(Phosphinyloxy)acyl Amino Acid Inhibitors of Angiotensin Converting Enzyme (ACE). 1. Discovery of (*S*)-1-[6-Amino-2-[[hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxohexyl]-L-proline, a Novel Orally Active Inhibitor of ACE

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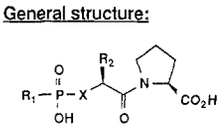
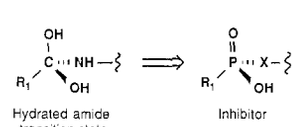
The synthesis of a series of orally active, phosphinyloxyacyl proline inhibitors of angiotensin converting enzyme (ACE) is described. The in vitro and in vivo ACE inhibitory activities are reported for each compound. The structure–activity relationship for this series of compounds in relation to the carboxyalkyl dipeptide ACE inhibitors as well as other types of hydroxyphosphinyl-containing ACE inhibitors (e.g., the corresponding nitrogen and carbon isosteres) is discussed. Within an isosteric series of phosphorus-containing inhibitors based on the lysylproline terminal dipeptide sequence, only the phosphonates (oxygen isosteres) show a high level of oral activity. Optimum potency and oral activity in the phosphonate series occurs with the (phenylbutyl)- and *n*-hexylphosphonate side chains. An aminobutyl side chain in the P_1' residue is an absolute requirement for full expression of oral activity. The most potent of these compounds, **8b** (SQ 29,852), has intravenous and oral activities superior in potency to those of captopril in the normotensive rat.

Angiotensin converting enzyme (ACE) is a dipeptidyl carboxypeptidase that catalyzes the hydrolysis of the decapeptide angiotensin I to the pressor octapeptide angiotensin II. It also deactivates the endogenous depressor substance bradykinin. Several potent inhibitors of this enzyme have been shown to be useful for the treatment of hypertension and congestive heart failure.[1a,b] Most notable are the orally active converting enzyme inhibitors captopril, enalapril, and lisinopril. We have completed a systematic study of inhibitors with the general structure **1** in which the hydroxyphosphinyl function is proposed to coordinate to the zinc ion at the active site of the enzyme and approximate the tetrahedral geometry of the hydrated amide transition state resulting from the addition of water to the scissile amide bond of the substrate. Within this series of compounds we have discovered a series of phosphonates (**1**, X = O) that are potent inhibitors of ACE in vitro and that exhibit remarkably high levels of inhibition of an angiotensin I induced pressor response after oral administration to normotensive rats. This activity profile is not shared by the phosphonamide (**1**, X = NH) or phosphinic acid (**1**, X = CH_2) isosteres. In this paper we describe the preparation and structure–activity relationships for a series of phosphonates (**1**, X = O). The structural requirements for optimal binding to the S_1 (e.g., R_1 in structure **1**) and S_1' (e.g., R_2 in structure **1**) subsites of ACE were found to parallel those observed in the carboxyalkyl dipeptide series (e.g., enalapril and lisinopril) of converting enzyme inhibitors.

General structure:

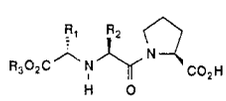


1 X = CH_2, O, NH



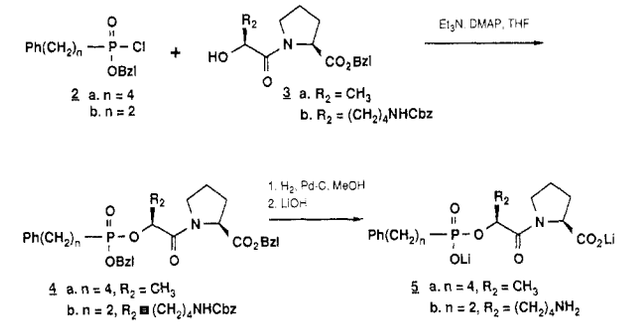
Hydrated amide transition state — Inhibitor

Carboxyalkyl dipeptides:

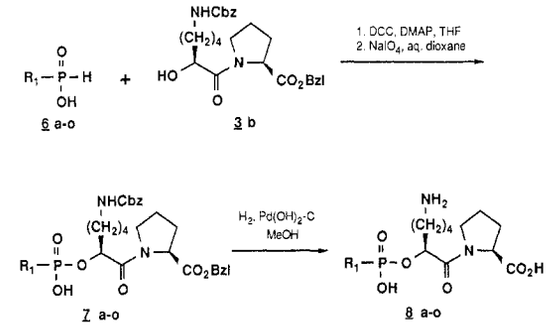


enalaprilat: R_1 = $Ph(CH_2)_2$; R_2 = CH_3; R_3 = H
enalapril: R_1 = $Ph(CH_2)_2$; R_2 = CH_3; R_3 = C_2H_5
lisinopril: R_1 = $Ph(CH_2)_2$; R_2 = $(CH_2)_4NH_2$; R_3 = H

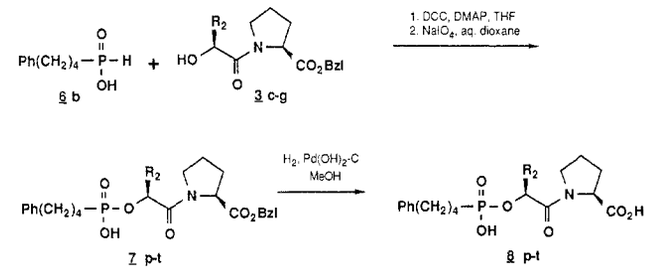
Scheme I



Scheme II



Scheme III



Chemistry

In our initial investigation, compounds in the phosphonate series were prepared by utilizing the methodology developed[2,3] for the synthesis of inhibitors of the phos-

(1) (a) Wyvratt, M. J.; Patchett, A. A. *Med. Res. Rev.* **1985**, *5*, 483. (b) Petrillo, E. W.; Ondetti, M. A. *Med. Res. Rev.* **1982**, *2*, 1.

(2) Thorsett, E. D.; Harris, E. E.; Peterson, E. R.; Greenlee, W. J.; Patchett, A. A.; Ulm, E. H.; Vassil, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 2716.

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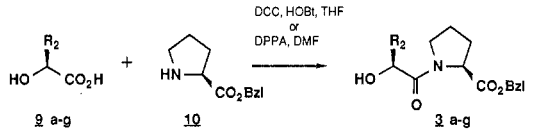
Scheme IV



phonamide type (Scheme I). In contrast to what we had observed in the phosphonamide series, phosphonochloridates **2a,b** would only react with (hydroxyacyl)prolines **3a,b** in the presence of (*N,N*-dimethylamino)pyridine (DMAP) to give moderate yields (40–60%) of the desired phosphonic diesters **4a,b**. Hydrogenolysis of the protecting groups (H_2, 10% Pd–C, MeOH) gave the desired diacid products **5a,b**.

Further exploration of the structure–activity relationships in the phosphonate series was facilitated by the development of a new method for the synthesis of monoesters of phosphonic acids[4] (see Schemes II and III). This method involves the *N,N'*-dicyclohexylcarbodiimide (DCC)/(*N,N*-dimethylamino)pyridine (DMAP) mediated coupling of phosphonous acids **6a–o** with (hydroxyacyl)-prolines **3b–g** and oxidation of the resulting phosphonous monoesters to phosphonic monoesters **7a–t** with sodium metaperiodate in aqueous dioxane. This method has proven to be very general and can be used to prepare phosphonic monoesters of even tertiary alcohols with phosphonous acids of a wide variety of structural types.[4] Scheme II illustrates the synthesis of a series of alkyl- and aralkylphosphonates **8a–o** based on the dipeptide lysyl-proline. These analogues were prepared in an effort to optimize binding of the phosphonate side chain to the S_1 subsite of ACE. Scheme III shows the synthesis of a series of (phenylbutyl)phosphonates **8p–t** that differ in the side chain α to the phosphonate oxygen. These analogues were prepared in an effort to optimize binding of the side chain to the S_1' subsite of the enzyme.

(Hydroxyacyl)prolines **3a–g** were prepared by either DCC/HOBT or diphenylphosphoryl azide (DPPA) mediated coupling of the known hydroxy acids **9a–g** to proline benzyl ester (**10**; Scheme IV).

Phosphonous acids **6a–o** were prepared either by hydrophosphorylation of terminal olefins **11a–m** under free-radical conditions[5] (method A) or by hydrolysis of phosphonous diesters **13a,b** prepared, in turn, by reaction of Grignard reagents with diethyl chlorophosphite[6a,b] (method B) (Scheme V).

Scheme VI shows a number of modifications of the aminoalkyl phosphonates **8b**, **8p**, and **8q**. The guanidinoalkyl analogues **15a–c** were prepared by treatment of the corresponding aminoalkyl compounds with 2-methyl-2-thiopseudourea in aqueous sodium bicarbonate. Reduction of phosphonate **8b** over Rh/Al_2O_3 gave the corresponding cyclohexylbutyl analogue **16**.

