Electronic Supplementary Information

Additional data

Gas chromatography (GC)

Irradiation of a non-equimolar mixture

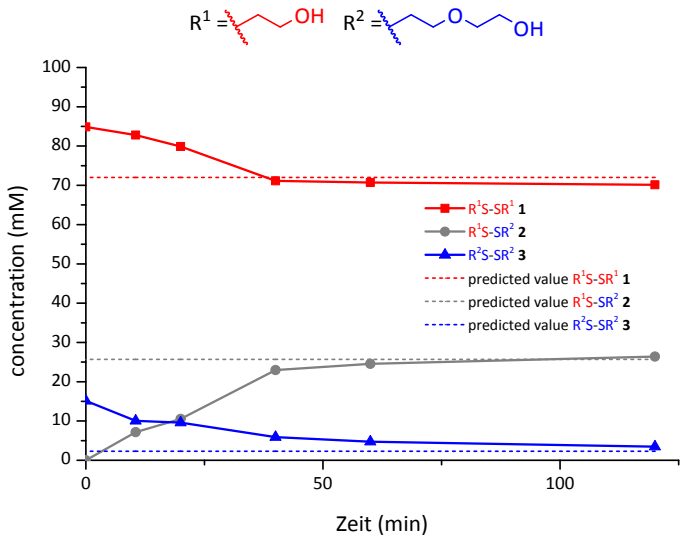


Fig. S1: Change in concentrations of $R^1S\text{-}SR^1$ 1, $R^1S\text{-}SR^2$ **2** and $R^2S\text{-}SR^2$ **3** from a solution of $R^1S\text{-}SR^1$ **1** (85 mM) and $R^2S\text{-}SR^2$ **3** (15 mM) in water upon irradiation with UV light. Values were determined by GC as described under **General Procedures**.

Irradiation of a heterodisulfide

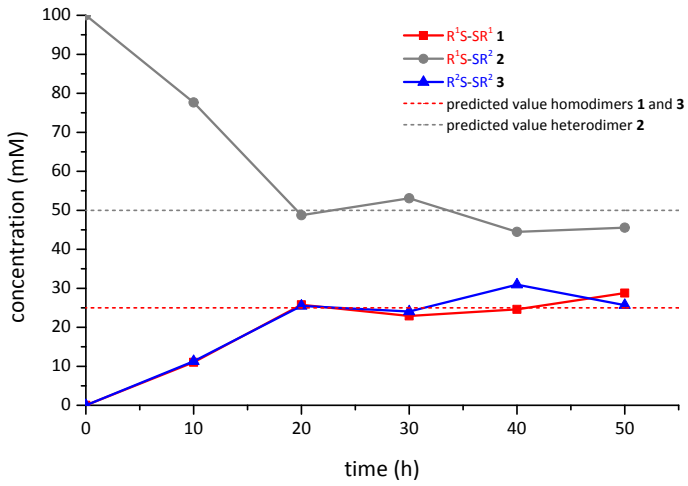


Fig. S2: Change in concentrations of $R^1S\text{-}SR^1$ **1**, $R^1S\text{-}SR^2$ **2** and $R^2S\text{-}SR^2$ **3** from a solution of $R^1S\text{-}SR^2$ **2** (100 mM) in water upon irradiation with UV light. Values were determined by GC as described under

High performance liquid chromatography (HPLC)

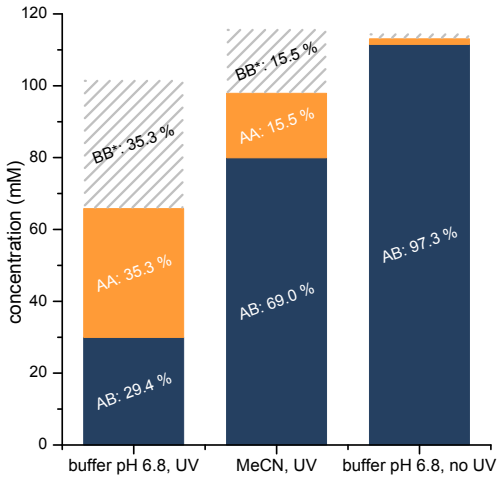


Fig. S3: Concentrations of AA, AB and BB after irradiating a solution of AB (100 mM) in phosphate buffer (250 mM, pH 6.8) with UV light for 6 h. Values were determined by HPLC as described under **General Procedures**.

Rheology

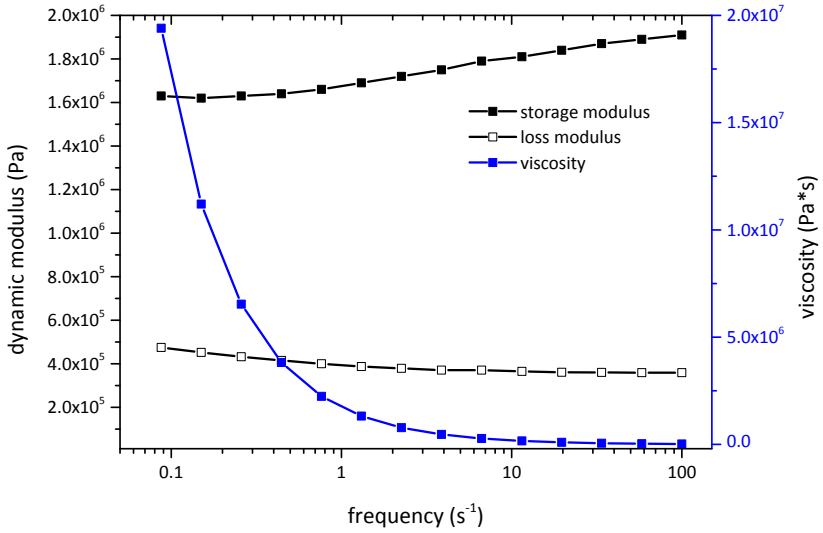


Fig. S4: Dynamic modulus and viscosity of a hydrogel prepared from AB (100 mM) in phosphate buffer (250 mM, pH 6.8) by irradiating with UV light for 18 h. Also see **General Procedures**.

General procedures

UV irradiation

Irradiation experiments were carried out using a filtered 8 W UV Lamp by *CAMAG* (*Camag Chemie-Erzeugnisse und Adsorptionstechnik AG*, Muttenz, Switzerland) using the mode with an emission maximum at λ = 254 nm. All samples were filled in *High Precision SUPRASIL* quartz glass cells (*Hellma GmbH & Co. KG*, Mühlheim, Germany) with a light path of 10 mm, flushed with Argon and sealed with a stopper. The samples were placed in front of the light source, keeping a 1 cm gap between filter plate and cuvette. Cuvettes were irradiated orthogonal to the usual light path.

Gas chromatography (GC) and sample preparation

The Sample size for the GC experiments was 60 µl. Aqueous Samples were dried *in vacuo* for 5 min. 300 µl of a 5mM solution of *n*-decane as internal standard in ethyl acetate was given to each sample. All samples containing free alcohols, amides or carboxylic acids were silylated by addition of 50 µl *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) and heating to 80 °C for 10 min. All samples were filtered over silica gel and diluted with ethyl acetate to a volume of approximately 2 ml.

Measurements were performed using a *Hewlett Packard 5890 Series II* gas chromatograph (today part of *Agilent Technologies Sales & Services GmbH & Co.KG, Life Sciences & Chemical Analysis*, Waldbronn, Germany) on an *Aglient DB-5* capillary column. 3 µl of sample solution were injected into a manual injector. The column was set to 50 °C for the first minute and then ramped up to 300 °C over a span of 25 min. The temperature was then held for 5 min. Data analysis was done by a *Hewlett Packard 6890 Series Integrator*.

