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Supporting Information

Bioluminescent Antibodies for Point-of-Care Diagnostics

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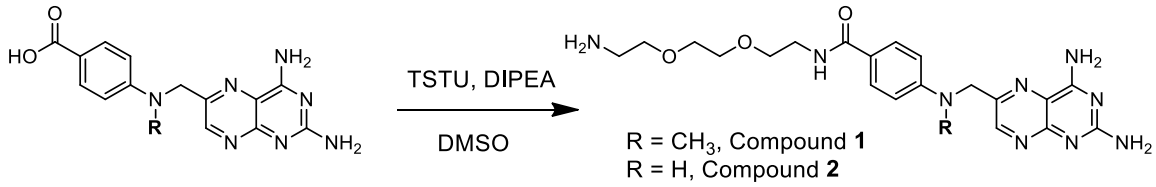
Chemistry

General considerations.

All reactions were carried out in oven-dried glassware under nitrogen atmosphere, unless stated otherwise. Chemicals were purchased from Sigma-Aldrich, Fisher Scientific, Merck, Alfa Aesar, Acros, Axon Lab or Reactolab and used without further purification.

Anhydrous solvents from Acros were used without further treatment. Flash column chromatography was performed with silica gel (230-400 mesh). Reverse-phase semi-preparative high-pressure liquid chromatography was performed on a Dionex system equipped with an an UltiMate 3000 pump and an UVD 170U UV-Vis detector for product visualization on a Waters SunFire™ Prep C18 column (5 μm, 10 × 150 mm). Buffer A: 0.1% v/v TFA in H_2O, Buffer B: acetonitrile. Reverse-phase preparative high-pressure liquid chromatography was performed on a Dionex system equipped with an an UltiMate 3000 pump and an UVD 170U UV-Vis detector for product visualization on a Waters SunFire™ Prep C18 OBD™ 5 μm 19 × 150 mm Column. Buffer A: 0.1% v/v TFA in H_2O, Buffer B: acetonitrile.

Nuclear magnetic resonance (NMR) spectra were recorded at room temperature on a Bruker Avance-III 400 or Bruker Avance 500 with chemical shifts (δ) reported in ppm relative to the solvent residual signals. $CDCl_3$: H 7.26 ppm, C 77.16 ppm, DMSO-d_6: H 2.5 ppm, C 39.52 ppm, CD_3CN: H 1.94 ppm, C 1.32 ppm, MeOD: H 3.31 ppm, C 49.0 ppm. Coupling constants are reported in Hz. High resolution mass spectra (HRMS) were measured on a 6530 Accurate Q-TOF LC/MS spectrometer with electrospray ionization (ESI).



Scheme S1. Synthesis of compound **1** and **2**.

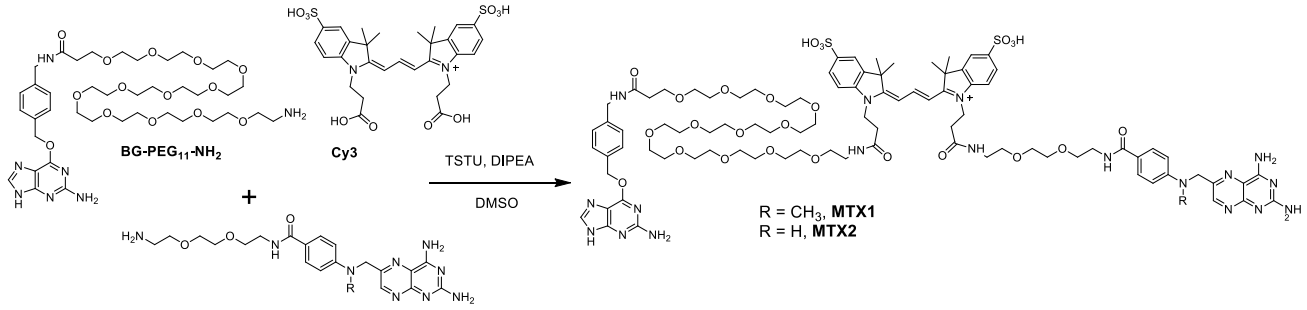
General procedures for Compounds 1 and 2.

To a 50 mM solution of deoxyaminopteroic acid (**DAMPA)** or 4-aminodeoxopteroic acid in DMSO (200 μL), DIPEA (5.3 μL, 3.0 eq) and a 50 mM solution of TSTU in dry DMSO (200 μL) were added. After 10 minutes at room temperature, the reaction was gradually added to a 200 μL DMSO solution of 1,2-bis(2-aminoethoxy)ethane (15 μL, 10.0 eq). After 15 minutes at room temperature, the reaction was quenched by addition of 100 μL H_2O and 30 μL acetic acid. The product was purified by RP-HPLC (0.1% TFA in H_2O/acetonitrile 10 - 50%, 42 min) and lyophilized to afford compound **1** and **2** as yellow solid.

Compound 1. Retention time 18 min, yield 56%. **^{1}H NMR** (400 MHz, MeOD) δ 8.64 (s, 1H), 7.73 (d, *J* = 8.72 Hz, 2H), 6.87 (d, *J* = 8.80 Hz, 2H), 4.92 (s, 2H), 3.69–3.64 (m, 8H), 3.56 (t, *J* = 5.72 Hz, 2H), 3.27 (s, 3H), 3.08 (t, *J* = 5.08 Hz, 2H). **^{13}C NMR** (400 MHz, MeOD) δ 170.2, 164.9, 157.8, 153.6, 152.8, 150.3, 146.9, 130.0, 123.3, 123.2, 112.7, 71.4, 71.3, 70.8, 67.9, 56.6, 40.6, 40.5, 39.7. **HRMS** (ESI, pos. mode) m/z calc. for $C_{21}H_{30}N_9O_3^+$ 456.2472, found 456.2461 $[M+H]^+$.

Compound 2. Retention time 16.5 min, yield 43%. **^{1}H NMR** (400 MHz, MeOD) δ 8.82 (s, 1H), 7.68 (d, *J* = 8.36 Hz, 2H), 6.76 (d, *J* = 8.48 Hz, 2H), 4.69 (s, 2H), 3.71–3.64 (m, 8H), 3.54 (t, *J* = 5.80 Hz, 2H), 3.08 (t, *J* = 5.04 Hz, 2H). **^{13}C NMR** (400 MHz, MeOD)

δ 170.4, 165.0, 157.9, 153.8, 152.4, 150.8, 147.0, 130.0, 123.5, 123.0, 113.0, 71.4, 71.3, 70.8, 67.9, 47.3, 40.6, 40.5. **HRMS** (ESI, pos. mode) m/z calc. for $C_{20}H_{28}N_9O_3^+$ 442.2315, found 442.2300 $[M+H]^+$.



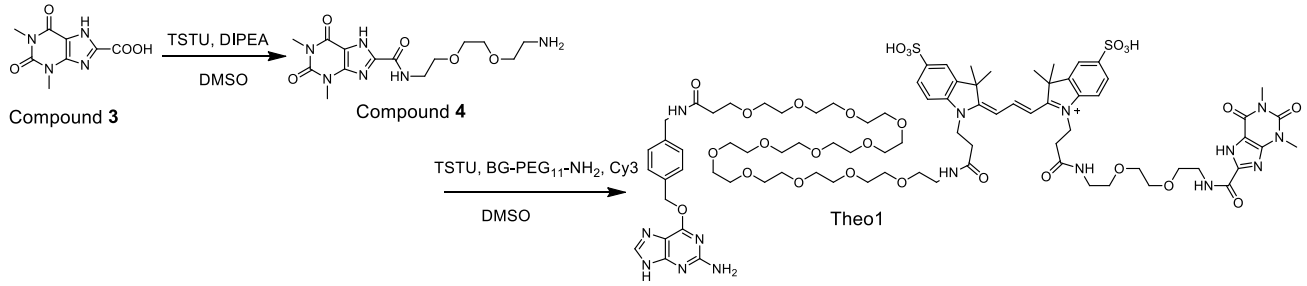
Scheme S2. Synthesis of **MTX1** and **MTX2**.

General procedures for MTX1 and MTX2.

The **MTX1** and **MTX2** were prepared according to our reported procedures.[1] A 15 mM solution of bis-carboxy-Cy3 derivative (1.5 μmol) in anhydrous DMSO was treated with 2 eq of DIPEA, 2 eq of TSTU and 1 eq of BG-PEG_{11}-NH_2 and shaken at room temperature for 30 min. Two additional eqalents of DIPEA were added, followed by 1.1 eq of the compound **1** or **2**. The reaction was shaken for further 15 min at room temperature, and then quenched with 3 volumes of H_2O. The solution was then purified by RP-HPLC (0.1% TFA in H_2O/acetonitrile 10 - 50%, 42 min).

MTX1. Retention time 23.3 min, yield 25%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.29 (s, 1H), 9.10 (s, 1H), 8.69–8.64 (m, 2H), 8.38–8.31 (m, 3H), 8.18–8.11 (m, 3H), 7.80 (s, 2H), 7.68–7.66 (m, 4H), 7.49 (d, J = 6.24 Hz, 2H), 7.34–7.32 (m, 2H), 7.29 (d, J = 6.24 Hz, 2H), 6.79 (d, J = 7.16 Hz, 2H), 6.46 (d, J = 10.6 Hz, 2H), 5.51 (s, 2H), 4.86 (s, 2H), 4.33 (m, 4H), 4.28 (d, J = 4.72 Hz, 2H), 3.74–3.26 (m, 54H), 3.23 (m, 5H), 3.14–3.09 (m, 4H), 2.59 (m, 4H), 2.36 (m, 4H), 1.67 (s, 12H). **HRMS** (ESI, pos. mode) m/z calc. for $C_{90}H_{126}N_{18}O_{25}S^{2+}$ 961.4286, found 961.4283 $[M+H]^{2+}$.

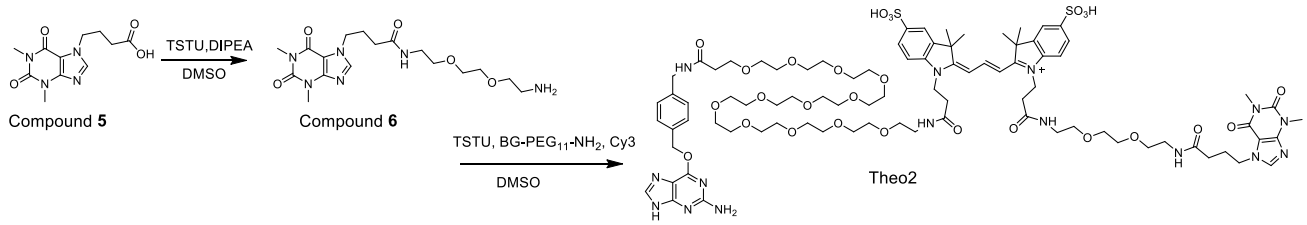
MTX2. Retention time 22.5 min, yield 35%. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 9.36 (s, 1H), 9.31 (s, 1H), 8.83 (s, 1H), 8.38–8.29 (m, 3H), 8.17 (q, J_1 = 5.68 Hz, J_2= 5.80 Hz, 2H), 8.08 (t, J = 5.56 Hz, 1H), 7.80 (s, 2H), 7.68–7.63 (m, 4H), 7.48 (d, J = 7.80 Hz, 2H), 7.35 (d, J = 8.48 Hz, 2H), 7.29 (d, J = 7.80 Hz, 2H), 6.88 (s, 1H), 6.69 (d, J = 8.36 Hz, 2H), 6.46 (d, J = 13.4 Hz, 2H), 5.50 (s, 2H), 4.60 (s, 2H), 4.33 (m, 4H), 4.28 (d, J = 5.8 Hz, 2H), 3.64–3.22 (m, 54H), 3.13 (m, 6H), 2.59 (m, 4H), 2.37 (m, 4H), 1.67 (s, 6H), 1.66 (s, 6H). **HRMS** (ESI, pos. mode) m/z calc. for $C_{89}H_{124}N_{18}O_{25}S^{2+}$ 954.4208, found 954.4202 $[M+H]^{2+}$.



Scheme S3. Synthesis of compounds **compound 4** and **Theo1**.

Compound 4. To a 50 mM solution of compound **3**[2] in DMSO (200 µL), DIPEA (5.3 µL, 3.0 eq) and a 50 mM solution of TSTU in dry DMSO (200 µL) were added. After 10 minutes at room temperature, the reaction was gradually added to a 200 µL DMSO solution of 1,2-bis(2-aminoethoxy)ethane (15 µL, 10.0 eq). After 15 minutes at room temperature, the reaction was quenched by addition of 100 µL H_2O and 30 µL acetic acid. The product was purified by RP-HPLC (0.1% TFA in H_2O/acetonitrile 10 - 50%, 42 min) and lyophilized to afford compound **4** as white solid. Retention time 18 min, yield 80 %. **^{1}H NMR** (400 MHz, DMSO) δ 14.39 (s, 1H), 8.63 (d, *J* = 5.76 Hz, 1H), 7.75 (s, 3H), 3.60–3.54 (m, 8H), 3.48–3.44 (m, 5H), 3.25 (s, 3H), 2.97 (m, 2H). **^{13}C NMR** (400 MHz, DMSO) δ 157.2, 154.4, 151.2, 147.1, 143.3, 108.8, 69.7, 69.4, 68.6, 66.7, 38.6, 29.9, 27.9. **HRMS** (ESI, pos. mode) m/z calc. for $C_{14}H_{23}N_6O_5^+$ 355.1730, found 355.1721 $[M+H]^+$.

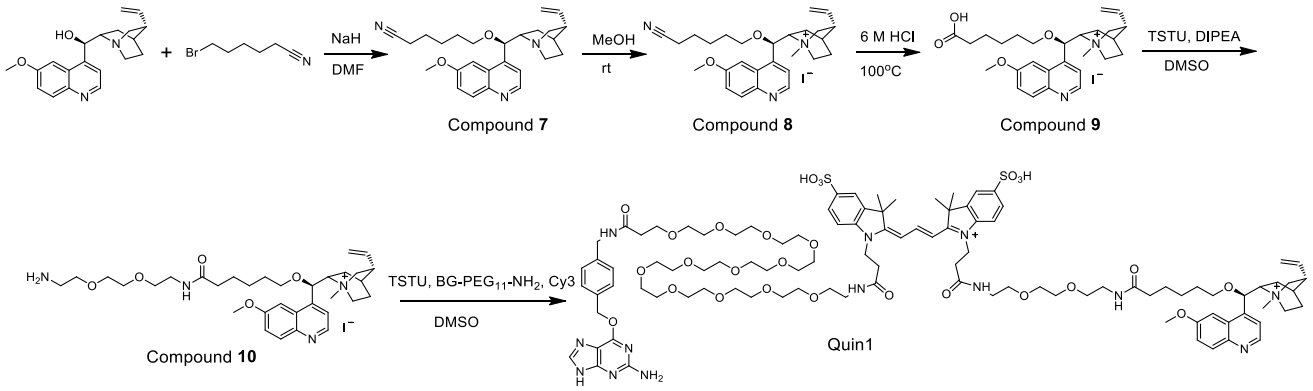
Theo1. **Theo1** was prepared according to the procedure for **MTX**. Retention time 23.5 min, yield 42%. **^{1}H NMR** (500 MHz, DMSO-d_6) 8.60-8.52 (m, 2H), 8.39-8.32 (m, 2H), 8.16 (m, 2H), 7.80 (s, 2H), 7.67 (d, J = 8.10 Hz, 2H), 7.49 (d, J = 7.80 Hz, 2H), 7.34 (d, J = 8.30 Hz, 2H), 7.29 (d, J = 7.8 Hz, 2H), 6.47 (d, J = 13.4 Hz, 2H), 5.53 (s, 2H), 4.43 (m, 4H), 4.28 (d, J = 5.9 Hz, 2H), 3.67-3.38 (m, 55H), 3.28 (d, J = 5.90 Hz, 4H), 3.23 (s, 3H), 3.13 (m, 4H), 2.59 (m, 4H), 2.37 (m, 4H), 1.69 (s, 12H). **HRMS** (ESI, pos. mode) m/z calc. for $C_{83}H_{119}N_{15}O_{27}S^{2+}$ 910.8915, found 910.8909 $[M+H]^{2+}$.



Scheme S4. Synthesis of compound **compound 6** and **Theo2**.

Compound 6. To a 50 mM solution of compound **5**[3] in DMSO (200 µL), DIPEA (5.3 µL, 3.0 eq) and a 50 mM solution of TSTU in dry DMSO (200 µL) were added. After 10 minutes at room temperature, the reaction was gradually added to a 200 µL DMSO solution of 1,2-bis(2-aminoethoxy)ethane (15 µL, 10.0 eq). After 15 minutes at room temperature, the reaction was quenched by addition of 100 µL H_2O and 30 µL acetic acid. The product was purified by RP-HPLC (0.1% TFA in H_2O/acetonitrile 10 - 50%, 42 min) and lyophilized to afford compound **6** as colorless oil. Retention time 17.5 min, yield 86%. **^{1}H NMR** (400 MHz, CD_3CN) δ 7.72 (s, 1H), 7.67 (s, 3H), 7.00 (s, 1H), 4.26 (t, *J* = 6.80 Hz, 2H), 3.69 (t, *J* = 5.04 Hz, 2H), 3.62–3.59 (m, 2H), 3.56–3.54 (m, 2H), 3.48 (t, *J* = 5.32 Hz, 2H), 3.46 (s, 3H), 3.31–3.27 (m, 5H), 3.12 (t, *J* = 5.12 Hz, 2H), 2.15–2.07 (m, 4H), **^{13}C NMR** (400 MHz, CD_3CN) δ 173.3, 156.1, 152.6, 150.0, 143.0, 107.7, 70.9, 70.8, 70.6, 67.3, 47.1, 40.4, 39.9, 33.0, 30.1, 28.2, 27.4. **HRMS** (ESI, pos. mode) m/z calc. for $C_{17}H_{29}N_6O_5^+$ 397.2199, found 397.2197 $[M+H]^+$.

Theo2. **Theo2** was prepared according to the procedure for **MTX**. Retention time 23 min, yield 45%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.61 (s, 1H), 8.39-8.33 (m, 2H), 8.17 (q, J_1 = 5.68 Hz, J_2= 5.80 Hz, 2H), 8.04 (s, 1H), 7.85 (t, J = 5.70 Hz, 1H), 7.80 (s, 2H), 7.67 (d, J = 8.20 Hz, 2H), 7.50 (d, J = 7.58 Hz, 2H), 7.35 (dd, J_1 = 8.45 Hz, J_2= 3.10 Hz, 2H), 7.30 (d, J = 7.80 Hz, 2H), 6.48 (d, J = 13.35 Hz, 2H), 5.54 (s, 2H), 4.34 (t, J = 6.90 Hz, 4H), 4.28 (d, J = 5.59 Hz, 2H), 4.23 (t, J = 6.60 Hz, 2H), 3.63-3.38 (m, 53H), 3.28-3.22 (m, 7H), 3.15-3.10 (m, 6H), 2.60 (m, 4H), 2.38 (m, 4H), 2.04-1.97 (m, 4H), 1.69 (s, 12H). **HRMS** (ESI, pos. mode) m/z calc. for $C_{86}H_{125}N_{15}O_{27}S^{2+}$ 931.9150, found 931.9154 $[M+H]^{2+}$.



Scheme S5. Synthesis of compound **Quin1**.

Compound 7. To the solution of quinine (0.32 g, 1 mmol) in dry DMF, NaH (0.12 g, 60 %suspension in mineral oil, 2.0 eq) was added. After the resulting mixture was stirred at room temperature for 2h, 6-bromohexanenitrile (0.153 mL, 1.1 eq) was added dropwisely via a syringe. The resulting mixture was stirred overnight. When the reaction was completed, brine (50 mL) was added carefully and the resulting mixture was extracted with EtOAc (30 mL). The organic phase was washed with brine (3 x 50 mL), dried over Na_2SO_4, and concentrated in vacuum. The residue was purified by flash chromatography (DCM+1% $NH_3 \bullet H_2O$ /0-2%MeOH) to give a yellow oil (0.25 g, 60% yield). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.76 (d, J = 4.48 Hz, 1H), 8.05 (d, J = 9.20 Hz, 1H), 7.41-7.33 (m, 3H), 5.74-5.69 (m, 1H), 4.97-4.90 (m, 2H), 3.96 (s, 3H), 3.40 (m, 3H), 3.11 (s, 2H), 2.74 (s, 1H), 2.65 (s, 1H), 2.37 (t, J = 6.64 Hz, 2H), 2.30 (s, 1H), 1.83-1.57 (m, 12H). **^{13}C NMR** (400 MHz, $CDCl_3$) δ 157.3, 147.0, 144.4, 144.2, 141.3, 131.3, 126.9, 121.2, 119.1, 118.3, 113.8, 100.9, 68.4, 59.7, 56.4, 55.3, 42.7, 39.4, 28.7, 27.4, 27.2, 25.0, 24.7, 16.5. **HRMS** (ESI, pos. mode) m/z calc. for $C_{26}H_{34}N_3O_2^+$ 420.2651, found 420.2645 $[M+H]^+$.

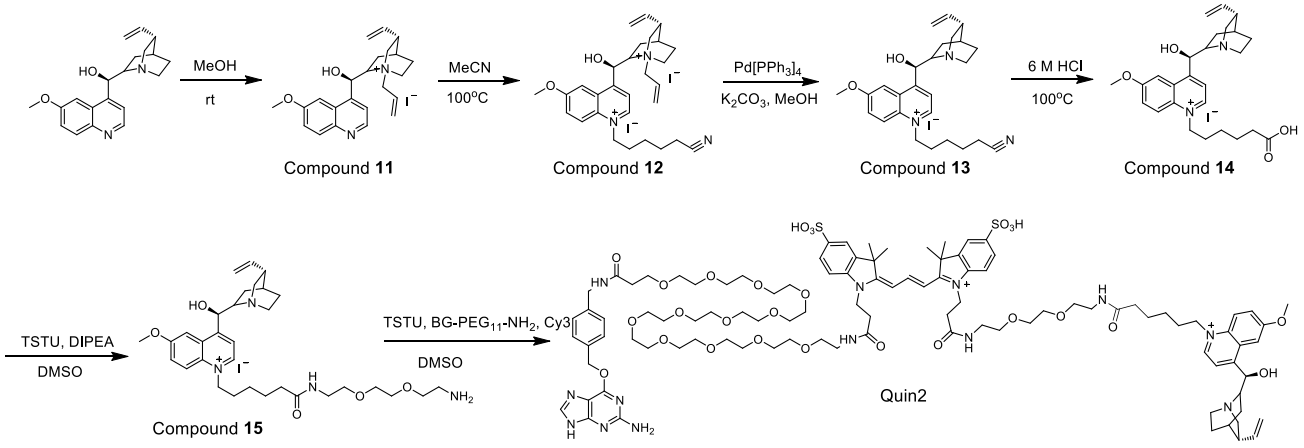
Compound 8. To the solution of compound **7** (0.10 g, 0.24 mmol) in 5 mL MeOH, CH_3I (45 uL, 3 eq) was added. The resulting mixture was stirred at room temperature for 24h. When the reaction was completed, the solvent was evaporated in vacuum. The residue was purified by RP-HPLC (0.1% TFA in H_2O/acetonitrile 15 - 70%, 42 min) to afford compound **8** as yellow oil. Retention time 20 min, yield 82%. **^{1}H NMR** (400 MHz, CD_3CN) δ 13.52 (s, 1H), 8.96 (d, J = 5.16 Hz, 1H), 8.34 (d, J = 9.28 Hz, 1H), 7.95 (d, J = 5.28 Hz, 1H), 7.70 (d, J = 9.36 Hz, 1H), 7.27 (s, 1H), 5.99 (s, 1H), 5.70-5.61 (m, 1H), 5.11-5.00 (m, 2H), 4.14-4.09 (m, 1H), 4.07 (s, 3H), 3.69-3.62 (m, 2H), 3.52-3.32 (m, 7H), 2.87-2.85 (m, 1H), 2.45 (t, J = 5.92 Hz, 2H), 2.26-1.98 (m, 4H), 1.80-1.50 (m, 7H). **^{13}C NMR** (400 MHz, CD_3CN) δ 160.7, 148.5, 143.5, 138.3, 128.8, 126.8, 126.7, 126.7, 121.7, 121.3, 117.6, 102.8, 73.9, 70.2, 68.0, 66.7, 57.0, 56.6, 50.7, 38.8, 29.5, 27.3, 26.0, 25.8, 25.8, 21.2, 17.4. **HRMS** (ESI, pos. mode) m/z calc. for $C_{27}H_{36}N_3O_2^+$ 434.2802, found 434.2796 $[M]^+$.

