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Stereoselective Synthesis of Stable Isotope-Labeled L-α-Amino Acids: Enantioselective Synthesis of ^{13}C-, ^{15}N-Labeled L-Proline Using Oppolzer's Glycine Template

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SUMMARY

We have developed a stereoselective route to the synthesis of stable isotope-labeled L-proline. Alkylation of (2*R*)-*N*-{*N'*-[*bis*(methylthio)methylidine]-glycyl}bornane-10,2-sultam with 3-chloro-iodopropane yielded (2*R*)-N-{(2'*S*)-2'-{[*Bis*(methylthio)methylidine]amino}-5-chloropentan-1-oyl}bornane-10,2-sultam. Cyclization to the imino acid occurred during the sequential removal of the α-amino protecting group and the chiral auxiliary.

Keywords: L-[1,2-^{13}C, ^{15}N]Proline, L-[2-^{13}C]Proline, L-[ε-^{15}N]Lysine

INTRODUCTION

Because L-Proline is an α-imino acid it imparts unique structural properties some to peptides and proteins. Peptide bonds formed with imino acids are conformationally heterogeneous because of the small enthalpy difference between the *cis* and *trans* conformers[1]. X-Pro peptide bonds in the *cis* configuration have been observed in proteins[2]. Some proteins exist in two folded states owing to the coexistence of *cis* and *trans* forms of the X-prolyl peptide bonds[2-5]. The barrier (~20 Kcal/mol) for the interconversion of *cis* and *trans* X-pro peptide bonds has significant implications for the kinetics of folding and unfolding of some proteins[2] and in peptide hydrolysis[6,7]. In addition, the *cis*-form of some proline-containing peptides has been implicated in biological recognition and activity[8-10]. Because of its important role in peptide and protein conformation and dynamics, we and others[11] have been interested in the stereoselective synthesis of isotope labeled L-proline.

Racemic proline and [^{15}N]proline have been prepared from a number of symmetrical compounds that are not readily adapted to specific carbon labeling[12,13]. L-Proline has been produced by several methods. Biosynthesis of proline from acetate has been reported[14];

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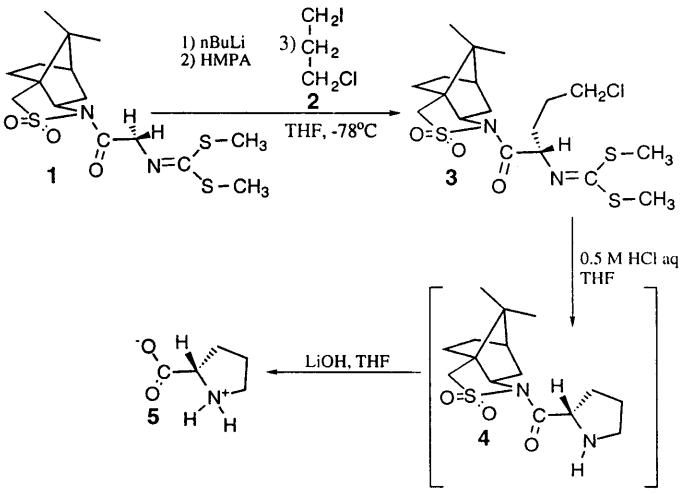
however, this use of biosynthesis for labeling would result in a complicated distribution of isotopomers[15,16]. Conceptually similar to the biosynthesis of L-proline, the chemical conversion of L-glutamate to L-proline has been described[17]. Carbon-13 labeled L-proline has been prepared from labeled glutamate[11]; however, the complicated methods required to label L-glutamate make these routes unattractive[15,16,18].

We have been interested in developing stereoselective methods for incorporating stable isotopes into L-amino acids[19-24]. The remarkable efficiency and stereoselectivity of Oppolzer's camphor-10,2-sultam-based strategies for the syntheses of D- or L-amino acids[25-29] make them attractive for stable isotope labeling[19,20,30,31]. Oppolzer's chiral glycine template (**1**) is an amino-protected glycine (N-[*bis*(methylthio)methylidine]-glycine) that is linked as an amide to camphor-10,2-sultam. This chiral glycine equivalent (**1**) is metallated by treatment with n-butyl lithium in THF at -78°C. Reaction of the lithium enolate with electrophiles is carried out in the presence of hexamethylphosphoramide. After deblocking, the product amino acid and the camphor sultam auxiliary are separated and recovered. Efficient alkylations with alkyl iodides or activated bromides occur with remarkable enantioselectivity (94.7 to 98.4 diastereomeric excess). In this manuscript we report the application of the Oppolzer glycine template to the synthesis of L-[2-^{13}C]- and L-[1,2-$^{13}C_2$, ^{15}N]proline. While the focus of this manuscript is the synthesis of proline, we report straightforward modifications of our route that lead to the synthesis of L-[ε-^{15}N]lysine.

