# Total Syntheses of (+)-Hypusine and Its (2S,9S)-Diastereomer

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*Received July 21, 1993*•

The total synthesis of (2S,9R)-hypusine dihydrochloride, an unusual amino acid constituent of the eukaryotic translation initiation factor eIF-4D, and its (2S,9S) diastereomer is described. The key step in the syntheses involves the Nε-alkylation of *Nε*-benzyl-*Nα*-(L)-lysine benzyl ester with (*R*)- or (*S*)-epichlorohydrin to give the respective (2S,9R) and (2S,9S) chlorohydrins. Subsequent displacement of the respective chlorides by cyanide ion provides the protected hypusine skeletons. The final step, hydrogenation over $PtO_2$ in AcOH followed by neutralization and reacidification, yielded the respective (2S,9S)- and (2S,9R)-hypusine dihydrochlorides in excess of 50% overall yield.

## Introduction

The unusual naturally-occurring amino acid, (+)-hypusine (1), was first isolated from bovine brain extracts by T. Shiba and co-workers.[1] Since its initial discovery, the post-translational formation of hypusine has been shown to occur on a precursor protein of the eukaryotic initiation factor 4D, eIF-4D.[2] In 1983, Folk and co-workers suggested that a hypusine-modified protein serves as an important initiation factor in all growing eukaryotic cells.[2] The possibilities of developing a hypusine antibody assay and influencing cellular protein synthesis by altering the structure of this critical initiation factor prompted the development of a flexible synthesis of hypusine and its derivatives.

The term hypusine was coined to denote the condensation product of *hy*droxy*put*rescine and ly*sine* (with elimination of ammonia). In 1982, Shiba was able to elucidate the absolute configuration of the two chiral centers present in the lysine and hydroxyputrescine fragments of naturally-occurring (+)-hypusine as being (2S,9R) respectively. This assignment was possible through a six step synthesis of the natural product.[3] While a number of hypusine syntheses have been executed all of them involve N7–C8 or C6–N7 bond formation, require four or more steps, and have overall yields between 10–15%.[3-6] As shown in Figure 1, retrosynthetic analysis of 1 suggests two synthons with opposite polarities. Formally path a, adopted by Ganem and by Knapp, requires a nucleophilic, chiral hydroxyputrescine substrate and an electrophilic L-lysine fragment for C6–N7 bond formation. Ganem employed a N1-functionalized hydroxyputrescine and a L-lysine-derived aldehyde as synthons,[4] while Knapp and co-workers utilized a D-asparagine amino derivative and a dibenzyl triazone protected L-lysine-derived aldehyde.[5]

Path b requires a nucleophilic L-lysine fragment and an electrophilic, chiral 4-amino-2-hydroxybutane precursor for N7–C8 bond formation. In earlier studies both Ganem and Shiba developed electrophilic 4-amino-2-hydroxy butane fragments. While Ganem's aldehydic isoxazolidine is elegant, the required separation of diastereomers can be somewhat cumbersome.[6] Furthermore, Shiba's (*R*)-4-[(benzyloxycarbonyl)amino]-1-bromo-2-butanol coupling with the lysine segment of the framework proceeds in very low yields.[3] We elected to explore alternative 4-amino-2-hydroxybutane precursors with the idea of developing methodologies which would allow easier access to (+)-hypusine, 9-epihypusine, and other analogues.