Scanning electron microscopy (SEM)

Hydrogel samples were applied to silicon wafer chips with a diagonal of less than 12 mm. Chip and sample were freeze-dried and fixed to a 12 mm aluminum sample holder using conductive carbon adhesive tape.

A *Zeiss Auriga Cross Beam Workstation* (*Carl Zeiss AG*, Oberkochen, Germany) was used for SEM imaging. The accelerating voltage of the electron beam was 3 keV and the working distance was around 2 mm. Image Processing was done using *ImageJ* v1.50i.

SEM was performed by Lukas Ibing at MEET in Münster.

NMR spectroscopy

NMR-Spectra were measured using a *Bruker AV 300* NMR spectrometer (*Bruker Corporation*, Billerica, USA) for 300 MHz (^{1}H) and 75 MHz (^{13}C) spectra or a *Bruker ARX 400 F* for 400 MHz (^{1}H) and 101 MHz (^{13}C) spectra. Residual solvent protons were used as Reference. *MestReNova* v9.0 was used for data analysis. Chemical shifts δ(ppm) where reported as relative differences to tetramethylsilane (δ = 0.00 ppm) as external standard. Multiplets were labelled with singlet (*s*), doublet (*d*), triplet (*t*), quartet (*q*) and multiplet (*m*) and suitable combinations of those. Coupling constants *J* were calculated in Hz.

Ultrapure water

Ultrapure water with a resistance higher than 18 MΩ was generated with an *ELGA PureLab UHQ* water purification system (*ELGA LabWater*, High Wycombe, United Kingdom).

Freeze-drying

A *Christ Alpha I-6* (*Martin Christ Gefriertrocknungsanlagen GmbH*, Osterode am Harz, Germany), equipped with a *Vacuumbrand RC4* Pump (*Vacuumbrand GmbH & Co. KG*, Wertheim, Germany), was used for freeze-drying.

Mass spectrometry

Mass spectra were recorded either on a *Bruker Daltonics micro TOF* mass spectrometer (*Bruker Daltonik GmbH*, Bremen, Germany) or on *a Thermo Scientific Orbitrap LTQ-XL* mass spectrometer (*Thermo Fisher Scientific GmbH*, Dreieich, Germany) using electrospray ionization (ESI) in Methanol and/or water as solvent.

High performance liquid chromatography (HPLC)

Concentrations of **AA** and **AB** discussed for the gelation experiments were determined using an Agilent *1100 Series* HPLC setup (*Agilent Technologies Sales & Services GmbH & Co.KG, Life Sciences & Chemical Analysis*, Waldbronn, Germany), which consisted of an *Agilent G1379A* degasser, an *Agilent G1311* quaternary pump, an *Agilent G1367A* well-plate sampler, an *Agilent G1316A* thermostatic column compartment, an *Agilent G1311A* diode array detector and an *Agilent G1328B* manual injector. Separation was done on an *Agilent Eclipse XDB-C18* (150 x 2.1 mm, 3.50 µm particle size) analytical HPLC column. Injection volume was 20 µl. *ChemStation for LC systems* Rev.B.01.03[204] was used for data processing.

UV-Absorption at 210 nm, 254 nm, 280 nm, 492 nm and 576 nm was measured. Injection volume was 25 µL. The mobile phase was a mixture of **A** water (0.1 % TFA) and **B** acetonitrile (0.1 % TFA) using to following gradient program:

Time (min)	A (%)	B (%)
0	95	5
3	95	5
20	20	80
22	20	80
25	95	5
30	95	5

All samples were dissolved and diluted 1:10 with acetonitrile and diluted again 1:20 with a mixture of 80 % **A** and 20 % **B** containing benzaldehyde (500 nM) as an internal standard. Samples containing compound **CC** and **DC** 100 mM, 80 mM, 60 mM, 40 mM and 20 mM each were treated accordingly and were used for a five-point calibration.

Optical density measurements

Optical density measurements were carried out on a *JASCO V-650* double beam spectrophotometer (*JASCO Germany GmbH*, Gross-Umstadt, Germany), equipped with a *JASCO PAC-743* automatic 6-position peltier cell changer. Samples were measured using fused silica cuvettes by *Hellma Analytics* (*Hellma GmbH & Co. KG*, Mühlheim, Germany) with a light path of 1 mm and were tempered at 20 °C by a *Julabo F250* thermocirculator. Data was analyzed using *Spectra Manager* v2.07.01. Samples for UV-irradiation experiments were irradiated outside the spectrometer.

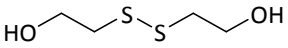
Rheology measurements

Rheology Measurements were performed on an Anton *Paar MCR101* rheometer (*Anton Paar*, *Ostfliedern*, *Germany*) equipped with a *CP25-1* cone plate (diameter d = 25 mm, cone angle = 2°). Temperature was held constant at 20 °C. Frequency tests were done at a deformation amplitude of 5 %.

We are grateful to Melanie Tekaat at the Institute of Physical Chemistry (WWU Münster) for help with the with rheology measurements.

Synthesis

Synthesis of di(2-hydroxyethyl)disulfide (R^1S-SR^1) (1)

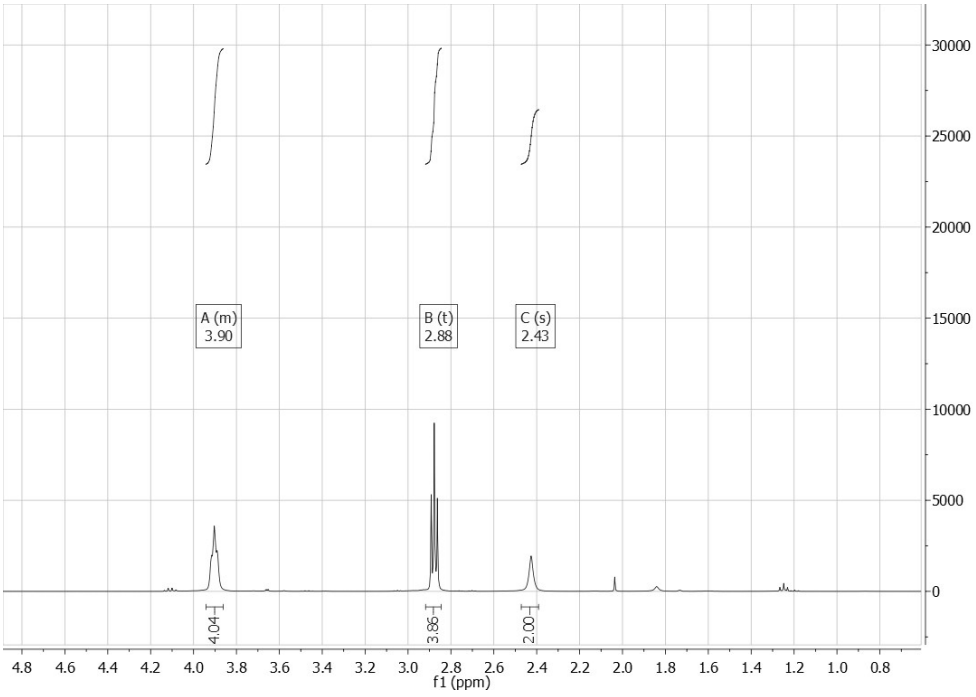


A 30% solution of H_2O_2 (750 µl, 6.6 mmol, 1.1 eq.) was given to a solution of mercaptoethanol (422 µl, 6 mmol, 1 eq.) and NaI (5 mg, 30 µmol, 0.5 %) in EtOAc (5 ml). After 20 min of stirring at r.t. the reaction was quenched by addition of sat. $Na_2S_2O_3$ solution until complete reduction of the excess iodine. The organic phase was washed with sat. Na_2CO_3 solution and brine. After drying over $MgSO_4$ and removal of the solvent *in vacuo*. **1** was obtained as colorless oil.

