

10-Step Asymmetric Total Synthesis and Stereochemical Elucidation of (+)-Dragmacidin D**

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In memory of Philip Joshua Chase Mabe

Abstract: The asymmetric synthesis of dragmacidin D (**1**) was completed in 10 steps. Its sole stereocenter was set by using direct asymmetric alkylation enabled by a C₂-symmetric tetramine and lithium *N*-(trimethylsilyl)-*tert*-butylamide as the enolization reagent. A central Larock indole synthesis was employed in a convergent assembly of the heterocyclic subunits. The stereochemical evidence from this work strongly supports the predicted *S* configuration at the 6''' position, which is consistent with other members of the dragmacidin family of natural products.

Dragmacidin D is a member of a family of heterocyclic bis(indole) natural products isolated from deep-water Caribbean sponges of the *Dragmacidon* and *Spongosorites* genera (Figure 1).^[1] Although the initially isolated sample displayed no optical activity,^[2] subsequent reisolation from a sponge specimen collected at 90 m depth along the coast of South Australia provided a sample of dragmacidin D with an $[\alpha]_D$ value of +12 (*c* 0.95, EtOH).^[3] These observations indicate a certain measure of ambiguity for the stereochemical identity of dragmacidin D and the configurational stability of its sole stereogenic center.^[1] Dragmacidin D, along with dragmacidin E, was found to be a potent inhibitor of the serine/threonine phosphatases PP1 and PP2A (PP₁, half maximal inhibitory concentration (IC₅₀) = 21.0 nM; PP_{2A}, IC₅₀ = 3.0 μM; PP_{2A}, IC₅₀ = 3.0 μM). Other biological activities reported for dragmacidins include antiviral, antibacterial, and antifungal activity, as well as in vitro cytotoxicity against P388 murine leukemia, A549 human lung, HCT-8 human colon, and MDAMB human mammary cancer cell lines, in addition to selective inhibition of neural nitric oxide synthase (bNOS) with EC₅₀ = 2.9 μM.

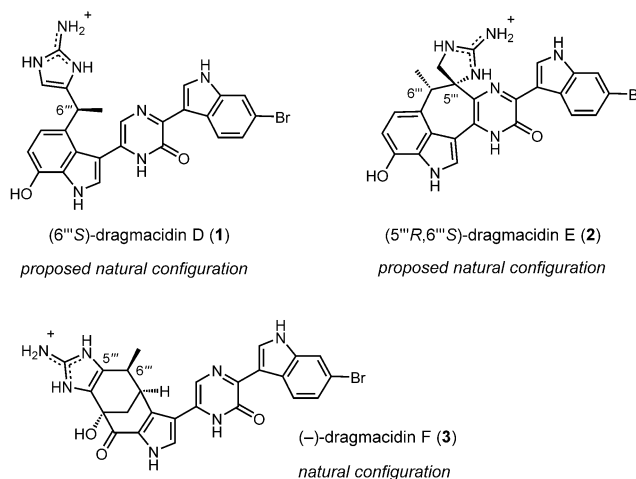


Figure 1. Structures of dragmacidins D, E, and F.

The distinctive structure of dragmacidin D combines a reactive central pyrazinone core with flanking indole substituents, one of which is further elaborated with an aminoimidazole unit bound by a stereogenic methine linker. In 2002, Stoltz and co-workers completed the first total synthesis of racemic dragmacidin D by effectively utilizing a series of sequential, temperature-controlled Suzuki cross-coupling reactions.^[4] The synthesis was completed in 17 steps, which reflect many intricacies at the late-stage installation of the polar aminoimidazole substituent. After completing the total synthesis of (-)-dragmacidin F (**3**) and thereby assigning its absolute configuration,^[5] the Stoltz group proposed the configuration of natural (+)-dragmacidin D and (-)-dragmacidin E to be 6'''*S* and 5'''*R*, 6'''*S*, respectively, postulating a common biogenesis.^[1] In 2011, Yamaguchi and Itami et al. reported the second total synthesis of (±)-**1**, which was completed in 12 steps by using a series of C–H cross-coupling reactions.^[6] This concise synthesis highlights the utility of C–H functionalization technology in complex total synthesis.^[7] Recently, a collaborative effort by the Jia and Capon groups culminated in the asymmetric total synthesis of (+)-**1** in 26 steps.^[8] This effort suggested a curious divergence in stereochemistry between dragmacidins D and F, and revises the stereochemistry of **1** to 6'''*R*. The authors noted that **1** has never been co-isolated with **3** but has been co-isolated with **2**, thus providing a plausible basis for this divergence, and reported that samples of **1** isolated by Capon and co-workers were either racemic or enantioenriched at 39% *ee*.

