Synthesis and Biological Activity of NK-1 Selective, N-Backbone Cyclic Analogs of the C-Terminal Hexapeptide of Substance P

Gerardo Byk,[†,§] David Halle,[‡] Irena Zeltser,[†] Gal Bitan,[†] Zvi Selinger,[‡] and Chaim Gilon*[,†]

Departments of Organic Chemistry and Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

Received February 26, 1996[⊗]

The application of the concept of backbone cyclization to linear substance P (SP) analogs is presented. We describe the synthesis, characterization, and biological activity of a series of backbone-to-amino-terminus cyclic analogs of the C-terminal hexapeptide of SP. These analogs were designed on the basis of NMR data and molecular modeling of the selective NK-1 analog WS-septide (Ac[Arg^6,Pro^9]SP$_{6-11}$). A series of peptides with the general formula: cyclo[-(CH$_2$)$_m$-NH-CO-(CH$_2$)$_n$-CO-Arg-Phe-Phe-N-]-CH$_2$-CO-Leu-Met-NH$_2$ (n = 2, 3, 6 and m = 2, 3, 4) was synthesized by solid phase methodology using Fmoc chemistry for the main chain and Boc chemistry for the building units [N^{α}-(ω-aminoalkyl)Gly] side chains. Cyclization was performed on the resin after removal of the Boc protecting group from the ω-aminoalkyl chain. Cyclic and precyclic analogs were compared. They were purified by HPLC and characterized by mass spectroscopy and NMR. Biological activity and selectivity to the NK-1 neurokinin receptor were found to depend on cyclization and the ring size: The most active and selective analog had a ring of 20 atoms. This analog was found to have enhanced metabolic stability in various tissue preparation compared to WS-septide.

Introduction

Tachykinins are a large family of peptides which share the common sequence Phe-Xaa-Gly-Leu-Met-NH$_2$ at their C-terminus and show a great variation in their N-terminal sequence. While most of the tachykinins are from nonmammalian origin, the three peptides called neurokinins: substance P (SP), neurokinin A (NKA), and neurokinin B (NKB), were identified in mammals (Figure 1).

Extensive studies implicated the neurokinins in a variety of physiological functions such as transmission of pain stimuli, exocrine gland secretion, intestinal motility, vasodilation, neuronally mediated inflammatory skin reaction, and behavioral responses.[1] The large variety of biological activities exerted by the neurokinins has indicated the existence of several receptors (or receptor subtypes). The three neurokinin receptors are NK-1, NK-2, and NK-3. Even though the neurokinin receptors are activated preferentially by each of the neurokinins (NK-1 by SP, NK-2 by NKA, and NK-3 by NKB), the selectivity of each neurokinin to its receptor is rather poor.[1,2] Thus, for example, NKB activates both the NK-1 and NK-3 receptors with almost the same EC$_{50}$ (4.2 and 1.3 nM, respectively). Since apparently each of the receptor subtypes mediates distinct physiological functions, it is essential to have receptor selective agonists and antagonists in order to relate the exact physiological function to each receptor. In our laboratory the design of selective neurokinin receptor agonists came out of a systematic study in which each of the peptide bonds in the C-terminal hexapeptide of SP was replaced by an N-methylated peptide bond. These studies revealed that N-methylation of specific peptide bonds resulted in selective loss of biological activity on two out of the three receptor subtypes, while activity on the third receptor remained unchanged. Thus, N-methylation of the Phe^7−Phe^8 peptide bone inferred selectivity to the NK-3 receptor whereas N-methylation of the Phe^8−Gly^9 peptide bone caused selectivity to the NK-1 receptors.[3] On the basis of structure-activity relationship studies, we prepared the NK-1 selective agonist WS-septide (Ac[Arg^6,Pro^9]SP$_{6-11}$ and the highly selective NK-3 agonist senktide (succ[Asp^6,N-MePhe^8]-SP$_{6-11}$;[4,5] (see Table 1). Senktide was found to be metabolically stable in many tissues,[6] whereas WS-septide was found to be metabolically unstable (Figure 3). This fact prompted us to design novel metabolically stable NK-1 agonists.

SP	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH$_2$
NKA	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH$_2$
NKB	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH$_2$

Figure 1. Amino acid sequences of the mammalian tachykinins (neurokinins).

