# Supplementary Information

## Systematic kinetic assay of phenylsulfonyl pyridine derivatives with free thiol for site-specific modification of proteins

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## 1 Synthesis of phenylsulfonyl pyridine tags

### 1.1 General information

Unless mentioned otherwise, all materials and solvents were commercially obtained and used without further purification. Thin–layer chromatography (TLC) was performed on 0.25 mm silica gel 60–F254. Column chromatography was carried out on silica gel (200-300 mesh). Visualization of compounds was carried out with 254 nm UV light or iodine. All the $^{1}$H–NMR spectra were recorded on Bruker NMR spectrometer at a magnetic field with a proton frequency of 400 MHz or 800 MHz and the $^{13}$C{$^{1}$H} NMR recorded on instrument of 101 MHz or 201 MHz. Chemical shifts were reported in ppm on the $\delta$ scale from $SiMe_4$ as an internal standard (NMR descriptions: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad). Coupling constants, *J*, are reported in Hz. HRMS was recorded on Agilent 1290 series HPLC system equipped with a 6545 series ESI-Q-TOF (Agilent, CA, USA).

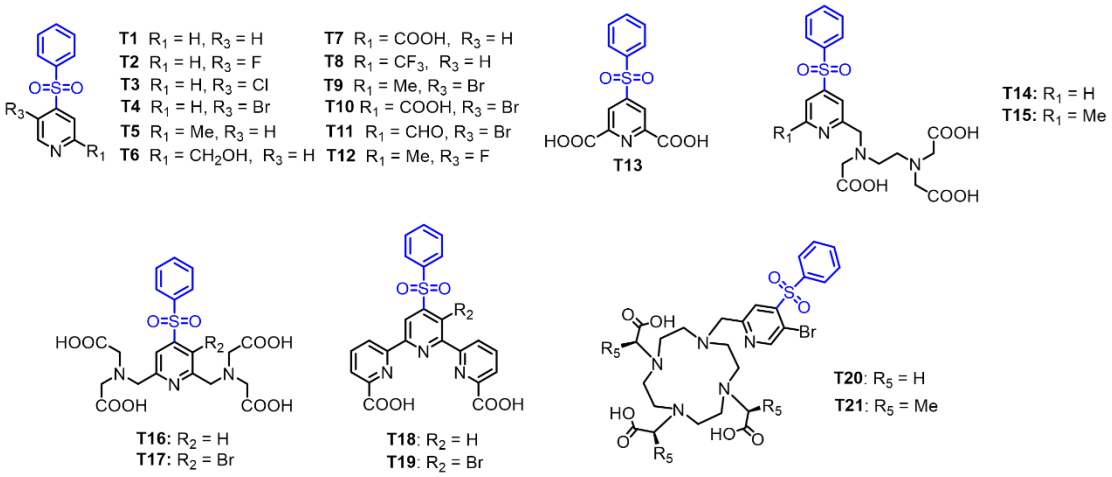
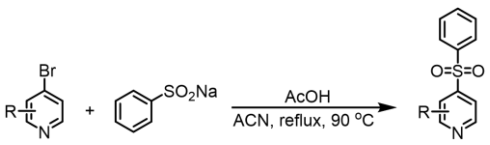



**Figure S1**. A summary of phenylsulfonyl pyridine tags in this work.

### 1.2 Synthetic routes of T1-T21



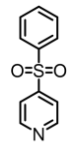

**General method A**: To a solution of 4-bromopyridine derivatives in acetonitrile was added benzensulfinic acid sodium salt and acetic acid. The mixture was refluxed under 90 °C for 12 h and then cooled down to room temperature. The precipitate was filtered and the solvent was removed under vacuum. The residue was purified by column chromatography to give the target compound.

**General method B**: To a solution of 4-bromopyridine derivatives in acetonitrile was added benzensulfinic

acid sodium salt and tetrabutylammonium bromide (TBAB). The mixture was refluxed under argon and then cooled down to room temperature. The precipitate was filtered and the solvent was removed under vacuum. The residue was purified by column chromatography to give the target compound.

The synthesis methods and character data of **T2**, **T12-T17**, **T20** and **T21** can be found in our previous work[1-6].

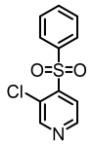
**Synthesis of T1:**



**T1**

**T1** was synthesized starting from 4-bromopydine as white solid according to the general method A. Yield: 96%. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.84 (d, *J* = 6.3 Hz, 1H), 7.98 (d, *J* = 7.4 Hz, 1H), 7.85 – 7.75 (m, 1H), 7.65 (t, *J* = 7.5 Hz, 1H), 7.57 (t, *J* = 7.6 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.2, 149.7, 139.6, 134.1, 129.6, 128.1, 120.6. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{11}H_{10}NO_2S$ 220.0427; Found: 220.0426.

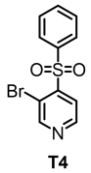
**Synthesis of T3:**



**T3**

**T3** was synthesized as white solid according to the general method A. Yield: 85%. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.77 (d, *J* = 5.0 Hz, 1H), 8.69 (s, 1H), 8.14 (d, *J* = 5.1 Hz, 1H), 8.04 – 7.96 (m, 2H), 7.71 – 7.65 (m, 1H), 7.57 (dd, *J* = 8.5, 7.2 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 152.1, 149.0, 146.0, 138.4, 134.4, 129.2, 129.0, 123.0. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{11}H_9ClNO_2S$ 254.0037; Found: 254.0048.

**Synthesis of T4:**

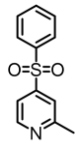


**T4**

**T4** was synthesized as white solid according to the general method A. Yield: 71%. $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.84 (s, 1H), 8.81 (d, *J* = 5.0 Hz, 1H), 8.18 (d, *J* = 5.0 Hz, 1H), 8.03 – 7.95 (m, 2H), 7.72 – 7.64

(m, 1H), 7.56 (dd, *J* = 8.5, 7.2 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 154.8, 149.5, 138.2, 134.3, 129.2, 129.1, 123.6, 118.0. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{11}H_9BrNO_2S$ 297.9532; Found: 297.9533.

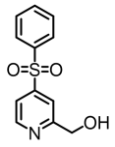
**Synthesis of T5:**



**T5**

**T5** was synthesized starting from 2-methyl-4-bromopydine as white solid according to the general method A. Yield: 86%. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.69 (d, *J* = 5.1 Hz, 1H), 7.97 (d, *J* = 7.8 Hz, 2H), 7.63 (t, *J* = 3.5 Hz, 2H), 7.60 – 7.53 (m, 3H), 2.64 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 160.7, 150.5, 149.9, 139.8, 134.0, 129.5, 128.1, 120.0, 117.7, 24.6. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{12}H_{12}NO_2S$ 234.0583; Found: 234.0581.

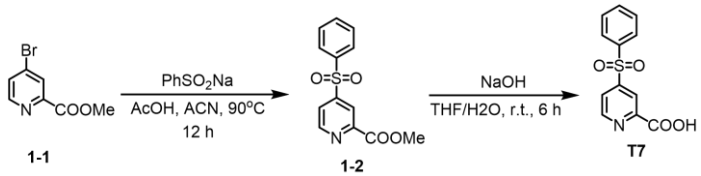
**Synthesis of T6:**



**T6**

**T6** was synthesized starting from 2-hydroxymethyl-4-bromopydine as white solid according to the general method A. Yield: 94%.$^1$H NMR (400 MHz, $CDCl_3$) δ 8.73 (d, *J* = 5.1 Hz, 1H), 8.02 – 7.94 (m, 2H), 7.83 (d, *J* = 1.8 Hz, 1H), 7.73 – 7.62 (m, 2H), 7.57 (dd, *J* = 8.5, 6.9 Hz, 2H), 4.84 (s, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 162.0, 150.1, 139.6, 134.2, 129.7, 128.2, 119.3, 117.6, 64.4. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{12}H_{12}NO_3S$ 250.0532; Found: 250.0535.

**Synthesis of T7:**



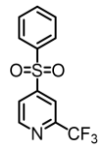

**1-1** **1-2** **T7**

**Synthesis of 1-2**: The **1-2** was synthesized by a modified method according to the reported literature[7]. The compound **1-2** was synthesized as white solid according to general method A, yield: 61%.

Characterization data correspond to the literature[7]. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.92 (d, *J* = 4.9 Hz, 1H), 8.51 (d, *J* = 1.7 Hz, 1H), 7.99 – 7.93 (m, 3H), 7.68 – 7.61 (m, 1H), 7.55 (dd, *J* = 8.5, 6.9 Hz, 2H), 4.00 (s, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 164.2, 151.5, 151.3, 149.7, 139.2, 134.5, 129.8, 128.3, 123.7, 122.2, 53.4.

**Synthesis of T7**: To a solution of compound **1-2** (2 mmol, 554 mg) in THF/$H_2O$ (30 mL, 2:1) was added NaOH (3 mmol, 120 mg). The mixture was stirred at room temperature for 6 hours followed by acidifying to pH 4-5. Filtering and collecting the precipitate afford the **T7** as white solid, yield 65%. $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.03 (d, *J* = 5.0 Hz, 1H), 8.38 (s, 1H), 8.19 (dd, *J* = 5.1, 1.7 Hz, 1H), 8.10 (d, *J* = 7.8 Hz, 2H), 7.81 (t, *J* = 7.4 Hz, 1H), 7.71 (t, *J* = 7.6 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) δ 165.3, 152.3, 150.9, 150.7, 139.2, 135.3, 130.6, 128.6, 124.1, 121.3. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{12}H_{10}NO_4S$ 264.0325; Found: 264.0328.

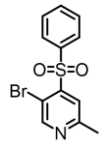
**Synthesis of T8:**



**T8**

**T8** was synthesized starting from 2-trifluoromethyl-4-bromopyridine as white solid according to the general method A. Yield: 74%.$^1$H NMR (400 MHz, $CDCl_3$) δ 8.95 (d, *J* = 5.0 Hz, 1H), 8.14 (s, 1H), 8.04 – 7.95 (m, 3H), 7.73 – 7.66 (m, 1H), 7.64 – 7.58 (m, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 151.9, 151.7, 150.0 (d, *J* = 36.0 Hz), 138.8, 134.7, 129.95, 128.3, 123.6, 120.7 (d, *J* = 274.6 Hz), 117.8 (d, *J* = 2.9 Hz). $^{19}$F{$^1$H} NMR (376 MHz, $CDCl_3$) δ -68.9. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{12}H_9F_3NO_2S$ 288.0301; Found: 288.0305.

**Synthesis of T9:**

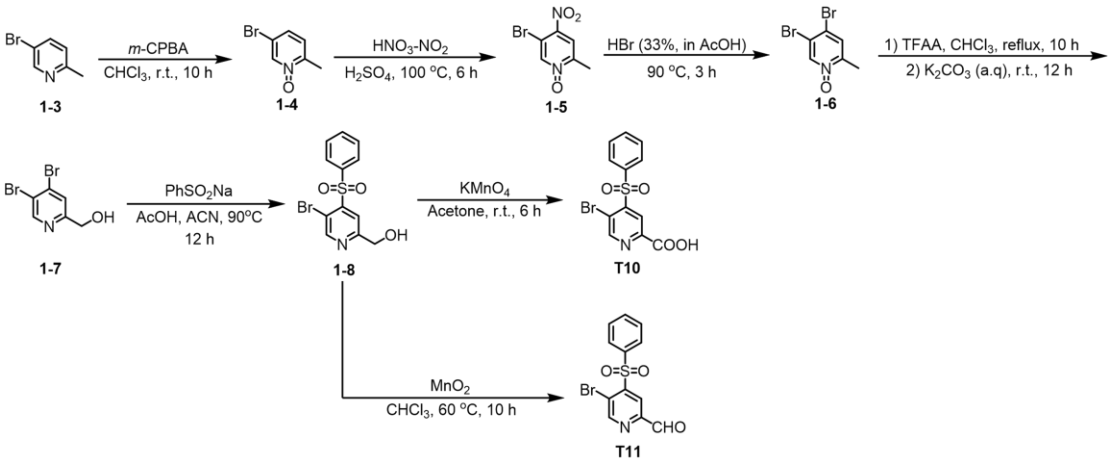


**T9**

**T9** was synthesized starting from 4,5-dibromo-2-methylpydine as white solid according to the general method A. Yield: 64%. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 1H), 8.05 (s, 1H), 8.01 – 7.95 (m, 1H), 7.69 – 7.64 (m, 1H), 7.55 (dd, *J* = 8.4, 7.1 Hz, 1H), 2.65 (s, 1H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 159.5, 154.1, 147.5, 138.4, 134.2, 129.1, 129.0, 123.4, 114.5, 24.1. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for

$C_{12}H_{11}BrNO_2S$ 311.9688; Found: 311.9679.

**Synthesis of T10, T11:**




**Synthesis of 1-4**: The **1-4** was synthesized by a modified method according to the reported literature[8]. *m*-CPBA (15.0 g, 87.2 mmol) was added to a solution of compound **1-3** (19.5 g, 95.9 mmol) in chloroform. The mixture was stirred at room temperature for 10 h. The formed precipitate was filtered and the filtrate was removed under vacuum. The crude product was purified by column chromatography to give the compound **1-4** as white solid (16 g, 98% yield). Characterization data correspond to the literature[8]. $^1$H NMR (600 MHz, $CDCl_3$) δ 8.42 (d, *J* = 1.7 Hz, 1H), 7.32 (dd, *J* = 8.4, 1.8 Hz, 1H), 7.15 (d, *J* = 8.4 Hz, 1H), 2.47 (s, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 148.1, 140. 7, 128.3, 126. 5, 117.3, 17.4.

**Synthesis of 1-5**: The **1-5** was synthesized by a modified method according to the reported literature[6]. To compound **1-4** (16 g, 85.1 mmol) was added fuming nitric acid (25 mL) and concentrated sulfuric acid (50 mL) under 0 °C and mixture was stirred at 100℃ for 6 h. Cool the mixture to room temperature and poured it into ice water. Extract the water phase with DCM (3×100 mL).The organic phase was washed with saturate sodium dicarbonate solution and dried over sodium sulfate followed by removing the solvent under vacuum. The residue was purified by column chromatography to give the compound **1-5** as brown oil (18 g, 91% yield). Characterization data correspond to the literature[6]. $^1$H NMR (600 MHz, $CDCl_3$) δ 8.54 (s, 1H), 8.01 (s, 1H), 2.52 (s, 4H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 149.5, 143.0, 142.5, 122.1, 111.1, 17.4.

**Synthesis of 1-6**: The **1-6** was synthesized by a modified method according to the reported literature[6]. To Hydrogen bromide in acetic acid (33%, 34 mL) was added compound **1-5** (18.0 g, 77.3 mmol). The mixture was stirred at 90 °C for 3 h and cooled to room temperature. Poured the solution into ice water

and adjusted the pH with sodium hydroxide to neutral. The water phase was extracted with DCM (3×100 mL). The organic phase was dried over sodium sulfate and filtered through celite. The residue was purified by column chromatography to give the compound **1-6** as yellow solid (19.5 g, 95% yield). Characterization data correspond to the literature[6]. $^{1}$H NMR (600 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.51 (s, 1H), 2.46 (s, 3H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 149.0, 141.4, 129.4, 121.3, 120.5, 17.2.

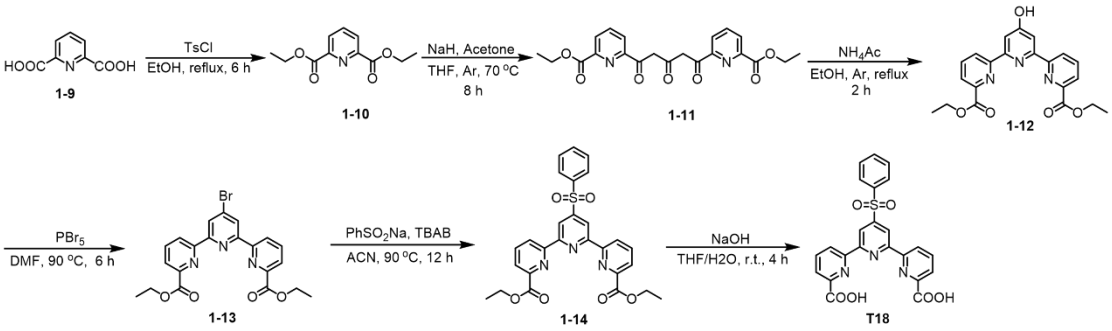
**Synthesis of 1-7**: The **1-7** was synthesized by a modified method according to the reported literature[6]. To a solution of **1-6** (19.5 g, 73.1 mmol) in chloroform was added TFAA (45 g, 214 mmol) dropwise. Then the mixture was stirred at 65℃ for 10 h. The solvent was removed under vacuum followed by adding saturate sodium decarbonate and stirred at room temperature overnight. The water phase was extracted with DCM (3×100 mL). The organic phase was dried over sodium sulfate and filtered through celite. The residue was purified by column chromatography to give the compound **1-7** as brown solid (6.0 g, 31% yield). Characterization data correspond to the literature[6]. $^{1}$H NMR (600 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.65 (s, 1H), 4.74 (s, 2H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 159.2, 151.2, 135.6, 125.5, 121.9, 63.7.

**Synthesis of 1-8**: The compound **1-8** was synthesized starting from **1-7** according to general method A as brown solid (6.3 g, 85 % yield). Characterization data correspond to the literature[6]. $^{1}$H NMR (600 MHz, $CDCl_3$) δ 8.78 (s, 1H), 8.25 (s, 1H), 8.01 (d, *J* = 7.9 Hz, 2H), 7.69 (t, *J* = 7.5 Hz, 1H), 7.58 (t, *J* = 7.7 Hz, 2H), 4.88 (s, 2H). $^{13}$C NMR (151 MHz, $CDCl_3$) δ 160.7, 153.8, 148.3, 138.2, 134.4, 129.2, 129.1, 120.8, 116.0, 64.12.

**Synthesis of T10**: To a solution of **1-8** (3.0 g, 9.5 mmol) in acetone was added $KMnO_4$. Then the mixture was stirred at room temperature for 6 h. Then the insoluble $KMnO_4$ was filtered through celite and the filtrate was concentrated under vacuum to afford the **T10** as white solid (2.7 g, 94% yield). $^{1}$H NMR (400 MHz, $CDCl_3$) δ 8.94 (s, 1H), 8.52 (s, 1H), 8.03 (d, *J* = 7.8 Hz, 2H), 7.75 (t, *J* = 7.5 Hz, 1H), 7.63 (t, *J* = 7.7 Hz, 2H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 157.0, 150.2, 137.9, 135.8, 134.4, 130.2, 130.1, 128.8, 123.3, 116.3. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{12}H_9BrNO_4S$ 341.9431; Found: 341.9428.

**Synthesis of T11**: To a solution of **1-8** (3.0 g, 9.5 mmol) in chloroform was added $MnO_2$. Then the mixture was stirred at room temperature for 6 h. Then the insoluble $MnO_2$ was filtered through celite and the filtrate was concentrated under vacuum to afford the **T11** as white solid (2.5 g, 91% yield). $^{1}$H NMR (400 MHz, CDCl3) δ 10.11 (s, 1H), 8.99 (s, 1H), 8.73 (s, 1H), 8.06 – 7.96 (m, 2H), 7.74 – 7.67 (m, 1H), 7.62 – 7.52 (m, 2H). $^{13}$C NMR (101 MHz, CDCl3) δ 190.9, 155.5, 152.5, 149.5, 137.7, 134.7, 129.4, 129.3, 123.1, 121.7. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{12}H_9BrNO_3S$ 325.9481; Found: 325.9485.

**Synthesis of T18:**




**Synthesis of compound 1-10**: The compound **1-10** was synthesized according to the reported literature[9]. A mixture of compound **1-9** (16 g, 100 mmol), *p*-toluenesufonic acid (861 mg, 5 mmol) and ethanol (150 mL) was stirred at 80 °C for 6 h. After cooling down to room temperature, the ethanol was removed under reduced pressure. The residue was diluted with ethyl acetate and washed with 2 M $K_2CO_3$ solution. The organic layer was dried over $Na_2SO_4$ followed by filtering and concentrating under vacuum to deliver compound **1-10** as white solid (21.2 g, 95% yield). The product directly used for the next step without further purification. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.29 (d, *J* = 7.8 Hz, 2H), 8.02 (t, *J* = 7.8 Hz, 1H), 4.50 (q, *J* = 7.1 Hz, 4H), 1.47 (t, *J* = 7.1 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 164.6, 148.6, 138.2, 127.8, 62.4, 14.2. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{11}H_{14}NO_4$ 224.0918; Found: 224.0922.

**Synthesis of compound 1-11:** The compound **1-11** was synthesized according to the reported literature[10]. Compound **1-10** (10 g, 44.8 mmol) and acetone (1.10 mL, 14.9 mmol) were dissolved in dry THF (50 mL) under argon. Then a suspension of NaH (60%, 1.80 g, 44.8 mmol) in dry THF (50 mL) was added dropwise. The mixture was stirred at 70 °C for 8 h to result in the formation of orange precipitate. Concentrated hydrochloric acid was added dropwise to neutralize the precipitate and then the residue was evaporated and dried under vacuum to give **1-11.** The crude product directly used for the next step without further purification. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{21}H_{21}N_2O_7$ 413.1344; Found: 413.1349.

**Synthesis of compound 1-12:** The compound **1-12** was synthesized according to the reported literature[10]. Ammonium acetate (10.3 g, 134 mmol) was added to a flask containing compound **1-11** (8.33 g, 19.2 mmol) and anhydrous ethanol (130 mL). The mixture was refluxed under argon for 2 h. The solvent was removed under reduced pressure and the resulting residue was washed with water (3 X 100 mL). The organic layer was dried over $Na_2SO_4$. Followed by filtering and concentrating to dryness. The crude product was purified by flash column chromatography ($SiO_2$, $CH_2Cl_2$-MeOH gradient 95 : 5) to give crude compound **1-12** (2.97 g, 38%) as a yellow solid. The crude product was used for next step without further

purification. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{21}H_{20}N_3O_5$ 394.1398; Found: 394.1382.

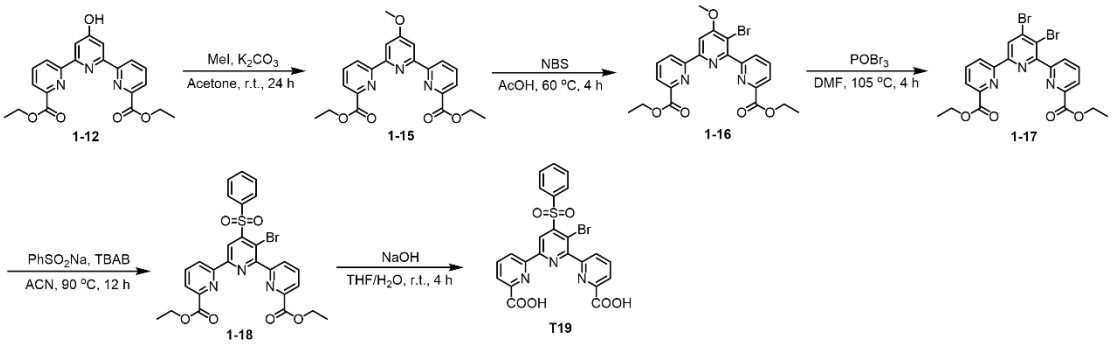
**Synthesis of compound 1-13:** The compound **1-13** was synthesized according to the reported literature[10]. To a solution of compound **1-12** (1.02 g, 2.58 mmol) in dry DMF (25 mL), $PBr_5$ (1.68 g, 3.88 mmol) in dry DMF (25 mL) was added under argon. And then the mixture was heated at 60 °C for 6 h. The resulting residue was dissolved in water and neutralized with $NaHCO_3$ solution. The aqueous layer was extracted with dichloromethane (4 X 50 mL). The combined organic layers were washed brine (100 mL), and dried over $Na_2SO_4$ followed by filtering and removing under vacuum. Purification of the crude product was achieved by flash column chromatography ($CH_2Cl_2$ as eluent) to provide **1-13** (1.02 g, 86%) as a white solid. Characterization data correspond to the literature[10]. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.78 (s, 2H), 8.73 (d, *J* = 7.9 Hz, 2H), 8.16 (d, *J* = 7.7 Hz, 2H), 8.00 (t, *J* = 7.8 Hz, 2H), 4.53 (q, *J* = 7.1 Hz, 4H), 1.50 (t, *J* = 7.1 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.1, 155.6, 155.0, 148.0, 137.9, 135.4, 125.5, 125.1, 124.4, 62.0, 14.3. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{21}H_{19}N_3O_4Br$ 456.0554; Found: 456.0551.

**Synthesis of compound 1-14:** Sodium benzene sulfinate (0.64 g, 3.87 mmol) and TBAB (0.42 g, 1.29 mmol) was added to a solution of compound **1-13** (0.51 g, 1.29 mmol) in dry acetonitrile. The mixture was stirred at 90 °C under argon for 12 h. Then the residue was filtered over celite and the solvent was evaporated under reduced pressure. The obtained crude product was purified by column chromatography (petroleum ether : ethyl acetate 1:1) to deliver the compound **1-14** (0.48 g, 72%) as a white solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.01 (s, 2H), 8.70 (d, *J* = 8.2 Hz, 2H), 8.16 (d, *J* = 7.6 Hz, 2H), 8.12 (d, *J* = 7.8 Hz, 2H), 7.99 (t, *J* = 7.9 Hz, 2H), 7.65 – 7.60 (m, 1H), 7.58 – 7.53 (m, 2H), 4.52 (dd, *J* = 7.3, 2.0 Hz, 4H), 1.50 (t, *J* = 7.0 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.1, 156.6, 154.6, 152.6, 148.4, 139.8, 138.0, 134.1, 129.6, 128.5, 125.7, 124.4, 118.92, 62.0, 14.3. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{27}H_{24}N_3O_6S$ 518.1381; Found: 518.1375.

**Synthesis of tag T18:** To a solution of compound **1-14** (0.48 g, 0.93 mmol) in THF/$H_2O$ (5 mL/5 mL) was added NaOH (112 mg, 2.78 mmol) and the mixture was stirred under room temperature for 4 h. THF was evaporate under vacuum and the residue was acidified by 2 M HCl solution to pH 4-5 resulting in precipitate. Collecting and drying the precipitate to give the tag **T18** as white solid (279 mg, 65% yield). $^1$H NMR (800 MHz, DMSO-$d_6$) δ 9.03 (s, 2H), 8.89 (d, *J* = 7.7 Hz, 2H), 8.24 (t, *J* = 7.7 Hz, 2H), 8.21 (d, *J* = 7.4 Hz, 2H), 8.14 (d, *J* = 7.9 Hz, 2H), 7.79 (t, *J* = 7.4 Hz, 1H), 7.72 (t, *J* = 7.7 Hz, 2H). $^{13}$C NMR (201 MHz, DMSO-$d_6$) δ 166.2, 156.9, 153.8, 152.4, 148.9, 139.8, 135.3, 130.7, 128.3, 126.5, 125.0, 118.4.

HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{23}H_{16}N_3O_6S$ 462.0755; Found: 462.0761.

**Synthesis of T19:**




**Synthesis of compound 1-15:** The **1-15** was synthesized by a modified method according the reported literature[11]. To a mixture of compound **1-12** (3.9 g, 10 mmol) and $K_2CO_3$ (2.7 g, 20 mmol) in acetone (50mL) was added $CH_3I$ (2.1 g, 15 mmol). And the mixture was stirred at room temperature for 24 h. Then the solvent was evaporated under reduced pressure. The obtained crude product was purified by column chromatography (DCM : MeOH 50:1) to deliver the compound **1-15** as a white solid (3.3 g, 82% yield). Characterization data correspond to the literature[11]. $^1$H NMR (400 MHz, $CDCl_3$) δ 8.76 (dd, *J* = 7.9, 1.1 Hz, 2H), 8.16 (s, 2H), 8.13 (dd, *J* = 7.7, 1.1 Hz, 2H), 7.97 (t, *J* = 7.8 Hz, 2H), 4.51 (q, *J* = 7.1 Hz, 4H), 4.06 (s, 3H), 1.48 (t, *J* = 7.1 Hz, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 168.0, 165.9, 156.3 (d, *J* = 4.3 Hz), 147.5, 137.8, 125.2, 124.6, 108.0, 55.7, 52.9. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{22}H_{22}N_3O_5$ 408.1554; Found: 408.1553.

**Synthesis of compound 1-16:** To a solution of compound **1-15** (3.3 g, 8.2 mmol) in acetic acid (30 mL) was added NBS (4.3 g, 24.6 mmol) and the mixture was stirred under 60 °C for 4 h in dark condition. After cooling to room temperature, the mixture was poured into ice water and neutralized with saturated $K_2CO_3$ solution. The residue was extracted with DCM (3x100 mL). The organic phase was dried over dried over $Na_2SO_4$ followed by filtering and removing under vacuum. Purification of the crude product was achieved by column chromatography (DCM : MeOH 100:1) to provide **1-16** as a white solid (3.6 g, 92% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.65 (d, *J* = 7.9 Hz, 1H), 8.20 (m, 2H), 8.13 (d, *J* = 7.7 Hz, 1H), 8.03 – 7.96 (m, 1H), 7.92 (m, 2H), 4.51 (q, *J* = 7.1 Hz, 4H), 4.17 (s, 3H), 1.53 – 1.40 (m, 6H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.16, 165.11, 163.84, 157.99, 156.42, 155.30, 155.07, 147.68, 147.62, 137.83, 137.23, 127.55, 125.30, 124.59, 110.75, 104.39, 61.90, 61.83, 56.75, 29.54, 14.32. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{22}H_{21}N_3O_5Br$ 486.0660; Found: 486.0677.

**Synthesis of compound 1-17:** To a solution of compound **1-16** (3.6 g, 7.5 mmol) in dry DMF (30 mL)

was added $POBr_3$ (4.3 g, 15 mmol) and the mixture was stirred under 105 °C for 4 h. After cooling to room temperature, the mixture was poured into ice water and neutralized with saturated $K_2CO_3$ solution. Then the residue was extracted with ethyl acetate. The organic phase was washed with brine and dried over $Na_2SO_4$ followed by filtering and removing under vacuum. Purification of the crude product was achieved by column chromatography (DCM : MeOH 100:1) to provide **1-17** as a white solid (3.2 g, 80% yield). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.88 (s, 1H), 8.60 (d, *J* = 7.9 Hz, 1H), 8.23 (d, *J* = 7.7 Hz, 1H), 8.15 (d, *J* = 7.6 Hz, 1H), 8.02 (td, *J* = 7.8, 1.0 Hz, 1H), 7.96 – 7.86 (m, 2H), 4.56 – 4.48 (m, 4H), 1.52 – 1.43 (m, 6H). $^{13}$C NMR (101 MHz, CDCl3) δ 165.01, 164.97, 157.87, 156.90, 154.11, 153.50, 148.03, 147.58, 138.68, 137.99, 137.53, 127.23, 126.23, 125.61, 124.93, 124.50, 123.59, 62.02, 62.01, 14.34. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{21}H_{18}N_3O_4Br_2$ 535.9639; Found: 535.9645.

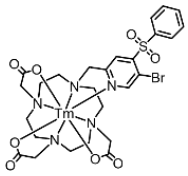
**Synthesis of compound 1-18:** Sodium benzene sulfinate (2.95 g, 18 mmol) and TBAB (0.65 g, 2 mmol) was added to a solution of compound **1-17** (3.2 g, 6 mmol) in dry acetonitrile. The mixture was stirred at 90 °C under argon for 12 h. After cooling to room temperature, the precipitate was filtered over celite. Then the solvent was evaporated under reduced pressure. The obtained crude product was purified by column chromatography (petroleum ether: ethyl acetate 1:1) to deliver the compound **1-18** (2.15 g, 60% yield) as a white solid. $^1$H NMR (400 MHz, $CDCl_3$) δ 9.38 (d, *J* = 1.6 Hz, 1H), 8.50 (d, *J* = 7.9 Hz, 1H), 8.17 – 8.09 (m, 2H), 7.99 (d, *J* = 8.2 Hz, 2H), 7.93 (td, *J* = 7.8, 1.4 Hz, 1H), 7.87 (td, *J* = 7.8, 1.4 Hz, 1H), 7.71 (d, *J* = 7.7 Hz, 1H), 7.62 – 7.55 (m, 1H), 7.52 – 7.45 (m, 2H), 4.47 (qd, *J* = 7.1, 1.4 Hz, 2H), 4.39 (qd, *J* = 7.1, 1.4 Hz, 2H), 1.48 – 1.41 (m, 3H), 1.33 (td, *J* = 7.1, 1.5 Hz, 3H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 165.08, 164.78, 158.61, 157.21, 155.05, 153.88, 150.65, 148.46, 147.74, 138.37, 138.10, 137.62, 134.21, 129.44, 129.12, 127.65, 125.94, 125.05, 124.56, 122.03, 117.69, 62.10, 14.34, 14.32. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{27}H_{23}N_3O_6BrS$ 596.0486; Found: 596.0485.

**Synthesis of tag T19:** To a solution of compound **1-18** (596 mg, 1 mmol) in THF/$H_2O$ (5 mL/5 mL) was added NaOH (112 mg, 2.78 mmol) and the mixture was stirred under room temperature for 4 h. THF was evaporate under vacuum and the residue was acidified 2 M HCl solution to pH 4-5 resulting in precipitate. Collecting and drying the precipitate to give the tag **T19** as yellow solid (378 mg, 70%).$^1$H NMR (800 MHz, DMSO-$d_6$) δ 9.36 (s, 1H), 8.57 (dd, *J* = 7.8, 1.1 Hz, 1H), 8.22 (dd, *J* = 7.7, 1.1 Hz, 1H), 8.21 – 8.14 (m, 3H), 8.08 (dd, *J* = 8.5, 1.3 Hz, 2H), 7.98 (dd, *J* = 7.5, 1.3 Hz, 1H), 7.86 – 7.79 (m, 1H), 7.70 (dd, *J* = 8.5, 7.4 Hz, 2H). $^{13}$C NMR (201 MHz, DMSO-$d_6$) δ 166.3, 159.5, 157.0, 154.8, 153.4, 150.1, 149.1, 148.2, 139.8, 139.1, 138.3, 135.3, 130.1, 129.2, 127.9, 126.6, 125.4, 124.7, 121.4, 117.6. HRMS (ESI/Q-TOF)

m/z: $[M + H]^+$ Calcd for $C_{23}H_{15}N_3O_6BrS$ 539.9860; Found: 539.9871.

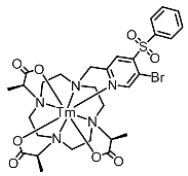
**Synthesis of T20-Tm and T21-Tm**

The **T20-Tm and T21-Tm** were synthesized according to our previous report.[5, 6].



T20-Tm

White solid, yield: 89%. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{26}H_{33}BrN_5O_8STm$ 824.0472; Found: 824.0482



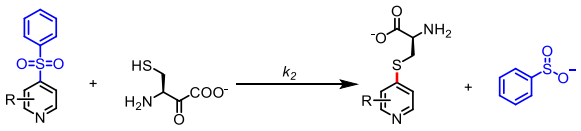
T21-Tm

Yellow solid, yield: 82%. HRMS (ESI/Q-TOF) m/z: $[M + H]^+$ Calcd for $C_{29}H_{39}BrN_5O_8STm$ 866.0941; Found: 866.0957.

**2 Kinetic assay of phenylsulfonyl pyridine tags towards *L*-cysteine**

**2.1 Determination of the second-order reaction rate constants**

For the mixture of phenylsulfonyl pyridine derivatives with *L*-cysteine, the reaction can be described as



$r = k_2[c_{tag}][c_{Cys}]$, in which the $[c_{tag}]$ and $[c_{Cys}]$ are the concentrations of phenylsulfonyl pyridine tag and *L*-cysteine in the reaction mixture at time point *t*, respectively, and $k_2$ is the second order reaction rate constant. Because of the diverse reactivity of the tags with L-cysteine, the second order reaction rate, $k_2$, was determined under different conditions. For the tags with higher reactivity to free thiols, equimolar concentrations of both reactants were employed and the reaction was considered as the second-order reaction (equation 1). For the tags with lower reactivity to free thiols, excess of *L*-cysteine was used and the reaction of the tag with L-cysteine was considered as pseudo-first reaction (equation 2).

The concentration of the reactant or product can be judged by the signal peak intensity in the $^1$H-NMR spectra recorded for the reaction mixture with time. Similarly, the concentration of the reactant or product can also be measured by the UV absorption. As to the excess of *L*-cysteine (generally 10 equivalents of

the tag) system, the reaction rate can be described as equation (2), and the determined pseudo-first reaction rate, $k_{obs}$, using linear-curve fitting as shown in equations (3) or (4). The second order reaction rate, $k_2$, can be calculated from $k_{obs}$ by dividing the concentration of L-cysteine with equation (5). As to the reactants with same initial concentrations, the second order reaction rate, $k_2$, can be simulated with non-linear curve fitting following equations (6) or (7).

$$r = k_2 c_{tag} c_{Cys} \quad (1)$$

$$r = k_{\mathrm{obs}} \mathrm{c_{tag}} \quad (2)$$

$$k_{\mathrm{obs}} = \frac{1}{t} \ln \frac{\mathrm{c_0}}{\mathrm{c_t}} \approx \frac{1}{t} \ln \frac{\mathrm{I_0}}{\mathrm{I_t}} \quad (3)$$

$$ln(\frac{C_0}{C_t}) = k_{obs} \mathrm{t} \quad (4)$$

$$k_2 = \frac{k_{\mathrm{obs}}}{\mathrm{C_{Cys}}} \quad (5)$$

$$\frac{1}{C_t} - \frac{1}{C_0} = k_2 t \quad (6)$$

$$c_t = \frac{1}{\frac{1}{c_0} + k_2 t} \quad (7)$$

Time-dependent concentration profiles were fitted to integrated rate laws through linear or nonlinear least-squares regression (OriginLab).

Note: $r$ is the reaction rate; $c_{tag}$ is the concentration of phenylsulfonylpyridine derivatives; $c_{Cys}$ is the concentration of *L*-cysteine; $c_0$ is the initial concentration of reactants, $c_t$ is the concentration of reactants at a given time t. $I_0$ is the initial integral peak intensity of reactants. $I_t$ is the integral peak intensity of reactants at a given time. $k_{obs}$ is the pseudo-first-order reaction rate constant. $k_2$ is the second-order reaction rate constant.

In the NMR experiment, the concentration of reactant or product was calculated by the ratio of integral area of characteristic peak, which can be described as following equation (8):

$$c_t = \frac{c_0 A_R}{A_R + A_P} \quad (8)$$

$A_R$: the integral area of characteristic peak of reactant; $A_P$: the integral area of characteristic peak of product. The kinetic reaction rate can be simulated similar to the equations mentioned above.

As to the Uv absorbance, the concentrations change of reactants or products were monitored by measuring absorbance variations (ΔA) using the Beer-Lambert Law (A = εcl), where absorbance (A) directly correlates with concentration (c) at a fixed wavelength and pathlength.

All the kinetic experiments were undertaken at 298 K.

**2.2 Kinetic assay of tags with *L*-cysteine by NMR without metal ions**

**T1-T21**: About 1 mM tag were prepared in 500 uL 20 mM PB containing 10% $D_2O$ at different pH (6.5, 7.5 and 8.5). $^1H$ NMR spectrum of free tags was recoded. Then 50 μL 100 mM *L*-cysteine (10 equivalents) aqueous solution was added (terminal volume and concentration: 550 μL, 0.9 mM). The above reaction mixture at different time was monitored by Bruker NMR spectrometer with a magnetic field with a proton frequency of 600 MHz. The second order reaction constant was fitted following the equation (4) and (6).

**2.3 Kinetic assay of tags with *L*-cysteine by NMR or UV-vis with metal ions**

**T18-Y**: 1.0 mM **T18** aqueous solution was prepared in 500 μL 20 mM Tris-HCl containing 10% $D_2O$ at different pH (6.5, 7.5 and 8.5). 1.2 μL 500 mM $Y(NO_3)_3 \cdot 6H_2O$ (1.2 equivalents) aqueous solution was then added to form the tag-metal complex. The $^1H$ NMR spectrum of free tag was recorded. Subsequently, 5.0 μL 100 mM *L*-cysteine (1.0 equivalent) aqueous solution was added (total volume: 506.2 uL). The reaction mixture at different time was monitored by Bruker NMR spectrometer with a magnetic field with a proton frequency of 600 MHz. The second order reaction constant was fitted following the equation (7).

**T19-Y**: 50 μM **T19-Y** was prepared in 500 uL 20 mM Tris-HCl following the method of **T18-Y**. The L-Cysteine was added reacting for 2 h. The ultraviolet-visible (UV-Vis) absorption spectrum of the reactant and product was recorded respectively across the wavelength range of 200–450 nm to confirm the characteristic peak (295 nm). 50 μM **T19-Y** was prepared in 500 uL 20 mM Tris-HCl. Then L-cysteine was added immediately and the absorbance of the reaction mixture at 295 nm was monitored by the UV-Vis spectrophotometer using quartz cuvette with 10 mm optical path length. The absorbance of the product concentration at different time course was to the concentration using the Beer-Lambert law. The second order reaction constant was fitted using the equation (7).

**T19-Zn**: 1.0 mM **T19-Zn** aqueous solution was prepared in 500 μL 20 mM Tris-HCl containing 10% $D_2O$ at different pH (6.5, 7.5 and 8.5). 2.0 μL 500 mM $ZnSO_4$ (2.0 equivalent) aqueous solution was then added to form the tag-metal complex. The $^1H$ NMR spectrum of free tag was recorded. Subsequently, 5.0 μL 100 mM *L*-cysteine (1.0 equivalents) aqueous solution was added (terminal volume: 507 uL). The reaction extent at different time was monitored by Bruker NMR spectrometer with a magnetic field with a proton frequency of 600 MHz.

**T20-Tm** and **T21-Tm**: 1 mM tag were prepared in 500 uL 20 mM PB containing 10% $D_2O$ at different pH (6.5, 7.5 and 8.5). Then 5 μL 1.0 mM *L*-cysteine (1.0 equivalents) aqueous solution was added (terminal volume and concentration: 550 μL, 0.9 mM). The reaction extent at different time was monitored by Bruker

NMR spectrometer with a magnetic field with a proton frequency of 600 MHz. The second order reaction constant was fitted following the equation (7)

**2.4 High performance liquid chromatography**

The analyses of the reaction of **T1** with *L*-cysteine were performed by HPLC on an analytical RP-C18 column (Phenomenex, Aqua®, 5 µm, 4.6 × 250 mm) with a gradient elution of acetonitrile (ACN; 10% to 100% in $H_2O$, containing 0.1 % formic acid) at a flow rate of 1 mL/min over 18 min. The products were monitored at 254 and 365 nm by UV detector.

**2.5 Interaction of T18 and T19 with $Y^{3+}$**

A stock of 100 mM $Y(NO_3)_3 \cdot 6H_2O$ was titrated gradually into 500 µL 20 mM MES buffer containing 1 mM tag **T18** or **T19** and 10% $D_2O$ in 5 mm NMR tube. The NMR spectra were recorded at a proton frequency of 600 MHz with increasing $Y(NO_3)_3 \cdot 6H_2O$ concentration.

**2.6 Selectivity experiment**

1 mM **T19-Y** were prepared in 500 uL 20 mM Tris-HCl buffer (containing 10% $D_2O$ and 1.2 mM $Y(NO_3)_3 \cdot 6H_2O$) at pH 8.5 in 5 mm NMR tube according to the above condition. $^1$H-NMR spectrum at zero point of free tag was obtained under 298 K. And then 50 uL 100 mM amino acid (10 equiv.) aqueous solution was added to the mixture. The NMR spectra were recorded at a proton frequency of 600 MHz after 5 hours.

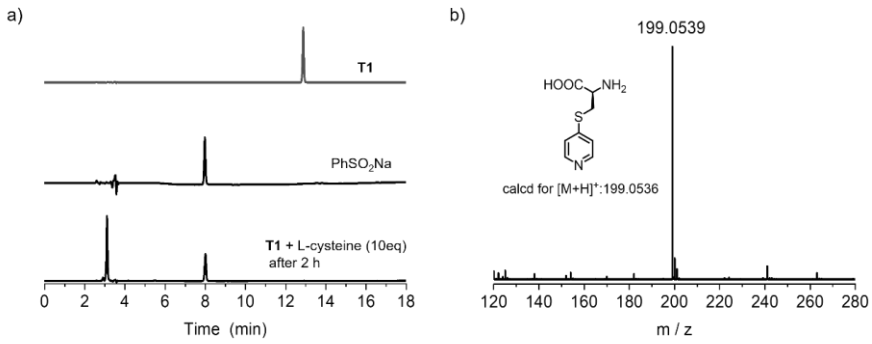



**Figure S2.** a) Reaction of 1.0 mM tag **T1** with 10.0 mM *L*-cysteine in 20 mM PB buffer at pH 7.5 monitored by HPLC; b) HRMS (ESI/Q-TOF) spectrum of the product of **T1** with *L*-cysteine in 20 mM PB at pH 7.5.

**Table 1.** A summary of second-order reaction rate constant of all tags towards *L*-cysteine in aqueous solution at different pH.

| Tag | $k_2$ / $M^{-1}\cdot h^{-1}$ | | |
|---|---|---|---|
| | **pH 6.5** | **pH 7.5** | **pH 8.5** |
| **T1** | 149.45 ± 1.10 | 169.23 ± 1.35 | 102.20 ± 1.10 |
| **T2** | 38.5 ± 5.3 | 36.2 ± 1.7 | 24.0 ± 1.1 |
| **T3** | 58.30 ± 6.60 | 48.40 ± 4.40 | 61.60 ± 1.10 |
| **T4** | 44.00 ± 0.62 | 66.10 ± 6.62 | 56.89 ± 1.30 |
| **T5** | 347.01 ± 6.65 | 335.20 ± 2.06 | 189.81 ± 2.71 |
| **T6** | 83.89 ± 0.82 | 94.77 ± 0.49 | 50.39 ± 0.41 |
| **T7** | 398.54 ± 22.22 | 309.59 ± 8.31 | 165.40 ± 6.06 |
| **T8** | --[a] | 3.39 ± 0.39 | 17.72 ± 0.58 |
| **T9** | 13.19 ± 0.16 | 14.04 ± 0.60 | 12.18 ± 0.37 |
| **T10** | 50.01 ± 1.66 | 41.71 ± 1.67 | 67.30 ± 15.06 |
| **T11** | 16.23 ± 0.5 | 35.14 ± 1.39 | 276.10 ± 46.39 |
| **T12** | 112.9 ± 6.2 | 102.1 ± 4.3 | 48.7 ± 4.4 |
| **T13** | 149.92 ± 1.49 | 67.93 ± 2.16 | 40.15 ± 0.72 |
| **T14** | 559.82 ± 13.42 | 601.38 ± 19.54 | 257.16 ± 4.29 |
| **T15** | 1441.22 ± 67.84 | 1310.30 ± 32.97 | 572.23 ± 12.91 |
| **T16** | 5.07 ± 0.33 | 154.94 ± 4.68 | 368.92 ± 10.81 |
| **T17** | 71.53 ± 2.35 | 389.45 ± 8.07 | 809.73 ± 37.46 |
| **T18** | --[a] | 5.27 ± 0.16 | 1.96 ± 0.10 |
| **T18-Y** | 917.95 ± 23.22 | 2363.55 ± 193.41 | 3402.99 ± 124.21 |
| **T19** | --[a] | 48.89 ± 2.52 | 310.96 ± 16.57 |
| **T19-Y** | 291666.67 ± 18055.56 | --[b] | --[b] |
| **T19-Zn** | 469.22 ± 34.47 | 514.61 ± 36.85 | 1877.51 ± 63.76 |
| **T20** | 145.91 ± 5.58 | 148.56 ± 6.56 | 207.15 ± 10.40 |
| **T20-Tm** | 38400.0 ± 10321.8 | --[b] | --[b] |
| **T21** | 2353.41 ± 97.70 | 1872.10 ± 51.32 | 823.69 ± 39.20 |
| **T21-Tm** | 44902.8 ± 17974.8 | --[b] | --[b] |

[a] The reaction was too slow to fit the reaction rate constant; [b] The reaction was too fast to fit the reaction rate constant;

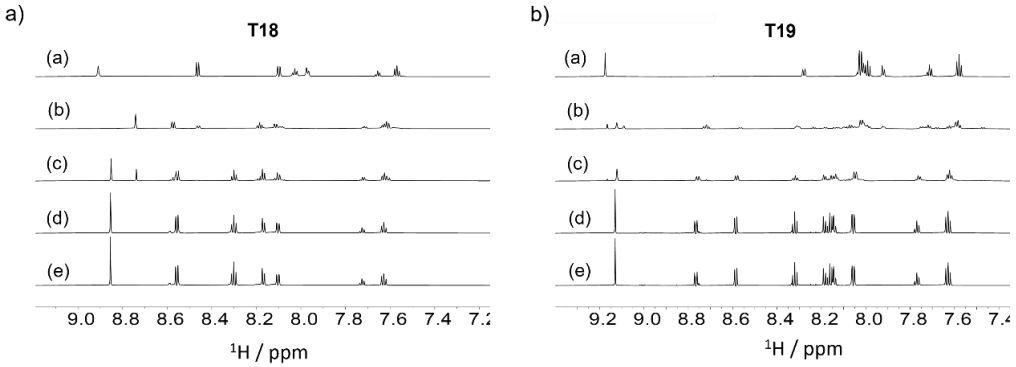



**Figure S3.** Interaction of **T18** (left) and **T19** (right) with different concentration of $Y^{3+}$ determined by high-resolution NMR spectroscopy. A aqueous solution of $Y(NO_3)_3\bullet 6H_2O$ was titrated into 500 µL 20 mM MES buffer containing 1 mM tag and 10% $D_2O$ in NMR tube. The NMR spectra were recorded at a proton frequency of 800 MHz with increasing $Y(NO_3)_3\bullet 6H_2O$ concentration: a) 0, b) 0.4, c) 0.8, d) 1.2 and e) 1.6 mM.

