Conformationally Constrained Substance P Analogues: The Total Synthesis of a Constrained Peptidomimetic for the Phe^7-Phe^8 Region

Yunsong Tong,[†] Yvette M. Fobian,[†] Meiye Wu,[‡] Norman D. Boyd,[‡] and Kevin D. Moeller*,[†]

The Department of Chemistry, Washington University, St. Louis, Missouri 63130, and The Department of Pharmacology and Experimental Therapeutics Neuropeptide Laboratory, Boston University School of Medicine, 80 East Concord Street, Boston, Massachusetts 02118-2394

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A lactam-based peptidomimetic for the Phe^7-Phe^8 region of substance P has been synthesized. The synthesis used an anodic amide oxidation to selectively functionalize the C_5-position of a 3-phenylproline derivative. The resulting proline derivative was coupled to a Cbz-protected phenylalanine, and an intramolecular reductive amination strategy used to convert the coupled material into a bicyclic piperazinone ring skeleton. The net result was a dipeptide building block that imbedded one of two proposed receptor bound conformations for the Phe^7-Phe^8 region of substance P into a bicyclic ring skeleton. The building block was then converted into a constrained substance P analogue with the use of solid-phase peptide synthesis. A similar intramolecular reductive amination strategy was used to synthesize a substance P analogue having only Phe^7 constrained, and the original 3-phenylproline was converted into a substance P analogue having only Phe^8 constrained. All of the analogues were examined for their ability to displace substance P from its NK-1 receptor.

Introduction

Ideally, constrained peptidomimetics for probing the biological relevance of a proposed peptide conformation can be designed by simply repacing spatially close hydrogens in the proposed conformation with an appropriate bridge. In this way, the backbone is imbedded into a polycyclic ring skeleton that fixes the conformation in place.[1] However, such a transformation on paper often leads to the suggestion of a peptidomimetic that is either difficult or impossible to synthesize in the laboratory. For this reason, we have been working to develop the methodology needed to simplify the syntheses of a variety of constrained amino acid building blocks.[2] As part of this effort, we have been building constrained mimetics for the Phe^7-Phe^8 region of substance P. Substance P is an 11 amino acid peptide having the structure Arg^1-Pro^2-Lys^3-Pro^4-Gln^5-Gln^6-Phe^7-Phe^8-Gly^9-Leu^{10}-Met^{11}-NH_2. It is a member of the mammalian tachykinin family of peptides and has been implicated in a number of disease states including arthritis, asthma, inflammatory bowel disease, and depression.[3] Recently, high-affinity antagonists for substance P/NK_1 binding have shown promise as antidepressant drugs in clinical trials.[3d]

Marshall and Nikiforovich have proposed two low energy conformations of substance P as potential models for the binding of substance P to the NK_1 receptor.[4] These two models were derived by determining the low energy conformations available to both substance P and a series of substance P analogues having a high affinity for the NK_1 receptor. Interestingly, the two proposed conformations differ greatly with respect to the conformation of the critical Phe^7-Phe^8 region of the peptide (Scheme 1, **1** and **2**).[5] This difference suggested that the Phe^7-Phe^8 region of substance P might provide a handle for beginning to assess the relevance of the two proposed models. For this reason, a pair of constrained peptide building blocks (**3** and **4**) for fixing the Phe7-Phe8 region of substance P into the desired conformations were designed. But, how could these building blocks and the corresponding substance P analogues be synthesized, and would the bridges interfere with the binding affinity and potency of the analogues constructed? We report here the synthesis of the first of these substance P analogues (**3**) and the initial evaluation of how each added constraint affects the activity of the analogue.

The Construction of a Bicyclic Piperazinone Building Block for Constraining Both Phe^7 and

[†] Washington University.

[‡] Boston University School of Medicine.

(1) For selected examples of this approach to other systems, see: (a) Wolf, J. P.; Rapoport, H. *J. Org. Chem.* **1989**, *54*, 3164 as well as refs 1−8 therein. (b) Freidinger, R. M.; Perlow, D. S.; Veber, D. F. *J. Org. Chem.* **1982**, *47*, 104. (c) Freidinger, R. M. *J. Org. Chem.* **1985**, *50*, 3631. (d) Zydowsky, T. M.; Dellaria, J. F., Jr.; Nellans, H. N. *J. Org. Chem.* **1988**, *53*, 5607 (e) Kempf, D. J.; Condon, S. L. *J. Org. Chem.* **1990**, *55*, 1390. (f) Kemp, D. S.; McNamara, P. E. *J. Org. Chem.* **1985**, *50*, 5834 and references therein. (g) Nagai, U.; Sato, K. *Tetrahedron Lett.* **1985**, *26*, 647, (h) Hinds, M. G.; Richards, N. G. J.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1447, (i) Paul, P. K. C.; Burney, P. A.; Campbell, M. M.; Osguthorpe, D. J. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 141, (j) Lombart, H. G.; Lubell, W. D. *J. Org. Chem.* **1996**, *61*, 9437.

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(3) (a) Payan, D. G. *Annu. Rev. Med.* **1989**, *40*, 341. (b) Ichinose, M.; Nakajima, N.; Takahashi, T.; Yamauchi, H.; Inote, H.; Takishima, T. *Lancet* **1992**, *340*, 1248. (c) Maggi, C. A.; Patacchini, R.; Roverro, P.; Giachetti, A. *J. Auton. Pharmacol.* **1993**, *13*, 23. (d) Kramer, M. S. et. al. *Science* **1998**, *281*, 1640.

(4) Private communication from Nikiforovich, G. V. and Marshall, G. R. The methods for the calculation have been published: (a) Nikiforovich, G. V. *THEOCHEM* **1986**, *134*, 325. (b) Nikiforovich, G. V. *Int. J. Peptide Protein Res.* **1994**, *44*, 513.

(5) (a) Fobian, Y. M.; d'Avignon, D. A.; Moeller, K. D. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 315. (b) Fobian, Y. M.; Moeller, K. D. In *Methods in Molecular Medicine: Peptidomimetic Protocols*; Kazmierski, W. M., Ed.; Humana Press Inc.: Totowa, NJ, 1998; p 259.

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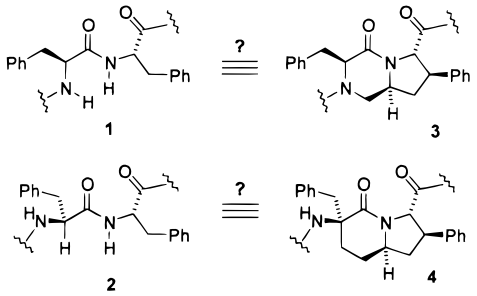
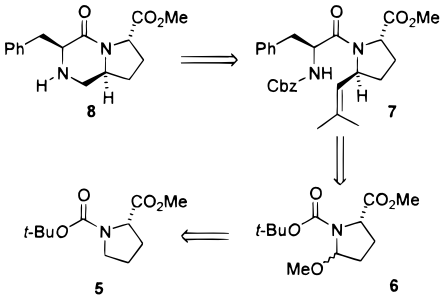
Scheme 1



Scheme 2



Phe[8] of Substance P. Work on the synthesis of constrained substance P analogues began with the development of an approach for constructing the bicyclic piperazinone ring skeleton needed for analogue **3**.[5] This approach (outlined in Scheme 2) utilized an electrochemical amide oxidation to selectively functionalize proline derivative **5**.[6] The resulting methoxylated product **6** was treated with $BF_3 \cdot Et_2O$ and the cuprate derived from 2-methylpropenyllithium to form a 5-vinyl-substituted proline. The Boc group was then removed and the resulting amine coupled to a Cbz protected phenylalanine to form **7**. The olefin in **7** was cleaved using an ozonolysis reaction and the subsequent aldehyde treated with reductive amination conditions to complete the desired ring system. The synthesis could be varied to employ either an allyl-substitued proline (derived from **6**) or an N-terminal β-amino acid in order to construct seven- and eight-membered ring lactam derivatives of the originally proposed ring system.

In principle, one of the strengths of this approach was that it could potentially allow for the use of a wide variety of existing amino acid starting materials. For example, the synthesis of a building block for making substance P analogues such as **3** would involve starting with the known 3(*R*)-phenyl-substituted proline **9**.[7] However, the addition of the phenyl ring was worrisome. The anodic oxidation of 3-substituted proline derivatives had not been studied, and previous amide oxidation reactions had proven to be sensitive to the presence of phenyl substituents.[8] In addition, it was not known how the presence of the phenyl ring would affect the cuprate addition

Scheme 3

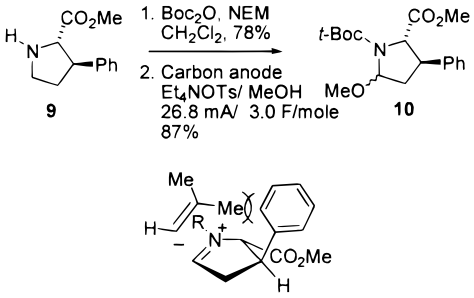


Figure 1.

reaction. Fortunately, the anodic oxidation reaction was not a problem (Scheme 3). The substrate for the electrolysis was obtained by protecting the nitrogen of the 3(*R*)-phenylproline derivative (**9**) with a *t*-Boc group. The carbamate was then subjected to oxidation using a carbon rod anode, a 0.03 M Et_4NOTS in methanol electrolyte solution, and a constant current of 26.8 mA (Scheme 3). A total of 3.0 F/mol was passed. Using these conditions,[9] the oxidation could be run on a multigram scale and led to the formation of an 87% isolated yield of the methoxylated product **10**.

Having successfully methoxylated the amide, attention was turned to the addition of a vinyl group to the substituted proline.[10] Initially, the *N*-acyliminium ion generated from **10** was treated with the vinyl cuprate derived from 2-methylpropenyllithium in analogy to Scheme 2. However, this reaction was not successful, and none of the desired 5-vinyl-substituted proline was obtained. Clearly, the phenyl ring interfered with the incoming nucleophile. This was surprising since earlier cuprate additions to *N*-acyliminium ions derived from proline had not been hindered by substituents, even when a substituent was placed at C_4 of the proline cis to the incoming nucleophile at C_5.[10b] One suggestion for explaining the result obtained from **10** is illustrated in Figure 1, where a complex between a copper atom, the methyl ester of the proline ring, and the double bond of the iminium ion would force the nucleophile to approach C_5 from the face of the ring opposite that of the methyl ester. This model for the reaction was initially forwarded by Wistrand and co-workers[10a] and has been consistent with the stereochemical observations made for a variety of related reactions.[10b] The fact that in the current example the addition was blocked by the phenyl ring argued for a scenario where the nucleophile approached the iminium ion from "over the ring". In this way, the allylic methyl group cis to the anion would encounter the phenyl ring leading to a large steric interaction that blocked the favored approach to the iminium ion. The net result was an unsuccessful addition reaction.