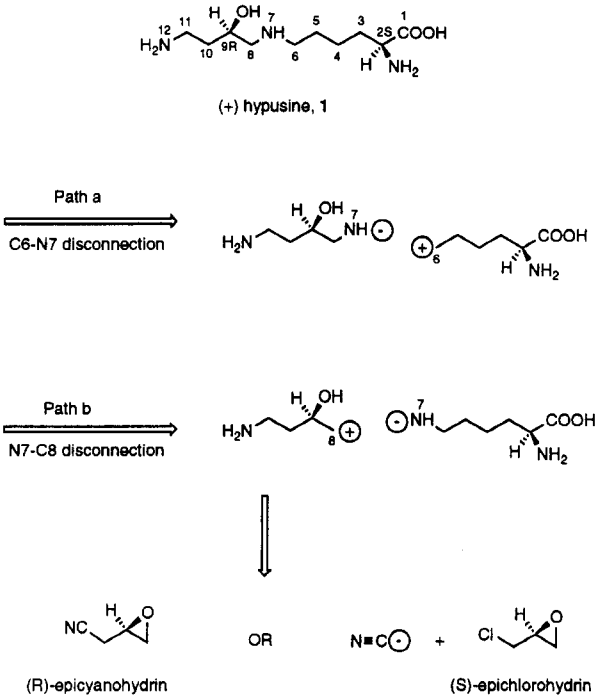


**Figure 1.** Retrosynthetic analysis of (+)-hypusine.

## Results and Discussion

Our strategy focused on the design of a new electrophilic 4-amino-2-hydroxybutane synthon as required for N7–C8 bond formation in path b, Figure 1. As shown in Figure 1, we envisioned epicyanohydrin as such a fragment. While Hartenstein and Pazschke reported the synthesis of epicyanohydrin by condensation of KCN and epichloro-

• Abstract published in *Advance ACS Abstracts*, October 15, 1993.

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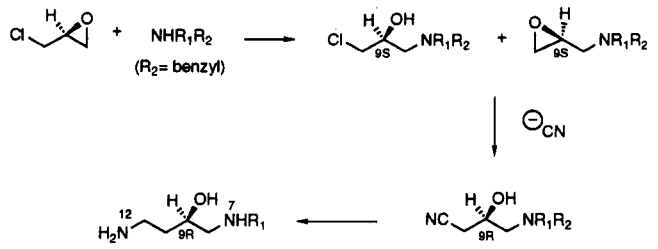
**Scheme I**

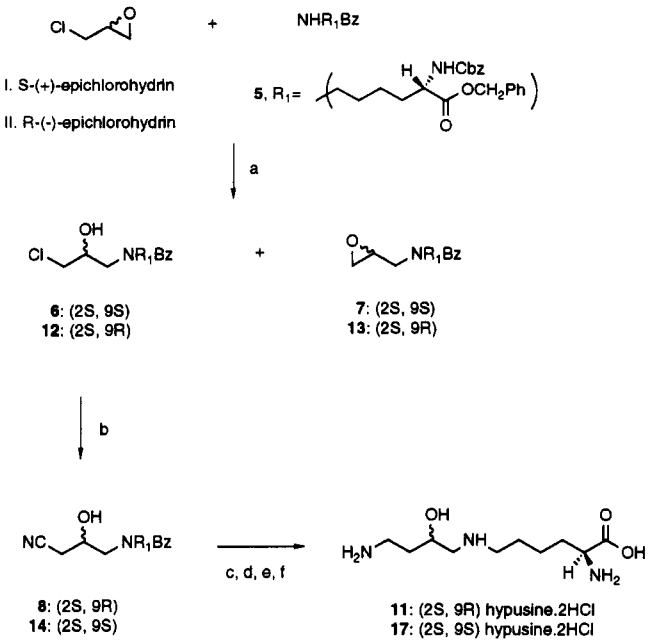



hydrin,[7,8] Wei and Butler later established that this condensation product was in fact a 2,5-bis(cyanomethyl)-1,4 dioxane dimer.[9] Although other methods of preparation have yielded epicyanohydrin, they were not readily amenable to our chiral synthesis.[10,11] Therefore, we focused on the use of the readily available (*R*)- and (*S*)-epi*chloro*hydrins as chiral epicyanohydrin precursors. Indeed, reaction of tri- and dialkylamines with chiral epichlorohydrins have been shown to give the desired 1-amino-3-chloro-2-hydroxypropane frameworks and chiral oxiranes with retention of configuration.[12,13] As illustrated in Scheme I, we envisioned further reaction with cyanide ion would give the desired 1-amino-3-cyano-2-hydroxypropane moiety, i.e. the skeletal framework of hypusine from N7 to N12.

As anticipated, reaction of (*S*)-epichlorohydrin with N$\alpha$-CBZ-L-lysine benzyl ester (**4**)[14] provided a number of products. The primary N$\epsilon$-amino group of N$\alpha$-CBZ-L-lysine benzyl ester (**4**) tends to polyalkylate, and the resulting 1-(alkylamino)-3-chloro-2-propanols are also somewhat unstable.[15] For these reasons **4** was converted to the N$\epsilon$-benzyl, N$\alpha$-CBZ-L-lysine benzyl ester (**5**)[16] by reaction with benzaldehyde followed by reduction with $NaBH_3CN$ in 52% yield.

