

SUPPORTING INFORMATION

Structure-Activity Study of New Inhibitors of Human Betaine-Homocysteine S-Methyltransferase

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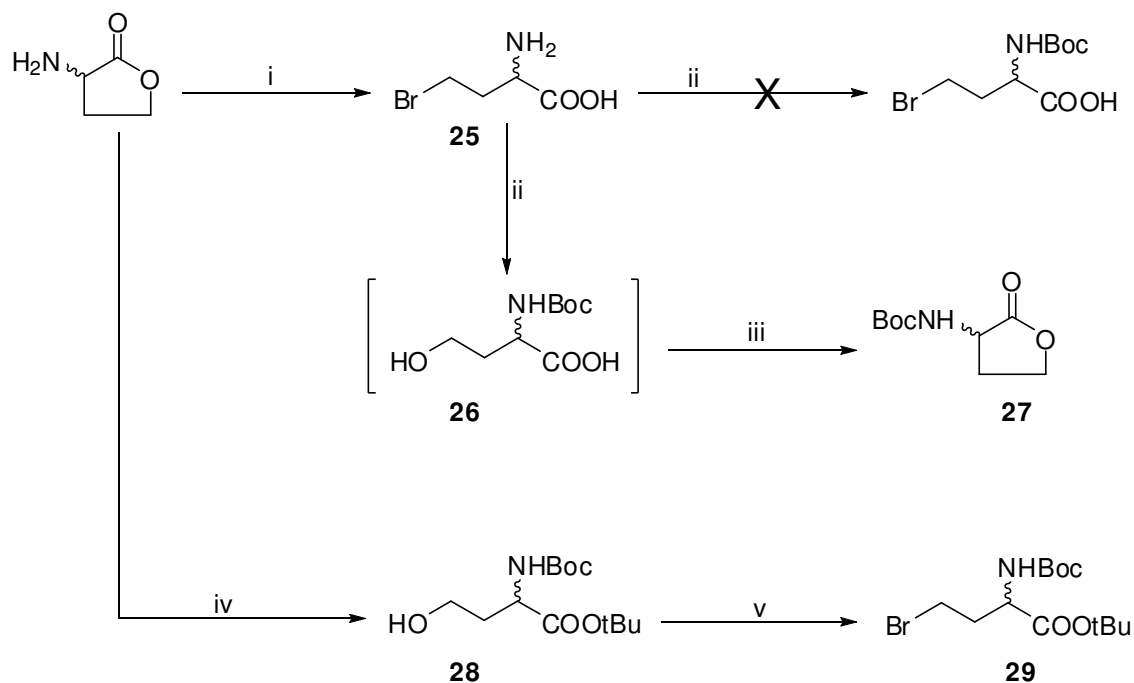
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1. Synthesis of compounds 27-29, 63 and 64.

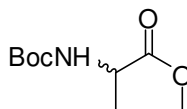
Scheme S1 describes the synthesis of intermediate **29**, which was proposed as a precursor for the synthesis of compounds **8** and **9**. Initially, we attempted a route that involved ring opening of DL- α -amino- γ -butyrolactone with hydrogen bromide, which gave bromide **25**. However, after reaction with Boc_2O using a standard procedure, only homoserine derivative **26** was obtained, which accounts for the rapid alkaline hydrolysis of the bromine from **25**. Later, we found that quantitative hydrolysis occurs even under mild conditions in 10% aqueous NaHCO_3 within less than 0.5 h. Moreover, the weak acidic conditions used during the partitioning of the reaction mixture between 10% aqueous citric acid and ethyl acetate led, in accordance with the literature,¹ to cyclization of **26**, affording lactonic compound **27**.



Scheme S1. Reagents and conditions: (i) HBr/AcOH, 100 °C, 1 d; (ii) 10% aq. NaHCO_3 , Boc_2O , dioxane, rt; (iii) 10% aq. citric acid/EtOAc; (iv) (a) aq. K_2CO_3 , rt, 1 h (b) aq. K_2CO_3 , Boc_2O , dioxane, rt; (v) CBr_4 , Ph_3P , CH_2Cl_2 , 0 °C.

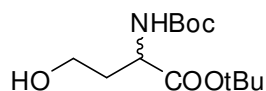
Thus, we developed an alternative route starting from DL- α -amino- γ -butyrolactone, which was, after the ring opening in alkaline solution, protected by a Boc group followed by esterification with *tert*-butyl bromide² under alkaline conditions. Final conversion of alcohol **28** to the desired bromide **29** was carried out according to the published procedure;³ this simple, almost one-pot reaction sequence appears to be a very efficient method for the preparation of bromide **29**.

(*RS*)-*N*-*tert*-Butoxycarbonyl- α -amino- γ -butyrolactone (27**)**



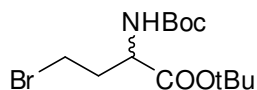
DL- α -Amino- γ -butyrolactone hydrobromide (5.0 g, 27.5 mmol) in 50 ml of a 33% soln. of HBr in glacial AcOH was heated in a sealed ampule for 30 h at 110 °C. After cooling of the reaction mixture, Et₂O (500 mL) was added, and the precipitate was filtered off, giving crude bromide **25** (6.54 g) as white crystals (a mixture of bromide **25** and unreacted lactone in a ratio of 83:17 as determined by NMR analysis). Boc₂O (6.0 g, ca. 1.1 eq.) in dioxane (150 mL) was added dropwise to a stirred solution of crude **25** (6.5 g) in 10% aqueous NaHCO₃ (300 mL) at 0 °C, and the reaction mixture was left to react at rt overnight. After the reaction was complete (TLC in H1, single spot), the dioxane was evaporated in vacuo, and the mixture was acidified to pH 3 with saturated citric acid solution, after which TLC analysis showed the formation of a new, less polar product and a decrease in the proportion of the former one. After extraction with EtOAc (6 x 100 mL), only the new, less polar product was obtained, which was later found to have the structure of **27** (4.64 g, 84% overall yield from the lactone). ¹H NMR (600 MHz, CDCl₃) 1.45 (s, 9H), 2.20 (m, 1H), 2.77 (m, 1H), 4.25 (m, 1H), 4.36 (m, 1H), 4.45 (m, 1H), 5.09 (b, 1H); ¹³C NMR (150.9 MHz, CDCl₃) 28.37 (3xC), 30.65, 50.16, 65.77, 80.60, 155.45, 175.32. HRMS (ESI) calcd for C₉H₁₆NO₄ [M+H]⁺ 202,1079; found: 202,1080.

(*RS*)-tert-Butyl 2-(tert-butoxycarbonylamino)-4-hydroxybutanoate (28)



DL- α -Amino- γ -butyrolactone hydrobromide (4.46 g, 24.5 mmol) was added to a stirred solution of K_2CO_3 (33.9 g, 245 mmol, 10 eq.) in H_2O (200 mL) at rt. Boc_2O (5.88 g, 27.0 mmol, 1.1 eq.) in dioxane (100 mL) was added dropwise to the solution, and the reaction mixture was left to react at rt overnight (TLC in H1). The solvents were evaporated in vacuo, and the residue was successively co-evaporated with EtOH (100 mL) and toluene (2x100 mL) to remove traces of water. Then, K_2CO_3 (54.2 g, 392 mmol, 16 eq.), TEBAC (5.58 g, 24.5 mmol, 1 eq.), tert-butyl bromide (132 mL, 48 eq.), and dimethylacetamide (185 mL) were added, and the slurry was intensively stirred at 55 °C for 24 h (TLC in H1). After cooling, the reaction mixture was partitioned between water (500 mL) and EtOAc (2x250 mL), the organic layer was separated, washed with water (2x100 mL), dried over Na_2SO_4 and concentrated in vacuo. The crude product was purified by flash chromatography on silica (elution with a linear gradient of EtOAc in toluene) to give compound **28** as a clear viscous oil (5.54 g, 82% overall yield). 1H NMR (600 MHz, $CDCl_3$) 1.45 (s, 9H), 1.47 (s, 9H), 1.53 (m, 1H), 2.14 (m, 1H), 3.64 (m, 1H), 3.38 (m, 1H), 3.70 (m, 1H), 5.33 (bd, 1H); ^{13}C NMR (150.9 MHz, $CDCl_3$) 27.99 (3xC), 28.27 (3xC), 36.63, 50.97, 58.34, 80.32, 82.29, 156.63, 172.00. HRMS (ESI) calcd for $C_{13}H_{26}NO_5$ $[M+H]^+$ 276,1811; found: 276,1800.

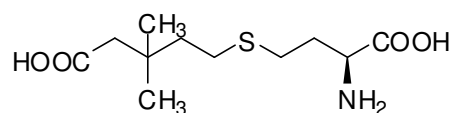
(*RS*)-tert-Butyl 4-bromo-2-(tert-butoxycarbonylamino)butanoate (29)



A stirred solution of alcohol **28** (1.93 g, 7.0 mmol) and CBr_4 (4.66 g, 14.1 mmol, 2 eq.) in dichloromethane (30 mL) was cooled to 0 °C. Triphenylphosphine (3.69 g, 14.1 mmol, 2 eq.) in CH_2Cl_2 (30 mL) was added dropwise over 5 min (TLC in T2). The resulting solution was stirred at rt for 2 h before being concentrated in vacuo to leave a brown solid which was triturated with ether-hexane (2:1, 100 mL) with sonication. The precipitated triphenylphosphine oxide was removed by filtration, and the

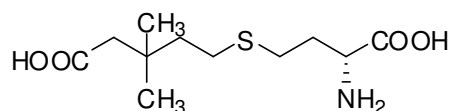
filtrate was evaporated in vacuo to leave a crude product, which was purified by flash chromatography on silica (elution with a linear gradient of EtOAc in toluene) to give compound **29** as a white crystalline solid (1.55 g, 65%). ¹H NMR (600 MHz, CDCl₃) 1.45 (s, 9H), 1.48 (s, 9H), 2.16 (m, 1H), 2.38 (m, 1H), 3.41 (m, 1H), 3.44 (m, 1H), 4.27 (m, 1H), 5.10 (bd, 1H); ¹³C NMR (150.9 MHz, CDCl₃) 27.98 (3xC), 28.29 (3xC), 28.37, 36.38, 53.18, 79.99, 82.52, 155.32, 170.78. HRMS (ESI) calcd for C₁₃H₂₅NO₄Br [M+H]⁺ 338,0967; found: 338,0958.