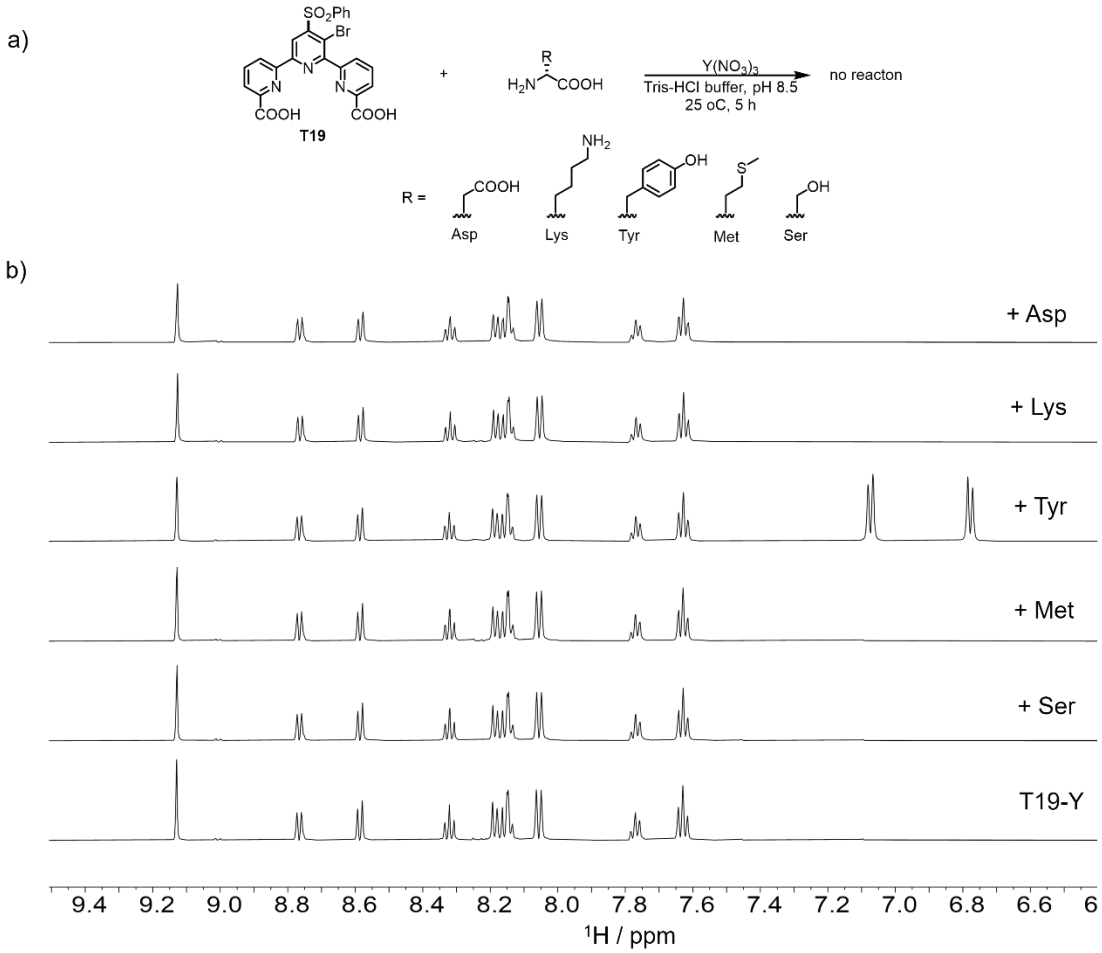



**Figure S4.** The reaction of **T19-Y** towards other amino acid contained nucleophilicity side chain towards. 1 mM tag were prepared in 500 uL 20 mM buffer (containing 10% $D_2O$ and 1.2 mM $Y(NO_3)_3$) in 5 mm NMR tube. $^1$H-NMR spectrum at zero point of free tag was obtained under 25 °C. And then 50 uL 100 mM amino acid (10 equiv.) was added to the sample. The NMR spectra were recorded at a proton frequency of 600 MHz after 5 hours.

## 3 Site-specifically tagging POIs with tag T1, T5 and T25

### 3.1 Protein expression and purification

**The amino acid sequences of the proteins used in this study**

The cysteine mutations for protein labeling are highlighted in red and native cysteine is marked in blue.

**GB1 K28C**

MQYKLILNGKTLKGETTTEAVDAATAECVFKQYANDNGVDGEWTYDDATKTFTVTE

**Ub E18C**

MQIFVKTLTGKTITLEVCPSDTIENVKAKIQDNEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLR

LRGG

**sfGFP N212C**

MSKGEELFTGVVPILVELDGDVNGHKFSVRGEGEGDATNGKLTLKFICTTGKLPVPWPTLVTTLTYGVQC

FSRYPDHMKRHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVCRIELKGIDFKEDGNILGH

KLEYNFNSHNVYITADKQKNGIKANFKIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSVLSK

DPCEKRDHMVLLEFVTAAGITHGMDELYKGSHHHHHH

**Hsp70 T427C**

MSKGPAVGIDLGTTYSCVGVFQHGKVEIIANDQGNRTTPSYVAFTDTERLIGDAAKNQVAMNPTNTVFD

AKRLIGRRFDDAVVQSDMKHWPFMVVNDAGRPKVQVEYKGETKSFYPEEVSSMVLTKMKEIAEAYLGK

TVTNAVVTVPAYFNDSQRQATKDAGTIAGLNVLRIINEPTAAAIAYGLDKKVGAERNVLIFDLGGGTFDVSI

LTIEDGIFEVKSTAGDTHLGGEDFDNRMVNHFIAEFKRKHKKDISENKRAVRRLRTACERAKRTLSSSTQ

ASIEIDSLYEGIDFYTSITRARFEELNADLFRGTLDPVEKALRDAKLDKSQIHDIVLVGGSTRIPKIQKLLQD

FFNGKELNKSINPDEAVAYGAAVQAAILSGDKSENVQDLLLLDVTPLSLGIETAGGVMTVLIKRNTTIPTKQ

TQCFTTYSDNQPGVLIQVYEGERAMTKDNNLLGKFELTGIPPAPRGVPQIEVTFDIDANGILNVSAVDKST

GKENKITITNDKGRLSKEDIERMVQEAEKYKAEDEKQRDKVSSKNSLESYAFNMKATVEDEKLQGKIND

EDKQKILDKCNEIINWLDKNQTAEKEEFEHQQKELEKVCNPIITKLYQSAGGMPGGMPGGFPGGGAPPS

GGASSGPTIEEVD

**BSA**

DTHKSEIAHRFKDLGEEHFKGLVLIAFSQYLQQCPFDEHVKLVNELTEFAKTCVADESHAGCEKSLHTLF

GDELCKVASLRETYGDMADCCEKQEPERNECFLSHKDDSPDLPKLKPDPNTLCDEFKADEKKFWGKY

LYEIARRHPYFYAPELLYYANKYNGVFQECCQAEDKGACLLPKIETMREKVLTSSARQRLRCASIQKFGE

RALKAWSVARLSQKFPKAEFVEVTKLVTDLTKVHKECCHGDLLECADDRADLAKYICDNQDTISSKLKEC

CDKPLLEKSHCIAEVEKDAIPENLPPLTADFAEDKDVCKNYQEAKDAFLGSFLYEYSRRHPEYAVSVLLRL

AKEYEATLEECCAKDDPHACYSTVFDKLKHLVDEPQNLIKQNCDQFEKLGEYGFQNALIVRYTRKVPQV

STPTLVEVSRSLGKVGTRCCTKPESERMPCTEDYLSLILNRLCVLHEKTPVSEKVTKCCTESLVNRRPCF

SALTPDETYVPKAFDEKLFTFHADICTLPDTEKQIKKQTALVELLKHKPKATEEQLKTVMENFVAFVDKCC

AADDKEACFAVEGPKLVVSTQTALA

**Protein expression and purification**

The plasmid used in this study was transformed into Escherichia coli BL21 (DE3) Codon Plus strain for protein expression.

**GB1 K28C and Ub E18C**: The protein expression of Ub E18C and GB1 K28C was similar. The cells transformed with the plasmid of interest were grown in 600 mL LB medium at 37 °C till OD600 of 0.7-0.9 was achieved. Protein expression was then induced by addition of 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and the cells were left to grow for 8-10 hours at 37 °C, 200 rpm. The cells were collected, lysed and then the supernatant was applied for FPLC purification. The protein was first purified through a DEAE column (DEAE Sepharose FF, Cytiva) and gel filtration. Pure protein was obtained through size exclusion chromatography (HiLoad 16/600 Superdex 75, GE Healthcare Biosciences).

**sfGFP N212C**: The cells transformed with the plasmid of interest were grown in 600 mL LB medium at 37 °C till OD600 of 0.7-0.9 was achieved. Protein expression was then induced by addition of 1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and then the cells were left to grow for 8-10 hours at 37 °C, 200 rpm. The protein was purified through a Ni-NTA column.

**Hsp70 T427C**: The cells transformed with the plasmid of interest were grown in 600 mL LB medium at 37 °C till OD600 of 0.7-0.9 was achieved. Protein expression was then induced by addition of 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) and the cells were incubated for 18-20 hours at 20 °C, 200 rpm. The protein was first purified through a Ni-NTA column, and followed by a Q-Sepharose anion exchange column (Q Sepharose HP, GE Healthcare Biosciences).

**BSA** was purchased from Sigma-Aldrich.

**3.2 Reactivity assay of cysteine-specific bioconjugation of proteins with T1, T5 and T19**

Protein of interests (0.1 mM) was mixed with 0.2 mM tris(2-carboxyethyl)phosphine (TCEP) in 20 mM

tris(hydroxymethyl)aminomethane (Tris) at pH 7.6 and the mixture was incubated at 30 °C about 1 h. Then three equivalents of tag molecule (stock: 100 mM in DMF) or pre-synthesized **T19-Y** were added to the protein solution and the pH was adjusted to 8.0 using 0.5 M NaOH. The reaction was carried out under 30 °C. The reaction process was monitored by ESI-TOF mass spectrometry. The preparation **T19-Y** was according to the kinetic assay of **T195-Y** towards *L*-cysteine.

Similar process was undertaken for other tags.

### 3.3 Characterization of POI-tag adducts by MS (ESI-Q-TOF)

Free protein and its crude product modified by the tags were analyzed on an Agilent 1290 series HPLC system equipped with a 6545 series ESI-Q-TOF (Agilent, CA, USA). The protein sample was injected into a C8 column (Agilent ZORBAX 300SB-C8, 2.1 X 150 mm) and run through by gradient elution at a column temperature of 70 °C. The mobile phases consisted $H_2O$ with 0.1% formic acid (buffer A) and acetonitrile containing 0.1% formic acid (buffer B). Protein mass deconvolution was conducted by Agilent MassHunter BioConfirm software (Agilent Technologies Inc., V10.0).

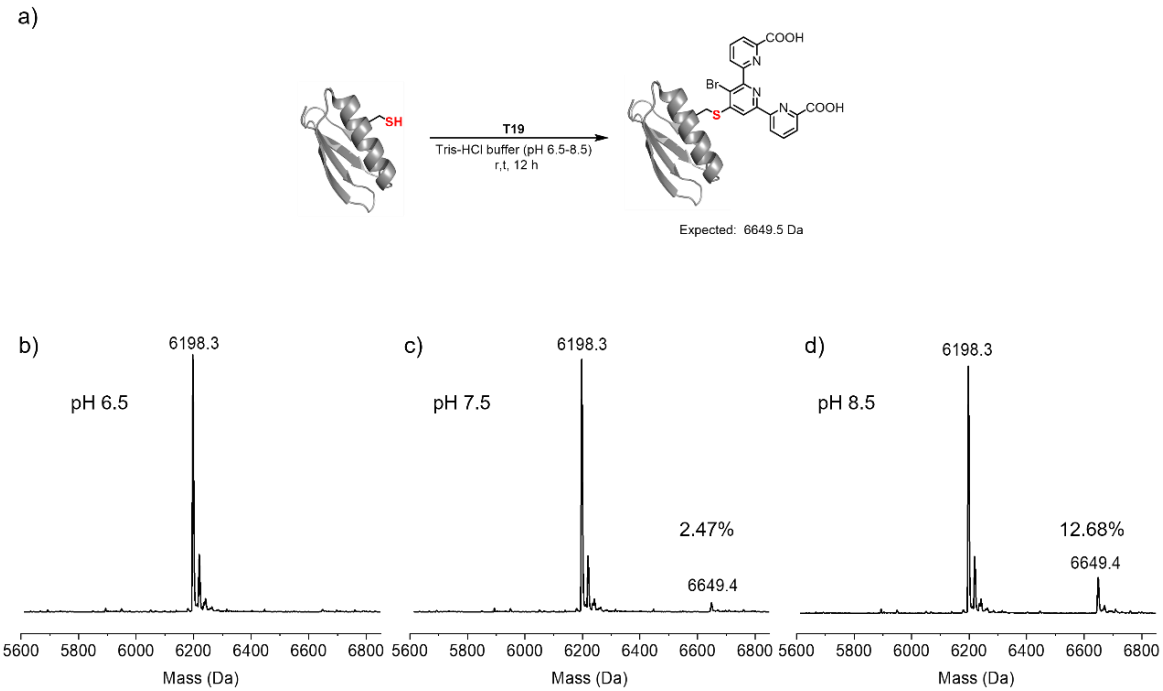



**Figure S5.** Cysteine-specific modification of GB1 K28C with **T19.** a) Schematic diagram for the labelling of GB1 K28C with tag **T19;** b) Deconvoluted ESI-Q-TOF mass spectrum of free protein and protein-tag adduct, 6198.3 Da is the molecular weight of GB1 K28C and 6649.4 is the molecular weight of GB1 K28C-T30 adduct.

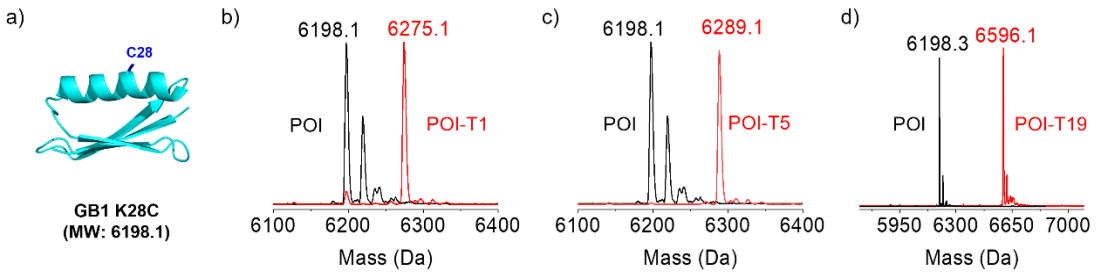



**Figure S6.** a) 3D structure of GB1 K28C; b) c) d) Deconvoluted ESI-Q-TOF mass spectrum of GB1 K28C (black) and GB1 K28C-tag adducts (red).

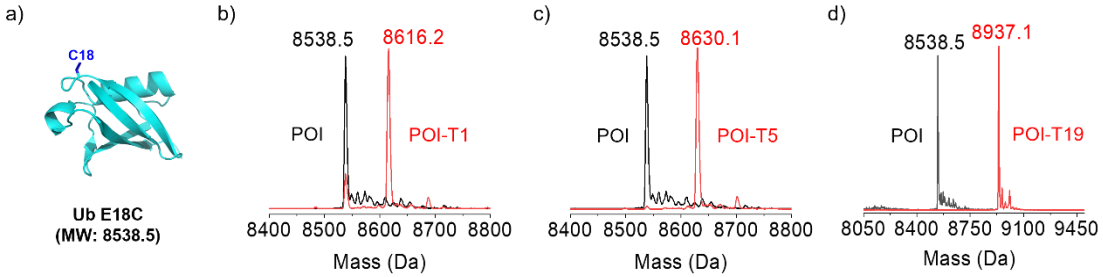



**Figure S7.** a) 3D structure of Ub E18C; b) c) d) Deconvoluted ESI-Q-TOF mass spectrum of Ub E18C (black) and Ub E18C-tag adducts (red).

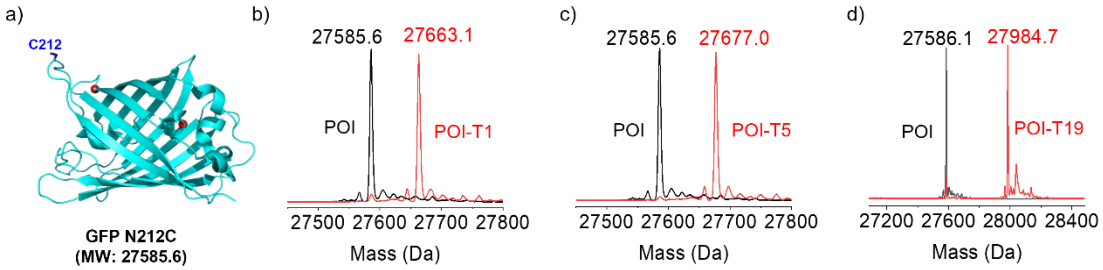



**Figure S8.** a) 3D structure of sfGFP N212C; b) c) d) Deconvoluted ESI-Q-TOF mass spectrum of GFP N212C (black) and GFP N212C-tag adducts (red).

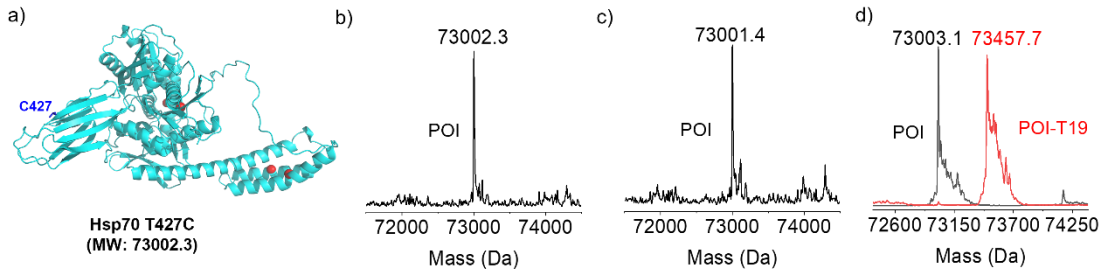



**Figure S9.** a) Structure of Hsp70 T427C; b) c) d) Deconvoluted ESI-Q-TOF mass spectrum of Hsp70 T427C (black) and Hsp70 T427C-tag adducts (red).

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**4 Kinetic time course data for determining second order rate constants**

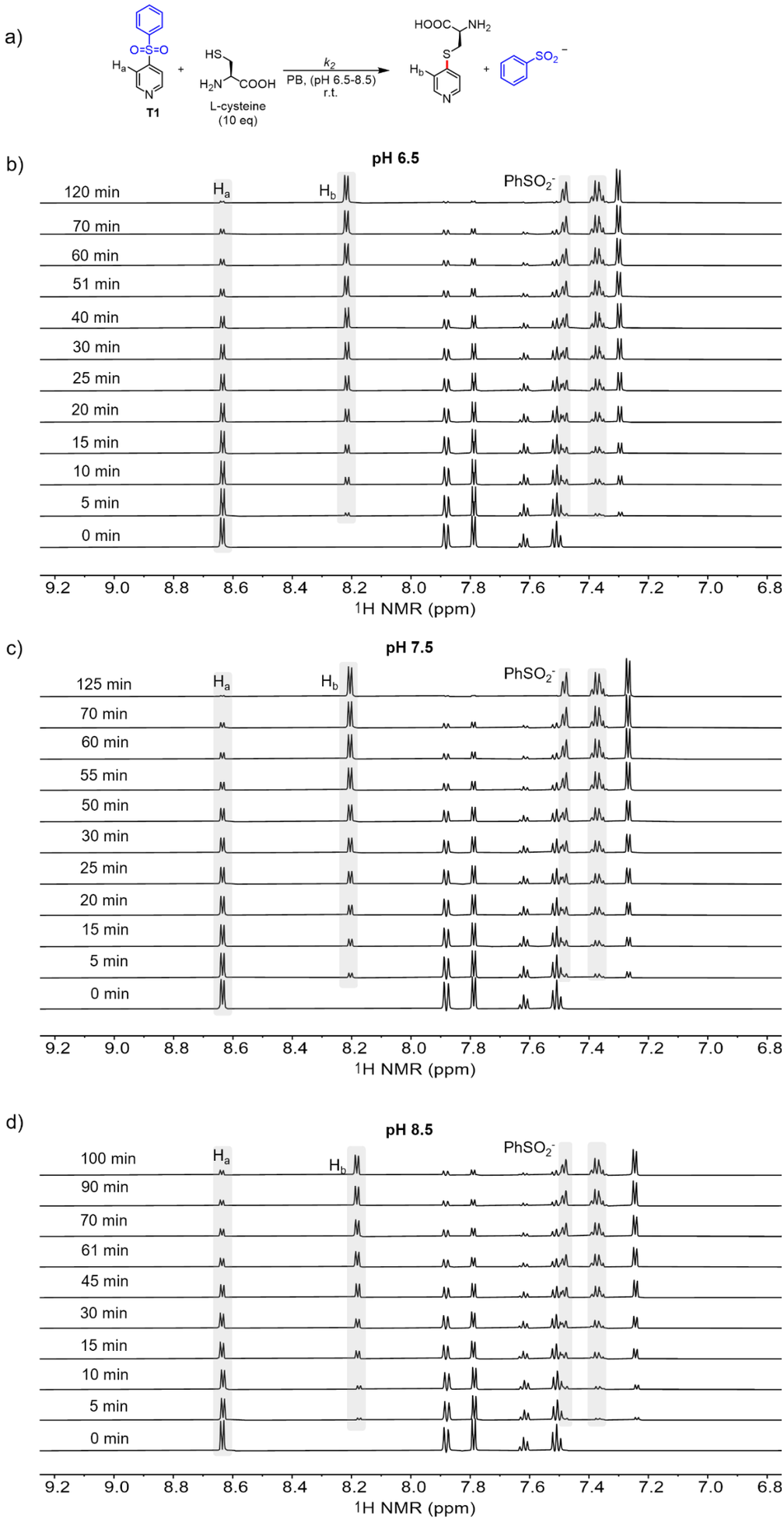



**Figure S10.** a) Reaction of **T1** with *L*-cysteine with pyridine protons assigned in reactant and product; b) c) d) Zoomed NMR spectra of **T1** in reaction with 10 equivalents of *L*-cysteine over 2 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S2.** The time-dependent concentration profiles of **T1** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constant determined by linear regression.

| **T1** + *L*-Cysteine (10 eq)<br>($c_0$: the initial concentration of **T1**; $c_t$: the concentration of **T1** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| T1 pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0 | 0.9091 | 0.0000 | y = 1.36x + 0.05<br>$R^2$ = 0.9990<br>$k_1^{obs}$ = 1.36 ± 0.01 $h^{-1}$<br>$k_2$ = 149.45 ± 1.10 $M^{-1}\cdot h^{-1}$ |
| | 5 | 0.0833 | 0.7639 | 0.1639 | |
| | 10 | 0.1667 | 0.6819 | 0.2775 | |
| | 15 | 0.2500 | 0.6075 | 0.3930 | |
| | 20 | 0.3333 | 0.5441 | 0.5033 | |
| | 25 | 0.4167 | 0.4746 | 0.6400 | |
| | 30 | 0.5000 | 0.4224 | 0.7565 | |
| | 40 | 0.6667 | 0.3367 | 0.9833 | |
| | 51 | 0.8500 | 0.2614 | 1.2364 | |
| | 60 | 1.0000 | 0.2141 | 1.4361 | |
| | 70 | 1.1667 | 0.1727 | 1.6510 | |
| | 120 | 2.0000 | 0.0579 | 2.7433 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0 | 0.9091 | 0.0000 | y = 1.54x + 0.03<br>$R^2$ = 0.9988<br>$k_1^{obs}$ = 1.54 ± 0.02 $h^{-1}$<br>$k_2$ = 169.23 ± 1.35 $M^{-1}\cdot h^{-1}$ |
| | 5 | 0.0833 | 0.7391 | 0.1969 | |
| | 15 | 0.2500 | 0.5822 | 0.4356 | |
| | 20 | 0.3333 | 0.5130 | 0.5622 | |
| | 25 | 0.4167 | 0.4549 | 0.6824 | |
| | 30 | 0.5000 | 0.4011 | 0.8082 | |
| | 50 | 0.8333 | 0.2468 | 1.2937 | |
| | 60 | 1.0000 | 0.1924 | 1.5429 | |
| | 70 | 1.1667 | 0.1529 | 1.7728 | |
| | 125 | 2.0833 | 0.0336 | 3.2889 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.93x + 0.02<br>$R^2$ = 0.9990<br>$k_1^{obs}$ = 0.93 ± 0.01 $h^{-1}$<br>$k_2$ = 102.20 ± 1.10 $M^{-1}\cdot h^{-1}$ |
| | 5 | 0.0833 | 0.8181 | 0.0954 | |
| | 10 | 0.1667 | 0.7507 | 0.1814 | |
| | 25 | 0.4167 | 0.6063 | 0.3951 | |
| | 30 | 0.5000 | 0.5501 | 0.4923 | |
| | 45 | 0.7500 | 0.4439 | 0.7067 | |
| | 61 | 1.0167 | 0.3369 | 0.9825 | |
| | 70 | 1.1667 | 0.2954 | 1.1142 | |
| | 90 | 1.5000 | 0.2148 | 1.4329 | |
| | 100 | 1.6667 | 0.1943 | 1.5332 | |

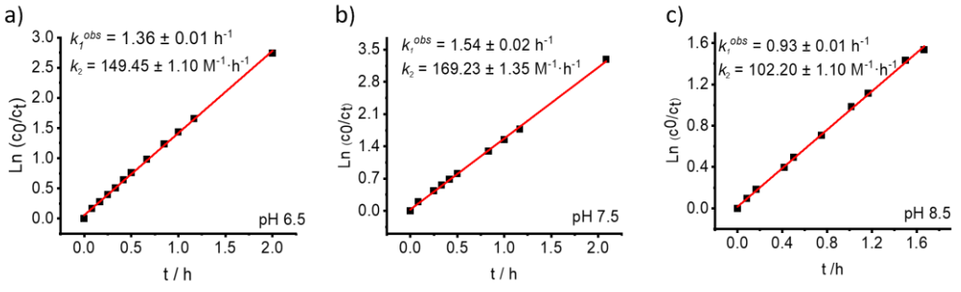



**Figure S11.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/$c_0$) as a function of time (hours) for the reaction of **T1** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T1** at t; $c_0$: concentration of **T1** at $t_0$.

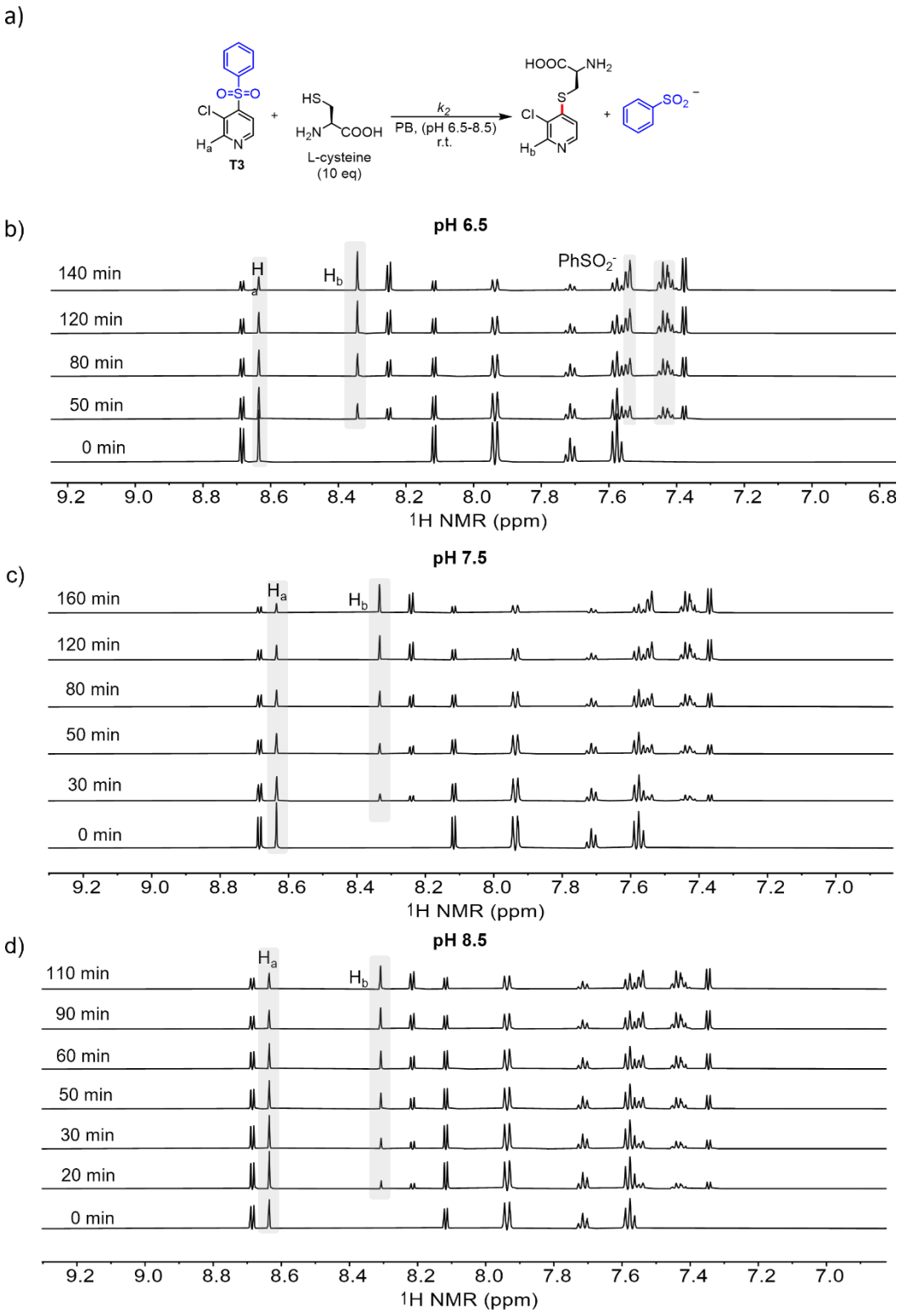



**Figure S12.** a) Reaction of **T3** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T3** in reaction with 10 equivalents of *L*-cysteine over 2 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S3.** The time-dependent concentration profiles of **T3** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T3** + *L*-cysteine (10 eq)<br>($c_0$: the initial concentration of **T3**; $c_t$: the concentration of **T3** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0 | 0.9091 | 0.0000 | y = 0.53x + 0.02<br>$R^2$ = 0.9652<br>$k_1^{obs}$ = 0.53 ± 0.06 $h^{-1}$<br>$k_2$ = 58.30 ± 6.60 $M^{-1}\cdot h^{-1}$ |
| | 50 | 0.8333 | 0.7639 | 0.1639 | |
| | 80 | 1.3333 | 0.6819 | 0.2775 | |
| | 120 | 2.0000 | 0.6075 | 0.3930 | |
| | 140 | 2.3333 | 0.5441 | 0.5033 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0 | 0.9091 | 0 | y = 0.44x + 0.07<br>$R^2$ = 0.9655<br>$k_1^{obs}$ = 0.44 ± 0.04 $h^{-1}$<br>$k_2$ = 48.40 ± 4.40 $M^{-1}\cdot h^{-1}$ |
| | 30 | 0.5 | 0.6407 | 0.3399 | |
| | 50 | 0.83333 | 0.5500 | 0.4926 | |
| | 80 | 1.33333 | 0.4465 | 0.7010 | |
| | 120 | 2 | 0.4000 | 0.8109 | |
| | 160 | 2.66667 | 0.2503 | 1.27973 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0 | 0.9091 | 0 | y = 0.56x - 0.02<br>$R^2$ = 0.9981<br>$k_1^{obs}$ = 0.56 ± 0.01 $h^{-1}$<br>$k_2$ = 61.60 ± 1.10 $M^{-1}\cdot h^{-1}$ |
| | 20 | 0.33333 | 0.7697 | 0.1564 | |
| | 30 | 0.5 | 0.6986 | 0.2533 | |
| | 50 | 0.83333 | 0.5950 | 0.4138 | |
| | 60 | 1 | 0.5282 | 0.5330 | |
| | 90 | 1.5 | 0.3942 | 0.8255 | |
| | 110 | 1.83333 | 0.3306 | 1.0017 | |

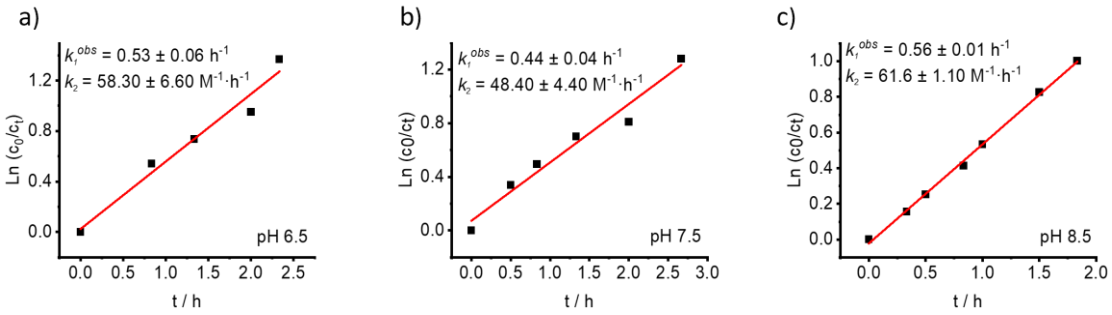



**Figure S13.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/$c_0$) as a function of time (hours) for the reaction of **T3** (0.9 mM) with *L*-Cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T3** at t; $c_0$: concentration of **T3** at $t_0$.

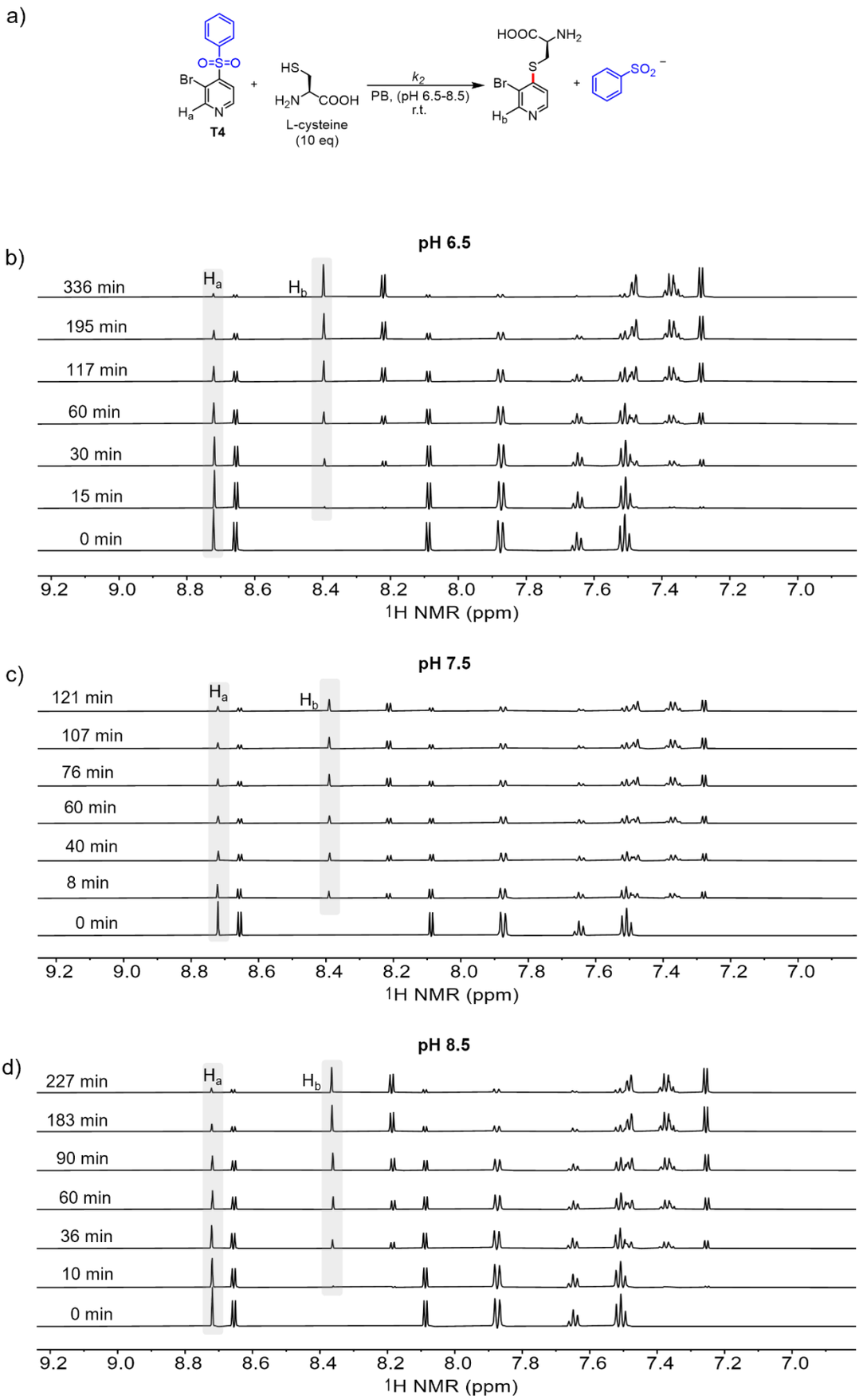



**Figure S14.** a) Reaction of **T4** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T4** in reaction with 10 equivalents of *L*-cysteine over 2 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S4.** The time-dependent concentration profiles of **T4** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T4** + *L*-Cysteine (10 eq)<br>($c_0$: the initial concentration of **T4**; $c_t$: the concentration of **T4** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0.000 | 0.9091 | 0.0000 | y = 0.40x - 0.01<br>$R^2$ = 0.9990<br>$k_1^{obs}$ = 0.40 ± 0.01 $h^{-1}$<br>$k_2$ = 44.00 ± 0.62 $M^{-1}\cdot h^{-1}$ |
| | 15 | 0.2500 | 0.8253 | 0.0967 | |
| | 30 | 0.5000 | 0.7322 | 0.2164 | |
| | 60 | 1.000 | 0.6264 | 0.3724 | |
| | 117 | 1.9500 | 0.4333 | 0.7411 | |
| | 195 | 3.2500 | 0.2592 | 1.2547 | |
| | 336 | 5.6000 | 0.0953 | 2.2553 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.60x + 0.17<br>$R^2$ = 0.9432<br>$k_1^{obs}$ = 0.60 ± 0.06 $h^{-1}$<br>$k_2$ = 66.10 ± 6.62 $M^{-1}\cdot h^{-1}$ |
| | 8 | 0.1333 | 0.7605 | 0.1785 | |
| | 40 | 0.6667 | 0.4269 | 0.7560 | |
| | 60 | 1.0000 | 0.3618 | 0.9215 | |
| | 75 | 1.2500 | 0.3321 | 1.0069 | |
| | 107 | 1.7833 | 0.2601 | 1.2513 | |
| | 121 | 2.0167 | 0.2401 | 1.3312 | |
| | 140 | 2.3333 | 0.2101 | 1.4647 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.52x + 0.05<br>$R^2$ = 0.9974<br>$k_1^{obs}$ = 0.52 ± 0.01 $h^{-1}$<br>$k_2$ = 56.89 ± 1.30 $M^{-1}\cdot h^{-1}$ |
| | 10 | 0.1667 | 0.7843 | 0.1477 | |
| | 36 | 0.6000 | 0.6247 | 0.3752 | |
| | 60 | 1.0000 | 0.5008 | 0.5963 | |
| | 90 | 1.5000 | 0.3891 | 0.8485 | |
| | 183 | 3.0500 | 0.1899 | 1.5658 | |
| | 227 | 3.7833 | 0.1188 | 2.0349 | |

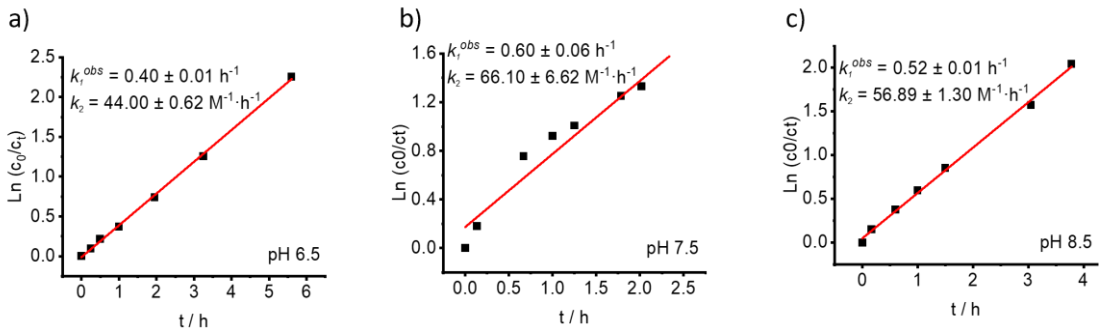



**Figure S15.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/$c_0$) as a function of time (hours) for the reaction of **T4** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T4** at t; $c_0$: concentration of **T4** at $t_0$.

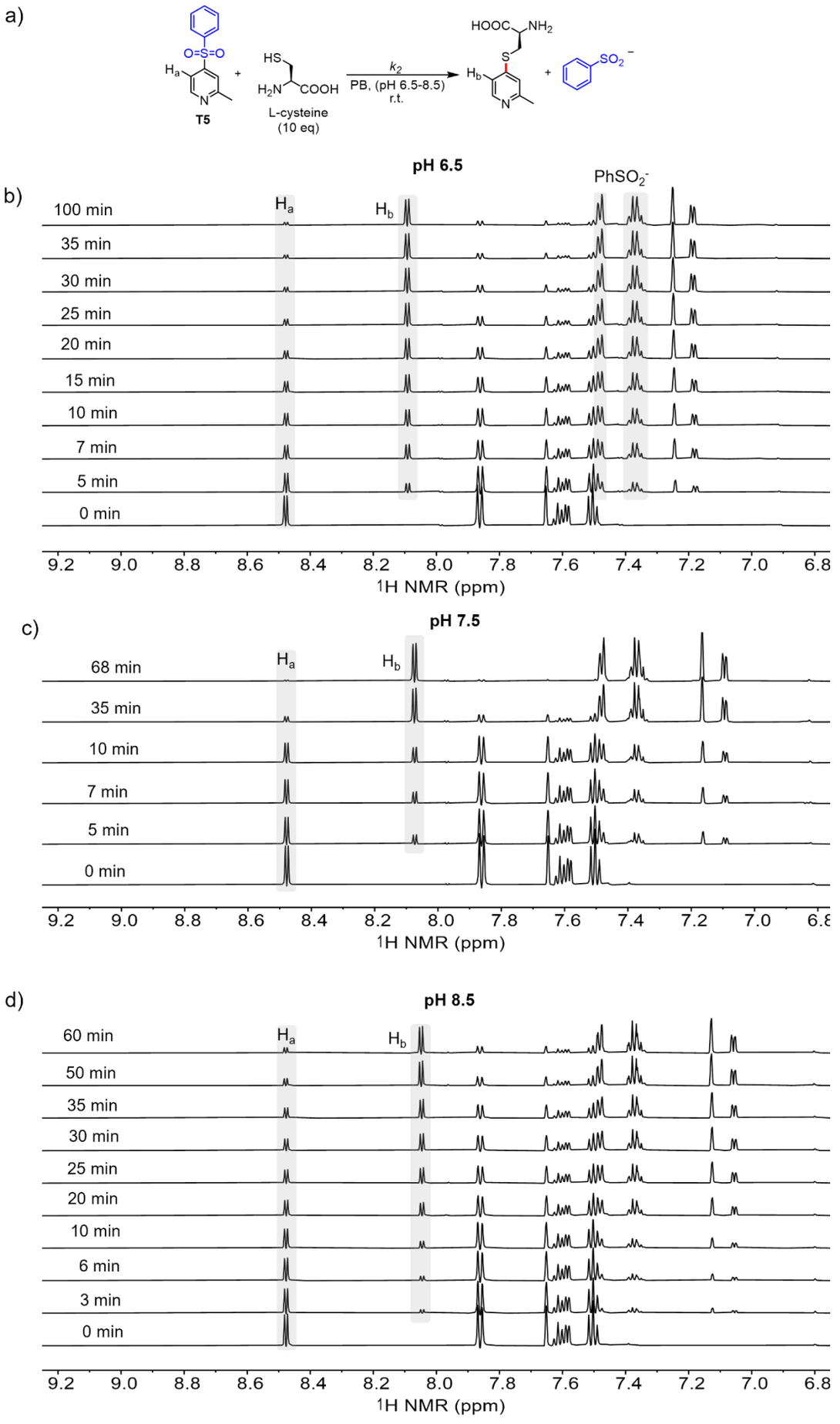



**Figure S16.** a) Reaction of **T5** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T5** in reaction with 10 equivalents of *L*-cysteine over 1 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S5.** The time-dependent concentration profiles of **T5** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T5** + *L*-Cysteine (10 eq)<br>($c_0$: the initial concentration of **T5**; $c_t$: the concentration of **T5** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 3.15x + 0.09<br>$R^2$ = 0.9971<br>$k_1^{obs}$ = 3.15 ± 0.06 $h^{-1}$<br>$k_2$ = 347.01 ± 6.65 $M^{-1}·h^{-1}$ |
| | 5 | 0.0833 | 0.6278 | 0.3703 | |
| | 7 | 0.1167 | 0.5619 | 0.4812 | |
| | 10 | 0.1667 | 0.4649 | 0.6707 | |
| | 15 | 0.2500 | 0.3704 | 0.8978 | |
| | 20 | 0.3333 | 0.2867 | 1.1539 | |
| | 25 | 0.4175 | 0.2225 | 1.4076 | |
| | 30 | 0.5000 | 0.1701 | 1.6760 | |
| | 35 | 0.5833 | 0.1331 | 1.9212 | |
| | 100 | 1.6667 | 0.1043 | 2.1651 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 1.54x + 0.05<br>$R^2$ = 0.9990<br>$k_1^{obs}$ = 1.54 ± 0.02 $h^{-1}$<br>$k_2$ = 335.20 ± 2.06 $M^{-1}·h^{-1}$ |
| | 5 | 0.0833 | 0.7079 | 0.2502 | |
| | 7 | 0.1167 | 0.6407 | 0.3499 | |
| | 10 | 0.1667 | 0.5438 | 0.5138 | |
| | 35 | 0.5833 | 0.1485 | 1.8117 | |
| | 68 | 1.1333 | 0.0291 | 3.4416 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 1.73x + 0.003<br>$R^2$ = 0.9998<br>$k_1^{obs}$ = 1.73 ± 0.02 $h^{-1}$<br>$k_2$ = 189.81 ± 2.71 $M^{-1}·h^{-1}$ |
| | 3 | 0.0500 | 0.7899 | 0.1406 | |
| | 6 | 0.1000 | 0.7456 | 0.1983 | |
| | 10 | 0.1667 | 0.6567 | 0.3253 | |
| | 20 | 0.3333 | 0.4949 | 0.6080 | |
| | 25 | 0.4167 | 0.4276 | 0.7542 | |
| | 30 | 0.5000 | 0.3816 | 0.8680 | |
| | 35 | 0.5833 | 0.3323 | 1.0063 | |
| | 50 | 0.8333 | 0.2014 | 1.5071 | |
| | 60 | 1.0000 | 0.1602 | 1.7359 | |

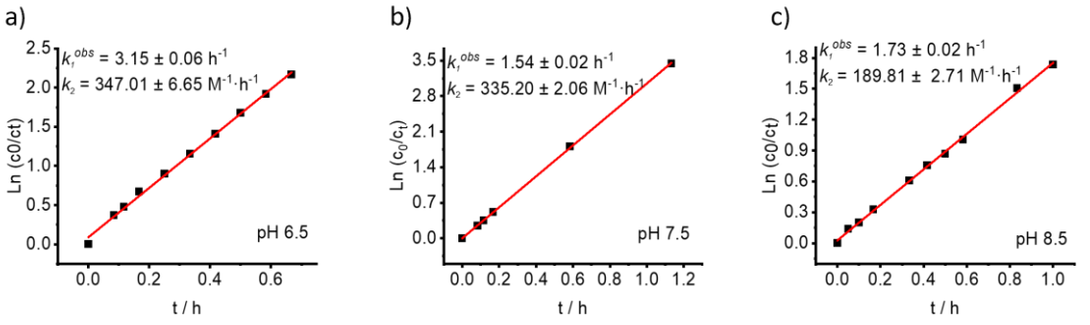



**Figure S17.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln($c/c_0$) as a function of time (hours) for the reaction of **T5** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T5** at t; $c_0$: concentration of **T5** at $t_0$.

