Supplementary Information

Combining local conformational preferences and solvophobic effects in helical aromatic oligoamide foldamers

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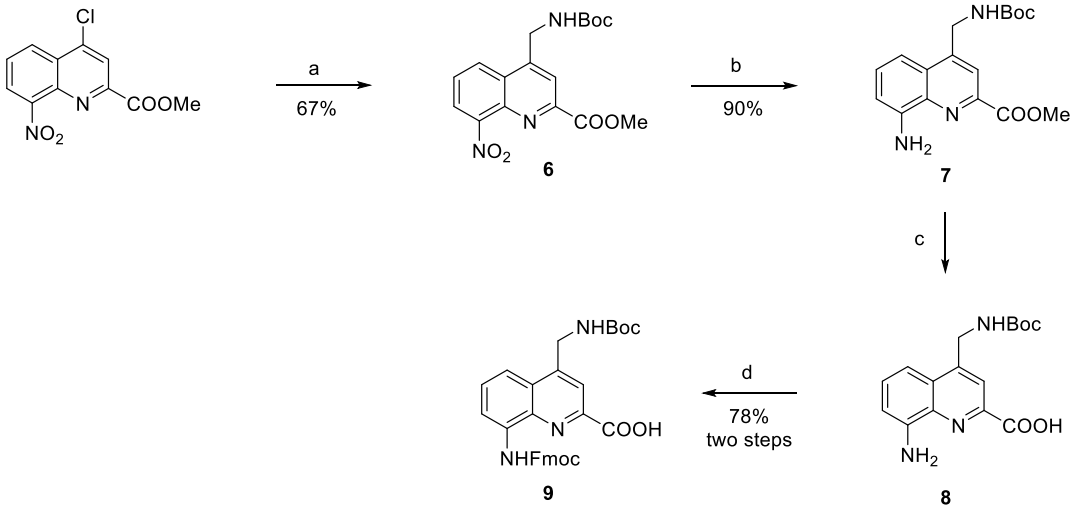
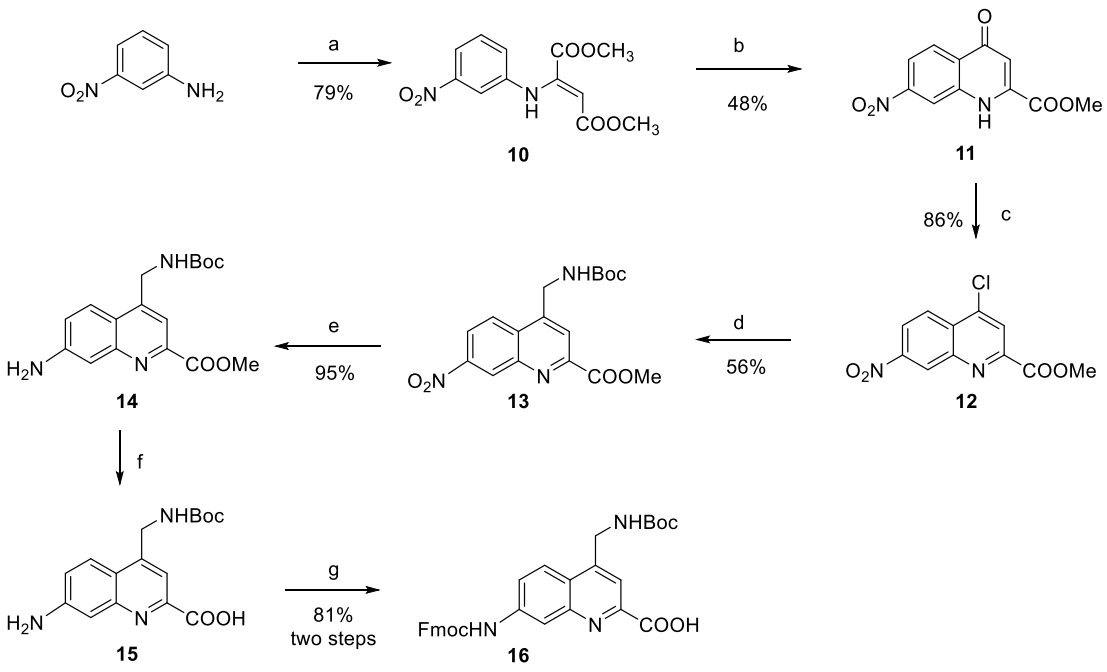
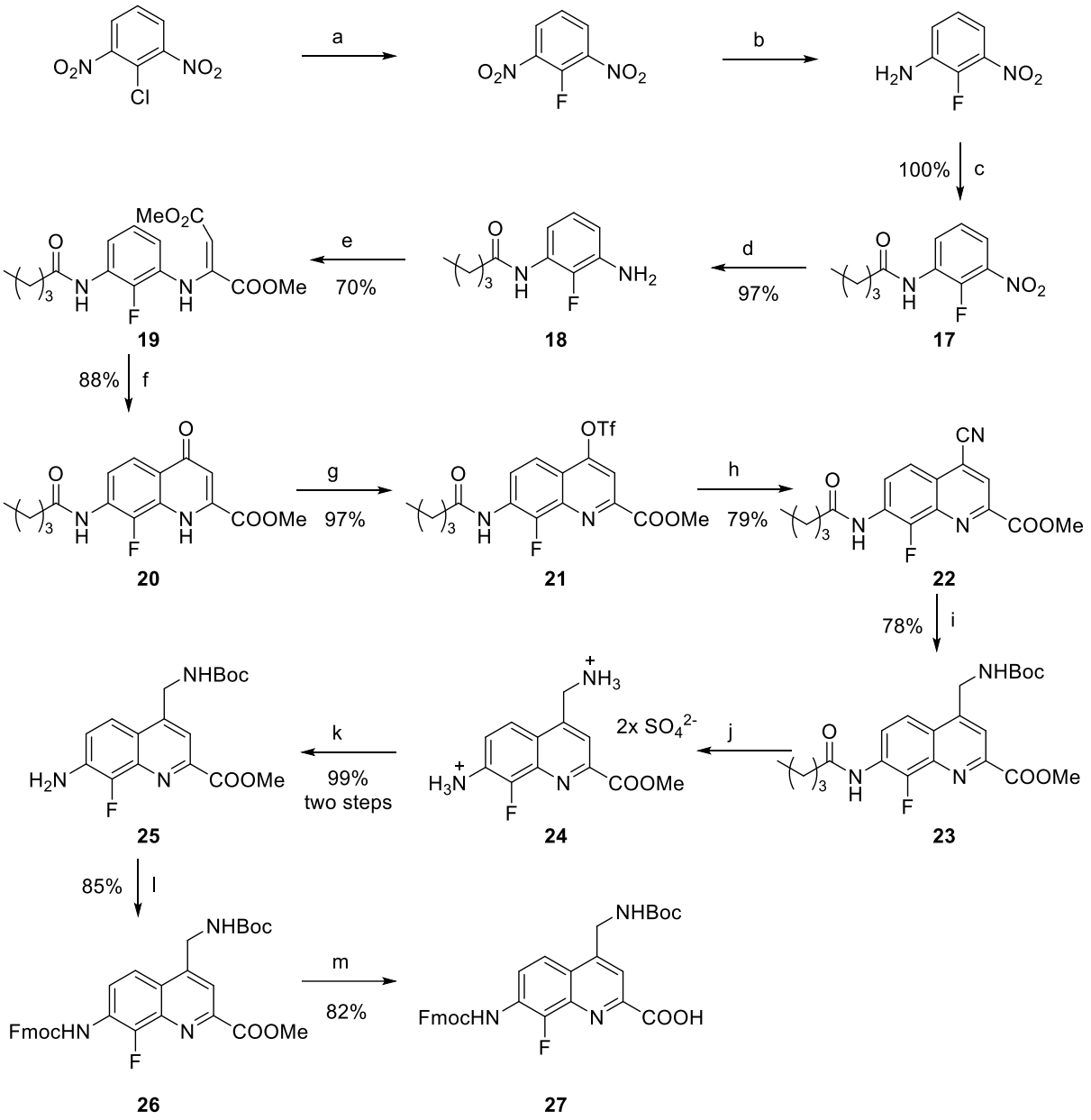
1 Supplementary Schemes and Figures



Scheme S1. Synthesis of **Q** monomer: (a) KF_3BCH_2NHBoc, $Pd(OAc)_2$, SPhos, K_2CO_3, toluene, H_2O, 85 °C; (b) Pd/C, H_2, THF, MeOH; (c) LiOH, THF, H_2O, 0 °C; (d) Fmoc-Cl, $NaHCO_3$, dioxane, H_2O, 0 °C to r.t..



Scheme S2. Synthesis of $\mathbf{Q^H}$ monomer: (a) Dimethyl acetylenedicarboxylate, MeOH; (b) PhOPh, reflux; (c) $POCl_3$, DMF; (d) KF_3BCH_2NHBoc, $Pd(OAc)_2$, SPhos, K_2CO_3, toluene, H_2O, 85 °C; (e) Pd/C, H_2, THF, MeOH; (f) LiOH, THF, H_2O, 0 °C; (g) Fmoc-Cl, $NaHCO_3$, dioxane, H_2O, 0 °C to r.t..



Scheme S3. Synthesis of $\mathbf{Q^F}$ monomer: (a) KF, dry DMSO; 100 °C; (b) Fe, MeOH, AcOH, 110 °C; (c) DIEA, valeric anhydride, 40 °C; (d) Pd/C, H_2, AcOEt; (e) Dimethyl acetylenedicarboxylate, MeOH; (f) PhOPh, reflux; (g) Tf_2O, Pyridine, DCM; (h) KCN, $Pd(PPh_3)_4$, toluene, 100 °C; (i) Boc_2O, NH_4HCOO, Pd/C, THF; (j) H_2SO_4, MeOH, reflux; (k) Boc_2O, $NaHCO_3$, DMF; (l) Fmoc-Cl, $NaHCO_3$, dioxane, H_2O, 0 ^oC to r.t.; (m) LiI, AcOEt.

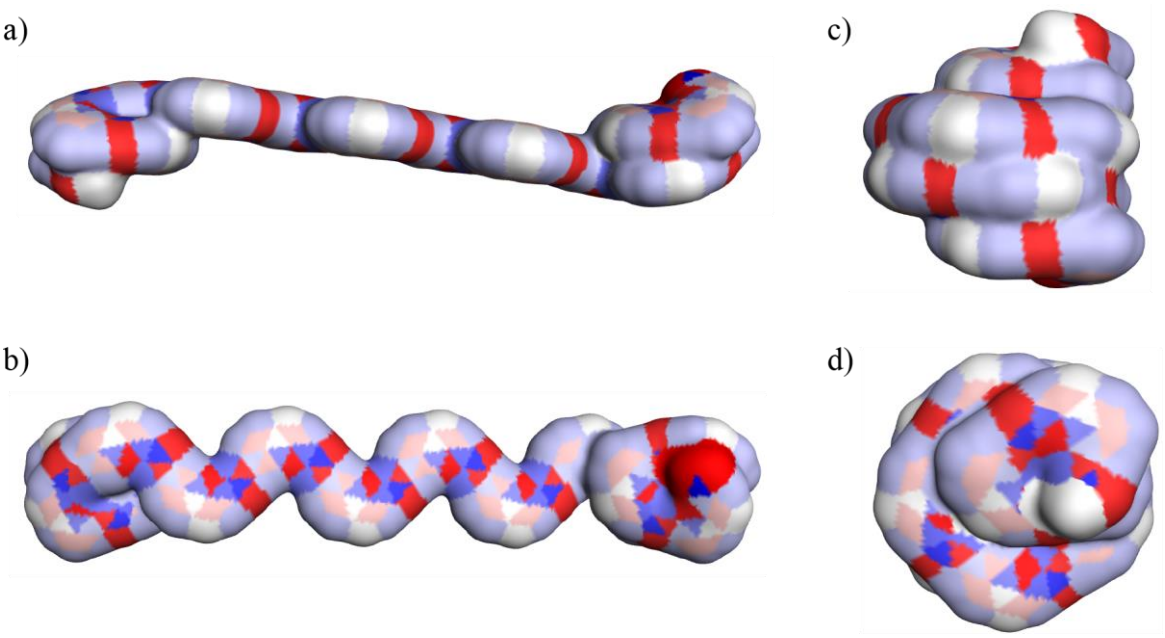


Figure S1. Solvent accessible surface of two conformations: (a) side view and (b) top view of extended **2**. Solvent accessible surface of: (c) side view and (d) top view of folded **2**. Different elements have been marked by different colors (hydrogen: white, carbon: grey, oxygen: red, nitrogen: blue). Solvent-accessible surface area was calculated by discovery studio software with a radius of 1.4 Å. Short side chains have been removed for the surface estimation. The calculated solvent accessible surface for **2** of extended and folded state were found to be 2578.08 $Å^2$ and 1717.48 $Å^2$, respectively. Thus, one can estimate that a folded **2** presents to the solvent about 67% of the surface presented by extended **2**.

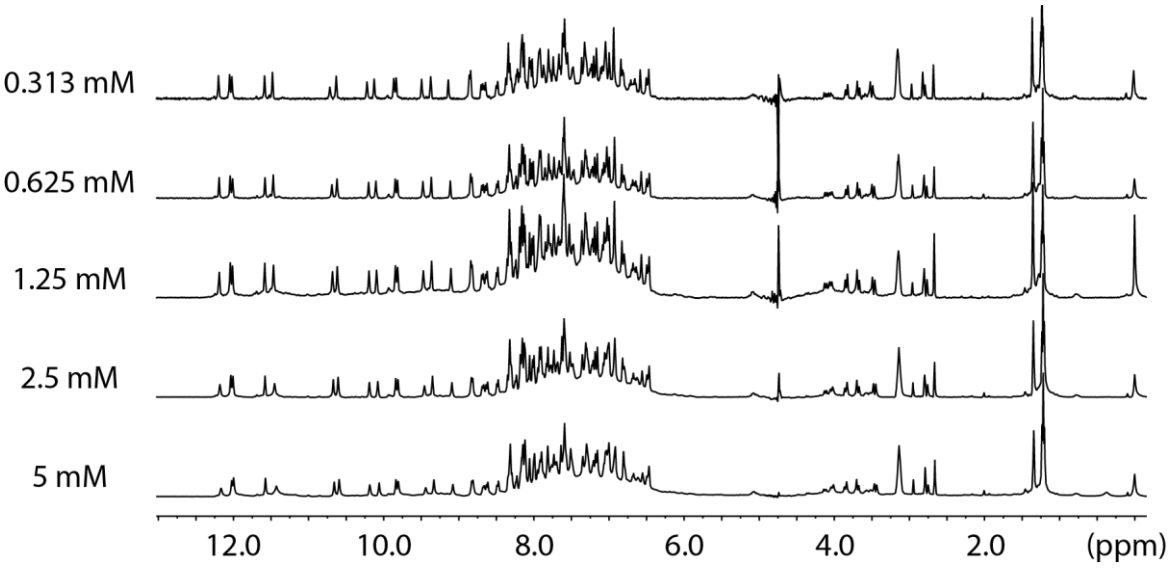


Figure S2. Variable concentration ^{1}H NMR spectra of compound 2. The spectra were recorded with a water suppression method (500 MHz, 10% D_2O in H_2O, 25 °C).

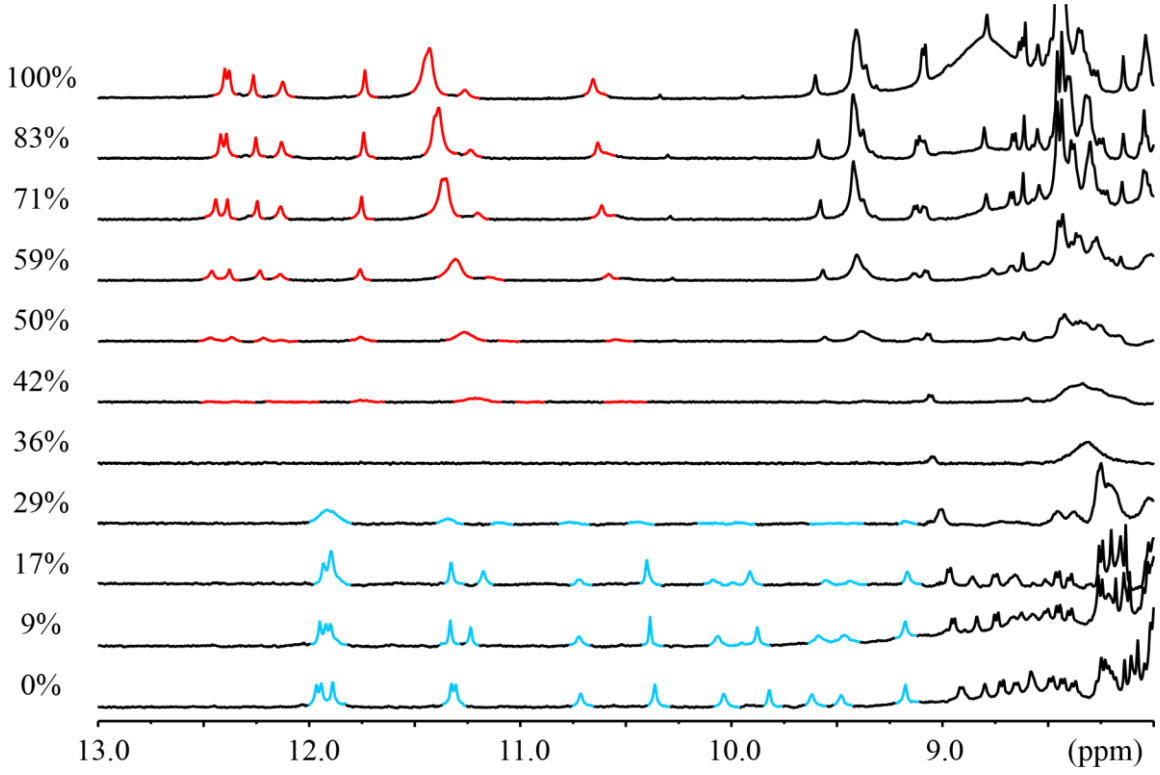


Figure S3. DMSO-d_6/CD_3OH variation ^{1}H NMR spectra of 2. Excerpts of ^{1}H NMR (500 MHz, water suppression) titration at 25 °C in DMSO-d_6/CD_3OH mixtures with different DMSO percentages. Note: 0% DMSO means that ^{1}H NMR spectrum (500 MHz) of compound **2** was performed in CD_3OH. Red and blue marked amide proton signals represent unfolded and folded conformations, respectively.

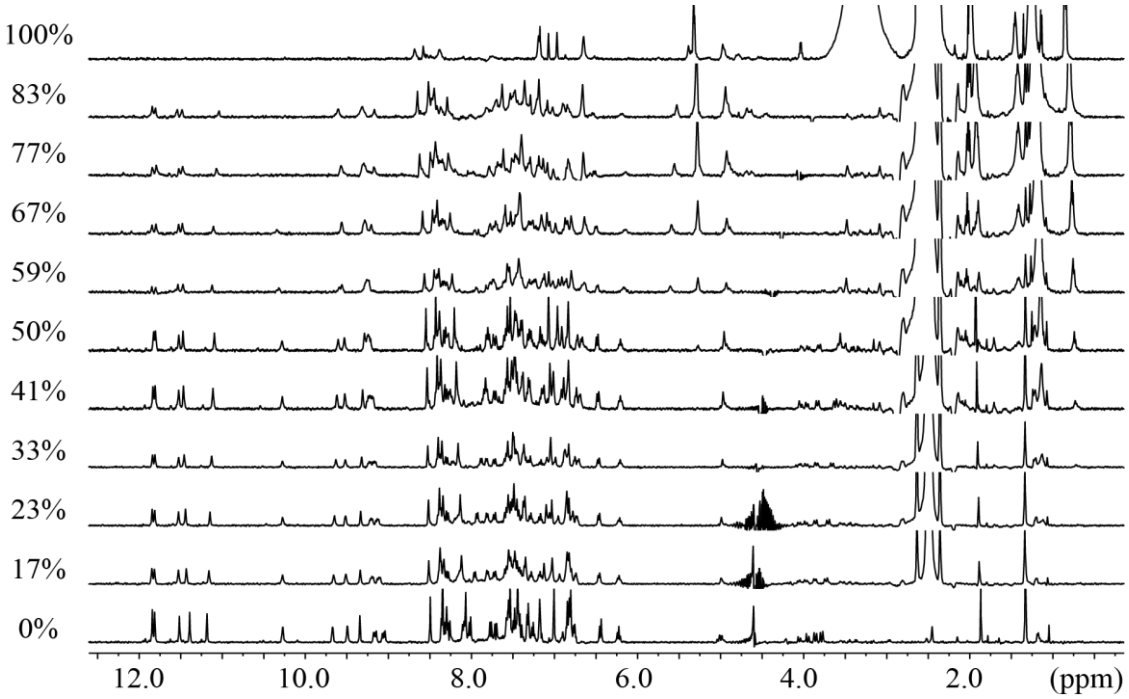


Figure S4. DMSO-d_6/H_2O variation ^{1}H NMR spectra of 5. ^{1}H NMR (500 MHz, water suppression) spectra at 25°C in DMSO-d_6/H_2O mixtures with different DMSO percentages. Note: 0% DMSO means that ^{1}H NMR (500 MHz) spectra of compound **5** were recorded in 90%H_2O+10%D_2O.

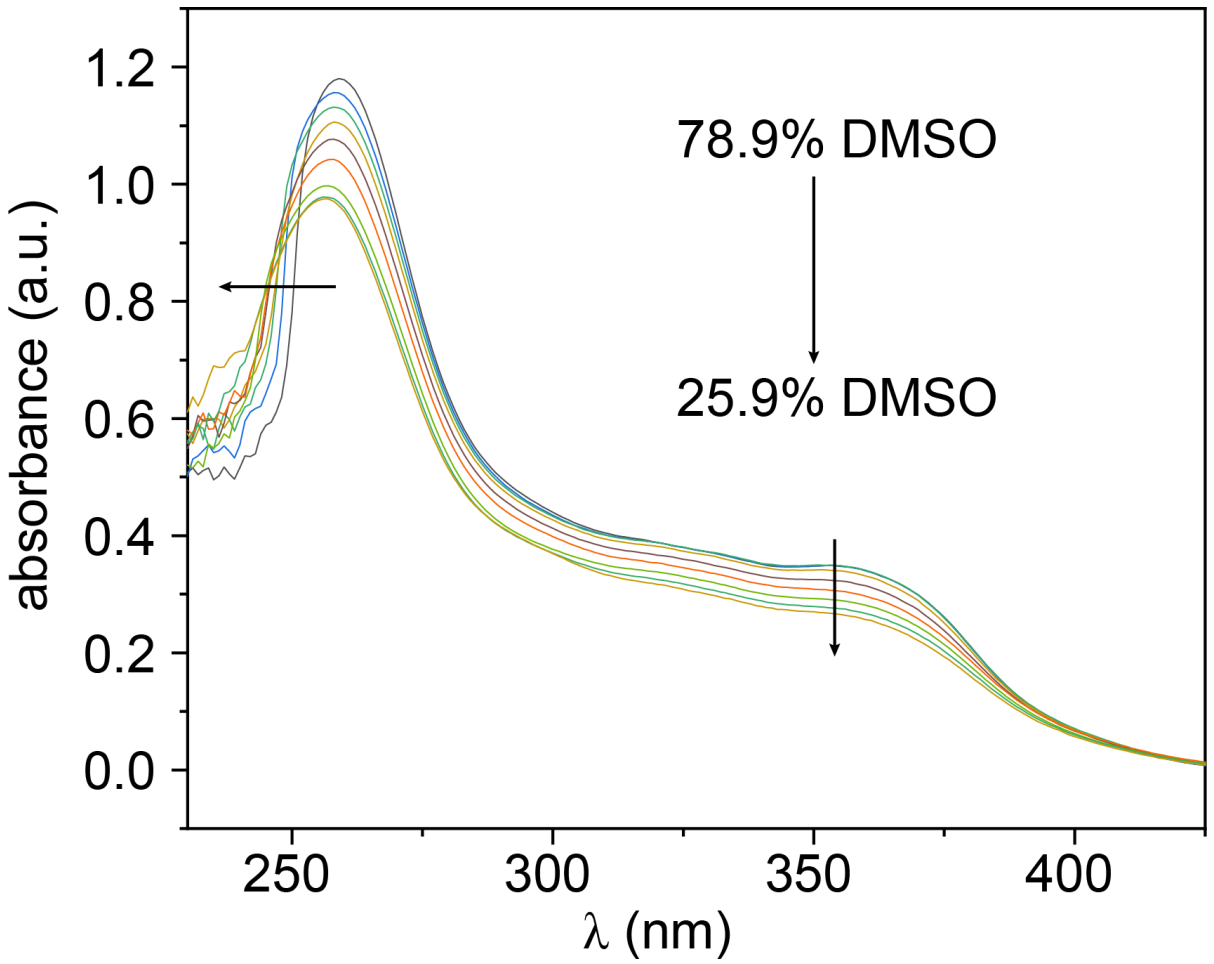


Figure S5. Water/DMSO proportion variation UV spectra of compound 1. UV spectra in different proportions of water/DMSO.

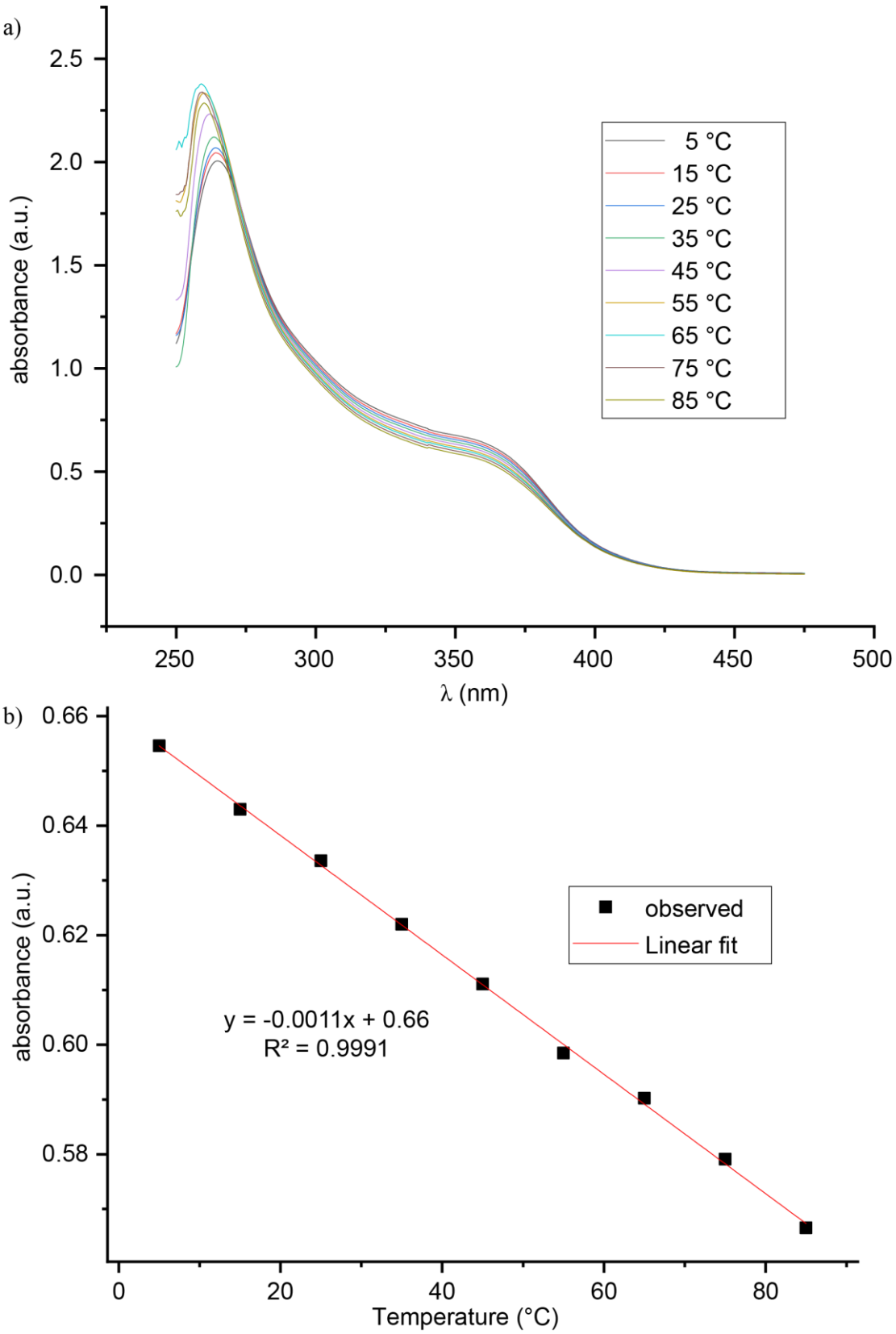


Figure S 6. Temperature variation UV of 2. (a) Variable temperature UV spectra of compound **2** in DMSO:H_2O (75.5:24.5, *v*/*v*). (b) Plot of the absorbance at 357 nm *vs* temperature.

