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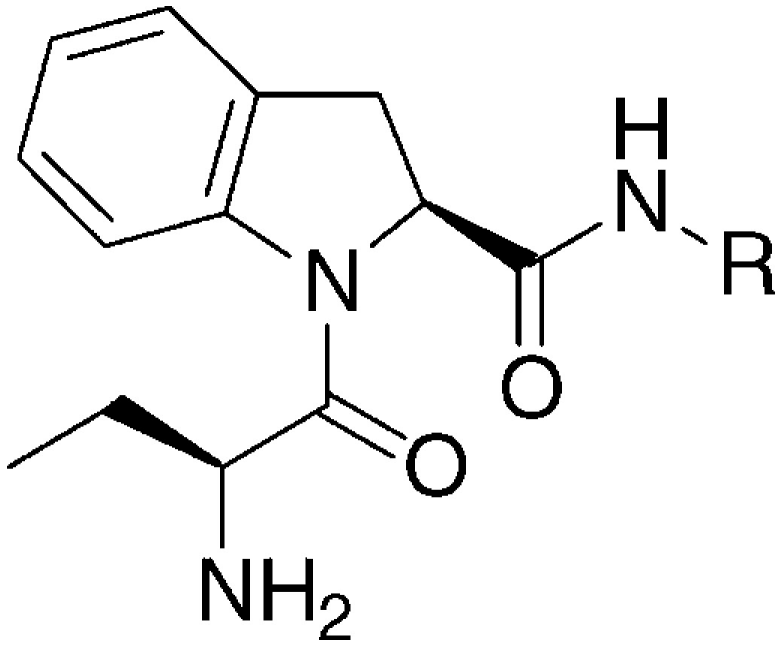

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Inhibitors of Tripeptidyl Peptidase II. 3. Derivation of Butabindide by Successive Structure Optimizations Leading to a Potential General Approach to Designing Exopeptidase Inhibitors

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The cholecystokinin-8 (CCK-8)-inactivating peptidase is a serine peptidase that has been shown to be a membrane-bound isoform of tripeptidyl peptidase II (EC 3.4.14.10). It cleaves the neurotransmitter CCK-8 sulfate at the Met-Gly bond to give Asp-Tyr(SO_3H)-Met-OH + Gly-Trp-Met-Asp-Phe-NH_2. Starting from Val-Pro-NHBu, a dipeptide of submicromolar affinity that had previously been generated to serve as a lead, successive optimization at P3, P1, and then P2 gave Abu-Pro-NHBu (**18**, K_i = 80 nM). Further transformation (by making a benzologue) gave the indoline analogue, butabindide (**33**) as a reversible inhibitor having nanomolar affinity (K_i = 7 nM). Retrospective analysis suggested the possibility of a general approach to designing exopeptidase inhibitors starting from the structure of the first hydrolysis product. Application of this approach to CCK-8 led to Abu-Phe-NHBu (**37**), but this only had K_i = 9.4 μM. Molecular modeling, to determine the minimum energy conformations and explain the 1000-fold better affinity of butabindide, indicated that **37** cannot access the likely active conformation of butabindide.

Introduction

The octapeptide cholecystokinin (CCK-8) in its sulfated (Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2) form functions as a neurotransmitter.[1] It is released in response to ingestion of food and is involved in the control of food digestion through regulation of gallbladder contraction,[2] pancreatic secretion,[2] and contraction of the pyloric sphincter to delay gastric emptying.[3] CCK-8 also functions as a satiety signal,[4] acting through CCK_1[5] (previously called CCK-A) receptors.

Exogenous CCK-8 has been shown to shorten meal duration and reduce meal size in several species[6] and in lean[7] and obese humans.[8] Use of CCK-8 as a satiety agent for assisting the treatment of obesity is, however, limited by its lack of oral activity and metabolic instability. Selective CCK-8 agonists for the CCK_1 receptor have been described, but so far none has yielded a clinically useful drug for treating obesity.[9] Previously, we have described[10] another approach to this problem, which is to design a selective peptidase inhibitor to protect the endogenous neuropeptide (CCK-8) from inactivation and hence potentiate its signal. Endogenous CCK-8 released from brain slices (from the rat) by K^+-induced depolarization was shown[11] to be inactivated by a peptidase successively cleaving at the two peptidic amide bonds where the carboxyl group is donated by a methionine residue.[12] The peptide is cleaved at first at the Met-Gly bond to give Asp-Tyr(SO_3H)-MetOH and CCK-5 (Figure 1) and then at the Met-Asp bond to give inactive fragments.[13]

Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

I

↓

Asp-Tyr(SO_3H)-Met-OH + Gly-Trp-Met-Asp-Phe-NH_2

II

Figure 1. Cleavage of sulfated CCK-8 (**I**) by the inactivating peptidase TPP II.

A simple peptidase was shown[10a] to effect both cleavages of CCK-8 and to correspond to tripeptidyl peptidase II (TPP II; EC 3.4.14.10). This is a subtilisin-like serine peptidase that was initially purified from cytosolic extracts of rat liver[14] and human erythrocytes,[15] but it is found in a variety of tissues.[16] It is an unusually large exopeptidase (subunits have a relative molecular mass 138 000[17]) that at neutral pH removes tripeptide from the free *N*-terminus of longer peptides.[16]

In seeking a peptidase inhibitor, our strategy[18] was to avoid including a serine-reactive group such as chloromethyl ketone or boronate ester since, although it should endow the inhibitor with high affinity (for inhibitory potency), it is also likely to react with other nucleophilic centers in the body in a rather nondiscriminatory fashion and thereby lose specificity. To maximize the therapeutic potential, the aim was therefore to seek a reversible inhibitor of the CCK-8 inactivating peptidase that did not possess a reactive group.

The approach taken[18] was to use molecular probes to look for noncovalent interactions surrounding the enzyme active site and to achieve closely matched ste-

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reospecific interactions between the enzyme and the putative inhibitor by empirical investigation. The method used was to first characterize the binding opportunities of the enzyme subsites using systematically varied dipeptides and tripeptides to determine the accessible volume for binding and to probe the potential for hydrophobic interaction. The compounds were studied[18] in vitro for their ability to inhibit the CCK-inactivating peptidase activity determined fluorometrically from the hydrolysis of the artificial substrate[15] Ala-Ala-Phe-amidomethylcoumarin (AAF-Amc; Amc = 4-methylcoumarin-7-ylamide).

We have previously described[18] the initial study of di- and tripeptides from which emerged the main lead Val-Pro-NHBu having sub-micromolar affinity (K_i = 0.57 μM) and clearly not possessing any obvious serine-reactive group. This served for structure optimization to yield the potent nanomolar reversible competitive inhibitor, butabindide.[10] The studies leading to this compound are described herein. Furthermore, a retrospective analysis of the derivation of butabindide led to the intriguing possibility of providing a general approach to designing expeptidase inhibitors given the structure of the first peptidic hydrolysis product.

Butabindide has since become available as a reagent[19] for inhibition of TPP II and has been used as a lead for the design of imidazole isosteres[20] (replacing the *N*-butyl carboxamide moiety) as TPP II inhibitors. As has been discussed by Breslin et al.,[20a] butabindide when unprotonated in solution can eliminate butylamine to form an indolinodiketopiperazine. They reported that 28% of butabindide had cyclized after 8 h at pH 7.0 at 38 °C. We can confirm that this has little consequence for the assay of butabindide during the time period of the assay and that the diketopiperazine was also inactive in our hands.

Chemistry

Formulas are given in Tables 1−4; melting points and crystallization solvents are given in Table 7 of the Supporting Information. The synthetic routes to compounds **1**−**27**, **29**, **30**, and **37** are given in Schemes 1−7 and 12, which are in the Supporting Information. Synthetic routes to compounds **28** and **31**−**36** are given in Schemes 8−11, respectively. The *N*-benzyloxycarbonyl (Cbz) and *N*-*tert*-butoxycarbonyl (*t*-Boc) protecting groups were used exclusively.

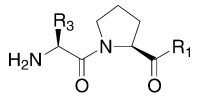
Two different strategies were employed in the preparation of the desired dipeptide amides, H−X_1−X_2−R_1. In the first, *N*-protected amino acid, Y−X_1−OH, was condensed with a second amino acid, H−X_2−OH, to give the protected dipeptide acid, Y−X_1−X_2−OH. This was converted to the corresponding amide, Y−X_1−X_2−R_1, by reaction with the amine, R_1−H, and then to the desired product, H−X_1−X_2−R_1, by deprotection. In the second strategy, the amino acid amide, H−X_2−R_1, was either synthesized or obtained commercially, condensed with Y−X_1−OH to form Y−X_1−X_2−R_1 and deprotected to give the desired product H−X_1−X_2−R_1.

Two principal methods of amide bond formation were used. In the first, a protected carboxylic acid succinimide ester was condensed with the required amine in a polar solvent; in the other, the protected carboxylic acid was converted in situ to a mixed anhydride, by reaction with isobutyl chloroformate, and then reacted with the amine. In both cases a protected amide was obtained.

Certain individual syntheses employed other methods of amide bond formation. In the preparation of compound **16** (Scheme 4), *N*-*t*-Boc-α-methyl-Ala-OH was reacted with H-Pro-NHBu in the presence of dicyclohexyl carbodiimide to give the desired intermediate, *N*-*t*-Boc-α-methyl-Ala-Pro-NHBu. In the preparation of compounds **26**, **27**, **28**, and **32** (Schemes 6, 8, and 10), *N*-protected (2*S*)-aminobutyric acid was activated using dicyclohexyl carbodiimide and 3-hydroxy-1,2,3-benzotriazin-4(3*H*)one prior to amide bond formation. In the preparation of compound **37** (Scheme 12), *N*-protected (2*S*)-aminobutyric acid was activated using bis(2-oxo-3-oxazolidinyl)phosphinic chloride.

