SUPPORTING INFORMATION

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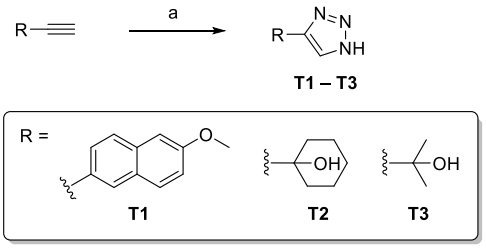
Synthetic procedures

General methods

Starting materials, reagents and solvents were purchased from commercial suppliers and used without further purification. All non-aqueous reactions were run under an inert atmosphere (nitrogen or argon) with rigid exclusion of moisture from reagents and all reaction vessels were oven-dried. Thin-layer chromatography (TLC) was carried out on pre-coated TLC plates with silica gel HSGF 254. Spots were visualized under UV at 254 nm, or stained by ninhydrin, $KMnO_4$ or phosphomolybdic acid. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a BRUKER AVANCE III 400 (or 500, 600) spectrometer or a Varian Mercury VX300 Fourier transform spectrometer using deuterated chloroform ($CDCl_3$) or DMSO (DMSO-d_6) as the solvent. Chemical shifts were reported in δ (ppm) using the δ 0.00 signal of TMS or δ 2.50 signal of DMSO-d_6 (^{1}H NMR) and the δ 77.23 signal of $CDCl_3$ or δ 39.52 signal of DMSO-d_6 (^{13}C NMR) as internal standards. Abbreviations for signal coupling are as follows: s, singlet; br, broad singlet; d, doublet; t, triplet; td, triple doublet; q, quartet; dd, double doublet; ddd, doublet of doublets of doublets; m, multiplet. Coupling constants (J) are given in Hz. Low-resolution mass data were obtained on an Agilent 6110 Single Quadrupole LC/MS System. High-resolution mass data were obtained on a MICROMASS Q-Tof Ultima™ spectrometer. The following abbreviations for solvents and reagents are used: N,N-dimethylformamide (DMF), dimethylsulfoxide (DMSO), sodium hydroxide (NaOH), dichloromethane (DCM), tetrahydrofuran (THF), ethyl acetate (EtOAc), petroleum ether (PE), N,N-diisopropylethylamine (DIPEA), 4-dimethylaminopyridine (DMAP).

Scheme S1. Synthesis of triazole building blocks



Reagents and conditions: (a) azidotrimethylsilane, $CuSO_4 \cdot 5H_2O$, ascorbic acid, DMF, 60°C, 16 hrs.

Triazole T1: The title compound was prepared according to the reported procedure with some modifications [1]. In a 50 mL round-bottom flask, 2-ethynyl-6-methoxynaphthalene (728 mg, 4.0 mmol) and azidotrimethylsilane (692 mg, 6.0 mmol) were dissolved in DMF (8.7 mL) at r.t., followed by the addition of 70 mM $CuSO_4 \cdot 5H_2O$ solution in DMF (11.5 mL, 0.80 mmol) and 199 mM ascorbic acid solution in DMF (24.1 mL, 4.8 mmol). The mixture was purged with argon three times and heated to 60°C overnight. The reaction was allowed to cool to room temperature and 0.5N HCl (20 mL) was added after removal of the solvents. Products were extracted with EtOAc (4 × 30 mL). Organic fractions were combined, washed with brine (30 mL), and dried over Na_2SO_4. After filtering and removal of the solvents, the crude material was recrystallized in hexane/EtOAc = 5/1 and hexane/EtOAc = 3/1 to give **T1** as a dark yellow solid (574 mg, 64%). ^{1}H NMR (500 MHz, DMSO-d_6): δ 14.96 (s, 1H), 8.34 (s, 1H), 8.32 (s, 1H), 7.97 (d, J = 8.5 Hz, 1H), 7.89 (dd, J = 8.6, 3.7 Hz, 2H), 7.35 (s, 1H), 7.20 (d, J = 6.8 Hz, 1H), 3.89 (s, 3H). MS [ESI+] m/z: calculated for $C_{13}H_{11}N_3O$ $[M + H]^+$ 226.1, found 226.1.

Triazole T2: The title compound was synthesized from 1-ethynylcyclohexan-1-ol (497 mg, 4.0 mmol) according to the similar procedures described for compound **T1**, and the crude product was purified by flash chromatography over silica gel (PE/EtOAc = 5/1) to give **T2** as a white solid (107 mg, 16%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.54 (br, 1H), 7.64 (s, 1H), 4.87 (br, 1H), 1.95 – 1.58 (m, 6H), 1.50 (s, 1H), 1.45 – 1.34 (m, 2H), 1.28 (t, J = 11.0 Hz, 1H). MS [ESI+] m/z: calculated for $C_8H_{13}N_3O$ $[M + H]^+$ 168.1, found 168.1.

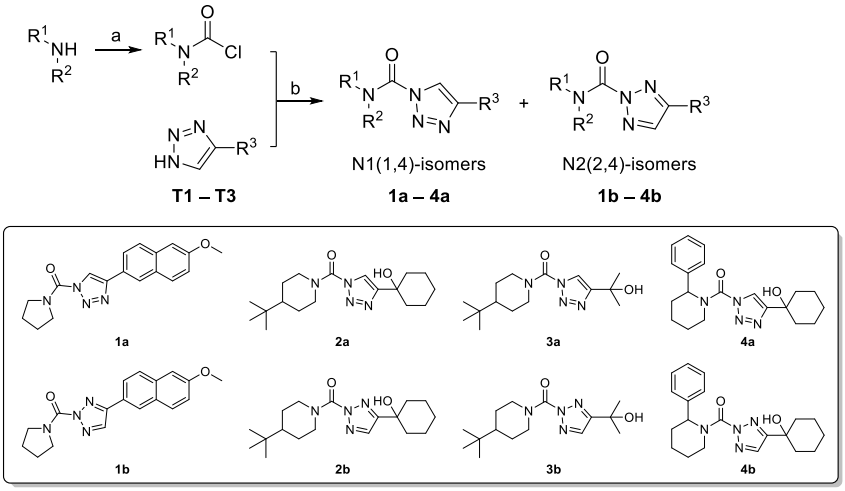
Triazole T3: The title compound was synthesized from 2-methylbut-3-yn-2-ol (337 mg, 4.0 mmol) according to the reported procedure [1] affording **T3** as a white solid (147 mg, 29%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 14.51 (br, 1H), 7.62 (s, 1H), 5.12 (s, 1H), 1.45 (s, 6H). ^{1}H NMR peaks indicated that the product **T3** (solution form in DMSO-d_6) is a mixture of three isomers with a ratio of 57:23:20. MS [ESI+] m/z: calculated for $C_5H_9N_3O$ $[M + H]^+$ 128.1, found 128.1.

Structural assignment of 4-substituted triazole urea regioisomers.

Assignment of regioisomeric structures for 4-substituted triazole ureas (**1a − 7b**) was made by ^{1}H-NMR according to the reported rules described by Adibekian et al.[2], which is the N2(2,4) regioisomer showed an upfield ^{1}H-NMR shift of the triazole ring proton and slower migration on the TLC plate relatively to the N1(1,4) regioisomer.

SUPPORTING INFORMATION

Scheme S2. Synthesis of triazole urea compounds



Reagents and conditions: (a) triphosgen, DIPEA, DCM, 0 °C, 30 mins; (b) DIPEA, DMAP, THF, 55 °C, 16 hrs.

Triazole Urea 1a: 1-pyrrolidinecarbonyl chloride was purchased from Sigma-Aldrich. Triazole **T1** (300 mg, 1.3 mmol) and TEA (673 mg, 6.7 mmol) were dissolved dry THF (8 mL), followed by the addition of DMAP (cat.) and 1-pyrrolidinecarbonyl chloride (355 mg, 2.7 mmol) at r.t., and the mixture was stirred for 16 hrs at 55 °C. The solvents were removed under vacuum to yield crude triazole urea as a mixture of N1- and N2-carbamoylated regioisomers. The N1-carbamoyl triazole was isolated by silica gel chromatography (DCM ~ DCM/MeOH = 200/1) to afford 1,4-triazole urea **1a** (57 mg, 20%) as a gray solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.55 (s, 1H), 8.33 (s, 1H), 7.91 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.81 (dd, *J* = 8.6, 2.6 Hz, 2H), 7.18 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.15 (d, *J* = 2.5 Hz, 1H), 4.10 (t, *J* = 6.6 Hz, 2H), 3.94 (s, 3H), 3.77 (t, *J* = 6.6 Hz, 2H), 2.12 – 1.96 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.35, 147.39, 146.83, 134.81, 130.01, 129.13, 127.71, 125.04, 124.99, 124.52, 120.23, 119.63, 106.01, 55.54, 50.53, 49.21, 26.77, 24.14. HRMS [ESI+] m/z: calculated for $C_{18}H_{18}N_4O_2$ $[M + Na]^+$ 345.1322, found 345.1322.

Triazole Urea 1b: The title compound was isolated from the mixture of compound **1a**. The N2-carbamoyl triazole regioisomer was purified by silica gel chromatography (DCM ~ DCM/MeOH = 200/1) to afford 2,4-triazole urea **1b** (220 mg, 51%) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.27 (d, *J* = 1.7 Hz, 1H), 8.15 (s, 1H), 7.96 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.81 (dd, *J* = 8.7, 3.2 Hz, 2H), 7.23 – 7.14 (m, 2H), 4.02 – 3.96 (m, 2H), 3.95 (s, 3H), 2.05 – 1.97 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.62, 149.48, 148.25, 135.25, 133.37, 130.02, 128.95, 127.78, 125.88, 124.69, 124.56, 119.78, 106.04, 55.56, 50.41, 48.92, 26.74, 24.36. HRMS [ESI+] m/z: calculated for $C_{18}H_{18}N_4O_2$ $[M + Na]^+$ 345.1322, found 345.1322.

Triazole Urea 2a: The title compound was prepared according to the reported procedure with minor modifications[1]**.** A solution of 4-(*tert*-butyl)piperidine hydrochloride (28 mg, 0.16 mmol) in dry THF (0.5 mL) was treated with DIPEA (0.11 mL, 0.64 mmol), triphosgen (29 mg, 0.096 mmol), and the reaction was stirred for 30 min at 0 °C. The mixture was poured into water and extracted with EtOAc (3 × 10 mL). The organic layer was washed with water, brine, dried over Na_2SO_4, filtered, and concentrated to give the carbamoyl chloride, which was used directly for the next step without further purification. The carbamoyl chloride was added to a solution of triazole **T2** (27 mg, 0.16 mmol), DIPEA (0.084 mL, 0.48 mmol), and DMAP (20 mg, 0.16 mmol) in dry THF at r.t. The mixture was stirred for 2 h at 60 °C and poured into a saturated aqueous NH_4Cl solution. The mixture was extracted with EtOAc (3 × 10 mL), washed with brine, dried over Na_2SO_4, and filtered. The solvents were removed under vacuum to yield the crude triazole urea as a mixture of N1-and N2-carbamoylated regioisomers. The N1-carbamoyl triazole was isolated by flash chromatography over silica gel (PE/EtOAc = 10/1 ~ 4/1) to afford 1,4-triazole urea **2a** (13 mg, 24%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01 (s, 1H), 4.55 (d, *J* = 13.1 Hz, 2H), 3.15-3.00 (br, 1H), 3.00 – 2.85 (br, 1H), 2.29 (s, 1H), 2.06 – 1.95 (m, 2H), 1.95 – 1.70 (m, 6H), 1.63 – 1.50 (m, 2H), 1.50 – 1.22 (m, 5H), 0.89 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.72, 148.35, 121.37, 69.69, 49.01, 46.75, 38.13, 32.51, 27.44, 26.96, 25.51, 22.10. HRMS [ESI+] m/z: calculated for $C_{18}H_{30}N_4O_2$ $[M + Na]^+$ 357.2261, found 357.2260.

Triazole Urea 2b: The title compound was isolated from the mixture of compound **2a**. The N2-carbamoyl triazole regioisomer was purified by flash chromatography over silica gel (PE/EtOAc = 10/1 ~ 4/1) to afford 2,4-triazole urea **2b** (23 mg, 44%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (s, 1H), 4.72 – 4.35 (br, 1H), 4.34 – 4.00 (br, 1H), 3.15 – 2.80 (br, 2H), 2.25 (s, 1H), 2.04 – 1.84 (m, 4H), 1.83 – 1.69 (m, 4H), 1.68 – 1.52 (m, 3H), 1.49 – 1.20 (m, 4H), 0.88 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.37, 149.37, 133.30, 69.97, 48.69, 46.78, 38.18, 32.48, 27.42, 27.15, 25.41, 21.95. HRMS [ESI+] m/z: calculated for $C_{18}H_{30}N_4O_2$ $[M + Na]^+$ 357.2261, found 357.2261.

Triazole Urea 3a: The title compound was synthesized from 4-(*tert*-butyl)piperidine hydrochloride (28 mg, 0.16 mmol) and triazole **T3** (20 mg, 0.16 mmol) according to the similar procedures described for compound **2a**, and the crude product was purified by flash chromatography over silica gel (PE/EtOAc = 10/1 ~ 3/1) to afford 1,4-triazole urea **3a** (13 mg, 27%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.00 (s, 1H), 4.54 (d, *J* = 13.1 Hz, 2H), 3.18 – 3.03 (br, 1H), 3.02

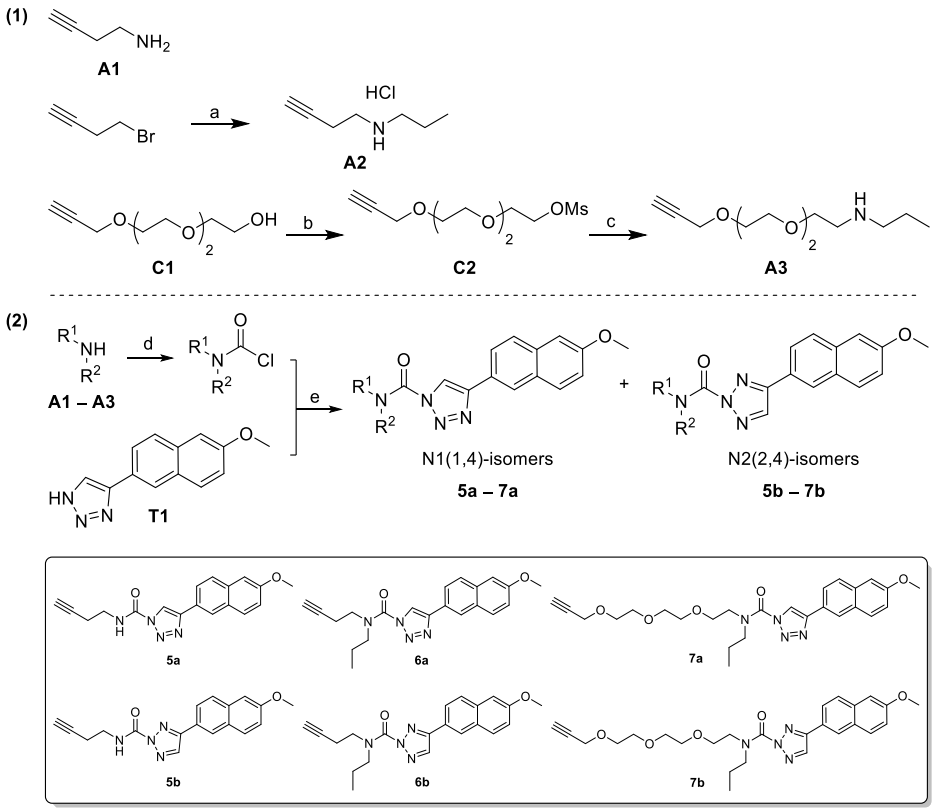
– 2.86 (br, 1H), 2.45 (s, 1H), 1.95 – 1.72 (br, 2H), 1.67 (s, 6H), 1.52 – 1.21 (m, 3H), 0.89 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.76, 148.31, 120.86, 68.63, 49.02, 46.74, 32.51, 30.45, 27.43, 26.88. HRMS [ESI+] m/z: calculated for $C_{15}H_{26}N_4O_2$ $[M + Na]^+$ 317.1948, found 317.1946.

Triazole Urea 3b: The title compound was isolated from the mixture of compound **3a**. The N2-carbamoyl triazole regioisomer was purified by flash chromatography over silica gel (PE/EtOAc = 10/1 ~ 3/1) to afford 2,4-triazole urea **3b** (20 mg, 43%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (s, 1H), 4.73 – 4.36 (br, 1H), 4.31 – 4.00 (br, 1H), 3.10 – 2.80 (br, 2H), 2.45 (s, 1H), 1.90 – 1.68 (m, 2H), 1.65 (s, 6H), 1.51 – 1.21 (m, 3H), 0.89 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.37, 149.33, 132.95, 68.93, 48.73, 46.78, 32.49, 30.56, 27.43, 26.79. HRMS [ESI+] m/z: calculated for $C_{15}H_{26}N_4O_2$ $[M + Na]^+$ 317.1948, found 317.1949.

Triazole Urea 4a: The title compound was synthesized from 2-phenylpiperidine (32 mg, 0.20 mmol) and triazole **T2** (33 mg, 0.20 mmol) according to the similar procedures described for compound **2a**, and the crude product was purified by flash chromatography over silica gel (PE/EtOAc = 10/1 ~ 4/1) to afford 1,4-triazole urea **4a** (18 mg, 25%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (s, 1H), 7.45 – 7.31 (m, 4H), 7.31 – 7.26 (m, 1H), 5.86 (s, 1H), 4.29 (d, *J* = 14.0 Hz, 1H), 3.13 (t, *J* = 13.2 Hz, 1H), 2.50 (d, *J* = 14.0 Hz, 2H), 2.11 (t, *J* = 13.8 Hz, 1H), 2.05 – 1.47 (m, 13H), 1.46 – 1.29 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.91, 149.74, 138.08, 129.11, 127.32, 126.75, 121.57, 69.65, 56.48, 43.91, 38.06, 27.96, 25.94, 25.48, 22.05, 19.47. HRMS [ESI+] m/z: calculated for $C_{20}H_{26}N_4O_2$ $[M + Na]^+$ 377.1948, found 377.1946.

Triazole Urea 4b: The title compound was isolated from the mixture of compound **4a**. The N2-carbamoyl triazole regioisomer was purified by flash chromatography over silica gel (PE/EtOAc = 10/1 ~ 4/1) to afford 2,4-triazole urea **4b** (31 mg, 44%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (s, 1H), 7.45 – 7.33 (m, 4H), 7.32 – 7.25 (m, 1H), 5.67 (s, 1H), 4.04 (s, 1H), 3.06 (t, *J* = 13.2 Hz, 1H), 2.48 (d, *J* = 14.5 Hz, 2H), 2.09 (t, *J* = 13.8 Hz, 1H), 2.01 – 1.47 (m, 13H), 1.46 – 1.20 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.49, 150.67, 138.18, 133.60, 128.97, 127.19, 126.91, 69.91, 56.28, 43.54, 38.11, 38.07, 27.80, 25.80, 25.36, 21.91, 19.53. HRMS [ESI+] m/z: calculated for $C_{20}H_{26}N_4O_2$ $[M + Na]^+$ 377.1948, found 377.1947.

Scheme S3. Synthesis of activity-based probes **5a** – **7b**



Reagents and conditions: (a) i) $PrNH_2$, K_2CO_3, MeCN, 70°C, 16 hrs; ii) HCl/EtOAc; (b) methanesulfonyl chloride, TEA, DCM, 0 °C ~ r.t., 2 hrs; (c) $PrNH_2$·HCl, TEA, MeCN, 60°C, 48 hrs; (d) triphosgen, DIPEA, DCM, 0 °C, 30 mins; (e) DIPEA, DMAP, THF, 55 °C, 16 hrs.

Compound A2: In a 15 mL cylindrical Bottom pressure vessel (Synthware), 4-Bromo-1-butyne (500 mg, 3.8 mmol), propylamine (267 mg, 4.5 mmol) were dissolved in MeCN (5 mL), then K_2CO_3 (1.04g, 7.52 mmol) was added. The mixture was stirred at 70 °C for 16 hrs. Then the mixture was poured into water (20 mL), extracted with DCM (3 × 10 mL) and concentrated. The crude product was purified by flash chromatography over silica gel (DCM ~ DCM/MeOH = 10 /1). The eluent containing the product was salified with 1N HCl/EtOAc (3 mL) and concentrated in vacuum. The solid was then

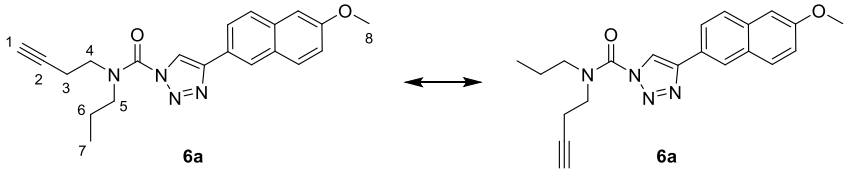
recrystallized in Et_2O to give compound **A2** (170 mg, 30.6%) as a light orange solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.66 (s, 2H), 3.22 – 3.10 (m, 2H), 3.03 – 2.93 (m, 2H), 2.89 (td, *J* = 7.8, 2.2 Hz, 2H), 2.12 (t, *J* = 2.5 Hz, 1H), 2.00 – 1.86 (m, 2H), 1.04 (t, *J* = 7.4 Hz, 3H). MS [ESI+] m/z: calculated for $C_7H_{13}N$ $[M + H]^+$ 112.1, found 112.1.

Compound A3: The Intermediate **C1** was prepared according to the reported procedure[3]. In a 25 mL round bottom flask, **C1** (377 mg, 2.0 mmol) was dissolved in DCM (12 mL), followed by the addition of TEA (0.84 mL, 6.0 mmol) and methanesulfonyl chloride (0.31 mL, 4.0 mmol) at 0 °C. The mixture was allowed to stirred at r.t. for 2 hrs. After the completion of the reaction monitored by TLC, the reaction mixture was poured into water (20 mL), extracted with EtOAc (3 × 15 mL), and washed with brine. The organic phase was dried over Na_2SO_4, filtered and concentrated to yield the methanesulfonate **C2** as a brown oil. In a 15 mL cylindrical Bottom pressure vessel (Synthware), **C2** (150 mg, 0.56 mmol) was added to a solution of $PrNH_2$·HCl (269 mg, 2.8 mmol) and TEA (0.12 mL, 0.85 mmol) in MeCN (5.6 mL) and heated to 60 °C for 2 days. The reaction was allowed to cool to r.t. and all the solvent was removed under vacuum. The residue was dissolved in water (5 mL) and made basic by addition of 1 N NaOH (pH = 9) and extracted with DCM (3 × 10 mL). The organic extracts were dried over Na_2SO_4, filtered and concentrated under vacuum. The crude product was purified by flash chromatography over silica gel (PE/EtOAc = 5/1 ~ 1/1) to give **A3** as a colorless oil (70 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.21 (d, *J* = 2.4 Hz, 2H), 3.77 – 3.57 (m, 10H), 2.81 (t, *J* = 5.2 Hz, 2H), 2.60 (t, *J* = 7.2 Hz, 2H), 2.50-2.40 (br, 1H), 2.45 (d, *J* = 2.4 Hz, 1H), 1.53 (q, *J* = 7.4 Hz, 2H), 0.92 (t, *J* = 7.4 Hz, 3H). MS [ESI+] m/z: calculated for $C_{12}H_{23}NO_3$ $[M + H]^+$ 230.2, found 230.2.

Probe 5a: The title compound was synthesized from 1-amino-3-butyne **A1** (14 mg, 0.20 mmol) and triazole **T1** (23 mg, 0.10 mmol) according to the similar procedures described for compound **2a**, and the crude product was purified by silica gel chromatography (hexane/EtOAc = 7/1 ~ 2/1) to afford probe **5a** (13 mg, 41%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.37 (t, *J* = 5.9 Hz, 1H), 9.19 (s, 1H), 8.50 (s, 1H), 8.06 (dd, *J* = 8.5, 1.7 Hz, 1H), 7.91 (d, *J* = 8.7 Hz, 2H), 7.36 (d, *J* = 2.6 Hz, 1H), 7.21 (dd, *J* = 8.9, 2.5 Hz, 1H), 3.89 (s, 3H), 3.48 (q, *J* = 6.8 Hz, 2H), 2.89 (t, *J* = 2.6 Hz, 1H), 2.53 (td, *J* = 7.1, 2.7 Hz, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 157.70, 147.29, 147.20, 134.23, 129.68, 128.42, 127.43, 124.71, 124.24, 124.21, 119.30, 119.23, 106.03, 81.54, 72.48, 55.24, 39.21, 18.48. HRMS [ESI+] m/z: calculated for $C_{18}H_{16}N_4O_2$ $[M + Na]^+$ 343.1165, found 343.1164.

Probe 5b: The title compound was isolated from the mixture of compound **5a**. The N2-carbamoyl triazole regioisomer was purified by silica gel chromatography (hexane/EtOAc = 7/1 ~ 2/1) to afford probe **5b** (9.8 mg, 30%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.20 (d, *J* = 1.7 Hz, 1H), 8.10 (s, 1H), 7.88 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.74 (dd, *J* = 8.7, 5.3 Hz, 2H), 7.44 (t, *J* = 6.2 Hz, 1H), 7.17 – 7.05 (m, 2H), 3.87 (s, 3H), 3.63 (q, *J* = 6.4 Hz, 2H), 2.54 (td, *J* = 6.5, 2.7 Hz, 2H), 2.03 (t, *J* = 2.6 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.81, 150.75, 147.80, 135.45, 134.65, 130.09, 128.92, 127.89, 126.20, 124.60, 124.04, 119.93, 106.07, 80.93, 70.88, 55.59, 39.69, 19.77. HRMS [ESI+] m/z: calculated for $C_{18}H_{16}N_4O_2$ $[M + Na]^+$ 343.1165, found 343.1167.

Probe 6a: The title compound was synthesized from secondary amine **A2** (33 mg, 0.22 mmol) and triazole **T1** (25 mg, 0.11 mmol) according to the similar procedures described for compound **2a**, and the crude product was purified by silica gel chromatography (hexane/EtOAc = 10/1 ~ 5/1) to afford probe **6a** (14 mg, 34%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.47 (s, 1H), 8.33 (s, 1H), 7.90 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.80 (dd, *J* = 8.7, 5.9 Hz, 2H), 7.18 (dd, *J* = 8.9, 2.5 Hz, 1H), 7.15 (d, *J* = 2.5 Hz, 1H), 3.94 (s, 4H, 8-CH_3, 4-CH), 3.79 (s, 1H, 5-CH), 3.72 (s, 1H, 4-CH), 3.59 (s, 1H, 5-CH), 2.71 (s, 2H, 3-CH_2), 2.04 (d, *J* = 16.0 Hz, 1H, 1-CH), 1.88 – 1.76 (m, 2H, 6-CH_2), 1.07 – 0.88 (m, 3H, 7-CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.37, 149.17, 146.99, 134.82, 130.00, 129.10, 127.74, 124.99, 124.86, 124.43, 121.00, 119.66, 106.00, 80.94 (C2), 70.87 (C1), 70.66 (C1), 55.54 (C8), 52.67 (C5), 51.71 (C5), 48.60 (C4), 22.26 (C6), 20.60 (C6), 19.39 (C3), 17.46 (C3), 11.40 (C7), 11.16 (C7). The resonance peaks in the high field region (δ < 5.0 in ^{1}H NMR; δ < 100 in ^{13}C NMR) were assigned from the COSY and HSQC spectra, indicating the cis-trans isomerization of the urea group in the probe **6a**, and this phenomenon was also observed in the ^{13}C NMR spectra of probes **6b**, **7a** and **7b**. HRMS [ESI+] m/z: calculated for $C_{21}H_{22}N_4O_2$ $[M + Na]^+$ 385.1635, found 385.1628.



6a **6a**

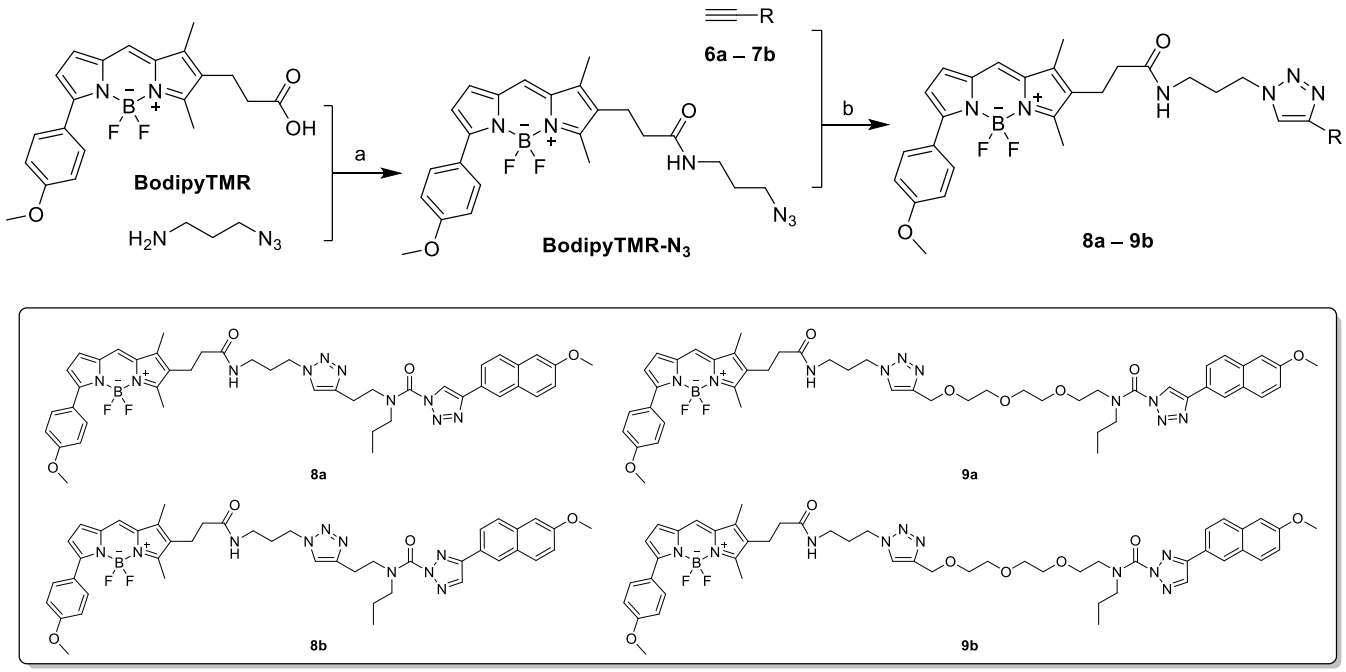
Probe 6b: The title compound was isolated from the mixture of compound **6a**. The N2-carbamoyl triazole regioisomer was purified by silica gel chromatography (hexane/EtOAc = 10/1 ~ 5/1) to afford probe **6b** (14 mg, 33%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (s, 1H), 8.17 (s, 1H), 7.95 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.81 (dd, *J* = 8.7, 5.4 Hz, 2H), 7.20 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.16 (d, *J* = 2.5 Hz, 1H), 3.95 (s, 3H), 3.74 (t, *J* = 7.3 Hz, 2H), 3.58 (t, *J* = 7.5 Hz, 2H), 2.76 (br, 2H), 2.05 (t, *J* = 2.7 Hz, 1H), 1.83 (q, *J* = 7.5 Hz, 2H), 0.97 (br, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.68, 150.13, 149.62, 135.29, 133.48, 130.05, 128.98, 127.85, 125.95, 124.64, 124.45, 119.83, 106.07, 81.13, 70.60, 55.58, 52.60, 51.38, 48.58, 22.23, 20.70, 19.26, 17.54, 11.35. HRMS [ESI+] m/z: calculated for $C_{21}H_{22}N_4O_2$ $[M + Na]^+$ 385.1635, found 385.1634.

