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N,O-Isopropylidenated Threonines as Tools for Peptide Cyclization: Application to the Synthesis of Mahafacyclin B

Nima Sayyadi, Danielle Skropeta, and Katrina A. Jolliffe*

School of Chemistry, The University of Sydney, 2006 NSW, Australia

jolliffe@chem.usyd.edu.au

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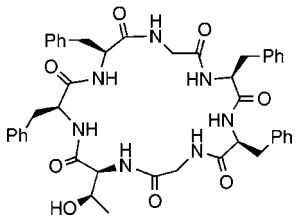
ABSTRACT



The influence of a single *N,O*-isopropylidenated threonine turn-inducer on the cyclization of a linear heptapeptide precursor to mahafacyclin B has been investigated. Incorporation of an *N,O*-isopropylidenated threonine more than doubles the head-to-tail cyclization yield. The *N,O*-isopropylidene grouping is then readily disassembled to give the antimalarial cyclic peptide in high yield.

Biologically active cyclic peptides have been isolated from a variety of sources including plants, bacteria, and marine organisms. These naturally occurring compounds exhibit a wide range of biological activities. This, together with the fact that they generally exhibit improved biological properties such as metabolic stability and receptor selectivity when compared to their linear counterparts, has led to much interest in the synthesis of cyclic peptides.[1−3] However, the head-to-tail cyclization of a linear peptide is often a slow, low-yielding procedure, accompanied by side reactions such as cyclodimerization and epimerisation.[4,5] We recently reported on the use of removable turn-inducers, in the form of *N,O*-isopropylidenated threonines, to facilitate head-to-tail peptide cyclization.[6] These threonine-derived acetals were recently introduced as solubilization aids for solid-phase peptide synthesis.[7,8] Their incorporation into linear peptide precursors was found to substantially increase cyclization yield, compared to cyclization of the same peptides having *O*-TBS-protected threonine residues.[6] After cyclization, the turn-inducers were readily removed to give the cyclic peptides bearing free threonine residues. We now report on the application of this methodology to the total synthesis of the naturally occurring cyclic peptide, mahafacyclin B.

Mahafacyclin B (**1**) is a cyclic heptapeptide [cyclo(Thr-Phe-Phe-Gly-Phe-Phe-Gly)] with antimalarial activity (IC_{50} = 2.2 μM), which has been isolated from the latex of *Jatropha mahafalensis* (Euphorbiaceae).[9] The structure and conformation of mahafacyclin B were recently elucidated and the structure confirmed by synthesis. The final step in the synthesis, namely, cyclization, was reported to occur in only 30% yield.[9] Since mahafacyclin B contains a single threonine residue, which can be readily *N,O*-isopropylide-

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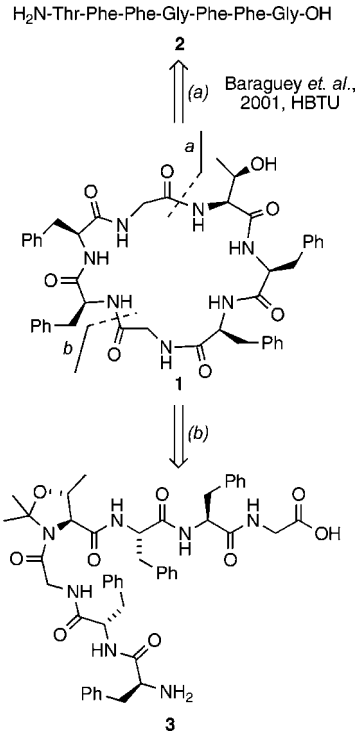
nated, it appeared an ideal target on which to test our *N*,*O*-acetal-assisted cyclization methodology.

In their previous synthesis of mahafacyclin B, Baraguey et al. chose the point of cyclization to be between Thr and Gly to give a linear precursor **2** with Thr as the *N*-terminal amino acid and Gly as the *C*-terminal amino acid (Scheme 1).[9]