All the amino acids H−X_1−OH and the majority of the amino acids H−X_2−OH were obtained commercially. The X_2 portion of compounds **28**, **31**, and **32** (Schemes 8−10) was obtained by synthesis. Treatment of (7*S*,-10*R*)-10-*tert*-butyl-9-methyl-1,9-diazobicyclo[5.3.0]decan-8-one afforded the azepine-2-carboxylic acid needed for the preparation of compound **28**. *N*-Benzyloxycarbonyl-

Table 1. Optimization of Val-Pro-NHBu (**1**): Variation of R_3 in P_3 and R_1 in P_1 (Formula **III**)

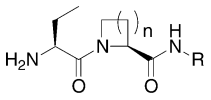


III

compd	R_3	R_1	K_i (μM)
1[a]	$(CH_3)_2CH$	$CH_3(CH_2)_3NH$	0.57
2[a,b]	$(CH_3)_2CH$	$CH_3(CH_2)_3NH$	190
3	$(CH_3)_2CH$	$CH_3(CH_2)_5NH$	0.9
4	$(CH_3)_2CH$	$PhCH_2NH$	3.3
5	$(CH_3)_2CH$	cyclohexylmethylamino	34
6	$(CH_3)_2CH$	β-naphthylamino	860
7	$(CH_3)_2CH$	piperidyl	57
8	$(CH_3)_2CH$	NH_2	6.0
9	CH_3	NH_2	2.1
10	CH_3CH_2	NH_2	0.57
11	$CH_3(CH_2)_2$	NH_2	1.6
12	$CH_3(CH_2)_3$	NH_2	2.0
13	$(CH_3)_2CHCH_2$	NH_2	2.1
14	$CH_3CH_2CH(CH_3)$	NH_2	4.3
15	$PhCH_2$	NH_2	71
16	*gem*-dimethyl	$CH_3(CH_2)_3NH$	5.0
17	CH_3CH_2	$CH_3(CH_2)_2NH$	0.08
18	CH_3CH_2	$CH_3(CH_2)_3NH$	0.08
19	CH_3CH_2	$CH_3(CH_2)_4NH$	0.27
20	CH_3CH_2	$(CH_3)_2CHCH_2NH$	0.32
21	CH_3CH_2	(*R*)-$C_2H_5CH(CH_3)NH$	4.5
22	CH_3CH_2	(*S*)-$C_2H_5CH(CH_3)NH$	20
23	CH_3CH_2	(*S*)-$C_2H_5CH(CH_3)CH_2NH$	0.5
24	CH_3CH_2	$CH_3S(CH_2)_3NH$	0.3

[a] Described in ref 18. [b] Compound **2** is Val-D-Pro-NHBu.

Table 2. Effect on Potency of Variation of Ring Size of the P_2 Residue; Variation of n and R in **IV**

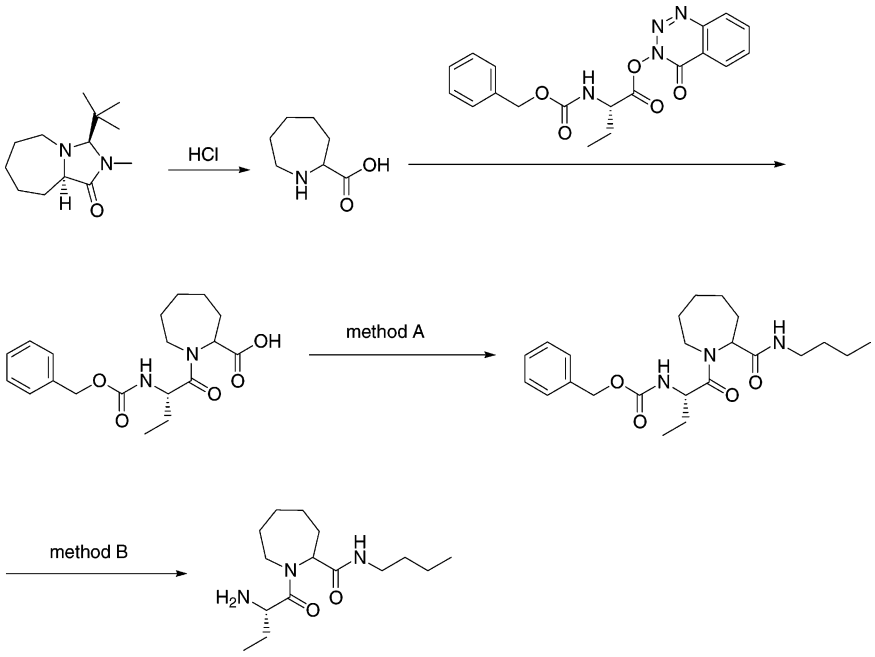


IV

compd	*n*	R	K_i (μM)	compd	*n*	R	K_i (μM)
25	1	H	20	**26**[a]	3	H	12
8	2	H	0.57	**27**	3	*n*-Bu	35
18	2	*n*-Bu	0.08	**28**	4	*n*-Bu	6

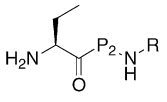
[a] The P_2 residue in **26** has D-stereochemistry.

Scheme 8

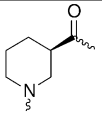
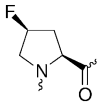
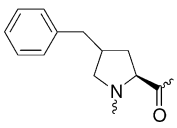


28

Table 3. Effect on Potency of Variations of P_2 in **V**



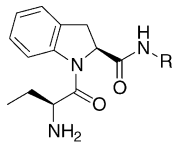
V

compd	P2	R	*Ki* (μM)
29		H	40
30		H	40
31		Bu	0.32
32		Bu	0.14

(4*R*)-hydroxy-L-proline methyl ester was the starting point for the preparation of both compounds **31** and **32**. The introduction of the fluorine atom (for compound **31**) was accomplished using diethylamino sulfur trifluoride and yielded the desired (4*S*)-isomer.[21] In the case of compound **32**, oxidation, Wittig reaction, and reduction gave the desired (4*R*)-benzylproline fragment.[22]

The NMR spectrum of **31** showed additional splitting of the signals assigned to the two methyl groups, the hydrogen atom geminal to the fluorine atom, and the hydrogen α to the proline carbonyl group. We attribute this to the presence of two distinct isomers formed by rotation about the Abu-Pro amide bond, as observed by Gerig and McLeod in the case of glycyl-*trans*-4-fluoro-L-prolyl-L-tryptophan.[23]

Table 4. Effect on Activity of Variation of R in **VI**



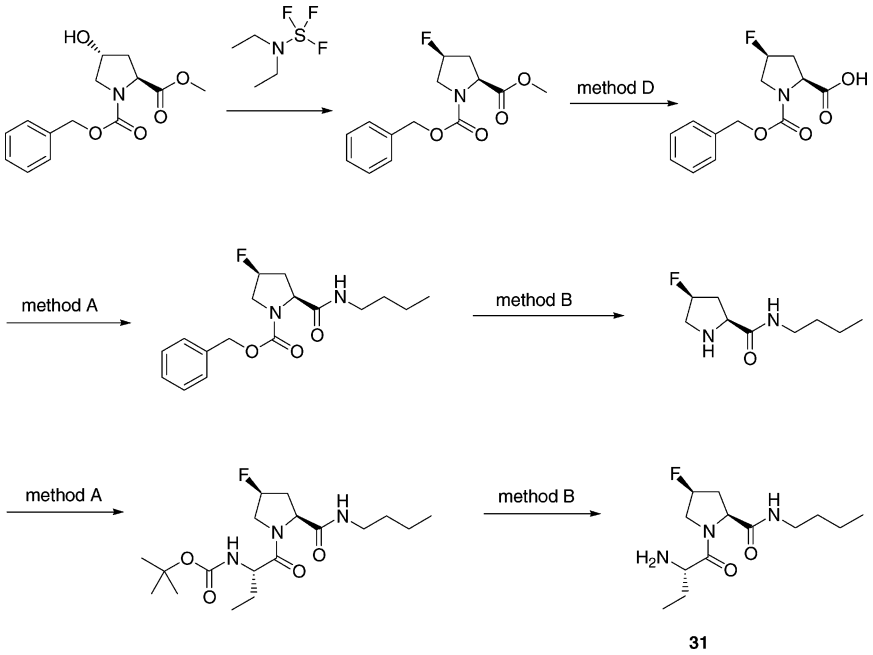
VI

compd	R	K_i (μM)	compd	R	K_i (μM)
33	*n*-Bu	0.007	**35**	Et	0.012
34	Me	0.340	**36**	*n*-Pr	0.006

