Resin-Bound Dynamic Combinatorial Chemistry

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Supplementary Data:

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Materials:

All materials (with the exception of 2-ethyl quinoline-3-carboxylic acid hydrochloride) used for solid phase synthesis of compounds **3-11** were purchased from Advanced Chemtech. All materials used in the synthesis of 2-ethyl quinoline-3-carboxylic acid hydrochloride, as well as 3-mercapto-1-propanol were obtained from Sigma Aldrich corporation.

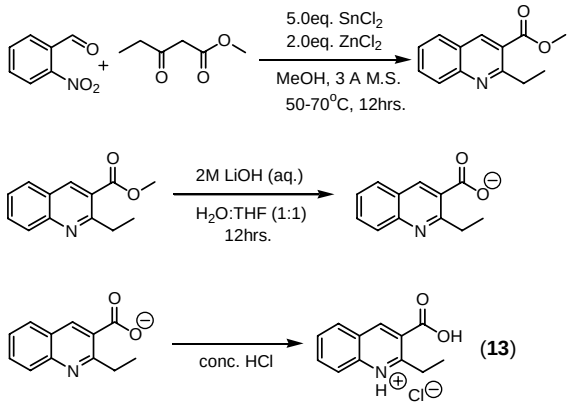
DNA was purchased from Integrated DNA Technologies (≥ 95% purity by HPLC)

Graphical and statistical analysis of data was carried out using Origin 7 (OriginLab Corporation).

NMR spectral data were processed and analyzed using MestReC v. 4.4.1.0 (Mestrelab Research).

I. Synthesis and HPLC analysis of library members:

Figure 1. Synthesis of 2-Ethyl-Quinoline-3-carboxylic acid Hydrochloride, **13**, and Solid phase synthesis of monomer compounds



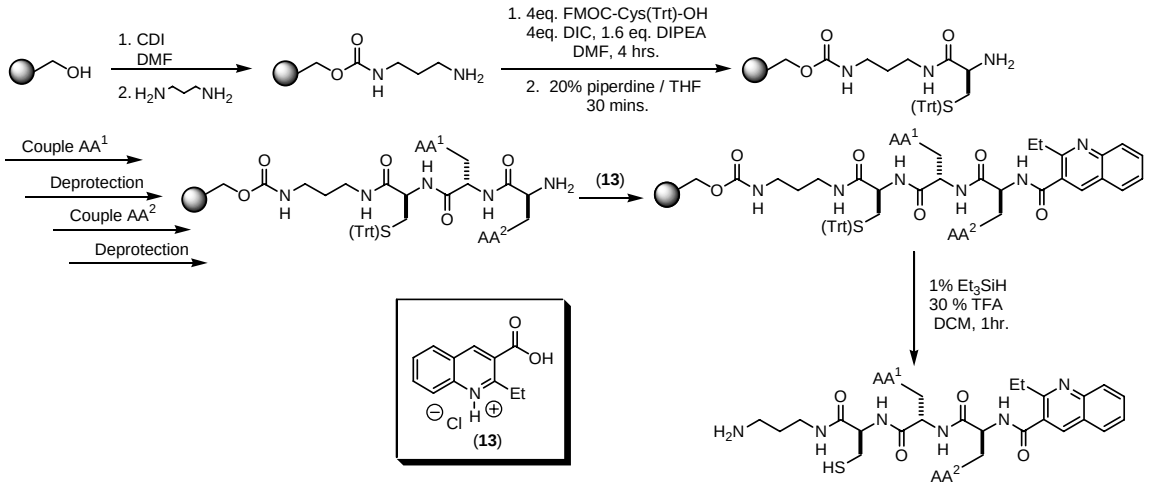
A flame dried 1000-ml, three necked, round bottom flask was equipped with a Teflon-coated magnetic stirbar, internal thermometer, glass stopper, and a reflux condenser fitted with nitrogen inlet. The flask was charged with 10 grams of 3Å molecular sieves, 2-nitrobenzaldehyde (10.0 g, 66.2 mmol), methyl propionylacetate (8.27 ml, 66.2 mmol), and zinc (II) chloride, (18.0 g, 132.4 mmol). To the flask was added 175 ml of methanol. The flask was then flushed with a stream of nitrogen, and heated with a heating mantle to an internal temperature of 50 ˚C for thirty minutes. Tin (II) chloride (62.7 g, 331 mmol) was then added to the flask, which was again flushed with a stream of nitrogen, heated to 70˚C, and stirred for an additional 11.5 hours. To a 500ml beaker was added potassium carbonate (45.7 g, 331 mmol), which was dissolved completely in 400 ml water. After coming to room temperature, the reaction solution was made alkaline (pH approximately 8 by pH paper) through the slow addition of the potassium carbonate solution, resulting in a light orange slurry. This slurry was subsequently filtered through a pad of lightly packed Celite (12 cm x 3 cm). An additional 75 ml of water was added to rinse the reaction vessel, and subsequently passed through the Celite. After all aqueous solution has filtered, the Celite was washed with ethyl ether (4 x 100). The organic layer was separated in a seperatory funnel, dried by the addition of 15 grams magnesium sulfate, filtered, and concentrated under rotary evaporation in a 500 ml round bottom flask. Complete removal of solvent resulted in an amber colored viscous oil, which was applied directly to a silica gel column, and eluted with 15% ethyl acetate/hexanes to give 10.9-11.2 g (82%-85%) of the methyl ester as a dark amber liquid.

The 500 ml round bottom flask containing the methyl ester was equipped with a Teflon-coated magnetic stirbar. The methyl ester was dissolved in 75 ml of THF. To the solution was added 2 M LiOH (aq.). After addition of LiOH solution, the round bottom was capped with a rubber septum, vented with a needle, and stirred at room temperature for 12 hours.

The THF was removed via rotary evaporation. The solution was made acidic, (pH of 1 as determined by pH paper) through the addition of 50 ml of 37% hydrochloric

acid, and stirred for 5 minutes. The solution was then chilled in a -20˚C freezer for 15 minutes, producing a precipitate. The solution was agitated by gentle manual shaking. Agitation caused a significant amount of additional precipitate to form, yielding a light yellow slurry. A ceramic filter (60 mm) was fitted with filter paper, and the slurry was filtered via vacuum filtration. Product remaining in the flask was taken up in 20 ml of 10% HCl (aq.) and filtered. Maintaining vacuum filtration, the precipitate was washed with chilled (0˚C) HPLC grade hexanes (2 x 20 ml). The remaining solid was transferred to a 100 ml round bottom flask and placed on a vacuum line (0.2 torr, 22 ˚C) for 24 hours to remove trace water. The result was 12.6-13.0 g (80%-83%) of **13** as a light yellow solid.

Solid-phase synthesis of library monomers 3-11:



Synthesis of solution phase monomers:

To a solid phase reaction vessel was added 100 mg of Wang Resin (100-200 mesh size, 1 mmol/g loading). The resin was washed one time each with dichloromethane, tetrahydrofuran, and dimethylformamide. To the vessel were added 1-1'-carbonyl-di-imidizole (162 mg, 1 mmol, 10 eq) in 5 mL of dimethylformamide. The suspension was agitated for 12 hours by roating on a LabQuake rotator. The vessel was then evacuated under vacuum and washed three times with 5 mL dichloromethane. Propane diamine (86 µL, 1 mmol, 10 eq) was added in 5 mL dimethylformamide and rotated for an additional 12 hours. Again, the vessel was evacuated under vacuum and washed three times each with 5 mL portions of dichloromethane, tetrahydrofuran, and dimethylformamide. After washing was completed, FMOC-Cys(Trt)-OH (234 mg, 0.4 mmol, 4 eq), HOBt hydrate (54 mg, 0.4 mmol, 4 eq), and di-isopropylcarbodiimide (62 µL, 0.4 mmol, 4 eq) were added to the resin which was suspended in approximately 5 ml dimethylformamide. The solution was rotated for 45 min., after which time Hünigs base (28 µL, 0.16 mmol, 1.6 eq) was added, and the solution was rotated for an additional 4 hours. Each coupling step was repeated twice to assure high yields. After coupling, FMOC was deprotected through the addition of 5ml of a 20% piperidine 80% tetrahydrofuran solution. The resin was rotated in the solution for 30 minutes and subsequently washed three times each with 5mL dichloromethane, tetrahydrofuran, and dimethylformamide. The coupling process

was repeated using the desired amino acid until the peptidic sequence was complete. Upon FMOC deprotection of the last amino acid, a solution containing 5 ml dimethylformamide, 3-carboxy-2-ethyl-3-quinolinium chloride (**13**; 94 mg, 0.4 mmol, 4 eq), Hünig's base (975 μL, 0.56 mmol, 5.6 eq), HOBt hydrate (54 mg, 0.4 mmol, 4 eq), and di-isopropylcarbodiimide (62 μL, 0.4 mmol, 4 eq) was added to the reaction vessel and rotated for 12 hours (Figure 1). The resin was washed thoroughly and cleaved in 5 mL of a 1% triethylsilane / 30% trifluoroacetic acid solution in dichloromethane for one hour. Compounds were purified by precipitation in chilled ether (-20ºC) followed by four washes with chilled ether (-20ºC).

Synthesis of resin bound monomers:

Synthesis of monomers attached to resin was conducted on Tentagel S resin (0.2 mmol / g, 80 – 100 um). The synthetic procedure is the exact same as described above. Conditions for RBDCC experiments with resin supported monomers are described below (p. S52)

HPLC and mass spectral analysis of library members:

All HPLC traces were acquired on a Shimadzu LC-2010A Liquid Chromatograph using a Shim-pack CLC-ODS-(M) C18 column.

Gradient: A 15 minute isocratic flow of 30 % acetonitrile (0.1 % triflouroacetic acid) in distilled water, followed by a linear concentration to 100 % acetonitrile (0.1 % triflouroacetic acid) at 30 minutes.

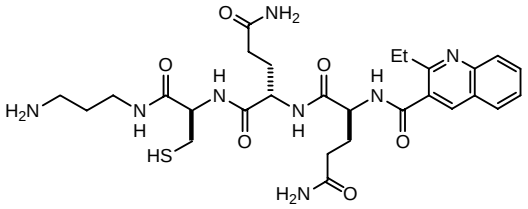
Flow: All HPLC analyses were performed using a flow rate of 0.5 ml/min.

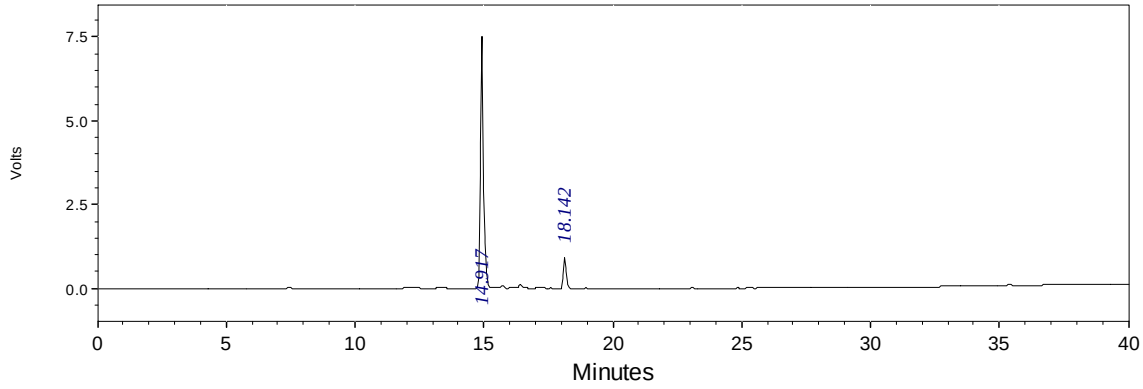
Detector: All HPLC analyses were performed with a UV detector setting of 235 nm

Mass spectra were acquired on an HP-1100 MSD LCMS system, using electrospray ionization in positive ion mode

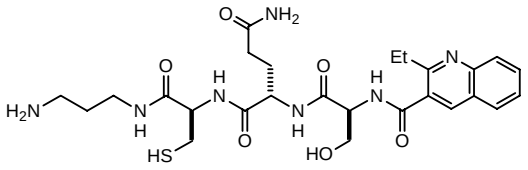
Monomers 3 – 11 (235 λ):

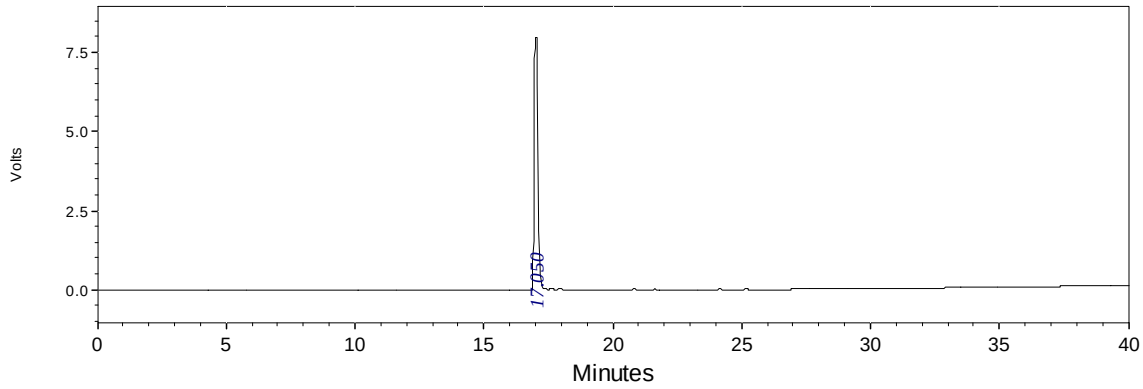
Compound 3:



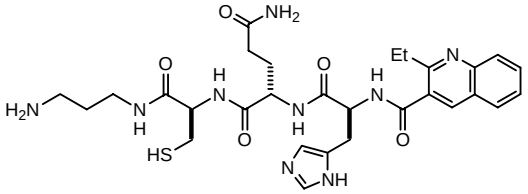


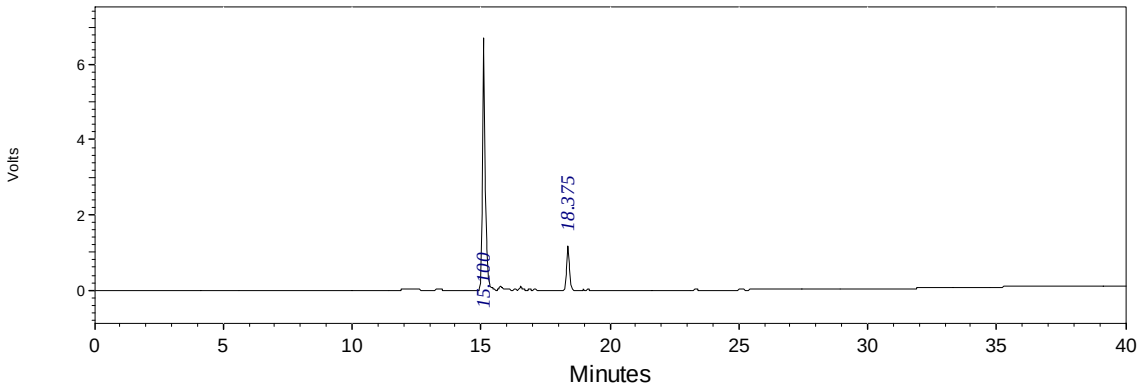
Compound 4:



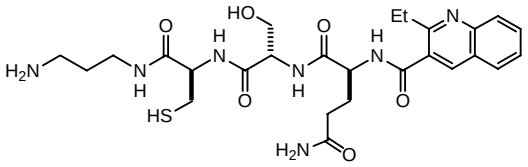


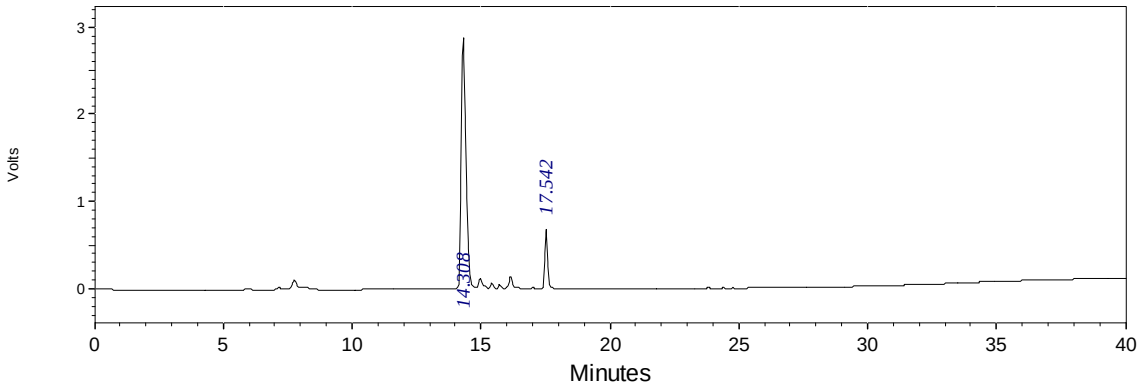
Compound 5:



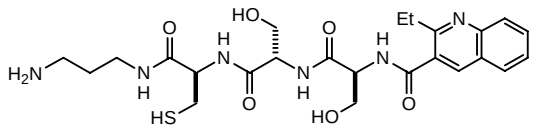


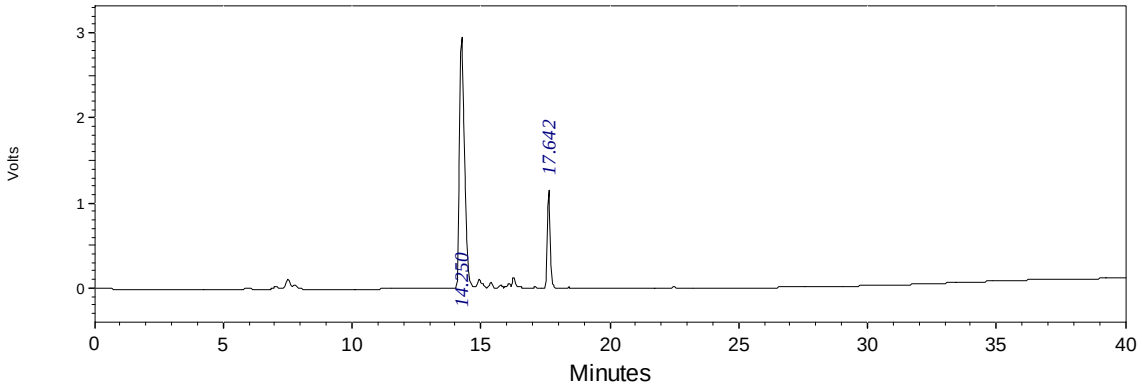
Compound 6:





Compound 7:





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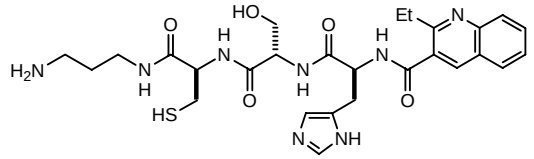
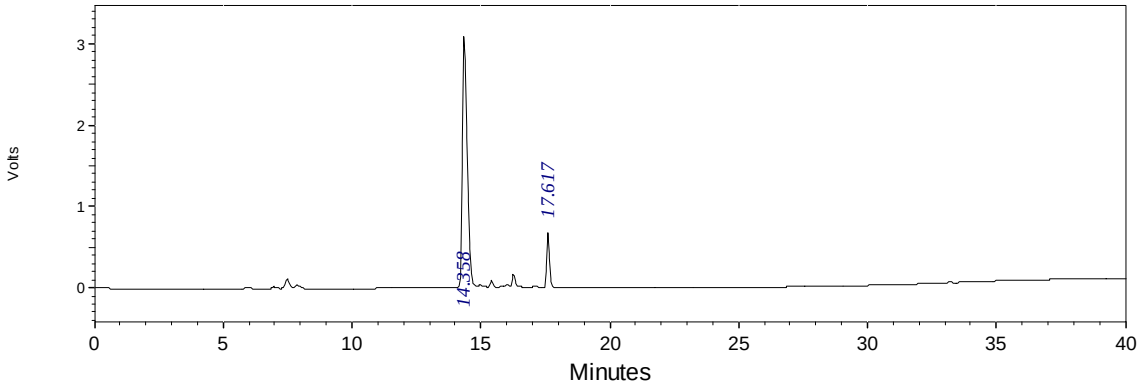
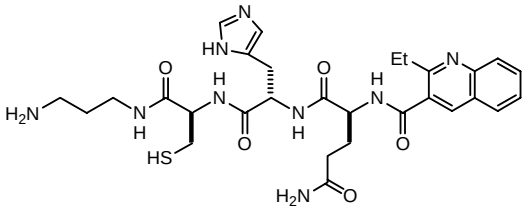
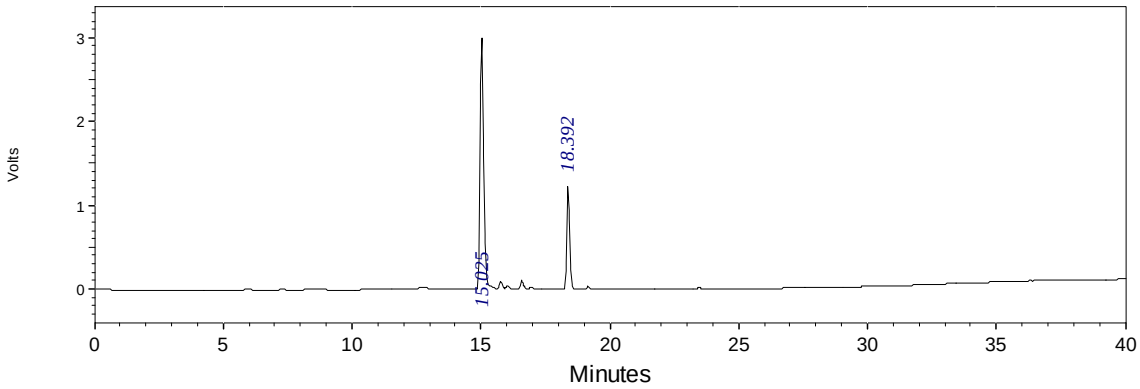




Compound 9:





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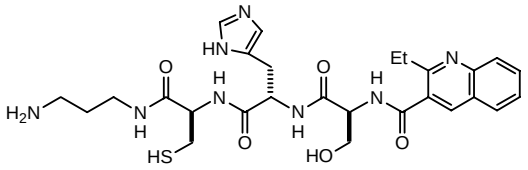

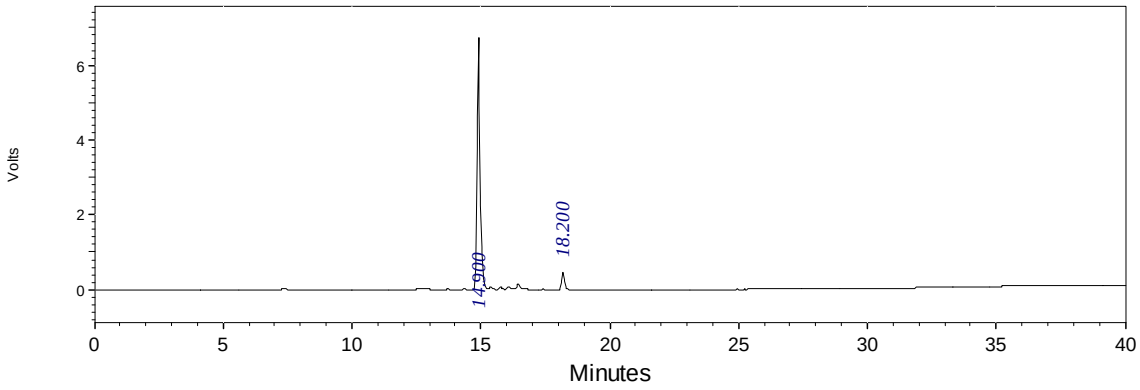

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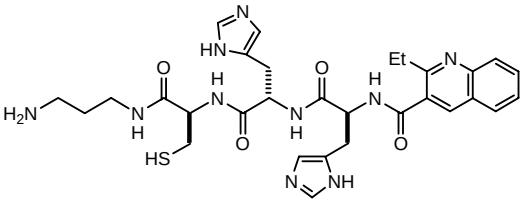
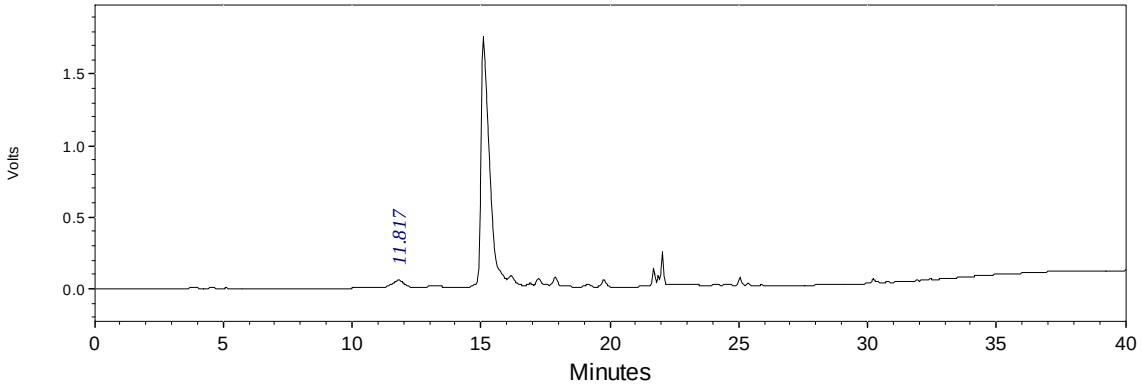
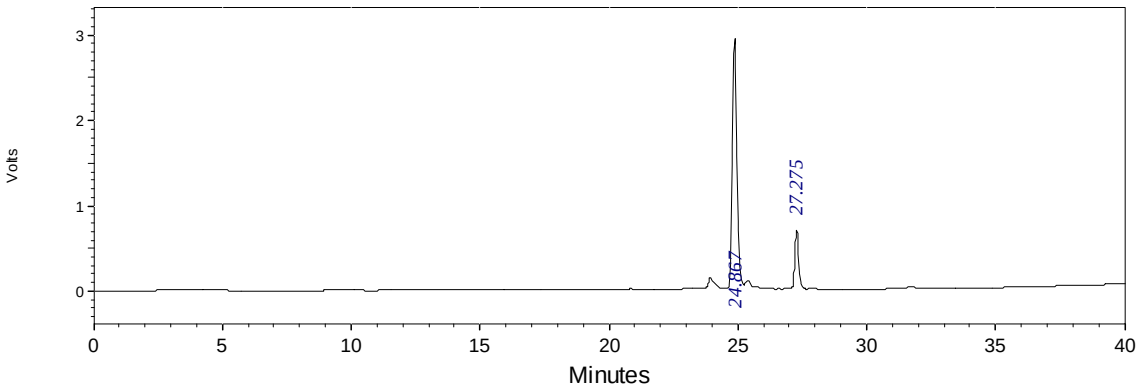




Homo-dimers 3-3 – 11-11 (235 λ):

