Divergent synthesis of *N*-hydroxy-l-indospicine, the carbon isostere of *N*-hydroxy-l-arginine, and *N*-hydroxy-l-homoarginine from l-glutamate

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Dedicated to Emeritus Professor Keiichiro Fukumoto on his 70th birthday

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Abstract

The carbon isostere of *N*-hydroxy-L-arginine, *N*-hydroxy-L-indospicine, and *N*-hydroxy-L-homoarginine, were prepared by divergent synthesis as possible NOS substrates, from L-glutamate.

Keywords: Nitric oxide, *N*-hydroxy-L-arginine, *N*-hydroxy-L-indospicine, *N*-hydroxy-L-homoarginine, benzyl *N*-butoxycarbonyl-γ-glutamate, synthesis

Introduction

Biologically important nitric oxide (NO)[1] is biosynthesized by nitric oxide synthases (NOSs) during the conversion of L-arginine (**1**) into L-citrulline (**3**) through *N*-hydroxy-L-arginine (L-NOHA) (**2**).[2] We have reported that *N*-hydroxyagmatine (**4**), a decarboxylated NOHA, caused the relaxation of cardiovascular muscle, albeit less effectively than does NOHA itself[3] and that NO was evolved when *N*-hydroxyphenethylguanidine (**5**) was subjected to photo-sensitized oxidation in a model study for biological NO generation.[4] In this paper we present the divergent synthesis of *N*-hydroxy-L-indospicine (L-NOHI) (**6**), the carbon isostere of L-NOHA, and *N*-hydroxy-L-homoarginine (L-homoNOHA) (**7**), as alternative NOS substrates, from a L-glutamate derivative.

Results and Discussion

We planned to synthesize L-NOHI (**6**) and L-homoNOHA (**7**) from the L-glutamate derivative **8**, through the norvaline derivative **9** as a common intermediate, as shown in Scheme 1. When we started our synthetic study, Feldman *et al*.[5] had just reported the preparation of L-indospicine itself from L-glutamate using a similar synthetic strategy, in which a protected L-NOHI **19** (see Scheme 3) was also prepared. Thus, we decided to follow their basic method for our purpose.

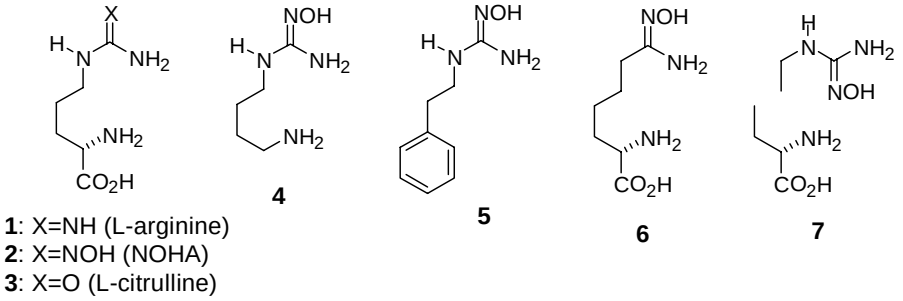
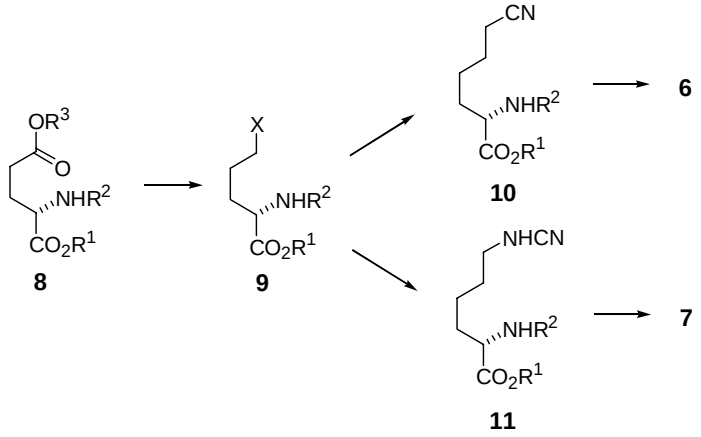


