duration of bleeding, is recorded. For each dose level, the percent of animals showing bleeding is also calculated. The subsequent evaluation of the compounds depends on the number and form of the dosages. When one or two dose levels are employed, the purpose is to ascertain the estrogenic action of the compound in the monkey. When several dose levels in increasing amounts are tested, the minimum effective dose that will cause withdrawal bleeding in all animals (MED_{100}) can be determined. When several dose levels of two or more compounds are tested, the ratios between their MED_{100} could be used to estimate their relative potencies in this test.

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Registry No. **1b**, 57-63-6; **7a**, 34111-53-0; **7b**, 4736-62-3; **7c**, 120574-27-8; **7d**, 120574-28-9; **7f**, 120476-04-2; **7g**, 16934-51-3; **7h**, 120476-05-3; **8a**, 59452-14-1; **8b**, 59077-04-2; **8c**, 59452-15-2; **8d**, 59452-16-3; **8e**, 65928-98-5; **8e** (17α isomer), 65929-00-2; **8i**, 120476-06-4; **9a**, 102177-29-7; **9b**, 84510-05-4; **10a**, 3342-64-1; **10b**, 5982-51-4; **10c**, 120476-07-5; **10d**, 7628-02-6; **11**, 60037-62-9; **12**, 1667-98-7; **13**, 120475-88-9; **14**, 120475-89-0; **15**, 99898-92-7; **16**, 97560-70-8; **17**, 120475-90-3; **18**, 120475-91-4; **19**, 120475-92-5; **20**, 73271-91-7; **21**, 17748-68-4; **22**, 120475-93-6; **23**, 95943-73-0; **24**, 120475-94-7; **25** (R = OH), 2487-49-2; **25** (R = Me), 10448-96-1; **26a**, 120475-95-8; **26b**, 120476-08-6; **26c**, 120476-09-7; **27a**, 120475-96-9; **27b**, 120476-10-0; **28**, 6803-21-0; **29a**, 120475-97-0; **29b**, 120476-11-1; **30**, 120475-98-1; **31**, 120475-99-2; **32**, 108887-34-9; **33**, 120476-00-8; **34**, 120476-01-9; **35**, 120476-02-0; **36**, 120476-03-1; PhLi, 591-51-5; estrone, 53-16-7; ethyltriphenylphosphonium bromide, 1530-32-1; propyltriphenylphosphonium bromide, 6228-47-3; butyltriphenylphosphonium bromide, 1779-51-7; trideuteriomethyl bromide, 1111-88-2; triphenylphosphine, 603-35-0; (trideuteriomethyl)triphenylphosphonium bromide salt, 1787-44-6; 3,21-diacetoxy-19-norpregna-1,3,5(10),17(20),20-pentaene, 120574-29-0; 6-dehydroestrone, 2208-12-0; methyltriphenylphosphonium bromide, 1779-49-3; (17(20)*Z*)-18-methyl-19-norpregna-1,3,5(10),17(20)-tetraen-3-ol, 120476-13-3; 3-acetoxy-17α-cyano-17β-hydroxyestra-1,3,5(10)-triene, 120661-79-2; 3-acetoxy-17-cyano-17-chloroestra-1,3,5(10)-triene, 120496-23-3; 2-hydrazinopyridine dihydrochloride, 62437-99-4; estrone acetate, 901-93-9; benzyl mercaptan, 100-53-8; estrone acetate bis(benzylthio) ketal derivative, 117864-98-9; triethylchlorosilane, 994-30-9; piperidinosulfur trifluoride, 33946-34-8; sulfur tetrafluoride, 7783-60-0; *N*-(trimethylsilyl)piperidine, 3768-56-7; 3-acetoxy-19-norpregna-1,3,5(10)-trien-20-one, 67530-18-1; 3-acetoxy-20,20-difluoro-19-norpregna-1,3,5(10)-triene, 120476-12-2; methyllithium, 917-54-4; (chloromethyl)trimethylsilane, 2344-80-1; estradiol, 50-28-2; 21-(triethylsilyl)-19-norpregna-1,3,5(10)-trien-20-yne-3,17β-diol, 50866-95-0; 3,11β,17β-trihydroxy-19-norpregna-1,3,5-(10)-trien-20-yne, 3762-05-8; 3,7α,17β-trihydroxy-19-norpregna-1,3,5(10)-trien-20-yne, 59903-16-1; 11β-methoxy-19-norpregna-1,3,5(10)-trien-20-yne-3,17β-diol, 34816-55-2.

Renin Inhibitors. Synthesis of Transition-State Analogue Inhibitors Containing Phosphorus Acid Derivatives at the Scissile Bond[1]

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The synthesis of five amino phosphorus derivatives, **1a–e**, is described. The derivatives were incorporated into a series (18) of analogues of the 5–14 portion of angiotensinogen, in most cases at the scissile Leu–Val bond. The resultant compounds were tested in vitro for their ability to inhibit human plasma renin. Replacement of the scissile bond with the phosphinic analogue of Leu[10]-Val[11] (**1b**) gave the most potent inhibitors, having $IC_{50} = 7.5 \times 10^{-8}$ M for H-Pro-His-Pro-Phe-His-(**1b**)-Ile-His-Lys-OH and $IC_{50} = 1.0 \times 10^{-7}$ M for Z-Arg-Arg-Pro-Phe-His-(**1b**)-Ile-His-NH_2. The shorter phosphonic acid sequence Z-Pro-Phe-His-(**1d**) retained biological activity with an $IC_{50} = 6.4 \times 10^{-6}$ M.

Renin is an aspartyl protease whose specific substrate is angiotensinogen. Cleavage of this substrate produces angiotensin I, which is further cleaved by angiotensin converting enzyme (ACE) to produce the pressor octapeptide hormone angiotensin II.[2] Interruption of this cascade has been shown to provide a means of lowering blood pressure in many hypertensive patients.[3-6] The interruption has been achieved specifically with the development of inhibitors of ACE,[7] and attention has now turned to the initial step in the cascade, the inhibition of renin action. Many laboratories have reported potent angiotensinogen analogue inhibitors of renin,[8] and most of these analogues possess putative transition-state dipeptide type mimics at the site of cleavage.

We report the synthesis and biological activities of a series of transition-state analogue inhibitors of renin,

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(1) Abbreviations follow the recommendations of the IUPAC–IUB Joint Commission on Biochemical Nomenclature for amino acids and peptides: *Eur. J. Biochem.* **1984**, *158*, 9–31. Additional abbreviations: ACE, angiotensin converting enzyme; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; Boc, *tert*-butoxycarbonyl; TFA, trifluoroacetic acid; TEA, triethylamine; HOBT, 1-hydroxybenzotriazole; Sta, (3*S*,4*S*)-4-amino-3-hydroxy-6-methylheptanoic acid; LPV or Leu^P-(CH_2)Val, 2(*R*,*S*)-[[hydroxy[1(*R*)-amino-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoic acid; LPG or Leu$^P(CH_2)$Gly, 3-[hydroxy[1(*R*)-amino-3-methylbutyl]phosphinyl]propanoic acid; Sta^P, 2-[hydroxy[1(*R*)-amino-3-methylbutyl]phosphinyl]acetic acid; Leu^{P}onic, [1(*R*)-amino-3-methylbutyl]-phosphonic acid; Leu^{P}inic, [1(*R*)-amino-3-methylbutyl]phosphinic acid; Leu^{Red}Val, *N*-(2-amino-4-methylpentyl)-1-carboxy-2-methylpropylamine; Leu^{C}Val, 2-isopropyl-4-hydroxy-5-amino-7-methyloctanoic acid.

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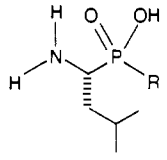
containing phosphorus acid mimics of the type **1a–e**.



1

1a: R = $(CH_2)_2CO_2H$	$Leu^P(CH_2)Gly$
b: R = $CH_2CH[CH(CH_3)_2]CO_2H$	$Leu^P(CH_2)Val$
c: R = CH_2CO_2H	Sta^P
d: R = OH	Leu^Ponic
e: R = H	Leu^Pinic

Analogues with potencies of up to 75 nM ($IC_{50} = 7.5 \times 10^{-8}$ M) for human renin were obtained.

Chemistry

Synthesis. Mimics. [1(*R*)-[(Benzyloxycarbonyl)-amino]-3-methylbutyl]phosphinic acid (**2**) (Scheme I) was synthesized as described by Baylis.[9] This was converted to the diastereoisomeric mixture of phosphinic esters (**3**) by using trimethyl phosphite. The mixture of diastereoisomers (**3**) that was obtained was used without further purification in subsequent reactions. The anion of **3** was generated by means of sodium methoxide in methanol in the presence of an acrylic ester, whereby conjugate addition of the anion to the acrylate ester gave phosphinic esters **4** (see also the recent publication of Parsons et al.[10]). In the case of the synthesis of **4a** from **3** an ethyl acrylate ester was used and the conjugate addition was accompanied by transesterification of the carboxylic ester function. Synthesis of the required amino-protected phosphinic acids **6a** and **6b** from **4a** and **4b** could be achieved either directly by base hydrolysis or, in the case of **6a**, more effectively by complete hydrolysis to **5a** and subsequent reprotection of the amino function. Selective hydrolysis of the phosphinic ester function of **4a** to give **7a** was by trimethylsilyl bromide. Phosphinic acid **2** was converted to the trivalent phosphonous ester (phosphonite) **8** by TMSC and base (esterification of racemic phosphonic ester **2** has been described elsewhere[11]). This underwent an Arbuzov reaction with bromoacetate esters, giving **9a** and **9b** after methanolysis. A similar approach was used by Giannousis[11] and Thottathil.[12] Base hydrolysis gave phosphastatine (**10**); hydrogenolysis selectively removed the amino protection, giving **11**.

Peptides. All the peptides described in this work were synthesized by solution procedures. The fragment condensation approach was adopted, and in the majority of instances couplings were achieved by using the DCC–HOBT method[13] in DMF without preactivation. This was the only method that we found to give reasonable quantities of required product when coupling phosphorus-containing fragments. The literature[14,15] indicates that couplings involving amino phosphorus acids may be accomplished in good yields provided that the phosphorus acid functionality possesses suitable covalent protection. There is evidence,[16] however, that couplings involving fragments not protected in this way are problematic. Such evidence is amply supported by this work. Coupling rates were very slow, and reactions took from 2 to 10 days for completion (assessed by TLC by the disappearance of starting materials) with additional equivalents of DCC and HOBT added during the course of the reaction. However, it was felt that the prevention of formation of additional diastereoisomers, which would be generated by such a strategy, justified the adopted approach. Any amidine formation[17] caused by the use of excess DCC in couplings involving histidine was reversed by treatment with methanol–acetic acid–water. Workup of the normally complex mixture of products was further complicated by the presence of the required product, in some cases, in two diastereoisomeric forms (e.g., from couplings involving **5a**, **6a**, and **7a**). The two products, where resolved, were assayed individually, and only the biologically more active isomer is quoted. HPLC resolution of the two diastereoisomeric forms of the $Leu^P(CH_2)Val$ mimic proved unsuccessful at the diacid stage (**6a**). However, analytical HPLC satisfactorily separated the four isomers of the diester **4a**, and semipreparative HPLC then allowed purification of two of these isomers. Subsequent conversion of the separated isomers to the Z diacids (**6a**) by the route already described provided the two isomers. Absolute configurations were not assigned, though one might expect the active isomer to possess the *R,S* configuration corresponding to the natural substrate. The far from ideal nature of the coupling reaction suggests the possibility of extensive racemization of the activated species. In all cases where a racemization test[18] was undertaken, a racemization

(2) For recent reviews and discussions see: (a) Vallotton, M. B. *Trends Pharmacol. Sci.* **1987**, *8*, 69. (b) Menard, J.; Corvol, P. *Clin. Invest. Med.* **1986**, *9*, 309. (c) Haber, E. *Hypertension* **1986**, *8*, 1093. (d) Greenlee, W. J. *Pharm. Res.* **1987**, *4*, 364. (e) Boger, J. In *Annual Reports in Medicinal Chemistry*; Bailey, D. M., Egan, R. W., Eds.; Academic: New York, 1985; Vol. 20, p 257.

