CHIRAL SYNTHESIS OF L-[^{14}C]PHENYLALANINE AND ITS INCORPORATION INTO THE RENIN INHIBITOR PD 132002

Helen T. Lee, James L. Hicks and Donald R. Johnson
Department of Chemistry
Parke-Davis Pharmaceutical Research Division
Warner-Lambert Company
Ann Arbor, Michigan 48105

SUMMARY

[1S-(1R*,2S*,3R*)]-3-[[1-(Cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-amino]-N-[N-(4-morpholinosulfonyl)-L-phenylalanyl]-3-oxo-DL-alanine methyl ester (PD 132002) was found to be a renin inhibitor and thus potentially useful for the treatment of hypertension. This peptidomimetic agent contains an L-phenylalanine residue which was chosen as the site for carbon-14 incorporation. The chiral synthesis using (4S)-4-(phenylmethyl)-2-oxazolidinone as a chiral auxiliary for asymmetric hydrazide formation was modified to make it amenable to carbon-14 synthesis.

L-[1-^{14}C]Phenylalanine was synthesized in seven steps from $^{14}CO_2$ in an overall yield of 33%. It was further converted to [^{14}C]PD 132002 in three more steps with an overall yield of 4.95%. The final specific activity was 35.5 mCi/mmol.

KEYWORDS: L-[1-^{14}C]Phenylalanine, renin inhibitor, PD 132002

INTRODUCTION

The renin-angiotensin system plays an important role in the regulation of blood pressure and has provided the basis of intensive efforts in the rational design of antihypertensive agents. Possible modes of intervention in this process include inhibition of renin formation for release, renin inhibition, angiotensin-converting enzyme (ACE) inhibition, and angiotensin II receptor antagonism. In the last several years, work on renin inhibitor design has yielded several classes of potent inhibitors.[1,2] Progress toward lower molecular weight inhibitors with oral absorption have been reported.[3,4] In our laboratory a series of renin inhibitors containing ester side chains at the P_2 subsite and the diol isostere at P1-P1′ has been synthesized. They are potentially useful

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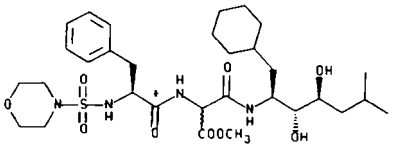
therapeutic agents. Among them, PD 132002,[5] a novel dipeptide renin inhibitor with oral antihypertensive activity has been labeled with carbon-14 to provide further pharmacokinetic and drug metabolism studies in vivo.

The building block around which PD 132002 was constructed was L-phenylalanine. Carbon-14 was introduced into PD 132002 using L-[1-^{14}C]phenylalanine. A chiral synthesis of L-[1-^{14}C]phenylalanine is described.

