Evaluation of Physicochemical Parameters Important to the Oral Bioavailability of Peptide-like Compounds: Implications for the Synthesis of Renin Inhibitors

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A series of radiolabeled compounds related to renin inhibitor structures was synthesized to represent a range of physicochemical properties. These compounds were tested in assays for intestinal absorption and hepatic clearance in order to define parameters conducive to optimizing bioavailability. In general, compounds with higher lipophilicity were better absorbed from the intestine. Absorption may also be dependent on molecular charge, as compounds with ionizable functionality were less well-absorbed than neutral compounds. Neutral compounds showed some dependency on molecular weight, with smaller compounds exhibiting better absorption. While uptake into hepatic cells was rapid regardless of partition coefficient or molecular weight, rate of appearance in bile was dependent on the molecular weight of the compounds.

Introduction

In an effort to understand factors important to the oral bioavailability of peptidomimetic drugs, we have studied the relationship between defined physicochemical parameters of renin inhibitor-like compounds and their absorption from the intestine and hepatic uptake and clearance properties.[1] The aim of this study was to identify optimal physicochemical parameters which maximize intestinal absorption and minimize hepatic extraction, leading to optimal bioavailability.[2] The compound series was designed to exemplify different physiochemical parameters with as little interrelation among variables as possible. Intestinal absorption and hepatic extraction were studied separately in order to more specifically define potential problems with oral availability of the compounds.

Compound Selection and Chemistry

Compound selection was initially based on a library of 243 compounds which contained the 2-amino-1-cyclohexyl-6-methylheptane-3,4-diol fragment (ACDMH, 1, Figure 1)[3] at the P1–P1′ position of compounds designed to fit the renin enzyme cleft.[4] This fragment was chosen because of the number of compounds available and the ability to generate a radiolabeled analog in the cyclohexyl ring.[5] The use of tritiated **1** allowed detection in biological matrices. The radiolabel was essential since some of the compounds possessed insufficient renin inhibition activity for bioassay, and many lacked a chromophore for UV/HPLC detection. Compound selection was also influenced by the need for minimal synthetic handling of intermediates and products which were radioactive. Receptor binding affinity was not considered in the selection process since the study was focused on structural features that would optimize bioavailability for the future design of lead series.

Within this framework of a consistent structural element, compounds were chosen to represent a broad range of physicochemical parameters, focusing primarily on molecular weight and partition coefficient. These two factors have been identified as influencing both intestinal absorption and hepatic clearance.[6] Compounds were selected by an iterative process, analyzing physicochemical properties within a correlation matrix of molecular weight, partition coefficient, hydrogen-bond donating and accepting capability, and the presence of basic and acidic functionalities (Figure 2). A low correlation (low number in Figure 2) was desired in order to minimize confounding of effects. Thus, if absorption was found to be related to molecular weight, any dependence on log *P* could be analyzed separately. In the final set of compounds, a separation of hydrogen bond-donating and -accepting groups with molecular weight was not achieved, but log *P* and molecular weight were well-separated (Figure 2).

A final compound set of nine analogs was advanced for biological testing. The molecular weight range was from 243 to 808, and the log *P* values were from 1.5 to 4.9 as measured by correlation method at pH 6.5 .[7] The structures of the compounds are shown in Figure 1. Their physicochemical values are given in Table 1.

Synthesis of the P3–P2 moiety is given in Schemes 1 and 2 for the precursors to **8** and **9**; the remaining P3–P2 fragments were commercially available. The synthesis of **4** has been published.[8] The final step(s) involved coupling of this fragment to the tritiated ACDMH **1**, followed by deprotection if necessary (Scheme 3). The specific activity of the final compounds was in the range of 6.43–128 mCi/mmol. Certain compounds required a higher specific activity because their solubility limited detection in the biological assays. Possible epimerization in the final coupling step was checked by purity determination of the final compounds, which was

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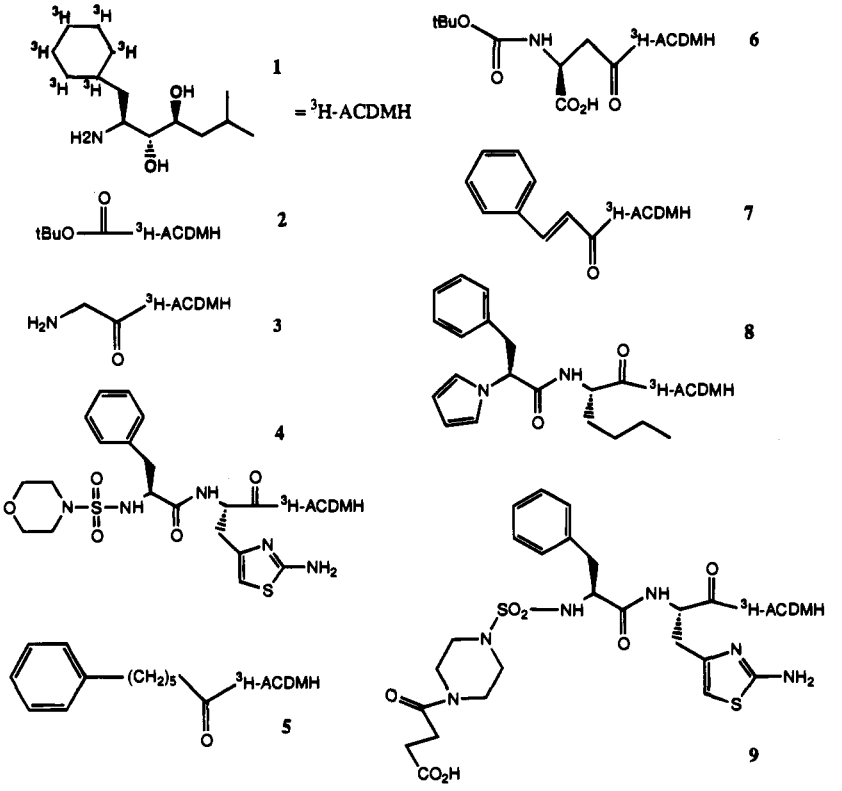


Figure 1. Structures of compounds.

	MW	log P	HBD	HBA	basic	acid
MW	1.00					
log P[a]	0.04	1.00				
HBD[b]	0.80	-0.55	1.00			
HBA[c]	0.86	-0.40	0.93	1.00		
basic[d]	0.24	-0.72	0.70	0.42	1.00	
acid[e]	0.50	-0.46	0.58	0.75	0.06	1.00

Figure 2. Correlation matrix of variables used (n = 9). [a] Distribution coefficient measured by HPLC at pH 6.5. [b] Count of potential H-bond donors. [c] Count of potential H-bond acceptors. [d] Indicator (=1) for presence of basic function, otherwise, 0. [e] Indicator (=1) for presence of acidic function, otherwise, 0.

Table 1. Molecular Weight, log P, pK_a, Hydrogen Bond-Donating and -Accepting Number, and Effective Permeabilities of Compounds 1–**9**

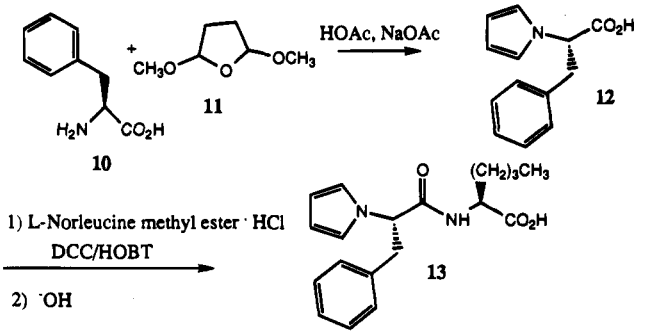
compd	MW	log P	pK_a	HBD	HBA	P_{eff}
1	243.0	1.86	9.3	4	3	1.28 ± 0.12
2	343.5	3.98		3	4	4.96 ± 0.53
3	300.5	1.52	7.5	5	4	0.62 ± 0.08
4	708.9	3.01	6.3	7	8	1.68 ± 0.25
5	417.6	4.66		3	3	5.75 ± 0.65
6	458.6	2.06	4.7	5	7	0.37 ± 0.06
7	373.5	3.61		3	3	4.77 ± 0.48
8	553.7	4.90		4	4	3.94 ± 0.67
9	808.0	2.03	5.9	8	10	0.21 ± 0.06

carried out by both radiochemical and HPLC/UV methods. Because of the relatively rapid rate of radioactive decomposition, a high initial purity was necessary for accurate biological testing results.

