Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

High-affinity recognition of the human C-reactive protein independent of phosphocholine.

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^{1}H NMR WaterLOGSY experiment.

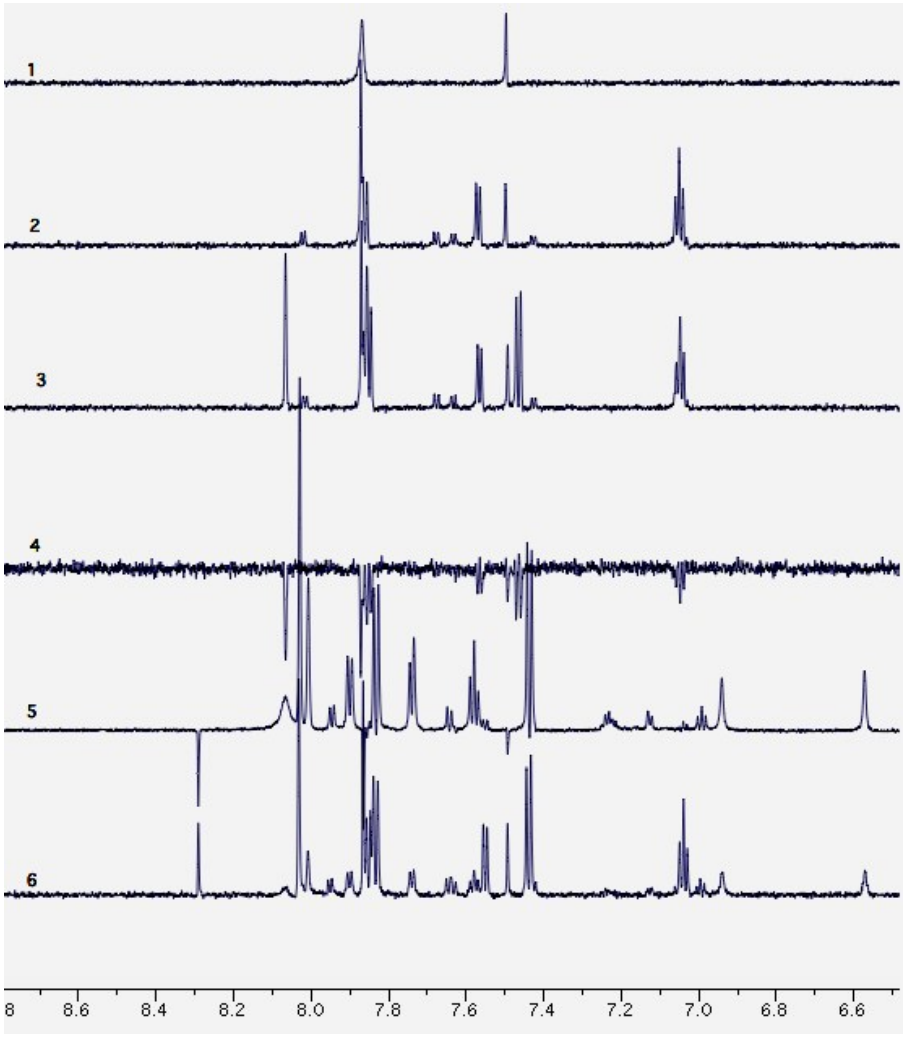
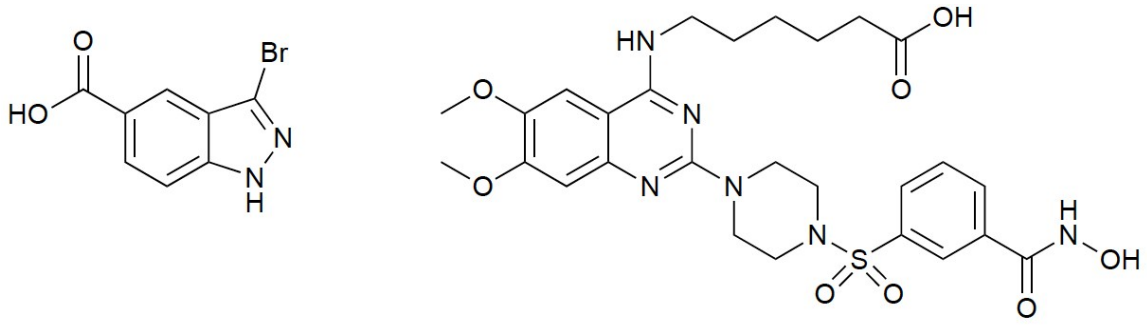


Figure S0. WaterLOGSY experiment disclosing affinities of compounds **1**, **2** and **3** for CRP. Protein concentration was 7 μM in PBS buffer. Spectrum 1 is **3**, spectrum 2 is **2** and **3**, spectrum 3 is **1**, **2** and **3.** Spectrum 4 is waterLOGSY experiment showing inverted peaks of **1**, **2** and **3** due to interactions with CRP. Spectrum 5 is waterLOGSY spectrum of compounds **1**, **2**, **3** and two compounds, see below, one of which binds. The structural

similarities between compounds **1**, **2** and **3,** guided the design. Spectrum 6 is ^{1}H NMR spectrum of compound mix.



Structures of compounds evaluated in waterLOGSY in mix with compounds **1**, **2** and **3.** Left, obtained from commercial sources and right, a gift from ModPro AB. Right compound binds but is too large to be used as fragment.

Small molecule synthesis of 5

*(E)-3-ethoxyprop-2-enoyl chloride (**6**).* Ethyl vinylether (0.97 ml, 10 mmol) was added to oxalyl chloride (1.30 mL, 15 mmol) while stirring and cooling in an ice bath. Stirring was continued in the ice bath for 2 hours, then at r.t. over night. The excess of oxalyl chloride was evaporated at reduced pressure. The residue was heated (120 ^{o}C) for 30 minutes with stirring and then purified by vacuum distillation (bp: 60–61 ^{o}C/4 mmHg), affording intermediate **6** as a colorless oil (738mg, 55 %). ^{1}H NMR (400 MHz): 7.78 (1H, d, 12.1 Hz,), 5.50 (1H, d,12.1 Hz), 4.05 (2H, q, 7.0 Hz), 1.39 (3H, t, 7.0 Hz, 3H). The ^{1}H NMR chemical shifts were identical to those previously reported.[1]

*Methyl 2-amino-5-bromobenzoate (**8**).* Anthranilic acid (11g, 80.2 mmol) was dissolved in DCM (100 mL) and thionyl chloride (25 mL) was dropwise added to the stirred solution. After 30 minutes DCM and excess of thionyl chloride were evaporated, methanol (100 mL) was added to the residue and stirring was continued overnight. The completion of the reaction was ascertained by TLC (9:1 PE/EA). The mixture was concentrated and the residue was partitioned between DCM/H_2O (1:1), the organic layer was washed with brine, dried with anhydrous sodium sulphate and concentrated. The residue was purified by column chromatography (9:1 PE/EA) to give pure ester **7** as a faintly yellow oil (6 g, 50 %). ^{1}H NMR (300 MHz): 7.85 (1H, dd, 8.0, 1.6 Hz), 7.31 – 7.20 (1H, m), 6.72 – 6.58 (2H, m), 5.73 (NH_2, broad), 3.87 (3H, s). The ^{1}H NMR chemical shifts were identical to those previously reported.[2]

A mixture of 7 (100 mg, 0.662 mmol), N-bromoscuccinimide (117 mg, 0.662 mmol) and dry THF (5 mL) was refluxed for 1 hour. The completion of the reaction was ascertained by TLC (9:1 PE/EA). The mixture was evaporated, and partitioned between water and DCM (1:1). The organic layer was dried with anhydrous sodium sulphate and concentrated. The residue was purified by column chromatography (9:1 PE/EA) to give pure 8 (70 mg, 46 %). ^{1}H NMR: 7.96 (1H, d, 2.4 Hz), 7.31 (1H, dd, 8.8, 2.4 Hz), 6.55 (1H, d, 8.8 Hz), 5.74 (NH_2, broad), 3.86 (3H, s). The ^{1}H NMR chemical shifts were identical to those previously reported. [3] ESI-MS calcd for $C_8H_8BrNO_2$ 228.9, 230.9; found 230.1, 232.1 $[M+H]^+$.

*7-Methoxylcarbonylheptylzinc bromide lithium chloride complex (**10**).*[4] 8-Bromooctanoic acid (4.0 g, 17.92 mmol) was dissolved in dry DCM (60 mL), then thionyl chloride (4.3 g, 35.80 mmol) was dropwise added. The reaction mixture was stirred for 30 minutes, then evaporated. Methanol (60 mL) was added to the residue and the reaction was stirred at room temperature overnight. The completion of the reaction was ascertained by TLC (6:1 PE/diethyl ether). The mixture was evaporated and the residue was partitioned between DCM and water 1:1. The organic layer was dried with anhydrous sodium sulphate and then concentrated. The residue was purified by column chromatography to give methyl ester 9 (4 g, 94 %). ^{1}H NMR (400 MHz): 3.66 (3H, s), 3.40 (2H, t, 6.8 Hz), 2.30 (2H, t, 7.5 Hz), 1.90 – 1.79 (2H, m), 1.68 – 1.54 (2H, m), 1.49 – 1.25 (6H, m). The ^{1}H NMR chemical shifts were identical to those reported.[5]

A flame dried flask was charged with anhydrous LiCl (870 mg, 21 mmol) and dried with a heat gun under high vacuum, then zinc dust (1.335 g, 21 mmol) was added, and the flask was dried again with the heat gun under high vacuum. Anhydrous THF (20 mL) was added and the mixture was activated: 1,2-dibromoethane and trimethylsilyl chloride. The 1,2-dibromoethane (69 μL) was added first, then the flask was heated until foaming, this procedure was repeated one more time, then trimethylsilyl chloride (18.9 μL) was added and the mixture was stirred for 10 minutes. After that, **9** (3.54 g, 15 mmol) and KI (2.49 g, 15 mmol) was sequentially added. The flask was evacuated and refilled with nitrogen gas, then the reaction mixture was stirred at 50 ^{o}C overnight. The obtained solution of **10** was directly used in the synthesis of **11**.

*Methyl 2-amino-5-(7'-methoxycarbonyheptyl)-benzoate(**11**).*[6] **8** (1.8 g, 7.89 mmol), $Pd(OAc)_2$ (53 mg, 3.0 %) and S-phos (194 mg, 6.0 %) were dissolved in anhydrous THF (10 mL). The reaction mixture was stirred for 10 minutes, then the solution of **10** was dropwise added and stirring was continued at room temperature for 48 hours. The completion of the reaction was ascertained by TLC (9:1 toluene/EA). The mixture was evaporated and the residue was partitioned between saturated aqueous NH_4Cl solution and EA. The organic layer was dried with anhydrous sodium sulphate

and concentrated. The residue was purified by column chromatography (9:1 toluene/EA) to give pure **11** (2.38 g, 98 %) .^{1}H NMR (400 MHz): 7.66 (1H, s), 7.11 (1H, d, 8.3 Hz), 6.67 (1H, d, 8.3 Hz), 3.87 (3H, s), 3.66 (3H, s), 2.48 (2H, t, 7.8 Hz), 2.29 (2H, t, 7.5 Hz), 1.69 – 1.50 (4H, m), 1.36 – 1.23 (6H, m). ^{13}C NMR (101 MHz): 174.27, 168.53, 147.44, 134.56, 131.29, 130.30, 117.26, 111.21, 51.53, 51.43, 34.83, 34.07, 31.50, 29.08, 29.05, 28.94 24.94. HRMS calcd for $C_{17}H_{25}NNaO_4$ 330.1676, found 330.1679 $[M+Na]^+$.

*(E)-Methyl 2-(3''-ethoxyprop-2''-enoylamido)-5-(7'-methoxycarbonylheptyl) benzoate (**12**).* Pyridine (0.75 mL, 9.3 mmol) and **6** (1.25 g, 9.3 mmol) were sequentially added to **12** (2.38 g, 7.75 mmol) dissolved in DCM (30 mL). The reaction mixture was stirred overnight at room temperature. The completion of the reaction was ascertained by TLC (9:1 toluene/EA). The DCM solution was washed with 1 M HCl, followed by brine wash once. The DCM layer was separated, concentrated, purified by column chromatography (9:1 toluene/EA) to give pure **12** (2.36 g, 75 %). ^{1}H NMR (400 MHz) 10.81 (NH), 8.66 (1H, d, 8.6 Hz), 7.80 (1H, d, 2.2 Hz), 7.65 (1H, d, 12.2 Hz), 7.34 (1H, dd, 8.6, 2.2 Hz), 5.38 (1H, d, 12.2 Hz), 3.99 – 3.90 (5H, m), 3.66 (3H, s), 2.56 (2H, t, 7.8 Hz), 2.29 (2H, t, 7.6 Hz), 1.66 – 1.52 (4H, m), 1.40 – 1.23 (9H, m). ^{13}C NMR (101 MHz): 174.23, 168.93, 165.51, 160.75, 140.01, 136.40, 134.75, 130.17, 120.50, 114.48, 100.06, 66.83, 52.16, 51.43, 35.09, 34.04, 31.26, 29.02, 28.91, 24.88, 14.54. HRMS calcd for $C_{22}H_{31}NNaO_6$ 428.2044, found 428.2051 $[M+Na]^+$.

*Methyl 6-(7'-carboxyheptyl)-2-oxo-1,2-dihydroquinoline-8-carboxylate (**13**).* **12** (1.2 g, 3.5 mmol) was mixed with concentrated 95 % sulfuric acid (5 ml) and the mixture was stirred overnight at room temperature. To monitor the progress of the reaction, an aliquot (10 μL) of the reaction mixture was mixed with concentrated 6 M NaOH (1 mL), then extracted with EA (500 μL). The EA layer was analyzed by TLC (20:1 DCM/Methanol) to ascertain reaction completion. The reaction mixture was poured onto ice, neutralized with NaOH pellets, then EA was used to extract the organic compounds from the aqueous mixture. The organic layer was washed by brine and dried with anhydrous sodium sulphate and concentrated. The residue was purified by column chromatography (30:1 DCM/Methanol and several drops of acetic acid) to

give pure mono-ester **13** (145 mg, 12 %). ^{1}H NMR (400 MHz): 11.65 (NH), 8.06 (1H, d, 2.0 Hz), 7.70 (1H, d, 9.6 Hz), 7.53 (1H, d, 2.0 Hz), 6.69 (1H, d, 9.6 Hz), 3.99 (3H, s), 2.68 (2H,t,7.7 Hz), 2.35 (2H, t, 7.5 Hz), 1.73 – 1.57 (4H, m), 1.42 – 1.30 (6H, m). ^{13}C NMR (101 MHz) 178.60, 167.12, 162.48, 140.23, 137.58, 136.08, 133.56, 133.06, 122.48, 120.70, 113.16, 52.52, 34.96, 33.83, 31.25, 28.99, 28.87, 28.80, 24.60. ESI-MS calcd for $C_{19}H_{23}NO_5$ 345.1, found 368.0 $[M+Na]^+$, 344.2 $[M-H]^-$.