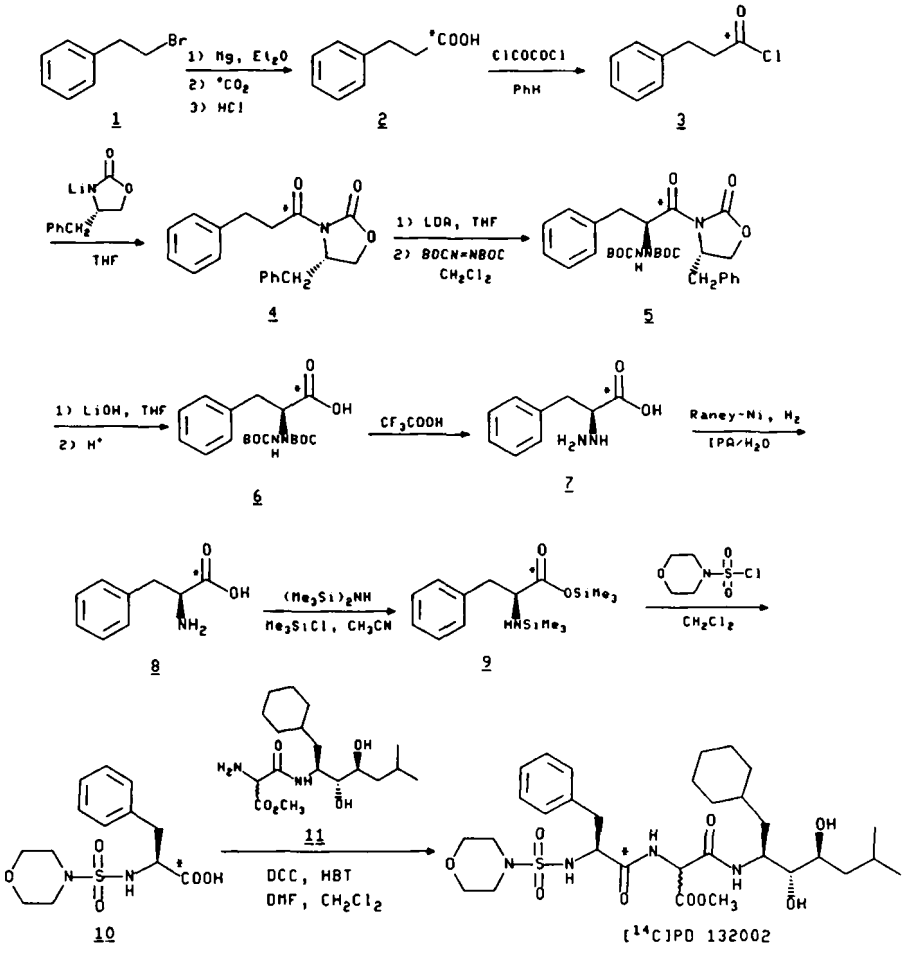


[^{14}C]PD 132002

RESULTS AND DISCUSSION

There are previous examples of the synthesis of carbon-14 labeled amino acids.[6] These examples result in the formation of a racemic mixture. Some report the resolution either by use of diastereomeric derivatives or enzymatic means. deKeczer and Parnes used hydrogenation with a chiral catalyst to produce a carbon-14 labeled L-phenylalanine derivative with 92.5% optical purity then enzymatic hydrolysis to gain 99.9% optical purity.[7] Hydrogenation of the appropriately substituted diketopiperazine was used to make L-[2-^{14}C]phenylalanine.[8] There are several examples in the literature for the preparation of chiral phenylalanine.[9] Some involve resolution and others are stereospecific synthesis. Resolution of radiolabeled racemate is wasteful as half of the radioactivity is lost in the formation of the wrong isomer. It is therefore more desirable to develop a stereospecific synthesis that would allow the incorporation of carbon-14 in good yield. Evans presented two syntheses which lead to L-phenylalanine.[10] The chiral auxiliary, (4S)-4-(phenylmethyl)-2-oxazolidinone was used to direct an asymmetric introduction of an amine precursor. The anion of 4 was treated with di-tert-butyl azodicarboxylate, or with azide. Evans noted good yields and enantiomeric purity with both methods. The azide route had fewer steps, and it was easy to reduce the azide to the amine at 15 psi. The hydrazide on the other hand needed 550 psi of H_2, conditions which required high-pressure apparatus not commonly available in radiosynthesis

laboratories. However, we found that in 2-propanol/water the hydrazide reduction was readily achieved at 50 psi of H_2. Furthermore, in spite of the extra steps as compared with the azide route, the hydrazide route gave a higher overall yield.



Scheme 1 Synthesis of [^{14}C]PD 132002 (-denotes position of carbon-14)*

The Grignard reagent derived from 1 and magnesium in diethyl ether was treated with $^{14}CO_2$ generated from barium [^{14}C]carbonate (1054 mCi). The resulting acid 2 was isolated in an 88% yield and 100% radiochemical purity (RCP). The acid chloride, 3, was formed using oxalyl chloride in benzene. The isolated 3 was added to the lithium salt of (4S)-4-(phenylmethyl)-2-oxazolidinone[11] in THF at -78°C to produce a quantitative yield of 4. The anion of 4 was generated by reaction with LDA at -78°C. Di-tert-butyl azodicarboxylate was then added to

give 357 mCi (46% yield) of 5. The chiral director group was removed by stirring 5 at 0°C in aqueous LiOH solution, giving a nearly quantitative yield of 6. The BOC group in 6 was removed by treatment with TFA, and N–N cleavage of the resulting hydrazine bond was accomplished by hydrogenolysis at 50 psi using Raney-Ni as a catalyst and 2-propanol/water as the solvent. This gave a 97% yield of L-[1-^{14}C]phenylalanine (8) at >90% chemical and radiochemical purity (HPLC). In parallel unlabeled runs, the comparable product was analyzed by a chiral HPLC column and found to consist of L-phenylalanine (retention time 6.27 min.) as the major product and < 1% of the D-isomer (retention time 4.27 min). To obtain compound 10, the carboxyl group was first protected by silylation with 1,1,1,3,3,3-hexamethyldisilazane and a catalytic amount of trimethylsilyl chloride in refluxing acetonitrile. After the solvent was removed, morpholinosulfonyl chloride and CH_2Cl_2 were added at 5°C and the solution was stirred at room temperature for 17 h. After work up, 10 was isolated in a 25% yield and 91% chemical and radiochemical purity (HPLC), with recovery of unreacted L-[1-^{14}C]phenylalanine. An equimolar mixture of 10, dicyclohexylcarbodiimide and 1-hydroxybenzotriazole in DMF was treated with a solution of 11 and Et_3N in CH_2Cl_2. The sample of 11 used was an equilibrated mixture of diastereomers with respect to one of the four chiral centers. After 62 h the crude product was isolated in 93% yield and 86% RCP. Further purification by chromatography gave [^{14}C]PD 132002 in 23% yield and >98% RCP.

