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TETRAHEDRON: *ASYMMETRY*

The first asymmetric syntheses of L-homocysteine and L-homocystine

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Abstract

Asymmetric syntheses of L-homocysteine **1** and L-homocystine **2** are described. Alkylation of the carbanion derived from Schöllkopf reagent **3** and ensuing hydrolyses gave *S*-triphenylmethyl-L-homocysteine **6**. Removal of the triphenylmethyl group gave L-homocysteine **1** and subsequent oxidation provided L-homocystine **2**.

1. Introduction

L-Homocysteine **1** is an established clinical marker.[1–3] A moderate increase in plasma or serum concentrations of **1** (hyperhomocysteinemia) constitutes an independent and reversible risk factor for cardiovascular,[4] cerebrovascular,[5] and peripheral vascular diseases.[6] Homocysteinuria, a rare inborn error marked by high levels of **1**, is a risk factor for premature cardiovascular disease in children and young adults.[7]

Vigneaud identified homocysteine in 1935 as a reduced form of homocystine,[8] which had been isolated in 1932 by treating methionine with concentrated sulfuric acid.[9] L-Homocysteine **1** and L-homocystine **2** were later found in the body (Fig. 1).[10,11] Most of the known preparations for these two compounds rely on chiral resolutions to afford L-homocysteine **1**,[8,12,13] which is then oxidized to give L-homocystine **2**. A recent preparation[14] employs HBr and H_2SO_4 to convert L-methionine to **2** as an improvement of a previously published method.[9] However, there are no published asymmetric syntheses of either L-homocysteine **1** or L-homocystine **2**.

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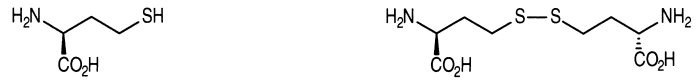
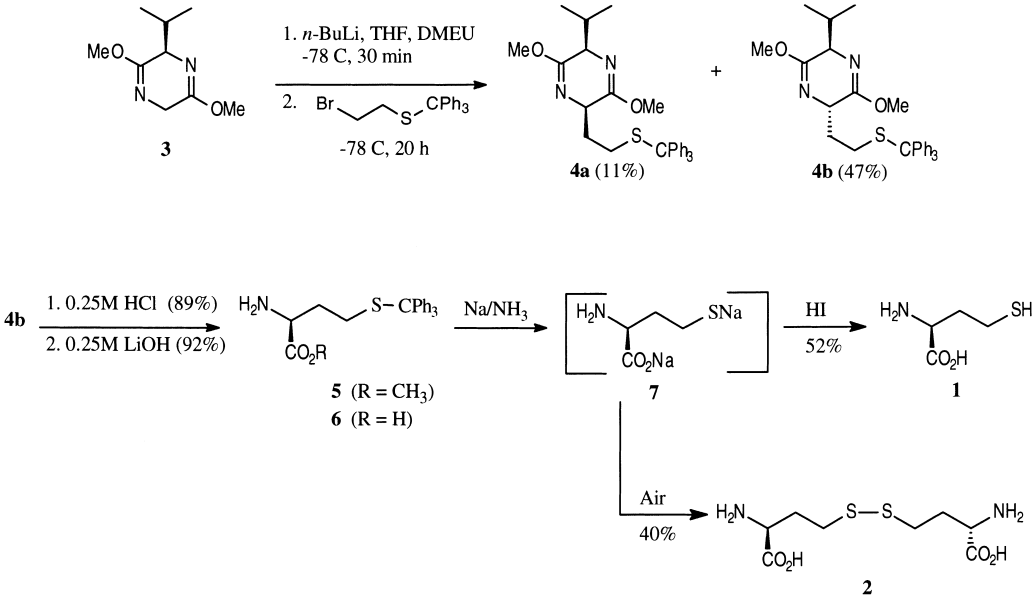


1 **2**

Figure 1.

