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Supplementary Information

Organoditelluride-mediated catalytic S-nitrosothiol decomposition

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Contents

(1) Synthetic experimental

Materials

2-Thiophene carboxylic acid, tellurium, dibutyltin dilaurate (DBTDL), hexamethylene diisocyanate (HMDI), and N,N'-dimethyl acetamide (DMAC), were purchased from Aldrich (Milwaukee, WI). N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS), glutathione (GSH) and cysteine (CySH) were products of Sigma (St. Louis, MO). Hydrophilic polyurethane (Tecophilic, SP-93A-100) was a gift from Thermedics Inc. (Wilmington, MA). Dipropylamine-PEO (DPA-400E, F.W.= 573.73) was a gift from Tomah products (Milton, WI). Other reagents from Aldrich, solvents from Fisher Scientific, and NMR reagents from Cambridge Isotope Laboratories, Inc. (Andover, MA) were used without further purification unless otherwise noted. Distillation was employed for the purification of HMDI, Et_3N, THF, and DMAC prior to use. DI water was prepared by a Milli-Q filter system (18 MΩ cm^{-1}: Millipore Corp., Billerica, MA, USA).

Measurements

^1H and ^{13}C NMR spectra were obtained on a Varian 400 or 500 MHz spectrometer. High-resolution (HR) mass spectra were recorded using a Waters Autospec. Ultima Magnetic Sector mass spectrometer with an electrospray interface. FTIR spectra were collected with a Perkin-Elmer spectrum BX FT-IR system. UV spectra were recorded by a Perkin-Elmer Lambda 35 UV/VIS spectrometer. Tellurium content of the polymers was measured by Inductively Coupled Plasma Emission-Mass Spectrometry (ICP-MS). Melting points were determined by Mel-Tempo® Laboratory Devices Inc. (USA). All NO measurements were made using a

Sievers Nitric Oxide Analyzer (NOA), model 280. The sample solution for the NOA was continuously bubbled with a nitrogen flow (50 mL/min), and thus the generated NO was purged into the detector cell in the NOA by vacuum (200 mL/min). The conversion constant for mole number of NO (mol/ppb·sec) was pre-calibrated using excess of KI and the known amount of $NaNO_2$ in a diluted sulfuric acid solution. Prior to experiment, each measurement was also calibrated by an internal two-point calibration (the nitrogen flow as zero gas and 45 ppm of NO gas flow). Therefore, the number of moles of NO evolved during the experiment can be calculated by multiplying the peak area (ppb·sec) and the pre-calibrated conversion constant (mol/ppb·sec) followed by the baseline correction.

A low NO background (ca. 1 – 5 ppb $NO_{(g)}$) is always observed when testing RSNO solutions using the very sensitive chemiluminescence NO detection system owing to light induced photodecomposition (even using well shielded cells) as well as the presence of trace Cu(II) even when EDTA is added to solution phase. The baseline of NO for every experiment varies depending on the specific reaction conditions (usually up to 5 ppb); however, the baseline of given GSNO solutions do not significantly change over 12 h in the presence of EDTA. Thus, to assess the amount of NO generated due to the presence of the RTe species, the initial stready-state NO amounts already present immediately before addition of the orgnaoditelluride species are substracted to obtain the levels produced by the presence of the RTe species.

Synthesis

5,5'-ditelluro-2,2'-dithiophenecarboxylic acid (organoditelluride **1**):

To a stirred solution of 2-thiophencarboxylic acid (2.0 g, 15.6 mmol) in THF (200 mL) was added NaH (95 %, 0.66 g, 15.7 mmol) at 0 °C. After 10 min, n-BuLi

(2.5 M solution in hexanes, 6.3 mL, 15.7 mmol) was slowly dropped into the above solution and stirred for 10 min. The reaction mixture was warmed to RT, then stirred for 50 min. Tellurium (1.9 g, 14.9 mmol) was quickly added into the reaction mixture under a strong stream of nitrogen. After stirring for 2 h, the mixture was concentrated under a reduced pressure to yield about 20 ml of reddish brown slurry. The slurry was poured into a solution of DI water (300 mL) and CH_2Cl_2 (200 mL) at 0 °C while adjusting the pH of solution to approx. 1 using 1.5 N HCl. The entire mixture was vigorously mixed by blowing air through it at 0 °C. The mixture was filtered to remove an undissolved solid and the filter cake was washed with CH_2Cl_2. The separated water layer was extracted 2 times more with CH_2Cl_2. The combined organic layer was dried with anhydrous Na_2SO_4, filtered and washed with CH_2Cl_2. The filtrate was concentrated to give a dark reddish solid under a reduced pressure. The crude residue was triturated with CH_2Cl_2 (50 mL), then filtered and washed with CH_2Cl_2 to give a reddish brown solid (0.93 g, 25 % yield).

Decomposition Temperature 168 - 172 °C; ^{1}H NMR (500 MHz, DMSO-d_6, 25 °C): δ=13.17 (bs, 2H; 2 COOH), 7.54 (d, J= 4.5 Hz, 2H; 2 CCH), 7.43 (d, J= 4.5 Hz, 2H; 2 TeCCH); ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C): δ=162.28, 142.13, 140.12, 134.53, 106.19.; ^{125}Te NMR (MHz, DMSO-d_6, 25 °C): δ=497.60; IR (KBr)= 3426 cm^{-1} (COO-H), 2959, 2554 cm^{-1} (=C-H), 1667 cm^{-1} (C=O), 1516 cm^{-1} (C=C), 1422 cm^{-1} (=C-H); HRMS(EI): *m*/*z*: $[M]^+$ calcd. for $C_{10}H_6O_4S_2Te_2$, 513.7832: found 513.7835.; Anal. Calcd for $C_{10}H_6O_4S_2Te_2$; C, 23.57; H, 1.19; O, 12.56; S, 12.59: Found C, 23.25; H, 1.23; S, 12.28.

5-Deuteriated 2-thiophene carboxylic acid; Identification for the regioisomer of organoditelluride **1**:

The product of organoditelluride **1** (50 mg) was put into a NaOD solution (1

mL, 40 %, w/w in D_2O) and stirred at 60 °C overnight. The solution color changed into a dark brown and then yellow color. After cooling to RT, the mixture was diluted with DI water. The product was extracted three times with $CHCl_3$ after the pH of solution was adjusted to 1 by adding diluted HCl at 0 °C. The combined organic layer was dried with anhydrous Na_2SO_4, filtered and washed with $CHCl_3$. The filtrate was concentrated under reduced pressure, then, dried with vacuum pump to give a partially deuterium-substituted product (see Fig. 1s).

