Solid-Phase Total Synthesis of Sandramycin and Its Analogues

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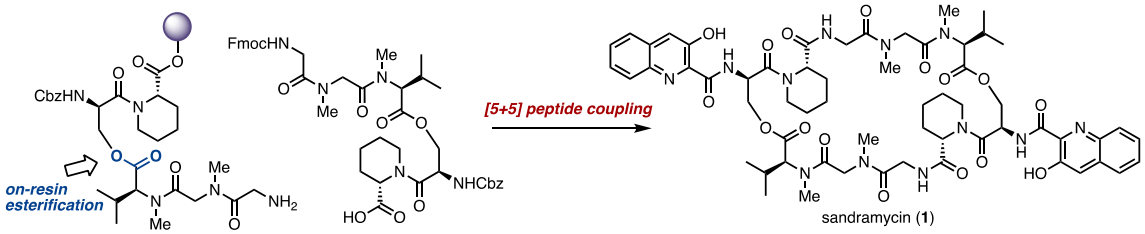


ABSTRACT: Solid-phase total synthesis of sandramycin (**1**), which is a C_2-symmetric cyclic decadepsipeptide natural product, and its analogues is described. On-resin ester formation and [5+5] peptide coupling allowed to prepare a range of de-symmetrized analogues. An amino acid residue which would not hamper the biological activity of **1** was successfully identified, and probe molecules and dimeric analogue were prepared based on the result of the structure-activity relationship study.

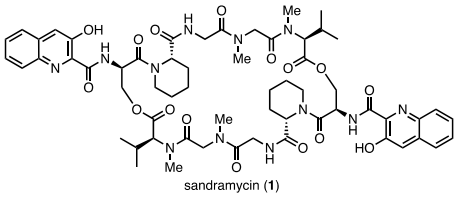
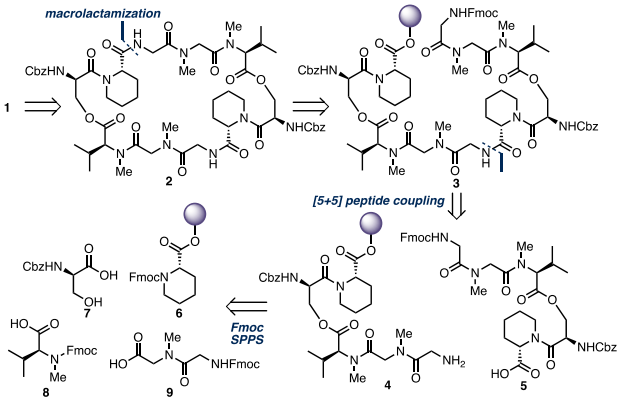


Figure 1. Structure of sandramycin (**1**)

Sandramycin (Figure 1, **1**) belongs to a family of bisintercalator natural products isolated from *Nocardioides* sp. (ATCC 39419).[1] It contains a C_2 symmetric cyclic decadepsipeptide moiety comprising glycine (Gly), sarcosine (Sar), *N*-methyl-L-valine (*N*-Me-Val), D-serine (D-Ser), and L-pipecolic acid (Pip) and possesses two 3-hydroxyquinoline-2-carboxylic acids as a chromophore. It binds to the minor groove of double-stranded DNA with the bisintercalation of its chromophores[2-4] and exhibits potent cytotoxicity against mouse leukemia cell lines *in vitro* and *in vivo*.[1,2] Because of the potent cytotoxicity of bisintercalator natural products, their derivatives have been examined as payloads for antibody-drug conjugates.[5] Hence, it is expected that **1** could be a lead compound as a novel anticancer agent. Two solution-phase total syntheses of **1** have been reported by Boger *et al.*[2] and us.[6] Both methods deployed the C_2 symmetry of **1** to reduce the number of required synthetic steps. Therefore, accessible analogues prepared using these synthetic methods have been limited to those with C_2 symmetry.[6-9] The same limitation has existed for the total synthesis of other bisintercalator natural products.[10-14] This synthetic limitation prevented us from examining the effects of altering single amino acids on the biological activity. Solid-phase peptide synthesis (SPPS) has an advantage of enabling the preparation of various analogues because the protocol simplifies the purification at each step in the linear synthesis and the amino acids contained in the peptide sequences can be easily exchanged for different amino acids by simply changing the condensed amino acids. Consequently, SPPS is easily extended to the parallel synthesis of analogues. Herein, we report solid-phase total syntheses of **1** and its analogues, which have not been previously elucidated. The method using SPPS allows preparation of desymmetrized analogues for structure–activity relationship studies. Furthermore, chemical probes, which could be used to investigate the mode of action of **1** and the dimeric analogue, were synthesized.

