CHEMMEDCHEM
Chemistry & Drug Discovery

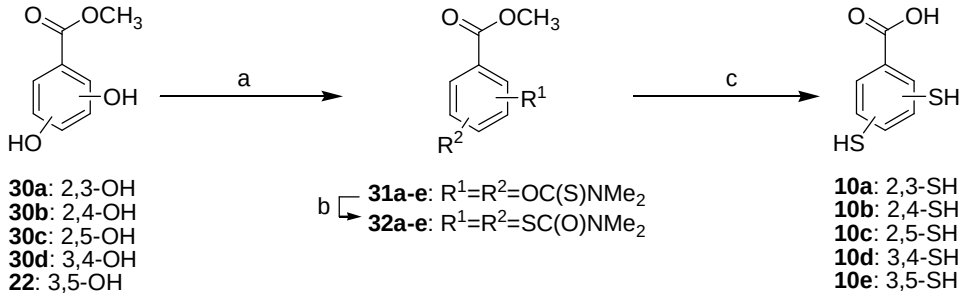
Supporting Information

Combined Mass Spectrometry and Dynamic Chemistry Approach to Identify Metallo-Enzyme Inhibitors

*Benoît M. R. Liénard, Nathalie Selevsek, Neil J. Oldham and Christopher J. Schofield**

1. Synthesis of dithiol support ligands **10a-e**

Dimercaptocarboxylates **10a-e** were synthesized from readily prepared diols **30a-e**, in three steps and in overall yields varying from 23 to 87 % (Scheme S1).



Scheme S1. *Conditions and reagents*: a) DABCO, $(CH_3)_2NC(S)Cl$, DMF, 82-87 %; b) Ph_2O, Δ, 36-99 %; c) NaOH (1N), 70°C, 79-99 %.

Thus, diols **30a-d** and **22** were dialkylated using dimethylthiocarbamoyl chloride to give *O*-aryl thiocarbamates **31a-eB,**[1, 2] then subjected to a Newman-Kwart rearrangement[3] to yield *S*-aryl thiocarbamates **32a-e**. Complete hydrolysis of compounds **32a-e** in aqueous sodium hydroxide afforded support ligand dithiols **10a-e** in high yields.

2. ESI-MS analyses of BcII(Zn^{II})$_2$ incubated with **10a-e**

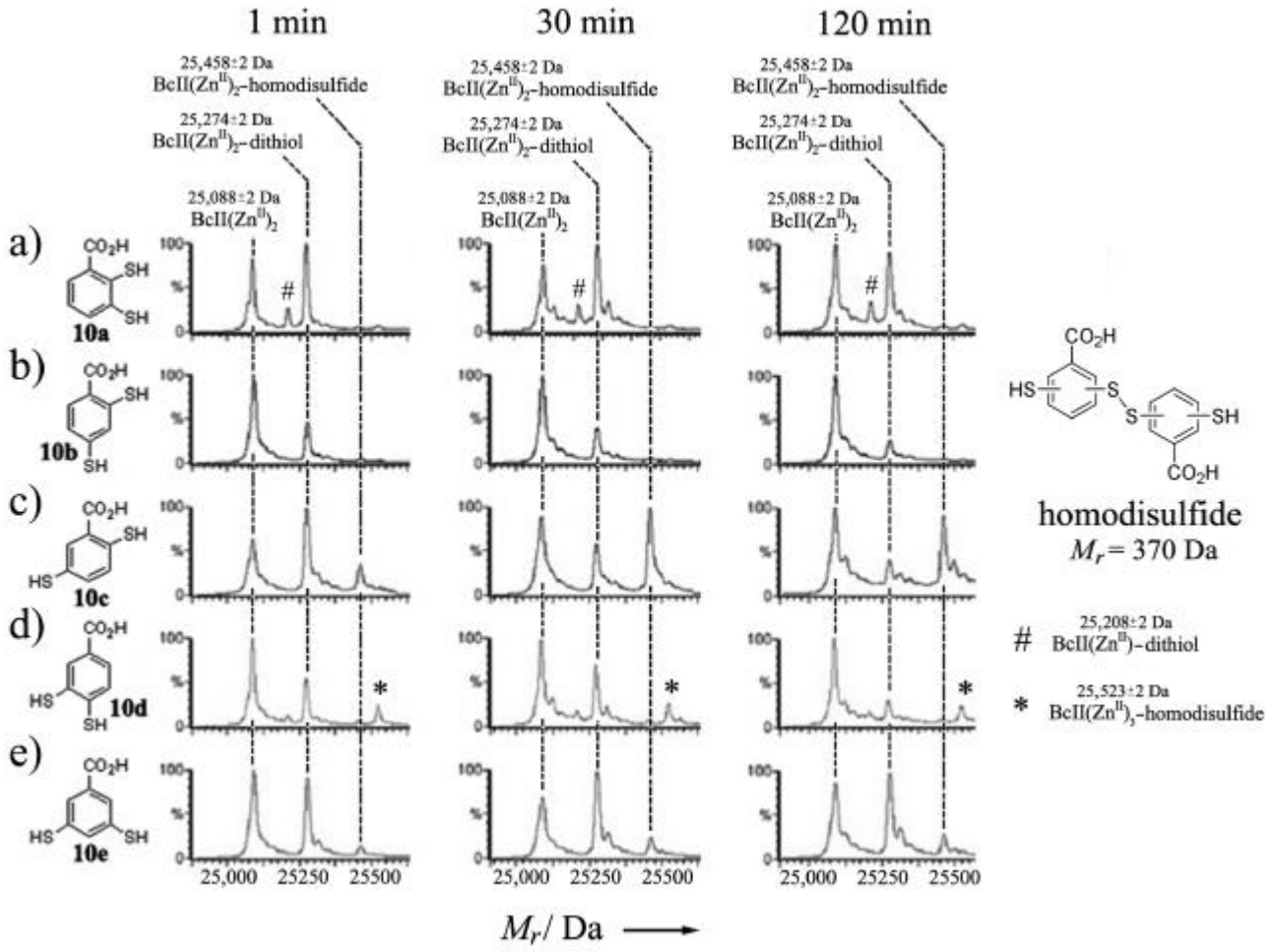


Figure S1. Deconvoluted ESI-MS spectra for BcII(Zn^{II})$_2$ in the presence of **10a-e** (a-e). An equimolar amount of the BcII(Zn^{II})$_2$ was used and reactions monitored over 8 h at 4°C and sample cone voltage 50 V. Selected time points t = 1 min, 30 min and 120 min after aerial exposure are shown.

3. Combined MS-DCL approach using the set of thiols **1**-**20** in the presence support ligands **10a** and **10d**

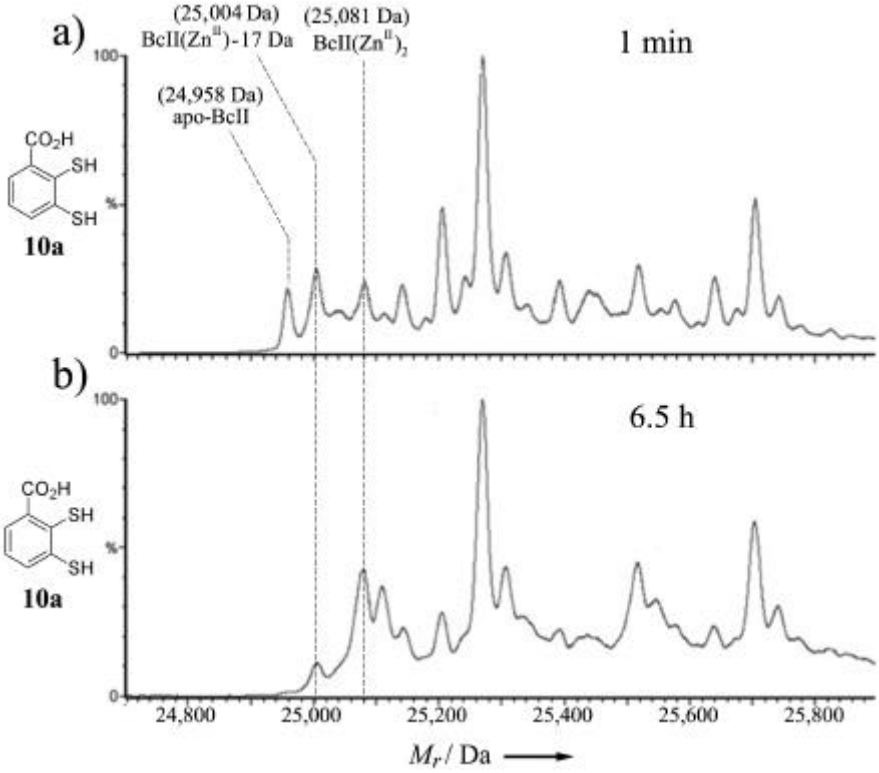


Figure S2. Deconvoluted ESI-MS spectra resulting from an equimolar mixture of 19 thiols (19 × 10 μM) and (a) BcII(Zn^{II})$_2$ (15 μM) + **10a** (30 μM) after 1 min and (b) 6.5 h of aerial exposure and sample cone voltage 50 V.

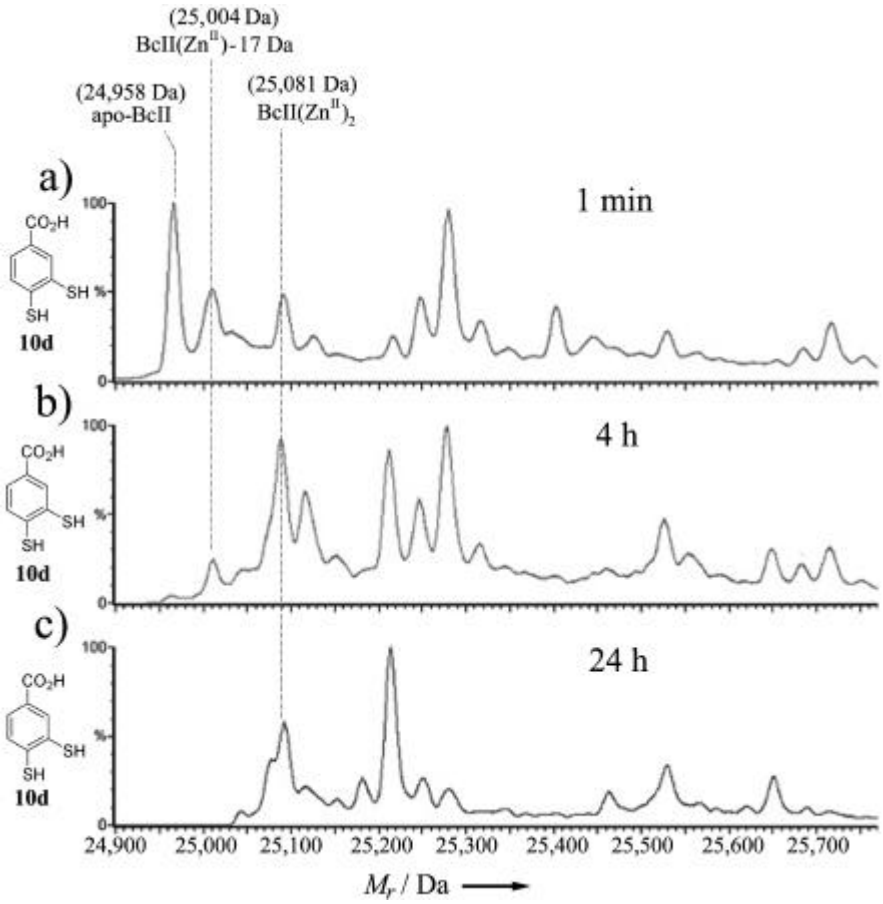


Figure S3. Deconvoluted ESI-MS spectra resulting from an equimolar mixture of 19 thiols (19 × 10 μM) and (a) BcII(Zn^{II})$_2$ (15 μM) + **10d** (30 μM) after 1 min, (b) 4 h and (c) 24 h of aerial exposure and sample cone voltage 50 V.

4. Knock-out experiments to identify preferentially binding disulfides

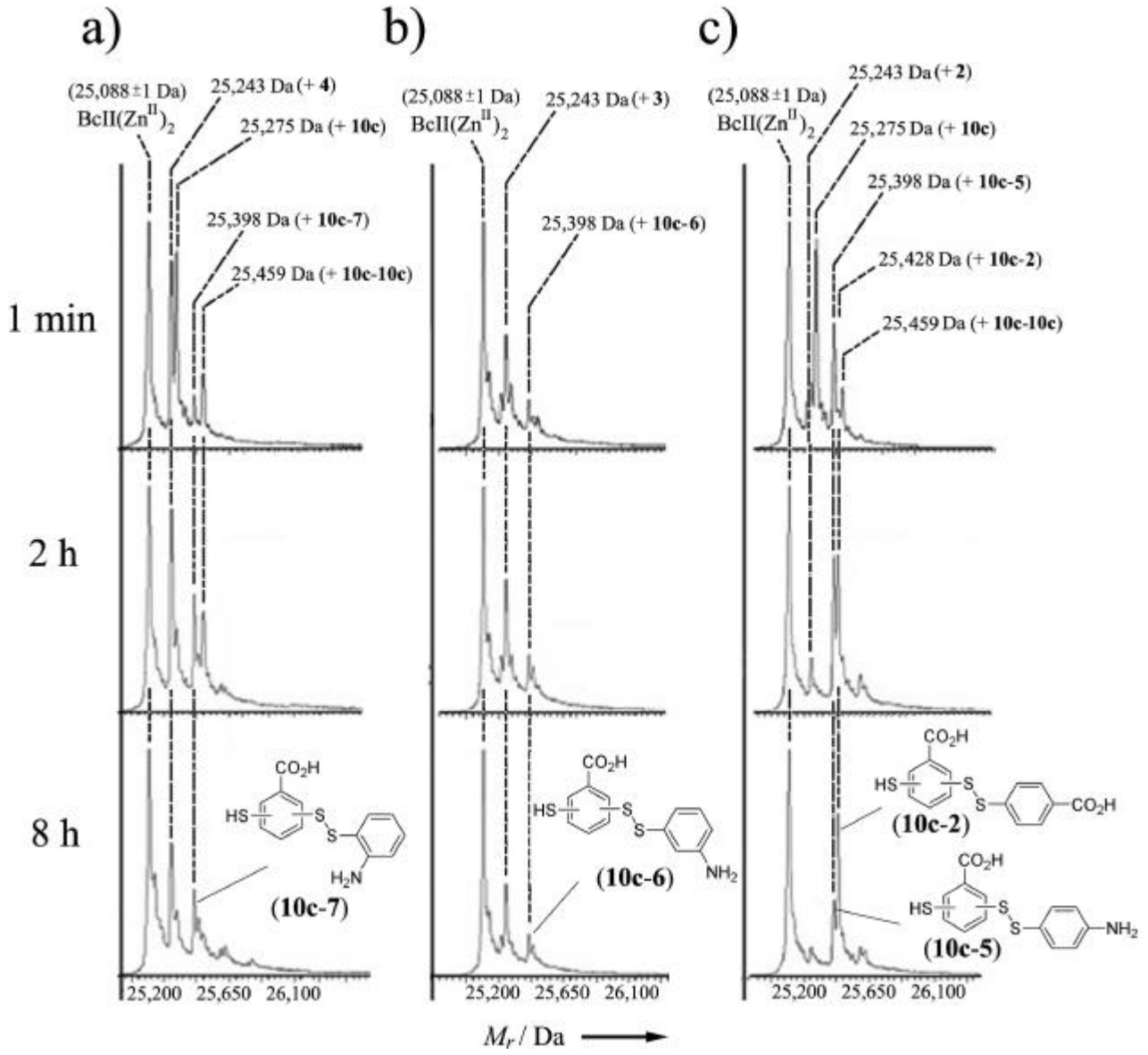
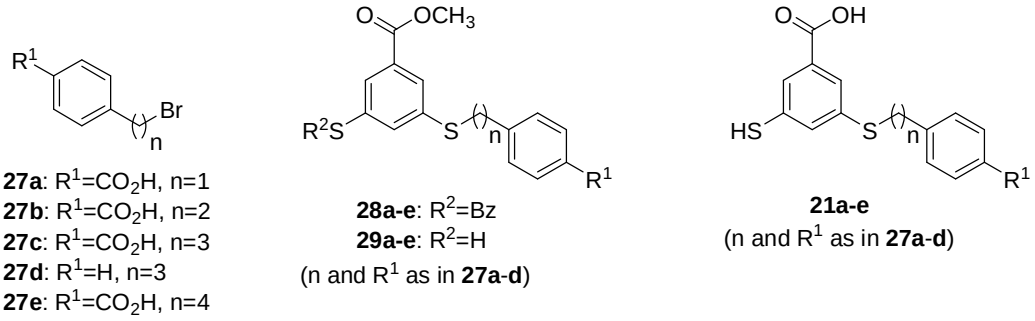


Figure S4. Deconvoluted ESI-MS spectra resulting from an equimolar mixture of a) compounds **10c**, **4** and **7** (15 μM each), b) compounds **10c**, **3** and **6** (15 μM each) and c) compounds **10c**, **2** and **5** (15 μM each) incubated with BcII(Zn^{II})$_2$ (15 μM) after 1min, 2 h and 8 h of aerial exposure under the anaerobic conditions and sample cone voltage of 50 V. The evolution of the system was monitored every minute and only selected time points are given.

