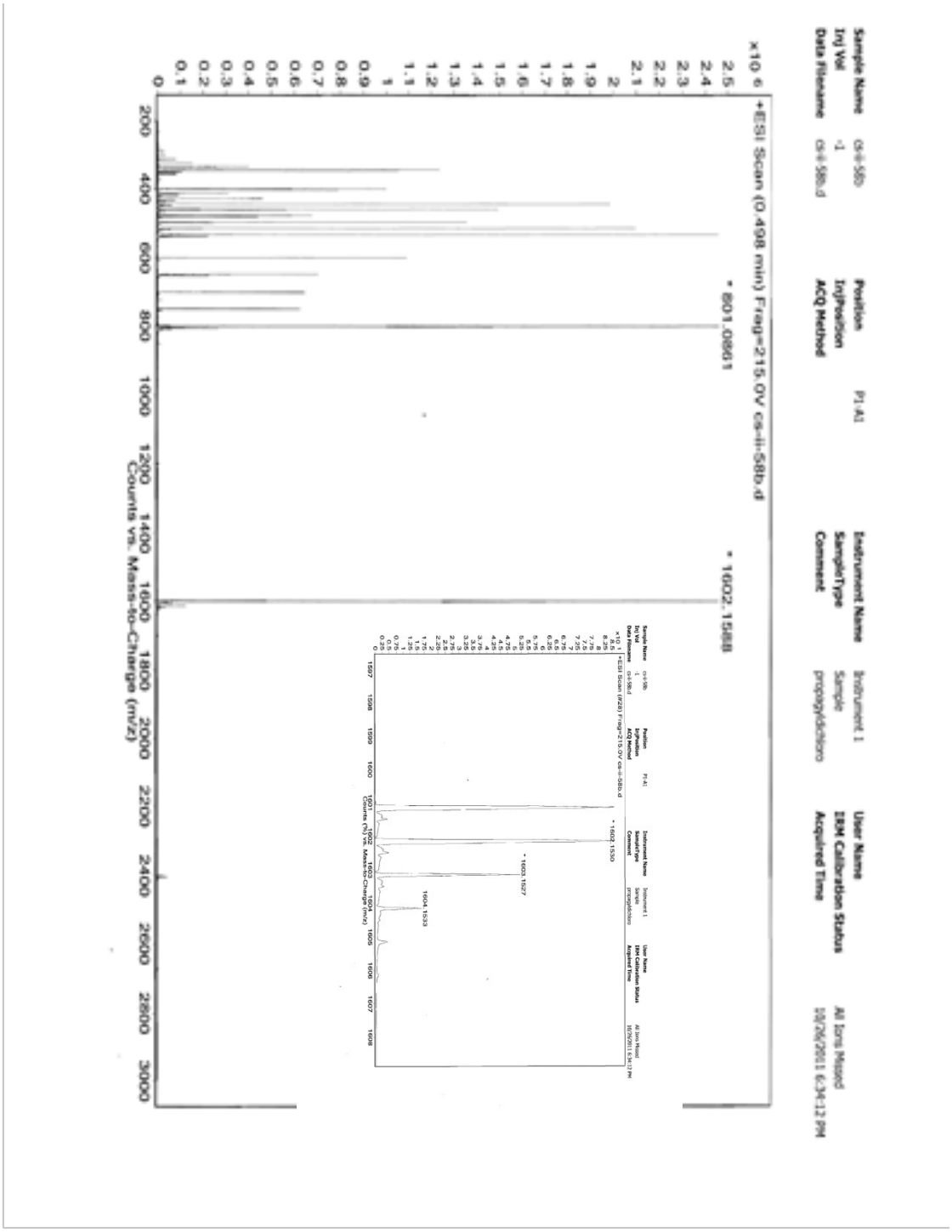


Figure S31. ^{1}**H NMR of 9.**

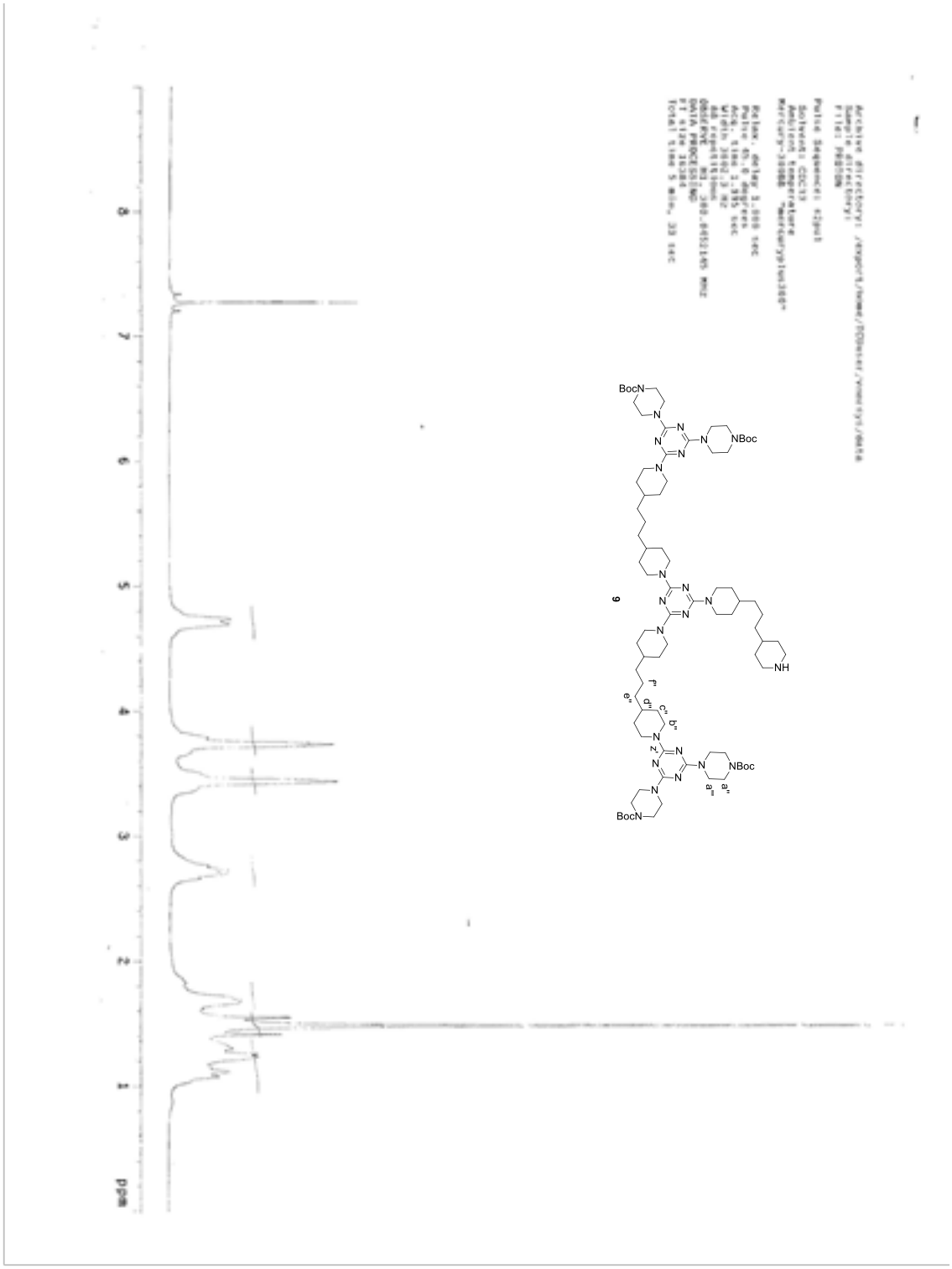
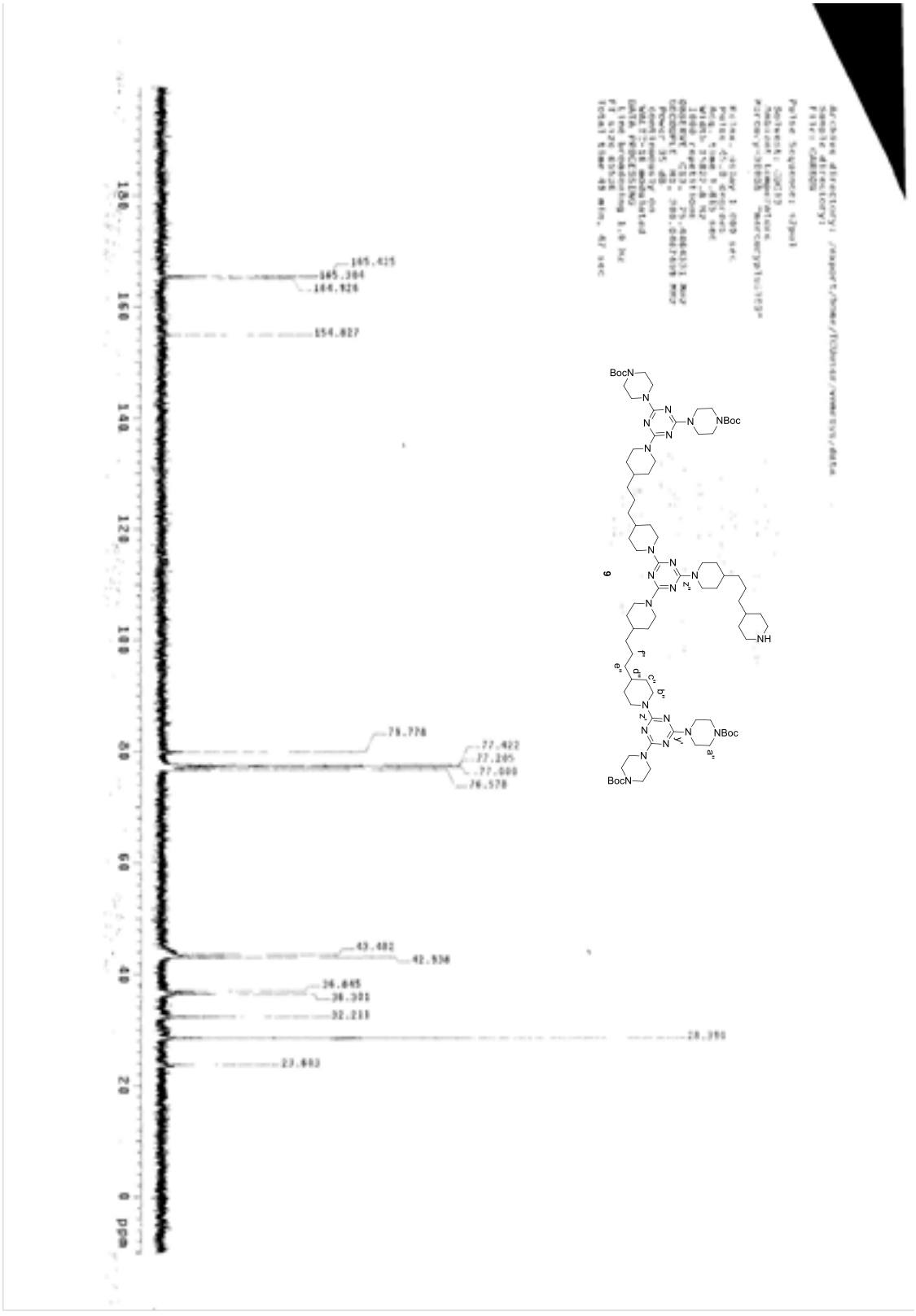
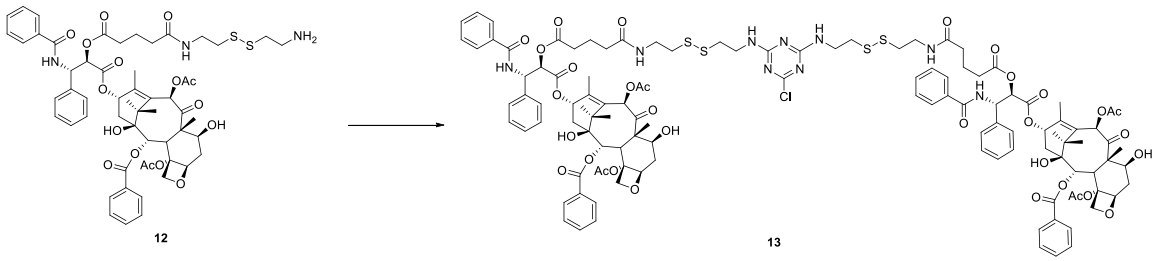


