Synthesis and Structure-Activity Relationships of a New Series of Antiarrhythmic Agents: 4,4-Disubstituted Hexahydro-3*H*-pyrido[1,2-*c*]pyrimidin-3-ones and Related Compounds

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A series of 4,4-disubstituted tetrahydro- and 4,4-disubstituted hexahydro-3*H*-pyrido[1,2-*c*]pyrimidin-3-ones (**4** and **5**, respectively) were prepared from 2-aryl-2-(2-piperidinyl)-4-[*N*,*N*-bis(1-methylethyl)amino]butanamides (**2**). Individual racemates of the piperidinyl amides **2** were converted to pure racemic diaza bicyclic compounds that were evaluated for antiarrhythmic activity in the Harris dog model and anticholinergic activity in a muscarinic receptor binding assay. Selected compounds were subsequently evaluated for hemodynamic effects in anesthetized dogs where blood pressure depression and negative inotropic activity were assessed. Of this group, **4a** (R = CH_3) and **5a** (R = CH_3) showed the most favorable pharmacological profiles; the former compound was chosen for toxicity testing over the latter due to its lack of noncompetitive inhibition of acetylcholine-induced contractions of guinea pig ileum segments. Clinical evaluation is now under way.

The use of effective cardiopulmonary resuscitative techniques, coronary care monitoring and therapy, and electrocardioversion has markedly increased the number of individuals who survive acute cardiac rhythm disturbances associated with myocardial infarcts.[1] Consequently, the prevalence of patients with chronic ventricular arrhythmias who require therapy has also increased. This need for effective, safe antiarrhythmic therapy has resulted in the clinical evaluation of a variety of agents with antiarrhythmic activity.[2] However, the adverse effects of these investigational drugs as well as of those antiarrhythmics currently marketed are well documented.[3] Thus, the goal of providing efficacious antiarrhythmics with fewer side effects remains.

Disopyramide phosphate (Norpace), an antiarrhythmic agent developed in our laboratories and marketed in 1977, has been successfully used to treat patients with a variety of ventricular and supraventricular arrhythmias.[4-6] However, it, like the other marketed Class I[7] agents used in the treatment of chronic ventricular arrhythmias, has significant side effects. Disopyramide possesses anticholinergic and negative inotropic activity; quinidine causes gastric disturbances and slows myocardial conduction; and procainamide produces a lupus erythematosus like syndrome and blood dyscrasias.[3] Furthermore, investigational Class I antiarrhythmics possessing subtle differences in cardiac pharmacology (compared to the preceding agents)[8] produce side effects associated with the central nervous system, e.g. tocainide and mexiletine.[3] Thus, in developing a second-generation drug, our aim was to produce a highly efficacious agent with an absence or minimization of side effects most frequently associated with drugs used to treat chronic ventricular arrhythmias.

Earlier work on modification of the disopyramide molecule **1a** showed that antiarrhythmic activity was retained upon substitution[9] or even replacement of the carboxamido group.[9,10] Since changes of, or around, the amide group did not necessarily diminish antiarrhythmic activity, we investigated the effect on biological activity of incorporating this amide into a bicyclic system. This change represented, to the best of our knowledge, the first example of an antiarrhythmic series with a conformationally restricted amide within a rigid framework.

Chemistry

Disopyramide[11] (**1a**) was hydrogenated in the presence of platinum oxide catalyst in aqueous sulfuric acid solution to afford the piperidine amide **2** as a mixture of racemates (Scheme I). Fractional recrystallization of this mixture (ca. 3:1 distribution of racemates) gave the preponderant racemate in 60–65% yield.[12] This diastereomeric pair was found to melt higher than the other racemate and, thus, was designated as the high-melting racemate **2a** (HMR). The other racemate, isolated in ca. 20% yield, was designated as the low-melting racemate **2a′** (LMR), before the relative stereochemistry of these diasteomers was assigned (vide infra).[13]

Treatment of **2a** with dimethylformamide diethyl acetal in DMF overnight at room temperature produced **4a** (R = H) in high yield (Scheme I, method A). Catalytic hydrogenation of **4a** (R = H) in the presence of palladium on carbon yielded the saturated bicyclic system **5a** (R = H), which gave **6** upon alkylation with methyl iodide in the presence of NaH in DMF. Similarly, **2a′**, the LMR, also afforded the bicyclic ring system **4a′** (R = H) upon DMF acetal treatment, albeit in lower yield. Catalytic hydrogenation of **4a′** (R = H), as described above, also gave saturated racemate **5a′** (R = H).

Precursors of diaza bicyclic system **4** possessing a substituent on the phenyl group were prepared via previously described procedures.[14] The appropriately substituted

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(1) Zipes, D. P. *Am. J. Cardiol.* **1978**, *41*, 975.
(2) Zipes, D. P. *New Eng. J. Med.* **1981**, *304*, 475.
(3) Schwartz, J. B.; Keefe, D.; Harrison, D. C. *Drugs* **1981**, *21*, 23.
(4) Vismara, L. A.; Mason, D. T.; Amsterdam, E. A. *Pharmacol. Ther.* **1974**, *16*, 330.
(5) Befeler, B.; Castellanos, A., Jr.; Wells, D. E. *Am. J. Cardiol.* **1975**, *35*, 282.
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(7) Vaughan Williams, E. M. *Prog. Pharmacol.* **1979**, *2*, 13 and references therein.
(8) Vaughan Williams, E. M. *J. Clin. Pharmacol.* **1984**, *24*, 129.
(9) Yen, C. H.; Lowrie, H. S.; Dean, R. R. *J. Med. Chem.* **1974**, *17*, 1131.
(10) Adelstein, G. W. *J. Med. Chem.* **1973**, *16*, 309.
(11) Cusic, J.; Sause, H. W. U.S. Patent 3 225 054, Dec 1965.
(12) These observations were originally made by P. K. Yonan of our laboratories who provided us with initial quantities of these racemates and to whom we are grateful.
(13) For the sake of simplicity, only a single enantiomer of each racemate is indicated in the schemes.