EXPERIMENTAL

Barium [^{14}C]carbonate was purchased from American Radiolabeled Chemical, St. Louis, MO. (4S)-4-(Phenylmethyl)-2-oxazolidinone was synthesized as described by Evans and Weber.[11] Radioactivity was determined with a Packard Tri-Carb 4530 liquid scintillation counter, using Beckman Ready-Gel as the counting medium. TLC plates, E. Merck silica gel 60 F-254, were scanned on a Berthold LB2832 automatic TLC linear analyzer. Column chromatography was performed using E. Merck silica gel, 230-400 mesh. HPLC was performed using a Spectra Physics SP8700 solvent delivery system, Kratos Spectroflow 773 variable wavelength UV detector, and Radiomatic Beta Flow 1 radioactivity flow detector.

^{1}H-NMR spectra were recorded on a Varian XL-200 (200 MHz) spectrometer. Chemical shifts are reported in δ units downfield from tetramethylsilane.

[1-^{14}C]Benzenepropanoic Acid (2): [^{14}C]Carbon dioxide generated from $Ba^{14}CO_3$ (3.739 g, 56.1 mCi/mmol, 1054 mCi) and H_2SO_4 was transferred under static vacuum to 2-phenylethyl magnesium bromide [made from magnesium (550 mg, 22.5 mmol) and 1 (4.18 g, 22.5 mmol)]; the mixture was then slowly warmed from -200°C to room temperature. After 3.5 h, 1 M HCl (30 mL) was added, and the ether layer was separated from the aqueous layer. The aqueous layer was extracted with ether (3 x 20 mL). The ether extracts were combined and extracted with 1 M NaOH (60 mL). The aqueous layer was washed with ether, acidified with concentrated HCl, and extracted with CH_2Cl_2 (5 x 20 mL). The extracts were combined, dried ($MgSO_4$), and evaporated to a white solid, 2.4764 g (16.5 mmol, 925 mCi, 88%). TLC: R_f=0.20; RCP=100%, (SiO_2, CH_2Cl_2:CH_3OH 19:1).

(4S)-4-(phenylmethyl)3-(3-Phenyl-1-[1-^{14}C]oxopropyl)-2-oxazolidinone (4): Oxalyl chloride (6 mL) was added to a solution of 2 in benzene (15 mL) under N_2. After 1.5 h at room temperature, the solution was heated to 55°C for 4 h. The solvent was then evaporated in vacuum and co-evaporated several times with toluene to give the acid chloride 3. n-BuLi (2.5 M, 7 mL, 17.5 mmol) was added to a cold THF solution (-78°C) of (4S)-4-(phenylmethyl)-2-oxazolidinone (3.01 g, 17 mmol) over 25 min. and the mixture was stirred at -78°C for 30 min. The acid chloride 3 prepared above, in THF (10 mL), was then added over 20 min. After 0.5 h, the solution was allowed to warm to room temperature. Saturated NH_4Cl solution was added after 2.25 h, the solvent was evaporated and the residue was extracted with CH_2Cl_2 (3 x 40 mL). The combined CH_2Cl_2 extracts were dried ($MgSO_4$) and evaporated to give 5.7477 g of brown oil. TLC R_f=0.70, RCP=97.6% (SiO_2, CH_2Cl_2:MeOH 19:1).

Bis(1,1-dimethylethyl) [S-(R*,R*)]-1-[2-oxo-2-[2-oxo-4-(phenylmethyl)-3-oxazolidinyl]-1-(phenylmethyl)[2-^{14}C]ethyl]-1,2-hydrazinedicarboxylate (5): A solution of 4 (7.725 g, 25 mmol) in THF (10 mL) was added to a freshly prepared LDA solution (26.25 mmol, from diisopropylamine and n-BuLi) at -78°C during 0.5 h. After 1 h at -78°C, di-tert-butylazodicarboxylate (6.62 g, 28.75 mmol) in THF (20 mL) was added, and stirring was continued for 0.5 h at this temperature.

The reaction was quenched with AcOH (5 mL), and the solvent was evaporated. The residue was distributed between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was separated, washed with brine and dried ($MgSO_4$). The product was purified by column chromatography (SiO_2, Hexane:EtOAc 4:1), to give 6.2 g of 5 (46%; 57.6 μCi/mg; 357 mCi).