Scheme 1. Retrosynthetic analysis of **1**



Our retrosynthetic analysis of **1** is illustrated in Scheme 1. As with the solution-phase syntheses, the chromophores would be attached to macrocycle **2** in the final stage of the synthesis. We planned to synthesize the macrocycle by macrolactamization of the linear peptide, which could be obtained from resin-bound

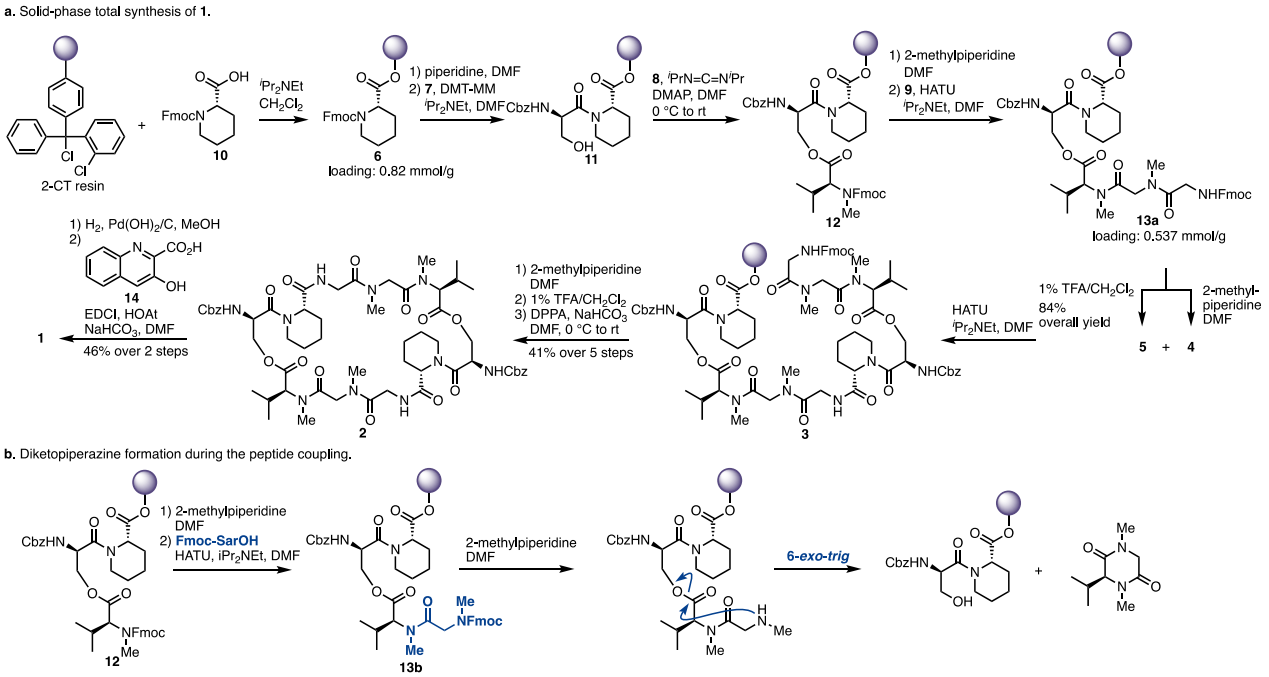
depsipeptide **3**. Considering the synthesis of desymmetrized analogues, we envisioned that the coupling of two pentadepsipeptide units[15-18] (hereafter [5 + 5] peptide coupling) would be a powerful method for the synthesis of **3**. The same fragment coupling methodologies on a solid support were reported in the syntheses of peptide and depsipeptide,[18] which reduced the number of synthetic steps compared with the standard solid-phase synthesis in which each amino acid residue is elongated one by one. The pentadepsipeptide units could be synthesized from resin-bound Fmoc-Pip **6** using SPPS, involving the ester-bond formation step. In this synthetic strategy, the ester formation step and on-resin [5 + 5] peptide coupling would be challenging because of the chemically labile ester bond and the reduced reactivity of pentadepsipeptide due to steric hindrance, respectively. Hence, the optimization of these steps was required to develop a synthetic method, which was applicable for the preparation of various analogues.

