Collective Asymmetric Total Synthesis of Cylindricines

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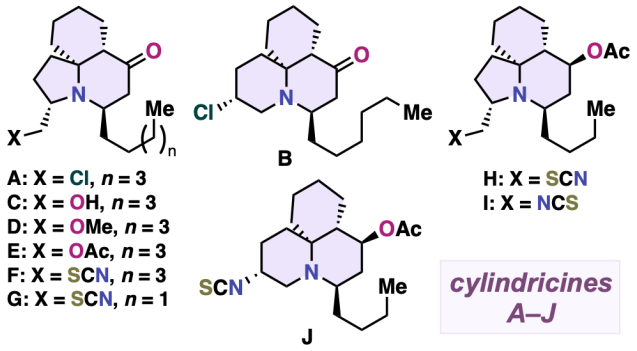
Supporting Information Placeholder



ABSTRACT: Collective asymmetric total synthesis of marine tricyclic alkaloids, cylindricines A–H, and the proposed structures of cylindricine I and J, was achieved in a concise manner from a single common spirocyclic pyrrolidine intermediate. A tandem chemoselective oxidation/intramolecular aza-Michael addition/epimerization was exploited to complete the tricyclic skeleton. This work provides a versatile synthetic entry to the cylindricine family of marine tricyclic alkaloids.

Marine alkaloids possessing a perhydropyrrolo[2,1-*j*]quinoline or perhydropyrido[2,1-*j*]quinoline skeleton have gained significant interest from the synthetic chemistry community for more than two decades because of their synthetically intriguing tricyclic structures.[1] This family of alkaloid natural products include cylindricines, fasicularin, and lepadiformines (Figure 1a). Cylindricines were isolated by Blackman and co-workers from the ascidian *Clavelina cylindrica* collected in Tasmania. The gross structure and relative configuration of cylindricines A (**1**) and B (**2**) were determined by single crystal X-ray crystallographic analysis.[2] These compounds were shown to exist as an equilibrating mixture in solution, possibly via generation of the corresponding aziridinium ion. Cylindricines C–J (**3**–**10**) were structurally characterized mainly by NMR spectroscopic analysis in comparison with **1** and **2**.[3,4] However, the specific rotation values of **1**–**10** were not reported, and the absolute configuration was left unassigned. While the absolute configuration of **1**–**10** cannot be determined unequivocally without re-isolation of authentic material, it is quite likely to be the same as that of lepadiformine A (**11**), which was isolated by Biard et al. from the Tunisian tunicate *Clavelina lepadiformis* Müller as a cytotoxic constituent (IC_{50} 16.8 μg/mL against KB cells).[5] The absolute configuration of **11** has been established through enantioselective total syntheses by Kibayashi[6] and Weinreb.[7]

A number of asymmetric and racemic total syntheses of cylindricines, fasicularin, and lepadiformines have been described to date.[8] Nonetheless, the synthetic development and biological evaluation of a collection of marine tricyclic alkaloids are yet to be achieved. Moreover, the biological activity of cylindricines has not been described so far except that a mixture of **1** and **2** was found toxic in brine shrimp bioassay.[2] Here we describe total syntheses of cylindricines A–H (**1**–**8**) and the proposed structures of cylindricines I and J (**9** and **10**) from a single common spirocyclic pyrrolidine derivative.

We have recently described an asymmetric total synthesis of lepadiformine A (**11**) by means of a tandem Au-catalyzed alkyne hydroamination/iminium formation/allylation reaction of **12** (Figure 1a).[9] The resultant spirocyclic pyrrolidine **13** was transformed into **11** over seven steps via the formation of the piperidine ring. In this work, we envisioned that **13** should serve as a pluripotent intermediate toward collective synthesis of **1**–**10**. Specifically, the perhydropyrrolo[2,1-*j*]quinolin-7-one skeleton **17** would be accessible through a tandem chemoselective oxidation/intramolecular aza-Michael addition/epimerization of amino alcohol **14** (Figure 1b). Epimerization at C5 position has been shown to be facile under acidic or basic conditions.[10]

The synthesis of cylindricines A–F (**1**–**6**) is summarized in Scheme 1. As described previously,[9] oxidative cleavage of the