Scheme V

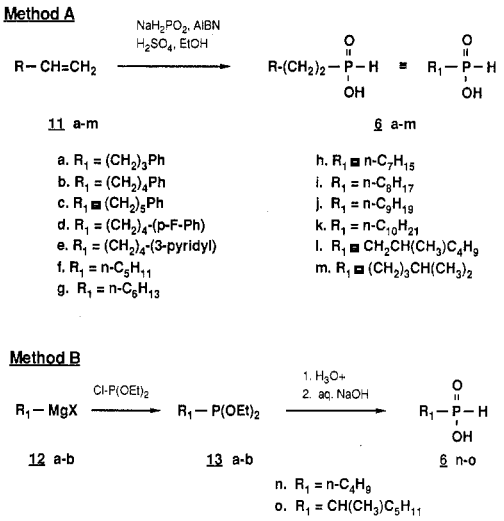
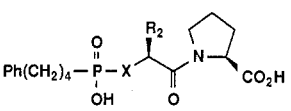


Table I



no.	X	R^2	ACE I_{50},[a] nM	normotensive rat AI challenge[a] ED_{50}, μmol/kg, iv	normotensive rat AI challenge[a] ED_{50}, μmol/kg, po
17a	NH	CH_3	12	0.050	25
5a	O	CH_3	59	0.750	>25
17b	CH_2	CH_3[b]	220	2.20	ND
17c	NH	$(CH_2)_4NH_2$	9.0	0.022	2.6
8b	O	$(CH_2)_4NH_2$	36	0.063	0.53
17d	CH_2	$(CH_2)_4NH_2$[b]	85	0.180	35
captopril			23	0.092	0.73
enalaprilat			4.3	0.033	5.13
enalapril					0.46

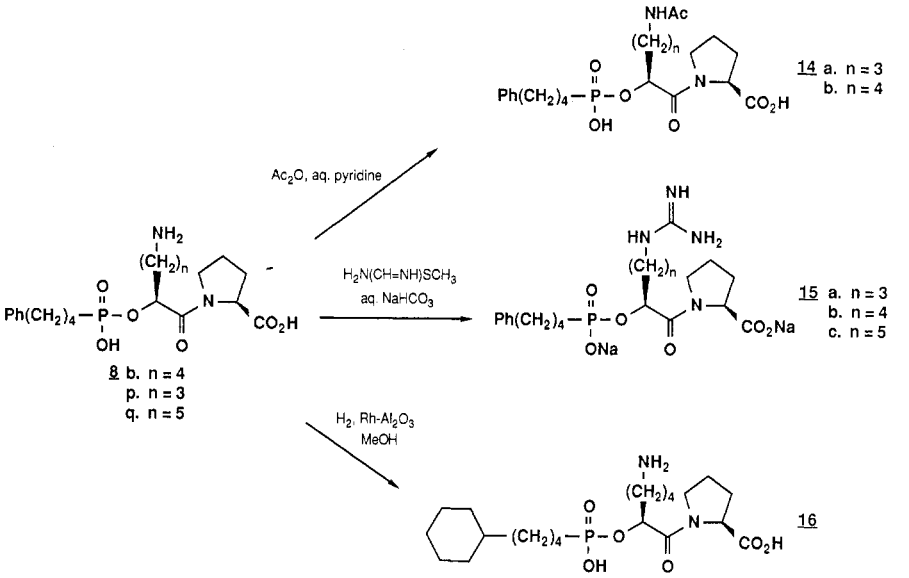
[a] See Experimental Section for description of biological assays. [b] *R,S* mixture.

Biological Results

Hydroxyphosphinyl-Containing Inhibitors: Discovery of 8b (SQ 29,852). The relationship among the various classes of hydroxyphosphinyl-containing ACE inhibitors is best illustrated by comparing two sets of isosteric inhibitors (Table I). ACE IC_{50}'s were determined against rabbit lung ACE with hippurylhistidylleucine as a substrate.[7] ED_{50}'s were determined from plots of percent maximal inhibition of the angiotensin I induced pressor response vs dose after either intravenous (iv) or oral (po) administration to the normotensive rat.[8] In general, ED_{50}'s were estimated from plots of at least three doses. Compound **17a** is a phosphonamide that incorporates the alanylproline terminal dipeptide sequence characteristic of many classes of ACE inhibitors, and **5a** and **17b** are the corresponding phosphonic ester and phosphinic acid isosteres, respectively. Compound **17c** incorporates ly-

(3) Petrillo, E. W.; Cushman, D. W.; Duggan, M. E.; Heikes, J. E.; Karanewsky, D. S.; Ondetti, M. A.; O'Reilly, B.; Rovnyak, G. C.; Schwartz, J.; Spitzmiller, E. R.; Wang, N.-Y. In *Peptides: Structure and Function—Proceedings of the Eighth American Peptide Symposium*; Hruby, V. J., Rich, D. H., Eds., Pierce Chemical Co.: Rockford, IL, 1983; p 541.
(4) Karanewsky, D. S.; Badia, M. C. *Tetrahedron Lett.* **1986**, *27*, 1751.
(5) (a) Nifantev, E. E.; Magdeva, R. K.; Shehepeteva, N. P. *J. Gen. Chem. USSR (Engl. Transl.)* **1980**, *50*, 1744. (b) Nifantev, E. E.; Koroteev, M. P. *J. Gen. Chem. USSR (Engl. Transl.)* **1967**, *37*, 1366.
(6) (a) Kabachnik, M. I.; Tsvetkov, E. N. *Dokl. Akad. Nauk SSSR* **1957**, *117*, 817. (b) Sander, M. *Chem. Ber.* **1960**, *93*, 1220.
(7) Cushman, D. W.; Cheung, H. S. *Biochem. Pharmacol.* **1971**, *20*, 1637.
(8) Rubin, B.; Laffin, R. J.; Kotter, D. G.; O'Keefe, E. H.; DeMaio, D. A.; Goldberg, M. E. *J. Pharmacol. Exp. Ther.* **1978**, *204*, 271.

Scheme VI



sylproline as the terminal dipeptide, and **8b** and **17d**[9] are the corresponding oxygen and carbon isosteres.

Comparison of the in vitro ACE-inhibitory activities of these compounds shows that the phosphonamides are the most potent inhibitors within each set of isosteres, and the phosphinic acids are the least potent. The replacement of the alanine side chain by a lysine side chain causes only a moderate increase in potency in each of the inhibitor types. The replacement of the phosphonamide nitrogen by an oxygen atom results only in a modest decrease in inhibitory potency (4–5-fold) in each of the inhibitor types. Apparently, the phosphonamide nitrogen does not play as crucial a role in binding to the enzyme as the secondary amino group of the carboxyalkyl dipeptide inhibitors. Replacement of the NH of enalaprilat by an oxygen atom has been reported[1a,10] to result in a substantial loss in potency.

An examination of the activity of these sets of isosteres as inhibitors of the angiotensin I induced pressor effect, however, reveals a surprising result. Within the alanine series, the order of potency in the normotensive rat after iv administration parallels the order of potency observed in vitro. However, there is a relatively large difference between equieffective oral and iv doses suggestive of poor bioavailability after oral administration. In the lysine series, the iv potency order parallels the in vitro situation and the compounds are considerably more potent than their alanine counterparts. However, the phosphonate **8b** is clearly and unexpectedly the most potent compound after oral administration. On the basis of its iv and po ED_{50}'s (0.063 μmol/kg, iv and 0.53 μmol/kg, po), phosphonate **8b** is significantly more potent than captopril in the normotensive rat. The decreased ratio of po to iv ED_{50} for this compound suggests that its excellent oral activity in rats may be due to improved oral bioavailability relative to its isosteres. Since phosphonamides are known to be rapidly hydrolyzed below pH 6, the poor oral activity displayed by **17c** may be due in part to hydrolysis in the acidic environment of the stomach. This is consistent with the observation that phosphonamide **17a** given at a dose

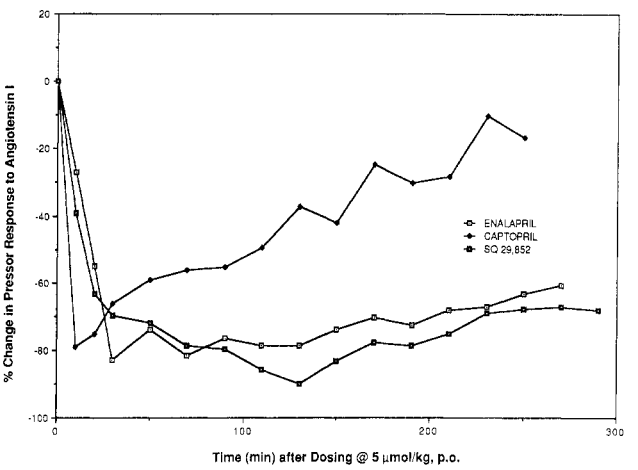


Figure 1. Inhibition of the AI pressor response versus time for captopril, enalapril, and SQ 29,852.

of 15 μmol/kg showed enhanced activity after either intraduodenal administration (71% inhibition, id) or after pretreatment with cimetidine (70% inhibition, po; cimetidine @ 5 mg/kg, iv) relative to **17a** given alone (26% inhibition, po). However, this does not explain the poor oral activity displayed by the carbon isostere **17d** since it does not suffer from chemical instability. Therefore, the enhanced oral activity of phosphonate **8b** relative to its carbon isostere **17d** is most likely due to increased oral absorption and/or increased metabolic stability. The oral activity enhancing effect of the aminobutyl side chain is well precedented in the carboxyalkyl dipeptide inhibitor series. Replacement of the alanine residue in enalaprilat by a lysine residue results in a compound (i.e., lisinopril) that, unlike enalaprilat, possesses substantial oral activity without recourse to prodrug design.[11]

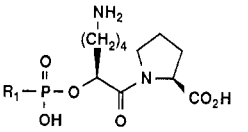
Figure 1 shows a plot of the percent inhibition of the angiotensin I induced pressor response vs time for captopril, enalapril, and phosphonate **8b** in the normotensive rat. At an oral dose of 5 μmol/kg, all three compounds show a maximal inhibition of 80–90%. After 140 min the response of the captopril-treated animals is already at

(9) Prepared by a method analogous to that used to prepare **17b** (see ref 3): Ryono, D. E., unpublished results.