We reasoned that if this suggestion was correct, then the addition reaction would benefit from the use of the

(6) For pioneering work, see: (a) Ross, S. D.; Finkelstein, M.; Peterson, C. *J. Am. Chem. Soc.* **1964**, *86*, 4139. (b) Ross, S. D.; Finkelstein, M.; Peterson, C. *J. Org. Chem.* **1966**, *31*, 128. (c) Ross, S. D.; Finkelstein, M.; Peterson, C. *J. Am. Chem. Soc.* **1966**, *88*, 4657. For reviews, see: (d) Shono, T.; Matsumura, Y.; Tsubata, K. In *Organic Synthesis*; Saucy, G., Ed.; Organic Synthesis Inc.: New York, 1984; Vol. 63, p 206 and references therein. (e) Shono, T. In *Topics in Current Chemistry*; Steckhan, E. Ed.; Springer-Verlag: Berlin, Heidelberg, New York, 1988; Vol. 148, p 131. (f) ref 1b and references therein.

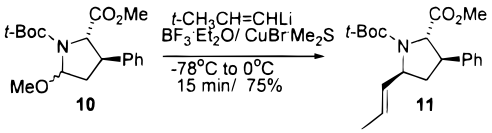
(7) Chung, J. Y. L.; Wasicak, J. T.; Arnold, W. A.; May, C. S.; Nadzan, A. M.; Holladay, M. W. *J. Org. Chem.* **1990**, *55*, 270.

(8) (a) Barrett, A. G. M.; Pilipauskas, D. *J. Org. Chem.* **1991**, *56*, 2787. (b) Li, W.; Hanau, C.; d'Avignon, A.; Moeller, K. D. *J. Org. Chem.* **1995**, *60*, 8155. (c) Fobian, Y. M. Ph.D. Thesis, Washington University, 1996. (d) Moeller, K. D.; Wang, P. W.; Tarazi, S.; Marzabadi, M. R.; Wong, P. L. *J. Org. Chem.* **1991**, *56*, 1058.

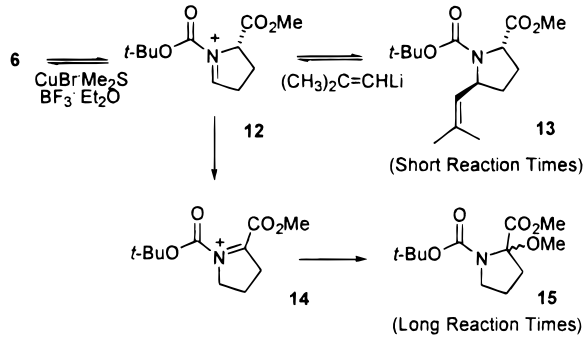
(9) (a) Wong, P. L.; Moeller, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 11434. (b) Alternatively, a 6 V lantern battery can be used for exploring a new electrolysis reaction: Frey, D. A.; Wu, N.; Moeller, K. D. *Tetrahedron Lett.* **1996**, *37*, 8317.

(10) Wistrand, L.-G.; Skrinjar, M. *Tetrahedron* **1991**, *47*, 573. (b) Collado, I.; Ezquerra, J.; Pedregal, C. *J. Org. Chem.* **1995**, *60*, 5011.

Scheme 4



Scheme 5

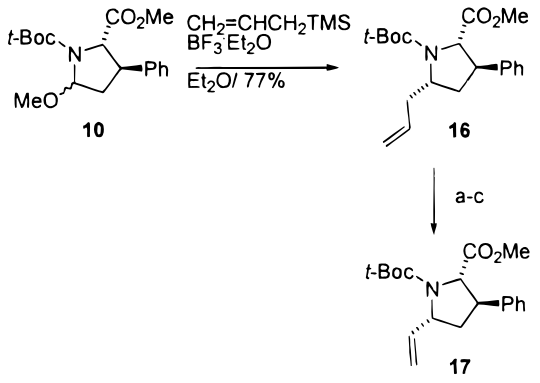


anion generated from *trans*-1-bromopropene. In this case, the nucleophile would have a proton cis to the anion and the size of the steric interaction with the phenyl ring would be greatly reduced. Since the synthesis called for a subsequent cleavage of the olefin, the change in substitution of the olefin product would not matter. In practice, the use of a cuprate derived from *trans*-1-bromopropene led to a 75% isolated yield of the desired addition product (Scheme 4). In addition, a small amount of a second isomer, assumed to be the cis product, was obtained. At this point, the isomers could not be separated. The stereochemistry of the major product was assigned with the use of 2-D NOE data after **11** was converted into the bicyclic building block (Scheme 7).[11] This assignment is discussed below. The formation of the 5(S) isomer in this reaction was consistent with the previous synthesis of **8** and the picture for the addition illustrated in Figure 1. It was clear from this work that the cuprate addition could not be made to afford a product where the incoming vinyl group and the methyl ester group wound up cis to each other. Depending on the size of the steric interaction, either the reactions afforded a high yield of the trans product, or the addition reaction failed.

On a final note, the short reaction time for the cuprate addition (15 min) was important. Longer reaction times led to the generation of a 2-methoxyproline-derived side product. The formation of this side product was consistent with what was observed for the cuprate-based addition of 2-methylpropene to the *N*-acyliminium ion derived from **6** (Scheme 5). In this case, short reaction times led to high yields of the vinyl-substituted product (**13**). When the reaction was run for 15 min, an 89% isolated yield of **13** was obtained.[5b] However, after 20 min the yield of the product decreased to 65−70%. This decrease in yield was accompanied by an increase in the formation of the 2-methoxyproline byproduct **15**. After 30 min, a 54% yield of the vinyl substituted product **13** was obtained along with 41% of **15**. Apparently, the formation of the 5-vinyl-substituted product **13** was reversible. Given enough time, the iminium ion isomerized from **12** to **14** and then trapped methoxide at C_2 to generate **15**. In the past, we

(11) See, for example: Kao, J.; Li, W.; Moeller, K. D. *Magn. Reson. Chem.* **1997**, *35*, 267.

Scheme 6[a]



[a] Reagents: (a) (i) O_3, MeOH, CH_2Cl_2, (ii) $NaBH_4$, 75%; (b) *o*-NO_2-Phe-SeCN, (*n*-Bu)$_3$P, THF, 72%; (c) (i) *m*-CPBA, CH_2Cl_2, −78 °C, (ii) Me_2S, Et_3N, −78 °C to rt, 92%.

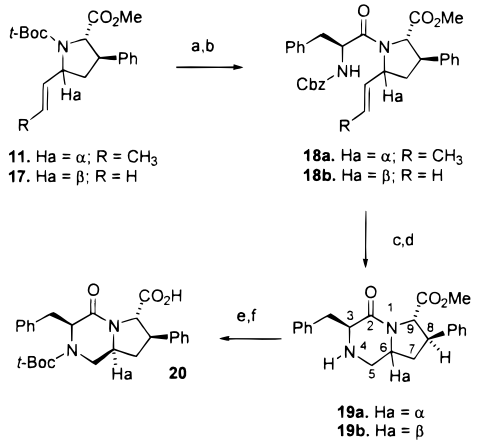
have found methoxylated amides such as **15** with an electron-withdrawing group on the carbon bearing the methoxy group do not afford iminium ions upon treatment with BF_3·Et_2O.[12] Stronger Lewis acids are required in these cases. In the current example, the inability to regenerate iminium ion **14** from **15** with BF_3·Et_2O meant that the equilibrium was "drained" toward the formation of **15**. Hence, longer reaction times increased the yield of **15** relative to the desired addition product **13**. Finally, no racemization at C_2 was observed in the desired vinyl-substituted product even when large amounts of the 2-methoxy derivative **15** were isolated. This result indicated that the addition of methanol to C_2 was nonreversible and fast enough so that an equilibrium was not established between the two iminium ions **12** and **14**. While this rearrangement reaction was less of a problem with the vinyl anion used in Scheme 4, the reaction times for the process were kept short in order to avoid the problem entirely.

In contrast to cuprate additions where the presence of a 3-phenyl substituent did not alter the stereochemistry of the product, the 3-phenyl substituent did control the stereochemistry of an allylsilane addition to the *N*-acyliminium derived from **10**. When **10** was treated with BF_3·Et_2O and allyltrimethylsilane, the nucleophile attacked the *N*-acyliminium ion from the α-face of the molecule. The product (**16**) having the allyl substituent trans to the phenyl ring and cis to the methyl ester at C_2 was formed (Scheme 6). None of the product having the opposite stereochemistry at C_5 of the proline ring was obtained. The stereochemistry of product **16** was assigned as being R at C_5 with a NOESY experiment that showed a cross-peak between the benzylic methine at C_3 and one of the allylic methylene protons. No cross-peak was observed for the methine at C_3 and the methine at C_5. These two observations indicated that the allyl group was on the same face of the molecule as the methine at C_3, and trans to the phenyl group.

The formation of **16** suggested that the chemistry being developed could be used to generate bicyclic piperazinone building blocks with either *S* or *R* bridgehead stereochemistry. For this reason, the 5-allylproline derivative **16** was converted into the (5*R*)-vinylproline derivative **17** by cleaving the double bond of the allyl group with an

(12) Unpublished results with Mr. Lawrence D. Rutledge. In this example, the treatment of a 5-methoxypyroglutamate derivative with BF_3·Et_2O led to complete recovery of the starting material.

Scheme 7[a]



[a] Reagents: (a) $BF_3 \cdot Et_2O$, THF; (b) Cbz-Phe-F, *N*-ethylmorpholine, CH_2Cl_2, yield **18a** = 60% over two steps, yield **18b** = 73% over two steps; (c) (i) O_3, MeOH, CH_2Cl_2, −78 °C, (ii) Me_2S, −78 °C to rt, yield from **18a** = 84%, yield from **18b** = 99%; (d) H_2, Pd on $BaSO_4$, MeOH, yield for **19a** = 75%, yield for **19b** = 66%; (e) Boc_2O, *N*-ethylmorpholine, 83%; (f) LiOH, THF/MeOH/H_2O, 62%.

ozonolysis reaction, reducing the resulting aldehyde to an alcohol, and then eliminating the alcohol to form the vinyl substituent.

Both complimentary vinyl-substituted prolines (**11** and **17**) were converted into bicyclic piperazinone derivatives by removing the *t*-Boc protecting group and coupling the resulting amine to a Cbz-protected phenylalanine using the acid fluoride derivative of the acid (Scheme 7).[13] In each case, the vinyl substituent was cleaved using an ozonolysis reaction. Following workup with dimethyl sulfide, both molecules spontaneously cyclized to form a six-membered ring hemiaminal. These products were reduced with hydrogen and Pd on barium sulfate in methanol in order to complete the synthesis of the bicyclopiperazine building blocks (**19a** and **19b**). The Cbz group on the N-terminus was removed during this step. The in situ removal of the Cbz group proved important for completion of the reductive amination reaction. When an analogous synthesis was attempted using a *t*-Boc-protected phenylalanine, the reductive amination reaction led to recovery of the cyclic *N*-α-hydroxyalkyl amide product from the ozonolysis without subsequent reduction by hydrogen.

At this point, the stereochemistry of the bridgehead carbon in **19a** was determined with the use of a NOESY experiment. While the stereochemistry could be established by systematically "walking" around the five-membered ring, two key long-range interactions were especially helpful. First, an NOE cross-peak for the interaction between the bridgehead methine at C_6 and the benzylic methine at C_8 was present. Second, a strong NOE cross-peak between the C_5 methylene proton on the *β*-face of the molecule and the C_7 methylene proton on the *β*-face of the molecule was present along with NOE cross-peaks for the interactions between the bridgehead methine at C_6 and the C_5 and C_7 methylene protons on the α-face of the molecule. This collection of NOE cross-peaks was only consistent with **19a** having the bridgehead methine proton on the α-face of the molecule.

