Angiotensin-Converting Enzyme Inhibitors: Synthesis and Biological Activity of Acyl Tripeptide Analogues of Enalapril

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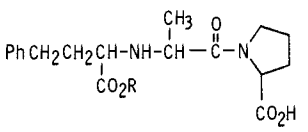
The synthesis and biological activity of a series of inhibitors of angiotensin-converting enzyme (EC 3.4.15.1) are described. Incorporation of the substituted *N*-carboxymethyl dipeptide design of enalapril (MK-421) into acyl tripeptides and larger peptides yielded potent inhibitors of the enzyme. These can be viewed as substrate analogues in which the carbonyl of the scissile peptide bond is replaced by a $CHCO_2H$ group. Several of the analogues described possess inhibitory potency equal to that of enalaprilat (MK-422), but none achieves an increase in potency which would demonstrate additional binding interactions contributed by the extended peptide chain. Application of the design described may be useful for inhibition of other metallopeptidases.

Previous reports from these laboratories have described the design and development of potent inhibitors of angiotensin-converting enzyme. Among these are enalapril (MK-421) and its parent diacid enalaprilat (MK-422)[1] as well as lactam inhibitors.[2] These substituted *N*-carboxymethyl dipeptide inhibitors are tripeptide analogues in which the carbonyl of the scissile –CO–NH– bond is replaced by a CH–CO_2H group.[1] In this view a hypothetical picture of the binding of enalaprilat to angiotensin-converting enzyme encompasses interactions with the S_1', S_2', and S_1 subsites of the active site[3] (Figure 1). The importance of the interaction of the $CHCO_2H$ group of the *N*-carboxymethyl dipeptides with the active site zinc atom of angiotensin-converting enzyme is indicated by the marked increase in inhibitor potency for enalaprilat relative to that for the inhibitory peptides *N*-benzoyl-Phe-Ala-Pro (Table I) and teprotide.[4] Thus far, no examples of tetrapeptides or larger inhibitors have been reported in the *N*-carboxymethyl dipeptide series.[4] If inhibitors of this type can be designed to interact with the S_2 and S_3 subsites, binding tighter than that observed for enalaprilat may result.



Enalapril (MK-421)

R = Et (maleate salt)

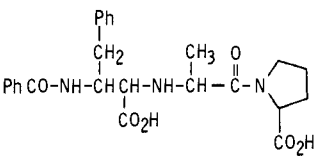
Enalaprilat (MK-422)

R = H

That interactions with the S_2 and S_3 subsites of the active site of angiotensin-converting enzyme can contribute to binding is indicated by the marked increase in inhibitor potency resulting from the addition of acylamino substituents to dipeptide analogues **2** (compounds **3–5**, Table I). A similar effect of an acylamino group was reported for ketone tripeptide analogues **6** and **7**.[5,6] In order to explore the possibility of a similar result for the *N*-carboxymethyl dipeptides, compound **1** and several other tripeptide analogues were studied. The synthesis and biological activities of these compounds are reported below.

Synthesis

The acyl tripeptide analogues were prepared by using a Strecker condensation as the key step.[7] The route



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followed is illustrated for compound **1** (Scheme I). As many of the compounds of interest possess one chiral center more than does enalapril, use of optically active aldehydes as starting materials was desirable. These were available in N-protected form by using published procedures. Aldehydes **8**, **13**, and **14** were prepared by reduction of the Boc-protected esters with diisobutylaluminum hydride.[8] The ester precursor of aldehydes **14** and **15** was obtained by standard Arndt–Eistert homologation of *N*-*t*-Boc-phenylalanine.[9] Aldehyde **16** was prepared by hydrolysis of the acetal.

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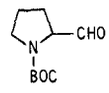

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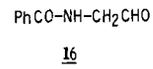

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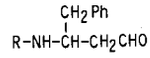


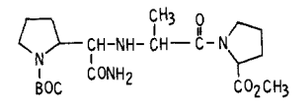
14 R = -BOC

15 R = -COPh

As illustrated in Scheme I, treatment of amino nitrile **9** with HCl-saturated methanol, followed by reblocking with di-*tert*-butyl dicarbonate, yielded a diastereomeric mixture of amido esters **10R**, **10S**. Treatment of the amido esters with Amberlyst 15 acidic resin in methanol[10] provided diesters **11R**, **11S**. After acylation with benzoyl chloride, the resulting diastereomeric diesters **12R**, **12S** were separated by chromatography on silica gel. Saponification of these afforded diacids **1R** and **1S**, which were isolated after ion-exchange chromatography. The absolute configuration at the newly formed chiral center in **1R** and **1S** was assigned by comparison of their in vitro activities (Table IV) and was based on the fact that enalaprilat, with the *S* configuration at this center, is 200-fold more active than the corresponding *R* diastereomer.[1] This relationship holds for other *N*-carboxymethyl dipeptides[11] and for lactam inhibitors.[12]

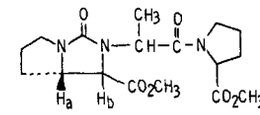
A similar sequence, starting with aldehyde **13**, was used to prepare amido esters **17R**, **17S** (see Experimental Section). As this structure was chosen for analogue studies, it was deemed important to establish with certainty the absolute configuration at the newly formed chiral center. This was accomplished by conversion of **17R** and **17S** to the cyclic ureas **18R** and **18S**. For the ureas, coupling constants in the ^1H NMR spectra allowed configurational assignment.[13] Standard acylation and peptide coupling procedures were used to prepare the acylated analogues listed in Table IV. Ester **44** (Table III) was prepared from the corresponding diester by selective acidic hydrolysis of the proline ester.

A synthetic sequence beginning with aldehyde **14** was troublesome due to the tendency for five-membered ring lactams to form when the primary amino group was unacylated (see Experimental Section). As a result, only one diastereomeric diacid (**25S**) was obtained (Table II). A modified synthesis starting with benzamido aldehyde **15** afforded a mixture (2:1 **25S**:**25R**) of diastereomers, although in low overall yield. A similar route with benzamido aldehyde **16** as starting material provided diacids **20** and **20S** in good yield. The absolute configuration of diacids **20S** and **25S** was assigned (as above) on the basis of their in vitro activities relative to that observed for the



17S (S, S, S, S)

17R (S, R, S, S)



18S J_{ab} = 10 Hz (S, S, S, S)

18R J_{ab} = 2 Hz (S, R, S, S)

diastereomeric mixtures (**20** and **25**).

Results and Discussion

The angiotensin-converting enzyme inhibitory activities for the acyl tripeptide analogues are shown in Table IV. In analogue **20S** the benzamidomethyl side chain was hoped to be directed into and make binding interactions in the S_2 substite of the enzyme. The moderate activity for this analogue demonstrated that binding contributed by the benzamidomethyl group does not compensate for the absence of the phenethyl side chain present in enalaprilat. Moreover, acyl tripeptide analogue **1S**, for which binding in both S_1 and S_2 subsites was expected, proved sixfold less active against angiotensin-converting enzyme than enalaprilat. An understanding of the origin of the reduced activity of **1S** is necessary in order to design more potent inhibitors with an acyl tripeptide-like structure.

One possible explanation for the reduced activity of **1S** was suggested by a strong intramolecular hydrogen-bonded amide NH observed in the IR spectrum of diester **12S** (3325 cm^{-1} in $CDCl_3$). If a similar hydrogen bond were to exist in analogue **1S** (Figure 2), energy would be required during binding in order to break this bond and permit interactions of the carboxyl and acylamino functions with the enzyme. In addition, it was felt that replacement of a peptide carbonyl of the inhibitory peptides in Table I by the $CHCO_2H$ moiety of the substituted *N*-carboxymethyl dipeptides might result in a regional displacement of the side chain interacting with the S_1 subsite from its position for the bound peptide inhibitors **3–5** (or bound substrates). While flexibility in the phenethyl side chain of enalaprilat might compensate for this alteration, in the case of **1S**, a misorientation of the acylamino side chain may be unavoidable.

Two approaches to more potent inhibitors were investigated. In acyl tripeptide analogue **32** (Table IV), intramolecular hydrogen bonding to the carboxyl is precluded. Proline is known to be accepted into the S_1 subsite of angiotensin-converting enzyme in substrates.[14] In acyl tripeptide homologue **25S** (Table IV) a longer and more flexible backbone is introduced, which we hoped would permit binding interactions with both S_1 and S_2 subsites. The phenylpropyl homologue of enalaprilat (1:1 mixture of diastereomers) is a potent inhibitor (I_{50} = 8.3 nM),[15] demonstrating the accommodation of the lengthened side chain in the S_1 subsite of the enzyme. Moreover, an intramolecular hydrogen bond (seven-membered) seemed unlikely for **25**.

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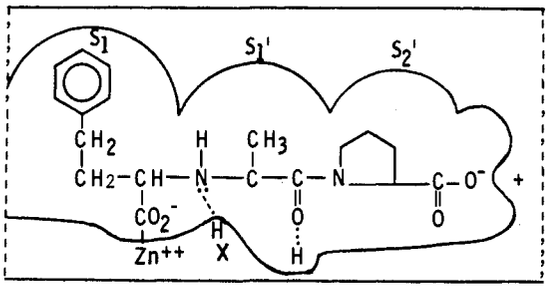
(14) For example, bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg).
(15) Compound prepared by T. Ikeler and tested as a mixture (*RS,S,S*) of diastereomers.



Enalaprilat (MK-422)

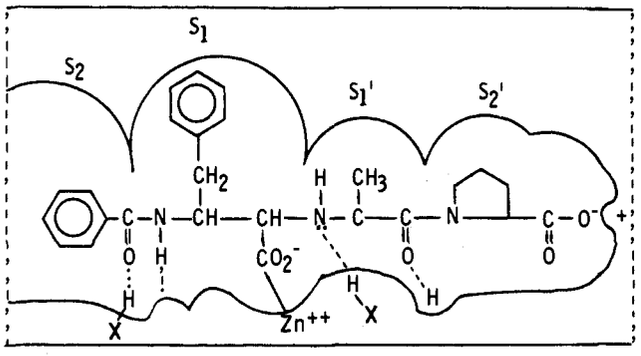


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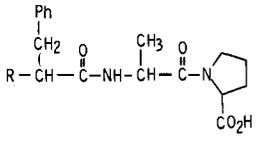
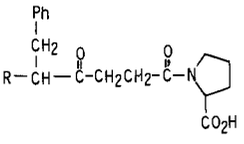
Figure 1. Binding of inhibitors to the hypothetical active site of angiotensin-converting enzyme.