(10) Roark, W. H.; Tinney, F. J.; Cohen, D.; Essenburg, A. D.; Kaplan, H. R. *Eighth American Peptide Symposium*, Tucson, AZ, May 1983, Abst. 6-18.

(11) Rotmensch, H. H.; Vlasses, P. H.; Swanson, B. N.; Irvin, J. D.; Harris, K. E.; Merril, D. G.; Ferguson, R. K. *Am. J. Cardiol.* **1984**, *53*, 116.

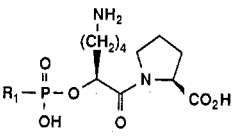
Table II



no.	R^1	formula[b]	mp, °C	$[\alpha]_D$, deg (*c*, MeOH)	ACE I_{50},[a] nM	normotensive rat AI challenge[a] ED_{50}, μmol/kg, iv	% inhibn (5.0 μmol/kg, po)	ED_{50}, μmol/kg, po
5b	$Ph(CH_2)_2$	$C_{19}H_{27}N_2O_6PLi_2 \cdot 1.1H_2O$	>200[c]	−43.0 (0.50)	190	0.217	65	1.39
8a	$Ph(CH_2)_3$	$C_{20}H_{31}N_2O_6P \cdot 1.4H_2O$	165–175 dec	−45.2 (0.50)	190	0.069	67	2.62
8b	$Ph(CH_2)_4$	$C_{21}H_{33}N_2O_6P \cdot 1.0H_2O$	190–195 dec	−47.5 (1.00)	36	0.063	90	0.53
8c	$Ph(CH_2)_5$	$C_{22}H_{35}N_2O_6P \cdot 1.5H_2O$	145–149	−41.6 (0.65)	14	0.067	65	ND
8d	*p*-$FPh(CH_2)_4$	$C_{21}H_{32}N_2O_6PF \cdot 1.6H_2O$	178–185 dec	−41.8 (0.50)	31	0.067	81	0.89
16	c-$C_6H_{11}(CH_2)_4$	$C_{21}H_{39}N_2O_6P \cdot 1.25H_2O$	188–192 dec	−44.9 (0.69)	55	0.180	68	2.30
8e	3-pyridyl$(CH_2)_4$	$C_{20}H_{32}N_3O_6P \cdot 0.8H_2O$	143–150	−45.3 (0.64)	94	0.190	56	ND

[a] See Experimental Section for description of biological assays. [b] All compounds had satisfactory C, H, N, and P elemental analyses (±0.4%) and exhibited IR and ^1H NMR spectra consistent with the structures. [c] Lyophilate (amorphous solid), melting point of this substance is the temperature at which the white solid became a colorless glass. ND = not determined.

Table III



no.	R^1	formula[b]	mp, °C	$[\alpha]_D$, deg (*c*, MeOH)	ACE I_{50},[a] nM	normotensive rat AI challenge[a] ED_{50}, μmol/kg iv	% inhibn (5.0 μmol/kg, po)	ED_{50}, μmol/kg, po
8n	*n*-C_4H_9	$C_{15}H_{29}N_2O_6P \cdot 0.5H_2O$	150–170	−66.8 (0.50)	96	0.091	27	ND
8f	*n*-C_5H_{11}	$C_{16}H_{31}N_2O_6P \cdot 1.2H_2O$	175–179	−65.0 (0.51)	27	0.071	57	ND
8g	*n*-C_6H_{13}	$C_{17}H_{33}N_2O_6P \cdot 1.0H_2O$	160–170 dec	−49.0 (0.50)	24	0.036	82	0.94
8h	*n*-C_7H_{15}	$C_{18}H_{35}N_2O_6P \cdot 0.8H_2O$	180–186	−54.4 (0.50)	19	0.120	75	0.62
8i	*n*-C_8H_{17}	$C_{19}H_{37}N_2O_6P \cdot 1.5H_2O$	168–172	−48.0 (0.50)	38	0.091	83	1.40
8j	*n*-C_9H_{19}	$C_{20}H_{39}N_2O_6P \cdot 0.9H_2O$	167–174	−46.9 (0.55)	31	0.130	23	ND
8k	*n*-$C_{10}H_{21}$	$C_{21}H_{41}N_2O_6P \cdot 1.45H_2O$	165–175 dec	−49.8 (0.50)	95	0.170	2	ND
8m	$(CH_3)_2CH(CH_2)_3$	$C_{17}H_{33}N_2O_6P \cdot 1.2H_2O$	170–190 dec	−54.4 (0.50)	150	0.095	69	ND
8o	*n*-$C_5H_{11}CH(CH_3)$[c]	$C_{18}H_{35}N_2O_6P \cdot 1.05H_2O$	130 dec[d]	−58.5 (1.00)	61	0.130	29	ND
8l	*n*-$C_4H_9CH(CH_3)CH_2$[c]	$C_{18}H_{35}N_2O_6P \cdot 2.25H_2O$	130 dec[d]	−50.6 (1.00)	40	0.140	68	ND

[a] See Experimental Section for description of biological assays. [b] All compounds had satisfactory C, H, N, and P elemental analyses (±0.4%) and exhibited IR and ^1H NMR spectra consistent with the structures. [c] *R*, *S* mixture. [d] Lyophilate (amorphous solid), melting point of this substance is the temperature at which the white solid became a colorless glass. ND = not determined.

half-maximal inhibition and after 240 min the response has returned to control values. In contrast, both the enalapril- and phosphonate **8b** treated animals are still giving near-maximal inhibition after 260 min. It appears that phosphonate **8b** is considerably longer acting than captopril in the normotensive rat.

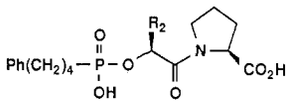
Phosphonate Side Chain Analogues of Phosphonate 8b. Tables II and III show the in vitro and in vivo testing results for a series of aralkyl- and alkylphosphonates based on the dipeptide lysylproline. As was previously observed in the carboxyalkyl dipeptide inhibitor series,[12] both aliphatic and aralkyl groups are consistent with good activity. In a series of homologues from phenethyl to phenylpentyl, the in vitro and in vivo activities of phenylbutyl and phenylpentyl side chain analogues **8b** and **8c**, respectively, appear to be optimum. While addition of a *p*-fluoro substituent to the aromatic ring (e.g., **8d**) has essentially no effect on inhibitory potency, reduction of the aromatic ring (e.g., **16**) or replacement by a pyridine ring (e.g., **8e**) appears to have a detrimental effect on potency. In contrast to the results of the iv assay, only the phenylbutyl (**8b**) and fluorophenylbutyl (**8d**) analogues exceed the oral activity of captopril. All of the other aralkyl analogues were significantly less active.

With the exception of the *n*-butyl and *n*-decyl compounds, all of the *n*-alkyl analogues were comparable in terms of in vitro potency. Branching (e.g., isohexyl analogue **8m**, 2-heptyl analogue **8o**, or 2-methylhexyl analogue **8l**) has a detrimental effect on the in vitro potency relative to the parent *n*-hexyl (**8g**) or *n*-heptyl (**8h**) compounds. This is in agreement with the SAR observed in the carboxyalkyl dipeptide series in which branching α to the zinc-binding function resulted in a substantial loss in inhibitory potency relative to the corresponding *n*-alkyl compound.[12]

The *n*-hexyl analogue (**8g**) is clearly the most potent compound on iv administration with an ED_{50} of 0.036 μmol/kg. The iv ED_{50}'s of the other alkylphosphonates range from 0.071 to 0.170 μmol/kg. After oral administration, the *n*-hexyl (**8g**), *n*-heptyl (**8h**), and *n*-octyl (**8i**) analogues all appear to be similar in potency to the phenylbutyl compound (**8b**). On the basis of single oral doses,

(12) Patchett, A. A.; Harris, E.; Tristram, E. W.; Wyvratt, M. J.; Wu, M. T.; Taub, D.; Peterson, E. R.; Ikeler, T. J.; tenBroeke, J.; Payne, L. G.; Ondeyka, D. L.; Thorsett, E. D.; Greenlee, W. J.; Lohr, N. S.; Hoffsommer, R. D.; Joshua, H.; Ruyle, W. V.; Rothrock, J. W.; Aster, S. D.; Maycock, A. L.; Robinson, F. M.; Hirschmann, R.; Sweet, C. S.; Ulm, E. H.; Gross, D. M.; Vassil, T. C.; Stone, C. A. *Nature (London)* **1980**, *288*, 280.

