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CONFORMATIONAL PROBES FOR ELUCIDATING THE NATURE OF SUBSTANCE P BINDING TO THE NK_1 RECEPTOR: INITIAL EFFORTS TO MAP THE PHE^7-PHE^8 REGION

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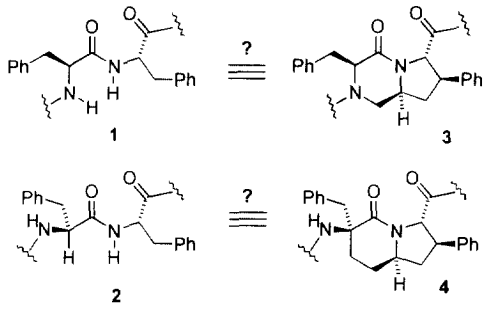
Abstract: Three substance P analogs with conformation constraints in the Phe^7-Phe^8 region have been prepared in connection with an effort to differentiate two families of potential conformations for the binding of substance P to its NK_1 receptor. While the analogs did not bind the NK_1 receptor with high affinity, the synthesis of the analogs demonstrated the utility of a general method for constructing piperazinone based peptidomimetics.

Substance P is an undecapeptide 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.[1] It belongs to the mammalian tachykinin family of peptides, whose members show potent biological effects on smooth muscle, glandular tissues, and the CNS. The NK_1 receptor has been implicated as the substance P binding site in several disease states including arthritis, asthma, and inflammatory bowel disease.[1a,1c] Recently, Marshall and Nikiforovich used energy calculations of substance P and its analogs having high affinity binding to the NK_1 receptor in order to deduce two main common types of low-energy conformers for each analog.[2] These two models for binding differed greatly in the Phe^7 region of substance P; a region known to play a vital role in the binding of substance P to its NK_1 receptor.[3] This difference is illustrated in Scheme 1 where structures **1** and **2** represent the Phe^7-Phe^8 region of substance P in the two proposed models.

Scheme 1



A variety of lactam based peptidomimetics have been used to probe the relationship between the predicted and actual biological activity of peptide conformations.[4] These peptidomimetics work by imbedding peptide backbones into polycyclic ring systems that hold the desired conformation in place.[5] For example, the conformations represented by **1** and **2** can in principle be mimicked with peptidomimetics **3** and **4**. While easy to design, the suggestion of analogs like **3** and **4** immediately raises questions about how the peptidomimetics can be synthesized, and whether or not the added bridges interfere with the binding and potency of the analogs. We

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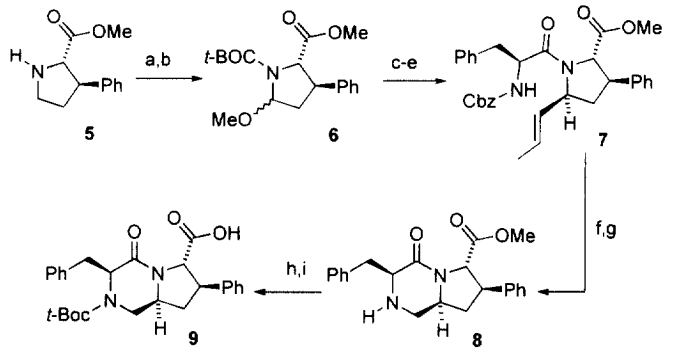
report here the synthesis and initial biological screening of substance P analogs having the Phe[7]-Phe[8] region constrained in a conformation consistent with **1**.

We have described a facile route to bicyclic piperazinone ring skeletons.[6] This route utilized a sequential anodic amide oxidation - reductive amination strategy to annulate the piperazinone ring onto a proline methyl ester. The application of this approach to the required building block for the synthesis of substance P analog **3** is outlined in Scheme 2. Two steps in this synthesis deserve comment. First, one of the strengths of the approach outlined is that it can allow for the use of a wide variety of amino acid starting materials. In this example, the annulation procedure was initiated by the functionalization of a 3-phenyl substituted proline derivative **5**. This substrate was synthesized using chemistry reported by Chung, Holladay, and coworkers.[7] The additional phenyl substituent on the proline ring did not interfere with the anodic oxidation reaction (step b), and an 87% isolated yield of the methoxylated amide **6** was obtained.

Scheme 2



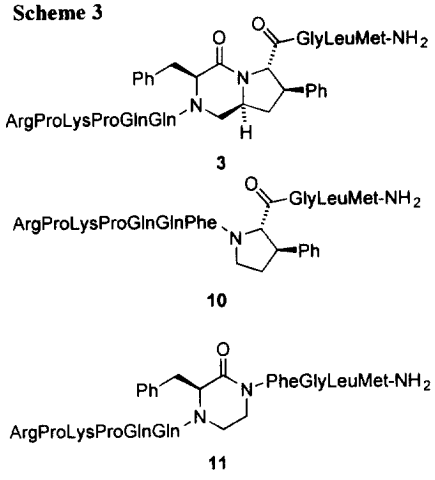
Reagents: (a) Boc_2O, NEM, CH_2Cl_2, 78%; (b) Carbon anode, Et_4NOTs, MeOH 26.8 mA, 3.0 F/mol, 87%; (c) *t*-CH_3CH=CHLi, CuBr·Me_2S, $BF_3·Et_2O$, -78 °C to 0 °C, 75%; (d) $BF_3·Et_2O$, Et_2O, rt., 81%; (e) Cbz-Phe-F, NEM, CH_2Cl_2, 75%; (f) i. O_3, MeOH, -78°C, ii. Me_2S, rt, 84%; (g) H_2/ Pd on $BaSO_4$, MeOH, 65%; (h) $(Boc)_2O$, NEM, CH_2Cl_2, 83%; i. LiOH, THF/MeOH/H_2O, 72%.