Figure S32. ^{13}C **NMR of 9.**



Preparation of 13



Compound 13. To a solution of compound **12** (399 mg, 0.36 mmol) and DIPEA (64 mg, 0.49 mmol) in THF (20 mL) a solution of cyanuric chloride (30 mg, 0.16 mmol) was added at 0^{o}C. After 30 min, the reaction warmed to room temperature then stirred for 24 h. The reaction was quenched with water (20 mL) and diluted with dichloromethane (30 mL). Organic layer was washed brine, dried over $MgSO_4$, and then concentrated. The crude product was purified by column chromatography (DCM:MeOH = 19:1; TLC R_f = 0.3 with DCM:MeOH = 19:1) to give **13** as a foam (338 mg, 89 %). The excess of paclitaxel-cystamine **12** was recovered from the purification. ^{1}H NMR (300 MHz, $CDCl_3$+MeOH-d_4): δ 8.14 (d, *J* = 6.9, 4H, i), 7.78 (d, *J* = 8.7, 4H, c), 7.62 (t, *J* = 7.5 Hz, 2H, g), 7.54-7.23 (m, 20H, a, b, d, e, f, h), 6.37 (s, 2H, y), 6.17 (t, *J* = 8.4, 2H, n), 5.95 (d, *J* = 3.9, 2H, l), 5.71 (q, *J* = 8.4, 2H, l), 5.45 (d, *J* = 3.9, 2H, m), 5.00 (d, *J* = 8.4, 2H, u), 4.40 (dd, *J* = 11.1, 6.9, 2H, w), 4.32 (d, *J* = 8.4, 2H, t), 4.23 (d, *J* = 8.4, 2H, t), 3.82 (d, *J* = 6.9, 2H, r), 3.69-3.66 (m, 4H, g'), 3.48 (t, *J* = 6.0 Hz, 4H, j'), 2.89-2.74 (m, 8H, h', i'), 2.56-2.43 (m, 6H, d', f', v), 2.43 (s, 6H, j), 2.31-2.11 (m, 14H, d', f', o), 2.22 (s, 6H, k), 1.98-1.86 (m, 6H, e' v), 1.96 (s, 6H, z), 1.69 (s, 6H, c'), 1.21 (s, 6H, b'), 1.17 (s, 6H, a'); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 204.2 (q'), 173.7(p'), 172.7(o'), 171.2(n'), 170.2(z'), 169.3(m'), 169.1(j'), 169.1(k'), 166.8(l'), 165.5(y'), 141.9(r'), 136.9(g'), 134.0(g), 133.7(h'), 133.4(s'), 132.1(d), 130.3(i), 129.8(i'), 129.4(b), 129.2(a), 128.8(e), 128.7(h), 127.5(c), 127.1(f), 84.8(u), 81.3(s), 78.1(p), 76.7(t), 75.9(y), 75.2(q), 74.4(m), 72.2(n), 71.6(w), 58.4(x), 53.4(l), 46.1(r), 43.5(t'), 40.1(x'), 38.4(u'), 37.5(v'), 37.5(w'), 36.1(o), 35.5(v), 34.9(f'), 33.0(d'), 26.6(b'), 22.7(j), 22.1(a'), 21.0(e'), 20.9(k), 14.6(z), 9.9(c'); HRMS (ESI) calcd for $C_{115}H_{133}ClN_9O_{32}S_4$ 2314.7628, found 2314.7006 $(M+H)^+$.

Figure S33. Mass Spectrum of 13. The line at 1158 corresponds to doubly-charged **13**. The line at 1493 is not assigned. It corresponds to loss of 823 (not paclitaxel at 853). This mass defect consistently appears in reactions that utilize trichlorotriazine.