5. Synthesis of carba-analogues **21a-e** – further details for Scheme 1

Thiols **21a**-**e** were synthesized from dithiol **10e**, in five to eight synthetic steps and in overall yields of 7 to 45 % (Figure S5).



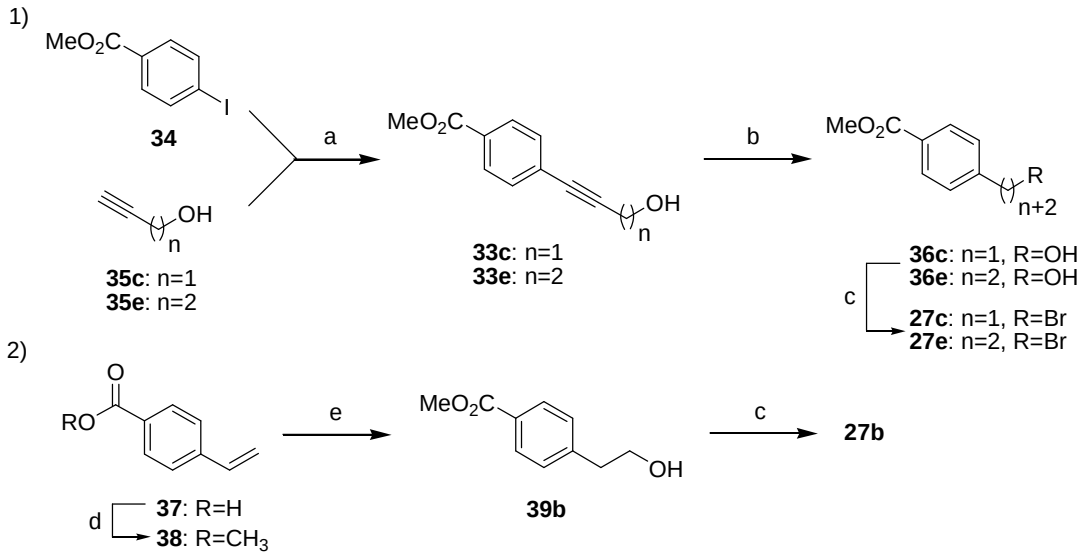
27a: R^1=CO_2H, n=1
27b: R^1=CO_2H, n=2
27c: R^1=CO_2H, n=3
27d: R^1=H, n=3
27e: R^1=CO_2H, n=4

28a-e: R^2=Bz
29a-e: R^2=H
(n and R^1 as in **27a**-**d**)

21a-e
(n and R^1 as in **27a**-**d**)

Figure S5. Carba-analogues and their synthetic intermediates.

Benzoic acid **10e** was first esterified using an acidic methanolic solution to give ester **25** in near quantitative yield. **25** was then dibenzoylated using benzoyl chloride and triethylamine in THF to afford **26** in excellent yield. The crude material of the reaction between compound **26** and sodium methoxide was used directly into the next step. The crude material was obtained by quenching the ice-cold reaction mixture with a 1 M solution of hydrochloric acid under a nitrogen atmosphere to pH 1. The addition of bromides **27a-e** onto a freshly prepared neat sample of crude **26**, followed by potassium carbonate under inert atmosphere afforded compounds **28a-e** in reasonable to good yields. Bromides **27a** and **27d** were commercially available whereas **27b**, **27c** and **27e** were generated from commercially available starting materials in three steps and in good overall yields (Scheme S2).



Scheme S2. *Reagents and conditions*: a) CuI, $Pd[P(Ph_3)]_4$, Et_3N, THF, 50°C, 72 % and 86 %; b) Pd-C, H_2, MeOH, 82 % and 84 %; c) NBS, PPh_3, DMF, 50°C, 89-95 %; d) Me_3OBF_4, Et_3N, CH_2Cl_2, 70 %; e) BH_3/THF then NaOH, H_2O_2, 58 %.

Alcohols **33c** and **33e** were obtained by Sonogashira coupling using aryl iodide **34** and alkynes **35c** and **35e**, respectively.[4] Subsequent palladium hydrogenation in methanol yielded alcohols **36c** and **36e**, followed by bromination using triphenylphosphine and *N*-bromosuccinimide afforded bromides **27c** and **27e** respectively.[5] Carboxylic acid **37** was converted into methyl ester **38** using trimethoxytetrafluoroborate then subjected to a hydroboration-oxidation sequence affording alcohol **39b** in good overall yield.[6] Final bromination using a reported method yielded bromide **27b**.[5] Full deprotection of the triesters **28a-e** using a 1 N solution of sodium hydroxide did not give satisfying results as difficulties during the purification step were encountered. Instead, compounds **28a-e** were first debenzoylated using a solution of sodium methoxide to yield intermediate thiols **29a-e** then subjected to saponification to afford the desired mercaptocarboxylates **21a-e**.

6. Affinity comparisons of compounds **3** and **21a-e** for BcII(Zn^{II})$_2$ using ESI-MS

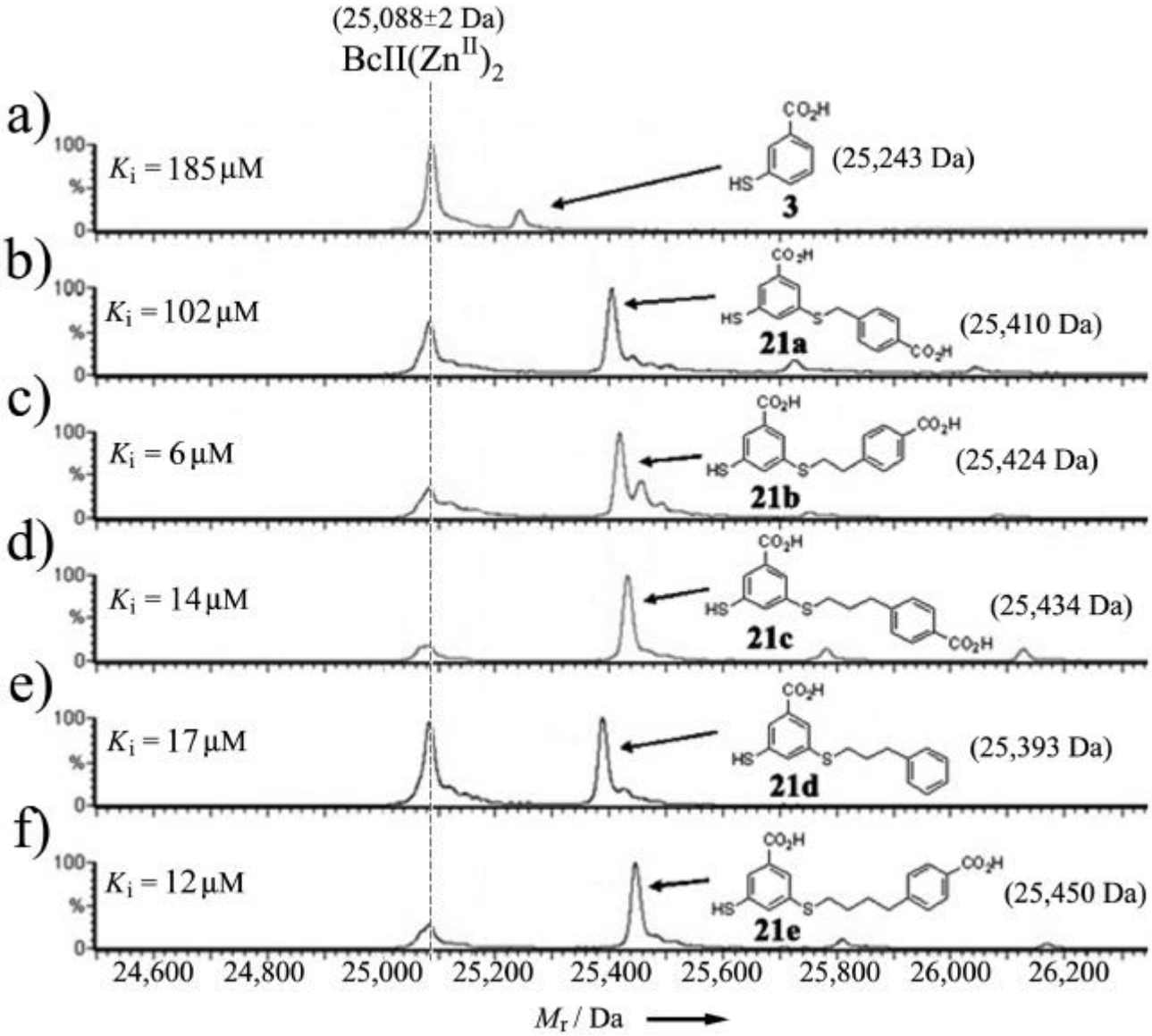


Figure S6. Deconvoluted ESI-MS spectra showing the equimolar mixture (15 μM) of a) **3**, b) **21a**, c) **21b**, d) **21c**, e) **21d** and f) **21e** with BcII(Zn^{II})$_2$ at 23°C and sample cone voltage 50 V. The incubation time of the derivatives with the enzyme prior to analysis was about 2 min.

7. View from the active site of BcII(Zn^{II})$_2$ from *Bacillus Cereus*

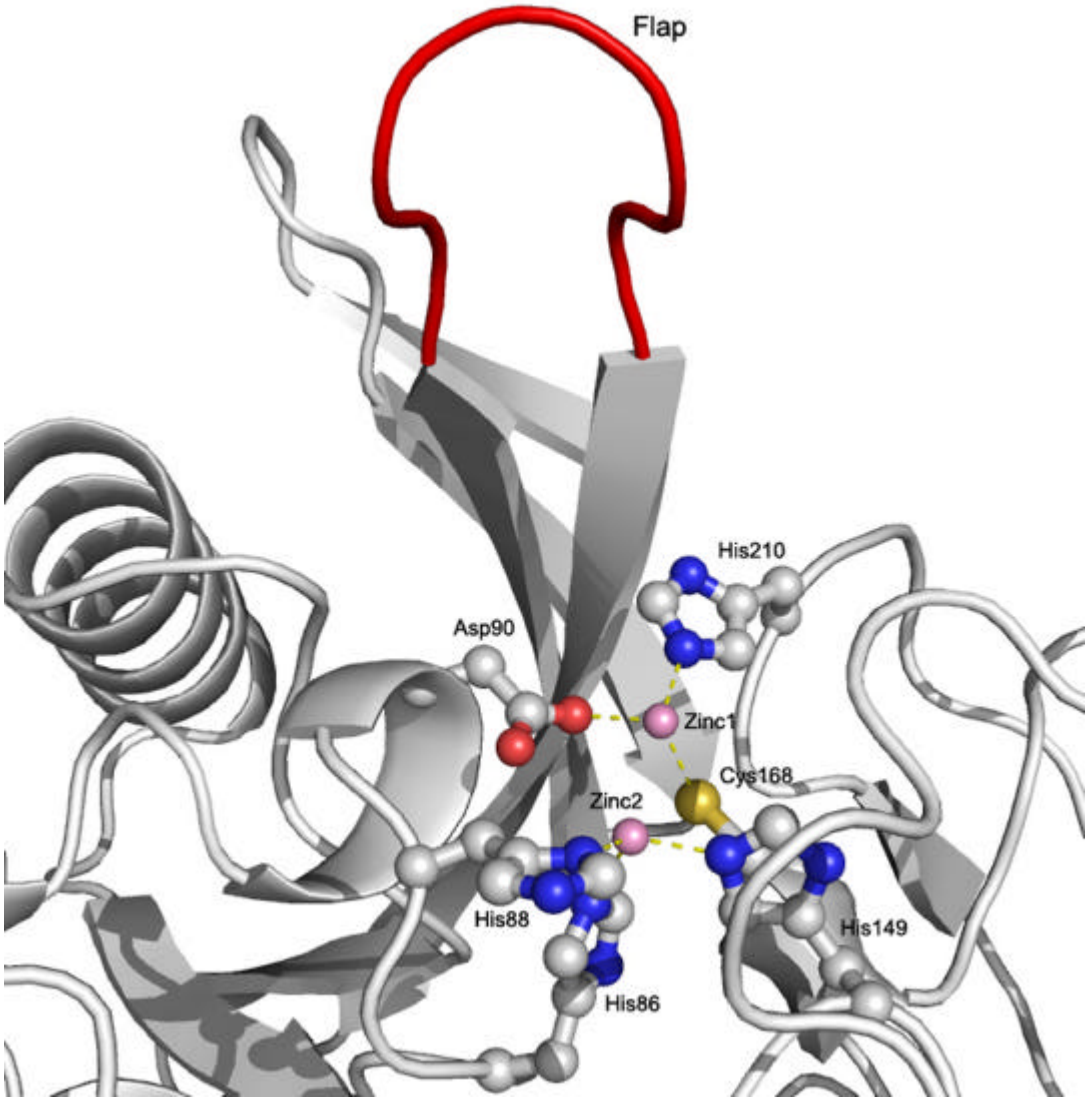
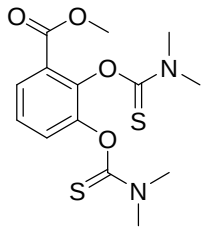


Figure S7. View from the active site of the dizinc BcII MBL. The flexible flap is shown in red. Figure generated from the coordinates from BcII-wild type crystal structure (PDB code 1BVT).[7] The derivative **21a** may be too short to allow the optimal loop closure, whereas longer linkers (*cf.* 2-4 methylenes) may enable productive interactions; **21b**, with 2 methylene groups, appears to be preferred from the tested compounds.

