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Convergence Leads to Success: Total Synthesis of the Complex Nonribosomal Peptide Polytheonamide B**

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Beyond the limited set of amino acids employed for ribosomal protein biosynthesis, numerous nonproteinogenic amino acids are used in nature for the generation of complex peptidic structures. In many cases, such nonribosomal peptides are assembled by the action of nonribosomal peptide synthetases (NRPS), large enzymes with modular organization. The interesting biological properties of nonribosomal peptides make them attractive targets for total synthesis, thus making studies of structure–activity relationships (SAR) possible.[1]

The polytheonamides A and B are possibly the largest and most complex nonribosomal peptides reported so far.[2] They were isolated from the marine sponge *Theonella swinhoei*, though they may potentially be produced by an unknown symbiotic microorganism. Polytheonamides A and B comprise 48 amino acid residues plus an N-terminal 5,5-dimethyl-2-oxohexanoyl cap (Figure 1). The amino acids display alternating L and D configuration, with the exception of eight glycine residues. Furthermore, 13 of the 19 different amino acid components constituting the polytheonamides are nonproteinogenic, including some β-methylated derivatives. One unique amino acid structure can be found: a sulfoxide amino acid bearing a stereogenic sulfur atom in its side chain (Figure 1). The only structural difference between polytheonamides A and B is the absolute configuration of this sulfoxide moiety, and so far, an assignment of the configuration (R_S/S_S) to the corresponding polytheonamide peptide has not been feasible.

Polytheonamides display pronounced cytotoxicity (e.g. polytheonamide B (**1**): $EC_{50} = 79$ pM). NMR studies indicate that **1** folds into a β helix stabilized by hydrogen bonds, thus forming a 30 Å long tubular structure.[3] Owing to its three-dimensional structure, the ability to conduct monovalent cations across lipid bilayers, and its voltage-gating properties, polytheonamide B has been proposed to act as an ion channel,[4] probably accounting for the highly cytotoxic effect of the peptide. While, for instance, gramicidin A is a rather thoroughly studied peptidic transmembrane channel,[5] molecular details of polytheonamide action remain to be elucidated.

Because of their interesting structural and biological properties, the polytheonamides should be considered attractive new targets for SAR investigations. SAR data might potentially broaden the molecular understanding of the polytheonamide mechanism and of channel formation and function in biological membranes in general. However, the total synthesis of complex peptide structures such as the polytheonamides still represents a significant challenge. The numerous unusual amino acid motifs make the synthetic assembly by standard solid-phase peptide synthesis (SPPS) difficult, and biosynthetic approaches often do not permit structural variations sufficient for detailed SAR studies. Inoue and co-workers have now reported the first total synthesis of polytheonamide B (**1**), which is based on a convergent synthetic strategy, and also the stereochemical assignment of the sulfoxide moiety.[6] They have dissected the structure of **1** into four peptide segments, each composed of 7–16 amino acids. These building blocks were designed in such a way that their late-stage coupling would occur through the activation of their C-terminal glycine residues, thus avoiding epimerization in the coupling steps (Figure 1). Eight of the 13 nonproteinogenic amino acids found in **1** were not commercially available. Consequently, their prepraration represented the initial synthetic challenge and was followed by assembly of the four peptide segments by SPPS.

The stereoselective synthesis of a 9-fluorenylmethoxycarbonyl(Fmoc)-protected derivative of the characteristic sulfoxide amino acid for SPPS was a key achievement in the total synthesis of **1**. Starting from suitably protected aspartate **2**, methylation in the β position gave **3**, which could be transformed into sulfide **4** in six steps. The diastereoselective oxidation of **4** was then performed under Katsuki conditions[7] with urea–hydrogen peroxide in the presence of the chiral Ti(salen) catalyst **5**. Thus, the Fmoc-protected sulfoxide amino acid ester **6** was obtained in an initial diastereoselectivity of 85% *de*, which could be improved to 96% *de* by column chromatography. Acidic deprotection then furnished target compound **7** (Scheme 1). The absolute configuration at