*2-oxo-6-(7'-pentafluorophenoxycarbonylheptyl)-1,2-dihydroquinoline 8-carboxylic acid (**5**).* **13** (145 mg, 0.413 mmol), LiOH (19.5 mg, 0.826 mmol) and t-butanol/water (1:1, 10 mL) were mixed in a 25 ml RB flask. The reaction was stirred under reflux for 2 hours. The completion of the reaction was ascertained by TLC (20:1 DCM/Methanol). 1 M HCl was added to adjust the pH to 7, and the mixture was extracted with EA. The organic layer was dried with anhydrous sodium sulphate, and concentrated to give the diacid intermediate **14** (137 mg, 100%). A small amount was taken to obtain ^{1}H NMR spectrum. ^{1}H NMR (400 MHz, DMSO-d6) 11.99 (NH), 8.02 (1H, d, 2.0 Hz), 7.96 (1H, d, 9.7 Hz), 7.80 – 7.75 (1H, m), 6.57 (1H, dd, 9.7, 2.0 Hz), 2.66 (2H, t, 7.6 Hz), 2.18 (2H, t, 7.4 Hz), 1.64 – 1.43 (4H, m), 1.34 – 1.21 (6H, m). ^{13}C spectrum was not recorded. HRMS calcd for $C_{18}H_{20}NO_5$ 330.1346, found 330.1347 $[M-H]^-$.

A 25 ml R.B. flask charged with **14** (137 mg, 0.413 mmol) and pentafluorophenol (76 mg, 0.413 mmol), was evacuated and refilled with N_2 three times. Anhydrous pyridine (10 mL) was added. The reaction was stirred for 5 minutes, and DIC (51.7 mg, 0.413 mmol) was added dropwise. The reaction was left stirring overnight at room temperature. The completion of the was ascertained by TLC (8:2 EA/toluene). The mixture was evaporated, and the residue was partitioned between EA and H_2O. The organic layer was first dried with anhydrous sodium sulphate and then concentrated. The residue was purified by column chromatography (8:2 EA/toluene) to give pure **5** (80 mg, 38%). ^{1}H NMR (400 MHz): 11.18 (NH), 8.28 (1H, d, 2.0 Hz), 7.75 (1H, d, 9.7 Hz), 7.70 (1H, d, 2.0 Hz), 6.73 (1H, d, 9.6 Hz), 2.76 (2H, t, 7.8 Hz), 2.66 (2H, t, 7.4 Hz), 1.83 – 1.66 (4H, m), 1.47 – 1.38 (6H, m). ^{13}C NMR (101 MHz) 171.15, 169.48, 162.00, 139.95, 138.67, 136.38, 135.12, 134.04, 123.21, 120.95, 109.87,

34.94, 33.25, 31.23, 29.68, 28.91, 28.87, 28.69, 24.65. HRMS calcd for $C_{24}H_{20}F_5NNaO_5$ 520.1154, found 520.1167 $[M+Na]^+$.

Small molecule synthesis of 4

*E-Methyl 2-(3'-ethoxyprop-2-enoylamido) benzoate (**15**).* Pyridine (0.83 mL) and **6** (1.41 g, 10.5 mmol) were sequentially added to **7** (1.33 g, 8.8 mmol) dissolved in DCM (30 mL). The same procedure was performed as that in the synthesis of **12** to give pure **15** (2.1 g, 95 %). ^{1}H NMR (400 MHz): 10.92 (NH), 8.77 (1H, d, 8.0Hz), 8.01 (1H, d, 8.0 Hz), 7.66 (1H, d, 12.2 Hz), 7.53 (1H, dd, 8.0 Hz), 7.04 (1H, dd, 8.0 Hz), 5.40 (1H, d, 12.2 Hz), 4.01 – 3.90 (5H, m), 1.36 (3H, t, 7.1 Hz). ^{13}C NMR (101 MHz): 168.87, 165.67, 160.98, 142.22, 134.61, 130.74, 121.90, 120.46, 114.53, 100.01, 66.90, 52.24. ESI-MS calcd for $C_{13}H_{15}NO_4$ 249.1, found 250.2 m/z $[M+H]^+$.

*2-Oxo-1,2-dihydroquinoline-8-carboxylic acid (**4**).* **15** (300 mg, 1.2 mmol) was mixed with 37 % acqueous hydrochloric acid (9 mL) and the mixture was stirred overnight at room temperature. The same procedure was performed as that in the synthesis of **13**. The residue was purified by chromatography (1:1 EA/PE) to give pure methyl ester intermediate **16** (100 mg, 40 %). ^{1}H NMR (400 MHz): 11.79 (NH), 8.25 (1H, d, 7.5 Hz), 7.80 – 7.70 (2H, m), 7.25 – 7.19 (1H, m), 6.71 (1H, d, 9.5 Hz), 3.99 (s, 3H). ^{13}C spectrum was not recorded. HRMS calcd for $C_{11}H_9NNaO_3$ 226.0475, found 226.0477 $[M+Na]^+$.

The ester **16** (10 mg, 0.05 mmol) was added to 5 mL 5 % NaOH. The reaction was stirred at room temperature for 2 hours. The completion of the reaction was ascertained by TLC (1:1 EA/PE). 1 M HCl was added to adjust the pH to 7, and the mixture was extracted with EA. The organic layer was dried by anhydrous sodium sulphate, and concentrated to give **4** (9.4 mg, 99%). ^{1}H NMR (400 MHz, DMSO-d6): 11.89 – 11.73 (NH), 8.20 (1H, dd, 7.8, 1 Hz), 8.04 (1H, d, 9.7 Hz), 7.99 (1H, dd, 7.8, 7.8 Hz), 7.31 (1H, dd, 7.8, 7.8 Hz), 6.62 (1H, d, 9.7Hz). The ^{1}H NMR chemical shifts were identical to those previously reported.[7] ESI-MS calcd for $C_{10}H_7NO_3$ 189.0, found 190.2 m/z $[M+H]^+$, 188.0 m/z $[M-H]^-$.

NMR spectra of intermediates and products **4**-**16**. Assignments shown in the Experimental section of the main article. No ^{13}C spectra were recorded for **4**, **6**, **7**, **8** and **9**, as their ^{1}H NMR spectra were in agreement with those previously reported.

(E)-3-ethoxyprop-2-enoyl chloride (**6**

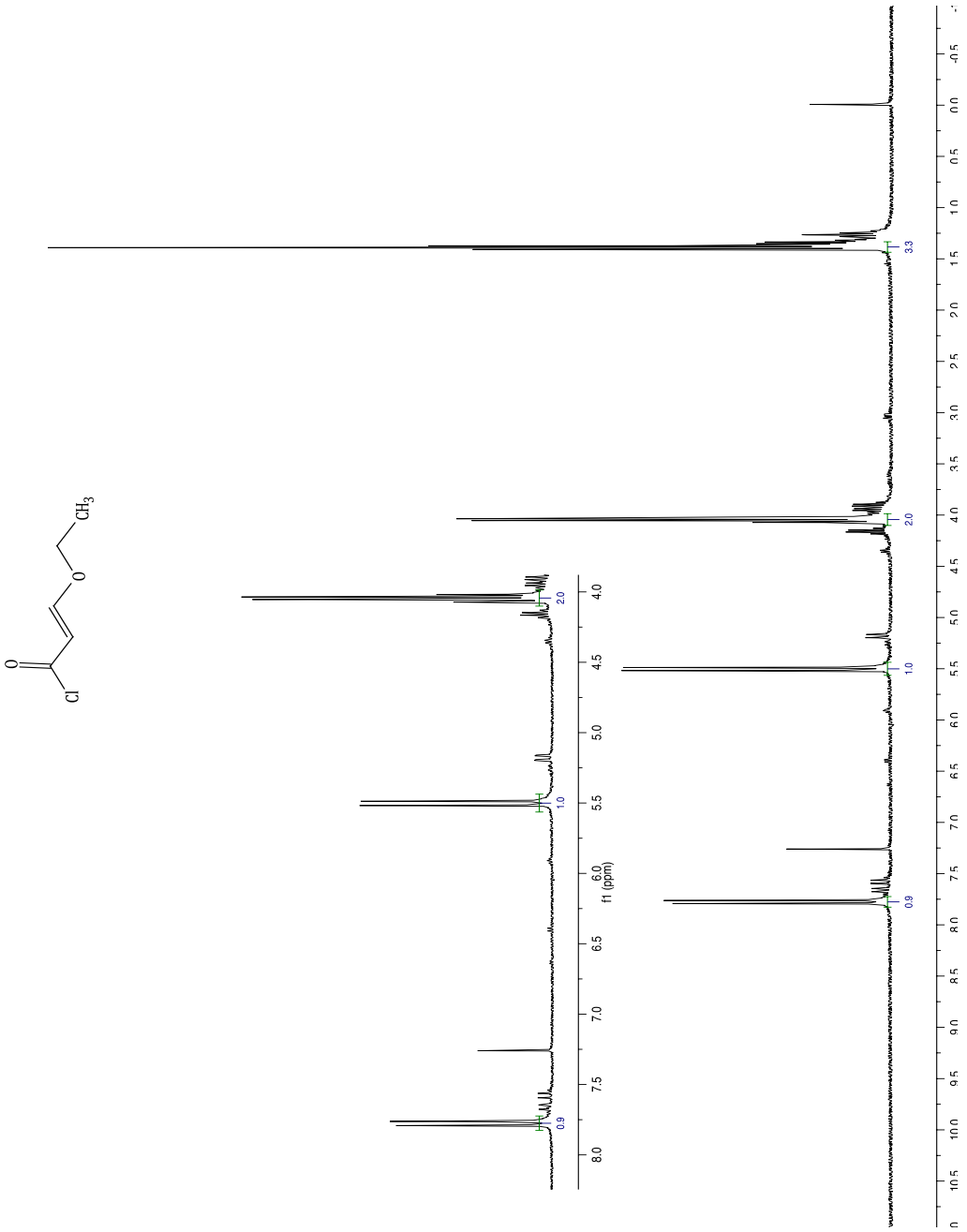


Figure S1. ^{1}H NMR spectrum of **6**.

Methyl 2-amino-benzoate (**7**)

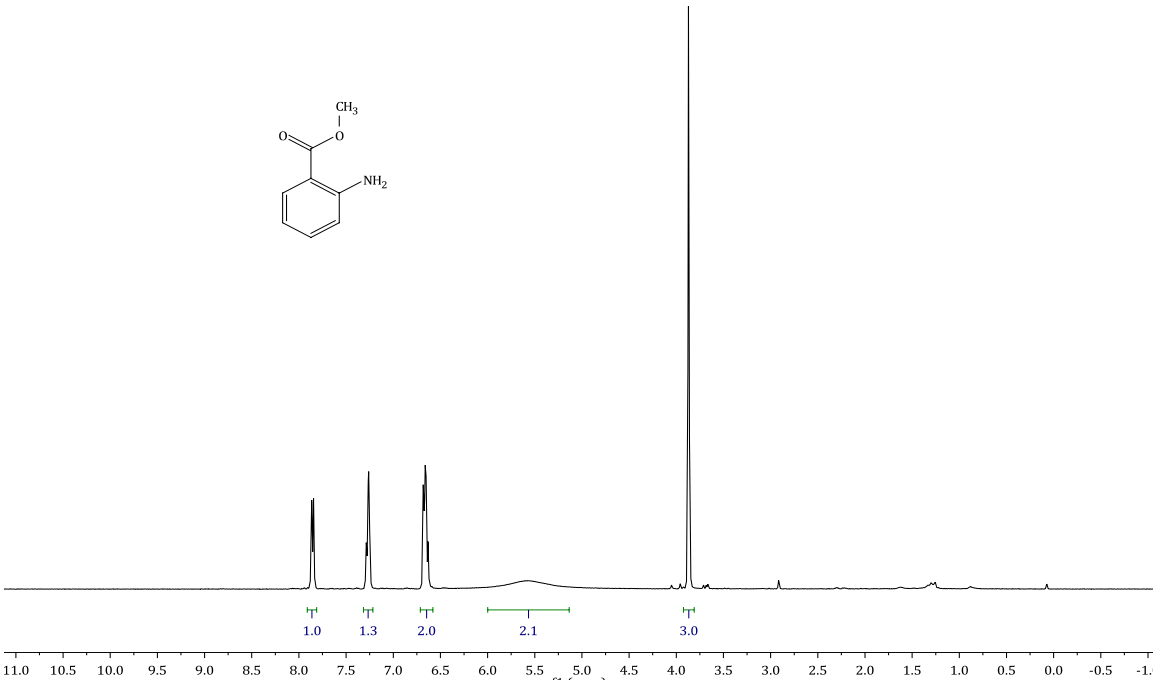


Figure S2. ^{1}H NMR spectrum of **7**.