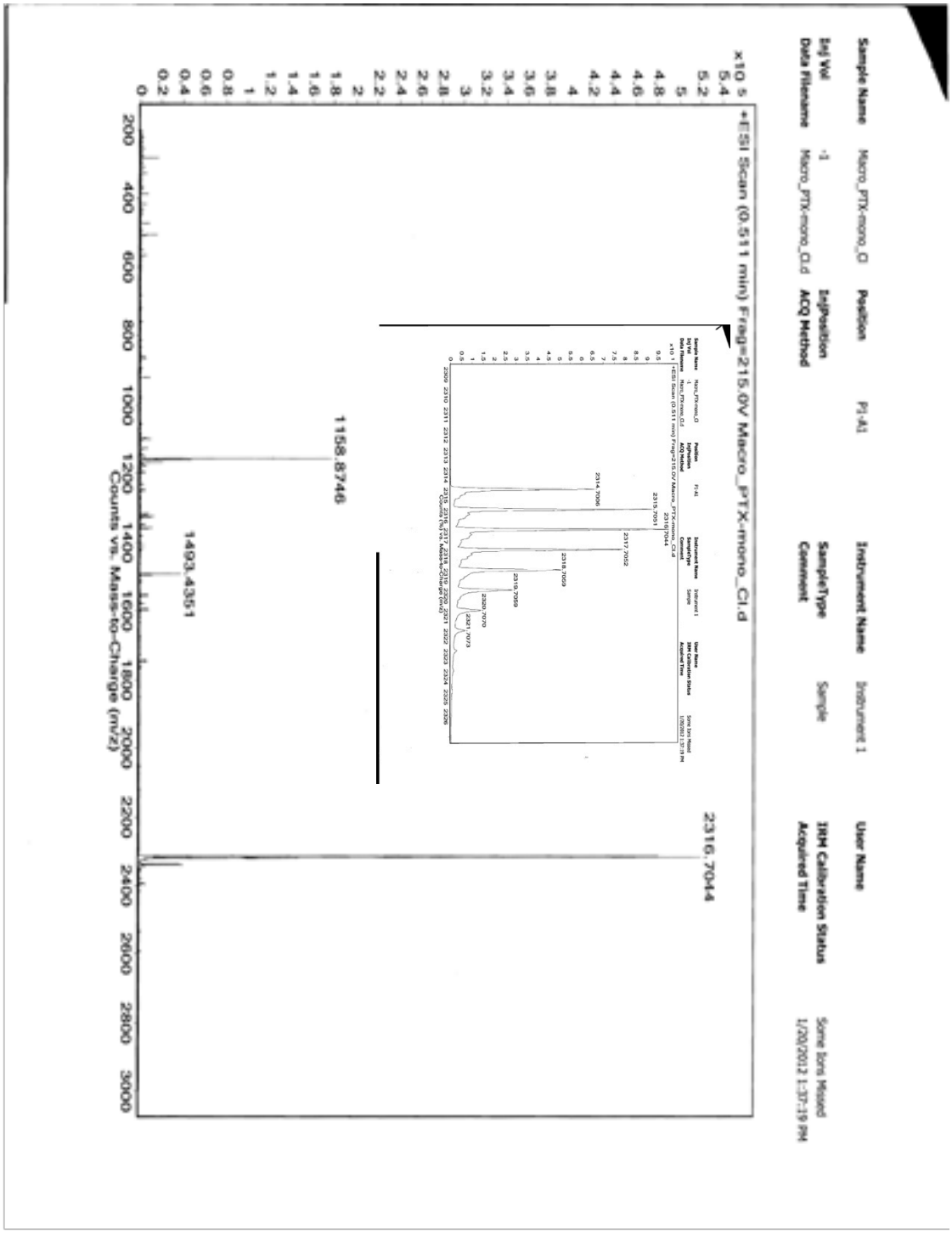


Figure S34. ^{1}H NMR Spectrum of 13.

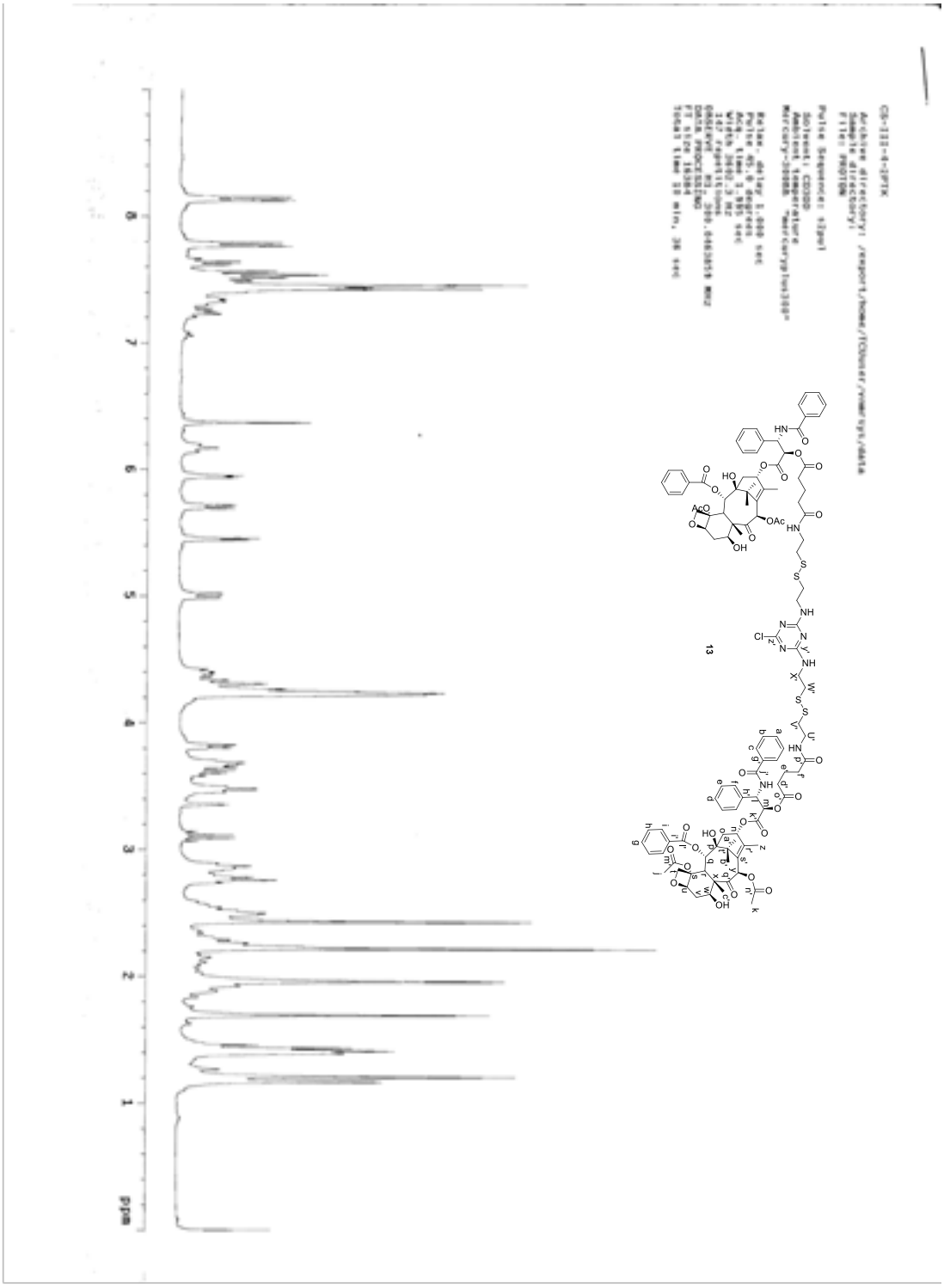
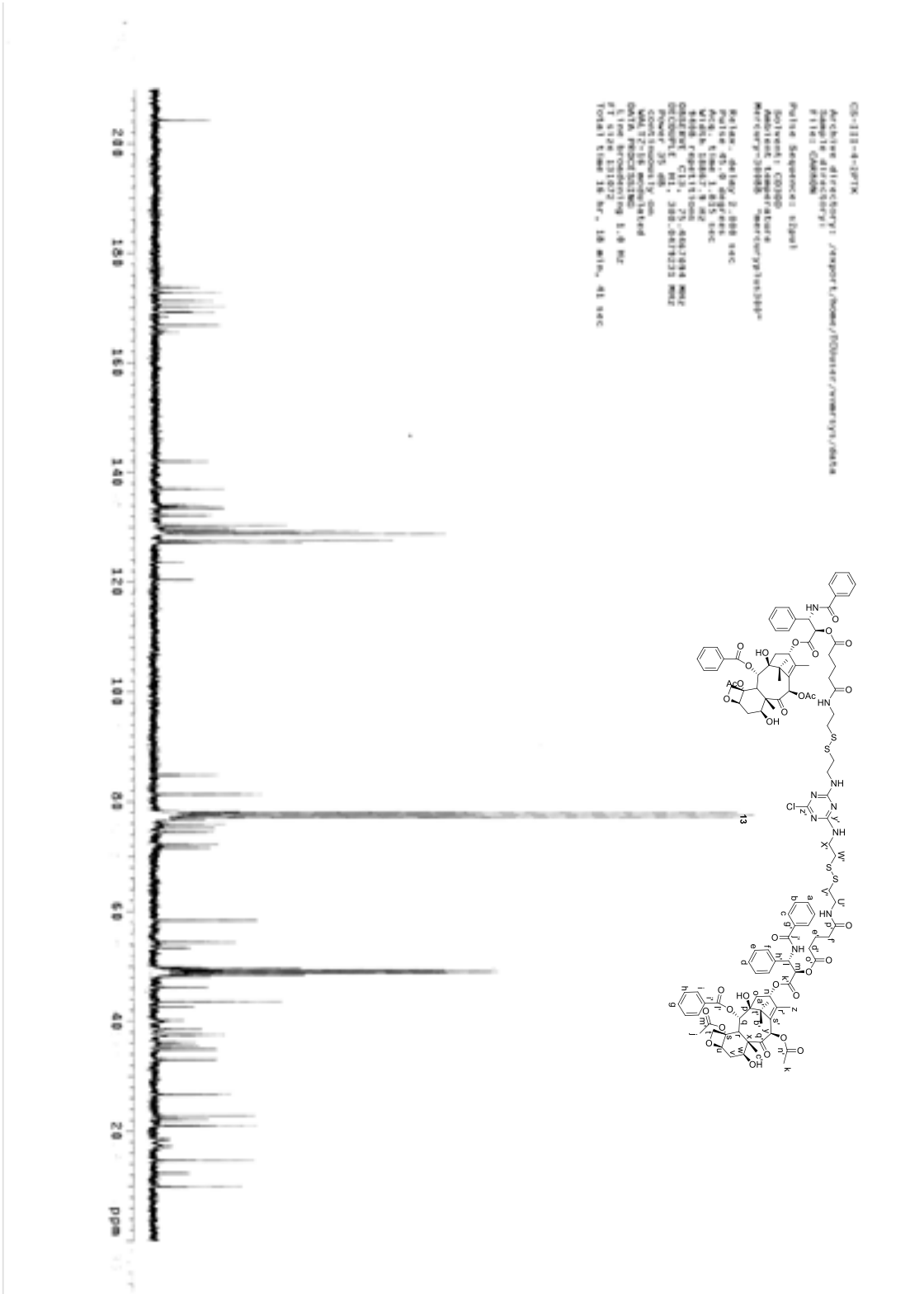
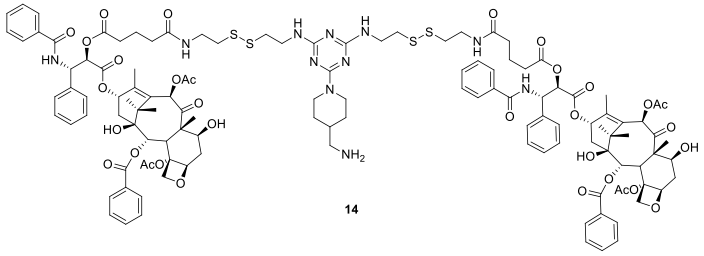