Having established that **19a** possesed the stereochemistry needed to complete the synthesis of the initially

(13) Vojkovsky, T. *Peptide Res.* **1995**, *6*, 316.

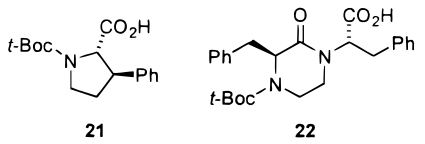
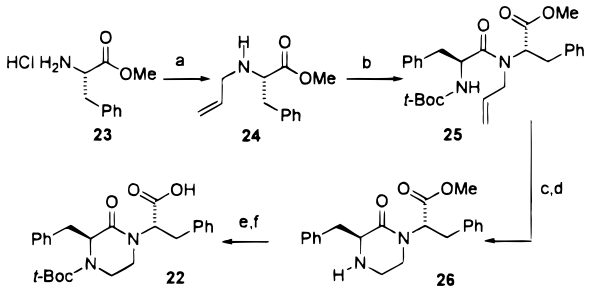


Figure 2.

Scheme 8[a]



[a] Reagents: (a) allyl bromide, Et_3N, DMF, 60%; (b) *t*-Boc-Phe-F, NEM, CH_2Cl_2, 75%; (c) (i) O_3, MeOH, −78 °C to rt, (ii) Me_2S, 88%; (d) Et_3SiH, TFA (15 equiv), CH_2Cl_2, 89%; (e) Boc_2O, NEM, 83%; (f) LiOH, THF/MeOH/H_2O, 72%.

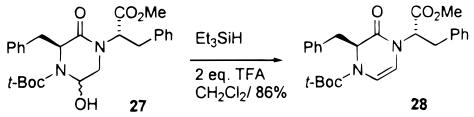
designed analogue,[4] the bicyclic piperazinone was converted into a building block for solid-phase peptide synthesis (**20**). This transformation was accomplished by reprotecting the proline nitrogen with a *t*-Boc group and saponifying the methyl ester with lithium hydroxide (Scheme 7).

Building Blocks for Constraining Either Phe[7] or Phe[8] of Substance P. Along with the bicyclic building block (**20**) needed for making a substance P analogue with both Phe[7] and Phe[8] constrained, monocyclic building blocks were desired for individually constraining either the Phe[7] or Phe[8] region of substance P. These analogues were needed so that the effects of each individual bridge could be determined.

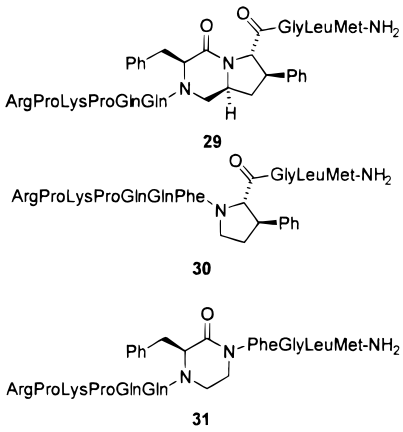
For constraining Phe[8], the known *t*-Boc-protected proline derivative **21** could be used (Figure 2).[7] However, the monocylcic piperazinone building block (**22**) required for constraining the Phe[7] group of substance P represented a larger synthetic challenge. Fortunately, the intramolecular reductive amination route used for constructing bicyclic building block **20** also proved useful for the synthesis of **22** (Scheme 8). This synthesis started with the selective monoallylation of the phenylalanine·HCl salt. To our suprise, this reaction could be accomplished by directly treating phenylalanine with allylbromide in the presence of triethylamine to afford 60% of the monoallylated amine along with only 5% of the doubly allylated byproduct. Attempts to monoallylate the *t*-Boc-protected phenylalanine using a variety of reaction conditions were not as successful. Once the allylated **24** was obtained, it was coupled to a second, *t*-Boc-protected phenylalanine group in a 75% yield by activating the carboxylic acid with cyanuric fluoride. We found that the use of an acid fluoride as the activated acid derivative was essential for this transformation.[13] For example, the use of EDC, HOBt conditions led to only recovered starting material and no coupling product.

Ozonolysis of **25** generated an aldehyde that underwent a spontaneous cyclization to form an *N*-α-hydroxyalkylamide in an 88% yield. The reductive amination was then completed with the use of triethylsilane and 15 equiv of TFA in dichloromethane. These conditions also

Scheme 9



Scheme 10



deprotected the N-terminal end of the amino acid. A reduction in the amount of TFA used did not lead to a reductive amination product having the *t*-Boc protecting group intact. For example, when 2 equiv of TFA was used, the reaction failed to produce any of the reductive amination product. Instead, the corresponding enamide **28** was formed (Scheme 9). Eventually, a route was settled on that used a large excess of TFA and then reprotected the N-terminal amino acid (Scheme 8). Following the reprotection, the methyl ester was saponified to complete the synthesis of building block **22**.

Substance P Analogue Syntheses. Once the building blocks were constructed, attention was turned toward synthesizing substance P analogues **29**−**31** (Scheme 10). In each case, the full substance P analogue was constructed using solid-phase peptide synthesis techniques. The solid support chosen was the MBHA (*p*-methylbenzhydrylamine·HCl). The monomers were protected on the N-terminus with a *t*-Boc group. Methionine, arginine, glutamine, and lysine monomers were "permanently" protected by oxide, 4-toluenesulfonyl (Tos), 9-xanthenyl (Xan), and 2-chlorobenzyloxycarbonyl (2-ClZ), respectively. During the synthesis, the *t*-Boc groups were removed with 50% TFA/dichloromethane. The coupling reactions were accomplished using the standard TBTU, HOBT, and DIEA conditions. Further details concerning the solid-phase peptide synthesis are given in the Experimental Section. After completion of each substance P analogue, thiophenol was used to remove the oxide protecting group on the methionine moiety. The remaining protecting groups were removed while cleaving the peptide from the resin with HF. The three substance P analogues were then purified using HPLC.

Equilibrium Displacement Binding of the Analogues.[14] All three substance P analogues were assayed for their ability to compete with radioiodinated SP binding to the NK-1 receptor expressing in CHO cells.[15] Analogues **29**−**31** inhibited binding only at high concentrations with IC_{50} values of 32, 80, and 5 μM, repectively. Under these binding assay conditions, the unmodified peptide Substance P exhibited an IC_{50} of 0.3 nM, indicating that the modification in the analogues reduced their affinity by approximately 4 orders of magnitude. Because of the low affinity of the analogues, the relative differences between their binding affinities may not be significant.

The most surprising of these results was the poor affinity of analogue **30** for the NK-1 receptor. This result was not anticipated because the 3-phenylproline used to constrain Phe[8] in this analogue was proposed for both model **1** and model **2** and because sterically bulky Phe[8] replacements have been compatible with the binding of an analogue to NK-1 in the past.[16] At this point, it is not clear whether the bridge sterically interfered with binding to the NK-1 receptor or whether the phenyl ring was not located in a position compatible with binding. What is clear is that until a suitable mimic for the Phe[8] region is found future work to examine the comformation of the Phe[7]-Phe[8] region of substance P responsible for binding NK-1 will need to be confined to analogues having just the Phe[7] constraint. The result obtained for the bicyclic analogue **29** was consistent with this observation, although other factors such as the wrong bridgehead stereochemistry could also contribute to the lack of analogue **29**/ NK-1 binding as well.

The poor affinity of constrained analogue **31** for the NK-1 receptor was most likely due to one (or a combination) of three problems. Either model **1** does not represent a viable backbone conformation for the bound Phe[7] region, the Phe[7] side chain in the analogue is not positioned correctly for binding to the receptor, or the added constraint in **31** sterically interferes with the analogues ability to bind the receptor. Clearly, a Phe[7] building block that constrains this region into a conformation consistent with model **2** for substance P/ NK-1 binding is needed in order to begin resolving this issue.

Conclusions

The synthetic methodology needed to rapidly construct both monocyclic and bicyclic piperazinone building blocks for use in the construction of constrained peptidomimetics has been developed. The analogues have been used to construct substance P analogues with conformational constraints in the Phe[7]Phe[8] region. In both cases, an intramolecular reductive amination reaction was utilized to complete the lactam ring used as the conformational constraint. In the case of the bicyclic building blocks, the intramolecular reductive amination reaction was used in a sequential strategy with an anodic amide oxidation to effect the net annulation of the piperazinone ring onto a proline derivative. The building blocks synthesized proved to be compatible with solid-phase peptide synthesis and were used to construct three constrained substance P analogues. None of the constrained analogues synthesized proved to be high affinity analogues for the NK-1 receptor, although analogue **31** did inhibit the binding of substance K to the receptor.

Finally, the biological data obtained identified a problem with the proposed constraint for the Phe[8] moiety of substance P and has triggered the search for a more

(14) The hard data are included in the Supporting Information.

(15) Kage, R.; Hershey, A. D.; Krause, J. E.; Boyd, N. D.; Leeman, S. E. *J. Neurochem.* **1995**, *64*, 316.

(16) Josien, H.; Lavielle, S.; Brunissen, A.; Saffroy, M.; Torrens, Y.; Beaujouan, J. C.; Glowinski, J.; Chassng, G. *J. Med. Chem.* **1994**, *37*, 1586.

effective constraint for this region. In analogy to the work described above, once a more effective constraint has been identified, the anodic amide oxidation − intramolecular reductive amination strategy should allow for the rapid synthesis of the corresponding bicyclic analogues directly from the constrained Phe[8] building block.

Experimental Section[17]

(3*R*)-*N*-*tert*-Butyloxycarbonyl-3-phenyl-L-proline Methyl Ester. In a 1 L flask was placed 8.13 g (24.2 mmol) of 2-α-methylbenzylamide-*trans*-3-phenyl-L-proline[6] along with 61 mL of glacial acetic acid and 245 mL of 8 N hydrochloric acid. The mixture was then heated at reflux for 20 h. When complete, the reaction mixture was concentrated in vacuo and the residue taken up in water and washed once with ethyl acetate. The aqueous layer was concentrated in vacuo to afford the *trans*-3-phenyl-L-proline hydrochloride. This crude product was dissolved in 50 mL of anhydrous methanol and saturated with hydrogen chloride gas. After 18 h, the reaction mixture was concentrated in vacuo. The residue was taken up in 1 N hydrochloric acid and washed twice with ethyl acetate. The aqueous layer was adjusted to pH 10 with 1 N sodium bicarbonate and then extracted three times with chloroform. The combined chloroform layers were dried over magnesium sulfate and concentrated in vacuo to afford the *trans*-3-phenyl-L-proline methyl ester **9**. The crude **9** was dissolved in 100 mL of dichloromethane with 4.89 g (42.5 mmol) of *N*-ethylmorpholine and cooled to 0 °C. To this mixture was added 4.61 g (21.1 mmol) of di-*tert*-butyl dicarbonate. After gas evolution had ceased, the reaction mixture was warmed to room temperature, stirred for 18 h, and then washed with 0.5 M citric acid, 5% sodium bicarbonate, and water. The organic layer was dried over magnesium sulfate and concentrated in vacuo. The crude product was chromatographed through 225 g of silica gel that was slurry packed with 10% hexane in ether. Elution with the same solvent afforded 5.75 g of the desired product, which was contaminated with some di-*tert*-butyl dicarbonate. The yield corrected for the contamination was 78%. The spectral data for the mixture of carbamate rotomers were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48−7.20 (m, 5H), 4.38 (d, J = 6.1 Hz, 0.33H), 4.25 (d, J = 6.8 Hz, 0.66H), 3.75 (m, 0.5H), 3.70 (s, 3H), 3.61 (m, 1.5H), 3.44 (m, 1H), 2.38−2.24 (m, 1H), 2.10−1.96 (m, 1H), 1.49 (s, 3H), 1.42 (s, 6H);^{13}C NMR (75 MHz, $CDCl_3$) δ 173.2, 153.6, 140.9, 140.5, 128.8, 127.2, 126.9, 80.2, 65.8, 65.1, 52.2, 52.0, 49.9, 48.7, 46.1, 46.0, 33.0, 32.3, 28.4, 28.2; FAB MS *m*/*z* (rel intensity) 306 (M + 1, 25), 304 (M − 1, 14), 289 (8), 279 (7), 251 (17), 250 (100), 246 (M^+ − CO_2CH_3, 10); HRMS FAB calcd for $C_{17}H_{24}NO_4$ (M + 1) 306.1705, found 306.1708.