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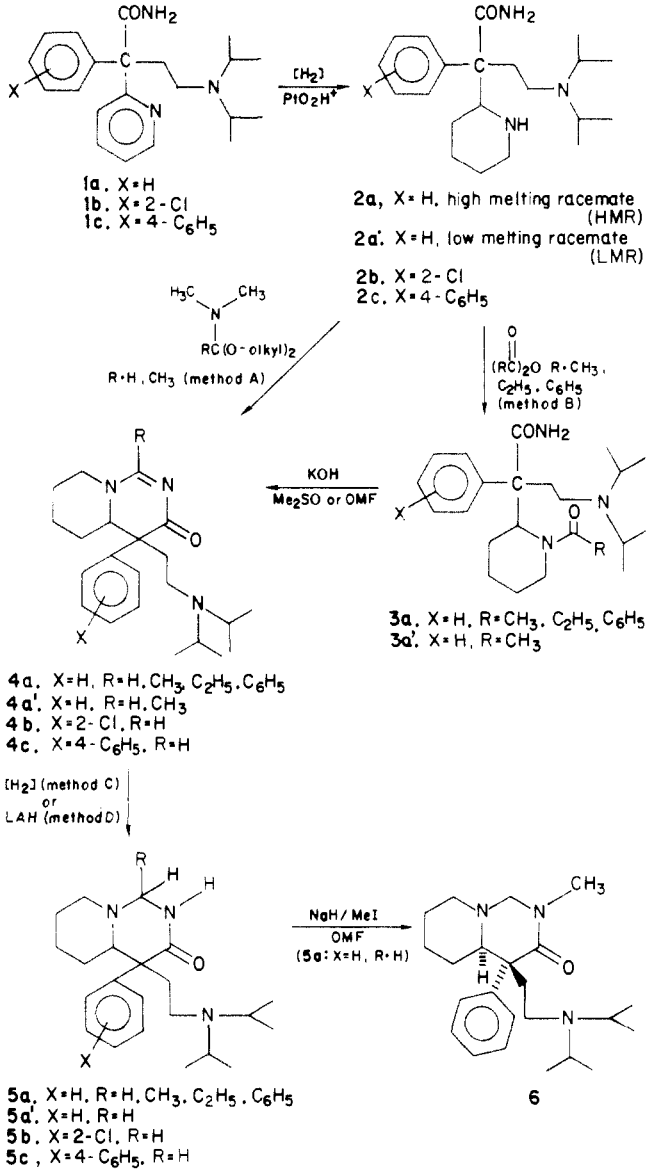
Scheme I[a]

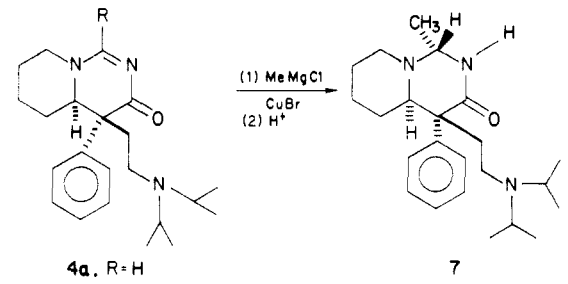


[a] The use of the prime symbol denotes the low melting racemates of this series.

phenylacetonitrile was alkylated with (*N*,*N*-diisopropylamino)ethyl chloride with sodamide in toluene or potassium hydride in DMF. The resultant nitriles were then treated with 2-bromopyridine in the presence of KH in DMF, and subsequent hydration of these pyridyl nitriles using potassium hydroxide in *tert*-butyl alcohol[15] gave the desired amides, **1b** and **1c**. These amides were hydrogenated, as previously described above, to afford a mixture of racemates that were again separated via fractional recrystallization. In each case, the preponderant less polar racemate (by TLC) **2b** or **2c** was isolated and converted to the unsaturated, **4b** and **4c**, and saturated, **5b** and **5c**, bicyclic systems as previously described. We assume these racemates have the same relative stereochemistry as the HMR **2a**. They were formed in preponderance during the hydrogenation and have the same relative polarity to the lesser racemate by TLC as **2a** has to **2a'**.

The ring-methylated homologue **4a** (R = CH_3) of the unsaturated diaza bicyclic system was initially prepared

Scheme II



from the HMR **2a** with dimethylacetamide dimethyl acetal. However, in contrast to the earlier reaction using formamide acetal, prolonged heating was necessary for formation of the desired compound, resulting in low yields (25–34%) of **4a** (R = CH_3) due to accompanying side reactions. Moreover, we found that **4a'** (R = CH_3), the methylated bicyclic system derived from the LMR, formed in even lower yields using this reagent. These problems were circumvented by the alternate sequence shown in Scheme I (method B) that allowed us to prepare these and related ring-substituted analogues in good yields.

Early attempts to acylate the piperidine nitrogen of **2a** without accompanying acylation or dehydration of the amide functionality were unsuccessful. These reactions, using acetyl chloride under a variety of conditions, produced multicomponent mixtures with evidence of nitrile formation. However, the use of alkyl or aryl acid anhydrides, neat or in ethyl acetate or DMF, produced the desired piperidyl amides **3** in high yields. Subsequent treatment of **3** with KOH in Me_2SO or DMF efficiently gave the unsaturated cyclized products **4**. Thus, this method provided a means of producing both ring-methylated compounds, **4a** (R = CH_3) and **4a'** (R = CH_3), in good overall yield as well as the ethyl, **4a** (R = C_2H_5), and phenyl, **4a** (R = C_6H_5), analogues.

Catalytic hydrogenation was an acceptable method of saturating the double bond of the bicyclic system when no substituent was present on the olefinic carbon of the ring. However, these molecules were resistant to hydrogenation when this position was occupied by an alkyl or phenyl group. In these cases LAH in THF reduced the double bond with no loss of carbonyl during this reaction. This reduction gave a single epimer at the carbon bridging the two nitrogens of **5** as indicated from ^1H and ^{13}C NMR spectra. Moreover, **7**, the opposite epimer of **5a** (R = CH_3), was produced when **4a** (R = CH_3) was treated with methylmagnesium chloride in the presence of cuprous iodide (Scheme II). This conjugate-addition reaction apparently proceeded from the least hindered side of the molecule, as did chemical hydrogenation, providing the opposite epimers.

Stereochemistry

A single-crystal X-ray study of **4a** (R = CH_3), derived from the HMR, indicated that the phenyl group and the methine proton at the ring juncture are on the same side of the bicyclic system. This study established **4a** (R = CH_3) and related analogues, as well as **5a** and the carboxamidopiperidine **2a** from which these bicyclic structures are derived, as the *R*,*R*:*S*,*S* pair. In the ball and stick drawing generated from the X-ray data (Figure 1[16]), the least hindered side of the molecule appears to be that

(14) Yonan, P. K.; Novotney, R. L.; Woo, C, M.; Prodan, K. A.; Hershenson, F. M. *J. Med. Chem.* **1980**, *23*, 1102 and references therein.
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(16) Single-crystal x-ray analysis was obtained from Dr. Oren Anderson, Colorado State University, Fort Collins, CO, using a Nicolet R3m-E Diffractometer; data are included with the supplementary material.