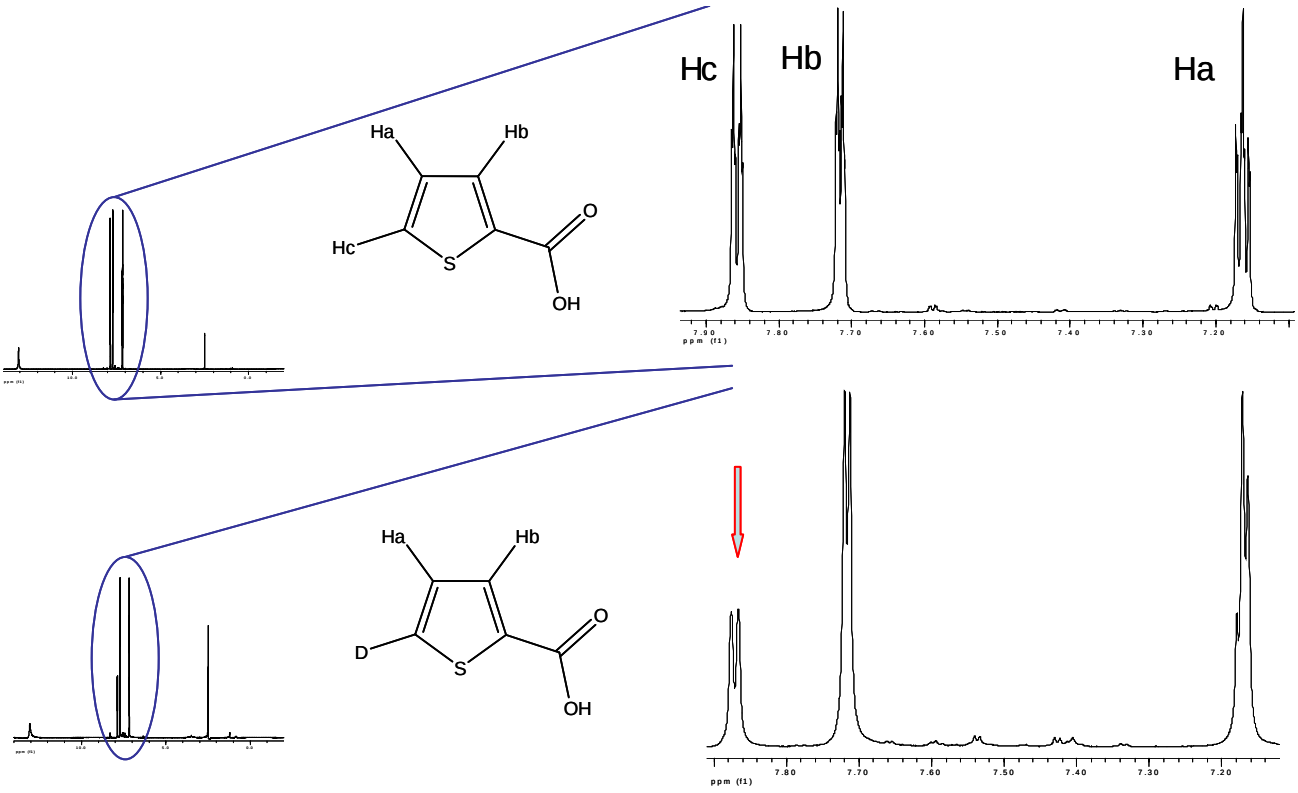


Figure 1s. The NMR identification for the regioisomer of organoditelluride **1** via the partial substitution of Te with a deuterum using NaOD.

HPU (Tecophilic, SP-93A-100);

Given our recent findings with RSe and Cu(II) species, this work was a more

fundamental effort to determine whether RTe species can also be useful for NO generation from RSNOs. At this point, it is too early to assess fully which approach would be best. We already know that immobilized Cu(II) sites are not immune from fouling by proteins, etc., and also exhibit different catalytic rates with different RSNO species. Hence, alternate species that can be linked to biomedical grade polymers is of general interest.

The polyurethane material used in this work is often employed as a material for biomedical devices such as catheters, vascular grafts, etc. It is a biomedical grade polyurethane, and hence it would be a good choice for potentially using the NO generation for demonstrating a practical medical application of this new chemistry. Further, because the HPU (Tecophilic, SP-93A-100) has very high water uptake, diffusion of RSNO and RSH species into the polymer should be quite good.

Aminated polymer **2**;

Hydrophilic polyurethane (Tecophilic, SP-93A-100) was purified by soxhlet extraction prior to use. A dried HPU (2.0 g, ca 4.8 mmole of urethane group) was dissolved in anhydrous DMAC (40 mL). This solution was added dropwise into a stirred solution of HMDI (3.89 ml, 24 mmole) and DBTDL (72 μL, 0.12 mmole) in DMAC (4 mL) at 40 - 45 °C for 3 h. After 1.5 d, the mixture was cooled to RT and then slowly added into anhydrous Et_2O (400 mL). The solid formed was filtered and washed with anhydrous Et_2O (600 mL). The filter cake was dried with N_2 blowing followed by vacuum drying to afford a white polymer. This polymer (1.86 g) was dissolved in anhydrous DMAC (30 mL), and then slowly added into a stirred solution of dipropylamine-PEO (10.4 g) in DMAC (12 mL) at 40 °C for 3 h. The mixture was stirred for 1 d at 40 °C and then slowly added into Et_2O (400 mL). The yellowish polymer formed was filtered and washed with Et_2O (600 mL). The filter

cake was soxhlet extracted with MeOH for 2 d. After cooling to RT, the solid cake was again washed with MeOH, then dried by vacuum pump for 2 d to yield polymer **2** (0.82 g). A solution of aminated polymer **2** in DMAC was titrated by a calorimetric method using bromophenol blue and p-toluenesulfonic acid in isopropanol (0.2 mmole of amine sites/g of polymer **2**).

IR (film on NaCl)= 3323 cm^{-1} (N-H), 2916, 2857 cm^{-1} (CH_2), 1715 cm^{-1} (C=O), 1614 cm^{-1} (HNCONH), 1529 cm^{-1} (C-N, N-H), 1102 cm^{-1} (CH_2-O-CH_2).

NMR data; see Appendix

Ditelluride polymer **3**:

Organoditelluride **1** (17 mg, 33 μmole) solution in THF (5 mL) was mixed with EDC·HCl (15 mg, 78 μmole) in DI water (5 mL). The cloudy mixture was stirred and became clear by adding Et_3N (20 mg, 198 μmole). Then, NHS (9 mg, 78 μmole) was added into the mixture at RT. Aminated polymer **2** (0.34 g, 68 μmole of free amine) solution in THF (12 mL) was then mixed with the above solution and stirred at RT overnight. The mixture was slowly added into Et_2O (900 mL) to form a slightly reddish yellow polymer. The solid was washed with Et_2O and DI water. The filter cake was stirred in MeOH at RT overnight. The residue was again filtered and washed with MeOH, then dried with a vacuum pump to give a yellowish polymer **3** (0.2 g). Based on the ICP-MS analysis, the total tellurium amounts in the prepared polymer was 5.3 mg in 1 g of the polymer, which is corresponding to 10.5 mg/cm^3 of organoditelluride species, if the density of polymer is 1 g/cm^3.

IR (film on NaCl)= 3320 cm^{-1} (N-H), 2915, 2849 cm^{-1} (CH_2), 1715 cm^{-1} (C=O), 1616 cm^{-1} (HNCONH), 1526 cm^{-1} (C-N, N-H), 1445 cm^{-1} (=C-H), 1099 cm^{-1} (CH_2-O-CH_2).

NMR data; see Appendix

Procedure for treatments of polymer **3** with the excess of GSH/GSNO;

A small film of polymer **3** (3.92 mg; size, 0.9 cm X 1.8 cm; thickness, 2.4 μm) was soaked in the solution of GSH/GSNO (100 μM/100 μM) in 10 mL of PBS buffer (10 mM), pH 7.4, containing 0.5 mM EDTA (the same PBS buffer used unless otherwise noted). After the mixture was shaken overnight at RT, the film was removed from the mixture. This film was again shaken in a fresh solution of GSH/GSNO (200 μM/200 μM) in 10 mL of PBS buffer for 6 h. The film was removed from the solution and then put into a fresh solution of 10 mL PBS buffer. The same procedure to wash the film was operated a couple of times to ensure the film is in a hydrated state immediately before use in the NOA experiments.

(2) NOA data

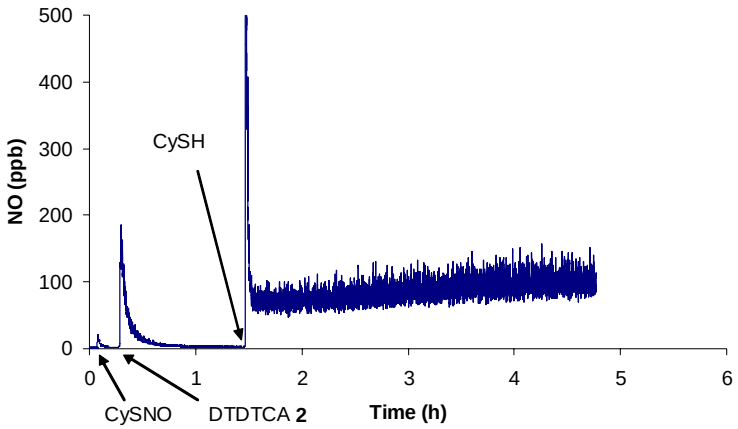


Figure 2s. The measurements of catalytic NO generation by 2.5 μM 5,5'-ditelluro-2,2'-dithiophenecarboxylic acid (organoditelluride **1**) in a solution of 50 μM CySNO and 100 μM CySH in 2 mL of 10 mM PBS buffer, pH 7.4, containing 0.5 mM EDTA, via chemiluminescence NO analyzer (NOA). Arrow indicates addition of each species into the test mixture.