The solid-phase total synthesis of **1** is illustrated in Scheme 2a. Fmoc-L-Pip was first loaded on a 2-chlorotrityl (2-CT) resin to afford resin-bound Fmoc-amino acid **6** (loading: 0.82 mmol/g). After the removal of the Fmoc group of **6** with piperidine in *N*,*N*-dimethylformamide (DMF), the free amine was condensed with **7**. In this coupling, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM)[19,20] was superior to suppress the undesired *O*-acylation on the unprotected hydroxy group of **7**, and the desired alcohol **11** was cleanly obtained using this coupling method. Alcohol **11** was condensed with **8** (diisopropylcarbodiimide, 4-dimethylaminopyridine (DMAP), and DMF at 0 °C to room temperature) to afford depsipeptide **12**. Subsequently, 2-methylpiperidine, which is less likely to cause the nucleophilic substitution of the ester due to its steric hindrance,[21-23] was used for Fmoc deprotection instead of piperidine. Removal of the Fmoc group of **12** with 2-methylpiperidine in DMF successfully afforded the corresponding amine without the undesired transamidation. The free *N*-methylamine was further coupled with dipeptide **9** (1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxide hexafluorophosphate (HATU), *N*,*N*-diisopropylethylamine (^{i}Pr$_2$NEt), and DMF) to afford resin-bound pentadepsipeptide **13a** (loading: 0.54 mmol/g, based on the UV measurement). Notably, the choice of carboxylic acid was crucial in this coupling. When Fmoc-Sar-OH was used instead of **9**, the coupling reaction proceeded smoothly to provide **13b**; however, the amine produced by the Fmoc removal of **13b** caused an unacceptable amount of diketopiperazine formation by the nucleophilic attack of the ester bond via a 6-*exo*-*trig* cyclization mechanism (Scheme 2b). The side reaction could be suppressed using dipeptide **9**, which did not produce the intermediate causing 6-*exo*-*trig* cyclization. The

