Tetrahedron Vol. 49, No. 17, pp. 3449-3466, 1993
Printed in Great Britain

0040-4020/93 $6.00+.00

Approaches to Peptidomimetics which serve as Surrogates for the *cis* Amide Bond: Novel Disulfide-Constrained Bicyclic Hexapeptide Analogs of Somatostatin.

Stephen F. Brady*, William J. Paleveda, Jr., Byron H. Arison§, Richard Saperstein†, Edward J. Brady†, Karen Raynor¶, Terry Reisine¶, Daniel F. Veber, and Roger M. Freidinger

Department of Medicinal Chemistry, Merck Research Laboratories, West Point, PA 19486-0004

§Departments of Animal and Exploratory Drug Metabolism and †Membrane Biochemistry and Biophysics, Merck Research Laboratories, Rahway, NJ 07065-0900

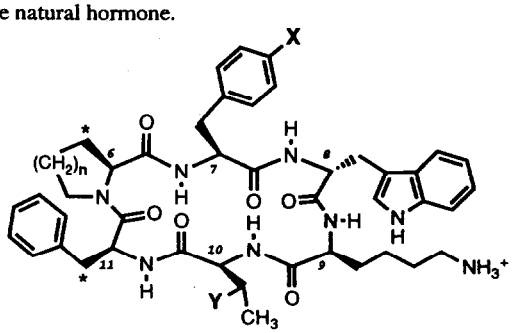
¶Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6084

(*Received in USA* 1 *March* 1993)

Abstract: In testing and refining our model of the receptor-bound conformation of the potent small-ring somatostatin analog *cyclo*-(Pro^6-Phe^7-*D*-Trp^8-Lys^9-Thr^{10}-Phe^{11}), we have investigated structures constrained within bicyclic systems. Specifically, we have incorporated the 8-membered -Cys-Cys- unit in place of the -Phe^{11}-Pro^6- segment, thus achieving two aims: 1) constraint of the 11->6 amide bond to the *cis* geometry established for the cyclic hexapeptide; 2) positioning of the disulfide in place of the position-11 phenyl group, to act as surrogate for phenyl in receptor binding. Synthetic methodology which provides ready access to this class of compounds is presented, along with results of NMR spectral studies of the bicyclic systems. Biological assays show retention of high potency, in confirmation of our view of *cyclo*-cystine as a good mimetic for *cis* amide. Other reported mimetics for the *cis* amide bond are reviewed from the perspectives of comparative ease of accessibility and approximation of various amide bond parameters.

INTRODUCTION

Efforts in these laboratories to develop reduced-size active analogs of somatostatin culminated in the synthesis of the highly potent cyclic hexapeptide **Ia**.[1] A model proposed for the solution conformation of **Ia** allows that the side chains of the tetrapeptide unit (-Phe-*D*-Trp-Lys-Thr-) closely approximate the key binding elements in the 7-10 segment of the receptor-bound hormone.[2] We have considered that these four residues, when induced to adopt the correct conformation, position receptor-interactive functions to express the total activity of the natural hormone.



Ia: n = 1, X = H, Y = OH **Ib:** n = 0(+2H), X = OH, Y = CH_3

We have pursued the constrained analog approach as a means to define that particular conformation in a peptide which triggers or blocks a biological response. Compound **Ia** represents a key milestone in our application of this approach and has sharpened our view of allowed structure at the somatostatin receptor. In the proposed receptor-bound conformation of **Ia** the residue pairs -*D*-Trp^8-Lys^9- and -Phe^{11}-Pro^6- comprise the *i*+ *1* and *i* + *2* residues of type II' and type VI reverse turns, respectively.[3] The role of the -Phe^{11}-Pro^6- dipeptide unit has been viewed largely as one of structural constraint, although an important component of ligand-receptor interaction *via* the phenyl nucleus has been recognized.[4] NMR studies on hexapeptide **Ia** have established that in solution the amide bond of this unit is essentially all *cis* and the phenyl nucleus is positioned close to the proline ring. Similar investigations of the analog N-Me-Ala^6-**Ia** have indicated a *cis*-Phe^{11}-N-Me-Ala^6- amide bond[5], and we have inferred the same to be the case in super-potent analog **Ib**.[6]

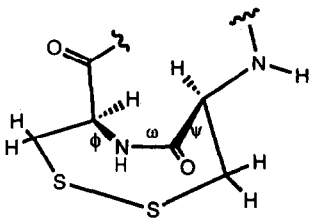
In seeking means to test and refine our model of the receptor-bound conformation of these small-ring somatostatin analogs, we were prompted to investigate structures made yet more rigid by connection of proximal β-carbons in residue pairs bridgable by disulfide linkages. Specifically, we viewed the joining of Phe^{11} and Pro^6 (asterisked carbons) in this manner, as an opportunity to direct the side chain of the position-11 residue toward that of the position-6 residue, thus simulating the proposed solution conformation. In addition, it seemed that the amide bond between residues 11 and 6, even though not a tertiary amide, would be fixed to the *cis* geometry in the eight-membered ring formed by closure of two neighboring cysteinyl residues, with minimal perturbation of stereoelectronic and physicochemical parameters. We also felt that such disulfide-linked analogs would be readily accessible by reliable and convenient synthetic procedures.[7] Herein we report analogs of somatostatin, and practical routes to their synthesis, in which the -Phe-X- segment in **Ia** (X = Pro) and **Ib** (X = N-Me-Ala) is replaced by -C$\overline{\text{ys-Cys}}$-, at once constraining the side chains of this segment and assuring fixation of the amide bond in the *cis* form.[8]

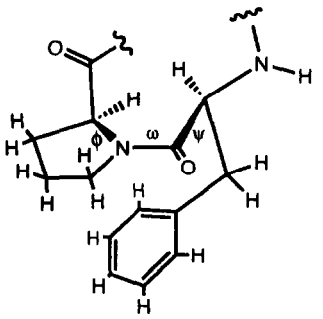
MODELLING RESULTS

Modelling of N-acetyl-*cyclo*-cystinyl-N-methyl amide was carried out to compare the structural parameters of -C$\overline{\text{ys-Cys}}$- with those of the -Phe-Pro- segment of the model (MM2) of **Ia**. Our results, as summarized in Figure 1, led us to conclude that: 1) attempts to construct the ring with a *trans* amide bond lead to an unacceptably high level of strain energy; 2) the backbone dihedral angles in -Phe-Pro- are close to those calculated for -C$\overline{\text{ys-Cys}}$-; 3) optimal separation of the β-carbons in -C$\overline{\text{ys-Cys}}$- would require only slight compression of the β-C(11)-β-C(6) distance from the value of 4.67 A calculated for **Ia**. These results are consistent with other energy calculations that predict the existence of *cyclo*-cystine in the *cis* form[9] and the fact that the *cis* amide has been confirmed for the solid state by single-crystal X-ray diffraction analysis.[10] Furthermore, replacement of -Phe-Pro- with -C$\overline{\text{ys-Cys}}$- in the model of cyclic hexapeptide **Ia** and energy minimization resulted in little overall change in the conformation of the cyclic hexapeptide.

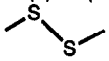
Figure 1. Comparison of Structural Parameters of -Cys-Cys- and-Phe-Pro- Segments

Segment:





Model: Ac-Cys-Cys-NHMe c-(Pro-Phe-D-Trp-Lys-Thr-Phe) (**Ia**)

Angles	Calc.[a]	X-Ray[b]	Model[c]	
ψ	134°	156°	148°	(131°)
ω	7°	-7°	-3°	(8°)
ϕ	-130°	-129°	-75°	(-82°)
$\beta_{C(6)}$-$\beta_{C(11)}$	4.2Å	4.1Å	4.67Å	
$\alpha_{H(6)}$-$\alpha_{H(11)}$	1.91Å	2.02Å	2.01Å	
	91°	94°	----	

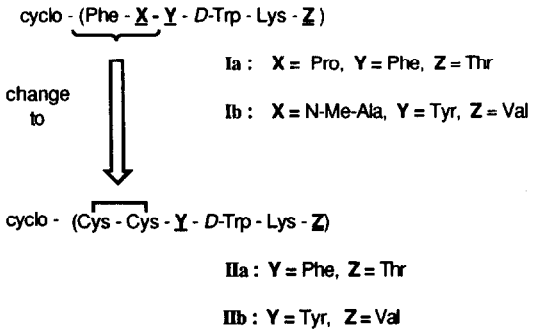
[a]Energy-minimized structure created using Merck Molecular Modeling System (MM2).