(S)-5-(3-Amino-3-carboxypropylthio)-3,3-dimethylpentanoic acid (63)



L-Methionine (0.500 g, 3.3 mmol) was dissolved in an intensively stirred liquid ammonia (about 20 mL) in a flask cooled to -33°C. Sodium (0.230 g, 10.0 mmol, 3 eq.) was slowly added in small pieces until the reaction mixture remained a consistent blue color. Ethyl 5-bromo-3,3-dimethylpentanoate **59** (0.825 mL, 3.68 mmol, 1.1 eq.) was then added, and the stirring was continued without cooling until the ammonia completely evaporated (1-2 h). The solid residue was dissolved in 30 mL of 1 M sodium hydroxide. After 2 h, the solution was passed through a Dowex 50W (H⁺) column, the resin was washed with water, and the compound was eluted with 2.5% ammonia. After evaporation, the crude product was dissolved in water and purified by the standard procedure to give **63** as a white solid (0.476 g, 54%). HRMS (ESI) calcd for C₁₁H₂₂NO₄S [M+H]⁺ 264,1270; found: 264,1268. The ¹H and ¹³C NMR spectra were identical to those recorded for the racemic **21**. α_D²⁰ +16.4 (c 0.310, 5 M HCl).

(R)-5-(3-Amino-3-carboxypropylthio)-3,3-dimethylpentanoic acid (64)



Using the procedure outlined for **63**, compound **64** was prepared from D-methionine (0.500 g, 3.3 mmol), sodium (0.230 g, 10.0 mmol, 3 eq.), and ethyl 5-bromo-3,3-dimethylpentanoate **59** (0.825 mL, 3.68 mmol, 1.1 eq.) as a white solid (0.690 g, 78%). HRMS (ESI) calcd for C₁₁H₂₂NO₄S [M+H]⁺ 264,1270; found: 264,1275. ¹H and ¹³C NMR spectra were identical to those recorded for racemic **21**. α_D^{20} -17.3 (c 0.742, 5 M HCl).

In order to eliminate any doubts about partial or complete racemization⁴ of our compounds, the optical purity of compounds **63** and **64** was determined using sodium [(*S*)-1,2-diaminopropane-*N,N,N',N'*-tetraacetato]-samarate(III), that is known to resolve enantiomer signals of α -amino acids in high-field ¹H and ¹³C NMR spectra⁵. We have checked successfully the use of this samarium chiral complex on the racemic mixture **21**. After addition of samarium complex (pH of substrate water solution was adjusted to 10.5 and molar ratio substrate : complex was about 2 : 1) we could observe resolved signal of H α protons ($\Delta\delta_{H\alpha}$ 0.118 ppm) in ¹H NMR spectrum and also fine splitting of some carbon signals in ¹³C NMR spectrum ($\Delta\delta_{C\alpha}$ 0.083, $\Delta\delta_{C\beta}$ 0.012 and $\Delta\delta_{C\gamma}$ 0.020 ppm). On the contrary, both compounds **63** and **64** did not show any detectable signal of second enantiomer after addition of the samarium complex. This observation indicates a high optical purity of both compounds.

2. Spectral data of non-target compounds **23a**, **23b**, **24**, **30-35**, **38-55** and **58-61**.

Diethyl 2-(3-chloropropyl)-2-acetamidomalonate (23a) and Diethyl 2-(3-bromopropyl)-2-acetamidomalonate (23b). NMR: Mixture of **23a** and **23b** (59 : 41); some ¹H and most of the ¹³C signals resolved. **23a**: ¹H NMR (600 MHz, CDCl₃) 1.27 (t, 6H), 1.62 (m, 2H), 2.05 (s, 3H), 2.48 (m, 2H), 3.51 (t, 2H), 4.26 (q, 4H), 6.79 (s, 1H); ¹³C NMR (150.9 MHz, CDCl₃) 13.95 (2xC), 23.02, 27.28, 31.13, 44.11, 62.70 (2xC), 65.99, 167.84 (2xC), 169.16. HRMS (ESI) calcd for C₁₂H₂₁NO₅Cl [M+H]⁺ 294,1108; found: 294,1114. **23b**: ¹H NMR (600 MHz, CDCl₃) 1.27 (t, 6H), 1.71 (m, 2H), 2.05 (s, 3H), 2.48 (m, 2H), 3.36 (t, 2H), 4.26 (q, 4H), 6.79 (s, 1H); ¹³C NMR (150.9 MHz, CDCl₃) 13.95 (2xC),

23.02, 27.48, 29.91, 33.38, 62.71 (2xC), 65.93, 167.81 (2xC), 169.16. HRMS (ESI) calcd for $C_{12}H_{21}BrNO_5$ $[M+H]^+$ 338,0603; found: 338,0593.

Diethyl 2-[3-(3-ethoxycarbonylpropylthio)propyl]-2-acetamidomalonate (24). 1H NMR (600 MHz, $CDCl_3$) 1.26 (t, 9H), 1.41 (m, 2H), 1.88 (m, 2H), 2.04 (s, 3H), 2.41 (t, 2H), 2.42 (m, 2H), 2.49 (m, 2H), 2.52 (t, 2H), 4.13 (q, 2H), 4.25 (q, 4H), 6.79 (bs, 1H); ^{13}C NMR (150.9 MHz, $CDCl_3$) 13.98 (2xC), 14.21, 23.05, 24.11, 24.64, 31.37, 31.44, 31.58, 32.98, 60.39, 62.60 (2xC), 66.23, 167.98 (2xC), 169.06, 173.08. HRMS (ESI) calcd for $C_{18}H_{32}NO_7S$ $[M+H]^+$ 406,1900; found: 406,1894.

Diethyl 2-[2-(2-chloroethoxy)ethyl]-2-acetamidomalonate (30). 1H NMR (600 MHz, $CDCl_3$) 1.26 (t, 6H), 2.06 (s, 3H), 2.67 (m, 2H), 3.51 (m, 2H), 3.54 (m, 2H), 3.59 (m, 2H), 4.21 (dq, 2H), 4.26 (dq, 2H), 6.95 (bs, 1H); ^{13}C NMR (150.9 MHz, $CDCl_3$) 13.89 (2xC), 23.02, 32.27, 42.66, 62.47 (2xC), 64.61, 66.43, 70.99, 168.13 (2xC), 169.31. HRMS (ESI) calcd for $C_{13}H_{22}O_6NCINa$ $[M+Na]^+$ 346,1028; found: 346,1031.

Diethyl 2-[2-(2-iodoethoxy)ethyl]-2-acetamidomalonate (31). 1H NMR (600 MHz, $CDCl_3$) 1.26 (t, 6H), 2.07 (s, 3H), 2.66 (m, 2H), 3.16 (t, 2H), 3.49 (m, 2H), 3.60 (t, 2H), 4.21 (dq, 2H), 4.26 (dq, 2H), 6.94 (bs, 1H); ^{13}C NMR (150.9 MHz, $CDCl_3$) 2.41, 13.92 (2xC), 23.08, 32.26, 62.51 (2xC), 64.59, 65.96, 71.62, 168.13 (2xC), 169.30. HRMS (ESI) calcd for $C_{13}H_{23}O_6NI$ $[M+H]^+$ 416,0565; found: 416,0562.

Diethyl 2-{2-[4-(methoxycarbonyl)butoxy]ethyl}-2-acetamidomalonate (32). 1H NMR (600 MHz, $CDCl_3$) 1.25 (t, 6H), 1.51 (m, 2H), 1.64 (m, 2H), 2.06 (s, 3H), 2.31 (t, 2H), 2.63 (m, 2H), 3.30 (t, 2H), 3.40 (m, 2H), 3.67 (s, 3H), 4.18 (dq, 2H), 4.24 (dq, 2H), 6.96 (bs, 1H); ^{13}C NMR (150.9 MHz, $CDCl_3$) 13.90 (2xC), 21.48, 23.00, 28.90, 32.38, 33.66, 51.51, 62.36 (2xC), 65.84, 70.51, 168.22 (2xC), 169.25, 173.94. HRMS (ESI) calcd for $C_{17}H_{30}O_8N$ $[M+H]^+$ 376,1966; found: 376,1966.

(*RS,RS*)-Di-*tert*-butyl 4,4'-diselanediybis[2-(*tert*-butoxycarbonylamino)butanoate] (33). 1H NMR (600 MHz, $CDCl_3$) 1.45 (s, 18H), 1.48 (s, 18H), 2.05 (m, 2H), 2.25 (m, 2H), 2.88 (m, 2H), 2.92 (m, 2H), 4.27 (bq, 2H), 5.12 (bd, 2H); ^{13}C NMR (150.9 MHz, $CDCl_3$) 24.95 (2xC), 28.01 (6xC), 28.31 (6xC),

34.75 (2xC), 53.90 (2xC), 79.80 (2xC), 82.23 (2xC), 155.33 (2xC), 171.17 (2xC). HRMS (ESI) calcd for C₂₆H₄₈O₈N₂NaSe₂ [M+Na]⁺ 699,1639; found: 699,1642.

(RS)-Ethyl 5-[3-*tert*-butoxycarbonyl-3-(*tert*-butoxycarbonylamino)propylselanyl]pentanoate (34). ¹H NMR (600 MHz, CDCl₃) 1.26 (t, 3H), 1.44 (bs, 9H), 1.47 (s, 9H), 1.69 (m, 2H), 1.72 (m, 2H), 1.95 (m, 1H), 2.14 (m, 1H), 2.32 (t, 2H), 2.54 (m, 2H), 2.57 (m, 2H), 4.13 (q, 2H), 4.25 (m, 1H), 5.10 (bd, 1H); ¹³C NMR (150.9 MHz, CDCl₃) 14.23, 18.79, 23.47, 25.10, 27.99 (3xC), 28.29 (3xC), 29.82, 33.72, 34.19, 54.25, 60.30, 79.75, 82.12, 155.32, 171.28, 173.36. HRMS (ESI) calcd for C₂₀H₃₇O₆NNaSe [M+Na]⁺ 490,1684; found: 490,1679.