8. Chemical characterization

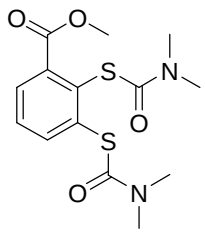
All solvents used were either anhydrous solvents purchased from Aldrich (Sigma-Aldrich Chemical Co., Dorset, UK) or dried by passing over an aluminate column under nitrogen pressure. Reagents were used as obtained from commercial sources unless otherwise stated. Measurement of pH was carried out using Prolabo Rota^{TM} pH 1-10 paper. Flash chromatography was performed using silica gel (0.125-0.25 mm, 60-120 mesh) as the stationery phase. Thin layer chromatography (TLC) was performed on aluminium plates pre-coated with silica gel (Merck silica gel 60 F_{254}), which were visualised by the quenching of UV fluorescence (using an irradiation wavelength ? = 254 nm), and/or by staining with iodine or 10% ammonium molybdate in 2 M sulphuric acid, followed by heating. Retention factors (R_f) are given to the nearest 0.05 decimal. Melting points (m.p.) were obtained using a Büchi 510 Cambridge Instruments Gallen III hot stage melting point apparatus. Infrared (IR) spectra were recorded as thin films between NaCl plates or as KBr discs on a Tensor 27 FT-IR Brüker spectrometer. Only selected absorbances are reported. Proton magnetic resonance spectra (^{1}H NMR) were recorded on a Brüker DPX 250 (250 MHz), Brüker DQX 400 (400 MHz), or Brüker AMX500 (500 MHz) spectrometers at ambient and variable temperature. ^{1}H NMR spectral assignments are supported by ^{1}H-^{1}H COSY experiments where necessary. Coupling constant values (J) are reported to the nearest 0.5 Hz. Carbon magnetic resonance spectra (^{13}C NMR) were recorded on a Brüker DPX 250 (62.9 MHz), Brüker DQX 400 (100.6 MHz) or Brüker AMX500 (125.8 MHz) spectrometers at ambient temperature. ^{13}C NMR assignments were made using DEPT-135 along with HMQC, and HMBC correlation experiments. High-resolution mass spectra were recorded on a VG Autospec spectrometer by chemical ionisation or on a Micromass LCT electrospray ionisation mass spectrometer operating at a resolution of 5000 full width half height. High performance liquid chromatography (HPLC) was operating on a Waters 996 photodiode array detector, a Waters 600E system controller and a Waters 717 plus autosampler, using a Phenomenex Synergy 4μ MAX RP80A (250 × 4.60 mm) as a column for analytical HPLC and a Phenomenex Luna 5μ C_{18} (250 × 4.60 mm) as a column for preparative HPLC. Analytical data is given for published compounds where comprehensive analytical data has not been previously reported.

Methyl 2,3-bis{[(dimethylamino)carbonothioyl]oxy}benzoate (31a)



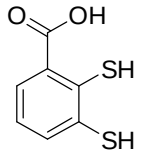
31a was prepared following a previously described method,[1] using **30a** (969 mg, 5.76 mmol) in DMF (10 mL), 1,4-diazabicyclo[2.2.2]octane (DABCO) (2.59 g, 23.1 mmol) and *N*,*N*-dimethylthiocarbamoyl chloride (2.85 g, 23.1 mmol). Purification by flash chromatography using a gradient of CH_2Cl_2/EtOAc [100:0] to [99:1] as eluent afforded 1.61 g (82 %) of the desired compound as a light orange sticky oil. R_f = 0.35 (silica gel, CH_2Cl_2/EtOAc 98:2); IR ν_{max} (film): 3020, 1725 (C=O), 1541 (C=S), 1216, 758 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.94 (dd, 1H, J = 7.5 Hz, J = 2 Hz, ArCH), 7.39 (dd, 1H, J = 8 Hz, J = 2 Hz, ArCH), 7.36-7.34 (m, 1H, ArCH), 3.85 (s, 3H, OCH_3), 3.45, 3.44, 3.33, 3.31 ppm (4 × br s, 4 × 3H, 4 × NCH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 186.7 (C=S), 186.3 (C=S), 164.1 (C=O), 146.9 (ArC), 145.9 (ArC), 128.9 (ArCH), 128.7 (ArCH), 125.9 (ArCH), 125.3 (ArC), 52.2 (OCH_3), 43.4 (2 × NCH_3), 39.0 (NCH_3), 38.9 ppm (NCH_3); HRMS (ES$^+$): $[M+H]^+$ calcd. for $C_{14}H_{19}N_2O_4S_2$, 343.0786; found, 343.0788.

Methyl 2,3-bis{[(dimethylamino)carbonyl]sulfanyl}benzoate (32a)



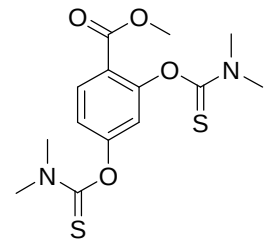
32a was prepared following a previously described method,[1] using **31a** (1.60 g, 4.68 mmol) in diphenyl ether (20 mL). Purification by flash chromatography using petroleum ether 40-60/CH_2Cl_2 [50:50] followed by CH_2Cl_2/EtOAc [95:5] to [70:30] afforded 572 mg (36 %) of the desired compound as a viscous orange oil. $R_f = 0.45$ (silica gel, CH_2Cl_2/EtOAc 1:1); IR ν_{max} (film): 3055, 2987, 1730 (C=O), 1667 (C=O), 1266, 739 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.83$ (dd, 1H, $J = 8$ Hz, $J = 1.5$ Hz, ArCH), 7.81 (dd, 1H, $J = 8$ Hz, $J = 1.5$ Hz, ArCH), 7.45 (t, 1H, $J = 8$ Hz, ArCH), 3.86 (s, 3H, OCH_3), 3.15-3.02 ppm (m, 12H, $4 \times NCH_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 167.3$ (C=O), 166.0 (C=O), 165.9 (C=O), 139.9 (ArCH), 137.9 (ArC), 137.4 (ArC), 134.4 (ArC), 131.0 (ArCH), 129.1 (ArCH), 52.4 (OCH_3), 37.1 ($4 \times NCH_3$) ppm; HRMS (ES^+): $[M+H]^+$ calcd. for $C_{14}H_{19}N_2O_4S_2$, 343.0786; found, 343.0784.

2,3-Disulfanylbenzoic acid (10a)



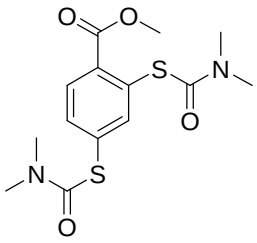
An aqueous solution of NaOH (1 N) (2.63 mL, 2.63 mmol, 9 eq) was added to **10a** (100 mg, 0.29 mmol). The resulting mixture was then heated at 70°C under a nitrogen atmosphere for 3.5 h. The reaction mixture was acidified with HCl (10 N). The white precipitate was collected by filtration, washed several times with H_2O and dried under high vaccum overnight to afford 43 mg (79 %) of the desired compound as a white solid. m.p. 177-178°C; IR ν_{max} (film): 1676 (C=O), 1419, 1308, 1259, 749 cm^{-1}; ^1H NMR (250 MHz, CD_3OD): $\delta = 7.88$ (dd, 1H, $J = 8$ Hz, $J = 1.5$ Hz, ArCH), 7.56 (dd, 1H, $J = 8$ Hz, $J = 1.5$ Hz, ArCH), 7.04 (t, 1H, $J = 8$ Hz, ArCH); ^{13}C NMR (62.9 MHz, CD_3OD): $\delta = 169.6$ (C=O), 137.3 (ArC), 134.5 (ArCH), 132.5 (ArC), 129.7 (ArCH), 127.7 (ArC), 124.1 ppm (ArCH); HRMS (ES^-): $[M-H]^-$ calcd. for $C_7H_5O_2S_2$, 184.9731; found, 184.9734.

Methyl 2,4-bis{[(dimethylamino)carbonothioyl]oxy}benzoate (31b)



31b was prepared following a previously described method,[1] using **30b** (738 mg, 4.39 mmol) in DMF (10 mL), DABCO (1.97 g, 17.6 mmol) and *N*,*N*-dimethylthiocarbamoyl chloride (2.17 g, 17.6 mmol). Purification by flash chromatography using petroleum ether 40-60/CH_2Cl_2 [20:80] then CH_2Cl_2/EtOAc [95:5] as eluent afforded 1.28 g (85 %) of the desired compound as a white sticky foam. $R_f = 0.3$ (silica gel, CH_2Cl_2/EtOAc 98:2); IR ν_{max} (film): 3020, 1723 (C=O), 1541 (C=S), 1216, 758 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.05$ (d, 1H, $J = 8.5$ Hz, ArCH), 7.05 (dd, 1H, $J = 8.5$ Hz, $J = 2.5$ Hz, ArCH), 6.91 (d, 1H, $J = 2.5$ Hz, ArCH), 3.83 (s, 3H, OCH_3), 3.46, 3.45, 3.39, 3.34 ppm ($4 \times$ br s, 4×3H, $4 \times NCH_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 187.0$ (C=S), 186.5 (C=S), 164.1 (C=O), 157.2 (ArC), 154.3 (ArC), 132.1 (ArCH), 121.5 (ArC), 120.6 (ArCH), 120.0 (ArCH), 52.1 (OCH_3), 43.3 ($2 \times NCH_3$), 39.0 ppm ($2 \times NCH_3$); HRMS (ES^+): $[M+H]^+$ calcd. for $C_{14}H_{19}N_2O_4S_2$, 343.0786; found, 343.0789.

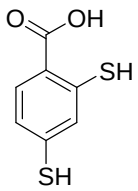
Methyl 2,4-bis{[(dimethylamino)carbonyl]sulfanyl}benzoate (32b)



32b was prepared following a previously described method,[1] using **31b** (700 mg, 2.05 mmol) in diphenyl ether (15 mL). Purification by flash chromatography using petroleum ether 40-60/CH_2Cl_2 [50:50] followed by CH_2Cl_2/EtOAc [80:20] to [50:50] afforded the compound as an oil. Co-evaporation using EtOH led to a solid which, recrystallised from EtOH, afforded 695 mg (99 %) of the desired compound as a white solid. $R_f = 0.30$ (silica gel, CH_2Cl_2/EtOAc 80:20); m.p. 84-85°C; IR ν_{max} (film): 3020, 1726 (C=O), 1666 (C=O), 1216, 758 cm^{-1}; ^1H NMR

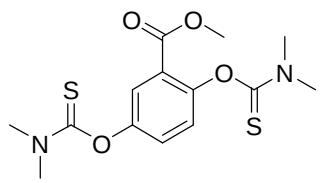
(400 MHz, $CDCl_3$): δ = 7.90 (d, 1H, *J* = 8 Hz, ArCH), 7.75 (d, 1H, *J* = 2 Hz, ArCH), 7.57 (dd, 1H, *J* = 8 Hz, *J* = 2 Hz, ArCH), 3.88 (s, 3H, OCH_3), 3.11-3.03 ppm (m, 12H, 4 × NCH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 166.6 (C=O), 165.9 (C=O), 165.4 (C=O), 143.1 (ArCH), 135.4 (ArCH), 134.9 (ArC), 133.2 (ArC), 130.7 (ArCH), 130.3 (ArC), 52.4 (OCH_3), 37.1 (2 × NCH_3), 37.0 (2 × NCH_3) ppm; HRMS (ES^+): $[M+H]^+$ calcd. for $C_{14}H_{19}N_2O_4S_2$, 343.0786; found, 343.0778.

2,4-Disulfanylbenzoic acid (10b)



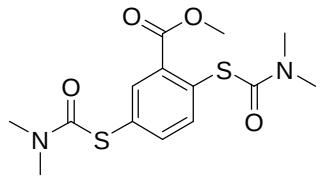
An aqueous solution of NaOH (1 N) (2.63 mL, 2.63 mmol) was added to **32b** (100 mg, 0.29 mmol). The resulting mixture was then heated at 70°C under a nitrogen atmosphere for 3 h. The reaction mixture was acidified with HCl (10 N). The white precipitate was collected by filtration, washed several times with H_2O and dried overnight to afford 45 mg (82 %) of the desired compound as a white solid. m.p. > 300°C (dec.); IR ν_{max} (disc): 3000, 2559, 1674 (br, 2 × C=O), 1580, 1272, 829, 771 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): δ = 7.87 (d, 1H, *J* = 8.5 Hz, ArCH), 7.31 (d, 1H, *J* = 2 Hz, ArCH), 7.05 (dd, 1H, *J* = 8.5 Hz, *J* = 2 Hz, ArCH); ^{13}C NMR (100.6 MHz, CD_3OD): δ = 168.5 (C=O), 140.2 (ArC), 139.7 (ArC), 132.4 (ArCH), 129.0 (ArCH), 124.1 (ArCH), 122.8 ppm (ArC); HRMS (ES^-): $[M-H]^-$ calcd. for $C_7H_5O_2S_2$, 184.9731; found, 184.9733.

Methyl 2,5-bis{[(dimethylamino)carbonothioyl]oxy}benzoate (31c)



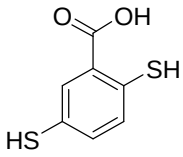
31c was prepared following a previously described method,[1] using **30c** (5.34 g, 31.8 mmol) in DMF (70 mL), DABCO (15.7 g, 0.13 mol) and *N*,*N*-dimethylthiocarbamoyl chloride (14.3 g, 0.13 mol). The crude yellow oil was crystallized from EtOH (400 mL) to afford 8.95 g (82 %) of the desired compound as a white solid. R_f = 0.35 (silica gel, CH_2Cl_2/EtOAc 98:2); m.p. 140-141°C; IR ν_{max} (film): 3054, 2987, 1731 (C=O), 1552 (C=S), 1266, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.71 (d, 1H, *J* = 2.5 Hz, ArCH), 7.28 (dd, 1H, *J* = 8.5 Hz, *J* = 2.5 Hz, ArCH), 7.14 (d, 1H, *J* = 8.5 Hz, ArCH), 3.82 (s, 3H, OCH_3), 3.47, 3.45, 3.39, 3.34 ppm (4 × brs, 4 × 3H, 4 × NCH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 187.3 (2 × C=S), 163.8 (C=O), 151.2 (ArC), 151.0 (ArC), 128.0 (ArCH), 125.7 (2 × ArCH), 124.4 (ArC), 52.2 (OCH_3), 43.4, 43.3, 38.9, 38.8 ppm (4 × NCH_3); HRMS (ES^+): $[M+H]^+$ calcd. for $C_{14}H_{19}N_2O_4S_2$, 343.0786; found, 343.0777.