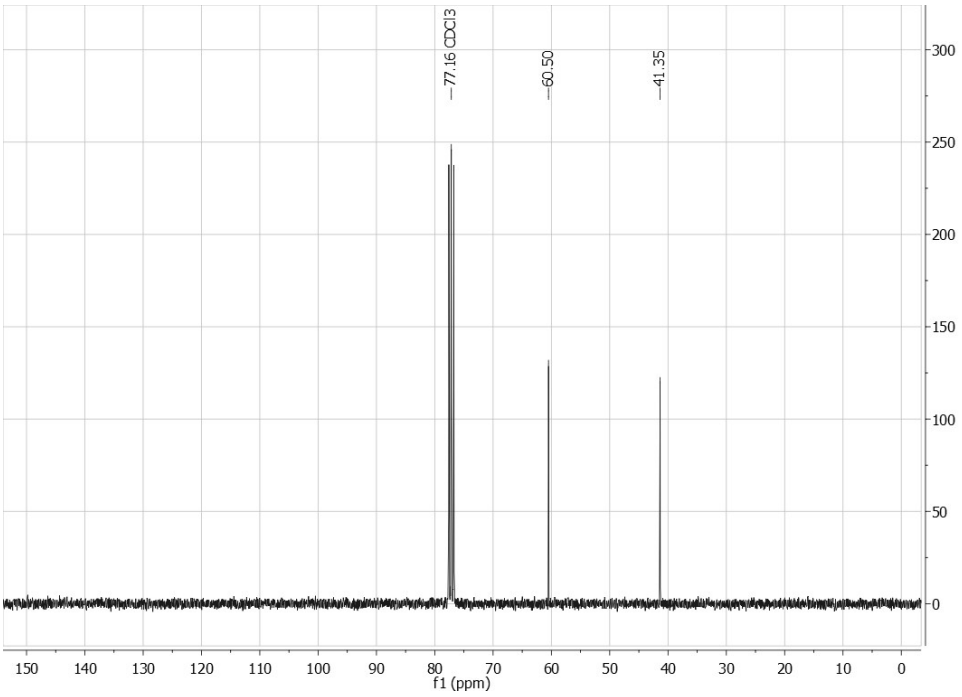
Yield: 414 mg (2.68 mmol, 89 %).

HR-MS: m/z =177.0022 $[M+Na]^+$ (calc. m/z = 177.0014).

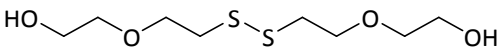
^{1}H-NMR: (400 MHz, $CDCl_3$, 300 K) δ(ppm) = 3.90 (t, J = 5.8 Hz, 4H, 1-CH_2), 2.88 (t, J = 5.8 Hz, 4H, 2-CH_2), 2.43 (s, 2H, OH).



^{13}C NMR: (75 MHz, $CDCl_2$) δ(ppm) = 60.5 (CH_2), 41.4 (CH_2).



Synthesis of di(2-(2-hydroxyethoxy)ethyl)disulfide (R^2S-SR^2) (3)

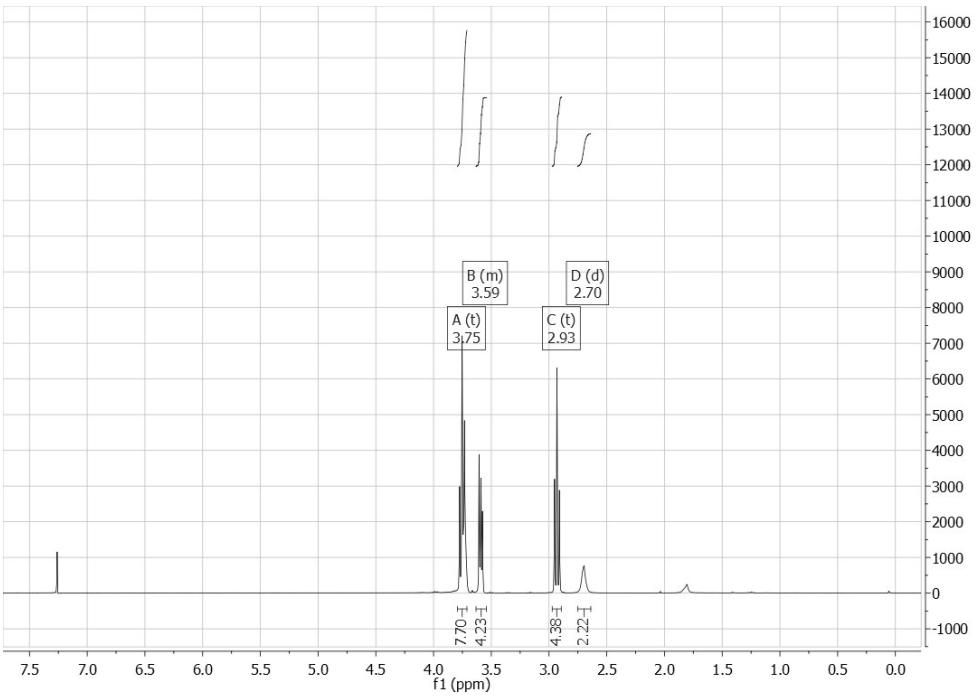


A 30% solution of H_2O_2 (450 µl, 4.4 mmol, 1.1 eq.) was given to a solution of 2-(2-mercaptoethoxy)ethanol (450 µl, 4 mmol, 1 eq.) and NaI (4.5 mg, 30 µmol, 0.5 %) in EtOAc (10 ml). After 20 min of stirring at r.t. the reaction was quenched by addition of sat. $Na_2S_2O_3$ solution until complete reduction of the excess iodine. The organic phase was washed with sat. Na_2CO_3 solution and brine. After drying over $MgSO_4$ and removal of the solvent *in vacuo*. **3** was obtained as colorless oil.

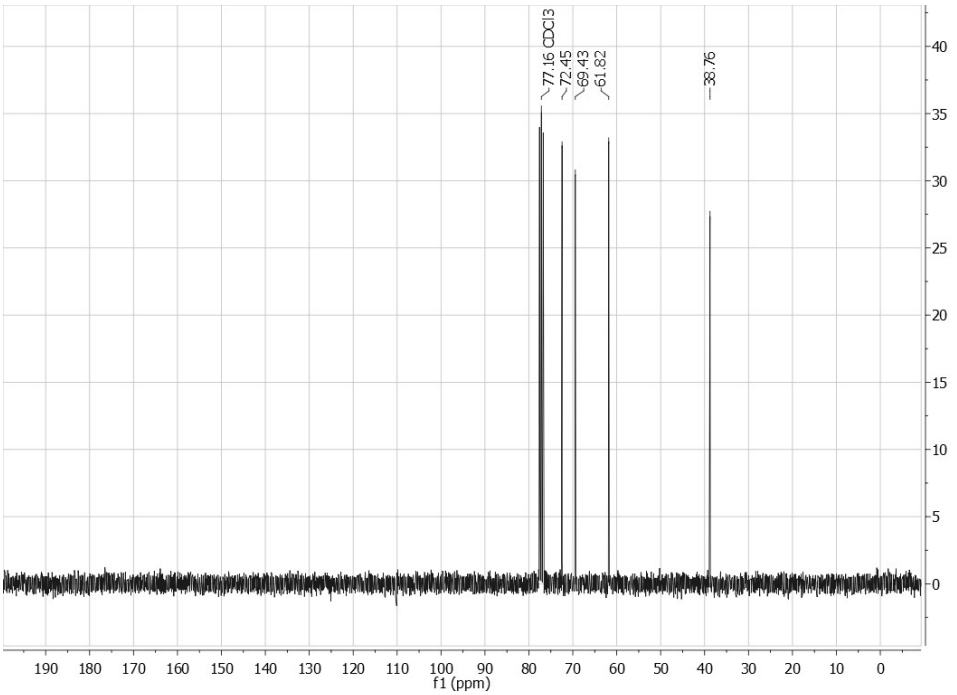
Yield: 418 mg (1.72 mmol, 86 %).