Diethyl 2-[(7-ethoxycarbonyl)heptyl]-2-acetamidomalonate (35). ¹H NMR (600 MHz, CDCl₃) 1.09 (m, 2H), 1.25 (t, 9H), 1.28 (m, 6H), 1.59 (m, 2H), 2.04 (s, 3H), 2.27 (t, 2H), 2.30 (m, 2H), 4.12 (q, 2H), 4.24 (q, 4H), 6.77 (s, 1H); ¹³C NMR (150.9 MHz, CDCl₃) 13.98 (2xC), 14.23, 23.07, 23.54, 24.84, 28.94, 29.02, 29.04, 32.02, 34.28, 60.17, 62.45 (2xC), 66.52, 168.23 (2xC), 168.90, 173.82. HRMS (ESI) calcd for C₁₉H₃₄NO₇ [M+H]⁺ 388,2335; found: 388,2345.

(RS,RS)-Di-*tert*-butyl 4,4'-(methylenedithio)-2,2'-di(*tert*-butoxycarbonylamino)dibutanoate (38). NMR: ¹H NMR (500 MHz, CDCl₃) 1.45 (bs, 18H), 1.48 (s, 18H), 1.90 (m, 2H), 2.08 (m, 2H), 2.65 (m, 4H), 3.66 (s, 2H), 4.28 (bq, 2H), 5.13 (bd, 2H); ¹³C NMR (125.7 MHz, CDCl₃) 26.20 (2xC), 28.00 (6xC), 28.32 (6xC), 32.50 (2xC), 35.12, 53.32 (2xC), 79.76 (2xC), 82.21 (2xC), 155.00 (2xC), 171.25 (2xC). HRMS (ESI) calcd for C₂₇H₅₁N₂O₈S₂ [M+H]⁺ 595,3087; found: 595,3068.

(RS,RS)-4,4'-(Methylenedithio)-2,2'(diamino)dibutanoic acid (39). ¹H NMR (500 MHz, D₂O) 2.20 (m, 2H), 2.27 (m, 2H), 2.82 (m, 4H), 3.84 (s, 2H), 4.08 (t, 2H); ¹³C NMR (125.7 MHz, D₂O) 25.50 (2xC), 29.24 (2xC), 33.34, 52.33 (2xC), 172.40 (2xC). HRMS (ESI) calcd for C₉H₁₉N₂O₄S₂ [M+H]⁺ 283,0786; found: 283,0791.

(RS)-1-[3-(*tert*-Butoxycarbonylamino)-3-(*tert*-butoxycarbonyl)propylthio]-*N*-(*tert*-butoxycarbonylmethyl)-*N,N*-dimethylmethan ammonium chloride (40). NMR: ¹H NMR (500 MHz, CDCl₃) 1.44 (s, 9H), 1.48 (s, 9H), 1.50 (s, 9H), 2.02 (m, 1H), 2.18 (m, 1H), 2.98 (m, 1H), 3.13 (m, 1H), 3.56 (s, 3H), 3.58 (s, 3H), 4.25 (m, 1H), 4.63 (d, 1H), 4.67 (d, 1H), 5.33 (bd, 1H), 5.39 (bd, 1H), 5.52

(bd, 1H); ^{13}C NMR (125.7 MHz, CDCl_3) 27.98 (6xC), 28.32 (3xC), 31.52, 33.52, 49.66 (2xC), 52.80, 60.17, 69.60, 80.00, 82.50, 85.82, 155.00, 163.60, 170.92. HRMS (ESI) calcd for $\text{C}_{22}\text{H}_{43}\text{N}_2\text{O}_6\text{S}$ $[\text{M}]^+$ 463,2842; found: 463,2860.

(RS)-1-[[3-(tert-Butoxycarbonyl)-3-(tert-butoxycarbonylamino)propylthio]methyl]-2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepin-1-ium chloride (41). ^1H NMR (500 MHz, CDCl_3) 1.44 (s, 9H), 1.48 (s, 9H), 1.83 (m, 4H), 1.91 (m, 3H), 2.07 (m, 1H), 2.19 (m, 2H), 2.73 (m, 1H), 2.76 (m, 1H), 3.01 (m, 2H), 3.72 (m, 2H), 3.75 (m, 2H), 3.83 (m, 2H), 4.30 (bq, 1H), 4.77 (s, 2H), 5.23 (bd, 1H); ^{13}C NMR (125.7 MHz, CDCl_3) 20.20, 22.86, 25.78, 27.98 (3xC), 28.17, 28.30 (3xC), 28.60, 29.47, 33.27, 47.29, 49.72, 52.87, 56.00, 56.02, 79.90, 82.58, 155.20, 167.61, 170.91. HRMS (ESI) calcd for $\text{C}_{23}\text{H}_{42}\text{N}_3\text{O}_4\text{S}$ $[\text{M}]^+$ 456,2891; found: 456,2890.

(RS)-2-Amino-4-[(2,3,4,6,7,8,9,10-octahydropyrimido[1,2-a]azepin-1-ium-1-yl)methylthio]butanoate (42). ^1H NMR (600 MHz, D_2O) 1.71 (m, 2H), 1.74 (m, 2H), 1.77 (m, 2H), 2.09 (m, 2H), 2.20 (m, 1H), 2.29 (m, 1H), 2.85 (m, 2H), 2.91 (m, 2H), 3.55 (m, 2H), 3.59 (m, 2H), 3.67 (m, 2H), 4.11 (t, 1H), 4.76 (s; 2H); ^{13}C NMR (150.9 MHz, D_2O) 22.21, 25.23, 27.89, 28.90, 30.67, 30.80, 32.72, 49.60, 51.86, 54.78, 57.29, 57.83, 170.00, 174.88. HRMS (ESI) calcd for $\text{C}_{14}\text{H}_{26}\text{N}_3\text{O}_2\text{S}$ $[\text{M}]^+$ 300,1740; found: 300,1745.

(RS)-tert-Butyl 2-(tert-butoxycarbonylamino)-4-[[tert-butoxycarbonylmethyl(methyl)amino]methylthio]butanoate (43). ^1H NMR (500 MHz, CDCl_3) 1.44 (s, 9H), 1.47 (s, 18H), 1.88 (m, 1H), 2.08 (m, 1H), 2.43 (s, 3H), 2.61 (m, 2H), 3.26 (s, 2H), 4.02 (d, 1H), 4.05 (d, 1H), 4.25 (m, 1H), 5.17 (bd, 1H); ^{13}C NMR (125.7 MHz, CDCl_3) 27.99 (3xC), 28.12 (3xC), 28.31 (3xC), 28.49, 33.64, 40.96, 53.44, 56.49, 62.06, 79.70, 81.17, 82.02, 155.00, 169.84, 171.32. HRMS (ESI) calcd for $\text{C}_{21}\text{H}_{41}\text{N}_2\text{O}_6\text{S}$ $[\text{M}+\text{H}]^+$ 449,2685; found: 449,2704.

Di-tert-butyl (RS,RS)-4,4'-(tert-butoxycarbonylmethylamino)bis(methylene)bis(sulfanediyl)bis[(2-tert-butoxycarbonylamino)butanoate] (44). ^1H NMR (500 MHz, CDCl_3) 1.44 (s, 27H), 1.47 (s, 18H), 1.88 (m, 2H), 2.06 (m, 2H), 2.38 (t, 2H), 2.57 (m, 4H), 2.94 (t, 2H), 4.00 (d, 2H), 4.03 (d, 2H), 4.25 (m, 2H),

5.18 (bd, 2H); ^{13}C NMR (125.7 MHz, CDCl_3) 27.69 (2xC), 28.01 (6xC), 28.12 (3xC), 28.33 (6xC), 33.49 (2xC), 34.32, 47.96, 53.42 (2xC), 56.55 (2xC), 82.02 (2xC), 81.99, 80.70, 80.59, 170.30 (5xC). HRMS (ESI) calcd for $\text{C}_{35}\text{H}_{67}\text{N}_3\text{O}_{10}\text{S}_2$ $[\text{M}+\text{H}]^+$ 752,4190; found: 752,4171.

(*RS*)-*tert*-Butyl 2-(*tert*-butoxycarbonylamino)-4-(2-chloroethylthio)butanoate (45). ^1H NMR (500 MHz, CDCl_3) 1.45 (bs, 9H), 1.48 (s, 9H), 1.88 (m, 1H), 2.09 (m, 1H), 2.60 (m, 2H), 2.86 (m, 2H), 3.63 (m, 2H), 4.28 (bq, 1H), 5.10 (bd, 1H); ^{13}C NMR (125.7 MHz, CDCl_3) 28.00 (3xC), 28.18, 28.30 (3xC), 33.32, 34.24, 42.95, 53.21, 82.36 (2xC), ~156.0, 171.20. HRMS (ESI) calcd for $\text{C}_{15}\text{H}_{29}\text{ClNO}_4\text{S}$ $[\text{M}+\text{H}]^+$ 354,1506; found: 354,1516.

(*RS*)-*tert*-Butyl 2-(*tert*-butoxycarbonylamino)-4-{2-[(*tert*-butoxycarbonylmethyl)(methyl)amino]ethylthio}butanoate (46). ^1H NMR (500 MHz, CDCl_3) 1.44 (s, 9H), 1.47 (s, 18H), 1.88 (m, 1H), 2.08 (m, 1H), 2.41 (s, 3H), 2.57 (m, 2H), 2.64 (m, 2H), 2.76 (m, 2H), 3.21 (s, 2H), 4.26 (m, 1H), 5.14 (bd, 1H); ^{13}C NMR (125.7 MHz, CDCl_3) 28.00 (3xC), 28.06, 28.17 (3xC), 28.32 (3xC), 29.91, 33.21, 41.92, 53.48, 56.42, 59.00, 79.90, 81.15, 82.10, 155.50, 170.06 (2xC). HRMS (ESI) calcd for $\text{C}_{22}\text{H}_{42}\text{N}_2\text{O}_6\text{S}$ $[\text{M}+\text{H}]^+$ 463,2842; found: 463,2809.