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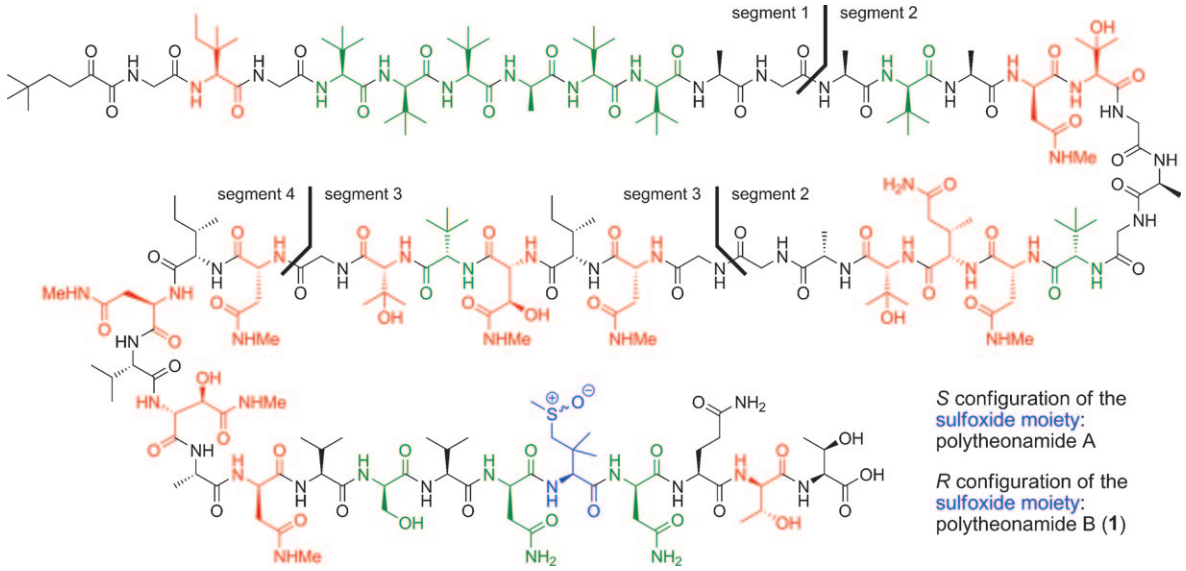
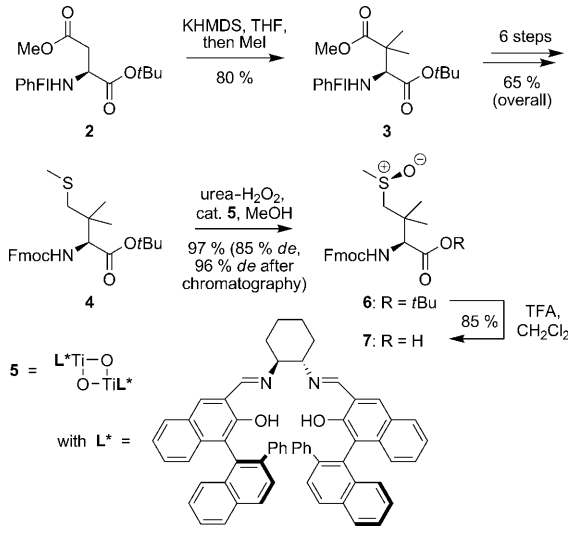


Figure 1. Structure of polytheonamides. Nonproteinogenic amino acids are highlighted in green, and those which required preparation in the course of the total synthesis of polytheonamide B (**1**) are highlighted in red. The sulfoxide amino acid is shown in blue. The four peptide segments conceived and prepared for the convergent total synthesis of **1** are indicated.



Scheme 1. Diastereoselective synthesis of the Fmoc-protected sulfoxide amino acid **7** for solid-phase peptide synthesis. KHMDS = potassium hexamethyldisilazanide, PhFl = 9-phenylfluoren-9-yl, TFA = trifluoroacetic acid.

the stereogenic sulfur atom of **7** was proven to be *R* by application of the NMR-based methodology of Kusumi and Yabuuchi after derivatization of the sulfoxide.[8]

The preparation of the four peptide segments was then feasible using standard SPPS chemistry. The main challenges at this stage, resulting from the amino acid sequence of **1**, were the numerous sterically hindered β-tetrasubstituted amino acids displaying low reactivity as well as the presence of many asparagine and glutamine derivatives, which tend to form interstrand aggregates in the peptide–resin matrix. Since the sequence length accessible by SPPS was limited to 16, the Inoue group developed the described segment-based convergent approach. Segments 1–3 (Figure 1) were synthesized on a Wang resin, and after cleavage from the resin, they were converted into thioester derivatives. The resultant peptide thioester building blocks could be isolated in overall yields from 9 to 13 %. The C-terminal segment 4 was synthesized on a 2-chlorotrityl resin and obtained in 7 % overall yield after 31 reaction steps. Thus, all reactants for the final convergent assembly of the complete peptide were prepared. Starting from N-terminally unprotected segment 4, coupling with the neighboring thioester segment 3 was achieved in the presence of silver(I) nitrate as introduced by Aimoto.[9] After basic Fmoc deprotection, the same coupling conditions with subsequent basic deprotection were applied for the reaction with thioester segments 2 and 1. The yields for each coupling procedure were in the range of 83 to 91 %. This resulted in the stepwise formation of protected polytheonamide B, and global acidic deprotection finally furnished target peptide **1**. As the obtained compound was spectroscopically and chromatographically identical with naturally occurring polytheonamide B, it was proven for the first time that polytheonamide B displays *R* configuration at the sulfoxide moiety, and polytheonamide A was therefore assigned to be *S*-configured in the side chain of amino acid 44.

In conclusion, the recently reported first total synthesis of the nonribosomal peptide polytheonamide B (**1**) is a prime example of the potential of state-of-the-art peptide chemistry. It is the latest in the series of impressive achievements in peptide synthesis, for example, the preparation of the membrane-pore-forming antibiotic alamethicin[10] and of the DNA-gyrase inhibitor microcin.[11] In the case of polytheonamide B (**1**), the stereoselective synthesis of nonproteinogenic amino acids in combination with solid-phase peptide synthesis and the coupling of peptide segments under mild conditions provided access to a complex peptidic structure of high biological relevance. The developed synthetic route also

demonstrates the versatility of convergent approaches in the preparation of longer oligopeptides. The stage is now set for the synthesis of polytheonamide analogues, SAR studies, and further structural as well as biophysical investigations.

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