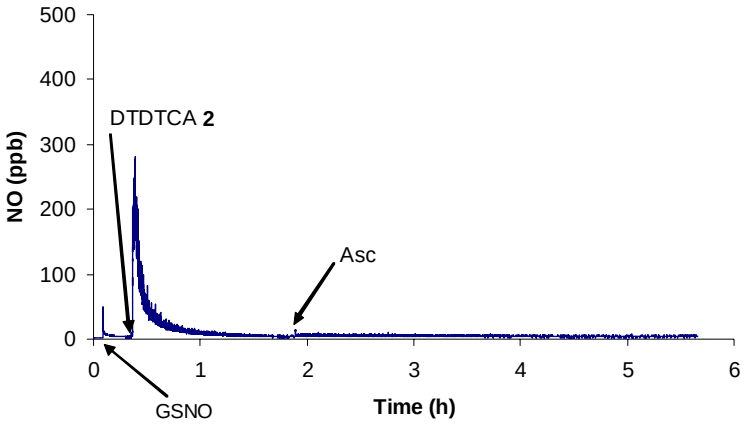


Figure 3s. The measurements of NO generation by 2.5 µM 5,5'-ditelluro-2,2'-dithiophenecarboxylic acid (organoditelluride **1**) in a solution of 50 µM GSNO and 100 µM Ascorbate (Asc) in 2 mL of 10 mM PBS buffer, pH 7.4, containing 0.5 mM EDTA.

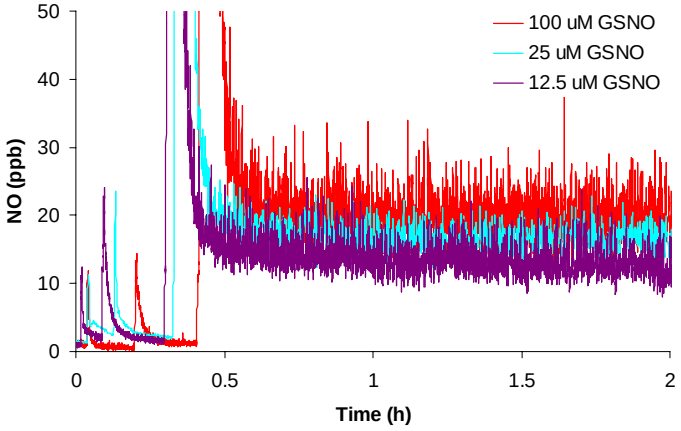


Figure 4s. GSNO concentration dependency. The NO generation of 2.5 µM 5,5'-ditelluro-2,2'-dithiophenecarboxylic acid (organoditelluride **1**) as a function of varied concentrations (12.5, 25, or 100 µM) of GSNO with 100 µM GSH in 2 mL of 10 mM PBS buffer, pH 7.4, containing 0.5 mM EDTA.

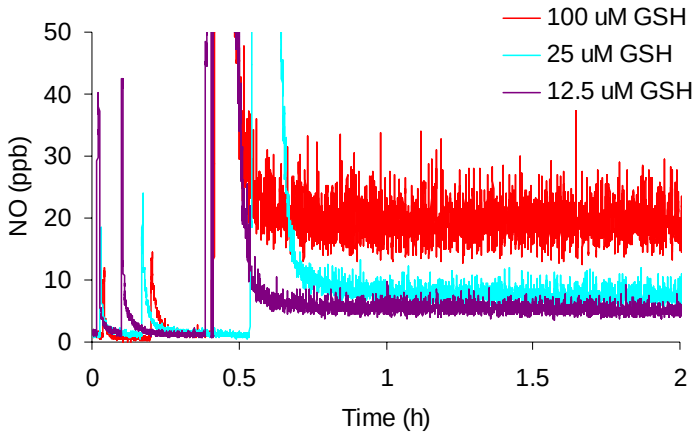


Figure 5s. GSH concentration dependency. The NO generation of 2.5 μM 5,5'-ditelluro-2,2'-dithiophenecarboxylic acid (organoditelluride **1**) depending on the various concentrations (12.5, 25, or 100 μM) of GSH in the 100 μM GSNO in 2 mL deoxygenated PBS buffer, pH 7.4, containing 0.5 mM EDTA.

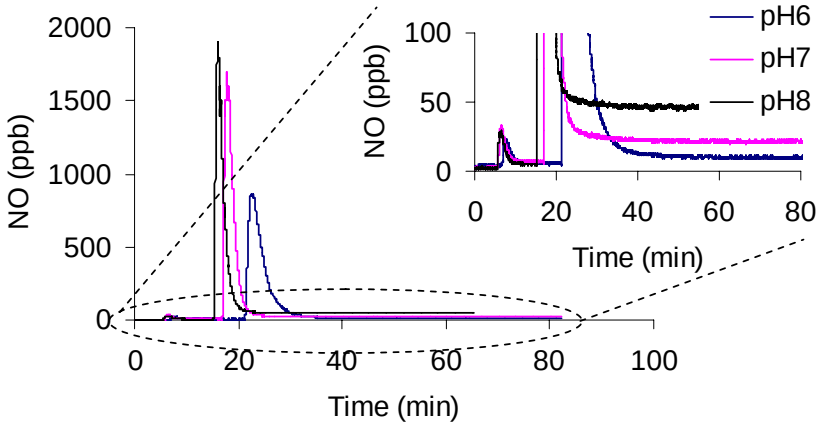


Figure 6s. pH dependency. The NO generation of 1.25 μM 5,5'-ditelluro-2,2'-dithiophenecarboxylic acid (organoditelluride **1**) depending on the various pH values of test solution (6, 7, or 8) in 2 mL buffer containing 0.5 mM EDTA with 25 μM GSH and GSNO.