3-3



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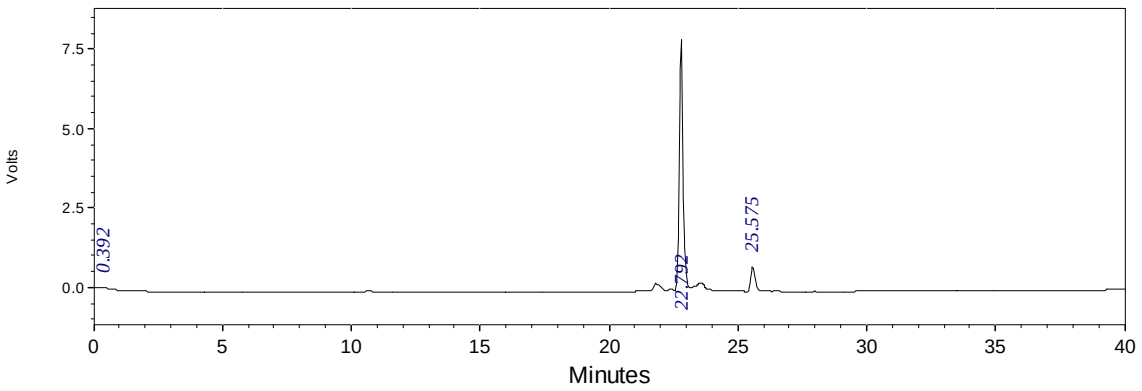
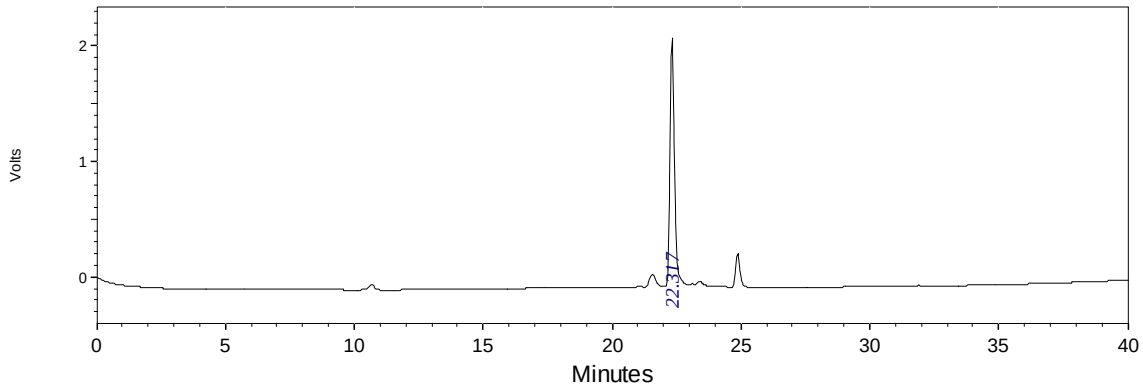


5-5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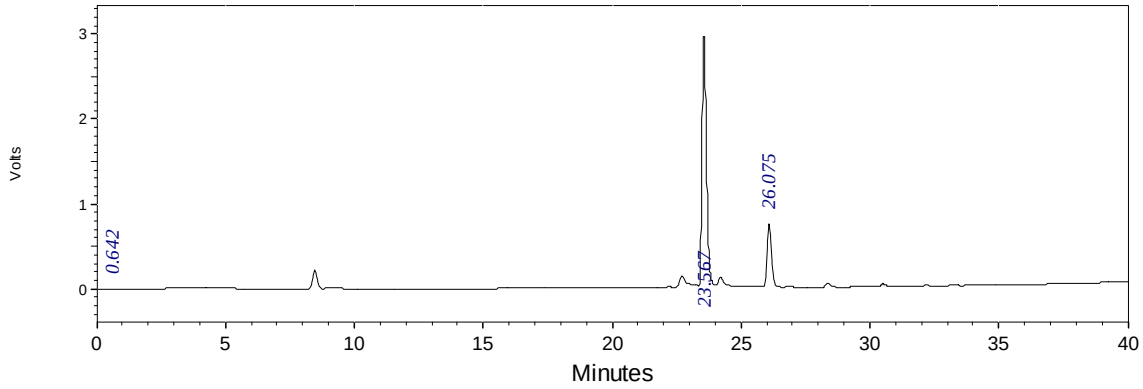
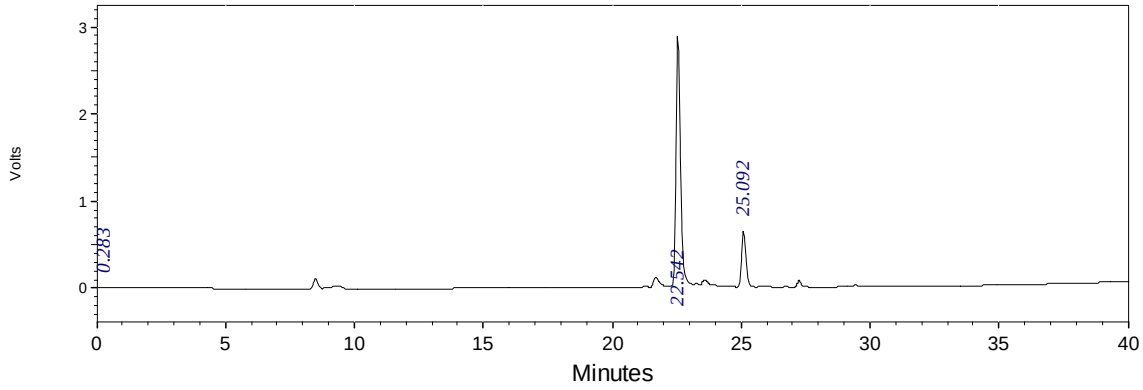
1
0
22.317
0
5
10
15
20
25
30
35
40
Minutes

6-6


Volts
3
2
1
0
0.642
23.567
26.075
0
5
10
15
20
25
30
35
40
Minutes

7-7


Volts
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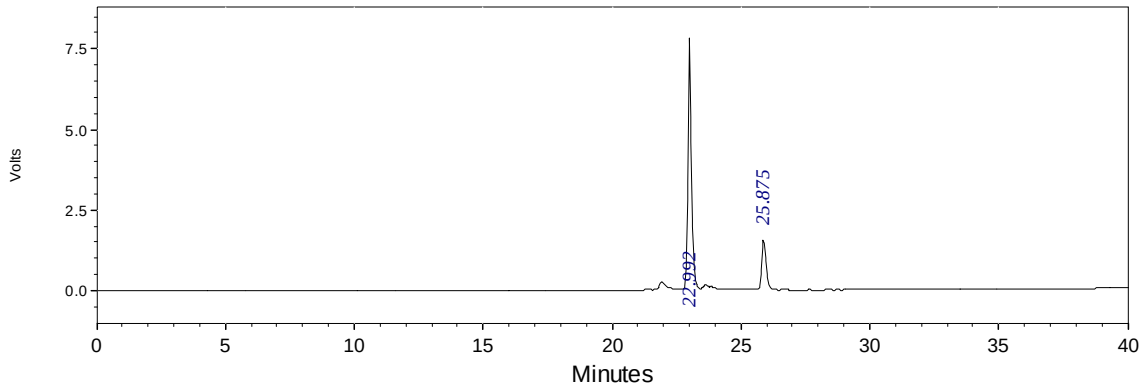
25.092
0
5
10
15
20
25
30
35
40
Minutes

8-8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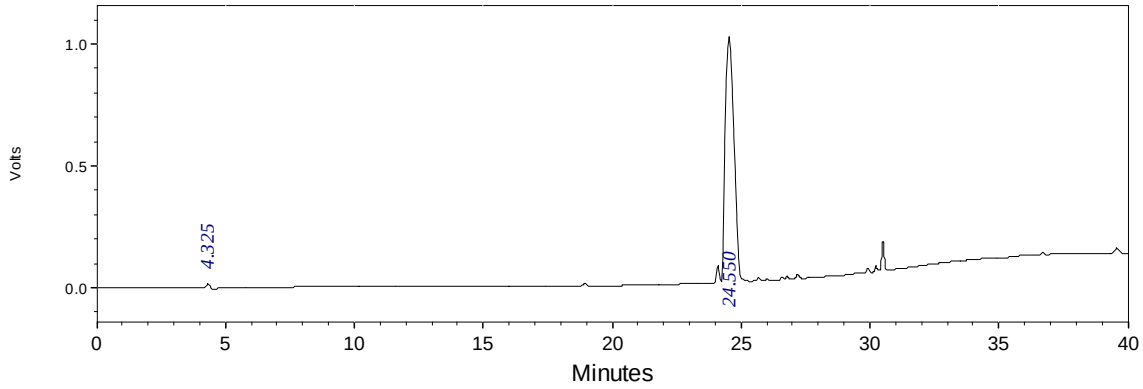
0
5
10
15
20
25
30
35
40
Minutes

9-9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5
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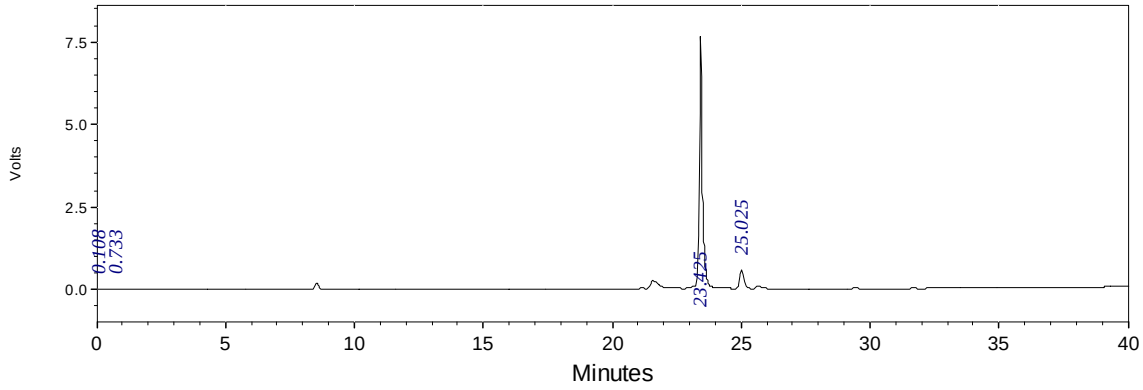
15
20
25
30
35
40
Minutes

10-10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2.5
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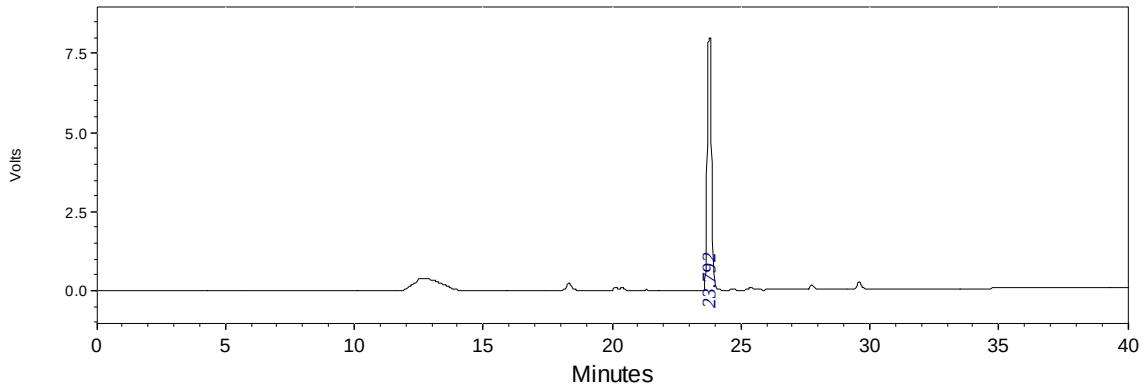
25.025
0
5
10
15
20
25
30
35
40
Minutes

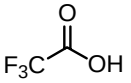
11-11



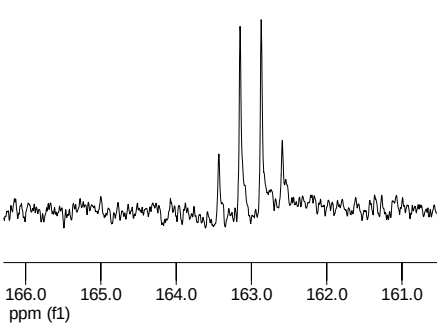
II. Spectral Analysis:

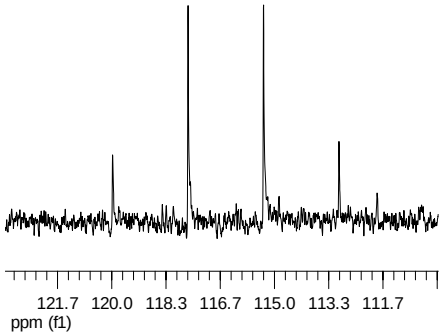
NOTE: Monomeric compounds **3**, **4**, …**11** partially dimerize immediately following resin cleavage to form disulfides. Therefore, spectral data were obtained on the dimers rather than on monomers (with the exception of HRMS data, which is reported for the monomers). Homodimers were prepared via oxidation analogous to that described in the DCC library experimental (*vida infra*). In brief, library members were allowed to undergo autoxidation in 10 mM phosphate buffer at a pH of 7.4 for a period of 7 days (a period sufficient to produce quantitative dimerization, as evidenced by HPLC). Solutions were then concentrated on a speed vacuum line and analyzed in D_2O.

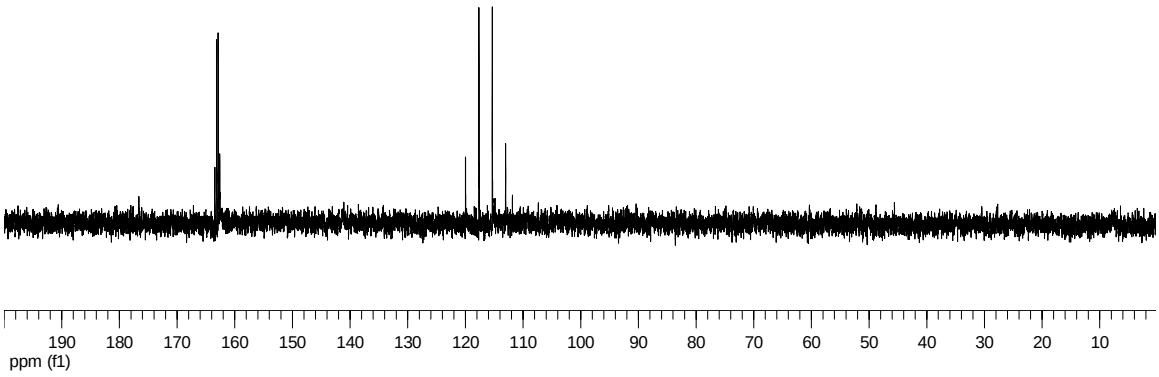
Due to cleavage conditions from the solid support, compounds exhibit peaks in the ^{13}C spectra that are indicative of trifluoroacetic acid (below); 163 ppm (q, J= 155 Hz), 116 ppm (q, J= 1125 Hz). These peaks are not listed in the spectral analysis below.

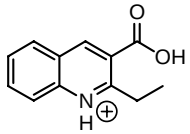


$C_2HF_3O_2$
Exact Mass: 113.99







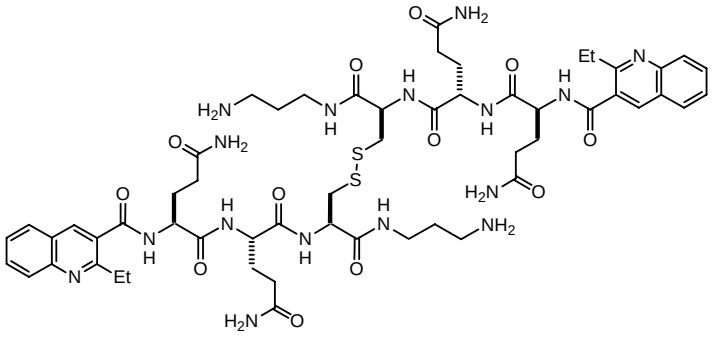


3-Carboxy-2-ethyl-quinolinium

$C_{12}H_{12}NO_2^+$

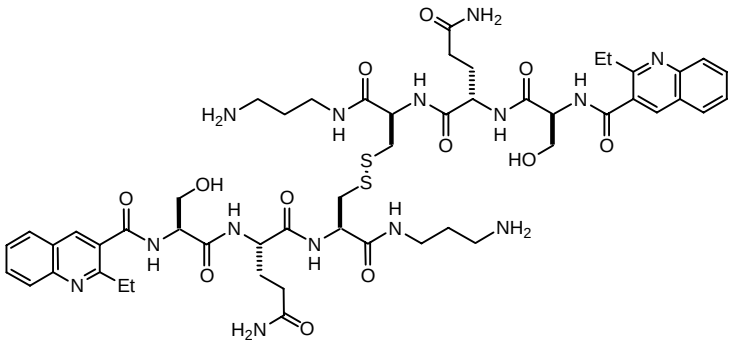
Exact Mass: 202.0868

IR (KBr pellet): 3323, 2709, 2360, 1696, 1645, 1287, 796, 772 cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 8.97 (s, 1H), 8.06 (d, 1H, J= 8 Hz), 7.95 (s, 2H), 7.75 (t, 1H, J= 6 Hz), 3.34 (q, 2H, J= 7.5 Hz), 1.34 (t, 3H, J= 7.5 Hz); ^{13}C NMR δ 176.5, 162.3, 161.1*, 146.7, 135.2, 133.1, 130.3, 127.9, 126.4, 126.1, 125.9, 29.3, 13.9 (*This peak is indicative of carbamic acid, which is used as an additive to aid in solvation) ; HRMS *m/z* calcd for (M^+ +H); 202.0868, found: 202.0821.



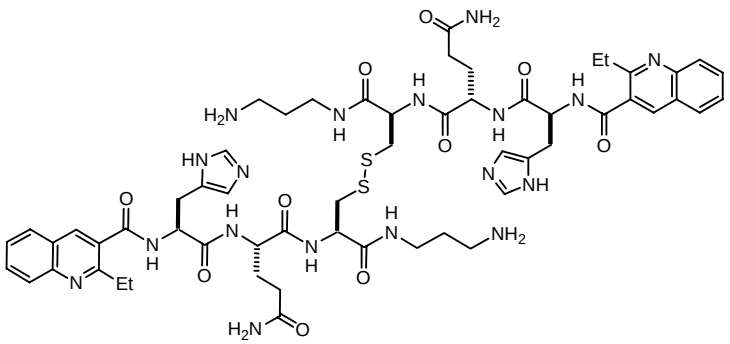
3-3

IR (thin film from MeOH): cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 9.09 (s, 1H), 8.23 (d, 2H, J= 8 Hz), 8.12, 7.89 (s, 2H), 7.89 (m, 1H), 4.55 (t, 1H, J= 7 Hz), 4.38 (m, 2H), 3.26 (m, 4H), 2.96 (t, 2H, J= 7 Hz), 2.85 (t, 2H, J= 6 Hz), 2.43 (t, 2H, J= 7 Hz), 2.38 (t, 2H, J= 8 Hz), 2.10 (m, 2H), 2.00 (m, 2H), 1.83 (t, 2H, J= 7 Hz), 1.35 (t, 3H, J= 8 Hz); ^{13}C NMR δ 177.7, 177.5, 173.0, 171.8, 166.8, 160.4, 145.5, 138.1, 136.4, 130.3, 129.5, 128.6, 119.6, 65.9, 56.0, 54.1, 53.2, 37.0, 36.2, 31.1, 30.9, 26.7, 26.7, 25.1, 13.5; dimer: LRMS *m/z* calcd for (M^+ +H); 1231.5, found:.1231.3; monomer: HRMS *m/z* calcd for (M^+ +H); 617.2792, found:.617.2856



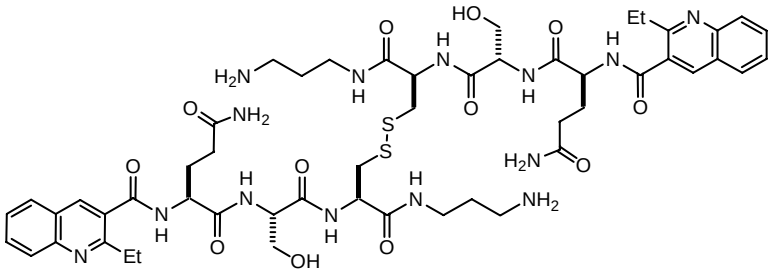
4-4

IR (thin film from MeOH): 3415, 2356, 2066, 1652, 1539, 1203, 1140cm^{-1}; ^1H NMR (500 MHz, D_2O) δ 9.16 (s, 1H), 8.24 (d, 1H, J = 8 Hz), 8.15 (s, 1H), 7.92 (m, 1H), 4.68 (m, 1H), 4.43 (t, 1H, J= 5.5 Hz), 4.38 (t, 1H, J= 6.7 Hz), 3.97 (d, 2H, J= 6 Hz), 3.29 (m, 4H), 2.97 (t, 2H, J= 7.3 Hz), 2.85 (t, 2H, J= 6 Hz), 2.41 (t, 2H, J= 7.3 Hz), 2.13 (m, 1H), 1.99 (m, 1H), 1.83 (t, 2H, J= 8 Hz), 1.39 (t, 3H, J= 8 Hz); ^{13}C NMR δ 177.8, 173.1, 171.9, 171.5, 167.0, 145.7, 138.0, 136.4, 130.2, 129.5, 128.7, 126.6, 119.6, 60.9, 56.4, 56.0, 53.2, 37.0, 36.2, 31.0, 26.6, 26.1, 25.0, 13.5; dimer: LRMS *m/z* calcd for (M^+ +H); 1149.4, found: 1149.2, monomer: HRMS *m/z* calcd for (M^+ +H); 576.2526, found:.576.2578.



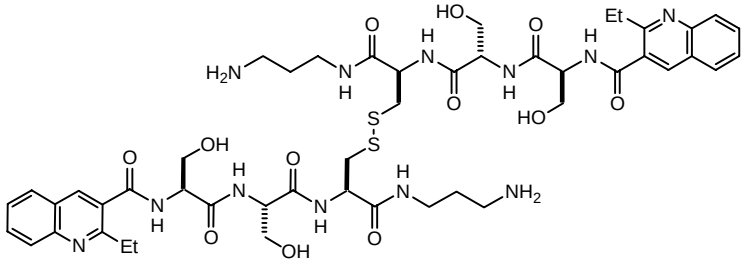
5-5

IR (thin film from MeOH): 3042, 1673, 1537, 1432, 1202, 1134, 837, 700, 721 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 8.99 (s, 1H), 8.63 (s, 1H), 8.18 (d, 1H, J= 8 Hz), 8.11 (s, 2H), 7.89 (m, 1H), 7.36 (s, 1H), 4.94 (t, 1H, J= 9.8 Hz), 4.41 (m, 2H), 3.30 (m, 4H), 3.16 (m, 2H), 2.96 (t, 3H, J= 8 Hz), 2.81 (t, 2H, J= 8.5 Hz), 2.38 (t, 2H, J= 8 Hz), 2.09 (m, 1H), 1.99 (m, 1H), 1.84 (t, 2H, J= 7.3 Hz), 1.28 (t, 3H, J= 7.9 Hz) ;^{13}C NMR δ 177.7, 172.9, 171.9, 171.1, 166.6, 160.2, 145.5, 138.0, 136.5, 133.8, 130.3, 129.5, 128.4, 128.2, 126.6, 119.6, 117.5, 56.1, 53.2, 53.1, 37.0, 36.2, 30.9, 26.8, 26.6, 26.3, 26.0, 25.1, 13.4; dimer: LRMS *m*/*z* calcd for (M^+ +H); 1249.5, found:1249.3, monomer: HRMS *m*/*z* calcd for (M^+ +H); 626.2795, found: 626.2879.



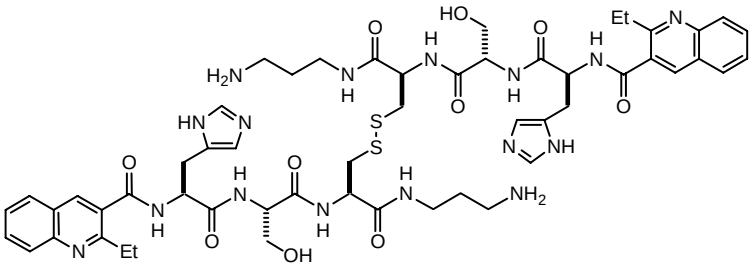
6-6

IR (thin film from MeOH): 3287, 1666, 1538, 1201, 1134 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 9.10 (s, 1H), 8.22 (d, 1H, J = 8.8 Hz), 8.13 (m, 2H), 7.91 (m, 1H), 4.64 (t, 1H, J = 7.0 Hz), 4.52 (t, 1H, J = 6.7 Hz), 4.45 (t, 1H, J = 7.0 Hz); 3.88 (m, 2H), 3.28 (m, 4H), 2.97 (t, 3H, J = 7.9 Hz), 2.88 (m, 2H), 2.47 (t, 2H, J = 7.0 Hz), 2.19 (m, 1H), 2.12 (m, 1H), 1.84 (m, 2H), 1.37 (t, 3H, J = 8.5 Hz) ^{13}C NMR δ 177.6, 172.9, 171.7, 166.8, 160.8, 160.4, 145.5, 138.0, 136.3, 130.2, 129.5, 128.6, 126.6, 119.6, 60.9, 55.9, 55.6, 54.0, 37.0, 36.2, 31.0, 26.9, 26.6, 26.1, 25.1, 13.5; LRMS *m*/*z* calcd for (M^+ +H); 1149.2, found 1149.1, monomer HRMS *m*/*z* calcd for (M^+ +H); 576.2526, found: 576.2600.



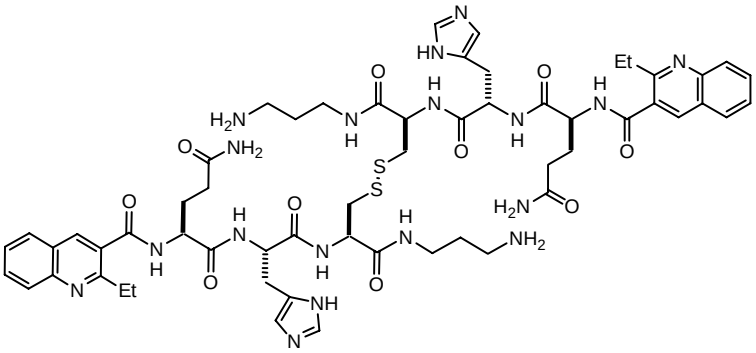
7-7

IR (thin film from MeOH): 3336, 1647, 1540, 1202, 1137, 840, 801, 761, 722 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 9.175 (s, 1H), 8.27 (d, 1H, J= 8 Hz), 8.16 (m, 2H), 7.94, (t, 1H, J= 8 Hz), 4.75 (m, 1H), 4.54 (t, 1H, J= 7.3 Hz), 4.42 (t, 1H, J = 5.78 Hz), 3.95 (m, 5H), 3.27 (m, 4H), 2.95 (t, 2H, J= 8 Hz), 2.87 (m, 2H), 1.87 (t, 2H, J= 6 Hz), 1.40 (t, 3H, J= 7.9 Hz); ^{13}C NMR δ 171.8, 171.7, 171.5, 166.9, 160.4, 145.7, 138.0, 136.3, 130.2, 129.5, 128.6, 126.6, 119.6, 61.0, 60.9, 56.2, 56.0, 55.6, 36.9, 36.2, 26.6, 26.1, 25.0, 13.5; LRMS *m*/*z* calcd for (M^+ +H); 1067.4, found: 1067.0, monomer HRMS *m*/*z* calcd for (M^+ +H); 535.2261, found: 535.2323.