Table IV



no.	R^2	formula[b]	mp, °C	$[\alpha]_D$, deg	ACE I_{50},[a] nM	normotensive rat AI challenge[a] ED_{50}, μmol/kg, iv	normotensive rat AI challenge[a] % inhibn (5.0 μmol/kg, po)
8b	$H_2N(CH_2)_4$	$C_{21}H_{33}N_2O_6P \cdot 1.0H_2O$	190–195 dec	−47.5 (1.00)[e]	36	0.063	90%
8p	$H_2N(CH_2)_3$	$C_{20}H_{31}N_2O_6P \cdot 0.74H_2O$	215 dec	−49.7 (0.34)[f]	390	1.00	28%[g]
8q	$H_2N(CH_2)_5$[c]	$C_{22}H_{35}N_2O_6P \cdot 0.5NH_3 \cdot 1.5H_2O$	105–120[d]	−36.5 (0.54)[f]	110	>1.50 (44%)	ND
5a	CH_3	$C_{18}H_{24}NO_6PLi_2 \cdot 2.5H_2O$	209–212[d]	−58.6 (0.50)[f]	59	0.64	ND
8r	$CH_3(CH_2)_3$	$C_{21}H_{30}NO_6PLi_2 \cdot 1.1H_2O$	230°C dec[d]	−56.9 (0.52)[e]	180	>1.40 (11%)	ND
8s	$(CH_3)_2CH\text{-}CH_2$	$C_{21}H_{30}NO_6PLi_2 \cdot 1.7H_2O$	145[d]	−64.6 (0.50)[e]	3,800	ND	ND
8t	$Ph(CH_2)_2$	$C_{25}H_{30}NO_6PLi_2 \cdot 2.5H_2O$	190[d]	−41.8 (0.50)[e]	640	29.0	ND
14b	$AcNH(CH_2)_4$	$C_{23}H_{33}N_2O_7PLi_2 \cdot 1.7H_2O$	160[d]	−38.6 (0.50)[e]	180	<1.40 (55%)	ND
14a	$AcNH(CH_2)_3$	$C_{22}H_{31}N_2O_7PLi_2 \cdot 1.55H_2O$	170–175[d]	−50.2 (0.55)[e]	42	0.250	4%
15b	$HN{=}C(NH_2)NH(CH_2)_4$	$C_{22}H_{33}N_4O_6PNa_2 \cdot 1.1H_2O$	180–190[d]	−49.2 (0.52)[f]	6.2	0.045	48%
15a	$HN{=}C(NH_2)NH(CH_2)_3$	$C_{21}H_{31}N_4O_6PNa_2 \cdot 0.3H_2O$	190–200[d]	−49.1 (0.57)[f]	19	0.120	40%
15c	$HN{=}C(NH_2)NH(CH_2)_5$[c]	$C_{23}H_{35}N_4O_6PNa_2$[h]	150–155 dec[d]	−25.6 (0.50)[f]	32	>1.50 (47%)	ND

[a] See Experimental Section for description of biological assays. [b] All compounds had satisfactory C, H, N, and P elemental analyses (±0.4%) and exhibited IR and ^1H NMR spectra consistent with the structures. [c] *R, S* mixture. [d] Lyophilate (amorphous solid), melting point of this substance is the temperature at which the white solid became a colorless glass. [e] Measured in MeOH. [f] Measured in H_2O. [g] Tested at 50.0 μmol/kg, po. [h] H: calculated, 6.48; found, 6.90. ND = not determined.

the *n*-butyl (**8n**), *n*-nonyl (**8j**), and *n*-decyl (**8k**) analogues were nearly inactive despite activity comparable to the other alkylphosphonates after iv administration. Similarly, the α-methylhexyl analogue (**8o**) appears to be considerably less active than either the β-methylhexyl (**8l**) or the *n*-heptyl (**8h**) analogues after oral administration despite the fact that all three compounds were equipotent after iv administration. Apparently, branching α to phosphorus has a detrimental effect on oral activity. These data strongly suggest that there is some optimum size and/or lipophilicity for the phosphonic acid side chain required for maximum oral bioavailability.

Acyloxy Side Chain Analogues of Phosphonate 8b. Table IV shows the testing results for a series of (phenylbutyl)phosphonates that differ in the side chain α to the phosphonate oxygen. As observed in the carboxyalkyl dipeptide series,[1a,11] hydrophobic residues larger than methyl do not enhance activity relative to the methyl analogue (compare **8r–t** with **5a**). Branching has a detrimental effect (compare **8s** and **8r**). As has been suggested in the captopril series, the activity-enhancing properties of this substituent is most likely of conformational origin.[13]

Addition of an amino group to the *n*-butyl analogue **8r** as in **8b** results in a 5-fold increase in in vitro potency. However, shortening or lengthening the aminobutyl side chain by one methylene (**8p** and **8q**, respectively) appears to be detrimental to biological activity relative to the aminobutyl analogue. This stands in contrast to the carboxyalkyl dipeptide series in which the aminopropyl, -butyl, and -pentyl analogues were all nearly equipotent.[1a] Acetylation of the aminoalkyl side chain led to a decrease in activity in the case of the lysine analogue (e.g., **14b**) but gave a 9-fold increase in potency in the case of the ornithine analogue (e.g., **14a**). On the other hand, all three guanidine analogues **15a–c** showed enhanced activity relative to their respective parents **8b** and **8p,q**. On iv administration, the only compound more potent than **8b** was the homoarginine analogue **15b**. Despite its enhanced iv potency, **15b** was less active than **8b** after oral administration.

Discussion

A variety of phosphorus-containing inhibitors of zinc metalloproteases have been described since Umezawa's discovery of phosphoramidon, a phosphoryl dipeptide of microbial origin that is a potent inhibitor of thermolysin.[14] X-ray studies of the thermolysin–phosphoramidon complex show that an ionized oxygen atom attached to phosphorus binds as a ligand to the zinc ion at the enzyme active site.[15] This mode of binding would be consistent with the structure–activity studies reported for a variety of phosphorus-containing inhibitors of angiotensin-converting enzyme. We[1b,3] and Thorsett et al.[2] have previously shown that, within isosteric series of ACE inhibitors, the nature of the other atoms bound to the hydroxyphosphinyl moiety have a substantial effect on inhibitory potency. We reported that phosphonamide **17a** binds more tightly to ACE than either its phosphonate (**5a**) or phosphinic acid isostere (**17b**), and the present work extends this observation to the compounds bearing an aminobutyl side chain in place of the methyl side chain of the latter compounds. However, the difference between the phosphonamides and phosphinic acids is at most 20-fold, while the phosphonates are only 4–6-fold less potent than the phosphonamides. Recently, Bartlett and Marlowe observed a fairly large (840-fold) difference in thermolysin binding affinity between a series of phosphonamides and the corresponding phosphonates.[16] Their attribution of the 4.0 kcal/mol difference in binding energy between the two series to the presence of a hydrogen bond between the phosphonamide N–H and an acceptor site on the enzyme was elegantly confirmed by an X-ray crystallographic study of the inhibitors.[17] If such a hydrogen bond exists between

(13) Cushman, D. W.; Cheung, H. S.; Sabo, E. F.; Ondetti, M. A. *Biochemistry* **1977**, *16*, 5484.

(14) Komiyama, T.; Suda, H.; Aoyagi, T.; Takeuchi, T.; Umezawa, H.; Fujimoto, K.; Umezawa, S. *Arch. Biochem. Biophys.* **1975**, *171*, 727.

(15) Weaver, L. H.; Kester, W. R.; Matthews, B. W. *J. Mol. Biol.* **1977**, *114*, 119.

(16) Bartlett, P. A.; Marlowe, C. K. *Science (Washington, D.C.)* **1987**, *235*, 569.

phosphonamide ACE inhibitors and angiotensin-converting enzyme, then it must not contribute as much binding energy to overall affinity as does the inhibitor–thermolysin hydrogen bond, since the similarity in binding affinity between **17a** and **5a** and **17c** and **8b** is much greater.

Within the isosteric series of phosphorus-containing ACE inhibitors with aminobutyl side chains, only the phosphonates (oxygen isosteres) show a high level of oral activity. Optimum potency and oral activity in the phosphonate series occurs with the (phenylbutyl)- and *n*-hexylphosphonate side chains. Branching of this side chain is detrimental to inhibitory potency. Branching α to phosphorus is very detrimental to oral activity. The data strongly suggest that there is some optimum size and/or lipophilicity for the phosphonic acid side chain required for maximum oral bioavailability. The aminobutyl acyloxy side chain is an absolute requirement for full expression of oral activity. The guanidinobutyl analogue shows enhanced intrinsic activity relative to the aminobutyl analogue, but this activity is not expressed orally. The improved oral activity of compounds possessing the [(aminobutyl)acyl]oxy side chain relative to the simple alkyl side chain analogues is reminiscent of the carboxyalkyl dipeptide series in which a similar modification of enalaprilat resulted in a compound (i.e., lisinopril) with enhanced oral bioavailability. It is unlikely that this effect is due solely to "charge balance" of the phosphonate anion by the protonated amino group since minor structural modifications can result in a substantial loss of oral activity without effecting in vitro or iv in vivo potencies. For example, the lack of significant oral activity displayed by the α-methylhexyl analogue **8o** relative to its isomeric analogues **8l** and **8h** would not be expected solely on the basis of net charge and overall lipophilicity considerations. One hypothesis is that an active or facilitated transport mechanism is responsible for the excellent oral activity displayed by these compounds. We have not investigated this possibility. This work culminated in the choice of (*S*)-1-[6-amino-2-[hydroxy(4-phenylbutyl)phosphinyl]-oxy]-1-oxohexyl]-L-proline (**8b**, SQ 29,852) for clinical study in humans. This compound represents a new class of potent inhibitors of ACE. Despite the fact that phosphonate **8b** contains a rather acidic functional group, the compound exhibits excellent oral activity in the normotensive rat without recourse to prodrug design.

Experimental Section

Proton NMR spectra were determined at 270 MHz on a JEOL FX-270 spectrometer. ^{13}C NMR spectra were determined at 15 MHz on a JEOL FX-60Q spectrometer. Chemical shifts (ppm) are reported relative to internal tetramethylsilane (^1H, 0.00 ppm), $CDCl_3$ (^{13}C, 77.0 ppm), CD_3OD (^{13}C, 49.0 ppm), CD_3CN (^{13}C, 1.30 ppm), or dioxane (^1H, 3.53 ppm; ^{13}C, 66.5 ppm) in the case of spectra run in D_2O. P–C coupling constants (hertz) are shown in parentheses. Infrared spectra were recorded on a Perkin-Elmer Model 137 spectrophotometer. Optical rotations were measured with a Perkin-Elmer Model 241 polarimeter. Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Tetrahydrofuran (THF) and diethyl ether were distilled from potassium benzophenone. Dichloromethane was distilled from P_2O_5. Flash chromatography was performed on Whatman LPS-1 silica gel (13–24 μm). MCI Gel CHP-20P is a highly porous polystyrene-divinylbenzene copolymer resin (75–150 μm) supplied by Mitsubishi Chemical Industries LTD. All compounds were prepared by methods identical with those described below.

