Supporting Information for

Contributions of pocket depth and electrostatic interactions to affinity and selectivity of receptors for methylated lysine in water

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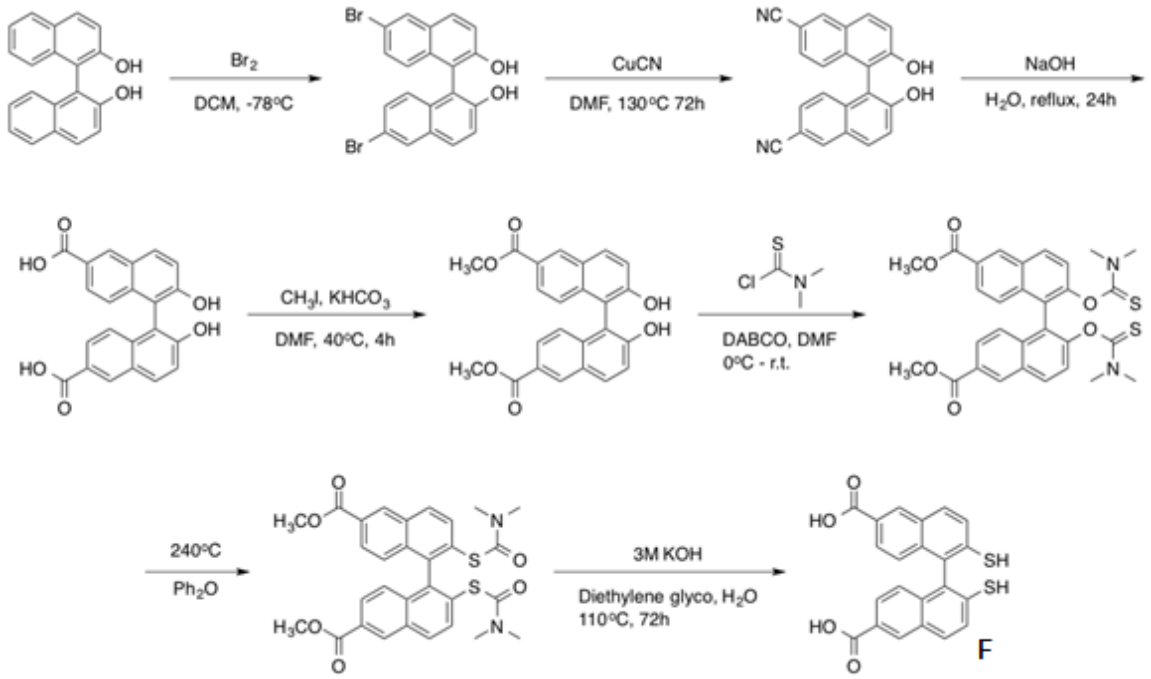
§ JEB and BCP contributed equally to this work.

Synthesis of Monomer F



Scheme S1. Synthesis of monomer **F**.

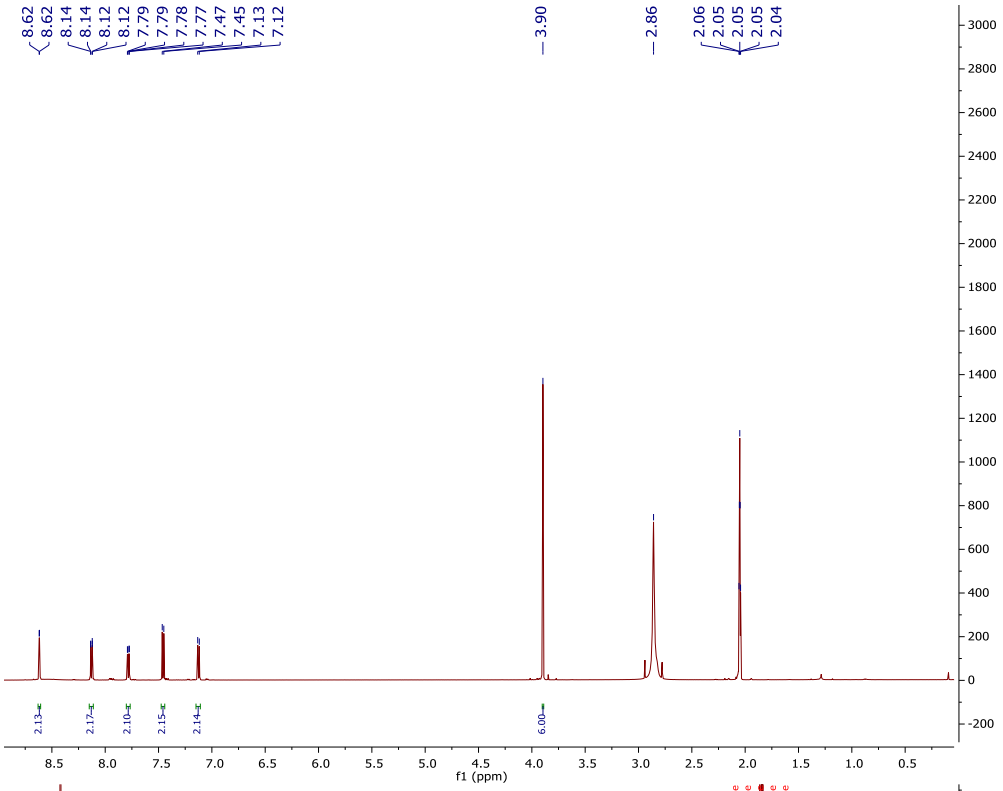
Monomer **F** was synthesized following a modified procedure for the synthesis of monomer **D**[1] and a modified synthesis of a dicarboxylic acid derivative of *rac*-Binol as described below.[2-4]

6,6'-dibromo-[1,1'-binaphthalene]-2,2'diol:[2] [1,1'-binaphthalene]-2,2'diol (10.0 g, 34.9 mmol) was added to a dry, nitrogen-flushed flask and the system purged with nitrogen. DCM (190 mL) was added to the flask and the solution was cooled to -78 °C in a dry ice, acetone bath. Bromine (14.9 g, 2.67 equivalents) was added dropwise to the solution and the reaction proceeded at -78 °C for 2.5 hours. The reaction was warmed to room temperature and reacted for an additional 30 minutes. The reaction was quenched upon the addition of sodium bisulfite (10% aqueous, 180 mL), and the organic phase collected and washed with brine (3x, 20 mL). The solution was dried over magnesium sulfate, filtered, and solvent removed under reduced pressure to afford an off-white solid. This solid was recrystallized in $CHCl_3$/hexanes to afford fluffy white crystals (15.1 g, 98% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ = 8.06 (s, 2H), 7.90 (d, 2H), 7.41 (d, 2H), 7.38 (dd, 2H), 6.97 (d, 2H), 5.014 (s, 2H). ^{13}C NMR ($CDCl_3$, 150 MHz): δ = 152.91, 131.80, 130.81, 130.67, 130.53, 130.40, 125.81, 118.91, 117.96, 110.52. MS (ESI-). Expected: 442.91 $[M-H]^-$; Observed: 442.86 $[M-H]^-$.

2,2'-dihydroxy-[1,1'binaphthalene]-6,6'-dicarbonitrile:[3] 6,6'-dibromo-[1,1'-binaphthalene]-2,2'diol (12.3g, 27.7 mmol) was dissolved in DMF (61.5 mL) and CuCN (6.2 g, 2.5 equivalents) was added. The system was flushed with nitrogen and heated to 130 °C for 4 days. Most of the DMF was removed under vacuum to afford a brown sludge. This reaction mixture was dissolved in ethyl acetate and water until only insoluble powder remained. The organic phase was washed with 5% NaCl (3x, 20 mL), brine (2x, 10 mL), dried over magnesium sulfate, filtered, and the solvent removed under reduced pressure to afford a tan powder (9.17 g, 98% yield). MS (ESI-). Expected: 335.08 $[M-H]^-$; Observed: 335.05 $[M-H]^-$.

2,2'-dihydroxy-[1,1'-binaphthalene]-6,6'-dicarboxylic acid:[4] 2,2'-dihydroxy-[1,1'binaphthalene]-6,6'-dicarbonitrile (2.3 g, 6.8 mmol) was suspended in aqueous sodium hydroxide (6N, 10.3 mL) and the solution heated to reflux for 22 hours. The solution was cooled to room temperature, diluted with H_2O (15 mL) and acidified with concentrated HCl (20 mL) to form a white precipitate. The precipitate was filtered to afford the product (2.34 g, 91% yield) as an off-white powder, which was used without further purification. ^{1}H NMR (DMSO-d_6, 600 MHz): δ = 12.77 (s, 2H,), 8.55 (d, 2H), 8.07 (d, 2H), 7.68 (d, 2H), 7.40 (d, 2H), 6.99 (d, 2H). ^{13}C (DMSO-d_6, 150 MHz): δ = 167.64, 155.32, 136.32, 130.95, 130.67, 127.10, 125.47, 124.55, 124.40, 119.35, 115.13. MS (ESI-). Expected: 373.07 $[M-H]^-$; Observed: 372.97 $[M-H]^-$.

Dimethyl 2,2'-dihydroxy-[1,1'-binaphthalene]-6,6'-dicarboxylate: 2,2'-dihydroxy-[1,1'-binaphthalene]-6,6'-dicarboxylic acid (1.15 g, 3.07 mmol) was dissolved in anhydrous DMF (10 mL) and cooled to 0 °C. Potassium bicarbonate (0.342 g, 3.38 mmol, 1.1 equivalents) was added and the solution stirred for 15 minutes. Methyl iodide (0.48 g, 3.4 mmol, 1.1 equivalents) was added dropwise and the solution heated to 40°C. After stirring for 3 hours, a second equivalent of potassium bicarbonate (0.34 g, 3.4 mmol) was added, followed by dropwise addition of methyl iodide (0.48 g, 3.4 mmol) and the solution was stirred for 15 hours at 40 °C. The reaction was quenched by the addition of H_2O (70 mL) to form a white precipitate. The precipitate was filtered and washed extensively with warm H_2O to afford a white powder (1.18 g, 96% yield), which was used without further purification. MS (ESI-). ^{1}H NMR (Acetone-d_6, 600 MHz): δ = 8.62 (s, 2H), 8.13 (d, 2H), 7.78 (d, 2H), 7.46 (d, 2H), 7.13 (d, 2H), 3.90 (s, 6H). ^{13}C NMR (Acetone-d_6, 150 MHz): δ =167.46, 156.85, 137.85, 132.37, 131.91, 128.89, 126.43, 125.50, 120.52, 115.07. Expected: 401.10 $[M-H]^-$; Observed: 401.09 $[M-H]^-$.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7.12
3.90
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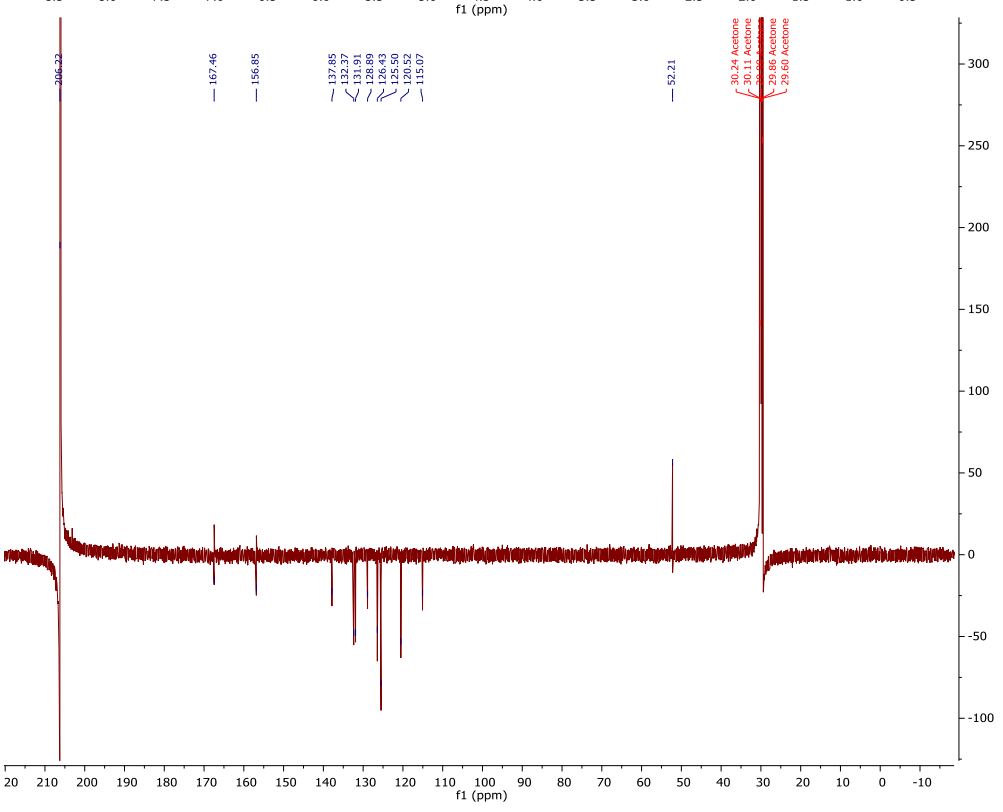
2.17
2.10
2.15
2.14
6.00
f1 (ppm)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132.37
131.91
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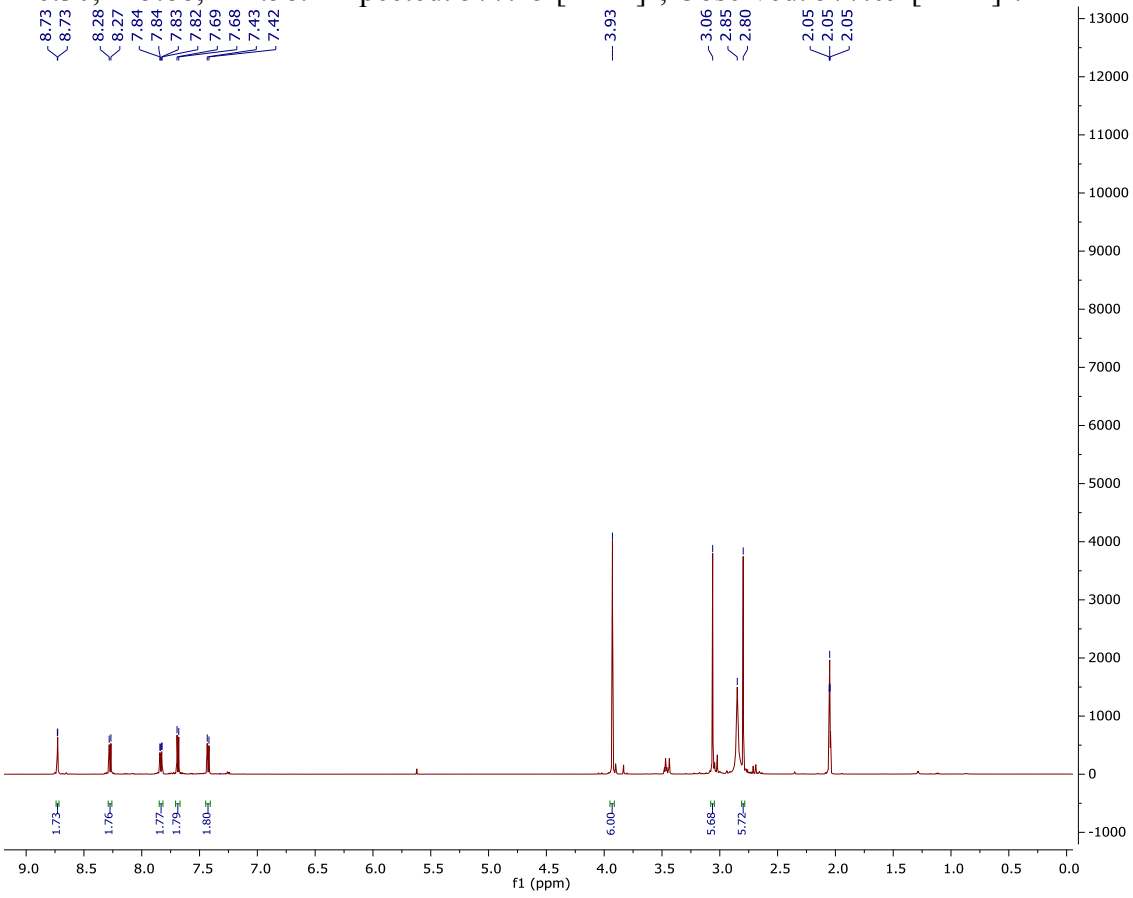
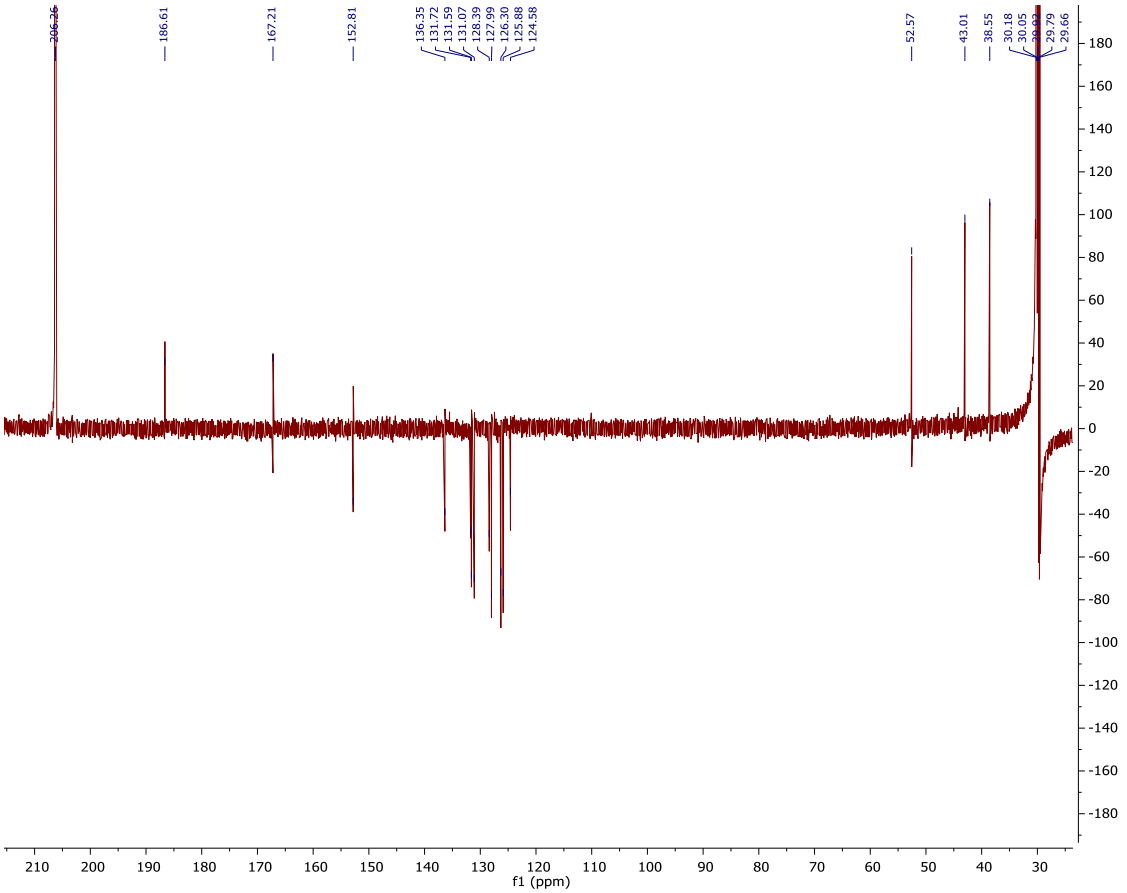
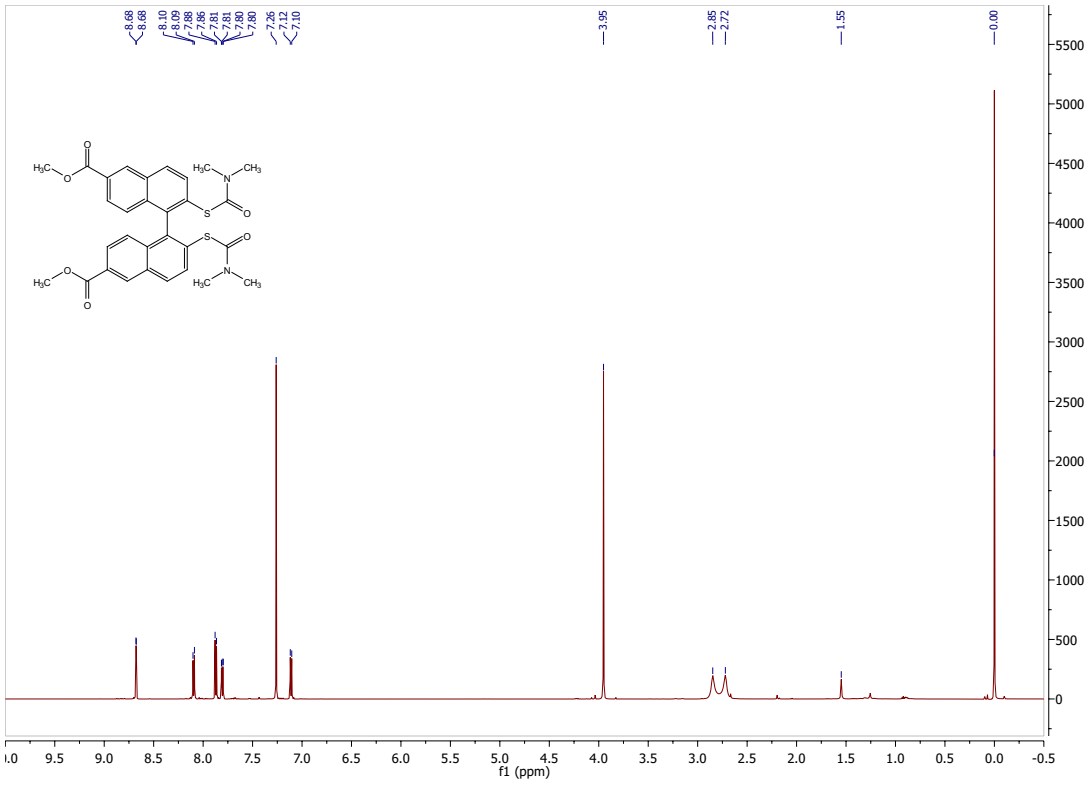
126.43
125.50
120.52
115.07
52.21
30.24 Acetone
30.11 Acetone
29.86 Acetone
29.60 Acetone
f1 (ppm)

