Supporting information

Cyclic telluride reagents with remarkable glutathione peroxidase-like activity for purification-free synthesis of highly pure organodisulfides

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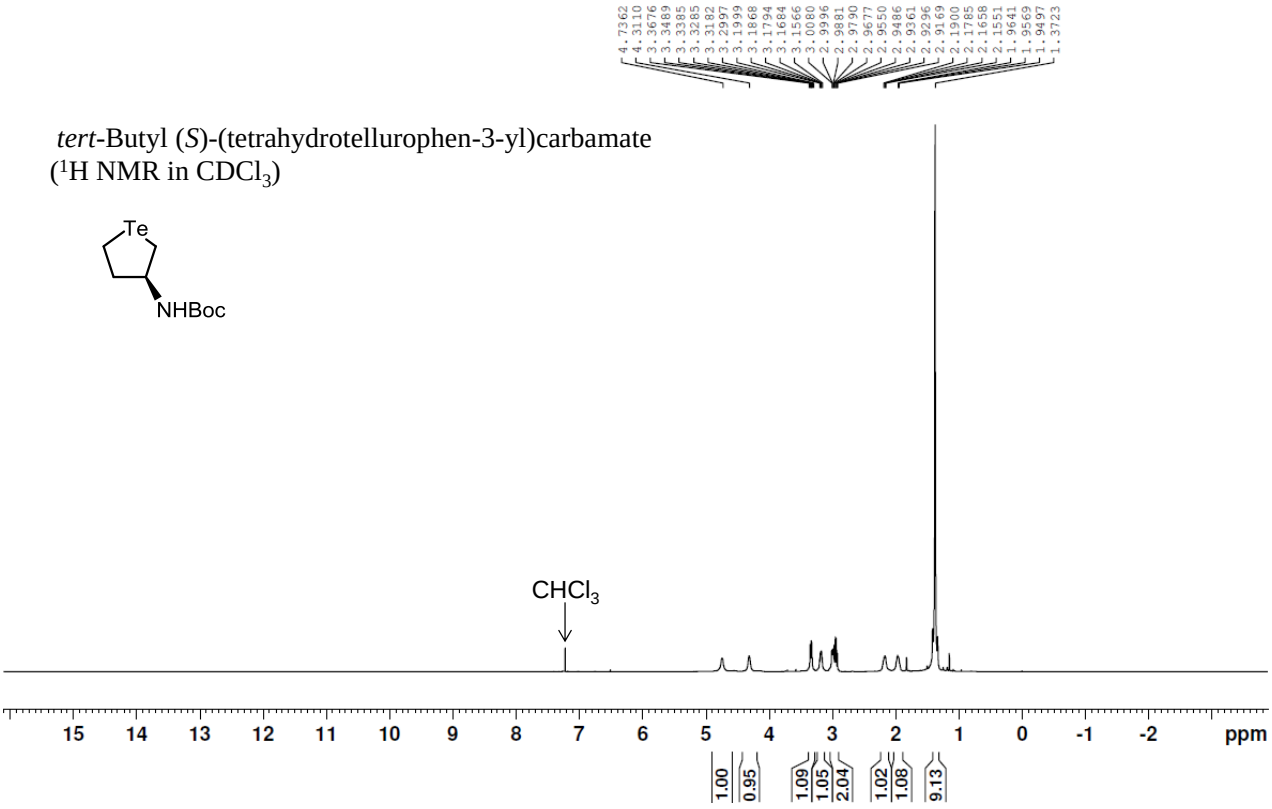
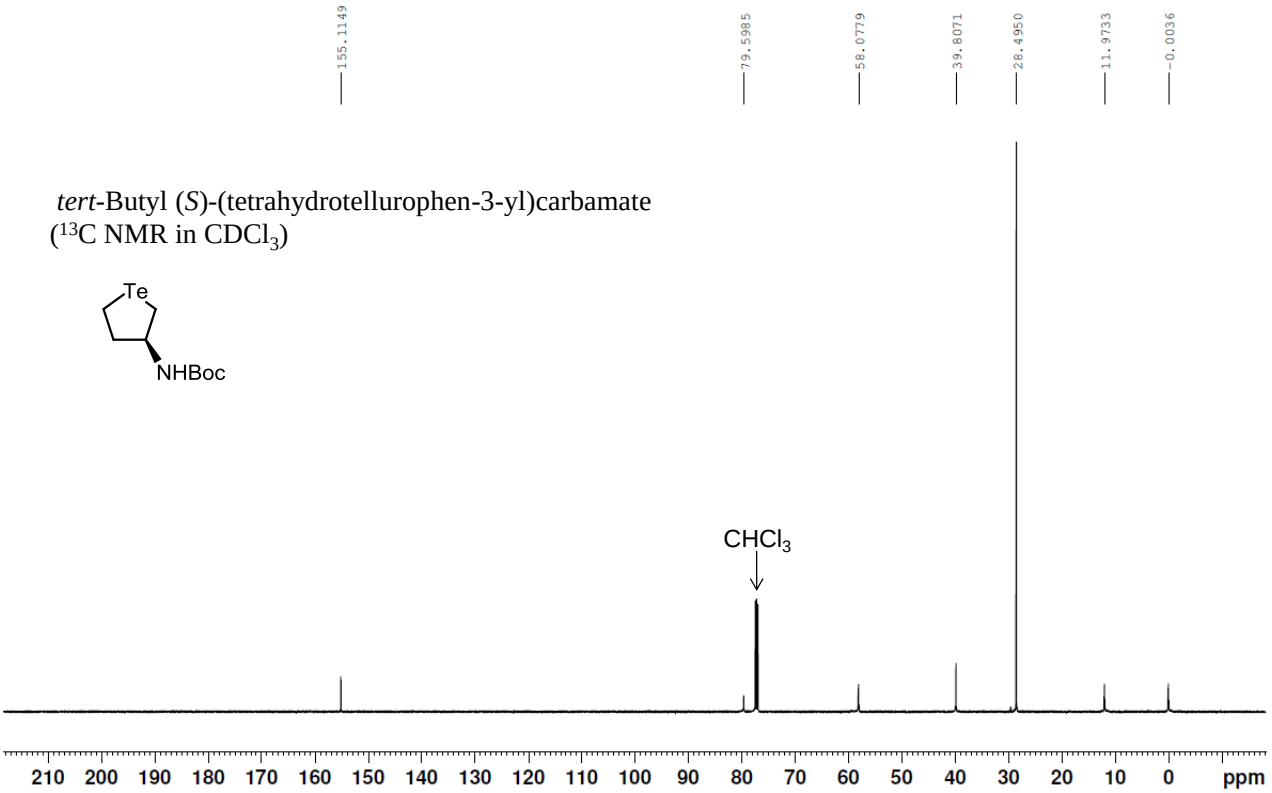
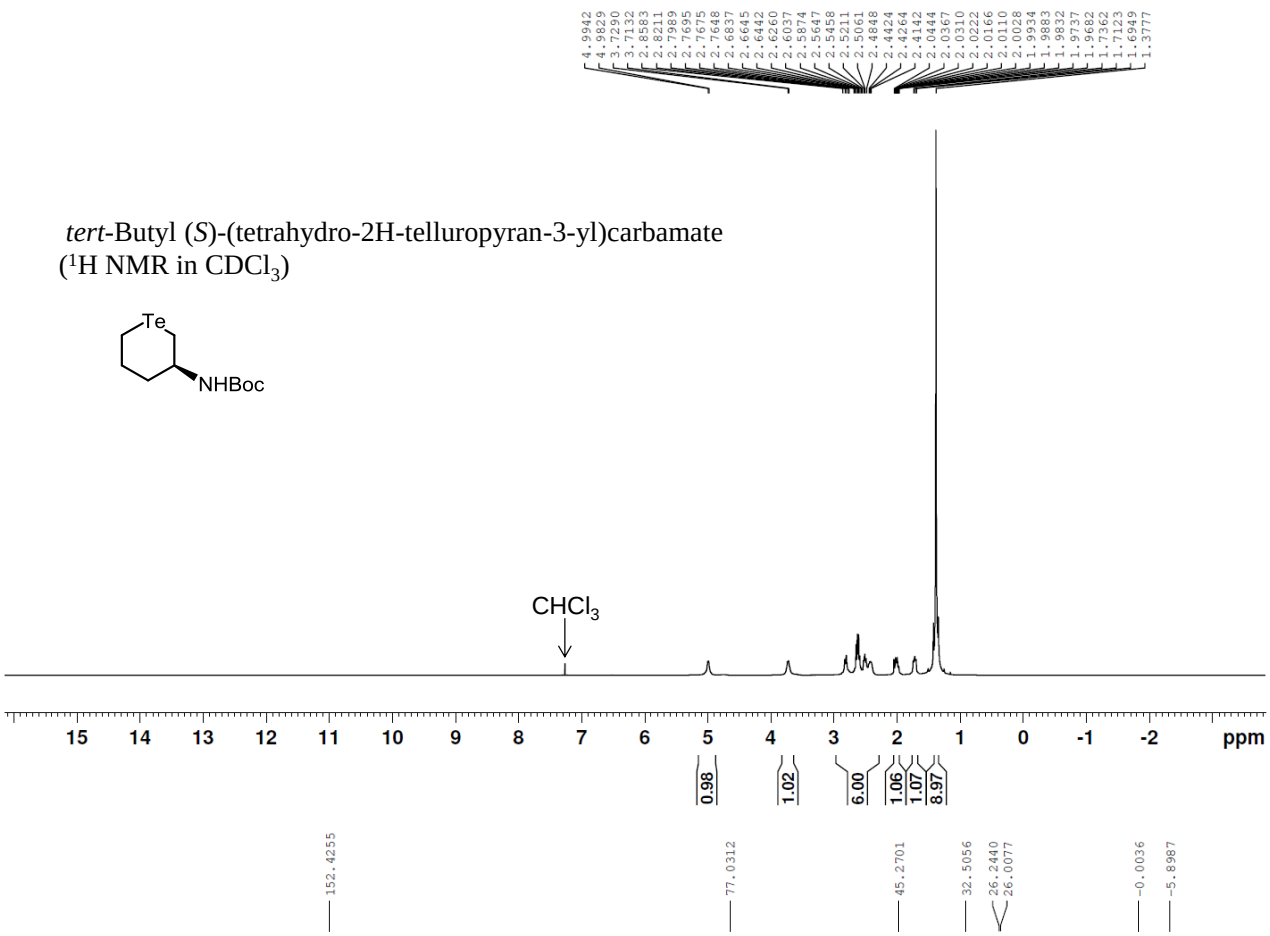
1. NMR spectra

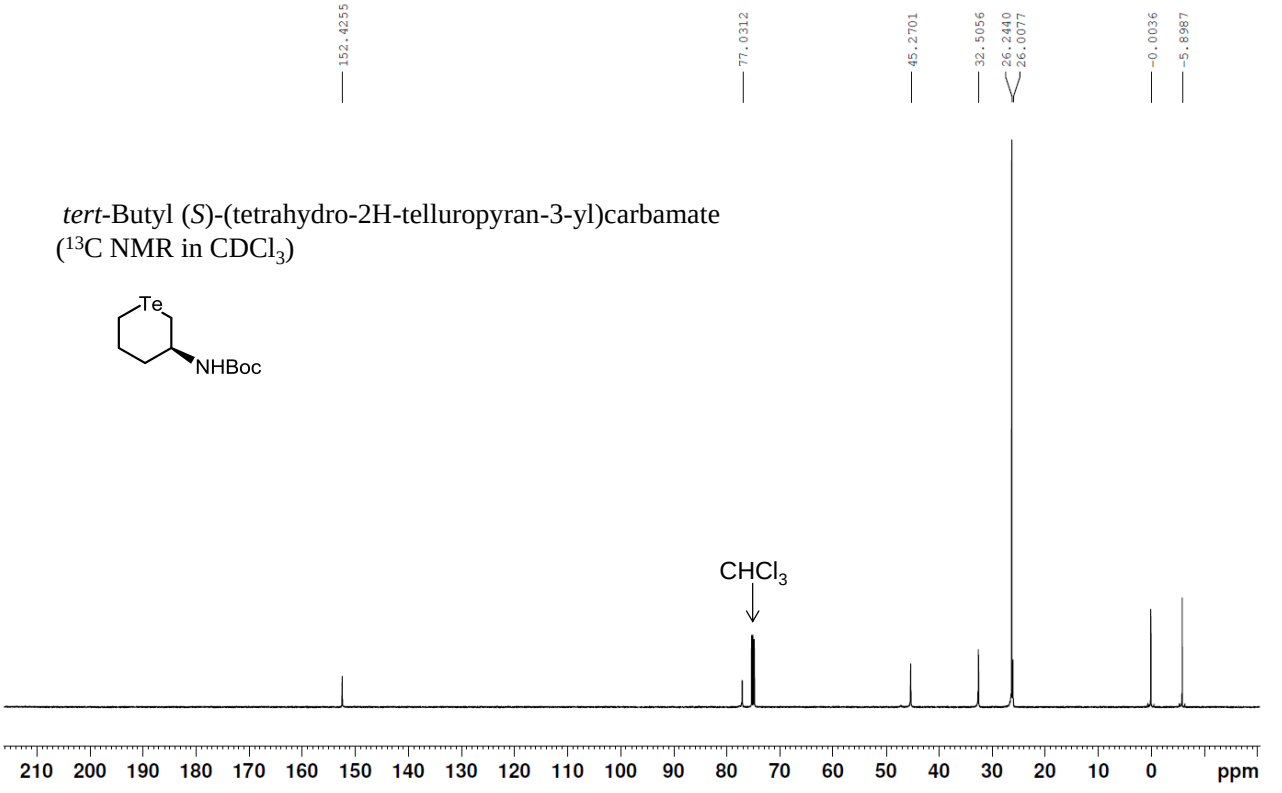
1.1 *tert*-Butyl (*S*)-(tetrahydrotellurophen-3-yl)carbamate (**7**)



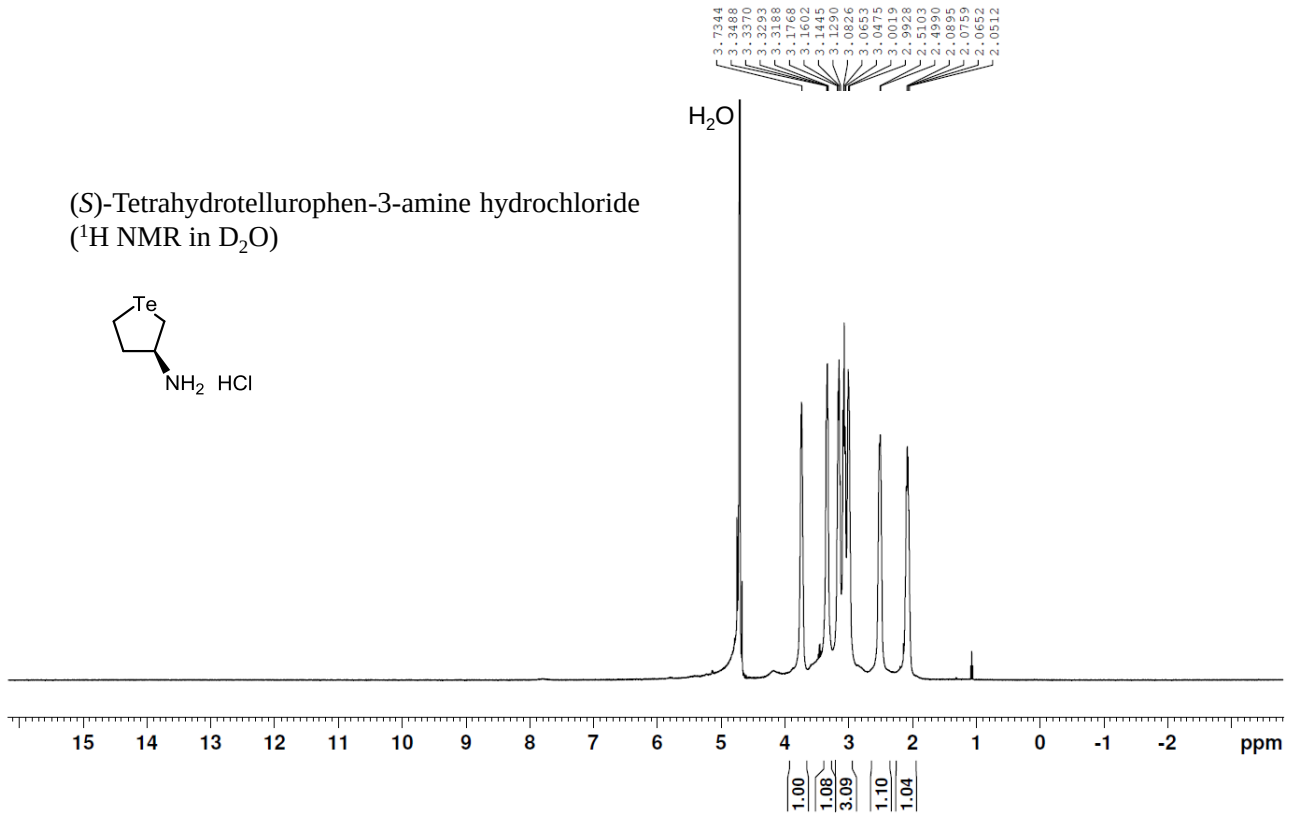


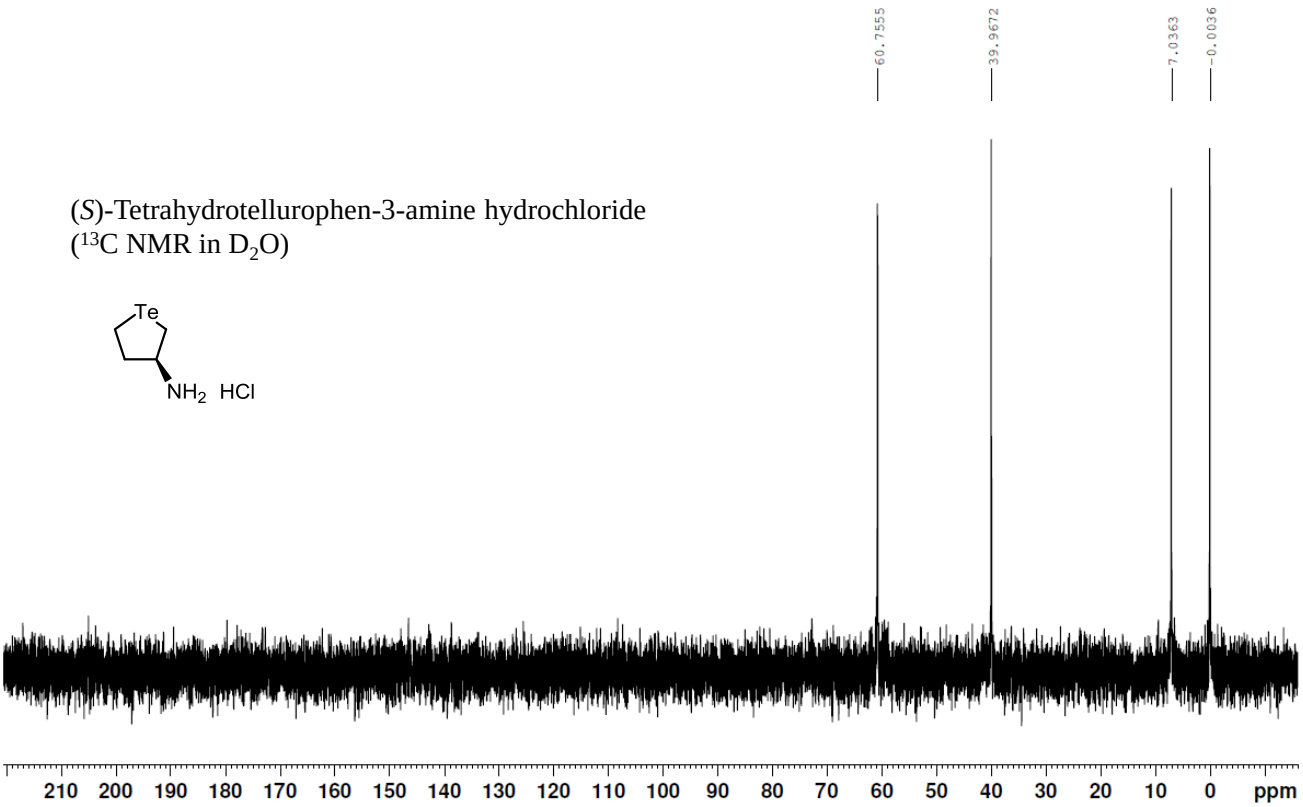
1.2 *tert*-Butyl (*S*)-(tetrahydro-2H-telluropyran-3-yl)carbamate (**8**)



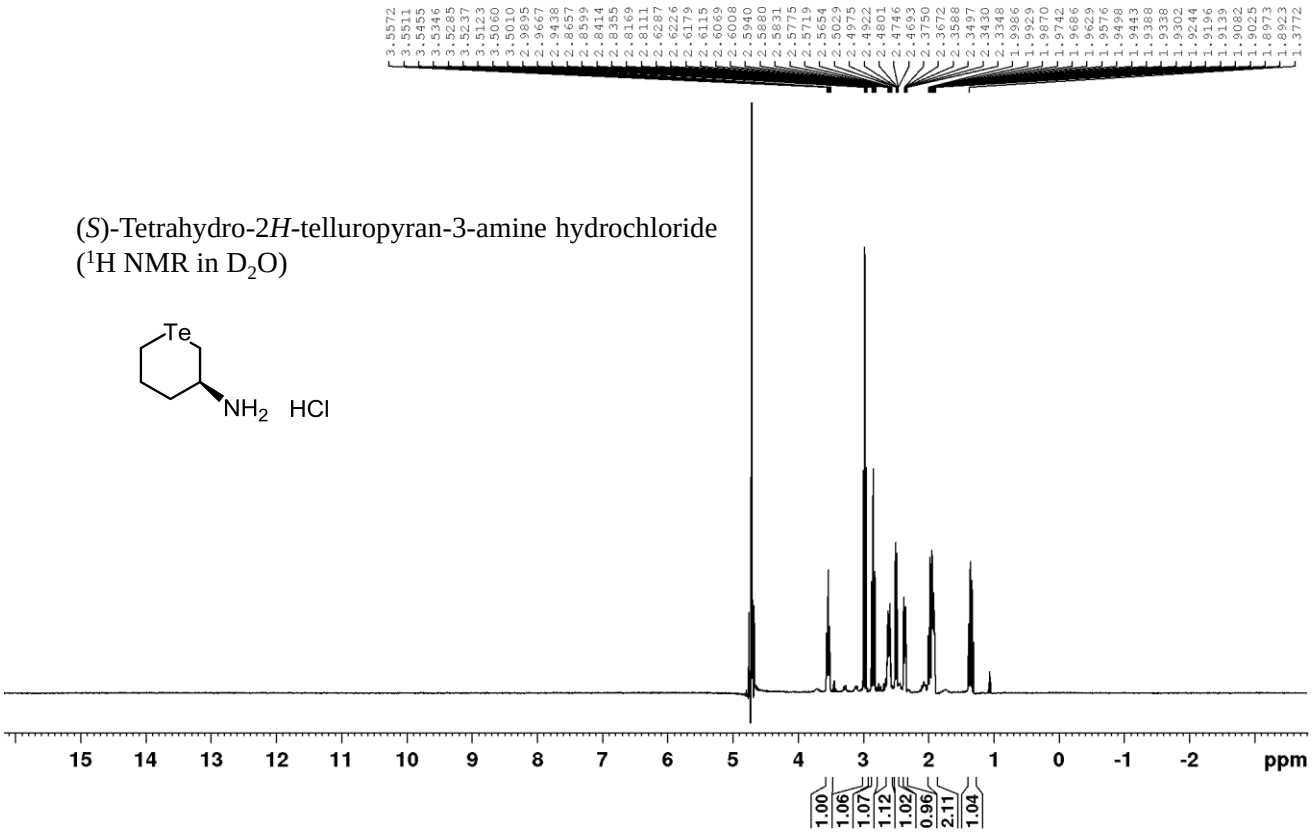


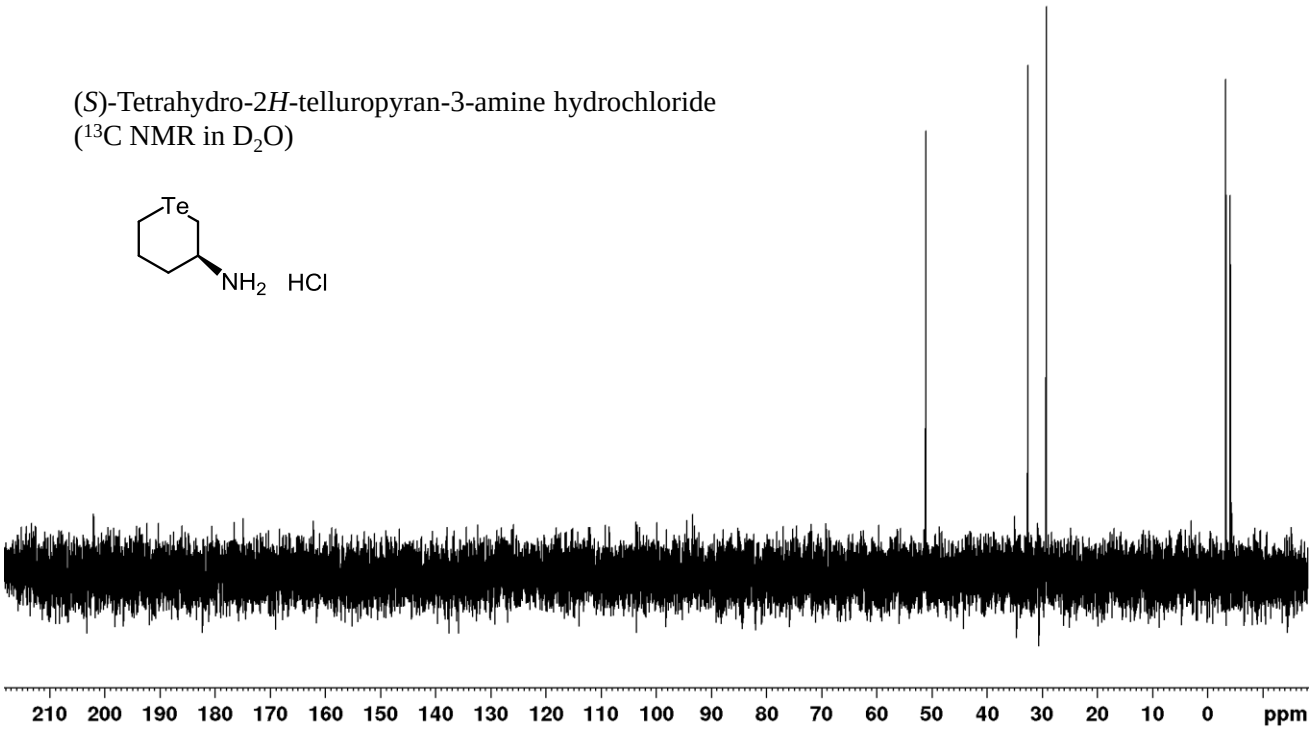
1.3 (*S*)-Tetrahydrotellurophen-3-amine hydrochloride (**4**)