(*RS*)-2-[3-(*tert*-Butoxycarbonylamino)-3-(*tert*-butoxycarbonyl)propylthio]-*N*-(*tert*-butoxycarbonylmethyl)-*N,N*-dimethylethanammonium iodide (47). ^1H NMR (500 MHz, CDCl_3) 1.44 (s, 9H), 1.48 (s, 9H), 1.52 (s, 9H), 1.93 (m, 1H), 2.04 (m, 1H), 2.75 (m, 2H), 3.01 (m, 2H), 3.64 (s, 6H), 4.10 (t, 2H), 4.33 (bq, 1H), 4.63 (s, 2H), 5.24 (bd, 1H); ^{13}C NMR (125.7 MHz, CDCl_3) 24.73, 28.03 (6xC), 28.37 (3xC), 28.99, 32.67, 52.33 (2xC), 52.49, 61.89, 64.24, 85.98 (2xC), 163.18, 170.05 (2xC). HRMS (ESI) calcd for $\text{C}_{23}\text{H}_{45}\text{N}_2\text{O}_6\text{S}$ $[\text{M}]^+$ 477,2998; found: 477,3006.

***tert*-Butyl 2-(*RS*)-(*tert*-butoxycarbonylamino)-4-[2-(*S*)-(1-*tert*-butoxycarbonylethylamino)ethylthio]butanoate (48).** ^1H NMR (500 MHz, CDCl_3) 1.27 (d, 3H), 1.44 (s, 9H), 1.47 (s, 18H), 1.88 (m, 1H), 2.08 (m, 1H), 2.51 (m, 1H), 2.61 (m, 1H), 2.63 (m, 1H), 2.69 (m, 2H), 2.79 (m, 1H), 3.23 (m, 1H), 4.25 (bq, 1H), 5.13 (bd, 1H); ^{13}C NMR (125.7 MHz, CDCl_3) 19.08, 27.60 + 27.68, 28.00 (3xC), 28.08 (3xC), 28.32 (3xC), 32.51, 33.11 + 33.14, 46.59 + 46.66, 53.47,

57.12, 80.98, 82.11 (2xC), 152.58, 174.83 (2xC). HRMS (ESI) calcd for C₂₂H₄₃N₂O₆S [M+H]⁺ 463,2842; found: 463,2835.

***tert*-Butyl** **2-(*RS*)-(tert-butoxycarbonylamino)-4-[2-(*R*)-(1-*tert*-butoxycarbonylethylamino)ethylthio]butanoate (49).** ¹H NMR (500 MHz, CDCl₃) 1.27 (d, 3H), 1.44 (s, 9H), 1.47 (s, 18H), 1.87 (m, 1H), 2.08 (m, 1H), 2.56 (m, 2H), 2.63 (m, 1H), 2.68 (m, 1H), 2.70 (m, 1H), 2.80 (m, 1H), 3.23 (q, 1H), 4.25 (bq, 1H), 5.12 (bd, 1H); ¹³C NMR (125.7 MHz, CDCl₃) 19.09, 27.57 + 27.65, 27.99 (3xC), 28.07 (3xC), 28.31 (3xC), 32.49, 33.10 + 33.13, 46.56 + 46.63, 53.45, 57.11, 79.75, 80.97, 82.10 + 82.12, 168.27, 171.20, 174.84. HRMS (ESI) calcd for C₂₂H₄₃N₂O₆S [M+H]⁺ 463,2842; found: 463,2836.

(*RS*)-*tert*-Butyl **2-(tert-butoxycarbonylamino)-4-[2-(tert-butoxycarbonylmethylamino)ethylthio]butanoate (50).** ¹H NMR (500 MHz, CDCl₃) 1.44 (s, 9H), 1.47 (s, 18H), 1.88 (m, 1H), 2.07 (m, 1H), 2.56 (m, 2H), 2.67 (m, 2H), 2.80 (m, 2H), 3.32 (s, 2H), 4.26 (m, 1H), 5.14 (bd, 1H); ¹³C NMR (125.7 MHz, CDCl₃) 27.66, 28.00 (3xC), 28.11 (3xC), 28.31 (3xC), 32.39, 33.14, 48.12, 51.38, 53.43, 81.23, 82.12 (2xC), 155.10, 171.30, 171.51. HRMS (ESI) calcd for C₂₁H₄₁N₂O₆S [M+H]⁺ 449,2685; found: 449,2693.

(*RS*)-*tert*-Butyl **2-(tert-butoxycarbonylamino)-4-[2-(2-*tert*-butoxycarbonylethylamino)ethylthio]butanoate (51)** NMR: ¹H NMR (500 MHz, CDCl₃) 1.44 (s, 9H), 1.45 (s, 9H), 1.47 (s, 9H), 1.88 (m, 1H), 2.08 (m, 1H), 2.44 (t, 2H), 2.55 (m, 2H), 2.67 (t, 2H), 2.81 (t, 2H), 2.85 (t, 2H), 4.26 (m, 1H), 5.15 (bd, 1H); ¹³C NMR (125.7 MHz, CDCl₃) 27.63, 27.98 (3xC), 28.10 (3xC), 28.29 (3xC), 32.27, 33.22, 35.90, 44.91, 48.39, 53.38, 79.76, 80.55, 82.14, 155.33, 171.30, 172.01. HRMS (ESI) calcd for C₂₂H₄₃N₂O₆S [M+H]⁺ 463,2842; found: 463,2825.

Di-*tert*-butyl **(*RS,RS*)-4,4'-[2,2'-(2-*tert*-butoxycarbonylethylaminodiyl)bis(ethane-2,1-diyl)bis(sulfanediyl)]bis[2-(*tert*-butoxycarbonylamino)butanoate] (52).** NMR: ¹H NMR (500 MHz, CDCl₃) 1.45 (s, 27H), 1.47 (s, 18H), 1.87 (m, 2H), 2.07 (m, 2H), 2.35 (t, 2H), 2.55 (m, 4H), 2.59 (m, 4H), 2.67 (m, 4H), 2.79 (t, 2H), 4.25 (m, 2H), 5.16 (bd, 2H); ¹³C NMR (125.7 MHz, CDCl₃) 28.01 (3xC), 28.12 (6xC), 28.23 (2xC), 28.32 (6xC), 30.10 (2xC), 33.26 (2xC), 33.87, 49.56, 53.46 (2xC),

53.90 (2xC), 79.70, 80.60 (2xC), 82.09 (2xC), 155.15 (2xC), 171.30 (2xC), 171.75. HRMS (ESI) calcd for C₃₇H₇₀N₃O₁₀S₂ [M+H]⁺ 780,4503; found: 780,4536.

(RS)-tert-Butyl 4-(3-bromopropylthio)-2-(tert-butoxycarbonylamino)butanoate (53). ¹H NMR (600 MHz, CDCl₃) 1.44 (bs, 9H), 1.47 (s, 9H), 1.88 (m, 1H), 2.08 (m, 1H), 2.11 (m, 2H), 2.53 (m, 1H), 2.57 (m, 1H), 2.67 (t, 2H), 3.52 (t, 2H), 4.28 (m, 1H), 5.11 (bd, 1H); ¹³C NMR (150.9 MHz, CDCl₃) 27.80, 27.99 (3xC), 28.30 (3xC), 30.14, 32.09, 32.11, 33.16, 53.31, 79.81, 82.22, 155.33, 171.29. HRMS (ESI) calcd for C₁₆H₃₁NO₄SBr [M+H]⁺ 412,1157; found: 412,1169.

(RS)-tert-Butyl 2-(tert-butoxycarbonylamino)-4-{3-[(tert-butoxycarbonylmethyl)(methyl)amino]propylthio}butanoate (54). ¹H NMR (600 MHz, CDCl₃) 1.44 (bs, 9H), 1.47 (s, 18H), 1.75 (m, 2H), 1.86 (m, 1H), 2.08 (m, 1H), 2.36 (s, 3H), 2.56 (m, 6H), 3.15 (s, 2H), 4.26 (m, 1H), 5.11 (bd, 1H); ¹³C NMR (150.9 MHz, CDCl₃) 27.44, 27.87, 27.98 (3xC), 28.15 (3xC), 28.30 (3xC), 29.79, 33.12, 42.11, 53.46, 55.68, 59.33, 79.73, 80.89, 82.09, 155.34, 170.22, 171.37. HRMS (ESI) calcd for C₂₃H₄₅N₂O₆S [M+H]⁺ 477,2998; found: 477,2988.

(RS)-3-[3-(tert-Butoxycarbonylamino)-3-(tert-butoxycarbonyl)propylthio]-N-(carboxymethyl)-N,N-dimethylpropan-1-ammonium iodide (55). ¹H NMR (600 MHz, CDCl₃) 1.44 (bs, 9H), 1.48 (s, 9H), 1.53 (s, 9H), 1.90 (m, 1H), 2.04 (m, 1H), 2.13 (m, 2H), 2.63 (m, 2H), 2.70 (m, 2H), 3.58 (s, 6H), 3.95 (m, 2H), 4.30 (bq, 1H), 4.48 (s, 2H), 5.15 (bd, 1H); ¹³C NMR (150.9 MHz, CDCl₃) 23.09, 28.04 (6xC), 28.36 (3xC), 28.53, 28.76, 33.06, 52.31 (2xC), 52.98, 62.14, 64.83, 79.80, 82.46, 86.19, 162.94, 171.20 (2xC). HRMS (ESI) calcd for C₂₄H₄₇N₂O₆S [M]⁺ 491,3155; found: 491,3147.

(RS)-Ethyl 5-bromo-3-methylpentanoate (58). ¹H NMR (600 MHz, CDCl₃) 0.98 (d, 3H), 1.27 (t, 3H), 1.76 (m, 1H), 1.93 (m, 1H), 2.18 (m, 2H), 2.31 (m, 1H), 3.40 (m, 1H), 3.46 (m, 1H), 4.14 (q, 2H); ¹³C NMR (150.9 MHz, CDCl₃) 14.25, 18.98, 29.15, 31.12, 39.29, 41.14, 60.33, 172.50. HRMS (ESI) calcd for C₈H₁₆BrO₂ [M+H]⁺ 223,0334; found: 223,0334.