Biological Assay

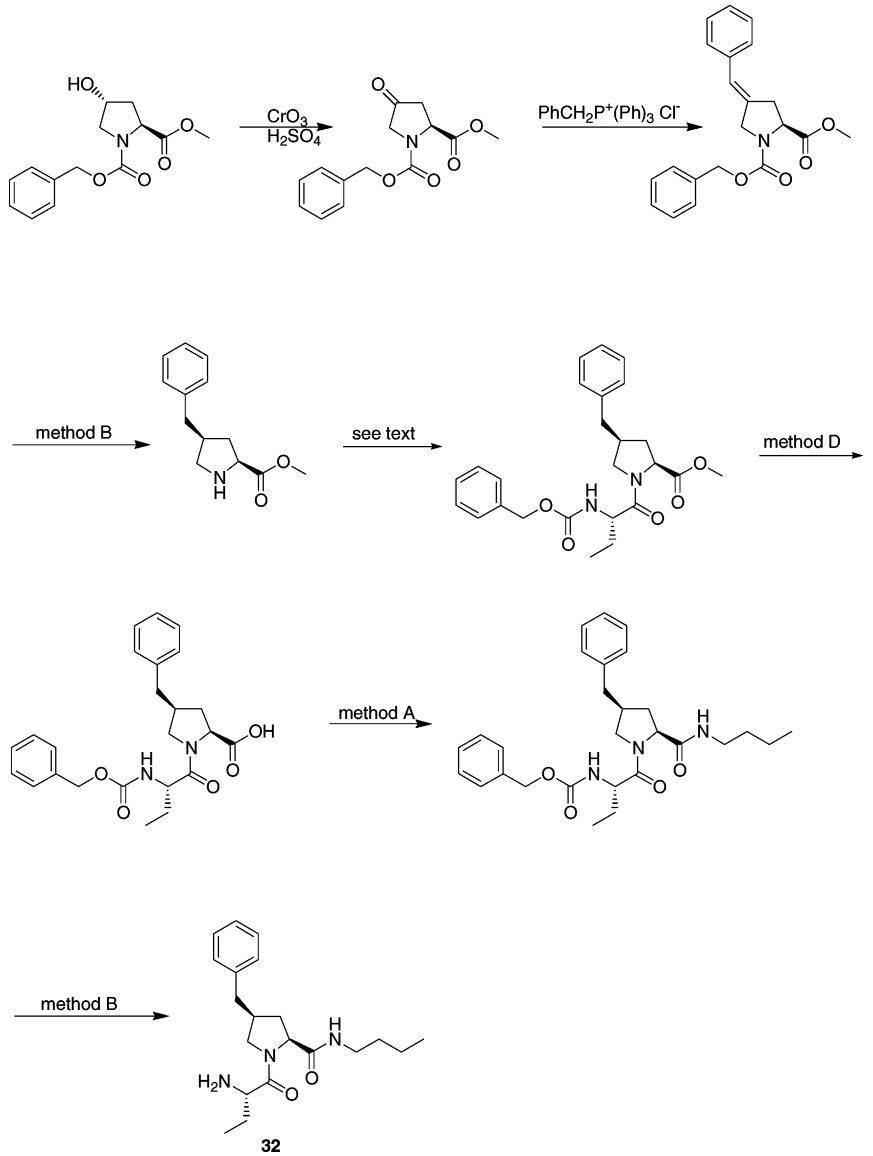
Rat cerebral membranes were prepared from cerebral cortex that was homogenized (Polytron) in 10 vol of 50 mM K_2/K phosphate buffer (pH 7.4) containing 10% glycerol. After centrifugation (500 000*g*), the pellet was washed three times in the same buffer and finally recovered in 50 mM K_2/K phosphate buffer containing 10% glycerol, 0.1% Brij 35, and 1 mM dithiothreitol, at a protein concentration of 25μg/mL. TPPII activity was evaluated in a final volume of 100 μL of K_2/K phosphate buffer containing 10^{-4} M bestatin, 10^{-7} M thiorphan, and 0.1% Brij 35, using 50 μM of the artificial substrate AAF-Amc and 25 μg of membrane proteins. Incubations were performed at 37 °C during 30 min, and the release of Amc was measured using a microfluor reader (Dynatech). When increasing concentrations of inhibitors were added, decreasing amounts of Amc were released, from which the IC_{50} was determined. Concentration−inhibition curves were established with two or three replicates. K_i values were derived from the IC_{50} using the Cheng−Prussoff[24] equation (K_m AAF-Amc = 25 μM); SEMs were generally around 10% of the mean.

Scheme 9



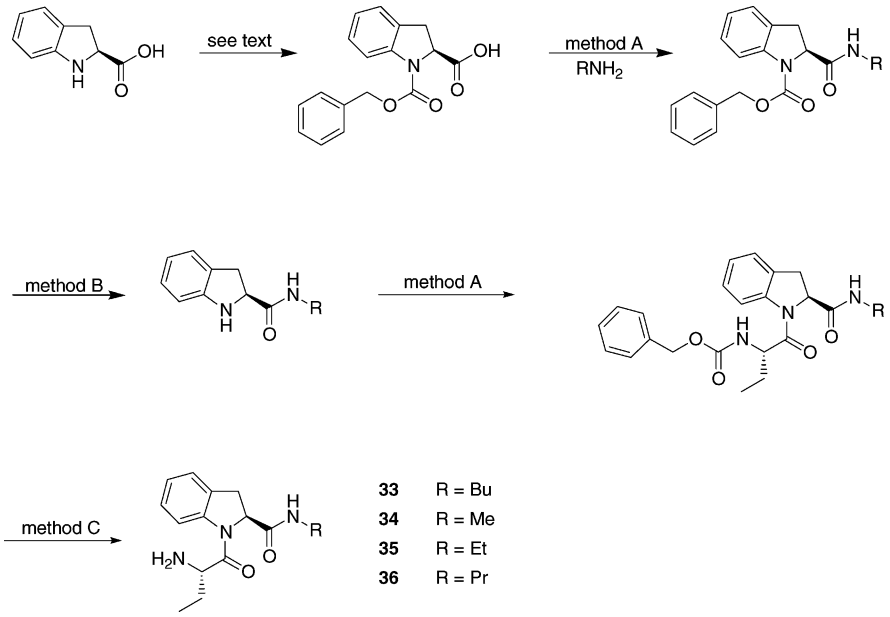
31

Scheme 10



32

Scheme 11



Results and Discussion

The search for an inhibitor of the cholecystokinin-8-inactivating peptidase led to a series of di-and tripeptides having various alkyl or aryl side chains that was studied to determine the accessible volume for binding and to probe the potential for hydrophobic interactions.[18] From this investigation the tripeptides Ile-Pro-Ile-OH (K_i = 1 μM) and Ala-Pro-Ala-OH (K_i = 3 μM) and the dipeptide amide Val-Nvl-NHBu (K_i = 3 μM) emerged as leads. Comparison of these structures led to the synthesis[18] of Val-Pro-NHBu (**1** Table 1) (K_i = 0.57 μM), which was selected as the basis for further optimization of the structure with respect to enzyme inhibitory activity.

Several features of **1** were identified as being necessary for effective binding as an enzyme inhibitor: (i) the absolute requirement for a nonsubstituted ammonium group; (ii) the dipeptide amide being P_3P_2 based on its derivation from the tripeptide $P_3P_2P_1$, the description being in accord with the convention[25] represented in Figure 2; and (iii) the favorable influence of a Pro residue in P_2.

Initially, various other amides of valyl proline (compounds **3**−**8**, Table 1) were synthesized and tested to explore whether a group larger than *n*-butyl might be accommodated at the active site; *n*-hexyl (**3**) and benzyl (**4**) amides were active (1−3 μM) but cyclohexylmethyl (**5**) and β-naphthyl (**6**) amides were much less active. The need for a secondary amide was also checked by testing the tertiary piperidine amide (**7**) and the primary amide (**8**); both of these were less active than **1** (approximately 100- and 10-fold respectively).

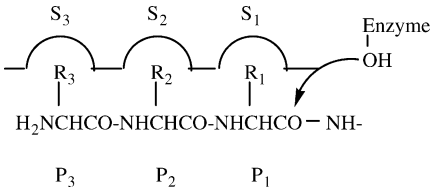


Figure 2. Designation of peptide sequence (P_1, P_2, P_3, ...) and enzyme subsites (S_1, S_2, S_3, ...) according to ref 25.

At this stage it seemed advisable to explore the dipeptide amide in a systematic manner. Taking the simplest example Ala-Pro-NH_2 (**9**), the P_3 residue was optimized. Thus, in formula **III** R_1 was maintained as NH_2 and R_3 as lower alkyl was varied from 2 to 4 carbon atoms. The most active of these inhibitors had $R_3 = CH_3$-CH_2 (**10**) with a K_i = 0.57 μM, i.e., equal to the tripeptide **1** in activity. Extending the ethyl chain to *n*-propyl (**11**), *n*-butyl (**12**), or isobutyl (**13**) reduced activity by 3−4-fold. However, branching the chain close to the peptide backbone to give *sec*-butyl (**14**) reduced the activity further still (K_i = 4.3 μM). A benzyl group, as in phenylalanine amide (**15**), was very poorly accepted. A second methyl group, as in α-methylalanine amide (**16**), was not well accepted (K_i = 5.0 μM).

Keeping $R_3 = CH_3CH_2$, the structure of R_1 was then optimized by making a series of monosubstituted amides of Abu. When R_1 = *n*-Pr or *n*-Bu, the compounds **17** and **18** were approximately 7 times more potent than **10**, having K_i = 80 nM. Lengthening the side chain (R_1 = *n*-amyl, **19**) or branching it distal to the amide *N* (*i*-Bu, **20**, 2′-amyl, **23**) reduced activity 3−6-fold; branching the side chain at the carbon atom adjacent to the amide *N*, however (*s*-Bu, **21** and **22**), had a profound effect and reduced activity 50−250-fold depending on the chirality of R_1. The group R_1 is lipophilic, but these results suggest that activity does not simply increase with increasing lipophilicity of R_1; rather it seems likely that R_1 is interacting with a lipophilic pocket that has quite limited dimensions and that when R_1 has the appropriate size it makes a profound contribution to binding, presumably by hydrophobic interactions. This is especially supported by the 4-fold difference in potency between the two chiral forms **21** and **22**. It is interesting to see from compound **24** that when R_1 corresponds to the Met side chain of the CCK-8 substrate, it is still not as effective for binding as when R_1 = *n*-Pr or *n*-Bu.

