Synthesis of N^2-[(*S*)-1-Carboxy-3-phenylpropyl]-L-lysyl-L-proline (Lisinopril)

M. T. Wu[x], A. W. Douglas, D. L. Ondeyka, L. G. Payne, T. J. Ikeler, H. Joshua, and A. A. Patchett

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Abstract □ The synthesis and some of the spectral properties of N^2-[(*S*)-1-carboxy-3-phenylpropyl]-L-lysyl-L-proline (lisinopril, MK-521) are described. This compound inhibits angiotensin-converting enzyme with an IC_{50} of 1.2×10^{-9} M.

Angiotensin-converting enzyme (peptidyldipeptide hydrolase, EC 3.4.15.1) generates the powerful vasoconstrictor angiotensin II by removing the *C*-terminal dipeptide from the precursor decapeptide angiotensin I. The enzyme also inactivates the vasodilating substance bradykinin. Thus, there is interest in inhibitors of the enzyme as potential antihypertensive drugs.

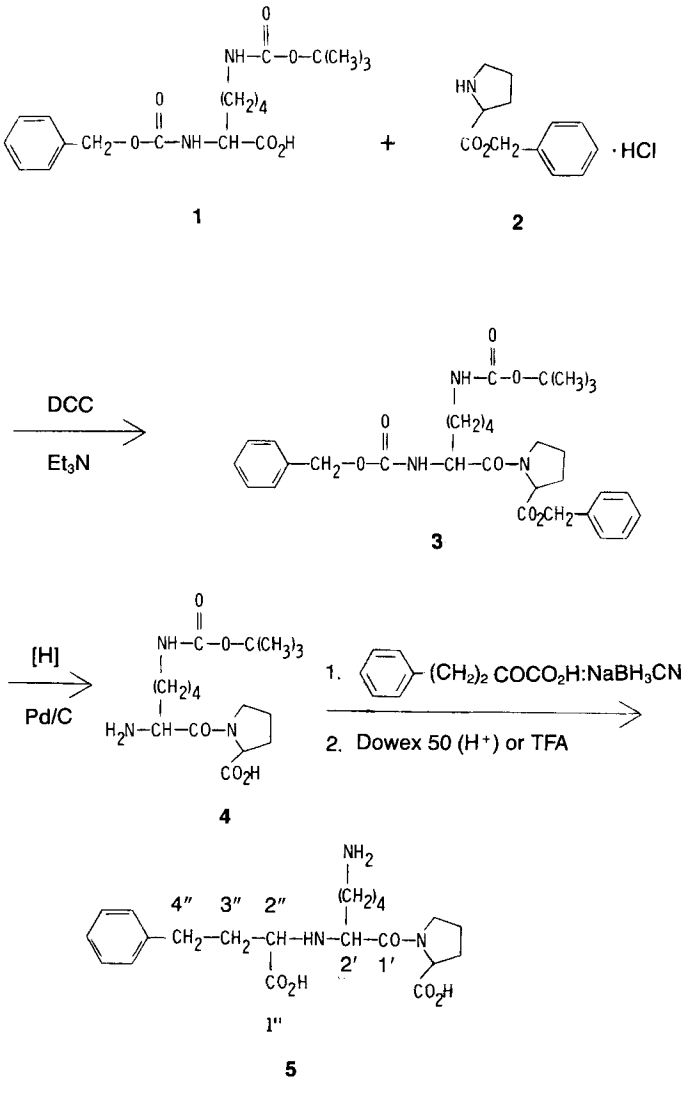
In 1980 we reported[1] a novel class of potent and specific inhibitors of angiotensin-converting enzyme (ACE). The compounds are substituted *N*-carboxymethyl dipeptides. The basic structural requirements for ACE inhibition by these compounds are (*a*) a carboxylic acid group, (*b*) a hydrophobic residue, and (*c*) a nonamide secondary NH.

Several of the compounds mentioned in this preliminary communication[1] were selected for in-depth clinical studies, including enalapril (MK-421), the prodrug form of enalaprilat (MK-422). More recently, extensive clinical studies have been undertaken with N^2-(1-carboxy-3-phenylpropyl)-L-lysyl-L-proline, since it displays excellent oral activity and a long duration of action in animal studies. The more active diastereoisomer of this compound has an IC_{50} of 1.2×10^{-9} M against the enzyme in vitro.[1] It was designated MK-521 and bears the nonproprietary name lisinopril.

The synthesis of analogues of MK-521 in which changes have been made at the lysine group has recently been reported.[2] We now wish to describe the synthesis and structural characterization of MK-521 (lisinopril) itself.

