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# SYNTHESIS OF CAPTOPRIL LABELED WITH $^{2}H$, $^{3}H$, $^{14}C$, OR $^{35}S$

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## SUMMARY

Three routes for the synthesis of labeled captopril are described. The preferred route can be selected with regard to the desired position of the label. The chemical and radiochemical stability of captopril is discussed. An isotope effect was found in the IR spectrum when the side chain carbonyl carbon was labeled with carbon-14.

Key Words: Captopril, Synthesis, Stability, Isotope Labeled

## INTRODUCTION

Captopril is the first of the orally active inhibitors of angiotensin-converting enzyme (1). Labeled captopril was required to study its absorption, excretion, and biotransformation. Captopril was initially labeled with $^{35}S$ because $H_2{}^{35}S$ was a readily available precursor. Subsequently, captopril was labeled in the proline ring with $^{2}H$, $^{3}H$, and $^{14}C$, and with $^{14}C$ in the carbonyl carbon of the side chain.

## RESULTS AND DISCUSSION

An earlier method for the synthesis of $^{14}C$-captopril, labeled in the carbonyl carbon of the side chain, has been published (2). The present report describes three methods for preparing captopril labeled with $^{2}H$, $^{3}H$, $^{14}C$, or $^{35}S$.

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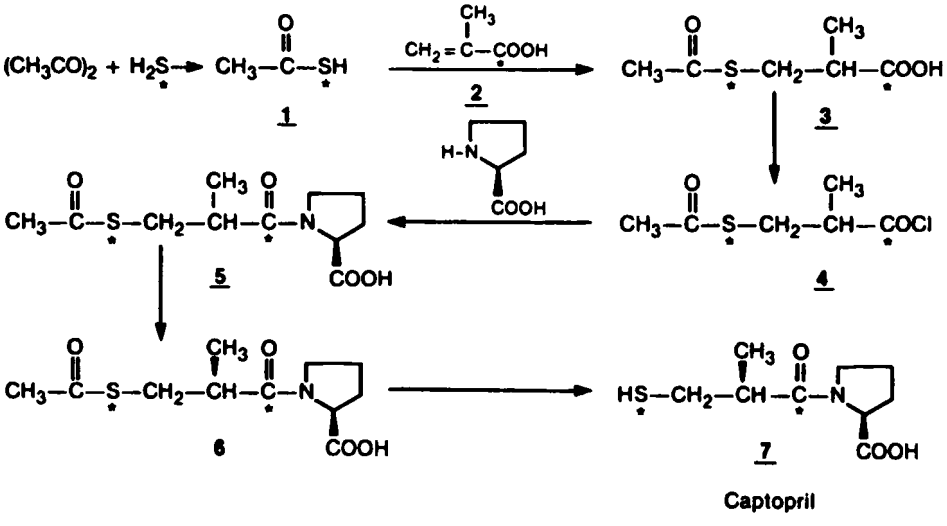
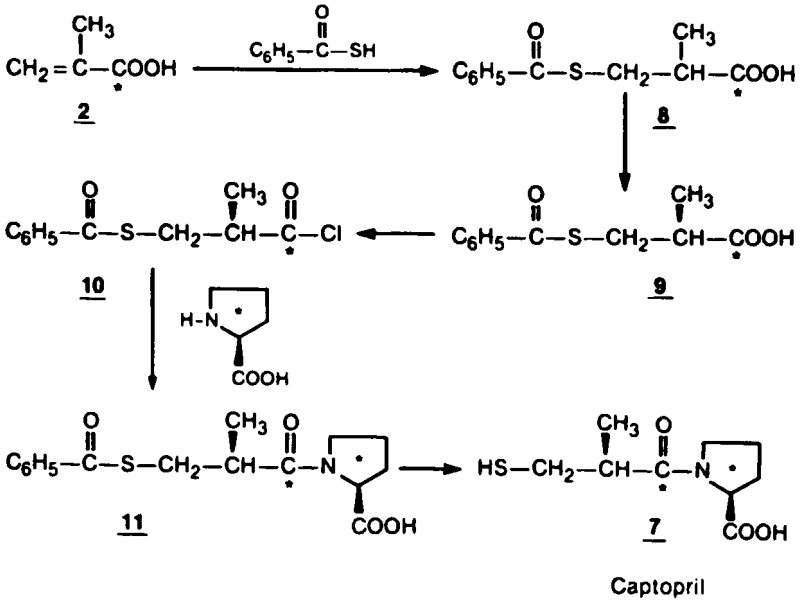
The synthesis of captopril labeled in the side chain is outlined in Schemes I and II. For introduction of the label with proline, Schemes II or III may be followed.