(3*R*)-*N*-*tert*-Butyloxycarbonyl-5-methoxy-3-phenyl-L-proline Methyl Ester (10). In a 100 mL three-neck round-bottom flask equipped with a carbon rod anode and a platinum wire cathode was dissolved 4.93 g (16.1 mmol) of (3*R*)-*N*-*tert*-butyloxycarbonyl-3-phenyl-L-proline methyl ester was dissolved in 33 mL of a 0.03 M solution of tetraethylammonium tosylate in methanol. The reaction mixture was degassed by sonication under a slow stream of nitrogen for 10 min and then electrolyzed at a constant current of 26.8 mA until 3.0 F/mol had passed. The solution was concentrated in vacuo, and the residue was chromatographed through 170 g of silica gel that was slurry packed with 20% hexane in ether. Elution with the same solvent afforded 4.95 g (87%) of the desired product (**10**). The product was a mixture of stereoisomers with respect to the methoxy group and carbamate rotomers: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80−7.40 (m, 5H), 5.41 (d, J = 4.5 Hz, 0.66H), 5.31 (d, J = 4.4 Hz, 0.33H), 4.42 (d, J = 9.3 Hz, 0.33H), 4.27 (d, J = 9.5 Hz, 0.66H), 3.80−3.59 (m, 1H), 3.68 (s, 3H), 3.51 and 3.49 (two s, 3H), 2.32−2.21 (m, 1H), 2.18−2.04 (m, 1H), 1.52 and 1.43 (two s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 172.5, 154.0, 153.9, 139.6, 139.2, 128.7, 128.4, 128.1, 127.4, 127.0, 126.9, 88.2, 87.9, 81.0, 80.9, 66.4, 65.5, 55.6, 55.2, 52.1, 52.0, 47.4, 46.6, 41.9, 40.6, 28.3, 28.1; FAB MS *m*/*z* (rel intensity) 334 (M − 1, 11), 305 (20), 304 (M^+ − OCH_3, 100), 276 (15), 260 (10), 250 (12), 235 (11), 234 (62), 220 (20). Due to stability issues, this product was carried on without further characterization.

(3*R*,5*S*)-*N*-*tert*-Butyloxycarbonyl-3-phenyl-5-(*trans*-1-propenyl)-L-proline Methyl Ester (11). In a flame-dried 25 mL flask under argon 150 mg (7.5 mmol) of lithium as a 30% dispersion in mineral oil was washed three times with hexane. Anhydrous ether (6 mL) was added, and the mixture was cooled to −20 °C. To this solution was added 359 mg (3.0 mmol) of *trans*-1-bromo-1-propene. After 2 h at −20 °C, the solution was cannulated into a 50 mL flask containing a heterogeneous mixture of 613 mg (3.0 mmol) of copper(I) bromide−dimethyl sulfide complex in 7.5 mL of anhydrous ether at −40 °C. The resulting dark brown solution was stirred at −40 °C for 1 h, cooled to −78 °C, and then treated with 424 mg (3.0 mmol) of boron trifluoride etherate. After 5 min, 501 mg (1.5 mmol) of **10** was added and the −78 °C bath removed. After 15 min, the reaction mixture was quenched with a 1:1 solution of ammonium hydroxide and saturated ammonium chloride and diluted with dichloromethane. The aqueous layer was extracted three times with dichloromethane. The organic layers were combined, dried over magnesium sulfate, and concentrated in vacuo. The crude product was chromatographed through 15 g of silica gel that was slurry packed with a 1:1 hexane in ether solution. Elution with the same solvent afforded 385 mg (75%) of the desired product (**11**). A small amount of a second stereoisomer (tentatively assigned as the cis compound) was also observed. For the trans isomer, the spectral data for the mixture of carbamate rotamers were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 5H), 5.69−5.23 (bm, 2H), 4.52−4.33 (m, 2H), 3.70 and 3.69 (two s, 3H), 3.38 (m, 1H), 2.49 (m, 1H), 1.94 (m, 1H), 1.65 (bs, 3H), 1.42 and 1.40 (two bs, 9H); FAB MS *m*/*z* (rel intensity) 346 (M + 1, 14), 291 (13), 290 (70), 247 (17), 246 (100), 245 (19), 244 (M^+ − $(CH_3)_3COCO$, 46), 230 (31); HRMS FAB calcd for $C_{20}H_{28}NO_4$ (M + 1) 346.2018, found 346.2011. In this case, the rate of equilibration between the carbamate rotomers was such that it was difficult to determine the purity of the product by NMR. For this reason, the product was deprotected and converted to **18a** prior to complete characterization.

(3*R*,5*R*)-*N*-*tert*-Butyloxycarbonyl-3-phenyl-5-allyl-L-proline Methyl Ester (16). To a −40 °C solution of 3.915 g (11.7 mmol) of **10** in 24 mL of ether was added 8.35 mL (53 mmol, 4.5 equiv) of allyltrimethylsilane and 1.48 mL (12 mmol, 1.03 equiv) of $BF_3{\cdot}Et_2O$. The cold bath was removed after the addition, and the reaction was stirred for 30 min. The resulting mixture was diluted with 20 mL of ether and extracted with 20 mL of $NaHCO_3$ solution. The aqueous layer was washed two more times with ether. The combined organic layers were dried over Na_2SO_4 and concentrated. The crude product (3.9 g) was chromatographed through 180 g of silica gel using ether/hexane (1:1) as the eluant to afford 3.093 g (77%) of pure product as a colorless oil. The spectral data for the mixture of carbamate rotamers were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34−7.21 (m, 5H), 5.89−5.79 (m, 1H), 5.16−5.05 (m, 2H), 4.37 (d, 0.34H, J = 8.3 Hz), 4.22 (d, 0.66H, J = 8.9 Hz), 4.17−4.10 (m, 0.66H), 4.04−4.01 (m, 0.34H), 3.68 (s, 3H), 3.56−3.47 (m, 1H), 2.82−2.66 (m, 1H), 2.40−2.30 (m, 1H), 2.17−2.13 (m, 2H), 1.49 (s, 3H), 1.41 (s, 7H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 153.3, 139.6, 128.7, 127.3, 127.2, 117.2, 80.3, 66.8, 66.1, 57.9, 57.8, 52.1, 51.9, 47.9, 47.1, 39.2, 38.4, 38.1, 36.7, 28.4, 28.3; IR (neat/NaCl) 3072, 1750, 1701 cm^{-1}; LRFAB MS *m*/*e* (rel intensity) 346 (MH^+, 24), 290 (MH^+ − C_4H_8, 46), 246 (MH^+−$C_5H_8O_2$, 89), 204 (MH^+−$C_8H_{12}O_2$, 100); HRFAB calcd for $C_{20}H_{28}NO_4$ (M + 1) 346.2018, found 346.2017. Anal. Calcd for $C_{20}H_{27}N_1O_4$: C, 69.54; H, 7.88. Found: C, 69.50; H, 7.99.

(3*R*,5*R*)-*N*-*tert*-Butyloxycarbonyl-3-phenyl-5-(2′-hydroxyethyl)-L-proline Methyl Ester. In a 100 mL flask, 3.043 g (8.8 mmol) of **16** was dissolved in 17 mL of anhydrous methanol and 17 mL of dichloromethane. Ozone was bubbled through the solution at −78 °C. The solution turned blue after 10 min, and the ozone bubbling was continued for another 10

(17) For a description of general experimental details, see ref 9a.

min. The ozone was stopped and then oxygen bubbled through the solution until the blue color disappeared. To this mixture was added 0.400 g (10.6 mmol, 1.2 equiv) of $NaBH_4$. The the flask was then capped with a septum and connected to a nitrogen bubbler. The dry ice bath was removed 10 min later, and the reaction was stirred at room temperature for 1 h before it was quenched with 50 mL of H_2O at 0 °C. The mixture was extracted two times (30 mL, 15 mL) with dichloromethane, and then the combined organic layers were dried over magnesium sulfate and concentrated in vacuo. The crude product (3.4 g) was chromatographed through 140 g of silica gel using ether/hexane (8:2) as the eluant to afford 2.314 g (75%) of pure product as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38−7.25 (m, 5H), 4.45−4.43 (m, 1H), 4.26 (d, 1H, J = 9.5 Hz), 3.81−3.71 (m, 3H), 3.67 (s, 3H), 3.58−3.49 (m, 1H), 2.35 (dt, 1H, J = 7.8, 12.4 Hz), 2.03 (dd, 1H, J = 6.6, 12.4 Hz), 1.86−1.79 (m, 1H), 1.42 (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.3, 155.2, 139.2, 128.8, 127.5, 127.3, 81.2, 66.8, 58.8, 54.5, 52.0, 48.3, 39.4, 38.2, 28.2; IR (neat/NaCl) 3459 br, 1744, 1697 cm^{-1}; LRFAB MS *m/e* (rel intensity) 350 (MH^+, 68), 294 ($MH^+ - C_4H_8$, 63), 250 ($MH^+ - C_5H_8O_2$, 100); HRFAB calcd for $C_{19}H_{28}N_1O_5$ (M + 1) 350.1967, found 350.1969. Anal. Calcd for $C_{19}H_{27}N_1O_5$: C, 65.31; H, 7.79. Found: C, 65.15; H, 7.90.

