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Synthesis of the cyclic heptapeptide Substance P antagonist, dihydro-WIN67689 and determination of the stereochemistry of the modified tyrosine moiety

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Abstract

The total synthesis of semi-synthetic cyclic heptapeptide dihydro-WIN67689, a Substance P antagonist 70 times more potent than the naturally occurring cyclic peptide WIN66306, established the stereochemistry of the β-OH group in the isoprenyltyrosine moiety in **I–III** as *R*.

Keywords: Substance P antagonist; cyclic heptapeptide; NMR solution structures.

Substance P (SP) is a well-known neurotransmitter peptide which binds to neurokinin 1 receptor (NK1R), a G protein-coupled receptor (GPCR).[1] Interest in molecular recognition between the receptor and the ligand has led to the development of a variety of NK1R peptide-analog ligands.[2] However, the structural basis for the interaction between the peptide ligands and the receptor still remains unclear due to flexibility of peptide backbone. Cyclic peptides have conformational constraints and the limited degree of freedom of the peptide structure enables study of the three-dimensional geometry necessary for the peptide–receptor interactions.

Recently, a novel cyclic heptapeptide **I** (WIN66306), isolated from a culture fermentation broth of *Aspergillus flavipes*, has been reported as a competitive antagonist to SP at the NK1R.[3] WIN67689 (**II**) is a semi-synthetic compound, which is obtained from **I** by methylation of the phenol group and is 70 times more potent than the native peptide **I**. In particular, the peptide **II** keeps the potent antagonist activity on the rat NK1R whereas the native peptide **I** does not show any activity on the rat receptor. Hydrogenation of the isoprenyl group of the peptide **II** gave dihydro-WIN67689 (**III**) which shows the same potency as that of **II** (Fig. 1). Although all the amino acid residues except for the glycine residues have been determined to have *S* configuration at the α-carbons, the stereochemistry of the β-hydroxy group of the isoprenyltyrosine moiety still remains unknown. Here we report the synthesis of the two stereoisomeric peptides **III** in order to determine the stereochemistry of the β-hydroxy group in the natural peptide and the solution structures of the peptide isomers.

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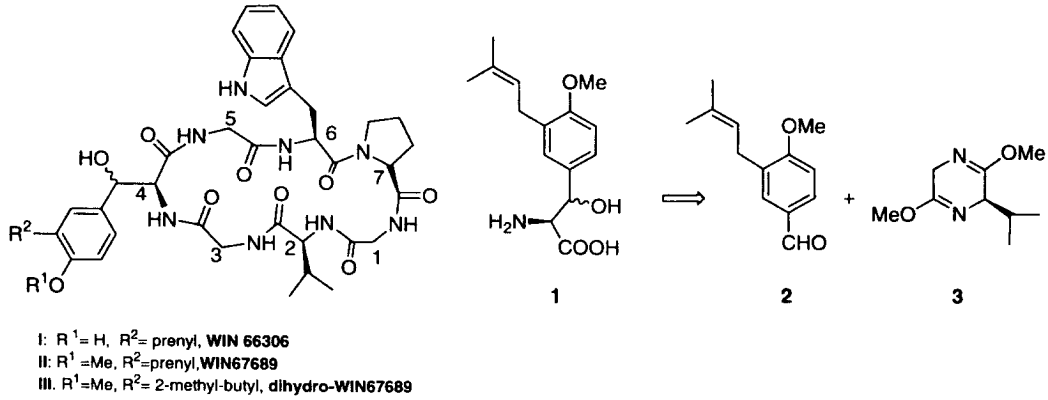
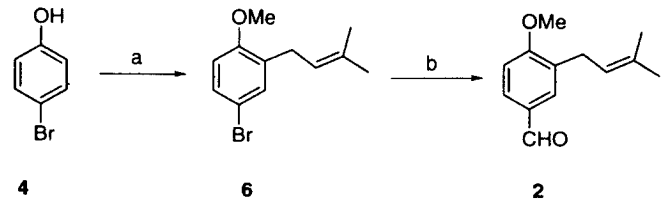


Figure 1.