SUPPORTING INFORMATION

Probe 7a: The title compound was synthesized from secondary amine **A3** (89 mg, 0.39 mmol) and triazole **T1** (44 mg, 0.19 mmol) according to the similar procedures described for compound **2a**, and the crude product was purified by silica gel chromatography (hexane/EtOAc = 10/1 ~ 5/1) to afford probe **7a** (30 mg, 32%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.47 (s, 1H), 8.33 (s, 1H), 7.90 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.80 (dd, *J* = 8.7, 2.7 Hz, 2H), 7.21 – 7.10 (m, 2H), 4.18 (d, *J* = 2.6 Hz, 2H), 3.93 (s, 3H), 3.87 – 3.72 (br, 4H), 3.72 – 3.47 (m, 10H), 2.42 (t, *J* = 2.4 Hz, 1H), 1.90 – 1.72 (m, 2H), 1.10 – 0.83 (br, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.29, 149.47, 146.84, 134.75, 129.96, 129.08, 127.68, 124.97, 124.90, 124.43, 121.03, 119.60, 105.96, 79.77, 74.75, 70.74, 70.59, 70.30, 69.25, 68.87, 58.55, 55.50, 52.92, 52.05, 49.54, 22.07, 20.53, 11.34. HRMS [ESI+] m/z: calculated for $C_{26}H_{32}N_4O_5$ $[M + Na]^+$ 503.2265, found 503.2265.

Probe 7b: The title compound was isolated from the mixture of compound **7a**. The N2-carbamoyl triazole regioisomer was purified by silica gel chromatography (hexane/EtOAc = 10/1 ~ 5/1) to afford probe **7b** (26 mg, 28%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.25 (s, 1H), 8.14 (s, 1H), 7.94 (dd, *J* = 8.6, 1.7 Hz, 1H), 7.81 (dd, *J* = 8.7, 2.0f Hz, 2H), 7.22 – 7.13 (m, 2H), 4.18 (d, *J* = 2.4 Hz, 2H), 3.95 (s, 3H), 3.88 – 3.74 (m, 4H), 3.73 – 3.55 (m, 10H), 2.41 (t, *J* = 2.4 Hz, 1H), 1.87-1.72 (m, 2H), 0.94 (br, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 158.63, 150.46, 149.41, 135.23, 133.21, 130.03, 128.97, 127.82, 125.86, 124.64, 124.56, 119.81, 106.05, 79.82, 74.73, 70.86, 70.80, 70.67, 70.38, 69.31, 69.10, 58.60, 55.58, 52.98, 52.04, 49.31, 22.06, 20.66, 11.34. HRMS [ESI+] m/z: calculated for $C_{26}H_{32}N_4O_5$ $[M + Na]^+$ 503.2265, found 503.2265.

Scheme S4. Synthesis of activity-based probes **8a, 8b, 9a, 9b**



Reagents and conditions: (a) EDC, HOBt, DIPEA, DCM, r.t., 16 hrs; (b) $CuSO_4 \cdot 5H_2O$, ascorbic acid, DMF, r.t., 16 hrs.

BodipyTMR-N_3: The intermediates BodipyTMR-OH and 3-azidopropan-1-amine **C3** were prepared according to the reported procedures [4, 5]. BodipyTMR-OH (46 mg, 0.12 mmol), EDC (27 mg, 0.14 mmol) and HOBt (22 mg, 0.14 mmol) were dissolved in DCM (3 mL), followed by the addition of DIPEA (22 mg, 0.23 mmol) and a solution of **C3** (14 mg, 0.14 mmol) in DCM (1 mL), and the mixture was stirred at r.t. for 16 hrs. After the completion of the reaction monitored by TLC, the reaction mixture was poured into water (10 mL) and extracted with DCM (3 × 15 mL) until the water phase became colorless. Organic fractions were combined, concentrated and purified by silica gel chromatography (DCM/MeOH = 80/1) to give the product as a sticky violet solid, and the further recrystallization (hexane/DCM = 20/1) afford the **BodipyTMR-N_3** as a red solid (47.2 mg, 84.7%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.88 (d, *J* = 8.8 Hz, 2H), 7.10 (s, 1H), 7.03 – 6.93 (m, 3H), 6.55 (d, *J* = 4.0 Hz, 1H), 5.54 (br, 1H), 3.86 (s, 3H), 3.38 – 3.22 (mf, 4H), 2.76 (t, *J* = 7.5 Hz, 2H), 2.52 (s, 3H), 2.28 (t, *J* = 7.4 Hz, 2H), 2.21 (s, 3H), 1.81 – 1.67 (m, 2H). HRMS [ESI+] m/z: calculated for $C_{24}H_{27}BF_2N_6O_2$ $[M + Na]^+$ 503.2149, found 503.2147.

Probe 8a: In a 4 mL vial, alkyne **6a** (2.8 mg, 7.8 μM) and BodipyTMR-N_3 (3.1 mg, 6.5 μM) were dissolved in DMF (0.55 mL) at r.t., followed by the addition of 70 mM $CuSO_4 \cdot 5H_2O$ solution in DMF (18 μL, 1.3 μmol) and 199 mM ascorbic acid solution in DMF (39 μL, 7.8 μmol). The mixture was purged with argon three times and stirred at r.t. for 16 hrs. After the completion of the reaction, the mixture was diluted with H_2O (5 mL) and extracted with EtOAc (3 × 10 mL). Organic fractions were combined and concentrated. The crude product was purified by silica gel chromatography (DCM/MeOH = 80/1 ~ 30/1) to give probe **8a** as a violet solid (4.0 mg, 73%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.47 – 7.36 (m, 1H), 8.29 (s, 1H), 7.87 (dd, *J* = 8.6, 1.9 Hz, 3H), 7.80

(d, *J* = 10.6 Hz, 2H), 7.44 (s, 1H), 7.18 (d, *J* = 9.1 Hz, 1H), 7.14 (s, 1H), 7.04 (d, *J* = 9.6 Hz, 1H), 6.97 (d, *J* = 8.7 Hz, 2H), 6.94 (d, *J* = 4.1 Hz, 1H), 6.54 (d, *J* = 4.1 Hz, 1H), 6.10 – 5.70 (m, 1H), 4.32 – 4.13 (m, 2H), 4.00 (s, 1H), 3.92 (s, 3H), 3.85 (s, 4H), 3.54 (t, *J* = 7.8 Hz, 2H), 3.16 (br, 4H), 2.74 (t, *J* = 7.4 Hz, 2H), 2.52 (s, 3H), 2.40 – 2.25 (m, 2H), 2.18 (d, *J* = 10.7 Hz, 3H), 2.01 (br, 2H), 1.88 – 1.72 (m, 2H), 1.05-0.80 (m, 3H). HRMS [ESI+] m/z: calculated for $C_{45}H_{49}BF_2N_{10}O_4$ $[M + Na]^+$ 865.3892, found 865.3888.

Probe 8b: The title compound was synthesized from alkyne **6b** (2.6 mg, 7.1 μM) and BodipyTMR-N_3 (2.9 mg, 5.9 μM) according to the similar procedures described for compound **8a**, and the crude product was purified by silica gel chromatography (DCM/MeOH = 80/1 ~ 30/1) to give probe **8b** as a violet solid (3.6 mg, 71%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.21 (s, 1H), 8.10 (s, 1H), 7.92 – 7.83 (m, 3H), 7.83 – 7.75 (m, 2H), 7.43 (s, 1H), 7.18 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.15 (d, *J* = 2.5 Hz, 1H), 7.04 (s, 1H), 6.97 (d, *J* = 8.3 Hz, 2H), 6.93 (s, 1H), 6.53 (s, 1H), 6.10 – 5.87 (m, 1H), 4.30 – 4.07 (m, 2H), 3.94 (s, 3H), 3.88 (s, 2H), 3.85 (s, 3H), 3.49 (s, 2H), 3.18 – 3.06 (m, 4H), 2.74 (t, *J* = 7.3 Hz, 2H), 2.52 (s, 3H), 2.28 (t, *J* = 7.3 Hz, 2H), 2.19 (s, 3H), 2.02 (br, 3H), 1.76 (br, 2H), 1.07 – 0.90 (s, 3H). HRMS [ESI+] m/z: calculated for $C_{45}H_{49}BF_2N_{10}O_4$ $[M + Na]^+$ 865.3892, found 865.3894.

Probe 9a: The title compound was synthesized from alkyne **7a** (3.8 mg, 7.9 μM) and BodipyTMR-N_3 (3.5 mg, 7.2 μM) according to the similar procedures described for compound **8a**, and the crude product was purified by silica gel chromatography (DCM/MeOH = 80/1 ~ 30/1) to give probe **9a** as a violet solid (5.3 mg, 77%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.21 (s, 1H), 8.11 (s, 1H), 7.92 – 7.86 (m, 3H), 7.78 (dd, *J* = 13.9, 8.7 Hz, 2H), 7.58 (s, 1H), 7.18 (dd, *J* = 8.8, 2.5 Hz, 1H), 7.15 (d, *J* = 2.5 Hz, 1H), 7.00 (s, 1H), 6.97 (d, *J* = 8.4 Hz, 2H), 6.91 (d, *J* = 4.1 Hz, 1H), 6.53 (d, *J* = 4.1 Hz, 1H), 6.17 (s, 1H), 4.61 (s, 2H), 4.25 (t, *J* = 6.7 Hz, 2H), 3.94 (s, 3H), 3.86 (s, 3H), 3.83 – 3.69 (m, 4H), 3.69 – 3.50 (m, 10H), 3.19 (q, *J* = 6.2 Hz, 2H), 2.72 (t, *J* = 7.5 Hz, 2H), 2.50 (s, 3H), 2.27 (t, *J* = 7.5 Hz, 2H), 2.16 (s, 3H), 2.06 – 1.98 (m, 2H), 1.82 – 1.73 (m, 2H), 1.03 – 0.87 (m, 3H). HRMS [ESI+] m/z: calculated for $C_{50}H_{59}BF_2N_{10}O_7$ $[M + Na]^+$ 983.4522, found 983.4527.

Probe 9b: The title compound was synthesized from alkyne **7b** (2.3 mg, 4.8 μM) and BodipyTMR-N_3 (2.1 mg, 4.4 μM) according to the similar procedures described for compound **8a**, and the crude product was purified by silica gel chromatography (DCM/MeOH = 80/1 ~ 30/1) to give probe **9b** as a violet solid (3.1 mg, 73%). ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.58-8.37 (m, 1H), 8.29 (br, 1H), 7.87 (d, *J* = 8.7 Hz, 3H), 7.78 (d, *J* = 8.5 Hz, 2H), 7.54 (s, 1H), 7.17 (d, *J* = 9.2 Hz, 1H), 7.14 (s, 1H), 7.05 (s, 1H), 6.97 (d, *J* = 8.8 Hz, 2H), 6.93 (d, *J* = 4.1 Hz, 1H), 6.53 (d, *J* = 4.1 Hz, 1H), 5.91 (s, 1H), 4.62 (s, 2H), 4.20 (s, 2H), 3.93 (s, 3H), 3.85 (s, 4H), 3.84 – 3.46 (m, 13H), 3.25-3.03 (m, 2H), 2z.74 (t, *J* = 7.4 Hz, 2H), 2.52 (s, 3H), 2.28 (t, *J* = 7.4 Hz, 2H), 2.19 (s, 3H), 2.09-1.87 (m, 2H), 1.82-1.73 (m, 2H), 1.05-0.88 (m, 3H). HRMS [ESI+] m/z: calculated for $C_{50}H_{59}BF_2N_{10}O_7$ $[M + Na]^+$ 983.4522, found 983.4533.

Experimental Procedures

Bacterial culture

An overview of all *S. aureus* strains used is presented in Table S1. All strains were routinely cultured in Tryptic Soy Broth (TSB) or on Tryptic Soy Agar (TSA). For late stationary phase cultures, 3 mL TSB in 14 mL snap-cap polypropylene tubes were inoculated with 2-3 colonies from a TSA plate and incubated at 37°C, 180 rpm for ~ 14 - 18 h. Alternatively bacteria were grown on TSA supplemented with 100 mM $MgCl_2$ (TSAMg) which has been reported to promote biofilm-like growth[8]. 10-20 colonies were collected with a 10 μL inoculation loop and struck out homogenously on TSAMg plates and grown at 37°C for 2 days.

Competitive Activity-based protein profiling screen

S. aureus ATCC35556 cells were struck out on TSAMg plates and incubated at 37°C for ~ 2 days. Bacteria were harvested with an inoculation loop and resuspended at high density in TSB to an OD_{600} ~ 16-20. To 0.5 μL of compounds (100x in DMSO), 49.5 μL of bacterial suspension were added. The samples were briefly vortexed and incubated at 37°C, 300 rpm for 60 min, before FP-TMR was added from a 100x stock solution in DMSO to a final concentration of 1 μM. Cells were vortexed and incubation at 37°C was continued for 30 min. Cells were spun down at 6,000 g for 5 min, 4°C and resuspended in 360 μL 0.1% SDS/PBS. Samples were transferred to o-ring tubes filled with 0.1 mm glass beads and lysed by bead-beating (3x 50 s) in a Biospec MiniBeadBeater 96 and centrifuged at 10,000 g for 5 min, 4°C to remove glass beads. The addition of SDS in the lysis buffer ensure extraction of proteins from the insoluble to the soluble fraction. Supernatants containing whole cell extracts were combined with 4x SDS-Sample Buffer and analysed on a 12% SDS-gel (80V for 30 min, then ~120V for 60-100 min). BodipyTMR-fluorescence and Cy5 -fluorescence (marker) were read on a Typhoon 9410 variable mode imager.

Band intensities were quantified using Image J. Data from compound-pretreated samples were normalized to the corresponding band intensities from the DMSO-pretreated control samples. In order to minimize distortions of the data due to differences in loading or inter-sample variations

in FP-TMR labeling, a calibration factor was introduced. The calibration factor represents the highest compound-to-control data ratio for any band within a sample (lane). If this factor is >1 it is indicative of higher enzyme activity/FP-TMR labeling in the compound-treated compared to the control sample and hence the data from all bands in this sample (lane) are normalized by this factor. If the highest compound-to-control data ratio within a sample is <1, no normalization was done, as it is possible that the test compound blocks FP-TMR labeling of all targets, and instead a calibration factor of 1 was used. %Inhibition values displayed in Figure S1 were calculated by the following formula: %inhibition=(1-(compound/control)/calibration factor)*100.

Probe-labeling experiments

Validation of hit compounds and newly synthesized probes was done by a streamlined labeling protocol. For competitive labeling experiments, cultures were preincubated with the test compounds at the indicated concentrations for 60 min, before addition of the FP-TMR (1 μM). For direct visualization of fluorescent and alkyne-probes, cultures were incubated with the chemical probes for 30min at 37°C without any preincubation step. For fluorescent probes, cells were directly transferred after labeling to 0.6 mL o-ring tubes filled with 0.1 mm glass beads and 310 μL 1.16xSDS-Loading Buffer. Cells were lysed by bead-beating (3x 45 s) and samples were boiled at 95°C for 10 min before centrifugation at 10,000 g for 5 min. After labelling with alkyne probes, 1 mL of ice-cold PBS was added to the cultures and cells were separated from the supernatant containing unreacted probe by centrifugation at 6,000 g for 5 min, 4°C. Supernatants were removed, cells were resuspended in 400 μL ice-cold PBS containing 0.1% SDS (to solubilize membrane proteins) and lysed by bead-beating. Samples were spun down at 8,000g, 5 min, 4°C to remove glass beads and click-reactions were performed in whole cell extracts as follows: A master mix containing 1 μL of 50 mM $CuSO_4$ in H_2O, 3 μL 1.7 mM BTTAA (in DMSO, ClickChemistryTools, Scottsdale, AZ, USA, or alternatively 3 μL of 1.7 TBTA in 1:4 tBuOH:DMSO, ClickChemistryTools), 1 μL 1 mM N_3-TMR in DMSO and 1 μL freshly prepared 50 mM TCEP in H_2O were added to 46 μL of lysate. Samples were vortexed and incubated at RT for 30 min, vortexed again and incubation continued for another 30 min. 4xSDS-Loading Buffer was added and samples were boiled and analysed by SDS-PAGE. In order to achieve reliable separation of FphE and FphH bands after FP-TMR labelling on a 12% gel, gels had to be run at reduced voltage (~80 V for 3-4 h).

Determination of IC_{50} values

To assess the inhibitory potency of compounds in the competitive activity-based protein profiling setup, band intensities were quantified using Image Studio Lite (V5.2.5, Li-Cor). The value obtained for each inhibitor concentration was divided by the value of the corresponding control condition (vehicle pretreated and labelled with FP-TMR) and multiplied by 100 to give a normalized '%labeling 'value. These values were then entered into Prism 7 (GraphPad Software), logarithmically transformed and analysed by non-linear regression ('log(inhibitor) vs. normalized response') to give IC_{50} values.

Fluorescence microscopy and flow cytometry analysis of probe-labeled bacteria.

Bacterial cultures were synchronized to compare cells in different growth stages including mid-exponential phase, late stationary phase, as well as after growth on TSAMg for 24-48h. Overnight cultures (~14 h) were diluted 1:100 and cells grown for 3-4 h to mid-exponential phase and labelled at an OD_{600} of 0.6-0.7. At the same time overnight cultures that had continued to grow for another ~4 h at 37°C were labelled without dilution as late stationary phase cultures (17-18 h). Cells from TSAMg were suspended in TSB and adjusted to an OD_{600} of ~20. To 0.3 μL of a stock solution of FP-TMR or **9a** in DMSO, 100 μL of bacterial culture was added. Samples were mixed by vortexing and incubated at 37°C for 30 min, 300 rpm. For each experimental condition three independent replicate cultures were prepared and the experiment was performed twice.

After probe-labeling, 100 μL of cell cultures were diluted with 1 mL PBS/0.05% Tween®20. Cells were pelleted by centrifugation at 6,000 g for 5 min. Cells were fixed in 2% PFA for 10 - 15 min and washed 2x with PBS. For confocal microscopy, bacteria were resuspended in PBS and added to an 8-well μ-Slide chamber (Ibidi, Planegg, Germany) coated manually with Poly-L-Lysine (Trevigen). Cells were imaged at 63x using a Zeiss LSM 700 confocal microscope in the BodipyTMR channel. Laser and gain settings were configured at the beginning of each acquisition series and constant settings used for all samples. For live imaging, cells were diluted 1:10 into PBS/0.05% Tween®20 after probe-labeling, transferred to a Poly-L-Lysine coated 8-well μ-Slide chamber (Ibidi, Planegg, Germany) and imaged on a confocal microscope.

For flow cytometry analysis, cells were resuspended in PBS and analyzed on a BD Accuri™ RUO Special Order System Flow Cytometer with BD CSampler software (BD Biosciences, San Diego, USA). FSC-A threshold was set to 5,000, and 100,000 ungated events were recorded.

BodipyTMR- and TAMRA fluorescence were detected using the FL-2 detector at 552 nm excitation in combination with a 586 nm emission filter. In order to determine levels of FphE-specific labeling for each experimental condition, mean-fluorescence intensity (MFI) values were normalized by subtracting MFI-values of probe-labeled *fph*E:Tn control cells.

Statistical analysis

Statistical analyses were performed using Prism 7 (GraphPad Software Inc., San Diego, CA, USA) employing unpaired, two-tailed Student´s t-test as specified in the corresponding figure legend.

Supplementary Results

Validation of the reactivity profile of screening hits

Isomers **1a** and **1b** (AA395) feature a pyrrolidinyl group at the urea and a 6-methoxynaphtalenyl substitution at the 4-position of the triazole urea ring. The original screening hit **1b** potently blocked FP-TMR labeling of a <36 kDa protein identified as FphB (IC_{50} = 128 nM) and showed a~ 4-5-fold selectivity over a ~ 50 kDa hydrolase as a secondary target (Fig. S1, S2) identified as FphA. The 1,4-regioisomer **1a** was even more potent against FphB (IC_{50} ~14 nM), but also targeted a >66.5 kDa and a ~40 kDa hydrolase (IC_{50}~ 19 nM), assigned to the pre-protein and matured form of SAL1 or SAL2 (**Fig. 1B**). Screening hit **2b** ($R_{1=}$ 4-*tert*butylpiperidin-1-yl, R_2=1-hydroxycyclohexyl) targeted FphA and the 28 kDa hydrolase FphH (which appears as a band of apparent molecular weight of ~ 33 kDa), with similar potency (IC_{50} (FphA) = 312 nM, IC_{50} (FphH) = 712 nM) , while the 1,4-regioisomer **2a** was ~ 10-fold more potent against both FphA and FphH and showed secondary activity against SAL2 (**Fig. 1B, Fig. S2**). Compound **3b** ($R_{1=}$ 4-*tert*butylpiperidin-1-yl, R_2=2-hydroxypropan-2-yl) had a similar activity profile as **2b**, while substitution of the 1-hydydroxycyclohexyl moiety in **2a** by the shorter hydroxypropan-2-yl moiety (**3a)** rendered this compound inactive against the *S. aureus* lipases. Similarly, substitution of the 4-*tert*butylpiperidin-1-yl moiety in **2a** by an aromatic 4-phenylpiperidin-1-yl (**4a**) invoked a major loss in activity against SAL1/2 while increasing activity against the upper of two hydrolases running as a double-band at ~ 29 kDa (IC_{50} ~ 25 nM) identified as FphF in both USA300 and Newman strains (Fig. 1b, Fig. S2). Of note, the FP-TMR labeling profile of strain USA300 revealed an additional hydrolase of an apparent molecular weight of ~30-31 kDa that was not observed in strains ATCC35556 or Newman. FP-TMR labeling of this unidentified hydrolase was blocked by compounds **1a**, **4a** and **4b** when used at 1 μM (Figure S2).

Validation of alkyne probes

Alkyne probes were validated on live cells and probe-labeling was visualized by SDS-PAGE analysis after conjugation to azide-functionalized fluorophores via bio-orthogonal 'click-chemistry' in bacterial lysates *in situ* (Figures S3A-H). As cells had to be separated from the culture supernatant containing unreacted probe, all labeled hydrolase targets are cell-associated, rather than secreted. Overall, the structural modifications introduced to install an alkyne-handle at the TU scaffold had dramatic effects on the selectivity profile of the resulting probes. Both regioisomers functionalized with a butynyl group (**5a**/**b**) showed preferential labeling of FphB (Figure S3B) comparable to the parent inhibitor. In contrast, alkyne probes **6a/b** (with an additional N-propyl substitution at the urea group) had a non-discriminatory selectivity profile (Figure S3C). The two regioisomers **7a** and probe **7b** (alkyne functionality after a PEG3-linker) showed very different labeling profiles. **7a** was non-discriminatory, but **7b** labeled FphA with a >30-fold selectivity over FphE as seen by both direct labeling (Figure S3E) and FP-TMR competition experiments (Figure S3F). As *in situ* click-labeling experiments did not account for any potential reaction with secreted lipases SAL1/SAL2, alkyne-probes were also tested in competition with FP-TMR labeling in bacterial cultures including supernatants. Under these conditions, the 1,4 regioisomers **6a** and **7a** showed potent labeling of SAL1/2 (Figure S3G).

SUPPORTING INFORMATION

Supplementary Tables and Figures

Table S1. List of bacterial strains

Strain	Information	Source/Reference
S. aureus ATCC35556	Restriction-deficient mutant derived from strain Rosenbach NCTC8325, MS-SA, known biofilm former	ATCC, [9] [10]
S. aureus Newman	Wild-type clinical osteomyelitis isolate, MS-SA	[11]
NWMN_gehA::φNΣ (NWMN_2569::φNΣ)	Transposon insertion mutant in NWMN_2569 (gehA/SAL1); Ery^R, $Linc^R$	[10]
NWMN_gehB:: φNΣ (NWMN_0262:: φNΣ)	Transposon insertion mutant in NWMN_0262 (gehB, SAL2); Ery^R, $Linc^R$	[10]
NWMN_*fph*A*:: φNΣ* (NWMN_2350*::φNΣ*)	Transposon insertion mutant in NWMN_2350 (*fph*A/*pnb*A); Ery^R, $Linc^R$	[10]
NWMN_fphB::φNΣ (NWMN_2434::φNΣ)	Transposon insertion mutant in NWMN_2434 (fphB); Ery^R, $Linc^R$	[10]
NWMN_fphC::φNΣ (NWMN_1210::φNΣ)	Transposon insertion mutant in NWMN_1210 (fphC); Ery^R, $Linc^R$	[10]
NWMN_fphD::φNΣ (NWMN_2092::φNΣ)	Transposon insertion mutant in NWMN_2092 (fphD); Ery^R, $Linc^R$	[10]
NWMN_*fph*E*::φNΣ* (WMN_2480*::φNΣ*)	Transposon insertion mutant in NWMN_2480 (*fph*E); Ery^R, $Linc^R$	[10]
NWMN_*fph*F*::φNΣ* (NWMN_2528*::φNΣ*)	Transposon insertion mutant in NWMN_2528 (*fph*F); Ery^R, $Linc^R$	[10]
NWMN_*fph*G*::φNΣ* (NWMN_1683*::φNΣ*)	Transposon insertion mutant in NWMN_1683 (*fph*G); Ery^R, $Linc^R$	[10]
NWMN_*fph*H*::φNΣ* (NWMN_0784*::φNΣ*)	Transposon insertion mutant in NWMN_0784 (*fph*H); Ery^R, $Linc^R$	[10]
S. aureus USA 300 LAC	Wild-type USA300 Los Angeles County (LAC) clone; multilocus sequence type 8, SCCmec type IV cured of antibiotic resistance plasmid, MR-SA strain	[12]
SAUSA300_*geh*A*::φNΣ* (SAUSA300_2603*::φNΣ*)	Transposon insertion mutant in SAUSA300_2603; Ery^R, $Linc^R$	Nebraska Transposon Mutant Library [13]
SAUSA300_*geh*B*::φNΣ* (SAUSA300_0320*::φNΣ*)	Transposon insertion mutant in SAUSA300_0320; Ery^R, $Linc^R$	Nebraska Transposon Mutant Library [13]
SAUSA300_*fph*A*::φNΣ* (SAUSA300_2396*::φNΣ*)	Transposon insertion mutant in SAUSA300_2396; Ery^R, $Linc^R$	Nebraska Transposon Mutant Library [13]
SAUSA300_*fph*B*::φNΣ* (SAUSA300_2473*::φNΣ*)	Transposon insertion mutant in SAUSA300_2473; Ery^R, $Linc^R$	Nebraska Transposon Mutant Library [13]
SAUSA300_*fph*C*::φNΣ* (SAUSA300_1194*::φNΣ*)	Transposon insertion mutant in SAUSA300_1194; Ery^R, $Linc^R$	Nebraska Transposon Mutant Library [13]
SAUSA300_*fph*D*::φNΣ* (SAUSA300_2148*::φNΣ*)	Transposon insertion mutant in SAUSA300_2148; Ery^R, $Linc^R$	Nebraska Transposon Mutant Library [13]
SAUSA300_fphE::φNΣ (SAUSA300_2518::φNΣ)	Transposon insertion mutant in SAUSA300_2518; Ery^R, $Linc^R$	Nebraska Transposon Mutant Library [13]
SAUSA300_fphF::φNΣ (SAUSA300_2564::φNΣ)	Transposon insertion mutant in SAUSA300_2564; Ery^R, $Linc^R$	Nebraska Transposon Mutant Library [13]
SAUSA300_fphG::φNΣ (SAUSA300_1733::φNΣ)	Transposon insertion mutant in SAUSA300_1733; Ery^R, $Linc^R$	Nebraska Transposon Mutant Library [13]
SAUSA300_fphH::φNΣ (SAUSA300_0763::φNΣ)	Transposon insertion mutant in SAUSA300_0763; Ery^R, $Linc^R$	Nebraska Transposon Mutant Library [13]

SUPPORTING INFORMATION

Figure S1. Results of competitive FP-TMR labeling screen.