The inhibitors wherein $R_3 = CH_3CH_2$ have an inherent stability, since the unnatural amino acid Abu (α-aminobutyric acid) is much less susceptible to proteolysis by the natural proteases; furthermore, proline at

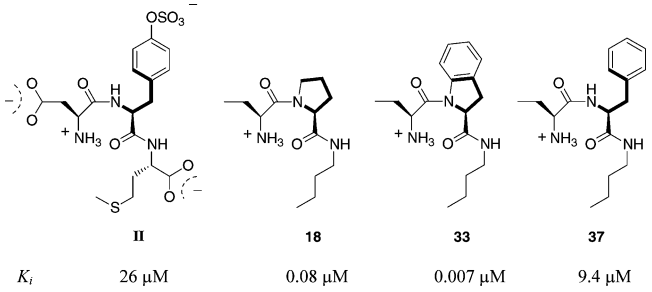


Figure 3. Comparison of the formulas of the first peptidic hydrolysis product Asp-Tyr(OSO_3H)-Met-OH (**II**) with the optimized inhibitor **18** (which lacks the polar groups CO_2H (×2), SO_3H, S, and (N)H of **II**), butabindide (**33**), and Abu-Phe-NHBu (**37**).

P_2 is itself resistant to TPP II, since this enzyme cannot cleave before or after proline residues.[14−16]

The possibilities for improving upon the proline at P_2 by changing the pyrrolidine ring size (structure **IV**) or introducing substituents (structure **V**) were also explored (Tables 2 and 3). Lower and higher homologues (Table 2, azetidine **25**, piperidine **26**, and azetepane **28**) were all found to be much less effective. The butylamide of pipecolinic acid **27** was less active than the primary amide **26**, suggesting that the fit into the enzyme active site was so poor that with this structure the butyl group did not provide additional binding through hydrophobic interactions. The isomeric 3-piperidinecarboxamide (the nipecotamide derivative **29**, Table 3), was also less active than **26**. Introducing a fluorine or benzyl substituent into the 4-position of the proline ring (butylamides **31** and **32**) also reduced activity, and the thiaproline amide **30** was weakly active. This last observation is especially interesting, since in other enzyme inhibitors, e.g., in the development of the ACE inhibitor cilazapril,[26] thiaproline was an effective replacement for proline. The present results suggest that the stereochemistry of the proline ring is critically important for good binding and lead to the inference that the binding to the enzyme active site is very specific.

At this stage of the structure−activity exploration a comparison of the structure of the optimized inhibitor **18** with that of the tripeptidic first hydrolysis product (Asp-Tyr(OSO_3H)-Met-OH, Figure 3) led to the surprising observation that the two molecules have a similar backbone structure. Although **18** was derived empirically through stepwise optimization, its structure closely resembles that of Asp-Tyr(OSO_3H)-Met-OH (**II**) except that various polar groups present in **II** are absent from **18**, namely, two carboxylates, a sulfonate, a thioether, and an amidic H. It seemed possible that there might be a subsite S_1 on the enzyme that interacts with the benzenoid ring of the tyrosinyl sulfate; this possibility led us to fuse a benzene ring to the proline in structure **18** in the hope of finding additional binding through hydrophobic interaction with the S_1 subsite. The requisite chiral *S*-indoline-2-carboxylic acid was available commercially and was derivatized to afford the corresponding Abu-indolinyl-2-*n*-butylcarboxamide [UCL 1397, butabindide, **33**, (**VI**, R = *n*-Bu) Table 4]. This strategy was highly successful, thereby giving a compound showing an increase in potency of an order of magnitude (K_i = 7 nM).

That butyl was an appropriate alkyl group for the amide moiety in butabindide was checked by comparing it with compounds having lower alkyl groups (**34**−**36**); the contribution to activity was *n*-Bu ∼ *n*-Pr > Et ≫ Me.

Successive increases in binding of the inhibitors during the design process appears to be largely based on hydrophobic interactions so that the interaction between butabindide and the enzyme is probably largely hydrophobic. However, the binding does not simply correlate with increased lipophilicity since, as already discussed, binding has been optimized with relatively small alkyl groups and stereochemistry is clearly very important. Thus, thiaproline or a six-membered ring was a poor replacement for proline, and affinity depends on chirality (compare compounds **1** with **2**, and **21** with **22**). There is no reason therefore to expect that butabindide will necessarily inhibit other enzymes through hydrophobic effects, and this is supported by the finding that butabindide is very selective. Thus, the K_i's for the following enzymes were >1 mM: subtilisin (*Bacillus licheniformis*), aminopeptidase (rat cortex membranes), dipeptidyl aminopeptidase IV, trypsin, chymotrypsin, and elastase (porcine pancreas and human leucocytes).

It is interesting that the interaction with the S_1 subsite should appear to be hydrophobic since the tyrosine sulfate residue is known to be important for the activity of CCK-8 (at least in the rat). However, this feature relates to its interaction with the CCK_1 receptor, whereas it is not known whether or how the sulfate residue interacts with the enzyme TPP II. Furthermore, the enzyme cleaves the substrate at the methionine amide linkage and it does this twice in succession, at the two methionines that are present in CCK-8. The residue corresponding to Tyr(SO_3H) for the second cleavage is Trp, which is hydrophobic and obviously has some resemblance to the indoline in butabindide.

In the series of imidazolylindolines described by Breslin et al.,[20b] there is some indication that a substituent may be accommodated at position-5 of the indoline ring, e.g., a 5-methoxy group reduced potency by a factor of approximately 2, whereas a 5-chloro group increased potency by 3-fold in one example or not at all in another. In the current series of compounds, the influence of substituents in the indoline ring has been investigated,[10b,c] and the results will be the subject of a further communication.

A Potential General Approach to Designing Exopeptidase Inhibitors. Comparison of the butabindide structure (**33**) with that of the first hydrolysis product **II** led to the intriguing possibility that, in retrospect, butabindide might have been derived directly from **II** by removal of the polar groups (vide supra) and formation of a second ring. It seems likely that after the enzyme has cleaved CCK-8 at the methionine amide bond to produce the first tripeptidic hydrolysis product **II**, the polar groups on **II** would facilitate its dissociation from the enzyme into the aqueous surroundings, thereby freeing the enzymic active site and regenerating the enzyme. This analysis suggests the possibility *of a general approach to designing exopeptidase inhibitors*, provided that the structure of the first hydrolysis product is known, namely, remove polar groups from the substrate hydrolysis product and increase affinity by optimizing hydrophobic interactions. There are of course several provisos: (1) which polar groups should

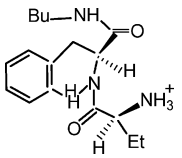


Figure 4. Abu-Phe-NHBu (**37**) in the butabindide minimum energy conformation showing the steric clash between the phenyl ortho hydrogen atom and the Phe N−H.

be removed to increase affinity would have to be determined, (2) it is necessary to identify suitable sites in the molecule which, when modified, would increase hydrophobic interactions, and (3) the enzyme must possess appropriate hydrophobic pockets in order to achieve high affinity binding through hydrophobic interactions.

The above approach was put to the test by reexamining the problem of designing de novo an inhibitor for TPP II. Removal of the polar groups CO_2H, OSO_3H and S from the tripeptidic hydrolysis product **II** results in Abu-Phe-NHBu (**37**). This compound was therefore synthesized, but when it was tested, the result was very disappointing; the $K_i = 9.4$ μM, was only a few fold better than that of the hydrolysis product **II** ($K_i = 26$ μM). Thus it appears that the notional cyclization of the Phe to indoline is critical to achieving the high potency for inhibition, and it results in an activity increase of some 1000-fold. This surprising result led to a further investigation using molecular modeling for a conformational analysis.

Molecular modeling studies were carried out on the butabindide structure using XED software[27] assuming an ion-pair $-NH_3^+X^-$ to avoid the asymmetry problem that would be caused by using $-NH_2$. In the global minimum energy conformation the indoline ring is predicted to be planar and the Abu amide bond is almost coplanar with the indoline and the Abu NH_3^+ group projects perpendicularly out of plane. The indoline carboxamide group also projects above the plane with the butylamide group in a cis conformation such that the butyl group (which is fully trans) extends over the indoline ring. This differs from the modeling results reported in ref 20a, where the butyl group is suggested to be all-trans but extended perpendicularly to the plane of the indoline ring. This latter result is derived by docking the molecule into the presumed active site of the enzyme TPP II based upon a homology model. Clearly in this case, butabindide is no longer in a minimum energy conformation, which may be acceptable if the binding energy is sufficient.

In contrast, for Abu-Phe-NHBu, the phenyl ring is almost perpendicular to Abu, and Phe carboxamide takes the usual amide trans conformation with the butyl group (which is fully trans) extending away from the phenyl ring.

Putting Abu-Phe-NHBu into the butabindide conformation is energetically extremely unfavorable and this is because the phenyl ortho hydrogen atom and the N-hydrogen atom of the Phe have to occupy the same position in space (Figure 4). This problem is solved in butabindide because in the indoline structure these two hydrogen atoms are replaced by a single bond that connects the phenyl ring to the nitrogen atom.

Thus it is clear that Abu-Phe-NHBu is not sufficiently active as an inhibitor because it cannot achieve the requisite conformation taken by butabindide. This leads to a fourth proviso in the proposed general approach to designing exopeptidase inhibitors, namely, (4) the proposed structures must be able to take up an appropriate conformation for effective binding to the enzyme. Since the structure of the enzyme is unknown, this leaves a condition whose solution is unpredictable, but requires careful exploration.