Figure 1

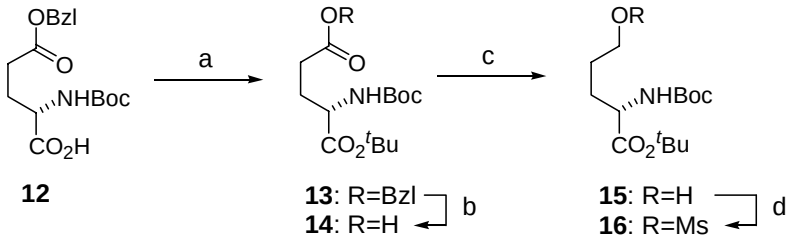


Scheme 1. Synthetic plan of L-NOHI (**6**) and L-homoNOHA (**7**) from the glutamate **8**.

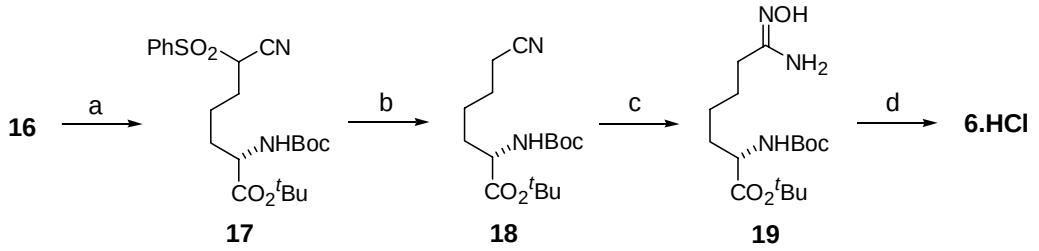
After modification of the reported method,[6] hydroxynorvaline **15** was prepared from γ-benzyl (Bzl) *N*-butoxycarbonyl (Boc)-L-glutamate (**12**) by the three steps of esterification with *N*,*N*-dimethylformamide di-t-butylacetal, catalytic hydrogenation over Pd–C, and reduction with $NaBH_4$ after conversion into a mixed anhydride with ethyl ortho-chloroformate. The alcohol **15** was then converted into its mesylate[5] **16** as a common precursor for the target compounds (Scheme 2).

At first, we focused on the preparation of L-NOHI (**6**) (Scheme 3). Feldman *et al.*[5] had reported the preparation of the cyanophenylsulfonyl hexanoate **17** from the mesylate **16** through an iodide. Although smooth conversion (>86%) of **16** to the iodide had been reported, we obtained an unsatisfactory result (24% yield). Examination of the reaction conditions led to an improvement of the yield to 63%, when ethanol was used as a solvent in place of acetonitrile.

However, a proline derivative – a cyclized product – was always produced as a side product, in *ca* 40% yield. Trials for the protection of the nitrogen function of **16** as a diBoc group failed.

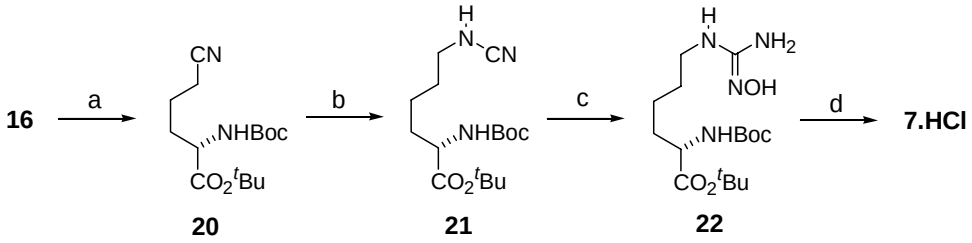


Scheme 2. Preparation of the common intermediate **16** from the L-glutamate **12**: (a). $(t\text{-}BuO)_2CHNMe_2$/PhH, reflux, 22 h (75%); (b). H_2/10% Pd–C/EtOH, RT, 20 h, (quant.); (c). (i) $NEt_3/ClCO_2Et$/THF, -10 °C, 50 min. (ii) $NaBH_4/H_2O$/THF, RT, 3 h (84% in 2 steps); (d). MsCl/pyridine/$CHCl_3$, RT, 2.5 h (95%).