A

Compd (1 µM)	Band A	Band B	Band C	Band D
AA261	0%	64%	0%	80%
AA2610	23%	92%	40%	96%
AA268	0%	95%	21%	55%
AA269	0%	93%	16%	79%
AA331	52%	88%	0%	82%
AA332	36%	89%	0%	45%
AA373	62%	96%	0%	54%
AA392	58%	97%	0%	62%
AA394	100%	100%	0%	89%
AA395	100%	100%	0%	61%
AA401	64%	97%	0%	90%
AA403	81%	100%	0%	47%
AA442	60%	97%	46%	54%
AA443	81%	99%	37%	88%
AA471	4%	97%	0%	55%
AA491	76%	93%	0%	86%
AA511	60%	98%	47%	79%
AA652	9%	13%	12%	73%
AA692	69%	10%	67%	14%
AA701	24%	95%	0%	59%
AA702	33%	98%	33%	70%
AA721	1%	76%	3%	43%
AA801 (1,4)	0%	30%	6%	2%
AA801 (2,4)	96%	88%	21%	47%
KT107	40%	85%	25%	36%
KT118	0%	73%	4%	84%
KT129	56%	37%	70%	90%
KT157	0%	26%	18%	89%
KT158	2%	28%	14%	81%
KT161	47%	53%	59%	96%
KT170	0%	13%	20%	64%
KT174	0%	19%	15%	69%
KT184	3%	13%	0%	58%
KT197	37%	0%	44%	73%
NHU4	0%	10%	46%	66%
NHU5	12%	29%	31%	57%
AA262	0%	66%	4%	54%
AA263	0%	16%	13%	43%
AA264	0%	25%	17%	32%
AA265	0%	31%	27%	48%
AA266	17%	22%	30%	43%
AA267	0%	24%	21%	55%
AA321	0%	30%	10%	12%
AA322	0%	49%	19%	23%
AA323	13%	29%	16%	26%
AA324	0%	51%	8%	31%
AA333	36%	87%	0%	22%
AA334	18%	90%	0%	33%
AA341	43%	14%	18%	37%
AA342	56%	22%	36%	36%
AA343	45%	25%	32%	23%

	Band A	Band B	Band C	Band D
AA344	30%	26%	11%	42%
AA371	20%	11%	0%	25%
AA381	34%	25%	0%	33%
AA382	24%	27%	8%	29%
AA383	43%	66%	0%	44%
AA391	0%	52%	23%	5%
AA393	42%	64%	0%	49%
AA402	6%	39%	0%	20%
AA432	46%	84%	0%	52%
AA472	32%	99%	21%	69%
AA501	20%	77%	20%	63%
AA621	0%	28%	7%	6%
AA622	31%	75%	20%	29%
AA642	59%	43%	42%	40%
AA691	87%	32%	29%	30%
AA722	0%	93%	13%	47%
AA741	8%	93%	0%	29%
KT106	30%	30%	33%	36%
KT109	39%	30%	35%	45%
KT110	0%	25%	15%	28%
KT111	0%	29%	16%	33%
KT112	30%	19%	0%	41%
KT113	25%	23%	0%	39%
KT114	36%	20%	0%	39%
KT115	28%	21%	0%	58%
KT116	11%	18%	12%	36%
KT117	21%	19%	1%	31%
KT119	36%	38%	25%	43%
KT120	16%	22%	4%	35%
KT121	26%	27%	0%	31%
KT122	28%	27%	0%	33%
KT123	20%	21%	0%	40%
KT124	26%	33%	36%	38%
KT125	35%	42%	40%	41%
KT127	24%	35%	31%	33%
KT128	17%	36%	29%	31%
KT130	5%	28%	20%	35%
KT131	30%	39%	47%	49%
KT133	23%	39%	45%	49%
KT135	11%	37%	36%	37%
KT136	39%	46%	55%	47%
KT137	47%	46%	56%	57%
KT138	21%	43%	38%	42%
KT139	0%	20%	10%	14%
KT140	0%	18%	4%	18%
KT141	5%	9%	14%	12%
KT142	0%	16%	5%	17%
KT144	0%	25%	7%	30%
KT146	2%	8%	2%	11%
KT147	14%	22%	20%	29%
KT148	0%	28%	17%	27%

	Band A	Band B	Band C	Band D
KT149	0%	20%	7%	11%
KT150	4%	10%	12%	17%
KT151	0%	53%	27%	28%
KT152	8%	22%	12%	19%
KT153	19%	18%	28%	43%
KT154	0%	20%	22%	24%
KT155	0%	14%	15%	20%
KT156	54%	41%	53%	72%
KT159	0%	19%	39%	21%
KT160	36%	27%	44%	62%
KT162	0%	29%	22%	81%
KT163	15%	26%	26%	62%
KT164	0%	23%	21%	72%
KT165	0%	5%	13%	39%
KT166	0%	9%	15%	19%
KT167	0%	4%	16%	43%
KT168	0%	17%	18%	19%
KT169	0%	15%	24%	36%
KT171	0%	14%	16%	35%
KT172	0%	21%	22%	32%
KT173	0%	26%	23%	35%
KT175	0%	21%	17%	34%
KT176	0%	11%	12%	26%
KT177	2%	2%	0%	18%
KT179	0%	23%	16%	37%
KT180	3%	3%	0%	15%
KT181	15%	0%	9%	27%
KT182	0%	19%	14%	40%
KT183	0%	6%	6%	27%
KT185	13%	10%	14%	17%
KT186	24%	5%	24%	28%
KT187	28%	3%	31%	34%
KT188	30%	5%	36%	50%
KT192	28%	0%	29%	38%
KT193	20%	0%	20%	28%
KT194	16%	1%	15%	31%
KT195	21%	0%	20%	28%
KT196	25%	1%	48%	54%
KT198	33%	0%	51%	50%
KT199	25%	0%	44%	49%
KT200	46%	1%	29%	56%
KT201	18%	0%	23%	26%
KT203	34%	0%	45%	48%
KT204	16%	26%	27%	0%
KT205	23%	21%	25%	48%
KT206	26%	17%	27%	50%
KT207	3%	12%	11%	0%
KT208	0%	10%	10%	11%
KT209	0%	17%	16%	12%
KT210	0%	19%	15%	17%
KT211	19%	12%	24%	23%

B

Compd (100nM)	Band A	Band B	Band C	Band D
AA261	0%	32%	33%	26%
AA2610	1%	19%	5%	8%
AA268	4%	26%	0%	22%
AA269	11%	58%	6%	18%
AA331	0%	25%	0%	8%
AA332	4%	25%	0%	12%
AA373	11%	58%	0%	7%
AA392	7%	17%	0%	5%
AA394	7%	68%	0%	14%
AA395	22%	89%	0%	11%
AA401	0%	39%	0%	11%
AA403	0%	15%	3%	12%

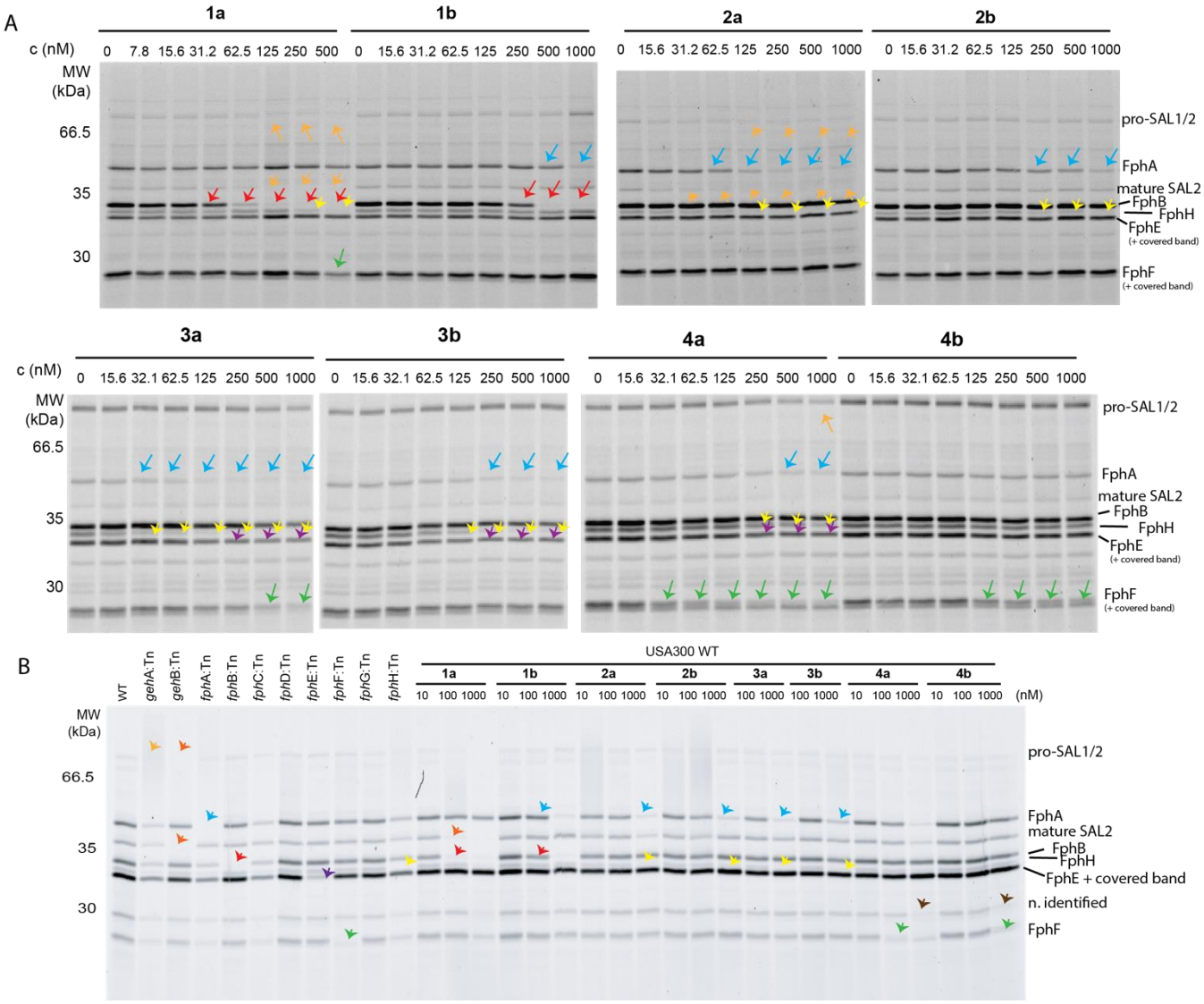
	Band A	Band B	Band C	Band D
AA442	12%	72%	0%	13%
AA443	3%	37%	0%	11%
AA471	3%	23%	0%	16%
AA491	3%	17%	0%	5%
AA511	10%	30%	8%	11%
AA652	6%	5%	15%	45%
AA692	5%	1%	16%	0%
AA701	0%	36%	0%	13%
AA702	2%	58%	0%	10%
AA721	0%	40%	2%	7%
AA801 (1,4)	0%	19%	9%	15%
AA801 (2,4)	12%	78%	0%	1%

	Band A	Band B	Band C	Band D
KT107	16%	23%	6%	9%
KT118	2%	15%	0%	19%
KT129	15%	1%	3%	33%
KT157	14%	6%	1%	30%
KT158	9%	8%	0%	43%
KT161	3%	8%	0%	59%
KT170	10%	6%	10%	34%
KT174	1%	0%	3%	20%
KT184	15%	0%	8%	21%
KT197	0%	4%	7%	5%
NHU4	1%	3%	0%	4%
NHU5	0%	12%	7%	9%

SUPPORTING INFORMATION

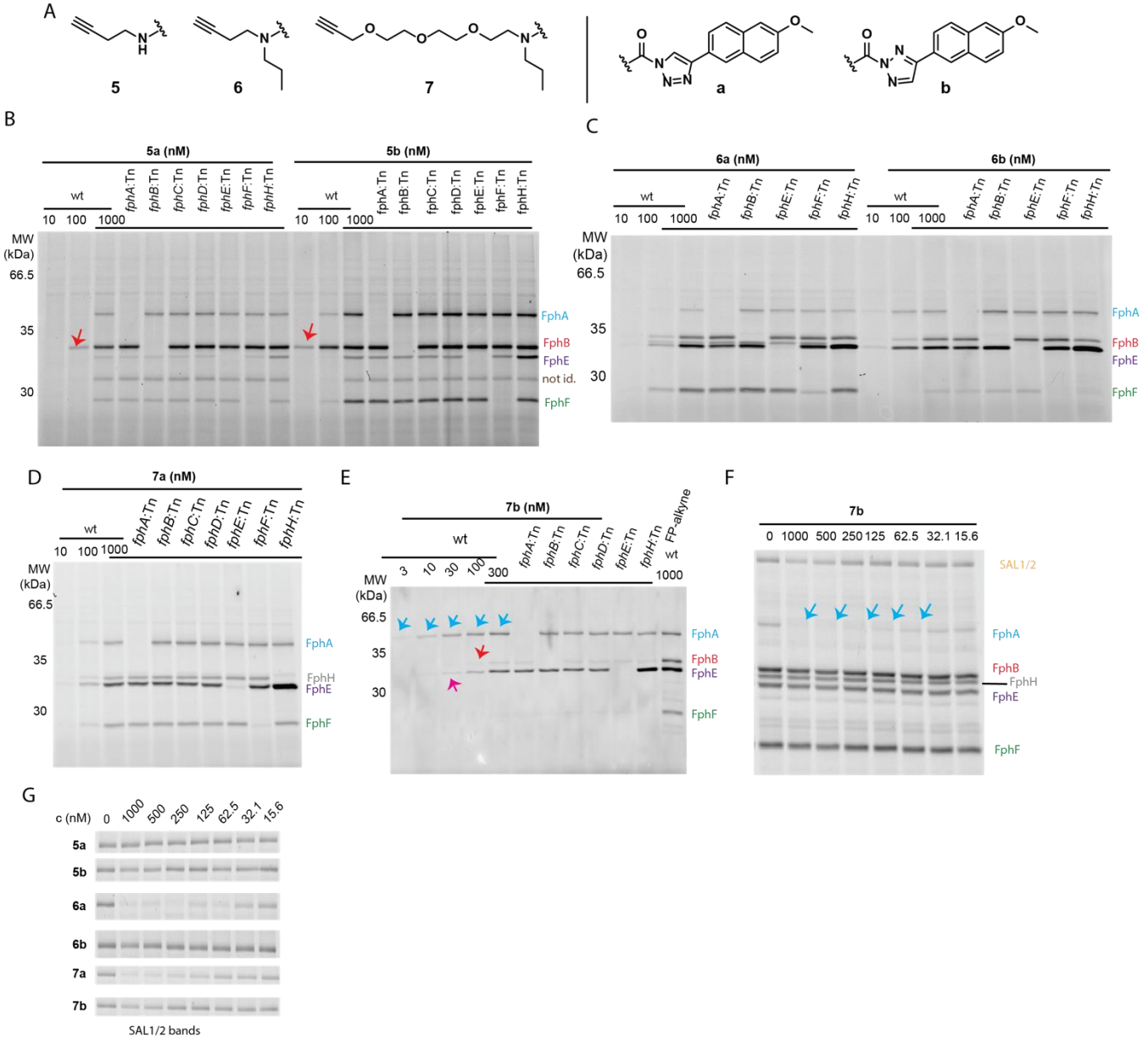
Heat-map representation of results of competitive FP-TMR labeling screen. FP-TMR labeling of bands A-D after pre-treatment with the indicated compounds was analyzed by SDS-PAGE, quantified and normalized to vehicle-pretreated control cells to achieve indicated % competition values. Results are shown for A) the primary screen performed at 1 µM of compound and B) of a rescreen of select compounds at 100 nM. Please note that based on the analysis of transposon mutant strains in Newman (Fig. 1) and USA300 (Fig. S2) background, we propose that the indicated bands correspond to the following enzymes: Band A: FphA, Band B: FphB, Band C: FphE and/or FphH, Band D: FphF.

Figure S2. FP-TMR competition labeling profiles of compounds **1-4 a/b**



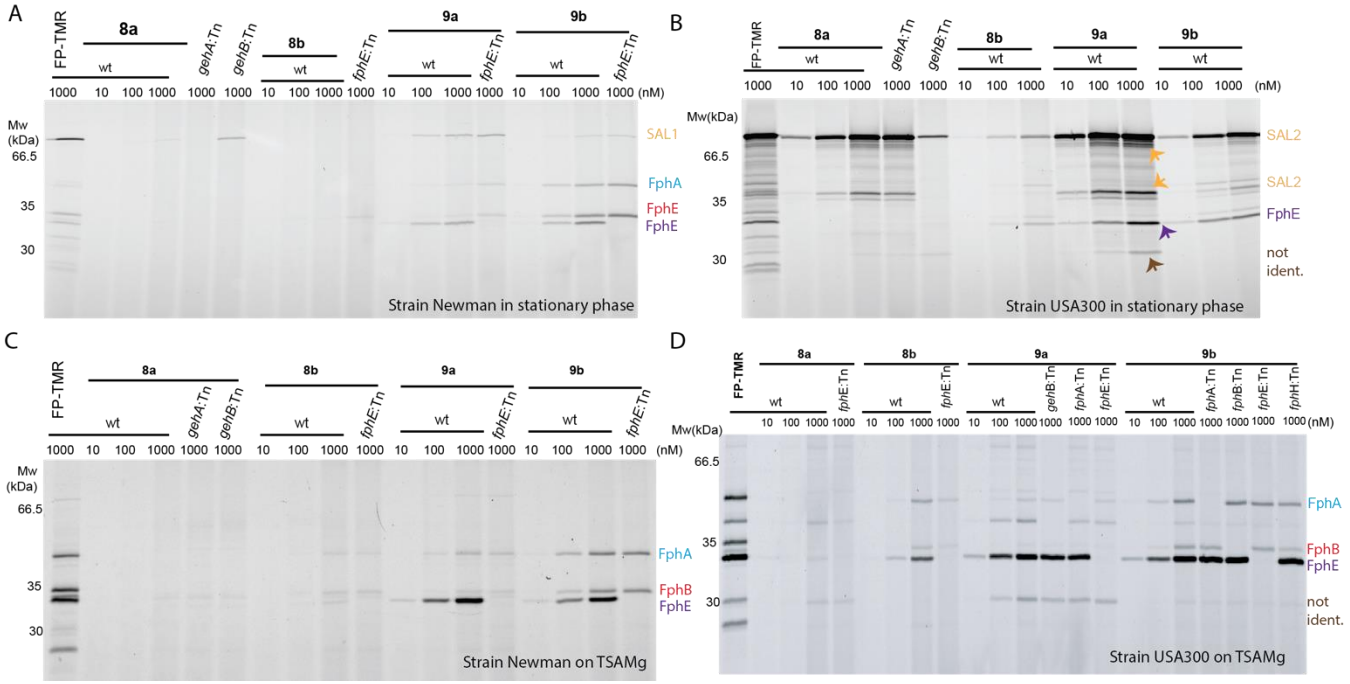
A) Competitive FP-TMR labelling profiles of *S. aureus* ATCC35556 after preincubation with compounds **1-4 a/b** at the indicated concentration. Cells were harvested from TSAMg plates, preincubated with inhibitors for 60 min, labelled with FP-TMR (1 µM), lysed and analyzed by SDS-PAGE/fluorescence scan. B) Competitive FP-TMR labelling profiles of *S. aureus* USA300 wt or indicated transposon mutant strains after preincubation with compounds **1-4 a/b** at the indicated concentration. All samples include full cultures with whole cell extracts and secreted culture supernatants. Identity of the hydrolases according to labelling of transposon mutant strains is highlighted by colored arrows: Orange: SAL1 or SAL2, blue: FphA, red: FphB, green: FphF, yellow: FphH, purple: FphE.

Figure S3. Alkyne triazole urea probes have distinct selectivity profiles.



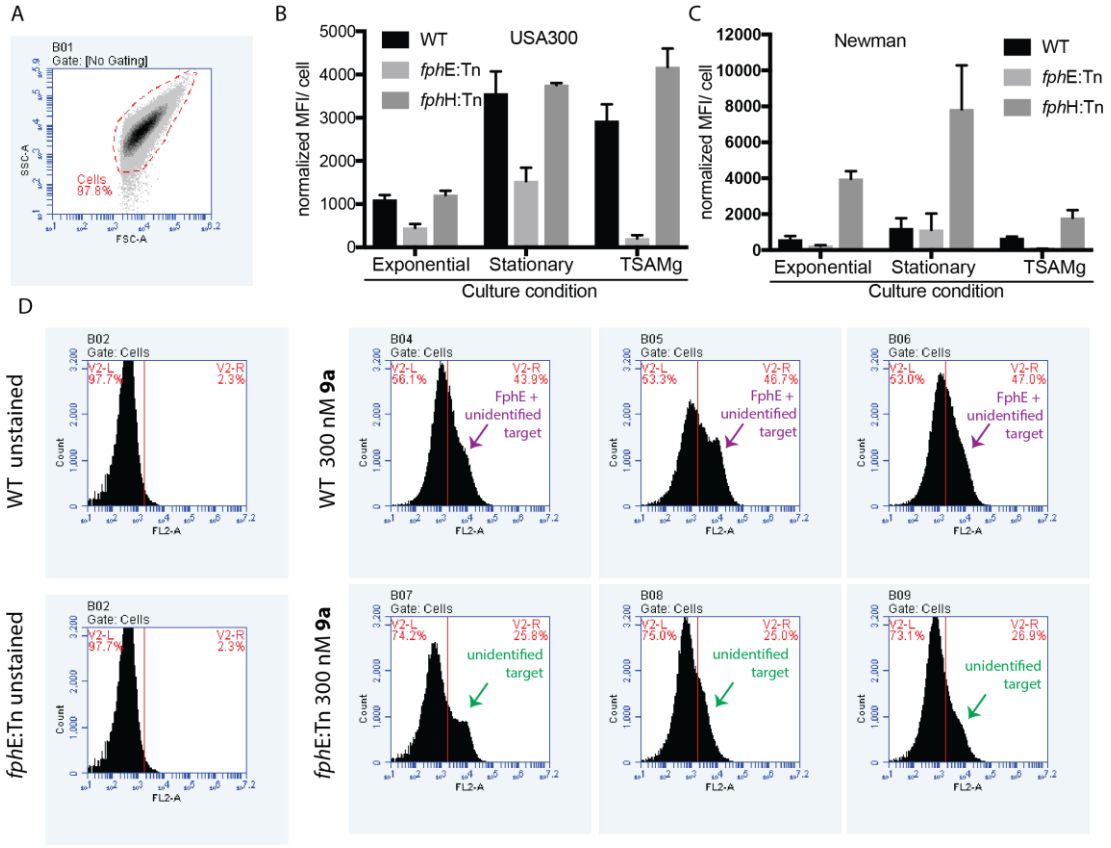
A) Chemical structures of clickable alkyne- TU probes **5**-**7 a/b**. B) Cellular labeling profiles of *S. aureus* Newman or its indicated transposon mutant with compounds **5a** and **5b**. After bacterial lysis, labeled proteins were fluorescently tagged by attachment of N_3-TAMRA by click-chemistry and analysed by SDS-PAGE. Red arrow indicates FphB. C) Cellular labeling profiles of *S. aureus* Newman cultures with compounds **6a** and **6b**. D) Cellular labeling profile of *S. aureus* Newman with compound **7a** and E) **7b**. Arrows indicate FphA (blue), FphB (red), and FphE (purple) and a not identified protein (brown). In B-E samples include whole cell extracts without culture supernatant containing secreted proteins. F) FP-TMR labelling of *S. aureus* Newman cultures in competition with different concentrations of **7b**. Samples include full cultures with whole cell extracts and secreted culture supernatants. G) FP-TMR labelling (1 µM) of secreted lipases SAL1/SAL2 in competition with alkyne-probes **5-7 a/b**.

Figure S4. Labeling profiles of fluorescent TU probes.



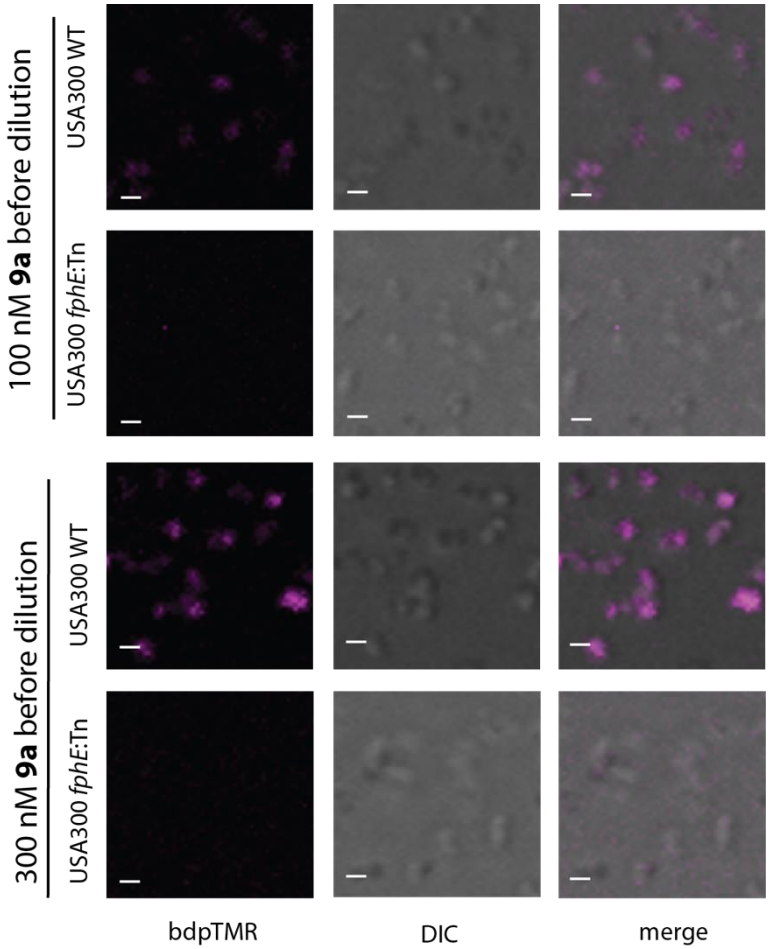
A-D show labeling profiles of *S. aureus* wt and its transposon mutant cultures with fluorescent triazole urea probes in different growth environments analyzed by SDS-PAGE. A,B) Late stationary phase cultures of A) strain Newman and B) strain USA300 cells labelled with FP-TMR, or probes **8a/b**, **9a/b** at the indicated concentrations. Colored arrows show labeling of FphE, SAL2 and a non-identified band by probe **9a** (at 1 μM). C,D) Cultures of strain Newman (C) and strain USA300 (D) cells harvested from TSAMg plates and labelled with FP-TMR, probes **8a/b**, **9a/b** at the indicated concentrations. All samples include full cultures with whole cell extracts and secreted culture supernatants.

Figure S5. Flow cytometry analysis of cellular FphE activity-levels.



A) Cell gate used for flow cytometry analysis of *S. aureus* cells. B,C) Plots of normalized mean fluorescence intensity (MFI) values of USA300 (B) or Newman (C) wt, *fph*E:Tn or *fph*H:Tn after labeling with 300 nM of probe **9a** for 30 min under the indicated culture conditions. MFI values for each sample were normalized to the average MFI of the corresponding unstained control cells. All conditions were tested in three biologically independent cultures per experiment. The graph is representative for 2 independent experiments. D) Histograms of USA300 cells labeled in stationary phase. 1 (out of 3) unstained control sample and the three probe **9a**-labeled replicates are shown indicating the presence of a **9a**-positive subset of ~25% of the cells in the fphE:Tn cell population. Arrows indicate **9a**-positive subpopulations and their FphE-dependent and FphE-independent (unidentified target) components.

Figure S6. Confocal microscopy of live cells.



Confocal micrographs of *S. aureus* USA300 wt or *fph*E:Tn transposon mutant cells. Cells were harvested from TSAMg and labelled with 300 nM or 100 nM of **9a** in TSB for 30 min and diluted 1:10 into PBS/0.05% Tween®20 before live imaging on a confocal microscope. Bodipy-TMR fluorescence is shown in magenta. Scale bar: 1 µm.

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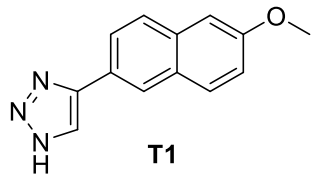
Author Contributions

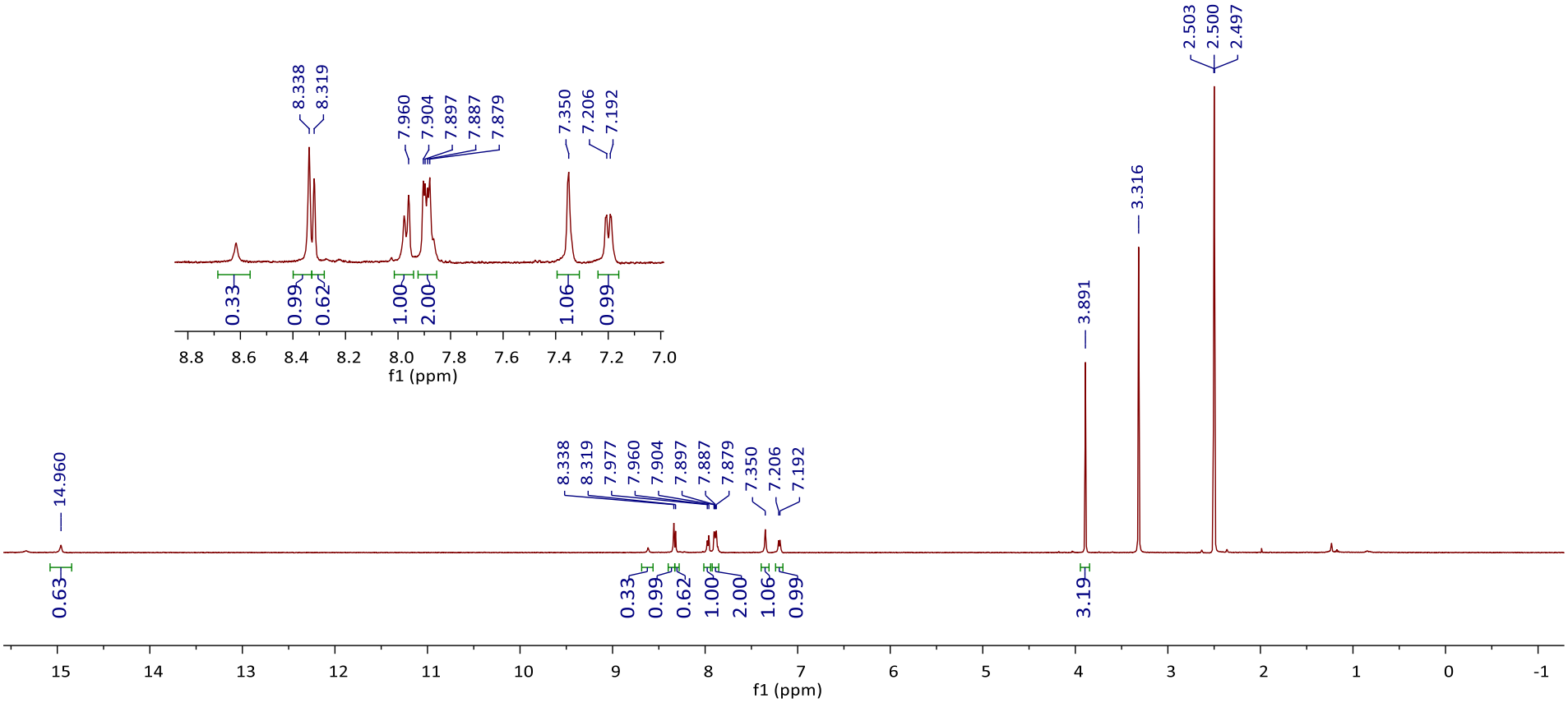
LC synthesized and characterized all compounds. LJK and CSL performed and analyzed biological experiments. EC performed the initial competitive activity-based protein profiling screen. MB and CSL conceived and supervised the project. LC, MB and CSL drafted the manuscript, all authors have edited the manuscript and have given approval for the final version of the manuscript.

