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A Late-Stage Synthetic Approach to Lanthionine-Containing Peptides via S-Alkylation on Cyclic Sulfamidates Promoted by Molecular Sieves

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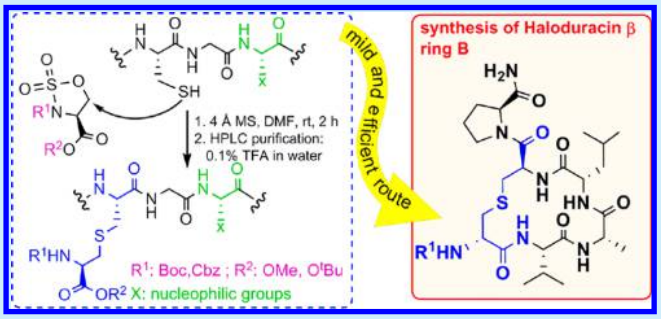
Supporting Information

ABSTRACT: A one-pot, high-yield procedure for synthesizing lanthionine-containing peptides was developed. It relies on the S-alkylation of cysteine-containing peptides with chiral cyclic sulfamidates. The key feature of this approach is the use of mild reaction conditions (only activated molecular sieves are employed as the catalyst), leading to good chemoselectivity and excellent stereochemical control. The potential of the new methodology has been investigated by synthesizing the thioether ring of a natural lantibiotic, Haloduracin *β*.



Lantibiotics show promise as a new class of antibacterial agents that exhibit an extremely potent activity against a broad spectrum of Gram-positive bacteria, including microorganisms resistant to conventional antibiotics.[1] They contain lanthionine (Lan), an unusual amino acid containing a thioether group generated by post-translational modification in ribosomes. Many efforts have been made to develop methodologies for the chemical synthesis of lanthionine-containing peptides, and one of the most challenging issues is the introduction of multiple thioether bridges between side chains, which is a unique structural feature of lantibiotics.[2] In this regard, the main hurdle is the generation of stereochemically pure lanthionine derivatives bearing protecting groups that are compatible with the standard peptide synthesis conditions and that allow the thioether ring formation. To this aim, the most successful and widely employed synthetic approach involves the direct incorporation of lanthionine (or methyl lanthionine) into the growing peptide by using an orthogonally protected lanthionine building block. Such a building block can be obtained by reacting a N-protected cysteine with an orthogonally protected cyclic sulfamidate in the presence of a base, such as Cs_2CO_3 or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).[3]

Cyclic sulfamidates are considered versatile precursors in organic synthesis for the preparation of various compounds of biological interest.[4] They are readily accessible to nucleophilic attack by a variety of substrates, including thiolates from cysteine groups.[5] The reaction occurs via the SN2 mechanism with inversion of the configuration at the sulfamidate O-bearing carbon, providing regioselective and stereoselective control of the process. The opening of the ring generates a N-sulfate derivative that is easily hydrolyzed under acidic conditions. When reacted with N- and C-protected cysteine under basic conditions, five-membered cyclic sulfamidates, derived from serine and threonine, yield protected derivatives of (methyl)lanthionine.[3] However, competing *β*-elimination on cyclic sulfamidates is known to yield the corresponding dehydroamino acid derivative. This side reaction represents a potential limitation of base-catalyzed SN2 reactions, as it might lead to an overall decrease of the reaction yield and to a significant racemization, because of the possible Michael addition of the sulfur nucleophile to the dehydrogenated product.[6]

In this paper, we describe a synthetic approach to promote the SN2 nucleophilic attack of a cysteine thiol group on cyclic sulfamidates while preventing any *β*-elimination side reaction, even in the presence of acidic hydrogens in the sulfamidate ring. Our aim has been to develop a novel synthetic strategy to introduce precursors within a linear peptide sequence able to generate a thioether bridge (i.e., the typical structural motif of lantibiotics). We rely on the S-alkylation of a cysteine residue