Compound 9. Compound **8** (0.10 g) was dissolved in 5 mL HCl (6 M). The resulting mixture was stirred at 100°C for 6h. Then the mixture was cooled down to room temperature and was further purified by RP-HPLC (0.1% TFA in H_2O/acetonitrile 15 - 70%, 42 min) to afford compound **9** as yellow oil. Retention time 18.5 min, yield 75%. **^{1}H NMR** (400 MHz, CD_3CN) δ 8.91 (d, J = 5.04 Hz, 1H), 8.29 (d, J = 9.36 Hz, 1H), 7.86 (d, J = 5.08 Hz, 1H), 7.64 (d, J = 9.28 Hz, 1H), 7.23 (s, 1H), 5.94 (s, 1H), 5.70-5.61 (m, 1H), 5.11-5.00 (m, 2H), 4.14-4.07 (m, 1H), 4.05 (s, 3H), 3.65-3.60 (m, 2H), 3.52-3.30 (m, 7H), 2.88-2.82 (m, 1H), 2.34 (t, J = 5.91 Hz, 2H), 2.26-2.00 (m, 4H), 1.78-1.44 (m, 7H). **^{13}C NMR** (400 MHz, CD_3CN) δ 175.6, 160.9, 149.7, 142.8, 138.3, 137.0,

129.0, 127.2, 125.9, 121.9, 117.6, 102.9, 73.9, 70.4, 67.9, 66.7, 57.0, 56.6, 50.7, 38.8, 34.3, 30.0, 27.3, 26.3, 25.8, 25.3, 21.2. **HRMS** (ESI, pos. mode) m/z calc. for $C_{27}H_{37}N_2O_4^+$ 453.2748, found 453.2758 $[M]^+$.

Compound 10. To a 50 mM solution of compound **9** in DMSO (200 µL), DIPEA (5.3 µL, 3.0 eq) and a 50 mM solution of TSTU in dry DMSO (200 µL) were added. After 10 minutes at room temperature, the reaction was gradually added to a 200 µL DMSO solution of 1,2-bis(2-aminoethoxy)ethane (15 µL, 10.0 eq). After 15 minutes at room temperature, the reaction was quenched by addition of 100 µL H_2O and 30 µL acetic acid. The product was purified by RP-HPLC (0.1% TFA in H_2O/acetonitrile 10 - 50%, 42 min) to afford compound **10** as yellow oil. Retention time 20 min, yield 90%. **^{1}H NMR** (400 MHz, CD_3CN) δ 8.94 (d, *J* = 5.00 Hz, 1H), 8.26 (d, *J* = 9.32 Hz, 1H), 7.85 (d, *J* = 5.04 Hz, 1H), 7.64-7.61 (m, 4H), 7.23 (s, 1H), 6.98 (s, 1H), 5.95 (s, 1H), 5.70-5.61 (m, 1H), 5.10-5.00 (m, 2H), 4.13-4.06 (m, 1H), 4.05 (s, 3H), 3.68-3.31 (m, 19H), 3.09 (s, 2H), 2.86-2.84 (m, 1H), 2.25-2.00 (m, 6H), 1.77-1.42 (m, 7H). **^{13}C NMR** (400 MHz, CD_3CN) δ 174.5, 160.4, 146.8, 144.7, 139.7, 138.3, 128.5, 128.1, 125.8, 121.6, 117.6, 102.6, 73.8, 70.9, 70.8, 70.6, 70.2, 68.1, 67.3, 66.6, 56.9, 56.5, 50.7, 40.3, 39.8, 38.8, 36.6, 30.0, 27.3, 26.4, 26.1, 25.8, 21.2. **HRMS** (ESI, pos. mode) m/z calc. for $C_{33}H_{51}N_4O_5^+$ 583.3854, found 583.3901 $[M]^+$.

Quin1. **Quin1** was prepared according to the procedure for **MTX**. Retention time 24 min, yield 24%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.83 (d, J = 4.50 Hz, 1H), 8.42-8.32 (m, 3H), 8.18 (m, 2H), 8.04 (d, J = 9.20 Hz, 1H), 7.83 (t, J = 5.65 Hz, 1H), 7.79 (d, J = 5.05 Hz, 2H), 7.67-7.65 (m, 2H), 7.60 (d, J = 4.55 Hz, 1H), 7.52-7.47 (m, 3H), 7.35-7.23 (m, 5H), 6.48 (d, J = 13.4 Hz, 2H), 6.05 (s, 1H), 5.78-5.71 (m, 1H), 5.52 (s, 2H), 5.13 (d, J = 17.2 Hz, 1H), 5.03 (d, J = 10.4 Hz, 1H), 4.34 (m, 4H), 4.28 (d, J = 5.85 Hz, 2H), 4.00 (m, 4H), 3.82-3.09 (m, 69H), 2.82 (m, 1H), 2.60 (m, 4H), 2.38 (m, 4H), 2.20-2.18 (m, 1H), 2.12-2.03 (m, 4H), 1.98-1.95 (m, 1H), 1.68-1.62 (m, 14H), 1.51-1.44 (m, 4H), 1.35-1.31 (m, 2H). **HRMS** (ESI, pos. mode) m/z calc. for $C_{102}H_{147}N_{13}O_{27}S_2^{2+}$ 1024.9980, found 1024.9984 $[M]^{2+}$.



Scheme S6. Synthesis of compound **Quin2**.

Compound 11. To the solution of quinine (0.32 g, 1 mmol) in 5 mL MeOH, allyl iodide (0.54 mL, 5 eq) was added. The resulting mixture was stirred at room temperature for 24h. When the reaction was completed, the solvent was evaporated in vacuum. The residue was recrystallized in DCM/ethyl acetate as yellow crystals. Yield 50%. **^{1}H NMR** (400 MHz, CD_3Cl) δ 8.76 (d, J = 4.52 Hz, 1H), 8.06 (d, J = 9.16 Hz, 1H), 7.74 (d, J = 4.56 Hz, 1H), 7.41 (d, J = 9.28 Hz, 1H), 7.14 (s, 1H), 6.50 (d, J = 5.68 Hz, 1H), 6.30-6.27 (m, 2H), 5.85 (t, J = 5.60 Hz, 1H), 5.59-5.50 (m, 1H), 5.44 (d, J = 6.28 Hz, 1H), 5.10-5.04 (m, 2H), 4.88 (s, 2H), 4.68 (t, J = 12.0 Hz, 1H), 4.04 (t, J = 10.72 Hz, 1H), 3.96 (s, 3H), 3.78-3.72 (m, 1H), 3.54 (t, J = 9.04 Hz, 1H), 3.29-3.24 (m, 1H), 2.86 (s,

1H), 2.24-2.01 (m, 4H), 1.39 (t, J = 10.56 Hz, 1H). **^{13}C NMR** (400 MHz, CD_3Cl) δ 158.1, 147.4, 143.9, 143.0, 136.6, 131.8, 129.9, 125.6, 124.5, 121.2, 120.6, 117.6, 101.4, 65.9, 64.0, 62.5, 61.7, 56.3, 54.1, 37.3, 26.3, 25.0, 21.5. **HRMS** (ESI, pos. mode) m/z calc. for $C_{23}H_{29}N_2O_2^+$ 365.2224, found 365.2237 $[M]^+$.

Compound 12. To the solution of compound **11** (0.10 g, 0.2 mmol) in 4 mL CH_3CN, NaI (0.3 g, 2 mmol) and 6-bromohexanenitrile (400 uL) were added. The resulting mixture was sealed and stirred at 100°C for 48h. The organic solvent was evaporated in vacuum and the residue was purified by RP-HPLC (0.1% TFA in H_2O/acetonitrile 15 - 70%, 42 min) to afford compound **12** as yellow oil. Retention time 18.5 min, yield 65%. **^{1}H NMR** (400 MHz, CD_3CN) δ 8.92 (d, J = 6.12 Hz, 1H), 8.37 (d, J = 9.84 Hz, 1H), 8.33 (d, J = 6.08 Hz, 1H), 7.88 (dd, J_1 = 9.72 Hz, J_2 = 2.64 Hz, 1H), 7.51 (d, J = 2.68 Hz, 1H), 6.81 (s, 1H), 6.24-6.16 (m, 1H), 5.94 (d, J = 16.84 Hz, 1H), 5.81 (d, J = 10.12 Hz, 1H), 5.72-5.64 (m, 1H), 5.12 (d, J = 17.2 Hz, 1H), 5.03 (d, J = 10.36 Hz, 1H), 4.88 (t, J = 7.6 Hz, 2H), 4.62-4.57 (m, 1H), 4.41-4.29 (m, 2H), 4.09 (s, 3H), 3.69-3.57 (m, 2H), 3.39-3.29 (m, 2H), 2.82 (m, 1H), 2.40 (t, J = 6.84 Hz, 2H), 2.21-2.02 (m, 6H), 1.71-1.41 (m, 5H). **^{13}C NMR** (400 MHz, CD_3CN) δ 161.1, 157.9, 146.0, 138.6, 134.4, 129.9, 129.3, 128.2, 126.3, 122.8, 122.3, 121.0, 117.5, 105.0, 67.5, 65.4, 63.9, 62.5, 59.0, 57.4, 54.0, 38.7, 29.8, 27.5, 26.1, 25.8, 25.5, 22.0, 17.3. **HRMS** (ESI, pos. mode) m/z calc. for $C_{29}H_{39}N_3O_2^{2+}$ 230.6516, found 230.6526 $[M]^{2+}$.

Compound 13. To the mixture of compound **12** (71 mg, 0.1 mmol), 1,3-dimethyluric acid (59 mg, 0.3 mmol), and $Pd(PPh_3)_4$ (23 mg, 0.02 mmol), 5 mL dry MeOH was added under N_2. The resulting mixture was stirred at room temperature for 24h. When the reaction was completed, the solvent was evaporated in vacuum. The residue was purified by RP-HPLC (0.1% TFA in H_2O/acetonitrile 15 - 70%, 42 min) to afford compound **13** as yellow oil. Retention time 19 min, yield 92%. **^{1}H NMR** (400 MHz, CD_3CN) δ 12.54 (s, 1H), 8.88 (d, J = 6.12 Hz, 1H), 8.33 (d, J = 10.40 Hz, 1H), 8.21 (d, J = 6.12 Hz, 1H), 7.82-7.81 (m, 2H), 6.47 (s, 1H), 5.72-5.66 (m, 1H), 5.10 (d, J = 17.16 Hz, 1H), 5.01 (d, J = 10.40 Hz, 1H), 4.90-4.84 (m, 2H), 4.14-4.10 (m, 4H), 3.55-3.49 (m, 2H), 3.24-3.17 (m, 2H), 2.76 (m, 1H), 2.40 (d, J = 6.84 Hz, 2H), 2.09-2.00 (m, 5H), 1.90 (m, 1H), 1.71-1.39 (m, 5H). **^{13}C NMR** (400 MHz, CD_3CN) δ 161.4, 158.2, 145.7, 139.4, 134.4, 130.1, 128.9, 121.9, 121.4, 121.0, 116.9, 104.4, 67.7, 60.2, 58.9, 57.8, 55.0, 44.6, 37.8, 29.8, 27.8, 26.1, 25.4, 24.8, 19.0, 17.3. **HRMS** (ESI, pos. mode) m/z calc. for $C_{26}H_{34}N_3O_2^+$ 420.2646, found 420.2648 $[M]^+$.

Compound 14. To the solution of compound **13** (0.10 g), 3 mL 6 M HCl was added. The resulting mixture was stirred at 100°C for 6h. Then the mixture was cooled down to room temperature and was further purified by RP-HPLC (0.1% TFA in H_2O/acetonitrile 15 - 70%, 42 min) to afford compound **14** as yellow oil. Retention time 18 min, yield 70%. **^{1}H NMR** (400 MHz, CD_3CN) δ 12.49 (s, 1H), 8.89 (d, J = 6.00 Hz, 1H), 8.31 (d, J = 9.40 Hz, 1H), 8.21 (d, J = 6.08 Hz, 1H), 7.79 (m, 2H), 6.50 (s, 1H), 5.74-5.65 (m, 1H), 5.09 (d, J = 17.2 Hz, 1H), 5.00 (d, J = 10.56 Hz, 1H), 4.87 (t, J = 7.56 Hz, 2H), 4.16-4.11 (m, 1H), 4.07 (s, 3H), 3.54-3.47 (m, 2H), 3.24-3.15 (m, 2H), 2.76 (m, 1H), 2.29 (t, J = 7.20 Hz, 2H), 2.09-2.00 (m, 5H), 1.89 (m, 1H), 1.65-1.61 (m, 2H), 1.47-1.41 (m, 3H). **^{13}C NMR** (400 MHz, CD_3CN) δ 175.2, 161.3, 158.1, 145.8, 139.4, 134.2, 129.9, 128.8, 121.9, 121.3, 116.9, 104.3, 67.6, 60.1, 59.0, 57.9, 54.9, 44.5, 37.8, 34.2, 30.1, 27.8, 26.3, 24.8, 18.9. **HRMS** (ESI, pos. mode) m/z calc. for $C_{26}H_{35}N_2O_4^+$ 439.2591, found 439.2588 $[M]^+$.

Compound 15. To a 50 mM solution of compound **14** in DMSO (200 µL), DIPEA (5.3 µL, 3.0 eq) and a 50 mM solution of TSTU in dry DMSO (200 µL) were added. After 10 minutes at room temperature, the reaction was gradually added to a 200 µL DMSO

solution of 1,2-bis(2-aminoethoxy)ethane (15 µL, 10.0 eq). After 15 minutes at room temperature, the reaction was quenched by addition of 100 µL H_2O and 30 µL acetic acid. The product was purified by RP-HPLC (0.1% TFA in H_2O/acetonitrile 10 - 50%, 42 min) and lyophilized to afford compound **15** as colorless oil. Retention time 19.5 min, yield 92%. **^{1}H NMR** (400 MHz, CD_3CN) δ 8.98 (d, *J* = 6.08 Hz, 1H), 8.34 (d, *J* = 10.4 Hz, 1H), 8.22 (d, *J* = 6.04 Hz, 1H), 7.82-7.79 (m, 4H), 7.04 (s, 1H), 6.46 (s, 1H), 5.74-5.66 (m, 1H), 5.09 (d, *J* = 17.16 Hz, 1H), 5.00 (d, *J* = 10.44 Hz, 1H), 4.93-4.86 (m, 2H), 4.15 (m, 1H), 4.10 (s, 3H), 3.67 (t, *J* = 5.12 Hz, 2H), 3.59-3.45 (m, 8H), 3.29-3.15 (m, 4H), 3.06 (t, *J* = 5.16 Hz, 2H), 2.77 (m, 1H), 2.15-1.97 (m, 7H), 1.89 (m, 1H), 1.63-1.56 (m, 2H), 1.46-1.37 (m, 3H). **^{13}C NMR** (400 MHz, CD_3CN) δ 174.1, 161.4, 158.0, 145.9, 139.4, 134.3, 130.1, 128.8, 122.0, 121.4, 116.9, 104.4, 70.8, 70.7, 70.6, 67.8, 67.3, 60.2, 59.1, 57.8, 55.0, 44.6, 40.3, 39.8, 37.8, 36.2, 30.2, 27.9, 26.4, 25.5, 24.8, 19.0. **HRMS** (ESI, pos. mode) m/z calc. for $C_{32}H_{49}N_4O_5^+$ 569.3697, found 569.3704 $[M]^+$.

Quin2. **Quin2** was prepared according to procedure for **MTX**. Retention time 23.5 min, yield 34%. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 10.15 (s, 1H), 9.40 (d, J = 6.15 Hz, 1H), 8.63 (d, J = 6.15 Hz, 1H), 8.37-8.32 (m, 2H), 8.23 (d, J = 6.15 Hz, 1H), 8.19-8.14 (m, 2H), 7.97 (dd, J_1 = 9.70 Hz, J_2 = 2.60 Hz, 1H), 7.81-7.78 (m, 3H), 7.68-7.63 (m, 3H), 7.47 (d, J = 7.85 Hz, 2H), 7.35-7.26 (m, 4H), 7.01 (d, J = 4.35 Hz, 1H), 6.48 (d, J = 13.4 Hz, 2H), 6.06 (d, J = 4.35 Hz, 1H), 5.82-5.75 (m, 1H), 5.49 (s, 2H), 5.13 (d, J = 17.15 Hz, 1H), 5.06-5.00 (m, 3H), 4.36 (m, 4H), 4.28 (d, J = 5.80 Hz, 2H), 4.07 (m, 4H), 3.65-3.28 (m, 54H), 2.75 (m, 1H), 2.59 (m, 4H), 2.36 (m, 4H), 2.06-2.02 (m, 4H), 1.92-1.87 (m, 4H), 1.68 (s, 12H), 1.51-1.47 (m, 4H), 1.32-1.27 (m, 2H). **HRMS** (ESI, pos. mode) m/z calc. for $C_{101}H_{145}N_{13}O_{27}S_2^{2+}$ 1018.4918, found 1018.4911 $[M]^{2+}$.

Biology

Sensor protein sequences

red: amino acid sequence of SNAP-tag

blue: amino acid sequence of NanoLuc luciferase

green: amino acid sequence of light chain on Fab

orange: amino acid sequence of heavy chain on Fab

purple: amino acid sequence of signal peptide for secretion expression

SNAP-Nluc-^{MTX}Fab heavy chain:

MAWMMLLLGLLAYGSGVDSDVQLQESGPGLVKPSQSLSLTCTVTGFSITSPYAWSWIRQFPGNTLEWMGYISYRGSTTYHPSLKSRISITRDT
SKNQFFLQLNSVTTEDTATYFCSSYGNYGAYSGQGTLVTVSAAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSGV
HTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRGPTIKPGPPGGSGGSGGSGGSDYKDDDDK

SNAP-Nluc-^{MTX}Fab light chain:

MDWTWRILFLVAAATGAHSASWSHPQFEKGGGGSMDKDCEMKRTTLDSPLGKLELSGCEQGLHEIIFLGKGTSAADAVEVPAPAAVLGGPE
PLMQATAWLNAYFHQPEAIEEFPVPALHHPVFQQESFTRQVLWKLLKVVKFGEVISYSHLAALAGNPAATAAVKTALSGNPVPILIPCHRVVQG
DLDVGGYEGGLAVKEWLLAHEGHRLGKPGLGEFPPPPPPPPPPPPPPPPPPPPPPPPPPPGGRMVFTLEDFVGDWRQTAGYNLDQVL
EQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVYPVDDHHFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAV
FDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGVTGWRLCERILADVLLTQIPLSLPVSLGDQASISCRSSQSIVHSNGNTYLEWYLQKPG
QSPKLLIYKVSTRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCFQGSHVPLTFGAGTQLELKRADAAPTVSIFPPSSEQLTSGGASVVCF
LNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEGAPGFSSISAHHHH
HHHHHH

SNAP-Nluc-^{Theo}Fab heavy chain:

MAWMMLLLGLLAYGSGVDSDVQLQESGPGLVKPSQTLSLTCTVSGYSITSDYAWNWIRQHPGKGLEWIGYIRYSGHTGYNPSLKSRVTISRD
TSKNQFSLKLSSVTAADTAVYYCARWVDYFDYWGQGTLVTVSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTWNSGSLSSG
VHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRGPTIKPGPPGGSGGSGGSGGSDYKDDDDK

SNAP-Nluc-^{Theo}Fab light chain:

MDWTWRILFLVAAATGAHSASWSHPQFEKGGGGSMDKDCEMKRTTLDSPLGKLELSGCEQGLHEIIFLGKGTSAADAVEVPAPAAVLGGPE
PLMQATAWLNAYFHQPEAIEEFPVPALHHPVFQQESFTRQVLWKLLKVVKFGEVISYSHLAALAGNPAATAAVKTALSGNPVPILIPCHRVVQG
DLDVGGYEGGLAVKEWLLAHEGHRLGKPGLGEFPPPPPPPPPPPPPPPPPPPPPPPPPPPGGRMVFTLEDFVGDWRQTAGYNLDQVL
EQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVYPVDDHHFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAV
FDGKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGVTGWRLCERILADVLLTQSPLSLPVTLGQPASISCRSSQSIVYNNRYTYLEWFQQRP
GQSPRLLIYGVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCYQGTHAPYTFGQGTKLEIKRTVADAAPTVSIFPPSSEQLTSGGAS
VVCFLNNFYPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEGAPGFSSISAH
HHHHHHHHH

SNAP-Nluc-^{Quin}Fab heavy chain:

MAWMMLLLGLLAYGSGVDSEVQLQQSGTVLARPGASVKMSCEASGYTFTSYWMHWLKKRPGQGLEWIGTIYPGNSDSSYNQRFKGKAKLT
AVTSTSTAYMELSSLTNEDSAVYYCTRERGLYYGGRSFDYWGQGTTLTVSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPVTVTW
NSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSETVTCNVAHPASSTKVDKKIVPRGPTIKPGPPGGSGGSGGSGGSDYKDDDDK

SNAP-Nluc-^{Quin}Fab light chain:

MDWTWRILFLVAAATGAHSASWSHPQFEKGGGGSMDKDCEMKRTTLDSPLGKLELSGCEQGLHEIIFLGKGTSAADAVEVPAPAAVLGGPEP
LMQATAWLNAYFHQPEAIEEFPVPALHHPVFQQESFTRQVLWKLLKVVKFGEVISYSHLAALAGNPAATAAVKTALSGNPVPILIPCHRVVQGDL
DVGGYEGGLAVKEWLLAHEGHRLGKPGLGEFPPPPPPPPPPPPPPPPPPPPPPPPPPPGGRMVFTLEDFVGDWRQTAGYNLDQVLEQ
GGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPYEGLSGDQMGQIEKIFKVVYPVDDHHFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAVFD
GKKITVTGTLWNGNKIIDERLINPDGSLLFRVTINGVTGWRLCERILADIQMTQTTSSLSASLGDRVTISCRASQDISNYLTWYQQKPDGTVKLLI
YYTSKLHSGVPSRFSGSGSGTDYSLTISNLEQEDVANYFCQQGNSLPPTFGGGTKLEIKRADADAAPTVSIFPPSSEQLTSGGASVVCFLNNF
YPKDINVKWKIDGSERQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEGAPGFSSISAHHHHHHHH
HH

Cloning and protein expression. The plasmid pVitro was already reported[4] and purchased from InvivoGen. The DNA encoding variable domains (VH and VL) of Fab fragments and mouse antibody constant domain (CH1 and CL) were synthesized by Thermo Fisher Scientific Inc. The PCR products of SNAP_p30 and NanoLuc were obtained by amplifying the coding sequence from our reported sensor.[1] These synthetic/PCR products were assembled into the pVitro plasmids by Gibson Assembly.

Suspension-adapted HEK-293E cells were routinely maintained in serum-free ExCell 293 medium with 4 mM glutamine as described.[5] On the day before transfection, cells were inoculated into fresh medium at a density of 1 x 10^6 cells/ml. The next day, 1 x 10^9 cells were harvested by centrifugation at 1,200 rpm for 5 min and re-suspended at a density of 2.0 x 10^7 cells in 50 ml of RPMI 1640 medium with 0.1% pluronic F68 in a TubeSpin® bioreactor 600 tube. Plasmid DNA (1.5 mg) and linear 25 kDa polyethylenimine (3.0 mg, 1 mg/ml in H_2O) were sequentially added and mixed. The culture was agitated by orbital shaking at 180 rpm at 37°C in the presence of 5% CO_2. After 60 min, the transfected culture was transferred to a 5 L glass bottle containing 950 ml of FreeStyle medium (Invitrogen) with 4 mM glutamine and 3.75 mM valproic acid (500 mM in H_2O). The culture was transferred to an incubator shaker at 37°C with 5% CO_2 with agitation at 120 rpm. At 7 d post-transfection, the cell culture medium was recovered by centrifugation at 2,500 rpm for 10 min and filtered through a 0.22 μm membrane. Then the supernatant was added with 5 mg $NiSO_4$ and subjected to Ni-NTA affinity chromatography according to the instructions of the supplier. The eluted fractions containing the target protein, were spinned down and concentrated using Amicon Ultra-0.5 50 kDa column. The proteins were further diluted into Tris buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.4) and purified by Flag-tag affinity chromatography. The final proteins were stored in 50 mM HEPES pH 7.2, 50 mM NaCl, 40% glycerol at -25°C. The protein concentration was determined using a Bradford assay with BSA as a standard.