Experimental Section

Melting points were determined using an Electrothermal melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL PMX60SI, Varian XL-200, or Varian VXR-400 NMR spectrometer. HPLC (analytical and preparative) was carried out on a Gilson binary gradient system using a UV detector set to 215 nm unless otherwise stated. Mass spectra were recorded on a VG 7070H double-focusing mass spectrometer with Finnigan Incos data system. Solvents requiring drying were purified by literature procedures.[28] Amino acids, *N*-protected amino acids, and their succinimide esters were obtained commercially or prepared by literature procedures.[29]

Method A. *N*-Protected dipeptide or amino acid (2.9 mmol) was dissolved in tetrahydrofuran (30 mL), cooled to −10 °C, and treated with isobutyl chloroformate (430 mg, 3.14 mmol) and *N*-ethylmorpholine (360 mg, 3.14 mmol). After 30 min, amine (230 mg, 3.14 mmol) was added and the mixture stirred at room temperature overnight. The mixture was diluted with ethyl acetate (200 mL); washed successively with dil HCl (50 mL), dil $NaHCO_3$ (50 mL), and water; and dried (Na_2SO_4), and the solvent was removed.

Method B. *N*-Benzyloxycarbonyl-protected product (3 g) was dissolved in methanol (100 mL), and 10% palladium on charcoal (1.2 g, 50% wet) was added. The mixture was hydrogenated at 30 psi and 25 °C for 3 h. The catalyst was removed and the solvent evaporated. The residue was purified.

Method C. *N*-Benzyloxycarbonyl-protected product (208 mmol) was dissolved in methanol (100 mL), and palladium on charcoal (300 mg) was added. The mixture was hydrogenated at 30 psi for 3 h, the catalyst was filtered off, and the solvent was removed to give a pale oil. Oxalic acid (182 mg, 202 mmol) in ethanol (5 mL) was added and the oil dissolved. Ether (50 mL) was added to give a turbid solution which was refrigerated. Filtration yielded the desired product.

Method D. Ester (4.57 mmol) was saponified in a mixture of NaOH (2 M, aqueous, 5 mL) and methanol (35 mL) at room temperature for 18 h. Water (100 mL) was added and the methanol evaporated under vacuum. The aqueous residue was made acid to pH 2−3 and extracted with $CHCl_3$ (3 × 75 mL). The extracts were combined and dried ($MgSO_4$), and the solvent was removed to give a residue which was purified.

***N*-Benzyloxycarbonyl-(2*S*)-aminobutyric Acid 3,4-Dihydro-4-oxo-1,2,3-benzotriazine Ester.** *N*-Benzyloxycarbonyl-(2*S*)-aminobutyric acid (2 g, 8.43 mmol) was dissolved in tetrahydrofuran at 0 °C. Dicyclohexylcarbodiimide (1.74 g, 8.43 mmol) was added followed by 3-hydroxy-1,2,3-benzotriazin-4(3*H*)-one (1.38 g, 8.43 mmol). The mixture was stirred at room temperature for 3 h and then the solvent was removed. Crystallization from ethyl acetate/petroleum 1:1 gave the product as pale yellow crystals: yield 2.33 g (72%); ^{1}H NMR (200 MHz $CDCl_3$) δ 8.3 (1H, d, Ar CH), 8.2 (1H, d, Ar CH), 8.0 (1H, dt, Ar CH), 7.8 (1H, dt, Ar CH), 7.3 (5H, m, Ar CH), 5.3 (1H, bd, NH), 5.1 (2H, s, $PhCH_2$), 4.8 (1H, q, NCHCO), 2.1 (2H, m, CH_2), 1.1 (3H, t, CH_3).

(2*R*/*S*)-Azepinecarboxylic Acid. (7*S*,10*R*)-10-*tert*-Butyl-9-methyl-1,9-diazobicyclo[5.3.0]decan-8-one[31] (1 g, 4.4 mmol) was dissolved in 6 M HCl in an autoclave and heated to 180 °C for 3 h. The mixture was cooled and extracted with methylene chloride, and the aqueous phase was concentrated. Preparative HPLC (Kromasil C-18; water/methanol/trifluoroacetic acid 80:20:0.1) gave 0.4 g (34%) of the desired product: ^{1}H NMR (200 MHz DMSO-d_6) δ (9.0, 1H, OH), 4.0 (1H, dd,

NCHCO), 3.1 (2H, m, CH_2), 2.1 (1H, m, CH(*H*)), 1.6 (7H, m, C*H*(H), 3 × CH_2); MS *m/z* 144 $[M + H]^+$.

***N*-Benzyloxycarbonyl-(2*S*)-aminobutyryl-(2*R/S*)-perhydroazepinecarboxylic Acid.** *N*-Benzyloxycarbonyl-(2*S*)-aminobutyric acid 3,4-dihydro-4-oxo-1,2,3-benzotriazine ester (0.63 g, 1.64 mmol) in dimethyl formamide (3 mL) was cooled to 0 °C. To this was added 2*R/S*-perhydroazepinecarboxylic acid (0.31 g, 1.198 mmol) in dimethylformamide (2 mL) containing triethylamine (0.2 mL). The mixture was stirred at room temperature for 5 days. The solvent was evaporated and the product chromatographed over silica gel using petroleum/ethyl acetate/acetic acid (5:4:1): yield 0.43 g (100%); ^{1}H NMR (200 MHz $CDCl_3$) δ 7.25 (5H, m, Ph), 6.0 (1H, d, NH), 5.0 (2H, q, $PhCH_2$), 4.8 (1H, m, NCHCO), 4.5 (1H, m, NCHCO), 3.6 (1H, m, NCH(*H*)), 3.1 (1H, m, NC*H*(H)), 2.3 (1H, m, CH_3-CH(*H*)), 1.6 (7H, m, CH_3C*H*(H), 3 × CH_2), 1.2 (2H, m, CH_2), 0.8 (3H, m, CH_3).

***N*-Benzyloxycarbonyl-(2*S*)-aminobutyryl-(2*R/S*)-perhydroazepinecarboxylic Acid *n*-Butylamide** was prepared by method A from *N*-benzyloxycarbonyl-(2*S*)-aminobutyryl-(2*R*/*S*)-perhydroazepinecarboxylic acid and purified by column chromatography over silica gel using petroleum/diethyl ether 7:3 and then 2:3 as eluant: yield 52%; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.3 (5H, m, Ph), 6.4 (1H, bd, NH), 5.3 (1H, bd, NH), 5.0 (2H, t, $PhCH_2$), 4.7 (1H, m, NCHCO), 4.5 (1H, m, NCHCO), 3.1 (4H, m, 2 × NCH_2), 0.8−1.8 (20H, m, 4 × CH_2 of azepine, 2 × CH_2 of butyl, CH_2 of Abu, 2 × CH_3); MS *m/z* 418 $[M + H]^+$.

(2*S*)-Aminobutyryl-(2*R/S*)-perhydroazepinecarboxylic Acid *n*-Butylamide Hydrogen Oxalate (28) was prepared by method B from *N*-benzyloxycarbonyl-(2*S*)-aminobutyryl-(2*R*/*S*)-perhydroazepinecarboxylic acid *n*-butylamide: yield 43%; ^{1}H NMR (200 MHz, CD_3OD) δ 8.0 (1H, bd, NH), 4.7 (1H, dd, NCHCO), 4.4 (1H, m, NCHCO), 3.8 (1H, m, NC*H*(H)), 3.5 (1H, m, NCH(*H*)), 3.2 (2H, m, NCH_2), 2.2 (2H, m, CH_2), 1.8 (6H, m, 3 × CH_2), 1.4 (6H, m, 3 × CH_2), 0.9 (6H, dt, 2 × CH_3); MS *m/z* 289 $[M + H]^+$; purity (Kromasil C-18; methanol/water/trifluoroacetic acid 40:60:0.1) 99.39%. Anal. ($C_{15}H_{29}N_3O_2$·1.25-$(COOH)_2$) C H N.

***N*-Benzyloxycarbonyl-(4*S*)-fluoro-L-proline** was prepared by method D from *N*-benzyloxycarbonyl-(4*S*)-fluoro-L-proline methyl ester: mp 123−124 °C; ^{1}H NMR (400 MHz $CDCl_3$) δ 7.26−7.38 (5H, m, Ar CH), 5.15−5.32 (3H, m, $PhCH_2$, CHF), 4.61 (1H, dd, NCHCO, J_{cis} = 27.6 Hz, J_{trans} = 9.6 Hz), 3.60−3.98 (2H, m, NCH_2), 2.54−2.78 (1H, m, NCHC*H*(H)), 2.24−2.52 (1H, m, NCHCH(*H*)); MS *m/z* 267 $[M]^+$.

***N*-Benzyloxycarbonyl-(4*S*)-fluoro-L-proline *n*-Butylamide** was prepared by method A from *N*-benzyloxycarbonyl-(4*S*)-fluoro-L-proline and purified by trituration with petrol: yield 88.9%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (5H, bs, Ar CH), 6.0−6.5 (1H, bd, NH), 5.21 (1H, dt, CHF, J_{HF} = 51.9 Hz, J_{HH} 3.4 Hz), 5.0−5.20 (2H, m, $PhCH_2$), 4.48 (1H, d, NCHCO, *J* = 1.4 Hz), 3.38−4.02 (2H, m, NCH_2 of Pro), 3.02−3.32 (2H, m, NCH_2 of butyl), 2.52−2.90 (1H, m, NCHC*H*(H) of Pro), 2.08−2.48 (1H, m, NCHCH(*H*) of Pro), 1.16−1.54 (4H, m, CH_3-$(CH_2)_2$), 0.87 (3H, bs, CH_3 of butyl).