Dimethyl 2,2'-bis((dimethylcarbamothioyl)oxy)-[1,1'binaphthalene]-6,6'-dicarboxylate: Dimethyl 2,2'-dihydroxy-[1,1'-binaphthalene]-6,6'-dicarboxylate (0.80 g, 1.9 mmol) was dissolved in anhydrous DMF (8 mL) and cooled to 0 °C. To this stirring solution, 1,4-diazabicyclo[2.2.2]octane (1.3 g, 11.9 mmol) was added in two equivalent portions and the resulting suspension stirred for 15 minutes. N,N-dimethylthiocarbamoyl chloride (1.5 g, 11.9 mmol) was dissolved in anhydrous DMF (4 mL) and added dropwise over 5 minutes at 0 °C. The solution was allowed to warm to room temperature under inert atmosphere and followed by TLC for 10 hours until reaction completion. The reaction mixture was poured into cold water (84 mL) and the resulting white precipitate was filtered and washed extensively with warm water to afford a white powder, **(**1.10 g, 95% yield) which was used without further purification. ^{1}H NMR (Acetone-d_6, 600 MHz): δ = 8.73 (d, 2H), 8.27 (d, 2H), 7.83 (dd, 2H), 7.68 (d, 2H), 7.43 (d, 2H), 3.93 (s, 6H), 3.06 (s, 6H), 2.80 (s, 6H). MS (ESI+).^{13}C NMR (Acetone-d_6, 150 MHz): δ =186.61, 167.21, 152.81, 136.35, 131.72, 131.59, 131.07, 128.39, 127.99, 126.30, 125.88, 124.58. Expected: 577.15 $[M+H]^+$; Observed: 577.09 $[M+H]^+$.





Dimethyl 2,2'-bis((dimethylcarbamoyl)thio)-[1,1'-binaphthalene]-6,6'-dicarboxylate: Dimethyl 2,2'-bis((dimethylcarbamothioyl)oxy)-[1,1'binaphthalene]-6,6'-dicarboxylate (0.30 g, 0.52 mmol) was suspended in diphenyl ether (3.0 mL) and heated to 250 °C and monitored by TLC for 6 hours. Upon reaction completion, the solution was cooled to 40°C and poured into warm hexanes (15 mL). The product was purified by column chromatography on silica gel to afford a white powder (0.24 g, 80% yield). ^{1}H NMR ($CDCl_3$, 600 MHz): δ = 8.68 (d, 2H), 8.10 (dd, 2H), 7.87 (d, 2H), 7.81 (dd, 2H), 7.14 – 7.09 (m, 2H), 3.95 (s, 3H) 2.85 (s, 6H), 2.72 (s, 6H). ^{13}C ($CDCl_3$, 150 MHz): δ = 166.99, 165.43, 140.06, 135.02, 133.92, 132.37, 131.55, 130.89, 129.53, 128.14, 126.92, 126.01, 52.30, 36.79. MS (ESI+). Expected: 577.15 $[M+H]^+$; Observed: 577.09 $[M+H]^+$.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8.68
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8.09
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7.86
7.81
7.80
7.80
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7.10
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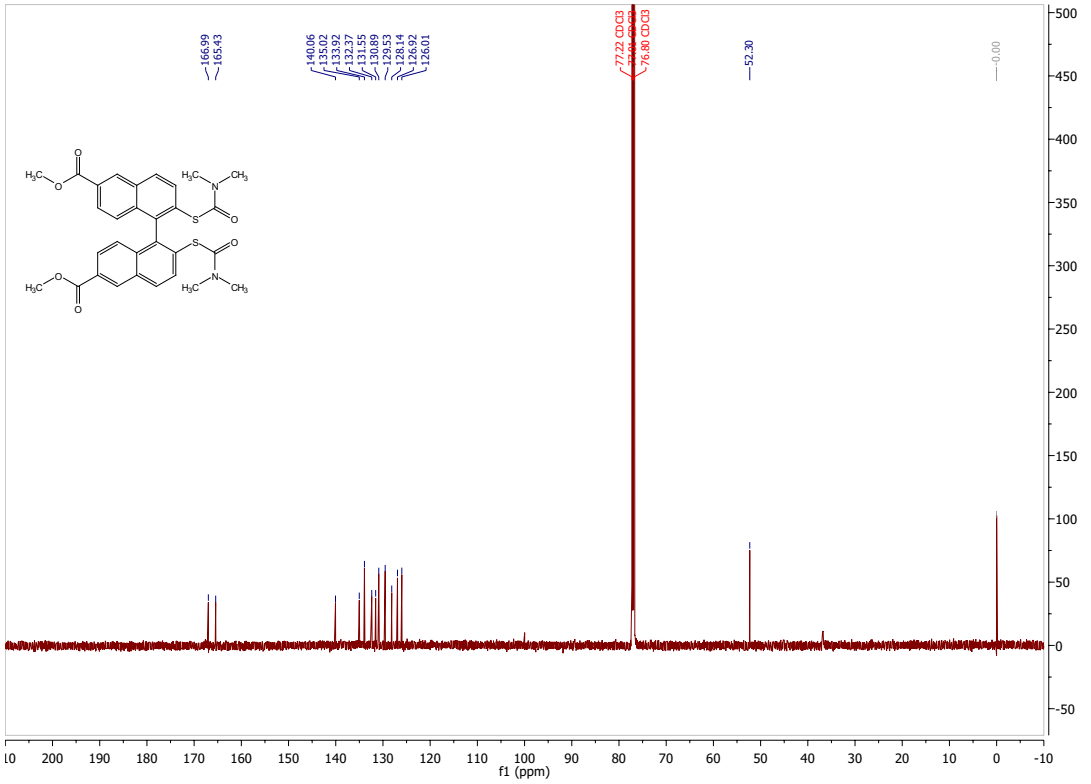
2.85
2.72
1.55
0.00
f1 (ppm)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126.92
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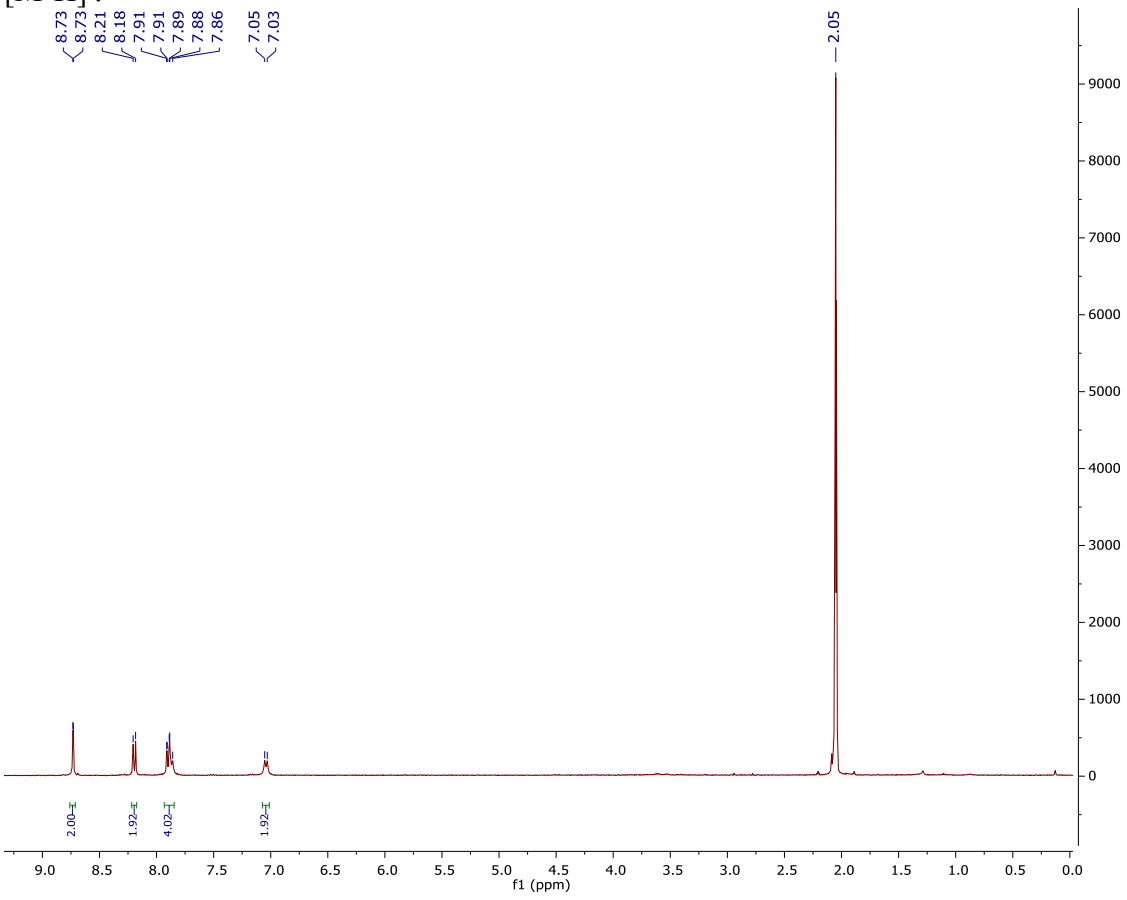
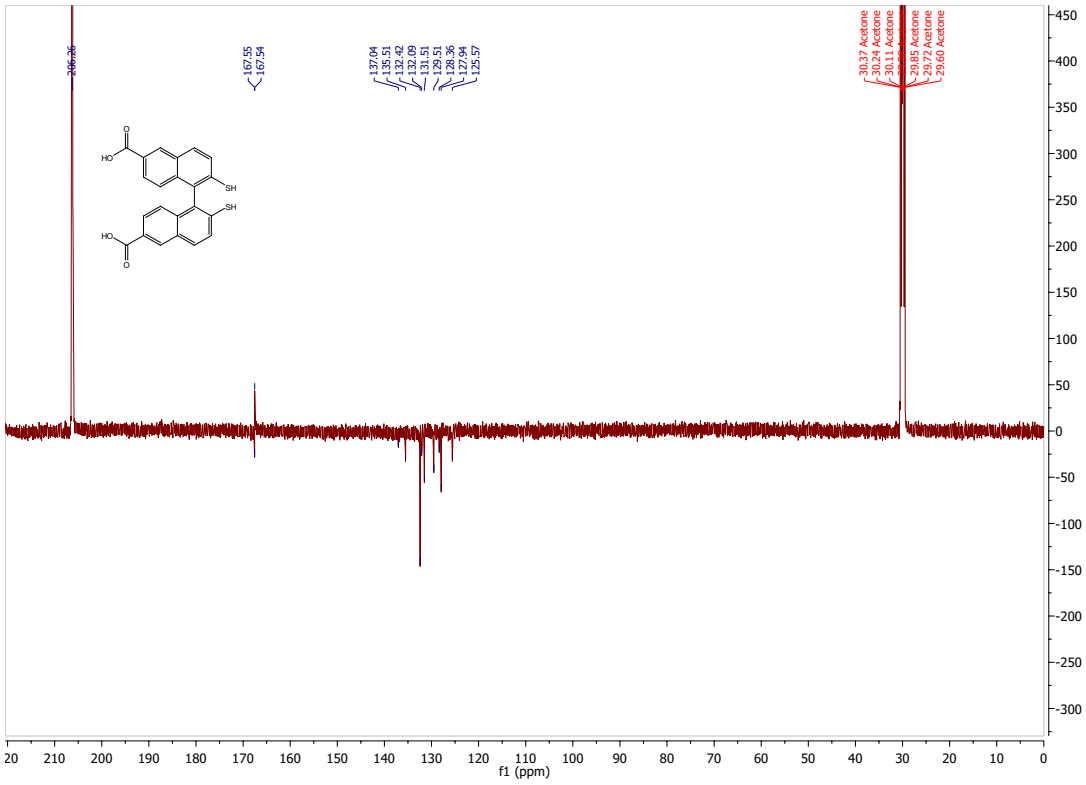
77.22 CDCl3
76.80 CDCl3
52.30
0.00
f1 (ppm)

2,2'-dimercapto-[1,1'-binaphthalene]-6,6'-dicarboxylic acid (F): Dimethyl 2,2'-bis((dimethylcarbamoyl)thio)-[1,1'-binaphthalene]-6,6'-dicarboxylate (0.035 g, 0.06 mmol) was suspended in a 1.75M potassium hydroxide solution (4.0 mL, 50% H_2O in diethylene glycol) which had been degassed under bubbling nitrogen for 2 hours. The solution was heated to 100 °C and stirred under positive nitrogen pressure for 72 hours to ensure reaction completion. The solution was cooled to room temperature and diluted with degassed H_2O (50 mL) and HCl (6 M) was added to afford a beige precipitate. The resulting powder was collected by centrifugation (6,000 rpm, 5 minutes) and washed by sonication in degassed water (3x, 25 mL) to remove diethylene glycol. The product was collected as a suspension in degassed water, frozen, and lyophilized to afford a pure beige powder (0.018 g, 73% yield). ^{1}H NMR (Acetone-d_6, 400 MHz): δ = 8.73 (s, 2H), 8.20 (d, 2H), 7.89 (m, 4H), 7.04 (d, 2H), ^{13}C (Acetone-d_6, 150 MHz) δ = 167.55, 137.04, 132.42, 131.51, 129.51, 128.36, 125.57. MS ESI(-). Expected: 405.03 $[M-H]^-$; Observed: 405.06 $[M-H]^-$.

