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Synthesis of the AviMeCys-Containing D-Ring of Mersacidin

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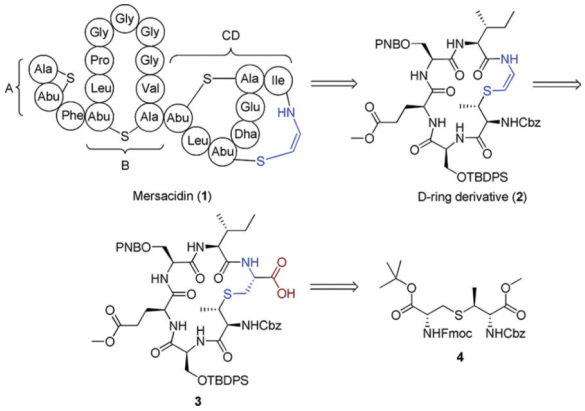
Abstract



A chemical synthesis of the D-ring of mersacidin is reported. The synthetic route relied upon development of a method for late-stage introduction of an unusual *S*-[(*Z*)-2-aminovinyl]-(3*S*)-3-methyl-D-cysteine (AviMeCys) functional group via an oxidative decarbonylation/decarboxylation reaction.

Mersacidin (**1**) is a 20-residue polycyclic lantibiotic peptide that possesses promising antibacterial activity against Gram-positive organisms. In addition, it has displayed good activity against problematic Gram-positive pathogens, in particular, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant entercocci (VRE), which have shown significant levels of resistance to commonly used members of the currently available antibiotic arsenal.[1,2]

Mersacidin is believed to inhibit the transglycosylation reaction in the bacterial cell wall biosynthetic pathway by forming a stoichiometric complex with lipid II, the final monomeric intermediate utilized by the bacterial cell for assembly of its cell wall. Sequestration of lipid II from the transglycosylases, enzymes that polymerize lipid II into

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Supporting Information Available. Experimental procedures describing the synthesis of all new compounds as well as the characterization data are provided. This material is available free of charge via the Internet at http://pubs.acs.org.

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the glycan strands that typify bacterial cell wall architecture, ultimately compromises the integrity of the bacterial cell wall and results in lysis and subsequent cell death.

Interestingly, mersacidin does not interact with the Lys-D-Ala-D-Ala portion of the stem peptide present in the lipid II intermediate and thus appears to target a site on lipid II that differs from the site utilized by vancomycin.[3] Considering the serious public health threat posed by bacterial resistance to glycopeptide antibiotics, mersacidin may represent a promising avenue for development of new antibacterial therapeutics with activity versus problematic resistant organisms.

The CD bicyclic fragment is believed to have a major role in the binding of mersacidin with lipid II.[4] However, the exact nature of this interaction is unknown. Structure–activity relationship studies (SAR) of mersacidin and its derivatives could reveal important insights into its mechanism of action and could contribute to the rational design of new lead compounds.[5]

As part of our effort directed toward the total synthesis of mersacidin, we initially focused our attention on the development of an efficient methodology that would introduce the synthetically challenging *S*-[(*Z*)-2-aminovinyl]-(3*S*)-3-methyl-D-cysteine (AviMeCys) into the polypeptide structure of a D-ring derivative. Thus, we report herein the synthesis of the D-ring derivative (**2**) containing the Avi-MeCys unit.

The (*Z*)-aminovinyl methyl sulfide bond found in mersacidin is the result of posttranslational modification mediated by the enzyme MrsD.[6] The proposed mechanism is believed to involve the formation of a thioaldehyde intermediate, which spontaneously decarboxylates to an enethiolate. This enethiolate intermediate subsequently carries out a 1,4-conjugate addition reaction to an adjacent dehydroamino acid to install the vinyl sulfide bond (Figure 1).[7]

Inspired by this mechanism, we set out to test different routes that would leverage a carboxyl handle for late-stage introduction of the enamide linkage from a readily available peptide precursor. Previous reports in the literature have described similar strategies for the synthesis of enamides. These include the oxidative decarbonylation of activated carboxylic acids[8] and the oxidative decarboxylation of amino acids using lead tetraacetate.[9] To the best of our knowledge, however, these methods have not been tested on cysteine containing peptides as would be required for the AviMeCys subunit of mersacidin.[10] In order to evaluate the potential utility of these methods for late-stage introduction of the AviMeCys subunit on a fully functionalized (C)D-ring precursor, a model study was carried out on a simplified β-methyllanthionine (MeLan) derivative **8**.