Figure S35. ^{13}C **Spectrum of 13.**



Synthesis of 14



14

Intermediate 14. To a solution of compound **13** (358 mg, 0.15 mmol) in THF (24 mL) a solution of 4-aminomethylpiperidine (529 mg, 4.6 mmol) in THF (2 mL) was added at room temperature. The solution was stirred under nitrogen for 19 h at room temperature. The volume of reaction was reduced to 5 mL *in vacuo* then diluted with dichloromethane (30 mL). The solution was washed two times with brine (20 mL), dried over $MgSO_4$, and then concentrated. The crude product was purified by column chromatography (DCM:MeOH = 10:1; TLC R_f = 0.3 with DCM:MeOH = 10:1) to give **14** as a foam (329 mg, 89 %). ^{1}H NMR (300 MHz, $CDCl_3$+MeOH-d_4): δ 8.14 (d, *J* = 6.9, 4H, i), 7.80 (d, *J* = 7.2, 4H, c), 7.65 (t, *J* = 7.5 Hz, 2H, g), 7.58-7.29 (m, 20H, a, b, d, e, f, h), 6.39 (s, 2H, y), 6.16 (t, *J* = 9.0, 2H, n), 5.93 (d, *J* = 4.2, 2H, l), 5.70 (d, *J* = 7.2, 2H, q), 5.46 (d, *J* = 4.2, 2H, m), 5.01 (d, *J* = 9.0, 2H, u), 4.8 (br s, 2H, h_e''), 4.39 (dd, *J* = 10.8, 6.6, 2H, w), 4.32 (d, *J* = 8.1, 2H, t), 4.25 (d, *J* = 8.1, 2H, t), 3.82 (d, *J* = 6.9, 2H, r), 3.69 (br s, 4H, g'), 3.49 (t, *J* = 6.3 Hz, 4H, j'), 3.39-3.34 (m, 2H, k''), 2.89-2.74 (m, 8H, h', i'), 2.56-2.43 (m, 8H, d', f', h_a'', v), 2.43 (s, 6H, j), 2.31-2.11 (m, 8H, d', f', o), 2.22 (s, 6H, k), 2.08-1.78 (m, 9H, e', j'',i_e'', i_a'', v), 1.96 (s, 6H, z), 1.69 (s, 6H, c'), 1.20 (s, 6H, b'), 1.17 (s, 6H, a'); ^{13}C NMR (75 MHz, $CDCl_3$+MeOH-d_4) δ = 205.0 (q'), 174.4(p'), 173.5(o'), 172.0(n'), 171.0(z'), 169.9(m'), 169.9(j'), 169.9(k'), 167.6(l'), 165.3(y'), 142.7(r'), 137.8(g'), 134.8(g), 134.5(h'), 134.2(s'), 132.9(d), 131.1(i), 130.6(i'), 130.0(b), 129.5(a), 129.5(e), 129.5(h), 128.3(c), 127.9(f), 85.6(u), 82.1(s), 78.9(p), 77.5(t), 76.6(y), 76.0(q), 75.2(m), 73.0(n), 72.4(w), 68.9 (h''), 59.2(x), 54.1(l), 46.9(r), 44.3 (k''), 44.0(t'), 40.5(x'), 39.2(u'), 38.8(v'), 38.3(w'), 36.9(o), 36.3(v), 35.7(f'), 33.8(d'), 30.5(i''), 27.5(j''), 26.5(b'), 23.5(j), 22.9(a'), 21.8(e'), 21.8(k), 15.5(z), 10.7(c'); HRMS (ESI) calcd for $C_{121}H_{146}N_{11}O_{32}S_4$ 2392.90182, found 2392.8223 $(M+H)^+$.

Figure S35. Mass Spectrum of 14. The line at 1197 corresponds to doubly-charged **14**.

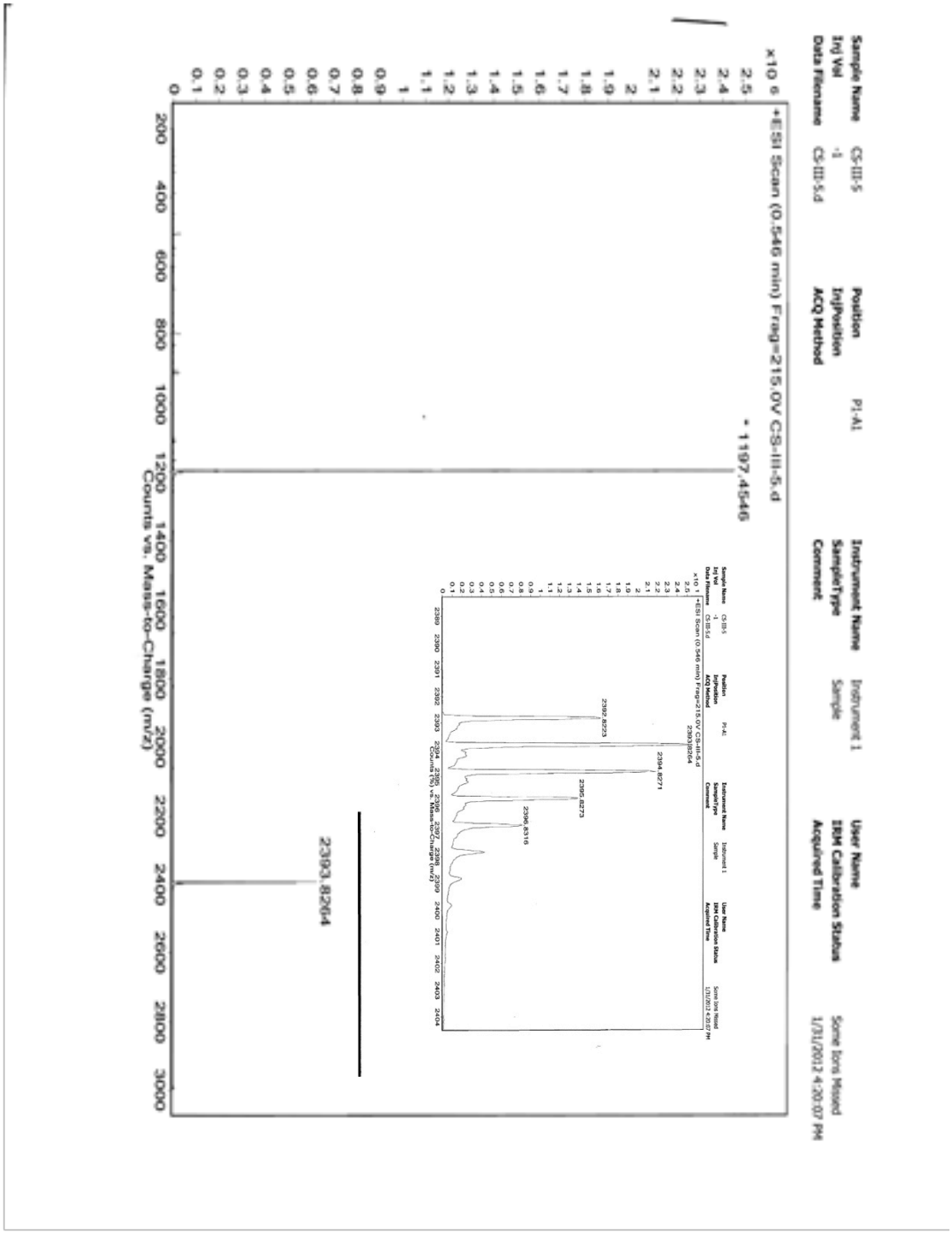


Figure S36. ^{1}H NMR Spectrum of 14.