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131.51
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127.94
125.57
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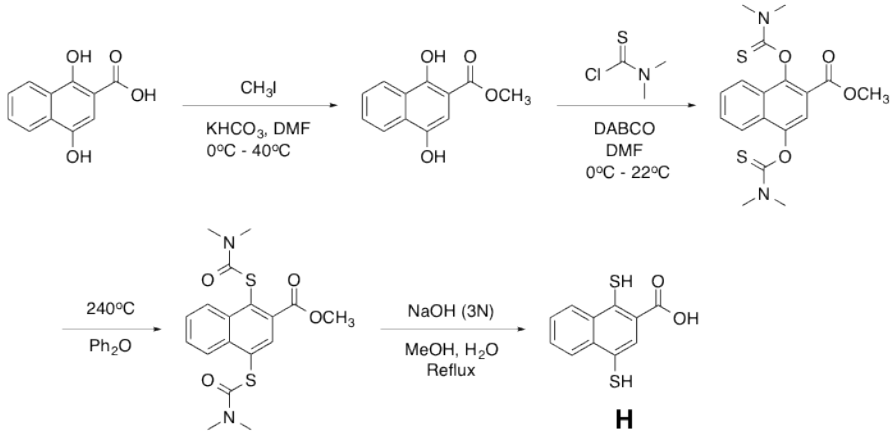
30.24 Acetone
30.11 Acetone
29.85 Acetone
29.72 Acetone
29.60 Acetone
HO
O
SH
HO
O
SH
f1 (ppm)

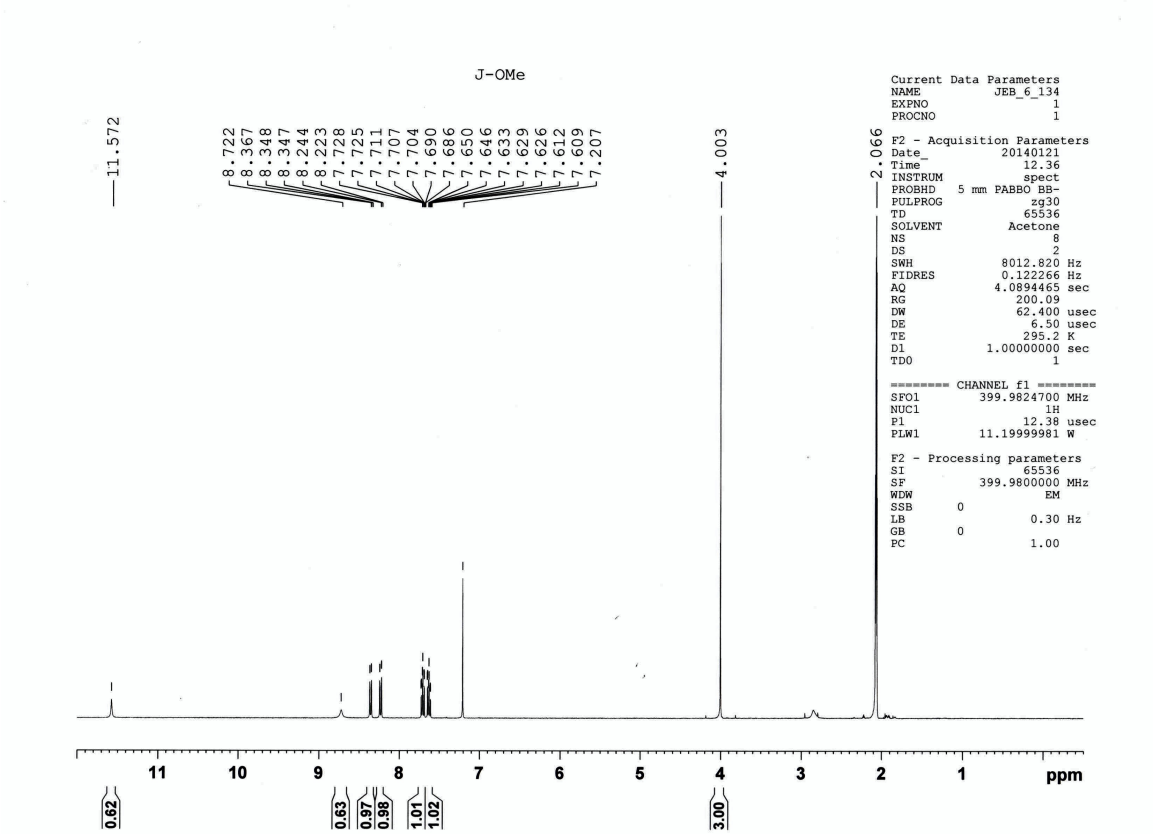
Synthesis of Monomer H.



Scheme S2. Synthesis of Monomer **H**.

Monomer H was synthesized following a modified procedure for the synthesis of monomer **D**[1] as described below.

Methyl 1,4-dihydroxy-2-naphthoate: A dry 100 mL flask was charged with 1,4-dihydroxy-2-naphthoic acid (1.99 g, 9.75 mmol) and flushed with nitrogen. To this flask, N,N-dimethylformamide (anhydrous, 20 mL) was added and the starting material dissolved. The solution was cooled to 0 °C and $KHCO_3$ (granular, oven-dried, 1.10g, 10.8 mmol, 1.1 equivalents) was added. The solution was allowed to warm to room temperature and stirred for 20 minutes. Methyl iodide (660 μL, 10.8 mmol, 1.1 equivalents) was added dropwise and the solution was warmed to 40 °C in a water bath. The reaction was monitored by TLC. After 4 hours, upon the disappearance of the starting material, the solution was cooled to room temperature and poured into water (140 mL) to precipitate product and dissolve potassium iodide. Sodium bicarbonate (saturated, 20 mL) was added to the solution to dissolve any unreacted starting material. The solution was stirred for 10 minutes, filtered, and the filtrate washed with water and subsequently dried to afford pure product as a brown powder (1.93 g, 91% yield). ^{1}H NMR (Acetone-d_6, 400 MHz): δ = 11.55 (s, 1H), 8.70 (s, 1H), 8.34 (d, 1H), 8.21 (d, 1H), 7.69 (m, 1H), 7.61 (m, 1H), 7.19 (s, 1H), 3.98 (s, 3H). ^{13}C NMR (150 MHz, Acetone-d_6): δ = 172.21, 155.72, 155.03, 129.72, 127.30, 126.44, 124.43, 123.23, 105.72, 105.11, 101.02, 53.00. MS (ESI-). Expected: 217.05 $[M-H]^-$; Observed: 217.00 $[M-H]^-$.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4.003
2.066
Current Data Parameters
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PROCNO 1
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FIDRES 0.122266 Hz
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RG 200.09
DW 62.400 usec
DE 6.50 usec
TE 295.2 K
D1 1.00000000 sec
TD0 1
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NUC1 1H
P1 12.38 usec
PLW1 11.19999981 W
F2 - Processing parameters
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SF 399.9800000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
11
10
9
8
7
6
5
4
3
2
1
ppm
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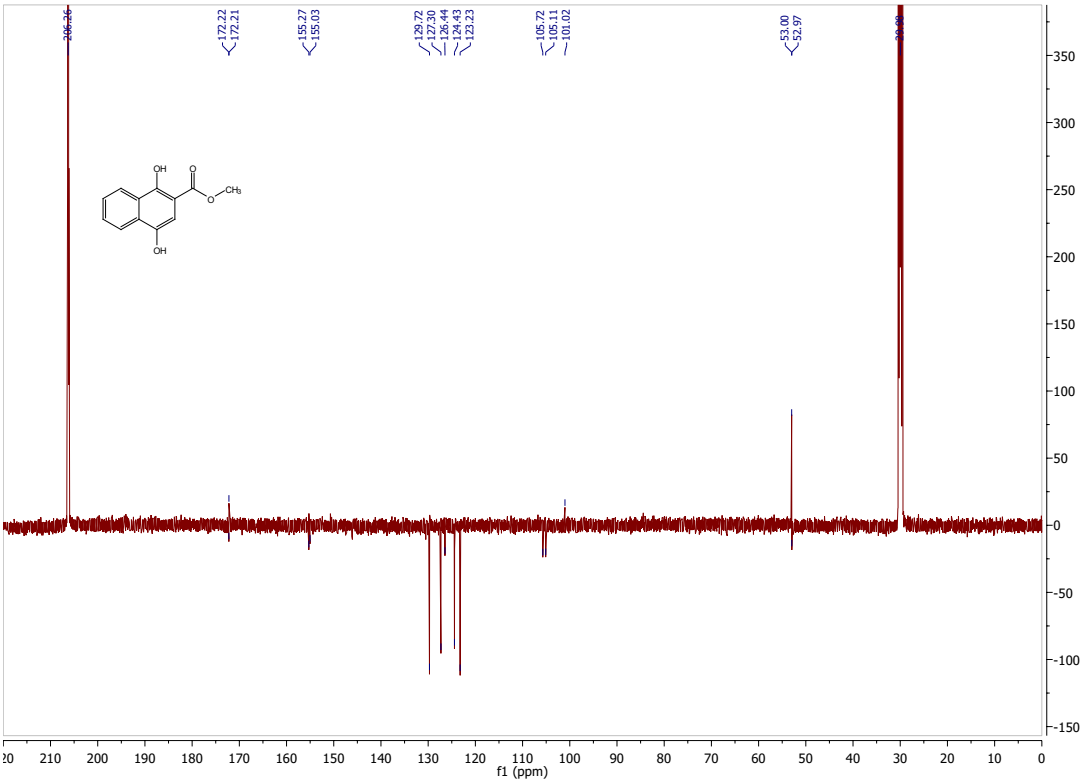
0.63
0.97
0.98
1.01
1.02
3.00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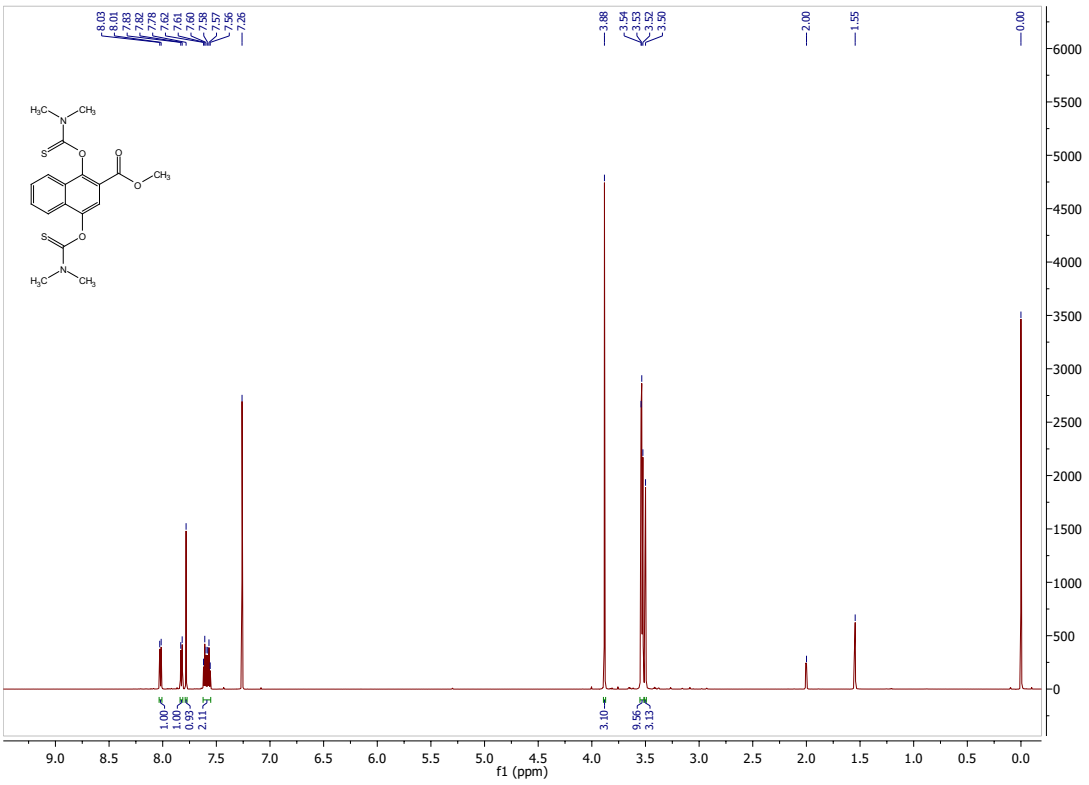
101.02
53.00
52.97
30.00
OH
O
O
CH3
OH
f1 (ppm)

