

Supplementary Information

Metallothionein-Inspired Prototype of Molecular Pincer

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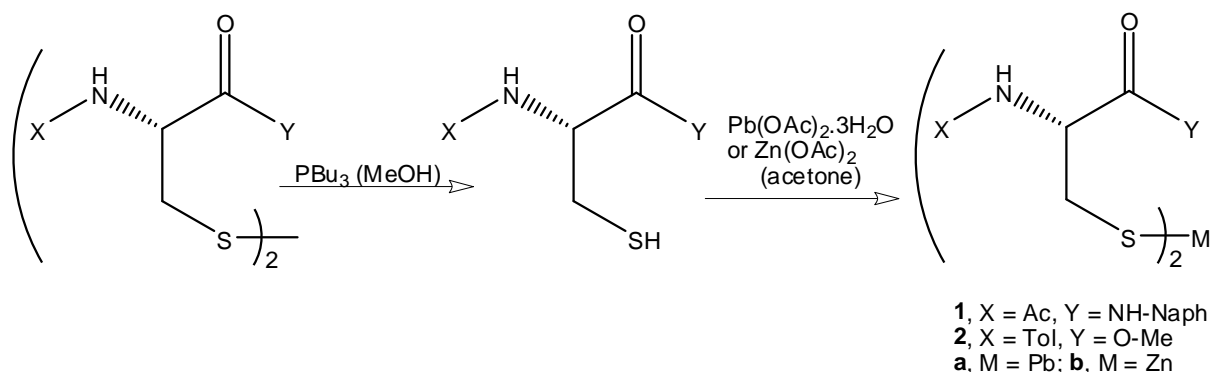
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Melting points were determined on a Stuart SMP 30 Melting Point Apparatus and are not corrected. NMR spectra (δ , ppm; J , Hz) were measured on Bruker Avance-400 instrument in hexadeuterated acetone (Ac), pentadeuterated pyridine (Py), hexadeuterated dimethyl sulfoxide (DMSO) or CDCl_3 and referenced to the solvent signal. Data are reported in the following order: chemical shifts; multiplicities are indicated br (broadened), s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), app (apparent). Elemental analyses (C, H, N, S) were performed on PE 2400 Series II CHNS/O (Perkin Elmer, USA, 1999) instrument, metal contents (Zn, Pb) on X-ray fluorescence spectrometer SPECTRO iQ II (RFA). Mass spectra were measured on a LTQ Orbitrap XL (Thermo Fisher Scientific). IR spectra were measured in solid phase on Bruker Alpha-P spectrometer. UPLC-MS was performed on Acquity UPLC Instrument (Waters Corporation). Solvents such as DMSO, THF, dioxane, dichloromethane were purchased from Aldrich, Fluka or Penta. Unless otherwise noted, they were dried over \AA molecular sieves and titrated for water level with a Karl Fischer Coulometer (Mettler Toledo DL 32) (water content below 10 ppm) and purged with dry Ar before using. Reagents were purchased from Aldrich or AlfaAesar and were used without further purification. 2-Substituted 5-nitro- or 5-[(4-methylbenzoyl) amino]benzo[*d*]-isothiazol-3(2*H*)-ones were prepared according to Ref.¹.

A. Cysteine Derivatives **1** and **2** – Synthesis and Releasing Experiments



1. Metal salts of *N*-acetyl-L-cysteine-2-naphthylamide (**1a** and **1b**)

Commercially available L-cystine-bis(2-naphthyl)amide (0.98 g, 2 mmol) was suspended in acetanhydride (14 mL) and stirred at RT for 1 h (UPLC control). The solvent was then evaporated to dryness, water (50 mL) added, resulting suspension filtered, washed with water and dried. Yield 1.14 g (99%) of the diacetyl derivative, which was used in the next step without further purification.

The diacetyl derivative (0.574 g, 1 mmol) was suspended in methanol (100 mL) and 5 min degassed. PBu_3 (97%, 0.229 g, 1.1 mmol) was then added and the reaction mixture stirred for 5 min (UPLC control). The solvent was evaporated under reduced pressure at 25 °C, the solid residue suspended in MeOH (5 mL), filtered, dried and crystallized from EtOH, yield 0.38 g (66 %) of the mercapto derivative, which was used in the metal salt preparation without further purification.

The mercapto derivative (0.13 g, 0.45 mmol) was dissolved in acetone (10 mL) and the solution of lead(II) acetate trihydrate (0.086 g, 0.225 mmol) or zinc(II) acetate (0.042 g, 0.225 mmol) in 15 mL acetone containing one drop of water was added. The lead sulfide **1a** precipitated immediately, the zinc sulfide **1b** was precipitated with ether.

Compound **1a**: Yield 0.119 g (68 %), m.p. 187-195 °C (dec.). Elemental analysis (%): For $\text{C}_{30}\text{H}_{30}\text{N}_4\text{O}_4\text{PbS}_2 \cdot 2\text{H}_2\text{O}$ (817.94) calculated: C, 44.05; H, 4.19; N, 6.85; found: C, 44.05; H, 3.75; N, 6.62. ^1H NMR spectrum (DMSO) (paragraph F): 1.89 s (3H), 3.75 m and 3.87 m (2xH), 4.53 dd (1H), 7.38 dd (1H), 7.47 dd (1H), 7.63 d (1H), 7.79 d (1H), 7.81 m (2H), 8.14 d (1H), 8.29 s (1H), 10.23 br s (1H).

Compound **1b**: Yield 0.083 g (56 %), m.p. 186.8-187.3 °C (dec.). Elemental analysis (%): For $\text{C}_{30}\text{H}_{30}\text{N}_4\text{O}_4\text{S}_2\text{Zn} \cdot \text{H}_2\text{O}$ (658.14) calculated: C, 54.75; H, 4.90; N, 8.51; found: C, 54.64; H, 4.79; N, 8.06. ^1H NMR spectrum (DMSO) (paragraph F): 1.89 s (3H), 2.74 m and 2.86 m (2xH), 4.41 dd (1H), 7.38 dd (1H), 7.41 dd (1H), 7.65 d (1H), 7.75 d (1H), 7.80 m (2H), 7.94 d (1H), 8.32 br s (1H), 10.12 br s (1H).

2. Metal salts of methyl *N*-*p*-toluoyl-L-cysteinate (**2a** and **2b**)

Commercially available L-cystine-dimethylester dihydrochloride (1.706 g, 5 mmol) was added to saturated Na₂CO₃ solution in water (25 mL) and stirred at RT for 1 min. *p*-Toluoyl chloride (1.38 mL, 2.52 mmol) was then added dropwise during 10 min and the reaction mixture was stirred at RT for 2 h (UPLC control). The resulting suspension was filtered, washed with water and dried. Yield 2.311 g (92%) of the bis(*p*-toluoyl) derivative, which was used in the next step without further purification.

