Organic & Biomolecular Chemistry

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| Journal: | *Organic & Biomolecular Chemistry* |
| Manuscript ID | OB-ART-12-2023-002037.R1 |
| Article Type: | Paper |
| Date Submitted by the Author: | 15-Jan-2024 |
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ARTICLE

# Synthesis of labionin and avionin precursors via nitrogen-centred-radical-triggered 1,5-HAT reaction of Tris derivatives

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Labionin and avionin are non-proteinogenic amino acids containing 2,4-diamino-2-(mercaptomethyl)pentanedioic acid that forms the core structures of spirocyclic peptides including labyrinthopeptin A2 and microvionin, respectively. We have developed a diastereoselective synthetic route to labionin and avionin precursors. This route highlights the formation of the quaternary carbon stereocenter of the *α*,*α*-disubstituted amino acid via regioselective 1,5-HAT reaction of the Tris derivative.

## Introduction

Lantipeptides are a subclass of ribosomally synthesized and post-translationally modified peptides (RiPPs) that characterized by thioether-cross-linked amino acids. These peptides are genetically encoded and expressed as large precursor molecules that undergo enzymatic processing to form a diverse array of polycyclic peptides with a wide range of bioactivities.[1] Among these, labyrinthopeptin A2[2] and microvionin[3] exhibit unique peptide topologies, each featuring a spirocyclic peptide centred on the non-proteinogenic amino acid, labionin (**1**) and avionin (**2**), respectively (Figure 1). Labionin (**1**) and avionin (**2**) are densely functionalized amino acids composed of 2,4-diamino-2-(mercaptomethyl)pentanedioic acid core structures. Labyrinthopeptin A2 is a spirocyclic peptide isolated from the actinomycete *Actinomadura namibiensis* DSM 6313.[2] The structure of labyrinthopeptin A2 was elucidated through X-ray crystallographic analysis. The labionin part of labyrinthopeptin A2 exhibits an *S* configuration at the junction chiral quaternary carbon, which is connected to an amino acid bearing (*S*)-α-carbon through a methylene bridge. In terms of biological activity, labyrinthopeptin A2 has potent antiallodynic activity in animal pain models. Microvionin is a hybrid lipopeptide composed of a spirocyclic peptide isolated from the actinomycete *Microbacterium arborescens* 5913.[3] The stereochemistries of the two chiral centres of the avionin motif have not been determined. Microvionin exhibits an antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and *Streptococcus pneumoniae*. Owing to their biological importance as well as structural appeal, these spirocyclic peptides have garnered attention as potential candidates for drug discovery.

In these contexts, numerous attempts have been undertaken to develop synthetic routes to labionin (**1**) and avionin (**2**) precursors to elucidate their stereochemistry and to expand the structure-activity relationships, leading to development of chemical tools and/or drug candidates. The primary challenges in the syntheses of labionin (**1**) and avionin (**2**) involve (1) constructing the quaternary carbon stereocenter of the α,α-disubstituted amino acid and (2) introducing a sulfur atom to the sterically cumbersome neopentyl position. In 2011, Süssmuth and co-workers reported the synthesis of an *α*,*α*-disubstituted amino acid building block,[4] employing Seebach's method with a serine derivative to stereoselectively construct the quaternary carbon stereocenter of labionin (**1**).[5] Subsequent transformations and diastereomer separation led to the preparation of sulfur-atom-free precursors for labionin synthesis. In 2021, the synthesis of orthogonally protected labionin was reported by Sani and co-workers, where the introduction of a sulfur atom and construction of the quaternary carbon stereocenter were achieved by thia-Michael addition to an (*S*)-pyroglutamic acid derivative followed by electrophilic azidation with moderate stereoselectivity.[6] These synthetic routes are based on chiral pooling methods that rely on the stereochemistry of the starting amino acids, necessitating diastereomeric separation to obtain the desired precursor in the diastereomerically pure form. Therefore, the development of enantioselective/diastereoselective synthetic routes to labionin and/or avionin motifs remains a challenging and worthwhile endeavour. Herein, we report the diastereoselective synthesis of labionin and avionin precursors, highlighting the construction of the quaternary carbon

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stereocenter via diastereoselective 1,5-hydrogen transfer (1,5-HAT) reaction of the tris(hydroxymethyl)aminomethane (Tris) derivative and the intermolecular nucleophilic attack of thiol to functionalized β-lactone.

