Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 2247–2252

BIOORGANIC & MEDICINAL CHEMISTRY

Enzymatic Synthesis of L-6-hydroxynorleucine

Ronald L. Hanson,[a,*] Mark D. Schwinden,[b] Amit Banerjee,[a] David B. Brzozowski,[a] Bang-Chi Chen,[c] Bharat P. Patel,[b] Clyde G. McNamee,[a] Gus A. Kodersha,[c] David R. Kronenthal,[b] Ramesh N. Patel[a] and Laszlo J. Szarka[a]

[a]*Department of Microbial Technology, Bristol-Myers Squibb, One Squibb Drive, New Brunswick, NJ 08903, USA*
[b]*Chemical Process Research, Bristol-Myers Squibb, P.O. Box 4000, Princeton, NJ 08540, USA*
[c]*Chemical Process Development, Bristol-Myers Squibb, One Squibb Drive, New Brunswick, NJ 08903, USA*

Received 24 June 1998

Abstract—L-6-Hydroxynorleucine, a key chiral intermediate used for synthesis of a vasopeptidase inhibitor, was prepared in 89% yield and >99% optical purity by reductive amination of 2-keto-6-hydroxyhexanoic acid using glutamate dehydrogenase from beef liver. In an alternate process, racemic 6-hydroxynorleucine produced by hydrolysis of 5-(4-hydroxybutyl)hydantoin was treated with D-amino acid oxidase to prepare a mixture containing 2-keto-6-hydroxyhexanoic acid and L-6-hydroxynorleucine followed by the reductive amination procedure to convert the mixture entirely to L-6-hydroxynorleucine, with yields of 91 to 97% and optical purities of >99%.

Introduction

L-6-Hydroxynorleucine is a chiral intermediate useful for the synthesis of a vasopeptidase inhibitor now in clinical trials[1] and for the synthesis of C-7 substituted azepinones as potential intermediates for other antihypertensive metalloprotease inhibitors.[2] It has also been used for the synthesis of siderophores,[3–5] indospicine,[6] and peptide hormone analogues.[7,8] Previous synthetically useful methods for obtaining this intermediate have involved synthesis of the racemic compound followed by enzymatic resolution. D-Amino acid oxidase has been used to convert the D-amino acid to the ketoacid leaving the L-enantiomer which was isolated by ion exchange chromatography.[9] In a second approach, racemic *N*-acetylhydroxynorleucine has been treated with L-amino acid acylase to give the L-enantiomer.[1,7] Both of these resolution methods give a maximum 50% yield and require separation of the desired product from the unreacted enantiomer at the end of the reaction. Enzymatic hydroxylation of L-norleucine by an activated form of phenylalanine hydroxylase has also been reported to give 6-hydroxynorleucine on an analytical scale, but not on a preparative scale.[10] A synthesis of L-6-hydroxynorleucine starting from lysine methyl ester has been reported, but very low reactant and product concentrations were required for success of one of the steps.[11]

Reductive amination of ketoacids using amino acid dehydrogenases has been shown to be a useful method for synthesis of natural and unnatural amino acids.[12,13] We have previously reported the synthesis of L-β-hydroxyvaline, an intermediate needed for the synthesis of the monobactam tigemonam, from the corresponding ketoacid using leucine dehydrogenase from *Bacillus* species.[14] We report here the synthesis and conversion of 2-keto-6-hydroxyhexanoic acid to L-6-hydroxynorleucine by reductive amination using beef liver glutamate dehydrogenase and glucose dehydrogenase from *Bacillus* sp. for regeneration of NADH. To avoid the lengthy chemical synthesis of the ketoacid, a second route was developed to prepare the ketoacid by treatment of racemic 6-hydroxynorleucine (readily available from hydrolysis of 5-(4-hydroxybutyl)hydantoin[15]) with D-amino acid oxidase from porcine kidney or *Trigonopsis variabilis* and catalase followed by the reductive amination procedure to convert the mixture to L-6-hydroxynorleucine.

Key words: L-6-Hydroxynorleucine; glutamate dehydrogenase; phenylalanine dehydrogenase; D-amino acid oxidase; 2-keto-6-hydroxyhexanoic acid.
* Corresponding author.

Results and Discussion

A variety of ketoacids can be converted to L-amino acids by treatment with a suitable amino acid dehydrogenase.