As shown in Scheme II, our synthesis of (+)-hypusine begins with the N$\epsilon$-alkylation of **5** with (*S*)-epichlorohydrin at rt to give the (2*S*,9*S*)-chlorohydrin **6** (75%) and (2*S*,9*S*)-oxirane **7** (8%). Subsequent displacement of the chloride in **6** by cyanide ion yielded the protected (2*S*,9*R*)-hypusine skeleton **8** in 83% yield. Attempted reductions of **8** over Pd-C in the presence of methanolic HCl resulted in removal of all the benzyl groups but incomplete reduction of the nitrile. Previous work in our laboratory suggested that reduction of *N*-benzylamino nitriles with $PtO_2$ in AcOH resulted in near quantitative yield of the debenzylated amine.[17] Consequently, the final deprotection of **8**, a quantitative reduction step, was effected by hydrogenation over $PtO_2$ in AcOH for 3 h. Neutralization of the triacetate salt **9** gave the hypusine free base **10**, and reacidification yielded (2*S*,9*R*)-(+)-hypusine dihydrochloride (**11**), in 80% isolated yield (53% overall).

**Scheme II.[a] Synthesis of (2*S*,9*R*)- and (2*S*,9*S*)-Hypusine**




[a] Reagents: (a) rt, neat; (b) KCN, 18-crown-6, refluxing dry $CH_3CN$; (c) $H_2/PtO_2/AcOH$; (d) 10% aqueous $NaHCO_3$; (e) column chromatography on $SiO_2$ ($CH_2Cl_2$/MeOH/30% $NH_4OH$); (f) 0.1 N HCl until pH = 5.2.

The 9-epimer, or (2*S*,9*S*)-hypusine, was also synthesized as shown in Scheme II. Condensation of (*R*)-epichlorohydrin and **5** at rt gave the (2*S*,9*R*)-chlorohydrin **12** (75%) and oxirane **13** (19%). Subsequent displacement of the chloride of **12** by cyanide ion yielded the protected (2*S*,9*S*)-hypusine skeleton **14** in 82% yield. Final deprotection was effected by hydrogenation over $PtO_2$ in AcOH for 3 h. Neutralization of the triacetate salt **15** gave the 9-epihypusine free base **16**, and careful reacidification yielded (2*S*,9*S*)-hypusine dihydrochloride (**17**) in 84% isolated yield (52% overall).

Optical rotation measurements of the final hypusine derivatives [**11**, +7.6° (*c* = 0.5, 6 N HCl), lit.[5] [$\alpha$] +7.8° (*c* = 0.52, 6 N HCl); **17**, +15.2° (*c* = 0.25, 6 N HCl, 23°C), lit.[6] [$\alpha$] +15° (*c* = 0.23, 6 N HCl)] confirmed the retention of stereochemical integrity of both chiral centers throughout the synthesis.

In summary, the synthetic strategies outlined in this investigation should allow facile access to a variety of hypusine derivatives for biological studies, including the development of a hypusine antibody assay.

## Experimental Section

**General.** All reagents were purchased from Aldrich Chemical Co. and were used without further purification. Fisher Optima-grade solvents were routinely used, and organic extracts were dried with anhydrous sodium sulfate. THF was distilled from sodium metal and benzophenone. Acetonitrile was distilled from $P_2O_5$. N$\alpha$-carbobenzoxyl(CBZ)-L-lysine benzyl ester *p*-toluenesulfonate was prepared by the method of Bezas and Zervas.[14] Silica gel (40 $\mu$m), obtained from J. T. Baker was used for flash column chromatography. NMR spectra were recorded on Varian EM-390, VXR-300, or QE-300 instruments and were run in $CDCl_3$ or $D_2O$ with chemical shifts given in parts per million downfield

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from an internal tetramethylsilane or sodium 3-(trimethylsilyl)-propionate standard, respectively. Mass spectra were carried out on a Kratos MS 80RFA or a Finnigan 4516 MS instrument. Optical rotations were run at 589 nm (the Na D-line) on a Perkin-Elmer 141 polarimeter, with $c$ expressed as g of compound per 100 mL. Elemental analyses were performed by Atlantic Microlabs, Norcross, GA. Melting points were uncorrected.