[b]From H-Cys-Cys-OH (see ref. 10a)).

[c]From ref. 1; values in parentheses are for energy-minimized structure (MM2).

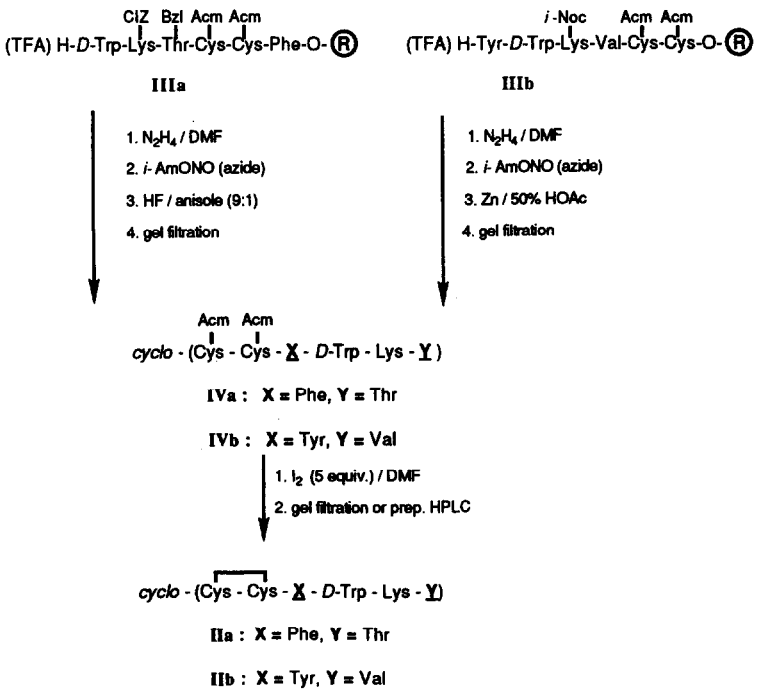
In the case of both analogs **Ia** and **Ib**, removal of Phe[11] was of special concern because the phenyl group had been identified as an important contributor to receptor binding.[4] Nonetheless, we theorized that disulfide might constitute an efficacious replacement for phenyl, based on literature reports that disulfide can form complexes with aromatic nuclei of a *pi* or charge-transfer type. Thus, disulfide could be capable of assuming a "surrogate" role for *pi* systems in receptor binding by means of its capacity for electron delocalization. Examples of sulfur-*pi* contact regions have been proposed for proteins based on X-ray data,[11] and sulfur-*pi* interactions are thought to contribute to structural stability in certain globular proteins.[12] Calculations by Scheraga and Némethy have revealed a specific favorable non-bonded interaction of approx. 0.8 kcal/mole between benzene and dimethyldisulfide.[13] Likewise, the existence of complexes between various divalent sulfur compounds and aromatic molecules has been observed spectrophotometrically.[14]

Thus, bicyclic analogs **IIa** and **IIb** replace **Ia** and **Ib**, respectively, with -Cys-Cys- in place of -Phe-X- (X = Pro or N-Me-Ala) and the disulfide moiety occupying the former position of the phenyl group. Herein we report readily-applied synthetic methodology to access these constrained peptides, along with the results of molecular modelling studies, biological evaluation, and characterization by NMR.

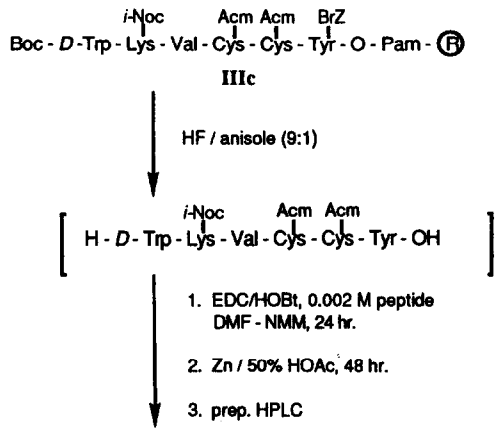


SYNTHETIC RESULTS

Synthesis of the two bicyclic somatostatin analogs **IIa** and **IIb** proceeded along parallel lines. Appropriate resin-bound sequences **IIIa** and **IIIb** were assembled according to standardized protocols, using Boc-protected amino acids for coupling and trifluoroacetic acid with 2% ethane dithiol as scavenger for Boc removal. Use of side chain unprotected tyrosine (**IIIb**) meant that liquid HF was not needed for protecting group removal; the *iso*-nicotinyloxycarbonyl (*i*-Noc) function[15] on lysine being readily cleaved reductively.



Thus in each example, following assembly and hydrazinolytic cleavage from the solid support, cyclization was accomplished after azide activation, in mildly basic media, to give fully-protected cyclic products.[16] In the one case (**IIIa**) treatment with liquid HF sufficed to cleave the Lys (2-chloro-Cbz) and Thr (benzyl) protecting groups; whereas in the other case (**IIIb**) activated powdered zinc in acetic acid was needed to remove the Lys (*i*-Noc) function. Gel filtration afforded the intermediate *bis*-acetamidomethyl (Acm) peptides **IVa** and **IVb**, respectively. Alternatively, monocyclic intermediate **IVb** could be handily synthesized starting from resin-bound precursor **IIIc**, assembled using optimized protocols on a fully-automated peptide synthesis instrument,[17] wherein cyclization after HF cleavage was implemented using water-soluble carbodiimide/ hydroxybenztriazole activation at high dilution.[18] Reductive removal of the *i* -Noc group with powdered zinc proceeded as above.



Formation of the bicyclic system was accomplished by oxidative removal of the Acm groups employing iodine. It was the application of DMF at high dilution (*ca.* 0.002 M peptide) which proved most reliable in assuring optimally efficient conversion to monomeric products.[19] Thus, *bis*-Acm precursor (**IVa/IVb**) undergoes rapid (< 5 min.) cyclization using a limited excess of iodine (5 equiv.) in DMF,[20] followed by removal of excess iodine by treatment with powdered zinc,[21] then concentration *in vacuo* and either gel filtration in 50% acetic acid or preparative HPLC. Monomeric product (**IIa/IIb**) was isolated by concentration and lyophilization of pooled fractions.

A key analytical finding (TLC, HPLC) was the presence of two components (70:30) in the bicyclic product **IIa**, which were shown to be conformation-related. The components were separable by preparative HPLC, but re-equilibrated by the time they were re-injected for analysis (see Experimental). Moreover, **IIa** was found to be stable only in acidic solution (aqueous acetic acid); a *p*H-stability study (ammonium acetate buffers) indicated decomposition in aqueous media at *p*H > 5, the rate increasing with higher *p*H. The possibility that trace amounts of sulfhydryl might be catalyzing polymerization was suggested by the presence of 2.5 mole % of free SH in **IIa** by quantitative Ellman assay.[22] Of several sulfhydryl-reactive reagents tested, N-ethyl maleimide (NEM)[23] completely prevented the degradation of peptide in aqueous solution, and treatment with NEM was

accordingly incorporated into the workup procedure for the I_2 cyclization. Alternatively, in the case of analog **IIb**, partitioning the excess I_2 into CCl_4 eliminated the need for zinc powder and afforded product with no free sulfhydryl.