Both the diastereoisomers of the novel tyrosine derivative **1** were synthesized by reaction of aldehyde **2** with Schöllkopf's reagent **3**.[4] Alkylation of 4-bromophenol (**4**) with isoprenylbromide (**5**) by a known procedure,[5] and subsequent methylation of the phenol group afforded **6** in 69% yield. Formylation of **6** gave aldehyde **2** in 85% yield (Scheme 1).

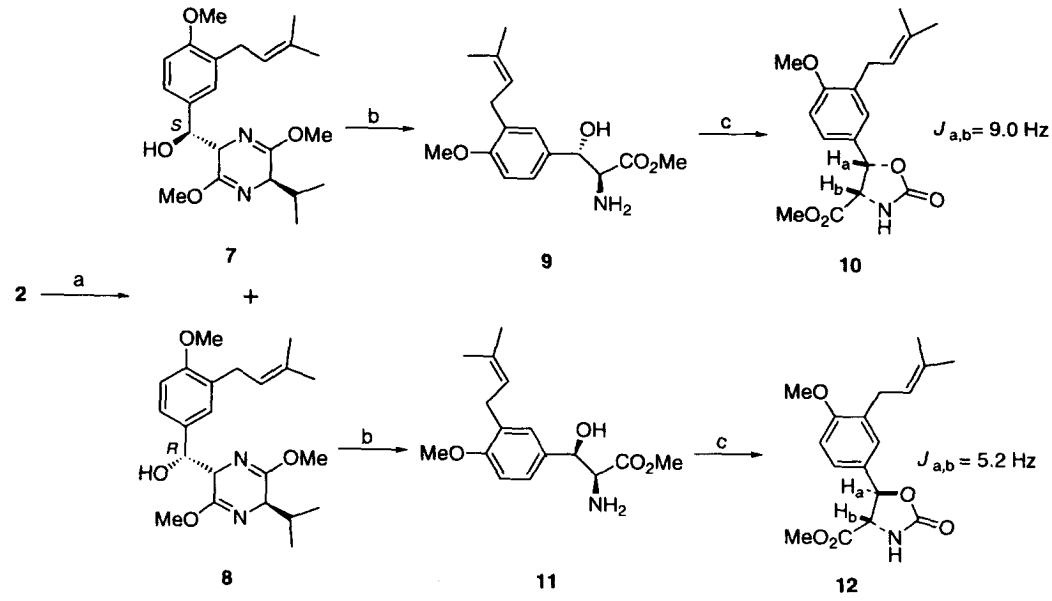


Scheme 1. Synthesis of benzaldehyde derivative **2**. (a) (1) Na, ether; (2) $(CH_3)_2C{=}CHCH_2Br$ (**5**), 74%; (3) MeI, K_2CO_3, acetone, reflux, 93%; (b) *t*-BuLi, DMF, THF, –78°C, 85%

Coupling of aldehyde **2** with the lithiated Schöllkopf's reagent-(*R*) (**3**) yielded a separable mixture of the diastereoisomers **7** and **8** in 74% in about a 1:1 ratio. The hydrolysis of **7** and **8** with 0.25N TFA solution provided the (*S*)-β-hydroxy- and (*R*)-β-hydroxy-ε-isoprenyl-*O*-methyltyrosine methyl esters **9** (in 56% yield) and **11** (in 55% yield), respectively. In order to confirm the stereochemistry of the β-amino alcohol portions of **9** and **11** were converted to the corresponding cyclic carbamates **10** and **12** by treatment with carbonyldiimidazole (CDI) in benzene. The large coupling constant (J=9.0 Hz) between the Cα-H and the Cβ-H in **10** established the *erythro* (*trans*) configuration of **9** while the smaller coupling constant (J=5.4 Hz) in **12** corresponds to the *threo* (*cis*) configuration of **11** (Scheme 2).[6]

