Ac-[3- and 4-Alkylthioproline³¹]-CCK₄ Analogs: Synthesis and Implications for the CCK-B Receptor-Bound Conformation

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It has been reported that substitution of the Met³¹ residue in Boc-CCK₄ (Boc-Trp³⁰-Met³¹-Asp³²-Phe³³-NH₂, CCK₃₃ numbering) by *trans*-3-propyl-L-proline yields a highly potent and selective CCK-B agonist. To further explore the structural requirements of the Met³¹ side chain in the receptor-bound conformation of CCK₄, we have synthesized several Ac-CCK₄ analogs containing substitution of Met³¹ by 3- and 4-(alkylthio)-substituted proline derivatives. To this end we have developed novel synthetic routes to enantiomerically pure *N*-Boc-4-*cis*- and -*trans*-(methylthio)prolines and racemic *N*-Boc-3-*cis* and -*trans*-[(4-methylbenzyl)thio]prolines. The protected mercaptoprolines were incorporated into Ac-CCK₄ analogs using SPPS and were alkylated using various electrophiles following cleavage from the solid support. Binding assays reveal that 3-(alkylthio)prolines analogs have higher affinities at the CCK-B receptor than the corresponding 4-(alkylthio)proline analogs, and that *trans*-3-(alkylthio)proline analogs had higher affinities than corresponding *cis*-3-(alkylthio)proline analogs. Within both the *cis*- and *trans*-3-(alkylthio)proline series, the order of potency was found to be Me < Et < *n*-Pr. The *trans*-3-(*n*-propylthio)-L-proline analog demonstrates a higher affinity than that reported for Boc-CCK₄[*trans*-3-propyl-L-Pro³¹]. Comparison of the low-energy structures calculated for several high-affinity Ac-CCK₄ analogs reveal a common geometry which we propose to be the CCK-B receptor-bound conformation. This model shows grouping of the hydrophobic side chains of Trp, Met, and Phe at one side of the molecule and the hydrophilic side chain of Asp and the C-terminal carboxamide at the other side.

Introduction

Cholecystokinin (CCK) is a 33-residue peptide hormone found in both the gastrointestinal (GI) tract and the central nervous system (CNS) and shown to govern various GI functions and act as a neuromodulator and/or neurotransmitter, respectively.[1] At least two receptor subtypes have been identified so far, the CCK-A receptors, which are found mostly in peripheral organs but also in discrete regions of the brain, and the CCK-B receptors, which are the predominant subtype found in the CNS.[2] These receptor subtypes can be differentiated by their relative affinities for various naturally occurring fragments of CCK. The minimal fragment which possesses nanomolar affinity for both the CCK-A and -B receptors is the sulfated form of the C-terminal octapeptide, CCK₈ (Asp²⁶-Tyr(SO₃H)²⁷-Met²⁸-Gly²⁹-Trp³⁰-Met³¹-Asp³²-Phe³³-NH₂), which is the predominant fragment found in the CNS. The desulfated form of CCK₈ and the C-terminal tetrapeptide, CCK₄, bind with nanomolar affinity only at the CCK-B receptors. There is a great deal of interest in designing highly potent and selective ligands for these receptors in order to elucidate the functions of CCK in the brain and develop CCK-based therapeutic agents. Thus far, CCK₄ has been found to induce anxiety attacks in humans,[3] and selective CCK-B antagonists have been reported to act as

potent anxiolytics in animal testing.[4] Also, CCK-B antagonists have been shown to potentiate morphine-induced analgesia[5] while CCK-B agonists reduced the antinociceptive effects of μ-opioid agonists.[6]

Numerous studies describing structure–activity relationships (SAR) for CCK₈ have appeared in the literature, some of the most interesting results on desamino analogs of the C-terminal heptapeptide of CCK[7] and A71380 (*desamino*-CCK₇[Nle²⁸,³¹]), the smallest synthetic fragments of CCK₈ possessing the full spectrum of biological activity.[8,9] *N*-Methylation of the α-amino group of Asp³² yielded A71378, a potent and selective CCK-A agonist,[10] whereas substitution of Nle³¹ by *N*-MeL yielded A72962, a potent and selective CCK-B agonist. Recently, several SAR studies on Boc-CCK₄ analogs have been reported incorporating highly constrained amino acid substitutions for the Met³¹ residue. Holladay *et al.*[11] reported that substitution by L-Pro gave an analog with 30-fold lower affinity than Boc-CCK₄ at the CCK-B receptor, while substitution by (2S,3S)-3-*n*-propylproline (*trans*-L-3PP) resulted in an analog with 13-fold higher affinity than Boc-CCK₄. *trans*-3PP can be viewed as a chimeric amino acid—a combination of Nle and Pro. Corringer *et al.*[12] reported that replacement of Met³¹ in Boc-CCK₄ by Nle, Phe, phenylglycine (Phg), and cyclohexylglycine (Chg) produced analogs with nanomolar affinity for the CCK-B receptor. They also reported that *N*-methylation of Nle³¹ increased binding by 4-fold, while *N*-methylation of the Phe, Phg, and Chg always resulted in approximately 10-fold reductions in affinity. Additional studies by Rodriguez

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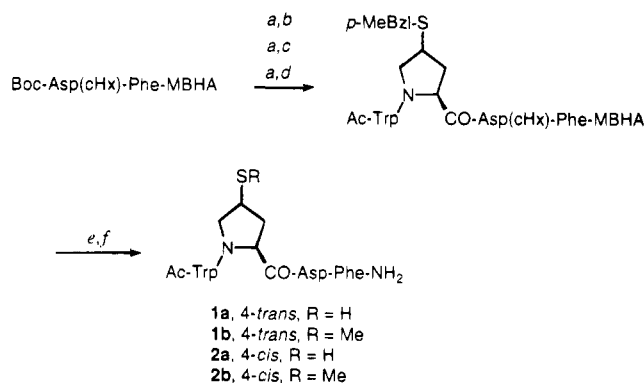§ Washington University.
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Scheme 1

^{a} Conditions: (a) 50% TFA/DCM; (b) Boc-4-L-MPt/c(p-MeBzl), TBTU, HOBt, DIEA; (c) Boc-Trp, TBTU, HOBt, DIEA; (d) Ac$_2$O, TEA; (e) HF/anisole/Me$_2$S (10:1:0.5); (f) (MeO)$_2$SO$_2$, NaOH, MeOH.

Scheme 2

^{a} Conditions: (a) SOCl$_2$, MeOH, -5 °C; (b) Boc$_2$O, Na$_2$CO$_3$, dioxane/H$_2$O, rt; (c) DIAD, PPh$_3$, HCO$_2$H, THF; (d) NaOH, MeOH; (e) DIAD, PPh$_3$, AcSH, THF; (f) 1 equiv NaOH, (MeO)$_2$SO$_2$, MeOH; (g) 2.5 equiv of NaOH.

et al.[13] reported that replacement of Met[28] and Met[31] in various fragments of CCK by lysine residues and cyclization through amidation using succinic acid yielded CCK-B-selective analogs. Taken together, the SAR studies on Met[31] substitutions imply that the side chains at this position may be interacting with a hydrophobic pocket in the CCK-B receptor.

In order to determine the structural requirements of the Met[31] side chain for a model of the CCK-B receptor-bound conformation of CCK$_8$, we have undertaken the synthesis of Ac-CCK$_4$ analogs with substitution of the Met[31] residue by all possible stereoisomers of 3- and 4-(alkylthio)-substituted prolines and analyzed these compounds in radioligand binding assays at CCK-A and -B receptors. Previous work from this laboratory described the use of *cis*- and *trans*-4-mercapto-L-proline (L-4-MPc and L-4-MPt) to develop highly potent bicyclic bradykinin and angiotensin II analogs.[14–16] Methylation of the thiol moieties of 4-MPc and 4-MPt creates chimeric amino acids which are a combination of Met and Pro. Such analogs would constrain the side chain of Met/Nle more than *cis*- and *trans*-3PP, since both χ_1 and χ_2 would be incorporated into the pyrrolidine ring. Alkylation of the thiol moiety of 3-MPc and 3-MPt would yield essentially the same types of chimeric amino acids as *cis*- and *trans*-3PP. We were interested in determining the effect that substitution of Met[31] in CCK$_4$ by all possible stereoisomers of 3- and 4-(alkylthio)proline would have on binding, since previous SAR studies on Boc-CCK$_4$ reported only L-amino acids. Molecular modeling studies on several of these analogs are described, and the results are compared to previously reported models of CCK$_8$.

Results

Synthesis. A. 4-MPt/c-Containing Peptides. The syntheses of Boc-L-4-MPt(MeBzl) and Boc-L-4-MPc-(MeBzl) have been previously described,[16] and these were incorporated into peptides **1a** and **2a**, respectively, using standard manual solid phase peptide synthesis (SPPS) techniques, as shown in Scheme 1. Methylation of the thiol moieties of **1a** and **2a** with dimethyl sulfate gave **1b** and **2b**, respectively. To obtain the corresponding D-4-MPt/c analogs, a novel, high-yielding synthetic strategy was developed (Scheme 2) which yielded the *S*-methylated derivatives, **6** and **9**. The key transformation of Scheme 2 is the reaction of **4** with thiolacetic
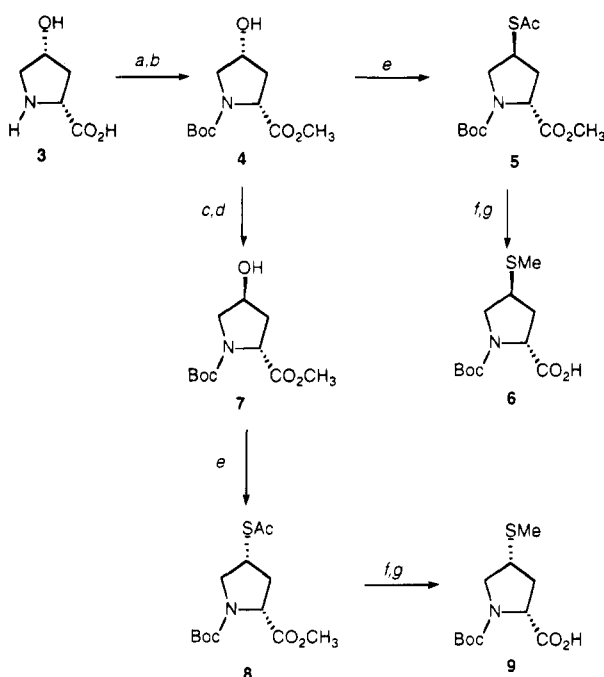
acid under Mitsunobu conditions to give **5** in an isolated yield of 85%.[17] This transformation was previously reported by Emmer *et al.*[18] for *N*-*p*-nitrobenzyloxycarbonyl-protected 4-hydroxyproline methyl esters. Selective hydrolysis of the thiolacetate of **5**, alkylation of the resulting thiol, and subsequent hydrolysis of the methyl ester to give **6** was accomplished in a one-pot sequence similar to that reported by Lago *et al.*[19] for the synthesis of β-mercaptophenylalanine. The isolated yield of **6** from the two-step process was 87%, making the overall yield of **6** 60% from the commercially available **3**. Synthesis of **9** requires epimerization of the C-4 carbon of **4**, which was accomplished by a Mitsunobu inversion using formic acid[18,20] followed by hydrolysis of the formate ester to give **7** in a yield of 64% for the two steps. Subsequent transformation as described for **4** gave **9** in an overall yield of 45% from **3**. Both **6** and **9** were incorporated into peptides **10** and **11**, respectively, using the SPPS strategy described in Scheme 1 for **1a** and **2a**. Because the methyl thioether moieties of **10** and **11** survived treatment by HF, subsequent methylation was not required.

B. 3-MPt/c-Containing Peptides. The synthesis of D,L-Boc-3-MPt(MeBzl) dicyclohexylamine salt (D,L-**20**·DCHA) is outlined in Scheme 3; the 2,3-dehydroproline derivative **18** was previously reported by Hausler.[21] Conjugate addition of 4-methylbenzyl mercaptan to **18** followed by deprotection of the *N*-acetyl group by acidolysis yielded **19** as the *trans*-diastereomer exclusively following recrystallization. The overall yield for the two steps was 52%. Protection of the amino group of **19** as the *N*-Boc derivative gave **20** in 92% yield. Because the product failed to crystallize, the DCHA salt was formed to facilitate purification. Synthesis of D,L-3-MPc(MeBzl) dicyclohexylamine salt (D,L-**23**·DCHA) is shown in Scheme 4; the 3-bromo-1,2-dehydroproline derivative **21** was previously reported by Hausler and

Scheme 3
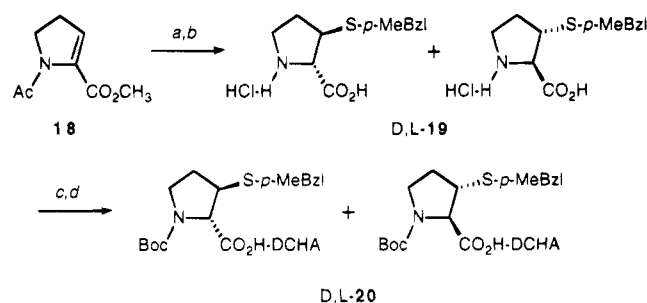
a Conditions: (a) 4-methylbenzyl mercaptan, NaH, MeOH, reflux; (b) HCl, reflux; (c) Boc₂O, TEA; (d) DCHA, Et₂O.

Scheme 4
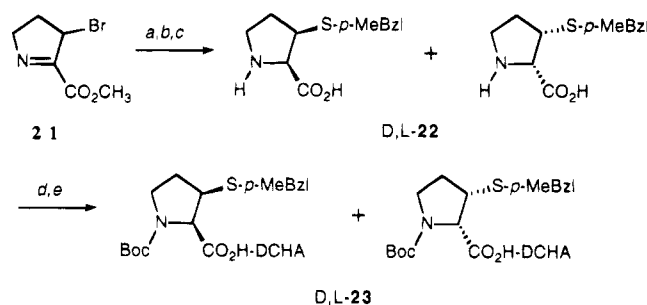
a Conditions: (a) NaOH, MeOH; (b) 4-methylbenzyl mercaptan; (c) NaBH₄; (d) Boc₂O, TEA; (e) DCHA, Et₂O.