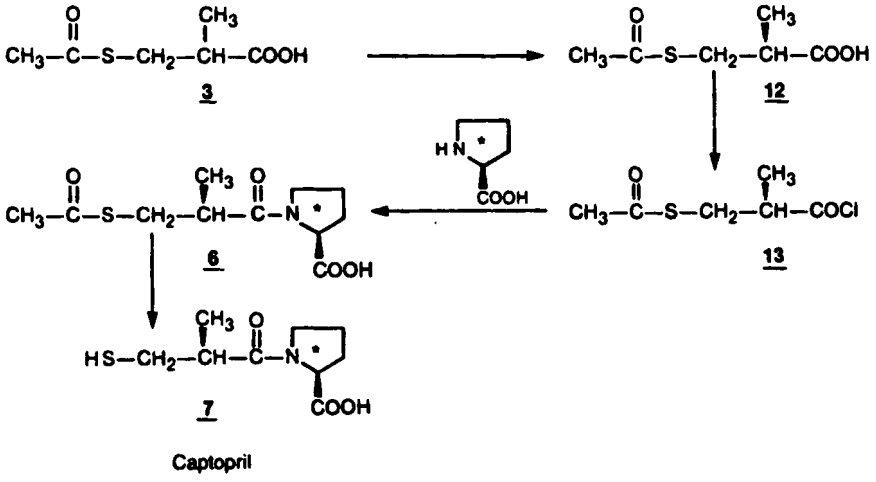
## Scheme I




## Scheme II



## Scheme III




In Scheme I, thioacetic acid (synthesized from acetic anhydride and either $H_2S$ or $H_2{}^{35}S$) is condensed with either labeled or unlabeled methacrylic acid to give the acetylated, racemic side chain acid. This is converted to the acid chloride and coupled with L-proline. Acetylated captopril is separated from its optical isomer by crystallization. Captopril is then obtained by hydrolysis.

In Scheme II, thiobenzoic acid is condensed with $^{14}C$-methacrylic acid. The benzoylated, racemic acid is then resolved by means of the dehydroabietylamine salt, followed by conversion to the acid chloride. The resolved acid chloride is coupled with L-proline (labeled or unlabeled), and the resulting product is hydrolyzed to captopril.

In Scheme III, the optically resolved acetylated side chain acid, if available, is first converted to the acid chloride. The resolved acid chloride is then coupled with either $^{2}H$-, $^{3}H$-, or $^{14}C$-L-proline, and the product is hydrolyzed to captopril.

Several problems were encountered in the synthesis of radiolabeled captopril. One problem was a radiation-induced polymerization of methacrylic acid. This was minimized by keeping labeled methacrylic acid in solution, by condensing it with the thio acid as soon as possible, and by keeping batch sizes to less than 100 mCi.

The acylation of L-proline with the side chain unexpectedly resulted in reduced yields. Typical yields for this reaction are about 90% (3); however, on a small scale (10 mmol), yields of only 60% to 80% were obtained, despite the use of either excess unlabeled L-proline or excess unlabeled side chain. Further attempts to increase the yield of this step were not successful because of the physical properties of the acid chloride.

The carboxyl group of L-proline presents another potential problem in that, in the presence of simple alcohols, esterification readily occurs. For example, in an earlier work-up procedure in which captopril was extracted from an aqueous solution (acidified with HCl to a pH of 2) using ethyl acetate, the presence of captopril ethyl ester was detected by TLC (3). The concentration of the captopril ethyl ester exceeded 3–5% in one case, and captopril could no longer be crystallized.

Another problem in the synthesis of captopril is related to the presence of the sulfhydryl group, which is readily oxidized to the disulfide. Therefore, the presence of oxygen should be avoided during the hydrolytic step and during the isolation of captopril.

During storage, labeled captopril will undergo radiolysis. Typically, when stored as a solid at -20°C, $^{14}$C-captopril (specific activity 5 μCi/mg) decomposed at the rate of 6% in 1 year. Therefore, at higher specific activities, or for prolonged storage, captopril is best stored as either the acetylthio- or benzoylthio-derivative. These derivatives of labeled captopril also undergo some radiodecomposition during storage, but are readily purified by chromatography just prior to their hydrolysis to captopril.