The NMR spectrum of both analogs **IIa** and **IIb** at 300 MHz clearly indicated their conformational heterogeneity, not seen in the monocyclic structures **Ia** and **Ib**. While the NMR spectrum of compound **IIa** proved to be too complex for ready interpretation, that of compound **IIb** clearly showed two isomeric forms in rapid equilibrium (*ca.* 55:45 in CD_3OD). In both bicyclic analogs two main components were resolved on analytical HPLC. By means of a ROESY experiment[24] we were able to discern Overhauser effects between the two α-protons of the -Cys[11]-Cys[6]- segment of both isomeric forms of compound **IIb**. This finding, definitive evidence for the *cis* amide bond in *both* isomers, led us to consider, alternatively, the interconversion of disulfide rotameric forms as the source of the observed isomerism. Specific precedent for such interconversion exists in the strained bicyclic system, *cyclo-bis*- cystine,[25] which has been shown to interconvert between P-helical and M-helical -S-S- isomers with an estimated energy barrier (ΔG^*) at 25° of 15.6-16.0 kcal./mole, too low to allow isolation but high enough to be evidenced on the NMR time scale. Our findings stand in contrast to the recent report of a monocyclic disulfide,[26] in which it was *cis*/*trans* amide bond isomerism that was invoked to account for observed conformational heterogeneity.[27]

For some time now we have applied acetamidomethyl to prepare a variety of peptides of biological interest. Initial procedures were devised to optimize yields of constrained small-peptide analogs of somatostatin, and more recently we have been able to improve and standardize our synthetic techniques in approaches to other peptides.[28] Typically, the *bis*-Acm peptide obtained upon HF-mediated cleavage of resin-bound precursor is subject to cyclization using the oxidative condition of iodine in DMF or aqueous acetic acid followed by brief slurrying with zinc dust or, in the case of more strained disulfides, partitioning with CCl_4 to remove excess iodine. An important goal achieved through our recent efforts has been the development of efficient routes to strained disulfide-bridged structures. Acetamidomethyl as protection for cysteine has proven ideal for this application, since the penultimate intermediate can be purified before the sensitive disulfide bond is formed in the final step.

BIOLOGICAL FINDINGS AND DISCUSSION

The results of biological assays of the somatostatin analogs are shown in Table I. The bridged bicyclic analogs **IIa** and **IIb** retain essentially the full potencies of the corresponding monocyclic compounds **Ia** and **Ib**, respectively, across both *in vivo* and *in vitro* biological parameters. Further assessment of analog **IIb** with respect to competitive binding to somatostatin receptors derived from a murine pituitary tumor cell line *vis-à-vis* ^{125}I-labelled **Ib**[29] showed IC_{50} = 4.4 nM, comparable to the affinity of both **Ib** (1.6 nM) and somatostatin (2.6 nM). Thus, the same receptor is implicated in interaction with somatostatin and both highly potent constrained analogs **Ib** and **IIb**. Overall, these findings support our proposition that the disulfide link constitutes a constraint which fixes the conformation of the monocyclic species in its bioactive form.

Table I. Biological Activities of Somatostatin Analogs[a]

Compound	Insulin	Glucagon	Growth Hormone
c-(Pro-Phe-D-Trp-Lys-Thr-Phe) **Ia**	5.2 (2.4,11)	8.0 (1.4,60)	1.7 (1.3,2.3)
c-(Cys-Phe-D-Trp-Lys-Thr-Cys) **IIa**	10.6 (5.5,19.9)	10 (3,26)	2.5 (1.7,3.8)
c-(NMe-Ala-Tyr-D-Trp-Lys-Val-Phe) **Ib**	70.0 (28,173)	117.0 (56,270)	52.3 (35,75)
c-(Cys-Tyr-D-Trp-Lys-Val-Cys) **IIb**	69.3 (21,214)	106.9 (37,265)	15.3 (8,29)
c-(Pro-Phe-D-Trp-Lys-Thr-Ala)[b]	0.05 (0.03,0.1)	0.08 (0.04,0.2)	0.08 (0.04,0.2)

[a]Potencies for inhibition of hormone release relative to somatostatin (=1); insulin and glucagon are *in vivo* (rats), growth hormone is an *in vitro* pituitary cell suspension. Bioassay methods are as reported in ref. 16; confidence limits in parentheses.
[b]ref. 4.

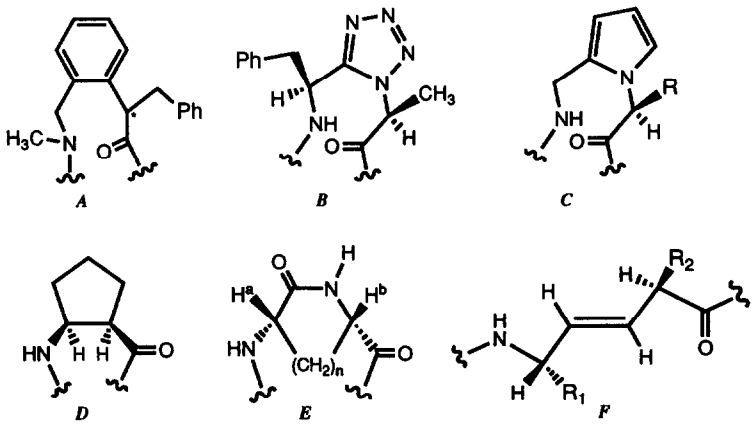
The much reduced potency of the Ala^{11} analog (last entry) highlights the importance of having a suitable hydrophobic receptor ligand at position 11. We conclude that the high potency of analogs **IIa** and **IIb** is a likely consequence of, not only constraint of the 11->6 amide bond to the *cis* form, but also of the role of disulfide as a surrogate for position-11 phenyl, which we infer must occupy the same position in analogs **Ia** and **Ib** as does disulfide in analogs **IIa** and **IIb** (*i.e.*, between the β-carbons of residues 6 and 11). Thus, the bicyclic analogs represent examples of substitution of disulfide for an aromatic moiety in key receptor-ligand interaction, in direct analogy to the aforementioned physico-chemical models.

Previous investigators have sought, as we have, to design and incorporate into bioactive molecules constraints which mimic features of peptidyl secondary structure (peptidomimetics, amide bond isosteres, peptide surrogates, *etc.*), while retaining or enhancing biological potency and if possible, introducing desirable properties such as enhanced oral absorption or *in vivo* half-life.[30] The field has been especially active with respect to amide bond replacements in analogs of somatostatin, with the incorporation of a variety of reverse-turn mimetics in place of the segment residues 7-10 designed to enforce a type II' β-turn.[31] Most of these structures have been devised to simulate the *trans* amide bond typically encountered within reverse turns of type I and II.

There have likewise been some, though fewer, reports of replacements for the -Thr^{10}-Phe^{11}-Pro^{6}-Phe^{7}- segment (see **Ia**) which incorporate mimetics of the *cis* amide bond between the Phe^{11} and Pro^{6} residues (see Figure 1). As one example, the -Phe-*D*-Trp-Lys-Thr- (7-10) sequence in **Ia** was subject to closure by means of the N-methyl-α-benzyl *o*-(aminomethyl) phenylacetyl (*o*-OMPA) unit ***A***, thus incorporating the probable key components of the secondary amine (N-Me) and the aromatic side chain (Phe) found in the highly potent Ala^{11}, N-Me-Phe^{6} analog of **Ia**.[32] One stereoisomer of moiety ***A*** incorporated in place of -Ala-N-Me-

Phe- conferred about 25% of the activity of the parent, thereby providing evidence that this entity is an appropriate mimetic for the dipeptide unit.

A functionality specifically designed as a surrogate for the *cis* amide bond is the 1,5-disubstituted tetrazole ring introduced by Marshall and co-workers[33] as a synthetic probe for the role of *cis/trans* isomerism of N-alkyl amide bonds in molecular recognition, and incorporated into a large number of structures of biological interest. As a test of the assumption that the presence of *cis* amide may be correlated with biological response,[34] the peptidomimetic segment ***B*** (-Phe-Ψ[CN_4]-Ala) was incorporated into analog **Ib** in place of -Phe^{11}-N-Me-Ala^{6}-.[35] On the basis of *in vitro* biological assays showing modest potency the authors



concluded that the tetrazole serves as a conformational mimic of the *cis* amide bond presumed to occur in the receptor-bound form. However, in recent work assessing the tetrazole mimetic in analogs of bradykinin[36] the authors observed loss of activity, which they ascribed either to a requirement for a *trans* amide bond, or perhaps as likely, to prevention of interaction with receptor due to steric bulk of the tetrazole. The latter speculation suggests that potential surrogates for the *cis* amide bond which occupy more space than the -CO-N< moiety do not ideally fit the spatial parameters of the amide bond. A recent report[37] describes a similar mimetic, the 1,2-disubstituted pyrrole ***C***, as a surrogate for a -Gly-*L*-Aaa- dipeptide with a *cis* amide bond. The synthesis, though convenient, is limited to introduction of the unbranched aminomethyl function.

