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First synthesis of segetalin A and analogous cyclohexapeptides

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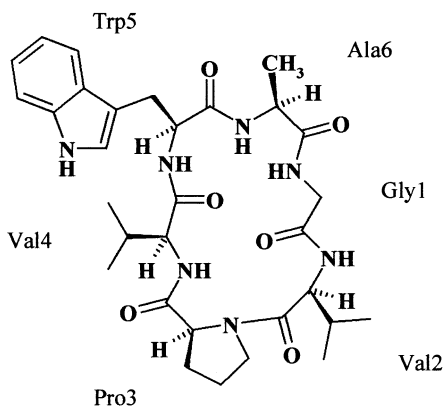
Abstract—The first synthesis of segetalin A and two analogues is described. Peptide cyclisation was carried out between L-glycine and L-alanine residues in the linear hexapeptide at the final step of the synthesis. Diphenylphosphoryl azide (DPPA) gave the best results as a coupling reagent without epimerisation. The synthesized segetalin A was completely identical to the natural compound with respect to its physicochemical properties.

Segetalins A and B have been isolated[1] from the seeds of *Vaccaria segetalis* (Caryophyllaceae) which have been used to activate blood flow and promote milk secretion and to treat amenorrhea and breast infections in China. Segetalin A (Scheme 1) was found to have a potent estrogen-like activity.[2] The cyclic structure and conformation of the segetalins were elucidated recently.[1,2]

In the course of a study of the structure–activity relationship of the segetalins, we report the first synthesis of segetalin A cyclo(Gly-Val-Pro-Val-Trp-Ala) and of two analogous cyclohexapeptides: cyclo(Gly-Val-Pro-Val-Ala-Ala) and cyclo(Gly-Val-Pro-Val-Val-Ala).

The linear peptide was obtained using standard automated continuous-flow SPPS methods. Sasrin resin was selected for the synthesis of the protected segetalins. Protected peptides can be liberated from the resin with dilute acid. The linear hexapeptides Sasrin-L-Ala-X-L-Val-L-Pro-L-Val-L-Gly (X = L-Trp(Boc), L-Ala, L-Val) were synthesized on an Applied Biosystems Model 433A peptide synthetiser. The synthesis of the segetalins described above starts from commercially available Fmoc-Ala-Sasrin. Coupling reactions were mediated by HBTU[3] and DIEA in NMP solvent using a standard ABI Fast-Moc protocol. The terminal Fmoc group of the growing peptide chain was removed with 20% piperidine in NMP. The peptide was then cleaved[4] from the resin using a solution of 1% trifluoroacetic acid in dichloromethane (Scheme 2).

In the course of experiments to prepare the three cyclohexapeptides, ring closure with pentafluorophenol[4] gave no results (Table 1). The crude linear peptide was cyclised between Gly and Ala with PyBrop in 10% yield (Table 1). However, ring closure using DPPA[5] gave a mixture of cyclohexa- and cyclododecapeptides (monomer/dimer: 1.5/1) at a concentration of 10^{-3} M in acetonitrile. Under conditions of high dilution (10^{-4}



Scheme 1. Segetalin A (Gly is numbered as the first amino acid).

Keywords: cyclohexapeptides; segetalin A; solid-phase peptide synthesis.

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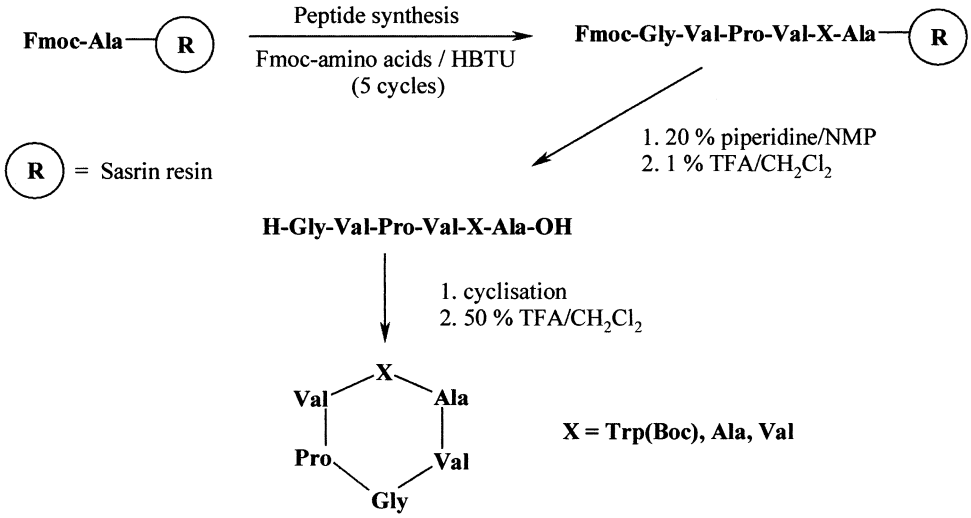

Scheme 2. Preparation of segetalin A and analogues.