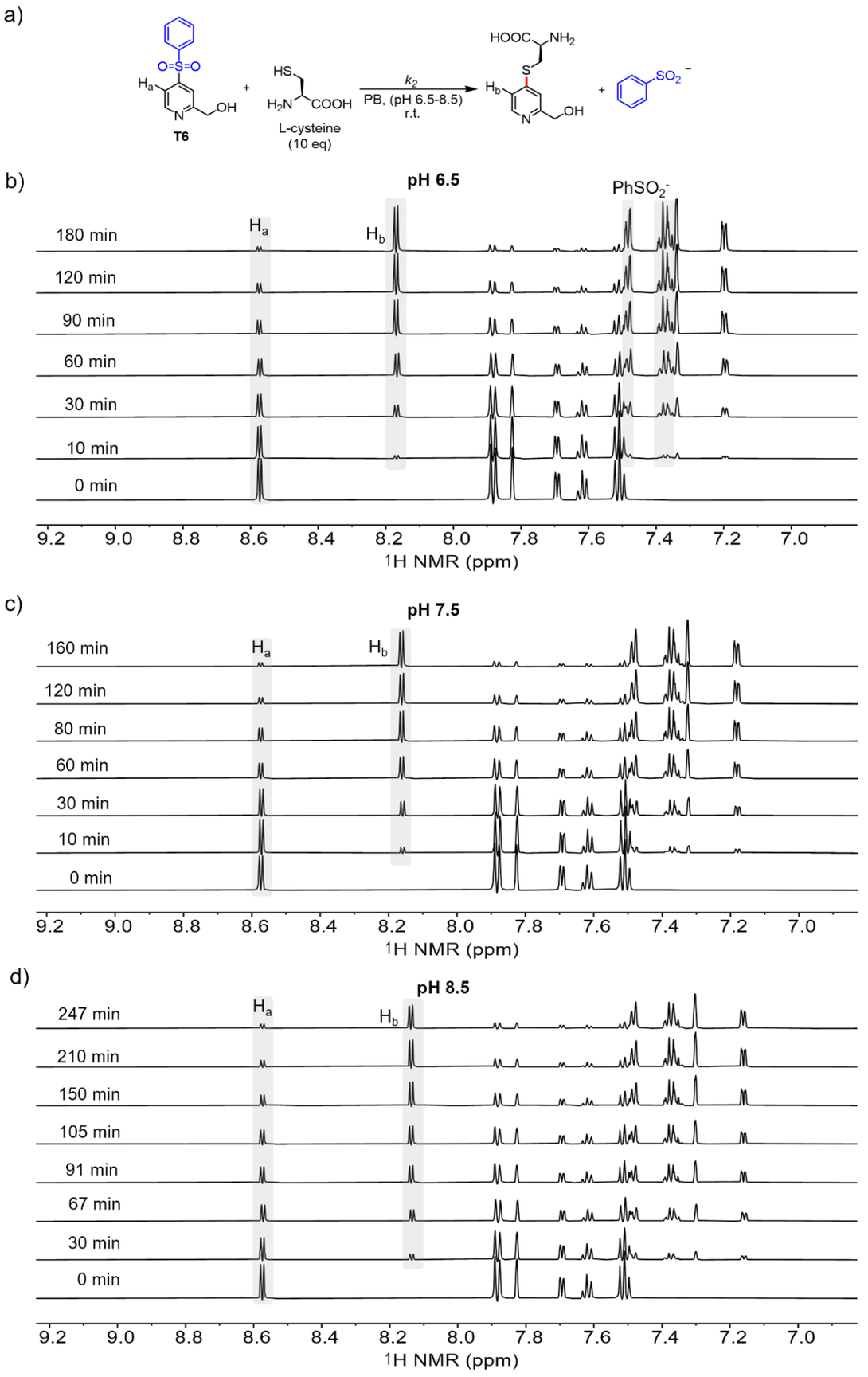



**Figure S18.** a) Reaction of **T6** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T6** in reaction with 10 equivalents of *L*-cysteine over 4 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S6.** The time-dependent concentration profiles of **T6** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T6** + *L*-Cysteine (10 eq)<br>($c_0$: the initial concentration of **T6**; $c_t$: the concentration of **T6** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.76x + 0.03<br>$R^2$ = 0.9995<br>$k_1^{obs}$ = 0.76 ± 0.01 $h^{-1}$<br>$k_2$ = 83.89 ± 0.82 $M^{-1}·h^{-1}$ |
| | 10 | 0.1667 | 0.7693 | 0.1670 | |
| | 30 | 0.5000 | 0.5976 | 0.4196 | |
| | 60 | 1.0000 | 0.4014 | 0.8176 | |
| | 90 | 1.5000 | 0.2775 | 1.1865 | |
| | 120 | 2.0000 | 0.1918 | 1.5559 | |
| | 180 | 3.0000 | 0.0905 | 2.3070 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.86x + 0.004<br>$R^2$ = 0.9998<br>$k_1^{obs}$ = 0.86 ± 0.004 $h^{-1}$<br>$k_2$ = 94.77 ± 0.49 $M^{-1}·h^{-1}$ |
| | 10 | 0.1667 | 0.7724 | 0.1630 | |
| | 30 | 0.5000 | 0.5946 | 0.4245 | |
| | 60 | 1.0000 | 0.3844 | 0.8607 | |
| | 80 | 1.3333 | 0.2844 | 1.1620 | |
| | 120 | 2.0000 | 0.1630 | 1.7186 | |
| | 160 | 2.6667 | 0.0905 | 2.3070 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.46x + 0.02<br>$R^2$ = 0.9996<br>$k_1^{obs}$ = 0.46 ± 0.004 $h^{-1}$<br>$k_2$ = 50.39 ± 0.41 $M^{-1}·h^{-1}$ |
| | 30 | 0.5000 | 0.7005 | 0.2607 | |
| | 67 | 1.1227 | 0.5306 | 0.5384 | |
| | 91 | 1.5167 | 0.4375 | 0.7313 | |
| | 105 | 1.7500 | 0.3918 | 0.8416 | |
| | 150 | 2.5000 | 0.2830 | 1.1669 | |
| | 210 | 3.5000 | 0.1803 | 1.6176 | |
| | 247 | 4.1167 | 0.1353 | 1.9051 | |

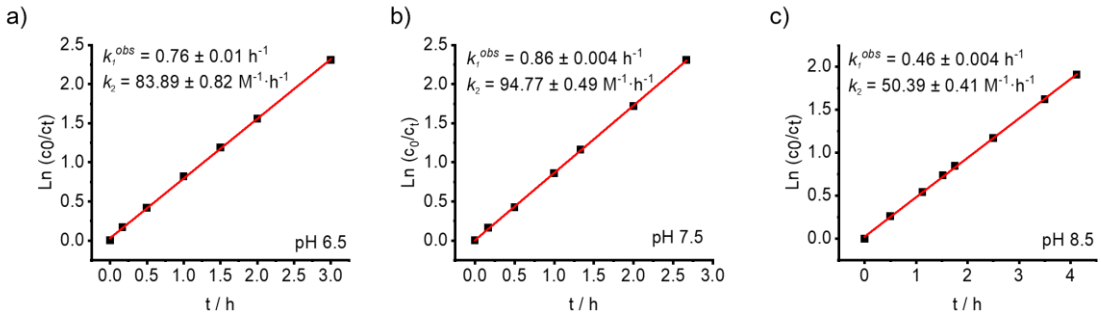



**Figure S19.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/$c_0$) as a function of time (hours) for the reaction of **T6** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T6** at t; $c_0$: concentration of **T6** at $t_0$.

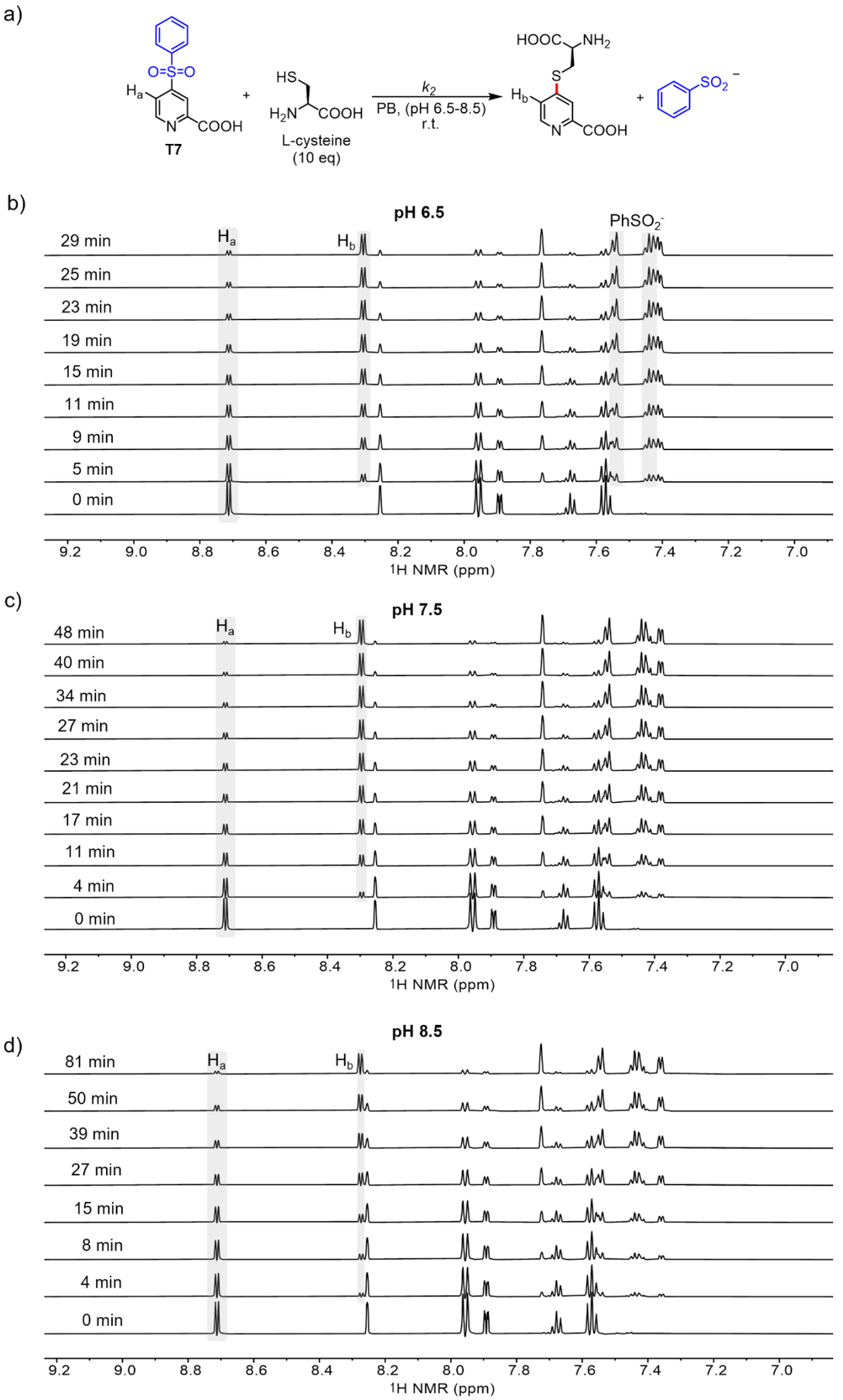



**Figure S20.** a) Reaction of **T7** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T7** in reaction with 10 equivalents of *L*-cysteine over 1 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S7.** The time-dependent concentration profiles of **T7** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T7** (1.0 mmol) + *L*-Cysteine (10 mmol)<br>($c_0$: the initial concentration of **T7**; $c_t$: the concentration of **T7** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 3.62x + 0.18<br>$R^2$ = 0.9787<br>$k_1^{obs}$ = 3.62 ± 0.20 $h^{-1}$<br>$k_2$ = 398.54 ± 22.22 $M^{-1}·h^{-1}$ |
| | 5 | 0.0833 | 0.5209 | 0.5570 | |
| | 9 | 0.1500 | 0.4073 | 0.8029 | |
| | 11 | 0.1833 | 0.3626 | 0.9192 | |
| | 15 | 0.2500 | 0.2912 | 1.1383 | |
| | 19 | 0.3167 | 0.2323 | 1.3646 | |
| | 23 | 0.3833 | 0.1922 | 1.5538 | |
| | 25 | 0.4167 | 0.1711 | 1.6702 | |
| | 29 | 0.4833 | 0.1416 | 1.8595 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 2.81x + 0.06<br>$R^2$ = 0.9943<br>$k_1^{obs}$ = 2.81 ± 0.08 $h^{-1}$<br>$k_2$ = 309.59 ± 8.31 $M^{-1}·h^{-1}$ |
| | 4 | 0.0667 | 0.7559 | 0.1845 | |
| | 11 | 0.1833 | 0.5186 | 0.5613 | |
| | 17 | 0.2833 | 0.3597 | 0.9273 | |
| | 21 | 0.3500 | 0.3057 | 1.0899 | |
| | 23 | 0.3833 | 0.2773 | 1.1873 | |
| | 27 | 0.4500 | 0.2269 | 1.3878 | |
| | 34 | 0.5667 | 0.1762 | 1.6406 | |
| | 40 | 0.6667 | 0.1356 | 1.9027 | |
| | 48 | 0.8000 | 0.0966 | 2.2417 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 1.64x + 0.10<br>$R^2$ = 0.9933<br>$k_1^{obs}$ = 1.64 ± 0.06 $h^{-1}$<br>$k_2$ = 165.40 ± 6.06 $M^{-1}·h^{-1}$ |
| | 4 | 0.0667 | 0.7391 | 0.2070 | |
| | 8 | 0.1333 | 0.6513 | 0.3336 | |
| | 15 | 0.2500 | 0.5446 | 0.5124 | |
| | 27 | 0.4500 | 0.3644 | 0.9142 | |
| | 39 | 0.6500 | 0.2656 | 1.2305 | |
| | 50 | 0.8333 | 0.2026 | 1.5010 | |
| | 81 | 1.3500 | 0.0969 | 2.2384 | |

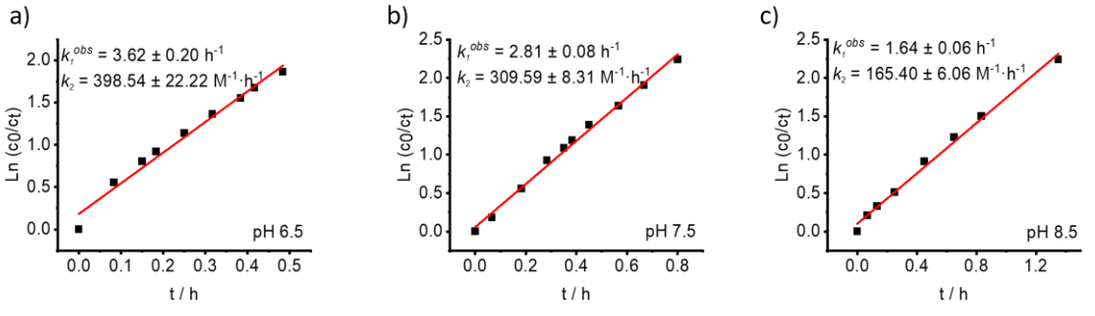



**Figure S21.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/$c_0$) as a function of time (hours) for the reaction of **T7** (0.9 mM) with *L*-Cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T7** at t; $c_0$: concentration of **T7** at $t_0$.

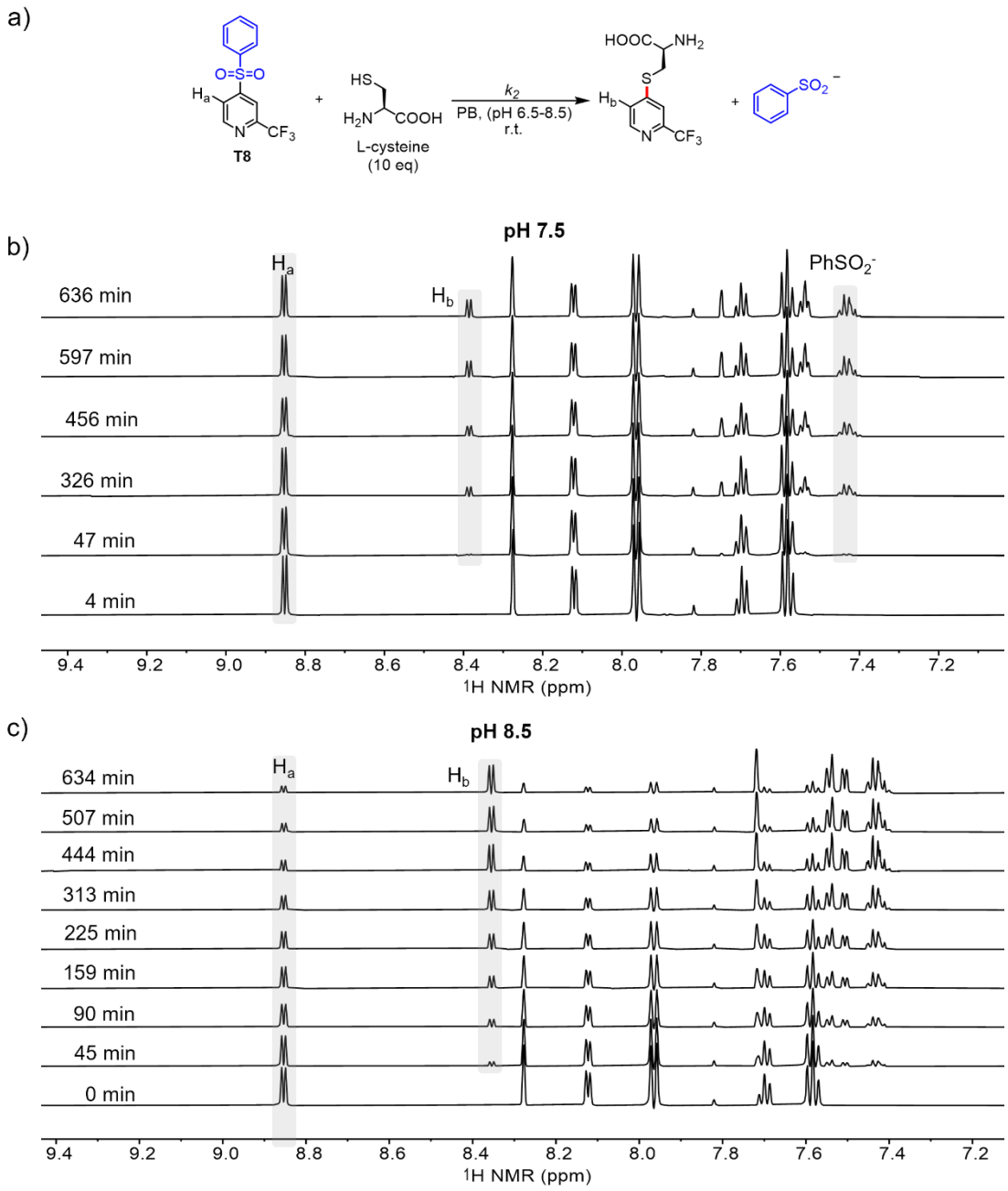



**Figure S22.** a) Reaction of **T8** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) Zoomed NMR spectra of **T8** in reaction with 10 equivalent *L*-cysteine over 10 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S8.** The time-dependent concentration profiles of **T8** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T8** + *L*-cysteine (10 eq)<br>($c_0$: the initial concentration of **T8**; $c_t$: the concentration of **T8** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 4 | 0.0667 | 0.8488 | 0.0686 | y = 0.03x + 0.11<br>$R^2$ = 0.9501<br>$k_1^{obs}$ = 0.03 ± 0.004 $h^{-1}$<br>$k_2$ = 3.39 ± 0.39 $M^{-1}·h^{-1}$ |
| | 47 | 0.7833 | 0.7516 | 0.1903 | |
| | 326 | 5.4333 | 0.6897 | 0.2762 | |
| | 456 | 7.6000 | 0.6443 | 0.3443 | |
| | 597 | 9.9500 | 0.5933 | 0.4268 | |
| | 636 | 10.6000 | 0.5886 | 0.4347 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.16x + 0.08<br>$R^2$ = 0.9925<br>$k_{obs}$ = 0.16 ± 0.01 $h^{-1}$<br>$k_2$ = 17.72 ± 0.58 $M^{-1}·h^{-1}$ |
| | 45 | 0.7500 | 0.7749 | 0.1597 | |
| | 90 | 1.5000 | 0.6242 | 0.3761 | |
| | 159 | 2.6500 | 0.5315 | 0.5368 | |
| | 225 | 3.7500 | 0.4451 | 0.7142 | |
| | 313 | 5.2167 | 0.3614 | 0.9226 | |
| | 444 | 7.4000 | 0.2456 | 1.3087 | |
| | 507 | 8.4500 | 0.2079 | 1.4753 | |
| | 634 | 10.5667 | 0.1653 | 1.7046 | |

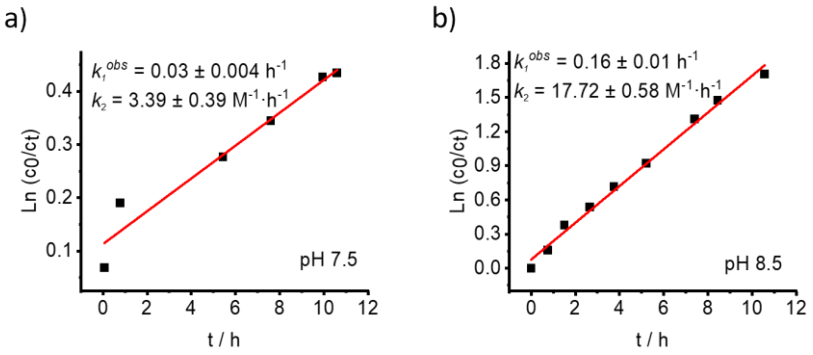



**Figure S23.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/$c_0$) as a function of time (hours) for the reaction of **T8** (0.9 mM) with *L*-Cysteine (10 eq) in PB (20 mM), at pH 7.5 (a), and 8.5 (b). $c_t$: concentration of **T8** at t; $c_0$: concentration of **T8** at $t_0$.

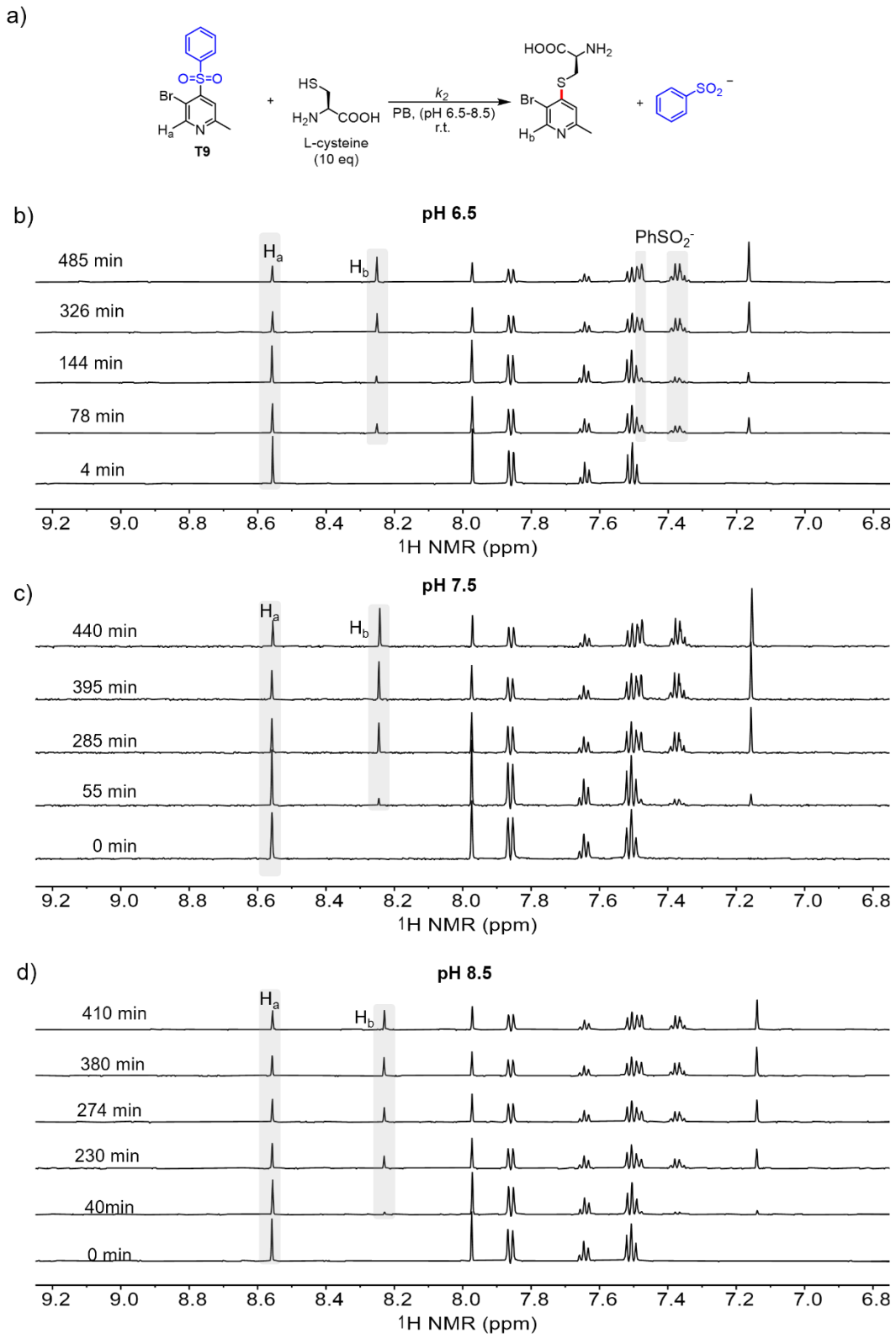



**Figure S24.** a) Reaction of **T9** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T9** in reaction with 10 equivalent *L*-cysteine over 8 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S9.** The time-dependent concentration profiles of **T9** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T9** + *L*-cysteine (10 eq)<br>($c_0$: the initial concentration of **T9**; $c_t$: the concentration of **T9** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.12x<br>$R^2$ = 0.9996<br>$k_1^{obs}$ = 0.12 ± 0.001 $h^{-1}$<br>$k_2$ = 13.19 ± 0.16 $M^{-1}\cdot h^{-1}$ |
| | 78 | 1.3060 | 0.7770 | 0.1570 | |
| | 144 | 2.4000 | 0.6849 | 0.2832 | |
| | 326 | 5.4333 | 0.4671 | 0.6659 | |
| | 485 | 8.0833 | 0.3473 | 0.9623 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.13x + 0.005<br>$R^2$ = 0.9946<br>$k_1^{obs}$ = 0.13 ± 0.01 $h^{-1}$<br>$k_2$ = 14.04 ± 0.60 $M^{-1}\cdot h^{-1}$ |
| | 55 | 0.9167 | 0.8107 | 0.1145 | |
| | 285 | 4.7500 | 0.4718 | 0.6560 | |
| | 395 | 6.5822 | 0.4069 | 0.8039 | |
| | 440 | 7.3333 | 0.3516 | 0.9501 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.11x - 0.0004<br>$R^2$ = 0.9964<br>$k_1^{obs}$ = 0.11 ± 0.003 $h^{-1}$<br>$k_2$ = 12.18 ± 0.37 $M^{-1}\cdot h^{-1}$ |
| | 40 | 0.6667 | 0.8610 | 0.0544 | |
| | 230 | 3.8333 | 0.5864 | 0.4385 | |
| | 274 | 4.5666 | 0.5444 | 0.5127 | |
| | 380 | 6.3333 | 0.4669 | 0.6663 | |
| | 410 | 6.8333 | 0.4233 | 0.7644 | |

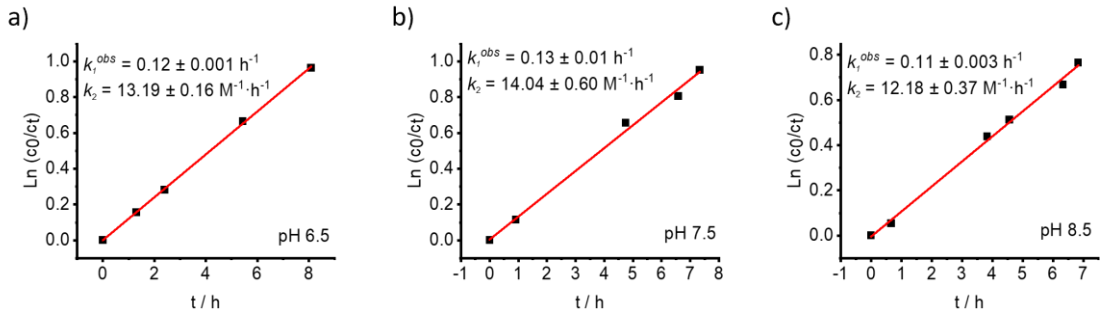



**Figure S25.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/$c_0$) as a function of time (hours) for the reaction of **T9** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T9** at t; $c_0$: concentration of **T9** at $t_0$.

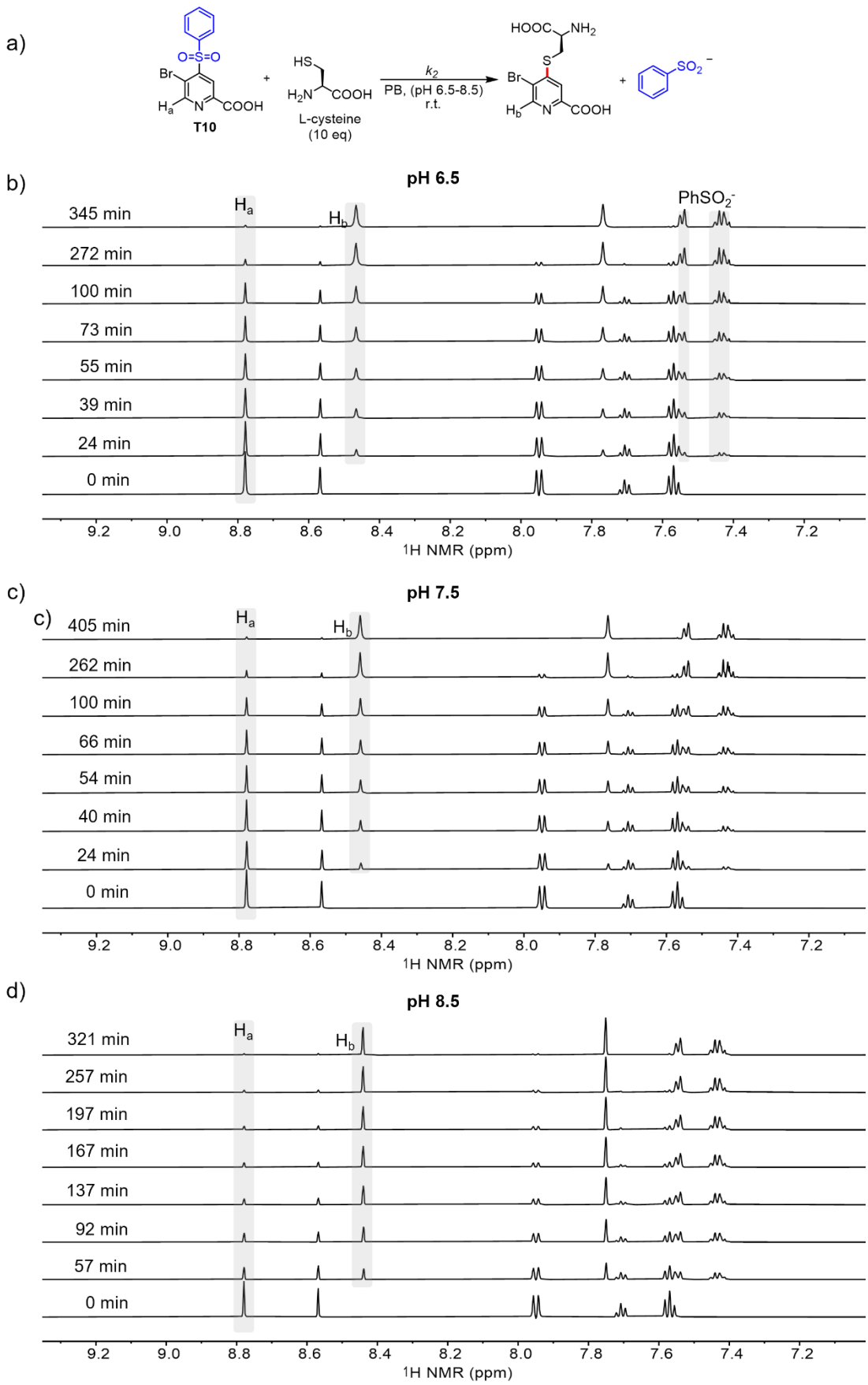



**Figure S26.** a) Reaction of **T10** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T10** in reaction with 10 equivalent *L*-cysteine over 6 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S10.** The time-dependent concentration profiles of **T10** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T10** + *L*-cysteine (10 eq)<br>($c_0$: the initial concentration of **T10**; $c_t$: the concentration of **T10** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.45x + 0.07<br>$R^2$ = 0.9934<br>$k_1^{obs}$ = 0.45 ± 0.02 $h^{-1}$<br>$k_2$ = 50.01 ± 1.66 $M^{-1}\cdot h^{-1}$ |
| | 24 | 0.4000 | 0.7143 | 0.2412 | |
| | 39 | 0.6500 | 0.6331 | 0.3618 | |
| | 55 | 0.9167 | 0.5584 | 0.4873 | |
| | 73 | 1.2167 | 0.4610 | 0.6790 | |
| | 100 | 1.6667 | 0.3604 | 0.9253 | |
| | 272 | 4.5333 | 0.1240 | 1.9920 | |
| | 345 | 5.7500 | 0.0578 | 2.7556 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.38x + 0.12<br>$R^2$ = 0.9905<br>$k_1^{obs}$ = 0.38 ± 0.02 $h^{-1}$<br>$k_2$ = 41.71 ± 1.67 $M^{-1}\cdot h^{-1}$ |
| | 24 | 0.4000 | 0.7306 | 0.2186 | |
| | 40 | 0.6667 | 0.6149 | 0.3910 | |
| | 54 | 0.9000 | 0.5587 | 0.4869 | |
| | 66 | 1.1000 | 0.5124 | 0.5734 | |
| | 100 | 1.6667 | 0.4033 | 0.8128 | |
| | 262 | 4.3667 | 0.1349 | 1.9081 | |
| | 405 | 6.7500 | 0.0698 | 2.5675 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.61x - 0.07<br>$R^2$ = 0.9970<br>$k_1^{obs}$ = 0.61 ± 0.01 $h^{-1}$<br>$k_2$ = 67.30 ± 15.06 $M^{-1}\cdot h^{-1}$ |
| | 57 | 0.9500 | 0.5527 | 0.4976 | |
| | 92 | 1.5333 | 0.4036 | 0.8119 | |
| | 137 | 2.2833 | 0.2589 | 1.2560 | |
| | 167 | 2.7833 | 0.1825 | 1.6055 | |
| | 197 | 3.2833 | 0.1255 | 1.9805 | |
| | 257 | 4.2833 | 0.0651 | 2.6367 | |
| | 321 | 5.3500 | 0.0382 | 3.1701 | |

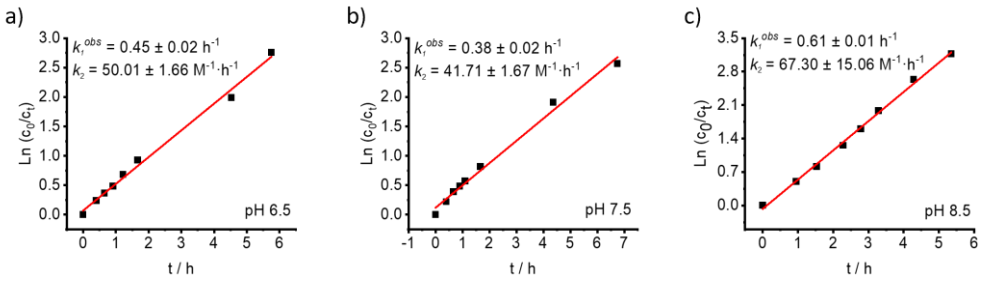



**Figure S27.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/c$_0$) as a function of time (hours) for the reaction of **T10** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T10** at t; $c_0$: concentration of **T10** at $t_0$.

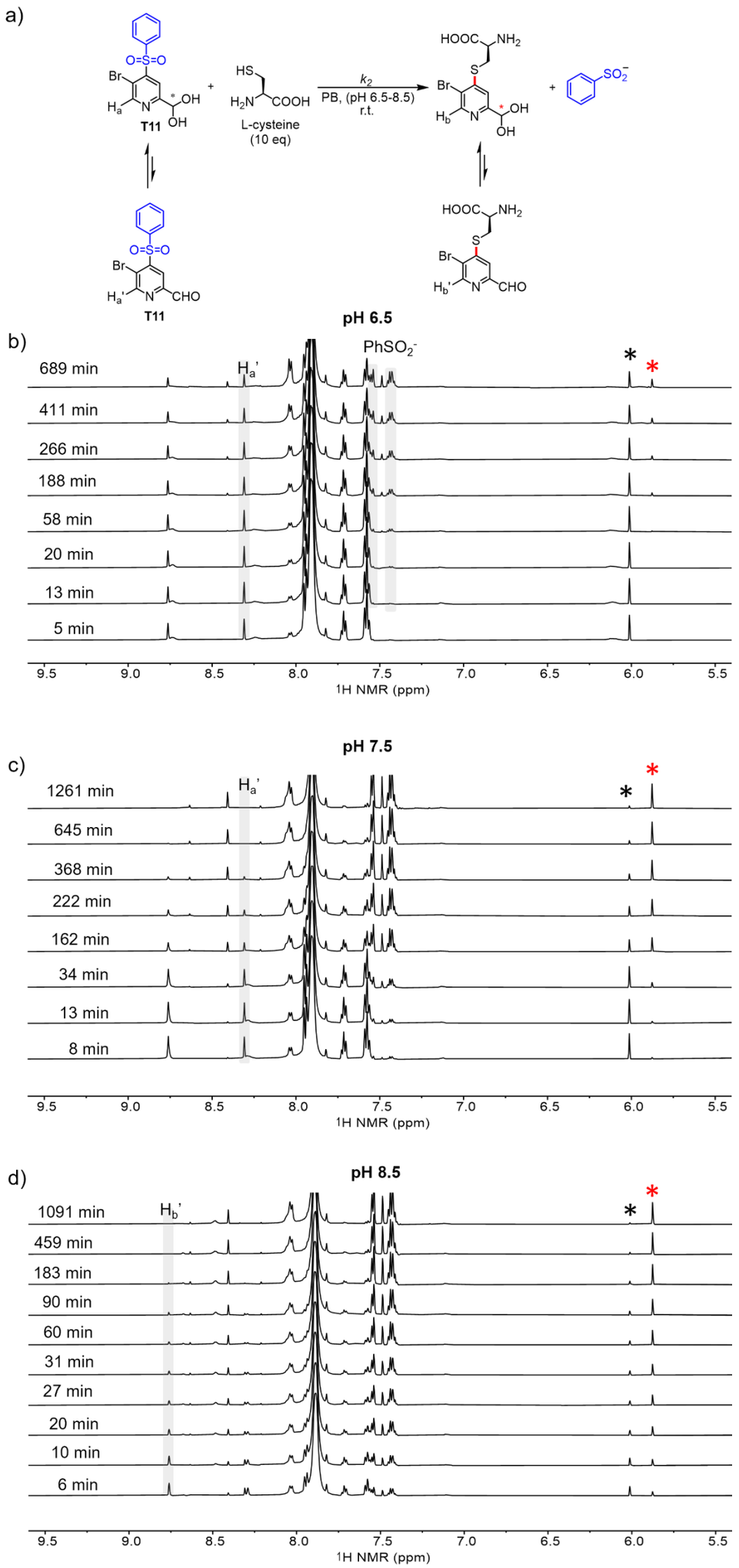



**Figure S28.** a) Reaction of **T11** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T11** in reaction with 10 equivalent *L*-cysteine over 18 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S11.** The time-dependent concentration profiles of **T11** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by nonlinear regression.

| **T11** + *L*-cysteine (10 eq)<br>($c_0$: the initial concentration of **T11**; $c_t$: the concentration of product at different time)<br>Fit equation: $ct = A\exp(-k_1^{obs}*t) + c_0$ | | | | |
|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Nonlinear Fit |
| pH 6.5 | 5 | 0.0833 | 0.05587 | $y = -0.65e^{-0.15x}+0.70$<br>$R^2 = 0.9992$<br>$k_1^{obs} = 0.15 \pm 0.01\ h^{-1}$<br>$k_2 = 16.23 \pm 1.01\ M^{-1}\cdot h^{-1}$ |
| | 13 | 0.2167 | 0.0636 | |
| | 20 | 0.3333 | 0.08643 | |
| | 58 | 0.9667 | 0.1412 | |
| | 188 | 3.1333 | 0.28956 | |
| | 266 | 4.4333 | 0.37085 | |
| | 411 | 6.8500 | 0.45814 | |
| | 689 | 11.4833 | 0.58696 | |
| pH 7.5 | t / min | t / h | $c_t$ / mM | Nonlinear Fit |
| | 8 | 0.1333 | 0.0947 | $y = -0.81e^{-0.32x}+0.88$<br>$R^2 = 0.9991$<br>$k_1^{obs} = 0.32 \pm 0.01\ h^{-1}$<br>$k_2 = 35.14 \pm 1.39\ M^{-1}\cdot h^{-1}$ |
| | 13 | 0.2167 | 0.1069 | |
| | 34 | 0.5667 | 0.2160 | |
| | 161 | 2.6833 | 0.5357 | |
| | 222 | 3.7000 | 0.6314 | |
| | 368 | 6.1333 | 0.7529 | |
| | 645 | 10.7500 | 0.8462 | |
| | 1261 | 21.0167 | 0.8875 | |
| pH 8.5 | t / min | t / h | $c_t$ / mM | Nonlinear Fit |
| | 6 | 0.1000 | 0.3422 | $y = -0.61e^{-2.51x}+0.85$<br>$R^2 = 0.9670$<br>$k_1^{obs} = 2.51 \pm 0.42\ h^{-1}$<br>$k_2 = 276.10 \pm 46.39\ M^{-1}\cdot h^{-1}$ |
| | 10 | 0.1667 | 0.4652 | |
| | 20 | 0.3333 | 0.6052 | |
| | 27 | 0.4500 | 0.6584 | |
| | 31 | 0.5167 | 0.6866 | |
| | 60 | 1.0000 | 0.7489 | |
| | 90 | 1.5000 | 0.7992 | |
| | 183 | 3.0500 | 0.8225 | |
| | 459 | 7.6500 | 0.8811 | |
| | 1091 | 18.1833 | 0.8970 | |

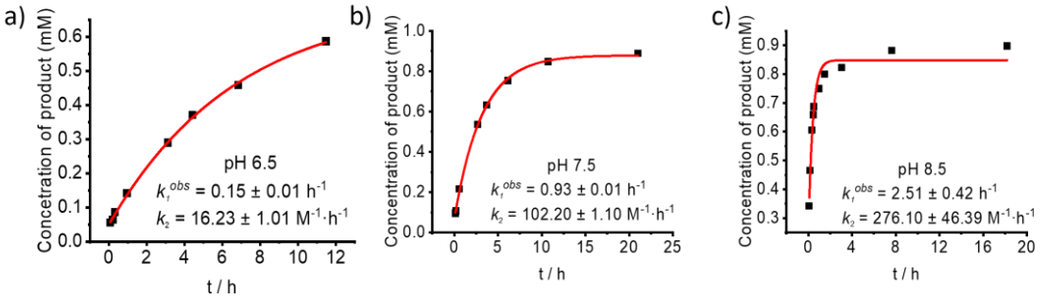



**Figure S29.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. $Ln(c/c_0)$ as a function of time (hours) for the reaction of **T11** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T11** at t; $c_0$: concentration of **T11** at $t_0$.