Ethyl 5-bromo-3,3-dimethylpentanoate (59). ¹H NMR (600 MHz, CDCl₃) 1.04 (s, 6H), 1.27 (t, 3H), 1.97 (m, 2H), 2.21 (s, 2H), 3.42 (m, 2H), 4.13 (q, 2H); ¹³C NMR (150.9 MHz, CDCl₃) 14.28, 27.22

(2xC), 28.63, 34.48, 45.30, 45.69, 60.20, 171.58. HRMS (ESI) calcd for C₉H₁₈BrO₂ [M+H]⁺ 237,0490; found: 237,0483.

Ethyl (RS,RS)-5-[3-(tert-butoxycarbonylamino)-3-(tert-butoxycarbonyl)propylthio]-3-methylpentanoate (60). NMR: Mixture of diastereoisomers – some carbon signals are doubled. ¹H NMR (600 MHz, CDCl₃) 0.96 (d, 3H), 1.26 (t, 3H), 1.45 (s, 9H), 1.47 (bs, 9H), 1.49 (m, 1H), 1.62 (m, 1H), 1.87 (m, 1H), 2.07 (m, 1H), 2.08 (m, 1H), 2.14 (m, 1H), 2.30 (m, 1H), 2.50 (m, 1H), 2.54 (m, 2H), 2.56 (m, 1H), 4.13 (q, 2H), 4.26 (m, 1H), 5.12 (bd, 1H); ¹³C NMR (150.9 MHz, CDCl₃) 14.26, 19.38, 27.76 + 27.69, 27.99 (3xC), 28.30 (3xC), 29.49, 29.68, 33.10, 36.20 + 36.23, 41.44, 53.43, 60.24, 79.76, 82.12, 155.40, 171.36, 172.80. HRMS (ESI) calcd for C₂₁H₄₀NO₆S [M+H]⁺ 434,2576; found: 434,2582.

Ethyl (RS)-5-[3-(tert-butoxycarbonylamino)-3-(tert-butoxycarbonyl)propylthio]-3,3-dimethylpentanoate (61). ¹H NMR (600 MHz, CDCl₃) 1.04 (s, 6H), 1.26 (t, 3H), 1.44 (s, 9H), 1.47 (bs, 9H), 1.61 (m, 2H), 1.89 (m, 1H), 2.08 (m, 1H), 2.20 (s, 2H), 2.48 (m, 1H), 2.52 (m, 1H), 2.55 (t, 2H), 4.13 (q, 2H), 4.26 (m, 1H), 5.19 (bd, 1H); ¹³C NMR (150.9 MHz, CDCl₃) 14.30, 26.76, 27.25, 27.31, 27.65, 27.98 (3xC), 28.31 (3xC), 33.04, 33.52, 41.86, 45.60, 53.51, 60.10, 79.71, 82.04, 155.42, 171.43, 171.93. HRMS (ESI) calcd for C₂₂H₄₂NO₆S [M+H]⁺ 448,2733; found: 448,2720.

3. HPLC purity data for target compounds.

HPLC analyses for target compounds were performed using two different HPLC systems.

Analytical **RP-HPLC** was performed using a Watrex (Nucleosil 120, 5 μm , C18, 25 x 0.46 cm; Prague, Czech Republic) column. For gradient RP-HPLC analysis, a Waters LC 625 System (Milford, MA, USA) was used. Different gradients of acetonitrile (% v/v) in water containing 0.1% (v/v) of TFA at 1 mL/min were used for elution of compounds: a) 0 min - 4%; 5 min - 4%; 25 min - 21.6%; 35 min - 80%; 36 min - 4%; b) 0 min - 0%; 5 min - 0%; 10 min - 1.6%; 15 min - 1.6%; 35 min - 17.6%; 45 min - 80%; 46 min - 0%; c) 0 min - 1.6%; 5 min - 1.6%; 25 min - 14.4%; 35 min - 64%; 36 min - 1.6%. The purity data resulted from integration of peaks at 218 nm and are summarized in Table S1.

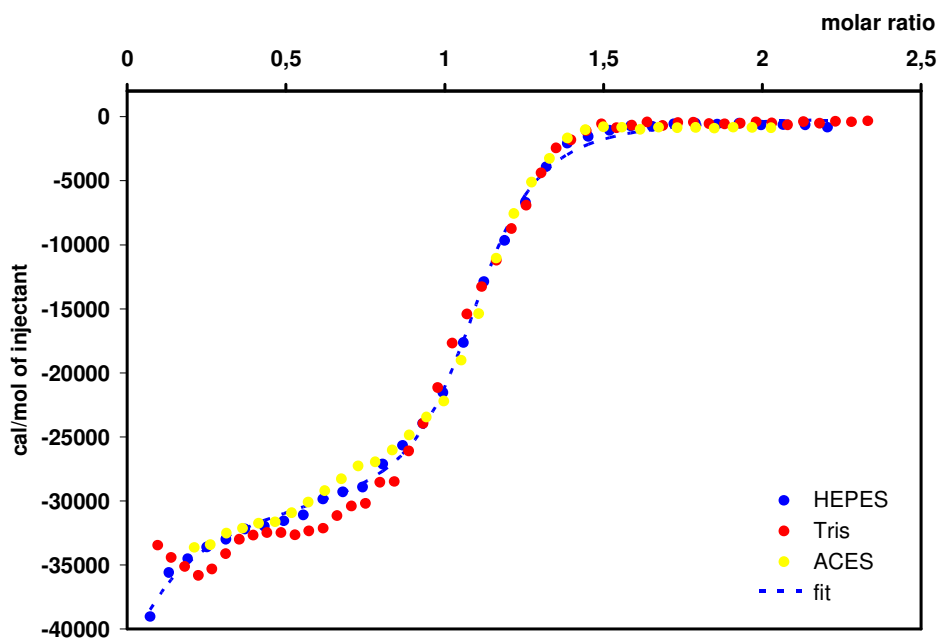
Analytical **ion-exchange chromatography** was performed at 0.25 mL/min using an AminoPac® PA10 (0.4 x 25 cm, Dionex Corporation, Sunnyvale, CA) column and BioLC system (GP50 gradient pump, ED50 electrochemical detector) from Dionex Corporation (Sunnyvale, CA). The following gradient of water (A), 0.25 M sodium hydroxide (B), 1 M sodium acetate (C) and 0.1 M acetic acid (D) was used for elution of compounds: 0 min - 76% A, 24% B; 2 min - 76% A, 24% B; 8 min - 64% A, 36% B; 11 min - 64% A, 36% B; 18 min - 40% A, 20% B, 40% C; 21 min - 44% A, 16% B, 40% C; 23 min - 14% A, 16% B, 70% C; 45 min - 4% A, 16% B, 70% C; 45.1 min - 100% D; 47.1 min - 100% D; 47.2 min - 20% A, 80% B; 49.2 min - 20% A, 80% B; 49.3 min - 76% A, 24% B; 74 min - 76% A, 24% B. The purity data resulted from integration of peaks after amperometric detection and are given in Table S1.

Table S1. HPLC purity data for target compounds. ^{a-c}Different gradients of acetonitrile in water.

Compound	RP-HPLC		Ion-exchange HPLC	
	E _t (min)	Purity (%)	E _t (min)	Purity (%)
5	25.85 ^a	98.2	32.48	95.6
6	20.86 ^a	99.9	44.49	95.3
7	35.59 ^b	97.6	37.86	94.7
8	20.51 ^c	99.8	32.08	98.5
9	29.07 ^c	99.5	42.51	97.7
10	39.56 ^b	99.3	40.63	99.1
11	4.02 ^b	98.1	7.93	98.2
12	5.01 ^b	98.6	7.82	93.5
13	4.37 ^b	99.1	32.94	95.6
14	4.70 ^b	97.3	31.94	96.5
15	4.64 ^b	98.2	31.88	98.1
16	3.80 ^b	98.7	32.54	98.9
17	4.47 ^b	98.9	31.93	93.0
18	7.46 ^b	98.8	7.57	97.4
19	6.08 ^b	99.3	33.74	98.8
20	39.98 ^b	99.2	40.65	95.8
21	44.27 ^b	98.4	45.02	97.1
63	44.11 ^b	98.6	44.83	97.5
64	44.39 ^b	98.1	44.96	98.4

4. Isothermal titration calorimetry (ITC).

Figure S1. Independent titrations of compound **21** to the solution of BHMT was performed in three buffers with different enthalpies of ionization (HEPES, $\Delta H_{\text{ion}} = 5.03 \text{ kcal.mol}^{-1}$; ACES, $\Delta H_{\text{ion}} = 7.51 \text{ kcal.mol}^{-1}$; Tris, $\Delta H_{\text{ion}} = 11.3 \text{ kcal.mol}^{-1}$). These experiments resulted in the same binding enthalpies as can be seen from the superposition of the individual titration curves. The titration performed in HEPES buffer was fitted using a model for two sets of sites in the Origin software (MicroCal Inc., Northampton, MA, USA).



5. Molecular modeling of inhibitor **21** into the active site of BHMT.

We modeled inhibitor **21** into the active site of BHMT on the basis of the available crystal structure⁶ of human BHMT with inhibitor **1** (PDB ID 1L8T, <http://www.rcsb.org/pdb/explore/explore.do?structureId=1L8T>) with the aim of analyzing possible interactions of inhibitor **21** with the enzyme. The modeling was also performed with the “reference” inhibitor **1**. The mutual distances and angles between Zn²⁺ and the interacting sulfur atoms of the cysteine residues of BHMT together with the sulfur atom of inhibitor **1** were fixed. Concerning the modeling of inhibitor **21**, both hydrogens in position 4 of inhibitor **1** were replaced by methyl groups. For ease of comparison, the complexes were modeled in a dimer of BHMT, where the first active site was occupied by inhibitor **1** and the second by inhibitor **21**. The dimer was surrounded by water molecules and energy minimization was carried out. Interaction energies were calculated for the given inhibitor and dimer of BHMT, whereas the second inhibitor and additional water molecules, which were present in the model during minimization, were excluded from the interaction energy calculation.