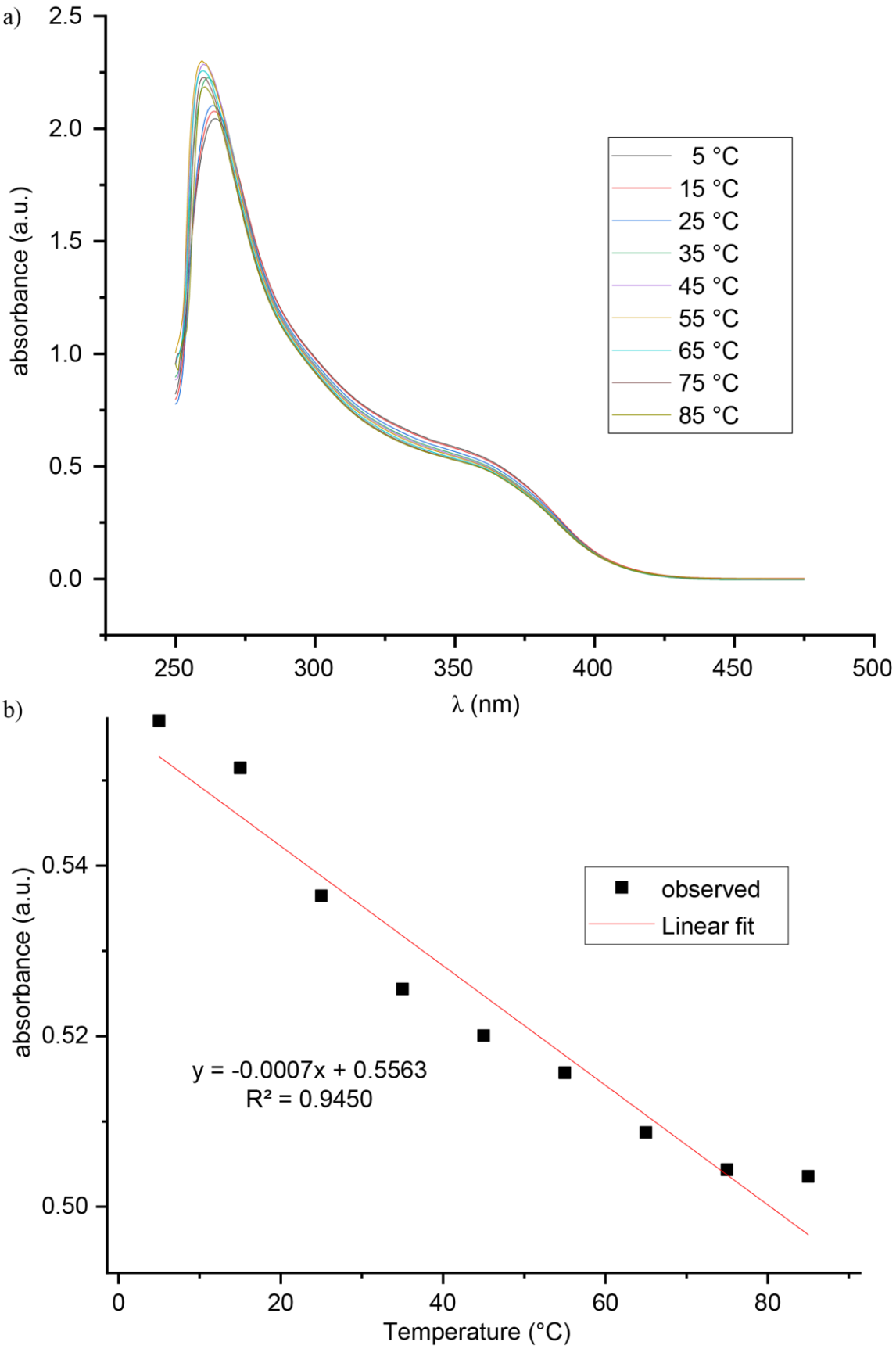


Figure S 7. Temperature variation UV of 3. (a) Variable temperature UV spectra of compound **3** in DMSO:H_2O (81.6:18.4, *v*/*v*). (b) Plot of the absorbance at 357 nm *vs* temperature.

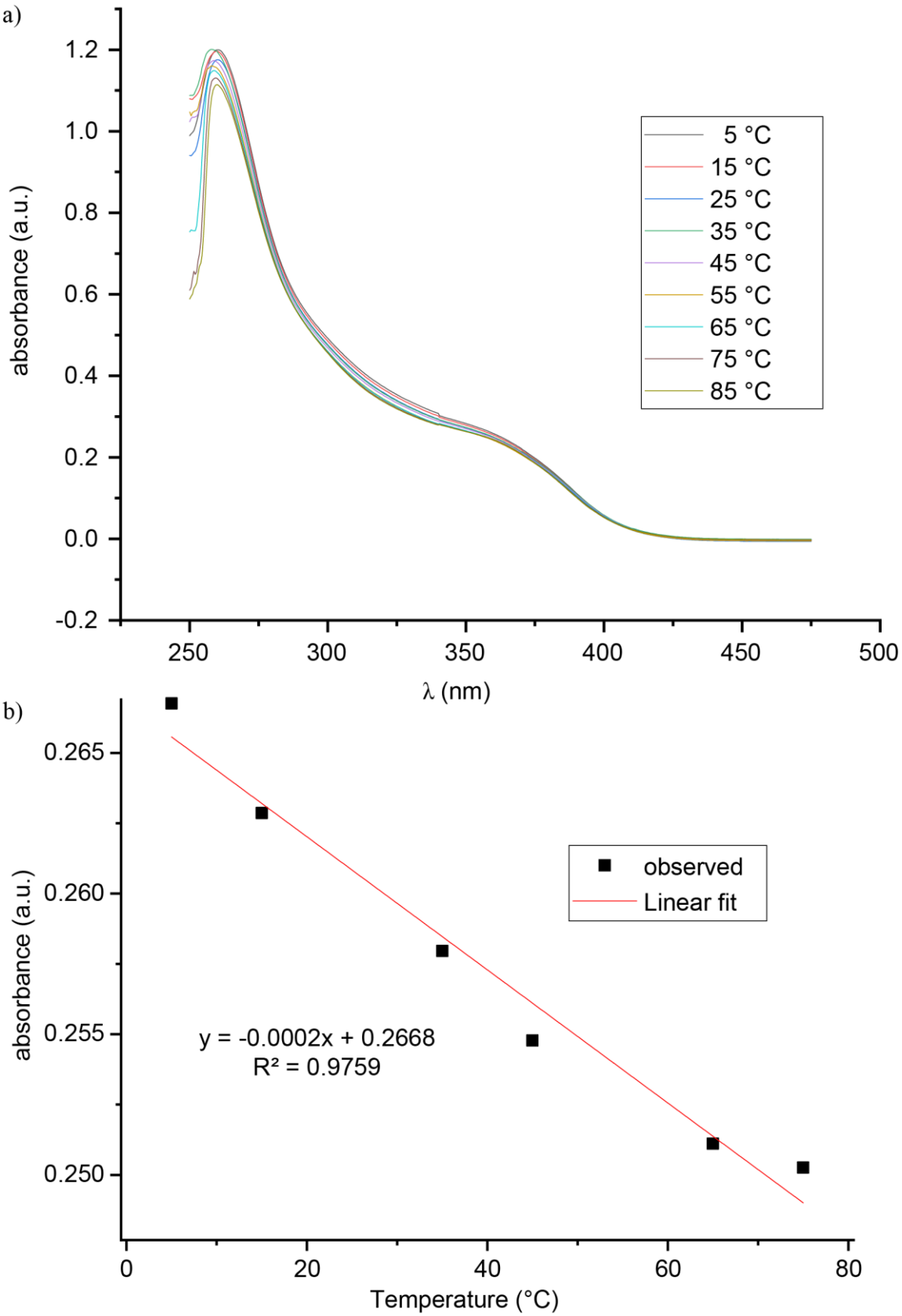


Figure S 8. Temperature variation UV of 4. (a) Variable temperature UV spectra of compound **4** in DMSO:H_2O (83.3:16.7, *v*/*v*). (b) Plot of the absorbance at 357 nm *vs* temperature.

2 Materials and Methods

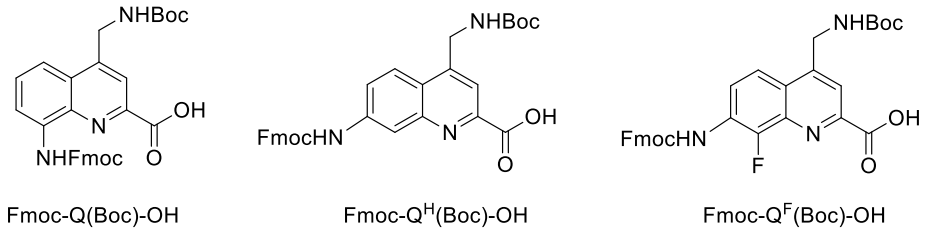


Figure S9. Fmoc-acid building blocks used in this study. For a detailed procedure to Fmoc-Q(Boc)-OH, Fmoc-Q^H(Boc)-OH and Fmoc-Q^F(Boc)-OH, see section 2.3.

2.1 General

General. Commercial reagents (Suppliers: Abcr, Fisher Scientific, Merck, Sigma-Aldrich, TCI, BLDpharm or VWR) were used without further purification unless otherwise stated. Wang resin LL (100–200 mesh) was purchased from Sigma-Aldrich. Peptide grade *N*,*N*-dimethylformamide (DMF) was purchased from Carlo Erba. Anhydrous chloroform, triethylamine (TEA) and *N*,*N*-diisopropylethylamine (DIEA) were obtained via distillation over CaH_2 prior to use. Anhydrous tetrahydrofuran (THF) and dichloromethane (DCM) were obtained via an MBRAUN SPS-800 solvent purification system. Ultrapure water was obtained via a Sartorius arium® pro VF ultrapure water system. Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60-F254 plates and observed under UV light. Column chromatography purifications were carried out on Merck GEDURAN Si60 (40–63 µm). Nuclear magnetic resonance (NMR) spectra were recorded on an Avance III HD 300 MHz Bruker BioSpin spectrometer or an Avance III HD 500 MHz Bruker BioSpin spectrometer equipped with a broad band observe 5-mm BB-H&FD CryoProbe™ Prodigy. Measurements were performed at 25 °C unless stated otherwise. Water suppression was performed with excitation sculpting method. Processing was done with MestReNova (v.12.0.0-20080) NMR processing software from Mestrelab Research. Chemical shifts are reported in ppm and calibrated via residual solvent signals or 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TMSP) when water suppression was applied. Signal multiplicities are abbreviated as s, singlet; d, doublet; t, triplet; q, quartet, and m, multiplet. Signals were assigned using ^{1}H-^{13}C HMQC and ^{1}H-^{13}C HMBC spectra. Electrospray ionization (ESI) mass spectra were recorded on Bruker microTOF II and Thermo Finnigan LTQ FT Ultra spectrometers. Electron ionization (EI) mass

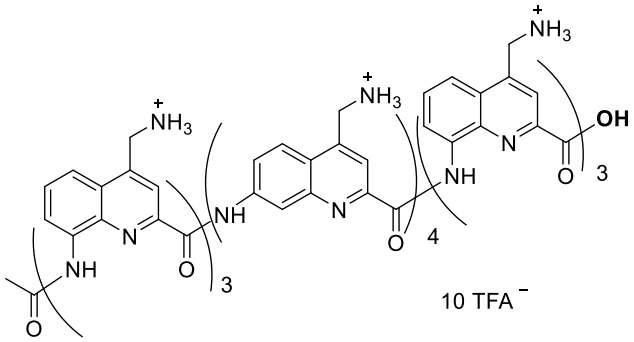
spectra were recorded on a Thermo Q Exactive GC Orbitrap or a Finnigan MAT 95 sector mass spectrometer. Analytical and semi-preparative reversed phase (RP) high performance liquid chromatography (HPLC) was performed on a Thermo Fisher Scientific Ultimate 3000 HPLC System using Macherey-Nagel Nucleodur C18 Gravity columns (4 × 100 mm, 5 µm and 10 × 250 mm, 5 µm) and Macherey-Nagel Nucleodur C8 Gravity columns (4 × 50 mm, 5 µm and 10 × 100 mm, 5 µm) with different gradients of 0.1% TFA water and 0.1% TFA acetonitrile. All ultraviolet–visible (UV/Vis) absorbance measurements were done with a Jasco V-750 spectrophotometer instrument using a 1 cm quartz cuvette. Measurements were performed at 20 °C if not stated otherwise. Microwave-assisted solid phase foldamer synthesis (SPFS) was performed via a CEM® Discover Bio manual microwave peptide synthesizer. The temperature within the reactor vessel was monitored with an optical fiber probe. Automated SPFS was done on a PurePep® Chorus synthesizer (Gyros Protein Technologies) by applying an induction heating.

2.2 Procedure for manual solid phase foldamer synthesis

Oligomers were synthesized according to previously reported manual SPFS protocols,[1] or automated SFPS,[2] as indicated, according to the procedures reported in the corresponding publications.

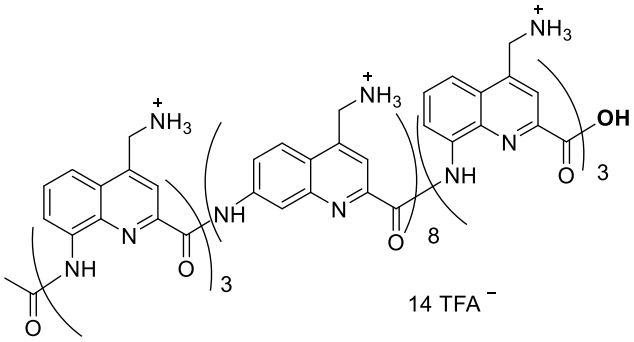
Final acetylation of the N-terminal aromatic amine: H_2N-$(Q)_n$-Wang resin and DIEA (6.0 equiv.) were suspended in anhydrous THF (1.25 mL) then acetyl chloride (4.0 equiv.) in anhydrous THF (1.25 mL) was added. The reaction vessel was then placed under microwave irradiation (25 W, ramp to 50 °C over 5 min, then hold at 50 °C for 15 min). The resin was filtered off and washed with anhydrous THF (2 x 3 mL). The coupling step was repeated twice using the same conditions and number of equivalents of coupling reagents.

Resin cleavage: The resin-bound foldamer was placed in a syringe equipped with a filter, washed with DMF (3 x 3 mL) and DCM (3 x 3 mL), and dried by passing N_2 flow through it. It was then suspended in a solution of TFA/iPr$_3$SiH/H_2O (95:2.5:2.5, *v*/*v*/*v*). The resin was next shaken for at least 2 h at room temperature. The resin was filtered off and rinsed one time with TFA. The foldamer was precipitated from the TFA cleavage solution by adding cold Et_2O and centrifugation to obtain a crude precipitate.



Compound 1: Compound **1** was synthesized starting from LL-Wang resin (0.44 mmol g^{-1}, 25 µmol scale) according to the manual synthesis method. Loading of the first monomer: 0.31 mmol g^{-1} (70%). Final acetylation was carried out via the general acetylation method. After precipitation in cold Et_2O, the crude mixture was purified by semi prep RP-HPLC to give the **1** as a yellow solid (15 mg, 19%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.40 (s, 2H), 12.24 (s, 1H), 12.12 (s, 1H), 11.70 (s, 1H), 11.45 (s, 1H), 11.32 (s, 1H), 10.64 (s, 2H), 9.62 (s, 1H), 9.47 (s, 1H), 9.42 (s, 1H), 9.12 (d, *J* = 7.6 Hz, 2H), 8.86 (s, 4H), 8.71 – 8.54 (m, 7H), 8.53 – 8.32 (m, 8H), 8.27 (m, 4H), 8.14 – 7.43 (m, 29H), 7.40 (d, *J* = 9.2 Hz, 1H), 7.23 (s, 1H), 4.99 – 4.34 (m, 27H).
HRMS: calcd. for $C_{112}H_{95}N_{30}O_{12}$ $[M+H]^+$ 2051.7740; found 2051.8066.

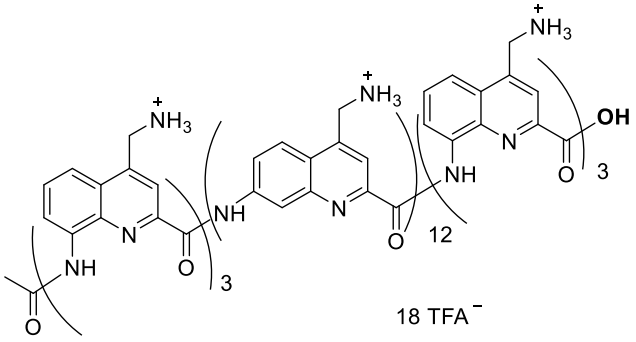


Compound 2: Compound **2** was synthesized starting from Wang resin (0.44 mmol g^{-1}, 17 µmol scale) according to the manual synthesis method. Loading of the first monomer: 0.27 mmol g^{-1} (62%). Final acetylation was carried out via the general acetylation method. After precipitation in cold Et_2O, the crude mixture was purified by semi prep RP-HPLC to give the **2** as a yellow solid (16 mg, 21%).

^{1}H NMR (500 MHz, H_2O/D_2O (9:1, *v*/*v*)) δ 12.19 (s, 1H), 12.04 (s, 1H), 12.01 (s, 1H), 11.58 (s, 1H), 11.47 (s, 1H), 10.69 (s, 1H), 10.62 (s, 1H), 10.20 (s, 1H), 10.10 (s, 1H), 9.85 (s, 1H), 9.82 (s, 1H), 9.48 (s, 1H), 9.37 (s, 1H), 9.11 (s, 1H), 8.83 (d, *J* = 9.3 Hz, 2H), 8.66 (m, 3H), 8.49 (d,

J = 9.7 Hz, 2H), 8.39 – 8.27 (m, 6H), 8.25 (d, *J* = 9.0 Hz, 3H), 8.15 (m, 10H), 8.09 – 7.98 (m, 6H), 7.98 – 7.42 (m, 39H), 7.39 – 7.12 (m, 18H), 7.12 – 6.41 (m, 29H).

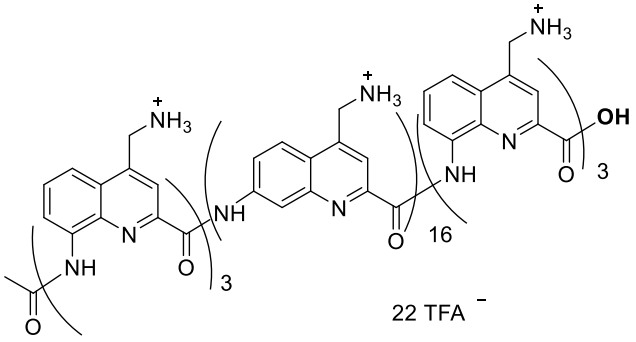
HRMS: calcd. for $C_{156}H_{132}N_{42}O_{16}$ $[M+2H]^{2+}$ 1424.5398; found 1424.5300.



Compound 3: Compound **3** was synthesized starting from Wang resin (0.44 mmol g^{-1}, 23 µmol scale) according to the manual synthesis method. Loading of the first monomer: 0.31 mmol g^{-1} (70%). Final acetylation was carried out via the general acetylation method. After precipitation in cold Et_2O, the crude mixture was purified by semi prep RP-HPLC to give the **3** as a yellow solid (42 mg, 32%).

^{1}H NMR (500 MHz, H_2O/D_2O (9:1, *v*/*v*)) δ 12.03 (s, 1H), 11.95 (s, 1H), 11.86 (s, 1H), 11.38 (s, 1H), 11.35 (s, 1H), 10.39 (s, 1H), 10.16 (s, 1H), 9.85 (s, 1H), 9.83 (s, 1H), 9.73 (s, 1H), 9.52 (s, 1H), 9.43 (s, 1H), 9.38 (s, 1H), 9.31 (s, 1H), 9.11 (s, 1H), 9.03 (d, *J* = 4.6 Hz, 3H), 8.69 (d, *J* = 8.1 Hz, 1H), 8.45 (d, *J* = 10.7 Hz, 1H), 8.40 – 8.30 (m, 3H), 8.16 – 8.00 (m, 4H), 7.99 – 7.87 (m, 6H), 7.88 – 7.78 (m, 6H), 7.78 – 7.57 (m, 20H), 7.56 – 7.30 (m, 18H), 7.26 – 7.10 (m, 5H), 7.08 – 6.75 (m, 18H), 6.68 (d, *J* = 9.1 Hz, 1H), 6.55 – 6.44 (m, 3H), 6.41 (s, 1H), 6.31 (d, *J* = 9.0 Hz, 1H), 4.19 (d, *J* = 14.6 Hz, 1H), 4.01 (d, *J* = 14.9 Hz, 1H), 3.70 – 3.55 (m, 2H), 3.47 (m, 1H).

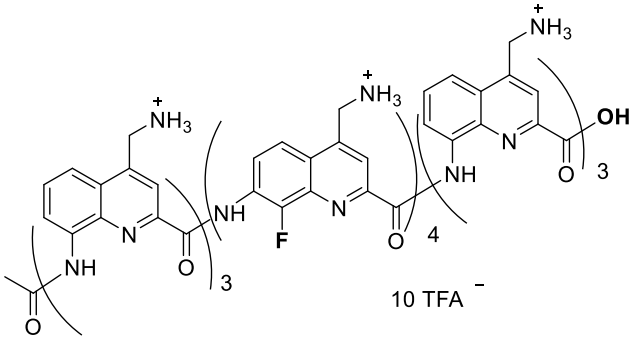
HRMS: calcd. for $C_{200}H_{168}N_{54}O_{20}$ $[M+2H]^{2+}$ 1822.6889; found 1822.7448.



Compound 4: Compound **4** was synthesized on Cl-MPA-Protide resin (0.17 mmol g^{-1}, 24 µmol scale) according to the automated synthesis method. Loading of the first monomer: 0.12

mmol g^{-1} (70%). Final acetylation was carried out via the general acetylation method. After precipitation in cold Et_2O, the crude mixture was purified by semi prep RP-HPLC to give the **4** as a yellow solid (25 mg, 15%).

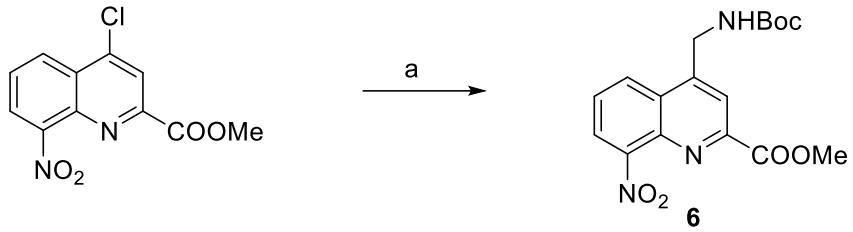
^{1}H NMR (500 MHz, H_2O/D_2O (9:1, *v*/*v*)) δ 11.81 (s, 2H), 11.76 (s, 1H), 11.19 (s, 1H), 11.15 (s, 1H), 10.26 (s, 1H), 10.08 (s, 1H), 9.98 (s, 1H), 9.80 (s, 1H), 9.60 (s, 1H), 9.58 (s, 1H), 9.52 (s, 1H), 9.48 (s, 1H), 9.35 (s, 1H), 9.32 (s, 1H), 9.25 (s, 1H), 9.12 (s, 1H), 9.01 (s, 1H), 8.98 (s, 1H), 8.97 (s, 1H), 8.94 (s, 1H), 8.84 (s, 1H), 8.41 (d, *J* = 8.1 Hz, 1H), 8.25 – 8.10 (m, 3H), 8.02 (d, *J* = 10.0 Hz, 1H), 7.92 – 7.74 (m, 6H), 7.73 – 7.50 (m, 26H), 7.50 – 7.40 (m, 14H), 7.40 – 7.27 (m, 15H), 7.20 (m, 18H), 7.11 – 6.92 (m, 10H), 6.92 – 6.53 (m, 18H), 6.53 – 6.07 (m, 8H), 3.44 (dd, *J* = 11.7, 4.3 Hz, 3H), 3.39 – 3.27 (m, 3H).
HRMS: calcd. for $C_{244}H_{205}N_{66}O_{24}$ $[M+3H]^{3+}$ 1480.8944; found 1480.9345.



Compound 5: Compound **5** was synthesized starting from LL-Wang resin (0.40 mmol g^{-1}, 15 µmol scale) according to the manual synthesis method. Loading of the first monomer: 0.33 mmol g^{-1} (76%). Final acetylation was carried out via the general acetylation method. After precipitation in cold Et_2O, the crude mixture was purified by semi prep RP-HPLC to give the **5** as a yellow solid (5 mg, 10%).

^{1}H NMR (500 MHz, H_2O/D_2O (9:1, *v*/*v*)) δ 11.94 (s, 1H), 11.92 (s, 1H), 11.62 (s, 1H), 11.49 (s, 1H), 11.28 (s, 1H), 10.37 (s, 1H), 9.77 (s, 1H), 9.60 (s, 1H), 9.45 (s, 1H), 9.31 – 9.22 (m, 1H), 9.20 – 9.11 (m, 1H), 8.60 (s, 1H), 8.49 – 8.31 (m, 5H), 8.23 – 8.14 (m, 3H), 8.12 (d, *J* = 8.4 Hz, 1H), 7.87 (d, *J* = 8.6 Hz, 1H), 7.81 (d, *J* = 10.5 Hz, 1H), 7.70 – 7.47 (m, 9H), 7.42 (d, *J* = 8.2 Hz, 2H), 7.36 (t, *J* = 8.2 Hz, 1H), 7.30 – 7.24 (m, 2H), 7.11 (s, 1H), 6.98 – 6.83 (m, 6H), 6.55 (d, *J* = 9.3 Hz, 1H), 6.33 (t, *J* = 8.7 Hz, 1H).
HRMS: calcd. for $C_{112}H_{92}F_4N_{30}O_{12}$ $[M+2H]^{2+}$ 1062.3718; found 1062.3538.