RESULTS and DISCUSSION

Alkylation of the Oppolzer chiral glycine template requires an alkylating reagent with a leaving group at least as good as iodide[26-29]. For example, methyl iodide alkylates the enolate in high yield at -78°C while methyl tosylate does not react, even at higher temperatures. This observation leads to the conclusion that alkylation with bifunctional halides such as 1-chloro-3-iodopropane would lead to a single product with the chloride left for further elaboration with other nucleophiles. Specifically, the internal displacement of chloride by the unmasked α-amino group would yield L-proline (Scheme 1). Indeed, Oppolzer and coworkers reported[25] the alkylation of the glycine template with bifunctional halides including 1-chloro-3-iodopropane. They developed the alkylation product (**3**) as a useful intermediate in the synthesis of optically active ornithine derivatives. In that



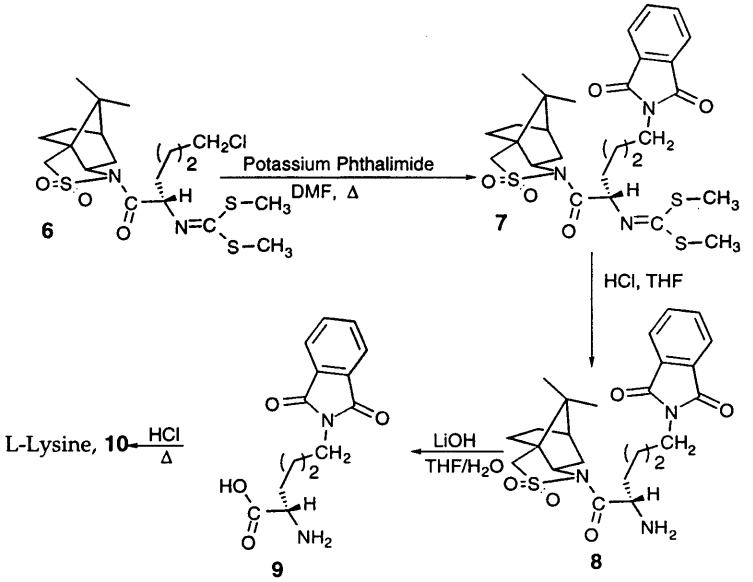
Scheme 1

manuscript Oppolzer and coworkers[25] speculated that the intramolecular displacement of chloride from **3** would provide a useful synthetic route to proline. We have confirmed their prediction.

Treatment of (2*R*)-N-{N-[*bis*-(methythio)methylidene]glycyl}bornane-10,2-sultam (**1**) with n-butyl lithium yielded the corresponding enolate. Three equivalents of HMPA were added followed by three equivalents of 1-chloro-3-iodopropane (**2**). The crude alkylated product (**3**) was isolated from the reaction mixture and used without further purification. Removal of the *bis*-(methythio)methylidene-blocking group was accomplished by treatment of **3** with dilute HCl/THF. Base hydrolysis of the resulting sultam was carried out using $LiOH/H_2O/THF$. Cyclization occurred spontaneously as these deblocking reactions were carried out. By starting with the appropriately labeled glycine sultam we prepared L-[2-^{13}C]proline (62% yield) and L-[1,2-$^{13}C_2$, ^{15}N]proline (75% yield). The overall process occurred with high diastereoselectivity which resulted in high overall enantioselectivity (e.e. = 97.3%).

The bimolecular displacement of the chloride from the appropriate protected amino acyl camphor-10,2-sultam with nucleophiles such as phthalimide, cyanide, guanidine, and methyl sulfide would yield useful amino acids. Described here is the displacement of the appropriate chloro-amino acylsultam (**6**) with phthalimide to ultimately yield L-lysine (Scheme 2). Alkylation of the (2*R*) sultam glycyl enolate with 1-chloro-4-iodobutane

yielded alkylation product **6**. Displacement of chloride from **6** with potassium phthalimide, followed by deblocking yielded L-lysine respectively. Using this route we have prepared L-[ε-^{15}N]lysine (95.0% e.e.).



Scheme 2

While this report focuses on the synthesis of proline, our approach is potentially more generally useful. The addition of one to four carbon bifunctional alkylating agents to the Oppolzer glycine enolate would yield functionalized intermediates useful for the synthesis of half of the protiogenic amino acids including serine, cysteine, aspartic acid, asparagine, methionine, glutamic acid, glutamine, proline, arginine, and lysine. We are developing strategies for the preparation of ^{13}C-labeled bifunctional alkylating agents that will make this approach useful for synthesis of side chain labeled amino acids.