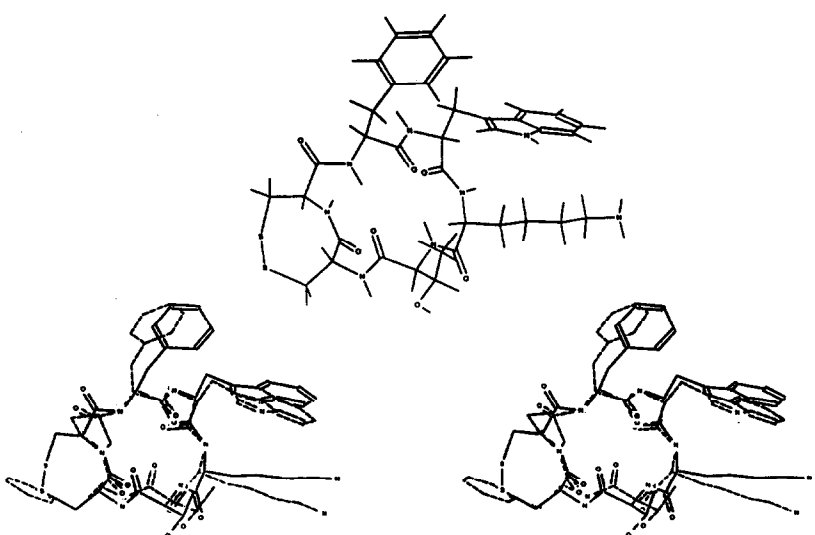
Another entity devised to force a *cis* amide bond in the backbone of a somatostatin analog is the *cis* -2-aminocyclopentyl carboxyl (2-Ac5c) moiety ***D***, incorporated in place of proline in analog **Ia**.[38] Although maintaining the preferred β-II' turn about the -*D*-Trp^{8}-Lys^{9}- segment, the bridged region retained the "normal" *trans* amide bond between Phe^{11} and 2-Ac5c, as evidenced by spectral data; and consistent with the loss of biological activity. In a similar design, the *L,L*- 3-amino-2-piperidone-6-carboxyl (*LL*-Acp) moiety (***E***: n = 2) was introduced by Kemp[39] to serve as a rigid analog of -*L*-Ala-*L*-Ala- in which the amide bond, constrained within the six-membered ring, must adopt the *cis* orientation. Kemp presented this structure as simulating a β-turn of the "rare *s-cis* type" observed in cyclic peptides containing N-alkyl amino acids. However, as

distinguished from -Cys-Cys-, in which the two $^{\alpha}$CH are definitively close to one another, the methine protons (H^a and H^b) were shown by NMR analysis to be axial and equatorial, respectively, thus too far apart to exhibit proximity (NOE) effects. In this context, we could consider that the *seven-* or *eight-* membered ring lactam (***E:*** n = 3 or 4) would be a closer mimic of a type VI (s-*cis*) turn.

Among important constraints incorporated into peptides as amide bond mimetics is the double bond isostere, shown as the *trans* (E) isomer ***F***. Substituting for a dipeptidyl unit of *L,L* configuration, this olefinic linker Ψ[E-CH=CH] has found a place in numerous pseudo-peptide structures.[40] Its similarity to the amide bond in regard to its effect on backbone structure was recognized early on, and a number of novel means for introduction of side chains R_1 and R_2 with high enantioselectivity have appeared in recent years,[41] expanding greatly the range of accessible surrogate amino acid residues. Difficulties associated with stability of the corresponding *cis* olefinic species have precluded assessment of the geometrically isomeric Ψ[Z-CH=CH] as a surrogate for *cis*-CO-NH.[42] However, the recent advances in synthetic methodology should be readily adaptable to structures having the C-methyl form Ψ[Z-CH=C(CH_3)] in place of -Aaa-Pro- or -Aaa-N-Me-Ala- in proposed type VI turns, thus providing a source of interesting structures.

Figure 2 depicts our structure proposed for the bioactive conformation of analog **IIa**, along with a superposition of this structure onto that proposed for analog **Ia**. The model overall is consistent with previous conceptions of the conformational preferences of this series of cyclic hexapeptides, and in addition demonstrates good overlap of the sulfur atoms in **IIa** with the phenyl nucleus in **Ia**. It is noteworthy that none of the above *cis* amide bond surrogates preserves the exact steric and electronic properties of -CO-N<. Thus, the close approximation of the cyclic disulfide structure to a dipeptidyl segment may be a significant factor in retention of biological response. This could be a key consideration in the design of amide bond mimetics.

Figure 2. Proposed Bioactive Conformation of Analog **IIa**, Derived Using Parameters From Energy-Minimized **Ia**; Superposition with Analog **Ia** (Dashed Structure)



EXPERIMENTAL

General. Unless otherwise noted, all solvents and reagents were obtained from commercial sources and used without further purification. DMF was degassed before use. ^{1}H-NMR spectra were recorded on a Varian 300, a Nicolet NT360, a VXR-400S, or a UNITY 400 instrument with chemical shifts (δ) reported in ppm relative to sodium TMS-propanesulfonate. Mass spectra were obtained on a Finnegen-MAT 731 spectrometer (FD) or a Fisons 7070E spectrometer (FAB). HPLC analyses were carried out on a Hewlett-Packard 1084-B liquid chromatography instrument with a DuPont ODS column using a pH 3.2 TMA-phosphate buffer -acetonitrile or -methanol gradient system (30 min.) or a Spectra-Physics SP8800 system having an SP4270 integrator with a Vydac Protein & Peptide C_{18} column, 300 A, 5μ, 150 x 4.6 mm. using a 0.1% TFA /H_2O-acetonitrile gradient system (30 min.), with detection by u.v. at 210 or 280 nm. Preparative HPLC was run on a Separations Technology ST/LAB 800B instrument fitted with a Waters 1000 PrepPak module and Delta-Pak C_{18} radial compression column, 300 A pore size, 15μ particle size, 13" x 2" i.d., eluting with a gradient system: 0.1% TFA-99.9% H_2O (solvent A) and 0.1% TFA-0.9% H_2O-99.0% CH_3CN (solvent B). Gel filtration was performed on Sephadex G25 SF (Pharmacia), eluting with 50% HOAc or 2N HOAc, monitoring by u.v. at 254 nm on an ISCO UA5 instrument. Thin layer chromatography (TLC) was performed on 250 mm 5 x 20 cm silica gel plates (Analtech), using u.v. light and/or *tert* -butyl hypochlorite / starch-iodine spray for visualization. Solvent systems employed were: $CHCl_3$-MeOH-H_2O (CMW); EtOAc-pyridine-HOAc-H_2O (EPAW); 1-Butanol-HOAc-H_2O (BAW). Amino acid analyses were performed on a Beckman Instruments amino acid analyzer. Molecular models were built using the Merck Molecular Modelling System (MMMS) and minimized using MM2.

Resin-bound precursors. Assembly of peptides on solid support was carried out using either a Beckman Instruments Model 990B peptide synthesizer (**IIIa** and **IIIb**) or an Applied Biosystems, Inc. Model 430A automated peptide synthesizer (**IIIc**). N^{α}-Boc amino acids were employed throughout, appropriately side-chain protected, from Bachem, Inc. or Applied Biosystems, Inc.

Assembly protocols were as follows: (**IIIa**) Starting from 1.65 g. (2.0 mmoles) of Boc-Phe-1% crosslinked polystyrene (Merrifield) resin, Boc removal (33% TFA/CH_2Cl_2), neutralization (Et_3N/CH_2Cl_2), and coupling with 5 mmoles of Boc amino acid (DCC/CH_2Cl_2), followed by recoupling each residue (DCC/HOBt), were implemented over 5 cycles. Thus, incorporated in order, were: Boc-Cys(Acm)-OH (2 cycles), Boc-Thr(Bzl)-OH, Boc-Lys(2-Cl-Cbz)-OH, and Boc-*D*-Trp-OH. Final resin weight was 4.00 g. (**IIIb**) Starting from 7.23 g. (6.0 mmoles) of Boc-Cys(Acm)-1% crosslinked polystyrene (Merrifield) resin, the above incorporation protocol was applied through 5 cycles: Boc-Cys(Acm)-OH, Boc-Val-OH, Boc-Lys(*i* -Noc)-OH, Boc-*D*-Trp-OH, and Boc-Tyr-OH (no side protection used). Final resin weight was 11.12 g. (**IIIc**) Starting from 0.80 g. (0.5 mmole) of Boc-Tyr(Br-Cbz)-PAM-resin (1% cross-linked), incorporation of the following residues was carried out according to protocols provided by Applied Biosystems, Inc. (each subjected to double coupling): Boc-Cys(Acm)-OH (2.0 mmoles); Boc-Cys(Acm)-OH (1.0 mmole); Boc-Val-OH; Boc-Lys(*i* -Noc)-OH (1.0 mmole); and Boc-*D*-Trp-OH. The Cys(Acm) and Lys(*i*-Noc) residues were

coupled using DCC/HOBt activation from DMF solution made up prior to assembly. Final resin weight was 1.34 g.