(3) UV data

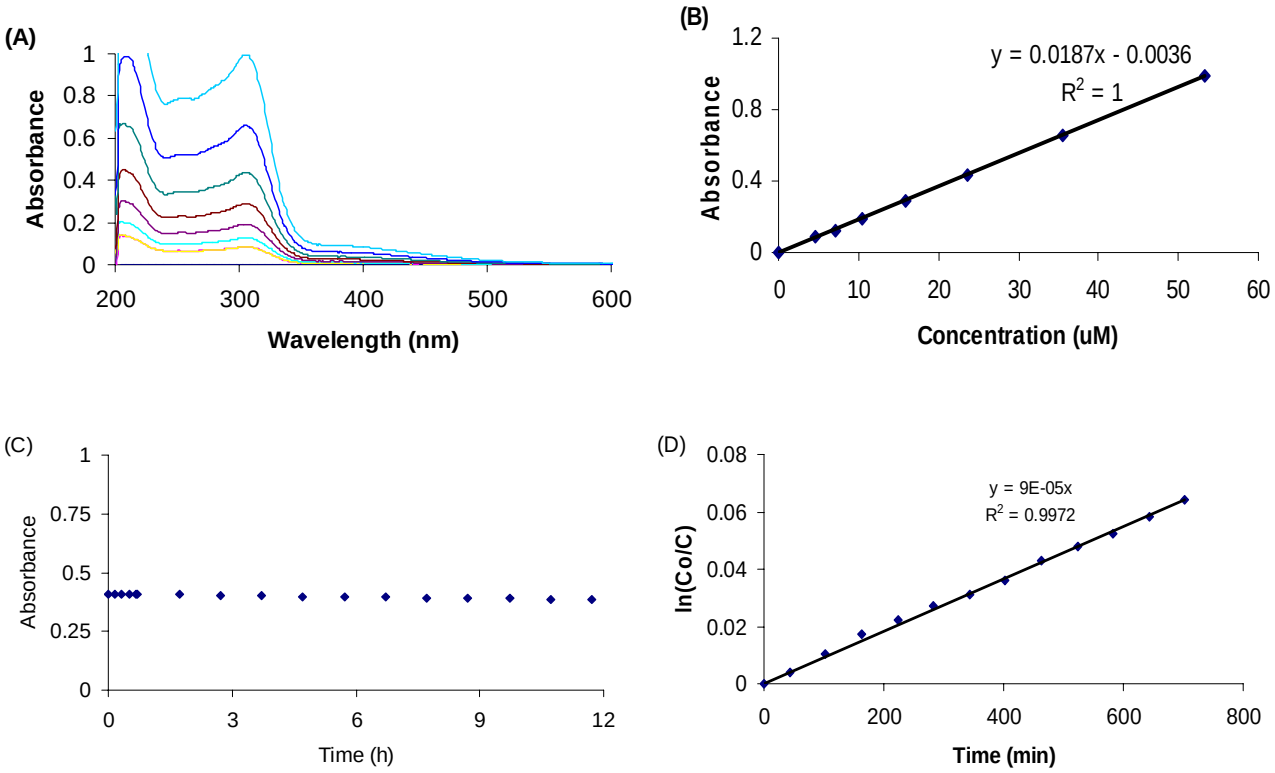


Figure 7s. (A) UV spectroscopy of 5,5'-ditelluro-2,2'-dithiophenecarboxylic acid (organoditelluride **1**) at different concentrations from 53.3 µM to zero in 10 mM PBS buffer, pH 7.4, containing 0.5 mM EDTA under ambient condition; (B) calibration curve at maximum absorbance (306 nm); (C) the stability test of 22 µM organoditelluride **1** in the same PBS buffer at RT; the change of absorbance at 306 nm is recorded as a function of time; (D) the calculated decomposition rate of organoditelluride **1** in the same PBS buffer; first oder, $k = 9 \times 10^{-5}$ min^{-1}.

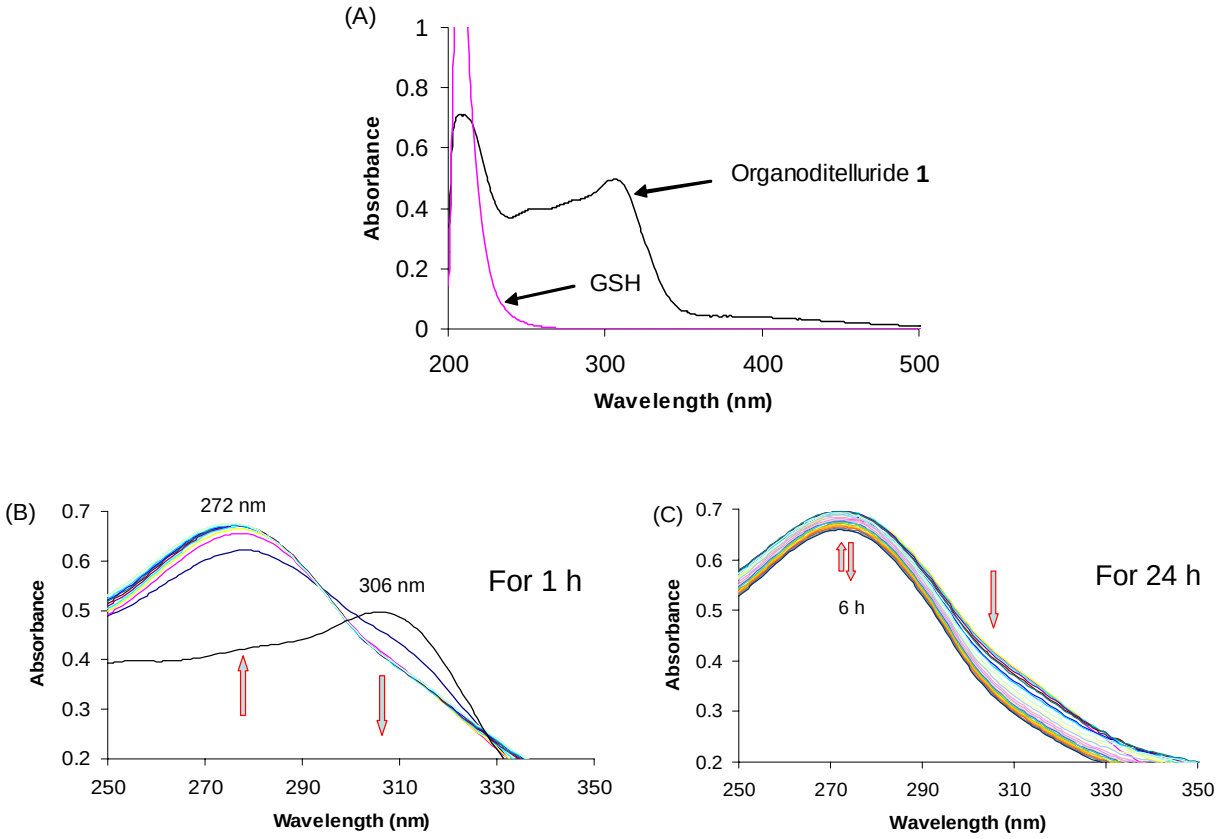


Figure 8s. (A) UV spectroscopy of 32 µM 5,5'-ditelluro-2,2'-dithiophenecarboxylic acid (organoditelluride **1**; ---) and 320 µM GSH (---) in 2.35 mL of 10 mM PBS buffer, pH 7.4, containing 0.5 mM EDTA under ambient conditions; (B) Absorbance change of solution as a function of time; the absorbance at 306 nm is quickly decreases while the absorbance at 272 nm increases within 1 h; (C) the peak at 306 nm slowly decreases for 24 h while the peak at 272 nm increases and then decreases slightly after 6 h.