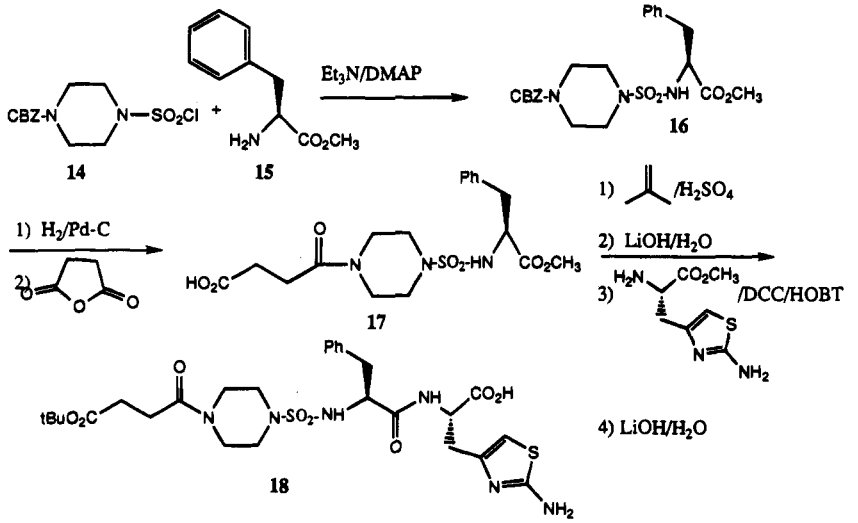
Intestinal Absorption

Intestinal absorption was examined with the single-pass perfusion model in rats (Figure 3). This model was chosen because of its high reproducibility and similarity to conditions in the intact animal. It also allowed measurement of absorption separately from other pharmacokinetic parameters. Compounds were first evaluated for stability in N-morpholinoethanesulfonic acid (MES) buffer which was used as the perfusion medium and in rat intestinal perfusate (generated by oscillating 12–15 mL of MES buffer in 15-cm rat jejunal segment at 30 mL/min for 90 min), and all had stability acceptable for study. All analogs were studied at concentrations below their solubility limits. The drug solution was perfused into the intestine of an anesthetized rat, and flux across the intestinal membrane was measured by the disappearance of drug from the perfusate effluent. The dimensionless effective permeability (P_{eff}) was calculated by mass balance analysis, and ranged from 0.21 to 5.75 (Table 1). Standard drugs with known human absorption values have been evaluated in this preparation,[9] and the results indicated that a compound with a P_{eff} value greater than 1.0 corresponds to a well-absorbed drug. All but three of the test compounds (**3**, **6**, and **9**) therefore appeared to cross the intestinal wall rapidly.

Scheme 1

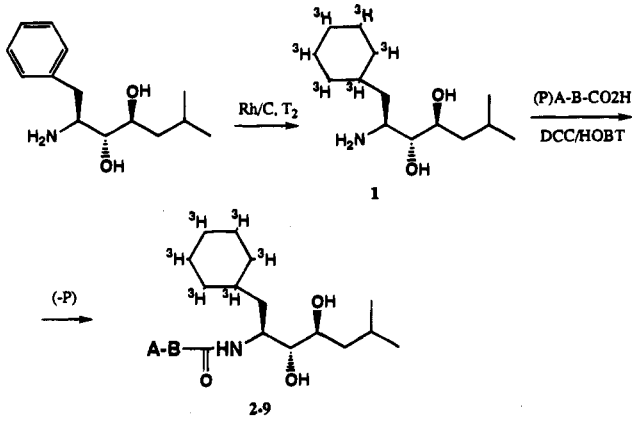


Scheme 2



[a] CBZ = benzyloxycarbonyl; Et_3N = triethylamine; DMAP = (dimethylamino)pyridine; DCC = dicyclohexylcarbodiimide; HOBT = hydroxybenzotriazole.

Scheme 3. Synthesis of Tritiated Compounds[a]



[a] A, B = various structures as shown in Figure 2; P = suitable protecting group.

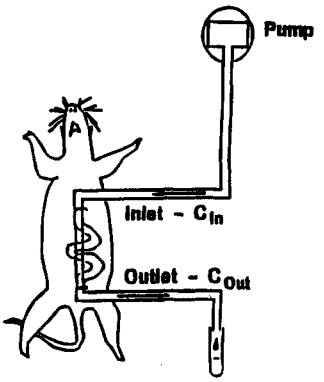


Figure 3. Single-pass intestinal perfusion in rat experimental setup.

Hepatic Processing

Hepatic processing of the analogs was examined in the isolated perfused rat liver model, using both the single-pass and recirculating modes (Figure 4). Initially the single-pass model was chosen to compare uptake rates among compounds. In this model, the isolated perfused rat liver was exposed to a constant concentration of tritiated test compound for 180 s. During this brief exposure to drug, hepatic uptake can be determined independently of elimination and storage capacity, as can the influence of nonhepatic factors such as plasma protein binding and circulating cellular components. BSP (sulfobromophthalein), an organic dye extensively extracted by the liver, was used as a reference.[10] BSP rapidly achieved a period of steady-state extraction with concentrations of 10–300 μM (ranging from 100 to 13% extracted at steady state, respectively).

The results with six of the compounds tested at a 1 μM concentration are shown in Figure 5. With all compounds a rapid initial uptake was observed, and there was either no detectable period of steady-state extraction (compounds **1**, **2**, and **3**) or steady-state extraction was greater than 90% at all the concentrations tested (compounds **4**, **5**, and **7**), illustrated with compound **5** in Figure 6. This narrow range did not allow discrimination among these test compounds, and compounds could not be differentiated using the single-pass hepatic extraction model during the 180 s.

To further profile the compounds, studies were performed in an isolated perfused recirculating liver preparation (Figure 4). This model exposes the liver to a single bolus of test compound and is monitored for 2 h. Under these conditions, the compound may be taken up into the liver, processed, and then released into the bile or refluxed back into the perfusate. Samples of perfusate and bile were collected for measurement of total radioactivity before bolus injection of compound and at several time points over the 2 h test period. At the conclusion of each experiment the hepatic uptake rate of 100 μM BSP was determined and compared to a series of historical controls.

A selected group of compounds was tested in this protocol at an initial concentration of 1 μM. As in the single-pass hepatic extraction model, all compounds disappeared rapidly from the perfusate after bolus injection. The compounds differed in their distribution profile between perfusate, liver, and bile as shown by a comparison between compounds **1** and **4** (Figure 7). With **4**, the radioactivity appeared primarily in the bile over time, while radioactivity from **1** was found to efflux back into the perfusate. The other compounds studied had distributions between perfusate, bile, and liver that were either similar or intermediate between **1** and **4** (Figure 8).

Compound **4** was studied under conditions of pro-

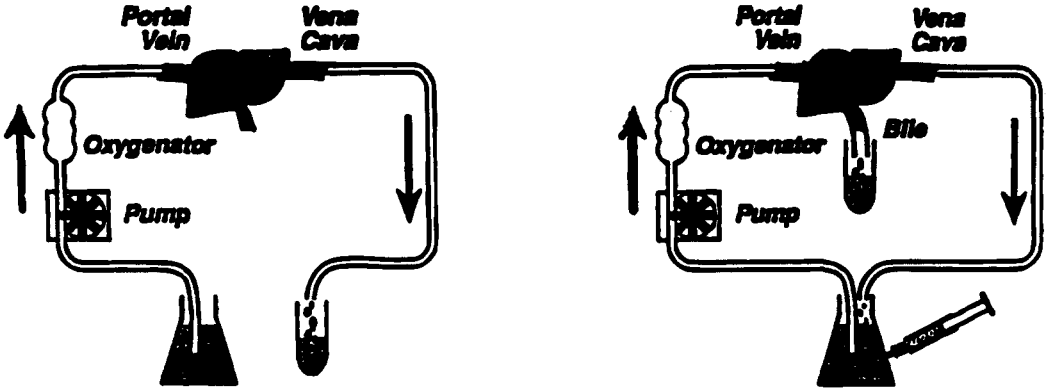


Figure 4. Experimental setup for single-pass (left) and recirculating (right) models of hepatic extraction in isolated perfused rat liver.

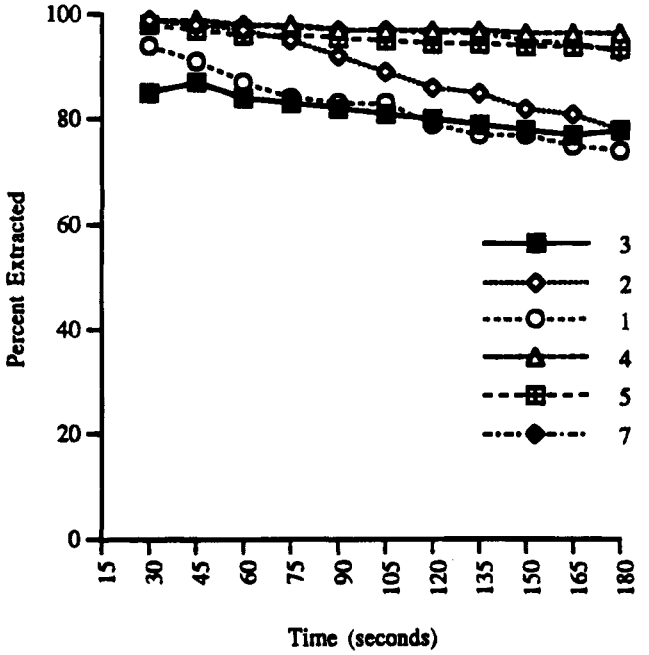


Figure 5. Single-Pass Data (1 μM). Values are the means of Four Livers. SEM values were within 3% of the mean.