Schmidt.[22] Reaction of **21** with 4-methylbenzyl mercaptan in the presence of aqueous sodium hydroxide and subsequent diastereoselective reduction of the imine using sodium borohydride gave predominantly the *cis*-isomer D,L-**22** in an overall yield of 37% from **21**. The amine was protected as the *N*-Boc derivative D,L-**23**, and ¹H NMR analysis revealed a trace amount of the trans isomer. To facilitate purification by recrystallization, the DCHA salt of D,L-**23** was formed. HPLC analysis of D,L-**23**·DCHA revealed less than 0.6% of the trans isomer after recrystallization.

Because both D,L-**20**·DCHA and D,L-**23**·DCHA were obtained as racemic mixtures, their incorporation in SPPS resulted in mixtures of two diastereomeric peptides (Scheme 5). In both cases, separation of the diastereomeric peptides was achieved by reversed-phase, high-pressure liquid chromatography (RP-HPLC), and the absolute stereochemistry was assigned based on chemical degradation to a product of known stereochemistry. Desulfurization of the thiol-peptides **24a**–**27a** with Raney nickel was found to afford either the L- or D-proline-containing analogs **13** or **14**. Comparison of HPLC retention times and ¹H NMR spectra of the reduction products with those of **13** and **14** allowed assignment of the absolute stereochemistry of **24a**–**27a**. The pure thiol peptides were alkylated with various electrophiles to give the desired peptides, as shown in Scheme 5 for **24a**.

Radioligand Binding Assays. A. 4-MPt/c-Containing Peptides. The results from the radioligand binding assays at both the CCK-A and CCK-B receptors are given in Table 2. Within the series of 4-(methylthio)proline analogs (**1b**, **2b**, **10**, and **11**), the analogs with the highest affinity (**1b** and **2b**) possessed an L-configuration at the α-carbon. The *cis*-analog (**2b**) had a 2.3-fold higher affinity than the *trans* (**1b**), in contrast to the report by Nadzan *et al.*[23] in which Boc-CCK₄-

Scheme 5
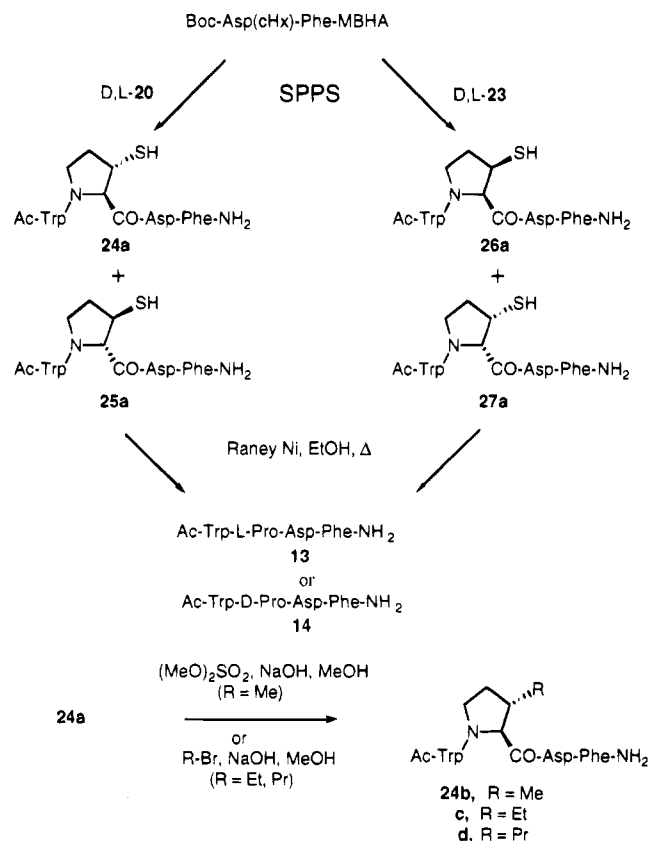
[Ctp³⁰,*trans*-L-3PP³¹] had a 43-fold higher affinity than Boc-CCK₄[Ctp³⁰,*cis*-L-3PP³¹]. The fact that **2b** is significantly less potent than Boc-CCK₄[*trans*-3PP³¹] at the CCK-B receptor (15-fold) indicates that the additional restriction of χ₁ imposed by 4-(methylthio)proline is not optimal for the receptor-bound conformation. The difference in binding affinity between **1b** and Boc-CCK₄-[*trans*-3PP³¹] does not seem to be the result of different substitution at the N-terminus, since the *N*-Ac compound **13** with L-Pro substitution for Met has nearly the same affinity at the CCK-B receptor (IC₅₀ = 600 nM) as the corresponding *N*-Boc analog reported by Abbott (IC₅₀ = 750 nM at CCK-B receptor[11]). Also, there are several analogs in the Ac-CCK₄ series that show very high affinity for the CCK-B receptor, e.g., compounds **24c**, **24d**, and **26d**. The lack of binding for analogs with a D configuration at the α-carbon of the residue at the 31-position is previously unreported in the CCK₄ series, and compounds **12** and **14** further demonstrate this phenomenon. The effect of α,α-dialkyl substitution at the 31-position was also explored, as compounds **15**, **16**, and **17** were synthesized and tested in the binding assay. None of these analogs showed high affinity for the CCK-B receptor.

B. 3-MPt/c-Containing Peptides. The results from substitution of Met³¹ by 3-(alkylthio)prolines were consistent with the binding results of the 4-(methylthio)-proline series in that analogs with a D-mercaptoproline at the 31-position (**25b**–**d** and **27b**–**d**) were unrecognized by the CCK-B receptor. In contrast to the results from the 4-(methylthio)proline series, the 3-L-MPt-containing analogs (**24b**–**d**) consistently showed higher affinities than the corresponding 3-L-MPc-containing analogs (**26b**–**d**), consistent with the report by Nadzan *et al.*[23] Affinity for the CCK-B receptor increased with

Table 1. Analytical and Physical Properties for Ac-CCK4[X31] Analogs

compd	-X-	amino acid analyses	MS(M + H)	HPLC[a] t_R (min)
1b	-L-4-MPt(Me)-	Asp (1) 1.07, 4-MPt(Me) (1) 1.00,Phe (1) 1.00	651	12.2
2b	-L-4-MPc(Me)-	Asp (1) 1.01, 4−MPc(Me) (1) 1.08,Phe (1) 1.00	651	12.0
10	-D-4-MPt(Me)-	Asp (1) 1.11, 4-MPt(Me) (1) 0.84, Phe (1) 1.00	651	13.5
11	-D-4-MPc(Me)-	Asp (1) 1.04, 4−MPc(Me) (1) 1.03, Phe (1) 1.00	651	12.5
12	-D-Nle-	Asp (1) 0.98, Nle (1) 0.96, Phe (1) 1.00	621	14.0
13	-L-Pro-	Asp (1) 1.00, Pro (1) 0.99, Phe (1) 1.00	605	8.8
14	-D-Pro-	Asp (1) 0.89, Pro (1) 1.02, Phe (1) 1.00	605	10.9
15	-MeA-	Asp (1) 0.97, MeA (1) 0.99, Phe (1) 1.00	593	10.7
16	-Cle-	Asp (1) 1.01, Cle (1) 1.04, Phe (1) 1.00	619	12.4
17	-D,L-MeL-	Asp (1) 1.07, MeL (1) 1.11, Phe (1) 1.00	635	15.7/16.1
24b	-L-3-MPt(Me)-	Asp (1) 1.15, Phe (1) 1.00	651	15.6[b]
25b	-D-3-MPt(Me)-	Asp (1) 1.04, Phe (1) 1.00	651	17.8[b]
26b	-L-3-MPc(Me)-	Asp (1) 0.95, Phe (1) 1.00	651	10.5
27b	-D-3-MPc(Me)-	Asp (1) 0.99, Phe (1) 1.00	651	11.8
24c	-L-3-MPt(Et)-	Asp (1) 0.94, Phe (1) 1.00	665	13.2
25c	-D-3-MPt(Et)-	Asp (1) 0.93, Phe (1) 1.00	665	15.1
26c	-L-3-MPc(Et)-	Asp (1) 0.99, Phe (1) 1.00	665	12.1
27c	-D-3-MPc(Et)-	Asp (1) 0.97, Phe (1) 1.00	665	13.5
24d	-L-3-MPt(Pr)-	Asp (1) 1.13, Phe (1) 1.00	679	16.6
25d	-D-3-MPt(Pr)-	Asp (1) 1.05, Phe (1) 1.00	679	18.5
26d	-L-3-MPc(Pr)-	Asp (1) 1.12, Phe (1) 1.00	679	15.4
27d	-D-3-MPc(Pr)-	Asp (1) 1.04, Phe (1) 1.00	679	17.2

[a]Gradient of 25−50% B over 25 min unless otherwise noted. [b]Gradient of 20−45% B over 25 min.

Table 2. Binding Affinities for Ac-CCK4[X31] Analogs

		IC_{50} (nM)[a]	
compd	-X-	pancreas (CCK-A)	jurkat cells (CCK-B)
1b	-L-4-MPt(Me)-	5 000	70
2b	-L-4-MPc(Me)-	>10 000	30
10	-D-4-MPt(Me)-	>10 000	>10 000
11	-D-4-MPc(Me)-	>10 000	>10 000
12	-D-Nle-	>10 000	>10 000
13	-L-Pro-	>1 000	600
14	-D-Pro-	>10 000	>10 000
15	-MeA-	>10 000	7 000
16	-Cle-	>10 000	4 000
17	-D,L-MeL-	>10 000	4 000
24b	-L-3-MPt(Me)-	>10 000	50
25b	-D-3-MPt(Me)-	>10 000	10 000
26b	-L-3-MPc(Me)-	10 000	300
27b	-D-3-MPc(Me)-	>10 000	10 000
24c	-L-3-MPt(Et)-	>10 000	1.8
25c	-D-3-MPt(Et)-	>10 000	2 500
26c	-L-3-MPc(Et)-	9 000	30
27c	-D-3-MPc(Et)-	>10 000	800
24d	-L-3-MPt(Pr)-	>1 000	0.45
25d	-D-3-MPt(Pr)-	>1 000	600
26d	-L-3-MPc(Pr)-	>1 000	3.3
27d	-D-3-MPc(Pr)-	>1 000	70

[a]Results are the means of at least three separate experiments in duplicate and the standard errors are within ±15%.

increasing chain length on the sulfur atom in the 3-L-MPt(R) series, as methyl (**24b**), ethyl (**24c**), and propyl (**24d**) displayed affinities of 50, 1.8, and 0.45 nM, respectively. The same trend appeared in the 3-L-MPc-(R) series, as methyl (**26b**), ethyl (**26c**), and propyl (**26d**) showed affinities of 300, 30, and 3.3 nM, respectively.

Molecular Modeling. Energy calculations revealed that the sets of low-energy backbone conformers (those satisfying the criterion of $\Delta E = E − E_{min} < 5$ kcal/mol) consisted of 16 structures for Ac-CCK4, 21 for Ac-[NMeNle31]-CCK4, 36 for Ac-[L-3-*trans*-PP31]-CCK4, 15 for Ac-[L-4-MPt(Me)31]-CCK4 (compound **1b**), 22 for Ac-[L-4-MPc(Me)31]-CCK4 (**2b**), 9 for Ac-[L-3-MPt(Me)31]-CCK4 (**24b**), and 8 for Ac-[L-3-MPc(Me)31]-CCK4 (**26b**). The criterion was raised to $\Delta E < 7$ kcal/mol for the D-mercaptoproline-containing analogs. Accordingly, the calculations yielded 26 low-energy conformers for Ac-

[D-4-MPt(Me)31]-CCK4 (compound **10**), and 30 for Ac-[D-4-MPc(Me)31]-CCK4 (**11**).

The results of binding assays (see Table 2 and the results of the Abbott group[8,11] and Corringer et al.[12]) allow one to roughly divide the discussed analogs into two groups: those with a high or moderate level of binding to CCK-B receptors (Boc-CCK4, Boc-[NMeNle31]-CCK4, Boc-[L-3-*trans*-PP31]-CCK4 and all L-mercapto-proline-containing Ac-CCK4 analogs) and those with low CCK-B affinity (all D-mercaptoproline-containing compounds). It is reasonable to expect that the analogs of the former group can possess similar geometrical shape-(s), which would correspond to the CCK-B-receptor-bound conformer(s). Therefore, all low-energy conformers obtained for all analogs of this group were compared to all low-energy conformers found for the "parent compound" (Ac-CCK4) with respect to the spatial arrangement of all C^{α}- and C^{β}-atoms. It appeared that only one type of the Ac-CCK4 low-energy backbone conformers possessed similar counterparts in the sets of low-energy conformers for each of the six others compounds with high or moderate level of binding to CCK-B receptors. This conformer is described in Table 3 together with some of its counterparts and is depicted in Figure 1. The shape of the peptide backbone for this conformer is reminiscent of a Z-like bend, which contains two distinct peptide chain reversals by *ca.* 90° at the C^{α}-atoms in residues 2 and 3. The conformer in question was also checked for possible geometrical similarity to the low-energy conformers for the D-mercaptoproline-containing analogs (compounds **10** and **11**). Only one similar conformation per molecule was found for both **10** and **11**, the relative energies of which exceed the limit of $\Delta E < 5$ kcal/mol. The spatial difference between the conformers of **10** and **11** that are most similar to the conformer of Ac-CCK4 from Table 3 is depicted in Figure 2. The most potent analogs, like **24c** and **24d** (those with ethyl and *n*-propyl substituents), were not investigated by molecular modeling and subsequent geometrical comparison, since the length of an alkyl chain does not influence the backbone conformer in which we were interested.

