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Comparison of Solution-Phase and Solid-Phase Syntheses of a Restrained Proline-Containing Analogue of the Nodularin Macrocycle

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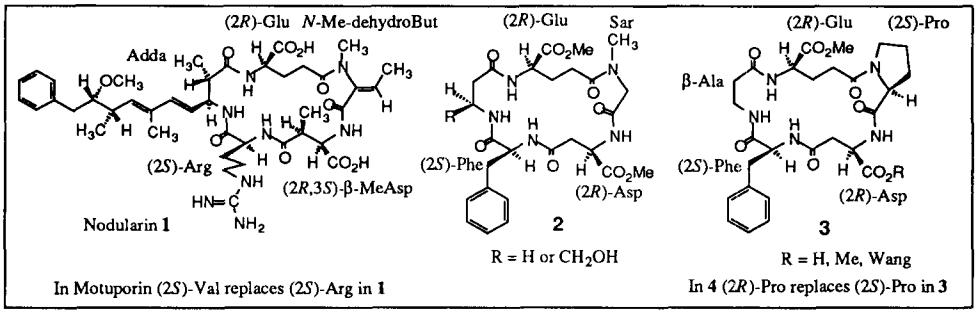
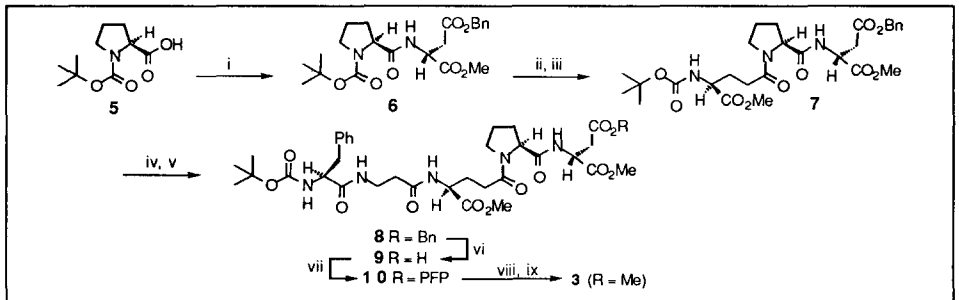
Abstract. The solution-phase synthesis of a restrained (2*S*)-proline-containing analogue of the nodularin macrocycle, *cyclo*-[β-Ala-(2*R*)-Glu(α-OMe)-γ-(2*S*)-Pro-(2*R*)-Asp(α-OMe)-β-(2*S*)-Phe-], is described and compared to two solid-phase syntheses of the same cyclic isopentapeptide diester; one in which Fmoc-(2*S*)-Phe-β-Ala-(2*R*)-Glu(α-OMe)-γ-(2*S*)-Pro-(2*R*)-Asp(α-O-Wang Resin)-β-OAllyl is deprotected and then cyclised on the resin and one in which this same precursor is removed from the resin prior to cyclisation. © 1997 Elsevier Science Ltd.

In mammals modulation of the phosphorylation of proteins is the principal mechanism by which cells respond to stimuli.[1,2] Ser-Thr protein phosphatases are collectively responsible for the dephosphorylation of phosphoserine and phosphothreonine residues within phosphoproteins. Several different types exist (*eg.* PP1, PP2A, PP2B and PP2C), usually in association with regulatory subunits. Microcystin and nodularin **1** are powerful subnanomolar inhibitors of both PP1 and PP2A but, as yet, there are no specific small molecule inhibitors for either type of enzyme. These phosphatases show considerable homology, particularly in regions that are associated with binding the two M^{2+} ion cofactors[3,4] indicating that the active-sites are highly conserved.[5] Other work has shown that the *N*-Me dehydroamino acid residue, a potential Michael acceptor present in both microcystin and nodularin **1**, is not required for biological activity.[6,7,8] and preparative studies have provided a new route to phosphopeptide substrates[9] and syntheses of both the 19-membered nodularin[6,10] and 25-membered microcystin ring system[11,12] in functionalised and stripped-down form.