The synthesis of protected **8** relied on the alkylation of β-MeCys **5** with bromoalanine **6**[11] following methodology previously developed in our laboratory[12] (Scheme 1). Cleavage of the Fmoc group under standard conditions (20% piperidine, DMF), followed by reprotection of the free amine with Cbz (BnOCOCl, $NaHCO_3$, EtOAc, H_2O), provided diester **7**. Finally, cleavage of the *tert*-butyl ester (TFA, CH_2Cl_2) provided the target MeLan derivative **8**.

Our initial results are summarized in Scheme 2. Exposure of **8** to the oxidative decarbonylation conditions (2.2 equiv of diphenylphosphoryl azide (DPPA), 2.2 equiv of TEA, toluene) resulted in the formation of protected AviMeCys **9** as a mixture of stereoisomers in good yields. The same occurred when subjecting **8** to 1 equiv of $Pb(AcO)_4$ in the presence of 1 equiv of $Cu(OAc)_2$ under oxidative decarboxylation conditions. Although, the Avi-MeCys derivative was obtained as a mixture of *Z* and *E* isomers, we were encouraged by these results and were hoping that conformational constraints imposed by the cyclic peptide substrate would facilitate preferential formation of the desired (*Z*)-AviMeCys moiety upon subjecting a suitably protected D-ring precursor to the optimized oxidative decarboxylation conditions.

Cyclic peptide **3** was selected as the precursor for introduction of the AviMeCys subunit. Since the AviMeCys unit is acid-sensitive, the side-chain protecting groups were selected in order to achieve the dual goals of mutual orthogonality while maintaining nonacidic cleavage conditions. Additionally, orthogonal protection for each of the serine residues is required since subsequent conversion into a CD-ring system requires that one must be converted into a didehydroalanine, while the other will be incorporated into the C-ring lanthionine bridge.

Our retrosynthetic analysis for **2** is shown in Scheme 3. Our macrocyclization precursor was selected with Ile at the *C*-terminus. This site was selected for amide bond formation since the Ile is less prone to epimerization than Ser or Glu. In addition, this site is proximate to the site at which MrsD catalyzes the cyclization reaction in mersacidin (Figure 1), thus presenting the possibility that, although in protected form, the peptide might exist in a conformation to facilitate macrocyclization.

Ile **16** and Glu **14** derivatives were commercially available, but the serine residues (**13** and **15**) and MeLan **12** had to be synthesized (Scheme 4). MeLan **12** was prepared by selective deprotection of the methyl ester of **4** using Nicolaou conditions ($(CH_3)_3SnOH$, 1,2-dichloroethane).[13] For the synthesis of **13**, Fmoc-Ser-OH **17** was converted to its allyl ester in excellent yields (allyl-Br, $CsCO_3$, DMF).[14] Protection of the side chain hydroxyl group of **18** as a TBDPS ether (TBDPS-Cl, imidazole, THF) followed by cleavage of the allyl ester ($Pd(OAc)_2$, PS-PPh_3, $PhSiH_3$, CH_2Cl_2) afforded the acid in 65% overall yield. Finally, serine derivative **15** was prepared by protection of alcohol **19** as a *p*-nitrobenzyl ether (Ag_2O, *p*-nitrobenzyl bromide, toluene) followed by cleavage of the methyl ester under standard conditions (LiOH, THF, H_2O).

Our synthesis of **3** began with the coupling of **16** and **15**, under standard peptide coupling conditions (DEPBT, DIEA, THF), affording **21** in 75% yield (Scheme 5). DEPBT (3-(diethoxyphosphoryloxy)-1,2,3-benzotriazin-4(3*H*)-one) was the coupling reagent of choice since its ability to suppress racemization at the adjacent stereocenter of activated carboxylates has been demonstrated.[15]

The *N*-terminal Boc protecting group found in **21** was cleanly removed (4 N HCl, dioxane), and the resulting free amine was coupled with Fmoc-Glu(OMe)-OH **14** (DEPBT, DIEA, THF) to provide **22** in 85% yield. Cleavage of the *N*-terminal Fmoc protecting group of **22**