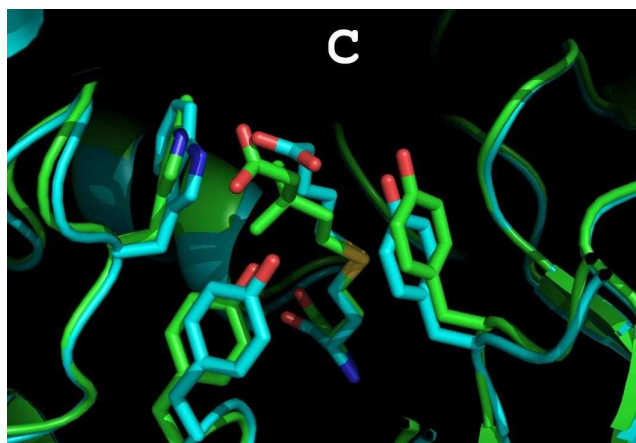
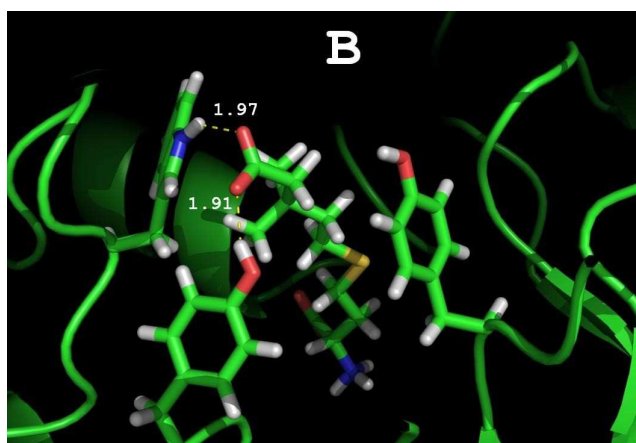
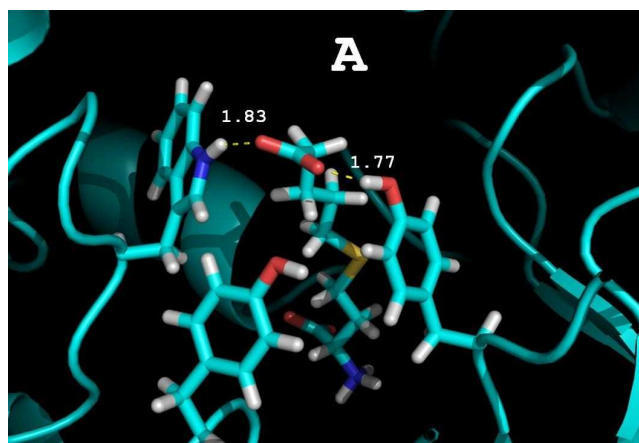
Modeling, minimization and interaction energy calculations were done in the YASARA program.⁷ Positioning of the inhibitors was done by energy minimization using the AMBER99 force-field.⁸ For interacting energy calculations, the YAMBER3 force-field was used. The force-field parameters for inhibitors were assigned using the AutoSMILES approach.⁹ In the first step, YASARA calculated semi-empirical AM1 Mulliken point charges that were corrected by assignment of AM1BCC atom types and improved AM1BCC charges by fragments of molecules with known RESP charges to more closely resemble the RESP charges. The corresponding bond, angle and torsion potential parameters were taken from the General AMBER force field.¹⁰

The final modeled structures are shown in Figure S2 (A-C). The computed structure of inhibitor **1** is the same as its crystal structure from Evans *et al.*⁶ However, the modeling of inhibitor **21** gave different results. The system of hydrogen bonds anchoring the carboxyl group of the “betaine part” of inhibitor **1** (Figure S2 A) is different from that of the complex of BHMT with inhibitor **21** (Figure S2

B). More specifically, the first carboxyl oxygen of inhibitor **21** interacts with the hydrogen of the hydroxyl group of Tyr77 instead of Tyr 160.

The distance between the second carboxyl oxygen and hydrogen on N^{ϵ} of Trp 44, which forms a hydrogen bond in the BHMT-inhibitor **1** complex, is 1.83 Å, but in the modeled complex of inhibitor **21**, it is 1.91 Å. Moreover, the mutual position of the hydrogen bond donor and acceptor is not ideal in the case of inhibitor **21**. Logically, the computed (predicted) interaction energy of the BHMT-inhibitor **21** complex is lower (-5.9 kcal.mol⁻¹) compared with that of the BHMT-inhibitor **1** complex (-8.5 kcal.mol⁻¹). Taking into account the stronger inhibition of BHMT by **21** compared to **1** (Table 1, main document), we conclude that this simple model of a complex of inhibitor **21** with BHMT is not correct, and that some substantial rearrangements in local BHMT structure occur to accommodate inhibitor **21**.

Figure S2. A. Computed interaction of inhibitor **1** with BHMT. **B.** Computed interaction of inhibitor **21** with BHMT. **C.** Superposition of computed structures of inhibitors **1** (blue) and **21** (green) in the active site of BHMT.



6. ^1H and ^{13}C NMR spectra measurements for pH titration of compounds **12**, **13** and **16**.

The dependence of ^1H and ^{13}C chemical shifts on the pH was measured for compounds **16**, **13** and **12**. For each compound, its solution in D_2O was divided into two parts - one part was used for the NMR measurements in the acidic region, and the second in the alkaline region. The pH was changed by the stepwise addition of a very small amount of DCl or NaOD solution. After each addition, the pH value was measured directly in the NMR tube and ^1H and ^{13}C NMR spectra were recorded with dioxane as an internal reference (using relations $d_{\text{H}}(\text{dioxane}) = 3.75$ and $d_{\text{C}}(\text{dioxane}) = 69.3$). The obtained pH dependencies of the chemical shifts are shown as NMR titration curves in Figures S3-S8. The largest changes in chemical shifts with pH were observed on the atoms of the ionizable group (carboxyl carbon atoms) and on the atoms in neighboring positions. The $\text{p}K$ values estimated from titration curves must be corrected using the known relation: $\text{pD} = \text{pH} (\text{meter reading}) + 0.40$ according to Glasoe *et al.*¹¹ All studied compounds (**16**, **13** and **12**) showed titration curves with two inflection points: the first around pH 2.5 corresponds to $\text{p}K_1$ for titration of both carboxyl groups ($\text{COOH} \rightarrow \text{COO}^-$), and the second around pH 9.5 corresponding to $\text{p}K_2$ for deprotonation of the amino groups ($-\text{NH}_3^+ \rightarrow -\text{NH}_2$, $>\text{NH}_2^+ \rightarrow >\text{NH}$ or $>\text{NH}(\text{CH}_3)^+ \rightarrow >\text{NCH}_3$). The estimated $\text{p}K_2 \sim 9.5$ for the secondary and tertiary amino groups in both **16** and **13** indicates that equilibrium between the protonated and deprotonated forms of their nitrogen atoms at pH 7.5, which was used in our enzymatic assay, is shifted strongly to the side of the protonated form. The dimethylamino group in compound **12** stays positively charged over the whole range of pH.

Figure S3. ^1H NMR titration of compound **16**.

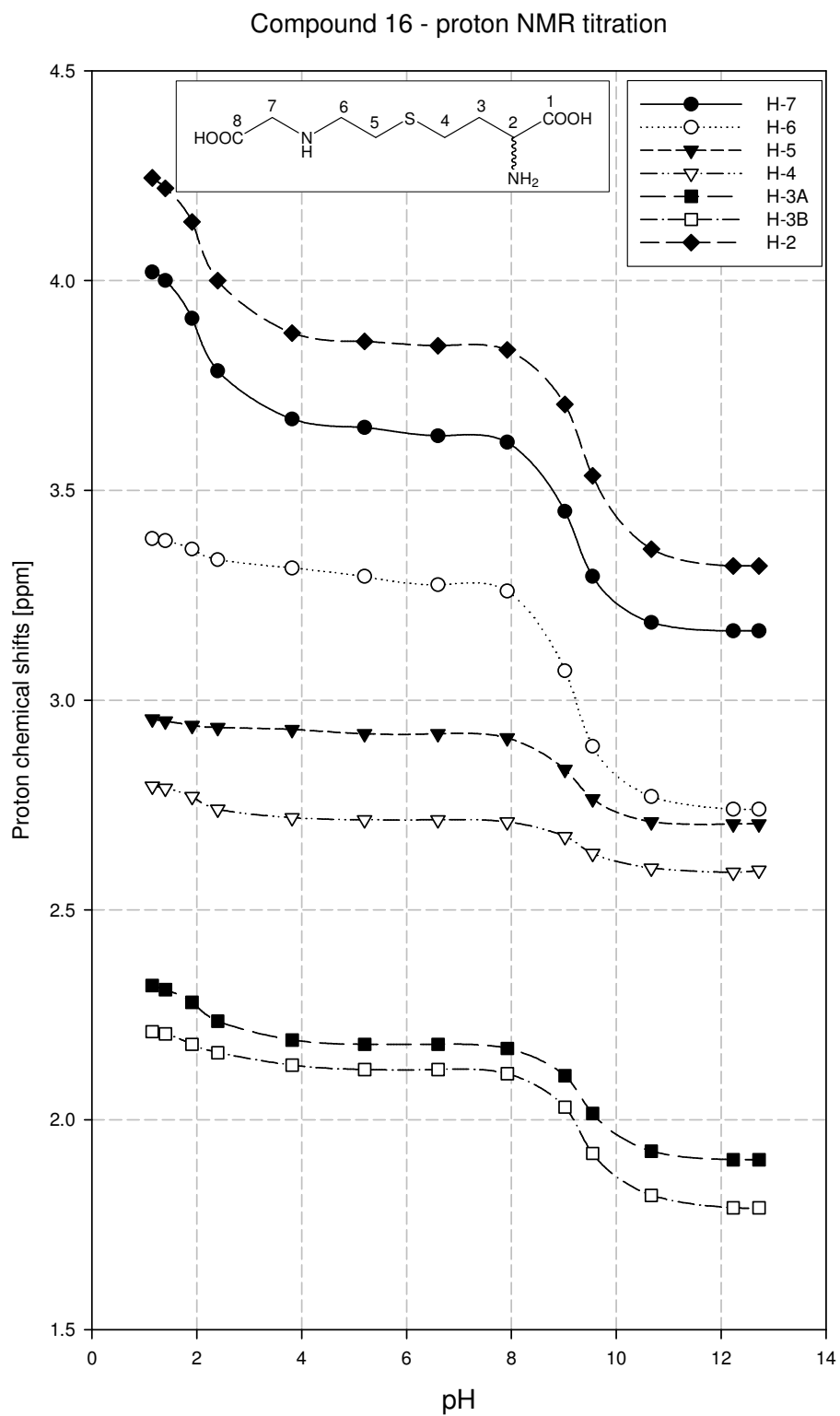


Figure S4. ^{13}C NMR titration of compound 16.