2.3 Monomer synthesis procedures



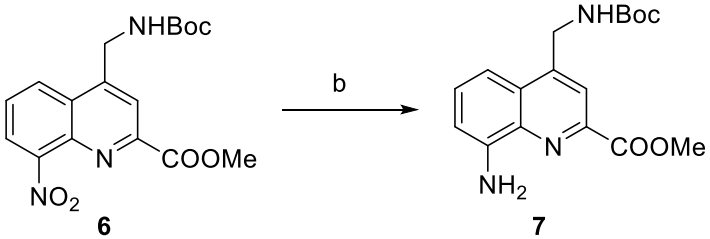
6

Compound 6. Methyl 4-chloro-8-nitroquinoline-2-carboxylate[3] (0.954 g, 0.0036 mol), KF_3BCH_2NHBoc (0.744 g, 0.0037 mol), $Pd(OAc)_2$ (0.036 g, 0.00016 mol), SPhos (0.148 g, 0.00036 mol) and K_2CO_3 (1.248 g, 0.011 mol) were added in a sealed tube and the mixture was purged 3 times with argon. Then toluene (9.6 mL) and water (2.4 mL) were added and the reaction mixture was heated to 85 °C for 18 h. After completion of the reaction that was confirmed by TLC, phosphate buffer (pH = 7) was added then the crude mixture was extracted with DCM. The combined organic layers were washed with water and brine, dried (Na_2SO_4) and then concentrated under reduced pressure. The resulting residue was purified by column chromatography to give 0.86 g (67%) of **6** as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.53 (dd, J = 8.6, 1.2 Hz, 1H), 8.39 (dd, J = 7.5, 1.2 Hz, 1H), 8.13 (s, 1H), 7.93 (dd, J = 8.6, 7.5 Hz, 1H), 7.77 (t, J = 6.0 Hz, 1H), 4.77 (d, J = 6.0 Hz, 2H), 3.96 (s, 3H), 1.43 (s, 9H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 164.6, 155.86, 149.0, 148.9, 148.6, 137.8, 128.0, 127.6, 127.5, 123.9, 119.7, 78.6, 54.9, 53.0, 28.1.

HRMS: calcd. for $C_{17}H_{19}N_3O_6Na$ $[M+Na]^+$ 384.1166; found 384.1180.



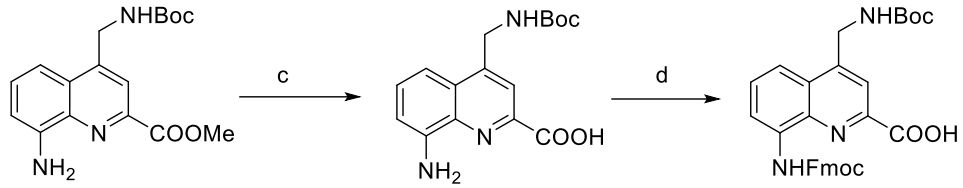
6 **7**

Compound 7. Quinoline **6** (2 g, 0.006 mmol) was dissolved in a mixture of THF (133 mL)/ MeOH (200 mL) under N_2. Then 10 wt.% Pd/C (1 g, 35% *w*/*w*) was added and N_2 was replaced by H_2. The mixture was stirred for 3 h at room temperature and the reaction was checked by TLC. After completion of the reaction, the mixture was filtered and concentrated then the residue was precipitated in diethyl ether to give 1.65 g (90%) of **7** as a yellow powder.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.92 (s, 1H), 7.63 (t, J = 6.1 Hz, 1H), 7.44 (dd, J = 8.3, 7.7 Hz, 1H), 7.22 (dd, J = 8.3, 1.1 Hz, 1H), 6.95 (dd, J = 7.7, 1.1 Hz, 1H), 6.14 (s, 2H), 4.58 (d, J = 6.0 Hz, 2H), 3.94 (s, 3H), 1.43 (s, 9H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 165.5, 155.9, 146.8, 146.4, 143.3, 136.2, 130.3, 127.7, 124.9, 117.7, 109.2, 108.6, 78.3, 52.5, 28.2.

HRMS: calcd. for $C_{17}H_{22}N_3O_4$ $[M+H]^+$ 332.1605; found 332.1600.



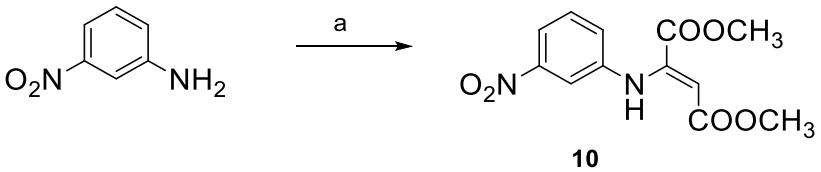
7 **8** **9**

Compound 9. The protocol was modified from the reference (X. Hu, et al., *Chem. Sci.*, 2017, **8**, 3741–3749.).[3] Compound **7** (3.43 g, 0.01 mol) was dissolved in dioxane (206 mL) then LiOH·H_2O (0.65 g, 0.015 mol) in H_2O (65 mL) was added slowly at 0 °C. The mixture was stirred for 1 h at 0 °C, then neutralized by 0.1 mol/L HCl. Then H_2O (80 mL) and $NaHCO_3$ (2.61 g, 0.031 mol) were added and cooled to 0 °C. Fmoc-Cl (3.48 g, 0.013 mol) was added slowly as a solution in dioxane. The resulting mixture was stirred at 0 ° for 1 h and let to stir overnight at room temperature. After completion of the reaction that was confirmed by TLC, the solution was acidified to pH=4 using a saturated citric acid solution. Then water was added and the aqueous layers were extracted with DCM. The combined organic layers were washed with water and brine, dried (Na_2SO_4) and then concentrated under reduced pressure. The residue was precipitated in acetonitrile, filtered and washed with a small amount of acetonitrile to give 4.36 g (78%) of **9** as a grey powder.

^{1}H NMR (500 MHz, DMSO-d_6) δ 13.60 (s, 1H), 10.49 (s, 1H), 8.10 (s, 1H), 7.93 (dt, J = 7.5, 0.9 Hz, 2H), 7.84 (d, J = 8.4 Hz, 1H), 7.78 (dd, J = 7.4, 1.0 Hz, 2H), 7.72 (t, J = 6.7 Hz, 2H), 7.44 (t, J = 7.5 Hz, 3H), 7.36 (td, J = 7.4, 1.2 Hz, 2H), 4.70 (d, J = 6.0 Hz, 2H), 4.62 (d, J = 6.9 Hz, 2H), 4.46 (t, J = 6.8 Hz, 1H), 1.43 (s, 9H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 165.5, 155.9, 153.5, 148.4, 144.9, 143.7, 140.8, 136.5, 136.3, 129.5, 127.8, 127.3, 127.2, 125.2, 120.3, 118.1, 117.6, 116.5, 116.1, 78.4, 66.4, 46.6, 28.2.

HRMS: calcd. for $C_{31}H_{30}N_3O_6$ $[M+H]^+$ 540.2129; found 540.2138.



10

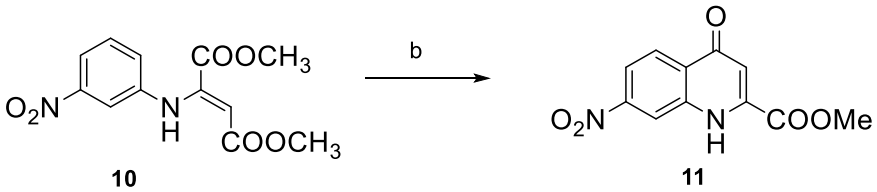
Compound 10. The protocol was modified from the reference (N. D. Heindel, et al., *J. Org. Chem.*, 1967, **32**, 4155–4157.).[4] 3-Nitroaniline (20 g, 0.145 mol) was dissolved in MeOH (350

mL) under N_2. The solution was cooled to 0 ˚C in an ice-bath and dimethyl acetylenedicarboxylate (26.7 mL, 0.217 mol) was added dropwise. The mixture was stirred at 0 ˚C for 30 min and then at room temperature for 23 h, during which time the product precipitated. The suspension was cooled to 0 ˚C to introduce additional precipitation. The product was then filtered off and washed with cold MeOH to give 32 g (79%) of **10** as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.70 (s, 1H), 7.86 (ddd, J = 8.3, 2.2, 0.9 Hz, 1H), 7.81 (t, J = 2.2 Hz, 1H), 7.55 (t, J = 8.1 Hz, 1H), 7.33 (ddd, J = 8.1, 2.3, 0.9 Hz, 1H), 5.57 (s, 1H), 3.71 (s, 3H), 3.67 (s, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 167.2, 164.2, 148.1, 144.5, 142.1, 130.2, 125.6, 117.4, 113.7, 98.1, 53.2, 51.3.

HRMS: calcd. for $C_{12}H_{12}N_2O_6$ $[M]^+$ 280.0690; found 280.0692.



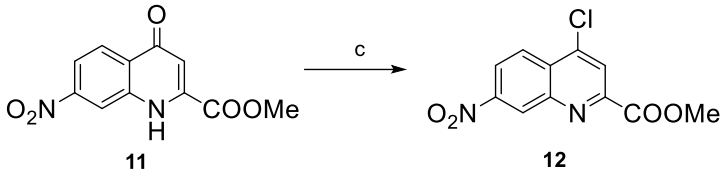
10 **11**

Compound 11. The protocol was modified from the reference (N. D. Heindel, et al., *J. Org. Chem.*, 1967, **32**, 4155–4157.).[4] Diphenyl ether (680 mL) was placed in a 1 L round bottom flask and heated using a heating mantle to its boiling point at 260 ˚C . Then compound **10** (32 g, 0.114 mol) was added to the boiling solvent by means of a glass funnel. The mixture was kept boiling for 20 min; no condenser was used. The reaction mixture was left to cool down to room temperature. The product was filtered off, and the solid was washed several times with cyclohexane then dried under reduced pressure. The product was recrystallized from hot DMF to give 13.5g (48%) of **11** as a golden solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.49 (s, 1H), 8.86 (d, J = 2.3 Hz, 1H), 8.27 (d, J = 8.9 Hz, 1H), 8.07 (dd, J = 8.9, 2.3 Hz, 1H), 6.73 (s, 1H), 3.99 (s, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 176.9, 162.2, 149.7, 139.8, 139.3, 128.7, 127.2, 117.5, 115.9, 111.5, 53.8.

HRMS: calcd. for $C_{11}H_8N_2O_5$ $[M]^+$ 248.0428; found 248.0437.

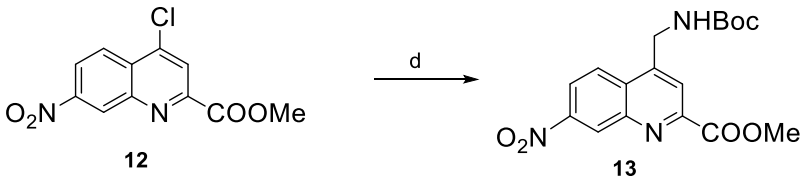


Compound 12. Compound **11** (8.86 g, 0.036 mol) was dissolved in dry DMF (93 mL) then $POCl_3$ (3.66 mL, 0.039 mmol) was added slowly. The reaction mixture was stirred for 15 min at 80 °C. After completion of the reaction that was confirmed by TLC, the mixture was poured into ice and neutralised with saturated $NaHCO_3$ solution. Then the mixture was extracted with DCM. The combined organic layers were washed with water and brine, dried (Na_2SO_4) and then concentrated under reduced pressure to give 8.18 g (86%) of **12** as a brown solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 9.01 (dd, J = 2.3, 0.6 Hz, 1H), 8.59 (dd, J = 9.2, 2.3 Hz, 1H), 8.54 (dd, J = 9.2, 0.6 Hz, 1H), 8.44 (s, 1H), 4.01 (s, 3H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 163.8, 150.0, 149.1, 146.6, 143.2, 129.6, 126.4, 126.0, 123.6, 123.3, 53.2.

HRMS: calcd. for $C_{11}H_7ClN_2O_4$ $[M]^+$ 266.0089; found 266.0086.

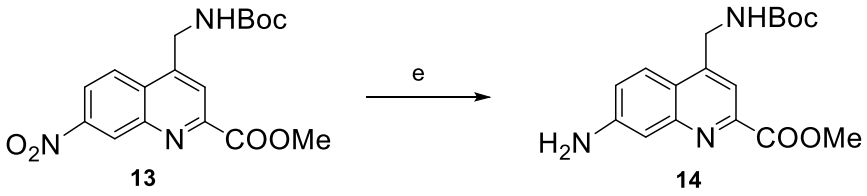


Compound 13. Compound **12** (0.954 g, 0.0036 mol), KF_3BCH_2NHBoc (0.744 g, 0.0037 mol), $Pd(OAc)_2$ (0.036 g, 0.00016 mol), SPhos (0.148 g, 0.00036 mol) and K_2CO_3 (1.248 g, 0.011 mol) were added in a sealed tube and the mixture was purged 3 times with argon. Then toluene (9.6 mL) and water (2.4 mL) were added and heated to 85 °C for 30 h. After completion of the reaction that was confirmed by TLC, pH = 7 phosphate buffer was added then the mixture extracted with DCM. The combined organic layers were washed with water and brine, dried (Na_2SO_4) and then concentrated under reduced pressure. The resulting residue was purified by column chromatography. The recovered quinoline derivative **12** was used to repeat the reaction again and the product that was obtained after column was precipitated in DCM/diethyl ether to give 0.729 g (56%) of **13** as white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 8.96 (d, J = 2.3 Hz, 1H), 8.52 (d, J = 9.2 Hz, 1H), 8.46 (dd, J = 9.2, 2.4 Hz, 1H), 8.15 (s, 1H), 7.78 (t, J = 5.9 Hz, 1H), 4.77 (d, J = 6.0 Hz, 2H), 3.99 (s, 3H), 1.43 (s, 9H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 165.3, 156.3, 150.2, 148.9, 148.8, 146.5, 130.6, 126.7, 126.5, 122.2, 121.1, 79.0, 53.5, 41.1, 28.7.

HRMS: calcd. for $C_{17}H_{19}N_3O_6Na$ $[M+Na]^+$ 384.1166; found 384.1170.

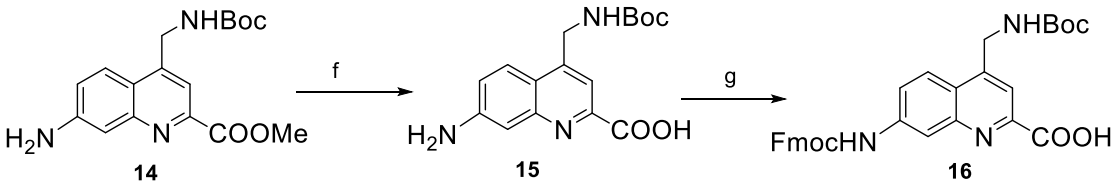


Compound 14. Compound **13** (2.91 g, 0.008 mmol) was dissolved in mixture of THF (260 mL) and MeOH(390 mL) under N_2. Then 10 wt.% Pd/C (1 g, 35% *w*/*w*) was added and N_2 was replaced by H_2. The mixture was stirred for 3 h at room temperature. After completion of the reaction that was confirmed by TLC, the mixture was filtered and concentrated then the residue was precipitated in diethyl ether to give 2.54 g (95%) of **14** as a yellow powder.

^{1}H NMR (500 MHz, DMSO-d_6) δ 7.90 (d, *J* = 9.0 Hz, 1H), 7.61 (s, 1H), 7.59 (t, *J* = 6.1 Hz, 1H), 7.14 (dd, *J* = 9.0, 2.3 Hz, 1H), 7.04 (d, *J* = 2.3 Hz, 1H), 5.95 (s, 2H), 4.56 (d, *J* = 6.1 Hz, 2H), 3.91 (s, 3H), 1.43 (s, 9H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 166.4, 156.3, 151.0, 145.0, 147.5, 146.7, 124.4, 121.4, 119.8, 114.2, 107.6, 78.7, 52.8, 40.8, 28.7.

HRMS: calcd. for $C_{17}H_{22}N_3O_4$ $[M+H]^+$ 332.1605; found 332.1608.



Compound 16. Compound **14** (2.54 g, 0.0077 mol) was dissolved in dioxane (153 ml) then LiOH H_2O (0.483 g, 0.0112 mol) in water (48 mL) was added slowly at 0 °C. The mixture was stirred for 1 h at 0 °C, then neutralized by 0.1 mol·L^{-1} HCl. Then water (14 ml) and $NaHCO_3$ (1.93 g, 0.023 mol) were added and cooled down to 0 °C by ice-bath. Fmoc-Cl (2.58 g, 0.01 mol) is added slowly as a solution in dioxane. The resulting mixture is stirred at 0 °C for 1 h and allowed to warm to room temperature overnight. After completion of the reaction that was confirmed by TLC, the solution was acidified to pH=4 using a saturated citric acid solution. Then water was added and the aqueous layers were extracted with DCM. The combined organic layers were washed with water and brine, dried (Na_2SO_4) and then concentrated under reduced

pressure. The residue was precipitated in acetonitrile then precipitated in DCM/diethyl ether to give 3.30 g (81%) of **16** as a yellow powder.

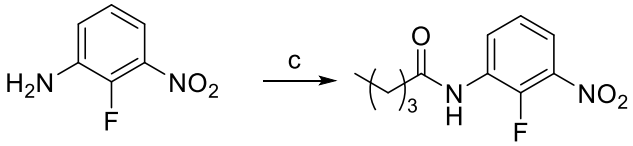
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.20 (s, 1H), 8.33 (s, 1H), 8.14 (d, J = 9.1 Hz, 1H), 7.93 (dt, J = 7.6, 0.9 Hz, 2H), 7.85 (s, 1H), 7.79 (dd, J = 7.5, 1.1 Hz, 3H), 7.65 (t, J = 6.1 Hz, 1H), 7.44 (tt, J = 7.5, 0.8 Hz, 2H), 7.37 (td, J = 7.5, 1.2 Hz, 2H), 4.65 (d, J = 6.1 Hz, 2H), 4.60 (d, J = 6.5 Hz, 2H), 4.36 (t, J = 6.5 Hz, 1H), 1.42 (s, 9H).

^{13}C NMR (126 MHz, DMSO-d_6) δ 166.9, 156.3, 153.9, 149.1, 148.2, 147.4, 144.2, 141.3, 141.1, 128.2, 127.6, 125.6, 124.6, 123.2, 121.9, 120.7, 116.9, 116.0, 78.8, 66.3, 47.1, 40.9, 28.7.

HRMS: calcd. for $C_{31}H_{30}N_3O_6$ $[M+H]^+$ 540.2129; found 540.2138.

2-fluoro-3-nitroaniline

2-fluoro-3-nitroaniline (CAS: 21397-11-5) was synthesized as previously described[5] (3 steps, only two are depicted in the scheme S1).

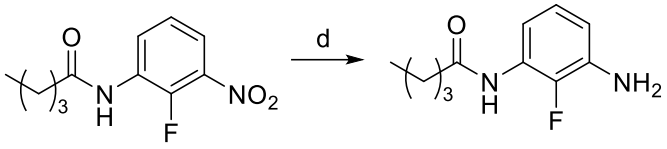


17

Compound 17: Dry DIEA (12.5 mL, 90.0 mmol, freshly distilled over CaH_2) was added to a suspension of 2-fluoro-3-nitroaniline (12.77 g, 81.8 mmol) in pentanoic anhydride (17.8 mL, 90.0 mmol) under inert atmosphere of N_2. The mixture was stirred at 40 ˚C for 19 h, after which complete conversion of the starting material was observed by TLC. The mixture was diluted with DCM, washed twice with 5% aqueous citric acid and several times with saturated aqueous $NaHCO_3$, dried over $MgSO_4$ and concentrated under reduced pressure, to obtain 19.66 g (quantitative) of compound **17** as a pale white solid.

^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.68 (ddd, J = 8.4, 6.6, 1.7 Hz, 1H), 7.73 (ddd, J = 8.6, 7.1, 1.7 Hz, 1H), 7.51 (s, 1H), 7.26 (td, J = 8.3, 1.8 Hz, 1H), 2.46 (t, J = 8.5, 2H), 1.73 (ddt, J = 8.5, 7.3, 6.4 Hz, 2H), 1.50 – 1.35 (m, 2H), 0.96 (t, J = 7.3 Hz, 3H).

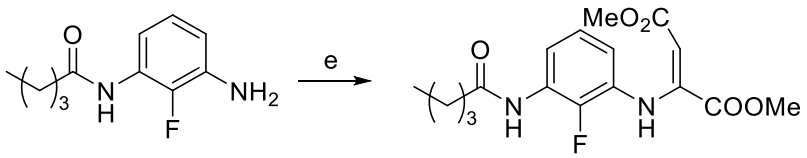
^{13}C NMR: (75 MHz, $CDCl_3$) δ 171.9, 145.6 (d, $J_{C\text{-}F}$ = 260.3 Hz), 137.4, 128.9 (d, $J_{C\text{-}F}$ = 9.3 Hz), 126.9 (d, $J_{C\text{-}F}$ = 2.3 Hz), 124.5 (d, $J_{C\text{-}F}$ = 5.2 Hz), 119.8 (d, $J_{C\text{-}F}$ = 3.1 Hz), 37.6, 27.5, 22.4, 13.9.
HRMS: calcd. for $C_{11}H_{14}FN_2O_3$ $[M+H]^+$ 241.0983; found 241.0985.



17 **18**

Compound 18: Compound **17** (10.92 g, 45.5 mmol) dissolved in 100 mL of degassed AcOEt was placed into a two-necked 500 mL round bottom flask equipped with a teflon-coated magnetic stirring bar. One of the necks was closed with a septum, N_2 was flushed and 250 mg of Pd/C (10%) catalyst was added to the solution. The reaction flask was placed under a H_2 atmosphere by alternately purging the flask under vacuum, shaking it and filling the flask with H_2 (3 times). The reaction mixture was vigorously stirred under H_2 atmosphere (balloon pressure) at room temperature for 24 h, after which complete conversion of the starting material was observed by TLC. The solution was filtered through Celite® and solvent was removed under reduced pressure, to give 9.29 g (97%) of compound **18** as a pale white solid.

^{1}H NMR: (300 MHz, $CDCl_3$) δ 7.65 (t, *J* = 7.4 Hz, 1H), 7.30 (s, 1H), 6.88 (td, *J* = 8.2, 1.7 Hz, 1H), 6.51 (td, *J* = 8.4, 1.6 Hz, 1H), 3.71 (s, 2H), 2.38 (t, *J* = 7.6 Hz, 2H), 1.80 – 1.62 (m, 2H), 1.40 (dq, *J* = 14.6, 7.3 Hz, 2H), 0.94 (t, *J* = 7.3 Hz, 3H).
^{13}C NMR: (75 MHz, $CDCl_3$) δ 171.5, 142.1 (d, $J_{C\text{-}F}$ = 234.9 Hz), 134.2 (d, $J_{C\text{-}F}$ = 11.4 Hz), 126.7 (d, $J_{C\text{-}F}$ = 8.1 Hz), 124.4 (d, $J_{C\text{-}F}$ = 4.4 Hz), 112.2, 111.5, 37.7, 27.7, 22.5, 13.9.
HRMS: calcd. for $C_{11}H_{16}FN_2O$ $[M+H]^+$ 211.1241; found 211.1243.



18 **19**

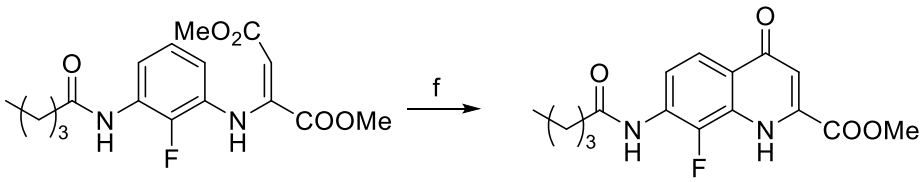
Compound **19**: Compound **18** (9.03 g, 42.9 mmol) was dissolved in 130 mL of degassed MeOH under inert atmosphere of N_2. The solution was cooled down to 0 ˚C in an ice-bath and dimethyl acetylenedicarboxylate (5.8 mL, 47.2 mmol) was added dropwise under stirring. The mixture was stirred at 0 ˚C for 30 min and then at room temperature for 22 h, during which time the

product precipitated. Volatiles were partially evaporated and the resulting concentrated suspension was cooled down to 5 ˚C to force the precipitation. The product was filtered off and washed with cold MeOH, to give 10.65 g (70%) of compound **19** as a white solid.

^{1}H NMR: (300 MHz, $CDCl_3$) δ 9.53 (s, 1H), 8.06 (t, *J* = 7.7 Hz, 1H), 7.34 (s, 1H), 7.00 (td, *J* = 8.2, 1.7 Hz, 1H), 6.59 (td, *J* = 8.0, 1.5 Hz, 1H), 5.54 (s, 1H), 3.75 (s, 3H), 3.72 (s, 3H), 2.40 (t, *J* = 7.6 Hz, 2H), 1.79 – 1.62 (m, 2H), 1.48 – 1.32 (m, 2H), 0.94 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR: (75 MHz, $CDCl_3$) δ 171.6, 169.9, 164.2, 147.1, 144.9 (d, $J_{C\text{-}F}$ = 241.6 Hz), 128.3 (d, $J_{C\text{-}F}$ = 10.0 Hz), 127.2 (d, $J_{C\text{-}F}$ = 8.9 Hz), 124.2 (d, $J_{C\text{-}F}$ = 4.7 Hz), 117.1 (d, $J_{C\text{-}F}$ = 19.9 Hz), 95.3, 53.0, 51.5, 37.7, 27.6, 22.4, 13.9.

HRMS: calcd. for $C_{17}H_{21}FN_2O_5Na$ $[M+Na]^+$ 375.1326; found 375.1389.



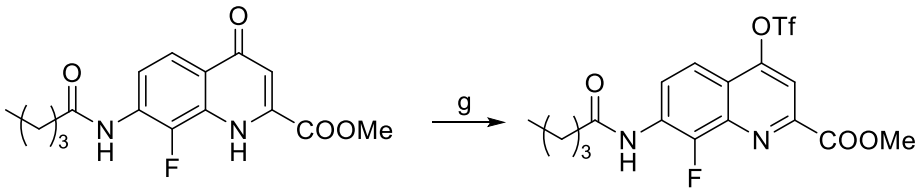
19 **20**

Compound 20: Diphenyl ether (415 mL) was placed in a 1 L round bottom flask and heated to its boiling point at 260 ˚C with a heating mantle and without any reflux condenser or stirring bar. Then compound **19** (16.60 g, 47.1 mmol) was added to the boiling solvent by means of a glass funnel. The addition was done stepwise during 10 min to have control over the MeOH bubbling produced each time a portion of the starting material is added. The mixture was kept boiling for 20 min leaving the reaction flask open to allow MeOH vapors out. Then the heating mantle was removed and the reaction mixture was left to cool down to approximately 40 ˚C, observing the partial precipitation of the product. The cooled suspension was transferred to a bigger flask and cyclohexane (700 mL) was added to force the precipitation. The product was filtered off, washed thoroughly with more cyclohexane and dried under reduced pressure, obtaining 13.33 g of compound **20** (88%) as a light-gray solid.

