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$a\beta$,7$a\beta$]-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

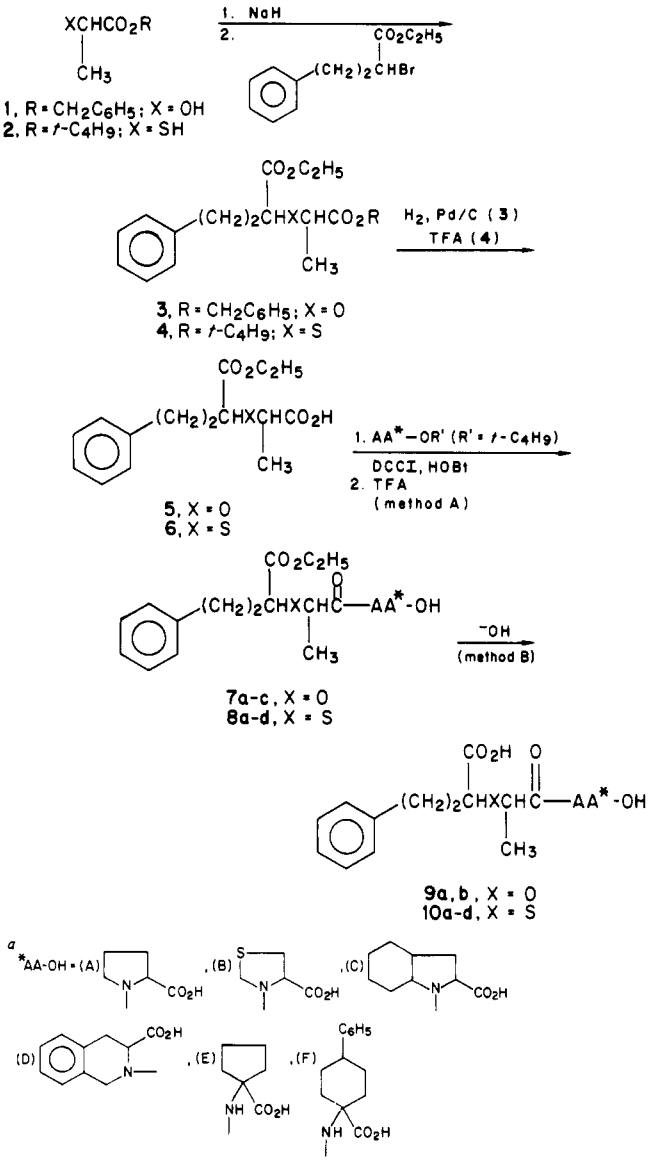

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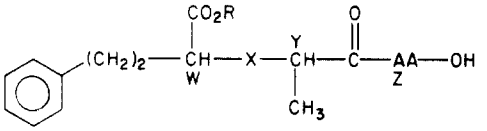
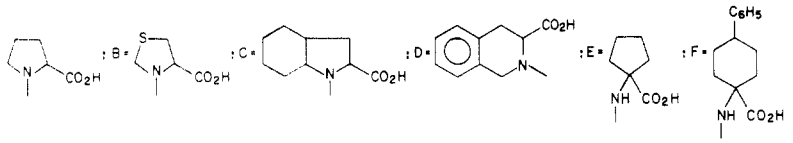
Table I. Chemical and Biological Data of Modified Peptide Inhibitors of Angiotensin-Converting Enzyme



