# Asymmetric Synthesis of *threo*-(2*S*,3*R*)-2-Amino-3-hydroxypentanedioic Acid 5-Amide (*threo*-β-Hydroxy-L-glutamine)

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A new method for the synthesis of *threo*-β-hydroxy-L-glutamine consists of epoxidation of (*S*)-3-benzyloxycarbonyl-5-oxo-4-vinyltetrahydro-1,3-oxazole (protected L-vinylglycine) and regioselective ring cleavage.

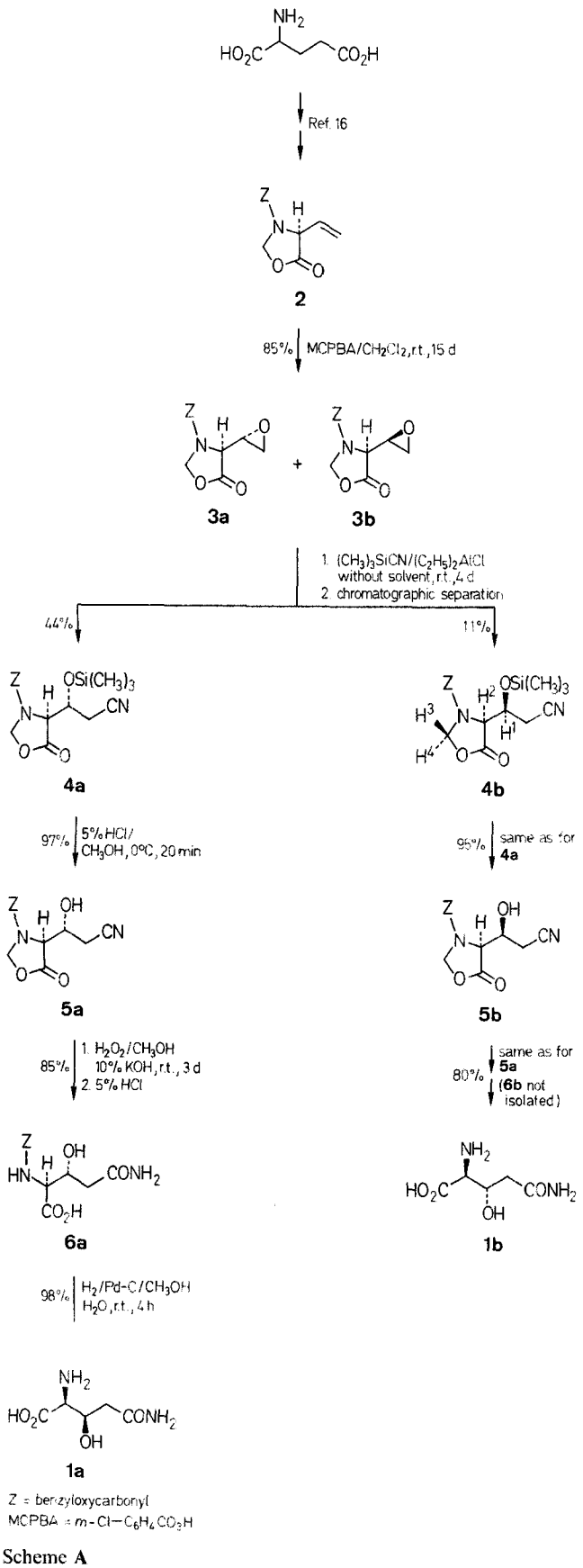
A large number of unusual amino acids have been isolated in the past fifty years[1]. Among them, α-amino-β-hydroxyacids[2] are derivatives of primary importance both as enzyme inhibitors and as starting materials for the synthesis of β-lactam antibiotics[3]. Although several synthesis of racemic α-amino-β-hydroxyacids[4–9] have been reported, only a few methods for the synthesis of the related optically active compounds are known[2,10,11].

*threo*[12]-β-Hydroxy-L-glutamine (**1a**)[13,14] is an interesting molecule because of its structural relation to several natural and biologically active compounds. One of them, *threo*-β-hydroxyglutamic acid, is active on cranial nerves and is useful in the synthesis of, for example, tricholomic acid[15].