Design of Backbone Cyclic Analogs

Theoretical energy calculations[7] indicated that the C-terminal hexapeptide of SP had a folded conformation stabilized by hydrogen bonding between the C-terminal carboxamide and the carboxamide of Gln^6. This model, which was later corroborated by NMR studied,[8,9] prompted researchers to prepare cyclic analogs of SP in order to stabilize the C-terminal hexapeptide in the predicted bent conformation. However, although some success was made in preparation of NK-2 antagonists,[10,11] all the cyclic analogs in which cyclization involved in the C-terminal hexapeptide were either inactive or 2−3 orders of magnitude less active as agonists than SP itself.[12−18]

There were two possible explanations for the lack of activity of these cyclic analogs: Structure−activity relationship studies indicated that only minor modifications were permissible in the structure or chirality of the side chains constituting the active region of SP-(-Phe-Phe-Gly-Leu-Met-NH$_2$).[1] It was, hence, plausible

† Department of Organic Chemistry.
‡ Department of Biological Chemistry.
§ Present address: UMR-133 CNRS/RPR, Rhône Poulenc Rorer, Centre de Recherche de Vitry-Alfortville, 13 Quai Jules Guesde, B.P. 14, 94403 Vitry-sur-Seine, France.
⊗ Abstract published in *Advance ACS Abstracts,* July 1, 1996.

S0022-2623(96)00154-9 CCC: $12.00

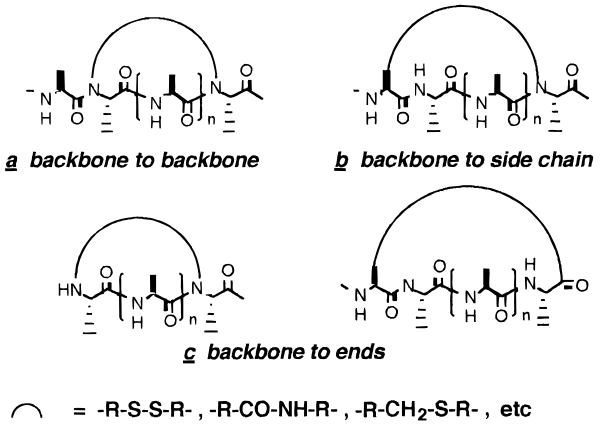

Figure 2. Various modes of N-backbone cyclization.

that the loss of activity of the cyclic peptides stemmed from alterations in the side chains or the C-terminal carboxamide necessary for receptor activation. It was also possible that the cyclization conferred constraints that prevented the peptides from attaining the active conformation. We attributed the loss of biological activity of the cyclic peptides to structural rather than conformational causes.

Conformational analysis of senktide and WS-septide using NMR studies in DMSO-d_6[19] revealed that each of these selective linear agonists had an exclusive predominant conformation in DMSO-d_6. The neurokinin NK-1 receptor selective analog WS-septide adopted mainly a type I β conformation, stabilized by hydrogen bonds between Phe^8 C═O and Met^{11} N−H and between Arg^6 N−H^G and Met^{11} carboxamide. The existence of a predominant conformation in this analog was attributed to the incorporation of Pro^9 which stabilized the conformation. We then faced the question: Was the predominant conformation of WS-septide found by NMR studies relevant to its biologically active conformation? This question could be answered by solving the 3D structure of the WS-septide:NK-1 complex. Since this information has not been available, the problem was approached by the design and synthesis of constrained WS-septide cyclic analogs based on the findings of the NMR studies.

In order to overcome the limitations of the classical cyclization methods, which led mostly to inactive peptides, we suggested a new general concept of backbone cyclization (BC) which would not necessarily alter either side chains or the carboxyl and amino termini. According to this method, cyclization can be accomplished by adjoining atoms in the peptide backbone rather than side chains or terminal groups (Figure 2.) N$^\alpha$ and/or C$^\alpha$ hydrogens are replaced by ω-functional alkyl chains, which can then be interconnected to form the desired ring.[21,22]

Results and Discussion

Molecular models of the predominant conformation of WS-septide based on the NMR data indicated that conformational restriction could be imposed on WS-septide by cyclization in which the nitrogen of the Phe^8−Pro^9 peptide bond would be attached to the amino terminal Arg^6 by a ring. Since the molecular models could not define the exact distance between the nitrogen of the terminal Arg and the nitrogen of the Phe−Pro bond, we prepared a series of six cyclic homologous peptides containing a lactam ring, which differed from each other by the number of atoms in the ring—ranging from 17 atoms (peptide **1**, Table 1) to 22 atoms (peptide **6**, Table 1).