2. Results and discussion

We have developed an asymmetric synthetic strategy that affords **1** or **2** in four steps. Our strategy utilized Schöllkopf reagent **3**,[15,16] a chiral glycine anion equivalent. Successful alkylation of the lithio derivative of Schöllkopf reagent **3** with 2-bromoethyl triphenylmethyl sulfide required the presence of two equivalents of *N*,*N*-dimethylethyleneurea (DMEU).[17] Alkylation at −78°C for 20 h gave two diastereomers, **4a** and **4b**, in a 1:4.3 ratio after chromatographic isolation as pure diastereomers (Scheme 1). Acid catalyzed hydrolysis of the pyrazine nucleus of the desired diastereomer **4b** gave *S*-triphenylmethyl-L-homocysteine methyl ester **5** in 89% yield after separation from valine methyl ester by flash chromatography. The Mosher amide derivative of **5** revealed only a single ^{19}F resonance, δ=−6.15 ppm vs α,α,α-trifluorotoluene.[18] Hydrolysis of **5** with lithium hydroxide in 1:1 water:dioxane afforded *S*-triphenylmethyl-L-homocysteine **6** in 92% yield. Removal of the triphenylmethyl group was achieved by reduction using excess sodium in liquid ammonia at −33°C to form an intermediate disodium salt **7**. L-Homocysteine **1** was isolated in 52% yield from **6** after adding acid to the disodium salt **7**.[19] Air oxidation of the intermediate **7** and ensuing acidic workup furnished L-homocystine **2** as a powder in 40% yield from **6**.



Scheme 1.

3. Conclusion

In conclusion, we have developed a synthetic route for the asymmetric syntheses of L-homocysteine **1** and L-homocystine **2**. This flexible strategy is amenable for the synthesis of unnatural amino acids, homologs of **1** and **2**, needed for biochemical studies, building blocks for combinatorial chemistry and assay development.

4. Experimental

All chemicals were purchased from Aldrich except (*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (**3**, Schöllkopf reagent) which was purchased from Fluka. THF was freshly distilled from sodium–benzophenone ketyl. ^{1}H, ^{13}C and ^{19}F NMR spectra were recorded on a Varian Gemini 2300 spectrometer using tetramethylsilane as internal standard (^{1}H, ^{13}C) or α,α,α-trifluorotoluene as external standard (^{19}F). Electrospray mass spectra (ESMS) were obtained on a PE Sciex API 100 system. Optical rotations were obtained on an AUTOPOL III automatic polarimeter. Melting points are uncorrected and obtained on an Electrothermal® melting point apparatus. Thin layer chromatography was carried out on silica gel (Whatman MK6F) plates purchased from VWR and spots located either by UV, PMA stain or I_2 visualization. Analytical HPLC runs were performed using a Waters μ-porasil 8×100 column, λ=225 nm, flow rate=1.8 mL/min.

*4.1. Preparation of (2*R*,5*R*)-2-isopropyl-3,6-dimethoxy-5-[2-(tritylsulfanyl)ethyl]-2,5-dihydropyrazine **4a** and (2*R*,5*S*)-2-isopropyl-3,6-dimethoxy-5-[2-(tritylsulfanyl)ethyl]-2,5-dihydropyrazine **4b***

n-Butyl lithium (*n*-BuLi, 2.5 M in hexanes, 22 mL, 55 mmol) was added to a −78°C solution of triphenylmethyl mercaptan (15.0 g, 54.3 mmol) in dry THF (300 mL) and stirred for 2 h. 1,2-Dibromoethane (22.0 mL, 255 mmol, 4.7 equiv.) was added all at once to the −78°C reaction solution. After 30 min, the reaction mixture was warmed to room temperature, stirred for 3 h and concentrated in vacuo. Residue was partitioned between ether (500 mL) and water (500 mL), the layers were separated and the aqueous layer was extracted with CH_2Cl_2 (500 mL). Organic extracts were combined, dried over Na_2SO_4, decanted and concentrated in vacuo to afford the desired 2-bromoethyl triphenylmethyl sulfide as a white solid (20.1 g, 97%). ^{1}H NMR ($CDCl_3$) δ 7.64–7.60 (m, 5H), 7.52–7.38 (m, 10H), 3.09–3.03 (m, 2H), 2.95–2.86 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 144.4, 129.5, 128.0, 126.9, 67.4, 34.1, 29.9.