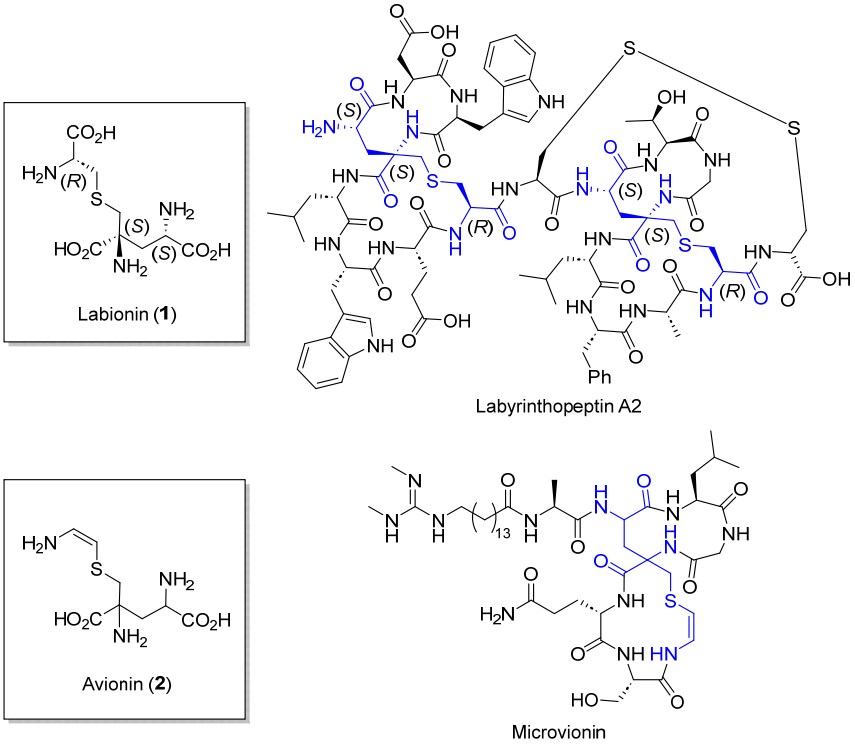
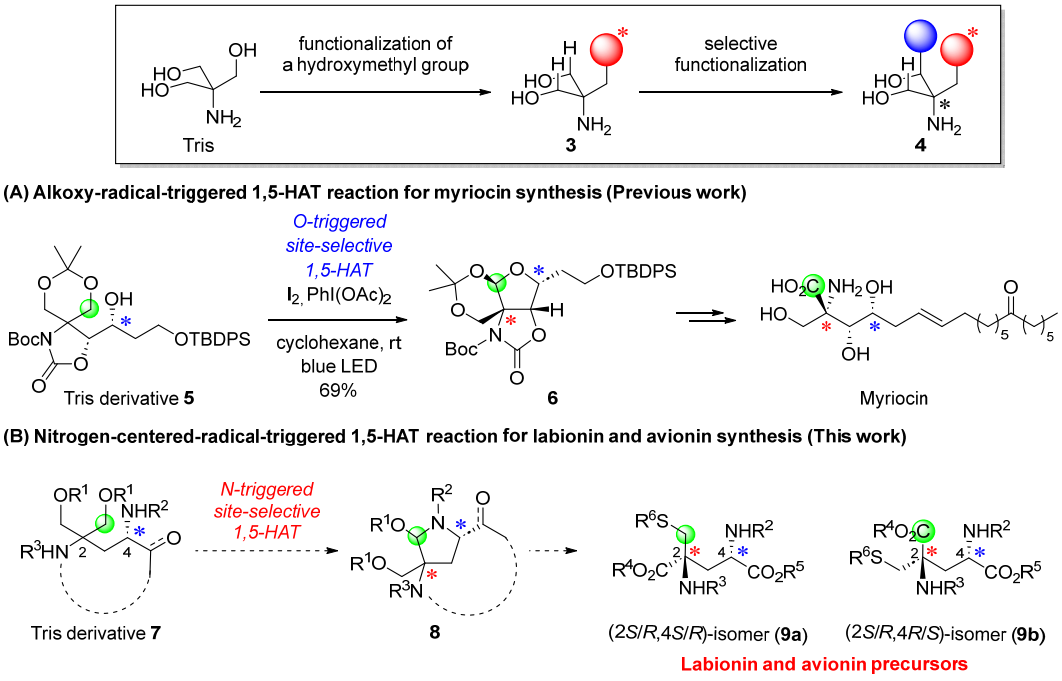