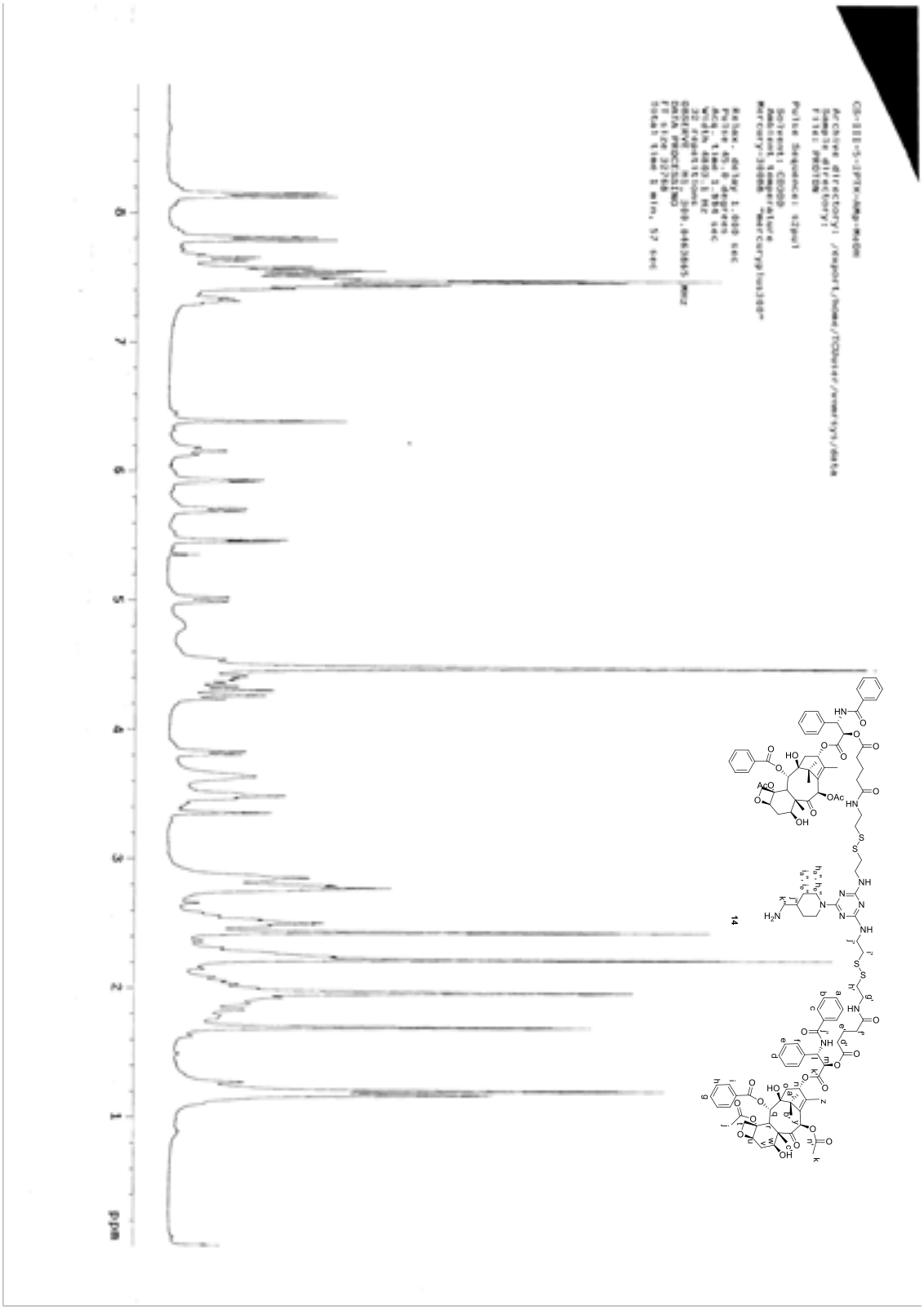
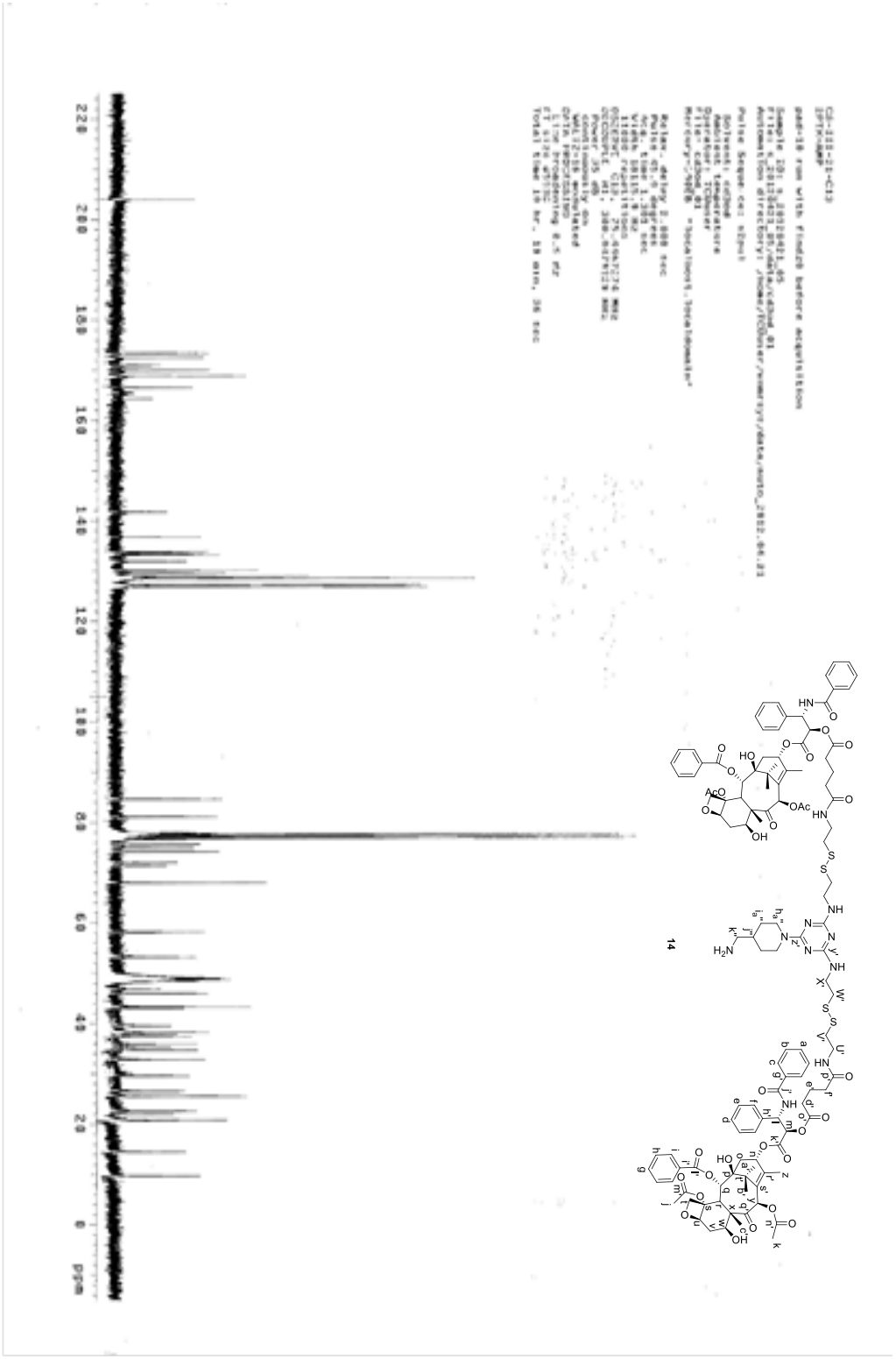
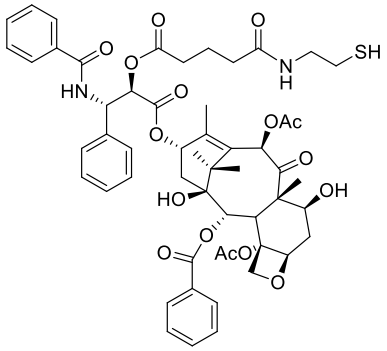


Figure S37. ^{13}C NMR Spectrum of 14.