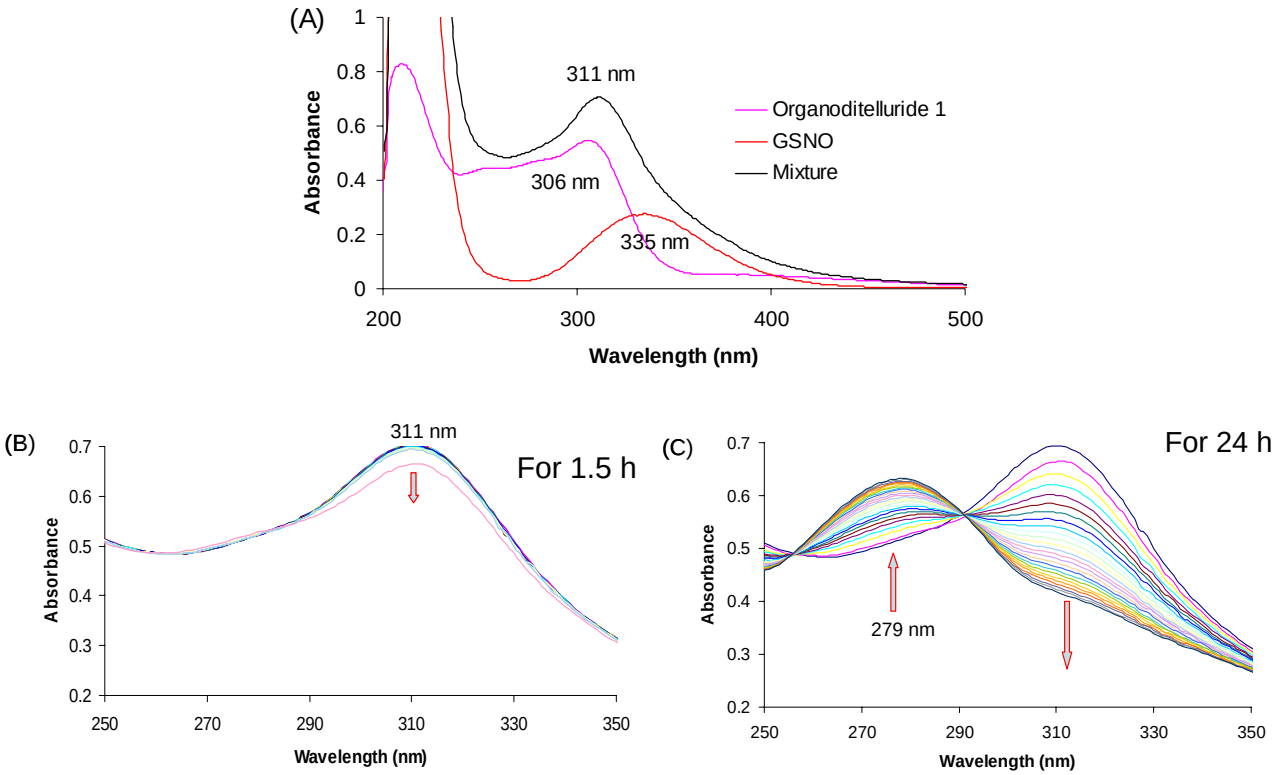


Figure 9s. (A) UV spectroscopy of 32 µM 5,5'-ditelluro-2,2'-dithiophenecarboxylic acid (organoditelluride **1**; ---, λmax 306 nm), 320 µM GSNO (---, λmax 335 nm), and a mixture of 32 µM organoditelluride **1** and 320 µM GSNO (---, λmax 311 nm) in 2.35 mL of 10 mM PBS buffer, pH 7.4, containing 0.5 mM EDTA under ambient conditions; (B) Absorbance change of the organoditelluride **1** and GSNO solution as a function of time; the absorbance at 311 nm slowly decreased within 1 h; (C) then, continuously decreases for 24 h while the peak at 279 nm is increases.

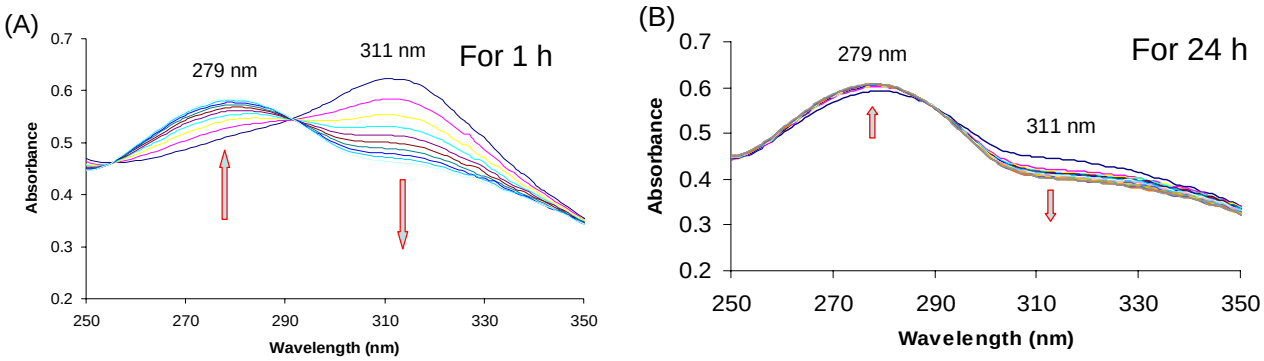


Figure 10s. (A) Absorbance change of the mixture of 32 µM 5,5'-ditelluro-2,2'-dithiophenecarboxylic acid (organoditelluride **1**), 320 µM GSH, and 320 µM GSNO as a function of time at UV spectrophotometric measurements; as the peak at 279 nm increases, the absorbance at 311 nm quickly decreases within 1 h; (B) then, very slowly changes for 24 h as the peak at 279 nm increases.

(4) Mass spectrometer data for tellurosulfide (ArTeSR)

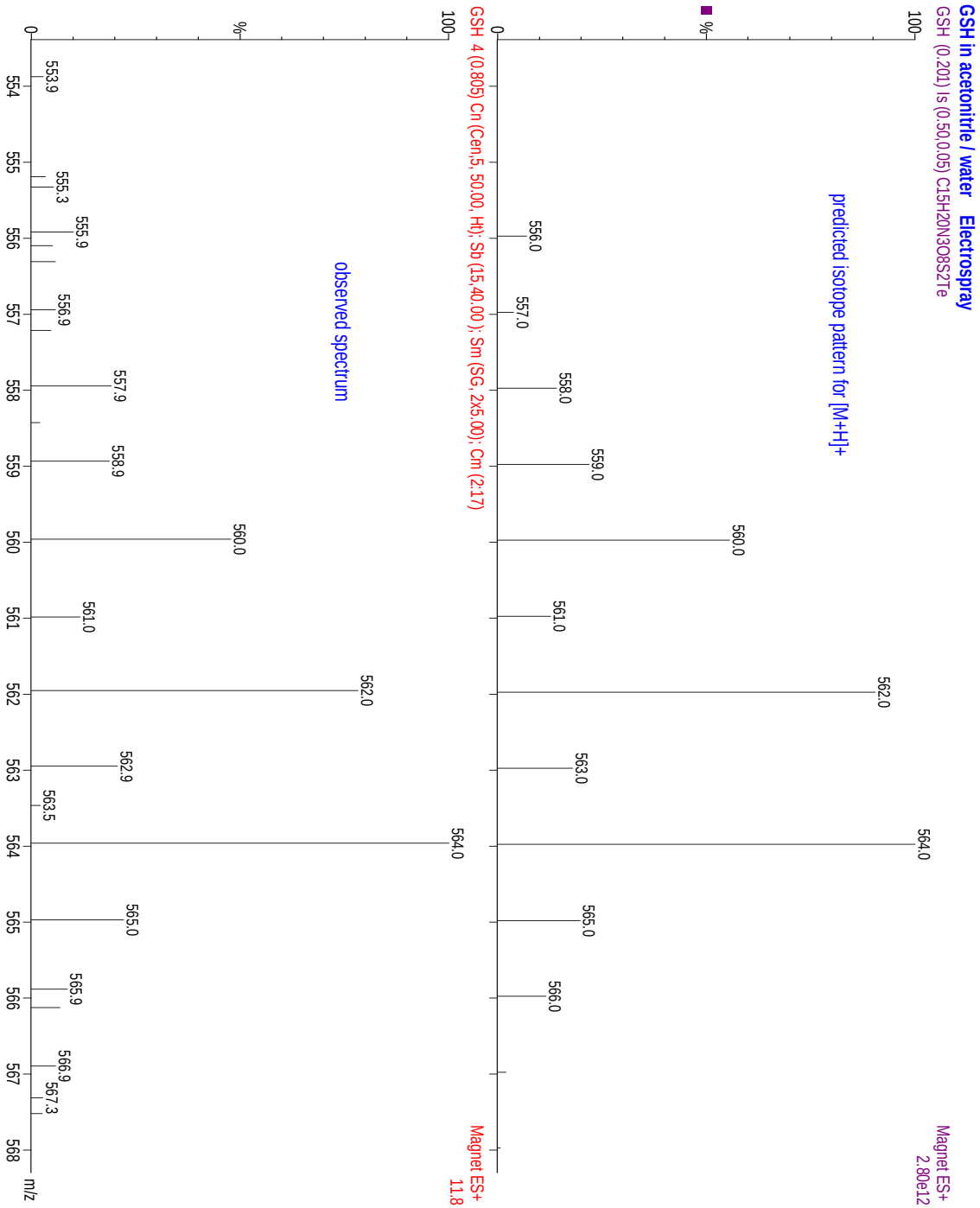


Figure 11s. ArTeSR species can be detected by mass spectrometry (electrospray) from a mixture of 5,5'-ditelluro-2,2'-dithiophenecarboxylic acid (organoditelluride **1**)/GSH (1/14, mole/mole) in CH_3CN/water (1/1, v/v).