Table 3. Geometrically Similar Low-Energy Backbone Conformers for Ac-CCK$_4$ and Its Analogs with High or Moderate Affinity to CCK-B Receptors

compd	Trp		Xxx		Asp		Phe	ΔE
	ψ_1	ω_{12}	ϕ_2	ψ_2	ϕ_3	ψ_3	ϕ_4	(kcal/mol)
Ac-CCK$_4$	169	180	−83	93	−71	149	−118	4.9
Ac-CCK$_4$[NMeNle[31]]	90	164	64	27	−69	148	−119	0.3
Ac-CCK$_4$[L-3-*trans*-PP[31]]	167	151	−75	−96	−147	143	−136	2.6
Ac-CCK$_4$[L-4-MPt(Me)[31]]	159	179	−75	87	−85	141	−132	3.0
Ac-CCK$_4$[L-4-MPc(Me)[31]]	162	175	−75	86	−71	149	−117	0.7
Ac-CCK$_4$[L-3-MPt(Me)[31]]	169	143	−75	141	−81	140	−121	4.6
Ac-CCK$_4$[L-3-MPc(Me)[31]]	168	142	−75	131	−88	153	−125	3.0
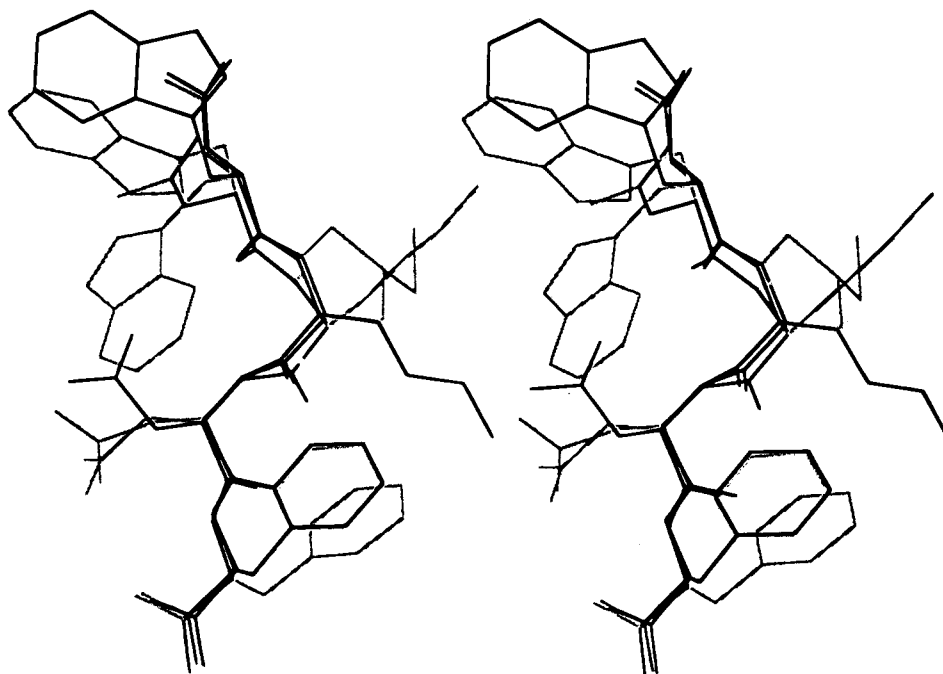
Figure 1. Stereoviews of conformers from Table 3 for Ac-CCK$_4$ (in bold), Ac-[*N*-MeNle[31]]-CCK$_4$ and Ac-[L-4-MPc(Me)[31]]-CCK$_4$ (peptide bonds shown in **bold**). All hydrogen atoms are omitted.

Discussion

An accurate model of the receptor-bound conformations of CCK$_8$ at the CCK-A and -B receptors would be a great advance toward the rational design of CCK-related drugs. In the process of refining our previously reported model of the CCK-B receptor-bound conformation of CCK$_8$,[24] we found that Ac-CCK$_4$ analogs with substitution of Met[31] by selected stereoisomers of 3- and 4-(alkylthio)prolines showed high affinity for the CCK-B receptor. Replacement of a native residue by a chimeric proline derivative results in conformational restrictions of both the peptide backbone and the side chain moiety. When this replacement yields an analog with high affinity, valuable information regarding the receptor-bound conformation of the peptide can be obtained. In using this type of approach, one should obtain all possible stereoisomers of the chimeric proline derivatives. The synthetic approach reported herein represents a general method for obtaining peptide analogs containing all stereoisomers of 3- and 4-mercaptoproline which can be derivatized with an appropriate electrophile to obtain the desired chimeric amino acid. Additionally, this approach allows one to generate an entire series of (alkylthio)proline-containing peptides from a single mercaptoproline-containing precursor. In all cases, the thiol moiety could be selectively alkylated in the presence of a free carboxyl group, which can be attributed to the higher nucleophilicity of the thiol group

compared to the carboxyl. Although only dimethyl sulfate, ethyl bromide, and propyl bromide were used in this study, other electrophiles could potentially react with the thiol moiety.

In order to correctly interpret the results from the binding assays, the absolute stereochemistry of the mercaptoproline residue in each analog must be known. In the case of 4-MPt/c, the synthetic precursor, 4-hydroxyproline, is commercially available as a single enantiomer, and the stereochemical integrity is preserved throughout the synthetic schemes we have developed. The synthetic approach in Scheme 2 represents an improvement over the previously reported synthesis,[16] because *N*-Boc protection can be used throughout the entire scheme and the introduction of the sulfur moiety under Mitsunobu conditions goes in high yield (85%). Thus, overall yields of protected 4-MPt/c analogs are higher from Scheme 2 than the previously reported route. We have found that Scheme 2 can also be used to obtain the *S-p*-methylbenzyl derivative of Boc-4-MPt/c by incorporating *p*-methylbenzyl bromide in the reaction mixture during hydrolysis of the thiolacetate group.

Because the syntheses of both **20** and **23** outlined in Schemes 3 and 4 yielded the racemic mixtures, two diastereomeric peptides resulted from their incorporation into a peptide sequence (see Scheme 5). It was found that these thiol-containing peptides could be
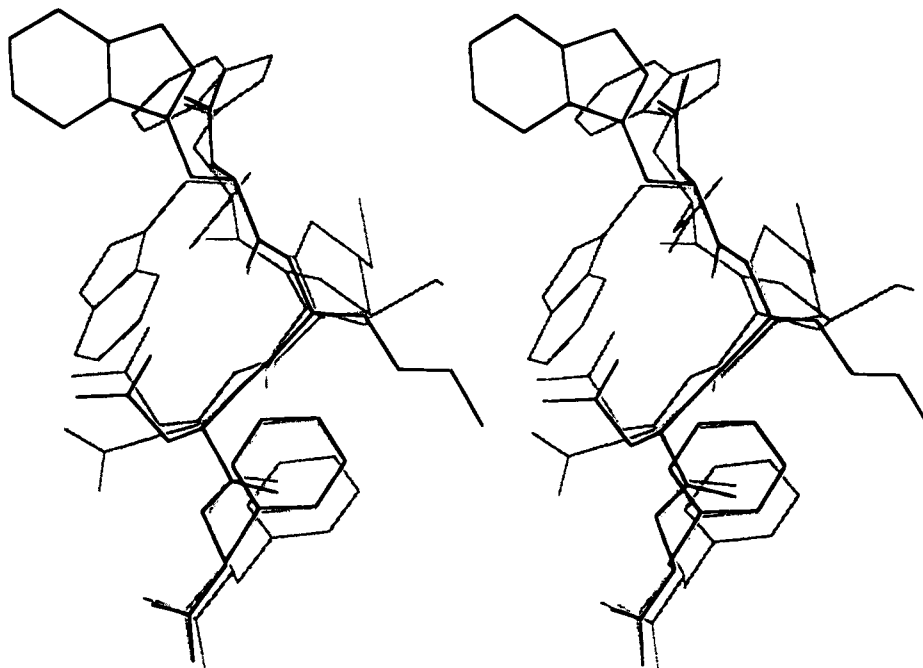
Figure 2. Stereoviews of conformers from Table 3 for Ac-CCK$_4$ (in bold), Ac-[L-4-MPc(Me)^{31}]-CCK$_4$ and Ac-[D-4-MPc(Me)^{31}]-CCK$_4$ (in shadow). All hydrogen atoms are omitted.

desulfurized with Raney nickel to the corresponding proline containing peptides. Because both Ac-CCK$_4$[L-Pro^{31}] (**13**) and Ac-CCK$_4$[D-Pro^{31}] (**14**) were made for this study, the physical properties of the products from reduction of **24a**−**27a** with Raney nickel could be compared with those of **13** and **14** in order to assign the absolute stereochemistry of **24a**−**27a**. We feel that this approach is valid, since the relative stereochemistry of the MPt/c residue in **24a**−**27a** is known and reduction with Raney nickel does not affect the chirality of the C-α atoms. A single reduction product was observed in each case, and the HPLC retention time corresponded to either **13** or **14**. Furthermore, ^{1}H NMR spectra of the products were indistinguishable from those of **13** or **14**. Clearly, the stereoselective syntheses of 3-MPt and 3-MPc derivatives suitably protected for SPPS would be an improvement for the general synthesis of 3-MPc- and 3-MPt-containing peptides, and this is currently being pursued in our lab.

The conformational geometries depicted in Table 3 can be regarded as a model of the "biologically active" conformer of CCK$_4$ at the CCK-B receptors. It is noteworthy that the 3D shapes of the conformers for the different peptides in Table 3 are similar (see Figure 1) despite the differences in the dihedral angle values for the residues in position 2. It is easy to notice that the backbone peptide bonds of each of the conformers depicted in Figure 1 lie within the same plane. This plane is the basis for an amphiphilic structure with the hydrophobic side chains of the Trp and Phe residues located "at one surface" of the molecule and the hydrophilic side chain of the Asp residue located "at the other surface". The hydrophobic side chain in position 2 can cluster together with the other hydrophobic side chains to fill a suggested hydrophobic pocket within the CCK-B receptor (Figure 1). The spatial orientation suitable for such clustering became more difficult when the residue in position 2 had a D-configuration (see Figure 2). However, it is clearly seen in Figure 2 that increasing the chain length of the alkyl substituent on the 3-mer-

captoproline residue (increased conformational flexibility) would allow the alkyl moiety to locate itself at the "hydrophobic" side of the molecule, even for the D-amino acids. (The same is true concerning the *trans-/cis*-isomers (cf. analogs **24d** and **26d**), despite the differences in restrictions imposed on the χ_1 and χ_2 values.) Consequently, the differences in affinities between the L- and D- or *trans-* and *cis*-mercaptoproline-containing compounds should become less significant with increasing chain length, which seems to be the tendency in Table 2. This tendency speaks in favor of the suggested model for the CCK-B "biologically active" conformer.

So far, few authors have presented models for the 3D structure of CCK$_4$ (or for the C-terminal tetrapeptide of CCK$_8$), which might be involved in binding with the CCK-B receptor. Nevertheless, the Z-like bend formed by the three peptide bonds of the Trp-Met-Asp-Phe sequence can be clearly viewed in the model suggested by the Roques group on the basis of NMR and fluorescence spectroscopy and Monte-Carlo simulations.[25,26] The same kind of bend is even more pronounced in the model of Boc-CCK$_4$[L-3-*trans*-PP^{31}] suggested by the Abbott group.[23] It is also clearly seen in the recent model of the CCK$_4$ conformation, which was proposed by NMR spectroscopy with subsequent molecular dynamics simulations.[40] On the other hand, the model reported by Pincus *et al.*,[27] which was produced by energy calculations on Ac-CCK$_4$, places the Trp and Phe side chains "at one side" of the molecule and the Met and Asp side chains "at the other side," contrary to the model in **Figure 1** and the other reported models discussed above. The model for the CCK-B "biologically active" conformer proposed by us earlier[24] also bears only slight resemblance to the model suggested by the present study. However, the present model can be regarded as a refinement of the previous one, since the number of compounds for mutual geometrical comparison was significantly increased in this study (nine *vs* three in the earlier paper[24]), and the requirements for geo-

metrical similarity were more strict (matching of eight atomic centers *vs* five in the earlier paper[24]).

Experimental Section

A. Synthesis. Materials. *N*-Boc protected amino acids and MBHA resin (0.62–0.69 mequiv/g) were purchased from Bachem (Torrance, CA). 2-(1*H*-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) was purchased from Richelieu Biotechnologies (St. Hyacinthe, Quebec, Canada). 1-Hydroxybenzotriazole hydrate (HOBT) and (2*R*,4*R*)-4-hydroxyproline (**3**) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Boc-L-4-MPt(MeBzl) and Boc-L-4-MPc-(MeBzl) were obtained as previously described,[16] and methyl *N*-Boc-(2*R*,4*R*)-4-hydroxyprolinate (**4**) was obtained from **3** using known procedures.[28] Tetrahydrofuran (THF) was reagent grade and distilled from sodium/benzophenone under argon prior to use. Dichloromethane (DCM) and dimethylformamide (DMF) were HPLC grade and stored over 4 Å molecular sieves prior to use; all other reagents were reagent grade and used as purchased.

General Methods: Solution Phase Chemistry. Progress of reactions were monitered by TLC on silica gel plates (Analtech, 250 *μ*) using the indicated developing solvent. Plates were visualized by UV irradiation and by placement in a chamber containing HCl vapors, spraying with a 0.5% ninhydrin solution in ethanol, and heating to 110 °C. Melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were obtained on a Perkin-Elmer FT-IR spectrometer (model 1710). ¹H and ¹³C NMR spectra were collected on a Varian Gemini instrument operating at 300 and 75 MHz, respectively. Proton chemical shifts (*δ*) are reported in ppm from TMS included as an internal standard, and coupling constants (*J*) are given in hertz. Carbon spectra are referenced to the center peak of the chloroform multiplet = 77.00 ppm. Optical rotations were measured on a Perkin-Elmer polarimeter (model 241). Elemental analyses were performed by M-H-W Laboratories, Phoenix, AR.