Synthesis of HPLC Standard 15



15

Compound 15. To a solution of **12** (12 mg, 0.011 mmol) in DCM (1.0 mL) a solution of dithiothreitol (8.4 mg, 0.054 mmol) in DCM (1.0 mL) was added at 0°C. The reaction warmed to room temperature then stirred for 6 h. After concentration, the crude product was purified by column chromatography (DCM:MeOH = 10:1; TLC R_f = 0.28 with DCM:MeOH = 10:1) to give **15** as a foam (9.5 mg, 85 %). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.15 (d, *J* = 7.8, 2H, i), 7.78 (d, *J* = 8.4, 2H, c), 7.62 (t, *J* = 7.2 Hz, 1H, g), 7.54-7.23 (m, 10H, a, b, d, e, f, h), 6.30 (s, 1H, y), 6.24 (t, *J* = 9.3, 1H, n), 6.19 (d, *J* = 6.3, 1H, NH), 5.98 (dd, *J* = 9.3, 3.6, 1H, l), 5.69 (d, *J* = 6.9, 1H, q), 5.49 (d, *J* = 8.4, 2H, m), 4.98 (d, *J* = 9.0, 1H, u), 4.47 (dd, *J* = 10.5, 6.0, 1H, w), 4.32 (d, *J* = 8.4, 1H, t), 4.20 (d, *J* = 8.4, 1H, t), 3.81 (d, *J* = 7.2, 1H, r), 3.66 (t, *J* = 4.8, 1H, SH), 3.34-3.28 (m, 2H, g'), 2.74-2.31 (m, 8H, h',d', f', v, o), 2.46 (s, 3H, j), 2.22 (s, 3H, k), 2.21-2.11 (m, 1H, o), 2.00-1.80 (m, 3H, e' v), 1.95 (s, 3H, z), 1.68 (s, 3H, c'), 1.25 (s, 3H, b'), 1.14 (s, 3H, a'); HRMS (ESI) calcd for $C_{54}H_{63}N_2O_{16}S$ $(M+H)^+$ 1027.3893, found 1027.5400.

Figure S38. Mass Spectrum of 15.

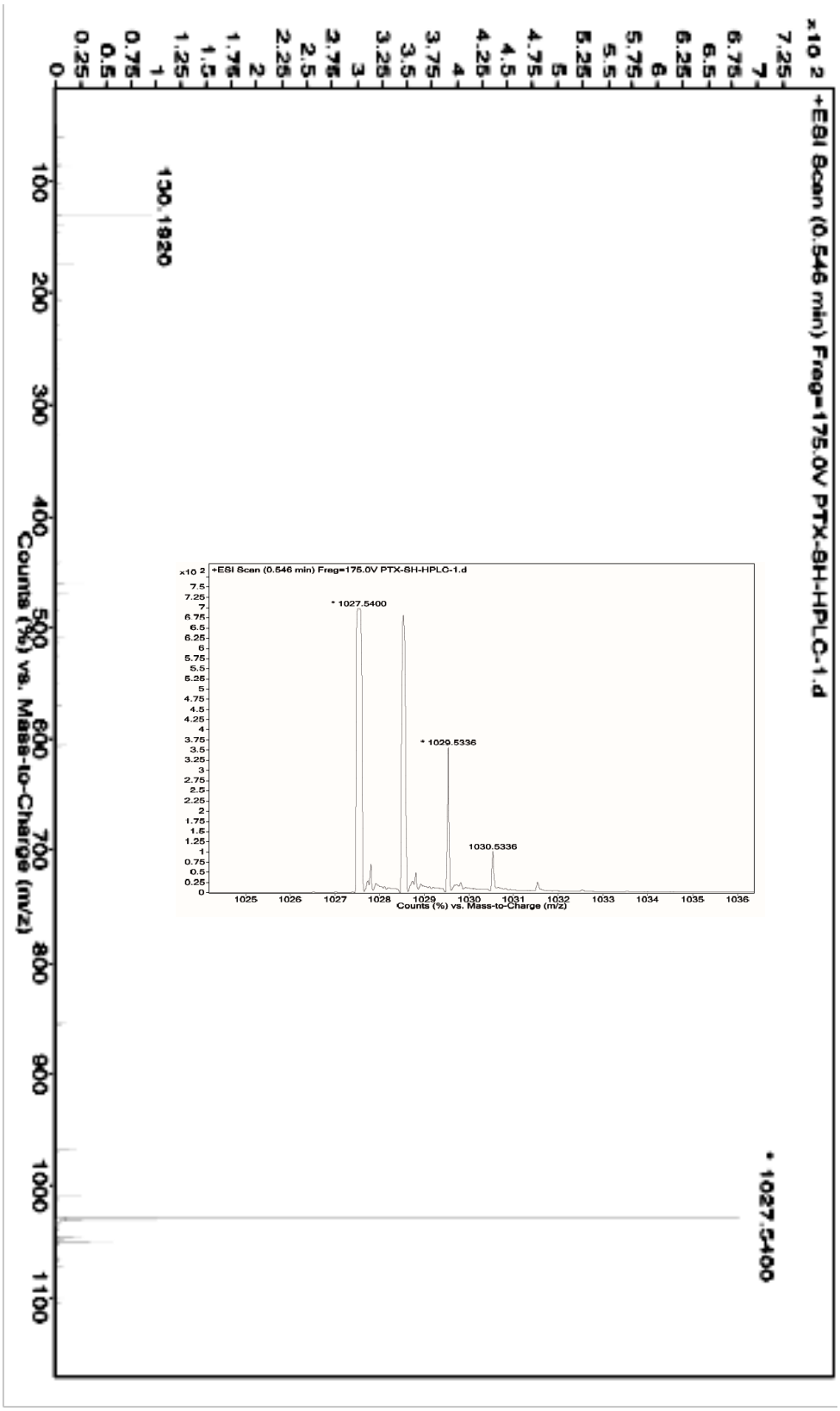


Figure S39. ^{1}H NMR Spectrum of 15.

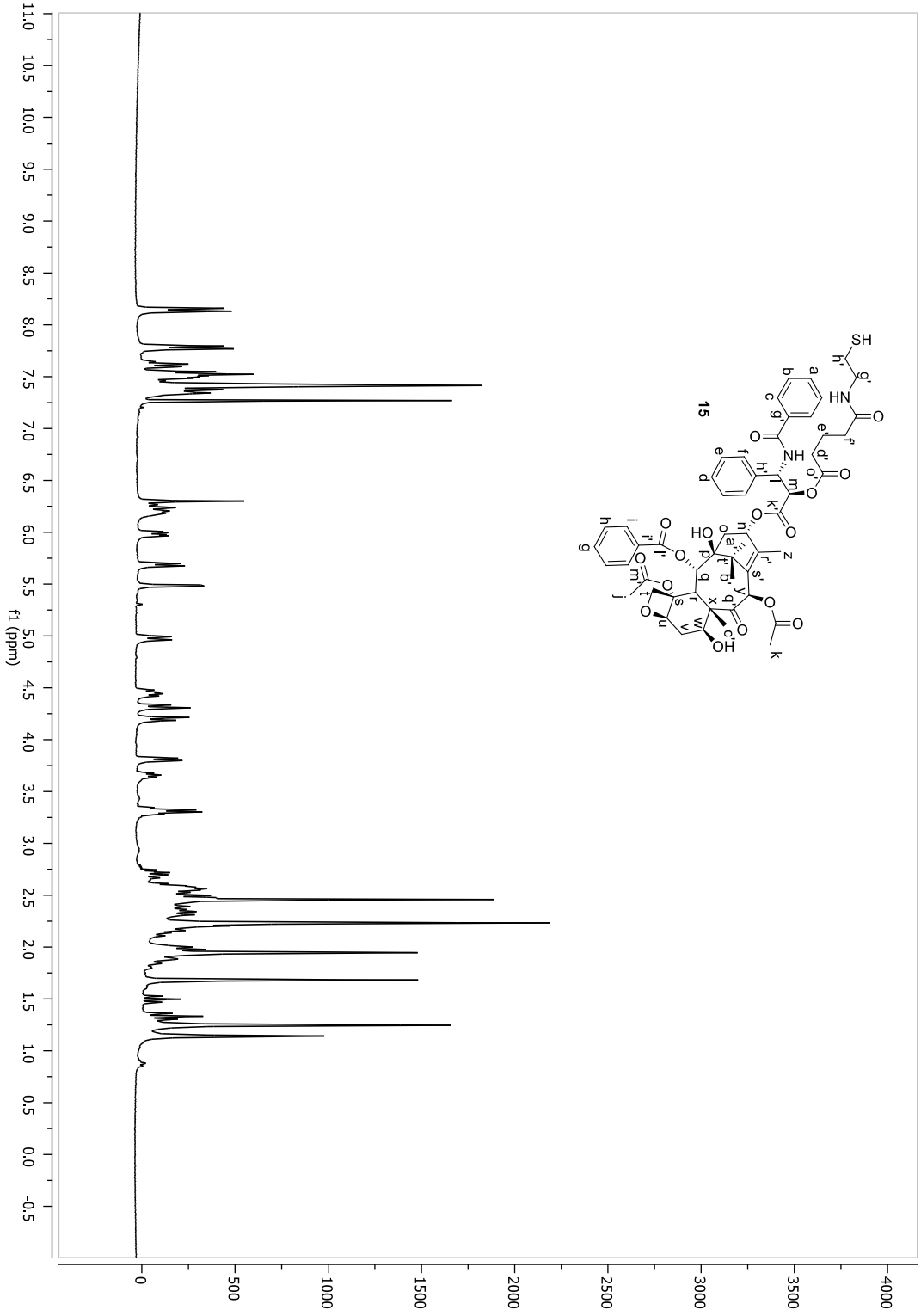


Figure S40. The light-scattering distribution analysis of **1.** Molecular modeling suggests a much smaller radius (3nm) than that measured >10nm. We hypothesize an equilibrium between a discrete aggregate of these dimensions and the 100 nm super-aggregates.