Scheme 3. Preparation of L-NOHI.HCl (**6.HCl**): (a).$PhSO_2CH_2CN$/NaH/THF, 50 °C, 23 h (quant.); (b). 5% Na–Hg/THF/MeOH, RT, 1.5 h (28%); (c). $NH_2OH.HCl/NaHCO_3/H_2O$/EtOH, 60 °C, 5d (30%); (d). HCl (g)/dioxane, RT 1.7 h (quant.).

Fortunately, direct elongation of **16** with phenylsulfonylacetonitrile in THF in the presence of sodium hydride gave a desired cyanophenylsulfonyl hexanoate **17**, quantitatively. Feldman *et al.*[5] reported successful desulfonylation of **17** with aluminum amalgam in a mixed solvent of THF–MeOH–H_2O; however, in our case, no reaction was observed, even after modification of the reaction conditions. Thus, we examined the conditions for desulfonylation of **17** using either sodium amalgam or Raney nickel. Treatment with 5% sodium amalgam in a mixture of THF and MeOH (5:1) gave a nitrile derivative **18**, albeit in moderate yield (28%). Oximation of **18** with hydroxylamine afforded a protected NOHI, **19**, in nearly the same yield (30%) as reported.[5] Deprotection of **19** with hydrogen chloride in dioxane smoothly afforded a desired NOHI (**6**) hydrochloride as a colorless viscous oil, which was characterized by spectroscopic means such as HRMS and NMR. The optical rotation, $[\alpha]_{589}^{18}$, showed +13.1 (*c* 2.1x10^{-3}, MeOH).



Scheme 4. Preparation of L-homoHONA.HCl (**7.HCl**): (a). NaCN/DMF, 60 °C, 1.5 h (76%): (b). (i) H_2/5%Rh–Al/10% NH_4OH/EtOH, RT, 2.5 kg/cm^3, 22h, (ii) BrCN/NaOAc/MeOH, -10°C, 3.2 h (13% in 2 steps); (c). $NH_2OH.HCl/NEt_3$/EtOH, RT, 50 min (88%); (d). See **d** in Scheme 2 (quant.).

Next, we tried to synthesize L-homoNOHA **(7)** from the mesylate **16** (Scheme 4). After conversion of **16** into a nitrile **20** by a conventional method, and reduction of the nitrile function to an amine, one method was examined under several conditions ($NaBH_4$ in the presence of CF_3COOH or $CoCl_2$, diborane, or catalytic hydrogenation using 5% Rh–Al_2O_3 or PtO_2 as catalyst). A desired lysine derivative was produced when **20** was hydrogenated over 5% Rh–Al_2O_3 in 10% NH_3–EtOH under a pressure of 2.5 kg/cm^2. Since isolation of the lysine had failed, the crude product was treated with cyanogen bromide without isolation to give the *N*-cyanamide **21** in 13% yield.

Oximation of **21**, followed by deprotection as described above, afforded a desired homoHONA (**7**) hydrochloride as a pale yellow viscous oil in 88% yield in two steps. This also was characterized by spectroscopic means, such as HRMS and NMR. The optical rotation, $[\alpha]_{589}^{19}$, was +12.8 (*c* 1.95x10^{-3}, MeOH).