NMR spectra

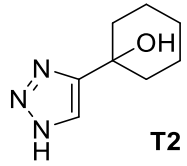
Compound T1 ^{1}H NMR (500 MHz, DMSO-d_6)



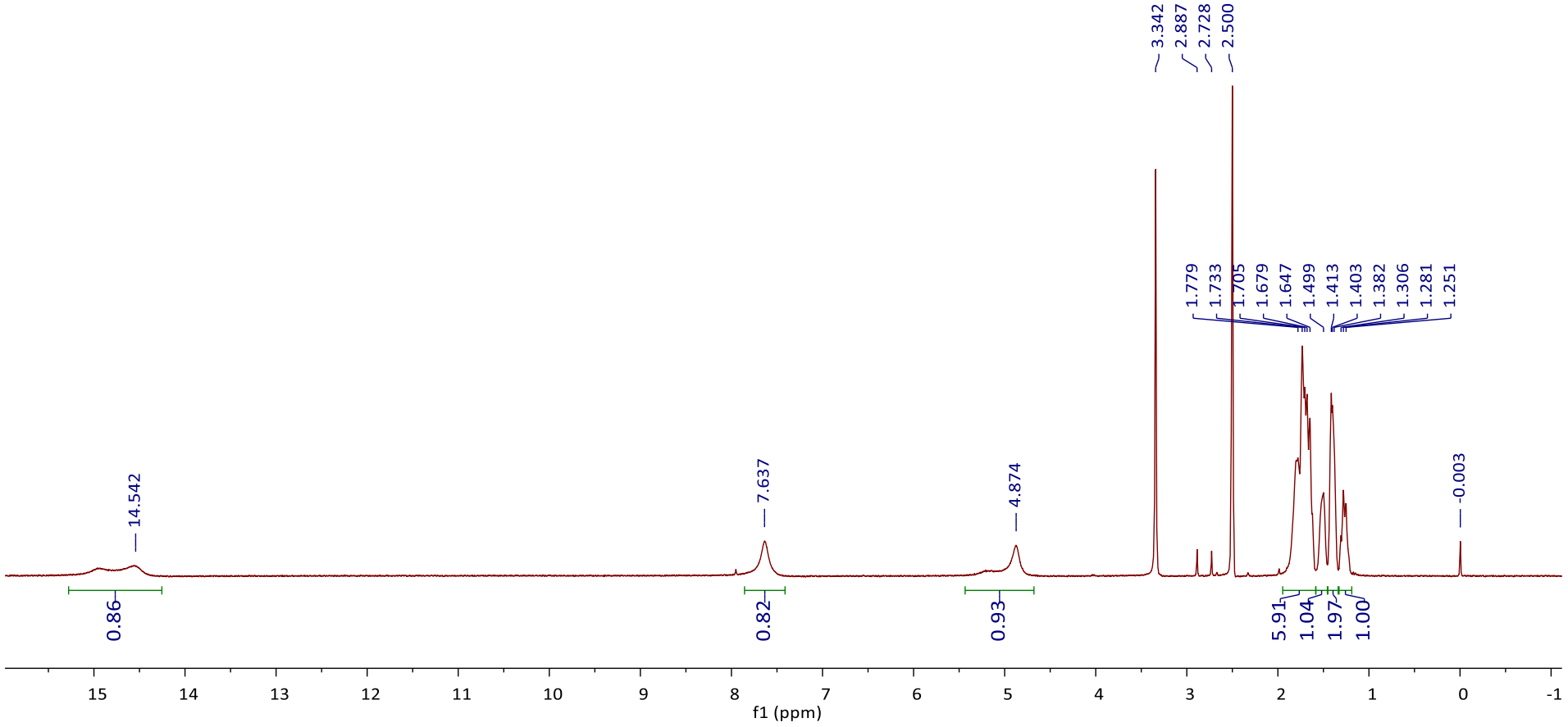
T1



Compound T2 ^{1}H NMR (400 MHz, DMSO-d_6)

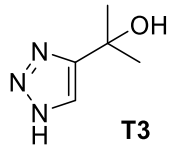


T2

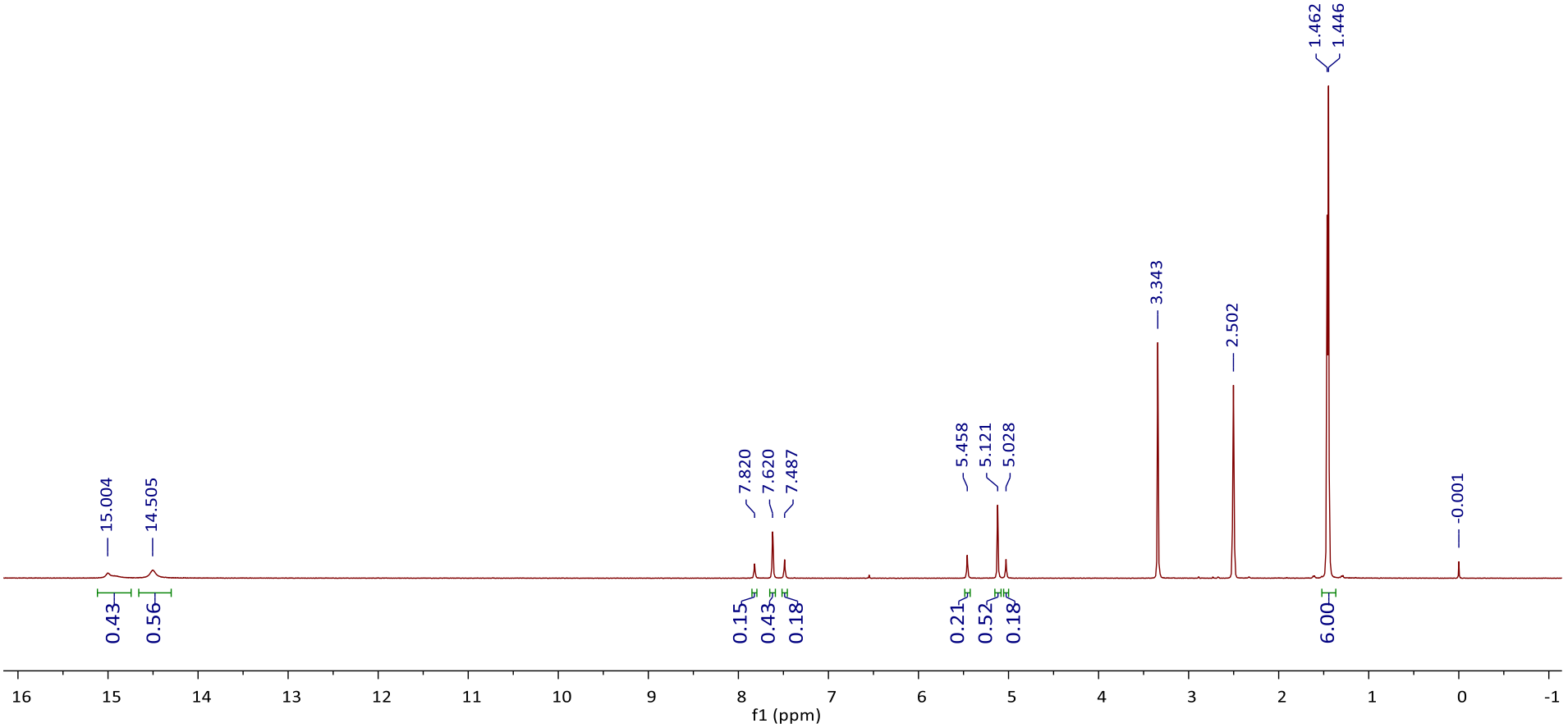


SUPPORTING INFORMATION

Compound T3 ^{1}H NMR (400 MHz, DMSO-d_6)

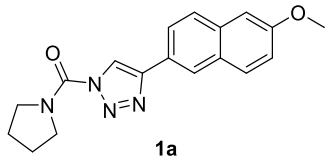


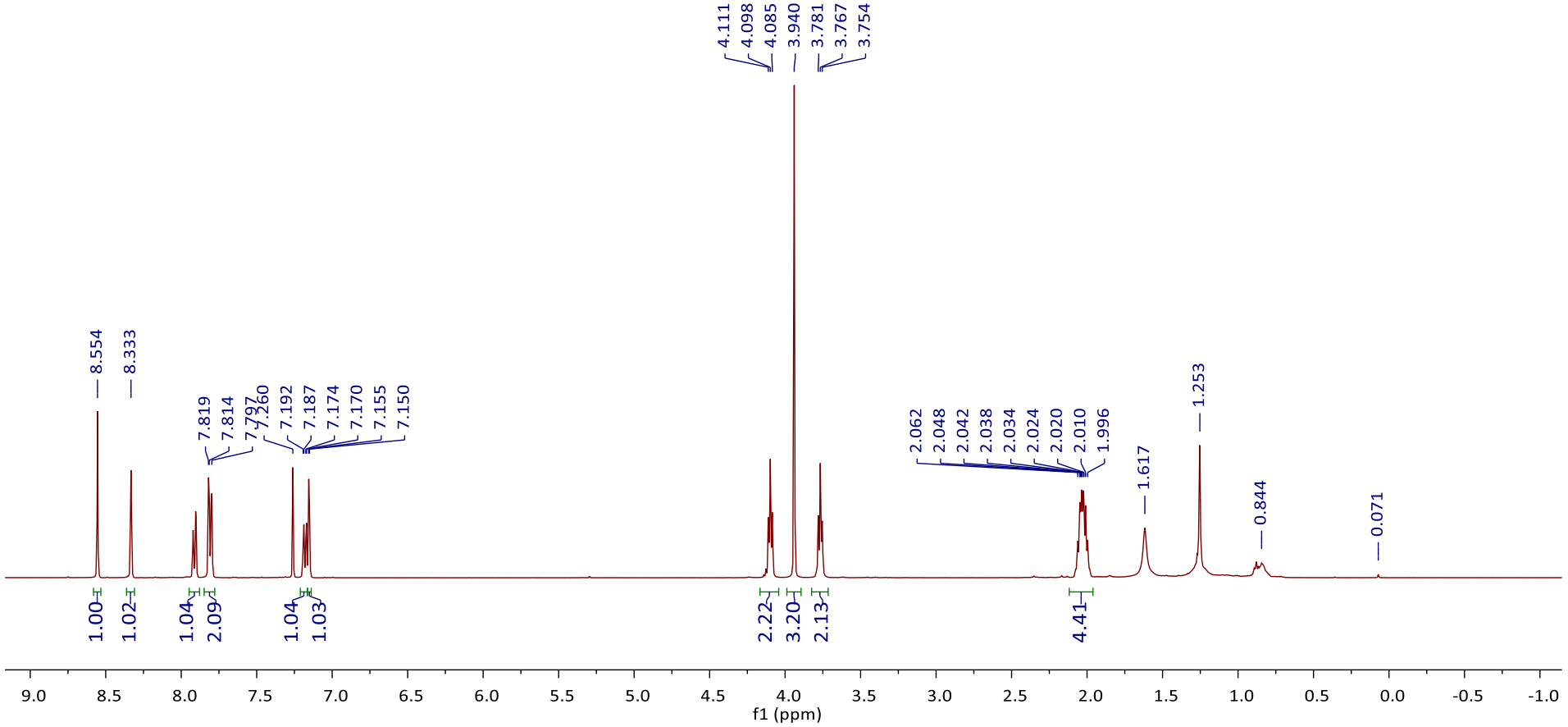
T3



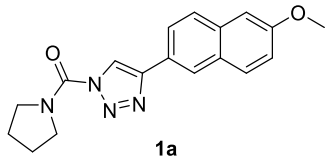
SUPPORTING INFORMATION

Compound 1a ^{1}H NMR (500 MHz, $CDCl_3$)

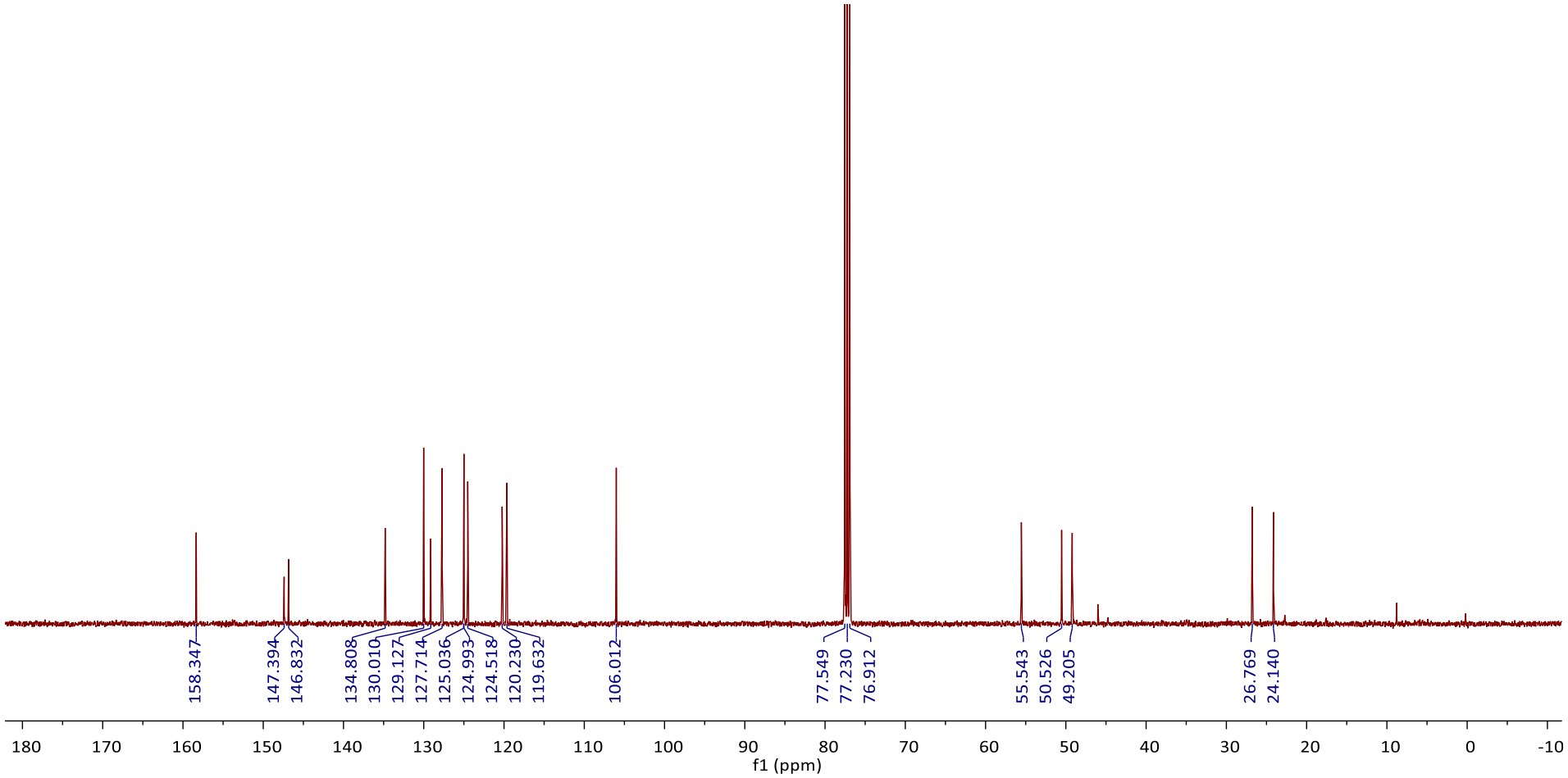




Compound 1a ^{13}C NMR (100 MHz, $CDCl_3$)

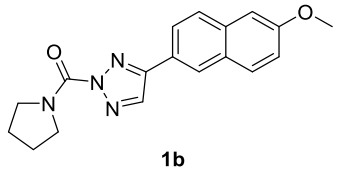


1a

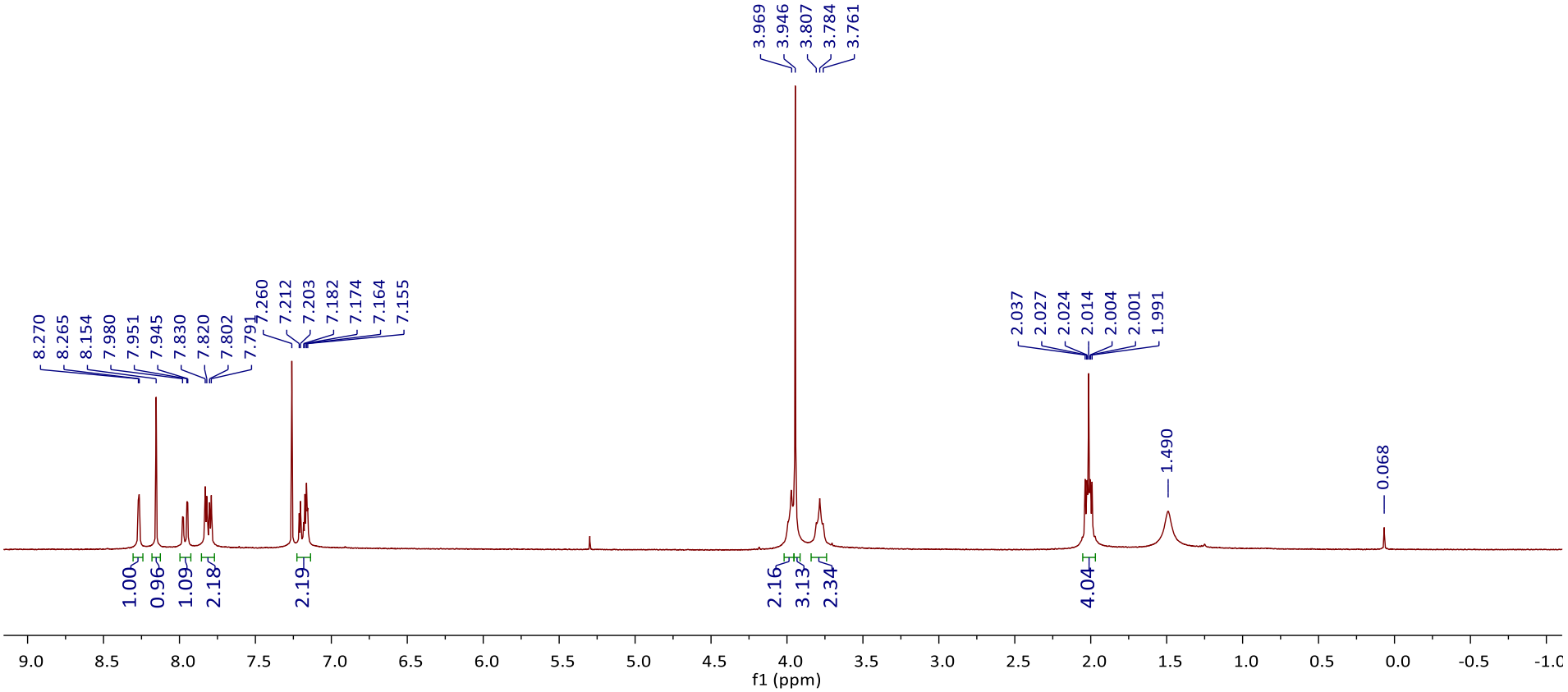


SUPPORTING INFORMATION

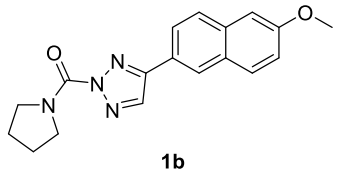
Compound 1b ^{1}H NMR (300 MHz, $CDCl_3$)



1b



Compound 1b ^{13}C NMR (100 MHz, $CDCl_3$)



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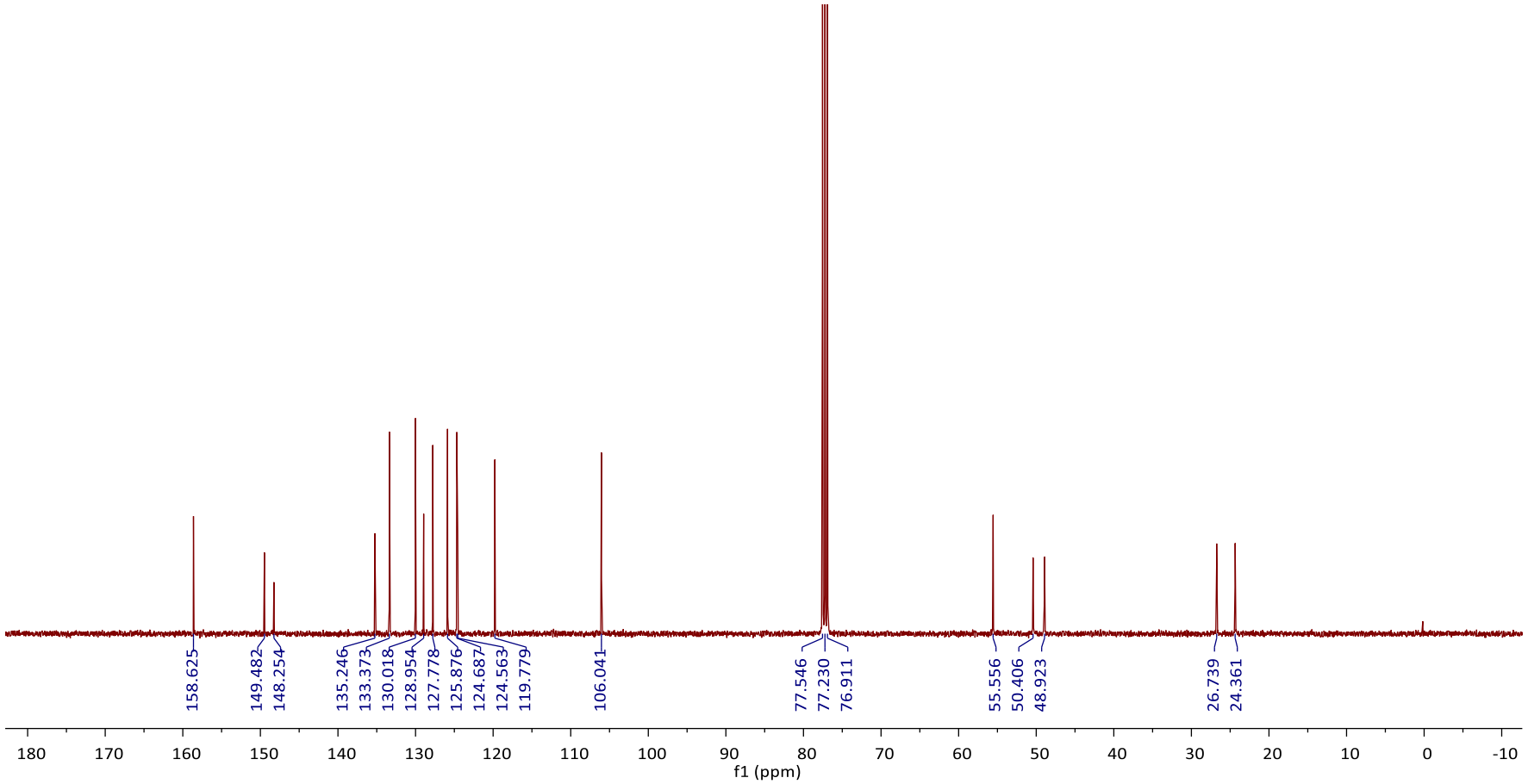
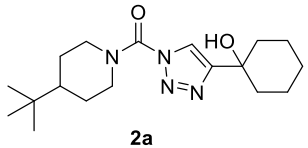
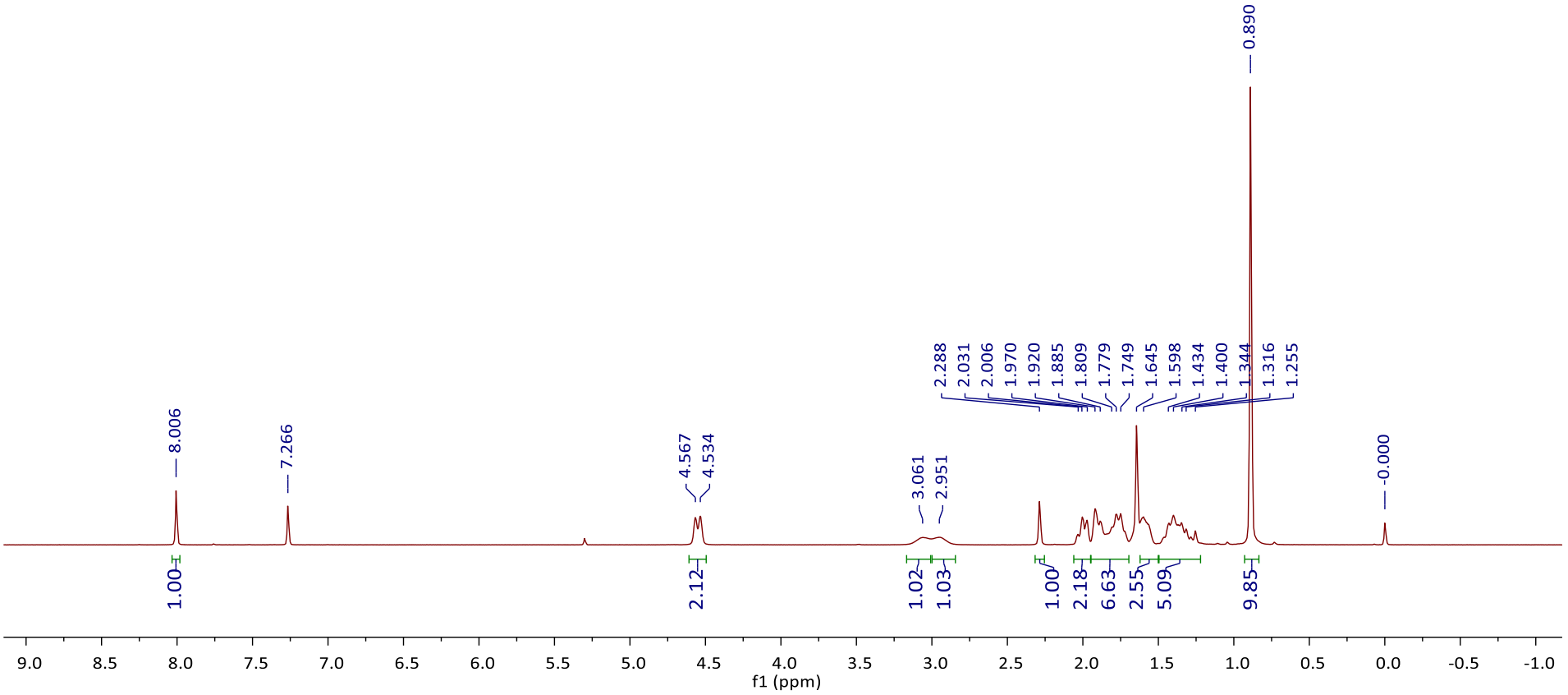


SUPPORTING INFORMATION

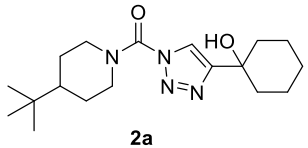
Compound 2a ^{1}H NMR (400 MHz, $CDCl_3$)



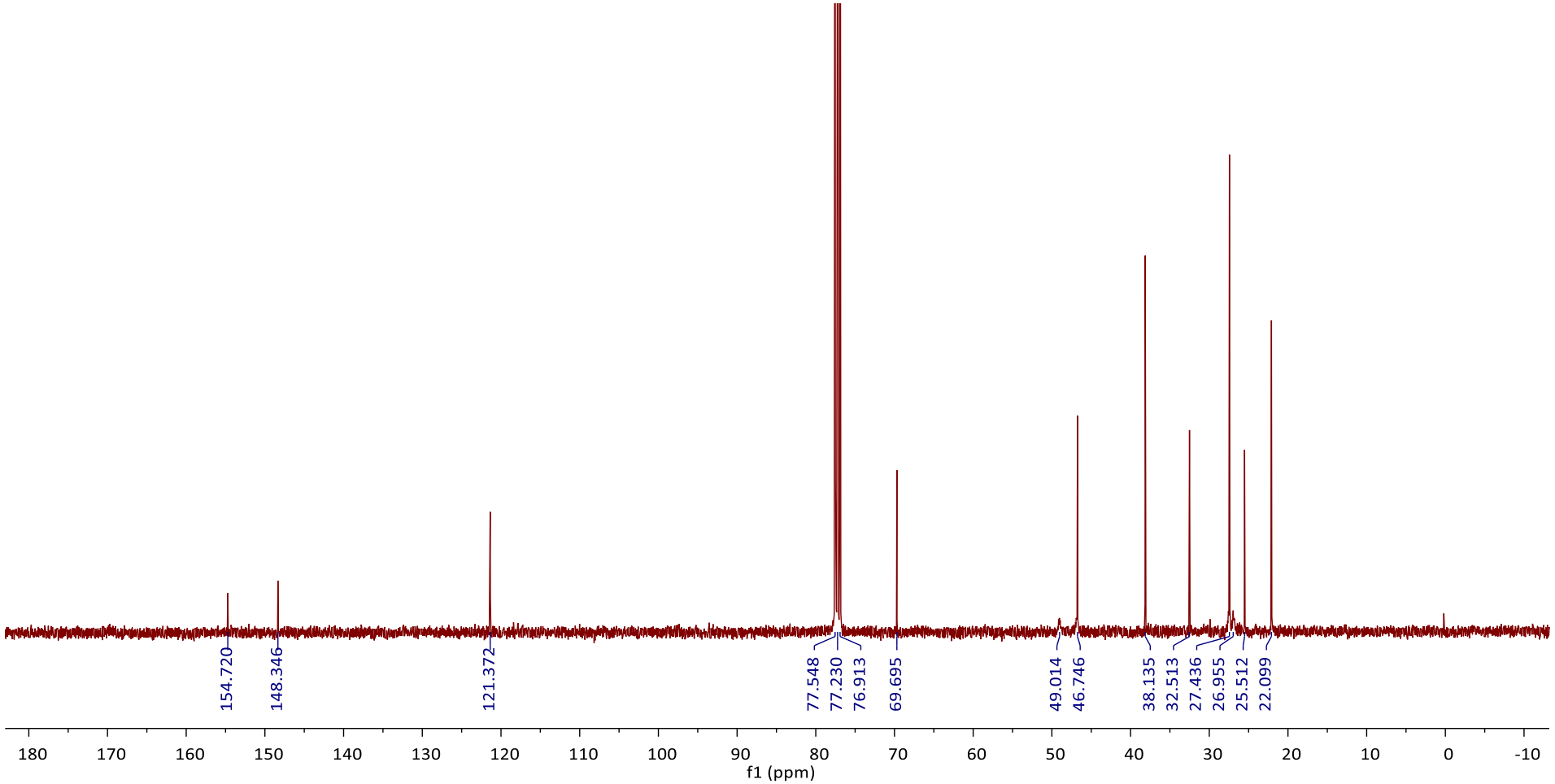
2a



Compound 2a ^{13}C NMR (100 MHz, $CDCl_3$)