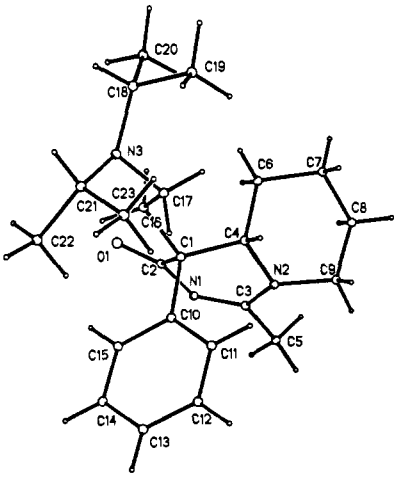

Figure 1. Structure of compound 4a (R = CH_3) as determined by X-ray analysis.

opposite the bulky (diisopropylamino)ethyl appendage. Since chemical hydrogenation of **4a** (R = CH_3) and conjugate addition of methyl Grignard to **4a** (R = H) gave opposite epimers (vide supra), both compounds apparently result from attack from the side opposite that of the bulkier appendage. Thus, racemate **7** would possess the relative stereochemistry shown in Scheme II, and **5a** (R = CH_3) would have the opposite configuration of the methyl group.

Support for the assignment of these epimers came from the proton NMR spectra of the saturated series.[17] In **5a** (R = H) only one of the methylene protons on the carbon between the two nitrogens is coupled to the lactam hydrogen. As expected, this coupling vanished upon D_2O exchange. This phenomenon is an example of the well-documented dependence of proton–proton coupling on the dihedral angle of the atoms involved.[18] The pseudoaxial proton forms an angle of about 90° with the lactam hydrogen and thus shows no vicinal coupling. The pseudoequatorial proton and the lactam hydrogen have a dihedral angle of about 0°, and thus vicinal coupling is observed. In each of the compounds where this saturated system was generated through chemical reduction, i.e., **5a** with R = CH_3, C_2H_5, or C_6H_5, the resulting methine proton was not coupled to the lactam hydrogen. Thus, hydrogenation from the less hindered face resulted in the pseudoaxial proton. In contrast, the corresponding methine proton in **7** did show coupling to this lactam proton, indicating a pseudoequatorial methine proton. Furthermore, the chemical shift of this methine proton in **7** was downfield from that of the corresponding methine proton of its epimer **5a** (R = CH_3) by ca. 0.7 ppm, also lending support to its pseudoequatorial configuration in **7**.[19]

Biology

All bicyclic compounds were evaluated for antiarrhythmic activity; most were evaluated for their binding affinity to the muscarinic cholinergic receptor. Hemodynamic activity (effects on blood pressure and myocardial contractile force) and muscarinic receptor blocking activity were assessed for compounds with good antiarrhythmic activity, an absence of observable behavioral effects during antiarrhythmic testing, and low affinity for the muscarinic receptor.

Antiarrhythmic activity was determined by using the unanesthetized 24-h Harris dog model.[20] A two-stage ligation of the left anterior descending coronary artery was performed on an anesthetized animal approximately 24 h before a compound was tested. Compounds were tested in animals where the following criteria were met: (1) abnormal ventricular (ectopic) rate of at least 130 beats/min; (2) ectopic complexes forming at least 75% of the total heart rate; (3) the total heart rate in each of five control readings varying by no more than 10% of the average of the five readings.

Compounds were administered intravenously as hydrochloride salt solutions using one of two dose regimens. Initially a 5-mg/kg dose was injected into two animals, and if the compound reduced the ventricular ectopic rate by ≥25% for a minimum duration of 10 min, the compound was rated active and tested in a lower dose regimen. If the compound was not active at the 5-mg/kg dose, additional 5-mg/kg injections were administered at 15-min intervals until activity was observed or a maximum dose of 20 mg/kg was given. Compounds active at the initial 5-mg/kg dose were tested at 1-mg/kg doses at 5-min intervals until activity was observed or a maximum dose of 6 mg/kg was administered, with the same criteria as described above necessary for an active rating.

Displacement of the muscarinic radioligand [^{3}H]-quinuclidinylbenzilate (QNB) in a rat brain homogenate was used to assess the muscarinic receptor binding affinity of the bicyclic compounds.[21] The muscarinic receptor binding affinity of disopyramide was the standard against which the activities of test compounds were compared. Relative affinities were determined by calculating the following ratio: IC_{50}(test compound)/IC_{50}(disopyramide).

The dose of compound required to decrease mean arterial blood pressure 50% was compared to the mean antiarrhythmic dose in order to assess general cardiovascular safety. If the mean antiarrhythmic dose was greater than 5 mg/kg, compounds were administered intravenously to anesthetized dogs in consecutive doses of 5 mg/kg for 5 min every 15 min until mean arterial blood pressure was depressed 50% or up to a maximum cumulative dose of 50 mg/kg. If the mean antiarrhythmic dose was 5 mg/kg or less, a compound was administered in consecutive doses of 1 mg/kg per min every 5 min until the depressor end point was reached or a maximum cumulative dose of 10 mg/kg was administered.

Negative inotropic activity was assessed by using the closed-chest anesthetized dog. Compounds were administered intravenously as hydrochloride salt solutions at the previously determined average effective antiarrhythmic dose. Left ventricular pressure was determined with a single-pressure sensor, transducer-tipped catheter that was inserted retrograde through the left common carotid artery into the left ventricle. The index of contractility was the maximum rate of rise of the left ventricular pressure (maximum dp/dt). These results as determined in five dogs were expressed as a percentage change from the average of the base-line readings. Compounds that depressed myocardial contractility ≤25% were arbitrarily considered

(17) The proposed argument for the assignment of the epimers of **5a** assumes that the diazacyclohexane ring is in a chair conformation. The single-crystal X-ray structure of **5a** (R = H) provided to us by Dr. J. Brown, Latticeworks, Inc., Crawford, NJ, confirmed this assumption.