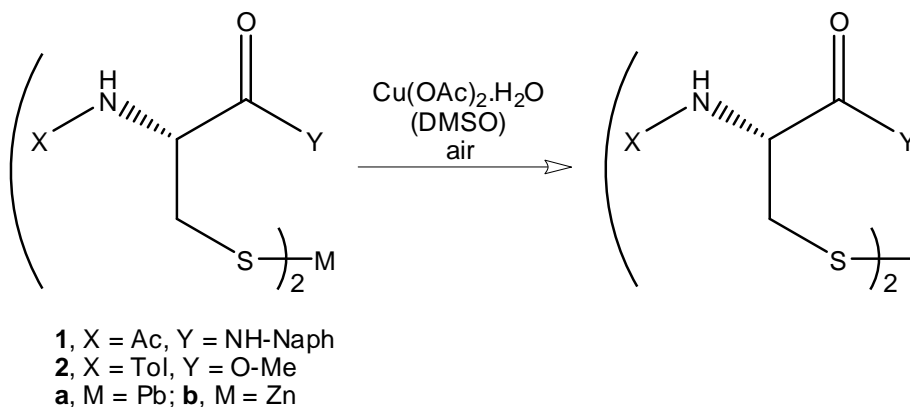
The bis(*p*-toluoyl) derivative (1.1 g, 2.18 mmol) was suspended in methanol (20 mL) and 5 min degassed. PBU₃ (97%, 882 mg, 4.36 mmol) was then added and the reaction mixture stirred for 5 min (UPLC control). The solvent was evaporated under reduced pressure at 25 °C, the solid residue dissolved in MeOH (5 mL), precipitated with hexane (10 mL), filtered and dried, yield 0.70 g (63 %) of the mercapto derivative, which was used in the metal salt preparation without further purification.

The mercapto derivative (0.17 g, 0.67 mmol) was dissolved in acetone (5 mL) and the solution of lead(II) acetate trihydrate (0.127 g, 0.335 mmol) or zinc(II) acetate (0.062 g, 0.335 mmol) in 15 mL acetone containing one drop of water was added. The lead sulfide **2a** precipitated immediately, the zinc sulfide **2b** was precipitated with ether/hexane and after drying a colourless crystals were obtained.

Compound **2a**: Yield 0.169 g (71 %), m.p. 176.7-178.0 °C. Elemental analysis (%): For C₂₄H₂₈N₂O₆PbS₂ (711.81) calculated: C, 40.50; H, 3.96; N, 3.94; found: C, 39.70; H, 3.98; N, 3.75. ¹H NMR spectrum (DMSO) (paragraph F): 2.34 s (3H), 3.63 s (3H), 3.86 m (2H), 4.53 dd (1H), 7.25 d (2H), 7.79 d (2H), 8.42 br d (1H).

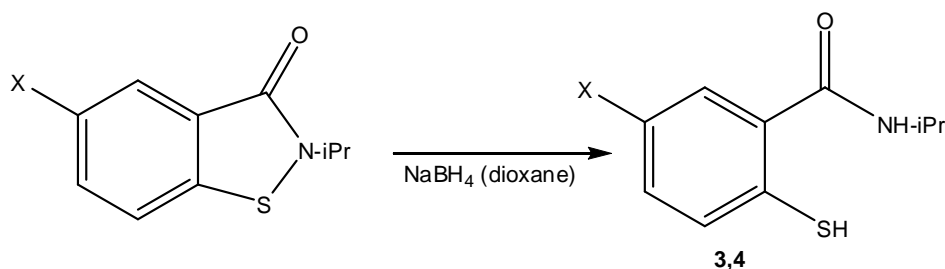
Compound **2b**: Yield 0.133 g (70 %), m.p. 199.4-202.0 °C. Elemental analysis (%): For C₂₄H₂₈N₂O₆S₂Zn (570.03) calculated: C, 50.57; H, 4.95; N, 4.91; found: C, 50.95; H, 5.05; N, 4.51. ¹H NMR spectrum (DMSO) (paragraph F): 2.33 s (3H), 2.87 m (2H), 3.60 s (3H), 4.44 dd (1H), 7.26 d (2H), 7.78 d (2H), 8.25 br d (1H).

3. Releasing Experiments of Cysteine Derivatives



Releasing experiments of compounds **1a**, **1b** and **2a**, **2b** were performed as given in Paragraph E. Only the corresponding cysteine derivative was formed.

B. Synthesis of Mercapto Derivatives **3,4**. General Procedure



Compound	X
3	NO ₂
4	4-MeBz-NH

2-Substituted 5-nitro- or 5-[(4-methylbenzoyl)amino]benzo[*d*]isothiazol-3(2*H*)-one (3 mmol) was dissolved in dioxane (20 mL) or dioxane-ethanol 2:1 (20 mL). Sodium borohydride (113.5 mg, 3 mmol) was then added and the reaction mixture was stirred at RT for ~2.5 h (UPLC control). After reaching complete conversion, the resulting suspension was poured to 10% HCl-ice mixture (250 mL). After warming to RT the product was filtered and dried. Yields, melting points and elemental analyses are given in Table 1, NMR spectra in Tables 3-4 and in Paragraph F. IR spectra are given in Table 6.

N-Isopropyl-2-mercapto-5-nitrobenzamide (**3**) (X = NO₂) was prepared according to the General Procedure from 2-isopropyl-5-nitrobenzo[*d*]isothiazol-3(2*H*)-one (0.714 g) in dioxane. ¹H NMR spectrum is given in Table 3.

N-Isopropyl-2-mercapto-5-[(4-methylbenzoyl)amino]benzamide (**4**) (X = 4-methylbenzoyl-amino) was prepared according to the General Procedure from 2-isopropyl-5-[(4-methylbenzoyl)amino]benzo[*d*]isothiazol-3(2*H*)-one (0.978 g) in dioxane-ethanol. ¹H NMR spectrum is given in Table 4.

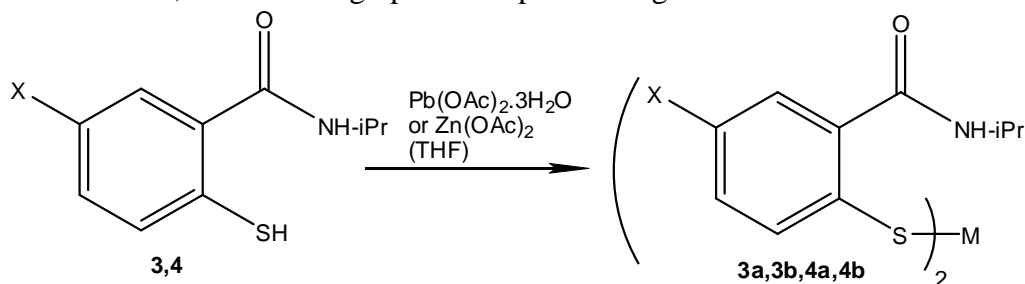
Table 1. Yields, melting points and elemental analyses of compounds **3,4**

Compound	Yield (%)	M.p. (°C)	%C calc/found	%H calc/found	%N calc/found
3	87	252.0-254.0	49.99/49.64	5.03/4.99	11.66/11.18
4	93	227.5-230.6	65.83/65.30	6.14/6.31	8.53/8.14

C. Synthesis of Metal(II) Sulfides **3a**, **3b**, **4a**, **4b**

Method *a*:

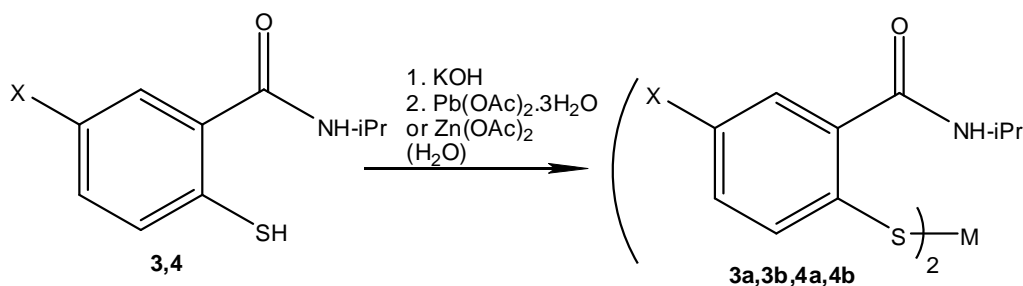
Thiol **3** or **4** (0.25 mmol) was dissolved in degassed THF (4 mL) and the corresponding metal acetate (0.12 mmol) was added. The reaction mixture was stirred at RT (22 °C) or slightly elevated temperature (35 °C) under Ar atmosphere for 16 h. The resulted microcrystalline precipitate was filtered, dissolved in small amount of acetone, re-precipitated with hexane, filtered and dried. Yields, elemental analyses and HRMS results are given in Table 2, NMR spectra in Tables 3,4 and in Paragraph **F**. IR spectra are given in Table 5.



Thiol	a	b
3 (X = NO ₂), 4 (X = 4-MeBz-NH)	Pb(II)	Zn(II)

Method *b*:

Thiol **3** or **4** (0.25 mmol) was suspended in degassed water (10 mL), a solution of freshly powdered KOH (14 mg, 0.25 mmol) in degassed water (9 mL) was added and the resulting orange solution (thiolate of **3**) or colorless solution (thiolate of **4**) was stirred under Ar atmosphere at RT for 5 min. Then metal acetate (0.12 mmol) dissolved in degassed water (20 mL) was added in one portion and the reaction mixture was stirred for 1 h. The resulting suspension was then centrifuged for 30 min at 3000 rpm, separated, the precipitated sulfide washed with water, dried and analyzed. Supernatant was used for scavenging experiments (See paragraph **D**). Yields of metal sulfides prepared by Method *b* were generally lower (30-60%) than yields of sulfides prepared by Method *a*.



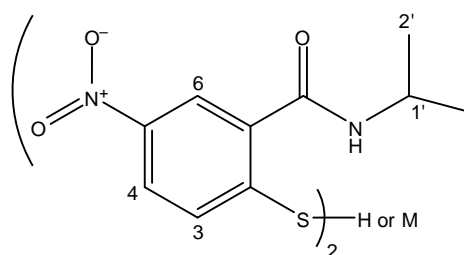
Thiol	a	b
3 (X = NO ₂), 4 (X = 4-MeBz-NH)	Pb(II)	Zn(II)

Table 2. Characterization data of compounds **3a-4b**

Compound	Yield ^a (%)	M.p. (°C)	%C calc/found	%H calc/found	%N calc/found	%S calc/found	% metal calc/found	
3a	74	185.2-186.4	35.03/35.06	3.23/3.21	8.17/7.62	n.d.	30.22/29.45	
3b	73	250.3-252.9	HRMS: For C ₂₀ H ₂₂ O ₆ N ₄ ZnS ₂ calc/found: 542.0272/542.0276					
4a	76	194.0-196.3	50.16/50.32	4.44/4.97	6.50/6.18	n.d.	24.04/24.05	
4b	77	194.4-196.6	60.03/59.47	5.32/5.36	7.78/7.48	8.90/8.32	9.08/8.78	

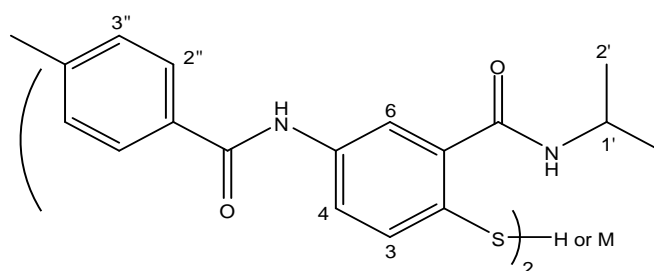
n.d. not determined; ^aIsolated yields – Method a.

Table 3. ¹H NMR spectra of compounds **3**, **3a**, **3b**



Compd	Solv	H-1'	H-2'	$J_{1'2'}$	H-3	H-4	H-6	NH	SH
3	Ac	4.24	1.27	6.4	7.67	8.11	8.39	8.10	5.76
3a	Ac	4.35	1.34	6.6	7.41	8.01	8.10	8.47	-
3b	Ac	4.25	1.32	6.4	7.74	8.02	8.27	9.07	-

Table 4. ¹H NMR spectra of compounds **4**, **4a**, **4b**



	Solv	H-1'	H-2'	NH Tol	H-2''	H-3''	Me	H-3	H-4	H-6	NH <i>i</i> Pr
4^a	Py	4.62	1.37	11.04	8.18	7.27	2.29	8.10	8.10	8.67	9.10
4a	DMSO	4.10	1.18	10.14	7.87	7.32	2.38	7.26	7.69	7.75	9.07
4b	Ac	4.23	1.30	9.44	7.88	7.31	2.39	7.47	7.57	7.91	8.57

^aSH signal at 5.25 ppm

Table 5. IR spectra of compounds **3,4** and **3a-4b**

Compound	IR spectrum (cm ⁻¹)
3	1345, 1529, 1632, 2360, 2972
3a	1048, 1325, 1503, 1591
3b	925, 1273, 1321, 1493, 1584
4	742, 1322, 1474, 1531, 1632, 2968
4a	743, 1305, 1462, 1502, 1569, 1463
4b	746, 1302, 1390, 1502, 1561

D. Scavenging Experiments

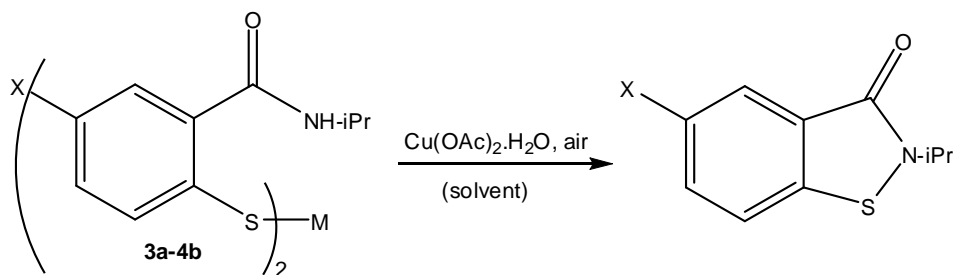
The scavenging experiments were performed in water according to the Method *b*. The metal content of supernatant was analyzed by RFA (Table 6).

