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Synthetic studies toward labionin, a new α,α-disubstituted amino acid from type III lantibiotic labyrinthopeptin A2

Georg M. Sambeth and Roderich D. Süssmuth*

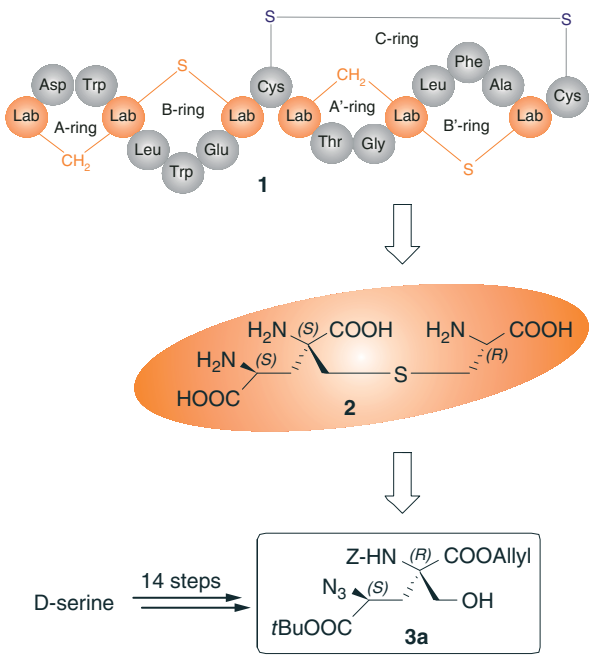
The labyrinthopeptins are a new class of lantibiotics containing two identical quaternary α,α-disubstituted amino acids, named labionin (Lab). The synthetic formation of this unique structural feature represents the key step in the total synthesis of these polycyclic peptides. In this report we describe the synthesis of an orthogonally protected α,α-disubstituted amino acid building block serving as labionin precursor for the future assembly of labyrinthopeptin A2 and of other labyrinthopeptin derivatives.

Supporting information may be found in the online version of this article

Keywords: peptide; lantibiotic; labyrinthopeptin; labionin; lanthionine; α,α-disubstituted amino acid

Introduction

The labyrinthopeptins are a new class of lantibiotics, which are produced by the actinomycete *Actinomadura namibiensis* DSM 6313 [1,2]. Recently our group elucidated their pentacyclic, globular structures alternating in ring size and amino acid composition and established the reconstitution of essential biosynthetic steps [3,4]. Labyrinthopeptin A2 (**1**, Scheme 1) shows an excellent efficacy against tactile allodynia in an *in vivo* mouse model (ED_{50} = 50 µg/kg) and is considered as a drug for potential applications in treatment of neuropathic pain. Its X-ray crystallographic structure published by our group and collaborators [4] revealed a new amino acid named labionin (Lab), assembled from two 2,3-didehydroalanines (Dha) and one cysteine in a consecutive double Michael-type addition reaction [3]. The resulting (2*S*,4*S*,8*R*)-configured structure of labionin (**2**) may be described as an α,α-disubstituted lanthionine, whose central quaternary carbon atom is linked to a further amino acid moiety by a methylene bridge. Compared with lanthionine (Lan) enabling the establishment of only one thioether bridge in peptide side chains, labionin facilitates an additional unusual carbacyclic ring linkage (Scheme 1).

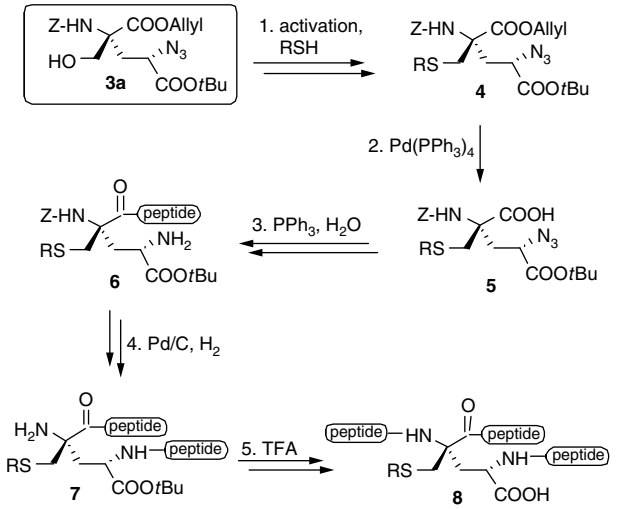


Scheme 1. Structures of labionin (**2**) and labionin precursor (**3a**) and retrosynthesis strategy for labyrinthopeptin A2 (**1**).

