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SCIENCE DIRECT
Tetrahedron 61 (2005) 6808–6815
Tetrahedron

Synthesis, structure, and biological aspects of cyclopeptides related to marine phakellistatins 7–9

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Received 27 January 2005; revised 18 April 2005; accepted 29 April 2005

Abstract—Phakellistatins 7, 8 and 9, three cyclic decapeptides naturally occurring in marine sponges of the genus *Phakellia* and characterized by the distinctive presence of Pro–Pro tracts, pose a non-trivial synthetic challenge, despite only containing coded amino acid residues. Their chemical synthesis was approached using a combination of solid and solution-phase techniques. As expected, our synthetic efforts yielded, for each cyclopeptide, a mixture of geometric isomers, owing to their *cis–trans* isomerism at Pro peptide linkages. A further complication arose because their synthesis yielded, together with the desired monomeric cyclopeptides, cyclodimeric species. In the case of phakellistatin 7 (originally determined as *cis*-Pro^2, *cis*-Pro^8) our synthetic product was chemically and spectrally identical to the natural one, whereas none of the different isomeric products obtained for both phakellistatins 8 and 9 resulted to be fully equivalent (with respect to Pro geometries) to their natural counterparts. Finally, all synthetic cyclopeptides were submitted to biological assays and, as noted before for other members of the 'proline rich' family, synthetic compounds did not fully reproduce the biological properties (in terms of in vitro cytotoxicity against a panel of cancer cell lines) originally found for the natural products.

1. Introduction

Homodetic cyclopeptides of 'proline rich' class, so named for their unusual high content of proline residues, are mainly distributed in marine environments,[1] but were found also in higher plants.[2] They have attracted great interest owing to their remarkable pharmacological activities, such as anti-proliferative and cytotoxic effects, and also due to their peculiar structural aspects that make more challenging the spectral analysis as well as their chemical synthesis.

In the course of our on-going studies on bioactive marine metabolites as potential candidates for the development of novel and more effective pharmaceuticals, we have recently focused our attention on several members belonging to the proline-rich cyclopeptides family;[3] we already reported the synthesis and the biological evaluation of yunnanins A and C, two cyclic heptapeptides isolated from the roots of *Stellaria yunnanensis*, and phakellistatins 1 and 10, a hepta- and octacyclopeptide, respectively, first isolated from marine sponges of genus *Phakellia*.[4] Also, in this case, in accordance with the typical behavior associated to such products, already observed by our own as well as by other research groups,[5] we found that the biological properties of the synthesized cyclopeptides significantly differed from those found for their natural counterparts. There seems to be a growing consensus on the fact that these products are endowed with a quite remarkable conformational profile, which likely results from combined effects due to several simultaneous *cis–trans* isomerisms (at Pro linkages) in a constrained macrocyclic ring. Intrigued by this puzzle, we decided to further explore the structural and the biological aspects of other members of this singular class of marine natural products,[6] in the hope to shed more light on the topic of their chemical and yet not biological equivalence. Herein, we describe our work towards the total synthesis of phakellistatins 7–9 (**1**–**3**, Fig. 1)[7] and our subsequent efforts aimed at a thorough exploration of their conformational and biological properties. In terms of synthetic challenge, phakellistatins 7–9, by virtue of their unusual sequences comprising two Pro–Pro tracts in somewhat constrained

Abbreviations: AcOH, acetic acid; AA, amino acid; DCM, dichloromethane; DIEA, diisopropylethylamine; DKP, diketopiperazine; DMEM, Dulbecco's modified Eagle's medium; DMF, *N*,*N*-dimethylformamide; ESIMS, electrospray ionization mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; HATU, *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium; HEPES, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid; HOBt, 1-hydroxy-1,2,3-benzotriazole; MeOH, methanol; 6-MP, 6-mercaptopurine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2*H*-tetrazolium bromide; NMM, *N*-methyl morpholine; ROESY, rotating-frame Overhauser effect spectroscopy; rt, room temperature; SDS, sodium dodecyl sulfate; SPG, side-chain protecting group; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol; TIS, triisopropylsilane.

Keywords: Cyclopeptides; Solid phase synthesis; Marine natural products; Cytotoxic.

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0040–4020/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2005.04.067