Results and Discussion

Chemistry—The synthetic sequence used to prepare **5a** (lisinopril, MK-521) is shown in Scheme I. N^2-Benzyloxycarbonyl-N^6-*tert*-butoxycarbonyl-L-lysine (**1**) was coupled with L-proline benzyl ester HCl (**2**) by means of dicyclohexylcarbodiimide (DCC) in the presence of triethylamine to give **3**, which was then hydrogenated to **4** over 10% palladium-on-carbon. Reductive condensation of this intermediate with 2-oxo-4-phenylbutyric acid utilizing sodium cyanoborohydride followed by acid hydrolysis afforded N^2-(1-carboxy-3-phenylpropyl)-L-lysyl-L-proline (**5**) as a mixture of two diastereoisomers (*S,S,S* and *R,S,S*). The diastereoisomers were separated by chromatography on an XAD-2 column using 3% acetonitrile in 0.1 M NH_4OH. The first isomer to be eluted was the more active enzyme inhibitor. It was assigned an (*S,S,S*)-configuration in analogy with enalaprilat (MK-422), whose stereochemistry was assigned by X-ray crystallography and by an independent synthesis using L-homophenylalanine. Attempts to obtain satisfactory crystals for a structure determination of lisinopril by X-ray crystallography have been unsuccessful to date. Accordingly, we have related the chemical shifts of the lisinopril diastereoisomers to those of MK-422 (*S,S,S*) and its less active (*R,S,S*)-diastereoisomer as a means of confirming the *S,S,S* conformational assignment of lisinopril.



Scheme I

Carbon-13 Nuclear Magnetic Resonance Spectra—Carbon-13 chemical shifts for all carbon atoms of the (*S,S,S*)- and (*R,S,S*)-isomers of both MK-422 and MK-521 are presented in Table I. Differential effects corresponding to the isomeric change (i.e., to inversion, conceptually, of the 4-phen-

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Table I—^{13}C Chemical Shifts (Major Conformer Only)[a]

Carbon No.	MK-422 (*S,S,S*)	Iso-MK-422 (*R,S,S*)	$\Delta\delta$[b]	MK-521 (*S,S,S*)	Iso-MK-521 (*R,S,S*)	$\Delta\delta$[b]
1	175.83	175.70	0.13	175.71	175.65	0.06
2	60.34	60.17	0.17	60.53	60.42	0.11
3	29.50	29.39	0.11	29.49	29.44	0.05
4	25.35	25.21	0.14	25.39	25.27	0.12
5	48.27	48.13	0.14	48.70	48.68	0.02
1′	168.36	168.16	0.20	167.32	167.39	−0.07
2′	55.83	54.24	1.59	59.73	58.06	1.67
3′	15.41	15.13	0.28	27.20	27.28	−0.08
4′	—	—	—	21.57	21.38	0.19
5′	—	—	—	30.02	30.00	0.02
6′	—	—	—	39.89	39.85	0.04
1″	171.47	171.58	−0.11	171.44	171.42	0.02
2″	59.71	58.01	1.70	60.11	58.91	1.20
3″	31.92	30.78	1.14	31.92	30.84	1.08
4″	31.14	31.28	−0.14	31.21	31.24	−0.03
iso	140.61	140.52	0.09	140.53	140.51	0.02
ortho	129.67	129.76	−0.09	129.65	129.78	−0.13
meta	129.44	129.61	−0.17	129.50	129.57	−0.07
para	127.56	127.66	−0.10	127.58	127.71	−0.13

[a] At 10% w/v in ~1 M DCl:D_2O. Chemical shift assignments for all four molecules were made independently with reference to literature shifts (ref. 3) and model studies of amino acids and simple peptides. Carbon–proton spin coupling and proton off-resonance decoupling experiments were also utilized. [b] Positive $\Delta\delta$ signifies greater shielding in "iso" form. [c] Assignments could be reversed.

ylbutyric asymmetric methine, 2″) appear in columns denoted as $\Delta\delta$. The differential shifts are significant for only three carbon atoms in each isomeric pairs, identified as 2′, 2″, and 3″ in **5**. All affected shifts belong to carbons situated no more than three bonds from the site controlling recognizability of an isomeric shielding effect. The unmistakable similarity in both magnitude and direction of isomeric differential effects on carbon-13 chemical shifts is strong evidence that the isomeric pair of MK-521 structures are correctly assigned.