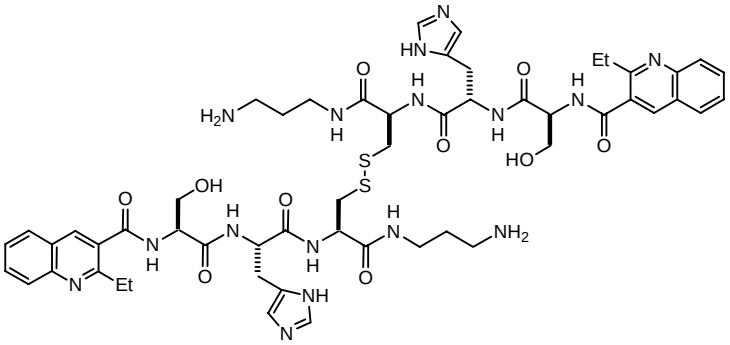
8-8

IR (thin film from MeOH): 3047, 2360, 1669, 1540, 1201, 1134, 668 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 9.00 (s, 1H), 8.64 (s, 1H), 8.22 (d, 1H, J= 8 Hz), 8.10 (m, 2H), 7.90 (t, 1H, J= 8 Hz), 7.37 (s, 1H), 5.01 (t, 1H, J= 7.9 Hz), 4.54 (t, 1H, J= 7.3Hz), 4.48 (t, 1H, J= 5.5 Hz), 3.90 (m, 2H), 3.28 (m, 4H), 3.16 (m, 2H), 2.96 (t, 2H, J= 8.5Hz), 2.85 (t, 2H, J= 5Hz)1.81 (t, 2H, J=7.9 Hz), 1.29 (t, 3H, J= 8.5 Hz); ^{13}C NMR δ 171.8, 171.6, 171.2, 166.6, 160.2, 145.6, 138.1, 136.5, 133.8, 130.0, 129.5, 128.4, 126.6, 119.6, 117.3, 61.0, 55.9, 55.4, 53.1, 37.0, 36.2, 26.6, 26.5, 26.0, 25.1, 13.3; LRMS *m*/*z* calcd for (M^+ +H); 1167.4, found: 1167.3; monomer HRMS *m*/*z* calcd for (M^+ +H); 585.2529, found: 585.2631.



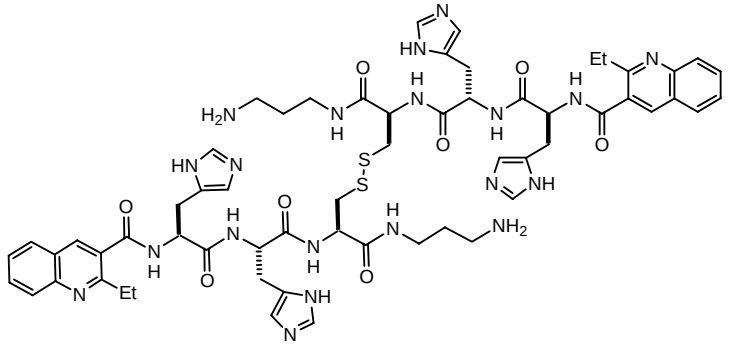
9-9

IR (thin film from MeOH): 3260, 1669, 1540, 1429, 1201, 1132, 837, 799, 721 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 9.10 (s, 1H), 8.59 (s, 1H), 8.27 (d, 1H, J= 8 Hz), 8.16 (m, 2H), 7.92 (t, 1H, J= 8 Hz), 7.32 (s, 1H), 4.77 (m, 1H), 4.56 (t, 1H, J= 7.1 Hz), 4.42 (t, 1H, J= 6 Hz), 3.27 (m, 5H), 2.98 (t, 2H, J= 7.8 Hz), 2.84 (t, 2H, J= 7.3 Hz), 2.44 (q, 2H, J= 7.3 Hz), 2.13 (m, 2H), 1.84 (m 2H), 1.34 (t, 3H, J= 6.7 Hz); ^{13}C NMR δ 177.4, 172.8, 171.6, 171.4, 166.8, 160.3, 145.5, 138.0, 136.4, 133.5, 130.3, 129.5, 128.6, 128.4, 126.6, 119.6, 117.4, 55.9, 54.0, 52.5, 37.0, 36.3, 31.0, 26.8, 26.6, 26.1, 26.1, 25.3, 13.5; LRMS *m*/*z* calcd for (M^+ +H); 1249.5, found: 1249.3; monomer HRMS *m*/*z* calcd for (M^+ +H); 626.2795, found: 626.2864.



10-10

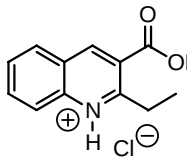
IR (thin film from MeOH): 3291, 1667, 1537, 1202, 1134, 722 cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 9.10 (s, 1H), 8.58 (s, 1H), 8.22 (d, 1H, J= 8 Hz), 8.11 (m, 2H), 7.92 (t, 1H, J= 8 Hz), 7.31 (s, 1H), 4.80 (m, 1H), 4.62 (t, 1H, J= 6.1 Hz), 4.40 (t, 1H, J= 6.1 Hz), 3.92 (t, 2H, J= 6.2 Hz), 3.26 (m, 5H), 2.95 (t, 3H, J= 7.3 Hz), 2.83 (t, 2H, J= 7.3 Hz), 1.85 (t, 2H, J= 8.5 Hz), 1.35 (t, 3H, J= 9.7 Hz); ^{13}C NMR δ 171.7, 171.4, 171.4, 166.9, 160.3, 146.6, 138.0, 136.4, 133.5, 130.3, 129.5, 128.6, 128.5, 126.6, 117.3, 61.0, 56.3, 56.0, 52.6, 37.0, 36.3, 26.6, 26.1, 26.0, 25.0, 13.4; LRMS *m*/*z* calcd for (M^+ +H); 1167.4, found: 1167.1; monomer HRMS *m*/*z* calcd for (M^+ +H);585.2529, found: 585.2573.



11-11

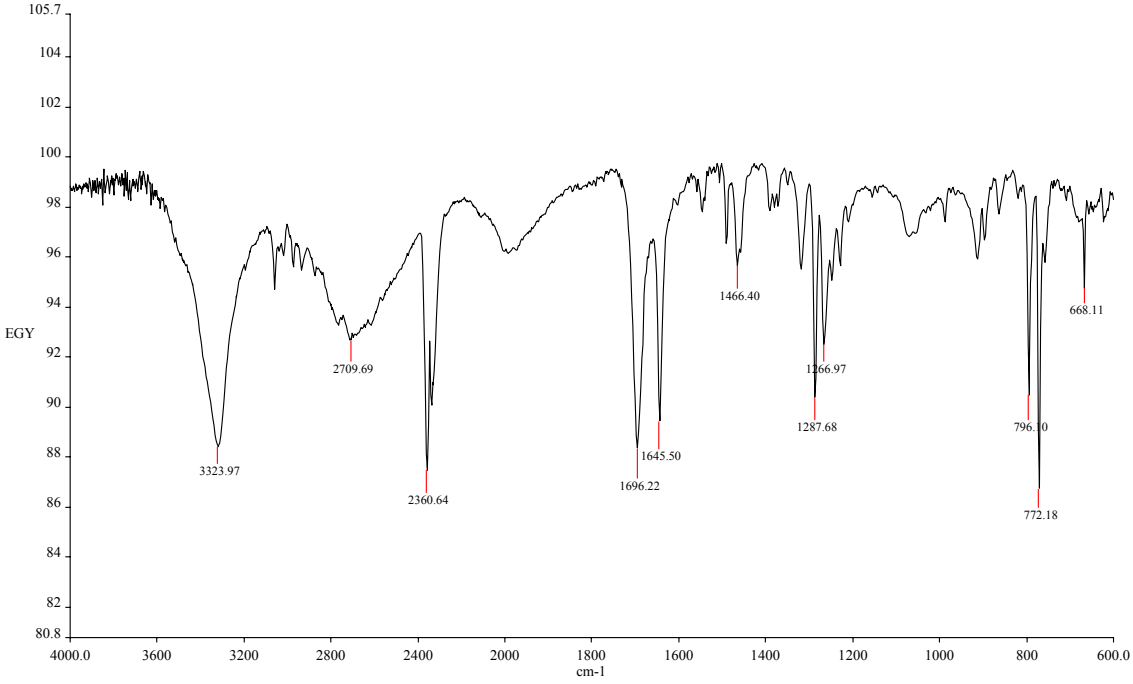
IR (thin film from MeOH): 3275, 1670, 1544, 1432, 1201, 1134, 836, 799, 721cm^{-1}; ^{1}H NMR (500 MHz, D_2O) δ 8.97 (s, 1H), 8.65 (s, 1H), 8.59 (s, 1H), 8.23 (d, 1H, J= 8 Hz), 8.13 (m, 2H), 7.91 (m, 1H), 7.37 (s, 1H), 7.29 (s, 1H), 4.92 (q, 1H, J= 6.7 Hz), 4.77 (m, 1H), 4.39 (t, 1H, J= 7 Hz), 3.21 (m, 8H), 2.97 (t, 2H, J= 7.9 Hz), 2.85 (t, 2H, J= 6.1 Hz), 1.82 (m, 2H), 1.22 (t, 3H, J= 8 Hz); ^{13}C NMR δ 171.6, 171.2, 171.1, 166.6, 160.1, 145.5, 138.0, 136.5, 133.8, 133.6, 130.4, 129.5, 128.3, 128.2, 126.6, 119.7, 117.4, 117.4, 65.9, 55.9, 53.2, 52.5, 37.0, 36.3, 26.6, 26.3, 25.9, 25.9, 25.3, 13.3; LRMS *m*/*z* calcd for (M^+ +H); 1267.5, found: 1267.4; monomer HRMS *m*/*z* calcd for (M^+ +H); 635.2798, found: 635.2897.

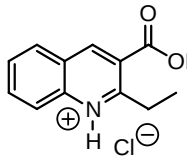
III. Spectra:



3-Carboxy-2-ethyl-quinolinium chloride

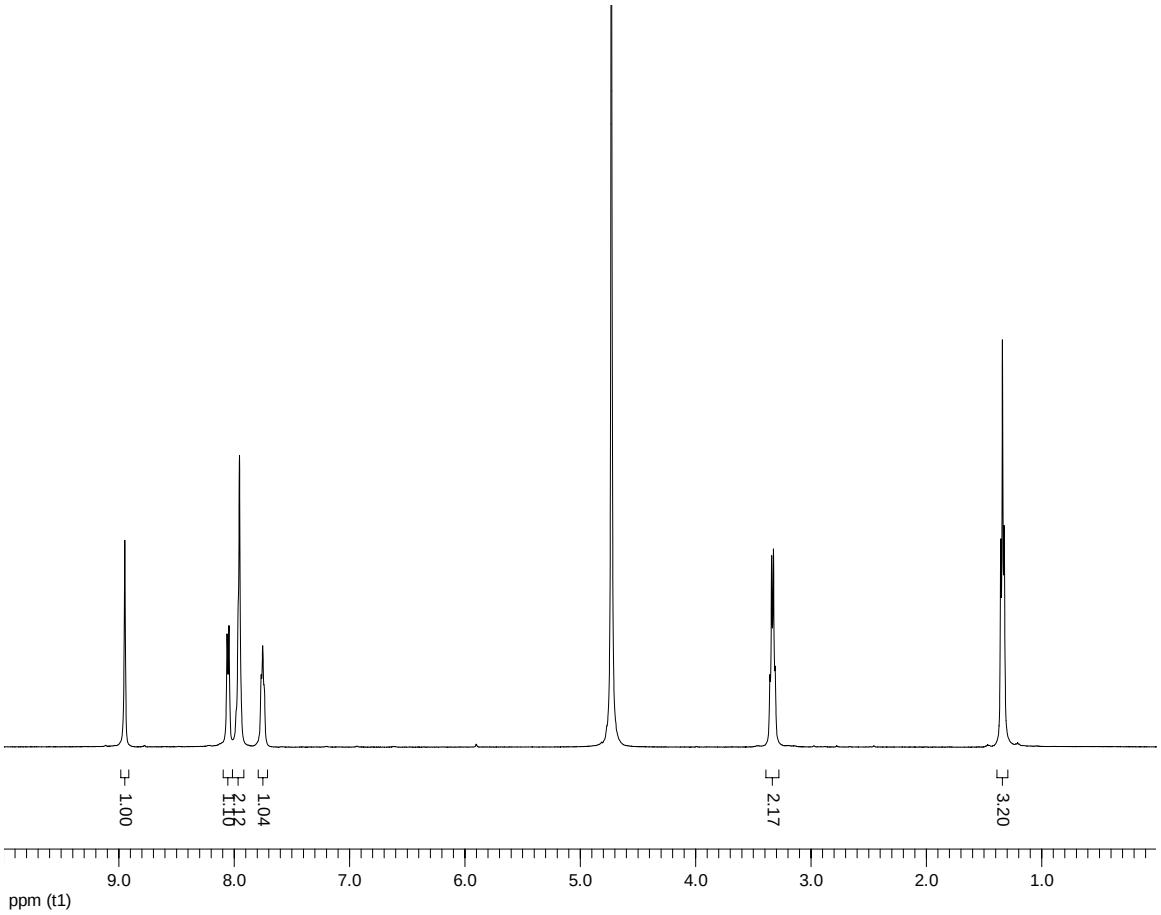
13

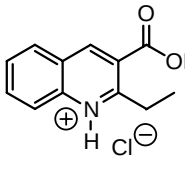




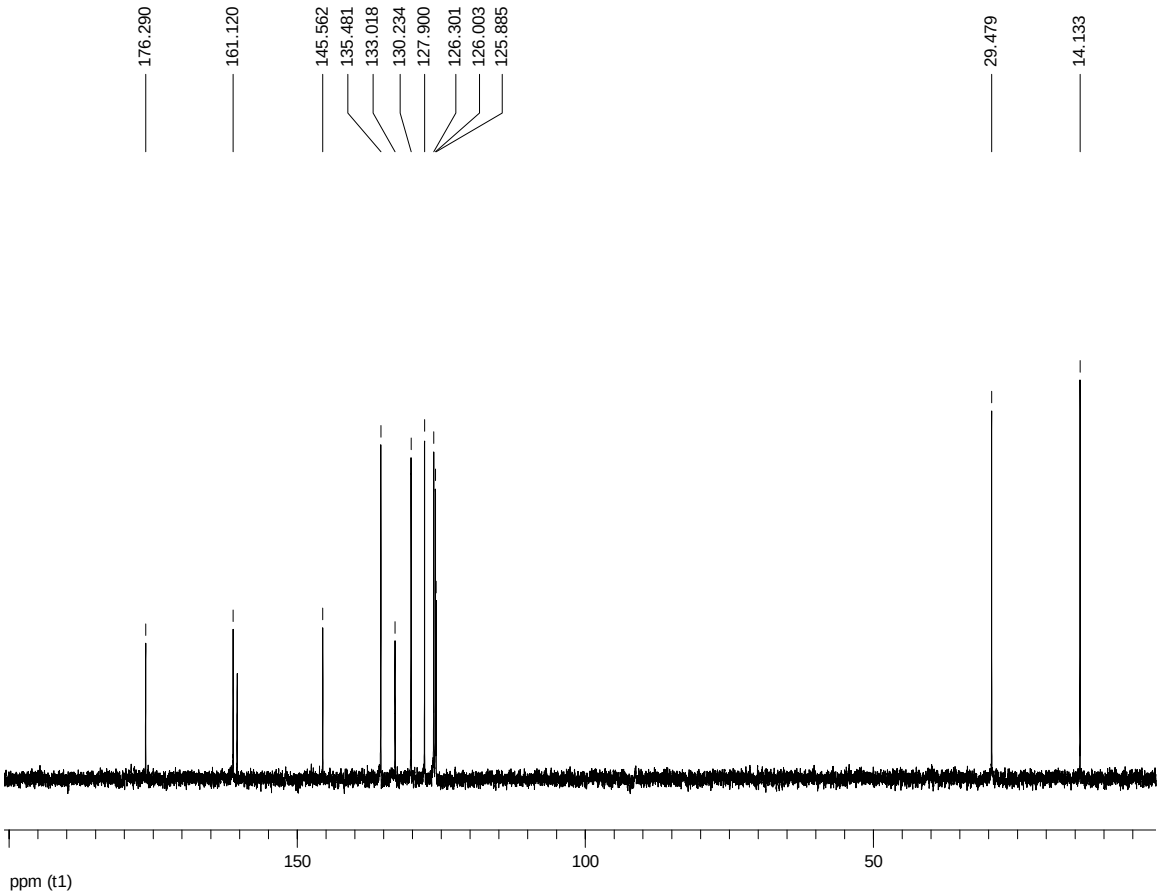
3-Carboxy-2-ethyl-quinolinium;chloride

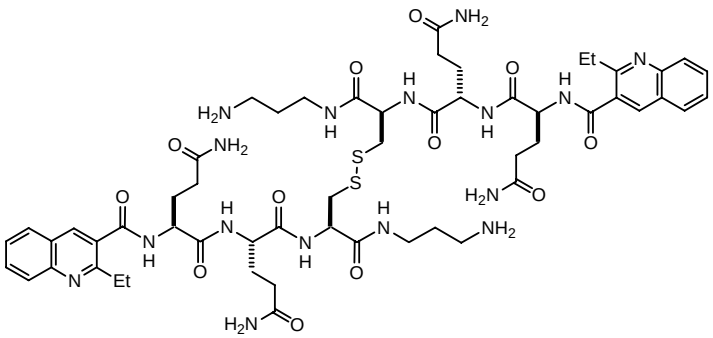
13



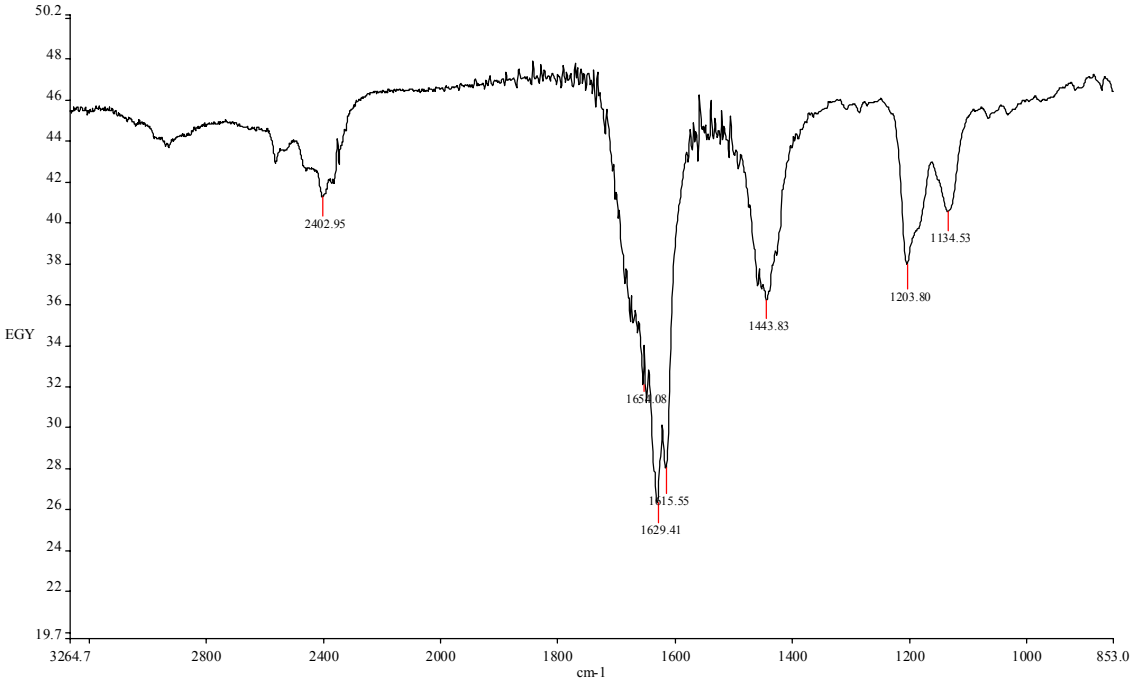


3-Carboxy-2-ethyl-quinolinium chloride





3-3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19.7
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1629.41
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1000
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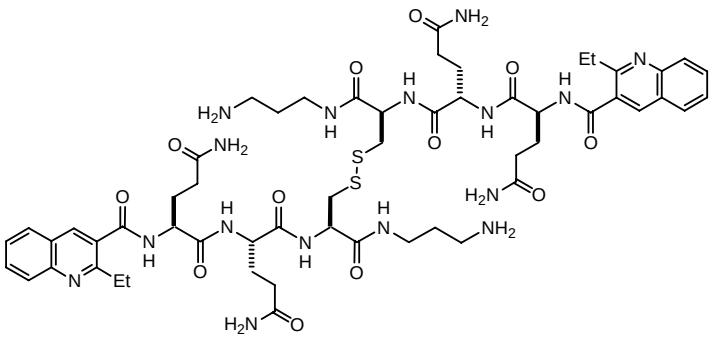
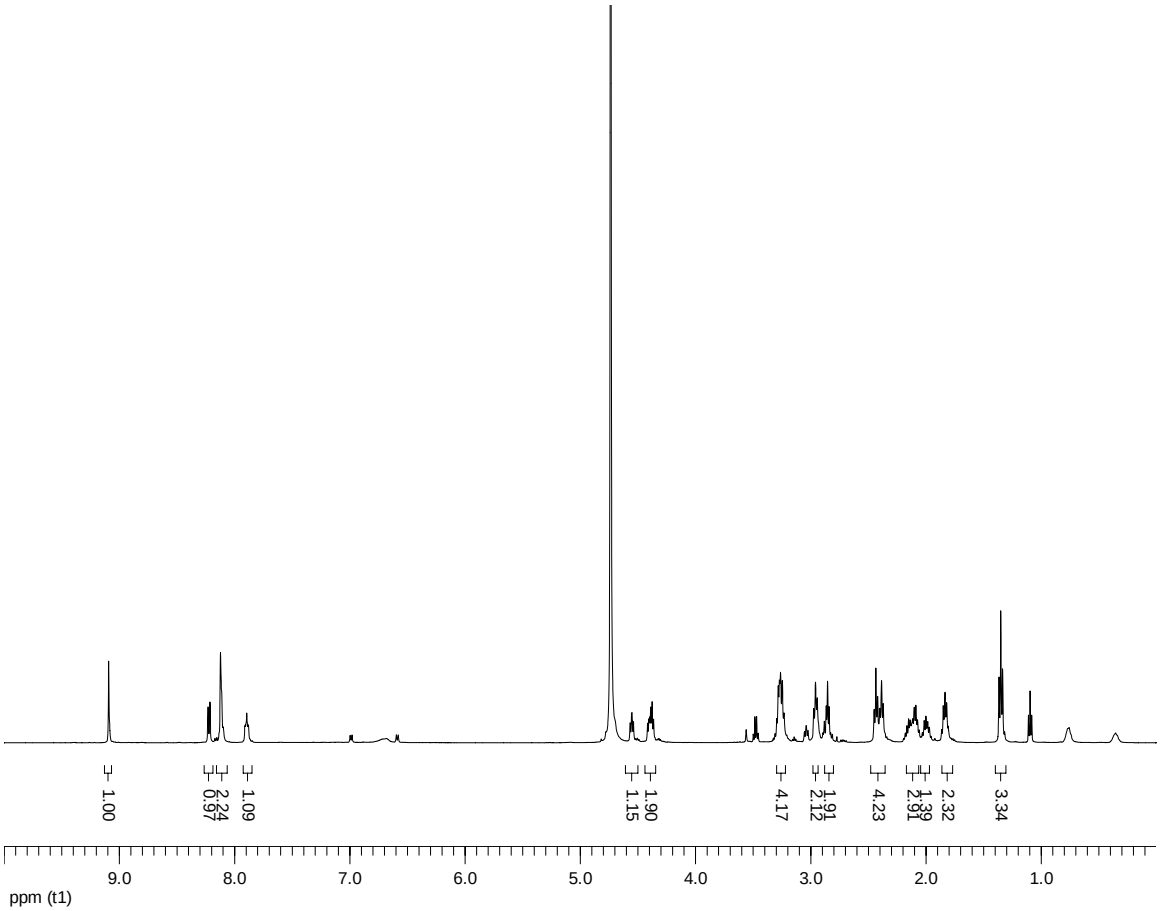
cm-1



3-3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1.91
2.12
4.23
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2.91
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3.34
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6.0
5.0
4.0
3.0
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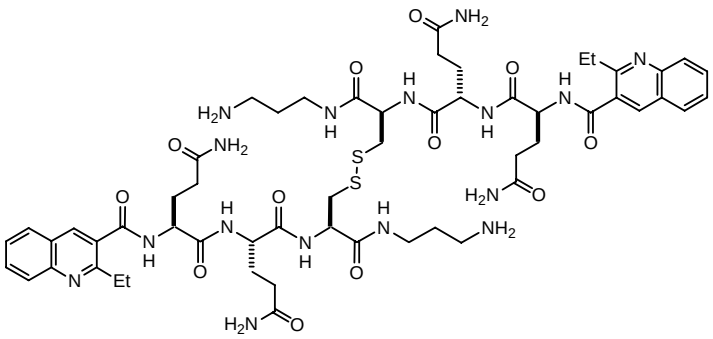
1.0
ppm (t1)



3-3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177.548
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119.619
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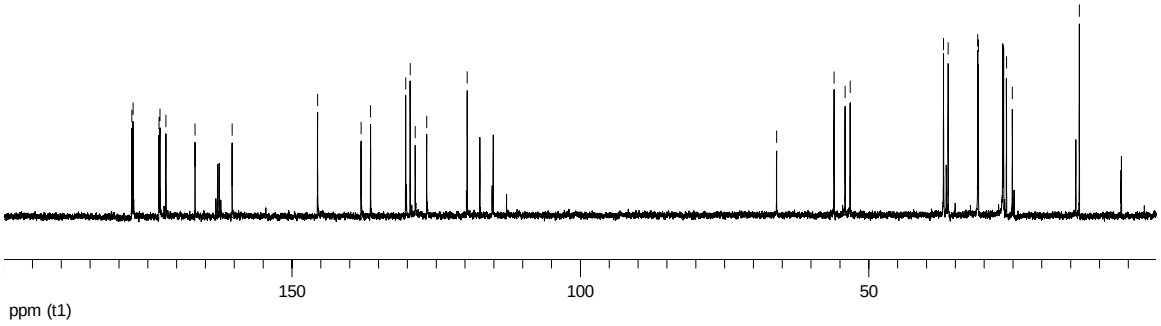
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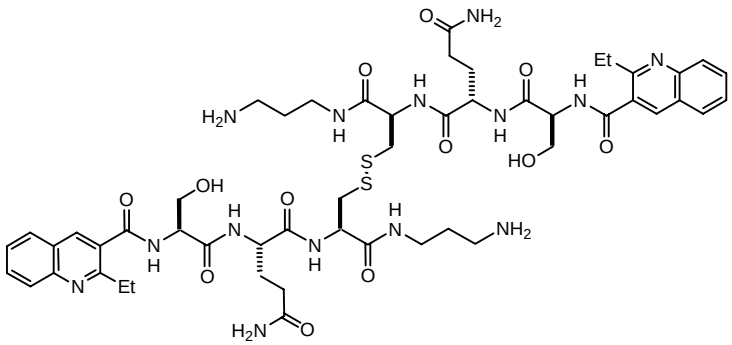
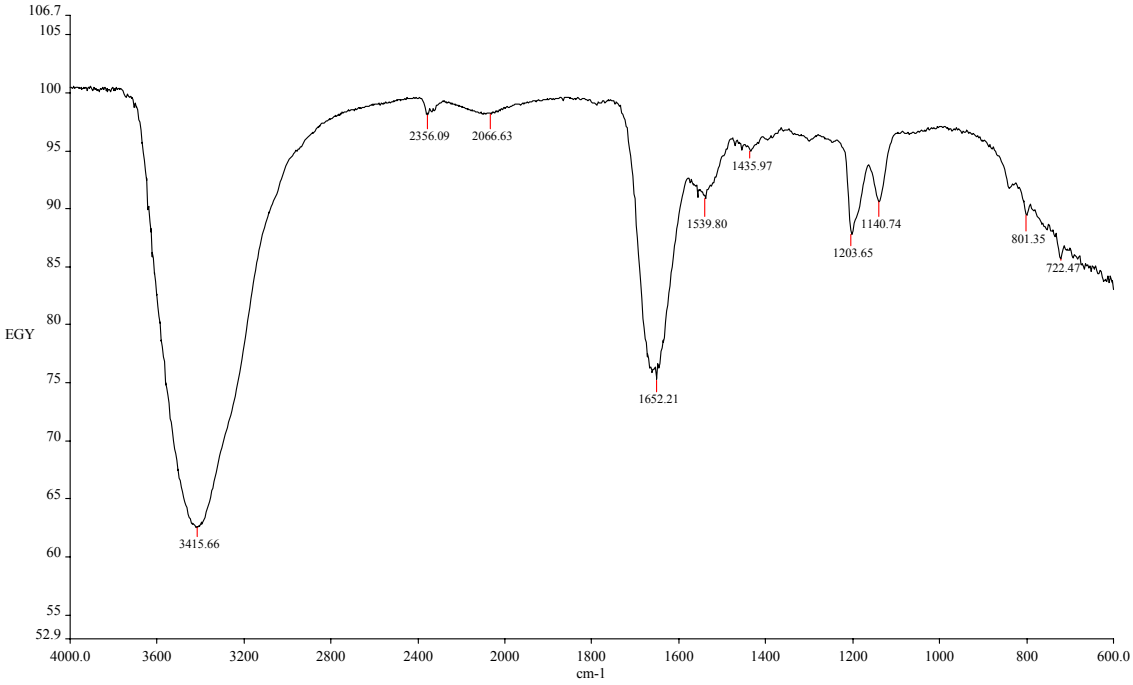
100
50
ppm (t1)