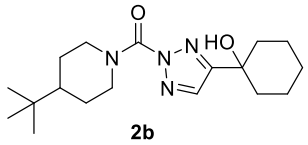


2a



SUPPORTING INFORMATION

Compound 2b ^{1}H NMR (400 MHz, $CDCl_3$)



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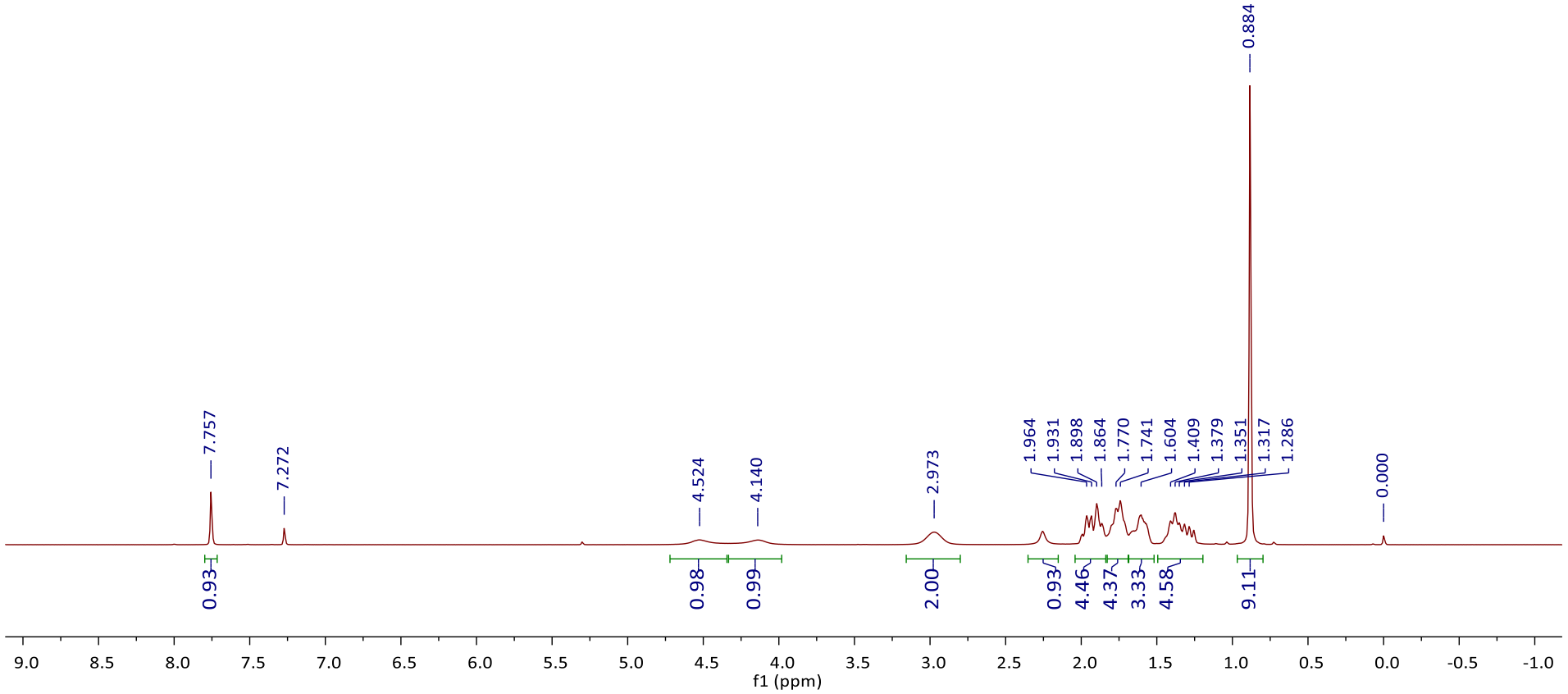
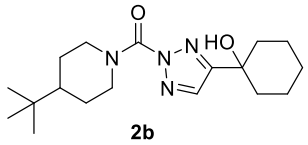
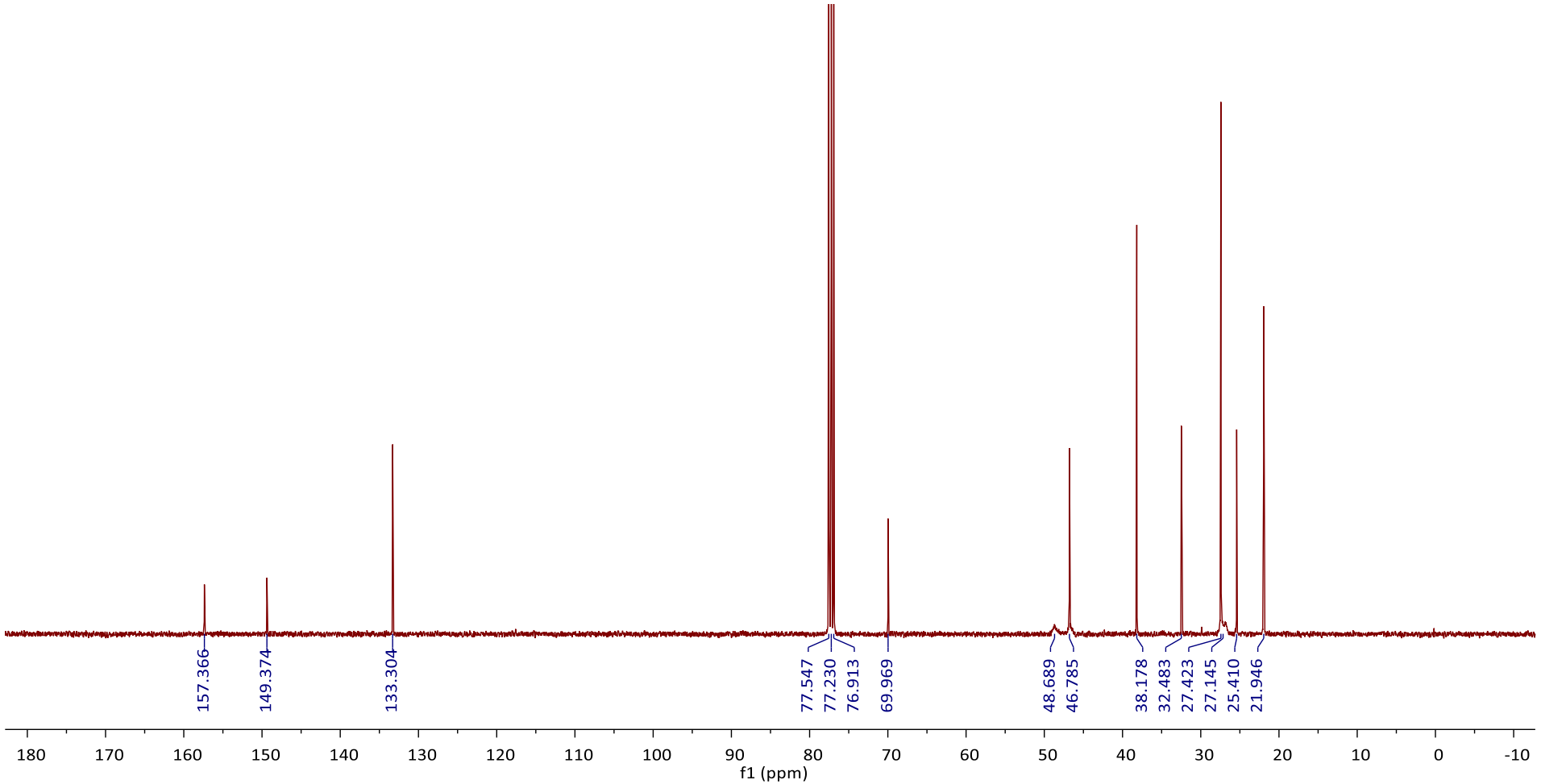


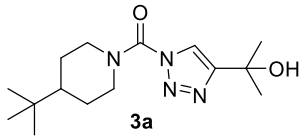
Compound 2b ^{13}C NMR (100 MHz, $CDCl_3$)



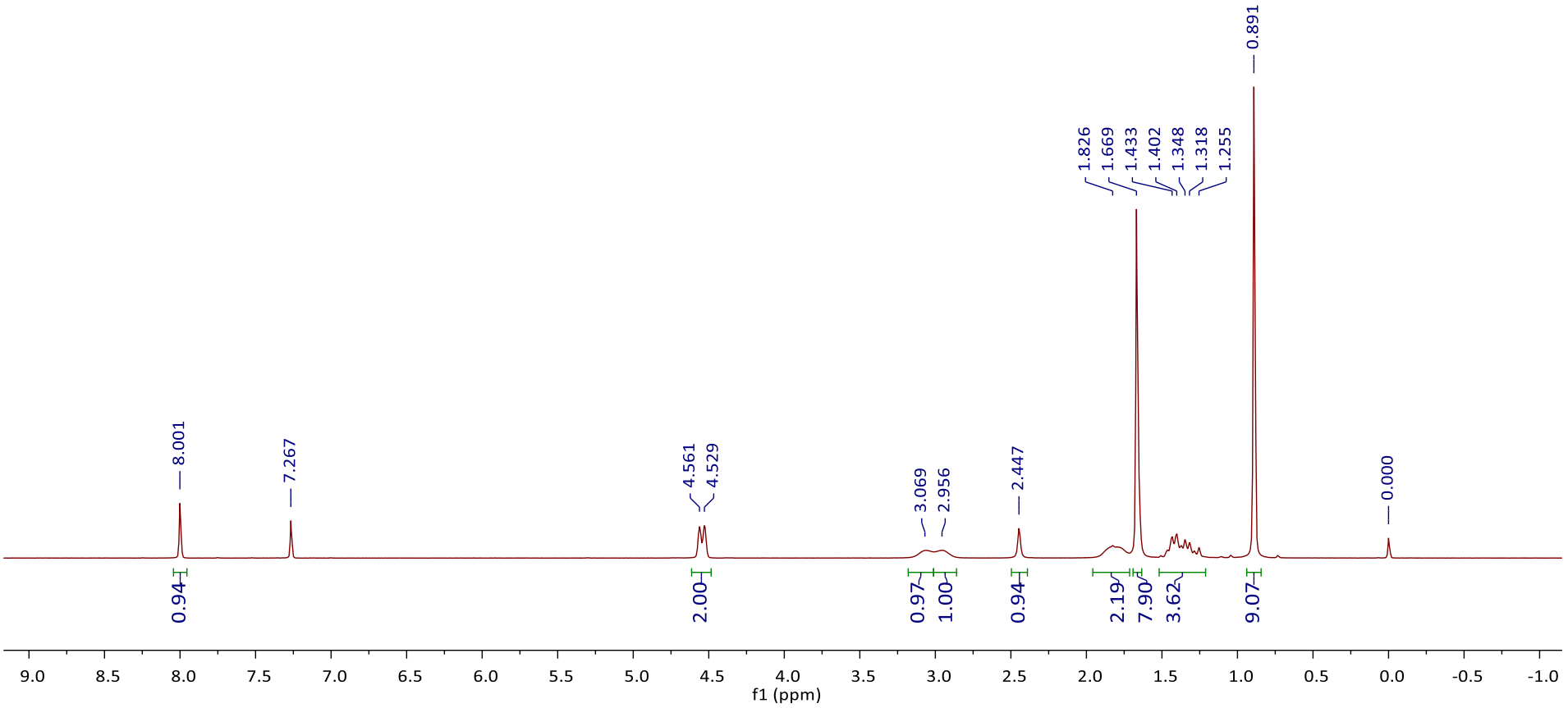
2b



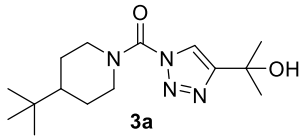
Compound 3a ^{1}H NMR (400 MHz, $CDCl_3$)



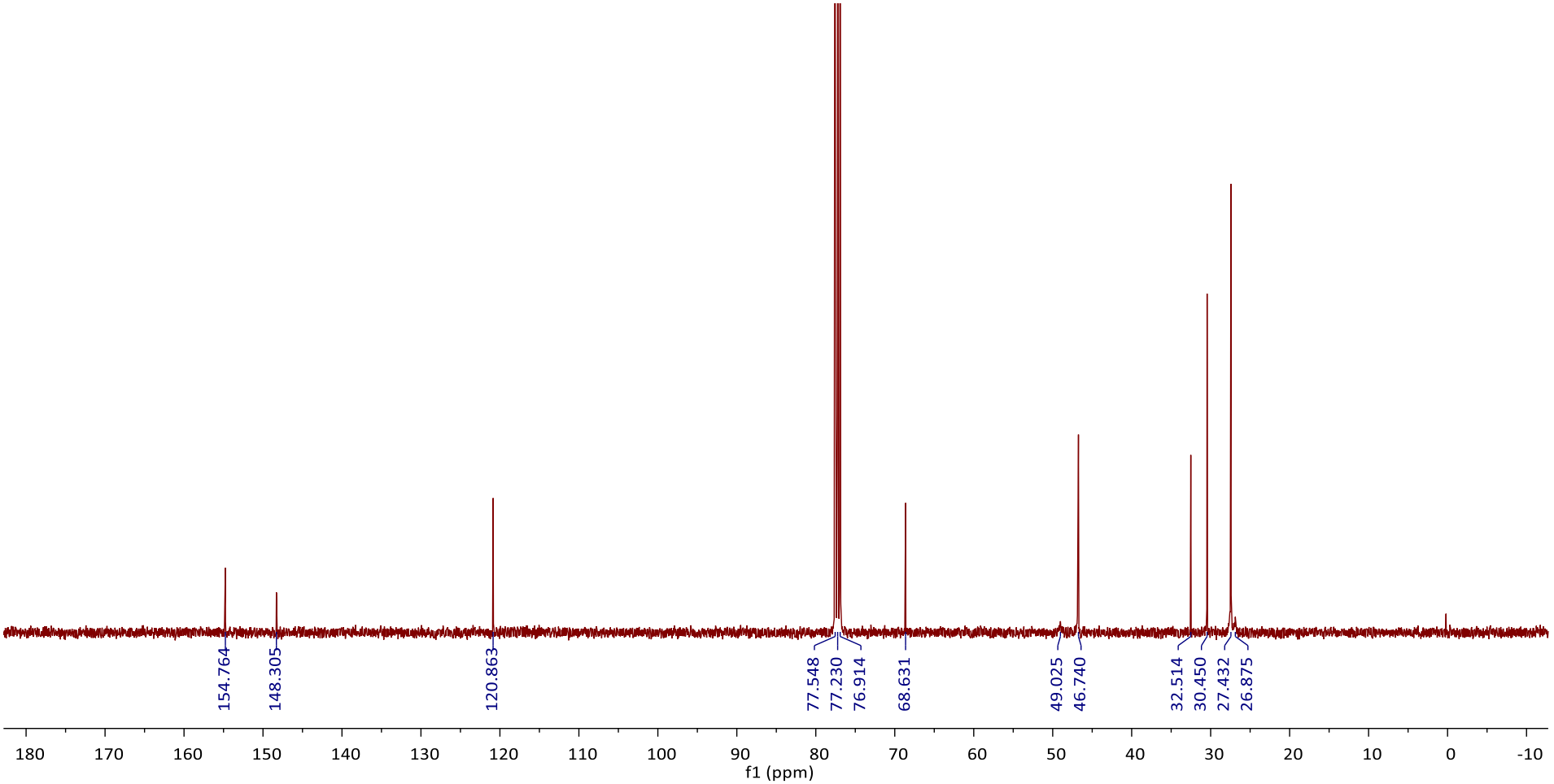
3a



Compound 3a ^{13}C NMR (100 MHz, $CDCl_3$)

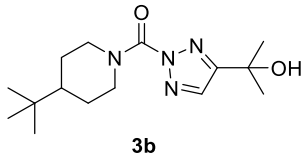


3a



SUPPORTING INFORMATION

Compound 3b ^{1}H NMR (400 MHz, $CDCl_3$)



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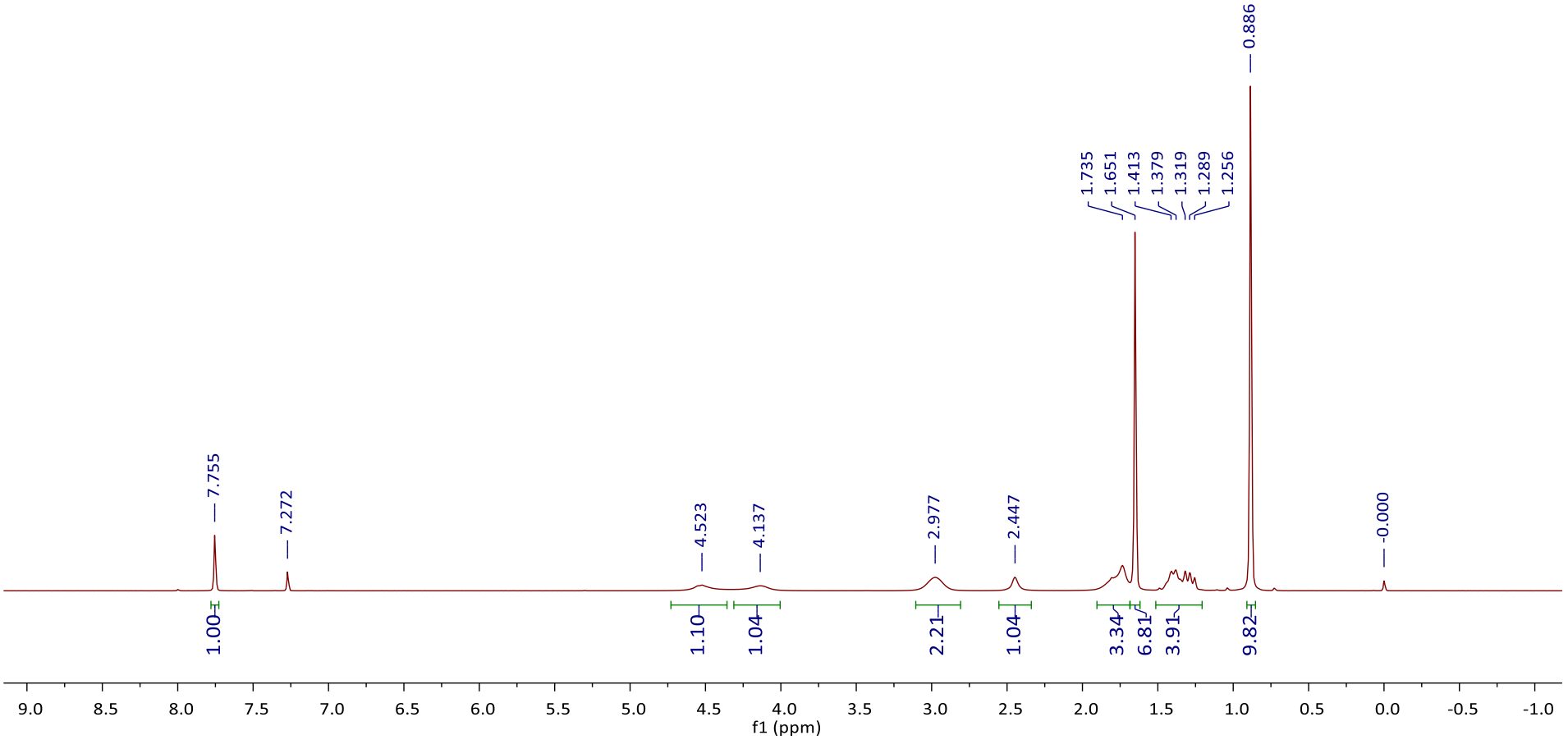
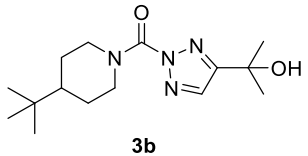


Compound 3b ^{13}C NMR (100 MHz, $CDCl_3$)



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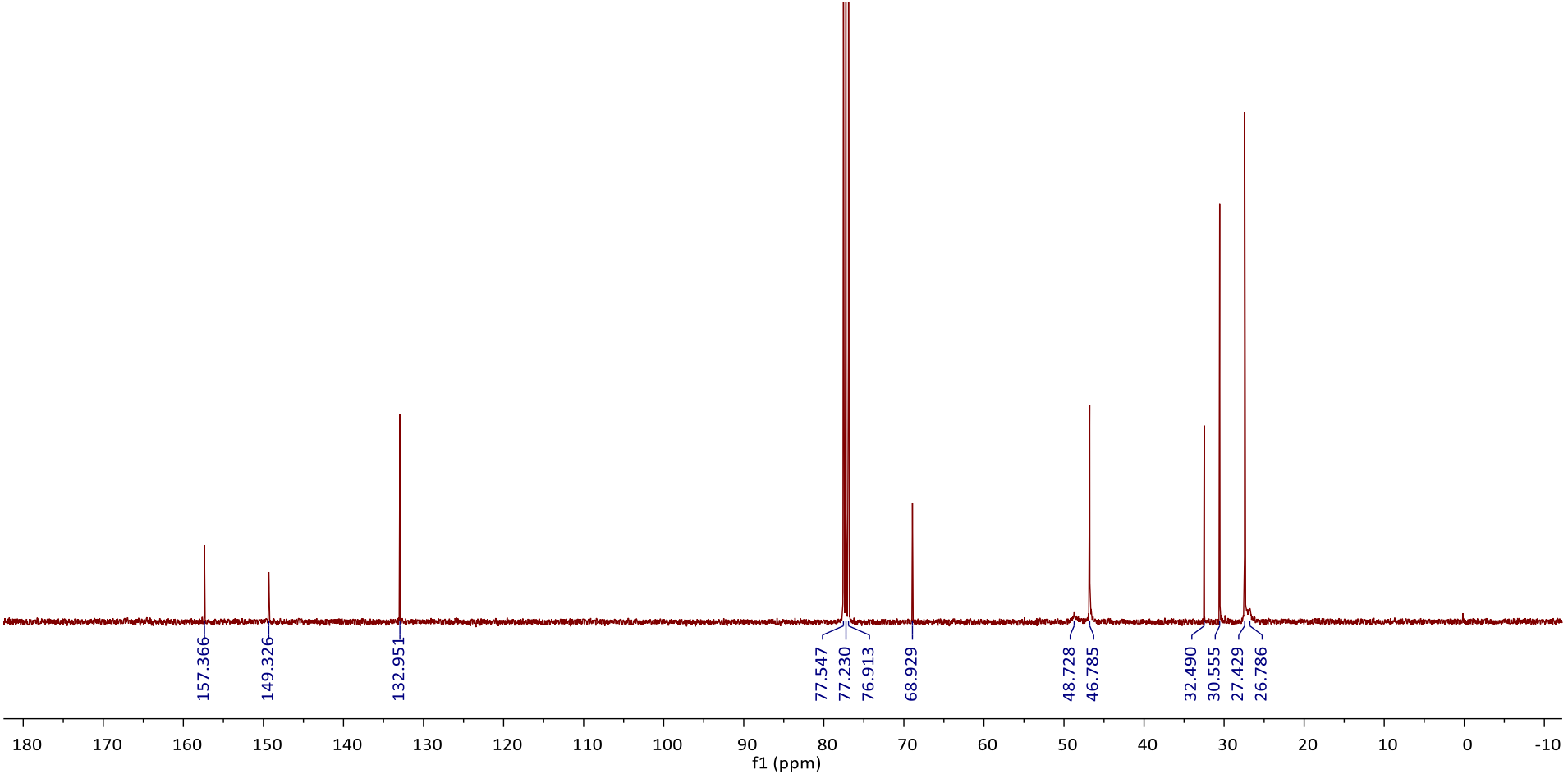
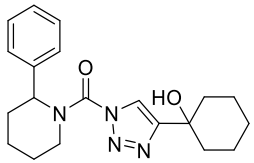


SUPPORTING INFORMATION

Compound 4a ^{1}H NMR (400 MHz, $CDCl_3$)



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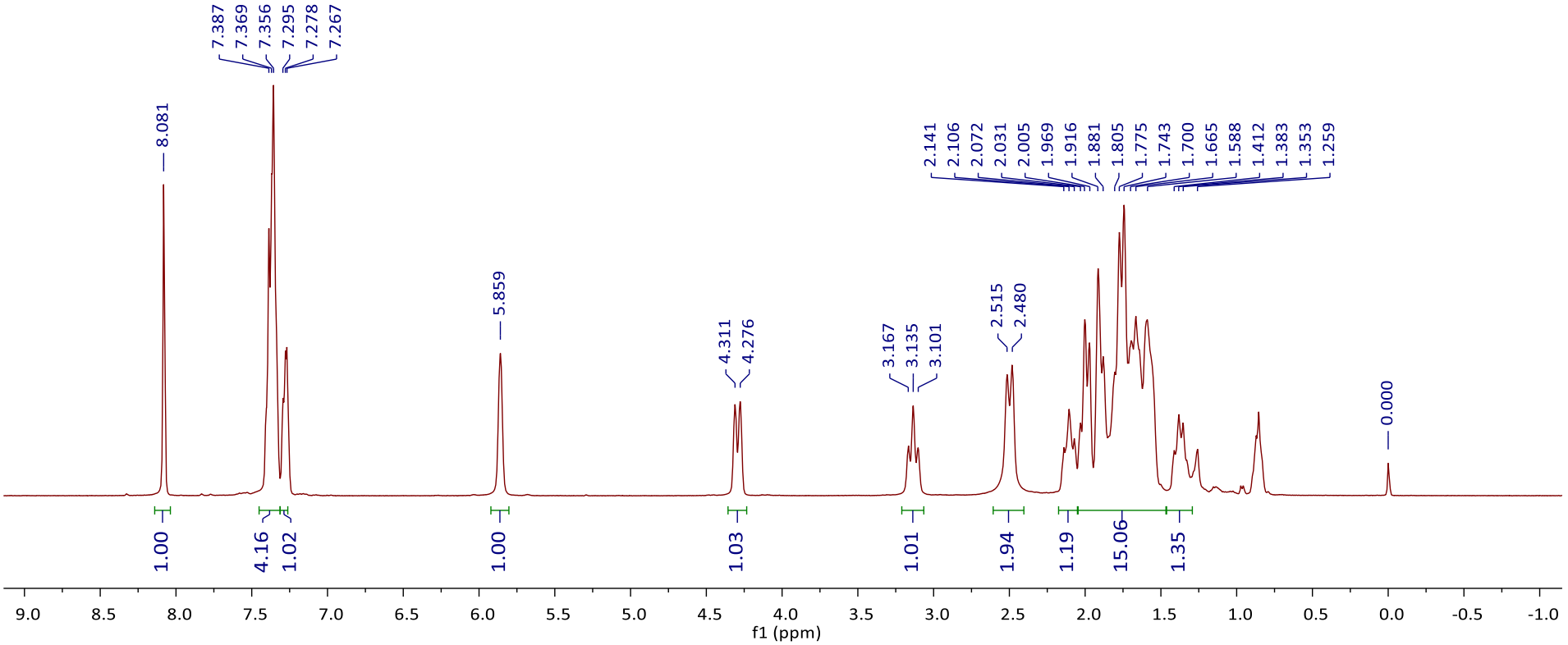
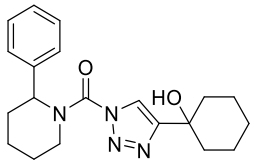


Compound 4a ^{13}C NMR (100 MHz, $CDCl_3$)



