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Authors

Jackson, Jeffrey J
Kobayashi, Hiroyuki
Steffens, Sophia D
et al.

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10 Step Asymmetric Total Synthesis and Stereochemistry of (+)-Dragmacidin D

Jeffrey J. Jackson[a], **Hiroyuki Kobayashi**[b], **Sophia D. Steffens**[a], and **Armen Zakarian**[a]

[a]Department of Chemistry and Biochemistry, University of California, Santa Barbara, CA 93106-9510 zakarian@chem.ucsb.edu

[b]Daiichi Sankyo Co., LTD, 1-2-58, Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan, kobayashi.hiroyuki.ef@daiichisankyo.co.jp

Abstract

The asymmetric synthesis of dragmacidin D (**1**) has been completed in 10 steps. Its sole stereocenter was set 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 heterocyclic subunits. The stereochemical evidence from this work strongly supports the predicted *S* configuration at 6‴ position consistent with other members of the dragmacidin family of natural products.

Keywords

indoles; asymmetric synthesis; dragmacidin; heterocycles; total synthesis

Dragmacidin D is a member of a family of heterocyclic bis(indole) natural products isolated from deep-water Caribbean sponges of *Dragmacidon* and *Spongosorites* sp. (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 $[\alpha]_D$ of +12 (*c* 0.95, EtOH). [3] These observations indicate a certain measure of ambiguity for the stereochemical identity of dragmacidin D and configurational stability of its sole stereogenic center. [1] Dragmacidin D, along with dragmacidin E, was found to be a potent inhibitor of serine-threonine phosphatases PP1 and PP2A (PP_1, IC_{50} = 21.0 nM; PP_2A_1, IC_{50} = 3.0 μM; PP_2A_2, IC_{50} = 3.0 μM). Other biological activity reported for dragmacidins include antiviral, antibacterial, antifungal, and *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.

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

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Correspondence to: Armen Zakarian.

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total synthesis of racemic dragmacidin D effectively utilizing a series of sequential, temperature-controlled Suzuki cross-coupling reactions. [4] The synthesis was completed in 17 steps, revealing 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 their common biogenesis.[1] In 2011, Yamaguchi and Itami et al. reported the second total synthesis of (±)-**1** completed in 12 steps 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, revising 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**, 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.

Here 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 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, extending methodology recently developed in our laboratory.[9]

The final synthesis plan that unlocked a path to success is outlined in Scheme 1. A concise elaboration of thioester to 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 convergency in the synthesis plan. This transformation was to be followed by Friedel-Crafts-type direct arylation with 6-bromoindole under acidic conditions, also utilized in the Itami/Yamaguchi synthesis.6 Bromoaniline **5** for the Larock indole synthesis was to arise from precursor **7**, identifying 4-methoxy-2-bromoacetic acid **8** as a straightforward starting material for its preparation by our direct alkylation method with the readily available tetramine (*R*)-**1TA** as the stereodirecting reagent (Scheme 1).[9] One of the challenging objectives was the preservation of the stereogenic center in **7** through the remaining operation 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 LDA or *n*-BuLi as the enolization reagents only led to decomposition of the starting material (Table 1, entries 1, 2).[9a] A clean methylation was observed with $LiN(SiMe_3)_2$ (entry 3). We postulated decomposition with the more basic reagents was due to competitive lithiation of the arene C—H bond of **8** at the C3 position forming benzyne species. This problem could be solved by a careful choice of base to prevent the arene lithiation and yet potent enough to be compatible 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*)-**TALi$_2$** to (*R*)-**^{1}TA**. Intact lithium amide (*R*)-**TALi$_2$** 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 stroke the right balance between steric bulk to prevent C3 lithiation and basicity (pK_a = 33 for *t*-BuNHSiMe$_3$; pK_a = 37 for *i*-Pr$_2$NH).[12] A preliminary experiment supported this assessment (entry 5). We were delighted to discover that LiN(^{t}Bu)SiMe$_3$ was an excellent choice, affording product **9** in 65% yield and 81% ee (entry 6).

The synthesis of (+)-**1** began with asymmetric direct alkylation of **8** with 0.9 equiv of iodomethane mediated by (*R*)-**^{1}TA** on scales up to 4.7 g, affording (*S*)-**9** in 65% yield and 81% ee (Scheme 2a). We found that excess of 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 $Cu(NO_3)_2$•$5H_2O$ in Ac_2O at −10 °C,[13] providing 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 treatment of **12** with N_2H_4•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 as illustrated in Scheme 2b.[15] The central heteroannulation between **13** and **14** using 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 observed upon the addition of tetra-*n*-butylammonium bromide (TBAB) to prevent Pd black formation. A variant of the indole synthesis with an alkyne reagent analogous to **14** bearing a preinstalled 6-bromoindole substituent was unproductive.