Table 6. Metal content in supernatant (ppm)

Compound	Initial metal conc. (ppm)	Metal content after scavenging (%)	
		3	4
a (Pb)	637	0 ^a	6.59
b (Zn)	201	5.97	6.97

^abelow the limit of detection

E. Releasing Experiments



1. In DMSO:

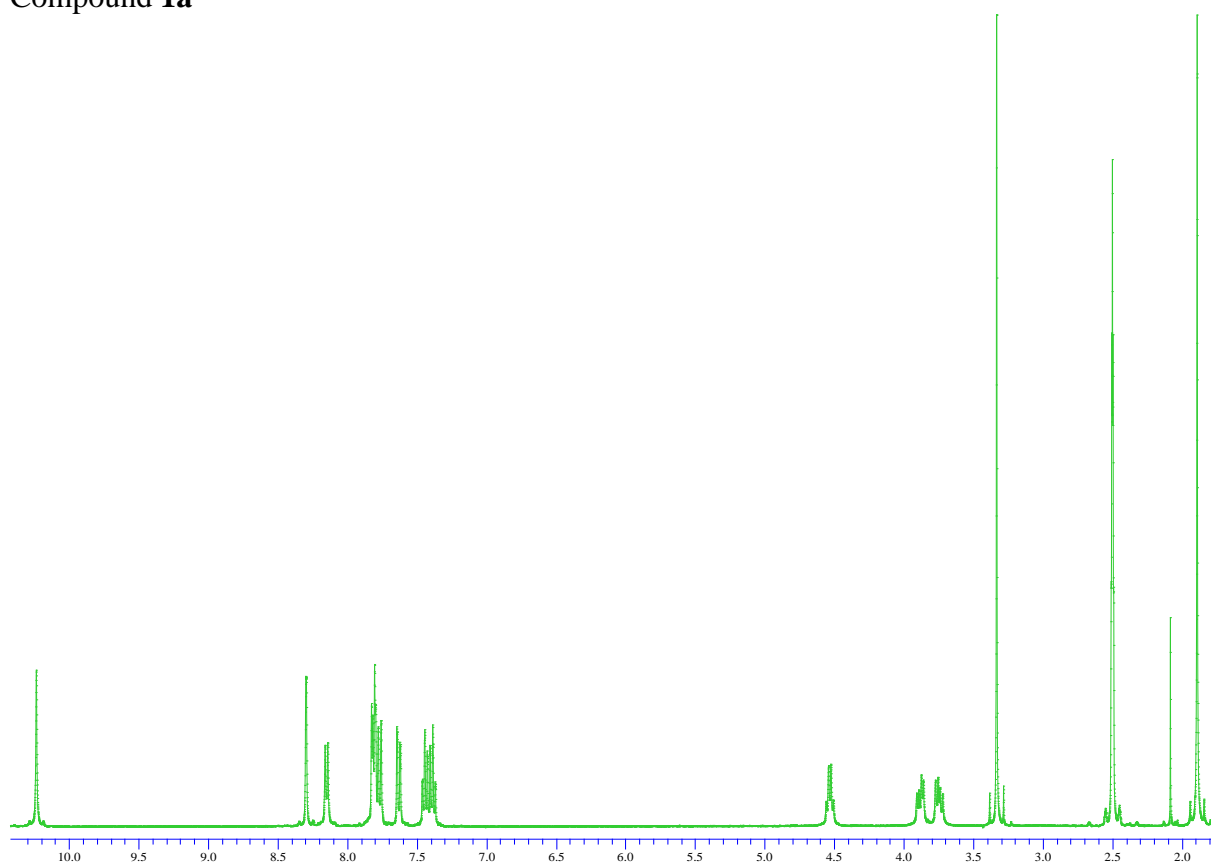
Metal sulfide **3a-4b** (20 μmol) was dissolved in DMSO (1 mL) and Cu(OAc)₂.H₂O (0.4 mg, 2 μmol, 10 %) was added. The reaction mixture was vigorously shaken with a laboratory vortex (900 rpm) for 20 h under air atmosphere. Samples were taken at 0.25, 2, 4, 6 and 20 h and analyzed with UPLC for corresponding substituted benzo[*d*]isothiazol-3(2*H*)-one content.

2. In water:

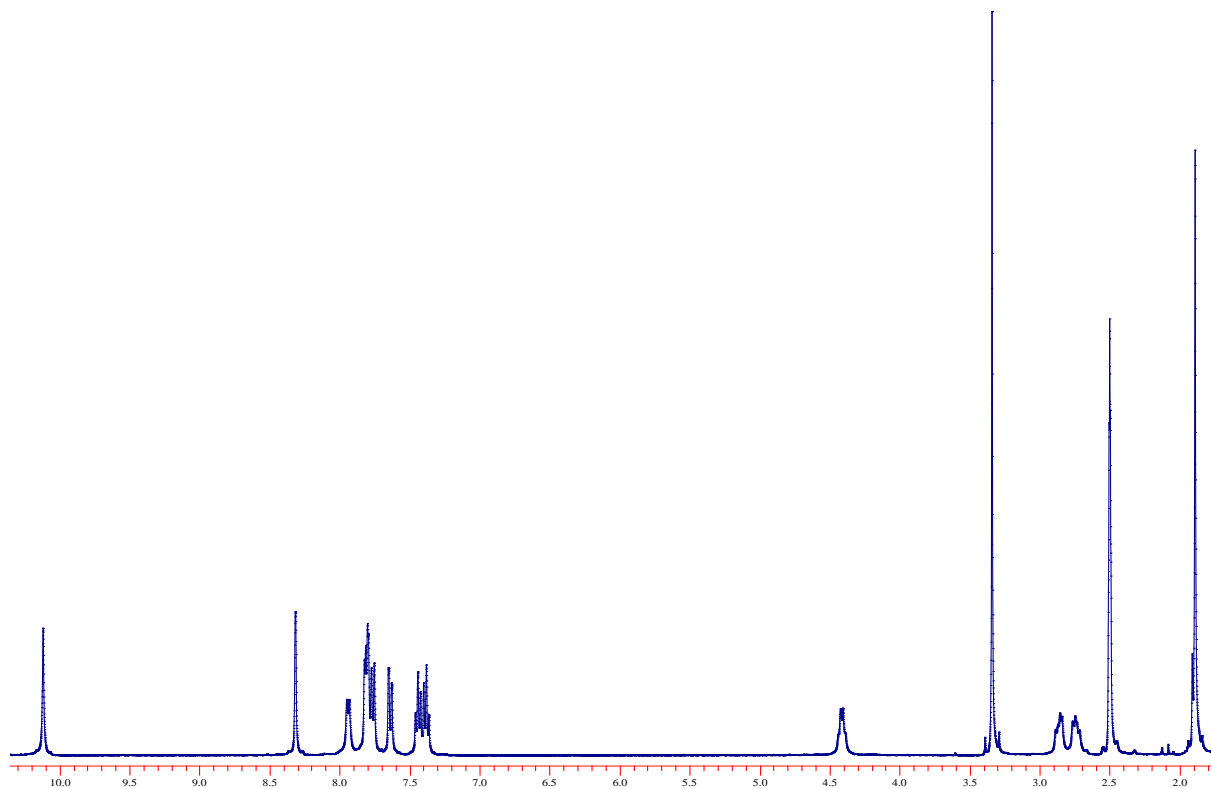
Metal sulfide **3a** (20 μmol) was suspended in water (2 mL) and Cu(OAc)₂.H₂O (0.4 mg, 2 μmol, 10 %) was added. The reaction mixture was vigorously shaken with a laboratory vortex (900 rpm) for 20 h under air atmosphere. Samples were taken at 0.25, 2, 4, 6 and 20 h and analyzed with UPLC for corresponding substituted benzo[*d*]isothiazol-3(2*H*)-one content. Graph of comparison of the releasing experiments of compound **3a** in DMSO and water see in Paragraph **G**.