**Fig. 1.** Structures of labionin (**1**), avionin (**2**), labyrinthopeptin A2 and microvionin



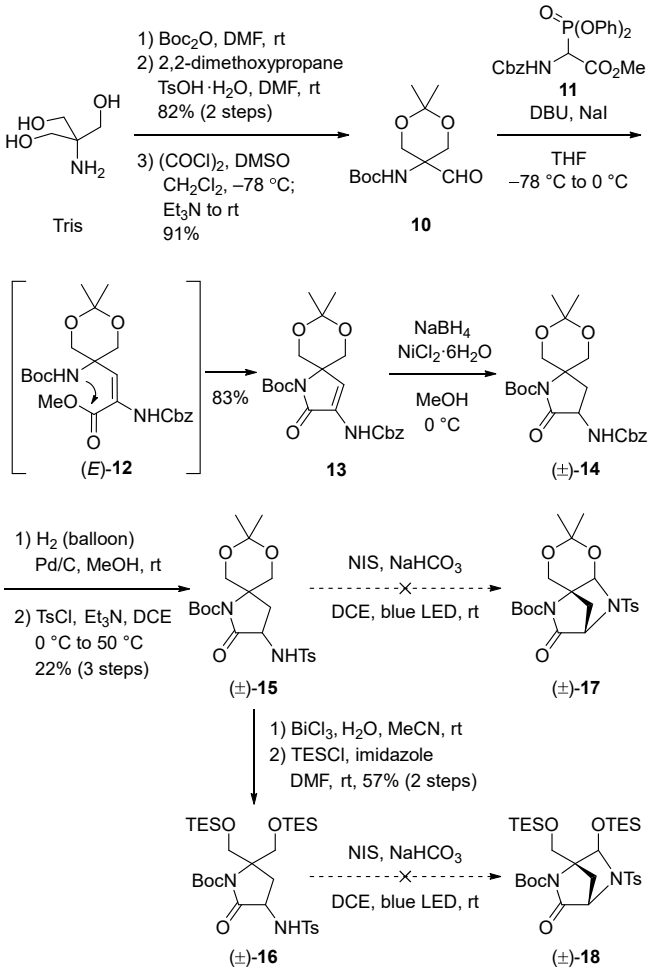

**Scheme 1.** Strategy for the contruction of the quaternary carbon stereocenter of an α-tertiary amine

## Results and Discussion

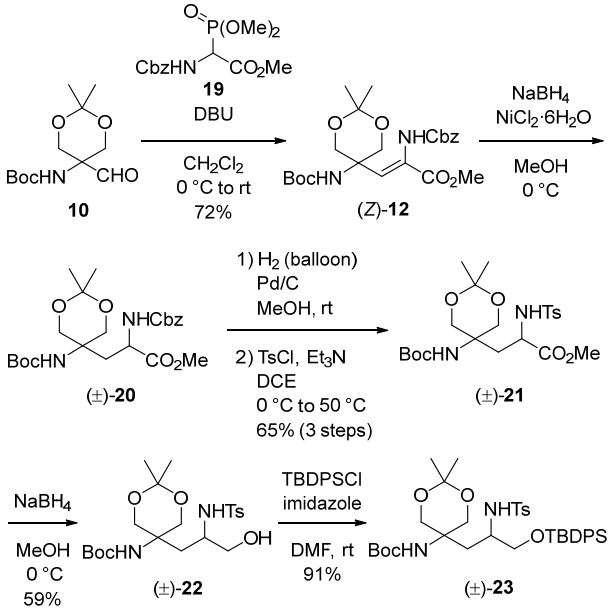
A variety of the methods for constructing the quaternary carbon stereocenter of *α*,*α*-disubstituted amino acids have been developed, including C–C/C–N bond formation and desymmetrization strategies.[7] Among these, desymmetrization strategy is an efficient approach to stereoselectivly construct *α*,*α*-disubstituted amino acids because the number of steps for C–C/C–N bond formation is reduced.[8] Recently, we have developed a strategy for the construction of chiral *α*,*α*-disubstituted amino acid precursors **4** from Tris derivatives **3** (Scheme 1). Namely, we have developed diastereoselective C-H functionalization involving an alkoxy-radical-triggered 1,5-HAT reaction of a conformationally fixed spiro-compound **5** from readily available Tris (Scheme 1A) to achieve an enantioselective total synthesis of myriocin.[9] Applying the methodology, in this study, we planned to construct a chiral *α*,*α*-disubstituted amino acid building block of the labionin and avionin moieties. We posited that a nitrogen radical-mediated 1,5-HAT reaction (Hofmann–Löffler–Freytag reaction, HLF reaction)[10] of 2,4-diamine substrate having two hydroxymethyl groups **7** would provide a hemiaminal **8** with required relative stereocenters for the preparation of labionin and/or avionin (Scheme 1B). In this reaction, selective functionalization of one of the two diastereotopic hydroxymethyl groups would be expected to be controlled by the preinstalled chiral amino group at the 4-position. The key to success of this tactic is the design of substrates accomplishing the diastereoselective functionalization of the desired C–H bond in the presence of various functional groups. Furthermore, we considered that the resulting hemiaminal **8** could be transformed to both diastereomers of labionin and avionin precursors [(2*S*/*R*,4*S*/*R*)- or (2*S*/*R*,4*R*/*S*)-isomer **9**] by regioselectively introducing sulfur atoms and adjusting the oxidation states.