0968-0896/99/$ - see front matter
PII: S0968-0896(99)00158-3

2-Keto-6-hydroxyhexanoic acid was prepared by the route described under Experimental. Initial screening using HPLC analysis, with formate dehydrogenase for regeneration of NADH, showed that phenylalanine dehydrogenase from *Sporosarcina* sp. and beef liver glutamate dehydrogenase converted 0.1 M 2-keto-6-hydroxyhexanoic acid, sodium salt mixture completely to L-6-hydroxynorleucine. Additional screening of amino acid dehydrogenases with spectrophotometric enzyme assays using the 2-keto-6-hydroxyhexanoic acid, sodium salt substrate mixture showed that glutamate dehydrogenases from *Candida utilis* and *Proteus* sp. were active using NADPH but not NADH. Leucine dehydrogenase partially purified from *Bacillus sphaericus* ATCC 4525[14] and alanine dehydrogenase from *Bacillus subtilis* were not active. Of 132 cultures screened, extract from *Thermoactinomyces intermedius* ATCC 33205 was the most active (Table 1). This strain has been shown to be a source of thermostable phenylalanine dehydrogenase[16] as well as leucine dehydrogenase.[17]

Beef liver glutamate dehydrogenase was used for preparative reactions at 10% total substrate concentration. As depicted in Scheme 1, 2-keto-6-hydroxyhexanoic acid, sodium salt, in equilibrium with 2-hydroxytetrahydropyran-2-carboxylic acid, sodium salt, is converted to L-6-hydroxynorleucine and the reaction requires ammonia and reduced nicotinamide adenine dinucleotide (NADH). Nicotinamide adenine dinucleotide (NAD) produced during the reaction was recycled to NADH by the oxidation of glucose to gluconic acid using glucose dehydrogenase from *Bacillus megaterium*. The optimum pH for glutamate dehydrogenase with this substrate was determined to be about 8.76. We previously reported that glucose dehydrogenase had a broad pH optimum centered at about 8.5.[14] The kinetics of the reaction are shown in Figure 1. Reaction was complete in about 3 h with reaction yields of 89–92%, and optical purity was >99%.

Chemical synthesis and isolation of 2-keto-6-hydroxyhexanoic acid required several steps. In a second more convenient process (shown in Scheme 2), the ketoacid was prepared by treatment of racemic 6-hydroxynorleucine (produced by hydrolysis of 5-(4-hydroxybutyl) hydantoin) with D-amino acid oxidase and catalase. After the optical purity of the remaining L-6-hydroxynorleucine had risen to >99%, the reductive amination procedure was used to convert the mixture containing 2-keto-6-hydroxyhexanoic acid and L-6-hydroxynorleucine entirely to L-6-hydroxynorleucine with yields of 91–97% and optical purities of >99%. Sigma porcine kidney D-amino acid oxidase and beef liver catalase or Trigonopsis variabilis whole cells (source of oxidase and catalase)[18] were used successfully for this transformation.

Table 1. 2-Keto-6-hydroxyhexanoic acid dehydrogenase in microbial strains

Strain	No.	Units/mL[a]
Bacillus licheniformis	SC12772	0.0099
Bacillus licheniformis	SC12148	0.0368
Rhodococcus sp	SC13810	0.0177
Sporosarcina ureae	ATCC 6473	0.0103
Sporosarcina ureae	ATCC 13888	0.0613
Thermoactinomyces intermedius	ATCC 33205	0.2161

[a] Cells from a 50 mL culture were assayed as described in Experimenal, except that *Thermoactinomyces intermedius* was grown at 53°C and assayed at 50°C.