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Table I. Antiarrhythmic Activity and Muscarinic Binding Affinity of Substituted Hexahydro- and Tetrahydro-3*H*-pyrido[1,2-*c*]pyrimidin-3-ones

no.	R	med, iv, mg/kg (dogs)[c]	musc recept[b] aff (disopyr = 1)
4a	H	10 (2)	1
4a′	H	20 (2)	$<^1/_{20}$
4b	H	15 (2)	$^1/_{80}$
4c	H	2.5 (2)	$^1/_{50}$
4a	CH_3	9 (6)	$^1/_5$
4a′	CH_3	10 (3)	$^1/_{16}$
4a	C_2H_5	12.5 (2)	$^1/_{16}$
4a	C_6H_5	1 (2)	$^1/_4$
5a	H	6 (5)	$^1/_{13}$
5a′	H	20 (2)	NT[c]
5b	H	7.5 (2)	$<^1/_{100}$
5c	H	5[d] (2)	$^1/_{16}$
5a	CH_3	2.5 (5)	$<^1/_{20}$
5a	C_2H_5	6 (5)	$^1/_{11}$
5a	C_6H_5	4 (4)	$^1/_{23}$
6		7.5 (2)	NT
7		10 (2)	$^1/_{23}$
1a (disopyramide)		10 (5)	1

[a] Mean effective dose required to suppress ventricular ectopic rate ≥25% for a minimum duration of 10 min. [b] Ratio of muscarinic receptor binding affinity (IC_{50}) of test compound to that of disopyramide (=1). IC_{50}s were determined by log-probit analysis after measuring the displacement of [^{3}H]QNB by test compound at three to six different concentrations in triplicate.[21] [c] Not tested. [d] Lethal in second animal.

to possess an acceptable degree of negative inotropic action.

Compounds of highest interest were tested for muscarinic receptor blocking activity against acetylcholine-induced contractions of the isolated guinea pig ileum. Concentration–effect curves for the activity of acetylcholine were generated in the absence and presence of compounds tested. Data were analyzed by comparing pA_2 values according to the method of Van Rossum.[22] Nonparallel shifts in the concentration–effect curve indicated noncompetitive antagonism. In this case, the negative log value was calculated from the concentration of test compound required to reduce maximum agonist response by 50%. Antimuscarinic activity of test compounds was compared with that of disopyramide.

Results and Discussion

All compounds in this diaza bicyclic series, with or without unsaturation, possessed antiarrhythmic activity in the Harris dog assay (Table I). However, our primary goal was to identify an antiarrhythmic agent with negligible myocardial depressant (decreased inotropic and dromotropic) activity in the antiarrhythmic dose range and minimal antimuscarinic activity. In addition, we wanted a compound with little central nervous system (CNS) activity. Changes in the behavior (e.g., vocalization, excessive limb movement) of conscious dogs during antiarrhythmia testing have been seen consistently with compounds such as aprindine and mexilitine (unreported observations) which have been reported to show CNS activity in humans.[3] Thus, the behavior of animals during antiarrhythmic testing was closely scrutinized for indications of potential CNS activity of test compounds.

Compounds that markedly depressed myocardial conduction (negative dromotropic activity), **4a** (R = C_2H_5) and **4a** (R = C_6H_5), or caused behavioral signs of CNS activity, **4a′** (R = CH_3) and **7**, or death, **5a** (R = H), were eliminated from further development. Since there was no potency advantage associated with diastereomers derived from the LMR, which was produced to a much lesser degree during the hydrogenation, we also disregarded **4a′** (R = H) and **5a′** (R = H) from further testing. One compound, **4a** (R = H), was eliminated because its muscarinic binding affinity and antiarrhythmic potency were similar to that of disopyramide. Both *o*-chlorophenyl compounds **4b** (R = H) and **5b** (R = H) caused emesis in dogs. Since this side effect was not observed with other compounds in this series, these analogues were also rejected. The *N*-methyl compound **6** appeared to be approximately equal in antiarrhythmic potency to its unsubstituted precursor **5a** (R = H), and the latter compound was chosen for further evaluation.

Thus, of the six compounds that appeared to possess minimal side effects in the Harris dog model, four (excluding **5a** (R = C_2H_5, C_6H_5)) were chosen for hemodynamic studies in the anesthetized dog. These included two unsaturated compounds, **4a** (R = CH_3) and **4c** (R = H), and two of the saturated analogues, **5a** (R = H, CH_3). The effects of these compounds on mean arterial blood pressure are shown in Table II. Compounds **4c** (R = H) and **5a** (R = H) possessed unacceptable hypotensive activity when compared to both **4a** (R = CH_3) and **5a** (R = CH_3). Negative inotropic activity of these latter two compounds was assessed in the anesthetized dog. The results (Table II) indicated that **4a** (R = CH_3) and **5a** (R = CH_3) administered intravenously at their mean antiarrhythmic dose decreased myocardial contractility insignificantly ($p > 0.05$, unpaired Student's t-test). In contrast, disopyramide, at its mean antiarrhythmic dose, decreased myocardial contractility by 56%.[23] Thus, both test compounds appeared to possess acceptable hemodynamic profiles in the dog.

To quantitate the potential anticholinergic activity of **4a** (R = CH_3) and **5a** (R = CH_3), both agents were evaluated for their ability to antagonize the contractile activity of acetylcholine on guinea pig ileum segments. These results, along with those of disopyramide, are shown in Table III. Compound **4a** (R = CH_3) had a pA_2 lower than disopyramide and possessed only about 7% of the anticholinergic activity of disopyramide. The saturated compound **5a** (R = CH_3) exhibited noncompetitive antagonism of acetylcholine-induced contractions, indicated by nonparallel shifts in the cumulative concentration–effect curves. In this case, the negative log of the concentration required to produce a 50% decrease in the efficacy of acetylcholine is reported. The noncompetitive property of the latter compound led us to favor **4a** (R = CH_3) as a more acceptable compound to develop. It is now undergoing clinical evaluation.

Experimental Section

Melting points were determined in a Thomas Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a Varian FT-80 spectrometer, and ^{13}C NMR spectra were obtained on a Varian XL-100 spectrometer in $CDCl_3$. UV and IR spectra were consistent with the structures described, and all spectra were recorded by A. J. Damascus. Elemental analyses were determined by E. Zielinski of G. D. Searle & Co., Skokie, IL, and are within ±0.4% of the calculated values, unless otherwise noted. Hydrogenations were carried out by M. Scaros.

(±)-α-[2-[Bis(1-methylethyl)amino]ethyl]-α-phenyl-2-pyridineacetamide (1a), **(±)-α-[2-[bis(1-methylethyl)-amino]ethyl]-α-(2-chlorophenyl)-2-pyridineacetamide** (1b), **and (±)-α-[2-[bis(1-methylethyl)amino]ethyl]-α-(4-phenyl-**

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(23) Stickney, J. L.; Frederick, L. G.; Stamm, M. H. *Fed. Proc.* **1983**, *42*, 634.