Table 1. Structure, Activity, and Selectivity of Linear, Backbone Cyclic and Precyclic SP Analogs

Peptide	Structure	Biological activity (EC_{50}, nM)		
		NK-1	NK-2	NK-3
1	CO-NH—(CH$_2$)$_2$ / (CH$_2$)$_2$-CO-Arg-Phe-Phe-N-Gly-Leu-Met-NH$_2$	4000	>50,000	>10,000
2	CO-NH—(CH$_2$)$_3$ / (CH$_2$)$_2$-CO-Arg-Phe-Phe-N-Gly-Leu-Met-NH$_2$	180	>50,000	>10,000
3	CO-NH—(CH$_2$)$_3$ / (CH$_2$)$_3$-CO-Arg-Phe-Phe-N-Gly-Leu-Met-NH$_2$	11	>50,000	>10,000
4	CO-NH—(CH$_2$)$_3$ / (CH$_2$)$_4$-CO-Arg-Phe-Phe-N-Gly-Leu-Met-NH$_2$	5	>50,000	>10,000
5	CO-NH—(CH$_2$)$_6$ / (CH$_2$)$_2$-CO-Arg-Phe-Phe-N-Gly-Leu-Met-NH$_2$	160	>50,000	1,000
6	CO-NH—(CH$_2$)$_6$ / (CH$_2$)$_3$-CO-Arg-Phe-Phe-N-Gly-Leu-Met-NH$_2$	60	>50,000	1,000
7	CO-NH—(CH$_2$)$_2$ / (CH$_2$)$_2$-CO-Gly-Arg-Phe-Phe-N-Gly-Leu-Met-NH$_2$	>10,000	>50,000	>10,000
8	(CH$_2$)$_3$-NH$_2$ / Ac-Arg-Phe-Phe-N-Gly-Leu-Met-NH$_2$	300	>100,000	2,000
9	CO-NH-CH$_3$ (CH$_2$)$_3$-NH-Ac / (CH$_2$)$_4$-CO-Arg-Phe-Phe-N-Gly-Leu-Met-NH$_2$	400	>100,000	2,000
WS-Septide	Ac-Arg-Phe-Phe-Pro-Leu-Met-NH$_2$	3	>200,000	>50,000
Senktide	Succ-Asp-Phe-MePhe-Gly-Leu-Met-NH$_2$	35,000	>200,000	0.5

Peptide Synthesis. Backbone cyclic peptides were synthesized by the solid phase peptide synthesis methodology using Boc chemistry for the first two amino acids (Leu and Met) and then Fmoc chemistry for the rest of the peptide. Boc was used for the protection of the ω-amino group of the N^α-(ω-aminoalkyl)Gly building units.[23,24] No special procedure was required for the coupling of the Fmoc-N^α-[ω-(Boc-amino)alkyl]Gly-OH building units to the growing peptide. After the removal of the Fmoc protecting group, repeated couplings with BOP were used to ensure coupling of Fmoc-Phe to the secondary α-amine of the Gly building units. Monitoring the coupling by Kaiser test was impossible. This difficultly was overcome using amino acid analysis of peptide-resin hydrolysates after every coupling to the secondary amine and checking its completion. Generally three repetitions were necessary to complete the condensation.

After the coupling of Fmoc-Arg and removal of the Fmoc protecting group, the peptides were reacted with dicarboxylic acid spacers (as their anhydrides) with a catalytic amount of DMAP. Then the Boc protecting group on the N^α-(ω-aminoalkyl)Gly unit was removed, and the peptide was cyclized on the resin. In the case of peptide **4** ($n = 3$, $m = 4$), the peptide-resin obtained was divided into two parts after the appropriate wash-

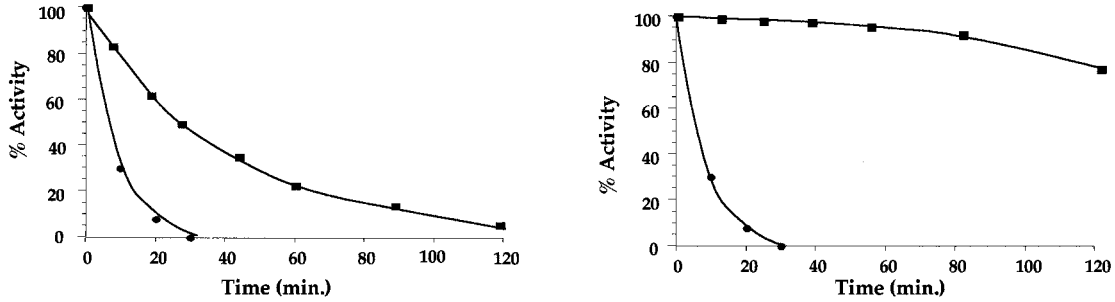

Figure 3. Time course of degradation of WS-septide (●) and cycloseptide (■) by slices of (left) liver and (right) parotid gland.