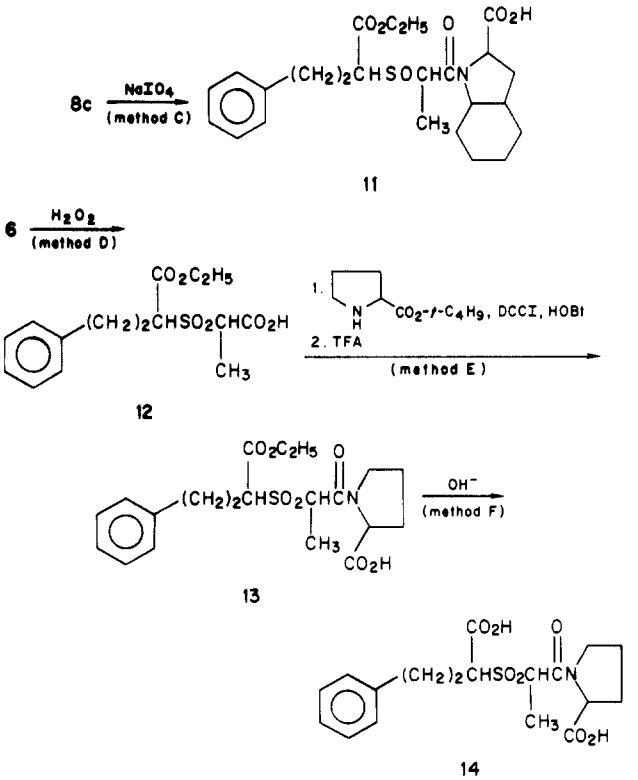
compd	R	X	AA-OH[a]	optical confign at W	Y	Z	method	yield, %	$[a]^{23}_D$, deg (*c*, MeOH)	formula	anal.	ACE IC_{50}[b]
7a	C_2H_5	O	A	*RS*	*S*	*S*	A	50	−67.5 (1.32)	$C_{20}H_{27}NO_6$	C, H, N	2.2×10^{-7}
7b	C_2H_5	O	C	*RS*	*S*	*S*	A	43	−98.6 (0.93)	$C_{24}H_{33}NO_6$	C, H, N	2.4×10^{-8}
7c	C_2H_5	O	D	*RS*	*S*	*S*	A	59	+35.2 (1.03)	$C_{25}H_{29}NO_6 \cdot 0.33H_2O$	C, H, N	1.4×10^{-8}
8a	C_2H_5	S	A	*RS*	*RS*	*S*	A	46	−39.3 (1.24)	$C_{20}H_{27}NO_5S \cdot 0.14CHCl_3$	C, H, N	2.9×10^{-6}
8b	C_2H_5	S	B[c]	*RS*	*RS*	*S*	A	52	−77.7 (0.54)	$C_{19}H_{25}NO_5S_2$	C, H, N	4.8×10^{-6}
8c	C_2H_5	S	C	*RS*	*RS*	*S*	A	21	−36.9 (1.05)	$C_{24}H_{33}NO_5S$	C, H, N	2.0×10^{-6}
8d	C_2H_5	S	D	*RS*	*RS*	*S*	A	26	−0.9 (1.12)	$C_{25}H_{29}NO_5S$	C, H, N	4.6×10^{-7}
9a	H	O	A	*RS*	*S*	*S*	B	49	−61.5 (1.11)	$C_{18}H_{26}NO_6 \cdot H_2O$	C, H, N	1.1×10^{-7}
9b	H	O	D	*RS*	*S*	*S*	B	27	+19.5 (0.50)	$C_{23}H_{25}NO_6$	C, H, N	8.6×10^{-8}
10a	H	S	A	*RS*	*RS*	*S*	B	67	−35.5 (1.07)	$C_{18}H_{23}NO_5S$	C, H, N	5.8×10^{-7}
10b	H	S	C	*RS*	*RS*	*S*	B	57	−21.0 (1.01)	$C_{22}H_{29}NO_5S$	C, H, N	1.4×10^{-7}
10c	H	S	E	*RS*	*RS*		B	83		$C_{19}H_{25}NO_5S$	C, H, N	N[d]
10d	H	S	F	*RS*	*RS*		B	34		$C_{26}H_{31}NO_5S$	C, H, N	N[d]
11	C_2H_5	SO[e]	C	*RS*	*RS*	*S*	C	21	+4.1 (1.08)	$C_{24}H_{33}NO_6S$	C, H, N	2.2×10^{-7}
13	C_2H_5	SO_2	A	*RS*	*RS*	*S*	E	54	−11.0 (0.85)	$C_{20}H_{27}NO_7S$	C, H, N	N[d]
14	H	SO_2	A	*RS*	*RS*	*S*	F	87	−18.9 (0.95)	$C_{18}H_{23}NO_7S$	C, H, N	N[d]
CI-906	C_2H_5	NH	D	*S*	*S*	*S*						8.3×10^{-9}
CI-906[f]	H	NH	D	*S*	*S*	*S*						2.8×10^{-9}
CI-907	C_2H_5	NH	C	*S*	*S*	*S*						1.0×10^{-7}
CI-907[f]	H	NH	C	*S*	*S*	*S*						2.6×10^{-9}
enalapril[g]	C_2H_5	NH	A	*S*	*S*	*S*						1.4×10^{-7}
MK-422[g]	H	NH	A	*S*	*S*	*S*						3.1×10^{-9}
captopril[h]												1.3×10^{-8}

[a] AA-OH: A =  [b] Molar concentration for 50% inhibition. [c] Prepared from imidazolide of **6** and disilyl derivative of 4-thiazolidine-carboxylic acid (Iwase, H.; Takeuchi, Y.; Murai, A. *Chem. Pharm. Bull.* **1979**, *27*, 1307). [d] N, no significant activity at 1.0×10^{-5}. [e] Optical confign at SO unknown. [f] Diacid. [g] Merck Sharpe and Dohme Research Laboratories. [h] Squibb Institute for Medical Research.