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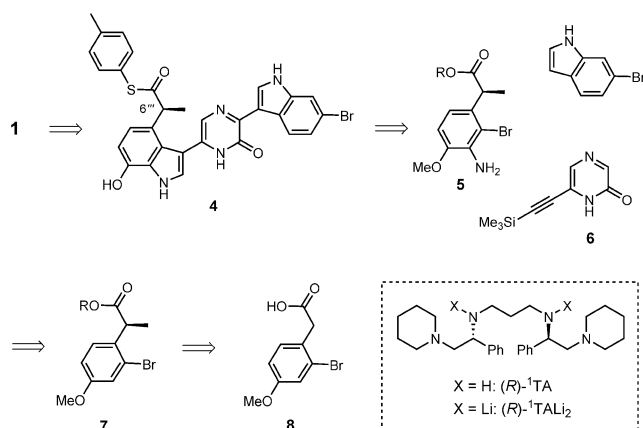
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Herein, we report a 10-step asymmetric total synthesis of (+)-dragmacidin D and provide what appears to be compelling evidence that its absolute configuration is indeed 6'''S, as originally forecasted by Stoltz, and is thus uniform with that of (–)-dragmacidin F. This short 10-step synthesis is enabled by direct early-stage enantioselective alkylation of commercially available 4-methoxy-2-bromophenylacetic acid, in an extension of the methodology recently developed in our laboratory.^[9]

The final synthesis plan that unlocked the path to success is outlined in Scheme 1. A concise elaboration of the thioester

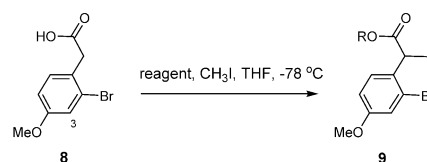


Scheme 1. Synthesis plan for dragmacidin D (**1**).

to the aminoimidazole was projected for the final operations of the synthesis.^[10] In contrast to all previous efforts that engaged a preassembled 7''-hydroxyindole found in **1**, we opted for the construction of the indole ring system by a Larock indole synthesis,^[11] thereby introducing a point of convergence in the synthesis plan. This transformation was to be followed by a Friedel–Crafts-type direct arylation with 6-bromoindole under acidic conditions, which was also utilized in the Itami/Yamaguchi synthesis.^[6] Bromoaniline **5** for the Larock indole synthesis was to be produced from precursor **7**, with 4-methoxy-2-bromoacetic acid **8** identified as a straightforward starting material for its preparation by our direct alkylation method with the readily available tetramine (*R*)-**1**TA as the stereodirecting reagent (Scheme 1).^[9] One of the challenging objectives was preservation of the stereogenic center in **7** through the remaining operations of the synthesis.

Direct α -alkylation of carboxylic acids occurs via dianionic enediolates as reactive intermediates. Our initial studies showed that **8** is a challenging substrate for this reaction. Attempts to obtain the α -methylation product with CH_3I and lithium diisopropylamide (LDA) or *n*BuLi as the enolization reagents only led to decomposition of the starting material (Table 1, entries 1 and 2).^[9a] Clean methylation was observed with $\text{LiN}(\text{SiMe}_3)_2$ (entry 3). We postulated that decomposition with the more basic reagents was due to competitive lithiation of the arene C–H bond of **8** at the C3 position to form benzyne species. This problem could be solved through careful choice of a base that would prevent the arene lithiation and yet be potent enough to be compatible

Table 1: Development of the direct stereoselective α -methylation of **8**.