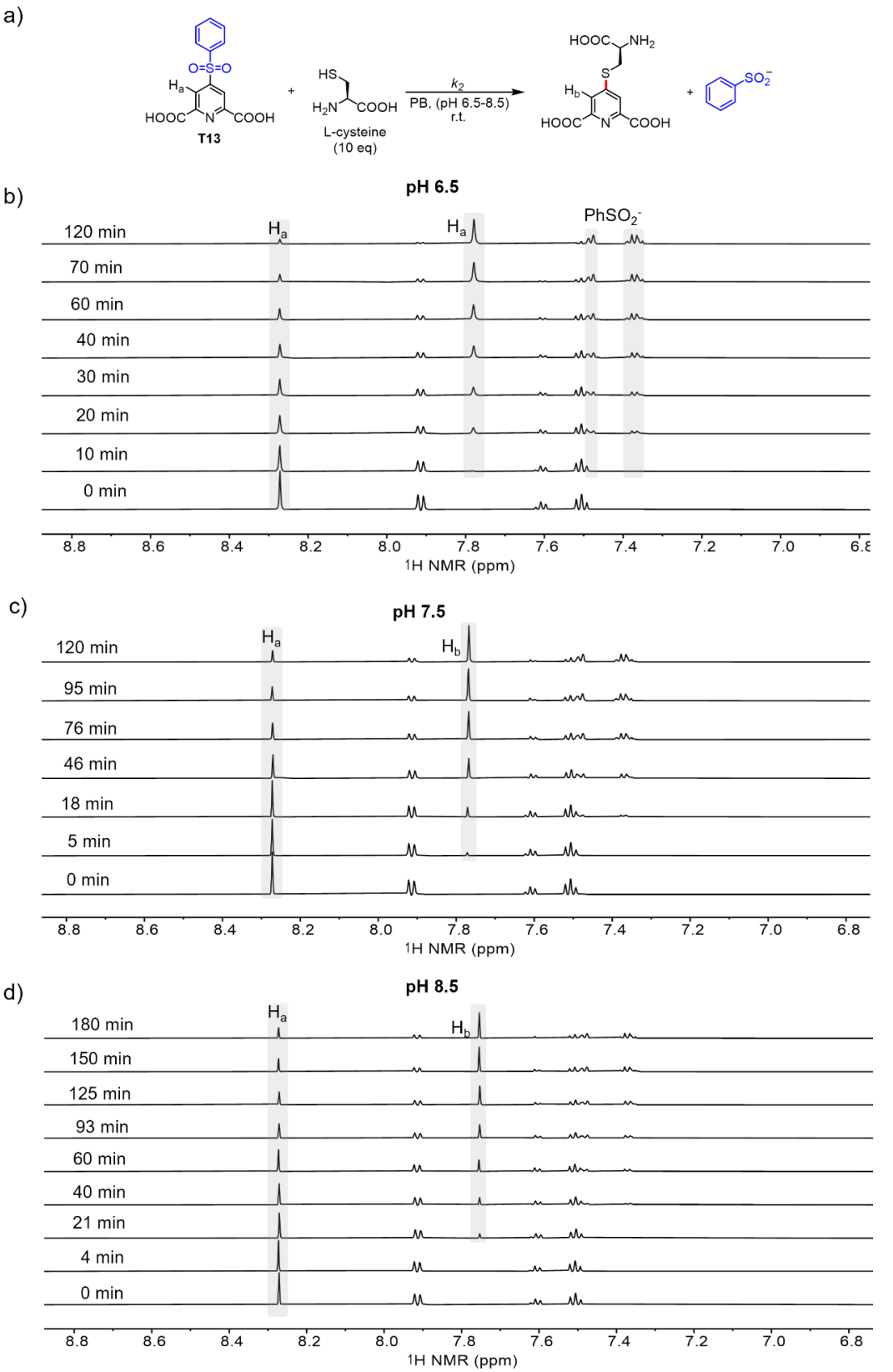



**Figure S30.** a) Reaction of **T13** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T13** in reaction with 10 equivalent *L*-cysteine over 3 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S12.** The time-dependent concentration profiles of **T13** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T13** + *L*-cysteine (10 eq)<br>($c_0$: the initial concentration of **T13**; $c_t$: the concentration of **T13** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 1.36x + 0.05<br>$R^2$ = 0.9990<br>$k_1^{obs}$ = 1.36 ± 0.01 $h^{-1}$<br>$k_2$ = 149.92 ± 1.49 $M^{-1}\cdot h^{-1}$ |
| | 10 | 0.1667 | 0.6888 | 0.2775 | |
| | 20 | 0.3333 | 0.5496 | 0.5033 | |
| | 30 | 0.5000 | 0.4266 | 0.7565 | |
| | 40 | 0.6667 | 0.3400 | 0.9834 | |
| | 60 | 1.0000 | 0.2162 | 1.4361 | |
| | 70 | 1.1667 | 0.1744 | 1.6510 | |
| | 120 | 2.0000 | 0.0585 | 2.7433 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.62x + 0.06<br>$R^2$ = 0.9950<br>$k_1^{obs}$ = 0.62 ± 0.02 $h^{-1}$<br>$k_2$ = 67.93 ± 2.16 $M^{-1}\cdot h^{-1}$ |
| | 5 | 0.0833 | 0.7990 | 0.1291 | |
| | 18 | 0.3000 | 0.6984 | 0.2637 | |
| | 46 | 0.7667 | 0.5240 | 0.5510 | |
| | 76 | 1.2667 | 0.3780 | 0.8775 | |
| | 95 | 1.5833 | 0.3221 | 1.0375 | |
| | 120 | 2.0000 | 0.2579 | 1.2598 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.36x + 0.04<br>$R^2$ = 0.9977<br>$k_1^{obs}$ = 0.36 ± 0.01 $h^{-1}$<br>$k_2$ = 40.15 ± 0.72 $M^{-1}\cdot h^{-1}$ |
| | 4 | 0.0600 | 0.8535 | 0.0631 | |
| | 21 | 0.3500 | 0.7536 | 0.1876 | |
| | 40 | 0.6667 | 0.6758 | 0.2966 | |
| | 60 | 1.0000 | 0.5954 | 0.4233 | |
| | 93 | 1.5455 | 0.4958 | 0.6063 | |
| | 125 | 2.0909 | 0.4061 | 0.8059 | |
| | 150 | 2.5000 | 0.3477 | 0.9611 | |
| | 180 | 3.0000 | 0.2976 | 1.1166 | |

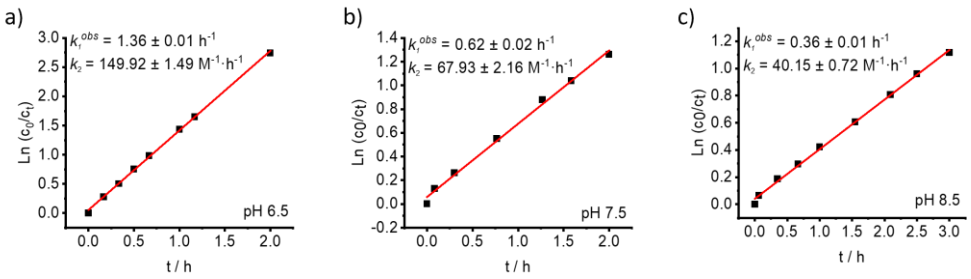



**Figure S31.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/c$_0$) as a function of time (hours) for the reaction of **T13** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T13** at t; $c_0$: concentration of **T13** at $t_0$

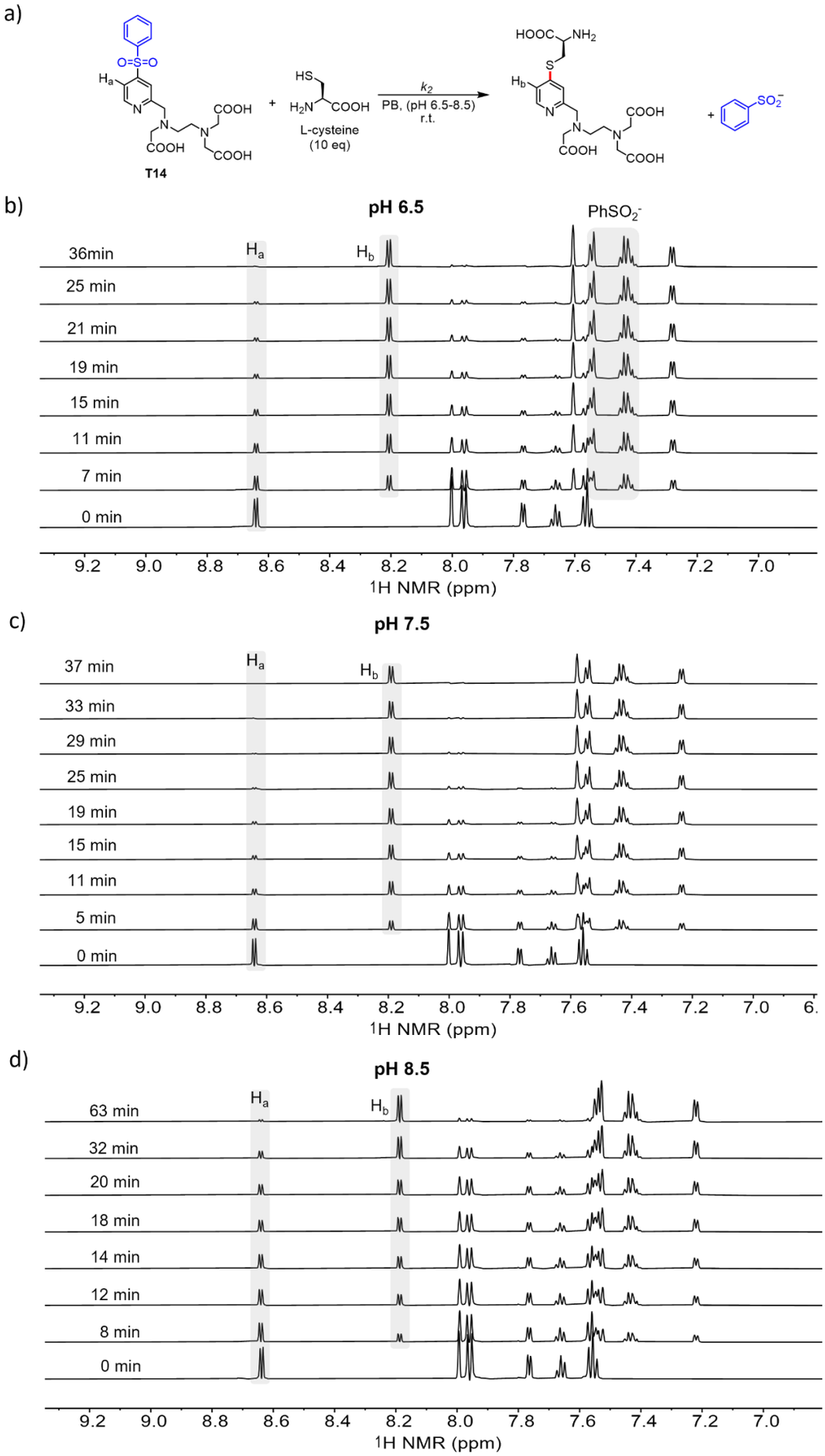



**Figure S32.** a) Reaction of **T14** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T14** in reaction with 10 equivalent *L*-cysteine over 8 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S13.** The time-dependent concentration profiles of **T14** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T14** + *L*-cysteine (10 eq)<br>($c_0$: the initial concentration of **T14**; $c_t$: the concentration of **T14** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 5.09x + 0.01<br>$R^2$ = 0.9966<br>$k_1^{obs}$ = 5.09 ± 0.12 $h^{-1}$<br>$k_2$ = 559.82 ± 13.42 $M^{-1}·h^{-1}$ |
| | 7 | 0.1167 | 0.5254 | 0.5482 | |
| | 11 | 0.1833 | 0.3604 | 0.9252 | |
| | 15 | 0.2500 | 0.2475 | 1.3010 | |
| | 19 | 0.3167 | 0.1685 | 1.6854 | |
| | 21 | 0.3500 | 0.1397 | 1.8732 | |
| | 25 | 0.4167 | 0.1047 | 2.1609 | |
| | 36 | 0.6000 | 0.0460 | 2.9845 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 5.46x + 0.19<br>$R^2$ = 0.9932<br>$k_1^{obs}$ = 5.46 ± 0.17 $h^{-1}$<br>$k_2$ = 601.38 ± 19.54 $M^{-1}·h^{-1}$ |
| | 5 | 0.0833 | 0.4588 | 0.6838 | |
| | 11 | 0.1833 | 0.2556 | 1.2687 | |
| | 15 | 0.2500 | 0.1737 | 1.6553 | |
| | 19 | 0.3167 | 0.1243 | 1.9896 | |
| | 25 | 0.4167 | 0.0730 | 2.5214 | |
| | 29 | 0.4833 | 0.0530 | 2.8427 | |
| | 33 | 0.5500 | 0.0404 | 3.1127 | |
| | 37 | 0.6167 | 0.0278 | 3.4883 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 2.34x - 00.01<br>$R^2$ = 0.9983<br>$k_1^{obs}$ = 2.34 ± 0.04 $h^{-1}$<br>$k_2$ = 257.16 ± 4.29 $M^{-1}·h^{-1}$ |
| | 8 | 0.1333 | 0.7104 | 0.2466 | |
| | 12 | 0.2000 | 0.5835 | 0.4434 | |
| | 14 | 0.2333 | 0.5229 | 0.5532 | |
| | 18 | 0.3000 | 0.4460 | 0.7122 | |
| | 20 | 0.3333 | 0.4041 | 0.8107 | |
| | 32 | 0.5333 | 0.2720 | 1.2068 | |
| | 63 | 1.0500 | 0.0790 | 2.4430 | |

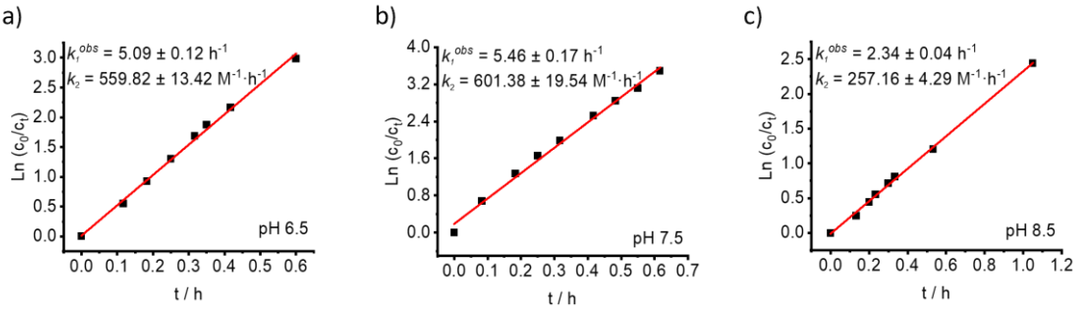



**Figure S33.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/$c_0$) as a function of time (hours) for the reaction of **T14** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T14** at t; $c_0$: concentration of **T14** at $t_0$.

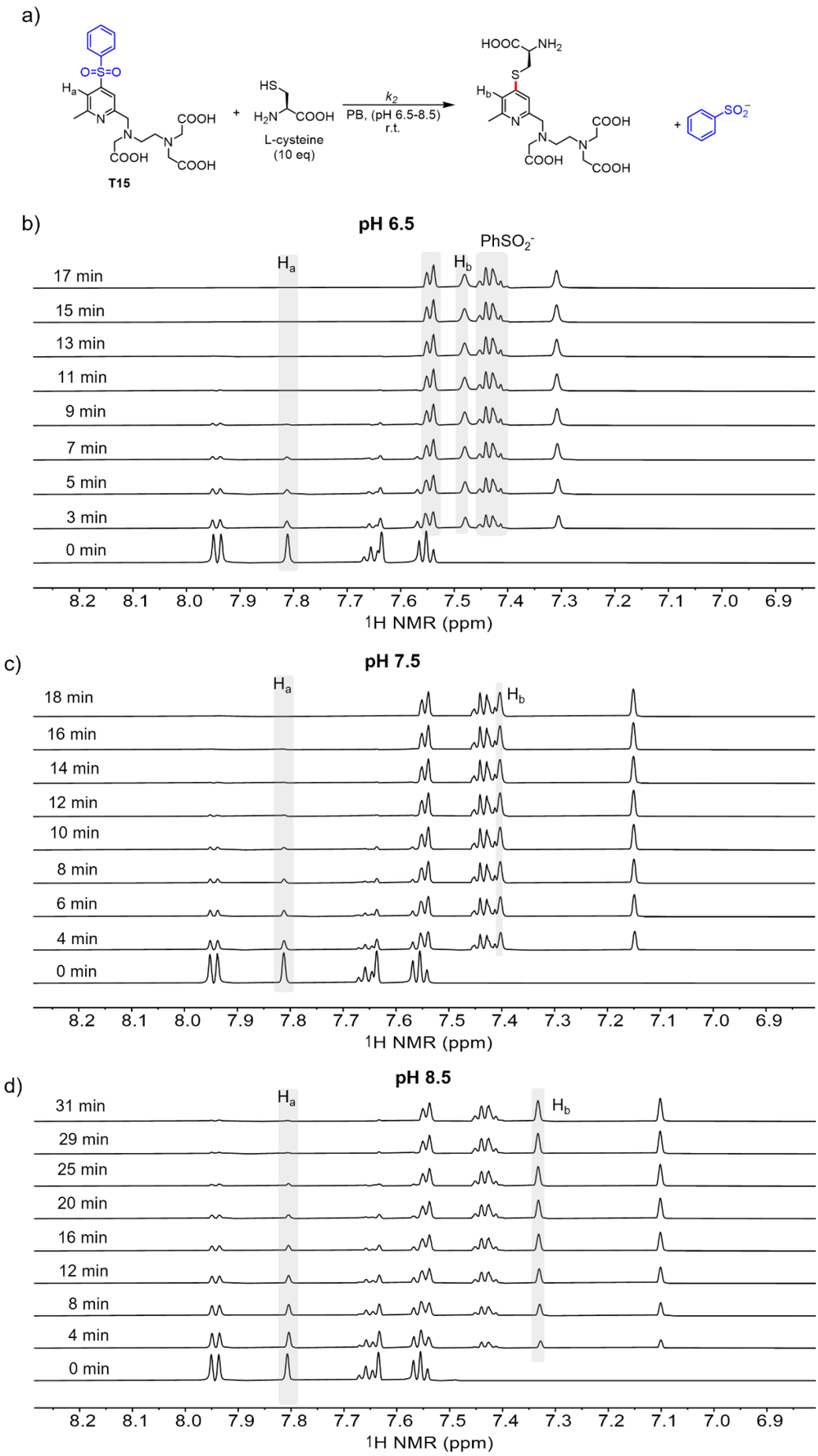



**Figure S34.** a) Reaction of **T15** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T15** in reaction with 10 equivalent *L*-cysteine over 30 min in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S14.** The time-dependent concentration profiles of **T15** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T15** + *L*-cysteine (10 eq)<br>($c_0$: the initial concentration of **T15**; $c_t$: the concentration of **T15** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 13.10x + 0.31<br>$R^2$ = 0.9847<br>$k_1^{obs}$ = 13.10 ± 0.62 $h^{-1}$<br>$k_2$ = 1441.22 ± 67.84 $M^{-1}·h^{-1}$ |
| | 3 | 0.0500 | 0.3136 | 1.0642 | |
| | 5 | 0.0833 | 0.1975 | 1.5268 | |
| | 7 | 0.1167 | 0.1282 | 1.9589 | |
| | 9 | 0.1500 | 0.0834 | 2.3892 | |
| | 11 | 0.1833 | 0.0542 | 2.8197 | |
| | 13 | 0.2167 | 0.0386 | 3.1584 | |
| | 15 | 0.2500 | 0.0277 | 3.4905 | |
| | 17 | 0.2833 | 0.0188 | 3.8795 | |
| pH 7.5<br>$c_0$ = 0.9091 mM | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | *k* |
| | 0 | 0.0000 | 0.9091 | 0.0000 | y = 11.91x + 0.15<br>$R^2$ = 0.9956<br>$k_1^{obs}$ = 11.91 ± 0.30 $h^{-1}$<br>$k_2$ = 1310.30 ± 32.97 $M^{-1}·h^{-1}$ |
| | 4 | 0.0667 | 0.3332 | 1.0039 | |
| | 6 | 0.1000 | 0.2216 | 1.4116 | |
| | 8 | 0.1333 | 0.1484 | 1.8129 | |
| | 10 | 0.1667 | 0.1016 | 2.1917 | |
| | 12 | 0.2000 | 0.0700 | 2.5646 | |
| | 14 | 0.2333 | 0.0478 | 2.9449 | |
| | 16 | 0.2667 | 0.0341 | 3.2846 | |
| | 18 | 0.3000 | 0.0236 | 3.6491 | |
| pH 8.5<br>$c_0$ = 0.9091 mM | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | *k* |
| | 0 | 0.0000 | 0.9091 | 0.0000 | y = 13.10x + 0.12<br>$R^2$ = 0.9965<br>$k_1^{obs}$ = 5.20 ± 0.12 $h^{-1}$<br>$k_2$ = 572.23 ± 12.91 $M^{-1}·h^{-1}$ |
| | 4 | 0.0667 | 0.5563 | 0.4912 | |
| | 8 | 0.1333 | 0.3851 | 0.8591 | |
| | 12 | 0.2000 | 0.2688 | 1.2187 | |
| | 16 | 0.2667 | 0.1940 | 1.5447 | |
| | 20 | 0.3333 | 0.1377 | 1.8876 | |
| | 25 | 0.4167 | 0.0935 | 2.2749 | |
| | 29 | 0.4833 | 0.0680 | 2.5928 | |
| | 31 | 0.5167 | 0.0568 | 2.7737 | |

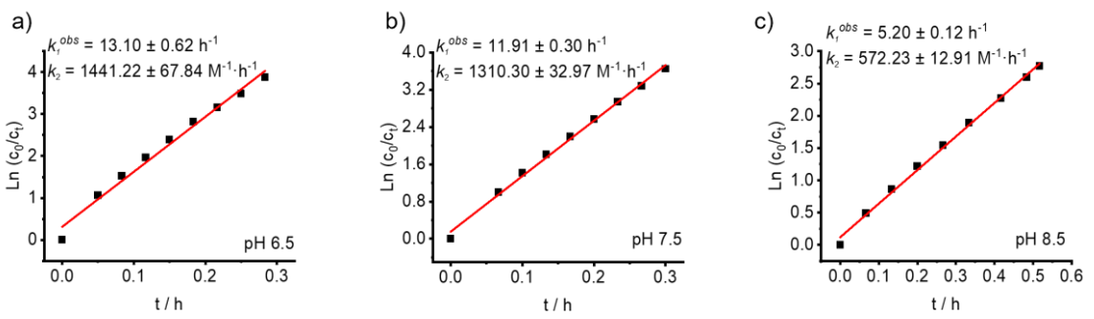



**Figure S35.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/$c_0$) as a function of time (hours) for the reaction of **T15** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T15** at t; $c_0$: concentration of **T15** at $t_0$.

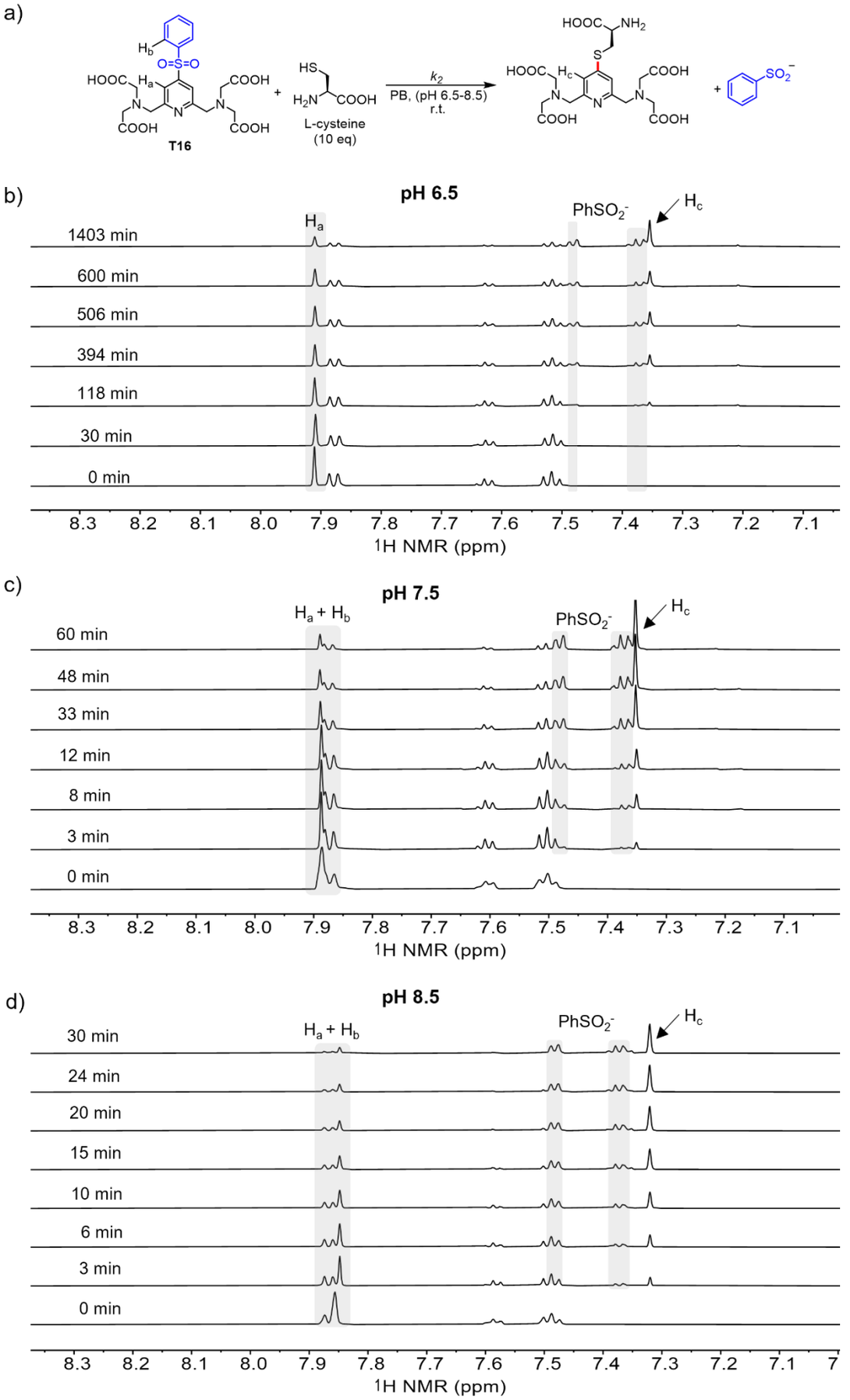



**Figure S36.** a) Reaction of **T16** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T16** in reaction with 10 equivalent *L*-cysteine over 20 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S15.** The time-dependent concentration profiles of **T16** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T16** + *L*-cysteine (10 eq)<br>($c_0$: the initial concentration of **T16**; $c_t$: the concentration of **T16** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.05x + 0.07<br>$R^2$ = 0.9787<br>$k_1^{obs}$ = 0.05 ± 0.003 $h^{-1}$<br>$k_2$ = 5.07 ± 0.33 $M^{-1}\cdot h^{-1}$ |
| | 30 | 0.5000 | 0.8562 | 0.0600 | |
| | 118 | 1.9667 | 0.7775 | 0.1564 | |
| | 394 | 6.5667 | 0.5936 | 0.4263 | |
| | 506 | 8.4333 | 0.5423 | 0.5167 | |
| | 600 | 10.0000 | 0.5074 | 0.5832 | |
| | 1403 | 23.3833 | 0.3056 | 1.0901 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 1.41x + 0.07<br>$R^2$ = 0.9955<br>$k_1^{obs}$ = 1.41 ± 0.04 $h^{-1}$<br>$k_2$ = 154.94 ± 4.68 $M^{-1}\cdot h^{-1}$ |
| | 3 | 0.0500 | 0.7663 | 0.1709 | |
| | 8 | 0.1333 | 0.6998 | 0.2616 | |
| | 12 | 0.2000 | 0.6217 | 0.3800 | |
| | 33 | 0.5500 | 0.3784 | 0.8764 | |
| | 48 | 0.8000 | 0.2735 | 1.2010 | |
| | 60 | 1.0000 | 0.2132 | 1.4501 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 3.35x + 0.08<br>$R^2$ = 0.9949<br>$k_1^{obs}$ = 3.35 ± 0.10 $h^{-1}$<br>$k_2$ = 368.92 ± 10.81 $M^{-1}\cdot h^{-1}$ |
| | 3 | 0.0500 | 0.6817 | 0.2879 | |
| | 6 | 0.1000 | 0.5839 | 0.4428 | |
| | 10 | 0.1667 | 0.4759 | 0.6473 | |
| | 15 | 0.2500 | 0.3704 | 0.8978 | |
| | 20 | 0.3333 | 0.2747 | 1.1967 | |
| | 24 | 0.4000 | 0.2114 | 1.4586 | |
| | 30 | 0.5000 | 0.1644 | 1.7100 | |

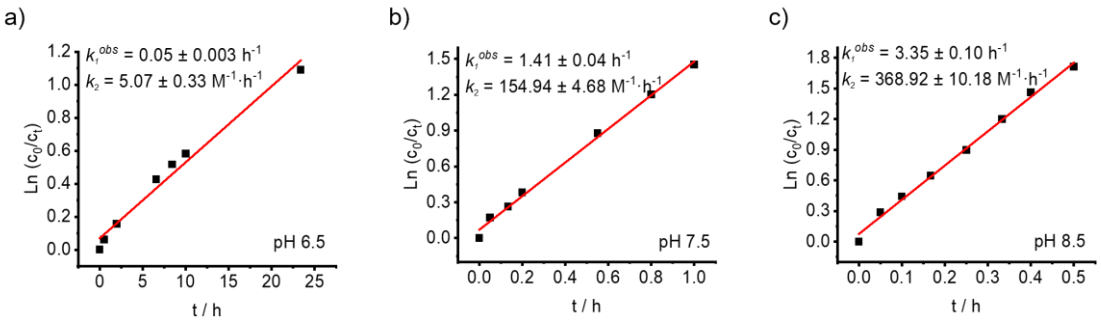



**Figure S37.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/$c_0$) as a function of time (hours) for the reaction of **T16** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T16** at t; $c_0$: concentration of **T16** at $t_0$.

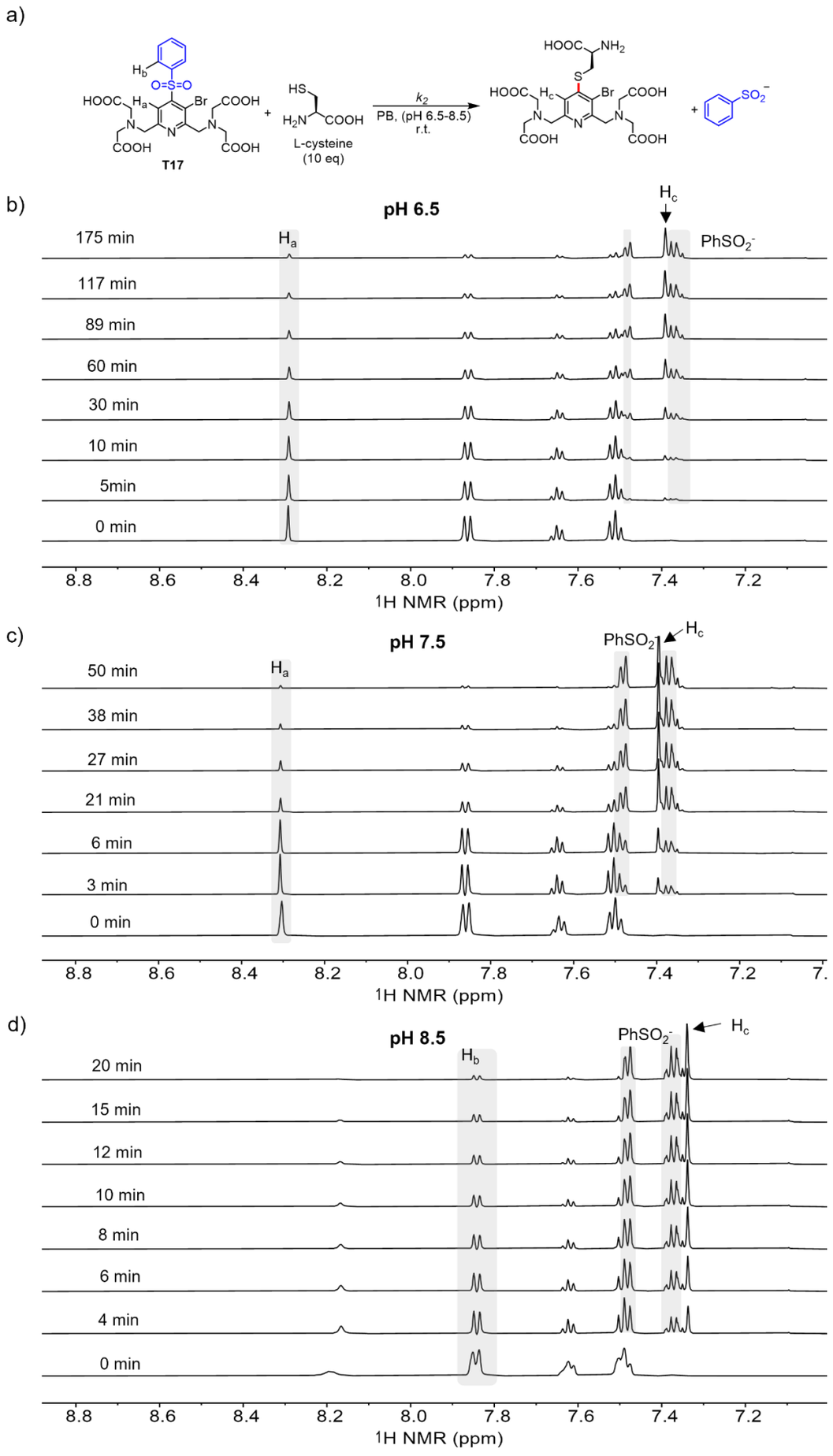



**Figure S38.** a) Reaction of **T17** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T17** in reaction with 10 equivalent *L*-cysteine over 3 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S16.** The time-dependent concentration profiles of **T17** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T17** + *L*-cysteine (10 eq)<br>($c_0$: the initial concentration of **T17**; $c_t$: the concentration of **T17** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.65x + 0.08<br>$R^2$ = 0.9936<br>$k_1^{obs}$ = 0.65 ± 0.02 $h^{-1}$<br>$k_2$ = 71.53 ± 2.35 $M^{-1}\cdot h^{-1}$ |
| | 5 | 0.0833 | 0.8046 | 0.1221 | |
| | 10 | 0.1667 | 0.7502 | 0.1921 | |
| | 30 | 0.5000 | 0.5939 | 0.4258 | |
| | 60 | 1.0000 | 0.4263 | 0.7572 | |
| | 89 | 1.4833 | 0.2966 | 1.1200 | |
| | 117 | 1.9500 | 0.2277 | 1.3843 | |
| | 175 | 2.9167 | 0.1361 | 1.8990 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 3.54x + 0.07<br>$R^2$ = 0.9979<br>$k_1^{obs}$ = 3.54 ± 0.07 $h^{-1}$<br>$k_2$ = 389.45 ± 8.07 $M^{-1}\cdot h^{-1}$ |
| | 3 | 0.0500 | 0.7103 | 0.2467 | |
| | 6 | 0.1000 | 0.5875 | 0.4366 | |
| | 21 | 0.3500 | 0.2251 | 1.3958 | |
| | 27 | 0.4500 | 0.1671 | 1.6938 | |
| | 38 | 0.6333 | 0.0899 | 2.3136 | |
| | 50 | 0.8333 | 0.0467 | 2.9686 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 7.36x – 0.13<br>$R^2$ = 0.9873<br>$k_1^{obs}$ = 7.36 ± 0.34 $h^{-1}$<br>$k_2$ = 809.73 ± 37.46 $M^{-1}\cdot h^{-1}$ |
| | 4 | 0.0667 | 0.6662 | 0.3109 | |
| | 6 | 0.1000 | 0.5188 | 0.5610 | |
| | 8 | 0.1333 | 0.4078 | 0.8018 | |
| | 10 | 0.1667 | 0.3033 | 1.0976 | |
| | 12 | 0.2000 | 0.2252 | 1.3954 | |
| | 15 | 0.2500 | 0.1850 | 1.5920 | |
| | 20 | 0.3333 | 0.0791 | 2.4416 | |

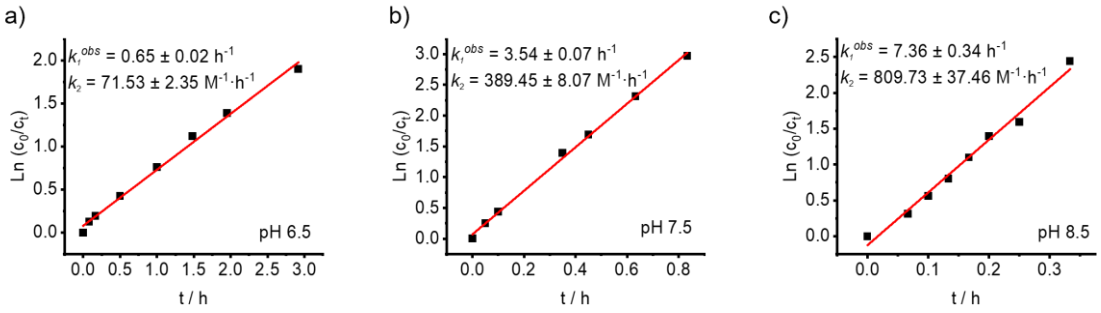



**Figure S39.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/$c_0$) as a function of time (hours) for the reaction of **T17** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T17** at t; $c_0$: concentration of **T17** at $t_0$.

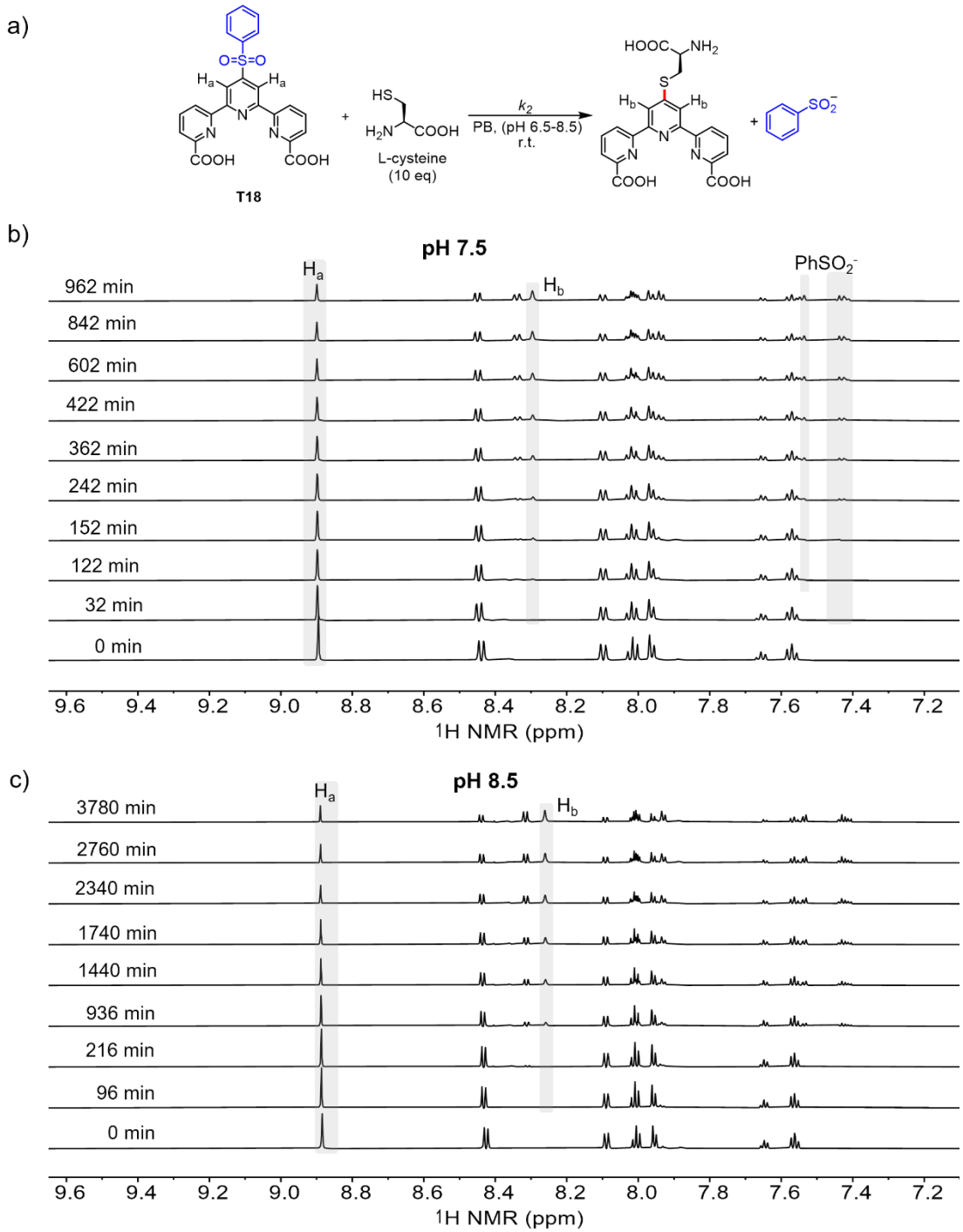



**Figure S40.** a) Reaction of **T18** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T18** in reaction with 10 equivalent *L*-cysteine over 60 h in 20 mM PB at pH 7.5 and 8.5, respectively.

**Table S17.** The time-dependent concentration profiles of **T18** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T18** + *L*-cysteine (10 eq)<br>($c_0$: the initial concentration of **T18**; $c_t$: the concentration of **T18** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.05x + 0.05<br>$R^2$ = 0.9930<br>$k_1^{obs}$ = 0.05 ± 0.001 $h^{-1}$<br>$k_2$ = 5.27 ± 0.16 $M^{-1}·h^{-1}$ |
| | 32 | 0.5333 | 0.8435 | 0.0749 | |
| | 122 | 2.0333 | 0.7794 | 0.1539 | |
| | 152 | 2.5333 | 0.7465 | 0.1970 | |
| | 242 | 4.0333 | 0.6980 | 0.2643 | |
| | 362 | 6.0333 | 0.6391 | 0.3524 | |
| | 422 | 7.0333 | 0.6130 | 0.3941 | |
| | 602 | 10.0333 | 0.5257 | 0.5477 | |
| | 842 | 14.0333 | 0.4495 | 0.7044 | |
| | 962 | 16.0333 | 0.4038 | 0.8116 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.02x + 0.09<br>$R^2$ = 0.9835<br>$k_1^{obs}$ = 0.02 ± 0.001 $h^{-1}$<br>$k_2$ = 1.96 ± 0.10 $M^{-1}·h^{-1}$ |
| | 96 | 1.6000 | 0.8552 | 0.0611 | |
| | 216 | 3.6000 | 0.8199 | 0.1033 | |
| | 936 | 15.6000 | 0.5964 | 0.4215 | |
| | 1440 | 24.0000 | 0.5172 | 0.5641 | |
| | 1740 | 29.0000 | 0.4853 | 0.6276 | |
| | 2340 | 39.0000 | 0.4040 | 0.8110 | |
| | 2760 | 46.0000 | 0.3615 | 0.9222 | |
| | 3780 | 63.0000 | 0.2893 | 1.1451 | |

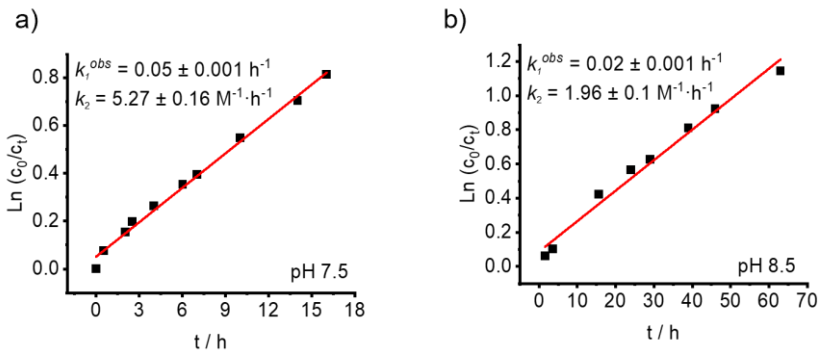



**Figure S41.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/$c_0$) as a function of time (hours) for the reaction of **T18** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 7.5 (a), 7.5 and (b). $c_t$: concentration of **T18** at t; $c_0$: concentration of **T18** at $t_0$.

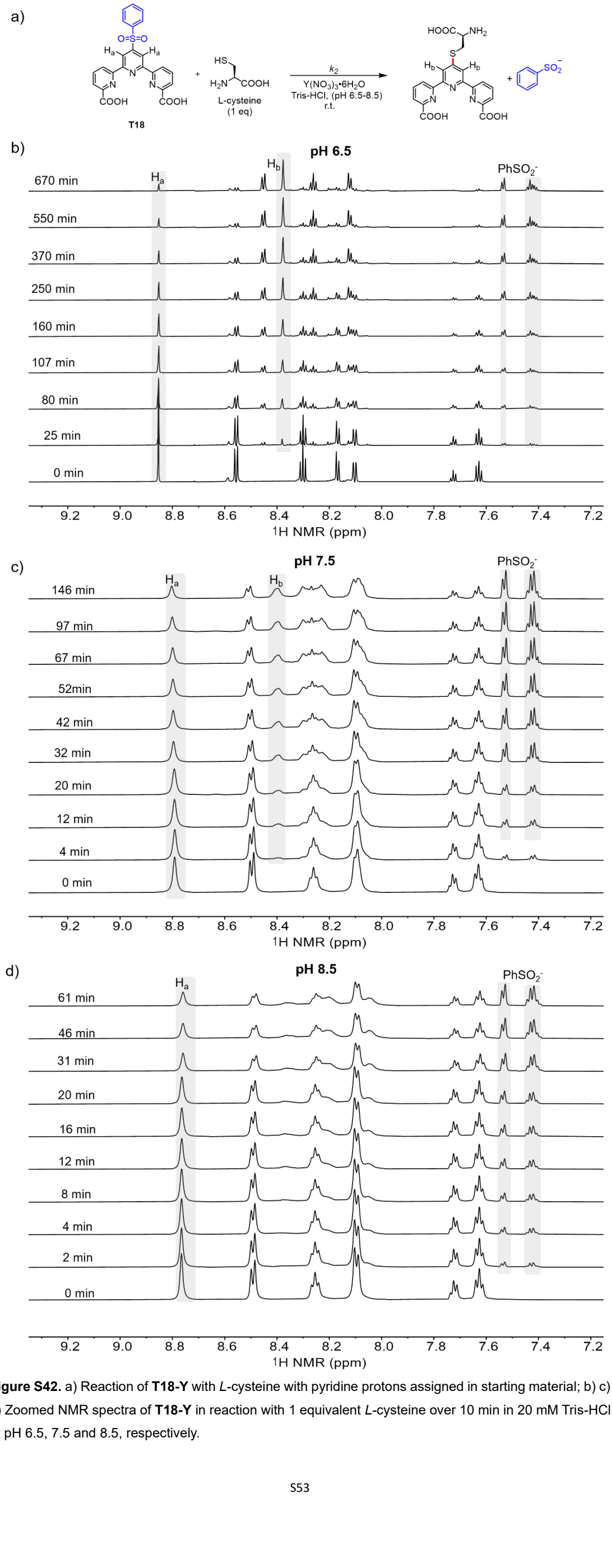



**Figure S42.** a) Reaction of **T18-Y** with *L*-cysteine with pyridine protons assigned in starting material; b) c) d) Zoomed NMR spectra of **T18-Y** in reaction with 1 equivalent *L*-cysteine over 10 min in 20 mM Tris-HCl at pH 6.5, 7.5 and 8.5, respectively.