HR-MS: m/z =265.0551 $[M+Na]^+$ (calc. m/z = 265.0539).

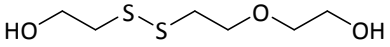
^{1}H-NMR: (300 MHz, $CDCl_3$) δ(ppm) = 3.75 (t, J = 6.3 Hz, 8H, 4 CH_2), 3.62 – 3.55 (m, 4H, 2 CH_2), 2.93 (t, J = 6.3 Hz, 4H, 2 CH_2), 2.70 (s, 2H, OH).



^{13}C-NMR: (75 MHz, $CDCl_3$) δ(ppm) = 72.5 (CH_2), 69.4 (CH_2), 61.8 (CH_2), 38.8 (CH_2).



Synthesis of (2-(2-hydroxyethoxy)ethyl)(2-hydroxyethyl)disulfide (R^1S-SR^2) (2)

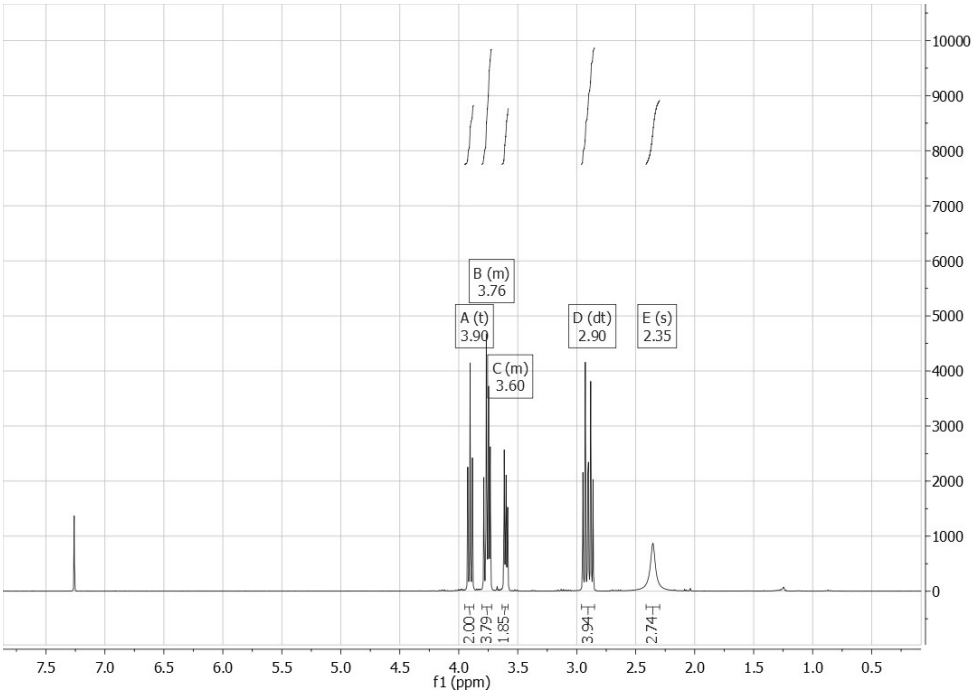


A 30% solution of H_2O_2 (450 µl, 4.4 mmol, 1.1 eq.) was given to a solution of 2-(2-mercaptoethoxy)ethanol (225 µl, 2 mmol, 1 eq.), mercaptoethanol (140.2 µl, 2 mmol, 1 eq.) and NaI (4.5 mg, 30 µmol, 0.5 %) in EtOAc (10 ml). After 20 min of stirring at r.t. the reaction was quenched by addition of sat. $Na_2S_2O_3$ solution until complete reduction of the excess iodine. The organic phase was washed with sat. Na_2CO_3 solution and brine. After drying over $MgSO_4$ and removal of the solvent *in vacuo*, the product **2** was isolated by flash chromatography (EtOAc : cyclohexane, 1 : 4) as a colorless oil.

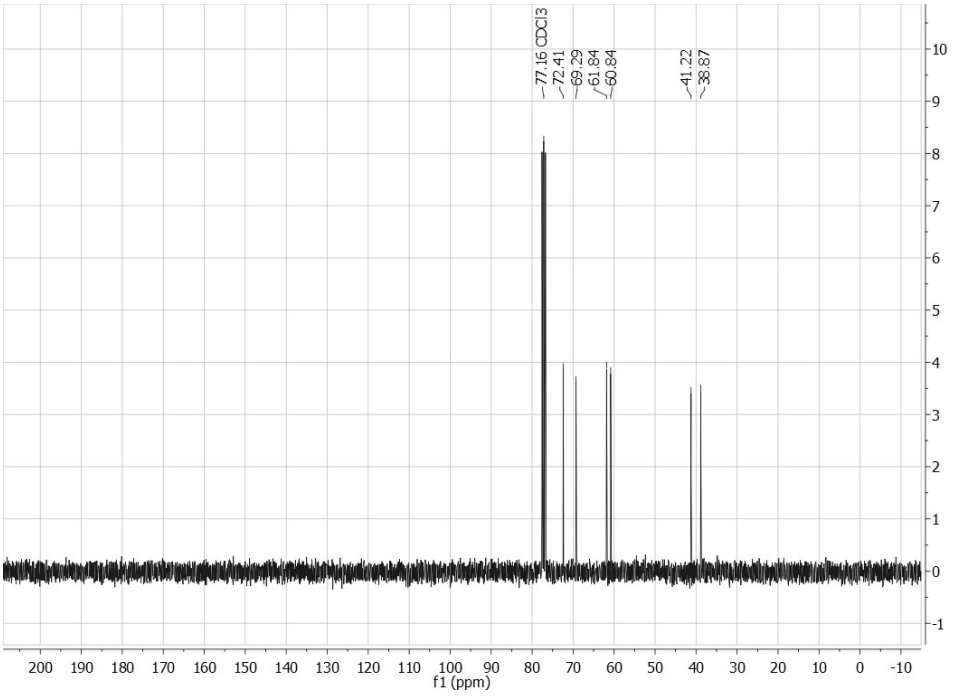
Yield: 95 mg (0.47 mmol, 23 %).

HR-MS: m/z = 221.0287 $[M+Na]^+$ (calc. m/z = 221.0287).

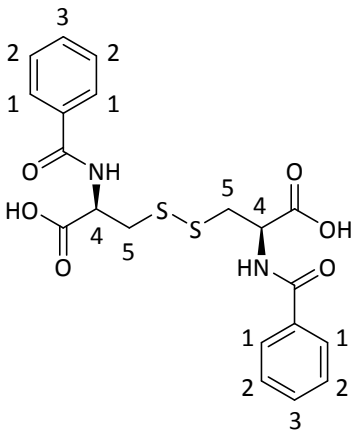
^{1}H-NMR: (300 MHz, $CDCl_3$) δ(ppm) = 3.90 (t, J = 5.8 Hz, 2H, CH_2), 3.80 – 3.72 (m, 4H, CH_2), 3.63 – 3.58 (m, 2H, CH_2), 2.90 (dt, J = 13.5, 6.0 Hz, 4H, CH_2), 2.35 (s, 2H, OH).