4-4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1140.74
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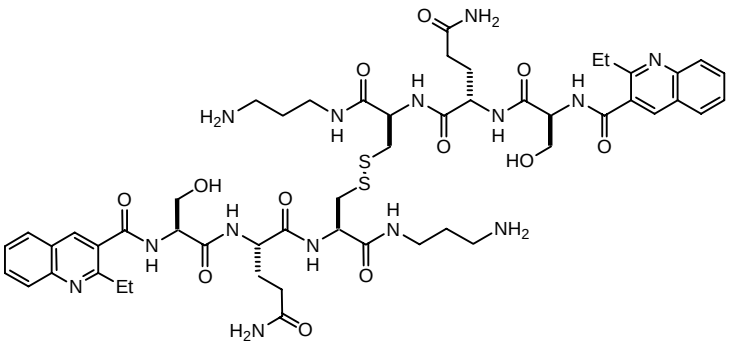
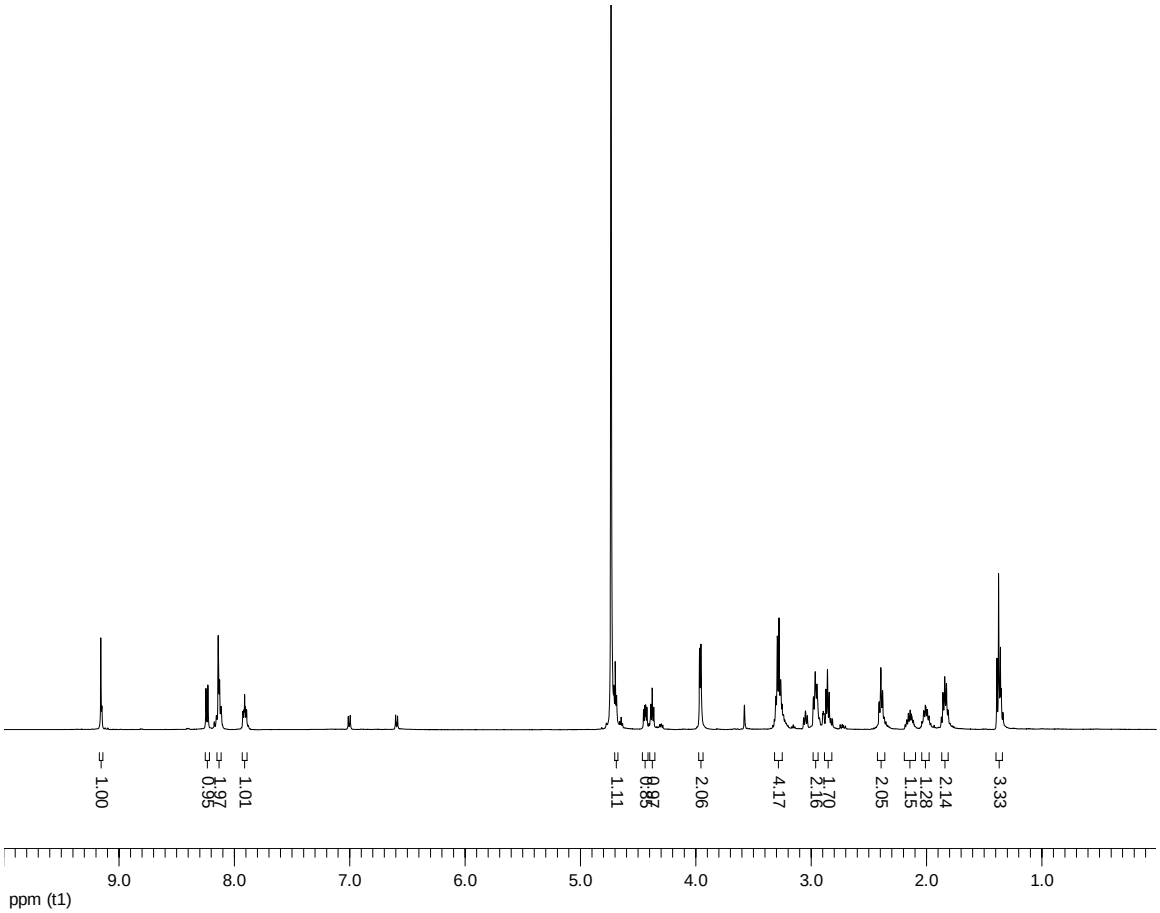
cm-1



4-4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5.0
4.0
3.0
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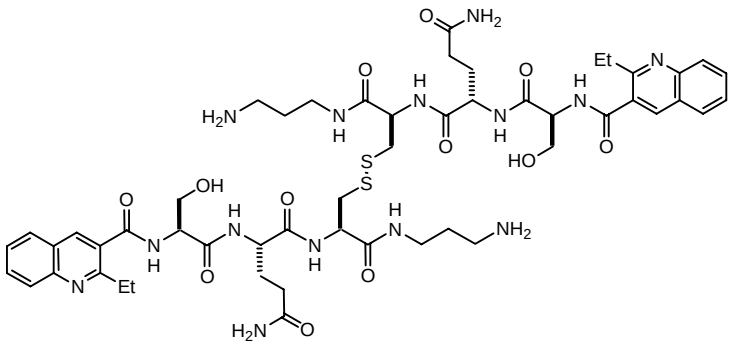
1.0
ppm (t1)



4-4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136.362
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129.535
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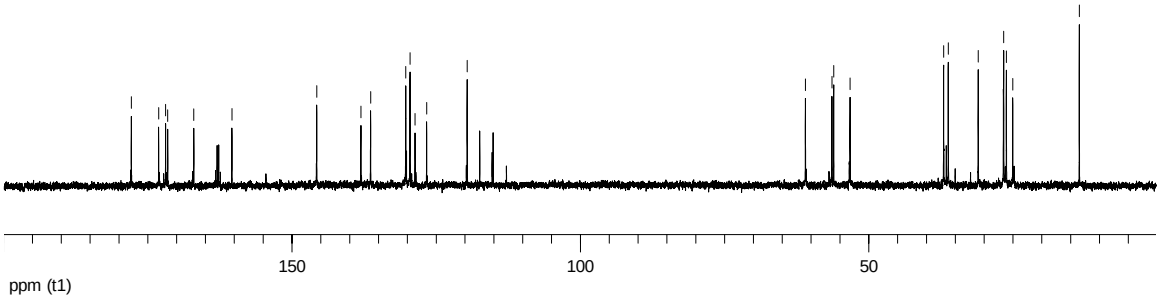
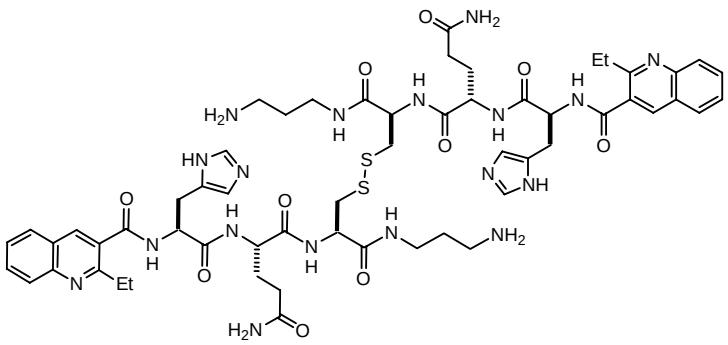
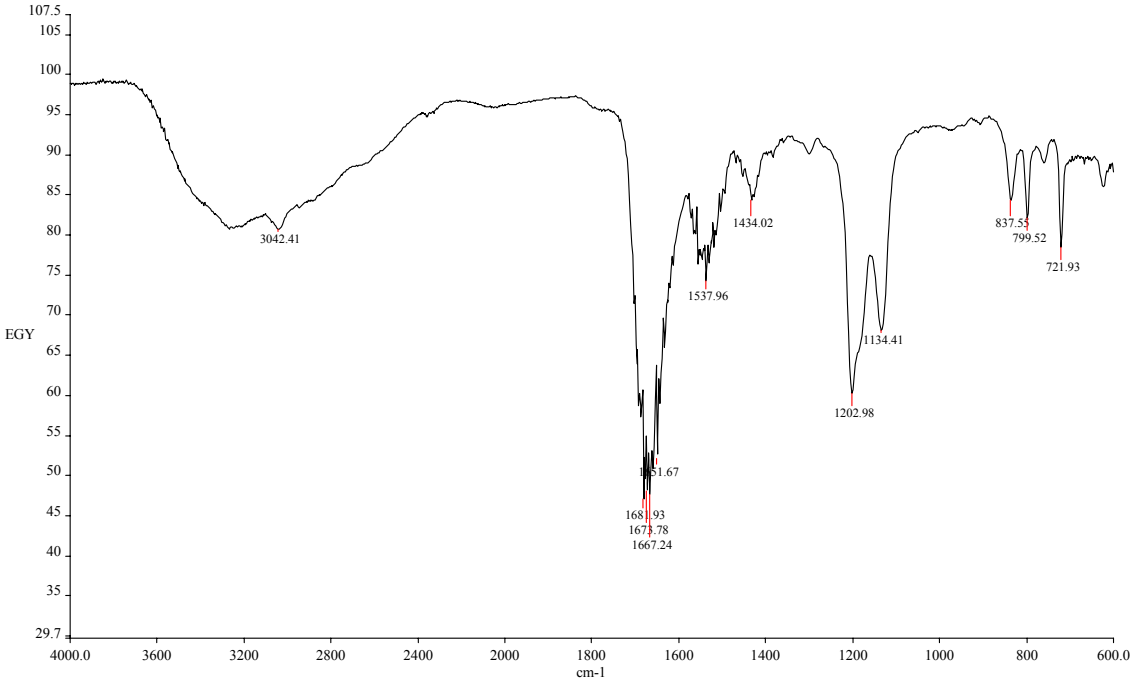
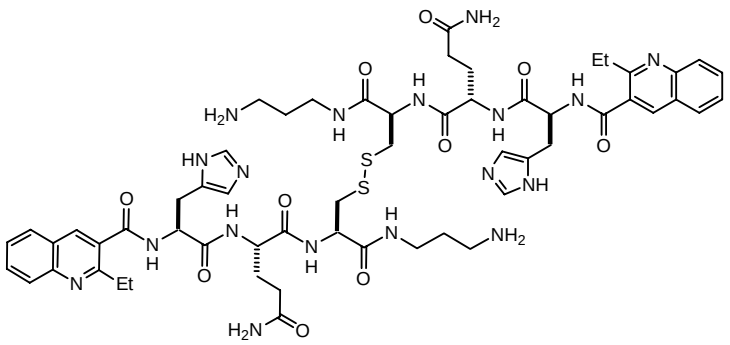
56.068
53.236
37.016
36.207
31.018
26.648
26.606
26.138
25.029
13.491


150
100
50
ppm (t1)

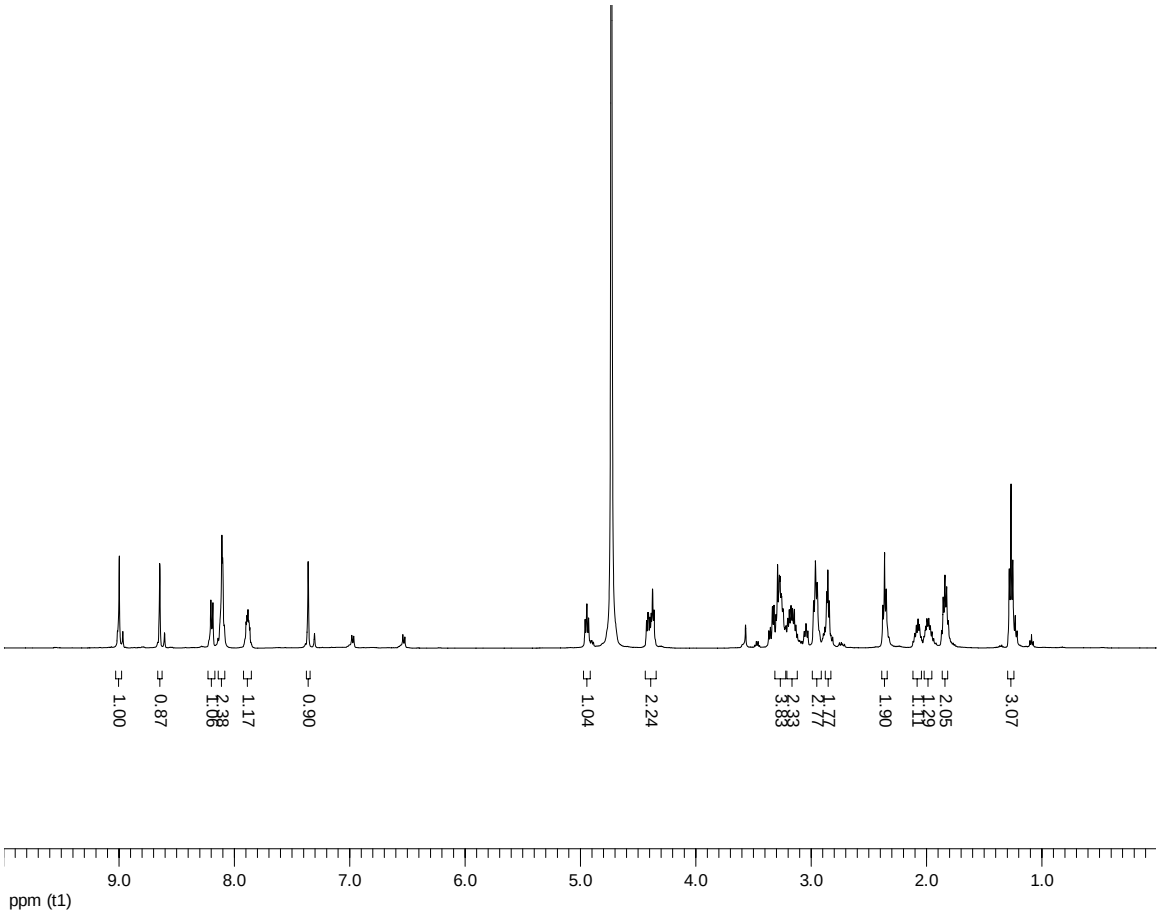


5-5


EGY
cm-1
3042.41
1681.93
1673.78
1667.24
1651.67
1537.96
1434.02
1202.98
1134.41
837.55
799.52
721.93



5-5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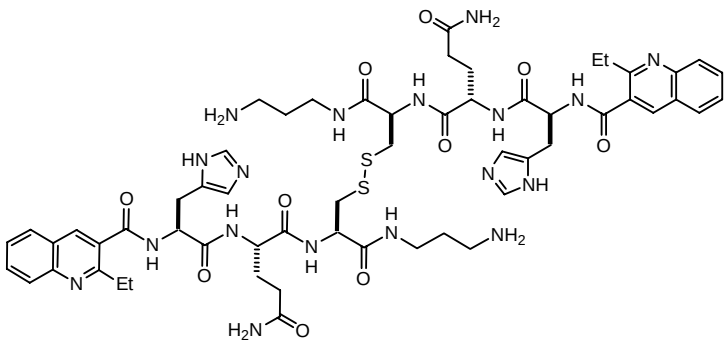
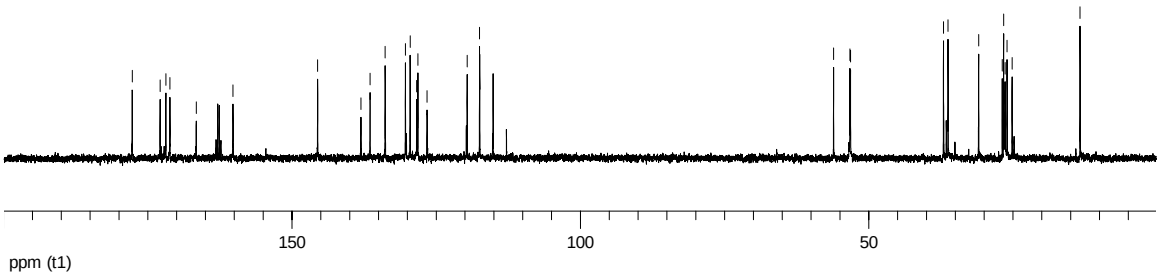
0.87
2.38
2.08
1.17
0.90
1.04
2.24
3.33
3.33
1.77
2.77
1.90
1.11
1.29
2.05
3.07
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)



5-5


177.690
172.852
171.860
171.146
166.585
160.220
145.540
138.029
136.448
133.835
130.316
129.512
128.375
128.161
126.569
119.622
117.476
56.087
53.269
53.157
37.038
36.246
30.942
26.853
26.613
26.343
26.021
25.146
13.371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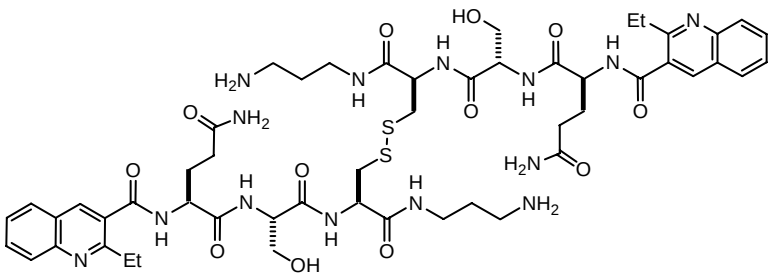
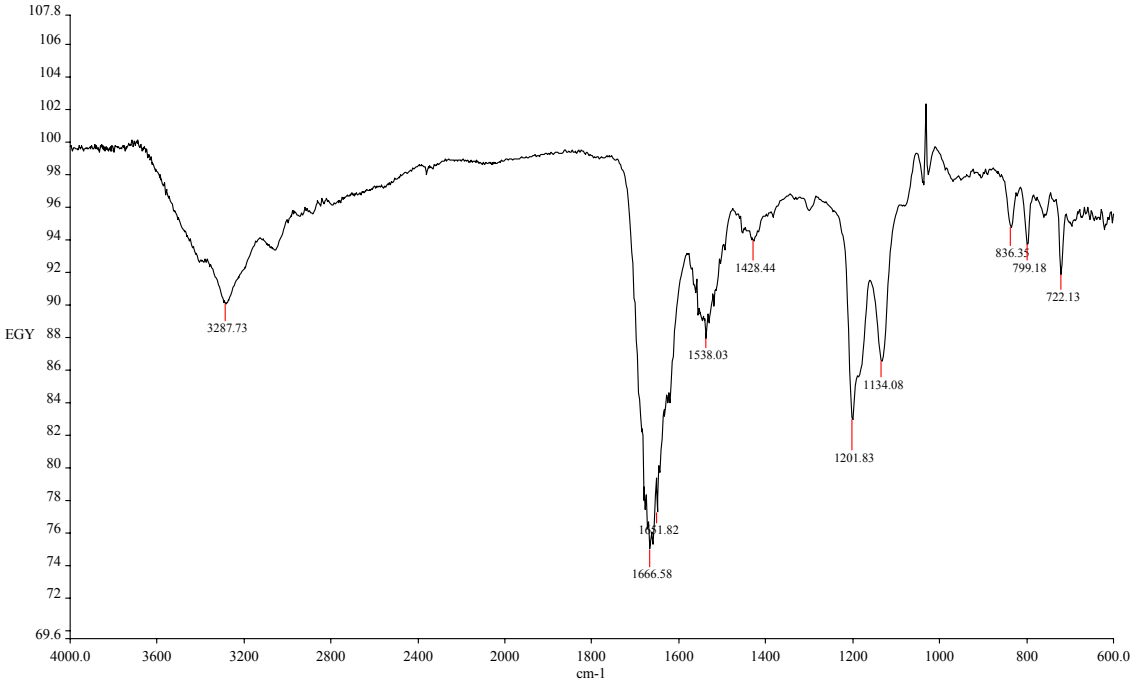
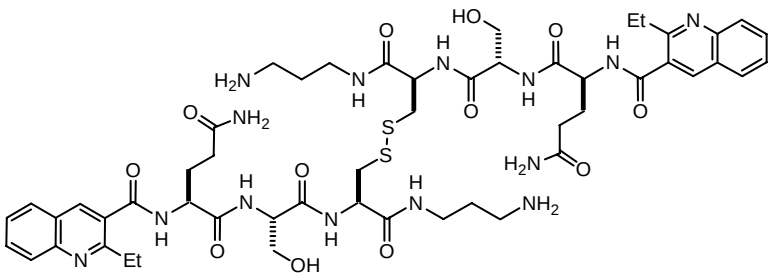
100
50
ppm (t1)

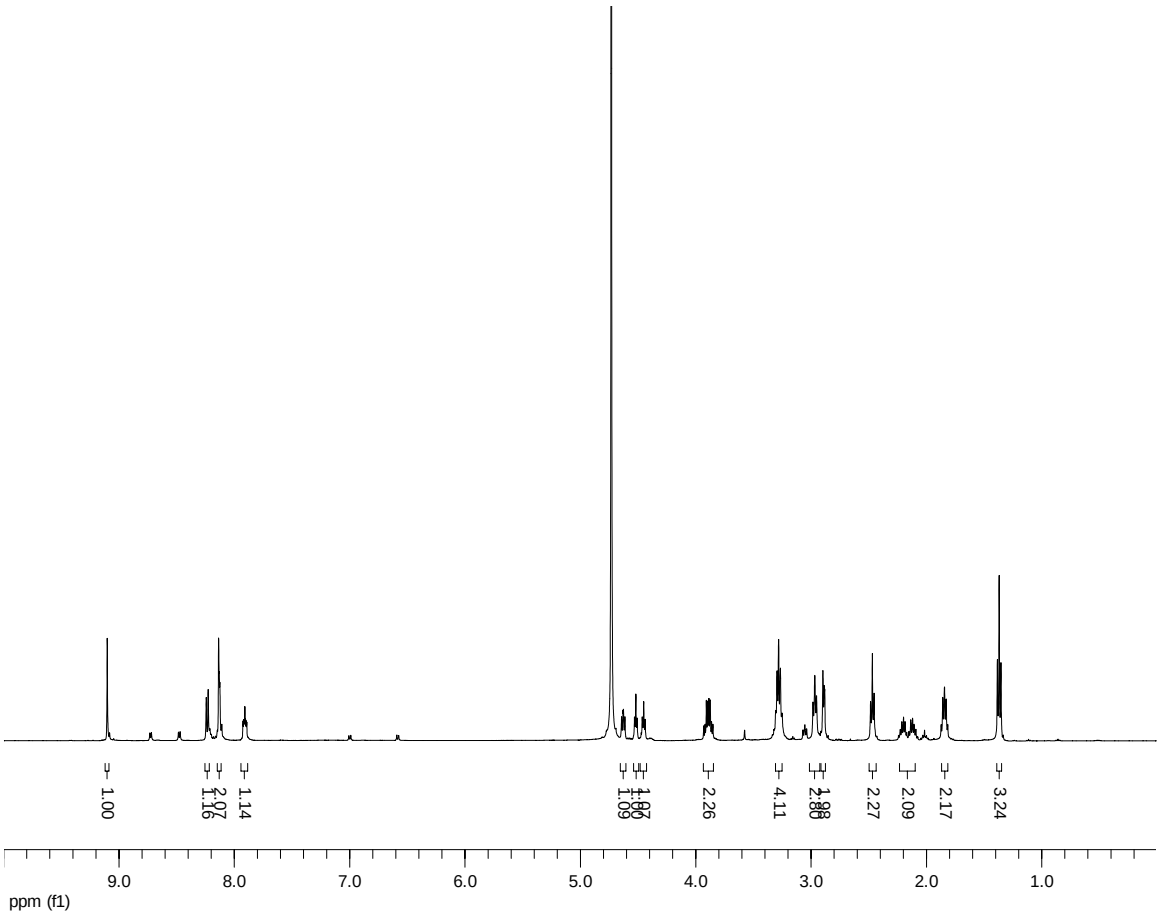


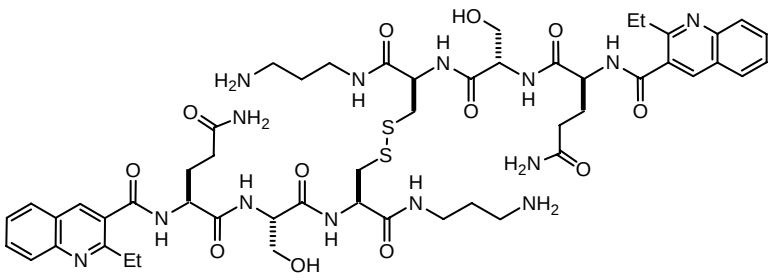
6-6


107.8
106
104
102
100
98
96
94
92
90
88
86
84
82
80
78
76
74
72
69.6
EGY
3287.73
1666.58
1651.82
1538.03
1428.44
1201.83
1134.08
836.33
799.18
722.13
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600.0
cm-1



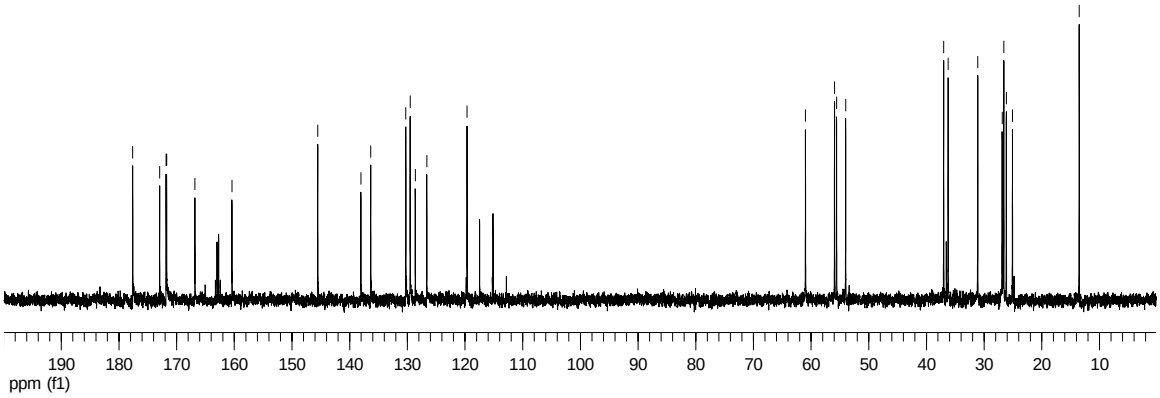
6-6

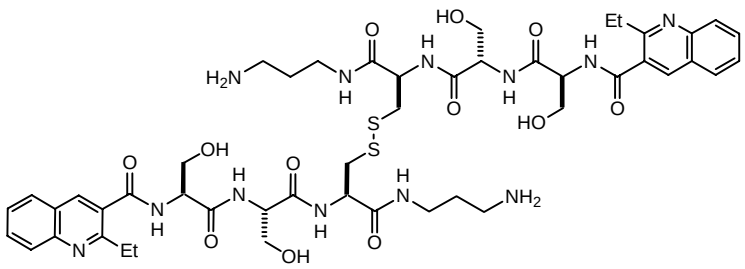

1.00
2.16
2.07
1.14
1.09
1.07
1.06
2.26
4.11
2.08
2.06
2.27
2.09
2.17
3.24
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (f1)