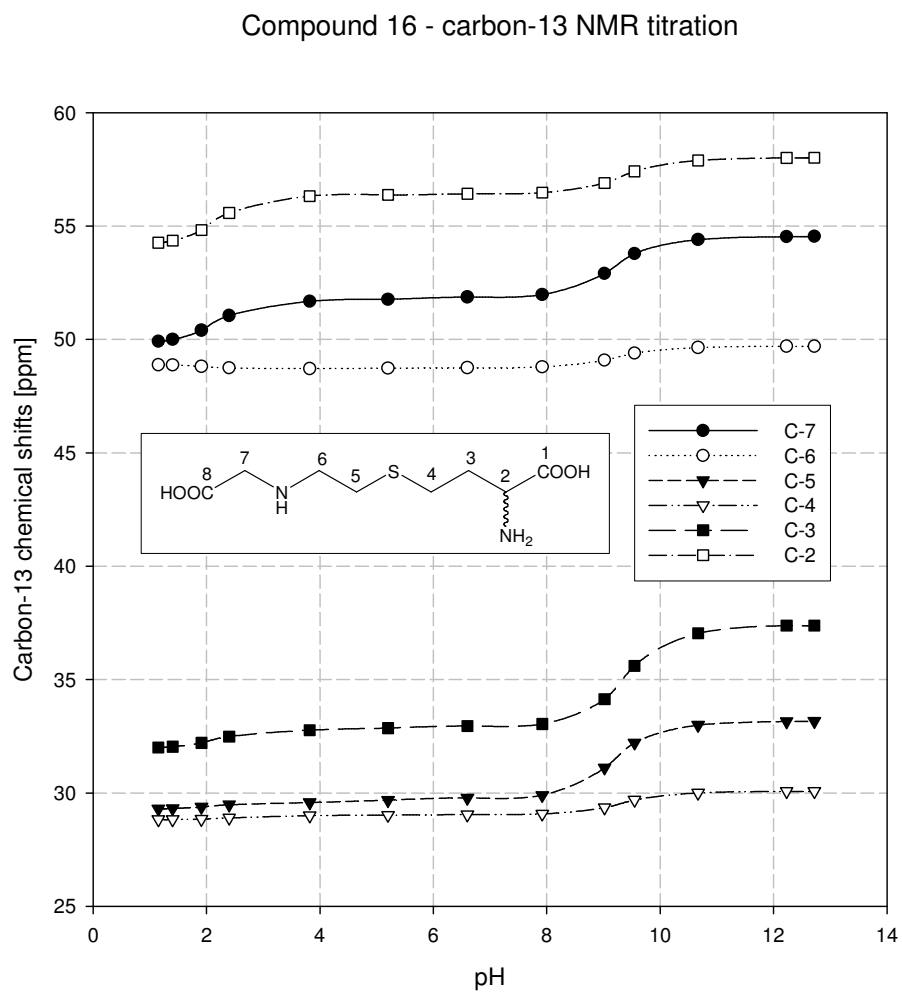


Figure S5. ¹H NMR titration of compound 13.

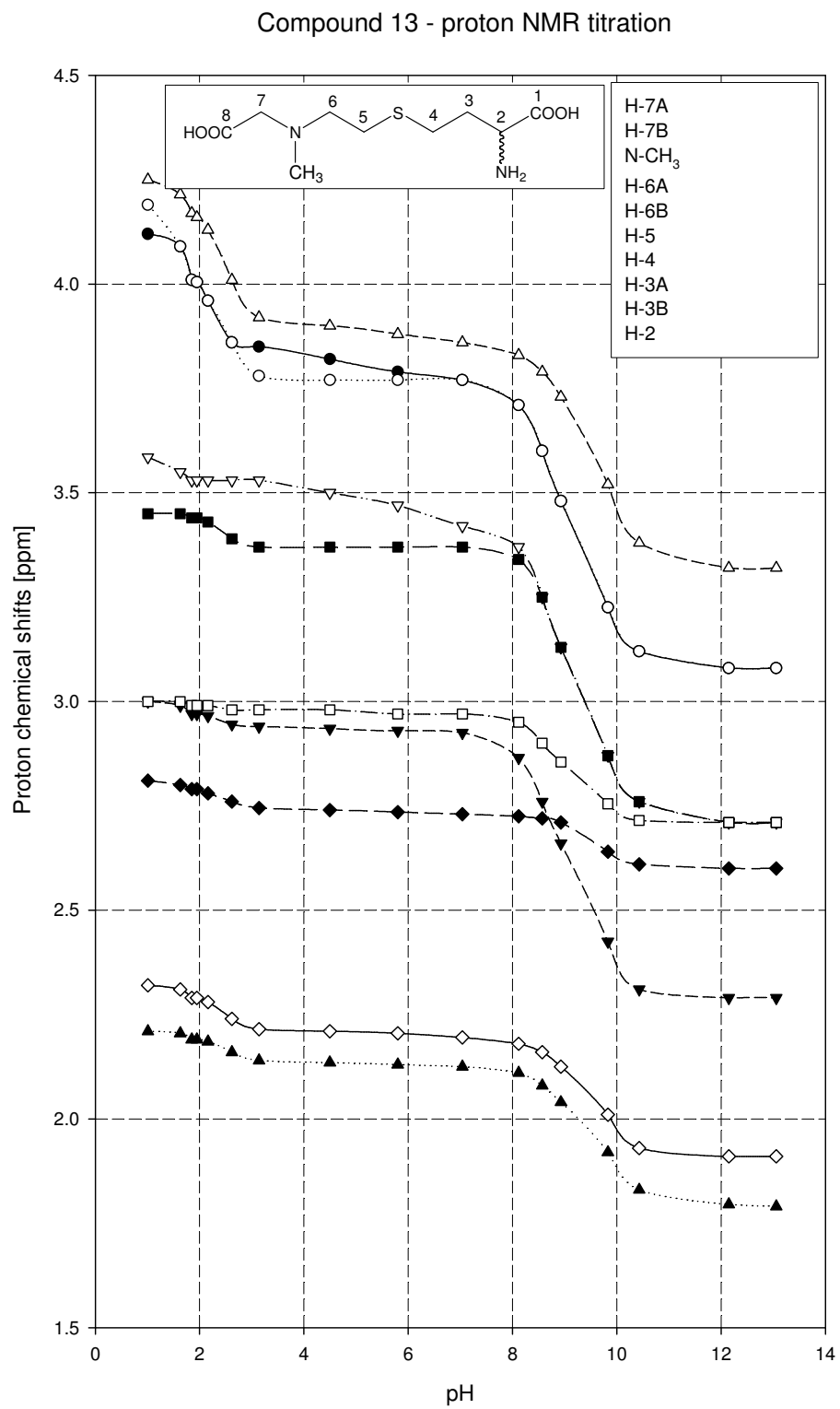


Figure S6. ^{13}C NMR titration of compound 13.

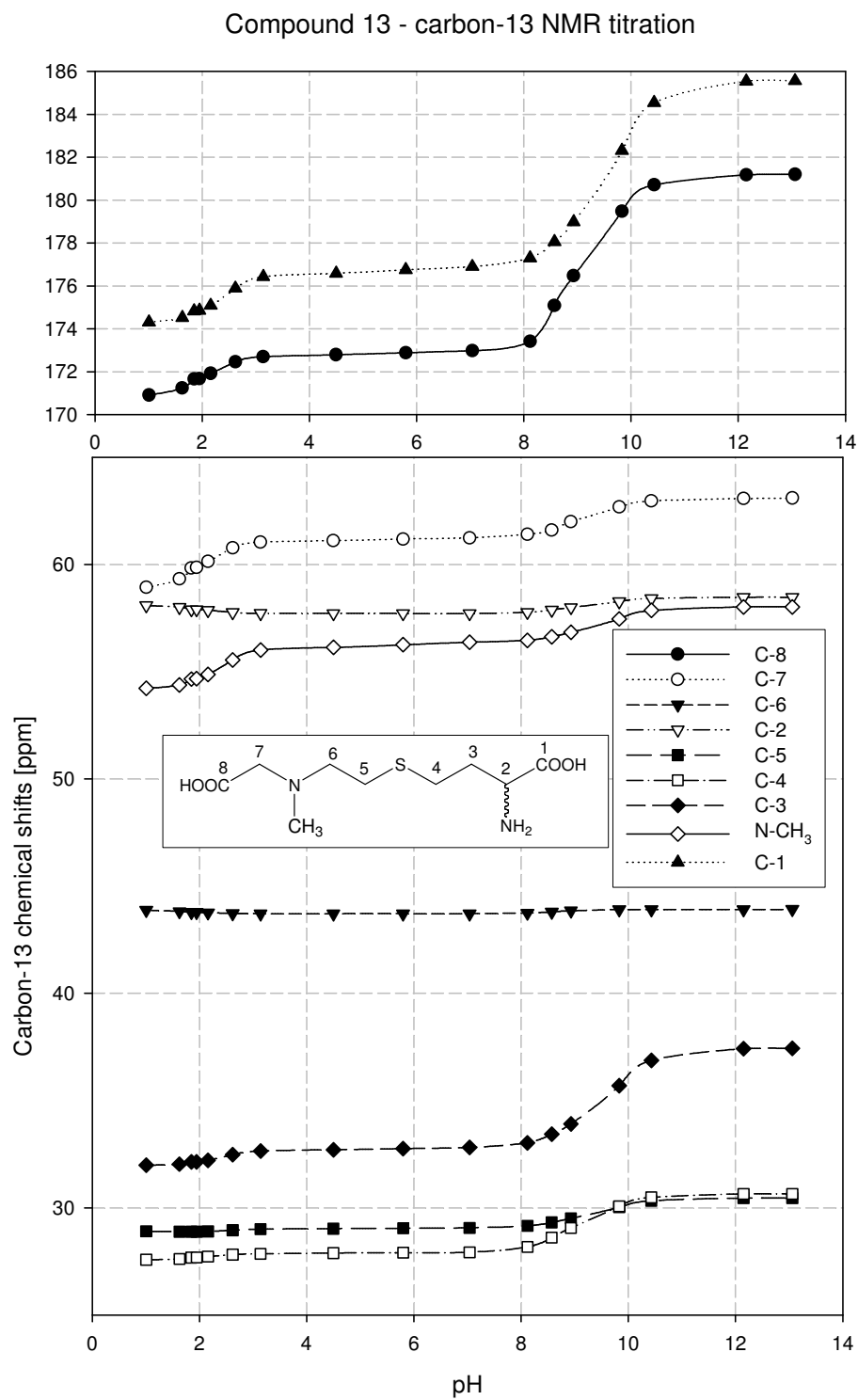


Figure S7. ^1H NMR titration of compound **12**.