Table 1. Cyclisation of segetalin A

Solvent	Activation	Concentration	Product (%)	
			Cyclic monomer	Cyclic dimer
$CHCl_3/NaHCO_3/H_2O$	Pentafluorophenol	–	No reaction	
CH_2Cl_2	PyBrop	–	10	0
CH_3CN	DPPA	10^{-3} M	30	20
CH_3CN	DPPA	10^{-3} M	45	0

M), cyclohexapeptide was the major product obtained with a yield about 45%. The bent conformation of the peptide in acetonitrile had permitted intramolecular or intermolecular cyclisation depending on concentration. We successfully prepared cyclo(Gly-Val-Pro-Val-Ala-Ala) and cyclo(Gly-Val-Pro-Val-Val-Ala) respectively in 35% and 40% yield (after purification by reverse phase HPLC). In the case of segetalin A, the cyclopeptide was obtained after cleavage of the protecting group using a solution of 50% trifluoroacetic acid in dichloromethane (30% yield).

Racemisation especially during 'head-to-tail' cyclisation is an ongoing problem in peptide chemistry.[6] Despite the fact that cyclisation required the activation via DPPA of the Ala carboxyl group, no racemisation was observed in our experimental conditions. Indeed, the electron withdrawing forces present in the activating group of the carbonyl lead to the increased acidity of the proton on the α-carbon. This could cause epimerization of the activated intermediate. Here, no diastereomers were detected by spectroscopy (^1H and ^{13}C NMR) and chromatography (HPLC).

^1H–^1H COSY, HMQC[7] and HMBC[8] experiments in [$^2H_6$]-DMSO permitted the complete assignments of the signals of the different cyclopeptides. Extracted segetalin A, described by Morita,[9] and the synthetic product[10] exhibited the same NMR spectra. The presence of a single stable conformer of segetalin A on the NMR time scale was displayed by the occurrence of well-resolved sharp signals. The ^{13}C chemical shifts (δ 31.20 and 21.35) of the β and γ positions in the Pro_3 residue suggested that the geometry of the proline amide bond was fixed in the *cis* conformation. The replacement of the aromatic amino acid Trp in segetalin A by Val or Ala allowed conformational modifications in the structure. In the case of the other cyclopeptides, NMR and HPLC analysis have shown the presence of two conformers, a major one (70%) and minor one (30%), which changed into a single peak by heating.

In conclusion, segetalin A and two analogues were synthesized in good yields without racemization. The best results were obtained using DPPA in high dilution as the coupling reagent. The sequence and conformation of Trp-Ala-Gly-Val are supposed to play an important role in the estrogen-like activity of segetalin A and the analogues synthesized here should allow further studies in structure and function relationships.

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3. Abbreviations agree with the recommendation of the IUPAC–IUB Commission on Biochemical Nomenclature. All amino acids are in the L configuration unless otherwise specified. Other abbreviations: BOC, *tert*-butyloxycarbonyl; DIEA, diisopropylethylamine; DPPA, diphenylphosphoryl azide; Fmoc, 9-fluorophenylmethoxy; HBTU, 2-(1-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HMBC, ^{1}H-^{13}C heteronuclear multiple-bond correlation; HMQC, ^{1}H-^{13}C heteronuclear multiple-quantum coherence; HPLC, high-performance liquid chromatography; NMP, *N*-methyl-2-pyrrolidone; PyBrop, bromo-tris-pyrrolidinophosphonium hexafluorophosphate; SPPS, solid-phase peptide synthesis; TFA, trifluoroacetic acid.
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10. ^{1}H NMR (500 MHz, [$^{2}H_6$]-DMSO, ppm δ relative to tetramethylsilane, J(Hz)): Gly[1], 3.32 (1H, H_α, m), 3.66 (1H, H_α, m), 7.44 (1H, H_{NH}, m). Val[2], 0.82 (3H, H_γ, d, 6.8), 0.95 (3H, H_γ, d, 6.7), 2.11 (1H, H_β, m), 4.51 (1H, H_α, dd, 8.7, 4.9), 7.42 (1H, H_{NH}, m). Pro[3], 1.68 (1H, H_γ, m), 1.91 (1H, H_γ, m), 1.94 (1H, H_β, m), 2.05 (1H, H_β, m), 3.57 (1H, H_δ, m), 3.59 (1H, H_δ, m), 4.35 (1H, H_α, d, 8.0). Val[4], 0.75 (3H, H_γ, d, 6.2), 0.77 (3H, H_γ, d, 6.2), 2.05 (1H, H_β, m), 4.13 (1H, H_α, t, 9.8), 7.31 (1H, H_{NH}, d, 9.8). Trp[5], 3.10 (1H, H_β, dd, 16.5, 7.7), 3.12 (1H, H_β, dd, 16.5, 7.7), 4.26 (1H, H_α, m), 6.98 (1H, H_6, t, 7.8), 7.07 (1H, H_5, t, 7.8), 7.14 (1H, H_2, s), 7.34 (1H, H_7, d, 7.8), 7.55 (1H, H_4, d, 7.8), 8.49 (1H, H_{NH}, d, 5.7), 10.88 (1H, $H_{1\text{-}NH}$, s). Ala[6], 1.14 (3H, H_β, d, 7.0), 3.69 (1H, H_α, m), 8.92 (1H, H_{NH}, d, 6.3).