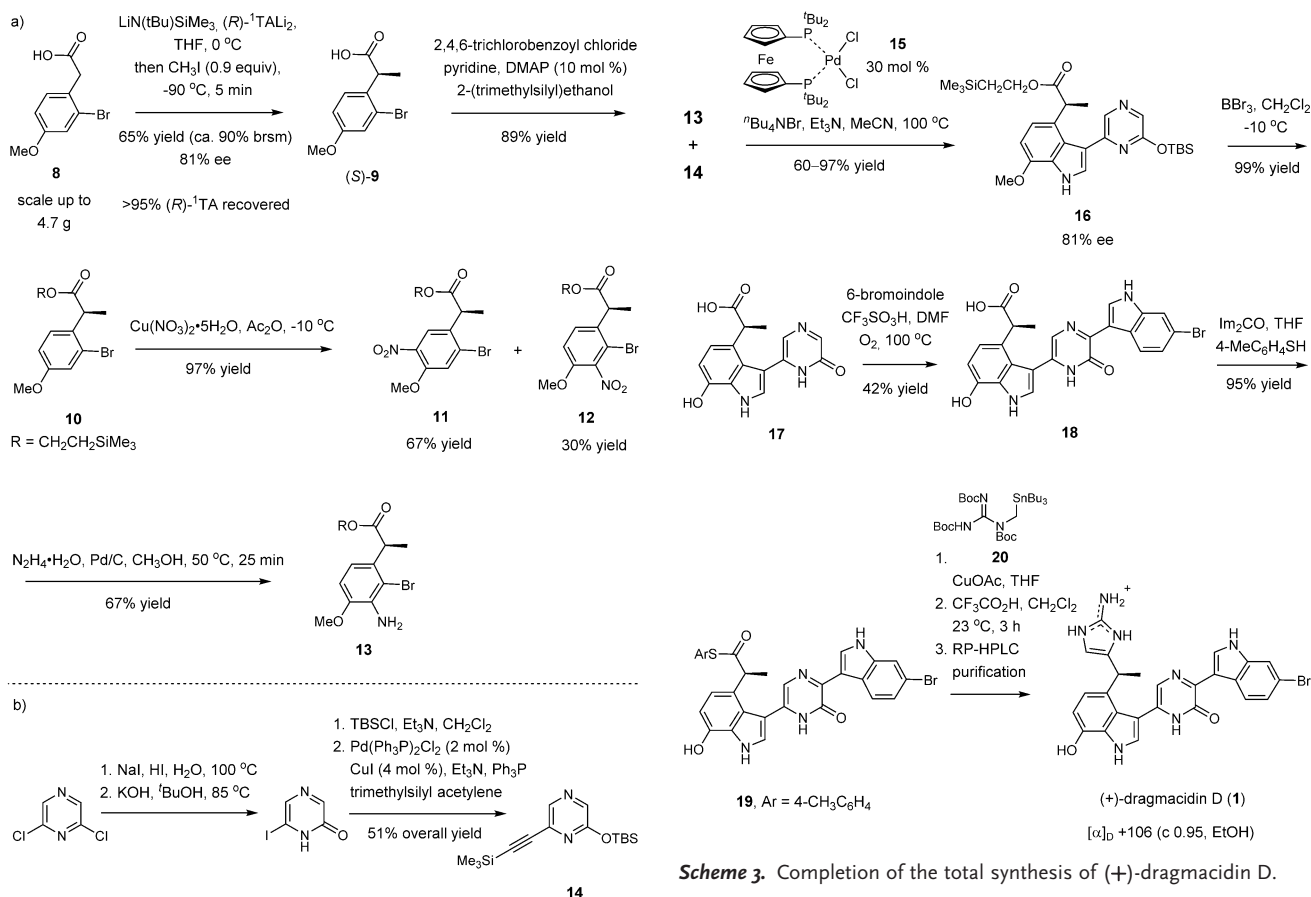


Entry	Reagent	Result
1	<i>n</i> BuLi	Complete decomposition
2	LDA	Complete decomposition
3	$\text{LiN}(\text{SiMe}_3)_2$	78 % conversion, clean
4	$\text{LiN}(\text{SiMe}_3)_2 + (R)\text{-}^1\text{TALi}_2$	67 % yield, 0 % ee
5	$\text{LiN}(t\text{Bu})\text{SiMe}_3$	99 % conversion, clean
6	$\text{LiN}(t\text{Bu})\text{SiMe}_3 + (R)\text{-}^1\text{TALi}_2$	65 % yield, 81 % ee

with the asymmetric alkylation protocol. Indeed, after enolization with $\text{LiN}(\text{SiMe}_3)_2$, alkylation with (*R*)-**1**TALi₂ resulted in racemic **9** (entry 4). It is likely that, after enolization, the higher acidity of $(\text{Me}_3\text{Si})_2\text{NH}$ ($\text{p}K_a = 26$) led to protonation of (*R*)-**1**TALi₂ to give (*R*)-**1**TA. Intact lithium amide (*R*)-**1**TALi₂ is a critical part of the chiral aggregate for stereoselective alkylation.^[9b] Investigation of various readily available amines drew our attention to *t*Bu(Me_3Si)NH, which in our assessment struck the right balance between steric bulk to prevent C3 lithiation, and basicity ($\text{p}K_a = 33$ for *t*BuNH– SiMe_3 ; $\text{p}K_a = 37$ for *i*Pr₂NH).^[12] A preliminary experiment supported this assessment (entry 5). We were delighted to discover that $\text{LiN}(t\text{Bu})\text{SiMe}_3$ was an excellent choice, affording product **9** in 65 % yield and 81 % ee (entry 6).

The synthesis of (+)-**1** began with direct asymmetric alkylation of **8** with 0.9 equiv of iodomethane mediated by (*R*)-**1**TA on scales up to 4.7 g, which afforded (*S*)-**9** in 65 % yield and 81 % ee (Scheme 2a). We found that excess iodomethane was detrimental to enantioselectivity. Esterification of (*S*)-**9** with 2-(trimethylsilyl)ethanol was accomplished under Yamaguchi conditions in