1.4 (*S*)-Tetrahydro-2H-telluropyran-3-amine hydrochloride (**5**)

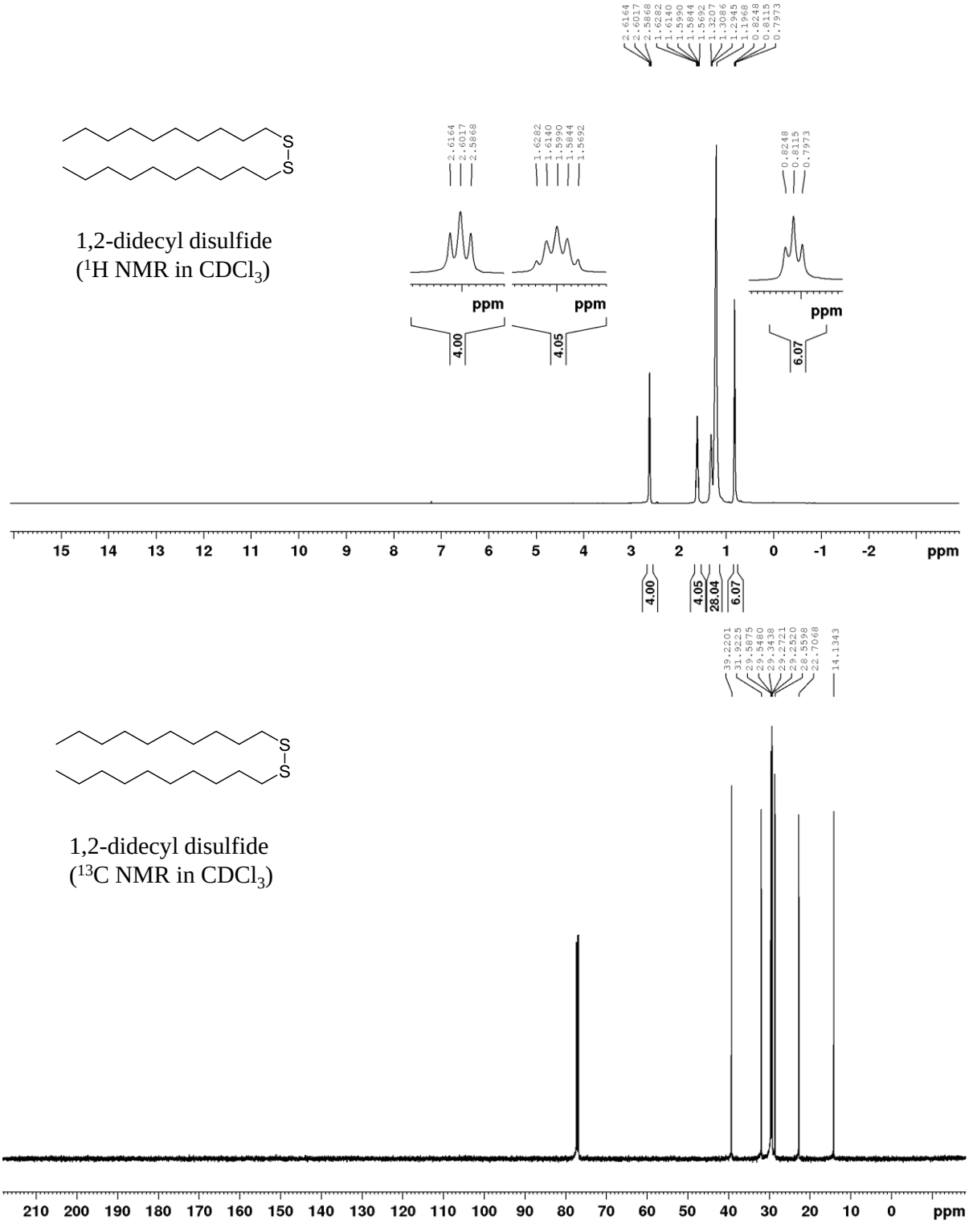




1.5 NMR spectra of disulfides (**16a–t**)

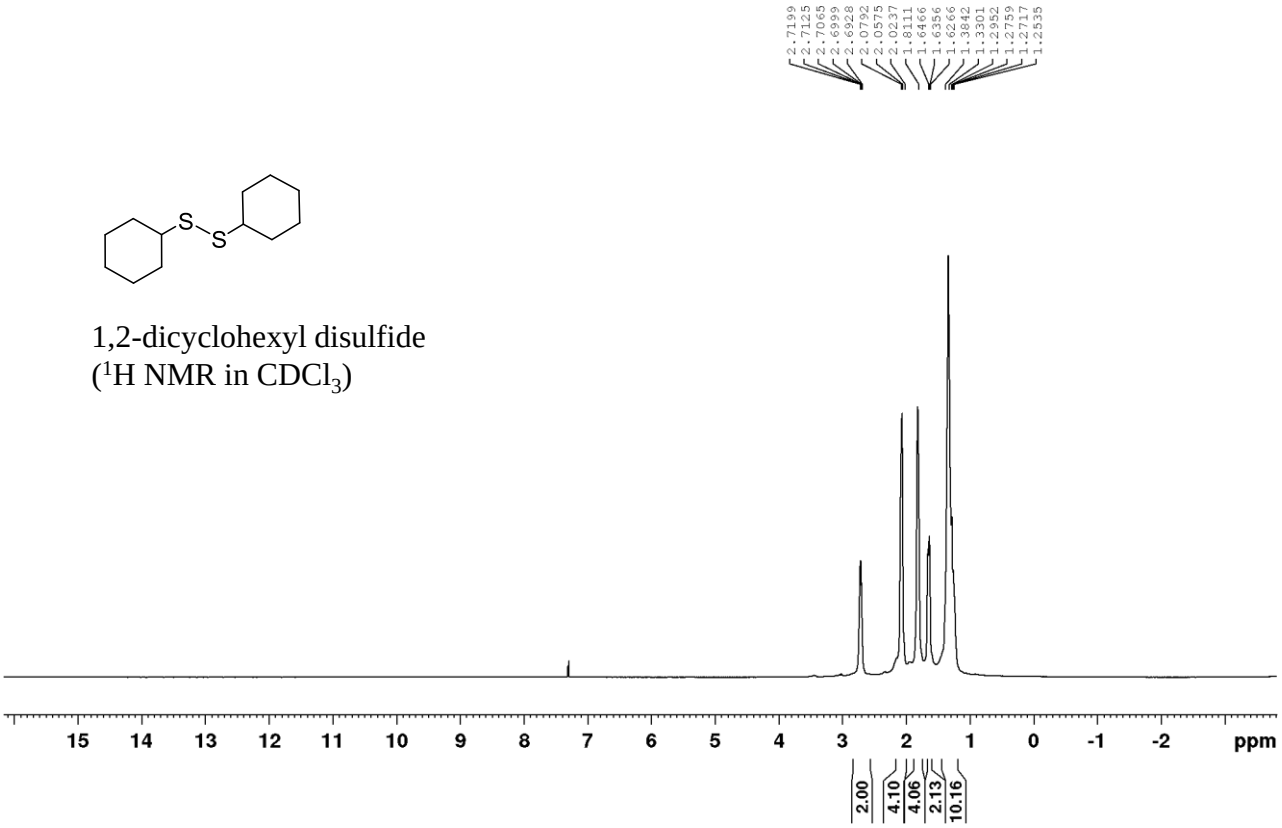
1,2-Didecyl disulfide (**16a**)

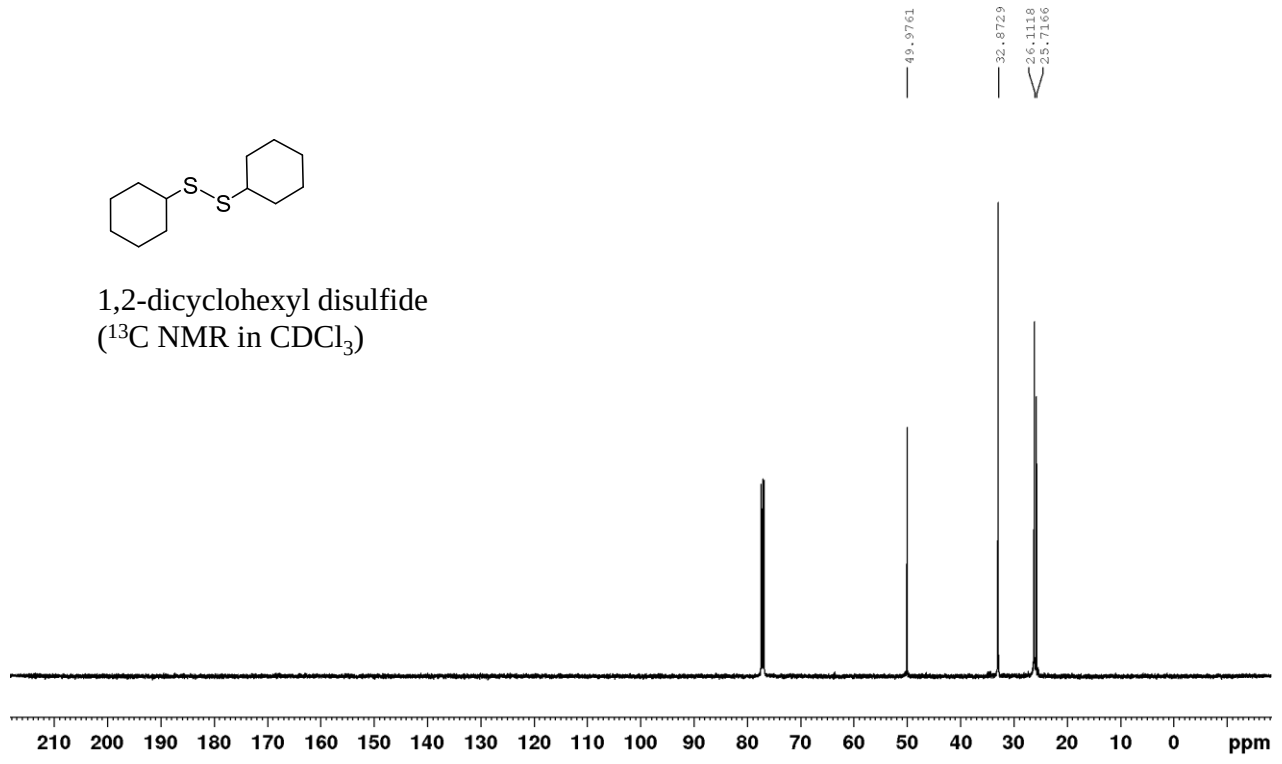
Colorless oil; Yield in batch: 38.7 mg (99%) and 38.5 mg (98%) using **4** and **6**, respectively; Yield in flow: 39.4 mg (quant.) using **4**; ^{1}H NMR ($CDCl_3$): δ = 2.60 (t, *J* = 7.4 Hz, 4H), 1.63–1.57 (m, 4H), 1.32–1.20 (m, 28H), 0.81 ppm (t, *J* = 6.7 Hz, 4H); ^{13}C NMR ($CDCl_3$): δ = 39.22, 31.92, 29.59, 29.55, 29.34, 29.27, 29.25, 28.56, 22.71, 14.13 ppm. Spectroscopic data are in accordance with the previously presented.[1]



1,2-dicyclohexyl disulfide (**16b**)

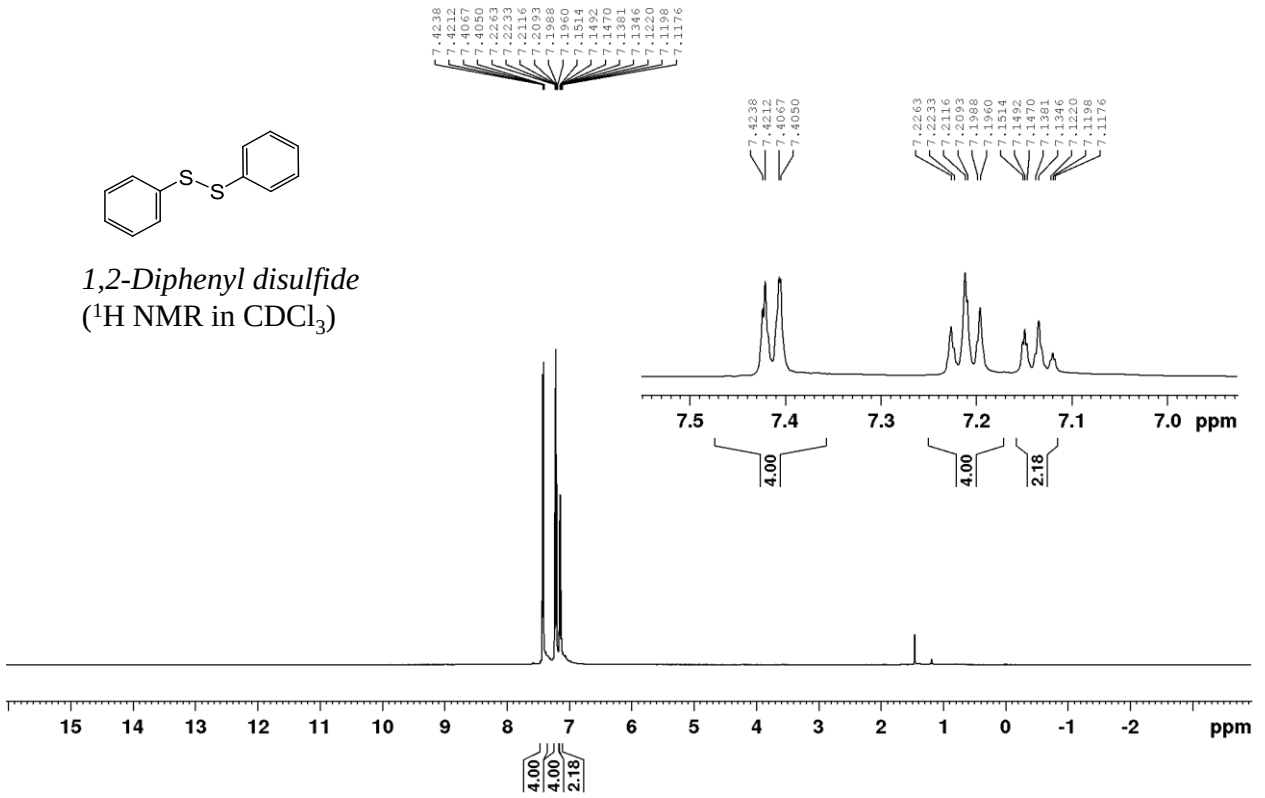
Colorless oil; Yield in batch: 26.1 mg (99%) and 25.4 mg (96%) using **4** and **6**, respectively; Yield in flow: 25.9 mg (quant.) using **4**, respectively; ^{1}H NMR ($CDCl_3$): δ = 2.72–2.69 (m, 2H), 2.08–2.02 (m, 4H), 1.84–1.77 (m, 4H), 1.65–1.63 (m, 2H), 1.38–1.25 ppm (m, 10H); ^{13}C NMR ($CDCl_3$): δ = 50.0, 32.9, 26.1, 25.7 ppm. Spectroscopic data are in accordance with the previously presented.[2]

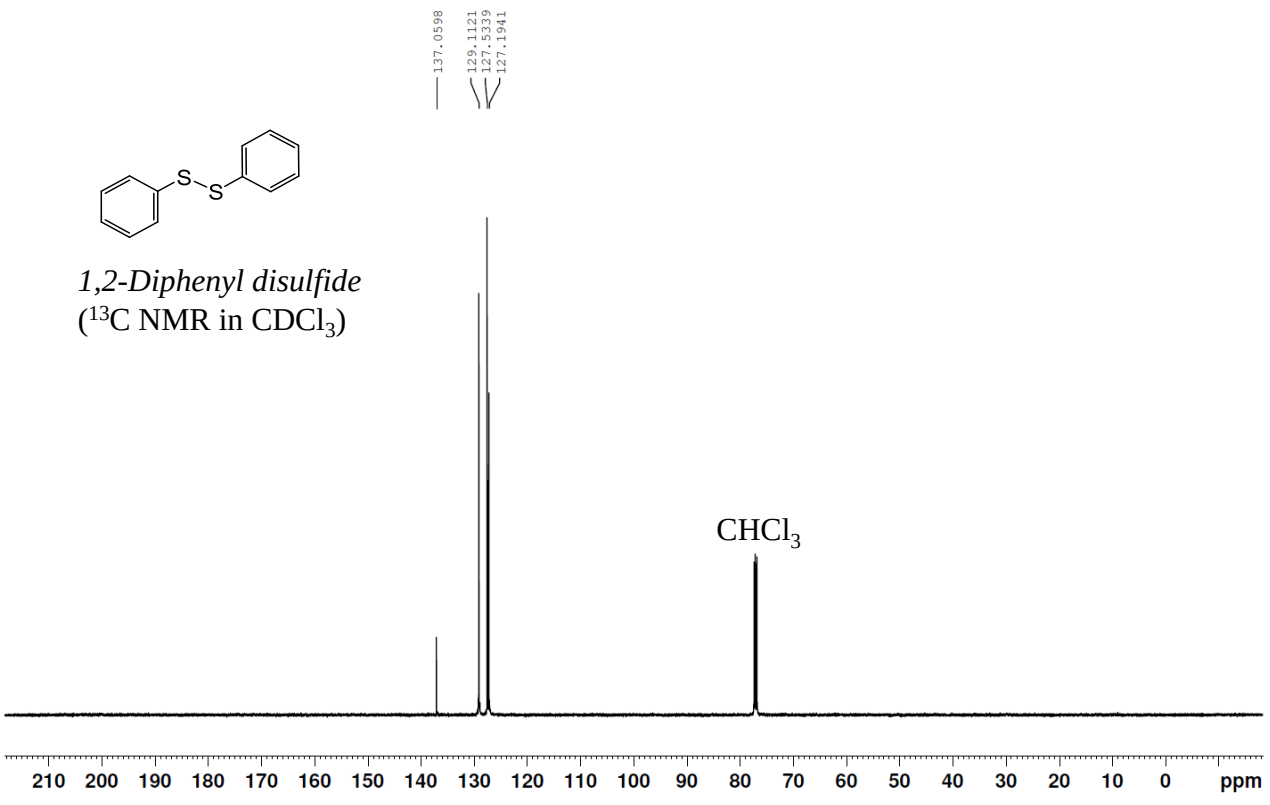




1,2-Diphenyl disulfide (**16c**)

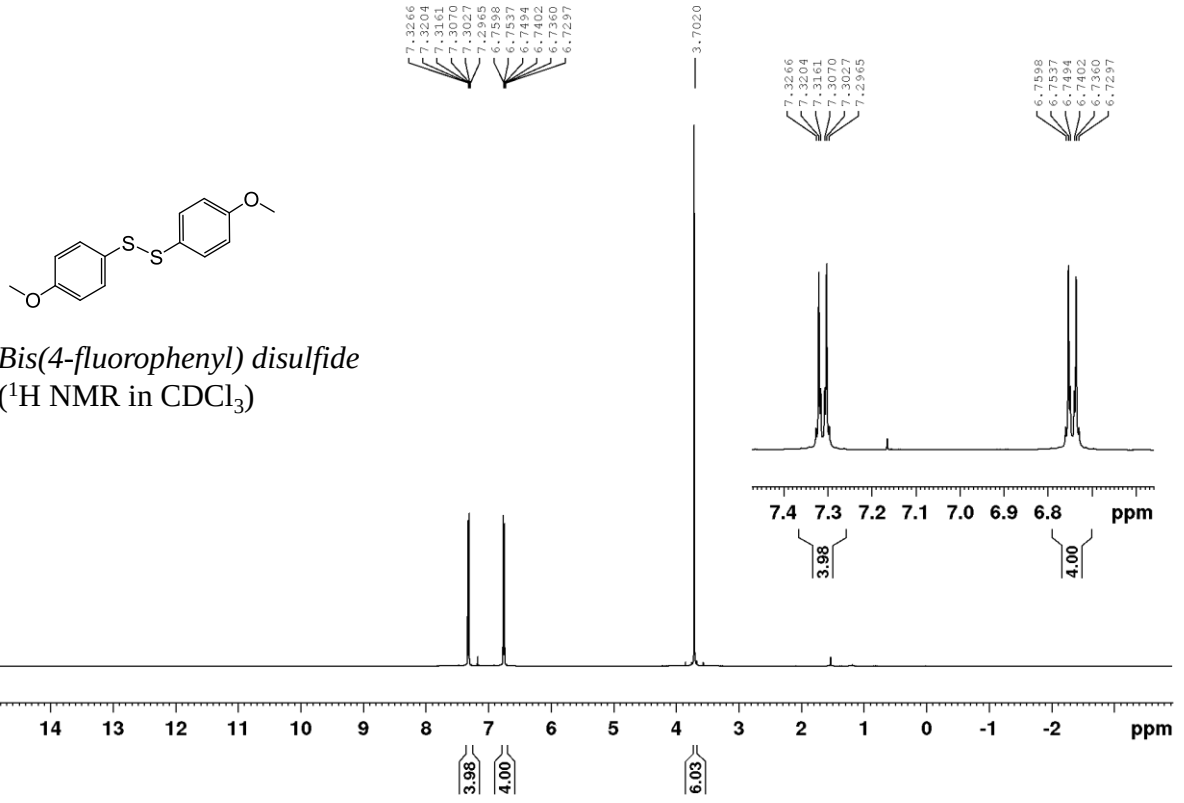
White solid; Yield in batch: 23.4 mg (95%) and 29.3 mg (quant.) using **4** and **6**; Yield in flow: 23.6 mg (95%) and 23.9 mg (96%) using **4** and **5**, respectively; ^{1}H NMR ($CDCl_3$): δ = 7.42–7.41 (m, 4H), 7.22–7.20 (m, 4H), 7.15–7.12 ppm (m, 2H); ^{13}C NMR ($CDCl_3$): δ = 137.1, 129.1, 127.5, 127.2 ppm. Spectroscopic data are in accordance with the previously presented.[3]



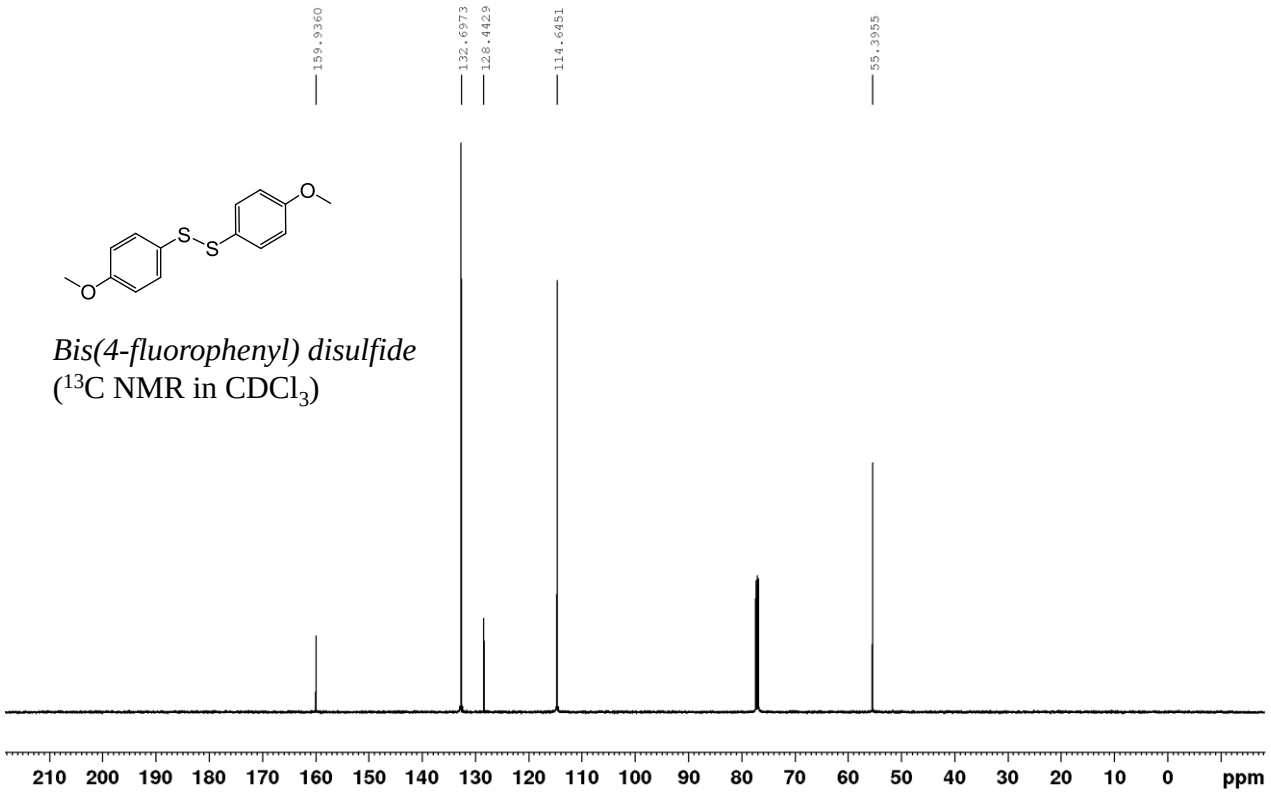


Bis(4-methoxyphenyl) disulfide (**16d**)