^{13}C NMR: (75 MHz, $CDCl_3$) δ(ppm) = 72.3 (CH_2), 69.2 (CH_2), 61.7 (CH_2), 60.7 (CH_2), 41.1 (CH_2), 38.7 (CH_2).



Synthesis of *N,N'*-dibenzoyl-L-cystine (4)



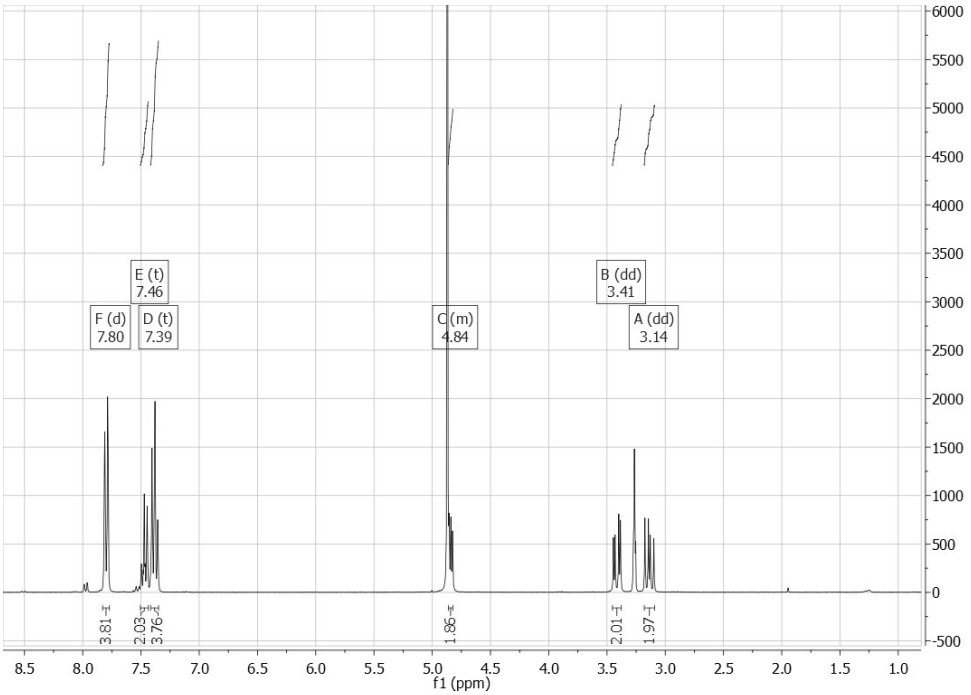
N,N'-Dibenzoyl-L-cystine **1** was synthesized according to literature with slight modifications.[1] A solution of L-cystine (2.40 g, 10 mmol, 1 eq.) in Et_2O (10 ml), EtOH (1ml), 2N NaOH (22 ml) and H_2O (30 ml) was cooled to 0 °C. Benzoylchloride (3.5 ml, 30 mmol, 3 eq.) was added dropwise. The mixture was allowed to heat up to r.t. and was left stirring overnight. The mixture was diluted with water to a volume of approximately 150 ml. 6N HCl was added until the mixture reached a pH of 2. The white precipitate was filtered and washed with hot water. Freeze-drying yielded product **4**, which was used without further purification.

Yield: 3.63 g (81 mmol, 81 %).

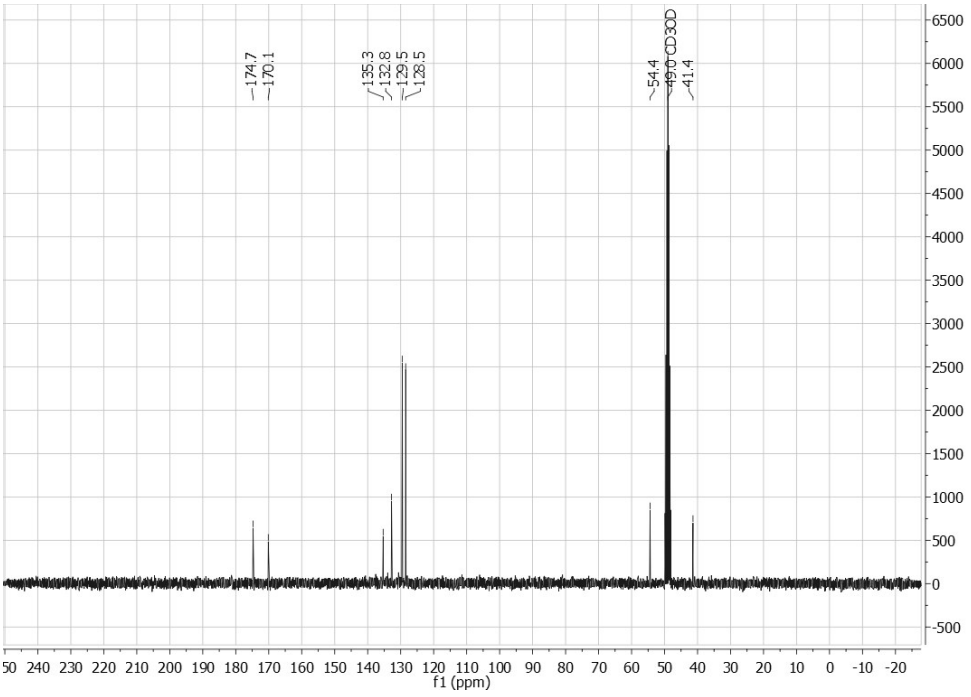
HR-MS: m/z = 471.0654 $[M+Na]^+$ (calc. m/z = 471.0655).
m/z = 447.0695 $[M-H]^-$ (calc. m/z = 447.0690).

[1] T. A. Martin, *J. Med. Chem.*, 1969, **12**, 950–953.

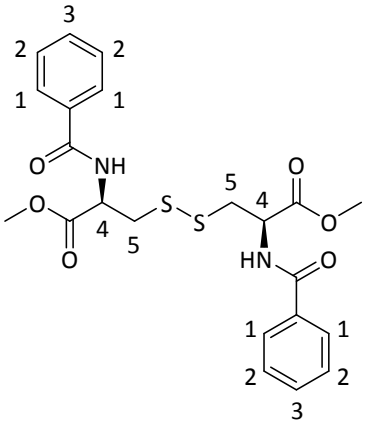
^{1}H-NMR: (300 MHz, MeOD) δ(ppm) = 7.80 (d, J = 7.2 Hz, 4H, 1-CH), 7.48 (t, J = 7.2 Hz, 2H, 3-CH), 7.39 (t, J = 7.3 Hz, 4H, 3-CH), 4.86 – 4.82 (m, 2H, 4-CH), 3.41 (dd, J = 13.8, 4.3 Hz, 2H, 5-CH_2), 3.14 (dd, J = 13.8, 9.1 Hz, 2H, 5'-CH_2).



^{13}C NMR: (75 MHz, MeOD) δ(ppm) = 174.7 (*C*OOH), 170.1 (*C*OAr), 135.3 (C_q(Ar)), 132.8 (*C*H), 129.5 (*C*H), 128.5 (*C*H), 54.4 (CH_2), 41.4 (*C*H).