Table II. Hemodynamic Effects of Selected Diaza Bicyclic Compounds

compd	dec in mean arterial pressure (MAP), % (no. of dogs; max dose admin, mg/kg)[a]	MED,[b] mg/kg	dec in myocardial contractility, % (no. of dogs; dose, mg/kg)[c]
4a (R = CH_3)	3 ± 3[d] (5; 50)[e]	9.1	14 ± 6[d] (5; 10)
4c (R = H)	27 ± 8 (2; 10)[f]	2.5	
5a (R = H)	54 ± 13[g] (5; 35)[e]	6.0	
5a (R = CH_3)	4 ± 3 (5; 10)[f]	2.5	21 ± 4 (5; 10)
disopyramide	57 ± 3[g] (4; 29)[e]	10.0	56 ± 2 (5; 10)[23]

[a] Maximum dose was 50 mg/kg for compounds with a mean antiarrhythmic dose (MED) of 5 mg/kg or greater and 10 mg/kg for compounds with MED of <5 mg/kg or that dose to decrease MAP by about 50%. [b] See Table I, footnote *a*. [c] Myocardial contractility determined by monitoring the maximum rate of rise of left ventricular pressure. [d] Mean percent decrease ± standard error of the mean. [e] Compound administered at 5 mg/kg bolus doses. [f] Compound administered at 1 mg/kg bolus doses. [g] Significant response, $p < 0.05$ (unpaired Student's t-test).

Table III. Antimuscarinic Activity of 1-Methyl-Substituted Pyrido[1,2-*c*]pyrimidinones **4a** and **5a** and Disopyramide in the Isolated Guinea Pig Ileum Assay

compd	pA_2[a,b]	compd	pA_2[a,b]
4a (R = CH_3)	4.88 ± 0.05	**5a** (R = CH_3)	4.22[c]
disopyramide	6.05 ± 0.03		

[a] Calculated by the method of Van Rossum.[22] [b] Values and standard error of the mean were determined from at least four separate experiments using at least three different concentrations of antagonist. [c] Compound exhibited noncompetitive antagonism. Value given is the negative log of the concentration of the test compound to inhibit the maximum agonist response by 50%.

Table IV. Physical Data of 4,4-Disubstituted Hexahydro-3*H*-pyrido[1,2-*c*]pyrimidin-3-ones and Related Compounds

compd (R)	method[a]	yield, %	mp, °C (solv)[b]	formula	% anal.[c]
1b	*a*	43	93–95 (F)	$C_{21}H_{28}N_3OCl$	CHNCl
1c	*a*	44	116–120 (F)	$C_{27}H_{33}N_3O$	CHN
2a	*a*	59	108–109.5 (F)	$C_{21}H_{35}N_3O$	CHN
2a′	*a*	19	93–95.5 (F)	$C_{21}H_{35}N_3O$	CHN
2b	*a*	36	152–154 (B)	$C_{21}H_{35}N_3OCl$	CHNCl
2c	*a*	45	158–159 (A)	$C_{27}H_{34}N_3O$	CHN
4a (H)	A	84	224–226 (E)	$C_{22}H_{33}N_3O$	CHN
4a′ (H)	A	69	155–158 (B)	$C_{22}H_{33}N_3O$	CHN
4a (CH_3)	A, B	29, 68	201–202 (B)	$C_{23}H_{35}N_3O$	CHN
4a′ (CH_3)	B	75	177–180 (A)	$C_{23}H_{35}N_3O$	CHN
4a (C_2H_5)	B	57	145–147 (B)	$C_{24}H_{37}N_3O$	CHN
4a (C_6H_5)	B	55	220–222 (A)	$C_{28}H_{37}N_3O$	CHN
4b (H)	A	79	129–149 (B)	$C_{22}H_{32}N_3OCl$	CHNCl
4c (H)	A	49	257–258 (C)	$C_{28}H_{37}N_3O$	CHN
5a (H)	C	70	208–209 (D)	$C_{22}H_{35}N_3O$	CHN
5a′ (H)	C	72	164–166 (B)	$C_{22}H_{35}N_3O$	CHN
5a (CH_3)	D	86	70–75 (E)	$C_{23}H_{37}N_3O$	CHN
5a (C_2H_5)	D	79	113–127 (F)	$C_{24}H_{39}N_3O$	CHN
5a (C_6H_5)	D	82	131.5–134 (A)	$C_{28}H_{39}N_3O$	CHN
5b (H)	D	65	234–237 (B)	$C_{22}H_{34}N_3OCl$	CHNCl
5c (H)	D	50	87–96 (E)	$C_{28}H_{39}N_3O$	C[d]HN
6	*a*	41	oil	$C_{23}H_{37}N_3O$	CHN[e]
7	*a*	40	142–145 (F)	$C_{23}H_{37}N_3O$	CHN

[a] See the Experimental Section. [b] Recrystallization solvent: A = Et_2O/Skellysolve-B; B = Et_2O; C = EtOAc; D = MeOH; E = none; F = Skellysolve-B. [c] Elemental analyses are within ± 0.4% of the calculated values unless otherwise noted. [d] Calcd, 77.56. Found, 77.11. [e] Calcd, 11.31. Found, 10.80.

phenyl)-2-pyridineacetamide (1c) were prepared by previously reported methods.[14,15]

(±)-α-(*R)-[2-[Bis(1-methylethyl)amino]ethyl]-α-phenyl-2(*R*)-piperidineacetamide (2a, High-Melting Racemate; 2a′, Low-Melting Racemate).** A solution of **1a** (200 g, 0.59 mol) in H_2SO_4 (37.5 mL) and water (360 mL) containing 5% PtO_2/C (20 g) was shaken in a Parr apparatus under a hydrogen atmosphere at 60 psi, for 4 h at 75 °C. The catalyst was removed by filtration, and the filtrate was added to a cold 1 N NaOH solution (1.5 L). The mixture was extracted with toluene (1.1 L) and the toluene solution was washed with H_2O (4 × 300 mL) and dried (anhydrous Na_2SO_4). The organic solution was filtered and evaporated, leaving 218 g of colorless damp solid, which was recrystallized from Skellysolve-B (500 mL) to give 125 g (59%) of **2a**, the high-melting racemate: ^1H NMR ($CDCl_3$) δ 7.18 (5 H, C_6H_5, s), 4.70 (1 H, CH, dd, J = 8 Hz), 1.20 (1 H, CH, t, J = 8 Hz), 0.78 (12 H, 4-CH_3, m);[24] ^{13}C NMR ($CDCl_3$) δ 63.78 (CH, d), 42.34 (CH_2, t), 39.89 (CH_2, t).

The filtrate was concentrated and the residue chromatographed on silica gel, eluting with CH_2Cl_2/MeOH/NH_4OH (85:14:1) to give 40.3 g (19%) of **2a′**, the low-melting racemate: ^1H NMR ($CDCl_3$) δ 7.25 (5 H, C_6H_5, m), 1.05 and 0.98 (12 H, 4-CH_3, d); ^{13}C NMR ($CDCl_3$) δ 61.45 (CH, d), 47.11 (CH_2, t), 41.10 (CH_2, t).