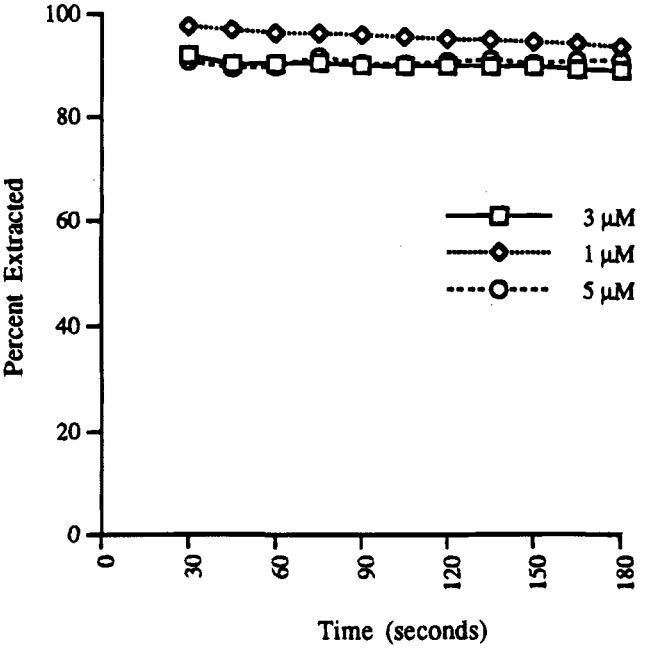


Figure 6. Single-pass data for **5** at three concentrations. Values are the mean of four livers. SEM values were within 3% of the mean.

longed exposure with the single-pass model. This compound was suitable for study because it represented a highly extracted compound with a demonstrable steady state of extraction. It also had renin inhibition activity so the presence of a cold compound could be detected by bioassay. A concentration of 10 μM cold **4** was infused for 120 min (Figure 9). Unlabeled drug concentrations were determined by bioassay.[11] As in the single-pass experiment with radiolabeled **4**, hepatic extraction of unlabeled drug was greater than 98% during the first 120 s of the experiment. Over the next 75 min, hepatic extraction fell markedly. A steady state was reached between 75 and 120 min, and at 120 min an apparent first-pass hepatic extraction of 24 ± 7% was observed. At this time the increased appearance of bioactivity in the perfusate could be the result of an increased reflux from the hepatocyte into the perfusate of parent **4**, active metabolites, or both parent and metabolites. Alternatively, actual uptake of **4** may have been reduced. To determine the actual uptake of **4** into the liver at that point, livers were exposed (beginning 118 min into the experiment) to radiolabeled **4**[12] for 150 s. This period, like the first 150 s of exposure, was brief enough to determine extraction without efflux of radiolabeled parent or formation of labeled metabolites. Actual extraction of radiolabeled **4** was found to be only 73%, as determined by scintillation counting. The reduction in extraction of **4** with prolonged exposure indicates that perhaps the hepatic processes involved in the handling of **4** had become saturated.

Discussion

The single-pass rat intestinal perfusion technique used in this study measured intestinal permeability of the compounds which was used as a gauge for intestinal absorption. All compounds tested had acceptable stability in an enzyme-rich blank intestinal perfusate; therefore, compound disappearance from the intestinal lumen during perfusion (at steady state) was due to drug permeability into the gut wall. This model does not address gut wall metabolism. In these intestinal absorption studies, L-phenylalanine, prednisolone, gabapentin, and D-mannitol were used as reference compounds with known values for the fraction of dose absorbed in humans of 1.0, 1.0, 0.74, and 0.05, respectively, corresponding to P_{eff} values of 3.57, 0.93, 0.46, and 0.05.[13,9b] Most of the tritiated analogs tested in this study exhibited P_{eff} of greater than unity, suggesting that the compounds would be well-absorbed from solution in the intestine. There were some trends for

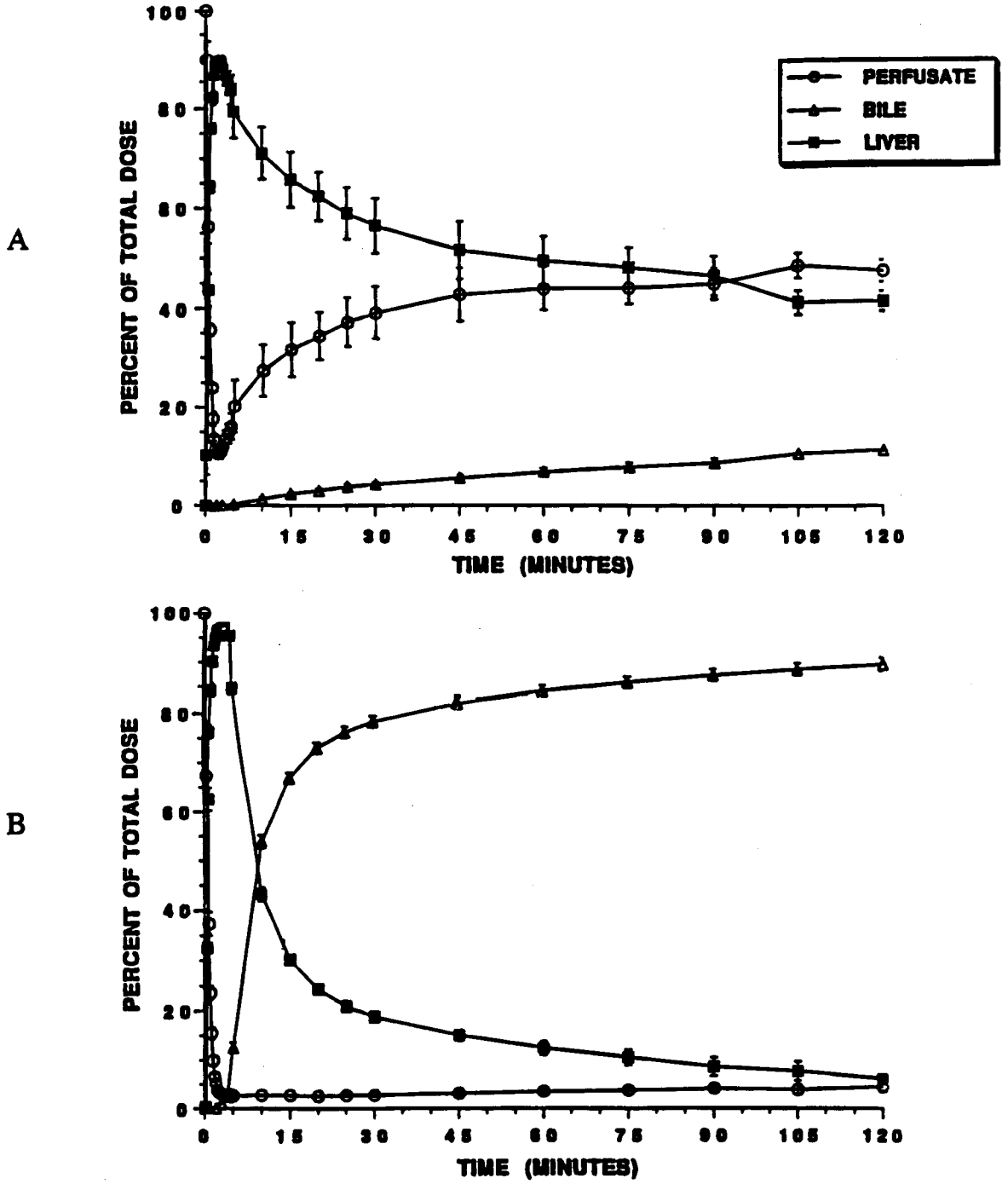


Figure 7. Recirculating isolated perfused rat liver. (A) **1**, 1 μM concentration, $n = 3$. (B) **4**, 1 μM concentration, $n = 4$.

intestinal permeability and physicochemical parameters that were seen upon examination of the data. Plotting P_{eff} versus molecular weight and versus log P (Figures 10 and 11) suggests a greater dependence of permeability on log P than on molecular weight. This dependence on lipophilicity is consistent with recent results reported with renin inhibitor compounds measuring portal concentration of drug after intraduodenal administration.[6b] It is also likely that the compounds are less well-absorbed when partially charged under assay conditions (e.g., compounds **3**, **6**, and **9** with a P_{eff} value of less than 1.0). Those compounds in the upper right portion of Figure 10 are those with no ionizable functionality, while those in the lower left contain acid or amino groups. Within the nonionizable compounds there is a trend toward decreased absorption with increasing molecular weight. Hydrogen-bonding functionality along the backbone of renin inhibitor compounds has been implicated as an important factor in intestinal transport in one study;[14] however, as noted, H-bonding parameters could not be separated from molecular weight in our analysis (Figure 2); an analysis of H-bonding and permeability yields a plot similar to Figure 11.

