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First synthesis of segetalins B and G: two cyclopentapeptides with estrogen-like activity

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Abstract—The first synthesis of two segetalins B and G is described. The corresponding linear peptides were synthesized using standard automated continuous-flow SPPS methods. Ring closure positions were investigated for segetalin B. The best ring closure result was obtained between Val and Gly. © 2003 Elsevier Science Ltd. All rights reserved.

In continuation of our work regarding the structure–activity relationships of the segetalins,[1] we report the first synthesis of segetalins B and G (Fig. 1), cyclic peptides previously isolated from the seeds of *Vaccaria segetalis* (Caryophyllaceae).[2] Segetalins A/B and G display an estrogenic activity in ovarectomized rats.[3] The conformations of the segetalins play an important role in their estrogenic activity (acyclic segetalins are inactive). This pharmacological property seems to be dependent on the sequence and the conformation of Trp-Ala-Gly-Val or Tyr-Ala-Gly-Val (Figs. 1 and 2).[4] Segetalin B adopts a β-turn structure[2b] between Trp[4] and Ala[5]. In the cyclopeptide segetalin A, two β-turns are found between Trp[5] and Ala[6] and between Val[2] and Pro[3]. The segetalins A, B, G and their structural analogues could be used to explore potential drug candidates in estrogenic or anti-angiogenic activities. Enhancement of the conformational rigidity of these segetalins could be a new approach for changing structure and properties of these cyclic peptides (Fig. 2) as exemplified, by the synthesis of peptidomimetic **1**.

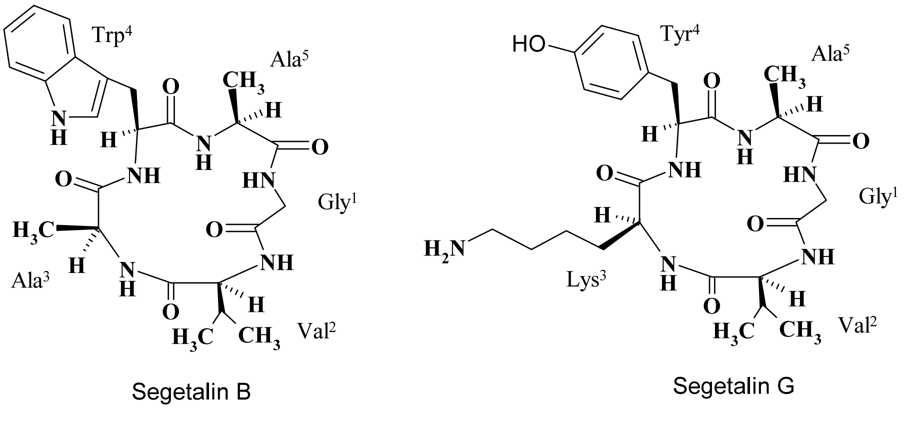

Figure 1. Structures of segetalins B and G (Gly is numbered as the first amino acid).

Keywords: cyclopentapeptides; segetalins; ring-closure reaction; solid-phase peptide synthesis.

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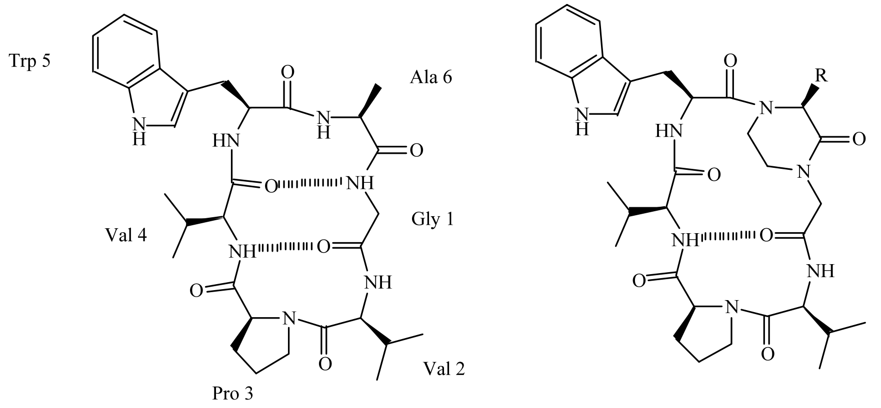


Segetalin A

1

Figure 2. Structures of segetalins A and peptidomimetic **1** (with intramolecular hydrogen bonds).