Table I. In Vitro Angiotensin-Converting Enzyme Inhibitory Activities



2-5

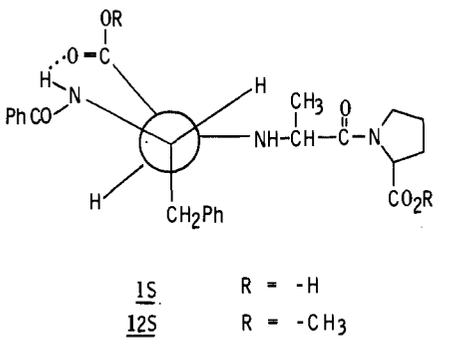


6,7

no.	R	config	I_{50}, M	ref
2	H	*S,S*	3.3×10^{-4} [a] 37%	16
3	NH_2	*S,S,S*	1.4×10^{-6}	17
4	PhCONH	*S,S,S*	2.7×10^{-6}	18
5	*p*-Glu-Lys-NH	*S,S,S,S,S*	5×10^{-6}	17
6	H	*S,S*	2.6×10^{-3}	5
7	PhCONH	*S,S*	1.2×10^{-6}	5

[a] The figure in parentheses refers to percent inhibition at the concentration listed.

Both acyl tripeptide analogues **32** and **25S** proved to be more potent inhibitors than **1S** but are still less active than enalaprilat. The strikingly low activity of the unacylated analogue **28** may reflect a lack of tolerance for a protonated amine, which might disrupt the interaction between the $CHCO_2H$ moiety of **28** and the active-site zinc atom. The protonated amine groups of **34** and **36** may be sufficiently removed for this not to occur. While the high activity of analogue **32**, which lacks the aromatic side chain of enalaprilat, is interesting, it appears that here, as in **1S**, the acylamino side chain does not occupy a position optimal for interactions in the S_2 subsite. Further support for this belief is provided by the extended tetra- and pentapeptide

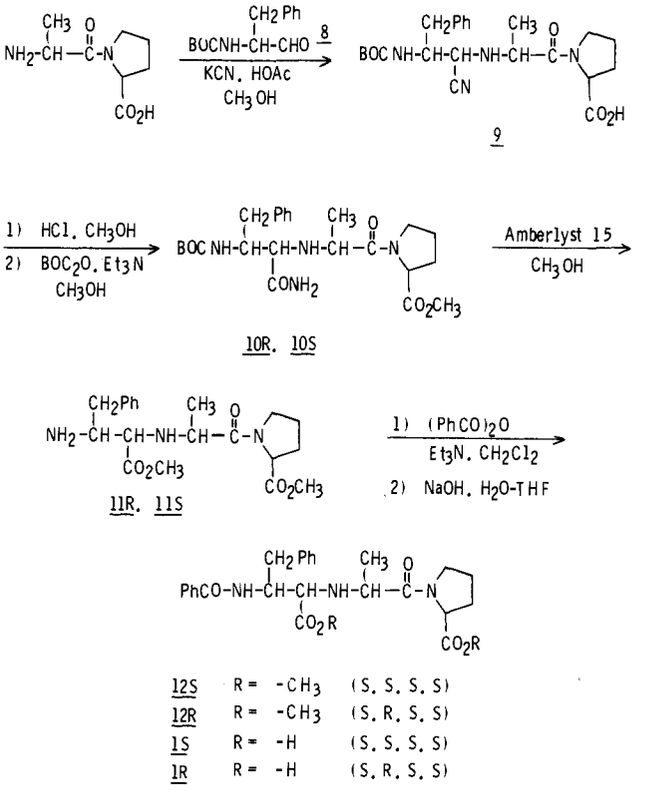


1S R = -H

12S R = -CH_3

Figure 2. Intramolecular hydrogen bonding of inhibitor **1S** and analogue **12S**.

Scheme I



analogues **34**, **36**, **38**, and **41**, which exhibited inhibitor potency no greater than that of benzamido analogue **32**.

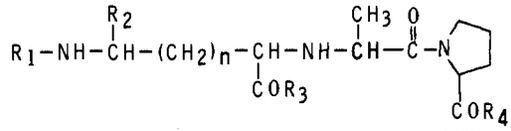
Conclusions

It is clear from the results presented that the acyl tripeptide analogues described possess geometry which does not permit contributions to binding from both a side chain directed to the S_1 subsite of angiotensin-converting enzyme and an acylamino substituent intended to participate in multiple interactions with the enzyme. It is hoped that other modifications of the substituted *N*-carboxymethyl dipeptide design will establish its usefulness in an internal position of a peptide with retention of binding interactions remote from the scissile bond. This possibility is currently under study.

Experimental Section

All optically active amino acids used were of the L configuration. Standard abbreviations were used for the peptide derivatives, i.e., Cbz = benzyloxycarbonyl, Boc = *tert*-butoxycarbonyl, Phe = phenylalanine, *p*-Glu = pyroglutamic acid, Ala = alanine, Pro = proline, Lys = lysine, and for the following: TFA = CF_3CO_2H, DPPA = diphenylphosphoryl azide. *N*-Hydroxysuccinimide esters of protected amino acids were purchased from Sigma Chemical

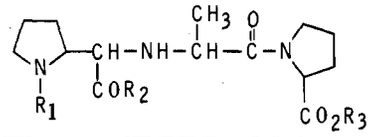
Table II. Physical Properties of *N*-[Carboxy(benzamidophenylalkyl)methyl]-L-alanyl-L-prolines and Analogues



no.	R_1	R_2	*n*	R_3	R_4	config	mp, °C	yield, %	formula[a]
19	PhCO	H	0	OCH_3	OCH_3	*RS,S,S*	oil	45	$C_{20}H_{27}N_3O_6 \cdot {}^1/_2H_2O$[b]
19S	PhCO	H	0	OCH_3	OCH_3	*S,S,S,*	104–105	45	$C_{20}H_{27}N_3O_6 \cdot {}^1/_2H_2O$
20	PhCO	H	0	OH	OH	*RS,S,S*	glass	86	$C_{16}H_{23}N_3O_6$[c]
20S	PhCO	H	0	OH	OH	*S,S,S*	glass	78	$C_{16}H_{23}N_3O_6$[d]
10R, 10S	Boc	CH_2Ph	0	NH_2	OCH_3	*S,RS,S,S*	177–183	44	$C_{24}H_{36}N_4O_6$
21S	Boc	CH_2Ph	0	OCH_3	OCH_3	*S,S,S,S*	75–76	37	$C_{25}H_{37}N_3O_7$
12S	PhCO	CH_2Ph	0	OCH_3	OCH_3	*S,S,S,S*	120–122	55	$C_{27}H_{33}N_3O_6$
12R						*S,R,S,S*	168–170		$C_{27}H_{33}N_3O_6$
1S	PhCO	CH_2Ph	0	OH	OH	*S,S,S,S*	214–216[e]	96	$C_{25}H_{29}N_3O_6$
1R						*S,R,S,S*	glass	90	$C_{25}H_{29}N_3O_6$
24	Boc	CH_2Ph	1	NH_2	OCH_3	*S,S,S,S*	oil	18	$C_{25}H_{39}N_3O_7$
25	PhCO	CH_2Ph	1	OH	OH	*S,RS,S,S*	glass	59	$C_{26}H_{31}N_3O_6 \cdot {}^3/_2H_2O$

[a] Unless otherwise indicated, all compounds gave satisfactory (±0.4%) combustion analyses (C, H, N). [b] Calcd: C, 57.96. Found: C, 58.53. [c] Calcd: C, 57.28. Found: C, 59.57. [d] Calcd: C, 57.28. Found: C, 58.59. [e] Decomposes.

Table III. Physical Properties of *N*-[Carboxy(*N*-alkanoyl-2-pyrrolidinyl)methyl]-L-alanyl-L-prolines



no.	R_1	R_2	R_3	config	mp, °C	yield	formula[a]
17S	Boc	NH_2	OCH_3	*S,S,S,S*	oil	29	$C_{20}H_{34}N_4O_6$
17R	Boc	NH_2	OCH_3	*S,R,S,S*	oil		$C_{20}H_{34}N_4O_6$
27	Boc	OCH_3	OCH_3	*S,S,S,S*	68–70	64	$C_{21}H_{35}N_3O_7 \cdot {}^1/_2H_2O$
28	H	OH	OH	*S,S,S,S*	216–220		$C_{14}H_{23}N_3O_5$
29	CH_3CO	OCH_3	OCH_3	*S,S,S,S*	118–119	71	$C_{16}H_{29}N_3O_6$
30	CH_3CO	OH	OH	*S,S,S,S*	glass	78	$C_{16}H_{25}N_3O_6$
31	PhCO	OCH_3	OCH_3	*S,S,S,S*	glass	64	$C_{23}H_{31}N_3O_6 \cdot {}^1/_4H_2O$
32	PhCO	OH	OH	*S,S,S,S*	glass	95	$C_{21}H_{27}N_3O_6 \cdot {}^1/_4NH_3$
33	Boc-GlyCO	OCH_3	OCH_3	*S,S,S,S*	122–124	76	$C_{23}H_{36}N_4O_6$
34	GlyCO	OH	OH	*S,S,S,S*	glass	90	$C_{16}H_{26}N_4O_6 \cdot {}^3/_2H_2O$
35	Boc-PheCO	OCH_3	OCH_3	*S,S,S,S,S*	oil	64	$C_{30}H_{44}N_4O_6$
36	PheCO	OH	OH	*S,S,S,S,S*	165–168	91	$C_{23}H_{32}N_4O_6$
37	PhCO-PheCO	OCH_3	OCH_3	*S,S,S,S,S*	68–70	89	$C_{32}H_{40}N_4O_7$
38	PhCO-PheCO	OH	OH	*S,S,S,S,S*	146–148	66	$C_{30}H_{36}N_4O_7 \cdot {}^1/_2H_2O$
41	*p*-Glu-LysCO	OH	OH	*S,S,S,S,S,S*	glass	53	$C_{25}H_{40}N_6O_6 \cdot {}^5/_2H_2O$
44	PhCO	OEt	OH	*S,S,S,S*	glass	94	$C_{23}H_{31}N_3O_6 \cdot HCl \cdot H_2O$

[a] All compounds gave satisfactory (±0.4%) combustion analyses (C, H, N).