Initially, we investigated the diastereoselective HLF reaction of Tris derivatives. The racemic Tris derivatives **15** and **16** bearing an amino group at the appropriate position were designed as substrates. The synthesis of substrates **15** and **16** is shown in Scheme 2. The Ando-type Horner–Wadsworth–Emmons olefination[11] of known aldehyde **10**[12], easily prepared from Tris, with *N*-Cbz-α-diphenylphosphonoglycine methyl ester **11**[13] gave alkene (*E*)-**12**, which underwent spontaneous cyclization between the amino group and the methyl ester to form α,β-unsaturated γ-lactam **13**. Treatment of **13** with $NaBH_4$ in the presence of $NiCl_2{\cdot}6H_2O$ provided γ-lactam **14**. However, this reaction was not completed, probably due to steric repulsion originating from the adjacent quaternary carbon. The removal of the Cbz group from **14** and Ts protection furnished the key substrate **15**. Furthermore, the removal of acetonide with $BiCl_3$[14] followed by TES protection of the resulting two primary alcohols afforded substrate **16**.

We went on to investigate the diastereoselective HLF reactions of substrates **15** and **16**. We chose a previously reported method using NIS to generate a nitrogen radical from Ts-protected amino group.[15] The reaction was carried out under LED irradiation in the presence of NIS, but the desired compounds **17** and **18** were not obtained, and only the starting materials were recovered. This might be attributed to the difficult approach of the nitrogen radicals to the desired C–H bond at the hydroxymethylene of the substrate due to conformational restriction.

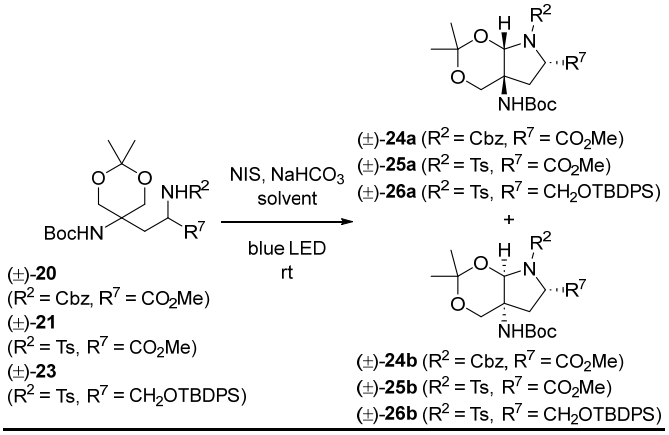



**Scheme 2.** Synthesis of Tris derivatives (±)-**15** and (±)-**16**




**Scheme 3.** Synthesis of Tris derivatives (±)-**20**, (±)-**21** and (±)-**23**

**Table 1**. Optimization of diastereoselective HLF reaction



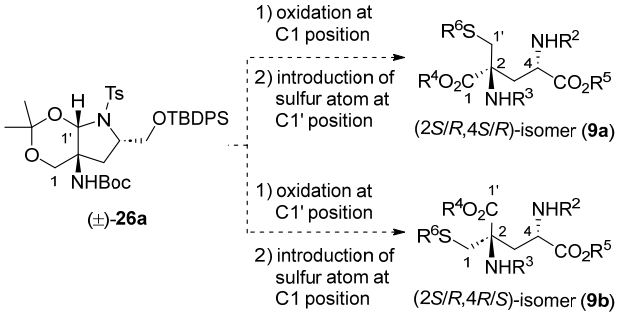

| entry | substrate | solvent | yield (%) | dr (**a**:**b**) |
|---|---|---|---|---|
| 1 | (±)-**20** | DCE | (±)-**24** (ND) | - |
| 2 | (±)-**21** | DCE | (±)-**25** (54%) | 78:22[a] |
| 3 | (±)-**23** | DCE | (±)-**26** (44%) | 89:11[b] |
| 4 | (±)-**23** | $CH_2Cl_2$ | (±)-**26** (50%) | 88:12[b] |
| 5 | (±)-**23** | MeCN | (±)-**26** (38%) | >95:5[b] |
| 6 | (±)-**23** | cyclohexane | (±)-**26** (61%) | 81:19[b] |