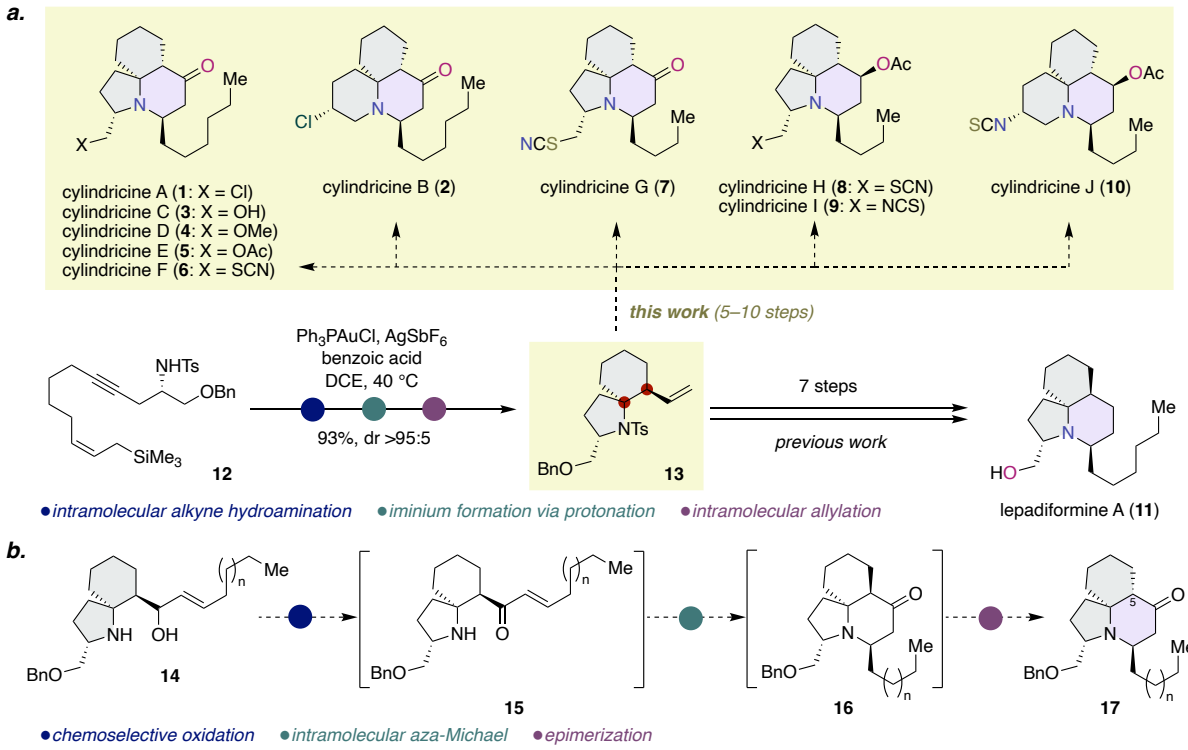
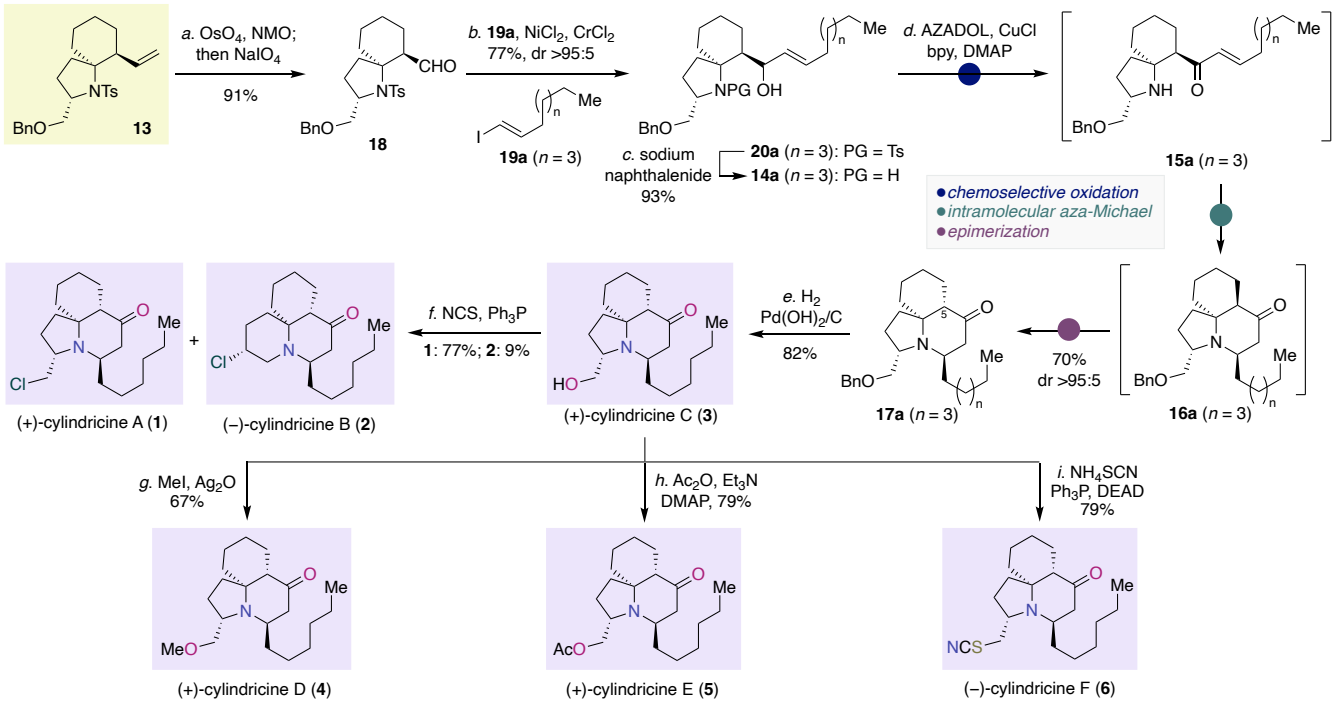