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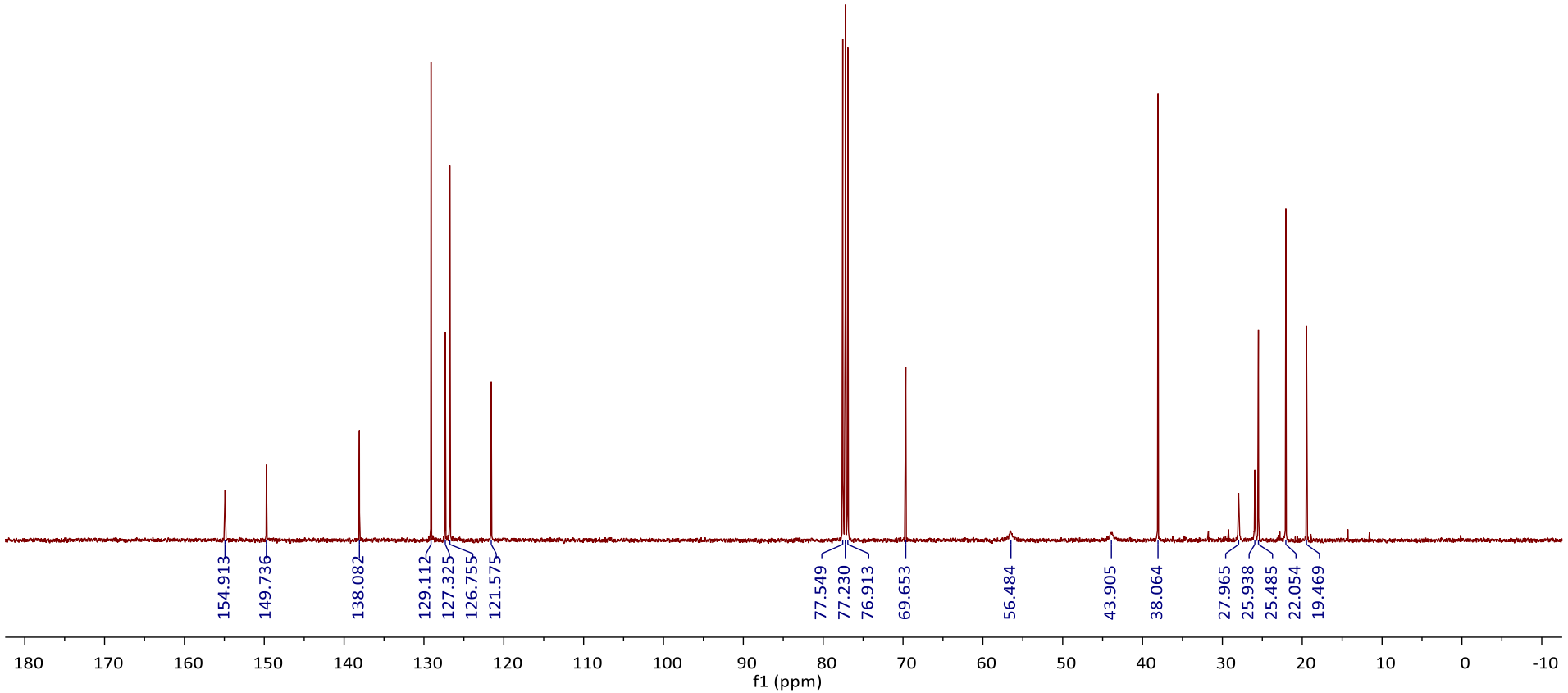
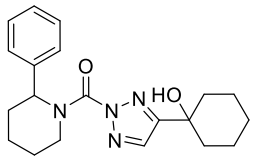


SUPPORTING INFORMATION

Compound 4b ^{1}H NMR (400 MHz, $CDCl_3$)



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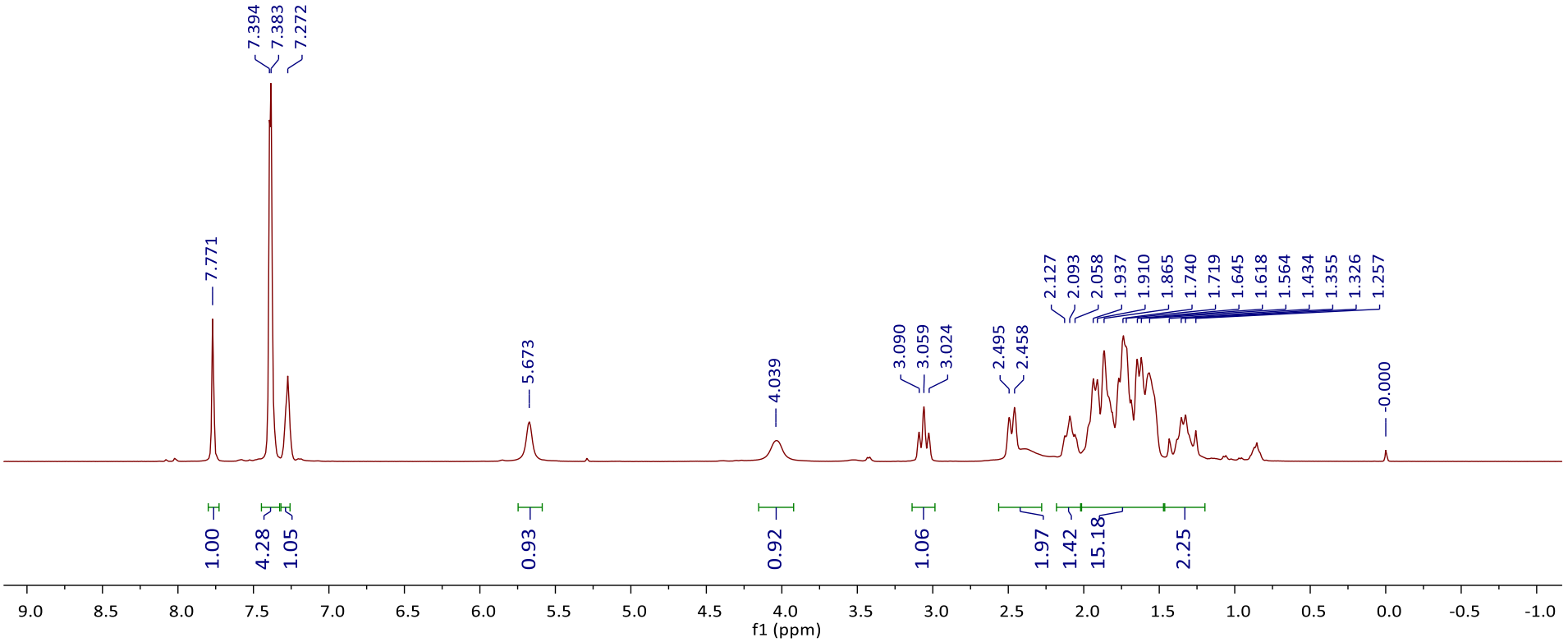
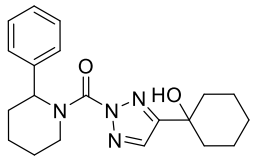
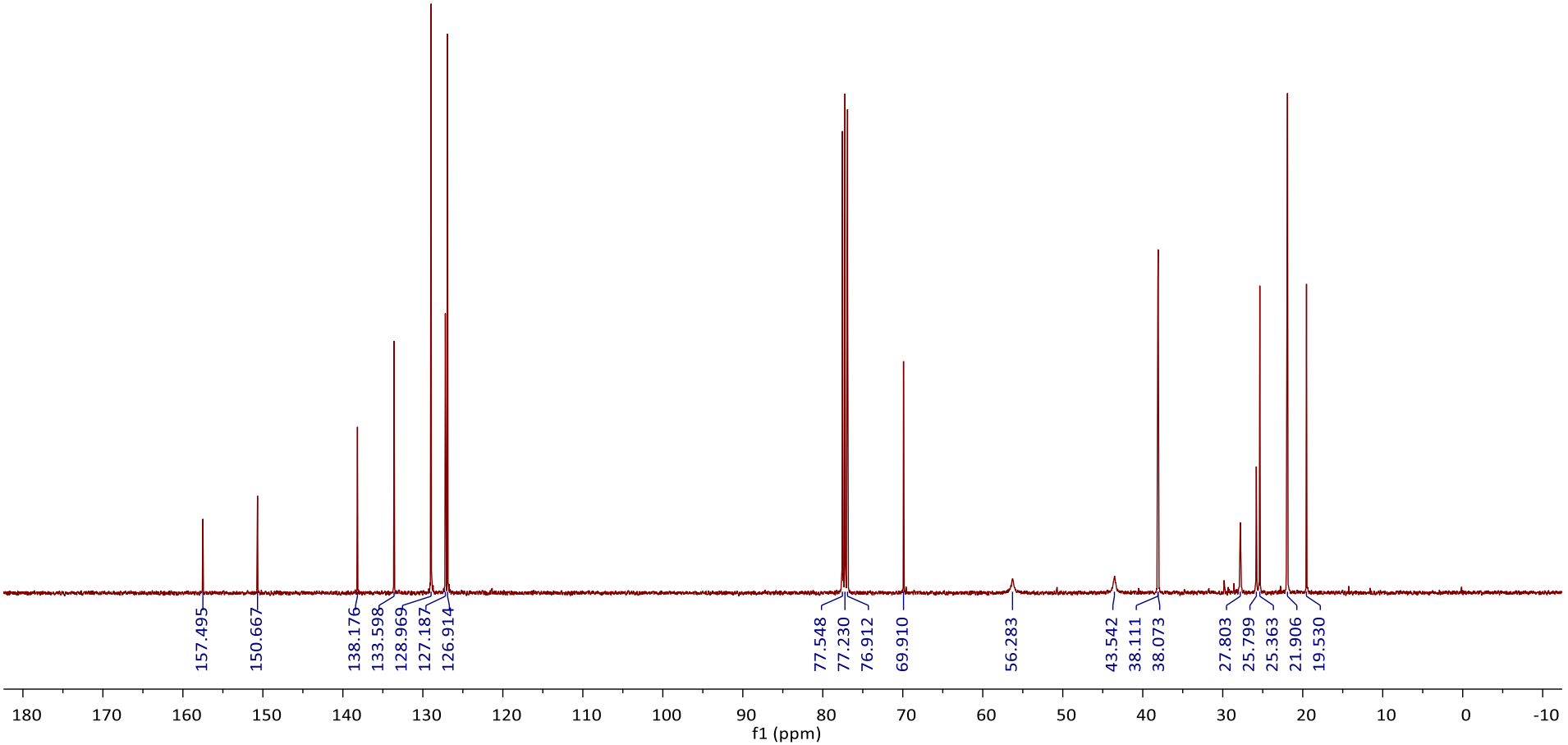


Compound 4b ^{13}C NMR (100 MHz, $CDCl_3$)

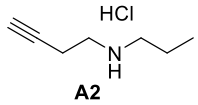


4b

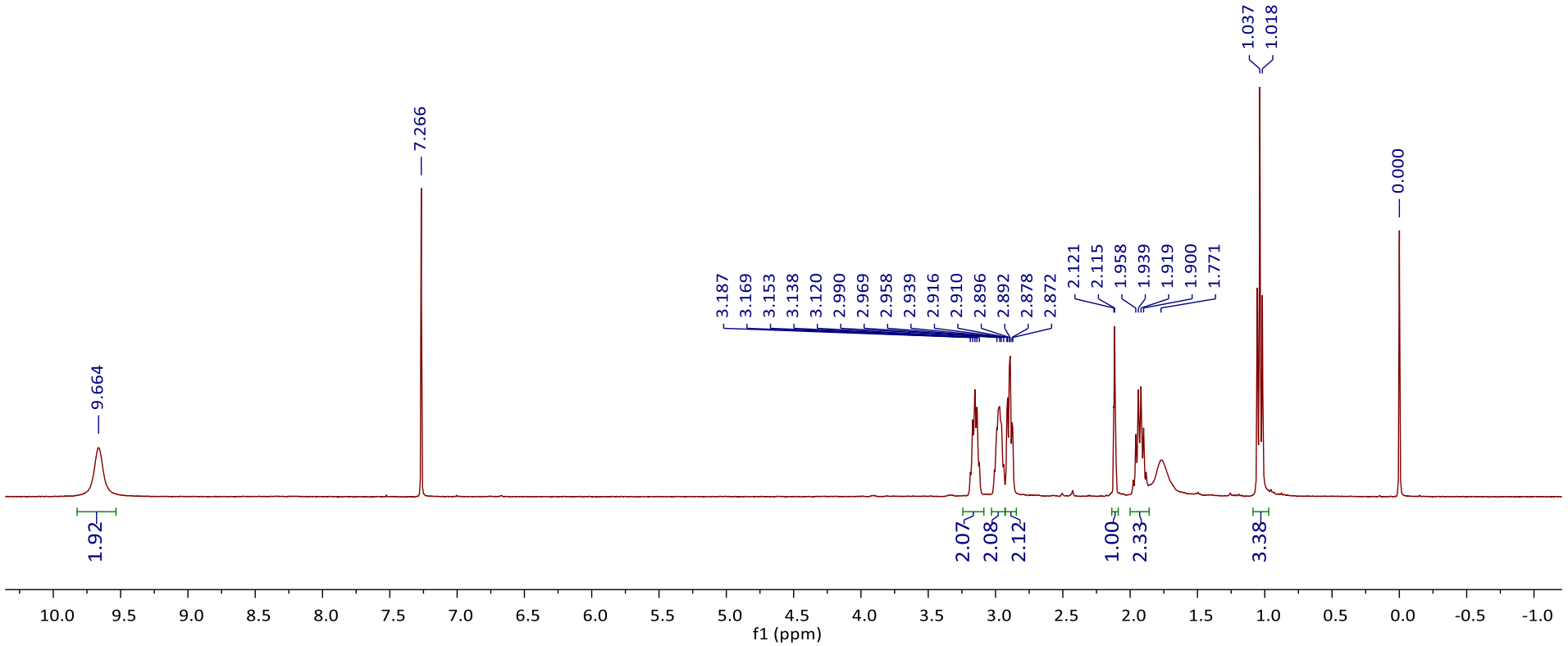


SUPPORTING INFORMATION

Compound A2 ^{1}H NMR (400 MHz, $CDCl_3$)

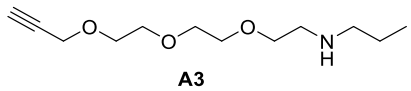


A2

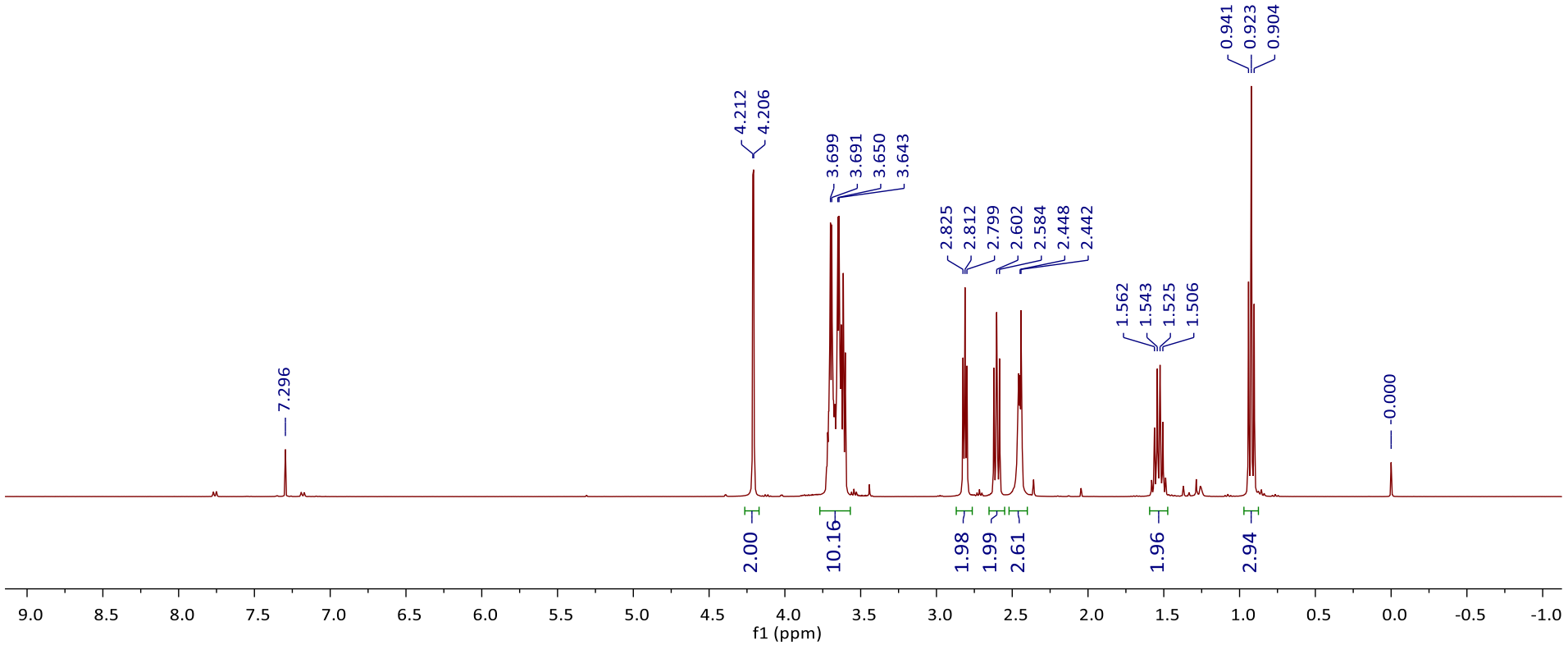


SUPPORTING INFORMATION

Compound A3 ^{1}H NMR (400 MHz, $CDCl_3$)

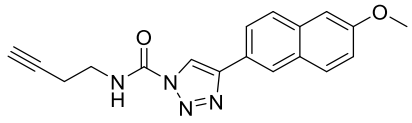


A3

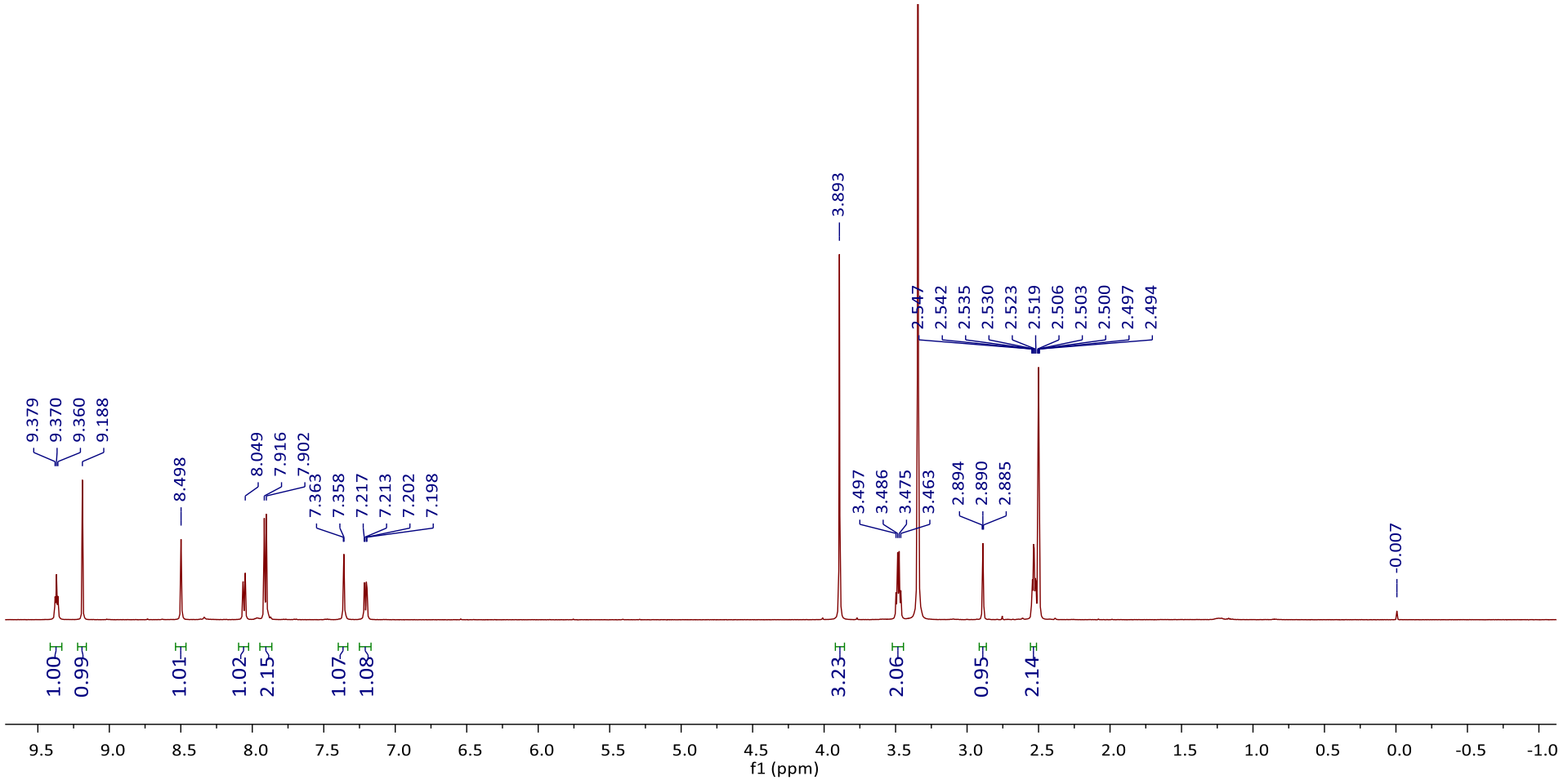


SUPPORTING INFORMATION

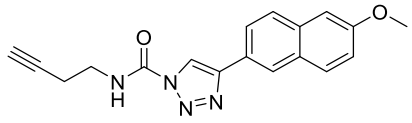
Compound 5a ^{1}H NMR (600 MHz, DMSO-d_6)



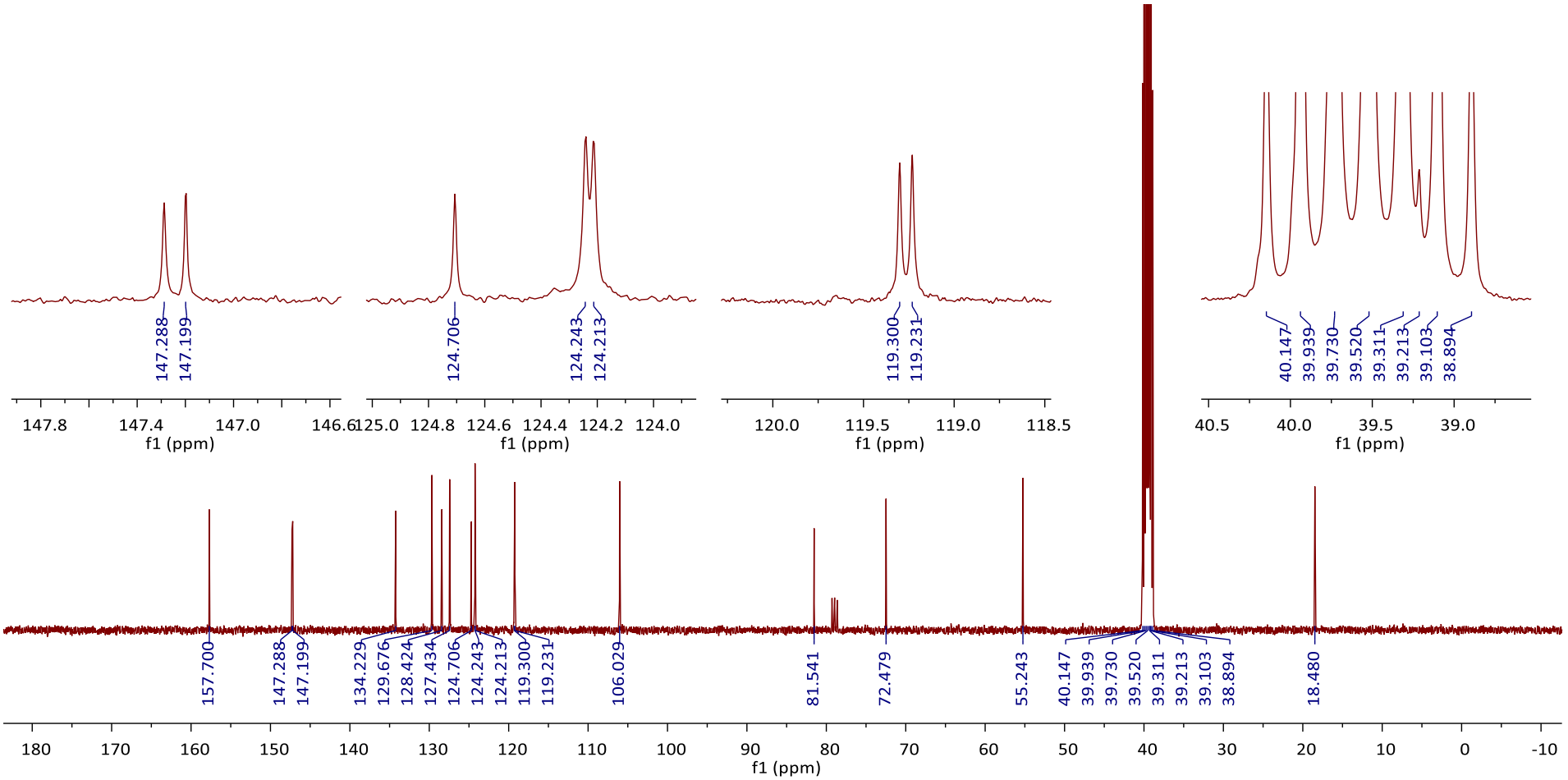
5a



Compound 5a ^{13}C NMR (100 MHz, DMSO-d_6)

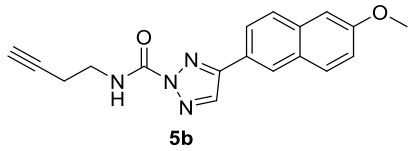


5a



SUPPORTING INFORMATION

Compound 5b ^{1}H NMR (600 MHz, $CDCl_3$)



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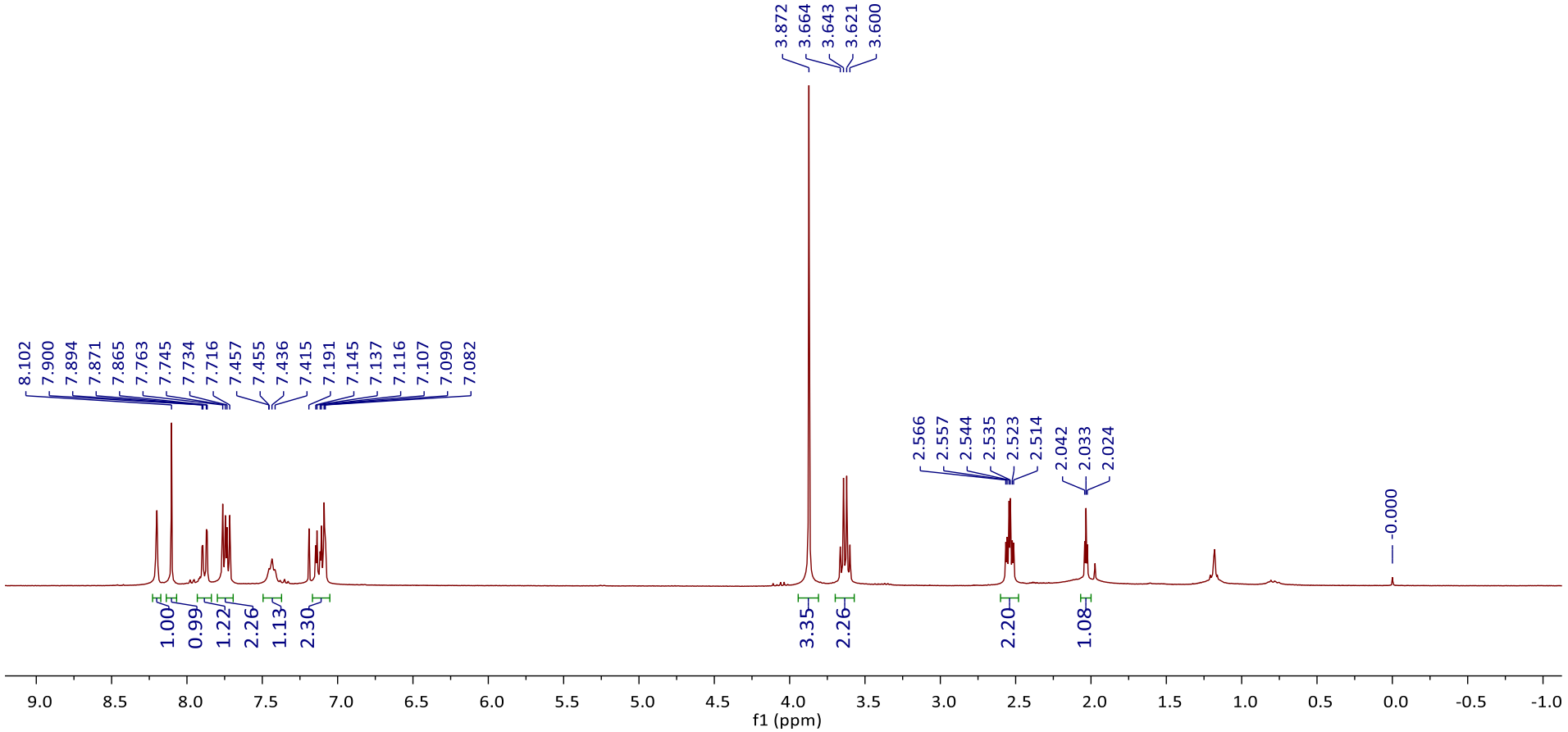
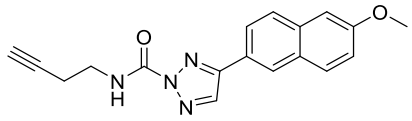
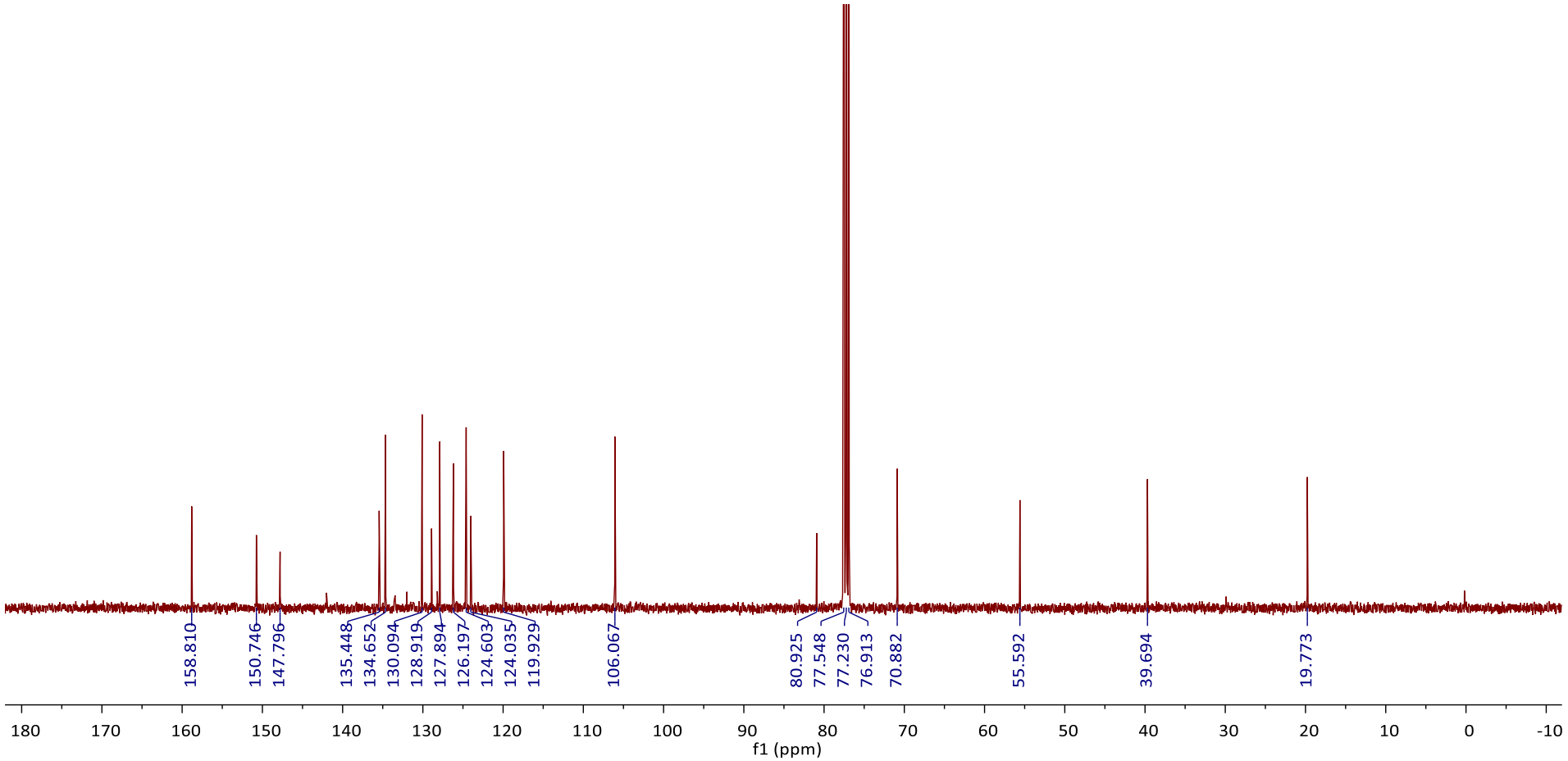


