

Total Synthesis of Ciliatamides A–C: Stereochemical Revision and the Natural Product-Guided Synthesis of Unnatural Analogues

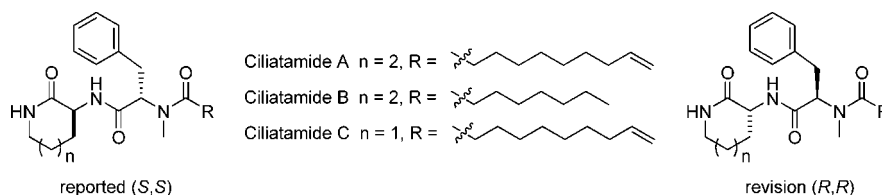
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ABSTRACT



The first total synthesis of Ciliatamides A–C was completed, leading to a revision of the reported stereochemistry from (*S,S*) to the (*R,R*) enantiomers. Due to the expedited route, a library of over 50 unnatural ciliatamide analogs was also prepared.

Leishmaniasis is a group of vector-borne diseases, caused by obligate intramacrophage parasites of the genus *Leishmania*, which is endemic in the tropics.^{1,2} The disease may manifest itself as either a cutaneous, a mucosal, a dermal, or as the deadly visceral variant.³ Second only to malaria, leishmaniasis afflicts more than 12 million people in 88 countries with an annual death toll exceeding 50,000.^{1–3} The current treatments for leishmaniasis are the pentavalent antimonials **1** and **2**, humatin **3**, amphotericin B **4**, pentamidine **5**, and miltefosine **6** (Figure 1); however, these drugs possess a number of drawbacks including cardiotoxic effects, parenteral administration, long treatment regimens, and high cost.^{1–3}

Earlier this year, Nakao and co-workers reported on the isolation of three lipopeptides, Ciliatamides A (**7**), B (**8**), and C (**9**), from the deep sea sponge *Aaptos ciliata* as the

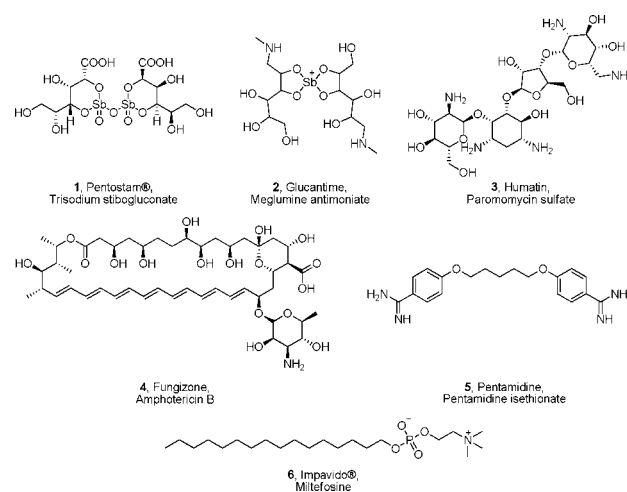


Figure 1. Structures of current antileishmaniasis treatments.

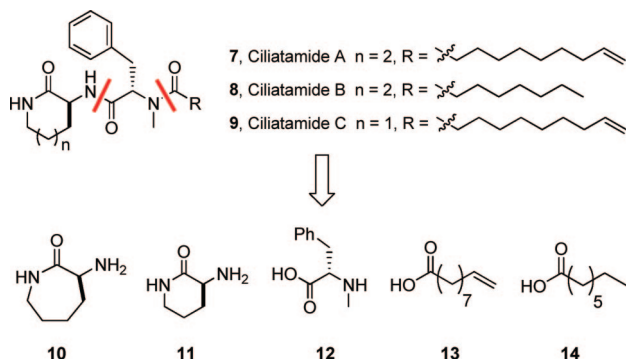
(*S,S*)-enantiomers (Scheme 1).⁴ Importantly, Ciliatamides A and B demonstrated significant antileishmanial activity and,