General Methods: Solid Phase Peptide Synthesis (SPPS). All peptides were synthesized by manual SPPS using an *N*-Boc amine protection strategy. Deprotection of the *N*-Boc group of the growing peptide-resin was achieved by reaction with 50% TFA in DCM for 20 min (1% indole was included after the incorporation of the Trp residue). Coupling of each amino acid was performed with a 3-fold excess of the *N*-Boc amino acid using TBTU (3 equiv) as the coupling reagent in the presence of HOBT (3 equiv) and *N*-methylmorpholine (6 equiv) in a minimal amount of a 1:1 mixture of DMF and DCM. The progress of the coupling reaction was followed by the Kaiser test.[29] Acetylation of the *N*-terminus was accomplished by reaction of the free amine with 10 equiv each of acetic anhydride and triethylamine in DMF. Cleavage of the peptide from the solid support was accomplished by reaction of the peptide-resin with a mixture of liquid HF, anisole, and dimethyl sulfide (10:1:0.5) for 60 min at 0 °C. The HF was evaporated, and the crude peptide was precipitated with ethyl ether, collected by vacuum filtration, and removed from the resin by extraction into glacial acetic acid. The purity of the crude peptide was assessed by analytical RP-HPLC on a Spectra Physics instrument using a Vydac C18 peptide/protein column (4.6 mm i.d. × 25 cm, 5 *μ*m) and the indicated gradient with solvent A = 0.05% aqueous TFA and B = 0.038% TFA in 9:1 acetonitrile/water. Purification of the crude peptide was accomplished by preparative RP-HPLC on a Rainin instrument using a Dynamax 300A C18 column (21.4 mm i.d. × 25 cm, 5 *μ*m) and the indicated gradient with solvent A = 0.1% aqueous TFA and B = 100% acetonitrile. Each peptide was characterized by mass spectroscopy using fast atom bombardment (FAB) ionization on a Finnigan 3300 instrument equipped with a capillarton gas gun from Phrasor Scientific (Duarte, CA) in a matrix of either acidified glycerol or 3-nitrobenzyl alcohol. Amino acid analysis was performed for each peptide and consisted of vapor phase hydrolysis of the peptide for 24 h at 110 °C with constant boiling 6 N HCl, dabsylation of the constituent amino acids, and RP-HPLC analysis on a Beckman

C18 Ultrasphere ODS-DABS column (4.6 mm i.d. × 25 cm, 5 *μ*m) using a Beckman System Gold instrument.

General Method for Reduction of 3-MPt/c-Containing Peptides. As outlined in the text, 3-MPt- and 3-MPc-containing peptides **24a−27a** were reduced with Raney nickel to the corresponding D- or L-proline-containing peptides to assign the absolute stereochemistry of the α-carbon of this residue. Prior to use, Raney nickel (Sigma, St. Louis, MO) was carefully washed with water (3×) followed by 80–90% ethanol (3×), never allowing the solid to dry completely. The general procedure for this reduction involved dissolving approximately 10–15 mg of the thiol-containing peptide in 2 mL ethanol, adding approximately 20 mg of activated Raney nickel, and refluxing the resulting slurry overnight (16–20 h) under Ar. The reaction was followed by RP-HPLC, and upon completion, the mixture was diluted with ethanol, filtered, and concentrated *in vacuo*. The crude reaction mixture consisted of one major component which corresponded to either **13** or **14** as judged by coinjection on RP-HPLC and by comparison of the ¹H NMR spectra of the reduction product with both **13** and **14**.

General Method for Alkylation of Thiol-Peptides. Thiol-containing peptides (**1a**, **2a**, and **24a−27a**) were alkylated by reaction with 1.2 equiv of either dimethyl sulfate, ethyl bromide, or propyl bromide (see experimental description of individual peptides for details) in the presence of 4 equiv of NaOH in a 1:2 mixture of methanol and water (previously deoxygenated by purging with Ar). The reactions were followed by RP-HPLC and required anywhere from 1 h (dimethyl sulfate) to 24 h (ethyl bromide and propyl bromide) to go to completion. Once judged complete, the reactions were worked up using the following procedure: the reaction mixture was acidified with 10% KHSO₄, volatiles were removed *in vacuo*, and the residue was partitioned between ethyl acetate and water. The ethyl acetate layer was washed with brine, dried over MgSO₄, and concentrated *in vacuo*. Peptides were purified by preparative RP-HPLC as previously described, giving the desired products in purities of >97%. The purified peptides were characterized by FAB mass spectroscopy, amino acid analysis, and ¹H NMR spectroscopy to assess the incorporation of the desired alkyl substituent.

Boc-Asp(cHx)-Phe-MBHA. MBHA resin (2.0 g, 1.24 mmol) was swollen in DCM for 2 h and then washed successively with 10% triethylamine in DCM (2×), DCM (3×), and DMF (3×). Then, the resin was reacted with Boc-Phe as described in the general synthetic procedures; two couplings were required to achieve a negative Kaiser test. Subsequent deprotection and coupling with Boc-Asp(cHx) were also accomplished as described in the general synthetic procedures to yield 2.47 g of Boc-Asp(cHx)-Phe-MBHA (0.50 mmol/g resin) after drying *in vacuo* overnight.

Ac-Trp-L-4-MPt(Me)-Asp-Phe-NH₂ (1b). The title compound was obtained by stepwise elongation of Boc-Asp(cHx)-Phe-MBHA (0.25 g, 0.125 mmol) using Boc-MPt(MeBzl) and Boc-Trp, acetylation of the *N*-terminus, and cleavage from the solid support using the procedures outlined in the general methods in a yield 56 mg (68% crude yield). Analytical RP-HPLC (25–50% B over 25 min) revealed a single major component at t_R = 10.9 min. Crude **1a** (55 mg, 0.0846 mmol) was dissolved in a mixture of methanol (0.17 mL) and 1 N NaOH (0.34 mL, 0.34 mmol) and cooled with an ice bath, and then dimethyl sulfate (8 *μ*L, 0.0846 mmol) was added dropwise. The solution was stirred with cooling for 60 min, after which time analytical RP-HPLC showed disappearance of **1a** and the appearance of a new component at t_R = 12.2 min. The reaction mixture was diluted with 10% KHSO₄ (10 mL) and extracted with ethyl acetate (10 mL × 3). The combined ethyl acetate layers were washed with water (10 mL × 2) and brine (10 mL × 1), dried over MgSO₄, and concentrated *in vacuo* to yield 48 mg of crude **1b** as a white solid. Purification of the crude by preparative RP-HPLC (25–40% B over 30 min) gave 16.7 mg of pure **1b** (21% overall yield from SPPS) as a white solid: analytical RP-HPLC (25–50% B over 25 min) t_R = 12.2 min; MS (FAB) *m/e* 651 (M + 1); [α]$_D$ = −44.8° (*c* = 0.54, DMF); amino acid analysis Asp (1) 1.07, 4−MPt(Me) (1) 1.00, Phe (1) 1.00, Trp (1) ND.

Ac-Trp-L-4-MPc(Me)-Asp-Phe-NH₂ (2b). As described for **1b**, the title compound was obtained in a yield of 14.4 mg (18% overall yield from SPPS) after purification by preparative RP-HPLC (25–40% B over 30 min): analytical RP-HPLC (25–50% B over 25 min) t_R = 12.0 min; MS (FAB) m/e 651 (M + 1); [α]$_D$ −34.1° (c = 0.44, DMF); amino acid analysis Asp (1) 1.01, Phe (1) 1.00, 4-MPc(Me) (1) 1.08, Trp (1) ND.

Methyl (2R,4S)-N-Boc-4-(acetylthio)prolinate (5). Diisopropyl azodicarboxylate (DIAD, 0.73 mL, 3.7 mmol) was added dropwise to a solution of triphenylphosphine (0.97 g, 3.7 mmol) in dry·THF (10 mL) at 0 °C under an Ar atmosphere. The reaction mixture was stirred at 0 °C for 30 min, and then a solution of **4** (0.45 g, 1.84 mmol), thiolacetic acid (0.27 mL, 3.7 mmol), and dry THF (3 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h after complete addition, then warmed to room temperature and stirred an additional hour. Volatiles were removed *in vacuo*, and the residual was purified by flash column chromatography (18% ethyl acetate in hexanes) to yield 0.44 g of pure **5** (79%) as a colorless oil: TLC (3:7 ethyl acetate/hexanes) R_f = 0.59; MS (FAB) m/e 304.5 (M + H), 248.5 (M − t-Bu), 204.5 (M − t-Boc); IR (thin film) 2977, 1751, 1698, 1397, 1367, 1206, 1180, 1158, 1124 cm⁻¹; ¹H NMR (CDCl₃) mixture of two conformers in a ratio of ca. 1:1 δ 1.41 (s, 9 H, one conformer), 1.46 (s, 9 H, other conformer), 2.10–2.47 (m, 2H), 2.34 (s, 3 H), 3.31 (dd, J = 6.2, 10.9 Hz, 1 H, one conformer), 3.43 (dd, J = 5.3, 11.2 Hz, 1 H, other conformer), 3.75 (s, 3 H), 3.91–4.10 (m, 2 H), 4.31 (dd, J = 5.4, 8.2 Hz, 1 H, one conformer), 4.40 (dd, J = 4.3, 8.5 Hz, 1 H, other conformer); ¹³C NMR (CDCl₃) mixture of two conformers δ 194.83, 194.72, 172.81, 172.53, 153.90, 153.26, 80.43, 58.35, 57.98, 52.35, 52.18, 51.87, 51.41, 39.62, 39.38, 36.76, 35.51, 30.56, 28.27, 28.15; [α]$_D$ +33.5° (c = 0.89, CHCl₃). Anal. (C₁₃H₂₁NO₅S) C, H, N, S.

(2R,4S)-N-Boc-4-(methylthio)proline (6). A solution of **5** (0.40 g, 1.32 mmol) in methanol (6.6 mL) was treated successively with 1 N NaOH (1.45 mL, 1.45 mmol) and dimethyl sulfate (0.14 mL, 1.45 mmol) at rt. After the mixture was stirred for 30 min, TLC analysis (3:7 ethyl acetate/hexanes) revealed the disappearance of starting material and the appearance of a new component of R_f = 0.62, which was non-UV quenching. Additional NaOH was added at this time (3.3 mL, 3.3 mmol), and the reaction mixture allowed to stir for an additional 2 h. TLC analysis (3:7 ethyl acetate/hexanes) revealed the disappearance of the component at R_f = 0.62 and a new component at the baseline. Volatiles were removed *in vacuo*, and the residual was partitioned between 10% KHSO₄ (50 mL) and ethyl acetate (50 mL). The aqueous layer was removed and reextracted with several portions of ethyl acetate (3 × 25 mL). The combined ethyl acetate layers were washed successively with water (2 × 50 mL) and brine (1 × 50 mL), dried over MgSO₄, and concentrated *in vacuo* to yield 0.32 g of crude product (93%) as a colorless oil. Purification of the crude by flash column chromatography (2% acetic acid in 1:4 ethyl acetate/hexanes) gave 0.30 g of pure product as a colorless oil: TLC (5% AcOH in 1:4 ethyl acetate/hexanes) R_f = 0.30; MS (FAB) m/e 262 (M + H), 284 (M + Na), 206 (M − t-Bu), 162 (M − t-Boc); IR (thin film) 2978, 1698, 1417, 1369, 1162 cm⁻¹; ¹H NMR (CDCl₃) mixture of two conformers in a ratio of ca. 1:1 δ 1.42 (s, 9 H, one conformer), 1.48 (s, 9 H, other conformer), 2.14 (s, 3 H, one conformer), 2.15 (s, 3 H, one conformer), 2.15–2.55 (m, 2 H), 3.23–3.45 (m, 2 H), 3.78–3.95 (m, 2 H), 4.39 (dd, J = 4.4, 8.4 Hz, 1 H, one conformer), 4.47 (dd, J = 2.9, 8.8 Hz, 1 H, other conformer); ¹³C NMR (CDCl₃) mixture of two conformers δ 177.77, 175.65, 155.14, 153.55, 81.30, 80.75, 58.51, 58.45, 52.23, 51.84, 41.93, 41.57, 36.63, 35.38, 28.26, 28.12, 14.38; [α]$_D$ + 38.3° (c = 0.46, DMF). Anal. (C₁₁H₁₉NO₄S) C, H, N, S.

Methyl (2R,4S)-N-Boc-4-hydroxyprolinate (7). Using the procedure outlined for **5**, reaction of **4** (1.00 g, 4.08 mmol) with formic acid (0.31 mL, 8.16 mmol) in place of thiolacetic acid yielded 0.76 g of the formate ester of **7** (76%) after flash column chromatography (20% ethyl acetate in hexanes). The product was characterized by ¹H NMR and used without further characterization: ¹H NMR (CDCl₃) mixture of two conformers in a ratio of ca. 2:3 δ 1.42 (s, 3 H, major conformer), 1.46 (s, 3 H, minor conformer), 2.15–2.30 (m, 1 H), 2.35–2.50

(m, 1 H), 3.55–3.80 (m, 5 H), 4.33–4.48 (m, 1 H), 5.38–5.48 (m, 1H), 8.03 (s, 1 H).

The formate ester of **7** obtained above (0.76 g, 3.1 mmol) was dissolved in methanol (14 mL) and cooled with an ice bath, and then 2.8 mL of 1 N NaOH (2.8 mmol) was added dropwise to the flask. TLC analysis (1:1 ethyl acetate/hexanes) 5 min after addition revealed disappearance of starting material and a new component at R_f = 0.21. The crude reaction mixture was acidified with 10% KHSO₄, the volatiles were removed *in vacuo*, and the residual was partitioned between 10% KHSO₄ (25 mL) and ethyl acetate (25 mL). The ethyl acetate was removed and the aqueous layer reextracted with ethyl acetate (2 × 25 mL). The combined ethyl acetate layers were washed with water (2×) and brine (1×), dried over MgSO₄, and concentrated *in vacuo* to yield 0.75 g of crude product as a colorless oil. Purification of the crude by flash column chromatography (1:1 ethyl acetate/hexanes) yielded 0.64 g of pure product as a colorless oil (64% overall yield): TLC (1:1 ethyl acetate/hexanes) R_f = 0.21; ¹H NMR (CDCl₃) mixture of two conformations in a ratio of ca. 2:1 δ 1.40 (s, 9 H, major conformer) 1.46 (s, 9 H, minor conformer) 2.00–2.11 (m, 1 H), 2.22–2.35 (m, 1 H), 3.40–3.66 (m, 2 H), 3.73 (s, 3 H), 4.35–4.50 (m, 2 H); ¹³C NMR (CDCl₃) mixture of two conformers δ 173.67, 173.45, 154.52, 153.99, 80.38, 80.23, 69.92, 69.17, 57.89, 57.44, 54.64, 54.58, 52.21, 52.02, 38.96, 38.33, 28.29, 28.16; [α]$_D$ +61.4° (c = 2.24, CHCl₃). Anal. (C₁₁H₁₉NO₅) C, H, N.

