Published in final edited form as:

Science. 2014 October 10; 346(6206): 219–224. doi:10.1126/science.1255677.

Asymmetric syntheses of sceptrin and massadine and evidence for biosynthetic enantiodivergence

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Abstract

Cycloaddition is an essential tool in chemical synthesis. Instead of using light or heat as a driving force, marine sponges promote cycloaddition with a more versatile but poorly understood mechanism in producing pyrrole–imidazole alkaloids sceptrin, massadine, and ageliferin. Through de novo synthesis of sceptrin and massadine, we show that sponges may use single-electron oxidation as a central mechanism to promote three different types of cycloaddition. Additionally, we provide surprising evidence that, in contrast to previous reports, sceptrin, massadine, and ageliferin have mismatched chirality. Therefore, massadine cannot be an oxidative rearrangement product of sceptrin or ageliferin, as is commonly believed. Taken together, our results demonstrate unconventional chemical approaches to achieving cycloaddition reactions in synthesis and uncover enantiodivergence as a new biosynthetic paradigm for natural products.

The dimeric pyrrole–imidazole alkaloids are structurally complex small molecules produced by marine sponges through reactions that are not well understood (1) (Fig. 1 and figs. S1 and S2). These natural products are highly polar, noncrystalline, redox labile, and pH sensitive because of their exceptionally high nitrogen content (~16% atomic composition or 15 to 30% by weight). The presence of multiple halogen atoms further contributes to their chemical instability and synthetic challenges (2–21). As depicted in Fig. 1, sceptrin (**1a**) (22), massadine (**2a**) (23, 24), and ageliferin (**3a**) (25, 26) are family members derived from

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www.sciencemag.org/content/346/6206/219/suppl/DC1

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formal [2+2], [3+2], and [4+2] cycloaddition reactions of oroidin (**4a**), hymenidin (**4b**), or their congeners. Our previous studies on the de novo synthesis of agelifेरins (**3**) (4, 14, 15) suggest that the biogenic dimerization of **4** is likely a radical reaction. We now describe the de novo syntheses of sceptrin (**1a**) and massadine (**2a**), using oxidative reactions that are suspected to be involved in their biosyntheses. These asymmetric syntheses along with two new crystal structures allow us to unambiguously assign the absolute stereochemistries of the pyrrole–imidazole dimers. We also demonstrate that the [3+2] dimers cannot be derived from the [4+2] or [2+2] dimers through a skeletal rearrangement reaction. Instead, a surprising enantiodivergent pathway must be involved in the biosynthesis of these alkaloids.

Sceptrin (**1a**) is the first dimeric pyrrole–imidazole alkaloid discovered in nature (22). Because there is insufficient light where the sponges live (20 to 30 m below the ocean surface), it is unlikely for hymenidin (**4b**) to undergo a photocycloaddition reaction to give **1a** biogenically (2, 22). In addition, a [2+2] photocycloaddition reaction of **4b** would lead to the formation of racemic **1a**, whereas the isolated **1a** was optically active. Based on results of meta-biosynthetic experiments, Molinski and Romo proposed an enzyme-promoted single-electron transfer (SET) mechanism for the biogenic dimerization of **4** (27). We believe that a SET oxidation of **4** would give radical cation **4**^{•+} that is highly reactive toward [2+2] (path a) and [4+2] (path b) cycloaddition reactions to afford **1**^{•+} and **3**^{•+}, correspondingly (Fig. 1). Subsequent back-SET would provide sceptrins (**1**) and “iso-agelifेरins” (**5**) that tautomerize to agelifेरins (**3**).

Massadine (**2a**) is a densely functionalized polycyclic [3+2] dimer that contains 8 contiguous stereocenters, 10 nitrogen atoms, and multiple sensitive functional groups. These structural features preclude the use of many traditional tools for its synthesis. Because enzymes that promote [3+2] cycloaddition reactions remain elusive, it is commonly believed that **2a** is derived from **5** through an oxidative skeletal rearrangement reaction (13, 28, 29). Oxidation of **5** would induce a ring-contraction reaction to give “pre-massadine” (**6**). Further oxidation of **6** would provide **2** and its congeners via different modes of cyclization (1, 30). This oxidative rearrangement hypothesis was first proposed by Scheuer for palau’amine (28) and recently modified by us (13), Al-Mourabit, and Romo (1) to account for its revised relative stereochemistry. The chemical viability of this skeletal rearrangement through two-electron oxidation has also been demonstrated by Romo, Lovely, and us (5, 12, 18, 19).