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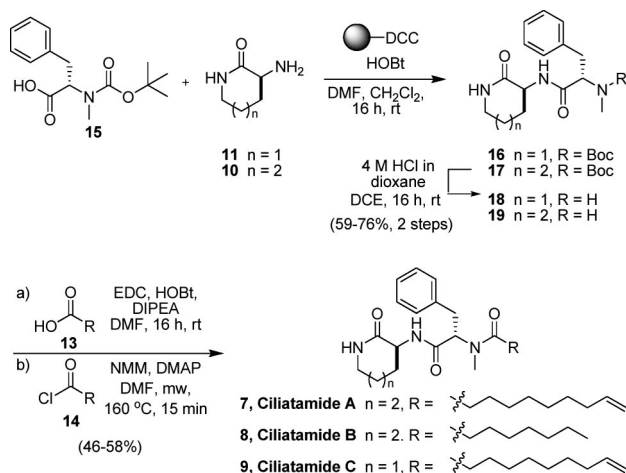
Scheme 1. Structures of Ciliatamides A (7), B (8), and C (9) and Retrosynthesis



thus, appeared as ideal targets for total synthesis and further biological evaluation as 7–9 are chemically far less complex than current antileishmanials 1–6.^{1–4} The retro-synthesis of 7–9 (Scheme 1) involved cleavage of the two amide bonds to provide the (*S*)-3-aminoazepan-2-one **10** (for 7 and 8) or the (*S*)-3-aminopiperid-2-one **11** (for 9), *L*-*N*-methyl Phe **12** and either decenoic acid **13** (for 7 and 9) or octanoyl chloride **14** (for 8). Surprisingly, all of the requisite precursors were commercially available.⁵

In the event, Boc-*L*-*N*-MePhe **15** was coupled to **10** with PS-DCC and HOBT, followed by treatment with HCl in dioxane to deliver the free base **19** in 59% yield for the two steps after ion-exchange chromatography. **19** then underwent a second amide coupling with **13** to afford 7, or with **14** to afford 8 in 56 and 58% yields, respectively. Following the same scheme (Scheme 2),⁵ but substituting (*S*)-3-aminopi-

Scheme 2. Synthesis of Ciliatamides A (7), B (8), and C (9)

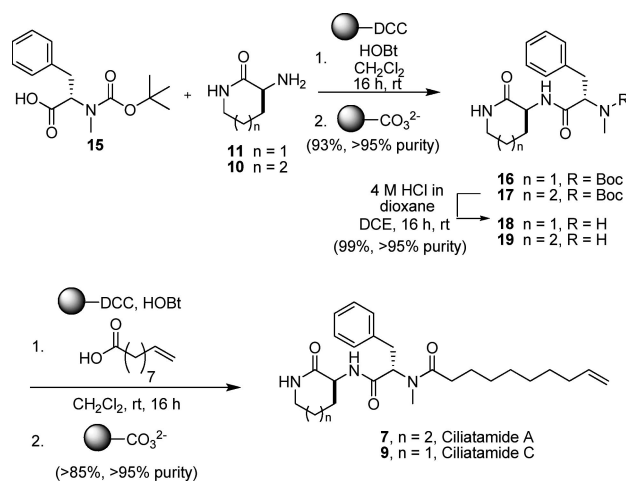


perid-2-one **10** for **11**, provided Ciliatamide C (**9**) in 44% yield over the three steps. The overall yields were lower than anticipated due to the physicochemical properties of 7–9, and poor chromatographic performance of these lipopeptides.