[a] The ratio was determined based on isolated yields. [b] The ratio was determined by $^1$H NMR spectroscopy. ND = not detected. DCE = 1,2-dichloroethane

Next, we designed substrates **20**, **21**, and **23** with flexible structures to facilitate the 1,5-HAT process. The synthesis of substrates **20**, **21**, and **23** is shown in Scheme 3. The Horner–Wadsworth–Emmons olefination of aldehyde **10** and *N*-Cbz-α-phosphonoglycine trimethyl ester **19**[16] gave the desired alkene (*Z*)-**12**, which was converted to alkane **20** with $NaBH_4$ and $NiCl_2{\cdot}6H_2O$. Removal of the Cbz group and Ts protection afforded ester **21**. The reduction of methyl ester **21** with $NaBH_4$, followed by TBDPS protection provided silyl ether **23**.

With precursors **20**, **21**, and **23** in hand, we examined the diastereoselective HLF reaction (Table 1). When the methyl ester **20** having a Cbz group was subjected to LED irradiation in the presence of NIS, $NaHCO_3$ and dichloroethane (DCE), the desired product **24** was not obtained (entry 1). Using the substrate having a Ts group instead of the Cbz group gave the bicyclic compound **25** in 54% yield with a diastereomeric ratio of 78:22 (entry 2). In the HLF reaction via in situ generation of nitrogen halide (improved method developed by Suárez et al.),[10b, 17] it is known that increasing the acidity of NH group facilitates the formation of nitrogen halide, which serves as the precursor for generating the nitrogen-centred radical. The variation in acidity resulting from the introduction of Cbz or Ts group could impact the reactivity. The reaction was carried out under similar conditions using the silyl ether **23** to give the bicyclic compound **26** in 44% yield as a diastereomeric mixture (**a**:**b** = 89:11, entry 3). The relative configurations of **25** and **26** were determined by X-ray crystallography of their derivatives (See Supporting Information, Scheme S1). Changing the solvent to $CH_2Cl_2$ gave **26** in 50% yield with a diastereomeric ratio of 88:12 (entry 4). Using acetonitrile as the solvent caused a decrease in the yield of **26**, but the diastereomeric ratio was improved (entry 5, 38%, **a**:**b** = >95:5). Conversely, the use of cyclohexane decreased the diastereomeric ratio but gave the desired product in 61% yield (entry 6, **a**:**b** = 81:19).

We then turned our attention to the conversion of **26a** to labionin and avionin precursors **9** (Scheme 4). The absolute configuration of labionin has been determined by X-ray crystallography, whereas the relative/absolute configuration of avionin remains unclear. Therefore, we planned to develop synthetic routes for both diastereomers **9**, and posited that these selective syntheses could be achieved by controlling the position of the introduced sulfur atom. Specifically, we expected to obtain (2*S*/*R*,4*S*/*R*)- or (2*S*/*R*,4*R*/*S*)-**9** by introducing a sulfur atom to the C1' or C1 position of compound **26a**, respectively.