Experimental Section

Melting points were determined on a Thomas-Hoover Unimelt apparatus and are uncorrected. Microanalyses were performed by the Analytical Section of these laboratories. Optical rotations were measured with a Perkin-Elmer 241 polarimeter in 10-cm cells at 25°C. ^1H NMR spectra were usually obtained on the Varian T-60A spectrometer using tetramethylsilane as an internal standard. For detailed proton assignment studies, a Bruker WM 250 spectrometer was employed to compare MK-521 and its (*R,S,S*)-isomer. ^{13}C NMR spectra were recorded on a Varian Associates XL100A spectrometer. The electron-impact MS were determined with a LKB-9000 mass spectrometer (70 eV) or a Varian MAT-731(FAB). TLC was performed on Analtech silica gel GF plates (250 μm) developed with EtOAc:*n*-BuOH:H_2O:HOAc (1:1:1:1). HPLC analyses were performed on a reversed-phase (RP-18) column at 50°C with a solvent mixture consisting of 13% acetonitrile in 0.005 M tetrabutylammonium phosphate (pH 7.2).

***N*-(*N*^{2}-Benzyloxycarbonyl-*N*^{6}-*tert*-butoxycarbonyl-L-lysyl)-L-proline Benzyl Ester (3)**—To a solution of *N*^{2}-benzyloxycarbonyl-*N*^{6}-*tert*-butoxycarbonyl-L-lysine (2.20 g, 5.8 mmol) in dichloromethane (25 mL) at 0°C was added L-proline benzyl ester hydrochloride (1.54 g, 6.4 mmol) and triethylamine (0.88 mL, 6.4 mmol). Dicyclohexylcarbodiimide (1.31 g, 6.4 mmol) was added in one portion to the stirred solution. After 1 h, the solution was allowed to warm to room temperature. After an additional 15 h of stirring, the white dicyclohexylurea was removed by filtration, and the filtrate was concentrated under reduced pressure to dryness. The gummy solid was thoroughly stirred with ethyl acetate (100 mL) and was filtered to remove insoluble triethylamine hydrochloride. The filtrate was washed with 1 M HCl (2 × 25 mL), saturated $NaHCO_3$ (2 × 25 mL), and saturated NaCl (2 × 25 mL). The solution was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give **3** (2.85 g, 79% yield), a colorless oil; ^1H NMR ($CDCl_3$): δ 1.41 (m, 15, *tert*-butyl, lysyl C-3, C-4, and C-5), 1.9–2.1 (br s, 4, proline C-3 and C-4), 2.9–3.2 (m, 2, lysyl C-6), 3.4–3.8 (bd m, 2, proline C-5), 4.4–4.7 (m, 2, —NRC*H*R′CO—), 5.07 (s, 2, benzyl CH_2), 5.14 (s, 2, CBZ CH_2), and 7.30 ppm (s, 5, ArH); MS: *m/z* 567 (M^+), 5.11 (M^+ − butyl), 494 (M^+ − *tert*-butoxy), and 467 (M^+ − *tert*-Boc).

***N*-(*N*^{6}-*tert*-Butoxycarbonyl-L-lysyl)-L-proline (4)**—Compound **3** (2.85 g, 5.0 mmol) was dissolved in ethanol (100 mL); acetic acid (0.5 g) and 10% Pd/C (0.5 g) were added, and the mixture was hydrogenated at room temperature and 40 psi for 15 h. The mixture was filtered, and the filtrate was concentrated to dryness to give **4** (1.35 g, 78%), a white solid; TLC: single spot, R_f 0.75; ^1H NMR (D_2O): δ 1.61 (br s, 15, *tert*-butyl, lysyl C-3, C-4, and C-5), 2.0–2.35 (m, 4, proline C-3 and C-4), 3.1–3.5 (m, 2, proline C-5), 3.5–4.0 (m, 2, lysyl C-6), and 4.4–4.7 ppm (m, 2, —NRC*H*R′CO—); MS (silylated): *m/z* 559 (trisilyl pdt.), 544 (559 − CH_3), 488 (disilyl + 1), 472 (487 − CH_3), and 343 (M^+).

***N*^{2}-(1-Carboxy-3-phenylpropyl)-L-lysyl-L-proline (5).** Compound **4** (2.68 g, 7.80 mmol) and 2-oxo-4-phenylbutyric acid (6.95 g, 39.0 mmol) were suspended in 70 mL of water and the pH was adjusted to 7.0 with dilute sodium hydroxide. An aqueous solution of sodium cyanoborohydride (1.47 g, 23.4 mmol, in 28 mL of H_2O) was added in 2-mL increments evenly spaced over 8 h. After stirring at room temperature overnight, the mixture was stirred with 200 mL (wet) of Dowex 50 (H^+, 50–100 mesh) for several hours until hydrogen evolution ceased. During the period, the pH dropped to 1–2, and a yellow gum (a mixture of hydroxy acid and excess ketoacid) separated and was removed. The clear aqueous solution and the resin were added to a 400-mL bed of fresh Dowex, which was washed until the pH of the eluate rose to ~5. The product was eluted with 2% pyridine in water. After the product began to elute, a total of 1700 mL was collected and concentrated under reduced pressure to give 2.75 g of **5** (87%) as a white foamy solid; ^1H NMR (D_2O) indicated no remaining *tert*-Boc and a structure consistent with **5**.