Conclusion

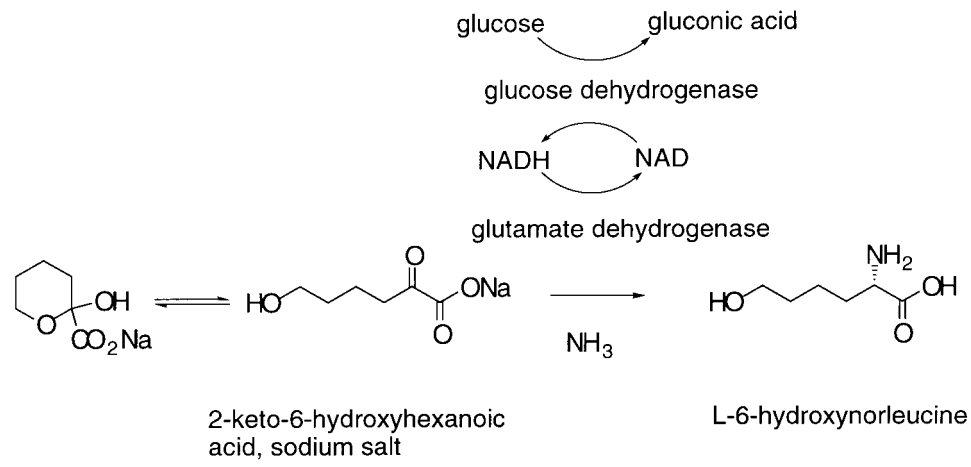
L-6-Hydroxynorleucine was readily prepared from the corresponding ketoacid by reductive amination using beef liver glutamate dehydrogenase. Phenylalanine dehydrogenase from *Sporosarcina* sp. or *Thermoactinomyces intermedius* extract were also effective. Either formate dehydrogenase or glucose dehydrogenase was useful for NADH regeneration. Preparation of the ketoacid required several steps, but a shorter route was found in which racemic 6-hydroxynorleucine was prepared by hydrolysis of 5-(4-hydroxybutyl)hydantoin. Conversion of the D-enantiomer to the ketoacid using D-aminoacid oxidase followed by in situ reductive amination gave nearly complete conversion to L-6-hydroxynorleucine.

Experimental

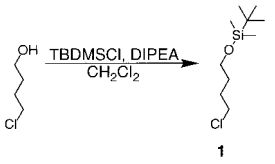
HPLC. Analysis of optical purity and amount of 6-hydroxynorleucine was performed with a Chiralpak WH (Daicel Chemical Industries, Ltd.) 25×0.46 cm column; mobile phase was 0.3 mM CuSO4; flow rate was 1 ml/min; temperature was 40°C; and detection was at 230 nm.

Screening of strains. Strains inoculated from frozen vials were shaken at 220 rpm at 28°C for 48 h in 50 mL medium containing (g/L): L-phenylalanine, 10; peptone, 10; yeast extract, 5; K_2HPO_4, 2; NaCl, 1; and $MgSO_4 .7H_2O$, 0.2. Cells were harvested by centrifugation, washed with 50 mM potassium phosphate buffer pH 7, and resuspended in 5 mL of the same buffer containing 1 mM dithiothreitol. Cells were sonicated for 2 min, then centrifuged for 20 min at 101,000 *g*. Extracts were assayed spectrophotometrically for reductive amination. The assay for amino acid dehydrogenase contained in a volume of 1 mL: 0.75 M NH_4OH adjusted to pH 8.75 with HCl, 0.4 mM NADH, 25 mg/mL 2-keto-6-hydroxyhexanoic acid salt mixture,[19] and extract. Absorbance change per min at 340 nm was used to calculate the activity of the enzyme.

Materials. 5-(4-Hydroxybutyl)hydantoin was obtained from Hampshire Chemical Company. Enzymes were purchased from the following sources: D-amino acid oxidase, catalase, glutamate, phenylalanine, and alanine dehydrogenases, Sigma; formate dehydrogenase, Boehringer Mannheim; glucose dehydrogenase, Amano.



Scheme 1. Conversion of 2-keto-6-hydroxyhexanoic acid to L-6-hydroxynorleucine.



4-*tert*-Butyldimethylsilyloxy-1-chlorobutane (1). A 2 L flask equipped with a mechanical stirrer, addition funnel, internal temperature probe and argon inlet was charged with *tert*-butyldimethylsilyl chloride (124.8 g, 82.78 mmol) and dichloromethane (350 mL). The solution was cooled to 0°C, and 4-chloro-1-butanol (86.4 g, 79.60 mmol) was added via the addition funnel. The addition funnel was rinsed twice with dichloromethane (2×50 mL), and the rinses were added to the reaction. *N*,*N*-Diisopropylethylamine (153 mL, 87.56 mmol) was added slowly over 1.75 h so that the internal temperature did not exceed 6°C. After the addition was complete, the reaction was stirred at 0°C for 1 h. The reaction was stirred at room temperature for 30 min. The homogeneous reaction mixture was concentrated in vacuo until a white solid precipitated. The mixture was slurried in hexane (1000 mL) and then filtered. The solid was washed twice with hexane (2×350 mL). The combined filtrates were concentrated in vacuo to a thick slurry. The mixture was diluted with hexane (800 mL) and filtered. The solid was washed with hexane (250 mL). The combined filtrates were washed with water (3×400 mL), saturated NaHCO3 (400 mL) and saturated NaCl (400 mL). The hexane layer was dried over $MgSO_4$, filtered and concentrated in vacuo to 182 g of a light yellow oil. The oil was distilled (75–78°C/2 mm Hg) through a 5 cm Vigreux column to give **1** (165 g, 93% yield) as a clear, colorless oil: TLC $R_f = 0.77$ (hexane:EtOAc, 3:1); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 3.60 (2H, t, $J = 5.9$ Hz, CH_2), 3.52 (2H, t, $J = 6.4$ Hz, CH_2), 1.80 (2H, m, CH_2), 1.62 (2H, m, CH_2), 0.84 (9H, s, $C(CH_3)_3$), 0.01 (6H, s, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 270 MHz) δ 62.2, 45.0, 30.0, 29.3, 25.9, 18.3, −5.4; CIMS *m*/*z* 223 ($M+H^+$, $C_{10}H_{23}ClSiO$ requires 223); Elem. Anal. calcd for $C_{10}H_{23}ClSiO$: C, 53.90; H, 10.40. Found: C, 54.53; H, 10.52; K-F moisture 0.20.