(±)-α-(*R)-[2-[Bis(1-methylethyl)amino]ethyl]-α-(2-chlorophenyl)-2(*R**)-piperidineacetamide (2b).** A solution of **1b** (28.5 g, 0.0762 mol) and PtO_2 (2.8 g) in HOAc (225 mL) was hydrogenated as described above to give 26.7 g of colorless solid. Recrystallization from Et_2O (2×) gave 10.3 g (36%) of **2b**.

(±)-α-(*R)-[2-[Bis(1-methylethyl)amino]ethyl]-α-(4-phenylphenyl)-2(*R**)-piperidineacetamide (2c).** A solution of **1c** (17.5 g, 0.0421 mol) and PtO_2 (1.75 g) in HOAc (200 mL) was hydrogenated as described above to give 8 g (45%) of **2c** after recrystallization from ether/Skellysolve-B.

(±)-*trans*-4-[2-[Bis(1-methylethyl)amino]ethyl]-4,4a,5,6,7,8-hexahydro-1-methyl-4-phenyl-3*H*-pyrido[1,2-c]-pyrimidin-3-one (4a, R = CH_3). Method A. A solution of **2a** (20 g, 0.058 mole in dimethylacetamide dimethyl acetal (22 g, 0.018 mol) was heated at 80 °C for 12.5 h. The reaction mixture was diluted with Et_2O, and the resulting precipitate was collected and washed with Et_2O to afford 5.6 g (26%) of **4a** (R = CH_3). An additional 0.7 g (29% total) of product was obtained after chromatography [SiO_2; CH_2Cl_2/MeOH/NH_4OH (85:14:1)].

Method B. A mixture of **2a** (R = CH_3; 10 g, 0.029 mole and Ac_2O (23 mL, 0.25 mol) in CH_2Cl_2 (100 mL) was stirred at room temperature for 20 h. The reaction mixture which had solidified was diluted with CH_2Cl_2 (30 mL) and poured into water (140 mL) containing concentrated NH_4OH solution (35 mL). After the mixture was stirred at room temperature for 1 h, the immiscible layers were separated and the aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL). The combined organic extracts were washed with saturated brine, dried (anhydrous $MgSO_4$), filtered, and concentrated to a thick oil: NMR ($CDCl_3$) δ [$(CH_3)_4Si$] 2.20 (CH_3).

The oil was taken up in Me_2SO (35 mL), and powdered KOH (1.9 g) was added with stirring. After 2 h, the reaction mixture was diluted with CH_2Cl_2 (35 mL) and poured into water (200 mL). The immiscible layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (4 × 35 mL) and dried (anhydrous Na_2SO_4). The combined organic extracts were evaporated, and the resulting residue was dissolved in PhMe (40 mL) on a steam bath. Hexane (40 mL) was added to the hot solution, and a solid immediately formed. After cooling, the solid was collected by filtration to afford **4a** (R = CH_3), 7.2 g (68%).

(±)-*trans*-4-[2-[Bis(1-methylethyl)amino]ethyl]octahydro-4-phenyl-3*H*-pyrido[1,2-c]pyrimidin-3-one (5a, R = H). Method C. A solution of **4a** (R = H) (1.0 g, 0.028 mol in 95% EtOH (70 mL) containing 5% Pd–C (0.3 g) was shaken under a hydrogen atmosphere at room temperature for 26 h. The catalyst was removed by filtration, and the filtrate was concen-

(24) ^1H NMR data on all new compounds has been included with the supplementary material.

trated to dryness. The solid residue was recrystallized from MeOH to give 0.71 g (70%) of **5a** (R = H): ^{1}H NMR ($CDCl_3$) δ 8.15 (1 H, NH, d, J = 4 Hz), 7.25 (5 H, C_6H_5, s), 4.00 (1 H, NCH*H*NH, dd, J = 4 Hz, J' = 8 Hz), 3.60 (1 H, NC*H*HNH, d, J' = 8 Hz), 1.13–1.00 (12 H, 4-CH_3, m).

(±)-*trans*-4-[2-[Bis(1-methylethyl)amino]ethyl]octahydro-1-methyl-4-phenyl-3*H*-pyrido[1,2-c]pyrimidin-3-one (5a, R = CH_3). Method D. To a suspension of $LiAlH_4$ (0.57 g, 0.014 mol) in anhydrous THF (40 mL) was added a solution of 4a (R = CH_3) (2.8 g, 0.0076 mol) in THF (15 mL) dropwise. After stirring at room temperature for 2 h, the mixture was cooled in an ice bath and the excess hydride decomposed by the sequential addition of water (1.2 mL), 15% NaOH solution (1.2 mL), and water (3 mL). After the mixture was stirred for 20 min, the precipitate was removed by filtration through a mixture of Celite and $MgSO_4$, and the filter cake was washed with THF. The filtrate was concentrated to dryness, affording 2.4 g (86%) of pure **5a** (R = CH_3): ^{1}H NMR ($CDCl_3$) δ 7.25 (5 H, C_6H_5, s), 6.93 (1 H, NH, s), 3.78 (1 H, NC*H*CH_3, q, J = 6 Hz), 1.31 (3 H, NCHC*H*$_3$, d, J = 6 Hz), 1.10–100 (12 H, 4-CH_3, m). ^{1}H NMR ($CDCl_3$) δ: for **5a** (R = C_2H_5) 3.75 (1 H, NC*H*CH_2, dd, J = 2 Hz); for **5a** (R = C_6H_5) 4.60 (1 H, NC*H*C_6H_5, s).

(±)-*trans*-4-[2-[Bis(1-methylethyl)amino]ethyl]octahydro-2-methyl-4-phenyl-3*H*-pyrido[1,2-c]pyrimidin-3-one (6). A solution of **5a** (R = H) (1.63 g, 4.6 mmol) in dry DMF (5 mL) was added dropwise to a stirred suspension of NaH (0.26 g, 5.7 mmol of 50% suspension in mineral oil, washed with Skellysolve-B and decanted) in dry DMF (25 mL) at room temperature. After gas evolution had ceased (~10 min), MeI (0.97 g, 6.8 mmol) was added dropwise to the reaction mixture and stirring continued for 40 min. Water (0.2 mL) was added and the mixture partitioned between H_2O and Et_2O/EtOAc (1:1). The aqueous phase was washed with additional EtOAc/Et_2O , and the combined organic solutions were extracted with 1 N HCl. The acidic solution was neutralized with NaOH and extracted (3×) with Et_2O, and the combined extracts were dried (anhydrous Na_2SO_4) and filtered. Solvent removal afforded 1.5 g of oil, which was chromatographed on silica gel [CH_2Cl_2/MeOH/NH_4OH (85:14:1)] to give 0.7 g of oil, which was taken up in Skellysolve-B, filtered, and concentrated to afford **6**, 0.66 g (41%), as a colorless oil.