We were interested in using chemical synthesis to evaluate the Molinski-Romo and Scheuer biosynthetic hypotheses. The prior synthesis of sceptrin (**1a**) by Birman (20) and us (2, 4, 5) involved the construction of the cyclobutane ring by forming the C9/C10 and C9'/C10' bonds using a [2+2] photocycloaddition reaction. The synthesis described herein involves the alternative formation of the C9/C9' and C10/C10' bonds (biogenic connection) via a SET-promoted [2+2] cycloaddition reaction (Molinski-Romo hypothesis). The synthesis of massadine (**2a**) has also been achieved by us, using an intramolecular aldol reaction (8) or a Pauson-Khand reaction (10) to construct the central cyclopentane ring. We now describe a complementary synthesis that involves an oxidative rearrangement (Scheuer hypothesis) of a dibromoagelifेरin derivative (14, 15), using an oxidative radical cyclization reaction as the key step.

We first set out to develop an asymmetric synthesis of scep trin (**1a**) using a SET-mediated [2+2] cycloaddition reaction to construct its core skeleton (Fig. 2). L-Glutamic acid (**7**) was used as the source of chirality to establish the absolute stereochemistry of our synthetic **1a**. Diazotization of **7** induced an anchimeric lactonization to give (*S*)-(+)-5-oxo-2-tetrahydrofurancarboxylic acid (**8**), a commercially available chiral building block. Dihydrofuran **9** was then prepared from **8** through reduction, silylation, and dehydration. Electrophilic selenation of **9** proceeded from the less hindered face, allowing alcohol **10** to approach from the more hindered face of **9** with excellent stereoselectivity. Subsequent oxidative elimination of the phenylselenenyl group and reduction of the azido group provided **11** with *cis*-C8/C11 stereochemistry, setting the stage for the key [2+2] cycloaddition reaction.

Whereas treating **11** with a variety of single-electron oxidants (31) resulted in complete decomposition, the photoredox method developed by Yoon (32) promoted the reversible SET reactions of **11** smoothly. Irradiation of a solution of **11** with a catalytic amount of Ir(ppy)₃ induced the [2+2] cycloaddition to give **12** [diastereomeric ratio (d.r.) 1.8:1 at C10']. Although this cycloaddition reaction could also proceed through an energy transfer mechanism (33), 9-fluorenone, which has the same triplet energy ($E_T = 55$ kcal/mol) as Ir(ppy)₃ (34, 35), could not catalyze this reaction. Therefore, we believe that the [2+2] cycloaddition of **11** was induced by reversible SET between Ir(ppy)₃ and the aminoimidazole group of **11**.

To complete the synthesis of scep trin (**1a**), we used transthioketalization to reveal the cyclobutane core skeleton in **12**. The requisite all-trans configuration of the central cyclobutane core was furnished by epimerization of the C9 stereogenic center after protecting the hydroxyl groups and hydrolyzing the dithiane group of **13**. Subsequent reduction of the aldehyde group and deacetylation provided triol **14**. With the scep trin core skeleton in hand, we then functionalized the side chains to install the second aminoimidazole group and the two pyrrole groups. The N7 and N7' groups were introduced by sequential mesylation, iodination, and azidation. Subsequent desilylation gave bisazide **15**. The second aminoimidazole group was constructed by oxidizing the hydroxyl group of **15** and treating the resulting unstable mesyl aldehyde with Boc-guanidine. The azido groups of **16** were next reduced, and the pyrrole groups were introduced to give **17**. Removal of the protecting groups of **17** afforded scep trin (**1a**) (0.4% yield from L-glutamic acid).