(20% piperidine, DMF) followed by coupling with **13** (DEPBT, DIEA, THF) provided tetrapeptide **23** in 75% yield. Fmoc deprotection (20% piperidine, DMF) of **23** and coupling with MeLan **12** (DEPBT, DIEA, THF) provided **11** in 75%yield. Cleavage of the *C*-terminal allyl ester ($Pd(OAc)_2$, PS-PPh_3, $PhSiH_3$, CH_2Cl_2) provided the free carboxylic acid in 98% yield. Deprotection of the *N*-terminal Fmoc protecting group (20% piperidine, DMF), followed by cyclization of the peptide (PyBOP, DMAP, DIEA, DMF, CH_2Cl_2), afforded D-ring precursor **10** in 65% overall yield.

In order to set the stage for investigation of reaction conditions for oxidative decarboxylation, the *C*-terminal *tert*-butyl ester of **10** was cleaved under standard conditions (50% TFA in CH_2Cl_2, 0 °C), affording carboxylic acid **3** in good yield. In addition, carboxylic acid **3** was easily converted to the corresponding thioester **24** under standard conditions (PhSH, PyBOP, DIEA, CH_2Cl_2, 0 °C to rt, 78%).

Our initial attempt to introduce the (*Z*)-AviMeCys subunit sought to take advantage of Ni(0)-mediated decarbonylation reactions as described in the preceding manuscript. Unfortunately, all efforts to facilitate oxidative decarbonylation of **24** under the optimized reaction conditions (1.2 equiv of $Ni(PPh_3)_4$, copper(I) 3-methylsalicylate (CuMeSal), dioxane) were unsuccessful as only complex product mixtures were obtained.[16] In response to this disappointing result, we then turned our attention to oxidative decarbonylation/ decarboxylation of carboxylic acid **3** via the reaction conditions described in Scheme 2.

As shown in Scheme 6 when **3** was exposed to the oxidative decarbonylation conditions (DPPA, TEA, toluene), the desired (major) product possessing the (*Z*)-olefin geometry was obtained, albeit in poor yield. The vicinal coupling constant found for the olefinic hydrogens was consistent with coupling constant values for 1,2-disubstituted double bonds of (*Z*)-geometry (J = 8 Hz). A 2D-NOESY experiment (see Supporting Information) also provided support for the *cis* geometry of the double bond.

Various bases and solvents were screened in an effort to improve the reaction yield. The combination of DPPA, DABCO, and 1,4-dioxane gave the best results, providing D-ring derivative **2** containing the desired (*Z*)-AviMeCys in 25–30% yield.

Acid **3** was also exposed to the oxidative decarboxylation conditions utilizing lead tetraacetate. However, when using 1 equiv of both $Pb(OAc)_4$ and $Cu(OAc)_2$, the reaction produced none of the desired product.Wefelt that this resultmay be due to the greater number of Lewis basic sites present in **3** relative to those inMeLan **8**, our test substrate. Thus, when 5 equiv of each reagent were added under the same conditions, the desired product **2** was formed in 25–30% yield.

In summary, we were able to synthesize the D-ring of mersacidin that contains the unusual amino acid *S*-[(*Z*)-2-aminovinyl]-(3*S*)-3-methyl-D-cysteine (AviMeCys). Our strategy took advantage of a *C*-terminal carboxyl group that enabled late-stage introduction of the enamide subunit via an oxidative decarbonylation reaction promoted by DPPA or an oxidative decarboxylation promoted by $Pb(OAc)_4$. These reactions produced the desired (*Z*)-enamide predominantly; only minor amounts of the (*E*)-enamide were detected. The

