

## High-yielding macrocyclization conditions used in the synthesis of novel Sansalvamide A derivatives

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**Abstract**—Described are the syntheses of nine Sansalvamide A derivatives using new, high-yielding, in situ deprotection–cyclization conditions that are general for this series of macrocycles, 55% average for both steps. This is 10-fold greater than previously reported yields.

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Sansalvamide A is composed of four hydrophobic amino acids and one hydrophobic hydroxy acid. Herein, we describe the synthesis of nine novel Sansalvamide A derivatives using conditions that give high yields for the in situ deprotections–cyclizations. Cyclizing large macrocycles is usually very challenging, typically the yields are low, and they often take long reaction times (2–4 days).<sup>1</sup> Peptide cyclization yields depend on the coupling agent and the linear precursor conformation.<sup>2</sup> Most cyclizations are reported as single examples, where the conditions were optimized for coupling agents, concentration conditions, and solvents. What is unique about the work reported here is that not only do we cyclize in relatively high yields, we do so for nine different substrates. Thus, despite structural and likely conformational differences, these new conditions produced nine macrocycles in reasonable yields. Furthermore, these conditions give approximately 10-fold greater yields when compared to our previous conditions. This work opens the possibility for the synthesis of focused libraries in practical quantities of material, which is critical for biological assays.

Initial in situ deprotection–cyclization conditions for the first- and second-generation Sansalvamide A derivatives (reaction conditions I, Table 1) involved deprotection of the amine on the pentapeptide using trifluoroacetic acid (TFA), which gave insoluble TFA salts. Subsequent deprotection of the acid using lithium hydroxide pro-

**Table 1.** Comparison of macrocyclization conditions

Compound #	Original yield (%) <sup>a</sup>	New yield (%) <sup>b</sup>
1	5	65
2	2	39
3	5 <sup>c</sup>	85
4	5 <sup>c</sup>	65
5	9	53
6	0.7	37
7	5 <sup>c</sup>	81
8	5 <sup>c</sup>	40
9	5 <sup>c</sup>	35

<sup>a</sup> Old cyclization conditions, referred to as conditions I.

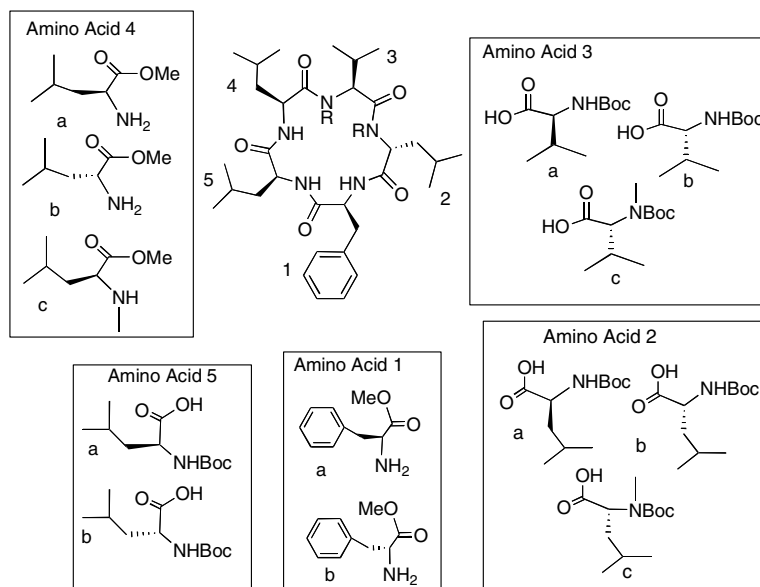
<sup>b</sup> New cyclization conditions, referred to as conditions II.

<sup>c</sup> The cyclization was only run under the new conditions. Reported is the average yield of all Sansalvamide A derivatives synthesized in our lab to date using the old conditions.<sup>7</sup>

duced insoluble salts with the acid of the linear pentapeptide. Investigation into more efficient in situ deprotection and cyclization conditions led to the discovery that HCl deprotected both the amine and the acid without forming insoluble salts, thus furnishing cleaner cyclization products in high yields (Fig. 2). The average yield for the new macrocyclization conditions (conditions II) is approximately 10-fold higher than the original cyclization conditions (conditions I). In addition, the new cyclization conditions are general for nine substrates, which is unusual for cyclizations of large macrocycles.

The synthesis of nine derivatives utilized the amino acids shown in Figure 1, where the amino acids were chosen

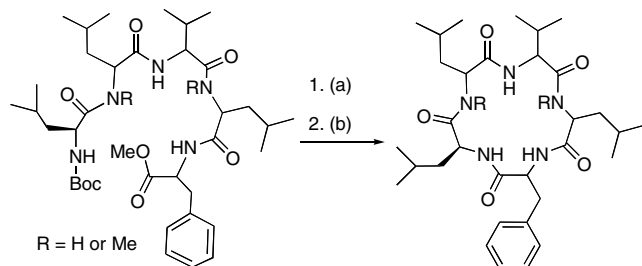
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**Figure 1.** Amino acids used in synthesis of nine derivatives.

based on the structure–activity relationship (SAR) of the first generation.<sup>3</sup>