Second, it was found that the presence of the phenyl ring did not alter the stereochemical outcome of the addition reaction. When **6** was treated with a vinyl cuprate in the presence of $BF_3·Et_2O$ the vinyl nucleophile attacked the incipient *N*-acyliminium ion from the face of the proline ring opposite the methyl ester at C2. The stereochemistry of the product was assigned with the use of a 2-D NOESY experiment following its conversion into the bicyclic compound **8**.[8] This stereochemical result was identical to that obtained previously with either unsubstituted proline rings or 4-alkoxy substituted prolines.[9] In these examples, the stereochemistry of the addition was explained by suggesting the presence of a complex between copper, the methyl ester and the iminium ion pi-system. Presumably, the nucleophile attacked from the face of the proline ring trans to the copper complex.

Once the vinyl substituted proline derivative **7** was obtained, it was readily converted into the bicyclic building block **9** using the chemistry reported previously.[6] The corresponding substance P analog was then synthesized using *t*-Boc based, solid-phase peptide synthesis techniques (HOBt, TbTu, DIEA). The only alteration from normal procedures was that the coupling reactions involving the sterically more hindered constrained Phe[7]-Phe[8] building block were slow and required reaction times of approximately 15 h for complete reaction to occur. While slower, the yields of these couplings were about the same as those obtained with the

unrestricted amino acid building blocks. The success of this chemistry let us quickly assemble the desired substance P analog **3** (Scheme 3).

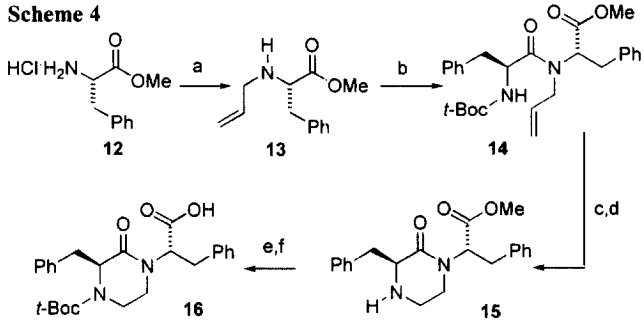
Scheme 3



With the substance P analog having both the Phe[7] and Phe[8] moieties constrained in hand, attention was turned to making the analogs having either the Phe[7] or the Phe[8] amino acid constrained (**10** and **11**). These analogs were needed so that the effects of each individual bridge could be determined. The synthesis of the analog having only the Phe[8] moiety constrained was straight forward. To this end, solid-phase peptide synthesis was used to construct a substance P analog having the Phe[8] amino acid replaced with the known 3-phenyl substituted proline derivative **5** used as a starting material above. As in the earlier analog synthesis, standard *t*-Boc based chemistry was employed in order to complete the synthesis of **10**.

The synthesis of a building block for restricting just the Phe[7] region of substance P (**11**) was potentially a more difficult challenge. Fortunately, the reductive amination route developed for the bicyclic ring skeleton proved to be general (Scheme 4). In this case, the initial step in the sequence involved the selective mono-allylation of phenylalanine methyl ester. To our surprise, this transformation could be accomplished by treating the hydrochloride salt of the starting material directly with triethylamine and allylbromide in DMF. Over alkylation of the nitrogen was not a problem, and a 60% yield of the desired product **13** was obtained. Once the allylated product was obtained, it was coupled to a second, *t*-Boc protected phenyl alanine. The double bond of the allyl group was cleaved using an ozonolysis reaction. As with the earlier bicyclic case, the aldehyde formed in the ozonolysis spontaneously cyclized to form a piperazinone ring having an *N*-α-hydroxyalkyl group. The hydroxyl group was removed with the use of triethylsilane and TFA. The synthesis of building block **16** was completed by protecting the N-terminus with a *t*-Boc group and then saponifying the methyl ester with lithium hydroxide. Building block **16** was converted into substance P analog **11** using solid phase peptide synthesis techniques as described above for the construction of **10**.

Scheme 4



Reagents: (a) allylbromide, 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%.

All three substance P analogs were tested for their ability to bind the NK_1 receptor.[10] These studies utilized rat substance P (NK_1) receptor expressed in Chinese hamster ovary cells. Competition curves were determined with the use of ^{125}I-BH-SP. The binding of ^{125}I-BH-SP was determined in the presence of known concentrations of the peptide. The first experiments were conducted at low concentrations of competing ligand and then repeated at higher concentration when no high affinity interaction was observed. This observation was true for all three of the synthesized ligands. In all three cases, the affinity of the analog for the NK_1 receptor was 10^3 to 10^4 times lower than the affinity obtained for substance P itself.

Two possible conclusions can be drawn from this result. Either proposed conformation **1** for the binding of substance P to its NK_1 receptor is wrong, or the bridges themselves are interfering with the binding of the analog to the receptor. This second possibility is particularly worrisome since the analog containing only the Phe^8 constraint (**17**) failed to bind with a high affinity. This constraint was proposed for use in both of the initial models (Scheme 1). What this suggests is that efforts to determine the biological relevance of the Phe^7 conformation in model **2** will need to focus on the use of a monocyclic peptidomimetic that restricts only Phe^7, at least until a constraint can be found for the Phe^8 region that is compatible with binding to the NK_1 receptor. Once such a constrained Phe^8 amino acid derivative is found, the generality of the anodic amide oxidation reaction should allow for its functionalization and incorporation into more fully constrained Phe^7-Phe^8 peptidomimetics.

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