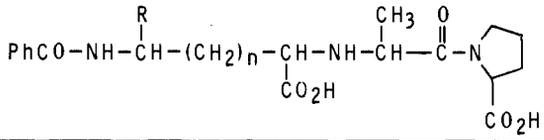
Co. and used as is. Melting points were recorded on a Bausch and Lomb hot-stage apparatus and are uncorrected. ^1H NMR spectra were taken on a Varian XL-200 FT spectrometer. Chemical shifts are reported in ppm (δ) downfield from Me_4Si as internal standard. In many cases, alanylproline cis–trans isomers were identified in the NMR spectra by a pair of alanyl methyl doublets. In these cases, both doublets are listed; the minor doublet (5–40% of the total) is upfield. IR spectra were taken on a Perkin-Elmer Model 297 spectrometer. The FT-IR spectrum of compound **12S** was recorded on a Nicolet 7000 spectrometer. Optical rotations were measured with a Perkin-Elmer 141 automatic polarimeter and were run in CH_3OH. Mass spectra (MS) were taken on a Varian 731 spectromater at 70 eV. Those marked FAB were taken by using the fast atom bombardment method. Elemental analyses were performed by J. Gilbert and his associates, Merck Sharp and Dohme Research Laboratories. The existence of solvents of crystallization was confirmed by ^1H NMR whenever possible. Thin-layer chromatography was performed on uniplates from Analtech coated with 250 μM of silica gel GF. Preparative-layer chromatography was carried out with 1.5- or 2-mm silica gel GF plates from Analtech. MPLC was performed with E. Merck Lobar columns or columns packed with E. Merck silica gel 60 (cat. no. 9385). The following abbreviations were used for chromatography solvents: CMWA = $CHCl_3$–CH_3OH–H_2O–HOAc, EBAW = EtOAc–BuOH–HOAc–H_2O, EPAW = EtOAc–Pyr:HOAc–H_2O. Ion-exchange chromatography was carried out on DOWEX 50W-X4 resin prewashed with H_2O. Unless otherwise indicated, elutions were performed with H_2O–Pyr (25:1) and products were isolated after freeze-drying. A description of our enzyme assay has been published.[1]

(*N*-Benzoylamino)acetaldehyde Diethyl Acetal. A mixture of aminoacetaldehyde diethyl acetal (6.66 g, 0.050 mmol), benzoyl chloride (7.03 g, 0.050 mmol), Et_3N (5.05 g, 0.050 mmol), and CH_2Cl_2 (100 mL) was stirred at 0 °C for 15 min and then at room temperature for 2 h. The mixture was filtered and evaporated. An EtOAc solution of the residue was washed with aqueous HCl (0.5 N), H_2O, saturated $NaHCO_3$, H_2O, and brine and dried ($MgSO_4$). Evaporation afforded the product (10.0 g), which was recrystallized from hexanes–EtOAc: mp 35–36 °C (lit.[19] mp 38 °C); TLC (EtOAc) R_f 0.70; NMR ($CDCl_3$) δ 1.37 (6 H, t, J = 7 Hz), 3.2–4.0 (4 H, m), 3.75 (2 H, d, J = 6 Hz), 4.75 (1 H, t, J = 6 Hz), 7.2–7.8 (5 H, m), 6.5 (1 H, br).

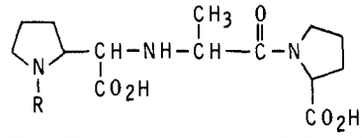
***N*-(2-Benzamido-1-carbomethoxyethyl)-Ala-Pro-OCH_3 (19R, 19S).** A mixture of the above-described acetal (2.0 g), aqueous HCl (2.0 N, 10 mL), and THF (10 mL) was stirred vigorously for 2.5 h. The mixture was then extracted several times

(16) Compound prepared by Dr. E. Harris.
(17) Cushman, D. W.; Pluscec, J.; Williams, N. J.; Weaver, E. R.; Sabo, E. F.; Kocy, O.; Cheung, H. S.; Ondetti, M. A. *Experimentia* **1973**, *29*, 1032.
(18) Compound prepared by R. D. Hoffsommer.
(19) Fisher, E. *Chem. Ber.* **1893**, *26*, 464.

Table IV. In Vitro Angiotensin-Converting Enzyme Inhibitory Activities



no.	R	*n*	config	I_{50}, nM
20	H	0	(*RS,S,S*)	40
20S	H	0	(*S,S,S*)	17
1S	CH_2Ph	0	(*S,S,S,S*)	7.7
1R	CH_2Ph	0	(*S,R,S,S*)	1400
25	CH_2Ph	1	(*S,RS,S,S*)	4.8
25S	CH_2Ph	1	(*S,S,S,S*)	3.0



no.	R	config	I_{50}, nM
28	H	*S,S,S,S*	700
30	CH_3CO	*S,S,S,S*	9.4
32	PhCO	*S,S,S,S*	2.9
34	NH_2CH_2CO	*S,S,S,S*	6.0
36	NH_2-Phe-CO	*S,S,S,S,S*	5.4
38	PhCONH-Phe-CO	*S,S,S,S,S*	2.9
41	*p*-Glu-Lys-CO	*S,S,S,S,S,S*	8.5
enalaprilat (MK-422)		*S,S,S*	1.2[1]
captopril		*S,S*	20[1]

with ether, and the combined organic portions were washed with H_2O and brine and dried ($MgSO_4$). Evaporation gave (*N*-benzoylamino)acetaldehyde (**16**) as a colorless oil (1.5 g).[20] TLC (EtOAc) R_f 0.50; NMR ($CDCl_3$) δ 4.25 (2 H, d, J = 5 Hz), 7.4–8.0 (5 H, m), 9.80 (1 H, s).

A mixture of the crude aldehyde (0.697 g, 4.27 mmol), Ala-Pro·$^1/_2H_2O$ (0.865 g, 3.89 mmol), KCN (0.277 g, 4.27 mmol), and CH_3OH (7.5 mL) was stirred for 3 days. The mixture was then cooled (ice bath) as anhydrous HCl was passed in until saturation. The mixture was tightly capped and stirred overnight at room temperature. After evaporation, the residue was taken up in CH_2Cl_2 (25 mL) and the mixture saturated with anhydrous NH_3. The mixture was immediately evaporated and the residue slurried in EtOAc and filtered. The residue after evaporation was combined with Amberlyst 15 resin[10] (20 g) and CH_3OH (50 mL). The mixture was warmed at 60 °C for 3 days. The product was eluted with CH_3OH–Et_3N (2:1) and purified on silica gel (EtOAc–EtOH, 20:1), giving a mixture (1:1) of diastereomers. These were separated by MPLC; more-mobile diastereomer (**19S**): NMR ($CDCl_3$) δ 1.30, 1.37 (3 H, 2 d, J = 7), 1.9–2.4 (4 H, m), 3.3–3.4 (1 H, m), 3.5–3.8 (5 H, m), 3.80 (6 H, s), 4.5–4.6, 4.6–4.7 (1 H, 2 m), 7.4–7.5 (3 H, m), 7.7–7.8 (1 H, br), 7.9–8.0 (2 H, m); IR ($CHCl_3$) 3330, 1740, 1650, 1530, 1435 cm^{-1}; MS (FAB), m/e 406 (M^+ + 1); less-mobile diastereomer (**19R**): NMR ($CDCl_3$) δ 1.23, 1.32 (3 H, 2 d, J = 7 Hz), 1.9–2.4 (4 H, m), 3.4–3.8 (6 H, m), 3.73, 3.75, 3.76, 3.77 (6 H, 4 s), 4.3–4.4, 4.5–4.6 (1 H, 2 m), 7.10 (1 H, br), 7.4–7.5 (3 H, m), 7.7–7.8 (2 H, m); IR ($CHCl_3$) 3450–3300, 1730, 1640, 1435 cm^{-1}; MS (FAB), m/e 406 (M^+ + 1).

***N*-(2-Benzamido-1-carboxyethyl)-Ala-Pro (20).** A mixture of diesters **19R**, **19S** (1:1 ratio; 68 mg, 0.17 mmol) and a 0.25 N NaOH solution (2.0 mL, 0.50 mmol) was stirred for 3 h. The mixture was neutralized by addition o 0.50 N HCl (1.0 mL) and placed onto Dowex 50W-X4 (3 g). The product was isolated as a white foam: TLC (3:1:1:1 EBAW) R_f 0.25, 0.30; NMR (CD_3OD) δ 1.53 (minor), 1.55, 1.59 (3 H, 3 d, J = 7 Hz), 1.9–2.4 (4 H, m), 4.3–4.9 (3 H, m), 4.4–4.6 (3 H, m), 7.2–7.4 (3 H, m), 7.8–7.9 (2 H, m); MS (FAB), m/e 406 (M^+ + 1). **Diacid 20S.** Diester **19S** (25 mg, 0.062 mmol) was treated as above, giving **20S** as a white foam: TLC R_f 0.030; NMR (CD_3OD) δ 1.53 (minor), 1.59 (3 H, 2 d, J = 7 Hz), 1.9–2.3 (4 H, m), 3.2–3.8 (3 H, m), 4.0–4.6 (3 H, m), 7.4–7.6 (3 H, m), 7.8–7.9 (2 H, m); MS (FAB), m/e 406 (M^+ + 1).

***N*-[1-Carboxamido-2-[(*tert*-butoxycarbonyl)amino]-3-phenylpropyl]-Ala-Pro-OCH_3 (10S, 10R).** L-*N*-*t*-Boc-phenylalaninal (**8**) was prepared by the procedure of Rich et al.,[8] with the exception that 2-propanol was used to quench the reaction mixture and the silica gel chromatography was omitted. The NMR spectrum of the crude aldehyde matched that reported except that traces of toluene and 2-propanol were present. The crude aldehyde was a sticky solid and was used immediately without purification. A mixture of aldehyde (5 g, 20 mmol), KCN (1.3 g, 20 mmol), Ala-Pro·$^1/_2H_2O$ (3.7 g, 19 mmol), and HOAc (1.2 mL, 20 mmol) in CH_3OH (35 mL) was stirred for 3 days. The mixture was saturated with anhydrous HCl at 0 °C and then tightly capped and allowed to stir for 24 h at room temperature. After evaporation to dryness, the residue was combined with di-*tert*-butyl dicarbonate (4.35 mL, 19 mmol), Et_3N (6.6 mL, 47.5 mmol), and CH_3OH (50 mL). The mixture was stirred for 3 h and then filtered and evaporated to a light-yellow oil. An EtOAc solution of the crude product was extracted with H_2O, dried ($MgSO_4$), and evaporated. The product was purified on silica gel (EtOAc–EtOH, 20:1) and recrystallized (EtOAc–CH_3OH): TLC R_f 0.45, 0.50; NMR ($CDCl_3$) δ 1.10, 1.17 (3 H, 2 d, J = 7 Hz), 1.25 (9 H, s), 1.8–2.0 (4 H, m), 2.6–3.1 (3 H, m), 3.30–3.5 (3 H, m), 3.83 (3 H, s), 4.0–4.1 (1 H, br), 4.40 (1 H, m), 5.01 (1 H, br d, J = 8 Hz), 5.6–5.7 (1 H, br), 7.1–7.3 (5 H, m); IR ($CHCl_3$) 3500–3300, 2980, 1740, 1690, 1650 cm^{-1}; MS, m/e 476 (M^+).