No pronounced distinction among our compounds could be made based on initial single-pass experiments, as all compounds disappeared from the perfusate within minutes. This is in contrast to results found with cholecystokinin analogs, where hydrophobicity was a major determinant of hepatic extraction.[15] Further studies in the recirculating model described differential handling for the analogs after uptake. Compounds exhibited different distributions between bile and perfusate after 2 h. Interpretation of these results is

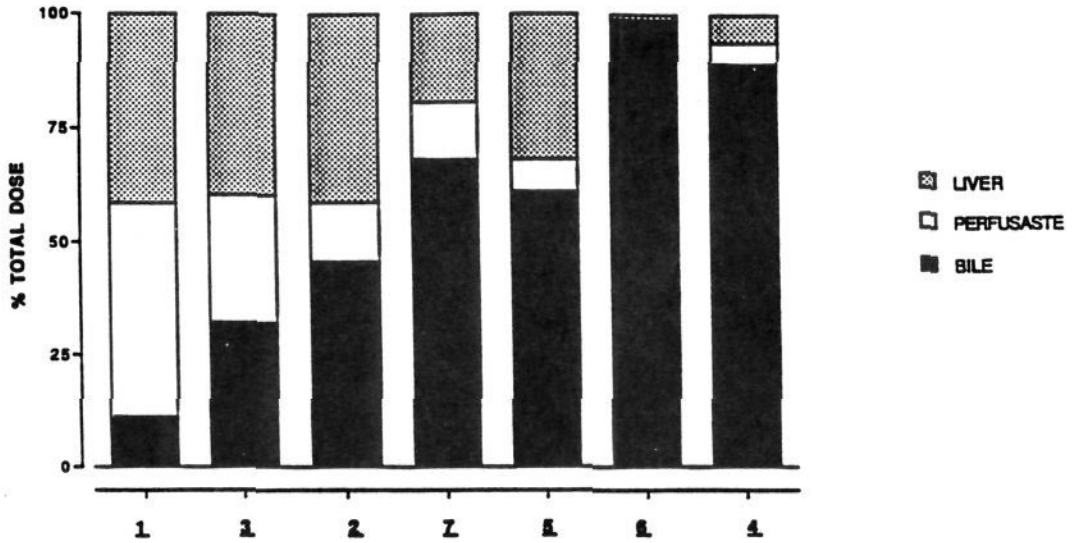


Figure 8. Distribution of radioactivity among perfusate, bile, and liver.

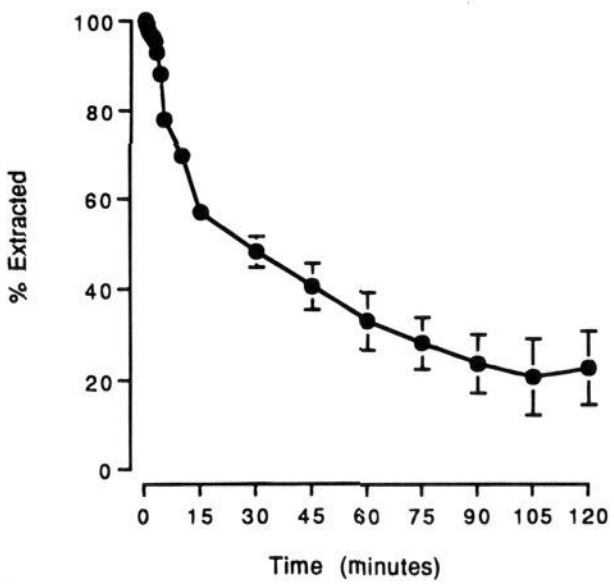


Figure 9. Constant infusion of **4** (10 μM). Concentrations of **4** were determined by bioassay. Values are expressed as mean ± SEM (n = 4).

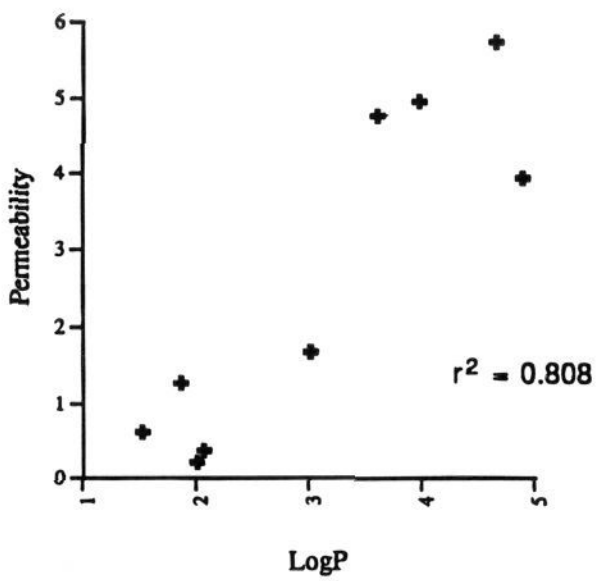


Figure 10. P_{eff} versus log P.

complicated by the potential for metabolism of the compounds within the liver. HPLC analysis of the perfusate from **1** indicated that the radioactivity seen was not associated exclusively with parent compound.[16] Additional experiments in the bile duct-cannulated rat with **1** and **4** following both IV and PO administration found that the majority of the radioactivity seen in the bile with **4** (as the primary route of elimination) and in the urine with **1** was not parent[17] although the metabolic product(s) was not identified.

It is important to note that the maximum rate of appearance of radioactivity in bile occurred between 5 and 10 min after bolus injection for all compounds (as illustrated by Figure 7). When the bile elimination rate (between 5 and 10 min) was examined as a function of molecular weight of parent compound, a significant relationship was observed (Figure 12). In contrast, no relationship was apparent between bile elimination rate and log P of parent compound (Figure 13).

This hepatic model employs a Krebs buffer as the perfusion media and thus does not take into account the possibility of protein binding or association with red blood cells as protective mechanisms for the compounds as they pass through the liver. In the intact animal these mechanisms may reduce the clearance of these molecules. Furthermore, the long-term exposure experiment with **4** indicates that perhaps hepatic processing may be saturable over time. This would be more analogous to oral administration of drug if the portal concentration achieved exceeds the saturation threshold.

Conclusions

In this study, compounds with higher lipophilicity were better absorbed from the intestine. Absorption may also be dependent on molecular charge, as compounds with ionizable functionality were, in general, less well absorbed than neutral compounds. Neutral compounds exhibited a dependence on smaller molecular weight for better absorption. All compounds were rapidly taken up into the liver regardless of log P or molecular weight; however, excretion into bile occurred at a more rapid rate with higher molecular weight compounds. These results are consistent with the

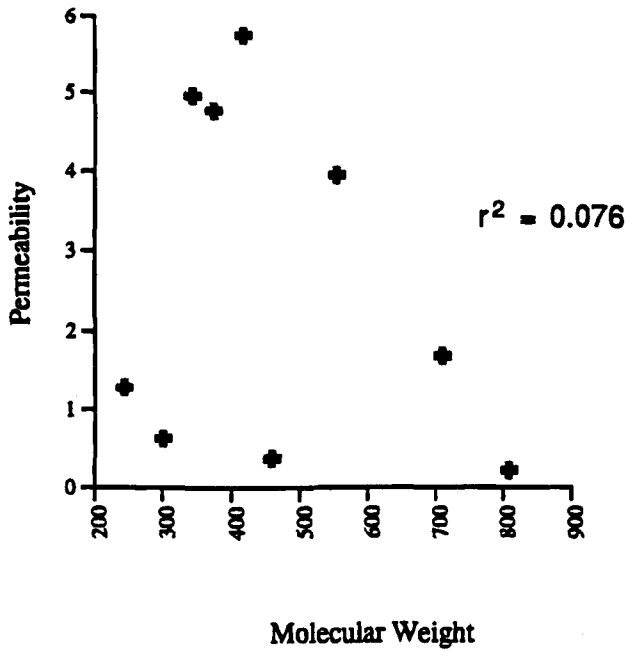


Figure 11. P_{eff} versus molecular weight.

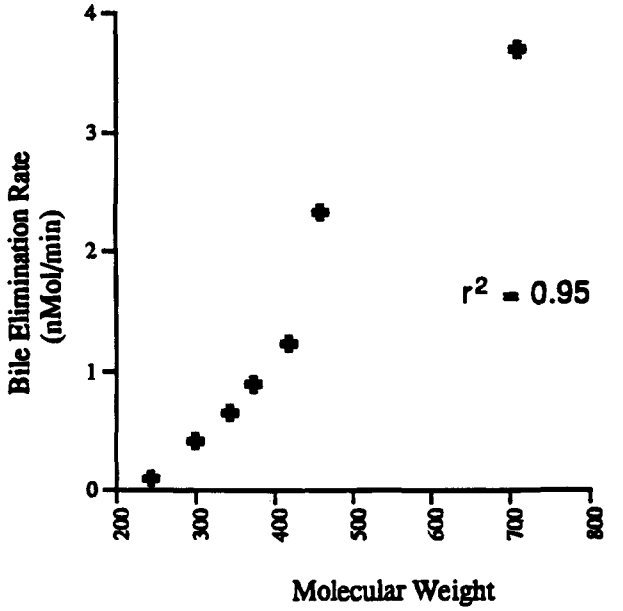


Figure 12. Molecular weight versus rate biliary clearance.

conventional wisdom that compounds with a higher log P and lower molecular weight offer the best opportunity of achieving good oral bioavailability.