Off yellow oil; Yield in batch: 35.5 mg (quant.) and 30.4 mg (97%) using **4** and **6**, respectively; Yield in flow: 32.0 mg (quant.) and 30.4 mg (97%) using **4** and **5**, respectively; ^{1}H NMR ($CDCl_3$): δ = 7.32–7.30 (m, 4H), 6.76–6.73 (m, 4H), 3.70 ppm (s, 6H); ^{13}C NMR ($CDCl_3$): δ = 159.9, 132.7, 128.4, 114.6, 55.4 ppm. Spectroscopic data are in accordance with the previously presented.[3]



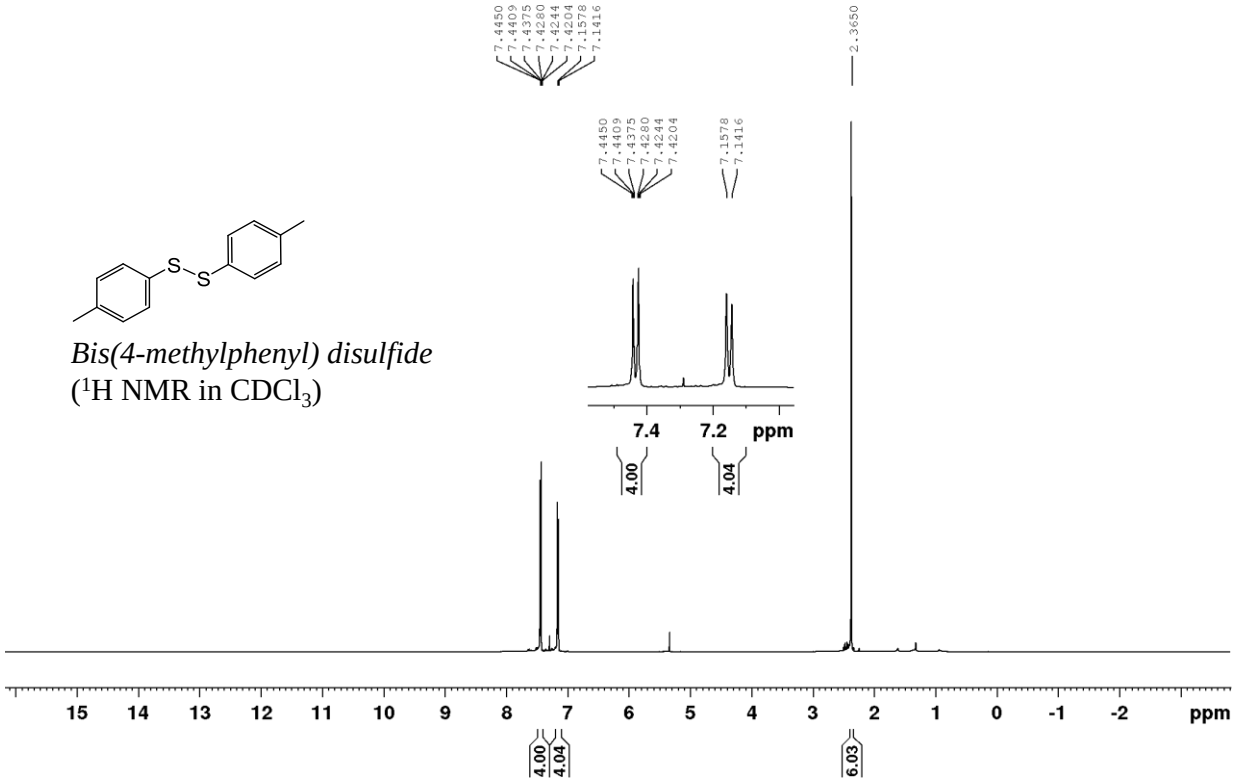
Bis(4-fluorophenyl) disulfide
(^{1}H NMR in $CDCl_3$)



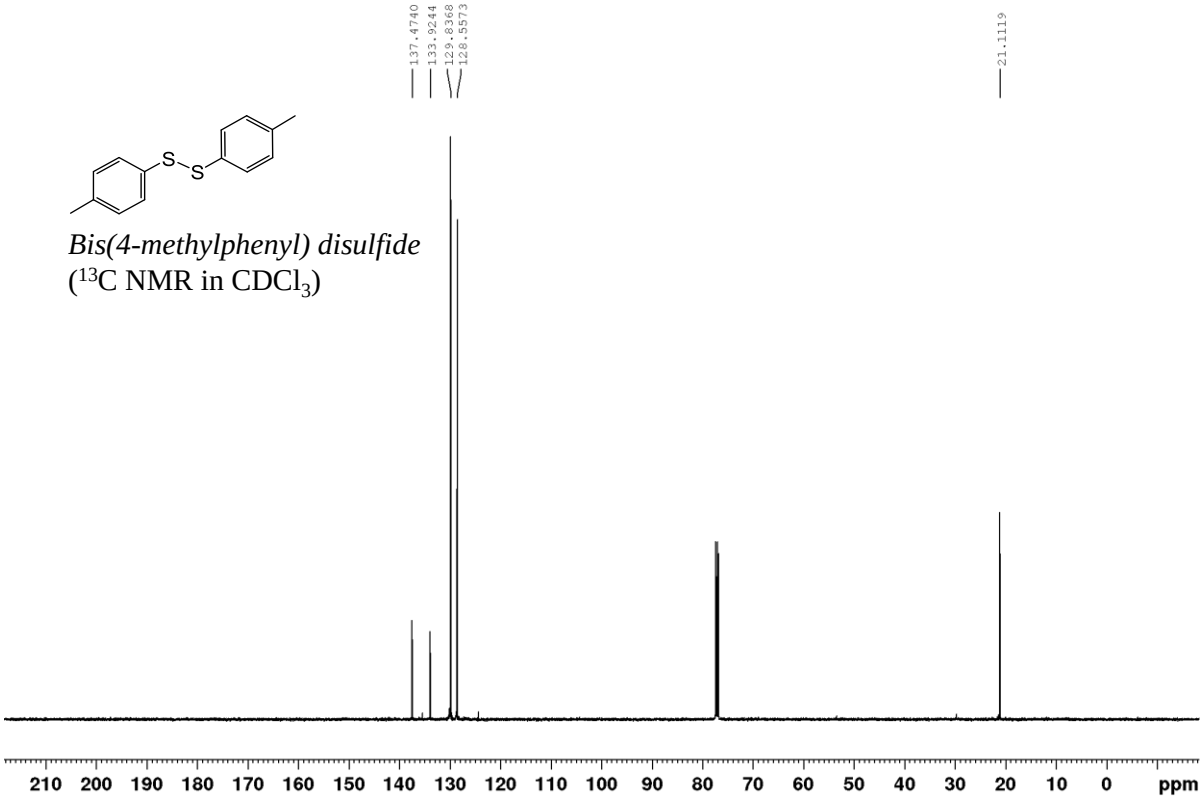
Bis(4-fluorophenyl) disulfide
(^{13}C NMR in $CDCl_3$)

Bis(4-methylphenyl) disulfide (**16e**)

White solid; Yield in batch: 27.3 mg (98%) and 29.2 mg (quant.) using **4** and **6**, respectively; Yield in flow: 27.1 mg (97%) and 27.2 mg (97%) using **4** and **5**, respectively; ^{1}H NMR ($CDCl_3$): δ = 7.44–7.14 (m, 4H), 7.15 (d, J = 8.1, Hz, 4H), 2.37 ppm (s, 6H); ^{13}C NMR ($CDCl_3$): δ = 137.5, 133.9, 129.8, 128.6, 21.1 ppm. Spectroscopic data are in accordance with the previously presented.[1]



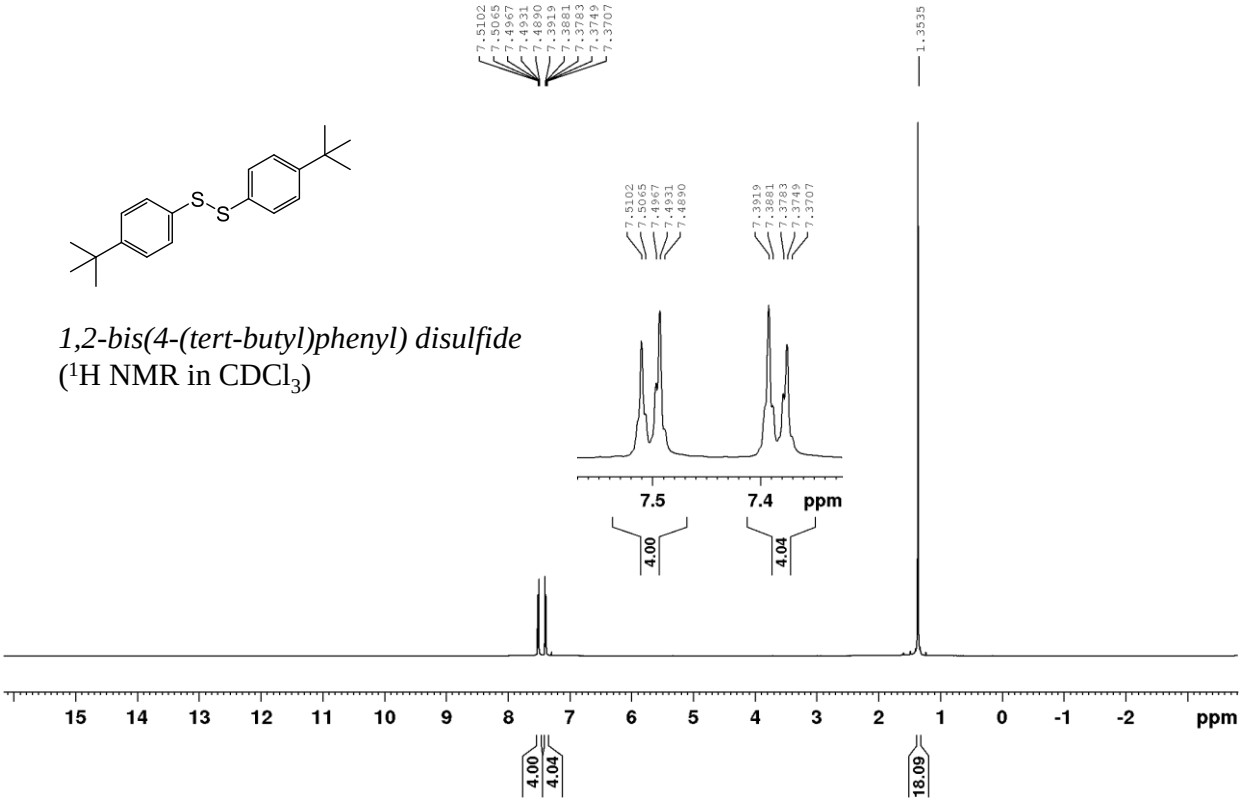
Bis(4-methylphenyl) disulfide
(^{1}H NMR in $CDCl_3$)



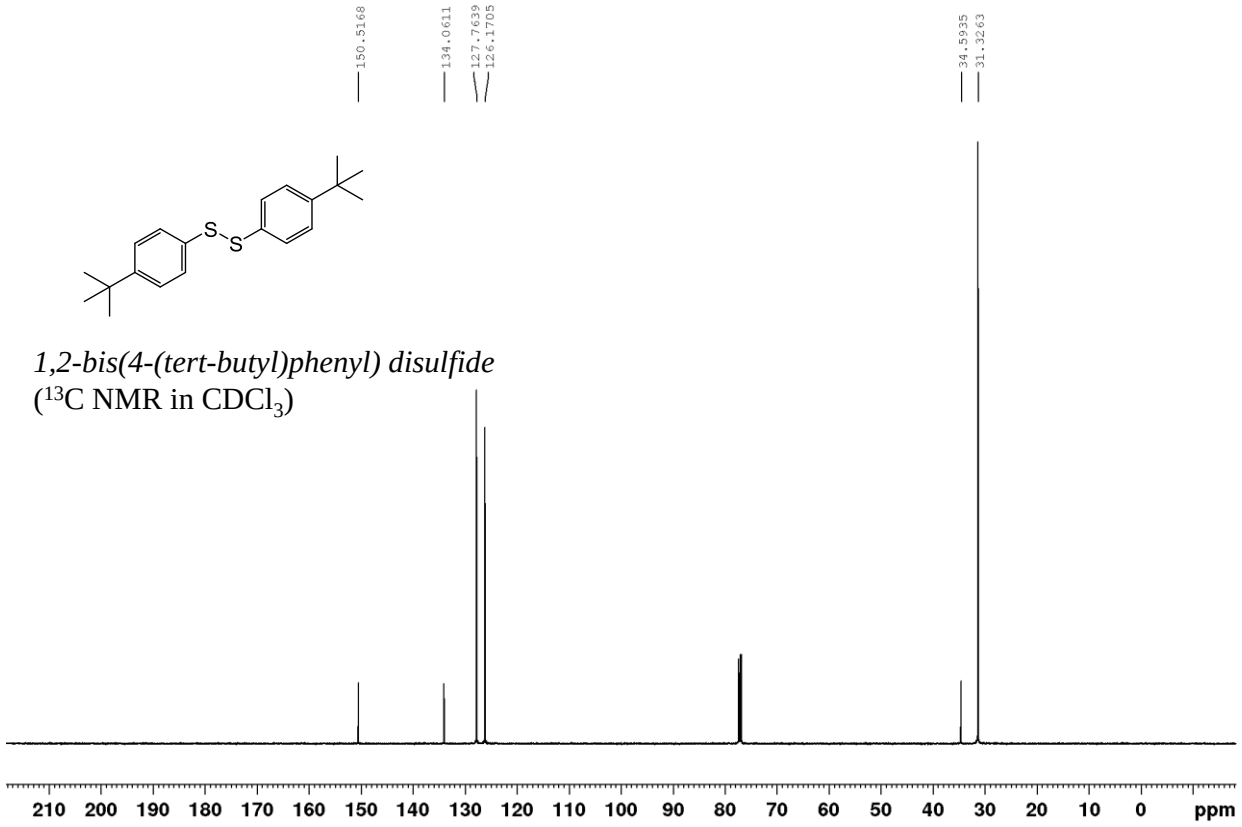
Bis(4-methylphenyl) disulfide
(^{13}C NMR in $CDCl_3$)

1,2-bis(4-(tert-butyl)phenyl) disulfide (**16f**)

White solid; Yield in batch: 37.4 mg (quant.) and 35.9 mg (96%) using **4** and **6**, respectively; Yield in flow: 37.6 mg (quant.) and 34.8 mg (93%) using **4** and **5**, respectively; ^{1}H NMR ($CDCl_3$): δ = 7.51–7.49 (m, 4H), 7.39–7.37 (m, 4H), 1.35 ppm (m, 18H); ^{13}C NMR ($CDCl_3$): δ = 150.5, 134.1, 127.8, 126.2, 34.6, 31.3 ppm. Spectroscopic data are in accordance with the previously presented.[4]



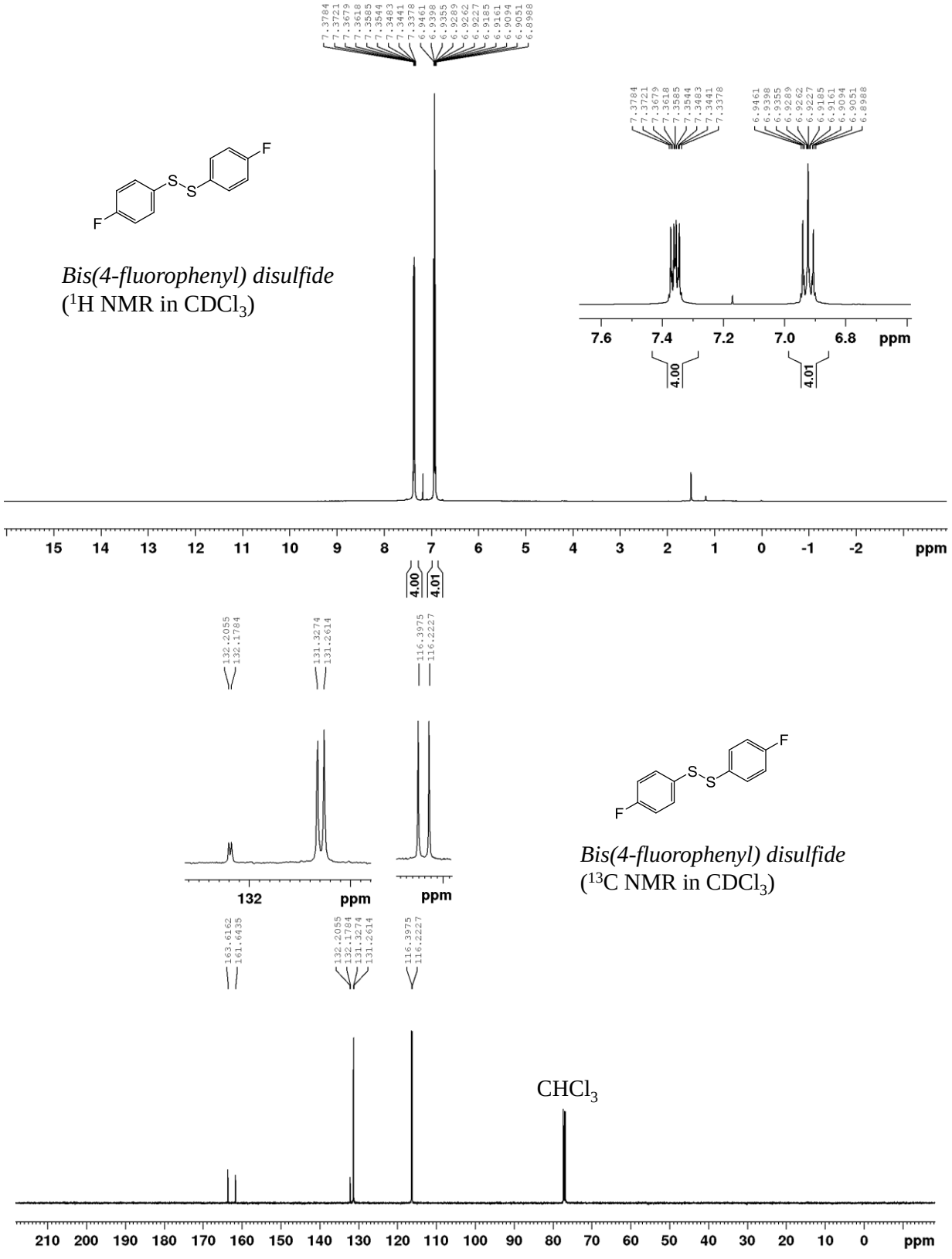
1,2-bis(4-(tert-butyl)phenyl) disulfide
(^{1}H NMR in $CDCl_3$)



1,2-bis(4-(tert-butyl)phenyl) disulfide
(^{13}C NMR in $CDCl_3$)

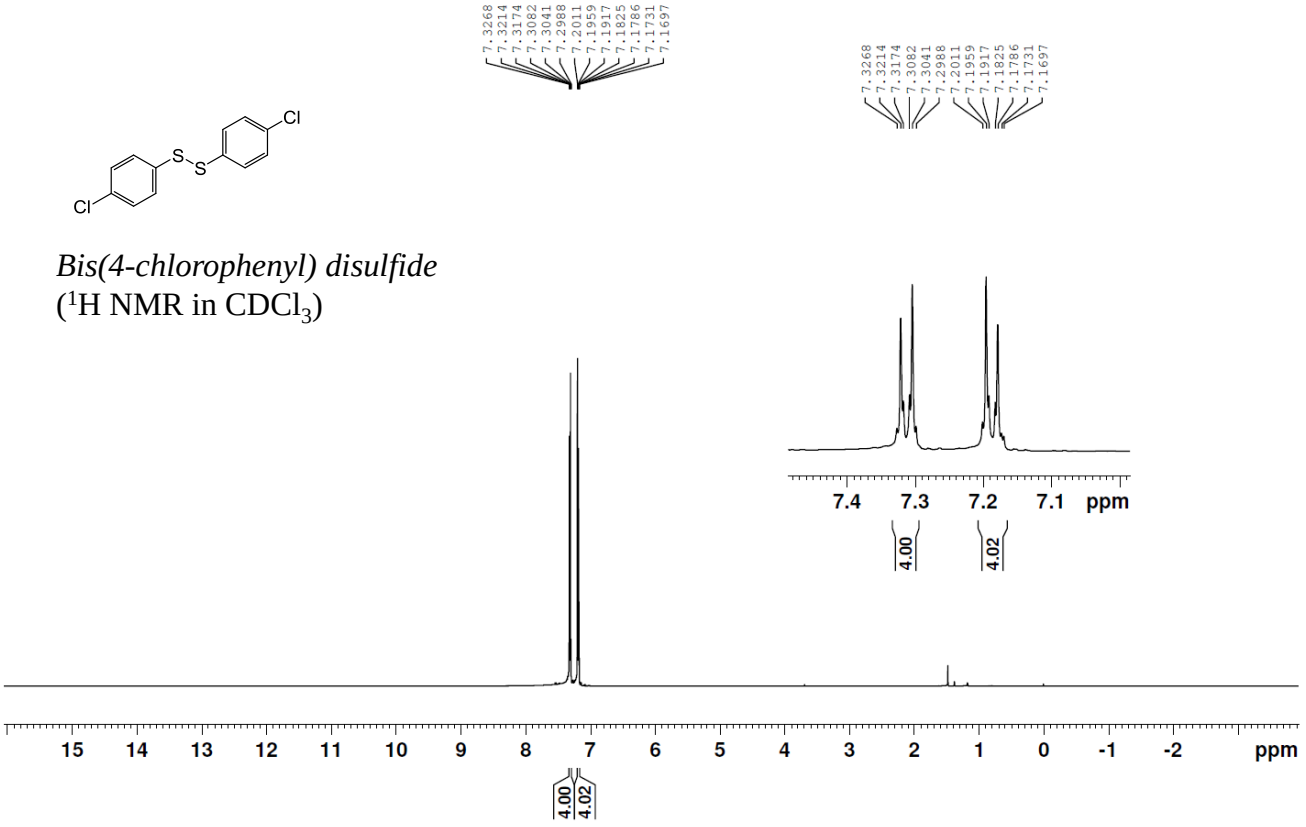
Bis(4-fluorophenyl) disulfide (**16g**)

Colorless oil; Yield in batch: 29.0 mg (quant.) and 28.2 mg (99%) using **4** and **6**, respectively, respectively; Yield in flow: 29.0 mg (quant.) and 27.9 mg (97%) using **4** and **5**, respectively; ^{1}H NMR ($CDCl_3$): δ = 7.38–7.34 (m, 4H), 6.95–6.90 (m, 4H); ^{13}C NMR ($CDCl_3$): δ = 163.62, 161.64, 132.19 (d, *J* = 3.4 Hz), 131.30 (d, *J* = 8.8 Hz), 116.31 ppm (d, *J* = 22.6 Hz). Spectroscopic data are in accordance with the previously presented.[1]

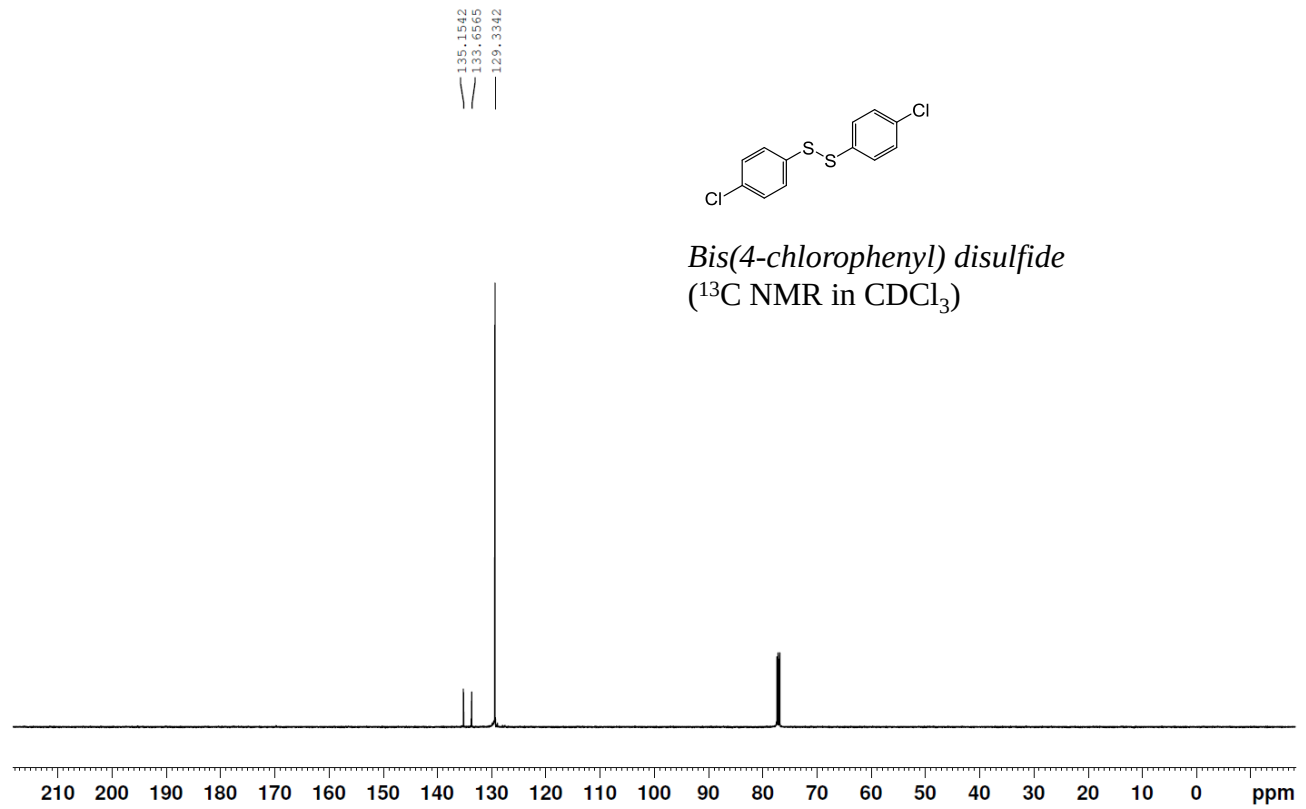


Bis(4-chlorophenyl) disulfide (**16h**)