SNAP-tag labeling. The sensor proteins were diluted to concentrations of 1 μM in buffer (50 mM HEPES, 50 mM NaCl, 1 mM DTT, pH 7.4,) containing 4 μM of the corresponding SNAP-tag substrate. After incubation at room temperature for 2 h, the labeled sensor constructs were used directly without further purification.

Sensor titration. The labeled sensors were diluted to concentrations of 10 nM in 25 μL human serum (H4522, Sigma) spiked with known concentrations of analyte in white nonbinding 96-well plates and another 25 μL Nano-Glo Luciferase assay substrate (Promega) diluted 250-fold in buffer (50 mM HEPES, 50 mM NaCl, pH 7.45) was added. After incubation at room temperature for 20 min, bioluminescence was measured on an EnVision Multilabel Reader (PerkinElmer). The signal was collected using an emission filter (wavelength: 460 nm, bandwidth: 25 nm) to record NanoLuc emission and a filter for Cy3 (wavelength: 595 nm, bandwidth: 60 nm) to record Cy3 emission. To obtain the concentration of half-maximal ratio change, C_{50}, the data were fitted to the following binding isotherm:

$$R = R_0 + \frac{R_s - R_0}{1 + \frac{C_{50}}{[analyte]}}$$

where R is the experimental emission ratio of NanoLuc emission vs Cy3 emission, [analyte] is the concentration of the free analyte, and R_0 and Rs are the emission ratio in the absence and presence of analyte, respectively. Fits were performed with free fit parameters C_{50}, R_0, and Rs. ΔR_{max} is calculated as (Rs / R_0) × 100%.

Emission spectra. Emission spectra were obtained essentially the same way as titration curves. Spectra were measured on an Infinite M1000 spectrofluorometer (Tecan) with a step size of 1 nm, a bandwidth of 20 nm and an integration time of 100 ms.

Paper device preparation and measurements using a digital camera. Black circles in the shape of the wells of a 96-well plate were printed onto Whatman No. 1 filter paper using a Xerox ColorQube 8580 solid ink printer. The paper was then heated in an oven at 90 °C for 6 min until the black circles (diameter of ~5 mm) were well visible from the opposite side. The paper (each test zone) was spotted with 5 μL BSA buffer (50 mM HEPES, 50 mM NaCl, 10 mg/mL BSA, 0.025% TWEEN, pH = 7.45) and was lyophilized overnight. The next day 5 μL HEPES buffer (50 mM HEPES, 50 mM NaCl, 1 mg/mL BSA, pH = 7.45) containing 30 nM sensor was spotted on the BSA-blocked paper and was further lyophilized overnight.

The human serum spiked with known concentrations of analyte and the HEPES buffer (50 mM HEPES, 50 mM NaCl, pH 7.45) with Nano-Glo luciferase assay substrate diluted 50-fold was mixed with 1:1 ratio. 5 μL of this buffer was directly spotted in the center of the circles printed on the paper. After 15 min, a picture of the paper was taken using a digital camera through a hole in a polystyrene ice box to prevent light from the environment to disturb the measurement.

For calculation of the correlation coefficient between the LUCID and UV/Vis spectrophotometric method, the drugs were randomly diluted into HEPES buffer (50 mM HEPES, 50 mM NaCl, pH 7.45) within the corresponding therapeutic concentrations. UV-Vis measurements were obtained with a Shimadzu UV-1800 spectrometer, using 1 cm quartz cuvettes. By measuring the absorbance (methotrexate: 372 nm, theophylline: 272 nm, quinine: 332 nm) at increasing concentrations of the drugs, the calibration curves were constructed. The concentrations of the spiked samples were measured by UV/Vis spectra. Then the samples were mixed with human serum (1:1, v/v) with Nano-Glo luciferase assay substrate diluted 50-fold. 5 μL of this buffer was directly spotted in the center of the circles printed on the paper. After 15 min incubation, a picture of the paper was taken using a digital camera through a hole in a polystyrene ice box to prevent light from the environment to disturb the measurement.

The human blood, containing ~5 mM EDTA as the anticoagulant, and HEPES buffer (50 mM HEPES, 50 mM NaCl, 5 mM EDTA, pH 7.45) with spiked analytes and Nano-Glo luciferase assay substrate diluted 90-fold was mixed with 1:9 ratio. 5 μL of this mixture was directly spotted in the center of the circles printed on the paper. After 15 min, a picture of the paper was taken using a digital camera through a hole in a polystyrene ice box to prevent light from the environment to disturb the measurement.

The pictures were taken using a Canon PowerShot G1X digital camera with the exposure time of 10 – 20 s, F value of 2.8 and ISO value of 6400. The pictures were saved into RAW format and were further converted into PNG format with optimized parameters (white balance: 40 K, color space: Adobe RGB 1998, gamma value = 1.0) by Adobe Camera Raw 8.0. The images were further processed by using the ImageJ-based java servlet[1] to calculate the ratio values between the average intensity per pixel in the blue and red color channel.

Table S1. Expression yield for the sensor proteins from 1L cell culture.

	SNAP-Nluc-^{MTX}Fab	SNAP-Nluc-^{Theo}Fab	SNAP-Nluc-^{Quin}Fab
Yield	0.51 mg	0.56 mg	0.44 mg

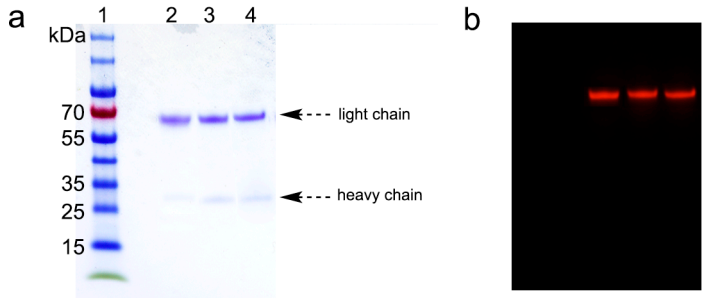


Figure S1. SDS-PAGE and in-gel fluorescence scanning of the labeled sensor proteins. Lane 1: MW, lane 2: SNAP-Nluc-^{MTX}Fab, lane 3: SNAP-Nluc-^{Theo}Fab, lane 4: SNAP-Nluc-^{Quin}Fab. (a) Coomassie staining. (b) BG-TMR (tetramethylrhodamine coupled to benzylguanine) fluorescence[6].

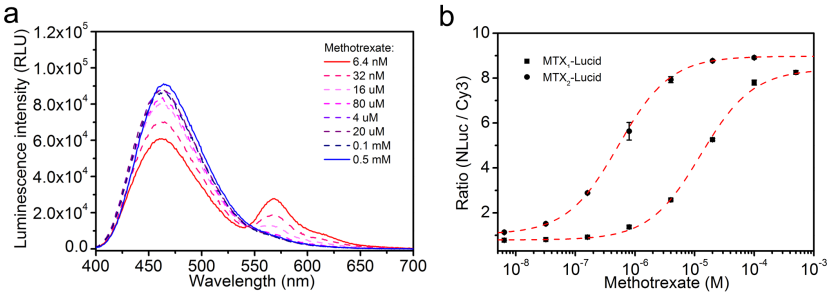


Figure S2. (a) Emission spectra of 5 nM MTX_2-Lucid in human serum (50 mM HEPES, 50 mM NaCl, pH = 7.45, 50% serum) spiked with known concentrations of methotrexate. (b) The ratio (NanoLuc/Cy3) responses of MTX_1-Lucid and MTX_2-Lucid as a function of methotrexate concentration. The data (mean ± SD) are fitted to a single-site binding isotherm (dashed line).

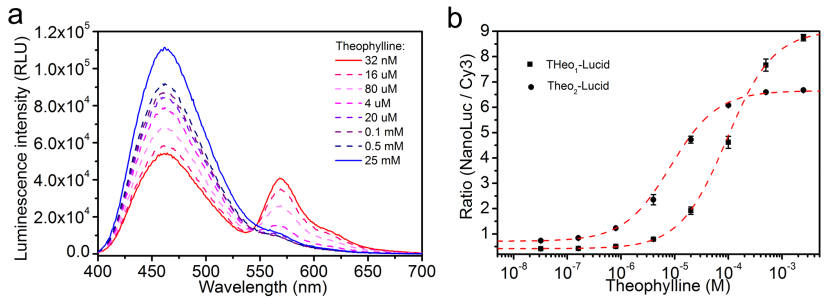


Figure S3. (a) Emission spectra of 5 nM $Theo_2$-Lucid labeled with $Theo_2$ in human serum (50 mM HEPES, 50 mM NaCl, pH = 7.45, 50% serum) spiked with known concentrations of theophylline. (b) The ratio (NanoLuc/Cy3) responses of $Theo_1$-Lucid and $Theo_2$-Lucid as a function of theophylline concentration. The data (mean ± SD) are fitted to a single-site binding isotherm (dashed line).

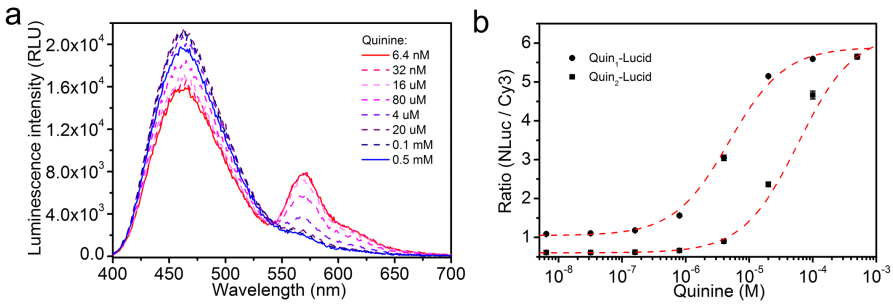


Figure S4. (a) Emission spectra of 5 nM $Quin_1$-Lucid in human serum (50 mM HEPES, 50 mM NaCl, pH = 7.45, 50% serum) spiked with known concentrations of quinine. (b) The ratio (NanoLuc/Cy3) responses of $Quin_1$-Lucid and $Quin_2$-Lucid as a function of quinine concentration. The data (mean ± SD) are fitted to a single-site binding isotherm (dashed line).

Table S2. ΔR_{max}, and C_{50} of the LUCIDs in different conditions.

Sensor	in buffer microplate reader[a]		on paper microplate reader[b]		on paper microplate reader[c]		on paper digital camera[d]	
	ΔR_{max}(%)	C_{50} (μM)	ΔR_{max}(%)	C_{50} (μM)	ΔR_{max}(%)	C_{50} (μM)	ΔR_{max}(%)	C_{50} (μM)
MTX_1-Lucid	1064	12.74 ± 1.55	856	20.64 ± 2.77	740	20.87± 2.35	1420	22.15 ± 2.62
MTX_2-Lucid	863	0.53 ± 0.05	570	1.14 ± 0.14	563	1.20 ± 0.12	982	1.01 ± 0.07
$Theo_1$-Lucid	2155	92.96 ± 5.02	1713	82.54 ± 8.31	1502	93.32 ± 13.06	2030	99.34 ± 4.62
$Theo_2$-Lucid	933	8.44 ± 0.17	839	8.86 ± 0.41	758	9.83 ± 1.02	1385	8.64 ± 0.84
$Quin_1$-Lucid	562	4.87 ± 0.90	535	1.28 ± 0.14	417	1.24 ± 0.06	747	1.43 ± 0.12
$Quin_2$-Lucid	1036	59.12 ± 8.51	937	35.78 ± 5.68	688	32.45 ± 1.72	1730	48.16 ± 1.19

[a] The measurement is performed in HEPES/serum buffer (50 mM HEPES, 50 mM NaCl, pH = 7.45, 50% (v/v) serum) and the signal is collected by EnVision Multilabel Reader.

[b] The measurement is performed on paper spotted with 5 μL HEPES/serum buffer (50 mM HEPES, 50 mM NaCl, pH = 7.45, 50% (v/v) serum) and the signal is collected by EnVision Multilabel Reader.

[c] The sensors are freeze-dried on paper for 35 days at room temperature. The measurement is performed on paper spotted with 5 μL HEPES/serum buffer (50 mM HEPES, 50 mM NaCl, pH = 7.45, 50% (v/v) serum) and the signal is collected by EnVision Multilabel Reader.

[d] The measurement is performed on paper spotted with 5 μL HEPES/serum buffer (50 mM HEPES, 50 mM NaCl, pH = 7.45, 50% (v/v) serum) and the signal is collected by Canon PowerShot G1X digital camera.

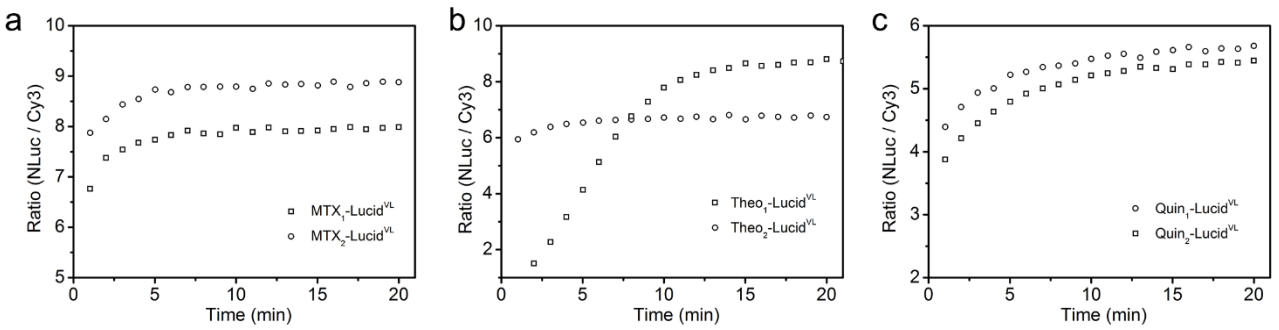


Figure S5. (a) Kinetics of 5 nM MTX_1-Lucid and MTX_2-Lucid in human serum (50 mM HEPES, 50 mM NaCl, pH = 7.45, 50% (v/v) serum) upon addition of 0.1 mM methotrexate. (b) Kinetics of 5 nM $Theo_1$-Lucid and $Theo_2$-Lucid in human serum upon addition of 2.5 mM theophylline. (c) Kinetics of 5 nM $Quin_1$-Lucid and $Quin_2$-Lucid in human serum upon addition of 0.5 mM quinine.

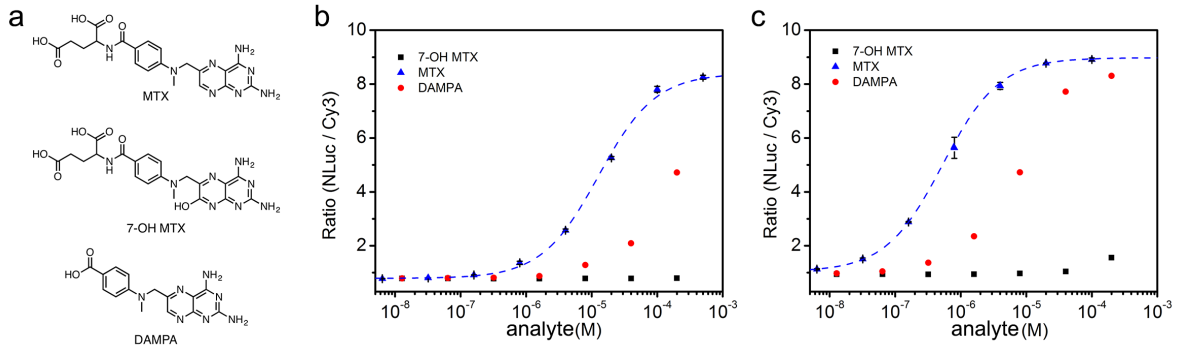


Figure S6. (a) The compounds used for measurements. (b) The ratio (NanoLuc/Cy3) responses of 5 nM MTX_1-Lucid as a function of different analyte concentration. (c) The ratio (NanoLuc/Cy3) responses of 5 nM MTX_2-Lucid as a function of different analyte concentration.

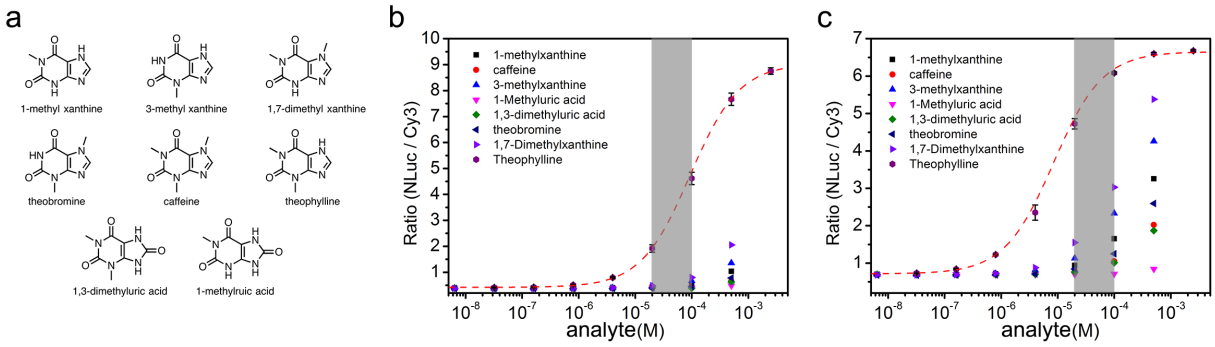


Figure S7. (a) The compounds used for measurements. (b) The ratio (NanoLuc/Cy3) responses of 5 nM $Theo_1$-Lucid as a function of different analyte concentration. (c) The ratio (NanoLuc/Cy3) responses of 5 nM $Theo_2$-Lucid as a function of different analyte concentration. The shaded area corresponds to the therapeutic range of theophylline.

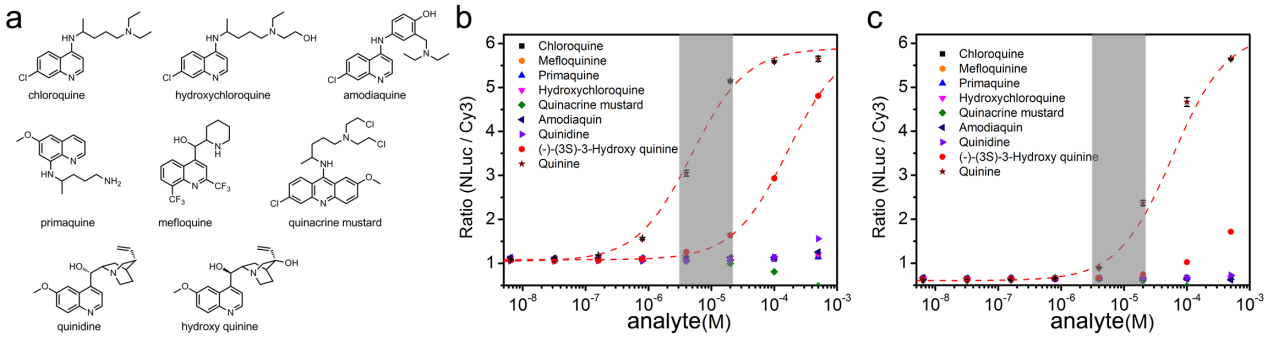


Figure S8. (a) The compounds used for measurements. (b) The ratio (NanoLuc/Cy3) responses of 5nM $Quin_1$-Lucid as a function of different analyte concentration. (c) The ratio (NanoLuc/Cy3) responses of 5 nM $Quin_2$-Lucid as a function of different analyte concentration. The shaded area corresponds to the therapeutic range of quinine.

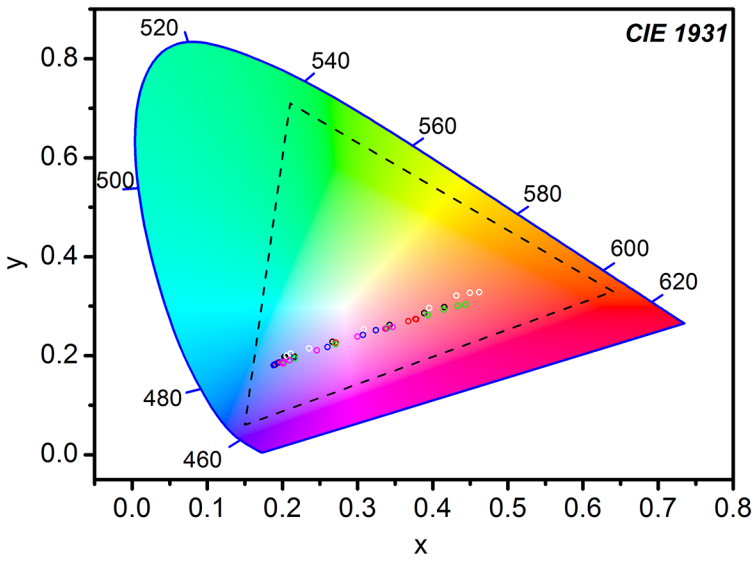


Figure S9. The RGB values of the pictures shown in Figure 4d-f in the main text were transformed on the CIE1931 xy chromaticity diagram. MTX_1-Lucid: red, MTX_2-Lucid: blue, $Theo_1$-Lucid: white, $Theo_2$-Lucid: black, $Quin_1$-Lucid: magente, $Quin_2$-Lucid: green. The dashed-triangle range represents the Adobe RGB 1998 color space[7].

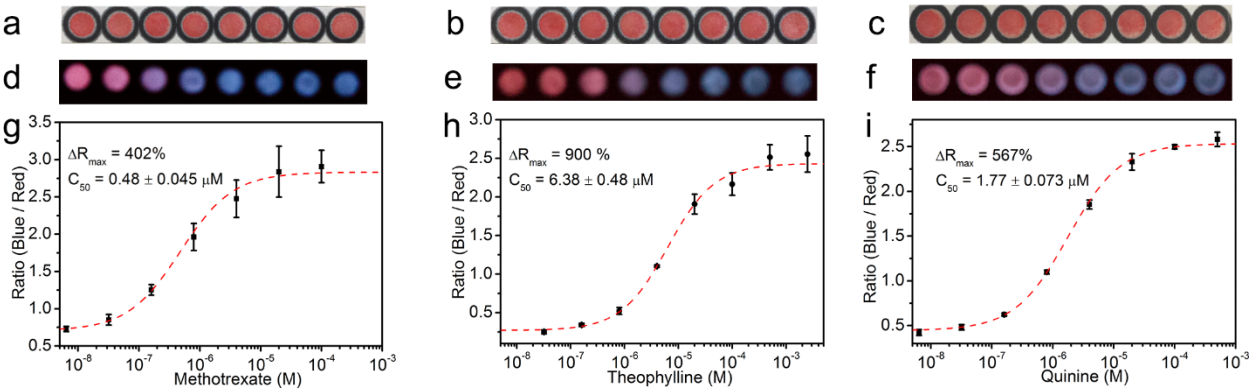


Figure S10. The human blood was diluted into HEPES buffer (50 mM HEPES, 50 mM NaCl, 5 mM EDTA, pH = 7.45, 1/10 dilution) and was spiked with known concentrations of analytes. (a-c) the bright-field picture of the paper containing 30 nM Lucid sensors with varying concentration of analyte in the blood sample. (d-f) the picture (a-c) was taken by the digital camera in dark in the presence of luciferase substrate. (g-i) the ratio responses between number of blue and red pixels of Lucids as a function of analyte concentrations. The data (mean ± SD) are fitted to a single-site binding isotherm (dashed line).

