CYCLIC (HYDROXYPHOSPHINYL)ACYL DIPEPTIDES: A NEW CLASS OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS

HAROLD N. WELLER* and MARY BETH ROM

The Squibb Institute for Medical Research, P.O. Box 4000, Princeton, New Jersey 08543–4000, USA

Conformationally constrained phenylbutyl(hydroxyphosphinyl)acyl dipeptides are potent inhibitors of angiotensin converting enzyme. The activity enhancement obtained by introducing conformational constraint into these molecules is greater than for related sulfhydryl and carboxyl analogs. The results are interpreted in terms of a binding model which optimally positions both zinc binding and hydrophobic groups for active site binding.

KEY WORDS: Angiotensin converting enzyme, inhibition, ACE, acyl dipeptides, subsite binding.

INTRODUCTION

Progress in the design of angiotensin-converting enzyme (ACE, EC 3.4.15.1) inhibitors has been guided by a hypothetical model of enzyme substrate interactions as initially proposed by Ondetti and Cushman.[1,2] For example, the model was used to aid development of potent ACE inhibitors such as 1^3, 2^4, and 3 (enalaprilat)[5], which make use of important binding interactions in the S_2', S_1', and S_1 regions of the enzyme active site (Figure 1). Importance of the S_1 binding group in those compounds has been shown by deletion or modification of that group, which causes significant loss of inhibitory potency.[3-5]

We recently reported a series of substituted succinyl dipeptides (for example 5 and 6) designed to similarly incorporate an appropriate S_1 binding group (Table I).[6] Those compounds, however, were not significantly more active than the unsubstituted parent (4), suggesting that S_1 subsite binding was not achieved. At the same time we reported that conformational constraint of the succinate carboxyl groups can result in a substantial increase in potency, with the optimal geometry resulting from introduction of a *trans*-substituted cyclohexane ring having the (R, R)-configuration (e.g., 7). That result led us to propose a binding model for 7 as shown in Figure 2 wherein the S_1 subsite is at least partially filled by the atoms of the contraining ring. In light of our inability to obtain apparent S_1 subsite binding from the unconstrained substituted succinyl derivatives, however, another possibility must also be considered wherein the S_1 subsite region remains unoccupied and the observed activity enhancement (relative to 4) is due to conformational constraint alone (Figure 3). In the latter case, there is opportunity for a further increase in potency through introduction of S_1 subsite interactions. We now wish to describe our efforts to introduce such potential
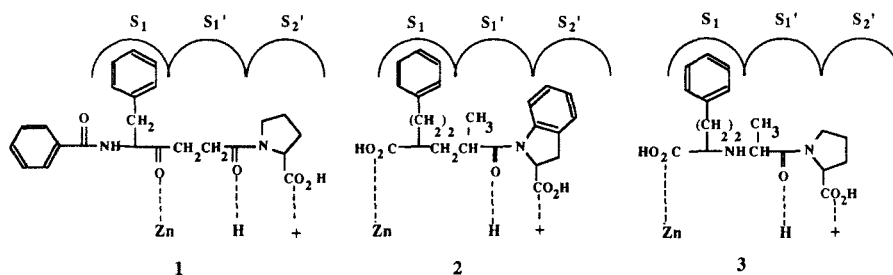
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* Correspondence.

FIGURE 1:   Hypothetical Binding Model for ACE Inhibitors.

S_1 subsite binding groups into functionalized conformationally constrained acyl dipeptides.

Compounds such as **8**, which incorporates a phosphinic acid as the "zinc binding" group, have been found to be potent ACE inhibitors.[7] Since the valence of the phosphinate group offers an additional site for attachment of substituents (versus carboxyl- or sulfhydryl-groups), we chose to study phosphinic acid bearing acyl
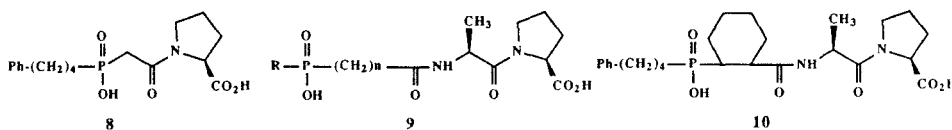
dipeptides (**9**) having various substituents (**R**) and spacings (**n**). We then chose the most active such derivative for incorporation of a *trans* six-membered ring (as in **7**), resulting in a new conformationally constrained compound (**10**) with potential for S_1 subsite interactions.

TABLE I
Substituted Succinyl Dipeptides

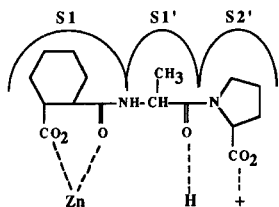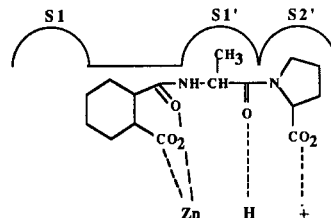| No.	R_1	R_2	I_{50} (µM)
4	H	H	37
5	H	CH_2Ph	11
6	CH_2Ph	H	110
7	$-CH_2CH_2CH_2CH_2-$		2.8

FIGURE 2:   Proposed Binding of **7**.

FIGURE 3:   Alternate Binding of **7**.

MATERIALS AND METHODS

General

All reagents and solvents were commercially available ACS reagent grade, except as noted. Terahydrofuran (THF) was distilled from sodium/benzophenone ketyl prior to use. Organic extracts were dried over anhydrous magnesium sulfate. Flash chromatography was performed as previously described[8] using Whatman LPS-1 silica gel. HP-20 referes to Mitubishi CHP20P polystyrene gel, 75–150 μ. Thin layer chromatography was performed using precoated silica gel glass plates from E.M. Science (number 5719). HPLC analysis was performed using two Beckman model 110A solvent pumps with a model 410 solvent programmer and a Perkin-Elmer LC-75 spectrophotometric detector. Preparative HPLC was performed using two Perkin-Elmer Series 10 solvent pumps (outfitted with preparative heads) with system controller and an LKB model 2151 variable wavelength monitor outfitted with a 0.4 mm flow cell. HPLC columns were commercially available as described from Whatman or YMC Corp. N.m.r. spectra were recorded on one of the following: JOEL GX-400 operating at 400 MHz (^{1}H), JOEL FX270 operating at 270 (^{1}H) or 67.8 (^{13}C) MHz, JOEL FX-60Q operating at 15 MHz (^{13}C). Mass spectra were recorded on a Finnigan MAT TSQ-4600 mass spectrometer (chemical ionization, CI) or a VG-ZAB-2F mass spectrometer (fast atom bombardment, FAB). High resolution mass spectra (HRMS) of dilithium salts were determined on the $(M + Li)^+$ ion using peak matching techniques versus PEG standards on a VG-ZAB-2F spectrometer. Optical rotations were measured using a Perkin-Elmer model 241 polarimeter and a 10 cm path length optical cell. Final target compounds were obtained as lyophilized dilithium salts which had the appearance of deliquescent while solids; microanalysis results were adjusted to obtain best fit assuming non-stoichiometric hydration.

