Note

Design, Synthesis and Biological Activity of 16,17-Dihydroheronamide C and *ent*-Heronamide C

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Abstract

16,17-Dihydroheronamide C (**8**) and *ent*-heronamide C (*ent*-**1**) were designed as probes for the mode-of-action analysis of heronamide C (**1**). These molecules were synthesized by utilizing a highly modular strategy developed in the preceding paper. Evaluation of the antifungal activity of these compounds revealed the exceptional importance of the C16-C17 double bond for the biological activity of heronamide C, and the existence of chiral recognition between heronamide C (**1**) and cell membrane components.

Introduction

In the preceding paper,[1] we developed a highly modular strategy for the synthesis of heronamide C-type polyene macrolactams[2-4] featuring modular Suzuki coupling utilizing MIDA boronate ester and borylcupration/protonation of internal alkynes. By utilizing the developed strategy, 8-deoxyheronamide C (**2**) and four heronamide C-like molecules named heronamidoids α–δ were successfully synthesized. Synthesis of heronamidoids enabled the conformational and reactivity

analysis of the 20-membered polyene macrolactams. As an application of the modular synthetic strategy, as well as a part of our program directed toward the mode-of-action study of heronamides, we herein report the design, synthesis, and biological activity of 16,17-dihydroheronamide C (**8**) and *ent*-heronamide C (*ent*-**1**) (Figure 1).

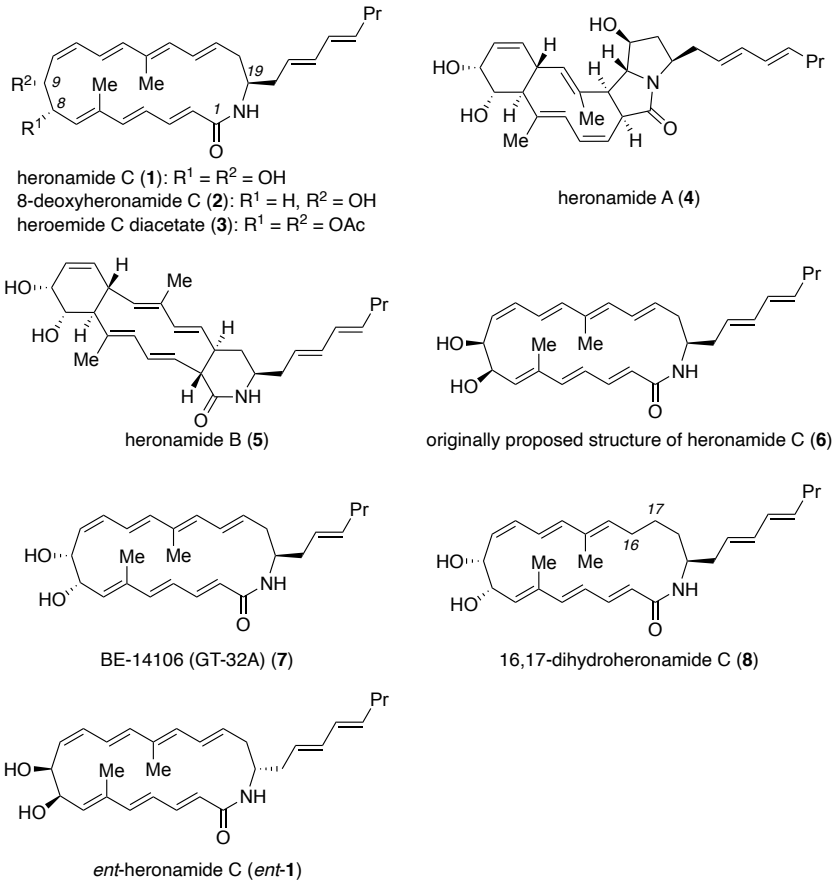


Figure 1. Structures of heronamides (selected) and their synthetic analogues

Results and Discussion

Design of molecular probes for the mode-of-action studies of heronamide C

Having established a unified strategy,[1] we decided to design and synthesize molecular probes for the mode-of-action studies of heronamide C (**1**). Previously, we showed that heronamide C (**1**) and 8-deoxyheronamide C (**2**) bind to liposomes consisting of lipids having saturated hydrocarbon chains in an irreversible manner.[5] In addition, structure-activity relationship studies revealed that, among the congeners **1**–**5** (Figure 1), only 20-membered macrolactams **1** and **2** having hydroxyl group(s) possess not only growth inhibition activity against fission yeast cells,[5,6] but also the ability to induce abnormal cell morphology of the yeast cells.[5] The reversal morphological effect of **1** on mammalian cells was also reported by Capon's group.[7] Importantly, heronamides A (**4**) and B (**5**), compounds derived from heronamide C (**1**) under aerobic/thermal and photochemical conditions,[5-7] respectively (Figure 2), lost all of these activities. Moreover, between the compounds **1** and **2**, compound **2** was shown to have a prominent selectivity profile against fission yeast cells: it inhibited the growth of wild-type fission yeast cells (MIC 5.9 μM) while mutant yeast cells lacking the ergosterol biosynthetic gene (*erg2*, *erg31* and *erg32*, *sts1/erg4*, or *erg5*) were tolerant to **2** up to a concentration of 46 μM.[5] It was later shown that both the originally proposed structure of heronamide C (**6**) and BE-14106 (a.k.a. GT-32A) (**7**), an analogue having a truncated side chain, have reduced antifungal activity when compared with **1**.[6,8]

These results suggested that heronamide C-type molecules interact with cell membrane lipids having saturated hydrocarbon chains and perturb the structure of the membrane in cells. Indeed,

molecular dynamic simulation of heronamides in a bilayer of 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine (DMPC) suggested that heronamide C (**1**) and 8-deoxyheronamide C (**2**) resided in the bilayer to form head-to-tail contacts with DMPC, as cholesterol does (Supporting Figure 1). However, the actual localization and fates of these reactive heronamide C-type macrolactams in lipid bilayers and living cells have not been studied experimentally.