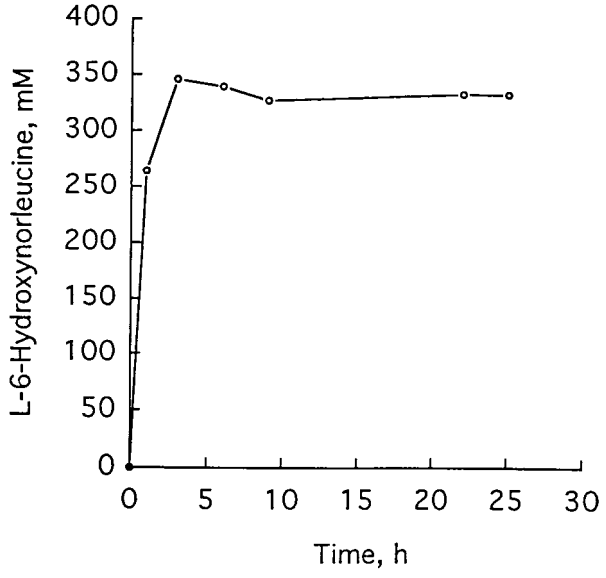
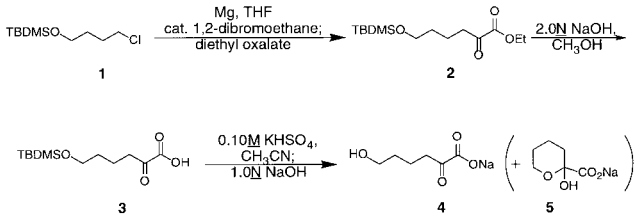
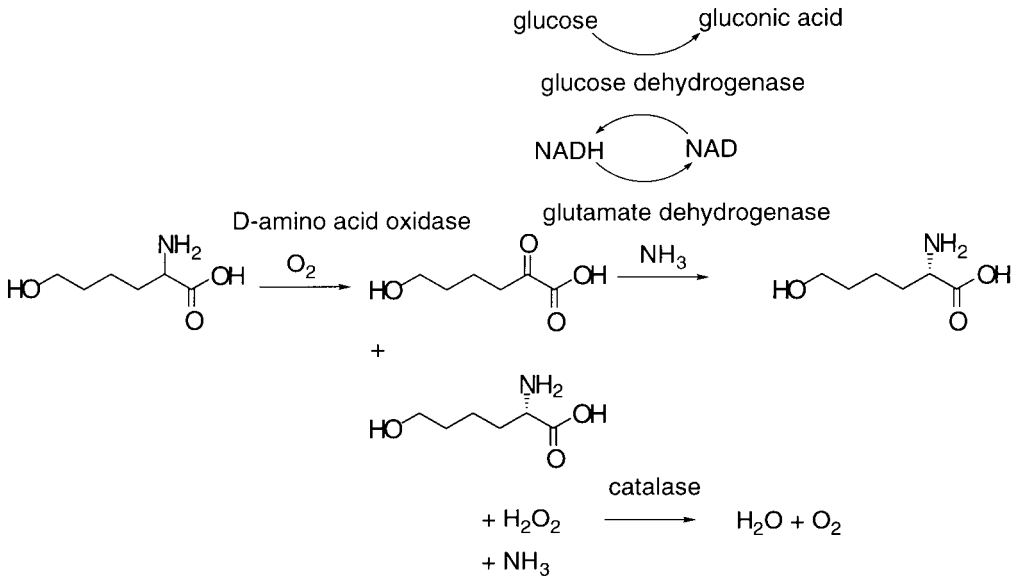


Figure 1. Kinetics of reductive amination using beef liver glutamate dehydrogenase. The reaction conditions are described under Experimental.