Here we report the asymmetric synthesis of *threo*-β-hydroxy-L-glutamine (**1a**) from L-glutamic acid, an inexpensive and commercially available starting material.

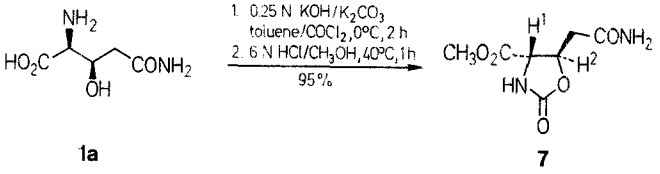
Epoxidation of the protected glycine **2**, obtained according to the procedure of Lit.[16], (see Scheme **A**; *m*-chloroperbenzoic acid/room temperature) provided smoothly epoxides **3a** and **3b** in a 4:1 ratio (85% yield). Determination of the diastereoisomeric ratio was readily accomplished by HPLC [VARIAN 5000, $S_1$-100 10 μ column (Brownlee-

Labs)] with hexane/ethyl acetate (75:25) as eluent and $^{13}$C-NMR analysis of the crude mixture. As compounds **3a** and **3b** were not separable by flash chromatography[17], the next reaction was performed with the diastereoisomeric mixture. The epoxides were treated with cyanotrimethylsilane and diethyl aluminium chloride[18,19] to afford a mixture of **4a** and **4b** (55% yield, not optimized) which was easily separated by flash chromatography[17] on silica gel using hexane/ethyl acetate (8:2) as eluent. $^1$H-NMR analysis of an analytical sample of the product mixture of **4a** and **4b** gave the same ratio as that of the starting epoxides **3a** and **3b**. The ratio was not affected by purification using flash chromatography[17]. The ratio was determined by integration of the AB system of N–C$\underline{H}_2$–O the two diastereoisomeric compounds. Deprotection of the optically pure major isomer **4a** (5% hydrochloric acid/methanol) gave **5a** in quantitative yield. Compound **5a** was hydrolyzed with hydrogen peroxide/10% potassium hydroxide to give amide **6a** (82% yield based on **4a**). Hydrogenolysis of the *N*-protecting benzyloxycarbonyl group (palladium-on-carbon/methanol-/water) led to *threo*-β-hydroxy-L-glutamine (**1a**).




Scheme A

Comparison of the coupling constants of the protons 1-H and 2-H of the *threo* (**1a**; $J$ = 4.80 Hz) and *erythro* (**1b**; $J$ = 3.66 Hz) isomers of β-hydroxy-L-glutamine allowed to determine the absoluted configuration at C-3 as *R* for the major isomer[20]. In order to confirm this assignment, isomer **1a** was converted into the corresponding oxazolidinone **7** (phosgene/toluene) (see Scheme **B**). The $^1$H-NMR spectrum of **7** showed a coupling constant $J_{1,2}$ = 4.70 Hz which points to the *trans* relation between the two protons 1-H and 2-H[8,20].




Scheme B

Silica gel (Merck, 270–400 mesh) was used for flash chromatography[17]. Optical rotations were measured in a 1 dm cell of 1 ml capacity using a Perkin-Elmer 241 polarimeter. Microanalyses were performed with a Perkin-Elmer 240 instrument. IR spectra were recorded with a Perkin-Elmer 457 spectrophotometer. $^1$H-NMR spectra were recorded with Bruker WP-80 (80 MHz) instrument. $^{13}$C-NMR spectra with Varian-XL-100 (25.1 MHz) instrument.

**(*S*)-3-Benzyloxycarbonyl-5-oxo-4-(1,2-epoxyethyl)-oxazolidine (3a and 3b):**

To a solution of the protected glycine **2** (608 mg, 2.46 mmol) in dichloromethane (4.9 ml), 90% *m*-chloroperbenzoic acid (753 mg, 3.94 mmol) in dichloromethane (10.6 ml) is added. The mixture is stirred for 15 days at room temperature and then quenched with 10% sodium sulfite solution (7 ml). After stirring for 30 min, the mixture is extracted with dichloromethane (3 × 6 ml) and the organic phase is washed with 5% aqueous sodium hydrogen carbonate solution (10 ml), water (7 ml), and saturated sodium chloride solution (7 ml). The combined organic layers are dried with sodium sulfate and evaporated under vacuum. The crude product is purified by flash chromatography[17] on silica gel with hexane/ethyl acetate (7:3) as eluent to afford pure **3a** and **3b** ($R_F$ = 0.38); yield: 550 mg (85%).

