

Synthesis and Stereochemistry of Hypusine, a New Amino Acid in Bovine Brain.¹⁾

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(Received July 25, 1981)

Hypusine, a new basic amino acid occurring in the homogenate of bovine brain tissue, was synthesized to determine the absolute structure. *N*^α-Benzyloxycarbonyl-L-lysine benzyl ester was coupled with (*S*)- or (*R*)-4-benzyloxycarbonylamino-1-bromo-2-butanol derived from L- or D-malic acid, respectively. The products were deprotected by catalytic hydrogenation. One of the synthetic compounds, *i.e.*, (2*S*,9*R*)-2,11-diamino-9-hydroxy-7-azaundecanoic acid, was completely identical with natural hypusine in all respects.

Hypusine (**1**) had been isolated from bovine brain and its unique structure was determined chemically and spectrometrically to be 2,11-diamino-9-hydroxy-7-azaundecanoic acid.²⁾ The term of hypusine came from hydroxyputrescine and lysine as composite moieties which joined together by an elimination of ammonia as shown in Fig. 1. Of the two asymmetric carbon atoms in hypusine molecule, the configuration of C-2 corresponding to α-carbon atom of lysine had been decided to be of L(*S*) form. This conclusion was given by the fact that periodic acid–permanganate oxidation of hypusine afforded L-lysine.²⁾ On the other hand, the configuration of C-9 in hydroxyputrescine moiety could not be ascertained yet. To clarify the stereochemistry of this carbon atom, we attempted to synthesize two possible isomers, namely (2*S*,9*S*)- and (2*S*,9*R*)-2,11-diamino-9-hydroxy-7-azaundecanoic acid.

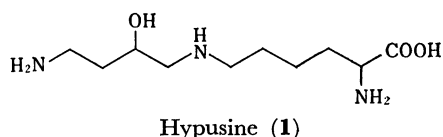
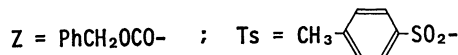
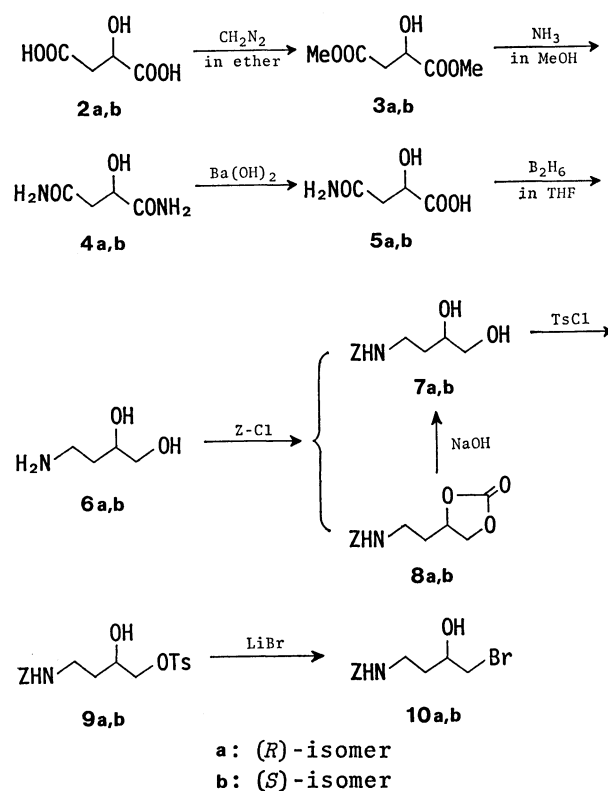


Fig. 1. The structure of hypusine.

A principle of the synthesis of this new amino acid is based on the coupling of L-lysine and 4-amino-1-bromo-2-butanol as shown in Scheme 4. Both enantiomers of the latter component can be obtained from L- and D-malic acid, respectively. While L-malic acid was commercially available, D-isomer had to be prepared by optical resolution of the racemic compound with *S*(–)-1-phenylethylamine.

