Asymmetric Synthesis

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

*Jeffrey J. Jackson, Hiroyuki Kobayashi, Sophia D. Steffens, and Armen Zakarian**

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 C_2-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 (PP_1, half maximal inhibitory concentration (IC_{50}) = 21.0 nM; PP_2A_1, IC_{50} = 3.0 μM; PP_2A_2, IC_{50} = 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 EC_{50} = 2.9 μM.

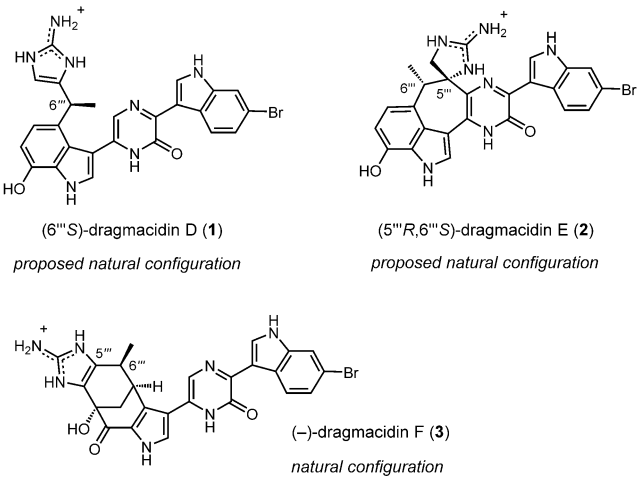

Figure 1. Structures of dragmacidins D, E, and F.

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*.

[*] J. J. Jackson, S. D. Steffens, Prof. Dr. A. Zakarian
Department of Chemistry and Biochemistry
University of California, Santa Barbara, CA 93106-9510 (USA)
E-mail: zakarian@chem.ucsb.edu
Dr. H. Kobayashi
Daiichi Sankyo Co., LTD
1-2-58, Hiromachi, Shinagawa-ku, Tokyo 140-8710 (Japan)

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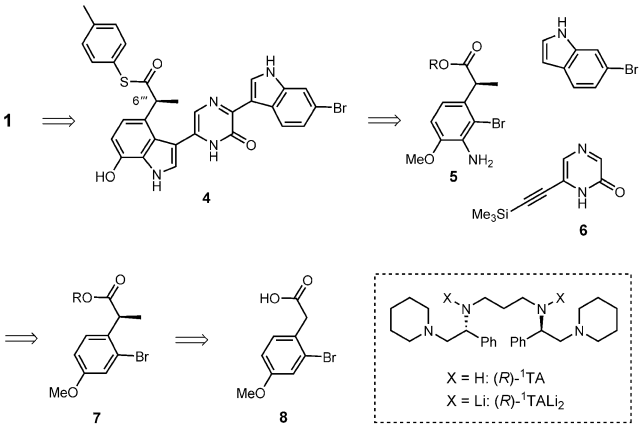
Herein, we report a 10-step asymmetric total synthesis of (+)-dragmacidin D and provide what appears to be compelling evidence that its absolute configuration is indeed 6‴*S*, as originally forecasted by Stoltz, and is thus uniform with that of (−)-dragmacidin F. This short 10-step synthesis is enabled 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

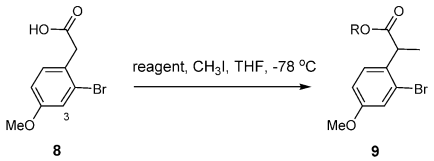


Scheme 1. Synthesis plan for dragmacidin D (**1**).

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 $LiN(SiMe_3)_2$ (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**.