(3) Laragh, J. H. *Am. J. Med.* **1984**, *77(2A)*, 1.

(4) Cleland, J. G. F.; Dargie, H. J.; Hadsman, G. P.; Bell, S. G.; Robertson, J. I. S.; Morton, J. J.; East, B. W.; Robertson, I.; Murray, G. D.; Gillen, G. *Br. Heart J.* **1984**, *52*, 530.

(5) Materson, B. J.; Freis, E. D. *Arch. Intern. Med.* **1984**, *144*, 1947.

(6) Ferguson, R. K.; Vlasses, P. H.; Rotmensch, H. H. *Am. J. Med.* **1984**, *77*, 690.

(7) Sweet, C. S.; Blaine, E. H. *Cardiovascular Pharmacology*; Antonaccio, M., Ed.; Raven Press: New York, 1984; p 119.

(8) (a) Boger, J.; Payne, L. S.; Perlow, D. S.; Lohr, N. S.; Poe, M.; Blaine, E. H.; Ulm, E.; Schorn, T.; LaMont, B. I.; Lin, Tsan-Yen; Kawai, M.; Rich, D. H.; Veber, D. F. *J. Med. Chem.* **1985**, *28*, 1779. (b) Johnson, R. L. *J. Med. Chem.* **1984**, *27*, 1351. (c) Luly, J. R.; Bolis, G.; BaMaung, N.; Soderquist, J.; Dellaria, J. F.; Stein, H.; Cohen, J.; Perun, T. J.; Greer, J.; Plattner, J. J. *J. Med. Chem.* **1988**, *31*, 532. (d) Salituro, F. G.; Agarwal, N.; Hofmann, T.; Rich, D. H. *J. Med. Chem.* **1987**, *30*, 286. (e) Thaisrivongs, S.; Pals, D. T.; Turner, S. R.; Kroll, L. T. *J. Med. Chem.* **1988**, *31*, 1369. (f) Bock, M. G.; DiPardo, R. M.; Evans, B. E.; Freidinger, R. M.; Rittle, K. E.; Payne, L. S.; Boger, J.; Whitter, W. L.; LaMont, B. I.; Ulm, E. H.; Blaine, E. H.; Schorn, T. W.; Veber, D. F. *J. Med. Chem.* **1988**, *31*, 1918. (g) Buhlmayer, P.; Caselli, A.; Fuhrer, W.; Goschke, R.; Rasetti, V.; Rueger, H.; Stanton, J. L.; Criscione, L.; Wood, J. M. *J. Med. Chem.* **1988**, *31*, 1839.

(9) Baylis, E.; Campbell, C.; Dingwall, J. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2845.

(10) Parsons, W. H.; Patchett, A. A.; Bull, H. G.; Schoen, W. R.; Taub, D.; Davidson, J.; Combs, P. L.; Springer, J. P.; Gadesbusch, H.; Weissberger, B.; Valiant, M. E.; Mellin, T. N.; Busch, R. D. *J. Med. Chem.* **1988**, *31*, 1772.

(11) Giannousis, P. P.; Bartlett, P. A. *J. Med. Chem.* **1987**, *30*, 1603.

(12) Thottathil, J. K.; Przybala, C. A.; Moniot, J. L. *Tetrahedron Lett.* **1984**, *25*, 4737. Thottathil, J. K.; Ryono, D. E.; Przybala, C. A.; Moniot, J. L.; Neubeck, R. *Tetrahedron Lett.* **1984**, *25*, 4741.

(13) Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788.

(14) Bartlett, P. A.; Kezer, W. B. *J. Am. Chem. Soc.* **1984**, *106*, 4282.

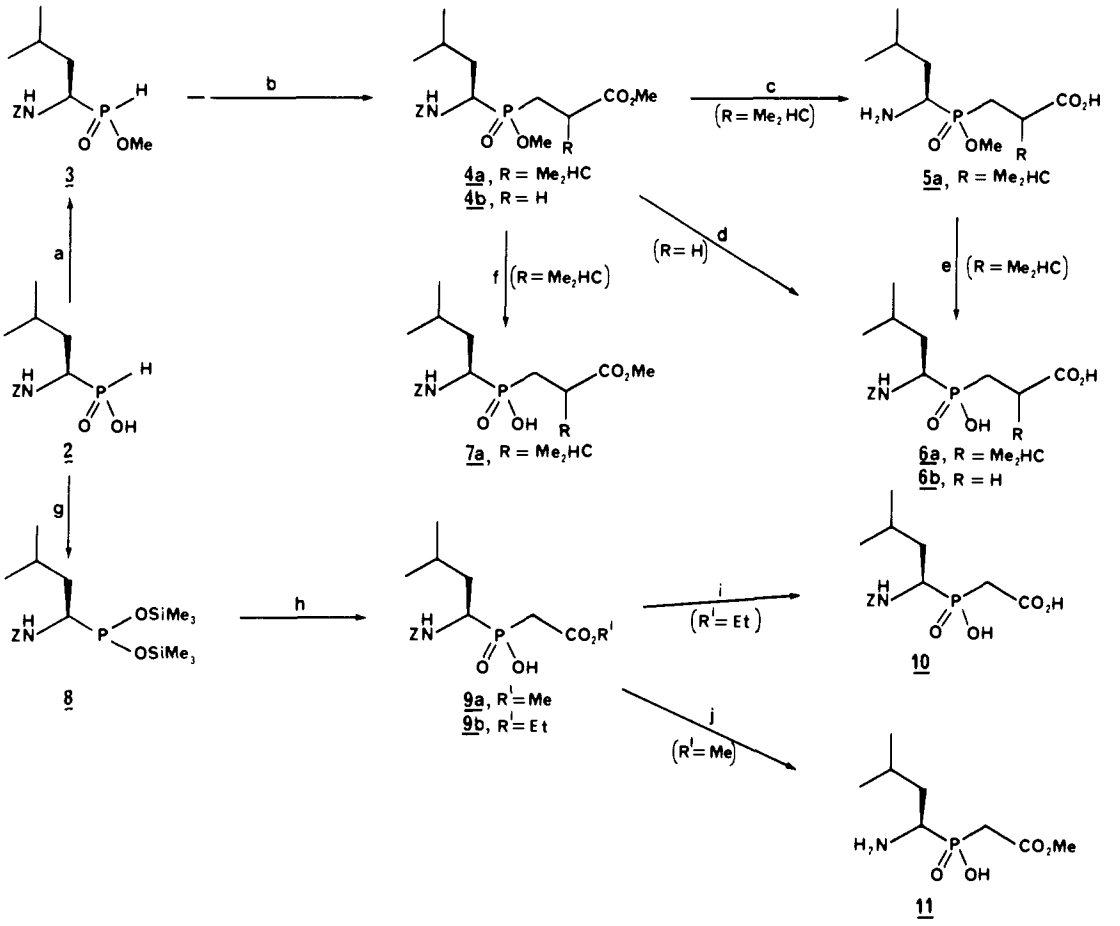
(15) Bartlett, P. A.; Acher, F. *Bull. Soc. Chim. Fr.* **1986**, *5*, 771.

(16) Kafarski, P.; Lejczak, B.; Mastalerz, P. *Beitr. Wirkstofforsch.* **1985**, *25*.

(17) Rink, H.; Riniker, B. *Helv. Chim. Acta* **1974**, *57*, 831.

(18) Manning, J.; Moore, S. *J. Biol. Chem.* **1968**, *243*, 5591.

Scheme I. Synthesis of Amino Phosphorus Acid Derivatives[a]



[a] (a) $(MeO)_3P$; (b) $CH_2{=}C(R)CO_2Me$; (c) aqueous HBr; (d) NaOH, H_2O, MeOH; (e) ZCl, NaOH; (f) Me_3SiBr; (g) Me_3SiCl, Et_3N; (h) $BrCH_2CO_2R'$; (i) NaOH, H_2O, MeOH; (j) H_2, Pd/C.

Table I. Characterization of Renin-Inhibitory Peptides

	amino acid analysis[b]																FAB-MS	
compd[a]	Ala	Arg	Asp	Gaba	Gly	His	Ile	Lys	Phe	Pro	Sta	LPV	LPG	Sta^P	Leu^P-inic	Leu^P-onic	(M′ + H$^+$), m/z	TLC, R_f[c]
12						2.93	0.96	1.00	1.02	2.04		0.81					1259	0.19A, 0.07B
13						2.07	0.96		1.00			1.01					933	0.14B, 0.40E
14						2.04	1.03		0.97				1.03				891	0.15E, 0.59G
15						2.03	1.01		1.00					1.03			877	0.21D, 0.20E
16						0.97	0.99		2.00			0.83					943	0.77D, 0.54E
17		1.00				1.02	1.02		1.00			1.02					1009	0.43E, 0.29H
18						1.05	1.02		1.97			1.03					909	0.39D, 0.26F
19						1.00	1.01		1.00			0.97					923	0.41D, 0.51E
20						1.00	1.03		1.00			0.95					913	0.33E, 0.20F
21						2.03	1.02		1.03	0.96		1.05					1030	0.60E, 0.25F
22					1.04	1.94	0.97		1.00			0.88					990	0.22E, 0.37F
23		2.08				2.02	0.99		1.00	1.03		0.96					1343	0.39B, 0.60C
24		2.03				2.00	0.95		1.04	0.95			1.05				1300	0.19A, 0.47C
25	1.01					1.03			0.98	1.02	0.78	0.83					1023	0.84E, 0.35F
26	0.98					1.02			1.00		0.24			0.95			870	0.26D, 0.26F
27						1.03			0.98		1.05	0.85					855	0.67E, 0.21F
28						1.04			0.98		0.97				0.96		667	0.28E, 0.11F
29						0.99			1.00		0.99					0.98	683	0.26E, 0.12F

[a] See Table II for sequence. [b] Following acid hydrolysis. Sta and LPV subject to variable decomposition during hydrolysis. [c] For solvent systems (A, B, etc.), see Experimental Section.

level no greater than 3% was detected—reflecting either the absence of racemization or the efficacy of the cleanup procedure. *N*-Acylurea formation was however a major problem in leading to reduced yields. The side products, however, were generally easily removed during the purification process. The products were assessed for homogeneity by TLC and analyzed by amino acid analysis (following acid hydrolysis) and FAB-MS. Elemental analysis of the lyophilized products was not routinely undertaken, since we have found[19] that the results obtained have little meaning for unprotected or partially protected peptides of the size discussed in this work. Protection of reactive functional groups during coupling was achieved by standard methods (e.g., urethane amino protection and

(19) Allen, M. C.; Brundish, D. E.; Wade, R. *J. Chem. Soc., Perkin Trans. 1* **1979**, 2057. Allen, M. C.; Brundish, D. E.; Wade, R. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1928.