Herein we report the synthesis of the α,α-disubstituted amino acid building block **3a** (Scheme 1) destined for a future assembly of new labyrinthopeptins in a total synthesis effort. Our particular interest in labionin synthesis is driven by the need for new labionin analogues which cannot be accessed by biosynthetic manipulations. Previously, there have been published a number of elegant strategies for the synthesis and assembly of lanthionines [5–7] and lantibiotics [8–11], respectively. However, none of those approaches seem to be applicable to the synthesis of labionin. The additional, sterically demanding incorporation of an amino acid moiety in a preferably stereoselective manner under establishment of an unusual quaternary α-carbon center afforded the development of a new strategy for the preparation of labionin-like structures. Therefore, the herein presented synthesis is considered as an important milestone toward the total synthesis of labyrinthopeptin A2.

* *Correspondence to: Prof. Dr. Roderich D. Süssmuth, Technische Universität Berlin, Institut für Chemie/FG Organische Chemie, Straße des 17. Juni 124/TC 2, D-10623 Berlin, Germany. E-mail: suessmuth@chem.tu-berlin.de*

Technische Universität Berlin, Institut für Chemie, Straße des 17. Juni 124, 10623 Berlin, Germany



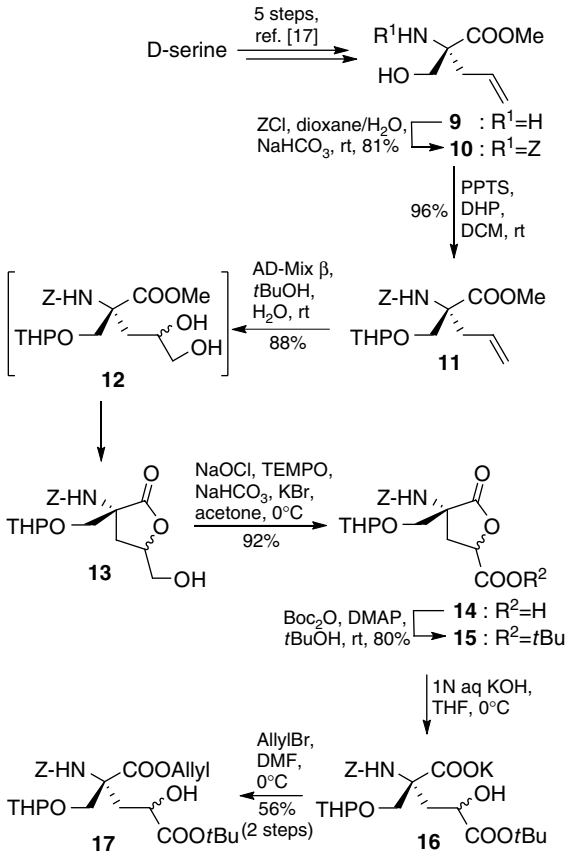
Scheme 2. Selective and sequential demasking and transformation of each of the five functional groups of labionin precursor **3a**.

Results and Discussion

Our primary aim was to prepare a labionin precursor already containing the quaternary carbon and the methylene bridge pre-established as found in labionin. Further requirements for the subsequent peptide assembly and ring formations are (i) correct stereochemistry, (ii) facile incorporation into a peptide chain, (iii) stability toward peptide coupling conditions, (iv) functionalization of the amino acid side chain into a thioether establishing the lanthionine moiety and (v) orthogonally masked functional groups, which could be addressed selectively. The latter turned out to be the most challenging part of the synthesis. Related, albeit less complex amino acids, like lanthionine or diaminoglutamic acid either contain only four functional groups instead of five, as in the present case of labionin, or are not protected orthogonally [8,12,13]. Additionally the high density of functionalities easily leads to mostly undesired and disturbing intramolecular ring closing reactions [14–16]. Hence, lactam and lactone formations could be observed, when inadequate strategies were applied.

We considered compound **3a** to be suitable as a labionin precursor, because it accomplishes all the above-mentioned criteria to a maximum extend. Selective demasking and transformation of each functionality (Scheme 2) would be achieved by the following sequence of reactions: (i) conversion of the alcohol **3a** into a thioether (**4**), (ii) removal of the allyl ester using Pd^0 (**5**), (iii) Staudinger reduction of the azide (**6**), (iv) hydrogenolysis of the Z group (**7**), (v) cleavage of the *tert*-butyl ester using TFA (**8**). Hence, each transformation or deprotection step liberates selectively one functional group for subsequent formation of the lanthionine thioether and the peptide assembly.