^{1}H NMR: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.94 (s, 1H), 8.35 (dd, *J* = 9.1, 7.1 Hz, 1H), 8.07 (dd, *J* = 9.1, 1.7 Hz, 1H), 7.52 (s, 1H), 7.26 (s, 1H), 6.92 (d, *J* = 1.8 Hz, 1H), 4.05 (s, 3H), 2.48 (t, *J* = 7.6 Hz, 2H), 1.84 – 1.67 (m, 2H), 1.52 – 1.34 (m, 2H), 0.97 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR: (126 MHz, $CDCl_3$) δ 178.3, 171.7, 163.3, 140.5 (d, $J_{C\text{-}F}$ = 242.8 Hz), 136.1, 129.5 (d, $J_{C\text{-}F}$ = 6.7 Hz), 128.9 (d, $J_{C\text{-}F}$ = 11.0 Hz), 123.0, 122.3 (d, $J_{C\text{-}F}$ = 4.2 Hz), 117.3, 112.5, 54.1, 37.7, 27.5, 22.5, 13.9.

HRMS: calcd. for $C_{16}H_{18}FN_2O_4$ $[M+H]^+$ 321.1245; found 321.1339.



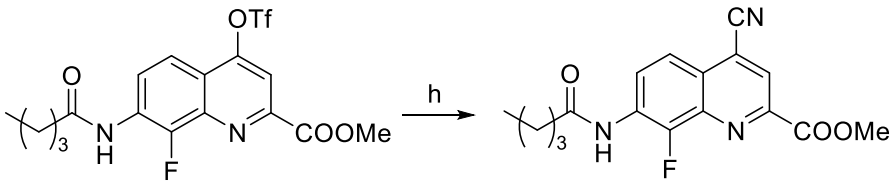
20 **21**

Compound 21: Dry pyridine (11.0 mL, 136 mmol) was added to a suspension of compound **20** (8.69 g, 27.1 mmol) in dry DCM (100 mL) under inert atmosphere of N_2. The slurry was cooled to –10 ˚C in an ice/NaCl-batch, and trifluoromethanesulfonic anhydride (Tf_2O, 6.8 mL, 40.4 mmol) was added dropwise under stirring. The resulting mixture was stirred at –10 ˚C for 2 h, after which complete conversion of the starting material was observed by TLC. Then the cold reaction mixture was poured into saturated aqueous NH_4Cl. The product was extracted with DCM, washed with 0.1 M aqueous HCl and once with saturated aqueous NH_4Cl, dried over $MgSO_4$ and concentrated under reduced pressure, obtaining 11.95 g (97%) of compound **21** as a white-gray solid.

^{1}H NMR: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.99 (dd, *J* = 9.4, 6.8 Hz, 1H), 8.16 (s, 1H),7.90 (dd, *J* = 9.4, 1.8 Hz, 1H), 7.71 (d, *J* = 3.6 Hz, 1H), 4.10 (s, 3H), 2.54 (t, *J* = 7.6 Hz, 2H), 1.88 – 1.69 (m, 2H), 1.55 – 1.33 (m, 2H), 0.99 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR: (75 MHz, $CDCl_3$) δ 172.1, 164.3, 153.4 (d, $J_{C\text{-}F}$ = 4.5 Hz), 149.8, 145.9 (d, $J_{C\text{-}F}$ = 254.8 Hz), 139.6 (d, $J_{C\text{-}F}$ = 11.4 Hz), 128.7 (d, $J_{C\text{-}F}$ = 8.1 Hz), 124.9, 120.9, 119.2, 116.6 (d, $J_{C\text{-}F}$ = 5.4 Hz), 111.8, 53.9, 37.8, 27.5, 22.5, 13.9.

HRMS: calcd. for $C_{17}H_{16}F_4N_2O_6SNa$ $[M+Na]^+$ 475.0557; found 475.0617.

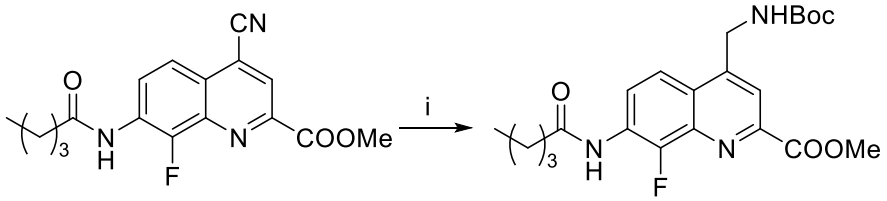


21 **22**

Compound 22: Compound **21** (4.00 g, 8.84 mmol) and KCN (3.46 g, 53.1 mmol) were suspended in 40 mL of dry THF under inert atmosphere of N_2. The slurry was sonicated until the suspended particles of solid became very small. Then the solvent was evaporated leaving a slightly wet solid residue in the reaction flask. Dry toluene (150 mL) and $Pd(PPh_3)_4$ (817 mg,

0.707 mmol) were added under inert atmosphere of N_2. The resulting suspension was stirred at 100 ˚C for 7 h, after which complete conversion of the starting material was observed by NMR. Volatiles were evaporated under reduced pressure and the solid residue was dissolved in AcOEt, washed with saturated aqueous $NaHCO_3$ and once with saturated aqueous NaCl, dried over $MaSO_4$ and concentrated under reduced pressure. The crude residue was suspended in MeOH, filtered off, washed with more MeOH and dried under reduced pressure, obtaining 2.31 g (79%) of compound **22** as a pale white solid.

^{1}H NMR: (300 MHz, $CDCl_3$) δ 9.05 (dd, *J* = 9.3, 6.8 Hz, 1H), 8.45 (s, 1H), 8.03 (dd, *J* = 9.3, 1.7 Hz, 1H), 7.77 (s, 1H), 4.11 (s, 3H), 2.55 (dd, *J* = 8.2, 6.9 Hz, 2H), 1.78 (ddt, *J* = 8.5, 7.4, 6.4 Hz, 2H), 1.53 – 1.38 (m, 2H), 0.98 (t, *J* = 7.3 Hz, 3H).
^{13}C NMR: (75 MHz, $CDCl_3$) δ 172.1, 164.2, 148.2, 146.0 (d, $J_{C\text{-}F}$ = 255.8 Hz), 137.6 (d, $J_{C\text{-}F}$ = 11.2 Hz), 128.7 (d, $J_{C\text{-}F}$ = 8.1 Hz), 125.7, 124.5, 123.7, 120.6 (d, $J_{C\text{-}F}$ = 5.5 Hz), 120.1 (d, $J_{C\text{-}F}$ = 3.9 Hz), 114.8, 53.9, 37.8, 27.4, 22.5, 13.9.
HRMS: calcd. for $C_{17}H_{16}FN_3O_3Na$ $[M+Na]^+$ 352.1068; found 352.1135.



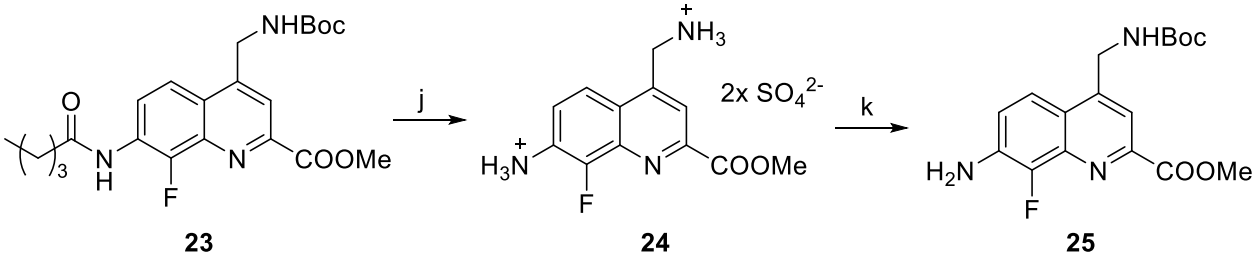
22 **23**

Compound 23: Pd/C (10%) (2.27 g) was added to a solution of compound **22** (3.25 g, 9.86 mmol), di-*tert*-butyl dicarbonate (8.61 g, 39.4 mmol) and ammonium formate (3.73 g, 59.1 mmol) in dry THF (190 mL), under inert atmosphere of N_2. After 4 h stirring at 40 ˚C, the complete conversion of the starting material was observed by TLC. The reaction mixture was filtered through Celite® using DCM to completely wash out the product from the filter. The crude product was concentrated under reduced pressure and purified by flash chromatography (SiO_2) using acetone/DCM as eluent (from 5% to 10% acetone) to give 3.32 g (78%) of compound **23** as a white solid.

^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.80 (dd, *J* = 9.4, 7.1 Hz, 1H), 8.12 (s, 1H), 7.81 (dd, *J* = 9.4, 1.7 Hz, 1H), 7.68 (d, *J* = 3.4 Hz, 1H), 5.07 (s, 1H), 4.85 (d, *J* = 6.2 Hz, 2H), 4.07 (s, 3H), 2.52 (t, *J* = 7.6 Hz, 2H), 1.85 – 1.69 (m, 2H), 1.48 (s, 9H), 1.43 (m, 2H), 0.98 (t, *J* = 7.3 Hz, 3H).

^{13}C NMR: (75 MHz, $CDCl_3$) δ 172.1, 165.8, 155.9, 148.5, 146.6 (d, $J_{C\text{-}F}$ = 253.5 Hz), 146.3, 137.7 (d, $J_{C\text{-}F}$ = 10.1 Hz), 126.8 (d, $J_{C\text{-}F}$ = 8.2 Hz), 124.6, 123.3, 118.5 (d, $J_{C\text{-}F}$ = 4.9 Hz), 118.4, 80.6, 53.5, 41.6, 37.7, 28.5, 27.5, 22.5, 14.0.

HRMS: calcd. for $C_{22}H_{29}FN_3O_5$ $[M+H]^+$ 434.2086; found 434.2147.



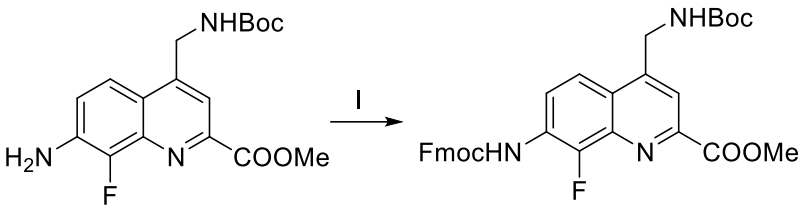
23 **24** **25**

Compound 25: Concentrated sulfuric acid (1.65 mL, 30.9 mmol) was added to a suspension of compound **23** (5.15 g, 11.9 mmol) in 110 mL of MeOH at room temperature under stirring. The mixture was refluxed for 16 h, during which time it turned from a white slurry to a red solution. The crude mixture was left to cool down to room temperature observing the formation of a red precipitate. The complete precipitation was forced by adding some Et_2O and the supernatant was separated by decantation. The precipitate was washed twice with more Et_2O, separating the supernatant by decantation, and dried under reduced pressure to obtain compound **24** as a red solid. Then di-*tert*-butyl decarbonate (2.65 g, 12.1 mmol), $NaHCO_3$ (2.00 g, 23.8 mmol) and 75 mL of dry DMF were added under inert atmosphere of N_2. The resulting solution was stirred at room temperature for 26 h, after which complete conversion of the starting material was observed by NMR. The mixture was diluted with AcOEt, washed several times with saturated aqueous $NaHCO_3$ to completely remove the DMF, dried over $MgSO_4$ and concentrated under reduced pressure, to obtain 4.11 g (99%) of compound **25** as a yellow solid.

^{1}H NMR: (300 MHz, DMSO-d_6) δ 7.78 (d, J = 9.1 Hz, 1H), 7.70 (s, 1H), 7.62 (t, J = 6.1 Hz, 1H), 7.32 (dd, J = 9.1, 8.1 Hz, 1H), 5.94 (s, 2H), 4.58 (d, J = 6.0 Hz, 2H), 3.93 (s, 3H), 1.42 (s, 9H).

^{13}C NMR: (126 MHz, DMSO-d_6) δ 165.7, 155.9, 147.2, 146.9, 142.2 (d, $J_{C\text{-}F}$ = 242.8 Hz), 138.3 (d, $J_{C\text{-}F}$ = 8.3 Hz), 136.5 (d, $J_{C\text{-}F}$ = 10.9 Hz), 121.7 (d, $J_{C\text{-}F}$ = 5.3 Hz), 119.2 (d, $J_{C\text{-}F}$ = 4.1 Hz), 119.1, 114.4, 78.3, 52.5, 40.5, 28.2.

HRMS: calcd. for $C_{17}H_{21}FN_3O_4$ $[M+H]^+$ 350.1511; found 350.1583.



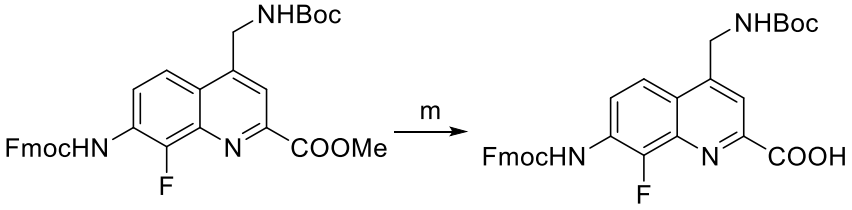
25 **26**

Compound 26: A suspension of compound **25** (4.11 g, 11.8 mmol) in 350 mL of water/1,4-dioxane (3:4, *v*/*v*) was cooled down to 0 ˚C in an ice-bath. Then a solution of 9-fluorenylmethoxycarbonyl chloride (Fmoc-Cl, 4.57 g, 17.6 mmol) in 50 mL of dioxane was added dropwise under stirring, during approximately 1 h. The reaction mixture was stirred at 0 ˚C for 2 h and then at room temperature for 5 h, after which some more Fmoc-Cl (1.52 g, 5.88 mmol) dissolved in 10 mL of dioxane was added dropwise under stirring. After 16 h stirring at room temperature, again some more Fmoc-Cl (1.52 g, 5.88 mmol) dissolved in 10 mL of dioxane was added dropwise under stirring. 1 h later the complete conversion of the starting material was finally observed by NMR. The reaction mixture was diluted with DCM, washed with water, dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified by flash chromatography (SiO_2) using acetone/DCM as eluent (from 0% to 10% acetone) to give 5.74 g (85%) of compound **26** as a white solid.

^{1}H NMR: (300 MHz, $CDCl_3$) δ 8.53 (s, 1H), 8.12 (s, 1H), 7.84 (s, 1H), 7.80 (dd, *J* = 7.4, 1.0 Hz, 2H), 7.65 (dd, *J* = 7.3, 1.0 Hz, 2H), 7.44 (tdd, *J* = 7.5, 1.2, 0.6 Hz, 2H), 7.35 (td, *J* = 7.4, 1.3 Hz, 2H), 7.27 (s, 1H), 5.04 (s, 1H), 4.85 (d, *J* = 6.2 Hz, 2H), 4.63 (d, *J* = 6.6 Hz, 2H), 4.33 (t, *J* = 6.6 Hz, 1H), 4.08 (s, 3H), 1.50 (s, 9H).

^{13}C NMR: (75 MHz, $CDCl_3$) δ 165.8, 155.9, 153.1, 148.7, 146.4 (d, $J_{C\text{-}F}$ = 253.9 Hz), 146.3 (d, $J_{C\text{-}F}$ = 2.3 Hz), 143.6, 141.5, 137.8 (d, $J_{C\text{-}F}$ = 9.5 Hz), 128.1, 127.4, 126.6 (d, $J_{C\text{-}F}$ = 8.5 Hz), 125.1, 124.3, 122.0, 120.3, 118.7 (d, $J_{C\text{-}F}$ = 4.5 Hz), 118.3, 80.6, 67.8, 53.4, 47.1, 41.6, 28.5.

HRMS: calcd. for $C_{32}H_{31}FN_3O_6$ $[M+H]^+$ 572.2191; found 572.2229.



26 **27**

Compound 27: LiI beads (778 mg, 5.82 mmol) were added to a solution of compound **26** (1.11 g, 1.94 mmol) in degassed AcOEt (110 mL, degassed by freeze-thaw). The solution was

protected from light and heated to reflux temperature under inert atmosphere of N_2. After stirring at reflux temperature for 19 h, the mixture was cooled down to room temperature, washed with 5% aqueous $Na_2S_2O_3$ and 2% aqueous citric acid, dried over $MgSO_4$ and concentrated under reduced pressure, washed twice with Et_2O and dried *in vacuo*, to obtain 891 mg (82%) of compound **27** as a white solid.

^{1}H NMR (500 MHz, DMSO-d_6) δ 13.55 (s, 1H), 10.04 (s, 1H), 8.03 (s, 1H), 7.99 (d, J = 7.4 Hz, 2H), 7.92 (d, J = 7.6 Hz, 2H), 7.80 (d, J = 7.5 Hz, 2H), 7.70 (t, J = 6.1 Hz, 1H), 7.44 (t, J = 7.4 Hz, 2H), 7.36 (t, J = 7.4 Hz, 2H), 4.68 (d, J = 6.0 Hz, 2H), 4.51 (d, J = 6.9 Hz, 2H), 4.35 (t, J = 6.9 Hz, 1H), 1.43 (s, 9H).
^{13}C NMR: (75 MHz, DMSO-d_6) δ 166.2, 155.9, 153.8, 148.9, 148.3 (d, $J_{C\text{-}F}$ = 256.5 Hz), 147.3, 143.7, 140.8, 137.5 (d, $J_{C\text{-}F}$ = 9.1 Hz), 127.7, 127.1, 125.8 (d, $J_{C\text{-}F}$ = 9.8 Hz), 125.3, 124.9, 124.5, 120.2, 118.8 (d, $J_{C\text{-}F}$ = 4.8 Hz), 117.9, 78.4, 66.4, 46.6, 40.6, 28.2.
HRMS: calcd. for $C_{31}H_{29}FN_3O_6$ $[M+H]^+$ 558.2035; found 558.2076.

3 NMR structure elucidation of 2 in water

Two dimensional NMR spectra were recorded on a Avance III HD 500 MHz Bruker BioSpin spectrometer equipped with a broad band observe 5-mm BB-H&FD CryProbe^{TM} Prodigy.

2D NOESY was recorded with a phase-sensitive pulse sequence with water suppression employing an excitation sculpting element from the Bruker pulse program library (noesyesfpgpphrs). Data acquisition was performed with 1K (F2) x 512 (F1) data points and a mixing time of 0.5 s. The recycling delay was 1.5 s and 16 transients per increment were applied at a sweep width of 8 kHz in both dimensions resulting in an acquisition time of 0.1204 s. The special acquisition parameters regarding the water suppression element of the pulse sequence were adopted from the optimized parameter set of the respective one-dimensional experiment. A 90° shifted sinesquare multiplication, an exponential window of 1.0 Hz as well as a gaussian window of 1 Hz in both dimensions prior to FT was applied.

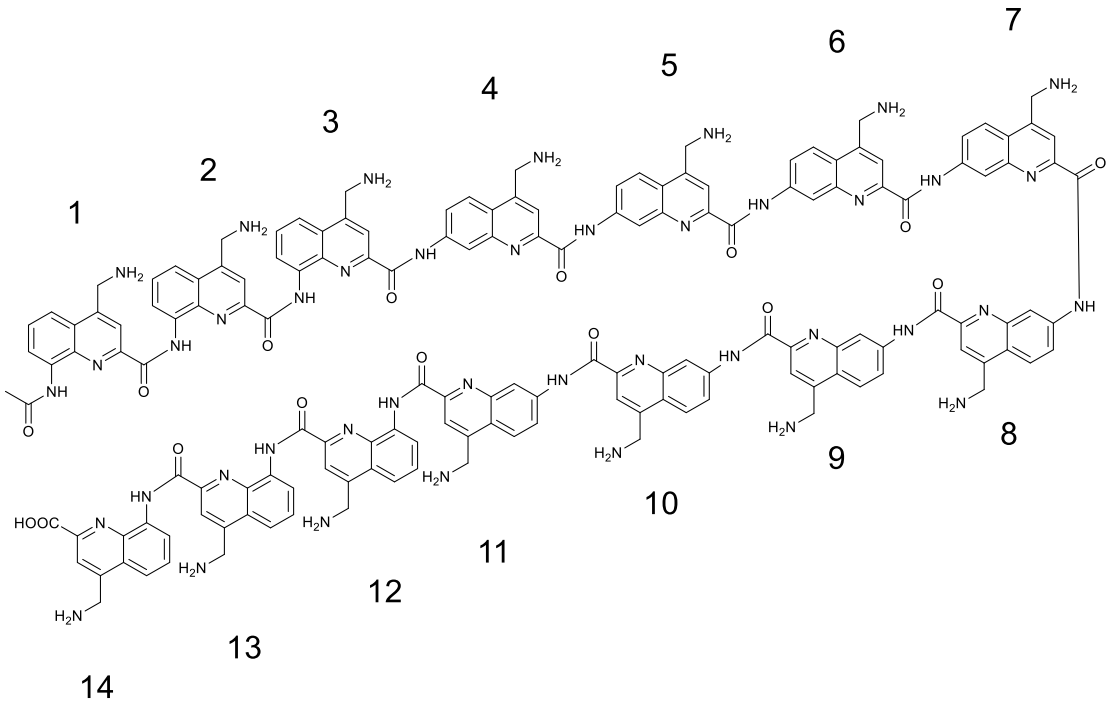
2D COSY was recorded using with water suppression using watergate W5 pulse sequence with gradients (cosygpphppw5) from the Bruker pulse program library. Data acquisition was performed with 2048 (F2) x 256 (F1) data points in States-TPPI mode. The recycling delay was 1.0 s and 128 transients per increment were applied at a sweep width of 8 kHz in both dimensions resulting in an acquisition time of 0.1204 s. The TOCSY mixing time was set to 80 ms. Special acquisition parameters regarding the water suppression element of the pulse

sequence were adopted from the optimized parameter set of a respective one-dimensional experiment with a watergate W5 pulse sequence. A 90° shifted sinesquare multiplication was applied in both dimensions prior to FT.

2D TOCSY was recorded with a phase-sensitive pulse sequence using composite pulse scheme MLEV with water suppression employing an excitation sculpting element (mlevesgpph) from the Bruker pulse program library. Data acquisition was performed with 2048 (F2) x 256 (F1) data points in States-TPPI mode. The recycling delay was 2.0 s and 8 transients per increment were applied at a sweep width of 8 kHz in both dimensions resulting in an acquisition time of 0.1283 s. The TOCSY mixing time was set to 80 ms. Special acquisition parameters regarding the water suppression element of the pulse sequence were adopted from the optimized parameter set of the respective one-dimensional experiment. A 90° shifted sinesquare multiplication was applied in both dimensions prior to FT.

^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC spectra were recorded using standard pulse sequences from the Bruker pulse program library applying standard processing parameters.

a)



b)

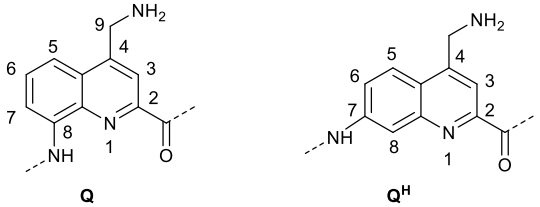


Figure S 10. a) Numbering of the units of compound **2** used in NMR assignment. b) Representative numbering of the carbon atoms in Q- and Q^H-units

Table S1. Assignment of the ^{1}H chemical shifts of compound **2** in H_2O/D_2O (9:1, *v*/*v*) at 25 °C (500 MHz).

Monomer	Atom	^{1}H (ppm)	Monomer	Atom	^{1}H (ppm)
Q1	H3	7.09	$Q^{H}8$	H3	7.50
	H5	7.38		H5	7.84
	H6	7.49		H6	8.21
	H7	7.21		H8	6.36
	NH	9.26		NH	9.00
	AcNH	1.25			
Q2	H3	8.05	$Q^{H}9$	H3	8.01
	H5	7.12		H5	8.24
	H6	6.95		H6	8.72
	H7	7.68		H8	7.92
	NH	11.94		NH	9.74
Q3	H3	6.82	$Q^{H}10$	H3	7.70
	H5	6.97		H5	8.21
	H6	6.59		H6	8.53
	H7	7.55		H8	7.63
	NH	11.90		NH	10.52
$Q^{H}4$	H3	7.90	$Q^{H}11$	H3	6.89
	H5	7.48		H5	7.81
	H6	7.21		H6	7.74
	H8	7.26		H8	6.47
	NH	10.09		NH	9.37
$Q^{H}5$	H3	8.03	Q12	H3	7.51
	H5	8.04		H5	6.69
	H6	8.38		H6	6.69
	H8	8.09		H7	6.92
	NH	9.71		NH	11.48
$Q^{H}6$	H3	8.21	Q13	H3	7.05
	H5	8.04		H5	6.39
	H6	8.38		H6	6.53
	H8	8.09		H7	8.73
	NH	10.58		NH	12.08
$Q^{H}7$	H3	7.95	Q14	H3	6.83
	H5	7.82		H5	7.48
	H6	8.58		H6	7.37
	H8	6.73		H7	7.57
	NH	9.99		NH	11.36

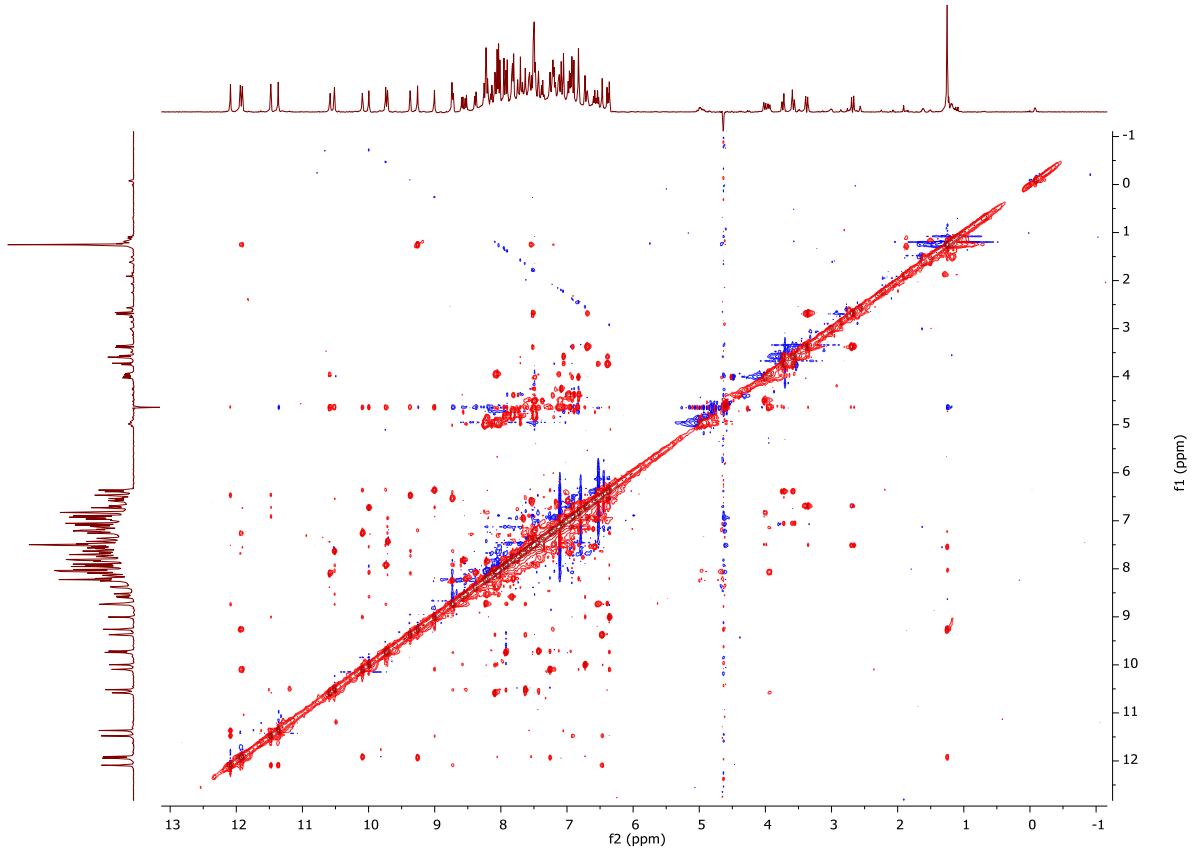


Figure S11. ^{1}H-^{1}H NOESY spectrum of **2** (0.5 mM, 500 MHz, H_2O/D_2O (9:1, *v*/*v*), 25 °C, 0.5 s mixing time).