Methyl 2-amino-5-bromobenzoate (**8**)

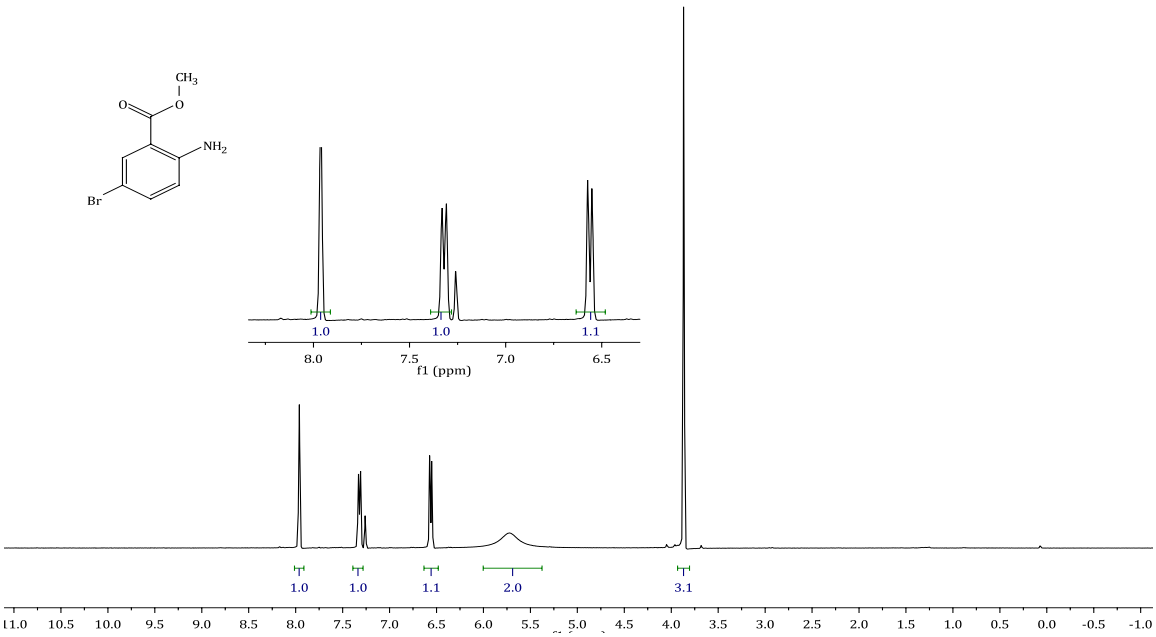


Figure S3. ^{1}H NMR spectrum of **8**.

7-Methoxylcarbonylheptyl bromide(**9**)

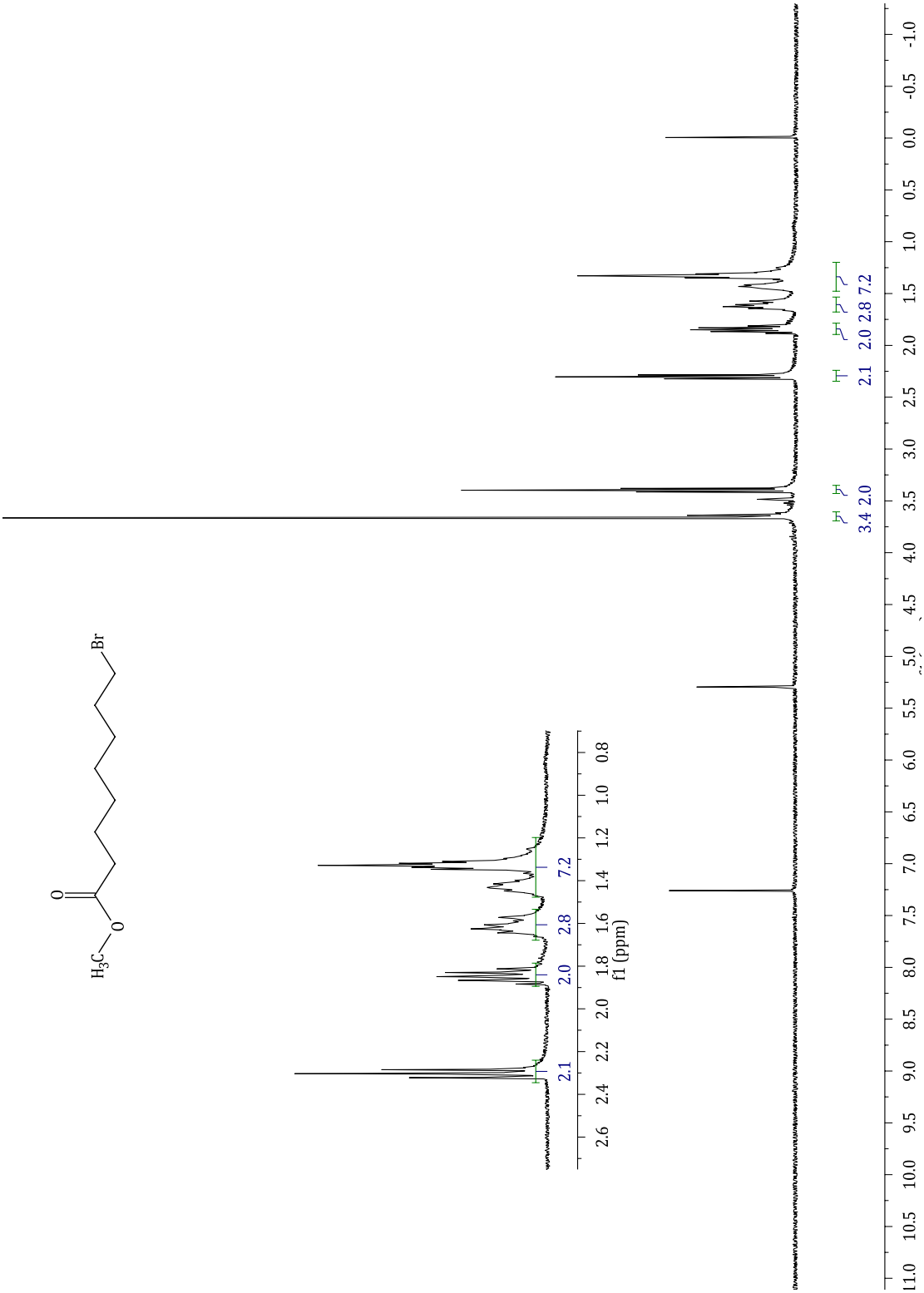
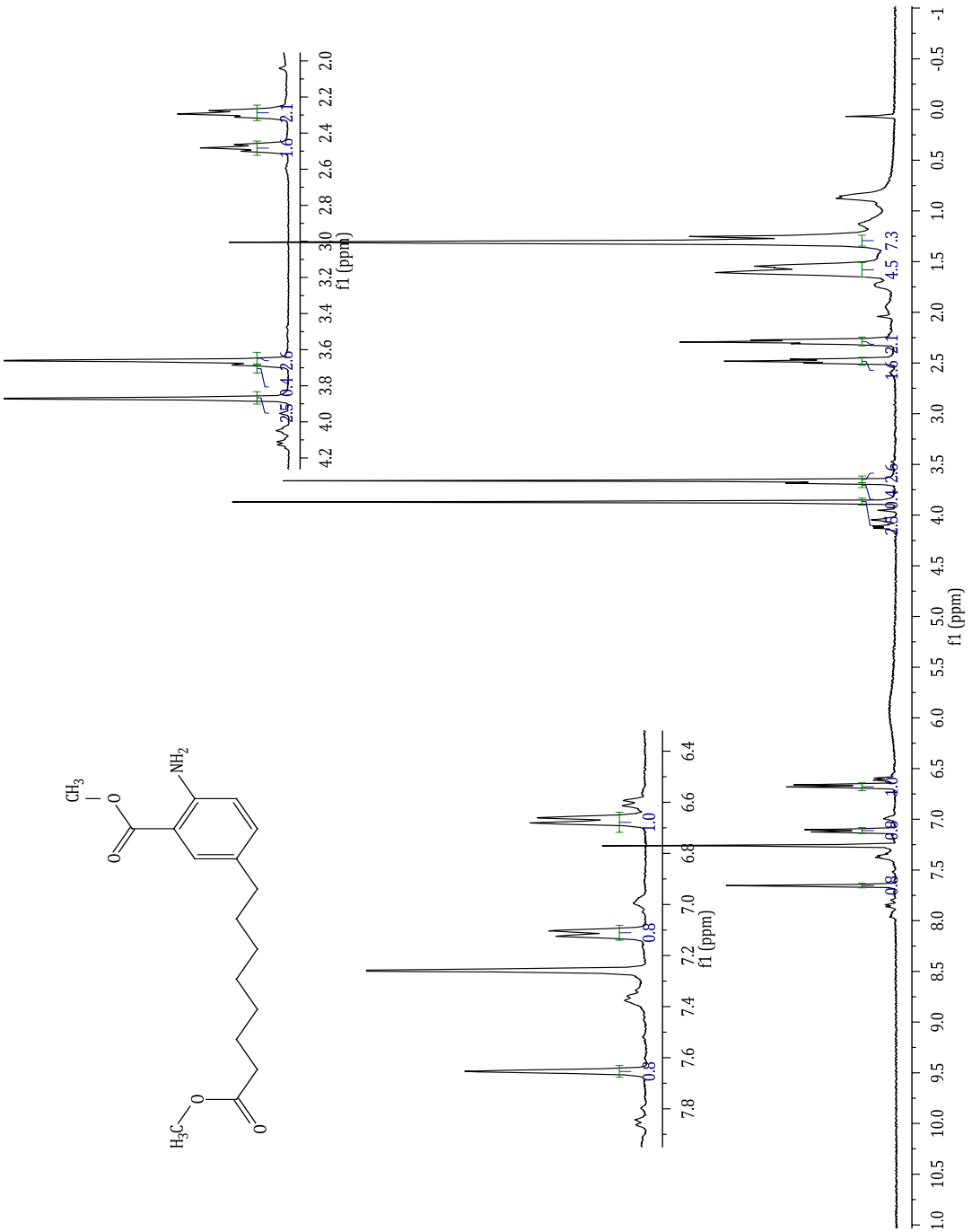


Figure S4. ^{1}H NMR spectrum of **9**.

Methyl 2-amino-5-(7’-methoxycarbonyheptyl)-benzoate(**11**)



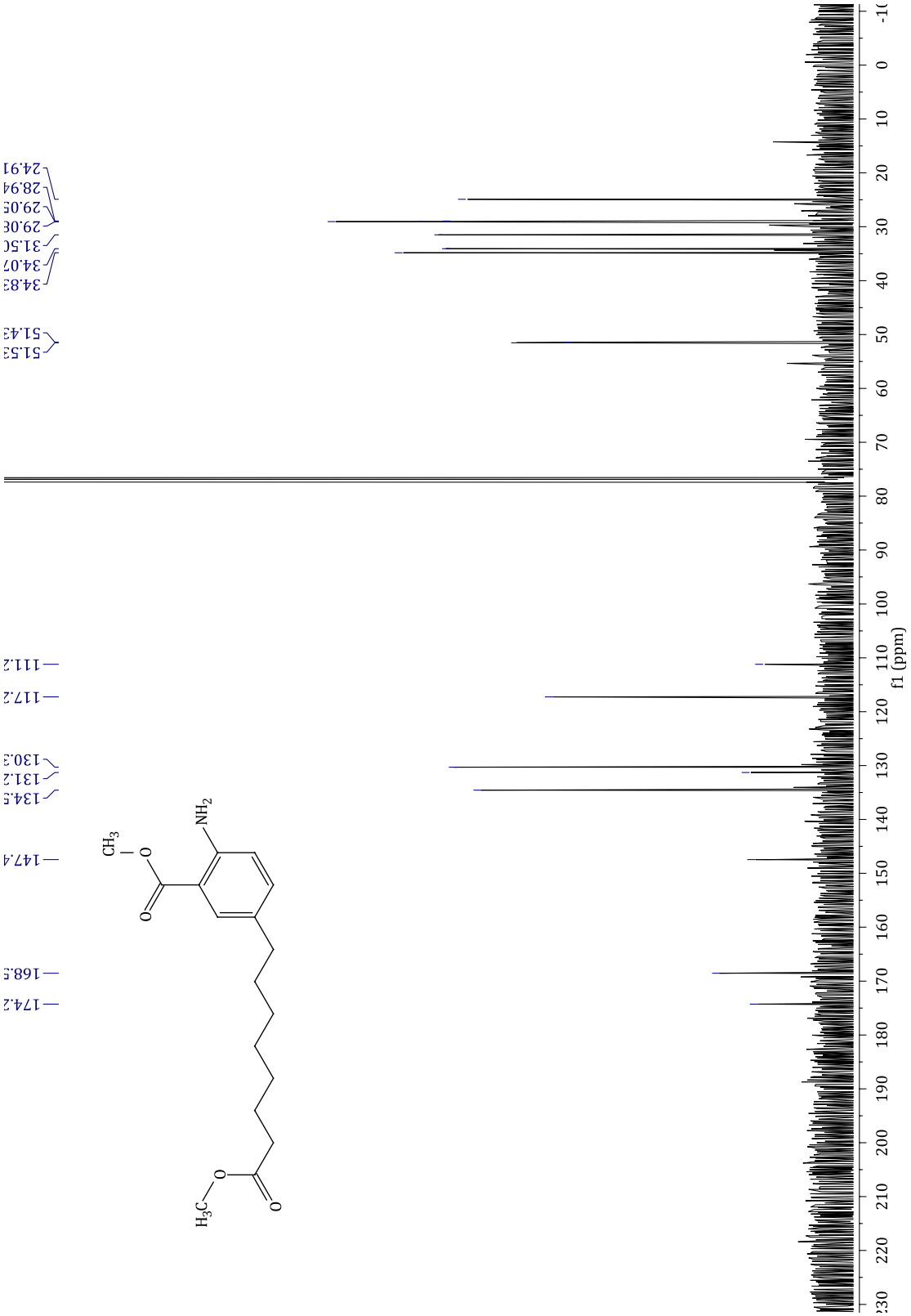
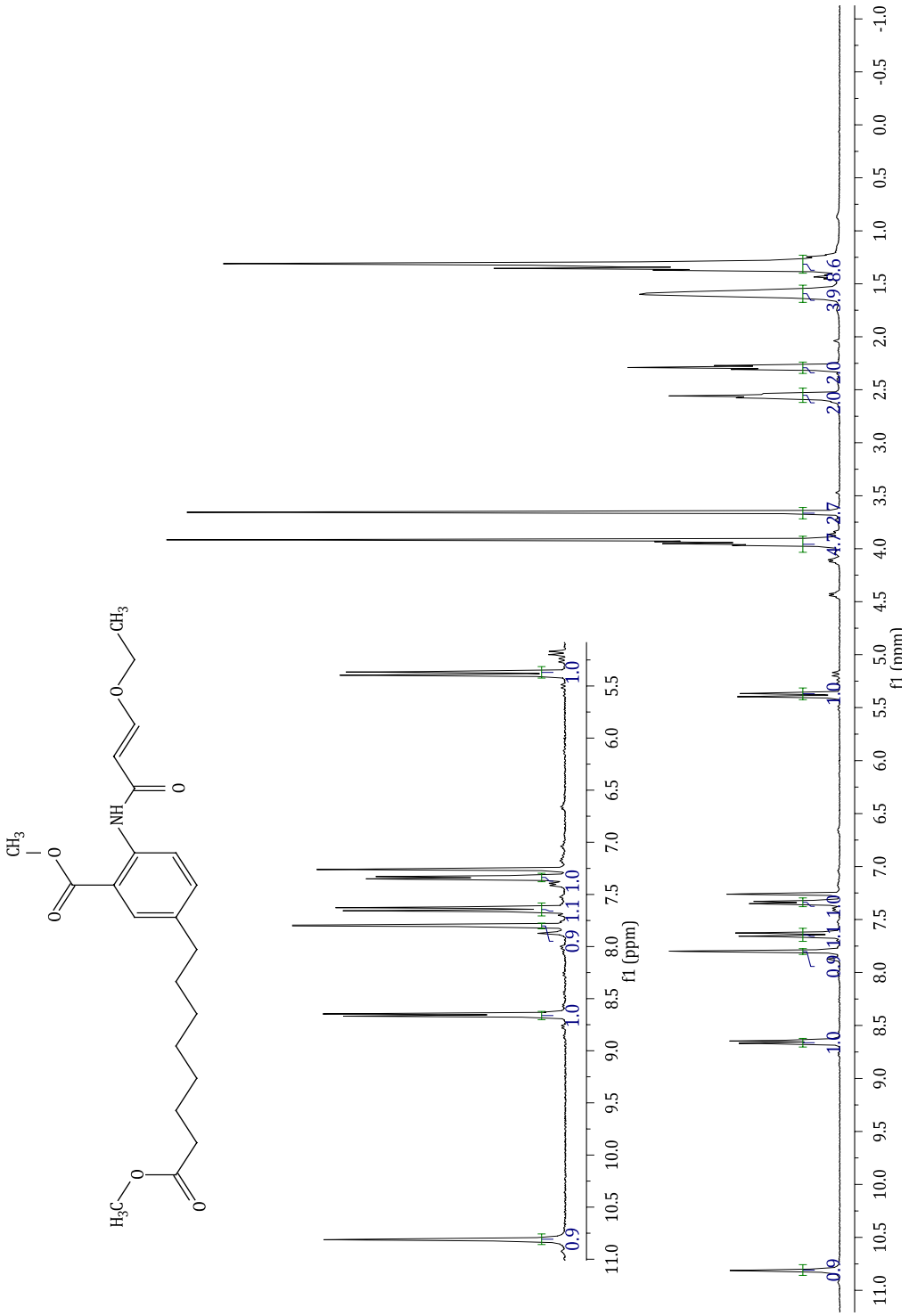