With renin inhibitors, the problem of maintaining sufficient receptor potency with a lower molecular weight compound is a particular challenge. X-ray crystallographic analyses of potent compounds bound to the renin enzyme show a linear alignment with a series of weak to medium interactions acting cooperatively to afford maximum receptor affinity.[18] Given the extended binding conformation required by the enzyme, a higher molecular weight compound may be required to span the contacts necessary for receptor affinity. There have been, however, a number of clinical candidates which were efficacious in primates and humans, despite low plasma concentrations.[19] Whether this is due to active metabolites, protection by plasma protein binding, selective tissue action such as in the kidney, or some other mechanism is not known. Incorporation of desirable physicochemical parameters adds a layer

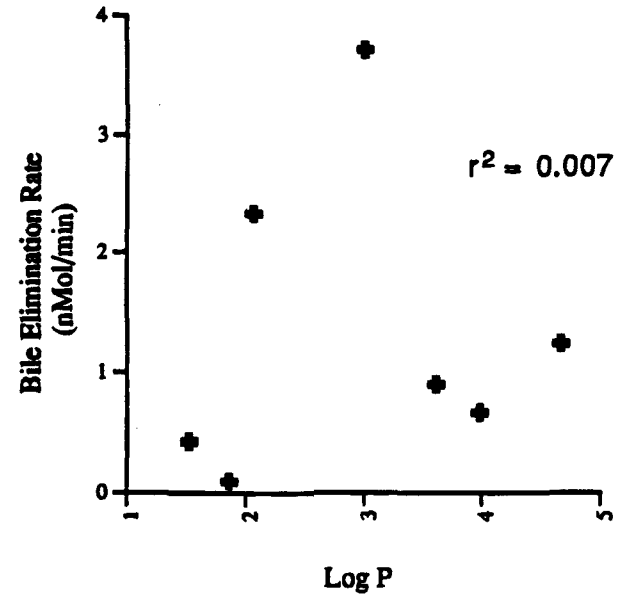


Figure 13. log P versus rate of biliary clearance.

of complexity to this problem that may only be achieved with a true non-peptide compound.

Experimental Section

Chemistry. For nonradioactive intermediates, all spectra and microanalysis data were obtained by the Parke-Davis Analytical Chemistry section. ^{1}H NMR spectra were obtained on either a Bruker AM250 or a Varian XL300 NMR spectrometer and were run in either $CDCl_3$ or d_6-DMSO. Chemical shifts are reported in δ units downfield from tetramethylsilane. Mass spectra were obtained on (a) Finnigan 4500 MS, (b) VG Ananlytical 7070E/HF MS, (c) VG Masslab Trio-2A MS, or (d) Finnigan TSQ-70 MS instruments. Flash chromatography was performed using E. Merck silica gel (230–400 mesh). Thin-layer chromatography was performed using E. Merck 60 F_{254} precoated silica gel plates (0.25 mm × 5 cm × 10 cm). Visualization was obtained by UV light. Solvents were interchangeabley dried according to Perrin and Armarego[20] or purchased dry and used fresh from suppliers.

For analysis of radioactive compounds, radioactivity was determined with a Packard Tri-Carb 4530 liquid scintillation counter using Beckman Ready-Gel as the counting medium. TLC plates, E. Merck silica gel 60 F_{254}, were scanned on a Berthold LB2832 automatic TLC linear analyzer. Column chromatography was performed using E. Merck silica gel (230–400 mesh). HPLC was performed using a Waters 600E solvent delivery system, a Waters 481 variable-wavelength UV detector, and Radiomatic Beta Flow 1 radioactivity flow detector. ^{1}H-NMR spectra were recorded on a Varian XL-200 (200 MHz) spectrometer. Chemical shifts are reported in δ units downfield from tetramethylsilane.

Syntheses of compounds **3** and **5–7** were conducted with commercially available precursors and radiolabeled **1** by typical peptide coupling reactions described below (methods A and B) and in Table 2. The synthesis of compound **2** is given. The synthesis of the precursor for **4**[8] has been described; the synthesis of the precursors to **8** and **9** is given, and these were coupled as described. All precursors had satisfactory NMR and MS spectra. A typical coupling reaction is described as method A when DCC (dicyclohexylcarbodiimide) was used and method B when EDAC (ethyl[3-(3-dimethylamino)propyl]carbodiimide hydrochloride salt) was used. A mixture of cold **1** and tritiated **1** was used in a predetermined ratio to give a desired final specific activity after coupling in both methods. Further deprotection reactions (compounds **3**, **6**, and **9**) are given. Table 2 cites sources and method used, yields and purity, specific activity, and HPLC (radiochemical and chemical) data of the products.

The tritiation reaction (as given in Scheme 3) yielding 2-amino-1-cyclohexyl-6-methyl-heptane-3,4-diol (ACDMH, **1**)

Table 2. Compound Source, Coupling Synthetic Method, Specific Activity and Purity

compd	source	method	yield (%)	specific activity (mCi/mmol)	HPLC radiochemical purity (%)	HPLC chemical purity (%)
1	*a*	–	–	131.6	98.89	–
2	*a*	–	84.6	24.72	98.84	–
3	*b*	B[g]	91.4	6.73	96.00	98.00
4	*c*	A	45.2	6.43	97.10	98.21
5	*d*	B	73.4	15.84	96.6	100.0
6	*e*	A[g]	67.5	22.32	100.0	89.66
7	*f*	B	42.9	16.51	91.91	100.0
8	–	A	52.3	128	94.79	54.47
9	–	A	43.4	59.5	91.81	78.86

[a] Parke-Davis Radiochemistry. [b] U.S. Biochemical Corp. [c] Reference 7. [d] Columbia Organic Chemial Corp. [e] Sigma. [f] Aldrich. [g] Given in the Experimental Section.

was carried out by Amershem Corp. (2636 Clearbrook Drive, Arlington Heights, IL 60005) and by Chemsyn Science Labs (13605 West 96th Terrace, Lenexa, KS 66215) and the product purified in-house.

General methods are illustrated with the compounds noted.

Method A ([1*S*-[1*R(*R**),2*S**,3*R**]]-2-[(*tert*-Butoxycarbonyl)amino]-*N*-(1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]succinamic Acid Benzyl Ester (Benzyl Ester-Protected 6).** An ice-cold mixture of 132.8 mg (0.4 mmol) of *N*-*t*-BOC-L-aspartic acid α-benzyl ester, 83 mg of 1, 17 mg of [^{3}H]1 (0.4 mmol), 59.4 mg (0.4 mmol) of HOBT, and 91.4 mg (0.4 mmol) of DCC in 2 mL of dichloromethane (CH_2Cl_2) was stirred at 0 °C for 2 h and for 18 h at ambient temperature. The mixture was filtered and the filtrate diluted with CH_2Cl_2. The organic layer was washed sequentially with 10% citric acid, saturated $NaHCO_3$, and brine, dried (Na_2SO_4), and concentrated under a N_2 stream to yield 249 mg of a white solid. The solid was purified by silica gel chromatography (25 g, 2% MeOH/CH_2Cl_2) to yield 152.2 mg (67.5%) of a white solid (benzyl ester of **6**; deprotection given below).

Method B ([1*S*-([1*R,2*S**,3*R**)]-[[[1-(Cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]carbamoyl]methyl]carbamic Acid Benzyl Ester (CBZ-Protected 3).** An ice-cold mixture of 43 mg (0.2 mmol) of CBZ-glycine (*N*-(benzyloxycarbonyl)glycine), 47.1 mg of ACDMH (1), 2.86 mg of [^{3}H]-1 (0.2 mmol), and 39.4 mg EDAC in 1 mL of CH_2Cl_2 was stirred under N_2 at 0 °C for 2 h and then for 18 h at ambient temperature. The mixture was diluted with CH_2Cl_2 and washed sequentially with 10% citric acid, brine, saturated $NaHCO_3$, and brine, and the organic layer was dried (Na_2SO_4). After filtering, the filtrate was concentrated under a N_2 stream and dried under high vacuum to yield 81.6 mg (0.19 mmol, 91.3%) of a white solid (CBZ-protected **3**; deprotection given below).

Carbamic acid, [1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-, 1,1-Dimethylethyl Ester, [1*S*-(1*R, 2*S**,3*R**)]- (2).** An ice-cold mixture of 42.2 mg of **1** and 7.8 mg of [^{3}H]-1 (0.2 mmol) in 1 mL of CH_2Cl_2 was treated with 50 mg (0.23 mmol) of di-*tert*-butyl dicarbonate, and the mixture was stirred overnight at room temperature under N_2. The solution was dried under a N_2 stream to yield a white solid which was purified by silica gel chromatography (8 g, MeOH/CH_2Cl_2 (1–2%) to yield 59.7 mg (84.6%) of **2** as a white solid.

Acetamide, *N*-[1-(Cyclohexylmethyl)-2,3-dihydro-5-methylhexyl]-2-amino-, [1*S*-[1*R,2*S**,3*R**)]- (3).** The solid from method B above was dissolved in methanol and treated with 5 mg of 20% Pd/C catalyst. The mixture was stirred under H_2 (1 atm) for 1.5 h, filtered through Celite, concentrated under a N_2 stream. The residue was dissolved in 5% MeOH/CH_2Cl_2 and chromatographed on silica gel (2 g) to yield 49 mg (86.9%) of **3** as a white solid.