White solid; Yield in batch: 33.5 mg (quant.) and 34.6 mg (quant.) using **4** and **6**, respectively; Yield in flow: 31.5 mg (97%) and 31.0 mg (95%) using **4** and **5**, respectively; ^{1}H NMR ($CDCl_3$): δ = 7.33–7.30 (m, 4H), 7.20–7.17 ppm (m, 4H); ^{13}C NMR ($CDCl_3$): δ = 135.2, 133.7, 129.3 ppm. Spectroscopic data are in accordance with the previously presented.[1]



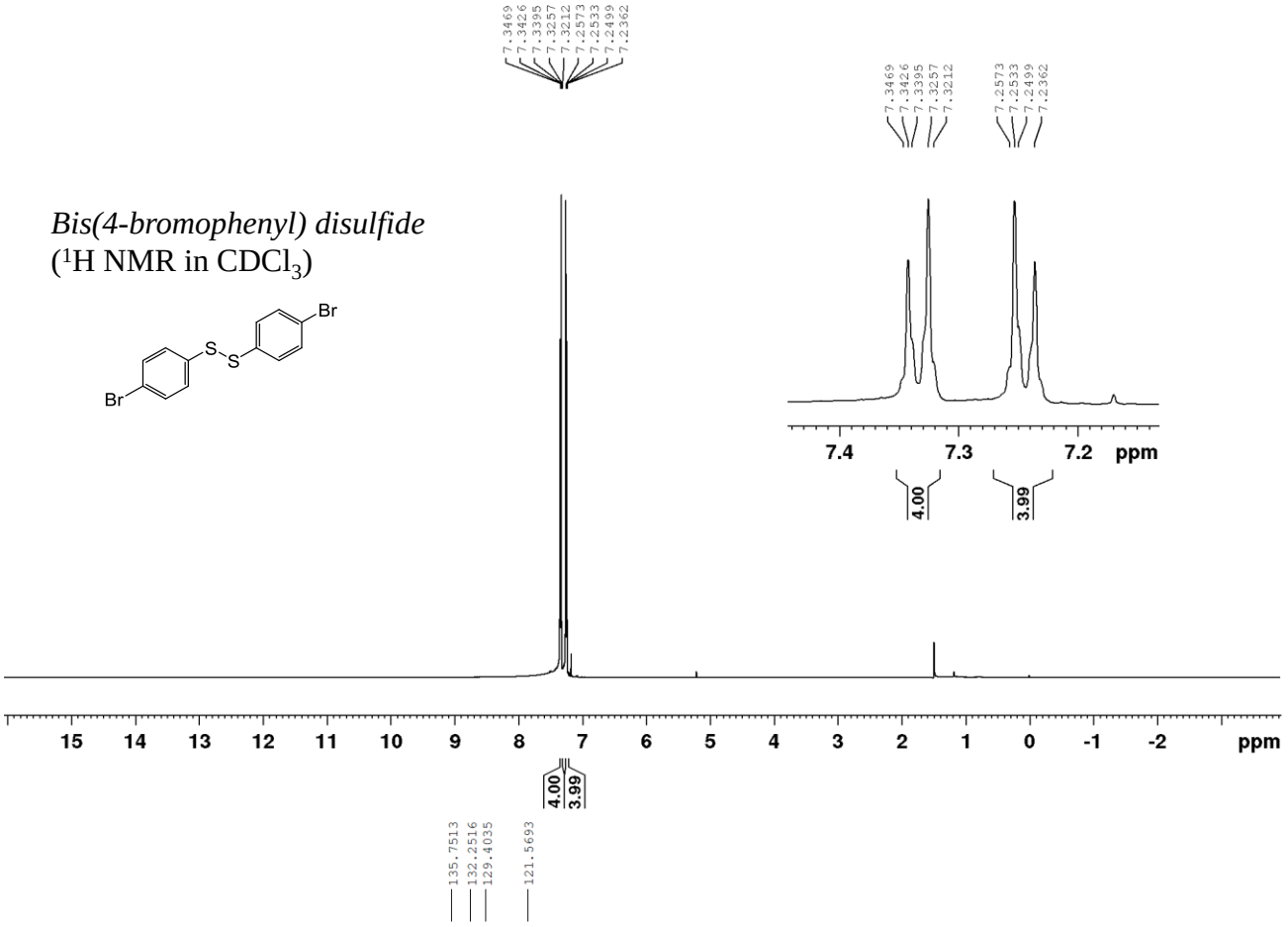
Bis(4-chlorophenyl) disulfide
(^{1}H NMR in $CDCl_3$)

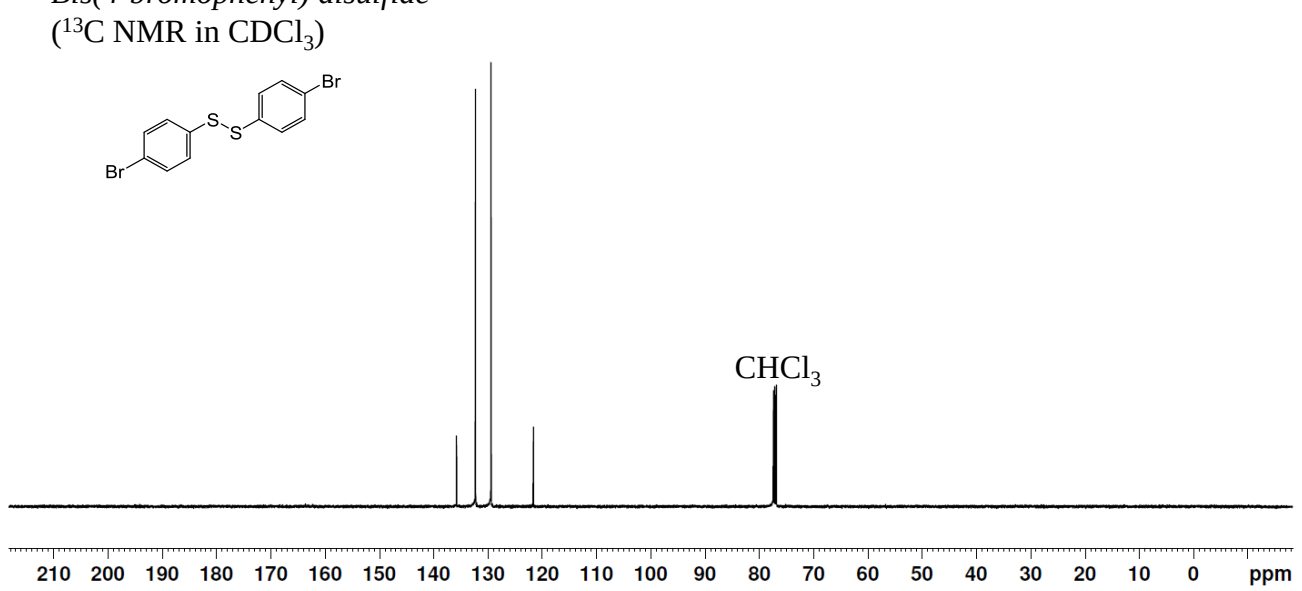


Bis(4-chlorophenyl) disulfide
(^{13}C NMR in $CDCl_3$)

Bis(4-bromophenyl) disulfide (**16i**)

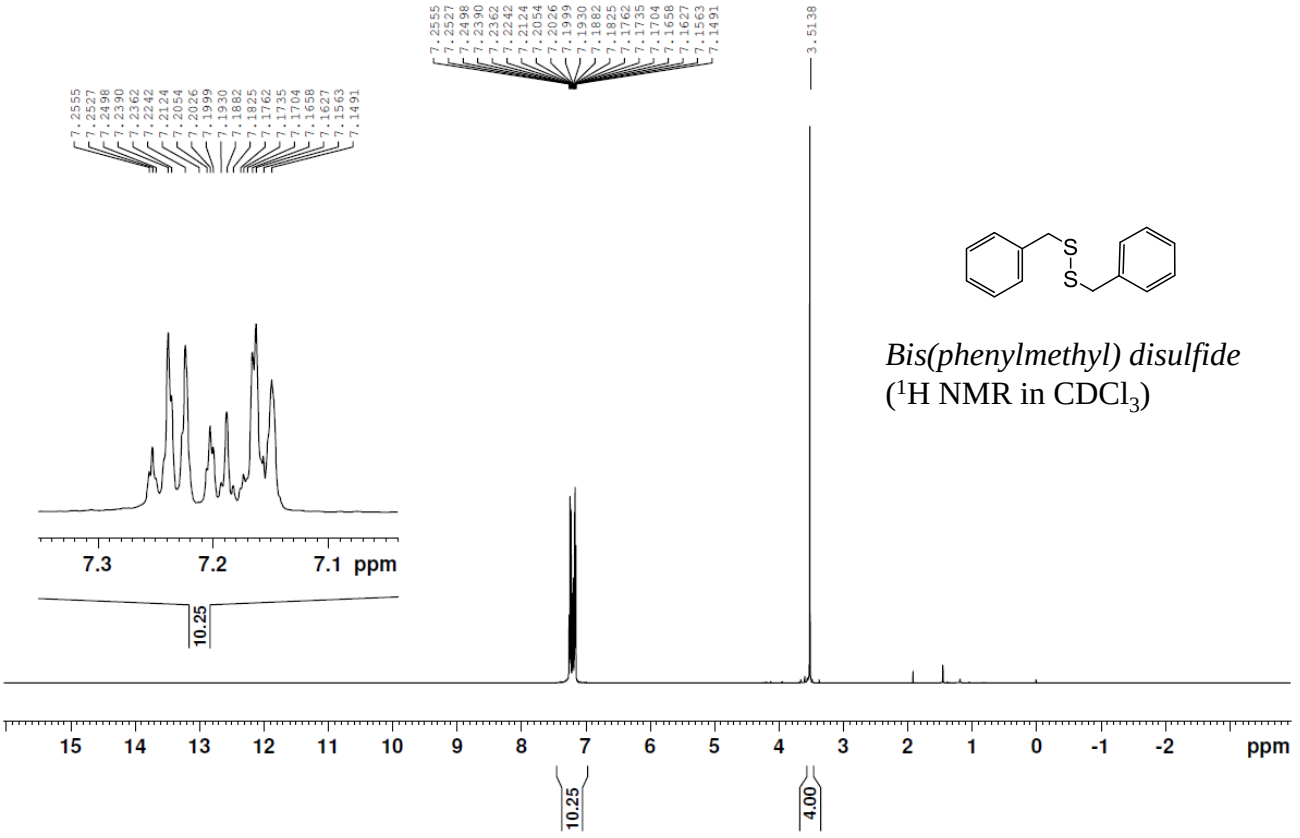
White solid; Yield in batch: 43.2 mg (quant.) and 41.1 mg (97%) using **4** and **6**, respectively; Yield in flow: 41.9 mg (99%) and 41.7 mg (98%) using **4** and **5**, respectively; ^{1}H NMR ($CDCl_3$): δ = 7.35–7.32 (m, 1H), 7.25–7.24 ppm (m, 1H); ^{13}C NMR ($CDCl_3$): δ = 135.8, 132.3, 129.4, 121.6 ppm. Spectroscopic data are in accordance with the previously presented.[1]



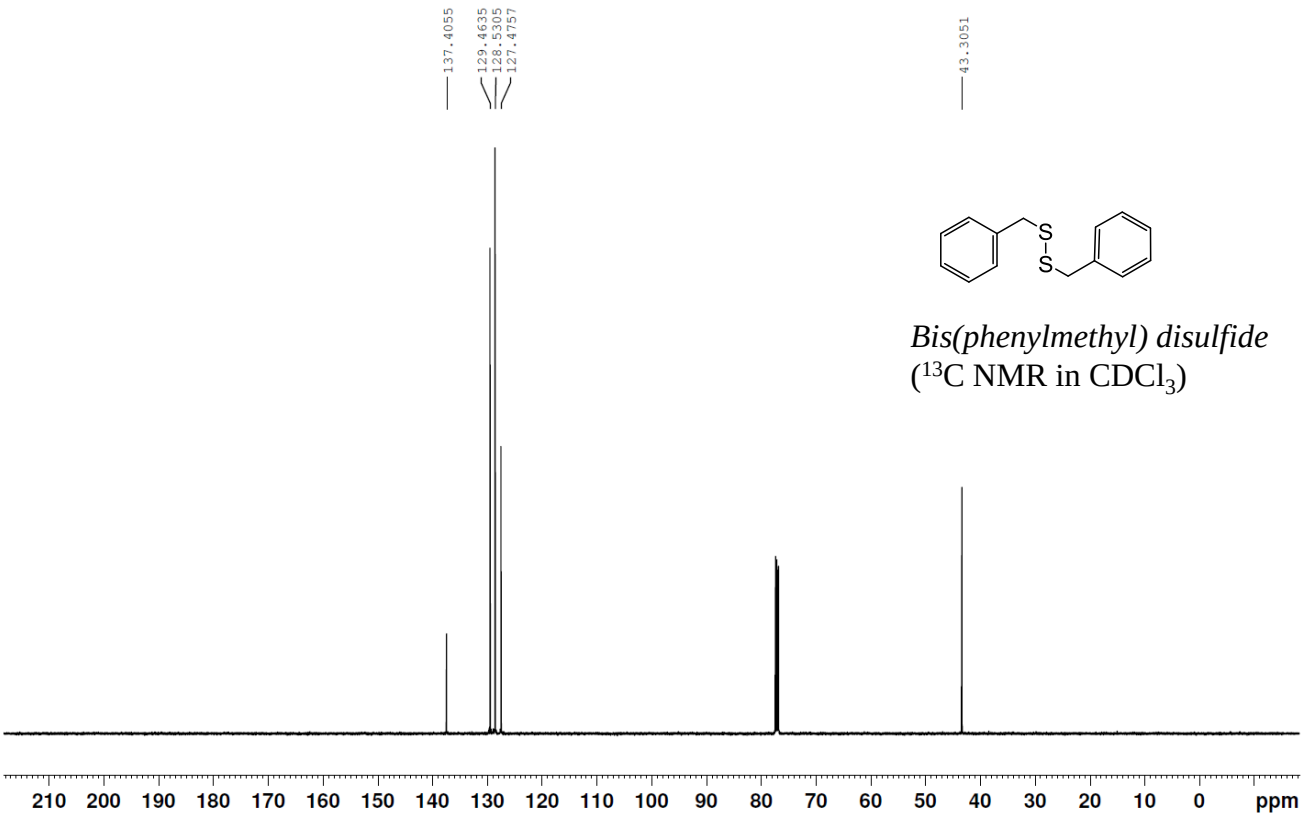


Bis(phenylmethyl) disulfide (**16j**)

White solid; Yield in batch: 26.3 mg (97%) and 29.3 mg (quant.) using **4** and **6**, respectively; Yield in flow: 27.5 mg (99%) and 27.5 mg (99%) using **4** and **5**, respectively; ^{1}H NMR ($CDCl_3$): δ = 7.26–7.15 (m, 10H), 3.51 ppm (s, 4H); ^{13}C NMR ($CDCl_3$): δ = 137.4, 129.5, 128.5, 127.5, 43.3 ppm. Spectroscopic data are in accordance with the previously presented.[1]



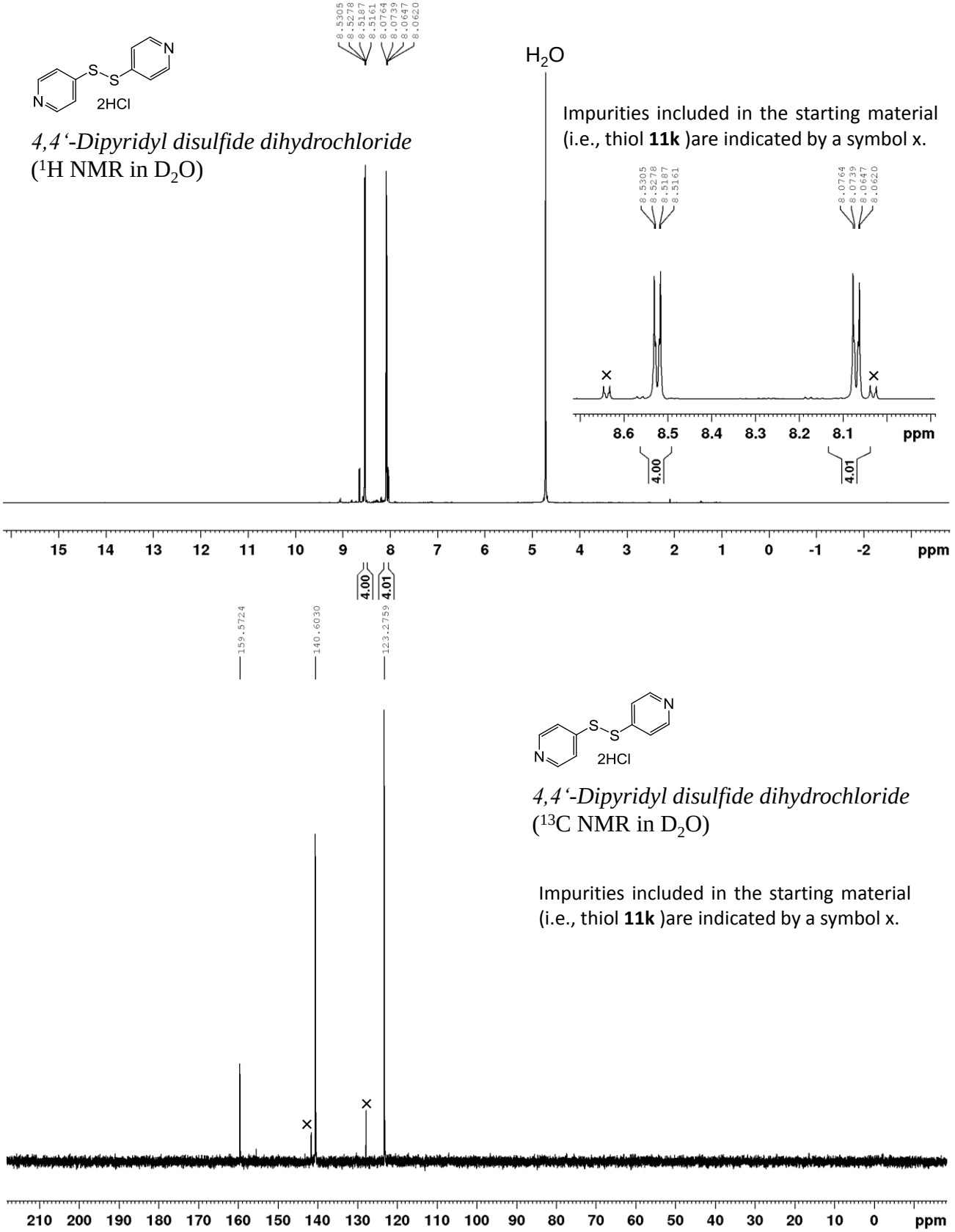
Bis(phenylmethyl) disulfide
(^{1}H NMR in $CDCl_3$)



Bis(phenylmethyl) disulfide
(^{13}C NMR in $CDCl_3$)

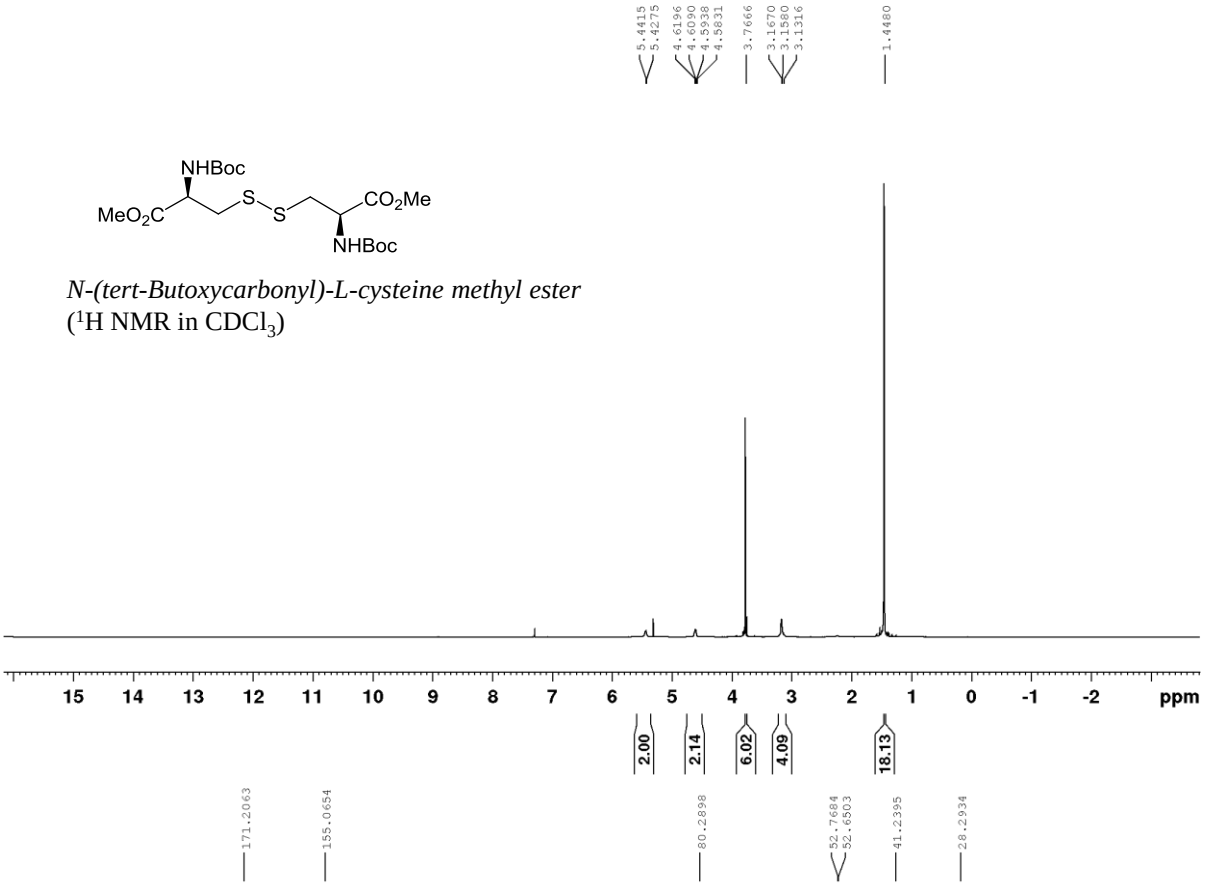
4,4'-Dipyridyl disulfide dihydrochloride (**16k**)

Off yellow solid; Yield in batch: 33.5 mg (quant.) using **7**; Yield in flow: 33.3 mg (quant.) using **7**; ^{1}H NMR (D_2O): δ = 8.53–8.52 (m, 4H), 8.08–8.06 ppm (m, 4H); ^{13}C NMR (D_2O): δ = 159.6, 140.6, 123.3 ppm. HRMS (ESI+): m/z calcd for $C_{10}H_9N_2S_2^+$: 221.0202 $[M+H-2HCl]^+$; found: 221.0205.