ing and drying. In one part the free amine was acetylated and the free carboxyl group was amidated with *N*-methylamine to yield the counterpart precyclic peptide **9** (Table 1). The second part of the peptide-resin was subjected to cyclization. Another precyclic peptide, peptide **8**, Ac[Arg^6,N^α-(ω-aminopropyl)Gly^9]SP$_{6-11}$, was obtained by acetylation of the N-terminus of the linear peptide before removal of the Boc protecting group. The cyclizations were carried out on the resin using BOP reagent. The time required for cyclization was ring size dependent. For example for the cyclization of peptide **1** (ring size = 17 atoms), the cyclization step was repeated six times to get almost negative Kaiser test (6 days). Cyclization times were significantly shorter—3 days for peptides **2**−**4**—coming to a few hours in the cases of peptides **5** and **6**. The quality of the crude products (after cleavage from the resin) depended inversely on the cyclization time. Shorter cyclization times gave crude peptides of higher quality.

It was previously suggested[25] that conformational factors dictated the speed and extent of cyclization. The difficulty in the cyclization step encountered in peptide **1** was probably due to intensified steric constraint exerted on the peptide upon cyclization. Moreover, the extension of cyclization time enhanced side reactions such as dimerization, partial cleavage from the resin, partial racemization, etc. (no data). After cyclization, the peptides were cleaved from the resin by HF and purified by RP-HPLC. Characterization of the purified cyclic and precyclic peptides was performed by fast atom bombardment (FAB) MS, amino acid analysis, and, in some cases, NMR. FAB MS/MS sequencing was used to unequivocally establish the structure of the cyclic peptides. Peptides **3**−**5** and **7** were fully characterized by NMR.[26,27]

Biological Activity and Metabolic Stability. The biological activity of the cyclic and precyclic peptides was assayed on three different smooth muscle preparations: guinea pig ileum (GPI), rat vas deferens (RVD), and rat portal vein (RPV). Assay of the activity of the different analogs in these systems provided quantitative determination of their selectivity toward the three neurokinin receptors (NK-1 and NK-3 receptors in GPI, NK-2 in RVD, and NK-3 in RPV). Blocking the action of acetylcholine, which is released by the neuronal NK-3 receptor, by atropine provided a selective GPI assay for the NK-1 receptor. The resistance of the peptide analogs to digestion by proteases was studied by incubating the peptides with slices or homogenates of liver and parotid gland and measuring the residual activity after incubation by the GPI assay.

The activity and selectivity of peptides **1**−**6** (Table 1) depended greatly on the ring size. Optimal activity and full selectivity to the NK-1 receptor were exhibited by the 20-membered ring analog **4** [EC_{50} (nM): NK-1, 5; NK-2, >200 000; NK-3, >10 000]. This particular cyclic analog, which we have termed *cycloseptide*, had comparable activity and selectivity to the linear parent peptide WS-septide. Peptide **1**, which had a smaller ring, was 3 orders of magnitude less active compared to cycloseptide. We attributed this loss of activity to enhanced conformational constraint which distorted the Arg^6-Phe^7-Phe^8 region. The decrease in selectivity of the larger rings containing analogs **5** and **6** (21 and 22 atoms, respectively) was attributed to ring flexibility which allowed activation of the NK-3 receptor.

In order to demonstrate that the high biological activity and selectivity of cycloseptide indeed resulted from cyclization, the activity and selectivity of two precyclic analogs of cycloseptide (analogs **8** and **9**) were compared to that of cycloseptide (see Table 1). The low activity and lack of selectivity of the precyclic linear analogs proved the importance of cyclization in achieving the conformational restriction required for activity and selectivity.

Cycloseptide also showed protracted activity in various tissues. In Figure 3 the time course of the degradation of cycloseptide is compared to that of WS-septide in liver (Figure 3, left) and parotid gland slices (Figure 3, right). The linear WS-septide was metabolically unstable, and its activity was lost already after a few minutes (half-life in liver slices was 4 min and in parotid slices 6 min). Cycloseptide retained about 80% of its original activity even after 120 min of incubation with parotid gland slices. A similar pattern of behavior could be seen with liver slices: 50% of the biological activity of cycloseptide was retained after 30 min of incubation with liver slices, whereas WS-septide was deactivated almost immediately.