Our experience has shown that some $^{14}$C-labeled carbonyl groups vibrate at a different infrared (IR) frequency than their carbon-12 analogs. Other examples of this phenomenon have been observed for cyclohexanone-1-$^{14}$C (4) and [4-(cyclopropylcarbonyl)phenyl]acetic-1-$^{14}$C acid (5). When captopril is labeled in the carbonyl carbon of the side chain, the same isotope effect was observed, i.e., a strong band at 1590 $cm^{-1}$ for carbon-12 and a band at 1520 $cm^{-1}$ for carbon-14. The shift is typically 70 $cm^{-1}$ to a higher frequency. A high-resolution IR

spectrum will also reveal the carbon-13 band (1.1% natural abundance) located between the carbon-12 and carbon-14 bands.

## EXPERIMENTAL

$H_2{}^{35}S$, L-[$^{14}$C(U)]proline and L-[$^{3}$H(U)] proline were obtained from DuPont NEN®, Boston, MA. Benzoylthio- and acetylthio-2-methyl-[1-$^{14}$C]propanoic acid were purchased from Pathfinder Laboratories, St. Louis, MO. L-Proline-$d_7$ was obtained from MSD Isotopes, Dorval P.Q. Canada. Non-labeled chemicals were purchased from Aldrich Chemical Co., Inc., Milwaukee, WI. Silica gel GF TLC plates were obtained from Analtech, Inc. (Newark, DE).

Radioactivity was determined with a RackBeta Model 1211 liquid scintillation spectrometer (LKB Instruments, Inc.; Gaithersburg, MD). Infrared spectra were recorded on a Sirius 100 IR spectrometer (Mattson Instruments, Inc.; Madison, WI). NMR spectra were generated on a Model FX-270 NMR spectrometer (JEOL, Inc.; Peabody, MA). Mass spectra were obtained by fast-atom bombardment on a VG-ZAB-2F mass spectrometer (VG Analytical, Inc.; Stamford, CT). Optical rotations were determined with a Model 1241 automatic polarimeter (Perkin-Elmer Corp.; Norwalk, CT).

Thioacetic acid-$^{35}$S (1): Acetic anhydride (50 mmol), sodium hydroxide powder (1.1 mmol), and a stirring bar were placed into a 25-ml flask. The flask was connected to a bridge on a vacuum line, cooled, and evacuated. The stirred mixture was heated at 55°C, and $H_2{}^{35}S$ (10 mmol, 200 mCi) was added, in portions, at slightly below atmospheric pressure; most of the $H_2{}^{35}S$ was absorbed after 2 hr. $H_2S$ was then added during 8 hr (total uptake 38 mmol), until the rate of the reaction had become very slow, and the mixture was distilled on a vacuum line to eliminate sodium salts. Compound 1 was distilled at 760 Torr (90-93°C) by means of a 12 in. Vigreux column to yield 2.7 g of 1 (35 mmol; 112 mCi).

3-Acetylthio-2-Methylpropanoic Acid (3): The synthesis of the labeled intermediate, methacrylic acid (2), has been reported (2). Thioacetic acid (18.4 mmol) was added to a solution of 2 (16 mmol) in 4 ml of hexane. The solution was heated and maintained at reflux for 5 hr. (When radioactivity in

excess of 100 mCi of $^{35}S$ or $^{14}C$ was used, polymerized methacrylic acid sometimes precipitated and was removed by filtration.) The solvent was removed, and 3 (2.1 g, 70%) was obtained as a white solid (MP of 40°C). Compound 3 was purified by crystallization from hexane (20 ml) at 5°C in a yield of 80-90%.

3-Acetylthio-2-Methylpropanoyl Chloride (4): 3-Acetylthio-2-methylpropanoic acid (15 mmol) was dissolved in 2 ml of thionyl chloride. One drop of dimethylformamide was added, and the solution was stirred for 24 hr at 20°C. Excess thionyl chloride was removed in vacuo, and 4 was distilled at 60-70°C (1-2 Torr) in a yield of 85%.