METHODS

Chemicals - Stable isotope labeled (2*R*)-*N*-{*N'*-[*bis*(methylthio)methylidine]glycyl}-bornane-10,2-sultam[31] (**1**) was prepared from [1,2-$^{13}C_2$, ^{15}N]- or [2-^{13}C]glycine essentially as described in the literature. Potassium [^{15}N]phthalimide was prepared from [^{15}N]ammonia[32]. 1-Chloro-3-iodopropane (**2**) (Aldrich Chemical Co.) and 1-chloro-4-iodobutane (Aldrich Chemical Co.) were obtained from commercial sources and dried with 3 Å molecular sieves. THF was distilled from K°/benzophenone. HMPA was distilled from CaH_2 and stored over activated 3 Å molecular sieves and under an argon atmosphere. Solvents were evaporated using Büchi rotary evaporators evacuated by direct-drive

mechanical pumps preceded by a trap cooled in liquid nitrogen. Ion chromatography was done on Dowex™ 50 using 0.1 M NH_3 to generate the zwitterionic form of the amino acid[21].

Analytical Methods - Melting points were determined using a Thomas-Hoover apparatus and are uncorrected. Melting points are compared to that of the unlabeled analogues[25].

Proton-decoupled ^{13}C FT-NMR spectra were obtained at 50.3 MHz using a Bruker AM-200 WB NMR spectrometer. Acquisition parameters were as follows: 10.869 KHz sweep width, 16 K data points, 0.75 s acquisition time, 2 s relaxation delay, 0.663 Hz/pt data point resolution, and 25°C. For the determination of isotopic enrichments, signal intensities were determined by Lorentzian line shape analysis. Chemical shifts are reported in ppm using the solvent as an internal reference ($CDCl_3$ = 77.00 ppm). The chemical shifts of the unlabeled enantiomers of many of the sultam derivatives have been reported[25].

The enantiomeric purity of the product amino acids was determined by either NMR spectroscopy of the corresponding Mosher's ester (proline) or gas chromatography (lysine). Mosher's esters were prepared according to method of Williams and coworkers [33]. Samples were analyzed by proton NMR using a Bruker AMX-500 NMR spectrometer. Integration was carried out using the FELIX software package (BIOSYM/Molecular Simulations, San Diego, CA). Gas chromatography was carried out with a Hewlett-Packard 5890A GC using a fused silica capillary column (50 meter) with a chiral stationary phase (Chirasil-Val lll, Alltech Associates). The amino acids were chromatographed as their N-trifluoroacetylamide isopropyl esters[34] and monitored using a flame ionization detector. Calibration samples of the D, L, and DL amino acids were prepared from commercially available amino acids. Enantiomeric excess (e.e.) was calculated from the integrated areas (Hewlett-Packard 3396 Series II integrator) of the peaks of the D- and L-isomers. To identify the D-isomer in our experimental samples, we spiked them with a small amount (1 to 3%) of derivatized D-amino acids.

(2*R*)-N-{(2'*S*)-2'-[1',2'-$^{13}C_2$,^{15}N]{[*Bis*(methylthio)methylidine]amino}-5-chloropentan-1-oyl}bornane-10,2-sultam (3). - A 500 mL flask containing a solution of the (2*R*)-[1',2'-$^{13}C_2$, ^{15}N]glycylsultam (**1**) (11.35 g, 29.89 mmol in 150 mL THF) and a magnetic stir bar and was