(4*S*)-Fluoro-L-proline *n*-Butylamide was prepared by method B from *N*-benzyloxycarbonyl-(4*S*)-fluoro-L-proline *n*-butylamide: yield 88.5%; mp 30 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (1H, bs, NH), 5.16 (1H, dt, J_{HF} =53.1 Hz, J_{HH} = 3.7 Hz, CHF), 3.82 (1H, dd, J_{cis} = 10.3 Hz, J_{trans} = 3.3 Hz, NCHCO), 3.10−3.4 (4H, m, 2 × NCH_2), 2.2−2.48 (2H, m, NCHCH_2), 2.12 (1H, bs, NH), 1.42−1.52 (2H, m, NCH_2CH_2), 1.28−1.37 (2H, m, CH_3CH_2), 0.91 (3H, t, *J* = 7.34 Hz, CH_3).

***N*-(*tert*-Butoxycarbonyl)-(2*S*)-aminobutyryl-(4*S*)-fluoro-L-proline *n*-Butylamide** was prepared by method A from (4*S*)-fluoro-L-proline *n*-butylamide. Purified by column chromatography over silica gel using an ethyl acetate/petrol gradient from 20:80 to 60:40: yield 51%; mp 67−68 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.55 & 7.52 (1H, 2 × bs, NH of cis and trans isomers), 5.23 & 5.31 (1H, 2 × dt, CHF of cis and trans isomers), 5.19 (1H, bd, NH), 4.43 & 4.76 (1H, 2 × d, NCHCO cis and trans isomers), 4.33 (0.63H, q, NCHCO of trans isomer), 3.76−4.09 (2.37H, m, NCH_2 of Pro, NCHCO of cis isomer), 2.85−3.46 (3H, m, NCH_2 of butyl, C*H*(H) of Abu), 2.07−2.34 (1H, m, CH(*H*) of Abu), 1.20−1.89 (15H, m, *t*-Bu, NCHCH_2 of Pro, $CH_3CH_2CH_2$ of butyl), 1.0 (3H, t, CH_3 of Abu), 0.98 (3H, t, CH_3 of butyl); MS *m/z* 374 $[M + H]^+$.

(2*S*)-Aminobutyryl-(4*S*)-fluoro-L-proline *n*-Butylamide Trifluoroacetate (31) was prepared by method B from *N*-(*tert*-butoxycarbonyl)-(2*S*)-aminobutyryl-(4*S*)-fluoro-L-proline *n*-butylamide: yield 90%; ^{1}H NMR (400 MHz CD_3OD) δ 5.34 (0.73H, m, CHF of trans isomer), 5.27 (0.27H, m, CHF of cis isomer), 4.65 (1H, dd, NCHCO of Abu), 4.13 (0.73H, dd, NCHCO of Pro of trans isomer), 3.63−4.05 (2H, m, NCH_2 of Pro, 0.27H, NCHCO of Pro of cis isomer), 3.08−3.28 (2H, m, NCH_2 of butyl), 2.25−2.68 (2H, m, CH_2 of Abu), 1.75−2.10 (2H, $CHCH_2CHF$), 1.42−1.54 (2H, m, $C_2H_5CH_2$), 1.29−1.40 (2H, m, $CH_3CH_2CH_2$), 1.11 (2.19H, t, CH_3 of Abu of trans isomer), 1.01 (0.81H, CH_3 of Abu of cis isomer), 0.94 (0.81H, t, CH_3 of butyl of cis isomer), 0.92 (2.19H, t, CH_3 of butyl of trans isomer); MS *m/z* 274 $[M + H]^+$; HPLC purity (Kromasil C-18, water/methanol/trifluoracetic acid 70:30:0.1) 99.9%. Anal. ($C_{13}H_{24}$-O_2N_3F·CF_3COOH·0.25H_2O) C H N.

***N*-Benzyloxycarbonyl-4-keto-L-proline Methyl Ester.** *N*-Benzyloxycarbonyl-4-hydroxy-L-proline methyl ester (5 g, 17.9 mmol) was dissolved in acetone (300 mL). With stirring, chromic acid in sulfuric acid (15 mL, approx 4 M) was added over 3 min. The mixture was allowed to react for 1 h and then 2-propanol (5 mL) added slowly over 20 min. The solvent was removed and the product dissolved in ether and filtered through Fluorisil. The solvent was removed and the residue purified by chromatography over silica gel using an ethyl acetate/petroleum gradient from 15:85 to 40:60 as eluant: yield 3.97 g (80%); ^{1}H NMR (200 MHz $CDCl_3$) δ 7.30 (5H, bs, Ar CH), 5.0−5.2 (2H, m, $PhCH_2$), 4,85 (1H, t, NCHCO), 3.95 (2H, bs, NCH_2), 3.6−3.7 (3H, d, OCH_3), 2.80−3.05 (1H, m, CHC*H*(H)), 3.50−3.65 (1H, m, CHCH(*H*)).

***N*-Benzyloxycarbonyl-4-benzylidine-L-proline Methyl Ester.** Benzyltriphenylphosphonium chloride (3.68 g, 9.46 mmol), suspended in dry tetrahydrofuran (30 mL), was added to a solution of sodium hydride (0.22 g, 9.65 mmol, 95%) in dry tetrahydrofuran (20 mL) under nitrogen. Dry dimethyl sulfoxide (25 mL) was added and the mixture heated at 70 °C until homogeneous (approx 4 h). The solution was cooled to 50 °C and treated with *N*-benzyloxycarbonyl-4-keto-L-proline methyl ester in dry tetrahydrofuran (10 mL) over 5 min. The mixture was returned to 70 °C for a further 16 h and then poured onto ice water containing $KHCO_3$ (1.4 g). The mixture was extracted with methylene chloride (2 × 150 mL). The extracts were combined and dried ($MgSO_4$), and the solvent was removed. The residue was purified by chromatography over silica gel using ethyl acetate/petroleum 15:85 then 35:65 as eluant: yield 2.19 g (70%); ^{1}H NMR (400 MHz $CDCl_3$) δ 7.05−7.50 (10H, m, Ar CH), 6.13−6.53 (1H, m, vinylic CH), 5.0−5.3 (2H, m, $PhCH_2$), 4.30−4.73 (3H, m, NCHCO, NCH_2), 3.50−3.80 (3H, dd, OCH_3), 3.10−3.33 (1H, m, CHC*H*(H)), 2.67−3.05 (1H, m, CHCH(*H*)).

4-*cis*-Benzyl-L-proline Methyl Ester was prepared by method B from *N*-benzyloxycarbonyl-4-benzylidine-L-proline methyl ester: yield 67%; ^{1}H NMR (400 MHz $CDCl_3$) δ 7.12−7.32 (5H, m, ArCH), 3.82 (1H, t, NCHCO), 3.74 (3H, s, MeO), 3.02−3.08 (1H, m, NCH(*H*)), 2.73−2.80 (1H, m, NC*H*(H)), 2.58−2.72 (2H, m, $PhCH_2$), 1.60−1.70 (0.1H, m, *trans*-$PhCH_2CH$), 1.50−1.59 (0.9H, m, *cis*-$PhCH_2CH$).

***N*-Benzyloxycarbonyl-(2*S*)-aminobutyryl-4-*cis*-benzyl-L-proline Methyl Ester** was prepared by the method of Anderson[b] from 4-*cis*-benzyl-L-proline methyl ester and purified by chromatography over silica gel ethyl acetate/petroleum 15/85 and then 35/65 as eluant: yield 74%; ^{1}H NMR (200 MHz $CDCl_3$) δ 9.95−7.50 (10H, m, Ar CH), 5.60 (1H, d, NH), 5.05 (2H, m, $PhCH_2O$), 4.35−4.50 (2H, m, 2 × NCHCO), 3.60−3.95 (4H, m, OCH_3, NC*H*(H)), 3.20−3.35 (1H, m, NCH(*H*)), 2.10−2.80 (4H, m, $PhCH_2CH$, $CHCH_2CH$ of Pro), 1.50−1.95 (3H, m, CH_2 of Abu, $PhCH_2CH$), 0.95 (3H, t, CH_3 of Abu).

***N*-Benzyloxycarbonyl-(2*S*)-aminobutyryl-4-*cis*-benzyl-L-proline** was prepared by method D from *N*-benzyloxycarbonyl-(2*S*)-aminobutyryl-4-*cis*-benzyl-L-proline methyl ester:

yield 83.5%; mp 56−57 °C; ^{1}H NMR (200 MHz $CDCl_3$) δ 7.0−7.5 (10H, m, Ar CH), 5.7 (1H, d, NH), 5.05 (2H, bs, $PhCH_2O$), 4.25−4.50 (2H, m, NCHCO), 3.80−4.00 (1H, m, NC*H*(H) of Pro), 3.10−3.30 (1H, m, NCH(*H*) of Pro), 2.10−2.80 (4H, m, $PhCH_2CH$, $CHCH_2CH$ of Pro), 1.40−1.90 (3H, m, CH_2 of Abu, $PhCH_2CH$), 0.9 (3H, t, CH_3)

***N*-Benzyloxycarbonyl-(2*S*)-aminobutyryl-4-*cis*-benzyl-L-proline *n*-Butylamide** was prepared by method A from *N*-benzyloxycarbonyl-(2*S*)-aminobutyryl-4-*cis*-benzyl-L-proline and partially purified by chromatography over silica gel using an ethyl acetate/petroleum gradient from 15:85 to 50:50 as eluant. Further purification was not attempted. Yield 76%.