**Table S18.** The time-dependent concentration profiles of **T18-Y** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by nonlinear regression.

| **T18-Y**<br>($c_t$: the concentration of T18-Y at different time; the initial concentration: [T18-Y] = [L-Cys])<br>Fit equation: $c_t = 1/(k_2*t + 1/c_0)$ | | | | |
|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Nonlinear Fit |
| pH 6.5<br>$c_0$ = 0.5 mM | 0 | 0.0000 | 0.5000 | y = 1/(0.92x + 1.98)<br>$R^2$ = 0.9984<br>$k_2$ = 917.95 ± 23.22 $M^{-1}·h^{-1}$ |
| | 25 | 0.4167 | 0.4268 | |
| | 80 | 1.3333 | 0.3095 | |
| | 107 | 1.7833 | 0.2813 | |
| | 160 | 2.6667 | 0.2272 | |
| | 250 | 4.1667 | 0.1788 | |
| | 370 | 6.1667 | 0.1279 | |
| | 550 | 9.1667 | 0.0874 | |
| | 670 | 11.1667 | 0.0723 | |
| | t / min | t / h | $c_t$ / mM | Nonlinear Fit |
| pH 7.5<br>$c_0$ = 1.0 mM | 0 | 0.0000 | 1.0000 | y = 1/(2.36x + 0.93)<br>$R^2$ = 0.9819<br>$k_2$ = 2363.55 ± 193.41 $M^{-1}·h^{-1}$ |
| | 4 | 0.0667 | 0.8696 | |
| | 12 | 0.2000 | 0.7114 | |
| | 20 | 0.3333 | 0.5812 | |
| | 32 | 0.5333 | 0.4335 | |
| | 42 | 0.7000 | 0.3424 | |
| | 52 | 0.8667 | 0.2697 | |
| | 67 | 1.1167 | 0.1970 | |
| | 97 | 1.6167 | 0.0959 | |
| | 146 | 2.4333 | 0.0215 | |
| | t / min | t / h | $c_t$ / mM | Nonlinear Fit |
| pH 8.5<br>$c_0$ = 1.0 mM | 0 | 0.0000 | 1.0000 | y = 1/(3.40x + 0.99)<br>$R^2$ = 0.9921<br>$k_2$ = 3402.99 ± 124.21 $M^{-1}·h^{-1}$ |
| | 2 | 0.0333 | 0.8854 | |
| | 4 | 0.0667 | 0.8216 | |
| | 8 | 0.1333 | 0.7072 | |
| | 12 | 0.2000 | 0.6106 | |
| | 16 | 0.2667 | 0.5407 | |
| | 20 | 0.3333 | 0.4770 | |
| | 31 | 0.5167 | 0.3424 | |
| | 46 | 0.7667 | 0.2446 | |
| | 61 | 1.0167 | 0.1755 | |

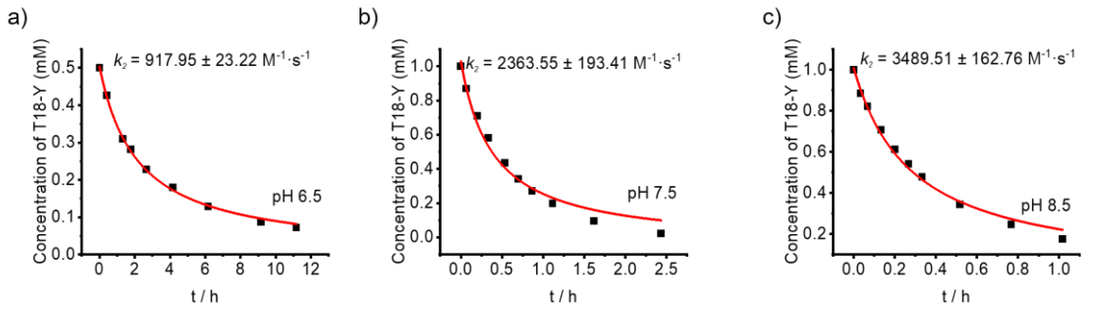



**Figure S43.** Determination of second order reaction $k_2$ of **T18-Y** with *L*-cysteine (1.0 equivalent) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c).

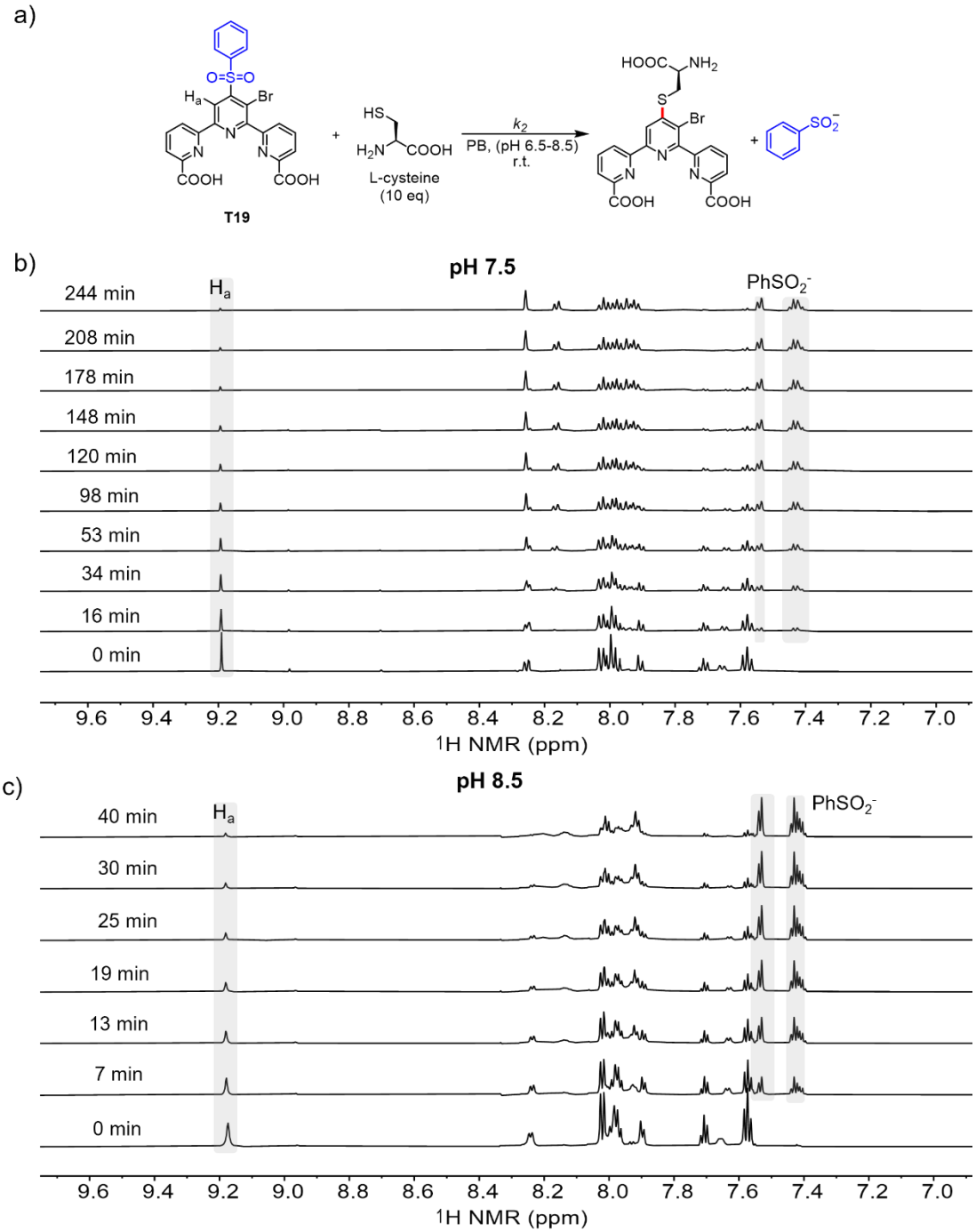



**Figure S44.** a) Reaction of **T19** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T19** in reaction with 10 equivalent *L*-cysteine over 4 h in 20 mM PB at pH 7.5 and 8.5, respectively.

**Table S19.** The time-dependent concentration profiles of **T19** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T19** + L-cysteine (10 eq)<br>($c_0$: the initial concentration of **T19**; $c_t$: the concentration of **T19** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 0.44x + 0.17<br>$R^2$ = 0.9791<br>$k_1^{obs}$ = 0.44 ± 0.02 $h^{-1}$<br>$k_2$ = 48.89 ± 2.52 $M^{-1}·h^{-1}$ |
| | 16 | 0.2667 | 0.7104 | 0.2466 | |
| | 34 | 0.5667 | 0.5696 | 0.4676 | |
| | 53 | 0.8833 | 0.4847 | 0.6289 | |
| | 98 | 1.6333 | 0.3365 | 0.9939 | |
| | 120 | 2.0000 | 0.2986 | 1.1135 | |
| | 148 | 2.4667 | 0.2368 | 1.3453 | |
| | 178 | 2.9667 | 0.1970 | 1.5293 | |
| | 208 | 3.4667 | 0.1710 | 1.6707 | |
| | 244 | 4.0667 | 0.1425 | 1.8533 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 2.83x + 0.12<br>$R^2$ = 0.9860<br>$k_1^{obs}$ = 2.83 ± 0.15 $h^{-1}$<br>$k_2$ = 310.96 ± 16.57 $M^{-1}·h^{-1}$ |
| | 7 | 0.1167 | 0.5718 | 0.4636 | |
| | 13 | 0.2167 | 0.4027 | 0.8143 | |
| | 19 | 0.3167 | 0.3099 | 1.0763 | |
| | 25 | 0.4167 | 0.2380 | 1.3401 | |
| | 30 | 0.5000 | 0.1942 | 1.5437 | |
| | 40 | 0.6667 | 0.1346 | 1.9098 | |

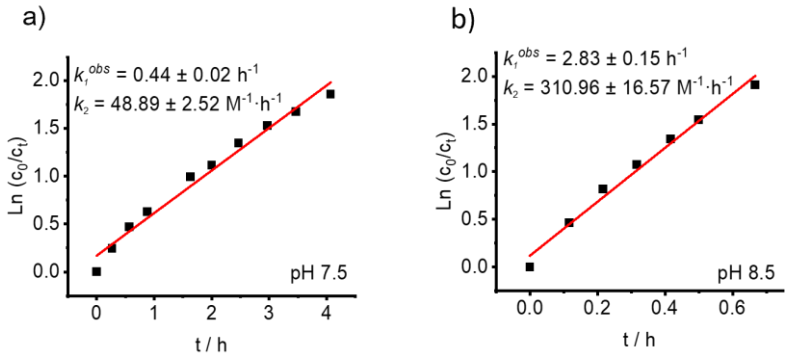



**Figure S45.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln(c/$c_0$) as a function of time (hours) for the reaction of **T19** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a) and 8.5 (b). $c_t$: concentration of **T18** at t; $c_0$: concentration of **T19** at $t_0$.

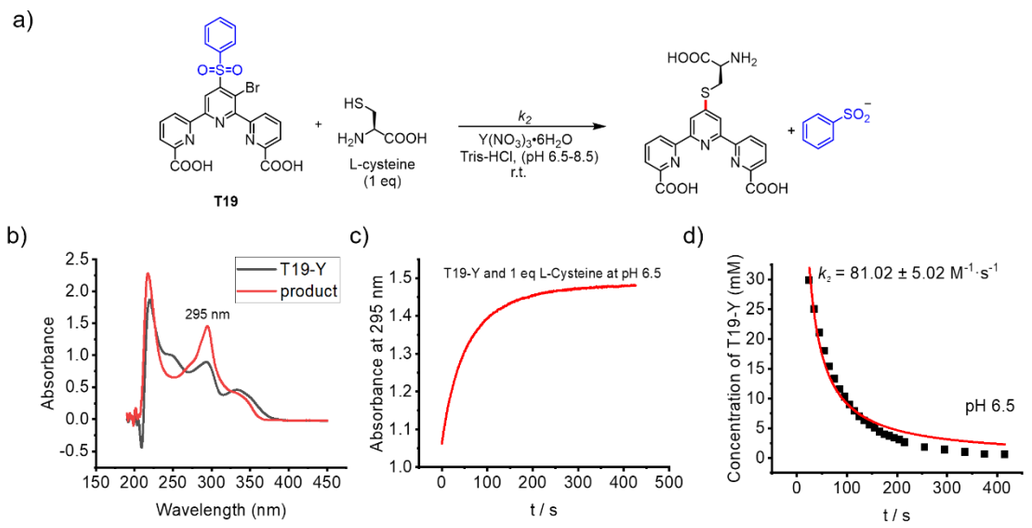



**Figure S46.** a) Reaction scheme of **T19** with *L*-cysteine assisted by $Y^{3+}$; b) UV-vis absorbance of **T19-Y** (black) and the product of **T19-Y** with *L*-cysteine (red); c) Absorbance for the reaction of **T19-Y** (50 μM) with *L*-cysteine (50 μM) in Tris-HCl (20 mM) at 295 nm as the reaction proceeded. d) Determination of second order reaction $k_2$ for the reaction of **T19-Y** (50 μM) with *L*-cysteine (50 μM) in Tris-HCl (20 mM). Note: the concentration can be calculated from the absorbance measurement using the Beer-Lambert law.

**Table S20.** The time-dependent concentration profiles of **T19-Y** calculated from the ultraviolet absorbance at 295 nm of the product and the reaction rate constants determined by nonlinear regression.

| **T19-Y**<br>($c_t$: the concentration of T18-Y at different time; the initial concentration: [T19-Y] = [L-Cys] = 50 μM)<br>Fit equation: $c_t = 1/(k_2 * t + 1/c_0)$ | | | | |
|---|---|---|---|---|
| | t / s | c[T19-Y] | c[product] | Nonlinear Fit |
| pH 6.5<br>$c_0$ = 50 μM | 25 | 29.8881 | 20.1119 | y = 1/(0.00105x + 0.00515)<br>$R^2$ = 0.9670<br>$k_2$ = 81.02 ± 5.02 $M^{-1} \cdot s^{-1}$<br>= 291666.67 ± 18055.56 $M^{-1} \cdot h^{-1}$ |
| | 35 | 24.9914 | 25.0086 | |
| | 45 | 21.0499 | 28.9501 | |
| | 55 | 17.9862 | 32.0138 | |
| | 65 | 15.3614 | 34.6386 | |
| | 75 | 13.3133 | 36.6867 | |
| | 85 | 11.5749 | 38.4251 | |
| | 95 | 10.2668 | 39.7332 | |
| | 105 | 8.9501 | 41.0499 | |
| | 115 | 7.9260 | 42.0740 | |
| | 125 | 6.9449 | 43.0551 | |
| | 135 | 6.2823 | 43.7177 | |
| | 145 | 5.5680 | 44.4320 | |
| | 155 | 5.1119 | 44.8881 | |
| | 165 | 4.4062 | 45.5938 | |
| | 175 | 3.9673 | 46.0327 | |
| | 185 | 3.7005 | 46.2995 | |
| | 195 | 3.3649 | 46.6351 | |
| | 205 | 3.0895 | 46.9105 | |
| | 215 | 2.6162 | 47.3838 | |
| | 255 | 1.7900 | 48.2100 | |
| | 295 | 1.3856 | 48.6144 | |
| | 335 | 0.9811 | 49.0189 | |
| | 375 | 0.6197 | 49.3803 | |
| | 415 | 0.5852 | 49.4148 | |

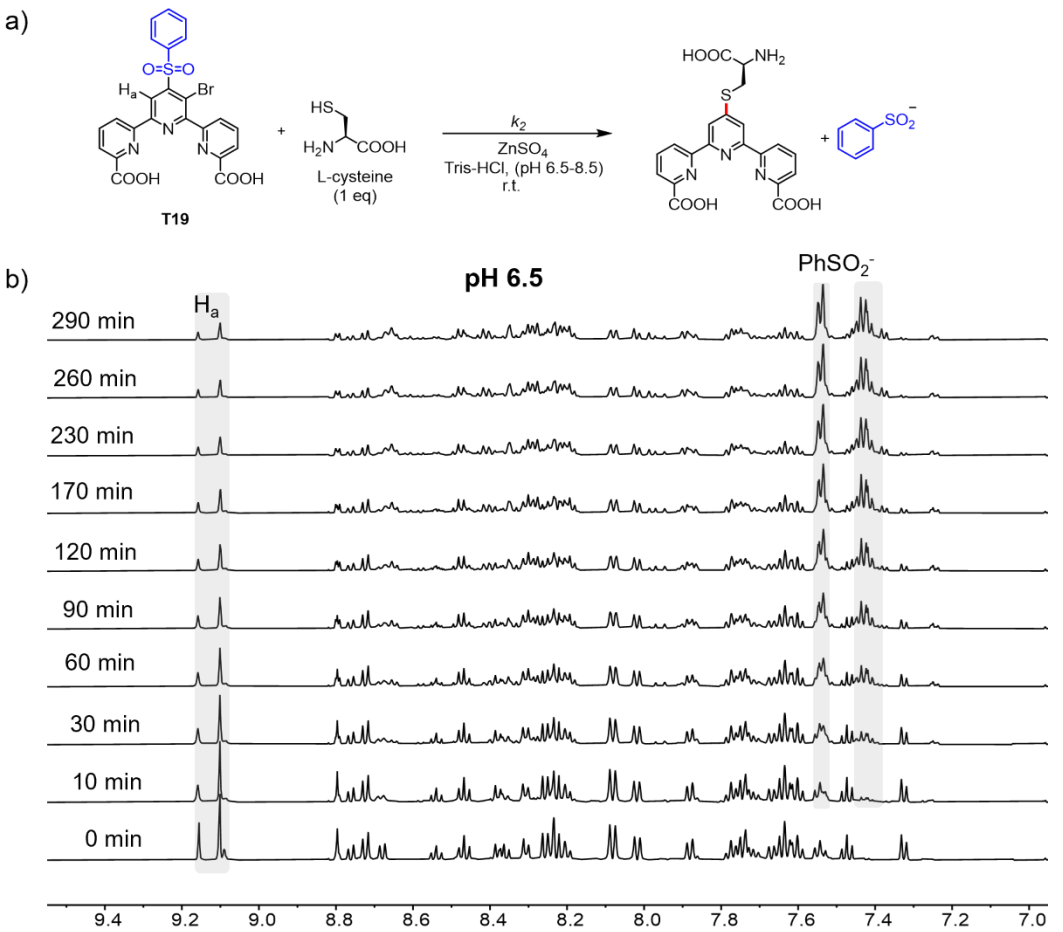



**Figure S47.** a) Reaction of **T19-Zn** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T19-Zn** in reaction with 1 equivalent *L*-Cysteine over 5 h in 20 mM Tris-HCl at pH 6.5, 7.5 and 8.5, respectively.

**Table S21.** The time-dependent concentration profiles of **T19-Zn** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by nonlinear regression.

| **T19-Zn**<br>($c_t$: the concentration of T18-Y at different time; the initial concentration: [T19-Zn] = [L-Cys])<br>Fit equation: $c_t = 1/(k_2 * t + 1/c_0)$ | | | | |
|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Nonlinear Fit |
| pH 6.5<br>$c_0$ = 1.0 mM | 0 | 0.0000 | 1.0000 | y = 1/(0.92x + 1.98)<br>$R^2$ = 0.9984<br>$k_2$ = 917.95 ± 23.22 $M^{-1}·h^{-1}$ |
| | 10 | 0.1667 | 0.8172 | |
| | 30 | 0.5000 | 0.7202 | |
| | 60 | 1.0000 | 0.6260 | |
| | 90 | 1.5000 | 0.5537 | |
| | 120 | 2.0000 | 0.4939 | |
| | 170 | 2.8333 | 0.4266 | |
| | 230 | 3.8333 | 0.3686 | |
| | 260 | 4.3333 | 0.3378 | |
| | 290 | 4.8333 | 0.3176 | |
| | t / min | t / h | $c_t$ / mM | Nonlinear Fit |
| pH 7.5<br>$c_0$ = 1.0 mM | 0 | 0.0000 | 1.0000 | y = 1/(2.36x + 0.93)<br>$R^2$ = 0.9819<br>$k_2$ = 2363.55 ± 193.41 $M^{-1}·h^{-1}$ |
| | 4 | 0.0667 | 0.8217 | |
| | 12 | 0.2000 | 0.7722 | |
| | 32 | 0.5333 | 0.6676 | |
| | 52 | 0.8667 | 0.5909 | |
| | 72 | 1.2000 | 0.5353 | |
| | 82 | 1.3667 | 0.5097 | |
| | 98 | 1.6333 | 0.4717 | |
| | 118 | 1.9667 | 0.4387 | |
| | 138 | 2.3000 | 0.4180 | |
| | t / min | t / h | $c_t$ / mM | Nonlinear Fit |
| pH 8.5<br>$c_0$ = 1.0 mM | 0 | 0.0000 | 1.0000 | y = 1/(3.40x + 0.99)<br>$R^2$ = 0.9921<br>$k_2$ = 3402.99 ± 124.21 $M^{-1}·h^{-1}$ |
| | 5 | 0.0833 | 0.8027 | |
| | 13 | 0.2167 | 0.6844 | |
| | 22 | 0.3667 | 0.5834 | |
| | 32 | 0.5333 | 0.5028 | |
| | 41 | 0.6833 | 0.4419 | |
| | 46 | 0.7667 | 0.4086 | |
| | 51 | 0.8500 | 0.3816 | |
| | 56 | 0.9333 | 0.3610 | |
| | 71 | 1.1833 | 0.3072 | |

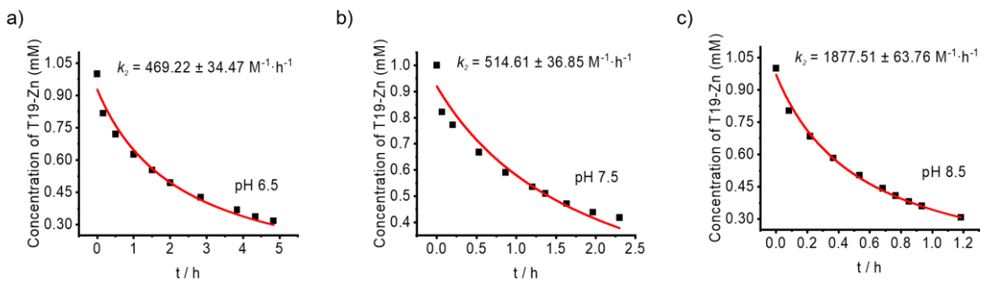



**Figure S48.** Determination of second order reaction $k_2$ of **T19-Zn** (1.0 mM) with *L*-cysteine (1.0 mM) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c).

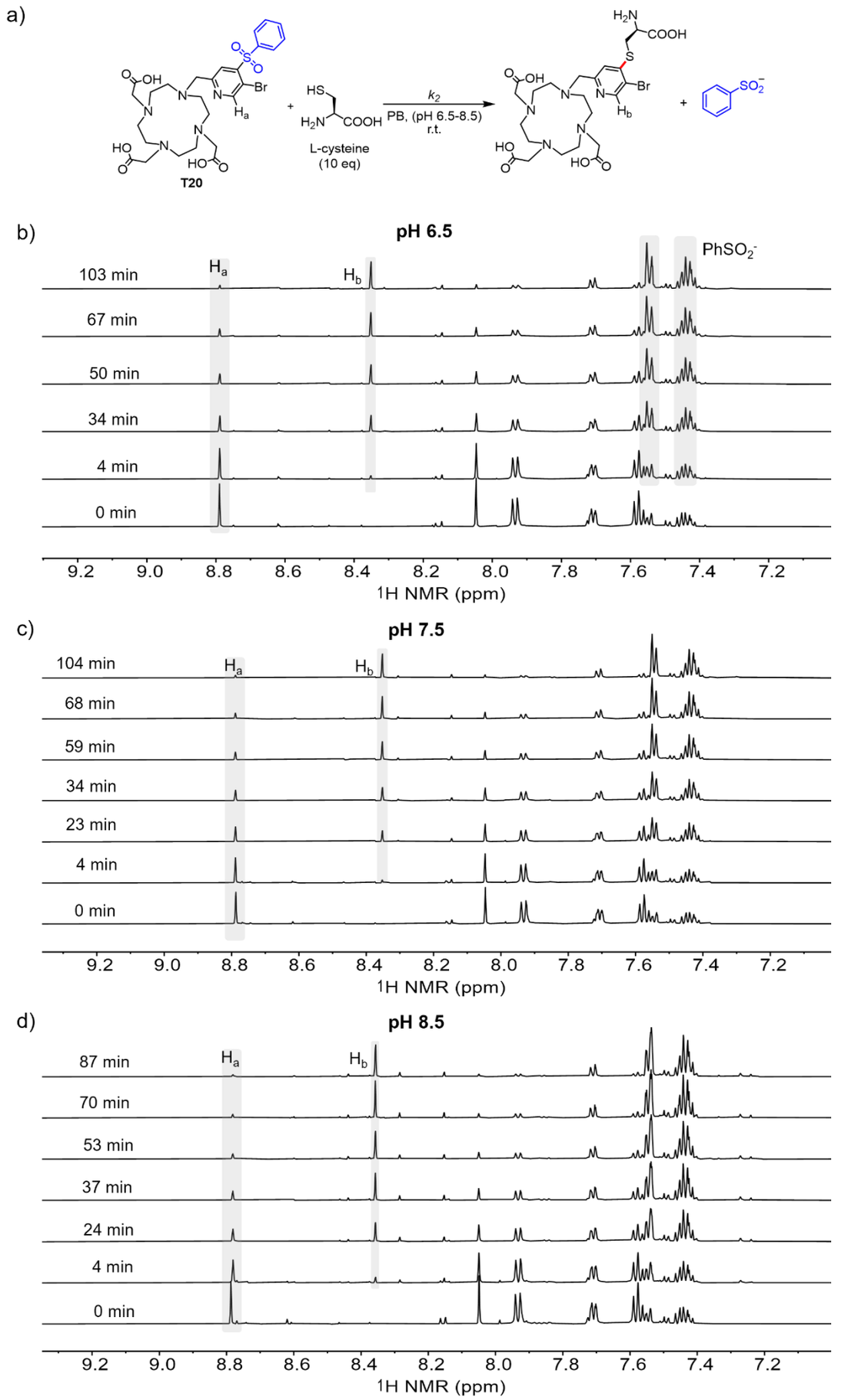



**Figure S49.** a) Reaction of **T20** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T20** in reaction with 10 equivalent *L*-cysteine over 2 h in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S22.** The time-dependent concentration profiles of **T20** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T20** + L-cysteine (10 eq)<br>($c_0$: the initial concentration of **T20**; $c_t$: the concentration of **T20** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 1.33x + 0.10<br>$R^2$ = 0.9942<br>$k_1^{obs}$ = 1.33 ± 0.05 $h^{-1}$<br>$k_2$ = 145.91 ± 5.58 $M^{-1}\cdot h^{-1}$ |
| | 4 | 0.0667 | 0.7311 | 0.2179 | |
| | 34 | 0.5667 | 0.3803 | 0.8714 | |
| | 50 | 0.8333 | 0.2502 | 1.2902 | |
| | 67 | 1.1167 | 0.1867 | 1.5830 | |
| | 103 | 1.7167 | 0.0896 | 2.3169 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 1.35x + 0.07<br>$R^2$ = 0.9904<br>$k_1^{obs}$ = 1.35 ± 0.06 $h^{-1}$<br>$k_2$ = 148.56 ± 6.56 $M^{-1}\cdot h^{-1}$ |
| | 4 | 0.0667 | 0.8043 | 0.1224 | |
| | 23 | 0.3833 | 0.4724 | 0.6547 | |
| | 34 | 0.5667 | 0.3445 | 0.9705 | |
| | 59 | 0.9833 | 0.2512 | 1.2862 | |
| | 68 | 1.1333 | 0.1858 | 1.5879 | |
| | 104 | 1.7333 | 0.0813 | 2.4145 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 1.88x + 0.19<br>$R^2$ = 0.9876<br>$k_1^{obs}$ = 1.88 ± 0.09 $h^{-1}$<br>$k_2$ = 207.15 ± 10.40 $M^{-1}\cdot h^{-1}$ |
| | 4 | 0.0667 | 0.5736 | 0.4605 | |
| | 24 | 0.4000 | 0.3200 | 1.0442 | |
| | 37 | 0.6167 | 0.2432 | 1.3184 | |
| | 53 | 0.8833 | 0.1333 | 1.9195 | |
| | 70 | 1.1667 | 0.0908 | 2.3033 | |
| | 87 | 1.4500 | 0.0482 | 2.9370 | |

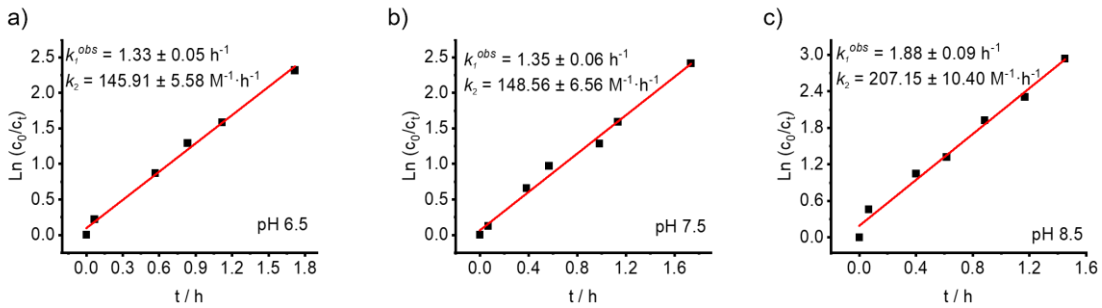



**Figure S50.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln($c/c_0$) as a function of time (hours) for the reaction of **T20** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T20** at t; $c_0$: concentration of **T20** at $t_0$.

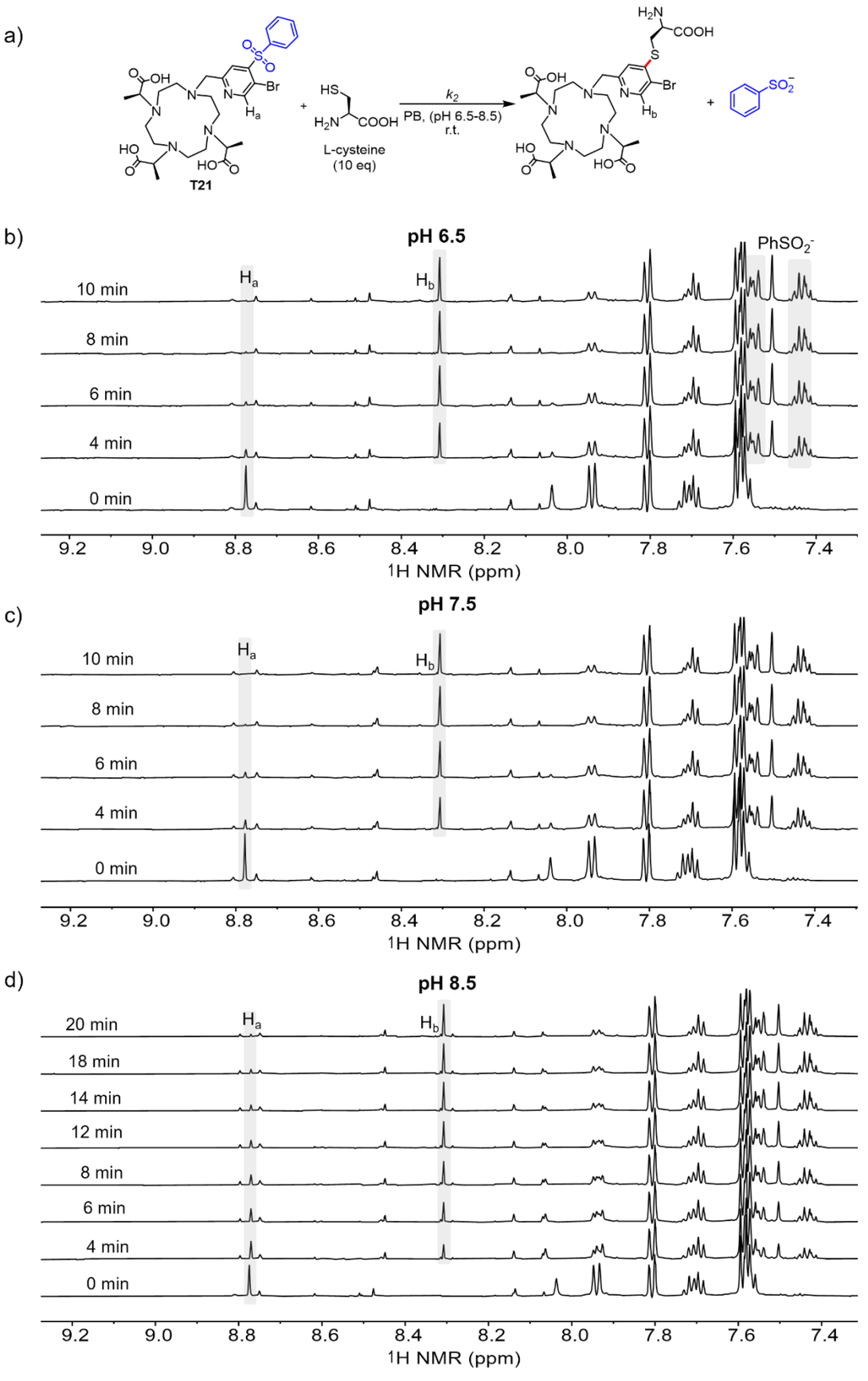



**Figure S51.** a) Reaction of **T21** with *L*-cysteine with pyridine protons assigned in the reactant and product; b) c) d) Zoomed NMR spectra of **T21** in reaction with 10 equivalent *L*-cysteine over 20 min in 20 mM PB at pH 6.5, 7.5 and 8.5, respectively.

**Table S23.** The time-dependent concentration profiles of **T21** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by linear regression.

| **T21** + L-cysteine (10 eq)<br>($c_0$: the initial concentration of **T21**; $c_t$: the concentration of **T21** at different time)<br>Fit equation: $Ln(c_0/c_t) = k_1^{obs}*t$ | | | | | |
|---|---|---|---|---|---|
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 6.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 21.39x + 0.09<br>$R^2$ = 0.9949<br>$k_1^{obs}$ = 21.39 ± 0.89 $h^{-1}$<br>$k_2$ = 2353.41 ± 97.70 $M^{-1}\cdot h^{-1}$ |
| | 4 | 0.0667 | 0.1862 | 1.5854 | |
| | 6 | 0.1000 | 0.0901 | 2.3118 | |
| | 8 | 0.1333 | 0.0453 | 2.9982 | |
| | 10 | 0.1667 | 0.0267 | 3.5286 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 7.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 17.2x + 0.01<br>$R^2$ = 0.9978<br>$k_1^{obs}$ = 17.2 ± 0.47 $h^{-1}$<br>$k_2$ = 1872.10 ± 51.32 $M^{-1}\cdot h^{-1}$ |
| | 4 | 0.0667 | 0.2840 | 1.1633 | |
| | 6 | 0.1000 | 0.1710 | 1.6707 | |
| | 8 | 0.1333 | 0.0859 | 2.3596 | |
| | 10 | 0.1667 | 0.0553 | 2.8005 | |
| | t / min | t / h | $c_t$ / mM | Ln ($c_0/c_t$) | Linear Fit |
| pH 8.5<br>$c_0$ = 0.9091 mM | 0 | 0.0000 | 0.9091 | 0.0000 | y = 7.49x – 0.16<br>$R^2$ = 0.9866<br>$k_1^{obs}$ = 7.49 ± 0.36 $h^{-1}$<br>$k_2$ = 823.69 ± 39.20 $M^{-1}\cdot h^{-1}$ |
| | 4 | 0.0667 | 0.7032 | 0.2568 | |
| | 6 | 0.1000 | 0.5383 | 0.5240 | |
| | 8 | 0.1333 | 0.4190 | 0.7746 | |
| | 10 | 0.1667 | 0.3071 | 1.0853 | |
| | 12 | 0.2000 | 0.2467 | 1.3043 | |
| | 14 | 0.2333 | 0.1807 | 1.6157 | |
| | 18 | 0.3000 | 0.1041 | 2.1676 | |

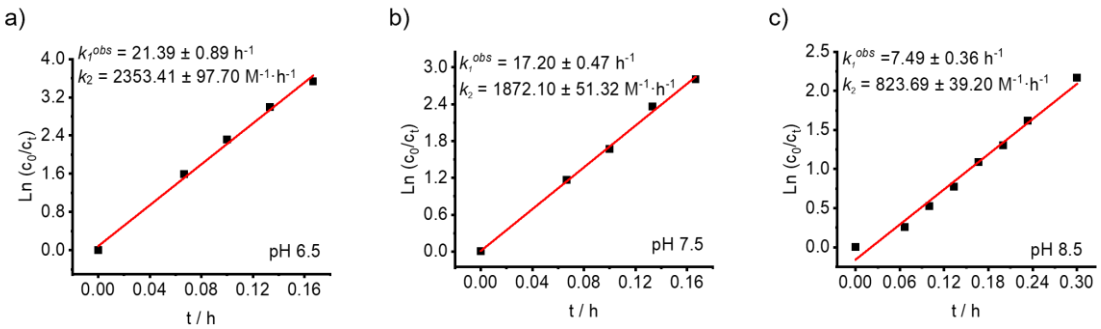



**Figure S52.** Determination of pseudo-first order reaction $k_1^{obs}$ and second order reaction $k_2$. Ln($c/c_0$) as a function of time (hours) for the reaction of **T21** (0.9 mM) with *L*-cysteine (10 eq) in PB (20 mM), at pH 6.5 (a), 7.5 (b) and 8.5 (c). $c_t$: concentration of **T21** at t; $c_0$: concentration of **T21** at $t_0$.

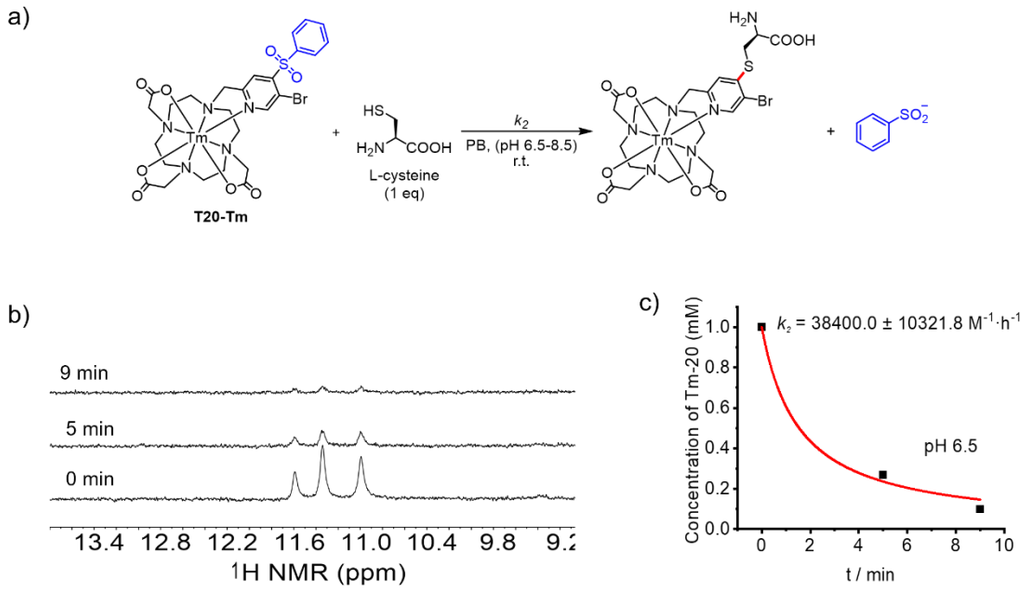



**Figure S53.** a) Reaction of **T20-Tm** with *L*-cysteine; b) Zoomed NMR spectra of **T20-Tm** in reaction with 1 equivalent *L*-cysteine over 10 min in 20 mM PB at pH 6.5. c) Determination of second order reaction $k_2$ of **T20-Tm** (1.0 mM) with *L*-cysteine (1.0 mM) in PB (20 mM), at pH 6.5.

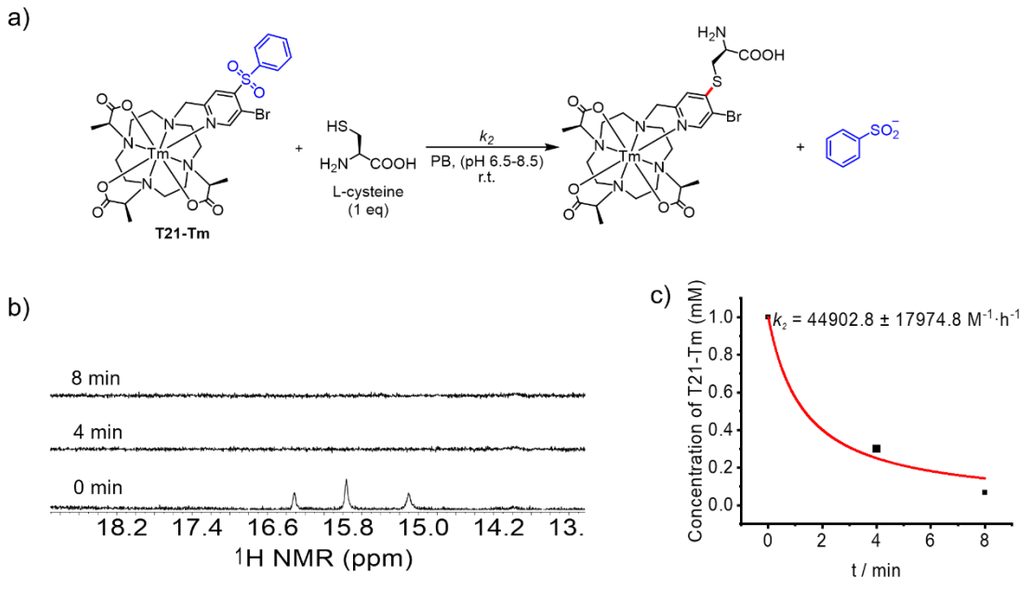


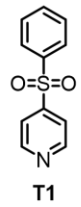

**Figure S54.** a) Reaction of **T21-Tm** with *L*-cysteine; b) Zoomed NMR spectra of **T21-Tm** in reaction with 1 equivalent *L*-cysteine over 10 min in 20 mM PB at pH 6.5. c) Determination of second order reaction $k_2$ of **T21-Tm** (1.0 mM) with *L*-cysteine (1.0 mM) in PB (20 mM), at pH 6.5.

**Table S24.** The time-dependent concentration profiles of **T20-Tm** and **T21-Tm** calculated from the integrated areas of the characteristic peaks of the reactant or product and the reaction rate constants determined by nonlinear regression.