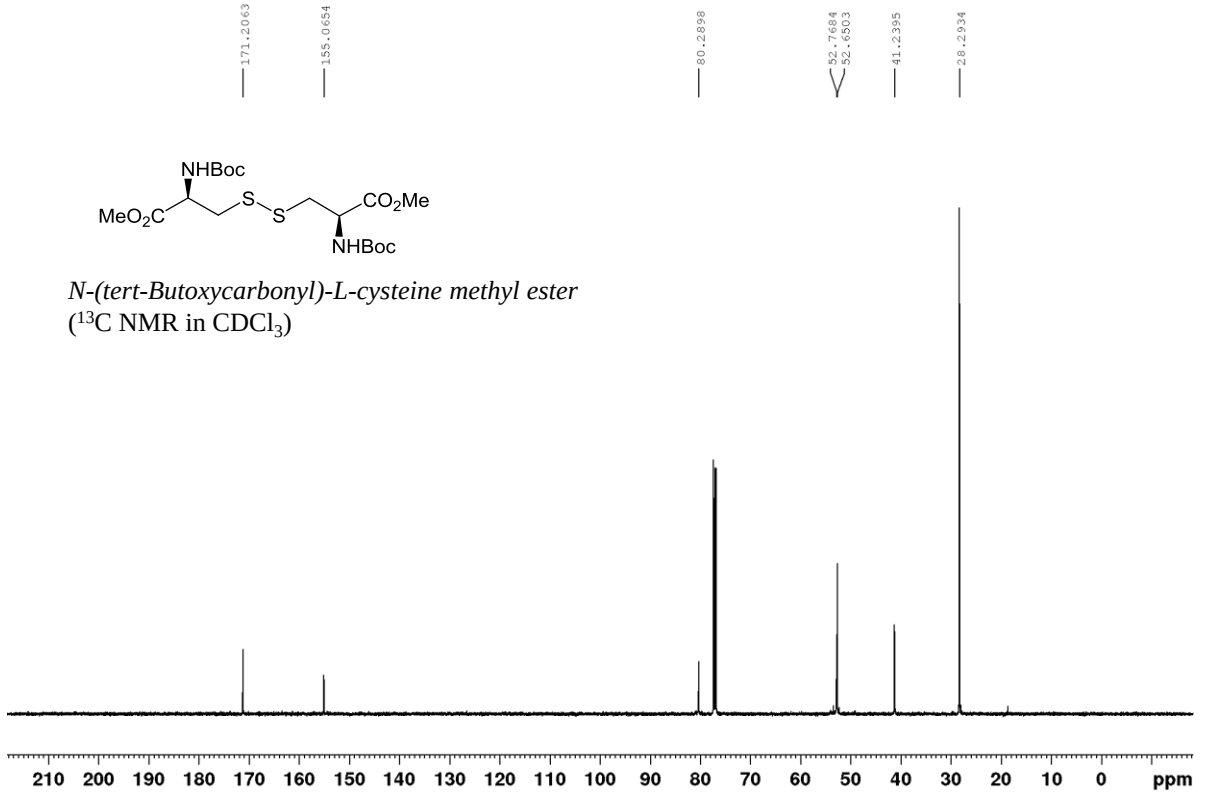


N-(tert-Butoxycarbonyl)-L-cysteine methyl ester (**16l**)

Colorless oil; Yield in batch: 54.2 mg (quant.) and 47.1 mg (89%) using **4** and **6**, respectively; Yield in flow: 50.7 mg (96%) and 51.2 mg (97%) using **4** and **5**, respectively; ^{1}H NMR ($CDCl_3$): δ = 5.43 (br d, *J* = 7.0 Hz, 2H), 4.62–4.58 (m, 2H), 3.77 (s, 6H), 3.17–3.13 (m, 4H), 1.45 ppm (s, 18H); ^{13}C NMR ($CDCl_3$): δ = 171.2, 155.1, 80.3, 52.8, 52.7, 41.2, 28.3 ppm; LRMS (APCI+): m/z calcd for $C_{18}H_{33}N_2O_8S_2^+$: 469.17 $[M+H]^+$; found: 469.19. Spectroscopic data are in accordance with the previously presented.[5]



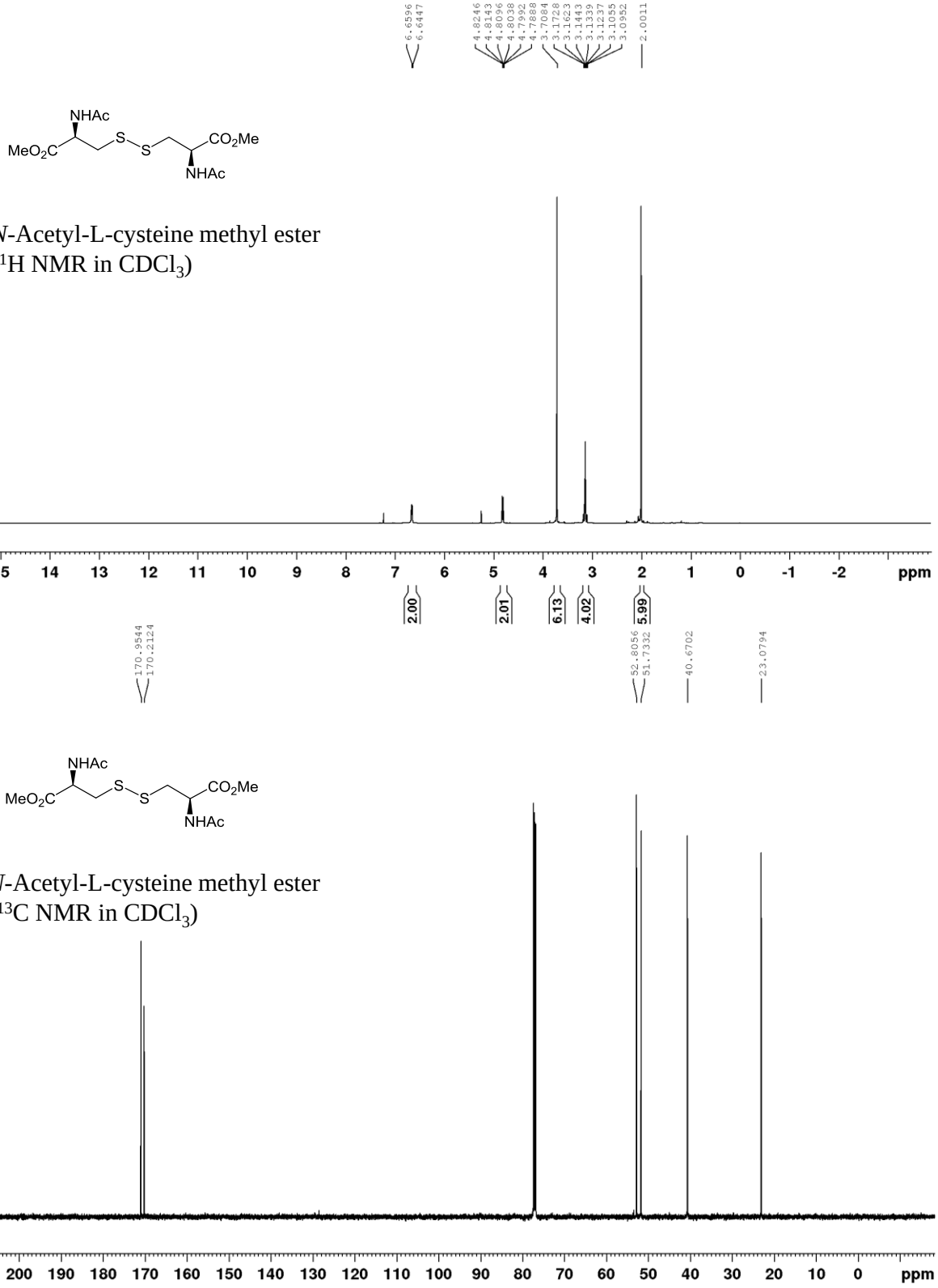
N-(tert-Butoxycarbonyl)-L-cysteine methyl ester
(^{1}H NMR in $CDCl_3$)



N-(tert-Butoxycarbonyl)-L-cysteine methyl ester
(^{13}C NMR in $CDCl_3$)

N-Acetyl-L-cysteine methyl ester (**16m**)

White solid; Yield in batch: 28.6 mg (72%) and 30.9 mg (78%) using **4** and **6**, respectively; Yield in flow: 28.5 mg (71%) and 27.0 mg (68%) using **4** and **5**, respectively; ^{1}H NMR ($CDCl_3$): δ = 6.65 (d, *J* = 7.45 Hz, 2H), 4.82–4.79 (m, 2H), 3.71 (s, 6H), 3.17–3.10 (m, 4H), 2.00 ppm (s, 6H); ^{13}C NMR ($CDCl_3$): δ = 170.9, 170.2, 52.8, 51.7, 40.7, 23.1 ppm; LRMS (APCI+): m/z calcd for $C_{12}H_{21}N_2O_6S_2^+$: 353.08 $[M+H]^+$; found: 353.10.[6]

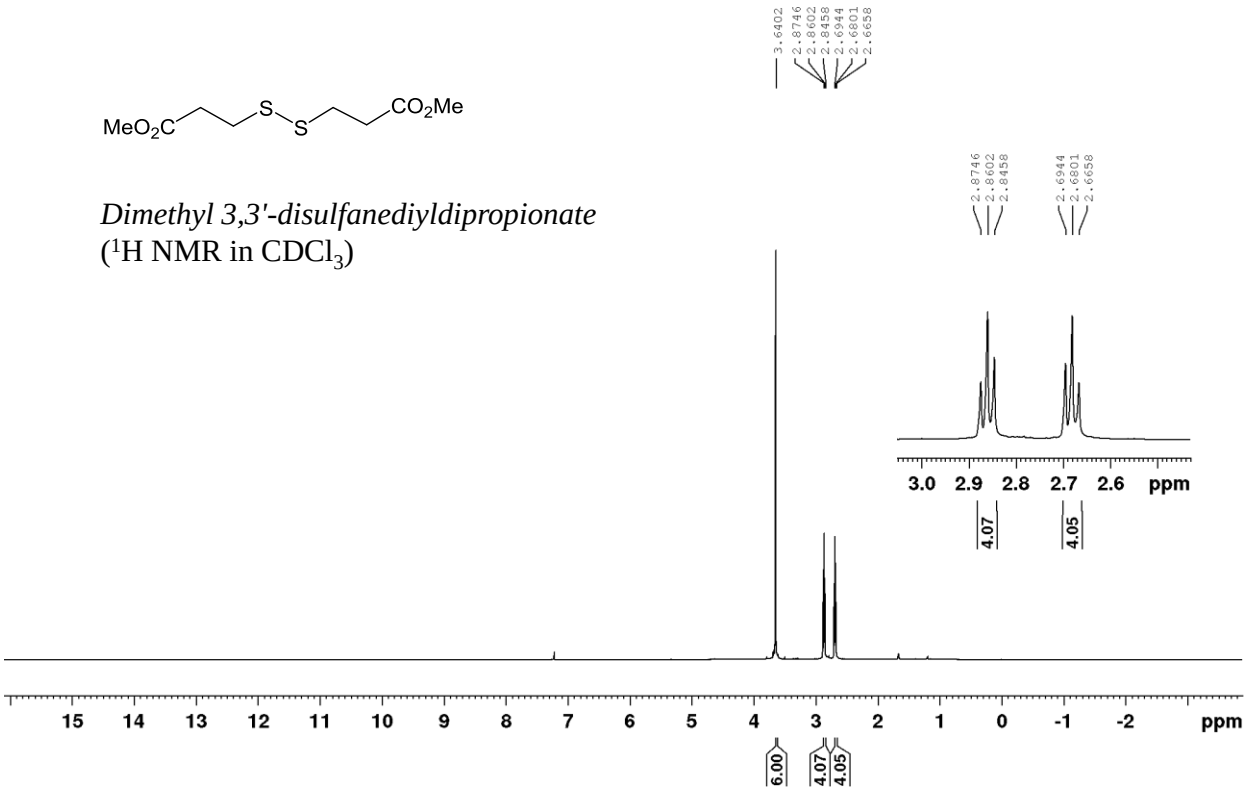


N-Acetyl-L-cysteine methyl ester (^{1}H NMR in $CDCl_3$)

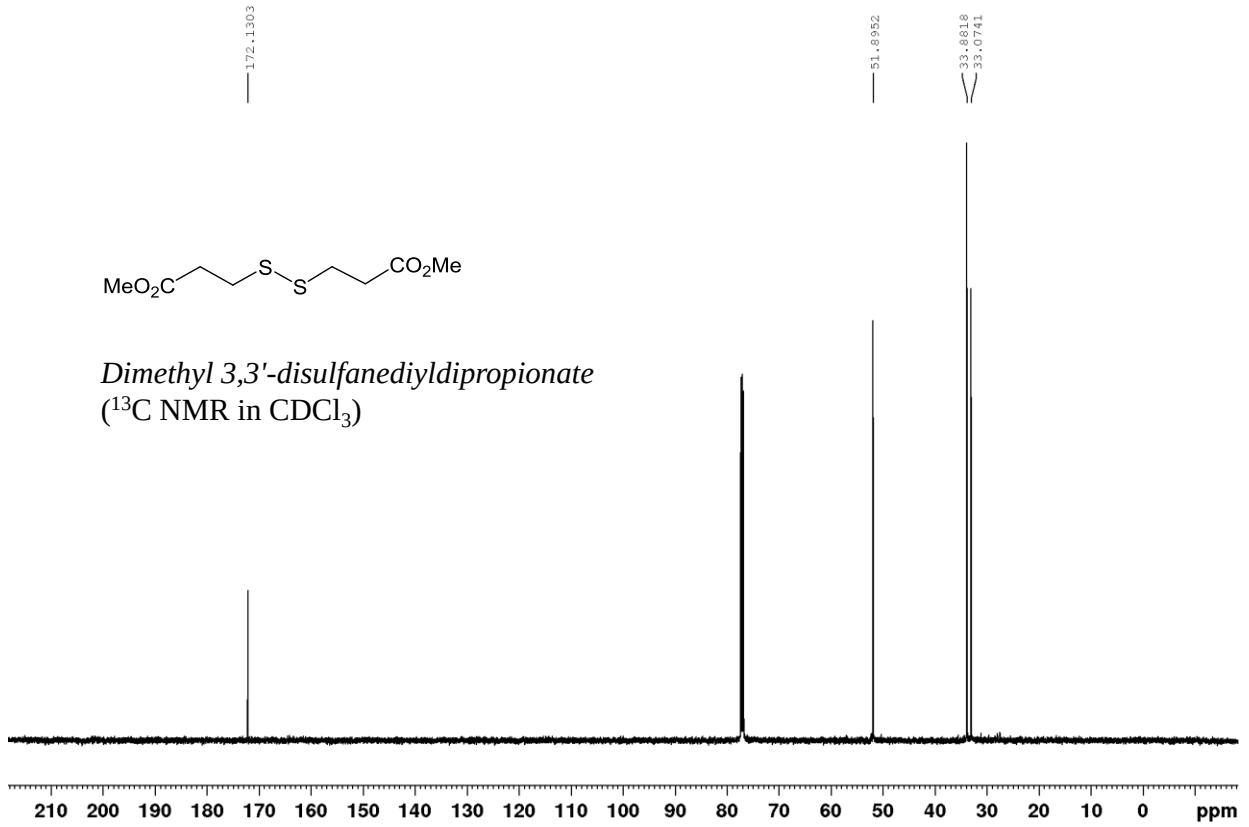
N-Acetyl-L-cysteine methyl ester (^{13}C NMR in $CDCl_3$)

Dimethyl 3,3'-disulfanediyldipropionate (**16n**)

Colorless liquid; Yield in batch: 26.6 mg (92%) and 21.5 mg (79%) using **4** and **6**, respectively; Yield in flow: 26.4 mg (98%) and 27.5 mg (quant) using **4** and **5**, respectively; ^{1}H NMR ($CDCl_3$): δ = 3.64 (s, 6H), 2.86 (t, *J* = 7.2 Hz, 4H), 2.68 ppm (t, *J* = 7.2 Hz, 4H); ^{13}C NMR ($CDCl_3$): δ = 172.1, 51.9, 33.9, 33.1 ppm; LRMS (APCI+): m/z calcd for $C_8H_{15}O_4S_2^+$: 239.04 $[M–H]^+$; found: 239.04. Spectroscopic data are in accordance with the previously presented.[7]



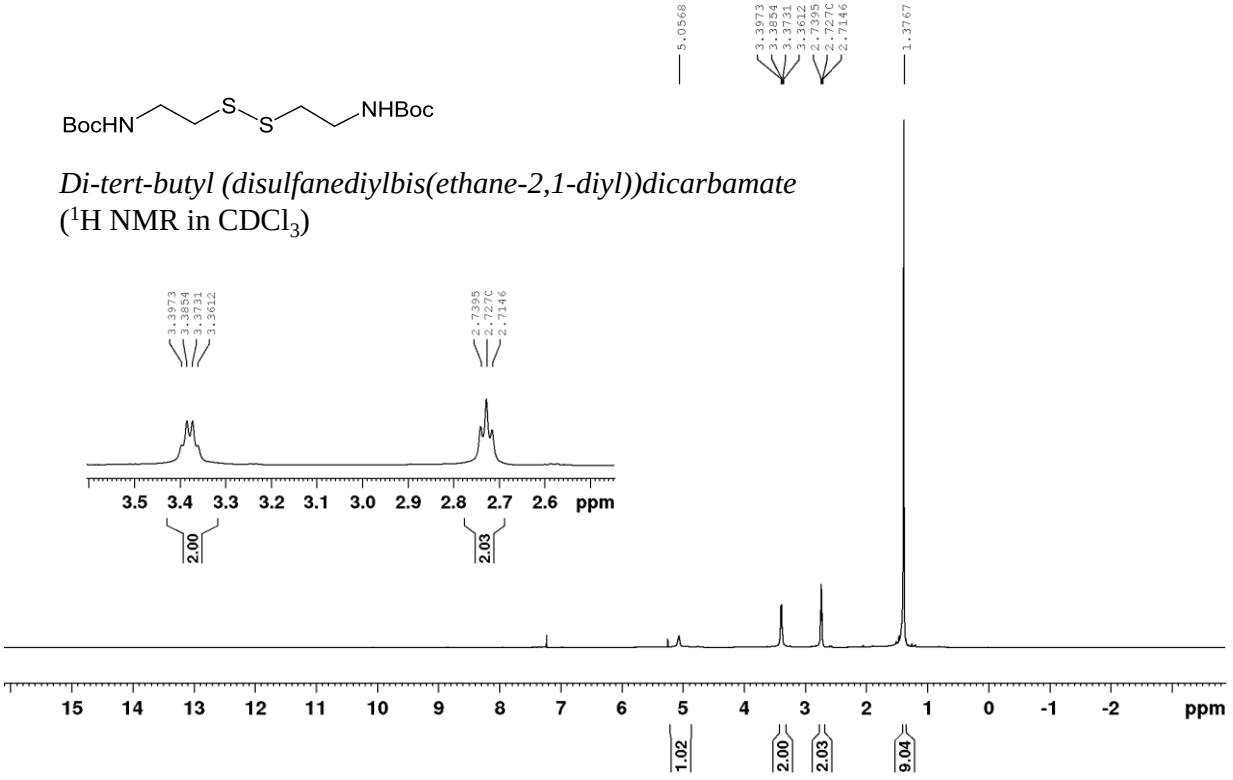
Dimethyl 3,3'-disulfanediyldipropionate
(^{1}H NMR in $CDCl_3$)



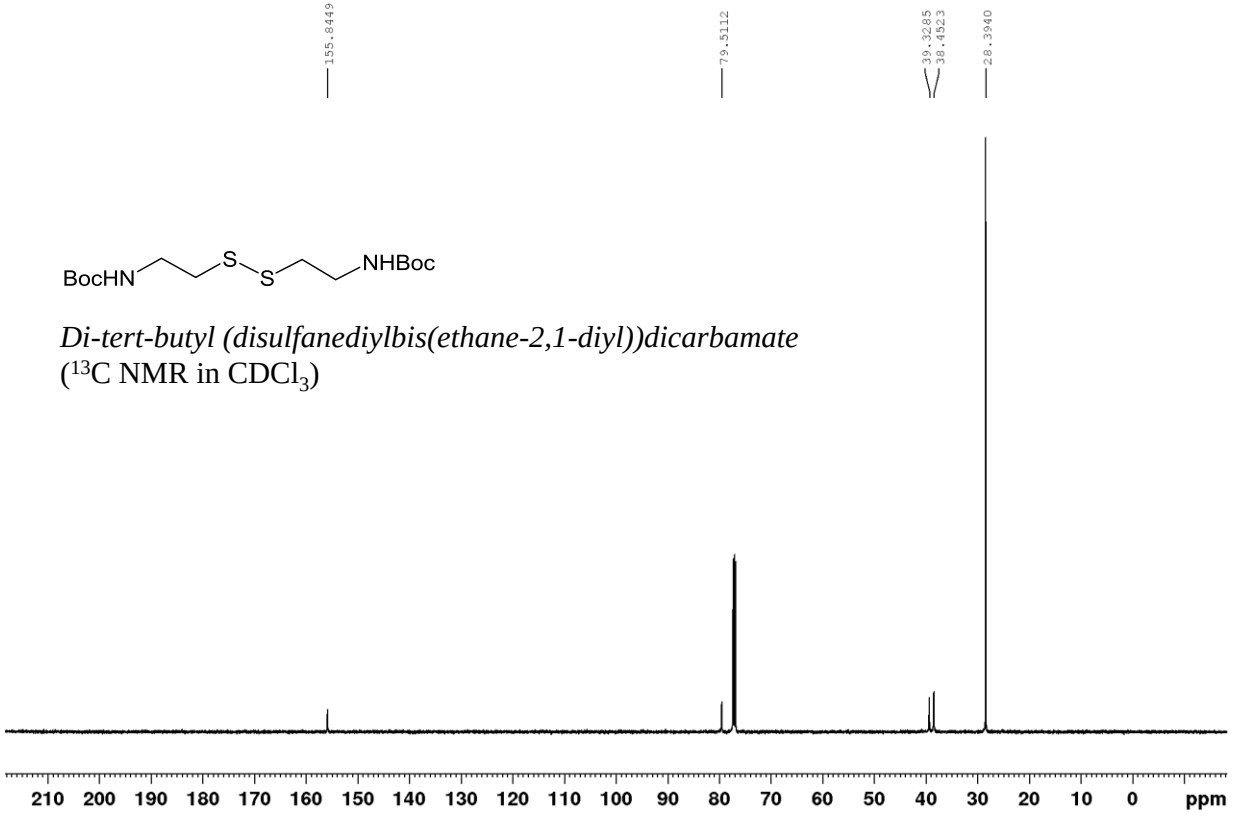
Dimethyl 3,3'-disulfanediyldipropionate
(^{13}C NMR in $CDCl_3$)

Di-tert-butyl (disulfanediylbis(ethane-2,1-diyl))dicarbamate (**16o**)

White solid; Yield in batch: 38.5 mg (95%) and 31.5 mg (80%) using **4** and **6**, respectively; Yield in flow: 37.0 mg (93%) and 37.2 mg (93%) using **4** and **5**, respectively; ^{1}H NMR ($CDCl_3$): δ = 5.06 (br s, 2H), 3.38 (q, *J* = 6.0 Hz, 4H), 2.73 (t, *J* = 6.3 Hz, 4H), 1.38 (s, 18H) ppm; ^{13}C NMR ($CDCl_3$): δ = 155.8, 79.5, 39.3, 38.5, 28.4 ppm; LRMS (APCI+): m/z calcd for $C_{14}H_{29}N_2O_4S_2^+$: 353.16 $[M–H]^+$; found: 353.15. Spectroscopic data are in accordance with the previously presented.[8]



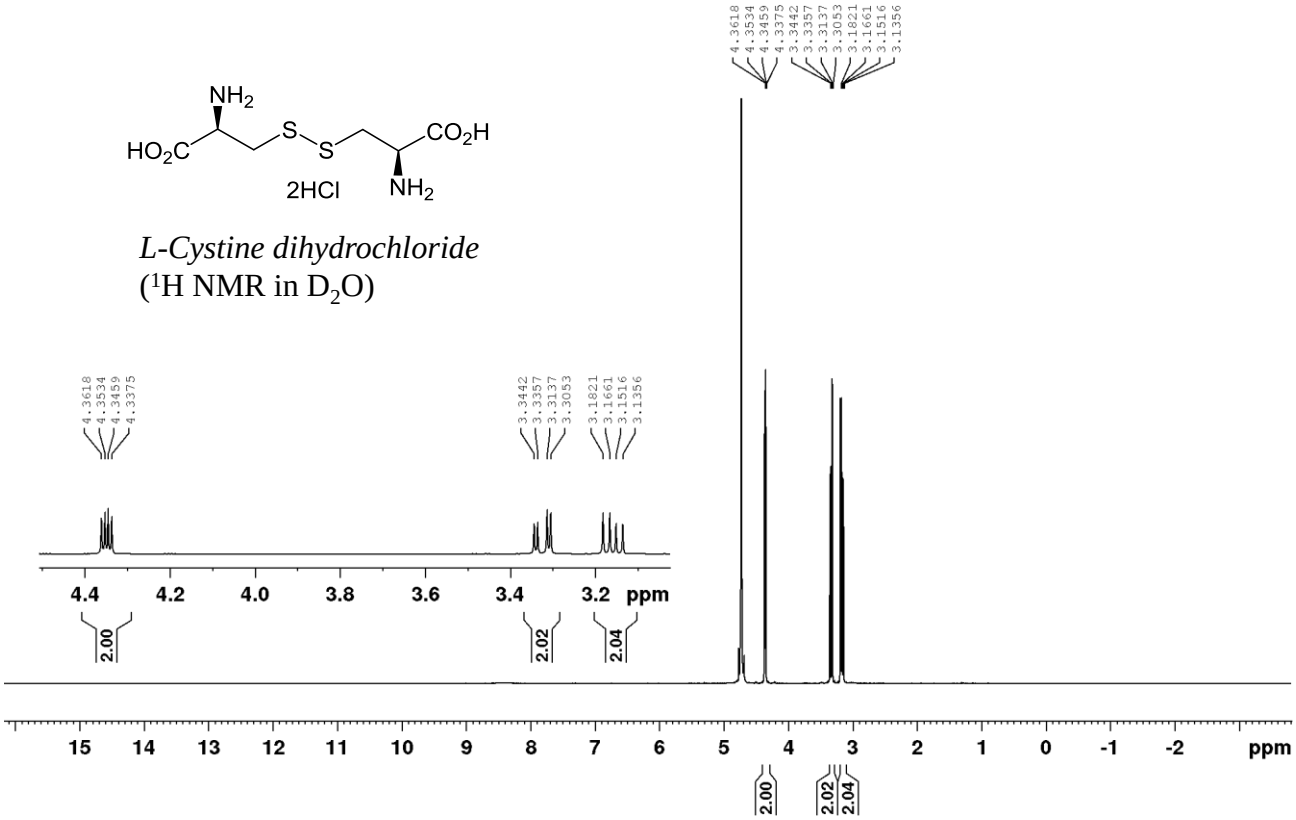
Di-tert-butyl (disulfanediylbis(ethane-2,1-diyl))dicarbamate (^{1}H NMR in $CDCl_3$)

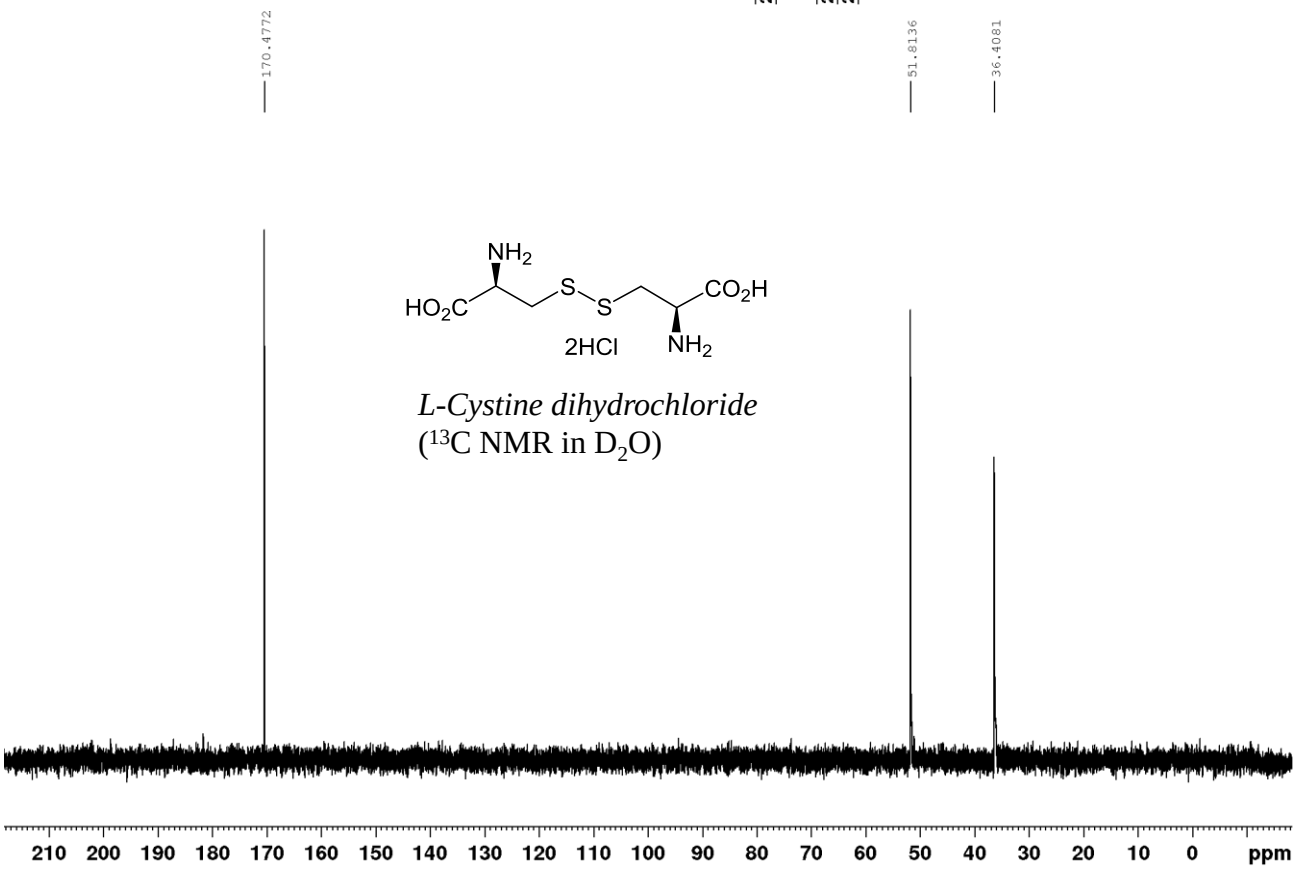


Di-tert-butyl (disulfanediylbis(ethane-2,1-diyl))dicarbamate (^{13}C NMR in $CDCl_3$)