R′ = C_2H_5) were hydrolyzed directly to **10c** and **10d**.

Reaction of **8c** with $NaIO_4$ afforded the sulfoxide **11**. Oxidation of **6** with H_2O_2 gave **12**, which was coupled to 1,1-dimethylethyl prolinate in the presence of DCCI and

Scheme II



(1) A preliminary report on this work has appeared: Roark, W. H.; Tinney, F. J.; Cohen, D.; Essenburg, A. D.; Kaplan, H. R., "Abstracts of Papers", 8th American Peptide Symposium, University of Arizona, Tucson, AZ, May 1983; Abstr 6-18.
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HOBt. Cleavage of the *tert*-butyl ester gave the sulfone **13**. Alkaline hydrolysis of **13** afforded the sulfone diacid **14**.

The target modified peptides shown in Table I were obtained as mixtures of diastereomers and tested without separation of the diastereomers.

Results and Discussion

Table I lists the ACE IC_{50} for the modified peptides along with those for CI-906, CI-907, enalapril, and captopril.

Previous studies from our laboratories[6-9] as well as reports[10-15] by others have shown that incorporation of bulky hydrophobic fused-ring amino acids in place of proline leads to potent inhibitors of ACE. Patchett et al.[5] reported that replacement of NH with CH_2 in the *N*-carboxymethyl dipeptides resulted in a marked reduction in inhibitory potency. This suggests that the NH moiety plays an important role in the biological activity of this class of ACE inhibitors. In contrast to this is the recent report of potent in vitro ACE inhibition for a series of 1-glutaryl-2,3-dihydro-1*H*-indole-2-carboxylic acid derivatives.[16] In this series of modified dipeptides, the NH in the side chain of the *N*-carboxymethyl dipeptides was replaced with CH_2 and the proline with the hydrophobic terminal amino acid (S)-2,3-dihydro-1*H*-indole-2-carboxylic acid.

We were interested in determining what the effect of incorporation of various isosteric moieties for the NH in CI-906 and CI-907 would have on the biological activity of these compounds.

In general, the compounds in which oxygen replaced NH were more potent inhibitors in vitro than those containing sulfur or sulfoxide at the same position. Compounds containing sulfone in place of NH did not show any appreciable inhibitory activity. In general, with the exception of **7c** and **9b**, the diacids **9a**, **9b**, and **10a–d** were more potent than the monoacids **7a–c** and **8a–d**. The lower potency of **9b** compared to that of **7c** may be due to enrichment in the less active isomer of **9b** during chromatographic purification. An approximate order of inhibitory potency when other amino acids were substituted for the proline residue was D > C > A > B. The spiro analogues containing amino acids E and F did not show any appreciable activity.

Compounds **7a–c**, **9a**, and **11** were tested orally in the renal hypertensive rat along with CI-906, CI-907, enalapril, and captopril (Table II). Compounds **7b** and **7c** showed modest activity at 30 mg/kg po but exhibited weak or insignificant activity at 3 mg/kg po. At 3 mg/kg po, CI-906, CI-907, or enalapril decreased blood pressure to normotensive levels in renal hypertensive rats.

Table II. Effects of Modified Peptide Inhibitors of Angiotensin-Converting Enzyme on Mean Arterial Pressure in Conscious Renal Hypertensive Rats

compd	% decrease in mean arterial pressure[a] at the following doses, mg/kg po	
	3	30
7a	N[b]	3
7b	N	12
7c	8	18
9a	N	9
11	N	10
enalapril[c]	55	
CI-906	54	
CI-907	46	
captopril[d]	49	

[a] Initial (predose) mean arterial pressures were ~190 mmHg. [b] N, no significant lowering of arterial pressure at this dose. [c] Merck Sharpe and Dohme Research Laboratories. [d] Squibb Institute for Medical Research.

It is interesting to note that **7b** and **7c**, in which one of the optical centers was racemic, had comparable in vitro potency to CI-906, CI-907, and enalapril. One possible explanation for the lack of significant oral activity in the isosteric modified peptides might be decreased bioavailability resulting from poor absorption.

Collectively, this series of modified dipeptides provides further information on the requirements for binding at the active site of ACE.