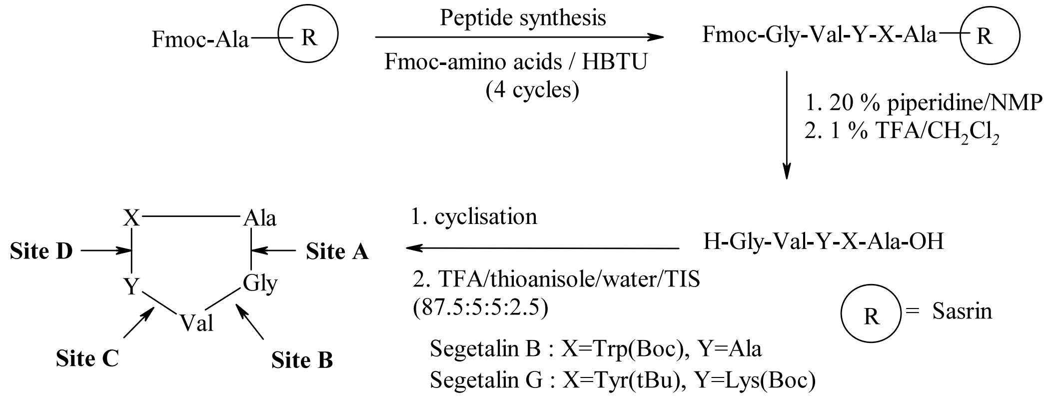
The linear peptides corresponding to segetalins B and G were synthesized on an Applied Biosystems Model 433A peptide synthetizer, using standard automated continuous-flow SPPS methods. Sasrin resin was selected for the synthesis of the protected segetalins because protected peptides can be cleaved from the resin with dilute acid solution. Synthesis of segetalins starts from commercially available Fmoc-Ala-Sasrin. Coupling reactions were mediated by HBTU[5] and DIEA in NMP using a standard Fast-Moc protocol. The terminal Fmoc group of the growing peptide chain was removed with 20% piperidine in NMP. The peptide was then cleaved[6] from the resin using a solution of 1% trifluoroacetic acid solution in dichloromethane (Scheme 1).

In this work, we have used DPPA diluted in acetonitrile at 2×10^{-4} M concentration for the peptide ring closures. This high dilution was necessary to avoid formation of the dimeric cyclodecapeptides. The crude linear peptides were cyclized to segetalins B and G, between Gly and Ala as previously described for the preparation of segetalin A[1], in low yields (55 and 39% respectively followed by HPLC, site A, Table 1). However, HPLC analysis did not reveal any cyclodecapeptides. No racemization and no diastereomers were observed by NMR spectroscopy and HPLC chromatography. Mass spectrometric investigations (electrospray MS) only revealed the presence of cyclopentapeptides.

Ring closures at other possible sites have also been explored (Scheme 1). The linear peptides were obtained with the procedure described above from the corresponding commercially available Fmoc-Sasrin aminoacid. For segetalin B, the best ring closure result was obtained between Ala and Trp (site D) which gave rise to a mixture of diastereomers (50/50). This phenomen was due to the racemization of L-Ala to D-Ala which occurred during the activation of the Ala carboxyl group via DPPA. No ring closure was detected between Ala and Val (Site C).

We successfully prepared the cyclopentapeptide B with a 90% yield, including the full-L-configuration, when the ring closure occurred between Val and Gly (site B). For segetalin G, we decided to apply this cyclization between Val and Gly by analogy to segetalin B. The recovery yield was 63% using this strategy (Scheme 1, Table 1).

As previously noted by Schmidt,[7] we assume that the transition state in ring closures very nearly corresponds conformationally to the cyclic product. In the case of segetalin B, hydrophobic interactions between Ala and



Scheme 1. Preparations of segetalins B and G.

Table 1. Ring closure sites and experimental procedures used for the cyclization of segetalins B and G

Cyclopeptide	Ring closure site	Solvent	Coupling reagent	Cyclization[a] (%)	Yield[b] (%)
Segetalin B	Site A	CH_3CN	DPPA	55	37
	Site B	CH_3CN	DPPA	90	50
	Site C	CH_3CN	DPPA	0	0
	Site D	CH_3CN	DPPA	99	49
	Site A	CH_3CN	DEPBT	0	0
	Site A	THF	DEPBT	0	0
Segetalin G	Site A	CH_3CN	DPPA	39	16
	Site B	CH_3CN	DPPA	63	25
	Site A	THF	DPPA	0	0
	Site A	THF	DEPBT	0	0

[a] The cyclization was monitored by HPLC, the yields of cyclic peptides are obtained after 18 h or reaction.