(*S*)-6-[[(Phenylmethoxy)carbonyl]amino]-2-hydroxyhexanoic Acid (9b). An aqueous solution of L-lysine hydrochloride (36.6 g, 0.20 mol) was passed through an AG3-X4A (100–200 mesh) ion-exchange column (OH^- form, 1000-mL bed volume) eluting with water. The ninhydrin-positive fractions were combined, acidified with 2 M H_2SO_4 (200 mL, 0.4 mol), and evaporated to dryness.

The crude L-lysine disulfuric acid was taken up in 10% H_2SO_4 (500 mL) and treated dropwise with a solution of sodium nitrite (51.8 g, 0.72 mol) in water (200 mL) at 45–50 °C (bath temperature) over a period of 2 h. When the addition was complete, the mixture was stirred at 45–50 °C for an additional 4.5 h, the excess nitrous acid decomposed with urea, and the mixture poured onto an AG-50-X8 ion-exchange column (H^+ form, 400-mL bed volume). The column was eluted with water and then with aqueous NH_4OH (concentrated NH_4OH/H_2O, 1:3) to elute the product. The ninhydrin-positive fractions were combined and evaporated to give a pink semisolid, which was recrystallized from H_2O/EtOH to give 6-amino-2(*S*)-hydroxyhexanoic acid (18.37 g, 62%) as white crystals: mp 197–199 °C, $[\alpha]_D$ −12.2° (*c* 1.2, H_2O) [lit.[18] mp 203–206 °C, $[\alpha]_D$ −12.1° (*c* 1.16, H_2O)]; TLC (*i*-PrOH/concentrated NH_4OH/H_2O, 7:2:1) R_f 0.16.

A solution of the amino hydroxy acid (11.25 g, 76.5 mmol) in 1 N NaOH solution (100 mL) at 0 °C (ice bath) was adjusted to pH 10 with concentrated HCl and treated with benzyl chloroformate (12.6 mL, 95%, 84 mmol) in 2-mL portions at 15-min intervals. Throughout the reaction, the pH was maintained at pH 9.8–10.2 by the addition of 1 N NaOH solution. When the addition was complete and pH had stabilized, the mixture was stirred at pH 10, 0 °C for an additional 45 min and then washed with one portion of Et_2O. The aqueous solution was acidified to pH 1 with concentrated HCl and extracted with EtOAc. The EtOAc extract was washed with saturated NaCl solution, dried over Na_2SO_4 and evaporated. Recrystallization of the crude product from EtOAc/hexane gave pure **9b** (20.2 g, 94%) as a white crystalline solid: mp 79–81 °C, $[\alpha]_D$ +4.5°, $[\alpha]_{365}$ +26.8° (*c* 1.1, $CHCl_3$), [lit.[19] mp 79–81 °C, $[\alpha]_{589}$ +2.7°, $[\alpha]_{365}$ +21.4° (*c* 1, $CHCl_3$)]; TLC (AcOH/MeOH/CH_2Cl_2, 1:1:20) R_f 0.19; ^{13}C NMR (CD_3CN) δ 22.9, 30.2, 34.5, 41.5, 67.0, 70.9 (CH), 158.0 (C), 176.6 (C).

The Cbz-amino hydroxy acids **9c** (21% overall from L-ornithine hydrochloride) and *d,l*-**9d** (36% overall from *d,l*-homolysine hydrochloride) were also prepared by this method.

1-[(*S*)-2-Hydroxy-1-oxo-6-[[(phenylmethoxy)carbonyl]-amino]hexyl]-L-proline Phenylmethyl Ester (3b). A mixture of hydroxy acid **9b** (4.20 g, 15.0 mmol), proline benzyl ester hydrochloride (4.00 g, 16.5 mmol), and triethylamine (2.28 mL, 16.5 mmol) in dry THF (45 mL) at 0 °C (ice bath) was treated with hydroxybenzotriazole (2.13 g, 15.8 mmol) and DCC (3.24 g, 15.7 mmol). The solution was stirred at 0 °C for 3 h, allowed to warm to room temperature, and stirred for an additional 1 h. The mixture was filtered, diluted with EtOAc, and washed successively with 5% $KHSO_4$, saturated $NaHCO_3$, and saturated NaCl, dried over Na_2SO_4, and evaporated. The residue was taken up in CCl_4, filtered to remove the last traces of DCU, and evaporated. The crude product was purified by flash chromatography on silica gel (100 g), eluting with EtOAc/hexane (2:1) to give pure **3b** (6.56 g, 94%) as a colorless, very viscous oil: TLC (MeOH/CH_2Cl_2, 5:95) R_f = 0.36; ^{13}C NMR ($CDCl_3$) δ 21.0, 24.6, 28.2, 29.0, 33.0, 40.2, 46.1, 58.7 (CH), 66.0, 66.5, 68.6 (CH), 156.2 (C), 171.2 (C), 172.8 (C).

The following (hydroxyacyl)prolines were also prepared by this method: **3c** (22%), **3d** (100%), **3e** (24%), and **3f** (87%). Yields in this reaction were variable due to a tendency for (hydroxyacyl)prolines **3b–f** to lactonize under the reaction conditions and on silica gel chromatography.

1-[(*S*)-2-Hydroxy-1-oxopropyl]-L-proline Phenylmethyl Ester (9a). To a mixture of L-sodium lactate (3.20 g, 15.0 mmol) and diphenylphosphoryl azide (3.60 mL, 16.5 mmol) in dry DMF (30 mL) was added triethylamine (2.1 mL, 16.5 mmol) and proline benzyl ester hydrochloride (3.60 g, 15.0 mmol). After stirring at room temperature for 24 h, the mixture was partitioned between EtOAc and H_2O. The organic phase was washed successively with

(17) Tronrud, D. E.; Holden, H. M.; Matthews, B. W. *Science (Washington, D.C.)* **1987**, *235*, 569.

(18) Aketa, K.; Terashima, S.; Yamada, S. *Chem. Pharm. Bull.* **1976**, *24*, 621.

(19) Haskell, T. H.; Rodebaugh, R.; Plessas, N.; Watson, D.; Westland, R. D. *Carbohyd. Res.* **1973**, *28*, 263.

5% $KHSO_4$ and saturated NaCl, dried over Na_2SO_4, and evaporated. The residue (5.0 g) was purified by flash chromatography on silica gel, eluting with EtOAc/hexane (1:1), to give **9a** (2.50 g, 60%) as a white, crystalline solid. A sample recrystallized from *i*-Pr_2O had mp 86–88 °C: TLC (EtOAc) R_f 0.40; ^{13}C NMR ($CDCl_3$) δ 19.7 (CH_3), 24.4, 28.1, 45.8, 58.5 (CH), 64.9 (CH), 66.1, 171.0 (C), 173.2 (C). Anal. ($C_{15}H_{19}NO_4$) C, H, N.

1-[(*S*)-2-Hydroxy-1-oxo-4-phenylbutyl]-L-proline Phenylmethyl Ester (3g). To a mixture of L-proline benzyl ester hydrochloride (2.64 g, 11.0 mmol) and *d,l*-2-hydroxy-4-phenylbutyric acid (**9g**; 1.80 g, 10.0 mmol) at 0 °C under argon were added triethylamine (1.52 mL, 11.0 mmol), dicyclohexylcarbodiimide (2.17 g, 10.5 mmol), and 1-hydroxybenzotriazole hydrate (1.41 g, 10.4 mmol). The resulting mixture was stirred at 0 °C for 3 h and then allowed to warm to room temperature. The mixture was filtered and the filter cake washed throughly with EtOAc. The combined filtrate was washed successively with 5% $KHSO_4$, saturated $NaHCO_3$, and saturated NaCl solutions, dried over $MgSO_4$, and evaporated to give 3.58 g of crude product as a pale yellow oil. TLC (EtOAc/hexane, 1:1) showed two product spots, R_f 0.30 and 0.22. Flash chromatography on silica gel gave (2*R*)-**3g** (0.73 g, 20%, R_f 0.30) and (2*S*)-**3g** (1.13 g, 31%, R_f 0.22) along with 0.44 g (12%) of mixed fractions. (2*R*)-**3g**: ^{13}C NMR ($CDCl_3$) δ 24.2, 28.4, 30.8, 35.5, 46.0, 59.1 (CH), 66.5, 68.3 (CH), 171.3 (C), 172.9 (C); $[\alpha]_D$ −36.0° (*c* 1.0, MeOH). (2*S*)-**3g**: ^{13}C NMR ($CDCl_3$) δ 24.6, 28.3, 30.6, 35.8, 46.0, 58.7 (CH), 66.5, 68.0 (CH), 171.2 (C), 172.8 (C); $[\alpha]_D$ −32.2° (*c* 1.0, MeOH).