Experimental Section

Biological Methods. In vitro ACE inhibitory activity was determined in unpurified guinea pig serum by a radioassay procedure reported previously.[17] Activity is reported as the IC_{50}, which is the approximate molar concentration of test compound causing a 50% inhibition of the control converting enzyme activity.

Selected compounds were tested for antihypertensive activity at 3 and 30 mg/kg po in conscious two kidney, one clip Goldblatt hypertensive rats ($N = 3$). This technique has been described previously.[18]

Chemistry. Infrared (IR) data were recorded on a Beckman IR-9 or IR-7 prism grating instrument on a Digilab FTS-14 interferometer. Optical rotations were measured on a Perkin-Elmer 141 polarimeter in a 1-dm tube. Nuclear magnetic resonance measurements (NMR) were made on a Bruker WH-90 pulsed Fourier transform instrument. Homogeneity of the products was determined by ascending thin-layer chromatography (TLC) on silica gel coated glass plates, using principally the following solvent systems: diisopropyl ether; toluene–MeCN–HOAc, 10-9-1. The TLC of the compounds described in Table I was homogeneous when visualized with UV and/or I_2 vapors. The IR and NMR spectra of the compounds were compatible with the structures. Microanalysis for C, H, and N gave results within 0.4% of theory unless otherwise indicated.

Ethyl α-[1-Methyl-2-oxo-2-(phenylmethoxy)ethoxy]-benzenebutanoate (3) (L-Form of Alanine Analogue; Remaining Center (±)). Sodium hydride (12.5 g of a 60% dispersion in mineral oil, 0.31 mol) was added portionwise to a stirred solution of phenylmethyl (*S*)-2-hydroxypropanoate (**1**; 61.7 g, 0.34 mol) in 500 mL of THF at 0 °C. The mixture was stirred for 0.5 h at 0 °C and (±)-ethyl α-bromobenzenebutanoate (92.9 g, 0.34 mol) was added with stirring to the previous mixture and the reaction was stirred at room temperature for 3 h. Water was carefully added to consume any excess NaH. The solution was

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dried over $MgSO_4$, filtered, and concentrated to a yellow liquid. The yellow liquid was chromatographed over silica gel and eluted with $CHCl_3$ to give 82.6 g of an oil. The oil was distilled and the material distilling at 178–205 °C (0.31 mm) was collected and chromatographed three times on silica gel and eluted with 50:30:10 hexane–CH_2Cl_2–diisopropyl ether. Combination of the appropriate fractions yielded 7.51 g (6%) of **3** as a light yellow oil: $[\alpha]^{23}_D$ −28.6° (*c* 0.91, CH_3OH); IR (neat, NaCl) 2990, 1751, 1139, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.29 (s, 5 H, Ar H), 7.13 (br s, 5 H, Ar H), 5.11 (s, 2 H, OCH_2 Ar), 4.10 (m, 4 H, OCH_2Me, 2 >CH), 2.76 (m, 2 H, CH_2), 2.11 (m, 2 H, CH_2), 1.45 (dd, 3 H, CH_3), 1.24 (t, 3 H, CH_3). Anal. ($C_{22}H_{26}O_5$) C, H, N.

Ethyl α-[[2-(1,1-Dimethylethoxy)-1-methyl-2-oxoethyl]thio]benzenebutanoate (4). Sodium hydride (1.88 g of a 60% dispersion in mineral oil, 47 mmol) was added to 125 mL of THF at 0 °C. (±)-1,1-Dimethylethyl 2-mercaptopropanoate (**2**; 7.7 g, 47 mmol) was added dropwise (much foaming), followed by dropwise addition of neat (±)-ethyl α-bromobenzenebutanoate (12.7 g, 47 mmol), and the mixture was stirred overnight at room temperature. The THF was removed in vacuo and the residue was partitioned between H_2O and Et_2O. The Et_2O layer was separated, dried over $MgSO_4$, filtered, and concentrated to yield 16.34 g (99%) of **4** as a clear oil. An analytical sample was prepared by column chromatography over silica gel with CH_2Cl_2 as eluant. IR (neat, NaCl) 2978, 1731, 1147, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.15 (br s, 5 H, ArH), 4.19 (dq, 2 H OCH_2), 3.52 (m, 2 H, 2 CH), 2.72 (br t, 2 H, CH_2 Ar), 2.12 (m, 2 H, CH_2), 1.2–1.6 (m, 15 H, *t*-Bu, 2-CH_3). Anal. ($C_{19}H_{28}O_4S$) C, H, N.

