methylene chloride/petrol as eluent to give **22**: 125 mg (44%); mp 64 °C. Anal. ($C_{11}H_8F_3Cl_3O$) C, H.

***trans*-5-(4,4-Dichlorophenyl)-3-hydroxy-3-(trifluoromethyl)pent-4-enamide (23).** To a solution of bis(trimethylsilyl)acetamide (0.92 mL; 3.7 mmol) in dry THF (50 mL) maintained at −78 °C was added *n*-butyllithium (2.3 mL of 1.6 M solution; 3.7 mmol), the mixture stirred for 30 min, a solution of *trans*-4-(3′,4-dichlorophenyl)-1,1,1-trifluorobut-3-en-2-one (**25**) (1 g,; 3.7 mmol) in dry THF (25 mL) added, and after stirring for an additional 30 min at −78 °C the reaction quenched with ammonium chloride solution, warmed to room temperature, and stirred for 17 h. Workup with EtOAc gave an oil that was purified by chromatography with a 1:1 mixture of EtOAc/petrol as eluent to give **23**: 0.4 g (33%); mp 126 °C. Anal. ($C_{12}H_{10}F_3Cl_2NO_2$) C, H, N.

***trans*-5-(3′,4′-Dichlorophenyl)-3-hydroxy-3-(trifluoromethyl)pent-4-en-2-one (24).** *n*-Butyllithium (3.5 mL of 1.6 M; 5.6 mmol) was added dropwise to a THF (50 mL) solution of (trimethylsilyl)acetylene (0.76 mL; 5.6 mmol) maintained at −78 °C, the mixture stirred for 1 h, a solution of *trans*-4-(3′,4′-dichlorophenyl)-1,1,1-trifluorobut-3-en-2-one (**25**) in THF (25 mL) added dropwise, and the reaction stirred for 20 min at −78 °C. After the mixture was quenched with saturated ammonium chloride solution and worked up with EtOAc, the resulting oil was dissolved in THF (15 mL), tetra-*N*-butylammonium fluoride (1.19 g; 5.6 mmol) added, and the mixture stirred at ambient temperature for 1 h, diluted with water, and worked up with EtOAc. The resulting oil was purified by chromatography with 40% methylene chloride/petrol as eluent to give *trans*-1-(3,4-dichlorophenyl)-3-(trifluoromethyl)pent-1-en-4-yn-3-ol (**27**) as an oil, 0.8 g (73%). Anal. ($C_{12}H_7F_3Cl_2O$) C, H, N. **27** (0.8 g; 2.7 mmol) dissolved in methanol (5 mL) was added to a mixture of mercuric oxide (0.6 g; 2.7 mmol) in 4% H_2SO_4 (50 mL) maintained at 60 °C and the reaction stirred at this temperature for 30 min, cooled to ambient temperature, and worked up with EtOAc. The resulting oil was purified by chromatography with 60% methylene chloride/petrol to give **24**, 180 mg (21%), as an oil. Anal. ($C_{12}H_9F_3Cl_2O_2$) C, H.

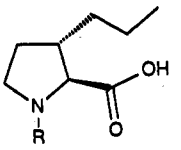
2-[1′-Hydroxy-1′-(trifluoromethyl)ethyl]-3-methylindole (19). *n*-Butyllithium (15 mL of 1.6 M solution; 24 mmol) was added dropwise to a solution of 1-(phenylsulfonyl)-3-methylindole (5.4 g; 20 mmol) (prepared from 3-methylindole by standard procedure) in THF at −78 °C, the mixture stirred for 0.5 h, trifluoroacetone (2 mL; 22 mmol) added, and the reaction stirred for an additional 1 h. Aqueous ammonium chloride was added followed by standard workup with Et_2O which gave a semisolid that was purified by chromatography with 30% methylene chloride in petrol as eluent to give **20**: 2.6 g (34%); mp 91–2 °C. Anal. ($C_{18}H_{16}F_3NO_3S$) C, H, N.

A solution of **20** (1.3 g; 3.3 mmol) in 1,4-dioxane (30 mL) was treated with KOH (22 mL; 0.22 mol) and stirred for 0.5 h at ambient temperature. It was diluted with water and extracted with Et_2O. The organic layer was washed with water, dried Na_2SO_4, and evaporated in vacuo. The residue was purified by chromatography with 70% methylene chloride/petrol as eluent to give **19**: 0.3 g (38%); mp. Anal. ($C_{12}H_{12}F_3NO$) C, H, N.