Synthesis of *N*,*N*′-benzoyl-L-cystine dimethylester (AA)



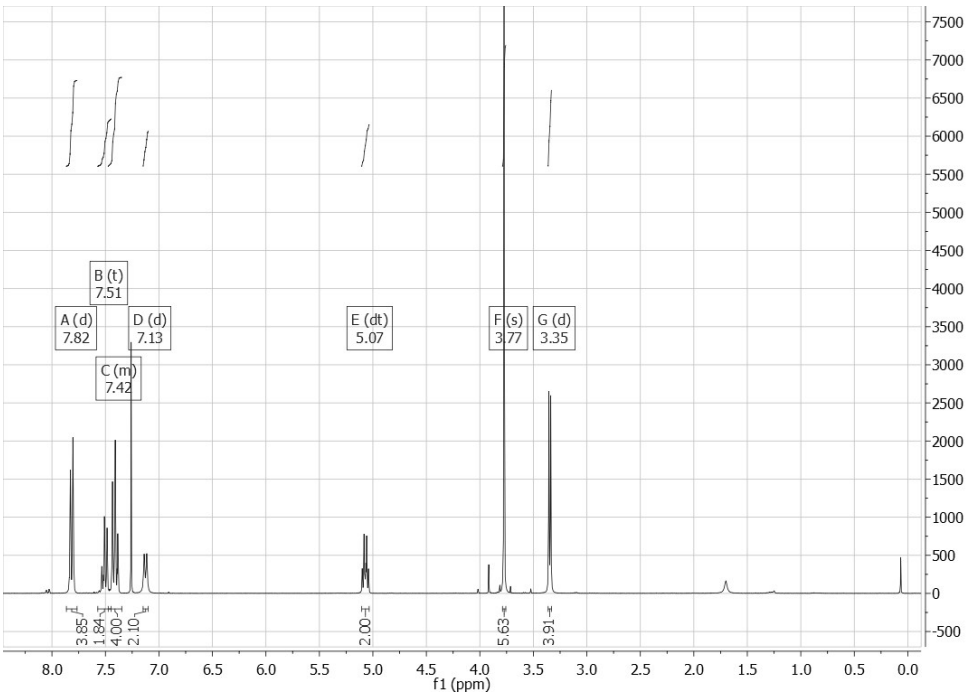
$SOCl_2$ (2.35 ml, 32.4 mmol, 4 eq.) was slowly added dropwise to abs. MeOH (150 ml) at 0 °C. After addition of *N*,*N*′-dibenzoyl-L-cystine **1** (3.66 g, 8.1 mmol, 1 eq.) the mixture was allowed to heat to r.t. and stirred overnight. The solvent was removed under reduced pressure and the raw product was dissolved in chloroform. The solution was washed with sat. Na_2CO_3 and brine. Drying over $MgSO_4$ and removal of the solvent *in vacuo* yielded **AA** as white solid and was used without further purification.

Yield: 3.36 g (7.04 mmol, 86 %).

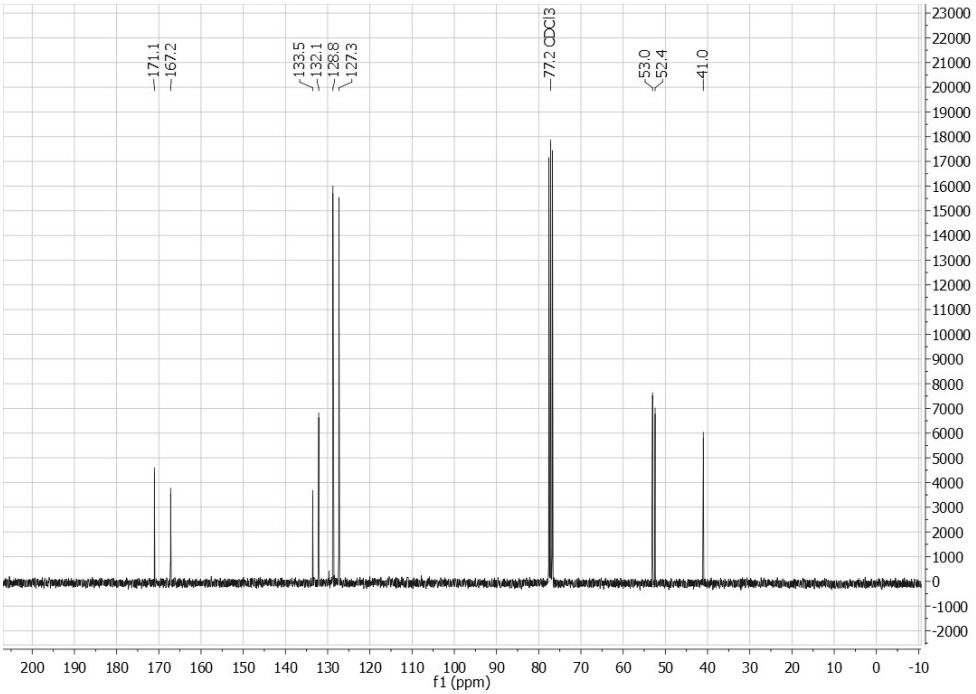
HR-MS: *m*/*z* = 499.0973 $[M+Na]^+$ (calc. *m*/*z* = 499.0968).

m/*z* = 975.1886 $[2M+Na]^+$ (calc. *m*/*z* = 975.2044).

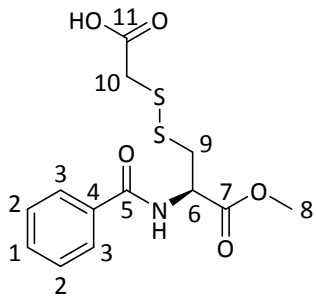
^{1}H-NMR: (300 MHz, $CDCl_3$) δ(ppm) = 7.82 (d, *J* = 7.0 Hz, 4H, 1-CH), 7.51 (t, *J* = 7.4 Hz, 2H, 3-CH), 7.44 – 7.38 (m, 4H, 2-CH), 7.13 (d, *J* = 7.3 Hz, 2H, NH), 5.07 (dt, *J* = 7.3, 5.1 Hz, 2H, 4-CH), 3.77 (s, 6H, OMe), 3.35 (d, *J* = 5.1 Hz, 4H, 5-CH).



^{13}C-NMR: (75 MHz, $CDCl_3$) δ(ppm) = 171.1 (*C*OOMe), 167.2 (*C*OAr), 133.5 (C_q(Ar)), 132.1 (*C*H), 128.8 (*C*H), 127.3 (*C*H), 53.0 (CH_3), 52.4 (CH_2), 41.0 (*C*H).



Synthesis of (*R*)-2-((2-benzamido-3-methoxy-3-oxopropyl)disulfanyl)acetic acid (AB)