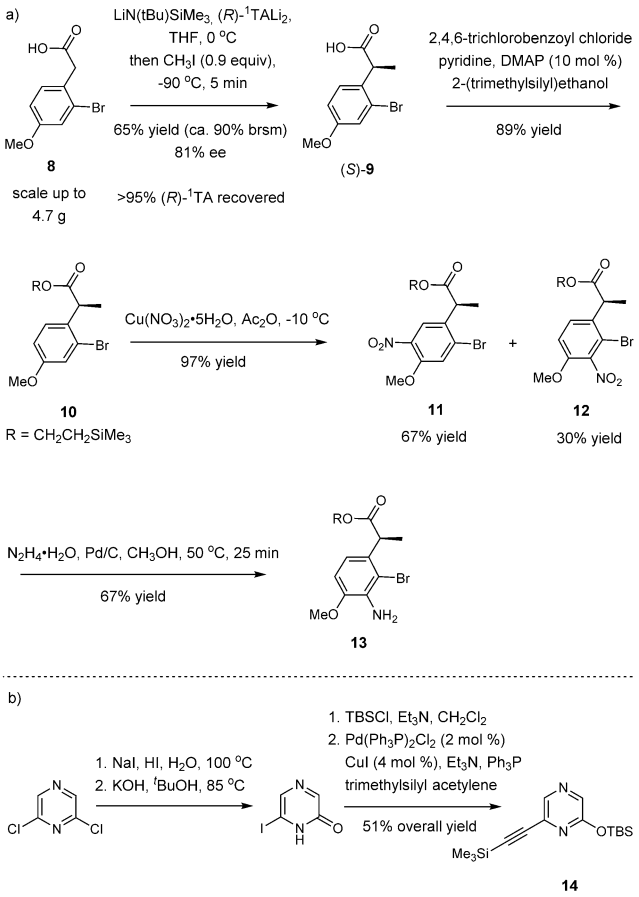


Entry	Reagent	Result
1	*n*BuLi	Complete decomposition
2	LDA	Complete decomposition
3	$LiN(SiMe_3)_2$	78% conversion, clean
4	$LiN(SiMe_3)_2$ + (*R*)-^{1}TALi$_2$	67% yield, 0% ee
5	$LiN(tBu)SiMe_3$	99% conversion, clean
6	$LiN(tBu)SiMe_3$ + (*R*)-^{1}TALi$_2$	**65% yield, 81% ee**

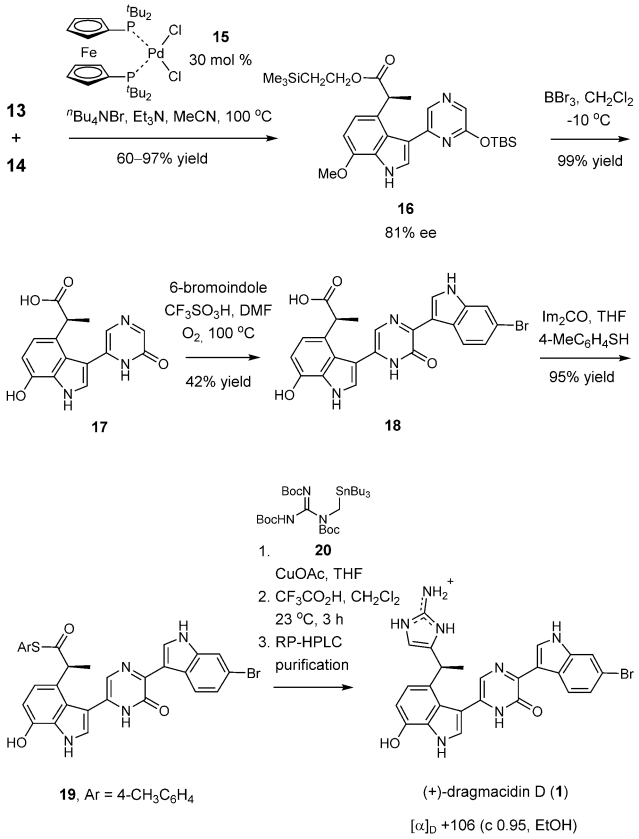
with the asymmetric alkylation protocol. Indeed, after enolization with $LiN(SiMe_3)_2$, alkylation with (*R*)-^{1}TALi$_2$ resulted in racemic **9** (entry 4). It is likely that, after enolization, the higher acidity of $(Me_3Si)_2NH$ ($pK_a = 26$) led to protonation of (*R*)-^{1}TALi$_2$ to give (*R*)-^{1}TA. Intact lithium amide (*R*)-^{1}TALi$_2$ is a critical part of the chiral aggregate for stereoselective alkylation.[9b] Investigation of various readily available amines drew our attention to $tBu(Me_3Si)NH$, which in our assessment struck the right balance between steric bulk to prevent C3 lithiation, and basicity ($pK_a = 33$ for *t*BuNH-$SiMe_3$; $pK_a = 37$ for iPr_2NH).[12] A preliminary experiment supported this assessment (entry 5). We were delighted to discover that $LiN(tBu)SiMe_3$ 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 $Cu(NO_3)_2 \cdot 5H_2O$ 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 $N_2H_4 \cdot H_2O$ 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]$PdCl_2$ 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.