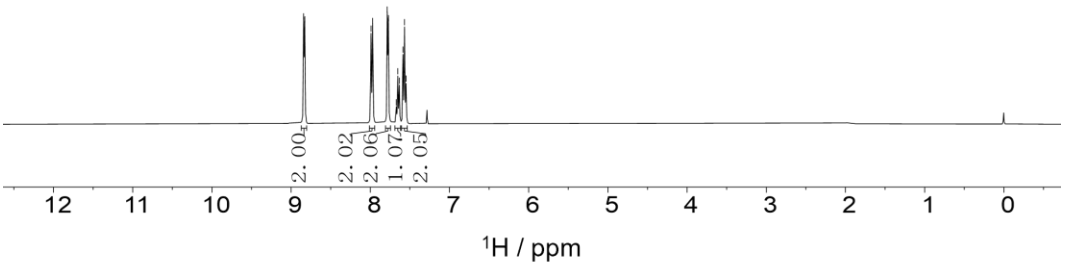
| ($c_t$: the concentration of tag at different time; the initial concentration:[tag] = [*L*-Cysteine])<br>Fit equation: $c_t = 1/(k_2*t + 1/c_0)$ | | | |
|---|---|---|---|
| **T20-Tm**<br>pH 6.5<br>$c_0$ = 1.0 mM | t / min | $c_t$ / mM | Nonlinear Fit |
| | 0 | 1.0 | y = 1/(0.92x + 1.98) |
| | 5 | 0.2689 | $R^2$ = 0.9984 |
| | 9 | 0.0988 | $k_2$ = 917.95 ± 23.22 M$^{-1}$·h$^{-1}$ |
| **T21-Tm**<br>pH 6.5<br>$c_0$ = 1.0 mM | t / min | $c_t$ / mM | Nonlinear Fit |
| | 0 | 1.0 | y = 1/(2.75x + 1.00) |
| | 4 | 0.2993 | $R^2$ = 0.9676 |
| | 8 | 0.0684 | $k_2$ = 2747.54 ± 275.54 M$^{-1}$·h$^{-1}$ |

## 5 $^{1}$HNMR and $^{13}$C NMR spectra of the tags synthesized in this work

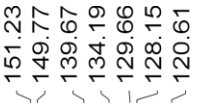
### $^{1}$H NMR (400 MHz, $CDCl_3$) of T1

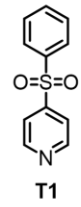




T1

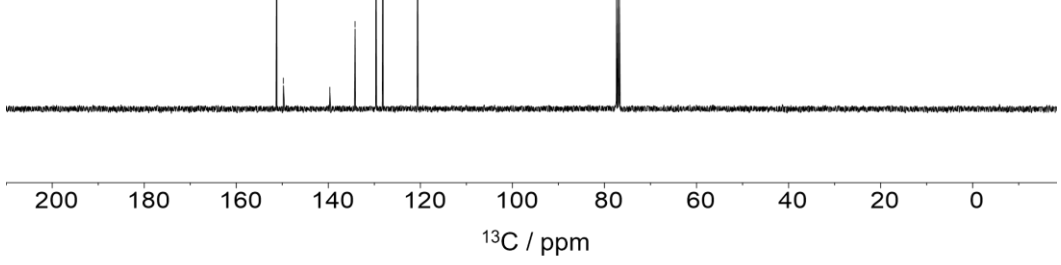



### $^{13}$C NMR (101 MHz, $CDCl_3$) of T1

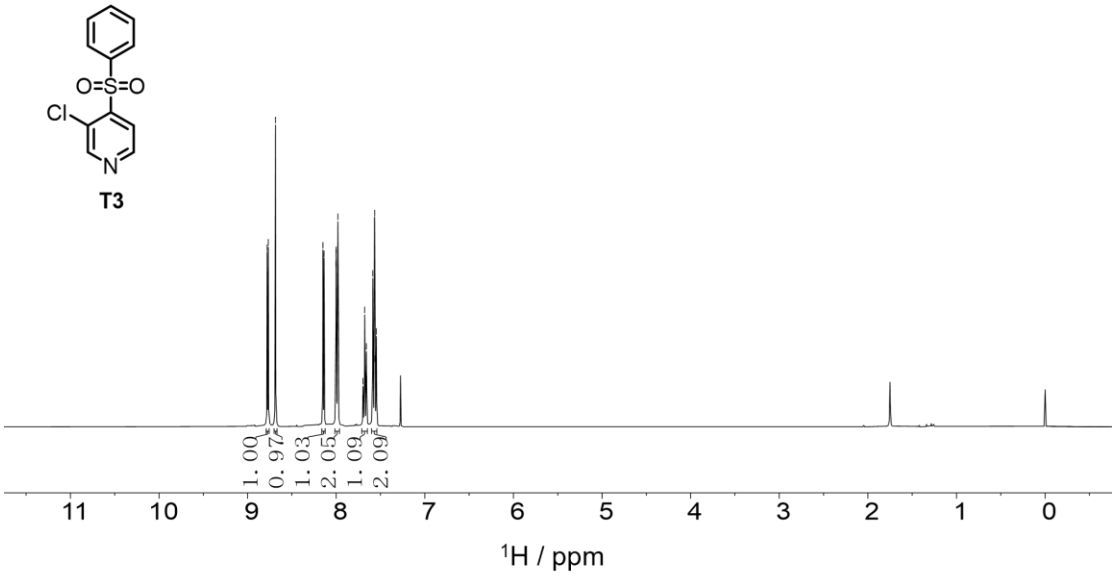


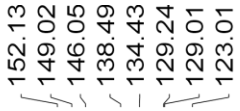

T1

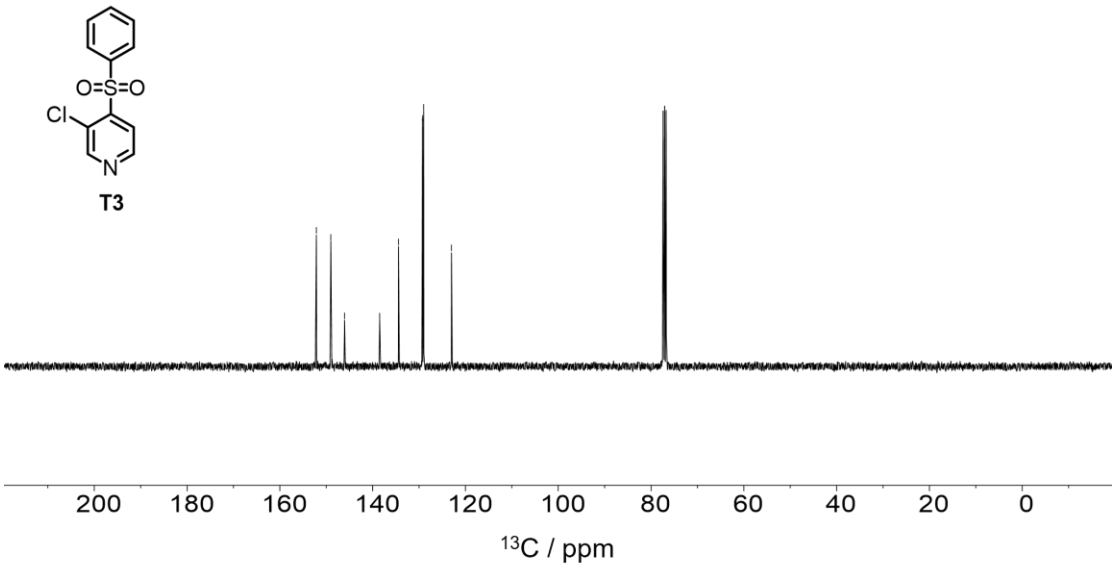


**$^{1}$H NMR (400 MHz, $CDCl_3$) of T3**

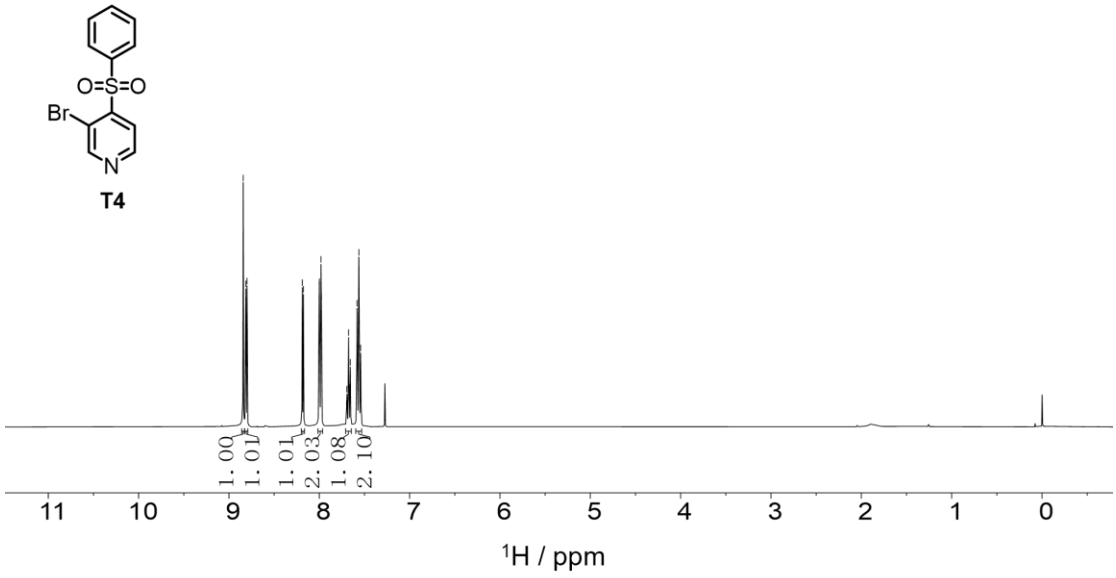




**$^{13}$C NMR (101 MHz, $CDCl_3$) of T3**

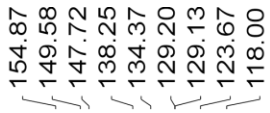


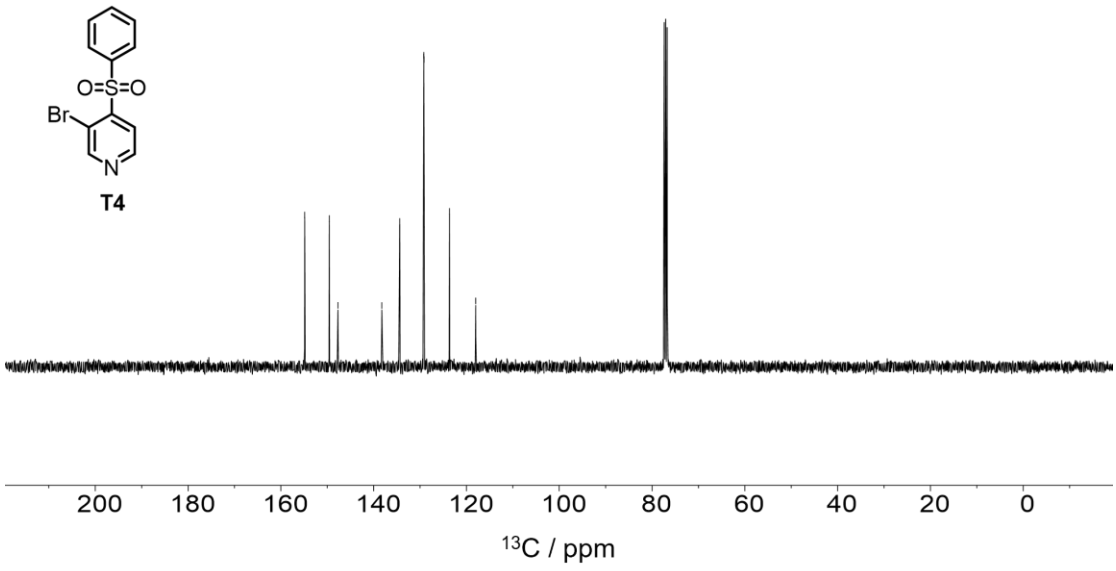



**$^{1}$H NMR (400 MHz, $CDCl_3$) of T4**

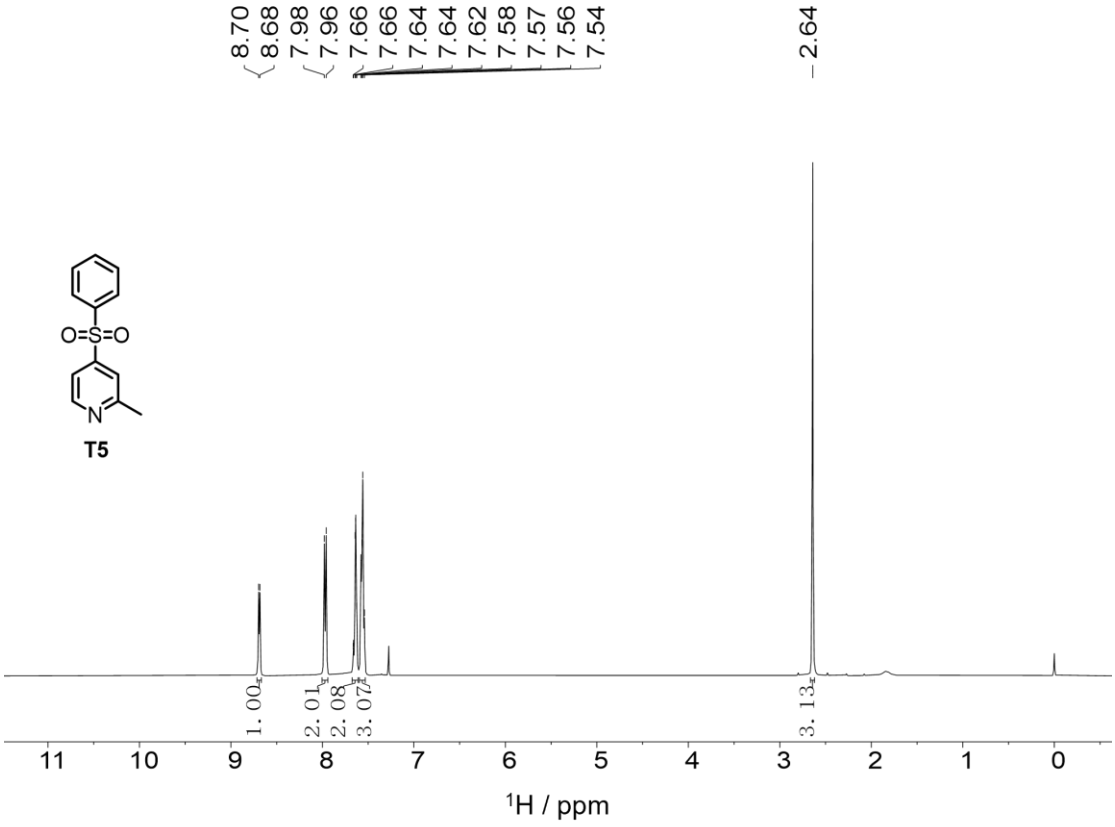




**$^{13}$C NMR (101 MHz, $CDCl_3$) of T4**

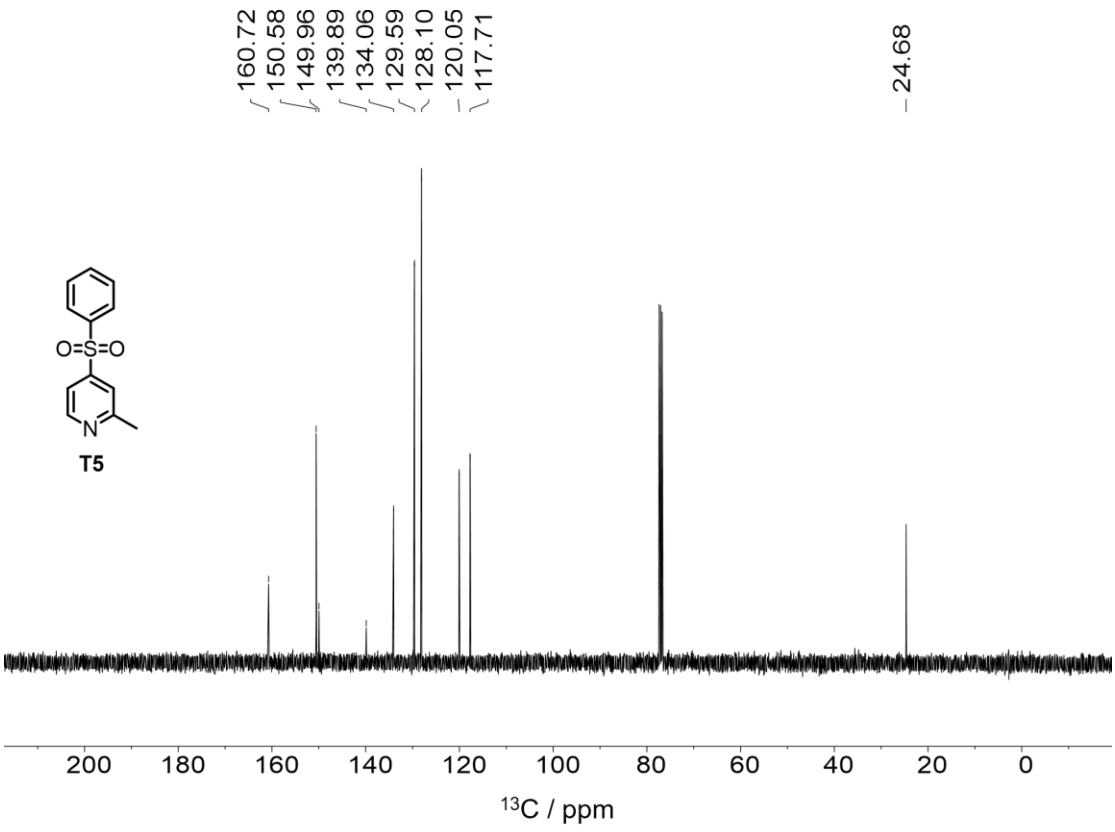


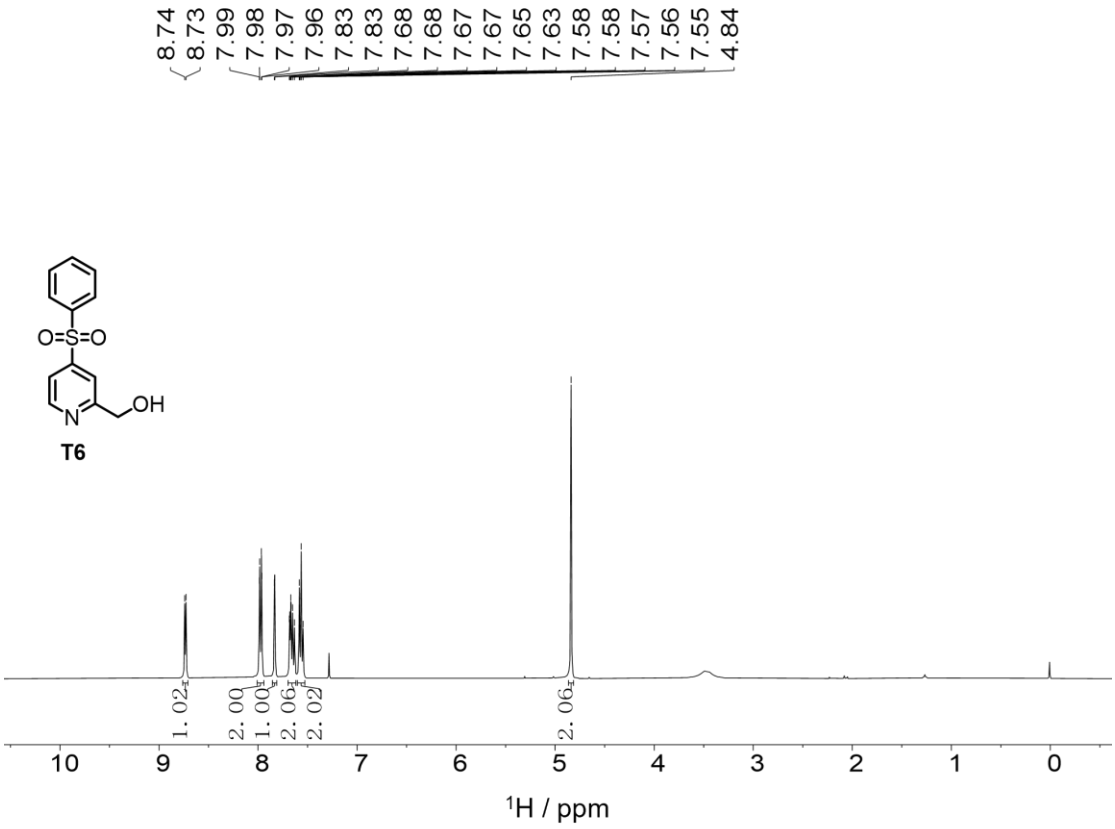
**$^{1}$H NMR (400 MHz, $CDCl_3$) of T5**



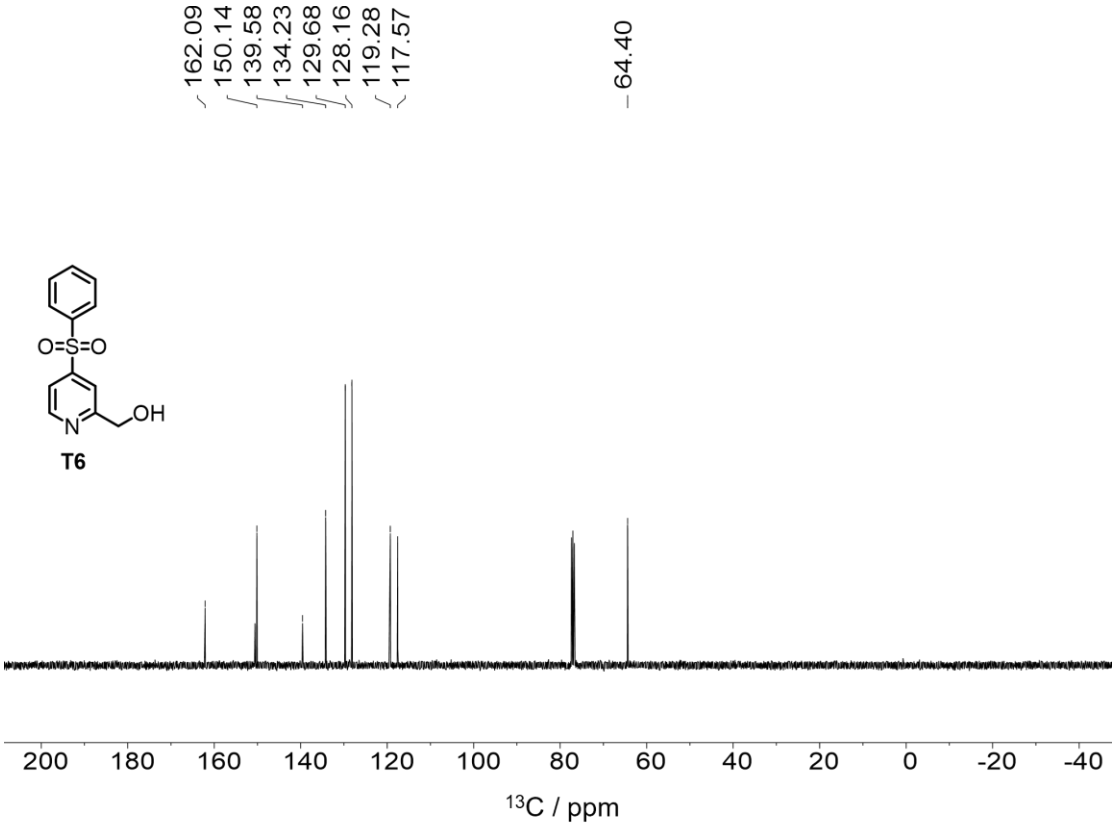

**$^{13}$C NMR (101 MHz, $CDCl_3$) of T5**



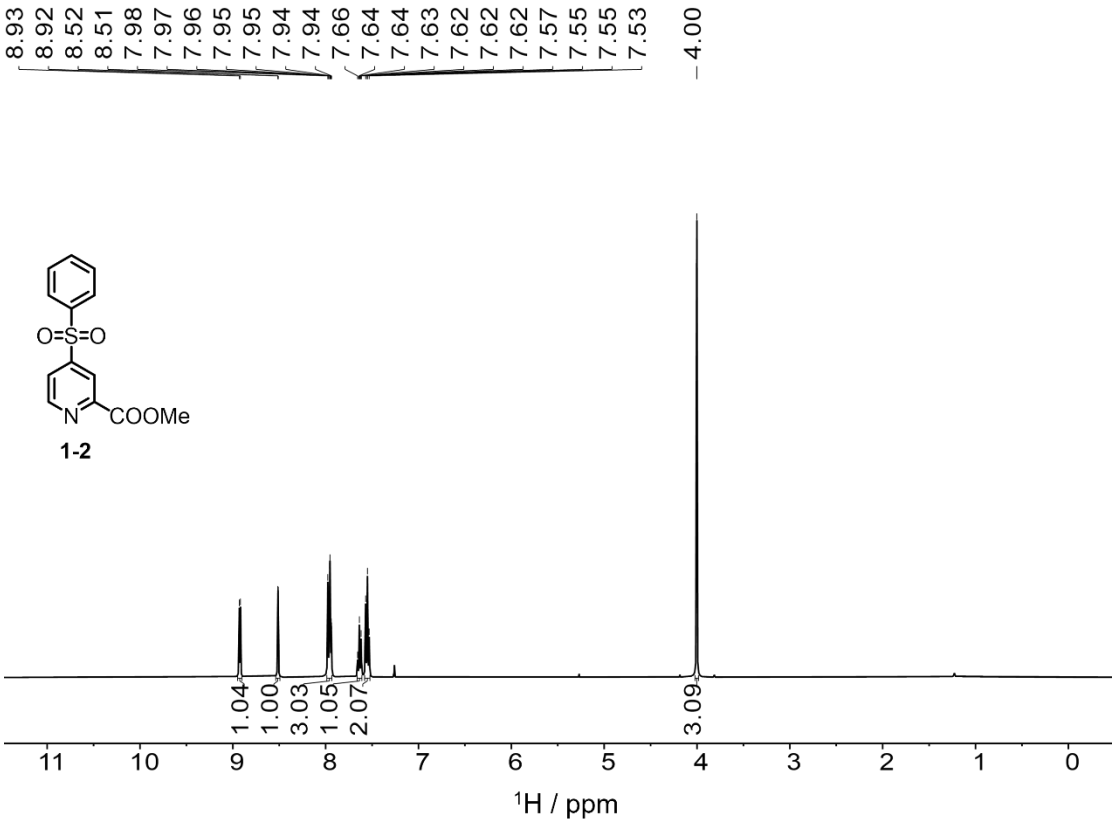
**$^{1}$H NMR (400 MHz, $CDCl_3$) of T6**



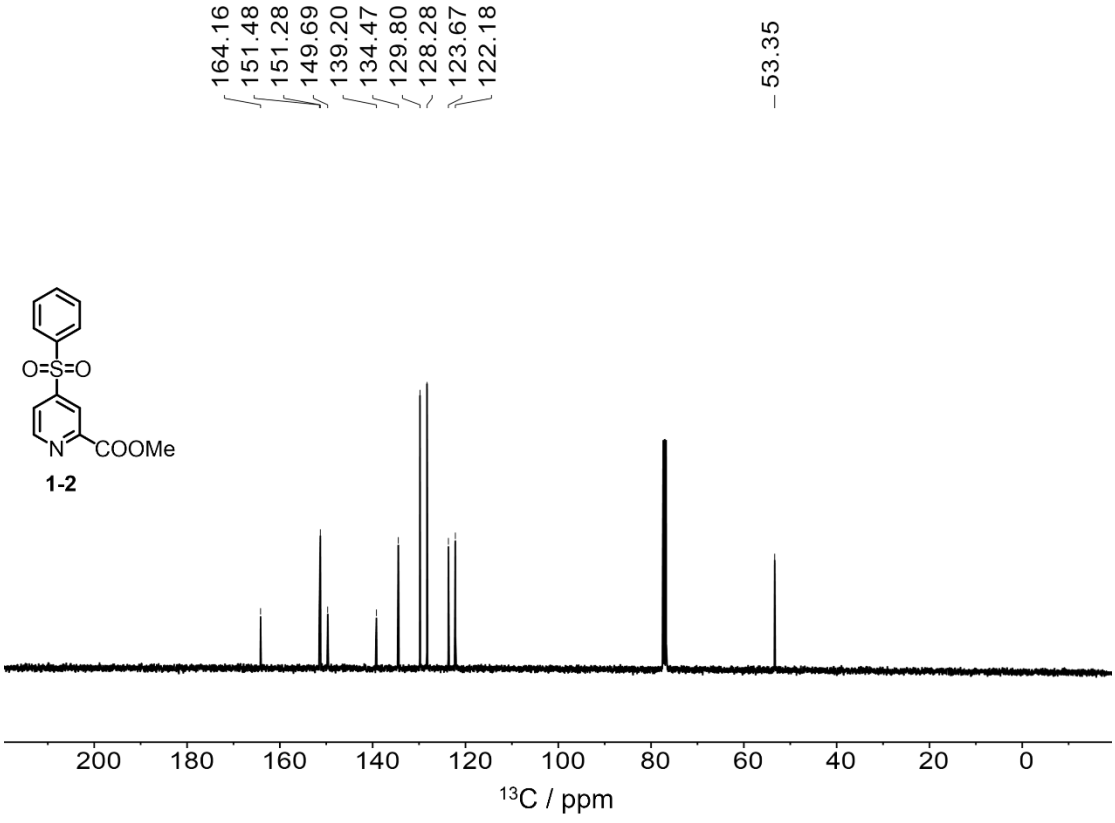

**$^{13}$C NMR (101 MHz, $CDCl_3$) of T6**



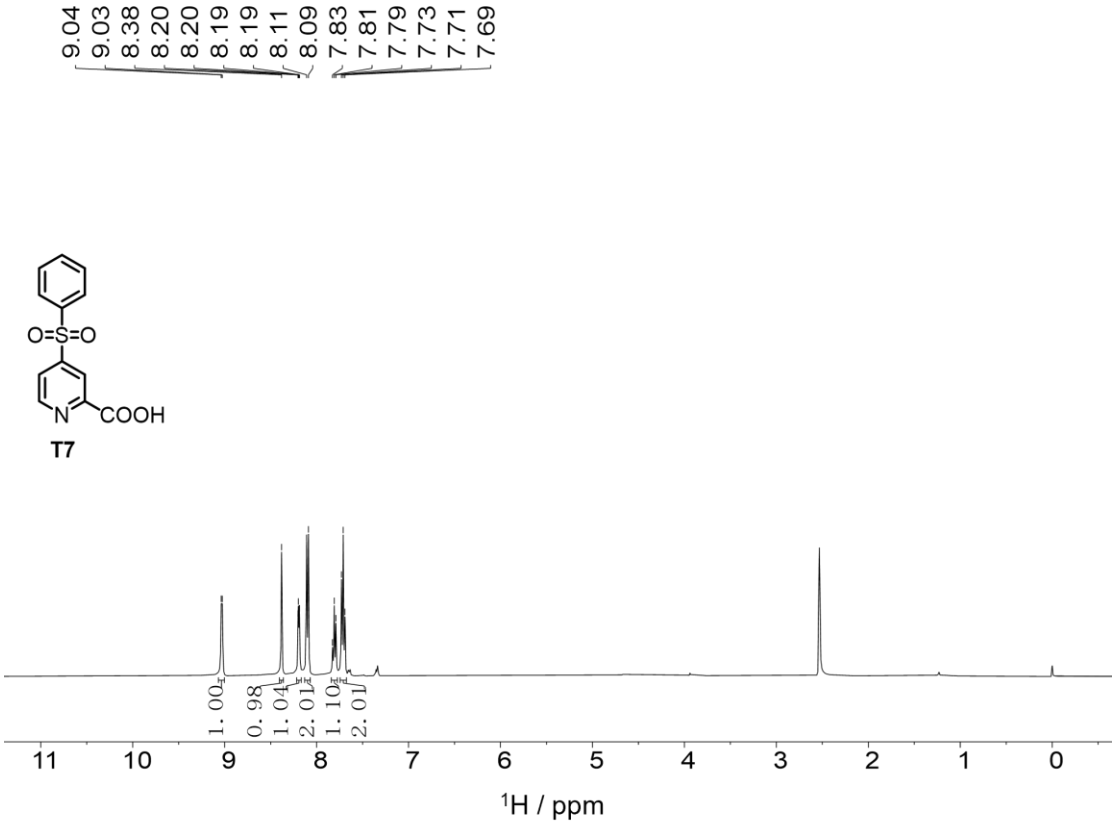
**$^{1}$H NMR (400 MHz, $CDCl_3$) of 1-2**



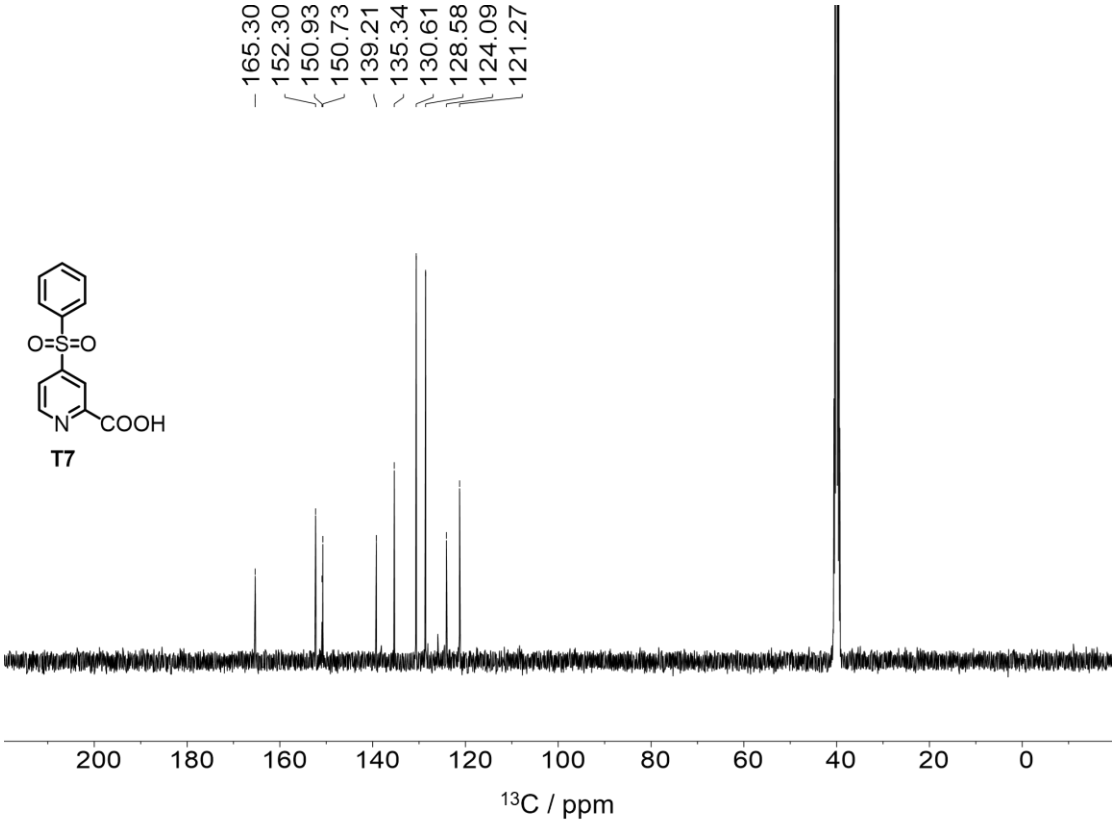

**$^{13}$C NMR (101 MHz, $CDCl_3$) of 1-2**



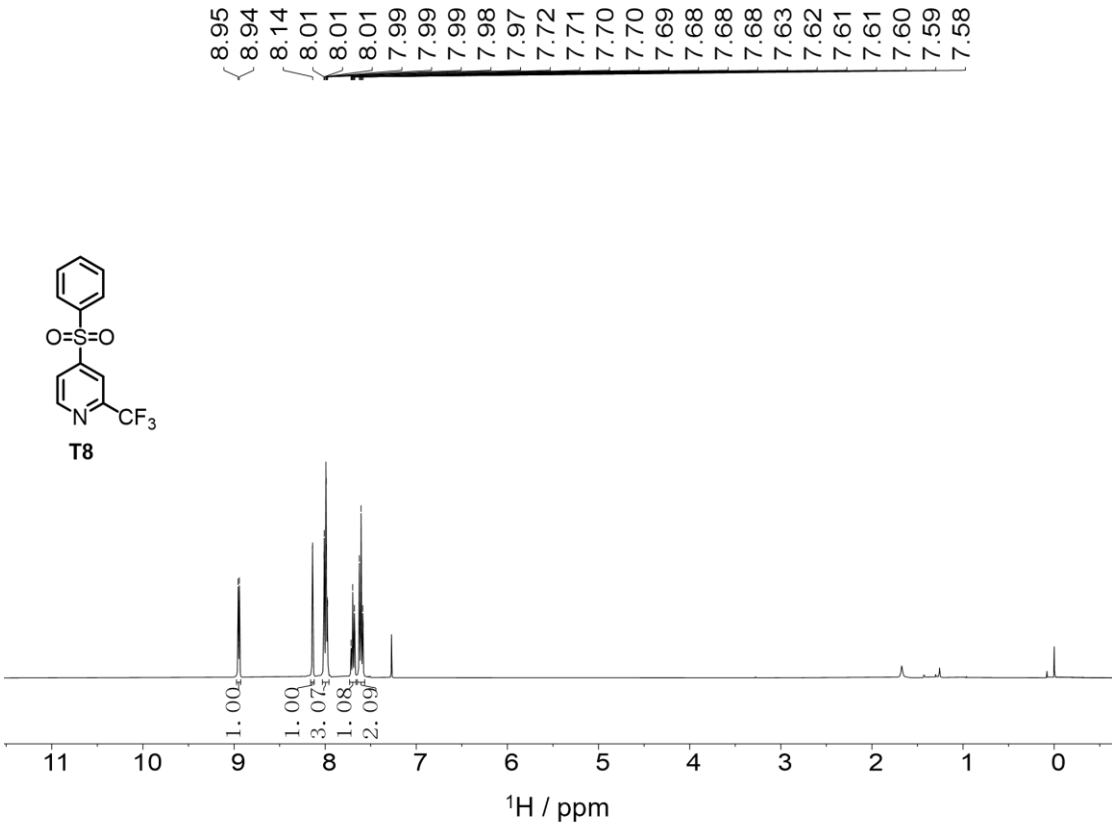
**$^{1}$H NMR (400 MHz, DMSO-$d_6$) of T7**



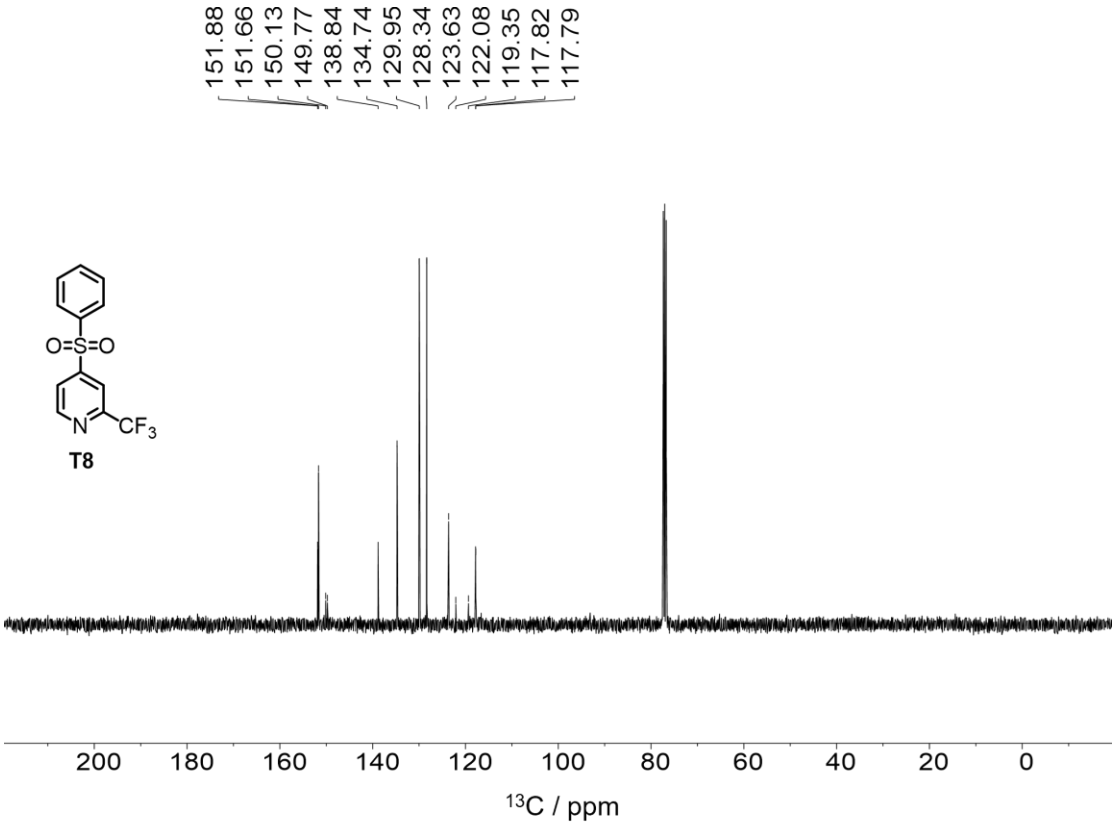

**$^{13}$C NMR (101 MHz, DMSO-$d_6$) of T7**



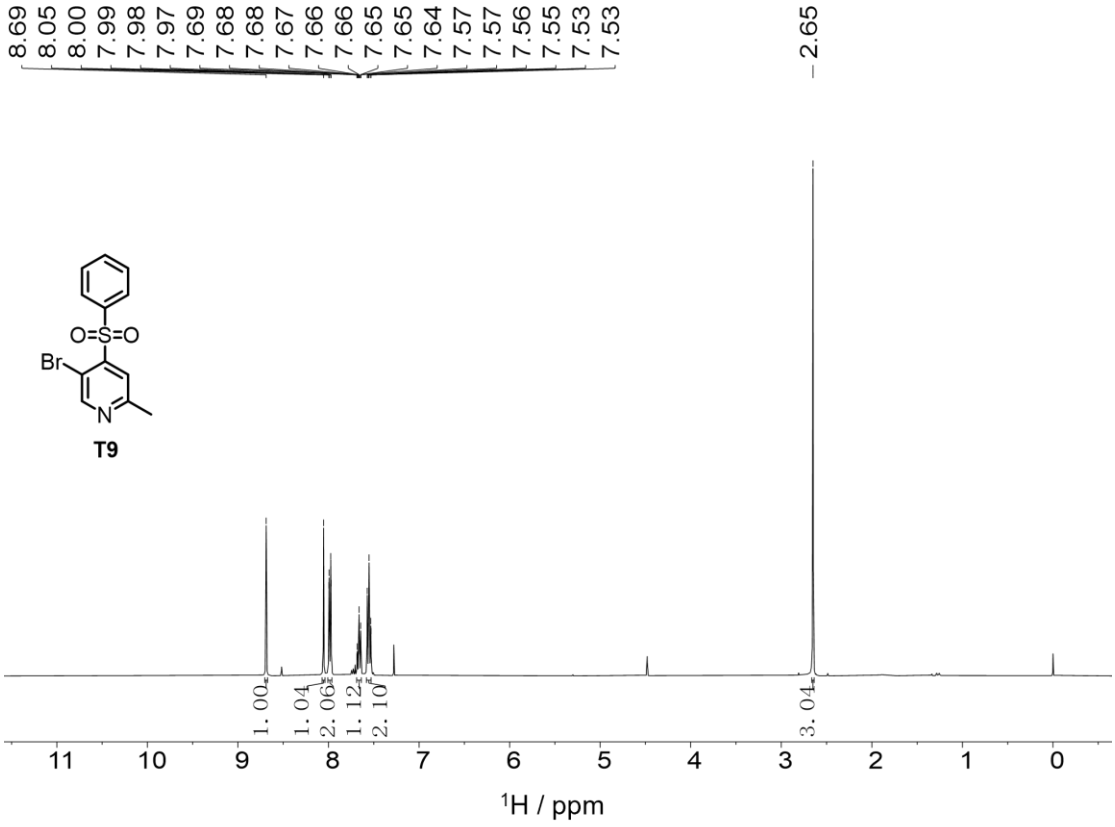
**$^{1}$H NMR (400 MHz, $CDCl_3$) of T8**



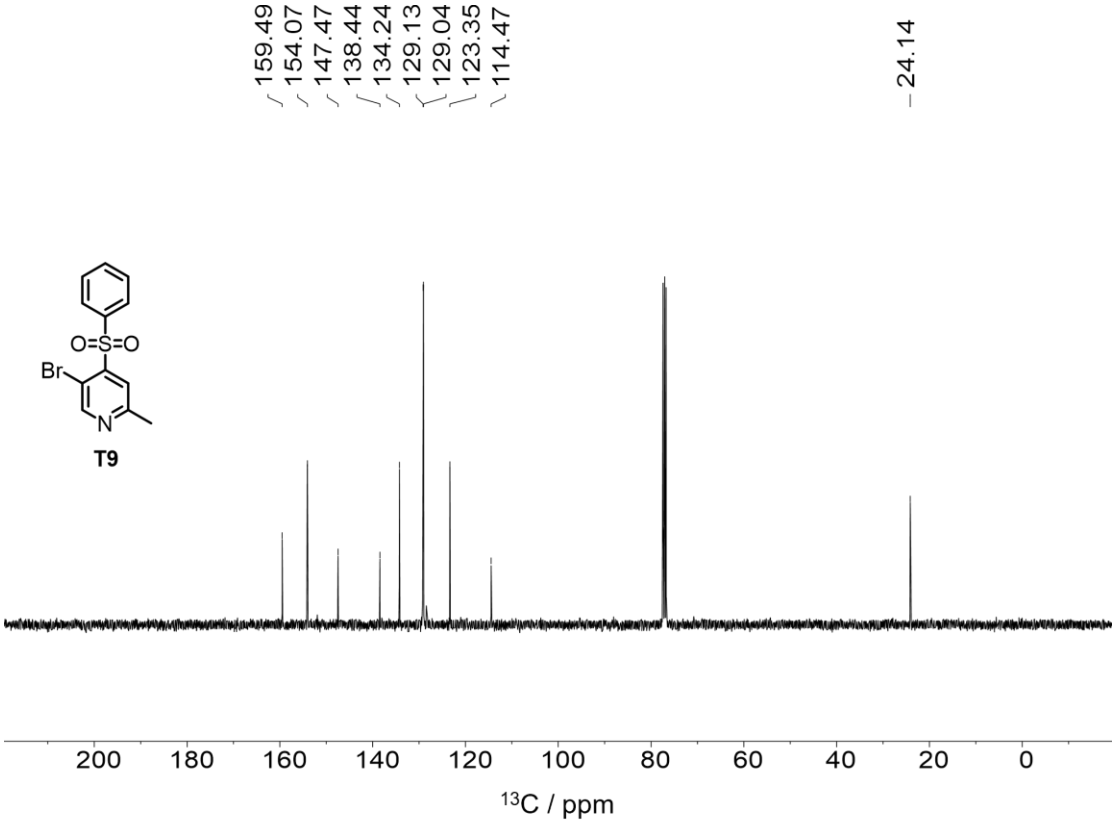

**$^{13}$C NMR (101 MHz, $CDCl_3$) of T8**



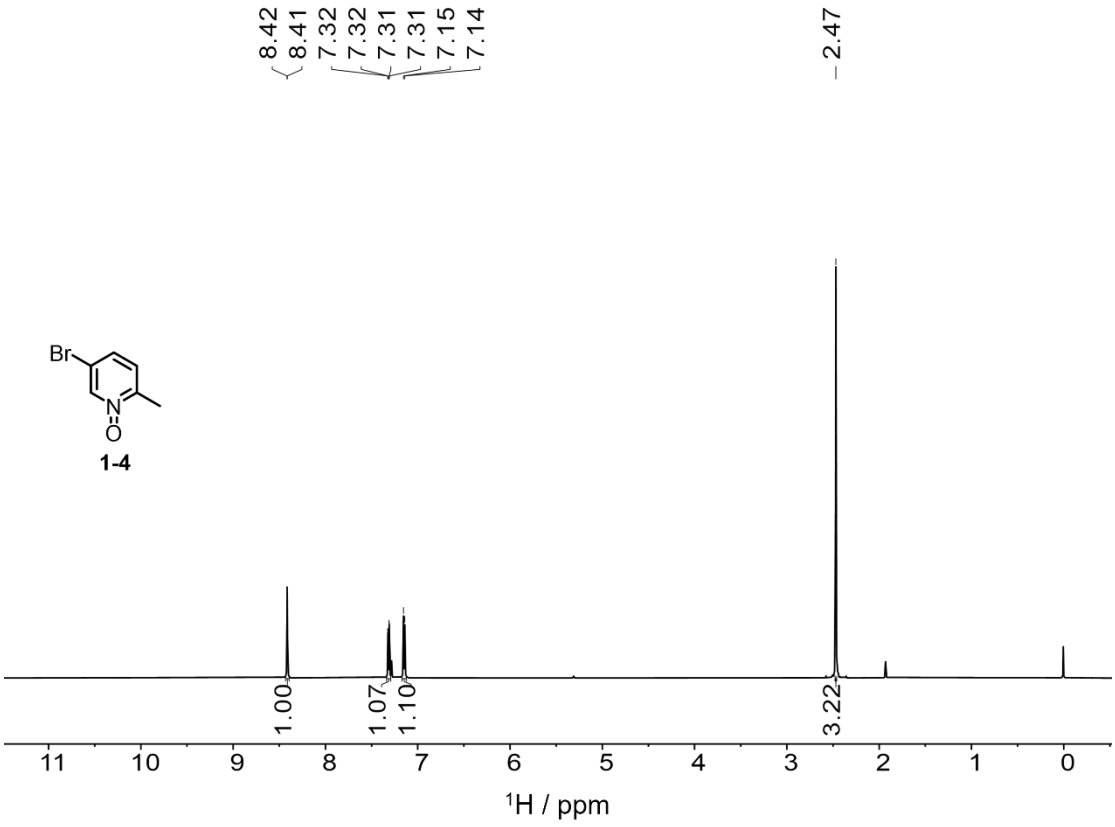
**$^{1}$H NMR (400 MHz, $CDCl_3$) of T9**