cyclo -(Cys(Acm)-Cys(Acm)-Phe-*D*-Trp-Lys-Thr) (**IVa**). *Hydrazinolysis.* A sample of 3.95 g. of resin-bound peptide **IIIa** suspended in 36 ml. of DMF was treated with 4.0 ml. of 95% hydrazine, followed by stirring for 1-1/2 hr. The reaction mixture was filtered to remove spent resin, and the filtrate was concentrated *in vacuo* . Trituration with 50 ml. of H_2O afforded a white solid, which was isolated by filtration and washing with 6 portions of H_2O, followed by drying *in vacuo* to give 2.46 g. of crude hydrazide: TLC R_f (CMW, 80-20-2) 0.17. *Cyclization.* A solution of hydrazide (2.41 g.) in 40 ml. of DMF, cooled to -20° under N_2, was treated withg 2.0 ml. of freshly prepared 5.0M HCl/THF (reaction pH = 1.5, moistened narrow-range pH paper), followed portion-wise by *iso*-amyl nitrite (total of 0.31 ml., 2.31 mmoles) over 45 min. Monitoring by starch-iodide paper was used to assure uptake of nitrite and complete azide formation. After 75 min.the solution was added to 1200 ml. of pre-cooled (-25°) DMF, followed by addition of diisopropyl ethyl amine (DIEA) to pH 7.5-8 (moistened narrow-range pH paper); cyclization took place over a period of 3 days at -15°, as determined by TLC. The reaction solution was concentrated *in vacuo*, and trituration with 50 ml. of H_2O gave a white solid, which was isolated by filtration and drying *in vacuo* to give 2.30 g. of crude cyclic product: TLC R_f (CMW, 80-20-2) 0.65. *HF Cleavage.* A sample of 2.03 g. of protected cyclic peptide was stirred with 2.0 ml. of anisole and 20 ml. of HF in a *Kel-F* reactor at 0° for 1 hr. After removal of the HF *in vacuo* , the peptide was isolated by precipitation with 50 ml. of ether, filtration, then drying *in vacuo* to give 1.87 g. of crude product. This sample was purified by gel filtration (50% HOAc), and fractions pooled on the basis of TLC were concentrated and lyophilized to give 1.17 g. (60% overall yield) of *bis*- Acm intermediate **IVa**: TLC R_f (EPAW, 10-5-1-3) 0.26; HPLC (95->5%, 30 min.) 88% (RT 9.9 min.); FAB-MS $^m/_e$ 912 (calc. M + H = 912); amino acid analysis (HCl, 70 hr.) Lys = 0.77, Phe = 0.77 μmole/mg., Cys, Thr, Trp decomp.; ^{1}H NMR (300 MHz, D_2O) δ 2.05,2.02 s,s (Cys CH_3-CO-, 2 x 3H), 4.35 s (Cys -HN-CH_2-S-, 2 x 2H), 7.66 d, 7.56 d (J = 9 Hz)(*D*-Trp H^4, H^7), 7.21 s (*D*-Trp H^2), 0.45, 0.26 (Lys γCH_2, 2H), 3.98 q (Thr βCH, 1H), 4.72 d (1), 4.62 q (1), 4.55 m (2), 4.26m (2) (6 αCH).

cyclo -(Cys(Acm)-Cys(Acm)-Tyr-*D*-Trp-Lys-Val) (**IVb**). Method A. *Hydrazinolysis.* A sample of 11.02 g. (equiv. to a 6.0-mmole run) of resin-bound peptide **IIIb** suspended in 96 ml. of anhydrous CH_3OH was treated with 24 ml. of 95% hydrazine, stirring for 1 1/2 hr. The mixture was filtered to remove the spent resin, which was washed with 3 portions of DMF to recover precipitated hydrazide. The solvent was removed *in vacuo* , and H_2O was added to give solid product, which was isolated by filtration, washing several times with H_2O, then dried *in vacuo* to give 3.38 g. of crude hydrazide: TLC R_f (EPAW, 10-5-1-3) 0.44. *Cyclization.* A solution of hydrazide (3.30 g.) in 33 ml. of DMF, cooled to -20° under N_2, was treated with 2.4 ml. of freshly-prepared 6.5 M HCl/THF, followed by *iso*-amyl nitrite portion-wise (total of 0.44 ml., 2.99 mmoles) over 45 min. After azide formation was deemed complete (see above), this solution was added to 1600 ml. of pre-cooled (-25°) DMF, followed by DIEA until pH ≥ 8; cyclization took place over a period of 3 days at -15°, as determined by TLC. The reaction solution was concentrated *in vacuo*, and 100 ml. of H_2O was added to give a tacky solid, which was triturated and isolated by filtration, washing with H_2O, and drying *in vacuo* to

give 2.39 g. of crude cyclic product. Gel filtration (50% HOAc) afforded 1.70 g. of purified intermediate (*i*-Noc-Lys), which was carried on without purification: TLC R_f (EPAW, 10-5-1-3) 0.58; (BAW, 65-10-25) 0.51. *Zinc treatment.* A solution of 1.58 g. of the *i*- Noc intermediate in 52 ml. of 50% HOAc was stirred with freshly-activated Zn (prepared from 8.5 g. of powdered zinc[43]) for a period of 20 hr. The reaction was followed by TLC to completion, the suspended solids were removed by filtration, and the filtrate was concentrated *in vacuo* and charged to gel filtration (50% HOAc). Concentration of pooled fractions and lyophilization afforded 1.25 g. (21% overall yield) of *bis*- Acm intermediate **IVb** (acetate): TLC R_f (BAW, 65-10-25) 0.43; HPLC (95->5%, 30 min.) 86% (RT 8.7 min.); amino acid analysis (HCl, 70 hr.) Lys = 0.91, Val = 0.92, Tyr = 0.89 μmole/mg., Cys, Trp decomp. Method **B**. *HF Cleavage / Cyclization* . A sample of 1.34 g. (equiv. to 0.5 mmole) of resin-bound peptide **IIIc**, suspended in 3.0 ml. of anisole, was treated with 30 ml. of HF in a *Kel-F* reactor, at 0° for 1 1/2 hr. After removal of the HF *in vacuo*, the product was isolated by precipitation with ether (60 ml.), filtration of the solids, and after brief drying, transfer to 300 ml. of DMF, addition of 750 mg. (4.9 mmoles) of HOBt·H_2O, then N-methyl morpholine (NMM) until pH 7 (moistened narrow-range pH paper); followed by 635 mg. (3.3 mmoles) of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC). The reaction, shown by TLC R_f (EPAW, 12-5-1-2) 0.36 (linear), 0.61(cyclic) to be complete in 24 hr., was worked up by evaporation of solvent *in vacuo* and partition of the residue with 100 ml. of *n*-butyl alcohol / 30 ml. of H_2O, then washing with dil. $KHSO_4$ ($pH \leq 2$), H_2O, dil. $NaHCO_3$ ($pH \sim 8$), and H_2O/NaCl (2x). The organic phase was concentrated to a volume of < 5 ml. and taken up in 20 ml. of 50% HOAc, followed by addition of freshly-activated Zn (prepared from 1.5 g. of powdered zinc 42) and stirring until *i*-Noc cleavage was shown to be complete (TLC). After 2 days the suspended solids were removed by filtration, and the residue after concentration *in vacuo* was taken up in 60 ml. of H_2O and charged to preparative HPLC (100 -> 60, 60 min.). Pooled fractions afforded, after lyophilization, 187 mg. (36% overall yield) of *bis*-Acm derivative **IVb** (trifluoroacetate): TLC R_f (EPAW, 12-5-1-2) 0.26; HPLC (100->60%, 30 min.) 98% (RT 18.6 min.); FAB-MS $^m/_e$ 925 (calc. M + H = 925); ^{1}H NMR (400 MHz, CD_3OD) δ 2.00, 1.98 s,s (Cys CH_3-CO-, 2 x 3H), 4.40, 4.32 s, s (Cys-HN-CH_2-S-, 2 x 2H), 7.50 d, 7.33 d (J = 9 Hz) (D-Trp H^4, H^7), 6.99 s (D-Trp H^2), 7.04 d (J = 9 Hz) (Tyr H^2, H^6), 6.71 d (J = 9 Hz) (Tyr H^3, H^5), 0.64 env. (Lys γ CH_2, 2H), 0.95 d/d (Val CH_3, 2 x 3H), 1.35 env. (Lys βCH_2, δ CH_2, 4H), 1.77 env (Val βCH, 1H), 4.52 m (1), 4.43 m (1), 4.25-4.35 env (2), 4.00-4.10 m (2) (6 αCH).