Although we targeted the synthesis of scep trin in its presumed natural enantiomeric form, the circular dichroism (CD) spectrum of our synthetic scep trin (fig. S3) was the reverse of the natural samples' spectrum (4, 36). In our synthesis, the absolute stereochemistry of scep trin was established by the intramolecular [2+2] cycloaddition reaction of **11**. The structure of the resulting pseudo-symmetric product **12** was determined by nuclear magnetic resonance (NMR) experiments. The rigid, cage-like structure of **12** rendered NMR a reliable tool for assigning its relative stereochemistry. We thus suspected that the original absolute stereochemistry of scep trin was misassigned.

The absolute stereochemistry of scep trin was determined in 1981 by Faulkner and Clardy through x-ray crystallographic analysis (fig. S1) (22). Although the Hamilton test favored

ent-**1a**, the difference in the *R* factors was small (0.090 versus 0.094). In our previous synthetic study (4), a crystal of intermediate **18** was obtained from co-crystallization with (*S*)- α -methylbenzylamine. Although it supports the original assignment, the acid (**18**) used had only 75% enantiomeric excess. In light of our above-described findings, the ambiguity associated with enantio-impure sample of **18**, and the unconvincing x-ray data by modern standards, we recrystallized a natural sample of sceptrin and used anomalous dispersion to determine its absolute stereochemistry. A suitable crystal was obtained with a slowly evaporating (4°C) biphasic mixture of ethyl acetate and water (1:10). With a Flack factor of $-0.002(5)$ for **1a**, we concluded that the previously reported absolute stereochemistry of sceptrin was incorrect. Because the absolute stereochemistry of ageliferin was determined based on that of sceptrin (4), this result suggests that a revision is also needed for ageliferin.

Previously, we targeted the synthesis of ageliferin via **19** using L-serine as the source of chirality (14, 15). However, we obtained *ent*-ageliferin instead of the expected *nat*-ageliferin (figs. S4 and S5). In light of the revision of the absolute stereochemistry of sceptrin (**1a**), we reinvestigated the structural assignment of **19** (Fig. 3). Reduction of the azido and carbonyl groups of **19** followed by oxidation with dimethyldioxirane (DMDO) induced the Scheuer-type rearrangement (5, 12, 18, 19) to give **20**. The absolute and relative stereochemistry of **20** was determined by x-ray crystallographic analysis of its crystalline derivative **21**. The C9/C10/C11 relative stereochemistry of **21** is consistent with that of **19** instead of **22**, a structure that would be consistent with the original *ent*-ageliferin (fig. S5). The absolute stereochemistry of **21** determined by anomalous x-ray scattering is also consistent with the chirality of L-serine. These experiments provide independent evidence that the absolute stereochemistry of ageliferin also needs to be revised.

After completing the biomimetic synthesis of sceptrin (**1a**), we turned our attention to the asymmetric synthesis of massadine (**2a**). We chose to carry out the Scheuer-type oxidation on **23**, a protected 10'-oxo-dibromoageliferin, to best mimic the putative Scheuer biosynthetic pathway (Fig. 4). Although we could induce the ring-contraction reaction with various oxidative methods, the undesired spiro-configuration was always obtained (15). We envisioned that the C10' oxygen functionality of **23** could be used as a directing group (37) to reverse this stereo-selectivity. Indeed, reduction of **23** with LiBHET₃ gave **24** bearing the desired hydroxyl configuration. Oxidation of **24** with Ti(O^{*i*}Pr)₄/TBHP induced the Scheuer-type ring contraction to give iminohydantoin **25** with the desired spiro configuration. This Ti(IV)-promoted oxidation was markedly chemo- and stereoselective. It only oxidized the more electron-rich aminoimidazole group in the presence of an unprotected aminoimidazole and two dibromopyrroles. It was also the only method that could provide the correct spiro configuration. Although alcohol **24** was only stable as an acid salt, the Ti(O^{*i*}Pr)₄/TBHP-oxidation reaction failed completely under even slightly acidic conditions. Therefore, we washed **24**•TFA with base and used it immediately to give **25** with good reproducibility. Without purification, **25** was reduced with Ca(BH₄)₂ to afford hemiaminal **26**. Subsequent removal of the protecting groups gave "pre-massadine" (**6**).