high yield with no racemization. Nitration of **10** was best achieved with $\text{Cu}(\text{NO}_3)_2 \cdot 5\text{H}_2\text{O}$ in Ac_2O at -10°C ^[13] to provide a mixture of nitration products **11** and **12** in 67 % and 30 % yield, respectively. The temperature of the reaction mixture had to be maintained at or below -10°C to avoid racemization. At this junction, we opted against further optimization of regioselectivity in favor of advancing the synthesis, given that multigram quantities of **12** could be produced in a concise fashion from **8** in 81 % ee. Reduction of the nitro group to aniline was accomplished by treating **12** with $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$ and Pd/C in CH_3OH at 50°C . Careful control of temperature and N_2H_4 stoichiometry was necessary to minimize overreduction. In this manner, **13** was obtained in 67 % yield.^[14]

The alkynyl pyrazine precursor **14** was prepared in 4 steps from 2,6-dichloropyrazine (Scheme 2b).^[15] The central heteroannulation between **13** and **14** with the [1,1'-bis(di-*tert*-butylphosphino)ferrocene]PdCl₂ catalyst **15**^[11] afforded the requisite 2,3,4,7-tetrasubstituted indole **16** in good yields and with no erosion of enantiomeric excess (Scheme 3). Yields were variable but the best results were obtained with freshly prepared substrates and freshly purified solvents. A dramatic improvement in reaction yield and reproducibility was



Scheme 2. Synthesis of precursors **13** and **14** for Larock indole synthesis.

observed upon the addition of tetra-*n*-butylammonium bromide (TBAB) to prevent the formation of Pd black. A variant of the indole synthesis with an alkyne reagent analogous to **14** but bearing a preinstalled 6-bromoindole substituent was unproductive.