Butanoic Acid, 4-[[1-(Cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]amino]-2-[[(1,1-dimethylethoxy)carbonyl]-amino]-4-oxo-, [1*S*-[1*R(1*R**)2*S**,3*R**]]- (6).** A mixture of 80.8 mg (0.15 mmol) of the solid from method A above and 10 mg of 20% Pd/C in 2 mL of MeOH was stirred under H_2 (1 atm) for 2 h. After filtering through Celite, the solution was concentrated under a N_2 stream to yield 86.2 mg of a gel. Purification by reverse phase preparative HPLC yielded 29.0 mg (40%) of **6** as a white solid.

[*S*-(*R,*R**)]-2-[(3-Phenyl-2-pyrrol-1-ylpropionyl)amino]-hexanoic Acid (13, α-Pyrrolophenylalanine–norleucine Precursor to 8, Scheme 1). 3-Phenyl-2-pyrrol-1-ylpropionic acid (α-Pyrrolophenylalanine (12)).** A solution of 29.6 g (0.36 mol, 6-fold excess) of anhydrous NaOAc in 250 mL of HOAc was heated to reflux and 10.0 g (0.06 mol) of L-phenylalanine (**10**) added. When all had dissolved, 7.9 mL (0.06 mol) of 2,5-dimethoxytetrahydrofuran (**11**) was added and the solution heated for 1 min. The dark solution was poured onto ice and extracted twice with EtOAc. The organic layers were washed with saturated NaCl, dried over $MgSO_4$, and concentrated under reduced pressure to afford 15.9 g of a dark oil. This was taken up in EtOH, treated with charcoal, and filtered and the solvent removed under reduced pressure, leaving 14.6 g of a dark oil. This was taken up in 100 mL of Et_2O, and 11.8 mL (0.06 mol) of dicyclohexylamine was added. A solid separated which was collected and recrystallized from absolute EtOH. There was obtained 13.0 g of the salt, mp 202–204 °C.[21] This salt was suspended in EtOAc and washed twice with 1 N citric acid and then with saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left 7.69 g (60%) of **12** as an oil which crystallized: ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.20–3.48 (m, 2H), 4.78 (m, 1H), 6.16 (m, 2H), 6.66 (m, 2H), 7.00 (m, 2H), 7.22 (m, 3H); MS (FAB, thioglycerol) *m*/*z* 216 (m + 1); $[\alpha]^{23}$ −76° (*c* 1.192 in MeOH). Anal. Calcd for $C_{13}H_{13}NO_2 \cdot 0.2H_2O$ (218.24): C, H, N.

α-Pyrrolophenylalanine–norleucine methyl ester was prepared: A solution of 5.0 g (27.5 mmol) of (*S*)-2-aminohexanoic acid methyl ester (L-norleucine methyl ester) hydrochloride, 5.93 g (27.5 mmol) of α-pyrrolophenylalanine (**12**), and 3.84 g (27.5 mmol) of 1-hydroxybenzotriazole in 60 mL of DMF was cooled in an ice bath and 3.84 mL (27.5 mmol) of triethylamine added, followed by a solution of 5.74 g (27.5 mmol) of DCC in 10 mL of DMF. The cooling bath was removed and the solution allowed to stir for 4 days. The mixture was then filtered and the DMF removed under high vacuum. The residue was taken up in EtOAc and filtered, and the filtrate was washed with 1 N citric acid, twice with H_2O, saturated $NaHCO_3$, and saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left 11.2 g of the product as a tan solid. Recrystallization from MeOH/H_2O using charcoal gave 8.11 g (86% yield) of the product as a light tan solid: mp 91–94 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.86 (t, 3H), 1.04–1.88 (m, 6H), 3.18–3.26 (m, 1H), 3.70 (s, 3H), 4.52 (m, 1H), 4.72 (m, 1H), 5.80 (d, 1H), 6.20 (broad s, 2H), 6.64 (broad s, 2H), 7.00 (m, 2H), 7.20 (m, 3H); MS *m*/*z* 343 (m + 1); $[\alpha]^{23}$ −48° (*c* 1.027 in MeOH). Anal. Calcd for $C_{20}H_{26}N_2O_3 \cdot 0.1\ H_2O$: C, H, N.

The ester was saponified: A solution of 7.88 g (23 mmol) of α-pyrrolophenylalanine–norleucine methyl ester in 75 mL MeOH was treated with 31 mL (31 mmol) of 1 N NaOH, warmed to effect solution, and then stirred at room temperature for 2 h. Some of the solvent was removed under reduced pressure, and the residue was diluted with H_2O and then washed with $CHCl_3$. The pH was brought to the Congo red end point with 1 N citric acid. The mixture was extracted twice with EtOAc, and these organics were washed with saturated NaCl. Drying over $MgSO_4$ and removal of the solvent under reduced pressure left 7.11 g (94%) of the product (**13**) as a light brown oil which could not be induced to crystallize: ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.84 (t, 3H), 1.08–1.40 (m, 4H), 1.48–1.70 (m, 1H), 1.72–1.94 (m, 1H), 3.20 (m, 1H), 3.64 (m, 1H), 4.52 (m, 1H), 4.72 (m, 1H), 5.80 (d, 1H), 6.18 (m, 2H), 6.64 (m, 2H), 7.00 (m, 2H), 7.18 (m, 3H), 9.00 (broad s, 1H); MS *m*/*z* 329 (m + 1). Anal. Calcd for $C_{19}H_{24}N_2O_3 \cdot 0.3$EtOAc: C, H, N.

4-[4-[[1-[2-(2-Aminothiazol-4-yl)-1-carboxyethyl]carbamoyl]-2-phenylethyl]sulfamoyl]-piperazin-1-yl]-4-oxo-(1,1-dimethylethoxy)butyric Acid (18, Precursor to 9, Scheme 2). (*S*)-4-[[1-(Methoxycarbonyl)-2-phenylethyl]-sulfamoyl]piperazine-1-carboxylic Acid Benzyl Ester (16). A mixture of 13.74 g of CBZ-piperazinesulfonyl chloride

(**14**, 43.1 mmol), L-phenylalanine methyl ester hydrochloride (**15**, 18.6 g, 86.2 mmol), triethylamine (12 mL, 86.1 mmol), and 4-(dimethylamino)pyridine (10 mg) was stirred in CH_2Cl_2 (400 mL) at room temperature for 4 days. The mixture was stirred at reflux for 8 h and the solvent evaporated. The residue was partitioned between EtOAc and 2 N aqueous HCl, and the organic layer was washed with 2 N Na_2CO_3 and saturated brine, dried ($MgSO_4$), filtered, and evaporated to give **16** as a brown oil (20.0 g, 100%): ^{1}H NMR (300 MHz, d_6-DMSO + drop D_2O) δ 2.40–3.90 (series of m, 14H), 5.08 (s, 2H), 7.20–7.40 (m, 5H).

(*S*)-4-[4-[[1-(Methoxycarbonyl)-2-phenylethyl]sulfamoyl)piperazin-1-yl]-4-oxo-butyric Acid (17). 16 (19.7 g, 42.7 mmol) was hydrogenated at 50 psi in MeOH (120 mL) with 20% Pd/C (2 g) for 18 h. The mixture was filtered and evaporated to give the intermediate ester as a white foam (13.7 g, 98%): ^{1}H NMR (300 MHz, d_6-DMSO + drop D_2O) δ 2.40–3.00 (series of m, 10H), 3.69 (s, 3H), 3.86 (dd, J = 9.2, 5.9 Hz, 1H), 7.05–7.40 (m, 10 H).

A mixture of 13.7 g (41.9 mmol) of this ester and succinic anhydride (4.61 g, 46.1 mmol) in CH_2Cl_2 (150 mL) was stirred at room temperature overnight. The solvent was evaporated to leave **17** as an off-white foam (18.1 g, 100%): ^{1}H NMR (300 MHz, d_6-DMSO + drop D_2O) δ 2.50–3.50 (series of m, 15H), 3.76 (s, 3H), 4.13 (m, 1H), 5.46 (d, J = 10.2 Hz, 1H), 7.20–7.40 (m, 5H), 8.45 (br s, 1H).

Acid **17** (4.67 g, 10.9 mmol) was stirred in CH_2Cl_2 (200 mL) and isobutylene (100 mL) with sulfuric acid (1 mL) at room temperature for 22 h in a sealed pressure vessel. The mixture was poured into 2 N $NaHCO_3$, the organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The extracts were combined, washed with saturated brine, dried ($MgSO_4$), filtered, and evaporated to leave a yellow oil. This oil was purified by chromatography on silica gel (100 g) eluting with 1% MeOH/CH_2Cl_2 to give the intermediate diester as a white foam (0.93 g, 18%): ^{1}H NMR (300 MHz, d_6-DMSO + drop D_2O) δ 1.36 (s, 9H), 2.25–2.60 (series of m, 9H), 2.74 (dd, J = 13.3, 10.4 Hz, 1H), 2.98 (dd, J = 13.6, 5.1 Hz, 1H), 3.22 (m, 4H), 3.73 (s, 3H), 3.88 (dd, J = 10.2, 4.9 Hz, 1H), 7.15–7.35 (m, 5H).