Conclusions

In conclusion, we have synthesized L-NOHI (**6**) and L-homoNOHA (**7**) from the L-glutamate derivative **12** through the hydroxynorvaline derivative **16** as a common synthetic precursor. The overall yields were 5% and 4.9%, respectively. NOHI has also been synthesized in a racemic form,[7,8] and the synthesis[8] of L-homoNOHA (**7**) from a lysine derivative was reported just before our work was finished. Thus, their possibilities as NOS substrates have been examined and weak activity had been observed on racemic NOHI, whereas L-homoHONA **(7)** showed significant NO production. Our products synthesized here will be tested independently to act as NOS substrates in our estimation system using aortic rinds.[3]

Experimental Section

General Procedures. All melting points were measured on a micro melting-point hot stage (Yanagimoto) and are uncorrected. IR spectra were recorded on a JASCO FT/IR-300E spectrophotometer. ^{1}H-, and ^{13}C-NMR spectra were recorded in $CDCl_3$ on a JEOL JNM-LA400 (400 MHz), and tetramethylsilane (0.00 ppm) and the middle peak of $CDCl_3$ (77.0 ppm) being used as internal standards. FAB MS and HR FAB MS were recorded on a JMS-HX110 with *m*-nitrobenzyl alcohol as a matrix. Optical rotations, $[\alpha]_{589}$, were measured with a JASCO J-20 spectropolarimeter. Reactions were monitored by TLC on Kieselgel 60 F254 (Merck, 5715). Column chromatography was performed on silica gel (Fuji Silysia, FL100D). RT denotes room temperature.

α-t-Butyl γ-Bzl-*N*-boc-L-glutamate (13). A mixture of **12** (6.50 g, 19.3 mmol) and *N*,*N*-dimethylformamide di-t-butylacetal (25 mL, 104 mmol) in dry benzene (43 mL) was heated at reflux for 22 h under Ar. After addition of H_2O the mixture was extracted with AcOEt. The organic solution was washed with H_2O, sat. aq. $NaHCO_3$, and brine, dried (K_2CO_3), and evaporated. Column chromatography of the residue with AcOEt–hexane (1:8) gave **13** as a colorless oil (5.89 g, 75%). υ ($CHCl_3$) 3433, 1729. δ_H 1.43, 1.46 (each 9H, s, t-Bu), 1.88–1.97 (1H, m, 3- or 4-H), 2.08–2.24 (1H, br s, 3- or 4-H), 2.36–2.53 (2H, m, 3-, 4-H), 4.20 (1H, br s, 2-H), 5.12 (3H, fine splitting, NH, OCH_2Ph), 7.32–7.36 (5H, m, Ph). FAB MS *m/z*: 394 (MH^+).

t-Butyl *N*-boc-L-glutamate (**14**). A solution of **13** (2.17 g, 5.51 mmol) in EtOH (21 mL) was hydrogenated over 10% Pd–C (0.124 g) at RT and 1 atm. for 20 h. After filtering off the catalyst, using a Celite pad, the filtrate was evaporated to give **14** as a pale yellow oil (1.76 g, quant.), which solidified in the refrigerator. ν ($CHCl_3$) 3431, 1712. δ_H 1.44, 1.47 (each 9H, s, t-Bu), 1.92, 2.15 (each 1H, m, 3-H), 2.44 (2H, m, 4-H), 4.22 (1H, br. s, 2-H), 5.17 (1H, d- like, NH, exchangeable).