placed under an argon atmosphere and cooled in a Dry Ice™/isopropyl alcohol bath. A 2.5 M solution of n-butyllithium in hexanes (Aldrich Chemical Co., 13.2 mL, 33.0 mmol) was added dropwise with stirring over 10 min. The characteristic yellow color of the enolate anion was immediately apparent. After an additional 15 min of stirring, a solution of 90.0 mmol (16.1 g, 15.7 mL) of HMPA in 15 mL of THF was added dropwise with stirring over 10 min. After an additional 15 min of stirring, 3-iodochloropropane (**2**) (90.0 mmol, 18.40 g, 9.66 mL) was added dropwise over 5 min. After stirring an additional 2 hr in the cold, the bath was removed and the now orange reaction solution was allowed to warm to room temperature. After evaporation of the THF the residual viscous orange oil was dissolved in 160 mL of EtOAc followed by the addition of 80 mL of hexane. The resulting turbid mixture was passed through 120 g of silica gel (Aldrich, suitable for flash chromatography) contained in a fritted glass funnel. The silica gel was rinsed with a total of 300 mL of 1/1 (v/v) EtOAc/hexane. This procedure effected the complete removal of the HMPA and most of the color. The pale yellow filtrate was evaporated, leaving 23.7 g of yellow oil. The excess alkylating agent was removed *in vacuo* overnight using a mechanical pump and a trap cooled with liquid nitrogen. This process left 14.2 g of a yellow oily solid, which represents an essentially quantitative crude yield of the expected product (**3**). The solid was taken up in 75 mL of EtOAc and 325 mL of hexane was added slowly with stirring, and a white solid precipitated. After filtration and washing with EtOAc/hexane, 6.102 g (13.38 mmol, m.p. 115-116°C Lit [25] 115-117°C) of 1',2'-$^{13}C_2$, ^{15}N-labeled **3** was obtained. ^{13}C NMR ($CDCl_3$): 171.01 ($^1J_{C\text{-}C}$ = 54.39 Hz), 162.62 (broad), 65.16, 64.08 ($^1J_{C\text{-}C}$ = 54.45 Hz), 52.96, 48.44, 47.71, 44.56 ($^3J_{C\text{-}C}$ = 3.76 Hz), 44.42, 38.32, 32.67, 32.12 ($^1J_{C\text{-}C}$ = 35.69 Hz), 28.95 (broad), 26.36, 20.63, 19.77, 15.15, 14.76 ($^3J_{C\text{-}N}$ = 5.11 Hz)†. This crystallized material was set aside for other work. The mother liquor was evaporated, leaving 8.1 g of a yellow solid (18 mmol crude) which was converted to L-[1,2-$^{13}C_2$, ^{15}N]proline as described below.

L-[1,2-$^{13}C_2$, ^{15}N]Proline (5) - The 8.1 g of the of the yellow solid above was dissolved in 180 mL of THF, followed by 180 mL of 1 M HCl. After stirring at room temperature for 26 hr, the solvents were evaporated, leaving a yellow oil/solid residue. This residue was partitioned between 100 mL of water and 100 mL of diethyl ether. After two additional

†The ^{13}C NMR chemical shifts reported here differ significantly from those reported by Oppolzer and coworkers[25] for an unlabeled sample. They report the imido carbon at 167.67 ppm. In both our labeled and unlabeled samples the imido carbon resonance is at 162.57 ppm. In addition, we report a resonance at 44.42 ppm which Oppolzer and coworkers did not observe. Finally, we did not observe the resonance at 13.06ppm.

washes with 100 mL of ether, the pale yellow aqueous phase was evaporated, leaving 4.72 g (15.0 mmol crude) of **4** as a yellow frothy residue. This residue was dissolved in 100 mL of THF, and 32 mL of 1 M LiOH was added. After stirring at room temperature for 3.25 hr, 33 mL of 1 M HCl was added and the resulting acidic solution was evaporated, leaving a yellow residue, which was partitioned between water and CH_2Cl_2. After two more extractions with CH_2Cl_2, the pale yellow aqueous phase was evaporated, leaving a yellow oily residue. After ion chromatography and evaporation of the ninhydrin-positive fraction, 1.46 g (12.4 mmol, 75.0% from the sultam glycinate) of L-[1,2-$^{13}C_2$, ^{15}N]proline was obtained. ^{13}C NMR (D_2O): 175.22 ($^1J_{C\text{-}C}$ = 53.42 Hz), 62.34 ($^1J_{C\text{-}C}$ = 53.53 Hz; $^1J_{C\text{-}N}$ = 5.38 Hz), 47.17 ($^1J_{C\text{-}N}$= 4.54 Hz), 30.07 ($^1J_{C\text{-}C}$ = 32.92 Hz), 24.85. e.e. = 97.3%

L-[2-^{13}C]Proline (5) - Prepared as above, without splitting out a portion of the alkylation product, in 62% yield from the (2*R*)-[2'-^{13}C]glycylsultam) ^{13}C NMR (D_2O): 175.65 ($^1J_{C\text{-}C}$ = 53.94 Hz), 62.26 ($^1J_{C\text{-}C}$ = 53.37 Hz), 47.10, 30.00 ($^1J_{C\text{-}C}$ = 32.99 Hz), 24.79. e.e. = 97.4%

(2*R*)-N-{(2'*S*)-2'-{[*Bis*(methylthio)methylidine]amino}-6-chlorohexan-1-oyl}-bornane-10,2-sultam (6) - Compound **6** was prepared by alkylation of **1** (1.00 mmol, 376 mg; Oxford Chirality) with 1-chloro-4-iodobutane (1.5 mmol, 328 mg) analogously to the preparation of **3**. After removing volatiles, the crude product **6** (480 mg, 1.03 mmol crude) was used without purification. ^{13}C NMR was consistent with the desired product. ^{13}C NMR ($CDCl_3$): 171.24, 162.02, 65.11, 64.45, 52.92, 48.32, 47.61, 44.63, 44.38, 38.24, 33.66, 32.60, 31.87, 26.28, 22.90, 20.56, 19.72, 15.09, 14.69.