n-BuLi (1.6 M in hexanes, 9.6 mL, 15.4 mmol) was added to a −78°C solution of (*R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (**3**, 2.5 mL, 14 mmol) and 1,3-dimethyl-2-imidazolidinone (DMEU, 3.1 mL, 28 mmol) in dry THF (140 mL). After 45 min, a solution of 2-bromoethyl triphenylmethyl sulfide (6.32 g, 16.5 mmol, 1.2 equiv.) in dry THF (30 mL) was added over 25 min. After 20 h at −78°C, the cherry colored reaction mixture was quenched with 5 mL of 100 mmol, pH 7.2 phosphate buffer and warmed to ambient temperature. The yellow solution was concentrated in vacuo and the resulting residue was partitioned between ethyl acetate (EtOAc, 100 mL) and water (100 mL). The aqueous layer was separated and extracted with EtOAc (50 mL). Combined organic extracts were dried over Na_2SO_4, decanted and concentrated in vacuo to give an oil. Purification by flash chromatography (5% EtOAc/hexanes) gave 0.37 g (11% based on recovered starting material) of (2*R*,5*R*)-2-isopropyl-3,6-dimethoxy-5-[2-(tritylsulfanyl)ethyl]-2,5-dihydropyrazine **4a**, 1.60 g (47% based on recovered starting material) of (2*R*,5*S*)-2-isopropyl-3,6-dimethoxy-5-[2-(tritylsulfanyl)ethyl]-2,5-dihydropyrazine **4b** and 3.64 g recovered 2-bromoethyl triphenylmethyl sulfide.

Compound **4a**: ^{1}H NMR ($CDCl_3$) δ 7.42–7.38 (m, 5H), 7.29–7.12 (m, 10H), 4.01–3.95 (m, 1H), 3.87 (dd, *J*=4.8, 3.9 Hz, 1H), 3.59 (s, 3H), 3.55 (s, 3H), 2.45–2.35 (m, 1H), 2.32–2.20 (m, 2H), 2.04–1.90 (m, 1H), 1.66–1.54 (m, 1H), 1.00 (d, *J*=6.6 Hz, 3H), 0.57 (d, *J*=6.9 Hz, 3H); ESMS $(M+2H)^{2+}$ 243.5. Analytical HPLC (2% EtOAc/hexanes): R_t=12.88 min (97%).

Compound **4b**: ^{1}H NMR ($CDCl_3$) δ 7.42–7.38 (m, 5H), 7.29–7.12 (m, 10H), 3.96 (ddd, *J*=7.2, 3.6, 3.6 Hz, 1H), 3.81 (dd, *J*=3.6, 3.6 Hz, 1H), 3.61 (s, 3H), 3.55 (s, 3H), 2.27–2.07 (m, 2H), 1.99–1.88 (m, 1H), 1.78–1.66 (m, 1H), 1.00 (d, *J*=6.6 Hz, 3H), 0.65 (d, *J*=6.9 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 163.67, 163.22, 144.98, 129.60, 127.75, 126.47, 66.51, 60.73, 54.52, 52.36, 33.19, 31.78, 27.75, 18.99, 16.64; ESMS $(M+2H)^{2+}$ 243.2. $[\alpha]_D^{20}$=+10.88 (c=0.06, $CHCl_3$). Analytical HPLC (2% EtOAc/hexanes): R_t=7.72 min (100%). Anal. calcd for $C_{30}H_{34}N_2O_2S$: C, 74.04; H, 7.04; N, 5.76. Found: C, 73.88; H, 6.96; N, 5.49.