Methyl 1,4-bis((dimethylcarbamothioyl)oxy)-2-naphthoate: Methyl 1,4-dihydroxy-2-naphthoate (1.0 g, 4.6 mmol) was dissolved in anhydrous DMF (8 mL) and cooled to 0 °C. To this solution was added 1,4-diazabicyclo[2.2.2]octane (2.1 g, 18 mmol) in two equivalent portions. To the resulting suspension, a solution of N,N-dimethylthiocarbamoyl chloride (2.3 g,18 mmol) in anhydrous DMF (5 mL) was added dropwise over 5 minutes at 0 °C. The solution was allowed to warm to room temperature under inert atmosphere and followed by TLC for 24 hours until reaction completion. The reaction mixture was poured into cold water (60 mL) and the resulting brown precipitate was filtered and washed with water. The crude product was recrystallized from acetonitrile and water to produce tan crystals (1.65 g, 92% yield). ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.02 (d, *J* = 7.9 Hz, 1H) 7.83 (d, *J* = 7.9 Hz, 1H), 7.78 (s, 1H), 7.59 (m, 2H), 3.88 (s, 3H), 3.49-3.55 (m, 12H). ^{13}C NMR (150 MHz, $CDCl_3$): δ = 187.28, 164.10, 149.09, 147.07, 130.44, 128.94, 127.36, 124.03, 121.92, 119.76, 52.21, 43.37, 39.09, 38.96. MS (ESI+). Expected: 393.09 $[M+H]^+$; Observed: 393.01 $[M+H]^+$.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8.01
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7.62
7.61
7.60
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7.57
7.56
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3.88
3.54
3.53
3.52
3.50
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1.00
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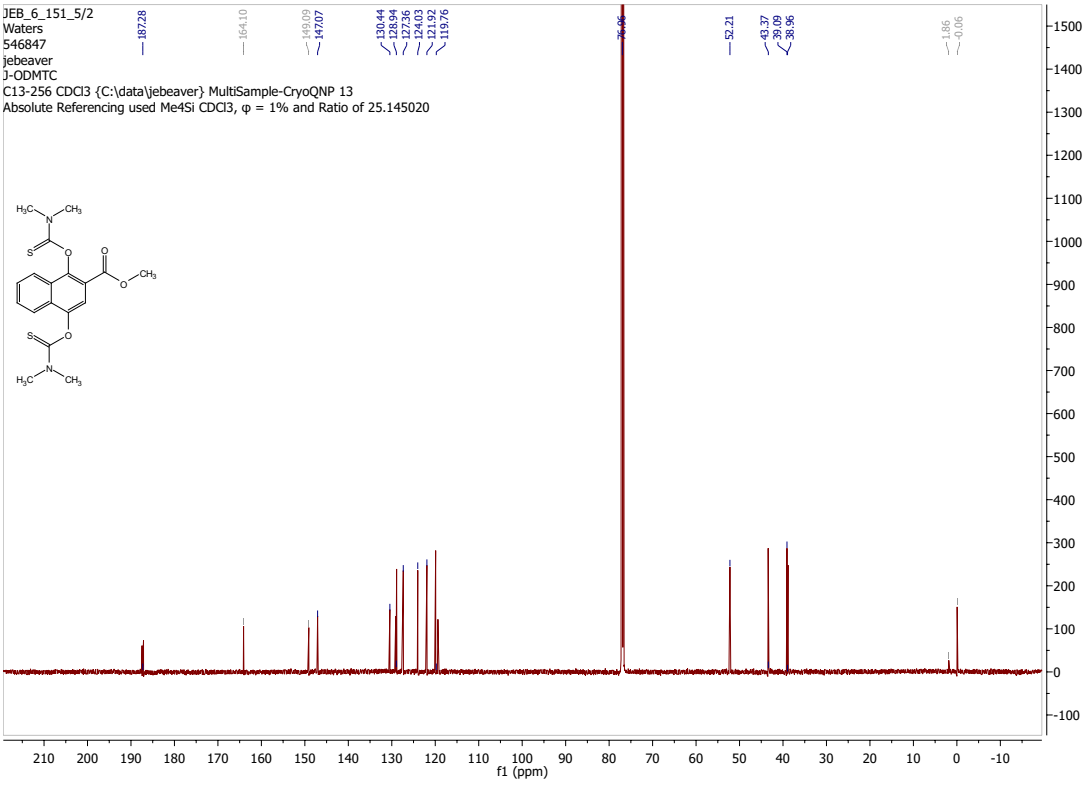
3.10
9.95
3.13
f1 (ppm)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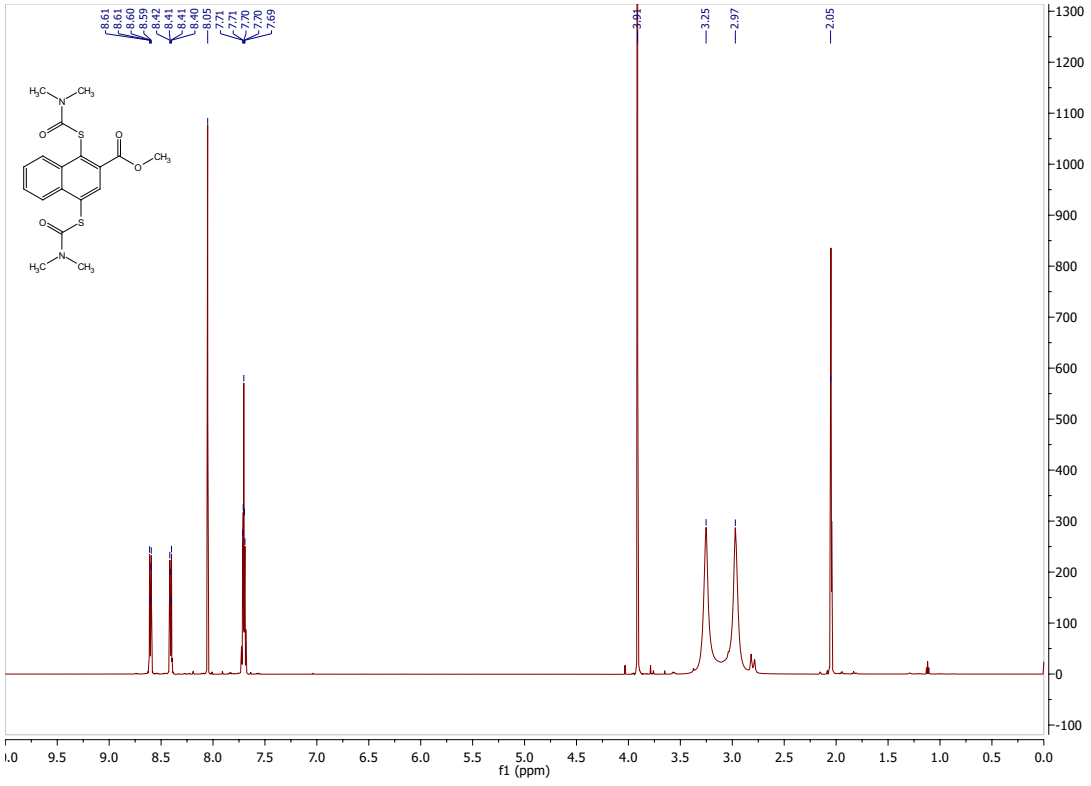
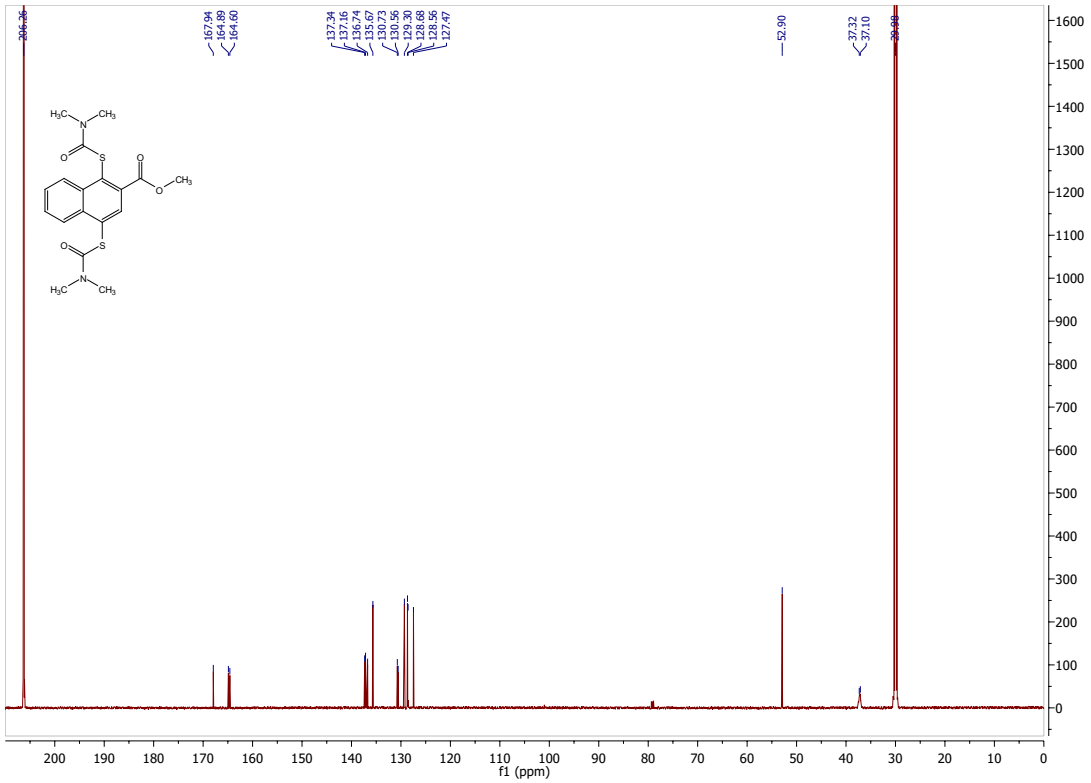
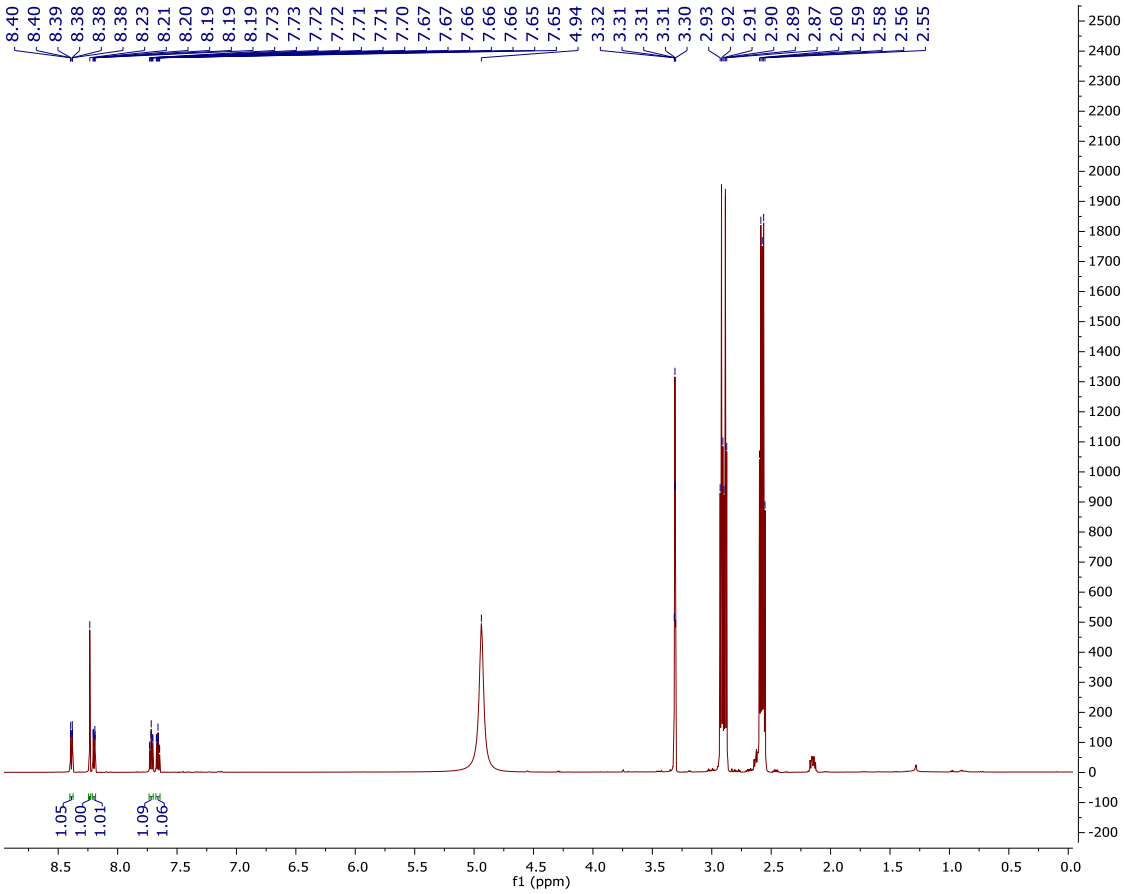
187.28
164.10
149.09
147.07
130.44
128.94
127.36
124.03
121.92
119.76
76.66
52.21
43.37
39.09
38.96
1.86
-0.06
f1 (ppm)

Methyl 1,4-bis((dimethylcarbamoyl)thio)-2-naphthoate: Methyl 1,4-bis((dimethylcarbamothioyl)oxy)-2-naphthoate (1.00 g, 2.54 mmol) was dissolved in degassed diphenyl ether (26 mL) and heated to 240 °C for 5 hours. The solution was cooled to room temperature and purified by flash column chromatography on silica gel. The diphenyl ether was eluted with hexanes and the product eluted with 65% ethyl acetate in hexanes to obtain a white crystalline solid (0.831 g, 83% yield). ^{1}H NMR (Acetone-d_6, 600 MHz): δ = 8.63-8.58 (m, 1H), 8.43-8.38 (m, 1H), 8.05 (s, 1H), 7.74-7.67 (m, 2H), 3.91 (s, 3H), 3.25 (s, 6H), 2.97 (s, 6H). ^{13}C NMR (Acetone-d_6, 150 MHz): δ = 166.92, 163.87, 163.58, 136.32, 136.14, 135.73, 134.66, 129.72, 128.29, 127.66, 127.54, 51.88, 36.32, 36.10. MS (ESI+). Expected: 393.09 $[M+H]^+$; Observed: 393.00 $[M+H]^+$.





1,4-dimercapto-2-naphthoic acid (H): Methyl 1,4-bis((dimethylcarbamoyl)thio)-2-naphthoate (0.1 g, 0.25 mmol) was dissolved in degassed methanol (1.0 mL). A degassed solution of 3M aqueous sodium hydroxide (8 mL) was added to the stirring reaction and the solution was purged with nitrogen for 5 minutes. The solution was heated to 100 °C for 24 hours to ensure complete global deprotection. The solution was cooled to room temperature and poured into degassed water (5 mL), followed by HCl (10%, 2 mL) to afford a bright yellow precipitate. The precipitate was filtered, washed with acidic water and dried under vacuum to give a pure yellow solid (0.024 g, 40 % yield). The monomer is used as is, but TCEP was added to the NMR for stability. ^{1}H NMR (CD3OD, 400 MHz): δ =8.39 (d, 1H), 8.23 (s, 1H), 8.20 (d, 1H), 7.72 (m, 1H), 7.66 (m, 1H). MS (ESI-). Expected: 234.99 $[M-H]^-$; Observed: 235.00 $[M-H]^-$.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8.40
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7.67
7.66
7.66
7.66
7.65
7.65
4.94
3.32
3.31
3.31
3.31
3.30
2.93
2.92
2.91
2.90
2.89
2.87
2.60
2.59
2.58
2.56
2.55
1.05
1.00
1.01
1.09
1.06
f1 (ppm)

Peptide synthesis

All peptide synthesis was performed on a Tetras Peptide Synthesizer using Peptides International CLEAR-Amide resin. Peptides were synthesized on a 0.06 mmol scale. All amino acids with functionality were protected during synthesis. Coupling reagents were HOBt/HBTU in DMF. For the dipeptides, the N-terminus was acylated with a solution of 5% acetic anhydride and 6% 2,6-lutidine in DMF. Cleavage was performed by hand with a cocktail of 95% TFA/2.5% triisopropylsilane/2.5% H_2O for 3 hours.

Methylated peptides were synthesized with either 2 equivalents of Fmoc-Lys(Boc)(Me)-OH purchased from BaChem or Fmoc-Lys(Me)2-OH•HCl purchased from Anaspec and coupled for 4 hours. The trimethyl lysine-containing peptides were synthesized by reacting the corresponding dimethylated peptides (0.6 mmol scale) prior to cleavage from the resin with MTBD (10.8 μL, 0.075 mmol) and methyl iodide (37.4 μL, 0.6 mmol) in DMF (5 mL) for 5 hours with bubbling N_2 in a peptide synthesis flask stoppered with a vented septum. After washing the resin with DMF (3x), CH_2Cl_2 (3x), and drying, the peptide was cleaved and purified as normal.

Peptides were purified by semipreparative reverse-phase HPLC on a C18 column at a flow rate of 4 mL/min. Peptides were purified with a linear gradient of A and B (A: 95% H_2O, 5% CH_3CN with 0.1% TFA, B: 95% CH_3CN, 5% H_2O with 0.1% TFA) and elution was monitored at 214 nm. Once purified, peptides were lyophilized to powder and characterized by ESI-MS. Peptides used in binding studies were desalted and repurified by semipreparative reverse-phase HPLC with a C-18 column and buffered mobile phase. Peptides were purified using an optimized gradient of A and B (A: 100% H_2O, 10 mM NH_4OAc; B: 90% CH_3CN, 10% H_2O, 10 mM NH_4OAc). The ammonium salts were removed under reduced pressure for three to five days after the samples were dry.

Dynamic Combinatorial Chemistry

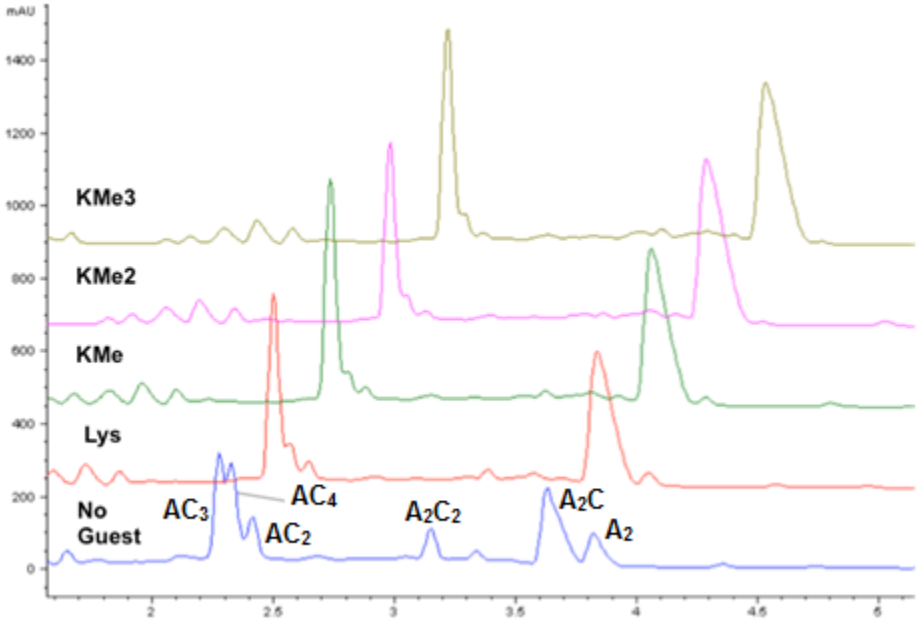

Figure S1. Overlaid HPLC traces at 254 nm of DCC libraries biased toward the formation of **A_2C** with monomers **A** (5 mM) and **C** (2.5 mM) and Ac-K(Me)$_n$G-NH_2 (7.5 mM).[5]

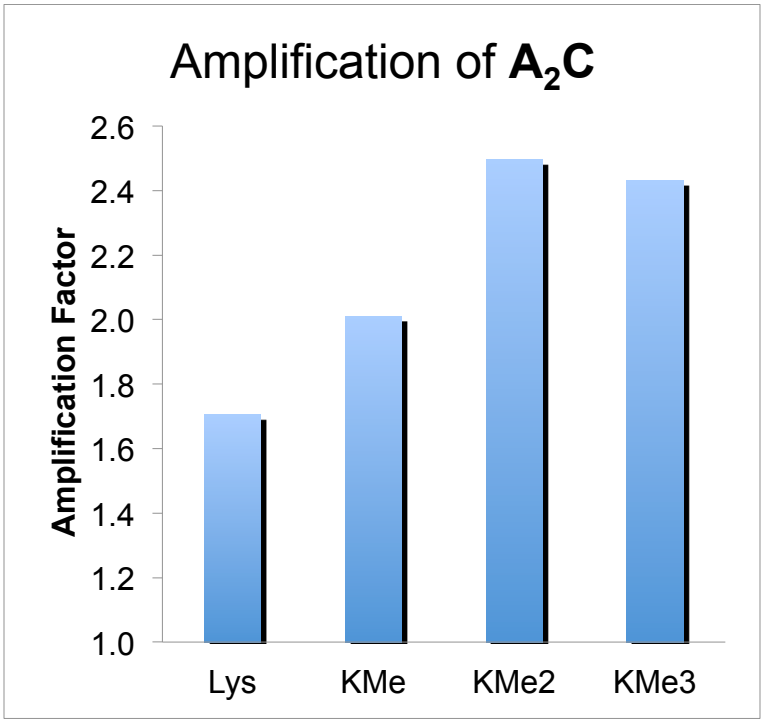

Figure S2. Amplification data for the low concentration DCC libraries biased toward the formation of **A_2C** with 0.5 mM **A**, 0.25 mM **C**, and 0.75 mM Ac-K(Me)$_n$G-NH_2.[6]

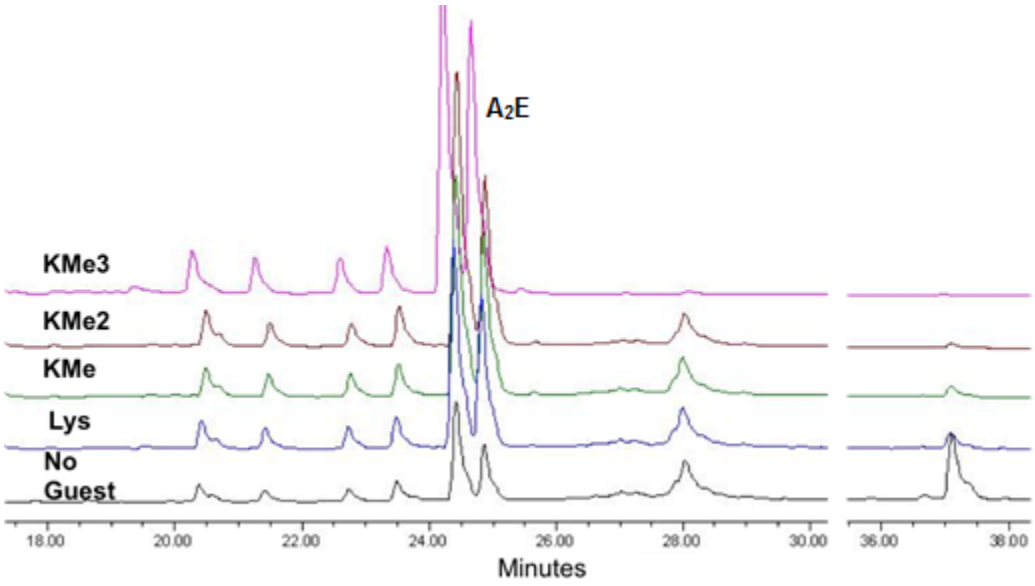

Figure S3. Overlaid HPLC traces at 280 nm of DCC libraries biased toward the formation of **A_2E** at low concentration, with monomers **A** (0.5 mM) and **E** (0.25 mM) and Ac-K(Me)$_n$GGY-NH_2 (0.75 mM). Two **A_2E** isomers eluted at 24.5 and 25 minutes as the major peaks in the library.