Methyl (2R,4R)-N-Boc-4-(acetylthio)prolinate (8). Using the procedure outlined for **5**, the title compound was obtained from **7** (0.51 g, 2.1 mmol) in a yield of 0.53 g (85%) after flash column chromatography (18% ethyl acetate in hexanes): TLC (1:1 ethyl acetate/hexanes) R_f = 0.55; MS (FAB) m/e 304.6 (M + H), 248.5 (M − t-Bu), 204.6 (M − t-Boc); IR (thin film) 2978, 1752, 1698, 1397, 1367, 1257, 1159, 1117 cm⁻¹; ¹H NMR (CDCl₃) mixture of two conformers in a ratio of ca. 1:1 δ 1.41 (s, 9 H, one conformer), 1.46 (s, 9 H, other conformer), 1.90–2.03 (m, 1 H), 2.35 (s, 3 H), 2.64–2.80 (m, 1 H), 3.27–3.41 (m, 1 H), 3.75 (s, 3 H, one conformer), 3.76 (s, 3 H, other conformer), 3.90–4.05 (m, 2 H), 4.29 (dd, J = 7.1, 8.0 Hz, 1 H, one conformer), 4.37 (dd, J = 6.7, 8.2 Hz, 1 H, other conformer); ¹³C NMR (CDCl₃) mixture of two conformers δ 194.98, 194.69, 172.75, 172.53, 153.75, 153.10, 80.43, 58.50, 58.04, 52.32, 52.10, 51.96, 51.17, 39.31, 38.58, 36.86, 35.55, 30.49, 28.27, 28.15; [α]$_D$ +43.3° (c = 1.41, CHCl₃). Anal. (C₁₃H₂₁NO₅S) C, H, N, S.

(2R,4R)-N-Boc-4-(methylthio)proline (9). Using the procedure described for the preparation of **6**, the hydrolysis of **8** (0.16 g, 0.52 mmol) gave 0.112 g of pure **9** (83%) after flash column chromatography (2% acetic acid in 1:4 ethyl acetate/hexanes) as a colorless oil (crystallized after long-term storage in refrigerator): TLC (5% acetic acid in 1:4 ethyl acetate/hexanes) R_f = 0.28; MS (FAB) m/e 262 (M + H), 284 (M + Na), 206 (M − t-Bu), 162 (M − t-Boc); IR (thin film) 3365, 2979, 1749, 1702, 1420, 1397, 1368, 1160 cm⁻¹; ¹H NMR (CDCl₃) mixture of two conformers in a ratio of ca. 1:1 δ 1.42 (s, 9 H, one conformer), 1.47 (s, 9 H, other conformer), 1.95–2.15 (m, 2 H), 2.13 (s, 3 H), 2.55–2.72 (m, 1 H), 3.17–3.35 (m, 2 H), 3.81–4.02 (m, 1 H), 4.29 (br t, J = 7.9 Hz, 1 H, one conformer), 4.38 (br t, J = 7.5 Hz, 1 H, other conformer), 9.50 (br s, 1 H); ¹³C NMR (CDCl₃) mixture of two conformers δ 177.65, 175.95, 154.87, 153.38, 81.17, 80.85, 58.61, 58.41, 52.34, 51.99, 42.34, 41.76, 36.58, 35.13, 28.24, 28.09, 14.28; [α]$_D$ +47.7° (c = 0.43, DMF). Anal. (C₁₁H₁₉NO₄S) C, H, N.

Trp-D-4-MPt(Me)-Asp-Phe-NH₂ (10). The title compound was obtained by elongation of Boc-Asp(cHx)-Phe-MBHA (0.25 g, 0.125 mmol) by successive coupling of **6** and Boc-Trp, acetylation of the N-terminus, and cleavage of the peptide as described in the general procedures in a crude yield of 50 mg. Preparative RP-HPLC of the crude (28–43% B over 30 min) gave 25.6 mg of pure **10** (32%) as a white solid: analytical RP-HPLC (25–50% B over 25 min) t_R = 13.5 min; MS (FAB) m/e 652 (M + 1); [α]$_D$ −11.6° (c = 0.86, DMF); amino acid analysis Asp (1) 1.11, Phe (1) 1.00, 4-MPt(Me) (1) 0.84, Trp (1) ND.

Ac-Trp-D-4-MPc(Me)-Asp-Phe-NH₂ (11). The title compound was obtained by the procedure described for **10**, using

9 in place of **6**, in a crude yield of 47 mg (57%). Preparative RP-HPLC of the crude (25–37% B over 30 min) yielded 30.3 mg of pure **11** (37%) as a white solid: analytical RP-HPLC (25–50% B over 25 min) t_R = 12.5 min; MS (FAB) m/e 651 (M + 1); $[\alpha]_D$= –3.8° (c = 0.43, DMF); amino acid analysis Asp (1) 1.04, 4-MPc (1) 1.03, Phe (1) 1.00, Trp (1) ND.

Ac-Trp-D-Nle-Asp-Phe-NH₂ (12). The title compound was obtained by the procedure described for **10**, using Boc-D-Nle in place of **6**, in a crude yield of 99 mg. Purification by preparative RP-HPLC (25–40% B over 30 min) yielded 41.5 mg of pure **12** (27%) as a white solid: analytical RP-HPLC (25–40% B over 15 min) t_R = 14.0 min; MS (FAB) m/e 621 (M + 1); $[\alpha]_D$ –10.0° (c = 0.17, AcOH); amino acid analysis Asp (1) 0.98, Nle (1) 0.96, Phe (1) 1.00, Trp (1) ND.

Ac-Trp-Pro-Asp-Phe-NH₂ (13). The title compound was obtained by the procedure described for **10**, using Boc-Pro in place of **6**, in a crude yield of 85 mg. Purification by preparative RP-HPLC (23–33% B over 30 min) yielded 40 mg of pure **13** (26%) as a white solid: analytical RP-HPLC (25–40% B over 15 min) t_R = 8.8 min; MS (FAB) m/e 605 (M + 1); ¹H NMR (DMSO-d_6) two conformations evident in spectrum in ratio of 3:1 δ 1.75 (s, 3 H, major conformer), 1.82 (s, 3 H, minor conformer), 1.75–2.05 (m, 2 H), 2.40–2.55 (m, 1 H), 2.60–3.20 (m, 5 H), 3.42–3.70 (m, 2 H), 4.25–4.40 (m, 2 H), 4.42–4.53 (m, 1 H), 4.68–4.80 (m, 1 H), 6.95–7.35 (m, 11 H), 7.43 (d, J = 7.7 Hz, 1 H, minor conformer), 7.52 (d, J = 7.8 Hz, 1 H, major conformer), 7.82 (d, J = 8.1 Hz, 1 H, minor conformer), 7.83 (d, J = 8.1 Hz, 1 H, major conformer), 8.21 (d, J = 7.7 Hz, 1 H, major conformer), 8.26 (d, J = 8.1 Hz, 1 H, major conformer), 8.33 (d, J = 7.8 Hz, 1 H, minor conformer), 8.48 (d, J = 6.1 Hz, 1 H, minor conformer), 10.84 (br s, 1 H, major conformer), 10.95 (br s, 1 H, minor conformer); $[\alpha]_D$ –67.2° (c = 0.12, AcOH); amino acid analysis Asp (1) 1.00, Pro (1) 0.99, Phe (1) 1.00, Trp (1) ND.

Ac-Trp-D-Pro-Asp-Phe-NH₂ (14). The title compound was obtained by the procedure described for **10**, using Boc-D-Pro in place of **6**, in a crude yield of 100 mg. Purification by preparative RP-HPLC (23–38% B over 30 min) yielded 67.1 mg of pure **14** (44%) as a white solid: analytical RP-HPLC (25–40% B over 15 min) t_R = 10.9 min; MS (FAB) m/e 605 (M + 1); ¹H NMR (DMSO-d_6) δ 1.20–1.45 (m, 1 H), 1.50–1.75 (m, 3 H), 1.87 (s, 3 H), 2.38 (dd, J = 8.8, 16.7 Hz, 1 H), 2.64 (dd, J = 4.8, 16.8 Hz, 1 H), 2.72–2.80 (m, 1 H), 2.90–3.14 (m, 4 H), 3.37–3.48 (m, 1 H), 4.02–4.11 (m, 1 H), 4.30–4.45 (m, 2 H), 4.61–4.71 (m, 1 H), 6.96–7.37 (m, 11 H), 7.53 (d, J = 8.0 Hz, 1 H), 7.86 (d, J = 8.3 Hz, 1 H), 8.20–8.30 (m, 2 H), 10.89 (br s, 1 H); $[\alpha]_D$ +36.4° (c = 0.17, AcOH); amino acid analysis Asp (1) 0.89, Pro (1) 1.02, Phe (1) 1.00, Trp (1) ND.

Ac-Trp-MeA-Asp-Phe-NH₂ (15). The title compound was obtained by the procedure described for **10**, using Boc-MeA in place of **6**, in a crude yield of 44 mg. Purification by preparative RP-HPLC (25–40% B over 30 min) yielded 21.1 mg of pure **15** (18%) as a white solid: analytical RP-HPLC (25–40% B over 15 min) t_R = 10.7 min; MS (FAB) m/e 593 (M + 1); $[\alpha]_D$ –29.4° (c = 0.17, AcOH); amino acid analysis Asp (1) 0.97, MeA (1) 0.99, Phe (1) 1.00, Trp (1) ND.

Ac-Trp-Cle-Asp-Phe-NH₂ (16). The title compound was obtained by the procedure described for **10**, using Boc-Cle in place of **6**, in a crude yield of 65 mg. Purification by preparative RP-HPLC (25–40% B over 30 min) yielded 18.2 mg of pure **16** (25%) as a white solid: analytical RP-HPLC (25–40% B over 15 min) t_R = 12.4 min; MS (FAB) m/e 619 (M + 1); $[\alpha]_D$ –33.3° (c = 0.24, AcOH); amino acid analysis Asp (1) 1.01, Cle (1) 1.04, Phe (1) 1.00, Trp (1) ND.

Ac-Trp-D,L-MeL-Asp-Phe-NH₂ (17). The title compound was obtained by the procedure described for **10**, using Boc-D,L-MeL in place of **6**, in a crude yield of 35 mg. Purification by preparative RP-HPLC (25–40% B over 30 min) yielded 12.5 mg of pure **17** (16%) as a mixture of two diastereoisomers: analytical RP-HPLC (25–50% B over 25 min) t_R = 15.7 and 16.1 min; MS (FAB) m/e 635 (M + 1); amino acid analysis Asp (1) 1.07, MeL (1) 1.11, Phe (1) 1.00, Trp (1) ND.

(2S,3S)- and (2R,3R)-N-Boc-3-(4-methylbenzyl)proline Dicyclohexylamine Salt (D,L-20·DCHA). To a solution of **18** (45.6 g, 0.27 mol) and 4-methylbenzyl mercaptan (35 mL) in MeOH (120 mL) was added 50% NaH in mineral oil (0.2g).

The mixture was heated to reflux for 3 h, after which TLC analysis (95:5 methanol/ethyl acetate) showed reaction to be complete. After cooling, the mixture was concentrated *in vacuo*, and the residue was taken up in 300 mL of water and 100 mL of concentrated HCl. The resulting solution was heated to reflux for 2 h. After cooling to rt, the aqueous solution was extracted with ethyl ether (2 × 150 mL); further cooling resulted in precipitation of a white solid. The solid was collected by vacuum filtration, yielding 40.7 g of **19** (52% yield from **18**) as a white solid. The ¹H NMR spectrum was consistent with the desired product, and this material was taken on into the next step without further characterization.

To a stirred solution of **19** (40 g, 0.139 mol) in DCM was added triethylamine (43 mL, 0.31 mol) and di-*tert*-butyl carbonate (33.4 g, 0.153 mol) portionwise over 5 min. The mixture was stirred for 90 min at room temperature, and then the solution was washed with 1 N HCl (250 mL), H₂O (100 mL), and brine (100 mL). The organic layer was concentrated *in vacuo*, the residue was taken up in 1 N NaOH (250 mL), and the aqueous layer was extracted with ethyl ether (2 × 150 mL). The aqueous layer was cooled with ice and acidified with concentrated HCl. The product was extracted with ethyl ether, dried over MgSO₄, and concentrated *in vacuo*. Purification by flash column chromatography (100% acetonitrile followed by 90:9:1 acetonitrile/water/acetic acid) yielded 45 g of **20** (92% yield) as a colorless oil: ¹H NMR (CDCl₃) two conformers present in ratio of 1:1.4 δ 1.41 (s, 3 H, minor conformer), 1.49 (s, 3 H, major conformer), 1.78–1.90 (m, 1 H), 2.15–2.30 (m, 1 H), 2.32 (s, 3 H), 3.34–3.65 (m, 3 H), 3.79 (br s, 2 H), 4.22 (d, J = 3.1 Hz, 1 H, minor conformer), 4.36 (d, J = 1 Hz, 1 H, major conformer), 7.11 (d, J = 8.1 Hz, 2 H), 7.25 (d, J = 8.0 Hz, 2 H), 10.40 (br s, 1 H).

The DCHA salt of **20** was formed by dissolving **20** (45 g, 0.128 mol) from above in ethyl ether (600 mL) and adding dicyclohexylamine (23 g, 0.128 mol) dropwise. After stirring for 30 min, no precipitate had formed, so the volatiles were removed *in vacuo*, and the residue was taken up in hexane resulting in a white precipitate. The solid was collected by vacuum filtration to yield 50 g of D,L-**20**·DCHA (66% yield) as a white solid: mp 130–131 °C; ¹H NMR (CDCl₃) δ 1.02–1.30 (m, 6 H), 1.35–1.85 (m, 19 H), 1.91–2.01 (m, 4 H), 2.11–2.28 (m, 2 H), 2.31 (s, 3 H), 2.85–2.97 (m, 2 H), 3.37–3.42 (m, 1 H), 3.49–3.67 (m, 2 H), 3.74–3.84 (m, 2 H), 4.11–4.18 (m, 1 H), 7.08 (d, J = 7.8 Hz, 2 H), 7.26 (d, J = 7.8 Hz, 2 H), 8.5 (br s, 1 H); ¹³C NMR (CDCl₃) δ 21.03, 24.74, 25.13, 28.44, 28.97, 29.18, 29.86, 30.60, 30.88, 35.62, 35.73, 44.75, 45.19, 46.72, 48.10, 52.28, 67.89, 67.98, 78.52, 78.63, 128.69, 128.78, 129.01, 135.22, 136.33, 154.41, 154.68, 175.50, 175.68. Anal. (C₃₀H₄₈N₂O₄S) C, H, N.