Ethyl α-(1-Carboxyethoxy)benzenebutanoate (5). Compound **3** (7.3 g, 20 mmol) was dissolved in 100 mL of THF, 1 g of 20% Pd/C added, and the mixture exposed to H_2 gas for 1.5 h. The mixture was filtered and the solvent evaporated. The residual oil was dissolved in saturated aqueous $NaHCO_3$ solution and extracted with Et_2O. The aqueous solution was separated and acidified with 1 N HCl, saturated aqueous NaCl solution was added, and the resulting solution was extracted with Et_2O (2 × 150 mL). The ether solution was dried over $MgSO_4$, filtered, and concentrated to give 4.08 g (74%) of **5** as a faint yellow oil. An analytical sample was prepared by chromatography over silica gel with 5% CH_3OH–CH_2Cl_2 and then 10% CH_3OH–CH_2CH_2 as eluant. $[\alpha]^{23}_D$ −20.5° (*c* 0.58, CH_3OH); IR (neat, NaCl) 2990, 1750, 1458, 1142, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.92 (br s, 1 H, OH), 7.18 (s, 5 H, Ar H), 4.12 (m, 4 H, OCH_2, 2 CH), 2.80 (m, 2 H, CH_2), 2.14 (m, 2 H, CH_2), 1.50 (dd, 3 H, CH_3), 1.27 (t, 3 H, CH_3). Anal. ($C_{15}H_{20}O_5$) C, H, N.

Ethyl α-[(1-Carboxyethyl)thio]benzenebutanoate (6). Compound **4** (4.0 g, 11 mmol) was added at 0 °C to 20 mL of TFA. After 1.5 h the TFA was evaporated in vacuo, Et_2O was added, and the mixture was evaporated in vacuo (two times). The residual oil was dissolved in saturated aqueous $NaHCO_3$ solution and extracted with Et_2O. The aqueous solution was separated, acidified with 1 N HCl (aqueous), and extracted with CH_2Cl_2. The organic layer was separated, dried over $MgSO_4$, filtered, and evaporated in vacuo to yield 2.68 g (80%) of **6** as a clear oil. IR (neat, NaCl) 2934, 1733, 1713, 1454, 1157 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.65 (br s, 1 H, OH), 7.13 (br s, 5 H, Ar H), 4.17 (dq, 2 H, OCH_2), 3.3–3.8 (m, 2 H, 2 CH), 2.70 (br t, 2 H, Ar CH_2), 1.8–2.3 (m, 2 H, CH_2), 1.43 (dd, 3 H, CH_3), 1.29 (t, 3 H, CH_3). Anal. ($C_{15}H_{20}O_4S$) C, H, N.

Representative general procedures for preparation of the compounds in Table I are listed as methods A–F. The reported yields for the products obtained were not maximized.

Method A. 1-[2-[1-(Ethoxycarbonyl)-3-phenylpropoxy]-1-oxopropyl]-L-proline (7a). A mixture of **5** (4.0 g, 14 mmol), HOBt (2.31 g, 17 mmol), and L-proline *tert*-butyl ester (2.9 g, 17 mmol) in 50 mL of DMF was cooled to 0 °C and DCCI (3.53 g, 17 mmol) was added dropwise as a solution in CH_2Cl_2. The mixture was stirred at 0 °C for 2 h and then overnight at room temperature. The mixture was diluted with two volumes of Et_2O, filtered, and washed with saturated aqueous $NaHCO_3$, 10% aqueous citric acid solution, and saturated aqueous NaCl solution. The organic layer was separated, dried over $MgSO_4$, filtered, and evaporated in vacuo to a yellow oil. The oil was chromatographed on silica gel with CH_2Cl_2 and then 5% CH_3OH–CH_2Cl_2 as eluant to yield 2.82 g (47%) of the diester as a clear oil. IR (neat, NaCl) 2980, 1740, 1661, 1154 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.21 (br s, 5 H, Ar H), 3.85–4.50 (m, 5 H, OCH_2, 3 CH), 3.53 (2 H, m, NCH_2), 2.80 (2 H, m, Ar CH_2), 1.8–2.3 (m, 6 H, 3 CH_2), 1.35–1.60 (m, 12 H, *t*-Bu, CH_3), 1.27 (t, 3 H, CH_3). Anal. ($C_{24}H_{35}NO_6$) C, H, N.