**$N\epsilon$-Benzyl-$N\alpha$-carbobenzoxy-L-lysine Benzyl Ester (5).**[16] A solution of $N\alpha$-carbobenzoxyl(CBZ)-L-lysine benzyl ester $p$-toluenesulfonate[14](1.08 g, 2 mmol) and sodium carbonate (0.21 g, 2 mmol) in 20 mL of absolute EtOH was stirred at 23 °C for 30 min, and 210 mg (20 μl, 2 mmol) of benzaldehyde was added by syringe. The solution was stirred for 30 min and 50 mg (0.8 mmol) of solid sodium cyanoborohydride was added. The reaction mixture was stirred at room temperature overnight and concentrated, and the residue was partitioned between 15 mL of chloroform and 50 mL of water. The aqueous layer was extracted with chloroform (3 × 20 mL). The combined organic layers were washed with 20 mL of brine, dried, and concentrated. Flash chromatography on silica gel (5% EtOH/$CHCl_3$) ($R_f$ = 0.32) afforded 480 mg (52.2%) of **5** as an oil. **5**: $^1$H NMR ($CDCl_3$) δ 1.1–1.85 (m, 6H), 2.48 (t, 2H), 3.75 (s, 2H), 4.33 (m, 1H), 5.05 (s, 2H), 5.11 (s, 2H), 5.29 (m, 1H), 7.21 (m, 16H); EI-MS $m/z$ 460 ($M^+$, 0.64% rel inten). Anal. Calcd for $C_{28}H_{32}N_2O_4$: C, 73.02; H, 7.00; N, 6.08. Found: C, 72.91; H, 6.97; N, 6.04.

**(2*S*,9*S*)-7-Benzyl-2-(carbobenzoxyamino)-10-chloro-9-hydroxy-7-azadecanoic Acid Benzyl Ester (6) and (2*S*,9*S*)-7-Benzyl-2-(carbobenzoxyamino)-9,10-epoxy-7-azadecanoic Acid Benzyl Ester (7).** A mixture of $N\epsilon$-benzyl-$N\alpha$-carbobenzoxy-L-lysine benzyl ester (**5**)(1.7 g, 3.7 mmol) and (*S*)-(+)-epichlorohydrin (342 mg, 3.7 mmol) was stirred at room temperature overnight. The resulting gummy oil was chromatographed on silica gel (75% hexane/ethyl acetate) to give 1.03 g of **6** (75%, $R_f$ = 0.37) and 0.25 g of **7** (8%, $R_f$ = 0.27). **6**: $^1$H NMR ($CDCl_3$) δ 1.1–1.85 (m, 6H), 2.3–2.63 (m, 4H), 3.25 (br s, 1H), 3.4–3.75 (m, 4H), 3.82 (m, 1H), 4.39 (m, 1H), 5.1 (s, 2H), 5.15 (d, 2H), 5.38 (m, 1H), 7.3 (m, 15H); $^{13}$C NMR ($CDCl_3$) δ 22.64, 26.17, 32.28, 47.06, 53.69, 53.76, 57.08, 58.79, 66.91, 67.03, 67.32, 127.33, 128.01, 128.09, 128.24, 128.40, 128.53, 128.70, 128.74, 128.90, 135.19, 136.13, 138.02, 155.80, 172.17; FAB-MS $m/z$ 553 ($M + 1^+$, 100% rel inten), 555 ($M + 3^+$, 37.2). Anal. Calcd for $C_{31}H_{37}N_2O_5$-Cl: C, 67.32; H, 6.74; N, 5.07; Cl, 6.41. Found: C, 67.24; H, 6.81; N, 5.08; Cl, 6.50. **7**: $^1$H NMR ($CDCl_3$) δ 1.1–1.85 (m, 6H), 2.4 (m, 4H), 3.4–3.75 (m, 5H), 4.38 (m, 1H), 5.1 (s, 2H), 5.13 (d, 2H), 5.38 (m, 1H), 7.3 (m, 15H); $^{13}$C NMR ($CDCl_3$) δ 22.75, 26.45, 32.27, 53.83, 53.93, 58.43, 59.03, 65.54, 66.91, 66.97, 67.01, 126.97, 127.94, 128.04, 128.09, 128.21, 128.39, 128.46, 128.54, 128.87, 135.27, 136.20, 139.06, 155.87, 172.23; FAB-MS $m/z$ 517 $(M + 1)^+$. Anal. Calcd for $C_{31}H_{36}N_2O_5$: C, 72.07; H, 7.02; N, 5.42. Found: C, 71.80; H, 6.99; N, 5.64.