Preparation of 1-[N-[(Ethoxymethylphosphinyl)acetyl]-L-alanyl]-L-proline, benzyl ester (14)

To a stirring solution of α-ethoxymethylphosphinyl acetic acid[9,10] (**11**, 4.06 g, 24.4 mmol) in THF (50 ml) at 0°C was added carbonyldiimidazole (4.09 g, 25.2 mmol). The solution was stirred for 1 hour at 0°C, after which L-alanyl-L-proline, benzyl ester p-toluene sulfonate[11] (10.7 g, 24.5 mmol) and triethylamine (6.7 ml, 48 mmol) were added. The resulting mixture was stirred at 25°C for 18 h after which it was diluted with ethyl acetate, washed with 1N hydrochloric acid, dried, and concentrated. The residue was flash chromatographed, eluting with chloroform:-methanol:acetic acid (25:1:1). Fractions containing the desired product ($R_f = 0.4$) were combined and concentrated. The residue was partitioned between chloroform

and water. The chloroform layer was dried and concentrated to give **14** as a colorless oil (1.48 g, 15%). ^{13}C n.m.r. (67.8 MHz, CDCl$_3$): 171.4 ($\underline{C}$=O) 170.8 ($\underline{C}$=O), 164.5 ($\underline{C}$=O), 66.6 (O-$\underline{C}$H$_2$-Ph), 60.4 (d, $\underline{C}$H$_2$-O-P), 58.7 ($\underline{C}$H-$\overline{C}$OO), 46.8 (N$\overline{H}$-$\underline{C}$H(CH$_3$)-$\overline{C}$O), 46.6 (proline $\overline{C}$5), 38.7 (d, j = $\overline{8}$1, P-$\underline{C}$H$_2$-CO)$\overline{,}$ 28.7 (proline C3), 24.$\overline{7}$ (proline C4), 17.3 ($\underline{C}$H$_3$), 16.3 (P-O-CH$_2$-$\underline{C}$H$_3$), 14.$\overline{5}$ (d, j = 78, $\underline{C}$H$_3$ − P). Mass spectrum (CI): 425$^+$ $\overline{(}$M + H)$^+$.

Preparation of 1-[N-[[Hydroxyl(4-phenylbutyl)phosphinyl]acetyl]-L-alanyl-L-proline, benzyl ester (15)

To a stirring solution of α-phenylbutylhydroxy phosphinyl acetic acid[10] (**12**, 0.51 g, 2.0 mmol) in THF (10 ml) at 0°C was added carbonyldiimidazole (0.34 g, 2.1 mmol). The solution was stirred for 1 h at 0°C, after which L-alanyl-L-proline benzyl ester p-toluenesulfonate[11] (0.89 g, 2.0 mmol) and triethylamine (1.4 ml, 2.0 mmol) were added. The resulting mixture was stirred at 25°C for 18 h, after which it was diluted with ethyl acetate, washed sequentially with 5% aqueous potassium hydrogen sulfate solution, water, and brine, dried, and concentrated. The residue was flash chromatographed, eluting with chloroform:methanol:acetic acid (34:1:1 to 24:1:1 gradient). Fractions containing the desired product (R_f = 0.12, chloroform:methanol:acetic acid, 24:1:1) were combined and concentrated to give **15** as a colorless oil (0.64 g, 62%). ^{13}C n.m.r. (15 MHz, CDCl$_3$): 171.6 ($\underline{C}$=O), 171.2 ($\underline{C}$=O), 168.0 ($\underline{C}$=O), 128.1 (aromatic), 127.8 (aromatic), 127.2 (aromatic), 126.7 (aromatic), 125.$\overline{5}$ (aromatic), 66.6 (O-$\underline{C}$H$_2$-Ph), 58.9 ($\underline{C}$H-COO), 46.9 (NH-$\underline{C}$H(CH$_3$)-CO), 46.9 (proline C5), 38.0 (d, j = $\overline{8}$4, P-$\underline{C}$H$_2$-CO)$\overline{,}$ 35.2 (Ph-$\underline{C}$H$_2$-CH$_2$)$\overline{,}$ 32.6 ($\underline{C}$H$_2$), 31.7 ($\underline{C}$H$_2$), 28.6 proline C3), 24.6 (proline C4), 21.2 ($\underline{C}$H$_2$)$\overline{,}$ 17.0 ($\underline{C}$H$_3$). Mass spectrum ($^+$/-FAB): 553$^+$ (M + K)$^+$/ 513$^-$ (M − H)$^-$.

Preparation of 1-[N-[(Hydroxymethylphosphinyl)acetyl]-L-alanyl]-L-proline, dilithium salt (16)

To a stirred solution of **14** (0.78 g, 1.84 mmol) in dichloromethane (6 ml) was added bromotrimethylsilane (0.49 ml). The resulting solution was stirred at 25°C under argon for 4 h, after which it was concentrated *in vacuo*. The residue was dissolved in ethyl acetate and washed with 0.5 N hydrochloric acid. The organic phase was dried and concentrated to a white foam (0.3 g, 41%). The foam was dissolved in a mixture of ethyl acetate (15 ml) and ethanol (15 ml) and was hydrogenated for 18 h at one atmosphere over 20% palladium hydroxide on carbon catalyst. The resulting mixture was filtered and concentrated to a clear, colorless oil. The residue was dissolved in 0.1 M lithium carbonate solution (7.2 ml) and chromatographed on HP-20 using water as eluant. Fractions containing the desired material (R_f 0.5, isopropanol:ammonium hydroxide:water, 7:4:1) were combined and lyophilized to give **16** as a white solid (0.12 g, 20%). ^{13}C n.m.r. (67.8 MHz, D$_2$O): 180.5 ($\underline{C}$=O), 173.3 ($\underline{C}$=O), 171.0 ($\underline{C}$=O), 63.1 ($\underline{C}$H-COOLi), 48.7 (NH-$\underline{C}$H(CH$_3$)-CO), $\overline{4}$8.5 (proline $\overline{C}$5), 42.1 (d, j = 78, P-$\underline{C}$H$_2$-$\overline{C}$O), 30.4 (proline C3), 25.$\overline{6}$ (proline C4), 16.7 ($\underline{C}$H$_3$), 16.9 (d, j = 98, $\underline{C}$H$_3$ − P). $\overline{M}$ass spectrum ($^+$/-FAB): 319$^+$ (M + H)$^+$/317$^-$ $\overline{(}$M − H)$^-$. HRMS: found 325.1299, C$_{11}$H$_{17}$N$_2$O$_6$PLi$_3$ requires 325.1304. [α]$_D$ = −64° (c = 1, CH$_3$OH). Found: C, 36.58; H, 5.67; N, 7.25; P, 8.70; C$_{11}$H$_{17}$N$_2$O$_6$PLi$_2$· 2.4 H$_2$O requires C, 36.58; H, 6.08; N, 7.76; P, 8.58%.

Preparation of 1-[N-[[Hydroxy(4-phenylbutyl)phosphinyl]acetyl]-
L-alanyl-L-proline, dilithium salt (17)

A solution of **15** (0.64 g, 1.2 mmol) in methanol (10 ml) was hydrogenated for 2 h at one atmosphere over 10% palladium on carbon catalyst. The resulting mixture was filtered and concentrated to a colorless glass. The residue was dissolved in 0.1 M lithium carbonate solution (6 ml) and chromatographed on HP-20, eluting with a gradient from water to methanol. Fractions containing the desired product (R_f 0.57, n-butanol:acetic acid:water:ethyl acetate, 1:1:1:1) were combined and concentrated. The residue was dissoved in water and applied to a column of AG-50-WX2 (Li$^+$ form) ion exchange resin, which was eluted with water. Fractions containing the desired material (TLC) were combined and lyophililzed to give **17** as a white solid (0.22 g, 42%), ^{13}C n.m.r. (67.8 MHz, D$_2$O): 178.4 (C=O), 173.7 (C=O), 170.8 (C=O), 144.1 (aromatic), 129.6 (aromatic), 126.9 (aromatic), 61.5 (CH-COOLi), 48.5 (NH-CH(CH$_3$)-CO), 48.7 (proline C5), 40.4 (d, j = 76, P-CH$_2$-CO), 35.6 (Ph-CH$_2$-CH$_2$), 33.3 (CH$_2$), 31.0 (d, j = 96, CH$_2$-P), 30.0 (proline C3), 25.6 (proline C4), 22.6 (CH$_2$), 16.6 (CH$_3$). $[\alpha]_D = -61°$(c = 0.5, CH$_3$OH). Mass spectrum ($^+$/-FAB): 437$^+$ (M + H)$^+$/435$^-$ (M − H)$^-$, 443$^+$ (M + Li)$^+$/429$^-$ (M − Li)$^-$. HRMS: found 443.2073, C$_{20}$H$_{27}$N$_2$O$_6$PLi$_3$ requires 443.2087. Found: C, 52.08; H, 6.53; N, 5.97; P, 6.60; C$_{20}$H$_{27}$N$_2$O$_6$PLi$_2$ 1.4 H$_2$O requires C, 52.08; H, 6.51; N, 6.07; P, 6.72%.