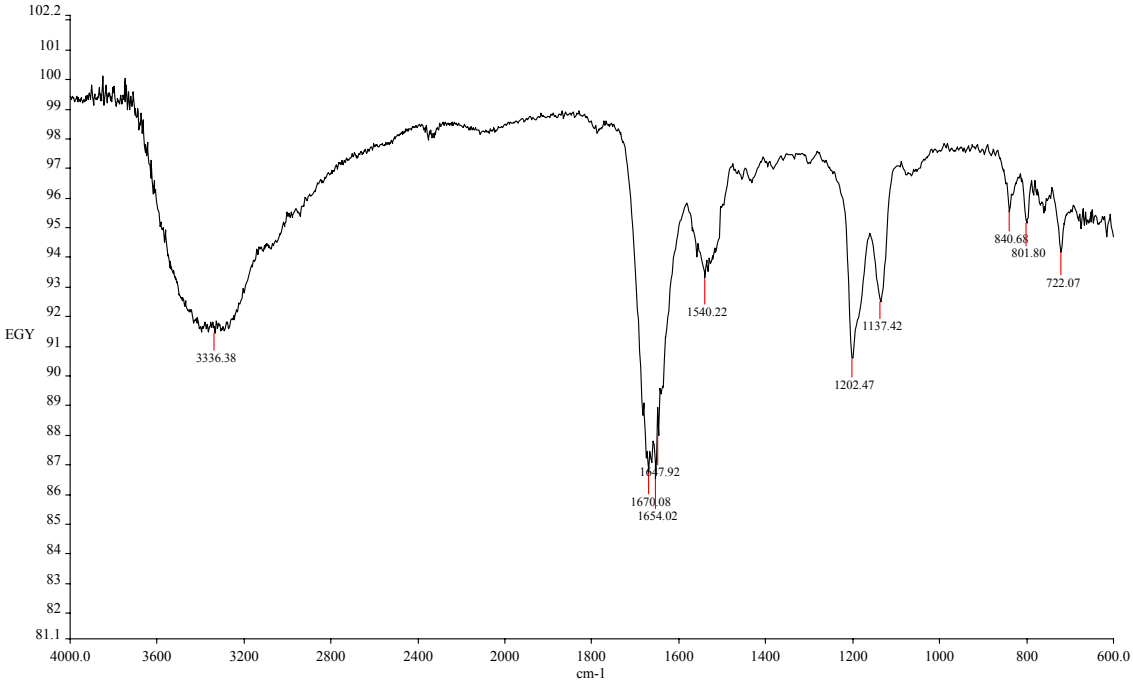
6-6

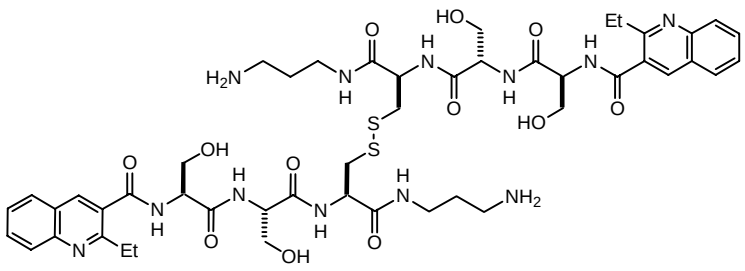

177.605
172.935
171.822
171.720
166.820
160.402
145.526
138.054
136.345
130.255
129.509
128.619
126.620
119.647
60.960
55.925
55.571
54.001
37.006
36.223
31.096
26.872
26.582
26.142
25.083
13.504


190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm (f1)

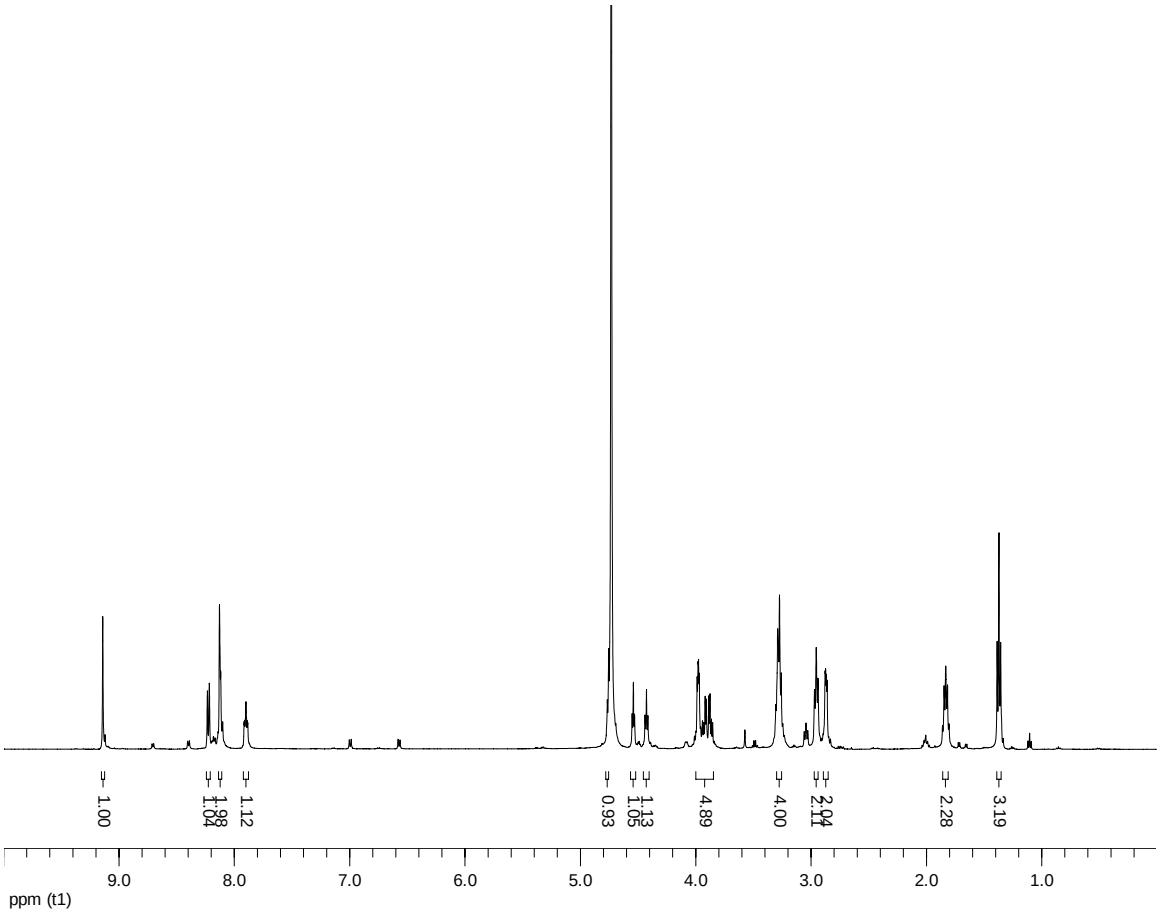


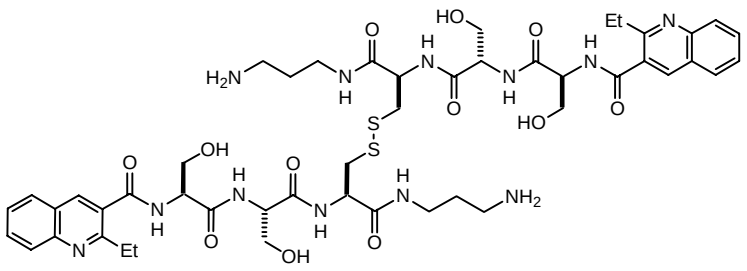
7-7


102.2
101
100
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
83
82
81.1
EGY
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600.0
cm-1
3336.38
1670.08
1654.02
1647.92
1540.22
1202.47
1137.42
840.68
801.80
722.07



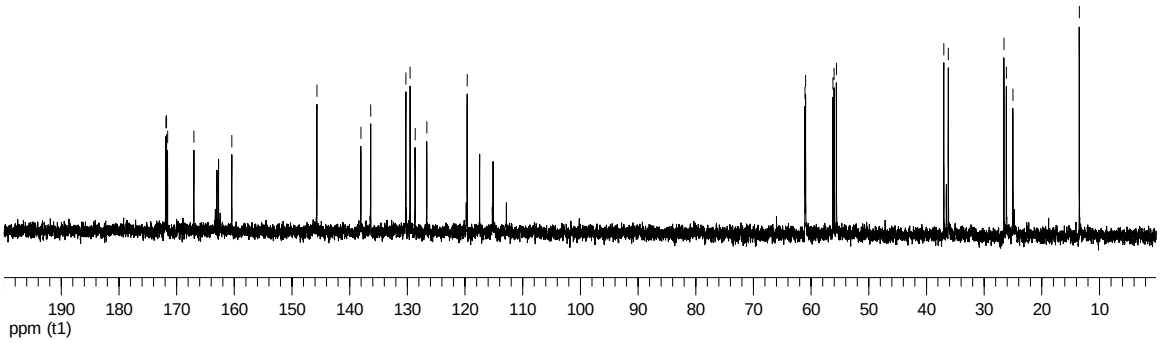
7-7

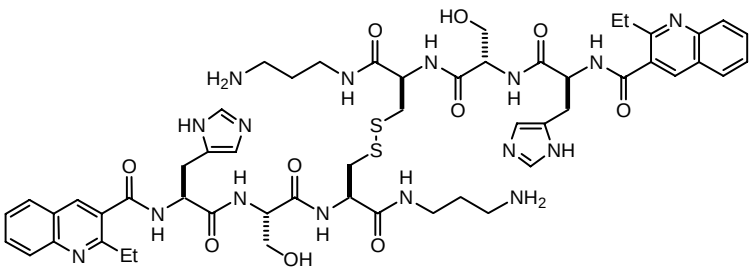

1.00
1.98
1.04
1.12
0.93
1.05
1.13
4.89
4.00
2.04
2.11
2.28
3.19
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)



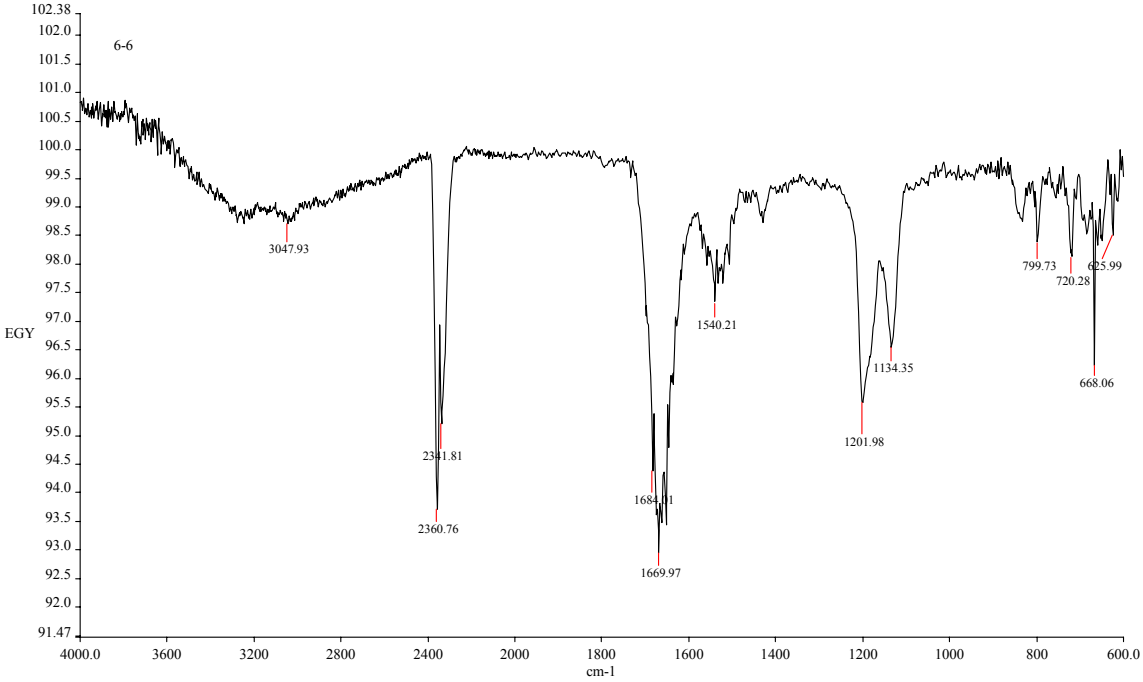
7-7

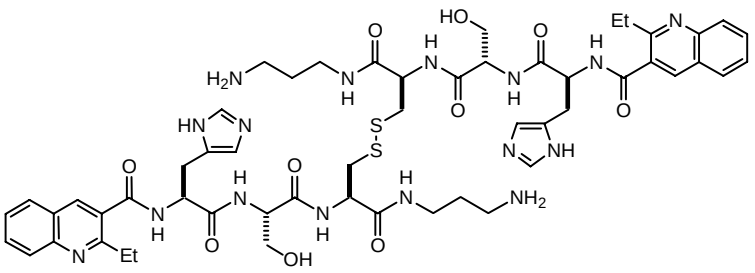

171.853
171.731
171.561
166.997
160.421
145.679
138.054
136.342
130.242
129.521
128.652
126.619
119.630
61.064
60.945
56.215
56.001
55.601
36.987
36.198
26.567
26.141
25.002
13.501


190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm (t1)

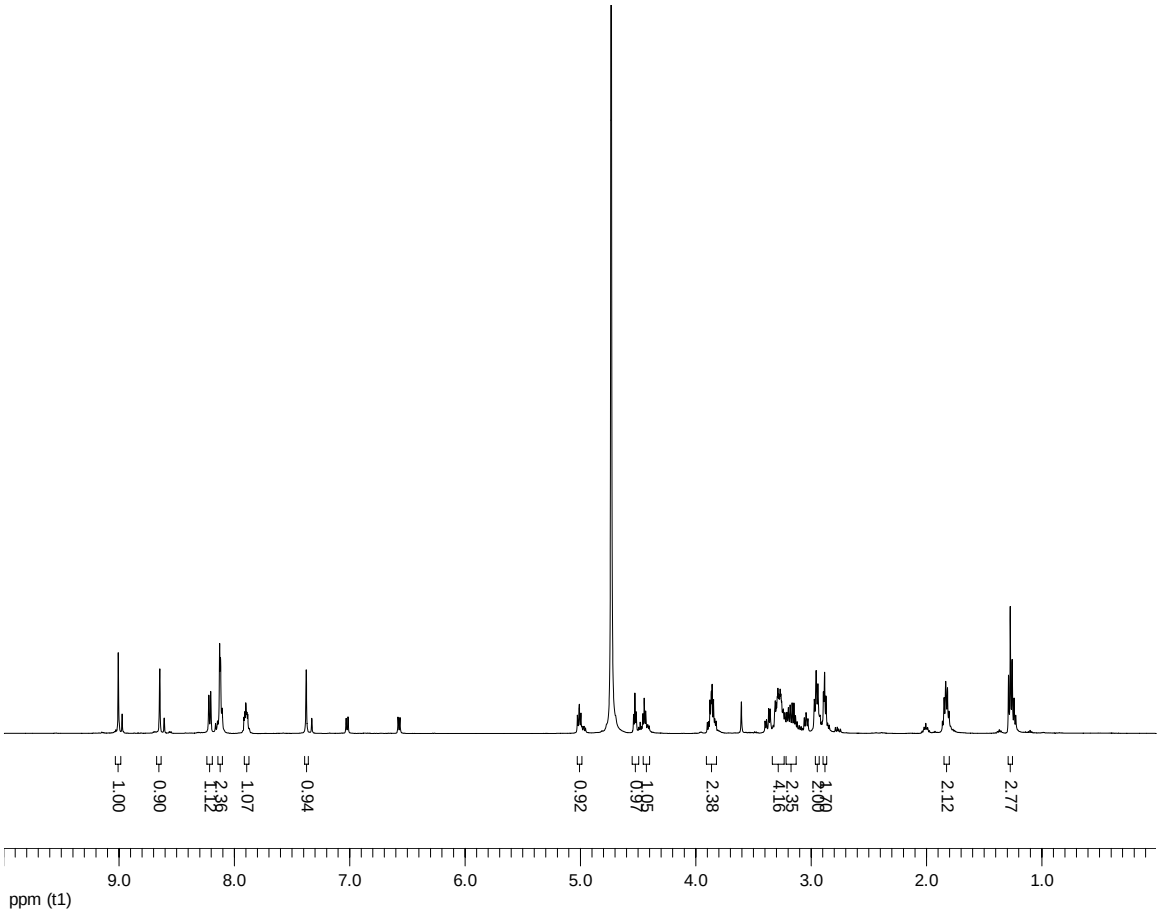


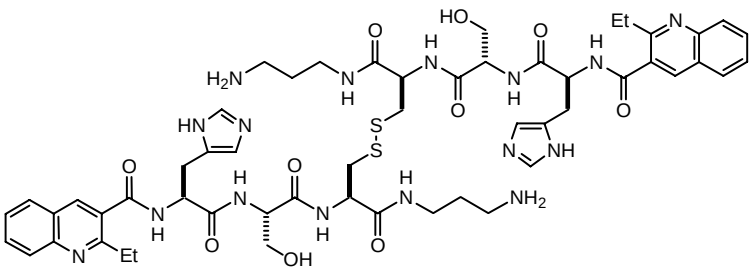
8-8


6-6
EGY
cm-1
3047.93
2361.81
2360.76
1684.11
1669.97
1540.21
1201.98
1134.35
799.73
720.28
625.99
668.06

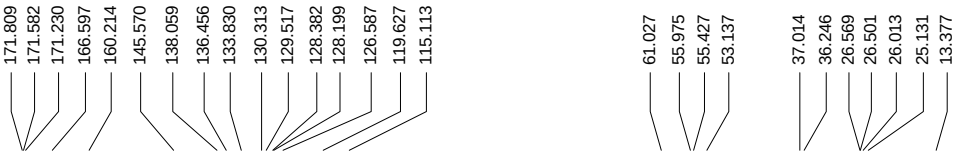


8-8


1.00
0.90
2.36
1.12
1.07
0.94
0.92
1.05
0.97
2.38
2.35
4.16
2.08
1.79
2.12
2.77
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)



8-8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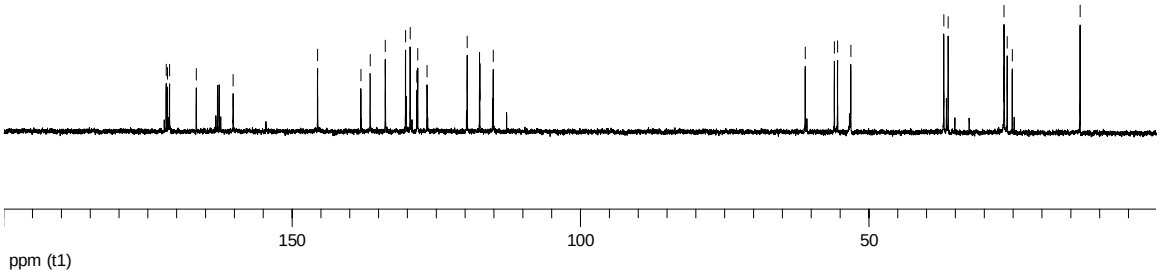
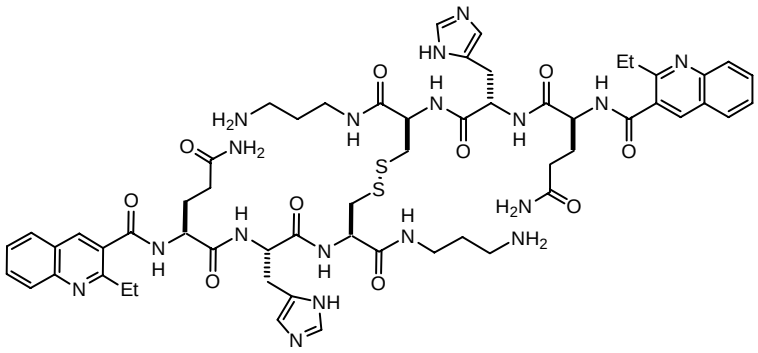
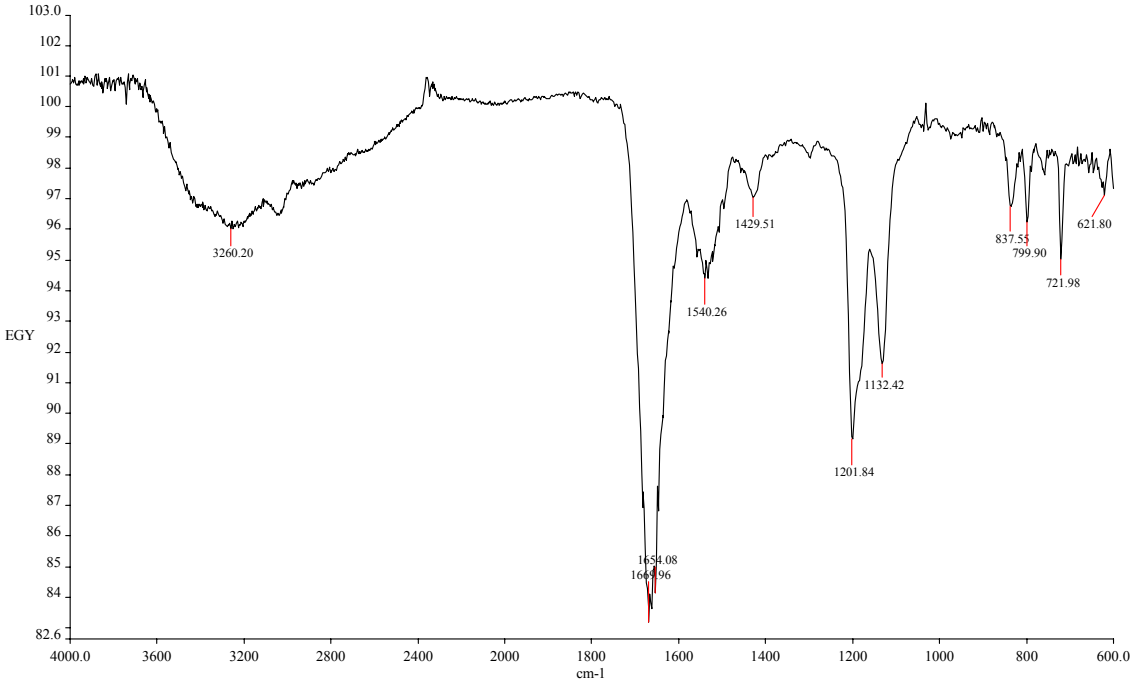
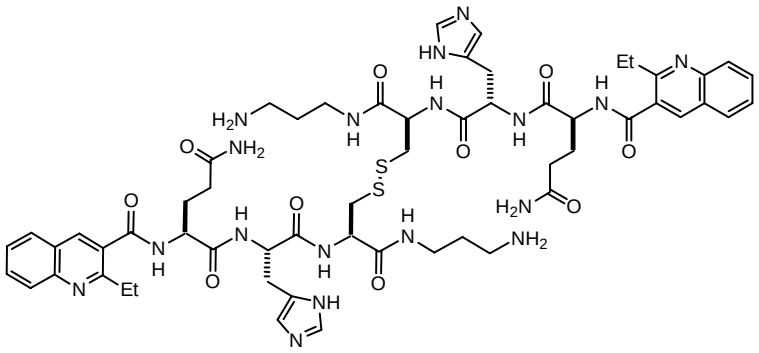
171.582
171.230
166.597
160.214
145.570
138.059
136.456
133.830
130.313
129.517
128.382
128.199
126.587
119.627
115.113
61.027
55.975
55.427
53.137
37.014
36.246
26.569
26.501
26.013
25.131
13.377


150
100
50
ppm (t1)

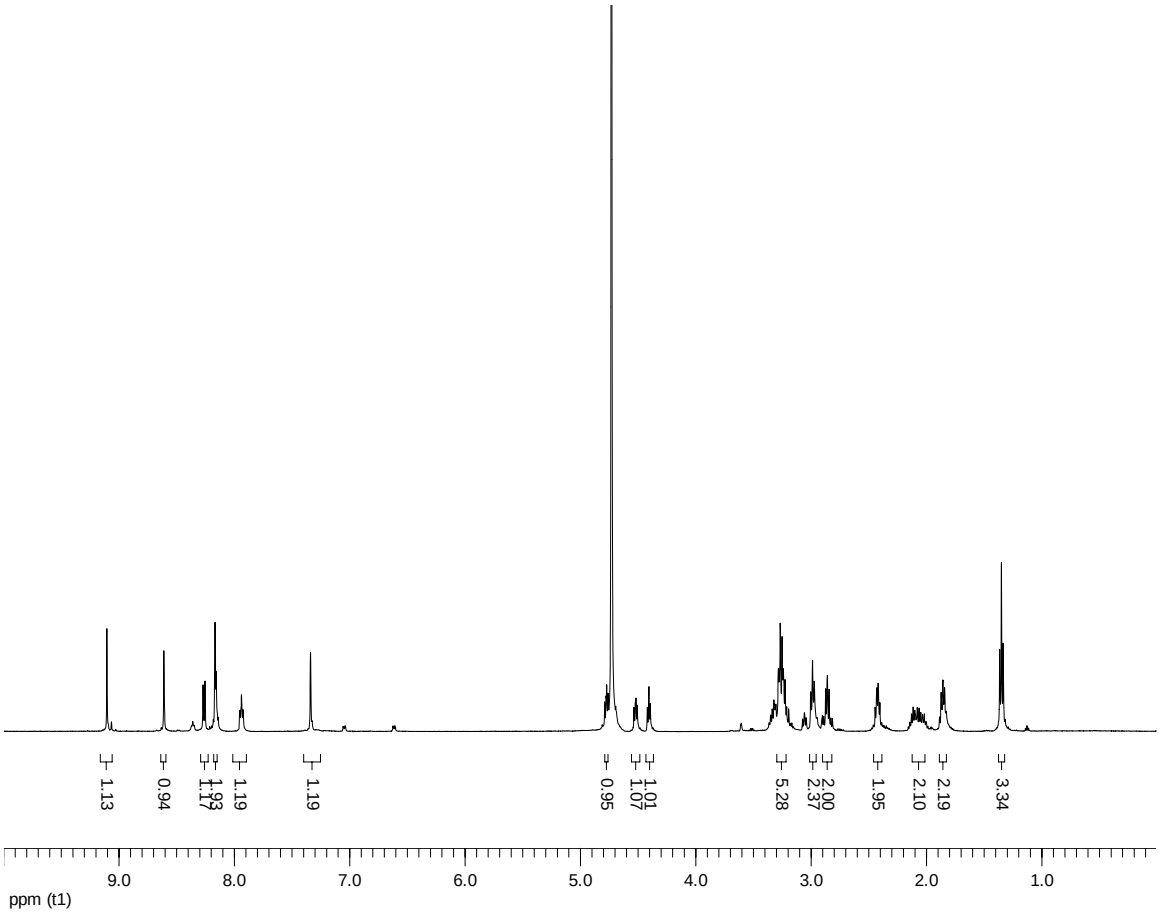


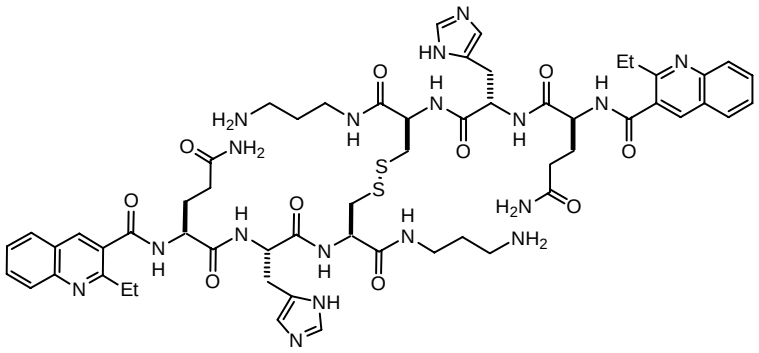
9-9


103.0
102
101
100
99
98
97
96
95
94
93
92
91
90
89
88
87
86
85
84
82.6
EGY
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600.0
cm-1
3260.20
1654.08
1668.96
1540.26
1429.51
1201.84
1132.42
837.55
799.90
721.98
621.80