Malic acid (**2**) of either configuration was esterified with diazomethane to give dimethyl ester **3**, which was converted to β-malamic acid (**5**) according to Freudenberg's procedure.³⁾ Hydrogenation of β-malamic acid (**5**) to 4-amino-1,2-butanediol (**6**) with LiAlH₄ failed because of a poor solubility of the acid **5** in ether or THF. However, reduction with diborane could be employed satisfactorily for this purpose. Thus, the suspension of β-malamic acid in THF was heated under reflux with diborane.⁴⁾ The product was purified by ion-exchange column chromatography of Dowex 50 W×2 (NH₄⁺ form) to isolate the desirable amino alcohol **6**. The amino group of the compound **6** was then protected with benzyloxycarbonyl group to give 4-benzyloxycarbonylamino-1,2-



Scheme 1.

butanediol (**7**).

Although, a small amount of cyclic carbonate derivative **8** was formed in this reaction as a by-product, the carbonate was quantitatively saponified to **7** giving the total yield of *N*-benzyloxycarbonylation of around 90%. Presumably, benzyloxycarbonylation to primary hydroxyl group occurred in a minor degree and a cyclization through an elimination of benzyl alcohol gave the carbonate **8**.

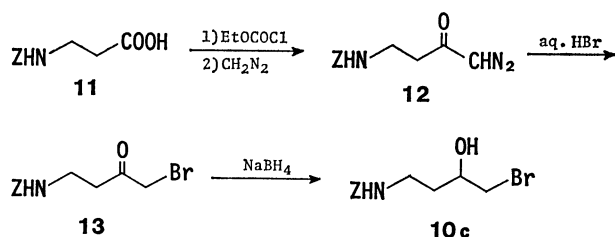
O-Tosylation of **7** in dichloromethane using pyridine as a base afforded predominantly the primary O-tosylate **9** along with formation of the ditosylate as a minor product. O-Tosylate **9** was finally converted into 4-benzyloxycarbonylamino-1-bromo-2-butanol (**10**) with LiBr. Thus, we could prepare successfully the carbon unit to construct hydroxyputrescine moiety.

According to this method, two optically active isomers were obtained starting from D- and L-malic acid, respectively.

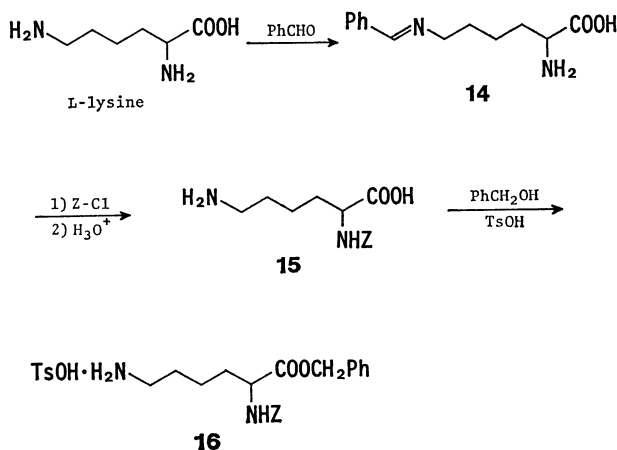
Parallel to the preparation of optically active **10**, a racemic compound was also synthesized for the preliminary experiment to find out the optimum coupling conditions with lysine residue. The racemic compound **10c** was prepared rather easily from *N*-benzyloxycarbonyl- β -alanine as shown in Scheme 2. Mixed carbonic carboxylic anhydride of *N*-benzyloxycarbonyl- β -alanine (**11**) was allowed to react with diazomethane to give the diazo ketone derivative **12**.⁵⁾ The diazo ketone in ether was treated with an equivalent of hydrobromic acid to afford the bromo ketone **13**. Sodium borohydride reduction of **13** gave the racemic bromo alcohol **10c**.

For modification of the lysine moiety to a suitable form for the coupling, L-lysine was first converted to *N* α -benzyloxycarbonyl-L-lysine (**15**) via *N* ϵ -benzylidene derivative **14**.⁶⁾ Since monoacylated lysine was scarcely soluble in both organic solvents and water, the compound **15** was changed to benzyl ester **16** in the usual way using benzyl alcohol and *p*-toluenesulfonic acid as a catalyst in benzene⁷⁾ (Scheme 3).