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[7] https://www.adobe.com/digitalimag/adobergb.html
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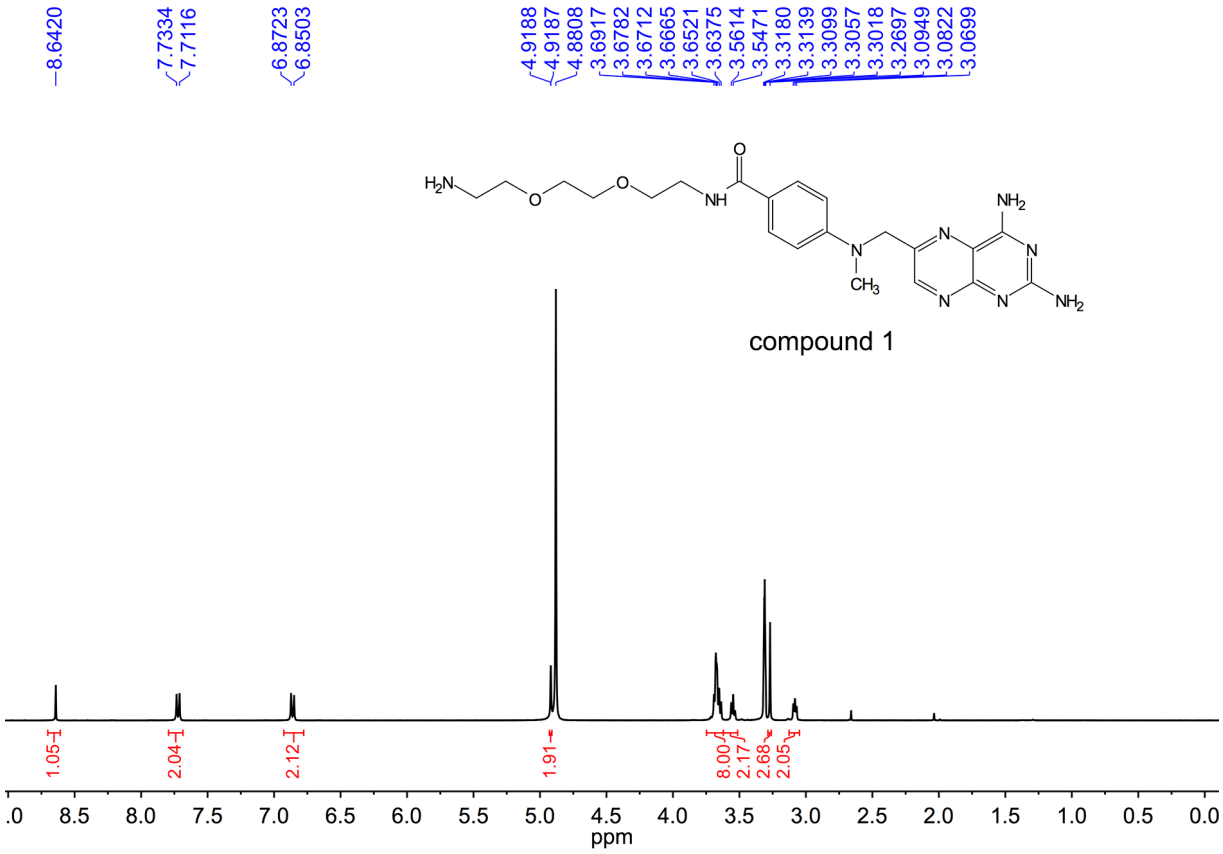
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3.6665
3.6521
3.6375
3.5614
3.5471
3.3180
3.3139
3.3099
3.3057
3.3018
3.2697
3.0949
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2.05
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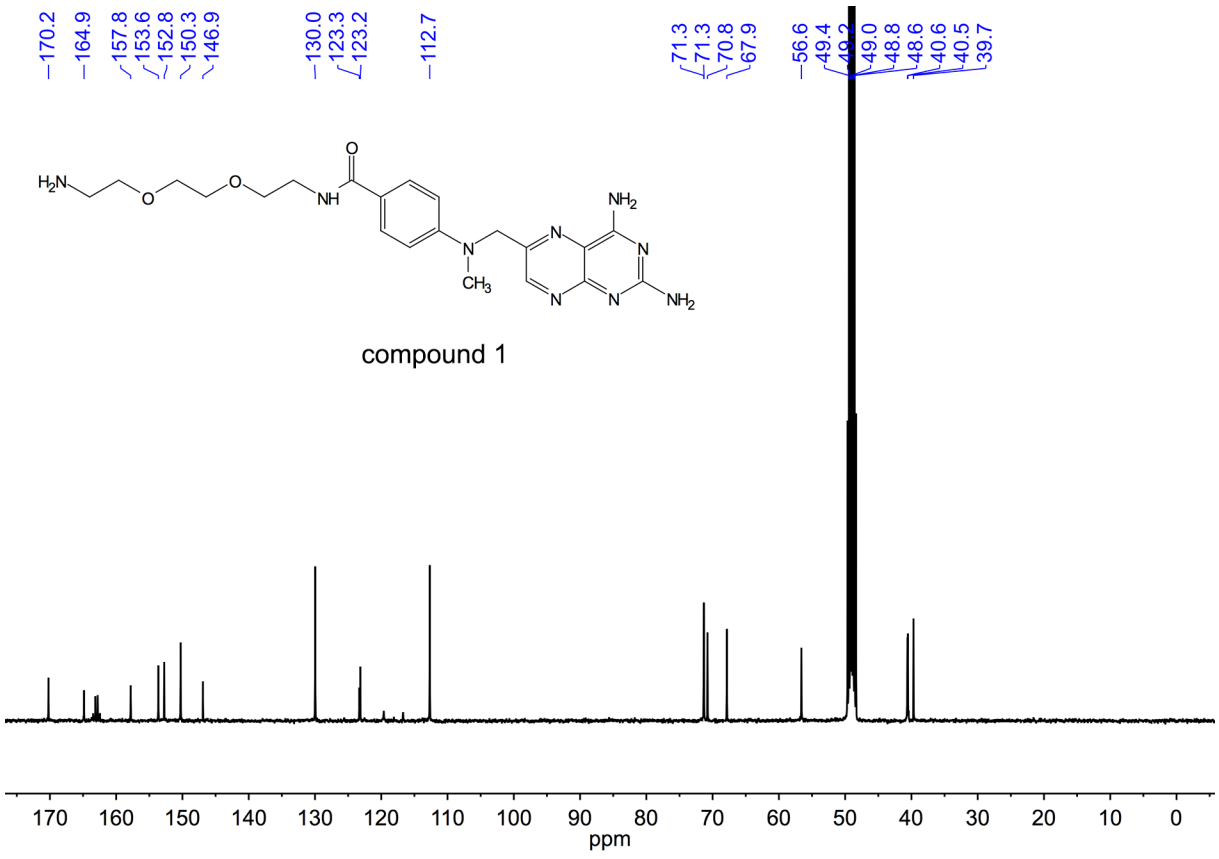
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71.3
70.8
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49.2
49.0
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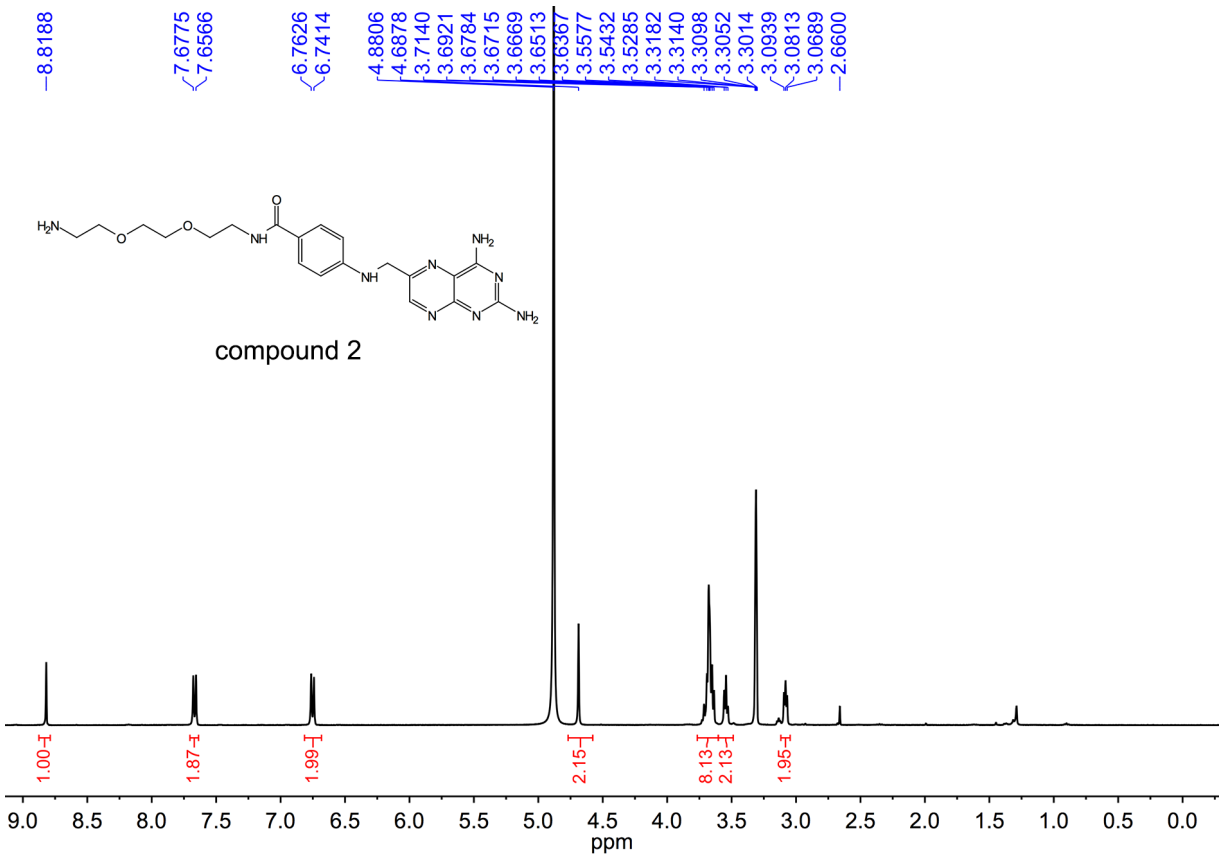
40.6
40.5
39.7
compound 1
ppm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1.95
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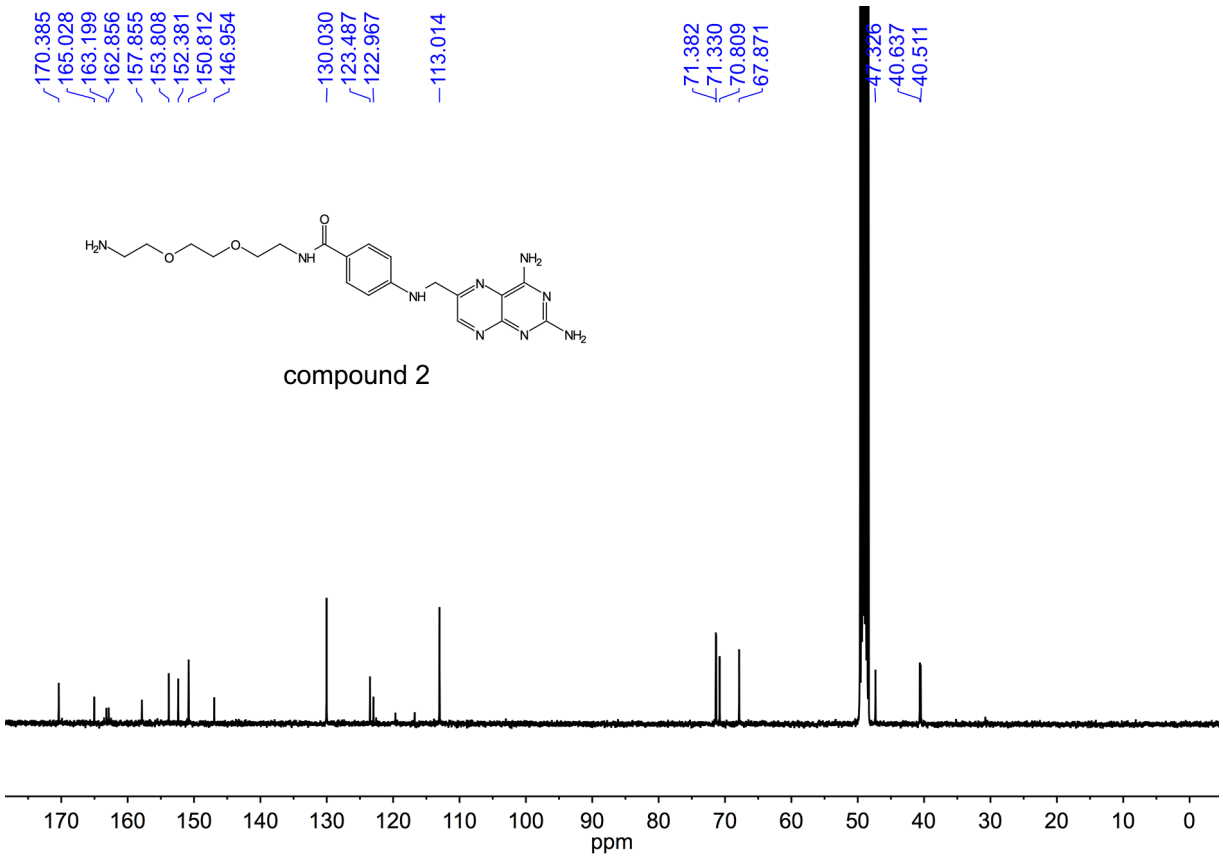
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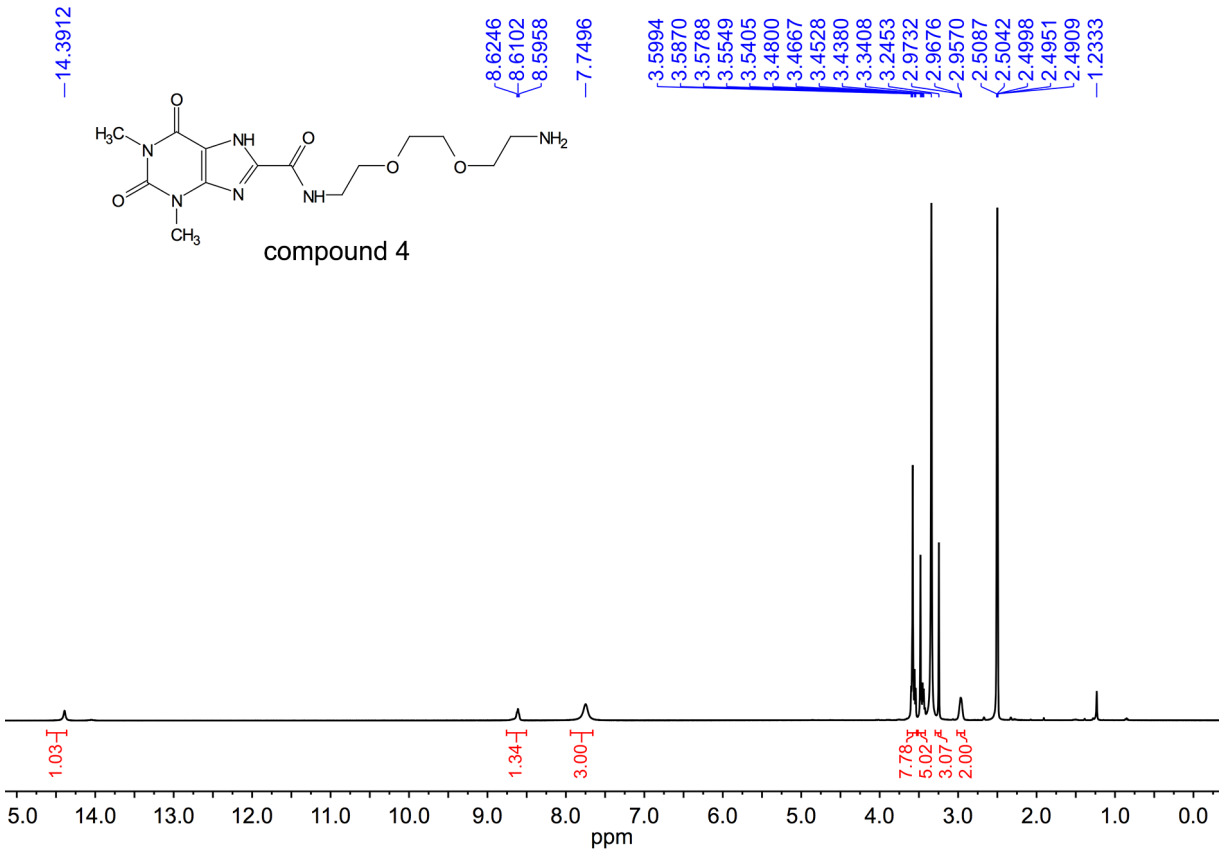
compound 2
ppm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3.4528
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3.3408
3.2453
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2.9676
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2.5042
2.4998
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2.00
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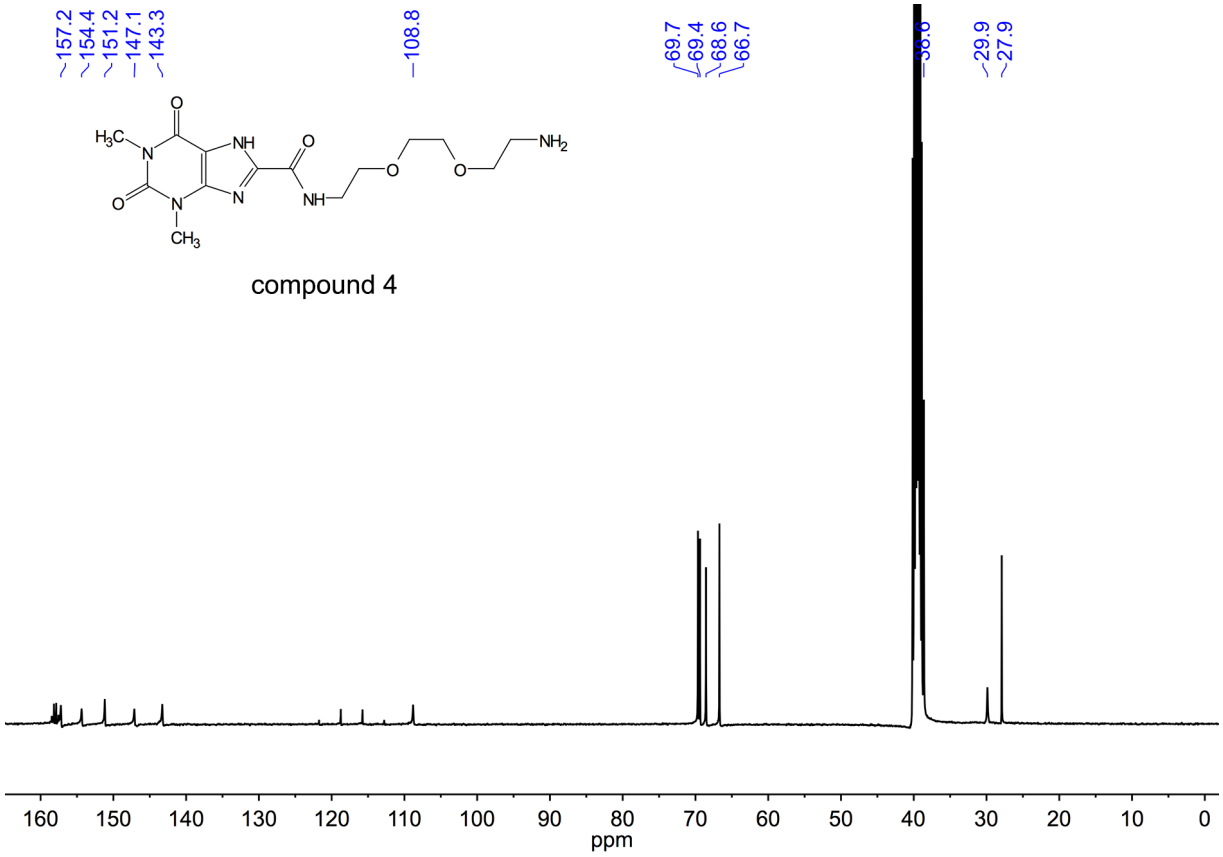
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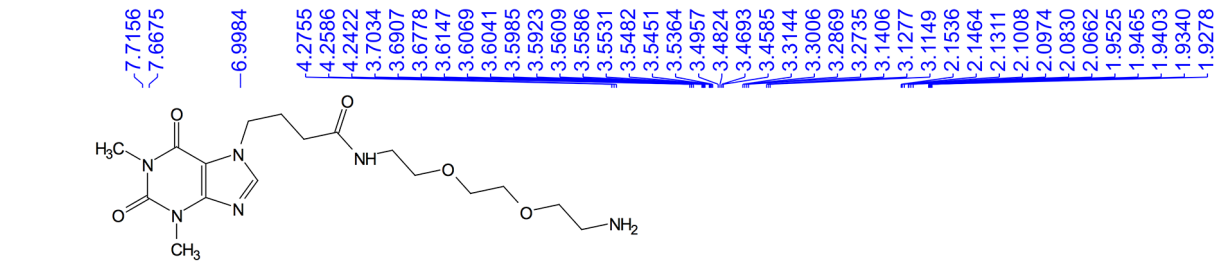

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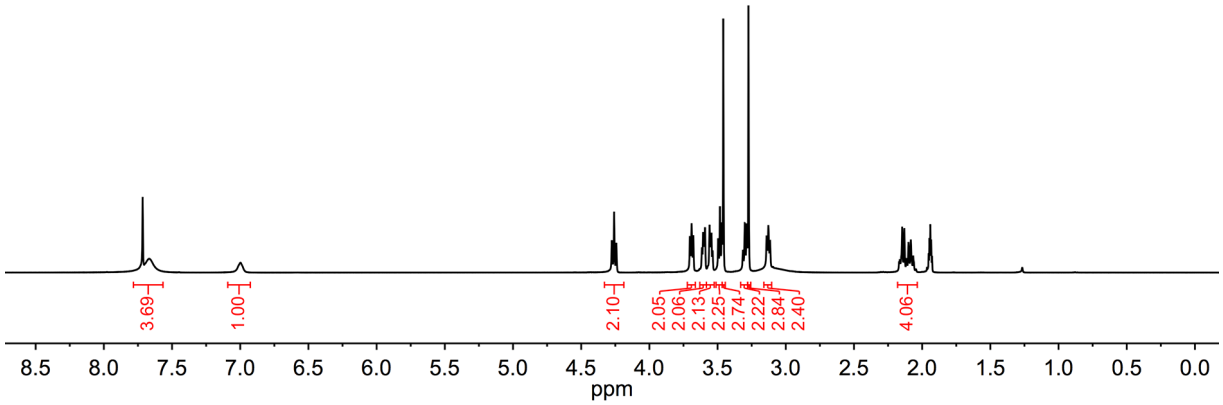