In our own work on the nodularin system **2** and the analogous microcystin system, which focused on the preparation of synthetic analogues, we opted to introduce lipophilic surrogates for the Adda side-chain (see structure **1**) after the construction of the macrocycle.[6,12] Solution phase chemistry was used to construct each macrocycle where, in view of its redundancy, the *N*-Me dehydroamino acid residue present in each natural product was replaced by a sarcosine (*N*-methylglycine) residue. In solution, NMR spectra indicated that both cyclic peptides existed in multiple conformations, for each of which we wished to determine the 3-D structure. As the Sar residue allows free movement about the C^{α}-N bond and possesses no N-H proton which can report on the torsional angle about that bond, syntheses of restrained analogues containing (2*S*)- and (2*R*)-Pro in place of Sar (**3** and **4**) were considered in order to circumvent these problems, and are reported on here.



Using similar solution-phase methods to those employed for the synthesis of the sarcosine containing macrocycle **2**,[6,12] the (2*S*)-Pro containing linear peptide analogue **8** was prepared starting from (2*R*)-Asp-α-OMe-β-OBn and t-Boc (2*S*)-Phe-β-AlaOH, both of which have been described previously,[6] see Scheme 1.

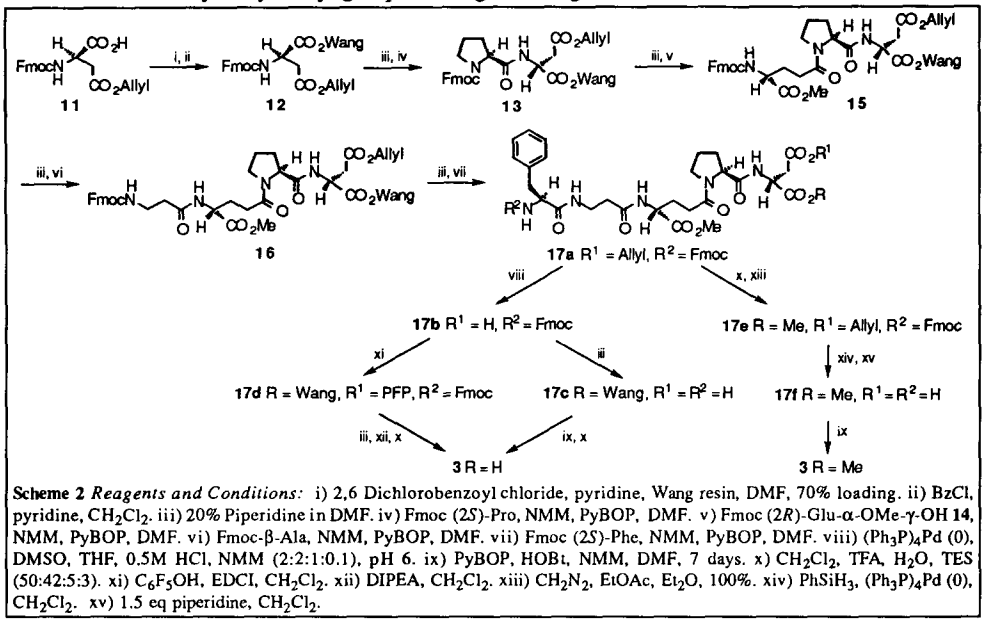


Scheme 1 *Reagents and Conditions:* i) IBCF, NMM, THF, DMF, -15 °C, then (2*R*)-Asp-α-OMe-β-OBn.HCl, NMM, THF, DMF, -15 °C→r.t., 12 h, 88%. ii) $HCl_{(g)}$, EtOAc, 0 °C→r.t., 4 h, 94%. iii) Boc-(2*R*)-Glu-α-OMe-γ-OH, IBCF, NMM, THF, DMF, -15 °C→r.t., 12 h, 82%. iv) $HCl_{(g)}$, EtOAc, 0 °C→r.t., 2 h, 95%. v) Boc-(2*S*)-Phe-β-AlaOH, IBCF, NMM, THF, DMF, -15 °C→r.t., 12 h, 92%. vi) H_2, Pd/C, EtOH, r.t., 12 h, 99%. vii) EDCI, C_6F_5OH, CH_2Cl_2, 0 °C→r.t., 12 h, 68%. viii) TFA, CH_2Cl_2, r.t., 45 min, 100%. ix) DIPEA, CH_2Cl_2, r.t., 7 days, 54%.