Coupling reaction of racemic 4-benzyloxycarbonyl-amino-1-bromo-2-butanol (**10c**) with *N* α -benzyloxycarbonyl-L-lysine benzyl ester (**16**) was carried out in several organic solvents. The yield of hypusine was estimated by amino acid analysis after hydrogenation of the coupling product without any purification procedure. From the results of this preliminary experiment as shown in Table 1, methanol, ethanol, and *t*-butyl alcohol seemed to be preferable solvents. However, we realized later on that the use of methanol



Scheme 2.



Scheme 3.

and ethanol caused undesirable ester exchange reaction. This disadvantage could be effectively overcome by employment of *t*-butyl alcohol as a reaction solvent.

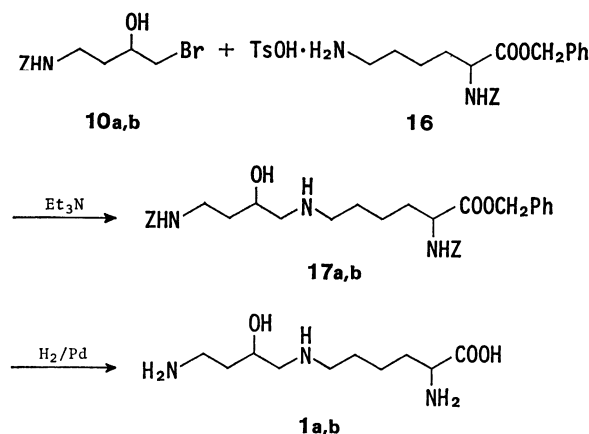
Based on the above results, optically active bromo alcohol **10** was condensed with *N* α -benzyloxycarbonyl-L-lysine benzyl ester *p*-toluenesulfonate (**16**) in *t*-butyl alcohol in the presence of two equivalents of triethylamine.⁸⁾ Protecting groups of the coupling product were then removed by catalytic hydrogenation in 50% *t*-butyl alcohol (Scheme 4). Crude hypusine thus obtained was purified by column chromatography of Dowex 50 W $\times$ 2 (NH₄⁺ form) with dilute NH₄OH eluent.

According to the above procedure, both (2*S*,9*S*)- and (2*S*,9*R*)-hypusine were obtained as crystalline dihydrochloride. Several comparisons of synthetic compounds with natural hypusine were achieved physicochemically, chromatographically, and spectrometrically. However, both isomers did not show significant differences in most respects. For example, NMR spectra (Fig. 2) and retention times in amino acid analyses of both synthetic products were virtually identical with those of natural one. Only melting point and

TABLE 1. EXAMINATION OF COUPLING CONDITIONS OF **10c** WITH **16**

Solvent	Base (equiv.)	Temperature	Time d	Yield ^{a)} %
Benzene	TEA ^{b)} (1.0)	reflux	5	0.6
	TEA (2.0)	reflux	5	6.0
	NMM ^{b)} (2.0)	reflux	5	1.0
CH ₃ CN	TEA (2.0)	reflux	5	6.7
DMF ²⁾	TEA (2.0)	73 °C	5	11
DMSO ³⁾	TEA (2.0)	73 °C	5	3.0
Methanol	K ₂ CO ₃ (2.0)	reflux	19 h	11
Ethanol	K ₂ CO ₃ (2.0)	reflux	5	1.0
	TEA (2.0)	reflux	5	20
<i>t</i> -Butyl alcohol	TEA (2.0)	reflux	5	20

a) Yield was obtained by amino acid analysis after hydrogenolysis of the coupling product without any purification. b) Abbreviations: TEA=triethylamine, NMM=*N*-methylmorpholine, DMF=*N,N*-dimethylformamide, DMSO=dimethyl sulfoxide.



Scheme 4.

specific rotation indicated that the natural compound should be (2*S*,9*R*)-isomer rather than (2*S*,9*S*)-one, though still ambiguously. Fortunately, IR spectra of synthetic compounds showed much difference each other and the spectrum of (2*S*,9*R*)-isomer was completely identical with that of natural hypusine (Fig. 3). Judging from these results thus obtained, we could

now determine the absolute structure of hypusine to be (2*S*,9*R*)-2,11-diamino-9-hydroxy-7-azaundecanoic acid.

The total synthesis of hypusine established in this investigation may contribute also to supply an enough amount of the sample of this unique brain amino acid for biological tests.