2-Keto-6-hydroxyhexanoic acid, sodium salt (4,5). A 2 L flask equipped with a large magnetic stir bar was charged with crushed magnesium turnings (15.23 g, 626.5 mmol). The flask was flame-dried under high vacuum and allowed to cool under argon. In a separate flame-dried 2 L flask, **1** (103.4 g, 464.1 mmol) was dissolved in THF (600 mL). A portion of the solution of **1** (~150 mL) was added via cannula into the reaction flask containing the magnesium. With stirring, 1,2-dibromoethane (4 mL, 46.4 mmol) was added dropwise (Caution!! Exothermic up to 45–50°C). With external

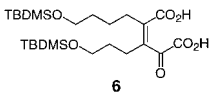


Scheme 2. Conversion of racemic 6-hydroxynorleucine to L-6-hydroxynorleucine.

heating to maintain a reflux, the remaining solution of **1** was added via cannula over 1 h. The flask containing the solution of **1** was rinsed with THF (100 mL), and the rinse was added to the reaction. After the addition was complete, the reaction was refluxed for 2 h. The reaction was then allowed to cool to room temperature.

A flame-dried 2 L flask equipped with a large magnetic stir bar was charged with diethyl oxalate (76 mL, 556.9 mmol) and THF (400 mL). The solution was cooled to −75°C. The Grignard reagent solution was added via cannula to the diethyl oxalate solution over 1 h (maximum internal temperature was −65°C). After the addition was complete, the reaction was kept at −60°C for 2 h. The cold reaction mixture was poured into a mixture of ice (530 g), 2*N* HCl (360 mL) and saturated NaCl (360 mL). The mixture was shaken and immediately extracted with EtOAc (1×1000 mL and 3×500 mL). The combined extracts were washed with half-saturated $NaHCO_3$ (780 mL) and saturated NaCl (780 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo to a light yellow oil. The oil was concentrated twice from hexane, and the residual solvents were removed under high vacuum (1 h) to give a yellow oil (152 g) which by ^{1}H NMR consisted of 82 wt% **2** (94% corrected crude yield), 3 wt% **1**, and 13 wt% diethyl oxalate: TLC R_f = 0.43 (hexane:EtOAc, 5:1); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 4.30 (m, CH_2 + diethyl oxalate), 3.58 (2H, t, J = 6.2 Hz, CH_2), 2.83 (2H, t, J = 7.3 Hz, CH_2), 1.65 (2H, m, CH_2), 1.52 (2H, m, CH_2), 1.32 (m, CH_3 + diethyl oxalate), 0.85 (9H, s, $C(CH_3)_3$), 0.0 (6H, s, $Si(CH_3)_2$); ^{13}C NMR ($CDCl_3$, 270 MHz) δ 194.8, 161.4, 63.3, 62.8, 39.2, 32.9, 26.1, 19.7, 18.5, 14.2, −5.2.