The indole synthesis was followed by a 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 success, 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, delivering bis(indole) carboxylic acid **18** in 42% yield.[6] Thioester **19** was produced in high yield with carbonyldiimidazole and 4-methylphenylthiol in THF. Again, we were unable to determine conditions to measure the ee of this compound. Therefore, the total synthesis was completed in two additional steps that included 1) CuOAc-mediated acyl cross-coupling of thioester **19** with stannane **20** bearing a guanidinyl substituent, and 2) cyclocondensation of the resulting α-guanidinyl methyl ketone under acidic condition 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 TFA salt of synthetic dragmacidin D were 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 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 using TFA. Potential instability of (+)-**1** to racemization could also be an issue (*vide infra*).

In light of uncertainties regarding the stereochemistry of **1** 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). The results reveal that **1** (61% ee) undergoes slow but steady epimerization, reaching 33% ee after 4 days and 4% ee in ~16 days (Figure 2). Notably, (+)-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 Jia, Capon and co-workers.8 First, the value of 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 the 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, consistent with the known configuration of natural dragmacidin F. The evidence comes from correlation of the reduction product of carboxylic acid (+)-**9** used as an intermediate in the total synthesis of (+)-**1** reported herein, to well-characterized alcohol (–)-**21** (Scheme 4).[16,17,18,19,20]

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 reagent (*R*)-1**TA**; 2) a Larock indole assembly at the convergence point of the total synthesis; and 3) a concise conversion of thioester to aminoimidazole at the concluding stage of the synthesis. As a result, 15 mg of (+)-dragmacidin D were produced in 61% ee, supporting the assignment of its sole stereogenic center at carbon 6‴ as *S*, in line with the original prediction by Stoltz and consistent with absolute stereochemistry of dragmacidin F, but in contrast to the 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 a curious context for the existence of the natural product in oceanic environments at high depth.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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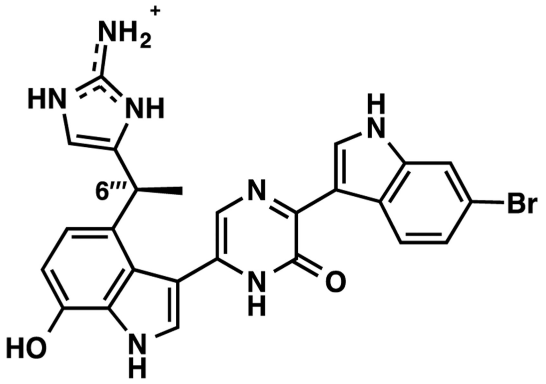
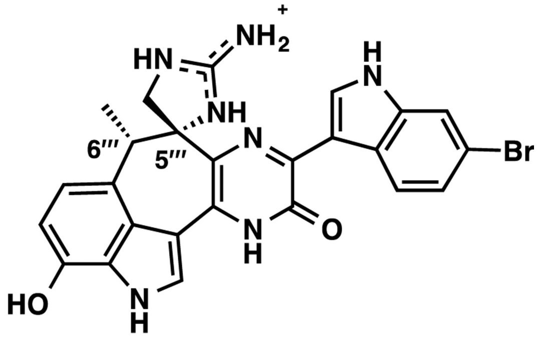


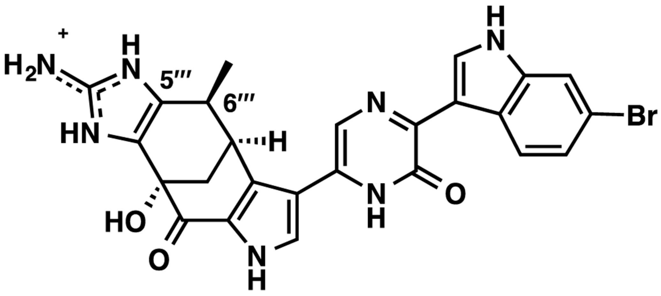
(6'''*S*)-dragmacidin D (**1**)

proposed natural configuration



(5'''*R*,6'''*S*)-dragmacidin E (**2**)

proposed natural configuration



(–)-dragmacidin F (**3**)

natural configuration

Figure 1.