(±)-4β-[2-[Bis(1-methylethyl)amino]ethyl]octahydro-(1*R*)-1α-methyl-4-phenyl-3*H*-4aα-pyrido[1,2-c]pyrimidin-3-one (7). To a cooled (CO_2(s)/CCl_4) mixture of anhydrous Cu_2I_2 (2.3 g, 12 mmol) in dry THF (20 mL) was added MeMgI (8.3 mL of 2.9 M in Et_2O, 24 mmol) dropwise with rapid stirring. A solution of 4a (R = H, 2.8 g, 8.0 mmol) in THF (60 mL) was then added dropwise to the reaction mixture, which was stirred for 1 h. After warming to room temperature for 1 h, the reaction mixture was cooled to 0 °C and acidified to pH 3 with 1 N HCl solution. The mixture was stirred for 0.5 h and brought to pH 12 with 2 N KOH. The organic phase was separated and the aqueous phase washed with Et_2O. The combined extracts were washed with brine and dried (anhydrous Na_2SO_4), filtered, and concentrated to dryness to give 3.1 g of yellow solid, mp 67–73 °C, as a mixture of **5a** (R = CH_3) and **7**. The solid was dissolved in CH_2Cl_2 and washed with 10% NH_4OH solution until the blue color in the aqueous phase was absent. The organic solution was dried (anhydrous Na_2SO_4) and concentrated to afford 1.2 g (40%) of pure crystalline **7**: ^{1}H NMR ($CDCl_3$) δ 7.35–7.18 (5 H, C_6H_5, m), 4.45 (1 H, C*H*CH_3, m [collapses to q, J = 6 Hz upon D_2O exchange], 1.10 (3 H, CHC*H*$_3$, d, J = 6 Hz), 0.95–0.85 (12 H, 4-CH_3, m).

Pharmacological Methods. Antiarrhythmic activity was determined for all compounds by using random-source beagle dogs of either sex. A two-stage ligation of the left anterior descending coronary artery was performed in animals anesthetized with pentobarbital sodium (30 mg/kg, intravenously) following the method of Harris.[20] Compounds were tested in the conscious animal approximately 24 h after surgery. Each compound was administered intravenously to at least two dogs. (Protocol was given above.)

Muscarinic receptor binding activity was determined for 15 of the 17 compounds. Whole rat brain (male Charles River CD rats) minus cerebellum was homogenized and centrifuged at 1000*g*. The resulting supernatant was incubated with 0.15 nM [^{3}H]quinuclidinylbenzilate ([^{3}H]QNB) in the absence and presence of standard (atropine, disopyramide) or test compound at 25 °C for 1 h. Activity was measured as the concentration of compound inhibiting [^{3}H]QNB binding by 50% (IC_{50}).[21]

Hemodynamic activity of selected compounds was assessed in either mongrel dogs or random-source beagle dogs of either sex anesthetized with pentobarbital sodium (30 mg/kg intravenously). The animals were allowed to stabilize for 30 min after the completion of surgery. A supplementary dose of pentobarbital (5 mg/kg) was administered intravenously after the first 15 min of the stabilization period. Rectal temperature was monitored with a Telethermometer and maintained at 38 °C with an electric heating pad.

In blood pressure depression studies, arterial blood pressure was recorded continuously with a fluid-filled catheter inserted into the right femoral artery and attached to a miniature pressure transducer. Mean arterial blood pressure was obtained by electronic averaging. The right femoral vein was catheterized for compound administration. A lead II electrocardiogram was recorded continuously. Data were analyzed at 5-min intervals. The base-line value in each experiment was the average of four readings taken at 5-min intervals beginning at the end of the stabilization period. (Protocol for compound administration was given above.)

The rate of development of left ventricular pressure was assessed via a single-pressure sensor, transducer-tipped catheter inserted retrograde through the left common carotid artery into the left ventricle. The maximum rate of development of left ventricular pressure was used as the index of myocardial contractility in these studies. Compounds were administered via a catheter in the right femoral vein. At the end of the stabilization period, five control readings were obtained at 1-min intervals 5 min before a compound was administered. Data were analyzed at 1-min intervals during compound administration and at 5-min intervals during the first hour after compound administration. The maximum negative inotropic response occurred during the last 5–10 min of compound administration. (Protocol for compound administration was given above.)

Antimuscarinic (anticholinergic) activity was quantitated for selected compounds. A 30-cm length of ileum (proximal to the ileocecal junction) was removed from an unconscious guinea pig, flushed with modified Tyrode's solution, and placed in a flask containing modified Tyrode's solution gassed with 95% O_2 and 5% CO_2, maintaining a pH of 7.4. Composition of modified Tyrode's solution (g/L): NaCl, 160.92; KCl, 4.00; $CaCl_2 \cdot 2H_2O$, 2.64; $MgCl_2 \cdot 6H_2O$, 4.26; $Na_2HPO_4 \cdot H_2O$, 2.30. Compounds were tested using 2.0-cm segments mounted in a bath of Tyrode's solution maintained at 30 °C and gassed as above. An initial tension of 0.5 g was placed on each segment. The tissue was allowed to equilibrate for 30 min with two bath changes at 10-min intervals.

A cumulative concentration–effect curve for the contractile activity of acetylcholine was generated. The tissue was rinsed for 10 min, with a change of solution at 5 min. At the end of 10 min, tension had decreased to base-line values. A predetermined concentration of standard (disopyramide) or test compound was attained in the bath and a complete cumulative concentration–effect curve for acetylcholine repeated. Each standard or test compound was evaluated with four concentrations at one-fourth log intervals; there were four replications of each concentration. Relative antimuscarinic activity was determined by comparing pA_2 values according to the method of Van Rossum.[22] Analysis of variance was used to statistically analyze the data. The level of significance was $p \leq 0.05$.

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Registry No. 1a, 74427-45-5; **1b**, 91257-11-3; **1c**, 91257-06-6; **2a**, 91256-96-1; **2a'**, 91256-97-2; **2b**, 96914-33-9; **2c**, 96914-34-0; **3a** (R = CH_3), 96914-35-1; **3a** (R = C_2H_5), 96914-36-2; **3a** (R =

C_6H_5), 96914-37-3; **3a'**, 96914-38-4; **4a** (R = H), 91256-98-3; **4a** (R = CH_3), 96914-39-5; **4a** (R = C_2H_5), 96914-41-9; **4a** (R = C_6H_5), 96914-42-0; 4a' (R = H), 91257-00-0; **4a'** (R = CH_3), 96914-40-8; **4b**, 91257-09-9; **4c**, 96914-43-1; **5a** (R = H), 91256-99-4; **5a** (R = CH_3), 96997-16-9; **5a** (R = C_2H_5), 96914-44-2; **5a** (R = C_6H_5), 96914-45-3; **5a'** (R = H), 91257-01-1; **5b**, 96914-46-4; **5c**, 96914-47-5; **6**, 96914-48-6; **7**, 96997-17-0; dimethylacetamide dimethyl acetal, 18871-66-4; dimethylformamide dimethyl acetal, 4637-24-5.