*4.2. Preparation of (2S)-2-amino-4-(tritylsulfanyl)butanoic acid **6***

A 0.50 M aqueous solution of hydrochloric acid (10.1 mL, 5.06 mmol, 2.1 equiv.) was added to a solution of (2*R*,5*S*)-2-isopropyl-3,6-dimethoxy-5-[2-(tritylsulfanyl)ethyl]-2,5-dihydropyrazine (**4b**, 1.17 g, 2.41 mmol) in dioxane (10.1 mL) and stirred for 6 h at ambient temperature. The solution was then adjusted to pH 10 with concentrated ammonium hydroxide and extracted with $CHCl_3$ (3×40 mL). Combined organic extracts were dried (Na_2SO_4), decanted and concentrated in vacuo. The residual oil was purified by flash column chromatography (5% MeOH/CH_2Cl_2; R_f=0.37) to afford methyl (2*S*)-2-amino-4-(tritylsulfanyl)butanoate **5** as a thick colorless oil (840 mg, 89%). ^{1}H NMR ($CDCl_3$) δ 7.43–7.39 (m, 5H), 7.31–7.18 (m, 10H), 3.64 (s, 3H), 3.43–3.38 (m, 1H), 2.30 (t, *J*=7.8 Hz, 2H), 1.80–1.70 (m, 1H), 1.58–1.46 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 175.85, 144.79, 129.59, 127.86, 126.62, 66.77, 53.48, 51.96, 33.95, 28.31. $[\alpha]_D^{20}$=+9.03 (c=0.0113, $CHCl_3$). Anal. calcd for $C_{24}H_{25}NO_2S$: C, 73.63; H, 6.44; N, 3.58; S, 8.19. Found: C, 73.20; H 6.58; N 3.70; S 7.95.

The Mosher amide of **5** was prepared by adding triethylamine (10.2 μL, 0.073 mmol) and (*R*)-(−)-(α)-methoxy-(α-trifluoromethyl)phenylacetic acid chloride (13.6 μL, 0.073 mmol) in succession to a solution of methyl (2*S*)-2-amino-4-(tritylsulfanyl)butanoate (**5**, 19 mg, 0.05 mmol) in anhydrous dichloromethane (1 mL) and stirred for 3 h at ambient temperature then quenched by addition of water (100 μL). After 15 min of stirring, dichloromethane (20 mL) was added and washed with water (20 mL). The organic layer was separated, dried (Na_2SO_4), decanted and concentrated in vacuo to afford an oil which was purified by flash chromatography to afford 22 mg (72%) of the Mosher amide of **5**. ^{1}H NMR ($CDCl_3$) δ 7.51–7.42 (m, 2H) 7.35–7.15 (m, 18H), 6.93 (d, *J*=8.4 Hz, 1H), 4.55–4.48 (m, 1H), 3.66 (s, 3H), 3.47 (d, *J*=1.5 Hz, 3H), 2.25–2.15 (m, 1H), 2.09–1.98 (m, 1H), 1.83–1.71 (m, 1H), 1.54–1.38 (m, 1H); ^{19}F NMR ($CDCl_3$) δ −6.15.

A 0.50 M LiOH solution (9.0 mL, 4.5 mmol, 2.02 equiv.) was added dropwise to a solution of ester **5** (772 mg, 1.98 mmol) in dioxane (9.0 mL) and stirred at room temperature for 3 h. The reaction mixture was concentrated in vacuo and the residual solid was suspended in water (20 mL). Careful adjustment to pH 8 with 1 M aqueous HCl afforded a precipitate. The white solid thus obtained was filtered and dried under high vacuum at 46°C over P_2O_5 to give (2*S*)-2-amino-4-(tritylsulfanyl)butanoic acid (**6**, *S*-triphenylmethyl-L-homocysteine, 687 mg, 92%). ^{1}H NMR (DMSO-d_6) δ 7.48–7.18 (m, 15H), 3.02 (t, *J*=6.3 Hz, 1H), 2.26 (t, *J*=5.1 Hz, 2H), 1.90–1.73 (m, 1H), 1.69–1.53 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 169.17, 144.51, 129.05, 127.93, 126.62, 65.90, 53.43, 30.40, 28.11; ESMS $(M-H)^-$ 376.