Lithium hydroxide (4.23 mL of 1M in water, 4.23 mmol) was added to the diester above (1.02 g, 21.2 mmol) in THF (20 mL) at 0 °C and stirred for 4 h at room temperature. The mixture was added to 5% citric acid (200 mL) and extracted with $CHCl_3$. The extracts were dried ($MgSO_4$), filtered, and evaporated to leave a yellow oil. This oil was purified by chromatography on silica gel (100 g of TLC grade) eluting with 2.5% methanol/1% HOAc/CH_2Cl_2 to give the monoacid as a colorless oil (0.76 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 9H), 2.58 (br s, 4H), 2.82 (br s, 5H), 3.18 (m, 1H), 3.30–3.60 (m, 4H), 4.16 (m, 1H), 5.30 (m, 1H), 7.20–7.40 (m, 5H), 9.60 (br s, 1H).

A mixture of the monoacid above (0.45 g, 0.95 mmol), 2-amino-3-(2-aminothiazol-4-yl)propionic acid methyl ester hydrochloride (0.27 g, 1.0 mmol), and HOBT (0.14 g, 1.05 mmol) in DMF (4 mL) was stirred at 0 °C and triethylamine (0.28 mL, 2.0 mmol) added. The mixture was stirred at 0 °C for 15 min and DCC (0.22g, 1.05 mmol) in EtOAc (10 mL) added over 30 min via syringe pump. The mixture was stirred at 0 °C for a further 15 min and then allowed to warm to room temperature and stirred overnight. The mixture was diluted with EtOAc, filtered, washed with H_2O, 2 N $NaHCO_3$, and saturated NaCl, dried ($MgSO_4$), filtered, and evaporated to leave a yellow oil. This oil was purified by column chromatography on SiO_2 (50 g), eluting with 2% and then 3% MeOH/CH_2Cl_2 to give the diester as a colorless glass (0.57 g, 92%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.82 (br s, 5H), 2.56 (br s, 4H), 2.80–3.60 (series of m, 12 H), 3.69 (s, 3H), 4.10 (m, 1H), 4.77 (m, 1H), 5.48 (br s, 3H), 6.12 (s, 1H), 7.20-7.40 (m, 5H), 8.13 (d, J = 7.9 Hz, 1H).

A mixture of diester above (0.46 g, 0.72 mmol) in THF 20 mL) at 0 °C was treated with lithium hydroxide (1.42 mL of 1.0M in H_2O, 1.42 mmol) and the mixture stirred at room temperature for 2 h. The mixture was added to 5% citric acid (100 mL) and extracted with EtOAc. The extracts were washed with saturated NaCl, dried ($MgSO_4$), filtered, and evaporated to leave a white solid. This was purified twice by column chromatography on SiO_2 (30 g), eluting with 10% MeOH/CH_2Cl_2 to give **18** (0.13 g, 28%) as a beige powder: ^{1}H NMR (300 MHz, d_6-DMSO + drop D_2O) δ 1.39 (s, 9H), 2.30–2.95 (series of m, 12H), 3.21 (m, 4H), 3.92 (m, 1H), 4.42 (m, 1H), 6.20 (s, 1H), 7.15–7.35 (m, 5H).

L-Alaninamide, *N*-[[4-(3-Carboxy-1-oxopropyl)-1-piperazinyl]sulfonyl]-L-phenylalanyl-3-(2-amino-4-thiazolyl)-*N*-[1-(cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl]-[1*S*-(1*R,2*S**,3*R**)]- (9).** Compound **18** was coupled by method A above to give the penultimate *tert*-butyl ester. The ester was removed: a solution of t-butyl ester of **9** (12.5 mg, 0.01 mmol) in 1.5 mL of CH_2Cl_2 was treated with 250 μL (3.2 mmol) of trifluoroacetic acid. After stirring at room temperature for 3 h the reaction was concentrated under a N_2 stream. The residue was taken up in CH_2Cl_2 and concentrated again to yield a film which was purified by reverse phase preparative HPLC (acetonitrile/water, 4:6) to give **9** (2.6 mg, 22.2%) as a solid.

Intestinal Perfusion Experiments. Stability Studies. Compounds were incubated at 37 °C for 6–8 h in modified MES buffer (pH 6.5) or rat intestinal perfusate. At selected time points, samples were combined with an equal volume of acetonitrile, vortexed, and centrifuged, and the supernatant was injected onto HPLC with UV and radiomatic detection.

Single-Pass Intestinal Perfusion in Rats. Male white Wistar rats (250–350 g) were fasted overnight with water ad libitum. Laparotomy was performed after onset of anesthesia and the upper jejunum was identified. Proximal and distal ends of a 3- to 15-cm segment of intestine were cannulated with tubing. Perfusion solutions of drug and a nonabsorbable marker, [^{14}C]PEG-4000, were prepared in iso-osmotic MES buffer (pH 6.5). Drug solution was perfused into the proximal intestine at a constant concentration, C_{in}, and flow rate, Q (0.125–0.25 mL/min), using a Harvard Apparatus Infusion Pump. Exiting perfusate was collected from the distal cannula at concentration C_{out}. Reference compounds, test compounds, and PEG-4000 concentrations were determined by liquid scintillation spectrometry. The ratio of outlet to inlet drug concentration, C_{out}/C_{in}, was normalized for Q, intestinal length, L, and calculated drug diffusivity, D, corrected for water flux using the nonabsorbable marker, and then used to calculate dimensionless, steady-state effective permeabilities:[9b]

$$P_{eff} = [\ln(C_{out}/C_{in})]/(-4Gz)$$

where the Graetz number, $Gz = \pi DL/2Q$.

Gravimetric measurements were made for all samples from the 45 intestinal perfusions for the nine compounds studied to confirm PEG 4000 served as a nonabsorbable marker under experimental conditions.

Hepatic Studies. Male Wistar rats (250–300 g, Charles River) were anesthetized by concurrent intramuscular administration of ketamine and xylazine. Livers were isolated and perfused using either a single-pass or recirculating model in a standard organ reperfusion apparatus. Livers were placed in a heated chamber (37 °C) and perfused through the portal vein with oxygenated Krebs bicarbonate buffer to pH 7.4. Flow was set to 40 mL/min with a roller pump. The livers were allowed to stabilize for 30 min before exposure to test compounds. During the stabilization period, blank perfusate was recycled through the liver. Liver viability was assessed throughout the experiment by visual examination of the liver for gross changes in appearance and by measurement of perfusion pressure, bile flow, and effluent pH, and the data were used only if these parameters were satisfactory. During the single-pass experiments samples were taken for measurement of total radioactivity before each exposure and every 15 s during exposure. Compounds were first dissolved in DMSO and then added to the Krebs bicarbonate buffer. The final concentration of DMSO (1%) was found to have no effect on first-pass hepatic extraction of BSP. First-pass hepatic extraction was calculated as a percentage ((1-radioactivity in the Vena cava per unit time/total radioactivity in portal vein per unit time) × 100). In the recirculating protocol, the total volume of the reservoir was 25 mL and the total volume of the recirculating system was 45 mL. After a 30 min acclimation, 45 nmol of tritiated compound was bolus injected into

the reservoir. This resulted in an initial concentration of 1 μM. Samples of perfusate and bile were collected for measurement of total radioactivity before bolus injection and at several time points over the next two hours. The percent of total radiolabel in the liver was determined by subtraction. Radioactivity in the samples was determined by adding 200 μL (recirculating studies) or 2 mL (single-pass studies) of perfusate to 18 mL of scintillation fluid (Ready Gel, Beckman Instruments Inc., Fullerton, CA). Samples were then counted in a liquid scintillation counter (Packard Tri-Carb 4530). Each sample was corrected for quenching by a standard curve. BSP was measured using a spectrometric procedure.[22] Unlabeled **4** was measured by bioassay.[11]

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Supplementary Material Available: NMR spectra of the final cold and hot compounds for this study (16 pages). Ordering information is given on any current masthead page.

References

(1) Hamilton, H.; Steinbaugh, B.; Blankley, C. J.; Taylor, M.; Chan, O. H.; Stewart, B.; Schroeder, R.; Ryan, M.; Rapundalo, S.; Cook,J.; Bernabei, A.; Stewart, C. Evaluation of the Intestinal Permeability and Hepatic Handling of Peptidomimetic Analogs. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 813–818.

(2) (a) Plattner, J. J.; Norbeck, D. W. Obstacles to Drug Development From Peptide Leads. In *Drug Discovery Technology*; Clark, C. R., Moos, W. H., Eds.; Horwood: Chichester, U.K., 1990; pp 92–126. (b) Humphrey, M. J.; Ringrose, P. S. Peptides and Related Drugs: A Review of Their Absorption, Metabolism, and Excretion. *Drug Metab. Rev.* **1986**, *17* (3 and 4), 283–310.

(3) Kleinert, H. D.; Luly, J. R.; Marcotte, P. A.; Perun, T. J., Plattner, J. J.; Stein, H. Renin Inhibitors Improvements in the Stability and Biological Activity of Small Peptides Containing Novel Leu-Val Replacements *FEBS Lett.* **1988**, *230*, 38–42.

(4) Subsite nomenclature (P_n) is described in the following: Schechter, I.; Berger, A. On the Size of the Active Site in Proteases. I. Papain. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157–162.