(2*S*)-Aminobutyryl-4-*cis*-benzyl-L-proline *n*-butylamide Hydrogen Oxalate (32) was prepared by method B from *N*-benzyloxycarbonyl-(2*S*)-aminobutyryl-4-*cis*-benzyl-L-proline *n*-butylamide. Prior to oxalate salt formation, the product was purified by chromatography over silica gel using ethyl acetate/petroleum 50:50 and then ethyl acetate/methanol 50:50 as eluants: yield of oxalate 25%; ^{1}H NMR (400 MHz CD_3OD) δ 7.15−7.32 (5H, m, Ar CH), 4.28−4.48 (1H, m, NCHCO), 4.10−4.22 (1H, m, NCHCO), 3.68−3.87 (1H, m, NC*H*(H) of Pro), 3.06−3.42 (3H, m, NCH(*H*) of Pro, NCH_2 of butyl), 2.44−2.84 (3H, $PhCH_2$, $CHCH$(H)CH of Pro), 2.10−2.36 (1H, m, CHCH(*H*)CH of Pro), 1.76−2.02 (2H, m, CH_2 of Abu), 1.26−1.67 (5H, m, $CH_3CH_2CH_2$, $PhCH_2CH$), 0.86−1.12 (6H, m, 2 × CH_3); MS *m/z* 346 $[M]^+$; HPLC purity (Kromasil C-18, acetonitrile/water/trifluoracetic acid 30:70:0.1) 88.9% cis isomer, 10.1% trans isomer. Anal. ($C_{20}H_{31}N_2O_2 \cdot 1.25(COOH)_2 \cdot 0.25H_2O$) C H N.

***N*-Benzyloxycarbonyl-(2*S*)-indolinecarboxylic Acid** was prepared from (2*S*)-indoline carboxylic acid and *N*-benzyloxycarbonyl chloride by the method of Anderson:[29] yield 99%; mp 117−118 °C; MS *m/z* 298 $[M + H]^+$; ^{1}H NMR (200 MHz $CDCl_3$) δ 6.90−8.30 (10H, m, Ar CH, COOH), 5.14−5.45 (2H, m, $PhCH_2$), 4.92−5.08 (1H, m, NCHCO), 3.15−3.65 (2H, m, CH_2 of indoline).

***N*-Benzyloxycarbonyl-(2*S*)-indolinecarboxylic Acid *n*-Butylamide** was prepared by method A from *N*-benzyloxycarbonyl-(2*S*)-indolinecarboxylic acid and purified by crystallization from methanol: yield 80%; mp 164−165 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.9−7.8 (9H, m, Ar CH), 5.2 (2H, m, $PhCH_2$), 4.9 (1H, m, NCHCO), 2.9−3.8 (4H, m, NCH_2 of butylamide, CH_2 of indoline), 1.0−1.5 (4H, m, $NCH_2(CH_2)_2$), 0.8−1.0 (3H, m, CH_3); MS *m/z* 351 $[M + H]^+$.

(2*S*)-Indolinecarboxylic Acid *n*-Butylamide was prepared by method B from *N*-benzyloxycarbonyl-(2*S*)-indolinecarboxylic acid *n*-butylamide; yield 80%; ^{1}H NMR (200 MHz, $CDCl_3$), δ 6.8−7.1 (4H, m, Ar CH), 4.3 (1H, m, NCHCO), 2.9−3.5 (4H, m, CH_2 of indoline, NCH_2), 1.0−1.9 (4H, m, $CH_3CH_2CH_2$), 0.9 (3H, t, CH_3); MS *m/z* 229 $[M + H]^+$.

***N*-Benzyloxycarbonyl-(2*S*)-aminobutyryl-(2*S*)-indolinecarboxylic Acid *n*-Butylamide** was prepared by method A from (2*S*)-indolinecarboxylic acid *n*-butylamide and purified by crystallization from carbon tetrachloride: yield 65%; mp 178−179 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.0−7.2 (9H, m, Ar CH), 4.9−5.3 (4H, m, 2 × NCHCO, $PhCH_2$), 3.5 (2H, m, CH_2 of indoline), 3.1 (2H, m, NCH_2 of butylamide), 1.0−2.0 (9H, m, $NCH_2(CH_2)_2$, CH_3CH_2 of Abu), 0.9 (3H, m, CH_3 of butyl); MS *m/z* 438 $[M + H]^+$.

(2*S*)-Aminobutyryl-(2*S*)-indolinecarboxylic Acid *n*-Butylamide Hydrogen Oxalate (33, butabindide) was prepared by method A from *N*-benzyloxycarbonyl-(2*S*)-aminobutyryl-(2*S*)-indoline carboxylic acid *n*-butylamide, which was prepared by method A from (2*S*)-indolinecarboxylic acid *n*-butylamide: yield 80%; ^{1}H NMR (200 MHz, CD_3OD) δ 8.25 (1H, m, NH), 7.25 (4H, m, Ar CH), 5.1 (2H, m, 2 × NCHCO, obscured), 3.8 (2H, m, $ArCH_2$), 3.5 (2H, m, CH_2 of indoline), 3.2 (2H, m, NCH_2), 2.0 (2H, m, CH_2 of Abu), 0.9−1.8 (10H, m, CH_3 of Abu, $CH_3CH_2CH_2$); MS *m/z* 304 $[M + H]^+$; HPLC purity (lichrosorb rp select b, methanol/water/trifluoroacetic acid 40:60:0.1) 99.8%. Anal. ($C_{17}H_{25}N_3O_2 \cdot (COOH)_2 \cdot 0.75H_2O$) C H; N calculated 10.33, found 9.84.

Molecular Modeling. The conformational search for each molecule involved exploration of the conformational space using torsional dynamics, employing the XEDYNIN module of XED/COSMIC[27] running on a Silicon Graphics O^2 workstation, with charges from an ab initio 6-31G* calculation. The temperature of the run was 1000 °C, the length was 400 ps, and the collection interval was 2 ps; i.e., 200 structures were stored from each run. The dynamics run was performed three times with a dielectric constant of 1, 4, or 78.5 to attenuate the influence of electrostatics which, in vacuo, might restrict the conformational preferences of the molecule. Each set of 200 structures was minimized using the XEDMININ module of XED with a dielectric constant of 1, 4, or 78.5. All structures resulting from the minimizations were placed in one file and filtered for duplicate conformers.

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Supporting Information Available: Synthesis Schemes 1−7 and 12 for compounds **1**−**27**, **29**, **30**, and **34**−**37**, experimental methods A−K, descriptions of experimental details and data, and spectroscopic data for all of the described compounds submitted for biological assay. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Dockray, G. J. Immunochemical Evidence of Cholecystokinin-like Peptides in Brain. *Nature* **1976**, *264*, 568−570.
(2) Anagnostides, A. A.; Chadwick, V. S.; Selden, A. C.; Barr, J.; Maton, P. N. Human Pancreatic and Biliary Responses to Physiological Concentrations of Cholecystokinin Octapeptide. *Clin. Sci.* **1985**, *69*, 259−263.
(3) (a) Moran, T. H., McHugh, P. R. Cholecystokinin Suppresses Food Intake by Inhibiting Gastric Emptying. *Am. J. Physiol*. **1982**, *242*, R491−R497. (b) Grider, J. Role of Cholecystokinin in the Regulation of Gastrointestinal Motility. *J. Nutr.* **1994**, *124*, 1334S-1339S. (c) Beglinger, C. Effect of cholecystokinin on Gastric Motility in Humans. *Ann. N.Y. Acad. Sci.* **1994**, *713*, 219−225.
(4) Smith, G. P.; Gibbs, J. Satiating Effect of Cholecystokinin. *Ann. N.Y. Acad. Sci.* **1994**, *713*, 236−241.
(5) Noble, F.; Wank, S. A.; Crawley, J. N.; Bradwejn, J.; Seroogy, K. B.; Hamon, M.; Roques, B. P. International Union of Pharmacology. XXI. Structure, Distribution, and Functions of Cholecystokinin Receptors. *Pharmacol. Rev*. **1999**, *51*, 745−781.
(6) (a) Della-Fera, M. A.; Baile, C. A. Cholecystokinin Octapeptide-Continuous Picomole Injections into the Cerebral Ventricles of Sheep Suppress Feeding. *Science* **1979**, *206*, 471−473. (b) Parrott, R. F.; Baldwin, B. A. Operant Feeding and Drinking in Pigs Following Intracerebroventricular Injection of Synthetic Cholecystokinin Octapeptide. *Physiol. Behav.* **1981**, *26*, 419−422. (c) Moran, T. H.; Sawyer, T. K.; Seeb, D. H.; Ameglio, P. J.; Lombard, M. A.; McHugh, P. R. Potent and Sustained Satiety Actions of a Cholecystokinin Octapeptide Analogue. *Am. J. Clin. Nutr.* **1992**, *55*, 286S− 290S.
(7) Kissileff, H. R.; Pi-Sunyer, F. X.; Thornton, J.; Smith, G. P. C−Terminal Octapeptide of Cholecystokinin Decreases Food Intake in Man. *Am. J. Clin. Nutr.* **1981**, *34*, 154−160.
(8) Pi-Sunyer, F. X.; Kissileff, H. R.; Thornton, J.; Smith, G. P. C−Terminal Octapeptide of Cholecystokinin Decreases Food Intake in Obese Men. *Physiol. Behav*. **1982**, *29*, 627−630.
(9) (a) Bignon, E.; Alonso, R.; Arnone, M.; Boigegrain, R.; Brodin, R.; Gueudet, C.; Heaulme, M.; Keane, P.; Landi, M.; Molimard, J. C.; Olliero, D.; Poncelet, M.; Seban, E.; Simiand, J.; Soubrie, P.; Pascal, M.; Maffrand, J. P.; Le Fur, G. SR146131: A New Potent Orally Active, and Selective Nonpeptide Cholecystokinin Subtype 1 Receptor Agonist. II. In Vivo Pharmacological Characterization. *J. Pharm. Exp. Ther*. **1999**, *289*, 752−761. (b) Simmons, R. D.; Kaiser, F. C.; Pierson, M. E.; Rosamund, J. R. ARL 15849: A Selective CCK-A Agonist with Anorectic Activity in the Rat and Dog. *Pharmacol. Biochem. Behav*. **1998**, *59*, 439−444. (c) Pierson, M. E.; Comstock, J. M.; Simmons, R. D.; Julien R.; Kaiser, F.; Rosamond, J. D. CCK Peptides with Combined Features of Hexa- and Tetrapeptide CCK-A Agonists. *J. Med. Chem.* **2000**, *43*, 2350−2355.