Figure S5. ^{1}H NMR & ^{13}C NMR spectra of **11**.

(E)-Methyl 2-(3’’-ethoxyprop-2”-enoylamido)-5-(7’-methoxycarbonylheptyl) benzoate (**12**)



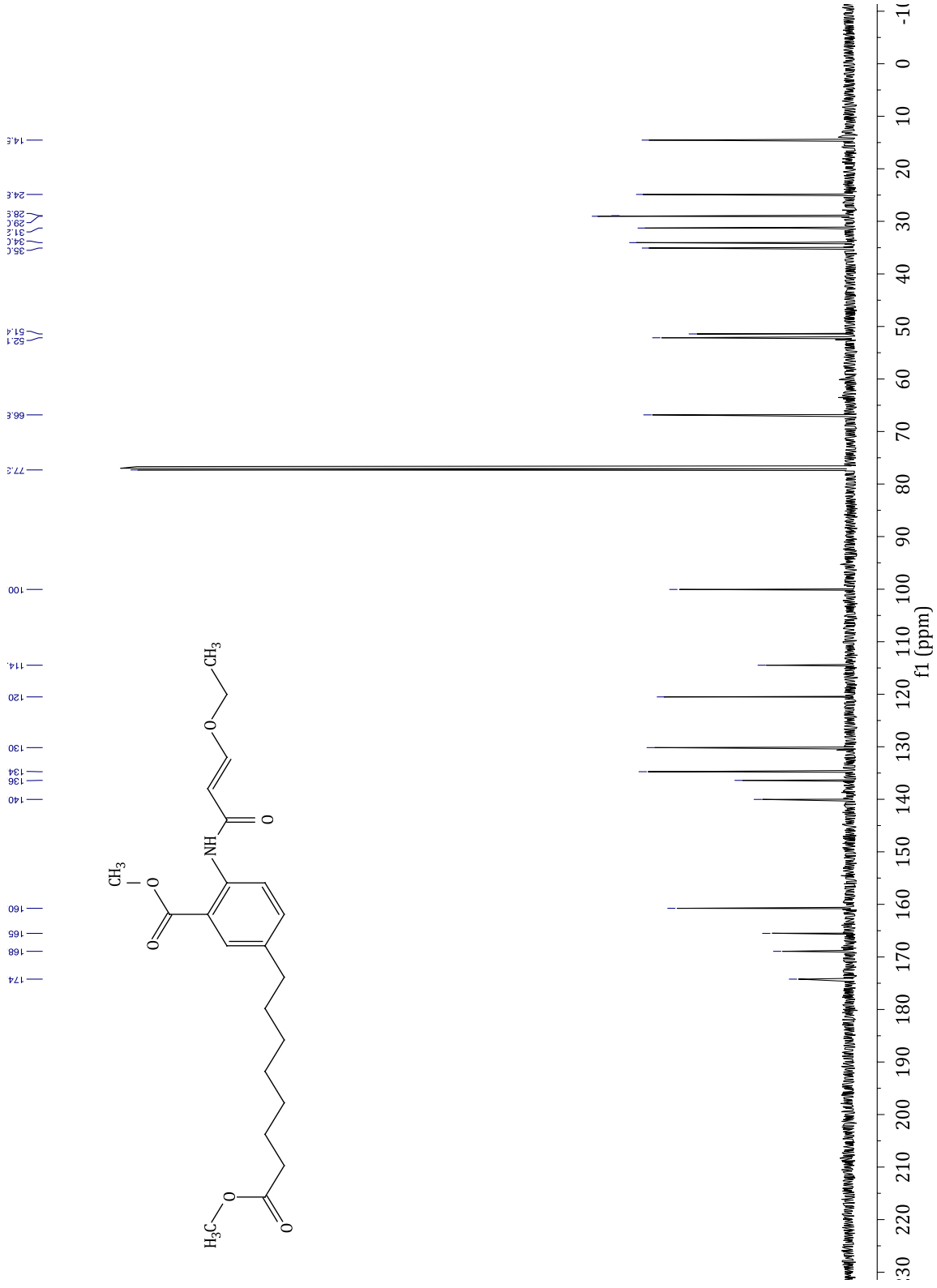
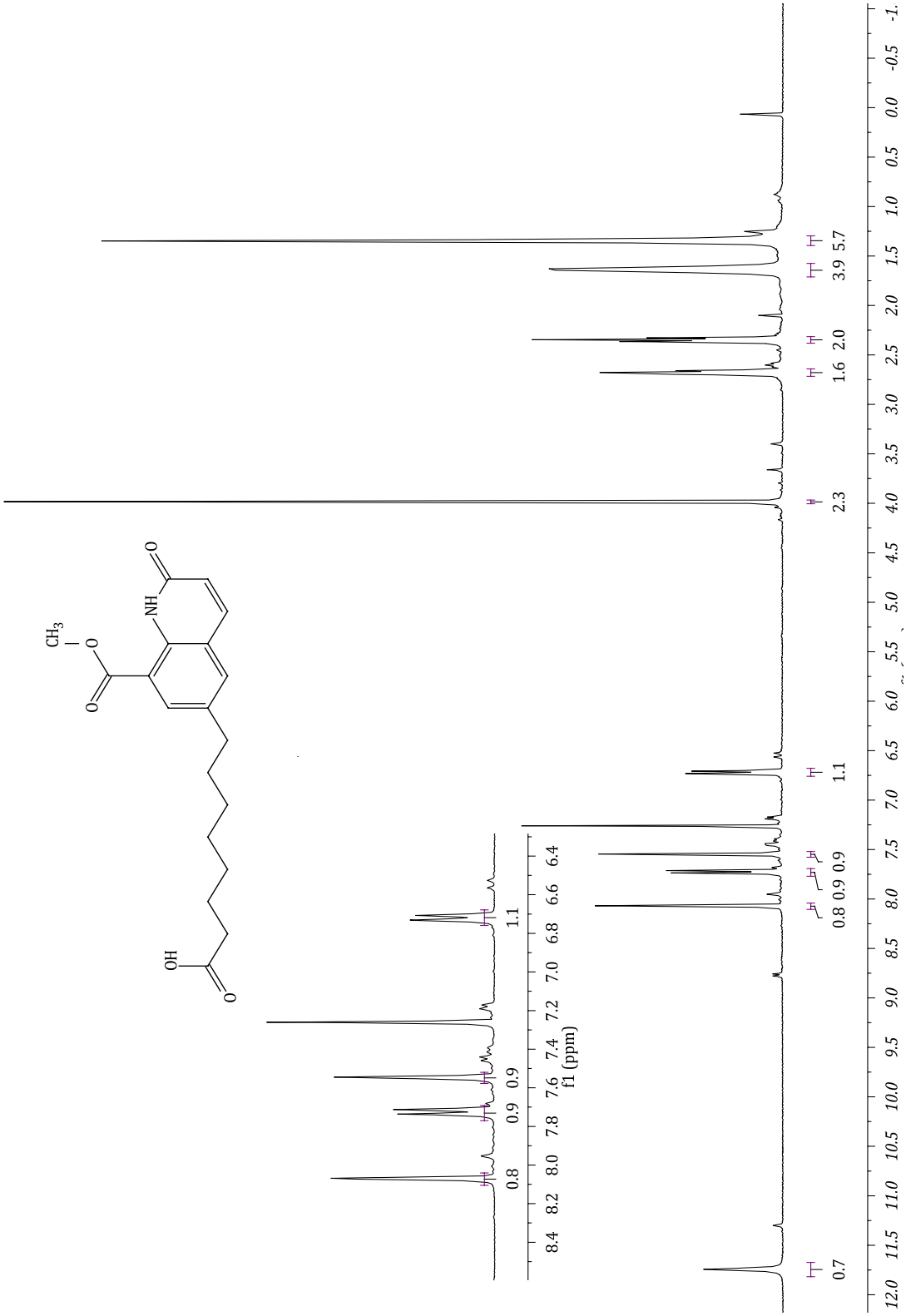


Figure S6. ^{1}H NMR & ^{13}C NMR spectra of **12**.

Methyl 6-(7’-carboxyheptyl)-2-oxo-1,2-dihydroquinoline-8-carboxylate (**13**)