(4-Phenylbutyl)phosphonous Acid (6b). To a suspension of sodium hypophosphite hydrate (60.0 g, 0.566 mol) in absolute ethanol (600 mL) were added concentrated H_2SO_4 (15 mL), 4-phenylbutene (**11b**; 25.0 g, 0.189 mol), and AIBN (3.0 g). The resulting mixture was refluxed for 6 h, treated with a second portion of AIBN (2.0 g), and refluxed for an additional 16 h. The cooled mixture was filtered and concentrated in vacuo. The residue was suspended in water (200 mL), made basic with 50% NaOH solution, and washed with two portions of Et_2O (200 mL each). The aqueous phase was acidified with concentrated H_2SO_4 and extracted with EtOAc. The EtOAc extract was washed with saturated NaCl, dried over Na_2SO_4, and evaporated to give crude phosphonous acid **6b** (34.5 g, 92%) as a colorless oil.

The crude phosphonous acid was purified by conversion to its 1-adamantanamine salt. Thus, the crude phosphonous acid **6b** (34.5 g) was taken up in Et_2O (200 mL) and treated with a solution of 1-adamantanamine (26.3 g, 0.174 mol) in Et_2O (200 mL). The resulting white precipitate was collected, washed with Et_2O, and dried in vacuo to give **6b**-adamantanamine salt (54.2 g, 82% overall from 4-phenylbutene) as a white solid, mp 192–200 °C.

To regenerate the free acid, the salt (10.5 g) was partitioned between EtOAc and 1 N HCl (150 mL each). The EtOAc phase was washed with 1 N HCl and saturated NaCl, dried over Na_2SO_4, and evaporated to give pure **6b** (5.75 g, 96%) as a colorless, viscous oil: TLC (*i*-PrOH/concentrated NH_4OH/H_2O, 7:2:1) R_f 0.67; ^{13}C NMR ($CDCl_3$) δ 20.2 (3), 28.9 (94), 31.9 (16), 35.2, 125.7 (CH), 128.2 (CH), 141.6 (C).

The following phosphonous acids were also prepared by this method: **6a** (81%), **6c** (67%), **6d** (89%), **6f** (93%), **6g** (87%), **6h** (87.5%), **6i** (86%), **6j** (85%), **6k** (95%), **6l** (56%), and **6m** (88%). Yields quoted are of the pure 1-adamantanamine salts.

[4-(3-Pyridyl)butyl]phosphonous Acid (6e). A mixture of 4-(3-pyridyl)-1-butene (**11e**; 1.153 g, 8.67 mmol), sodium hypophosphite hydrate (2.75 g, 25.9 mmol), and di-*tert*-butyl peroxide (0.2 mL) in absolute methanol (5 mL) was heated in a sealed tube at 135 °C (bath temperature) for 6 h. An additional portion (0.2 mL) of di-*tert*-butyl peroxide was added and heating continued for an additional 16 h. TLC indicated a small amount of starting olefin still remained; a third portion of di-*tert*-butyl peroxide (0.2 mL) was added and heating continued for an additional 4 h. The mixture was then concentrated in vacuo, taken up in water, and adjusted to pH 2 with concentrated HCl. The aqueous solution was applied to an AG50W-X8 (H^+ form) column (20-mL bed volume), eluting first with water and then finally with concentrated NH_4OH/water (1:3) to elute the product. The product containing fractions were combined and evaporated to dryness, and the residue was azeotroped with CH_3CN (3×) and CH_2Cl_2 (2×) to give phosphonous acid **6e** (1.23 g, 71%) as a waxy semisolid; TLC (*i*-PrOH/concentrated NH_4OH/H_2O, 7:2:1) R_f 0.59; ^{13}C NMR (CD_3OD) δ 22.5 (2), 33.1 (15), 33.1 (91), 33.4, 125.6 (CH), 139.7 (CH), 140.5 (C), 146.2 (CH), 148.6 (CH).

***n*-Butylphosphonous Acid Diethyl Ester (13a).** A 2.0 M solution of *n*-butylmagnesium bromide in Et_2O (80 mL, 160 mmol) was added dropwise over 2 h to a solution of diethyl chlorophosphite (23.1 mL, 160 mmol) in dry Et_2O (60 mL) under argon while the internal temperature was maintained at <10 °C. After the addition was completed, the white suspension was heated at 50 °C (bath temperature) for 1 h, cooled, and filtered through dried Celite under argon. The Et_2O was removed by distillation at atmospheric pressure and the residue distilled under reduced pressure to give phosphonous diester **13a** (14.01 g, 49%) as a clear, colorless liquid: bp 39–40 °C (0.5 mmHg); ^{13}C NMR (CD_3CN) δ 14.2 (CH_3), 17.6 (CH_3, 6), 24.8 (12), 25.2 (17), 34.7 (17), 63.4 (13).

Phosphonous diester **13b** (20% from 2-bromoheptane) was also prepared by this method.

***n*-Butylphosphonous Acid (6n).** To a vigorously stirred mixture of phosphonous diester **13a** (13.80 g, 77.5 mmol) and H_2O (12 mL) was added concentrated HCl (6 drops). An exothermic reaction was observed. After being stirred at room temperature for 15 min, the mixture was extracted with EtOAc. The organic phase was washed with saturated NaCl, dried over Na_2SO_4, and evaporated. The crude phosphonous monoester was taken up in 2.0 N NaOH (40 mL) and stirred at room temperature for 1 h. The aqueous mixture was washed with Et_2O, acidified with concentrated HCl (pH 1), and extracted with EtOAc. The EtOAc was washed with saturated NaCl, dried over Na_2SO_4, and evaporated to give phosphonous acid **6n** (6.467 g, 68%) as a clear, colorless oil: TLC (CH_2Cl_2/MeOH/AcOH, 20:1:1) R_f 0.19; ^{13}C NMR ($CDCl_3$) δ 13.2 (CH_3), 22.5 (4), 23.2 (17), 28.6 (94).

Phosphonous acid **6o** (60% from **13b**) was also prepared by this method.

1-[(*S*)-2-[[(Phenylmethoxy)(2-phenethyl)phosphinyl]oxy]-1-oxo-6-[[(phenylmethoxy)carbonyl]amino]hexyl]-L-proline Phenylmethyl Ester (4b). To a solution of dibenzyl phenethylphosphonate (1.48 g, 4.04 mmol) in dry benzene (15 mL) was added phosphorus pentachloride (0.952 g, 4.57 mmol) and the resulting mixture refluxed under argon for 2.5 h. The clear solution was evaporated to dryness (room temperature, 0.5 mmHg), taken up in benzene (10 mL), and evaporated again. The resulting phosphonochloridate **2b** was taken up in dry CH_2Cl_2 (15 mL), cooled in an ice bath, and treated successively with (hydroxyacyl)proline **3b** (1.26 g, 2.69 mmol), triethylamine (0.56 mL, 4.04 mmol), and DMAP (0.049 g, 0.40 mmol). After being stirred at 0 °C for 15 min and at room temperature for 1 h, the mixture was washed with 5% $KHSO_4$ and saturated NaCl solutions, dried over Na_2SO_4, and evaporated. The crude product was purified by flash chromatography on silica gel, eluting with acetone/hexane (3:7), to give phosphonate **4b** (0.916 g, 47%) as a colorless oil: TLC (acetone/hexane, 7:3) R_f 0.73; ^{13}C NMR ($CDCl_3$) δ 21.0, 24.5, 27.8 (139), 28.0 (5), 28.1, 28.8, 31.7 (7), 40.1, 46.3, 58.6 (CH), 65.9, 66.0 (6), 66.3, 72.2 (CH, 6), 156.1 (C), 168.5 (C, 4), 171.3 (C).

Phosphonate **4a** (66% based on (hydroxyacyl)proline **3a**) was also prepared by this method.

1-[(*S*)-6-Amino-2-[[hydroxy(2-phenethyl)phosphinyl]oxy]-1-oxohexyl]-L-proline Dilithium Salt (5b). A suspension of 10% Pd/C (0.184 g) in a solution of phosphonate **4b** (0.916 g, 1.26 mmol) and triethylamine (0.53 mL, 3.8 mmol) in methanol (10 mL) was stirred under hydrogen for 5 h. The catalyst was removed by filtration through Celite and the filtrate evaporated to dryness. The residue was taken up in 1 N LiOH (5.0 mL) and chromatographed on an CHP-20P column (200-mL bed volume, 25-mm-diameter colum) eluting with a linear gradient of H_2O (100%)/CH_3CN (100%). The product-containing fractions were pooled and evaporated. The residue was taken up in water (50 mL), filtered through a polycarbonate membrane, and lyophilized to give dilithium salt **5b** (0.373 g, 70%) as a granular, white solid: mp >200 °C; TLC (*i*-PrOH/concentrated NH_4OH/H_2O, 7:2:1) R_f 0.17; $[\alpha]_D$ −43.0° (*c* 0.50, MeOH); ^{13}C NMR (D_2O) δ 22.0, 25.4, 27.5, 30.0 (137), 30.1, 30.1, 33.1 (5), 40.3, 48.4, 63.0 (CH), 72.2 (CH, 6), 172.1 (C, 3), 180.3 (C); ^1H NMR (CD_3OD) δ 1.50–2.25 (12 H, m), 2.90 (4 H, m), 3.62 (1 H, m), 3.87 (1 H, m), 4.41 (1 H, dd), 5.00 (1 H, dt), 7.25 (5 H, m). Anal. ($C_{19}H_{27}N_2O_6PLi_2 \cdot 1.1H_2O$) C, H, N, P.

Dilithium salt **5a** (91.5%) was also prepared by this method.