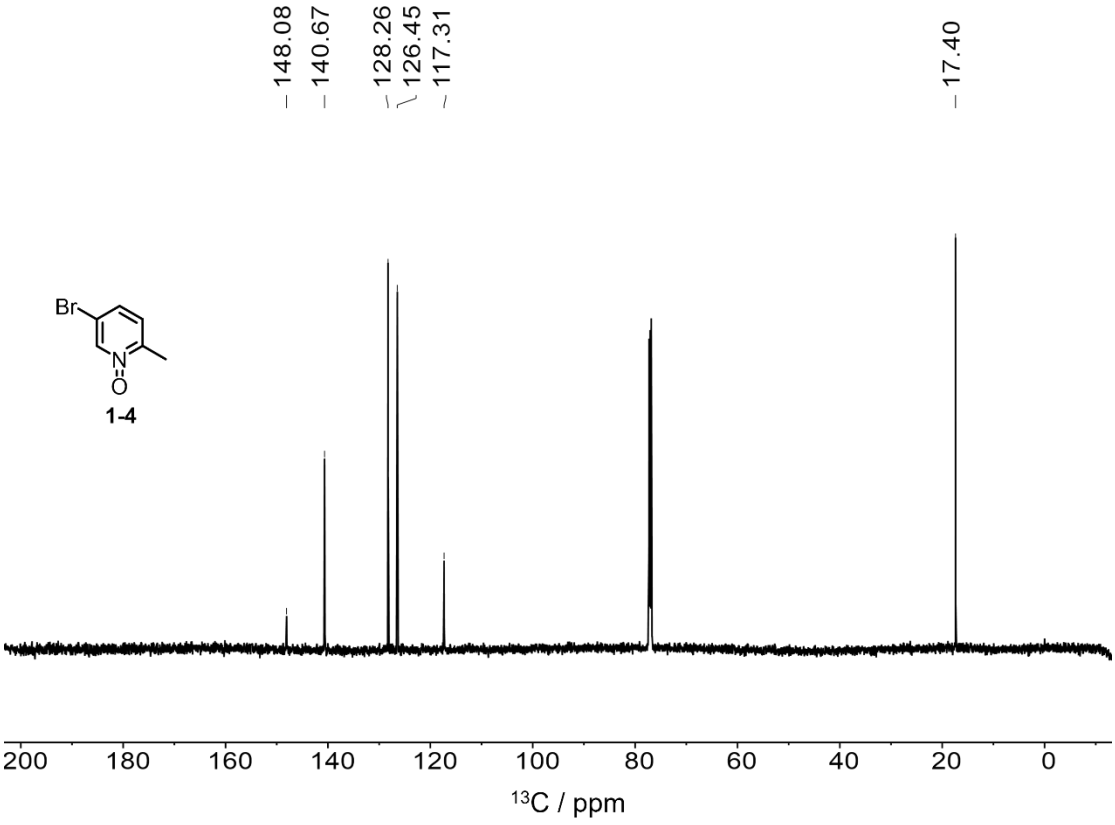

**$^{13}$C NMR (101 MHz, $CDCl_3$) of T9**



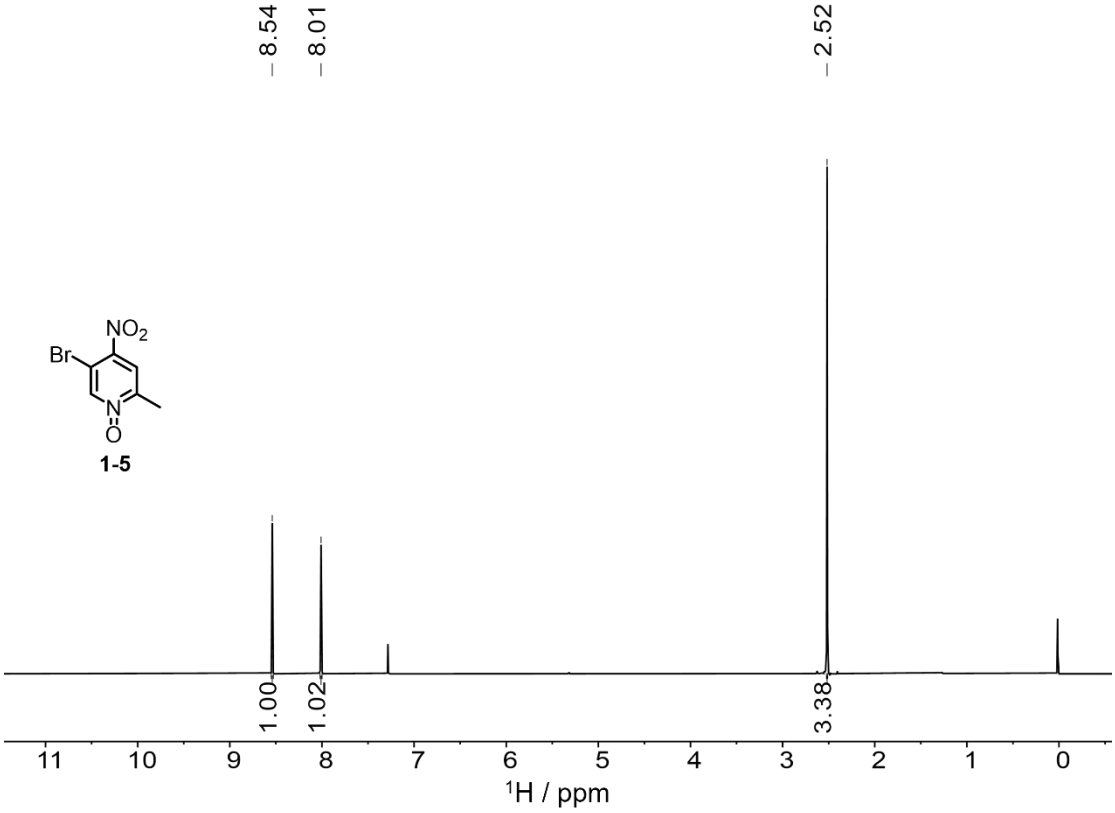
**$^{1}$H NMR (400 MHz, $CDCl_3$) of 1-4**



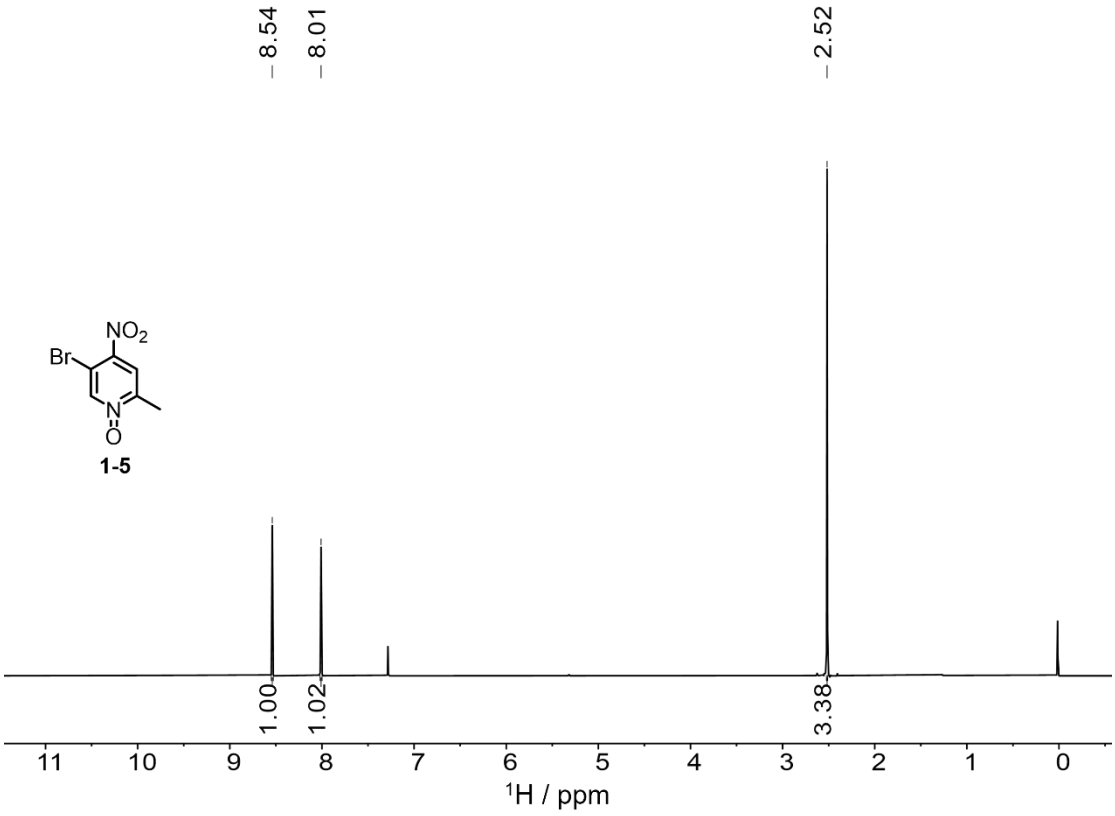

**$^{13}$C NMR (101 MHz, $CDCl_3$) of 1-4**



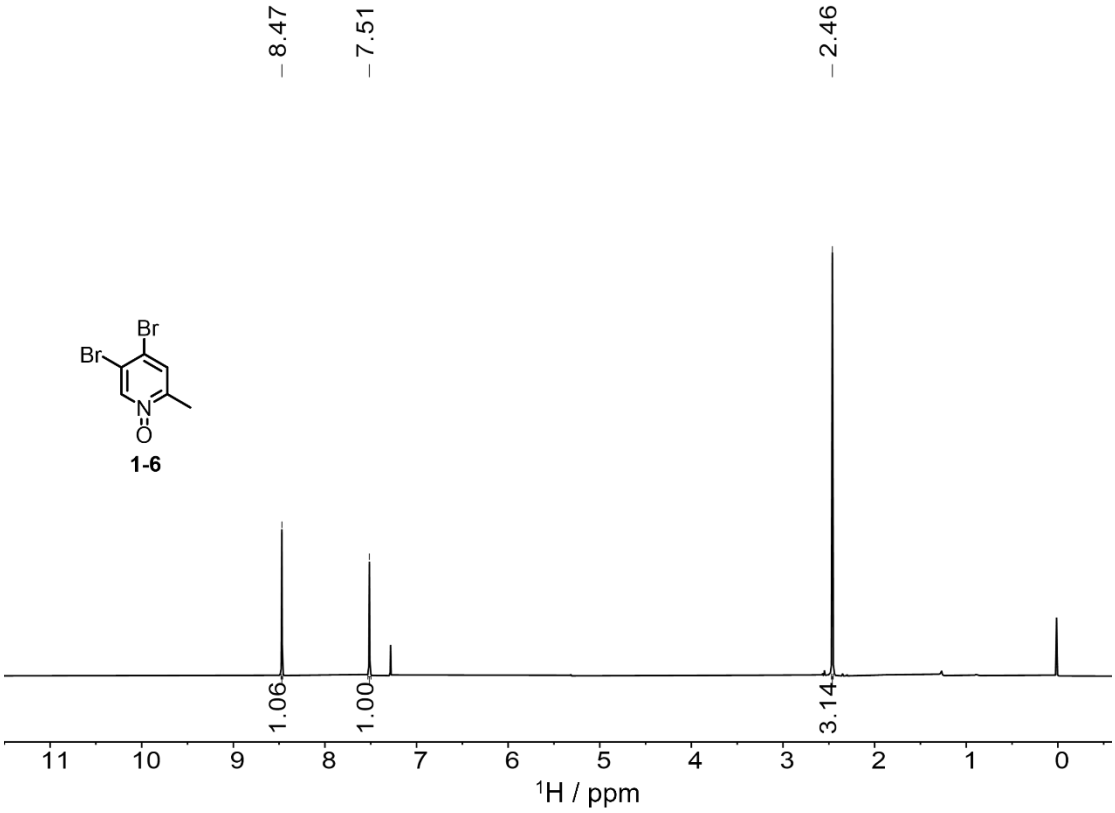
**$^{1}$H NMR (400 MHz, $CDCl_3$) of 1-5**



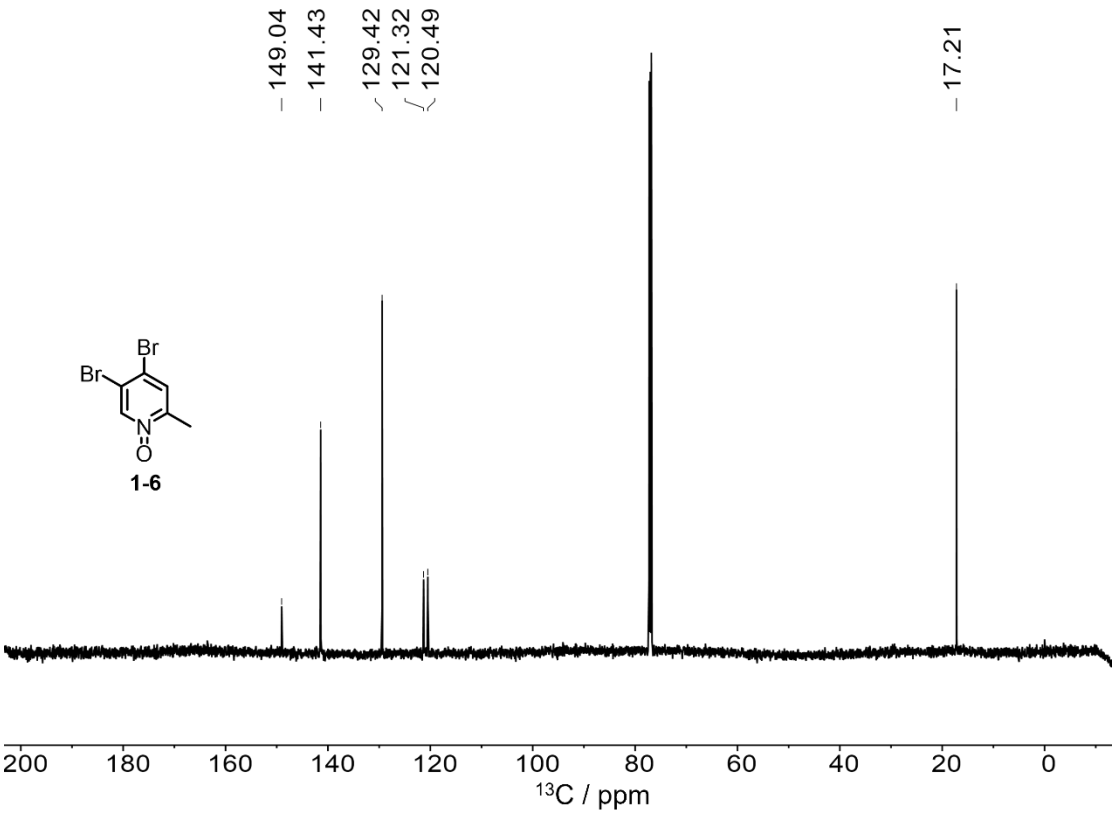

**$^{13}$C NMR (101 MHz, $CDCl_3$) of 1-5**



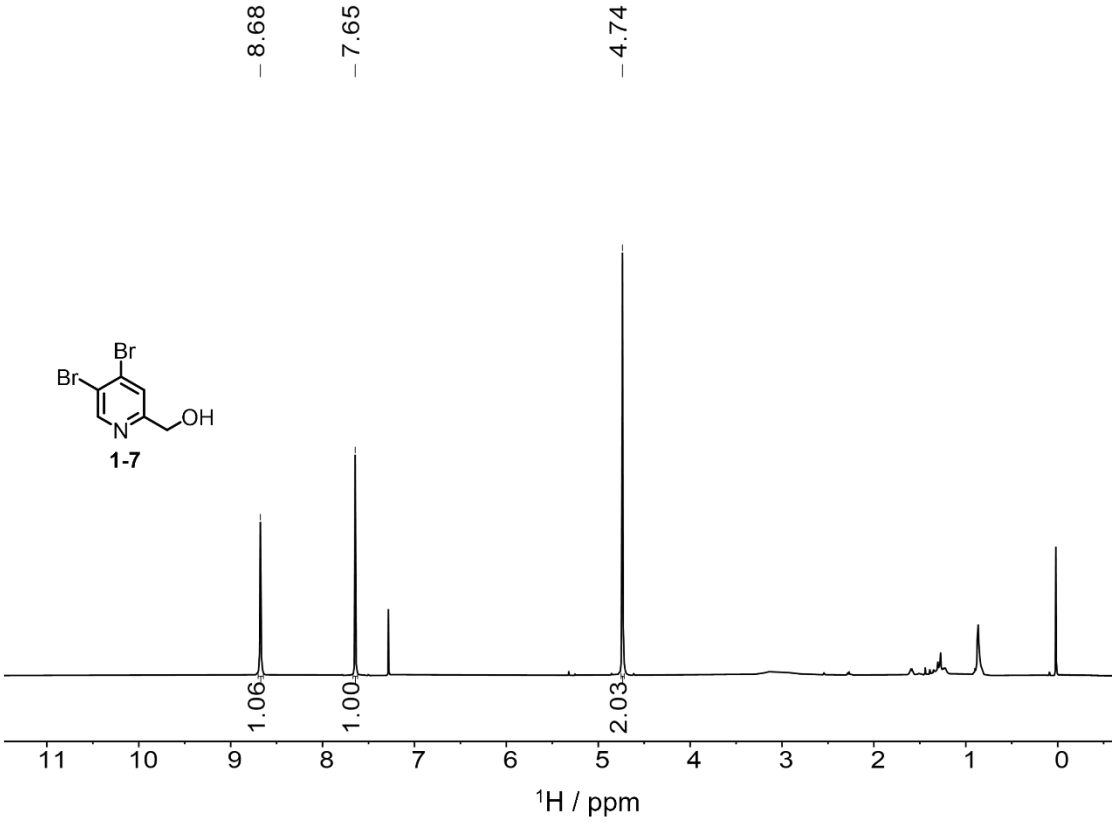
**$^{1}$H NMR (400 MHz, $CDCl_3$) of 1-6**



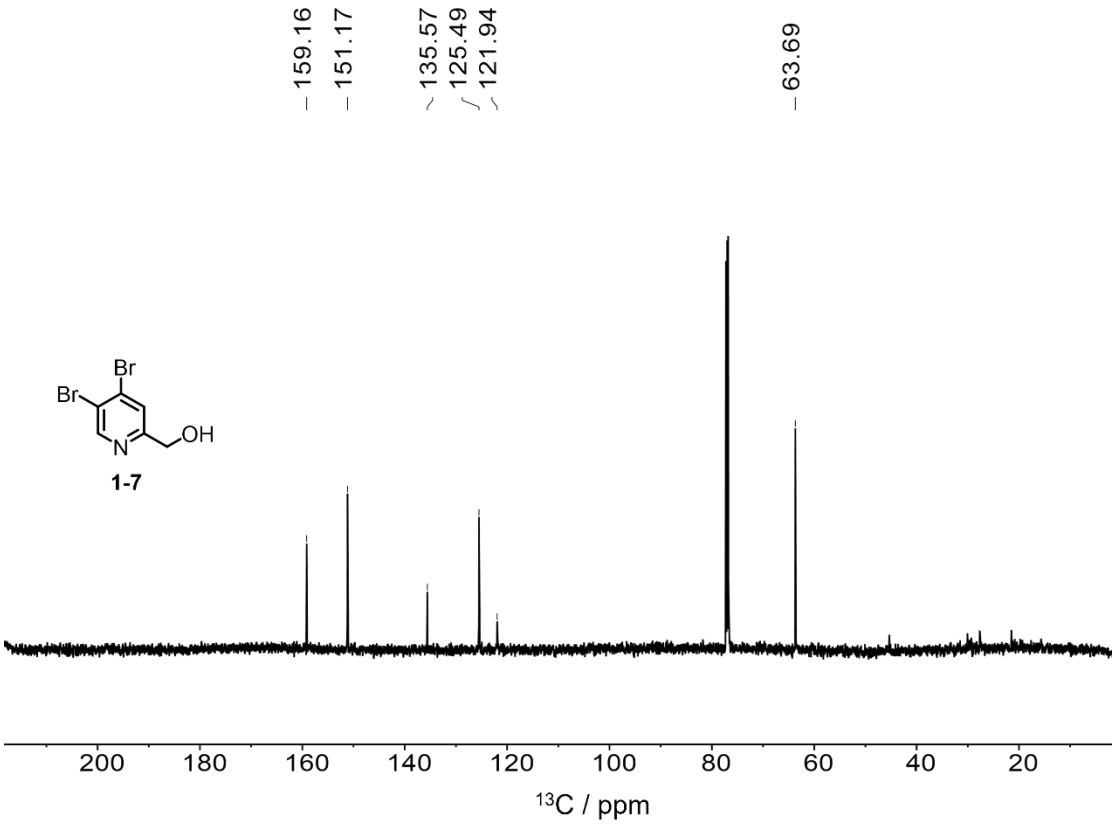

**$^{13}$C NMR (101 MHz, $CDCl_3$) of 1-6**



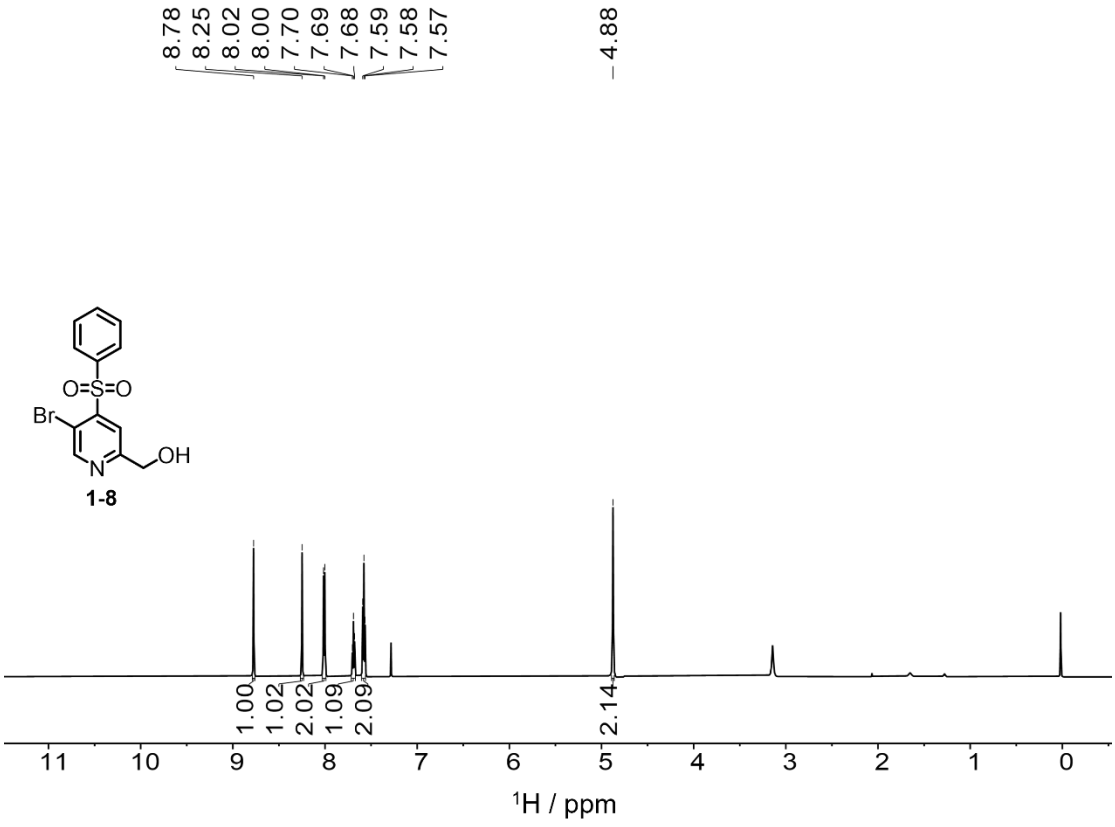
**$^{1}$H NMR (400 MHz, $CDCl_3$) of 1-7**



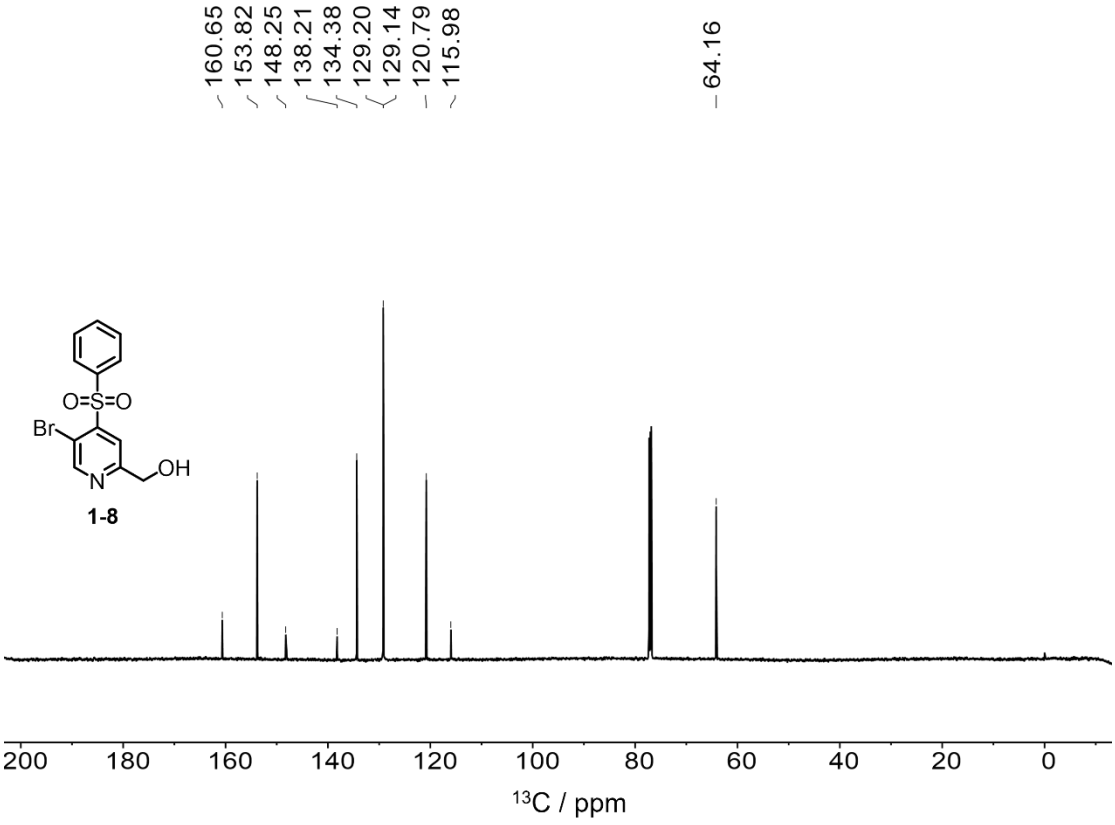

**$^{13}$C NMR (101 MHz, $CDCl_3$) of 1-7**



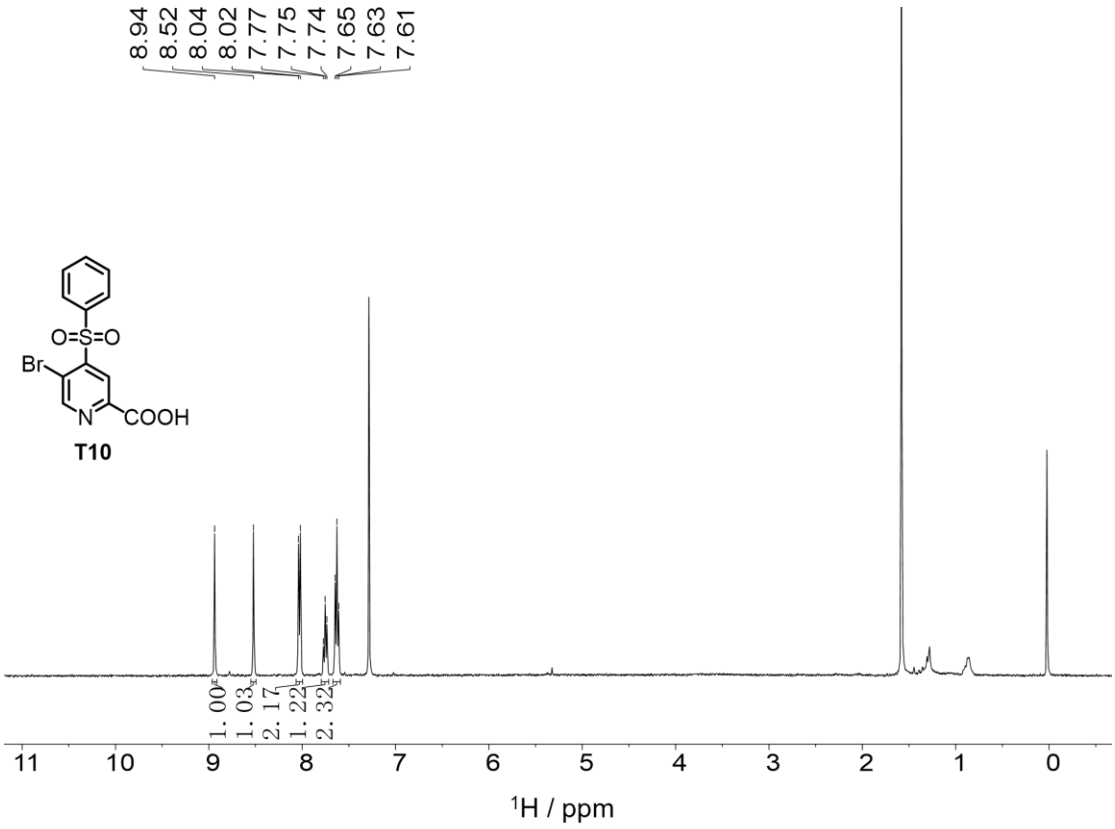
**$^{1}$H NMR (400 MHz, $CDCl_3$) of 1-8**



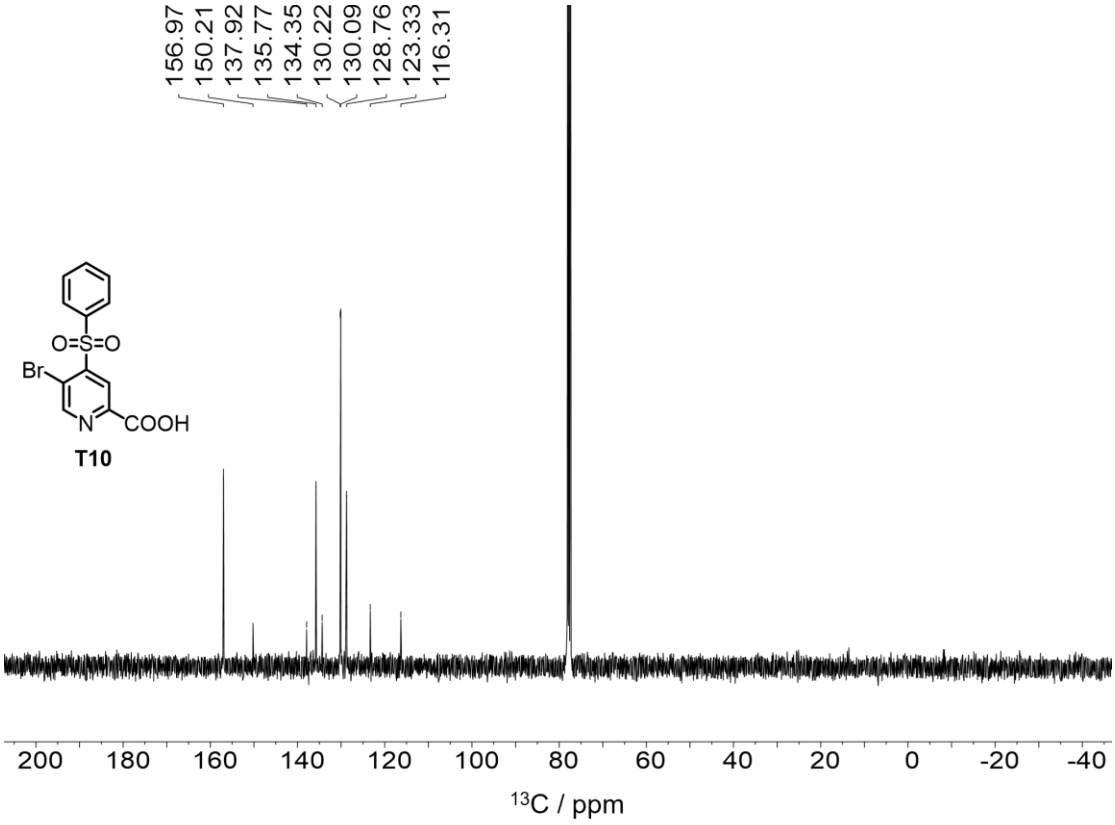

**$^{13}$C NMR (101 MHz, $CDCl_3$) of 1-8**



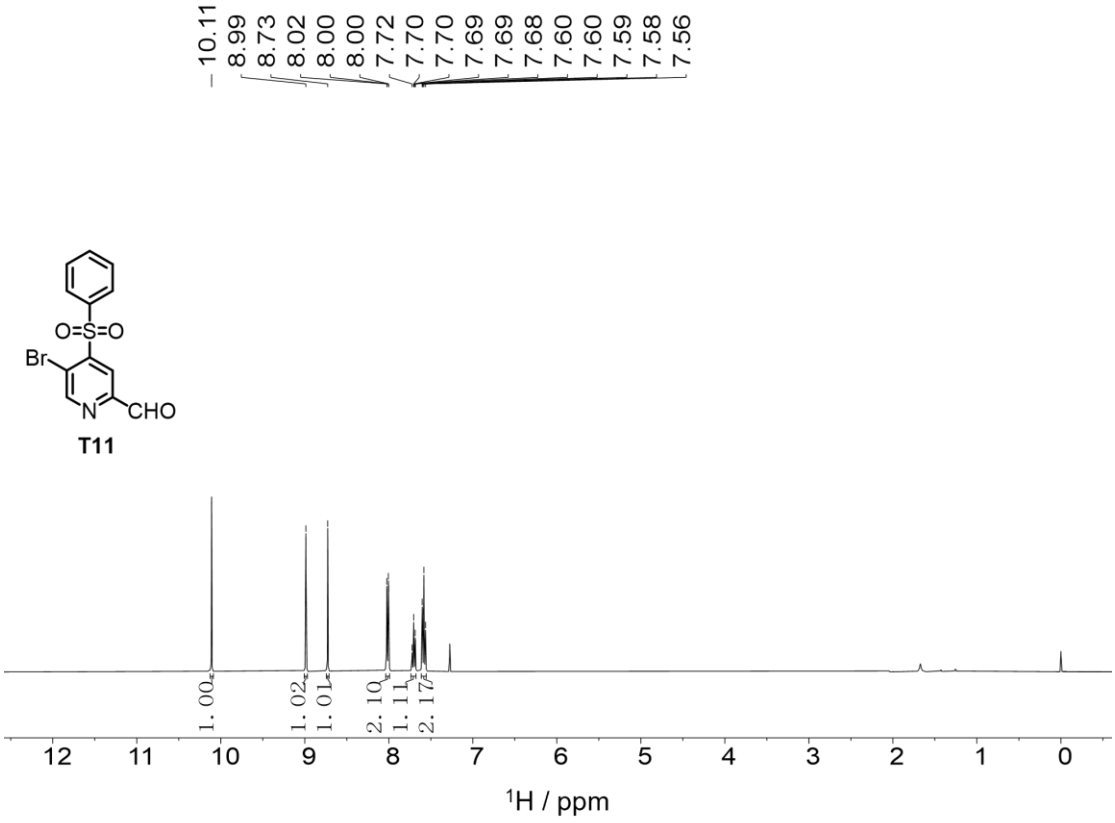
**$^{1}$H NMR (400 MHz, $CDCl_3$) of T10**



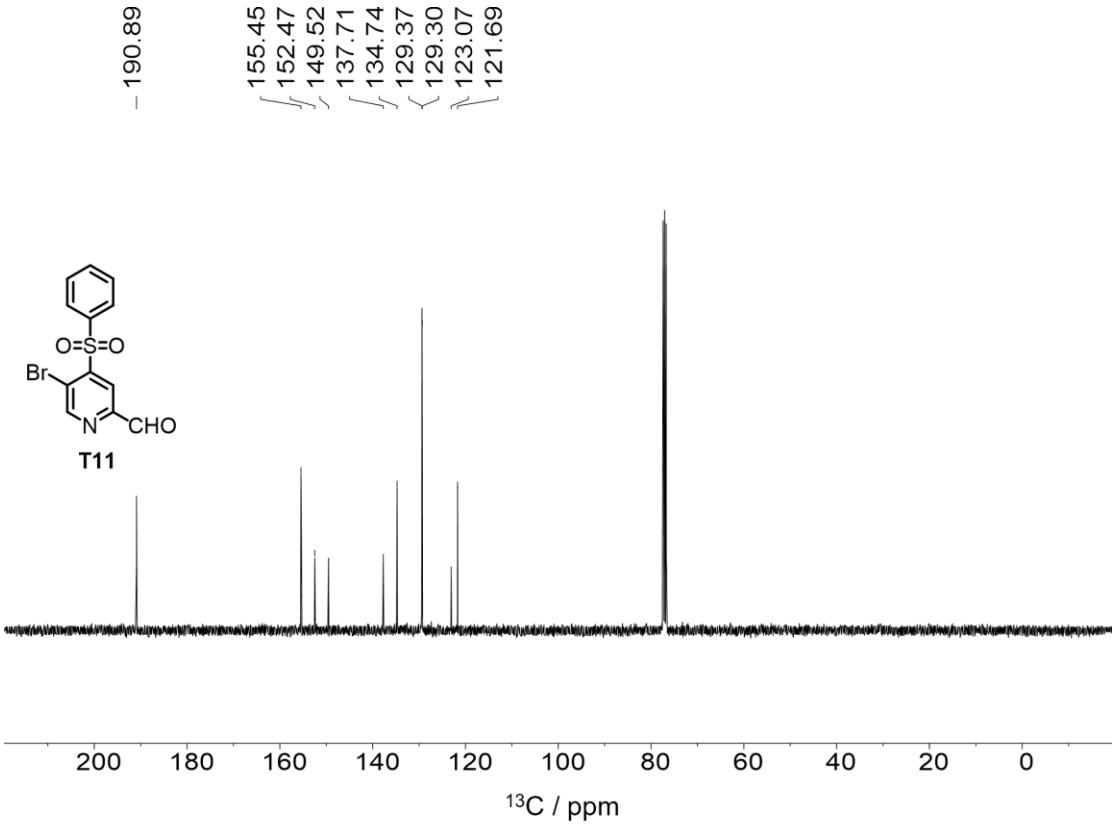

**$^{13}$C NMR (101 MHz, $CDCl_3$) of T10**



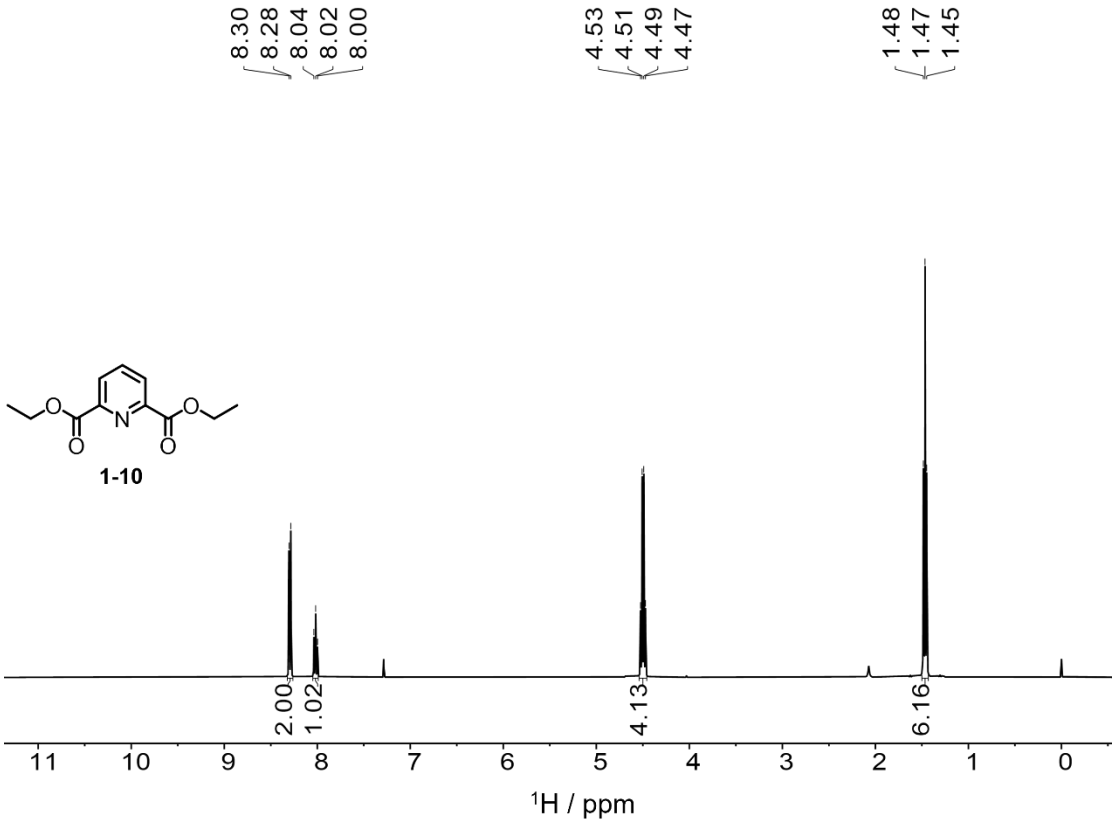
**$^{1}$H NMR (400 MHz, $CDCl_3$) of T11**



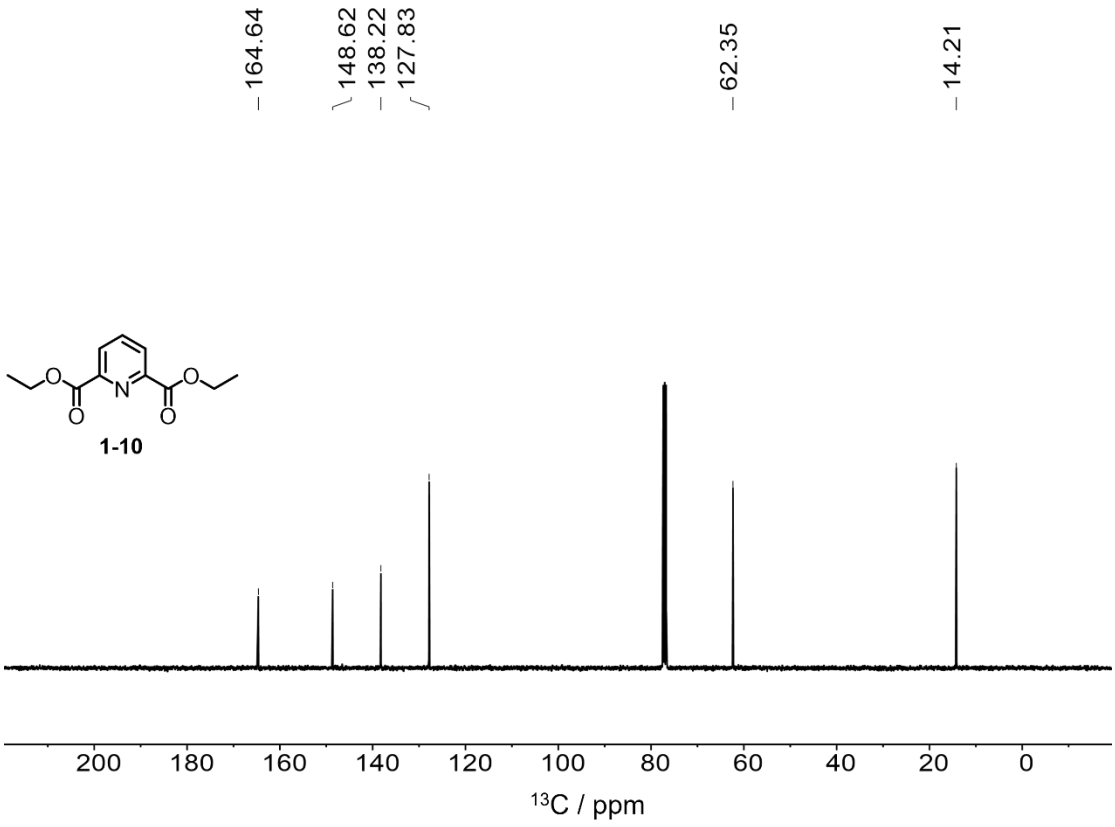

**$^{13}$C NMR (101 MHz, $CDCl_3$) of T11**



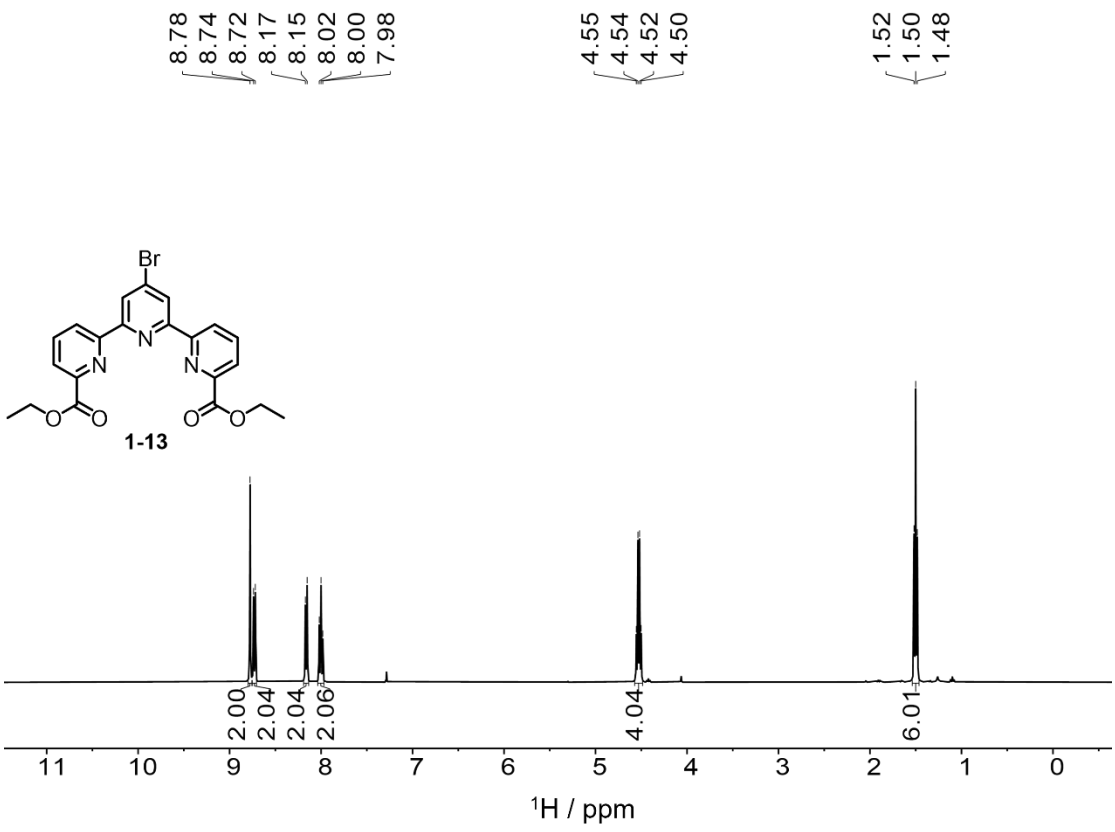
**$^{1}$H NMR (400 MHz, $CDCl_3$) of 1-10**