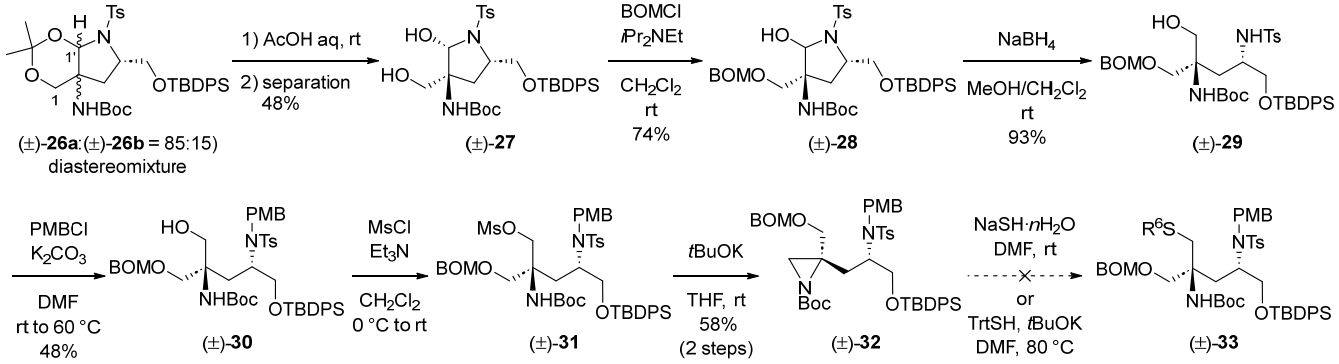

**Scheme 4.** Synthetic strategy for labionin and avionin precursors **9**

We first investigated the introduction of a sulfur atom to the C1' position using aziridine **32** as a substrate (Scheme 5). A diastereomeric mixture of **26** was subjected to acetic acid/water followed by separation of the resulting diastereomeric mixture using column chromatography to give alcohol **27** as a sole diastereomer. BOM protection of the alcohol of **27** provided compound **28**, which was reduced with $NaBH_4$ to afford alcohol **29**. Alcohol **29** was converted to *N*-PMB-protected mesylate **31**, which was treated with *t*BuOK to afford aziridine **32**. Then, nucleophilic attack by thiols was investigated. In the case using $NaSH{\cdot}nH_2O$ as a nucleophile, the desired product was not obtained. When TrtSH was used in the presence of *t*BuOK, the starting material was consumed, but multiple undesired products were observed.

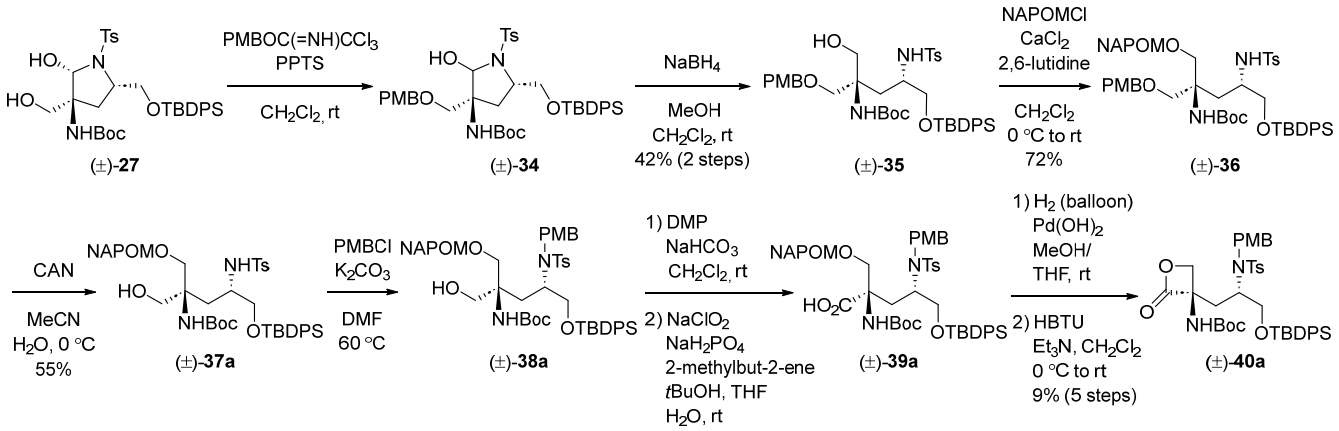
Goodman and co-workers reported that an intermolecular nucleophilic attack of thiol proceeded smoothly using *β*-lactones derived from sterically hindered serine derivatives.[18] Accordingly, we examined the introduction of the sulfur atom via *β*-lactone **40a** as an electrophile. The synthesis of *β*-lactone **40a** is shown in Scheme 6. The PMB protection of the primary alcohol of **27** followed by reduction with $NaBH_4$ afforded alcohol **35**. The primary alcohol of compound **35** was protected with a 2-naphtylmethoxymethyl (NAPOM) group[19] to give compound **36**. Removal of the PMB group by treatment with CAN followed by PMB protection of the tosylamide furnished compound **38a**. Dess-Martin oxidation and Pinnick oxidation provided the carboxylic acid **39a**. The NAPOM group was removed using $Pd(OH)_2$ under a hydrogen atmosphere, and treatment with HBTU provided the desired *β*-lactone **40a**.