The indole synthesis was followed by simultaneous cleavage of the 2-(trimethylsilyl)ethyl ester, phenolic methyl ether, and pyrazine silyl ether upon exposure to BBr₃ in dichloromethane at −10 °C for 1 h.^[4] The resulting carboxylic acid was isolated by reverse-phase column chromatography in a nearly quantitative yield. Since we could not identify a procedure to determine its enantiomeric excess, the material was advanced further. Friedel–Crafts-type arylation with 6-bromoindole was achieved in the presence of CF₃SO₃H in DMF at 100 °C under an atmosphere of oxygen to deliver bis(indole) carboxylic acid **18** in 42% yield.^[6] Thioester **19** was produced in high yield with carbonyldiimidazole and 4-methylphenylthiol in tetrahydrofuran (THF). Again, we were unable to determine conditions to measure the *ee* of this compound. Therefore, the total synthesis was completed with two additional steps: 1) CuOAc-mediated acyl cross-coupling of thioester **19** with stannane **20**, which bears a guanidinyldimethyl ketone under acidic conditions with CF₃CO₂H in CH₂Cl₂ at 23 °C for 3 h. After purification by reverse-phase

Scheme 3. Completion of the total synthesis of (+)-drarmacidin D.

preparative HPLC, 15 mg of the trifluoroacetic acid (TFA) salt of synthetic drarmacidin D was isolated as a brownish red foam (44% overall yield from thioester **19**).

With **1** now available in sufficient supply (as well as the racemic sample prepared analogously), we were able to identify an effective chiral-phase HPLC method to measure its enantiomeric excess, which turned out to be 61% (Figure 2). Clearly there had been some erosion of *ee* between pyrazine-indole intermediate **16** and **1**. The most likely origins of the erosion in our assessment are either the multiple-group cleavage reaction with BBr₃, the Friedel–Crafts indolization, or, less likely, the final aminoimidazole formation with TFA. The potential instability of (+)-**1** to racemization could also be an issue (see below).

In light of uncertainties regarding the stereochemistry of **1**, as discussed in the literature, we measured the rate of racemization of a solution of (+)-drarmacidin D in water (Fisher Scientific W5-4, HPLC grade, pH 6.8, 1 mg mL^{−1}). The results reveal that **1** (61% *ee*) undergoes slow but steady epimerization, reaching 33% *ee* after 4 days and 4% *ee* in approximately 16 days (Figure 2). Notably, the (+)-drarmacidin D TFA salt is configurationally stable upon storage at −20 °C as a mixture with benzene for at least 40 days with no change in enantiomeric excess. When aqueous **1** was exposed to light at 23 °C, rapid decomposition occurred.

Perhaps more intriguingly, our work provides evidence for the absolute configuration of (+)-drarmacidin D that appears to contradict the recent results of the Jia, Capon, and co-