Methyl 2,5-bis{[(dimethylamino)carbonyl]sulfanyl}benzoate (32c)



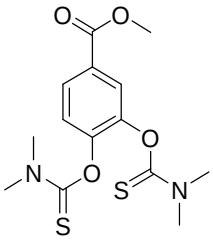
32c was prepared following a previously described method,[1] using **31c** (8.95 g, 26.2 mmol) in diphenyl ether (100 mL). Purification by flash chromatography using petroleum ether 40-60/CH_2Cl_2 [50:50] followed by CH_2Cl_2/EtOAc [80:20] to [50:50] afforded the compound as an oil. Co-evaporation using EtOH led to a solid which, upon recrystallization from EtOH, afforded 6.60 g (74 %) of the desired compound as a white solid. R_f = 0.3 (silica gel, CH_2Cl_2/EtOAc 4:1); m.p. 118-119°C; IR ν_{max} (film): 3055, 1730 (C=O), 1668 (C=O), 1266, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.04 (d, 1H, *J* = 2 Hz, ArCH), 7.63 (d, 1H, *J* = 8 Hz, ArCH), 7.60 (dd, 1H, *J* = 8 Hz, *J* = 2 Hz, ArCH), 3.87 (s, 3H, CH_3), 3.13-3.03 ppm (m, 12H, 4 × NCH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 166.2 (C=O), 165.7 (C=O), 165.7 (C=O), 138.1 (ArCH), 137.3 (ArCH), 137.2 (ArCH), 134.7 (ArC), 131.6 (ArC), 130.1 (ArC), 52.4 (OCH_3), 37.1 ppm (4 × NCH_3); HRMS (ES^+): $[M+H]^+$ calcd. for $C_{14}H_{19}N_2O_4S_2$, 343.0786; found, 343.0781.

2,5-Disulfanylbenzoic acid (10c)



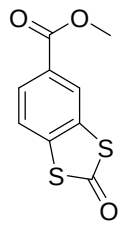
An aqueous solution of NaOH (1 N) (174 mL, 0.17 mol) was added to **32c** (6.60 g, 19.3 mmol). The resulting mixture was then heated at 70°C under a nitrogen atmosphere for 7 h. The reaction mixture was acidified with HCl (10 N). The white precipitate was collected by filtration, washed several times with H_2O and dried overnight to afford 3.55 g (99 %) of the desired compound as a white solid. m.p. 178-179°C; IR ν_{max} (film): 1686 (br, 2 × C=O), 1315, 1261 cm^{-1}; ^1H NMR (250 MHz, acetone-d6): δ = 8.02-8.00 (m, 1H, ArCH), 7.40-7.39 (m, 2H, 2 × ArCH); ^{13}C NMR (62.9 MHz, acetone-d6): δ = 167.2 (C=O), 135.9 (ArC), 133.5 (ArCH), 132.3 (ArCH), 131.9 (ArCH), 128.1 (ArC), 127.2 ppm (ArC); HRMS (ES^-): $[M-H]^-$ calcd. for $C_7H_5O_2S_2$, 184.9731; found, 184.9734.

Methyl 3,4-bis{[(dimethylamino)carbonothioyl]oxy}benzoate (31d)



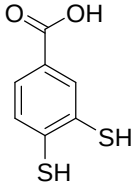
31d was prepared following a previously described method,[1] using **30d** (964 mg, 5.73 mmol) in DMF (10 mL), DABCO (2.57 g, 22.9 mmol) and *N*,*N*-dimethylthiocarbamoyl chloride (2.84 g, 22.9 mmol). Purification by flash chromatography using CH_2Cl_2/EtOAc [99:1] as eluent afforded 1.70 g (87 %) of the desired compound as a white solid. R_f = 0.3 (silica gel, CH_2Cl_2/petroleum 40-60 9:1); m.p. 57-59°C; IR ν_{max} (film): 3020, 1723 (C=O), 1541 (C=S), 1216, 909, 760 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.00 (dd, 1H, J = 8.5 Hz, J = 2 Hz, ArCH), 7.86 (d, 1H, J = 2 Hz, ArCH), 7.25 (d, 1H, J = 8.5 Hz, ArCH), 3.90 (s, 3H, OCH_3), 3.43, 3.42, 3.30, 3.29 ppm (4 × br s, 4 × 3H, 4 × NCH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 186.4 (C=S), 186.0 (C=S), 165.6 (C=O), 149.3 (ArC), 145.5 (ArC), 128.7 (ArC), 128.2 (ArCH), 125.9 (ArCH), 124.4 (ArCH), 52.3 (OCH_3), 43.4, 43.3, 38.9, 38.9 ppm (4 × NCH_3); HRMS (ES^+): $[M+H]^+$ calcd. for $C_{14}H_{19}N_2O_4S_2$, 343.0786; found, 343.0780.

Methyl 2-oxo-1,3-benzodithiole-5-carboxylate (32d)



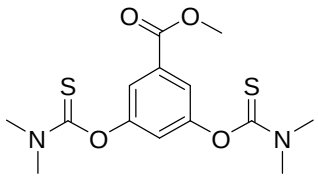
32d was prepared following a previously described method,[1] using **31d** (1.70 g, 4.97 mmol) in diphenyl ether (25 mL). Purification by flash chromatography using petroleum ether 40-60/CH_2Cl_2 [100:0] followed by petroleum ether 40-60/CH_2Cl_2 [95:5] to [50:50] afforded 710 mg (42 %) of the desired compound as a white solid. R_f = 0.3 (silica gel, CH_2Cl_2/petroleum 40-60 1:1); m.p. 139-140°C; IR ν_{max} (film): 3060, 2985, 1725 (C=O), 1665 (C=O), 1266, 739 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.18 (d, 1H, J = 1.5 Hz, ArCH), 7.98 (dd, 1H, J = 8.5 Hz, J = 1.5 Hz, ArCH), 7.57 (d, 1H, J = 8.5 Hz, ArCH), 3.95 ppm (s, 3H, CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 189.0 (SC=OS), 165.7 (ArC=O), 137.9 (ArC), 133.0 (ArC), 129.1 (ArC), 127.8 (ArCH), 124.1 (ArCH), 122.9 (ArCH), 52.6 ppm (OCH_3); HRMS (Cl^+): $[M]^+$ calcd. for $C_9H_6O_3S_2$, 225.9758; found, 225.9752.

3,4-Disulfanylbenzoic acid (10d)



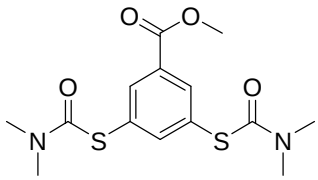
An aqueous solution of NaOH (1 N) (12.0 mL, 12.0 mmol) was added to **32d** (300 mg, 1.33 mmol). The resulting mixture was then heated at 70°C under a nitrogen atmosphere for 4 h. The reaction mixture was acidified with HCl (10 N). The white precipitate was collected by filtration, washed several times with H_2O and dried overnight to afford 245 mg (99 %) of the desired compound as a white solid. m.p. 218-220°C; IR ν_{max} (film): 1685 (C=O), 1584, 1426, 1313, 1250, 760 cm^{-1}; ^1H NMR (250 MHz, CD_3OD): δ = 8.03 (d, 1H, J = 2 Hz, ArCH), 7.70 (dd, 1H, J = 8 Hz, J = 2 Hz, ArCH), 7.47 (d, 1H, J = 8 Hz, ArCH); ^{13}C NMR (62.9 MHz, CD_3OD): δ = 167.9 (C=O), 139.4 (ArC), 131.9 (ArCH), 130.4 (ArC), 129.5 (ArCH), 128.5 (ArC), 127.4 ppm (ArCH); HRMS (ES^-): $[M-H]^-$ calcd. for $C_7H_5O_2S_2$, 184.9731; found, 184.9733.

Methyl 3,5-bis{[(dimethylamino)carbonothioyl]oxy}benzoate (23)



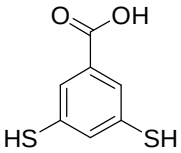
23 was prepared following a previously described method,[1, 2] using **22** (10.0 g, 59.5 mmol) in DMF (200 mL), DABCO (26.7 g, 0.24 mol) and *N*,*N*-dimethylthiocarbamoyl chloride (29.4 g, 0.24 mol). Recrystallization from EtOH afforded 18.9 g (93 %) of the desired compound as a white solid. R_f = 0.2 (silica gel, CH_2Cl_2); m.p. 100-102°C (lit.[8] 129-132°C); IR ν_{max} (film): 3052, 2953, 1725, 1540, 1266, 737 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.66 (s, 2H, 2 × ArCH), 7.07 (s, 1H, ArCH), 3.90 (s, 3H, CH_3), 3.45, 3.36 ppm (2 × brs, 2 × 6H, 4 × NCH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 186.9 (C=O), 165.4 (2 × C=S), 153.9 (2 × ArC), 131.6 (ArC), 122.9 (ArCH), 121.7 (2 × ArCH), 52.4 (OCH_3), 43.4 (2 × NCH_3), 38.9 ppm (2 × NCH_3); MS (ES^+): m/z (%): 343 (100) $[M+H]^+$, 365 (50) $[M+Na]^+$.

Methyl 3,5-bis{[(dimethylamino)carbonyl]sulfanyl}benzoate (24)



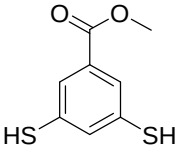
24 was prepared following a previously described method,[1, 2] using **23** (18.9 g, 55.3 mmol) in diphenyl ether (200 mL). Purification by flash chromatography using petroleum ether 40-60/CH_2Cl_2 [50:50] following by CH_2Cl_2/EtOAc [80:20] afforded the compound as an oil. Co-evaporation using EtOH led to a solid which, when recrystallised from EtOH, afforded 18.3 g (97 %) of the desired compound as a white solid. R_f = 0.35 (silica gel, CH_2Cl_2/EtOAc 4:1); m.p. 120-122°C (lit.[8] 134-137°C); IR ν_{max} (film): 3055, 2988, 1724 (C=O), 1671 (C=O), 1422, 1367, 1266, 740, 705 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.17 (d, 2H, *J* = 1.5 Hz, 2 × ArCH), 7.83 (t, 1H, *J* = 1.5 Hz, ArCH), 3.90 (s, 3H, CH_3), 3.07, 3.04 (2 × brs, 2 × 6H, 4 × NCH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 165.8 (2 × NC=OS), 165.6 (ArC=O), 146.3 (ArCH), 137.2 (2 × ArCH), 131.3 (ArC), 130.2 (2 × ArC), 52.4 (OCH_3), 37.0 ppm (4 × NCH_3); MS (ES^+): m/z (%): 342.83 (5) $[M+H]^+$, 364.85 (30) $[M+Na]^+$, 401.00 (100) $[M+CH_3CN+NH_4]^+$.

3,5-Disulfanylbenzoic acid (10e)



An aqueous solution of NaOH (1 N) (481 mL, 0.48 mol) was added to **24** (18.3 g, 53.5 mmol). The resulting mixture was then heated at 70°C under a nitrogen atmosphere for 8 h. The reaction mixture was acidified with HCl (10 N). The white precipitate was collected by filtration, washed several times with H_2O and dried overnight to afford 9.52 g (96 %) of the desired compound as a white solid. R_f = 0.75 (silica gel, EtOAc/AcOH 100:2); m.p. 156-158°C; IR ν_{max} (disc): 1694 (C=O), 1567, 1443, 1299, 769, 716 cm^{-1}; ^{1}H NMR (200 MHz, CD_3OD): δ = 7.68 (d, 2H, *J* = 1.5 Hz, 2 × ArCH), 7.43 (t, 1H, *J* = 1.5 Hz, ArCH); ^{13}C NMR (50.3 MHz, CD_3OD): δ = 167.5 (C=O), 134.2 (2 × ArC), 132.4 (ArC), 132.1 (ArCH), 126.4 ppm (2 × ArCH); HRMS (ES^-): $[M-H]^-$ calcd for $C_7H_5O_2S_2$, 184.9736; found, 184.9725.

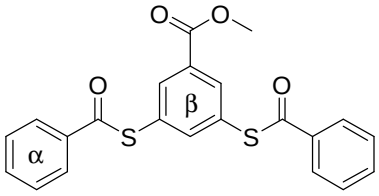
Methyl 3,5-disulfanylbenzoate (25)



To a solution of **10e** (5.00 g, 26.9 mmol) in MeOH (500 mL) under a nitrogen atmosphere was added concentrated H_2SO_4 (1.44 mL, 26.9 mL). The mixture was heated at reflux for 12 h then cooled to 22°C and concentrated to dryness. The solid residue was taken up in $CHCl_3$ (100 mL) and washed with H_2O (2 × 100 mL), brine (100 mL), dried over $MgSO_4$ and concentrated *in vacuo* to afford 5.28 g (98 %) of the desired compound as a white solid. m.p. 60-62°C (lit.[9] 61-62°C); IR ν_{max} (film): 3055, 2987, 1726 (2 × C=O), 1570, 1266, 736 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.71 (d, 2H, *J* = 1.5 Hz, 2 × ArCH), 7.34 (t, 1H, *J* = 1.5 Hz, ArCH), 3.91 (s, 3H, CH_3), 3.54 ppm (s, 2H, 2 × SH); ^{13}C NMR (100.6 MHz, $CDCl_3$):

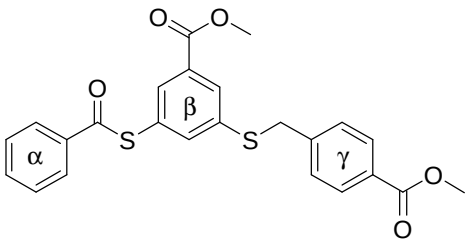
δ = 165.8 (C=O), 132.9 (ArCH), 131.6 (2 × ArC), 131.2 (ArC), 127.2 (2 × ArCH), 52.5 ppm (OCH_3); HRMS (ES^-): $[M-H]^-$ calcd. for $C_8H_7O_2S_2$, 198.9887; found, 198.9893.

Methyl 3,5-bis(benzoylsulfanyl)benzoate (26)



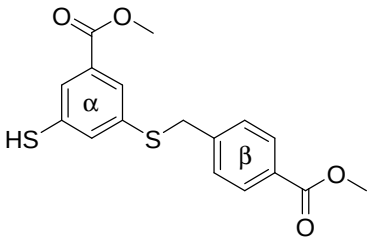
26 was prepared following a previously described method,[10] using **25** (6.70 g, 33.5 mmol), benzoyl chloride (11.3 mL, 0.10 mol) and triethylamine (13.6 mL, 0.10 mol) in THF (200 mL). The crude oil was crystallized under high vaccum and the resulting solid was triturated in cold hexane and frittered off (repeated 3 times) to afford 12.4 g (91 %) of the desired compound as a white solid. $R_f = 0.45$ (silica gel, CH_2Cl_2/petroleum 40-60 1:3); m.p. 79-80°C; IR ν_{max} (film): 3055, 2987, 1728 (C=O), 1683 (C=O), 1266, 897, 737 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.28 (d, 2H, J = 1.5 Hz, 2 × ArβCH), 8.04-8.02 (m, 4H, 4 × ArαCH), 7.89 (t, 1H, J = 1.5 Hz, ArβCH), 7.65 (t, 2H, J = 7.5 Hz, 2 × ArαCH), 7.52 (t, 4H, J = 8 Hz, 4 × ArαCH), 3.96 ppm (s, 3H, OCH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 188.9 (2 × SC=O), 165.4 (ArβC=O), 145.3 (ArβCH), 137.0 (2 × ArβCH), 136.2 (2 × ArC), 134.0 (2 × ArαCH), 132.1 (ArC), 129.3 (2 × ArC), 128.9 (4 × ArαCH), 127.6 (4 × ArαCH), 52.6 ppm (OCH_3); HRMS (ES^+): $[M+Na]^+$ calcd. for $C_{22}H_{16}NaO_4S_2$, 431.0388; found, 431.0382.