***N*-[1-Carbomethoxy-2-[(*tert*-butoxycarbonyl)amino]-3-phenylpropyl]-Ala-Pro-OCH_3 (21S, 21R).** A mixture of amido esters **10S**, **10R** (0.800 g, 1.68 mmol), Amberlyst 15 resin[10] (12 g), and CH_3OH (40 mL) was warmed at 60 °C for 10 days. The residue (0.574 g) remaining after rinsing of the resin with CH_3OH–Et_3N (2:1) and evaporation was treated in CH_2Cl_2 (5 mL) with di-*tert*-butyl dicarbonate (0.35 mL, 1.5 mmol). The product was purified on silica gel (EtOAc), giving the more-mobile (0.125 g) and less-mobile (0.304 g) diastereomers. The more-mobile diester (**21R**), R_f 0.40, was accompanied by impurities and was not characterized. The less-mobile diester (**21S**), R_f 0.35, showed the following properties: NMR ($CDCl_3$) δ 1.10, 1.17 (3 H, 2 d, J = 7 Hz), 1.25 (9 H, s), 1.8–2.0 (4 H, m), 2.6–3.1 (3 H, m), 3.3–3.5 (3 H, m), 3.83 (3 H, s), 4.0–4.1 (1 H, br), 4.40 (1 H, m), 5.01 (1 H, br d, J = 8 Hz), 5.6–5.7 (1 H, br), 7.1–7.3 (5 H, m); MS, m/e 491 (M^+). Diester **21S**, when treated first with TFA and then with benzoyl chloride and triethylamine in CH_2Cl_2, afforded a diester identical in all respects with **12S**.

***N*-(2-Benzamido-1-carbomethoxy-3-phenylpropyl)-Ala-Pro-OCH_3 (12S, 12R).** Amido esters **10S**, **10R** (0.944 g, 1.98 mmol) were combined with Amberlyst 15 resin[10] (15 g) and CH_3OH (25 mL) and the mixture warmed (60 °C) for 10 days. The residue remaining after elution of the product with CH_3OH–Et_3N (2:1) and evaporation (0.663 g) was divided and one portion (0.455 g) was combined with benzoic anhydride (0.262 g, 1.16 mmol), Et_3N (0.16 mL, 1.2 mmol), and CH_2Cl_2 (4 mL). The mixture was stirred for 3 h and then diluted with EtOAc. The solution was extracted with aqueous $NaHCO_3$, H_2O, and brine and dried ($MgSO_4$). Chromatography on silica gel afforded the product (0.378 g, 39%) as a mixture (6:1) of diastereomers. Separation of these by MPLC (EtOAc) gave the following. More-mobile diastereomer **12S**: TLC R_f 0.45; NMR ($CDCl_3$) δ 1.30, 1.38 (3 H, 2 d, J = 7 Hz), 1.8–2.2 (4 H, m), 2.94 (2 H, ABX, J_{AB} = 10, δ_{AB} = 52, J_{AX} = 5, J_{BX} = 6), 3.26, 3.36, 3.61, 3.74 (6 H, 4 s), 3.28 (1 H, d, J = 3 Hz); 3.4–3.6 (3 H, m), 4.3–4.4 (1 H, m), 4.8–4.9 (1 H, m), 7.1–7.8 (11 H, m); IR ($CHCl_3$) 3350, 1730, 1635, 1430 cm^{-1}; MS, m/e 495 (M^+). Less-mobile diastereomer **12R**: TLC R_f 0.40; NMR ($CDCl_3$) δ 1.15, 1.23 (3 H, 2 d, J = 7 Hz), 1.8–2.2 (4 H, m), 2.94 (2 H, ABX, J_{AB} = 14, δ_{AB} = 32, J_{AX} = 6, J_{BX} = 8), 3.4–3.6 (4 H, m), 3.70 (3 H, s), 3.74 (3 H, s), 4.4–4.5 (1 H, m), 4.6–4.8 (1 H, m), 7.1–7.8 (10 H, m); IR ($CHCl_3$) 3425, 1730, 1640, 1430 cm^{-1}; MS, m/e 495 (M^+).

***N*-(2-Benzamido-1-carboxy-3-phenylpropyl)-Ala-Pro (1S, 1R).** Diester **12S** (77.1 mg, 0.156 mmol) was combined with aqueous NaOH (0.25 N, 1.9 mL) and the mixture was stirred for 24 h. Addition of aqueous HCl (0.50 N, 0.90 mL) and CH_3OH (1 mL) gave a clear solution which was placed onto Dowex 50. Elution of the product with H_2O–CH_3OH (5:1) and then H_2O–Pyr (25:1) gave **1S**, which was recrystallized from EtOAc–CH_3OH: TLC (EBAW; 3:1:1:1) R_f 0.50; NMR (CD_3OD) δ 1.55 (3 H, br d,

(20) Bendall, M. R.; Cartwright, I. L.; Chark, P. I.; Lowe, G.; Burse, D. *Eur. J. Biochem.* **1977**, *79*, 201.

J = 7 Hz), 1.9–2.4 (4 H, m), 3.16 (2 H, J = 8 Hz), 3.3–3.8 (2 H, m), 4.3–4.5 (2 H, m), 4.7–4.9 (1 H, m), 7.2–7.8 (10 H, m); MS, m/e 468 (M^+). **Diacid 1R**: Diester **12R** (22.4 mg) was treated as for **12S**, giving the corresponding diacid as a white foam: TLC (EBAW, 3:1:1:1) R_f 0.50; NMR (CD_3OD) δ 1.50, 1.59 (3 H, 2 d, J = 7 Hz), 1.8–2.4 (4 H, m), 2.8–3.8 (4 H, m), 4.2–4.9 (3 H, m), 7.2–7.8 (10 H, m); MS, m/e 468 (M^+).

3-[(*tert*-Butoxycarbonyl)amino]-1-diazo-4-phenyl-2-butanone (22). To a cooled (−15 °C) mixture of *N*-*t*-Boc-phenylalanine (24 g, 0.091 mol), Et_3N (12.7 mL, 0.091 mol), and ether (200 mL) was added $ClCO_2Et$ (8.7 mL, 0.091 mol) with vigorous stirring. After 30 min at −15 °C, excess ethereal diazomethane was added over 30 min. Stirring was continued for 2 h and then the mixture was concentrated first with a stream of nitrogen and then on the rotary evaporator. The residue was slurried in EtOAc and filtered. The filtrate was washed with saturated $NaHCO_3$ and H_2O and then dried ($MgSO_4$) and concentrated. The residue was recrystallized from hexanes–EtOAc (15.4 g, 59%); mp 91–93 °C; TLC (CH_2Cl_2–CH_3OH, 10:1) R_f 0.50; NMR ($CDCl_3$) δ 1.44 (9 H, s), 2.9–3.1 (2 H, d of d, J = 7, 9 Hz), 4.2–4.6 (1 H, d of t, J = 9, 7 Hz), 5.2–5.5 (1 H, br d, J = 8 Hz), 5.3 (1 H, s), 7.2 (5 H, s); IR ($CHCl_3$) 3440, 3000, 2125, 1705, 1636, 1490, 1375, 1165 cm^{-1}; MS, m/e 220 (M^+ − 69); $[\alpha]^{20}_D$ −32.5° (CH_3OH). Anal. ($C_{14}H_{18}N_2O_3$) C, H, N.

Methyl 3-[(*tert*-Butoxycarbonyl)amino]-4-phenylbutanoate (23). To a solution of diazo ketone **22** (15.4 g, 0.053 mol) in CH_3OH (180 mL) was added a solution of silver benzoate (0.25 g) in Et_3N (5 mL) and the mixture was stirred until nitrogen evolution ceased. Activated charcoal (1 g) was added and the mixture filtered and evaporated. An EtOAc solution of the crude product was extracted with H_2O and brine, then dried ($MgSO_4$), and evaporated to a yellow oil. Recrystallization from hexane gave a white solid (11.9 g, 76%): mp 54–56 °C; TLC (CH_2Cl_2–CH_3OH, 10:1) R_f 0.70; NMR ($CDCl_3$) δ 1.45 (9 H, s), 2.51 (2 H, d, J = 6 Hz); 2.90 (2 H, d of d, J = 6, 8 Hz), 3.72 (3 H, s), 3.9–4.0 (1 H, m), 5.06 (1 H, br d, J = 8 Hz), 7.23 (5 H, s); IR ($CHCl_3$ 3440, 2975, 1710, 1495, 1370, 1165 cm^{-1}; MS, m/e 220 (M^+ − 73); $[\alpha]^{20}_D$ −20.0° (CH_3OH). Anal. ($C_{16}H_{19}NO_4$) C, H, N.

3-[(*tert*-Butoxycarbonyl)amino]-4-phenylbutanaldehyde (14). A cooled (−78 °C) suspension of ester **23** (1.04 g, 3.54 mmol) in ether (10 mL) was stirred vigorously as diisobutylaluminum hydride (1 M in hexane, 8.8 mL) was added over 30 min. After an additional hour at −78 °C, CH_3OH (1 mL) and then a saturated solution of Rochelle salt (1 mL) were added. The mixture was allowed to warm to room temperature and then diluted with ether (50 mL) and Rochelle salt solution (20 mL). After extraction of the aqueous layer with ether, the combined organic portions were washed with H_2O and brine and dried ($MgSO_4$). Evaporation gave the crude product which was purified on silica gel (hexane–EtOAc; 5:1) and then recrystallized (hexane–EtOAc), giving a white solid (0.69 g, 75%): mp 88–89 °C; TLC R_f 0.40; NMR ($CDCl_3$) δ 1.43 (9 H, s), 2.49 (1 H, d, J = 6 Hz), 2.52 (1 H, d, J = 6 Hz), 2.81 (1 H, d, J = 7 Hz), 2.85 (1 H, d, J = 7 Hz), 4.0–4.3 (1 H, m), 4.98 (1 H, br d, J = 8 Hz), 7.2 (5 H, s), 9.60 (1 H, t, J = 2 Hz); IR ($CHCl_3$) 3400, 2950, 1700, 1470, 1370, 1260, 1160, 1040 cm^{-1}; MS, m/e 263 (M^+); $[\alpha]^{20}_D$ −14.8° (CH_3OH). Anal. ($C_{14}H_{21}NO_3$) C, H, N.

***N*-[3-[(*tert*-Butoxycarbonyl)amino]-1-carboxamido-4-phenylbutyl]-Ala-Pro-OCH$_3$ (24).** A mixture of aldehyde **14** (0.347 g, 1.32 mmol), Ala-Pro·$^1/_2H_2O$ (0.259 g, 1.32 mmol), KCN (0.086 g, 1.32 mmol), HOAc (0.076 mL, 1.32 mmol), and CH_3OH (3 mL) was stirred for 48 h and then cooled (ice bath) and saturated with anhydrous HCl. The flask was tightly capped and the mixture was allowed to stir at room temperature for 72 h. The residue after evaporation was combined with di-*tert*-butyl dicarbonate (0.30 mL, 1.32 mmol), Et_3N (0.70 mL, 5.28 mmol), and CH_2Cl_2 (3 mL). The mixture was stirred (3 h), then diluted with EtOAc (25 mL), extracted with H_2O and brine, and dried ($MgSO_4$). The residue after evaporation was purified on silica gel (EtOAc–EtOH, 20:1), giving the product as a single diastereomer (0.117 g, 18%): TLC (EtOAc–EtOH, 10:1) R_f 0.50; NMR ($CDCl_3$) δ 1.30 (3 H, d, J = 7 Hz), 1.40 (9 H, s), 1.7–2.3 (7 H, m), 2.7–3.2 (3 H, m), 3.3–3.6 (2 H, m), 3.73 (3 H, s), 4.5–4.6 (3 H, m), 7.2 (5 H, s); IR ($CHCl_3$) 3400, 2960, 1640, 1430 cm^{-1}; MS (FAB), m/e 491 (M^+ + 1). Also isolated was a single diastereomeric lactam (135 mg, 21%) with the following properties: TLC (EtOAc–EtOH, 10:1) R_f 0.10; NMR ($CDCl_3$) δ 1.35 (3 H, d, J = 7 Hz), 1.9–2.2 (7 H, m), 2.6–2.9 (3 H, m), 3.5–3.7 (3 H, m), 3.73 (3 H, s), 5.7 (2 H, br s), 6.6–6.8 (1 H, m), 7.2 (5 H, s); MS (FAB); m/e 374 (M^+ + 1).