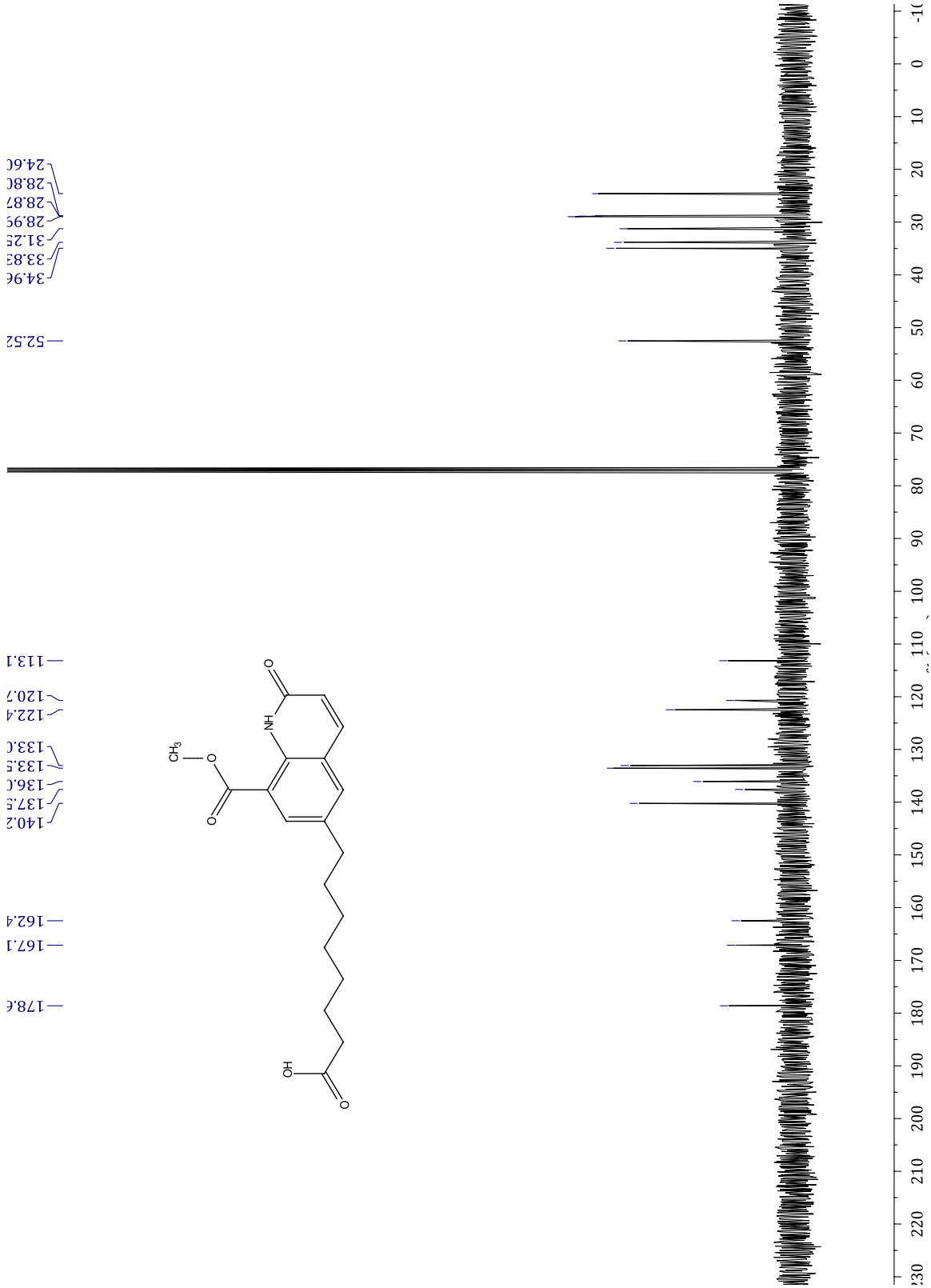
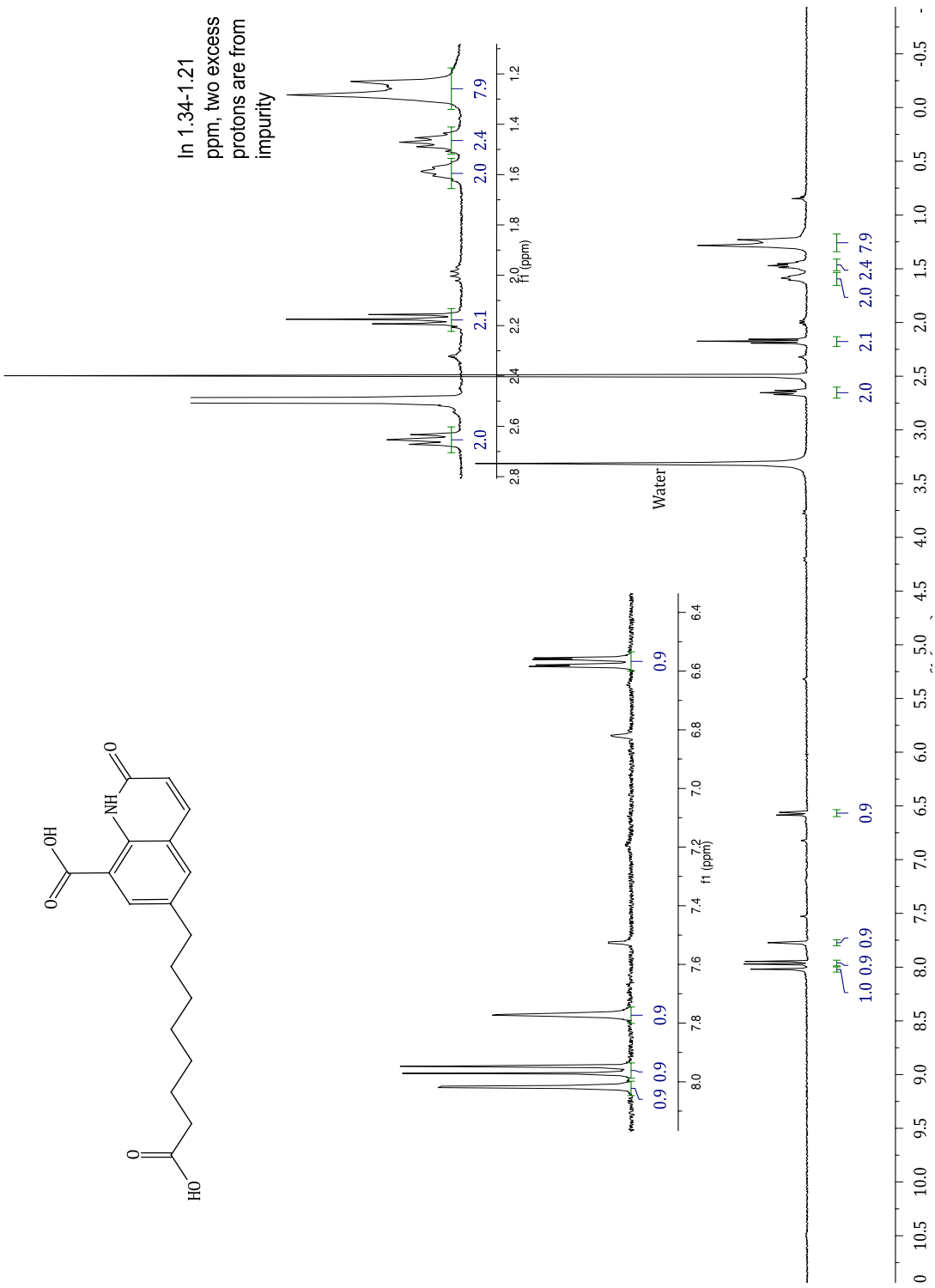
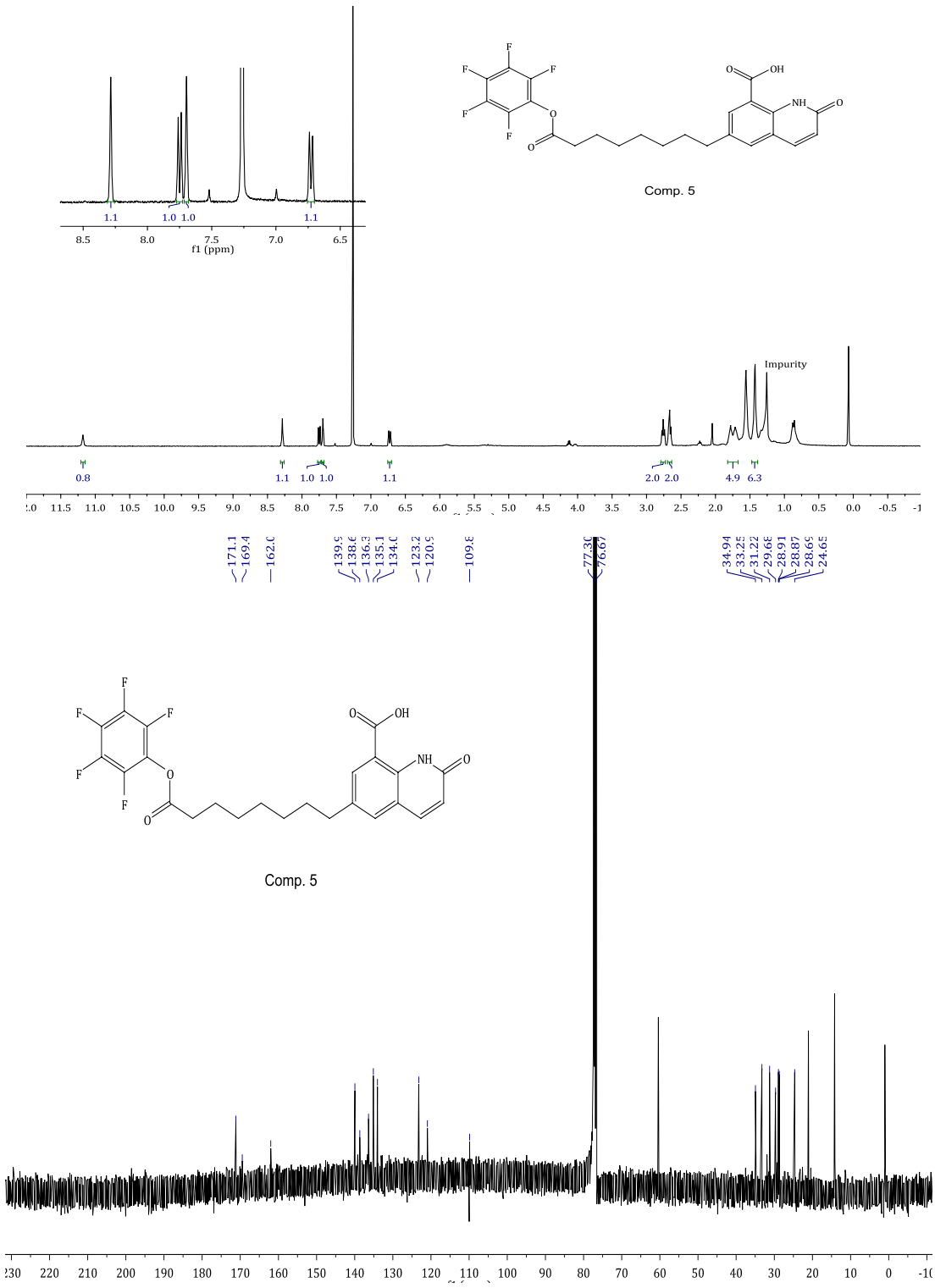


Figure S7. ^{1}H NMR & ^{13}C NMR spectra of **13**.



2-oxo-6-(7'-carboxylheptyl)-1,2-dihydroquinoline 8-carboxylic acid (**14**)

Figure S8. ^{1}H NMR spectrum of **14**.



2-oxo-6-(7’-pentafluorophenoxycarbonylheptyl)-1,2-dihydroquinoline 8-carboxylic acid (**5**)

Figure S9. ^{1}H NMR & ^{13}C NMR spectra of **5**.

E-Methyl 2-(3'-ethoxyprop-2-enoylamido) benzoate (**15**)

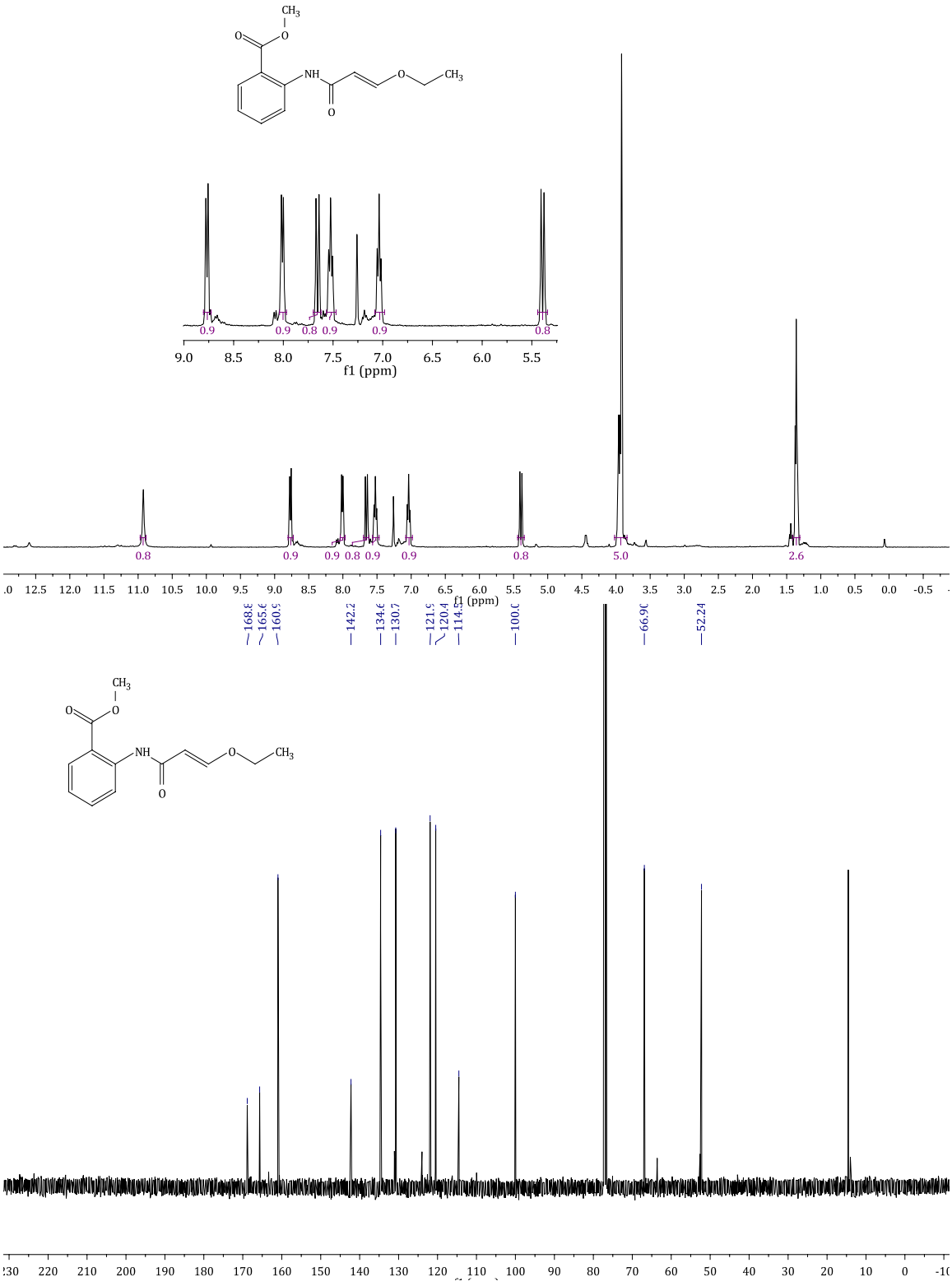


Figure S10. ^{1}H NMR & ^{13}C NMR spectra of **15**.

Methyl 2-Oxo-1,2-dihydroquinoline-8-carboxylic ester(**16**)

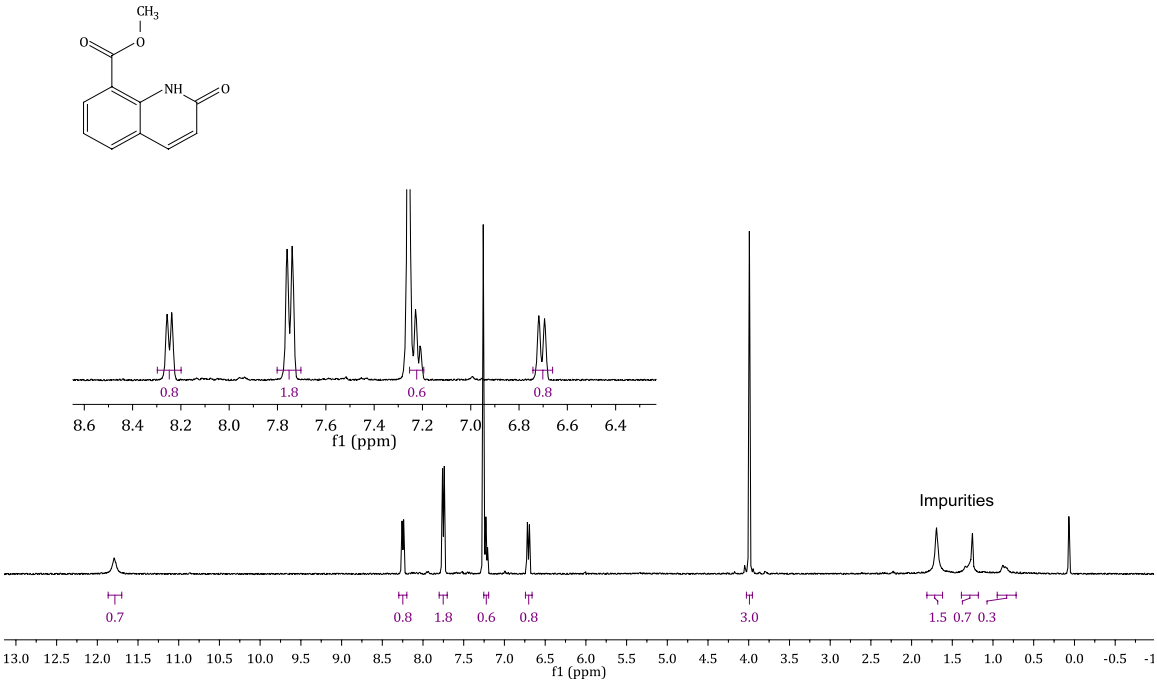


Figure S11. ^{1}H NMR spectrum of **16**.