The compound **8** [m.p. 118–119 °C, $[\alpha]_D$ +31.67 (MeOH)] gave satisfactory analytical data and existed in both the *trans*- and *cis*- γ-Glu-Pro amide rotomeric forms.[6,12] Peptide bond-forming reactions were achieved in each case using the mixed isobutyl carbonic anhydride method and all compounds and intermediates displayed the expected properties. The final cyclisation reaction was performed using the activated PFP ester **10** to afford the required macrocyclic diester **3** (R = Me) in 54% yield. The epimer **4** (R = Me), containing a (2*R*)-Pro residue, was prepared similarly and was obtained in 16% overall yield. Both macrocycles gave satisfactory analytical data and NMR spectra revealed that each existed as a mixture of the *trans*- and *cis*- γ-Glu-Pro amide rotomers. In each case the replacement of the Sar residue by Pro allowed the 3-D structures of each conformer to be deduced, as required. Details are provided in a following article.[13]

While the synthetic route to the nodularin macrocycle was proving to be efficient and robust, the time required to prepare such compounds (and characterise and purify the synthetic intermediates) was too long to employ for the preparation of the hundreds of structural variants needed for a full SAR study. Thus, solid-phase routes were considered in which; **a**) the entire isopentapeptide would be prepared and cyclised on the resin, and; **b**) the solid-phase assembled isopentapeptide would be removed from the resin prior to cyclisation. Accordingly, the generic route, Scheme 1, in which cyclisation was achieved through the reaction of a (2*S*)-Phe residue with an activated (2*R*)-Asp β-PFP ester in the linear precursor **10**,[6,12] was adapted for use with Wang resin, Fmoc protection and PyBOP activation protocols. Thus, *N*-Fmoc (2*R*)-Asp β-allyl ester **11** was

prepared [m.p. 110°C, $[\alpha]_D$ +3.04 (MeOH)] and was activated as its 2,6-dichlorobenzoic anhydride,[14] and then reacted with the 4-hydroxymethyl group of Wang resin to give the immobilised diester **12**, Scheme 2.



Scheme 2 *Reagents and Conditions:* i) 2,6 Dichlorobenzoyl chloride, pyridine, Wang resin, DMF, 70% loading. ii) BzCl, pyridine, CH_2Cl_2. iii) 20% Piperidine in DMF. iv) Fmoc (2*S*)-Pro, NMM, PyBOP, DMF. v) Fmoc (2*R*)-Glu-α-OMe-γ-OH **14**, NMM, PyBOP, DMF. vi) Fmoc-β-Ala, NMM, PyBOP, DMF. vii) Fmoc (2*S*)-Phe, NMM, PyBOP, DMF. viii) $(Ph_3P)_4Pd$ (0), DMSO, THF, 0.5M HCl, NMM (2:2:1:0.1), pH 6. ix) PyBOP, HOBt, NMM, DMF, 7 days. x) CH_2Cl_2, TFA, H_2O, TES (50:42:5:3). xi) C_6F_5OH, EDCI, CH_2Cl_2. xii) DIPEA, CH_2Cl_2. xiii) CH_2N_2, EtOAc, Et_2O, 100%. xiv) $PhSiH_3$, $(Ph_3P)_4Pd$ (0), CH_2Cl_2. xv) 1.5 eq piperidine, CH_2Cl_2.