Preparation of 1-[N-[3-Ethoxymethylphospinyl]-1-oxopropl-L-alanyl]-L-proline,
benzyl ester (25)

To a solution of 3-(ethoxymethylphosphinyl)propionic acid[12] (**23**, 0.87 g, 4.8 mmol) and *N*-methyl morpholine (0.55 ml, 5.0 mmol) in the THF (20ml) under argon at −30°C was added isobutyl chloroformate (0.65 ml, 5.0 mmol). The resulting mixture was stirred at −30°C for 30 minutes, after which L-alanyl-L-proline benzyl ester p-toluenesulfonate[11] (**13**, 2.2 g, 4.9 mmol) and *N*-methyl morpholine (0.55 ml, 5.0 mmol) were added. The mixture was allowed to warm to 25°C and was stirred for 18 h. The solution was then poured in to excess 1 N aqueous hydrochloric acid and was extracted with chloroform. The extract was washed with saturated sodium bicarbonate solution, dried, and concentrated. The residue was flash chromatographed (eluting with chloroform:methanol:acetic acid, 25:1:1) to give as the major product (R_f = 0.5 in the same solvent) **25** (2.1 g, 100%). ^{13}C n.m.r. (15 MHz, CDCl$_3$): 173.3 (C = 0), 171.3 (C = 0), 170.8 (d, j = 14, C = 0), 135.2 (aromatic), 128.6 (aromatic), 128.1 (aromatic), 127.8 (aromatic), 66.4 (O-CH$_2$-Ph), 60.1 (d, j = 6, P-O-CH$_2$-CH$_3$), 58.6 (CH-COOLi), 46.3 (proline C5), 46.3 (CH-CH$_3$), 28.1 (d, j = 12, P-CH$_2$-CH$_2$-CO-), 27.7 (proline C3), 24.5 (proline C4), 24.5 (d, j = 96, P-CH$_2$-CH$_2$), 17.0 (alanine CH$_3$O, 16.3 (PO-CH$_2$CH$_3$), 13.1 (d, j = 85, P-CH$_3$). Mass sprectrum (CI): 439$^+$ (M + H)$^+$, 347$^+$ (M − CH$_2$Ph)$^+$.

Preparation of 1-[N-[3-[Ethoxy(4-phenylbutyl)phosphinyl]-1-oxopropyl]-
L-alanyl]-L-proline, benzyl ester (26)

To a solution of 3-(ethoxy(4-phenylbutyl)phosphinyl)propionic acid[13] (**24**, 1.4 g, 4.6 mmol) and *N*-methyl morpholine (0.53 ml, 4.8 mmol) in THF (15 ml) under argon at −30°C was added isobutyl chloroformate (0.62 ml, 4.8 mmol). The resulting mixture was stirred at −30°C for 30min, after which L-alanyl-L-proline benzyl ester p-toluenesulfonate[11] (**13**, 2.1 g, 4.7 mmol) and *N*-methyl morpholine (0.53 ml, 4.8 mmol) were added. The mixture was allowed to warm to 25°C and was stirred for

18 h. The solution was then poured into excess 1 N aqueous hydrochloric acid and was extracted with dichloromethane. The extract was washed with saturated sodium bicarbonate solution, dried, and concentrated. The residue was flash chromatographed, eluting with chloroform:methanol:acetic acid, 50:1:1. Fractions containing the desired product (R_f 0.5, chloroform:methanol:acetic acid, 25:1:1) were combined and concentrated to give **26** as a colorless oil (2.4 g, 93%). ^{13}C n.m.r. (15 MHz, CDCl$_3$): 174.1 ($\underline{C}$=O), 171.3 ($\underline{C}$=O), 171.0 (d, j = 14, $\underline{C}$=O), 141.3 (aromatic), 135.1 (aromatic), $\overline{1}$28.0 (aromatic), 127.8 (aromatic), 127.$\overline{6}$ (aromatic), 125.4 (aromatic), 66.4 (O-$\underline{C}$H$_2$Ph), 60.4 (d, j = 7, P-O-$\underline{C}$H$_2$-CH$_3$), 58.6 ($\underline{C}$HCOO, 46.6 (proline C5), 46.3 ($\underline{CH}$CH$_3$), 34.8 (Ph-$\underline{C}$H$_2$-CH$_2$) 31.9 (d, j = 15, P-$\underline{C}$H$_2$-CH$_2$-CO), 28.4 (proline C3), 2$\overline{8}$.0 (d, j = 68, P-$\underline{C}$H$_2$-CH$_2$-CO), 27.6 ($\underline{C}$H$_2$), 24.3 (proline C4), 22.0 (d, j = 71, P-$\underline{C}$H$_2$CH$_2$CH$_2$), 20.$\overline{8}$ (d, j = 4, P-CH$_2$-$\underline{C}$H$_2$-CO), 16.9 (alanine $\underline{C}$H$_3$), 16.1 (d, j = 6, P-$\overline{O}$-CH$_2$-$\underline{C}$H$_3$). Mass spectrum ($^+$/-FA$\overline{B}$): 557$^+$ (M + H)$^+$/ 5$\overline{27}$$^-$ (M-C$_2$H$_5$)$^-$.

Preparation of 1-[N-[3-Hydroxymethylphosphinyl]-1-oxopropyl-L-alanyl]-L-proline, dilithium salt (27)

To a solution of **25** (1.1 g, 2.5 mmol) in dichloromethane (5 ml) under argon was added bromotrimethylsilane (1.0 ml, 7.5 mmol). The mixture was stirred for 6 h, after which it was concentrated *in vacuo*. The residue was dissolved in ethyl acetate, washed with 0.5 N hydrochloric acid, dried and concentrated. The residue was dissolved in ethyl acetate:ethanol (1:1, 50 ml) and hydrogenated for 18 h at one atmophere over 20% palladium hydroxide on carbon catalyst. The resulting mixture was filtered and concentrated. The residue was dissolved in water; lithium carbonate solution was added until the pH of the solution was 7.0. The mixture was then chromatographed on HP-20, eluting with water. Fractions containing the desired product (R_f 0.4, isopropanol:ammonium hydroxide:water 7:4:1) were combined and lyophilized to give **27** as a white solid (275 mg, 33%). ^{13}C n.m.r. (67.8 MHz, D$_2$O): 175.8 (d, j = 15, $\underline{C}$=O), 179.9 ($\underline{C}$=O), 172.8 ($\underline{C}$=O), 62.5 ($\underline{C}$H-COOLi), 48.0 (proline C5), 47.9 (alanine $\underline{C}$H-C$\overline{H}$$_3$O, 29.6 (d, $\overline{j}$ = 25, P-$\underline{C}$H$_2$-CH$_2$-CO-), 29.8 (proline C3), 25.0 (proline $\overline{C}$4), 27.7 (d, j = 93, P-$\underline{C}$H$_2$-CH$_2$), 1$\overline{5}$.9 (alanine $\underline{C}$H$_3$), 15.7 (d, j = 93, P-$\underline{C}$H$_3$). Mass spectrum ($^+$/-FA$\overline{B}$): 333$^+$ (M + H)$^+$/33$\overline{1}$$^-$ (M – H)$^-$, 339$^+$ ($\overline{M}$ + Li)$^+$/325$^-$ (M – Li)$^-$. HRMS: found 339.1470, C$_{12}$H$_{19}$N$_2$O$_6$PLi$_3$ requires 339.1461. [α]$_D$ = –85° (c = 1, H$_2$0). Found: C, 32.84; H, 6.50; N, 5.97; P, 6.70; C$_{12}$H$_{19}$N$_2$O$_6$PLi$_2 \cdot$ 6 H$_2$0 requires C. 32.74; H, 7.10; N, 6.36; P, 7.00%.
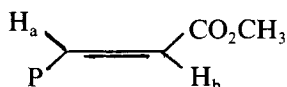
Preparation of 1-[N-[3-[Hydroxyl(4-phenylbutyl)phosphinyl]-1-oxopropyl]-L-alanyl]-L-proline, dilithium salt (28)