Communications to the Editor

trans-3-*n*-Propyl-L-proline Is a Highly Favorable, Conformationally Restricted Replacement for Methionine in the C-Terminal Tetrapeptide of Cholecystokinin. Stereoselective Synthesis of 3-Allyl- and 3-*n*-Propyl-L-proline Derivatives from 4-Hydroxy-L-proline

The introduction of conformational constraints into peptides has been an important approach toward studying bioactive conformations of peptides.[1,2] There are, for example, numerous cases in which conformational information has been gained by replacement of a native peptide residue with the constrained amino acid proline.[3,4] However, when such a substitution leads to a reduction in biological activity, it is not clear whether this result is more properly attributable to conformational and steric considerations or to loss of important interactions associated with the side chain of the original amino acid residue. Therefore, we have undertaken the synthesis of proline derivatives that incorporate potentially important amino acid side-chain functionality, with the expectation that replacement of appropriate residues in biologically active peptides with these analogues could lead to important conformational information and offer the potential to discover peptide analogues with improved selectivity, stability, or bioavailability. For example, *trans*-3-*n*-propyl-L-proline (3PP, 1) can be viewed as a constrained analogue of norleucine which has a two-carbon bridge from the β-carbon to the α-nitrogen. Here we wish to report the stereoselective synthesis of 1 from *trans*-4-hydroxy-L-proline and the relative effects of replacing the methionine residue in Boc-CCK$_4$ (Boc-Trp-Met-Asp-Phe-NH_2)[5] with each of alanine, norleucine, proline, and 3PP. The results indicate that replacement of Met with 3PP, which possesses conformational rigidity together with an appropriate side-chain moiety, gives a highly potent analogue that is significantly more active than either of the corresponding Nle or Pro replacement analogues.



1 R = H

2 R = Boc

We wished to prepare the N-protected derivative **2** of 3PP in a way that would allow unequivocal assignment of absolute stereochemistry at the α-position. This was successfully accomplished with *N*-Boc-4-oxo-L-proline methyl ester (**3**) as the starting material (readily prepared from 4-hydroxy-L-proline[6]) by the route shown in Scheme I. Formation of the enamine from **3** and pyrrolidine was conducted according to the procedure of Taguchi and Westheimer (room temperature, 20 h).[7,8] During allylation

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(2) Freidinger, R. M. *J. Org. Chem.* **1985**, *50*, 3631.
(3) Marshall, G. R. In *Chemical Recognition in Biological Systems*; Creighton, A. M., Turner, S., Eds.; Chemical Society: London, 1982; p 278.
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(5) Abbreviations used are as follows: Boc, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; 4-DMAP, 4-(*N*,*N*-dimethylamino)-pyridine; AIBN, azobisisobutyronitrile; EDAC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBt, 1-hydroxybenzotriazole.
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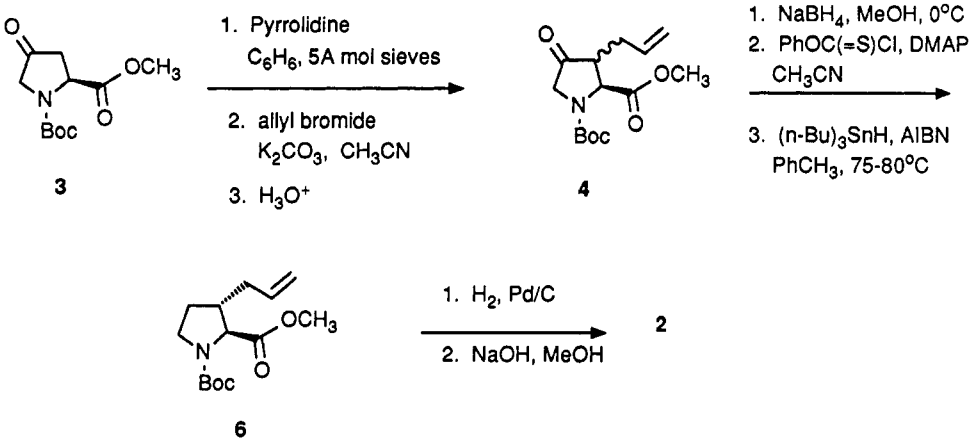
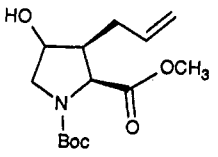
Scheme I



3 4 6 2

of the enamine with allyl bromide, it was necessary to quench the reaction before consumption of starting material was complete (room temperature, 21–24 h) to avoid overformation of diallylated products. Thus, the desired monoallylated product **4** was obtained in 44% yield (2:1 mixture of trans:cis isomers),[10] together with 11% diallylated product and 22% recovered starting material. Among a number of methods examined for reduction of the ketone to methylene, a Barton-type deoxygenation procedure via the secondary alcohol was the most promising. Thus, the ketone was reduced to a mixture of alcohols, consisting predominantly of a single 2,3-trans isomer (C-4 stereochemistry unknown) and a single 2,3-cis isomer (**5**) (C-4 stereochemistry unknown) in a ca. 4:1 ratio.