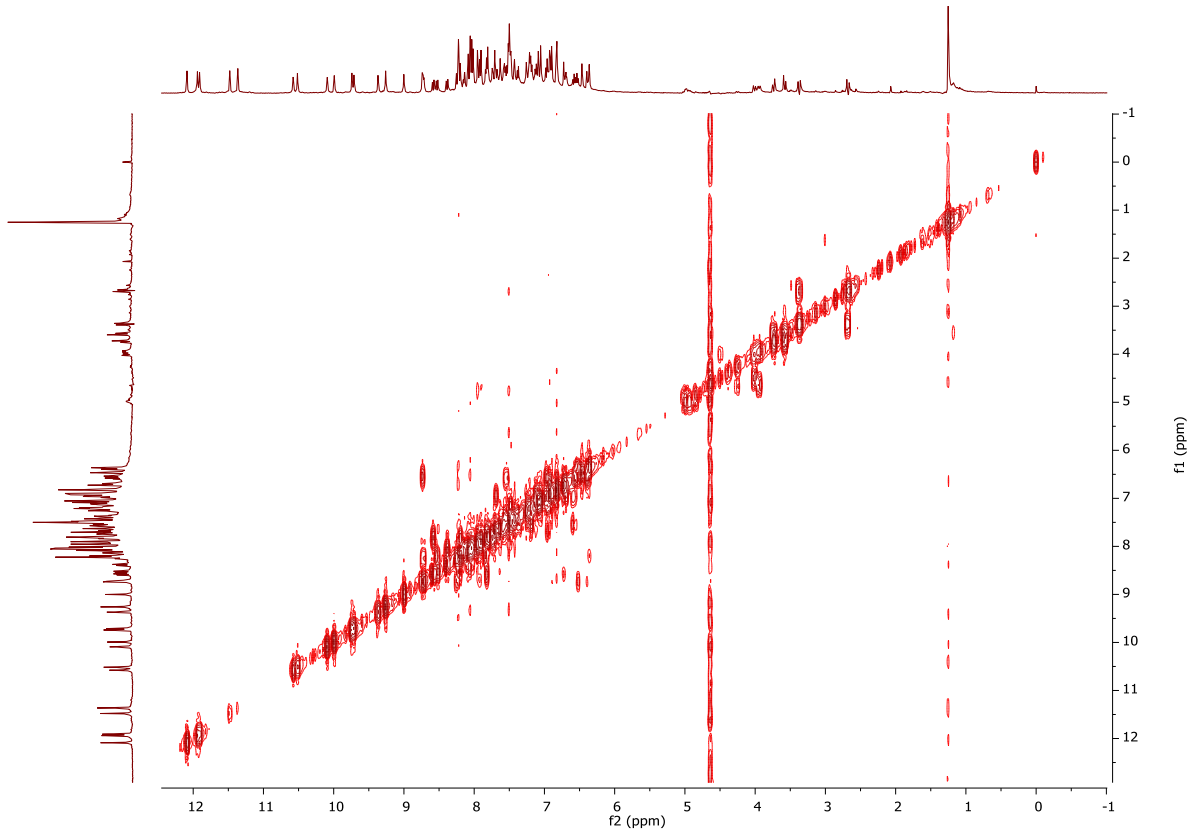


Figure S12. ^{1}H-^{1}H COSY spectrum of **2** (0.5 mM, 500 MHz, H_2O/D_2O (9:1, *v*/*v*), 25 °C).

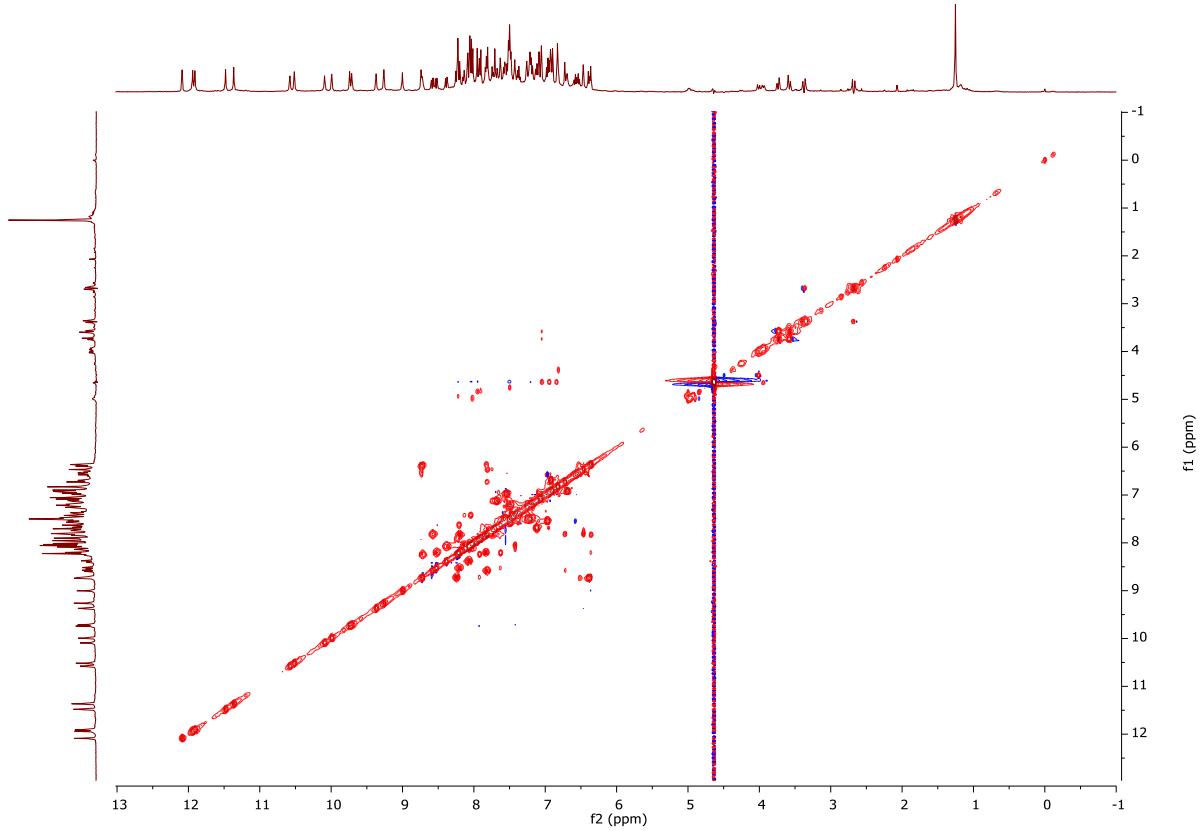


Figure S13. ^{1}H-^{1}H TOSY spectrum of **2** (0.5 mM, 500 MHz, H_2O/D_2O (9:1, *v*/*v*), 25 °C).

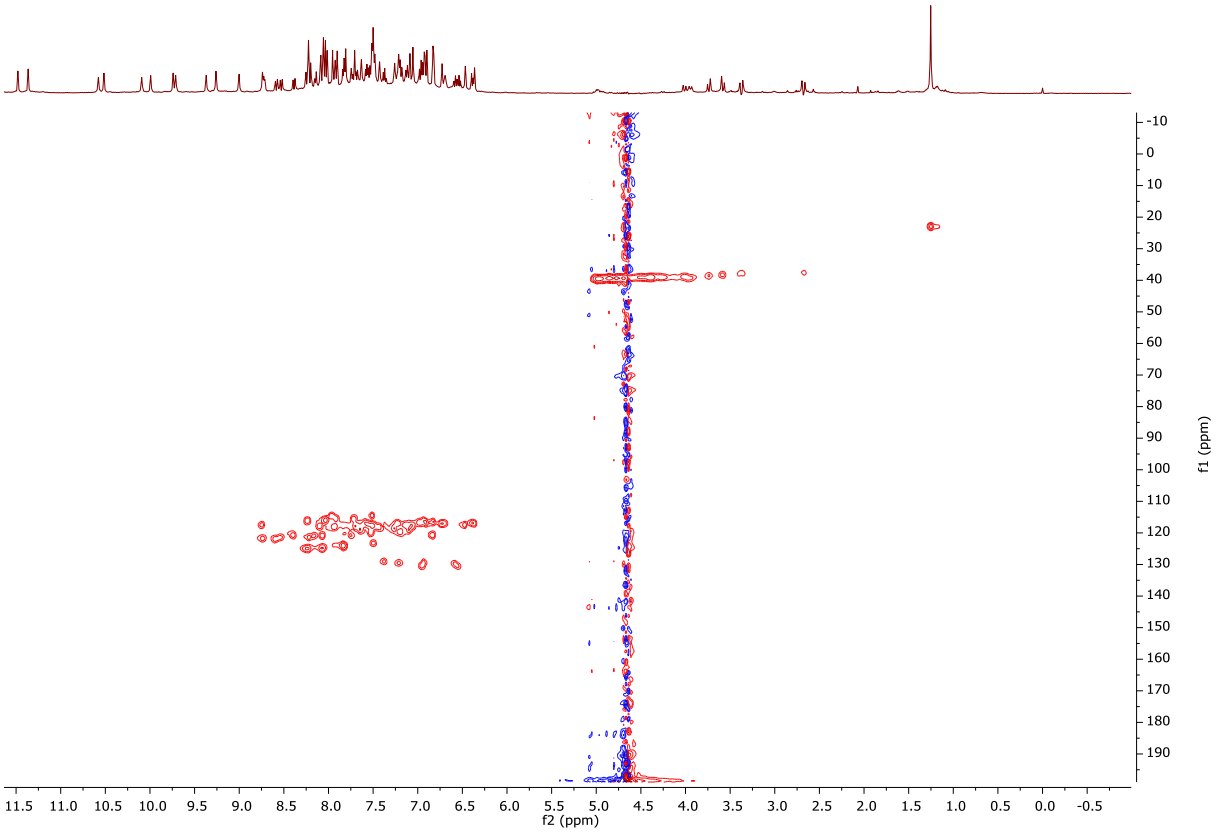


Figure S14. ^{1}H-^{13}C HSQC spectrum of **2** (0.5 mM, 500 MHz, H_2O/D_2O (9:1, *v*/*v*), 25 °C).

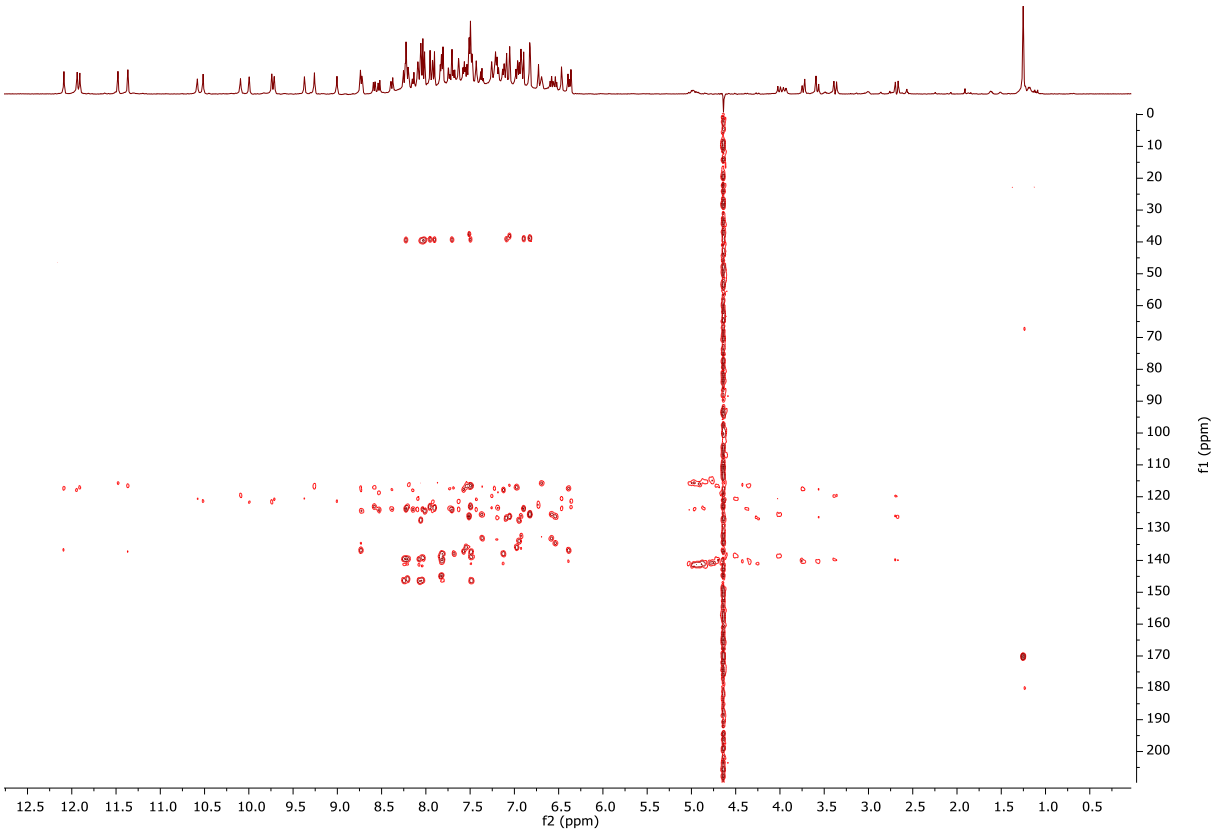


Figure S15. ^{1}H-^{13}C HMBC spectrum of **2** (0.5 mM, 500 MHz, H_2O/D_2O (9:1, *v*/*v*), 25 °C).

4 Spectra and chromatograms of new compounds

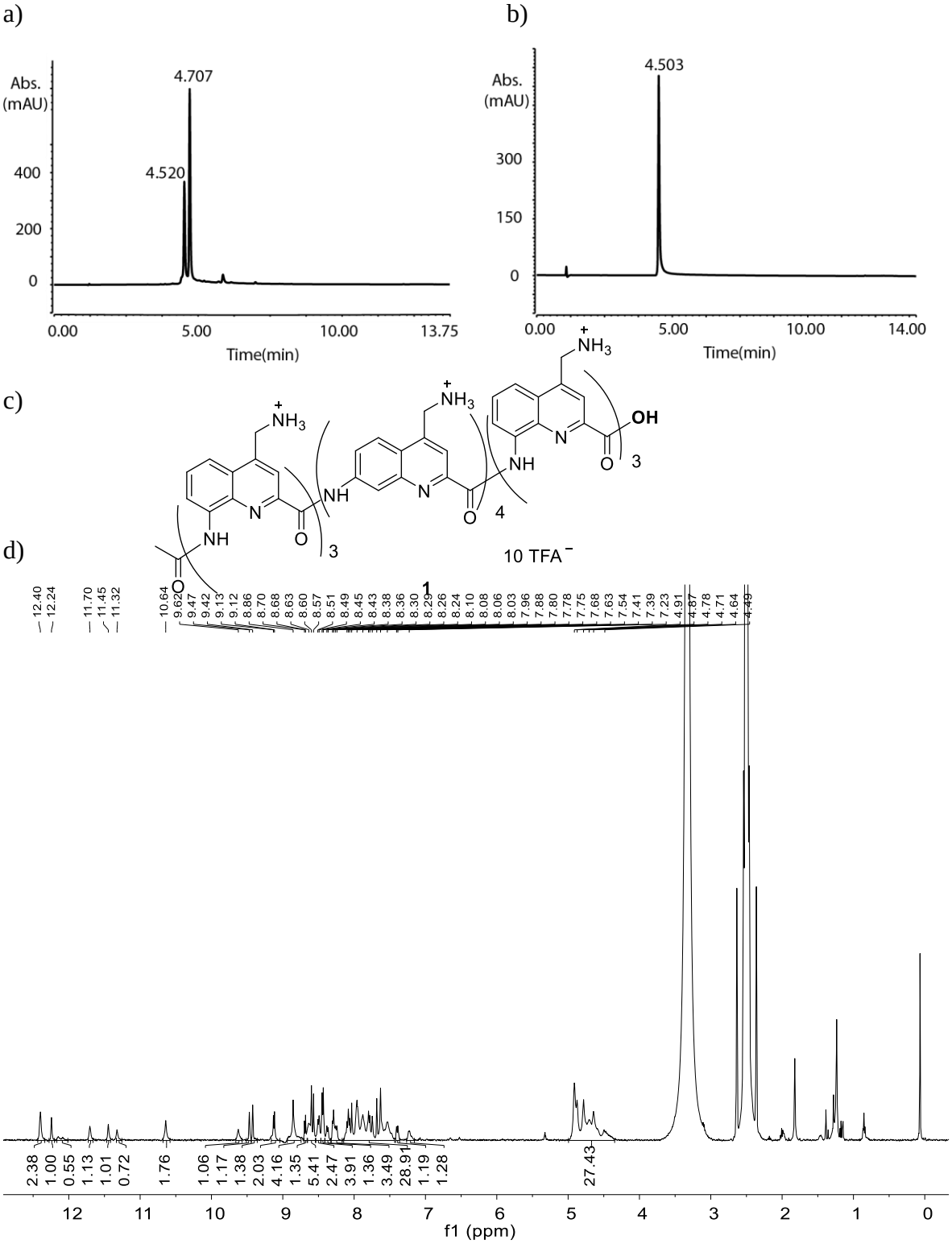


Figure S16. Analytical data of compound **1**. HPLC chromatograms (a) after cleavage from the resin (C18, 5 to 60 B% over 10 min, λ = 300 nm) and (b) after purification (C18, 5 to 60 B% over 10 min, λ = 300 nm); A: 0.1% TFA water, B: 0.1% TFA acetonitrile. (c) Chemical structure of compound **1**. (d) ^{1}H NMR spectrum (500 MHz, DMSO-d_6, 25 °C).

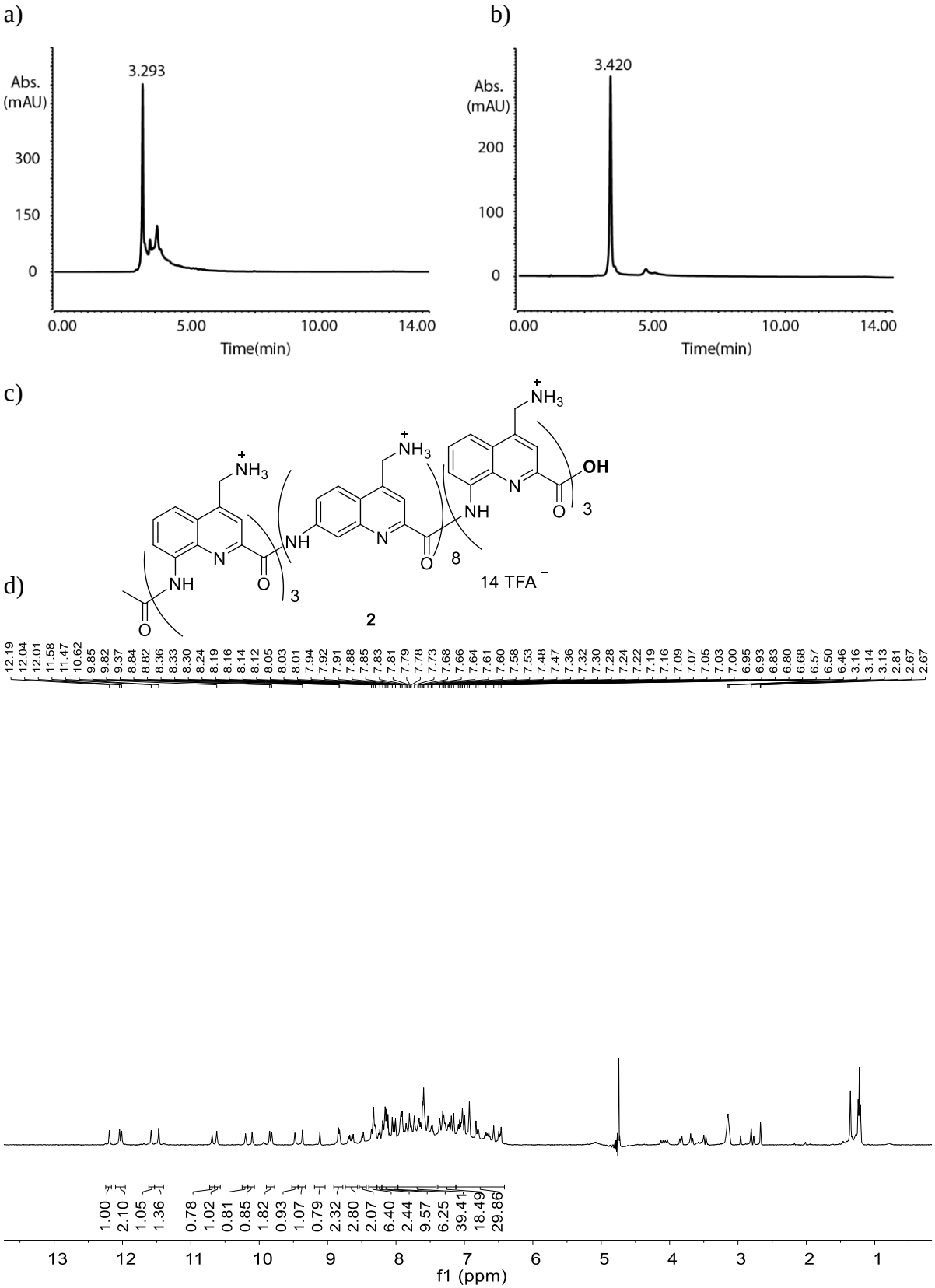


Figure S17. Analytical data of compound **2**. HPLC chromatograms (a) after cleavage from the resin (C18, 5 to 70 B% over 10 min, λ = 300 nm) and (b) after purification (C18, 10 to 40 B% over 10 min, λ = 300 nm); A: 0.1% TFA water, B: 0.1% TFA acetonitrile. (c) Chemical structure of compound **2**. (d) ^{1}H NMR spectrum with water suppression (500 MHz, H_2O/D_2O = (9:1, *v*/*v*), 25 °C).

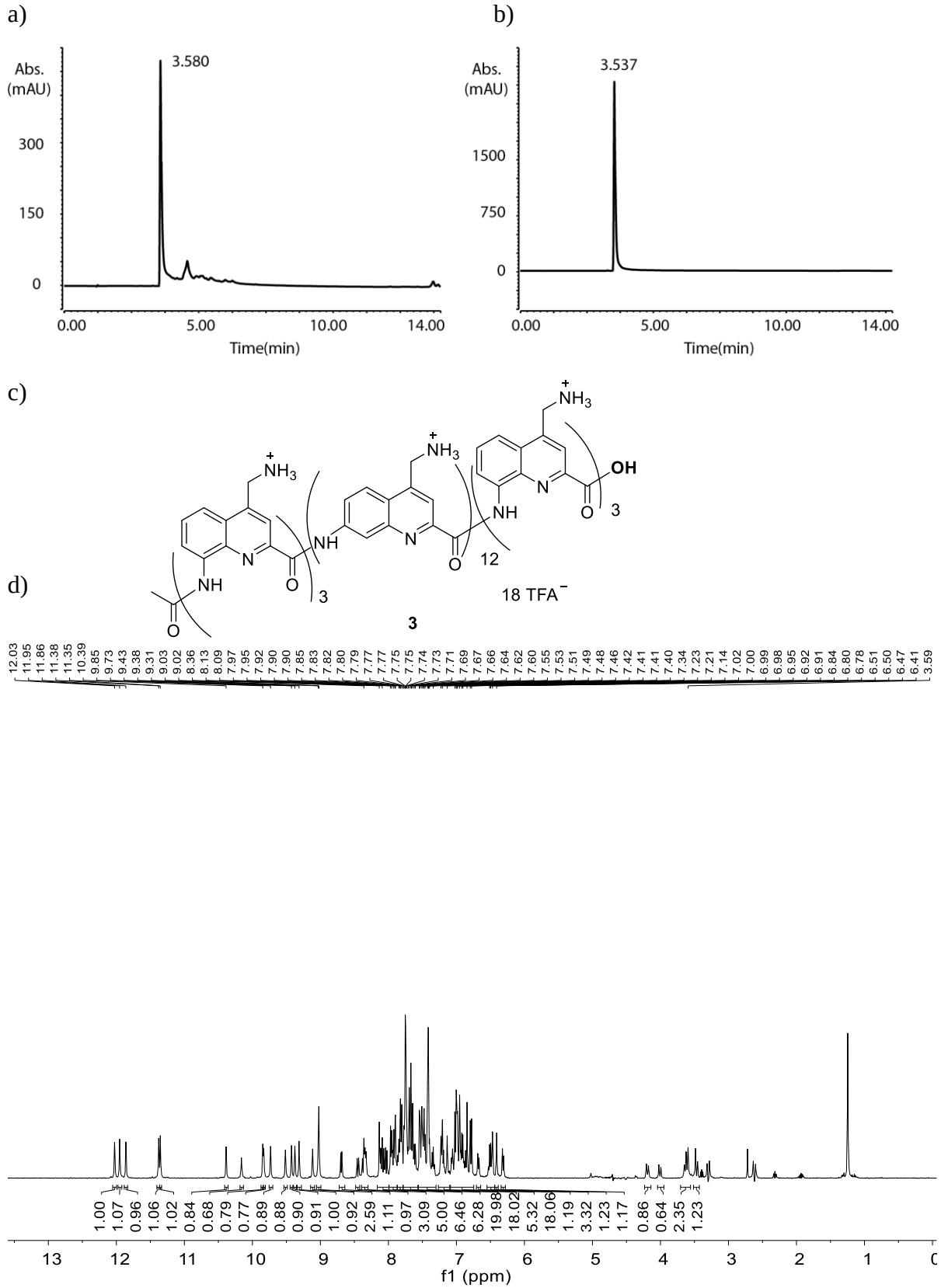


Figure S18. Analytical data of compound **3**. HPLC chromatograms (a) after cleavage from the resin (C18, 5 to 60 B% over 10 min, λ = 300 nm) and (b) after purification (C18, 5 to 60 B% over 10 min, λ = 300 nm); A: 0.1% TFA water, B: 0.1% TFA acetonitrile. (c) Chemical structure of compound **3**. (d) ^{1}H NMR spectrum with water suppression (500 MHz, H_2O/D_2O (9:1, *v*/*v*), 25 °C).