Structures of dragmacidins D, E, and F.

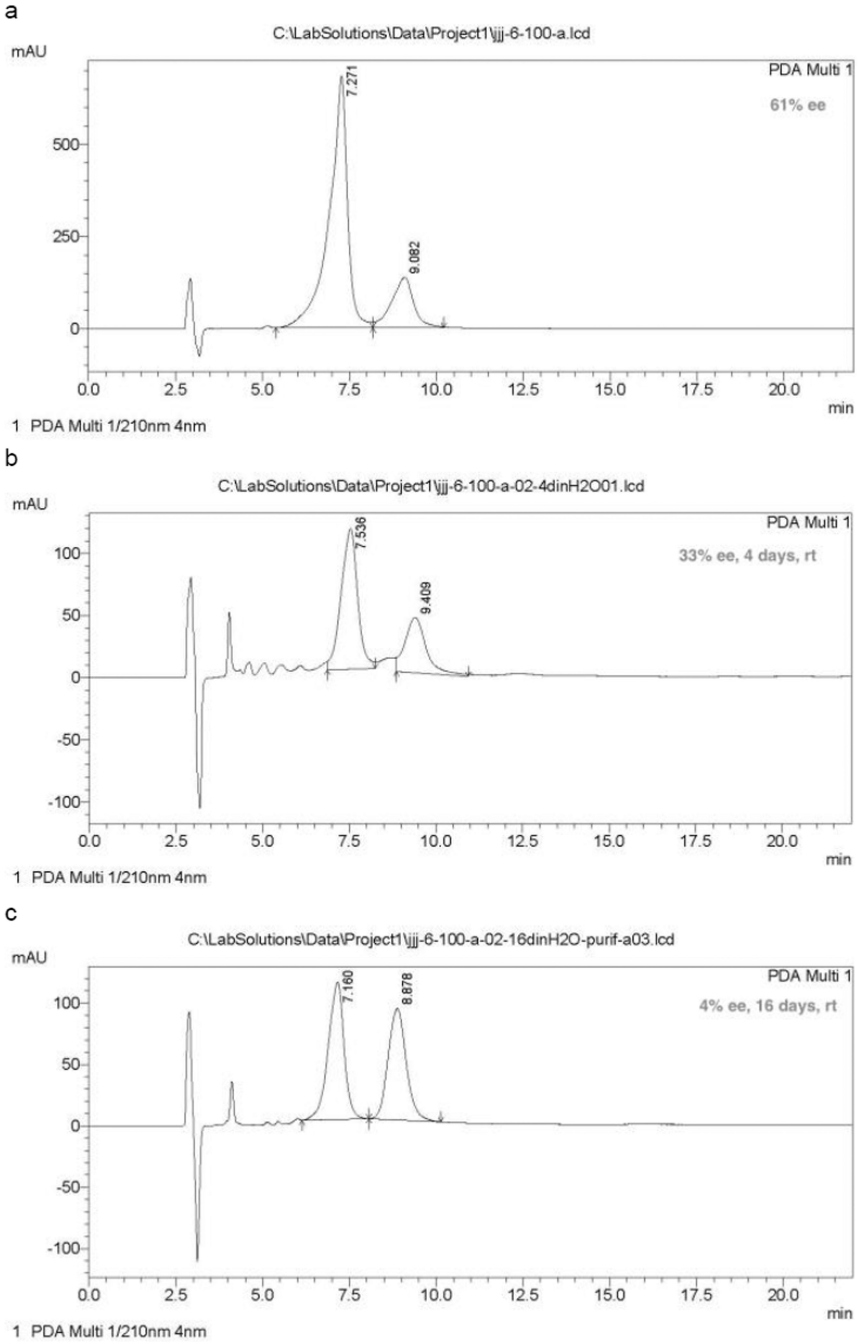