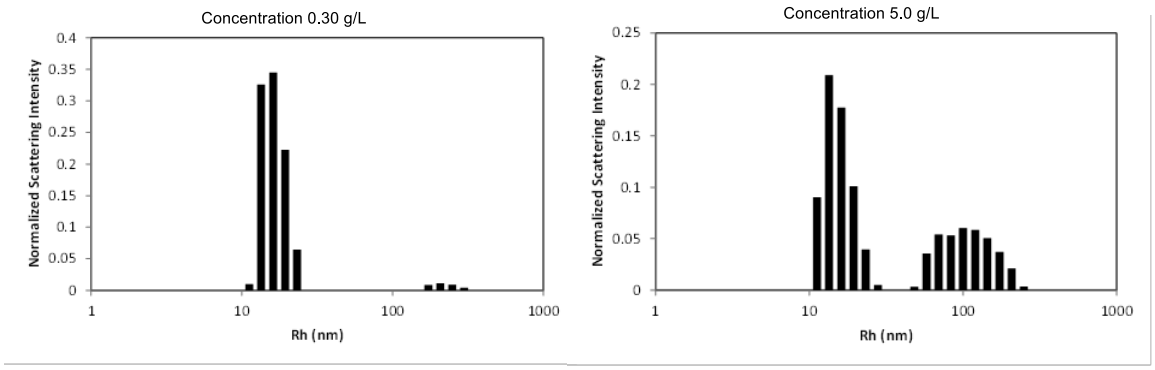


Figure S41. Cumelative release of paclitaxel from **1.** The slow release observed here compared with the >10% release observed with prodrug **3** makes this construct less attractive.

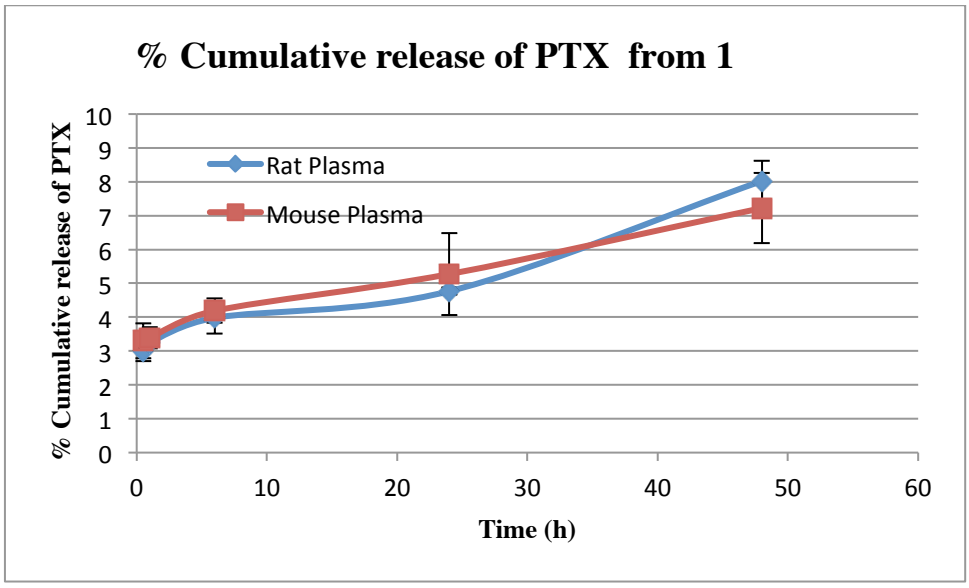


Figure S42. Release of PTX from prodrug 1 in plasma derived from mouse and rat. The broad peaks of the traces and negligible amount of release makes interpretation challenging.

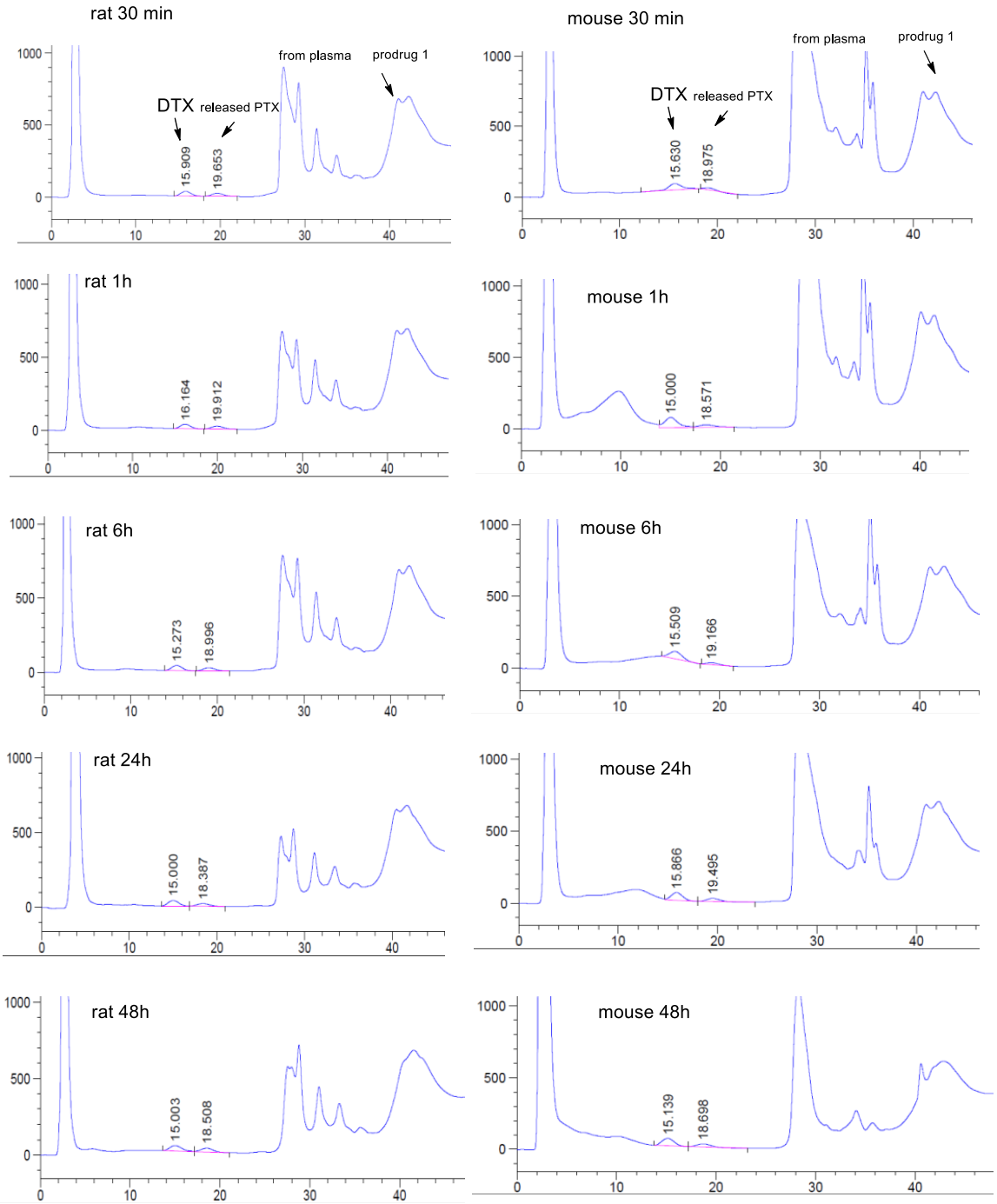


Figure S43. Model Release Studies by Disulfide Cleavage of 1, 12, and Model 16. Top traces: Addition of DTT to **1** leads to disulfide cleavage and a peak corresponding to model compound **15** and shift in the position of the dendrimer. Middle traces: Intermediate **12** also produces **15** on incubation with DTT. Bottom: A standard to establish where the amide hydrolysis product, acid **16**, appears. Paclitaxel appears around 15 minutes in these conditions.