Loadings ranged from 0.6-0.8 mmol g^{-1} resin. Removal of the Fmoc group using 20% piperidine in DMF followed by reaction with PyBOP-activated Fmoc-(2*S*)-Pro gave the resin-bound diester **13** which was treated with piperidine to remove the Fmoc group. Fmoc-(2*R*)-Glu-α-OMe-γ-OH **14** [m.p. 130 °C, $[\alpha]_D$ +19.5 (MeOH)] was prepared in 62% yield in 3 steps from (2*R*)-Glu-α-OH-γ-OAllyl.HCl,[15] and was activated with PyBOP then added to the free amine derived from diester **13** to give the immobilised Fmoc-tripeptide triester **15**. Removal of the Fmoc group followed by reaction with PyBOP-activated Fmoc-β-alanine gave the tetrapeptide triester **16** which was deprotected and reacted with PyBOP-activated Fmoc-(2*S*)-Phe to give the Fmoc-pentapeptide triester **17a**. The β-allyl ester group of the Asp residue was unmasked using $(Ph_3P)_4Pd$ (0) to give pentapeptide diester **17b** (R = Wang) and the N-terminal Fmoc group of this material was removed to give the resin-bound amino acid **17c** (R = Wang). Treatment with PyBOP and HOBt in the presence of DIPEA in DMF for 7 days gave the cyclic peptide **3** (R = Wang) which was removed from the resin with TFA solution to afford the crude macrocyclic monoester **3** (R = H) in 78% overall recovery. This displayed one major peak by reverse-phase HPLC analysis (*ca.* 50 % of the total) and several close running peaks which were subsequently removed by HPLC. The pure material (obtained in 30% yield) eluted as a single peak and gave the expected ES mass spectrum (574 Da, $[M+H]^+$). ^1H and ^{13}C NMR spectra recorded in DMSO and analysed using COSY, TOCSY and HSQC techniques revealed the presence of two major conformers, as expected,[12] corresponding to the *trans-* and *cis-* Glu-γ-Pro rotomers.[13] Saponification of the monoester **3** (R = H) gave a diacid which displayed an identical ES mass spectrum (560 Da, $[M+H]^+$) to that derived from the saponification of the diester **3** (R = Me). Treament of a sample of the crude material **3** (R = H) with diazomethane gave the crude diester **3** (R = Me) which displayed several NMR spectral signals coincident with those for the pure solution-phase synthesised material, Scheme 1, and further analysis indicated that crude **3** (R = Me) was *ca.* 50 % pure in keeping with the HPLC analysis of the precursor **3** (R = H).

In an attempt to improve upon the overall yield of 30%, the diester **17b** was converted to the PFP triester **17d** and was treated briefly with piperidine, immediately washed (to minimise piperidine amide formation) and then treated with DIPEA to give the macrocycle **3** (R = Wang resin). Removal from the resin gave crude **3** (R = H) in 80% recovery which was of similar purity to the material **3** (R = H) prepared using PyBOP-activation. In order to discover the cause of the low purity of the cyclised materials, the Fmoc-pentapeptide triester **17a** was removed from the resin and the free Asp α-carboxy group was methylated. The crude product **17e** was > 90% pure and gave the expected analytical and spectroscopic data, showing that the solid-phase cyclisation itself was the cause of the problem. The allyl and Fmoc groups were then sequentially removed (Scheme 2) to give the amino acid **17f** (R = Me) which was cyclised through activation of the carboxy group with PyBOP to afford crude **3** (R = Me) in quantitative recovery. HPLC, MS and NMR spectroscopic analysis indicated that this material was at least 85% pure and identical to the material obtained from the solution-phase synthesis, Scheme 1. Thus, the resin-based synthesis gives low yields for the cyclisation step, compared to the situation in solution, but offers significant advantages in the construction of the linear isopentapeptide precursor. We are now using this optimised approach to synthesise structural variants for conformational and biological SAR studies. The 3-D structures of **3** (R = H), **3** (R = Me), **4** (R = Me) and some related compounds are given in the following article.

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Abbreviations DIPEA, N,N-diisopropyl ethylamine; EDCI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide methiodide; IBCF, isobutylchloroformate; NMM, N-methyl morpholine; PyBOP, Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate; TES, triethylsilane; TFA, trifluoroacetic acid; Wang, Wang resin.

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