1-[(*S*)-2-[[Hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxo-6-[[(phenylmethoxy)carbonyl]amino]hexyl]-L-proline Phenylmethyl Ester (7b). To a solution of phosphonous acid **6b** (0.67 g, 3.38 mmol) and (hydroxyacyl)proline **3b** (1.00 g, 2.14 mmol) in dry THF (8.0 mL) at room temperature under argon were added DCC (0.67 g, 3.25 mmol) and DMAP (0.10 g). After being stirred for 2 h, the mixture was filtered, diluted with EtOAc (75 mL), washed successively with 5% $KHSO_4$, saturated $NaHCO_3$, and saturated NaCl solutions, dried over Na_2SO_4, and evaporated. The crude phosphonous monoester was filtered through a pad of SiliCAR CC7 (10 g) eluting with acetone/hexane (1:1). Evaporation of the product-containing fractions gave phosphonous monoester (1.42 g, 102%) as a colorless oil: TLC (acetone/hexane, 1:1) R_f 0.18 (R_f of **3b** = 0.28). This phosphonous monoester is somewhat unstable and has been observed to hydrolyze on prolonged exposure to silica gel. It is best used immediately for the next step.

The above phosphonous ester (1.42 g) was taken up in dioxane (10.0 mL), treated with a solution of $NaIO_4$ (0.50 g, 2.34 mmol) in water (6.0 mL), and stirred at room temperature for 16 h. The orange mixture was then partitioned between EtOAc and 1% $KHSO_4$ solution. The organic phase was washed successively with water, dilute $NaHSO_3$, and saturated NaCl, dried over Na_2SO_4, and evaporated. The crude phosphonic monoester was taken up in a small volume of EtOAc (5 mL), diluted with Et_2O (20 mL), and treated with a solution of 1-adamantanamine (0.34 g, 2.25 mmol) in Et_2O (4 mL). The white precipitate was collected, washed with Et_2O, and dried in vacuo to give pure **7b**·1-adamantanamine salt (1.50 g, 86%) as a white solid, mp 129–140 °C.

The adamantanamine salt (1.50 g) was partitioned between EtOAc and 1 N HCl (75 mL each). The organic phase was washed with 1 N HCl and saturated NaCl, dried over Na_2SO_4, and evaporated to give pure phosphonic monoester **7b** (1.195 g, 98%) as a glassy solid: TLC (AcOH/MeOH/CH_2Cl_2, 1:1:20) R_f 0.24; ^{13}C NMR ($CDCl_3$) δ 21.3, 21.8 (5), 24.8, 28.4, 29.1, 26.1 (143), 31.7 (16), 32.1 (6), 35.1, 40.5, 46.7, 59.1 (CH), 66.2, 66.6, 72.4 (CH, 7), 156.4, 168.8, 171.4.

The following phosphonic monoesters were also prepared by this method: **7a** (72%), **7c** (84%), **7d** (85%), **7f** (94%), **7g** (91.5%), **7h** (50%), **7i** (63%), **7j** (76%), **7k** (78%), **7l** (71%), **7m** (85%), **7n** (94%), **7o** (69%), **7p** (50%), **7q** (100%), **7r** (82%), **7s** (77%), and **7t** (62%). The yields quoted are of the corresponding 1-adamantanamine salts and are based on starting (hydroxyacyl)prolines **3b–g**.

1-[(*S*)-6-Amino-2-[[hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxohexyl]-L-proline (8b, SQ 29,852). A suspension of 20% $Pd(OH)_2/C$ (1.50 g) in a solution of phosphonate **7b** (9.56 g, 14.4 mmol) in methanol (50 mL) was stirred under a hydrogen atmosphere for 3 h. The catalyst was removed by filtration through Celite and the filtrate evaporated to dryness. The residue (6.50 g) was taken up in hot H_2O (70 mL), filtered though a glass frit, and concentrated to a small volume (25 mL). The amino diacid **8b** (4.54 g, 69%) separated on cooling as white crystals, mp 187–195 °C dec. Concentration of the mother liquor gave a second crop of **8b** (0.57 g, total yield 5.11 g, 78%). Recrystallization of 4.00 g of this material from 15 mL of H_2O gave 3.50 g of pure **8b**: mp 190–195 °C dec; TLC (*i*-PrOH/concentrated NH_4OH/H_2O, 7:2:1) R_f 0.24; $[\alpha]_D$ −47.5° (*c* 1.00, MeOH); ^{13}C NMR (D_2O) δ 20.9, 22.3, 24.5, 26.3, 27.0 (140), 28.6, 31.6, 32.2 (17), 34.7, 39.2, 47.3, 59.9 (CH), 71.1 (CH), 171.6 (C), 176.2 (C); ^1H NMR (CD_3OD) δ 1.45–2.10 (15 H, m), 2.20 (1 H, m), 2.62 (2 H, t), 2.94 (2 H, t), 3.60 (1 H, m), 3.86 (1 H, m), 4.45 (1 H, dd), 4.91 (1 H, m), 7.16 (5 H, m). Anal. ($C_{21}H_{33}N_2O_6P$·1.0H_2O) C, H, N, P, H_2O (KF).

The following phosphonates were also prepared by this method: **8a** (97.5%), **8c** (97%), **8d** (88%), **8e** (91%), **8f** (54%), **8g** (83%), **8h** (52%), **8i** (81%), **8j** (67%), **8k** (66%), **8l** (55%), **8m** (67%), **8n** (80%), **8o** (78%), **8p** (72%), **8q** (26%), **8r** (62%), **8s** (76%), and **8t** (95%). Phosphonate **8q** required an additional purification on an AG-50X8 (H^+ form) column eluting with H_2O and then concentrated NH_4OH/H_2O (15:85) to remove a nonbasic impurity carried over from the previous step.

1-[(*S*)-2-[[Hydroxy[4-(3-pyridyl)butyl]phosphinyl]oxy]-1-oxo-6-[[(phenylmethoxy)carbonyl]amino]hexyl]-L-proline Phenylmethyl Ester (7e). To a solution of phosphonous acid **6e** (0.70 g, 3.52 mmol), (hydroxyacyl)proline **3b** (1.00 g, 2.14 mmol), and pyridine hydrochloride (0.40 g, 3.46 mmol) in dry pyridine (8.0 mL) at room temperature under argon were added DCC (0.67 g, 3.25 mmol) and DMAP (0.10 g). After being stirred for 2 h, the mixture was diluted with CH_2Cl_2 (75 mL) and filtered and the filtrate evaporated to dryness. The residue was taken up in CH_2Cl_2, filtered, and washed with saturated $NaHCO_3$ and saturated NaCl solutions, dried over Na_2SO_4, and evaporated. The crude phosphonous monoester was filtered through a pad of SiliCAR CC7 (10 g), eluting first with acetone/CH_2Cl_2 (3:7) and then with methanol/CH_2Cl_2 (1:9). Evaporation of the product-containing fractions gave phosphonous monoester as a colorless oil: TLC (MeOH/CH_2Cl_2, 1:9) R_f 0.44 (R_f of **3b** = 0.59). This phosphonous monoester is somewhat unstable and has been observed to hydrolyze on prolonged exposure to silica gel. It is best used immediately for the next step.

The crude phosphonous ester was taken up in dioxane (10.0 mL), treated with a solution of $NaIO_4$ (0.50 g, 2.34 mmol) in water (5.0 mL), and stirred at room temperature for 18 h and at 55 °C (bath temperature) for 4 h. The mixture was evaporated to dryness and the residue taken up in 1% $NaHCO_3$ (75 mL) and washed with Et_2O (2 × 50 mL) to remove a small amount of **3b**. The aqueous phase was acidified to pH 2.5–3.0 with 2 M H_2SO_4 and extracted with CH_2Cl_2. The extract was washed with dilute $NaHSO_3$ solution and water, dried over Na_2SO_4, and evaporated to dryness to give phosphonic monoester **7e** (0.74 g, 52%) as a pale yellow glass: TLC (AcOH/MeOH/CH_2Cl_2, 1:1:8) R_f 0.34; ^{13}C NMR (CD_3OD) δ 23.0, 23.8 (4), 26.0, 28.3 (143), 29.7, 30.4, 32.6 (16), 33.1, 33.8 (5), 41.5, 48.2, 60.7 (CH), 67.2, 67.8, 72.8 (CH, 6), 127.3 (CH), 142.0 (CH), 143.1 (C), 144.1 (CH), 145.1 (CH), 158.6 (C), 172.4 (C), 173.2 (C).

1-[(*S*)-6-Amino-2-[[hydroxy(4-cyclohexylbutyl)phosphinyl]oxy]-1-oxohexyl]-L-proline (16). A suspension of 5% Rh/Al_2O_3 (0.20 g) in a solution of amino diacid **8b** (0.385 g, 0.875 mmol) in methanol (30 mL) was hydrogenated in a Parr apparatus at a pressure of 50 psi for 24 h. The catalyst was removed by filtration through Celite and the filtrate evaporated to dryness. The residue was taken up in H_2O, filtered through a polycarbonate membrane, and again evaporated to dryness. The crystalline residue was triturated with CH_3CN, collected, and dried in vacuo to give **16** (0.369 g, 95%) as a white, crystalline solid: mp 188–192 °C dec; $[\alpha]_D$ −44.9° (*c* 0.69, MeOH); TLC (*i*-PrOH/concentrated NH_4OH/H_2O, 7:2:1) R_f 0.26; ^1H NMR (CD_3OD) δ 0.90 (2 H, m), 1.10–2.10 (26 H, m), 2.24 (1 H, m), 2.96 (2 H, t), 3.61 (1 H, m), 3.87 (1 H, m), 4.45 (1 H, dd), 4.94 (1 H, dt). Anal. ($C_{21}H_{39}N_2O_6P$·1.25H_2O) C, H, N, P.