Due to instability of the β-hydroxyl groups of **9** and **11** against reaction conditions necessary for Boc deprotection, the benzyl group (Bzl) was chosen for the C-terminal protection and the carboxybenzyl group (Cbz) for the N-terminal protection in the synthesis of the linear precursors. The intramolecular cyclization of the linear heptapeptides occurred only at N-terminal glycine1 and C-terminal proline7 using pentafluorophenyl diphenylphosphinate (FDPP).[7] Solution structures of the cyclic peptides **19** and **20** clearly indicate the type-II β-turn at glycine3 and modified tyrosine4 which may lead to the proximity of glycine1 and proline7 (Fig. 2).

The synthesis of the cyclic heptapeptide **III** is shown in Scheme 3. Hydrogenation of the isoprenyl groups of **9** and **11** and the subsequent coupling with tripeptide Cbz-Gly-Val-Gly-OH (**13**) gave the tetrapeptides **14** and **15** in 87 and 90% yields, respectively. After alkaline saponification of **14** and **15**, the tetrapeptides were coupled with the TFA salt of tripeptide Gly-Trp-Pro-Bzl (**16**) to furnish the linear heptapeptides **17** and **18** in 78 and 75% yields, respectively. Simultaneous deprotection of the N- and C-



Scheme 2. Determination of the stereochemistry of the amino alcohols **9** and **11**. (a) (*R*)-**3**, BuLi, THF, −78 to −20°C, 74%, **7**:**8**=1:1; (b) TFA (0.25N), 56% for **9**, 55% for **11**; (c) CDI, benzene, 60% for **10**, 56% for **12**

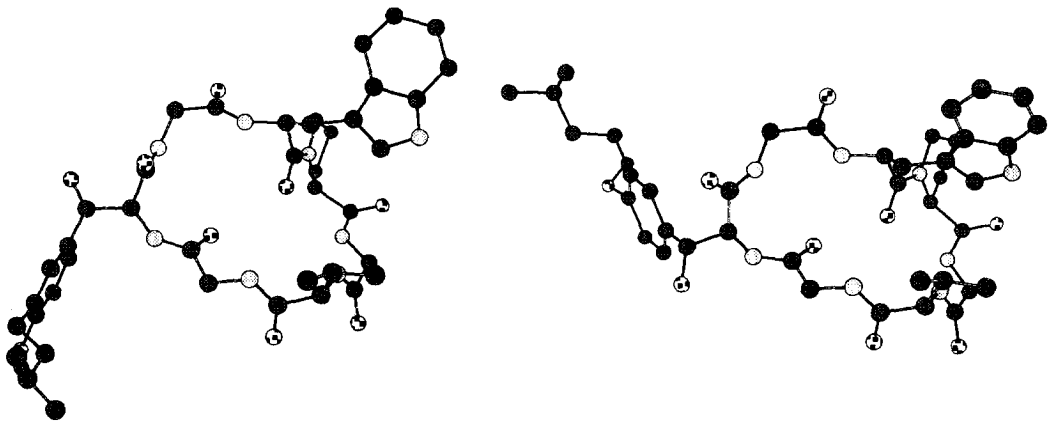
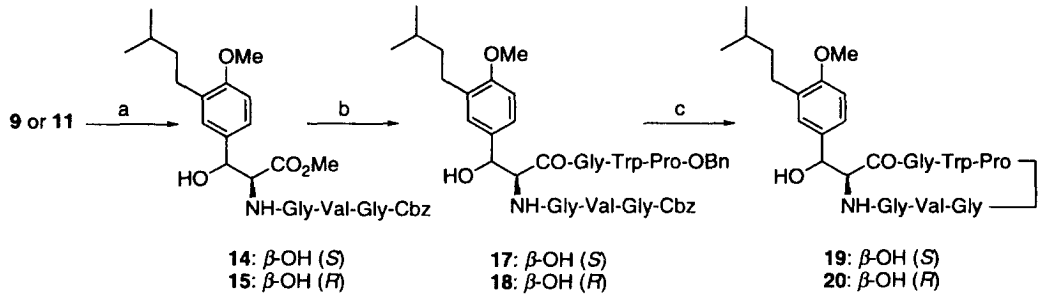