(5) The amperometric NO sensor data

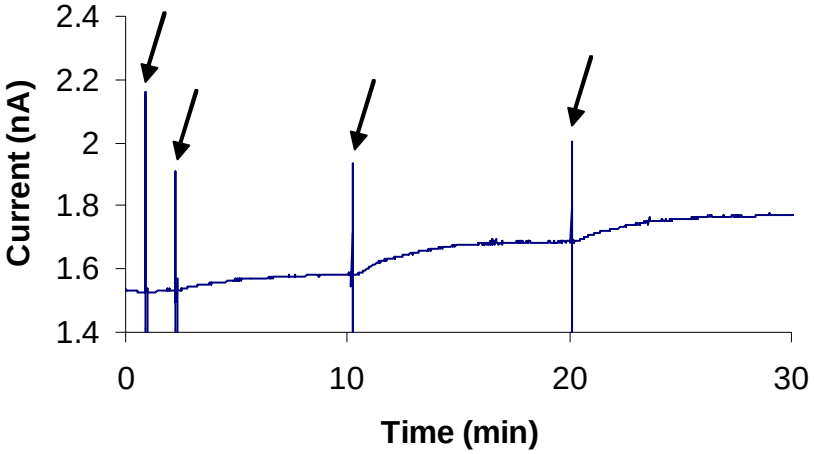
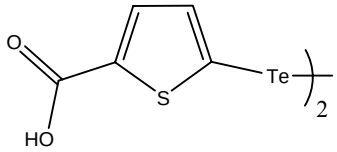
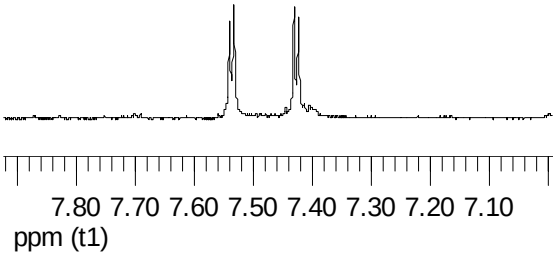


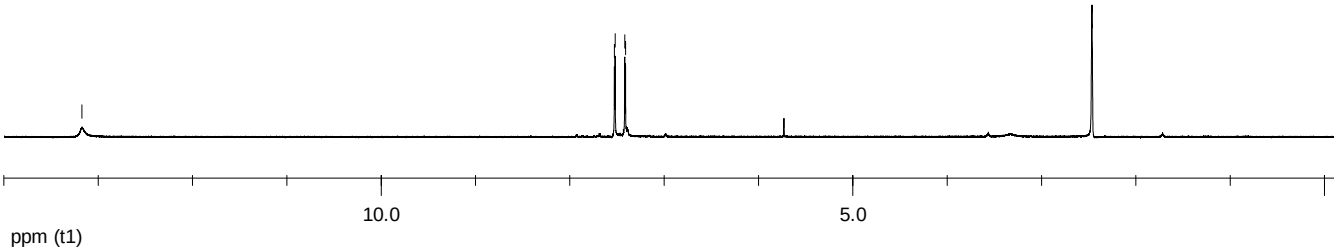
Fig. 12s. Owing to the catalytic GSNO decomposition of organoditelluride **1** in the outer membrane of amperometic NO sensor, the current change (NO signal) is correlated to the RSNO concentration in a given mixture. The NO response could be achieved via this amperometric NO sensor in proportion to the added 20 μL of 5 mM GSNO solutions as the arrow indicated in the graph into 100 ml PBS buffer containing 50μM GSH and 0.1 mM EDTA under ambient oxygen.