1-[3-(Acetylthio)-2-Methyl-1-Oxopropyl]-L-Proline (5): Unlabeled L-proline (28 mmol) was dissolved in 15 ml of water and added to the 4-$^{14}C$ (14 mmol, 50 mCi), followed immediately by solid $NaHCO_3$ (58 mmol). The mixture was stirred well at 20°C for 2 hr, the solution was washed with 15 ml of dichloromethane, and acidified to a pH of 2 with 10 N HCl. Crude Compound 5 was extracted with three 15-ml portions of dichloromethane, the solution was dried with magnesium sulfate, filtered, and the solvent was removed in vacuo. (This isolation procedure removed any unreacted L-proline). Crude 5 (3.0 g) was found to contain 20% of 3 by TLC (developed in benzene-glacial acetic acid (3:1, v/v, $R_f$ = 0.4).

Compound 3 interferes with the resolution, by crystallization, of the optical isomers of 5; therefore, 3 was removed by the following procedure. A Waters Prep 500A HPLC system was used with one PrepPak®-500/silica gel cartridge. Four liters of benzene-glacial acetic acid (3:1 v/v) were prepared, and the cartridge was washed with 1 liter of solvent. The remaining 3 liters of solvent were recirculated until a stable baseline of 10 Relative Response Units was obtained on the refractive index detector. This was attained after five column volumes (CV). When one cartridge is used, one CV is equal to about 500 ml. The 3 g of crude 5 was dissolved in 5 ml of solvent and injected onto the column. Compound 3 eluted between 1.0 and 1.8 CV and was discarded. Compound 5 eluted between 1.8 and 3.2 CV, and the solvent was removed in vacuo. Compound 5 was obtained with a purity of > 95% in a yield of 58% (2.1 g, 8.1 mmol, 29 mCi).

1-[3-(Acetylthio)-2-D-Methyl-1-Oxopropyl]-L-proline (6): The resolution of

the optical isomers of 6 was accomplished by crystallization. Compound 5 (8.1 mmol, 29 mCi) was dissolved in 24 ml of water to which sodium bicarbonate (1 g) was slowly added. The pH was then adjusted to 2.3 with 10 N HCl, and the solution was stirred and seeded with 6; the resulting slurry was stirred at 20°C for 1 hr, and then in an ice-bath for 2 hr. The crystals were harvested by filtration, washed with 5 ml of ice-cold water, and dried *in vacuo*. Unlabeled 6 was added to the mother liquors at two separate times (700 mg each) to increase the radiochemical yield. Compound 6 was crystallized, each time as described above, to yield the three batches shown in Table 1. A total of 11.6 mCi of 6 was obtained. TLC was performed as for 5; 6 was > 98% pure. The chemical and optical purity of 6 can be increased by crystallization as follows: Impure solid 6 (1.5 g) was dissolved in dichloromethane (10 ml), the solvent was removed with a stream of nitrogen, and the oil which remained was dissolved in ethyl acetate (1 ml). After crystallization for 1 hr, hexane (10 ml) was added in portions, during 2 hr, to increase the yield; a recovery of 90% or greater was obtained.

Table 1: Yield of 6

| Batch No. | Amount (mg) | Optical Rotation $[\alpha]_D^{20}$ (0.5% in ethanol) | Specific Activity (μCi/mg) | Total Activity (mCi) |
|---|---|---|---|---|
| I | 642 | -155.2° | 12.1 | 7.77 |
| II | 710 | -161.1° | 3.98 | 2.83 |
| III | 729 | -157.7° | 1.33 | 0.97 |

(S)-1-(3-Mercapto-2-Methyl-1-Oxopropyl)-L-Proline, $^{14}$C-Captopril (7): The hydrolysis of 6 was performed with argon-purged reagents and under an atmosphere of argon. Compound 6 (1.3 g) was dissolved in 7.5 ml of 2.5 N NaOH and stirred for 2 hr. The solution was neutralized to a pH of 7 with 10 N HCl and washed with 10 ml of dichloromethane. Then, the solution was acidified to a pH of 2.0, and crude 7 was extracted with three 12-ml portions of dichloromethane. The extracts were combined, dried with magnesium sulfate, filtered, and the solvent was removed *in vacuo*. The oil which remained was dissolved in 1 ml of ethyl acetate, and 7 was crystallized by the addition of 10 ml of hexane

during 2 hr. The white crystals were washed with 15 ml of hexane and dried in vacuo. Compound 7 was obtained in a yield of 75-90%, depending on the purity of 6.