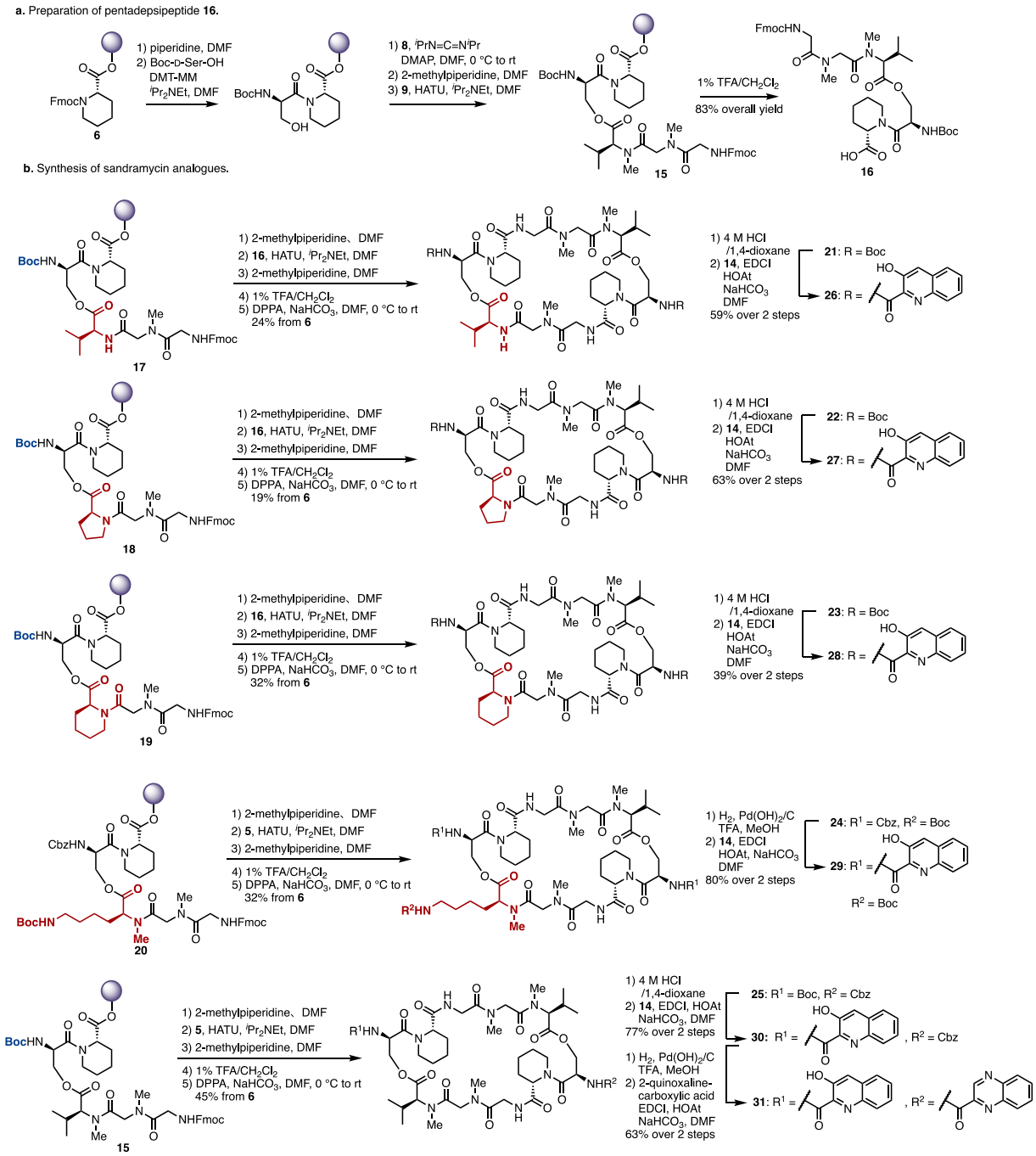
resin-bound depsipeptide **13** could be used as a common intermediate of **4** and **5**. Specifically, the Fmoc removal of **13** with 2-methylpiperidine afforded **4**, and cleavage from the solid support under a weak acidic condition yielded carboxylic acid **5** in 84% overall yield (1% trifluoroacetic acid (TFA)/ CH_2Cl_2). The on-resin [5 + 5] peptide coupling between **4** and **5** with HATU and ^{i}Pr$_2$NEt in DMF smoothly proceeded to afford **3**. The removal of the Fmoc group on the *N*-terminus of the peptide (2-methylpiperidine, DMF), followed by cleavage from the resin (1% TFA/CH_2Cl_2) yielded the cyclization precursor, which was successfully cyclized in the presence of diphenylphosphoryl azide (DPPA) and $NaHCO_3$ in DMF to afford macrocycle **2** in 41% yield over five steps from **13**. After the removal of the Cbz groups on the α-nitrogen of the serine residues in **2**, carboxylic acid **14** was condensed to liberate the amines [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI), 1-hydroxy-7-azabenzotriazole (HOAt), $NaHCO_3$, and DMF] and produce **1** in 46% yield over two steps. The analytical data of **1** agreed well with those reported.

With the concise synthetic method in hand, we applied the method to the synthesis of various analogues, particularly the unsymmetrical ones. The synthesis of unsymmetrical analogues is illustrated in Scheme 3. A differently protected pentadepsipeptide, **16**, with Boc-protected D-Ser residues, was prepared using the established SPPS protocol (Scheme 3a). Specifically, Cbz-D-Ser **7** in Scheme 2 was changed to Boc-protected D-Ser to produce resin-bound pentadepsipeptide **15**, and cleavage from the resin afforded Boc- and Fmoc-protected pentadepsipeptide **16**. Similarly, resin-bound pentadepsipeptides **17–20** containing L-Val, L-Pro, L-Pip, and *N*-Me-L-Lys residues instead of *N*-Me-L-Val, respectively, were prepared (Scheme 3b). Resin-bound pentadepsipeptide **15** was also used to achieve a chromophore modification. After the Fmoc removal of **15** and **17**–**20** with 2-methylpiperidine in DMF, the free amines were coupled with Boc-protected depsipeptide **16** or Cbz-protected depsipeptide **5** to afford resin-bound decadepsipeptides. Sequential Fmoc deprotection, cleavage from the resins, and macrolactamization with DPPA yielded cyclic peptides **21**–**25**, respectively. Two Boc groups of **21**–**23** were removed using 4 M HCl in 1,4-dioxane to afford amines, and the coupling reaction

Scheme 2. Total synthesis of sandramycin (**1**)



Scheme 3. Synthesis of sandramycin analogues



between the amines and **14** produced unsymmetrical analogues **26**–**28**, respectively. Similarly, the amine prepared by the Cbz removal of **24** was also condensed with **14** to afford analogue **29** in 80% over two steps. The Boc removal of **25** followed by installation of 3-hydroxyquinoline-2-carbonyl group on the amine gave **30**, and further installation of quinoxaline-2-carbonyl group yielded chromophore-modified analogue **31**. All unsymmetrical analogues **26-29** and **31** were purified by silica gel column chromatography, their chemical structures were confirmed by NMR analyses, and their purity was assured by LC-MS analysis to be sufficient for the following biological evaluation (see the Supporting Information).