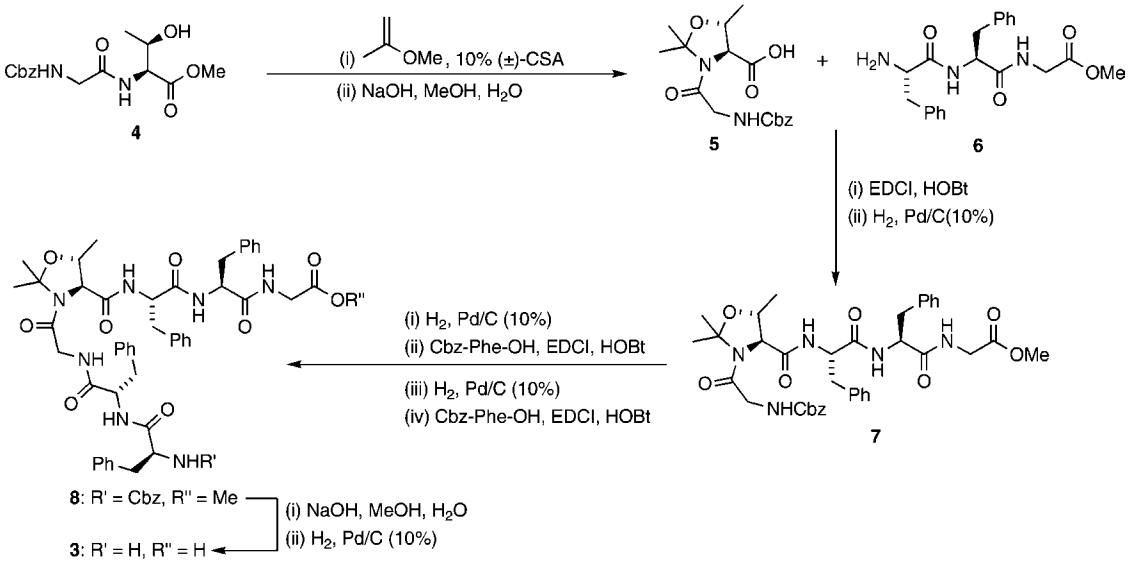
Scheme 1. Different Linear Precursors Required for the Synthesis of Mahafacyclin B



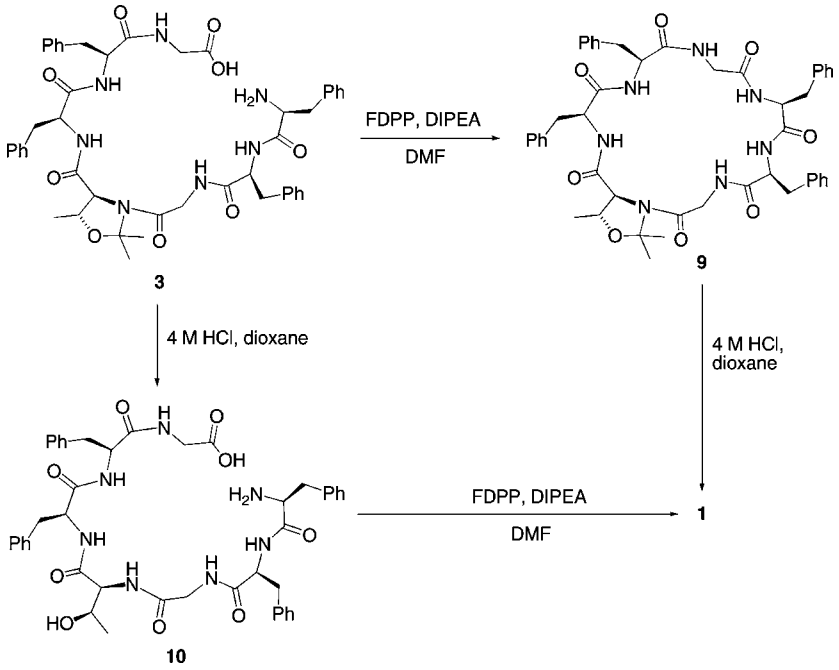
This removed the problem of racemization during the cyclization step. For an *N*,*O*-acetal-assisted cyclization, it was necessary to choose a different cyclization point so that the Thr residue was not positioned at or near the *N*-terminus of the linear peptide precursor. Therefore, we chose to cyclize between the Phe and Gly residues, which gave us the linear precursor **3** (Scheme 1), again with Gly as the *C*-terminal residue.