The conversion of *β*-lactone **40a** to protected labionin **45a** is shown in Scheme 7. The *β*-lactone **40a** was subjected to TrtSH in the presence of *t*BuOK to yield the desired thioether **41a**. The compound **41a** was converted to the methyl ester **42a** by treatment with TMS diazomethane, and removal of the Boc group and azidation[20] led to azide **44a**. The TBDPS group was removed with TBAF, accompanied by simultaneous hydrolysis of the methyl ester. Thus, TMS diazomethane was used to convert it back to the methyl ester form. These transformations produced some unidentified side products, although the exact causes remain elusive. Finally, the primary alcohol was oxidized by PDC[21] to obtain the desired protected labionin **45a**.

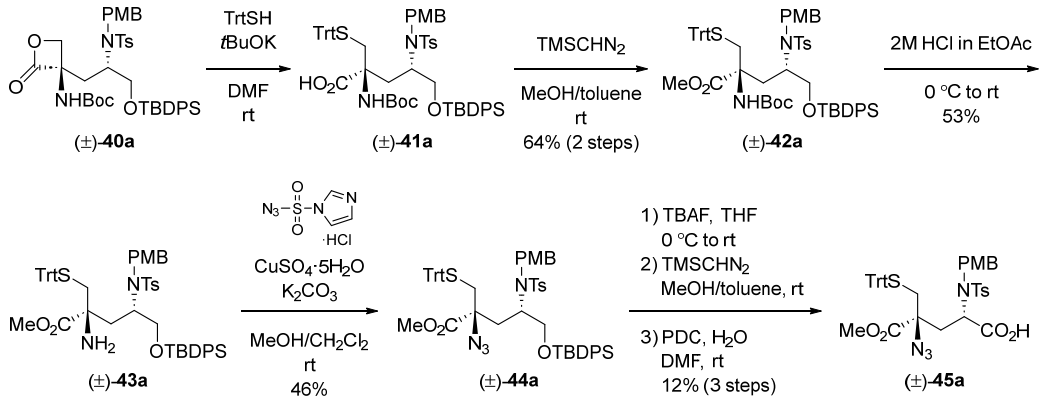
Next, we attempted to synthesize protected labionin **45b**, the diastereomer of carboxylic acid **45a**. In this synthesis, β-lactone