The cell growth inhibitory activities of the synthesized analogues are summarized in Table 1 (for details, see the Supporting Information). All side chain-modified analogues **26**–**29** and chromophore-modified analogue **31** retained the activity, indicating that the substitution of the *N*-Me-Val to other amino acids did not hamper the cell growth inhibitory activity. Notably, analogue **29** possessed a Boc-protected Lys side chain, and the bulky substituent acceptably exhibited the activity. The result motivated us to use the protected amino group as a starting point for further modifications (Scheme 4a). After the removal of the Boc group of **29**, the amine was acylated with dicarbonate **32** to produce an active ester intermediate. The active ester was further treated with biotin-PEG2-amine **33** to afford biotinylated probe **34**. The free amine corresponding to **31** was also acylated with **35** to produce **36**, which contained Yao's minimalist clickable photo-crosslinker.[24] The chromophore modification shown in Scheme 3b could also be applied to prepare probe molecules. The amine derived from **30** was

condensed with Hosoya's diazide carboxylic acid **37**[25] to afford diazide probe **38**. Analogues **34**, **36** and **38** retained the biological activity of **1** (IC_{50} = 29, 1.3 and 2.7 nM, respectively); hence, these probe molecules could be used to investigate the mode of action of **1**.

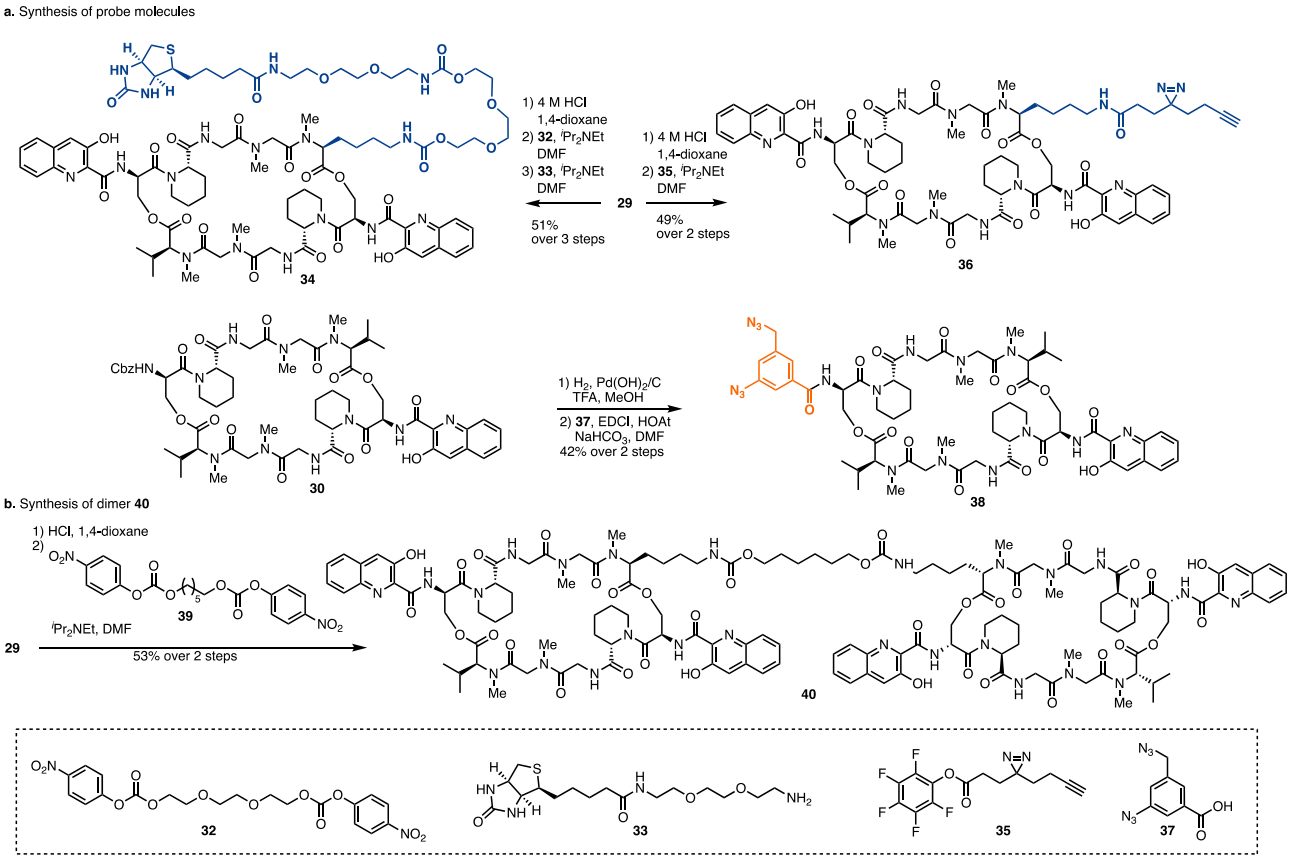
Table 1. Cell growth inhibitory activities of **1** and its analogues.