Experimental

All melting points are uncorrected. The specific rotations were measured with a Perkin-Elmer 141 polarimeter. NMR spectra were obtained with a Varian XL-100-15 spectrometer and the chemical shifts were given in δ values (ppm) from sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). IR spectra were taken in KBr disk with a Hitachi EPI-G21 spectrophotometer. Amino acid analyses were carried out on a 15 cm column at 55 °C with a Hitachi KLA-3 amino acid analyzer. A buffer solution of 0.7 M (1 M = 1 mol dm⁻³) sodium citrate, pH 5.28, containing 3% NaCl was used for the analysis.

Preparation of D-Malic Acid by Optical Resolution. To a solution of DL-malic acid (131 g, 0.98 mol) in 650 ml of water was added *R*(+)-1-phenylethylamine (118 g, 0.98 mol). The mixture was allowed to stand for 2 d and *R*(+)-amine salt of L-malic acid precipitated was filtered off. The filtrate was concentrated *in vacuo* until a small amount of crystals appeared. After allowing to stand overnight, the second precipitate was filtered again. This procedure was repeated three times and the precipitates combined were recrystallized from water.

The filtrate after taking off fourth precipitate and the mother liquor of recrystallization were combined and concentrated *in vacuo*. To the residue was added 310 ml of 4 M NaOH, and then *R*(+)-amine freed was extracted with benzene three times. Aqueous layer was neutralized with 4 M HCl and concentrated *in vacuo*. Solid residue was triturated with acetone several times to extract malic acid and acetone extract was concentrated *in vacuo*. The residue (56 g) was dissolved in 280 ml of water and *S*(-)-1-phenylethylamine (67 g, 0.54 mol) was added. The precipitate of *S*(-)-amine salt of D-malic acid was obtained by the same procedure as mentioned above and crude salt was recrystallized from water. The pure salt (58 g) was dissolved in 120 ml of 4 M NaOH and *S*(-)-amine was extracted with benzene. Aqueous layer was neutralized with 4 M HCl and concentrated *in vacuo*. D-Malic acid was extracted with acetone by the same treatment as mentioned above and recrystallized from anhydrous ether, yield 17 g (13%), mp 97–99 °C,⁹⁾ $[\alpha]_D^{25} +6.4^\circ$ (*c* 1.9, acetone).⁹⁾ When *S*(-)-1-phenylethylamine was first used for the resolution, actually, optically active D-malic acid in a satisfactorily pure state was not obtained.

β -Malamic Acid (5). Optically active malic acid dimethyl ester, was converted to β -malamic acid according to Freudenberg's method.⁹⁾

(*R*)-4-Amino-1,2-butanediol (6a). Powdered D- β -malamic acid (5a) (9.0 g, 0.068 mol) was suspended in 600 ml of anhydrous tetrahydrofuran (THF). To this suspension was introduced diborane, which was generated from boron trifluoride etherate (60 g, 0.42 mol) in diglyme by addition of small portions of sodium borohydride (12.5 g, 0.33 mol) with a gentle stream of nitrogen under cooling in an ice bath. The suspension was then heated under reflux for 3 d and the clear solution obtained was cooled in an ice bath. To the reaction mixture was added water