methodology reported above is currently being applied toward the synthesis of the CDring system of mersacidin, with the goal of gaining a better understanding of how this subunit interacts with lipid II. The results of these studies will be presented in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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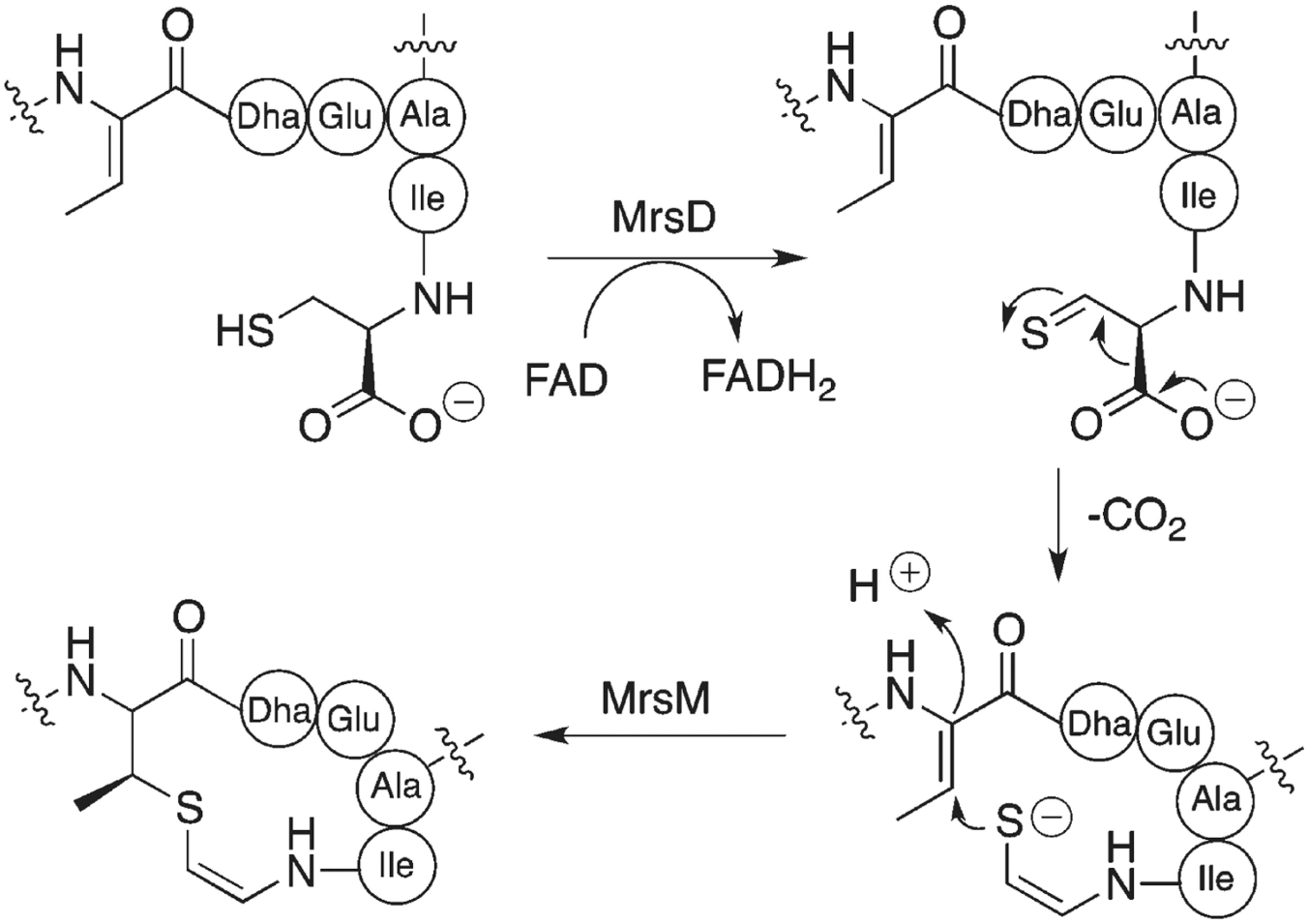
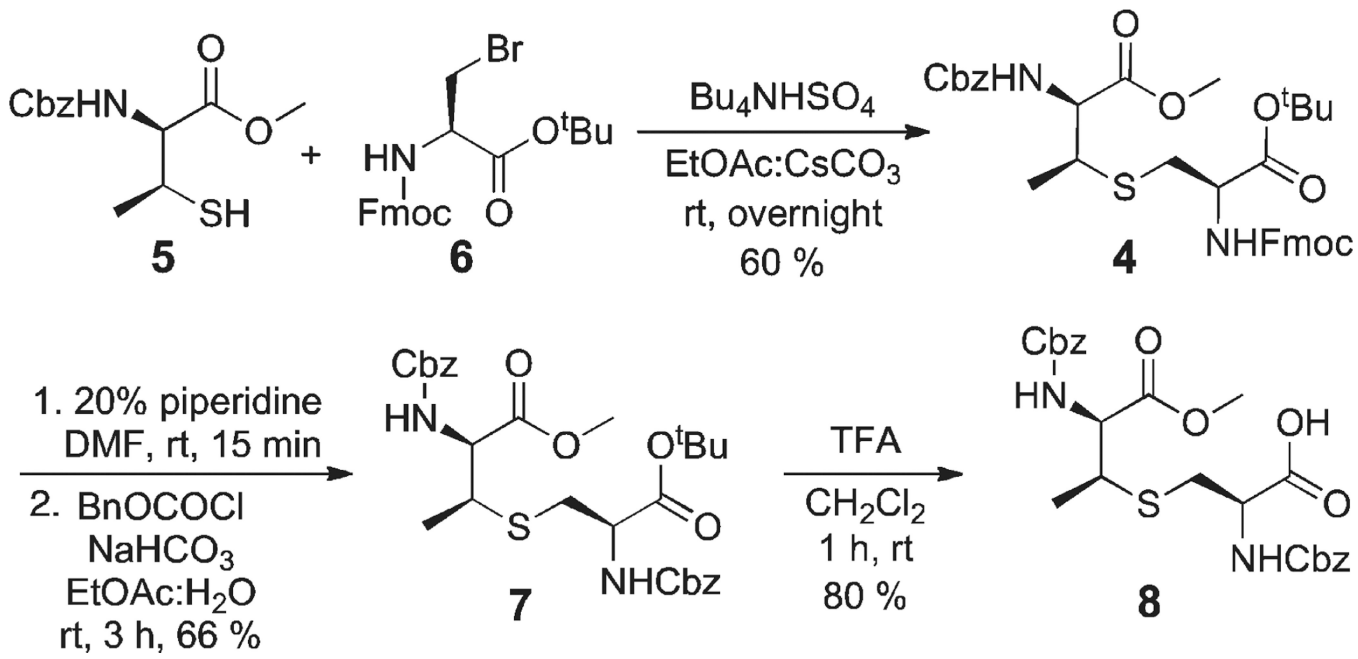
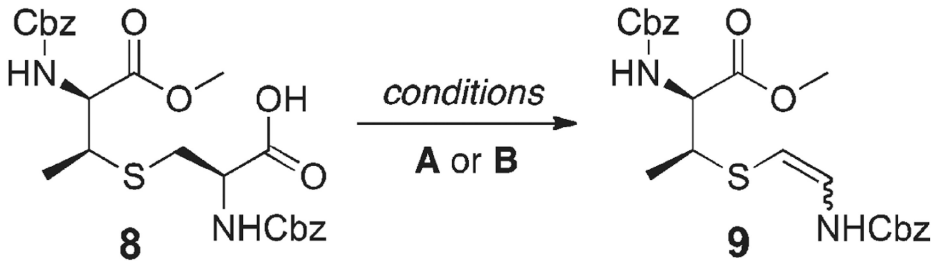