[b] Isolated after purification by reverse phase HPLC.

Val lead to a conformation separating the N- and the C-terminal which appears to have inhibited ring closure.

Due to the low solubility in acetonitrile of the linear peptides H-Gly-Val-Ala-Trp(Boc)-Ala-OH and H-Gly-Val-Lys(Boc)-Tyr(*t*Bu)-Ala-OH (sites A), the ring closures were achieved in THF but in no case, any cyclization took place (Table 1). In the course of our investigations to prepare the two cyclopentapeptides B and G, ring closures (site A) with a novel coupling reagent, such as DEPBT[8] gave no results (Table 1).

Cyclopeptides B and G were obtained with an overall yield of 23 and 12% respectively (ring closure site B) after cleavage of the protecting group using a mixed solution of trifluoroacetic acid with scavengers (TFA/thioanisole/water/TIS: 87.5/5/5/2.5). Our synthetic products exhibited the NMR spectra[9] of natural B and G segetalins, described by Itokawa.[2b,3b]

The presence of a single stable conformer of segetalin B on the NMR time scale was displayed by the occurrence of well-resolved sharp signals.

In conclusion, segetalins B and G were synthesized in low yields without racemization. The best ring closure results were obtained between Val and Gly (site B) using DPPA 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 segetalins A and B. The replacement of the amino acids Ala^6 and Gly^1 in segetalin A (compound **1**, Fig. 2) or Ala^5 and Gly^1 in segetalin B by a 3-substituted-2-oxopiperazine ring[10] is under investigation.

Acknowledgements

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5. 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; DEPBT, 3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one; DIEA, diisopropylethylamine; DPPA, diphenylphosphonic azide; Fmoc, 9-fluorenylmethoxycarbonyl; HBTU, *O*-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HPLC, high performance liquid chromatography; NMP, *N*-methyl-2-pyrrolidone; SPPS, solid-phase peptide synthesis; *t*Bu, *tert*-butyl; TFA, trifluoroacetic acid; TIS, triethylsilane.
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9. ^{1}H NMR and ^{13}C of segetalin B (500 and 125 MHz, [$^2H_6$]-DMSO, ppm δ relative to tetramethylsilane, J(Hz)): Gly^1, 3.30 (1H, H_α, dd, 14.4, 4.9), 4.10 (1H, H_α, dd, 14.4, 4.9), 8.42 (1H, H_{NH}, t, 4.9), 44.3 (C_α), 170.5 (C=O); Val^2, 0.88 (3H, H_γ, d, 6.4), 0.89 (3H, H_γ, d, 6.4), 1.97 (1H, H_β, m), 3.92 (1H, H_α, dd, 8.4, 7.5), 7.72 (1H, H_{NH}, d, 8.4), 18.9 (C_γ), 20.0 (C_γ), 30.5 (C_β), 60,3 (C_α), 171.2 (C=O); Ala^3, 1.24 (3H, H_β, d, 7.1), 4.08 (1H, H_α, m), 7.97 (1H,

H_{NH}, d, 8.4), 17.8 (C_β), 50.5 (C_α), 172.2 (C=O); Trp[4], 3.19 (1H, H_β, dd, 14.5, 6.1), 3.24 (1H, H_β, dd, 14.5, 8.2), 4.22 (1H, H_α, m), 6.98 (1H, H_6, t, 7.9), 7.07 (1H, H_5, t, 7.9), 7.12 (1H, H_2, d, 2), 7.34 (1H, H_7, d, 7.9), 7.55 (1H, H_4, d, 7.9), 7.99 (1H, H_{NH}, d, 8.4), 10.84 (1H, $H_{1\text{-}NH}$, s), 27.2 (C_β), 57.1 (C_α), 110.8 (C_3), 112.2 (C_7), 119.1 (C_4), 119.2 (C_6), 121.8 (C_5), 124.4 (C_2), 128.1 (C_9), 136.9 (C_8), 171.9 (C=O); Ala[5], 1.21 (3H, H_β, d, 6.9), 4.22 (1H, H_α, m), 7.92 (1H, H_{NH}, d, 8.4), 17.9 (C_β), 49.3 (C_α), 173.0 (C=O); electrospray-MS: *m*/*z* calcd for $C_{24}H_{33}N_6O_5$ ($[M+H]^+$): 485.25, found 485.30.

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