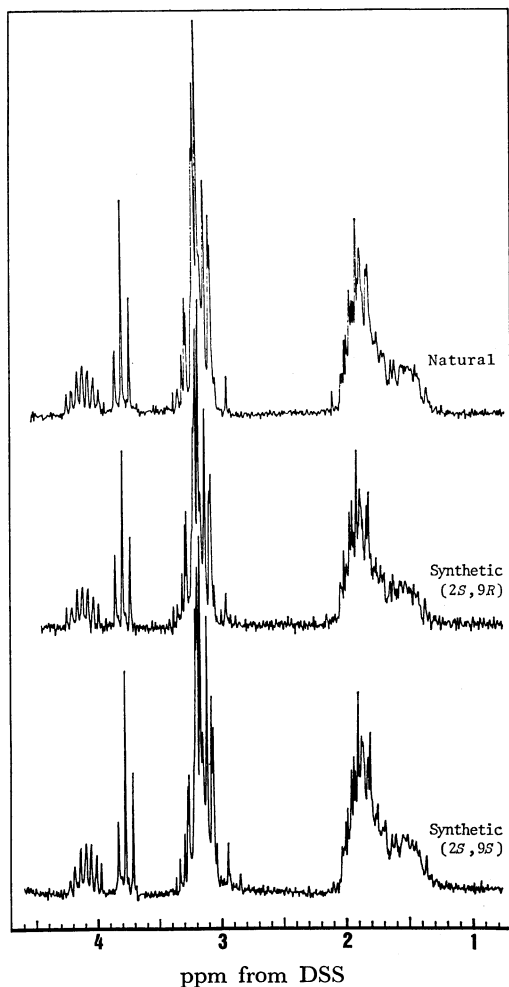


Fig. 2. NMR spectra of natural hypusine and synthetic compounds in D₂O.

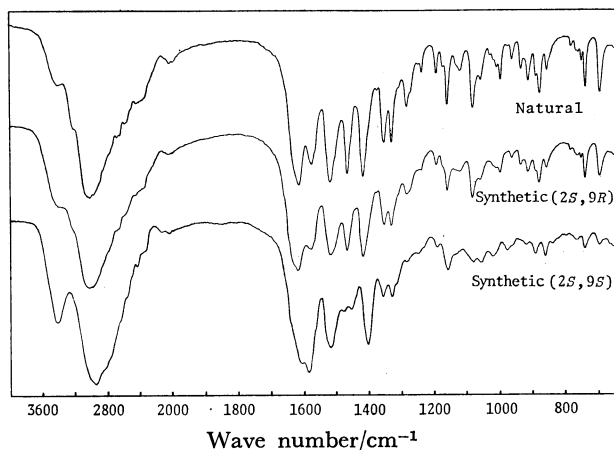


Fig. 3. IR spectra of natural hypusine and synthetic compounds (KBr disk).

carefully until no gas generation occurred. The solution was acidified to pH 4 with hydrochloric acid and concentrated *in vacuo*. The residue was dissolved in cold water and insoluble material was filtered off. The filtrate was concentrated *in vacuo* again and the residue was chromatographed on Dowex 50W $\times$ 2 (200–400 mesh, NH_4^+ form) column, which was first washed with 1 l of water. Elution with 2.8% NH_4OH gave a fraction of positive ninhydrin reaction, which was then concentrated *in vacuo*. (*R*)-4-Amino-1,2-butanediol was obtained as highly hygroscopic oil, yield 4.3 g (61%).

(*S*)-4-Amino-1,2-butanediol (**6b**). L- β -Malamic acid (**5b**) gave (*S*)-amino alcohol **6b** with the same procedure as in the preparation of **6a** in a yield of 56%. A part of (*S*)-amino alcohol was distilled to obtain an analytical sample, bp 172–175 $^\circ\text{C}/0.10$ – 0.15 mmHg,[†] $[\alpha]_D^{25} -23.0^\circ$ (c 1.87, CH_3OH). Found: C, 41.53; H, 10.40; N, 11.85%. Calcd for $\text{C}_4\text{H}_{11}\text{NO}_2 \cdot 3/5\text{H}_2\text{O}$: C, 41.43; H, 10.61; N, 12.08%.

(*R*)-4-Benzoyloxycarbonylamino-1,2-butanediol (**7a**). To a solution of (*R*)-4-amino-1,2-butanediol (**6a**) (4.20 g, 40.0 mmol) in 20 ml of water and 30 ml of ether were added benzoyloxycarbonyl chloride (7.50 g, 44.0 mmol) and 11 ml of 4 M NaOH dropwise with vigorous stirring at 0 $^\circ\text{C}$. After addition of reagents, the reaction mixture was stirred at room temperature overnight and neutralized with 2 M HCl. The product was extracted with ether several times. The extract was dried over magnesium sulfate and concentrated *in vacuo*. Oily residue obtained was purified by silica-gel column chromatography. Elution was carried out with chloroform and methanol (95:5 v/v) to separate (*R*)-4-benzoyloxycarbonylamino-1,2-butanediol (**7a**) and its carbonate **8a**. The main product **7a** was recrystallized from chloroform and petroleum ether, yield 7.30 g (76%), mp 67–68 $^\circ\text{C}$, $[\alpha]_D^{25} +5.8^\circ$ (c 1.9, CHCl_3), and IR 3300 cm^{-1} . Found: C, 60.09; H, 7.15; N, 5.90%. Calcd for $\text{C}_{12}\text{H}_{17}\text{NO}_4$: C, 60.24; H, 7.16; N, 5.85%.