Figure 1.
Biosynthetic pathway for introduction of AviMeCys.

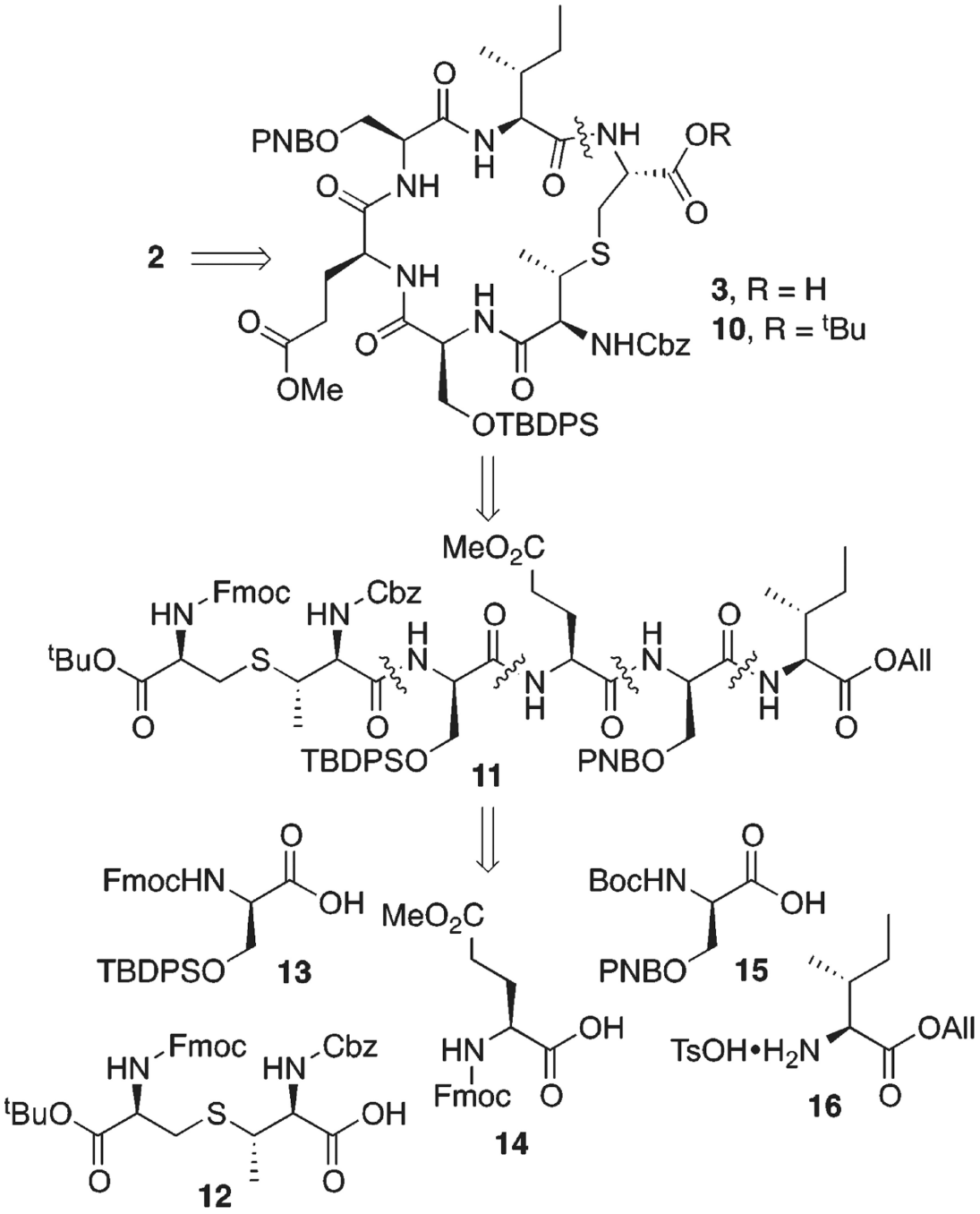


Scheme 1.