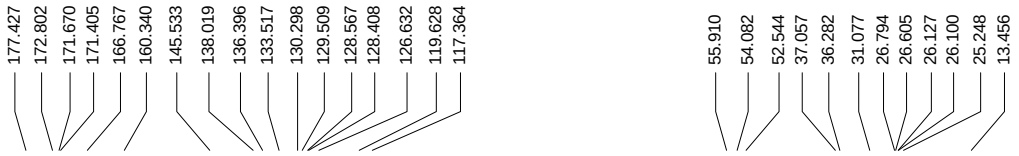


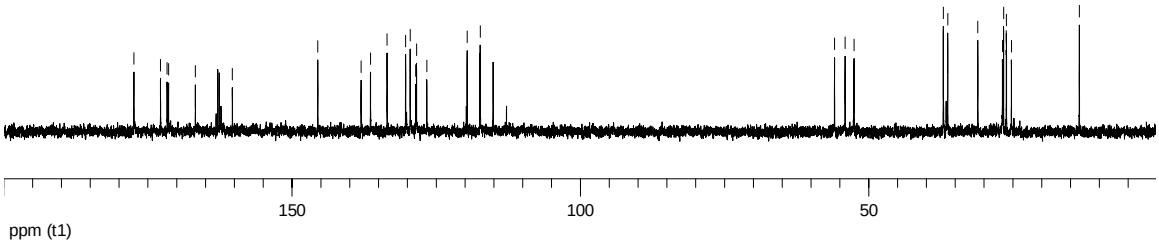
9-9

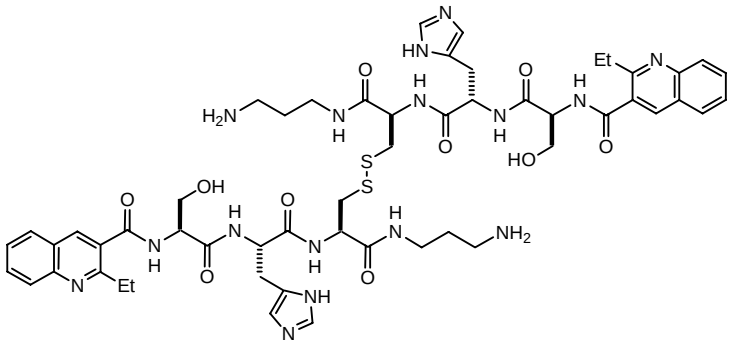

1.13
0.94
1.93
1.17
1.19
1.19
0.95
1.01
1.07
5.28
2.00
2.37
1.95
2.10
2.19
3.34
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)



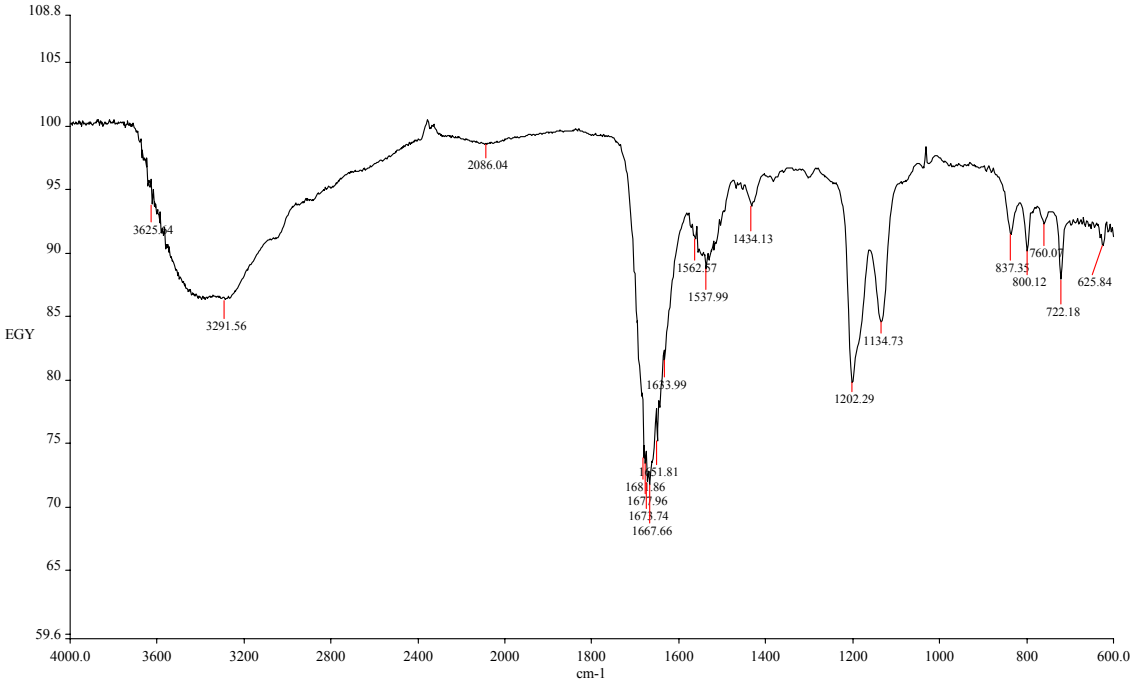
9-9

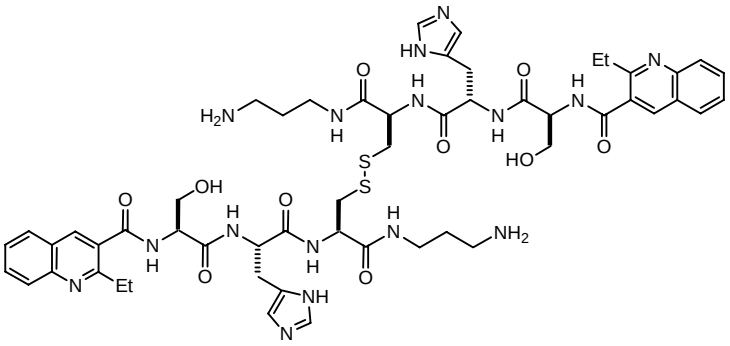

177.427
172.802
171.670
171.405
166.767
160.340
145.533
138.019
136.396
133.517
130.298
129.509
128.567
128.408
126.632
119.628
117.364
55.910
54.082
52.544
37.057
36.282
31.077
26.794
26.605
26.127
26.100
25.248
13.456


150
100
50
ppm (t1)

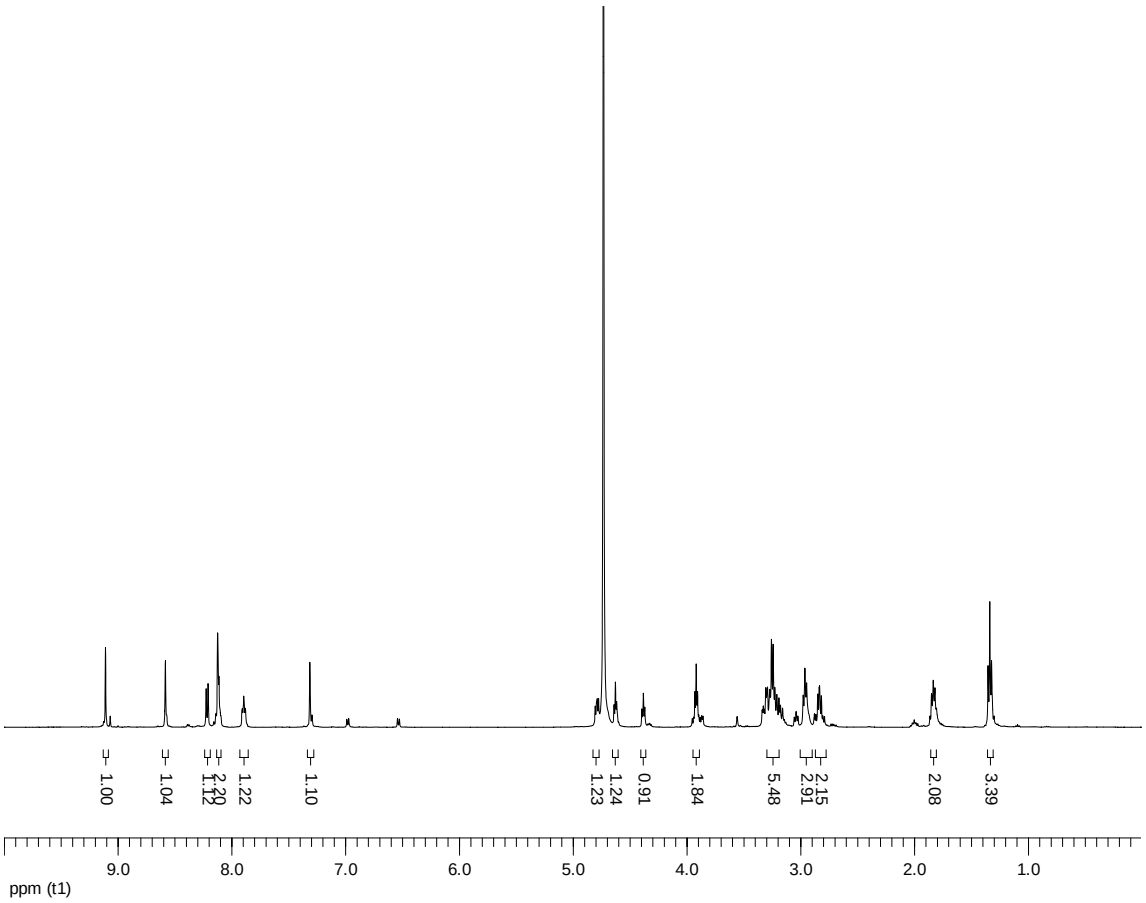


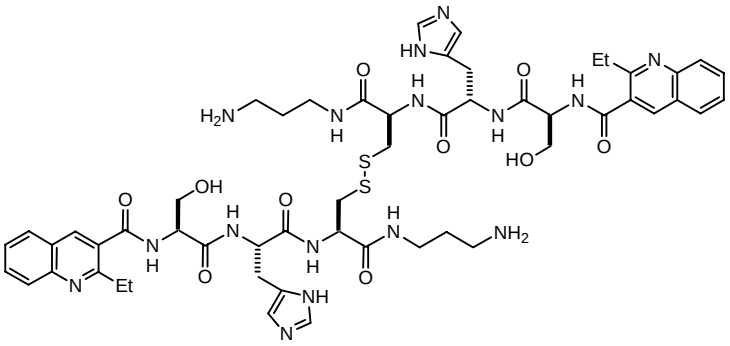
10-10


108.8
105
100
95
90
85
80
75
70
65
59.6
EGY
4000.0
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600.0
cm-1
3625.04
3291.56
2086.04
1633.99
1651.81
1681.86
1677.96
1673.74
1667.66
1562.57
1537.99
1434.13
1202.29
1134.73
837.35
800.12
760.07
722.18
625.84

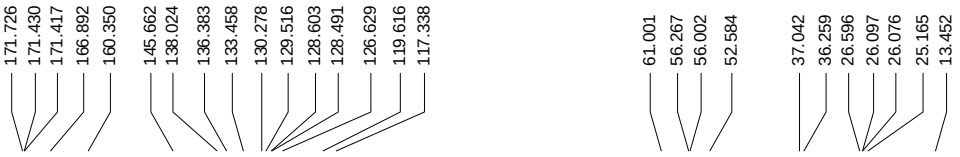


10-10


1.00
1.04
2.29
1.12
1.22
1.10
1.23
1.24
0.91
1.84
5.48
2.91
2.15
2.08
3.39
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
ppm (t1)



10-10


171.726
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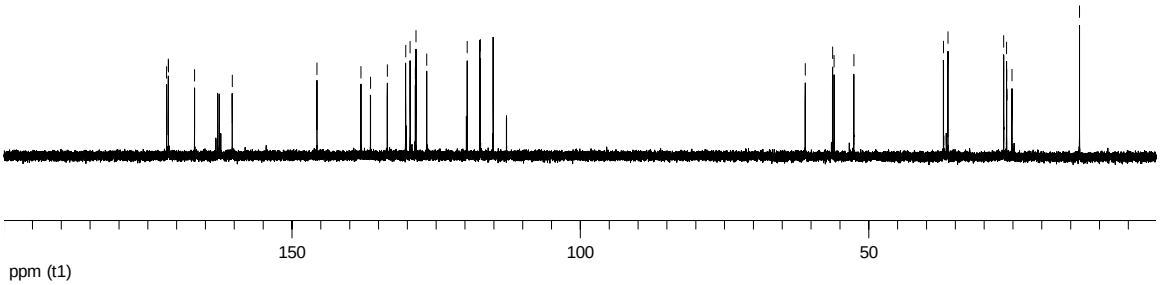
171.417
166.892
160.350
145.662
138.024
136.383
133.458
130.278
129.516
128.603
128.491
126.629
119.616
117.338
61.001
56.267
56.002
52.584
37.042
36.259
26.596
26.097
26.076
25.165
13.452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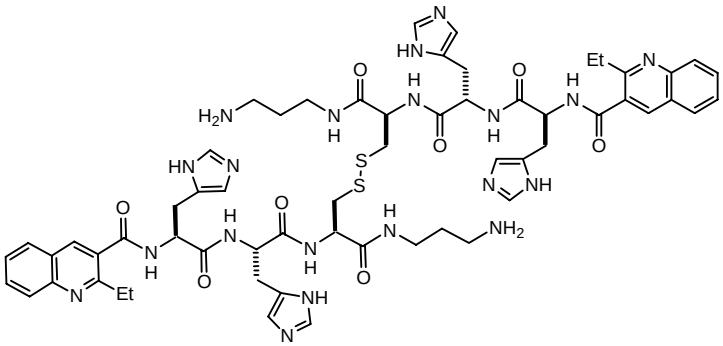
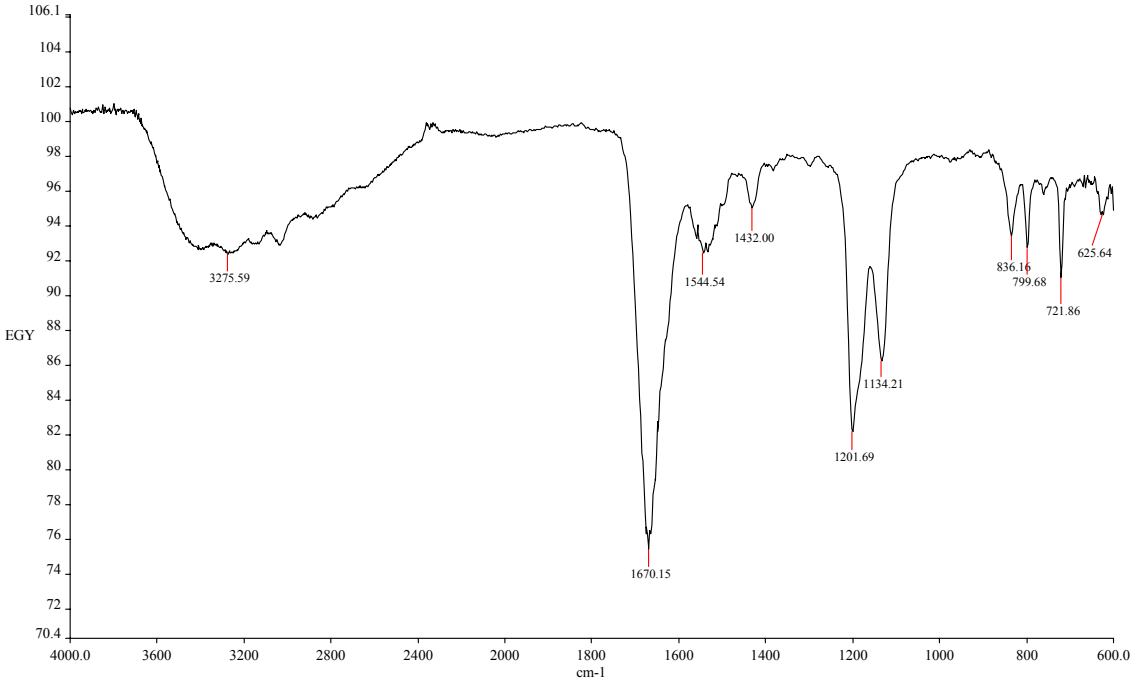
100
50
ppm (t1)



11-11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102
100
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96
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92
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84
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80
78
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3275.59
1670.15
1544.54
1432.00
1201.69
1134.21
836.16
799.68
721.86
625.64
4000.0
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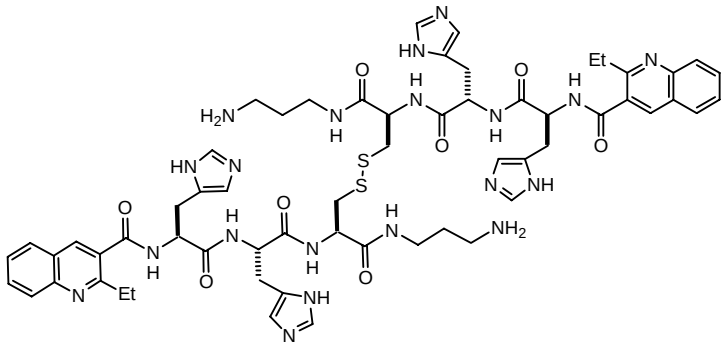
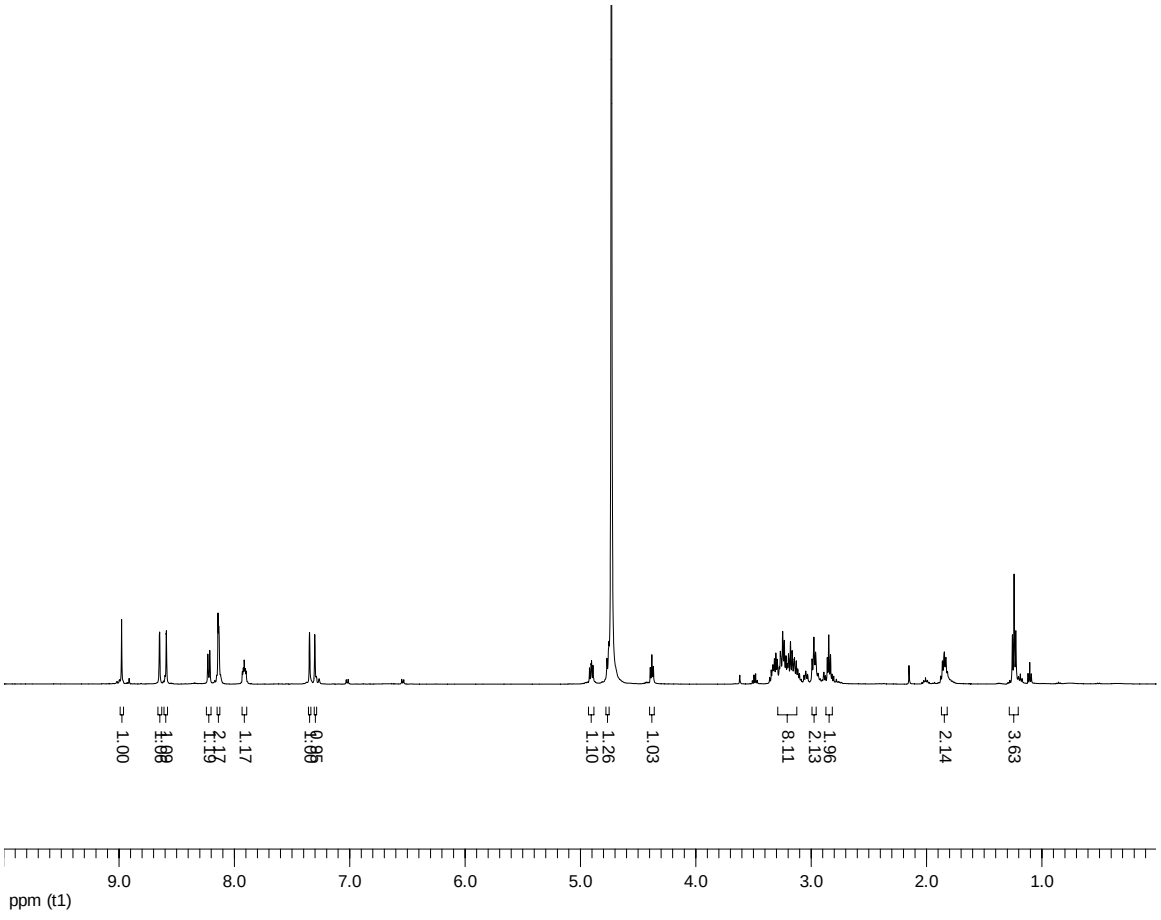
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600.0
cm-1



11-11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1.98
2.17
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0.86
1.10
1.26
1.03
8.11
2.13
1.96
2.14
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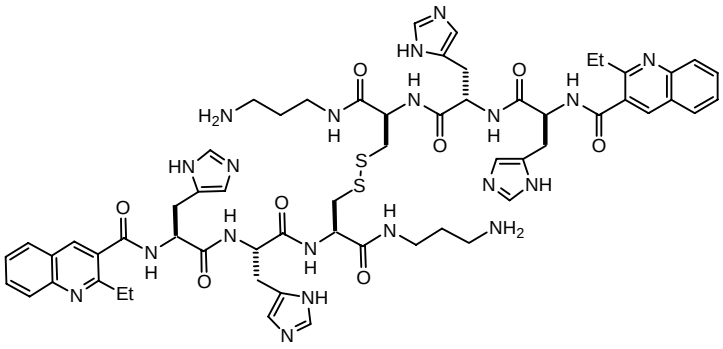
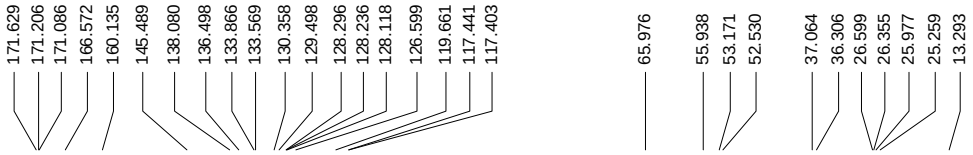
3.0
2.0
1.0
ppm (t1)



11-11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138.080
136.498
133.866
133.569
130.358
129.498
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128.236
128.118
126.599
119.661
117.441
117.403
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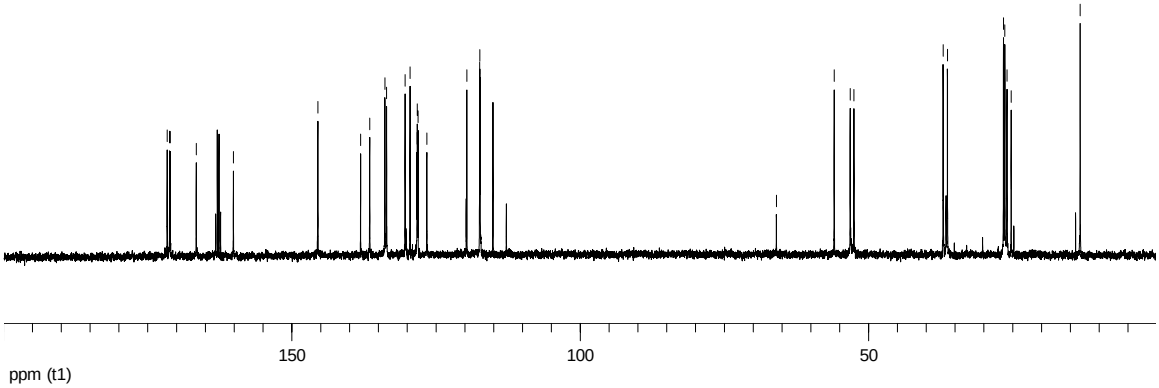
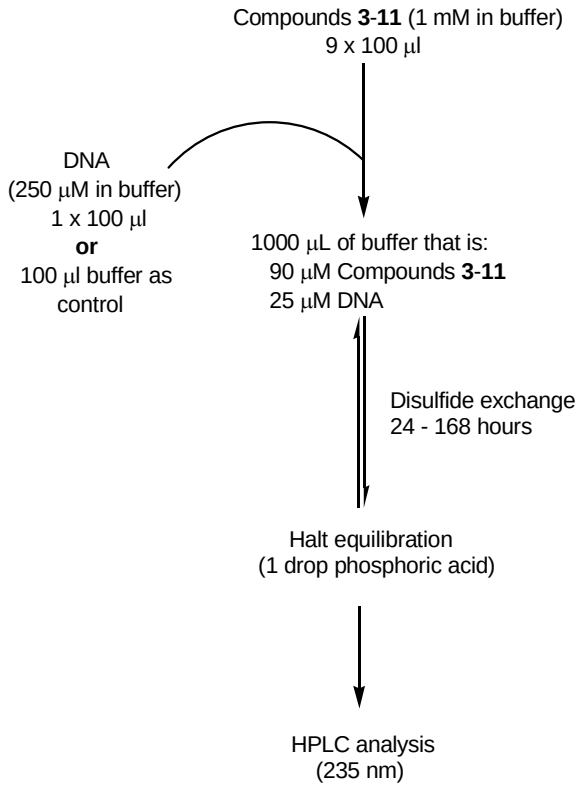
36.306
26.599
26.355
25.977
25.259
13.293


150
100
50
ppm (t1)

IV. Creation and analysis of solution phase dynamic combinatorial libraries:

The solution phase DCL experiment is illustrated schematically in Scheme 1.