Our synthesis utilized a convergent approach and standard conditions to reach the linear pentapeptide.<sup>3</sup> The high yielding macrocyclization conditions (II) involved dissolving the linear pentapeptide in THF (0.05 M, Fig. 2), addition of two equivalents of anisole, and adding approximately eight drops concentrated HCl per 0.3 mmol of linear pentapeptide. The reaction mixture was stirred at room temperature for 24 h, whereupon LCMS usually indicated that the acid and amine were partially deprotected. At this point, four drops of HCl per 0.3 mmol of peptide were added, and reactions were monitored by LCMS. Typically deprotection reactions were completed within four days.<sup>4</sup> Upon completion, the reaction was concentrated in vacuo, and dried on the high-vac. The dried, crude linear pentapeptide was dissolved in a 2:2:1 ratio of THF/CH<sub>3</sub>CN/CH<sub>2</sub>Cl<sub>2</sub> (0.007 M), addition of DIPEA (6 equiv), and three coupling agents (HATU, DEPBT, and TBTU 0.7 equiv ea) to reaction gave a clear solution. Reactions were usually completed in 1–2 h, which is significantly faster than most published cyclization conditions.<sup>1,5</sup>



**Figure 2.** New cyclization conditions (conditions II): (a) HCl in THF (0.05 M), anisole (2 equiv); (b) HATU (0.7 equiv), DEPBT (0.7 equiv), TBTU (0.7 equiv), DIPEA (6 equiv), THF/CH<sub>3</sub>CN/DCM (2:2:1) 0.007 M.

**Table 1** shows the macrocyclization yields using our new method (conditions II). Although there is some variation in these yields, the average yield is 55%. By comparison, the average yield using our previous cyclization conditions is 5% (this includes both first and second-generation data). The cyclization yields for compounds **3**, **4**, **7**, **8**, and **9** were not available using the old approach (I) because these compounds were synthesized after realization that the new method substantially improved yields.<sup>6</sup> LCMS data of crude cyclization reactions show only one product peak, which is around 6.1–7 min.<sup>5,8</sup> HPLC purification gave yields in **Table 1** ('new yields'). The nine macrocycles are shown in **Figure 3**.

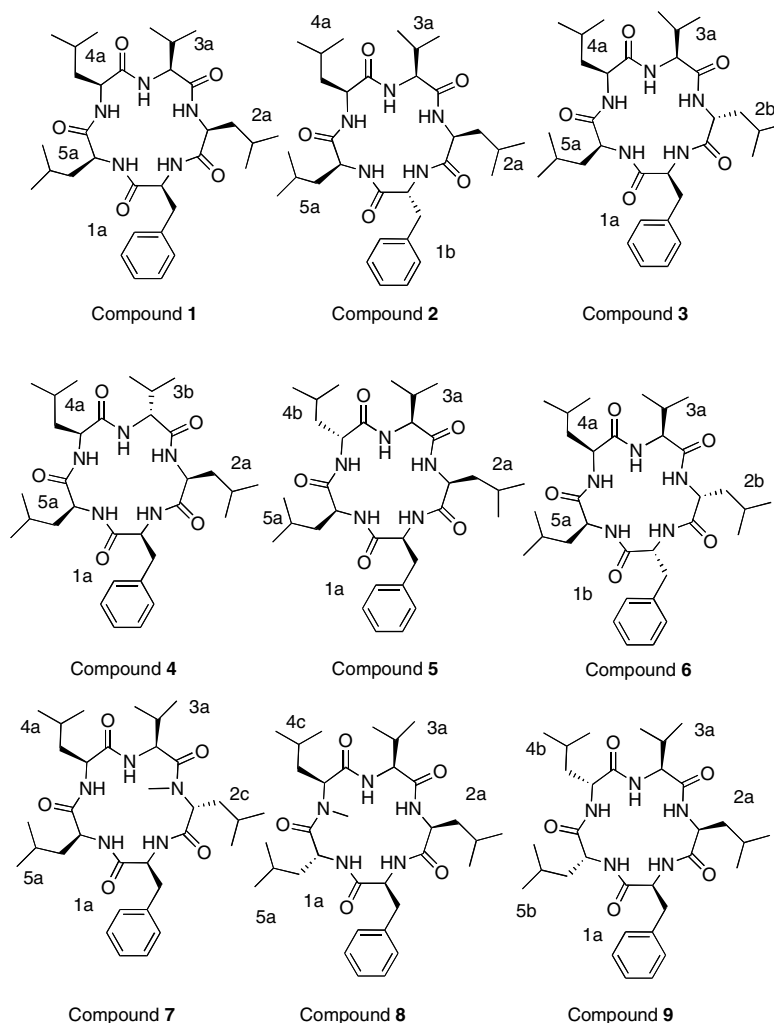
In summary, new cyclizations conditions have provided nine novel, Sansalvamide A derivatives in high yields. These yields are ~10-fold greater than previously reported. The LCMS data of the crude reactions show the product is the primary peak in all examples.<sup>8</sup> Most importantly, these cyclization conditions appear to be general for this series of macrocycles. Recent assay data of these nine compounds<sup>9</sup> have provided insight into their SAR, and these results will be reported in due course.

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### Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.tetlet.2005.11.056](https://doi.org/10.1016/j.tetlet.2005.11.056).



**Figure 3.** Compounds synthesized via new conditions.

### References and notes

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- For details on the reaction conditions see supplementary data.
- It was straightforward to follow the reactions via LCMS as the starting material double deprotected linear precursor would appear at 5.0–5.5 min, the cyclized product would appear between 6.1 and 7.0 min.
- Because of the high yields achieved with the cyclizations using the new conditions (B), we had enough material for bioassays. Thus, it was not necessary to resynthesize these compounds using a low yielding conditions (the old approach A) in order to recognize the value of this cyclization reaction.
- Yields previously reported for cyclizations in *Org. Lett.* **2005**, *7*, 3481 were yields after silica gel flash chromatography.
- The nine macrocyclic peptides have LCMS spectra and yields given in the supplementary data. In addition, several representative NMRs are shown.
- Unpublished results.