The protracted activity of cycloseptide in parotid and liver slices was attributed solely to cyclization. Both cycloseptide and WS-septide contain N-alkylated amino acids in position 9 [N^α-(ω-aminoalkyl)Gly unit and Pro, respectively]. In both analogs the amino and carboxy terminals are blocked, and both have an Arg−Phe bond susceptible to degradation by trypsin or trypsin-like enzymes. Nevertheless, cycloseptide was found much more stable to degradation the WS-septide.

Structure−Activity Relationships Studies. Taking into account the biological activity and selectivity of the resulting backbone cyclic peptides **1**−**6**, we selected the highly active and selective NK-1 agonist cycloseptide and synthesized a closely related cyclic peptide in which the chain −$(CH_2)_3$-NH-CO-$(CH_2)_4$-CO-Arg− in cycloseptide was replaced by the chain −$(CH_2)_2$-NH-CO-$(CH_2)_2$-CO-NH-CH_2-CO-Arg− in peptide **7** (see

Table 2. Physical Data of Cyclic and Precyclic Analogs **1**−**9**

peptide	*m*	*n*	ring size	FAB MS (calcd MW)	amino acid analysis					
					Arg	Phe	Leu	Met	Gly unit	Gly
1	2	2	17	894 (894.1)	1.0	2.0	1.0	0.9	1.0	
2	2	3	18	908 (908.1)	0.9	2.0	1.0	1.0	0.9	
3	3	3	19	923 (922.2)	1.0	2.0	1.0	1.0	1.1	
4	4	3	20	937 (936.2)	1.0	2.0	1.0	0.9	1.0	
5	2	6	21	951 (950.2)	0.9	2.0	1.0	1.0	1.0	
6	3	6	22	964 (964.2)	0.9	2.0	1.0	1.0	1.0	
7	2	2	20	951 (951.2)	0.7	1.8	1.0	0.9	1.1	1.1
8[a]		3		856 (855.5)	0.8	2.0	2.0	0.9	1.1	
9[a]	4	3		955 (955.3)	0.9	1.8	1.0	0.9	1.0	

[a] Precyclic analog.

Table 1). Both analogs had the same sequence, the same location of the ring, and the same ring size (20 atoms). The two analogs differed in the number of methylene groups vs amide bonds in the ring: Analog **7** had five methylene groups and six amide bonds, whereas cycloseptide had seven methylene groups and five amide bonds. Peptide **7** was found to be completely inactive toward all of the neurokinin receptors (Table 1).

We assumed that the loss of activity in peptide **7** was due to excessive conformational constraint: The backbone of the ring of peptide **7** adopted a conformation which precluded the bioactive conformation essential for the binding and activation of any of the neurokinin receptors. This assumption was validated by detailed NMR studies and MD calculations of analogs **3**−**5** and **7**.[27] Comparison of the conformations of the active analogs **4** and **5** to that of the inactive analog **7** revealed that while the ring conformations of the active analogs were very similar, they differed considerably from that of the inactive analog **7**.[27] These results prompted us to propose the bioactive conformation for the backbone of the sequence −Arg-Phe-Phe− in cycloseptide.

Conclusions

In this article we demonstrated the feasibility of backbone cyclization as a tool for generation of selective and metabolically stable peptidomimetic analogs of linear peptides, by the synthesis of potent cyclic analogs of the C-terminal hexapeptide of SP. One of these analogs, cycloseptide (peptide **4**, Table 1), showed compared activity and selectivity to the most active and NK-1 selective linear analog, WS-septide. Moreover, cycloseptide was metabolically stable, as indicated by incubation with different tissue preparations, whereas WS-septide was not.

Precyclic linear analogs of cycloseptide (peptides **8** and **9**) showed low biological activity and selectivity, thus suggesting that the high biological activity and selectivity of cycloseptide resulted directly from the cyclization of the peptide. Comparative studies of cycloseptide and its inactive analog **7** demonstrated that the ring conformations of the active and nonactive analogs were different and that the backbone in the ring portion of cycloseptide was stabilized in the bioactive conformation essential for the binding and activation of the NK-1 receptor, or very close to it.