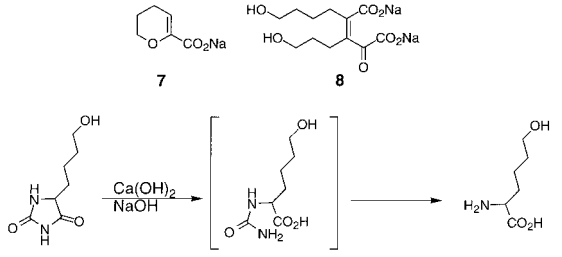
A 2 L flask equipped with a large magnetic stir bar and an internal temperature probe was charged with methanol (700 mL) and cooled to 0°C. 2.0 N NaOH (345 mL, 690 mmol) was added slowly, and the solution was allowed to cool back to 0°C. A solution of crude **2** (70.7 g, 201.7 mmol of **2** + 63.4 mmol of diethyl oxalate; see above) in methanol (300 mL) was added dropwise over 1 h (maximum internal temperature was 2°C). The flask containing the solution of **2** was rinsed with methanol (50 mL), and the rinse was added to the reaction. After the addition was complete, the reaction was stirred for 20 min. The pH of the reaction mixture was adjusted to 7.15 with 2 M $KHSO_4$ (195 mL). The methanol was removed in vacuo, and the mixture was transferred into a separatory funnel with a water rinse (100 mL) of the reaction flask. Water (740 mL) was added to the mixture, and the resulting solution was washed with MTBE (2×425 mL). The aqueous layer was transferred into a clean 3 L flask and cooled to < 5°C. The pH of the aqueous layer was adjusted to 4 with 2 M $KHSO_4$. EtOAc (565 mL) was added with stirring, and the pH of the aqueous layer was further adjusted to 2.45 with 2 M $KHSO_4$ (a total of 176 mL of 2 M $KHSO_4$ was used). The EtOAc layer was separated, and the aqueous layer was further extracted with EtOAc (2×565 mL). The combined EtOAc extracts were dried over $MgSO_4$, filtered and concentrated in vacuo to give a yellow oil (46 g) which by ^{1}H NMR consisted of 87 wt% **3** (76% corrected crude yield), 2 wt% **6**, and 11 wt% EtOAc: ^{1}H NMR (DMSO-d_6, 270 MHz) δ 3.55 (2H, t, J = 6.2 Hz, CH_2), 2.77 (2H, t, J = 7.3 Hz, CH_2), 1.46 (4H, m, CH_2CH_2), 0.84 (9H, s, $C(CH_3)_3$), 0.0 (6H, s, $Si(CH_3)_2$); ^{13}C NMR (DMSO-d_6, 270 MHz) δ 197.8, 164.2, 63.6, 61.1, 39.4, 32.8, 27.1, 19.3, −4.04.



6

A 1 L flask equipped with a magnetic stir bar, internal temperature probe, and addition funnel was charged with crude **3** (45.2 g, 150 mmol of **3** + 1.7 mmol of **6**; see above) and CH_3CN (450 mL). The solution was cooled

to 2°C. The addition funnel was charged with 0.10 M $KHSO_4$ (150 mL, 15 mmol). The $KHSO_4$ solution was added to the reaction over 3–5 min. The maximum temperature of the reaction during addition was 2.7°C. The reaction was stirred 10 min more, and then was warmed to room temperature over 1 h. After stirring for 1 h at room temperature the pH was adjusted to 7.85 with 1 N NaOH (180 mL). The CH_3CN was removed in vacuo, and the resulting heterogeneous mixture was transferred into a separatory funnel with an MTBE rinse (150 mL) and two water rinses (2×50 mL). The mixture was shaken, and the aqueous layer (now pH 7.25) was separated and extracted again with MTBE (1×150 mL). The aqueous layer was transferred to a clean 1 L flask and placed on a rotary evaporator for 45 min to remove volatile organics. The aqueous solution was frozen at −78°C and lyophilized for 22 h to give a white solid (28.5 g) which consisted of 81 wt% 2-keto-6-hydroxyhexanoic acid, sodium salt (91% corrected crude yield; 33 wt% keto form **4** and 48wt% hemiketal form **5** (^{1}H NMR)), 2.5 wt% **7**, 6 wt% **8** (both by ^{1}H NMR), and 8 wt% $NaKSO_4$ (according to theory): mp (uncorrected) 139–141°C (decomp.); ^{1}H NMR (D_2O, 270 MHz) δ 3.86 (0.6H, m, OCH hemiketal form), 3.68 (0.6H, m, OCH hemiketal form), 3.59 (0.8H, t, OCH_2 keto form), 2.75 (0.8H, t, CH_2 keto form), 1.6 (5.2H, complex m); ^{13}C NMR (D_2O, 270 MHz) δ 209, 179, 172, 98, 64, 63, 41, 34, 33, 26, 21, 20; IR (KBr pellet ν_{max} 3400, 2932, 1734 (weak), 1628, 1423 cm^{-1}; FAB–MS *m/z* 167 (M-H, $C_6H_9O_4Na$ requires 167); K-F moisture 2.30; Elem. Anal. calcd for $C_6H_9O_4Na$•0.03 $C_6H_7O_3Na$•0.04 $C_{12}H_{16}O_7Na_2$•0.27 H_2O•0.11 $NaKSO_4$: C, 38.55; H, 5.05; Na, 13.49. Found: C, 38.05; H, 4.90; Na, 13.70.



