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

Hiroyo Kabasawa, Makoto Yoshida, and Tsutomu Ishikawa*

Graduate School of Pharmaceutical Sciences, Chiba University, 1-33 Yayoi, Inage, Chiba 263-8522, Japan
E-mail: benti@p.chiba-u.ac.jp

Dedicated to Emeritus Professor Keiichiro Fukumoto on his 70th birthday
(received 25 May 03; accepted 14 Aug 03; published on the web 29 Aug 03)

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-$\gamma$-glutamate, synthesis

Introduction

Biologically important nitric oxide (NO)\textsuperscript{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).\textsuperscript{2} We have reported that $N$-hydroxyagmatine (4), a decarboxylated NOHA, caused the relaxation of cardiovascular muscle, albeit less effectively than does NOHA itself\textsuperscript{3} and that NO was evolved when $N$-hydroxyphenethylguanidine (5) was subjected to photo-sensitized oxidation in a model study for biological NO generation.\textsuperscript{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. 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](image)

**Figure 1**

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

After modification of the reported method, 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 NaBH₄ after conversion into a mixed anhydride with ethyl ortho-chloroformate. The alcohol 15 was then converted into its mesylate 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. 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-BuO)₂CHNMe₂/PhH, reflux, 22 h (75%); (b). H₂/10% Pd–C/EtOH, RT, 20 h, (quant.); (c). (i) NEt₃/ClCO₂Et/THF, -10 °C, 50 min. (ii) NaBH₄/H₂O/THF, RT, 3 h (84% in 2 steps); (d). MsCl/pyridine/CHCl₃, RT, 2.5 h (95%).

Scheme 3. Preparation of L-NOH·HCl (6·HCl): (a).PhSO₂CH₂CN/NaH/THF, 50 °C, 23 h (quant.); (b). 5% Na–Hg/THF/MeOH, RT, 1.5 h (28%); (c). NH₂OH.HCl/NaHCO₃/H₂O/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–H₂O; 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, [α]₅₈⁹¹⁸, 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$_4$OH/EtOH, RT, 2.5 kg/cm$^3$, 22h, (ii) BrCN/NaOAc/MeOH, -10°C, 3.2 h (13% in 2 steps); (c). NH$_2$OH.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$_3$COOH or CoCl$_2$, diborane, or catalytic hydrogenation using 5% Rh–Al$_2$O$_3$ or PtO$_2$ as catalyst). A desired lysine derivative was produced when 20 was hydrogenated over 5% Rh–Al$_2$O$_3$ 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, [α]$_{589}^{19}$, 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 tetramethysilane (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.

\(\alpha\)-t-Butyl \(\gamma\)-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\(_2\)O the mixture was extracted with AcOEt. The organic solution was washed with H\(_2\)O, sat. aq. NaHC\(_2\)O, and brine, dried (K\(_2\)CO\(_3\)), and evaporated. Column chromatography of the residue with AcOEt–hexane (1:8) gave 13 as a colorless oil (5.89 g, 75%). \(\nu\) (CHCl\(_3\)) 3433, 1729. \(\delta\)\(_H\) 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\(_2\)Ph), 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. \(\nu\) (CHCl\(_3\)) 3431, 1712. \(\delta\)\(_H\) 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\(_2\)O (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., 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%). \(\nu\) (CHCl\(_3\)) 3436, 1708. \(\delta\)\(_H\) 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\(_2\)O The organic solution was washed with sat. aq.CuSO\(_4\), sat. aq. NaHCO\(_3\), and brine, dried (K\(_2\)CO\(_3\)), and evaporated. Column
chromatography (Merck, 7734) of the residue with CHCl₃–AcOEt (20:1) gave 16 as a colorless oil (0.845 g, 95%). v (CHCl₃) 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 C₁₅H₂₀NO₇S: 368.1743).

**t-Butyl 2-(boc-amino)-6-cyanohexanoate (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 H₂O, the organic solution washed with H₂O and brine, dried (MgSO₄), 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 H₂O (0.12 mL) was successively added NH₂OH.HCl (0.009 g, 0.131 mmol) and NaHCO₃ (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 H₂O, the organic solution washed with H₂O and brine, dried (MgSO₄), and evaporated. Preparative TLC of the residue with CHCl₃–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), 1.50–1.85 (4H, m, 3-, 5-H), 2.14 (2H, t, J=7.6 Hz, 6-H), 4.17 (1H, q-like, J=7.3 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, 30%). δ H (D₂O) 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 (D₂O) 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 C₇H₁₆N₃O₃: 190.1192). [α]D+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 H₂O. The organic solution was washed with
H₂O and brine, dried (K₂CO₃), and evaporated. Column chromatography of the residue with AcOEt–hexane (1:8) gave 20 as a colorless oil (0.090 g, 76%). ν (CHCl₃) 3433, 2250, 1708. δH 1.45, 1.48 (each 9H, s, t-Bu), 1.67–1.98 (4H, 3- or 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 C₁₅H₃₀NO₇S: 299.1971).

t-Butyl 2-(boc amino)-6-cyanoaminohexanoate (21). A solution of 20 (0.273 g, 0.913 mmol) in 10% NH₃–EtOH (1.6 mL) was hydrogenated over 5% Rh–Al (0.152 g) at RT for 22 h under hydrogen pressure (2.5 kg/cm²). 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 H₂O. The organic solution was washed with H₂O and brine, dried (K₂CO₃), 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(CHCl₃) 3433, 3247, 2224, 1707. δH 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 C₁₆H₃₀N₃O₄: 328.2236).

t-Butyl Nα-boc-Nω-hydroxyhomoarginine (22). To a solution of 21 (0.039 g, 0.119 mmol) in EtOH (0.4 mL) was added NH₂OH.HCl (0.019 g, 0.267 mmol) and NEt₃ (1 drop) and the whole was stirred at RT for 40 min. After evaporation, column chromatography of the residue with CHCl₃–MeOH (4:1) gave 22 as a colorless oil (0.038 g, 88%). ν(neat) 3420, 3320, 3260, 3156, 1699, 1662. δH 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 C₁₆H₃₃N₄O₅: 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.) δH (D₂O) 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-H₂), 4.03 (1H, t, J=6.1 Hz, 2-H). δC (D₂O) 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 C₇H₁₇N₄O₃: 205.1300). [α]₃₉⁺19+12.8 (c 1.95x10⁻³, MeOH).

References