(2S,3R)- and (2R,3S)-N-Boc-3-(4-methylbenzyl)proline Dicyclohexylamine Salt (D,L-23·DCHA). A solution of **21** (9.8 g, 48 mmol) in 0.5 N NaOH (95 mL) was stirred for approximately 15 min (slight warming occurred) under an Ar atmosphere. Then, 4-methylbenzyl mercaptan (8 mL) was added via syringe, followed by 2 N NaOH (26 mL). After a few minutes, a thick precipitate formed. The mixture was stirred for 70 min, then sodium borohydride (1.6 g) was added, and the resulting mixture stirred overnight at rt. The reaction mixture was adjusted to pH 6 with 3 N HCl. The zwitterionic amino acid **22** precipitated and was collected by vacuum filtration. The solid was washed with ether and dried to obtain 4.45 g of crude **22** as a white solid. The compound was characterized by ¹H NMR and carried on without further purification: ¹H NMR (DMSO-d_6) δ 1.75–2.70 (m, 5 H), 3.15–4.05 (m, 5 H), 4.51–4.75 (m, 1 H), 7.00–7.45 (m, 4 H).

Crude **22** from above (4.0 g, 15.9 mmol) was suspended in DCM (50 mL) and then treated with triethylamine (5 mL, 36 mmol). The mixture was cooled with an ice bath, and then di-*tert*-butyl dicarbonate (4.4 g, 20 mmol) was added. The ice bath was removed, and the reaction mixture was stirred at room temperature overnight. The mixture was filtered to remove a slight amount of insoluble material, and then the solution was washed with 30 mL of 1 N HCl and 30 mL of brine. The solution was concentrated *in vacuo*, and the residue taken up in 2 N NaOH. The solution was washed with ethyl ether (2 × 30 mL), chilled to <5 °C, and acidified with 6 N

HCl. The product was extracted with ethyl ether (2 × 75 mL), dried over MgSO$_4$, and concentrated *in vacuo* to yield a foamy residue which quickly solidified. Trituration with pentane yielded 4.5 g of white solid. ^{1}H NMR of this material revealed a trace of trans isomer **20**: ^{1}H NMR (CDCl$_3$) *cis*-isomer, mixture of two conformers in ratio of 1:2 δ 1.44, 1.45 (2 s, 9 H), 1.92–2.17 (m, 2 H), 2.33 (s, 3 H), 3.17–3.34 (m, 2 H), 3.57–3.89 (m, 3 H), 4.40 (d, J = 8.1 Hz, 1 H, major conformer), 4.54 (d, J = 8.1 Hz, 1 H, minor conformer), 7.12 (d, J = 7.9 Hz, 2 H), 7.22 (d, J = 8.0 Hz, 2 H).

Crude **23** (4.33 g) was then dissolved in ethyl ether (125 mL), and dicyclohexylamine (2.33 g) in 10 mL of ethyl ether was added. The resulting solution was allowed to stand at 0 °C overnight. A layer of dense crystals had formed on the bottom of the flask. These were broken up and stirred in the supernatant for 30 min at rt, then collected by vacuum filtration, washed with pentane, and dried to yield 5.0 g of D,L-**23**·DCHA as a white solid (75%). RP-HPLC analysis (Alltech ADS C18 5 μm column, isocratic elution with 1:1 0.05 M triethylammonium citrate, pH 5.3/acetonitrile, λ = 250 nm) revealed <0.6% *trans* compound; mp 156–158 °C; ^{1}H NMR (CDCl$_3$) δ 1.08–1.30 (m, 6 H), 1.36–1.85 (m, 19 H), 1.90–2.22 (m, 6 H), 2.32 (s, 3 H), 2.86–3.05 (m, 2 H), 3.15–3.34 (m, 2 H), 3.56–3.82 (m, 2 H), 3.88–3.99 (m, 1 H), 4.22–4.30 (m, 1 H), 7.10 (d, J = 7.8 Hz, 2 H), 7.24 (d, J = 7.8 Hz, 2 H), 9.0 (br s, 1 H); ^{13}C NMR (CDCl$_3$) δ 21.00, 24.81, 25.04, 28.41, 28.67, 28.83, 30.15, 30.69, 35.80, 35.93, 43.22, 43.97, 45.13, 45.59, 52.11, 65.02, 78.38, 78.70, 128.64, 128.96, 135.28, 136.27, 136.34, 153.92, 154.28, 174.17, 174.35. Anal. (C$_{30}$H$_{48}$N$_2$O$_4$S) C, H, N.

Ac-Trp-3-MPt-Asp-Phe-NH$_2$ (24a and 25a). The title compounds were obtained by the procedure described for **10**, using D,L-**20** in place of **6**, in a crude yield of 480 mg (77%) as a mixture of **24a** and **25a**. Preparative RP-HPLC of the crude (25–40% B over 30 min) resulted in separation of the diastereomers:

Ac-Trp-L-3-MPt-Asp-Phe-NH$_2$ (24a): 99.9 mg of a white solid (16% isolated yield); analytical RP-HPLC (25–50% B over 25 min) t_R = 10.9 min; MS (FAB) *m/e* 637 (M + 1); ^{1}H NMR (DMSO-d_6) two conformations evident in spectrum in ratio of 1:3.3 δ 1.73 (s, 3 H, minor conformer), 1.76 (s, 3 H, major conformer), 1.8–1.9 (m, 1 H), 2.00–2.30 (m, 1 H), 2.45–2.55 (m, 1 H), 2.60–2.75 (m, 1 H), 2.80–2.95 (m, 2 H), 2.97 (d, J = 6.5 Hz, 1 H, major conformer), 3.00–3.15 (m, 2 H), 3.30–3.55 (m, 2 H), 3.80–3.93 (m, 1 H), 4.11–4.16 (m, 1 H, minor conformer), 4.22 (d, J = 3.3 Hz, 1 H, major conformer), 4.32–4.78 (m, 3 H), 6.95–7.05 (m, 2 H), 7.12–7.35 (m, 8 H), 7.54 (d, J = 7.7 Hz, 1 H), 7.91 (d, J = 8.1 Hz, 1 H-major conformer), 8.07 (d, J = 8.2 Hz, 1 H, minor conformer), 8.21 (d, J = 7.6 Hz, 1 H, minor conformer), 8.29 (d, J = 7.7 Hz, 1 H, major conformer), 8.42 (d, J = 7.7 Hz, 1 H, major conformer), 8.52 (d, J = 7.8 Hz, 1 H, minor conformer), 10.86 (s, 1 H), 12.40 (br s, 1 H); [α]$_D$ −13.1° (c = 0.13, AcOH); amino acid analysis Asp (1) 1.10, 3-MPt (1) ND, Phe (1) 1.00, Trp (1) ND. Raney nickel reduction of **24a** (10 mg) using the procedure described in the general methods resulted in a new component by RP-HPLC at t_R = 8.8 min (25–50% B over 25 min). Co-injection with a pure sample of **13** confirmed the stereochemistry of the L-Pro^{31} residue, and the ^{1}H NMR spectrum was indistinguishable from that of **13**.

Ac-Trp-D-3-MPt-Asp-Phe-NH$_2$ (25a): 103.4 mg of a white solid (17% isolated yield); analytical RP-HPLC (25–50% B over 25 min) t_R = 13.2 min; MS (FAB) *m/e* 637 (M + 1); ^{1}H NMR (DMSO-d_6) δ 1.23–1.37 (m, 1 H), 1.86 (s, 3 H), 2.02–2.08 (m, 1 H), 2.09 (s, 3 H), 2.22 (d, J = 7.4 Hz, 1 H), 2.41 (dd, J = 8.9, 16.8 Hz, 1 H), 2.64 (dd, J = 4.6, 16.8 Hz, 1 H), 2.96–3.23 (m, 7 H), 3.96 (d, J = 3.8 Hz, 1 H), 4.31–4.45 (m, 2 H), 4.65–4.77 (m, 1 H), 6.97–7.28 (m, 11 H), 7.33 (d, J = 7.8, 1 H), 7.56 (d, J = 7.7 Hz, 1 H), 7.88 (d, J = 8.7 Hz, 1 H), 8.31 (d, J = 6.6 Hz, 1 H), 8.36 (d, J = 7.6 Hz, 1 H), 10.93 (s, 1 H), 12.4 (br s, 1 H); [α]$_D$ −7.8° (c = 0.21, AcOH); amino acid analysis Asp (1) 0.90, 3-MPt (1) ND, Phe (1) 1.00, Trp (1) ND. Raney nickel reduction of **25a** (12 mg) using the procedure described in the general methods resulted in a new component by RP-HPLC at t_R = 10.9 min (25–50% B over 25 min). Co-injection with a pure sample of **14** confirmed the stereochemistry of the

D-Pro^{31} residue, and the ^{1}H NMR spectrum was indistinguishable from that of **14**.

Ac-Trp-L-3-MPt(Me)-Asp-Phe-NH$_2$ (24b). Methylation of **24a** (15.3 mg, 0.024 mmol) with dimethyl sulfate as described in the general procedure yielded 22 mg of crude **24b** as a yellow viscous oil. Purification of the crude by preparative RP-HPLC (25–40% B over 30 min) yielded 9.5 mg (61%) of pure **24b** as a white solid. Analytical RP-HPLC (20–45% B over 25 min) t_R = 15.6 min; MS (FAB) *m/e* 651(M + 1); ^{1}H NMR (DMSO-d_6) two conformations evident in spectrum in ratio of 1:3.6 δ 1.73 (s, 3 H, minor conformer), 1.75 (s, 3 H, major conformer), 1.80–1.95 (m, 1 H), 1.96 (s, 3 H, minor conformer), 2.09 (s, 3 H, major conformer), 2.20–2.32 (m, 1 H), 2.44–2.55 (m, 1 H), 2.60–2.75 (m, 1 H), 2.80–2.95 (m, 2 H), 3.00–3.15 (m, 2 H), 3.20–3.27 (m, 1 H), 3.40–3.60 (m, 1 H), 3.70–3.81 (m, 1H), 4.23 (d, J = 3.5 Hz, 1 H), 4.31–4.42 (m, 1 H), 4.43–4.53 (m, 1 H), 4.60–4.80 (m, 1 H), 6.92–7.10 (m, 2 H), 7.14–7.35 (m, 9 H), 7.51 (d, J = 6.6 Hz, 1 H, minor conformer), 7.53 (d, J = 7.7 Hz, 1 H, major conformer), 7.84 (d, J = 8.2 Hz, 1 H, major conformer), 8.05 (d, J = 8.1 Hz, 1 H, minor conformer), 8.20 (d, J = 7.9 Hz, 1 H, minor conformer), 8.30 (d, J = 8.2 Hz, 1 H, major conformer), 8.48 (d, J = 7.6 Hz, 1 H, major conformer), 8.56 (d, J = 8.1 Hz, 1 H, minor conformer), 10.85 (s, 1 H), 12.35 (br s, 1 H); [α]$_D$ −12.6° (c = 0.27, AcOH); amino acid analysis Asp (1) 1.15, Phe (1) 1.00, 3-MPt(Me) (1) ND, Trp (1) ND.

Ac-Trp-D-3-MPt(Me)-Asp-Phe-NH$_2$ (25b). As described for **24b**, methylation of **25a** (14.5 mg, 0.023 mmol) yielded 20 mg of crude **25b** as a yellow oil. Purification by preparative RP-HPLC (25–40% B over 30 min) yielded 10.7 mg of pure **25b** (71%) as a white solid: analytical RP-HPLC (20–45% B over 25 min) t_R = 17.8 min; MS (FAB) *m/e* 651 (M + 1); ^{1}H NMR (DMSO-d_6) δ 1.30–1.37 (m, 1 H), 1.87 (s, 3 H), 1.98 (s, 3 H), 2.00–2.33 (m, 1 H), 2.37 (dd, J = 9.2, 16.7 Hz, 1 H), 2.65 (dd, J = 4.4, 16.9 Hz, 1 H), 2.95–3.18 (m, 7 H), 4.01 (d, J = 4.8 Hz, 1 H), 4.33–4.46 (m, 2 H), 4.70–4.79 (m, 1 H), 6.97–7.35 (m, 11 H), 7.55 (d, J = 7.6 Hz, 1 H), 7.95 (d, J = 8.7 Hz, 1 H), 8.22 (d, J = 7.0 Hz, 1 H), 8.64 (d, J = 7.7 Hz, 1 H), 10.91 (s, 1 H), 12.40 (br s, 1 H); [α]$_D$ −0.48° (c = 0.42, AcOH); amino acid analysis Asp (1) 1.04, Phe (1) 1.00, 3-MPt(Me) (1) ND, Trp (1) ND.