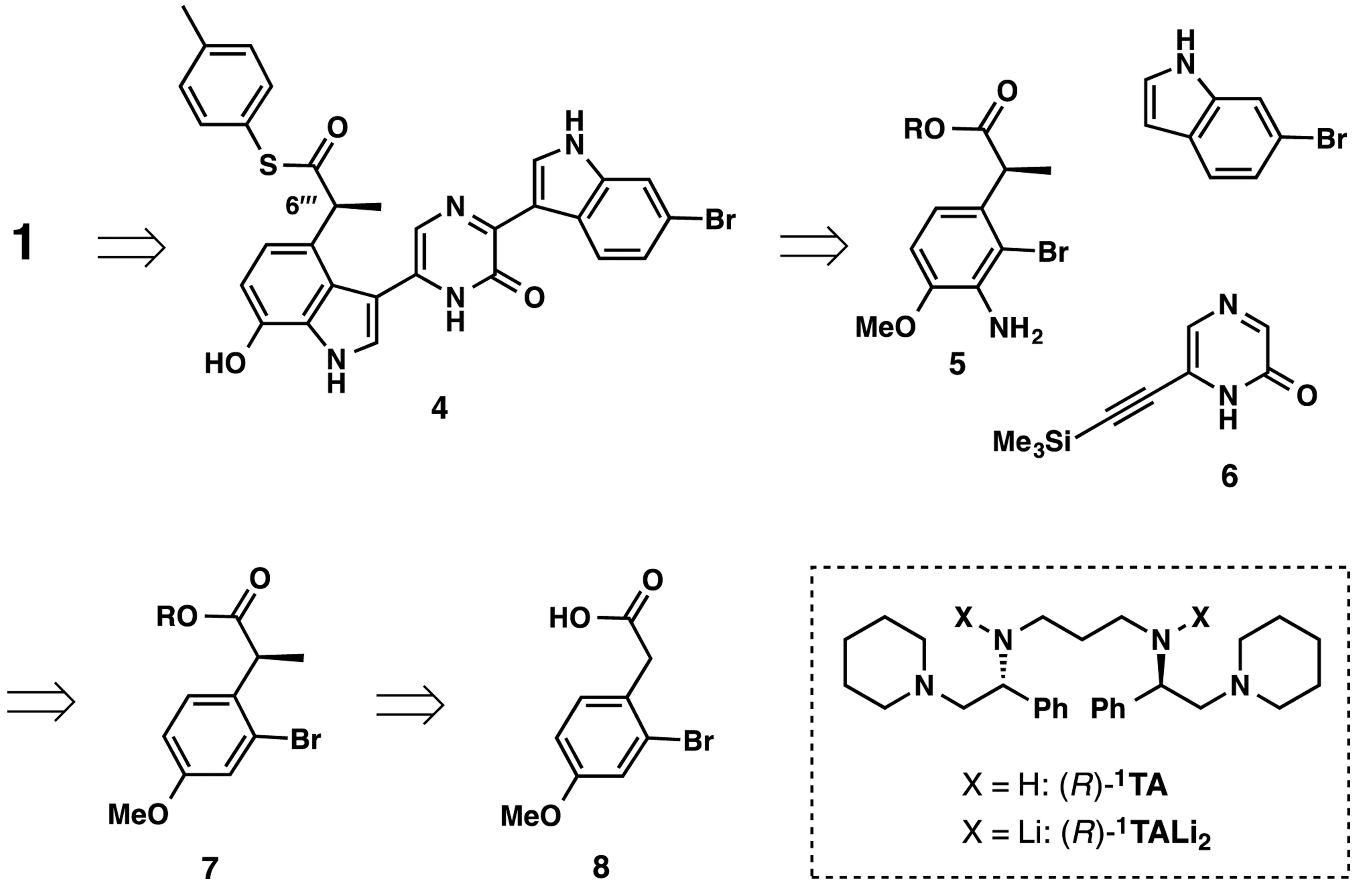