**(2*S*,9*R*)-7-Benzyl-2-(carbobenzoxyamino)-10-cyano-9-hydroxy-7-azadecanoic Acid Benzyl Ester (8).** A mixture of (2*S*,9*S*)-7-benzyl-2-(carbobenzoxyamino)-10-chloro-9-hydroxy-7-azadecanoic acid, benzyl ester **6** (1.4 g, 2.5 mmol), dry KCN (1.65 g, 25 mmol) and catalytic 18-crown-6 (67 mg, 0.25 mmol) in 25 mL of dry acetonitrile was refluxed with vigorous stirring overnight. The reaction mixture was cooled, filtered, and concentrated. Flash column chromatography on silica gel (50% hexane/ethyl acetate) ($R_f$ = 0.49) gave the (2*S*,9*R*)-nitrile **8** as an oil (910 mg, 83%) and recovered starting material (290 mg). **8**: $^1$H NMR ($CDCl_3$) δ 1.1–1.9 (m, 6H), 2.2–2.6 (m, 6H), 3.42 (dd, 1H, $J$ = 13.3 Hz, $J$ = 2.8 Hz), 3.7 (d, 1H, $J$= 13.3 Hz), 3.80 (m, 1H), 4.38 (dd, 1H), 5.09 (s, 2H), 5.15 (d, 2H, $J$ = 5.2 Hz), 5.38 (d, 1H), 7.33 (m, 15H); $^{13}$C NMR ($CDCl_3$) δ 22.62, 23.05, 26.22, 32.29, 53.63, 53.79, 53.84, 58.71, 63.49, 66.92, 67.05, 117.07, 127.40, 127.89, 128.01, 128.10, 128.25, 128.45, 128.53, 128.68, 128.82, 135.17, 136.11, 138.02, 155.81, 172.15; FAB-MS $m/z$ 544 ($M + 1^+$, 100% rel inten). Anal. Calcd for $C_{32}H_{37}N_3O_5$: C, 70.70; H, 6.86; N, 7.73. Found: C, 70.68; H, 6.88; N, 7.72.

**(2*S*,9*R*)-2,11-Diamino-9-hydroxy-7-azaundecanoic Acid (Hypusine) Dihydrochloride (11).** (2*S*,9*R*)-7-Benzyl-2-(carbobenzoxyamino)-10-cyano-9-hydroxy-7-azadecanoic acid benzyl ester (**8**)(100 mg, 0.18 mmol) was added to a suspension of platinum oxide (25 mg, 25% by weight) in glacial acetic acid (4.5 mL) and stirred under a hydrogen atmosphere for 3 h. The reaction mixture was then filtered and evaporated to give the triacetate as a glass: 76 mg (100%). The residue was neutralized with 10% aqueous sodium bicarbonate and concentrated to dryness. The residue was chromatographed with 1:2:1 $CH_2Cl_2$/MeOH/ammonium hydroxide (30%) ($R_f$ = 0.24) as the eluant to give 36 mg (88.7%) of hypusine as the free base **10**. The compound was dissolved in 0.6 mL of water, and the pH was adjusted to 5.2 by 0.1 N hydrochloric acid. The solvent was removed, and the residue recrystallized in 90% methanol to afford 45 mg (80% yield) of (2*S*,9*R*)-(+)-hypusine **11** as its dihydrochloride. **11**: mp 234–236 °C dec (lit.[3] mp 234–238 °C, lit.[4] mp 235–238 °C, lit.[5] mp 237–238 °C, lit.[6] mp 239–241 °C dec); $^1$H NMR ($D_2O$) δ 1.2–1.45 (m, 2H), 1.5–1.85 (m, 6H), 2.86–3.1 (m, 6H), 3.57 (t, 1H, $J$ = 6.1 Hz), 3.87 (m, 1H); CI-MS $m/z$ 234 (M + 1, 86.24% rel inten); optical rotation $[\alpha]_D$ +7.6° ($c$ = 0.5, 6 N HCl, 23 °C), lit.[5] [α] +7.8° ($c$ = 0.52, 6 N HCl). Anal. Calcd for $C_{10}H_{25}N_3O_3Cl_2$: C, 39.22; H, 8.23; N, 13.72; Cl, 23.15. Found: C, 38.98; H, 8.30; N, 13.49; Cl, 22.89.