2-Oxo-1,2-dihydroquinoline-8-carboxylic acid (**4**)

Figure S12. ^{1}H NMR spectrum of **4**.

HR-MS for **15**, **16**, **18**, **5**, and **20.**

Figure S13. HRMS of **15** (calcd for $C_{17}H_{25}NNaO_4$ 330.1676, found 330.1679 $[M+Na]^+$).

Figure S14. HRMS of **16** (calcd for $C_{22}H_{21}NNaO_6$ 428.2044, found 428.2051 $[M+Na]^+$).

Figure S15. [illegible]).

Figure S16.

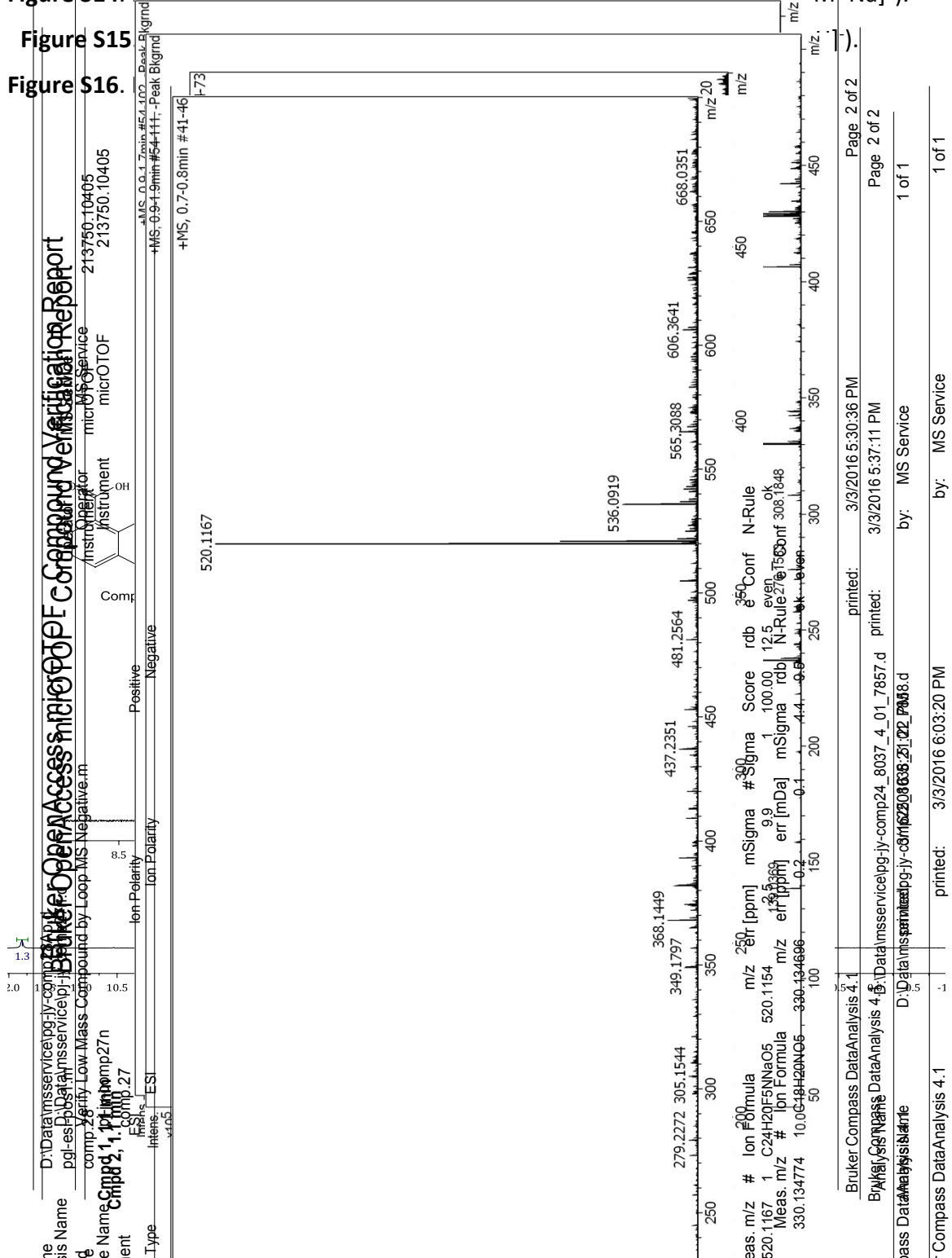


Figure S17. ESI-MS of **11**.(calcd for $C_8H_8BrNO_2$ 228.9, 230.9; found 230.1, 232.1 $[M+H^+]$, 252.0, 254.0 $[M+Na^+]$).

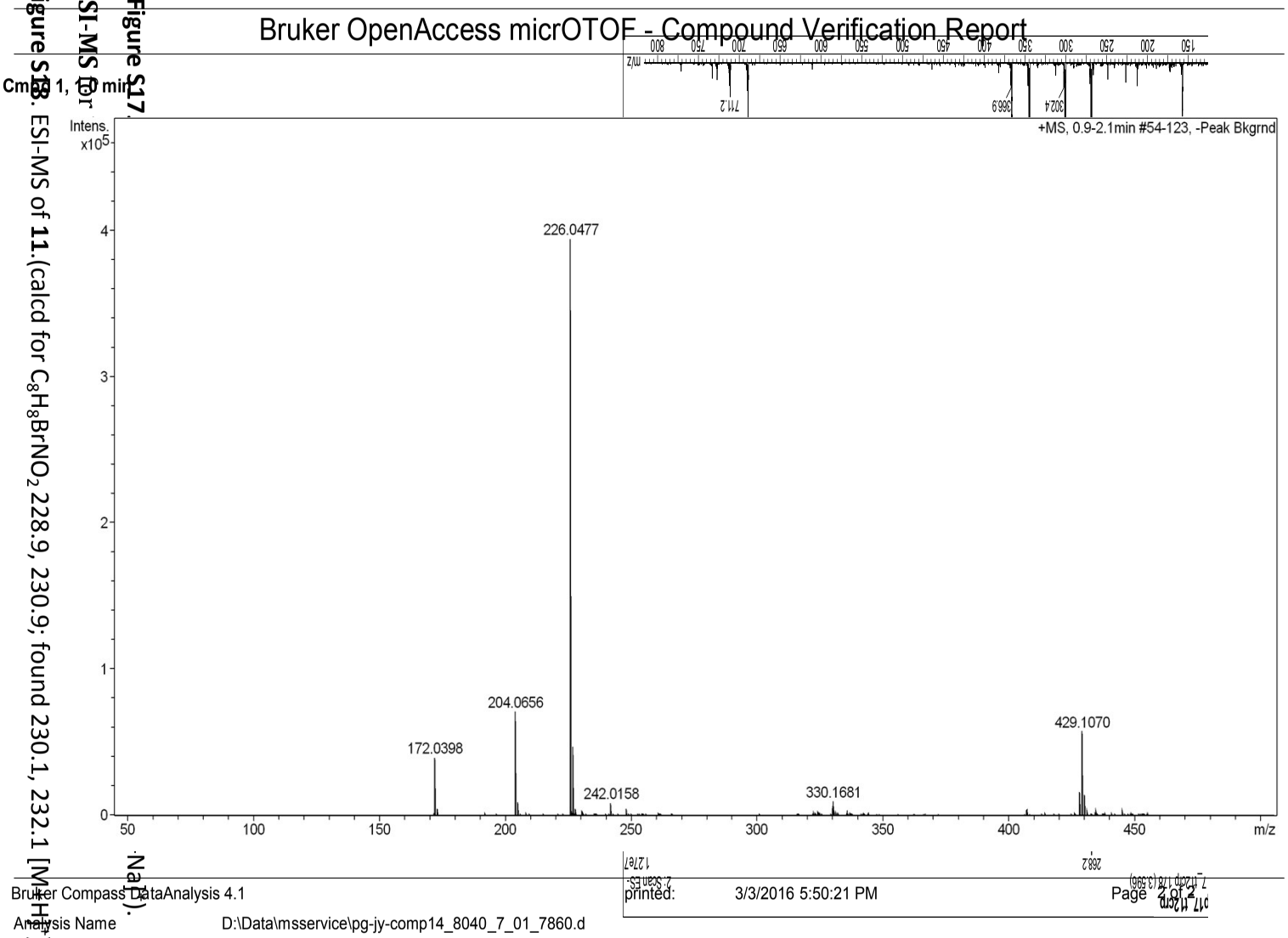


Figure S18. ESI-MS of **11**.(calcd for $C_8H_8BrNO_2$ 228.9, 230.9; found 230.1, 232.1 $[M+H^+]$).

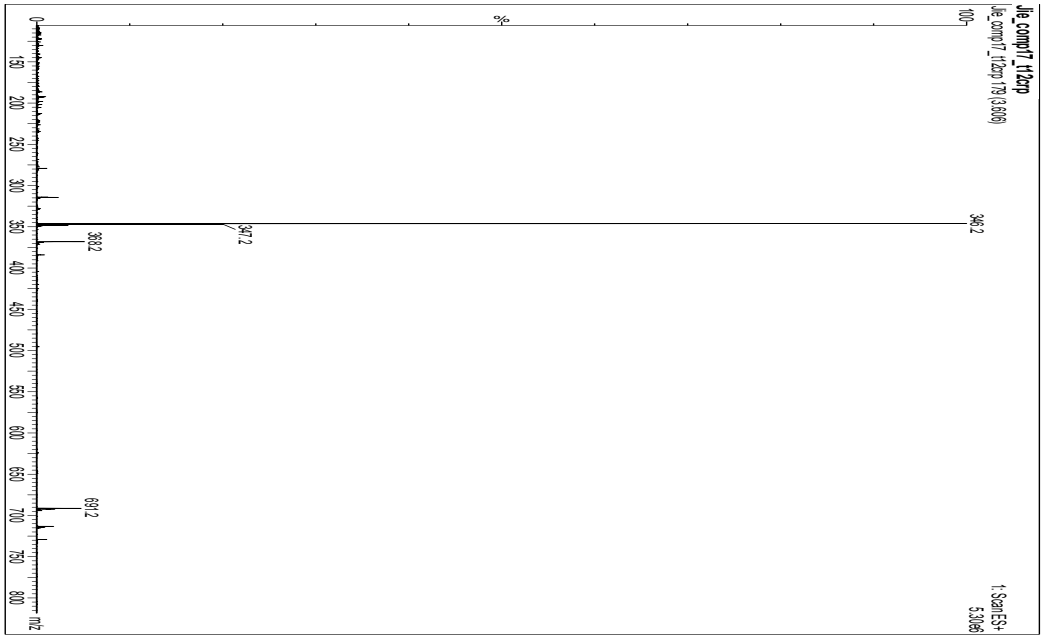


Figure S19. ESI-MS of **17**.($C_{19}H_{23}NO_5$ 345.1, found 346.2 $[M+H]^+$, 344.2 $[M-H]^-$).