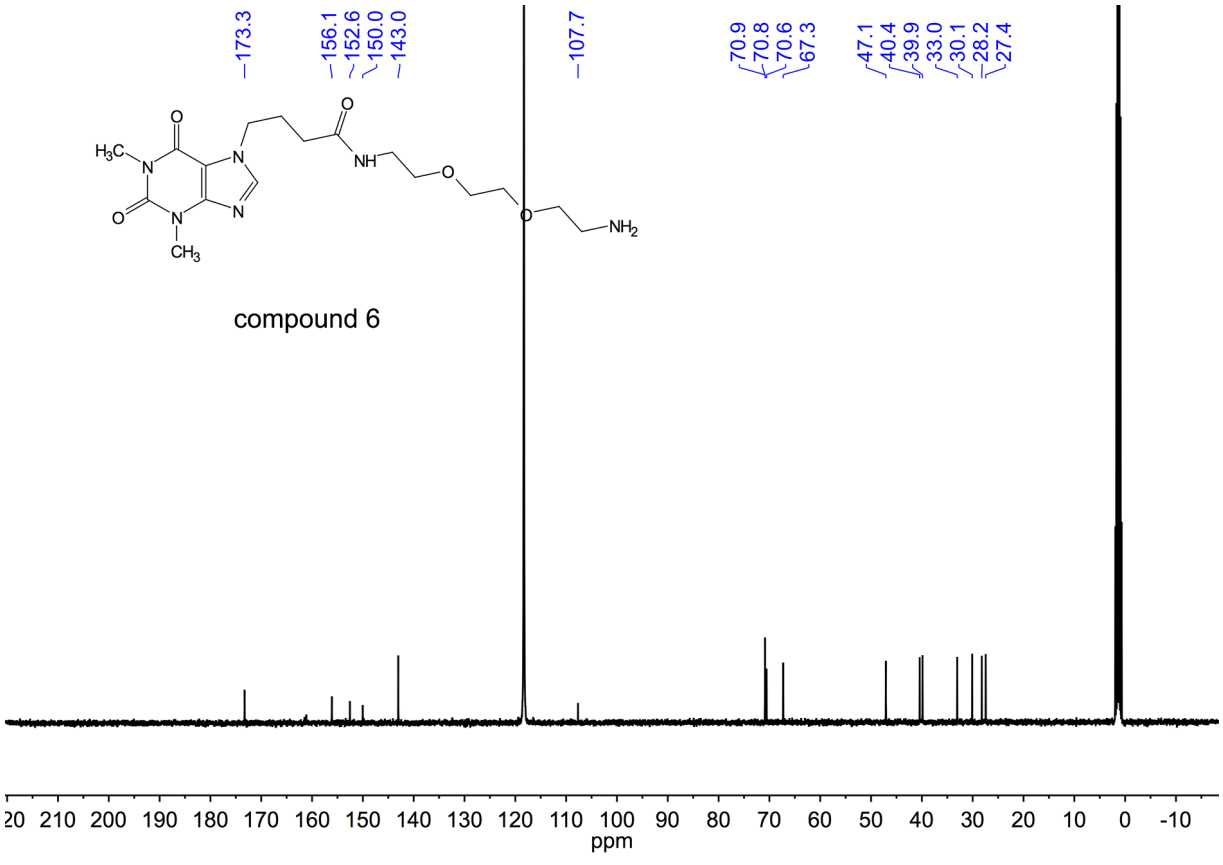

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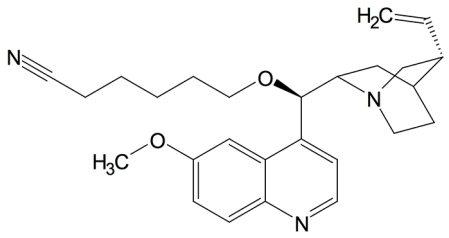
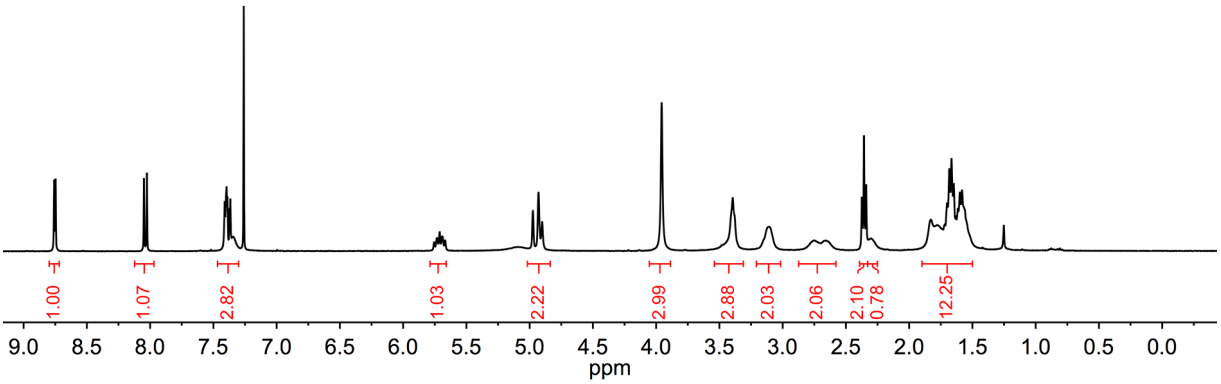
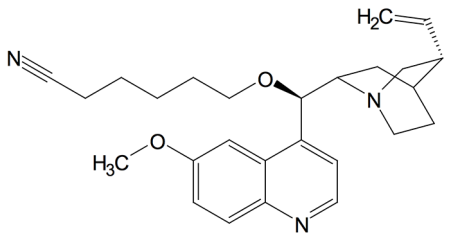
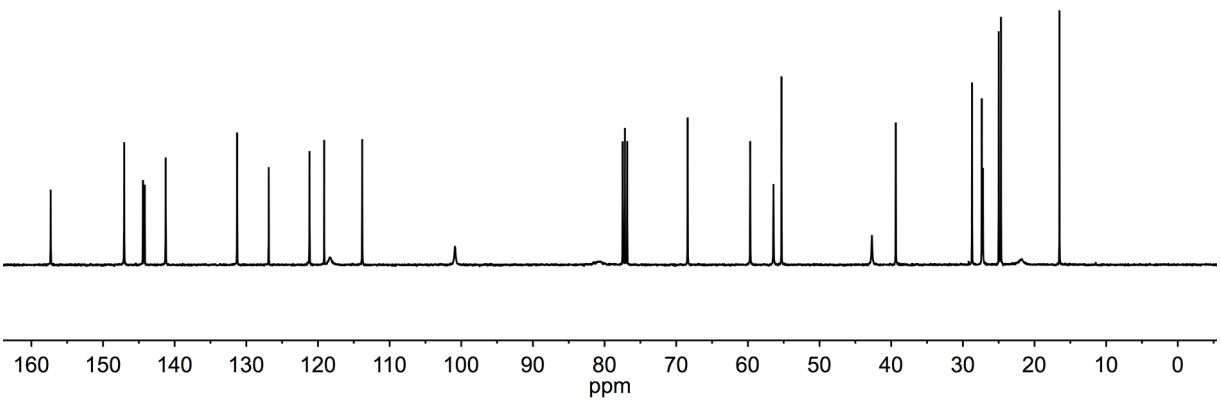


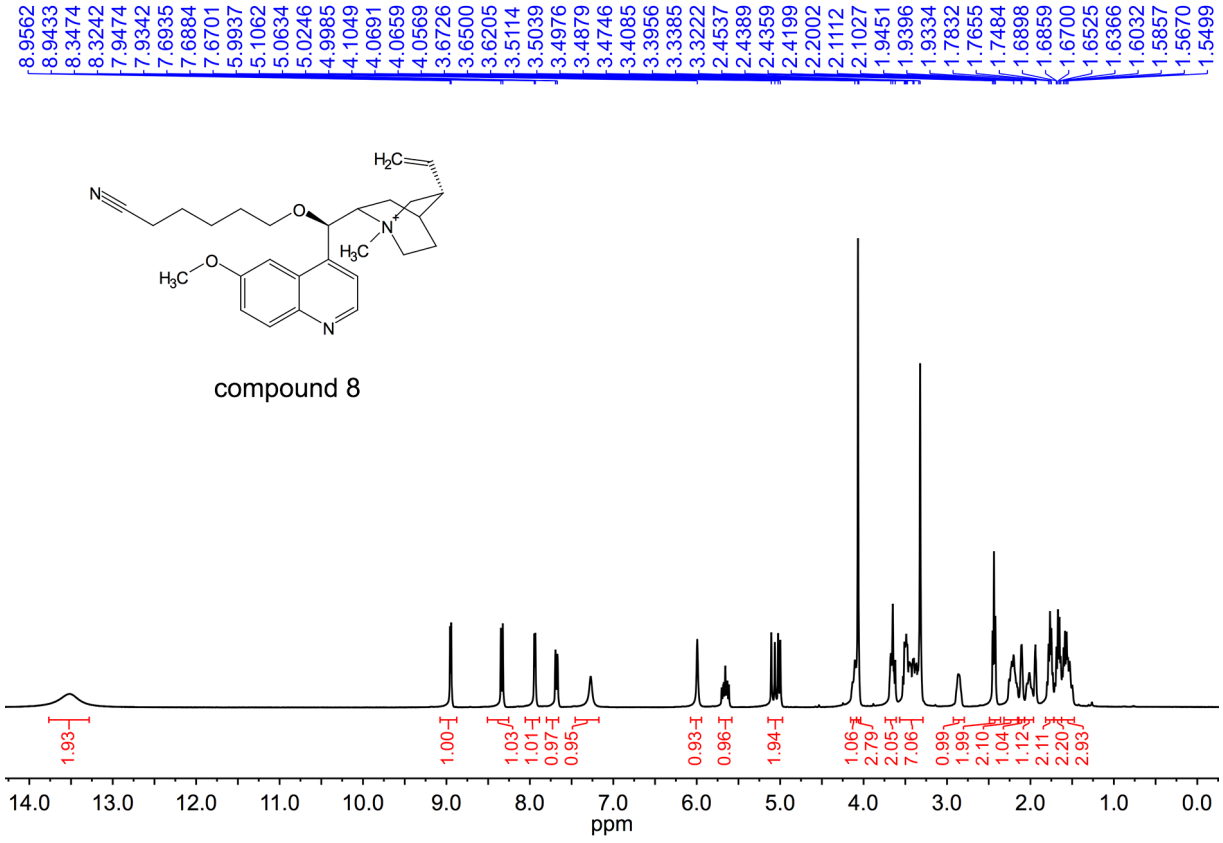
compound 7





compound 7

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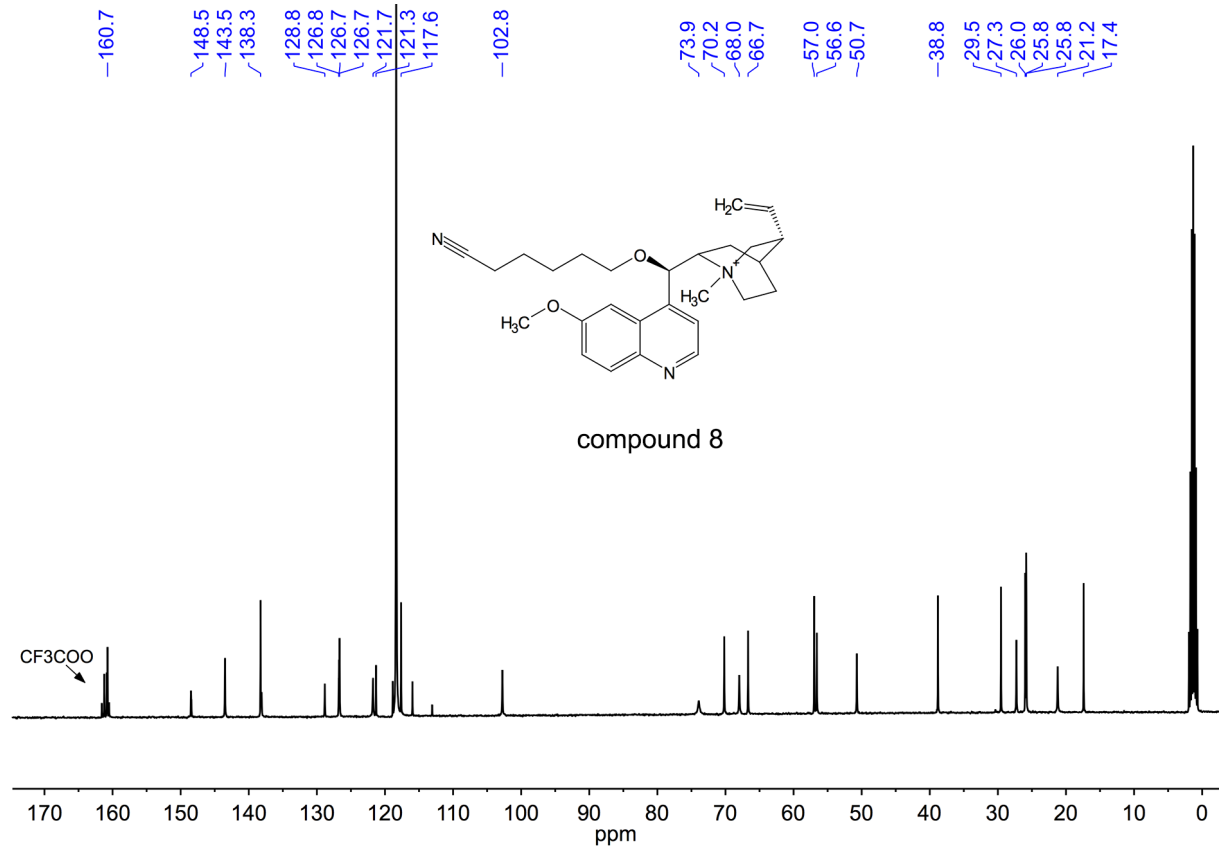
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66.7
57.0
56.6
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27.3
26.0
25.8
25.8
21.2
17.4
compound 8
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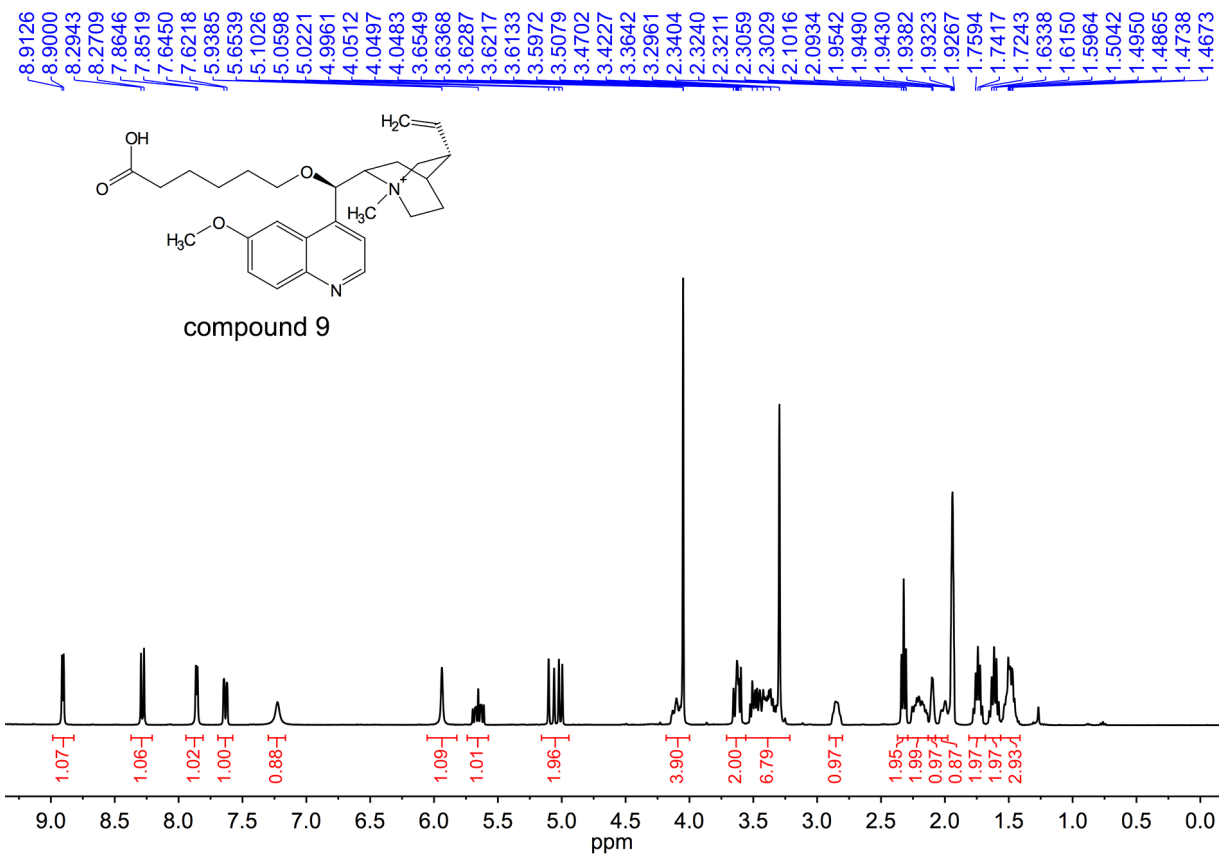
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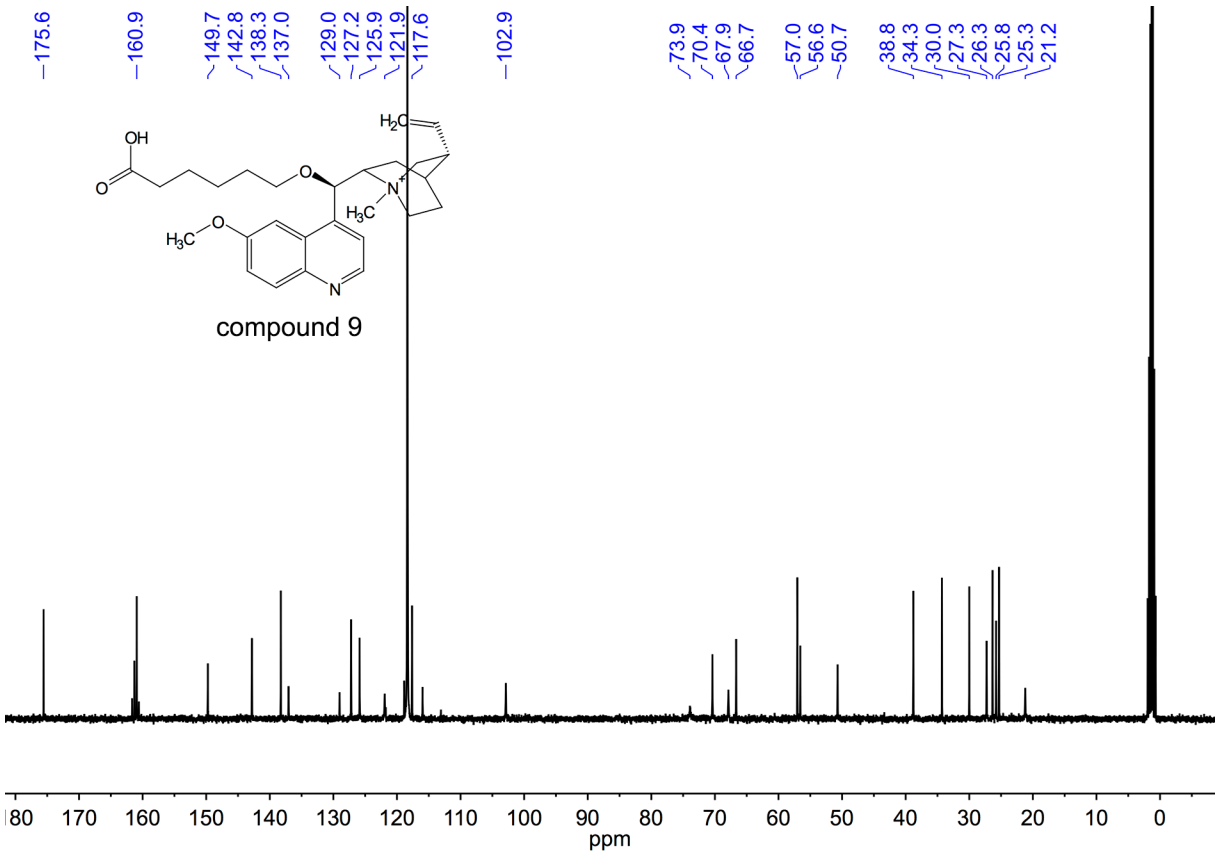
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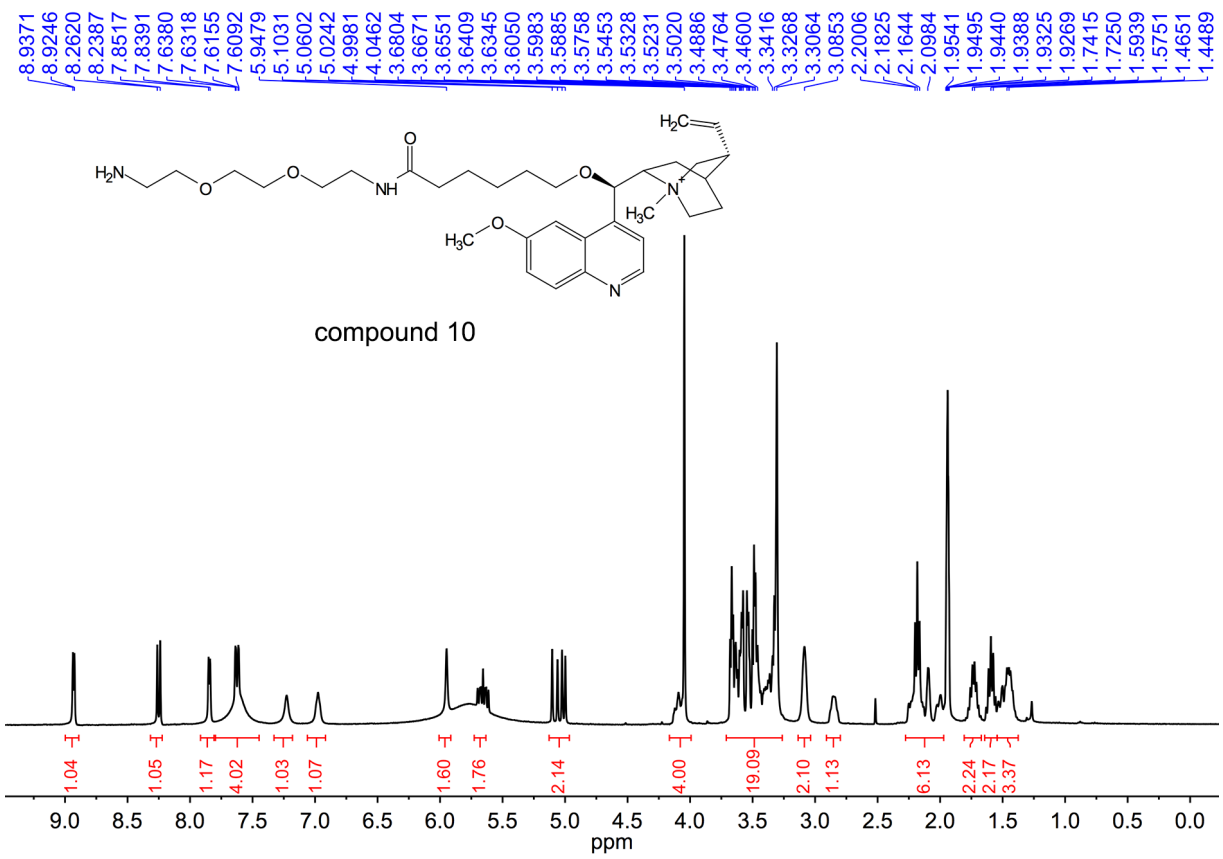
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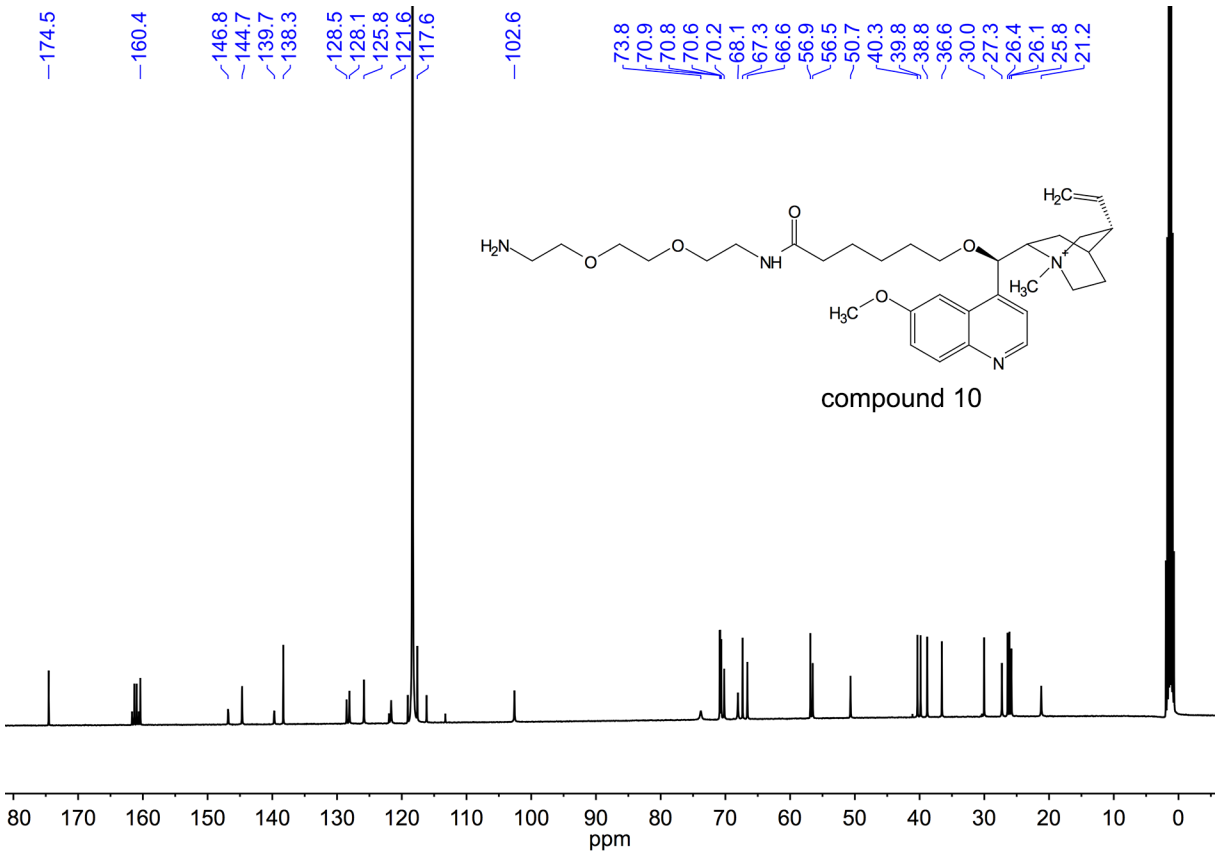
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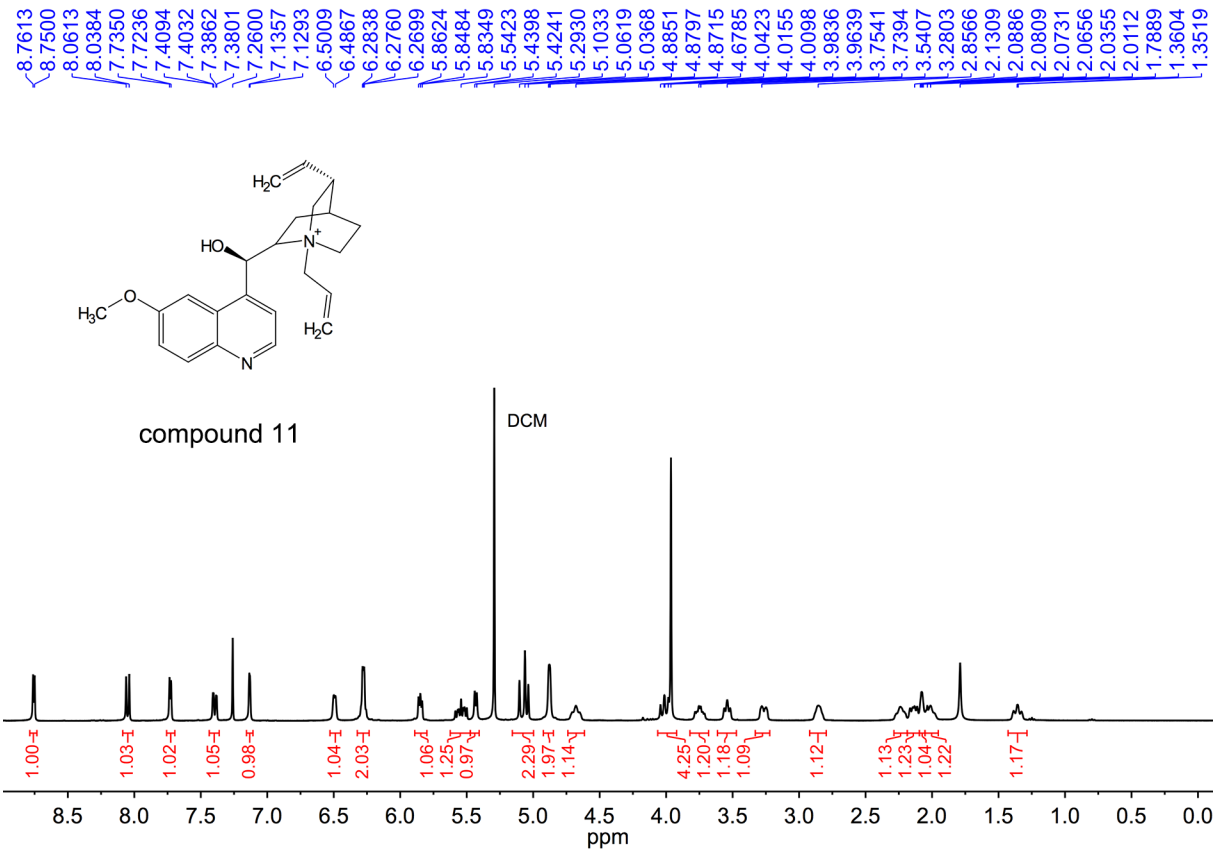
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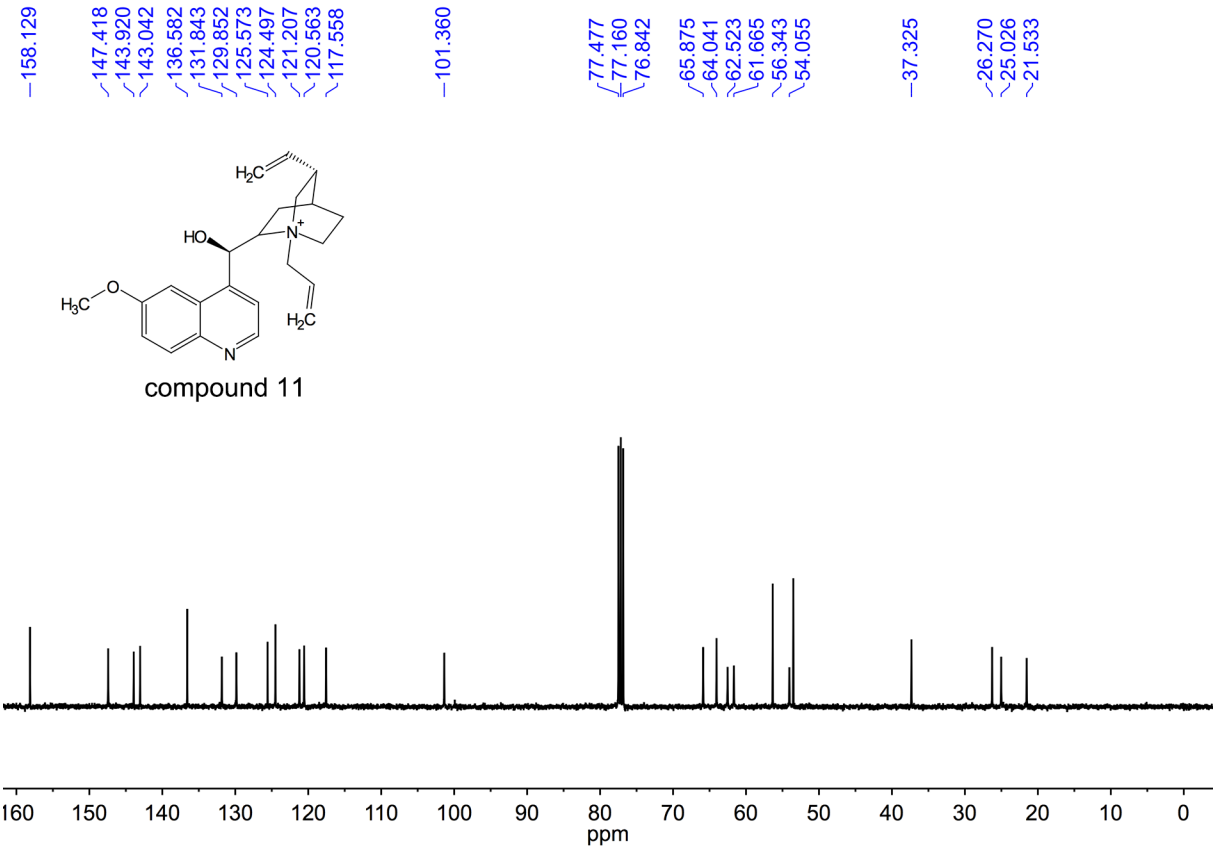
4.0098
3.9836
3.9639
3.7541
3.7394
3.5407
3.2803
2.8566
2.1309
2.0886
2.0809
2.0731
2.0656
2.0355
2.0112
1.7889
1.3604
1.3519
compound 11
DCM
1.00
1.03
1.02
1.05
0.98
1.04
2.03
1.06
1.25
0.97
2.29
1.97
1.14
4.25
1.20
1.18
1.09
1.12
1.13
1.23
1.04
1.22
1.17
ppm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147.418
143.920
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136.582
131.843
129.852
125.573
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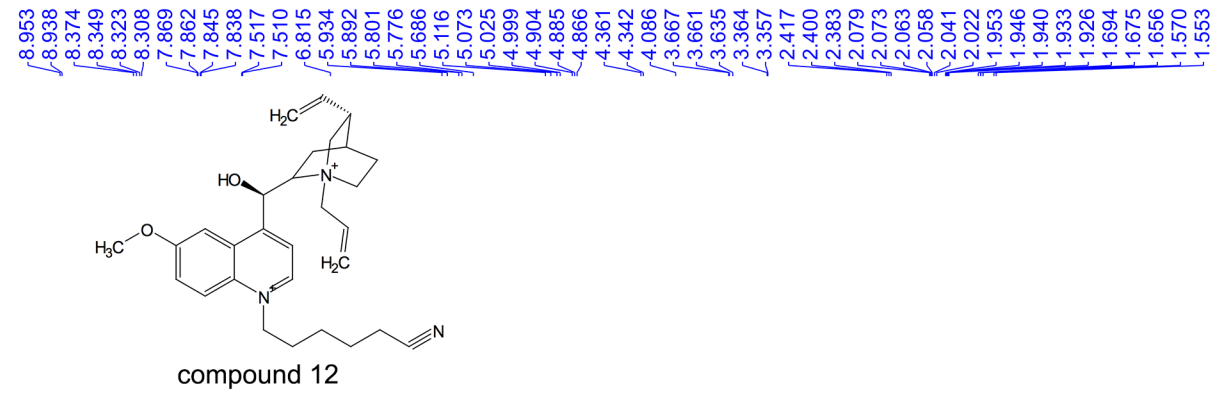
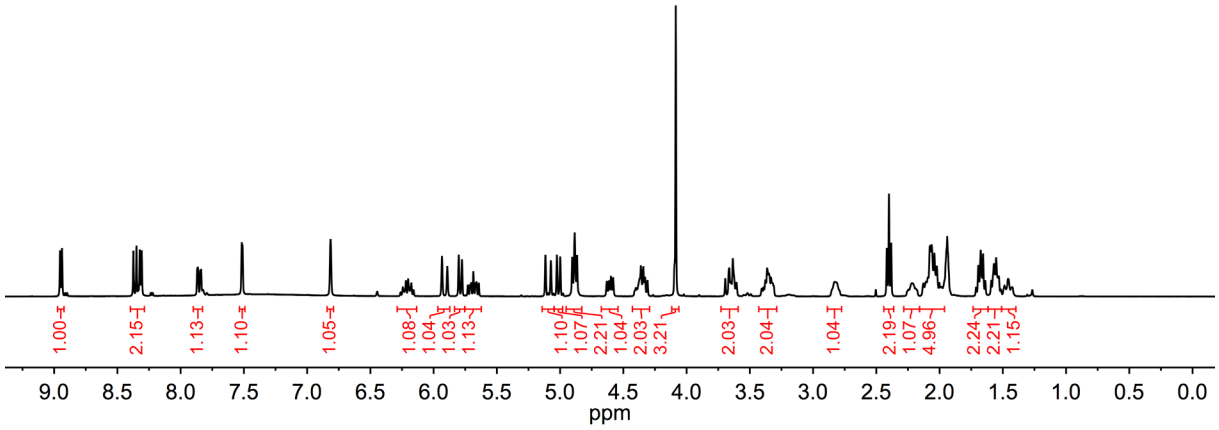
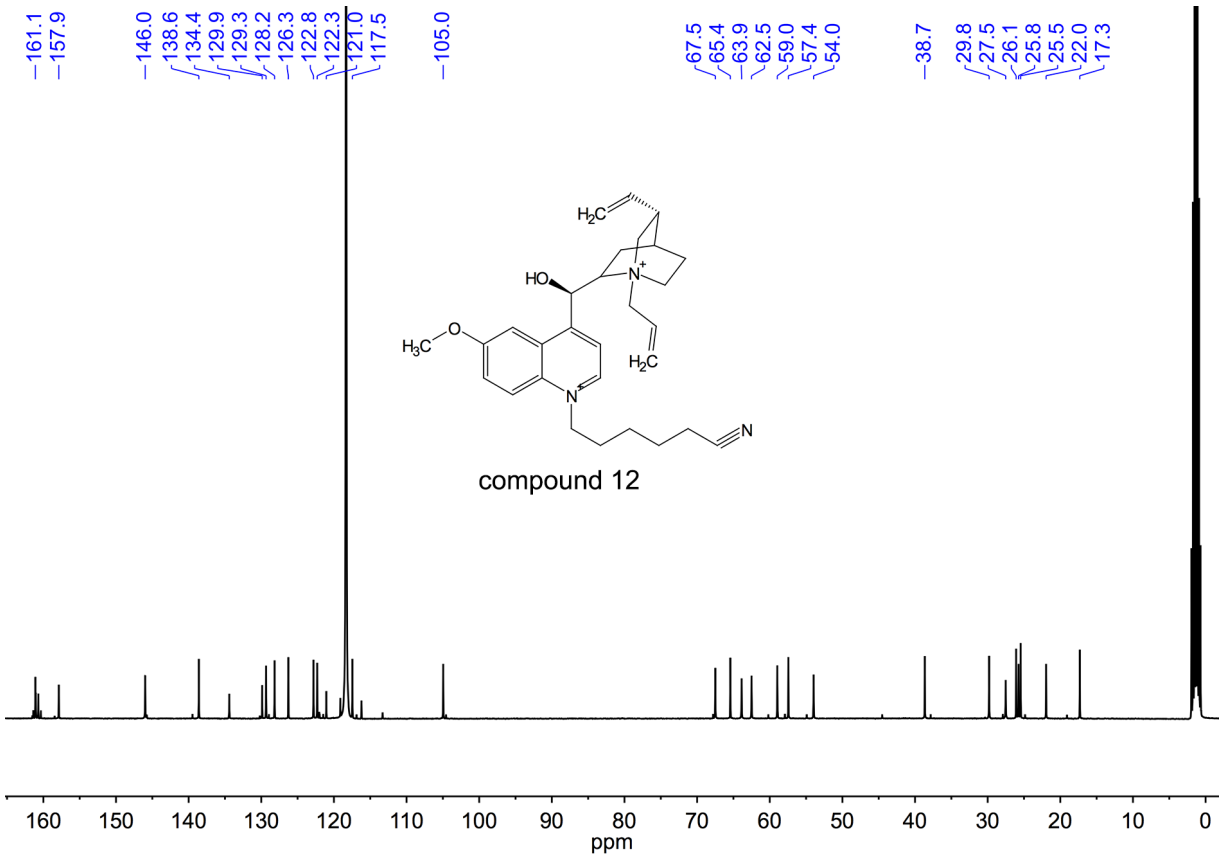
121.207
120.563
117.558
101.360
77.477
77.160
76.842
65.875
64.041
62.523
61.665
56.343
54.055
37.325
26.270
25.026
21.533
compound 11
ppm