Despite this, the NMR spectra obtained were in complete accord with those reported by Nakao and co-workers; however, the original spectra were recorded under very dilute conditions to minimize conformer populations (see Supporting Information).⁴

On the basis of these results, we then explored a solution phase parallel synthesis approach for the synthesis of Ciliatamides A (**7**) and C (**9**) employing only polymer-supported reagents, scavengers and ion-exchange chromatography to avoid either normal or reverse-phase chromatography.⁶ This strategy afforded improved results, providing 7 and 9 in overall yields in excess of 75% for the three steps with >95% purity (Scheme 3).⁵

Scheme 3. Solution Phase Parallel Synthesis of Ciliatamides A (7) and C (9)



Due to the expedited route to 7–9, in parallel, we prepared a 42-member solution phase library of unnatural ciliatamide analogs **20a–n**, **21a–n** and **22a–n**. The library employed three scaffolds **18**, **19**, and the unnatural (*R,S*) congener of **19**, and a collection of 14 different acid chlorides (Figure 2). All final compounds, including additional copies of 7–9 were purified to >98% by mass-directed preparative HPLC and afforded yields ranging from <5 to 60% for the three steps; however, we obtained sufficient quantities for biological evaluation in every case.^{5,7}

As we were compiling the final characterization data for 7–9, a discrepancy was noted with respect to the reported optical rotations, $[\alpha]_D^{20} = +40$ ($c = 0.05$, MeOH), $[\alpha]_D^{20} = +55$ ($c = 0.1$, MeOH), $[\alpha]_D^{26} = +74$ ($c = 0.05$, MeOH), for the natural products 7–9, respectively.⁴ While the ¹H and ¹³C NMR spectra of our synthetic 7–9 overlaid with the natural products, the optical rotations were of comparable magnitude, but opposite sign, that is, $[\alpha]_D^{20} = -35$ ($c = 0.05$, MeOH), $[\alpha]_D^{20} = -44$ ($c = 0.1$, MeOH), $[\alpha]_D^{26} = -43$ ($c = 0.1$, MeOH) for synthetic 7–9, respectively.⁵ On the basis of these results, we synthesized the four possible stereoisomers ((*S,S*), (*S,R*), (*R,S*), and (*R,R*)) of Ciliatamide A and Ciliatamide B, employing the route depicted in Scheme 2, and compared NMR spectra and obtained optical

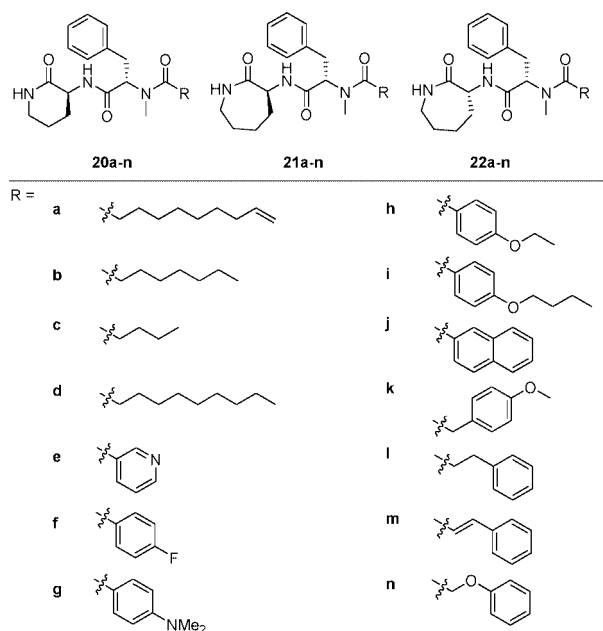


Figure 2. Three $\times$ 14 library of unnatural ciliatamide analogs.

rotations (Figure 3).⁵ For Ciliatamide A (**7**), reported to be the (*S,S*)-enantiomer, the NMRs of diastomeric pairs **23** (*S,R*) and **24** (*R,S*), as anticipated, did not match **7**; however, the (*R,R*)-enantiomer **25** was in complete accord with the published spectral data and possessed an optical rotation ($[\alpha]_{\text{D}}^{20} = +42$ ($c = 0.05$, MeOH)) that matched the literature report as well. Similarly, the (*R,R*)-enantiomer of Ciliatamide B (**28**) and overlaid with the reported NMR spectra of **8** as well as provided optical rotations in agreement with those reported by Nakao and co-workers, ($[\alpha]_{\text{D}}^{20} = +49$ ($c = 0.1$, MeOH)) for **28**.