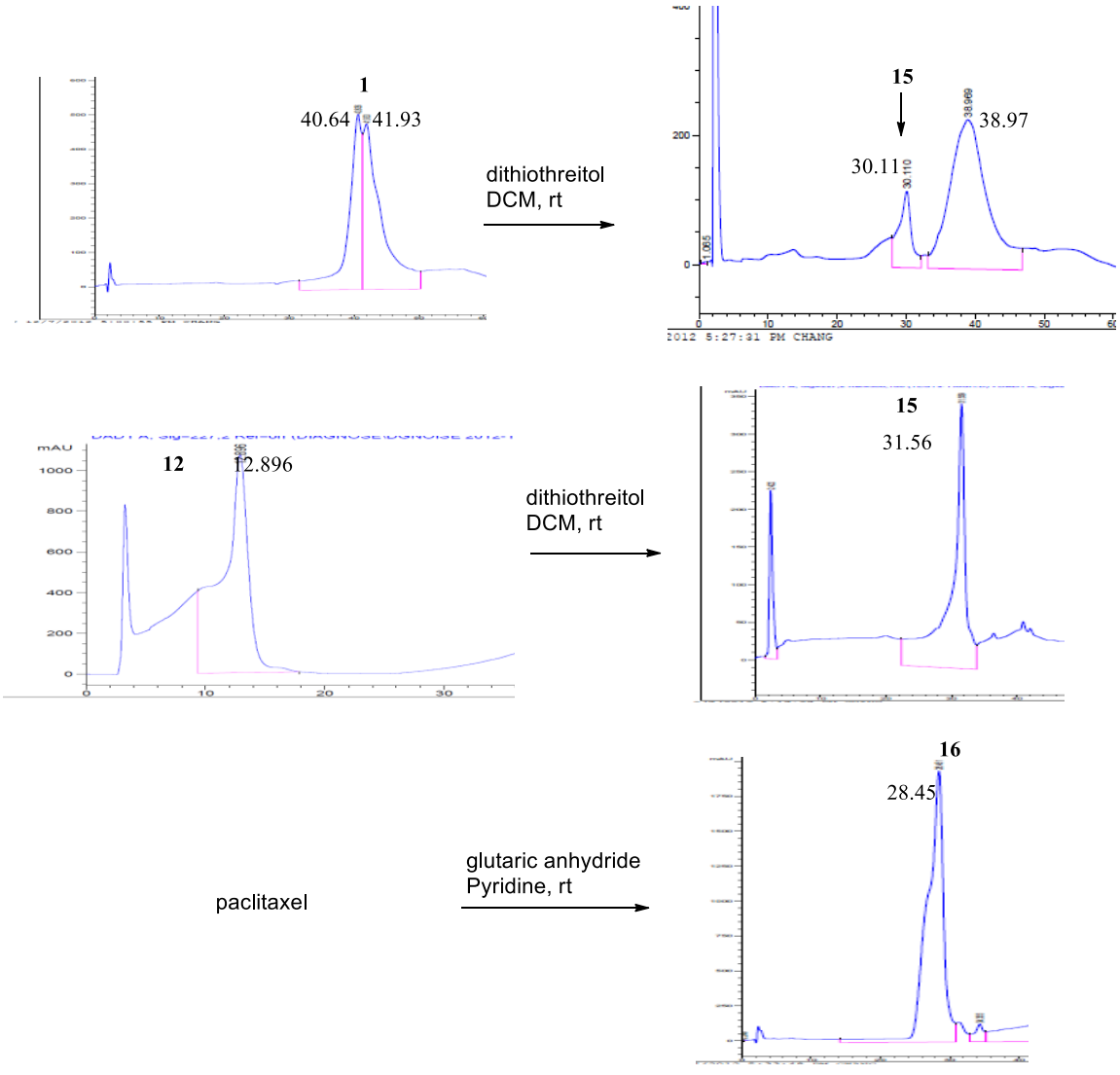
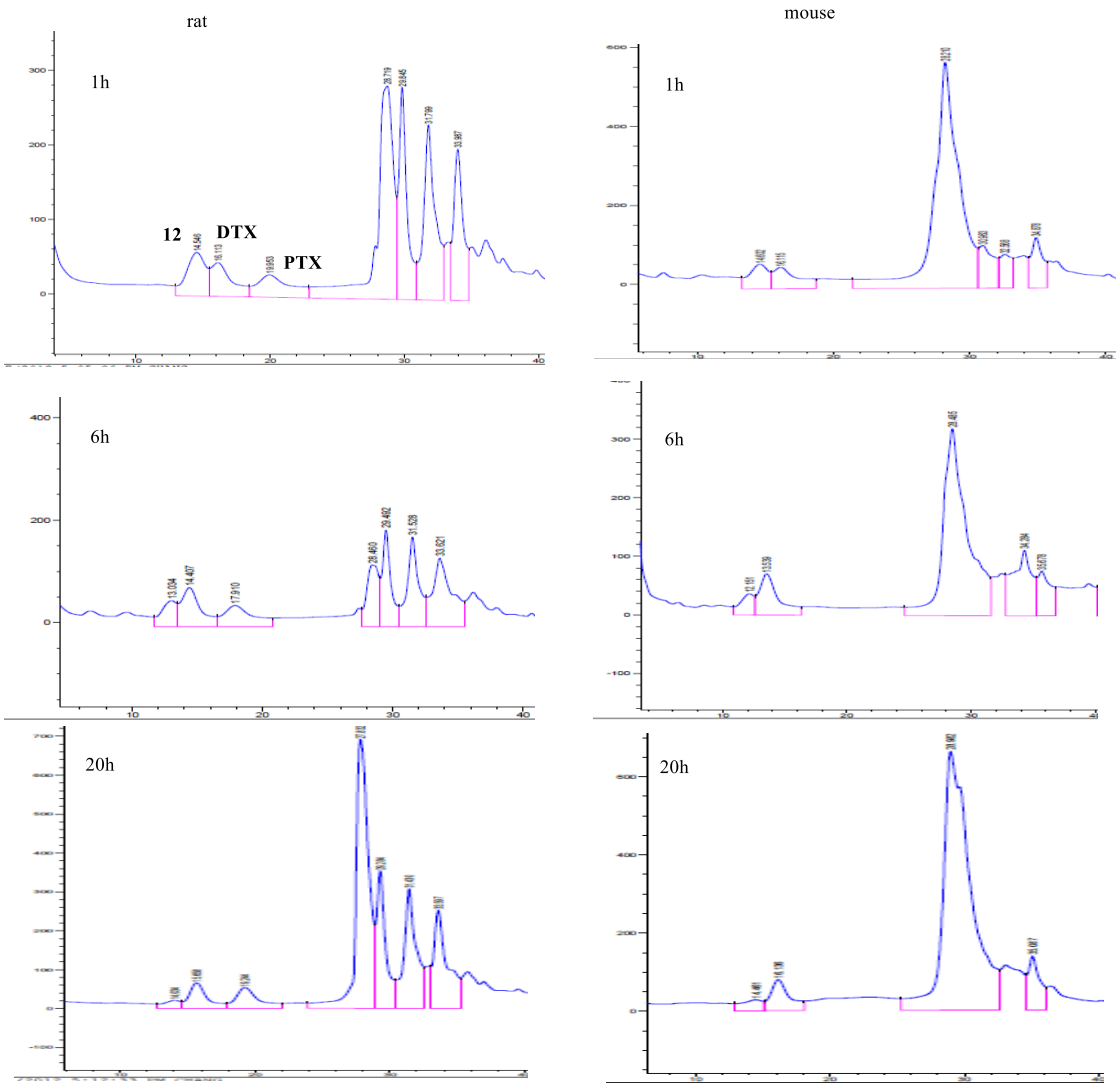


Figure S44. Plasma release with Model 12. Compound **12** appears rapidly degraded in plasma (in comparison to **1**) with appearance of paclitaxel in rat plasma (left). Disappearance of **12** in mouse plasma occurs at a similar rate to rat plasma, but paclitaxel is not observed. Plasma peaks do not allow us to quantitate the appearance (if any) of thiol **15** and the acid intermediate **16**.



Supporting Table 1. Cumulative Excretion Data of 1 and 3 from Urine and Feces.

Prodrug	%ID/g in urine		%ID/organ in feces	
	24h	48h	24h	48h
1	4.25	5.47	0.27	0.36
3	42.7	49.7	2.7	3.3

Supporting Table 2. The elimination and distribution half-lives of 1 and 3.

		paclitaxel		PEG		Half-lives (h)	
Prodrug	MW	number	linker	size	linker	$t_{1/2\alpha}$	$t_{1/2\beta}$
1	61	16	ester/disulfide	5KDa	ester	1.1	38
3	37.8	12	ester/disulfide	2Kda	ester	0.4	19.3±2.1

Figure S45. Biodistribution at 48 for 1 in SCID-PC-3 mice. Data are presented as %ID/g ± s.d. (n = 4).