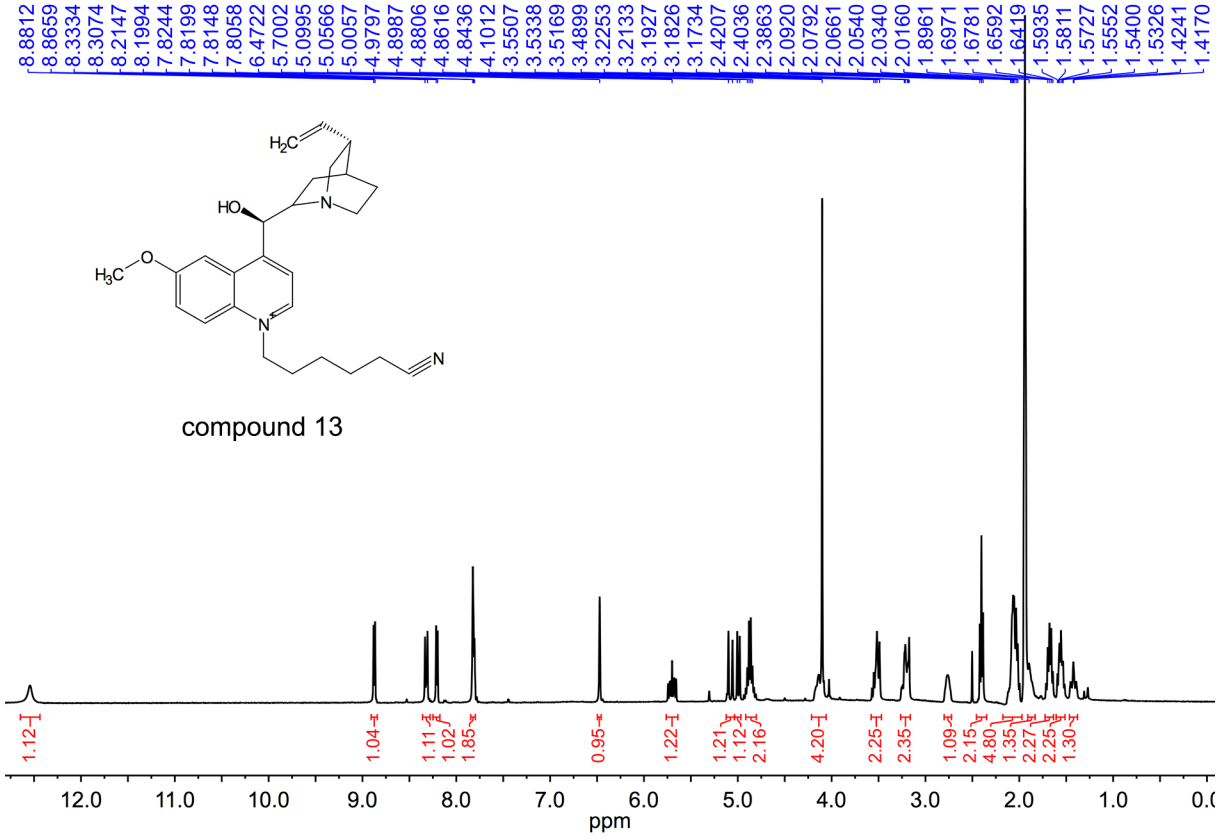


compound 12





compound 12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8.3334
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8.2147
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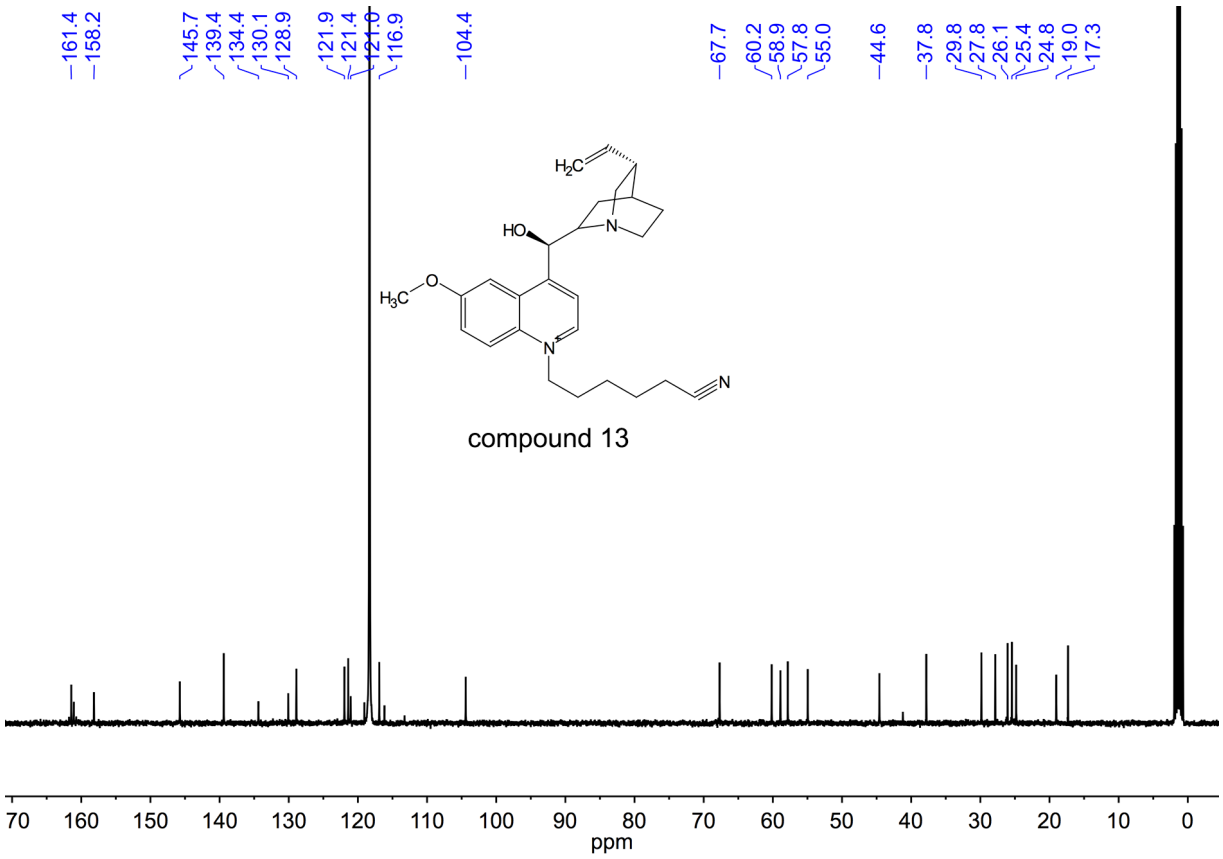
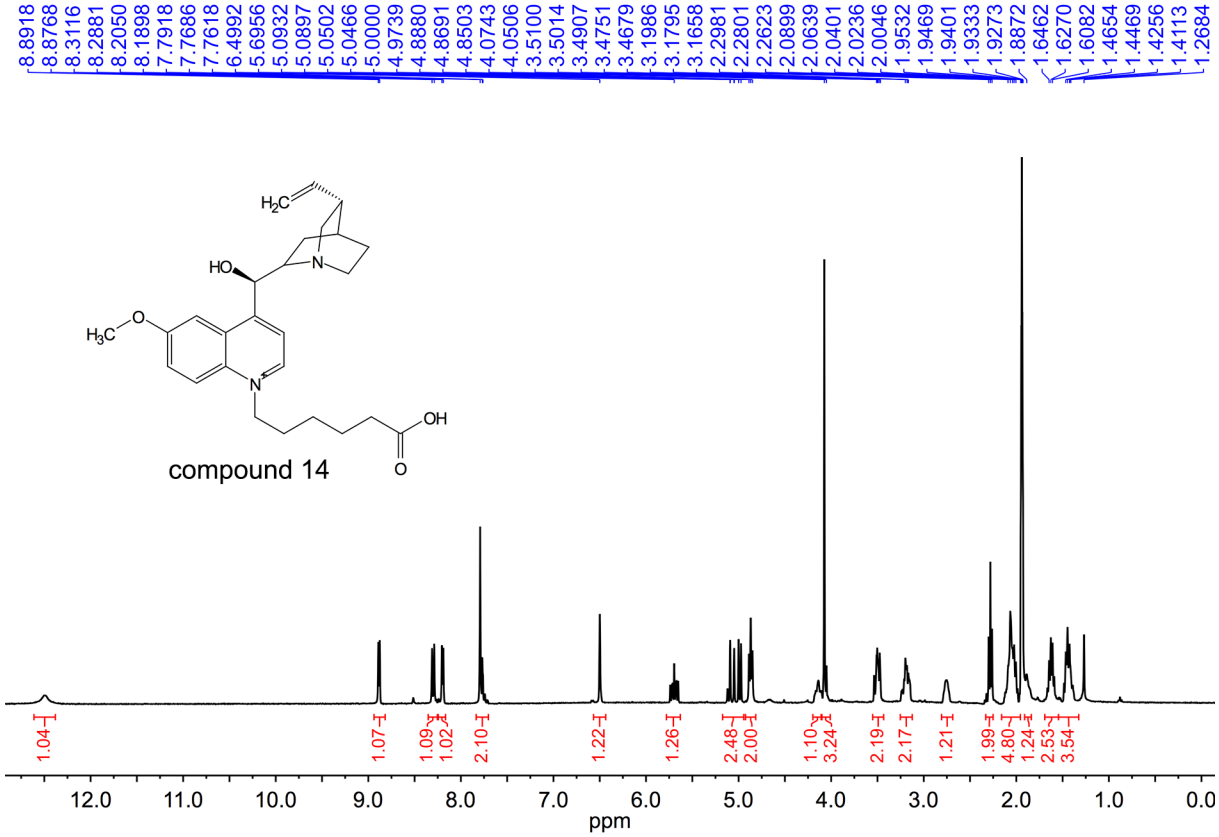
7.8244
7.8199
7.8148
7.8058
6.4722
5.7002
5.0995
5.0566
5.0057
4.9797
4.8987
4.8806
4.8616
4.8436
4.1012
3.5507
3.5338
3.5169
3.4899
3.2253
3.2133
3.1927
3.1826
3.1734
2.4207
2.4036
2.3863
2.0920
2.0792
2.0661
2.0540
2.0340
2.0160
1.8961
1.6971
1.6781
1.6592
1.6419
1.5935
1.5811
1.5727
1.5552
1.5400
1.5326
1.4241
1.4170
H2C
HO
N
H3C
O
N+
N
compound 13
1.12
1.04
1.11
1.02
1.85
0.95
1.22
1.21
1.12
2.16
4.20
2.25
2.35
1.09
2.15
4.80
1.35
2.27
2.25
1.30
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm


161.4
158.2
145.7
139.4
134.4
130.1
128.9
121.9
121.4
121.0
116.9
104.4
67.7
60.2
58.9
57.8
55.0
44.6
37.8
29.8
27.8
26.1
25.4
24.8
19.0
17.3
H2C
HO
N
H3C
O
N+
N
compound 13
70
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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8.2050
8.1898
7.7918
7.7686
7.7618
6.4992
5.6956
5.0932
5.0897
5.0502
5.0466
5.0000
4.9739
4.8880
4.8691
4.8503
4.0743
4.0506
3.5100
3.5014
3.4907
3.4751
3.4679
3.1986
3.1795
3.1658
2.2981
2.2801
2.2623
2.0899
2.0639
2.0401
2.0236
2.0046
1.9532
1.9469
1.9401
1.9333
1.9273
1.8872
1.6462
1.6270
1.6082
1.4654
1.4469
1.4256
1.4113
1.2684
compound 14
1.04
1.07
1.09
1.02
2.10
1.22
1.26
2.48
2.00
1.10
3.24
2.19
2.17
1.21
1.99
4.80
1.24
2.53
3.54
12.0
11.0
10.0
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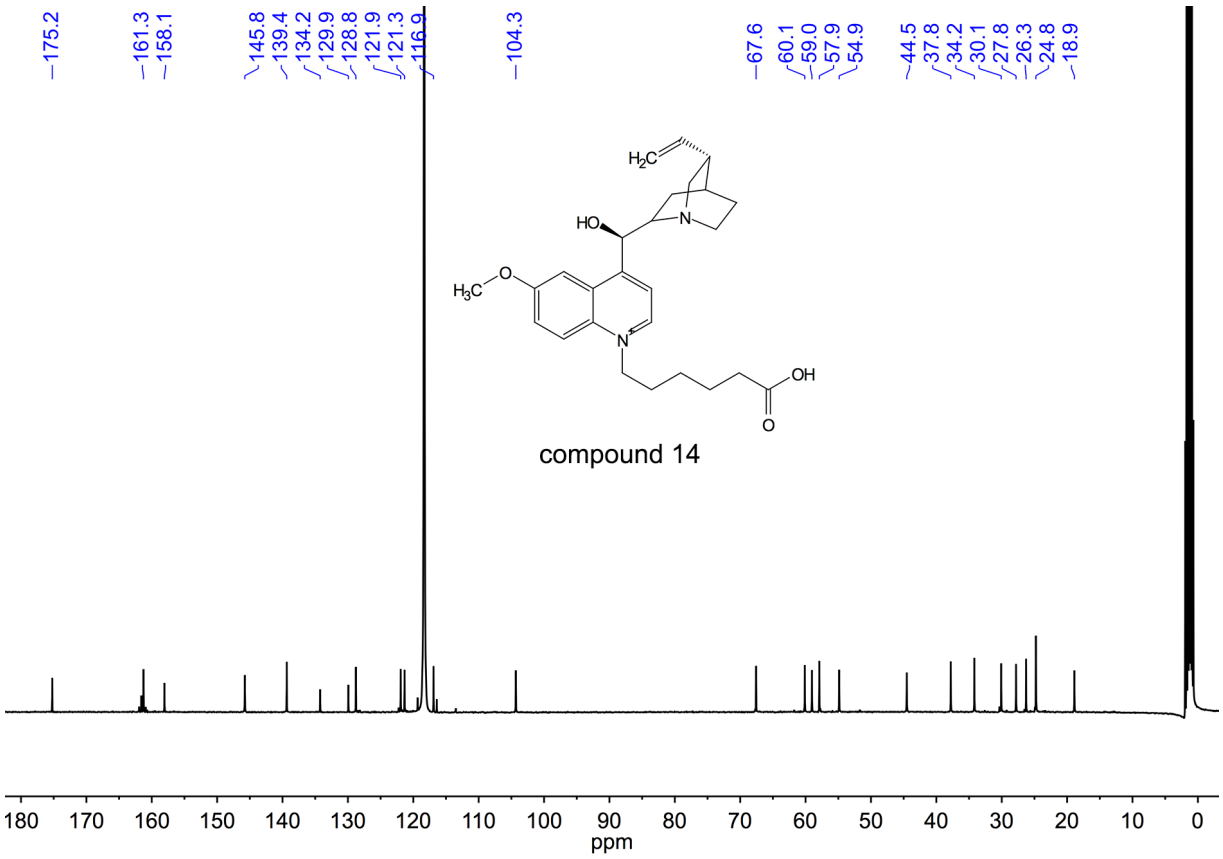
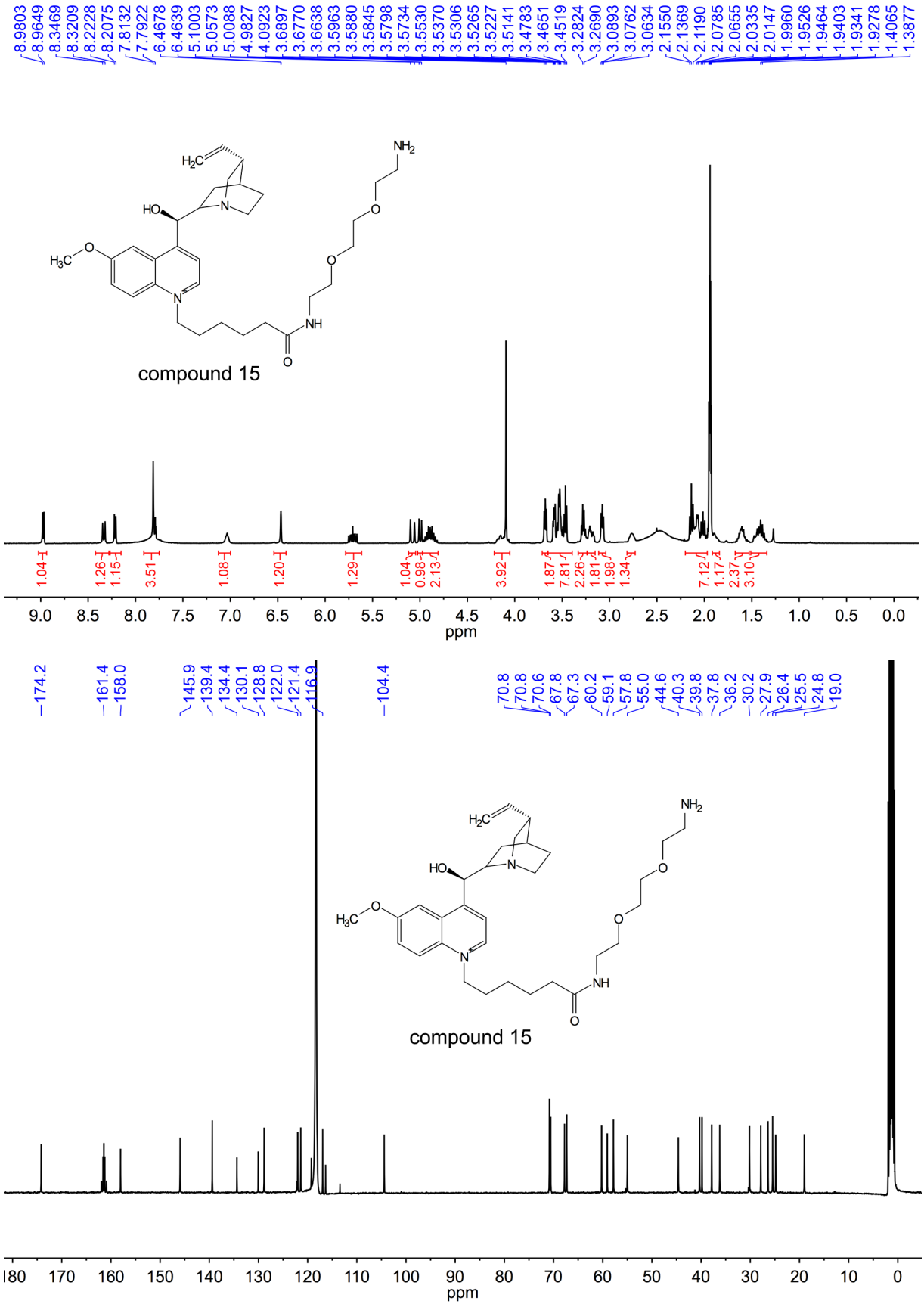
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
ppm


175.2
161.3
158.1
145.8
139.4
134.2
129.9
128.8
121.9
121.3
116.9
104.3
67.6
60.1
59.0
57.9
54.9
44.5
37.8
34.2
30.1
27.8
26.3
24.8
18.9
compound 14
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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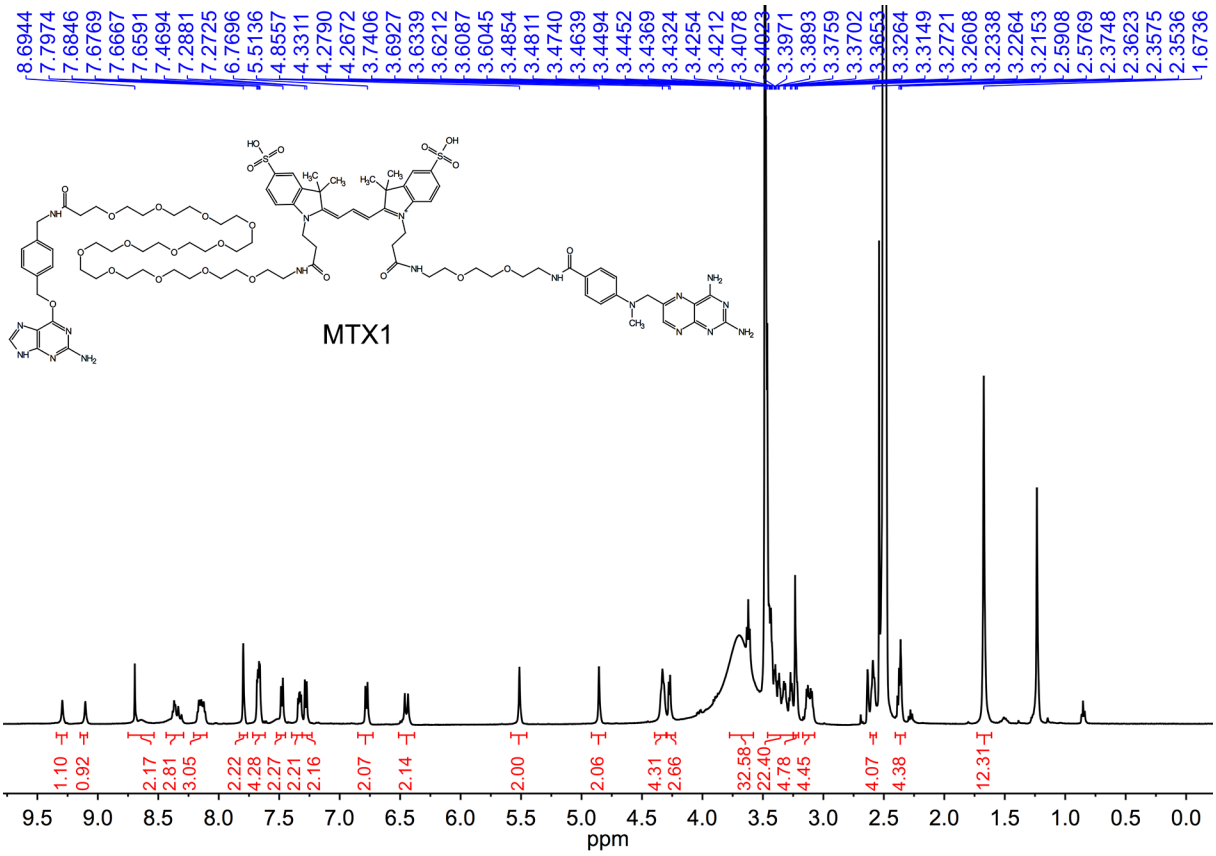
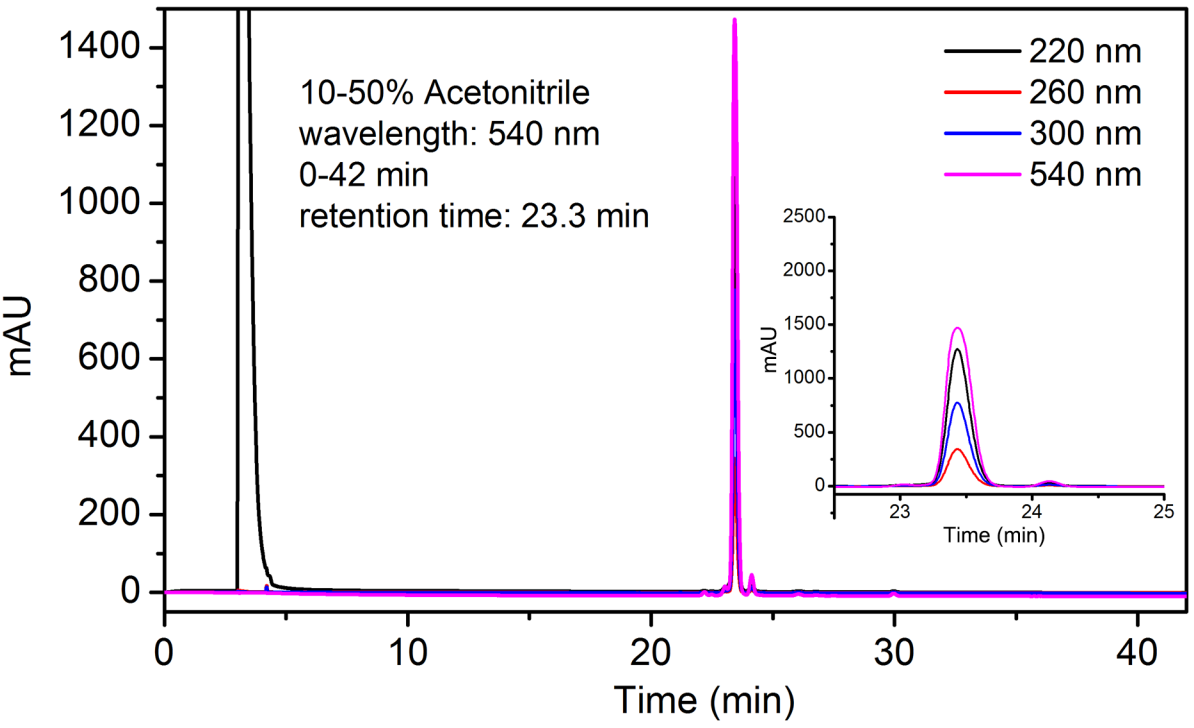
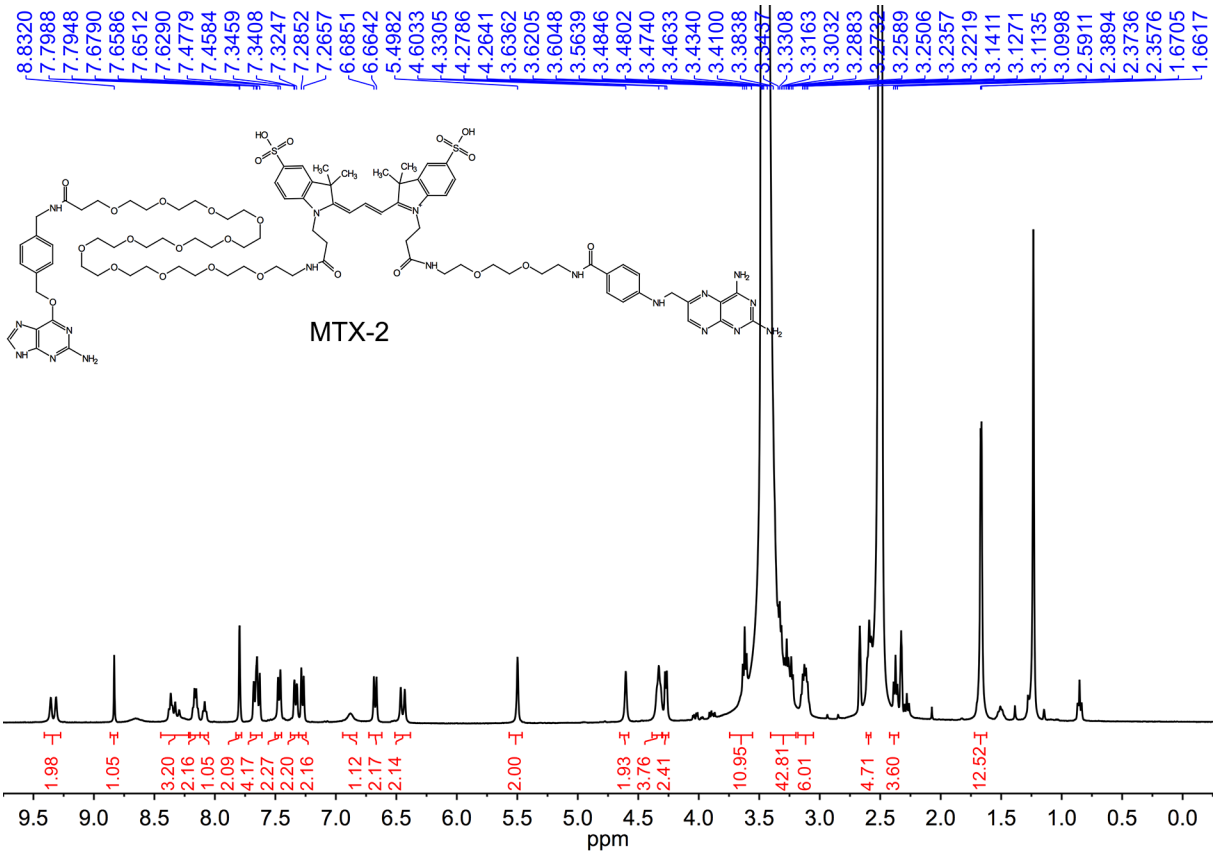
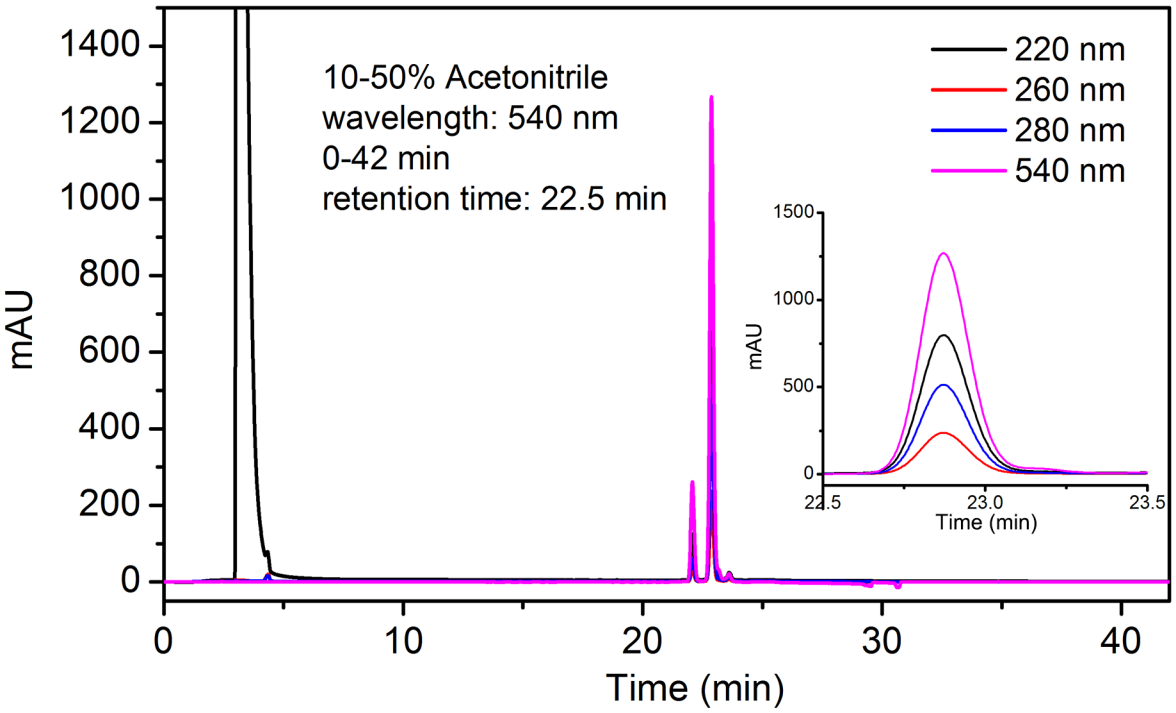
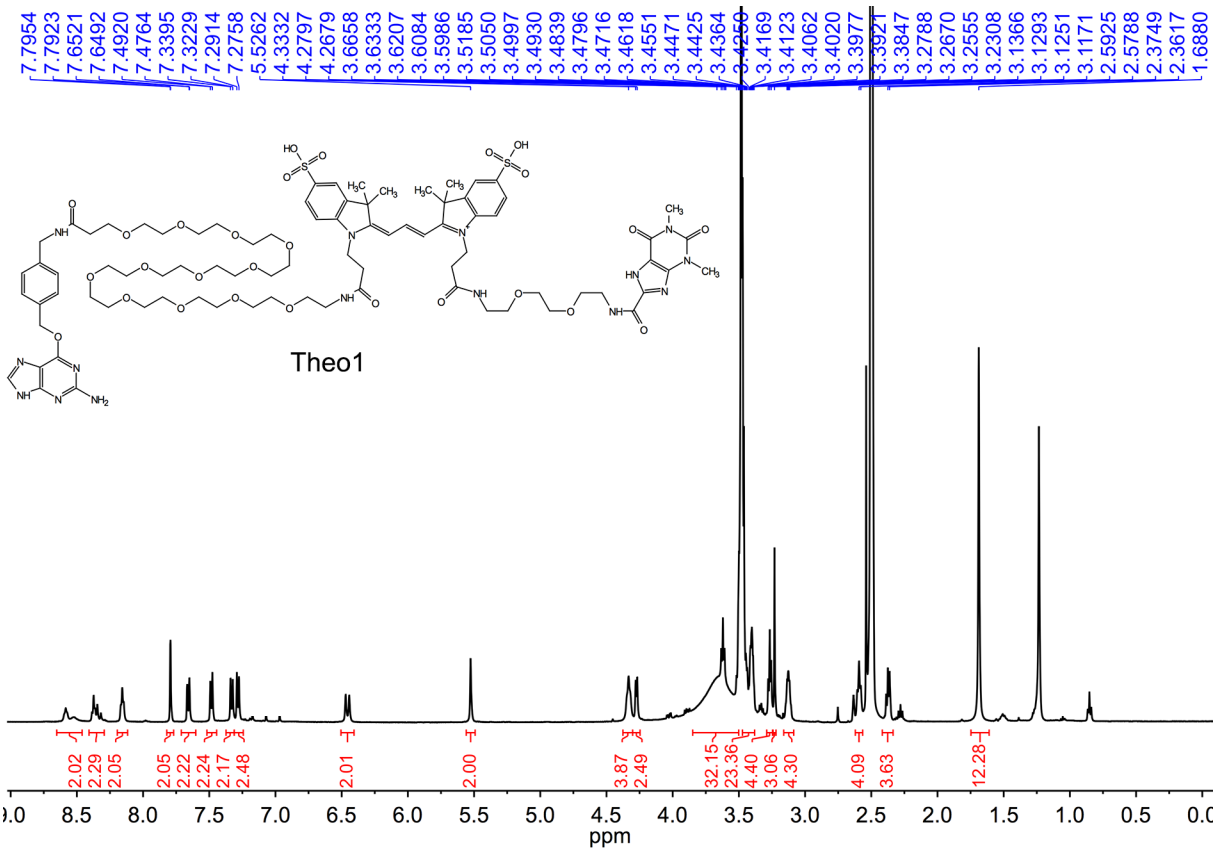
8.3209
8.2228
8.2075
7.8132
7.7922
6.4678
6.4639
5.1003
5.0573
5.0088
4.9827
4.0923
3.6897
3.6770
3.6638
3.5963
3.5880
3.5845
3.5798
3.5734
3.5530
3.5370
3.5306
3.5265
3.5227
3.5141
3.4783
3.4651
3.4519
3.2824
3.2690
3.0893
3.0762
3.0634
2.1550
2.1369
2.1190
2.0785
2.0655
2.0335
2.0147
1.9960
1.9526
1.9464
1.9403
1.9341
1.9278
1.4065
1.3877
compound 15
1.04
1.26
1.15
3.51
1.08
1.20
1.29
1.04
0.98
2.13
3.92
1.87
7.81
2.26
1.81
1.98
1.34
7.12
1.17
2.37
3.10
9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
ppm
174.2
161.4
158.0
145.9
139.4
134.4
130.1
128.8
122.0
121.4
116.9
104.4
70.8
70.8
70.6
67.8
67.3
60.2
59.1
57.8
55.0
44.6
40.3
39.8
37.8
36.2
30.2
27.9
26.4
25.5
24.8
19.0
compound 15
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