Attempts to prepare the (*S,R*), (*R,S*) and (*R,R*) stereoisomers of Ciliatamide C following the route depicted in Scheme 2 led to significant racemization of the (*R*)-piperidin-2-one, which was not observed within the Ciliatamide A and B series or the (*S*)-3-aminopiperidin-2-one **11**. Therefore, after several approaches, we developed an alternate route that avoided racemization and afforded pure stereoisomers (Scheme 4). In this instance, the carbodimide coupling in Scheme 2 was replaced with a HATU/collidine system for the coupling of pure (*S*)- or (*R*)-piperidin-2-one, **10** and **29**, respectively, with either enantiopure Boc-L-*N*-MePhe **15** or Boc-D-*N*-MePhe **30**. The milder acidolysis of the Boc deprotection of **31–33** with 5–7% TFA in CH_2Cl_2 in an ice bath instead of 10 equiv of HCl was used to deliver isomers **34–36**. Finally, a second HATU coupling with decenoic acid provided the remaining stereoisomers of Ciliatamide C **37** (*S,R*), **38** (*R,S*), and **39** (*R,R*). As in the case of Ciliatamides A and B, both NMR spectra and optical rotation ($[\alpha]_{\text{D}}^{20} = +56$ ($c = 0.1$, MeOH)) for **39** confirm a stereochemical reassignment of the natural product to the (*R,R*)-enantiomer.

For Ciliatamides A–C, the optical rotations were positive, and in agreement with the literature report, only when the unnatural D-MePhe was employed. Nakao and co-workers

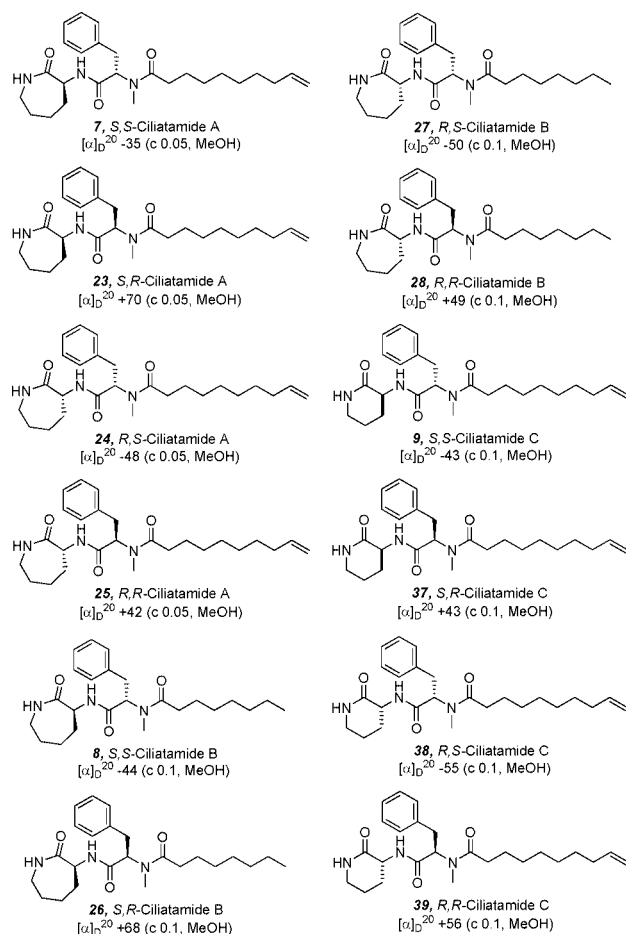
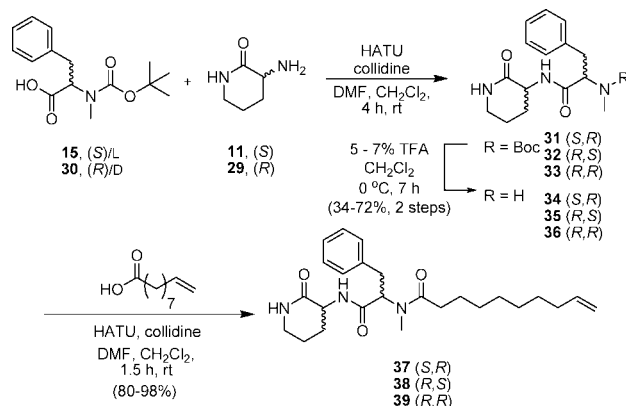