To the previous diester (1.0 g, 2.3 mmol) was added at room temperature 10 mL of TFA. After 1 h the TFA was evaporated in vacuo, Et_2O added, and the mixture evaporated in vacuo (three times). The residual oil was dissolved in saturated aqueous $NaHCO_3$ solution and extracted with Et_2O. The aqueous solution was separated, acidified with 1 N HCl, and extracted with Et_2O. The Et_2O solution was separated, dried over $MgSO_4$, filtered, and evaporated in vacuo to an oil. The oil was chromatographed on silica gel and eluted with 1% CH_3OH–CH_2Cl_2. The yield of **7a** was 0.43 g (50%). IR (neat, NaCl) 2981, 1748, 1722, 1640, 1180 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.28 (br s, 1 H, OH), 7.11 (m, 5 H, Ar H), 4.47 (br t, 1 H, CH), 3.8–4.3 (m, 4 H, OCH_2, 2 CH), 3.48 (m, 2 H, NCH_2), 2.75 (m, 2 H, Ar H), 1.7–2.3 (m, 6 H, 3-CH_2), 1.3 (d, 3 H, CH_3), 1.23 (t, 3 H, CH_3). Anal. ($C_{20}H_{27}NO_6$) C, H, N.

Method B. [2*S*-(2α,3aβ,7aβ)]-1-[2-[(1-Carboxy-3-phenylpropyl)thio]-1-oxopropyl]octahydro-1*H*-indole-2-carboxylic Acid (10b). Compound **8c** (1.6 g, 3.5 mmol) was added to a solution of 0.2 N methanolic KOH solution at room temperature. Water (10 mL) was added and the solution was stirred overnight. The solvent was evaporated in vacuo, the residue was dissolved in H_2O (100 mL) and washed with Et_2O (200 mL), the aqueous layer was separated and acidified with 1 N HCl, and the aqueous layer extracted with Et_2O (200 mL). The Et_2O layer was separated, dried over $MgSO_4$, filtered, and evaporated to give 0.86 g (57%) of **10b** as a fluffy white solid. IR (KBr) 2930, 1717, 1605, 1201 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.56 (s, 2 H, 20 H), 7.13 (br s, 5 H, Ar H), 4.42 (br t, 1 H, CH), 3.2–3.9 (m, 3 H, 3 CH), 2.72 (br t, 2 H, CH_2), 1.3–2.4 (m, 16 H, CH, 6 CH_2, CH_3). Anal. ($C_{22}H_{29}NO_5S$) C, H, N.

Method C. [2*S*-(2α,3aβ,7aβ)]-1-[2-[[1-(Ethoxycarbonyl)-3-phenylpropyl]sulfinyl]-1-oxopropyl]octahydro-1*H*-indole-2-carboxylic Acid (11). Compound **8c** (3.17 g, 7.1 mmol) was dissolved in methanolic Na_2CO_3 (aqueous) solution, $NaIO_4$ (1.6 g, 7.4 mmol) added, and the solution stirred overnight at 64 °C. The solution was acidified with 1 N HCl, saturated with aqueous NaCl solution, and extracted with Et_2O. The Et_2O solution was separated, dried over $MgSO_4$, filtered, and evaporated in vacuo to an orange solid. The solid was chromatographed on silica gel with CH_2Cl_2 and then 2% CH_3OH–CH_2Cl_2 as eluant. The sulfoxide **11** (0.69 g 21%) was obtained as a fluffy white solid. IR (KBr) 3500, 2939, 1739, 1605, 1069 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.83 (br s, 1 H, OH), 7.20 (s, 5 H, Ar H), 4.43 (br t, 1 H, CH), 3.3–3.9 (m, 5 H, OCH_2, 3 CH), 2.73 (br t, 2 H, Ar CH_2), 1.1–2.4 (m, 19 H, 1 CH, 6 CH_2, 2 CH_3). Anal. ($C_{24}H_{33}NO_6S$) C, H, N.

Method D. Ethyl α-[(1-Carboxyethyl)sulfonyl]benzenebutanoate (12). To a mixture of **6** (3.6 g, 12 mmol) in 30 mL of HOAc was added 9 mL of H_2O_2 (30%) and the mixture stirred overnight. The mixture was then poured into 150 mL of H_2O and extracted with $CHCl_3$ (2 × 100 mL). Saturated NaCl solution was then added and the aqueous solution extracted with $CHCl_3$ (2 × 100 mL). The combined extracts were dried over $MgSO_4$, filtered, and concentrated to a foul-smelling oil. The oil was chromatographed on silica gel and eluted with 10% CH_3OH–CH_2Cl_2 to give 3.32 g (83%) of the crude sulfone **12**, which was used without further purification. IR (neat, NaCl) 3300, 1740, 1324, 1159, 701 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.79 (br s, 1 H, OH), 7.19 (m, 5 H, Ar H), 3.95–4.60 (m, 4 H, 2 CH, OCH_2), 2.78 (m, 2 H, CH_2), 2.48 (m, 2 H, CH_2), 1.64 (dd, 3 H, CH_3), 1.34 (t, 3 H, CH_3).