(3*R*,5*R*)-*N*-*tert*-Butyloxycarbonyl-3-phenyl-5-(2′-*o*-nitrophenylseleno)ethyl-L-proline Methyl Ester. To a solution of 2.274 g (6.5 mmol) of (3*R*,5*R*)-*N*-*tert*-butyloxycarbonyl-3-phenyl-5-(2′-hydroxyethyl)-L-proline methyl ester in 65 mL of THF was slowly added 1.923 g (8.5 mmol, 1.3 equiv) of *o*-nitrophenyl selenocyanate and 2.12 mL (8.5 mmol, 1.3 equiv) of tributylphosphine. The reaction was stirred overnight, and then the mixture was concentrated. The crude product was chromatographed through 120 g of silica gel using gradient hexane to hexane/ether (7:3) as the eluant. The product (3.4 g) was mixed with some impurities. The material collected from the first column was chromatographed again through 150 g of silica gel using gradient hexane/ether (8:2 to 1:1) as the eluant to afford 2.5 g (72%) of pure product as a yellow solid. The spectral data for the mixture of carbamate rotamers were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.31 (d, 1H, J = 8.2 Hz), 7.70 (d, 1H, J = 8.2 Hz), 7.59−7.53 (m, 1H), 7.37−7.24 (m, 6H), 4.45−4.22 (m, 2H), 3.69 (s, 3H), 3.58−3.47 (m, 1H), 3.16−3.03 (m, 2H), 2.40−2.25 (m, 2H), 2.14−2.03 (m, 2H), 1.48 (s, 3H), 1.43 (s, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 173.4, 153.9, 146.8, 139.7, 139.3, 133.7, 129.2, 128.8, 127.5, 127.1, 126.5, 125.3, 125.2, 80.6, 66.7, 66.1, 58.4, 58.2, 52.3, 52.0, 48.3, 47.4, 39.3, 38.6, 34.5, 34.0, 28.5, 28.2, 22.9, 22.6; IR (neat/NaCl) 1744, 1694 cm^{-1}; LRFAB MS *m/e* (rel intensity) 535 (MH^+, 19), 435 ($MH^+ - C_5H_8O_2$, 100), 276 ($MH^+ - C_8H_6NO_4Se$, 76), 185 (60); HRFAB calcd for $C_{25}H_{31}N_2O_6Se$ (M + 1) 535.1347, found 535.1331. Anal. Calcd for $C_{25}H_{30}N_2O_6Se$: C, 56.29; H, 5.67. Found: C, 56.62; H, 5.93.

(3*R*,5*R*)-*N*-*tert*-Butyloxycarbonyl-3-phenyl-5-vinyl-L-proline Methyl Ester (17). To a −78 °C solution of 0.115 g (0.22 mmol) of the selenium compound made above in 24 mL of dichloromethane was added 0.074 g of *m*-CPBA (57−86%). After 90 min, methyl sulfide (0.79 mL, 50 equiv) and triethylamine (0.78 mL, 26 equiv) were added to the mixture, and the reaction was allowed to warm to room temperature. After 5 h, 10 mL of a saturated $NaHCO_3$ solution was added to the reaction. The layers were separated, and the aqueous layer was extracted with dichloromethane. The combined organic layers were dried over magnesium sulfate and concentrated in vacuo. The crude product was chromatographed through silica gel using gradient hexane/ether (8:2 to 1:1) as the eluant to afford 0.066 g (92%) pure product as a yellow oil. The spectral data for the mixture of carbamate rotamers were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36−7.22 (m, 5H), 6.03−5.93 (m, 1H), 5.57−5.39 (m, 1H), 5.30−5.17 (m, 1H), 4.61−4.59 (m, br, 0.6H), 4.47−4.46 (m, br, 0.4H), 4.40 (d, 0.4H, J = 7.8 Hz), 4.26 (d, 0.6H, J = 8.7 Hz), 3.67 (s, 3H), 3.54−3.45 (m, 1H), 2.36−2.25 (m, 1H), 2.14−2.08 (m, 1H), 1.46 (s, 3.3H), 1.42 (s, 5.7H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 173.1, 153.8, 139.1, 138.3, 137.7, 128.7, 127.4, 127.3, 127.2, 115.6, 115.3, 80.4, 66.6, 65.9, 60.1, 59.7, 52.1, 51.9, 47.8, 46.9, 39.8, 39.0, 28.3, 28.2; IR (neat/NaCl) 1757, 1701 cm^{-1}; LRFAB MS *m/e* (rel intensity) 332 (MH^+, 16), 276 ($MH^+ - C_4H_8$, 77), 232 ($MH^+ - C_5H_8O_2$, 100), 215 (58); HRFAB calcd for $C_{19}H_{26}N_1O_4$ (M + 1) 332.1862, found 332.1867. Anal. Calcd for $C_{19}H_{25}N_1O_4$: C, 68.86; H, 7.60; N, 4.23. Found: C, 68.86; H, 7.65; N, 4.21.

(3*R*,5*S*)-*N*-Benzyloxycarbonyl-L-phenylalanine-3-phenyl-5-(*trans*-1-propenyl)-L-proline Methyl Ester (18a). In a 10 mL flask, 361 mg (1.04 mmol) of **11** was dissolved in 2.1 mL of anhydrous ether. To this mixture was added 156 mg (1.10 mmol) of boron trifluoride etherate. The reaction was then stirred at room temperature for 18 h. When complete, the crude reaction mixture was washed with 5% sodium bicarbonate and concentrated in vacuo. The crude product, which contained some of the cis-substituted proline, was carried on to the coupling step without purification. The spectral data for the crude amine were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (m, 5H), 5.74−5.41 (m, 2H), 3.87 (m, 2H), 3.69 (s, 3H), 3.40 (m, 1H), 2.62 (bs, 1H), 2.31 (m, 1H), 1.76 (app q, $J \approx$ 11 Hz, 1H), 1.70 (d, J = 6.4 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.5, 142.7, 132.9, 132.5, 128.5, 127.3, 126.8, 126.7, 126.2, 67.6, 66.6, 61.2, 60.9, 55.3, 52.1, 49.9, 42.5, 42.4, 41.2, 17.7, 13.3; FAB MS *m/z* (rel intensity) 247 (M + 2, 10), 246 (M + 1, 100); HRMS FAB calcd for $C_{15}H_{20}NO_2$ (M + 1) 246.1494, found 246.1491.

For the coupling step, a solution of 305 mg (1.03 mmol) of *N*-carbobenzyloxy-L-phenylalanine and 83 mg (1.05 mmol) of pyridine in 2.6 mL of dichloromethane were placed in a 25 mL flask and cooled to −20 °C. To this mixture was added 698 mg (5.16 mmol) of cyanuric fluoride. After 1.5 h, the reaction was quenched by the addition of ice and extracted twice with dichloromethane. The organic layer was dried over magnesium sulfate and concentrated in vacuo. The resulting residue was taken up in 2.0 mL of dichloromethane and added to a solution of 202 mg (0.82 mmol) of the amine derived from **11** and 179 mg (1.55 mmol) of *N*-ethylmorpholine in 2.0 mL of dichloromethane at room temperature. After 18 h, the reaction mixture was washed with 0.5 M citric acid and 1 N sodium bicarbonate, dried over magnesium sulfate, and concentrated *in vacuo*. The crude product was chromatographed through 13 g of silica gel that was slurry packed with a 2:1 ether in hexane solution. Elution with the same solvent afforded 218 mg (60% over two steps) of the desired product (**18a**). The spectral data for the major rotamers were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.10 (m, 15H), 5.84−5.30 (m, 2.5H), 5.20 (app q, $J \approx$ 8.5 Hz, 0.5H), 5.02 (m, 2.5H), 4.84 (m, 0.5H), 4.60−4.35 (m w/ dd at δ 4.52, J = 8.7, 14.6 Hz, 1.5H), 3.91 (app q, $J \approx$ 7.2 Hz, 0.5H), 3.67 and 3.66 (two s, 3H), 3.40−3.20 (m, 1H), 3.10 (m, 1H), 2.89 (app dd, $J \approx$ 13.8, 6.9 Hz, 1H), 2.52−2.30 (m, 1H), 2.16−1.76 (m, 1.5H), 1.71 (app d, $J \approx$ 6.0 Hz, 1.5H), 1.67−1.44 (m, 1.5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.1, 171.6, 155.3, 138.4, 138.3, 136.5, 136.4, 136.3, 131.0, 129.7, 129.5, 129.4, 129.3, 128.7, 128.6, 128.5, 128.3, 128.2, 128.1, 128.0, 127.9, 127.6, 127.4, 127.1, 127.0, 126.8, 126.7, 126.6, 66.7, 66.6, 66.4, 66.3, 65.9, 61.2, 55.8, 53.3, 53.1, 52.0, 46.6, 46.4, 41.9, 41.1, 39.3, 39.2, 31.5, 22.5, 17.5 14.0; IR (neat/NaCl) 3295, 3063, 3030, 3004, 1721, 1641 cm^{-1}; FAB MS *m/z* (rel intensity) 659 (13), 529 (M + 3, 12), 528 (M + 2, 39), 483 ($M^+ - CH_2CHCH_2$, 10), 336 (9), 246 (100), 244 (20); HRMS FAB calcd for $C_{32}H_{35}N_2O_5$ (M + 1) 527.2546, found 527.2538.

(3*S*,6*S*,8*R*,9*S*)-*N*-Benzyloxycarbonyl-1,4-diaza-3-benzyl-9-carbomethoxy-5-hydroxy-8-phenyl-2-oxobicyclo[4.3.0]-nonane. To a 25 mL round-bottom flask was added 0.358 g (0.68 mmol) of **18a** along with 4 mL of anhydrous methanol and 4 mL of dichloromethane. Ozone was bubbled through the solution at −78 °C until a blue color persisted. After an additional 10 min, the bubbling of ozone was stopped and oxygen was bubbled through the solution until the blue color disappeared. The reaction flask was then capped with a septum and connected to a nitrogen bubbler, and 0.17 mL (2.4 mmol, 3.5 equiv) of methyl sulfide was added. The resulting mixture was allowed to warm to room temperature and stirred overnight. The reaction mixture was then concentrated in vacuo, and the crude product was chromatographed through 25 g of silica gel using ether/hexane (8:2) as the eluant to afford 0.293 g (84%) of the product as a white solid. Two products that were isomeric about the newly formed hydroxyl group

were obtained in a roughly 2.2:1 ratio. Both isomers were converted into **19a**. The spectral data for the major isomer were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39−7.09 (m, 15H), 4.98−4.95 (m, 3H), 4.51 (d, 1H, J = 9.4 Hz), 4.35 (x of abx, 1H, $J_{ax} = J_{bx}$ = 8.1 Hz), 4.08 (ddd, 1H, J = 5.0, 8.6, 13.4 Hz), 3.71 (s, 1H), 3.41 (ddd, 1H, J = 6.2, 9.2, 15.6 Hz), 3.28 (a of abx, 1H, J_{ax} = 4.3, J_{ab} = 13.2 Hz), 3.15 (b of abx, 1H, J_{bx} = 8.2, J_{ab} = 13.9 Hz), 2.58−2.50 (m, 1H), 1.73 (ddd, 1H, J = 12.2 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 166.0, 156.5, 138.5, 136.8, 134.9, 130.1, 129.6, 128.9, 128.8, 128.5, 128.4, 128.0, 127.7, 127.3, 127.1, 82.7, 67.7, 65.7, 63.0, 60.4, 52.5, 47.4, 38.4, 37.2; IR (neat/NaCl) 3396 br, 3030, 1750, 1708, 1665 cm^{-1}; LRFAB MS *m/e* (rel intensity) 515 (MH^+, 3), 167 (22), 149 (100); HRFAB calcd for $C_{30}H_{31}N_2O_6$ (M + 1) 515.2182, found 515.2166. Anal. Calcd for $C_{30}H_{30}N_2O_6$: C, 70.02; H, 5.88. Found: C, 69.58; H, 6.11.