Figure 1. (a) Structures of cylindricines A–J and lepadiformine A. (b) Tandem chemoselective oxidation/intramolecular aza-Michael addition/epimerization for construction of tricyclic skeleton of cylindricines A–J.

Scheme 1. Synthesis of Cylindricines A–F[a]



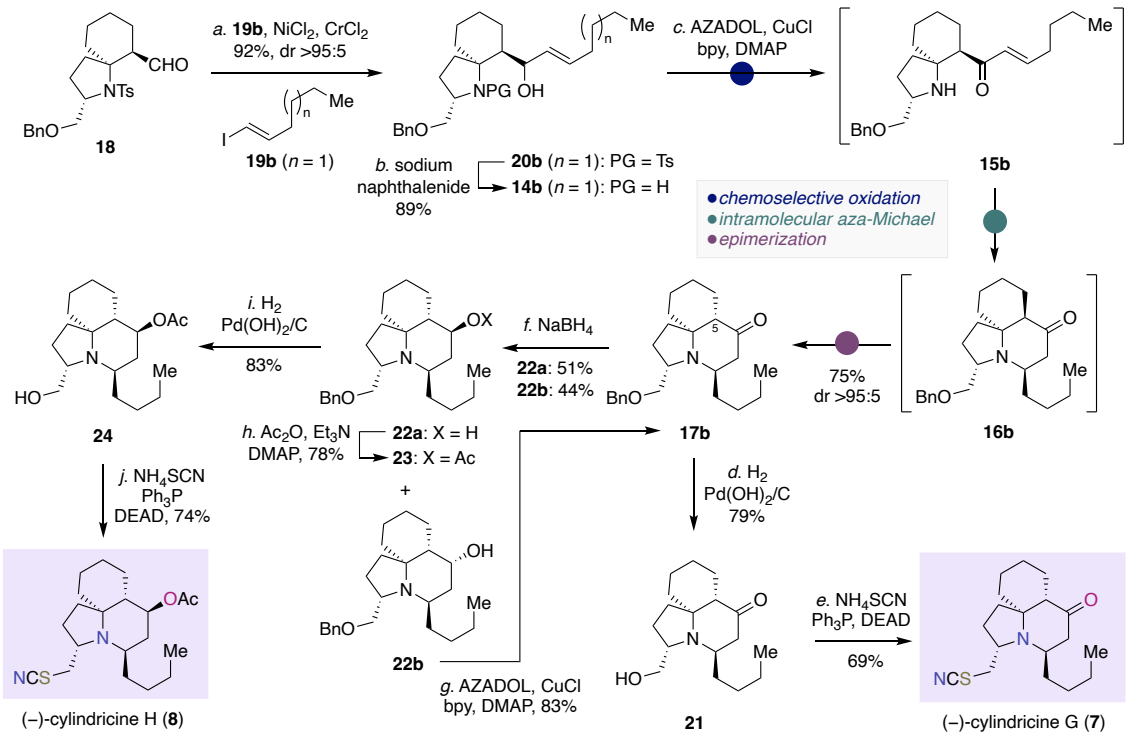
[a]Reagents and conditions: (a) OsO_4, NMO, THF/H_2O, rt; then $NaIO_4$, rt, 91%; (b) **19a**, $NiCl_2$, $CrCl_2$, DMSO, rt, 77%, dr >95:5; (c) sodium naphthalenide, DME, −65 °C, 93%; (d) AZADOL, CuCl, bpy, DMAP, CH_3CN, 0 °C, air; then silica gel, 70%, dr >95:5; (e) H_2, $Pd(OH)_2$/C, MeOH, rt, 82%; (f) NCS, Ph_3P, imidazole, DCE, 0 °C to rt, 77% for **1**, 9% for **2**; (g) MeI, Ag_2O, CH_3CN, rt, 67%; (h) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 79%; (i) NH_4SCN, DEAD, Ph_3P, CH_2Cl_2, 0 °C to rt, 79%.