Scheme 1: Solution Phase Dynamic Combinatorial Library

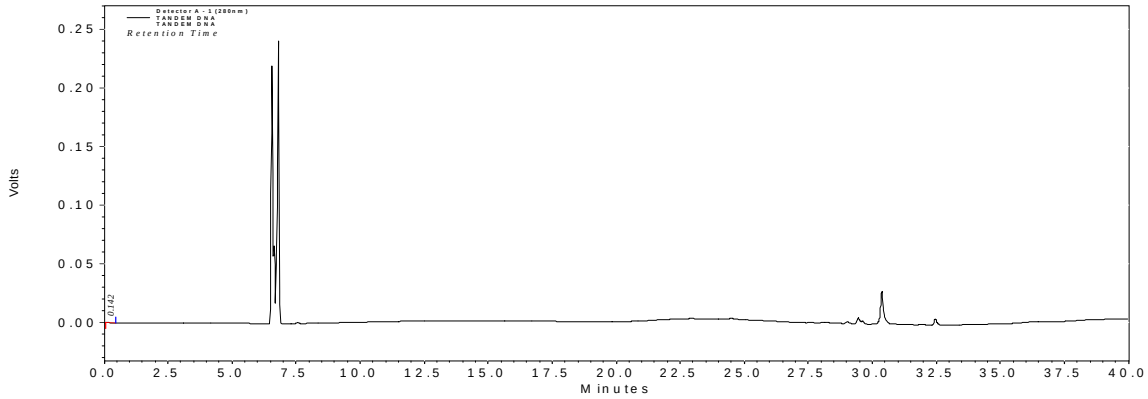


1 mM stock solutions of compounds **3-11** were prepared in phosphate buffer.[1] 100 μL of each stock solution were added to two vials. To the control vial was added 100 μL of buffer, while 100 μL of 250 μM DNA was added to the other. The solutions were stirred for a period of time (described in the main text) to allow for equilibration. After such time, equilibration (disulfide exchange) was halted by acidification of the solution through the addition of 1 drop phosphoric acid. The solution was immediately analyzed via HPLC as above with UV monitoring at 235 nm.

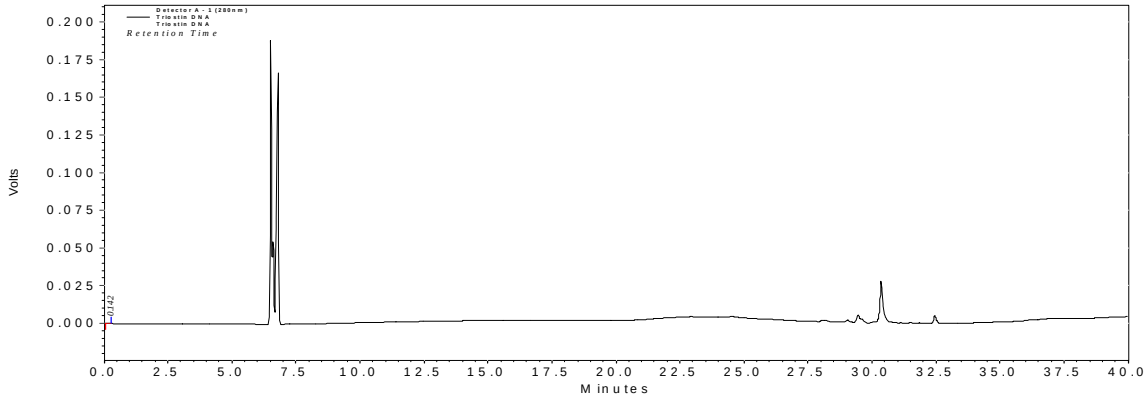
Solution phase DCC HPLC Analysis:

Underlined nucleotides are previously reported by other researchers to be bound by triostin A (**DNA_1**) and TANDEM (**DNA_2**).

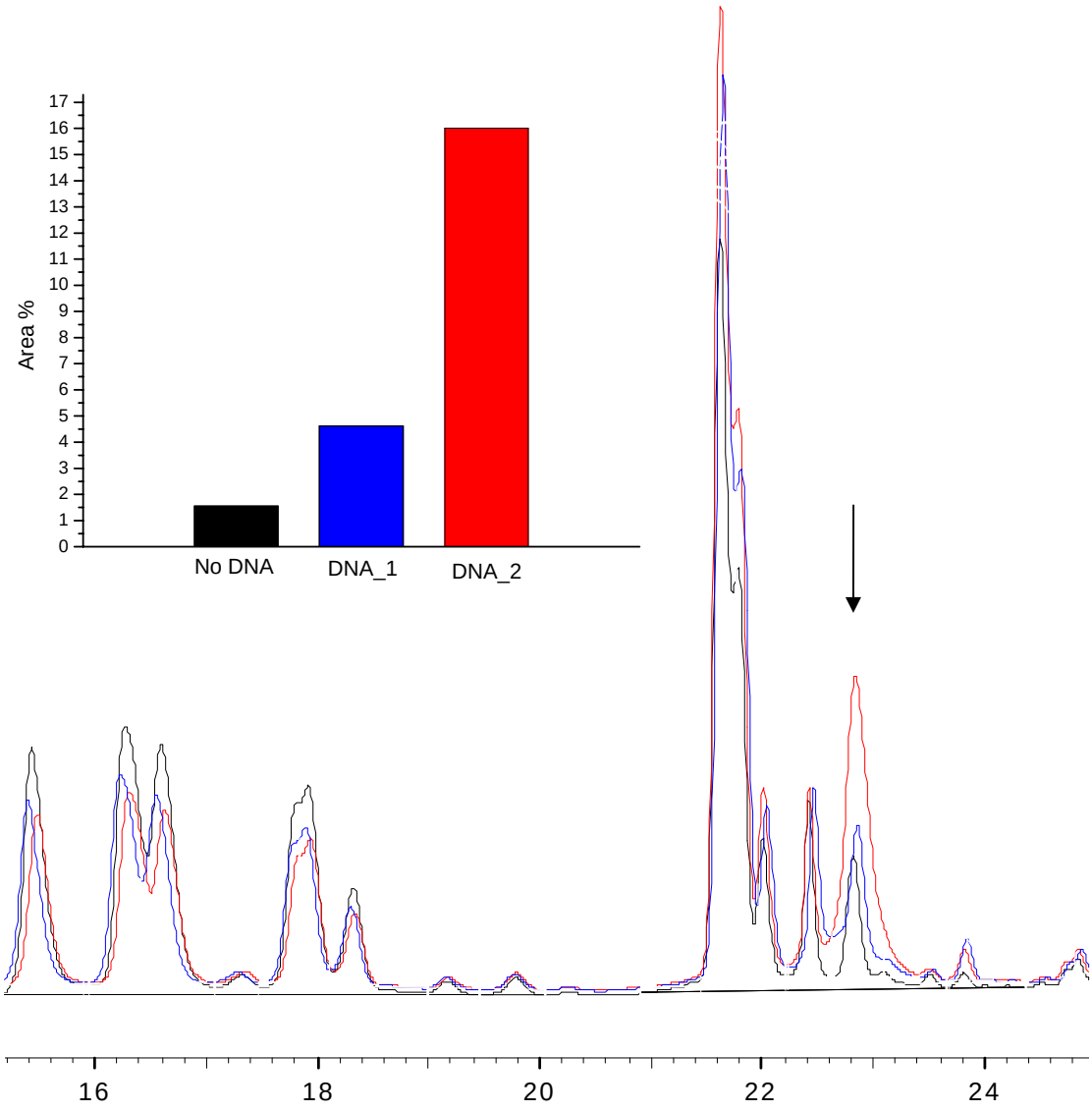
250 μM **DNA_1** in buffer (280 nm):**5'-TCTAGACGTC-3'**
3'-AGATCTGCAG-5'



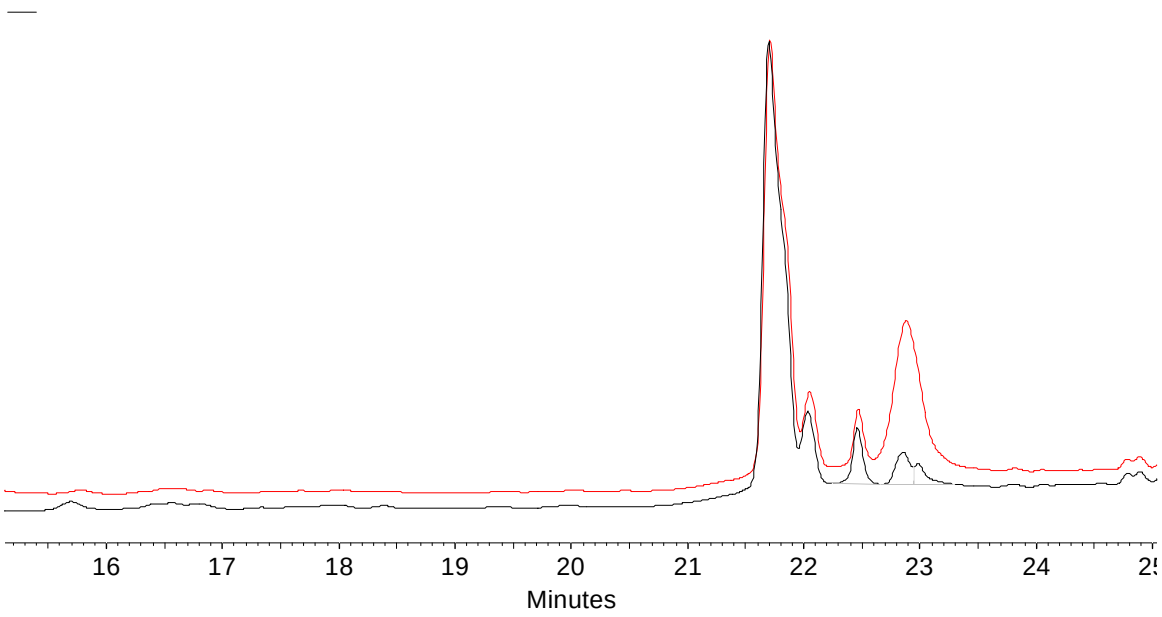
250 μM **DNA_2** in buffer (280 nm) **5'-CCATGATATC-3'**
3'-GGTACTATAG-5'



HPLC analysis of DCL after 24 hours equilibration: (—) no DNA added, (—) **DNA_1**, (—) **DNA_2**. Inset shows change in area % for the peak indicated by the arrow.



HPLC analysis of DCL after 7 days equilibration: (—) no DNA added, (—) **DNA_2**



V. On Bead Screening:

Fluorescence analyses were performed on an Olympus BX-50 microscope equipped with a QI-CAM FAST 1394 CCD (Q Imaging), and a fluorescent filter (Edmund Optics) with a characteristic wavelength of λ_{char} = 562 nm, an excitation wavelength of λ_{excite} = 531 nm, and an emission wavelength of λ_{emit} = 593 nm.

DNA_2* = 5'-CCATGATATC-3'
3'-GGTACTATAG-(C6Am)-(TAMRA)-5'

Image Analysis: All fluorescence images in the following section were generated with NIH-ImageJ v. 1.32 from raw CCD data. The same upper and lower intensity cutoffs are used for each image. We have included background images for reference even in cases where there is no apparent intensity, in order to provide a clear basis for comparison to other images.

Experimental setup and background images:

In all solid phase experiments, 20 mg TentaGel S Resin, onto which a single species of compound **3-11** had been synthesized, was transferred into a 1 ml solid phase reaction vessel. The resin has a loading of 0.2 mmol/g, and therefore the 20 mg aliquot carries approximately 4 μmoles monomer. After synthesis of the quinoline peptide, Trt protecting groups were deprotected by the addition of a 1% triethylsilane, 30% trifluoroacetic acid mixture in dichloromethane for 30 minutes at room temperature. Analysis of the resulting solution by mass spectrometry showed presence of the trityl group. It should be noted that TentaGel S Resin is stable in 30% trifluoroacetic acid.

Resin capped in *3*-mercapto-1-propanol was prepared as follows: 20 mg monomeric resin (4 μmoles) was mixed with 1ml of 200 mM 3-mercapto-1-propanol - phosphate buffer solution (50 eq., 200 μmoles) for 24 hours. The resin was washed 10 times each with tetrahydrofuran and water.

Upon being subjected to fluorescently tagged DNA, beads were washed as follows. The resin was drained under vacuum and washed with 1 mL of buffer for 5 minutes. This volume was drained under vacuum and the resin was rinsed with an additional 1mL.

After washing, resin was immediately positioned on a microscope slide and subjected to 100 msec. exposure under a fluorescent filter. Experiments were repeated in triplicate, and were found to be in agreement with one another.

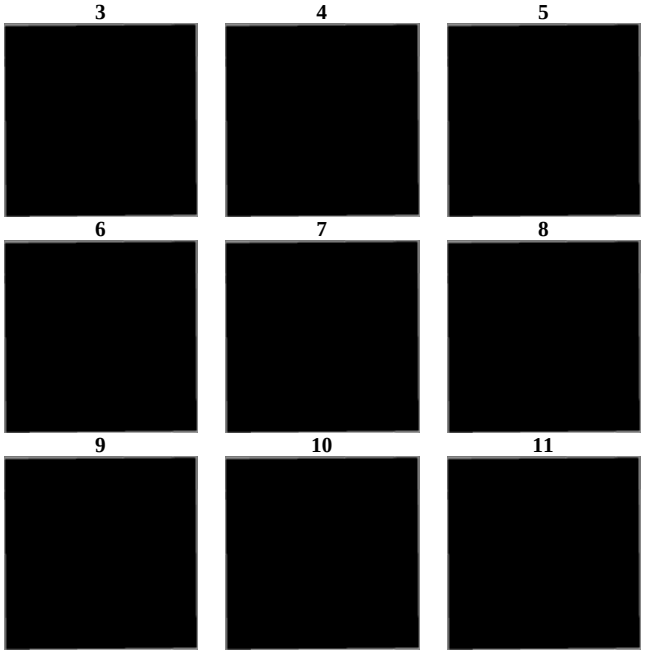
Tentagel resin, resin containing **3-11**, and resin incubated in 5 μM labeled DNA for 24 hours and washed as described above was examined under the same conditions and were found not to emit an observable fluorescence (data shown below).

Note that in the library analysis, an exposure time (100 ms) was chosen for imaging that provided the greatest differentiation between "bright" and "less bright" beads. One could of course vary this as needed depending on the level of affinity displayed by the tightest-binding library member.

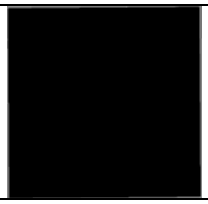
Background images:

Brightfield Image of Bead 3

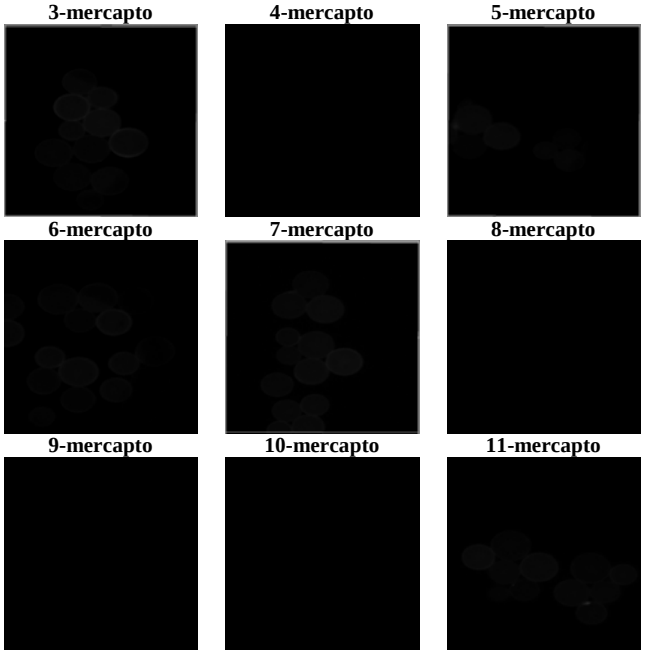
*Background Fluorescence images of beads bearing monomers **3-11**:*



Background images of Tentagel™ resin, and Tentagel™ resin after incubation in 5 µM DNA_2 then washed.*

Tentagel resin	Tental gel resin incubated in DNA_2*
	

*Background images of resin **3-11** capped with 3-mercapto-1-propanol*
After 24 hr incubation in DNA_2 and washing*

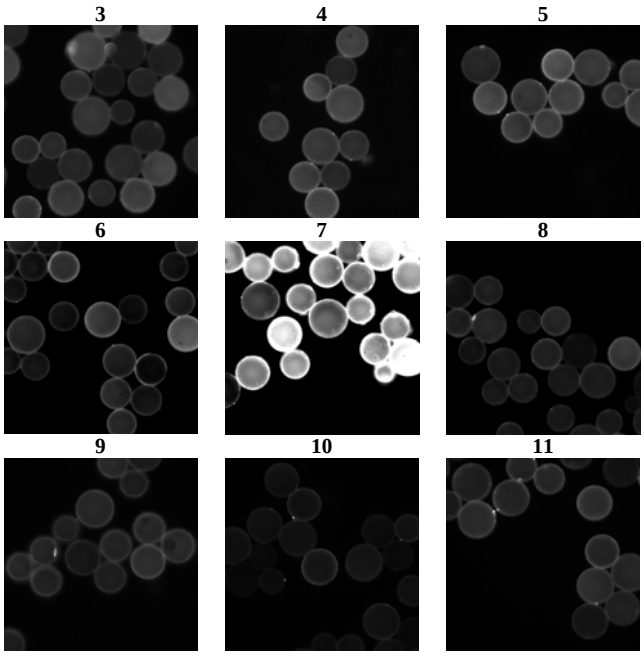


<u>Resin Bound Experiment No. 1</u>: Resin Bound Dynamic Combinatorial Chemistry ("Exchange")

In order to investigate target induced dynamics between solution phase monomers (45 μM total concentration) and di-sulfide capped monomeric resin (4 mM total concentration), we subjected 20 mg monomeric resin (4 μmoles) to 1 ml of 200 mM 3-mercapto-1-propanol - phosphate buffer solution (50 eq., 200 μmoles) for 24 hours. The resin was washed 10 times each with tetrahydrofuran and water. Next, the resin was subjected to 100 μL of 50 μM solutions of each monomer (45 μM total monomer, 900 μL total volume), as well as 100 μL of 50 μM **DNA_2*** (final DNA concentration 5 μM) and allowed to undergo exchange for a period of 7 days.

After such time the resin was drained under vacuum and washed as previously described. The resin was positioned on a microscope slide and subjected to 100 msec. exposure under a fluorescent filter. Images are shown below.

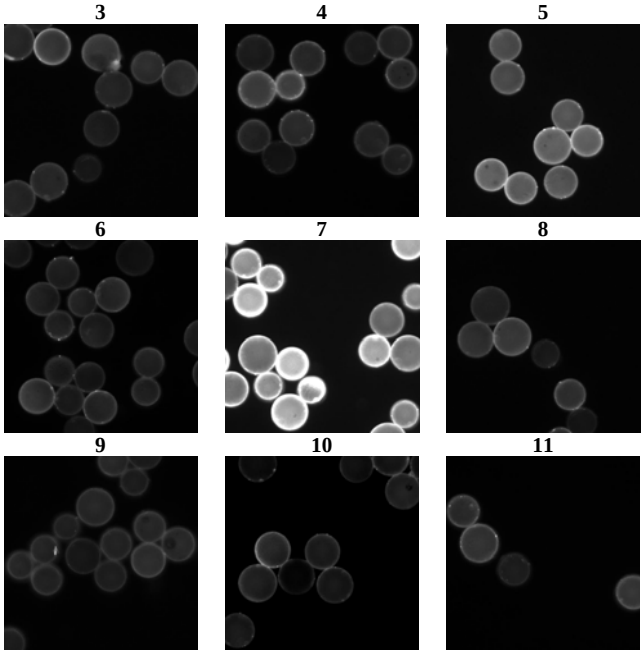
Resin bearing monomer capped with 3-mercapto-1-propanol and subjected to 45 μM solution phase monomers and 5 μM DNA_2 for 7 days.*



Resin Bound Experiment No. 2: Resin Capture

In order to test the time dependence of the resin capture, we subjected 20mg of monomeric resin (4 μmoles) to 45 μM solution phase monomers, and 5 μM DNA_2* for 24 hours. After a washing sequence identical to that described above the resin was monitored with a 100 msec. exposure under a fluorescent filter. Images are shown below.

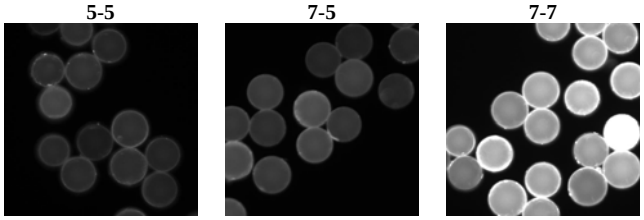
*Monomeric resin subjected to **3-11** and DNA_2* for24 hours*



Resin Bound Experiment No. 3: Confirmation of 7-7 as highest affinity binder by comparison with compounds 7-5 and 5-5.

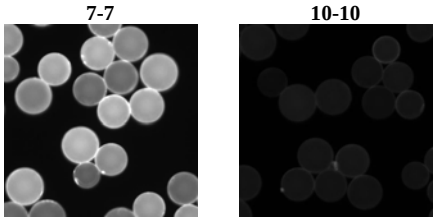
As discussed in the main text, resin bearing compound **7** was the most highly fluorescent following the resin-capture library screen, leading to the conclusion that homodimer **7-7** is the highest affinity binder. Resin bearing compound **5** displayed the next highest fluorescence. Therefore, in order to confirm that **7-7** was indeed the highest affinity ligand, a comparison study was done only using resin containing **5-7**, **7-7**, and **5-5**. While resin containing only monomeric **7** and **5** showed negligible fluorescence upon incubation in **DNA_2*** (as seen in background images), all dimers showed some affinity. Consistent with the prior screen, compound **7-7** however showed the most fluorescence after incubation with the target DNA (**DNA_2***), confirming its status as the highest affinity library member. These results are shown below.

*Confirmation of **7-7** as highest affinity ligand*



In addition, in order to further compare the difference in observed fluorescence to observed dissociation constants (vide infra) for compounds **7-7** (a compound selected by the screen) and **10-10** (a compound not selected by the screen), resin bearing each respective homodimer was incubated in labeled DNA under identical conditions to those described above for 4 hours. After a washing cycle identical to that described above the compounds were observed by fluorescence microscopy as in the library screen. The results, showing intensities entirely consistent with the full library screen, are shown below.

*Resin bearing only homodimers **7-7** and **10-10** after incubating in DNA_2* for 7 days*



Calculations designed to test whether amplification occurs in the "exchange" experiment.

Given the measured binding constants for selected ligands, we consider two primary hypothetical variants of the "Exchange" experiment.

Case 1: Amplification.

As discussed in the main text, ligand **7-7** was found to have a dissociation constant of ~3 μM:

$K_D = (L_T - x)\,(S_T - x) / (x)$; where x is the bound ligand and substrate to make (L·S)

$x^2 - x(L_T + S_T + K_d) + L_T S_T = 0$

$x^2 - L_T x + S_T x + K_d x + L_T S_T = 0$
(a) (b) (c)
The quadratic equation is then used to solve for x.

The following concentrations are used:

[DNA*] = 5μM
[Dimer] = 5 μM
K_d = 3 μM

Because monomer in bead is in much higher concentration than solution phase components (4 mM vs. 45 μM total, or 5 μM in solution for any individual monomer)

When we plug in the above concentrations, we find that 2.3 μM, or 2.3 nmoles of DNA is bound to the resin. This is an example of the best binder (bead 7), so there is no competition from solution phase members.

We now examine the other possibility: a resin bound ligand with a K_D of ~ 11 μM.

In the solution will be higher affinity ligands, such as the one discussed above that are not represented on the resin. We are adding 5 μM of each monomer, so 2.5 μM of each homodimer exists in solution. If we plug in the following concentrations

[DNA*] = 5μM
[Dimer] = 2.5 μM (solution phase)
K_D = 3 μM

We find that 1.37 μM, or 1.37 nmoles of DNA is bound in solution, leaving 3.63 nmoles of DNA (5 nmoles-1.37 nmoles) remaining in solution. In reality this would be even lower due to other competing solution phase dimers that would not be represented on the resin. If we continue with the remaining DNA:

[DNA*] = 3.63 μM
[Dimer] = 5 μM (resin bound)
K_d = 11 μM

We find that 0.97 μM, or 0.97 nmoles DNA are bound when resin dimer has K_D = 11μM, a 58% reduction in DNA found on the resin.

.
To conclude, allowing the system to equilibrate in the presence of DNA we find the following:

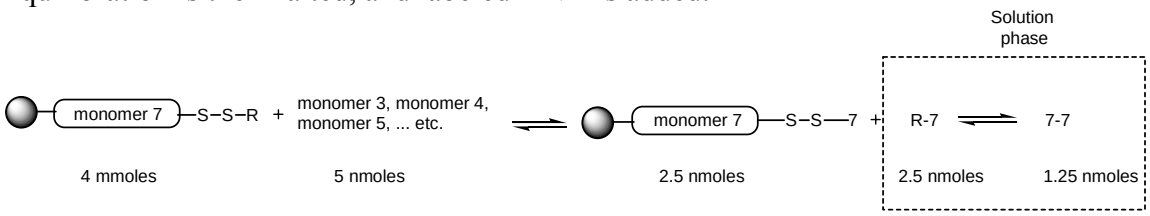
K_D = 3 μM = 2.3 nmoles DNA bound; K_D = 11 μM = 0.97 nmoles bound (note that this will actually be slightly lower because of other solution phase competition).

Since **10-10**, a compound subsequently determined to have a K_D = 11 μM, gave no observable fluorescence in either the "capture" or "exchange" experiment, we conclude that this amount of resin-bound DNA is not observable given our exposure cutoff.

Note also that we ignore differences in bound DNA after washes; this will cause an overall reduction in the quantity of DNA bound (systematic error).

Case 2: Static conditions.

Consider now a static condition, where the mercapto-propanol capped resin is fully equilibrated with solution phase monomers (45 nmoles total, 5 nmoles each monomer). Equilibration is then halted, and labeled DNA is added.



After equilibration, 2.5 nmoles of homodimer **7-7** is present on the resin. Without competition from solution phase **7-7**, resin bound homodimer with a K_D of 3 μM will bind 1.37 nmoles of DNA. This is 0.93 nmoles *less* DNA than is bound in the amplification experiment discussed above. We can go on to consider competition from solution phase dimers, namely the 1.25 nmoles of solution phase **7-7** generated from the equilibrium depicted in the dashed box. 1.25 nmoles of solution phase **7-7** (with a K_D of 3 μM) binds 0.73 nmoles of DNA. So, considering competition we find that the resin bound dimer binds 0.64 nmoles of DNA (1.37 – 0.73). This amount of DNA is *not* observable using the exposure cutoff used in the current analysis. Resin bearing dimer with a dissociation constant of 11 μM in the amplification experiment was calculated to bind 0.97 nmoles of DNA, which was not observable experimentally.

Conclusions: Amplification, resulting in observable fluorescence, is occurring in the "exchange" experiment.

	Experiment	Ligand K_D (μM)	$DNA_{initial}$ (nmoles)	$DNA_{resin\ bound}$ (nmoles)	Observable?
1	Amplification	3	5	2.3	Yes
2	Amplification	11	5	0.97	No
3	Static	3	5	0.64	No

VI. NMR Titration:

Homodimer **7-7** was prepared by stirring monomeric **7** in a phosphate buffer solution (10 mM sodium phosphate, pH = 7.4). After a period of 7 days, HPLC analysis revealed quantitative conversion of monomeric thiol to disulfide. Solutions were concentrated under vacuum, and diluted to a concentration of 2 mM in D_2O.

An NMR tube was washed thoroughly with water, then acetone, and dried in a 150 ºC oven for 24 hours. 400 µL of a 1 mM solution of compound **7-7** was added into the NMR tube. To this solution was added a 2 mM solution of **DNA_2**, and allowed to equilibrate for a period of five minutes. The ^{1}H NMR spectrum was then acquired using 64 scans on a Bruker AVANCE-400 operating at 400 MHZ. The titration is described below (Table 1):

Table 1. Experimental procedure for NMR titrations

DNA added (µL)	**DNA (nmoles)**	**DNA concentration (µM)**	**DNA : Ligand molar ratio (%)**
0	0	0	0
20	40	95	10
20	80	182	20
20	120	261	30
20	160	333	40
20	200	400	50
50	300	545	75
50	400	666	100

Small molecule-DNA interaction is most easily observed in this case by monitoring the singlet proton on the quinoline which is labeled H_A. Fortunately, this proton is located downfield of resonances associated with DNA (Figure 2). As a control, the experiment was repeated titrating D_2O into the NMR sample (Figure 3).