***N*-(3-Benzamido-1-carboxy-4-phenylbutyl)-Ala-Pro (25S).** A solution of amido ester **24** (117 mg, 0.239 mmol) in TFA (2 mL) was allowed to stand for 18 h and then evaporated to dryness. To the residue were added Et_3N (0.13 mL, 0.96 mmol), benzoyl chloride (0.29 mL, 0.25 mmol), and CH_2Cl_2 (1 mL), and the mixture was stirred for 18 h. A slurry of the mixture in EtOAc was filtered and then washed with H_2O, $NaHCO_3$ solution, and brine and dried ($MgSO_4$). Purification on silica gel (EtOAc–EtOH, 3:1) gave N-(3-benzamido-1-carboxamido-4-phenylbutyl)-Ala-Pro-OCH$_3$ (36 mg, 0.072 mmol, 30%): TLC R_f 0.5; NMR ($CDCl_3$) δ 1.29 (3 H, d, J = 8 Hz), 1.8–2.3 (5 H, m), 2.8–3.2 (5 H, m), 3.3–3.6 (5 H, m), 3.73 (3 H, s), 4.3–4.7 (3 H, m), 5.6–5.7 (2 H, m), 6.72 (1 H, d, J = 8 Hz), 7.1–7.2 (1 H, m), 7.2–7.9 (10 H, m); MS (FAB), m/e 495 (M^+ + 1). The amido ester was combined with Amberlyst 15 resin (0.27 g) and CH_3OH (1 mL) and the mixture warmed at 60 °C for 6 days. Elution of the product from the resin (CH_3OH–Pyr, 2:1) and purification on silica gel (EtOAc–EtOH, 10:1) gave *N*-(3-benzamido-1-carbomethoxy-4-phenylbutyl)-Ala-Pro-OCH$_3$ (18 mg, 0.035 mmol, 48%); TLC R_f 0.5; NMR ($CDCl_3$) δ 1.23 (3 H, d, J = 7 Hz), 1.6–2.4 (6 H, m), 2.81 (2 H, ABX, J_{AB} = 14, δ_{AB} = 34, J_{AX} = 8, J_{BX} = 6), 3.03 (1 H, q, J = 7 Hz); 3.4–3.6 (2 H, m), 3.62 (3 H, s), 3.70 (3 H, s), 4.2–4.3 (1 H, m), 4.4–4.6 (2 H, m), 7.2–7.9 (10 H, m).

A solution of the diester in aqueous NaOH (0.25 N, 0.56 mL) was stirred for 4 h and then diluted with aqueous HCl (0.50 N, 0.28 mL). The product was purified on Dowex 50 (1 g), giving **25S** (10 mg, 0.021 mmol) as a white foam: TLC (3:1:1:1 EBAW) R_f 0.5; NMR (CD_3OD) δ 1.2–1.8 (5 H, m), 2.0–2.5 (5 H, m), 3.0–3.2 (2 H, m), 3.6–3.9 (3 H, m), 4.4–4.7 (2 H, m), 7.2–7.9 (10 H, m); MS (FAB), m/e 482 (M^+ + 1).

3-Benzamido-4-phenylbutanal (15). To an ice-cold solution of ester **23** (1.33 g, 4.5 mmol) in toluene (20 mL) was added a solution (1 M) of Dibal-H in hexane (18.5 mL). The mixture was stirred 1 h and then treated as described for aldehyde **14**, affording after chromatography on silica gel 3-[(*tert*-butoxycarbonyl)-amino]-4-phenyl-1-butanol (0.425 g, 1.60 mmol, 36%) as a white solid: mp 57–59 °C; NMR ($CDCl_3$) δ 1.40 (9 H, s), 1.6–1.9 (2 H, m), 2.83 (2 H, d, J = 7 Hz), 3.5–3.9 (3 H, m), 5.23 (1 H, d, J = 7 Hz), 7.2 (6 H, s). Anal. ($C_{15}H_{23}NO_3$) C, H, N. A solution of the alcohol (0.181 g, 0.683 mmol) in TFA (5 mL) was allowed to stand for 2 h. The residue after evaporation was combined with benzoic anhydride (0.170 g, 0.751 mmol) and Et_3N (0.29 mL, 2.1 mmol) in CH_2Cl_2 (5 mL) and the mixture was stirred for 2 h. The mixture was diluted with EtOAc (25 mL) and extracted with dilute $NaHCO_3$ (3 × 5 mL). Chromatography on silica gel (40:1 CH_2Cl_2–CH_3OH) provided 3-benzamido-4-phenyl-1-butanol (97.1 mg, 0.361 mmol, 53%) as a colorless oil: NMR ($CDCl_3$) δ 1.3–2.0 (2 H, m), 3.03 (2 H, d, J = 7 Hz), 3.6–3.9 (2 H, m), 4.3–4.9 (1 H, m), 5.1 (1 H, br s), 6.7–7.0 (1 H, m), 7.2–7.8 (5 H, m); MS, m/e 269 (M^+). A solution of this alcohol (97.1 mg, 0.361 mmol) in CH_2Cl_2 (1 mL) was added to a slurry of pyridinium chlorochromate (0.12 g, 0.54 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 18 h, then diluted into EtOAc (50 mL), and filtered through Florisil. Silica gel chromatography (2:1 hexane–EtOAc) afforded pure aldehyde (37.5 mg, 0.140 mmol, 39%): mp 107–108 °C; NMR ($CDCl_3$) δ 2.76 (2 H, d, J = 6 Hz), 2.9–3.2 (2 H, m), 3.6–3.8 (1 H, m), 4.5–4.9 (1 H, m), 6.72 (1 H, d, J = 6 Hz), 7.2–7.8 (10 H, m), 9.80 (1 H, s); MS, m/e 267 (M^+).

***N*-(3-Benzamido-1-carboxy-4-phenylbutyl)-Ala-Pro (25).** A mixture of aldehyde **15** (35 mg, 0.13 mmol), Ala-Pro·$^1/_2H_2O$ (24.2 mg, 0.13 mmol), KCN (8.5 mg, 0.13 mmol), HOAc (7.4 μL, 0.13 mmol), and CH_3OH (0.5 mL) was stirred for 3 days, then cooled (ice bath), and saturated with anhydrous HCl. The flask was tightly capped and the mixture stirred for 18 h at room temperature. The residue after evaporation was taken up in CH_2Cl_2 and treated with anhydrous NH_3. An EtOAc slurry of the product was filtered to remove NH_4Cl and then silica gel chromatography (40:1 CH_2Cl_2–CH_3OH) provided *N*-(3-benzamido-1-carboxamido-4-phenylbutyl)-Ala-Pro-OCH$_3$ (33 mg, 0.067 mmol, 51%): TLC R_f 0.40; MS, m/e 494 (M^+). The amido ester (30 mg, 0.061 mmol) was combined with Amberlyst 15 resin (0.45 g) and CH_3OH (1 mL) and the mixture was stirred for 8 days at

60 °C. Elution of the product with Et_3N and purification on silica gel (10:1 EtOAc–EtOH) gave *N*-(3-benzamido-1-carbomethoxy-4-phenylbutyl)-Ala-Pro-OCH_3 (16.4 mg, 0.032 mmol, 53%) as a mixture (2:1) of diastereomers: TLC R_f 0.50 (major), 0.55; NMR ($CDCl_3$) δ 1.23 (major), 1.28 (3 H, 2 d, J = 7 Hz), 3.64 (major), 3.72 (major), 3.60, 3.70 (6 H, 4 s); remainder as for **25S**; MS, m/e 509 (M^+). A solution of the diester (14 mg, 0.028 mmol) in aqueous NaOH (0.25 N, 0.45 mL) was stirred for 2 h and then diluted with aqueous HCl (0.50 N, 0.22 mL). The product was purified on Dowex 50 (1.5 g), providing **25** (12 mg, 0.025 mmol) as a white foam: TLC (3:1:1:1 EBAW) R_f 0.50 (major), 0.55; MS (FAB), m/e 482 ($M^+ + 1$).

***N*-[Carboxamido[*N*-(*tert*-butoxycarbonyl)-2-pyrrolidinyl]methyl]-Ala-Pro-OCH_3 (17S, 17R).** A solution of *N*-*t*-Boc-Pro-OCH_3 (9.0 g, 41.4 mmol) in toluene (150 mL) was cooled (dry-ice bath) as a solution (1 M in hexane) of diisobutylaluminum hydride (62.1 mL) was added over 30 min. After an additional 30 min, CH_3OH (3 mL) was added slowly. Then Rochelle salt (25 g) was added with vigorous stirring. The resulting thick gel was vacuum filtered and rinsed with EtOAc. The filtrate was washed with saturated Rochelle salt solution, H_2O, and brine and dried ($MgSO_4$). The residue after evaporation (7.8 g) was dissolved in CH_3OH (60 mL), and Ala-Pro·$^1/_2H_2O$ (5.6 g, 28.7 mmol), KCN (2.0 g, 30 mmol), and HOAc (1.8 g, 30 mmol) were added. The resulting solution was allowed to stir for 4 days and then cooled (ice bath) and saturated with anhydrous HCl. The flask was tightly stoppered and the mixture allowed to stir for 4 days at room temperature. After evaporation and reconcentration from CH_3OH, the residue was taken up in CH_3OH (150 ml) and treated with Et_3N (10 mL) and di-*tert*-butyl dicarbonate (6.6 mL) for 24 h. After evaporation to dryness, the residue was slurried in EtOAc and filtered. The filtrate was washed with H_2O and brine and dried ($MgSO_4$). Evaporation provided the product as an oil. Silica gel chromatography gave pure material (3.5 g, 8.2 mmol, 29%) as a mixture of diastereomers (2:1). Separation of diastereomers was carried out by MPLC (EtOAc–EtOH, 10:1). More-mobile isomer (**17S**): TLC (EtOAc–EtOH, 10:1) R_f 0.30; NMR ($CDCl_3$) δ 1.26 (3 H, d, J = 7 Hz), 1.44 (9 H, s), 1.8–2.4 (8 H, m), 3.2–3.4 (2 H, m), 3.5–3.7 (2 H, m); 3.71 (3 H, s); 4.1–4.2 (2 H, m); 4.5–4.6 (1 H, m), 5.7 (1 H, br), 7.2 (1 H, br); IR ($CHCl_3$) 3500, 2950, 1730, 1680, 1620 cm^{-1}; MS, m/e 426 (M^+). Less-mobile isomer (**17R**): TLC R_f 0.25; NMR ($CDCl_3$) δ 1.26 (3 H, d, J = 8 Hz), 1.44 (9 H, s), 1.8–2.4 (8 H, m), 3.2–3.4 (2 H, m), 3.5–3.7 (2 H, m), 3.71 (3 H, s), 4.2–4.4 (2 H, m), 4.5–4.6 (1 H, m), 5.5 (1 H, br), 6.1 (1 H, br); IR ($CHCl_3$) 3500, 2950, 1735, 1680, 1620 cm^{-1}; MS, m/e (M^+).