Method E. 1-[2-[[1-(Ethoxycarbonyl)-3-phenylpropyl]sulfonyl]-1-oxopropyl]-L-proline (13). To a solution of **12** (3.32 g, 10 mmol), L-proline *tert*-butyl ester (1.82 g, 11 mmol), and HOBt (1.37 g, 10 mmol) in 30 mL of DMF at 0 °C was added DCCI (2.09 g, 10 mmol). The mixture was stirred at 0 °C for about 2 h and then overnight at room temperature. The solution was diluted with Et_2O, filtered, and washed with saturated aqueous $NaHCO_3$ solution, and the Et_2O layer was separated, dried over $MgSO_4$, filtered, and concentrated to a yellow oil. The oil was chromatographed on silica gel and eluted with 2% CH_3OH–CH_2Cl_2 to give 2.18 g of a light yellow oil, which was rechromatographed on silica gel and eluted with 10% CH_3CN–CH_2Cl_2 to give 1.61 g (28%) of the diester as a viscous oil. Anal. ($C_{24}H_{35}NO_7S$) C,

H, N. The diester (1.29 g, 2.7 mmol) was added at 0 °C to 15 mL of neat TFA. After 1.5 h at 0 °C and then 2 h at room temperature, the TFA was removed in vacuo, Et_2O was added, and the mixture was evaporated in vacuo (two times). The residual oil was dissolved in saturated aqueous $NaHCO_3$ solution and washed with CH_2Cl_2. The aqueous layer was separated, acidified with 1 N HCl, and extracted with CH_2Cl_2. The CH_2Cl_2 layer was separated, dried over $MgSO_4$, filtered, and evaporated to a yellow oil. The oil was chromatographed on silica gel and eluted with 13% CH_3OH–$CHCl_3$ to give 0.62 g (54%) of **13** as a viscous oil. IR (neat, NaCl) 2981, 1740, 1652, 1320, 1159, 1134 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ 7.17 (m, 5 H, Ar H), 3.95–4.80 (m, 5 H, 3 CH, OCH_2), 3.64 (m, 2 H, NCH_2), 3.48 (m, 2 H, CH_2), 1.7–2.8 (m, 6 H, 3 CH_2), 1.46 (dd, 3 H, CH_2), 1.23 (dt, 3 H, CH_3). Anal. ($C_{20}H_{27}NO_7S$) C, H, N.

Method F. 1-[2-[(1-Carboxy-3-phenylpropyl)sulfonyl]-1-oxopropyl]-L-proline (14). A solution of **13** (0.46 g, 1.1 mmol) in 0.25 N methanolic NaOH was allowed to stand at room temperature overnight. Water (3 mL) was added and the reaction allowed to proceed an additional night. The mixture was concentrated in vacuo and 1 N HCl was added until a precipitate formed. The aqueous solution was extracted with EtOAc. The EtOAc layer was separated, dried over $MgSO_4$, filtered, and evaporated to give 0.38 g (87%) of the diacid **14** as a fluffy white solid. IR (KBr) 2947, 1743, 1625, 1160, 1135 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.46 (s, 2 H, OH), 7.18 (br s, 5 H, Ar H), 3.3–4.6 (m, 5 H, 3 CH, NCH_2), 1.8–3.0 (m, 8 H, 4 CH_2), 1.63 (br d, 3 H, CH_3). Anal. ($C_{18}H_{23}NO_7S$) C, H, N.

Acknowledgment. We thank F. A. MacKellar, E. Schoeb, R. B. Scott, and S. Uhlendorf for the physical measurements and V. Brown and G. Sekerak for the microanalyses.