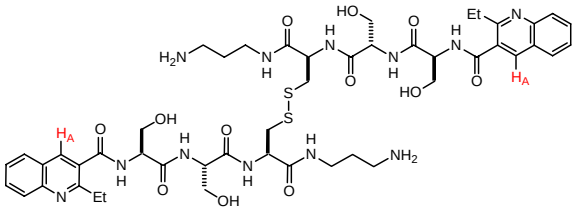


Figure 2a. ^{1}H NMR titration of DNA_2 into **7-7**.

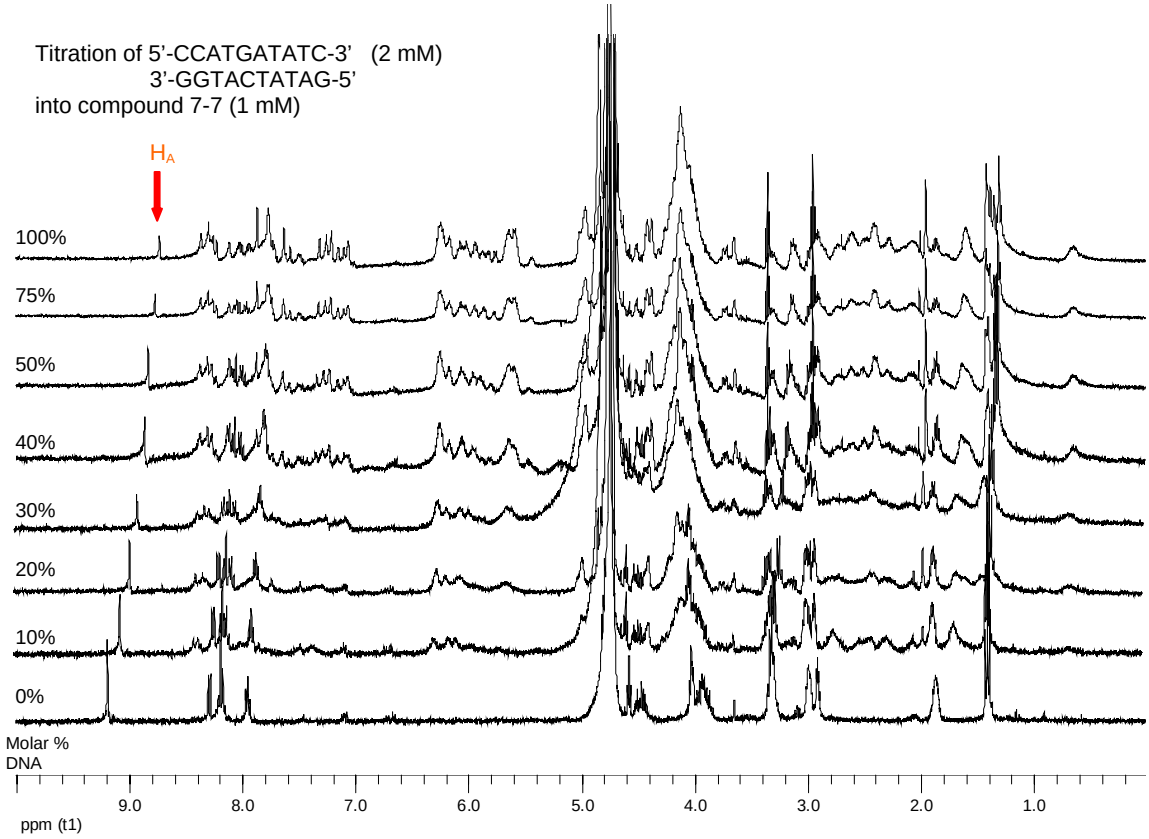
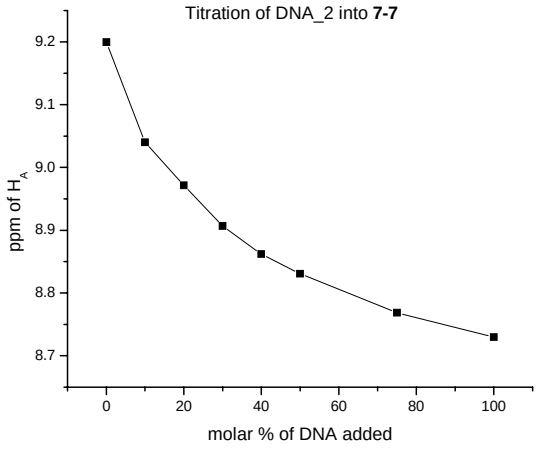


Figure 2b. Change in H_A as a function of molar % **DNA_2**.



Chemical shift of H_A in **7-7** as a function of addition of DNA solution.

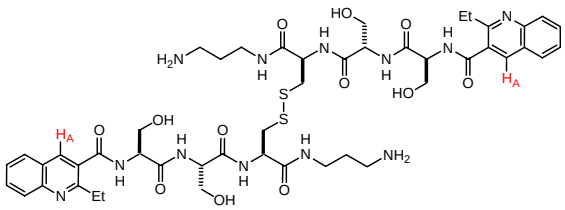


Figure 3a: ^{1}H NMR titration of D_2O into compound **7-7**

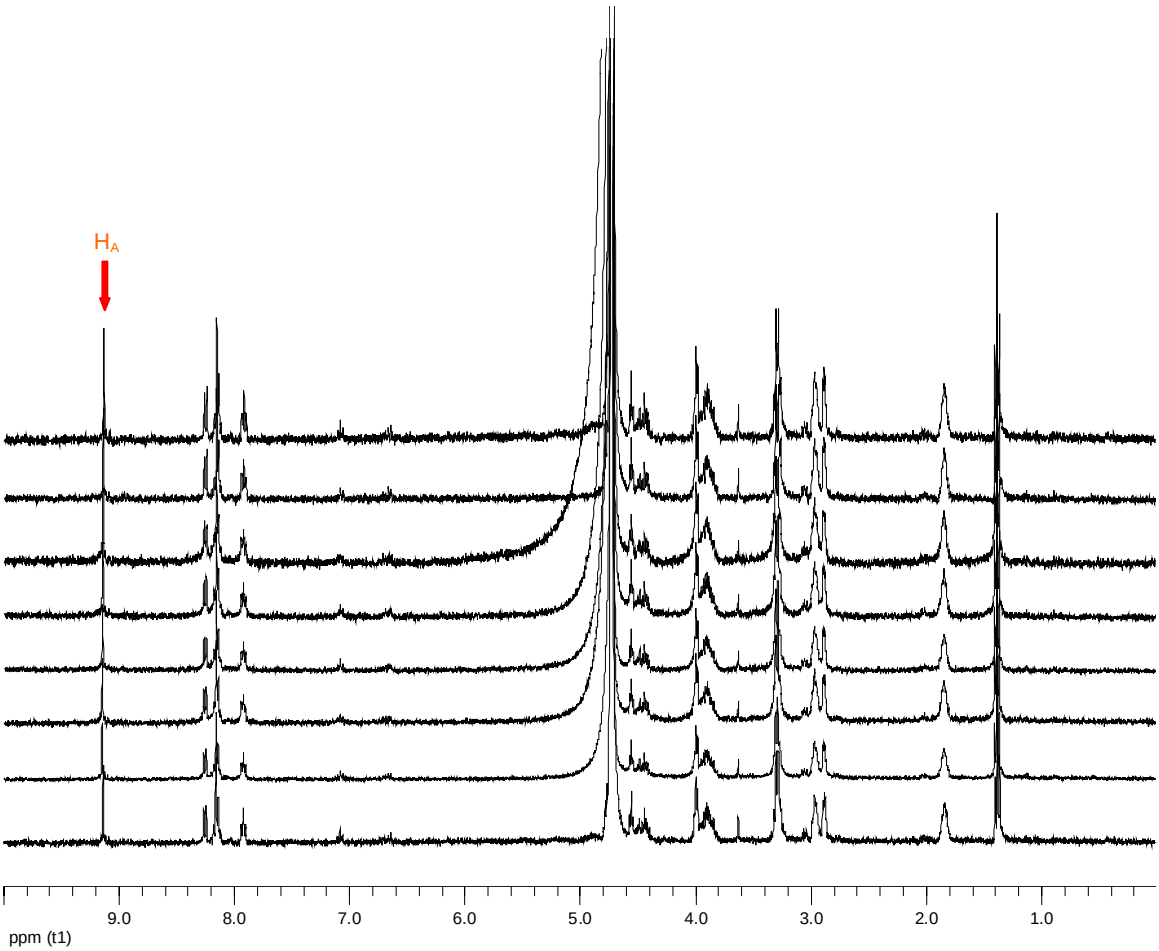
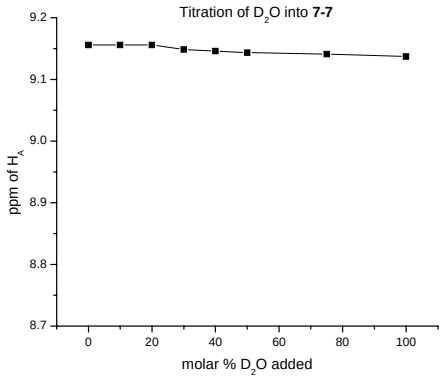


Figure 3b: Δppm of proton H_A vs. DNA concentration



Chemical shift of H_A in **7-7** as a function of addition of D_2O

VII. Equilibrium Dialysis:[2]

All data was recorded using a Shimadzu UV-1601PC UV-visable spectrophotometer at 235 nm. Solutions were monitored in a quartz cell with a pathlength of 1 cm.

A 100 μM solution of **7-7** was prepared in buffer solution and its absorbance, (A_i) was documented.

Following Beer's law;

$$A = \varepsilon\, b\, C \quad \text{(eq. 6.1)}$$

Where, A = absorbance, ε = extinction coefficient, b = pathlength, c = concentration

As an example, if we look at the 100 μM solution;

$$4.032 = \varepsilon\, (1)\, (100 \times 10^{-6})$$

Therefore, for **7-7**:

$$\varepsilon = 4.032 / (1)\, (100 \times 10^{-6})$$
$$\varepsilon = 40320$$

The 100 μM solution was diluted to 50 μM, 20 μM, 10 μM, 5 μM, 1 μM, and 500 nM. These solutions were subsequently analyzed by UV-vis. Using the above extinction coefficient, solution concentrations were verified using equation 6.1

To a 15 mL polypropylene conical tube was added 1 mL of ligand solution (external solution). A 2 mL glass vial was then equipped with membrane dialysis tubing (limit 8 kD) after being filled with 1 mL of buffer (internal solution). The membrane was secured and the vial inverted and placed in the exterior solution. The internal and external solutions were shaken and allowed to equilibrate for 5 days at room temperature. Solutions were subsequently removed and monitored by UV-vis to determine retention of ligand by the dialysis membrane. The 100 μM sample will be shown as an example.

After equilibration, the external solution was found to have an absorbance of 2.002,

$$2.002 = (40320)\, (1)\, (C)$$
$$C_{ext} = 49.6528\mu M$$

In a 1 mL volume;

$$(100 \times 10^{-6} \text{ moles / L}) \times (1.00 \times 10^{-3} \text{ L}) = \mathbf{m_{ext}} = 49.6528 \times 10^{-9} \text{ moles}$$

The internal solution was found to have an absorbance of 1.9998

$$1.9998 = (40320)\, (1)\, (C)$$
$$C_{int} = 49.5982\mu M$$

In a 1 mL volume;

$$(49.5982 \times 10^{-6} \text{ moles / L}) \times (1.00 \times 10^{-3} \text{ L}) = \mathbf{m_{int}} = 49.5982 \times 10^{-9} \text{ moles}$$

This calculation was repeated to find the moles of ligand present in the internal and external compartments after equilibration for all concentrations.

Subtracting initial moles ($\mathbf{m_i}$), from the moles found in the external **($\mathbf{m_{ext}}$)**, and internal solutions **($\mathbf{m_{int}}$)**, we are left with the number of moles bound by the membrane **($\mathbf{m_{mem}}$)**.

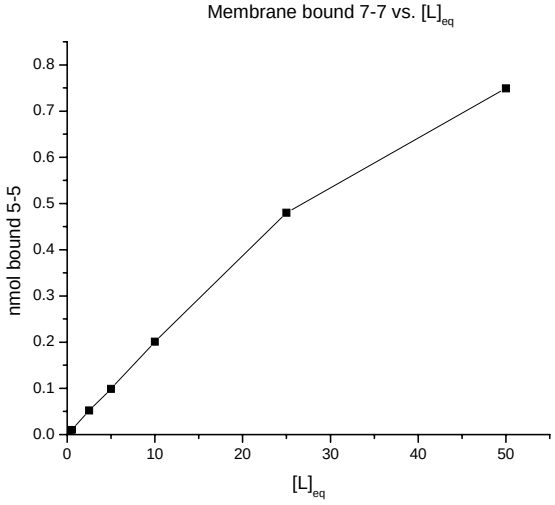
These calculations were repeated for all solutions, the result of which is shown below in Table 2.

Table 2. Analysis of internal and external concentrations, as well as membrane bound ligand by dialysis equilibration for **7-7**.

	100µM	**50µM**	**20µM**	**10µM**	**5µM**	**1µM**	**500nM**
A_i	4.032	2.019	0.8079	0.4041	0.2017	0.0404	0.02019
Initial moles of 7-7 (m_i), (10^{-9})	100	50	20	10	5	1	0.5
A_{ext}	2.002	0.9986	0.3993	0.1997	0.0998	0.0200	0.0098
External moles of 7-7 (m_{ext}) (10^{-9})	49.6528	24.7726	9.9033	4.9529	2.4752	0.4960	0.2431
A_{int}	1.9998	0.9981	0.3990	0.1995	0.0997	0.0199	0.0101
Internal moles of 7-7 (m_{int}), (10^{-9})	49.5982	24.7475	9.8958	4.9479	2.4727	0.4940	0.2505
Moles 7-7 bound by the membrane (m_{mem}), (10^{-9})	0.7490	0.4799	0.2009	0.0992	0.0521	0.0100	0.0064

A calibration curve can then be prepared (Figure 4).

Figure 4. Calibration curve of bound **7-7** to dialysis membrane



DNA used in membrane dialysis experiments is shown below. The sequence used in solution phase and multi-phase studies is effectively doubled, with an AA/TT spacer (highlighted in yellow). This was done to increase the molecular weight of the DNA, so that it does not cross the dialysis membrane (8 kD limit).

DNA_2b

5'-CCA TGA TAT CAA CCA TGA TAT C-3'
3'-GGT ACT ATA GTT GGT ACT ATA G-5'

In order to make sure that DNA was not passing through the dialysis tubing, a 2 mL vial was charged with 1 mL 5 μM DNA, fitted with membrane dialysis tubing and inverted in buffer solution for 5 days. No change in buffer absorbance was noted at 280 nm, leading us to the conclusion that no DNA was passing through the membrane filter.

To a series of 15 mL polypropylene conical tubes containing 1 mL of **7-7** solution at concentrations as above (external solution) was added an inverted 2 mL vial filled with 1 mL of 5 μM DNA solution and equipped with a dialysis membrane. The solution was shaken on a rotating platform and allowed to equilibrate for 5 days. Solutions were subsequently removed and monitored by UV-vis. As a comparison, the same experiment was performed on compound **10-10** (Table 3a and 3b).

Analysis of equilibrium dialysis experiments:

Table 3a. Analysis of bound **7-7** to **DNA_2b** from dialysis equilibration

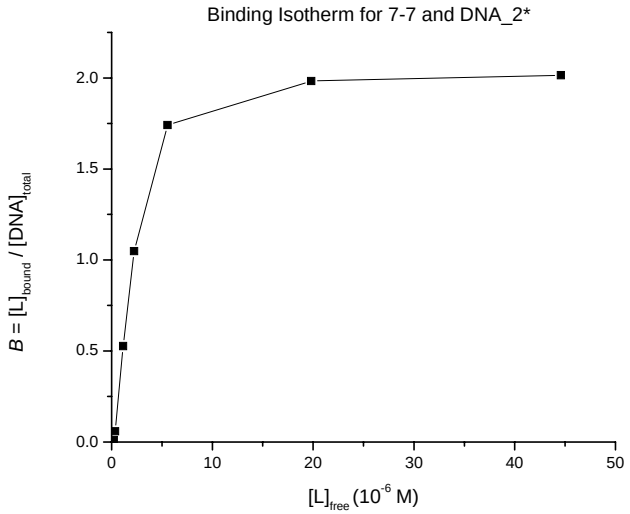
	100μM	**50μM**	**20μM**	**10μM**	**5μM**	**1μM**	**500nM**
A_i	4.0320	2.0190	0.8079	0.4041	0.2017	0.0404	0.0202
Total moles 7-7 (m_t), (10^{-9})	100	50	20	10	5	1	0.5
A'_{ext}	1.7978	0.7985	0.2237	0.0939	0.0466	0.0140	0.0089
External moles 7-7 (m'_{ext}), (10^{-9})	44.5883	19.8041	5.5481	2.3280	1.1557	0.3472	0.2207
Moles 7-7 bound by membrane (m_{mem}), (10^{-9})	0.7490	0.4799	0.2009	0.0992	0.0521	0.0100	0.0064
Moles 7-7 bound to DNA_2b (10^{-9})	10.0744	9.9119	8.7029	5.2448	2.6365	0.2956	0.0522

Using the above data, a binding isotherm is created by plotting $[L]_{free}$ versus the binding coefficient (B), which is defined in equation 6.2.

$$B = [L]_{bound} / [DNA]_{total} \quad \text{(eq. 6.2)}$$

The binding isotherm for compound **7-7** to **DNA_2b** is shown below in Figure 5.

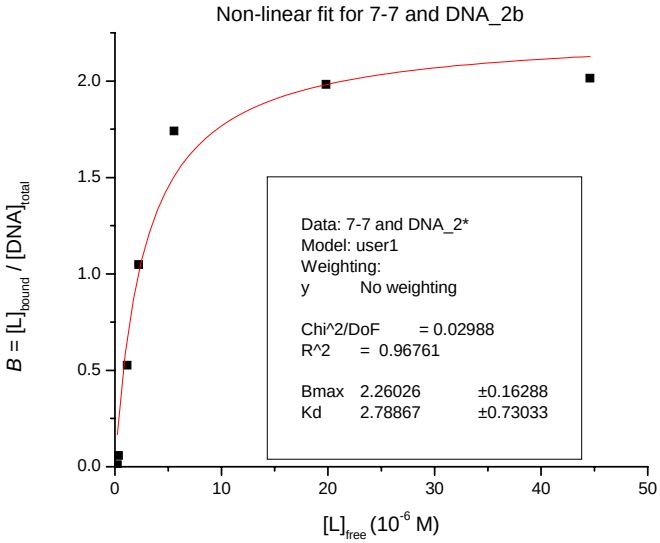
Figure 5: Binding Isotherm of **7-7** to **DNA_2b**



Binding isotherm data was fitted using non-linear regression (eq. 6.3), providing maximum number of binding sites (B_{max}), and the dissociation constant (K_d).

$$Y = (B_{max} \bullet x) / (K_d + x) \quad \text{(eq. 6.3)}$$

Figure 6. Non-linear regression analysis of **7-7** and **DNA_2b**



Analysis of 10-10 dialysis equilibration with DNA_2b

Figure 7. Calibration curve of bound **10-10** to dialysis membrane

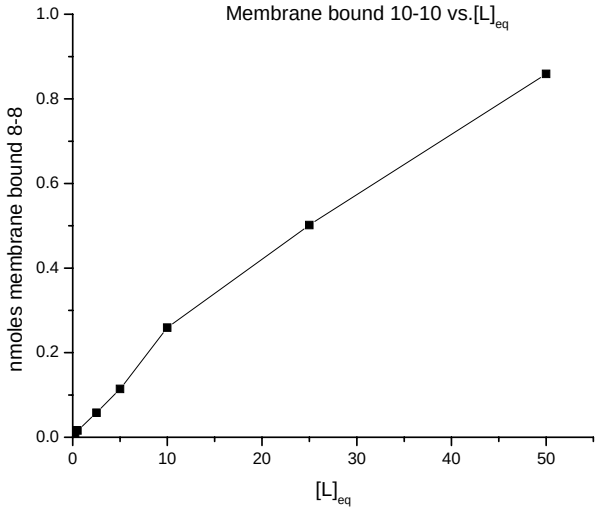


Table 3b. Analysis of bound **10-10** to DNA_2b from dialysis equilibration

	100µM	**50µM**	**20µM**	**10µM**	**5µM**	**1µM**	**500nM**
A_i	4.1630	2.0818	0.8320	0.4261	0.2134	0.0428	0.0213
Total moles 10-10 (m_t), (10^{-9})	100	50	20	10	5	1	0.5
A'_{ext}	1.9083	0.8988	0.3255	0.1542	0.0810	0.0179	0.0097
External moles 10-10 (m'_{ext}), (10^{-9})	45.8395	21.5782	7.8198	3.7041	1.9457	0.4299	0.2330
Moles 10-10 bound by membrane (m_{mem}), (10^{-9})	0.8792	0.5019	0.2589	0.1144	0.0578	0.0161	0.0068
Moles 10-10 bound to DNA_2b (10^{-9})	7.4418	6.3417	4.1015	2.4774	1.0508	0.1241	0.0272

Figure 7. Binding Isotherm for **10-10** and DNA_2b

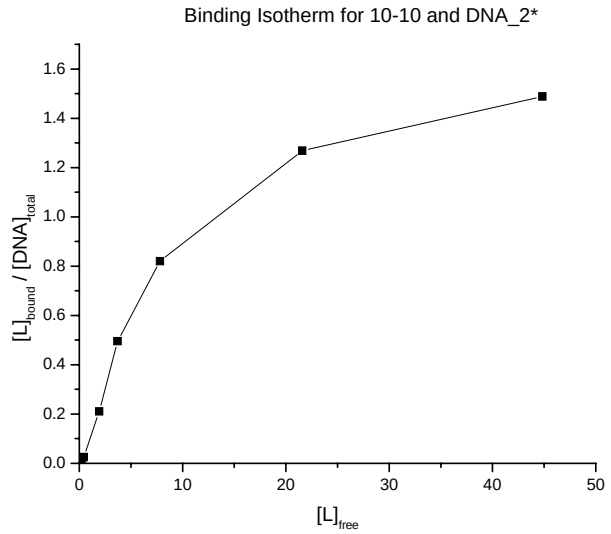
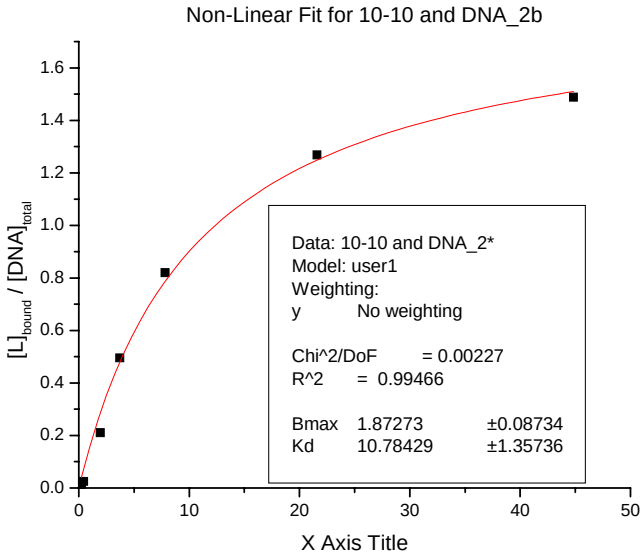


Figure 8. Non-linear regression analysis of **10-10** and DNA_2b



In order to ascertain whether ligand binding was subject to cooperative effects, or simply followed a statistical model, we plotted the ratio of moles total DNA to moles bound ligand *versus* the reciprocal of free ligand (10^{-6} M) for 1,5,10, and 20μM ligand solutions. Klotz *et al.* have shown that if a linear relationship of such a graph is obtained, statistical factors predominate.[3] Figures 9 and 10 show such a graph for ligands **7-7** and **10-10** respectively. While the number of data points is insufficient to unequivocally rule out any cooperative effects, the plots are clearly more consistent with statistical (nocooperative) binding.

Figure 9. Validity of statistical interpretation of binding by **7-7** to DNA

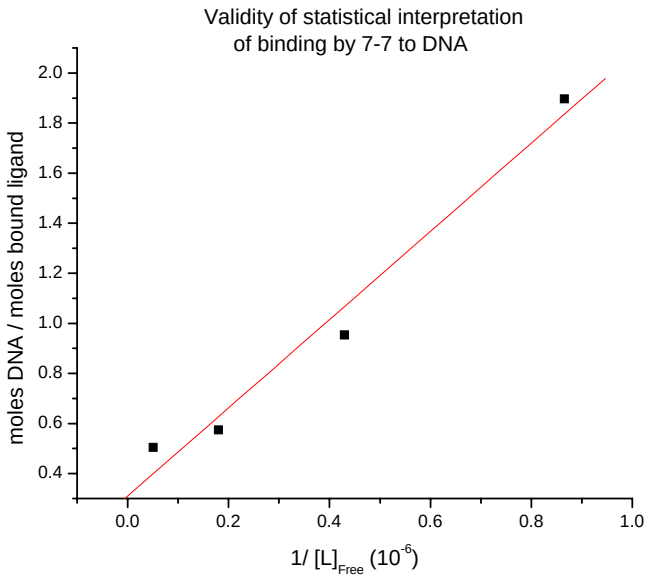
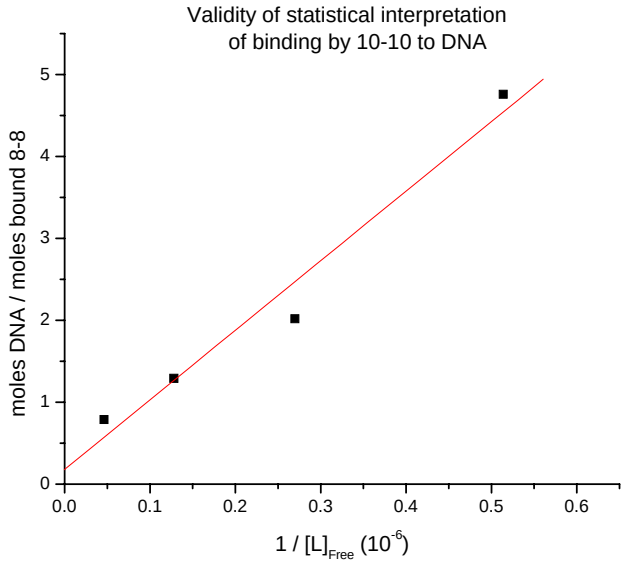


Figure 10. Validity of statistical interpretation of binding by **10-10** to DNA



VIII. References:

(1) The buffer used in all experiments is 10 mM phosphate buffer that is 250 mM NaCl and pH 7.5. See the following citation for its use in facilitating disulfide formation: Whitesides, G.M.; Lees, W.J. *J. Org. Chem.* **1993**, *58*, 642.
(2) (a) Klotz, I.M. *Ligand Receptor Energetics;* Wiley & Sons: New York, 1997, Appendix A1. (b) Connors, K.A. *Binding Constants*; Wiley & Sons: New York, 1987, Chapter 10.
(3) Klotz, I.M.; Walker, F.M.; Pivan, R.B. *J. Am. Chem. Soc.* **1946**, *68*, 1486.