(3*S*,6*S*,8*R*,9*S*)-1,4-Diaza-3-benzyl-9-carbomethoxy-8-phenyl-2-oxobicyclo[4.3.0]nonane (19a). To a solution of 1.557 g (3.03 mmol) of the α-hydroxyamide generated from **18a** in 27 mL of dry methanol was added 0.389 g (1/4 wt of the starting material) of 5% palladium/$BaSO_4$. The reaction was run under a hydrogen balloon for 26 h, and then the reaction mixture filtered through Celite and concentrated. The crude product was chromatographed through 120 g of silica gel using ether/methanol (9:1) as the eluant to afford 0.824 g (75%) of pure product as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38−7.22 (m, 10H), 4.60 (d, 1H, J = 8.9 Hz), 4.04−3.97 (m, 1H), 3.79−3.75 (m, 1H), 3.71 (s, 1H), 3.46−3.37 (m, 1H), 3.27 (dd, 1H, J = 3.3, 13.7 Hz), 3.14 (dd, 1H, J = 3.8, 12.6 Hz), 2.97 (dd, 1H, J = 10.3, 13.6 Hz), 2.65 (dd, 1H, J = 9.9, 12.3 Hz), 2.39−2.32 (m, 1H), 1.85 (s, br, 1H), 1.70 (dd, 1H, J = 11.7, 23.2 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.1, 169.2, 139.4, 138.7, 129.3, 128.7, 128.5, 127.4, 127.1, 126.5, 64.8, 60.2, 58.1, 52.2, 47.5, 44.0, 39.4, 37.9; IR (neat/NaCl) 3334, 1746, 1633 cm^{-1}; LRFAB MS *m/e* (rel intensity) 365 (MH^+, 100), 307 ($MH^+ - C_2H_2O_2$, 12), 273 ($M^+ - C_7H_7$, 79), 154 (92); HRFAB calcd for $C_{22}H_{25}N_2O_3$ (M + 1) 365.1865, found 365.1863. Anal. Calcd for $C_{22}H_{24}N_2O_3$: C, 72.51; H, 6.64; N, 7.69. Found: C, 72.84; H, 6.19; N, 7.06.

(3*S*,6*S*,8*R*,9*S*)-*N*-*tert*-Butyloxycarbonyl-1,4-diaza-3-benzyl-9-carbomethoxy-8-phenyl-2-oxobicyclo[4.3.0]nonane. To a 0 °C solution of 0.108 g (0.30 mmol) of **19a** in 1 mL of dichloromethane was added 0.13 mL (1.0 mmol, 3.5 equiv) of 4-ethylmorpholine and 0.258 g (1.18 mmol, 4 equiv) of di-*tert*-butyl dicarbonate dissolved in 1.5 mL of dichloromethane. The reaction mixture was warmed to room temperature and stirred overnight. Since TLC still showed the starting material, the reaction mixture was heated to reflux for another 9 h. The solution was concentrated in vacuo, and the crude product was chromatographed through 30 g of silica gel using ether as the eluant to afford 0.103 g (75%) of pure product as a white crystal. The spectral data for the mixture of carbamate rotamers were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38−7.17 (m, 10H), 4.91 (m, 0.2 H), 4.81−4.77 (m, 0.8H), 4.53 (d, 1H, J = 9.2 Hz), 4.40 (dd, 1H, J = 4.0, 13.6 Hz), 3.99−3.91 (m,1H), 3.72 (s, 3H), 3.44−3.35 (m, 1H), 3.23 (d, 2H, J = 5.7 Hz), 2.35−2.27 (m, 1H), 2.14 (dd, 1H, J = 10.8, 13.4 Hz), 1.91−1.78 (m, 0.5H), 1.58−1.50 (m, 0.5H), 1.47 (s, 3H), 1.32 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 166.8, 153.6, 138.9, 137.5, 129.8, 129.7, 128.8, 128.4, 128.2, 127.6, 127.0, 126.8, 80.8, 65.0, 58.2, 57.8, 52.4, 47.5, 42.9, 38.8, 37.5, 28.3, 28.0; IR (neat/NaCl) 1744, 1694, 1659 cm^{-1}; LRFAB MS *m/e* (rel intensity) 465 (MH^+, 42), 409 ($MH^+ - C_4H_8$, 100), 349 (22), 273 ($MH^+ - C_5H_9O_2 - C_7H_7$, 30), 154 (100); HRFAB calcd for $C_{27}H_{33}N_2O_5$ (M + 1) 465.2389, found 465.2383. Anal. Calcd for $C_{27}H_{32}N_2O_5$: C, 69.81; H, 6.94; N, 6.03. Found: C, 70.04; H, 6.84; N, 5.97.

(3*S*,6*S*,8*R*,9*S*)-*N*-*tert*-Butyloxycarbonyl-1,4-diaza-3-benzyl-9-carboxy-8-phenyl-2-oxobicyclo[4.3.0]nonane (20). To a 0 °C solution of 0.700 g (1.5 mmol) of the *t*-Boc-protected derivative of **19a** in 25.5 mL of THF/MeOH/H_2O (12:4:1) was added 0.164 g (3.9 mmol, 2.6 equiv) of lithium hydroxide monohydrate. After 3 h at 0 °C, the reaction mixture was acidified to pH = 3 using saturated $NaHSO_4$ solution. The solution was then concentrated, and the residue was dissolved in 9 mL of brine and 18 mL of ethyl acetate. After separation, the aqueous layer was washed twice with ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated. The crude product was chromatographed through 50 g of silica gel using ether/methanol (9:1) as the eluant to afford 0.423 g (62%) of pure product as a white solid. The spectral data for the mixture of carbamate rotamers were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.41−7.13 (m, 10H), 4.96−4.92 (m, 0.2H), 4.83−4.80 (m, 0.8H), 4.65−4.62 (m, 0.25H), 4.57 (d, 0.75 H, J = 8.9 Hz), 4.49−4.46 (m, 0.22H), 4.41 (dd, 0.78H, J = 4.1, 13.6 Hz), 3.92−3.61 (m, 1.5H), 3.48−3.38 (m, 0.5H), 3.23 (d, 2H, J = 5.6 Hz), 2.37−2.27 (m, 1H), 2.18−2.10 (m, 1H), 1.94−1.85 (m, 0.5H), 1.73−1.63 (m, 0.5H), 1.46 (s, 2H), 1.32 (s, 7H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.3, 169.2, 153.4, 139.1, 137.1, 130.2, 129.7, 128.9, 128.6, 127.6, 127.3, 127.0, 81.2, 66.2, 58.6, 57.7, 45.4, 42.7, 38.1, 37.3, 28.3, 28.0; IR (neat/NaCl) 3451 br, 1694, 1645 cm^{-1}; LRFAB MS *m/e* (rel intensity) 473 (MNa^+, 28), 451 (MH^+, 10), 395 ($MH^+ - C_4H_8$, 100), 303 (13), 277 (54), 213 (16), 154 (33); HRFAB calcd for $C_{26}H_{31}N_2O_5$ (M + 1) 451.2233, found 451.2214.

(3*R*,5*R*)-*N*-Benzyloxycarbonyl-L-phenylalanine-3-phenyl-5-(*cis*-1-vinyl)-L-proline Methyl Ester (18b). Using the same deprotection and coupling procedure outlined for the synthesis of **18a**, 0.586 g (1.77 mmol) of **17** was converted into 0.668 g (73%) of the coupled product **18b**. The proton NMR data for the intermediate amine were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36−7.24 (m, 5H), 5.96 (ddd, 1H, J = 7.1, 10.1, 17.1 Hz), 5.25 (d, 1H, J = 17.0 Hz), 5.11 (d, 1H, J = 10.4 Hz), 3.96−3.91 (m, 1H), 3.88 (d, 1H, J = 7.0 Hz), 3.69 (s, 3H), 3.45 (dd, 1H, J = 6.9, 15.5 Hz), 2.37 (s, 1H), 2.37−2.01 (m, 2H). The spectral data for **18b** were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.16 (m, 15H), 6.05 (ddd, 1H, J = 6.0, 10.2, 16.8 Hz), 5.60 (d, 1H, J = 16.8 Hz), 5.30−5.27 (m, 2H), 5.11−4.96 (m, 3H), 4.78−4.71 (m, 1H), 4.53 (d, 1H, J = 9.3 Hz), 3.67 (s, 3H), 3.66−3.45 (m, 1H), 3.14 (dd, 1H, J = 5.1, 13.8 Hz), 2.84 (dd, 1H, J = 9.0, 13.8 Hz), 2.49−2.38 (m, 1H), 2.20−2.13 (m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 156.0, 138.6, 138.1, 136.4, 136.2, 129.5, 128.8, 128.5, 128.4, 128.1, 127.8, 127.5, 127.2, 126.8, 117.2, 66.9, 66.0, 60.6, 52.8, 52.2, 46.0, 40.7, 38.4; IR (neat/NaCl) 3282 br, 3064, 3029, 1743, 1707, 1645 cm^{-1}; LRFAB MS *m/e* (rel intensity) 513 (MH^+, 81), 332 ($MH^+ - C_{14}H_{13}$, 40), 276 (94), 232 ($MH^+ - C_{17}H_{15}NO_3$, 100); HRFAB calcd for $C_{31}H_{33}N_2O_5$ (M + 1) 513.2389, found 513.2398. Anal. Calcd for $C_{31}H_{32}N_2O_5$: C, 72.64; H, 6.29. Found: C, 72.66; H, 6.42.

(3*S*,6*R*,8*R*,9*S*)-*N*-Benzyloxycarbonyl-1,4-diaza-3-benzyl-9-carbomethoxy-5-hydroxy-8-phenyl-2-oxobicyclo[4.3.0]-nonane. Using the same ozonolysis procedure described above for converting **18a** into (3*S*,6*S*,8*R*,9*S*)-*N*-benzyloxycarbonyl-1,4-diaza-3-benzyl-9-carbomethoxy-5-hydroxy-8-phenyl-2-oxobicyclo[4.3.0]nonane, 0.613 g (1.2 mmol) of **18b** was converted into 0.6104 g (99%) of the (3*S*,6*R*,8*R*,9*S*)-*N*-benzyloxycarbonyl-1,4-diaza-3-benzyl-9-carbomethoxy-5-hydroxy-8-phenyl-2-oxobicyclo[4.3.0]nonane. The molecule was obtained as a 2:1 mixture of stereoisomers about the *N*-α-hydroxy amide group. Both isomers were readily converted into the reduced **19b** below. The spectral data for the major isomer were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43−7.09 (m, 15H), 5.35 (d, 1H, J = 8.2 Hz), 4.99−4.94 (m, br, 1H), 4.82−4.80 (m, br, 1H), 4.77−4.73 (m, br, 1H), 4.66−4.63 (m, br, 1H), 3.78−3.71 (m, 4H), 3.61 (d, 1H, J = 7.1 Hz), 3.15 (dd, 1H, J = 4.8, 13.6 Hz), 3.03 (dd, 1H, J = 9.2, 13.5 Hz), 2.46−2.36 (m, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 171.1, 167.5, 156.0, 141.0, 136.1, 135.1, 129.9, 129.1, 128.4, 128.2, 127.4, 126.9, 126.2, 82.2, 67.9, 63.8, 60.3, 57.1, 52.8, 45.9, 38.6, 37.5; IR (neat/NaCl) 3444 br, 3036, 1743, 1673 cm^{-1}; LRFAB MS *m/e* (rel intensity) 537 (MNa^+, 4), 515 (MH^+, 87), 497 (M^+-OH, 100), 460 (28), 425 ($MH^+ - C_7H_6$, 23), 386 (36); HRFAB calcd for $C_{30}H_{31}N_2O_6$ (M + 1) 515.2182, found 515.2188. Anal. Calcd for $C_{30}H_{30}N_2O_6$: C, 70.02; H, 5.88. Found: C, 69.52; H, 6.01.