Starting material for the preparation of quaternary building block **3** was olefin **9** (Scheme 3), which was synthesized from D-serine as published by Palomo *et al*. [17] according to a protocol of Seebach *et al*. [18] in five steps and an overall yield of 36%. For a rapid establishment of the side chain functionalities in amino acid **3** we considered Sharpless asymmetric aminohydroxylation (AA) [19,20] as the method of choice. However, all efforts to transform olefin **11** into the corresponding amino alcohol failed. AA seems not to be applicable to terminal olefins with aliphatic substituents, probably because of insufficient stabilization of transition states by electron donating groups. Missing examples in the literature



Scheme 3. Synthesis of allyl ester **17** from D-serine via quaternary α,α-disubstituted amino acid **9** as an intermediate compound.

appear to underline this assumption. Alternatively, we chose the longer but feasible route via Sharpless asymmetric dihydroxylation (AD). For this purpose the *N*-terminus of amino acid **9** (Scheme 3) was Z-protected with ZCl and $NaHCO_3$ as a base in dioxane at room temperature (rt) to give alcohol **10** in 81% yield. The hydroxyl function of **10** was converted into 2-tetrahydropyranyl (THP) ether **11** using 3,4-dihydro-2*H*-pyran and pyridine *p*-toluenesulfonate (PPTS) in DCM at rt in 96% yield. Owing to the generation of a chiral center upon THP protection, we obtained serine derivative **11** as diastereomeric mixture. Subsequent AD afforded diol **12** in 88% yield as an inseparable mixture of four diastereomers. To our delight, we observed an *in situ* lactone formation between the secondary alcohol and the methyl ester affording alcohol **13**, which facilitated the selective functionalization of the primary hydroxyl group without additional protecting and deprotecting steps. NMR-data of the obtained lactone **13** indicated that the stereoselectivity of the AD was only moderate. Comparable results for related model compounds have been reported by Iwashima *et al*. [21,22]. Then, alcohol **13** was directly subjected to 2,2,6,6-tetramethyl-1-piperidinyloxy (TEMPO)/NaOCl oxidation with $NaHCO_3$ and KBr in acetone at 0 °C [20] to afford carboxylic acid **14** in 92% yield. The resulting carboxyl group was protected as *tert*-butyl ester by treatment with Boc_2O and DMAP as a base in *t*BuOH at rt [23] affording fully protected lactone **15** in 80% yield. To establish the amino function in the side chain, the lactone ring had to be opened by treatment with aqueous KOH in THF. The hydrolysis was performed at 0 °C to avoid saponification of the *tert*-butyl ester, that has been observed at higher temperatures.

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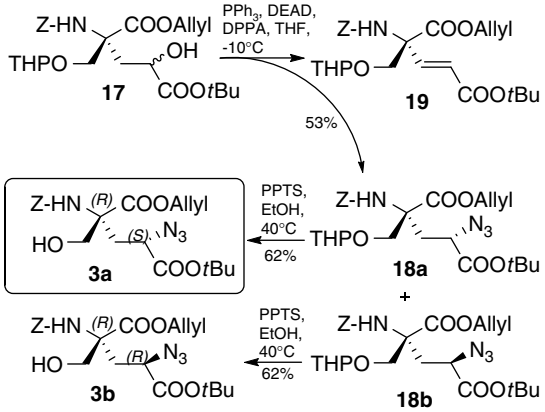
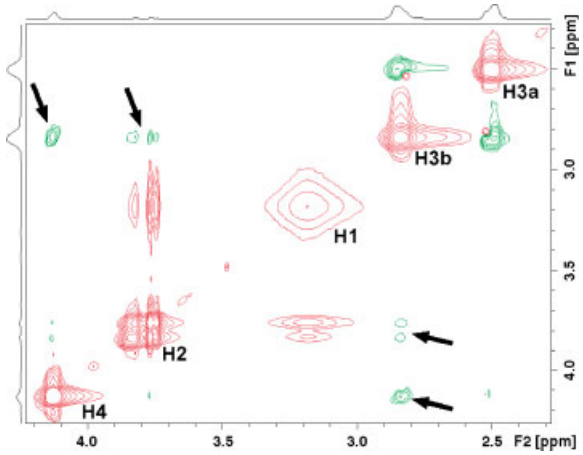


Scheme 4. Final synthesis steps for the assembly of α,α-disubstituted labionin precursor **3**.