To a solution of **26** (1.2 g, 2.1 mmol) in dichloromethane (5 ml) under argon was added bromotrimethylsilane (0.56 ml, 4.3 mmol). The resulting orange mixture was stirred for 16 h, after which it was concentrated *in vacuo*. The residue was dissolved in ethyl acetate, washed with 0.5 N hydrochloric acid, dried and concentrated. The residue was dissolved in ethyl acetate:ethanol (1:1, 50 ml) and hydrogenated for 18 h at one atmophere over 20% palladium hydroxide on carbon catalyst. The resulting mixture was filtered and concentrated. The residue was dissolved in water; 0.1 M lithium carbonate solution was added (17 ml), and the mixture was chromatographed on HP-20, eluting with water to methanol gradient. Fractions containing the desired product (R_f 0.53, isopropanol:ammonium hydroxide:water 7:4:1) were combined

and concentrated *in vacuo*. The residue was lyophilized to give **28** as a white solid (540 mg, 55%). ^{13}C n.m.r. (67.8 MHz, D$_2$O): 180.4 (C=O), 176.2 (d, j = 15, C=O), 173.3 (C=O), 144.1 (aromatic), 129.6 (aromatic), 126.8 (aromatic) 63.1 (CHCOO), 48.5 (proline C5), 48.5 CHCH$_3$, 35.7 (Ph-CH$_2$-CH$_2$), 33.4 (d, j = 15, P-CH$_2$-CH$_2$-CO), 30.4 (proline C3), 30.5 (d, j = 93, P-CH$_2$-CH$_2$—CO), 29.8 (CH$_2$), 25.6 (proline C4), 26.4 (d, j = 91, P-CH$_2$-CH$_2$), 22.8 (P-CH$_2$CH$_2$), 16.6 (alanine CH$_3$). Mass spectrum ($^+$/-FAB): 457$^+$ (M + Li)$^+$/443$^-$ (M — Li). HRMS: found 457.2243, C$_{21}$H$_{29}$N$_2$O$_6$PLi$_3$ requires 457.2243. [α]$_D$ = — 5.8° (c = 1, CH$_3$OH). Found: C, 51.17; H, 6.80; N, 5.38; P, 6.50; C$_{21}$H$_{29}$N$_2$O$_6$PLi$_2$· 2.35 H$_2$0 requires C, 51.20; H, 6.90; N, 5.69; P, 6.29%.

Preparation of (E)-3-[Ethoxy(4-phenylbutyl)phosphinyl]-2-propenoic acid, methyl ester (30)

To a solution of (4-phenylbutyl)phosphinic acid, ethyl ester[14] (**19**, 8.0 g, 35.4 mmols) in chloroform (40 ml) were added trimethylsilyl chloride (13.5 ml, 106 mmols) and triethylamine (19.7 ml, 142 mmols). The resulting mixture was stirred at 25°C for 2.5 h, after which it was concentrated *in vacuo*. To the residue were added methyl α-chloroacrylate[15] (20 g, 0.16 mole) and triethylamine (9.0 ml, 64 mmol). The resulting yellow mixture was stirred overnight at 65°C, resulting in an orange semisolid. The mixture was diluted with ethyl acetate and washed sequentially with 1N hydrochloric acid and saturated sodium bicarbonate solution, dried, and concentrated to an orange oil. The oil was flash chromatographed, eluting with a gradient from 1:1 hexane:ethyl acetate to ethyl acetate, to give **30** (3.72 g, 35%, R$_f$ 0.6 (4:1 toluene:acetic acid)). ^{1}H n.m.r. (400 MHz, CDCl$_3$): 7.1-7.4 (5H, m, aromatic); 6.94 (1H, d (J$_{Ha-P}$ = 21 Hz), d (J$_{Ha-Hb}$ = 17 Hz), *Ha*); 6.76 (1H, d (J$_{P-Hb}$ = 17 Hz), d (J$_{Ha-Hb}$ = 17 Hz), *Hb*); 4.0 (2H, m, OC*H$_2$*CH$_3$); 3.80 (3H, s, CO$_2$C*H$_3$*); 2.60 (2H, d, d C*H$_2$*Ph); 1.5-2.0(6H, m, —CH$_2$-); 1.30 (3H, t, CH$_2$C*H$_3$*). ^{13}C n.m.r. (67.8 MHz, CDCl$_3$): 165 (d, j = 25, C=O), 141.6 (aromatic), 137.3 (vinyl), 136.5 (vinyl), 134.8 (aromatic), 128.2 (aromatic), 125.7 (aromatic), 60.8 (O-CH$_2$CH$_3$), 52.2 (CO$_2$CH$_3$), 35.2 (Ph-CH$_2$), 32.2 (d, j = 16, P-CH$_2$CH$_2$), 28.7 (d, j = 103, P-CH$_2$), 21.0 (CH$_2$), 16.4 (O-CH$_2$CH$_3$). Mass spectrum (CI): 311$^+$ (M + H)$^+$. Found: C, 60.50; H, 7.38; P, 9.40; C$_{16}$H$_{23}$O$_4$-P·0.4 H$_2$O requires C, 60.50; H, 7.55; P, 9.75%.

$$\text{H}_a \diagdown \underset{\text{P}}{\diagup} = \underset{\text{H}_b}{\diagup} \diagdown \text{CO}_2\text{CH}_3$$

Preparation of trans-6-[Ethoxy(4-phenylbutyl)phosphinyl]-
3-cyclohexene-1-carboxylic acid, methyl ester (31)

A solution of **30** (3.72 g, 12.0 mmol) in a minimum amount of toluene (*ca* 5 ml) was transferred to a 25 × 150 mm glass test tube which served as a liner of a stainless steel Parr pressure bomb. The bomb and its contained tube were cooled to — 78°C and a stream of butadiene was bubbled through the mixture until the glass tube was nearly full. The pressure bomb was then quickly sealed and allowed to warm to room temperature. The bomb and its contents were then heated to 100°C and maintained at that temperature for 24 h, after which they were cooled to room temperature. The pressure was released from the bomb and methanol was added to the contents, resulting in a gelatinous precipitate. The precipitate was removed by filtration and the filtrate was concentrated to a clear oil (3.52 g). The oil was flash chromatographed,

eluting with ethyl acetate, to give **31** as a clear oil (2.6 g, 59%, R_f 0.2). ^{1}H n.m.r. (270 MHz, $CDCl_3$): 7.1–7.4 (5H, m, aromatic H); 5.70 (2H, s, vinyl H); 3.9–4.2 (2H, m, OCH_2CH$_3$); 3.70 (3H, s, CO$_2$CH_3); 1.5–2.9 (14 H, m); 1.25 (3H, t, OCH$_2$CH_3). ^{13}C n.m.r. (67.8 MHz, $CDCl_3$): 175.1 (C=O), 141.9 (aromatic), 128.3 (aromatic + vinyl), 125.8 (aromatic + vinyl), 124.7 (aromatic + vinyl), 60.4 (P-O-CH$_2$CH$_3$), 51.9 (CO$_2$CH$_3$, 39.3 (CHCO$_2$CH$_3$), 35.4 (PhCH$_2$), 20.9 (Ph-CH$_2$CH$_2$), 16.5 (P-O-CH$_2$CH$_3$), 38.9, 34.9, 34.4, 33.6, 32.6, 32.5, 28.3, 27.6, 27.1, 25.8, 24.1, 23.2, 21.3 (unassigned multiplet). Mass spectrum (CI): 365$^+$ (M + H)$^+$.