By-product **8a** was recrystallized from ethyl acetate and hexane, yield 1.38 g (13%), mp 36–38 $^\circ\text{C}$, $[\alpha]_D^{25} +27.6^\circ$ (c 1.20, AcOEt), and IR 1790 cm^{-1} . Found: C, 59.07; H, 5.73; N, 5.25%. Calcd for $\text{C}_{13}\text{H}_{15}\text{NO}_5$: C, 58.86; H, 5.70; N, 5.28%.

The carbonate **8a** was quantitatively saponified to **7a** in methanol with two equivalents of 2 M NaOH.

(*S*)-4-Benzoyloxycarbonylamino-1,2-butanediol (**7b**). The same procedure as in the preparation of **7a** was applied to get (*S*)-isomer (**7b**). Carbonate **8b** was also obtained in 10% yield, mp 37–39 $^\circ\text{C}$, $[\alpha]_D^{25} -27.4^\circ$ (c 1.10, AcOEt). Found: C, 58.99; H, 5.72; N, 5.48%. It was quantitatively saponified to **7b** and the total yield of diol **7b** was 80%, mp 66–67 $^\circ\text{C}$, $[\alpha]_D^{25} -6.0^\circ$ (c 2.2, CHCl_3). Found: C, 59.87; H, 7.13; N, 5.94%.

(*R*)-4-Benzoyloxycarbonylamino-1-*p*-tolylsulfonyloxy-2-butanol (**9a**). (*R*)-4-Benzoyloxycarbonylamino-1,2-butanediol (**7a**) (2.00 g, 8.36 mmol) was dissolved in 25 ml of dichloromethane and pyridine (1:1 v/v). To the solution was added *p*-toluenesulfonyl chloride (1.75 g, 9.18 mmol) by portions in 15 min at 5 $^\circ\text{C}$. The reaction mixture was stirred for 21 h at the same temperature. The product was extracted with chloroform several times after an addition of water. The extract was washed with water three times, dried over magnesium sulfate, and concentrated *in vacuo*. Oily residue was chromatographed on silica-gel column. Elution was carried out with benzene–ethyl acetate (9:1 v/v) to separate ditosylate (0.55 g, 12%) and monotosylate **9a** which was recrystallized from ethyl acetate and hexane, yield 2.22 g

(68%), mp 68–69 $^\circ\text{C}$, $[\alpha]_D^{25} +3.1^\circ$ (c 1.1, AcOEt). Found: C, 57.93; H, 5.97; N, 3.63; S, 8.00%. Calcd for $\text{C}_{19}\text{H}_{23}\text{NO}_6$: S, C, 58.00; H, 5.89; N, 3.56; S, 8.15%.

(*S*)-4-Benzoyloxycarbonylamino-1-*p*-tolylsulfonyloxy-2-butanol (**9b**). (*S*)-Monotosylate (**9b**) was prepared similarly to (*R*)-isomer in a yield of 65%, mp 67–69 $^\circ\text{C}$, $[\alpha]_D^{25} -3.0^\circ$ (c 1.1, AcOEt). Found: C, 57.69; H, 5.89; N, 3.55; S, 8.14%.

(*R*)-4-Benzoyloxycarbonylamino-1-bromo-2-butanol (**10a**).

The mixture of (*R*)-monotosylate **9a** (300 mg, 0.76 mmol) and $\text{LiBr} \cdot \text{H}_2\text{O}$ (250 mg, 2.40 mmol) was heated under reflux in 8 ml of acetone for 5 h. Solvent was evaporated *in vacuo* and the residue was dissolved in ethyl acetate. Insoluble material was once filtered off and the filtrate was washed with water several times. Organic layer was dried over magnesium sulfate and concentrated *in vacuo*. Oily residue was chromatographed on silica-gel column using an eluent of benzene–ethyl acetate (4:1 v/v). Bromo alcohol **10a** was obtained in a yield of 95% (220 mg), $[\alpha]_D^{25} +15^\circ$ (c 1.6, AcOEt). Found: C, 47.22; H, 5.36; N, 4.62; Br, 26.14%. Calcd for $\text{C}_{12}\text{H}_{16}\text{NO}_3\text{Br}$: C, 47.69; H, 5.34; N, 4.64; Br, 26.45%.