6-Hydroxynorleucine from 5-(4-hydroxybutyl)hydantoin. In a 400 mL Parr pressure reactor was placed (34.4 g, 0.2 mol) of 5-(4-hydroxybutyl)hydantoin, 80 mL of DI water, (14.8 g, 0.2 mol) of calcium hydroxide and (8 g, 0.2 mol) of sodium hydroxide. The reaction mixture was sealed, heated to 140°C and stirred for 5 h. After cooling to room temperature, the salt was filtered and the cake was washed with 2×35 mL of deionized water. To the filtrate was added 20 g of ammonium carbonate. The mixture was heated to reflux for 10 min, cooled to room temperature and filtered. The cake was washed with 20 mL of water. The pH of the filtrate was adjusted from 9.8 to 5.9 using acetic acid. Water was then removed on rotary evaporator to give about 70 g wet solid. 200 mL of absolute ethanol was added. The slurry was stirred for 30 min and filtered. The white cake was washed with 2×50 mL absolute ethanol and dried to afford 18.1 g (56%) of racemic 6-hydroxynorleucine.

L-6-Hydroxynorleucine from ketoacid. NH_4OH (2.02 mL of 14.8 M, 30 mmoles) in 10 mL of water was adjusted to pH 8.75 with 12.1 M HCl. Glucose (5.404 g, 30 mmol), substrate[19] (3 g, 13.12 mmol), NAD (20.56 mg, 30 μmol), and dithiothreitol (4.62 mg, 30 μmol) were added, and the volume was brought to 27 mL with water. Glucose dehydrogenase (2 mg containing 72.9 units/mg) and beef liver glutamate dehydrogenase (3 mL containing 19 mg protein/mL, 40 units/mg) were added to start the reaction. The reaction was carried out at 30°C and the pH was maintained at 8.75 by addition of 3 M NH_4OH using a Brinkmann pH stat. HPLC analysis indicated that the reaction was complete after 3 h (Fig. 1). After 25 h the reaction was stopped, and the yield, determined by HPLC analysis, was 11.62 mmol (88.6%). After the reaction was complete, the mixture was heated at 100°C for 4–5 min, then centrifuged at 5000 *g* for 15 min to remove precipitated proteins. The volume was then reduced by removing water and ammonium hydroxide at 25–28 psi and 58–68°C. The pH of the concentrated solution was adjusted to 2 with 6 N HCl, followed by chromatography over a Dowex-50W X8-200 column (4×42 cm). L-6-Hydroxynorleucine was eluted from the column with 1M NH_4OH. The product was isolated from the combined rich fractions by removing water and NH_4OH. The isolated yield was 80% and the optical purity was >99%. ^{1}H NMR (D_2O) δ 1.64 (m, 2 H), 1.82 (m, 2H), 2.05 (m, 2H), 3.82 (t, 2H), 3.91 (t, 1H); ^{13}C NMR (D_2O) δ 19.0, 27.5, 29.2, 44.6, 56.5, 172.5: ms, *m/z*: 148 (m + H), 295 (2m + H)

L-6-Hydroxynorleucine from racemic 6-hydroxynorleucine via porcine kidney oxidase. Racemic 6-hydroxynorleucine (0.5 g) in 20 mL 50 mM potassium phosphate buffer (pH 7) was treated with porcine kidney D-amino acid oxidase (49 u, 350 mg) and beef liver catalase (182 u, 8 mg) to give a mixture of ketoacid and 0.236 g L-6-hydroxynorleucine with 97% o.p. The enzymes were added in increments and the reaction took 10 days to complete. The reductive amination was then carried out as described above. 0.484 g of 6-hydroxynorleucine was produced (97% yield) with >99% o.p. by HPLC assay.

L-6-Hydroxynorleucine from racemic 6-hydroxynorleucine via *Trigonopsis* oxidase. *Trigonopsis variabilis* ATCC10679 was grown in a 250 L fermentor at 28°C on the medium containing (g/L): KH_2PO_4, 5; $MgSO_4$. $7H_2O$, 1; $CaCl_2$, 0.5; H_3BO_3, 0.1; $(NH_4)_2MoO4$, 0.04; $MnSO_4.4H_20$, 0.04; $ZnSO_4.7H_2O$; 0.04; $CuSO_4.5H_2O$; 0.045; $FeSO_4.7H_2O$, 0.025; DL-methionine, 3; biotin, 0.02; thiamine, 0.1; cysteine, 0.72; cerelose hydrate, 22. Cell paste (129 g) was harvested 50 h after inoculation and stored frozen. Racemic 6-hydroxynorleucine (0.514 g) in 70 mL 50 mM potassium phosphate buffer, pH 7, was shaken with 14 g washed *Trigonopsis variabilis* ATCC 10679 cells for 21 h at 28°C and 200 rpm to give L-6-hydroxynorleucine with o.p. >99%. Cells were removed by centrifugation, and the supernatant was treated with glutamate dehydrogenase, glucose dehydrogenase, NH_3, glucose and NAD as described above. L-6-Hydroxynorleucine (0.469 g, 91% yield) was obtained with >99% o.p. After the completion of the