***N*-[Carbomethoxy(2-pyrrolidinyl)methyl]-Ala-Pro-OCH_3 (26).** A mixture of amido ester **17S** (0.75 g, 1.76 mmol), Amberlyst 15 acidic resin (10 g), and CH_3OH (20 mL) was stirred gently and warmed at 60 °C for 7 days. The product was eluted from the resin with CH_3OH–Et_3N (2:1) and purified on silica gel (CMWA, 100:20:3:0.5), giving the product as the acetate salt. A CH_2Cl_2 solution of this was saturated with anhydrous NH_3 and then immediately evaporated to dryness. The residue was taken up in EtOAc and filtered. Evaporation then provided the product as a thick oil (0.35 g, 1.0 mmol): TLC (CMWA, 85:30:5:1) R_f 0.50; NMR ($CDCl_3$) δ 1.34 (3 H, d, J = 8 Hz), 1.5–2.3 (8 H, m), 3.0–3.2 (2 H, m), 3.5–3.7 (2 H, m), 3.73 (3 H, s), 3.76 (3 H, s), 4.5–4.6 (1 H, m), 5.8 (2 H, br); MS (FAB), m/e 432 ($M^+ + 1$); IR ($CHCl_3$) 3350, 2960, 1730, 1630 cm^{-1}.

***N*-[Carbomethoxy[*N*-(*tert*-butoxycarbonyl)-2-pyrrolidinyl]methyl]-Ala-Pro-OCH_3 (27).** Amido ester **17S** (0.75 g) was treated as described above with Amberlyst 15 resin. A mixture of the crude product, di-*tert*-butyl dicarbonate (0.38 g, 1.8 mmol) and CH_2Cl_2 (5 mL) was allowed to stir for 2 h. The product was purified on silica gel: TLC (EtOAc) R_f 0.35; NMR ($CDCl_3$) δ 1.20, 1.26 (3 H, 2 d, J = 7 Hz), 1.46 (9 H, s), 1.8–2.3 (8 H, m), 3.3–3.7 (5 H, m), 3.71 (3 H, s), 3.72 (3 H, s), 3.7–3.9 (1 H, m), 4.2–4.3 (1 H, m), 4.5–4.6 (1 H, m); MS, m/e 441 (M^+); IR ($CHCl_3$) 2975, 2900, 1740, 1680, 1650, 1410 cm^{-1}.

***N*-[Carboxy(2-pyrrolidinyl)methyl]-Ala-Pro (28).** A solution of diester **27** (55 mg, 0.125 mmol) in aqueous NaOH (2 mL, 0.25 N) was stirred vigorously for 4 h. Aqueous HCl (1 mL, 0.50 N) was added and the mixture was evaporated to dryness. The residue was then treated with TFA (4 mL) for 4 h. The product after evaporation was purified on Dowex 50 (3 g); TLC (EPAW, 5:5:1:3) R_f 0.30; NMR (D_2O) δ 1.28, 1.36 (3 H, 2 d, J = 7 Hz), 1.6–2.2 (8 H, m), 3.1–3.3 (2 H, m), 3.3–3.5 (3 H, m), 3.7–3.9 (1 H, m), 4.0–4.2 (2 H, m); MS, m/e 314 ($M^+ + 1$).

***N*-[Carbomethoxy(*N*-acetyl-2-pyrrolidinyl)methyl]-Ala-Pro-OCH_3 (29).** A mixture of diester **26** (0.19 g, 0.56 mmol), Ac_2O (0.085 g, 0.83 mmol), Et_3N (0.084 g, 0.83 mmol), and CH_2Cl_2 (2 mL) was stirred for 4 h and then evaporated to dryness. The residue was taken up in EtOAc and filtered. The product was purified on silica gel (EtOAc–EtOH, 10:1) and recrystallized (hexanes–EtOAc): TLC (EtOAc–EtOH, 5:1) R_f 0.2; NMR ($CDCl_3$) δ 1.25 (3 H, d, J = 7 Hz), 1.8–2.3 (8 H, m), 2.1 (3 H, s), 2.4–2.6 (4 H, m), 3.71 (3 H, s), 3.73 (3 H, s), 3.83 (1 H, d, J = 6 Hz), 4.3–4.4 (1 H, m), 4.5–4.6 (1 H, m), 4.9 (1 H, br); MS, m/e 383 (M^+); IR ($CHCl_3$) 2960, 2890, 1740, 1430 cm^{-1}.

***N*-[Carboxy(*N*-acetyl-2-pyrrolidinyl)methyl]-Ala-Pro (30).** A solution of diester **29** (50 mg, 0.13 mmol) in aqueous NaOH (0.25 N, 2.1 mL) was stirred overnight. The solution was neutralized by addition of aqueous HCl (0.50 N, 1 mL) and then placed directly onto Dowex 50 (2.5 g): TLC (EBAW, 3:1:1:1) R_f 0.15; NMR (D_2O) δ, 1.48, 1.56 (3 H, 2 d, J = 7 Hz), 1.7–2.3 (8 H, m), 2.06 (3 H, s), 3.4–3.7 (4 H, m), 3.8–4.0 (1 H, m), 4.3–4.5 (2 H, m); MS (FAB), m/e 356 ($M^+ + 1$).

***N*-[Carbomethoxy(*N*-benzoyl-2-pyrrolidinyl)methyl]-Ala-Pro-OCH_3 (31).** A mixture of diester **26** (110 mg, 0.275 mmol), Et_3N (0.076 mL, 0.550 mmol), benzoic anhydride (62 mg, 0.275 mmol), and CH_2Cl_2 (5 mL) was stirred for 16 h. The product was purified on silica gel (EtOAc–EtOH, 3:1): TLC R_f 0.45; NMR ($CDCl_3$) δ 1.24, 1.31 (3 H, 2 d, J = 7 Hz), 1.8–2.2 (8 H, m), 3.4–3.6 (4 H, m), 3.72 (3 H, s), 3.77 (3 H, s), 4.02 (1 H, d, J = 6 Hz), 4.14 (1 H, q, J = 6 Hz), 7.4–7.7 (5 H, m); MS, m/e 445 (M^+).

***N*-[Carboxy(*N*-benzoyl-2-pyrrolidinyl)methyl]-Ala-Pro (32).** To a solution of diester **31** (79.5 mg, 0.179 mmol) in H_2O–CH_3OH (6:4, 1.8 mL) was added aqueous NaOH (1 N, 0.72 mL). The mixture was stirred for 16 h and then placed directly onto Dowex 50; TLC (CMWA, 70:30:6:4) R_f 0.40; NMR (CD_3OD) δ 1.50, 1.53 (3 H, 2 d, J = 7 Hz), 1.8–2.4 (8 H, m), 3.3–3.6 (4 H, m), 3.9–4.1 (1 H, m), 4.3–4.5 (2 H, m), 4.5–4.7 (1 H, m), 7.4–7.6 (5 H, m); MS, m/e 561 [M^+ – bis(trimethylsilyl)].

***N*-[Carbomethoxy[[*N*-(*tert*-butoxycarbonyl)glycyl]-2-pyrrolidinyl]methyl]-Ala-Pro-OCH_3 (33).** A mixture of diester **26** (71.9 mg, 0.211 mmol), L-*t*-Boc-glycine *N*-hydroxysuccinimide ester (69 mg, 0.25 mmol), Et_3N (0.035 mL, 0.25 mmol), and CH_2Cl_2 (2 mL) was stirred for 2 h and then evaporated to dryness. The product was purified on silica gel (ether–CH_3OH, 10:1) and recrystallized (hexanes–EtOAc): TLC (ether–CH_3OH, 20:1) R_f 0.15; NMR ($CDCl_3$) δ 1.21, 1.25 (3 H, 2 d, J = 7 Hz), 1.46 (9 H, s), 1.7–2.2 (8 H, m), 2.5–2.7 (2 H, m), 3.4–3.5 (3 H, m), 3.73 (3 H, s), 3.74 (3 H, s), 3.8–3.9 (2 H, m), 4.2 (1 H, m), 4.5 (1 H, m), 5.5 (1 H, m); IR ($CHCl_3$) 3430, 2980, 1730, 1710, 1430 cm^{-1}; MS, m/e 425 (M^+ – O-*t*-Bu).

***N*-[Carboxy(*N*-glycyl-2-pyrrolidinyl)methyl]-Ala-Pro (34).** A mixture of diester **33** (40 mg, 0.080 mmol) and aqueous NaOH (0.25 N, 1.3 mL) was stirred for 24 h, diluted with aqueous HCl (0.50 N, 0.50 mL), and evaporated to dryness. The residue was taken up in concentrated HCl (5 mL) and stirred overnight. The mixture was diluted with H_2O (15 mL) and then evaporated to dryness. The product was purified on Dowex 50: TLC (CMWA, 85:30:5:1) R_f 0.3; NMR (D_2O) δ 1.31, 1.44 (3 H, 2 d, J = 7 Hz), 1.6–2.2 (8 H, m), 3.2–3.5 (5 H, m); 3.7–3.9 (1 H, m), 3.80 (2 H, s), 4.1–4.2 (1 H, m), 4.2–4.4 (1 H, m).

***N*-[Carbomethoxy[*N*-[(*tert*-butoxycarbonyl)phenylalanyl]-2-pyrrolidinyl]methyl]-Ala-Pro-OCH_3 (35).** A mixture of diester **26** (130 mg, 0.381 mmol), L-*t*-Boc-phenylalanine *N*-hydroxysuccinimide ester (145 mg, 0.400 mmol), Et_3N (0.055 mL, 0.400 mmol), and CH_2Cl_2 (3 mL) was stirred for 3 h and then evaporated to dryness. The product was purified on silica gel (CH_2Cl_2–CH_3OH, 10:1): TLC R_f 0.5; NMR ($CDCl_3$) δ 1.08, 1.20 (3 H, 2 d, J = 7 Hz), 1.26, 1.38 (9 H, 2 s), 1.8–2.2 (8 H, m), 2.6–2.9 (2 H, m), 2.9–3.1 (2 H, m), 3.2–3.5 (5 H, m), 3.63, 3.69, 3.65, 3.67 (6 H, 4 s), 4.2–4.4 (3 H, m), 4.5–4.7 (1 H, m), 5.19, 5.34 (1 H, 2 br d, J = 8 Hz); IR ($CHCl_3$) 2960, 1730, 1710, 1640, 1490, 1430 cm^{-1}.