cyclo-(Cys-Cys-Phe-*D*-Trp-Lys-Thr) (**IIa**). To a briskly-stirred solution of 182 mg. (0.20 mmole) of *bis*-Acm derivative **IVa** in 90 ml. of DMF was added all at once a solution of 254 mg. (1.0 mmole) of I_2 in 90 ml. of DMF. After 3.5 min. 600 mg. of powdered Zn was added, followed by 40 ml. of cold (0°) 50% HOAc, and the mixture was filtered immediately after decolorization (1-2 min.). The filtrate was concentrated *in vacuo* to a small volume, redissolved in 10 ml. of 50% HOAc, and charged to gel filtration (50% HOAc). Pooled fractions were concentrated *in vacuo*, and lyophilization from 10% HOAc yielded 67 mg. (44% yield) of product showing two components: TLC R_f (EPAW, 10-5-1-3) 0.31/0.33; HPLC (95->5%, 30 min.) 98% (RT 10.4/11.3 min., 29:71). A sample submitted to semipreparative HPLC (0.1% TFA/70:30 H_2O/CH_3CN isocratic) afforded fractions containing the separated components, each of which reverted to the starting mixture

in 12-24 hr., indicating that the constituents were interconverting. A study of the pH dependence of stability revealed significant decomposition to more polar constituents, determined as polymeric by gel filtration, at pH > 3 (0.1N NH_4OAc buffers). Addition of 0.5 molar equivalent of N-ethylmaleimide (NEM)[23] was shown to stabilize an aqueous solution at (ambient) pH 5 for > 24 hr. Excess NEM was separated by gel filtration, Ellman assay[22] showed free SH to have fallen from *ca.* 1% before to < 0.2% after NEM treatment, and lyophilization afforded a pH-stable sample: amino acid analysis (HCl / performic acid, 20 hr.) Lys = 0.87, Thr = 0.97, Phe = 0.97, $Cys(SO_3H)$ = 1.91, Trp (u.v. at 280 nm) = 0.96 μmole/mg.; FAB-MS $^m/_e$ 767 (calc. M + H = 767); ^{1}H-NMR (300 MHz, CD_3OD/1% CD_3COOD) complex (multicomponent), e.g. δ 1.10 d (J ~ 7 Hz) (minor Thr CH_3) coupled with 4.02 (Thr βCH); 1.18 d (J ~ 7 Hz) (major Thr CH_3) coupled with 4.66 (Thr βCH).

cyclo -(Cys-Cys-Tyr-D-Trp-Lys-Val) (**IIb**). Method **A**. To a briskly-stirred solution of 463 mg. (0.50 mmole) of *bis*-Acm derivative **IVb** in 420 ml. of DMF was added all at once a solution of 635 mg. (2.50 mmoles) of I_2 in 40 ml. of DMF. After 3.0 min. 1.5 g. of powdered Zn was added, followed by 100 ml. of cold (0°) 50% HOAc, and after decolorization (1-2 min.) the mixture was filtered, and the filtrate was concentrated *in vacuo*. The residue was submitted to gel filtration (50% HOAc), and pooled fractions containing product were concentrated *in vacuo*, and lyophilization gave 141 mg. (36% yield) of white lyophilizate, Ellman analysis of which showed *ca.* 3% free SH. Treatment of a solution in 1.0 ml. of H_2O with 11 mg. of NEM for 20 min., followed by addition of 0.25 ml. of HOAc and gel filtration (2N HOAc), pooling of fractions and lyophilization, afforded 101 mg. (72% recovery) of white fluffy powder: Ellman assay <0.4% free SH; TLC R_f (EPAW, 10-5-1-3) 0.41; HPLC (95->5%, 30 min.) 96% (RT 9.1 min.); amino acid analysis (HCl, 70 hr.) Lys = 1.03, Val = 1.01, Tyr = 1.02, Trp (u.v. at 280 nm) = 0.98 μmole/mg. (HCl/performic acid, 20 hr.) Lys = 0.99. Val = 0.99, $Cys(SO_3H)$ = 2.14 μmole/mg.; FAB-MS $^m/_e$ 781 (calc M + H = 781); ^{1}H NMR (360 MHz, CD_3OD) 2 components (*ca.* 2:3) δ 5.50 d (J = 10.5 Hz) : 5.14 d (J = 12.6 Hz) (Cys^x αCH), 5.27 d (J = 12.4 Hz) : 5.05 d (J = 11.0 Hz) (Cys^y αCH), 4.36/4.15 split d (J ~ 8 Hz) (Val αCH), 3.80/3.63 d/d (J ~ 12, 3 Hz) (Lys αCH), 4.45-4.70 m (D-Trp, Tyr αCH), 0.5 env. (Lys γCH_2). Method **B**. To a briskly-stirred solution of 126 mg. (0.121 mmole) of *bis*-Acm derivative **IVb** in 115 ml. of DMF was added all at once a solution of 173 mg. (0.68 mmole) of I_2 in 13 ml. of DMF. After 3.0 min. 410 mg. of powdered Zn was added, followed by 28 ml. of cold (0°) glacial HOAc, and after decolorization (2 min.) the mixture was filtered, and the filtrate was concentrated *in vacuo* to a volume of < 5 ml., to which was added 60 ml. of H_2O. HPLC (100->60%, 30 min.) indicated about 75% of the product **IIb** as a double peak, RT 18.8/19.7 min. (*ca.* 4:1) and 25% of a contaminant, RT 20.8 min.[44] The crude product was purified by preparative HPLC (100->60%, 60 min.), no resolution of the double peak being seen, and pooled fractions afforded, after concentration and lyophilization, 31 mg. (29% yield) of a white, fluffy powder: Ellman assay < 0.1% free SH; TLC R_f (EPAW, 12-5-1-3) 0.53; HPLC (100->60%, 30 min.) 99% (RT 18.8/19.8 min., 80:20); FAB-MS $^m/_e$ 781 (calc. M + H = 781). The ^{1}H NMR spectrum (500 MHz, CD_3OD), indistinguishable from that of **IIb** prepared by Method **A**, was assigned as follows: 2 components (ca. 2:3) δ 5.47 d (J = 10 Hz): 5.11 dd (J = 11.8, 4.1 Hz) (Cys^x αCH), 5.24 dd (J = 11.8, 4.1 Hz): 5.02 dd (J = 11.2, 1.8 Hz) (Cys^y αCH), 4.66 dd (J = 8.5, 4.8 Hz): 4.61

dd (J = 8.9, 4.4 Hz) (Tyr $^{\alpha}$CH), 4.52 dd (J = 9.0, 5.0 Hz): 4.49 dd (J = 11.0, 5.0 Hz) (Trp $^{\alpha}$CH), 4.35 dd (J = 9.1,* 5.2 Hz): 4.14 dd (J = 9.0*, 6.7 Hz) (Val $^{\alpha}$CH, incl. J* NH-CH), 3.80 dd (J = 10.8, 3.5 Hz): 3.63 dd (J = 10.5, 3.9 Hz) (Lys $^{\alpha}$CH), 7.53 d (J = 8.0) (D-Trp H^4), 7.33d (J = 8.0 Hz) (D-Trp H^7), 7.11 t (J = 7Hz) (D-Trp H^5), 7.10/7.09d (J = 8.5 Hz)(Tyr H^2, H^6), 6.71/6.70 d (J = 8.5 Hz) (Tyr H^3, H^5), 6.98s (D-Trp H^2), 7.11t/7.05t (D-Trp H^5, H^6), 3.51 dd (J = 13.1, 3.9 Hz) /3.50 br. (Cys βCH_2), 2.8 - 3.3 (Cys, Tyr, D-Trp βCH_2), 2.60m (Lys εCH_2), 2.00/1.89 env. (Val βCH), 1.60/1/52m,1.2-1.3m (Lys βCH_2, δCH_2), 0.92d/0.80d: 0.89d/0.79d (Val CH_3), 0.53/0.40m (Lys γCH_2).