t-Butyl 2-(boc-amino)-5-hydroxypentanoate (15). To a solution of **14** (1.53 g, 5.06 mmol) and NEt_3 (3.5 mL, 25.1 mmol) in THF (21 mL) was added ethyl chloroformate (2.5 mL, 26.1 mmol) at -10 °C. The mixture was stirred at -10 °C for 50 min, and insoluble materials were filtered off. The filtrate was added to a solution of $NaBH_4$ (0.956 g, 25.3 mmol) in a solution of THF (20 mL) and H_2O (5 mL) under ice-cooling and the whole was stirred at RT for 5 h. After acidification with dil. HCl (to pH *ca* 2), the mixture was extracted with AcOEt. The organic solution was washed with 10% NaOH aq., H_2O, then brine, dried ($MgSO_4$), and evaporated. Column chromatography of the residue with AcOEt–hexane (1:3) gave **15** as a pale yellow oil (1.08 g, 74%). υ ($CHCl_3$) 3436, 1708. δ_H 1.45, 1.47 (each 9H, s, t-Bu), 1.66 (3H, m, 3-, 4-H), 1.89 (1H, m, 4- or 3-H), 3.69 (2H, q, *J*=5.6 Hz, 5-H), 4.22 (1H, br. d, *J*=7.2 Hz, 2-H), 5.14 (1H, br. d, *J*=7.2 Hz, NH). FABMS *m/z*: 290 (MH^+).

t-Butyl 2-(boc-amino)-5-mesyloxypentanoate (16). To a solution of **15** (0.70 g, 2.43 mmol) and pyridine (0.58 mL, 7.24 mmol) in $CHCl_3$ (5 mL) was added mesyl chloride (0.38 mL, 4.91 mmol) under ice-cooling and the whole was stirred at RT for 2.5 h. After evaporation of solvent, the residue was partitioned with AcOEt and H_2O The organic solution was washed with sat. aq.$CuSO_4$, sat. aq. $NaHCO_3$, and brine, dried (K_2CO_3), and evaporated. Column

chromatography (Merck, 7734) of the residue with $CHCl_3$–AcOEt (20:1) gave **16** as a colorless oil (0.845 g, 95%). ν ($CHCl_3$) 3434, 1709. δ_H 1.37, 1.41 (each 9H, s, t-Bu), 1.75 (4H, m, 3-, 4-H), 2.95 (3H, s, OMe), 4.17 (1H, br., 2-H), 4.19 (2H, t, *J*=6.4 Hz, 5-H), 5.02 (1H, br. d, *J*=7.3 Hz, NH). HR FAB MS *m/z*: 368.1749 (Calcd for $C_{15}H_{30}NO_7S$: 368.1743).

t-Butyl 2-(boc-amino)-6-cyano-6-phenylsulfonylhexanoate (17). A mixture of cyanomethyl benzenesulfinate (0.33 g, 1.8 mmol) and NaH (60%, 0.05 g, 1.24 mmol) in THF (1.5 mL) was stirred at RT for 1.3 h. To this mixture was added a solution of **16** (0.30 g, 0.82 mmol) in THF (1.5 mL) and the whole was stirred at 50 C for 23 h. The reaction mixture was partitioned with AcOEt and H_2O, the organic solution washed with H_2O and brine, dried ($MgSO_4$), and evaporated. Column chromatography of the residue with AcOEt–hexane (1:2) gave **17** as a colorless oil (0.372 g, quant.). ν (neat) 3385, 2247, 1715. δ_H 1.43, 1.47 (each 9H, s, t-Bu), 1.50–2.04 (5H, m, 3-, 4-, 5-H), 2.23 (1H, m, 5-H), 3.95 (0.5 H, dd, *J*=10.8, 4.7 Hz, 6-H), 4.02 (0.5 H, dd, *J*=11.1, 3.8 Hz, 6-H), 4.17 (1H, br. d, *J*=5.9 Hz, 2-H), 5.12 (1H, t-like, NH), 7.66 (2H, t, *J*=7.8 Hz, 3'-H), 7.78 (1H, t, *J*=7.8 Hz, 4'-H), 8.02 (2H, m, 2'-H). FABMS *m/z*: 453 (MH^+).