double bond of **13** gave aldehyde **18**. Nozaki–Hiyama–Kishi reaction[11] of **18** with iodoolefin **19a**[12] ($NiCl_2$, $CrCl_2$, DMSO) proceeded cleanly to deliver allylic alcohol **20a** in 77% yield as a single diastereomer. Deprotection of the tosyl group using sodium naphthalenide provided amino alcohol **14a** (93%). Chemoselective oxidation of the allylic alcohol of **20a** under

Iwabuchi conditions (AZADOL, CuCl, bpy, DMAP, CH_3CN, 0 °C, air)[13] generated α,β-unsaturated ketone **15a**, which underwent intramolecular aza-Michael addition to afford ketone **16a**. Upon adsorption of **16a** on silica gel followed by purification by flash column chromatography, epimerization at the C5 position took place cleanly to furnish ketone **17a** in 70% yield from **20a** as a single diastereomer. Hydrogenolysis of the benzyl ether of **17a** provided cylindricine C (**3**) in 82% yield ($[\alpha]_D^{19}$ +61.5 (*c* 0.48, CH_2Cl_2); lit.[8g] $[\alpha]_D^{24}$ +63.8 (*c* 0.50, CH_2Cl_2)). The ^1H and ^{13}C NMR spectra and specific rotation of our synthetic **3** matched those reported by Sato and Chida.[8g]

Cylindricines A (**1**), B (**2**), D (**4**), E (**5**), and F (**6**) were synthesized from (+)-cylindricine C (**3**). Chlorination of **3** with NCS/Ph_3P afforded (+)-cylindricine A (**1**) in 77% yield ($[\alpha]_D^{22}$ +18.3 (*c* 0.13, CH_2Cl_2)), along with (−)-cylindricine B (**2**) in 9% yield ($[\alpha]_D^{23}$ −20.1 (*c* 0.10, CH_2Cl_2)), which were separable by flash column chromatography using silica gel. The ^1H and ^{13}C NMR spectra of our synthetic **1** matched that reported by Snider and Liu.[15] While the ^1H NMR chemical shift data of our synthetic **2** showed small deviations from those of the authentic material, the ^{13}C NMR spectroscopic data of synthetic **2** matched excellently with those of natural **2**.[2] Methylation of **3** with MeI/Ag_2O gave (+)-cylindricine D (**4**) in 67% yield ($[\alpha]_D^{23}$ +28.4 (*c* 0.19, CH_2Cl_2); lit.[14] $[\alpha]_D^{25}$ +21.5 (*c* 0.08, CH_2Cl_2)). Acetylation of **3** under standard conditions delivered (+)-cylindricine E (**5**) in 79% yield ($[\alpha]_D^{20}$ +27.3 (*c* 0.17, CH_2Cl_2); lit.[14] $[\alpha]_D^{25}$ +28.67 (*c* 0.13, CH_2Cl_2)). The ^1H and ^{13}C NMR spectroscopic data of our synthetic **4** and **5** were in accordance with those reported previously by Trost.[14] Mitsunobu reaction[16] of **3** with NH_4SCN (DEAD, Ph_3P) led to (−)-cylindricine F (**6**) in 79% yield ($[\alpha]_D^{22}$ −20.9 (*c* 0.37, CH_2Cl_2)). The ^1H and ^{13}C NMR signals of synthetic **6** were in accordance with those of natural cylindricine F.