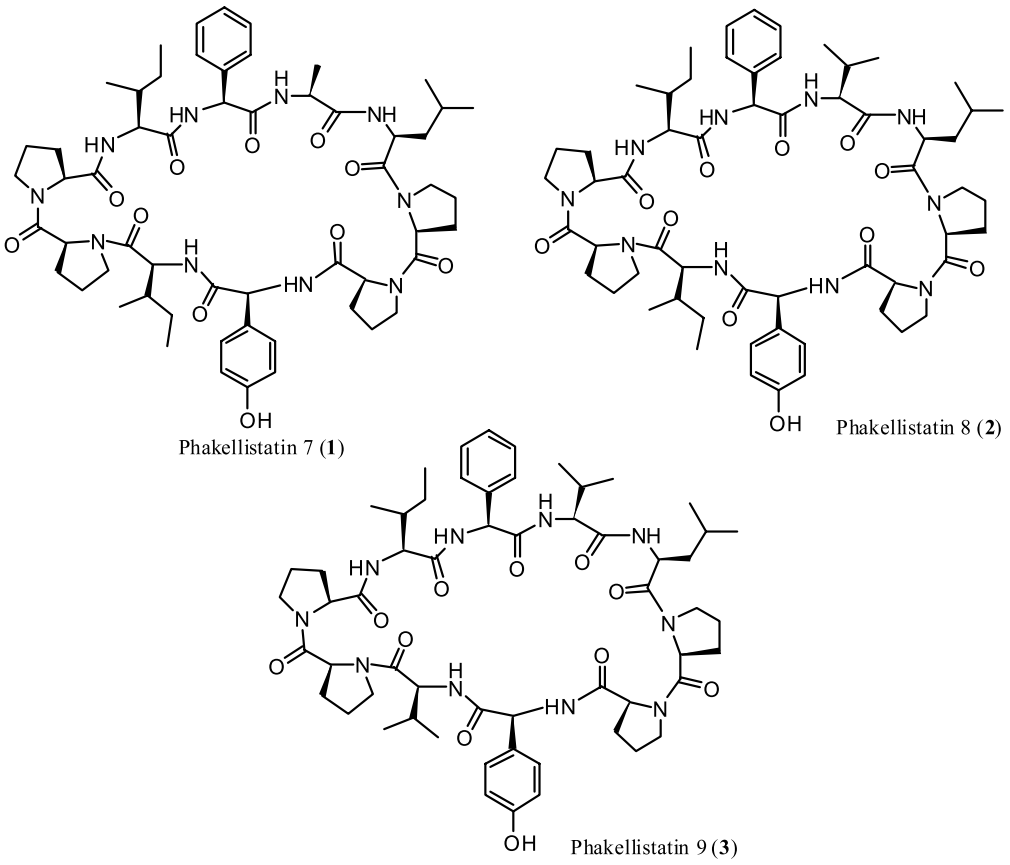

Figure 1. Chemical structures of natural cyclopeptides **1**–**3**.

decapeptidic macrocyclic frameworks, have to be considered relatively demanding targets, even though their structures just encompass proteinogenic amino acids. Not surprisingly, difficulties in obtaining the desired geometric isomers for all the target phakellistatins, have in fact emerged, preventing us from obtaining substantial amounts of compounds identical in all structural respects to the naturally occurring phakellistatins 8 (**2**) and 9 (**3**). Remarkably, in the case of phakellistatin 7, the compound with correct Pro–Pro geometry was obtained as unique product. We wish to point out that rather subtle (and fully conservative) amino acidic substitutions in the sequence of these related compounds, have translated into quite dramatic differences in the final outcome of our synthesis.

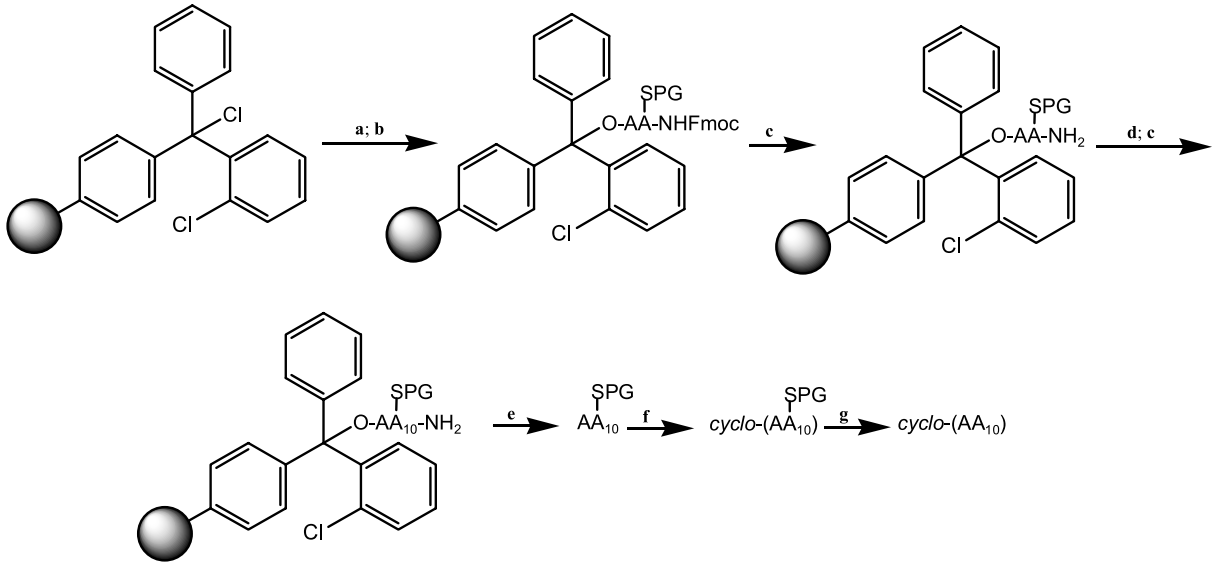

Figure 2. Synthetic scheme: (a) loading with C-terminal amino acid: Fmoc-AA, DIEA, DCM, 2 h; (b) capping: DCM/MeOH/DIEA; (c) N^{α}-deprotection: 20% piperidine in DMF; (d) cycles of nine amino acid couplings: HOBt, HBTU, NMM, DMF, 2 h; (e) cleavage: AcOH/TFE/DCM (2:2:6), 2 h; (f) cyclization: HATU, DIEA, DCM; (g) side-chain deprotection: TFA/TIS/H_2O (95.2.5:2.5).