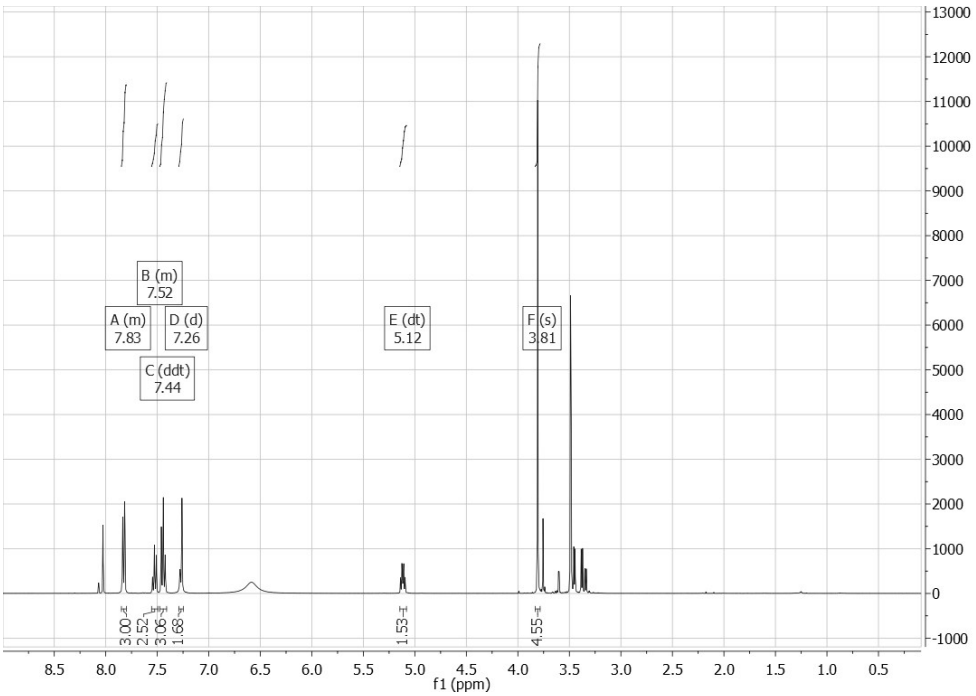


A solution of *N*,*N*'-dibenzoyl-L-cystine dimethylester **AA** (953 mg, 2 mmol, 1 eq), thioglycolic acid (556 µl, 2 mmol, 1 eq.) and Et_3N (1.940 µl, 14 mmol, 7 eq.) in MeOH (60 ml) was stirred in an open flask overnight. The solvent was removed *in vacuo* and the crude product was purified by flash-chromatography (DCM : MeOH : formic acid, 100 : 5 : 0.5). **AB** was obtained as colorless oil.

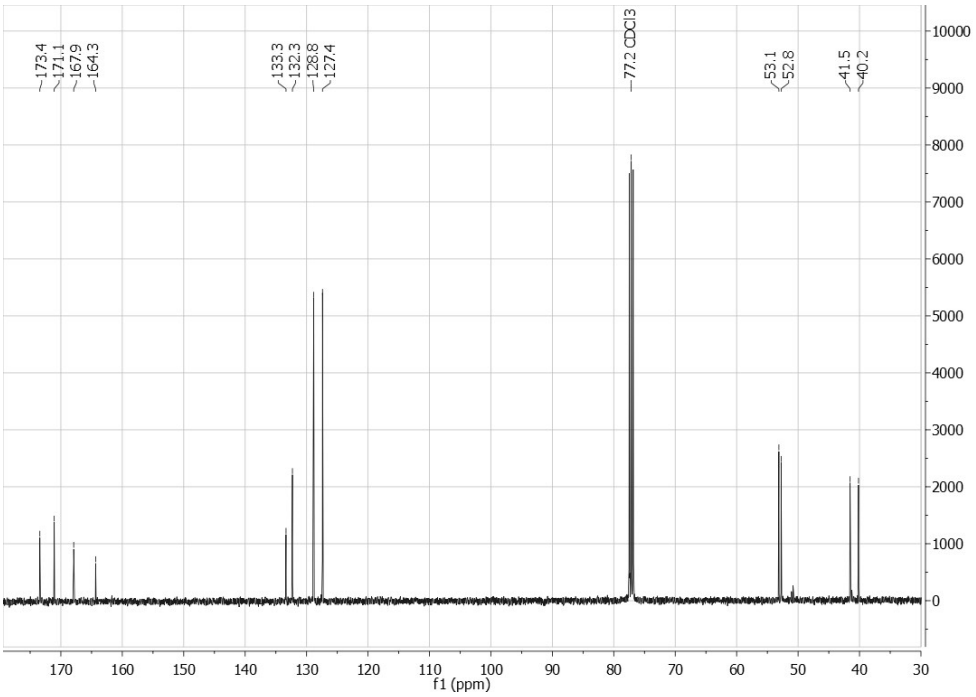
Yield: 555 mg (1.68 mmol, 42 %).

HR-MS: *m*/*z* = 352.0294 $[M+Na]^+$ (calc. *m*/*z* = 329.0392)

^{1}H-NMR: (400 MHz, $CDCl_3$) δ(ppm) = 7.85 – 7.79 (m, 2H, 3-CH), 7.56 – 7.49 (m, 1H, 1-CH), 7.44 (ddt, *J* = 8.2, 6.6, 1.3 Hz, 2H, 2-C), 7.27 (d, *J* = 7.3 Hz, 1H, NH), 5.12 (dt, *J* = 7.3, 5.1 Hz, 1H, 6-CH), 3.81 (s, 3H, 8-CH_3), 3.42 (ddd, *J* = 42.9, 14.3, 5.5 Hz, 2H, 9-CH_2), 3.39 (s, 2H, 10-CH_2).



^{13}C-NMR: (101 MHz, $CDCl_3$) δ(ppm) = 173.4 (C11), 171.1 (C7), 167.9 (C5), 133.4 (C4), 132.3 (C1), 128.8 (C2), 127.4 (C3), 53.1 (C8), 52.8 (C6), 41.53 (C10), 40.2 (C9).