Scheme 2. Synthesis of Cylindricines G and H[a]



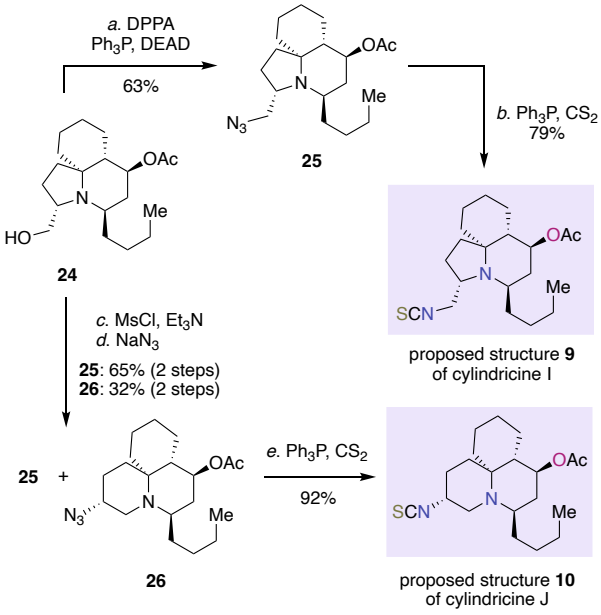
[a]Reagents and conditions: (a) **19b**, $NiCl_2$, $CrCl_2$, DMSO, rt, 92%, dr >95:5; (b) sodium naphthalenide, DME, −65 °C, 89%; (c) AZADOL, CuCl, bpy, DMAP, CH_3CN, 0 °C, air; then silica gel, 75%, dr >95:5; (d) H_2, $Pd(OH)_2/C$, MeOH, rt, 79%; (e) NH_4SCN, Ph_3P, DEAD, CH_2Cl_2, 0 °C to rt, 69%; (f) $NaBH_4$, MeOH, −78 °C, 51% for **22a**, 44% for **22b**; (g) AZADOL, CuCl, bpy, DMAP, CH_3CN, 0 °C, 83%; (h) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 78%; (i) H_2, $Pd(OH)_2/C$, MeOH, rt, 83%; (j) NH_4SCN, Ph_3P, DEAD, CH_2Cl_2, 0 °C to rt, 74%.

The synthesis of cylindricines G and H (**7** and **8**) is illustrated in Scheme 2. Nozaki–Hiyama–Kishi reaction of aldehyde **18** with iodoolefin **19b**[12] provided allylic alcohol **14b** (92%), from which the tosyl group was removed to give amino alcohol **20b** (89%). Tandem Iwabuchi oxidation/intramolecular aza-Michael addition/epimerization of **20b** afforded ketone **17b** in 75% yield as a single diastereomer. Debenzylation of **17b** (H_2, $Pd(OH)_2/C$) gave alcohol **21** (79%). Mitsunobu reaction of **21** with NH_4SCN by the action of DEAD/Ph_3P furnished (−)-cylindricine G (**7**) in 69% yield ($[\alpha]_D^{22}$ −12.1 (*c* 0.19, CH_2Cl_2)). The ^1H and ^{13}C NMR spectroscopic data of synthetic **7** were in accordance with those of the natural product. Next, our attention turned to cylindricine H (**8**). However, reduction of ketone **17b** was problematic with respect to its diastereoselectivity. Treatment of **17b** with $NaBH_4$ in MeOH at −78 °C provided alcohol **22a** (51%) and its diastereomer **22b** (44%). Other reaction conditions examined all failed to provide alcohol **22a** with correct configuration as the major diastereomer, presumably because the α-face of **17b** was sterically shielded by the tricyclic skeleton. The undesired diastereomer **22b** could be transformed back into **17b** via Iwabuchi oxidation (83%). Acetylation of **22a** led to acetate **23** (78%), which was debenzylated by hydrogenolysis to afford alcohol **24** (83%). Mitsunobu reaction of **24** using NH_4SCN,