t-Butyl 2-(boc amino)-6-cyanohexanoate (18). A mixture of **17** (0.64 g, 1.41 mmol) and 5% Na–Hg (0.763 g) in a mixed solution of THF (6.4 mL) and MeOH (1.3 mL) was stirred at RT for 1.5 h. After removal of inorganic materials by filtration, the filtrate was evaporated. Column chromatography of the residue with AcOEt–hexane (1:3) gave **18** as a colorless oil (0.122 g, 28%). ν (neat) 3372, 2247, 1715. δ_H 1.45, 1.48 (each 9H, s, t-Bu), 1.50–1.86 (6H, m, 3-, 4-, 5-H), 2.35 (2H, t, *J*=7.2 Hz, 6-H), 4.19 (1H, q-like, *J*=7.3 Hz, 2-H), 5.07 (1H, d, *J*=7.3 Hz, NH). FABMS *m/z*: 313 (MH^+).

t-Butyl 2-(boc amino)-6-hydroxyamidinohexanoate (19). To a solution of **18** (0.02 g, 0.06 mmol) in a mixed solution of EtOH (0.3 mL) and H_2O (0.12 mL) was successively added $NH_2OH.HCl$ (0.009 g, 0.131 mmol) and $NaHCO_3$ (0.011 g, 0.129 mmol). The whole was stirred at 70 C for 3 days and then at 50 C for 2 days. The reaction mixture was partitioned with AcOEt and H_2O, the organic solution washed with H_2O and brine, dried ($MgSO_4$), and evaporated. Preparative TLC of the residue with $CHCl_3$–MeOH (8:1) gave **19** as a colorless oil (0.007 g, 30%). ν (neat) 3489, 3370, 2979, 1705, 1662. δ_H 1.44, 1.46 (each 9H, s, t-Bu), 1.33–1.52 (2H, m, 4-H), 1.52–1.85 (4H, m, 3-, 5-H), 2.14 (2H, t, *J*=7.6 Hz, 6-H), 4.17 (1H, q-like, *J*=7.8 Hz, 2-H), 4.56 (2H, s, NH), 5.17 (1H, d, *J*=7.8Hz, NH). FABMS *m/z*: 346 (MH^+).

L-NOHI.2HCl; (6.2HCl). A protected NOHI **19** (0.022 g, 0.063 mmol) was dissolved in dry dioxane (0.31 mL) saturated with HCl gas and the whole was kept at RT for 1.7 h. The solvent was removed by decantation, followed by drying under the stream of Ar to give **6.**2HCl as a colorless viscous oil (0.018 g, quant.) δ_H (D_2O) 1.41–1.57 (2H, m, 4-H), 1.74 (2H, quint., *J*=7.6 Hz, 5-H), 1.87–2.01 (2H, m, 3-H), 2.49 (2H, t, *J*=7.6 Hz, 6-H), 3.97 (1H, s- like, 2-H). δ_C (D_2O) 24.2 (C4), 26.3 (C5), 29.0 (C3), 30.2 (C6), 54.1 (C2), 165.2 (C=N), 173.6 (CO). HR FAB MS *m/z*: 190.1197 (Calcd for $C_7H_{16}N_3O_3$: 190.1192). $[\alpha]_{589}^{18}$ +13.1 (c 2.1x10^{-3}, MeOH).

t-Butyl 2-(boc amino)-5-cyanopentanoate (20). A mixture of the mesylate **16** (0.145 g, 0.39 mmol) and NaCN (0.058 g, 1.18 mmol) in DMF (1.4 mL) was stirred at 60 C for 1.5 h. The reaction mixture was partitioned with AcOEt and H_2O. The organic solution was washed with

H_2O and brine, dried (K_2CO_3), and evaporated. Column chromatography of the residue with AcOEt–hexane (1:8) gave **20** as a colorless oil (0.090 g, 76%). ν ($CHCl_3$) 3433, 2250, 1708. δ_H 1.45, 1.48 (each 9H, s, t-Bu), 1.67–1.98 (4H, m, 3-, 4-H), 2.41 (2H, m, 5-H), 4.20 (1H, d- like, 2-H), 5.09 (1H, d- like, NH). HR FAB MS *m/z*: 299.1971 (Calcd for $C_{15}H_{30}NO_7S$: 299.1971).