Table II. Renin Inhibition by Substrate Analogues Containing $Leu^P(CH_2)Val$, $Leu^P(CH_2)Gly$, and Sta^P [a]

no.	compound	renin inhibition, human plasma (pH 7.2) (IC_{50}), × 10^{-6} M
12	H-Pro-His-Pro-Phe-His-$Leu^P(CH_2)$Val-Ile-His-Lys-OH	0.075
13	Z-Phe-His-$Leu^P(CH_2)$Val-Ile-His-NH_2	2.0
14	Z-Phe-His-$Leu^P(CH_2)$Gly-Ile-His-NH_2	>30.0
15	Z-Phe-His-Sta^P-Ile-His-NH_2	>30.0
16	Z-Phe-His-$Leu^P(CH_2)$Val-Ile-Phe-NH_2	5.0
17	Z-Phe-His-$Leu^P(CH_2)$Val-Ile-Arg-OBu^t	>30.0
18	Boc-Phe-Phe-$Leu^P(CH_2)$Val-Ile-His-NH_2 [b]	6.0
19	Boc-Phe-MePhe-$Leu^P(CH_2)$Val-Ile-His-NH_2 [b]	>30.0
20	Boc-Phe-MeHis-$Leu^P(CH_2)$Val-Ile-His-NH_2 [b]	>30.0
21	Z-Pro-Phe-His-$Leu^P(CH_2)$Val-Ile-His-NH_2	0.60
22	Z-Gly-Phe-His-$Leu^P(CH_2)$Val-Ile-His-NH_2 [b]	0.80
23	Z-Arg-Arg-Pro-Phe-His-$Leu^P(CH_2)$Val-Ile-His-NH_2	0.10
24	Z-Arg-Arg-Pro-Phe-His-$Leu^P(CH_2)$Gly-Ile-His-NH_2	10.0
25	Z-Pro-Phe-His-$Leu^P(CH_2)$Val-Ala-Sta-OMe	0.90
26	Z-Phe-His-Sta-Ala-Sta^P-OMe	0.04
27	Z-Phe-His-Sta-$Leu^P(CH_2)$Val-OMe	>30.0
28	Z-Pro-Phe-His-Leu^Pinic	>30.0
29	Z-Pro-Phe-His-Leu^Ponic	6.40

[a] Abbreviations: $Leu^P(CH_2)Val$, 2(*R,S*)-[[hydroxy[1(*R*)-amino-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoic acid; $Leu^P(CH_2)Gly$, 3-[hydroxy[1(*R*)-amino-3-methylbutyl]phosphinyl]propanoic acid; Sta^P, 2-[hydroxy[1(*R*)-amino-3-methylbutyl]phosphinyl]acetic acid; Leu^Ponic, [1(*R*)-amino-3-methylbutyl]phosphonic acid; Leu^Pinic, [1(*R*)-amino-3-methylbutyl]phosphinic acid. [b] Ca. 1:1 mixture of isomers at the valyl α-carbon.

alkyl ester carboxyl protection), except for the phosphinic acid function, which was protected as its triethylammonium salt throughout. Characterization data are given in Table I.

In Vitro Enzyme Inhibition. The synthetic peptides were tested for their ability to inhibit human plasma renin by using the in vitro assay system described previously.[20] The concentration of peptide that inhibited plasma renin activity by 50% (IC_{50}) was determined and is displayed in Table II.

Results and Discussion

The use of phosphinic acid derivatives in peptidic enzyme inhibitors has, until recently, been confined to phosphonamidate-containing inhibitors of zinc and serine peptidases, e.g., carboxypeptidase A,[21] thermolysin,[22] and chymotrypsin.[23] Petrillo and co-workers have described the synthesis of a series of (phosphinyloxy)acyl[24] and phosphinylalkanoyl[25] amino acid derivatives as components in a series of ACE inhibitors—with good effect. The concept has been further extended by Bartlett and coworkers[14,15] with the synthesis of the phosphinic acid analogue of statine and its inclusion in a series of peptides subsequently shown to have a potent inhibitory effect on the action of the aspartyl protease pepsin. More recently, reports have been published describing the synthesis and use of phosphinic acid derivatives as inhibitors of collagenase,[26] D-alanyl-D-alanine ligase,[10] and leucine aminopeptidase.[11]

We have applied this approach to another aspartyl protease, renin, with a view to preparing antihypertensive renin-inhibitory angiotensinogen analogues. In addition to the preparation of a statine phosphinic acid analogue (**1c**),[14] phosphinic acid analogues (**1a** and **1b**) of the natural sequence around the scissile bond, i.e., Leu–Val and also Leu–Gly, have been synthesized. During the preparation of this paper, a patent[27] was published describing the synthesis of similar phosphinic acid dipeptide mimics for similar purposes, though the synthetic approaches used both for preparing the mimics and for their inclusion in peptide structures differed significantly from our own. Additionally, no examples of peptides containing the mimics detailed in our work were described in the patent.

The initial decapeptide sequence examined contained amino- and carboxy-terminal substitutions originally suggested by Burton.[28] Inclusion of mimic **1b** in place of native Leu^{10}-Val^{11} gave a peptide (**12**) with greatly enhanced renin-inhibitory effect—and of the same order as comparable sequences containing other transition-state mimics (e.g., statine, $Leu^{Red}Val$, and $Leu^{C}Val$).[29] Reduction in length of the sequence to a hexapeptide (**13**) also containing mimic **1b** gave a compound with significant, if reduced, activity and became the initial lead compound. Replacement of the mimic **1b** with either **1a** or **1c** gave peptides **14** and **15**, respectively, with potencies below the range of the assay. This is a little surprising in the case of the phosphastatine (Sta^P) analogue **15** in view of the reported[14] ability of Sta^P to replace Sta in analogues of pepstatin as inhibitors of pepsin. The differences observed, however, may be attributable, in part, to the different pH optima of the enzymes. Pepsin has its pH optimum in the very acidic range, reflected by the pH (3.5) of the assay described. It is probable therefore that the reported compounds are relatively weak renin inhibitors because the hydroxy function at the phosphorus is largely deprotonated at pH 7.2 and therefore not ideal to bind to the aspartates at the active site of renin.

(20) Wood, J. M.; Gulati, N.; Forgiarini, P.; Fuhrer, W.; Hofbauer, K. G. *Hypertension* **1985**, *7*, 797.
(21) Jacobsen, N. A.; Bartlett, P. A. *J. Am. Chem. Soc.* **1981**, *103*, 654.
(22) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1983**, *22*, 4618.
(23) Bartlett, P. A.; Lamden, L. A. *Bioorg. Chem.* **1986**, *14*, 356.
(24) Karenewsky, D. S.; Badia, M. C.; Cushman, D. W.; DeForrest, J. M.; Dejneka, T.; Loots, M. J.; Perri, M. G.; Pertrillo, E. W.; Powell, J. R. *J. Med. Chem.* **1988**, *31*, 204.
(25) Petrillo, E. W.; Cushman, D. W.; Duggan, M. E.; Heikes, J. E.; Karanewsky, D. W.; Ondetti, M. A.; O'Reilly, B.; Rovnyak, G. O.; 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.
(26) Broadhurst, M.; Handa, B. K.; Johnson, W. H.; Lawton, G.; Machin, P. J. Europ. Pat. 276436A, 1988.
(27) Patchett, A. A.; Greenlee, W. J.; Parsons, W. H. Europ. Pat. 210545, 1987.
(28) Burton, J.; Gody, R. J.; Herd, J. A.; Haber, E. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5476.
(29) Unpublished data.

Replacement of the histidine residues (positions 9 and 13) with phenylalanine (compounds **16** and **18**) conserved the inhibitory effect at a slightly lower level, while substitution of His(9) by its *N*-methyl derivative in **20** or by *N*-methylphenylalanine in **19** was not tolerated—reflecting a possible need for a bulky or aromatic side chain at positions 9 and 13 and the need for hydrogen bonding at the nitrogen of residue 9. An increase in the chain length at the amino terminus by a single residue led to a significant increase in inhibitory activity—whether by addition of the native proline or by glycine. Replacement of statine by mimic **1b** in the nonapeptide analogue CGP 29287[20] gave a compound (**23**) with appreciable though diminished activity. Inhibitory activity was virtually abolished however, by the incorporation of the mimic **1a**—compound **24**.

In the analogues so far discussed only those containing mimic **1b** possessed any appreciable degree of inhibitory activity. In addition, a progressive loss of activity was observed as the chain length decreased—a trend that is reflected in other series[30] and has serious implications for any potential clinically useful renin inhibitor. Incorporation of a partial dipeptide sequence (Ala-Sta) of pepstatin on the carboxy-terminal side of the **1b** mimic gave material (**25**) with a greater inhibitory activity as compared to the original hexapeptide (**13**) while replacement of the mimic with statine and incorporation of the phosphinic acid derivative of the above partial pepstatin sequence gave material (**26**) with enhanced activity as compared to **13**, but of similar potency as compared to the non-phosphorus-containing analogue.[29]

Applying the arguments of Kokubu and his co-workers,[31] we prepared the 7–11 tetrapeptide sequences of angiotensinogen possessing carboxy-terminal phosphinic (**28**) and phosphonic (**29**) groups in place of the carboxylic function of leucine. The literature reveals[32] that simple substitution of phosphonate for carboxylate in enzyme inhibitors does not guarantee better binding and that correct alignment of functional groups with the active or binding site appears to be more important. It is interesting to note that the phosphonic acid derivative (**29**) displayed a relatively high level of inhibitory activity when compared to the phosphinic acid derivative (**28**). This may be an indication that, in this instance at least, the degree of ionization of the phosphorus hydroxyls is not the major contributing factor to the inhibitory activity of **29**. It may be possible to conclude that the phosphonic acid function is a superior transition-state mimic in this situation. The activity shown by the tetrapeptide phosphonic acid derivative (**29**) does indicate the prospect of small peptidelike inhibitors of renin and suggests a future area for fruitful investigation.

Experimental Section

Thin-layer chromatography (TLC) was performed on silica gel plates (E. Merck, silica gel 60 F-254), and components were visualized by chlorine–starch–KI reagents[33] (for NH), Pauly spray[34] (for His), Sakaguchi spray[35] (for Arg), and Phospray[36] (for P). Systems used in TLC were as follows: 1-butanol–pyridine–acetic acid–H_2O, 42:24:4:30 (A); ethyl acetate–1-butanol–acetic acid–H_2O, 1:1:1:1 (B); $CHCl_3$–CH_3OH–H_2O–acetic acid, 85:13:1.5:0.5 (C), 80:20:3:3 (D), 75:27:5:0.5 (E); $CHCl_3$–CH_3OH–concentrated aqueous NH_3, 40:10:1 (F); 41:41:18 (G); $CHCl_3$–CH_3OH, 98:2 (H); 1-butanol–acetic acid–H_2O, 10:1:3 (J); 1-butanol–pyridine–acetic acid–H_2O, 5:5:1:3 (L); $CHCl_3$–CH_3OH–acetic acid, 88:7:5 (M); ethyl acetate–pyridine–acetic acid–H_2O, 62:21:6:11 (P); $CHCl_3$–CH_3OH–acetic acid–H_2O, 70:40:0.5:10 (Q); 1-butanol–pyridine–acetic acid–H_2O, 38:24:8:30 (R). Column chromatography was performed on silica gel 60, 0.04–0.063 mm (E. Merck), eluting under a positive pressure of ca. 20 psi of nitrogen ensuring a flow rate of ca. 5 mL/min with systems C–F and H or suitable modifications thereof. NMR spectra (^1H) were recorded on a Varian EM-360 spectrometer and are expressed in parts per million (ppm) from tetramethylsilane as internal standard. NMR spectra (^{31}P) were obtained with a Jeol FX 90Q spectrometer with 80% H_3PO_4 as external reference. Amino acid analyses were performed on a Beckman 120C analyzer following acid hydrolysis in 6 N HCl for 16 h at 115 °C. Quantitative determination of Sta and LPV revealed variable degrees of degradation during hydrolysis. Fast atom bombardment mass spectrometry (FAB-MS) was performed on a VG-7070E spectrometer with a 11250 data system.