compounds	IC_{50} (nM)
1	0.13
26	1.1
27	3.2
28	1.4
29	0.53
31	0.36
34	29
36	1.3
38	2.7

Dimerization of natural products is an attractive strategy for enhancing their biological activity;[26-29] therefore, we synthesized a homo dimer of **1**. Because the modification of the Lys side chain could be used for further derivatization without the loss of activity, we planned to conjugate two molecules of **1** using the amino groups as linking points (Scheme 4b). After removing the Boc group of **29**, the free amine was coupled with almost a half equivalent of linker compound **39** to afford dimeric analogue **40**. This example illustrates that our SPPS-based synthetic route is a powerful method for synthesizing various types of analogues of **1**. Analyses of the biological activity and the effect of dimerization on the biological activity of **40** are currently in progress.

In conclusion, we developed a method for synthesizing **1** and its analogues using SPPS. The method, involving on-resin [5 + 5] peptide coupling, can be used for the efficient preparation of various analogues. The desymmetrization realized by the synthesis enables the pinpointing modification of the C_2 symmetric macrocycle of **1**, and the synthesis illustrated here is the first example of the synthesis of a asymmetric analogue of **1**. The structure–activity relationships of the analogues of **1** demonstrated that *N*-Me-Val could be converted to another amino acid residue without the loss of activity. We also prepared chemical probes: two photoaffinity probes, a biotin analogue, and a dimeric analogue, which will be useful to elucidate the mode of action of **1**.

Scheme 4. Synthesis of probe molecules **34, 36** and **38**, and dimer **40**



ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information Statement

The Supporting Information is available free of charge on the ACS Publications website.

Experimental details; ^1H, ^{13}C NMR and mass spectra, and LC-MS trace (PDF)

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest.

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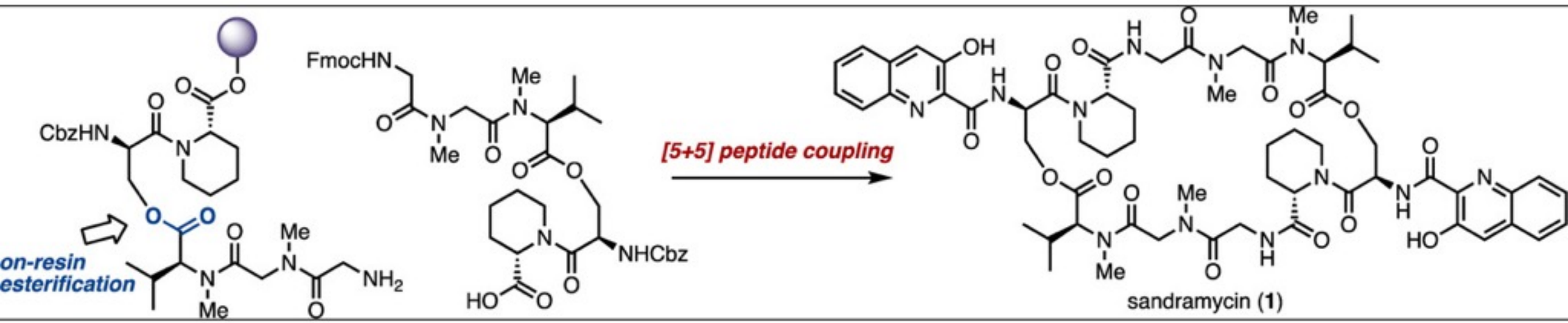
on-resin
esterification
[5+5] peptide coupling
sandramycin (1)