Figure 3. Library of all possible stereoisomers of Ciliatamides A–C and the corresponding optical rotations.

utilized Marfey's analysis⁸ to establish the L-configuration of the caprolactams and the MePhe in **7–9**; however, the

Scheme 4. Synthesis of the (*S,R*), (*R,S*), and (*S,S*)-stereoisomers of Ciliatamide C, **37**, **38**, and **39**, respectively



data presented herein suggests that either the reported optical rotations were incorrect, or the stereochemical assignments

were incorrect.⁴ It is also possible that the natural products, under the forcing acidic conditions of the Marfey's analysis⁸ racemized. We have noted that the (*R,R*)-analogs are prone to acid catalyzed racemization to the (*S,S*)-enantiomers. Thus, based on our data, we propose a stereochemical revision for Ciliatamides A–C from the (*S,S*)-**7**, **8**, and **9** to the (*R,R*)-**25**, **28**, and **39** (Figure 4).

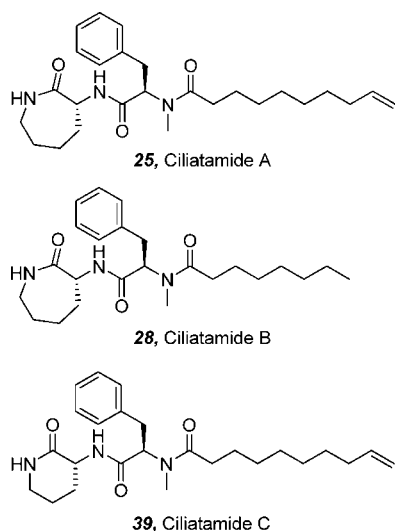


Figure 4. Revised structures of the natural lipopeptides Ciliatamides A–C after stereochemical revision.

We are in the process of developing an antileishmanial assay, which we hope will further validate the stereochemical reassignment of Ciliatamides A–C based on biological activity. However, once a validated assay is in place, we will evaluate all of the unnatural analog ciliatamide libraries in an attempt to develop structure–activity relationships (SAR). We are also in the process of identifying discrete

molecular targets, as we have done previously for other marine natural products,⁹ for Ciliatamides A–C that might afford a mechanistic understanding of their antileishmanial activities.

In summary, we have completed the first total synthesis of Ciliatamides A–C, originally reported as the (*S,S*)-enantiomers **7–9**, employing both traditional organic synthesis and solution phase parallel synthesis. Based on spectral and optical rotation data for all the possible stereoisomers of **7–9**, we propose a stereochemical revision for Ciliatamides A–C to the (*R,R*)-enantiomers **25**, **28**, and **39** respectively. Due to the expedited route, we also prepared a library of 42 unnatural ciliatamide analogs **20a–n**, **21a–n**, and **22a–n**, and when combined with the unnatural stereoisomers, the library exceeds 50 unnatural analogs. Assay development and biological evaluation for both the natural products and the unnatural ciliatamides are in progress and will be reported in due course.

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Supporting Information Available: Experimental procedures, characterization data, and ¹H and ¹³C NMR spectra for all new compounds, **7–9**, **16–19**, **23–28**, **31–39**, and a cross section of library members **20a–n**, **21a–n**, and **22a–n**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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(5) For full experimental details, see Supporting Information.

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