General Synthetic Procedures. General Procedure A. Coupling Reactions Involving Dicyclohexylcarbodiimide and 1-Hydroxybenzotriazole.[13] The amino component hydrochloride (1.0 mmol), the carboxy component (1.0 mmol), and HOBT (1.3 mmol) were dissolved, at 0 °C, in DMF (15 mL) containing TEA (1.0 mmol). The solution was treated with DCC (1.2 mmol) for 1 h at 0 °C and for 24 h at room temperature. Two further portions of DCC (2 × 1.2 mmol) were administered to the reaction mixture at 24-h intervals. On reaction completion (assessed by TLC by consumption of both starting materials) the mixture was evaporated to dryness in vacuo and the residue dissolved in methanol–acetic acid–H_2O, 94:3:3 (40 mL), and stirred for 1 h at 60 °C. The solution was evaporated to dryness and the residue suspended in methanol (5 mL), stored at 0 °C for 15 min, and filtered. The filtrate was evaporated to dryness and chromatographed.

General Procedure B. Coupling Reactions Involving Dicyclohexylcarbodiimide and 1-Hydroxybenzotriazole.[13] The amino component (1.0 mmol), the carboxy component (1.0 mmol), and HOBT (1.3 mmol) were dissolved, at 0 °C, in the minimum volume of DMF (between 5 and 25 mL) containing TEA (1.0 mmol). The solution was treated with DCC (2.4 mmol) for 1 h at 0 °C and 40 h at room temperature. The mixture was evaporated to dryness and worked up as described in general procedure A.

General Procedure C. Removal of Benzyloxycarbonyl Groups by Catalytic Hydrogenation. The *N*-(benzyloxycarbonyl) compound was dissolved in the minimum volume of 90% aqueous methanol (typically to a concentration of 10 mM). The mixture was vigorously stirred over 10% palladium-on-carbon catalyst under an atmosphere of hydrogen at ca. 16 psi for 1–2 h. Reaction completion was assessed by TLC. The mixture was filtered and evaporated in vacuo.

General Procedure D. Saponification of Peptide Esters. The peptide ester (1.0 mmol) was dissolved in ethanol, methanol, or a methanol–DMF mixture (ca. 5 mL), to which was added 1 N NaOH (1.2 mmol). On reaction completion (TLC, usually 1–2 h) the solution was cooled to 0 °C, neutralized with 1 N HCl, and evaporated in vacuo. The residue was dissolved in H_2O and washed with ethyl acetate and the aqueous layer cooled to 0 °C and acidified (pH 2) with 1 N citric acid, before extracting with 1-butanol–ethyl acetate, 1:1 (v/v). The organic layer was washed with water, dried (Na_2SO_4), and evaporated to dryness.

[1(*R*)-[(Benzyloxycarbonyl)amino]-3-methylbutyl]phosphinic acid (2) was prepared as described by Baylis et al.[9]

(30) Dann, J. G.; Stammers, D. K.; Harris, C. J.; Arrowsmith, R. J.; Davies, D. E.; Hardy, G. W.; Morton, J. A. *Biochem. Biophys. Res. Commun.* **1986**, *134*, 71.
(31) Kokubu, T.; Hiwada, K.; Sato, Y.; Iwata, T.; Imamura, Y.; Matsueda, R.; Yabe, Y.; Kogen, H.; Yamazaki, M.; Iijima, Y.; Baba, Y. *Biochem. Biophys. Res. Commun.* **1984**, *118*, 929.
(32) Galardy, R. E.; Kontoyiannidou-Ostrem, V.; Kortylewicz, Z. P. *Biochemistry* **1983**, *22*, 1990. Brand, L. B.; Lowenstein, J. B. *Biochemistry* **1978**, *17*, 1363.
(33) Rydon, H. N.; Smith, P. W. G. *Nature (London)* **1953**, *169*, 922.
(34) Smith, I. In *Chromatographic and Electrophoretic Techniques*; Smith, I., Ed.; Vol. 1, William Heinemann: London, 1960; p 96.
(35) Bugard, E.; Mauron, J. *J. Chromatogr.* **1966**, *21*, 19.
(36) Phospray reagent, available from Supelco chromatography supplies.
(37) Guttman, S.; Pless, J.; Huguenin, R.; Sandrin, E.; Bossert, H.; Zehnder, K. *Helv. Chim. Acta* **1969**, *52*, 1789.

Methyl [1(*R*)-[(Benzyloxycarbonyl)amino]-3-methylbutyl]phosphinate (3). A mixture of the phosphinic acid **2** (8.6 g, 30.1 mmol) and trimethyl phosphite (50 mL, 0.42 mmol) was stirred in an inert atmosphere at 60 °C for 1 h. The excess trimethyl phosphite and dimethyl phosphite were removed by evaporation in vacuo. Trituration of the residue with diethyl ether and pentane and allowing the mixture to stand at −10 °C for several days gave a crystalline solid which was collected, washed, and dried. The product **3** (8.2 g, 91%) was shown to be a mixture of diastereoisomers [^{31}P NMR ($CHCl_3$) δ 38.8, 38.1] and was used in further synthesis without purification. Giannousis and Bartlett[11] describe the synthesis of racemic esters **3** from racemic acid **2** and report ^{31}P NMR (CD_3OD) δ 38.3, 37.0.

Methyl 2(*R*,*S*)-[[(*R*,*S*)-Methoxy[1(*R*)-[(benzyloxycarbonyl)amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoate (4a). A solution of sodium methoxide in methanol [made by dissolving sodium (0.87 g, 0.038 mol) in methanol (10 mL)] was added dropwise over 3 h to a cooled (0 °C), stirred solution of ester **3** (11.25 g, 0.038 mol) and methyl 3-methyl-2-methylenebutanoate (5.5 g, 0.043 mol) in methanol (30 mL). The reaction mixture was allowed to warm to room temperature and stirred for 16 h before evaporation of the solvent in vacuo. The resulting viscous oil was partitioned between water (50 mL) and diethyl ether (100 mL), the separated aqueous layer extracted—again with ether (100 mL)—and the combined organic extracts washed with 5% KH_2PO_4 solution, dried ($MgSO_4$), and evaporated. The product, **4a** (12.1 g, 75%), was obtained as a viscous oil and shown to be a mixture of four diastereoisomers. ^{31}P NMR ($CDCl_3$) δ 55.8, 54.5, 54.3, 53.8. Partial separation of the diastereoisomers was effected by preparative HPLC on a column (21 × 250 mm) of Zorbax ODS eluting with MeCN–H_2O, 55:45 (v/v), at 5 mL/min, with UV detection at 220 nm.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[(benzyloxycarbonyl)amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoic Acid (6a). (a) A mixture of phosphinic ester **4a** (5.0 g, 11.7 mmol) and 48% aqueous HBr (50 mL) was heated under reflux for 6 h and then cooled and evaporated to dryness under reduced pressure. The phosphinic acid **5a** was obtained as the hydrobromide salt in quantitative yield. (b) The hydrobromide salt (19.0 g, 67 mmol) was dissolved in water (75 mL) and adjusted to pH 9.5 with 4 N NaOH before cooling to 0 °C. Benzyl chloroformate (10.3 g, 60 mmol) was added dropwise with stirring over 30 min with simultaneous addition of 4 N NaOH to maintain pH 9.5. After being warmed to room temperature, the solution was washed with ether and the aqueous phase cooled to 0 °C before acidifying with 10.5 N HCl. The precipitated oil was extracted into ethyl acetate, dried ($MgSO_4$), and evaporated to give the product **6a** (14.0 g, 65%). Pure product was obtained by crystallization: mp 154–156 °C (ethyl acetate/diethyl ether); ^{1}H NMR ($CDCl_3$) δ 11.2 (s, 2 H), 7.4 (s, 5 H), 6.2–5.4 (m, 2 H), 5.2 (s, 2 H), 4.3–3.7 (m, 1 H), 3.0–1.3 (m, 8 H), 1.1–0.7 (m, 12 H); ^{31}P NMR ($CHCl_3$/DMSO) δ 52.4, 51.6. Anal. ($C_{19}H_{30}NO_6P$) C, H, N.

Methyl 2(*R*,*S*)-[[Hydroxy[1(*R*)-[(benzyloxycarbonyl)amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoate (7a). A mixture of phosphinic ester **4a** (2.14 g, 5.0 mmol) and trimethylsilyl bromide (0.84 g, 5.5 mmol) in methylene chloride (25 mL) was stirred at room temperature for 3 h and then evaporated at reduced pressure. The residue was dissolved in ethyl acetate and an excess of methanol added to decompose the phosphinylsilyl ester. Evaporation of the solvent gave product **7a**. Pure product was obtained by crystallization in 42% yield: mp 123–124 °C; ^{1}H NMR ($CDCl_3$) δ 11.3 (s, 1 H), 7.4 (s, 5 H), 5.4 (d, 1 H) 5.2 (s, 2 H), 4.3–3.9 (m, 1 H), 3.7 (s, 3 H), 3.0–1.3 (m, 7 H), 1.2–0.7 (m, 12 H); ^{31}P NMR ($CDCl_3$) δ 55.8, 54.8. Anal. ($C_{20}H_{32}NO_6P$) C, H, N, P.

3-[Hydroxy[1(*R*)-[(benzyloxycarbonyl)amino]-3-methylbutyl]phosphinyl]propanoic Acid (6b). A solution of sodium methoxide [made from sodium (0.36 g, 15.6 mmol) and methanol (10 mL)] was added to a stirred, cooled (0 °C) mixture of phosphinic ester **3** (4.5 g, 15.2 mmol) and methyl acrylate (1.72 g, 20.0 mmol) in methanol (20 mL). The resulting solution was allowed to warm to room temperature and evaporated and the residue partitioned between water and ethyl acetate. The organic layer was washed with KH_2PO_4 solution, dried ($MgSO_4$), and evaporated, giving phosphinic ester **4b** (3.4 g, 59%). This ester was dissolved in ethanol (10 mL), and 2 N NaOH (10 mL) was added. After the solution was stirred at ambient temperature for 16 h, the ethanol was evaporated off in vacuo and the residue acidified with 2 N HCl. The mixture was extracted with ethyl acetate, and the organic solution ($MgSO_4$) was evaporated. The residue was stirred with a little diethyl ether and the product **6b** collected in 64% yield: mp 188–190 °C; $[\alpha]^{23}_D$ −20.8° (*c* 1.0, DMF); ^{1}H NMR (DMSO) δ 9.4 (s, 2 H), 7.55 (d, 1 H), 7.38 (s, 5 H), 5.10 (s, 2 H), 4.1–3.45 (m, 1 H), 2.75–2.2 (m, 2 H), 2.1–1.2 (m, 5 H), 0.85 (t, 6 H); ^{31}P NMR (DMF) δ 48.0. Anal. ($C_{16}H_{24}NO_6P$) C, H, N, P.