Preparation of trans-6-[Hydroxy(4-phenylbutyl)phosphinyl]-
3-cyclohexene-1-carboxylic acid (32)

A mixture of **31** (1.4 g, 3.85 mmol), 1 N sodium hydroxide solution (4.0 ml, 4.0 mmols), and methanol (4.0 ml) was stirred at 100°C for 24 h after which it was diluted with water. The mixture was acidified by addition of hydrochloric acid and extracted with ethyl acetate. The extract was dried and concentrated to give a glassy oil (1.1 g). HPLC analysis of the residue (1.0 ml/min of 90% aqueous methanol containing 0.2% phosphoric acid eluant on a Whatman ODS-2 analytical column with UV detection at 220 nm) showed three major components of retention times 3.5, 4.0 and 4.8 min respectively, and in a ratio of 27:53:17. The major component (R_t 4.0 min) was isolated by by preparative reverse phase HPLC (whatman ODS-2 "Magnum 20" preparative column eluting with 80% aqueous methanol containing 0.05% trifluoroacetic acid) to give **32** as a colorless oil (850 mg, 69%). ^{1}H n.m.r. (270 MHz, $CDCl_3$): 7.0–7.4 (5H, m, aromatic H); 5.7 (2H, s, vinyl H); 1.0–3.0 (14 H, m). ^{13}C n.m.r. (67.8 MHz, $CDCl_3$): 141.9 (aromatic), 128.2 (aromatic + vinyl), 125.6 (aromatic + vinyl), 124.8 (aromatic + vinyl), 124.6 (aromatic + vinyl), 39.0 (CHCO$_2$CH$_3$), 35.3 (PhCH$_2$), 32.4 (d, j = 15, P-CH$_2$CH$_2$), 20.9 (Ph-CH$_2$CH$_2$), 17.9–27.9 (unassigned multiplet). Mass spectrum (CI): 305$^+$ (M + H − H$_2$0)$^+$. Found: C, 62.49; H, 7.29; P, 9.1; $C_{17}H_{23}O_4P \cdot 0.25 H_2O$ requires C, 62.49; H, 7.25; P, 9.48.

Preparation of trans-1-[N-[[6-[Hydroxy(4-phenylbutyl)phosphinyl]-
*3-cyclohexen-1-yl]carbonyl]-L-alanyl]-L-proline, benzyl ester, isomers **a** and **b** (33a
and 33b)*

To a solution of **32** (550 mg, 1.71 mmol) in THF (5 ml) at 25°C was added carbonyliimidazole (450 mg, 2.7 mmol). The resulting mixture was stirred at 25°C for 1 h, after which L-alanyl-L-proline benzyl ester tosylate salt[11] (1.2 g, 2.7 mmol) and triethylamine (400 μl, 2.8 mmol) were added. The resulting mixture was stirred at 25°C for 18 h after which it was poured into excess 1 N hydrochloric acid. The mixture was extracted with ethyl acetate (3 ×); the extract was dried and concentrated to give a colorless glass (1.13 g). HPLC analysis (YMC A-302 ODS column, 4.6 × 150 mm, eluting with 1.0 ml/min of 78% aqueous methanol containing 0.2% phosphoric acid, UV monitoring at 220 nm) showed two major products (R_t = 6.03 and 6.60 min) in a ratio of 1:1. The products were separated by preparative HPLC (YMC S-15 ODS column, 20 × 500 mm, eluting with 22 ml/min of aqueous methanol containing 0.5% trifluoroacetic acid, retention times 12.8 min and 14.8 min, respectively) to give **33a** (420 mg, 42%) and **33b** (360 mg, 36%). **For 33a:** ^{1}H n.m.r. (400 MHz, $CDCl_3$): 7.0 (1H, br s, NH), 7.1–7.4 (10 H, m, aromatic H), 5.7 (3H, br s, vinyl H + P-OH), 5.05–5.25 (2H, d,d, OCH$_2$Ph), 4.8 (1H, m, NH-CH-CO), 4.6 (1H, m, NH-CH-CO), 1.35 (3H, d, ala

CH$_3$), 3.75 (1H, m, cyclohexyl methine), 3.60 (1H, m, cyclohexyl methine), 1.5–2.8 (18 H, m, methylene). ^{13}C n.m.r. (67.8 MHz, CDCl$_3$): 176.0 (C=O), 172.2 (C=O), 171.1 (C=O), 141.7 (aromatic + vinyl), 135.2 (aromatic + vinyl, 128.5 (aromatic + vinyl), 128.4 (aromatic + vinyl), 128.2 (aromatic + vinyl), 128.1 (aromatic + vinyl), 125.7 (aromatic + vinyl), 124.9 (aromatic + vinyl), 124.3 (aromatic + vinyl), 67.1 (OCH$_2$Ph), 59.3 (CHCO$_2$), 47.3 (proline C5), 47.1 (CHCH$_3$), 40.7 (P-CHR-CHR-CONH-), 34.5 (d, j = 150, P-CHR-CHR-CONH-), 35.2 (Ph-CH$_2$), 32.4 (d, j = 15, P-CHR-CH$_2$-), 28.7 (proline C3), 27.0 (d, j = 115, P-CH$_2$), 29.0 (P-CH$_2$CH$_2$), 24.0 (CH$_2$CHR-CONH-), 24.6 (proline C4), 20.7 (Ph-CH$_2$CH$_2$), 16.4 (CH$_3$). Mass spectrum ($^+$/-FAB): 581$^+$ (M + H)$^+$/579$^-$ (M − H)$^-$. **For 33b**: ^{1}H n.m.r (400 MHz, CDCl$_3$): 7.7 (1H, br s, NH), 7.1–7.4 (10 H, m, aromatic H), 5.6 – 5.9 (2H, br m, vinyl H), 5.3 – 5.9 (1H, m, P–OH), 5.05 – 5.25 (2H, d,d, OCH$_2$Ph), 4.7 (1H, m, NH-C*H*-CO), 4.35 (1H, m, NH-C*H*-CO), 1.35 (3H, d, ala CH$_3$), 3.80 (1H, m, cyclohexyl methine), 3.60 (1H, m, cyclohexyl methine), 1.5 – 2.8 (18 H, m, methylene). ^{13}C n.m.r. (67.8 MHz, CDCl$_3$): 174.7 (C=O), 173.0 (C=O), 170.9 (C=O), 141.6 (aromatic + vinyl), 135.3 (aromatic + vinyl), 128.5 (aromatic + vinyl), 128.2 (aromatic + vinyl), 128.1 (aromatic + vinyl), 125.7 (aromatic + vinyl), 125.4 (aromatic + vinyl), 128.1 (aromatic + vinyl), 67.0 (OCH$_2$Ph), 59.4 (CHCO$_2$), 47.1 (proline C5), 46.9 (CHCH$_3$), 39.0 (P-CHR-CHR-CONH-), 35.6 (d, j = 75, P-CHR-CONH-), 35.3 (Ph-CH$_2$), 32.4 (d, j = 15, P-CHR-CH$_2$-), 28.5 (proline C3), 26.6 (d, j – 115, P-CH$_2$), 27.8 (d, j = 20, P-CH$_2$CH$_2$), 25.2 (CH$_2$CHR-CONH-), 24.6 (proline C4), 20.7 (Ph-CH$_2$CH$_2$), 15.2 (CH$_3$). Mass spectrum ($^+$/-FAB): 581$^+$(M + H)$^+$/ 579$^-$ (M − H)$^-$.