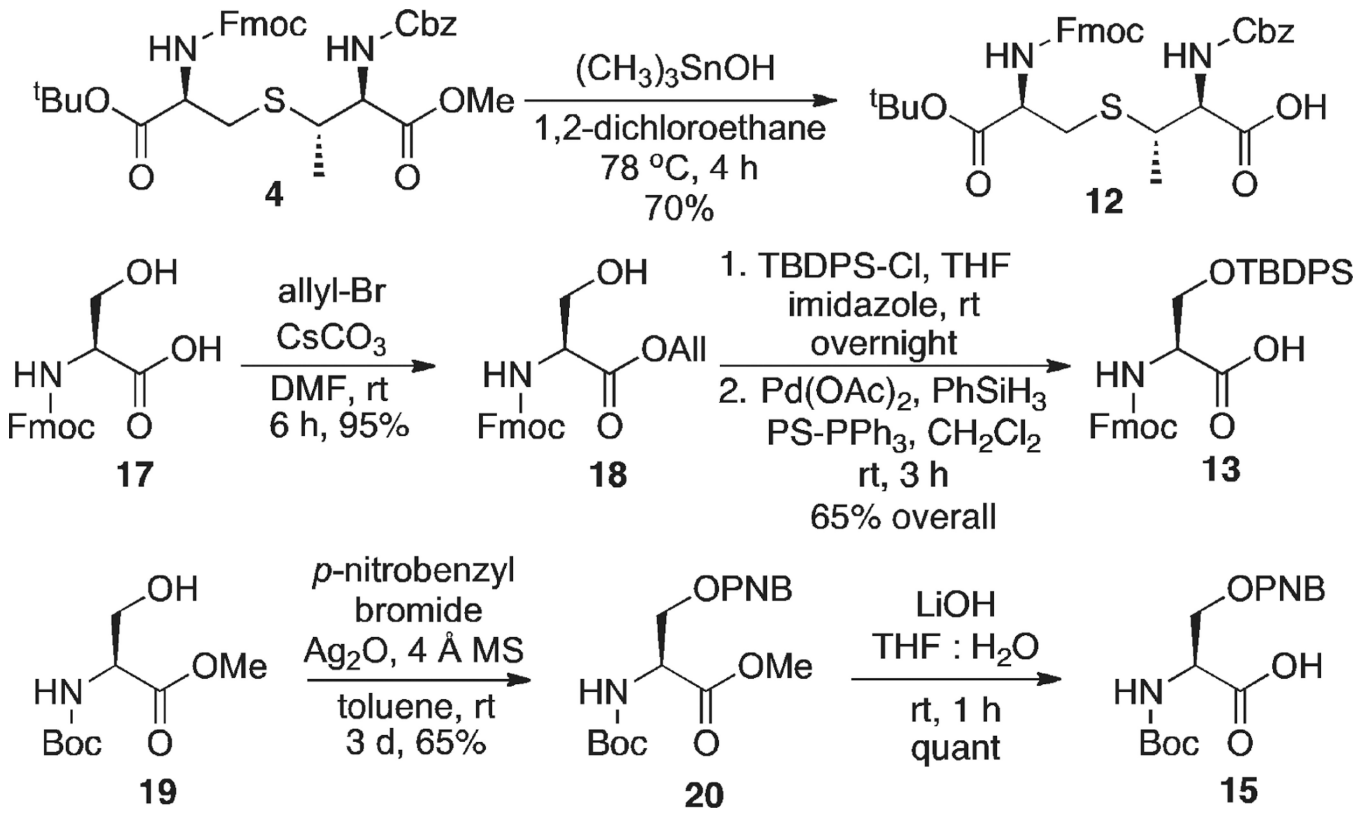


conditions	% yield	*Z*:*E*
A. DPPA, TEA, toluene, 94 °C, overnight	65	2:1
B. 1. $Pb(OAc)_4$, $Cu(OAc)_2$, pyr, THF, 0-25 °C, 2.5 h 2. TEA in EtOAc	35	1:1

Scheme 2.



Scheme 3.