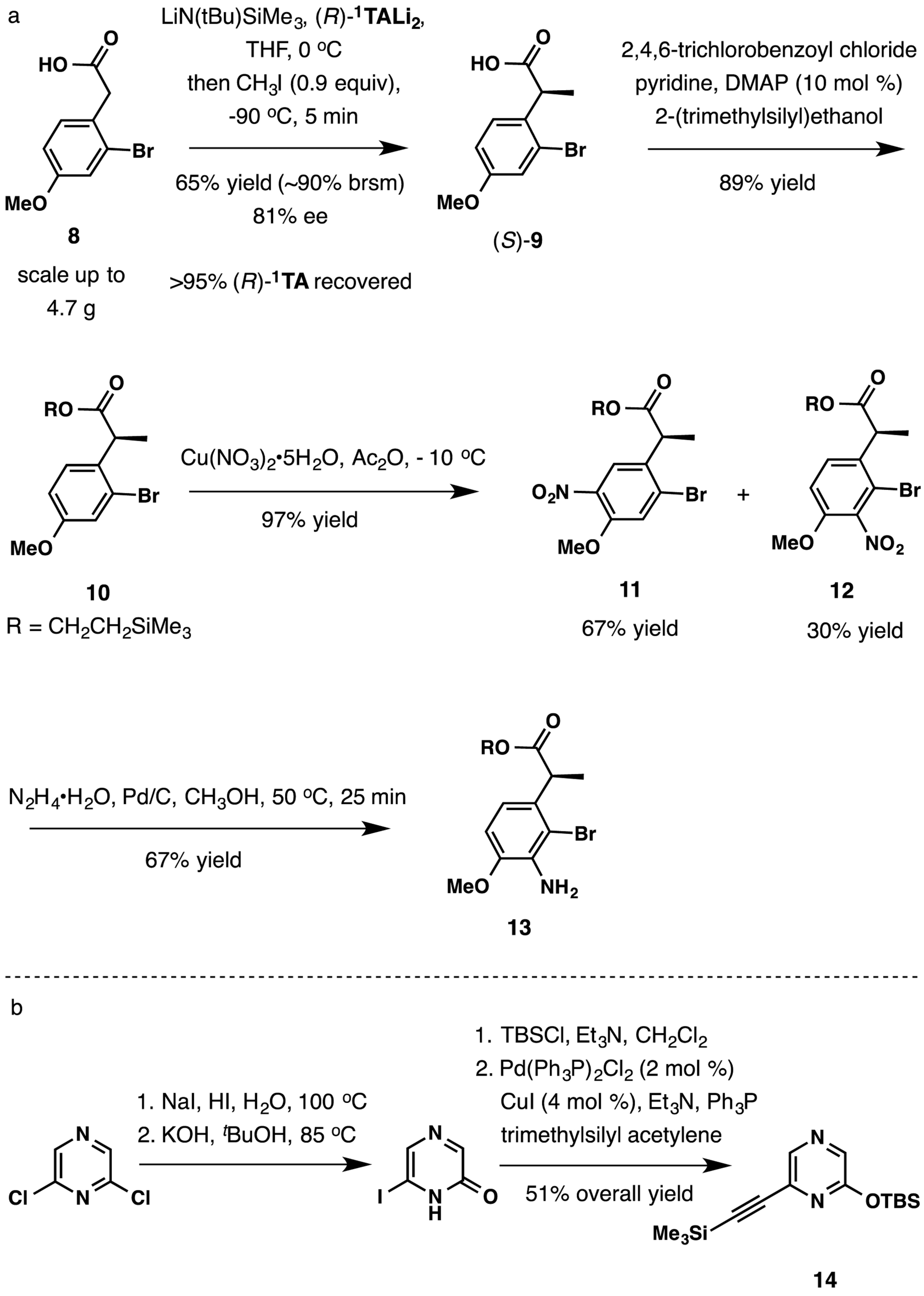
Figure 2.