F. NMR Spectra

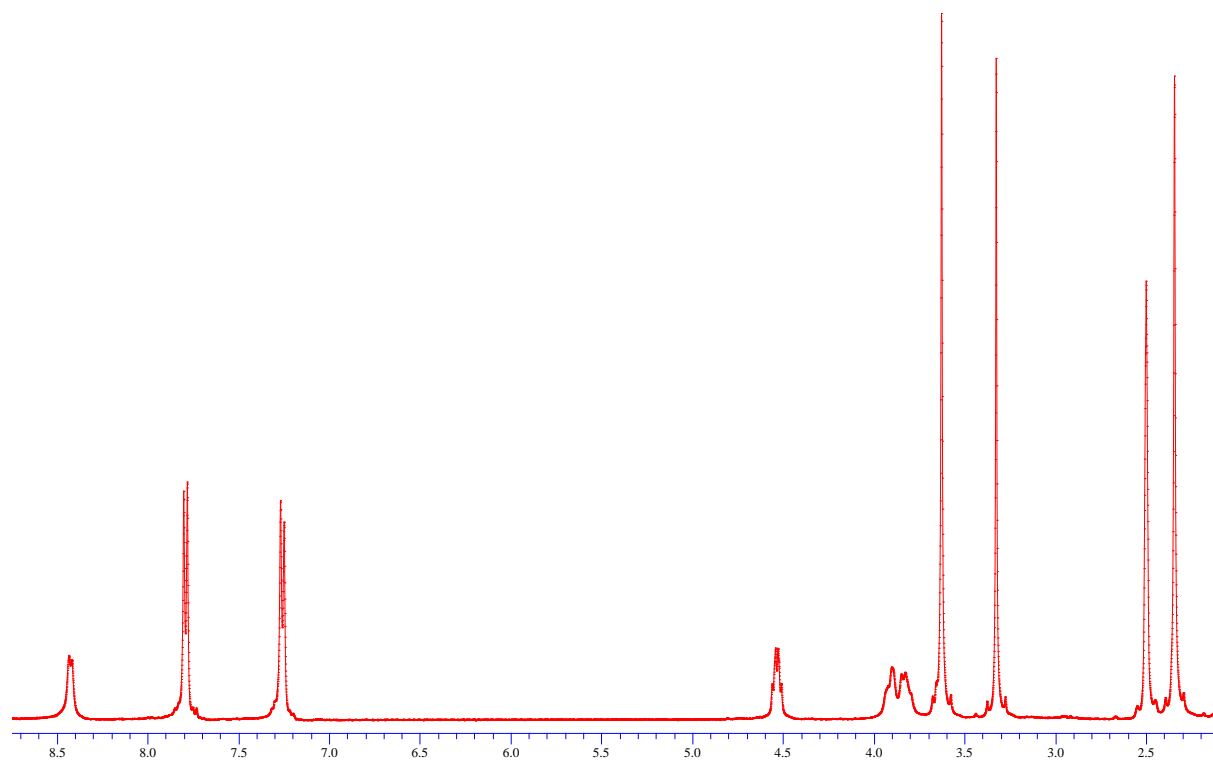
Compound **1a**



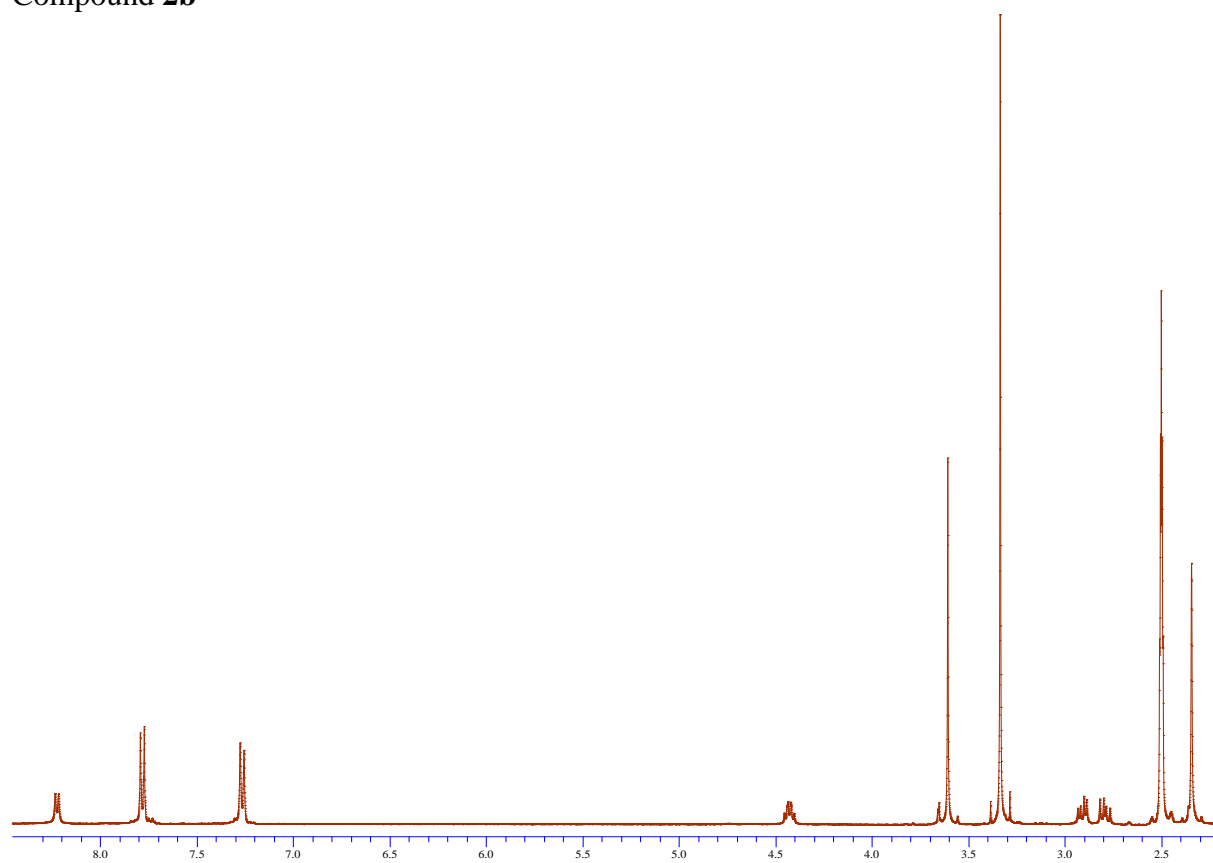
Compound **1b**



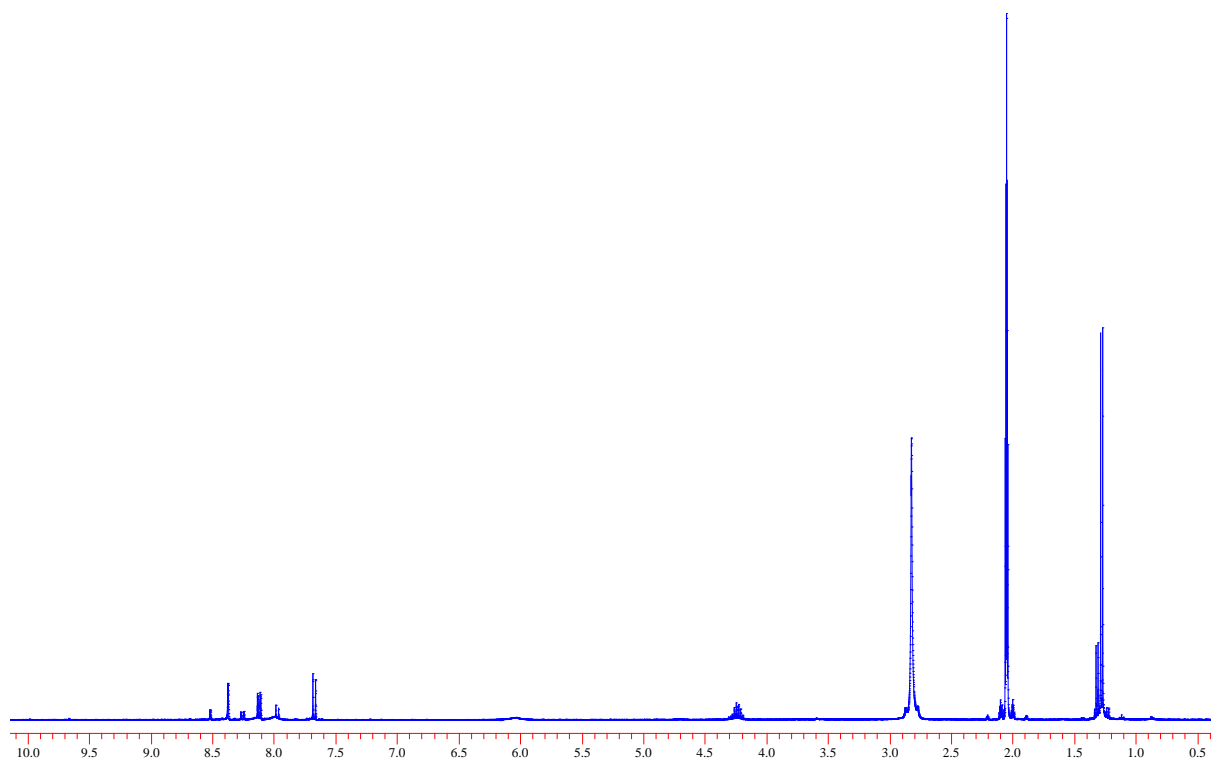