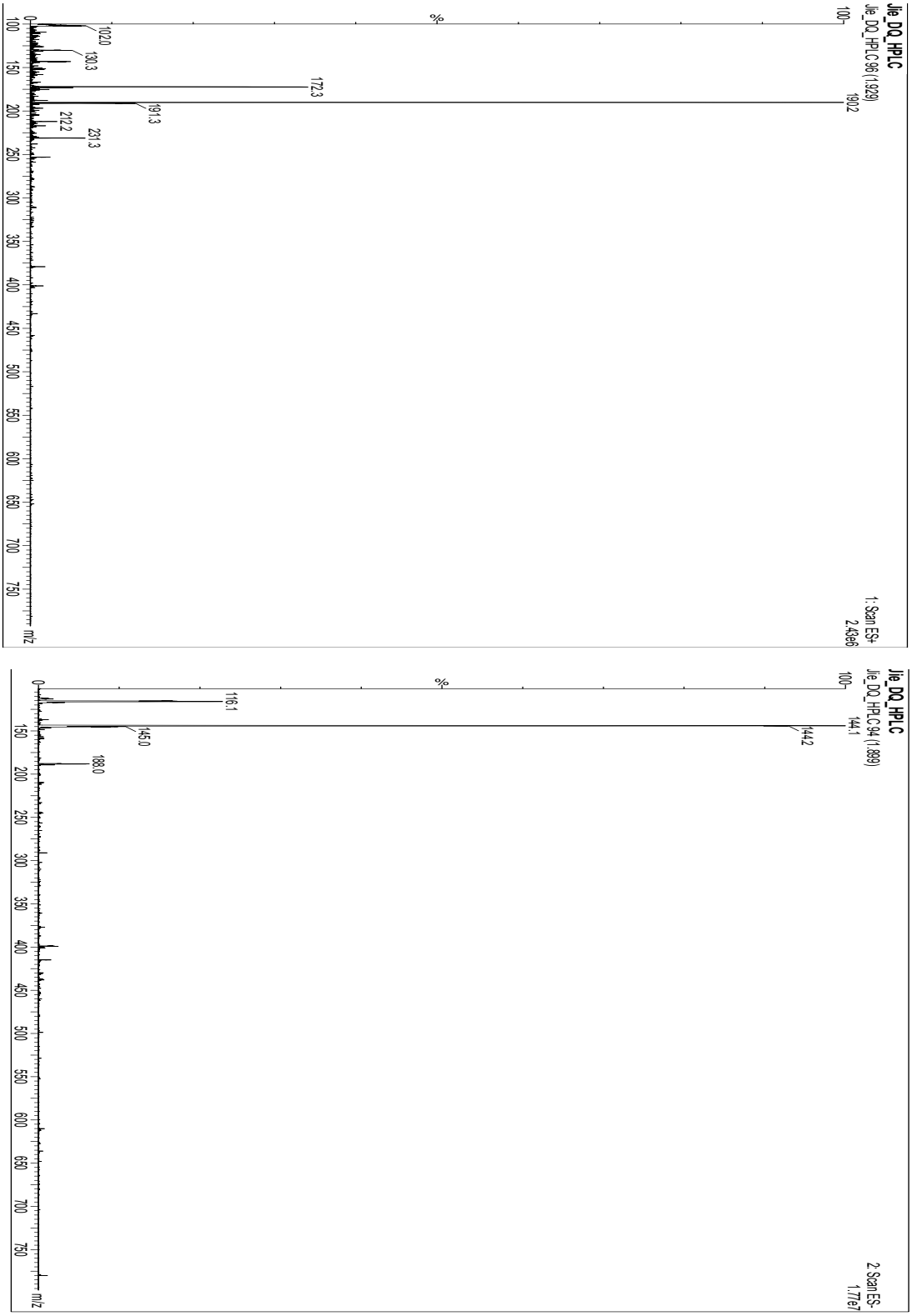
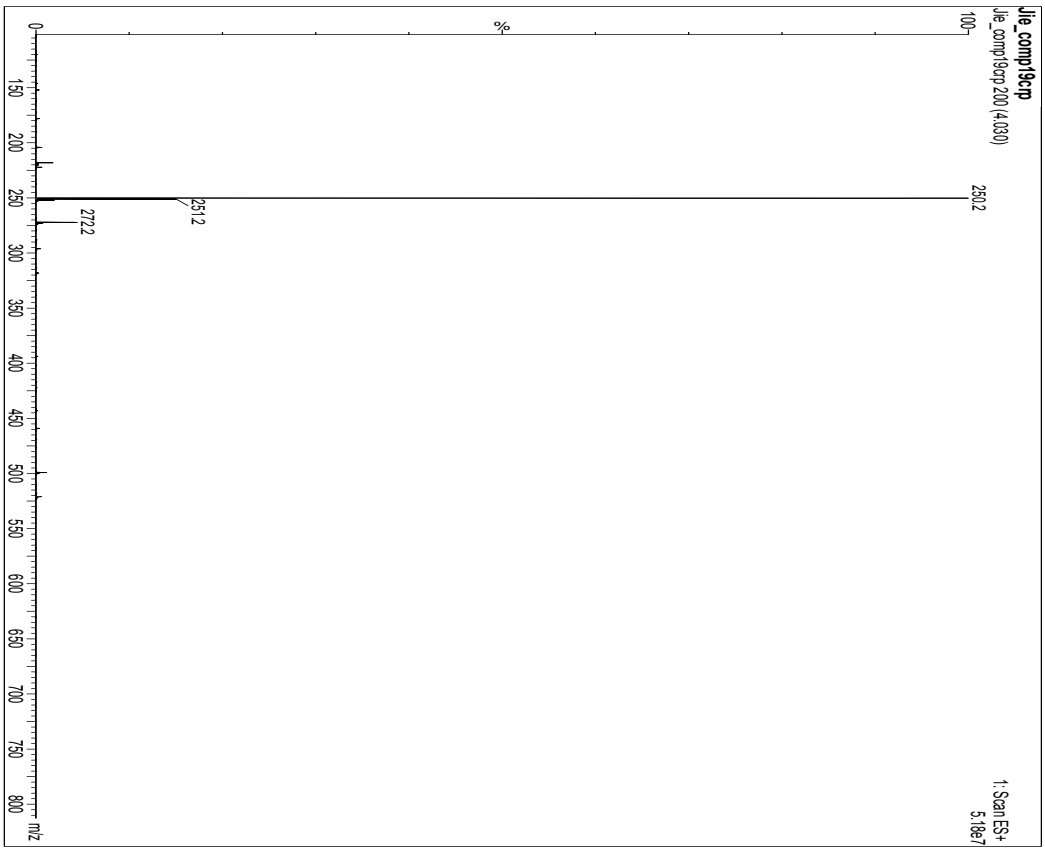


Figure S20. ESI-MS of **4**(calcd for $C_{10}H_7NO_3$ 189.0, found 190.2 m/z $[M+H]^+$, 188.0 m/z



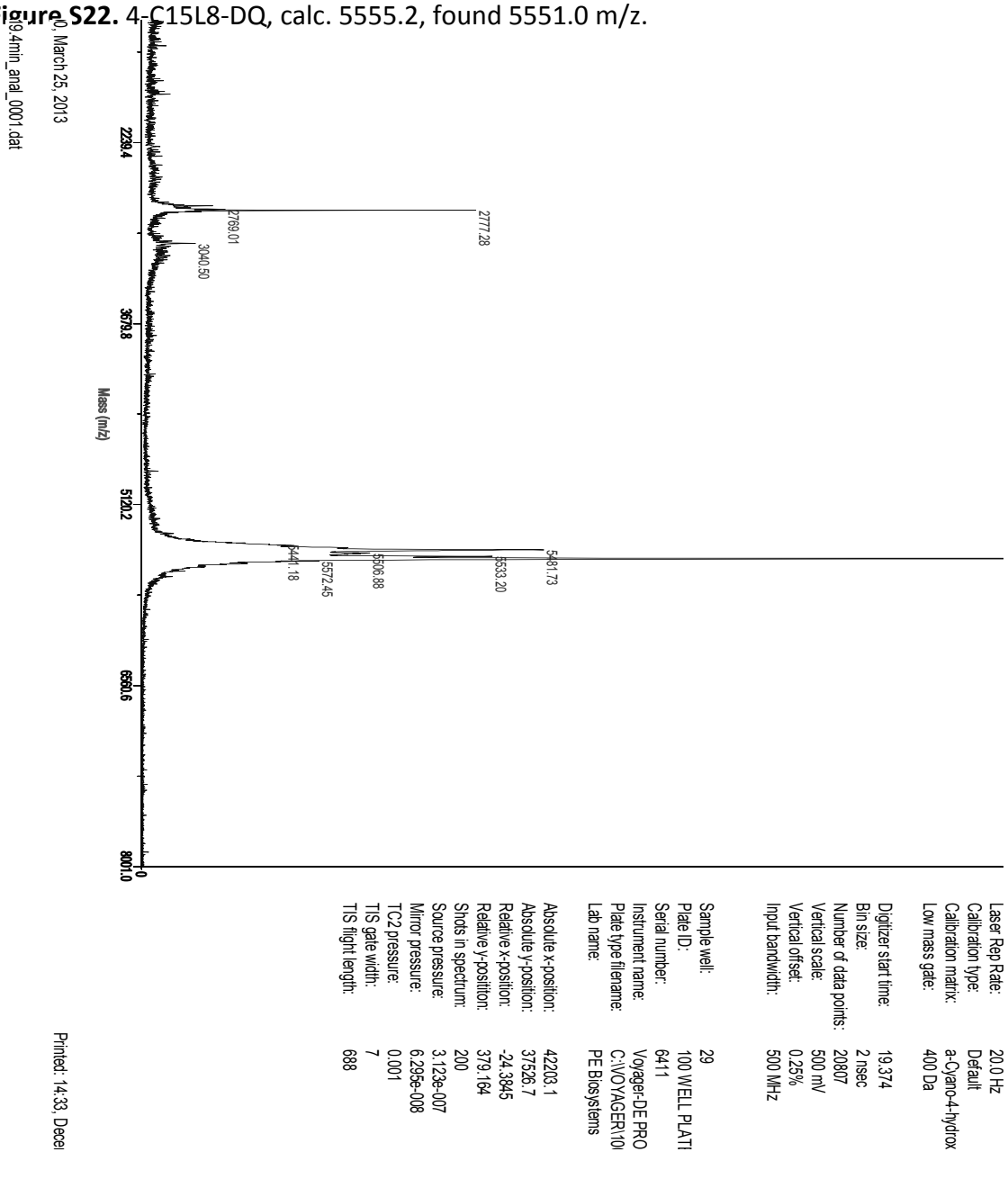
$[M-H]^-$).

Figure S21. ESI-MS of **19** (calcd for $C_{13}H_{15}NO_4$ 249.1, found 250.2 m/z $[M+H]^+$).

MALDI-TOF MS for peptide conjugates:

(Mass spectra of 4-C15L8-DQ, 4-C10L17-DQ, 4-C25L22-DQ, 4-C37L34-DQ, 4-C25L22-S^{24}H). Instrument calibration was done at regular intervals, however, factors such as the plate position of the sample slightly affected the obtained m/z values. This and the limited resolution of the linear mode gave an estimated $\pm$ 5 units (0.1 %) maximum error.

Figure S22. 4-C15L8-DQ, calc. 5555.2, found 5551.0 m/z.



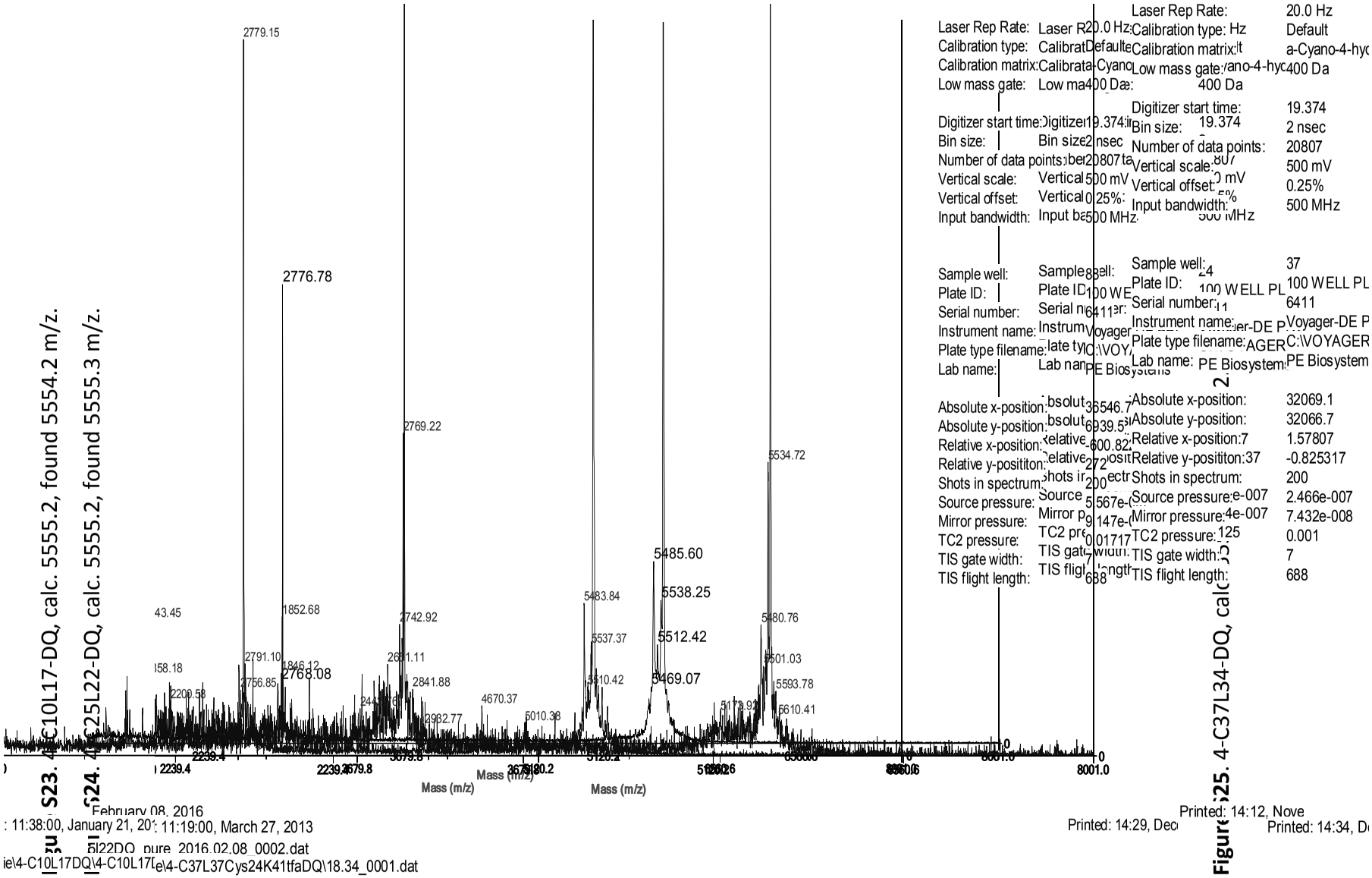


Figure S23. 4-C10L17-DQ, calc. 5555.2, found 5554.2 m/z.

Figure S24. 4-C25L22-DQ, calc. 5555.2, found 5555.3 m/z.