***N*-[Carbomethoxy[*N*-(benzoylphenylalanyl)-2-pyrrolidinyl]methyl]-Ala-Pro-OCH_3 (37).** A solution of diester **35** (64 mg, 0.109 mmol) in TFA (3 mL) was allowed to stand for 3 h and then evaporated to dryness. A mixture of the residue

with benzoyl chloride (17 mg, 0.12 mmol), Et_3N (0.075 mL, 0.54 mmol), and CH_2Cl_2 (1 mL) was stirred overnight and then evaporated to dryness. The residue was slurried in EtOAc and filtered. The product was purified on silica gel, giving a white solid: TLC (ether–CH_3OH, 10:1) R_f 0.3; NMR ($CHCl_3$) δ 1.16, 1.28 (3 H, 2 d, J = 7 Hz), 1.5–1.7 (2 H, m), 1.8–2.2 (6 H, m), 2.9–3.1 (1 H, m), 3.2–3.6 (6 H, m), 3.6–3.8 (1 H, m), 3.68 (major), 3.76 (major), 3.62, 3.70 (6 H, 4 s), 4.3–4.4 (1 H, m), 4.4–4.5 (1 H, m), 5.1–5.2 (1 H, m), 6.92 (major), 7.18 (1 H, 2 br d, J = 8 Hz), 7.2–7.8 (10 H, m); MS, m/e 592 (M^+).

N-[Carboxy[N-(benzoylphenylalanyl)-2-pyrrolidinyl]methyl]-Ala-Pro (38). A mixture of diester **35** (57.5 mg, 0.0971 mmol), aqueous NaOH (0.25 N, 1.55 mL), and CH_3OH (0.5 mL) was stirred overnight and then neutralized by addition of aqueous HCl (0.50 N, 0.75 mL). The residue after evaporation was purified on Dowex 50 (H_2O–CH_3OH–pyr, 10:10:1): TLC (EBAW, 3:1:1:1) R_f 0.45; NMR (D_2O) δ 1.07, 1.14 (3 H, 2 d, J = 7), 1.4–1.9 (8 H, m), 2.4–2.9 (2 H, m), 3.1–3.3 (2 H, m), 3.4–3.6 (2 H, m), 3.8–4.1 (3 H, m), 4.2–4.4 (1 H, m), 4.5–4.7 (1 H, m), 6.8–7.2 (10 H, m); MS, m/e 565 (M^+ + 1).

N-[Carboxy(N-phenylalanyl-2-pyrrolidinyl)methyl]-Ala-Pro (36). A mixture of diester **35** (65.8 mg, 0.112 mmol), aqueous NaOH (0.25 N, 1.8 mL), and THF (1.8 mL) was stirred for 24 h and then diluted with aqueous HCl (0.50 N, 0.90 mL). The residue after evaporation was dissolved in TFA and allowed to stand for 3 h. The residue after evaporation was purified on Dowex 50: TLC (EPAW, 5:5:1:3) R_f 0.50; NMR (D_2O) δ 1.20, 1.30 (3 H, 2 d, J = 7 Hz); 1.4–2.0 (8 H, m), 2.5–2.7 (1 H, m), 2.9–3.3 (5 H, m), 3.4–3.7 (2 H, m), 3.8–4.0 (1 H, m), 4.0–4.2 (2 H,); MS (FAB), m/e 461 (M^+ + 1).

N^α-Pyroglutamyl-N^ϵ-carbobenzoxy-Lys-O-t-Bu (39). A solution of N^ϵ-Cbz-Lys-O-t-Bu·HCl (1.00 g, 2.68 mmol) in DMF (6 mL) was cooled (–20 °C), and i-Pr_2NEt (0.47 mL, 2.70 mmol), pyroglutamic acid (0.346 g, 2.68 mmol), and DPPA (0.87 mL, 4.0 mmol) were added in order. Then $NaHCO_3$ (1.3 g, 15 mmol) was added. The mixture was stirred at –20 °C for 10 min, at 0 °C for 1 h, and then at room temperature for 18 h. The mixture was diluted with EtOAc (50 mL) and filtered. The filtrate was washed with H_2O, saturated $NaHCO_3$, aqueous HCl (0.5 N), H_2O, and brine, and dried ($MgSO_4$). The product was purified on silica gel (EtOAc–EtOH, 20:1), giving a white solid: mp 84–86 °C (1.10 g, 2.45 mmol; 91%); TLC (EtOAc–EtOH, 20:1) R_f 0.35; NMR ($CDCl_3$) δ 1.3–1.9 (4 H, m), 1.46 (9 H, s), 2.1–2.5 (4 H, m), 2.8 (1 H, br s), 2.93 (1 H, d, J = 14 Hz), 3.1–3.3 (2 H, m), 4.1–4.2 (1 H, m), 4.4–4.6 (1 H, s), 5.12 (2 H, s), 5.2–5.4 (1 H, br), 7.24 (1 H, br s), 7.38 (5 H, s); MS, m/e 447 (M^+). Anal. ($C_{23}H_{33}H_3O_6$), C, H, N.

N-[Carbomethoxy(N^α-pyroglutamyl-N^ϵ-carbobenzoxylysyl-2-pyrrolidinyl)methyl]-Ala-Pro-OCH_3 (40). A solution of ester **39** (0.155 g, 0.335 mmol) in TFA (5 mL) was stirred for 6 h. The residue was purified on silica gel (CMWA, 85:30:5:1), giving N^α-pyroglutamyl-N^ϵ-Cbz-Lys (52 mg, 0.13 mmol; 40%) as a colorless oil: TLC (CH_2Cl_2–CH_3OH, 10:1) R_f 0.50; NMR (D_2O) δ 1.3–1.8 (4 H, m), 2.2–2.6 (4 H, m), 3.1–3.2 (2 H, br s), 3.3–3.4 (2 H, br s), 4.2–4.3 (1 H, m), 5.10 (2 H, s), 7.36 (5 H, s). This material (49.2 mg, 0.126 mmol) was combined with N-methylmorpholine (0.0139 mL, 0.126 mmol) and THF (1 mL) under nitrogen. The solution was cooled (–15 °C) as isobutyl chloroformate (0.0163 mL, 0.126 mmol) was added. After 2 min, a solution of diester **26** (42.9 mg, 0.126 mmol) in THF (1 mL) was added. The mixture was stirred at –15 °C for 1 h and then diluted with EtOAc (50 mL) and extracted with saturated $NaHCO_3$, H_2O, and brine. The solution was dried ($MgSO_4$) and concentrated to a colorless oil, which was purified on silica gel: TLC (EtOAc–EtOH, 3:1) R_f 0.20; NMR ($CDCl_3$) δ 1.23 (3 H, d, J = 7 Hz), 1.4–2.5 (16 H, m), 3.1–3.3 (2 H, m), 3.4–3.8 (9 H, m), 3.68 (3 H, s), 3.69 (3 H, s), 4.2–4.3 (1 H, m), 4.4–4.5 (1 H, m), 4.3–4.4 (1 H, m), 4.6–4.7 (1 H, br), 5.11 (2 H, s), 5.6–5.7 (1 H, br), 7.37 (5 H, s), 7.61 (1 H, br d, J = 7 Hz); IR ($CHCl_3$) 1730, 1720, 1690, 1630, 1420 cm^{-1}; MS, m/e 714 (M^+).

N-[1-Carboxy-1-(N^α-pyroglutamyllysyl-2-pyrrolidinyl)methyl]-Ala-Pro (41). A solution of diester **40** (25.0 mg, 0.0352 mmol) in CH_3OH (2 mL) was hydrogenated with 10% Pd(C) catalyst (10 mg) for 3 h at 1 atm. Removal of catalyst by filtration and evaporation gave a colorless residue (16.2 mg), which was combined with aqueous NaOH (0.25 N, 0.56 mL). The mixture was stirred for 4 h and then neutralized with aqueous HCl (0.50 N, 0.21 mL). The product was purified on Dowex 50: TLC (EBAW, 1:1:1:1) R_f 0.20; NMR (D_2O) δ 1.53, 1.60 (3 H, 2 d, J = 7 Hz), 1.4–2.7 (18 H, m), 3.02 (2 H, t, J = 8 Hz), 3.3–4.0 (5 H, m), 3.88 (1 H, d, J = 2 Hz); 4.00 (1 H, q, J = 7 Hz), 4.2–4.5 (2 H, m), 4.5–4.7 (1 H, m); MS (FAB), m/e 553 (M^+ + 1).

Compound 18S. To a solution of diester **26** (34.3 mg, 0.100 mmol) in CH_2Cl_2 (1 mL) were added Et_3N (20.2 mg, 0.200 mmol) and a solution of phosgene (1.1 M) in toluene (0.100 mmol, 0.090 mL). The mixture was stirred for 30 min and then evaporated. A slurry of the residue in EtOAc was filtered and evaporated. The product was purified on silica gel (EtOAc) and recrystallized (hexane–EtOAc), giving a white solid (28.5 mg, 78%): mp 110–112 °C; TLC (EtOAc) R_f 0.25; NMR ($CDCl_3$) 1.26 (3 H, d, J = 7 Hz), 1.3–2.3 (7 H, m), 3.12 (1 H, ddd, J = 4, 6, 11 Hz); 3.56 (1 H, dt, J = 11, 6 Hz), 3.6–3.9 (2 H, m), 3.71 (3 H, s), 3.76 (3 H, s), 3.98 (1 H, td, J = 9, 6 Hz), 4.30 (1 H, dd, J = 6, 9 Hz), 4.48 (1 H, d, J = 10 Hz); 4.94 (1 H, q, J = 9 Hz); IR ($CHCl_3$) 2960, 1750, 1690, 1650, 1415 cm^{-1}; MS, m/e 367 (M^+). Anal. ($C_{17}H_{25}N_3O_6$) C, H, N.