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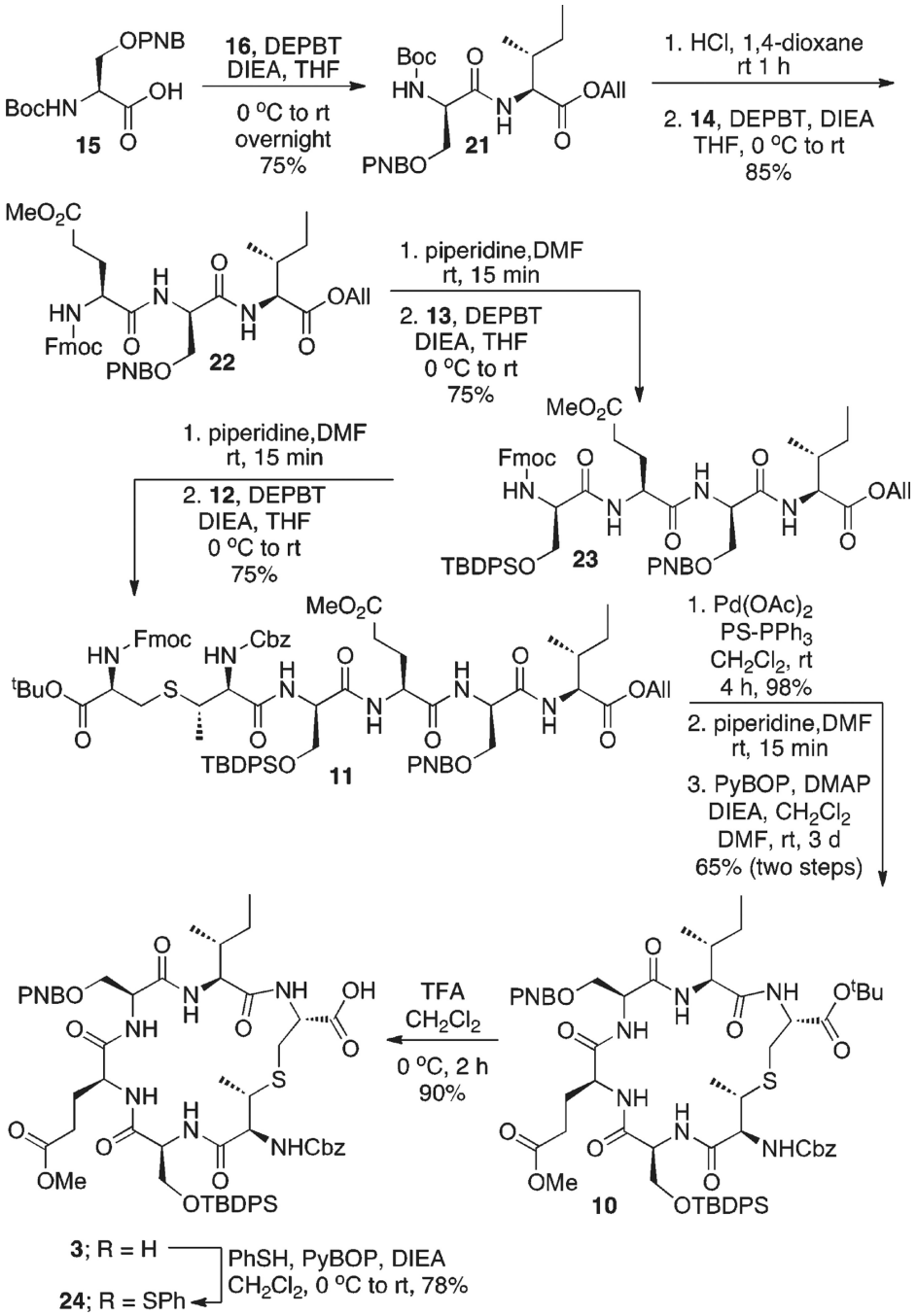
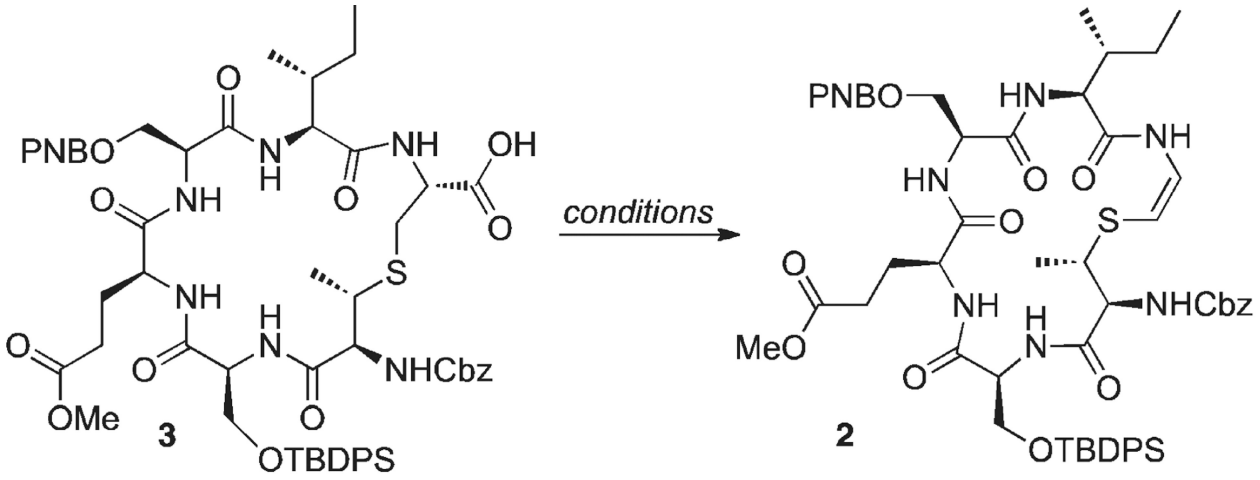


Scheme 5.



	conditions	% yield
A.	DPPA, TEA, toluene, 94 °C, overnight	10–12% Z
B.	DPPA, DABCO, 1,4-dioxane, 94 °C, overnight	25-30% Z
C.	1. $Pb(OAc)_4$ (1 equiv), $Cu(OAc)_2$ (1 equiv), pyridine, THF, 0-25 °C, 3 h 2. TEA in EtOAc	N.R.
D.	1. $Pb(OAc)_4$ (5 equiv),$Cu(OAc)_2$ (5 equiv), pyridine (excess), THF, 0-25 °C, 3 h 2. TEA in EtOAc	25-30% Z

Scheme 6.