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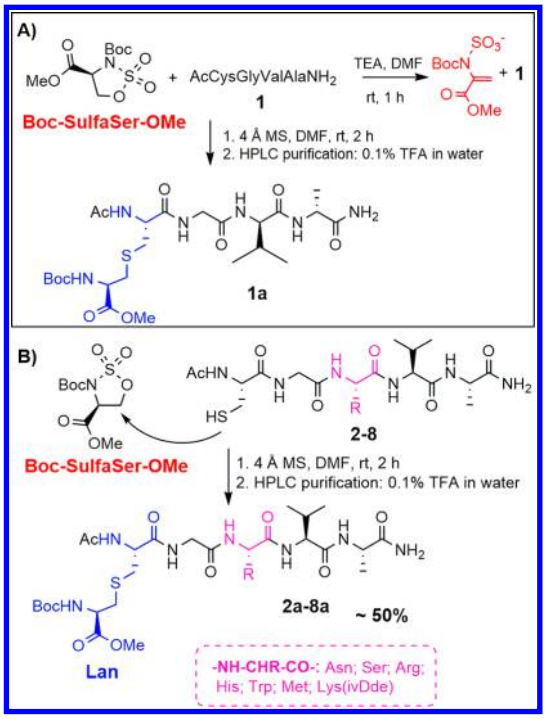
already inserted in a peptide sequence by reaction with a cyclic sulfamidate. The reaction is promoted by activated molecular sieves (MS). Recently, we have shown that the MS-based approach is very efficient for post-synthetic peptide modification via S-alkylation of cysteine-containing peptide sequences.[7] The dried aluminosilicate framework of the activated MS may likely assist in sulfhydryl proton removal.[7,8]

To demonstrate this concept, peptide **1** (bearing N-acetyl protection at the N-terminus and amide protection at the C-terminus, to mimic a simple peptide sequence devoid of potentially competing nucleophiles) was reacted with Boc-SulfaSer-OMe, either in the presence of triethylamine (TEA), as the base, or in the presence of activated MS (see Scheme 1).

Scheme 1. Incorporation of Lanthionine in Peptide Sequences



Boc-SulfaSer-OMe is a protected sulfamidate derived from L-serine that should convert the cysteine residue into an N- and C-protected lanthionine residue. Under basic conventional conditions (TEA, DMF, 1 h reaction time, rt), complete β-elimination to the dehydroalanine derivative was observed, while peptide **1** was recovered unreacted.

On the other hand, when model peptide **1** was reacted with Boc-SulfaSer-OMe in the presence of activated 4 Å MS (T = 280 °C for 4 h under vacuum), the expected product was recovered with 95% yield, as estimated by integration of the HPLC peak of **1a**, compared with that of the starting peptide (see Table 1). The reaction conditions included DMF under an argon atmosphere as the solvent, 1.2 equiv of Boc-SulfaSer-OMe, stirring for 2 h at room temperature. The high reaction yield confirmed the high reactivity exhibited by the cyclic sulfamidate toward the thiol group even under the mild reaction conditions we employed. It is important to underline that the mild acidic conditions of the final purification step (0.1% TFA in water) were sufficient to promote the complete hydrolysis of the sulfate ester.[9] Therefore, the incorporation of protected lanthionine can be considered a one-pot synthesis.

Table 1. Efficiency of the S-Alkylation Reaction To Introduce Lanthionine in Peptides[a]

entry	peptide	yield (%)	time (h)
1a	Ac**Lan**GlyValAlaNH$_2$	95	2
2a	Ac**Lan**GlyAsnValAlaNH$_2$	>95	2
3a	Ac**Lan**GlySerValAlaNH$_2$	>95	2
4a	Ac**Lan**GlyArgValAlaNH$_2$	>95	2
5a	Ac**Lan**GlyHisValAlaNH$_2$	95	2
6a	Ac**Lan**GlyTrpValAlaNH$_2$	>95	2
7a	Ac**Lan**GlyMetValAlaNH$_2$	>95	2
7b	Ac**Lan***GlyMetValAlaNH$_2$	90	2
8a	Ac**Lan**GlyLysValAlaNH$_2$	65	2
9a	AcGlyTrp**Lan**HisValAlaNH$_2$	95	2
10b	ValAlaLeu**Lan***ProNH$_2$ (S)	80	2

[a]Legend: Lan = a lanthionine with Boc and OMe protections. Lan* = a lanthionine with Cbz protection.

Next, we considered compounds **2a**–**7a** (Scheme 1, panel B) to assess whether or not the conversion of cysteine to lanthionine by our protocol could be performed in a chemoselective way. As a matter of fact, these peptides bear unprotected side-chain nucleophilic groups that might potentially compete with cysteine S-alkylation. The excellent yields of monoalkylated peptides **2a**–**7a** (see Table 1) were the first indication of the high chemoselectivity for S-alkylation.