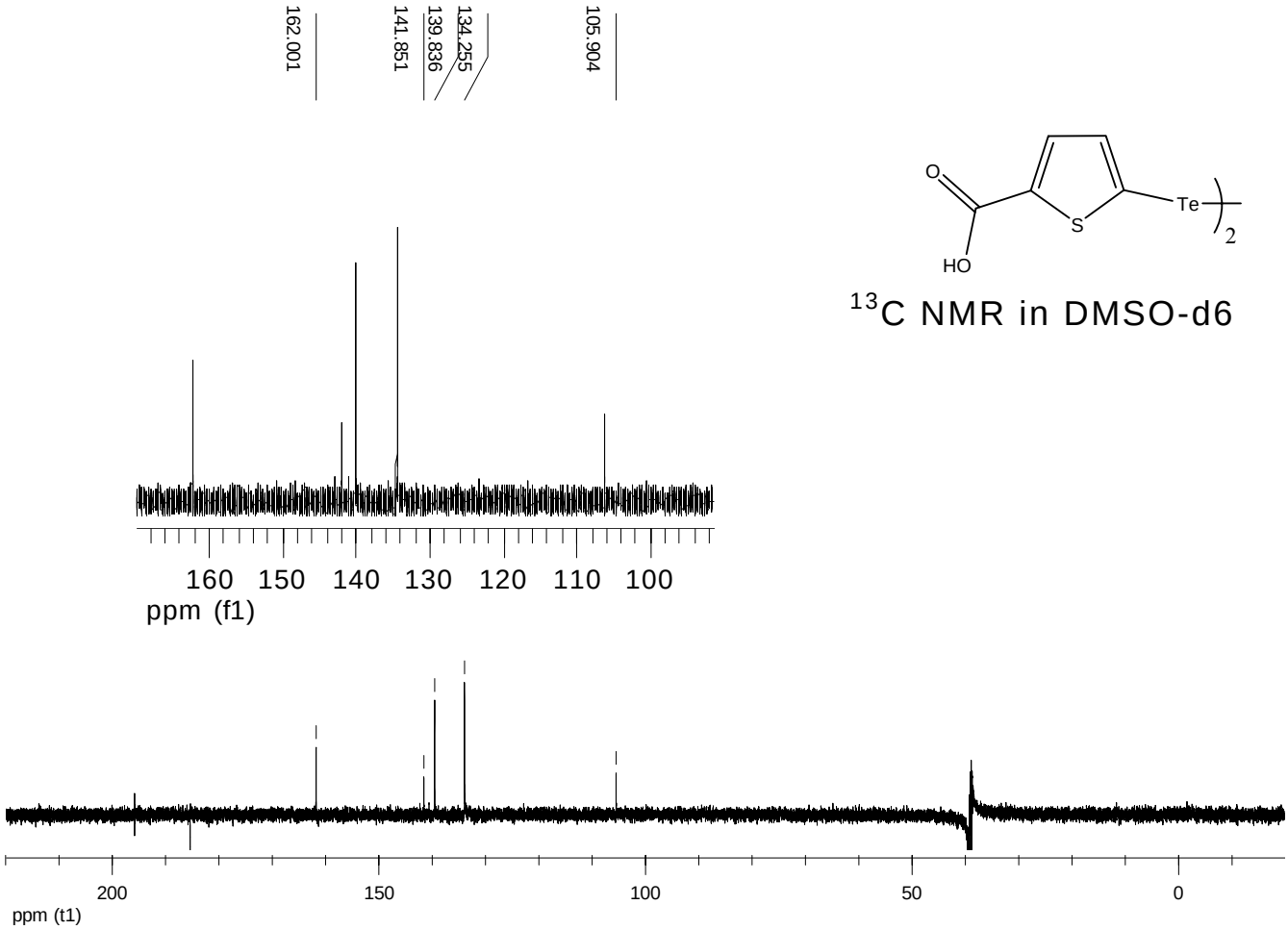
(6) Appendix for NMR, IR



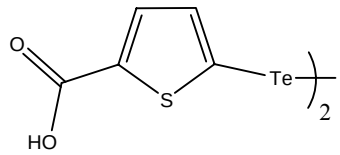
1H NMR in DMSO-d6



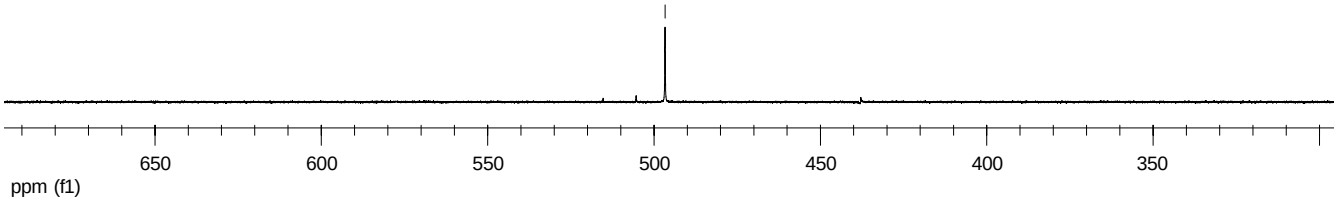




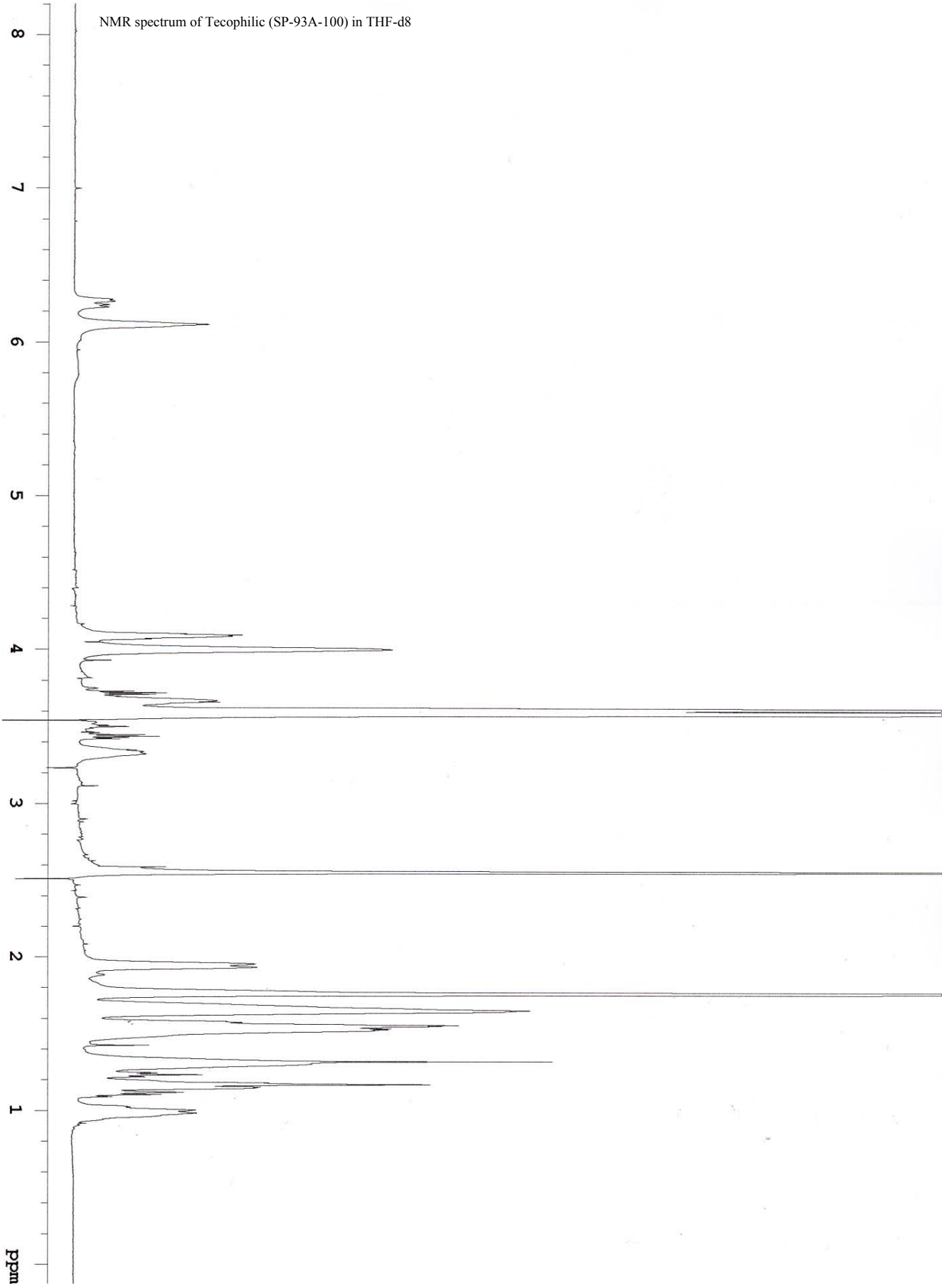
497.353



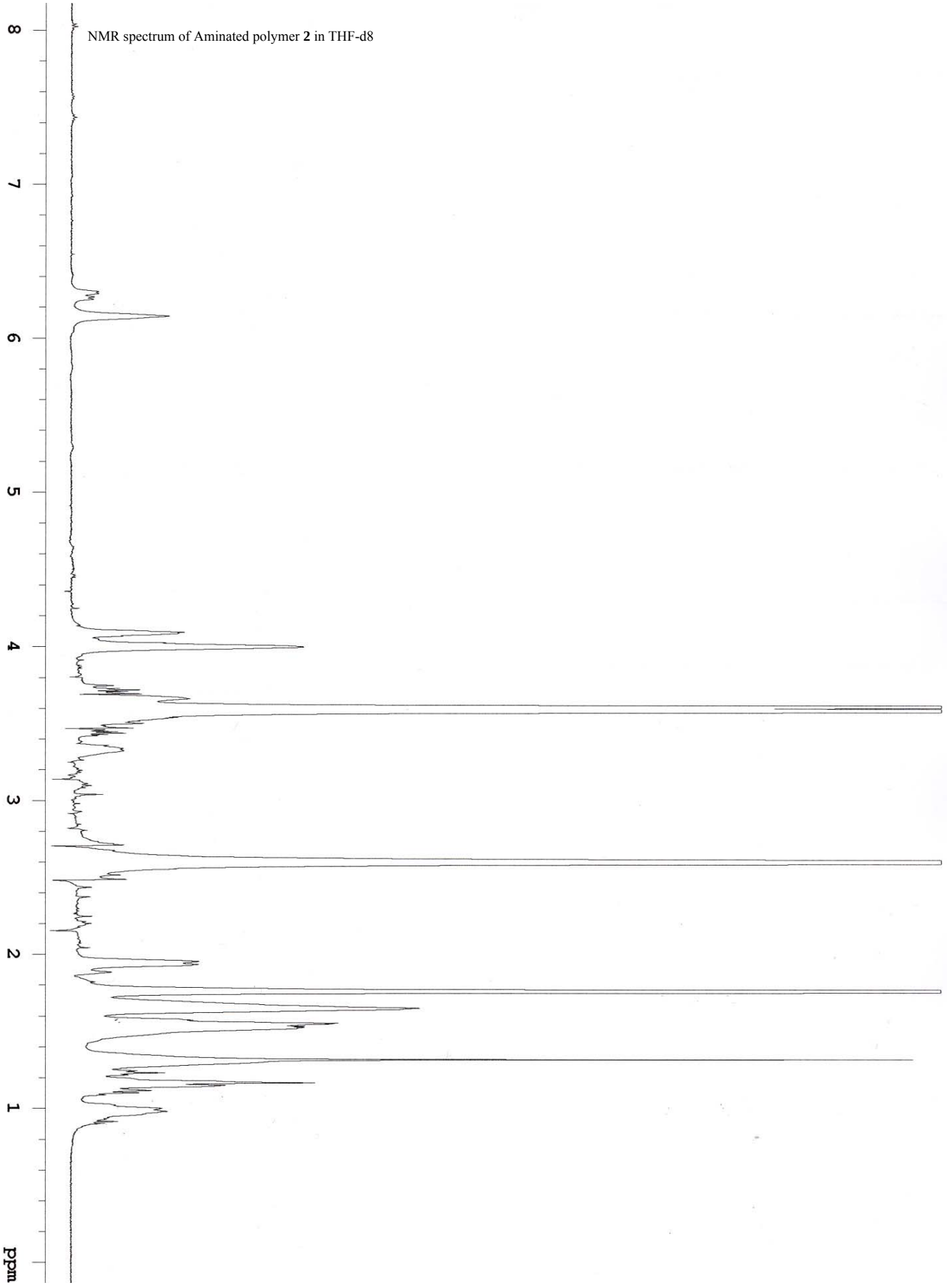
^{125}Te NMR in DMSO-d6