Fractions containing only the higher RT by-product were pooled, concentrated and lyophilized to give 18 mg. (17% yield) of a white powder: Ellman assay $\geq$ 50% free SH; HPLC (100->60%, 30 min.) 97% (RT 20.8 min.); FAB-MS $^{m}/_{e}$ 783 (calc. M + H = 783 for *bis*- SH-**IIb**).

Receptor binding assays. Mouse AtT-20/D16-16 cells (originally subcloned by Dr. S. Sabol, National Institutes of Health) were grown and subcultured in DMEM with 10% bovine serum as previously described.[45] For radioligand binding assays, cells were harvested in 50 mM Tris-HCl (pH 7.8) and centrifuged at 20,000 x g for 15 min at 4°C. The pellet was homogenized in Tris buffer containing 1 mM ethylene glycol *bis*(β-aminoethyl ether)-N,N'-tetraacetic acid, 5 mM $MgCl_2$, 10μg/ml leupeptin, 10 μg/ml pepstatin, 200 μg/ml bacitracin and 0.5 μg/ml aprotinin (buffer 1) using a Polytron (Brinkmann, setting 2.5, 30 sec) and this membrane preparation was used for the radioligand binding and photocrosslinking studies. AtT-20 cell membranes (20-30 μg protein) were incubated with ^{125}I-**Ib** (0.05 nM, specific activity 2200 Ci/mmol) in a final volume of 200 μL for 60 min at 25°C in the presence or absence of competing peptides. Nonspecific binding was defined as the radioactivity remaining bound in the presence of 100 nM SRIF. The binding reaction was terminated by the addition of ice-cold Tris-HCl buffer and rapid filtration over Whatman GF/C glass fiber filters. The filters were then washed with 12 mL of ice-cold Tris-HCl buffer and the bound radioactivity counted in a gamma counter (80% efficiency). Data from radioligand binding studies were used to generate inhibition curves. IC_{50} values were obtained from curve-fitting performed by the mathematical modeling program FITCOMP available on the National Institutes of Health-sponsored PROPHET system.

ACKNOWLEDGMENT

We extend our thanks to a number of individuals for their assistance in this work: Mr. Carl F. Homnick for HPLC analyses; Ms. Susan S. Fitzpatrick for amino acid analyses; Mr. Jack L. Smith and Mr. Arthur B. Coddington for mass spectral determinations; Dr. David W. Cochran and Ms. Joan S. Murphy for NMR spectra; and Ms. Jean F. Kaysen for preparation of the manuscript. We also thank Drs. Graham M. Smith and Chris Culberson for help in molecular modelling and graphics presentation. Special thanks go to Dr. Ruth F. Nutt for valuable suggestions and commentary and to Dr. Kenneth Kopple for thoughtful consultation. We are also grateful to Dr. Paul Anderson for his long-term support of this research endeavor.

REFERENCES AND NOTES

1. Veber, D. F.; Freidinger, R. M.; Perlow, D. S.; Paleveda, W. J. Jr.; Holly, F. W.; Strachan, R. G.; Nutt, R. F.; Arison, B. H.; Homnick, C.; Randall, W. C.; Glitzer, M. S.; Saperstein, R.; Hirschmann, R. *Nature* **1981**, *292*, 55-58.
2. Veber, D. F. In *Peptides - Synthesis-Structure-Function: Proceedings of the Seventh American Peptide Symposium*: Rich, D. H.; Gross, E. Eds.; Pierce Chemical Co.: Rockford, Illinois, 1981; pp. 685-694.
3. Freidinger, R. M.; Veber, D. F. In *ACS Symposium Series, No. 251: Conformationally Directed Drug Design*: Vida, J. A.; Gordon, M. Eds.; 1984; pp. 169-187.
4. Veber, D. F.; Randall, W. C.; Nutt, R. F.; Freidinger, R. M.; Brady, S. F.; Perlow, D. S.; Paleveda, W. J.; Strachan, R. G.; Holly, F. W.; Saperstein, R. In *Peptides 1982. Proceedings of the 17th European Peptide Symposium*, Blaha, K., Malon, P. Eds.; W. de Gruyter & Co. 1983, pp. 789-792.
5. Freidinger, R. M.; Perlow, D. W.; Randall, W. C.; Saperstein, R.; Arison, B. H.; Veber, D. F. *Int. J. Peptide Protein Res.* **1984**, *23*, 142-150; see also Mierke, D. F.; Pattaroni, C.; Delaet, N.; Toy, A.; Goodman M.; Tancredi, T.; Motta, A.; Temussi, P. A.; Moroder, L.; Bovermann, G.; Wünsch, E. *Int. J. Peptide Protein Res.* **1990**, *36*, 418-432.
6. Veber, D. F.; Saperstein, R.; Nutt, R. F.; Freidinger, R M.; Brady, S. F.; Curley, P.; Perlow D. S.; Paleveda, W. J.; Colton, C. D.; Zacchei, A. G.; Tocco, D. J.; Hoff, D. R.; Vandlen, R. L.; Gerich, J. E.; Hall, L.; Mandarino, L.; Cordes, E. H.; Anderson, P. S.; Hirschmann, R. *Life Sci.* **1984**, *34*, 1371-1378.
7. (a) Nutt, R. F.; Brady, S. F.; Lyle, T. M.; Ciccarone, T. M.; Paleveda, W. J.; Colton C. D.; Veber, D. F.; Winquist, R. J. *Proceedings, Protides of the Biological Fluids Colloquium.* 1986, 34, 55-58. (b) Brady, S. F.; Paleveda, W. J.; Nutt, R. F. In *Peptides - Chemistry and Biology: Proceedings of the Tenth American Peptide Symposium* Marshall, G. R. Ed. ESCOM Science Publishers, B.V., 1988, pp 192-194. (c) Bogusky, M. J.; Naylor, A. M.; Pitzenberger, S. M.; Nutt, R. F.; Brady, S. F.; Colton, D. C.; Sisko, J. T.; Anderson, P. S.; Veber, D. F. *Int. J. Peptide Protein Res.* **1992**, *39*, 63-76.
8. Portions of this work have already been disclosed: see Veber, D. F. In *Peptides - Chemistry and Biology, Proceeding of the Twelfth American Peptide Symposium*, Smith, J. A.; Rivier, J. E., Eds.; ESCOM Science Publishers, B.V., 1992; pp 3-14, and ref. therein; also Brady, S. F. Eur. Pat. Appl. EP113,029 (11 July 1984); U.S. Pat no. 4,663,435 (05 May 1987). *Chem. Abstr.* **1984**, 101(25), 211734j.
9. Varughese, K. I.; Lu, C. T.; Kartha, G. *Int. J. Peptide Protein Res.* **1981**, *18*, 88-102.
10. (a) Capasso, S.; Mattia, C.; Mazzarella, L.; Puliti, R. *Acta Cryst.* **1977**, *B33*, 3561-3564. (b) Hata, Y.; Matsura, Y.; Tanaka, N.; Ashida, T.; Kakudo, M. *Acta Cryst.* **1977**, *B33*, 2080-2083.
11. Summers, L.; Wistow, G.; Narebor, M.; Moss, D.; Lindley, P.; Slingsky, C.; Blundell, T. L.; Bartunik, H.; Bartels, K. *Peptide Protein Rev.* **1984**, *3*, 147-168.
12. Morgan, R. S.; McAdon, J. M. *Int. J. Peptide Protein Res.* **1980**, *15*, 177-180.
13. Némethy, G.; Scheraga, H. A. *Biochem. Biophys. Res. Commun.* **1981**, *98(2)*, 482-487.