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

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 BBr_3 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 CF_3SO_3H 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 guanidinyl substituent, and 2) cyclocondensation of the resulting guanidinylmethyl ketone under acidic conditions with CF_3CO_2H in CH_2Cl_2 at 23 °C for 3 h. After purification by reverse-phase preparative HPLC, 15 mg of the trifluoroacetic acid (TFA) salt of synthetic dragmacidin 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 BBr_3, 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 (+)-dragmacidin 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 (+)-dragmacidin 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 (+)-dragmacidin D that appears to contradict the recent results of the Jia, Capon, and co-

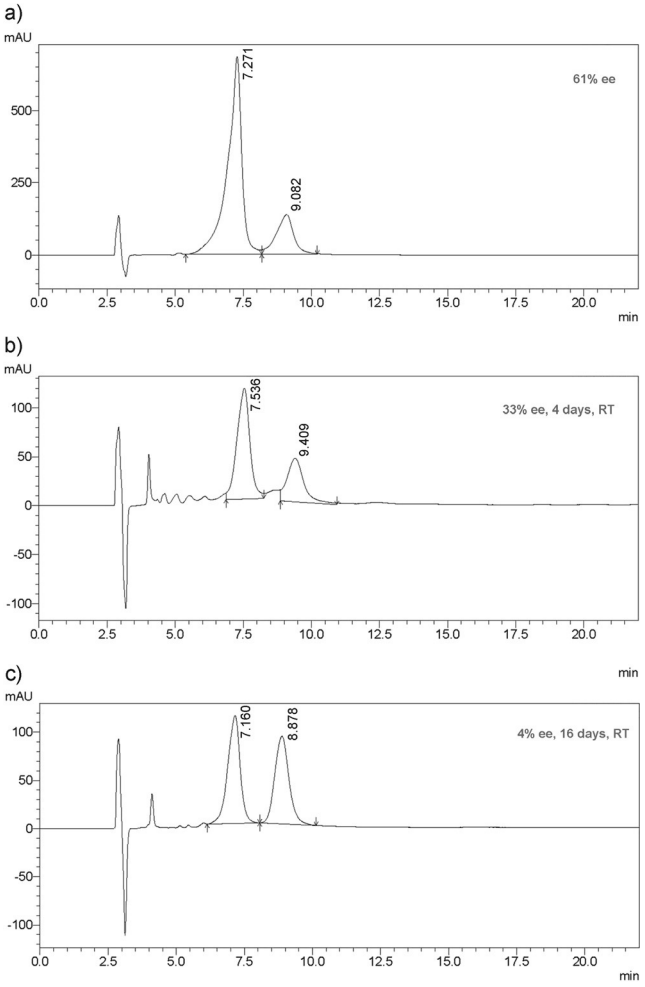
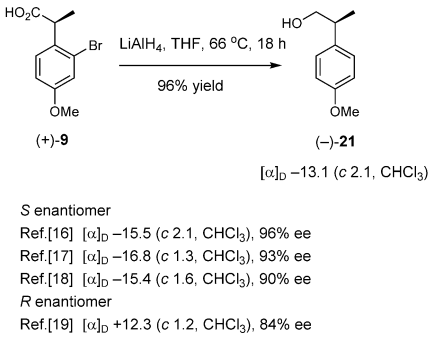


Figure 2. Enantiomeric excess of (+)-dragmacidin D trifluoroacetate solution in water at pH 6.8, as measured by chiral-phase HPLC. a) freshly prepared synthetic (+)-**1**, 61 % *ee*. b) after 4 days at 23 °C, 33 % *ee*. c) after 16 days, 4 % *ee*.

workers.[8] First, the specific rotation of our sample at 61 % *ee* ($[\alpha]_D$ +106° (*c* 0.95, EtOH); $[\alpha]_D$ +95° (*c* 0.10, EtOH) is notably higher at both concentrations than reported previously ($[\alpha]_D$ +12° (*c* 0.95, EtOH) at 39 % ee; ($[\alpha]_D$ +18° (*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 CH_3I mediated by the reagent (*R*)-



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

[1]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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