Compound 2a



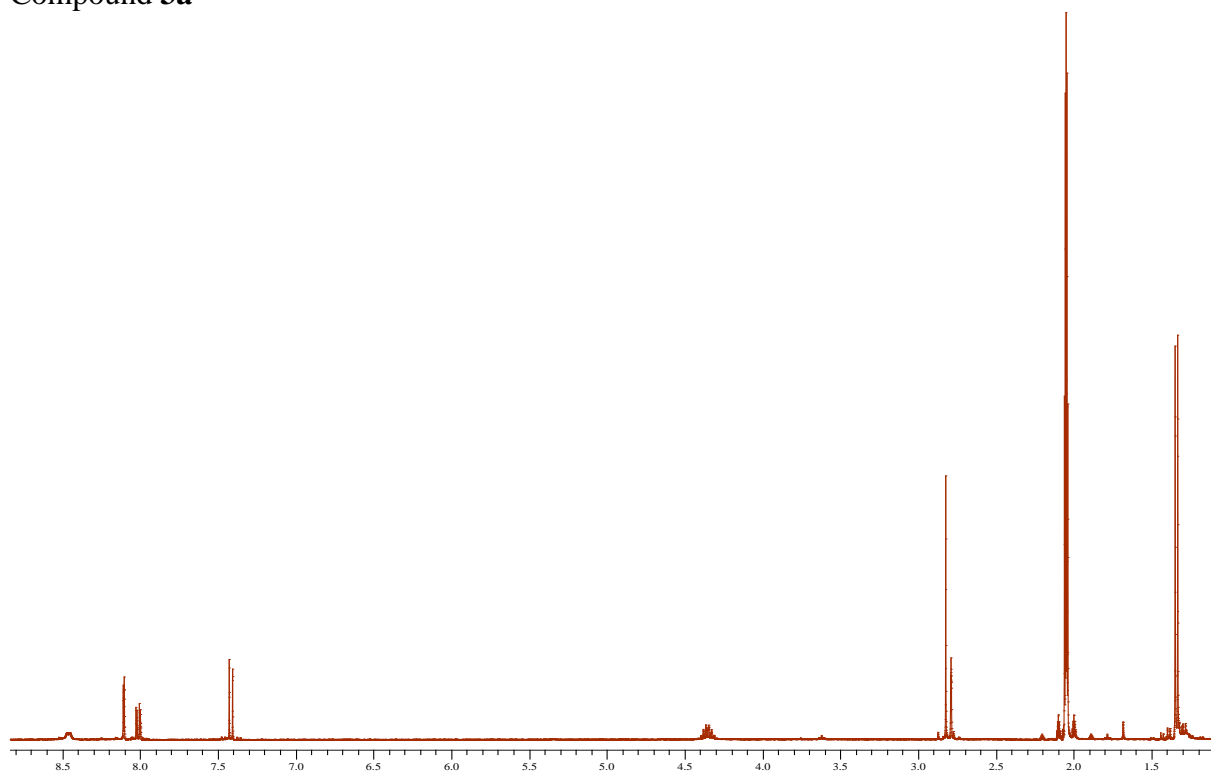
Compound 2b



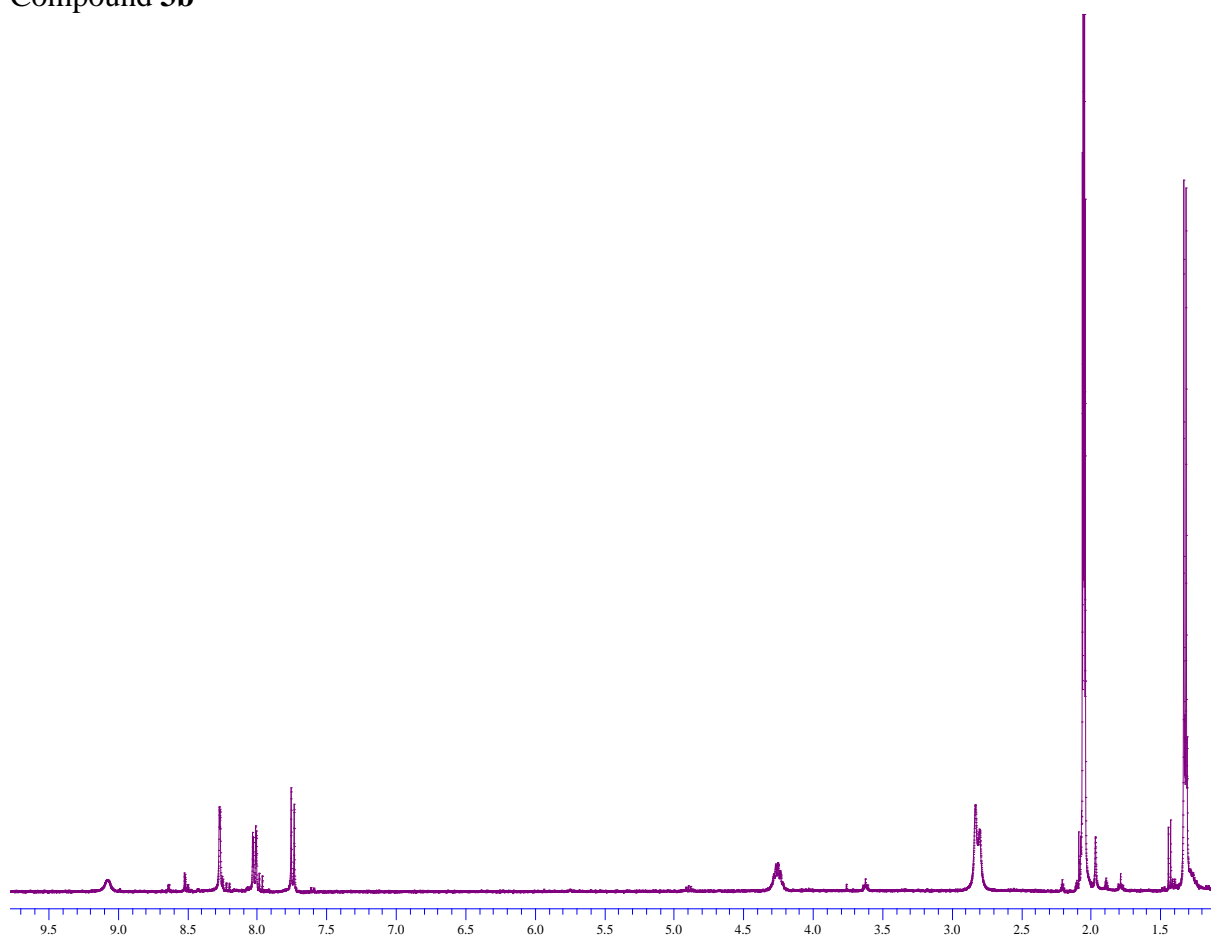
Compound 3



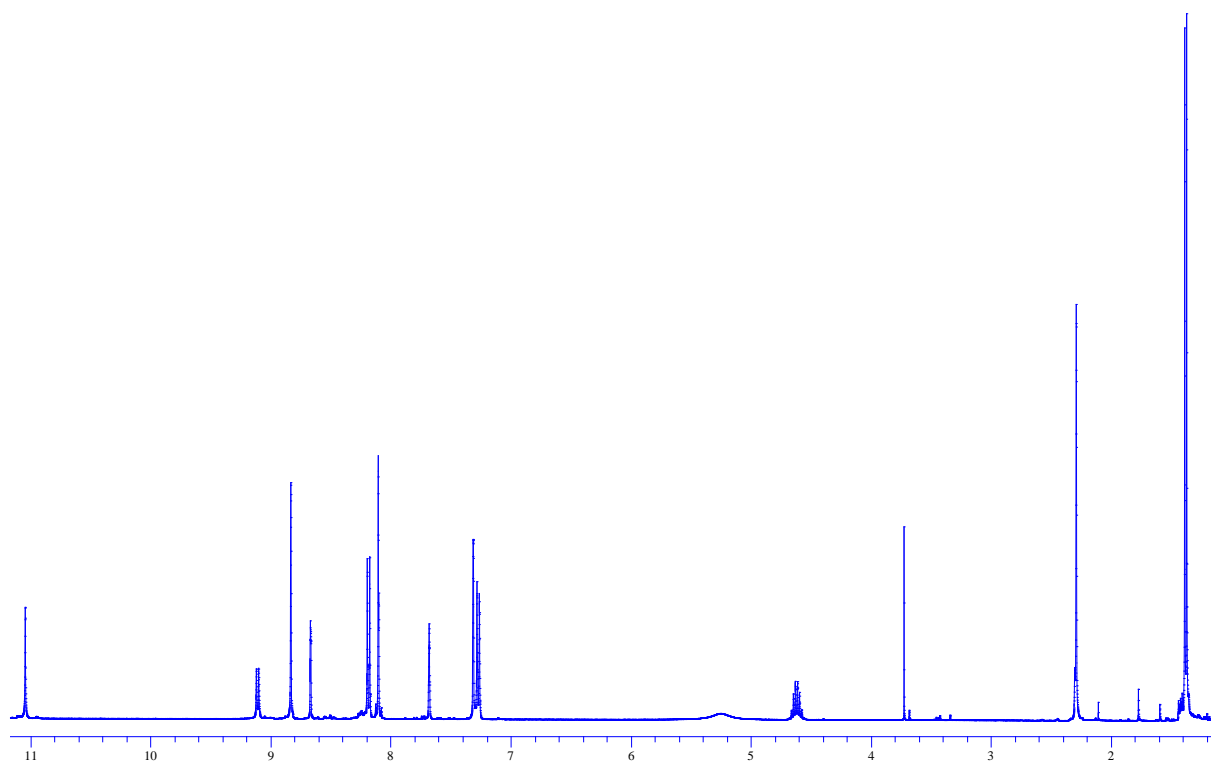