Figure 2. Solution structures of the cyclic heptapeptides **19** (left) and **20** (right). Hydrogens are omitted for clarity

terminal protecting groups by Pd/C catalyzed hydrogenation and the following intramolecular cyclization using FDPP afforded **19** and **20** in 60 and 58% yields, respectively.

Comparison of the synthesized cyclic peptides **19** and **20** with dihydro-WIN67689 (**III**) revealed that the ^{1}H and ^{13}C NMR spectra as well as the specific optical rotation of **20** were completely identical with those observed for **III**. On the other hand, the ^{1}H NMR spectrum of **19** shows significant differences from that of **III**.[8] The three-dimensional structures of the cyclic peptides **19** and **20** shown in Fig. 2 were built through a high temperature (900 K) simulated annealing simulation using the distance and dihedral restraints derived from NMR data of **19** and **20**.[9] The two isomers **19** and **20** show almost identical backbone structures except for the hydroxytyrosine moieties which may be crucial for the receptor-binding activity.

In conclusion, the accomplished synthesis of **19** and **20** enabled us to determine the stereochemistry



Scheme 3. Synthesis of dihydro-WIN67689 and the stereoisomer (**19** and **20**). (a) (1) H_2, Pd/C, MeOH; (2) Cbz-Gly-Val-Gly-OH (**13**), HOBt, EDCI, DIPEA, CH_2Cl_2, 87% for **14**, 90% for **15**; (b) (1) LiOH, CH_3CN; (2) TFA Gly-Trp-Pro-OBn (**16**), HOBt, EDCI, DIPEA, CH_2Cl_2, 78% for **17**, 75% for **18**; (c) (1) H_2, Pd/C (10%), MeOH; (2) FDPP, DMF, DIPEA, 60% for **19**, 58% for **20**

of the β-hydroxy group in the novel tyrosine moiety in the SP antagonists **I–III** as *R* and to elucidate the solution structures of the diastereomeric cyclic peptides.

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8. ^{1}H NMR data for the β-hydroxytyrosine moiety of **19** (750 MHz, DMSO-d_6): δ 3.89 (dd, 1H, *J*=5.2 and 8.9 Hz, Cα-H), 4.87 (dd, 1H, *J*=3.4 and 8.9 Hz, Cβ-H), 5.60 (d, *J*=3.8 Hz, 1H, β-OH), 6.90 (d, *J*=8.5Hz, 1H, Cε-H), 7.09 (d, *J*=1.7 Hz, 1H, $C\delta_1$-H), 7.17 (dd, *J*=8.5 and 1.7 Hz, 1H, Cε-H), 8.54 (b s, 1H, NH); $[\alpha]_D^{31}$=–7.2 (*c* 0.077, MeOH) for **19**. For the β-hydroxytyrosine moiety of **20**: δ 4.03 (d, 1H, *J*=7.6 Hz, Cα-H), 4.75 (d, 1H, *J*=7.6 Hz, Cβ-H), 5.69 (d, *J*=5.1 Hz, 1H, β-OH), 6.86 (d, *J*=8.5Hz, 1H, Cε-H), 7.06 (s, 1H, $C\delta_1$-H), 7.12 (dd, *J*=8.5 and 1.7 Hz, 1H, $C\delta_1$-H), 8.72 (b s, 1H, NH); $[\alpha]_D^{27}$=–3.96 (*c* 0.058, MeOH) for **20**.
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