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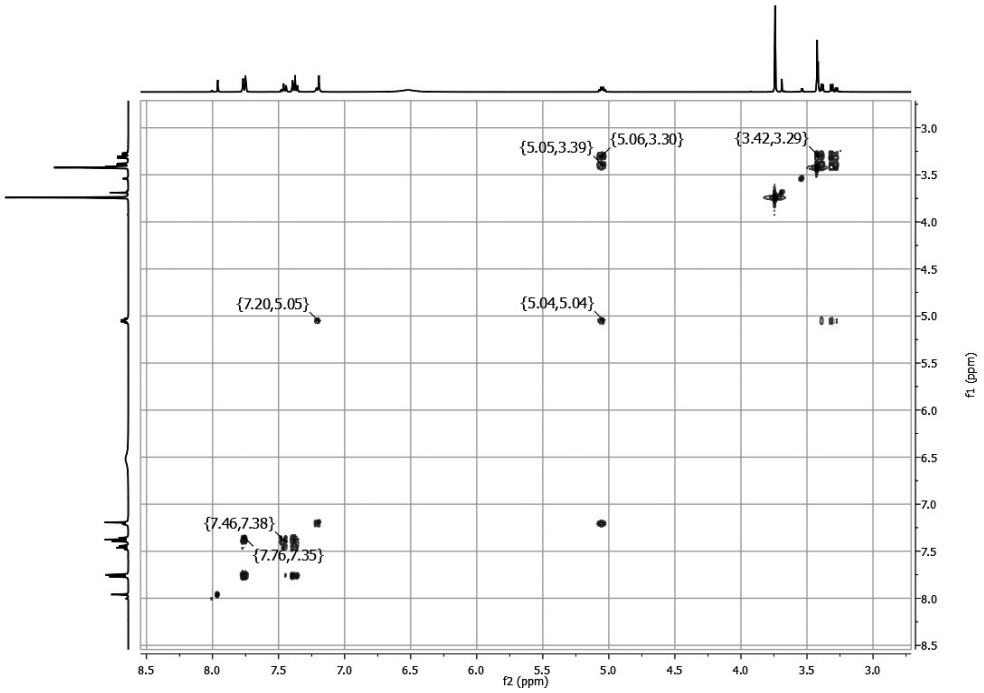
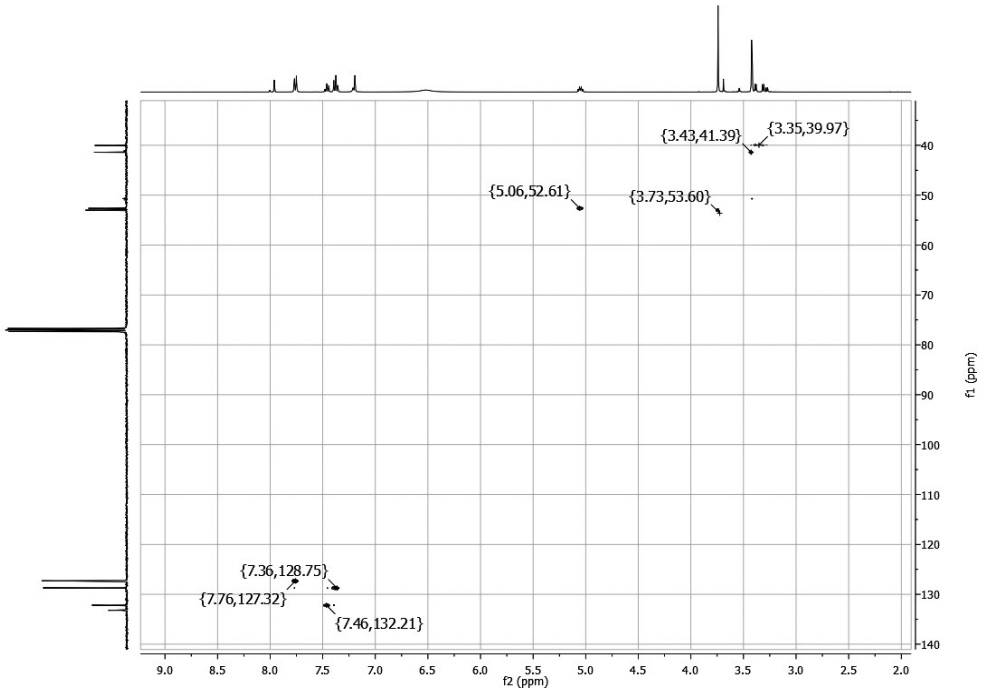
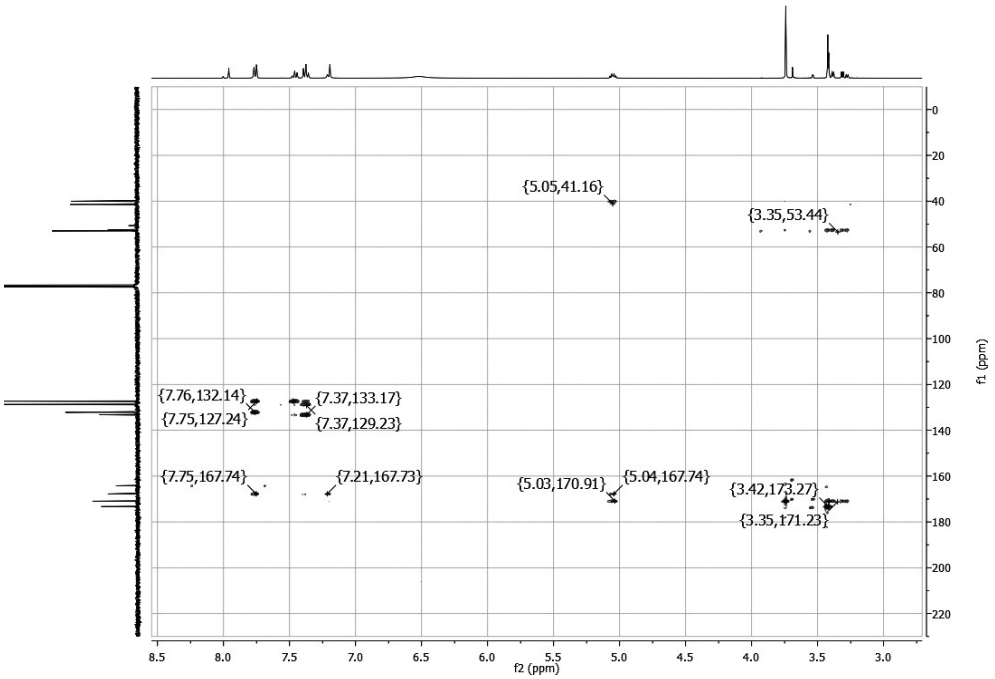

{5.05,3.39}
{5.06,3.30}
{3.42,3.29}
{7.20,5.05}
{5.04,5.04}
{7.46,7.38}
{7.76,7.35}
f2 (ppm)
f1 (ppm)

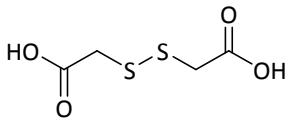
gHSQC:


{3.43,41.39}
{3.35,39.97}
{5.06,52.61}
{3.73,53.60}
{7.36,128.75}
{7.76,127.32}
{7.46,132.21}
f2 (ppm)
f1 (ppm)

gHMBC:



Synthesis of 2,2'-disulfanediyldiacetic acid (BB)

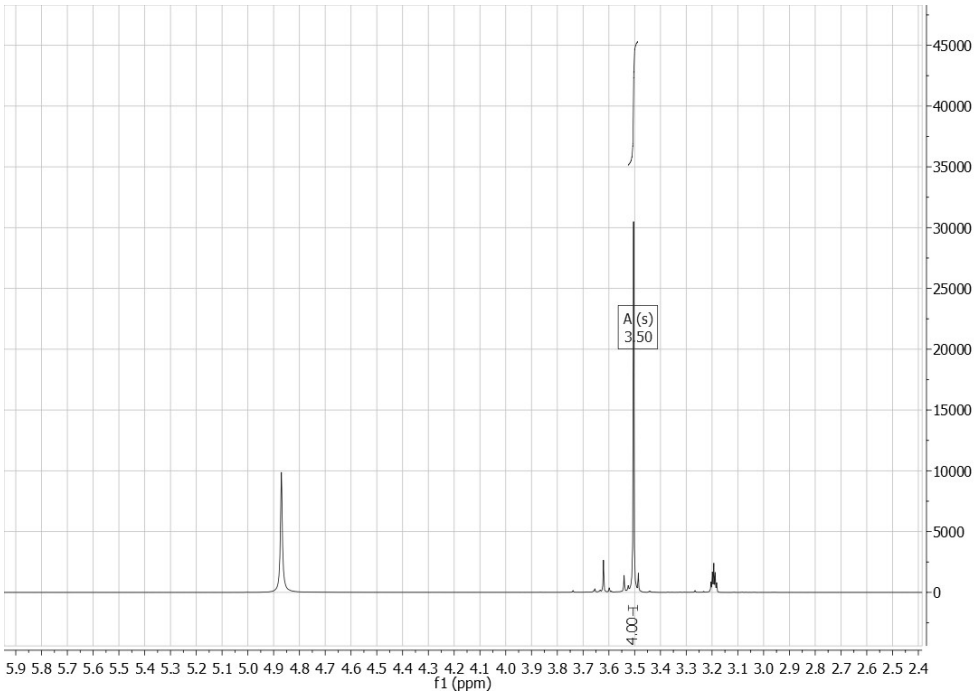


2,2’-Disulfanediyldiacetic acid **BB** was obtained as a byproduct in an additional fraction of the flashchromatography of the synthesis of **AB**. Removal of the solvent *in vacuo* yielded a white solid.

Yield: 176 mg (0.97 mmol, 24 %).

HR-MS: m/z = 180.96340 $[M-H]^-$ (calc. m/z = 180.96347)

^{1}H-NMR: (300 MHz, MeOD) δ(ppm) = 3.50 (s, 4H, CH_2).



^{13}C-NMR: (75 MHz, MeOD) δ(ppm) = 173.0 (C_q), 42.0 (CH_2).