The required linear heptapeptide **3** was synthesized by solution-phase peptide synthesis using a benzyloxycarbonyl (Cbz)/OMe protection strategy and EDCI (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride) in the presence of HOBt (1-hydroxybenzotriazole) as the coupling reagent. The *N*,*O*-isopropylidene-containing dipeptide **5** was prepared in excellent yield from Cbz-Gly-Thr-OMe **4** by treatment with 2-methoxypropene in the presence of a catalytic amount of (±)-10-camphorsulfonic acid, followed by hydrolysis of the methyl ester under standard conditions (Scheme 2). Tripeptide **6** was also prepared using standard procedures. The two fragments **5** and **6** were then coupled to give the pentapeptide **7**. Sequential addition of the two phenylalanine residues gave protected heptapeptide **8**, and subsequent deprotection of both the *C*- and *N*-termini of the peptide under standard conditions gave the linear heptapeptide **3** which was purified by preparative reversed-phase HPLC. For the *N*,*O*-isopropylidene-containing dipeptide **5** and the methyl ester precursor to **5**, a major and minor set of resonances in a ratio of 85:15 were clearly observed in the ^{1}H and ^{13}C NMR spectra.[10] The major conformer was determined to be that with a *cisoid* amide bond on the basis of the typical NOE cross-peaks observed by 2D NMR ROESY and NOESY experiments (i.e., $\alpha H_{i-1} - \alpha H_i$ and $\alpha H_{i-1} - \beta H_i$ cross-peaks).[11] For the *N*,*O*-isopropylidenated threonine-containing peptides **7**, **8**, and **3**, only a single set of resonances are observed in the ^{1}H and ^{13}C NMR spectra. In all cases, these were assigned to be the conformers with a *cisoid* amide bond preceding the *N*,*O*-isopropylidenated threonine residue on the basis of the typical NOE cross-peaks

Scheme 2



Scheme 3



observed by 2D NMR ROESY and NOESY experiments (i.e., $\alpha H_{i-1} - \alpha H_i$ and $\alpha H_{i-1} - \beta H_i$ cross-peaks).[11]

Cyclization of **3** was performed using both pentafluorophenyl diphenylphosphinate (FDPP) and HBTU [(*O*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate] as coupling reagents under high dilution (0.005 M) in DMF to give the cyclic peptide **9** in 63% and 58% yield, respectively, twice the yield obtained by Baraguey for cyclization of the linear precursor **2**.[9] The ^{1}H NMR spectrum of **9** indicated two conformers present in a ratio of 83:17. While overlapping of the signals from different rotamers makes it difficult to observe the characteristic NOE cross-peaks for the conformer in which the amide bond preceding the *N*,*O*-isopropylidenated threonine residue is *cisoid*, it can be assumed by analogy with the other peptides discussed here that this is the major conformer. Upon treatment of **9** with trifluoroacetic acid for 24 h, deprotection of the *N*,*O*-isopropylidenated threonine residue gave cyclic peptide **1**, in 90% yield (Scheme 3). This material was found to have physical and spectral properties identical to those reported for mahafacyclin B.[9]

The sequence of a peptide is known to have a strong influence on cyclization yield.[12,13] Therefore, to determine whether the higher yield obtained from our *N*,*O*-isopropylidenated threonine-containing precursor **3** was attributable to the presence of the turn-inducer, or simply a result of peptide sequence, we prepared and cyclized the linear heptapeptide **10**. Heptapeptide **10** has an identical sequence to **3**, but lacks the *N*,*O*-isopropylidene, and was readily prepared upon treatment of **3** with 4 M HCl in dioxane for 10 min. Cyclization of **10** was investigated using conditions identical to those described above to give the major cyclic peptide product, mahafacyclin B (**1**), in 23% yield. The material obtained via this route was identical, in all respects, to that obtained upon cyclization and deprotection of **3**. Notably, cyclization of **10** required more than 72 h to reach completion, as determined by following both the consumption of the starting material and the appearance of the product by HPLC, while the cyclization of **3** was complete in less than 3 h. The much lower yield and longer reaction time required for cyclization of **10** compared to **3** indicates that the presence of the *N*,*O*-isopropylidenated threonine residue is assisting **3** to adopt a conformation amenable to cyclization.

In summary, we have shown that the introduction of a single *N*,*O*-isopropylidenated threonine turn-inducer into a linear peptide precursor significantly increases the head-to-tail cyclization yield of that peptide. This method has successfully been applied to the total synthesis of mahafacyclin B, a naturally occurring cyclic heptapeptide and will find future application in the synthesis of a wide range of cyclic peptides.

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Supporting Information Available: Experimental procedures and characterization of all compounds. ^{1}H and ^{13}C NMR spectra of compounds **1**, **3**, **4**, and **7**−**10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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