Registry No. 1, 56777-24-3; 2, 59854-13-6; 3, 96965-37-6; 4, 96965-38-7; 5, 96965-39-8; 6, 96965-40-1; 7a (isomer 1), 96965-41-2; 7a (isomer 2), 97058-35-0; 7a (R^1 = *t*-Bu, isomer 1), 96965-53-6; 7a (R^1 = *t*-Bu, isomer 2), 97058-38-3; 7b (isomer 1), 96965-42-3; 7b (isomer 2), 97058-36-1; 7c (isomer 1), 97059-08-0; 7c (isomer 2), 96965-59-2; 8a, 96965-43-4; 8b, 96965-44-5; 8c, 96965-45-6; 8d, 96965-46-7; 8e (R^1 = Et), 96965-55-8; 8f (R^1 = Et), 96965-56-9; 9a (isomer 1), 96965-47-8; 9a (isomer 2), 97059-09-1; 9b (isomer 1), 96965-48-9; 9b (isomer 2), 97058-37-2; 10a, 79625-83-5; 10b, 96998-96-8; 10c, 96965-49-0; 10d, 96965-50-3; 11, 96998-97-9; 12, 96965-57-0; 13, 96965-51-4; 13 (prolyl *tert*-butyl ester), 96965-58-1; 14, 96965-52-5; *AA-OR^1 (*AA = A, R^1 = *t*-Bu), 2812-46-6; *AA-OR^1 (*AA = C, R^1 = *t*-Bu), 80876-00-2; *AA-OR_1 (*AA = D, R^1 = *t*-Bu), 77497-74-6; *AA-OR^1 (*AA = B, R^1 = *t*-Bu), 96965-54-7; ACE, 9015-82-1; (±)-ethyl α-bromobenzenebutanoate, 80828-27-9.

Ring-Substituted 1,1,2,2-Tetraalkylated 1,2-Bis(hydroxyphenyl)ethanes. 4. Synthesis, Estrogen Receptor Binding Affinity, and Evaluation of Antiestrogenic and Mammary Tumor Inhibiting Activity of Symmetrically Disubstituted 1,1,2,2-Tetramethyl-1,2-bis(hydroxyphenyl)ethanes

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The syntheses of symmetrically 2,2′-disubstituted derivatives of 1,1,2,2-tetramethyl-1,2-bis(4-hydroxyphenyl)ethane (**1**) and of 5,5′-, and 6,6′-disubstituted derivatives of 1,1,2,2-tetramethyl-1,2-bis(3-hydroxyphenyl)ethane (**6**) are described (**1** and **6** are strong antiestrogens with mammary tumor inhibiting activity exhibiting only slight estrogenic properties): (2,2′-substituents) F (**2**), Cl (**3**), OCH_3 (**4**), CH_3 (**5**); (5,5′-substituents) Cl (**7**); (6,6′-substituents) F (**8**), Cl (**9**), OCH_3 (**10**), CH_3 (**11**). The synthesis of **1**–**11** was accomplished by reductive coupling of the corresponding 2-phenyl-2-propanols with $TiCl_3$ and $LiAlH_4$. The binding affinity of the compounds to the calf uterine estrogen receptor was measured relative to that of [^{3}H]estradiol by a competitive binding assay. With the exception of **7** and **10** all other compounds showed relative binding affinity (RBA) values between 0.5 and 6.4% that of estradiol, **2** (RBA value 6.4), and **8** and **9** (4.0 and 3.5), exceeding those of the corresponding unsubstituted **1** and **6** (3.6 and 3.0). Compounds exhibiting RBA values of >2.5% were evaluated in the mouse uterine weight test. The substituted derivatives showed an increase in uterotrophic and a decrease in antiuterotrophic activity compared to **1** and **6**. Compound **2** showed a strong, dose-dependent inhibition on the DMBA-induced hormone-dependent mammary tumor of the SD-rat, exceeding that of the parent compound **1**. At a dose of 5 mg/kg per day, **2** reduced total tumor area by 47% and caused a complete remission in 74% of the tumors.

In the search for new structures of mammary tumor inhibiting antiestrogens, modifications on the synthetic estrogen hexestrol were performed. Displacement of the phenolic OH groups,[1] variation of the alkyl chains in the 1,2-positions,[2] and tetraalkylation in the 1,1,2,2-positions of the 1,2-diphenylethane skeleton[3] led to a number of active compounds. The most effective representatives are metahexestrol, metabutestrol, tetramethylHES (**1**), and metatetramethylHES (**6**) (Chart I). The tetramethylated 1,2-diphenylethanes exhibited the strongest antiestrogenic activity.[3] In contrast to metahexestrol they showed only slight[3] or no[4] estrogenic properties, depending on the test system. Compounds **1** and **6** are of great interest for the treatment of hormone-dependent breast cancer, for they showed marked inhibitory activity on the established DMBA-induced mammary carcinoma of the SD-rat.[3]

In contrast to the partial antiestrogens metahexestrol and tamoxifen (Nolvadex), tetramethylHES (**1**) and metatetramethylHES (**6**) seem to unfold their mammary tumor inhibiting activity by means of their antiestrogenic potency. They antagonized the tumor growth stimulating

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