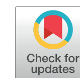
Tetrahedron 74 (2018) 4247–4258

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Thioether macrocycles of the microbisporicins via reductive desulfurization



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ARTICLE INFO

Article history:
Received 26 May 2018
Received in revised form
17 June 2018
Accepted 19 June 2018
Available online 23 June 2018

Keywords:
Lantibiotic
Thioether
Peptide synthesis
Desulfurization

ABSTRACT

The microbisporicins are the most potent lantibiotics isolated to-date. Cyclic tetra-, hexa- and octa-peptides, inspired by this family of antimicrobial agents, have been synthesized from linear peptides. Generalized reaction conditions are reported for the two-step conversion of linear peptides to thioether macrocycles: formation of a disulfide followed by reductive desulfurization. ^{1}H NMR analysis of the reduction reaction mixture indicated the intermediacy of a dehydroalanine when excess hexamethylphosphorus triamide (HMPT) was employed for the reduction. Maintaining a stoichiometric amount of HMPT, in dilute methanolic solution, gave the corresponding thioethers, retaining stereochemical integrity.

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1. Introduction

Lantibiotics are lanthionine-containing peptides with antibiotic properties (*e.g.*, **1**, Fig. 1A) [1]. These compounds are synthesized ribosomally and undergo substantial post-translational modifications, characterized by the formation of the bridging *bis*-amino acids, *meso*-lanthionine (Lan, Ala-S-Ala, Fig. 1B) and *meso*-β-methyl-lanthionine (MeLan, Abu-S-Ala) [2,3]. Microbisporicins A_1 (with a dihydroxylated proline at position 14) and A_2 constitute antibiotic NAI-107, demonstrated to have submicromolar activity against MRSA [4,5].

A major challenge in the chemical synthesis of lantibiotics is the formation of the array of thioether bridges with the correct topology [6]. While a number of approaches to such macrocycles have been explored [7–10], the incorporation of a preformed lanthionine unit [11], in combination with solid-phase peptide synthesis (SPPS), has gained momentum, with cyclizations involving peptide bond formation rather than manipulation of the thioether linkage. In 2010–11, there were some pioneering reports of the total synthesis of lantibiotics via SPPS protocols. Notwithstanding these significant accomplishments, this linear approach results in lengthy syntheses and limited quantities of peptides that can be difficult to purify. Vederas reported the first SPPS of lactocin S, in 10% overall yield for 71 steps [12]. Tabor's group reported the first synthesis of a lantibiotic fragment with overlapping lanthionine bridges via SPPS [13], reporting that “the peptide proved difficult to purify by HPLC due to tailing of impurities” and “a yield of less than 0.1 mg of the peptide was obtained, which was not sufficient for detailed NMR analysis.” Vederas reported a similar approach to both components of lacticin 3147 [14], largely via SPPS although “the *N*-terminal peptide containing ring A was synthesized in solution because of its extensive and densely packed modifications.” The van der Donk group has pursued both chemical [15] and bioengineering [16] avenues to produce lantibiotics (*e.g.*, their work on epilancin 15X) signifying that neither approach is superior and complementary approaches are valuable, even within the same research group. Since 2012, the chemical synthesis of lantibiotics has slowed and literature searches indicate production of these peptides mostly via microbial systems.

With a view to a more convergent, scalable approach to medium-sized peptide fragments bearing thioethers, we sought to explore the reductive desulfurization of disulfide macrocycles (Fig. 1B). Harpp and Gleason originally described the desulfurization of cystine to lanthionine using aminophosphines [17]. Reductive desulfurization has also been accomplished under alkaline conditions, with the caveat that a dehydroalanine intermediate surrenders control over stereochemistry [18]. The Harpp-Gleason approach was applied to the first total synthesis of the prototypical lantibiotic nisin by Shiba and coworkers in the early 1990s [19–23]. Considerable optimization of reaction conditions was

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https://doi.org/10.1016/j.tet.2018.06.050
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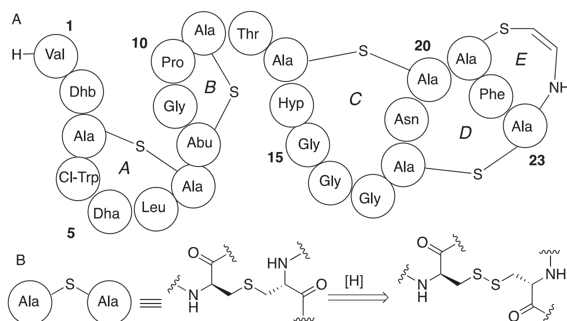


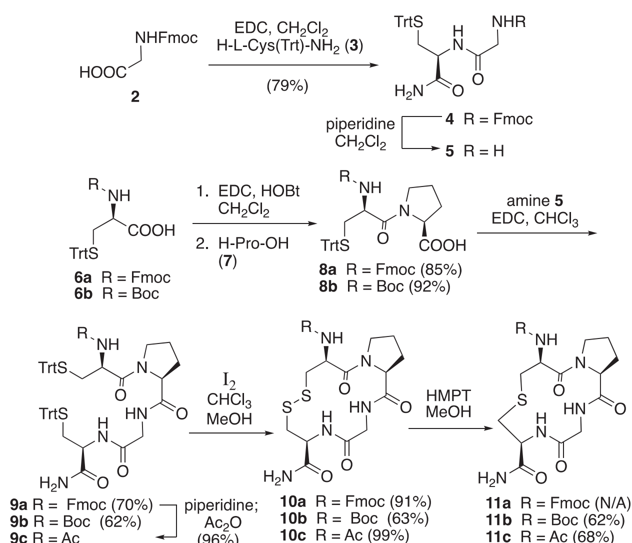
Fig. 1. (A) Microbisporicin A_2 (**1**). Dhb - dehydrobutyrine, ClTrp – 5-chlorotryptophan, Dha = dehydroalanine, Abu = α-aminobutyric acid, Hyp = 4-hydroxyproline. (B) Retrosynthetic analysis of the thioether macrocycle.

required for each peptide. Little attention has been paid to this approach in the intervening years. We sought to explore the reductive desulfurization approach with a view to identifying generalized reaction conditions that ought to be applicable to a range of peptides and their synthesis on a useful scale.