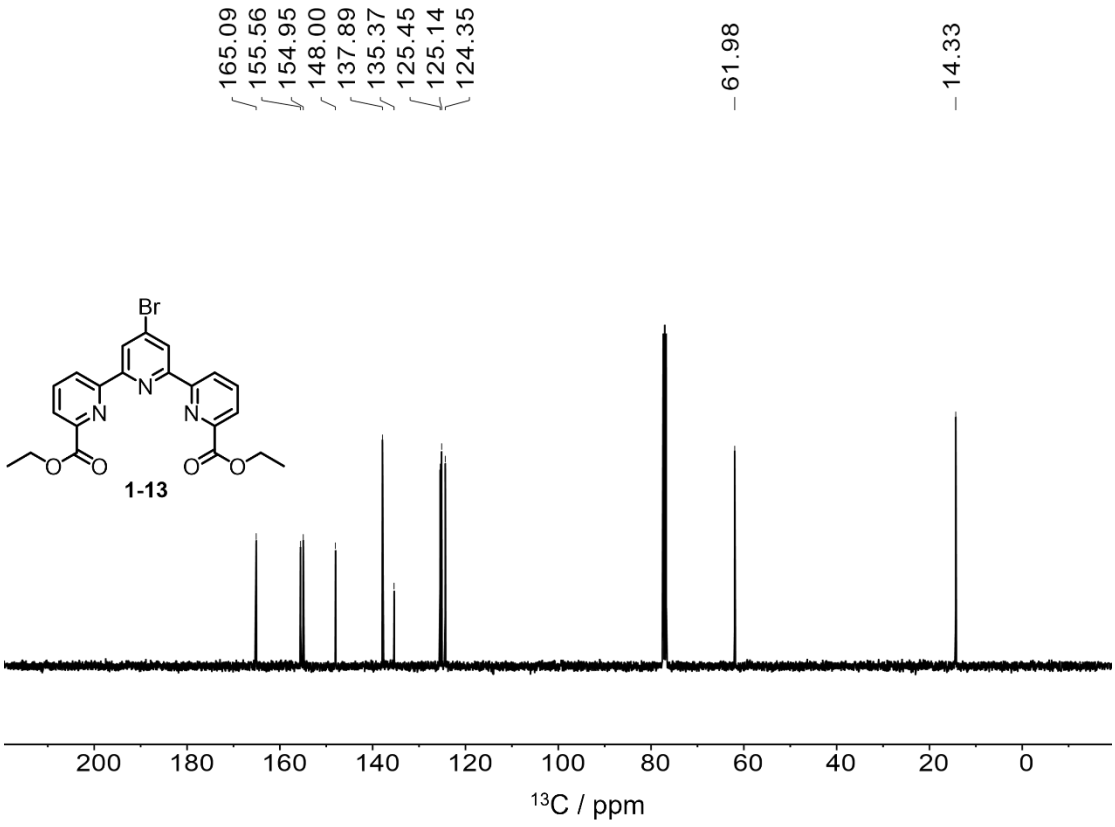

**$^{13}$C NMR (101 MHz, $CDCl_3$) of 1-10**



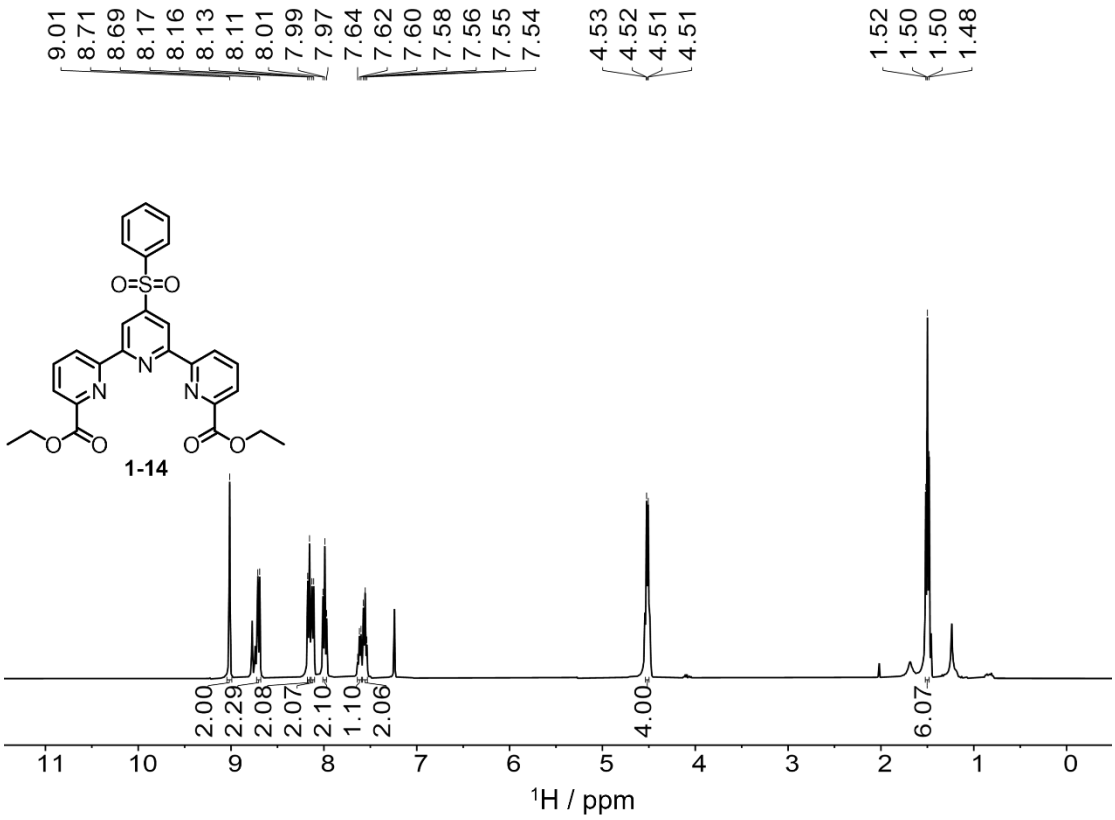
**$^{1}$H NMR (400 MHz, $CDCl_3$) of 1-13**



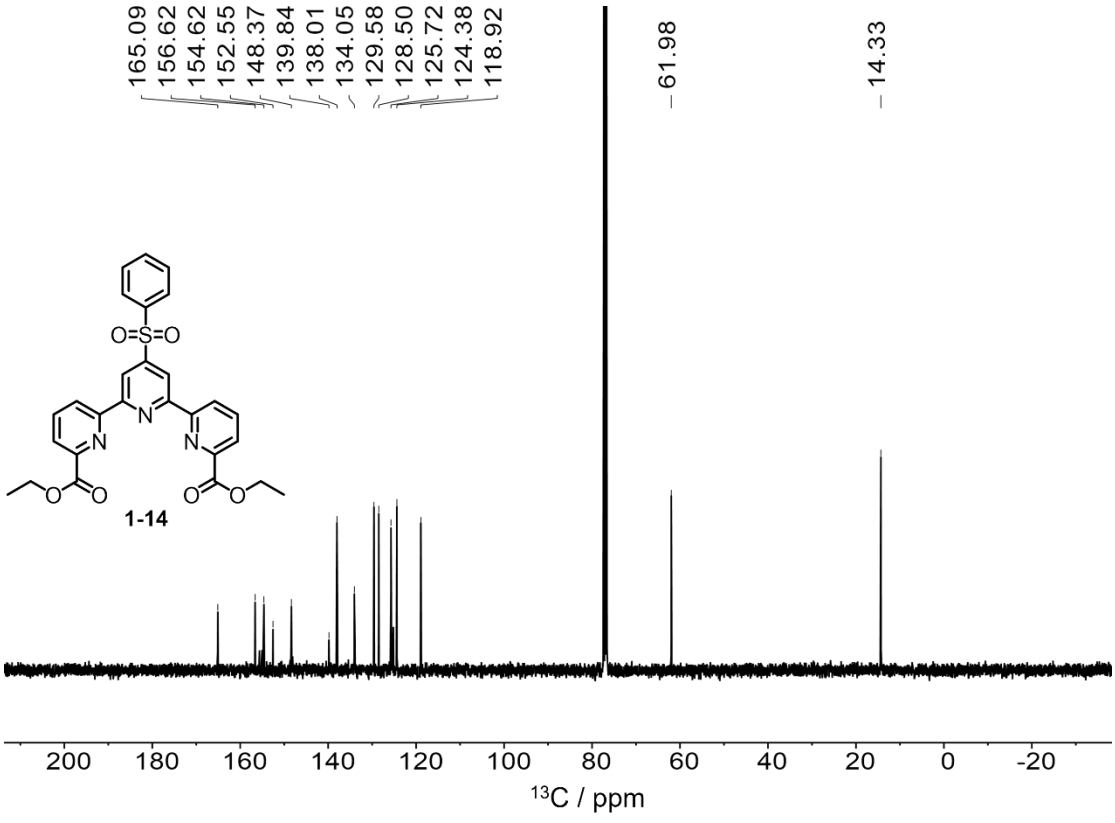

**$^{13}$C NMR (101 MHz, $CDCl_3$) of 1-13**



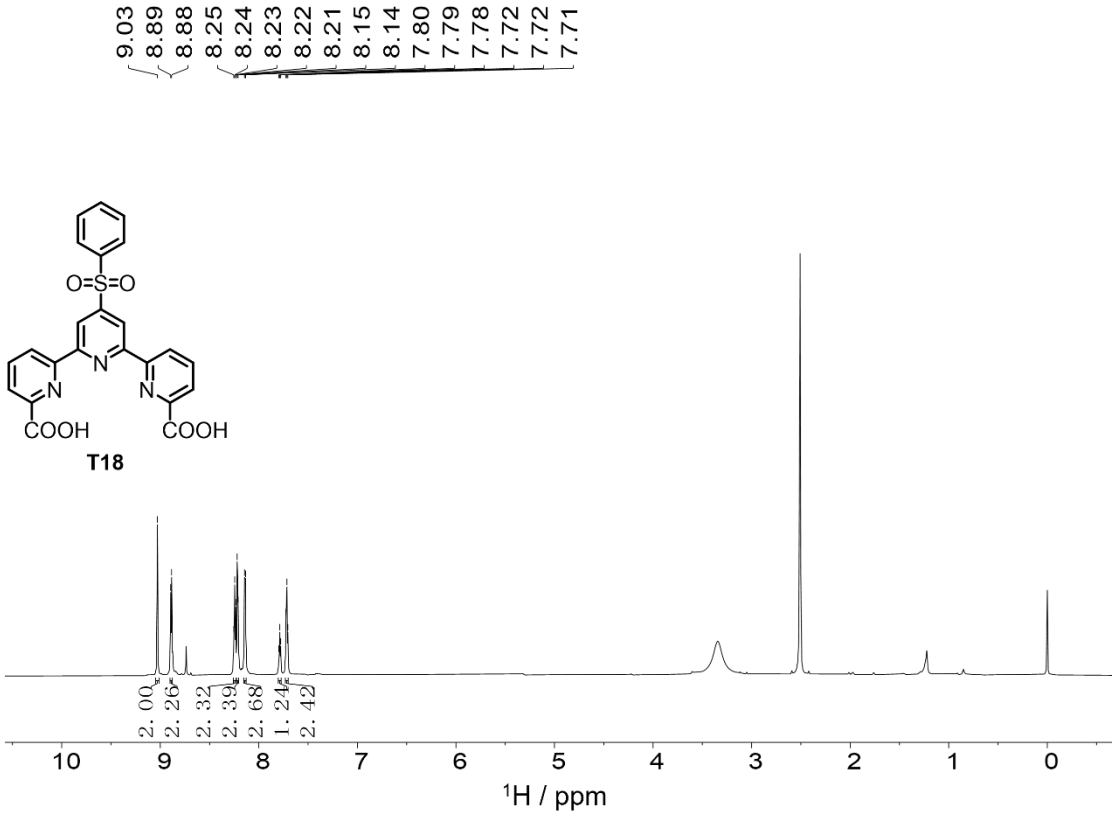
**$^{1}$H NMR (400 MHz, $CDCl_3$) of 1-14**



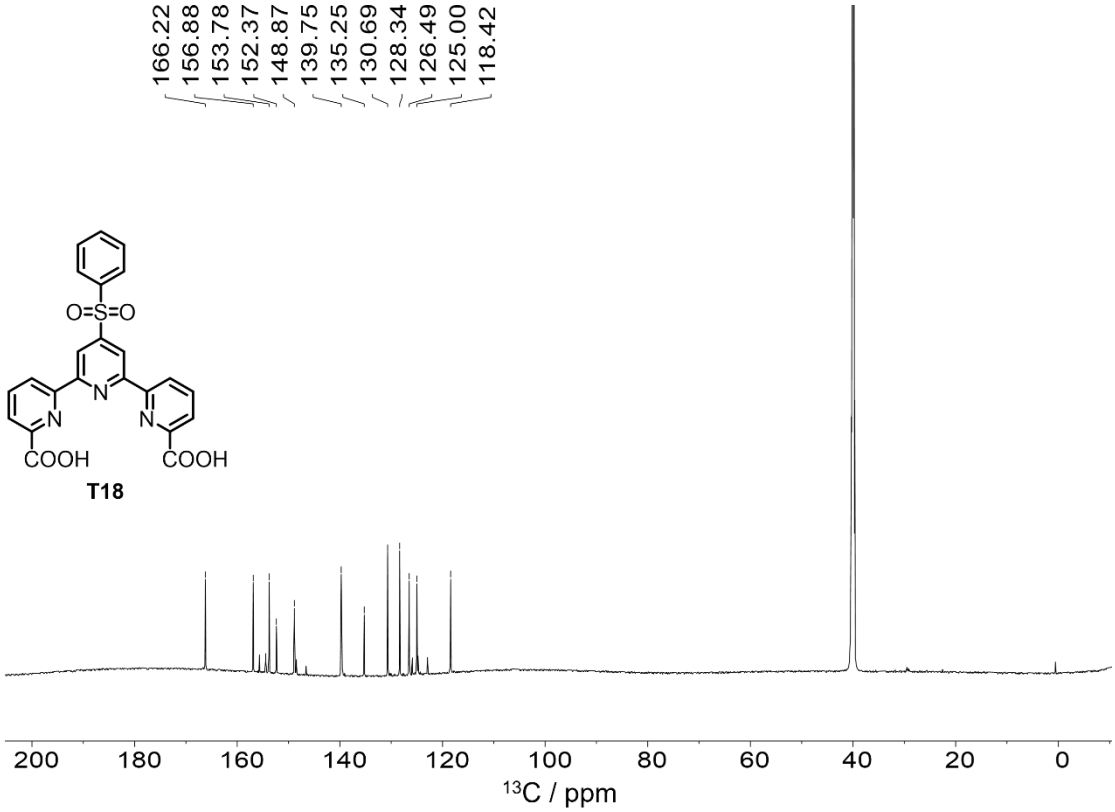

**$^{13}$C NMR (101 MHz, $CDCl_3$) of 1-14**



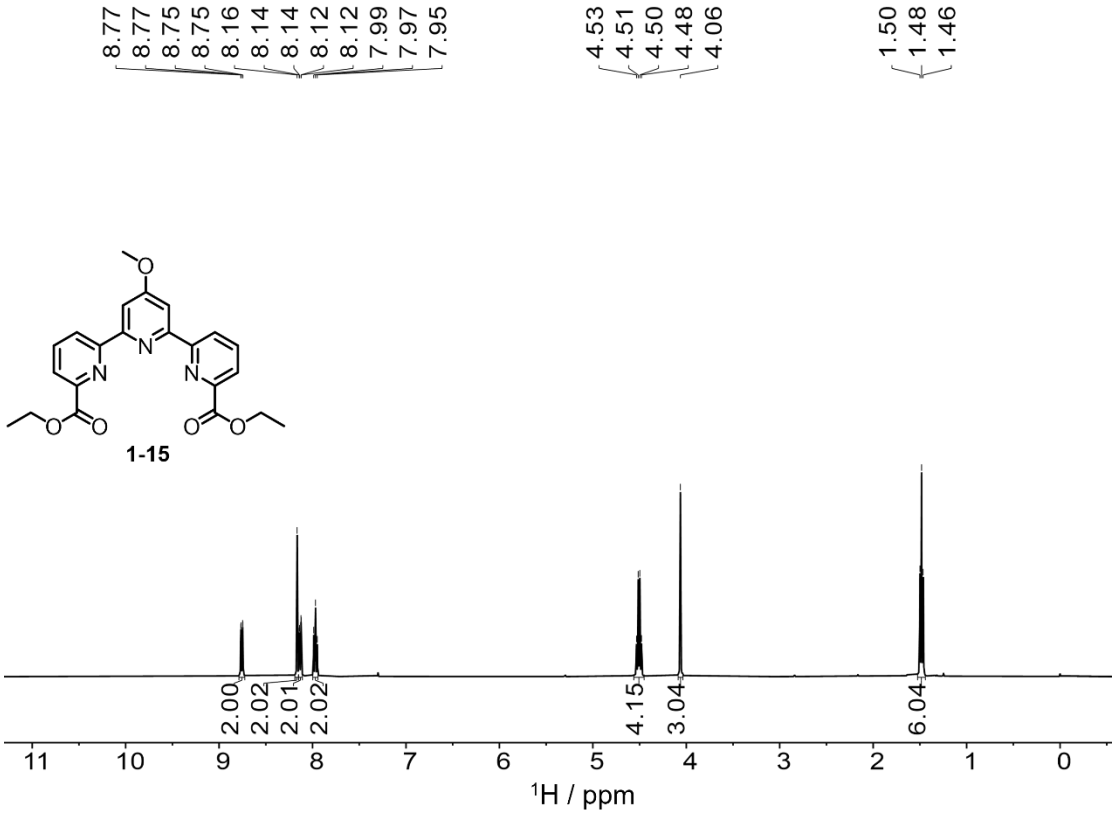
**$^{1}$H NMR (800 MHz, DMSO-$d_6$) of T18**



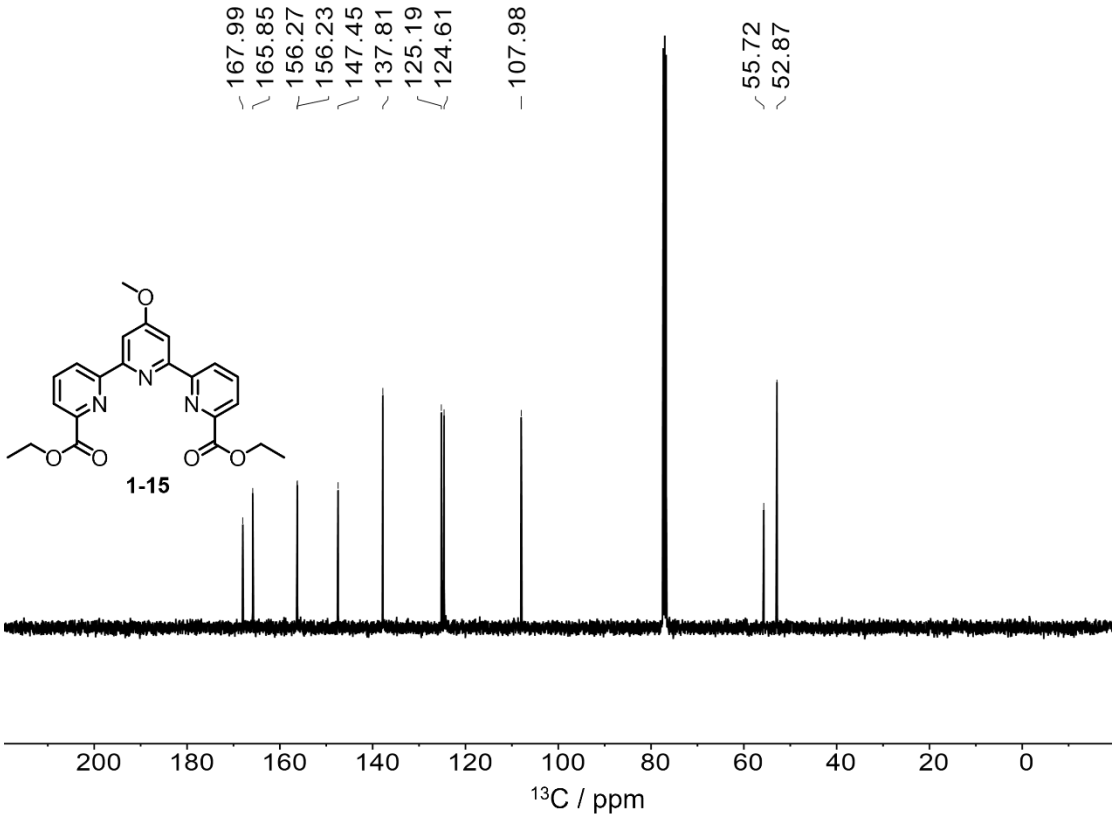

**$^{13}$C NMR (800 MHz, DMSO-$d_6$) of T18**



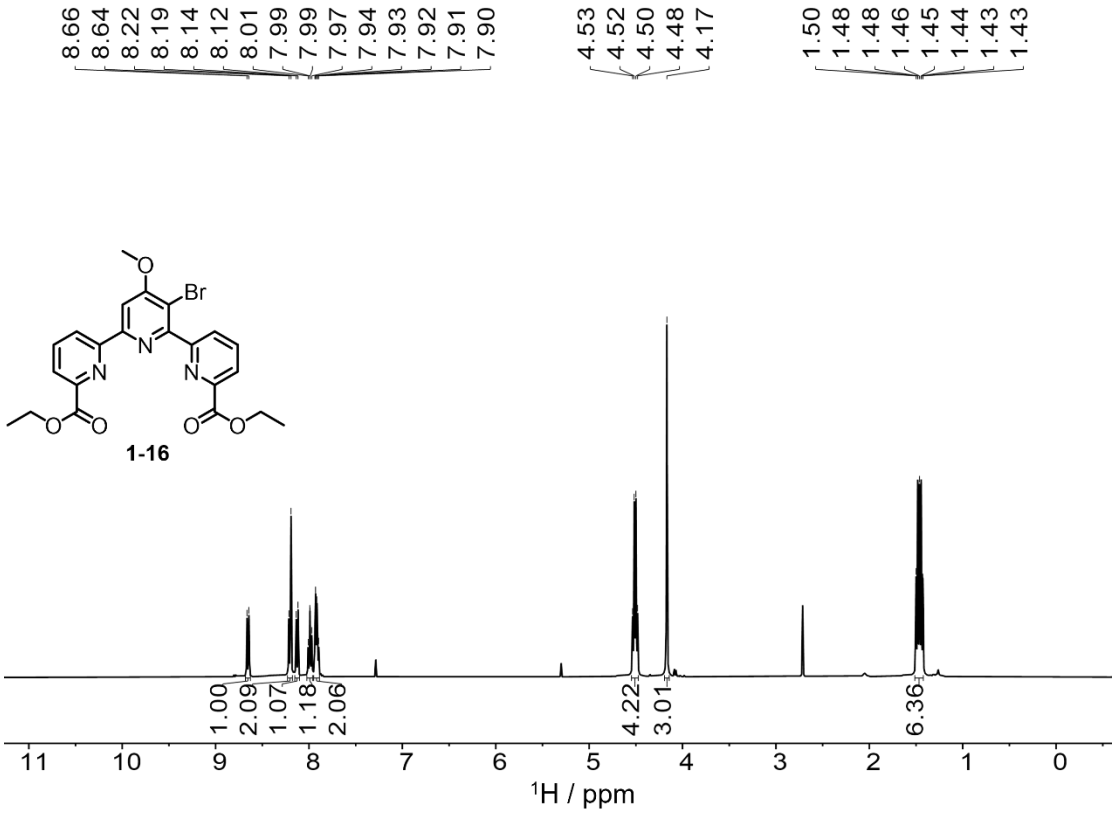
**$^{1}$H NMR (400 MHz, $CDCl_3$) of 1-15**



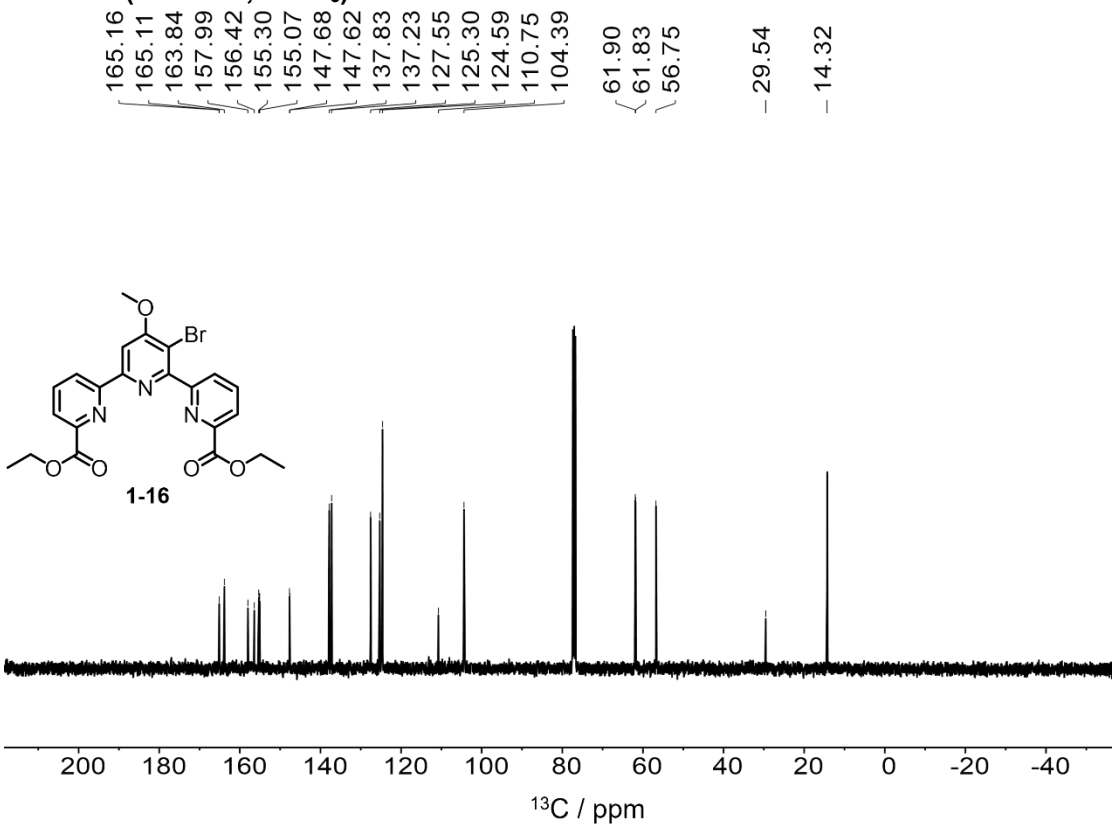

**$^{13}$C NMR (101 MHz, $CDCl_3$) of 1-15**



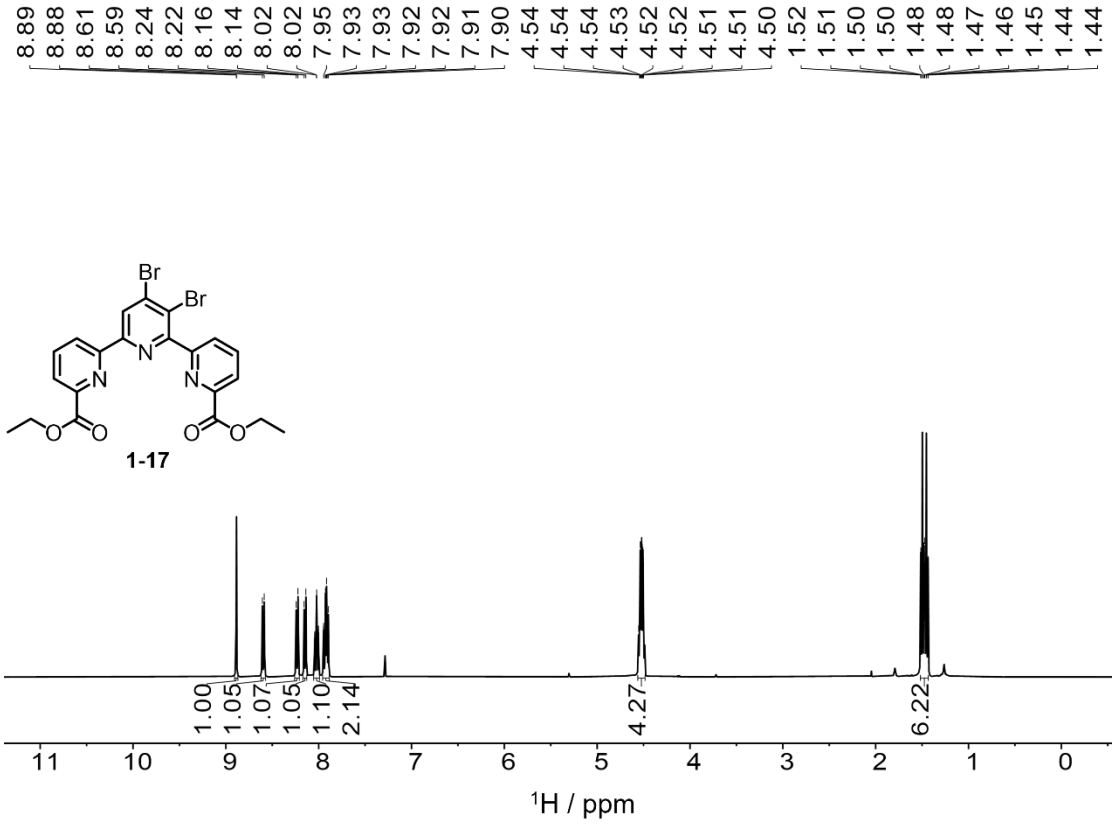
**$^{1}$H NMR (400 MHz, $CDCl_3$) of 1-16**



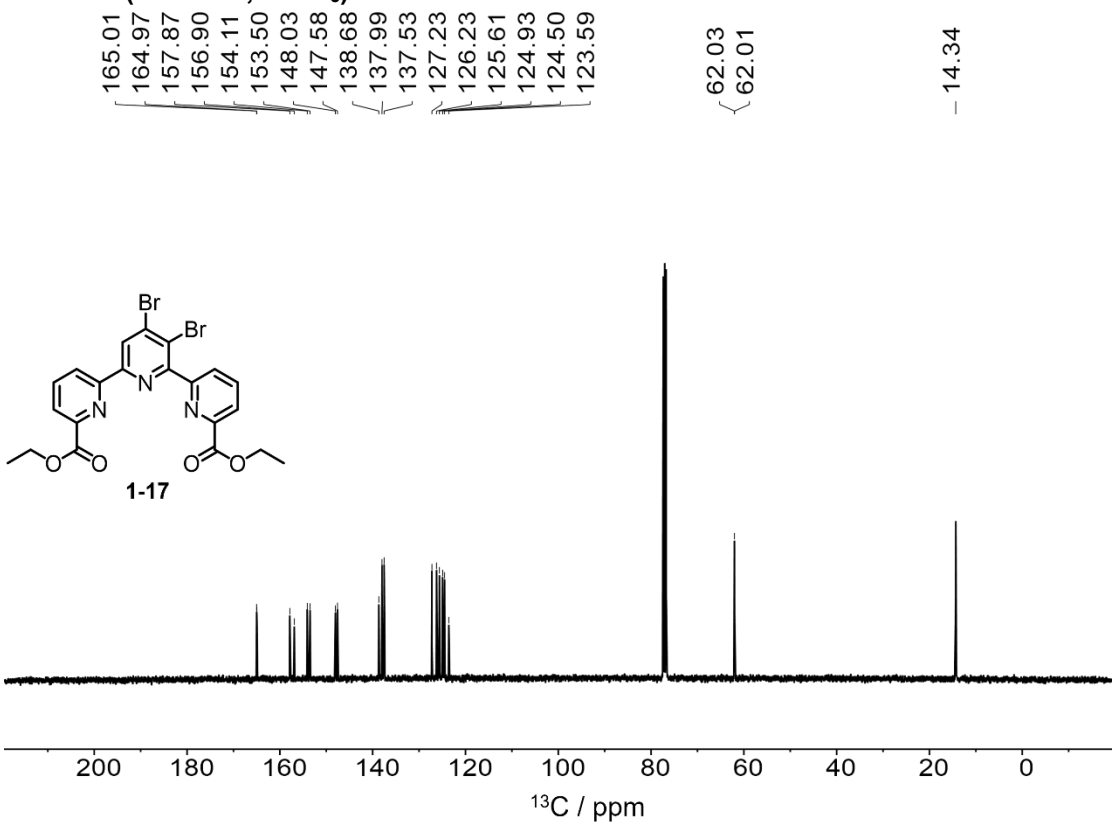

**$^{13}$C NMR (101 MHz, $CDCl_3$) of 1-16**



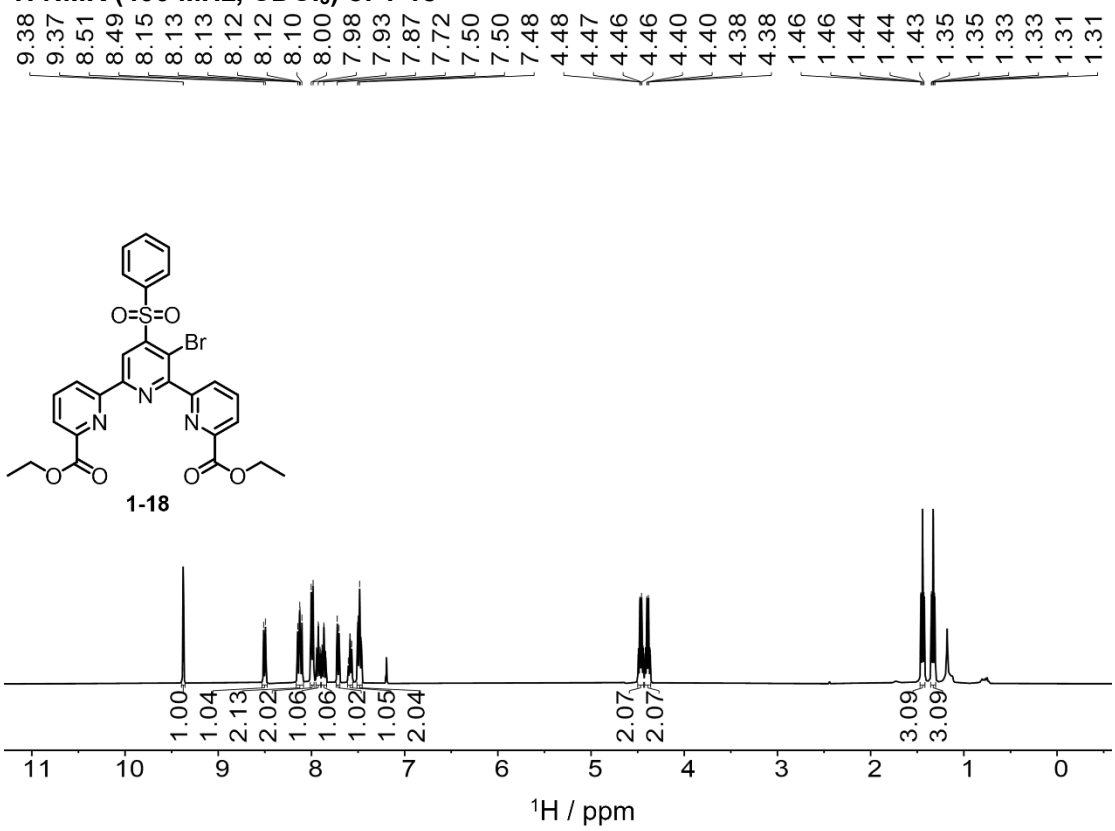
**$^{1}$H NMR (400 MHz, $CDCl_3$) of 1-17**



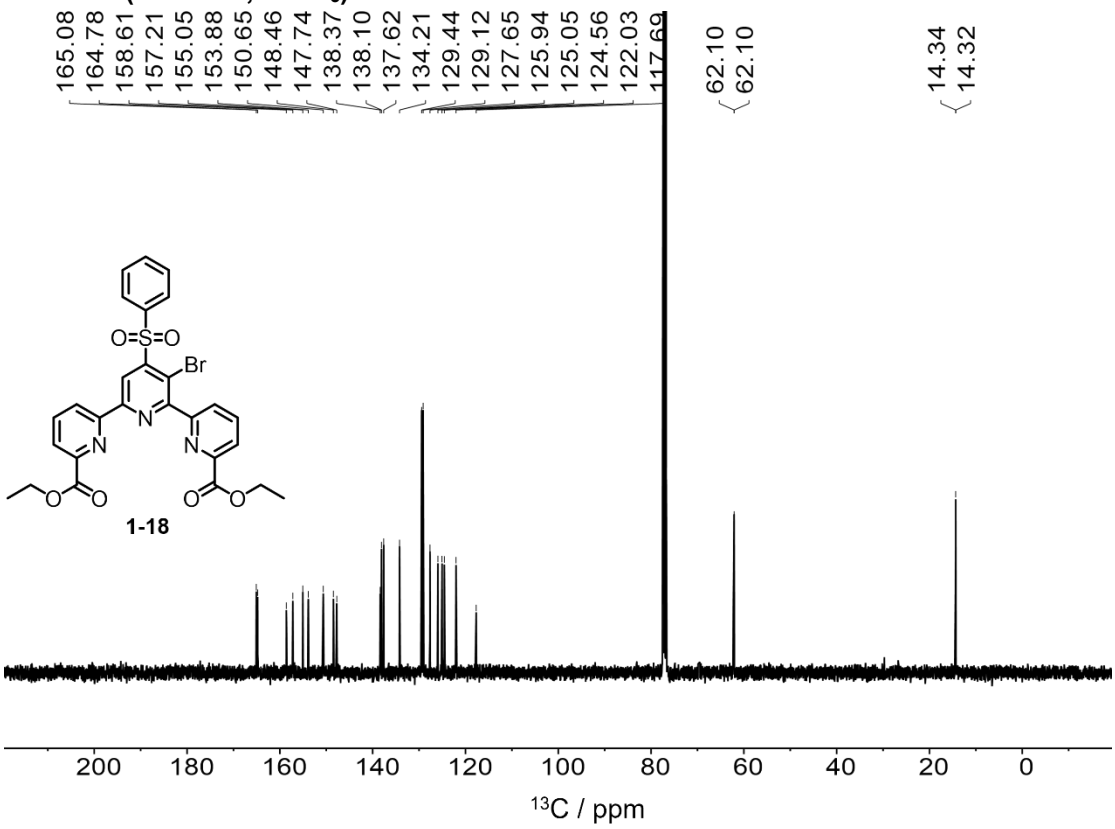

**$^{13}$C NMR (101 MHz, $CDCl_3$) of 1-17**



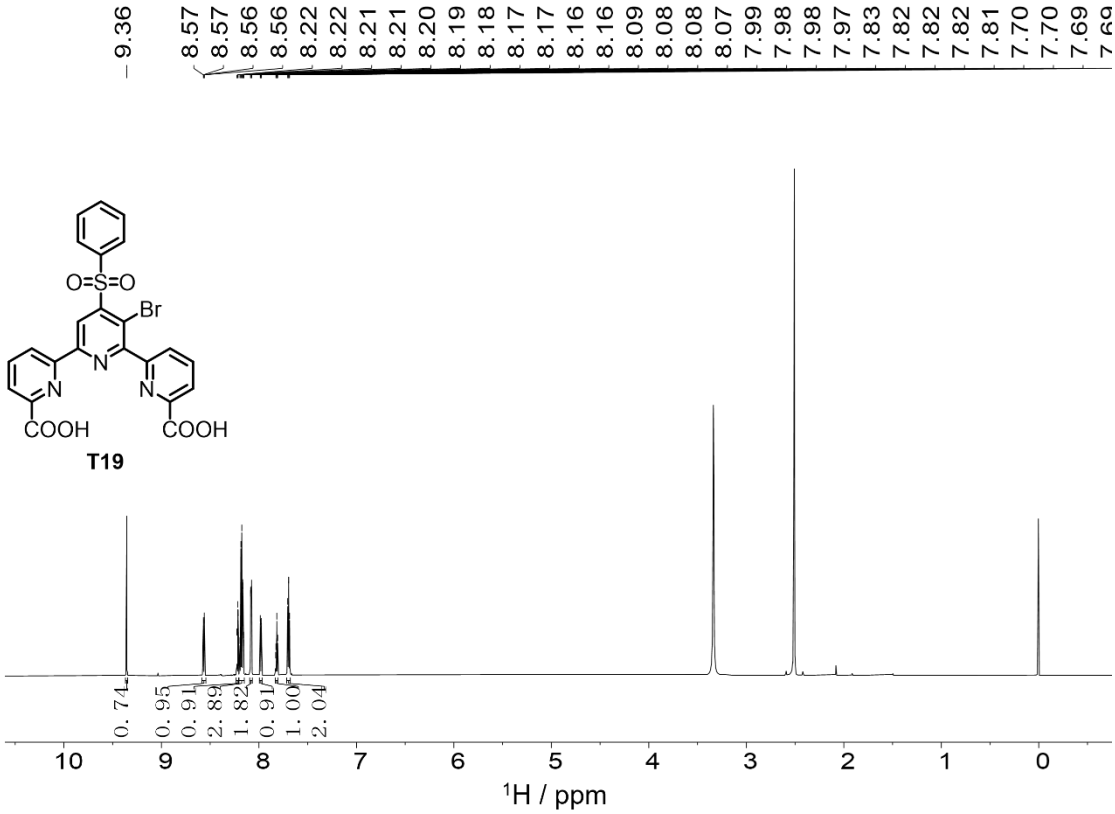
**$^{1}$H NMR (400 MHz, $CDCl_3$) of 1-18**



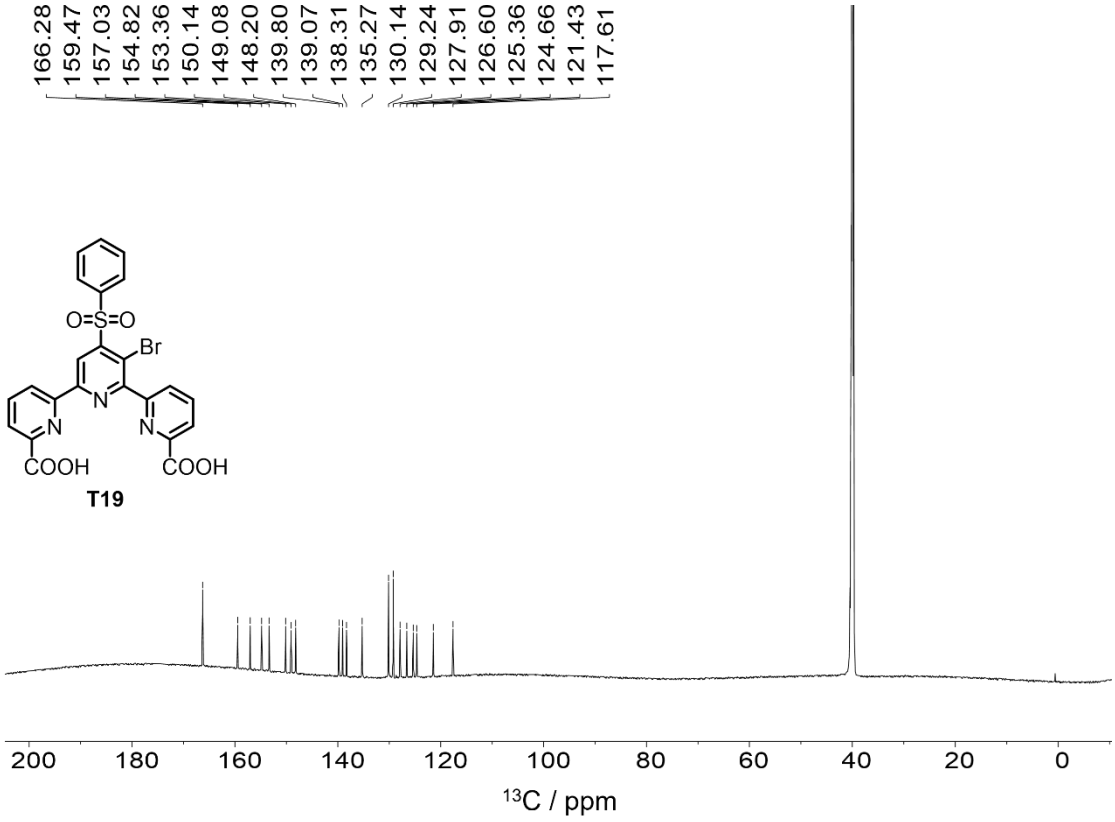

**$^{13}$C NMR (101 MHz, $CDCl_3$) of 1-18**



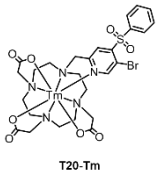
**$^{1}$H NMR (800 MHz, DMSO-$d_6$) of T19**



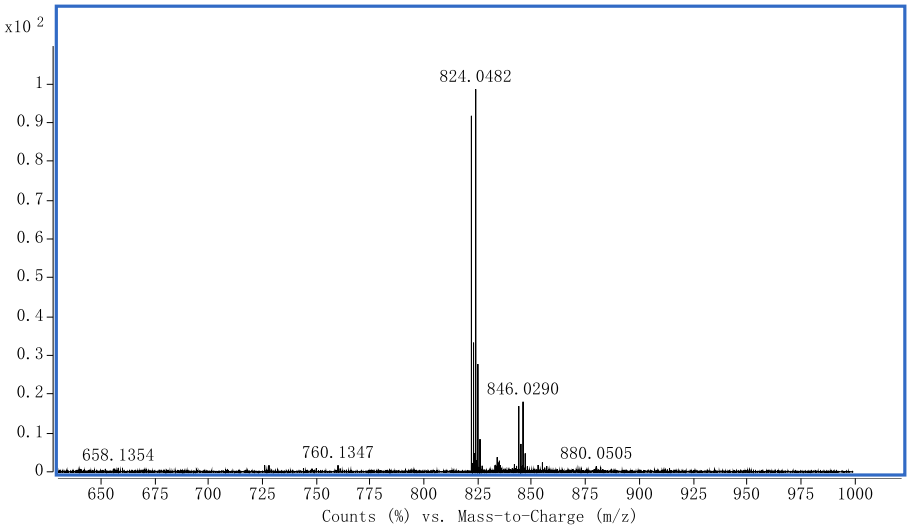

**$^{13}$C NMR (201 MHz, DMSO-$d_6$) of T19**



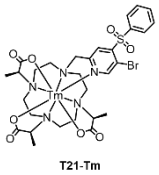
## HRMS (ESI-Q-TOF) of T20-Tm



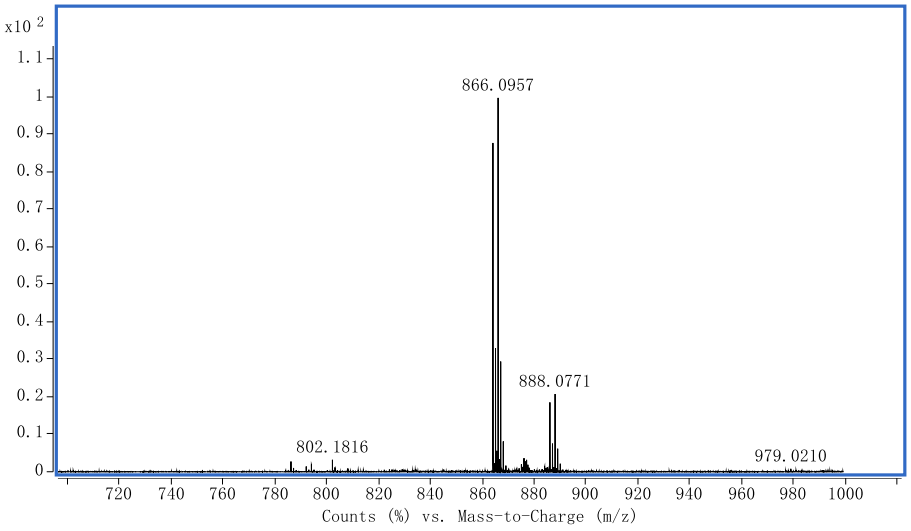

Calcd for $[C_{26}H_{32}BrN_5O_8STm + H]^+$ : 824.0472, found: 824.0482




## HRMS (ESI-Q-TOF) of T21




Calcd for $[C_{29}H_{38}BrN_5O_8STm + H]^+$ : 866.0941, found: 866.0957



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