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



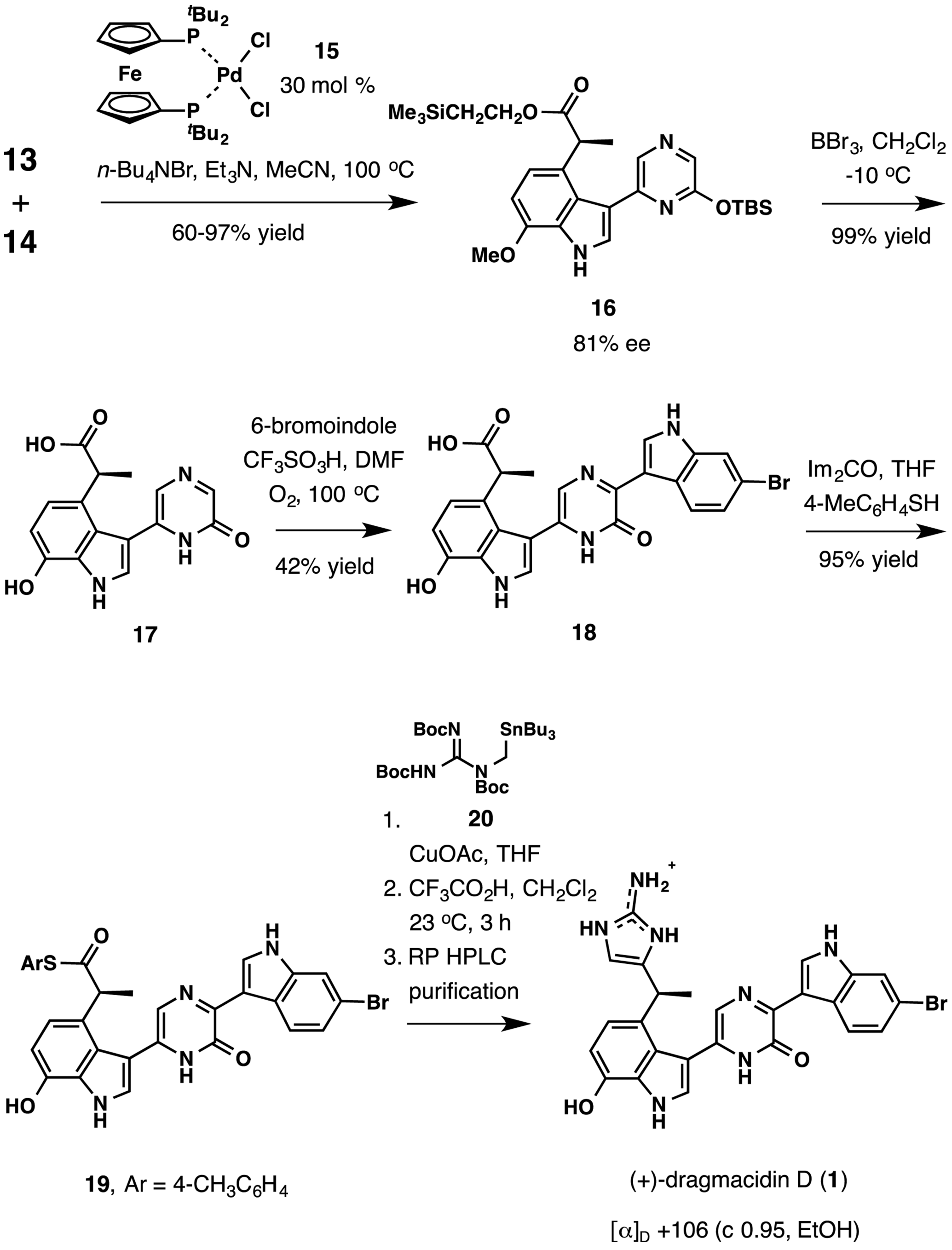
Scheme 1.

Synthetic plan for dragmacidin D (1).



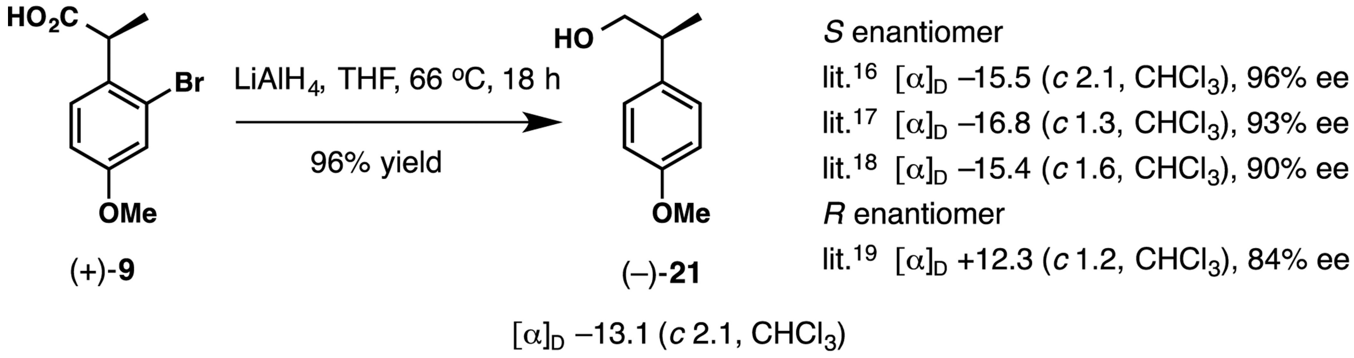
Scheme 2.

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



Scheme 3.

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

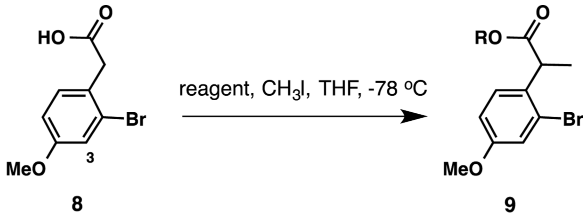


Scheme 4.

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

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**