t-Butyl 2-(boc amino)-6-cyanoaminohexanoate (21). A solution of **20** (0.273 g, 0.913 mmol) in 10% NH_3–EtOH (1.6 mL) was hydrogenated over 5% Rh–Al (0.152 g) at RT for 22 h under hydrogen pressure (2.5 kg/cm^2). After removal of the catalyst by filtration (Celite pad) the filtrate was evaporated. The crude oil (0.276 g) was dissolved in anhydrous MeOH (5 mL). To the solution was added a solution of BrCN (95%, 0.116 g, 1.04 mmol) in anhydrous MeOH (0.5 mL) at -10 °C in the presence of anhydrous AcONa (0.149 g, 1.82 mmol) and the whole was stirred at -10 °C for 3.2 h. After evaporation the residue was partitioned with AcOEt and H_2O. The organic solution was washed with H_2O and brine, dried (K_2CO_3), and evaporated. Purification of the residue by column chromatography with AcOEt–hexane (1:2) followed by preparative TLC with AcOEt–hexane (2:3) gave **21** as a colorless oil (0.038 g, 13%).ν ($CHCl_3$) 3433, 3247, 2224, 1707. δ_H 1.45, 1.47 (each 9H, s, t-Bu), 1.55–1.70 (4H, m, 3- or 4-H), 1.74–1.90 (2H, m, 5-H), 3.01–3.20 (2H, m, 6-H), 4.13 (2H, s- like, 2-H, NH), 5.11 (1H, d- like *J*=7.8 Hz, NH). HR FAB MS *m/z*: 328.2225 (Calcd for $C_{16}H_{30}N_3O_4$: 328.2236).

t-Butyl N^{α} -boc-N^{ω}-hydroxyhomoarginine (22). To a solution of **21** (0.039 g, 0.119 mmol) in EtOH (0.4 mL) was added $NH_2OH.HCl$ (0.019 g, 0.267 mmol) and NEt_3 (1 drop) and the whole was stirred at RT for 40 min. After evaporation, column chromatography of the residue with $CHCl_3$–MeOH (4:1) gave **22** as a colorless oil (0.038 g, 88%). ν (neat) 3420, 3320, 3260, 3156, 1699, 1662. δ_H 1.43, 1.46 (each 9H, s, t-Bu), 1.56–1.90 (6H, m, 3-, 4-, 5-H), 3.30 (2H, br. s, 6-H), 4.08 (1H, d like, *J*=5.6 Hz, 2-H), 5.11 (1H, d like, *J*=7.6 Hz, NH). HR FAB MS *m/z*: 361.2446 (Calcd for $C_{16}H_{33}N_4O_5$: 361.2451).

L-Homo-NOHA.2HCl (6.2HCl). Treatment of **22** (0.026 g, 0.071 mmol) with the HCl-saturated dioxane (0.36 mL) described above gave **6**.2HCl as a pale yellow viscous oil (0.026 g, quant.) δ_H (D_2O) 1.41–1.55 (2H, m, 4-H), 1.67 (2H, quintet, *J*=7.1 Hz, 5-H), 1.88–2.03 (2H, m, 3-H), 3.25 (2H, t, *J*=6.1 Hz, 6-H_2), 4.03 (1H, t, *J*=6.1 Hz, 2-H). δ_C (D_2O) 22.3 (C4), 28.2 (C5), 30.2 (C3), 41.4 (C6), 53.7 (C2), 159.6 (C=N), 173.1 (CO). HR FAB MS *m/z*: 205.1288 (Calcd for $C_7H_{17}N_4O_3$: 205.1300). $[\alpha]_{589}^{19}$ +12.8 (c 1.95x10^{-3}, MeOH).

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