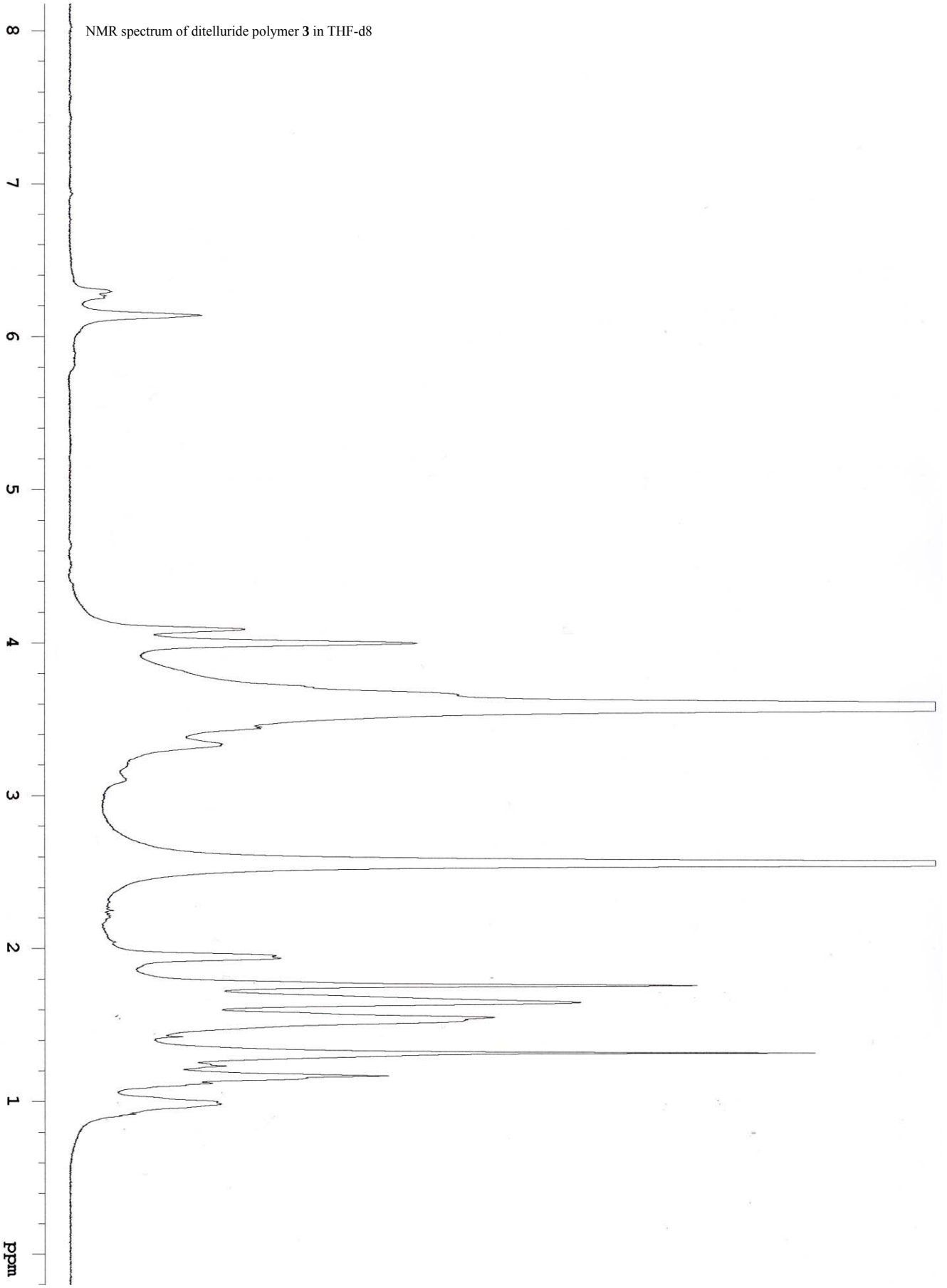
NMR spectrum of Tecophilic (SP-93A-100) in THF-d8

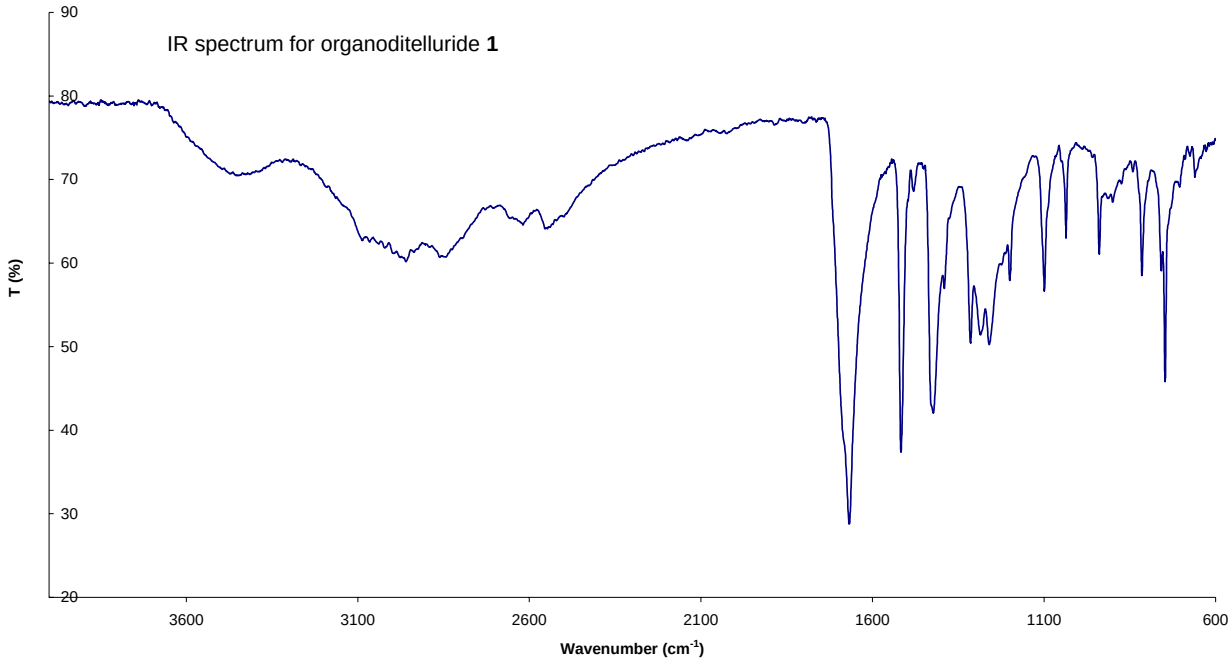


NMR spectrum of Aminated polymer **2** in THF-d8



NMR spectrum of ditelluride polymer **3** in THF-d8





IR spectra for HPU (Tecophilic, Sp-93A-100), Isocyanated HPU and Aminated polymer **2**