$C_{13}H_{13}NO_5$ (263.3) calc. C 59.31 H 4.98 N 5.32
found 59.28 5.00 5.27

IR ($CHCl_3$): $\nu$ = 3000, 1790, 1710, 1400, 1220, 1170 cm$^{-1}$.

$^1$H-NMR ($CDCl_3$): $\delta$ = 2.6–2.9 (m, 2 H, CHC$\underline{H}_2$–O); 3.4–3.5 (m, 1 H, C$\underline{H}$O); 4.5–4.7 (m, 1 H, C$\underline{H}$N); 5.0–5.7 (m, 4 H, OC$\underline{H}_2$–N and C$\underline{H}_2$–$C_6H_5$); 7.40 ppm (s, 5 H, $H_{arom}$).

$^{13}$C-NMR ($CDCl_3$), selected values: $\delta$ = 44.2 (**3a**), 44.4 (**3b**); 49.7 (**3b**), 50.7 (**3a**); 55.9 (**3a**), 55.6 (**3b**) ppm.

**(*S*)-2-Benzyloxycarbonyl-5-oxooxazolidine-4-(β-trimethylsiloxypropanenitrile (4a + 4b):**

The mixture of epoxy compounds **3a** + **3b** (2 g, 7.60 mmol) is placed under nitrogen in a two-necked round-bottom flask equipped with a reflux condenser. To this are added, dropwise with stirring, cyanotrimethylsilane (2.10 ml, 16.73 mmol) and a 1 molar solution (0.304 ml, 0.304 mmol) of diethylaluminum chloride in hexane. The mixture is stirred at room temperature for 5 days; during this time, cyanotrimethylsilane (1.05 ml, 8.37 mmol) and 1 molar diethylaluminum chloride in hexane (0.152 ml, 0.152 mmol) are added 3 times (every 40 h). The mixture is then cooled and water (5 ml) is added dropwise. The resultant mixture is extracted with dichloromethane (3 × 20 ml). The combined organic layers are dried with sodium sulfate and evaporated under vacuum. The crude product is purified by flash chromatography[17] on silica gel using hexane/ethyl acetate (8:2) to afford pure **4a** ($R_F$: 0.35) and **4b** ($R_F$: 0.28).

*Isomer* **4a**; yield: 1.210 g (44%); $[\alpha]_D^{25}$: +120.9° (c = 1.1, chloroform).

*Isomer* **4b**; yield: 303 mg (11%); $[\alpha]_D^{25}$: +92.7° (c = 0.9, chloroform).

| | | | | | |
|---|---|---|---|---|---|
| $C_{17}H_{22}N_2O_5Si$ | calc. | C | 56.33 | H 6.12 | N 7.73 |
| (362.5) | found | **(4a)** | 56.30 | 6.10 | 7.75 |
| | | **(4b)** | 56.35 | 6.10 | 7.77 |

IR ($CHCl_3$) of **4a**: ν = 2220, 1800, 1715, 1410, 1250 $cm^{-1}$.

IR ($CHCl_3$) of **(4b)**: ν = 2220, 1800, 1715, 1410, 1250 $cm^{-1}$.

$^1$H-NMR ($CDCl_3$) of **4a**: δ = 0.15 [s, 9H, $(CH_3)_3Si$]; 2.5–2.7 (m, 2H, $CH_2CN$); 4.3–4.6 (m, 2H, CHOSi, CHN); 5.20 (s, 2H, $CH_2-C_6H_5$); 5.76, 5.21 (AB system, 2H, $J_{AB}$ = 5.30 Hz, $OCH_2N$); 7.38 (s, 5 $H_{arom}$).

$^1$H-NMR ($CDCl_3$) of **4b**: δ = 0.05 [s, 9H, $(CH_3)_3$ Si]; 2.7–2.9 (m, 2H, $CH_2CN$); 4.2–4.3 (m, 1H, CHN); 4.3–4.7 (m, 1H, CHOSi); 5.20 (s, 2H, $CH_2-C_6H_5$); 5.16 and 5.49 (AB system, 2H, $J_{AB}$ = 4.00 Hz, $OCH_2N$); 7.34 (s, 5 $H_{arom}$).