L-Cystine dihydrochloride (**16p**)

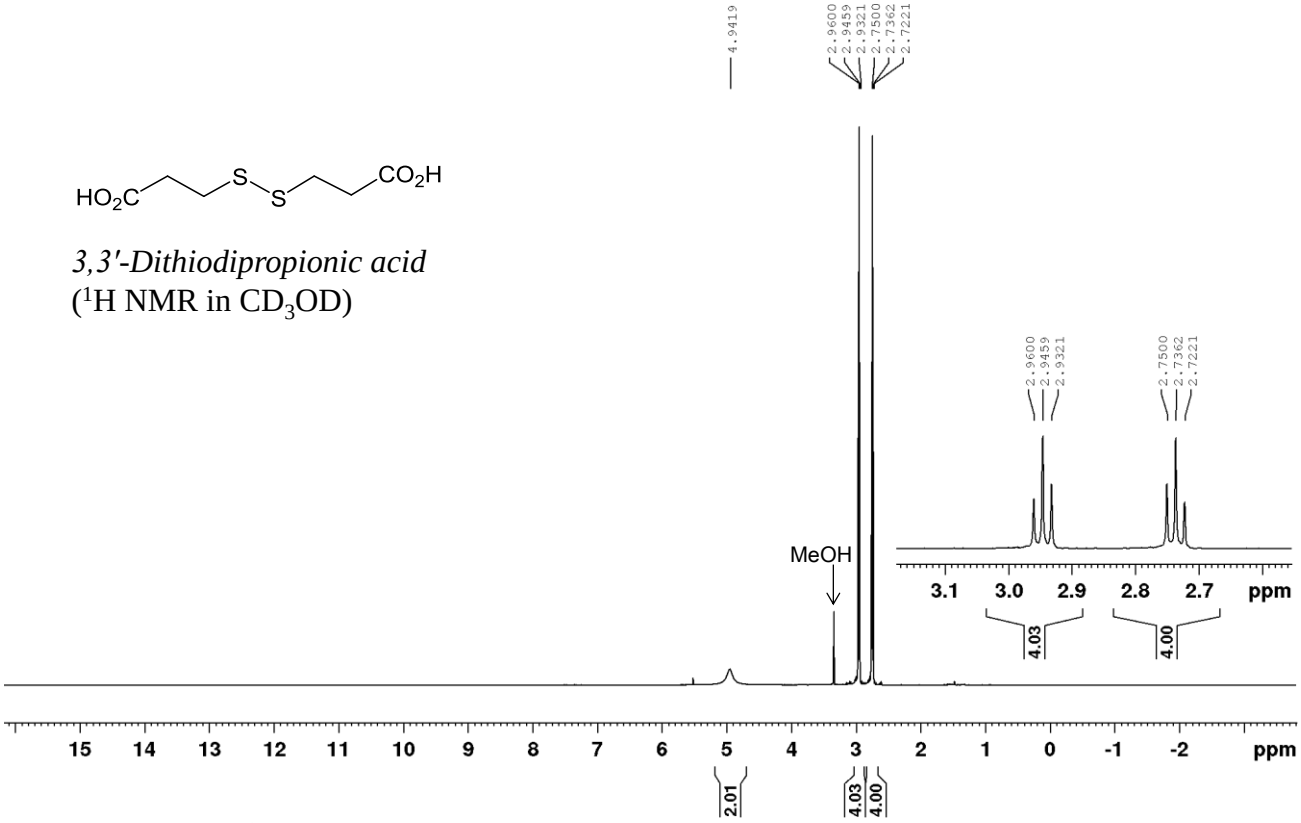
White solid; Yield in batch: 33.2 mg (94%); Yield in flow: 35.5 mg (quant.); ^{1}H NMR (D_2O): δ = 4.35 (dd, *J* = 4.2, 8.0 Hz, 2H), 3.32 (dd, *J* = 4.3, 15.2 Hz, 2H), 3.16 ppm (dd, *J* = 8.0, 15.3 Hz, 2H); ^{13}C NMR (D_2O): δ = 170.5, 51.8, 36.4 ppm; LRMS (APCI+): m/z calcd for $C_6H_{13}N_2O_4S_2^+$: 241.03 [M+H–2HCl]$^+$; found: 241.06. Spectroscopic data are in accordance with the previously presented.[9]



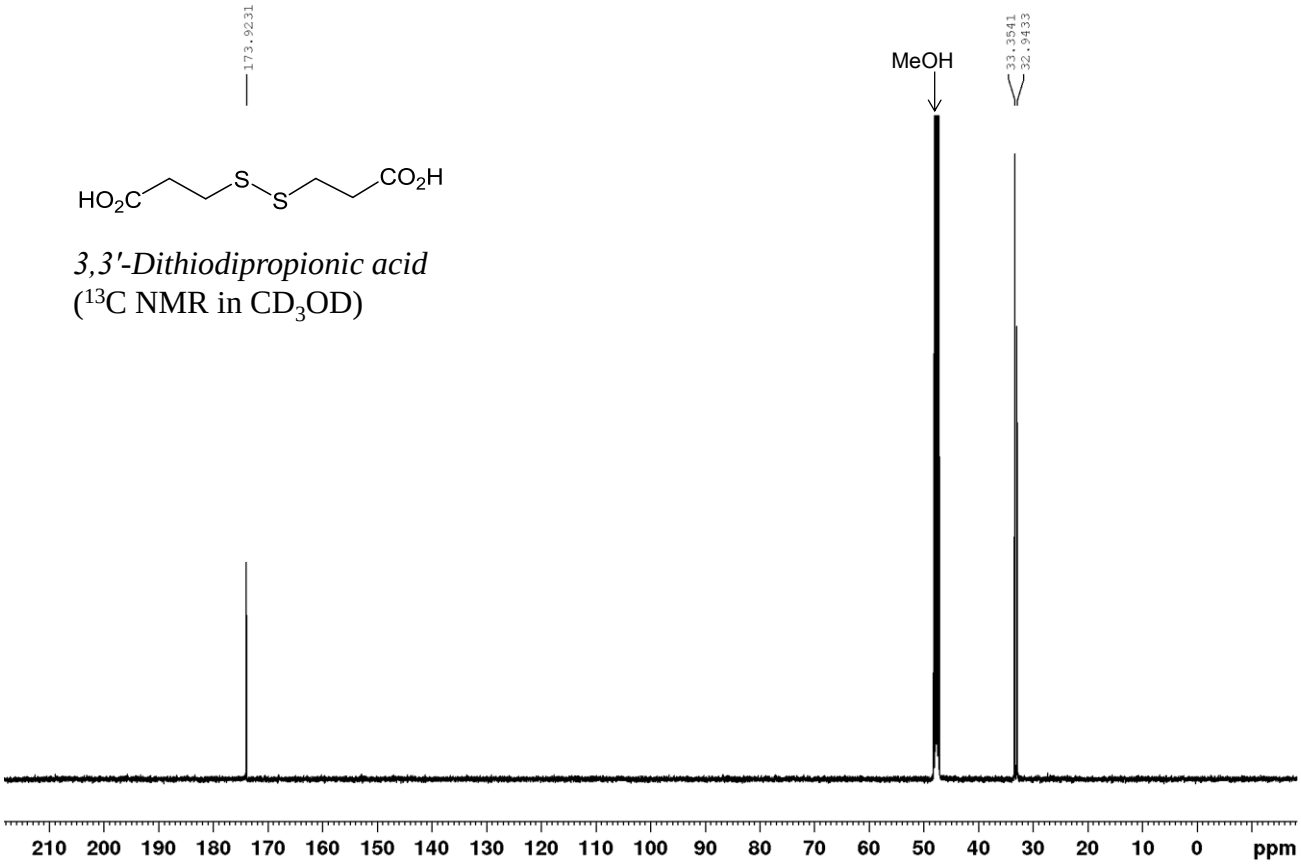


3,3′-Dithiodipropionic acid (**16q**)

White solid; Yield in batch: 23.2 mg (98%); Yield in flow: 23.1 mg (98%); ^{1}H NMR (CD_3OD): δ = 4.94 (br s, 1H), 2.95 (t, *J* = 7.1 Hz, 4H), 2.74 ppm (t, *J* = 6.9 Hz, 4H); ^{13}C NMR (CD_3OD): δ = 173.9, 33.4, 32.9 ppm; LRMS (APCI−): m/z calcd for $C_6H_9O_4S_2^-$: 208.99 $[M–H]^+$; found: 208.98. Spectroscopic data are in accordance with the previously presented.[10]



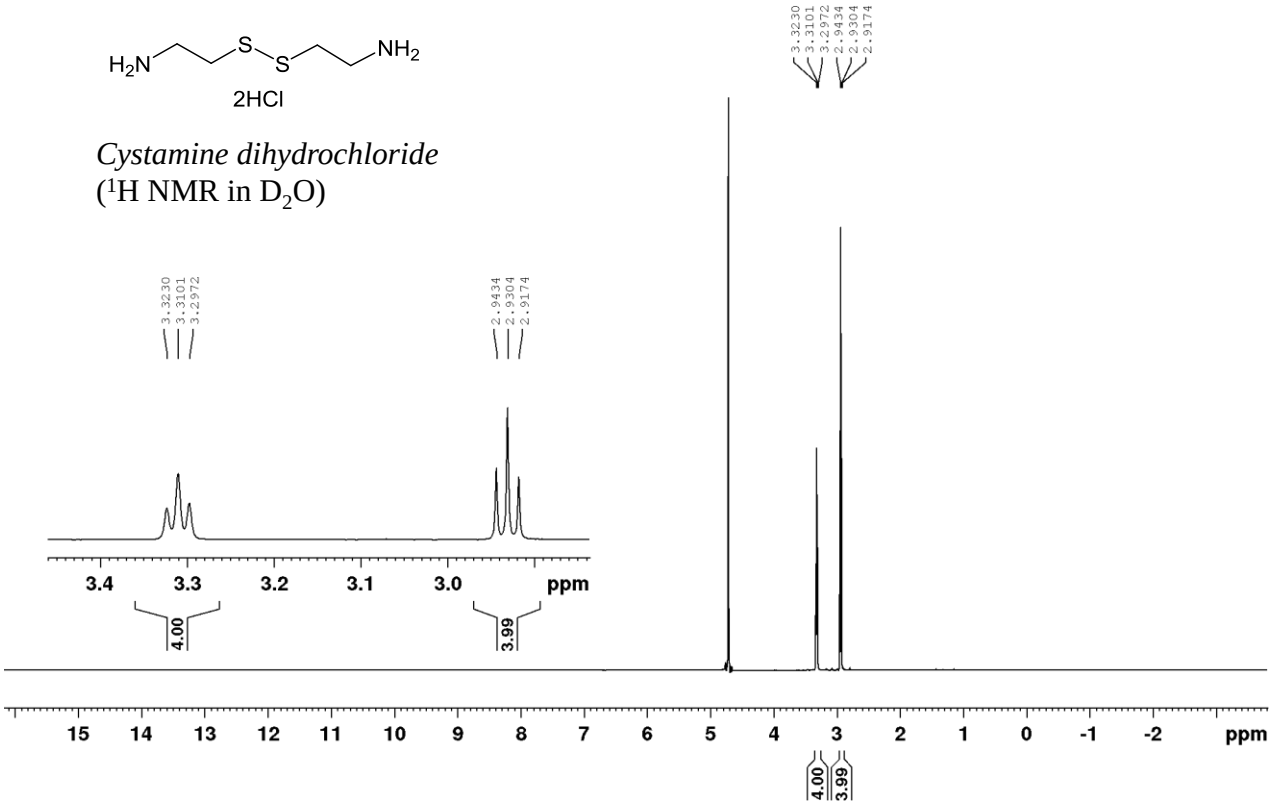
3,3′-Dithiodipropionic acid
(^{1}H NMR in CD_3OD)

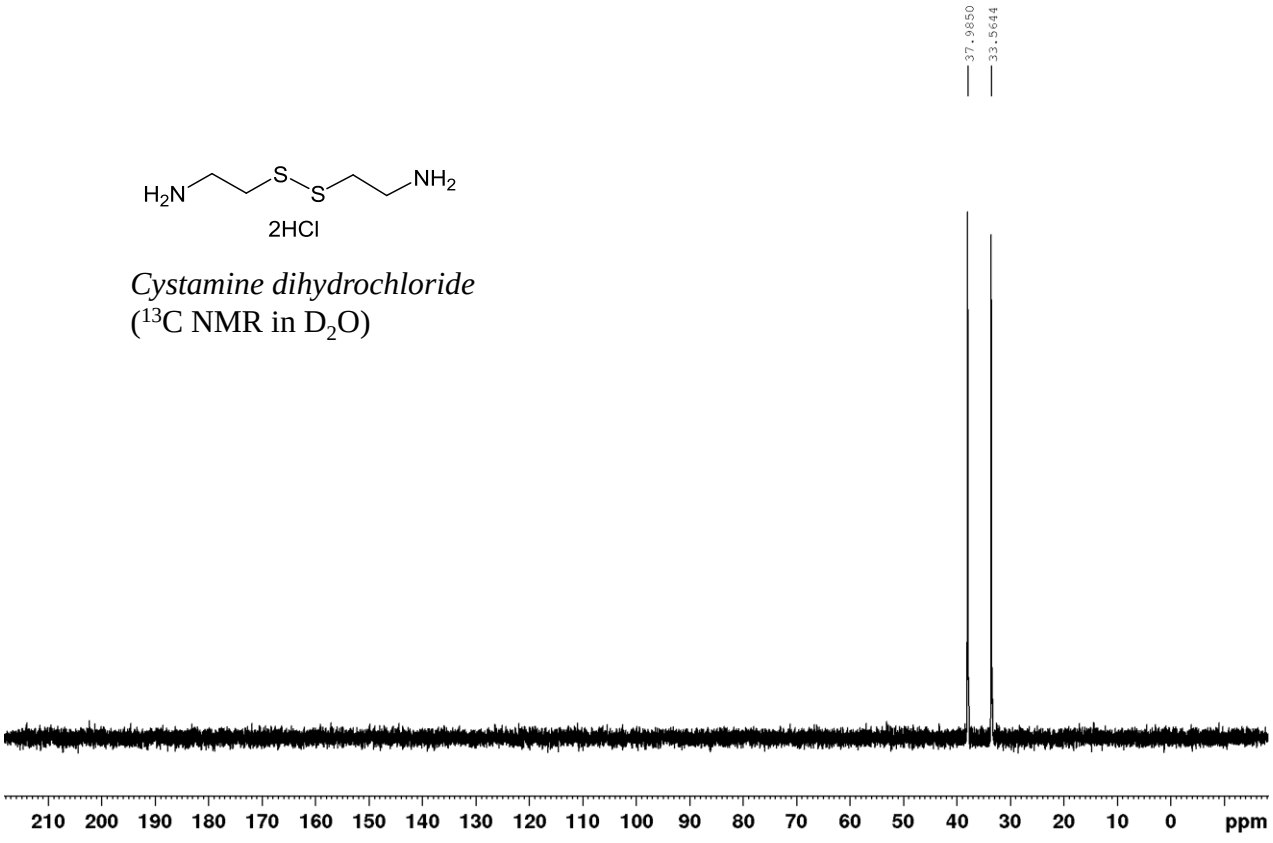


3,3′-Dithiodipropionic acid
(^{13}C NMR in CD_3OD)

Cystamine dihydrochloride (**16r**)

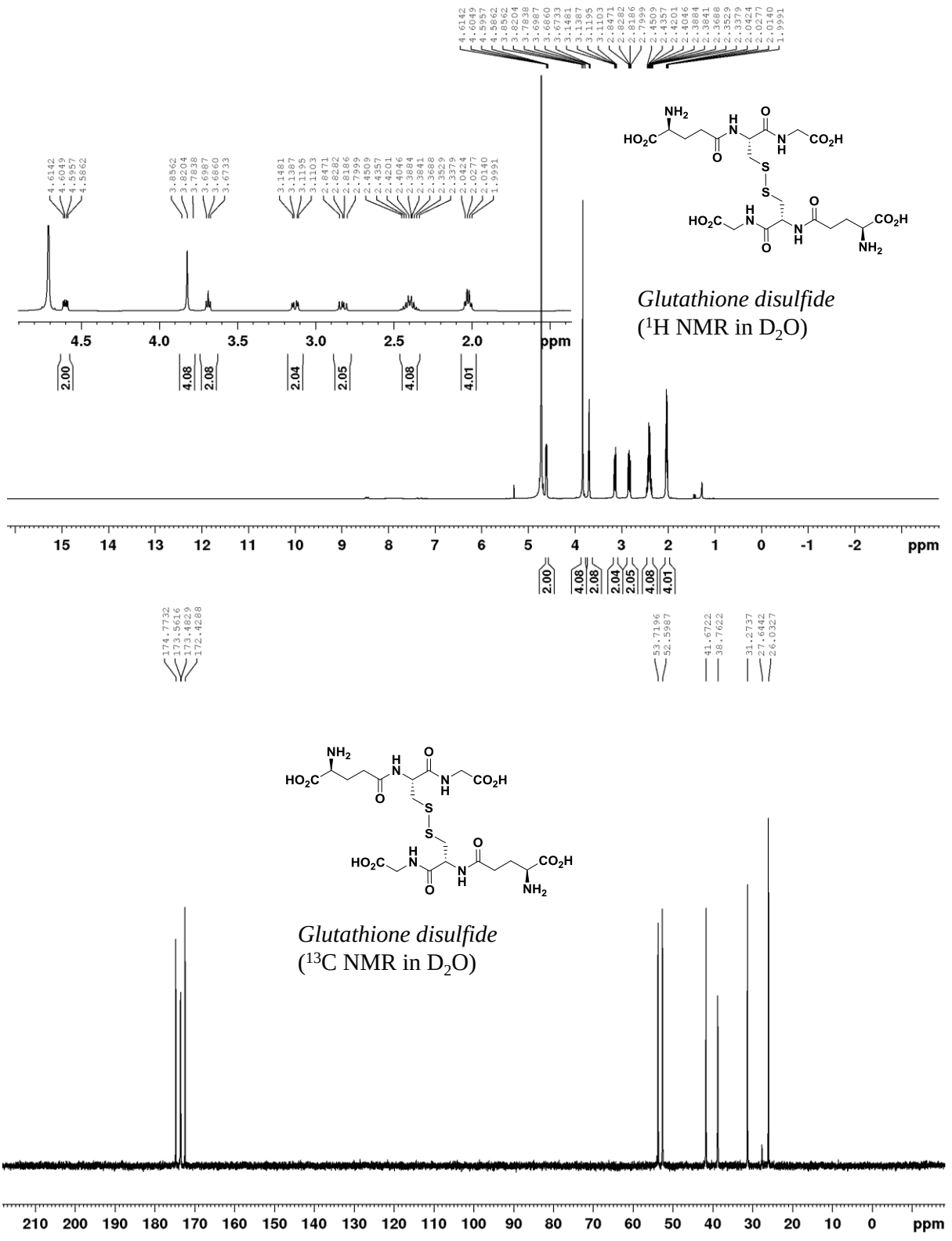
White solid; Yield in batch: 24.3 mg (96%); Yield in flow: 25.4 mg (quant.); ^{1}H NMR (D_2O): δ = 3.31 (t, *J* = 6.5 Hz, 2H), 2.92 (t, *J* = 6.5 Hz, 2H), 2.68 ppm (t, *J* = 7.2 Hz, 4H); ^{13}C NMR ($CDCl_3$): δ = 38.0, 33.6 ppm; LRMS (APCI+): m/z calcd for $C_4H_{13}N_2S_2^+$: 153.05 [M+H–2HCl]$^+$; found: 153.05. Spectroscopic data are in accordance with the previously presented.[11]





Glutathione disulfide (**16s**)

White solid; Yield in batch: 69.7 mg (quant.); Yield in flow: 69.0 mg (quant.); in a flow system; ^{1}H NMR (D_2O): δ = 4.60 (dd, *J* = 4.65, 9.35 Hz, 2H), 3.86–3.78 (m, 4H), 3.69 ppm (t, *J* = 5.9 Hz, 2H) , 3.13 ppm (dd, *J* = 4.7, 14.2 Hz, 2H), 2.84–2.80 ppm (m, 2H), 2.45–2.34 ppm (m, 4H), 2.04–2.00 ppm (m, 4H); ^{13}C NMR (D_2O): δ = 174.7, 173.6, 173.5, 172.4, 53.7, 52.6, 41.7, 38.8, 31.3, 27.6, 26.0 ppm; LRMS (APCI +): m/z calcd for $C_{20}H_{33}N_6O_{12}S_2^+$: 613.16 $[M+H]^+$; found: 613.23. Spectroscopic data are in accordance with the previously presented.[9]



2. Supplemental Figures and Tables

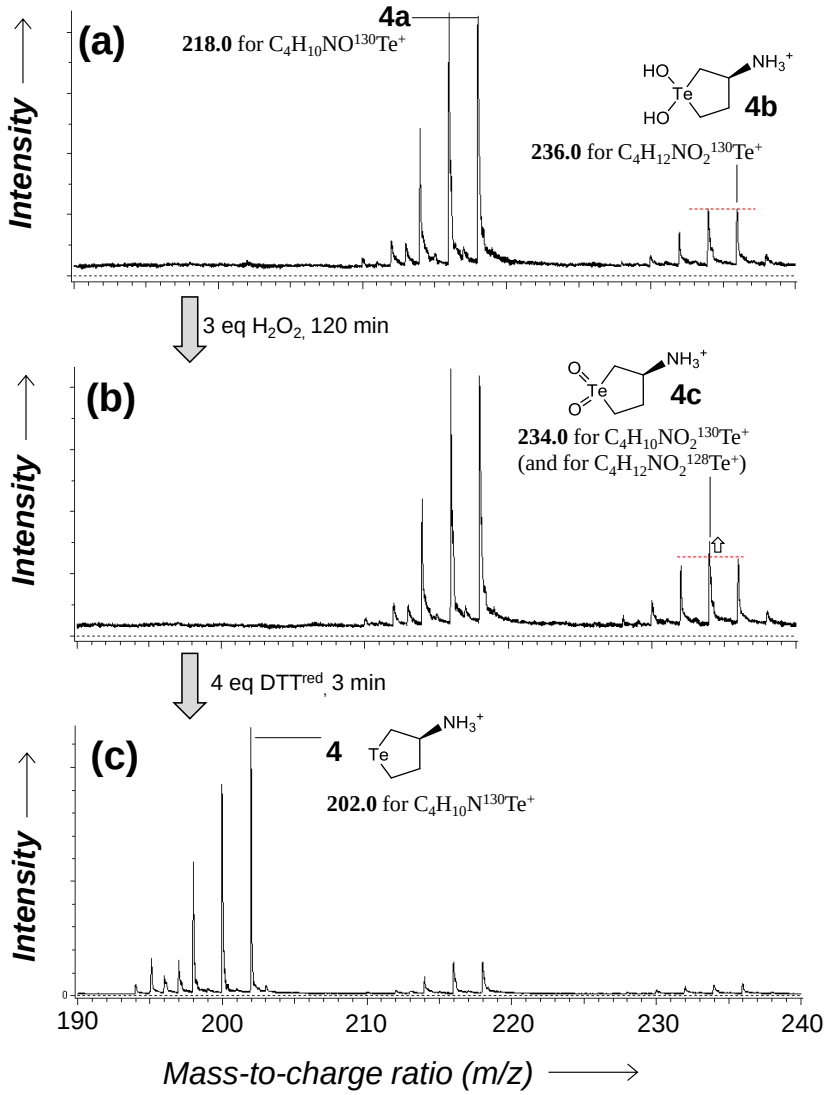


Fig. S1 ESI(+)-MS spectral changes upon the oxidation and subsequent reduction of **4** in H_2O at 25 °C. Reaction conditions: (**a**) **4** (0.47 μmol) and H_2O_2 (0.47 μmol) were mixed in H_2O. (**b**) to **a** was added H_2O_2 (1.41 μmol); (**c**) to **b** was added DTT^{red} (1.88 μmol).