To complete the synthesis of massadine (**2a**), we would need to selectively oxidize the aminoimidazole group of **6** and promote the intra-molecular cyclization to form the oxygen

bridge between the two cyclic guanidine groups of **2a**. Both the oxidation and cyclization steps were pH-sensitive. Oxidation of **6** under acidic conditions resulted in considerable decomposition because the reactivity of aminoimidazole toward oxidation was suppressed when protonated and competing oxidation of other functionalities occurred. Although oxidation of **6** under neutral or slightly basic conditions proceeded more smoothly, the undesired *N*-cyclization occurred spontaneously to give the axinellamine skeleton (**8**). Eventually, we found that oxidation of **6** with *N*-bromosuccinimide (NBS) proceeded smoothly and cleanly in methanol to give a diastereomeric mixture of massadine-methanol adducts (**11**). Upon treatment with aqueous HCl, this mixture of oxidation products slowly converted into massadine (**2a**) (0.014% yield from L-serine) and 3,7-*epi*-massadine (**27**) (**8**).

The CD spectrum of our synthetic **2a** suggested that we had obtained the natural enantiomer of massadine (fig. S6), confirming the correct assignment of its absolute stereochemistry by Fusetani (**23**) and us (**11**) previously. Because of our revision of the absolute stereochemistry of ageliferins (**3**), **2** and **3** have mismatched absolute stereochemistries. To verify this surprising result, we repeated the syntheses of **2a** and **3c**, using the same batch of common intermediate **23**. We confirmed that oxidation and deprotection of **23** gave *nat*-**2a**, whereas deoxygenation and deprotection gave *ent*-**3c**. These experiments provided direct evidence that natural massadine (**2a**) and natural dibromoageliferin (**3c**) are antipodal, and an enantio-divergent pathway must be involved in their biosynthesis.

Sceptrins (**1**), massadines (**2**), and ageliferins (**3**) were previously thought to be derived from a common biosynthetic intermediate and have matched absolute stereochemistries (**11**). We have now shown that massadine (**2a**) and dibromoageliferin (**3c**) are antipodal. Therefore, **2a** cannot be produced from a biogenic oxidation of **3c** or **5**. Considering that both enantiomeric forms of phakellin, a monomeric pyrrole-imidazole alkaloid, have been found in nature (**38**), it is possible that sponges also produce both enantiomers of **1–3**. However, literature reports suggest that all the cyclic pyrrole-imidazole dimers exist in only one enantiomeric form. Because both the sign and magnitude of the optical rotation of **1–3** change with the salt form (**4**, **26**), CD spectroscopy is currently the only reliable method to study their absolute stereochemistries. Ageliferins isolated from different species of sponges (two *Agelas*, one *Astrosclera*, and one *Stylissa* sponge) in the Caribbean and Pacific oceans all have matched CD spectra (table S1). Similarly, massadines, stylissadines, axinellamines, and donnazoles isolated from different species of sponges (one *Agelas*, two *Axinella*, and two *Stylissa* sponges) in the Caribbean, Pacific, Indian, and Southern oceans all show a positive exciton splitting centered at ~275 nm (table S1). The only two reported CD spectra of sceptrin are also consistent with one another (**4**, **36**). Currently, there is no evidence that both enantiomers of cyclic pyrrole-imidazole dimers exist in nature, although it has also been shown that enantiomeric natural products can arise from a single species or from different genera and/or species (**39**). Because massadine (**2a**) and dibromoageliferin (**3c**) are antipodal, they have to be produced by independent bio-synthetic pathways.

On the basis of our previous computational and experimental studies (**12**, **15**), we believe that sceptrin (**1a**) and ageliferin (**3a**) are derived from two hymenidin (**4b**, X=H) molecules whereas massadine (**2a**) is assembled from one oroidin (**4a**, X=H) and one dispacamide A (**4d**, X=OH) molecule (Fig. 1). The homodimerization (path a and b) and heterodimerization

(path c) of **4** are enantiomeric pathways. The pathway selectivity is determined by the stability of the radical intermediates. The redox-neutral, reversible SET-promoted heterodimerization of **4a** and **4d** would give “pre-massadines” (**6**) with a C9 aminal group that is present in all [3+2] pyrrole–imidazole dimers (fig. S2). Although enantiomeric biosynthesis that produces both enantiomers of a natural product has been reported (39), enantiodivergent biosynthesis that produces opposite enantiomers of natural products as congeners described herein is unknown. This discovery may serve as a new guiding principle for protein engineering and catalyst design.