Experimental Section

Equipment and Materials. Fmoc-amino acids were purchased from Chemical Dynamics Corp. Boc-amino acids were purchased from Nova Biochem, Switzerland. BOP reagent was purchased from Richelieu Biotechnologies Inc., Canada. The Fmco-N^{α}-[ω-(Boc-amino)alkyl]Gly-OH building units were synthesized according to procedures previously described.[23,24]

FAB MS was performed on a ZAB-3HF FAB/tandem mass spectrometer or on an API-III LC/MS/MS instrument. Amino acid analysis was performed on LKB-4400 apparatus equipped with an SP-4100 computing integrator and a double channel detector at 440 and 570 nm. Amino acids were detected by ninhydrin after postcolumn derivatization. Hydrolysis of the peptides: 1 mg of the peptide was heated at 110 °C in the presence of 6 N HCl in a sealed tube for 24 h. After the hydrolysis the samples were dried over KOH and dissolved in sodium citrate buffer, 3.3 M (pH = 2.2).

Peptides were synthesized by the manual solid phase method using MBHA resin (1% cross-linked 80−180 mesh, 0.9 mequiv/g substitution level) obtained from Peninsula, U.K. They were cleaved from the resin with dry HF in a Peptide Institute, Inc. (Japan) apparatus Type I. All peptides were purified by semipreparative reverse phase HPLC on a Merck-Hitachi 655A apparatus equipped with an LC-5000 gradient pump and a UV−vis detector set at 220 nm. The columns for the analytical HPLC were Lichrospher RP-8 and RP-18 from Merck (5 × 250 mm) and for the semipreparative purifications HIBAR RP-8 and RP-18 (9 × 250 mm). The purity of the peptides was checked by analytical HPLC using MeCN (0.085% TFA)/TDW (0.1% TFA) gradients. Final products were obtained as lyophilisates. Based on the amount of amine on the resin, the yields for the cyclic analogs ranged between 10% and 40%.

General Procedures. 4-Methylbenzhydrylamine resin was used for synthesis of all peptides. Coupling of Boc- or Fmoc-amino acids was performed using 6-fold excess of the protected amino acid, 6-fold excess of BOP, and 12-fold excess of diisopropylethylamine (DIEA) in DMF for 2 h, after preactivation of the protected amino acid and the coupling reagent for 5 min. After coupling of the first amino acid to the resin, the residual free amines were capped using 10-fold excess of acetic anhydride, 10-fold excess of DIEA, and 1 equiv of 4-(dimethylamino)pyridine (DMAP). Fmoc was deprotected with 20% piperidine in DMF for 30 min. Boc was deprotected with 55% TFA in DCM for 2 min and then for an additional 30 min followed by neutralization with 5% DIEA in DMF. Acylation with succinic and glutaric anhydrides was performed in DMF using 6-fold excess of anhydride and 1 equiv of DMAP. Adipic acid anhydride was prepared by preactivation of 6 equiv of the dicarboxylic acid for 30 min with 6 equiv of diisopropylcarbodiimide (DIC) in DMF and then addition of this mixture to the peptide-resin together with 6 equiv of DIEA and 1 equiv of DMAP.

Cyclization was performed by repeated reaction cycles with 6-fold excess of BOP and DIEA in DMF. Peptides were cleaved from the resin and completely deprotected by treatment with HF. The reaction was carried out for 90 min at −5 °C with 20 mL of HF/g of resin in the presence of 1 mL of anisole/g of resin. After evaporation of the HF, the resin was washed six times with hexane and six times with ether, and the dried resin-peptide was extracted four times with 10 mL of 30% acetic acid. Lyophilization of the combined acetic acid extracts yielded the crude products which were purified as described above. Physical data for peptides **1**−**9** are given in Table 2.

Biological Assays. Guinea pig ileum (GPI) assay was performed according to the procedures described by Wormser et al.[3] Rat vas deferens (RVD) assay was performed according to the procedures described by Chorev et al.[28] Rat portal vein (RPV): Rat was sacrificed by decapitation. The abdomen was cut open, and all the internal organs were moved to the side. The portal vein was tied at both ends within the animal and cleaned from all surrounding tissues. The cleaned tissue was immersed in a bath containing Tirode solution, aerated with a mixture of CO_2:O_2 (5:95). One of the tied ends was attached to a glass hook and the other end to a transducer lever, in order to measure the contraction. Tension was about 0.5 g. The tissue was left in the bath at 37 °C for 1 h; then the peptides were added at 20 min intervals, to prevent desensitization of the receptor. Resistance to digestion by proteases assay was performed according to the procedures described by Chorev et al.[28]

Abbreviations: Fmoc, 9-fluorenylmethoycarbonyl; BOP, benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate; DIEA, diisopropylethylamine; DMAP, 4-(dimethylamino)pyridine; DMSO, dimethyl sulfoxide; TFA, trifluoroacetic acids; SPPS, solid phase peptide synthesis.