SUPPORTING INFORMATION

Compound 5b ^{13}C NMR (100 MHz, $CDCl_3$)

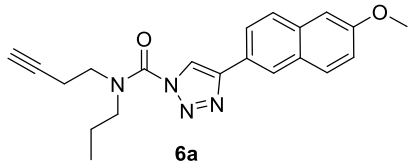


5b

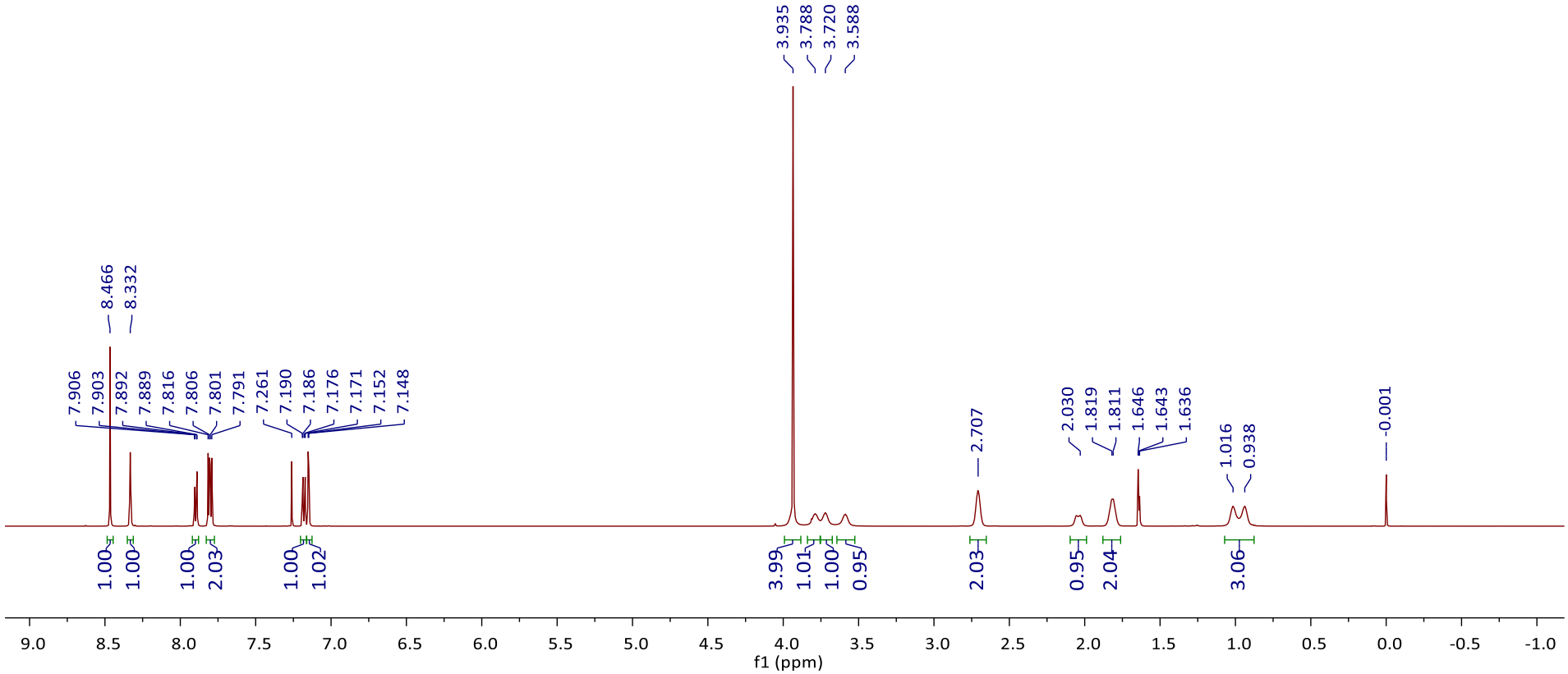


SUPPORTING INFORMATION

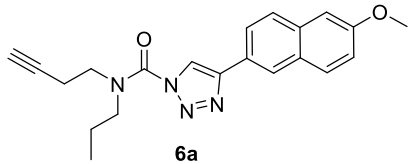
Compound 6a ^{1}H NMR (600 MHz, $CDCl_3$)



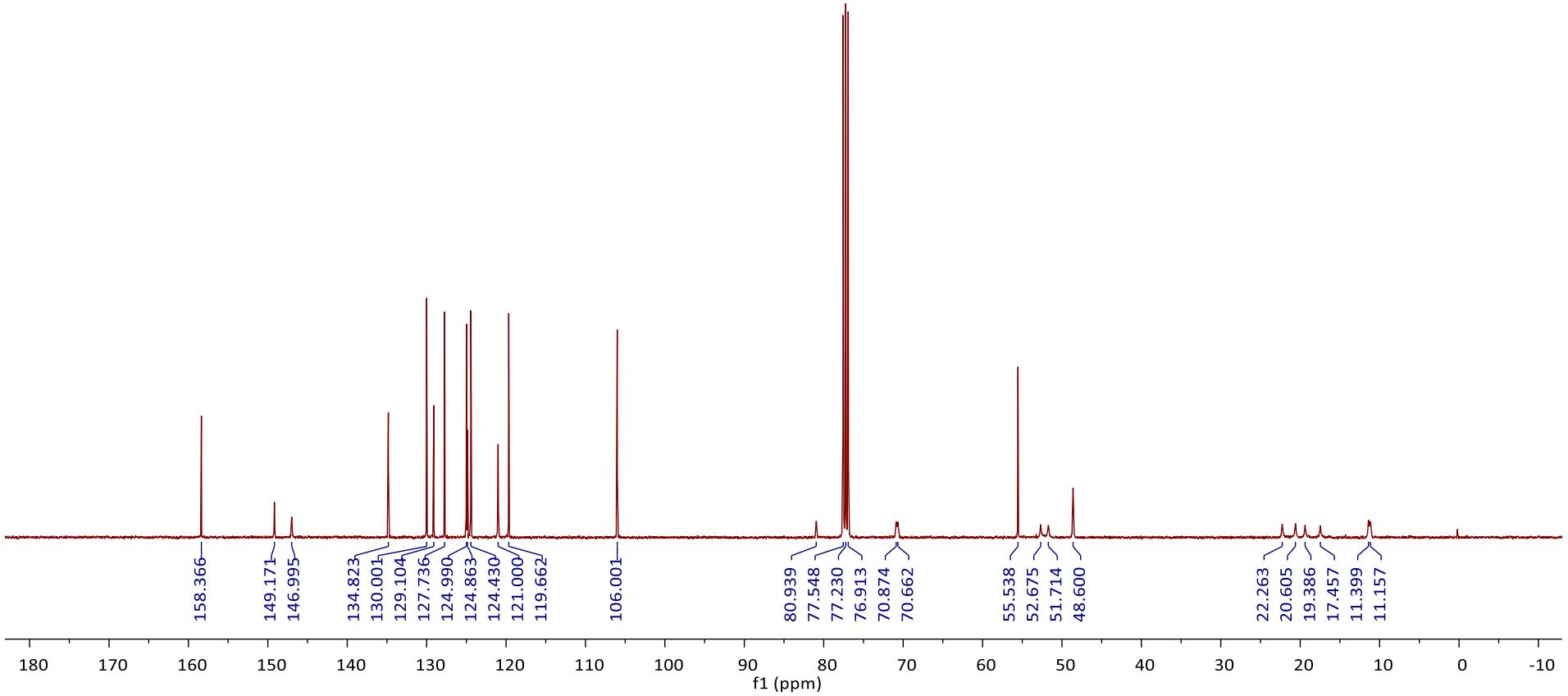
6a



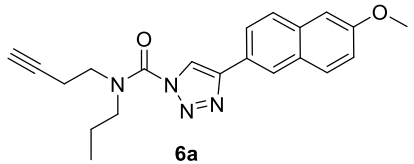
Compound 6a ^{13}C NMR (100 MHz, $CDCl_3$)



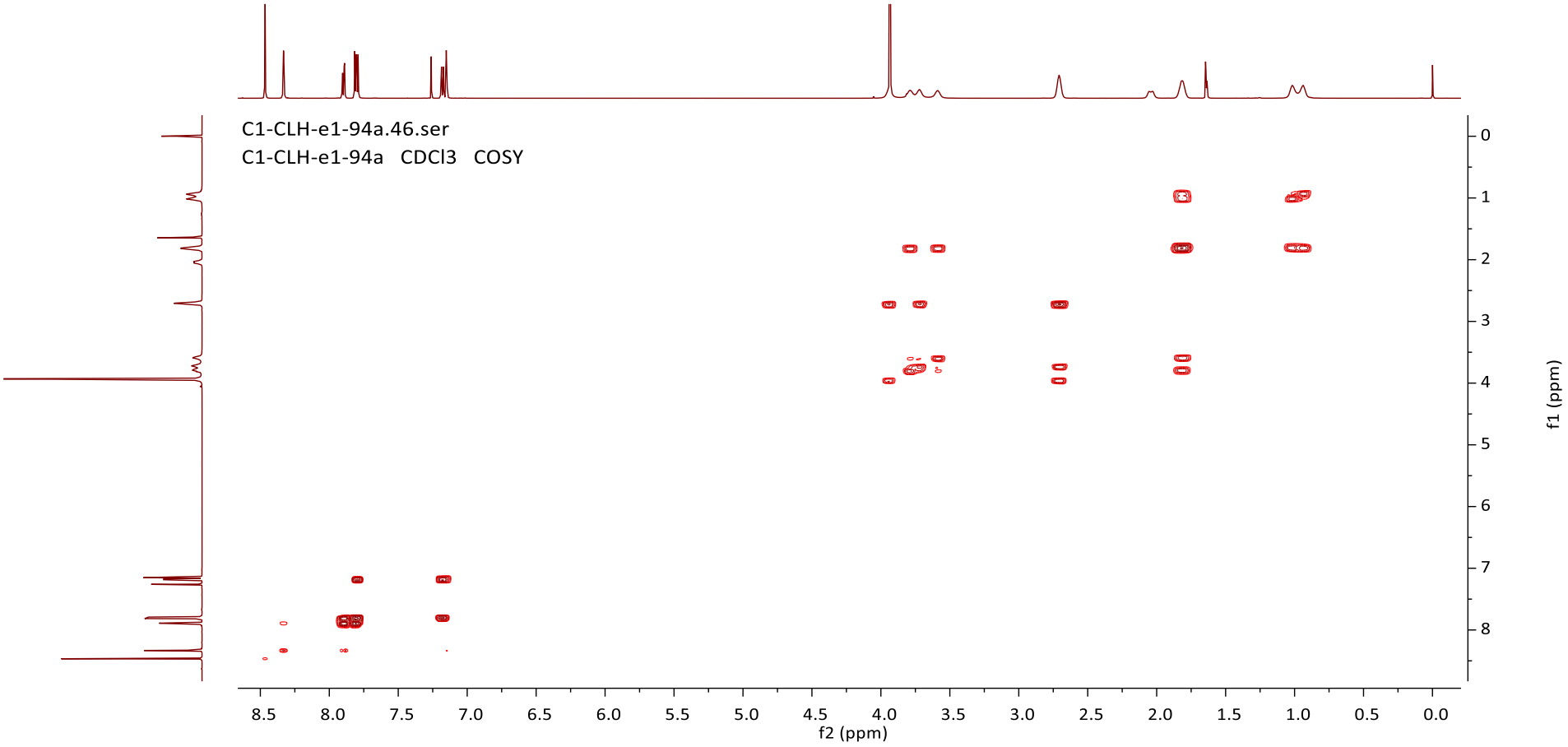
6a



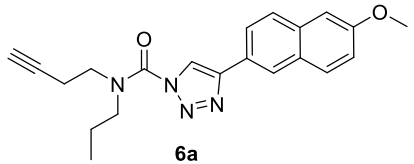
Compound 6a H-H COSY



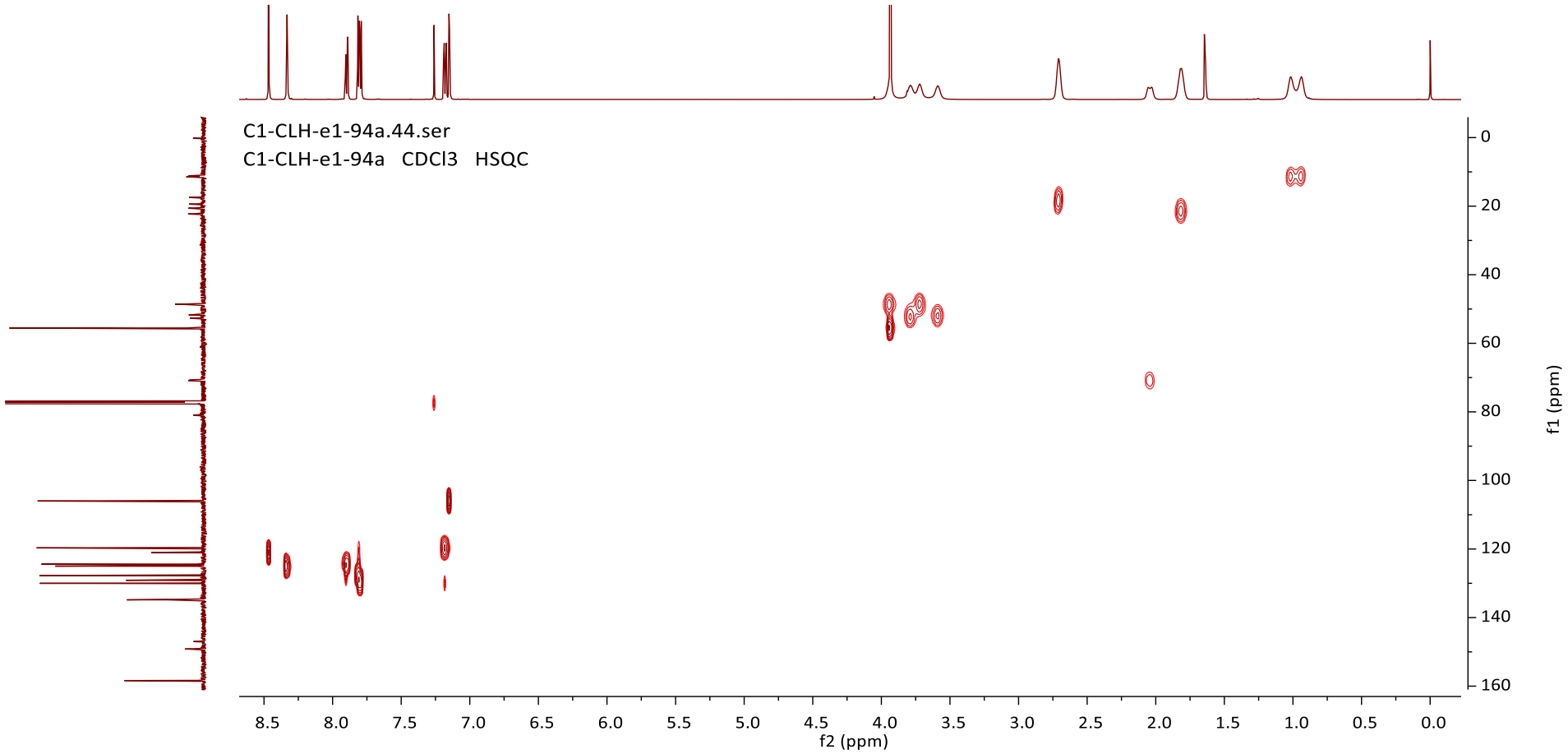
6a



Compound 6a HSQC

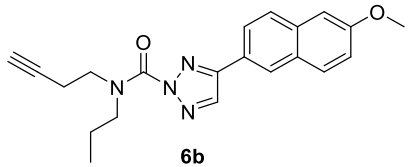


6a

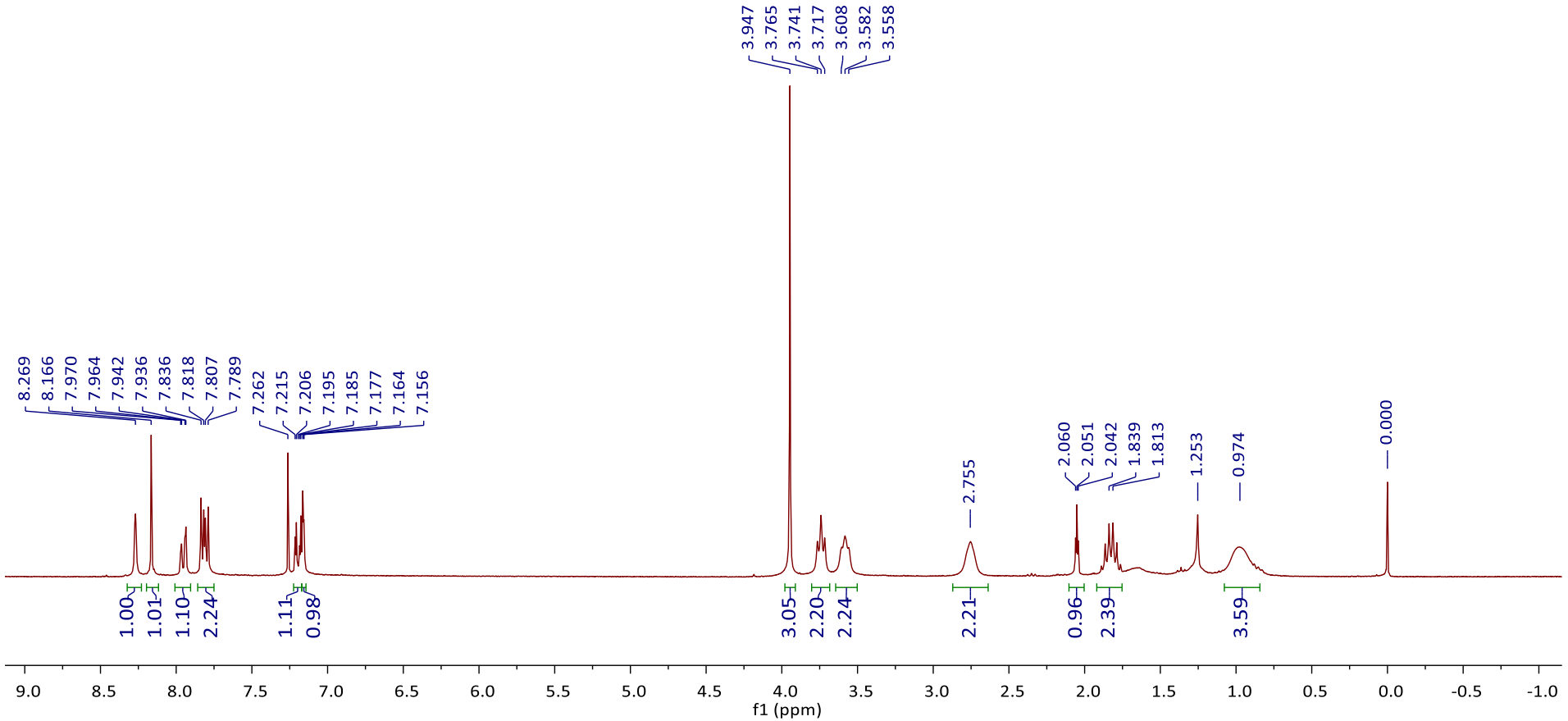


SUPPORTING INFORMATION

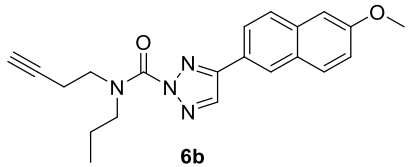
Compound 6b ^{1}H NMR (300 MHz, $CDCl_3$)



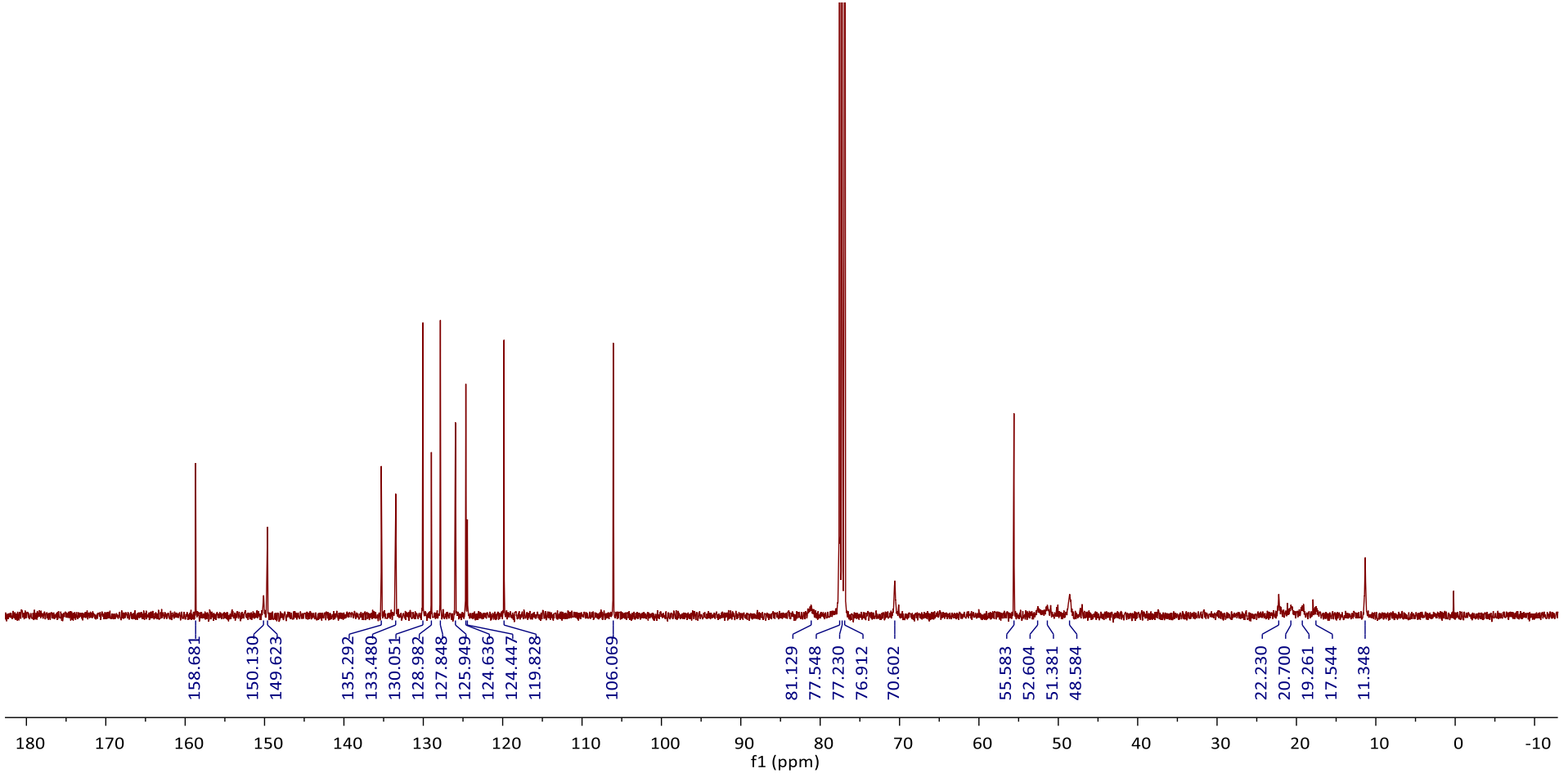
6b



Compound 6b ^{13}C NMR (100 MHz, $CDCl_3$)

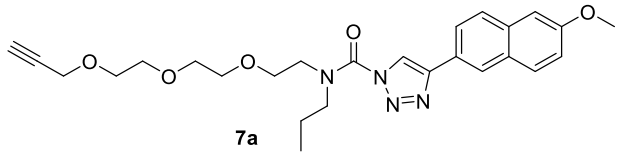


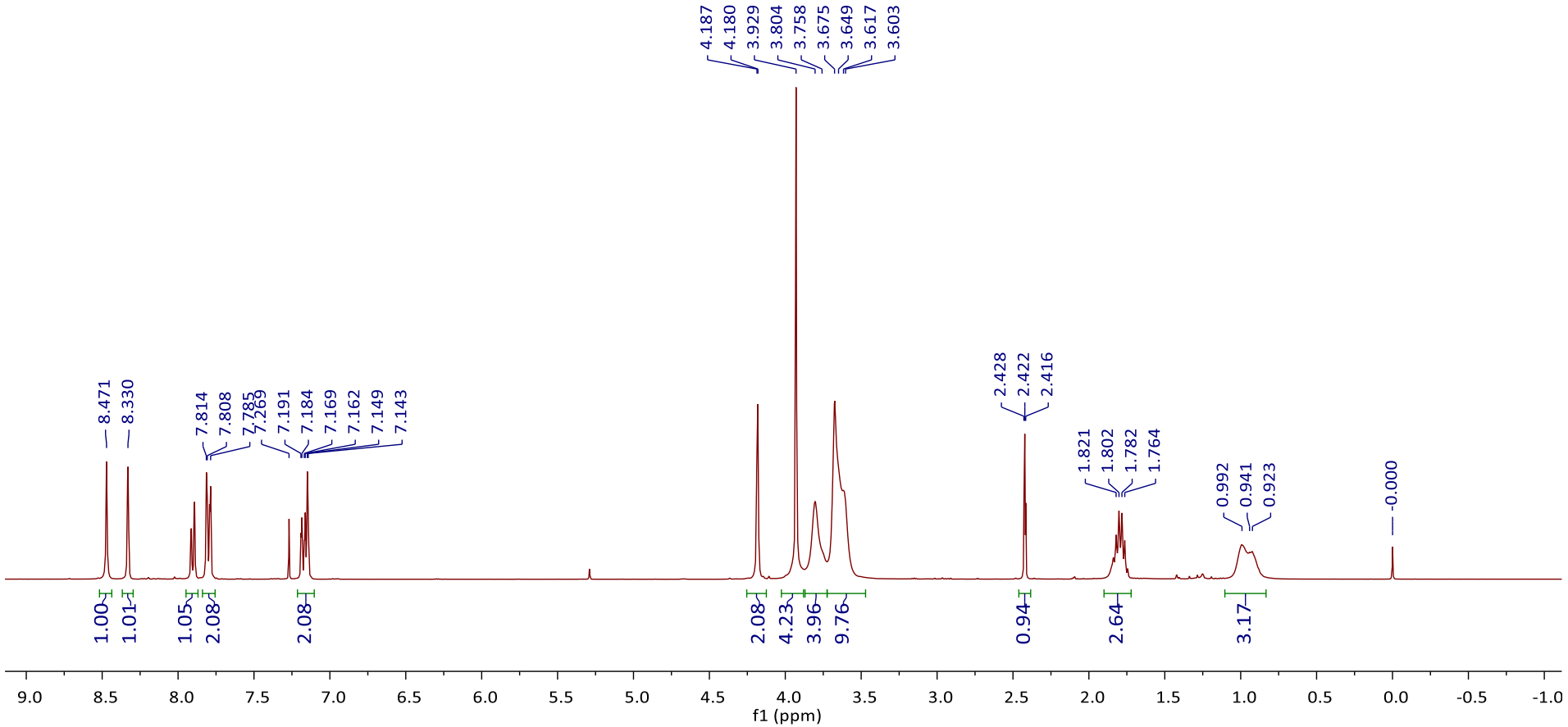
6b



SUPPORTING INFORMATION

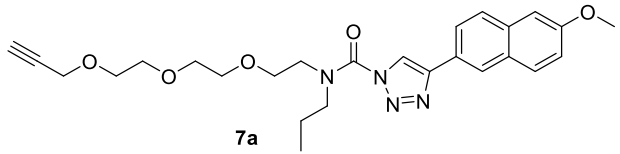
Compound 7a ^{1}H NMR (400 MHz, $CDCl_3$)