(3*S*,6*R*,8*R*,9*S*)-1,4-Diaza-3-benzyl-9-carbomethoxy-8-phenyl-2-oxobicyclo[4.3.0]nonane (19b). Using the procedure described above for the synthesis of **19a**, 0.376 g (0.73 mmol) of the *N*-α-hydroxyamide derived from **18b** was converted into 0.175 g (66%) of **19b**: ^{1}H NMR (300 MHz, $CDCl_3$)

δ 7.36−7.05 (m, 10H), 4.71 (s, 1H), 3.80 (s, 3H), 3.79−3.76 (m, 1H), 3.61−3.52 (m, 1H), 3.48−3.44 (m, 1H), 3.32 (dd, 1H, J = 3.7, 14.6 Hz), 3.16 (dd, 1H, J = 3.4, 12.2 Hz), 3.09 (dd, 1H, J = 8.0, 13.6 Hz), 2.76 (dd, 1H, J = 10.3, 12.2 Hz), 2.17 (ddd, 1H, J = 7.3, 12.0, 12.0 Hz), 1.85 (dd, 1H, J = 5.3, 12.2 Hz), 1.62 (s, br, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 172.1, 169.8, 142.0, 137.9, 129.5, 128.8, 128.6, 127.1, 126.8, 126.6, 64.3, 59.8, 57.9, 52.5, 48.1, 45.5, 38.2, 36.3; IR (neat/NaCl) 3641, 3331 br, 3072, 3030, 1743, 1651 cm^{-1}; LRFAB MS *m/e* (rel intensity) 365 (MH^+, 100), 307 ($MH^+ - C_2H_2O_2$, 62), 273 ($M^+ - C_7H_7$, 92); HRFAB calcd for $C_{22}H_{25}N_2O_3$ (M + 1) 365.1865, found 365.1856. Anal. Calcd for $C_{22}H_{24}N_2O_3$: C, 72.51; H, 6.64; N, 7.69. Found: C, 71.93; H, 6.79; N, 7.54.

***N*-Allyl-L-phenylalanine Methyl Ester (24).** To a solution of 1.224 g (5.7 mmol) of l-phenylalanine methyl ester hydrochloride **23** in 18 mL of *N*,*N*-dimethylformamide was added 2.77 mL (19.9 mmol, 3.5 equiv) of triethylamine at 0 °C. Allyl bromide (1.23 mL, 14.2 mmol, 2.5 equiv) was added to the mixture, and the reaction was warmed to room temperature. After 47 h of stirring, the reaction was diluted with 15 mL of water and extracted three times with 20 mL of ether. The combined organic layers were washed with saturated sodium chloride solution and then dried over sodium sulfate and concentrated. The crude product (1.3 g) was chromatographed through 60 g of silica gel (slurry prepared by ethyl acetate with 15 drops of triethylamine) using ethyl acetate as the eluant to afford 0.750 g (60%) of the purified product as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32−7.16 (m, 5H), 5.80 (ddt, 1H, J = 10.3, 17.1, 6.0 Hz), 5.14−5.05 (m, 2H), 3.64 (s, 3H), 3.56 (x of abx, 1H, $J_{ax} = J_{bx}$ = 6.8 Hz), 3.26 (a of abx, 1H, J_{ab} = 14.0, J_{ax} = 5.9 Hz), 3.15 (b of abx, 1H, J_{ab} = 14.0, J_{bx} = 6.2 Hz), 2.96 (d, 1H, J = 6.9 Hz), 1.59 (s, br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.0, 137.1, 136.1, 129.1, 128.4, 126.7, 116.4, 62.0, 51.6, 50.6, 39.7; IR (neat/NaCl) 3331 br, 3083, 3063, 1736, 1643 cm^{-1}; LRFAB MS *m/e* (rel intensity) 220 (MH^+, 100), 160 ($M^+ - C_2H_3O_2$, 58), 128 ($M^+ - C_7H_7$, 42); HRFAB calcd for $C_{13}H_{18}NO_2$ (M + 1) 220.1337, found 220.1336.

***t*-Boc-Phe-F.** To a −15 °C (ethylene glycol/dry ice bath) solution of 4.996 g (0.0188 mol) of *t*-Boc-Phe-OH in 45 mL of dichloromethane was added 1.52 mL (0.0188 mol, 1 equiv) of pyridine and 8.48 mL (0.0942 mol, 5 equiv) of cyanuric fluoride. The reaction was stirred at −15 °C for 1 h and then diluted with a large amount of ice−water. The aqueous layer was extracted four times with dichloromethane. The combined organic layers were dried over $MgSO_4$ and concentrated to afford 3.7 g (74%) of product as a white solid. The product was used in the next step without further purification: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.39−7.18 (m, 5H), 4.86−4.74 (m, br, 1H), 3.18−3.17 (m, 2H), 1.43 (s, 9H).

***N*-Allyl-*tert*-butyloxycarbonyl-L-phenylalaninyl-L-phenylalanine Methyl Ester (25).** To a room-temperature solution of 2.580 g (11.8 mmol) of **24** in 25 mL of dichloromethane was added 1.72 mL (13.5 mol, 1.15 equiv) of *N*-ethylmorpholine. To this mixture was cannulated 3.775 g (14.1 mol, 1.2 equiv) of *t*-Boc-Phe-F dissolved in 20 mL of dichloromethane. The reaction mixture was stirred for 2 days and then diluted with 15 mL of dichloromethane followed by extraction with 20 mL of 5% citric acid and 40 mL of 5% sodium bicarbonate. The crude product was chromatographed through 300 g of silica gel using ethyl acetate/hexane (1:1) as the eluant to afford 4.122 g (75%) of the purified product as a white crystal: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32−7.15 (m, 8H), 7.06−6.99 (m, 2H), 5.48−5.39 (m, 1H), 5.14−5.02 (m, 3H), 4.74 (dd, 1H, J = 7.2, 16.5 Hz), 4.41 (dd, 1H, J = 5.9, 9.1 Hz), 3.79 (dd, 1H, J = 4.8, 16.8 Hz), 3.68 (s, 3H), 3.47−3.30 (m, 3H), 3.07−2.99 (m, 2H), 2.90−2.82 (m, 1H), 1.38 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.0, 170.6, 154.9, 137.7, 136.6, 133.0, 129.6, 129.2, 128.4, 126.8, 118.3, 79.7, 60.7, 52.2, 51.8, 51.0, 39.6, 35.0, 28.3; IR (neat/NaCl) 3318 br, 3028, 1711, 1650 cm^{-1}; LRFAB MS *m/e* (rel intensity) 467 (MH^+, 30), 411 ($MH^+ - C_4H_8$, 9), 379 (16), 367 ($MH^+ - C_5H_8O_2$, 42), 246 ($M^+ - C_{13}H_{18}O_2N$, 4), 220 ($C_{13}H_{18}O_2N$, 100), 160 (63), 136 (27), 120 (75); HRFAB calcd for $C_{27}H_{35}N_2O_5$ (M + 1) 467.2546, found 467.2547. Anal. Calcd for $C_{27}H_{34}N_2O_5$: C, 69.51; H, 7.34; N, 6.00. Found: C, 69.65; H, 7.29; N, 6.03.

(3*S*)-3-Benzyl-4-*tert*-butyloxycarbonyl-5-hydroxy-1-(1′*S*-benzyl-1′-carbomethoxy)methyl-1,4-diaza-2-oxocyclohexane. In a 250 mL flask was dissolved 1.964 g (4.21 mmol) of **25** in 30 mL of anhydrous methanol. Ozone was bubbled through the solution at −78 °C. The solution turned blue 20 min later. After an additional 15 min, the addition of ozone was stopped, and oxygen was bubbled through the solution until the blue color disappeared. The reaction flask was then capped with a rubber septum and connected to a nitrogen bubbler, and 1.1 mL (15 mmol, 3.5 equiv) of methyl sulfide was added. The reaction was allowed to warm to room temperature and stirred overnight. The reaction mixture was then concentrated in vacuo. The crude product was chromatographed through 160 g of silica gel using ether/hexane (9:1) as the eluant to afford 1.736 g (88%) of the purified product as a white solid. Two isomers were obtained with respect to the *N*-α-hydroxyamide moiety. The isomers were combined and then used in the next reaction as a mixture: ^{1}H NMR (300 MHz, $CDCl_3$, major isomer) δ 7.30−7.11 (m, 10H), 5.53−5.41 (m, 2H), 4.51 (dd, 1H, J = 3.4, 11.0 Hz), 4.36 (s, 1H), 3.79 (s, 3H), 3.74−3.68 (m, 1H), 3.51−3.33 (m, 2H), 3.00 (dd, 1H, J = 13.1, 14.4 Hz), 2.48−2.29 (m, 2H), 1.04 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$, major isomer) δ 170.8, 169.4, 154.8, 136.9, 135.8, 130.0, 129.8, 129.1, 128.9, 128.6, 128.5, 128.2, 127.2, 127.1, 126.5, 81.3, 75.0, 59.7, 56.3, 52.6, 45.6, 38.1, 35.5, 28.2, 27.5; IR (neat/NaCl, major isomer) 3448 br, 1743, 1687 cm^{-1}; LRFAB MS (major isomer) *m/e* (rel intensity) 491(MNa^+, 6), 469 (MH^+, 22), 468 (M^+, 5), 451 ($M^+ - OH$, 100), 438 (7); HRFAB (major isomer) calcd for $C_{26}H_{33}N_2O_6$ (M + 1) 469.2338, found 469.2328; LRFAB MS (minor isomer) *m/e* (rel intensity 491 (MNa^+, 3), 469 (MH^+, 8), 451 ($M^+ - OH$, 100), 438 (3); HRFAB (minor isomer) calcd for $C_{26}H_{33}N_2O_6$ (M + 1) 469.2338, found 469.2340.