(5) Gresk, C. J.; Dorn, C. R.; Markos, C. S. Tritium Labeled SC-46944. *International Isotope Society, Third US Central Regional Meeting*, Indianapolis, IN, Oct 19, 1990.

(6) (a) Seydel, J. K. Quantitative Structure-Pharmacokinetics Relationships and Their Importance in Drug Design, Possibilities and Limitations. *Meth. Find. Exptl. Clin. Pharmacol.* **1984**, *6*, 571–581. (b) Rosenberg, S. H.; Kleinert, H. D.; Stein, H. H.; Martin, D. L.; Chekal, M. A.; Cohen, J.; Egan, D. A.; Tricarico, K. A.; Baker, W. R. Design of a Well-Absorbed Renin Inhibitor. *J. Med. Chem.* **1991**, *34*, 469–471.

(7) Haky, J. E.; Young, A. M. Evaluation of a Simple HPLC Correlation Method for the Estimation of the Octanol-Water Partition Coefficients of Organic Compounds. *J. Liq. Chromatogr.* **1984**, *7*, 675–689.

(8) (a) Belmont, D. T.; Henderickson, V.; Hoekman, M. Process for the Preparation of (1S-(1R*,2S*,3R*))-N-(4-morpholinylsulfonyl)-L-Phenylalanyl-3-(2-amino-4-thiazolyl-N-((cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl)-L-alaninamide. US Patent 5,220,031. (b) Belmont, D. T.; Hendrickson, V.; Hoekman, M. Process for the Preparation of (1S-(1R*,2S*,3R*))-N-(4-morpholinylsulfonyl)-L-Phenylalanyl-3-(2-amino-4-thiazolyl-N-((cyclohexylmethyl)-2,3-dihydroxy-5-methylhexyl)-L-alaninamide. US Patent 5,089,616.

(9) (a) Lu, R. H.; Chan, O. H.; Hamilton, H. W.; Reyner, E. L.; Schmid, H. L.; Steinbaugh, B.; Stewart, B. H. Discrimination Between the Intestinal Permeabilites of Renin Inhibitor Analogs as a Function of Lipophilicity and Molecular Weight Using a CACO-2 Cell Model: Comparison with a Rat Intestinal Perfusion Model. *Pharm. Res.* **1993**, *10*, S-179. (b) Amidon, G. L.; Sinko, P. J.; Fleisher, D. Estimating Human Oral Fraction Dose Absorbed: a Correlation Using Rat Intestinal Membrane Permeability for Passive and Carrier-Mediated Compounds. *Pharm. Res.* **1988**, *5*, 651–654.

(10) The K_m and V_{max} calculated for BSP in these experiments were similar to literature values: Hironor, T.; Aoyama, N.; Busch, N.; Mancuso, D. J.; Holzbach, R. J. Hepatic Extraction of Organic Anions in the Rat Depends of Ligand Hydrophobicity. *Hepatology* **1991**, *13*, 62–67.

(11) (a) Rosenberg, S. H.; Woods, K. W.; Kleinert, H. D.; Stein, H.; Nellans, H. N.; Hoffman, D. J.; Spanton, S. G.; Pyter, R. A.; Cohen, J.; Egan, D. A.; Plattner, J. J.; Perun, T. J. Azido Glycols: Potent, Low Molecular Weight Renin Inhibitors Containing an Unusual Post Scissile Site Residue. *J. Med. Chem.* **1989**, *32*, 1371–8. (b) Ruwart, M. J.; Sharma, S. K; Harris, D. W.; Lakings, D. B.; Rush, B. D.; Wilkinson, K. F.; Cornette, J. C.; Evans, D. B.; Friis, J. M.; Cook, K. J.; Johnson, G. A. Development of a Sensitive Activity Assay for High-Volume Evaluation of Human Renin Inhibitory Peptides in Rat Serum: Results with U-71,038. *Pharm. Res.* **1990**, *7*, 407–10.

(12) The concentration of administered **4** was maintained at 10 μM, containing 1% radiolabel.

(13) Stewart, B. H.; Kugler, A. R.; Thompson, P. R.; Bockbrader, J. N. A Saturable Transport Mechanism in the Intestinal Absorption of Gabapentin is the Underlying Cause of the Lack of Proportionality Between Increasing Dose and Drug Levels in Plasma. *Pharm. Res.* **1993**, *10*, 276–281.

(14) (a) Conradi, R. A.; Hilgers, A. R.; Ho, N. F. H.; Burton, P. S. The Influence of Peptide Structure on Transport Across Caco-2 Cells II. Peptide Bond Modification Which Results in Improved Permeability. *Pharm. Res.* **1992**, *9*, 435–439. (b) Karls, M. S; Rush, B. D.; Wilkinson, K. F.; Vidmar, T. J.; Burton, P. S.; Ruwart, M. J. Desolvation Energy: A Major Determination of Absorption, But Not Clearance, of Peptides in Rats. *Pharm. Res.* **1991**, *8*, 1477–1481. (c) Smith, A. B.; Hirschmann, R.; Pasternak, A. P.; Akaishi, R.; Guzman, M. C.; Jones, D. R.; Keenan, T. P.; Sprengeler, P. A.; Darke, P. L.; Emini, E. A.; Hollosay, M. K.; Schleif, W. A. Design and Synthesis of Peptidomimetic Inhibitors of HIV-1 Protease and Renin. Evidence for Improved Transport. *J. Med. Chem.* **1994**, *37*, 215–218.

(15) Hunter, E. B.; Powers, S. P.; Kost, L. J.; Pinon, D. I.; Miller, L. J.; LaRusso, N. F. Physicochemical Determinants in Hepatic Extraction of Small Peptides. *Hepatology* **1990**, *12*, 76–82.

(16) Rapundalo, S. Unpublished results.

(17) Stewart, B. H.; Lu, R. H. H.; Chan, O. H. Absorption, Biotransformation, and Excretion of the Renin Inhibitor, CI-992, and Its P1-P1′ Isostere in the Bile Duct-Cannulated (BDC) Rat. *Pharm. Res.* **1992**, *9*, S295.

(18) (a) Lunney, E. L.; Hamilton, H. W.; Hodges, J. C.; Kaltenbronn, J. S.; Repine, J. T.; Badasso, M.; Cooper, J. B.; Dealwis, C.; Wallace, B. A.; Lowther, W. T.; Dunn, B. M.; Humblet, C. Analyses of Ligand Binding in Five Endothiapepsin Crystal Complexes and Their Use in the Design and Evaluation of Novel Renin Inhibitors. *J. Med. Chem.* **1993**, *36*, 3809–3820. (b) Badasso, M.; Frazao, C.; Sibanda, B. L.; Dhanaraj, V.; DeAlwai, C.; Cooper, J. B.; Wood, S. P.; Blundell, T. L.; Murakami, K.; Miyazaki, H.; Hobart, P. M.; Geoghegan, K. F.; Ammirati, M. J.; Lanzetti A. J.; Danley, D. E.; O'Connor, B.A.; Hoover, D. J.; Sueiras-Diaz, J.; Jones, D. M.; Szelke, M. Crystallization and Preliminary X-ray Analysis of Complexes of Peptide Inhibitors with Human Recombinant and Mouse Submandibular Renins. *J. Mol. Biol.* **1992**, *223*, 447–453.

(19) (a) Patt, W. C.; Hamilton, H. W.; Ryan, M. J.; Painchaud, C. A.; Taylor, M. D.; Rapundalo, S. T.; Batley, B. L.; Connolly, C. J. C.; Taylor, D. G. A Novel Orally Active Renin Inhibitor Containing 2-Amino-4-Thiazoyl-Alanine at P2 with a Long Duration of Action in a Primate Model of Hypertension. *Med. Chem. Res.* **1992**, *2*, 10–15. (b) Kleinert, H. D.; Rosenberg, S. H.; Baker, W. R.; Stein, H. H.; Klinghofer, V. K.; Barlow, J.; Spina, K.; Polakowski, J.; Kover, P.; Cohen, J.; Denissen, J. Discovery of a Peptide-Based Renin Inhibitor with Oral Bioavailability and Efficacy. *Science* **1992**, *257*, 1940–1943. (c) Fischli, W.; Clozel, J. P.; Amrani, K. E.; Wostl, W.; Neidhart, W.; Stadler, H.; Branca, Q. Ro 42-5892 Is a Potent Orally Active Renin Inhibitor in Primates. *Hypertension* **1991**, *18*, 22–31. (d) Abernethy, D. R.; Wilner, K. D.; Lazar, J. D. Inhibition of Furosemide Stimulated Plasma Renin Activity After Oral Administration of CP-80,794, A Putative Renin Inhibitor. *Clin. Pharmacol. Ther.* **1990**, *47*, 141.

(20) Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: New York, 1966.

(21) Lit. mp 198–200.5 °C: Gloede, J.; Poduska, K.; Gross, H.; Rudinger, J. Amino Acids and Peptides. LXXIX. α-Pyrrolo Analogues of α-Amino Acids. *Collect. Czech. Chem. Commun.* **1968**, *33*, 1307–1314.

(22) Seligson, D.; Marino, J.; Dodson, E. Determination of Sulfobromophthalein in Serum. *Clin. Chem.* **1957**, *3*, 638–645.

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