(10) (a) Rose, C.; Vargas, F.; Facchinetti, P.; Bourgeat, P.; Bambal, R. B.; Bishop, P. B.; Chan, S. M. T.; Moore, A. N. J.; Ganellin, C. R.; Schwartz, J.-C. Characterization and Inhibition of a Cholecystokinin-Inactivating Serine Peptidase. *Nature (London)* **1996**, *380*, 403−409. (b) Rose, C.; Vargas, F.; Bourgeat, P.; Schwartz, J.-C.; Bishop, P. B.; Bambal, R. B.; Ganellin, C. R.; Leblond, B.; Moore, A. N. J.; Zhao, L.; Chan S. Tripeptidyl Peptidase Inhibitors. U.S. Patent 2002, 6,403,561 (WO 96/35805). (c) Schwartz, J.-C.; Rose, C.; Vargas, F.; Ganellin, C. R.; Zhao, L.; Samad, S.; Chen, Y Tripeptidyl Peptidase Inhibitors. U.S. Patent 2002, 6,335,630 (WO 99/33801).

(11) Rose, C.; Camus, A.; Schwartz, J.-C. A Serine Peptidase Responsible for the Inactivation of Endogenous Cholecystokinin in Brain. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 8326−8330.

(12) Rose, C.; Camus, A.; Schwartz, J.-C. Protection by Serine Peptidase Inhibitors of Endogenous Cholecystokinin Released from Brain Slices. *Neuroscience* **1989**, *29*, 583−594.

(13) Camus, A.; Rose, C.; Schwartz, J.-C. Role of a Serine Endopeptidase in the Hydrolysis of Exogenous Cholecystokinin by Brain Slices. *Neuroscience* **1989**, *29*, 595-*602*.

(14) Bålöw, R. M.; Ragnarsson, U.; Zetterquist, Ö. Tripeptidyl Aminopeptidase in the Extralysosomal Fraction of Rat Liver. *J. Biol. Chem*. **1983**, *258*, 11622−11628.

(15) Bålöw, R. M.; Tomkinson, B.; Ragnarsson, U.; Zetterquist, Ö. Purification, Substrate Specificity and Classification of Tripeptidyl Peptidase II. *J. Biol. Chem*. **1986**, *261*, 2409−2417.

(16) Tomkinson, B. Handbook of Proteolytic Enzymes, Barrett, A., Rawlings, N. D., Woessner, J. F., Eds.; Academic Press: New York, 1998; pp 338−340.

(17) Geier, E.; Pfeifer, G.; Wilm, M.; Lucchiari-Hartz, M.; Baumeister, W.; Eichmann, K.; Niedermann, G. A Giant Protease with Potential to Substitute for Some Functions of the Proteasome. *Science* **1999**, *283*, 978−981.

(18) Ganellin, C. R.; Bishop, P. B.; Bambal, R. B.; Chan, S. M. T.; Law, J. K.; Marabout, B.; Mehta Luthra, P.; Moore, A. N. J.; Peschard, O.; Bourgeat, P.; Rose, C.; Vargas F.; Schwartz J.-C. Inhibitors of Tripeptidyl Peptidase II. Part 2. Generation of the First Novel Lead Inhibitor of the Cholecystokinin-8-Inactivating Peptidase. A Strategy for the Design of Peptidase Inhibitors. *J. Med. Chem*. **2000**, *43*, 664−674.

(19) Tocris: http://www.tocris.com/index.html. Life Science Catalogue. Cat No. 1323 Butabindide oxalate.

(20) (a) Breslin, H. J.; Miskowski, T. A.; Kukla, M. J.; Leister, W. H.; De Winter, H. L.; Gautier, D. A.; Somers, M. V.; Peeters, D. C. G.; Roevens, P. W. Design, Synthesis, and Tripeptidyl Peptidase II Inhibitory Activity of a Novel Series of (*S*)-2,3-Dihydro-2-(4-alkyl-1*H*-imidazol-2-yl)-1*H*-indoles. *J. Med. Chem*. **2002**, *45*, 5303−5310. (b) Breslin, H. J.; Miskowski, T. A.; Kukla, M. J.; De Winter, H. L.; Somers, M. V.; Roevens, P. W.; Kavash, R. W. Tripeptidyl-Peptidase II (TPP II) Inhibitory Activity of (S)-2,3-Dihydro-2-(1*H*-imidazol-2-yl)-1*H*-indoles, a Systematic SAR Evaluation. Part 2. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4467−4471.

(21) Smith, E. M.; Swiss, G. F.; Neustadt, B. R.; Gold, E. H.; Sommer, J. A.; Brown, A. D.; Chiu, P. J. S.; Moran, R.; Sybert, E. J.; Baum, T. Angiotensin-Converting Enzyme Inhibitors, Mercaptan, Carboxyalkyl Dipeptide and Phosphinic Acid Inhibitors Incorporating 4-Substituted Prolines. *J. Med. Chem.* **1988**, *31*, 875−885.

(22) Krapcho, J.; Turk, C.; Cushman, D. W.; Powell, J. R.; DeForrest, J. M.; Spittzmiller, E. R.; Karanewsky, D. S.; Duggan, M.; Rovnvack, G.; Schwartz, J.; Natarajan, S.; Godfrey, J. D.; Ryono, D. E.; Neubeck, R.; Atwal, K. S.; Petrillo, E. W. Synthesis and Pharmacological Activity of Angiotensin Converting Enzyme Inhibitors: *N*-(Mercaptoacyl)-4-substituted-(*S*)-prolines. *J. Med. Chem*. **1988**, *31*, 1148−1160.

(23) Gerig, J. T.; McLeod, R. S.; Conformations of Glycyl-*trans*-4-Fluoro-L-Prolyl-L-Tryptophan in Aqueous Solution. *J. Am. Chem. Soc.* **1976**, *98*, 3970−3975.

(24) Cheng Y. C.; Prussoff, W. H. Relationship between the Inhibitor Constant (K_i) and the Concentration of Inhibitor Which Causes 50% Inhibition (IC_{50}) of the Enzymatic Reaction. *Biochem. Pharmacol*. **1973**, *22*, 3099−3108.

(25) Schechter, I.; Berger, A. On the Size of the Active Site in Proteases. I. Papain *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157−162.

(26) Skiles, J. W.; Suh, J. T.; Williams, B. E.; Menard, P. R.; Barton, J. N., Loev, B.; Jones, H.; Neiss, E. S.; Schwab, A.; Mann, W. S.; Khandwala, A.; Wolf, P. S. Weinryb, I. Angiotensin-converting Enzyme Inhibitors: New Orally Active 1,4-Thiazepine-2,5-diones, 1,4-Thiazine-2,5-diones, and 1,4-Benzothiazepine-2,5-diones Possessing Antihypertensive Activity. *J. Med. Chem.* **1986**, *29*, 784−796.

(27) (a) Vinter, J. G.; Davis, A.; Saunders, M. R. Strategic Approaches to Drug Design. I. An Integrated Software Framework for Molecular Modeling. *J. Comput.-Aided Mol. Des.* **1987**, *1*, 31−51. (b) Morley, S. D.; Abraham, R. J.; Haworth, I. S.; Jackson, D. E.; Saunders, M. R.; Vinter, J. G. Cosmic (90)-An Improved Molecular Mechanism Treatment of Hydrocarbons and Conjugated Systems. *J. Comput.-Aided Mol. Des.* **1991**, *5*, 475−504.

(28) Perrin, D. D.; Armarego, W. L. F.; Perrin D. R. *Purification of Laboratory Chemicals*; 2nd ed.; Pergamon: Elmsford, NY, 1987.

(29) Anderson G. W.; Zimmerman J. E.; Callahan F. M. The Use of Esters of *N*-Hydroxysuccinimide in Peptide Synthesis. *J. Am. Chem. Soc.* **1964**, *86*, 1839−1842.

(30) Koenig, W.; Geiger, R. Racemization in Peptide Synthesis. *Chem. Ber.* **1970**, *103*, 2024−2033.

(31) Seebach, D.; Dziadulewicz, E.; Beherendt, L.; Cantoreggi, S.; Fitz, F. Synthesis of Non-Pproteogeneic (*R*)- or (*S*)-Amino Acids Analogues of Phenylalanine, Isotopically Labelled and Cyclic Amino Acids from *tert*-Butyl-2-(*tert*-butyl)-3-methyl-4-oxo-1-imidazolinecarboxylate (Boc-BMI). *Liebigs Ann. Chem.* **1989**, 1215−1232.

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