5

This product ratio is consistent with the occurrence of partial epimerization at the 3-position under the reduction conditions to afford a larger proportion of product with the more stable 2,3-trans stereochemistry. Application of the deoxygenation sequence of Robins et al.[11] resulted in the production of a separable 9:1 mixture of trans isomer **6** and the corresponding cis isomer in a combined 40% overall yield from **4**, together with 17% of unconsumed **5**, which reacted sluggishly with the thionochloroformate.[12]

Table I. Binding Affinities of CCK Tetrapeptide Analogues at Guinea Pig Cortical CCK-B Receptors and Pancreatic CCK-A Receptors

Boc-Trp-X-Asp-Phe-NH_2		IC_{50},[a] nM	
compd	X	cortex	pancreas
Boc-CCK_4	Met	25 ± 4.5 (6)	1800 ± 630 (5)
7	Ala	3840 ± 592 (3)	10300 ± 440 (3)
8	Nle	65 ± 14 (8)	4000 ± 810 (6)
9	Pro	750 ± 78 (4)	2100 ± 330 (4)
10	3PP	1.9 ± 0.43 (4)	2700 ± 620 (5)

[a] IC_{50} values were determined as described.[16,17] Values represent the mean ± SE. The number of determinations is indicated in parentheses. Each determination was conducted in duplicate with less than 10% sample variability.

Thus, the lower reactivity of the 2,3-cis isomer **5** in this sequence led to further enhancement of the proportion of the desired trans isomer in the final product mixture. Saturation of the olefin, followed by saponification of the methyl ester, completed the preparation of **2**[13] in straightforward fashion (70%, two steps). Full synthetic details for this sequence are provided in the supplementary material. The chiral purity of **2** was assessed by condensation (EDAC, HOBt, CH_2Cl_2) with both (*R,S*)-(±)- and (*S*)-(−)-α-methylbenzylamine, followed by examination of the products by HPLC (Vydac C_{18}, CH_3CN/0.1% aqueous CF_3CO_2H, gradient elution over 15 min from 40% to 70% CH_3CN). Less than 2% of undesired isomer was observed in the product derived from **2** and the pure *S*-amine. The route described above offers the potential for preparation of other 3-substituted proline derivatives via elaboration of the 3-allyl substituent. In related work, procedures suitable for large-scale preparation of *trans*-3-*n*-propylproline and *trans*-3-phenylproline via achiral synthesis and resolution of the racemates have been developed in these laboratories.[14,15]

(8) That enamine formation favored the Δ-3,4 isomer was suggested by the studies of Friary et al.[9] who had converted the enamine from morpholine and 1,2-bis(ethoxycarbonyl)-4-oxopyrrolidine to the corresponding Δ-3,4-dehydroproline derivative via hydroboration. NMR studies conducted during the present work on the enamine from **3** and pyrrolidine confirmed an essentially complete preference for formation of the Δ-3,4 isomer.

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(10) For all intermediates described, assignments of the relative C2–C3 stereochemistry is based on $J_{2,3}$ values from ^1H NMR spectra, for which the larger values (8–9 Hz) are attributed to the cis isomer and the smaller values (2–6 Hz) are attributed to the trans isomer: Mauger, A. B.; Irrevere, F.; Witkop, B. *J. Am. Chem. Soc.* **1966**, *88*, 2019. Assignment of trans stereochemistry to **2** was confirmed by the observation that the precursor methyl ester was selectively saponified to the acid (NaOH, MeOH, room temperature) in the presence of the cis methyl ester.

(11) Robins, M. J.; Wilson, J. S.; Hansske, F. *J. Am. Chem. Soc.* **1983**, *105*, 4059.

(12) Interestingly, a similar deoxygenation sequence carried out on *N*-Cbz-3-*n*-propyl-4-hydroxyproline methyl ester proceeded without a marked difference in reaction rate among the stereoisomers. Subsequent to the development of this route, a related method for dehydration was applied to Boc-4-oxoproline methyl ester (ref 6).