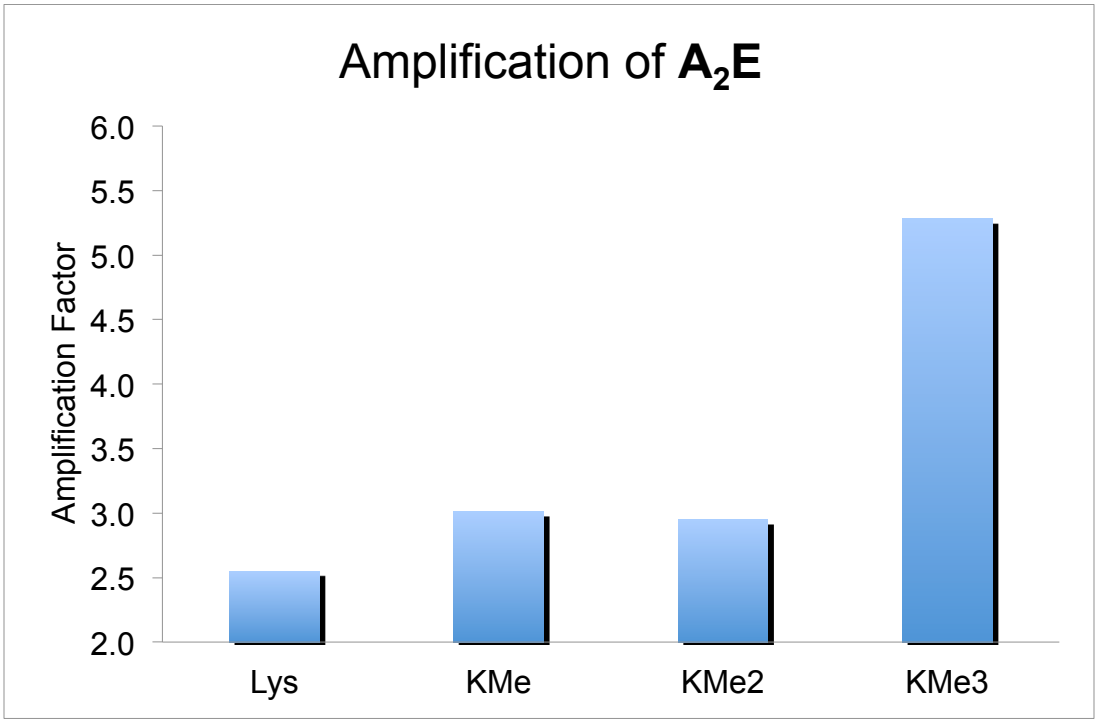

Figure S4. Amplification data for the low concentration DCC libraries biased toward the formation of **A_2E** with 0.5 mM **A**, 0.25 mM **E**, and 0.75 mM Ac-K(Me)$_n$GGY-NH_2.

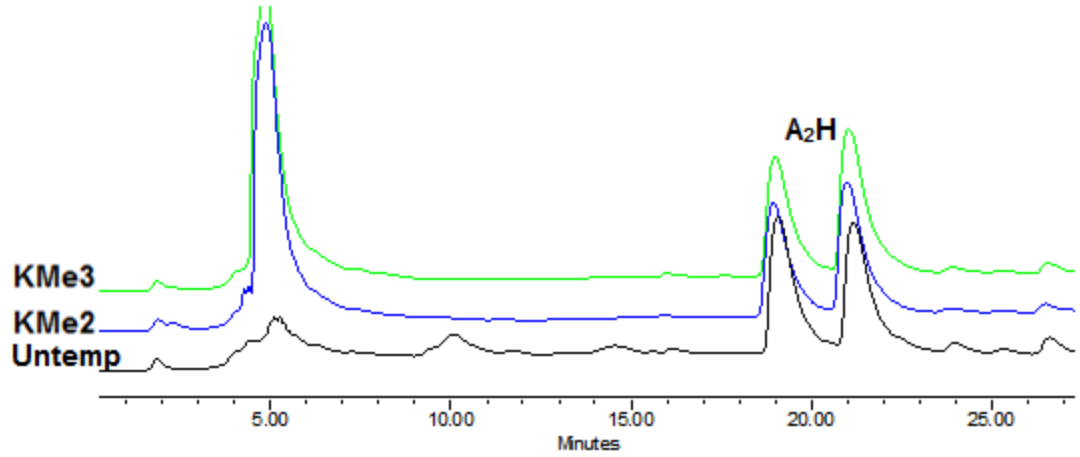

Figure S5. Overlaid HPLC traces at 254 nm of DCC libraries biased toward the formation of **A_2H** with monomers **A** (5 mM) and **H** (2.5 mM) and Ac-K(Me)$_n$GGY-NH_2 (7.5 mM).

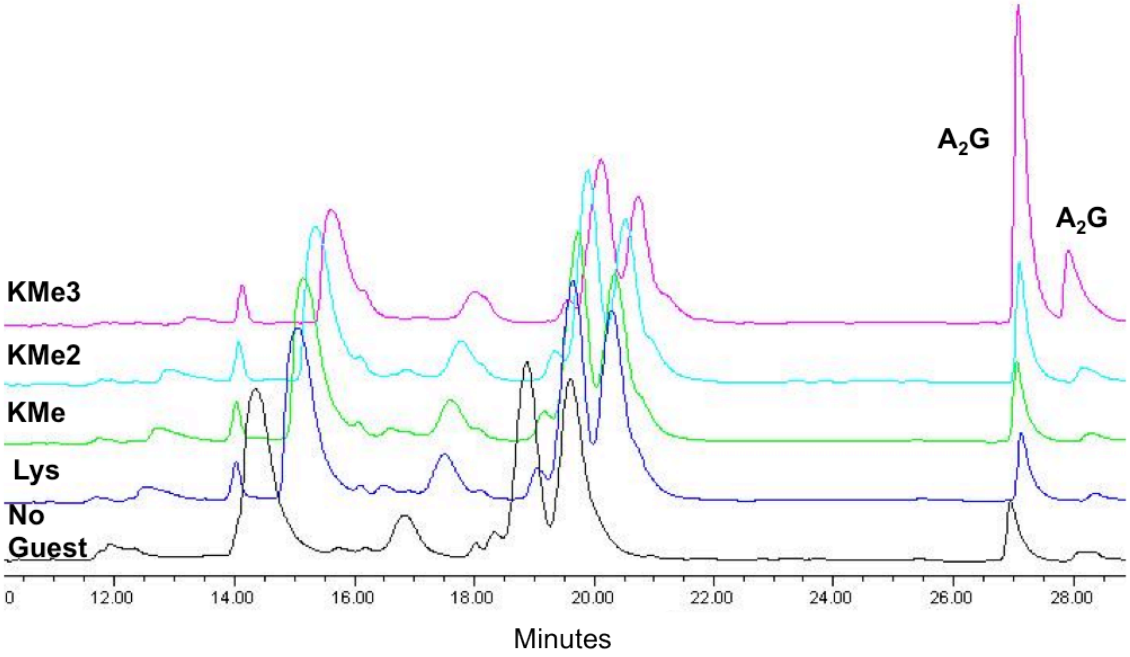

Figure S6. Overlaid HPLC traces at 254 nm of DCC libraries biased toward the formation of **A_2G** with monomers **A** (0.5 mM) and **G** (0.25 mM) and Ac-K(Me)$_n$GGY-NH_2 (0.75 mM).

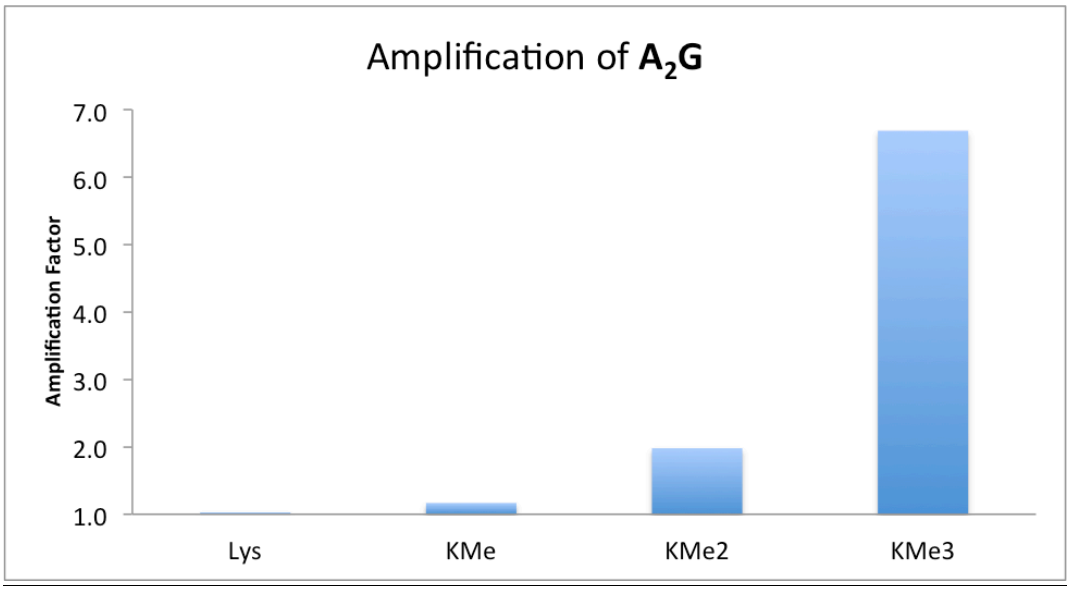

Figure S7. Amplification of **A_2G** in DCC libraries biased toward the formation of **A_2G** with monomer **A** (0.5 mM), monomer **G** (0.25 mM) and Ac-K(Me)$_n$GGY-NH_2 (0.75 mM).

Preparative synthesis of A_2C

A_2C was synthesized using a preparative scale, biased DCC library with a 1:1 ratio of monomer **A** (2 mM) to monomer **C** (2 mM) and five equivalents of commercially available, tetramethylammonium hydroxide (**NMe_4OH**) (5 mM) in 50 mM sodium borate buffer, pH = 8.5. After the library reached equilibrium, the library was filtered and purified using semi-preparative HPLC (solvent A: 10mM NH4OAc in H2O; solvent B: 10 mM NH4OAc in 10% H2O, 90% CH3CN)

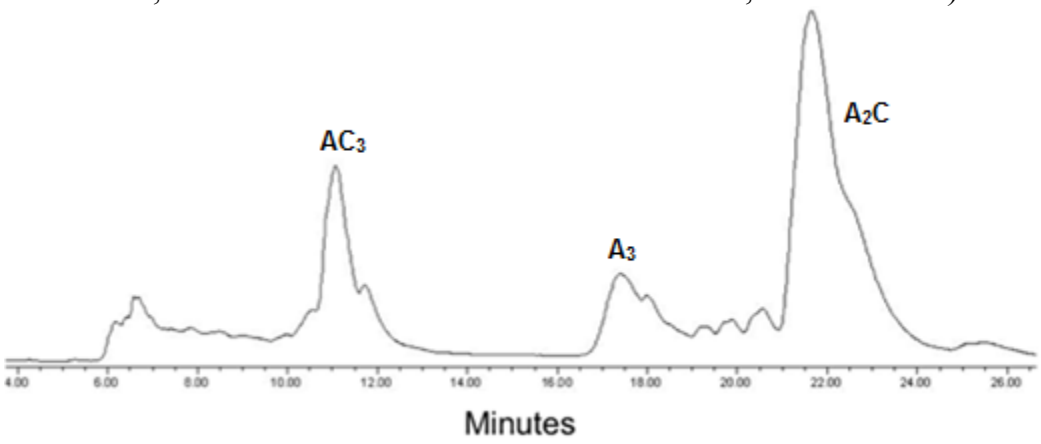

Figure S8. Reverse phase HPLC trace of the preparative DCC library for the synthesis of **A_2C**, monitored at 254nm.

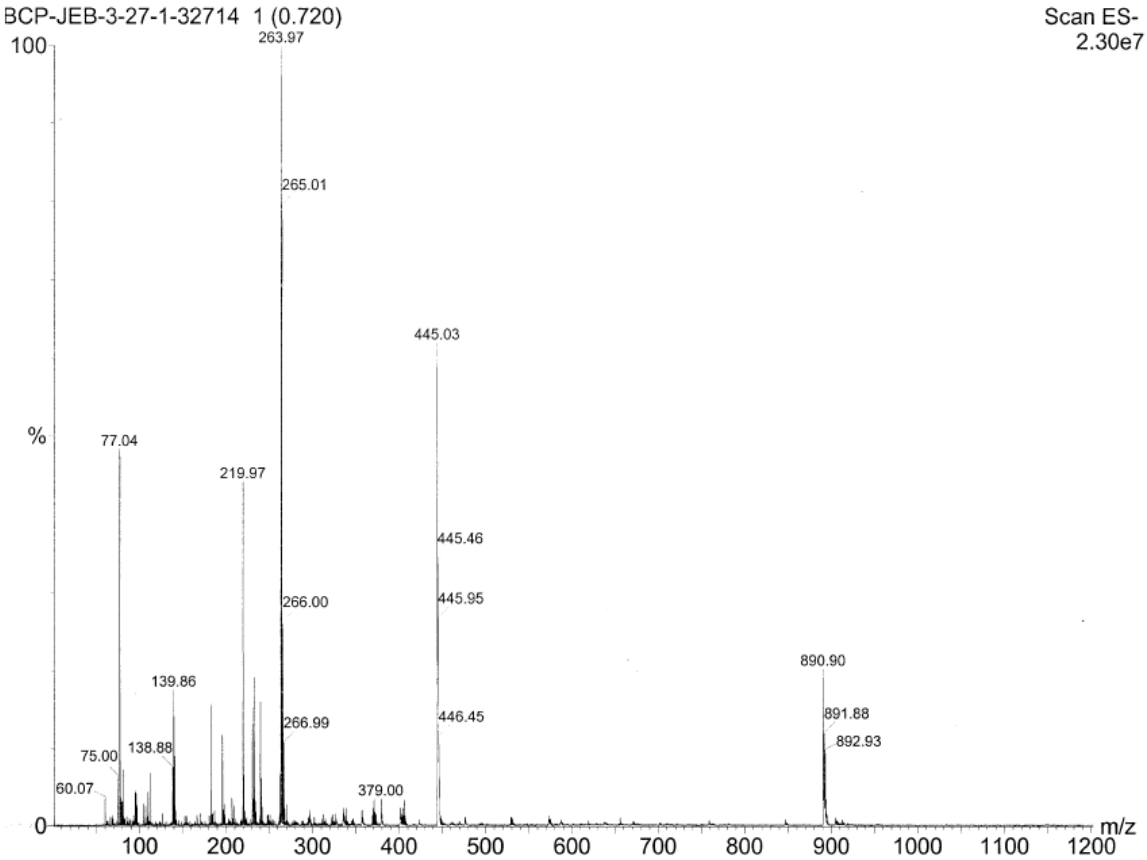

Figure S9. Mass spectrum of **A_2C** (-ESI). $[M-H]^{-1}$ at 890.90 and $[M-2H]^{-2}$ at 445.03.

Preparative Synthesis of A_2E

A_2E was synthesized using a preparative scale DCL, with monomer **A** (2.0 mM), monomer **E** (2.0 mM) and **Acetylcholine Chloride (AcCH)** (8.0 mM) in 50 mM sodium borate buffer, pH = 8.5. **AcCH** was used because it is commercially available and amplified **A_2E** under preparative library conditions. After five days, the library was filtered and purified using semi-preparative HPLC (solvent A: 10mM NH_4OAc in H_2O; solvent B: 10 mM NH_4OAc in 10% H_2O, 90% CH_3CN) in a linear gradient. Clean separation of the isomers was not possible, so the trace as reported was used for further experiments.