Ac-Trp-L-3-MPt(Et)-Asp-Phe-NH$_2$ (24c). Alkylation of **24a** (18 mg, 0.028 mmol) with ethyl bromide as described in the general procedures yielded 25 mg of crude **24c** as a yellow oil. Purification by preparative RP-HPLC (25–40% B over 30 min) yielded 14.1 mg (74%) of pure **24c** as a white solid: analytical RP-HPLC (25–50% B over 25 min) t_R = 13.2 min; MS (FAB) *m/e* 665 (M + 1); ^{1}H NMR (DMSO-d_6) two conformations evident in spectrum in ratio of 1:3.1 δ 1.10 (t, J = 7.5 Hz, 3 H, minor conformer), 1.16 (t, J = 7.4 Hz, 3 H, major conformer), 1.72 (s, 3 H, minor conformer), 1.75 (s, 3 H, major conformer), 1.80–2.00 (m, 1 H), 2.22–2.38 (m, 1 H), 2.40–2.76 (m, 4 H), 2.80–2.96 (m, 2 H), 3.00–3.14 (m, 2 H), 3.20–3.30 (m, 1 H), 3.40–3.62 (m, 1 H), 3.70–3.82 (m, 1 H), 4.22 (br d, J = 3.2 Hz, 1 H), 4.30–4.53 (m, 2 H), 4.60–4.80 (m, 1 H), 6.92–7.11 (m, 2 H), 7.15–7.35 (m, 9 H), 7.49–7.56 (m, 1 H), 7.82 (d, J = 8.1 Hz, 1 H, major conformer), 8.02 (d, J = 8.2 Hz, 1 H, minor conformer), 8.21 (d, J = 7.8 Hz, 1 H, minor conformer), 8.28 (d, J = 8.1 Hz, 1 H, major conformer), 8.44 (d, J = 7.6 Hz, 1 H, major conformer), 8.53 (d, J = 8.1 Hz, 1 H, minor conformer), 10.85 (br s, 1 H, major conformer), 10.91 (br s, 1 H, minor conformer), 12.40 (br s, 1 H); [α]$_D$ −8.8° (c = 0.29, AcOH); amino acid analysis Asp (1) 0.94, Phe (1) 1.00, 3-MPt-(Et) (1) ND, Trp (1) ND.

Ac-Trp-D-3-MPt(Et)-Asp-Phe-NH$_2$ (25c). As described above for **24c**, ethylation of **25a** (15 mg, 0.024 mmol) with ethyl bromide and purification by RP-HPLC (25–40% B over 30 min) yielded 11.1 mg of pure **25c** as a white solid: analytical RP-HPLC (25–50% B over 25 min) t_R = 15.1 min; MS (FAB) *m/e* 665 (M + 1); ^{1}H NMR (DMSO-d_6) δ 1.11 (t, J = 7.4 Hz, 3 H), 1.25–1.38 (m, 1 H), 1.86 (s, 3 H), 2.05–2.11 (m, 1 H), 2.33–2.48 (m, 3 H), 2.65 (dd, J = 4.2, 16.9 Hz, 1 H), 2.96–3.15 (m, 6 H), 3.18–3.26 (m, 1 H), 3.47–3.57 (m, 1 H), 4.00 (d, J = 4.6 Hz, 1 H), 4.32–4.46 (m, 2 H), 4.70–4.79 (m, 1 H), 6.97–7.35 (m, 11 H), 7.55 (d, J = 7.7 Hz, 1 H), 7.93 (d, J = 8.4 Hz, 1 H), 8.21 (d, J = 6.7 Hz, 1 H), 8.60 (d, J = 7.6 Hz, 1 H), 10.89 (s,

1 H), 12.34 (s, 1 H); [α]$_D$ −0.91° (c = 0.11, AcOH); amino acid analysis Asp (1) 0.93, Phe (1) 1.00, 3-MPt(Et) (1) ND, Trp (1) ND.

Ac-Trp-L-3-MPt(Pr)-Asp-Phe-NH₂ (24d). As described in the general procedures, alkylation of **24a** (16.1 mg, 0.025 mmol) with propyl bromide and subsequent purification by RP-HPLC (27−42% B over 30 min) yielded 9.6 mg (57%) of pure **24d** as a white solid: analytical RP-HPLC (25−50% B over 25 min) t_R = 16.6 min; MS (FAB) m/e 679 (M + 1); ¹H NMR (DMSO-d₆) two conformations evident in spectrum in ratio of 1:4.7 δ 0.79 (t, J = 7.3 Hz, 3 H, minor conformer), 0.85 (t, J = 7.4 Hz, 3 H, major conformer), 1.38−1.54 (m, 2 H)1.66 (s, 3 H, minor conformer), 1.66 (s, 3 H, major conformer), 1.72−1.88 (m, 1 H), 2.16−2.40 (m, 2 H), 2.51−2.74 (m, 5 H), 2.76−2.92 (m, 2 H), 2.94−3.08 (m, 2 H), 3.50−3.85 9m, 2 H), 4.14 (d, J = 2.9 Hz, 1 H, major conformer), 4.17−4.23 (m, 1 H, minor conformer), 4.22−4.35 (m, 1 H), 4.35−4.48 (m, 1 H), 4.52−4.77 (m, 1 H), 6.85−7.01 (m, 2 H), 7.08−7.30 (m, 9 H), 7.43−7.56 (m, 1 H), 7.82−7.90 (m, 1 H), 8.00 (d, J = 8.4 Hz, 1 H, minor conformer), 8.18 (d, J = 7.9 Hz, 1 H, minor conformer), 8.27 (d, J = 8.3 Hz, 1 H, major conformer), 8.47−8.53 (m, 1 H), 10.89 (s, 1 H), 12.40 (br s, 1 H); [α]$_D$ −9.5° (c = 0.42, AcOH); amino acid analysis: Asp (1) 1.13, Phe (1) 1.00, 3-MPt(Pr) (1) ND, Trp (1) ND.

Ac-Trp-D-3-MPt(Pr)-Asp-Phe-NH₂ (25d). As described in the general procedures, alkylation of **25a** (16.9 mg, 0.027 mmol) with propyl bromide and subsequent purification by RP-HPLC (27−42% B over 30 min) yielded 6.8 mg (39%) of pure **25d** as a white solid: analytical RP-HPLC (25−50% B over 25 min) t_R = 18.5 min; MS (FAB) m/e 679 (M + 1); ¹H NMR (DMSO-d₆) δ 0.88 (t, J = 7.4 Hz, 3 H), 1.20−1.68 (m, 4 H), 1.84 (s, 3 H), 2.00−2.12 (m, 2 H), 2.38−2.45 (m, 2 H), 2.56−2.68 (m, 2 H), 2.87−3.18 (m, 6 H), 3.98 (d, J = 4.4 Hz, 1 H), 4.30−4.43 (m, 2 H), 4.65−4.74 (m, 1 H), 6.92−7.35 (m, 11 H), 7.52 (d, J = 7.8 Hz, 1 H), 7.95 (d, J = 8.8 Hz, 1 H), 8.28 (d, J = 6.6 Hz, 1 H), 8.65 (d, J = 7.1 Hz, 1 H), 11.01 (s, 1 H); [α]$_D$ +13.8° (c = 0.14, AcOH); amino acid analysis Asp (1) 1.05, Phe (1) 1.00, 3-MPt(Pr) (1) ND, Trp (1) ND.

Ac-Trp-3-MPc-Asp-Phe-NH₂ (26a and 27a). Boc-Asp-(cHx)-Phe-MBHA (2.0 g, 0.98 mmol) was elongated by successive couplings with D,L-**23** and Boc-Trp and acetylated as described in the general procedures to yield 2.30 g of peptide-resin. HF cleavage of 1.15 g of this peptide resin as described in the general procedures yielded 256 mg of crude peptide (82%) as a mixture of **26a** and **27a**. Preparative RP-HPLC of 202 mg of the crude (25−40% B over 30 min) resulted in separation of the diastereomers.

Ac-Trp-L-3-MPc-Asp-Phe-NH₂ (26a): 39 mg of a white solid (18% isolated yield); analytical RP-HPLC (25−50% B over 25 min) t_R = 11.0 min; MS (FAB) m/e 637 (M + 1); ¹H NMR (DMSO-d₆) two conformations evident in spectrum in ratio of 1:3.3 δ 1.74 (s, 3 H, major conformer), 1.77 (s, 3 H, minor conformer), 1.90−2.20 (m, 1 H), 2.20−2.30 (m, 1 H), 2.45 (d, J = 8.3 Hz, 1 H), 2.56−2.75 (m, 2 H), 3.35−3.60 (m, 3 H), 4.00 (d, J = 7.5 Hz, 1 H, minor conformer), 4.31−4.39 (m, 1 H), 4.45 (d, J = 7.8 Hz, 1 H, major conformer), 4.48−4.54 (m, 1 H), 4.66−4.74 (m, 1 H), 6.90−7.35 (m, 11 H), 7.45 (d, J = 7.8 Hz, 1 H, minor conformer), 7.50 (d, J = 7.7 Hz, 1 H, major conformer), 7.82 (d, J = 8.2 Hz, 1 H, major conformer), 7.97 (d, J = 8.7 Hz, 1 H, minor conformer), 8.23−8.29 (m, 1 H), 8.45 (d, J = 7.1 Hz, 1 H, major conformer), 8.52 (d, J = 6.3 Hz, 1 H, minor conformer), 10.87 (s, 1 H, major conformer), 10.92 (s, 1 H, minor conformer), 12.50 (br s, 1 H); [α]$_D$ −7.69° (c = 0.13, AcOH); amino acid analysis Asp (1) 0.98, Phe (1) 1.00, 3-MPc (1) ND, Trp (1) ND. Raney nickel reduction of **26a** (13 mg) using the procedure described in the general methods resulted in a new component by RP-HPLC at t_R = 8.8 min (25−50% B over 25 min). Co-injection with a pure sample of **13** confirmed the stereochemistry of the L-Pro³¹ residue. Additionally, the ¹H NMR spectrum of the crude material was indistinguishable from that of **13**.

Ac-Trp-D-3-MPc-Asp-Phe-NH₂ (27a): 32 mg of a white solid (16% isolated yield); analytical RP-HPLC (25−50% B over 25 min) t_R = 12.8 min; MS (FAB) m/e 637 (M + 1); ¹H NMR (DMSO-d₆) δ 1.85 (s, 3 H), 1.85−2.0 (m, 2 H), 2.24 (d, J = 8.7 Hz, 1 H), 2.39 (dd, J = 5.7, 17.0 Hz, 1 H), 2.66 (dd, J = 4.6,

16.8 Hz, 1 H), 2.85−3.18 (m, 6 H), 3.65−3.75 (m, 1 H), 4.28 (d, J = 8.0 Hz, 1 H), 4.34−4.42 (m, 1 H), 4.46−4.53 (m, 1 H), 4.75−4.83 (m, 1 H), 6.95−7.38 (m, 11 H), 7.54 (d, J = 7.7 Hz, 1 H), 7.82 (d, J = 8.3 Hz, 1 H), 8.16 (d, J = 7.7 Hz, 1 H), 8.71 (d, J = 7.1 Hz, 1 H), 10.89 (s, 1 H), 12.40 (br s, 1 H); [α]$_D$ +7.14° (c = 0.14, AcOH); amino acid analysis Asp (1) 1.02, 3-MPc ND, Phe (1) 1.00, Trp (1) ND. Raney nickel reduction of **27a** (10 mg) using the procedure described in the general methods resulted in a new component by RP-HPLC at t_R = 10.9 min (25−50% B over 25 min). Co-injection with a pure sample of **14** confirmed the stereochemistry of the L-Pro³¹ residue. Additionally, the ¹H NMR spectrum was indistinguishable from that of **14**.

Ac-Trp-L-3-MPc(Me)-Asp-Phe-NH₂ (26b). As described in the general procedures, methylation of **26a** (10.2 mg, 0.016 mmol) with dimethyl sulfate and subsequent RP-HPLC purification yielded 8.3 mg of pure **26b** (80% isolated yield) as a white solid: analytical RP-HPLC (25−50% B over 25 min) t_R = 10.5 min; MS (FAB) m/e 651 (M + 1); ¹H NMR (DMSO-d₆) two conformations evident in spectrum in ratio of 1:4.2 δ 1.52−1.66 (m, 1 H), 1.68 (s, 3 H, minor conformer), 1.72 (s, 3 H, major conformer), 1.81 (s, 3 H, minor conformer), 1.91−2.00 (m, 1 H, minor conformer), 2.06 (s, 3 H, major conformer), 2.10−2.22 (m, 1 H, major conformer), 2.53−2.72 (m, 2 H), 2.80−3.13 (m, 6 H), 3.55−3.62 (m, 1 H), 3.84 (d, J = 6.5 Hz, 1 H, minor conformer), 4.25−4.49 (m, 2 H), 4.54 (d, J = 8.0 Hz, 1 H), 4.64−4.74 (m, 1 H), 6.92−7.37 (m, 11 H), 7.41 (d, J = 7.8 Hz, 1 H, minor conformer), 7.50 (d, J = 7.7 Hz, 1 H, major conformer), 7.73 (d, J = 8.3 Hz, 1 H, major conformer), 7.89 (d, J = 7.9 Hz, 1 H, minor conformer), 8.29 (d, J = 8.2 Hz, 1 H, major conformer), 8.35 (d, J = 7.6 Hz, 1 H, minor conformer), 8.47 (d, J = 7.3 Hz, 1 H, minor conformer), 8.60 (d, J = 7.0 Hz, 1 H, major conformer), 10.85 (s, 1 H, major conformer), 11.00 (s, 1 H, minor conformer), 12.40 (br s, 1 H); [α]$_D$ +19.7° (c = 0.37, AcOH); amino acid analysis Asp (1) 0.95, Phe (1) 1.00, 3-MPc(Me) (1) ND, Trp (1) ND.

Ac-Trp-D-3-MPc(Me)-Asp-Phe-NH₂ (27b). As described for **24b**, methylation of **27a** (10.2 mg, 0.016 mmol) with dimethyl sulfate yielded 16.6 mg of crude **27b** as a yellow solid. Purification of the crude by RP-HPLC (25−40% b over 30 min) yielded 8.2 mg of pure **27b** (79%) as a white solid: analytical RP-HPLC (25−50% B over 25 min) t_R = 11.8 min; MS (FAB) m/e 651 (M + 1); ¹H NMR (DMSO-d₆) δ 1.70−1.93 (m, 5 H), 2.02 (s, 3 H), 2.35 (dd, J = 8.6, 16.6 Hz, 1 H), 2.66 (dd, J = 5.0, 16.5 Hz, 1 H), 2.72−3.19 (m, 6 H), 3.65−3.75 (m, 1 H), 4.30−4.46 (m, 3 H), 4.72−4.82 (m, 1 H), 6.97−7.36 (m, 11 H), 7.53 (d, J = 7.7 Hz, 1 H), 7.78 (d, J = 8.7 Hz, 1 H), 8.17 (d, J = 7.7 Hz, 1 H), 8.79 (d, J = 7.3 Hz, 1 H), 10.90 (s, 1 H), 12.35 (br s, 1 H); [α]$_D$ −0.94° (c = 0.32, AcOH); amino acid analysis Asp (1) 0.99, Phe (1) 1.00, 3-MPc(Me) (1) ND, Trp (1) ND.