14. Lebl, M.; Sugg, E. E.; Hruby, V. J. *Int. J. Peptide Protein Res.* **1987**, *29*, 40-45.
15. Veber, D. F.; Paleveda, W. J. Jr.; Lee, Y. C.; Hirschmann, R. *J. Org. Chem.* **1977**, *42*, 3286-3288.
16. Veber, D. F.; Holly, F. W., Paleveda, W. J.; Nutt, R. F.; Bergstrand, S. J.; Torchiana, M.; Glitzer, M.; Saperstein, R.; Hirschmann, R. *Proc. Natl. Acad. Sci. USA* **1978**, *75*, 2636-2641.
17. Geiser, T.; Beilan, H.; Bergot, B. J.; Otteson, K. M. In *Macromolecular Sequencing and Synthesis*, Schlesinger, D. H. Ed.; , Alan R. Liss, New York, 1988, pp. 199-218.
18. Nutt, R. F.; Ciccarone, T. M.; Brady, S. F.; Williams T. M.; Colton, C. D.; Winquist, J. Veber D. F. In *Peptides 1988. Proceedings of the 20th European Peptide Symposium*, Jung, G.; Payer, E., Eds.; W. de Gruyter & Co., pp. 574-576.
19. Veber, D. F.; Holly, F. W.; Nutt, R. F.; Bergstrand, S. J.; Brady, S. F.; Hirschmann, R.; Glitzer, M. S.; Saperstein, R. *Nature* **1979**, *280*, 512-514.
20. In contrast to previous results with analogs of atrial natriuretic factor (Napier, M. A.; Vandlen, R. L.; Albers-Schonberg, G.; Nutt, R. F.; Brady, S.; Lyle, T.; Winquist, R.; Faison, E. R.; Heinel, L. A.; Blaine, E. H. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 2640-2644), no iodination of tyrosine in **Ib** was detected under these conditions.
21. Lyle, T. A.; Brady, S. F.; Ciccarone, T. M.; Colton, C. D.; Paleveda, W. J.; Veber, D. F.; Nutt, R. F. *J. Org. Chem.* **1987**, *52*, 3752-3759.
22. Ellman, G. L. *Arch. Biochem. Biophys.* **1959**, *82*, 70-77; see also Moroder, L.; Gemeiner, M.; Goehring, W.; Jaeger, E.; Thorum, P.; Wünsch, E. *Biopolymers* **1981**, *20*, 17-37.
23. *Methods in Enzymology*. (Hirs and Timasheff, Eds.) Academic Press 1972, Vol. XXV, Part.B, p. 449.
24. (a) Bax, A.; Davis, D. G.; *J. Magn. Reson.* **1985**, *63*, 207-213; (b) Kessler, H.; Griesinger, C.; Kerssebaum, R.; Wagner, K.; Ernst, R. *J. Am. Chem. Soc.* **1987**, *109*, 607- 609.
25. Jung, G.; Ottnad, M. *Angew. Chem. Int. Ed. Engl.* **1974**, *13*, 818-819.
26. Sukumaran, D. K.; Prorok, M.; Lawrence, D. S. *J. Am. Chem. Soc.* **1991**, *113*, 706-707.
27. Full interpretation of the spectrum of **IIb**, which includes analysis of the $^{\alpha}$NH to $^{\alpha}$CH coupling constants, has indicated minimal difference in backbone conformation between the two proposed rotameric forms (see ref. 8).
28. Brady, S. F.; Paleveda, W. J.; Nutt, R. F. *Peptides - Chemistry and Biology: Proceedings of the Tenth American Peptide Symposium*, Marshall, G. R. Ed., ESCOM Science Publishers B.V., 1988, pp. 192-194.
29. Raynor, K.; Reisine, T. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 510-517.
30. Recent reviews: (a) Morgan, B. A.; Gainor, J. A. *Ann. Rpts. Med. Chem.* **1989**, *24*, 243-252; (b) Hoelzemann, G. *Kontakte (Darmstadt)*, **1991**, (1), 3-12; (2), 55-63; (c) Toniolo, C. *Int. J. Peptide Protein Res.* **1990**, *35*, 287-300.
31. Hirschmann, R. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1278-1301; see also ref. 29(a), pp. 246-248.
32. (a) Elseviers, M.; Van Der Auwera, L.; Pepermans, H.; Tourwé, D.; Van Binst, G. *Biochem. Biophys. Res. Commun.* **1988**, *154*, 515-521; (b) Elseviers, M.; Van Der Auwera, L.; Tourwé, D.; Pepermans,

H.; Van Binst, G. In *Peptide Chemistry 1987*, Shiba, T.; Sakakibara, S., Eds. Protein Research Foundation 1988, pp. 607-610.

33. Zabrocki, J.; Smith, G. D.; Dunbar, J. B., Jr.; Iijima, H.; Marshall, G. R. *J. Am. Chem. Soc.* **1988**, *110*, 5875-5880.
34. Smith, G. D.; Zabrocki, J.; Flak, T. A.; Marshall, G. R. *Int. J. Peptide Protein Res.* **1991**, *37*, 191-197.
35. Zabrocki, J.; Slomczynska, U.; Marshall, G. R. In *Peptides - Chemistry, Structure and Biology: Proceedings of the Eleventh American Peptide Symposium*, Rivier, J.; Marshall, G. R., Eds.; ESCOM Science Publishers, B. V. 1990; pp. 195-197.
36. Zabrocki, J.; Dunbar, J. B.; Marshall, K. W.; Toth, M. V.; Marshall, G. R. *J. Org. Chem.* **1992**, *57*, 202-209.
37. Abell, A. D.; Hoult, D. A.; Jamieson, E. J.; *Tetrahedron Lett.* **1992**, *33(39)*, 5831-5832.
38. Yamazaki, T.; Huang, Z.; Pröbstl, A.; Goodman, M. In *Peptides 1990. Proceedings of the 21st European Peptide Symposium*, Giralt, E.; Andreu, D., Eds., ESCOM Science Publishers B.V., 1991, pp. 389-392; see also Mierke, D. F.; Nossner, G.; Schiller, P. W.; Goodman, M. *Int. J. Peptide Protein Res.* **1990**, *35*, 35-45.
39. Kemp, D. S.; Sun E. T. *Tetrahedron Lett.* **1982**, *23(37)*, 3759-3760.
40. (a) Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. *J. Chem. Soc. Chem. Commun.* **1980**, 234-235; (b) Cox, M. T.; Heaton, D. W.; Horbury, J. *J. Chem. Soc. Chem. Commun.* **1980**, 799-800; Cox, M. T.; Gormley, J. J.; Hayward, C. F.; Petter, N. N. *ibid.* **1980**, 800-802; (c) Bol, K.; Liskamp, R. M. J. *Tetrahedron* **1992**, *48(31)*, 6425-6438, and ref. therein.
41. (a) Spaltenstein, A.; Carpino, P. A.; Miyake, F.; Hopkins, P. B.; *Tetrahedron Lett.* **1986**, *27(19)*, 2095-2098; (b) Kempf, D. J.; Wang, X. C.; Spanton, S. G.; *Int. J. Peptide Protein Res.* **1991**, *38*, 237-241 and ref. therein; (c) Fujii, N.; Habashita, H.; Shigemori, N.; Otaka, A.; Ibuka, T.; Tanaka, M.; Yamamoto, Y. *Tetrahedron Lett.* **1991**, *32(37)*, 4969-4972; (d) Ibuka, T.; Habashita, H.; Otaka, A.; Fujii, N.; Oguchi, T.; Uyehara, T.; Yamamoto, Y. *J. Org. Chem.* **1991**, *56*, 4370-4382; (e) Ikuda, T.; Habashita, H.; Funakoshi, S.; Fujii, N.; Oguchi, Y.; Uyehara, T.; Yamamoto, Y. *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 801-803.
42. Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. *J. Chem. Soc. Perkin I* **1982**, 307-314; see also Kaltenbronn, J. S.; Hudspeth, J. P.; Lunrey, E. A.; Michniewicz, B. M.; Nicolaides, E. D.; Repine, J. T.; Roark, W. H.; Stier, M. A.; Tinney, F. J.; Woo, P. K. W.; Essenburg, A. D. *J. Med. Chem.* **1990**, *33*, 838-845; for a report of isolation of a Z-CH=CH isostere as the minor component of a 10:1 E/Z mixture, with comparison of biological activities.
43. Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*, J. Wiley & Sons, Inc. 1967, p. 1276; thus-activated zinc was washed with 3 portions of 50% HOAc before use.
44. Alternatively, when the reaction was worked up by partitioning into an equal volume of 1:1 CCl_4 / H_2O, washing with CCl_4 3 times, and concentrating the aqueous layer, HPLC showed none of the 20.8-min.

peak; which has been identified (see below) as *bis*- SH-IIb presumed to arise from partial reduction of **IIb** during the Zn workup)

45. (a) Thermos, K.; Reisine, T. *Mol. Pharmacol.* **1988**, *33*, 370-377; (b) Reisine, T. *Endocrinology* **1985**, *116*, 2259-2266.