Methyl 3-(benzoylthio)-5-(4-(methoxycarbonyl)benzylthio)benzoate (28a)



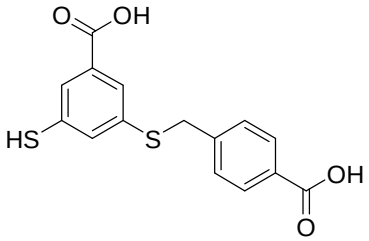
To an ice-cold solution of **26** (2.00 g, 4.90 mmol) under a nitrogen atmosphere in anhydrous THF (100 mL) was added a 30 % solution of MeONa in MeOH (965 μL, 5.15 mmol). The resulting mixture was stirred for 1 h at 0°C then quenched with a dilute aqueous solution of HCl (1 N) (100 mL) at 0°C and extracted with CH_2Cl_2 (3 × 300 mL). The combined organic extracts were washed with H_2O (3 × 300 mL), brine (300 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude residue was kept under a nitrogen atmosphere, diluted with acetone (100 mL) and methyl 4-(bromomethyl)benzoate (2.24 g, 9.80 mmol), 18-crown-6 (259 mg, 0.98 mmol) and potassium carbonate (1.36 g, 9.80 mmol) were added. The resulting mixture was heated at reflux for 14 h. The reaction mixture was then cooled to 22°C and concentrated to dryness under reduced pressure. The solid residue was taken up in CH_2Cl_2 (100 mL), washed with H_2O (2 × 100 mL), brine (100 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by flash chromatography using CH_2Cl_2 as eluent afforded 1.36 g (61 %) of the desired compound as a white solid. $R_f = 0.25$ (silica gel, CH_2Cl_2); m.p. 80-81°C; IR ν_{max} (film): 2954, 1720 (C=O), 1681 (C=O), 1282, 909, 734 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.01-7.98 (m, 4H, 2 × ArαCH + 2 × ArβCH), 7.96 (d, 2H, J = 8 Hz, 2 × ArγCH), 7.62 (t, 1H, J = 7.5 Hz, ArαCH), 7.59 (t, 1H, J = 1.5 Hz, ArβCH), 7.49 (t, 2H, J = 8 Hz, 2 × ArαCH), 7.52 (d, 2H, J = 8 Hz, 2 × ArγCH), 4.20 (s, 2H, CH_2), 3.90 (s, 3H, OCH_3), 3.88 ppm (s, 3H, OCH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 189.0 (ArαC=O), 166.7 (ArC=O), 165.6 (ArC=O), 142.0 (ArC), 140.0 (ArβCH), 137.7 (ArC), 136.2 (ArC), 134.0 (2 × ArCH), 131.7 (ArC), 131.5 (ArCH), 129.9 (2 × ArγCH), 129.3 (ArCH), 129.0 (2 × ArC), 128.9 (2 × ArCH), 127.6 (2 × ArCH), 52.5 (OCH_3), 52.1 (OCH_3), 38.6 ppm (CH_2); HRMS (ES^+): $[M+Na]^+$ calcd. for $C_{24}H_{20}NaO_5S_2$, 475.0650; found, 475.0644.

Methyl 3-{[4-(methoxycarbonyl)benzyl]sulfanyl}-5-sulfanylbenzoate (29a)



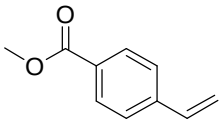
To an ice-cold solution of **28a** (500 mg, 1.11 mmol) under a nitrogen atmosphere in anhydrous THF (10 mL) was added a 30 % solution of MeONa in MeOH (228 µL, 1.22 mmol). The resulting mixture was stirred for 1 h at 0°C then quenched with a dilute aqueous solution of HCl (1 N) (10 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with H_2O (3 × 50 mL), brine (50 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by flash chromatography using CH_2Cl_2 as eluent afforded 319 mg (83 %) of the desired compound as a colorless oil. R_f = 0.5 (silica gel, CH_2Cl_2/EtOAc 98:2); IR ν_{max} (film): 1719 (br, 2 × C=O), 1282, 909, 735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.97 (d, 2H, *J* = 8.5 Hz, 2 × ArβCH), 7.73-7.72 (m, 2H, 2 × ArαCH), 7.36 (d, 2H, *J* = 8.5 Hz, 2 × ArβCH), 7.30 (t, 1H, *J* = 1.5 Hz, ArαCH), 4.16 (s, 2H, CH_2), 3.90 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.51 ppm (s, 1H, SH); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 166.7 (C=O), 165.8 (C=O), 142.0 (ArC), 137.5 (ArC), 133.6 (ArαCH), 132.7 (ArC), 131.5 (ArC), 129.9 (2 × ArβCH), 129.3 (ArC), 128.9 (2 × ArβCH), 128.0 (ArαCH), 127.4 (ArαCH), 52.5 (OCH_3), 52.2 (OCH_3), 38.3 ppm (CH_2); HRMS (ES^+): $[M+Na]^+$ calcd. for $C_{17}H_{16}NaO_4S_2$, 371.0388; found, 371.0382.

3-[(4-Carboxybenzyl)sulfanyl]-5-sulfanylbenzoic acid (21a)



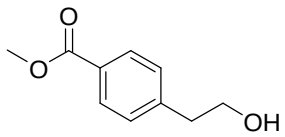
An aqueous solution of NaOH (1 N) (5.00 mL, 5.00 mmol) was added to **29a** (290 mg, 0.83 mmol). The resulting mixture was then heated at 70°C under a nitrogen atmosphere for 4 h. The reaction mixture was acidified with HCl (10 N). The white precipitate was collected by filtration, washed several times with H_2O and dried overnight to afford 265 mg (99 %) of the desired compound as a white solid. m.p. 177-179°C; IR ν_{max} (disc): 1693 (br, 2 × C=O), 1567, 1427, 1289 cm^{-1}; ^1H NMR (500 MHz, DMSO-d6): δ = 13.08 (brs, 2H, 2 × OH), 7.86-7.46 (m, 7H, 2 × ArCH), 4.37 ppm (s, 2H, CH_2); ^{13}C NMR (125.8 MHz, DMSO-d6): δ = 167.0 (C=O), 166.2 (C=O), 142.2 (ArC), 137.5, 134.9, 132.1, 130.9, 129.6 (ArC), 129.5 (2 × ArCH), 129.0 (2 × ArCH), 126.3, 124.9, 35.9 ppm (CH_2); HRMS (ES^-): $[M-H]^-$ calcd. for $C_{15}H_{11}O_4S_2$, 319.0099; found, 319.0093.

Methyl 4-vinylbenzoate (38)



38 was prepared following a previously described method,[6] using **37** (2.00 g, 13.5 mmol), trimethyloxonium tetrafluoroborate (2.50 g, 16.9 mmol) and triethylamine (2.07 mL, 14.9 mmol) in CH_2Cl_2 (200 mL). Purification by flash chromatography using CH_2Cl_2 as eluent afforded 1.44 g (70 %) of the desired compound as a white solid. R_f = 0.55 (silica gel, CH_2Cl_2/petroleum ether 40-60 1:1); ^1H NMR (400 MHz, $CDCl_3$): δ = 8.00 (d, 2H, *J* = 8.5 Hz, 2 × ArCH), 7.47 (d, 2H, *J* = 8.5 Hz, 2 × ArCH), 6.76 (dd, 1H, *J* = 17.5 Hz, *J* = 11 Hz, C*H*=CH_2), 5.87 (d, 1H, *J* = 17.5 Hz, CH=C*H*$_2$), 5.39 (d, 1H, *J* = 11 Hz, CH=C*H*$_2$), 3.93 ppm (s, 3H, OCH_3).

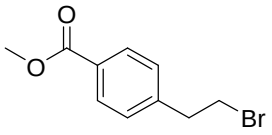
Methyl 4-(2-hydroxyethyl)benzoate (39b)



39b was prepared following a previously described method,[6] using **38** (1.24 g, 7.64 mmol), 1 M borane (BH_3) in THF (15.3 mL, 15.3 mmol) then 1 N NaOH (22.9 mL, 22.9 mmol) and 35 % H_2O_2 (12.6 mL, 0.13 mol). Purification by flash chromatography using a gradient of CH_2Cl_2/EtOAc [100:0] to [95:5] as eluent afforded 803 mg (58 %) of the desired compound as a colorless oil. R_f = 0.3 (silica gel, CH_2Cl_2/EtOAc 9:1); ^1H NMR (400 MHz, $CDCl_3$): δ = 7.99 (d, 2H, *J* = 8 Hz, 2 ×

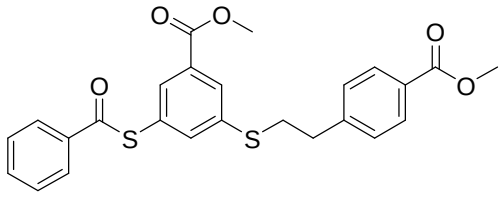
ArCH), 7.31 (d, 2H, J = 8 Hz, 2 × ArCH), 3.90 (m, 5H, OCH_2 + OCH_3), 2.93 (t, 2H, J = 6.5 Hz, $ArCH_2$), 1.63 (s, 1H, OH).

Methyl 4-(2-bromoethyl)benzoate (27b)



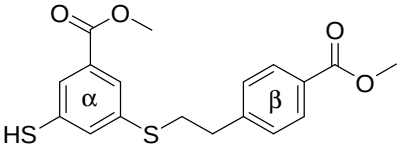
27b was prepared following a previously described method,[5] using **39b** (800 mg, 4.44 mmol, 1 eq), N-bromosuccinicimide (NBS) (1.58 g, 8.89 mmol) and triphenylphosphine (Ph_3P) (2.33 g, 8.89 mmol) in DMF (30 mL). Purification by flash chromatography using a gradient of CH_2Cl_2/petroleum ether 40-60 [10:90] to [50:50] as eluent afforded 962 mg (89 %) of the desired compound as a colorless oil. R_f = 0.55 (silica gel, CH_2Cl_2/petroleum ether 40-60 1:1); IR ν_{max} (film): 3060, 2985, 1740 (C=O), 1266 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.01 (d, 2H, J = 8 Hz, 2 × ArCH), 7.30 (d, 2H, J = 8 Hz, 2 × ArCH), 3.92 (s, 3H, OCH_3), 3.59 (t, 2H, J = 7.5 Hz, CH_2), 3.23 ppm (t, 2H, J = 7.5 Hz, CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 166.9 (C=O), 144.0 (ArC), 129.9 (2 × ArCH), 128.9 (ArC), 128.7 (2 × ArCH), 52.1 (OCH_3), 39.1 (CH_2), 32.2 ppm (CH_2); HRMS (CI^+): $[M+H]^+$ calcd. for $C_{10}H_{12}O_2Br$, 243.0021; found, 243.0027.

Methyl 3-(benzoylthio)-5-(4-(methoxycarbonyl)phenethylthio)benzoate (28b)



To an ice-cold solution of **26** (900 mg, 2.21 mmol) under a nitrogen atmosphere in anhydrous THF (50 mL) was added a 30 % solution of MeONa in MeOH (434 µL, 2.32 mmol). The resulting mixture was stirred for 1 h at 0°C then quenched with a dilute aqueous solution of HCl (1 N) (50 mL) at 0°C and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic extracts were washed with H_2O (3 × 150 mL), brine (150 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude residue was kept under a nitrogen atmosphere, diluted with acetone (50 mL) and **27b** (1.07 g, 4.41 mmol), 18-crown-6 (117 mg, 0.44 mmol) and K_2CO_3 (610 mg, 4.41 mmol) were added. The resulting mixture was heated at reflux for 14 h. The reaction mixture was then cooled to 22°C and concentrated to dryness under reduced pressure. The solid residue was taken up in CH_2Cl_2 (50 mL), washed with H_2O (2 × 50 mL), brine (50 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by flash chromatography using a gradient of CH_2Cl_2/petroleum ether 40-60 [20:80] to [100:0] then EtOAc/CH_2Cl_2 [10:90] as eluent afforded 410 mg (40 %) of the desired compound as a white solid. R_f = 0.4 (silica gel, CH_2Cl_2); m.p. 64-65°C; IR ν_{max} (film): 3055, 2987, 1721 (C=O), 1681 (C=O), 1266, 909, 741 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.01-7.97 (m, 6H, 6 × ArCH), 7.64-7.63 (m, 2H, 2 × ArCH), 7.51 (t, 2H, J = 7.5 Hz, 2 × ArCH), 7.31-7.29 (m, 2H, 2 × ArCH), 3.94 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.26 (t, 2H, J = 7.5 Hz, CH_2), 3.04 ppm (t, 2H, J = 7.5 Hz, CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 189.1 (ArC=OS), 166.9 (C=O), 165.7 (C=O), 145.0 (ArC), 139.1 (ArCH), 138.3 (ArC), 136.2 (ArC), 134.0 (ArCH), 133.3 (ArCH), 131.7 (ArC), 130.6 (ArCH), 129.9 (2 × ArCH), 128.9 (3 × ArCH), 128.7 (ArCH), 128.6 (2 × ArC), 127.6 (2 × ArCH), 52.5 (OCH_3), 52.1 (OCH_3), 35.4 (CH_2), 34.5 ppm (CH_2); HRMS (ES^+): $[M+Na]^+$ calcd. for $C_{25}H_{22}NaO_5S_2$, 489.0806; found, 489.0801.

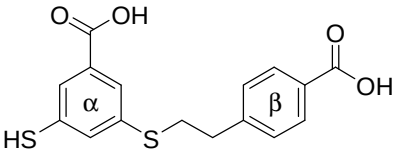
Methyl 3-({2-[4-(methoxycarbonyl)phenyl]ethyl}sulfanyl)-5-sulfanylbenzoate (29b)



To an ice-cold solution of **28b** (286 mg, 0.61 mmol) under a nitrogen atmosphere in anhydrous THF (10 mL) was added a 30 % solution of MeONa in MeOH (126 µL, 0.68 mmol). The resulting mixture was stirred for 1 h at 22°C then quenched with a dilute aqueous solution of HCl (1 N) (10 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with H_2O (3 × 50 mL), brine (50 mL), dried

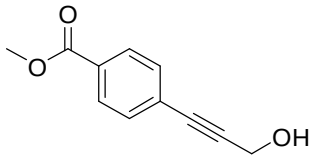
over $MgSO_4$ and the solvent removed under reduced pressure. Purification by flash chromatography using a gradient of CH_2Cl_2/petroleum ether 40-60 [50:50] to [100:0] as eluent afforded 150 mg (68 %) of the desired compound as a colorless oil. $R_f = 0.5$ (silica gel, CH_2Cl_2); IR ν_{max} (film): 1719 (br, 2 × C=O), 1283, 910, 735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.98$ (d, 2H, $J = 8.5$ Hz, 2 × ArβCH), 7.74 (t, 1H, $J = 1.5$ Hz, ArαCH), 7.73 (t, 1H, $J = 1.5$ Hz, ArαCH), 7.35 (t, 1H, $J = 1.5$ Hz, ArαCH), 7.28 (d, 2H, $J = 8.5$ Hz, 2 × ArβCH), 3.92 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.55 (s, 1H, SH), 3.22 (t, 2H, $J = 7.5$ Hz, CH_2), 3.00 ppm (t, 2H, $J = 7.5$ Hz, CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 166.9$ (C=O), 165.9 (C=O), 145.0 (ArC), 138.2 (ArC), 132.8 (ArαCH), 132.7 (ArC), 131.5 (ArC), 129.9 (2 × ArβCH), 128.6 (2 × ArβCH), 128.6 (ArC), 127.6 (ArαCH), 126.6 (ArαCH), 52.5 (OCH_3), 52.1 (OCH_3), 35.4 (CH_2), 34.4 ppm (CH_2); HRMS (FI^+): $[M]^+$ calcd. for $C_{18}H_{18}O_4S_2$, 362.0647; found, 362.0663.