Preparation of trans-*1-[*N-*[[2-[Hydroxy(4-phenylbutyl)phosphinyl]-cyclohexyl]-carbonyl]-L-alanyl]-L-proline,* **isomer a,** *dilithium salt (10a)*[16]

A mixture of **33a** (420 mg, 0.72 mmol) and palladium hydroxide (100 mg of 20% on carbon) in methanol (10 ml) was hydrogenated at 25°C and one atmosphere for 48 h, after which it was filtered and concentrated to give a glassy residue (245 mg, subsequently identified as the methyl ester resulting from transesterification). The residue was dissolved in methanol (0.7 ml) and 1.0 N lithium hydroxide solution was added (0.7 ml). The mixture was stirred at 25°C for 31 h, after which it was diluted with water and washed with ether. The pH of the aqueous solution was adjusted to approximately 6.0 by addition of AG-50 ion exchange resin; the mixture was filtered and lyophilized to give a fluffy white solid (110 mg, 32%). This material was chromatographed on HP-20, eluting with a water to methanol gradient. Fractions were monitored by HPLC (YMS A302 ODS column, 1.0 ml/min of 70% aqueous methanol containing 0.2% phosphoric acid, UV monitoring at 220 nm); those containing the desired product (R$_t$ = 4.88 min) were combined and concentrated. The residue was dissolved in water and lyophilized to give **10a** (50 mg, 15%) as a fluffy white solid. ^{1}H n.m.r. (400 MHz, CD$_3$OD): 7.0–7.4 (6 H, m, aromatic H + NH), 4.55 (1H, q, NH-CH-CO), 4.35 (1H, m, NH-CH-CO), 3.75 (1H, m, cyclohexyl methine), 3.60 (1H, q, NH-CH-CO), 4.35 (1H, m, NH-CH-CO), 3.75 (1H, m, cyclohexyl methine), 3.60 (1H, m, cyclohexyl methine), 1.5 – 2.8 (22 H, m, methylene), 1.35 (3H, d, ala CH$_3$). ^{13}C n.m.r. (67.8 MHz, CD$_3$OD): 177.3 (C=O), 175.6 (C=O), 173.5 (C = O), 143.4 (aromatic), 129.4 (aromatic), 126.8 (aromatic), 60.4 (CHCO$_2$), 48.3 (proline C5), 48.3 (CHCH$_3$), 45.1 (P-CHR-CHR-CONH-), 39.0 (d, j = 90, P-CHR-CHR-CONH-), 36.3 (Ph-CH$_2$), 33.9 (d, j = 15, P-CHR-CH$_2$), 32.1 (CH$_2$), 30.0 (proline C3), 29.0 (d, j = 90,

P-C$\underline{H}_2$), 26.4 (d, j = 20, P-CH$_2$C$\underline{H}_2$C$\underline{H}_2$), 26.0 (C$\underline{H}_2$CHR-CONH-), 25.8 (proline C$\overline{4}$), 25.6 (C$\underline{H}_2$), 22.2 (Ph-C$\overline{H}_2$C$\overline{H}_2$), 16.7 (C$\overline{H}_3$). Mass spectrum ($^+$/-FAB): 511$^+$(M + $\overline{Li}$)$^+$. HRMS: found 511.2710 C$_{25}$H$_{35}$N$_2$O$_6$Li$_3$P requires 511.2713. [α]$_D$ = −72° (c = 0.2, CH$_3$OH). Found: C, 52.27; H, 7.12; N, 4.83; P, 4.97; C$_{25}$H$_{35}$N$_2$O$_6$Li$_2$P · 4.0 H$_2$O requires C, 52.09; H, 7.52; N, 4.86; P, 5.37.

Preparation of trans-*1-[N-[[2-[Hydroxy(4-phenylbutyl)phosphinyl]-cyclohexyl]-carbonyl]-L-alanyl]-L-proline,* **isomer b,** *dilithium salt* **(10b)**

A mixture of **33b** (360 mg, 0.62 mmol) and palladium hydroxide (100 mg of 20% on carbon) in ethyl acetate (10 ml) was hydrogenated at one atmosphere and 25°C for 21 h, after which it was filtered and concentrated. The residue was dissolved in water and 1 N lithium hydroxide solution was added (1.2 ml). The mixture was applied to a column of HP-20 and eluted with a water to methanol graident. Fractions were monitored by HPLC (YMS A302 ODS column, 1.0 ml/min of 70% aqueous methanol containing 0.2% phosphoric acid, UV monitoring at 220 nm); those containing the major product (R$_t$ 5.3 min) were combined and concentrated. The residue was dissolved in water, charcoal filtered, and lyophilized to give **10b** (130 mg, 41%) as a fluffy white solid. ^{1}H n.m.r. (400 MHz, CD$_3$OD): 7.0–7.4 (6 H, m, aromatic H + NH), 4.60 (1H, q, NH-CH-CO), 4.30 (1H, m, NH-CH-CO), 3.75 (1H, m, cyclohexyl methine), 3.65 (1H, m, cyclohexyl methine), 1.5 – 2.8 (22 H, m, methylene), 1.35 (3H, d, ala CH$_3$). ^{13}C n.m.r. (CD$_3$OD).[17] 179.6/178.8 (C=O), 178.6 (C=O), 173.7/173.0 (C = O), 143.8/143.6 (aromatic), 129.4 (aromatic), 129.2 (aromatic), 126.8 (aromatic), 63.5/62.9 (C̲HCO$_2$), 48.2 (proline C5), 48.1 (C̲HCH$_3$), 46.1/44.3 (P-CHR-CHR-CONH-), 39.8 (d, j = 91, P-CHR-CHR-CONH-), 36.8/36.6 (Ph-C̲H$_2$), 33.9/34.4 (d, j = 15, P-CHR-C̲H$_2$), 33.1 (C̲H$_2$), 30.5 (proline C3), 30.5 (d, j = 178, P-C̲H$_2$), 27.2 (d, j = 20, P-C̲H$_2$CH$_2$CH$_2$), 26.9 (C̲H$_2$CHR-CONH-), 26.6 (proline C4), 25.8 (C̲H$_2$), 23.3 (Ph-CH$_2$C̲H$_2$), 17.2/17.1 (C̲H$_3$). Mass spectrum ($^+$/-FAB): 511$^+$(M + $\overline{Li}$)$^+$/ 497$^-$(M − $\overline{Li}$)$^-$. HRMS: found 511.2720 C$_{25}$H$_{35}$N$_2$O$_6$Li$_3$P requires 511.2713. [α]$_D$ = −44° (c = 0.6, CH$_3$OH). Found: C, 52.47; H, 7.49; N, 5.04; P, 5.26; C$_{25}$H$_{35}$N$_2$O$_6$Li$_2$P · 2.7 H$_2$O requires C, 54.29; H, 7.36; N, 5.07; P, 5.60.