DEAD, and Ph_3P furnished (−)-cylindricine H (**8**) in 74% yield ($[\alpha]_D^{22}$ −5.3 (*c* 0.38, MeOH); lit.[8a] $[\alpha]_D^{20}$ −8.5 (*c* 0.47, MeOH)). The ^1H and ^{13}C NMR spectra of synthetic **8** were in full agreement with those reported by Amat et al.[8a]

Mitsunobu reaction of **24** using DPPA, Ph_3P, and DEAD provided azide **25** (63%), which upon exposure to Ph_3P/CS_2 underwent Staudinger–aza-Wittig reaction to deliver cylindricine I (**9**) in 79% yield ($[\alpha]_D^{21}$ +2.2 (*c* 0.32, $CHCl_3$)) (Scheme 3). Meanwhile, mesylation/azidation of **24** provided a mixture of azides **25** (65%) and **26** (32%), the latter possibly being formed via an aziridinium ion intermediate. After separation by flash column chromatography using silica gel, subjection of **26** to Staudinger–aza-Wittig reaction afforded cylindricine J (**10**) in 92% yield ($[\alpha]_D^{23}$ −59.3 (*c* 0.37, $CHCl_3$)). However, the ^1H and ^{13}C NMR spectroscopic data of synthetic **9** and **10** showed deviations from those of natural cylindricines I and J, respectively. The structures of synthetic **9** and **10** were unambiguously confirmed by detailed NMR analyses and further supported by theoretical NMR chemical shift calculations.[12,17]

Scheme 3. Synthesis of Proposed Structures of Cylindricines I and J[a]



[a]Reagents and Conditions: (a) DPPA, Ph_3P, DEAD, THF, rt, 63%; (b) Ph_3P, CS_2, THF, 50 °C, 79%; (c) MsCl, Et_3N, CH_2Cl_2, 0 °C; (d) NaN_3, DMF, 50 °C, 63% (2 steps) for **25**, 32% (2 steps) for **26**; (e) Ph_3P, CS_2, THF, 50 °C, 92%.

Finally, we briefly examined the cytotoxic activity of synthetic cylindricines in human cancer cells. Compounds **1**, **2**, and **6** showed moderate activity in human cervix carcinoma HeLa cells and/or human T lymphocyte Jurkat cells.[12] Noticeably, compounds **9** and **10** were significantly more potent than other compounds, suggesting the importance of the electrophilic isothiocyanate group for activity.

Table 1. Cytotoxic activity of synthetic cylindricines[a]

Cpd.	IC_{50}/μM		Cpd.	IC_{50}/μM	
	HeLa	Jurkat		HeLa	Jurkat
1	49.5	21.8	**6**	98.3	15.2
2	30.5	15.3	**7**	>100	27.9
3	>100	62.7	**8**	>100	23.2
4	>100	53.0	**9**	13.0	1.0
5	97.3	>100	**10**	10.6	2.7

[a]Cell culture experiments were performed in RPMI1640 supplemented with 10% fetal bovine serum at 37 °C under 5% CO_2/air for 48 h (Jurkat) or 72 h (HeLa). Cell viability was assessed by WST-8 assay. See the Supporting Information for details.