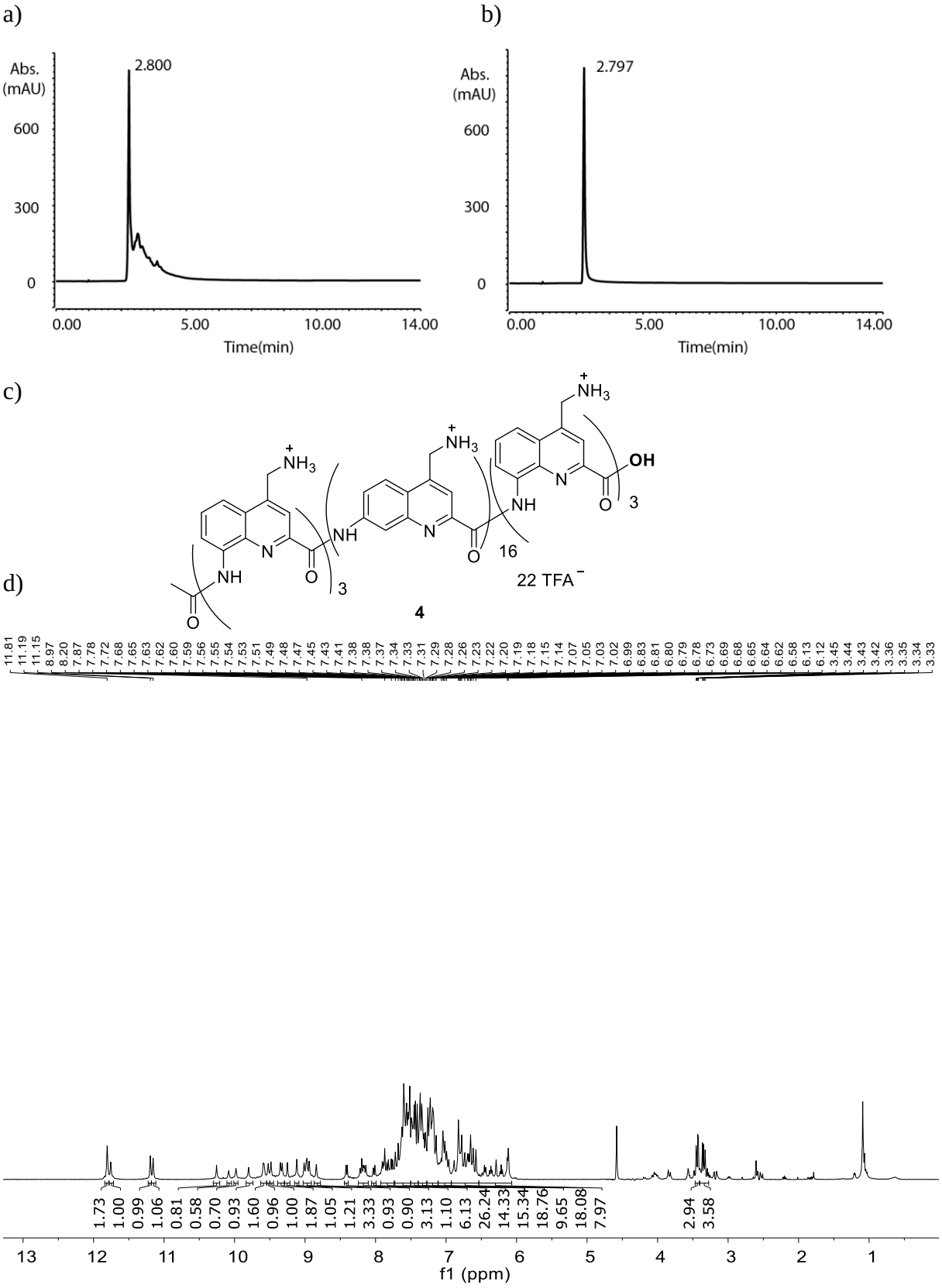


Figure S19. Analytical data of compound **4**. HPLC chromatograms (a) after cleavage from the resin (C18, 5 to 100 B% over 10 min, λ = 300 nm) and (b) after purification (C18, 5 to 100 B% over 10 min, λ = 300 nm); A: 0.1% TFA water, B: 0.1% TFA acetonitrile. (c) Chemical structure of compound **4**. (d) ^{1}H NMR spectrum with water suppression (500 MHz, H_2O/D_2O (9:1, *v*/*v*), 25 °C).

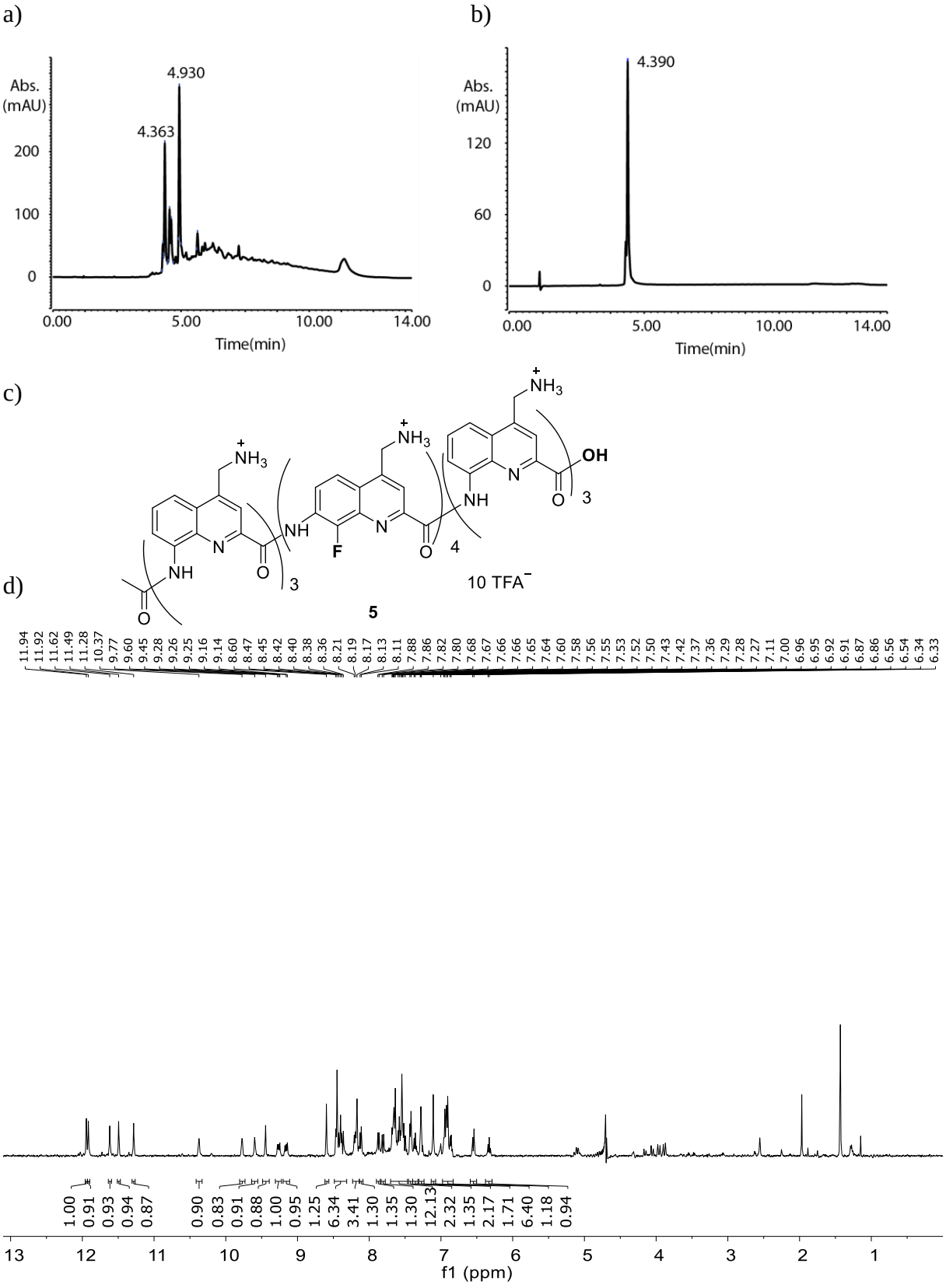


Figure S20. Analytical data of compound **5**. HPLC chromatograms (a) after cleavage from the resin (C18, 5 to 40 B% over 10 min, λ = 300 nm) and (b) after purification (C18, 5 to 40B% over 10 min, λ = 300 nm; A: 0.1% TFA water, B: 0.1% TFA acetonitrile). (c) Chemical structure of compound **5**. (d) ^{1}H NMR spectrum with water suppression (500 MHz, H_2O/D_2O (9:1, *v*/*v*), 25 °C).

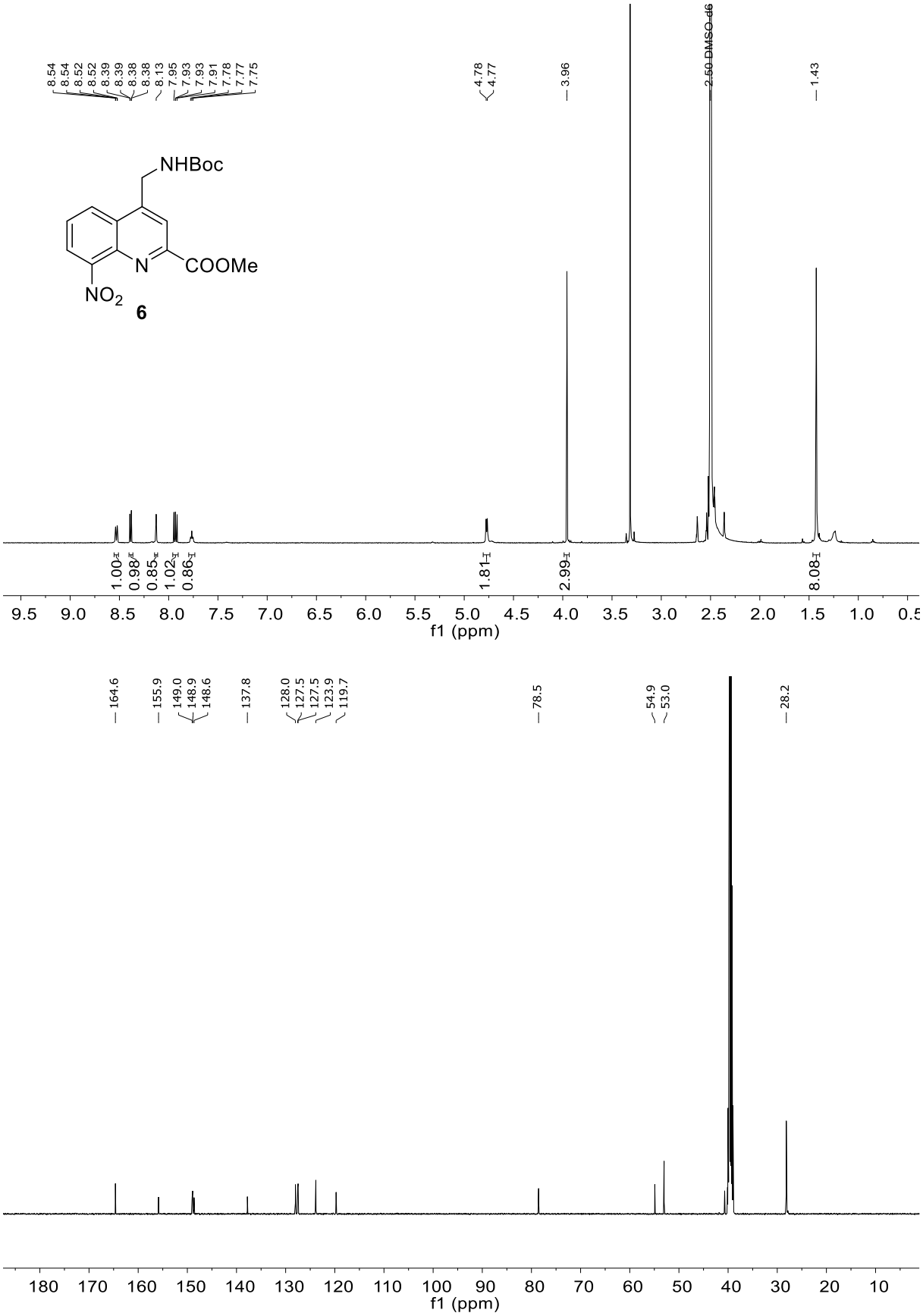


Figure S21. NMR spectra of compound **6**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (126 MHz, DMSO-d_6).

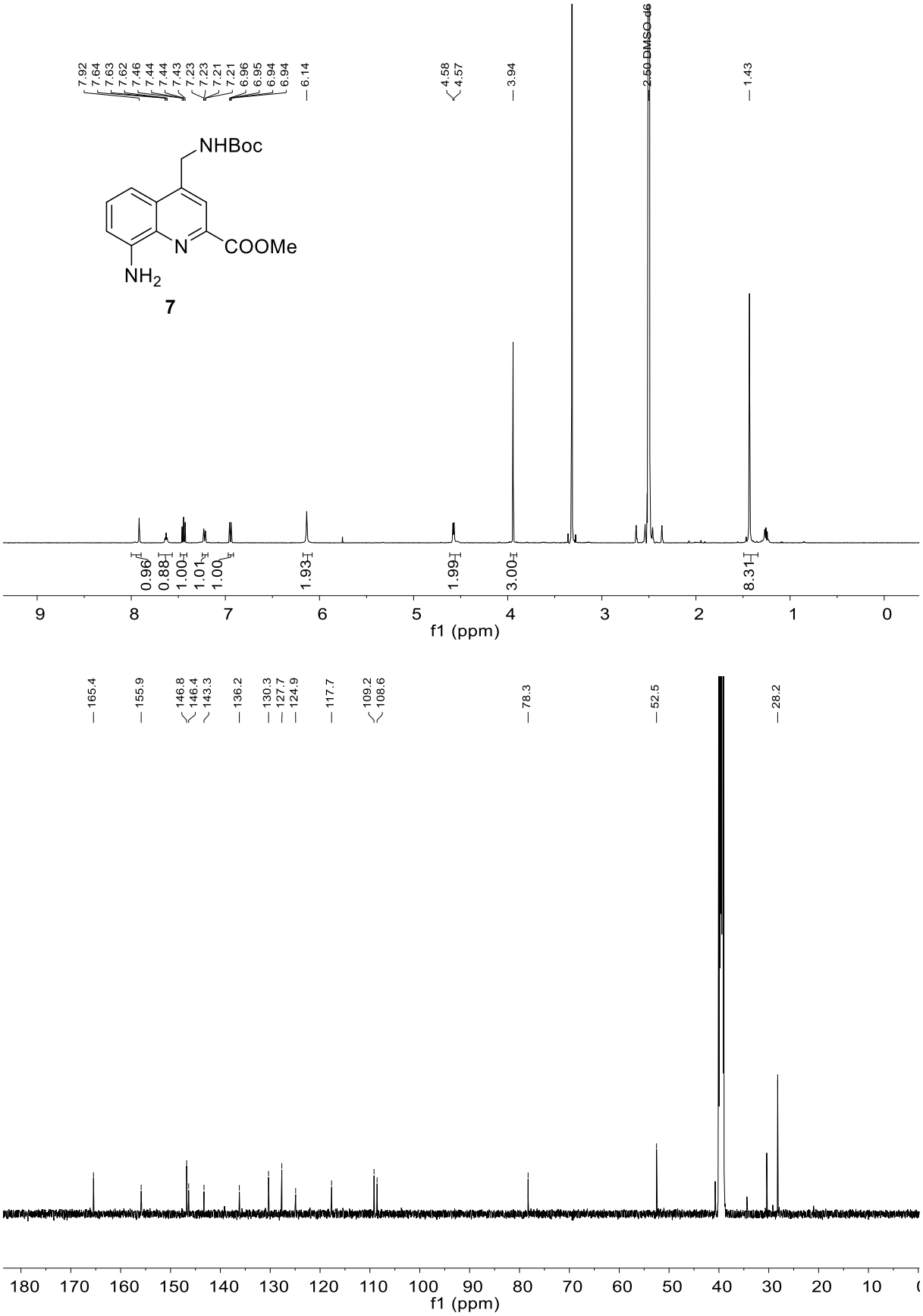


Figure S22. NMR spectra of compound **7**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (126 MHz, DMSO-d_6).

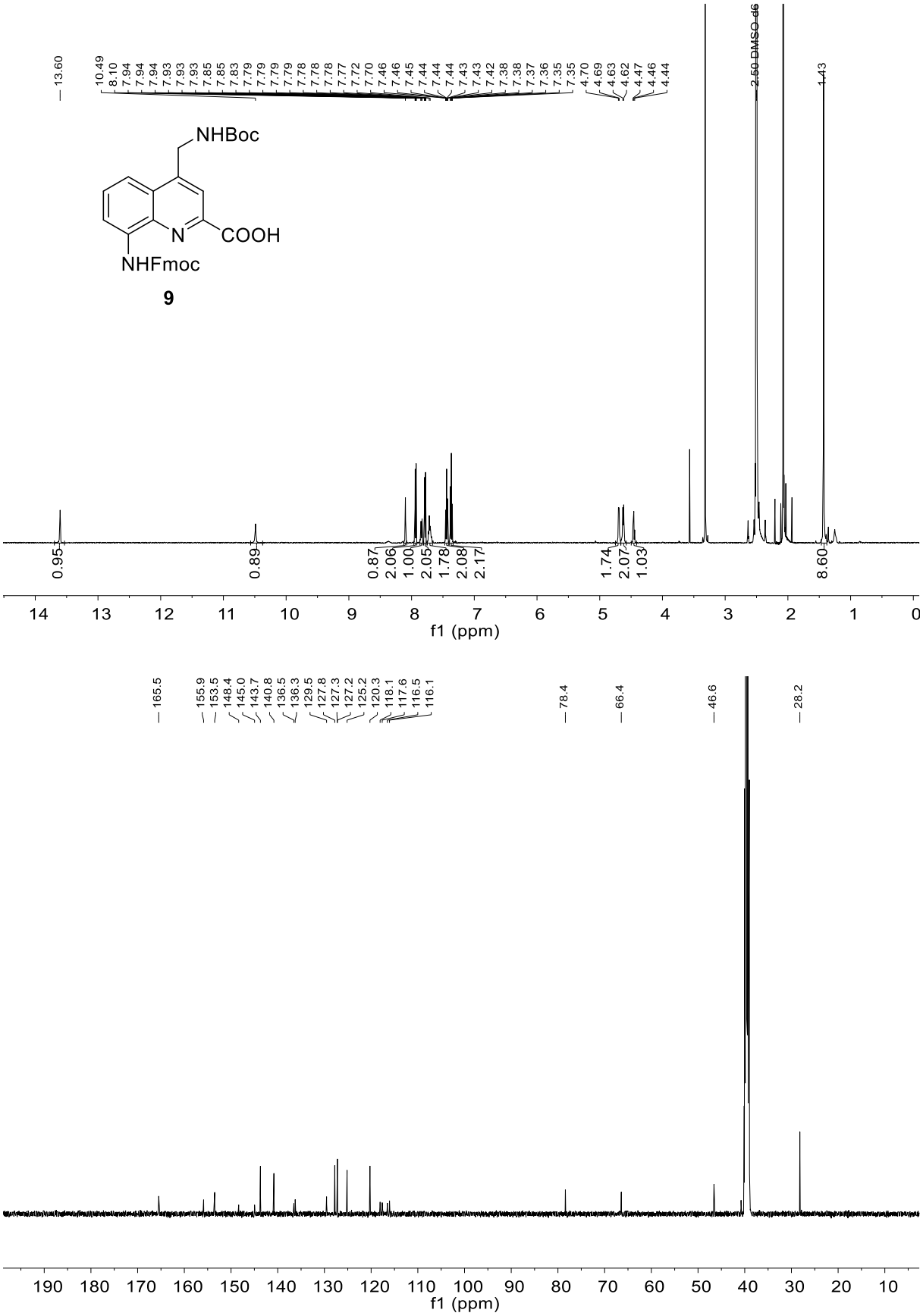


Figure S23. NMR spectra of compound **9**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (126 MHz, DMSO-d_6).

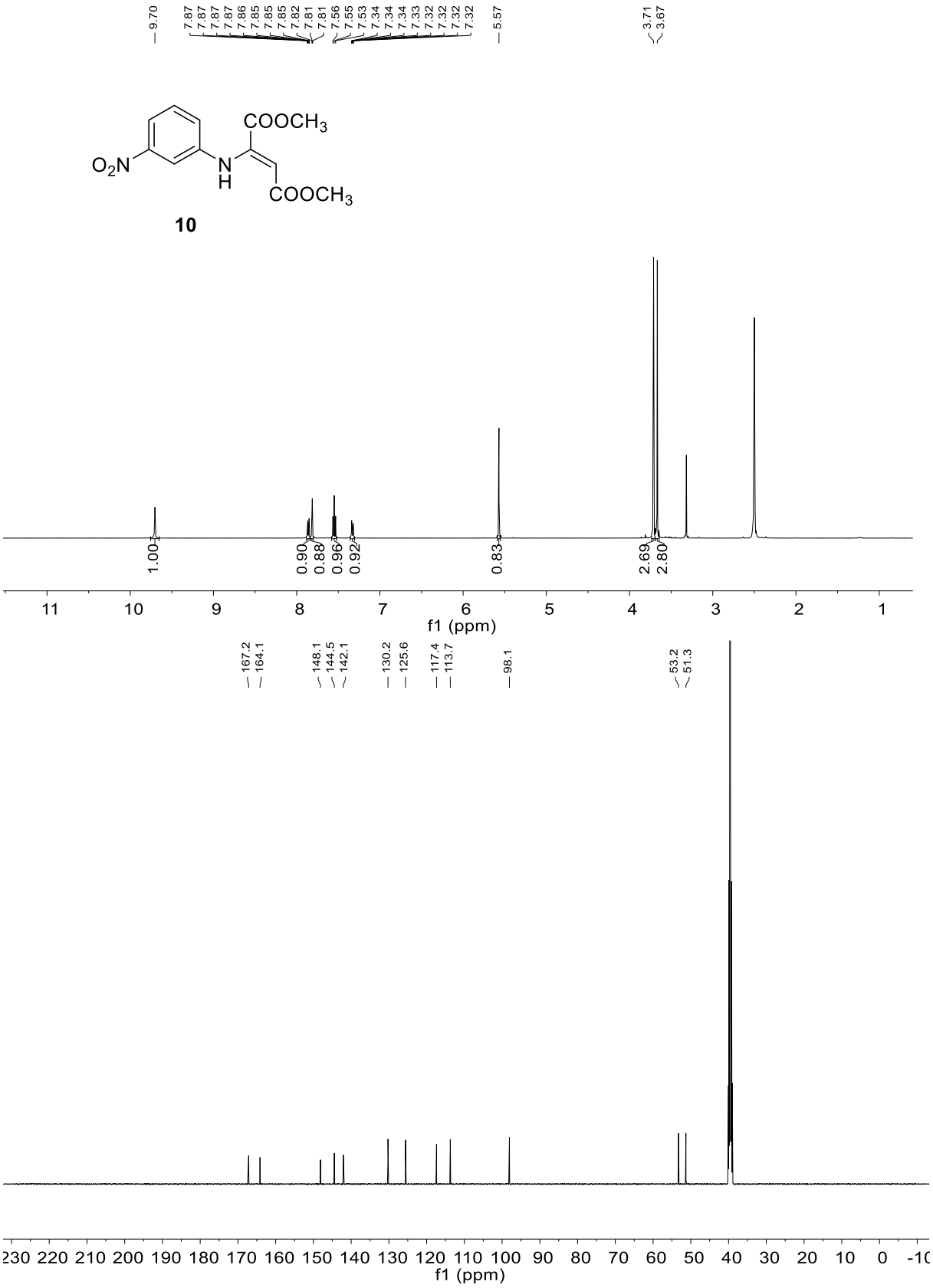


Figure S24. NMR spectra of compound **10**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (126 MHz, DMSO-d_6).

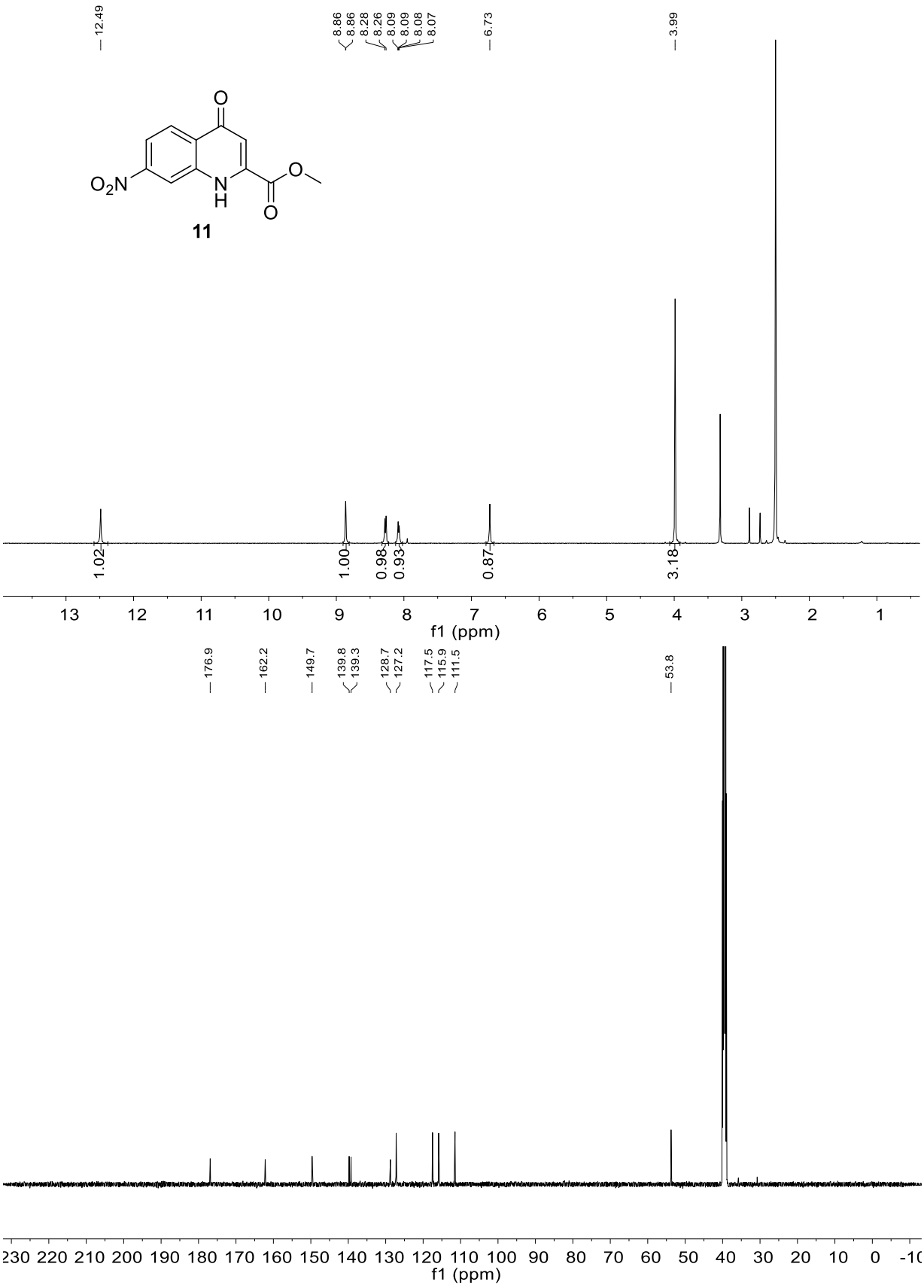


Figure S25. NMR spectra of compound **11**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (126 MHz, DMSO-d_6).