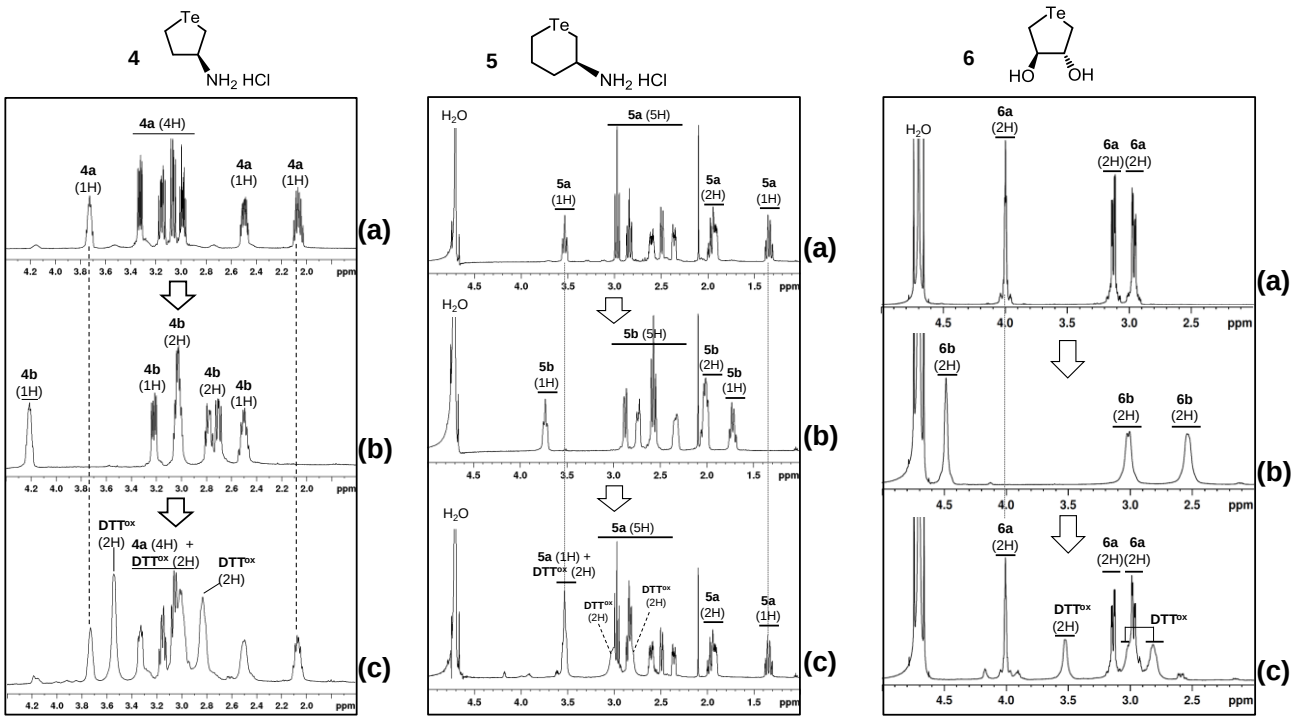


Fig. S2 ^{1}H NMR spectral changes upon oxidation and subsequent reduction of tellurides in D_2O at 25 °C. **Left panel:** (a), **4** (24 μmol) in D_2O (500 μL); (b), (a) + H_2O_2 (24 μmol); (c), (b) + DTT^{red} (24 μmol). **Middle panel:** (a), **5** (24 μmol) in D_2O (500 μL); (b), (a) + H_2O_2 (24 μmol); (c), (b) + DTT^{red} (24 μmol). **Right panel:** (a), **6** (24 μmol) in D_2O (500 μL); (b), (a) + H_2O_2 (24 μmol); (c), (b) + DTT^{red} (24 μmol). All sample solutions were incubated for 5 min at 25°C before the NMR measurements.

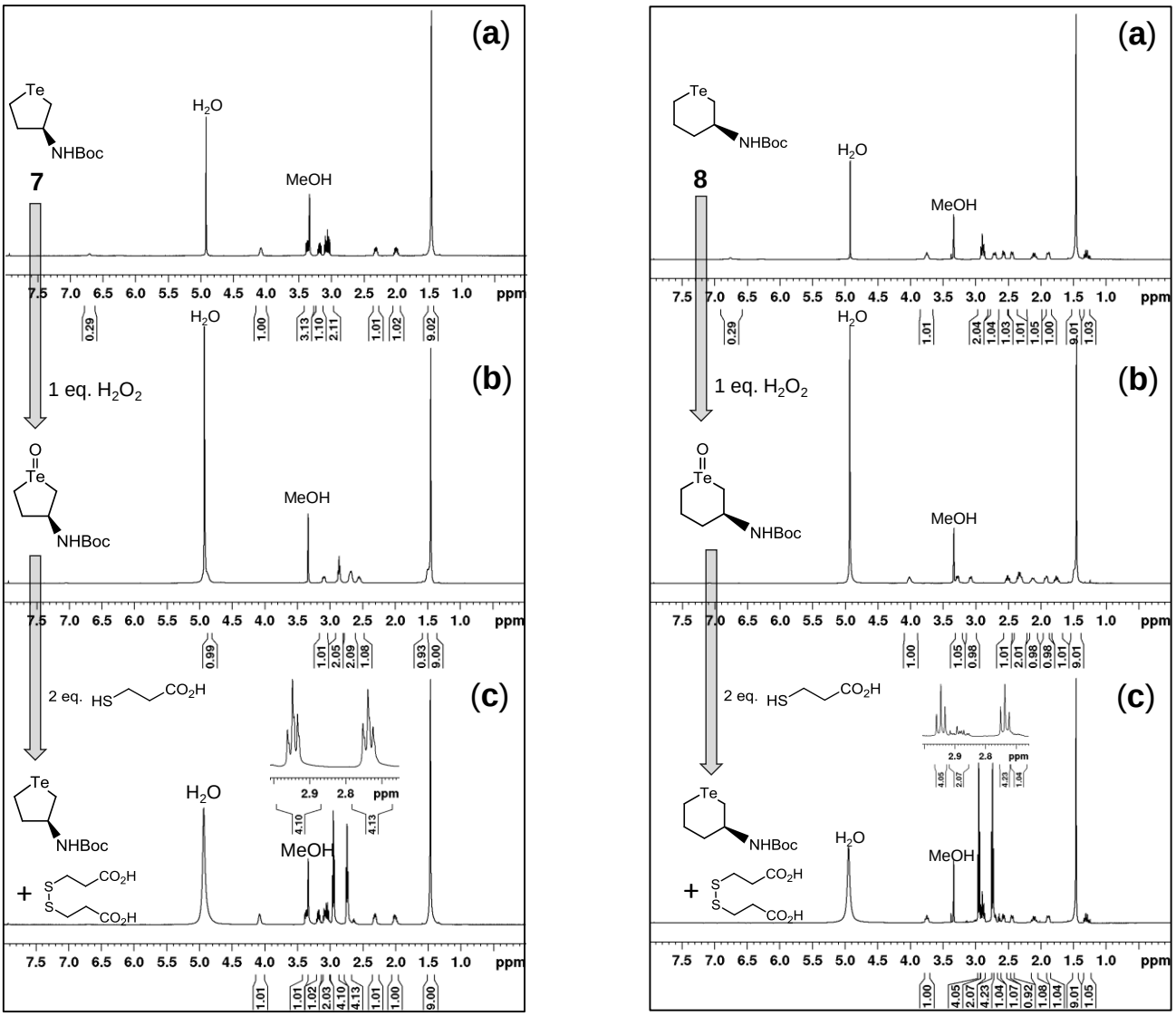


Fig. S3 ^{1}H NMR spectral changes upon oxidation and subsequent reduction of tellurides **7** and **8** in CD_3OD at 25 °C. **Left panel:** (a), **7** (23.4 μmol) in CD_3OD (550 μL); (b), (a) + H_2O_2 (23.4 μmol); (c), (b) + 3-mercaptopropionic acid (46.8 μmol). **Right panel:** (a), **8** (24 μmol) in CD_3OD (550 μL); (b), (a) + H_2O_2 (24 μmol); (c), (b) + 3-mercaptopropionic acid (48 μmol). All sample solutions were incubated for 5 min at 25°C before the NMR measurements.

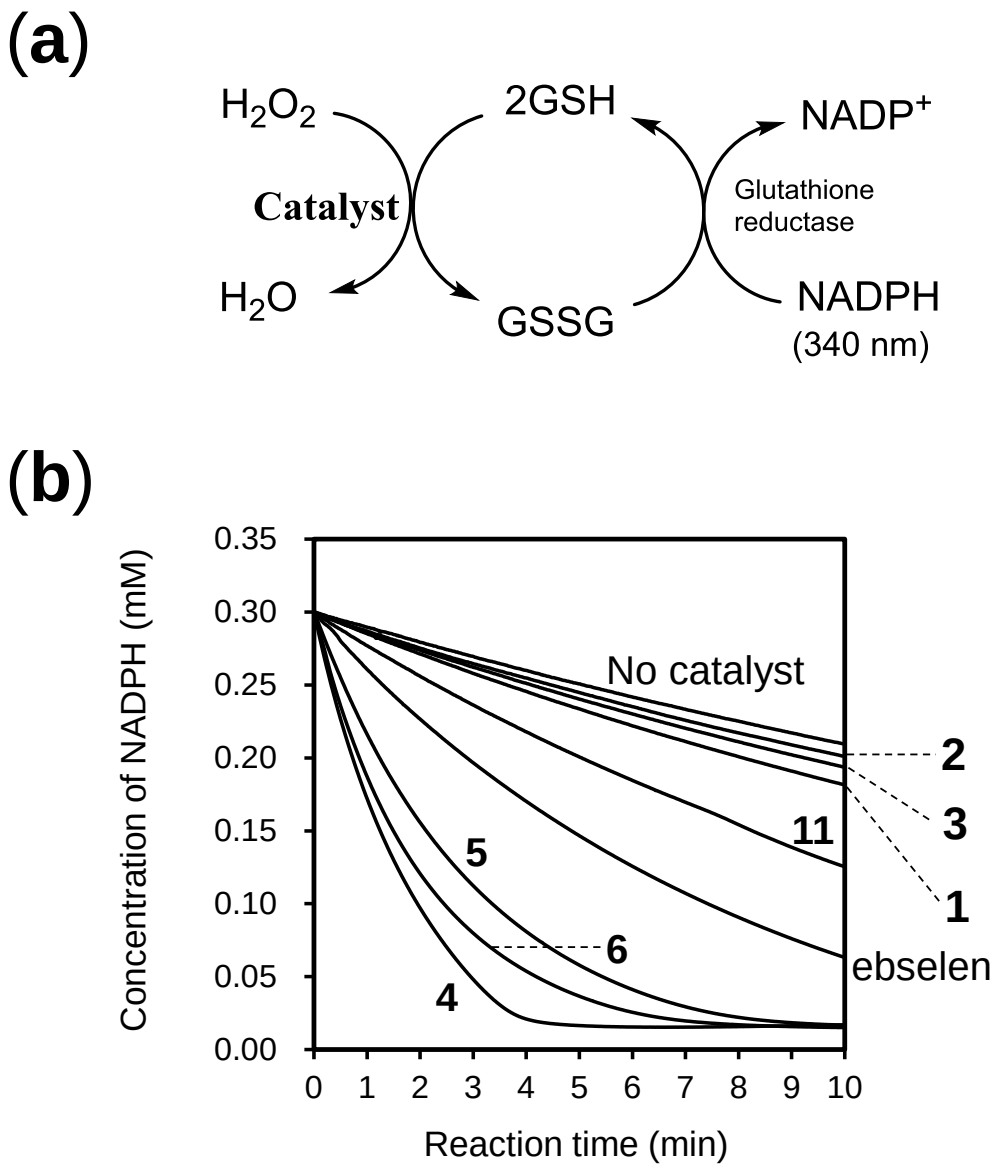


Fig. S4 (a) GPx-like activity coupled with reduction of GSSG by NADPH in the presence of glutathione reductase (GR). (b) GPx-like activity assay for selenides **1**–**3**, tellurides **4**–**6**, and ebselen. Reaction conditions were $[GSH]_0$ = 4 mM, $[H_2O_2]_0$ = 0.25 mM, $[NADPH]_0$ = 0.3 mM, [GR] = 4 units/mL, and [catalyst] = 25 μM in pH 7.2 phosphate buffer at 25 °C.

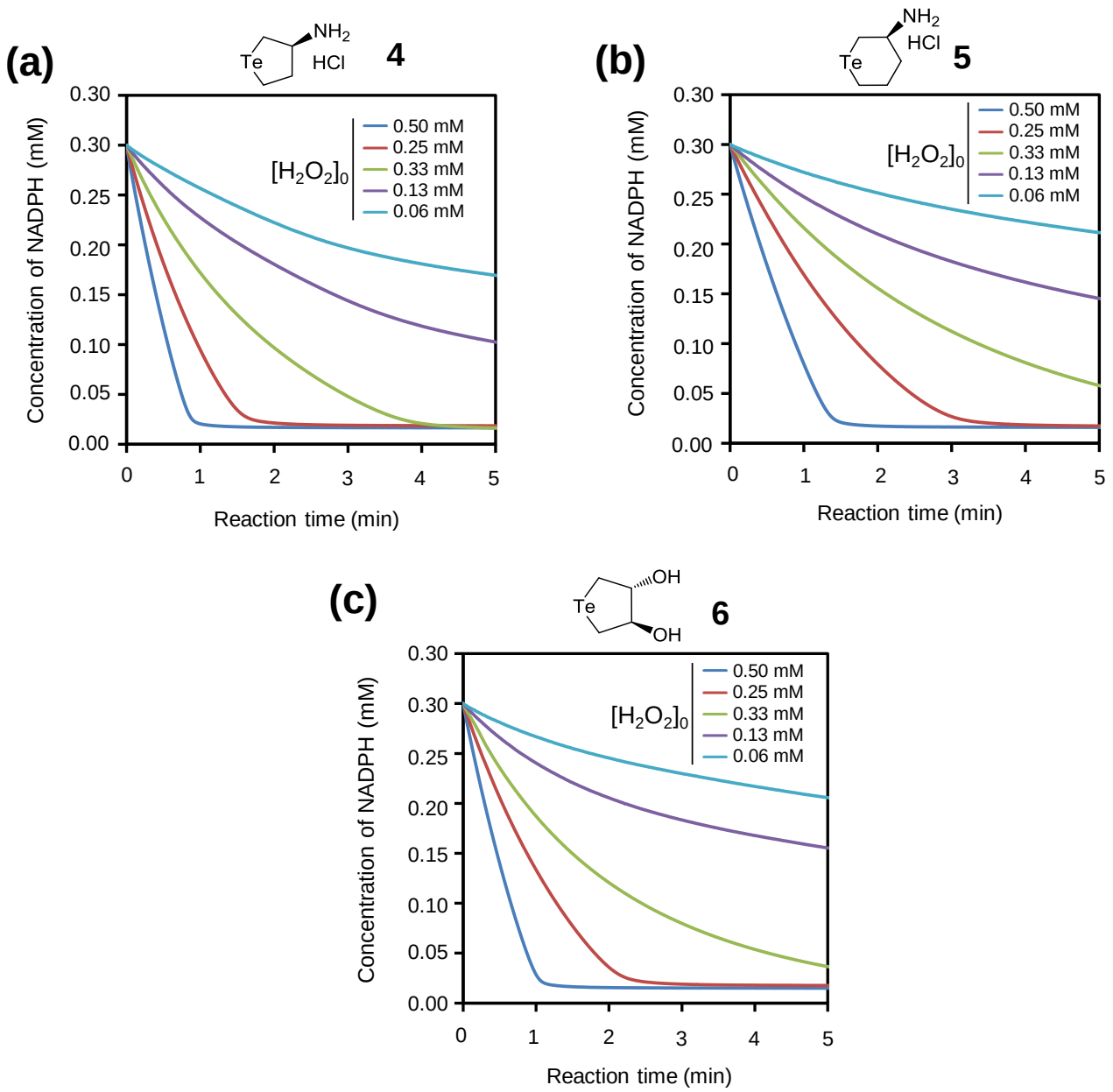


Fig. S5 Effect of H_2O_2 concentration on GPx-like activity. (a)–(c): GPx-like activity assay for tellurides **4**–**6**. Reaction conditions were $[GSH]_0$ = 4 mM, $[H_2O_2]_0$ = 0.06–0.25 mM, $[NADPH]_0$ = 0.3 mM, [GR] = 4 units/mL, and [catalyst] = 25 μM in pH 7.2 phosphate buffer at 25 °C.

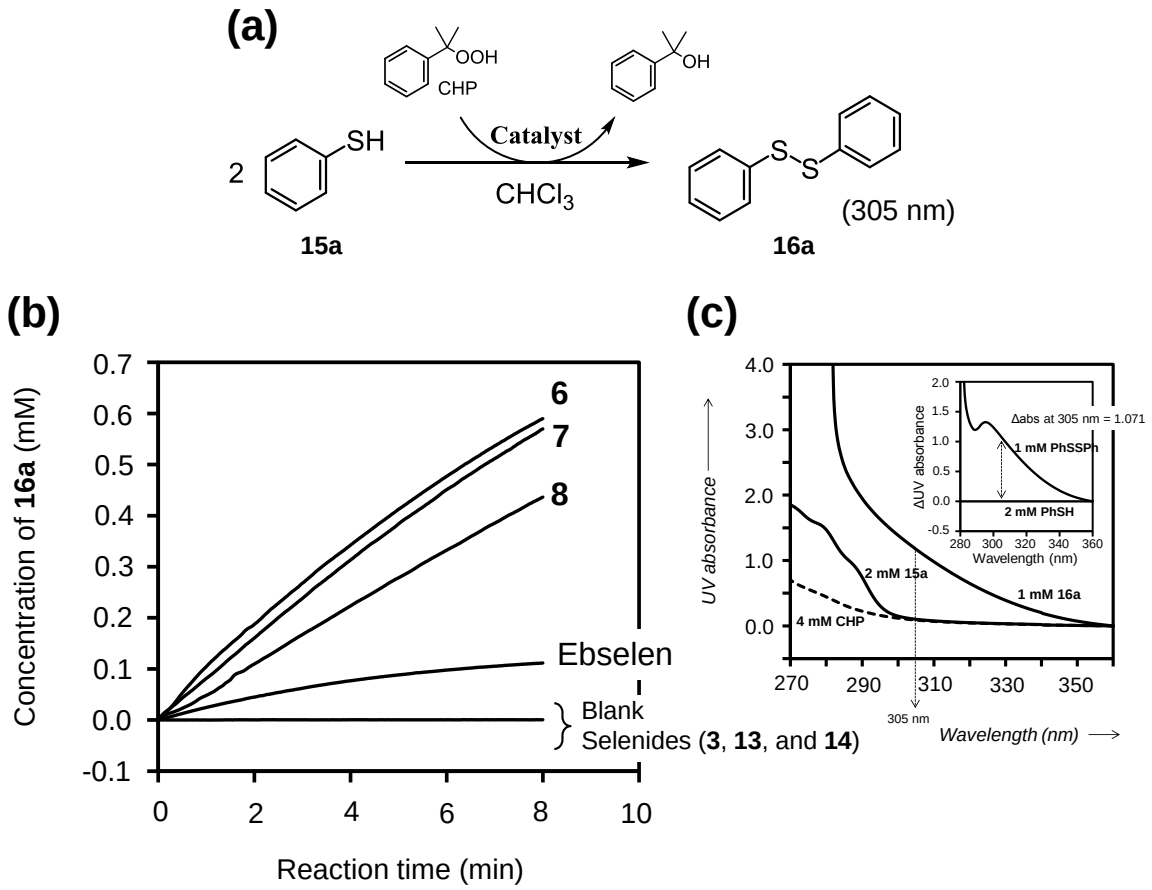


Fig. S6 (a) GPx-like activity coupled with oxidation of benzenethiol (**16a**) in $CHCl_3$. (b) GPx-like activity assay for selenides **3**, **13**, and **14**, tellurides **6**–**8**, and ebselen. Reaction conditions were $[\mathbf{15a}]_0$ = 2 mM, $[CHP]_0$ = 2 mM, [catalyst] = 0.04 mM in $CHCl_3$ at 25 °C. (c) UV spectra and the UV differential spectra of **15a** (2 mM) and **16a** (1 mM) in $CHCl_3$.

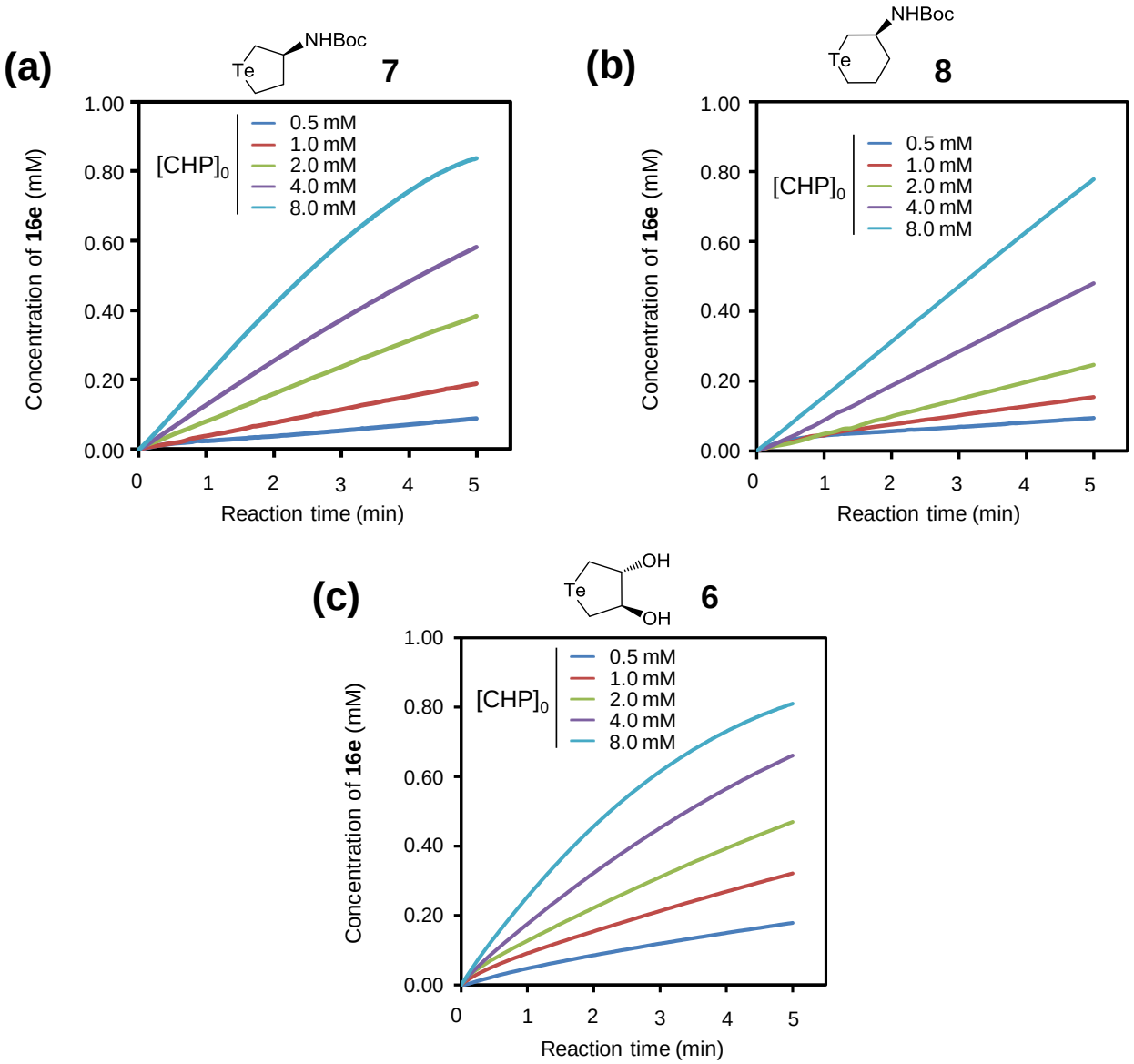


Fig. S7 Effect of CHP concentration on GPx-like activity in $CHCl_3$. (a), (b), and (c): GPx-like activity assay for tellurides **7**, **8**, and **6**, respectively. Reaction conditions were [**15a**]$_0$ = 2 mM, [CHP]$_0$ = 0.5–8.0 mM, and [catalyst] = 0.04 mM in $CHCl_3$ at 25 °C.

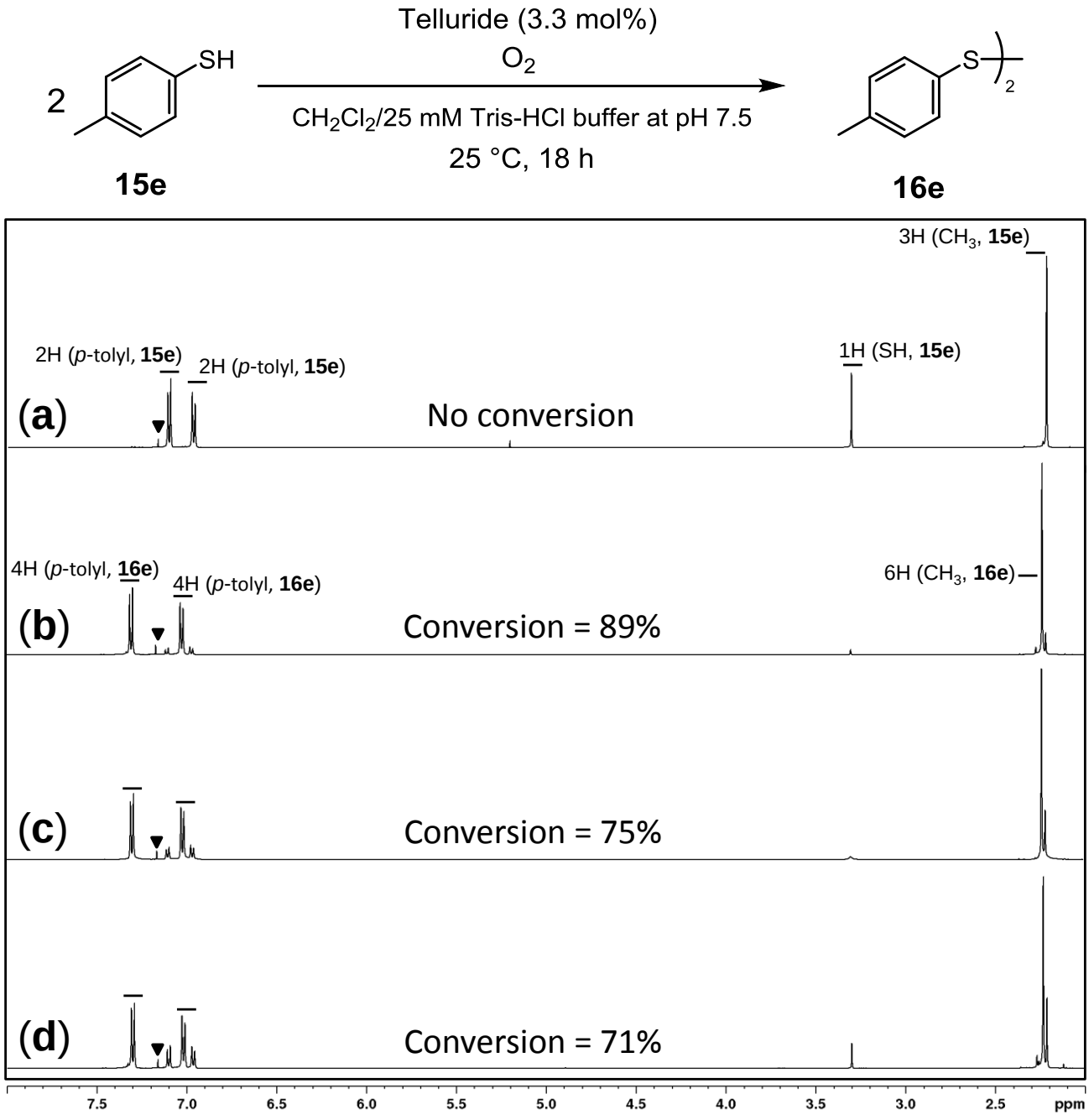


Fig. S8 ^{1}H NMR spectra obtained from aerial oxidation experiments of **15e** by using cyclic telluride catalysts. Reaction conditions: **15e** (0.23 mmol) and catalyst (3.3 mol%, 7.6 μmol) in CH_2Cl_2 (4 mL)/water (4 mL) at 25 °C for 18 h in the absence (a) or presence of telluride **4** (b), **5** (c), or **6** (d).