**(*S*)-3-Benzyloxycarbonyl-5-oxooxazolidine-4-[(*R*)-β-hydroxypropanenitrile] (5a):**

A solution of compound **4a** (562 mg, 1.55 mmol) in methanol (18 ml) is treated dropwise at 0 °C with 5% hydrochloric acid (3 ml). After 20 min, the solution is diluted with water (10 ml) and extracted with ethyl acetate (3 × 20 ml). The combined organic layers are dried with sodium sulfate and evaporated under vacuum. The crude product is purified by flash chromatography[17] of silica gel with hexane/ethyl acetate (1:1) as eluent ($R_F$: 0.32); yield: 436 mg (97%); $[\alpha]_D^{25}$: +146.0° (c = 1, chloroform).

| | | | | |
|---|---|---|---|---|
| $C_{14}H_{14}N_2O_5$ | calc. | C 57.93 | H 4.86 | N 9.65 |
| (290.3) | found | 57.89 | 4.83 | 9.66 |

IR ($CHCl_3$): ν = 3590, 3410, 3000, 2220, 1795, 1715, 1405 $cm^{-1}$.

$^1$H-NMR ($CDCl_3/D_2O$): δ = 2.5–2.7 (m, 2H, $CH_2CN$); 4.3–4.6 (m, 2H, CHOH, CHN); 5.21 (s, 2H), $CH_2-C_6H_5$); 5.48, 5.54 (AB system, 2H, $J_{AB}$ = 4.62 Hz, $OCH_2N$); 7.38 (s, 5H, $H_{arom}$).

**(*S*)-3-Benzyloxycarbonyl-5-oxooxazolidine-4-[(*S*)-β-hydroxypropanenitrile] (5b):**

Compound **5b** is prepared from **4b** (407 mg, 1.12 mmol) as described above for **5a**; yield: 310 mg (95%); m.p. 94–96 °C; $[\alpha]_D^{25}$: +114.0° (c = 0.85, chloroform).

| | | | | |
|---|---|---|---|---|
| $C_{14}H_{14}N_2O_5$ | calc. | C 57.93 | H 4.86 | N 9.65 |
| (290.3) | found | 57.91 | 4.85 | 9.64 |

IR ($CHCl_3$): ν = 3610, 3430, 2420, 2220, 1795, 1715, 1405 $cm^{-1}$.

$^1$H-NMR ($CDCl_3/D_2O$): δ = 2.7–2.9 (m, 2H, $CH_2CN$); 4.29 (dd, 1H, $J_{1,2}$ = 2.55 Hz, $J_{2,3\text{ or }2,4}$ = 0.85 Hz, CHN); 4.3–4.6 (m, 1H, CHOH); 5.21 (s, 2H, $CH_2-C_6H_5$); 5.30 (dd, 1H, $J_{3,4}$ = 4.00 Hz, $J_{3,2\text{ or }4,2}$ = 0.85 Hz, $H^3$ or $H^4$); 5.52 (d, 1H, $J_{3,4}$ = 4.00 Hz, $H^3$ or $H^4$); 7.38 (s, 5H, $H_{arom}$).

**(2*S*,3*R*)-*N*-Benzyloxycarbonyl-3-hydroxyglutamine (6a):**

To a solution of nitrile **5a** (710 mg, 2.45 mmol) and 30% aqueous hydrogen peroxide (0.08 ml, 0.73 mmol) in methanol (2.65 ml), aqueous 10% potassium hydroxide is added till pH 8. The mixture is allowed to stand at room temperature for 3 days during which time aqueous 30% hydrogen peroxide (total amount: 0.40 ml; 3.65 mmol) is added in five portions, the pH being maintained at 8.0 with aqueous 10% potassium hydroxide solution. Then, aqueous 10% sodium sulfite (1.5 ml) is added. After 30 min, the mixture is acidified to pH 1 with 5% hydrochloric acid and evaporated to dryness under vacuum. The residue is treated with methanol (15 ml), the resultant mixture is filtered, and the filtrate is evaporated under vacuum. The crude product **6a** is purified by flash chromatography[17] on silica gel using chloroform/methanol (75:25)/2% glacial acetic acid ($R_F$: 0.38); yield: 617 mg (85%); m.p. 62–64 °C; $[\alpha]_D^{25}$: +19.5° (c = 1.1, methanol).

| | | | | |
|---|---|---|---|---|
| $C_{13}H_{16}N_2O_6$ | calc. | C 52.70 | H 5.44 | N 9.45 |
| (296.3) | found | 52.72 | 5.50 | 9.39 |

IR (KBr): ν = 3360, 2910, 1740, 1660, 1600, 1260 $cm^{-1}$.