The analytical data (IR, mass, and proton NMR spectra; optical rotation; melting point; etc.) for captopril have been reported (6). [$^{14}C$,$^{35}S$]Captopril was obtained with a purity of > 98%. As expected, the major impurity, about 1%, was the disulfide of 7. When captopril was labeled with $^{14}C$ in the carbonyl group of the side chain, the IR spectrum (KBr pellet) showed an extra, small band at 1520 $cm^{-1}$, which represented the $^{14}C$-carbonyl band; the corresponding $^{12}C$-carbonyl band was found at 1590 $cm^{-1}$. The size of the $^{14}C$ carbonyl band is proportional to the specific radioactivity of 7 (5).

3-Benzoylthio-2-Methyl-[1-$^{14}C$]propanoic Acid (8): (Methacrylic acid-1-$^{14}C$ at a specific activity of greater than 0.5 mCi/mmol is very unstable and readily polymerizes; it is best stored in a suitable solvent and used as soon as possible.) Thiobenzoic acid (31 mmol) was added to an ethereal solution (25 ml) of methacrylic acid-1-$^{14}C$ (28 mmol, 140 mCi.) Hexane (30 ml) was added, and the ether was removed by distillation. The remaining solution was heated to reflux and maintained there for 16 hr. The solution was cooled to 20°C, and 8 was crystallized for 2 hr, isolated by filtration, washed with hexane (10 ml), and dried in vacuo. Compound 8 (4.5 g, 20 mmol) was obtained in a yield of 70% (MP: 59-62°C).

(S)-3-Benzoylthio-2-Methyl-[1-$^{14}C$]propanoic Acid (9): The optical isomers of 8 were resolved by crystallization of the dehydroabietylamine salt of 9. Compound 8 (20 mmol, 100 mCi) was dissolved in 38 ml of isopropanol-water (9:1 v/v; Solvent A) at 50°C and stirred. Then, a solution of dehydroabietylamine (22 mmol), dissolved in 21 ml of Solvent A, was added, and the solution was seeded with 3 mg of the dehydroabietylamine salt of 9. When the solution became turbid (5-20 min.), stirring was discontinued, and the product was allowed to crystallize for 1 hr at 50°C and then for 16 hr at 20°C. The crystals were isolated by filtration, washed with 10 ml of Solvent A, and slurried in 15 ml of isopropanol-water (8:2 v/v; Solvent B); the slurry was heated briefly to the boiling point, and stirred for 2 hr at 20°C. The crystals were isolated by

filtration, washed with Solvent B (4 ml), and dried *in vacuo*. The radiochemical yield of **9** was increased by the addition of carrier (10 mmol) to the concentrated mother liquors; this mixture was processed as above. The yields and analytical data of the two batches are presented in Table 2.

Table 2: Dehydroabietylamine Salt of **9**

| Batch No. | Amount (g) | Amount (mmol) | (mCi) | Specific Activity (μCi/mg) | Molar Specific Activity (mCi/mmol) | Optical Rotation[a] |
|---|---|---|---|---|---|---|
| 1 | 3.66 | 7.18 | 33.9 | 9.25 | 4.71 | 0° |
| 2 | 4.45 | 8.73 | 10.4 | 2.34 | 1.19 | 0° |
| Total | 8.11 | 15.91 | 44.3 | 5.47[b] | 2.78[b] | 0°[b] |

[a] $[\alpha]_D^{20}$: 1% in methanol. The optical rotation should be <+1°.
[b] Calculated value.

The dehydroabietylamine salt of **9** (15.9 mmol) was slurried in a two-phase system of water (60 ml) and dichloromethane (20 ml). Dehydroabietylamine was liberated by the dropwise addition, during 1 hr, of 1 N NaOH (16 ml); the pH should not be allowed to exceed 10.5. The dichloromethane layer was removed, the aqueous layer was washed with two 15-ml portions of dichloromethane, and any residual dichloromethane was removed *in vacuo*.

Compound **9** was crystallized by adjusting the aqueous solution to a pH of 5.7 by the addition of 1 N HCl, followed by seeding with **9**; the resulting slurry was stirred for 30 min. During the next hr, additional acid (for a total of 16 ml) was added to adjust the slurry to a pH of 2.5, and the slurry was stirred in an ice-bath for 1 hr. The white crystals were isolated by filtration, washed with water (20 ml), and dried *in vacuo* to yield **9** (41.2 mCi, 14.8 mmol). TLC in benzene-glacial acetic acid (3:1 v/v, $R_f$ = 0.8) and exposure to iodine vapors showed the presence of a single spot. The optical rotation (1% in methanol) was $[\alpha]_D^{20}$: -40.8°.