Acknowledgment. The authors are grateful to Peptor Co. Rehovot, the GIF, and the Center for Pain, The Hebrew University, Jerusalem, for their financial support. The authors are also grateful to Dr. J. W. Metzger and Prof. G. Jung from the Eberhard-Karls Universität, Tübingen, Germany, for performing the API LC/MS/MS determinations and to Dr. K. Eckart and Prof. H. Schwartz from the Technical University, Berlin, Germany, for performing the FAB MS measurements.

References

(1) Regoli, D.; Boudon, A.; Fouchere, J.-L. Receptors and Antagonists for Substance P and Related Peptides. *Pharmacological Rev.* **1994**, *46*, 551−599.
(2) Laufer, R.; Wormser, U.; Friedman, Z. Y.; Gilon, C.; Chorev, M.; Selinger, Z. Neurokinin B is a preferred agonist for neuronal Substance P receptor and its action is antagonized by enkephalin. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7444−7448.
(3) Wormser, U.; Laufer, R.; Hart, Y.; Chorev, M.; Gilon, C.; Selinger, Z. Highly selective agonists for Substance P receptor subtypes. *EMBO J.* **1986**, *5*, 2805−2808.
(4) Laufer, R.; Gilon, C.; Chorev, M.; Selinger, Z. Characterization of a neurokinin B receptor site in rat brain using a highly selective radioligand. *J. Biol. Chem.* **1986**, *261*, 10257−10263.
(5) Laufer, R.; Gilon, C.; Chorev, M.; Selinger, Z. Desensitization with a Selective Agonist Discriminates between Multiple Tachykinin Receptores. *Pharmacol. Exp. Ther.* **1986**, *245*, 639−643.
(6) Halle, D. Ph.D. Thesis, submitted to the Senate of the Hebrew University, October 1993.
(7) Manavalan, P.; Momany, F. A. Conformational Energy Calculations on Substance P. *Int. J. Pept. Protein Res.* **1982**, *20*, 351−356.
(8) Chassaing, G.; Convert, O.; Laville, S. Preferencial Conformation of Substance P in Solution. *Eur. J. Biochem.* **1986**, *154*, 77−85.
(9) Young, J. K.; Anklin, C.; Hicks, R. P. NMR and Molecular Modeling Investigations of the Neuropeptide Substance P in the Presence of 15 mM Sodium Dodecyl Sulfate Micelles. *Biopolymers* **1994**, *34*, 1449−1462.
(10) Williams, B. J.; Curtis, N. R.; McKnight, A. T.; Maguire, J.; Foster, A.; Tridgett, R. Development of NK-2 Selective Antagonists. *Regul. Pept.* **1988**, *21*, 189P.
(11) Maggi, C. A.; Quartara, L.; Giuliani, S.; Patacchini, R. Tachykinin NK-2 Receptor Antagonists. *Drugs Future* **1993**, *18*, 155−158.
(12) Neubert, K.; Bergmann, J.; Mansfield, J.; Hartrodt, B.; Barth, A.; Jakubke, H. D. Synthesis of Cyclic and Cyclically Branched Tachykinin Partial Sequences. 1. Synthesis of the HomodeticCyclic Eledoisin$_{6-11}$ Hexapeptide. *Pharmazie* **1985**, *40*, 456−459.
(13) Neubert, K.; Hartrodt, B.; Mehlis, B.; Ruger, M.; Bergmann, J.; Jakubke, H. D.; Barth, A. Synthesis of Cyclic and Cyclically Branched Tachykinin Partial Sequences. 2. Synthesis of the HomodeticCyclic Substance P$_{6-11}$ Hexapeptide. *Pharmazie* **1985**, *40*, 532−535.
(14) Chassaing, G.; Lavielle, S.; Ploux, O.; Julien S.; Convert, O.; Marquet, A.; Beaujouan, J. C.; Torrens, Y.; Glowinsky, J. Biological and Conformational Studies of Cyclic Analogs of Substance P. In *Peptides 1984*; Ragnarsson, U., Ed.; Almquist & Wiksell: Stockholm, 1985; pp 345−348.
(15) Sandberg, B. E. B.; Bishai, W. R.; Hanna, P. Synthesis of Cyclic Analogs Based on Substance P. In *Peptides 1984*; Ragnarson, U., Ed.; Almquist & Wiksell: Stockholm, 1985; pp 369−372.