The alkylation site was assessed by nuclear magnetic resonance (NMR) spectroscopy. All peptide resonances, including those from the lanthionine side chain, could be assigned by means of 2D TOCSY and 2D ROESY NMR spectra (see Figure 1 for representative NMR spectra, and SI for comprehensive NMR data). The formation of lanthionine was confirmed unambiguously by the detection of intra residue ROE cross-peaks between Lan Hα and Hδ_1/Hδ_2, and between Hε and Hβ_1/Hβ_2 (see Figure 1 for lanthionine atom nomenclature). Integration of the signal of N-Boc and C-OMe confirmed that all peptides, but **7a** retained full protection on the lanthionine side chain.

Unlike the other compounds, compound **7a** (containing methionine) was obtained as mixture of the Boc-protected (~20%) and deprotected (80%) lanthionine side chain. Compound **7b** was obtained by a reaction with the sulfamidate bearing the more robust Cbz (benzyloxycarbonyl) protection on the amino group, and O^{t}Bu protection on the carboxyl group. The final product **7b** fully retained the lanthionine N-Cbz protection. However, as much as 66% of the product missed the carboxyl O^{t}Bu protection (see the Supporting Information (SI)). Further investigation is needed to assess whether this effect is dependent on the lanthionine and methionine relative position within the peptide sequence.

The lysine amino group and, to a lesser extent, the aspartic/glutamic acid carboxylate group are known to be good nucleophiles. Therefore, S-alkylation to introduce lanthionine in the presence of these amino acids must be performed on peptides bearing suitable masking groups. As an example, we have synthesized compound **8a**, starting from a peptide precursor containing one lysine residue protected with ivDde

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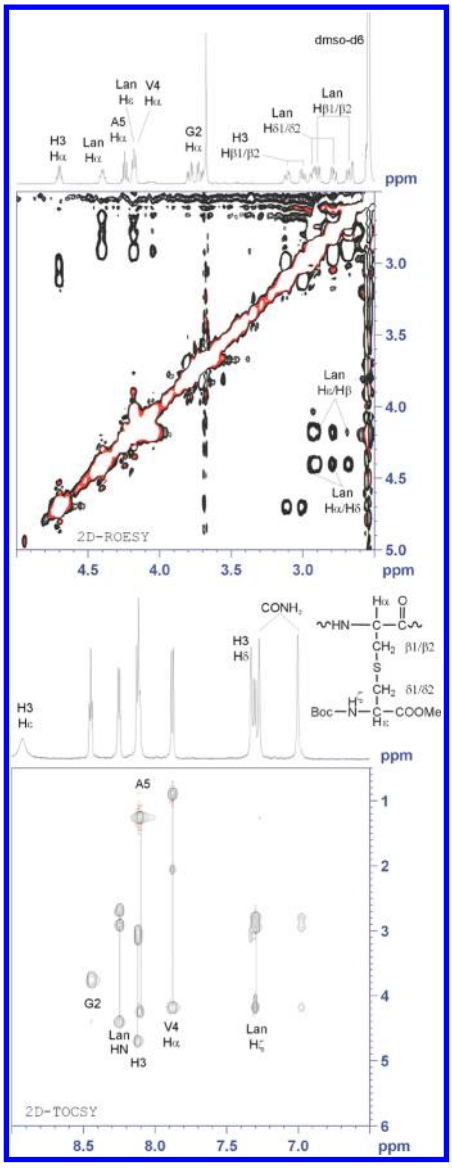
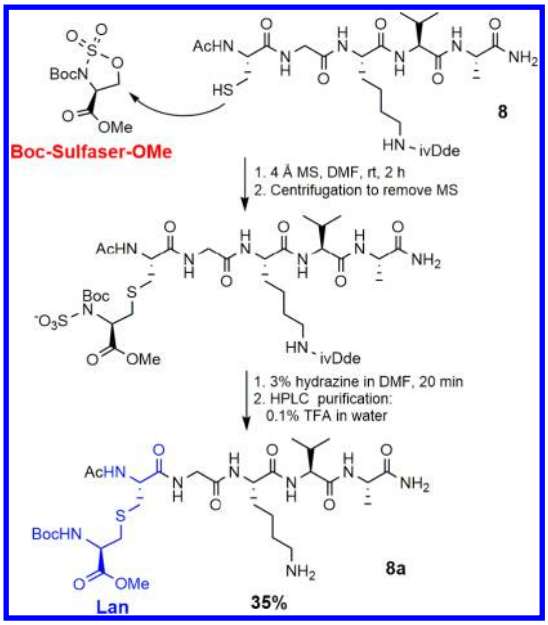


Figure 1. Expansions of 2D-TOCSY (bottom) and 2D ROESY (top) NMR spectra of compound **5a** with resonance assignment (DMSO-d_6, 300 K). The formation of lanthionine after S-alkylation is demonstrated by the intraresidue ROE peaks as labeled in the ROESY spectrum. An analogous pattern of lanthionine intraresidue ROE cross-peaks was found for all compounds (see the Supporting Information for complete NMR data).