4.3. Preparation of L-homocysteine **1**

Sodium metal (61 mg, 2.65 mmol) was added to a suspension of *S*-triphenylmethyl-L-homocysteine (**6**, 270 mg, 0.72 mmol) in 20 mL of liquid ammonia at −33°C. After 1 h, the reaction mixture was warmed to ambient temperature and concentrated in vacuo. (Caution: the product is extremely air sensitive. Exposure to air should be kept to a minimum.) The resulting solid was suspended in deaerated water (10 mL) and extracted with deaerated ether (10 mL) to remove triphenylmethane. The aqueous layer was adjusted to pH 6 by careful addition of deaerated 47% aqueous HI. This solution was concentrated in vacuo and any remaining HI was removed by azeotroping with deaerated water (3×10 mL). The residue was stirred in deaerated hot ethanol for 15 min, cooled to ambient temperature under nitrogen and filtered. The filtered solid was isolated and dried under high vacuum for 15 h at 56°C to afford 51 mg (52%) of L-homocysteine **1**. ^{1}H NMR (D_2O) δ 3.73 (dd, *J*=7.4, 5.7 Hz, 1H), 2.60–2.42 (m, 2H), 2.12–1.88 (m, 2H); ^{13}C NMR (D_2O) δ 174.61, 53.97, 34.98, 20.15; ESMS $(M-H)^-$ 134. $[\alpha]_D^{24}$=+27.8 (c=0.0027, 1N HCl) [lit.[12] $[\alpha]_D^{25}$=+26.9 (c=1, 1N HCl)].

4.4. Preparation of L-homocystine **2**

Sodium metal (141 mg, 6.13 mmol) was added to a suspension of **6** (200 mg, 0.53 mmol) in 40 mL of liquid ammonia at −33°C and stirred for 3.5 h. Unreacted sodium metal was quenched by the addition of a few crystals of NH_4Cl. Ammonia was allowed to evaporate by warming to ambient temperature and any residual ammonia was removed in vacuo. Deaerated water (50 mL) was poured into the flask and the mixture was extracted with deaerated ether (2×25 mL). The aqueous layer was adjusted to pH 7 by the careful addition of deaerated concentrated HCl, the mixture treated with decolorizing carbon, filtered through Celite® and the filter pad washed with deaerated water (15 mL). The resulting filtrate was flushed with air for 1 h and left sitting open to air for 12 h. Filtration removed trace suspended solids and the filtrate was concentrated in vacuo. The residual solid was dissolved in water (3 mL) and filtered. Filtrate pH was adjusted from 9.8 to 5.5 by careful addition of concentrated HCl and allowed to stand for 30 min. The solid thus obtained was washed with water (3×2 mL) and dried under high vacuum over P_2O_5 at 46°C to give 28 mg (40%) of L-homocystine **2** as an off-white solid. ^{1}H NMR (D_2O/NaOD) δ 3.15 (dd, *J*=7.4, 5.8 Hz, 2H), 2.58 (t, *J*=8.0 Hz, 4H), 1.90–1.64 (m, 4H); ^{13}C NMR (D_2O/NaOD) δ 183.13, 55.40, 34.78, 34.73; ESMS $(M-H)^-$ 267; mp: 266–268°C (uncorrected) [Aldrich material: 270–272°C]. $[\alpha]_D^{24}$=+69.3 (c=0.00284, 1N HCl) [lit.[13] $[\alpha]_D^{24}$=+70–73 (c=1, 1N HCl)]. Anal. calcd for $C_8H_{16}N_2O_4S_2$: C, 35.81; H, 6.01; N, 10.44; S, 23.89. Found: C, 35.88; H, 6.02; N, 10.42; S, 23.56.

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18. Mosher amide of **5** was prepared by literature procedure [Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc*. **1969**, *34*, 2543] as described in the Experimental and analyzed on a Varian Gemini 2300 NMR, ^{19}F NMR (282 MHz).
19. Trace amounts of **2** could be found in our **1** as well as in material purchased from Sigma (^{1}H NMR, D_2O).