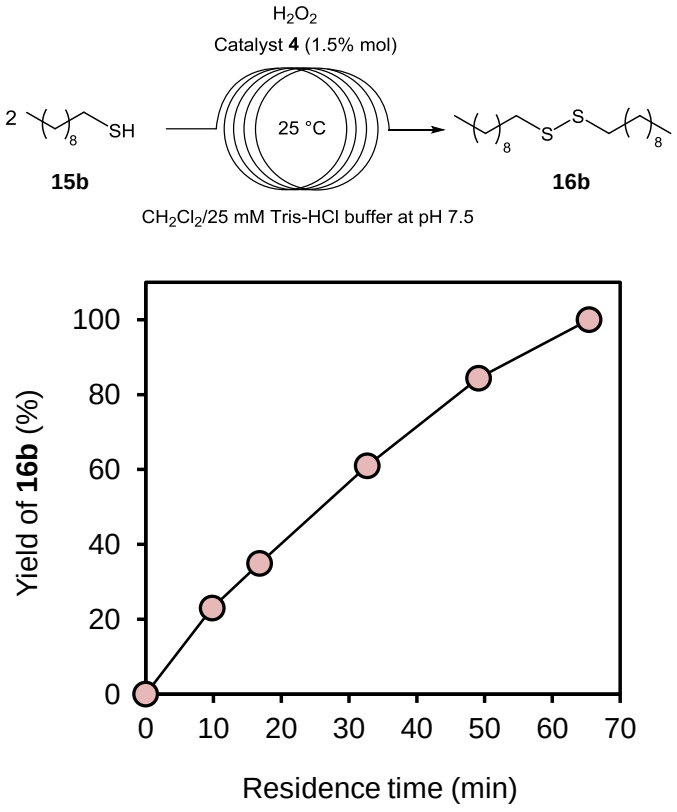


Fig. S9 Residence time effect on the catalytic oxidation of **15b** in a segmented-microflow system. Reaction conditions: **15b** (0.23 mmol), H_2O_2 (0.23 mmol), and catalyst (3.45 μmol) in CH_2Cl_2/25 mM Tris-HCl buffer solution at pH 7.5 (1:1 = *v*/*v*) at 25 °C. The yields were estimated by ^{1}H NMR.

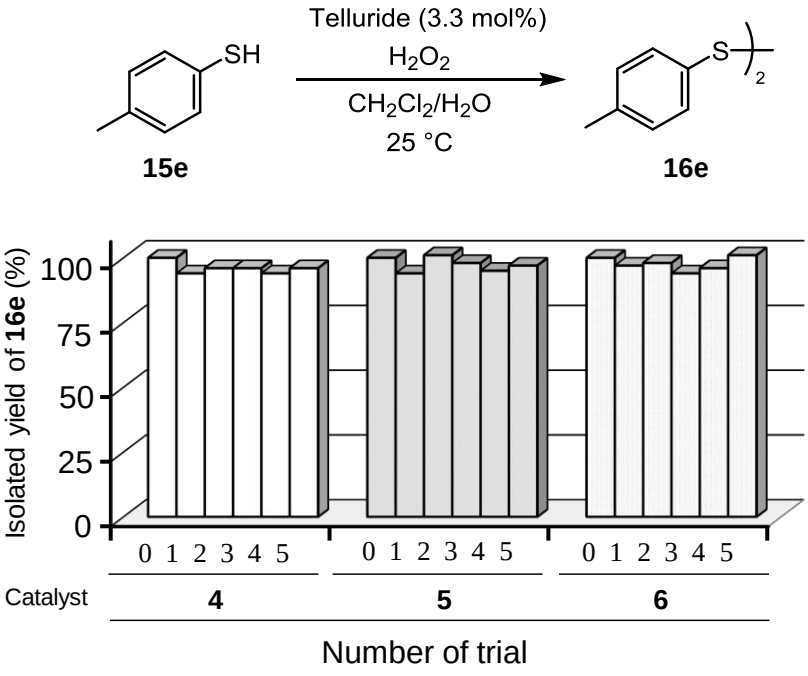


Fig. S10 Isolated yield of **16e** during recycle of telluride catalysts **4**–**6** in five times. Reaction conditions: **15e** (0.23 mmol), H_2O_2 (0.23 mmol), and catalyst (7.6 μmol) in CH_2Cl_2 (4 mL) and H_2O_2 (4 mL) at 25 °C for 180 min. The reaction was progressed with vigorously stirring.

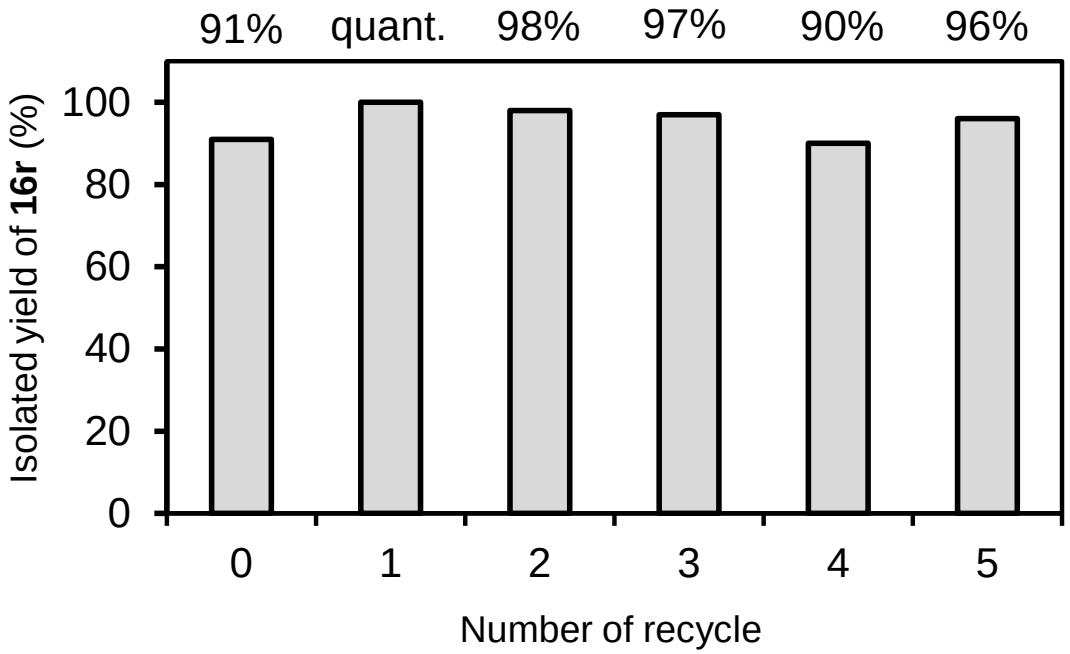


Fig. S11. Isolated yield of **16r** during six consecutive cycles of catalyst **7**. Reaction conditions: **15r** (0.23 mmol), H_2O_2 (0.23 mmol), and catalyst (7.6 μmol) in CH_2Cl_2 (4 mL) and H_2O_2 (4 mL) at 25 °C for 6 h. The reaction was progressed with vigorously stirring.

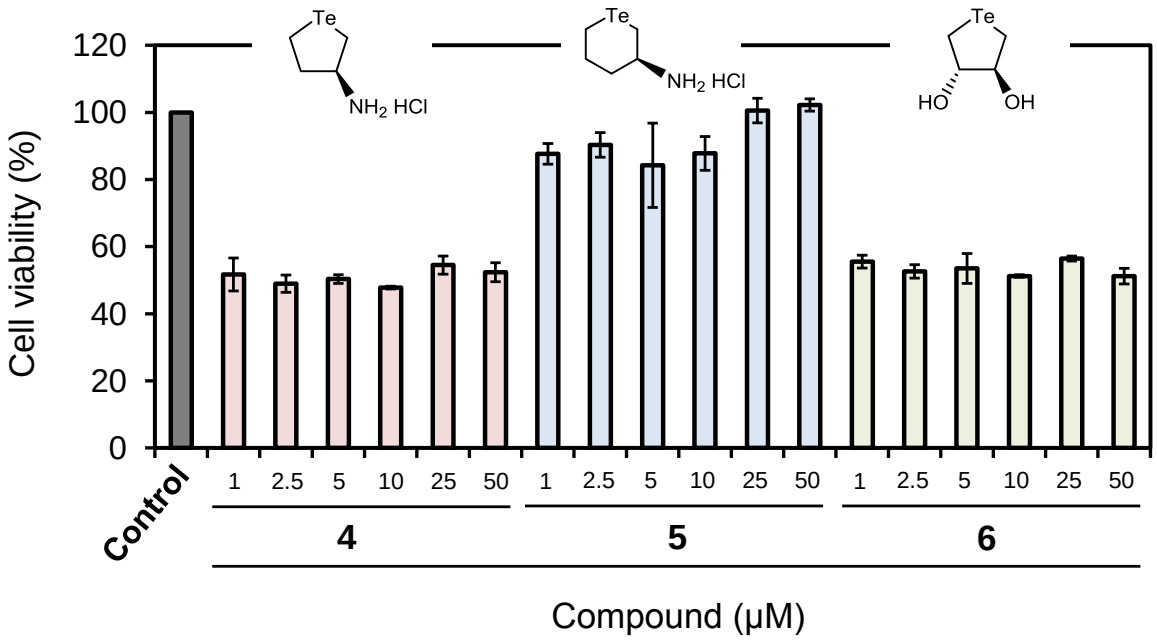


Fig. S12. Cytotoxicity of compounds **4**–**6** at different concentrations determined by MTT assay with HeLa cells. HeLa cells (1×10^4 cell/well) was cultured for 4 h in the presence of compounds **4**–**6** (1−50 μM) at 37 ˚C and 5% CO_2. Bars are shown as means ±SEM (*n*=4).

Experimental: Each well in a 96-well plate was seeded with HeLa cells (1×10^{-4} cells/well) in Dulbecco's Modified Eagle's Medium supplemented with 5% fetal bovine serum. After culturing in a humidified incubator at 37 °C with 5% CO_2 for 24 h, the cells were treated with 1−50 μM of compounds 4−6 in medium (100 μL) without fetal bovine serum and incubated for 4 h at 37 °C with 5% CO_2. The medium was then removed, and the cells were washed with phosphate-buffered saline. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (100 μL, 0.5 mg/mL in medium) was then added, and the cells were incubated for 4 h at 37 °C with 5% CO_2. MTT solution was then removed and dimethyl sulfoxide (100 μL) was added to each well to dissolve the formazan crystals, and the cells were incubated at 25 °C with shaking at 350 rpm. The absorbance at 530 nm was measured using a microplate reader. The control cells, which were treated with the medium containing no compound, were assumed to be 100% viable. The percentage viability of the treated cells was calculated from eq. (1).

$$\%viability = \frac{Abs^{535}_{sample} - Abs^{535}_{blank}}{Abs^{530}_{control} - Abs^{530}_{blank}}. \qquad \cdots(1)$$

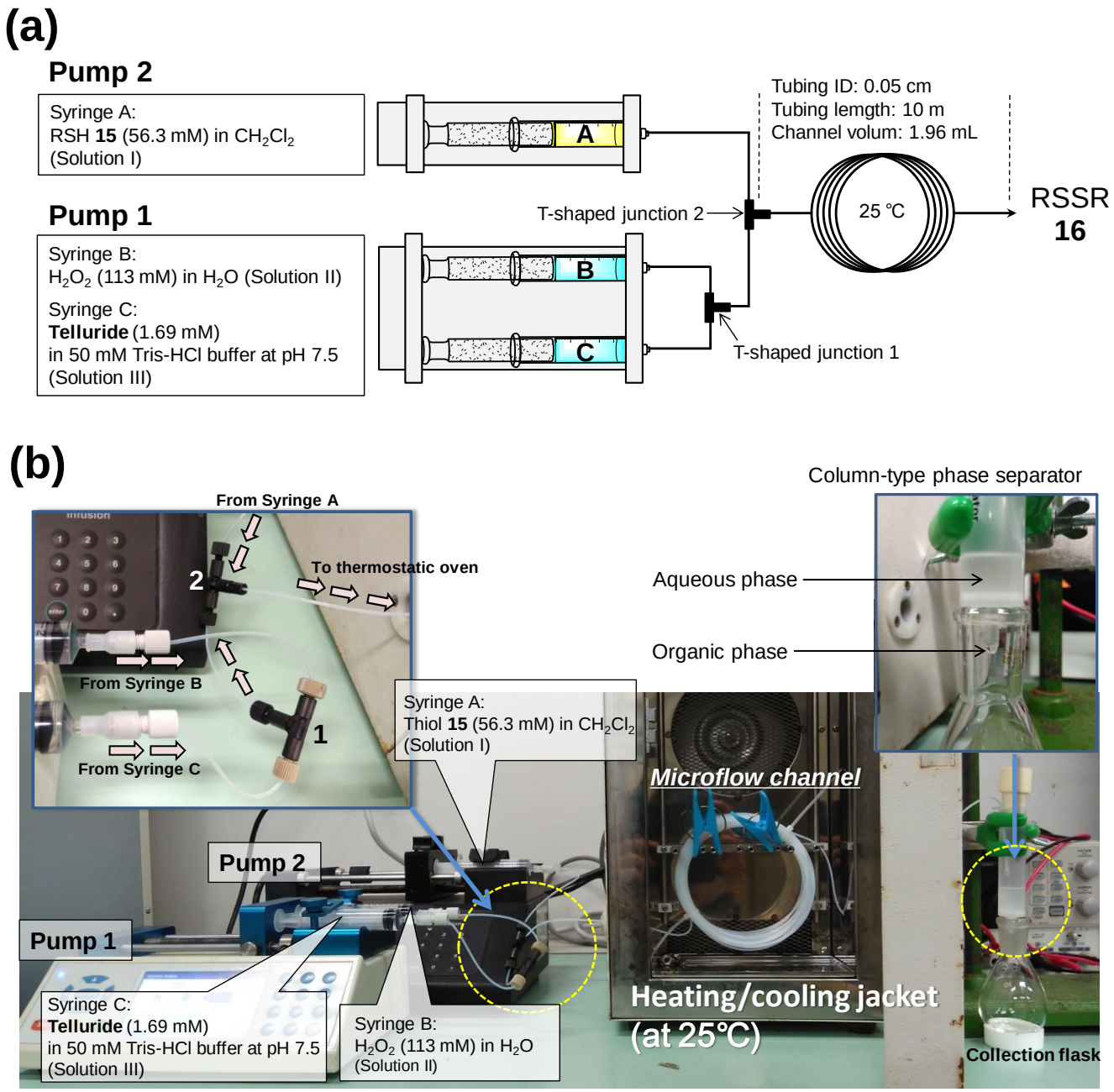


Fig. S13 Schematic (a) and photographs (b) of flow setup for the oxidation of fat-soluble thoils.

Note: Plastic T-shape junctions were purchased from EYELA. (Inner diameter: 0.5 mm). Teflon® tubes (ID = 0.5 mm) and PEEK fittings were purchased from GL Science Co., Ltd. Solutions were introduced to microflow channels with syringe pumps (Pump 1: Fusion Touch 100 from ISIS Co., Ltd and Pump 2: PHD 2000 syringe pump infusion 100 from Harvard apparatus) equipped disposable plastic syringes which include no rubber in the material.

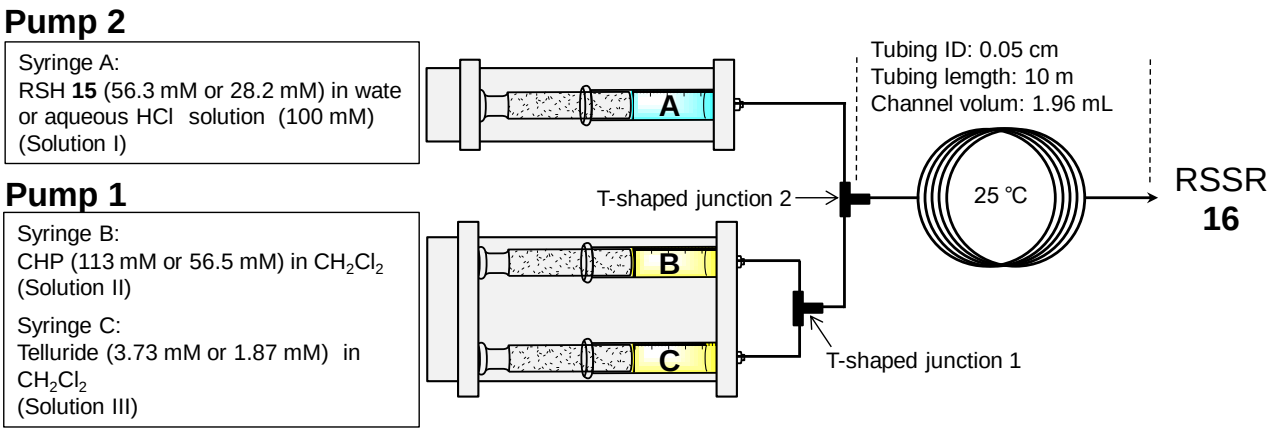
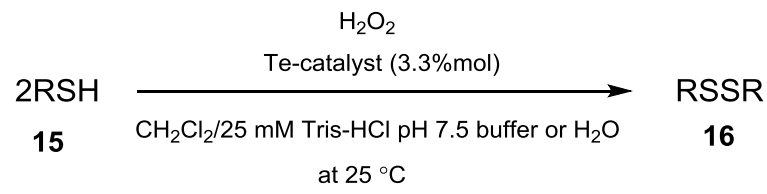
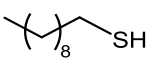
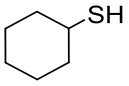
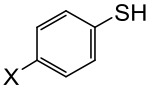
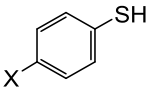
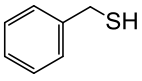
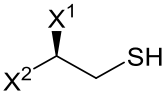


Fig. S14 Schematic of flow setup for the oxidation of water-soluble thiols.

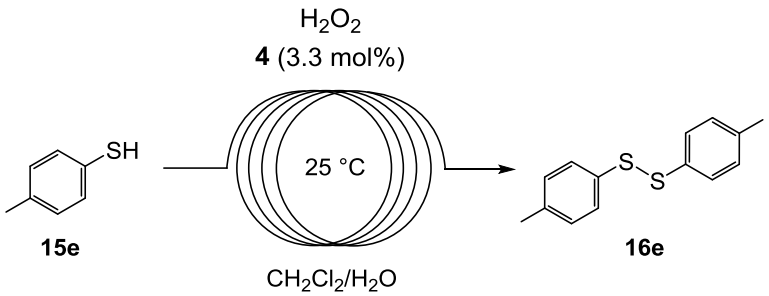
Table S1. Catalytic oxidation of thiols in a biphasic organic-aqueous system under a batch condition.



Thiol **15**	Yield of disulfide **16**[a]	
	Catalyst **4**[b]	Catalyst **6**[c]
 **15b**	**16b** (99%)	**16b** (98%)
 **15c**	**16c** (99%)	**16c** (96%)
 **15a** X = H **15d** X = OMe **15e** X = Me **15f** X = *t*Bu	**16a** (95%) **16d** (quant.) **16e** (98%) **16f** (quant.)	**16a** (quant.) **16d** (97%) **16e** (quant.) **16f** (96%)
 **15g** X = F **15h** X = Cl **15i** X = Br	**16g** (quant.) **16h** (quant.) **16i** (quant.)	**16g** (99%) **16h** (quant.) **16i** (97%)
 **15j**	**16j** (97%)	**16j** (quant.)
 **15l** X^1 = NHBoc, X^2 = CO_2Me **15m** X^1 = NHAc, X^2 = CO_2Me **15n** X^1 = H, X^2 = CO_2Me **15o** X^1 = H, X^2 = NHBoc	**16l** (quant) **16m** (72%) **16n** (92%) **16o** (95%)	**16l** (89%) **16m** (78%) **16n** (79%) **16o** (80%)

Reaction conditions: **15** (0.23 mmol), H_2O_2 (0.23 mmol), and catalyst (3.3 mol%, 7.6 μmol), CH_2Cl_2/25 mM Tris-HCl buffer solution (1:1 = *v*/*v*) at pH 7.5 and 25 °C. [a]Isolated yield. [b]25 mM Tris-HCl buffer solution was used as an aqueous solvent. [c]Distilled water was used as an aqueous solvent.

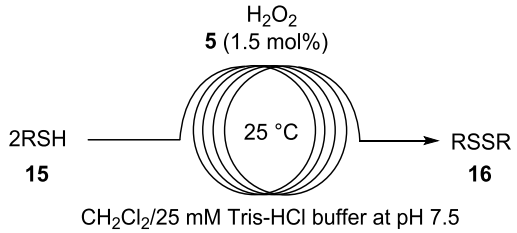
Table S2. Optimization of tubing ID of microflow channel.

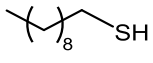
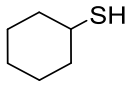
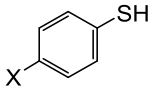
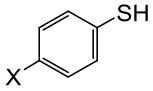
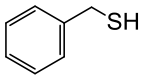
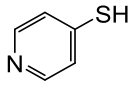
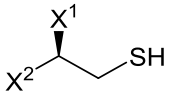


Entry	Tubing length (m)	Tubing ID (mm)	Residence time (min)[a]	Amount of catalyst **4** (mol%)	Conversion (%)[b]
1	4.8	1.0	18.8	3.3	78
2	4.8	1.0	37.6	3.3	87
3	4.8	1.0	9.4	3.3	67
4	10	0.05	19.6	3.3	100
5	10	0.05	16.8	3.3	100
6	10	0.05	16.8	1.5	100
7	10	0.05	16.8	0	0

[a] Defined as volume of tube (mL)/ flow rate (mL/min). [b]Estimated by ^{1}H NMR analysis.

Table S3. Catalytic oxidation of thiols in biphasic organic-aqueous system under a segmented microflow condition.



Thiol **15**	Residence time [a]	Yield of disulfide **16**[b]
 **15b**	65.4 min	**16b** (85% conversion)[c]
 **15c**	65.4 min	**16c** (86% conversion)[c]
 **15a** X = H **15d** X = OMe **15e** X = Me **15f** X = *t*Bu	32.7 min	**16a** (96%) **16a** (96%) **16e** (97%) **16e** (97%)
 **15g** X = F **15h** X = Cl **15i** X = Br	19.6 min	**16g** (97%) **16h** (95%) **16i** (98%)
 **15j**	32.7 min	**16j** (99%)
 **15k**	ND[d]	**16k** (ND)[d]
 **15l** X^1 = NHBoc, X^2 = CO_2Me **15m** X^1 = NHAc, X^2 = CO_2Me **15n** X^1 = H, X^2 = CO_2Me **15o** X^1 = H, X^2 = NHBoc	32.7 min	**16l** (97%) **16m** (68%) **16n** (quant.) **16o** (93%)

Reaction conditions: **15** (0.23 mmol), H_2O_2 (0.23 mmol), and catalyst (3.45 or 7.6 μmol), CH_2Cl_2/25 mM Tris-HCl buffer solution (1:1 = *v*/*v*) at pH 7.5 and 25 °C. [a] Defined as the channel volume (mL)/flow rate (mL/min). [b]Isolated yield. [c] Estimated by ^{1}H NMR analysis. [d] Not determined due to the low solubility in CH_2Cl_2 [f].

3 References

1 A. F. Vaquer, A. Frongia, F. Secci and E. Tuveri, *RSC Adv.*, 2015, **5**, 96695–96704.

2 E. Rattanangkool, W. Krailat, T. Vilaivan, P. Phuwapraisirisan, M. Sukwattanasinitt and S. Wacharasindhu, *Eur. J. Org. Chem.*, 2014, **2014**, 4795–4804.

3 A. Talla, B. Driessen, N. J. W. Straathof, L.-G. Milroy, L. Brunsveld, V. Hessel and T. Noël, *Adv. Synth. Catal.*, 2015, **357**, 2180–2186.

4 H. Huang, J. Ash and J. Y. Kang, *Org. Biomol. Chem.*, 2018, **16**, 4236–4242.

5 P. Mampuys, Y. Zhu, S. Sergeyev, E. Ruijter, R. V. A. Orru, S. Van Doorslaer and B. U. W. Maes, *Org. Lett.*, 2016, **18**, 2808–2811.

6 M. Lo Conte and K. S. Carroll, *Angew. Chem. Int. Ed.*, 2012, **51**, 6502–6505.

7 H.-Y. Huang and C.-F. Liang, *Asian J. Org. Chem.*, 2018, **7**, 955–963.

8 F. Ishikawa, R. W. Haushalter and M. D. Burkart, *J. Am. Chem. Soc.*, 2012, **134**, 769–772.

9 V. Rathore, A. Upadhyay and S. Kumar, *Org. Lett.*, 2018, **20**, 6274–6278.

10 S. S. Shah, S. Karthik and N. D. P. Singh, *RSC Adv.*, 2015, **5**, 45416–45419.

11 M. Oba, K. Tanaka, K. Nishiyama and W. Ando, *J. Org. Chem.*, 2011, **76**, 4173–4177.