(1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl, a protecting group routinely used in solid-phase peptide synthesis (see Scheme 2). The removal of the ivDde protecting group from lysine after S-alkylation could be achieved by adding 3% hydrazine directly in the reaction mixture used for the S-alkylation (DMF), after a quick centrifugation step to remove molecular sieves. Although the deprotection of lysine to obtain the final product does not require a specific additional step, the final yield is somewhat lower, compared to that of compounds **1a**−**7a** (recall Table 1).

Peptide **9a** containing the cysteine residue sandwiched between a histidine and a tryptophan residue was synthesized to assess whether or not the close proximity of multiple competing nucleophilic groups, such as the aromatic nitrogen atoms of tryptophan and histidine, could affect the chemoselectivity for cysteine. As shown in Table 1, we obtained a

Scheme 2. Incorporation of Lanthionine into a Lysine-Containing Peptide



single final product having the expected mass, with a yield comparable to that of compounds **1a**−**7a**. ^{1}H NMR characterization revealed a single final species retaining the full protection on the lanthionine side chain. The detection of intraresidue Lan Hα/Hδ and Lan Hε/Hβ ROE correlations confirmed the formation of the lanthionine structure; thus, there is a very high reaction chemoselectivity for cysteine alkylation.

Once we assessed the high efficiency and chemoselectivity of our approach to introduce lanthionine derivatives into peptide sequences, we focused on the chemical synthesis of Haloduracin β ring B. This molecule is a fragment of a lantibiotic peptide from the Gram-positive bacterium *Bacillus halodurans*. The native lantibiotic structure consists of two post-translationally processed peptides, Halα and Halβ, acting in synergy to provide bactericidal activity.[10]

The synthesis of Halβ ring B was first performed by a solution-phase S-alkylation approach (see Scheme 3). The precursor peptide Dde-VALCP-NH2 (**10**) was reacted with a cyclic sulfamidate derived from D-serine, in order to match the configuration of the natural Halβ, and was protected with O^{t}Bu at the carboxyl group and with Cbz at the amino group (Cbz-SulfaSer-O^{t}Bu). Cbz is widely used as an α-amino-protecting group, for its stability to bases and mild acid treatments and also for its versatile removal conditions; moreover, the aromatic ring simplified the HPLC as well as the NMR characterization of the final product. Peptide **10** Dde-VALCP-NH2 was protected at the N-terminus with Dde instead of Fmoc, to decrease the hydrophobicity of the protected peptide. The S-alkylation reaction was performed in dimethyl formamide (DMF) and in the presence of activated molecular sieves as previously described. After the reaction, MS were removed by mild centrifugation, and the DMF solution was treated with 3% hydrazine to remove the peptide N-terminus protection. The carboxylic function of the sulfamidate was rescued from the *tert*-butyl group with 50% TFA in DCM. After purification of the linear peptide, cyclization was