Labeled **9** (3.30 g, 41.2 mCi, $[\alpha]_D^{20}$: -40.8°) and unlabeled **9** (807 mg, $[\alpha]_D^{20}$: -42.5°) were dissolved in boiling hexane (70 ml). This step increased the optical purity, and, at the same time, adjusted the specific activity from 2.78 to 2.20 mCi/mmol. The volume of hexane was reduced to 50 ml with a stream of

nitrogen, and 9 was allowed to crystallize at 20°C for 2 hr. The white crystals were isolated by filtration, washed with hexane (15 ml), and dried in vacuo. Compound 9 (3.77 g, 37.0 mCi), when subjected to TLC as above, showed the presence of one spot and had an optical rotation of $[\alpha]_D^{20}$: -42.5°, the same as a standard of unlabeled 9.

(R)-3-Benzoylthio-2-Methyl-[1-$^{14}$C]propanoyl Chloride (10): Compound 9 (2.0 g, 19.7 mCi) was dissolved in toluene (5 ml) and thionyl chloride (1 ml), 2 drops of dimethylformamide were added, and the solution was heated at 35-40°C for 1 hr. Excess reagent and solvent were removed with a stream of nitrogen at 35°C, and the oil which remained was used in the next step.

(S)-1-[3-(Benzoylthio)-2-Methyl-1-Oxo-[1-$^{14}$C]propyl]-L-Proline (11): L-Proline (10.9 mmol), dissolved in water (12 ml), was added to labeled 10 (8.9 mmol, 19.7 mCi). The mixture was stirred vigorously and immediately adjusted to a pH of 9.5 with 5 N NaOH; the pH was maintained there for 30 min by the further addition of base. The solution was washed with two 10-ml portions of dichloromethane and then acidified to a pH of 1.5 with 10 N HCl. Crude 11 was extracted with three 15-ml portions of dichloromethane, the combined extracts were dried (magnesium sulfate), filtered, and the solvent was removed in vacuo. Compound 11 was crystallized from xylene (8 ml) by seeding with 11 and then maintained at 5°C for 20 hr. The white crystals were isolated by filtration, washed with xylene (4 ml), and dried in vacuo. Compound 11 was obtained in a yield of 65% (1.91 g, 13.2 mCi) and had an optical rotation of $[\alpha]_D^{20}$: -143° (1% in ethanol), which was similar to that of a standard of 11.

Compound (7) from 11: The hydrolysis of 11 was performed with argon purged reagents and under an atmosphere of argon. Compound 11 (5.95 mmol) was dissolved in 10 ml of 2.5 N NaOH and stirred for 2 hr. The solution was then acidified to a pH of 6 with 10 N HCl, and the benzoic acid which precipitated was removed by filtration and discarded. The solution was further acidified to a pH of 2, and 7 was extracted with three 17-ml portions of dichloromethane. The extracts were combined, dried (magnesium sulfate), filtered, and the solvent was removed in vacuo. The oil which remained was crystallized from toluene (3 ml) at 5°C for 2 hr. The white crystals were isolated by filtration, washed with toluene

(1 ml), and dried in vacuo. Compound 7 ($^{14}$C-captopril) was obtained in a yield of 70%.

(R)-3-(Acetylthio)-2-Methylpropanoyl Chloride (13): The preparation of 12 from 3 has been described (7). The acid chloride, 13, was synthesized in the same manner as racemic 4.

Compound (6) from 13: L-Proline ($^{2}$H ,$^{3}$H, or $^{14}$C; 17 mmol) was dissolved in water (10 ml). Solid sodium bicarbonate (60 mmol) and 13 (27 mmol) were added, the mixture was stirred for 2 hr, and the resulting solution was washed with dichloromethane (15 ml). The aqueous solution was then acidified to a pH of 2 with 10 N HCl. Crude 6 was extracted with three 15-ml portions of dichloromethane, the extracts were combined, dried (magnesium sulfate), filtered, and the solvent was removed with a stream of nitrogen. Crude 6 (6 g) contained about 40% of 3. Compound 6 was purified by HPLC, as described in the preparation of 5. Compound 6 was obtained in a yield of 60-70% and was recrystallized in the same manner as described for the preparation of 6. It was then hydrolyzed to 7.

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