(13) **2**: mp 88–90 °C; $[\alpha]^{25}_D$ = −40.6° (*c* 1, $CHCl_3$). NMR showed conformational isomers in $CDCl_3$ at room temperature: Boc, δ 1.42 and 1.47; α-H, δ 3.85 (d, *J* = 6 Hz) and 3.97 (d, *J* = 4.5 Hz). In Me_2SO-d_6 at 138 °C, a single set of resonances was observed: δ 0.92 (t, *J* = 7.5 Hz, 3 H), 1.38 (m, 2 H), 1.41 (s, 9 H), 1.53 (m, 2 H), 2.02 (m, 1 H), 2.20 (m, 1 H), 3.32 (m, 1 H), 3.46 (m, 1 H), 3.77 (d, *J* = 5 Hz, 1 H); MS (DCI/NH_3) 275 (M + NH_4^+), 258 (M + H^+). Anal. Calcd for $C_{13}H_{23}NO_4$ (C, H, N).

The preparation of tetrapeptides Boc-Trp-X-Asp-Phe-NH_2 (X = Ala (7), Nle (8), Pro (9), and 3PP (10)) was accomplished by using standard solution methods. For the condensation of Boc-Trp-OH with imino nitrogens, symmetrical anhydride couplings with benzyl ester side chain protection at Asp were used. After Asp deprotection by catalytic hydrogenolysis, the final products were purified by silica gel chromatography (EtOAc/pyridine/H_2O/HOAc systems) and characterized by mass spectrometry, NMR, and combustion analysis. Radioligand binding assays were conducted as described previously.[16,17]

Binding affinities for Boc-CCK_4 and **7–10** to membranes from guinea pig cortex, which contain CCK-B receptors, and guinea pig pancreatic acini, which contain CCK-A receptors, are shown in Table I. The data for cortical receptors indicates the dramatic enhancement in affinity for the CCK-B receptor of the 3PP analogue **10** compared with the Nle analogue **8** and, particularly, the Pro analogue **9**. With regard to functional activity, compound **10** is a full agonist relative to CCK_8 and Boc-CCK_4 in stimulating calcium mobilization in NCI-H345 cells, which express CCK-B/gastrin receptors.[17]

It is interesting to consider the activity of **10** in terms of the individual contributions by the *n*-propyl side chain and the constraint imposed by the proline ring of the 3PP residue. Relative to Ala analogue **7**, the addition of the *n*-propyl substituent to give Nle analogue **8** results in a 60-fold improvement in binding affinity to the CCK-B receptor, whereas incorporation of the bridging ethylene unit to give Pro analogue **9** results in a 5-fold improvement in binding affinity. The combination of the two modifications results in a 2000-fold improvement in binding affinity compared to **7**, ca. 7-fold higher than might be expected on the basis of results for the individual modifications.[18] It is interesting to note that incorporation of *N*-methyl residues at this position in similar tetrapeptide and pentapeptide series has a similarly beneficial effect on receptor binding relative to the corresponding unmethylated analogues.[19,20] The conformational restrictions imposed by the 3PP residue provide useful information on the bioactive conformation of CCK at this receptor; in particular, the ϕ and χ_1 angles of 3PP are restricted to a narrow range, which should closely approximate the angles found in the same region of CCK_4 when it is bound to this receptor.

Largely by virtue of its higher affinity for the CCK-B receptor, compound **10** shows substantially improved cortical selectivity (ca. 1400-fold) relative to Boc-CCK_4 (ca. 74-fold). Incorporation of N-methylated residues at the corresponding position in other CCK analogues also improves selectivity for the cortical receptor.[20-22] Cyclic analogues of CCK_7 and CCK_8 with high selectivity for the B receptor also have been described recently.[23,24]

In related work which will be described separately, we have incorporated 3PP into sulfated heptapeptide analogues of CCK to obtain analogues that bind potently to both pancreatic and cortical CCK receptors.

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Supplementary Material Available: Full experimental details (5 pages). Ordering information is given on any current masthead page.

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Dual Antagonists of Platelet Activating Factor and Histamine. Identification of Structural Requirements for Dual Activity of *N*-Acyl-4-(5,6-dihydro-11*H*-benzo[5,6]cyclohepta-[1,2-*b*]pyridin-11-ylidene)piperidines[1]

Platelet activating factor (PAF)[2] is a biologically active ether phospholipid which is released from a variety of cells[3] involved in the pathogenesis of the allergic and inflammatory response. It produces a variety of biological effects including bronchoconstriction, chemotaxis, and vascular permeability.[3] Consequently, it has been implicated as a mediator in a variety of respiratory and inflammatory diseases. Furthermore, PAF may play a major role in asthma,[4] especially since it has been shown to cause bronchial hyperreactivity in man,[5] a common characteristic of this disease.[6]

(1) This work was presented in part at the Third International Conference On Platelet-Activating Factor and Structurally Related Alkyl Ether Lipids, Tokyo, Japan, May 1989; I2, and at the 198th National Meeting of the American Chemical Society, Division of Medicinal Chemistry, Miami, FL, September 1989; MEDI 56 and 88.
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