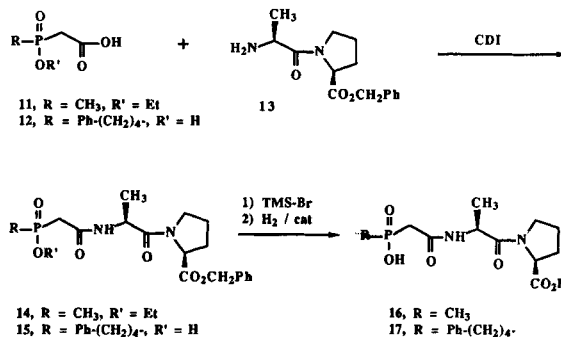*Biological Studies*

Compounds **16, 17, 27, 28, 10a,** and **10b** were tested by spectrophotometric assay for inhibition of rabbit lung ACE activity.[18] Results are reported as the molar concentration of test compound required for 50 percent inhibition of ACE activity (I$_{50}$). Inhibition of the angiotensin I pressor response in normotensive rats by compound **10a** was measured as previously described (4 animals/dose)[19].
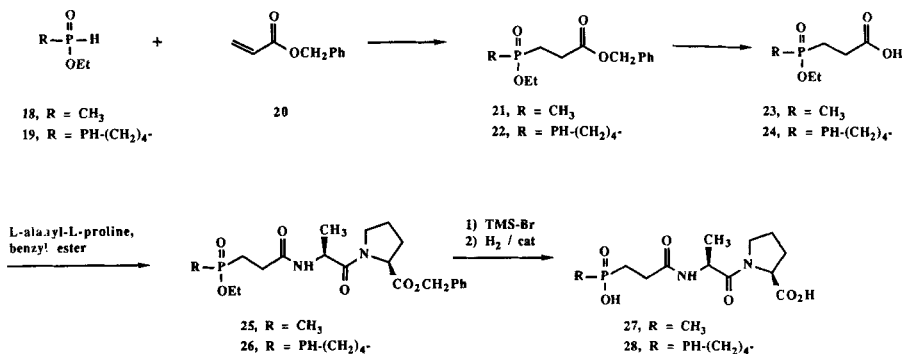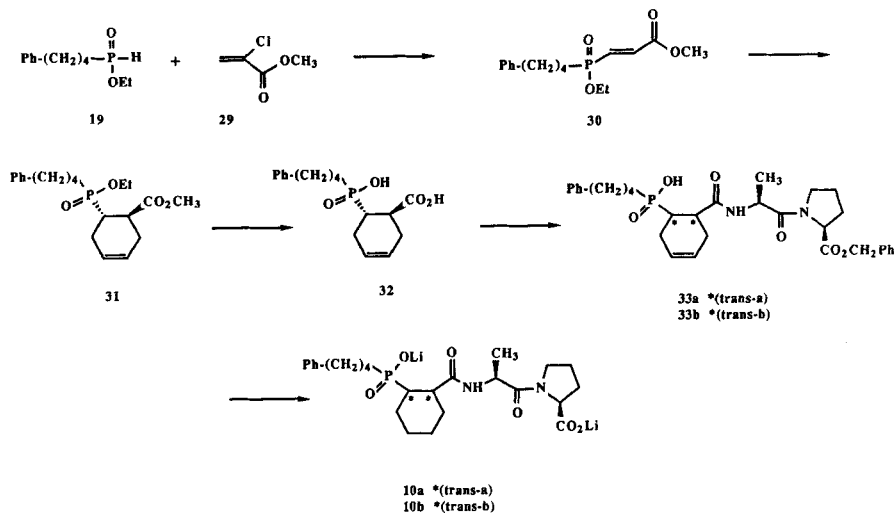
RESULTS

Chemistry

Acyclic derivatives of general structure **9** were prepared as outlined in Schemes 1 and 2. Phosphinyl acetyl dipeptides **16** and **17** were prepared by acylation of L-alanyl-L-proine benzyl ester with phosphinyl acetic acid derivatives **11** and **12**,[10] repsectively, followed by ester group removal (Scheme 1). Phosphinyl propionic acids **23** and **24** were prepared by Michael addition of phosphonous esters **18** and **19** to benzyl

11, R = CH₃, R' = Et
12, R = Ph-(CH₂)₄-, R' = H

13

14, R = CH₃, R' = Et
15, R = Ph-(CH₂)₄-, R' = H

16, R = CH₃
17, R = Ph-(CH₂)₄-

SCHEME 1: Synthesis of **16** and **17**

18, R = CH₃
19, R = PH-(CH₂)₄-

20

21, R = CH₃
22, R = PH-(CH₂)₄-

23, R = CH₃
24, R = PH-(CH₂)₄-

L-alanyl-L-proline,
benzyl ester

25, R = CH₃
26, R = PH-(CH₂)₄-

27, R = CH₃
28, R = PH-(CH₂)₄-

SCHEME 2: Synthesis of **27** and **28**

19   29   30

31   32   33a *(trans-a)
                        33b *(trans-b)

10a *(trans-a)
10b *(trans-b)

SCHEME 3: Synthesis of **10**

acrylate[13], followed by hydrogenolysis of the benzyl ester groups. Acylation of L-alanyl-L-proline benzyl ester with **23** and **24** led to diesters **25** and **26**. The phosphonic and carboxylic esters were removed by sequential treatment with trimethylsilyl bromide and catalytic hydrogenolysis, respectively, to yield the targets **27** and **28**. All final acyl dipeptides were converted to the corresponding dilithium salts and purified by chromatography on HP-20 polystyrene resin.

Synthesis of the cylic analog **10** is outlined in Scheme 3. Attempted Michael addition of phosphonous ester **19**[14] to cyclohexene carboxylic acid (or to the corresponding benzyl ester) failed to provide the expected addition product necessary for synthesis of target **10**, thus necessitating an alternate approach. Darling and Brandes have reported the use of phosphinyl substituted dienophiles in the Diels-Alder reaction,[20] while Coover *et al.* have reported synthesis of phosphonoacrylates by addition-elimination of triethyl phosphite with 2-chloroacrylates.[21] Those combined results suggested he pathway shown in Scheme 3. Addition-elimination of phosphonous monoester **19**[14] to methyl-2-chloroacrylate (**29**) led to the *trans* phosphinyl acrylate **30**, which underwent Diels-Alder reaction with butadiene to form the desired adduct **31** containing the requisite carbon-phosphorus skeleton and the desired *trans* relative sterochemistry. Ester saponification, followed by coupling to the dipeptide fragment, led to a diasteromeric mixture of product isomers **33** which bear the *trans* relative stereochemistry about the cyclohexene ring, but differ in their absolute configuration at the ring centers. The isomers **33a** and **33b** were separated by preparative reverse phase liquid chromatography. Hydrogenolysis of the double bond with simultaneous removal of the benzyl ester protecting group then gave the desired targets **10a** and **10b**, which were purified and characterized as the corresponding dilithium salts.

Biological Studies

The acyclic hydroxy-phosphinyl dipeptides were found to have modest ACE inhibitory potency as shown in Table II. While the effect of chain length (**n**) on ACE