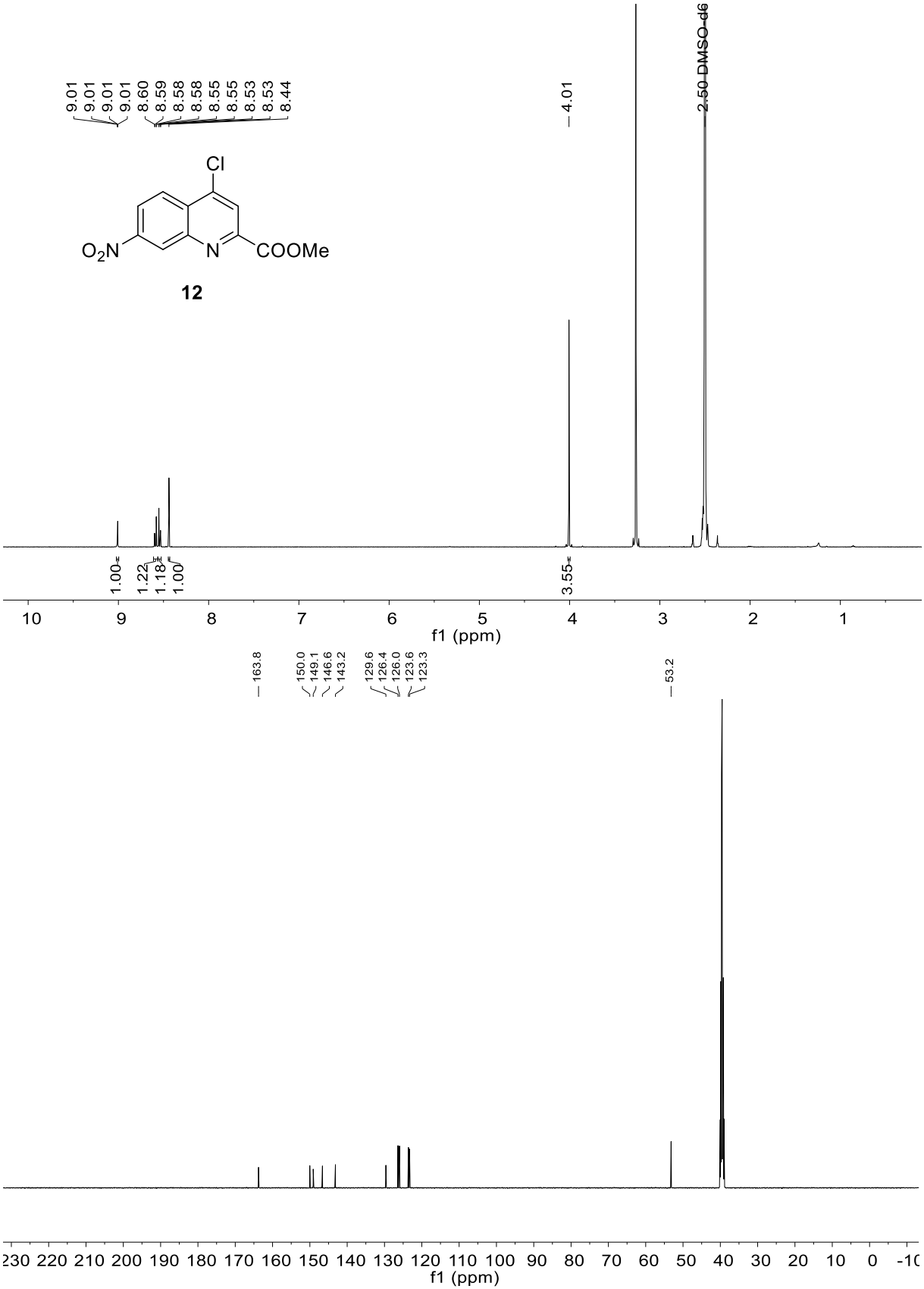


Figure S26. NMR spectra of compound **12**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (126 MHz, DMSO-d_6).

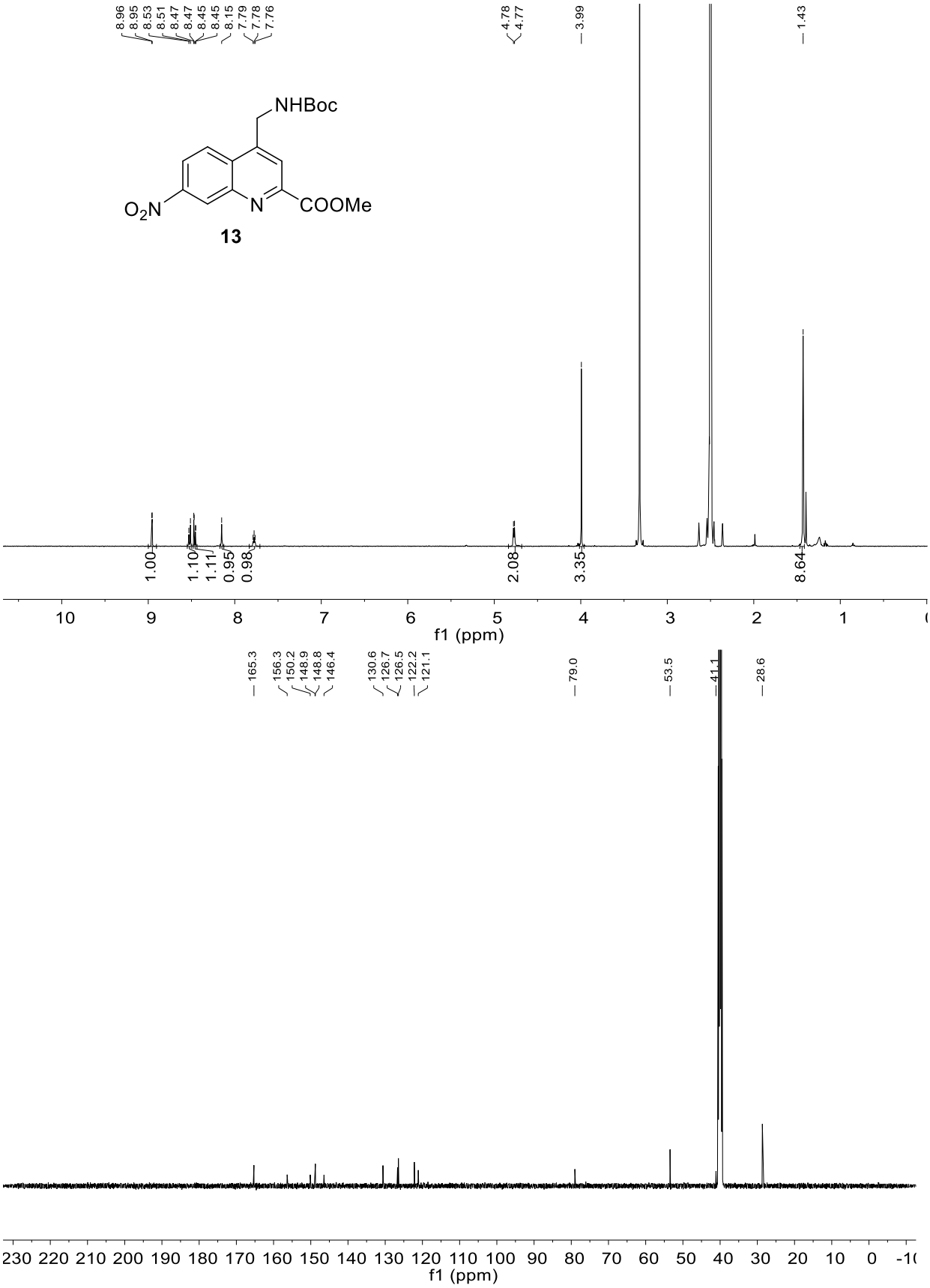


Figure S27. NMR spectra of compound **13**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (126 MHz, DMSO-d_6).

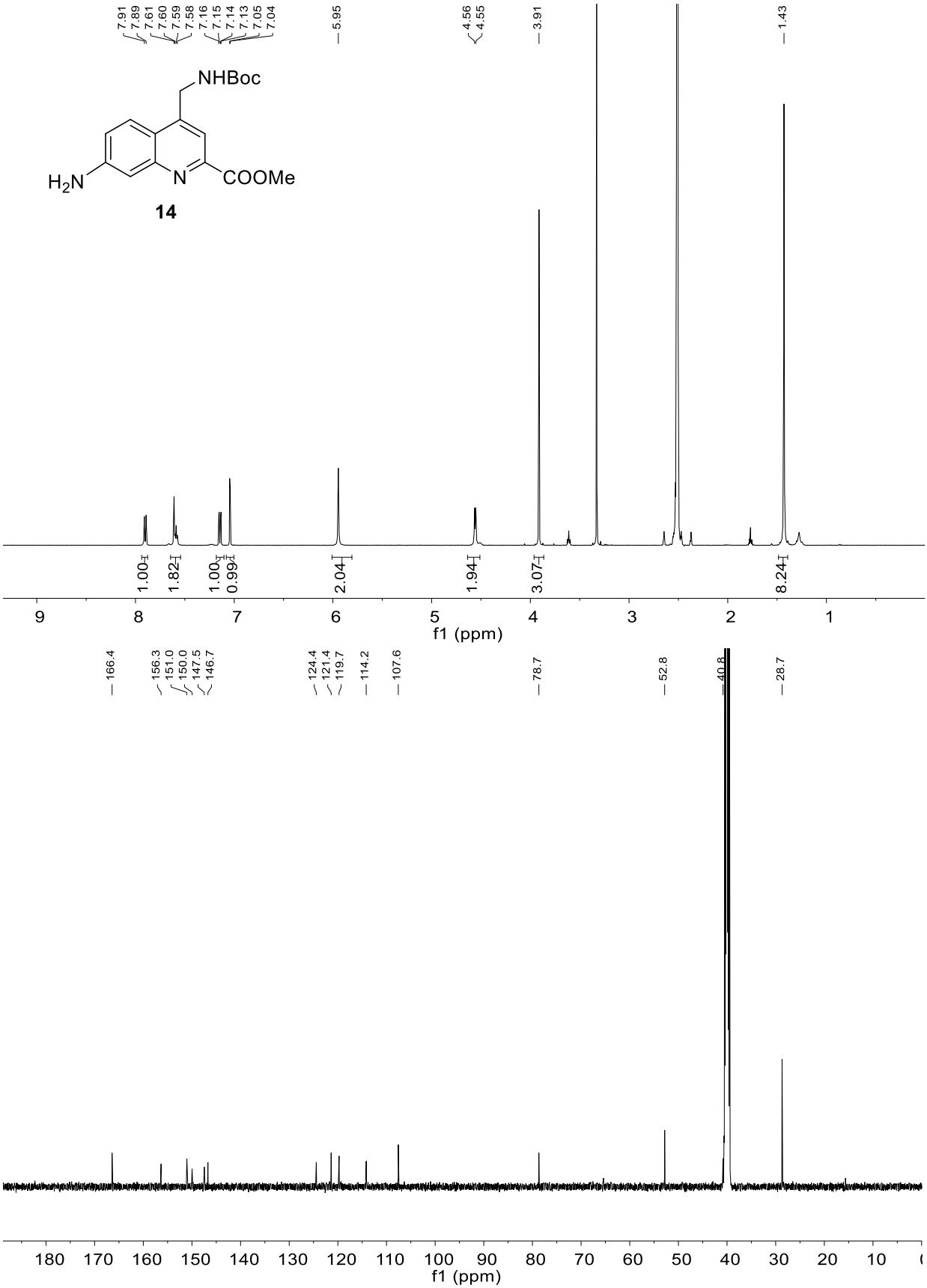


Figure S28. NMR spectra of compound **14**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (126 MHz, DMSO-d_6).

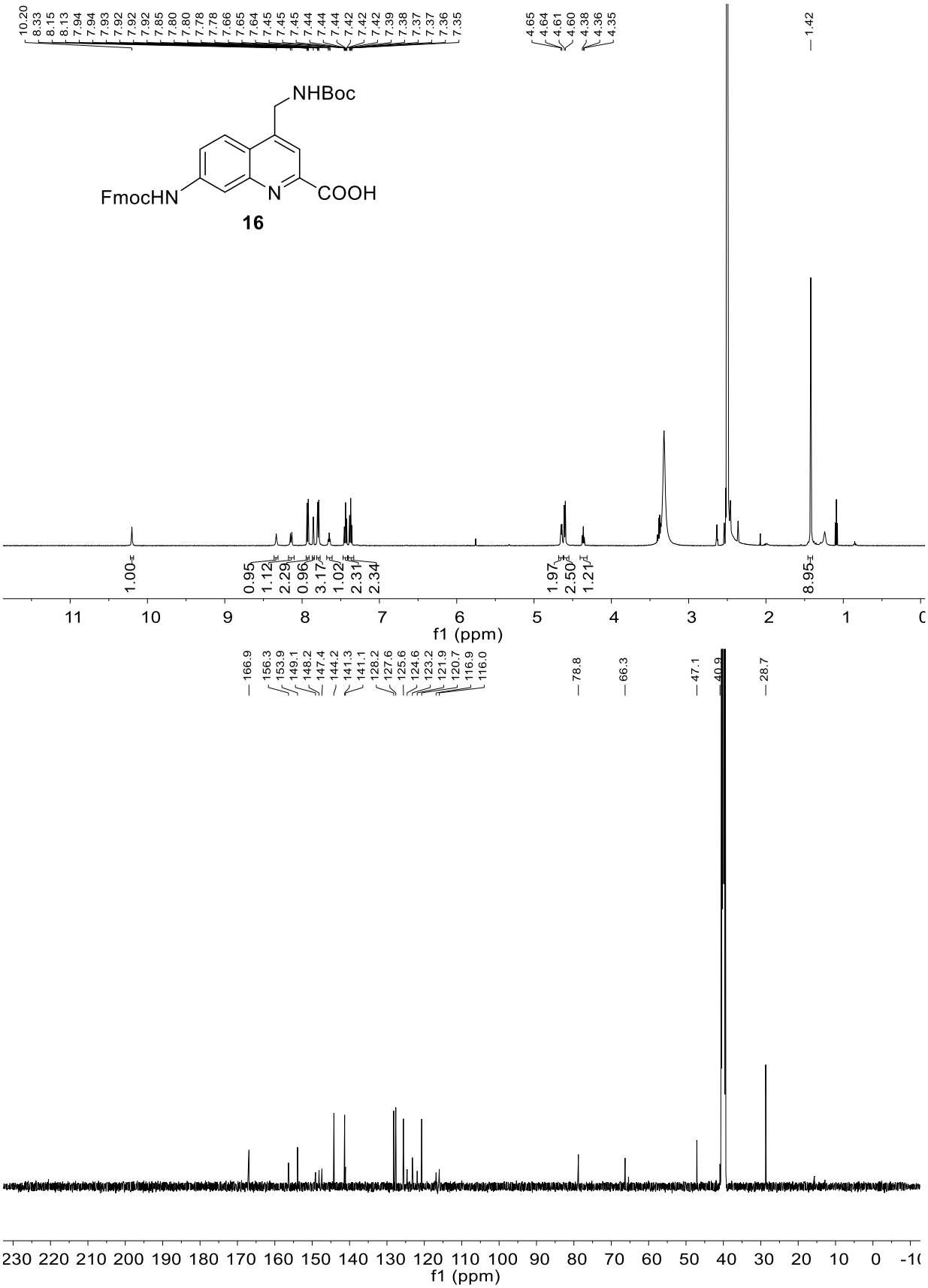


Figure S29. NMR spectra of compound **16**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (126 MHz, DMSO-d_6).

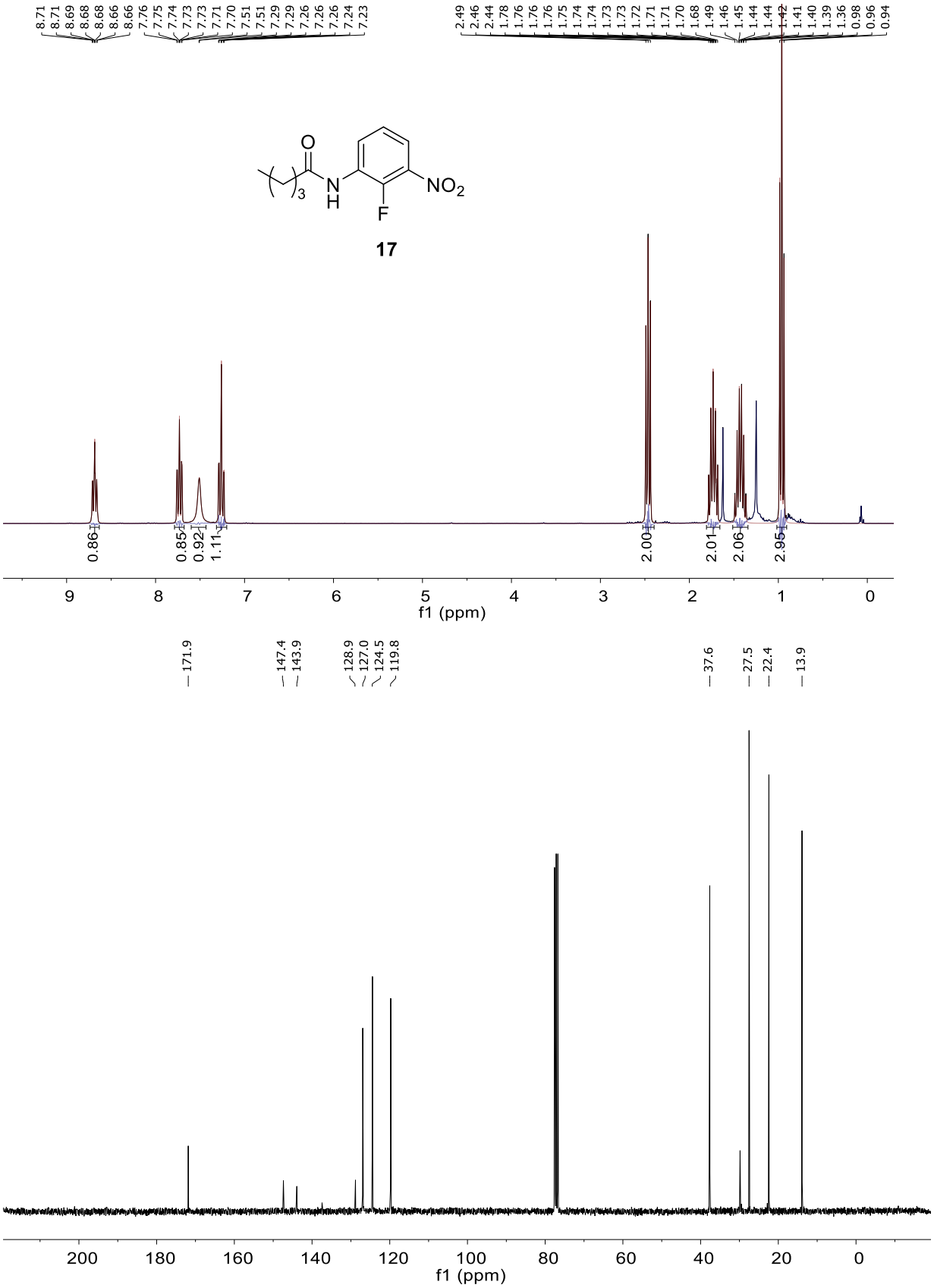


Figure S30. NMR spectra of compound **17**: ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$).

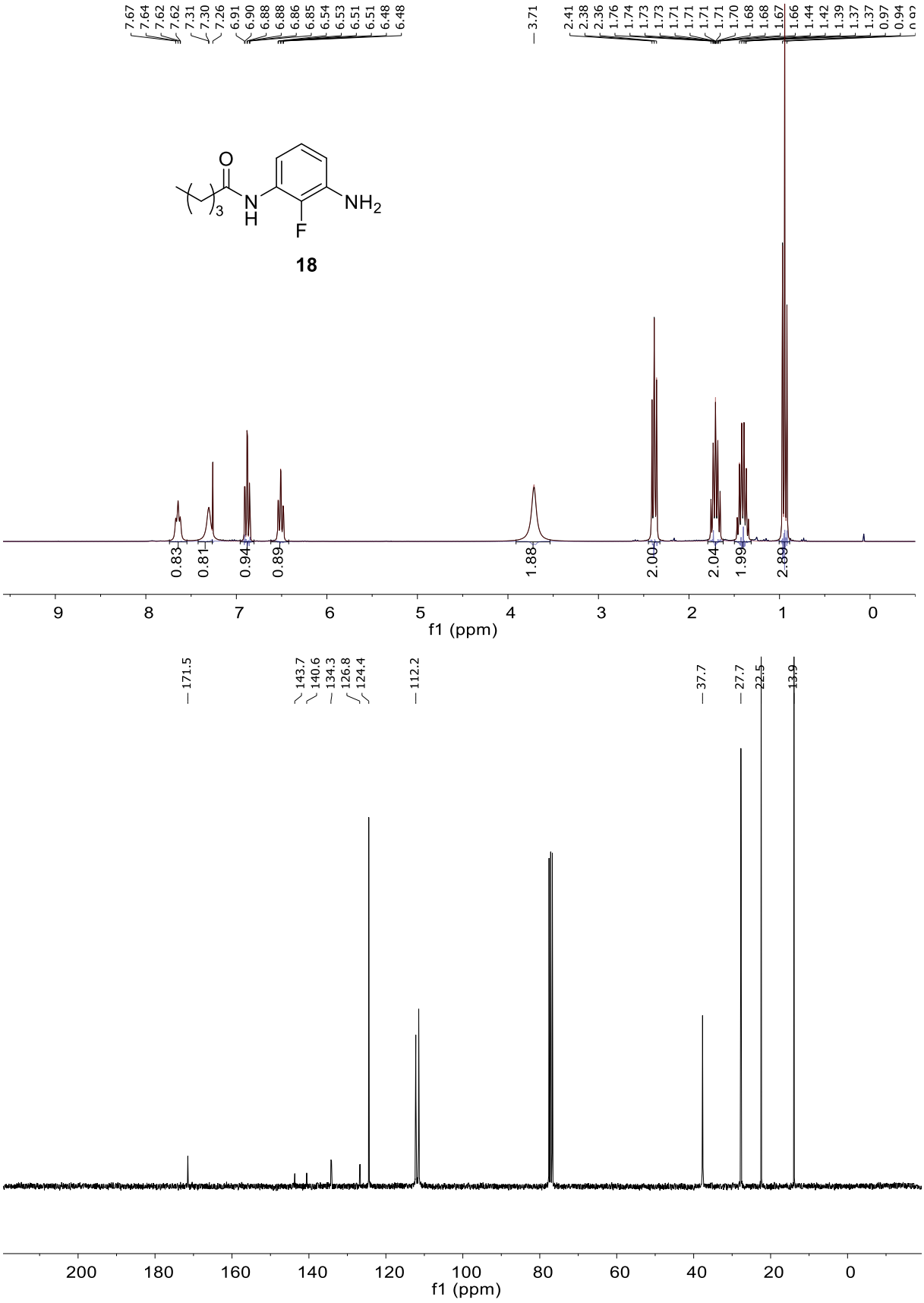


Figure S31. NMR spectra of compound **18**: ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$).

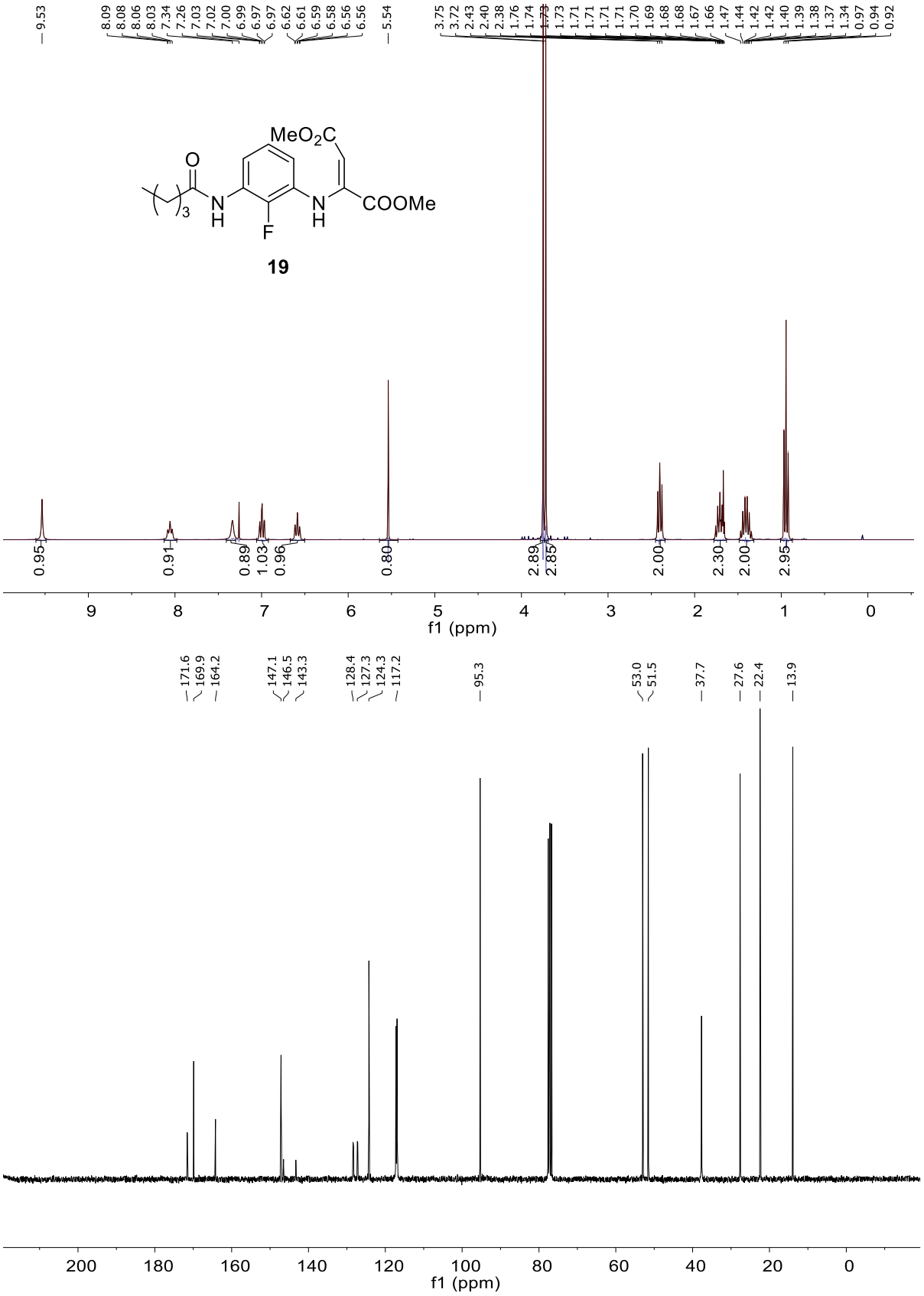


Figure S32. NMR spectra of compound **19**: ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$).