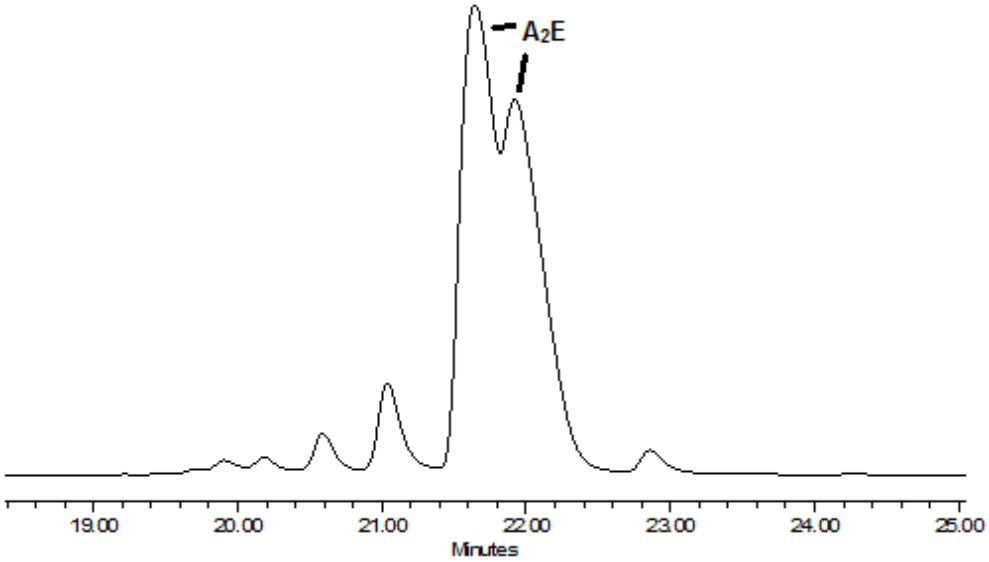

Figure S10. Reverse phase HPLC trace of the preparative DCC library for the synthesis of **A_2E**, monitored at 254nm.

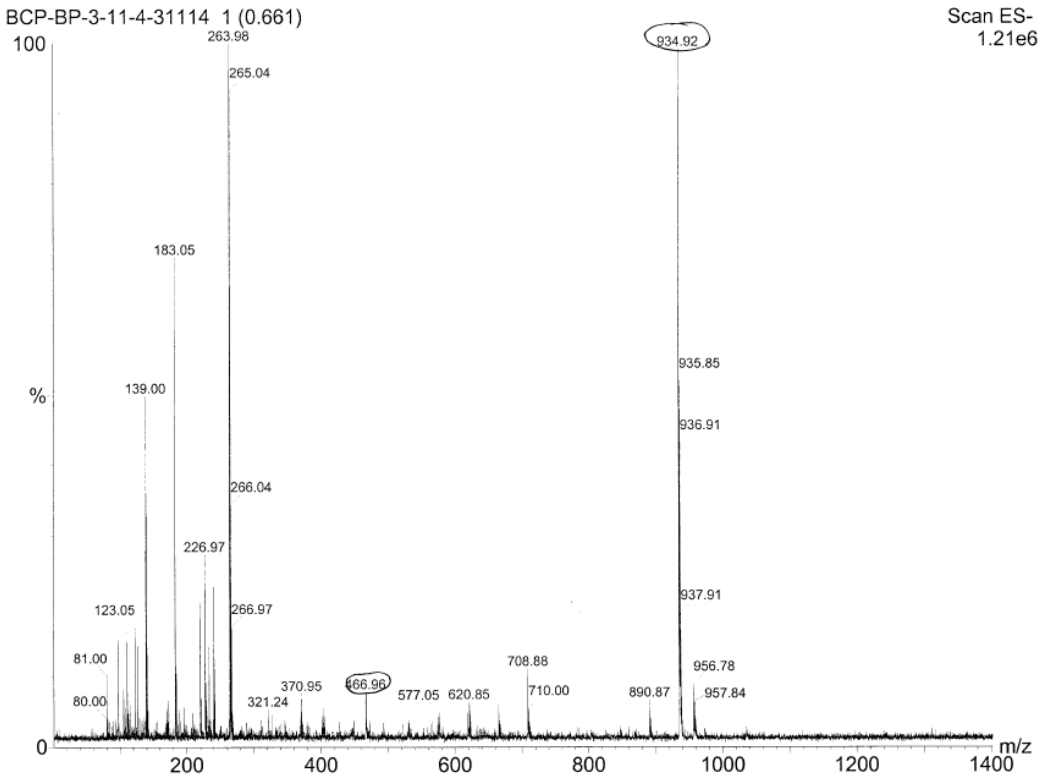

Figure S11. Mass spectrum of **A_2E** (-ESI). $[M-H]^{-1}$ at 934.92 and $[M-2H]^{-2}$ at 466.96.

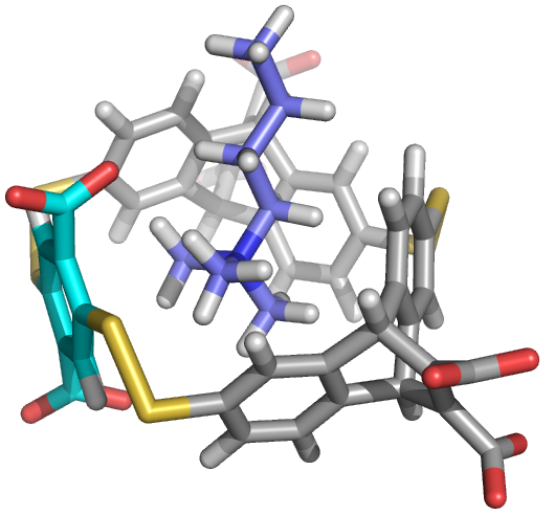

Figure S12. Molecular modeling of **A_2E** bound to butyl-trimethyl ammonium. The position of the thiols turns the carboxylates out of the plane of the aromatic ring, potentially including both into the binding pocket and increasing contacts to the bound guest.

Synthesis of A_2H

A_2H was synthesized using a preparative scale DCL, with monomer **A** (2.0 mM), monomer **H** (1.0 mM) and **Acetylcholine Chloride (AcCH)** (3.0 mM) in 50 mM sodium borate buffer, pH = 8.5. After reaching equilibration, the library was filtered and purified using semi-preparative HPLC (solvent A: 10mM NH_4OAc in H_2O; solvent B: 10 mM NH_4OAc in 10% H_2O, 90% CH_3CN).

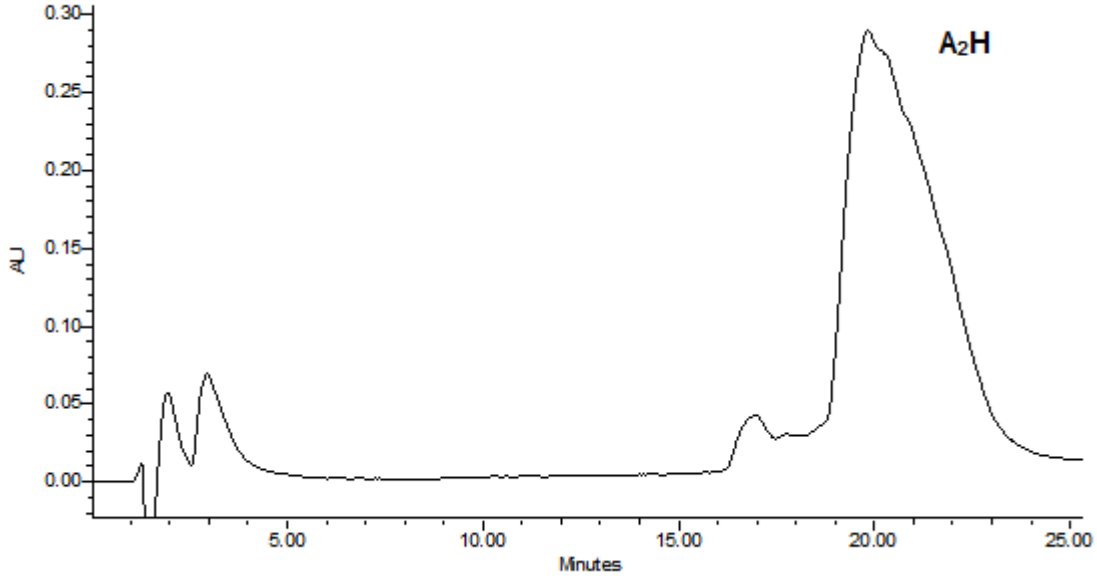

Figure S13. Reverse phase HPLC trace of the preparative DCC library for the synthesis of **A_2H** which was purified as a mixture of isomers.

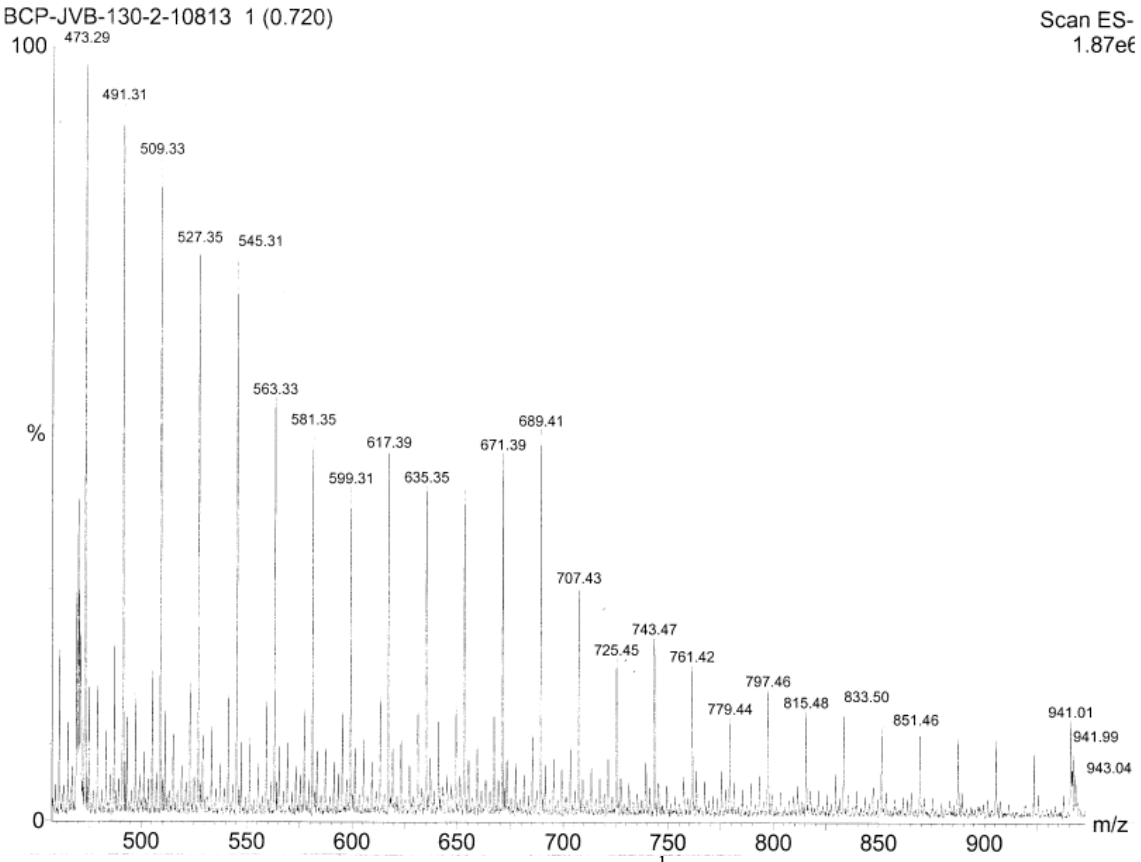

Figure S14. Mass spectrum of $\mathbf{A_2H}$ (-ESI). $[M-H]^{-1}$ at 941.01.

Preparative synthesis of A_2G

$\mathbf{A_2G}$ was synthesized using a preparative scale, biased DCC library with a 2:1 ratio of monomer **A** (2.0 mM) to monomer **G** (1.0 mM) and $\mathbf{BuNMe_3I}$ (5.0 mM) in 50 mM sodium borate buffer, pH = 8.5 (Figure). $\mathbf{BuNMe_3I}$ was used because it is commercially available and amplified $\mathbf{A_2G}$ under preparative library conditions. After the library reached equilibrium, the library was filtered and purified using semi-preparative HPLC (solvent A: 10mM NH4OAc in H2O; solvent B: 10 mM NH4OAc in 10% H2O, 90% CH3CN). $\mathbf{A_2G}$ was isolated as a mixture of isomers.

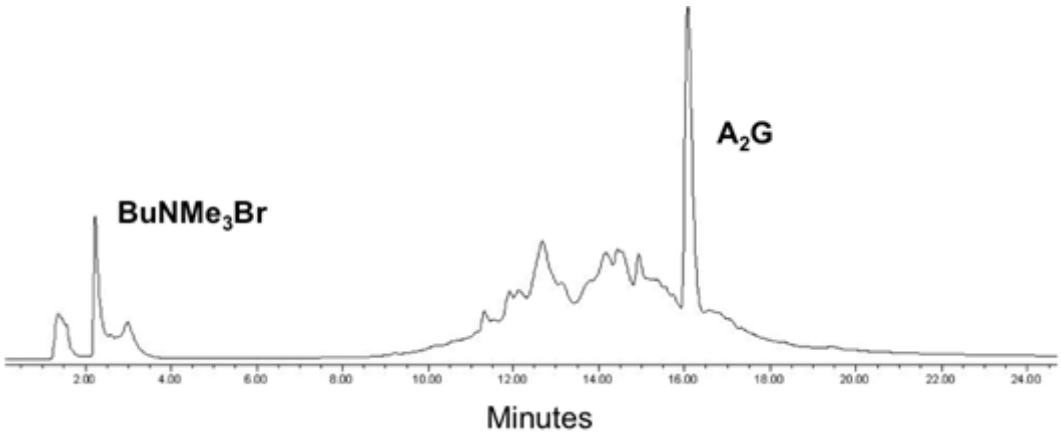

Figure S15. Reverse phase HPLC trace of the preparative DCC library for the synthesis of **A_2G** which was purified as a mixture of isomers.

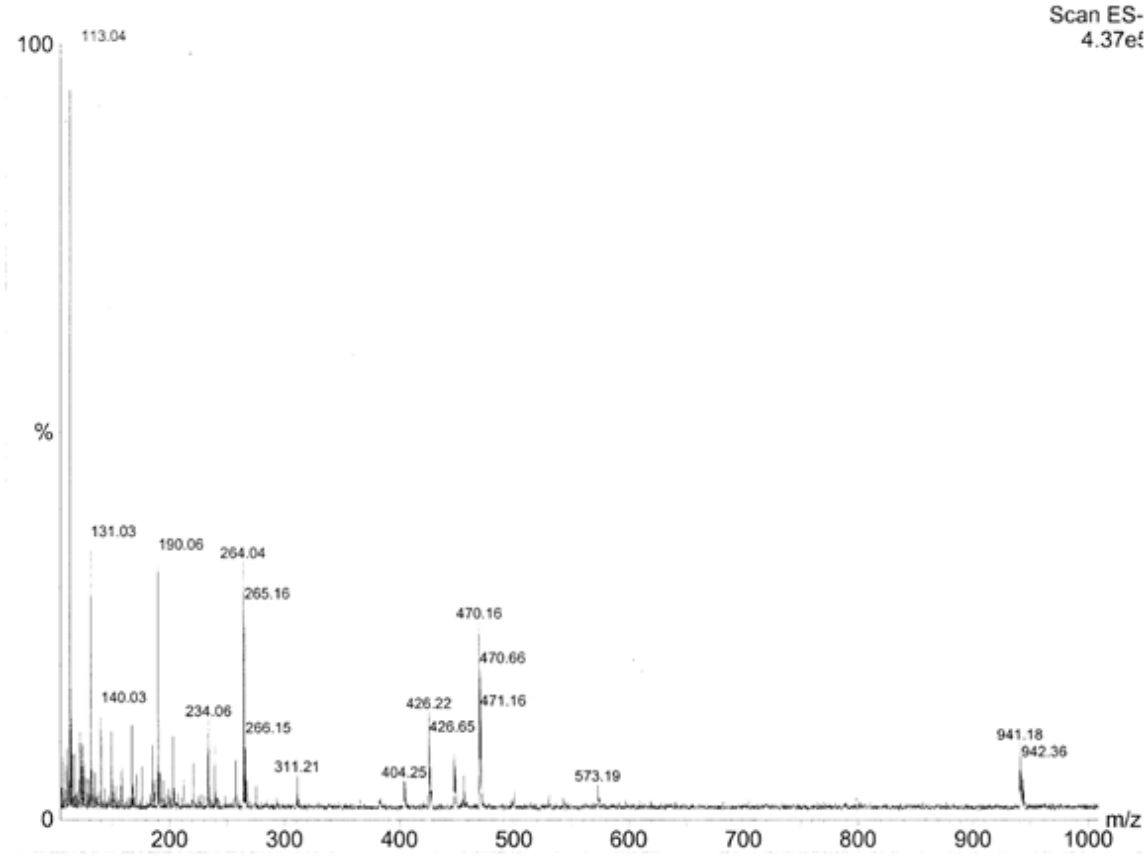

Figure S16. Mass spectrum of **A_2G** (-ESI). $[M-H]^{-1}$ at 941.18, $[M-H]^{-2}$ at 470.16.

Extinction Coefficient Determination

In order to have reproducible concentrations of receptor, extinction coefficients were determined using mixtures of isomers from **A_2C** and **A_2E**. For receptors **A_2H** and **A_2G**, the extinction coefficient was assumed to be similar to a receptor **A_2D** determined previously.[1] After purification using NH_4OAc buffered solvents, the receptors were lyophilized for at least one week to ensure removal of the volatile NH_4Oac salts. The dry sample was then taken up into anhydrous methanol and filtered with a 0.33 um filter into a tared vial. The methanol was evaporated and then the sample further dried under vacuum. After determining the mass, the dry sample was dissolved in 10 mM sodium borate buffer, pH 8.5 and diluted to 1 mM. Serial dilutions were performed to give ten samples which were analyzed at a variety of wavelengths. The extinction coefficient for **A_2C** determined to be 9,646 M^{-1} cm^{-1} at 297 nm and **A_2E** was 4,812 M^{-1} cm^{-1} at 325 nm.