(16) Theodoropoulos, D.; Poulos, C.; Gatos, D.; Cordopatis, D.; Escher, E.; Mizrahi, J.; Regoli, D.; Dalietos, D.; Furst, A.; Lee, T. D. Conformationally Restricted C-Terminal Peptides of Substance P. Synthesis, Mass Spectral Analysis and Pharmacological Properties. *J. Med. Chem.* **1985**, *28*, 1536−1539.
(17) Darman, P. S.; Landis, G. C.; Smits, J. R.; Hirning, L. D.; Gulya, K.; Yamamura, H. I.; Burks, T. F.; Hruby, V. J. Conformationally Restricted Cyclic Analogues of Substance P: Insight into the Receptor Binding Process. *Biochem. Biophys. Res. Commun.* **1985**, *127*, 656−662.
(18) Mutulis, F. K.; Mutule, I. E.; Maurops, G. H.; Sekacis, I. P.; Grigoyeva, V. D.; Kukaine, E. M.; Golubeva, V. V.; Myshliakova, N. V.; Klusha, V. E.; Chipens, G. I. Substance P - a New Type of Cyclic Analogs. *Bioorg. Khim.* **1985**, *11*, 1276−1278.
(19) Levian-Teitelbaum, D.; Kolodny, N.; Chorev, M.; Selinger, Z.; Gilon, C. ^{1}H-NMR studies on receptor-selective Substance P analogs reveal distinct predominant conformations in DMSO-d_6. *Biopolymers* **1989**, *28*, 51−64.
(20) Gilon, C.; Halle, D.; Chorev, M.; Selinger, Z.; Byk, G. Backbone Cyclization: A New Method for Conferring Conformational Constraint on Peptides. *Biopolymers* **1991**, *31*, 745−750.
(21) Gilon, C.; Halle, D.; Chorev, M.; Selinger, Z.; Byk, G. SAR Studies of Cycloseptide: Effects of Cyclization and Charge at Position 6. In *Peptides 1992*; Smith, J. A., Rivier, J., Eds.; ESCOM Science BV: The Netherlands, 1993; pp 476−477.
(22) Gilon, C.; Zeltser, I.; Rashti-Behar, V.; Muller, D.; Bitan, G.; Halle, D.; Bar-Akiva, G.; Selinger, Z.; Byk, G. Backbone cyclization as a tool for imposing conformational constraint on peptides. In *Peptide Chemistry 1992*; Yanaihara, N., Ed.; ESCOM Science BV: The Netherlands, 1993; pp 482−485.
(23) Byk, G.; Gilon, C. Building Units for N-Backbone Cyclic Peptides. 1. Synthesis of Protected N-(ω-Aminoalkylene)amino Acids and Their Incorporation into Dipeptide Units. *J. Org. Chem.* **1992**, *57*, 5687−5691.
(24) Muller, D.; Zeltser, I.; Bitan, G.; Gilon, C. Building Units for N-Backbone Cyclic Peptides. 3. Synthesis of Protected N-(ω-Aminoalkylene)amino Acids and N-(ω-Carboxylkylene)amino Acids. *Tetrahedron*, submitted for publication.
(25) Schiller, P. W.; Nguyen, T. M.-D.; Lamieur, C. Synthesis and OpioidActivity of Cyclic Dynorphin Analogs. *Tetrahedron* **1988**, *44*, 733−743.
(26) Saulitis, J.; Mierke, D. F.; Byk, G.; Gilon, C.; Kessler, H. Conformation of Cyclic Analogues of Substance P: NMR and Molecular Dynamics in Dimethyl Sulfoxide. *J. Am. Chem. Soc.* **1992**, *114*, 4818−4827.
(27) Golic-Grdadolnik, S.; Mierke, D.; Byk, G.; Zeltser, I.; Gilon, C.; Kessler, H. Comparison of the Conformation of Active and Nonactive Backbone Cyclic Analogs of Substance P as a Tool to Elucidate Features of the Bioactive Conformatio: NMR and Molecular Dynamics in DMSO and Water. *J. Med. Chem.* **1994**, *37*, 2145−2152.
(28) Chorev, M.; Rubini, M.; Hart, Y.; Gilon, C.; Wormser, U.; Selinger, Z. Metabolically Stable Analogues of Substance P: Persistant Action of Partially Modified Retro-Inverso Analogues of Substance P on Rat Parotid and Hypothalamic Slices. *Eur. J. Pharmacol.* **1986**, *127*, 187−195.

JM960154I