2-[Hydroxy[1(*R*)-[(benzyloxycarbonyl)amino]-3-methylbutyl]phosphinyl]acetic Acid (10). (a) A solution of phosphinic acid **2** (14.25 g, 50 mmol) and TEA (10.1 g, 0.1 mol) in THF (50 mL) was stirred at room temperature in an atmosphere of nitrogen while trimethylsilyl chloride (12.5 g, 0.1 mol) was added dropwise over 1 h. The precipitated triethylamine hydrochloride was removed by filtration and ethyl bromoacetate (8.5 g, 50 mmol) added dropwise to the stirred filtrate. Stirring was continued for 2 h before evaporating and partitioning the residue between water and ethyl acetate. The organic solution was washed successively with 2 N HCl and water and then dried ($MgSO_4$) and evaporated. Trituration of the residual oil with diethyl ether gave phosphinic acid **9b** in 78% yield, crystallized from ethyl acetate/diethyl ether: $[\alpha]^{23}_D$ −33.5° (*c* 1.0, EtOH); ^{1}H NMR ($CDCl_3$) δ 9.65 (s, 1 H), 7.33 (s, 5 H), 5.50 (d, 1 H), 5.10 (s, 2 H), 4.15 (q, 2 H), 4.2 (m, 1 H), 2.98 (d, 2 H), 1.8–1.4 (m, 3 H), 1.25 (t, 3 H), 0.93 (d, 6 H); ^{31}P NMR ($CDCl_3$) δ 45.1. (b) The above phosphinic acid **9b** (10.0 g, 27 mmol) in ethanol (100 mL) was added to a solution of NaOH (2.5 g, 62.5 mmol) in water (75 mL) and the mixture stirred at room temperature for 16 h. Evaporation followed by acidification of the residue with 2 N HCl and addition of ethyl acetate provided crystalline material **10** in 78% yield: mp 166–167 °C; $[\alpha]^{23}_D$ −30.2° (*c* 1.0, DMF); ^{1}H NMR (DMSO) δ 8.9 (s, 2 H), 7.38 (s, 5 H), 7.3 (d, 1 H), 5.1 (s, 2 H), 3.9 (m, 1 H), 2.78 (d, 2 H), 1.8–1.2 (m, 3 H), 0.87 (m, 6 H); ^{31}P NMR (DMSO) δ 38.7. Anal. ($C_{15}H_{22}NO_6P·H_2O$) C, H, N.

Methyl 2-[Hydroxy[1(*R*)-amino-3-methylbutyl]phosphinyl]acetate (11). (a) Methyl ester **9a** was prepared by a method similar to that described for the ethyl ester **9b** above, but with methyl bromoacetate in place of ethyl bromoacetate. The product was crystallized from methanol/water: $[\alpha]^{24}_D$ −35.8° (*c* 1.6, MeOH); ^{1}H NMR (CD_3OD) δ 7.06 (s, 5 H), 4.84 (s, 2 H), 4.72 (s, 3 H), 3.85 (m, 1 H), 3.40 (s, CD_3OH), 2.7 (d, 2 H), 1.36 (m, 3 H), 0.66 (m, 6 H); ^{31}P NMR (CD_3OD) δ 40.6. (b) Ester **9a** (4.5 g, 12.6 mmol) in methanol (50 mL) was stirred over 5% palladium-on-charcoal catalyst (0.5 g) under an atmosphere of hydrogen for 6 h at room temperature. The catalyst was removed by filtration and the solvent evaporated in vacuo. Trituration of the residue with diethyl ether gave product **11**, in 96% yield: mp 203–204 °C; $[\alpha]^{24}_D$ −16.6° (*c* 1.2, MeOH); ^{1}H NMR (D_2O) δ 3.74 (s, 3 H), 3.9 (m, 1 H), 2.98 (d, 2 H), 1.69 (m, 3 H), 0.95 (m, 6 H); ^{31}P NMR (D_2O) δ 26.0. Anal. ($C_8H_{18}NO_4P$) C, H, N, P.

Z-Ile-His-Lys(Boc)-OMe (30). A solution of Z-His-Lys(Boc)-OMe[35] (1.6 g, 3.0 mmol) in methanol (33 mL) containing 1 N HCl (3 mL) was hydrogenated according to general procedure C. The crude product was dissolved in DMF (10 mL) and TEA (0.41 mL, 3.0 mmol) and stirred with Z-Ile-OSu[36] (1.32 g, 3.6 mmol) for 8 h at room temperature. Diisopropyl ether (15 mL) was added and the precipitate filtered, dissolved in methanol (15 mL), and reprecipitated at 0 °C by the addition of $NaHCO_3$ solution (23 mL). The precipitate was filtered, washed (H_2O), dried, and reprecipitated from methanol–ethyl acetate–hexane: mp 203–204 °C; $[\alpha]^{28}_D$ +34.9° (*c* 1.0, MeOH); TLC, single spot, R_f(P) 0.65, R_f(D) 0.48. Anal. ($C_{32}H_{48}N_6O_8$) C, H, N.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[N^{α}-(benzyloxycarbonyl)amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-histidyl-N^{ϵ}-(*tert*-butoxycarbonyl)-L-lysine Methyl Ester (31). Z-Ile-His-Lys(Boc)-OMe (8 g, 12.4 mmol) was hydrogenated in methanol (80 mL), water (8 mL), and trifluoroethanol (80 mL) according to general procedure C. The residue, obtained in 100% yield, was crystallized: mp 129–130 °C (acetonitrile); TLC, single spot, R_f(D) 0.12, R_f(P) 0.14. Compound **6a** (156 mg, 0.39 mmol) and the base H-Ile-His-Lys(Boc)-OMe (200 mg, 0.39 mmol) were coupled according to general procedure A. The reaction mixture was purified by silica gel chromatography (100 g), first in solvent system D followed by

solvent system N. The product was isolated as a mixture of diastereomers in a yield of 76%: TLC, double spot, R_f(E) 0.51, 0.54, R_f(F) 0.38, 0.43. Amino acid analysis: $LPV_{0.96}$, $Ile_{0.98}$, $His_{1.00}$, $Lys_{1.01}$.

Boc-Pro-His-Pro-Phe-His-OH (32). (a) Boc-Pro-OSu[38] (1.25 g, 4.0 mmol) and H-His-OH (0.68 g, 4.3 mmol) were suspended in H_2O (4 mL) and dioxane (2 mL). NaOH (2 N) was added to maintain a pH of 9.0 (pH stat). After 45 min 4 N HCl was added to bring the solution to pH 5.0 and the solution concentrated to ca. 5 mL. Ion-exchange chromatography on a column (1.6 × 20 cm) of Diaion HP-20, eluting with 20% aqueous 2-propanol, gave fractions containing homogeneous product. These were combined and evaporated, and the residue was lyophilized from H_2O to yield the dipeptide: TLC, single spot, R_f(Q) 0.45, R_f(J) 0.21. (b) Z-Pro-Phe-His-OMe[39] (7 g, 12.8 mmol) was decarbobenzoxylated according to general procedure C to provide the tripeptide ester: TLC, single spot, R_f(Q) 0.21. (c) DCC (4.8 g, 23.3 mmol) was added to a cold (0 °C) solution of Boc-Pro-His-OH (6.6 g, 18.7 mmol), H-Pro-Phe-His-OMe (7.0 g, 16.9 mmol), and HOBT (5.6 g, 36.6 mmol) in DMF (35 mL). The solution was stirred for 6 h at 0 °C and for 16 h at room temperature, before cooling again and filtering. The filtrate was evaporated and the residue triturated with diisopropyl ether (200 mL) and dried. The residue was treated with methanol–acetic acid–water (94:3:3; 200 mL) for 1 h at 60 °C and concentrated to 40 mL before adding diisopropyl ether (400 mL) and cooling to 0 °C. After 1 h the solid was collected by filtration and dried: TLC, single spot, R_f(J) 0.17, R_f(Q) 0.51. (d) The protected pentapeptide (17.2 g, 23.0 mmol) was dissolved in methanol (54 mL) and treated with 0.1 N NaOH (650 mL) for 15 min at 25 °C before neutralizing with 0.1 N HCl (650 mL) and evaporating to dryness. The residue was dissolved in H_2O (120 mL) and subjected to counter-current chromatography (500 transfers, K = 2.8) in 1-butanol–H_2O (1:1). Fractions containing purified product were combined, evaporated, and lyophilized from H_2O: TLC, single spot, R_f(J) 0.15; R_f(R) 0.42; R_f(Q) 0.25. Amino acid analysis: $Pro_{2.04}$, $Phe_{1.00}$, $His_{2.04}$.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[*N*$^{\alpha}$-[(benzyloxycarbonyl)-L-prolyl-L-histidyl-L-prolyl-L-phenylalanyl-L-histidyl]amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-histidyl-*N*$^{\epsilon}$-(*tert*-butoxycarbonyl)-L-lysine Methyl Ester (33). (a) Compound **31** (40 mg, 0.045 mmol) was hydrogenated according to general procedure C to provide the free amine in 100% yield: TLC, single spot, R_f(C) 0.31, R_f(D) 0.17. (b) The free amine (32 mg, 0.04 mol) and pentapeptide acid **32** (37 mg, 0.04 mmol) were coupled according to general procedure A. The product was purified by silica gel chromatography (30 g) first using solvent system F, and then solvent system D, to provide the product in 69% yield: TLC, single spot, R_f(D) 0.23, R_f(F) 0.28. Amino acid analysis: $Pro_{1.91}$, $His_{2.95}$, $Phe_{1.00}$, $Ile_{0.96}$, $Lys_{1.09}$, $LPV_{1.07}$; FAB-MS, M + H at 1473.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[*N*$^{\alpha}$-(L-prolyl-L-histidyl-L-prolyl-L-phenylalanyl-L-histidyl)amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-histidyl-L-lysine (12). (a) Compound **33** (37 mg, 0.03 mmol) was treated with 95% aqueous TFA (0.2 mL) for 30 min at room temperature before adding diisopropyl ether (1.75 mL), at 0 °C, collecting the precipitate by centrifugation, and drying in vacuo: TLC, single spot, R_f(A) 0.41, R_f(B) 0.19. (b) The residue was dissolved in H_2O (1.45 mL), adjusted to pH 5.7 with 0.25 M NH_4OH (0.11 mL), and treated with a fresh aqueous solution (0.5%) of trypsin (15 μL). The pH was maintained at 5.0 by the addition of 0.25 M NH_4OH (25 μL). The solution was readjusted to pH 3 with 1 M acetic acid (0.5 mL) and stirred for 2 min in boiling water before lyophilization. The residue was dissolved in H_2O (1.0 mL) and passed through a column (1 × 12 cm) of Dowex-1 (OAc^- form) ion-exchange resin, eluting with H_2O. The eluate was lyophilized to yield the free peptide in 61% yield.