Compound 3a



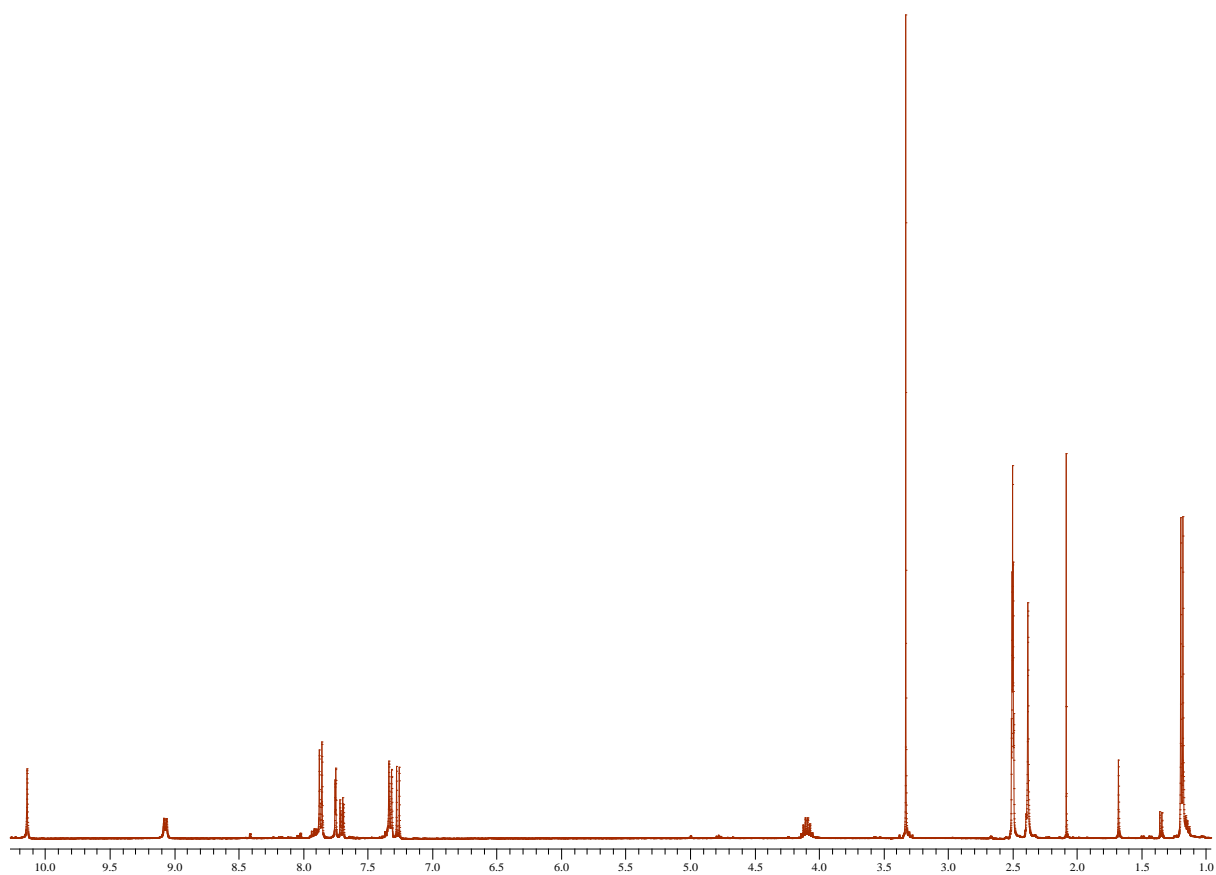
Compound 3b



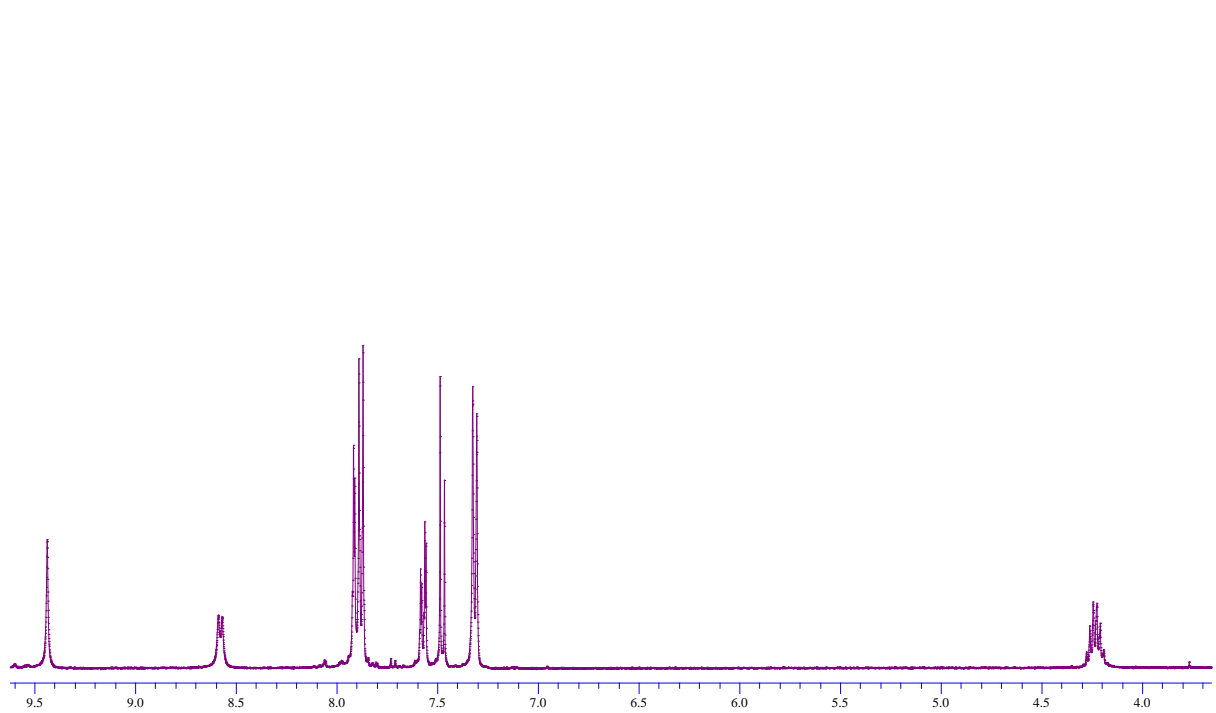
Compound 4



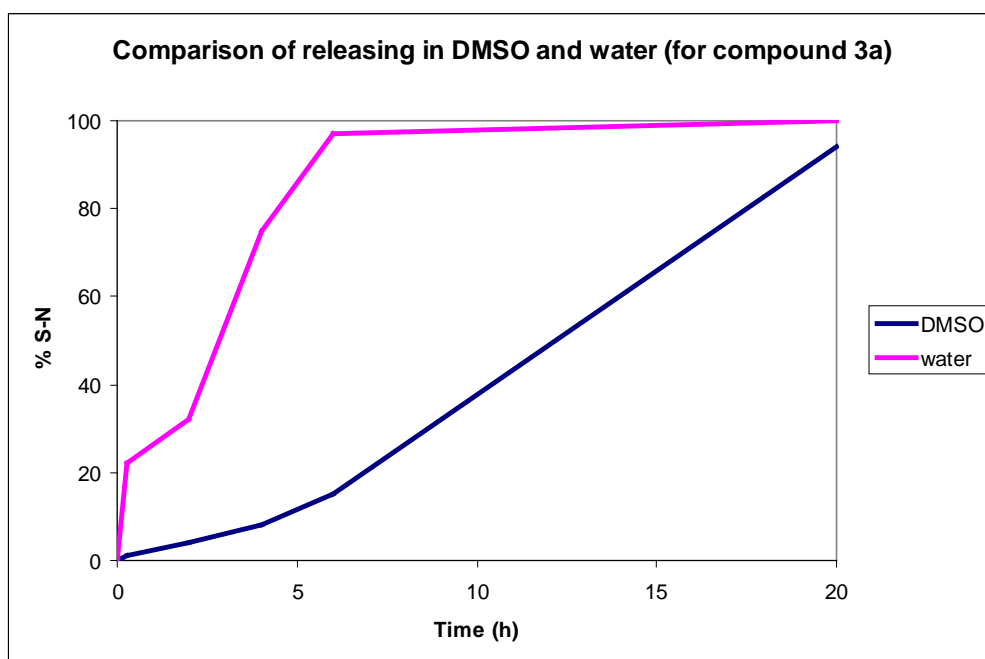
Compound **4a**



Compound **4b**



G. Releasing experiments



H. References

1 Henke A., Srogl J.: *Chem. Commun. (London)* 2010, 46, 6819–6821.