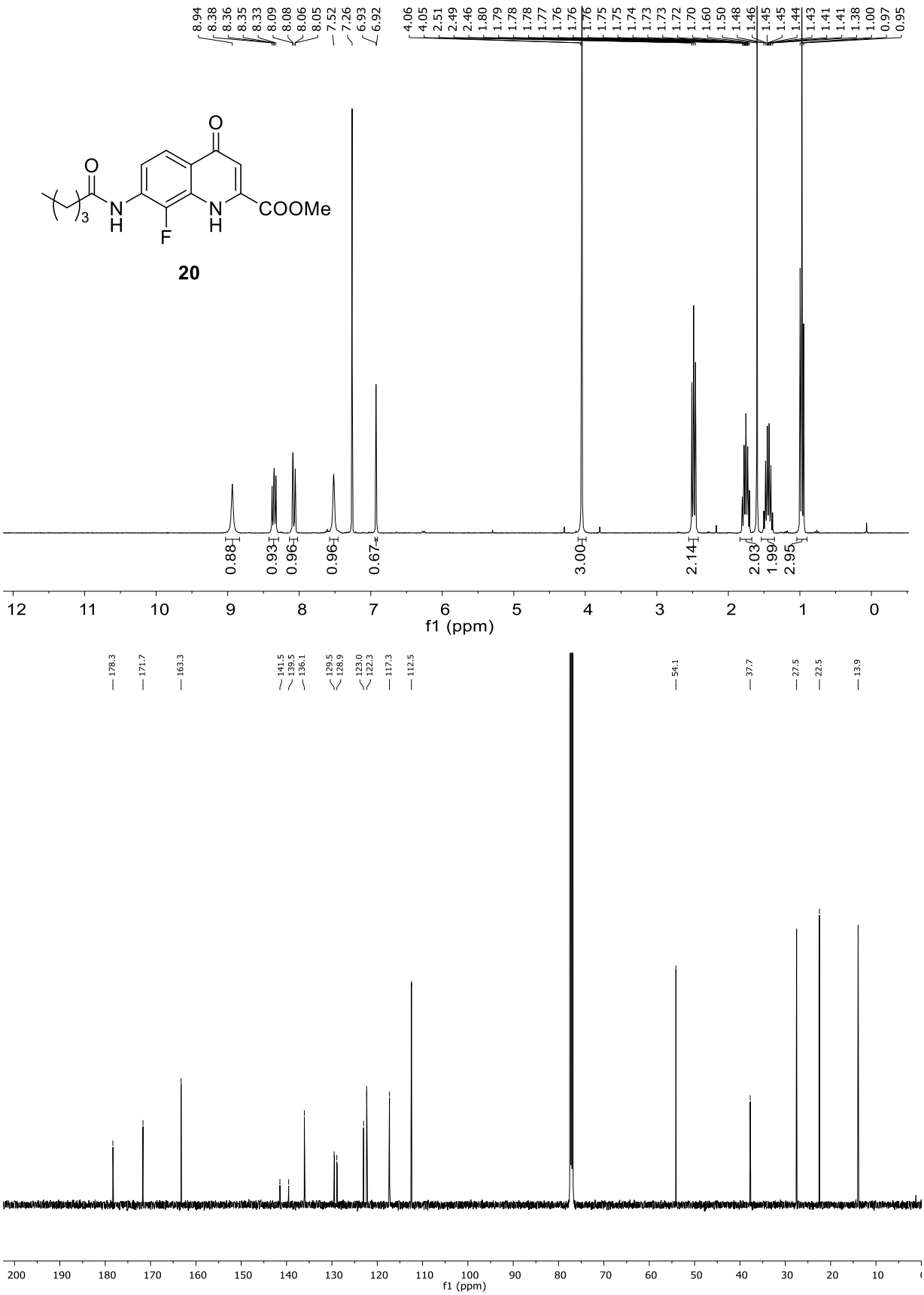


Figure S33. NMR spectra of compound **20**: ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$).

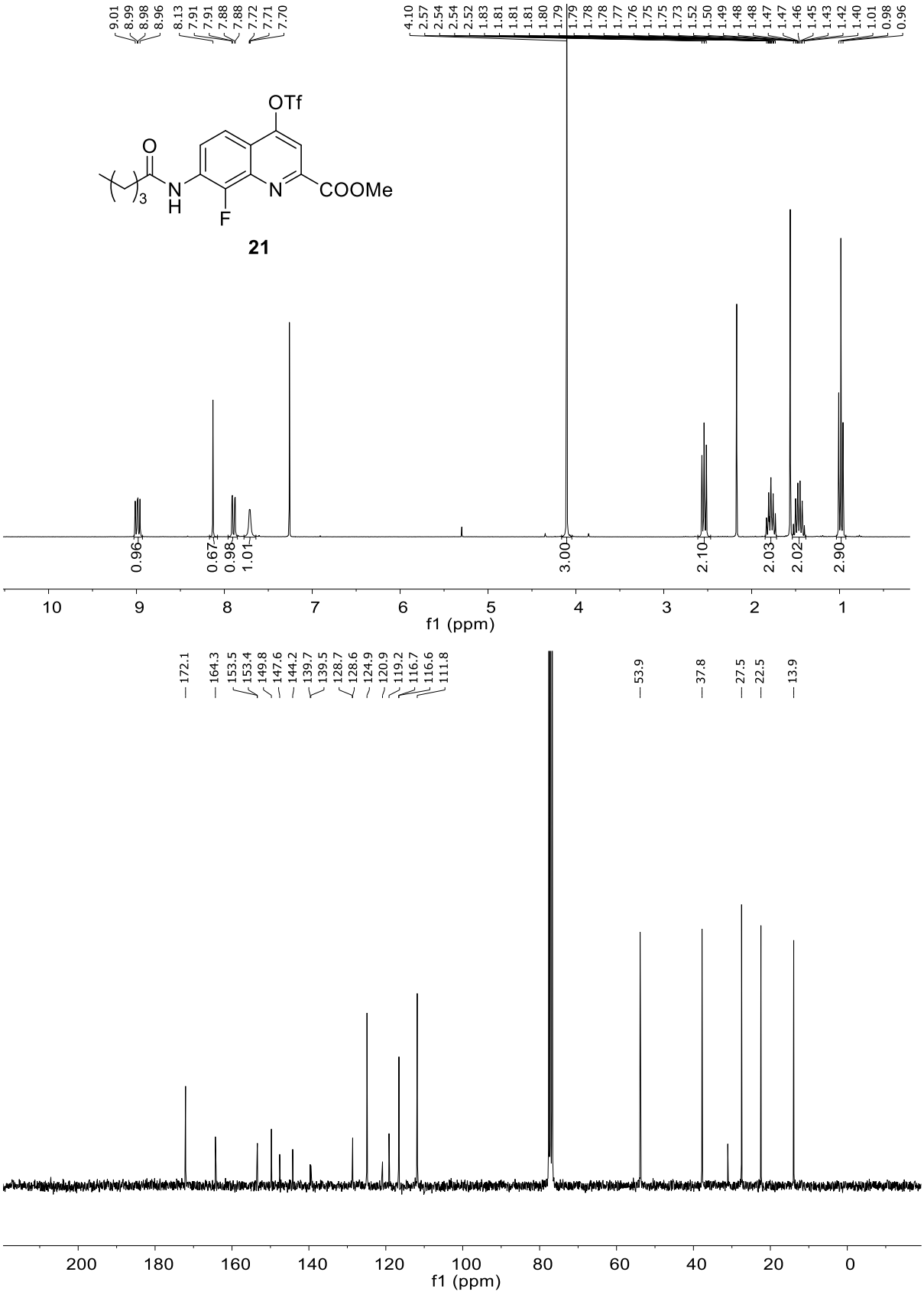


Figure S34. NMR spectra of compound **21**: ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$).

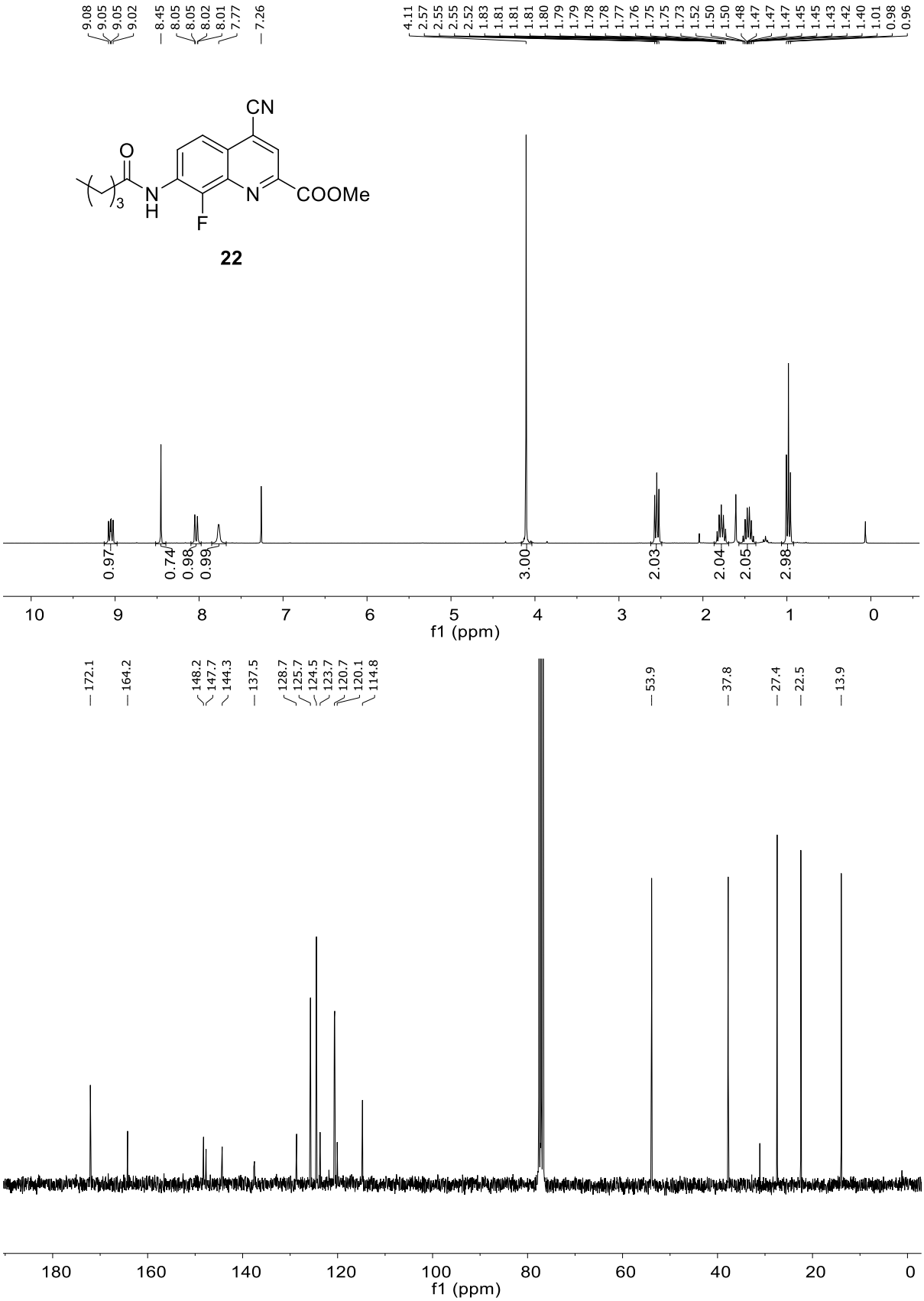


Figure S35. NMR spectra of compound **22**: ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$).

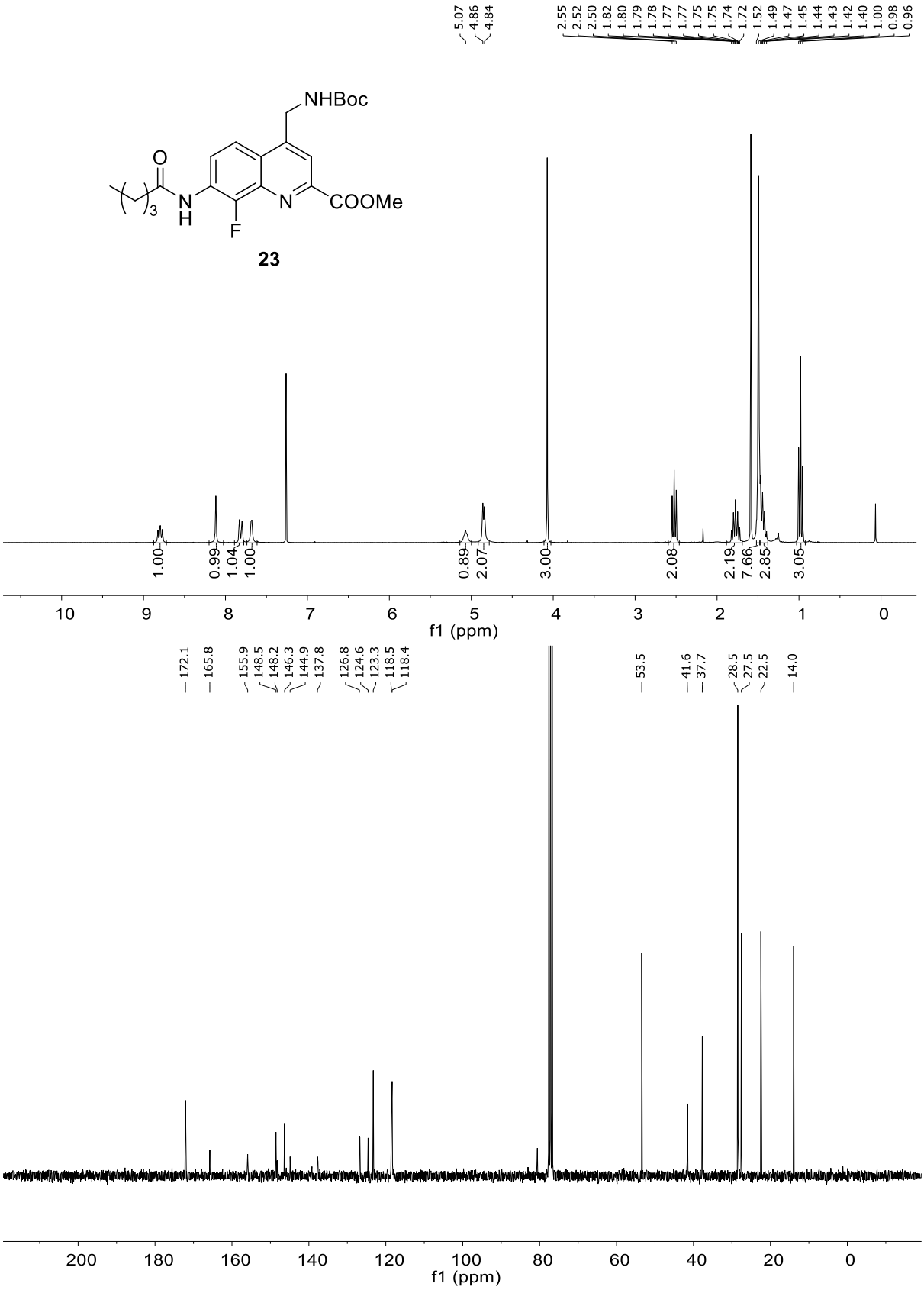


Figure S36. NMR spectra of compound **23**: ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$).

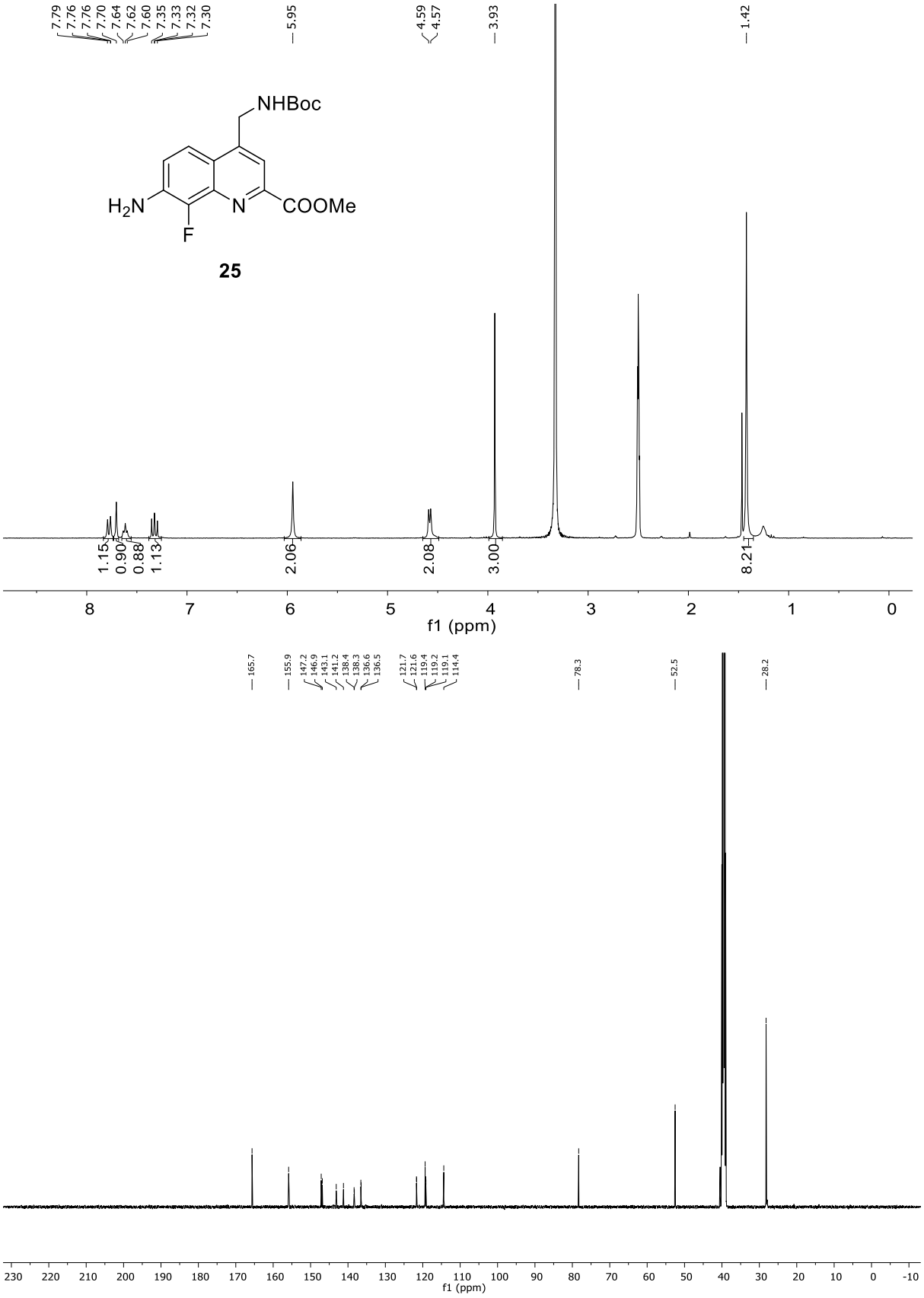


Figure S37. NMR spectra of compound **25**: ^{1}H NMR (300 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6).

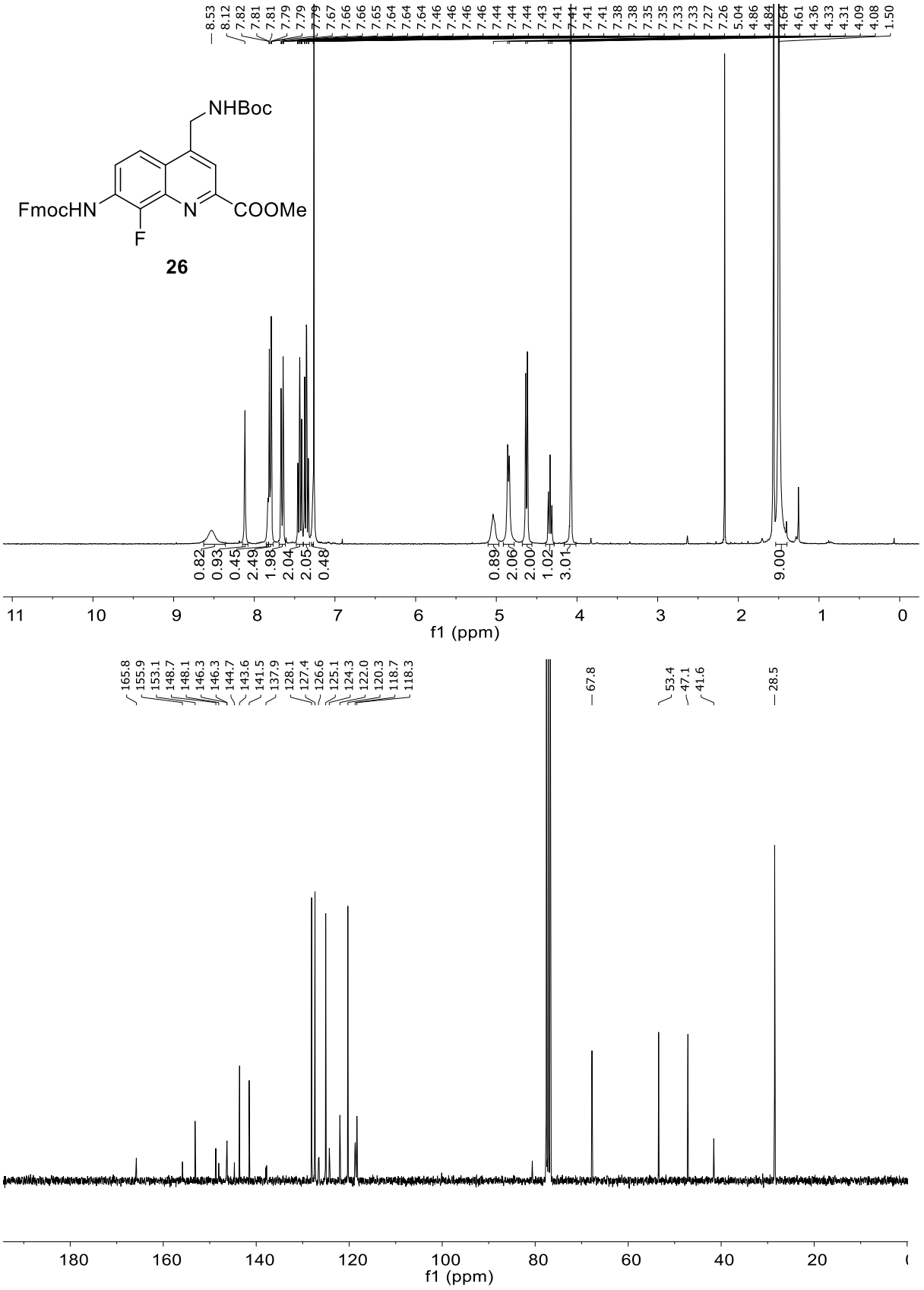


Figure S38. NMR spectra of compound **26**: ^{1}H NMR (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$).

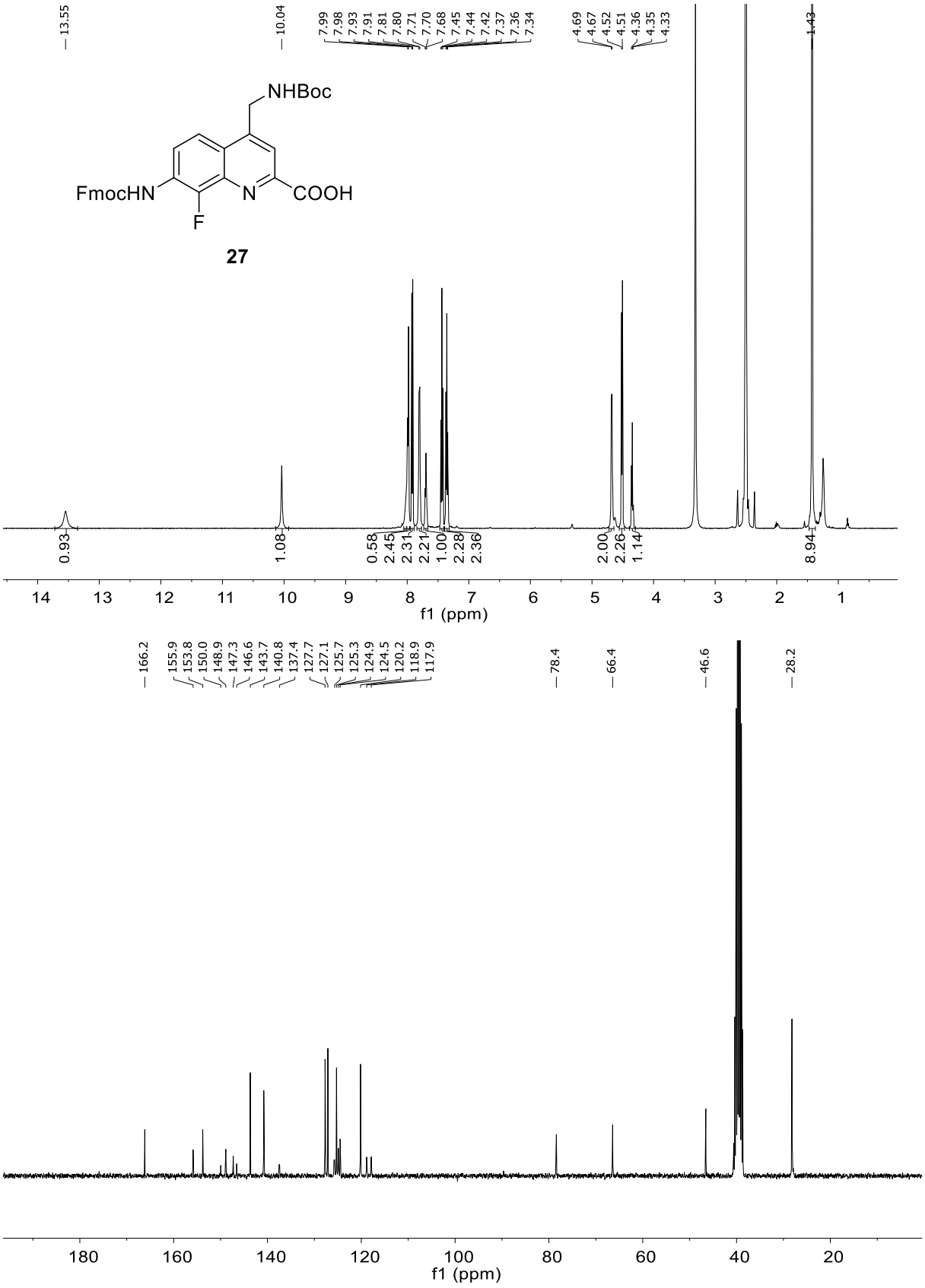


Figure S39. NMR spectra of compound **27**: ^{1}H NMR (500 MHz, DMSO-d_6) and ^{13}C NMR (75 MHz, DMSO-d_6).

5 References

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