Supplementary Material

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Acknowledgments

We thank M. Köck (Alfred Wegener Institute) for providing the natural samples of sceptrin and massadine, and J. Muñoz (Alfred Wegener Institute) for providing a copy of the CD spectrum of natural sceptrin•TFA. We also thank V. Lynch (University of Texas at Austin), J. Clardy (Harvard University), and T. Molinski (University of California, San Diego) for helpful discussions. The CD experiments were performed by H. Shi [University of Texas (UT) Southwestern]. Financial support was provided by NIH (NIGMS R01-GM079554 and R01-GM073949), the Welch Foundation (I-1596), and UT Southwestern. Metrical parameters for the structures of natural sceptrin (**1a**) and **21** are available free of charge from the Cambridge Crystallographic Data Centre under reference numbers CCDC-995387 (**1a**), 1014159 (**21**), and 1016848 (**21**).

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Fig. 1. The enantiodivergent biosynthetic pathways for the dimeric pyrrole-imidazole alkaloids
The biosyntheses of the [2+2] dimers sceptrins (**1**) and the [4+2] dimers ageliferrins (**3**) involve a homo-dimerization of oroidin (**4a**) or hymenidin (**4b**). SET oxidation of **4** promotes a [2+2] cycloaddition reaction of **4**^{•+}/4 to give **1**^{•+} (path a), or a [4+2] cycloaddition reaction to give **3**^{•+} (path b). Subsequent SET reduction yields **1** and **5** that aromatize to **3**. In contrast, the biosyntheses of the [3+2] dimers massadines (**2**) involve a heterodimerization of dispacamide A (**4d**) and oroidin (**4a**). SET oxidation of **4d** promotes a [3+2] cycloaddition reaction of **4d**^{•+}/**4a** to give **6**^{•+}. Subsequent SET reduction, protonation, and trapping with water or chloride provide “pre-massadines” (**6**). Oxidative cyclization of **6** gives massadines (**2**). The homodimerization (path a and b) and heterodimerization (path c) of **4** are enantiomeric pathways.

Fig. 2. Biomimetic synthesis of sceptrin and revision of its absolute stereochemistry

The key step involves a SET-mediated [2+2] cycloaddition reaction that mechanistically mimics the biogenic dimerization of hymenidin (**4b**). *ent*-Sceptrin was obtained while targeting the originally reported absolute stereochemistry. A new crystal structure of natural sceptrin (shown in ORTEP representation) confirms that its absolute stereochemistry needs to be revised. TIPS, triisopropylsilyl; DIBAL, diisobutylaluminium hydride; Ms, methanesulfonyl; BOM, benzyloxymethyl; CFL, compact fluorescent lamp; DMAP, 4-(dimethylamino)pyridine; Boc, *tert*-butoxycarbonyl; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, 1-hydroxybenzotriazole.

Fig. 3. Revision of the absolute stereochemistry of ageliferin (3a)

Oxidative ring-contraction of **19**, the key intermediate in our ageliferin synthesis, gave **20** that bears a massadine core skeleton. The crystal structure of its crystalline derivative **21** (shown in ORTEP representation) confirms that the absolute stereochemistry of ageliferin needs to be revised. DMDO, dimethyldioxirane; TFA, trifluoroacetic acid; Br-Bz, 4-bromobenzoyl.

Fig. 4. Asymmetric synthesis of massadine and evidence against the Scheuer biosynthetic hypothesis

Deoxygenation and deprotection of 10'-oxo-dibromoageliferin (**23**) gave *ent*-dibromoageliferin (*ent*-**3c**) (the unnatural enantiomer), whereas skeletal rearrangement and oxidation of **23** provided *nat*-massadine (**2a**) (the natural enantiomer). TBHP, *tert*-butyl hydroperoxide; NBS, *N*-bromosuccinimide.