(*S*)-4-Benzoyloxycarbonylamino-1-bromo-2-butanol (**10b**).

(*S*)-Bromo compound **10b** was prepared similarly in a yield of 97%, $[\alpha]_D^{25} -15^\circ$ (c 1.7, AcOEt). Found: C, 47.53; H, 5.43; N, 4.56; Br, 26.30%.

4-Benzoyloxycarbonylamino-1-bromo-2-butanone (**13**).

To a solution of *N*-benzyloxycarbonyl- β -alanine (9.4 g, 0.042 mol) in 300 ml of chloroform were added triethylamine (5.8 ml, 0.042 mol) and ethyloxycarbonyl chloride (4.0 ml, 0.042 mol) under cooling in an ice bath. The reaction mixture was stirred for 1 h at 0 $^\circ\text{C}$ and then a large amount of anhydrous ether was added to precipitate triethylamine hydrochloride. The precipitate was quickly filtered off and the filtrate was added to an excess of diazomethane in ether which was precooled in an ice bath. The reaction was continued for 1 h at 0 $^\circ\text{C}$. Since a small amount of triethylamine hydrochloride was precipitated again during the reaction, the reaction mixture was once filtered. The filtrate was then warmed at 30 $^\circ\text{C}$ to remove unreacted diazomethane and concentrated *in vacuo*. The crude product was purified by silica-gel column chromatography using chloroform as an eluent to give the diazo ketone **12** (5.7 g, 60%).

To a solution of the diazo ketone (1.1 g, 4.5 mmol) in 30 ml of ether was added 0.78 ml of 48% hydrobromic acid (4.5 mmol) dropwise in 1 h under vigorous stirring in an ice bath. After stirring for more 1 h, the reaction mixture was neutralized with aqueous sodium hydrogencarbonate and extracted with ethyl acetate. The extract was dried over magnesium sulfate and concentrated *in vacuo* to obtain 4-benzoyloxycarbonylamino-1-bromo-2-butanone (**13**), yield 1.3 g (95%). The crude product was recrystallized from ethanol and petroleum ether, yield 1.0 g (73%), mp 42–43 $^\circ\text{C}$. Found: C, 48.36; H, 4.80; N, 4.84; Br, 26.92%. Calcd for $\text{C}_{12}\text{H}_{14}\text{NO}_3\text{Br}$: C, 48.02; H, 4.70; N, 4.67; Br, 26.62%.

($\pm$)-4-Benzoyloxycarbonylamino-1-bromo-2-butanol (**10c**).

4-Benzoyloxycarbonylamino-1-bromo-2-butanone (**13**) (900 mg, 3.0 mmol) was dissolved in methanol and sodium borohydride (38 mg, 1.1 mmol) was added to the solution under cooling in an ice bath. After stirring for 2 h, the reaction mixture was neutralized to pH 7 by an addition of 5% acetic acid and concentrated *in vacuo*. Ethyl acetate was added to the residue and an insoluble material was filtered off. The solution was washed with water, dried over magnesium sulfate, and concentrated *in vacuo*. Oily residue was pu-

[†] 1 mmHg $\approx$ 133.322 Pa.

rified by silica-gel column chromatography and racemic 4-benzyloxycarbonylamino-1-bromo-2-butanol (**10c**) was eluted with benzene-ethyl acetate (4:1 v/v) in a quantitative yield (900 mg) as colorless oil. Found: C, 47.55; H, 5.30; N, 4.50; Br, 26.15%. Calcd for $C_{12}H_{16}NO_3Br$: C, 47.69; H, 5.34; N, 4.64; Br, 26.45%.