Bis(1,1-dimethylethyl) (S)-1-(1-[^{14}C]carboxy-2-phenylethyl)-1,2-hydrazinedicarboxylate (6): An ice-cold solution of lithium hydroxide hydrate (1.11 g in 23 mL of H_2O) was added to a solution of 5 in THF (46 mL) at 0°C. The mixture was stirred at 0°C for 3 h and refrigerated overnight. The solvent was evaporated, and the residue was distributed between H_2O and EtOAc. The aqueous layer was acidified with 1 M $NaHSO_4$. The product was extracted with EtOAc (5 x 20 mL), and the combined EtOAc extracts were dried ($MgSO_4$) and evaporated. The crude product was used for the next step without further purification.

L-[1-^{14}C]-Phenylalanine (8): Trifluoroacetic acid (200 mL) was added to a solution of 6 in CH_2Cl_2 (200 mL) under N_2. After stirring at room temperature for 4 h, the solvent was evaporated and again co-evaporated twice with toluene (2 x 20 mL) to give (S)-α-hydrazinobenzenepropanoic acid (7). Compound 7 was transferred to a 250 mL hydrogenating bottle, together with isopropyl alcohol (50 mL), H_2O (50 mL) and Raney-Ni (5 g), and hydrogenated (50 psi) for 21 h. The mixture was filtered and extracted with CH_2Cl_2 (3 x 50 mL). The aqueous layer was evaporated, and the product was used for the next step without further purification.

N-(4-Morpholinosulfonyl)-L-[1-^{14}C]phenylalanine (10): A suspension of 8 in CH_3CN (50 mL) was treated with 1,1,1,3,3,3-hexamethyldisilazane (16.8 g, 104 mmol), a few drops of Me_3SiCl, and heated at reflux under N_2. After 3 h, the solvent was removed and co-evaporated with toluene to remove the last traces of NH_3 and the disilazane. The residue, O-(trimethylsilyl)-N-(trimethylsilyl)-L-phenylalanine (9), was redissolved in CH_3CN (50 mL) and then cooled to 5°C and treated with a solution of the N-morpholinosulfonyl chloride (5.3 g, 28.7 mmol) in CH_2Cl_2 (2 mL). The reaction was stirred for 17 h at room temperature and then evaporated. The residue was partitioned between CH_2Cl_2 and water. The organic

layer was then washed with 1 M HCl, and the product was extracted from the organic layer with 0.5 M NaOH. The basic aqueous extract was acidified to pH 1, and the product was extracted with ethyl acetate (3 x 20 mL). The combined organic extract was washed with brine, dried ($MgSO_4$) and evaporated to a colorless solid (680 mg, 25%): HPLC [1% aqueous $MeSO_3H$:CH_3CN (95:5); CN column; flow rate: 1 mL/min; UV: 214 nm] showed that both chemical and radiochemical purities were 90%.

[^{14}C]PD 132002: A solution of 10 (340 mg, 1.08 mmol), 1-hydroxybenzotriazole hydrate (146 mg, 1.08 mmol), dicyclohexylcarbodiimide (222 mg, 1.08 mmol) and DMF (3 mL) was stirred at 15-20°C for 20 min. A solution of 11 in CH_2Cl_2 (5 mL) containing Et_3N (0.2 mL) was added. The resulting slurry was stirred for 62 h at 25°C. The reaction mixture was filtered, and the filtrate was evaporated under high vacuum. The residue was dissolved in EtOAc and evaporated again to remove the last traces of DMF. The resulting gum was partitioned between CH_2Cl_2 and 0.5 M $NaHCO_3$, and the organic layer was dried ($MgSO_4$) and concentrated at reduced pressure to give 695 mg of yellow foam. TLC: R_f=0.55, two partially resolved spots, 86.5% radiochemical purity (SiO_2, $CHCl_3$:MeOH 9:1). The crude product was purified by SiO_2 column (3.5 x 45 cm) three times (5% MeOH/$CHCl_3$; 5% MeOH/$CHCl_3$; 1% MeOH/$CHCl_3$). Fractions containing the product were collected, combined and lyophilized to give 174.2 mg (9.67 mCi), of [^{14}C]PD 132002 as a white solid (yield 23%). Specific activity: 55.6 μCi/mg, 35.7 mCi/mmol; TLC: R_f=0.58, RCP = 100% (SiO_2, $CHCl_3$:MeOH 9:1); HPLC: t_R = 8.8 and 9.5 min (ratio of diastereomers 2:3) RCP and chemical purity > 98%; ^{1}H-NMR ($CDCl_3$): δ 7.53(dd,1H), 7.41-7.24(m,5H), 6.91(dd,1H), 5.43(dd,1H), 5.18(t,1H), 4.41-4.30(m,1H), 4.11-4.00(m,1H), 3.91-3.86(m,1H), 3.83(s,3H), 3.51-3.49(m,3H), 3.43-3.11(m,3H), 2.99-2.68(m,5H), 2.30-2.13(m,1H), 1.93-1.58(m,8H), 1.42(d,2H), 1.34-1.03(m,4H), 0.96-0.86(m,6H).

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