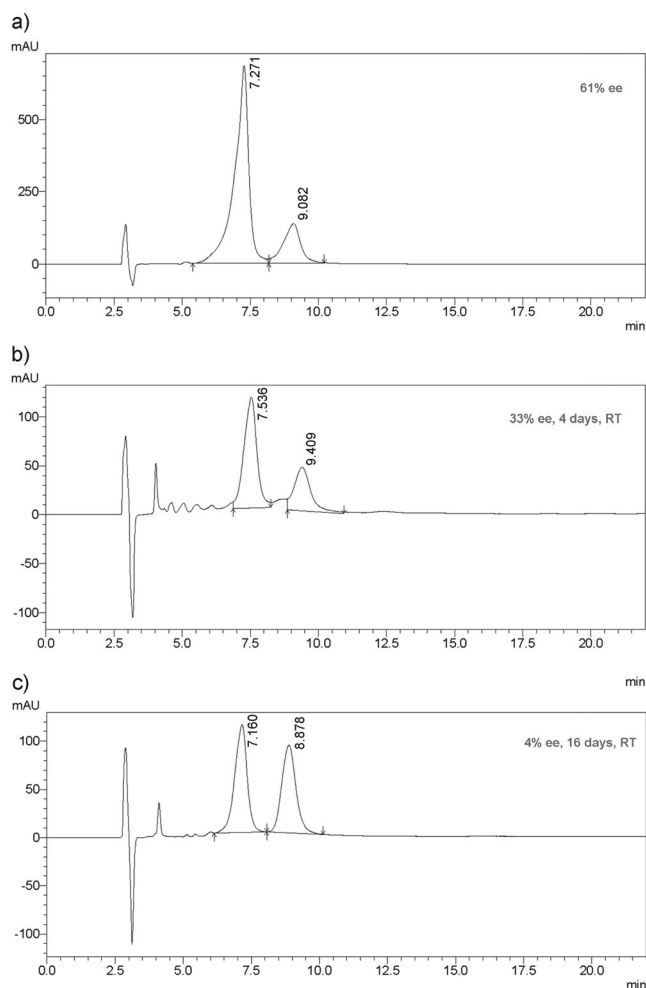
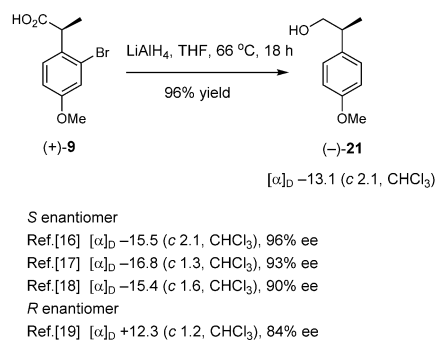


Figure 2. Enantiomeric excess of (+)-dragmacidin D trifluoroacetate solution in water at pH 6.8, as measured by chiral-phase HPLC. a) freshly prepared synthetic (+)-1, 61 % ee. b) after 4 days at 23 °C, 33 % ee. c) after 16 days, 4 % ee.

workers.^[8] First, the specific rotation of our sample at 61 % ee ($[\alpha]_D +106^\circ$ (c 0.95, EtOH); $[\alpha]_D +95^\circ$ (c 0.10, EtOH)) is notably higher at both concentrations than reported previously ($[\alpha]_D +12^\circ$ (c 0.95, EtOH) at 39 % ee; ($[\alpha]_D +18^\circ$ (c 0.10, EtOH), ee not reported)).^[8] Importantly, the precision of our enantiomeric excess measurement is supported by clear baseline separation in the HPLC traces (Figure 2). Second, the absolute configuration of (+)-1 has been recently reassigned based on total synthesis to 6'''R,^[8] in contrast to the biosynthetic prediction by Stoltz and co-workers.^[1] The present work, however, clearly supports the 6'''S configuration, which is consistent with the known configuration of natural dragmacidin F. The evidence comes from correlation of the reduction product of carboxylic acid (+)-9, which was used as an intermediate in the total synthesis of (+)-1 reported herein, to the well-characterized alcohol (–)-21 (Scheme 4).^[16–20]

In summary, we have completed a 10-step asymmetric total synthesis of the marine alkaloid dragmacidin D (1). Key transformations include: 1) a direct asymmetric methylation of carboxylic acid 8 with CH₃I mediated by the reagent (R)-



Scheme 4. Confirmation of the absolute stereochemistry of (+)-9.

TA; 2) a Larock indole assembly at the convergence point of the total synthesis; and 3) a concise conversion of a thioester into an aminoimidazole at the concluding stage of the synthesis. As a result, 15 mg of (+)-dragmacidin D were produced in 61 % ee, thus supporting the assignment of its sole stereogenic center at carbon 6''' as S. This result is in line with the original prediction by Stoltz and consistent with the absolute stereochemistry of dragmacidin F but contrasts with recent results by Jia, Capon, and co-workers. Additional studies revealed that dragmacidin D in solution in water at room temperature undergoes racemization within about 16 days and decomposes rapidly when exposed to light at room temperature. However, (+)-1 is chemically and configurationally stable at –20 °C in the dark. Collectively, these observations provide an interesting context for the existence of this natural product in oceanic environments at high depth.

Keywords: asymmetric synthesis · dragmacidin · heterocycles · indoles · total synthesis

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