Coupling Reaction of 10c and 16. A solution of ($\pm$)-4-benzyloxycarbonylamino-1-bromo-2-butanol (**10c**) (30 mg, 0.10 mmol), N^α -benzyloxycarbonyl-L-lysine benzyl ester *p*-toluenesulfonate (**16**) (54 mg, 0.10 mmol), and triethylamine (21 mg, 0.20 mmol) in 10 ml of benzene was refluxed for 5 d. The reaction mixture was concentrated *in vacuo*. A solution of the residue in 50% *t*-butyl alcohol, added with 2 M hydrochloric acid, was hydrogenated with Pd catalyst. After the completion of hydrogenation, catalyst was filtered off. The filtrate was concentrated *in vacuo* and the residue was dissolved in water. The solution was applied to an amino acid analysis.

(2S,9R)-2,11-Diamino-9-hydroxy-7-azaundecanoic Acid (Hypusine) (**1a**) Dihydrochloride. A solution of (*R*)-4-benzyloxycarbonylamino-1-bromo-2-butanol (**10a**) (0.82 g, 2.7 mmol), N^α -benzyloxycarbonyl-L-lysine benzyl ester *p*-toluenesulfonate (1.5 g, 2.8 mmol), and triethylamine (0.76 ml, 5.5 mmol) in *t*-butyl alcohol was refluxed for 5 d. The reaction mixture was concentrated *in vacuo* and the residue was dissolved in 10 ml of 50% *t*-butyl alcohol. It was hydrogenated to remove the protecting groups in the presence of Pd black under a gentle stream of hydrogen. The solution was kept acidic by an addition of 1 M hydrochloric acid through the hydrogenation reaction. After the completion of reduction, catalyst was filtered off and the filtrate was concentrated *in vacuo*. The crude product was purified by column chromatography on Amberlite IRC-50 (CG-Type 1, 1×68 cm, NH_4^+ form) using 0.28% NH_4OH as an eluent. Fractions containing hypusine were collected and concentrated *in vacuo*. The residue was dissolved in water and pH of the solution was adjusted to 5.2 with dilute hydrochloric acid. It was concentrated *in vacuo* to obtain hypusine (**1a**) dihydrochloride, which was recrystallized from 90% methanol, yield 172 mg (21%),⁸⁾ mp 234–236 °C (decomp),¹⁰⁾ $[\alpha]_D^{25} +6.8^\circ$ (*c* 0.12, 6 M HCl).¹⁰⁾ Found: C, 38.10; H, 8.24; N, 13.27; Cl, 22.79%. Calcd for $C_{10}H_{25}N_3O_3Cl_2 \cdot 1/2 H_2O$: C, 38.10; H, 8.31; N, 13.33; Cl, 22.49%.

(2S,9S)-2,11-Diamino-9-hydroxy-7-azaundecanoic Acid (**1b**) Dihydrochloride.

This was prepared by the same procedure as described above. The hydrochloride was recrystallized from 90% methanol and ethanol in a yield of 21%, mp 238–240 °C (decomp),¹⁰⁾ $[\alpha]_D^{25} +16^\circ$ (*c* 0.10, 6 M HCl).¹⁰⁾ Found: C, 39.14; H, 8.28; N, 13.44; Cl, 23.02%. Calcd for $C_{10}H_{25}N_3O_3Cl_2$: C, 39.22; H, 8.23; N, 13.72; Cl, 23.16%.

The authors wish to express their thanks to Professor Yasuo Kakimoto of Ehime University Medical School and Dr. Teruo Nakajima of Osaka University Medical School for supply of natural hypusine. The present work was partially supported by Grant-in-Aid for Scientific Research No. 921107 from the Ministry of Education, Science and Culture.

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- 8) Based on the preliminary experiments, the reaction was stopped before formation of by-products to make easy the isolation of hypusine from the reaction mixture at the sacrifice of the yield. Both starting materials, **10** and **16**, still remained after 5 d reaction indicating that they are relatively stable even under the basic condition mentioned above unless participated to the desired coupling.
- 9) Authentic L-malic acid showed mp 98–99 °C and $[\alpha]_D^{25} -6.3^\circ$ (*c* 1.9, acetone).
- 10) Natural hypusine dihydrochloride showed mp 234–238 °C (decomp) and $[\alpha]_D^{25} +9.9^\circ$ (*c* 0.12, 6 M HCl).