$^1$H-NMR ($CD_3OD$): δ = 2.3–2.5 (m, 2H, $CH_2-CONH_2$); 4.2–4.4 (m, 1H, CHN); 4.4–4.6 (m, 1H, CHOH); 5.15 (s, 2H, $CH_2-C_6H_5$); 7.40 (s, 5H, $H_{arom}$).

***threo*-β-Hydroxy-L-glutamine (1a):**

A solution of compound **6a** (187 mg, 0.63 mmol) in methanol/water (4.7 ml/1.5 ml) is hydrogenated over 10% palladium-on-carbon (67 mg) for 4 h at room temperature. Filtration and evaporation to dryness gives **1a** as a colorless solid; yield: 100 mg (98%); m.p. 185 °C (dec); $[\alpha]_D^{25}$: +14.4 (c = 1.1, water).

| | | | | |
|---|---|---|---|---|
| $C_5H_{10}N_2O_4$ | calc. | C 37.04 | H 6.22 | N 17.28 |
| (162.1) | found | 37.06 | 6.25 | 17.27 |

IR (KBr): ν = 1730, 1665, 1630, 1390 $cm^{-1}$.

$^1$H-NMR ($D_2O$): δ = 2.4–2.8 (m, 2H, $CH_2CONH_2$); 3.71 (d, 1H, $J_{2,3}$ = 4.80 Hz, 2-H = CHN); 4.3–4.7 (m, 1H, 3-H = CHOH).

***erythro*-β-Hydroxy-L-glutamine (1b):**

This compound is prepared from **5b** (300 mg, 1.03 mmol) by the same procedure as for **1a**, without purification of the intermediates. After hydrogenolysis, the crude product is purified by ion-exchange chromatography on DOWEX 50W ($H^+$) (eluting sequentially with water till pH = 7 and then with 5% aqueous ammonia); yield: 133 mg (80%); m.p. 177° (dec); $[\alpha]_D^{25}$: −3.0° (c = 0.25, water).

| | | | | |
|---|---|---|---|---|
| $C_5H_{10}N_2O_4$ | calc. | C 37.04 | H 6.22 | N 17.28 |
| (162.1) | found | 37.00 | 6.20 | 17.29 |

IR (KBr): ν = 1730, 1665, 1610 $cm^{-1}$.

$^1$-H-NMR ($D_2O$): δ = 2.4–2.8 (m, 2H, $CH_2CONH_2$); 3.93 (d, 1H, $J_{2,3}$ = 3.66 Hz, 2-H = CHN); 4.4–4.7 (m, 1H, 3-H = CHOH).

**Methyl (4*S*,5*R*)-5-Aminocarbonylmethyl-2-oxooxazolidine-4-carboxylate (7):**

Compound **1a** (90 mg, 0.555 mmol) is dissolved in 0.25 normal aqueous potassium hydroxide (2.37 ml), potassium carbonate (314 mg, 2.27 mmol) is added to the solution, and the mixture is covered with toluene (0.950 ml). Then, a 20% solution of phosgene in toluene (0.930 ml) is added dropwise with stirring at 0 °C. The mixture is stirred for 2 h and then excess phosgene is evaporated. The solution is extracted with ethyl acetate (3 ml). The aqueous phase is acidified (pH 2) with 6 normal hydrochloric acid, and evaporated under vacuum. The crude product is treated with 6 normal hydrogen chloride in methanol (3 ml). The mixture is allowed to stand at 40 °C for 1 h, and then evaporated to dryness under vacuum. The product is purified by flash chromatography[17] on silica gel using chloroform/methanol (8:2) containing 2% glacial acetic acid ($R_F$: 0.4).

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