**(2*S*,9*R*)-7-Benzyl-2-(carbobenzoxyamino)-10-chloro-9-hydroxy-7-azadecanoic Acid Benzyl Ester (12) and (2*S*,9*R*)-7-Benzyl-2-(carbobenzoxyamino)-9,10-epoxy-7-azadecanoic Acid Benzyl Ester (13).** Compounds **12** and **13** were prepared from reaction of **5** (1.15 g, 2.5 mmol) and (*R*)-(−)-epichlorohydrin (231 mg, 2.5 mmol) using the same procedure as described for **6** and **7**. **12**: 1.03 g, 75% yield; ($R_f$ = 0.37); $^1$H NMR ($CDCl_3$) δ 1.1–1.85 (m, 6H), 2.3–2.62 (m, 4H), 3.45–3.75 (m, 4H), 3.82 (m, 1H), 4.25 (br, 1H), 4.39 (m, 1H), 5.1 (s, 2H), 5.15 (d, 2H), 5.38 (m, 1H), 7.3 (m, 15H); FAB-MS $m/z$ 553 (M + 1, 76.99% rel inten), 555 (M + 3, 22.94). Anal. Calcd for $C_{31}H_{37}N_2O_5Cl$: C, 67.32; H, 6.74; N, 5.07; Cl, 6.41. Found: C, 67.26; H, 6.75; N, 5.07; Cl, 6.34. **13**: 0.25 g, 19.4% yield; ($R_f$ = 0.27); $^1$H NMR ($CDCl_3$) δ 1.1–1.85 (m, 6H), 2.4 (m, 4H), 3.4–3.75 (m, 5H), 4.38 (m, 1H), 5.1 (s, 2H), 5.13 (d, 2H), 5.38 (m, 1H), 7.3 (m, 15H); FAB-MS $m/z$ 517 $(M + 1)^+$. Anal. Calcd for $C_{31}H_{36}N_2O_5$: C, 72.07; H, 7.02; N, 5.42. Found: C, 71.82; H, 6.94; N, 5.65.

**(2*S*,9*S*)-7-Benzyl-2-(carbobenzoxyamino)-10-cyano-9-hydroxy-7-azadecanoic Acid Benzyl Ester (14).** This compound was prepared from **12** (123 mg, 0.22 mmol), dry KCN (58 mg, 0.9 mmol), 18-crown-6 (6 mg, 0.023 mmol), and 5 mL of dry $CH_3CN$ in 82% yield using the same procedure as described for **8**. **14**: ($R_f$ = 0.49, 50% hexane in EtOAc); $^1$H NMR ($CDCl_3$) δ 1.1–1.9 (m, 6H), 2.2–2.6 (m, 6H), 3.34–3.9 (m, 3H), 4.38 (dd, 1H), 5.09 (s, 2H), 5.15 (s, 2H), 5.38 (m, 1H), 7.3 (m, 15H); FT-IR ($cm^{-1}$) 3434, 2254, 1794, 1721, 1603, 1509; FAB-MS $m/z$ 544 (M + 1, 100% rel inten). Anal. Calcd for $C_{32}H_{37}N_3O_5$: C, 70.70; H, 6.86; N, 7.73. Found: C, 70.69; H, 6.91; N, 7.70.

**(2*S*,9*S*)-2,11-diamino-9-hydroxy-7-azaundecanoic Acid (9-Epihypusine) Dihydrochloride (17).** The triacetate salt **15** was prepared from **14** in 100% yield by using the same procedure as described for **11**. After neutralization and chromatography on silica, the white solid was brought to pH 5.2 in aqueous solution to afford **17** in 84% yield. **17**: mp 235–237 °C dec [lit.[3] mp 238–240 °C dec]; $^1$H NMR ($D_2O$) δ 1.2–1.45 (m, 2H), 1.5–1.85 (m, 6H), 2.86–3.1 (m, 6H), 3.57 (t, 1H, $J$ = 6.1 Hz), 3.87 (m, 1H)]; CI-MS $m/z$ 234 (M + 1, 100% rel inten; optical rotation $[\alpha]_D$ +15.2° ($c$ = 0.25, 6 N HCl, 23°C), lit.[6] [α] +15° ($c$ = 0.23, 6 N HCl). Anal. Calcd for $C_{10}H_{25}N_3O_3Cl_2$: C, 39.22; H, 8.23; N, 13.72; Cl, 23.15. Found: C, 38.94; H, 8.17; N, 13.45; Cl, 22.93.