H-Ile-His-NH$_2$ (34). (a) Z-Ile-His-OMe[40] (3.0 g, 7.2 mmol) in 5.4 N methanolic ammonia (120 mL) was stirred for 15 h at room temperature. Solvent and excess ammonia were removed by evaporation, and the resulting solid was treated with methanol several times to yield Z-Ile-His-NH_2 in 100% yield: TLC, single spot, R_f(E) 0.53. (b) The amide (2.9 g, 7.2 mmol) was hydrogenated according to general procedure C and the crude product purified by silica gel chromatography (160 g) in solvent system F: TLC, single spot, R_f(F) 0.10.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[*N*-(benzyloxycarbonyl)amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-histidine Amide (35). Compound **6a** (399 mg, 1.0 mmol) was coupled to compound **34** (269 mg, 1.0 mmol) according to general procedure A. The crude product was purified by silica gel chromatography (300 g) eluting twice with solvent system A followed by treatment on a 150 g column eluting with 75:25:1 and 70:30:1 chloroform/methanol/concentrated ammonia solution (v/v). Appropriate fractions were pooled and evaporated to yield the product in 32% yield: TLC, single spot, R_f(E) 0.38, R_f(F) 0.12. Amino acid analysis: $Ile_{0.96}$, $His_{1.00}$, $LPV_{0.90}$.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[*N*-[(benzyloxycarbonyl)-L-phenylalanyl-L-histidyl]amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-histidine Amide (13). (a) Compound **35** (203 mg, 0.31 mmol) was deprotected according to general procedure C to yield the N-decarbobenzoxylated product in 100% yield: TLC, single spot, R_f(E) 0.07, R_f(D) 0.04. (b) This material (90 mg, 0.18 mmol) and Z-Phe-His-OH[41] (83 mg, 0.19 mmol) were coupled according to general procedure B. The crude product was purified by silica gel chromatography (40 g) eluting with 5:3:1 chloroform/methanol/concentrated ammonia solution (v/v), followed by a 20-g column eluting with solvent system B. The product was isolated as a mixture of diastereomers in 38% yield. [The diastereomers could subsequently by separated by silica gel chromatography (250 g) eluting with solvent system A: TLC, double spot, R_f(E) 0.40 and 0.51].

3-[Hydroxy[1(*R*)-[*N*-(benzyloxycarbonyl)amino]-3-methylbutyl]phosphinyl]propanoyl-L-isoleucyl-L-histidine Amide (36). Compound **6b** (340 mg, 1.0 mmol) and compound **34** (267 mg, 1.0 mmol) were coupled according to general procedure A. The product was purified by silica gel chromatography (150 g) eluting with solvent system E in 59% yield: TLC, single spot, R_f(E) 0.51.

3-[Hydroxy[1(*R*)-[*N*-[(benzyloxycarbonyl)-L-phenylalanyl-L-histidyl]amino]-3-methylbutyl]phosphinyl]propanoyl-L-isoleucyl-L-histidine Amide (14). (a) Compound **36** (153 mg, 0.25 mmol) was deprotected according to general procedure C to yield the decarbobenzoxylated product in 97% yield: TLC, single spot, R_f(E) 0.14, R_f(D) 0.03. (b) This material (118 mg, 0.25 mmol) was coupled to Z-Phe-His-OH[41] (109 mg, 0.25 mmol) according to general procedure B. The product was purified by silica gel chromatography (150 g) eluting first with A, followed by 55:35:6:4 chloroform/methanol/concentrated ammonia solution/water (v/v), solvent system A, and finally a stepped gradient of 40:10:1, 40:15:1, 40:20:1 chloroform/methanol/concentrated ammonia solution (v/v). Lyophilization of the appropriate fractions gave the product in 16% yield: TLC, single spot, R_f(E) 0.15, R_f(G) 0.59.

2-[Hydroxy[1(*R*)-[*N*-(benzyloxycarbonyl)amino]-3-methylbutyl]phosphinyl]acetyl-L-isoleucyl-L-histidine Amide (37). Compound **10** (363 mg, 1.0 mmol) and compound **34** (267 mg, 1.0 mmol) were coupled according to general procedure B. Silica gel chromatography (150 g) with solvent system D gave homogeneous material which was lyophilized from *tert*-butyl alcohol to give the product in 88% yield: TLC, single spot, R_f(E) 0.29, R_f(D) 0.16. Amino acid analysis: $Sta^P_{1.05}$, $Ile_{0.97}$, $His_{1.00}$.

2-[Hydroxy[1(*R*)-[*N*-[(benzyloxycarbonyl)-L-phenylalanyl-L-histidyl]amino]-3-methylbutyl]phosphinyl]acetyl-L-isoleucyl-L-histidine Amide (15). Compound **37** (510 mg, 0.86 mmol) was decarbobenzoxylated according to general procedure C to yield the free amine in 100% yield: TLC, single spot, R_f(E) 0.07. This material (115 mg, 0.25 mmol) was coupled to Z-Phe-His-OH[41] (109 mg, 0.25 mmol) by general procedure B. Chromatography on silica gel (150 g) eluting with solvent A gave the product in 35% yield: TLC, single spot, R_f(D) 0.16, R_f(E) 0.16.

(38) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1964**, *86*, 1839.
(39) DeWald, H.; Nicolaides, E. *J. Med. Pharm. Chem.* **1964**, *7*, 50.
(40) Rittel, W.; Iselin, B.; Kappeler, H.; Riniker, B.; Schwyzer, R. *Helv. Chim. Acta* **1957**, *40*, 614.
(41) Losse, G.; Muller, G. *Chem. Ber.* **1961**, *94*, 2768.

Boc-Ile-Phe-NH_2 (38). *N*-(*tert*-Butoxycarbonyl)-L-isoleucine (3.5 g, 15 mmol) and L-phenylalanine amide (2.4 g, 14.6 mmol) were coupled according to general procedure B (except in the use of 16 mmol of DCC). The crude product was purified by silica gel chromatography (300 g, 70–230 mesh) eluting with chloroform under gravity flow. The appropriate fractions were combined and evaporated in vacuo, and the residue was crystallized to provide the pure dipeptide in 23% yield: mp 183–185 °C (methanol/water); $[\alpha]^{23}_D$ −40.2° (*c* 1.24, MeOH); TLC, single spot, R_f(H) 0.11. Anal. ($C_{20}H_{31}N_3O_4$) C, H, N.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[*N*-(benzyloxycarbonyl)-amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-phenylalanine Amide (39). Compound **38** (377 mg, 1.0 mmol) in a solution of 2.9 N HCl in ethyl acetate (5 mL) was stirred at room temperature for 45 min. Excess reagent was removed under reduced pressure and the residue dried in vacuo over KOH to yield the amine hydrochloride in 100% yield: TLC, single spot, R_f(D) 0.29. This material (314 mg, 1.0 mmol) was coupled to compound **6a** (399 mg, 1.0 mmol) in the presence of additional TEA (0.14 mL, 1.0 mmol), according to general procedure A. Chromatography on silica gel (120 g) eluting first with 80:20:1 chloroform/methanol/concentrated ammonia solution and then with 80:10:1 of the same solvents gave homogeneous product. Lyophilization from *tert*-butyl alcohol gave the product in 38% yield: TLC, single spot, R_f(D) 0.52, R_f(F) 0.13. Amino acid analysis: $LPV_{0.85}$, $Ile_{1.00}$, $Phe_{1.00}$.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[*N*-[(benzyloxycarbonyl)-L-phenylalanyl-L-histidyl]amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-phenylalanine Amide (16). Compound **39** (286 mg, 0.36 mmol) was deprotected according to general procedure C to yield the free amine in 100% yield: TLC, double spot (diastereomers), R_f(E) 0.22 and 0.45. This material (65 mg, 0.124 mmol) and Z-Phe-His-OH[41] (54 mg, 0.124 mmol) were coupled according to general procedure B. The crude product was chromatographed on silica gel (150 g) eluting with solvent system A to provide product in 18% yield following lyophilization from *tert*-butyl alcohol: TLC, single spot, R_f(E) 0.54, R_f(D) 0.77.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[*N*-(benzyloxycarbonyl)-amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-arginine *tert*-Butyl Ester (40). **(a) Z-Arg(NO_2)-OBu^t.** DCC (6.2 g, 30 mmol) and copper(II) chloride (61 mg) in *tert*-butyl alcohol (2.6 mL) were stirred for 65 h. The mixture was diluted with ethyl acetate (20 mL), and Z-Arg(NO_2)-OH[42] (1.76 g, 5 mmol) in ethyl acetate (10 mL) was added and stirred for 65 h. The mixture was filtered through silica gel (100 g, 70–230 mesh) eluting with ethyl acetate (250 mL). The eluant was concentrated to ca. 50 mL, washed with concentrated citric acid solution and 10% aqueous NaCl, dried (Na_2SO_4), and evaporated in vacuo. The residue was chromatographed on silica gel (200 g) eluting with 97:3 chloroform/methanol (v/v), to give the protected amino acid as an oil in 49% yield: TLC, single spot, R_f(J) 0.07, R_f(B) 0.84.

(b) H-Ile-Arg-OBu^t·HCl. The oil (500 mg, 1.2 mmol) was hydrogenated according to general procedure C in the presence of acetic acid (0.21 mL, 3.6 mmol). The filtered, evaporated residue in water (3 mL) was passed through Dowex-1 (Cl^- form, 10 g) ion-exchange resin and eluted with water (60 mL). The eluant was evaporated and dried in vacuo to yield H-Arg-OBu^t·2HCl in 61% yield: TLC, single spot, R_f(J) 0.22. This material (258 mg, 0.85 mmol), Z-Ile-OH (225 mg, 0.85 mmol), and HOBT (130 mg, 0.85 mmol) in DMF (4.5 mL) were cooled to 0 °C and treated with diisopropylethylamine (0.15 mL, 0.85 mmol) followed by DCC (210 mg, 1.02 mmol) and stirred for 1 h at 0 °C and for 16 h at 20 °C. The mixture was filtered, evaporated in vacuo, and chromatographed on silica gel (150 g) and solvent system A to provide the protected dipeptide in 68% yield: TLC, single spot, R_f(E) 0.49, R_f(K) 0.17. This material (350 mg, 0.67 mmol) was decarbobenzoxylated in the presence of 1 N HCl (0.67 mL) by general procedure C to yield the amine hydrochloride in 72% yield: TLC, single spot, R_f(E) 0.16.

(c) Formation of Product 40. Compound **6a** (282 mg, 0.71 mmol), H-Ile-Arg-OBu^t·HCl (301 mg, 0.71 mmol), and HOBT (142 mg, 0.93 mmol) in DMF (4.5 mL) were treated at −10 °C with TEA (0.20 mL, 1.42 mmol) and DCC (343 mg, 1.66 mmol) and stirred for 1 h at 0 °C and 90 h at 5 °C. DCC (171 mg, 0.83 mmol) was added and stirring continued for 24 h at 5 °C, followed by further DCC (171 mg, 0.83 mmol) and further stirring (24 h). The mixture was filtered and evaporated and the residue chromatographed on silica gel (150 g), eluting with 90:10:1:0.5 chloroform/methanol/water/acetic acid (v/v) to give product in 69% yield: TLC, single spot, R_f(E) 0.50, R_f(D) 0.44.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[*N*-[(benzyloxycarbonyl)-L-phenylalanyl-L-histidyl]amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-arginine *tert*-Butyl Ester (17). Compound **40** (158 mg, 0.21 mmol) was deprotected according to general procedure C to give the amine in 97% yield: TLC, single spot, R_f(E) 0.19. This material (128 mg, 0.21 mmol) and Z-Phe-His-OH[41] (95 mg, 0.21 mmol) were coupled according to general procedure B, and the product was purified by chromatography on silica gel (200 g) eluting with 35:15:1 chloroform/methanol/concentrated ammonia solution (v/v) followed by repeated chromatography on silica gel (100 g) eluting with 75:25:1 then 40:10:1 chloroform/methanol/concentrated ammonia solution (v/v) and finally 90:10:1:0.5 chloroform/methanol/water/acetic acid (v/v) to provide the product in 11% yield: TLC, single spot, R_f(E) 0.43, R_f(D) 0.44, R_f(F) 0.29.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[*N*-[(*tert*-butoxycarbonyl)-L-phenylalanyl-L-phenylalanyl]amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-histidine Amide (18). Boc-Phe-Phe-OH[43] 130 mg, 0.31 mmol) and H-Leu^P(CH_2)Val-Ile-His-NH_2 (from compound **35**, 162 mg, 0.31 mmol) were coupled according to general procedure B. The crude product was dissolved in chloroform (50 mL), filtered, washed with concentrated $NaHCO_3$ solution, and evaporated in vacuo before chromatographing on silica gel (200 g) with solvent system D to provide the product in 54% yield: TLC, single spot, R_f(D) 0.38.