Purification of 5 by LH-20 Gel Filtration Chromatography—The aforementioned product (2.75 g) was dissolved in 10 mL of methanol. A white crystalline precipitate formed that was removed by filtration and was washed with methanol. The filtrate was concentrated to a small volume, causing precipitation of a more crystalline solid. This procedure was repeated a total of four times to give 1.34 g of a methanol-insoluble solid, which was 89% the less active diastereoisomer **5b** by HPLC. The methanol-soluble portion (1.41 g in 5 mL of CH_3OH) was added to an LH-20 column (2.5 × 230 cm) packed in methanol and 20-mL fractions were collected at pump setting 2.8. The desired material was eluted in fractions 21–33 as detected by UV (LKB-UVICORD control unit, type 4701A), to give 1.16 g of white foam-like solid. TLC showed two overlapping spots, R_f 0.45 and 0.41, and HPLC indicated a 50:50 diastereoisomeric mixture (**5a**, *S,S,S*:**5b**, *R,S,S*).

Separation Chromatography–Isolation of *N*^{2}-[(*S*)-1-Carboxy-3-phenylpropyl]-L-lysyl-L-proline— A water-jacketed 2 in × 6 ft. (~5 cm × 1.9 m) column (3.5 L) was packed with 200–325 mesh XAD-2 polystyrene resin [previously defined by repeated (7×) slurrying in ethanol, settling for 1 h, and decanting] in 3% CH_3CN:0.1 M NH_4OH, and the temperature was raised to 50°C. The purified concentrate from the LH-20 column equivalent to 10 g of isomer mixture **5** was charged to the XAD-2 column and eluted with 3% CH_3CN:0.1 M NH_4OH at a flow rate of 80 mL/min. The desired isomer appeared in the first major UV peak at 3.0–4.0 min and was isolated (removal of the solvent under reduced pressure and freeze-drying) to yield 2.5 g of **5a**. The product was recrystallized from CH_3OH:EtOAc, mp 159–160°C; $[\alpha]_D^{25}$ −23.3° (*c* 1.0, CH_3OH); TLC: R_f 0.46; MS (FAB): *m/z* 406 (M^+);

^{1}H NMR (D_2O): δ 1.4–2.4 (m, 12, lysyl, C-3, C-4, and C-5, $PhCH_2CH_2$—, proline C-3 and C-4), 2.5–3.15 (m, 4, $PhCH_2$— and $\geq NCH_2$—), 3.15–3.7 (m, 3, $\geq NCH_2$— and —NC*H*RCO—), 4.0–4.2 (m, 2, —NC*H*RCO—), and 7.19 ppm (s, 5, ArH).

Anal.—Calc. for $C_{21}H_{31}N_3O_5 \cdot H_2O$: C, 59.56; H, 7.85; N, 9.92. Found: C, 59.56; N, 7.88; N, 9.77.

References and Notes

1. Patchett, A. A.; Harris, E.; Tristram, E. W.; Wyvratt, M. J.; Wu, M. T.; Taub, D.; Peterson, E. R.; Ikeler, T. J.; ten Broeke, J.; Payne, L. G.; Ondeyka, D. L.; Thorsett, E. D.; Greenlee, W. J.; Lohr, N. S.; Hoffsommer, R. D., Jr.; Joshua, H.; Ruyle, W. V.; Rothrock, J. W.; Aster, S. D.; Maycock, A. L.; Robinson, F. M.; Hirschmann, R.; Sweet, C. S.; Ulm, E. H.; Gross, D. M.; Vassil, T. C.; Stone, C. A. *Nature*, **1980**, *288*, 280.
2. Wu, M. T.; Ikeler, T. J.; Payne, L. G.; Ondeyka, D. L.; Hoffsommer, R. D., Jr.; Pachett, A. A.; Ulm, E. H.; Lamont, B. I. "Modified Lysine Analogs of N^2-[1-(*S*)-Carboxy-3-phenylpropyl]-L-lysyl-L-proline (Lisinopril, MK-521)"; American Chemical Society, St. Louis, MO, April 1984, Abstr. p. 15, MEDI.
3. Stothers, J. B. "Carbon 13 NMR Spectroscopy"; Academic Press: New York, 1972; p 479.

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