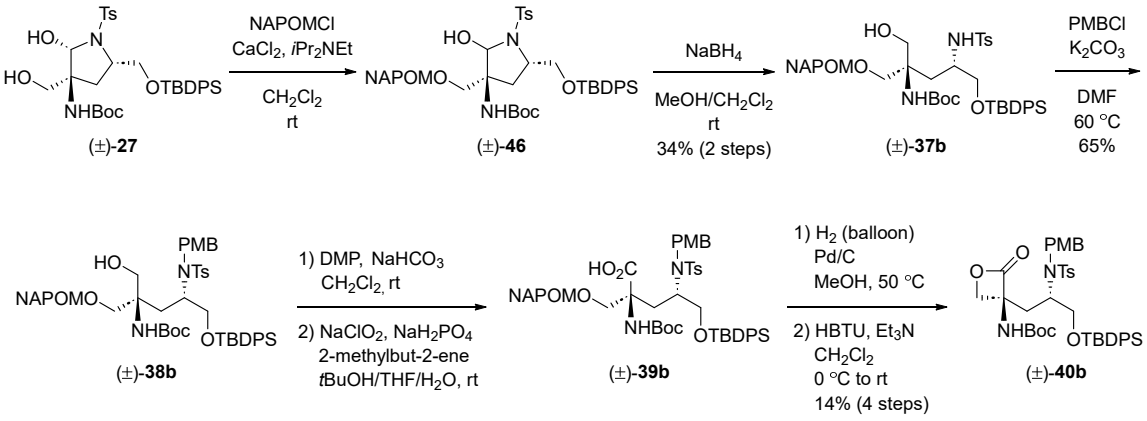

**Scheme 5.** Attempt to introduction of sulfur atom using aziridine **32**



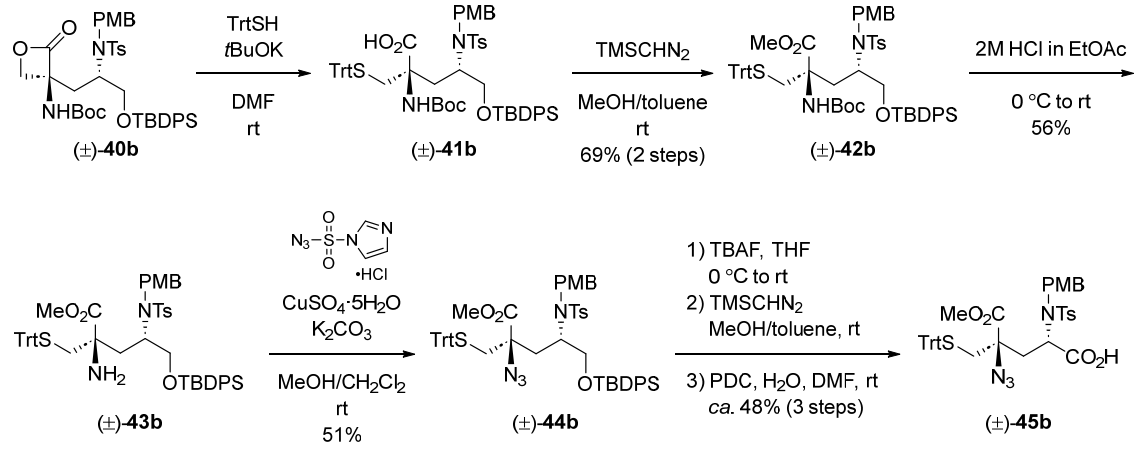

**Scheme 6.** Synthesis of *β*-lactone (±)-**40a**




**Scheme 7.** Synthesis of protected labionin (±)-**45a**




**Scheme 8.** Synthesis of *β*-lactone (±)-**40b**




**Scheme 9.** Synthesis of (±)-**45b**

**40b** was used to introduce a sulfur atom to the C1 position of compound **26a**. The synthesis of *β*-lactone **40b** is shown in Scheme 8. NAPOM protection of the primary alcohol of **27** followed by reduction with $NaBH_4$ afforded alcohol **37b**. *β*-Lactone **40b** was synthesized from **37b** in the same manner as that shown in Scheme 6.

The synthesis of **45b** is shown in Scheme 9. *β*-Lactone **40b** was treated with TrtSH in the presence of *t*BuOK to produce **41b**,[22] which was converted to methyl ester **42b**. Removal of the Boc group and azidation led to azide **44b**. It is worth noting that a different pathway involving the removal of the TBDPS group of **42b** and oxidation of the primary alcohol **S2** gave the undesired lactam **S3** (See: Supporting Information, Scheme S2). Finally, removal of the TBDPS group, methylesterification, and oxidation afforded the desired protected labionin diastereomer **45b**.

## Conclusions

In summary, we achieved the diastereoselective synthesis of labionin and avionin precursors containing 2,4-diamino-2-(mercaptomethyl)pentanedioic acid core structures. The key features of our synthesis include the construction of the quaternary carbon stereocenter of the *α*,*α*-disubstituted amino acid via regioselective 1,5-HAT reaction of the Tris derivative and the intermolecular nucleophilic attack of a sulfur group to the *β*-lactone. Regarding the 1,5-HAT reaction, the use of substrates **21** and **23** with flexible tethers facilitated the diastereoselective 1,5-HAT process. This synthetic route takes advantage of achiral Tris as a starting material and has the potential for application in enantioselective synthesis, enabling the divergent construction of all conceivable stereoisomers of labionin and avionin. Furthermore, the obtained carboxylic acids **45a** and **45b** could be useful precursors in the synthesis of spirocyclic peptide analogues, in addition to the synthesis of natural peptides such as labyrinthopeptin A2 and microvionin. Further investigation of improved synthetic methods for labionin and avionin precursors and synthetic studies of labyrinthopeptin A2 and microvionin are now in progress in our laboratory.

## Data availability

All experimental data, and detailed experimental procedures are available in the ESI.†

## Conflicts of interest

There are no conflicts to declare.

## Acknowledgements

This work was supported by JSPS KAKENHI (Grant Numbers 23K17975 and 23H02603), Research Support Project for Life Science and Drug Discovery [Basis for Supporting Innovative Drug Discovery and Life Science Research (BINDS)] from AMED (JP22ama121042), GteX Program Japan Grant (JPMJGX23B3), Takeda Science Foundation and Mochida Memorial Foundation for Medical and Pharmaceutical Research.

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22 The use of cysteine derivative as a nucleophile was investigated. The reaction of lactone (±)-**40b** with L-cysteine derivative and $Cs_2CO_3$ gave the corresponding amino acid derivative as a diastereomeric mixture. However, the diastereomeric mixture was difficult to separate and the relative configuration was not determined.

 