***N*-*t*-Boc-L-Phe-L-Phe(N^α-Me)-OH (41).** Boc-Phe-OH (1.28 g, 4.8 mmol) and HOBT (0.74 g, 4.8 mmol) were dissolved in DMF (8 mL) cooled to 0 °C and treated with a solution of H-Phe-(N^α-Me)-OMe (1.1 g, 4.8 mmol) and diisopropylethylamine (0.83 mL, 4.8 mmol) in DMF (8 mL). DCC (1.19 g, 5.8 mmol) was added, the mixture stirred for 65 h at 5 °C and filtered, and the filtrate evaporated in vacuo. The residue was dissolved in 2:1 ethyl acetate/1-butanol (50 mL, v/v), washed with saturated aqueous $NaHCO_3$, and dried (Na_2SO_4) before chromatographing on silica gel (300 g) eluting with chloroform to give the dipeptide in 69% yield: TLC, single spot, R_f(K) 0.46. This material (810 mg, 1.84 mmol) was saponified according to general procedure D to give the product, following crystallization in 48% yield: mp 175–176 °C (benzene/hexanes); TLC, R_f(K) 0.21. Anal. ($C_{24}H_{30}N_2O_5$) C, H, N.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[*N*-[(*tert*-butoxycarbonyl)-L-phenylalanyl-L-phenylalanyl(N^α-methyl)]amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-histidine Amide (19). Compound **41** (97.8 mg, 0.23 mmol) was coupled to H-Leu^P(CH_2)Val-Ile-His-NH_2 (from compound **35**, 162 mg, 0.23 mmol) according to general procedure B. The crude product dissolved in chloroform (50 mL) was washed with saturated aqueous $NaHCO_3$ and water, dried (Na_2SO_4), evaporated, and chromatographed on silica gel (75 g) eluting with solvent system C to give the product, following lyophilization from *tert*-butyl alcohol in 35% yield: TLC, single spot, R_f(C) 0.54, R_f(D) 0.72.

***N*-*t*-Boc-L-Phe-L-His(N^α-Me)-OMe (42).** Boc-Phe-OH (620 mg, 2.34 mmol) and HOBT (357 mg, 2.34 mmol) were dissolved in DMF (4.5 mL), cooled to 0 °C, and treated with H-His(N^α-Me)-OMe·2HCl (598 mg, 2.34 mmol) and diisopropylethylamine (0.38 mL, 2.34 mmol) in DMF (6.5 mL). DCC (578 mg, 2.8 mmol) was added, the mixture stirred for 65 h at 5 °C and filtered, and the filtrate evaporated to dryness. The residue was taken up in 2:1 ethyl acetate/1-butanol (50 mL, v/v), washed with saturated aqueous $NaHCO_3$, and dried (Na_2SO_4) before chromatographing on silica gel (150 g) and eluting with 95:5 chloroform/methanol

(42) Gibian, H.; Schroder, E. *Annalen* **1961**, *624*, 145.

(43) Chang, J. K.; Shimizu, M.; Wang, S. S. *J. Org. Chem.* **1976**, *41*, 3255.

(v/v), to provide the protected dipeptide in 60% yield: TLC, single spot, R_f(E) 0.67, R_f(L) 0.54. Anal. ($C_{22}H_{30}N_4O_5$) C, H, N.

N-t-Boc-L-Phe-L-His(N^{α}-Me)-OH (43). Compound **42** (400 mg, 0.93 mmol) was saponified in ethanol according to general procedure D to give the dipeptide acid in 54% yield: TLC, single spot, R_f(E) 0.32, R_f(L) 0.37. Anal. ($C_{21}H_{28}N_4O_5 \cdot H_2O$) C, H, N.

2(R,S)-[[Hydroxy[1(R)-[N-[(tert-butoxycarbonyl)-L-phenylalanyl-L-histidyl(N^{α}-methyl)]amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-histidine Amide (20). Compound **43** (129 mg, 0.31 mmol) was coupled to H-Leu^{P}(CH_2)Val-Ile-His-NH_2 (from compound **35**, 146 mg, 0.29 mmol) according to general procedure B and the product purified by chromatography on silica gel (150 g) and solvent B to give the product in 7% yield: TLC, single spot, R_f(E) 0.32, R_f(F) 0.19.

2(R,S)-[[Hydroxy[1(R)-[N-[(benzyloxycarbonyl)-L-prolyl-L-phenylalanyl-L-histidyl]amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-histidine Amide (21). Z-Pro-Phe-His-OMe[39] (135 mg, 0.25 mmol) in dioxane (4.8 mL) and water (1.0 mL) was cooled to 0 °C, treated with 2 N NaOH (0.15 mL), and stirred for 90 min at room temperature. Neutralization (0.09 mL, 2 N HCl) and evaporation gave a residue which was distributed between ethyl acetate (10 mL) and water (5 mL), cooled to 0 °C, and brought to pH 3.0 with 2 N HCl (0.06 mL). The aqueous phase was separated and extracted with 1:1 1-butanol/ethyl acetate (3 × 30 mL, v/v). The organic phases were combined, washed with water, dried (Na_2SO_4), and evaporated to give the free acid in 95% yield: TLC, single spot, R_f(E) 0.29 [cf. R_f(E) for ester 0.69]. This material (96 mg, 0.18 mmol) was coupled to H-Leu^{P}(CH_2)Val-Ile-His-NH_2 (from compound **35**, 82.5 mg, 0.16 mmol) according to general procedure B. The product was purified by chromatography on silica gel (200 g) with solvent D followed by chromatography on silica gel (50 g) eluting with 70:30:3:3 chloroform/methanol/acetic acid/water (v/v) to give the product in 15% yield: TLC, single spot, R_f(D) 0.53, R_f(E) 0.54, R_f(F) 0.21.

Z-Gly-L-Phe-L-His-OMe (44). Z-Phe-His-OMe[44] (4.5 g, 10 mmol) was decarbobenzoxylated in the presence of 1 N HCl (20 mL) according to general procedure C to give the amine hydrochloride in 97% yield: TLC, single spot, R_f(M) 0.01 [cf. R_f(M) for ester 0.11]. This material (2.1 g, 6 mmol) was coupled to Z-Gly-OH (1.25 g, 6 mmol) in a manner exactly analogous to that described for compound **42**. Following workup the crude product was crystallized to give the tripeptide in 62% yield: mp 193–195 °C (methanol/water); TLC, R_f(J) 0.35, R_f(E) 0.54. Anal. ($C_{26}H_{29}N_5O_6$) C, H, N.

2(R,S)-[[Hydroxy[1(R)-[N-[(benzyloxycarbonyl)-glycyl-L-phenylalanyl-L-histidyl]amino]-3-methylbutyl]-phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-histidine Amide (22). Compound **44** (254 mg, 0.5 mmol) was deesterified according to procedure D to give the free acid in 100% yield: TLC, single spot, R_f(E) 0.26. This material (205 mg, 0.42 mmol) was coupled to H-Leu^{P}(CH_2)Val-Ile-His-NH_2 (from compound **35**, 269 mg, 0.42 mmol) according to general procedure B. Chromatography first on silica gel (75 g) with solvent D, followed by silica gel (50 g) and a stepped gradient of chloroform/methanol/concentrated ammonia solution 40:10:1–80:30:1, v/v) gave the homogeneous product in 10% yield: TLC, single spot, R_f(E) 0.49, R_f(F) 0.18.

N^{α}-Z-L-Arg-L-Arg-L-Pro-L-Phe-L-His-OH (45). Z-Arg-OH (486 mg, 1.58 mmol) was suspended in DMF and treated with 5 N HCl in dioxane (0.32 mL, 1.58 mmol) before coupling to H-Pro-Phe-His-OMe (from preparation of compound **32**, 500 mg, 1.21 mmol) with DCC (325 mg, 1.58 mmol) and HOBT (185 mg, 1.21 mmol) in a manner analogous to that used for compound **32**. The worked-up product was purified by counter-current distribution (1-butanol/water) and isolated in 32% yield as the dihydrochloride: TLC, single spot, R_f(Q) 0.45, R_f(R) 0.55. This material (2.5 g, 3.5 mmol) was decarbobenzoxylated according to procedure C in 100% yield: TLC, single spot, R_f(Q) 0.05, R_f(R) 0.3. This material (1.9 g, 3.0 mmol) was coupled to Z-Arg-OH (1.27 g, 4.1 mmol) by the method described above for the tetrapeptide. The crude pentapeptide was purified (in 47% yield) by chromatography on silica gel (120 g, 70–230 mesh) eluting with 140:80:20:1 chloroform/methanol/water/acetic acid (v/v), under gravity. This material (1.3 g, 1.4 mmol) was dissolved in water (26 mL) and treated with 1 N NaOH (6.5 mL) for 5 min at 25 °C before bringing to pH 2.5 with 2 N HCl and lyophilizing. The material obtained in 91% yield was used without further purification: TLC, R_f(R) 0.3. Amino acid analysis: $Pro_{1.02}$, $Phe_{1.00}$, $His_{1.03}$, $Arg_{2.03}$.

2(R,S)-[[Hydroxy[1(R)-[N-[(benzyloxycarbonyl)-L-arginyl-L-arginyl-L-prolyl-L-phenylalanyl-L-histidyl]amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-isoleucyl-L-histidine Amide (23). Compound **45** (137 mg, 0.15 mmol), H-Leu^{P}(CH_2)Val-Ile-His-NH_2 (from compound **35**, 77 mg, 0.15 mmol), and HOBT (27 mg, 0.18 mmol) in DMF (6.7 mL) were cooled to 0 °C and treated with TEA (20.5 μL, 0.15 mmol) followed by DCC (153 mg, 0.74 mmol). The mixture was stirred for 1 h at 0 °C and for 81 h at room temperature before filtering and evaporating. The residue was dissolved in 94:3:3 methanol/acetic acid/water (10 mL, v/v) and stirred for 1 h at 60 °C and evaporated and the residue purified by counter-current distribution in 4:1:5 1-butanol/acetic acid/water to give the product in 35% yield: TLC, single spot, R_f(B) 0.39, R_f(L) 0.60.