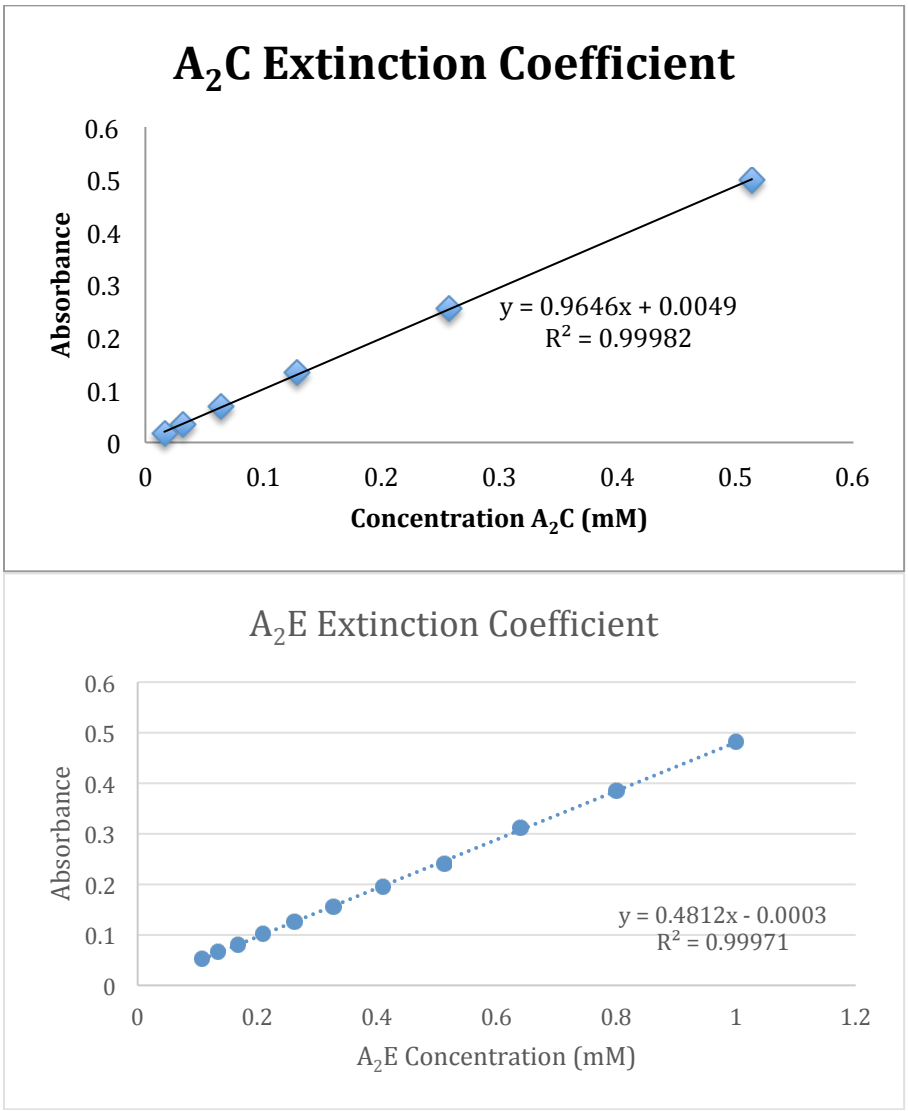

Figure S17. Extinction coefficient determination of **A_2C** (top) and **A_2E** (bottom). The extinction coefficient determined as the slope of the linear regression

Isothermal Titration Calorimetry Binding Experiments

Depending on the system studied, ITC titrations were performed with a range of ~0.7-2 mM solution of peptide into ~60-200 uM of receptor. For weaker interactions, the c-value is low, so there is a higher degree of error. While one-site binding is assumed, n-values do deviate from 1, which can be attributed to both the error in accurately determining receptor concentration as well as the complexity of the interaction being studied.

Heat of dilution titrations were measured on a Microcal AutoITC200 at 298K. In parallel to binding measurements by ITC, peptide (1-2 mM) was titrated into sodium borate buffer (10 mM, pH 8.5) using 2.0 μL increments every 3 minutes. The resulting data was manually integrated to reduce error in automatic baseline calculations. The resulting normalized changes in enthalpy (NDH) measurements were normalized for peptide concentration and subtracted directly from NDH measurements for all subsequent ITC titrations from that peptide stock solution.

Binding ITC titrations for some experiments generated at least 5 data points post receptor saturation (the change in slope minimized and began to approach zero). The heats of dilution titrations for receptors was negligible, so the heat produced during the final 5 measurements were determined to be the heat of dilution of the system. The last 5 points were averaged and subtracted from each point in the titration prior to fitting to a one-site binding model. This subtraction method is noted in the ITCs where it applies.

Table S1. Thermodynamic binding data obtained for binding of the receptors to Ac-WGGG-QTARK(Me)nSTG-NH2 as measured by ITC.[a]

Entry	Receptor[b]	Peptide	K_d[c] (μM)	S. Factor[d]	ΔG[c] (kcal/mol)	ΔH[c] (kcal/mol)	TΔS[c] (kcal/mol)
1[e]	**A_2B**	KMe3	2.6 ± 0.1	-	-7.63 ± 0.03	-11.26 ± 0.05	-3.61 ± 0.05
2[e]	**A_2B**	KMe2	6.3 ± 0.3	2.4	-7.10 ± 0.07	-11.65 ± 0.09	-4.5 ± 0.1
3[e]	**A_2B**	KMe	13.9 ± 0.1	5.4	-6.64 ± 0.01	-9.65 ± 0.06	-3.00 ± 0.07
4[e]	**A_2B**	Lys	22 ± 1	8.3	-6.38 ± 0.02	-9.2 ± 0.2	-2.9 ± 0.3
5[e]	**A_2B**	R8GKMe3	17.1 ± 0.1		-6.52 ± 0.01	-12.37 ± 0.01	-5.84 ± 0.02
6	**A_2C**	KMe3	2.3 ± 0.1	-	-7.69 ± 0.02	-10.4 ± 0.1	-2.7 ± 0.1
7	**A_2C**	KMe2	2.8 ± 0.2	1.2	-7.57 ± 0.04	-9.52 ± 0.08	-1.9 ± 0.1
8	**A_2C**	KMe	13.8 ± 0.7	6.0	-6.63 ± 0.03	-10.25 ± 0.01	-3.61 ± 0.04
9	**A_2C**	Lys	22 ± 1	9.6	-6.34 ± 0.03	-10.1 ± 0.4	-3.7 ± 0.4
10	**A_2C**	R8GKMe3	29 ± 3		-6.17 ± 0.05	-12.1 ± 0.4	-5.9 ± 0.4
11[f]	**A_2E**	KMe3	0.191 ± 0.002	-	-9.16 ± 0.01	-14.0 ± 0.3	-4.8 ± 0.3
12[f]	**A_2E**	KMe2	0.5 ± 0.1	2.6	-8.5 ± 0.1	-12.67 ± 0.05	-4.1 ± 0.1
13[f]	**A_2E**	KMe	1.6 ± 0.2	8.4	-7.92 ± 0.08	-11.92 ± 0.07	-3.9 ± 0.4
14[f]	**A_2E**	Lys	6.7 ± 0.1	35	-7.05 ± 0.01	-11.26 ± 0.02	-4.16 ± 0.02
15[f]	**A_2E**	R8GKMe3	2.7 ± 0.3		-7.59 ± 0.06	-12.2 ± 0.1	-4.5 ± 0.2
16[e]	**A_2N**	KMe3	0.30 ± 0.04	-	-8.91 ± 0.07	-12.0 ± 0.5	-3.1 ± 0.5
17[e]	**A_2N**	KMe2	4.1 ± 0.5	14	-7.36 ± 0.04	-12.5 ± 0.4	-5.1 ± 0.4
18[e]	**A_2N**	KMe	40 ± 4	130	-6.01 ± 0.06	-12.0 ± 0.5	-6.0 ± 0.5
19[e]	**A_2N**	Lys	10.5 ± 0.9	35	-6.80 ± 0.05	-7.3 ± 0.3	-0.5 ± 0.3
20[e]	**A_2N**	R8GKMe3	1.3 ± 0.2		-8.05 ± 0.08	-13.4 ± 0.5	-5.3 ± 0.6
21[f]	**A_2G**	KMe3	1.4 ± 0.1	-	-8.00 ± 0.05	-9.9 ± 0.2	-1.8 ± 0.2
22[f,g]	**A_2G**	KMe2	13.2 ± 2.4	10	-6.6 ± 0.1	-11.5 ± 0.1	-4.9 ± 0.1
23[f]	**A_2G**	KMe	15 ± 1	11	-6.57 ± 0.04	-10.2 ± 0.4	-3.6 ± 0.4
24[f,h]	**A_2G**	Lys	>58	>40	< -5.8	-	-
25[fg]	**A_2G**	R8GKMe3	5.4 ± 0.1		-7.19 ± 0.01	-9.0 ± 0.1	-1.8 ± 0.1

(a) All data determined by ITC, fit to one-site binding model; Conditions: 26 °C, in 10 mM sodium borate buffer, pH 8.5. (b) All receptors are mixtures of isomers except *rac*-**A_2B** and *meso*-**A_2N**. (c) Errors are from averages of three trials, unless noted otherwise. (d) S. factor is selectivity, which is calculated as the fold difference in affinity for KMe3 over the designated methylation state of the peptide in that row. (e) Data reported by Pinkin and Waters.[7] (f) Average of two trials. (g)Error determined by propagation from curve fitting and averages. (h)These values are approximate because the c-value for these experiments was <1.

A_2C Example Titrations

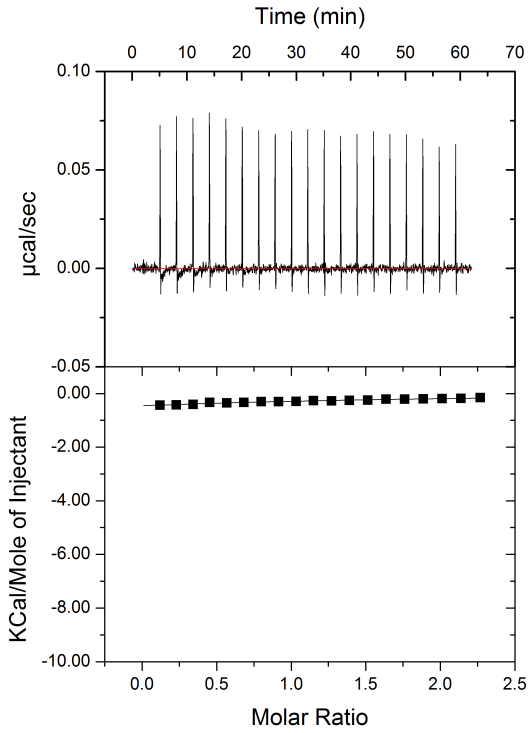

Figure S18. ITC titration of R8GK9G into **A_2C**. Analysis reveals that the receptor does not interact with the peptide in a measurable capacity.

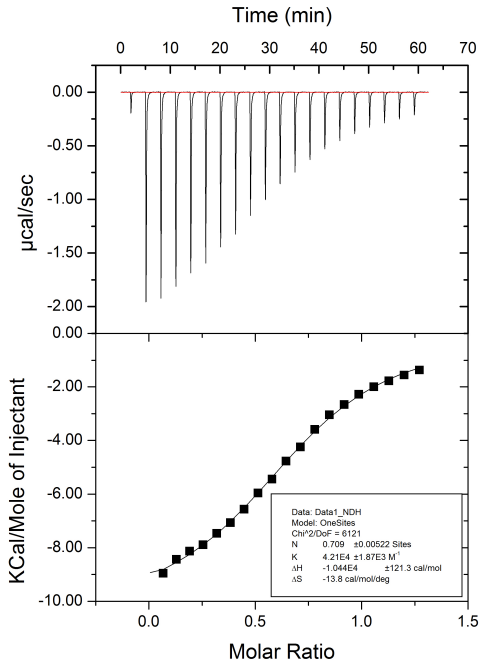

Figure S19. One of three trials of Lys (Ac-WGGG-QTARKSTG-NH_2) (1.25 mM) into **A_2C** (200 uM) at 26°C in 10 mM borate buffer, pH 8.5.

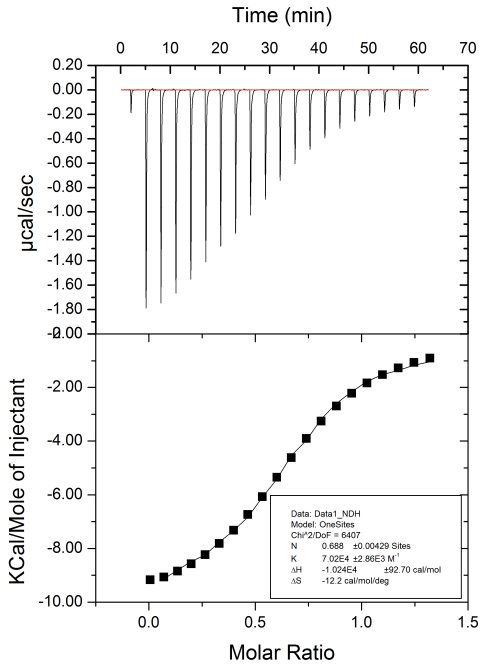

Figure S20. One of two trials of KMe (Ac-WGGG-QTARKMeSTG-NH_2) (1.16 mM) into $\mathbf{A_2C}$ (179 uM) at 26°C in 10 mM borate buffer, pH 8.5.

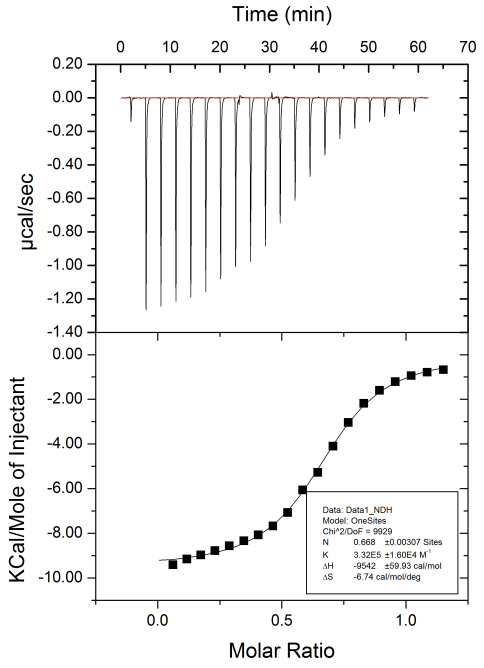

Figure S21. One of three trials of KMe2 (Ac-WGGG-QTARKMe_2STG-NH_2) (0.707 mM) into $\mathbf{A_2C}$ (125 uM) at 26°C in 10 mM borate buffer, pH 8.5.

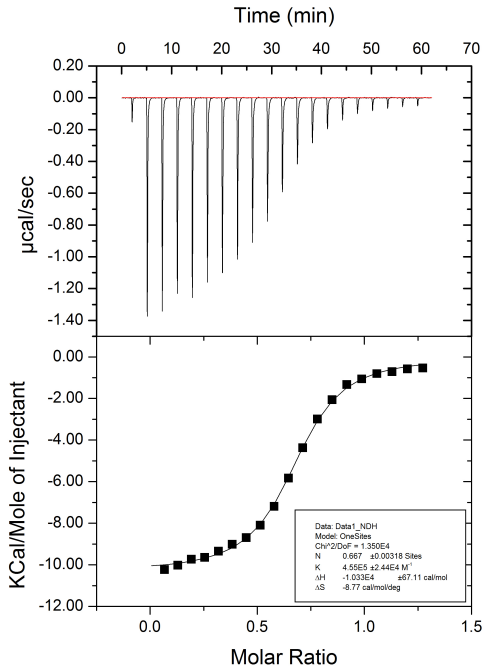

Figure S22. One of three trials of KMe3 (Ac-WGGG-QTARKMe_3STG-NH_2) (0.713 mM) into $\mathbf{A_2C}$ (114 uM) at 26°C in 10 mM borate buffer, pH 8.5. Heat of dilution was determined from the average of the last four data points and subtracted prior to fitting to a one-site binding curve.

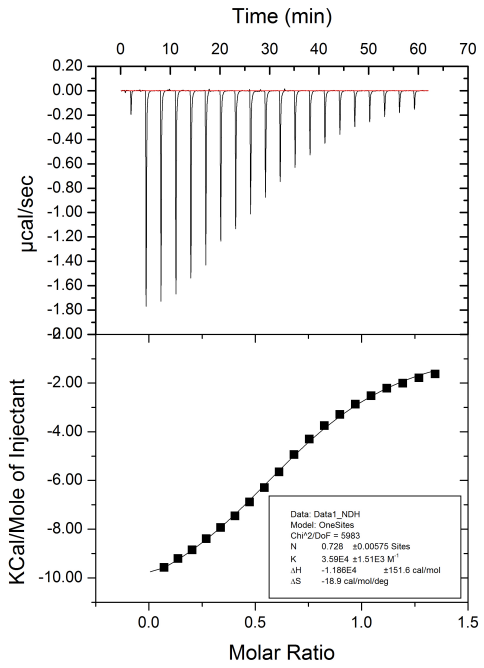

Figure S23. One of two trials of R8GKMe3 (Ac-WGGG-QTAGKMe_3STG-NH_2) (1.18mM) into $\mathbf{A_2C}$ (178 uM) at 26°C in 10 mM borate buffer, pH 8.5. Heat of dilution was determined from the average of the last four data points and subtracted prior to fitting to a one-site binding curve.