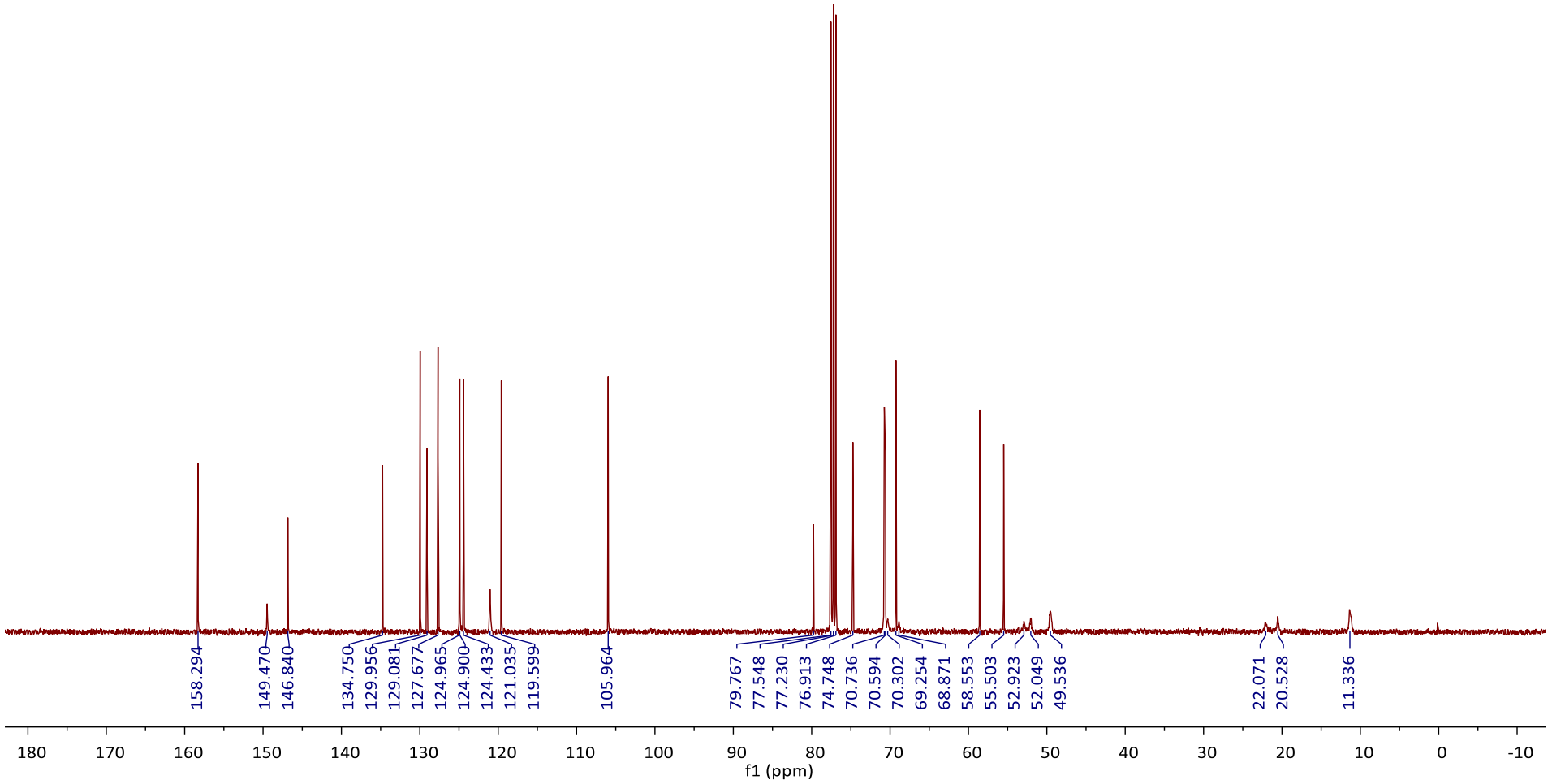


SUPPORTING INFORMATION

Compound 7a ^{13}C NMR (100 MHz, $CDCl_3$)

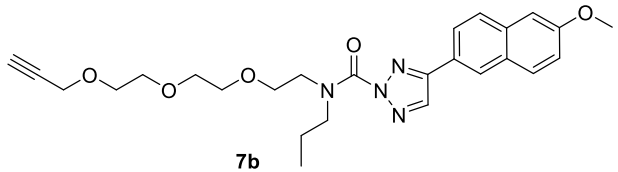


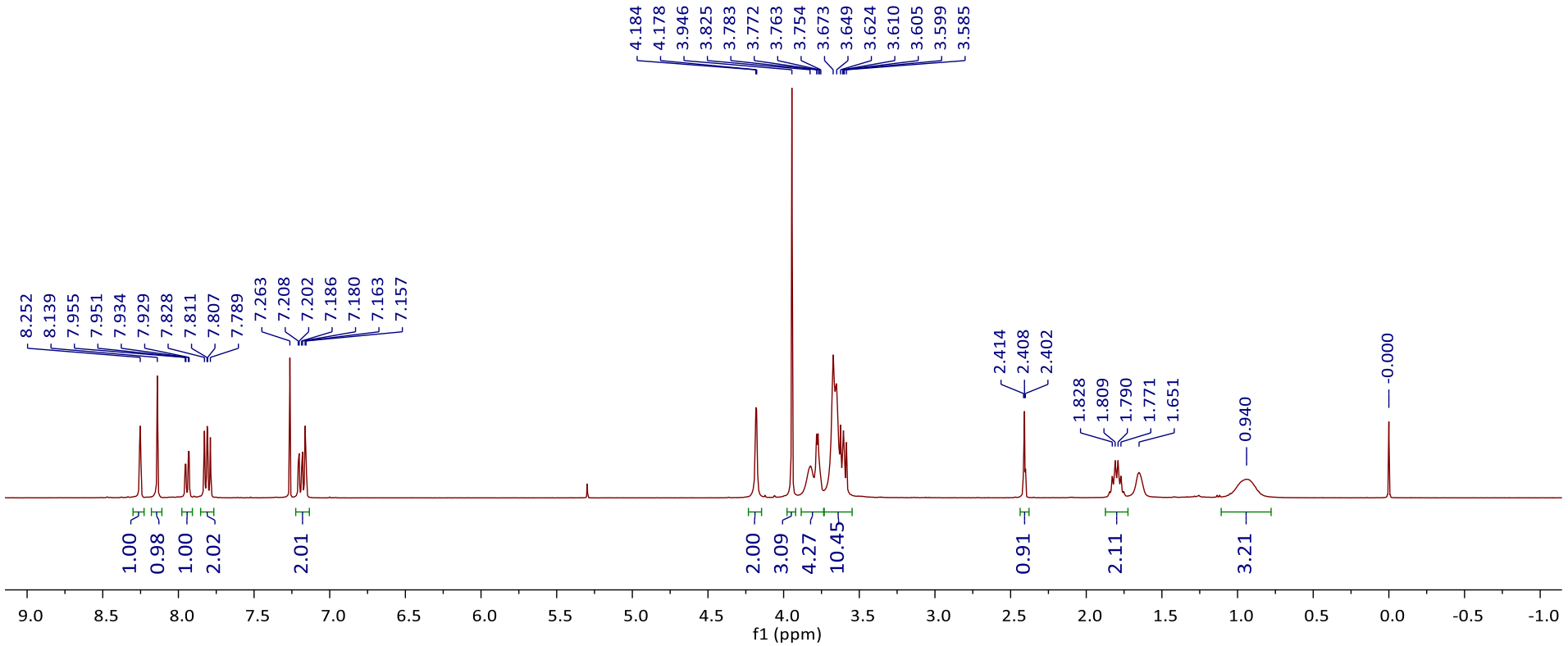
7a



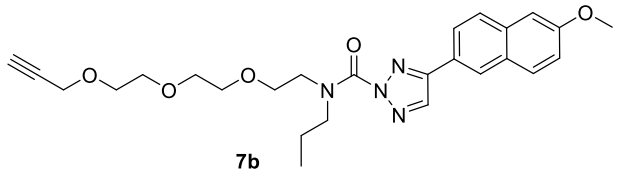
SUPPORTING INFORMATION

Compound 7b ^{1}H NMR (400 MHz, $CDCl_3$)

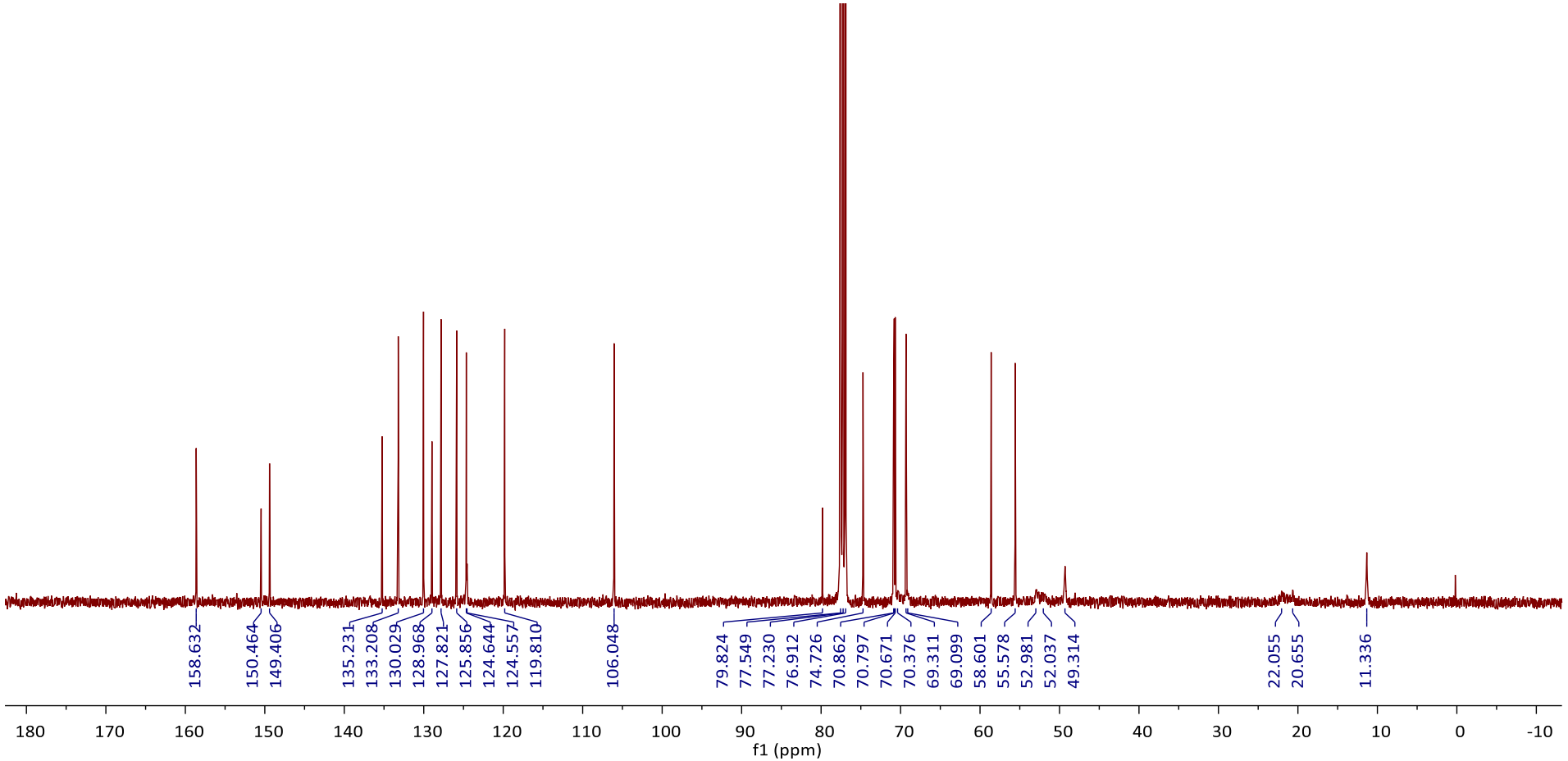




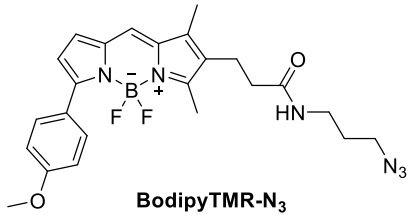
Compound 7b ^{13}C NMR (100 MHz, $CDCl_3$)



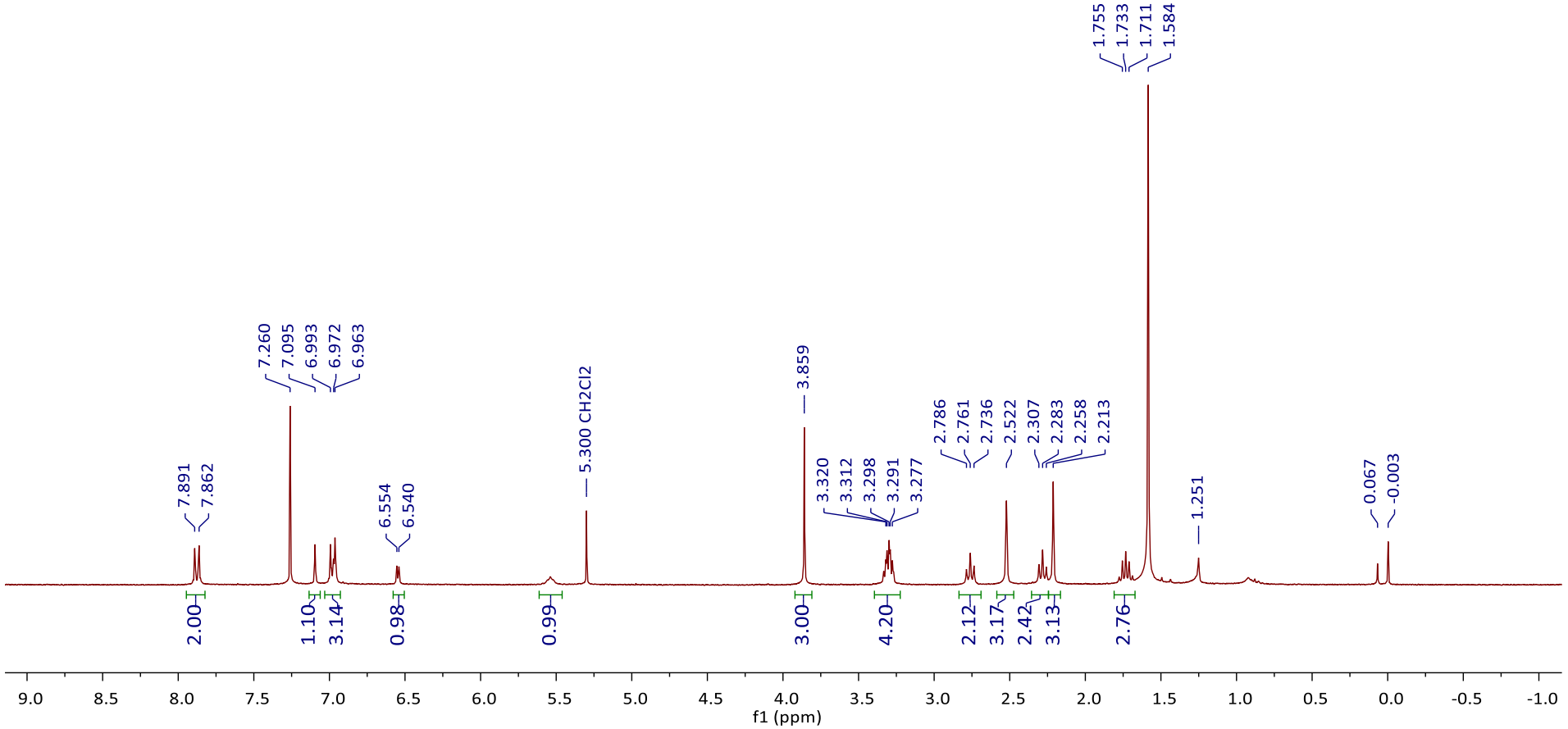
7b



BodipyTMR-N_3 (300 MHz, $CDCl_3$)

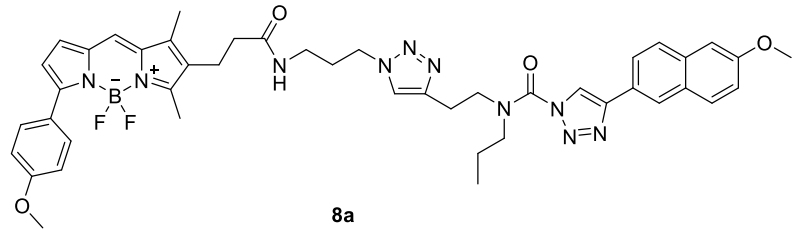


BodipyTMR-N_3

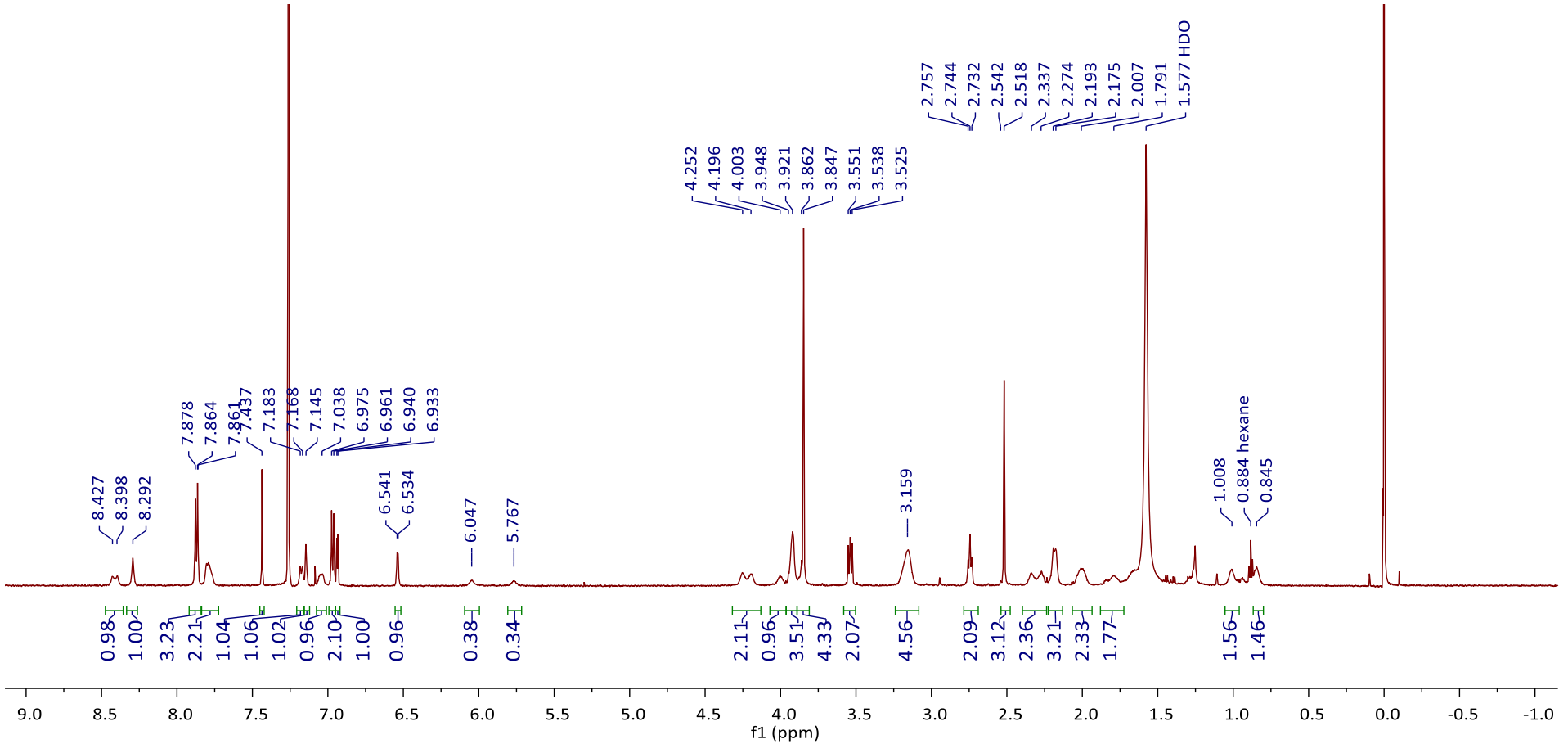


SUPPORTING INFORMATION

Compound 8a ^{1}H NMR (600 MHz, $CDCl_3$)

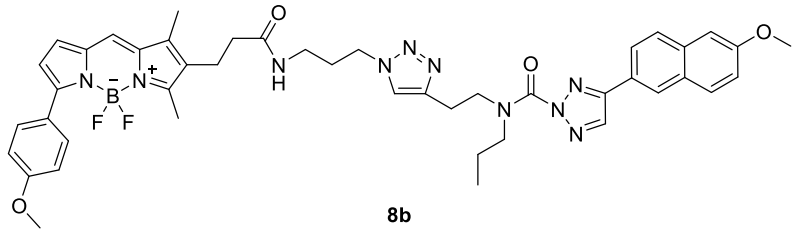


8a



SUPPORTING INFORMATION

Compound 8b ^{1}H NMR (600 MHz, $CDCl_3$)



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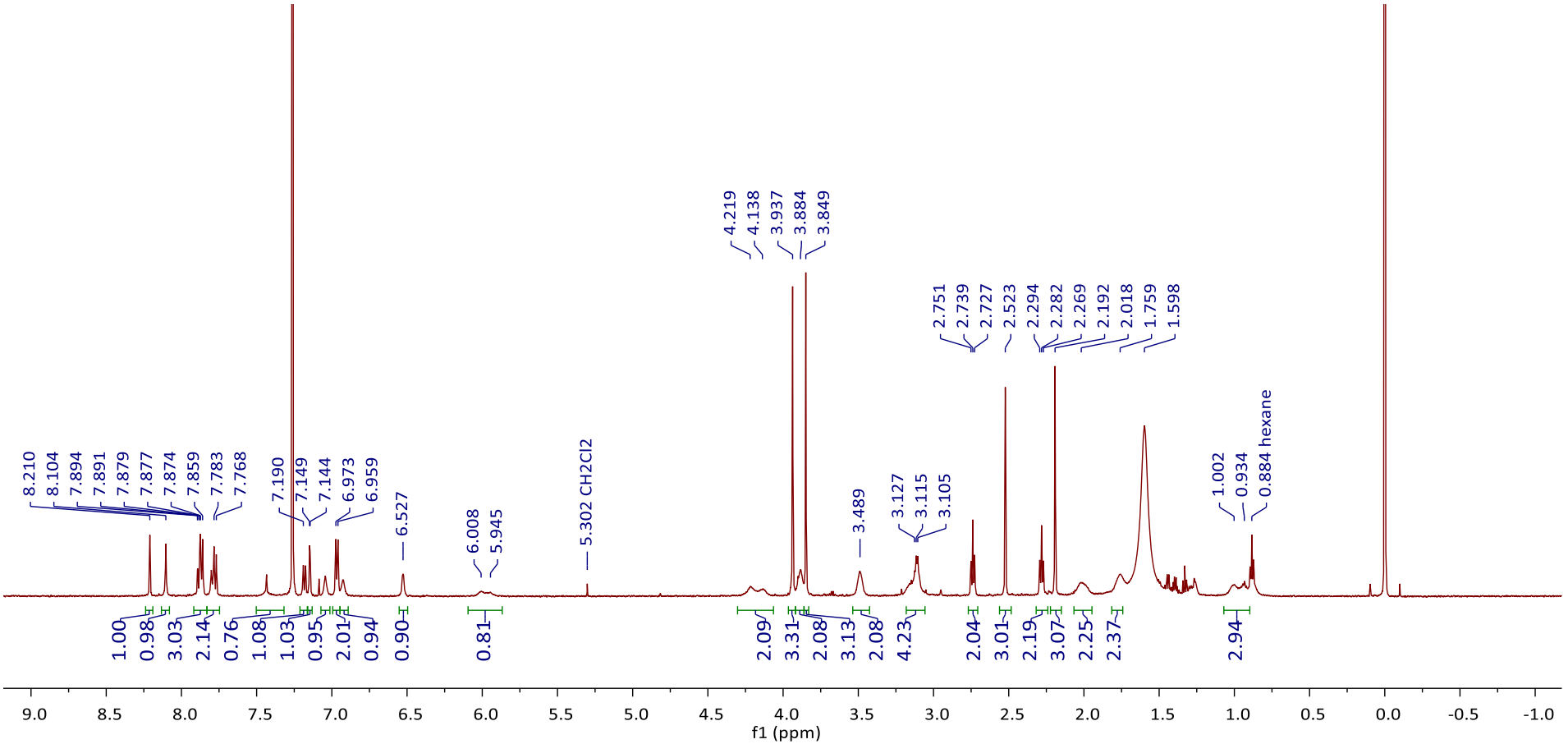
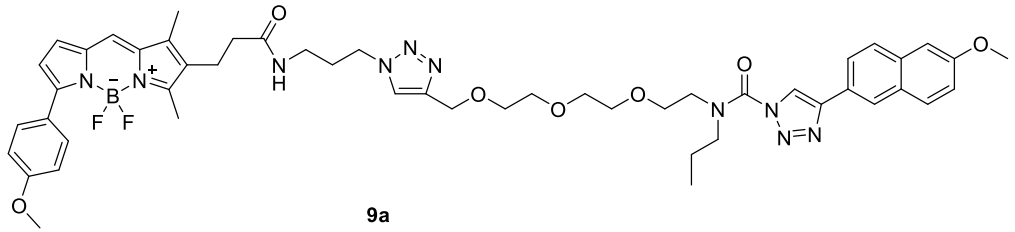
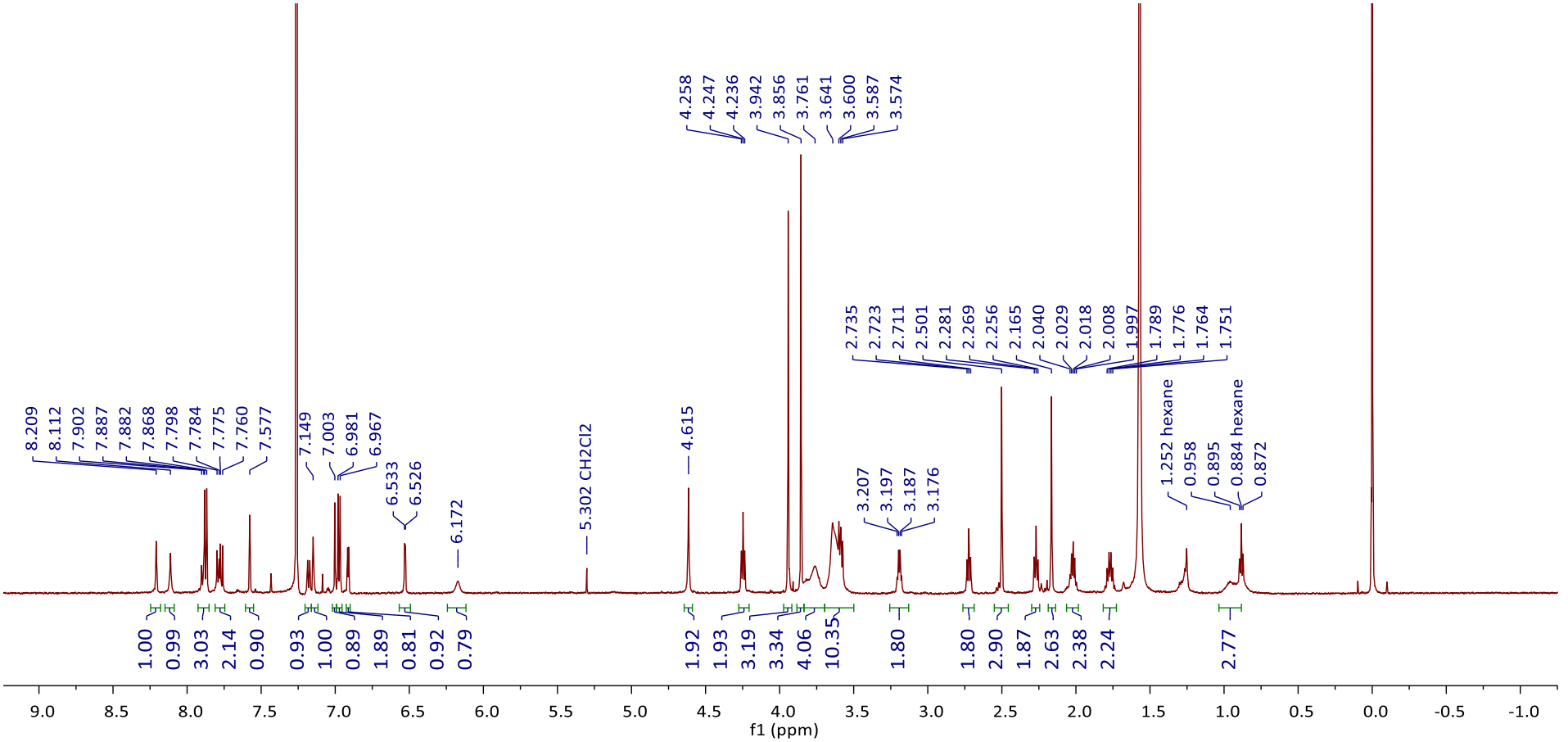


SUPPORTING INFORMATION

Compound 9a ^{1}H NMR (600 MHz, $CDCl_3$)

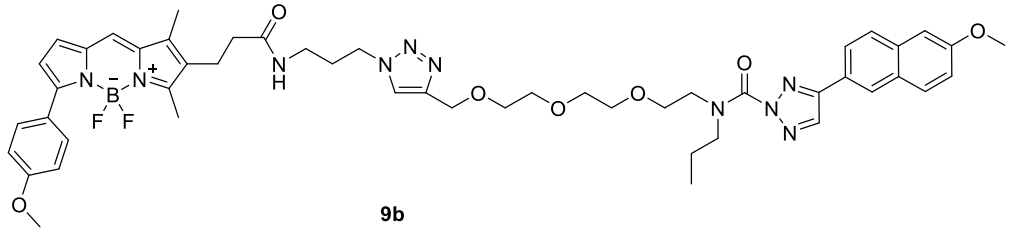


9a



SUPPORTING INFORMATION

Compound 9b ^{1}H NMR (600 MHz, $CDCl_3$)



9b