Compound 18R. Amido ester **17R** (0.442 g, 1.29 mmol) was combined with Amberlyst 15 resin (6 g) and CH_3OH (10 mL) and the mixture was warmed at 60 °C for 5 days. Elution of the resin with CH_3OH–Et_3N (2:1) gave the crude diester, which was purified on silica gel (CMWA; 100:20:3:0.5). A solution of the resulting acetate salt was dissolved in CH_2Cl_2 (10 ml) and neutralized with anhydrous NH_3. A portion of the resulting oil (87.2 mg) was treated as described above for diester **26**, affording **18R** (16.1 mg, 0.044 mmol, 17%). Recrystallization (hexane–EtOAc) gave a white solid: mp 97–99 °C; TLC (EtOAc) R_f 0.35; NMR ($CDCl_3$) δ 1.36 (1 H, d, J = 7), 1.5–2.3 (7 H, m), 3.03 (1 H, ddd, J = 4, 7, 11 Hz), 3.4–3.8 (4 H, m), 3.71 (3 H, s), 3.79 (3 H, s), 4.52 (1 H, dd, J = 4, 9 Hz), 4.81 (1 H, q, J = 7 Hz), 4.84 (1 H, d, J = 2 Hz); IR ($CHCl_3$) 2970, 1745, 1695, 1650, 1420 cm^{-1}; MS, m/e 367 (M^+). Anal. ($C_{17}H_{25}N_3O_6$) C, H, N.

N-[Carboethoxy(N-benzoyl-2-pyrrolidinyl)methyl]-Ala-Pro-OEt (42). A mixture of amido ester **17S** (97.8 mg, 0.230 mmol), Amberlyst XN-1010 acidic resin[10] (1.5 g), and anhydrous EtOH (4 mL) was warmed at 70 °C for 9 days. Elution with EtOH–Et_3N (2:1) gave a red-colored oil, which was chromatographed on silica gel (CMWA, 85:30:5:1) and then treated briefly with anhydrous NH_3 in CH_2Cl_2, affording N-[carboethoxy(2-pyrrolidinyl)methyl]-Ala-Pro-OEt (**43**) as a colorless oil (35.3 mg, 0.0957 mmol, 42%): TLC (CMWA, 85:30:5:1) R_f 0.55; NMR ($CDCl_3$) δ 1.2–1.4 (9 H, m), 1.5–2.3 (8 H, m), 3.2–3.4 (1 H, m), 3.4–3.8 (5 H, m), 4.1–4.3 (5 H, m), 4.6–4.7 (1 H, m); IR ($CHCl_3$) 2975, 1720, 1630 cm.$^{-1}$ This diester was combined with benzoic anhydride (22 mg), Et_3N (0.013 mL, 0.099 mmol), and CH_2Cl_2 (2 mL). The mixture was stirred overnight and then diluted with EtOAc (50 mL) and filtered. Solvent was evaporated and the product purified on silica gel (25 mg, 0.053 mmol, 55%): TLC (CH_2Cl_2–CH_3OH, 20:1) R_f 0.30; NMR ($CDCl_3$) δ 1.2–1.4 (9 H, m), 1.5–2.3 (8 H, m), 3.3–3.7 (5 H, m), 4.0–4.2 (5 H, m), 4.42 (1 H, d of d, J = 4, 8 Hz), 4.5–4.6 (1 H, m), 7.3–7.6 (5 H, m); IR ($CHCl_3$) 2975, 2880, 1720, 1630, 1420 cm^{-1}; MS, calcd for $C_{25}N_{35}N_3O_6$ 473.2524, found 473.2494.

N-[Carboethoxy(2-pyrrolidinyl)methyl]-Ala-Pro·HCl (44). A solution of diester **42** (43.8 mg, 0.0926 mmol) in concentrated HCl (3 mL) was stirred overnight, then diluted with H_2O (50 mL), and evaporated to a white solid. Purification on silica gel (CMWA, 85:30:5:1) gave a white foam, which was taken up in H_2O and filtered through Celite. Concentrated HCl (1 mL) was added to the filtrate, which was then evaporated to a white solid (42.1 mg, 0.0874 mmol, 94%): TLC (CMWA, 85:30:5:1) R_f 0.75; NMR (D_2O) δ 1.34 (3 H, t, J = 7 Hz), 1.57 (3 H, d of d, J = 4, 10 Hz), 1.7–2.4 (8 H, m), 3.4–3.7 (4 H, m), 4.03 (1 H, q, J = 7 Hz); 4.2–4.4 (4 H, m), 4.6–4.8 (1 H, m), 7.5–7.7 (5 H, m).

Acknowledgment. We thank Dr. J. Stevenson for FT-IR measurements and J. Smith for mass spectral analyses.

Registry No. 1R, 94730-82-2; **1S**, 94730-83-3; **2**, 84712-38-9; **3**, 72460-59-4; **4**, 69677-91-4; **5**, 35084-74-3; **6**, 82001-60-3; **7**, 74075-33-5; **8**, 72155-45-4; **9R**, 94670-41-4; **9S**, 94730-84-4; **10R**, 94670-42-5; **10S**, 94730-85-5; **11R**, 94798-95-5; **11S**, 94670-43-6; **12R**, 94670-44-7; **12S**, 94730-86-6; **13**, 69610-41-9; **14**, 94670-45-8;

15, 94670-46-9; **16**, 6542-76-3; **17R**, 94670-47-0; **17S**, 94730-87-7; **18R**, 94670-48-1; **18S**, 94730-88-8; **19R**, 84799-92-8; **19S**, 84759-76-2; **20R**, 94730-89-9; **20S**, 94730-90-2; **21R**, 94706-12-4; **21S**, 94798-96-6; **22**, 60398-41-6; **23**, 60398-42-7; **24**, 94706-13-5; **25R**, 94670-49-2; **25S**, 94730-91-3; **26**, 94670-50-5; **27**, 94670-51-6; **28**, 94670-52-7; **29**, 94670-53-8; **30**, 94670-54-9; **31**, 94670-55-0; **32**, 94670-56-1; **33**, 94670-57-2; **34**, 94670-58-3; **35**, 94670-59-4; **36**, 94670-60-7; **37**, 94670-61-8; **38**, 94706-14-6; **39**, 94670-62-9; **40**, 94670-63-0; **41**, 94670-64-1; **42**, 94670-65-2; **43**, 94670-66-3; **44**, 94670-67-4; aminoacetaldehyde diethyl acetal, 645-36-3; benzoyl chloride, 98-88-4; (*N*-benzoylamino)acetaldehyde diethyl acetal, 56459-72-4; di-*tert*-butyl dicarbonate, 24424-99-5; benzoic anhydride, 93-97-0; *N*-Boc-phenylalanine, 13734-34-4; diazomethane, 334-88-3; *N*-((3-benzamido-1-carboxamido-4-phenyl)butyl)-Ala-Pro-OCH_3, 94670-68-5; *N*-((3-benzamido-1-carbomethoxy-4-phenyl)butyl)-Ala-Pro-OCH_3, 94670-69-6; 3-[(*tert*-butoxycarbonyl)amino]-4-phenyl-1-butanol, 94670-70-9; 3-benzamido-4-phenyl-1-butanol, 94706-15-7; Boc-glycine *N*-hydroxysuccinimide, 3392-07-2; (L)-Boc-phenylalanine *N*-hydroxysuccinimide, 3674-06-4; pyroglutamic acid, 98-79-3; phosgene, 75-44-5; angiotensin converting enzyme, 9015-82-1; (*R*)-*N*-((3-benzamido-1-carbomethoxy-4-phenyl)butyl)-Ala-Pro-OCH_3, 94730-92-4; (*S*)-*N*-((3-benzamido-1-carbomethoxy-4-phenyl)butyl)-Ala-Pro-OCH_3, 94730-93-5; Ala-Pro, 13485-59-1; *N*-*t*-Boc-Pro-OCH_3, 59936-29-7; N^{ϵ}-Cbz-Lys-O-*t*-Bu·HCl, 5978-22-3; N^{α}-pyroglutamyl-N^{ϵ}-Cbz-Lys, 94670-71-0.

Synthesis and Platelet Aggregation Inhibitory Activity of 4,5-Bis(substituted)-1,2,3-thiadiazoles

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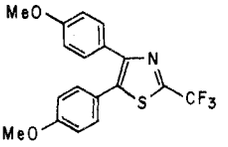
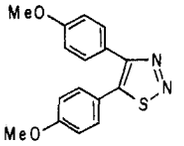
Routine screening of compounds for inhibition of collagen-induced platelet aggregation in vitro revealed 4,5-bis-(4-methoxyphenyl)-1,2,3-thiadiazole (**2**) was active and it represents the first example of a 1,2,3-thiadiazole with possible antithrombotic activity. In order to devleop a structure–activity relationship for this heterocycle, a number of new 4(5)-mono- and -disubstituted 1,2,3-thiadiazoles were synthesized. These were tested in our screen and a number of additional active compounds were found. The most active compounds (**2**, **5a**, **5b**, and **6c**) were those in which the heterocycle was substituted with benzene rings possessing para electron-donating groups.

A direct correlation has been drawn between platelet function and the development of cardiovascular disease states such as atherosclerosis[1] and its complications of myocardial infarction, transient ischemic attacks, and stroke.[2-4] Cyclooxygenase inhibitors, which inhibit platelet aggregation induced by collagen and arachidonic acids, have been explored to alter these disease states.[5,6] Yet, none of the platelet drugs has been overwhelmingly successful.[7] Since platelets may form a thrombus when exposed to collagen as a result of an arterial injury, we feel compounds that inhibit collagen-induced platelet aggregation would be of therapeutic interest.

Recently one of us and others have reported a very interesting platelet-inhibitory compound **1**[8] that has been shown to be active in vivo in humans.[9] During the course of study with this compound, another antithrombotic compound was synthesized, thiadiazole **2**. No one had previously shown that this heterocyclic system possessed platelet inhibitory activity.



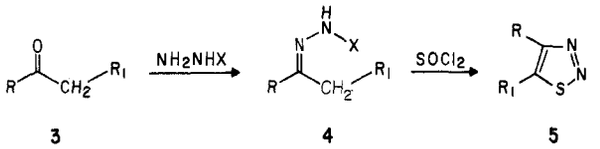
1



2

In order to understand the structure–activity relationship of this heterocycle, we planned to make a series of analogues and test them in vitro by the method of Born and Cross.[10] By synthesizing a number of 4- and 5-substituted 1,2,3-thiadiazoles, we discovered additional active drugs and the results are reported herein.

Scheme I



3 4 5

X = C(O)OEt or $SO_2C_6H_4Me$

We became interested in making analogues of the lead 1,2,3-thiadiazole due to its high biological activity, the stability of this heterocycle, the ease of its synthesis, and the novelty of these compounds. 1,2,3-Thiadiazoles are thermally stable below 200 °C, they are stable in strong acid (HCl), and 4,5-disubstituted thiadiazoles are stable to reducing conditions.[11] Although there are several methods available for the synthesis of this heterocyclic system, there are not many examples in the literature of structurally complex 1,2,3-thiadiazoles. We chose the method of Hurd and Mori[12] to synthesize 1,2,3-thiadiazoles in which α-methylene ketones or aldehydes are starting substrates (Scheme I).

Many of the ketones and aldehydes used in this report were commercially available and some of those not commercially available were synthesized by standard Friedel–Crafts reaction conditions[13] (Table I).

Appropriate acid chlorides reacted with electron-rich phenyl groups, in the presence of aluminum chloride, to afford the corresponding ketones. Stannic chloride was

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