Ac-Trp-L-3-MPc(Et)-Asp-Phe-NH₂ (26c). Alkylation of **26a** (10 mg, 0.016 mmol) with ethyl bromide as described for **24c** and purification by preparative RP-HPLC yielded 6.0 mg of pure **26c** (57%) as a white solid: analytical RP-HPLC (25−50% B over 25 min) t_R = 12.1 min; MS (FAB) m/e 665 (M + 1); ¹H NMR (DMSO-d₆) two conformations evident in spectrum in ratio of 1:2.3 δ 0.97 (t, J = 7.4 Hz, 3 H, minor conformer), 1.14 (t, J = 7.4 Hz, 3 H, major conformer), 1.57−1.68 (m, 1 H, minor conformer), 1.75 (s, 3 H, major conformer), 1.81 (s, 3 H, minor conformer), 1.92−2.05 (m, 1 H, major conformer), 2.10−2.25 (m, 1 H), 2.54−3.15 (m, 8 H), 3.48−3.60 (m, 3 H), 3.83 (d, J = 7.4 Hz, 1 H, minor conformer), 4.24−4.47 (m, 2 H), 4.56 (d, J = 8.0 Hz, 1 H, major conformer), 4.60−4.76 (m, 1 H), 6.92−7.35 (m, 12 H), 7.42 (d, J = 7.6 Hz, 1 H, minor conformer), 7.50 (d, J = 7.7 Hz, 1 H, major conformer), 7.70 (d, J = 8.4 Hz, 1 H, major conformer), 7.88 (d, J = 8.1 Hz, 1 H, minor conformer), 8.27 (d, J = 8.3 Hz, 1 H, major conformer), 8.30 (d, J = 7.7 Hz, 1 H, minor conformer), 8.42 (d, J = 6.8 Hz, 1 H, minor conformer), 8.57 (d, J = 6.6 Hz, 1 H, major conformer), 10.84 (s, 1 H, major conformer), 10.99 (s, 1 H, minor conformer), 12.35 (br s, 1 H); [α]$_D$ +18.6° (c = 0.14, AcOH); amino acid analysis Asp (1) 0.99, Phe (1) 1.00, 3-MPc(Et) (1) ND, Trp (1) ND.

Ac-Trp-D-3-MPc(Et)-Asp-Phe-NH₂ (27c). Alkylation of **27a** (10.2 mg, 0.016 mmol) with ethyl bromide as described for **24c** and purification by preparative RP-HPLC (25−40% B

over 30 min) yielded 5.4 mg of pure **27c** (51%) as a white solid: analytical RP-HPLC (25–50% B over 25 min) t_R = 13.5 min; MS (FAB) *m/e* 665 (M + 1); ¹H NMR (DMSO-d_6) δ 1.12 (t, *J* = 7.4 Hz, 3 H), 2.25–2.49 (m, 2 H), 2.60–3.16 (m, 8 H), 3.65–3.75 (m, 1 H), 4.30–4.45 (m, 2 H), 4.35 (d, *J* = 7.8 Hz, 1 H), 4.72–4.81 (m, 1 H), 6.97–7.36 (m, 11 H), 7.53 (d, *J* = 7.7 Hz, 1 H), 7.78 (d, *J* = 8.8 Hz, 1 H), 8.15 (d, *J* = 7.3 Hz, 1 H), 8.73 (d, *J* = 7.6 Hz, 1 H), 10.89 (s, 1 H), 12.32 (br s, 1 H); [α]$_D$ −10.5° (*c* = 0.22, AcOH); amino acid analysis Asp (1) 0.97, Phe (1) 1.00, 3-MPc(Et) (1) ND, Trp (1) ND.

Ac-Trp-L-3-MPc(Pr)-Asp-Phe-NH₂ (26d). Alkylation of **26a** (13 mg, 0.020 mmol) with propyl bromide as described for **24d** and purification by preparative RP-HPLC yielded 9.8 mg of pure **26d** (72%) as a white solid: analytical RP-HPLC (25–50% B over 25 min) t_R = 15.4 min; MS (FAB) *m/e* 679 (M + 1); ¹H NMR (DMSO-d_6) two conformations evident in spectrum in ratio of 1:2.4 δ 0.85 (t, *J* = 7.3 Hz, 3 H, minor conformer), 0.89 (t, *J* = 7.3 Hz, 3 H, major conformer), 1.23–1.67 (m, 2 H), 1.75 (s, 3 H, major conformer), 1.81 (s, 3 H, minor conformer), 1.85–2.20 (m, 2 H), 2.39–2.72 (m, 4 H), 2.85–3.15 (m, 2 H), 3.40–3.60 (m, 3 H), 3.84 (d, *J* = 7.6 Hz, 1 H, minor conformer), 4.24–4.46 (m, 2 H), 4.56 (d, *J* = 8.1 Hz, 1 H, major conformer), 4.60–4.77 (m, 1 H), 6.93–7.35 (m, 11 H), 7.42 (d, *J* = 8.1 Hz, 1 H, minor conformer), 7.50 (d, *J* = 7.7 Hz, 1 H, major conformer), 7.70 (d, *J* = 8.6 Hz, 1 H, major conformer), 7.88 (d, *J* = 8.1 Hz, 1 H, minor conformer), 8.20–8.32 (m, 1 H), 8.41 (d, *J* = 6.8 Hz, 1 H, minor conformer), 8.57 (d, *J* = 7.0 Hz, 1 H, major conformer), 10.84 (s, 1 H, major conformer), 10.97 (s, 1 H, minor conformer), 12.38 (br s, 1 H); [α]$_D$ +13.6° (*c* = 0.22, AcOH); amino acid analysis Asp (1) 1.12, Phe (1) 1.00, 3-MPc(Pr) (1) ND, Trp (1) ND.

Ac-Trp-D-3-MPc(Pr)-Asp-Phe-NH₂ (27d). Alkylation of **27a** (13.1 mg, 0.021 mmol) with propyl bromide as described in the general procedures and purification by preparative RP-HPLC (25–40% B over 30 min) yielded 7.5 mg of pure **27d** (54%) as a white solid: analytical RP-HPLC (25–50% B over 25 min) t_R = 17.2 min; MS (FAB) *m/e* 679 (M + 1); ¹H NMR (DMSO-d_6) δ 0.91 (t, *J* = 7.1 Hz, 3 H), 1.40–1.52 (m, 2 H), 1.72–1.93 (m, 5 H), 2.33–2.47 (m, 2 H), 2.55–2.65 (m, 1 H), 2.70–2.85 (m, 2 H), 2.92–3.20 (m, 5 H), 3.63–3.73 (m, 1 H), 4.34 (d, *J* = 7.6 Hz, 1 H), 4.35–4.48 (m, 2 H), 4.70–4.81 (m, 1 H), 6.97–7.10 (m, 4 H), 7.19–7.36 (m, 7 H), 7.53 (d, *J* = 7.7 Hz, 1 H), 7.80 (d, *J* = 8.3 Hz, 1 H), 8.17 (d, *J* = 7.3 Hz, 1 H), 8.75 (d, *J* = 7.3 Hz, 1 H), 8.75, *J* = 6.2 Hz, 1 H), 10.90 (s, 1 H), 12.30 (br s, 1 H); [α]$_D$ −20.0° (*c* = 0.16, AcOH); amino acid analysis Asp (1) 1.04, Phe (1) 1.00, 3–MPc(Pr) (1) ND, Trp (1) ND.

B. Radioligand Binding Assays. General. Male Wistar rats (180–200 g) were obtained from Effa-Credo (Saint Germain l'Arbesle, France). Hepes was obtained from Boehringer-Mannheim; purified collagenase from Serva (Garden City Park, NY); soybean trypsin inhibitor from Sigma (St. Louis, MO); Eagle's basal amino acid medium (100 times concentrated) was from GIBCO (Grand Island, NY); essential vitamin mixture 100 times concentrated) was from Microbiological Associates (Bethesda, MD); bovine plasma albumin (fraction V) was from Miles Laboratories (Elkhart, IN); ¹²⁵I-labeled *N*-succinimidyl-3-(4–hydroxyphenyl)propionyl-CCK₈ ([¹²⁵I]BH-CCK₈) was from Amersham Corp. (Buckinghamshire, UK). Unless otherwise stated, the standard incubation solutions contained 24.5 mM Hepes (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM NaH₂PO₄, 5 mM sodium pyruvate, 5 mM sodium fumarate, 5 mM sodium glutamate, 2 mM glutamine, 11.5 mM glucose, 0.5 mM CaCl₂, 1 mM MgCl₂, 0.5 mg/mL bacitracin, 0.2% (w/v) albumin, 0.03% (w/v) soybean trypsin inhibitor, 1% (w/v) essential amino acid mixture, and 1% (v/v) essential vitamin mixture. The incubation solution was equilibrated with 95% O₂/5% CO₂ in the gas phase. Dispersed acini from rat pancreas were prepared according to the previously described modifications[30] of the method of Peikin *et al.*[31]

Binding of [¹²⁵I]BH-CCK₈ to rat pancreatic acini was performed as previously described.[32] Briefly, samples (0.5 mL containing ≈ 1 mg/mL protein) were incubated with the appropriate peptide concentrations for 30 min at 37 °C in the presence of 10 pM of [¹²⁵I]BH-CCK₈ plus various concentrations of Boc-CCK₇[Nle²⁸,Nle³¹]. After centrifugation at 10000*g* for

10 min and washing, the radioactivity associated with the acinar pellet was measured. Values are expressed as the percentage of the value obtained with labeled CCK₈ alone. The specific activities of the ligand used in our experiments was 2000 Ci/mmol. Acini from the three rat pancreata were suspended in 100 mL of standard incubation solution. Specific binding in the absence of any unlabeled CCK-peptide was 13 ± 3.5% of the total radioactivity present in the sample. Nonspecific binding was determined in the presence of 1 μM Boc-CCK₇[Nle²⁸,³¹] and was always less than 15% of the total binding. Results are the means of three independent experiments in duplicate and the standard errors were within ±15%.

Binding of [¹²⁵I]-BH-CCK₈ to JURKAT cells was performed according to Lignon *et al.*[33,34] Cells were harvested by centrifugation at 1500*g* for 5 min and washing twice with the binding incubation medium consisting of 24.5 nM HEPES (pH 7.4), 98 mM NaCl, 6 mM KCl, 2.5 mM NaHPO₄, 5 mM sodium pyruvate, 5 mM sodium fumarate, 5 mM sodium glutamate, 2 mM glutamine, 11.5 mM glucose, 1.5 mM CaCl₂, 1 mM MgCl₂, 0.5 mg/mL bacitracin, 0.01% (w/v) soybean trypsin inhibitor, 1% (v/v) essential amino acid mixture, and 1% (v/v) vitamin mixture. Binding studies were carried out at 37°C for 60 min, using 10 pM [¹²⁵I]BH-CCK₈, plus appropriate drug concentrations, in a final volume of 500 mL containing 4 × 10⁶ cells/mL. The reaction was stopped by the addition of fresh standard medium plus 2% BSA and centrifugation for 10 min at 10000*g*. Nonspecific binding was measured in the presence of 1 μM CCK₈ and was always less than 10% of the total binding. Results are the means of three separate experiments in duplicate and the standard errors were within ±15%.

C. Molecular Modeling. Energy calculations were performed for 11 compounds. They were Ac-CCK₄ and eight of its analogs with substitutions of the Met³¹ residue, namely Ac-[NMeNle³¹]-CCK₄, Ac-[L-3-*trans*-PP³¹]-CCK₄, the four stereoisomers of Ac-[L/D-4-MPt/c(Me)³¹]-CCK₄ (compounds **1b, 2b, 10,** and **11**), and the two diastereomers Ac-[L-3-MPt/c(Me)³¹]-CCK4 (**25b** and **27b**). The ECEPP potential field[35,36] was used for conformational energy calculations. Dihedral angles were the only variables in the process of energy minimization, since rigid valence geometry with a planar *trans*-peptide bonds was assumed (both *trans*- and *cis*-peptide bonds were examined for *N*-Me-amino acid residues; in these cases the ω_{12} dihedral angle also was allowed to rotate). The valence geometry and atomic charges at the methylmercaptoprolines and *trans*-3-*n*-propylproline were calculated by the use of the SYBYL program. Aliphatic and aromatic hydrogens were generally included in united atomic centers of CH$_n$ type; only H$^\alpha$ atoms and H$^\delta$ atoms of prolines were described explicitly. The calculation scheme for all compounds considered all possible combinations of local minima for the peptide backbone for each amino acid residue. These minima were of *E*, *C*, and *A* types (according to the notation in ref 37) for Ac-Trp³⁰ residue, *trans*- and *cis*- for the ω_{12} angle, of *F*, *C*, and *A* for any of L-prolines³¹ (*F**, *C**, and *A** for D-prolines³¹), of *E*, *F*, *C*, *A*, and *A** types for the Asp³², and of *E*, *F*, and *A** types for the Phe³³-NH₂ residues. Totally, 270 backbone conformers were considered for each compound, except Ac-[NMeNle³¹]-CCK₄, where *E*, *F*, *C*, *A*, and *A** types of local minima were considered for the NMeNle³¹ residue, resulting in 450 backbone conformers considered for this analog. The dihedral angle values of side chain groups (χ_i's) and of the terminal groups of the backbone (ϕ_1 and ψ_4) were optimized before energy minimization to achieve their most favorable spatial arrangements according to an algorithm described elsewhere.[38] After that, energy minimization involved all dihedral angles.

Geometric comparison of a pair of conformers belonging to different molecules included an assessment of the best fit[39] of the spatial arrangement of the all C$^\alpha$ and C$^\beta$ atoms (eight atomic centers). Two conformers were regarded as geometrically similar when the corresponding rms value was less than 1.0 Å.

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