TABLE II
ACE Inhibition by Hydroxyl-phosphinyl Dipeptides

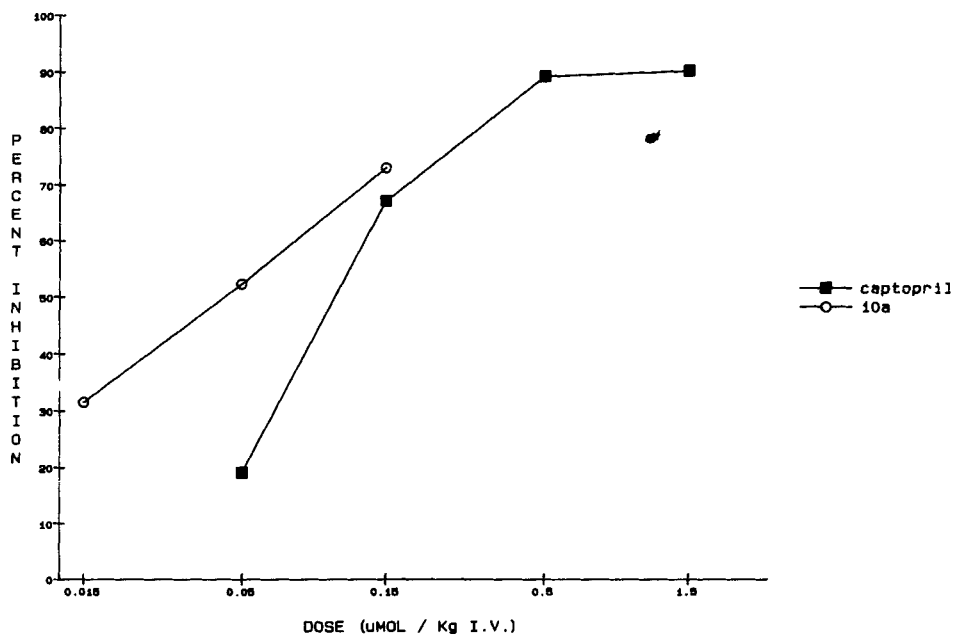| Cmpd. No.	R	n	I_{50} (μM)	Cmpd. No.	I_{50} (μM)
16	CH_3	1	300	10a	0.007
17	Ph-$(CH_2)_4$-	1	98	10b	0.2
27	CH_3	2	3,600		
28	Ph-$(CH_2)_4$-	2	10		
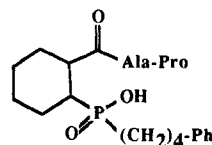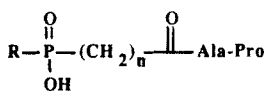
FIGURE 4: Inhibition of the Angiotensin I Induced Pressor Response by I.V. Admininstration of Captopril and **10a** in Conscious Normotensive Rats.
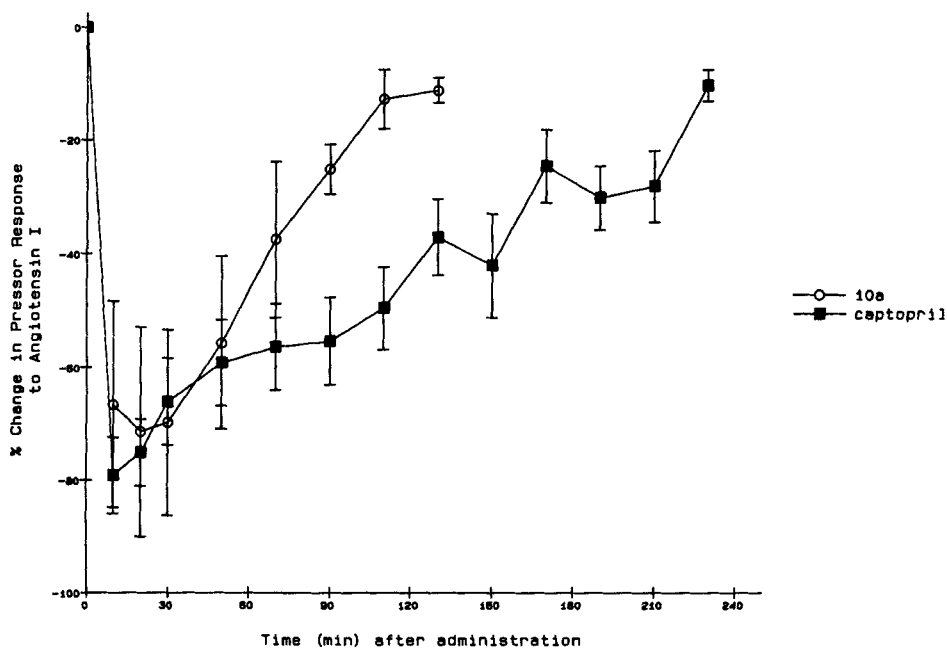

FIGURE 5: Effect of Oral **10a** and Captopril on Vasopressor Responses in Conscious Normotensive Rats (5 μMole/kg).
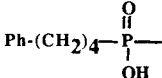
inhibition was somewhat ambiguous, both acyclic compounds bearing a phenylbutyl substituent (e.g., **17** and **28**) were found to be more active than their counterparts bearing only a methyl group (**16** and **27**, respectively). The most active compound of the group is **28**, which bears a phenylbutyl substituent and has a spacing of two methylene groups between the phosphorus atom and acyl dipeptide group. We thus chose **28** as the prototype for introduction of conformational constraint. The resulting isomeric constrained compounds **10a** and **10b** both contain the *trans* relative configuration about the cyclohexane ring. As shown in Table 2, both isomers were found to be significantly more potent than the unconstrained analogs, with the more potent constrained isomer (**10a**) having $I_{50} = 0.007 \mu M$.

The *in vivo* effects of **10a** relative to the well known ACE inhibitor captopril (2-D-methyl-3-mercaptopropanoyl-L-proline, $I_{50} = 0.023 \mu M$)[1,2,18,19] are shown in Figures 4 and 5. Intravenous administration incremental doses of **10a** or captopril caused dose-dependent inhibition of the angiotensin-I induced pressor response in conscious, sodium deplete, normotensive rats (Figure 4). The activity of **10a** was found to be equal to or slightly great than that of captopril. Oral administration of **10a** (5 μMole/Kg, n = 4) caused 71% maximal inhibition of the vasopressor response after 20 min, with return to control after about 2 h; captopril (5 μMole/Kg) showed similar maximal inhibition, but slightly longer duration of effect (Figure 5).

DISCUSSION

Our previous work[6] demonstrated that conformational constraint of functionalized acyl dipeptides can result in significant increases in potency and that the result is extremely sensitive to the geometry imposed by the constraining ring. We interpreted that result in terms of a bidentate binding model as shown in Figure 2 whereby the activity enhancement (constrained vs. unconstrained) may be due to proper positioning of the zinc binding group or to occupation of the S_1 enzyme subsite by the constraining ring, or both. In our present work both the putative zinc binding phosphinate group and the hydrophobic phenylbutyl group were constrained by introduction of a *trans* cyclohexane ring (e.g., **10a**). If the S_1 subsite is occupied by the cyclohexane ring of **10a** (as in Figure 2), then the phenylbutyl group presumably cannot also occupy that site and one might predict activity enhancement (due to constraint) comparable to that obtained for the related carboxyl and sulfhydryl compounds. Furthermore, there is possibility for highly unfavourable steric interactions involving a poorly positioned phenylbutyl group that could negate the advantage gained by constraining the phosphinate group. Conversely, if the activity enhancements observed in the carboxyl and sulfhydryl series were due to constraint of the zinc binding group alone and the S_1 subsite remained unoccupied (as in Figure 3), then introduction of a *properly positioned* phenylalkyl group, along with constraint of the zinc binding group, might lead to potent new inhibitors. Conformational constraint of **28** led to **10a**, which is a potent ACE inhibitor ($I_{50} = 7$ nM). The activity enhancement obtained by constraining **28** (ca. 1,400-fold) is thus greater than for the corresponding carboxyl (**4** and **7**)[6] and sulfhydryl[6] (**34** and **35**) analogs as shown in Table III. While the flexibility of the phenylbutyl chain and unknown torsional angles about the exocylic bonds prevent complete 3-dimensional definition of the bound conformation of **10a**, a reasonable schematic hypothesis can be made and is shown in Figure 6. According to the proposed model, conformational constraint positions

TABLE III
Activity Enhancement upon Conformational Constraint

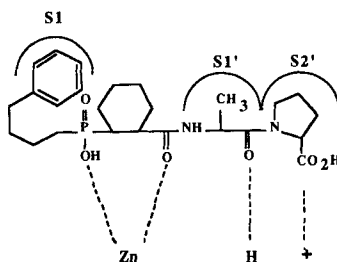| X	Cmpd. No.	I_{50} (µM)	Cmpd. No.	I_{50} (µM)	Ratio*
CO_2H	4	37	7	2.8	13
SH	34	2.6	35	0.003	860
Ph-(CH$_2$)$_4$—P— OH	28	10	10a	0.007	1,400

*Ratio = I50 (Acylic) / I50 (Cyclic)

FIGURE 6:   Proposed Binding Mode of **10a**.

the phosphinate group for binding to the zinc atom at the active site, while the phenylbutyl group is properly positioned for interaction with the S_1 subsite of the enzyme. Direct interaction between the cycloalkyl group and the S_1 subsite may not be a major contributor to the binding of **10a**. This hypothesis is consistent with our earlier failure to observe activity enhancements with substituted, but unconstrained, succinyl dipeptides (Table I).[6] Further structure-activity studies will be aimed at testing the above hypothesis and at more precise definition of the 3-dimensional nature of the ACE active site.

The *in vivo* activty of **10a** after I.V. administration is similar to or slightly greater than that of captopril, roughly reflecting the difference in their *in vitro* activity. The good oral activity of **10a**, however, is suprising in light of the relative inactivity of other known phosphinic acid ACE inibitors after oral adminstration[22] (except in prodrug form) and points out the difficulty in predicting oral activity based on structural generalizations. Though the factors that determine oral activity are not well understood, compound **10a** may be considered to be the prototype for a new class of potent, orally active, ACE inhibitors.

Acknowledgement

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