(2*R*)-N-{(2'*S*)-2'-{[*Bis*(methylthio)methylidine]amino}-6'-N-[^{15}N]phthalimidohexan-1-oyl}bornane-10,2-sultam (7) - Compound **6** (480 mg, 1.03 mmol crude) was dissolved in 10 mL of dry DMF (Aldrich) and 1.5 mmol (279 mg) of potassium [^{15}N]phthalimide was added. After establishing an Ar atmosphere, the suspension was magnetically stirred and heated at 80° for 12 hr. The DMF was evaporated from the cooled reaction mixture, and the resulting residue was taken up in 40 mL of CH_2Cl_2. After washing with water, drying with Na_2SO_4, filtering, and evaporating, a yellow residue was obtained. This residue was flash chromatographed on 16 g of silica gel (Aldrich) using 30 % EtOAc/hexane as the developer. After pooling and evaporating the fractions containing the desired product, 0.330 g (0.570 mmol, 57 % yield from the sultam glycinate) of white solid was obtained. ^{13}C NMR ($CDCl_3$): 171.35, 168.14 ($^1J_{C\text{-}N}$ = 13.10 Hz),162.02, 133.68, 132.08 ($^1J_{C\text{-}N}$ = 7.34

Hz),122.99, 65.14, 64.60, 52.94, 48.37, 47.66, 44.44, 38.29, 37.73, ($^1J_{C-N}$ = 9.15 Hz), 34.06, 32.66, 28.01, 26.34, 23.01, 20.58, 19.76, 15.13, 14.68.

(2*R*)-N-{(2'*S*)-2'Amino-6'-[^{15}N]phthalimidohexan-1-oyl}bornane-10,2-sultam (8) - Compound **7** was dissolved in 6 mL of THF and 6 mL of 1 M HCl was added. After stirring at room temperature for 25 hr, the reaction was titrated to about pH = 7 with 1 M NaOH. The solid residue remaining after evaporation to dryness was partitioned between dilute HCl and diethyl ether. The aqueous phase was extracted with 25 mL of diethyl ether, then neutralized with the addition of solid $NaHCO_3$. As the solution became alkaline, a white solid came out of solution. The resulting suspension was extracted with CH_2Cl_2, which in turn was dried with Na_2SO_4, filtered, and evaporated, leaving 206 mg (0.43 mmol) of white solid product. ^{13}C NMR ($CDCl_3$): 176.30, 168.24 ($^1J_{C-N}$ =13.20 Hz), 133.73, 132.10 ($^1J_{C-N}$ = 7.71 Hz), 123.06, 65.01, 54.43, 52.94, 48.55, 47.69, 44.55, 38.26, 37.65 ($^1J_{C-N}$ = 9.16 Hz), 34.94, 32.74, 28.15, 26.36, 22.88, 20.67, 19.78.

L-[ε-^{15}N]Lysine (10) - The product above (**8**) was dissolved in 5 mL of THF, followed by 1 mL of 1 M LiOH. After magnetically stirring for 3 hr at room temperature, 2 mL of 1 M HCl was added, followed by evaporation of the volatiles to dryness. The residue was partitioned between CH_2Cl_2 and water. After separation, the water phase was extracted with three 25 mL portions of CH_2Cl_2, and evaporated to dryness. Examination of the residue (^{13}C NMR) showed that there had been only partial hydrolysis of the phthalimido group. The residue was heated at reflux for two days in 3 M HCl. After cooling and extraction with CH_2Cl_2, the volatiles were evaporated, leaving a residue, which was ion chromatographed. The ninhydrin-positive fraction yielded 47 mg (0.32 mmol, 32% from the starting glycyl sultam) of L-[ε-^{15}N]-lysine. ^{13}C NMR (D_2O): 182.86, 56.59, 40.48 ($^1J_{C-N}$ = 4.88 Hz), 34.32, 28.44, 22.99 ($^2J_{C-N}$ = 1.06 Hz). e.e. = 95.0%.

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