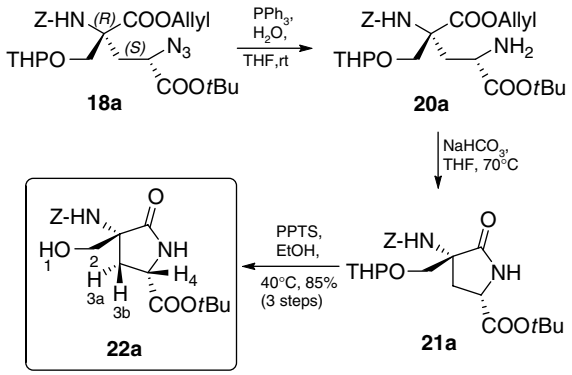


Scheme 6. 2D-NOESY-NMR spectrum of lactam **22a** and crosspeaks indicative of the assignment of the (*S*)-configuration.

The resulting potassium carboxylate salt **16** was directly esterified to the allyl ester **17** by treatment with allyl bromide in DMF. The moderate yield is because of partial back-formation of lactone **14**, which could be subjected to renewed ring opening by KOH. A 50-fold excess of allyl bromide and temperatures below 0 °C were necessary to suppress this competing lactonisation reaction.

Conversion of alcohol **17** into the corresponding azide **18** (Scheme 4) was performed under typical Mitsunobu conditions [24,25] in yields of 53%. The moderate yield is a result of elimination of H_2O under formation of compound **19** as a byproduct, which was identified by NMR spectroscopy (disappearance of the characteristic CH_2-signals between 2 and 3 ppm) and HPLC-ESI-MS analysis. A 15-fold excess of DPPA and low reaction temperatures led to a significant improvement but not to a full suppression of this elimination reaction. After separation of the two diastereomeric pairs by flash column chromatography, we obtained **18a** and **18b** in a 1.2 : 1 ratio. Then, cleavage of the THP ethers with PPTS in DCM at 40 °C accomplished the synthesis of the enantiomerically pure alcohols **3a** and **3b** in 62% yield each. Thus, target compound **3a** was obtained in an overall yield of 2% from D-serine in 14 steps.

Assignment of the relative and absolute stereochemistry of the tertiary stereocenter was achieved by 2D-NMR spectroscopy. Therefore compound **18a** was converted into lactam **22a** by Staudinger reduction of the azide to **20a**, subsequent lactam formation under heating at 70 °C affording compound **21a** and final cleavage of the THP ether to yield **22a** in 85% yield over three steps (Scheme 5). Likewise to Scheme 5, diastereomeric lactam **22b** (see Supporting Information) has been synthesized accordingly from **18b** in a yield of 70% over three steps. The lower yield compared to **22a** is probably because of less favored ring formation to lactam **21b** applying the same reaction conditions. Remarkably, formation of the five membered lactam did not occur spontaneously at rt, as for instance observed for lactonization of intermediate **12** to compound **13**.



Scheme 5. Synthesis of lactam **22a** for assignment of the absolute stereochemistry of the tertiary stereocenter of labionin precursor **3**.

As the stereochemistry of the quaternary α-carbon of amino acid **9** had been known from previous syntheses and assignments of the absolute configuration [17,18], only the newly generated stereocenter had to be assigned. The rigid structure of the five membered ring of lactam **22a** facilitated NOESY measurements showing correlations between the protons H2 and H3b as well as between H3b and H4 (Scheme 6). NOESY data of lactam **22b** confirmed these results by correlations between the protons H2 and H3b as well as between H3a and H4 (see Supporting Information). Hence, the configuration of the newly generated stereocenter of **3a** was determined as (*S*), the configuration of **3b** as (*R*).

Conclusions

In conclusion we succeeded in the first synthesis of the α, α-disubstituted building block **3** as a precursor to the amino acid labionin (**2**). Given numerous previous attempts in performing the synthesis of such labionin precursors in our laboratory, and within the limited options for installing protecting or masking groups this is a first step into the direction of a total synthesis of labyrinthopeptins. In this context, it was important to clarify and assign the configuration of the newly generated stereocenter by derivatization and subsequent NOESY experiments, as the installment of the second stereocenter could not be performed in a diastereoselective manner with an unambigous establishment of the desired configuration. Apart from this obstacle, a considerable advantage of the present synthesis strategy is, that compound **3** contains selectively addressable protecing groups and functional groups, respectively. Therefore, we are confident that **3** constitutes a valuable derivative, not only for the total synthesis of labyrinthopeptins, but also for the preparation of unusual and complex polycyclic peptides.

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