Figure S25. 4-C37L34-DQ, calc

February 08, 2016

: 11:38:00, January 21, 20; 11:19:00, March 27, 2013

5l22DQ_pure_2016.02.08_0002.dat

ie\4-C10L17DQ\4-C10L17D e\4-C37L37Cys24K41tfaDQ\18.34_0001.dat

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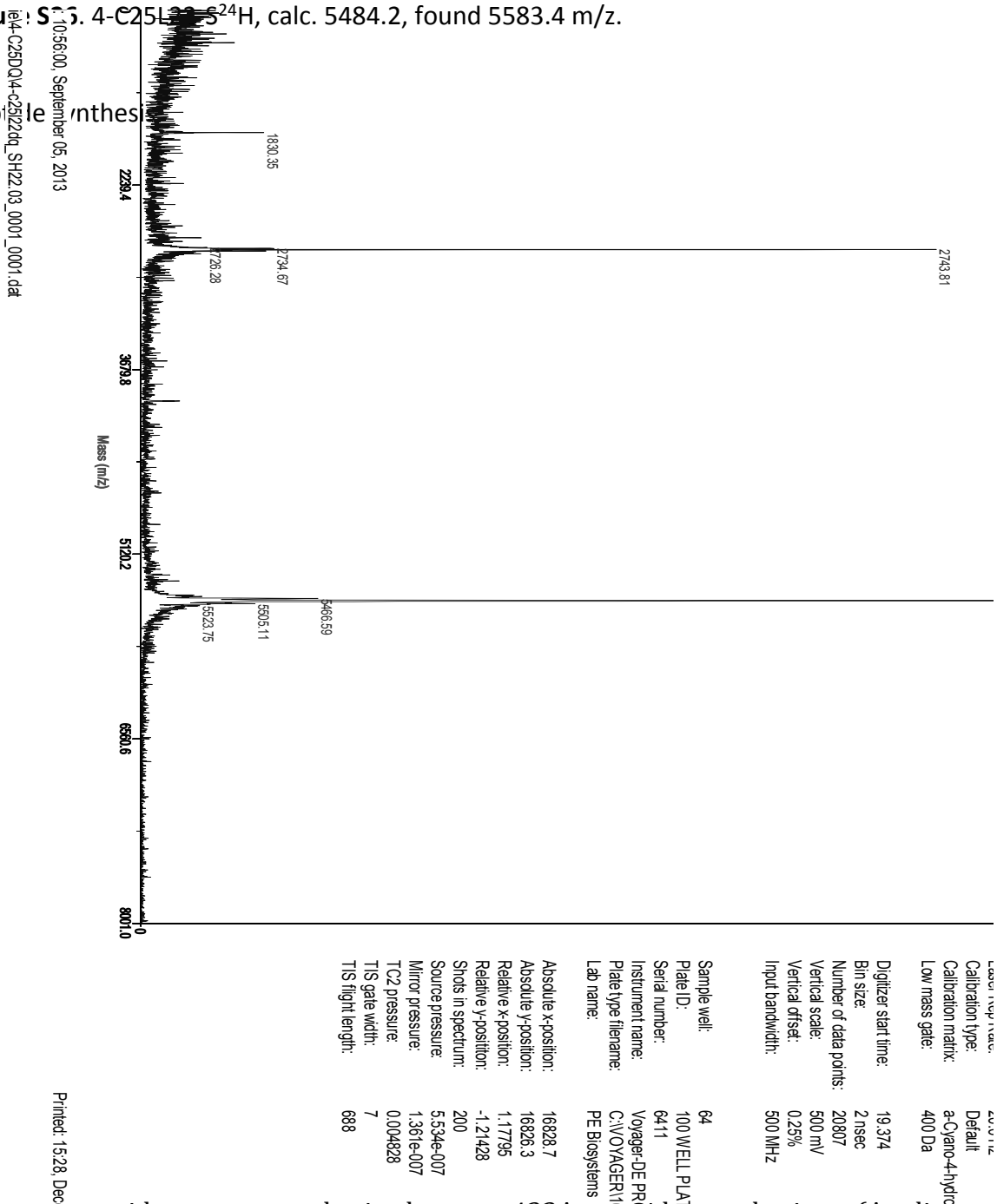
Figure S6. 4-C25L38S^{24}H, calc. 5484.2, found 5583.4 m/z.



Peptide synthesis

The peptides were synthesized on a 433A peptide synthesizer (Applied Biosystems, Carlsbad, CA, USA) using standard Fmoc chemistry with HCTU (Iris Biotech GmbH, Marktredwitz, Germany) and DIPEA (Iris Biotech GmbH) as activating agents. Fmoc- deprotection of the amino terminal was performed using 20% piperidine in NMP. The synthesis was performed on a 0.1 mmol scale using

Fmoc-PAL-polyethyleneglycol-polystyrene resin (Fmoc-PAL-PEG-PS, Applied Biosystems) with a substitution level of 0.23 mmol/g. A 10-fold excess of amino acid was used in each coupling, and activation was with a mixture of HCTU (0.5 M in DMF) and DIPEA (2 M in NMP). The side chains of the amino acids (Iris Biotech GmbH) were protected by either base-stable groups: tert-butyl ester (Asp and Glu), trt (His, Asn, and Gln), 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Arg) and acetamidomethyl (Cys) or by Tfa (Lys) which is stable during peptide synthesis, cleavage and conjugation.

For the site-selective incorporation of a fluorescent probe and ligands, the side chains of lysine residues were N-protected by an Alloc group, which can be selectively deprotected by treatment with tetrakis-(triphenylphosphine) palladium (0) (Pd(PPh3)4), or by a Boc group, which can be deprotected by TFA. The N-terminal of the polypeptide was capped manually with a mixture of acetic anhydride, DIPEA, and NMP (v/v/v 10:5:85) for 20 minutes. After complete machine synthesis, the resin was rinsed with DMF and DCM and dried in a desiccator under vacuum.

Before cleavage of the peptide from the resin, the Alloc-protected lysine was deprotected by three equivalent of tetrakis(triphenylphosphine) palladium (0) in a mixture of acetic acid, N-methylmorpholine, and chloroform (v/v/v 2:1:17; 10 mL/g of resin) at room temperature under N2 for 2.5 h. The resin was then sequentially washed with 30 mM DIPEA in DMF, 20 mM diethyldithiocarbamic acid in DMF (4 mL/g), DMF and then DCM. The fluorescence probe 7-methoxycourmarin-3-carboxylic acid was coupled to the amino group of lysine side chain was performed in DMF (10 mL) for 2 hours at room temperature with two equivalents of 7-methoxycourmain-3-carboxylic acid, two equivalents of HCTU and four equivalents of DIPEA. The resin was then washed with DMF and DCM, then the resin was desiccated. The peptide was cleaved from the resin using a mixture of TFA, TIS, and water (v/v/v 95:2.5/2.5; 10 mL/g of resin) for 2 h at room temperature. The mixture was filtered, and TFA was then evaporated from the filtrate using a stream of nitrogen. The polypeptide was precipitated by the addition of cold ether, centrifuged, the pellet was dissolved in MQ water, and lyophilized. The crude

peptide was purified by RP-HPLC and characterized by MALDI-TOF MS.

General method for acm-deprotection on Cys24 in peptide.

The peptide (1.7 mg) was dissolved in TFA (500 µL) and anisol (10 µL) was added to the peptide solution. The solution was stirred and cooled to 0 oC. Silver triflate (AgOTf, 13 mg) was added to the cold peptide solution. The solution was stirred at 0 oC for 1 hour and the temperature was allowed to come to room temperature and the stirring was continued over night. The silver salt of the peptide was precipitated and centrifuged. The supernatant was discarded and the residue was dissolved in 35 % acetic acid (700 µL). DTT (5mg) was added to the peptide solution, which was then stirred for 2 hours. The reaction mixture was centrifuged and the peptide was purified by RP-HPLC (25 % - 60 % acetonitrile in 30 minutes). The acm-deprotected peptides were then used in selectivity study. (Mass spectra of purified peptide was available in the ESI file, S26.)

Fluorescence screening

Titration of binders with CRP was carried out by fluorescence spectroscopy using a GeminiXPS platereader. BRANDplates®96 pureGrade non-sterile plates were coated with Pluronic® F108NF Prill poloxamer338 (BASF) before use. The plates were incubated with a 10 mg/L water solution of Pluronic® F108NF Prill overnight followed by washing with water. Tris Buffer (50mM, 5 mM CaCl2, 150 mM NaCl, pH 7.0) was used in the fluorescence screening experiment.

385 µL of CRP protein solution (3 mg/mL) (Millipore) was diluted in 615 µL buffer to make 10 µM CRP stock solution. 1 mg of peptide was dissolved in MQ water (500 µL). 40 µL thereof was dissolved in buffer (960 µL) to make a 10 µM peptide stock solution.

In each well, 500 nM peptide was used together with 0, 500, 1000, 1500 nM of CRP. Buffer was used as blank. The 7-methoxycoumarin probe of each peptide was excited at 350 nm using a high pmt setting (to maximize the signal detection) and the emission was recorded at 380-500 nm. All measurements were performed at 25

oC and were made in triplicates. Data was read after 30 minutes incubation.

SPR

CRP (3.0 mg/mL) preserving buffer was switched to the coupling buffer (sodium acetate, pH 5.5) with a desalting PD-10 column. The amide coupling standard protocol was then performed to immobilize CRP on a CM5 chip with HBS-P as running buffer. The flow rate was set at 10 µL/min. The chip was activated for 7 minutes, then human CRP (100 µg/mL) was injected for 15 minutes, after that 1 M ethanolamine hydrochloride-NaOH (pH 8.5) was injected for 7 minutes to deactivate the rest of unreacted NHS active ester. The reference channel was subjected to the same procedure but without CRP injection. Final response in human CRP immobilized channel was 8051 Ru.

Selectivity

HEPES buffer 1 (10 mM HEPES, 10 mM KCl, 10 mM EDTA, pH 7.9), HEPES buffer 2 (10 mM HEPES, 5 mM CaCl2, pH 7.4). LDS buffer was purchased from Invit.

Polystyrene beads (35 µL, 0.96 µM) were washed with 1 mL MQ water three times and incubated with 10 mg/mL Pluronic F108-PDS overnight at room temperature under constant shaking. This treatment introduces free SH groups onto the bead surface. The beads suspension was centrifugated at 14000 rpm for 10 minutes. The beads were then washed once with 300 µL MQ water and twice with HEPES buffer 1.

The beads were suspended in 50 µL HEPES buffer 1, and 400 µL acm-deprotected polypeptide (1 mg/mL) in buffer was added. The mixture was incubated overnight with gentle shaking at room temperature. The beads were spun down and washed three times with 200 µL HPEPES buffer 2, and then suspended in 200 µL HEPES buffer 2.

Sample a. preparation: 200 µL serum (CRP free) was added to the beads suspension (200 µL) and incubated for 30 minutes at room temperature under gentle shaking.

Sample b. preparation: 200µL serum (CRP 10 mg/L) was added to the beads suspension (200 µL) and incubated for 30 minutes at room temperature under gentle shaking.

Sample c. preparation: 200µL serum (CRP 10 mg/L) was added to the unfunctionalized

beads and incubated for 30 minutes at room temperature under gentle shaking.

The beads suspensions were centrifuged and washed 5 times with HEPES buffer 2 (300 µL each), then resuspended in 80 µL HEPES 2 respectively.

DTT (15 µL, 200 mM in HEPES buffer 2) was added and the mixture was incubated for 20 minutes to cleave the peptide-protein complexes from the beads. The beads were spun down at 14000 rpm for 10 minutes and the supernatant was collected and analyzed by SDS-PAGE loading 5 µL LDS buffer and 15 µL of the sample into each wells. The gel was run for 35 minutes at 200 V.

SPR sensorgrams from PCh competition and DQ competition experiments.

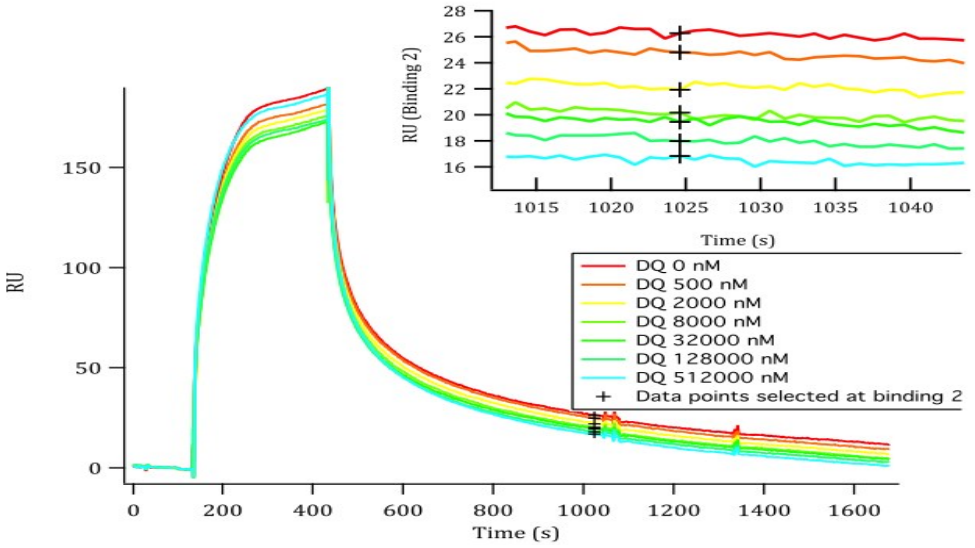


Figure S27. Sensorgram for DQ inhibition assay in the absence of Ca^{2+} ions. The data points at time point 2 (1046 s) were used for evaluation of DQ inhibition of 4-C25L22-DQ-protein binding. The reason is that precipitation may happen at the injection of 0.512 mM DQ with 500 nM 4-C25L22-DQ. Therefore, instead of readouts from time point 1 (10 s before association phase end), the readouts from binding 2 (1046 s) were used for competition evaluation. This is because the precipitation effect was eliminated in the dissociation time.

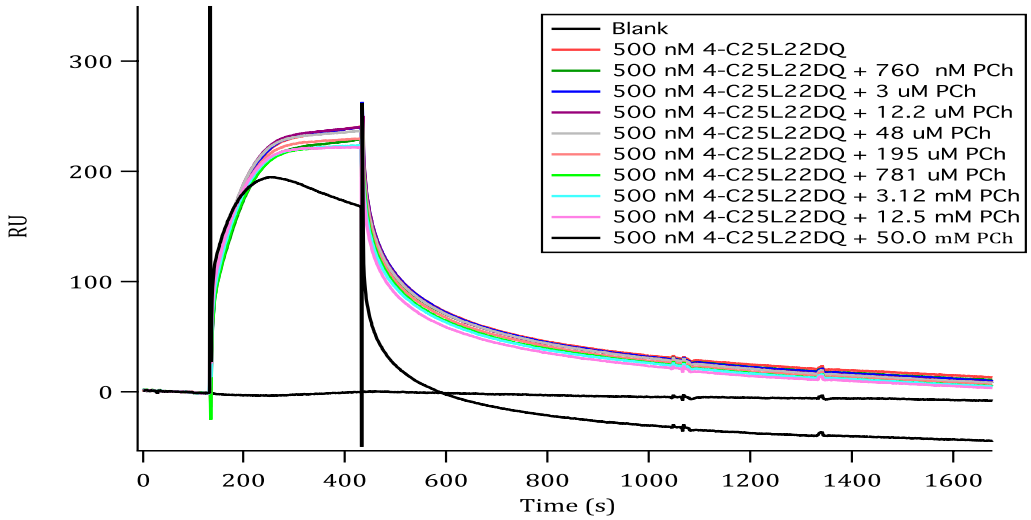


Figure S28. Sensorgram for PCh inhibition assay in 10 mM Ca^{2+}. The data points at time point 1 (10 s before association phase ends) were used for evaluation of PCh inhibition of 4-C25L22-DQ-protein binding.

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