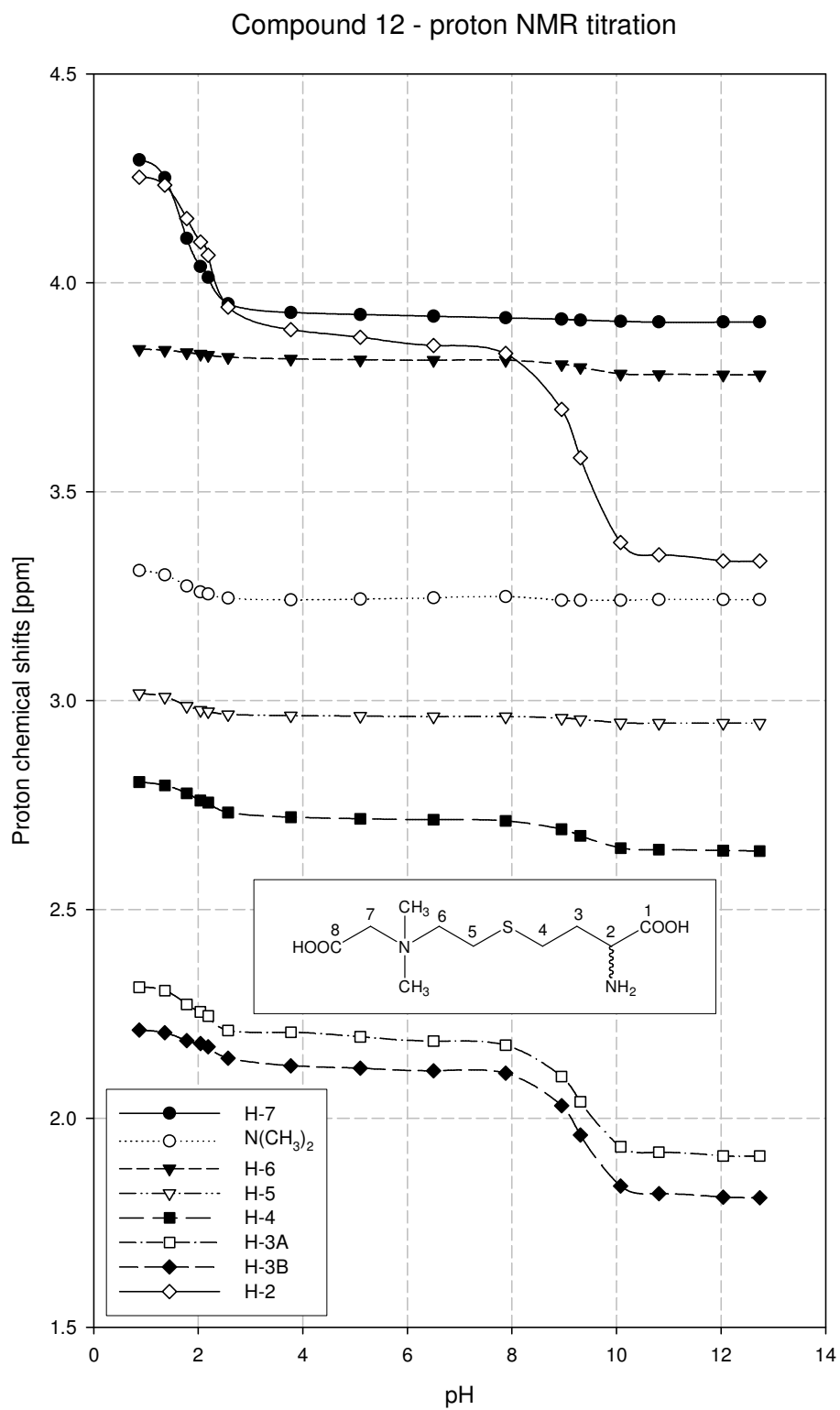
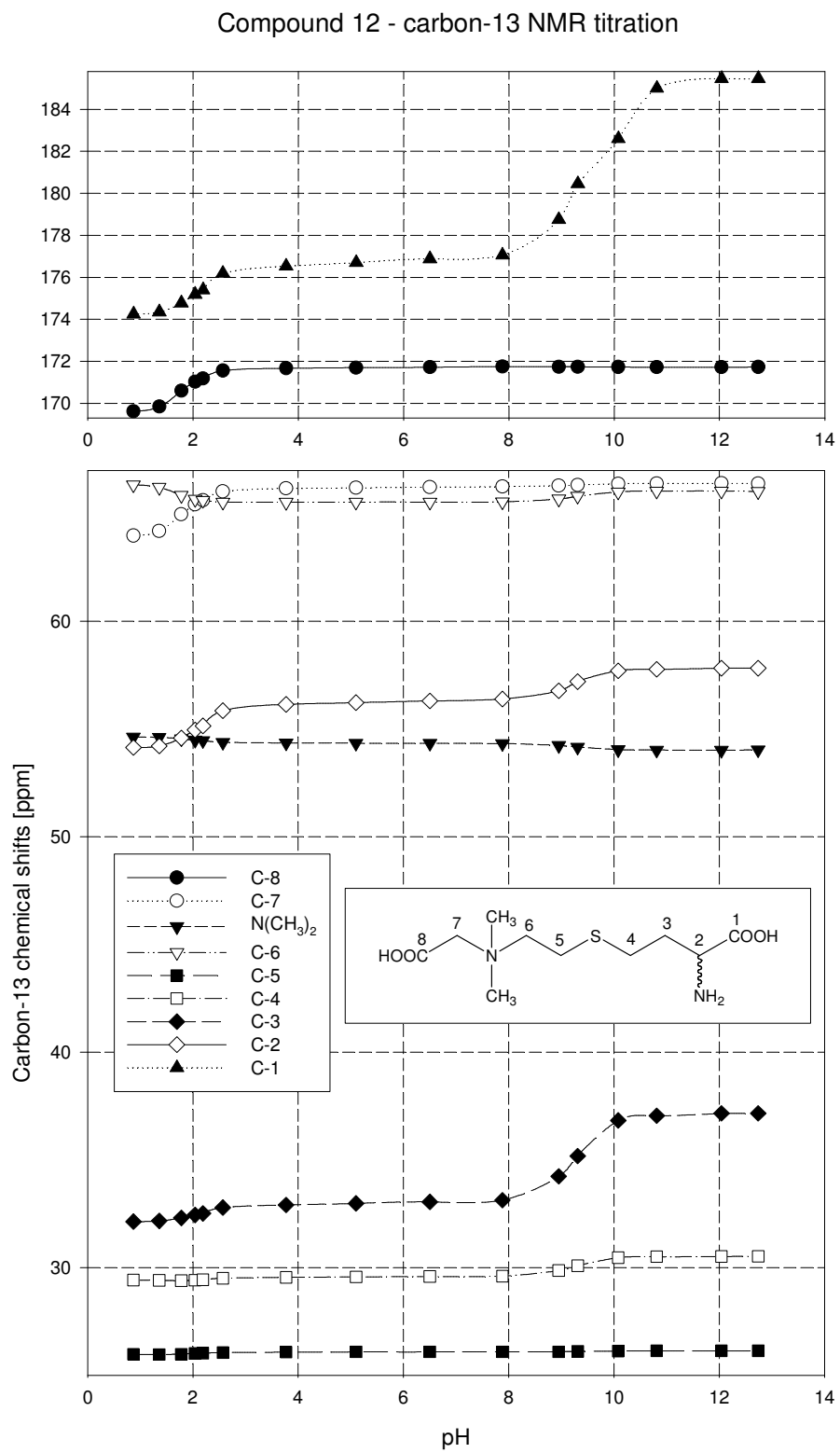


Figure S8. ^{13}C NMR titration of compound **12**.



7. Inhibition experiments with compounds **63** and **64**.

We determined the IC_{50} values of the inhibitors **63** (*S*-enantiomer of compound **21**) and **64** (*R*-enantiomer of compound **21**), and we found values of $0.064 \pm 0.006 \mu\text{M}$ and $0.701 \pm 0.099 \mu\text{M}$, respectively. These data indicate that the *S*-enantiomer (compound **63**) behaves as a stronger inhibitor than the racemic *R,S*-compound **21** (IC_{50} value of $0.084 \mu\text{M}$, Table 1 in the main document). The *R*-enantiomer (compound **64**) is a significantly weaker inhibitor of BHMT than compound **63**, with at least a 10-fold lower inhibitory potency (IC_{50} $0.701 \mu\text{M}$ versus $0.064 \mu\text{M}$, respectively). These data are in good agreement with the findings of Evans et al.⁶ who provided structural reasons for the stereospecificity of BHMT for L-Hcy (as discussed in the main document). Our data are also in a good agreement with Garrow¹², who reported that BHMT is able to use D-Hcy as a substrate, but only at about 15% of the rate observed for L-Hcy. When BHMT was assayed¹² with saturating concentrations of both D-Hcy and L-Hcy, the rate of methionine production was 95% of that observed with L-Hcy alone. These data indicate that D-Hcy has a very low affinity for the enzyme and that L-Hcy is preferentially methylated when a mixture of the enantiomers is used. Therefore, we suppose that inhibitors **63** and **64** behave towards BHMT in a similar manner as L-Hcy and D-Hcy and that only the *S*-enantiomer binds to BHMT when the inhibitor is assayed as a racemic mixture (compound **21**).

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