A_2E Example Titrations

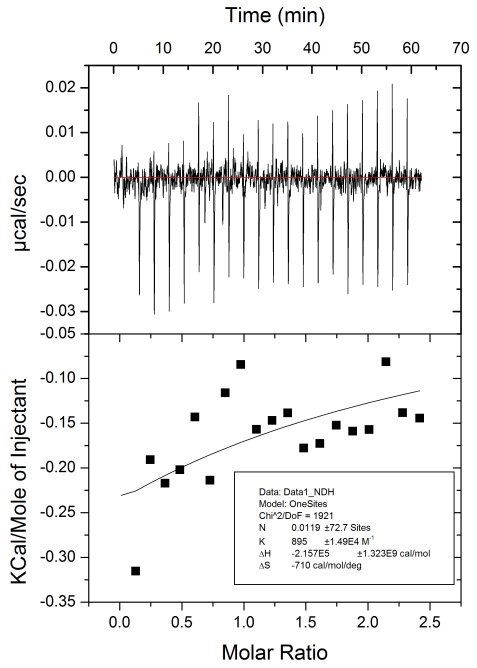

Figure S24. ITC of R8GK9G (Ac-WGGG-QTAGGSTG-NH_2) (1.2 mM) into **A_2E** (120 uM) at 26°C in 10 mM borate buffer, pH 8.5.

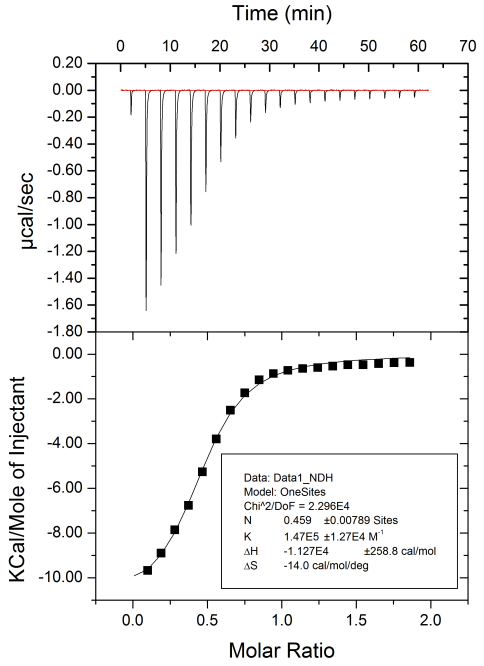

Figure S25. One of two trials of Lys (Ac-WGGG-QTARKSTG-NH_2) (0.996 mM) into **A_2E** (109 uM) at 26°C in 10 mM borate buffer, pH 8.5.

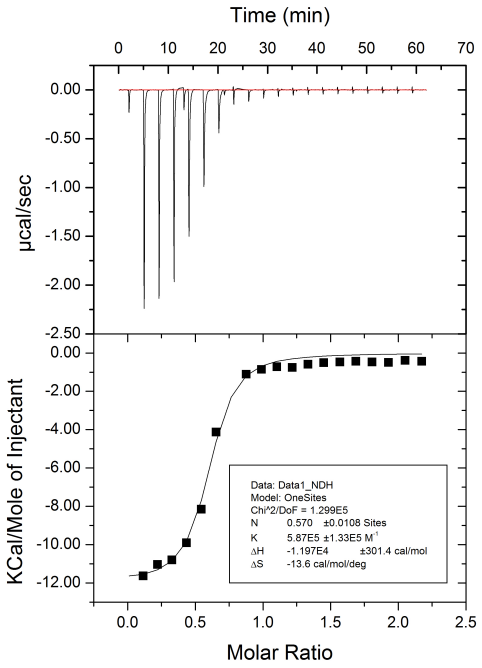

Figure S26. One of two trials of KMe (Ac-WGGG-QTARKMeSTG-NH_2) (1.1 mM) into **A_2E** (103 uM) at 26°C in 10 mM borate buffer, pH 8.5.

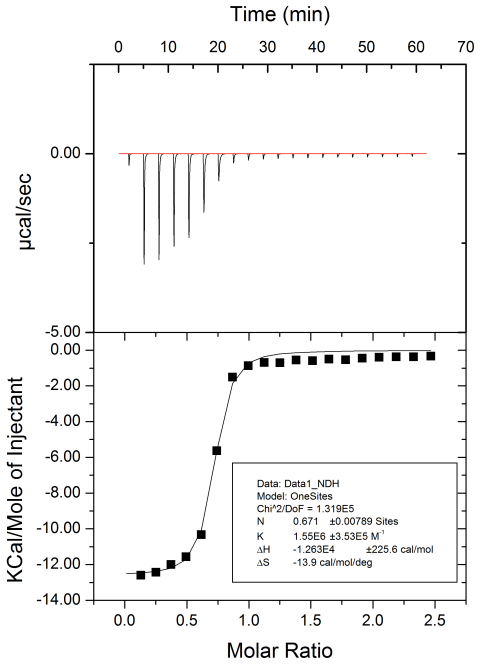

Figure S27. One of two trials of KMe2 (Ac-WGGG-QTARKMe$_2$STG-NH_2) (1.2 mM) into **A_2E** (99 uM) at 26°C in 10 mM borate buffer, pH 8.5.

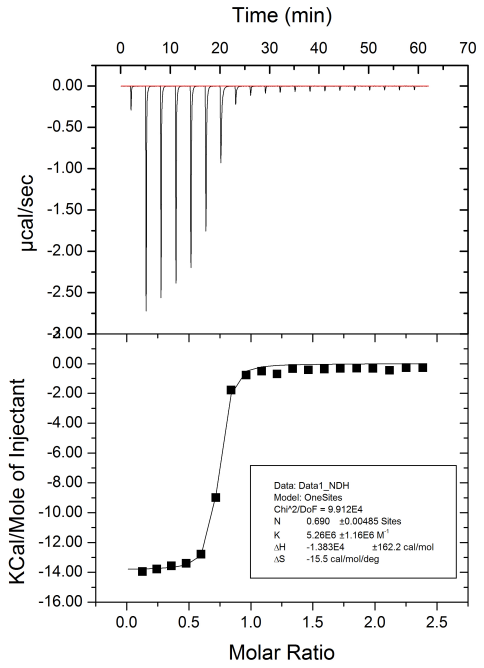

Figure S28. One of two trials of KMe3 (Ac-WGGG-QTARKMe$_3$STG-NH$_2$) (0.95 mM) into **A$_2$E** (80 uM) at 26°C in 10 mM borate buffer, pH 8.5.

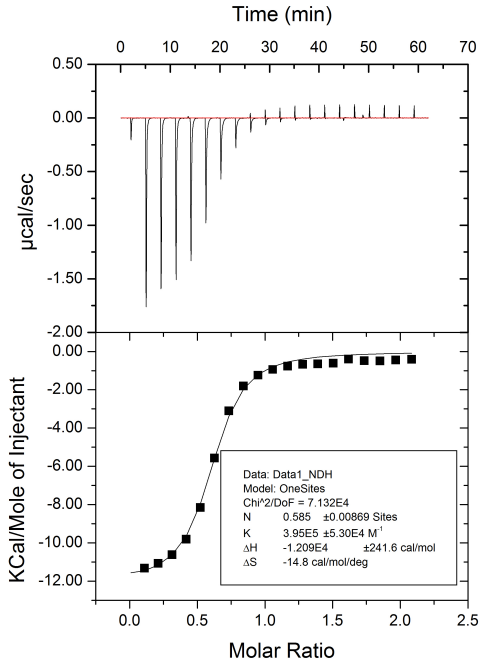

Figure S29. One of two trials of R8GKMe3 (Ac-WGGG-QTAGK(Me)$_3$STG-NH$_2$) (1.001 mM) into **A$_2$E** (98 uM) at 26°C in 10 mM borate buffer, pH 8.5.

A_2H Example Titrations

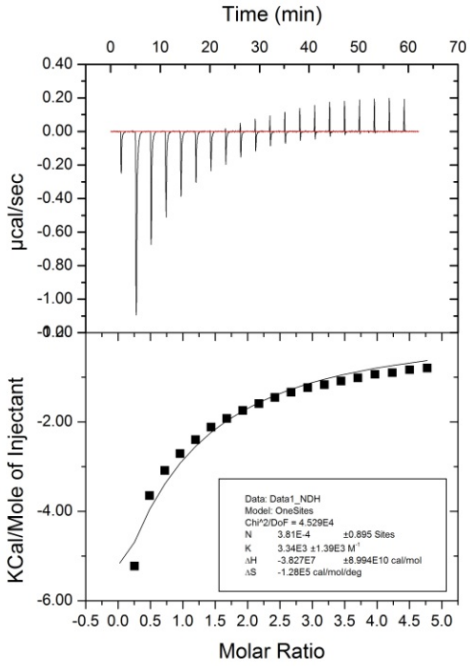

Figure S30. ITC of R8GK9Me (Ac-WGGG-QTAGKMeSTG-NH_2) (2.5 mM) into **A_2H** (214 uM) at 26°C in 10 mM borate buffer, pH 8.5.

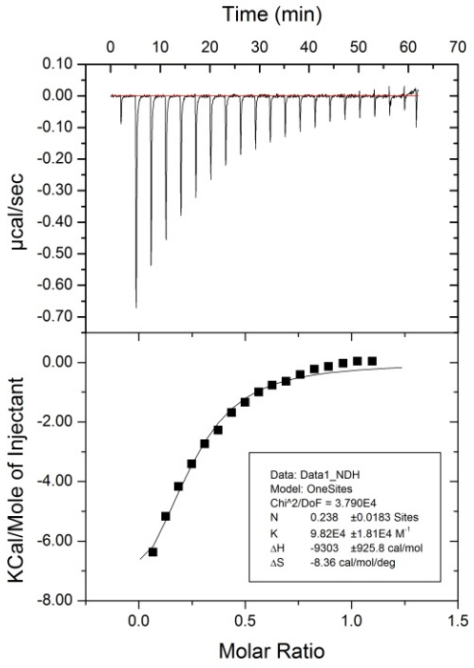

Figure S31. ITC of R8GKMe3 (Ac-WGGG-QTAGKMe_3STG-NH_2) (0.65 mM) into **A_2H** (107 uM) at 26°C in 10 mM borate buffer, pH 8.5.

A_2G Example Titrations

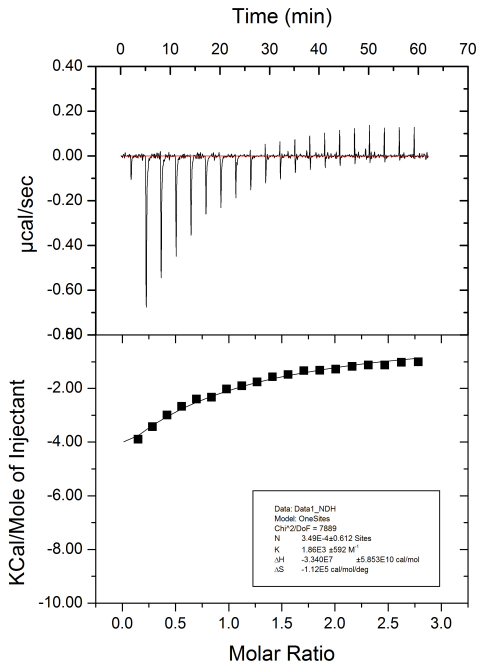

Figure S32. One of two trials of R8GK9 (Ac-WGGG-QTAGKSTG-NH_2) (1.5 mM) into **A_2G** (152 uM) at 26°C in 10 mM borate buffer, pH 8.5.

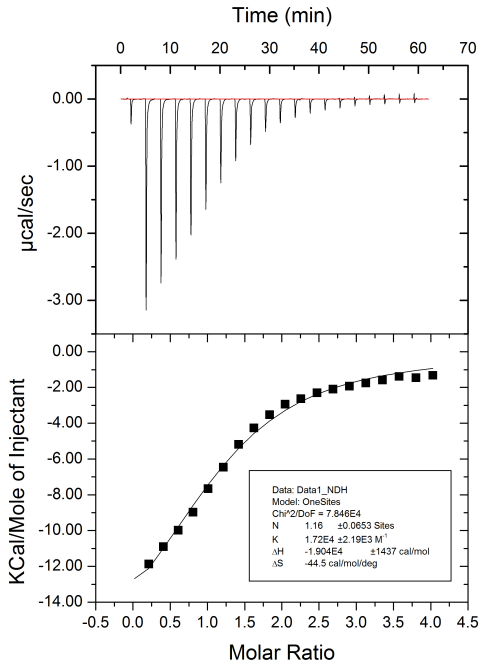

Figure S33. One of two trials of Lys (Ac-WGGG-QTARKSTG-NH_2) (2 mM) into **A_2G** (101 uM) at 26°C in 10 mM borate buffer, pH 8.5.

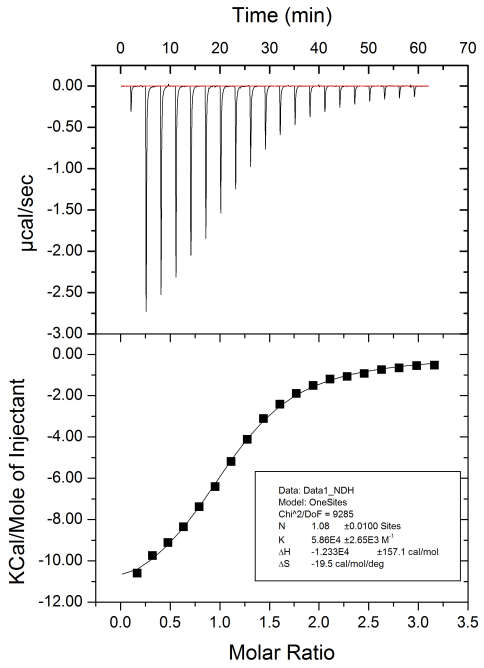

Figure S34. One of two trials of KMe (Ac-WGGG-QTARK(Me)STG-NH_2) (1.57 mM) into **A_2G** (101 uM) at 26°C in 10 mM borate buffer, pH 8.5.

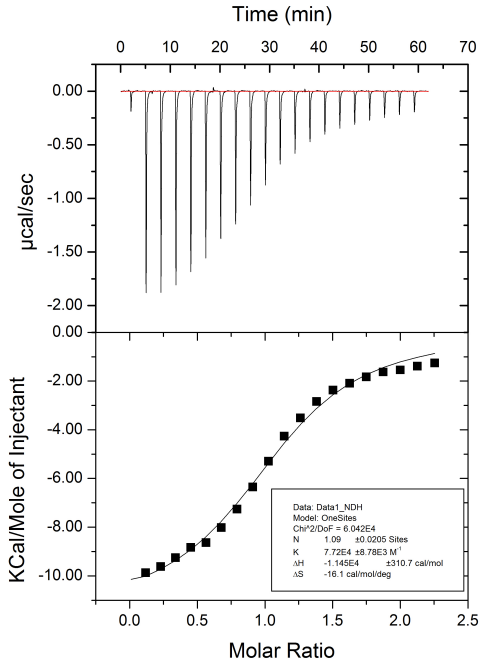

Figure S35. One of two trials of KMe2 (Ac-WGGG-QTARKMe_2STG-NH_2) (1.03 mM) into **A_2G** (93 uM) at 26°C in 10 mM borate buffer, pH 8.5.

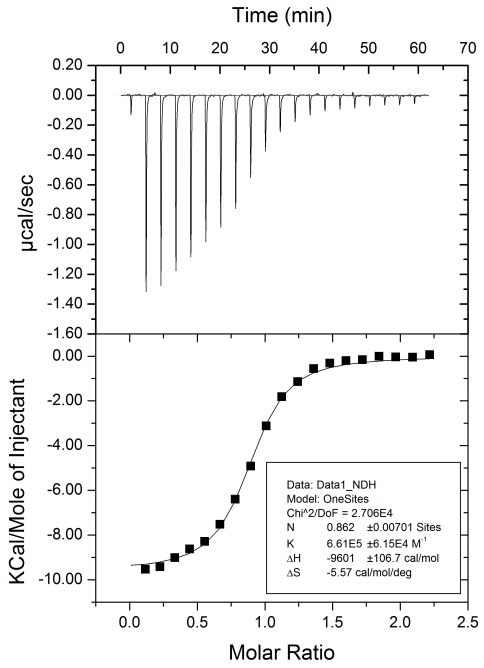

Figure S36. One of two trials of KMe3 (Ac-WGGG-QTARKMe_3STG-NH_2) (0.75 mM) into **A_2G** (75 uM) at 26°C in 10 mM borate buffer, pH 8.5. Heat of dilution was determined from the average of the last four data points and subtracted prior to fitting to a one-site binding curve.

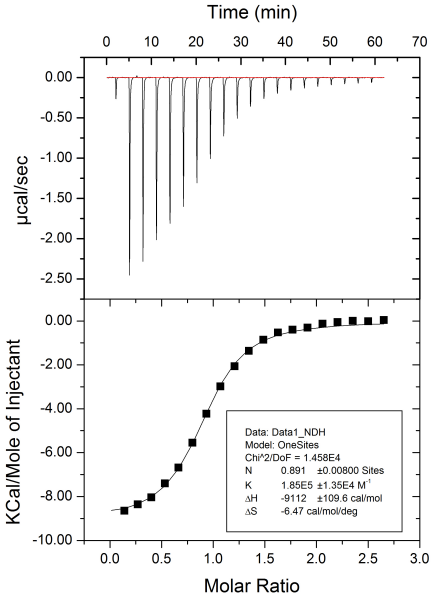

Figure S37. One of two trials of R8GKMe3 (Ac-WGGG-QTAGKMe_3STG-NH_2) (1.42 mM) into **A_2G** (109 uM) at 26°C in 10 mM borate buffer, pH 8.5. Heat of dilution was determined from the average of the last five data points and subtracted prior to fitting to a one-site binding curve.

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