3-{[2-(4-Carboxyphenyl)ethyl]sulfanyl}-5-sulfanylbenzoic acid (21b)



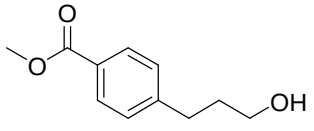
An aqueous solution of NaOH (1 N) (829 µL, 0.83 mmol) was added to **29b** (50.0 mg, 0.14 mmol). The resulting mixture was then heated at 70°C under a nitrogen atmosphere for 4 h. The reaction mixture was acidified with HCl (10 N). The white precipitate was collected by filtration, washed several times with H_2O and dried overnight to afford 38 mg (83 %) of the desired compound as a white solid. m.p. 199-200°C; IR ν_{max} (disc): 1686 (br, 2 × C=O), 1566, 1435, 1297 cm^{-1}; ^1H NMR (500 MHz, DMSO-d6): $\delta = 13.02$ (s, 2H, 2 × OH), 7.88 (d, 2H, $J = 8$ Hz, 2 × ArβCH), 7.65 (s, 1H, ArαCH), 7.55 (s, 1H, ArαCH), 7.50 (s, 1H, ArαCH), 7.40 (d, 2H, $J = 8$ Hz, 2 × ArβCH), 5.88 (brs, 1H, SH), 3.33 (m, 2H, CH_2), 2.72 (t, 2H, $J = 7.5$ Hz, CH_2); ^{13}C NMR (125.8 MHz, DMSO-d6): $\delta = 167.2$ (C=O), 166.4 (C=O), 145.0 (ArC), 137.9 (ArC), 134.8 (ArC), 132.1 (ArC), 130.3 (ArαCH), 129.4 (2 × ArβCH), 128.9 (ArC), 128.8 (2 × ArβCH), 125.8 (ArαCH), 124.4 (ArαCH), 34.2 (CH_2), 32.6 ppm (CH_2); HRMS (ES^-): $[M–H]^-$ calcd. for $C_{16}H_{13}O_4S_2$, 333.0255; found, 333.0270.

Methyl 4-(3-hydroxyprop-1-yn-1-yl)benzoate (33c)



33c was prepared following a previously described method,[4] using **35c** (3.38 mL, 57.2 mmol), copper iodide (73.0 mg, 0.38 mmol), tetrakis(triphenylphosphine)palladium(0) (220 mg, 0.19 mmol), **34** (5.00 g, 19.1 mmol, 1 eq), Et_3N (10.7 mL, 76.3 mmol, 4 eq) in anhydrous THF (100 mL). Purification by flash chromatography using CH_2Cl_2 as eluent afforded 2.62 g (72 %) of the desired compound as a white solid. $R_f = 0.15$ (silica gel, CH_2Cl_2); m.p. 73-74°C (lit.[11] 81-82°C); IR ν_{max} (film): 3055, 2987, 1721 (C=O), 1266, 739 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): $\delta = 7.95$ (d, 2H, $J = 8.5$ Hz, 2 × ArCH), 7.45 (d, 2H, $J = 8.5$ Hz, 2 × ArCH), 4.51 (s, 2H, CH_2), 3.90 (s, 3H, OCH_3), 2.54 (br s, 1H, OH); ^{13}C NMR ($CDCl_3$, 100.6 MHz): $\delta = 166.6$ (C=O), 131.6 (2 × ArCH), 129.6 (ArC), 129.5 (2 × ArCH), 127.3 (ArC), 90.4 (C=), 84.7 (C=), 52.3 (OCH_3), 51.5 ppm (CH_2); HRMS (FI^+): $[M]^+$ calcd. for $C_{11}H_{10}O_3$, 190.0630; found, 190.0636.

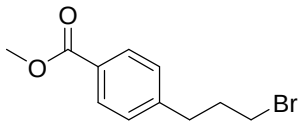
Methyl 4-(3-hydroxypropyl)benzoate (36c)



36c was prepared following a previously described method,[11] using **33c** (700 mg, 3.68 mmol), 10 % Pd-C (391 mg, 3.68 mmol) in MeOH (30 mL). Purification by flash chromatography using CH_2Cl_2/EtOAc [90:10] as eluent afforded 583 mg (82 %) of the desired compound as a colorless oil. $R_f = 0.2$ (silica gel, CH_2Cl_2/EtOAc 9:1); IR ν_{max} (film): 3417, 2950, 1719 (C=O), 1611, 1437, 1284, 1113, 737 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.94$ (d, 2H, $J = 8$ Hz, 2 × ArCH), 7.25 (d, 2H, $J = 8$ Hz, 2 × ArCH), 3.88 (s, 3H, OCH_3), 3.65 (t, 2H, $J = 6.5$ Hz, CH_2OH), 2.75 (t, 2H, $J = 6.5$ Hz, $ArCH_2$), 2.16 (br s, 1H, OH), 1.92-1.85 ppm (m, 2H, CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 167.2$ (C=O), 147.5 (ArC),

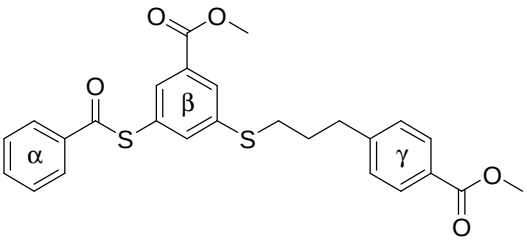
129.7 (2 × ArCH), 128.5 (2 × ArCH), 127.8 (ArC), 61.9 (CH_2OH), 52.0 (OCH_3), 33.8 (CH_2), 32.1 ppm ($ArCH_2$); HRMS (CI^+): $[M+H]^+$ calcd. for $C_{11}H_{15}O_3$, 195.1021; found, 195.1016.

Methyl 4-(3-bromopropyl)benzoate (27c)



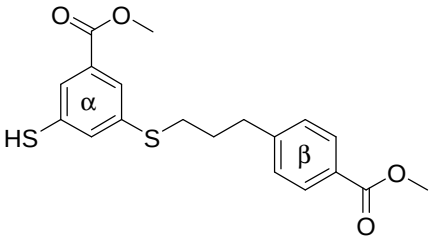
27c was prepared following a previously described method,[5] using **36c** (500 mg, 2.58 mmol), NBS (1.83 g, 10.3 mmol), Ph_3P (2.71 g, 10.3 mmol), in DMF (30 mL). Purification by flash chromatography using CH_2Cl_2/petroleum ether 40-60 [50:50] as eluent afforded 628 mg (95 %) of the desired compound as white solid. $R_f = 0.65$ (silica gel, CH_2Cl_2/petroleum ether 40-60 1:9); m.p. 38-39°C; IR ν_{max} (film): 3054, 2953, 1719 (C=O), 1266, 739 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.97$ (d, 2H, $J = 8.5$ Hz, 2 × ArCH), 7.27 (d, 2H, $J = 8.5$ Hz, 2 × ArCH), 3.91 (s, 3H, OCH_3), 3.39 (t, 2H, $J = 6.5$ Hz, CH_2Br), 2.84 (t, 2H, $J = 6.5$ Hz, $ArCH_2$), 2.22-2.15 ppm (m, 2H, CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 167.0$ (C=O), 146.0 (ArCH), 129.9 (2 × ArCH), 128.6 (2 × ArCH), 128.2 (ArCH), 52.1 (OCH_3), 34.0 ($ArCH_2$), 33.7 (CH_2), 32.8 ppm (CH_2Br); HRMS (CI^+): $[M+H]^+$ calcd. for $C_{11}H_{14}O_2Br$, 257.0177; found, 257.0173.

Methyl 3-(benzoylthio)-5-(3-(4-(methoxycarbonyl)phenyl)propylthio) benzoate (28c)



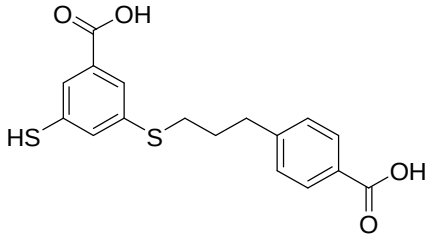
To an ice-cold solution of **26** (1.20 g, 2.94 mmol) under a nitrogen atmosphere in anhydrous THF (70 mL) was added a 30 % solution of MeONa in MeOH (0.58 mL, 3.09 mmol). The resulting mixture was stirred for 1 h at 0°C then quenched with a dilute aqueous solution of HCl (1 N) (70 mL) at 0°C and extracted with CH_2Cl_2 (3 × 200 mL). The combined organic extracts were washed with H_2O (3 × 200 mL), brine (200 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude residue was kept under a nitrogen atmosphere, diluted with acetone (70 mL) and **27c** (1.51 g, 5.88 mmol), 18-crown-6 (156 mg, 0.59 mmol) and potassium carbonate (813 mg, 5.88 mmol) were added. The resulting mixture was heated at reflux for 12 h. The reaction mixture was then cooled to 22°C and concentrated to dryness under reduced pressure. The solid residue was taken up in CH_2Cl_2 (50 mL), washed with H_2O (2 × 50 mL), brine (50 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by flash chromatography using a gradient of CH_2Cl_2/petroleum ether 40-60 [30:70] to [50:50] as eluent afforded 773 mg (55 %) of the desired compound as a white solid. $R_f = 0.15$ (silica gel, EtOAc/petroleum ether 40-60 1:9); m.p. 68-69°C; IR ν_{max} (film): 3055, 2987, 1720 (C=O), 1681 (C=O), 1266, 897, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 8.03$-8.01 (m, 3H, 2 × ArαCH + ArβCH), 7.97-7.95 (m, 3H, 2 × ArγCH + ArβCH), 7.65 (tt, 1H, $J = 7.5$ Hz, $J = 1$ Hz, ArαCH), 7.60 (t, 1H, $J = 1.5$ Hz, ArβCH), 7.52 (t, 2H, $J = 7.5$ Hz, 2 × ArαCH), 7.27-7.25 (m, 2H, 2 × ArγCH), 3.93 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 2.99 (t, 2H, $J = 7$ Hz, CH_2), 2.84 (t, 2H, $J = 7.5$ Hz, CH_2), 2.08-2.00 ppm (m, 2H, CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 189.2$ (ArαC=O), 167.1 (C=O), 165.8 (C=O), 146.5 (ArC), 138.8 (ArβCH), 138.7 (ArC), 136.2 (ArC), 134.0 (ArαCH), 133.2 (ArβCH), 131.7 (ArC), 130.5 (ArβCH), 129.8 (2 × ArγCH), 128.9 (2 × ArγCH), 128.8 (ArC), 128.6 (2 × ArαCH), 128.1 (ArC), 127.6 (2 × ArαCH), 52.5 (OCH_3), 52.0 (OCH_3), 34.6 (CH_2), 32.6 (CH_2), 30.0 ppm (CH_2); HRMS (ES^+): $[M+Na]^+$ calcd. for $C_{26}H_{24}NaO_5S_2$, 503.0963; found, 503.0957.

Methyl 3-({3-[4-(methoxycarbonyl)phenyl]propyl}sulfanyl)-5-sulfanylbenzoate (29c)



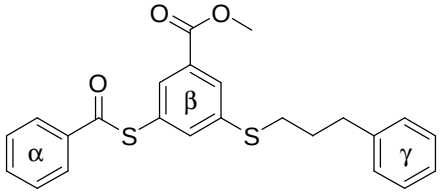
To an ice-cold solution of **28c** (500 mg, 1.04 mmol) under a nitrogen atmosphere in anhydrous THF (10 mL) was added a 30 % solution of MeONa in MeOH (293 µL, 1.56 mmol). The resulting mixture was stirred for 1 h at 0°C then quenched with a dilute aqueous solution of HCl (1 N) (10 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with H_2O (3 × 50 mL), brine (50 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by flash chromatography using CH_2Cl_2/petroleum ether 40-60 [50:50] as eluent afforded 332 mg (85 %) of the desired compound as a colorless oil. $R_f = 0.5$ (silica gel, CH_2Cl_2); IR ν_{max} (film): 3055, 1719 (br, 2 × C=O), 1266, 740 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.97 (d, 2H, J = 8.5 Hz, 2 × ArβCH), 7.72 (d, 2H, J = 1.5 Hz, 2 × ArαCH), 7.32 (t, 1H, J = 1.5 Hz, ArαCH), 7.25 (d, 2H, J = 8.5 Hz, 2 × ArβCH), 3.91 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.54 (s, 1H, SH), 2.94 (t, 2H, J = 7 Hz, CH_2), 2.82 (t, 2H, J = 7.5 Hz, CH_2), 2.01-1.97 ppm (m, 2H, CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 167.0 (C=O), 166.0 (C=O), 146.5 (ArC), 138.5 (ArC), 132.6 (ArC), 132.6 (ArαCH), 131.5 (ArC), 129.9 (2 × ArβCH), 128.5 (2 × ArβCH), 128.1 (ArC), 127.4 (ArαCH), 126.5 (ArαCH), 52.4 (OCH_3), 52.1 (OCH_3), 34.5 (CH_2), 32.4 (CH_2), 30.0 ppm (CH_2); HRMS (ES^-): $[M-H]^-$ calcd. for $C_{19}H_{19}O_4S_2$, 375.0730; found, 375.0719.

3-{[3-(4-Carboxyphenyl)propyl]sulfanyl}-5-sulfanylbenzoic acid (21c)



An aqueous solution of NaOH (1 N) (4.15 mL, 4.15 mmol) was added to **29c** (260 mg, 0.69 mmol). The resulting mixture was then heated at 70°C under a nitrogen atmosphere for 4 h. The reaction mixture was acidified with HCl (10 N). The white precipitate was collected by filtration, washed several times with H_2O and dried overnight to afford 240 mg (99 %) of the desired compound as a white solid. $R_f = 0.5$ (silica gel, CH_2Cl_2/EtOAc/MeOH/AcOH 50:45:5:1); m.p. 194-195°C; IR ν_{max} (disc): 1693 (br, 2 × C=O), 1567, 1435, 1292 cm^{-1}; ^1H NMR (500 MHz, DMSO-d6): δ = 13.06 (brs, 2H, 2 × OH), 7.84 (d, 2H, J = 8 Hz, 2 × ArβCH), 7.84 (s, 1H, ArαCH), 7.67 (t, 1H, J = 1.5 Hz, ArαCH), 7.61 (t, 1H, J = 1.5 Hz, ArαCH), 7.26 (d, 2H, J = 8 Hz, 2 × ArβCH), 2.97 (t, 2H, J = 7 Hz, CH_2), 2.72 (t, 2H, J = 7.5 Hz, CH_2), 1.83 ppm (m, 2H, CH_2); ^{13}C NMR (125.8 MHz, DMSO-d6): δ = 167.2 (C=O), 165.9 (C=O), 146.3 (ArC), 139.1 (ArC), 137.2 (ArC), 132.7 (ArC), 129.5 (2 × ArβCH), 128.6 (ArαCH), 128.6 (ArC), 128.4 (2 × ArβCH), 127.0 (ArαCH), 124.2 (ArαCH), 33.8 (CH_2), 31.0 (CH_2), 29.5 ppm (CH_2); HRMS (ES^-): $[M-H]^-$ calcd. for $C_{17}H_{15}O_4S_2$, 347.0412; found, 347.0416.