In summary, we developed a versatile synthetic entry to the cylindricine family of marine tricyclic alkaloids from a single common intermediate. We also revealed non-identity of the proposed structures of cylindricines I and J to the corresponding natural products. The asymmetric total synthesis of cylindricines A, B, F, G and the proposed structures of cylindricines I and J was achieved for the first time. Our synthesis took advantage of an appropriately functionalized spirocyclic pyrrolidine intermediate and completed in 10–15 steps from commercially available, oct-7-en-1-ol. A tandem chemoselective oxidation/intramolecular aza-Michael addition/epimerization was successfully implemented to complete the tricyclic skeleton of cylindricines in a succinct fashion.[18]

ASSOCIATED CONTENT

Data Availability

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

Detailed NMR data of natural and synthetic cylindricines, configurational assignment of important compounds, experimental procedure, compound characterization data, theoretical NMR calculation data, and NMR spectra (PDF).

The Supporting Information is available free of charge on the ACS Publications website.

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The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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REFERENCES

1. (a) Kaga, A.; Chiba, S. Synthesis of Tricyclic Marine Alkaloids, Cylindricines, Lepadiformines, Fasicularin, and Polycitorols: A Recent Update. *Synthesis* **2018**, *50*, 685–699; (b) Weinreb, S. M. Studies on Total Synthesis of the Cylindricine/Fasicularin/Lepadiformine Family of Tricyclic Marine Alkaloids. *Chem. Rev.* **2006**, *106*, 2531–2549.
2. Blackman, A. J.; Li, C.; Hockless, D. C. R.; Skelton, B. W.; White, A. H. Cylindricines A and B, Novel Alkaloids from the Ascidian *Clavelina cylindrica*. *Tetrahedron* **1993**, *49*, 8645–8656.
3. Li, C.; Blackman, A. J. Cylindricines C–G, Perhydropyrrolo[2,1-*j*]quinlin-7-one Alkaloids from the Ascidian *Clavelina cylindrica*. *Aust. J. Chem.* **1994**, *47*, 1355–1361.
4. Li, C.; Blackman, A. J. Cylindricines H–K, Novel Alkaloids from the Ascidian *Clavelina cylindrica*. *Aust. J. Chem.* **1995**, *48*, 955–965.
5. Biard, J. F.; Guyot, S.; Roussakis, C.; Verbist, J. F.; Vercauteren, J.; Weber, J. F.; Boukef, K. Lepadiformine, a new Marine Cytotoxic Alkaloid from *Clavelina lepadiformis* Müller. *Tetrahedron Lett.* **1994**, *35*, 2691–2694.
6. Abe, H.; Aoyagi, S.; Kibayashi, C. Total Synthesis of the Natural Enantiomer of (−)-Lepadiformine and Determination of Its Absolute Stereochemistry. *Angew. Chem. Int. Ed.* **2002**, *41*, 3017–3020.
7. Sun, P.; Sun, C.; Weinreb, S. M. Stereoselective Total Syntheses of the Racemic Form and the Natural Enantiomer of the Marine Alkaloid Lepadiformine via a Novel *N*-Acyliminium Ion/Allylsilane Spirocyclization Strategy. *J. Org. Chem.* **2002**, *67*, 4337–4345.