1-[(*S*)-5-(Acetylamino)-2-[[hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxopentyl]-L-proline Dilithium Salt (14a). To a solution of amino diacid **8p** (0.426 g, 1.0 mmol) in pyridine (1.2 mL)/H_2O (0.4 mL) at room temperature was added acetic anhydride (1.23 mL, 13.0 mmol) in one portion. After being stirred at room temperature for 1 h, the mixture was evaporated to dryness. The residue (0.514 g) was dissolved in CH_2Cl_2 (5 mL), treated with triethylamine (0.4 mL, 2.9 mmol), and concentrated in vacuo. The residue was dissolved in H_2O and passed down an AG-50X8 (Li^+ form) column (20-mL bed volume) eluting with H_2O. The product-containing fractions were combined and lyophilized to give **14a** dilithium salt (0.468 g, 92%) as a white solid: mp 170–175 °C; TLC (*i*-PrOH/concentrated NH_4OH/H_2O, 7:2:1) R_f 0.53; ^{13}C NMR (D_2O) δ 21.8 (CH_3), 22.4 (4), 23.7, 24.5, 26.9 (136), 29.1, 29.9 (4), 32.1 (17), 34.6, 39.0, 47.5, 61.8 (CH), 71.0 (CH); ^1H NMR (D_2O) δ 1.15–2.05 (14 H, m), 1.75 (3 H, s), 2.41 (2 H, t), 2.97 (2 H, m), 3.35 (1 H, m), 3.55 (1 H, m), 4.06 (1 H, dd), 4.61 (1 H, m), 7.09 (5 H, m); $[\alpha]_D$ −50.2° (*c* 0.55, MeOH). Anal. ($C_{22}H_{31}N_2O_7PLi_2$·1.55H_2O) C, H, N, P.

Phosphonate **14b** (47% yield from **8b**) was also prepared by this method.

1-[(*S*)-5-[(Aminoiminomethyl)amino]-2-[[hydroxy(4-phenylbutyl)phosphinyl]oxy]-1-oxopentyl]-L-proline Disodium Salt (15a). To a solution of amino diacid **8p** (0.426 g, 1.0 mmol) and $NaHCO_3$ (0.504 g, 6.0 mmol) in H_2O (4.0 mL) at room temperature was added 2-methyl-2-thiopseudourea sulfate (0.280 g, 1.0 mmol). After 3 h, additional portions of 2-methyl-2-thiopseudourea sulfate (0.140 g, 0.5 mmol) and $NaHCO_3$ (0.084 g, 1.0 mmol) were added, and the mixture was stirred for an additional 1.5 h at 70 °C (bath temperature). The mixture

was evaporated to dryness and the residue chromatographed on a CHP-20P column (200-mL bed volume, 1-in.-diameter column) eluting with a linear gradient of H_2O (100%)/CH_3CN (100%). The product-containing fractions were pooled and evaporated. The residue was taken up in water, filtered (millipore), and lyophilized to give disodium salt **15a** (0.310 g, 60%) as a white solid: mp 190–200 °C; TLC (*i*-PrOH/concentrated NH_4OH/H_2O, 4:2:1) R_f 0.52; ^{13}C NMR (D_2O) δ 22.5, 23.4, 24.6, 25.8 (138), 29.1, 31.5, 32.1 (17), 34.7, 40.7, 47.5, 62.1 (CH), 70.8 (CH, 5), 156.7 (C), 171.0 (C), 179.0 (C); ^1H NMR (D_2O) δ 1.15–2.10 (14 H, m), 2.40 (2 H, t), 3.01 (2 H, t), 3.35 (1 H, m), 3.55 (1 H, m), 4.07 (1 H, dd), 4.68 (1 H, m), 7.12 (5 H, m); $[\alpha]_D$ −49.1° (*c* 0.57, H_2O). Anal. ($C_{21}H_{31}N_4O_6PNa_2 \cdot 0.3H_2O$) C, H, N, P.

Guanidino phosphonates **15b** (28% from **8b**) and **15c** (16% from **8q**) were also prepared by this method.

In Vitro Inhibition of Angiotensin Converting Enzyme from Rabbit Lung. The conditions for the assay of inhibition of ACE are those we reported previously[7] in which hippuric acid liberated from the synthetic substrate hippurylhistidylleucine by rabbit lung ACE is quantitated by a spectrophotometric method (see ref 7 for details).

Angiotensin Converting Enzyme Inhibitor Screen in Vivo. Male Sprague–Dawley rats (225–275 g) were equipped with indwelling abdominal aorta and vena caval catheters by using a modification of the method of Weeks and Jones.[20] The animals were allowed to recover for at least 2 weeks before experimentation, during which time they were housed individually and maintained on rat chow and tap water ad libitum. On the day of experimentation aortic blood pressures were monitored directly by pressure transducers and recorded on a Beckman Dynograph. The venous catheter was used for drug injections. During all experiments the rats were conscious and unrestrained in their cages. Pressor responses were obtained for angiotensin I (310 ng/kg, iv) and angiotensin II (100 ng/kg iv) before administration of the compounds. For intravenous testing, compounds were administered in 0.1 mL of water or 5% $NaHCO_3$, and angiotensin I and II pressor responses were evaluated for up to 70 min. For oral testing, compounds were administered in 0.1 mL of water, 5% $NaHCO_3$, or 1% agar suspension and angiotensin I and II pressor responses were evaluated for up to 280 min. Maximum percent inhibition was determined as the mean of the responses for four animals per dose. The dose required to produce 50% inhibition of the response (ED_{50}) was estimated by interpolation of a plot of maximum inhibition versus dose.

(20) Weeks, J. R.; Jones, J. A. *Proc. Soc. Exp. Bioc. Med.* **1960**, *104*, 646.

Structural Factors of Importance for 5-Hydroxytryptaminergic Activity. Conformational Preferences and Electrostatic Potentials of 8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) and Some Related Agents

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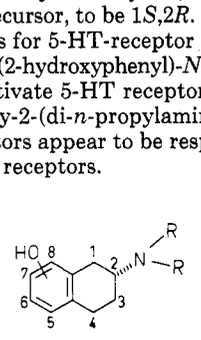
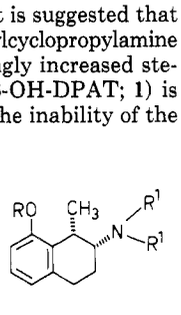
The conformational characteristics of two series of 5-hydroxytryptamine (5-HT) receptor agonists, monophenolic N,N-dialkylated 2-aminotetralins and *trans*-2-phenylcyclopropylamines, have been studied by a combination of experimental (NMR spectroscopy) and theoretical (molecular mechanics and MNDO calculations) methods. In addition, molecular electrostatic potentials have been calculated for selected conformations and the absolute configuration of the potent 5-HT-receptor agonist (+)-*cis*-8-hydroxy-1-methyl-2-(di-*n*-propylamino)tetralin (**2**) has been determined, by X-ray crystallography of the synthetic precursor, to be 1*S*,2*R*. Results obtained are discussed in terms of conformational, steric, and electronic requirements for 5-HT-receptor activation. It is suggested that different conformations of the 5-HT-receptor agonists (1*R*,2*S*)-2-(2-hydroxyphenyl)-*N*,*N*-di-*n*-propylcyclopropylamine [(1*R*,2*S*)-**4**] and its 3-hydroxy isomer (1*R*,2*S*)-**5** are able to activate 5-HT receptors. The strongly increased stereoselectivity of **2**, **4**, and **5** as compared to that of 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT; **1**) is rationalized on the basis of steric factors. Conformational factors appear to be responsible for the inability of the *trans*-C1-methyl-substituted derivative of **1** to activate 5-HT receptors.

8-Hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT; **1**) is a potent centrally active 5-hydroxytryptamine (5-HT) receptor agonist[1,2] with high selectivity for $5\text{-}HT_{1A}$-binding sites.[3,4] The *R* enantiomer of **1** is twofold more potent than the antipode.[1,5] Introduction of a *cis*-C1-methyl group in **1** led to the 5-HT agonist (+)-**2**,[6] which was found to be approximately equipotent to **1**. Interestingly, (−)-**2** and trans isomer (±)-**3** were found to be inactive.[6]

It has been shown that also monophenolic N,N-dialkylated derivatives of *trans*-2-phenylcyclopropylamine possess 5-HT receptor stimulating properties.[7] These compounds displayed high stereoselectivity, thereby being





(2R)-**1**: 8-OH; R = n-C_3H_7
(2R)-**6**: 7-OH; R = n-C_3H_7
(2R)-**10**: 8-OH; R = CH_3

(1S,2R)-**2**: R = H; R^1 = n-C_3H_7
(1S,2R)-**7**: R = CH_3; R^1 = n-C_3H_7
(1S,2R)-**11**: R = H; R^1 = CH_3

similar to the *cis*-C1-methylated derivative of **1**. The 5-HT-receptor activity of **4** and **5** resides in the 1*R*,2*S* en-

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(1) Arvidsson, L.-E.; Hacksell, U.; Nilsson, J. L. G.; Hjorth, S.; Carlsson, A.; Lindberg, P.; Sanchez, D.; Wikström, H. *J. Med. Chem.* **1981**, *24*, 921.
(2) Hjorth, S.; Carlsson, A.; Lindberg, P.; Sanchez, D.; Wikström, H.; Arvidsson, L.-E.; Hacksell, U.; Nilsson, J. L. G. *J. Neural Transm.* **1982**, *55*, 169.

0022-2623/88/1831-0212$01.50/0