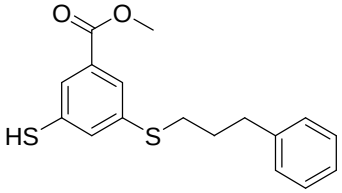
Methyl 3-(benzoylthio)-5-(3-phenylpropylthio)benzoate (28d)



To an ice-cold solution of **26** (580 mg, 1.42 mmol) under a nitrogen atmosphere in anhydrous THF (30 mL) was added a 30 % solution of MeONa in MeOH (293 µL, 1.49 mmol). The resulting mixture was stirred for 1 h at 0°C then quenched with a dilute aqueous solution of HCl (1 N) (30 mL) at 0°C and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were washed with H_2O (3 × 100 mL), brine (100 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude residue was kept under a nitrogen atmosphere, diluted with acetone (40 mL) and (3-bromopropyl)benzene (473 µL, 3.11 mmol), 18-crown-6 (82.0 mg, 0.31

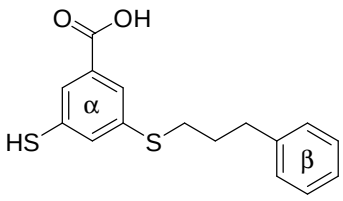
mmol) and potassium carbonate (430 mg, 3.11 mmol) were added. The resulting mixture was heated at reflux for 14 h. The reaction mixture was then cooled to 22°C and concentrated to dryness under reduced pressure. The solid residue was taken up in CH_2Cl_2 (50 mL), washed with H_2O (2 × 50 mL), brine (50 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by flash chromatography using a gradient of petroleum ether 40-60/EtOAc [95:5] to [90:10] as eluent afforded 337 mg (51 %) of the desired compound as a white solid. R_f = 0.15 (silica gel, EtOAc/petroleum 40-60 5:95); m.p. 55-56°C; IR ν_{max} (film): 3055, 2987, 1640 (br, 2 × C=O), 1266, 896, 737 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.04-8.02 (m, 3H, 2 × ArαCH + ArβCH), 7.97 (t, 1H, *J* = 1.5 Hz, ArβCH), 7.65 (t, 1H, *J* = 7.5 Hz, ArαCH), 7.59 (t, 1H, *J* = 1.5 Hz, ArβCH), 7.52 (t, 2H, *J* = 7.5 Hz, 2 × ArαCH), 7.28-7.27 (m, 2H, 2 × ArγCH), 7.21-7.19 (m, 3H, 3 × ArγCH), 3.94 (s, 3H, OCH_3), 3.00 (t, 2H, *J* = 7.5 Hz, CH_2), 2.79 (t, 2H, *J* = 7.5 Hz, CH_2), 2.07-2.00 ppm (m, 2H, CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 189.2 (ArαC=OS), 165.8 (ArβC=O), 141.0 (ArC), 139.0 (ArC), 138.6 (ArβCH), 136.3 (ArC), 134.0 (ArαCH), 133.1 (ArβCH), 131.6 (ArC), 130.3 (ArβCH), 128.9 (2 × ArαCH), 128.7 (ArC), 128.5 (2 × ArγCH), 128.5 (2 × ArγCH), 127.6 (2 × ArαCH), 126.1 (ArγCH), 52.5 (OCH_3), 34.6 (CH_2), 32.5 (CH_2), 30.3 ppm (CH_2); HRMS (CI^+): $[M+NH_4]^+$ calcd. for $C_{24}H_{26}NO_3S_2$, 440.1354; found, 440.1360.

Methyl 3-[(3-phenylpropyl)sulfanyl]-5-sulfanylbenzoate (29d)



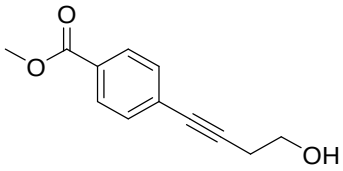
To an ice-cold solution of **28d** (200 mg, 0.47 mmol) under a nitrogen atmosphere in anhydrous THF (10 mL) was added a 30 % solution of MeONa in MeOH (293 µL, 1.04 mmol). The resulting mixture was stirred for 2 h at 22°C then quenched with a dilute aqueous solution of HCl (1 N) (10 mL) and extracted with CH_2Cl_2 (3 × 50 mL). The combined organic extracts were washed with H_2O (3 × 50 mL), brine (50 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by flash chromatography using petroleum ether 40-60/EtOAc [80:20] as eluent afforded 144 mg (95 %) of the desired compound as a colorless oil. R_f = 0.25 (silica gel, EtOAc/petroleum 40-60 5:95); IR ν_{max} (film): 1721 (C=O), 1279, 909, 734 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.73-7.72 (m, 2H, 2 × ArCH), 7.33-7.27 (m, 3H, 3 × ArCH), 7.24-7.18 (m, 3H, 3 × ArCH), 3.92 (s, 3H, OCH_3), 3.53 (s, 1H, SH), 2.95 (t, 2H, *J* = 7.5 Hz, CH_2), 2.78 (t, 2H, *J* = 7.5 Hz, CH_2), 2.03-1.95 ppm (m, 2H, CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 166.0 (C=O), 141.0 (ArC), 138.8 (ArC), 132.5 (ArC), 132.4 (ArCH), 131.4 (ArC), 128.5 (4 × ArCH), 127.2 (2 × ArCH), 126.4 (2 × ArCH), 126.1 (2 × ArCH), 52.4 (OCH_3), 34.6 (CH_2), 32.3 (CH_2), 30.4 ppm (CH_2); HRMS (ES^-): $[M-H]^-$ calcd. for $C_{17}H_{17}O_2S_2$, 317.0675; found, 317.0664.

3-[(3-Phenylpropyl)sulfanyl]-5-sulfanylbenzoic acid (21d)



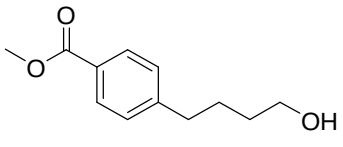
An aqueous solution of NaOH (1 N) (1.89 mL, 1.89 mmol) was added to **29d** (100 mg, 0.31 mmol). The resulting mixture was then heated at 70°C under a nitrogen atmosphere for 3 h. The reaction mixture was acidified with HCl (10 N). The white precipitate was collected by filtration, washed several times with H_2O and dried overnight to afford 94 mg (98 %) of the desired compound as a white solid. R_f = 0.4 (silica gel, CH_2Cl_2/EtOAc/AcOH 50:50:1); m.p. 75-76°C; IR ν_{max} (disc): 1695 (C=O), 1568, 1442, 1297 cm^{-1}; ^1H NMR (500 MHz, DMSO-d6): δ = 13.20 (br s, 1H, OH), 7.65 (s, 1H, ArαCH), 7.52 (s, 1H, ArαCH), 7.46 (s, 1H, ArαCH), 7.29 (m, 2H, 2 × ArβCH), 7.19 (m, 3H, 3 × ArβCH), 5.88 (br s, 1H, SH), 3.00 (t, 2H, *J* = 7.5 Hz, CH_2), 2.72 (t, 2H, *J* = 7.5 Hz, CH_2), 1.90-1.87 ppm (m, 2H, CH_2); ^{13}C NMR (125.8 MHz, DMSO-d6): δ = 166.4 (C=O), 141.1 (ArC), 138.1 (ArC), 134.7 (ArC), 132.1 (ArC), 130.3 (ArαCH), 128.4 (2 × ArβCH), 128.3 (2 × ArβCH), 125.9 (ArβCH), 125.8 (ArαCH), 124.4 (ArαCH), 33.9 (CH_2), 31.2 (CH_2), 30.1 ppm (CH_2); HRMS (ES^-): $[M-H]^-$ calcd. for $C_{16}H_{15}O_2S_2$, 303.0519; found, 303.0508.

Methyl 4-(4-hydroxybut-1-yn-1-yl)benzoate (33e)



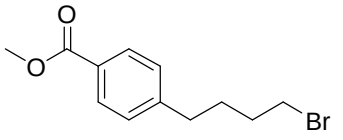
33e was prepared following a previously described method,[4] using **35e** (1.73 mL, 22.9 mmol), copper iodide (29.0 mg, 0.15 mmol), tetrakis(triphenylphosphine)palladium(0) (176 mg, 0.15 mmol), methyl-4-iodobenzoate (2.00 g, 7.63 mmol) and Et_3N (4.27 mL, 30.5 mmol) in anhydrous THF (40 mL). Purification by flash chromatography using a gradient of CH_2Cl_2/EtOAc [100:0] to [90:10] as eluent afforded 1.34 g (86 %) of the desired compound as an off-white solid. $R_f = 0.1$ (silica gel, CH_2Cl_2); m.p. 60-62°C (lit.[12] 95.3-96.3°C); IR ν_{max} (film): 1719 (C=O), 1279, 909, 735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.96$ (d, 2H, $J = 8.5$ Hz, 2 × ArCH), 7.47 (d, 2H, $J = 8.5$ Hz, 2 × ArCH), 3.91 (s, 3H, OCH_3), 3.84 (m, 2H, OCH_2), 2.72 (t, 2H, $J = 6.5$ Hz, CH_2), 1.97 (t, 1H, $J = 5.5$ Hz, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 166.6$ (C=O), 131.6 (2 × ArCH), 129.4 (2 × ArCH), 129.2 (ArC), 128.1 (ArC), 89.9 (C=), 81.8 (C=), 61.0 (CH_2OH), 52.2 (OCH_3), 23.9 ppm (CH_2); HRMS (Cl^+): $[M+H]^+$ calcd. for $C_{12}H_{13}O_3$, 205.0865; found, 205.0857.

Methyl 4-(4-hydroxybutyl)benzoate (36e)



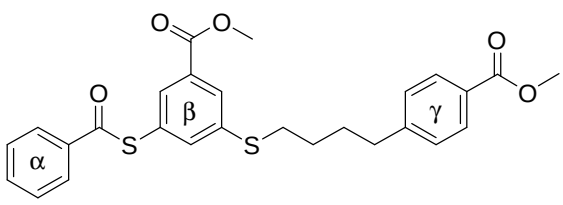
36e was prepared following a previously described method,[11] using **33e** (1.30 g, 6.37 mmol) and 10 % Pd-C (678 mg, 6.37 mmol) in MeOH (40 mL). Purification by flash chromatography using a gradient of EtOAc/petroleum ether 40-60 [30:70] to [50:50] as eluent afforded 1.12 g (84 %) of the desired compound as a colorless oil. $R_f = 0.2$ (silica gel, EtOAc/petroleum ether 40-60 3:7); IR ν_{max} (film): 1715 (C=O), 1285, 911, 742 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.95$ (d, 2H, $J = 8.5$ Hz, 2 × ArCH), 7.25 (d, 2H, $J = 8.5$ Hz, 2 × ArCH), 3.90 (s, 3H, OCH_3), 3.66 (t, 2H, $J = 6.5$ Hz, CH_2OH), 2.70 (t, 2H, $J = 7.5$ Hz, CH_2), 1.76-1.68 (m, 2H, CH_2), 1.64-1.57 ppm (m, 3H, OH + CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 167.2$ (C=O), 147.9 (ArC), 129.7 (2 × ArCH), 128.4 (2 × ArCH), 127.8 (ArC), 62.6 (CH_2OH), 52.0 (OCH_3), 35.7 (CH_2), 32.2 (CH_2), 27.3 ppm (CH_2); HRMS (Cl^+): $[M+H]^+$ calcd. for $C_{12}H_{17}O_3$, 209.1178; found, 209.1173.

Methyl 4-(4-bromobutyl)benzoate (27e)



27e was prepared following a previously described method,[5] using **36e** (1.05 g, 5.05 mmol), NBS (1.80 g, 10.1 mmol) and Ph_3P (2.65 g, 10.1 mmol) in DMF (60 mL). Purification by flash chromatography using EtOAc/petroleum ether 40-60 [5:95] as eluent afforded 1.24 g (91 %) of the desired compound as a colorless oil. $R_f = 0.3$ (silica gel, EtOAc/petroleum ether 40-60 5:95); IR ν_{max} (film): 1716 (C=O), 1285, 910, 731 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.97$ (d, 2H, $J = 8$ Hz, 2 × ArCH), 7.25 (d, 2H, $J = 8$ Hz, 2 × ArCH), 3.91 (s, 3H, OCH_3), 3.42 (t, 2H, $J = 6.5$ Hz, CH_2), 2.70 (t, 2H, $J = 7.5$ Hz, CH_2), 1.93-1.86 (m, 2H, CH_2), 1.84-1.76 ppm (m, 2H, CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 167.1$ (C=O), 147.3 (ArC), 129.8 (2 × ArCH), 128.4 (2 × ArCH), 128.0 (ArC), 52.0 (OCH_3), 35.0 (CH_2), 33.4 (CH_2), 32.1 (CH_2), 29.5 ppm (CH_2); HRMS (Cl^+): $[M+H]^+$ calcd. for $C_{12}H_{16}O_2Br$, 271.0334; found, 271.0338.