(3*S*)-*N*-(1′*S*-Benzyl-1′-carbomethoxy)methyl-3-benzyl-1,4-diaza-2-oxocyclohexane (26). To a room-temperature solution of 1.321 g (2.82 mmol) of the *N*-α-hydroxyamide derived from **25** in 20 mL of dichloromethane was added 0.90 mL (5.6 mmol, 2 equiv) of triethylsilane and 3.26 mL (42.3 mmol, 15 equiv) of trifluoroacetic acid. The reaction mixture was stirred for 1 day and then concentrated. The residue was dissolved in 10 mL of dichloromethane and 10 mL of triethylamine at 0 °C and stirred for 1 h at room temperature, and the solution was concentrated. The residue was dissolved in 20 mL of 20% sodium bicarbonate and 20 mL of dichloromethane. After separation, the aqueous layer was extracted two more times with dichloromethane. The crude product was chromatographed through 70 g of silica gel (slurry prepared in 95% ether/methanol with 30 drops of triethylamine) using ether/methanol (9.5:0.5) as the eluant to afford 0.82 g (83%) of pure product as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34−7.16 (m, 10H), 5.08 (dd, 1H, J = 5.4, 10.9 Hz), 3.76 (s, 3H), 3.63 (dd, 1H, J = 3.6, 9.7 Hz), 3.42−3.26 (m, 3H), 3.14 (dd, 1H, J = 10.9, 14.5 Hz), 2.97−2.89 (m, 2H), 2.80−2.71 (m, 1H), 2.61 (dd, 1H, J = 9.8, 13.4 Hz), 1.51 (s, br, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.0, 169.8, 138.3, 137.0, 129.3, 128.9, 128.6, 128.5, 126.8, 126.6, 60.6, 58.7, 52.4, 46.3, 41.8, 38.2, 34.2; IR (neat/NaCl) 3495 br, 3323, br, 3028, 1745, 1644 cm^{-1}; LRFAB MS *m/e* (rel intensity) 391 (MK^+, 6), 353 (MH^+, 100), 325 (25), 289 (17), 261 ($M^+ - C_7H_7$, 75); HRFAB calcd for $C_{21}H_{25}N_2O_3$ (M + 1) 353.1865, found 353.1865. Anal. Calcd for $C_{21}H_{24}N_2O_3$: C, 71.57; H, 6.86; N, 7.95. Found: C, 70.76; H, 6.64; N, 7.98.

(3*S*)-3-Benzyl-4-*tert*-butyloxycarbonyl-1-(1′*S*-benzyl-1′-carbomethoxy)methyl-1,4-diaza-2-oxocyclohexane. To a 0 °C solution of 0.105 g (0.30 mmol) of **26** in 1 mL of dichloromethane was added 0.057 mL (0.45 mmol, 1.5 equiv) of 4-ethylmorpholine and 0.162 g (0.742 mmol, 2.5 equiv) of di-*tert*-butyl dicarbonate dissolved in 2.5 mL of dichloromethane. The reaction was allowed to warm to room temperature. After 16 h, starting material was still evident by TLC. To the reaction mixture was added 0.034 mL of 4-ethylmorpholine and 0.097 g of di-*tert*-butyl dicarbonate. The reaction was then heated to reflux. Six hours later, the reaction was stopped, and the mixture was concentrated in vacuo. The residue was chromatographed through 30 g of silica gel using ether/hexane (9:1) as the eluant to afford 0.120 g (89%) of pure

product as a colorless oil. The spectral data for the mixture of carbamate rotamers were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33−7.21 (m, 8H), 7.04−7.02 (m, 2H), 5.29−5.26 (m, br, 1H), 4.66−4.65 (m, br, 1H), 3.96 (d, br, 0.7H), 3.77 (s, 3H), 3.3.66−3.53 (m, br, 0.3H), 3.41−3.35 (m, 1H), 3.19−2.94 (m, 4.2H), 2.72−2.64 (m, 1.8H), 1.36 (s, br, 2H), 1.19 (s, 7H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.5, 168.3, 153.4, 137.3, 136.4, 129.7, 128.8, 128.6, 128.3, 127.0, 126.5, 80.4, 58.8, 57.9, 52.4, 44.0, 37.4, 37.0, 34.1, 27.9; IR (neat/NaCl) 3023, 1744, 1694, 1652 cm^{-1}; LRFAB MS *m/e* (rel intensity) 453 (MH^+, 7), 397 ($MH^+ - C_4H_8$, 100), 337 (36), 265 (27), 146 (15); HRFAB calcd for $C_{26}H_{33}N_2O_5$ (M + 1) 453.2389, found 453.2392. Anal. Calcd for $C_{26}H_{32}N_2O_5$: C, 69.01; H, 7.13; N, 6.19. Found: C, 68.91; H, 7.10; N, 6.08.

(3*S*)-3-Benzyl-4-*tert*-butyloxycarbonyl-1-(1′*S*-benzyl-1′-carboxy)methyl-1,4-diaza-2-oxocyclohexane (22). To a 0 °C solution of 0.852 g (1.9 mmol) of (3*S*)-3-benzyl-4-*tert*-butyloxycarbonyl-1-(1′*S*-benzyl-1′-carbomethoxy)methyl-1,4-diaza-2-oxocyclohexane in 30.6 mL of THF/MeOH/H_2O (12:4:1) was added 0.197 g (4.7 mmol, 2.5 equiv) of lithium hydroxide monohydrate. The reaction was kept at 0 °C and stirred for 2 h. When complete, the reaction mixture was acidified to pH = 3 using saturated sodium bisulfate solution (about 3 mL). The solution was concentrated and the residue dissolved in 10 mL of brine and 20 mL of ethyl acetate. After separation, the aqueous layer was extracted two more times with ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated. The crude product was chromatographed through 50 g of silica gel using gradient ether/methanol (9:1 to 8:2) to afford 0.591 g (72%) of pure product as a white solid. The spectral data for the mixture of carbamate rotamers were as follows: ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.32 (s, br, 1H), 7.32−7.20 (m, 8.5H), 7.05−7.02 (m, 1.5H), 5.13 (s, br, 0.75H), 4.95 (s, br, 0.25H), 4.85 (s, br, 0.25H), 4.85, (s, br, 0.75H), 3.96 (d, 0.7H, J = 13.0 Hz), 3.67−3.64 (m, 0.3H), 3.49−3.36 (m, 1.5H), 3.28−3.11 (m, 2H), 3.08−2.93 (m, 1.5H), 2.80−2.65 (m, 2H), 1.35 (s, br, 2H), 1.12 (s, 7H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 173.6, 169.0, 153.6, 137.2, 136.3, 129.7, 128.6, 128.7, 128.4, 127.1, 126.6, 80.7, 59.3, 58.8, 45.0, 37.4, 36.9, 33.9, 27.9; IR (neat/NaCl) 2945 (br), 1735, 1701, 1665 cm^{-1}; LRFAB MS *m/e* (rel intensity) 439 (MH^+, 17), 383 ($MH^+ - C_4H_8$, 100), 337 ($M^+ - C_5H_9O_2$, 27), 265 (19), 154 (100); HRFAB calcd for $C_{25}H_{31}N_2O_5$ (M + 1) 439.2233, found 439.2223. Anal. Calcd for $C_{25}H_{30}N_2O_5$: C, 68.48; H, 6.90; N, 6.39. Found: C, 68.20; H, 7.16; N, 6.26.

General Procedure for Solid-Phase Peptide Synthesis. Substance P analogues **29**−**31** were made using standard solid-phase peptide synthesis techniques. The amine group of each monomer added was protected with a *t*-Boc group. Four of the eleven amino acids were also protected by a "permanent" protecting group. To this end, oxide, 4-toluenesulfonyl (Tos), 9-xanthenyl (Xan), and 2-chlorobenzyloxycarbonyl (2-LZ) protecting groups were used to protect the side chain functionality in the methionine, arginine, glutamine, and lysine residues, respectively.

In each case (**29**−**31**), the synthesis began with 0.571 g (0.4 mmol) of *t*-Boc-Met(O) that had already been linked to the MBHA resin (*p*-methylbenzyhydrylamine-resin·HCl, 0.7 mmol/g). Prior to the coupling sequence, the polymer was swelled by allowing it to stand in dichloromethane for 30 min. The *t*-Boc group was removed with the use of a freshly prepared 50% TFA/dichloromethne solution containing 2−5% of anisole as a Boc scavenger. Following deprotection, the resin was neutralized by washing it with 5% DIEA (diisopropylethylamine).

The coupling steps were accomplished with the common TbTu, HOBt, and DIEA conditions in DMF solvent. For coupling reactions using a primary amine, the reactions were allowed to stir for 30 min. The subsequent washing process involved DMF (1 min), 2-propanol (1 min), and dichloromethane three times (1 min each). The Kaiser test was used to ensure that the coupling reactions proceeded to complete conversion.

The coupling of the constrained building blocks proved to be slightly more difficult because of the more sterically demanding secondary amine. In these cases, the reactions were allowed to stir for 15−18 h. Due to the secondary amine, the Kaiser test was not useful for determining if the coupling reactions had proceeded to completion. For this reason, the reactions were monitored for completeness using the chloranil (tetrachlorobenzoquinone)−acetaldehyde procedure.[18]

After the final amino acid, arginine, was coupled to the peptide, the "permanent" protecting groups were removed. To this end, the oxide protecting the methionine moiety was removed by treatment of the solid-phase peptide with thiophenol in DMF. The *t*-Boc group was removed from the *N*-terminal Arg using the conditions described above. The peptide was then cleaved from the resin and the "permanent" protecting groups removed from the Arg, Lys, and Gln residues with the use of anisole, methyl sulfide, and a dithioethanol in HF solution. The reactions were performed at 0 °C using an HF reaction apparatus (model I) made by Immuno Dynamics, Inc.

The analogues were purified by filtering away the resin and then isolating the analogue by HPLC. The preparative HPLC purification of the crude peptide was carried on a Rainin HPXL solvent delivery system with a Rainin Dynamax UV-1 UV/Visible Absorbance Detector. A Vydac C18 (5 μm, 10 × 250 mm) column was used with a 20% to 80% acetonitrile/water linear gradient containing 0.1% (by volume) of TFA. The elution rate was 15 mL/min and the UV detection wavelength was set at 220 nm. The retention times for analogues **29**, **30**, and **31** were 12.9 min (50.9% acetonitrile), 12.4 min (49.9% acetonitrile), and 10.9 min (46.4% acetonitrile), respectively. The only major byproduct for each analogue came off the column about 2 min earlier. Using HRMS, the byproduct was shown to be the peptide with an oxidized Met side chain. Apparently, some of the Met side chain was oxidized during the final *t*-Boc deprotection and/or HF cleavage process. The purity of the peptide analogues was confirmed by analytical HPLC using a Hewlett-Packard 1100 series system with a Xpertek (C18, 5 μm, 4.6 × 250 mm) column. The gradient elution solvent conditions were the same as for the preparative purification, while the elution rate was changed to 1.4 mL/min. The retention times for analogues **29**, **30**, and **31** were 14.2, 12.9, and 13.5 min respectively. The mass spectrum evidence for analogue **29** is as follows: LRFAB MS (rel intensity) 1385.6 (MH^+, 100), 693.6 (35); HRFAB calcd for $C_{66}H_{101}N_{18}O_{13}S_1$ (M + 1) 1385.7516, found 1385.7452. The mass spectral data for analogue **30** is as follows: LRFAB MS (rel intensity) 1373.3 (MH^+, 100), 687.4 (25), 492.2 (25); HRFAB calcd for $C_{65}H_{101}N_{18}O_{13}S_1$ (M + 1) 1373.7516, found 1373.7495. The mass spectral data for analogue **31** is as follows: LRFAB MS (rel intensity) 1373.7 (MH^+, 100), 687.4 (25); HRFAB calcd for $C_{65}H_{101}N_{18}O_{13}S_1$ (M + 1) 1373.7516, found 1373.7491.

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Supporting Information Available: Included are the COSY and NOESY spectra for products **16** and **19a**, the biological data for analogues **29**−**31**, and the hard NMR spectral data (as evidence of purity) for all compounds lacking CHN analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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