reaction, L-6-hydroxynorleucine was isolated as described above. The isolated yield was 0.394 g (76.6%) and the optical purity was 99%. The ^{1}H NMR spectrum agreed with the structure.

References and Notes

1. Robl, J. A.; Sun, C.; Stevenson, J.; Ryono, D. E.; Simpkins, L. M.; Cimarusti, M. P.; Dejneka, T.; Slusarchyk, W. A.; Chao, S.; Stratton, L.; Misra, R. N.; Bednarz, M. S.; Asaad, M. M.; Cheung, H. S.; Aboa-Offei, B. E.; Smith, P. L.; Mathers, P. D.; Fox, M.; Schaeffer, T. R.; Seymour, A. A.; Trippodo, N. C. *J. Med. Chem.* **1997**, *40*, 1570.
2. Robl, J. A.; Cimarusti, M. P. *Tetrahedron Lett.* **1994**, *35*, 1393.
3. Maurer, P. J.; Miller, M. J. *J. Org. Chem.* **1981**, *46*, 2835.
4. Maurer, P. J.; Miller, M. J. *J. Am. Chem. Soc.* **1982**, *104*, 3096.
5. Maurer, P. J.; Miller, M. J. *J. Am. Chem. Soc.* **1983**, *105*, 240.
6. Culvenor, C. C. J.; Foster, M. C.; Hegarty, M. P. *Aust. J. Chem.* **1971**, *24*, 371.
7. Bodanszky, M.; Martinez, J.; Priestly, G. P.; Gardner, J. D.; Mutt, V. *J. Med. Chem.* **1978**, *21*, 1030.
8. Dreyfuss, P. *J. Med. Chem.* **1974**, *17*, 252.
9. Kern, B. A.; Reitz, R. H. *Agric. Biol. Chem.* **1978**, *42*, 1275.
10. Kaufman, S.; Mason, K. *J. Biol Chem.* **1982**, *257*, 14667.
11. Belyaev, A. A. *Tetrahedron Lett.* **1995**, *36*, 439.
12. Bommarius, A. S. In *Enzyme Catalysis in Organic Synthesis*; Drauz, K.; Waldmann, H., Eds.; VCH: Weinheim, 1995; Vol II, pp 633–641 and cited references.
13. Galkin, A.; Kulakova, L.; Yoshimura, T.; Soda, K.; Esaki, N. *Appl. Environ. Microbiol.* **1997**, *63*, 4651.
14. Hanson, R. L.; Singh, J.; Kissick, T. P.; Patel, R. N.; Szarka, L. J.; Mueller, R. H. *Bioorganic Chemistry* **1990**, *18*, 116.
15. Gaudry, R. *Can. J. Res. (B)* **1948**, *26*, 387.
16. Oshima, T.; Takada, H.; Yoshimura, T.; Esaki, N.; Soda, K. *J. Bacteriol.* **1991**, *173*, 3943.
17. Oshima, T.; Nishida, N.; Bakthavatsalam, S.; Kataoka, K.; Takada, H.; Yoshimura, T.; Esaki, N.; Soda, K. *Eur. J. Biochem.* **1994**, *222*, 305.
18. Wei, C.; Huang, J.; Tsai, Y. *Biotechnol. Bioeng.* **1988**, *34*, 570.
19. This material is a mixture containing 6.6 wt% 3,4-dihydro-2H-pyran-6-carboxylic acid, sodium salt, 48.4 wt% 2-hydroxy-tetrahydropyran-2-carboxylic acid, sodium salt, 25.1 wt% 2-keto-6-hydroxyhexanoic acid, sodium salt, 14 wt% NaCl, and 6 wt% $NaHCO_3$. The actual substrates for the enzyme reaction are underlined and comprise 73.5% of this mixture.