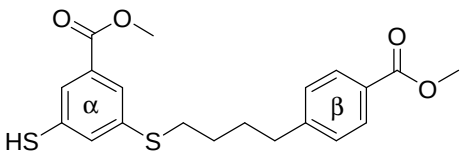
Methyl 3-(benzoylthio)-5-(4-(4-(methoxycarbonyl)phenyl)butylthio)benzoate (28e)



To an ice-cold solution of **26** (1.00 g, 2.45 mmol) under a nitrogen atmosphere in anhydrous THF (50 mL) was added a 30 % solution of MeONa in MeOH (483 µL, 2.57 mmol). The resulting mixture was stirred for 1 h at 0°C then quenched with a dilute aqueous

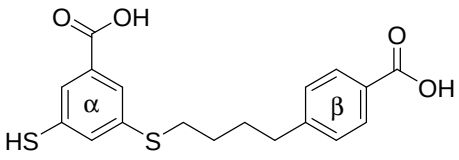
solution of HCl (1 N) (50 mL) at 0°C and extracted with CH_2Cl_2 (3 × 150 mL). The combined organic extracts were washed with H_2O (3 × 150 mL), brine (150 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude residue was kept under a nitrogen atmosphere, diluted with acetone (50 mL) and **27e** (1.32 g, 4.90 mmol), 18-crown-6 (129 mg, 0.49 mmol) and potassium carbonate (677 mg, 4.90 mmol) were added. The resulting mixture was then heated to reflux for 12 h. The reaction mixture was then cooled to 22°C and concentrated to dryness under reduced pressure. The solid residue was taken up in CH_2Cl_2 (50 mL), washed with H_2O (3 × 50 mL), brine (50 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by flash chromatography using CH_2Cl_2 as eluent afforded 378 mg (31 %) of the desired compound as a white solid. R_f = 0.3 (silica gel, CH_2Cl_2); m.p. 62-64°C; IR ν_{max} (film): 1719 (C=O), 1680 (C=O), 1282, 909, 736 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 8.03-8.01 (m, 3H, 2 × ArαCH + ArβCH), 7.97-7.94 (m, 3H, ArβCH + 2 × ArγCH), 7.65 (tt, 1H, *J* = 7 Hz, *J* = 1 Hz, ArαCH), 7.60 (t, 1H, *J* = 1.5 Hz, ArβCH), 7.51 (t, 2H, *J* = 7.5 Hz, 2 × ArαCH), 7.24 (d, 2H, *J* = 8.5 Hz, 2 × ArγCH), 3.93 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.02 (t, 2H, *J* = 7 Hz, CH_2), 2.70 (t, 2H, *J* = 7.5 Hz, CH_2), 1.85-1.70 ppm (m, 4H, 2 × CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 189.2 (ArαC=O), 167.1 (C=O), 165.8 (C=O), 147.5 (ArC), 139.1 (ArC), 138.7 (ArβCH), 136.3 (ArC), 134.0 (ArαCH), 133.0 (ArβCH), 131.6 (ArC), 130.2 (ArβCH), 129.7 (2 × ArγCH), 128.9 (2 × ArαCH), 128.7 (ArC), 128.4 (2 × ArγCH), 127.9 (ArC), 127.6 (2 × ArαCH), 52.5 (OCH_3), 52.0 (OCH_3), 35.4 (CH_2), 33.1 (CH_2), 30.0 (CH_2), 28.3 ppm (CH_2); HRMS (ES^+): $[M+Na]^+$ calcd. for $C_{27}H_{26}NaO_5S_2$, 517.1119; found, 517.1114.

Methyl 3-({4-[4-(methoxycarbonyl)phenyl]butyl}sulfanyl)-5-sulfanylbenzoate (29e)



To an ice-cold solution of **28e** (200 mg, 0.41 mmol) under a nitrogen atmosphere in anhydrous THF (5 mL) was added a 30 % solution of MeONa in MeOH (84.0 µL, 0.45 mmol). The resulting mixture was stirred for 1 h at 0°C then quenched with a dilute aqueous solution of HCl (1 N) (5 mL) and extracted with CH_2Cl_2 (3 × 25 mL). The combined organic extracts were washed with H_2O (3 × 25 mL), brine (25 mL), dried over $MgSO_4$ and the solvent removed under reduced pressure. Purification by flash chromatography using CH_2Cl_2 as eluent to afford 122 mg (77 %) of the desired compound as a colorless oil. R_f = 0.65 (silica gel, CH_2Cl_2/EtOAc 98:2); IR ν_{max} (film): 1718 (2C=O), 1282, 909, 735 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.95 (d, 2H, *J* = 8 Hz, 2 × ArβCH), 7.71 (d, 2H, *J* = 1.5 Hz, 2 × ArαCH), 7.33 (t, 1H, *J* = 1.5 Hz, ArαCH), 7.23 (d, 2H, *J* = 8 Hz, 2 × ArβCH), 3.91 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.54 (s, 1H, SH), 2.96 (t, 2H, *J* = 7 Hz, CH_2), 2.69 (t, 2H, *J* = 7.5 Hz, CH_2), 1.80 (m, 2H, CH_2), 1.69 ppm (m, 2H, CH_2); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 167.1 (C=O), 166.0 (C=O), 147.4 (ArC), 138.8 (ArC), 132.5 (ArαCH), 131.4 (ArC), 129.7 (2 × ArβCH), 128.4 (2 × ArβCH), 127.9 (ArC), 127.3 (ArαCH), 126.4 (ArαCH), 52.4 (OCH_3), 52.0 (OCH_3), 35.4 (CH_2), 33.0 (CH_2), 30.0 (CH_2), 28.3 ppm (CH_2); HRMS (ES^+): $[M+Na]^+$ calcd. for $C_{20}H_{22}NaO_4S_2$, 413.0857; found, 413.0852.

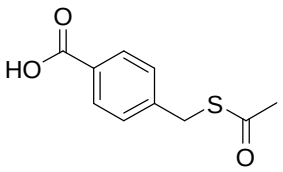
3-{[4-(4-Carboxyphenyl)butyl]sulfanyl}-5-sulfanylbenzoic acid (21e)



An aqueous solution of NaOH (1 N) (1.54 mL, 1.54 mmol) was added to **29e** (100 mg, 0.26 mmol). The resulting mixture was then heated at 70°C under a nitrogen atmosphere for 4 h. The reaction mixture was acidified with HCl (10 N). The white precipitate was collected by filtration, washed several times with H_2O and dried overnight to afford 91 mg (99 %) of the desired compound as a white solid. m.p. 198-199°C; IR ν_{max} (disc): 1688 (broad, 2 × C=O), 1566, 1428, 1291 cm^{-1}; ^1H NMR (500 MHz, DMSO-d6): δ = 12.99 (br s, 2H, 2 × OH), 7.85 (d, 2H, *J* = 7 Hz, 2 × ArβCH), 7.67-7.47 (m, 3H, 3 × ArαCH), 7.32-7.27 (m, 2H, 2 × ArβCH), 5.88 (br s, 1H, SH), 3.04-3.00 (m, 2H, CH_2), 2.68-2.60 (m, 2H, CH_2), 1.73-1.53 ppm (m, 4H, 2 × CH_2); ^{13}C NMR (125.8 MHz, DMSO-d6): δ = 167.3 (C=O), 166.4 (C=O), 147.3

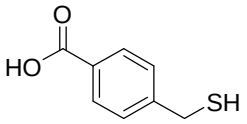
(ArC), 138.3 (ArC), 134.7 (ArC), 132.1 (ArC), 130.2 (ArαCH), 129.4 (2 × ArβCH), 128.5 (2 × ArβCH), 128.4 (ArC), 125.7 (ArαCH), 124.3 (ArαCH), 34.4 (CH_2), 31.4 (CH_2), 29.5 (CH_2), 27.8 ppm (CH_2); HRMS (ES^-): $[M–H]^-$ calcd. for $C_{18}H_{17}O_4S_2$, 361.0574; found, 361.0563.

4-[(Acetylsulfanyl)methyl]benzoic acid (40)



40 was prepared following a previously described method,[13] using 4-bromomethylbenzoic acid (500 mg, 2.33 mmol) and potassium thioacetate (265 mg, 2.33 mmol) in EtOAc (10 mL) to afford 395 mg (81 %) of the desired compound as a white crystalline solid. $R_f = 0.2$ (silica gel, CH_2Cl_2/AcOH 100:1); m.p. 143-145°C (lit.[14] 144-145°C); IR ν_{max} (film): 3055, 1265, 749 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): $\delta = 11.88$ (br s, 1H, OH), 8.05 (d, 2H, J = 8 Hz, 2 × ArCH), 7.41 (d, 2H, $J = 8$ Hz, 2 × ArCH), 4.17 (s, 2H, CH_2), 2.38 ppm (s, 3H, CH_3); MS (ES^-): m/z (%): 209 (100) $[M–H]^-$.

4-(Sulfanylmethyl)benzoic acid (41)



41 was prepared following a previously described method, [14] using **40** (200 mg, 0.95 mmol) and 1 N KOH in MeOH (2.86 mL, 2.86 mmol) in MeOH (3 mL) to afford 158 mg (99 %) of the desired compound as a gold colored solid. $R_f = 0.4$ (silica gel, CH_2Cl_2/EtOAc/AcOH 80:20:1); m.p. 144-146°C (lit.[14] 155.5-157°C); IR ν_{max} (disc): 1685 (C=O), 1609, 1427, 1317, 1291 cm^{-1}; ^1H NMR (400 MHz, CD_3OD): $\delta = 7.97$ (d, 2H, J = 8 Hz, 2 × ArCH), 7.44 (d, 2H, $J = 8$ Hz, 2 × ArCH), 4.91 ppm (s, 2H, CH_2); MS (ES^-): m/z (%): 167 (100) $[M–H]^-$.

9. Enzyme kinetics

The zinc-*ß*-lactamase from *Bacillus cereus* 569/H/9 was prepared as described.[15] Protein concentration was calculated from spectrophotometric data using the extinction coefficient e_{280} = 30,500 M^{-1} cm^{-1}.[15] Imipenem was provided by Merck Sharp and Dohme (Haar, Germany). HEPES buffer was purchased from Roth (Karlsruhe, Germany) and $ZnCl_2$ (98 % enriched) from Sigma-Aldrich (Steinheim, Germany). Water was purified with a Millipore (Bedford, MA, USA) water purification system. Kinetic parameters were determined in 15 mM HEPES, pH 7 at 24°C using a Lambda 9, (Perkin-Elmer instruments) spectrophotometer equipped with thermostatically controlled cells.

Imipenem hydrolysis was followed by monitoring the change in absorbance at 300 nm ($?e^{300}_{(imipenem)}$ = -9000 M^{-1} cm^{-1}). The final BcII enzyme concentration was 10 nM. The substrate concentration was 160 µM and equal to K_m value of the enzyme for imipenem. The inhibitors were dissolved in 4-10 % DMSO, HEPES 15 mM, pH 7 at 1-2 mg/ml and then diluted. Two different inhibitor concentrations were used for the K_i determinations. The presence of 0.5 % DMSO in the final solution did not show any effect on the activity (data not shown). K_i values were determined using the following equation for competitive inhibition:

$$v_o/v_i = [K_m (1 + I/K_i) + S] / K_m + S$$

v_o Initial rate in absence of inhibitor
v_i Initial rate in presence of inhibitor
S Substrate concentration
I Inhibitor concentration
K_m Michaelis Menten constant
K_i Inhibition constant

For inhibition pattern determination, kinetic analyses were performed in 15 mM HEPES, pH 7 at constant temperature. Substrate concentration was varied between 40 and 200 µM and the BcII concentration was fixed by 10 nM. Three analyses were performed for each inhibitor, where the inhibitor concentration [I] was varied ([I] = 0; [I] = K_i; [I] =2 $\times$ K_i).

10. Native ESI-MS

10.1 Protein preparation

The enzyme samples were desalted using Microcon YM-10 (cut-off = 10,000 Da) centrifugal filters (Millipore, Bedford MA, USA) in 15 mM ammonium acetate buffers (pH 6.5, 7.0 or 7.5). Seven dilution/concentration steps were performed at 4°C and 14,000 g. The stock enzyme solution was diluted in NH_4OAc buffer to a final concentration of 100 μM.

10.2 Sample preparation for the different DCL

Prior to each run, under an oxygen-free atmosphere, individual thiol members of the C-SAR were freshly dissolved in DMSO at a final concentration of 100 mM. Each monothiol was then diluted to 75 μM into the same mixture in 15 mM ammonium acetate buffer pH 7.5. The pH of the resulting mixture was then adjusted to the required value with a 2.8 % aqueous solution of NH_4OH using a pH-meter stick HI 1290 Piccolo. Each dithiol molecule was diluted to a concentration of 100 μM in 15 mM ammonium acetate buffer at pH 7.5. The experimental samples were prepared by mixing the appropriate volumes of the monothiols, dithiol and the enzyme stock solution in 15 mM ammonium acetate at pH 7.5. The final concentrations for each monothiol, dithiol and the enzyme were 15 μM, 45 μM and 15 μM respectively. An aliquot of this mixture was placed in a 96 well-plate sealed with adhesive aluminium foil and was subsequently taken out of the oxygen-free environment to be analysed.

10.3 Automated Nanoelectrospray Mass Spectrometry (additional information)

Samples were then infused to the Q-TOF through the ESI chip (estimated flow rate *ca.* 100 nL/min). Typically a spraying voltage of 1.70 kV ± 0.1 kV depending on the "sprayability" of the sample and a sample pressure of 0.25 psi were applied. The instrument was equipped with a standard Z-spray source block. Clusters of $Cs_{(n+1)}I_n$ (1mg/ml CsI in 100 % methanol) were used for calibration. Calibration and sample acquisitions were performed in the positive ion mode in the range of m/z 500-5000. Operating conditions for the mass spectrometer were: sample cone voltage (varied) between 20 to 200 V, source temperature 20°C. Acquisition and scan time were 30 s and 1 s, respectively. The pressure at the interface between the atmospheric source and the high vacuum region was fixed at 6.6 mbar (measured with the roughing pump Pirani gauge) by throttling the pumping line using an Edwards Speedivalve to provide collisional cooling. Data were smoothed by the Savitzky Golay method (smooth windows: 20, number of smooth: 4) the background subtracted and the masses finally calculated by centering. The standard deviation reported for all the calculated masses represents the precision of the mass calculation from m/z values reported from the ESI mass spectrum. Data were processed using MassLynx software v. 4.0.

11. References

[1] L. Field, P. R. Engelhardt, *J. Org. Chem.* 1970, *35*, 3647-3655.
[2] S. Otto, R. L. Furlan, J. K. Sanders, *Science* 2002, *297*, 590-593.
[3] M. S. Newman, H. A. Karnes, *J. Org. Chem.* 1966, *31*, 3980-3984.
[4] D. Xu, Z. Lib, S. Ma, *Tetrahedron: Asymmetry* 2003, *14*, 3657–3666.
[5] H. Nomura, H. Akimoto, T. Miwa, *EP0340905* 1989.
[6] W. Chu, Z. Tu, E. McElveen, J. Xu, M. Taylor, R. R. Luedtke, R. H. Mach, *Bioorg. Med. Chem.* 2005, *13*, 77-87.
[7] A. Carfi, E. Duee, M. Galleni, J. M. Frere, O. Dideberg, *Acta Crystallogr. D. Biol. Crystallogr.* 1998, *54*, 313-323.
[8] H. J. Kurth, U. Kraatz, F. Korte, *Chem. Ber.* 1973, *106*, 2419-2426.
[9] H. Miki, T. Nakahama, S. Yokoyama, S. Mashiko, *U.S. Pat. Appl. Publ.* 2002.
[10] B. W. Fausett, L. S. Liebeskind, *J. Org. Chem.* 2005, *70*, 4851-4853.
[11] M. D. Varney, C. L. Palmer, W. H. Romines, 3rd, T. Boritzki, S. A. Margosiak, R. Almassy, C. A. Janson, C. Bartlett, E. J. Howland, R. Ferre, *J. Med. Chem.* 1997, *40*, 2502-2524.
[12] A. Gangjee, Y. Zeng, J. J. McGuire, F. Mehraein, R. L. Kisliuk, *J. Med. Chem.* 2004, *47*, 6893-6901.
[13] G. J. Bodwell, J. N. Bridson, S. L. Chen, R. A. Poirier, *J. Am. Chem. Soc.* 2001, *123*, 4704-4708.
[14] H. Okuno, K. Uoto, T. Tomohiro, M.-T. Youinou, *J. Chem. Soc., Dalton Trans.* 1990, 3375-3381.
[15] R. Paul-Soto, R. Bauer, J. M. Frere, M. Galleni, W. Meyer-Klaucke, H. Nolting, G. M. Rossolini, D. de Seny, M. Hernandez-Valladares, M. Zeppezauer, H. W. Adolph, *J. Biol. Chem.* 1999, *274*, 13242-13249.