8. For recent examples not covered in ref. 1, see: (a) Wang, Y.-T.; Wu, J.-L.; Chiou, W.-H. Total Synthesis of (±)-Fasicularin through Double Consecutive Epimerizations. *Org. Lett.* **2022**, *24*, 5957–5961. (b) Piccichè, M.; Pinto, A.; Griera, R.; Bosch, J.; Amat, M. Total Synthesis of (−)-Cylindricine H. *Org. Lett.* **2022**, *24*, 5356–5360. (c) Huang, Y.-H.; Liu, Z.-J.; Huang, P.-Q. Enantioselective total syntheses of marine natural products (+)-cylindricines C, D, E and their diastereomers. *Org. Chem. Front.* **2022**, *9*, 58–63. (d) Minamikawa, R.; Fukaya, K.; Kobayashi, A.; Komiya, Y.; Yamamoto, S.; Urabe, D.; Chida, N.; Sato, T. Development of a Chiral *N*-Alkoxyamide Strategy and Application to the Asymmetric Total Synthesis of Fasicularin. *Synthesis* **2021**, *53*, 4621–4635. (e) Wu, J.-L.; Chiou, W.-H. Diastereocontrolled Formal Syntheses of (±)-Lepadiformines A, B, and C, and the Divergent Synthesis of 2-*epi*-Lepadiformine C through Unexpected Double Consecutive Epimerizations. *J. Org. Chem.* **2020**, *85*, 9051–9063. (f) Shimomura, M.; Sato, M.; Azuma, H.; Sakata, J.; Tokuyama, H. Total Synthesis of (−)-Lepadiformine A via Radical Translocation–Cyclization Reaction. *Org. Lett.* **2020**, *22*, 3313–3317. (g) Takashima, K.; Hayakawa, D.; Gouda, H.; Toyooka, N. Formal Syntheses of (−)-Lepadiformines A, C, and (−)-Fasicularin. *J. Org. Chem.* **2019**, *84*, 5222–5229. (h) Hiraoka, S.; Matsumoto, T.; Matsuzaka, K.; Sato, T.; Chida, N. Approach to Fully Substituted Cyclic Nitrones from *N*-Hydroxylactam Derivatives: Development and Application to the Total Synthesis of Cylindricine C. *Angew. Chem. Int. Ed.* **2019**, *58*, 4381–4385.
9. Yoshimura, A.; Hanzawa, R.; Fuwa, H. Stereoselective Tandem Synthesis of Pyrrolidine Derivatives under Au Catalysis: An Asymmetric Synthesis of (−)-Lepadiformine A. *Org. Lett.* **2022**, *24*, 6237–6241.
10. (a) Pandey, G.; Janakiram, V. Aza-Quaternary Scaffolds from Selective Bond Cleavage of Bridgehead-Substituted 7-Azabicyclo[2.2.1]heptane: Total Synthesis of (+)-Cylindricines C–E and (−)-Lepadiformine A. *Chem. Eur. J.* **2015**, *21*, 13120–13126. (b) Liu, J.; Swidorski, J. J.; Peters, S. D.; Hsung, R. P. An *N*-Acyliminium Cyclization Approach to a Total Synthesis of (+)-Cylindricine C. *J. Org. Chem.* **2005**, *70*, 3898–3902.
11. Jin, H.; Uenishi, J.; Christ, W. J.; Kishi, Y. Catalytic Effect of Nickel(II) Chloride and Palladium(II) Acetate on Chromium(II)-Mediated Coupling Reaction of Iodo Olefins with Aldehydes. *J. Am. Chem. Soc.* **1986**, *108*, 5644–5646.
12. See the Supporting Information for details.
13. Sasano, Y.; Nagasawa, S.; Yamazaki, M.; Shibuya, M.; Park, J.; Iwabuchi, Y. Highly Chemoselective Aerobic Oxidation of Amino Alcohols into Amino Carbonyl Compounds. *Angew. Chem. Int. Ed.* **2014**, *53*, 3236–3240.
14. Trost, B. M.; Rudd, M. T. Chemoselectivity of the Ruthenium-Catalyzed Hydrative Diyne Cyclization: Total Synthesis of (+)-Cylindricine C, D, and E. *Org. Lett.* **2003**, *5*, 4599–4602.
15. Snider, B. B.; Liu, T. Synthesis of (±)-Cylindricines A, D, and E. *J. Org. Chem.* **1997**, *62*, 5630–5633.
16. (a) Mitsunobu, O. The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis* **1981**, 1–28. (b) Swamy, K. C. K.; Kumar, N. N. B.; Balaraman, E.; Kumar, K. V. P. P. Mitsunobu and Related Reactions: Advances and Applications. *Chem. Rev.* **2009**, *109*, 2551–2651.
17. NMR calculations were performed according to our previously published procedure: Murata, K.; Mori, H.; Fuwa, H. GIAO NMR Calculation-Driven Stereochemical Assignment of Marine Macrolide Natural Products: Assessment of the Performance of DP4 and DP4+ Analyses and Assignment of the Relative Configuration of Leptolyngbyalide A–C/Oscillariolide Macrolactone. *Bull. Chem. Soc. Jpn.* **2022**, *95*, 1775–1785.