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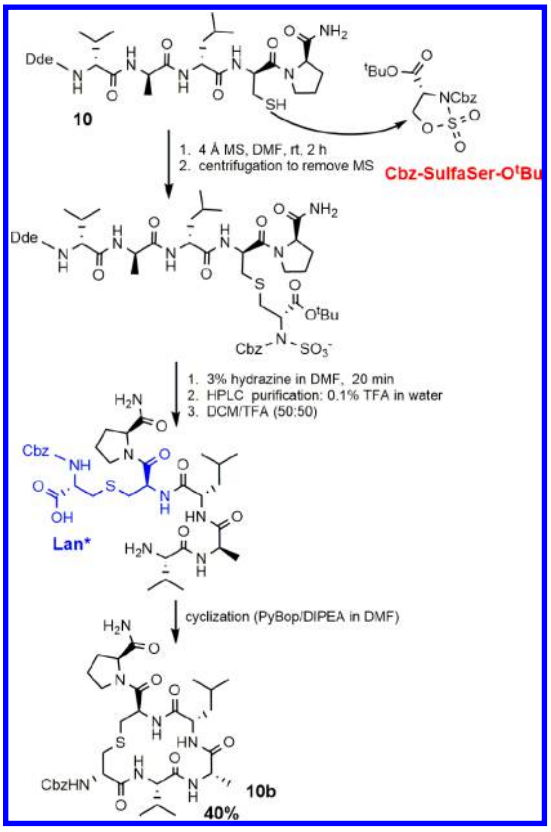
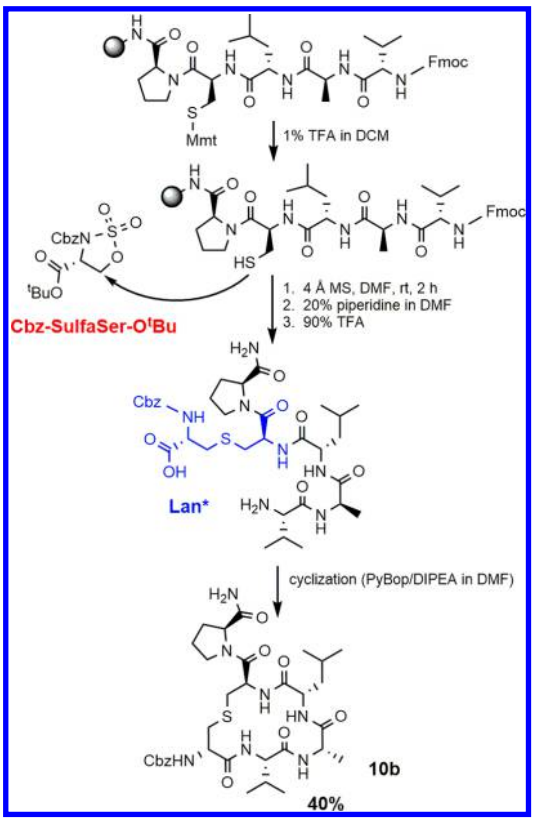
Scheme 3. Solution-Phase Approach for Preparing Halβ Ring B



Scheme 4. Solid-Phase Approach for Preparing Halβ Ring B



performed with standard PyBOP/DIPEA chemistry in DMF (see Scheme 3).[11]

With the aim of implementing the developed new strategy in compliance with solid-phase peptide synthesis, we have performed S-alkylation on a peptide precursor anchored on resin (see Scheme 4).

The cysteine to be alkylated was protected with a highly acid labile group, and the 4-methoxytrityl (Mmt), was easily removed by treatment of the peptidyl resin with dilute trifluoroacetic acid (1% TFA in DCM). Then, the peptidyl resin, suspended in anhydrous DMF and in the presence of activated 4 Å molecular sieves, was alkylated by adding 10 equiv of Cbz-SulfaSer-O^{t}Bu under stirring at room temperature for 15 h. The final product (compound **10b**) was obtained with a yield comparable to that of the solution-phase approach (Table 1). However, a much larger excess of the Cbz-SulfaSer-O^{t}Bu (10 equiv) was required by the solid-phase approach,[7d] if compared with that employed for the solution approach (1.2 equiv). Thus, we conclude that the latter approach is to be preferred, because it is much less demanding, in terms of the required amount of cyclic sulfamidate, especially if considering that it is not commercially available.

In conclusion, an efficient, highly chemoselective, and stereochemically controlled procedure to introduce lanthionine derivatives in preformed peptide sequences has been developed. It relies on the post-synthetic cysteine S-alkylation by cyclic sulfamidates in the presence of activated molecular sieves as the catalyst. The reaction conditions are mild enough to prevent competing β-elimination that leads to dehydroalanine formation. Moreover, they are compatible with a range of orthogonal peptide protecting groups on the sulfamidate derivatives. This is a key feature of the proposed procedure, since the selective removal of such orthogonal protections is required to perform the subsequent cyclization step that leads to the formation of a complete thioether ring structural motif. As a demonstration of the proposed methodology, the synthesis of Halβ ring B was successfully performed. It might serve as a template for the design of synthetic approaches to more-complex lantibiotics, having either sequential or interlocking rings. Further work to probe the potential of this methodology is currently in progress. Namely, we are testing sterically more demanding and less reactive sulfamidates, which generally require a higher temperature to react with thiolates.[3a]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b03254.

Experimental section, HPLC profiles, MS spectra, NMR chemical shift, and spectra of lanthionine-containing peptides (PDF)

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Notes

The authors declare no competing financial interest.

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