2. Results and discussion

Our investigation began with the synthesis of the tetrapeptides depicted in Scheme 1. This tetrapeptide occurs in several lantibiotics, including nisin and subtilin, where it constitutes the B-ring. Short peptides containing this B-ring have been prepared previously via diastereoselective conjugate addition of a liberated Cys thiol to an appropriately positioned dehydroalanine (Dha). Toogood (H-Ala-Dha-Pro-Gly-L-Cys-Ala-OBn) [24], Bradley (Boc-Ile-Dha-Pro-Gly-L-Cys-Val-Gly-O-resin) [25] and van der Donk (H-Leu-Dha-Pro-Gly-L-Cys-Val-Gly-OH) [26] all reported the formation of a single compound, corresponding to *meso*-lanthionine.

Our synthesis of the B-ring tetrapeptides is summarized in Scheme 1. Dipeptide **4** was prepared by condensation of Fmoc-Gly-OH (**2**) and H-L-Cys(Trt)-NH_2 (**3**). Peptide **4** was deprotected at the N-terminus to give amine **5**, a common intermediate in all the B-ring tetrapeptides. We synthesized dipeptide acids **8a** and **8b**, Fmoc- and Boc-protected respectively, at the *N*-terminus. Our usual approach to dipeptide acids [27], using DCC/NHS, was less effective



Scheme 1. Synthesis of B-ring tetrapeptides.

than EDC/HOBT, presumably due to the hindered nature of the prolyl amino component. Coupling of each dipeptide acid independently with amine **5** gave tetrapeptides **9a** and **9b**. The *N*-acetyl derivative **9c** was prepared from **9a** via standard methods. Treatment of each peptide with iodine led to concomitant removal of the trityl ethers and disulfide formation, *viz.* disulfide macrocycles **10a-c**. The excess iodine was typically quenched using sodium thiosulfate, followed by aqueous work-up. However, for peptides with considerable water-solubility (*e.g.*, **10c**), work-up was better achieved using Amberlite® IRA-400A resin in its chloride form [28] to avoid water. A single crystal of **10b** was suitable for X-ray diffraction analysis, which revealed the prolyl amide bond to be in the *cis*-conformation.

Desulfurization studies began with disulfide **10a**. Important reaction parameters such as desulfurization agent, solvent and concentration were considered. No reaction was observed with PPh_3 or $P(OEt)_3$ [29] over several days, whereas $P(^nBu)_3$ [30] led to the dithiol reduction product. Shiba and coworkers employed hexaethylphosphorus triamide (HEPT) [19–23], which we found to be incompatible with the Fmoc group. Cleavage could be attributable to diethylamine present as an impurity, although the phosphorus triamide itself is likely basic enough [31] to promote the β-elimination of dibenzofulvene. Indeed, dipeptide **4** could be converted to amine **5** in 48% yield on exposure to 6.1 mM HEPT (5 equivalents) in methanol. With a shift in focus to disulfide **10c**, a range of solvents were explored (PhH, PhMe, CH_2Cl_2, $CHCl_3$, THF, MeOH, MeCN and DMF), using 20 equivalents of HEPT (30 mM). For tetrapeptides **10b** and **10c**, the best results for contraction to the thioether, vis-à-vis minimizing byproducts and reaction time, were obtained with methanol as solvent.

Although less reagent was required for the reduction of **10c** to **11c** with Verkade's base [32], and the reaction was faster, epimerization was exacerbated (*vide infra*). A change from HEPT to HMPT was advantageous for two reasons. Firstly, dimethylamine is more volatile than diethylamine and thus the amine impurity is more readily removed by evaporation. Separation of the peptide products from the more polar byproducts derived from HMPT was typically also more facile. The high stoichiometric ratio of phosphorus triamide to peptide, employed by Shiba et al. [19–23], compensated for the high dilution (0.3–3.0 mM) invoked to encourage intramolecular thioether formation. In our hands, while the desired thioether formation was observed in the presence of 5–40 equivalents of HMPT, the reaction was cleaner, albeit slower, at lower concentrations. The impact of HMPT concentration was studied by ^1H NMR (Fig. 2) leading to insights into the mechanism of the reaction (Scheme 2). At ~50 mM HMPT, signals appeared at δ5.6 and 6.1 ppm, indicative of a dehydroalanine intermediate. When a stoichiometric amount of HMPT was employed, this intermediate was not observed.

Harpp and Gleason proposed that the reaction proceeds via S_N2 attack of a thiolate anion at the β-position of a Cys-derived phosphonium salt (Scheme 2, path a). Oku et al. reported the stabilization of such a phosphonium salt in a rotaxane [33]. The stereochemistry at Cα is unperturbed and the configuration at Cβ is inverted in the case of β-methylcysteine. In the presence of base, including an excess of HMPT, elimination of thiophosphoramide (path b) generates dehydroalanine (Dha, IV) which renders the Cα stereogenic center susceptible to scrambling. Others have reported the intermediacy of dehydroalanine intermediates [34,35].

Shiba and coworkers probed the stereochemical integrity of the lanthionine residues in the macrocyclic peptide fragments they synthesized via their case-by-case optimized reaction conditions [19–23]. Acidic degradation, followed by amino acid analysis, showed that “racemization” increased with the concentration of

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