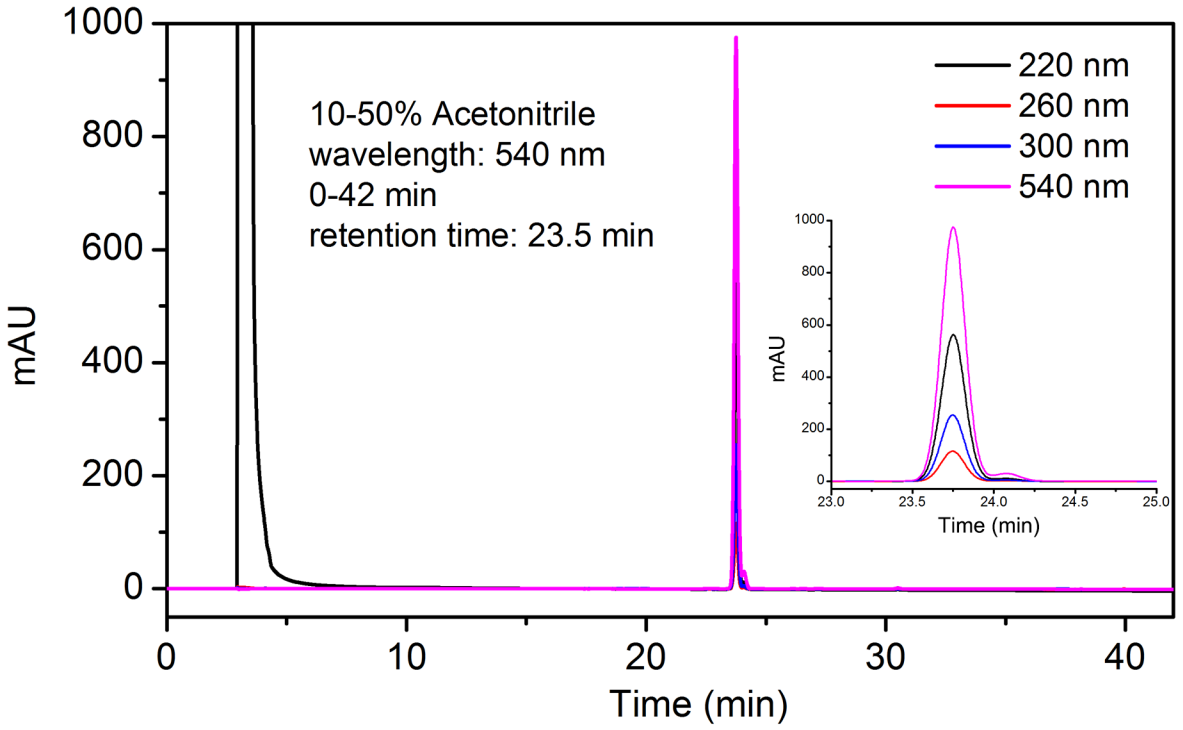

MTX1
ppm

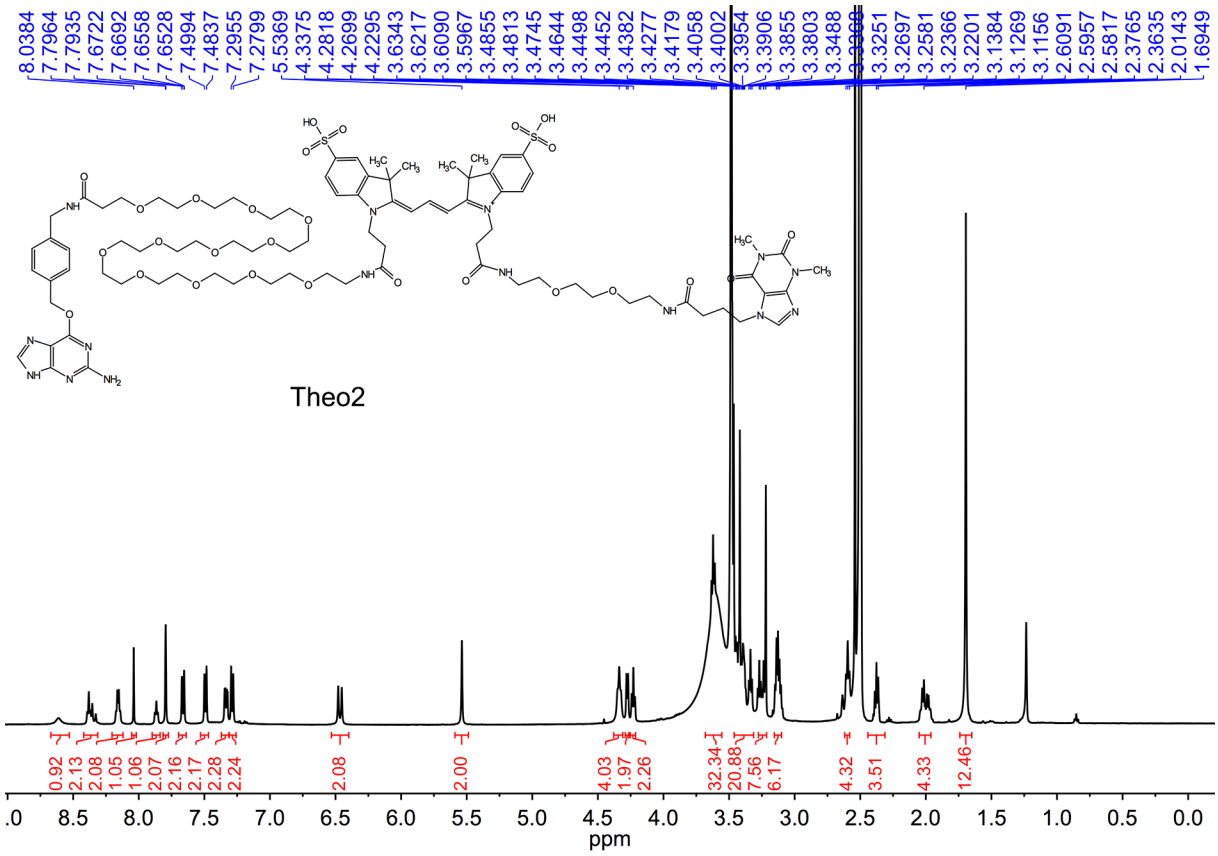

10-50% Acetonitrile
wavelength: 540 nm
0-42 min
retention time: 23.3 min
220 nm
260 nm
300 nm
540 nm
mAU
Time (min)

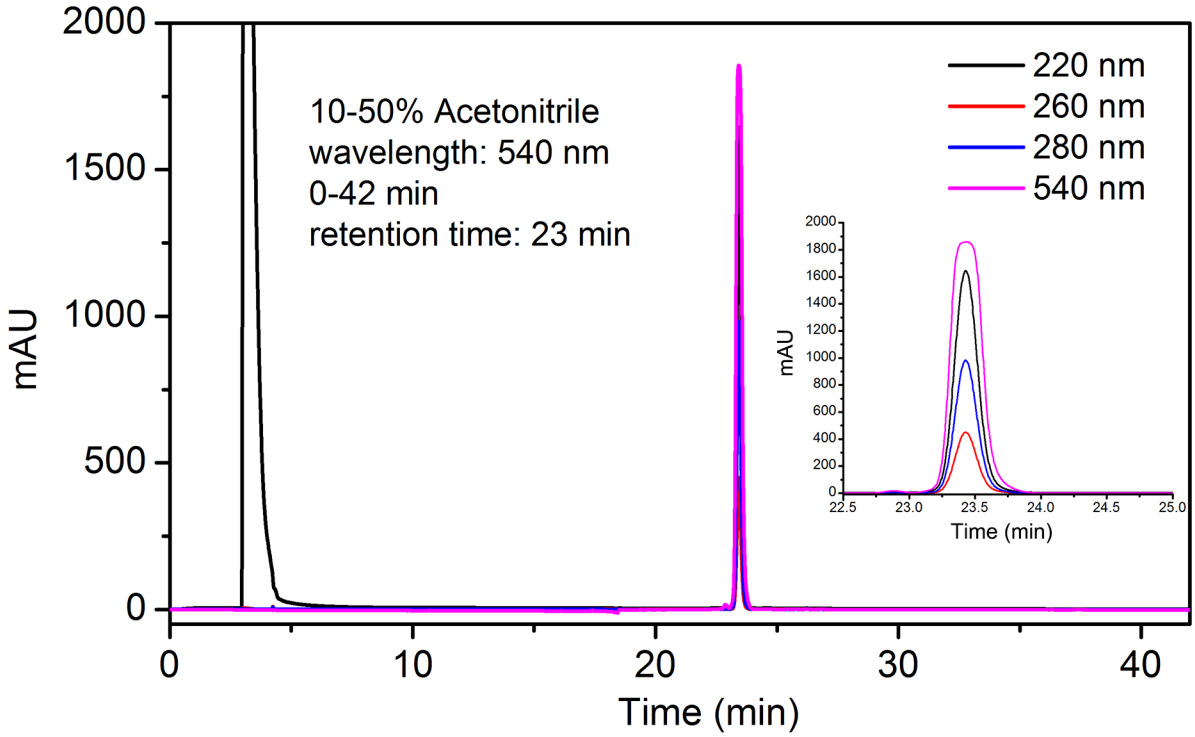

MTX-2
ppm

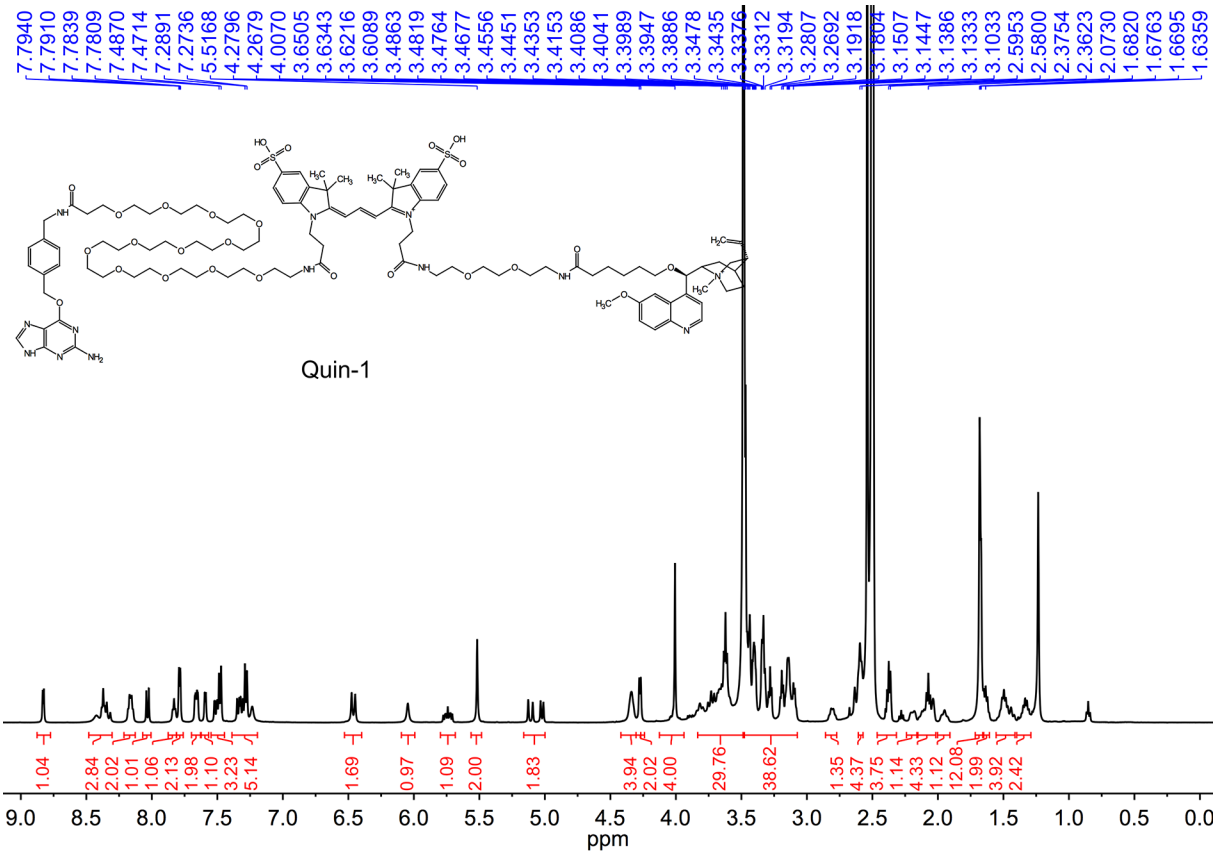

10-50% Acetonitrile
wavelength: 540 nm
0-42 min
retention time: 22.5 min
220 nm
260 nm
280 nm
540 nm
mAU
Time (min)

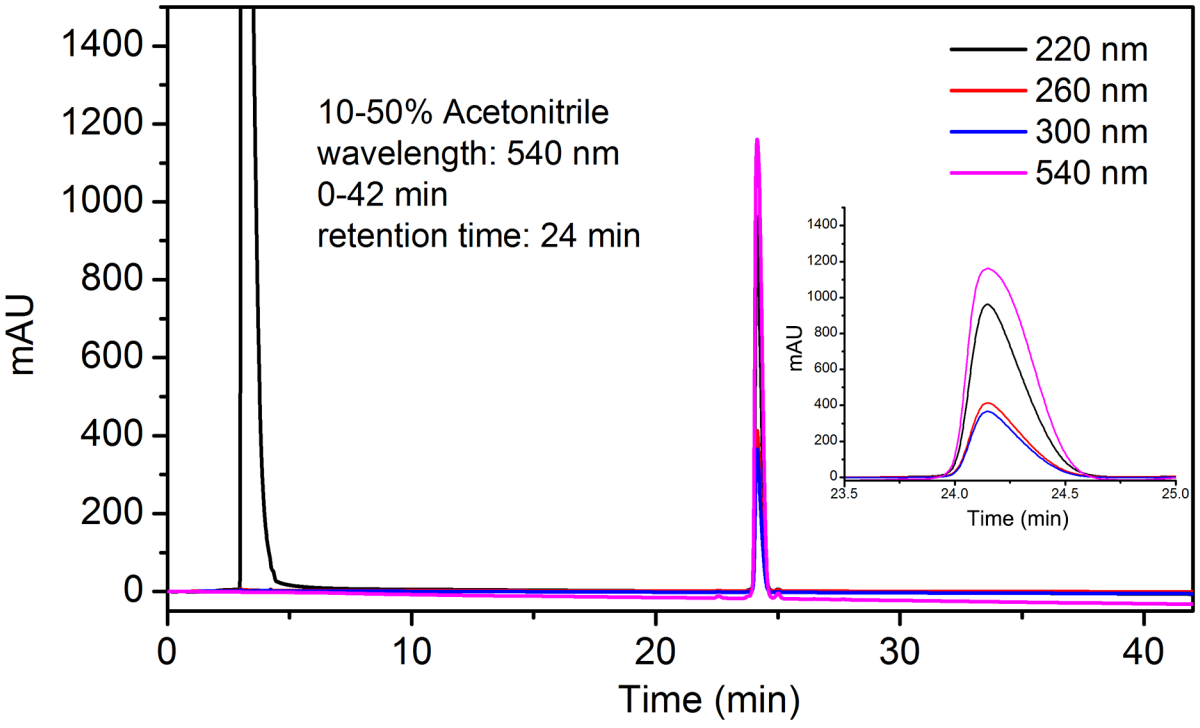

Theo1
ppm

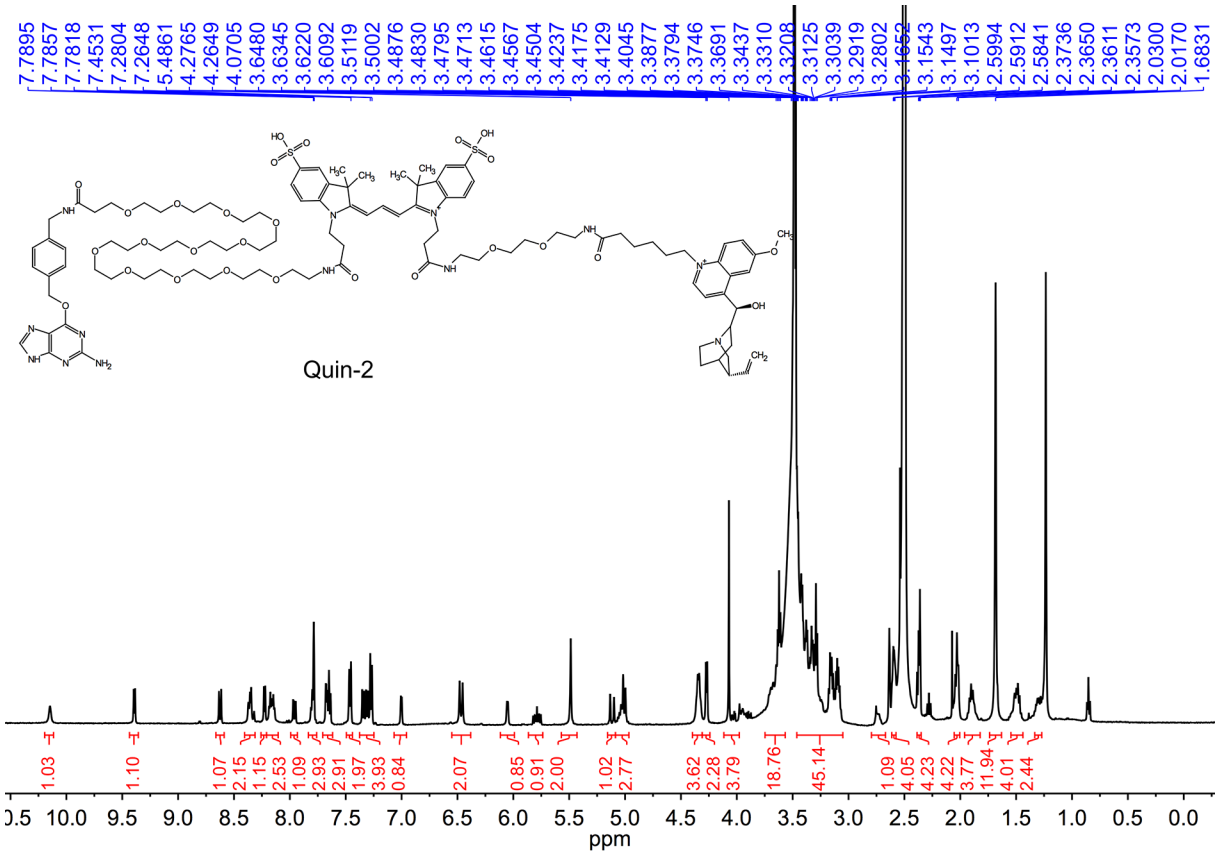

10-50% Acetonitrile
wavelength: 540 nm
0-42 min
retention time: 23.5 min
220 nm
260 nm
300 nm
540 nm
mAU
Time (min)

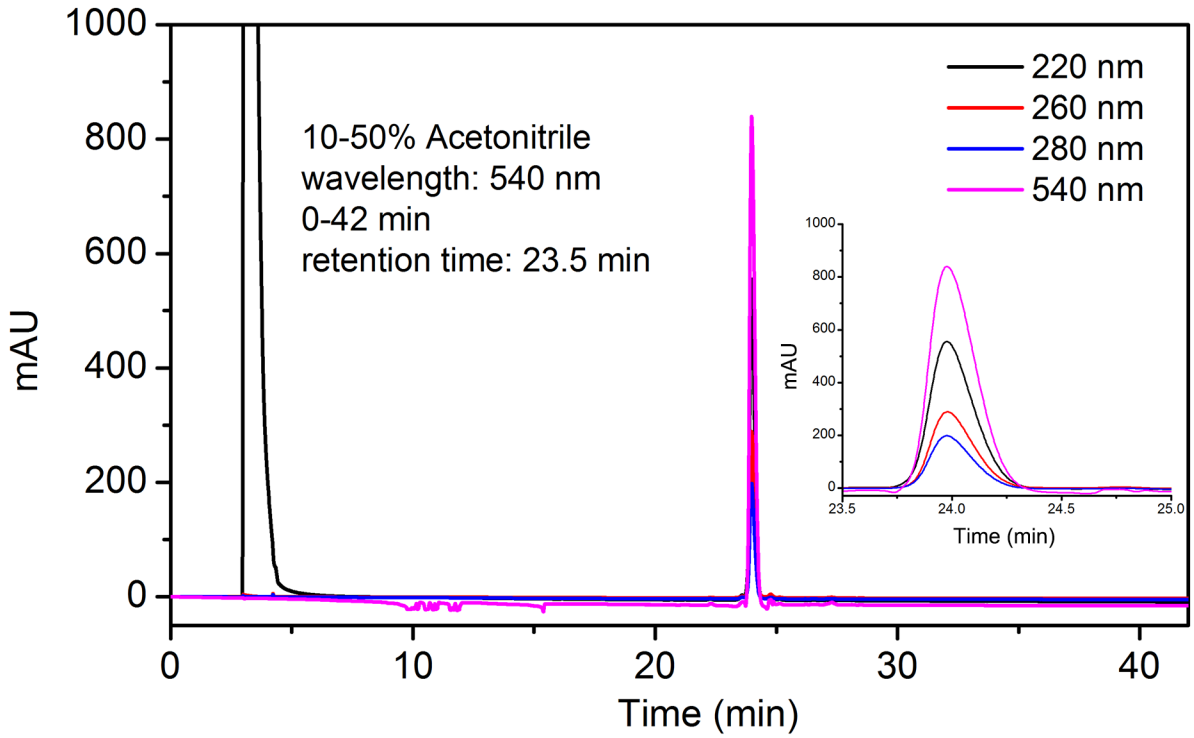

Theo2
ppm


10-50% Acetonitrile
wavelength: 540 nm
0-42 min
retention time: 23 min
220 nm
260 nm
280 nm
540 nm
mAU
Time (min)


Quin-1
ppm


220 nm
260 nm
300 nm
540 nm
10-50% Acetonitrile
wavelength: 540 nm
0-42 min
retention time: 24 min
mAU
Time (min)


Quin-2
ppm


10-50% Acetonitrile
wavelength: 540 nm
0-42 min
retention time: 23.5 min
220 nm
260 nm
280 nm
540 nm
mAU
Time (min)