3-[Hydroxy[1(R)-[N-[(benzyloxycarbonyl)-L-arginyl-L-arginyl-L-prolyl-L-phenylalanyl-L-histidyl]amino]-3-methylbutyl]phosphinyl]propanoyl-L-isoleucyl-L-histidine Amide (24). Compound **45** (219 mg, 0.24 mmol) and H-Leu^{P}(CH_2)Gly-Ile-His-NH_2 (from compound **36**, 115 mg, 0.24 mmol) were coupled in a manner exactly analogous to that described for compound **23**. Workup and purification were also effected in an identical manner to that described for compound **23** and gave the product, following lyophilization from *tert*-butyl alcohol in 4% yield; TLC, single spot, R_f(A) 0.19, R_f(L) 0.47.

2(R,S)-[[Hydroxy[1(R)-[N-(benzyloxycarbonyl)-amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-alanyl-(3S,4S)-statine Methyl Ester (46). Z-Ala-(3*S*,4*S*)-Sta-OMe[45] (217 mg, 0.55 mmol) was decarbobenzoxylated according to general procedure C to provide the free amine in 93% yield. This material (135 mg, 0.51 mmol) was coupled to compound **6a** (207 mg, 0.51 mmol) according to general procedure B. The crude product was purified by repeated chromatography on silica gel (150 g) eluting with solvent systems C, E, and F to give the protected peptide in 14% yield: TLC, single spot, R_f(E) 0.35. Amino acid analysis: $LPV_{1.00}$, $Ala_{1.00}$, $Sta_{0.98}$.

2(R,S)-[[Hydroxy[1(R)-[N-[(benzyloxycarbonyl)-L-prolyl-L-phenylalanyl-L-histidyl]amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoyl-L-alanyl-(3S,4S)-statine Methyl Ester (25). Compound **46** (120 mg, 0.19 mmol) was decarbobenzyloxylated according to general procedure C to yield the amine in 78% yield. This material (74 mg, 0.15 mmol) was coupled to Z-Pro-Phe-His-OH (from preparation of compound **21**, 78 mg, 0.15 mmol) by general procedure B. Purification by chromatography on silica gel (100 g) and solvent system B and lyophilization from *tert*-butyl alcohol gave the product in 31% yield: TLC, single spot, R_f(E) 0.84, R_f(F) 0.35.

2-[Hydroxy[1(R)-[N-[(benzyloxycarbonyl)-L-alanyl]-amino]-3-methylbutyl]phosphinyl]acetic Acid Methyl Ester (47). Z-L-Ala-OH (1.69 g, 7.6 mmol) and compound **11** (1.7 g, 7.6 mmol) were coupled according to general procedure B and purified by repeated chromatography on silica gel (300 g), first with solvent system D and in six subsequent runs with solvent system C. Lyophilization of the evaporated fractions gave the product in 12% yield: TLC, single spot, R_f(E) 0.27, R_f(D) 0.34. Amino acid analysis: $Ala_{1.00}$, $Sta^{P}_{0.98}$. Anal. ($C_{19}H_{29}N_2O_7P \cdot CH_3CO_2H \cdot 1.5H_2O$) C, H, N, P.

2-[Hydroxy[1(R)-[N-[(benzyloxycarbonyl)-(3S,4S)-statyl-L-alanyl]amino]-3-methylbutyl]phosphinyl]acetic Acid Methyl Ester (48). Compound **47** (296 mg, 0.68 mmol) was deprotected according to general procedure C to give the dipeptide amine in 100% yield (after drying in vacuo over KOH and P_2O_5).

(44) Gawne, G.; Kenner, G. W.; Sheppard, R. C. *J. Am. Chem. Soc.* **1969**, *91*, 5669.

(45) Guegen, R.; Diaz, J.; Cazaubon, C.; Beaumont, M.; Carlet, C.; Clement, J.; Dennarne, H.; Mellet, M.; Richaud, J.; Segondy, D.; Vedel, M.; Gagnol, J.; Roncucci, R.; Castro, B.; Corvol, P.; Evin, G.; Roques, B. *J. Med. Chem.* **1986**, *29*, 1152.

This material (203 mg, 0.68 mmol) was coupled to Z-Sta-OH (210 mg, 0.68 mmol) by general procedure B and chromatographed on silica gel (60 g), eluting initially with solvent system A and in a second run with solvent system D to provide the protected peptide in 47% yield: TLC, single spot, R_f(E) 0.68. Amino acid analysis: $Sta_{0.98}$, $Al_{1.00}$, $Sta^P_{0.90}$.

2-[Hydroxy[1(*R*)-[*N*-[(benzyloxycarbonyl)-L-phenylalanyl-L-histidyl-(3*S*,4*S*)-statyl-L-alanyl]amino]-3-methylbutyl]phosphinyl]acetic Acid Methyl Ester (26). Compound 48 (185 mg, 0.31 mmol) was decarbobenzoxylated according to general procedure C to give the amine in 100% yield. This material (143 mg, 0.31 mmol) was coupled to Z-Phe-His-OH[41] (137 mg, 0.31 mmol) according to general procedure B. Chromatography on silica gel (75 g) with solvent system D gave homogeneous material, which was lyophilized from *tert*-butyl alcohol, in 45% yield: TLC, single spot, R_f(D) 0.26.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[*N*-[(benzyloxycarbonyl)-(3*S*,4*S*)-statyl]amino]-3-methylbutyl]phosphinyl]-methyl]-3-methylbutanoic Acid Methyl Ester (49). Compound **7a** (413 mg, 1.0 mmol) was decarbobenzoxylated according to general procedure C in 97% yield. This material (270 mg, 0.97 mmol) was coupled to Z-Sta-OH (299.7 mg, 0.97 mmol) according to general procedure **B**. Chromatography on silica gel (100 g), eluting with solvent system B, gave the product in 70% yield: TLC, single spot, R_f(E) 0.54, R_f(F) 0.21.

2(*R*,*S*)-[[Hydroxy[1(*R*)-[*N*-[(benzyloxycarbonyl)-L-phenylalanyl-L-histidyl-(3*S*,4*S*)-statyl]amino]-3-methylbutyl]phosphinyl]methyl]-3-methylbutanoic Acid Methyl Ester (27). Compound 49 (216 mg, 0.38 mmol) was decarbobenzoxylated according to general procedure C in 88% yield. This material (145 mg, 0.33 mmol) was coupled to Z-Phe-His-OH[41] (145 mg, 0.33 mmol) according to general procedure B, except in the use of 4.5 equiv of DCC in three portions over 10 days. The product was purified by chromatography on silica gel (100 g) eluting first via 80:20:1 and then 85:15:1 chloroform/methanol/concentrated ammonia solution (v/v). Appropriate fractions were combined, evaporated, and lyophilized from *tert*-butyl alcohol to give the product in 26% yield: TLC, single spot, R_f(E) 0.67, R_f(F) 0.21.

(1*R*)-[1-[*N*-[(Benzyloxycarbonyl)-L-prolyl-L-phenylalanyl]amino]-3-methylbutyl]phosphinic Acid (28). *N,N,N',N'*-Tetramethylguanidine (41.5 μL, 0.33 mmol) was added to a suspension of Z-Pro-Phe-His-OH (from preparation of compound **21**, 176.5 mg, 0.33 mmol), [(1*R*)-amino-3-methylbutyl]phosphinic acid[9] (50 mg, 0.33 mmol), and HOBT (66 mg, 0.43 mmol) in DMF (3 mL) and stirred until solution was obtained. The solution was cooled to 0 °C, treated with DCC (150 mg, 0.73 mmol), and stirred for 1 h at 0 °C and 25 h at room temperature. The mixture was filtered and evaporated and the residue dissolved in 94:3:3 methanol/acetic acid/water (5 mL, v/v) and stirred for 60 min at 60 °C before evaporating to dryness. The crude product was chromatographed on silica gel (100 g), eluting with solvent system C, and provided the product in 37% yield: TLC, single spot, R_f(E) 0.26.

(1*R*)-[1-[*N*-[(Benzyloxycarbonyl)-L-prolyl-L-phenylalanyl]amino]-3-methylbutyl]phosphonic Acid (29). Z-Pro-Phe-His-OH (from preparation of compound **21**, 176.5 mg, 0.33 mmol) and [(1*R*)-amino-3-methylbutyl]phosphonic acid[9] (50 mg, 0.33 mmol) were coupled as described for compound **28**, except for the use of 2 equiv of base (83 μL, 0.66 mmol) and 5.25 equiv of DCC administered in two portions 24 h apart. Workup was as described for compound **28**. The crude product was purified by repeated chromatography on silica gel (100 g) first eluting with solvent system C and finally with solvent system A. Appropriate fractions were combined, evaporated, and lyophilized from *tert*-butyl alcohol to provide the product in 13% yield: TLC, single spot, R_f(D) 0.28, R_f(F) 0.27.

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Book Reviews

Molecular Neurobiology. Edited by N. G. Bazan and D. C. U'Prichard. Humana Press, Clifton, NJ. 1988. 398 pp. 22 × 28.5 cm. ISBN 0-89603-152-7. $85.00.

The volume is a glossy, hard-bound collection of reviews which are somewhat more general than the popular "Annual Reviews" series but are reasonably current. Readers of *Journal of Medicinal Chemistry* will find the volume generally well written, and the individual reviews do not suffer from the burdensome complexity of jargon which affect many current reviews. The editors have done a good job selecting a balanced view of current research topics in neurobiology. Potential readers, unacquainted with molecular biology, should not be deterred by the title. It is intended to imply the reduction of this discipline to the molecular events underlying neural phenomena. Certain chapters do deal with the applications of molecular biology to neurobiology; however, these sections are usually well explained and are readily followed. In this view the reader will not be disappointed with the contents. The contributors include a number of internationally recognized figures who are at the forefront of their fields. Each chapter begins with an abstract and an informative table of contents. The volume is, however, unnecessarily divided into four subvolumes differing in the season of 1987 when they were prepared.

The volume begins with a short preface by Dr. Soloman Snyder which is, true to form, informative and well written. In his short introduction, Dr. Snyder highlights the avenues of research in which the fastest progress is being achieved. If the reader desires a brief overview this section will be appreciated.

Dr. Barnstable addresses the process of cell migration in the developing retina. Anatomical and physiological events are effectively explained in juxtaposition with selected biochemical events. Considerable attention is directed to the role of cell surface markers and their role in guiding neural migration.

Drs. Soreq and Gnatt (Molecular Biological Search for Human Genes Encoding Cholinesterases) provide a textbook example of gene cloning as applied to neurobiology. The importance of cholinesterase in human disease is, however, somewhat overstated.

Dr. Greengard, a pioneer in the field of protein phosphorylation, gives a very good description of neuronal kinases and phosphoproteins. As specific examples, synapsin-1 and the 32-kDa dopamine receptor phosphoprotein (DARPP-32) are discussed in detail. The information is clear, and the tabular presentation is effective at providing a concise summary. Attention is also directed toward the dephosphorylation of phosphoproteins by specific phosphatases. Lastly, the physio- and pathophysiological roles of these proteins are discussed in sufficient detail as to impress the reader with the importance of this field.

Drs. Sibley and Lefkowitz contribute a chapter entitled "β-Adrenergic Receptor-Coupled Adenylate Cyclase. Biochemical Mechanisms of Regulation". The chapter is an excellent and current review of the regulation of β-receptor function. The phenomena of heterologous and homologous desensitization are presented effectively. Receptor phosphorylation, sequestration, and coupling to G-proteins are discussed in detail. However, the physiological importance of these phenomena are not discussed.

In contrast, the chapter by Duman and colleagues, "Molecular Biology of Inhibitory Amino Acid Receptors", is something of a