Supplementary Material Available: Tables of ^1H NMR data, atomic coordinates, bond lengths and angles, and temperature factors (8 pages). Ordering information is given on any current masthead page.

Synthesis and Biological Activity of Modified Peptide Inhibitors of Angiotensin-Converting Enzyme[1]

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A series of non-sulfhydryl modified dipeptides related to CI-906, CI-907, and enalapril was prepared in which various isosteric moieties (O, S, SO, SO_2) have been substituted for the amino group and in which the proline residue has been replaced with various hydrophobic amino acids. The compounds were evaluated in vitro for inhibition of angiotensin-converting enzyme and in vivo for antihypertensive activity. Compound **7c**, the most potent member of this series, had an in vitro IC_{50} of 1.4×10^{-8} M and showed modest oral antihypertensive activity at 30 mg/kg in conscious, two kidney, one clip Goldblatt hypertensive rats. Structure–activity relationships are discussed.

Angiotensin-converting enzyme (ACE) is responsible for the conversion of the decapeptide angiotensin I (A-I) to angiotensin II (A-II), a potent vasoconstrictor octapeptide, and for the hydrolysis of the C-terminal dipeptide from the hypotensive nonapeptide bradykinin. These combined effects of ACE result in an overall pressor effect.[2] Inhibition of ACE results in a concomitant antihypertensive effect.[3]

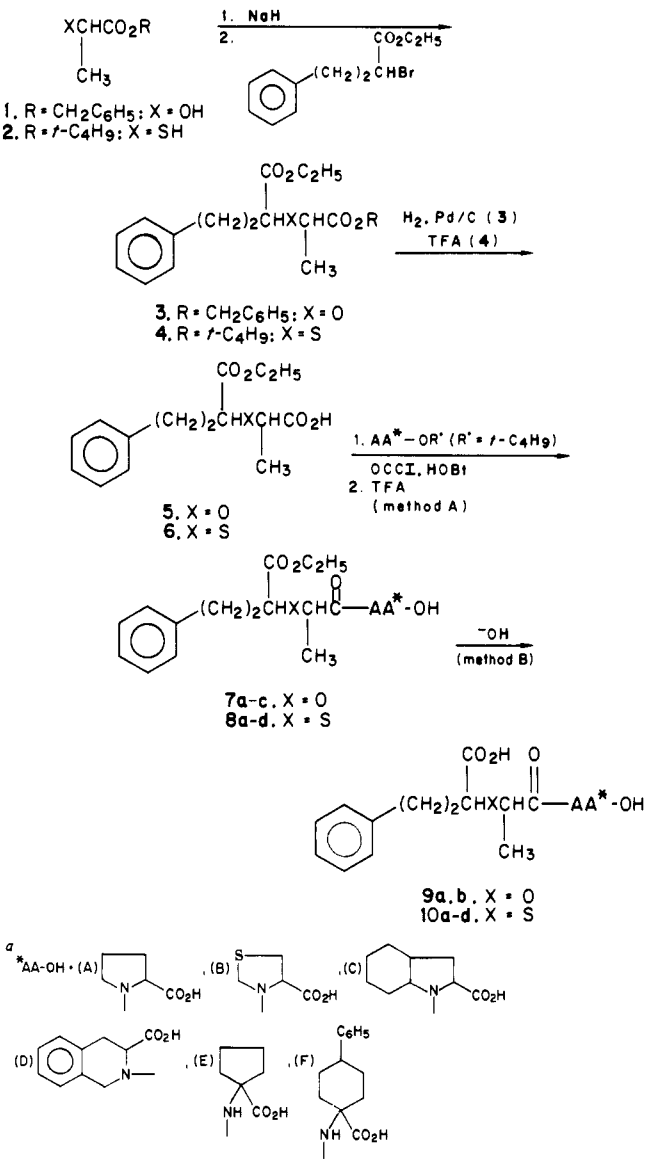
The discovery of 1-[(2*S*)-3-mercapto-2-methyl-1-oxopropyl]-L-proline (captopril), an orally effective ACE inhibitor and clinically effective antihypertensive agent by Ondetti et al.,[4] has generated considerable interest in the development of additional ACE inhibitors. Recently, Patchett et al.[5] reported a series of non-sulfhydryl *N*-carboxymethyl dipeptides that were orally active ACE inhibitors. This resulted in the development of enalapril.

Previous reports from our laboratories[6-9] have described CI-906, [3*S*-[2[*R**(*R**)]],3*R**]-2-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid monohydrochloride, and CI-907, [2*S*-[1[*R**(*R**)]],2*R**,2α,3$\alpha\beta$,7$\alpha\beta$]-1-[2-[[1-(ethoxycarbonyl)-3-phenylpropyl]amino]-1-oxopropyl]octahydro-1*H*-indole-2-carboxylic acid monohydrochloride, two new potent antihypertensive agents.

We now report a novel series of modified peptides related to CI-906, CI-907, and the *N*-carboxymethyl dipeptides in which various isosteric moieties (O, S, SO, SO_2) have been substituted for the amino group and in which the proline residue has been replaced with various hydrophobic amino acids (Table I).

Chemistry. The modified peptides were prepared according to the routes shown in Schemes I and II. Reaction of ethyl α-bromobenzenebutyrate with either phenylmethyl 2-hydroxypropanoate (**1**) or 1,1-dimethylethyl 2-mercaptopropanoate (**2**) in the presence of NaH gave **3** and **4**, respectively. Catalytic hydrogenolysis of **3** or TFA cleavage of **4** resulted in **5** and **6**, which were subsequently coupled with the appropriate amino acid esters (see Scheme I, A-F) in the presence of DCCI (*N*,*N*'-dicyclohexylcarbodiimide) and HOBt (1-hydroxybenzotriazole) to give the diesters. Cleavage of the *tert*-butyl esters afforded **7a–c** and **8a–d**. Subsequent alkaline hydrolysis afforded **9a**, **9b**, and **10a–d**. In the case of compounds containing amino acids E and F, the diesters (Scheme I,

Scheme I



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