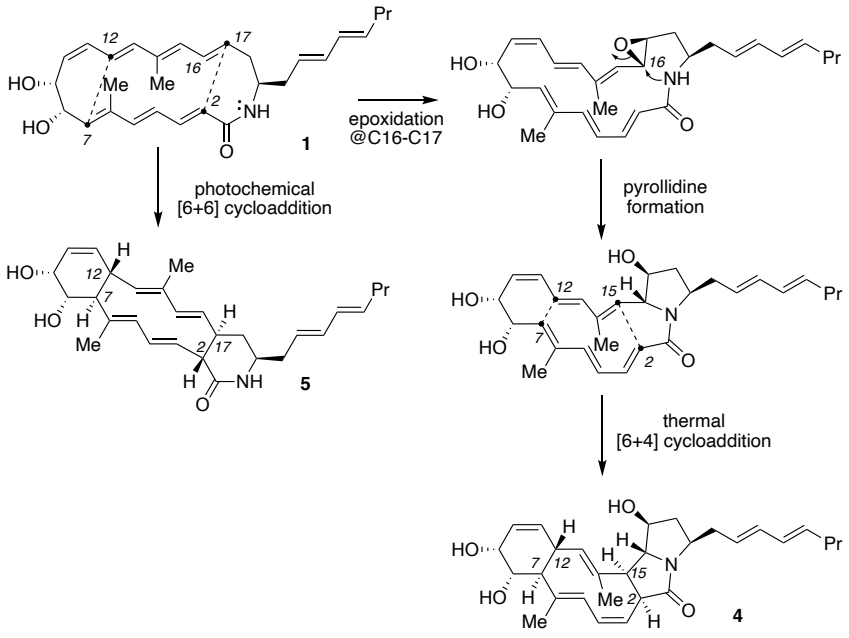


Figure 2. Biogenetic transformation of heronamide C (**1**) to heronamides A (**4**) and B (**5**).

To understand the localization and fates of **1** in living cells, we planned live cell time–course experiments of **1** by using a Raman imaging technique.[9] Usually, alkyne tags are used for visualization of small molecules in live cell Raman imaging,[10] but a recent study showed that polyenes could be

observed in cells.[11] Indeed, a strong Raman signal at ~1600 cm^{-1} was observed when heronamide C (**1**) on a quartz substrate was scanned by using a Raman microscope with 532 nm excitation (Supporting Figure 2). However, the signal intensity diminished as the measurement was repeated, suggesting laser-induced degradation and/or a photochemical reaction, possibly including [6π+6π] cycloaddition (Figure 2). We therefore decided to design a stabilized heronamide C analog that behaves similarly to heronamide C (**1**) in the lipid membrane but does not degrade/react like heronamide C (**1**).

Based on the molecular mechanics (MM) calculation, we herein designed 16,17-dihydroheronamide C (**8**) (Figure 1). The designed compound **8** lacks a C16-C17 olefin that is needed not only for the [6π+6π] cycloaddition leading to heronamide B (**5**), but also for C16-C17 epoxidation, the first step of the reaction sequence leading to heronamide A (**4**) (Figure 2). Importantly, the calculated macrocycle conformation of the 16,17-dihydro analogue was found to be very similar to that of heronamide C (**1**) (Figure 3). MD simulation also showed that 16,17-dihydroheronamide C (**8**) in a DMPC lipid bilayer behaved in the same manner as heronamide C (**1**) and 8-deoxyheronamide C (**2**) (Supporting Figure 1-3).

In addition to 16,17-dihydroheronamide C (**8**), we also designed *ent*-heronamide C (*ent*-**1**) as an enantiomeric probe of heronamide C (**1**). We have shown that diastereomeric analogue **6** (Figure 1) having C8,C9-diol functionalities possesses 80-fold less-potent antifungal activity than that of

heronamide C (**1**).[6] We also showed in the preceding paper[1] that truncated compounds named heronamidoids γ and δ, which have the same relative stereochemistries as **1** and **6**, respectively, differ in their stable confirmation indicating that the stable conformation of compounds **1** and **6** also differ significantly. Thus, we are interested in whether *ent*-**1** possesses antifungal activity. If there is a difference in the antifungal activity between heronamide C (**1**) and *ent*-**1**, chiral interaction of **1** with cell membrane component(s) would be strongly suggested.

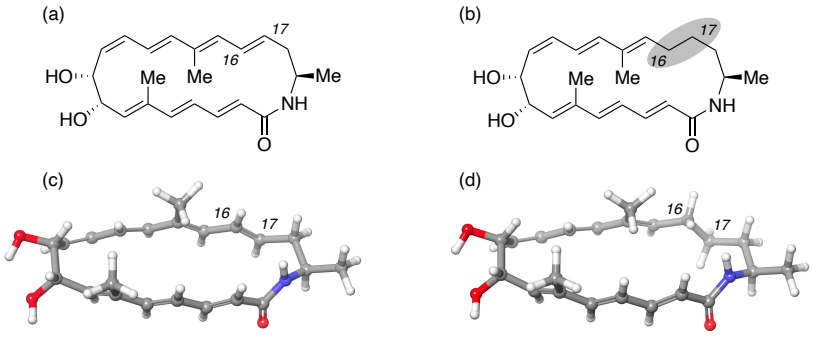
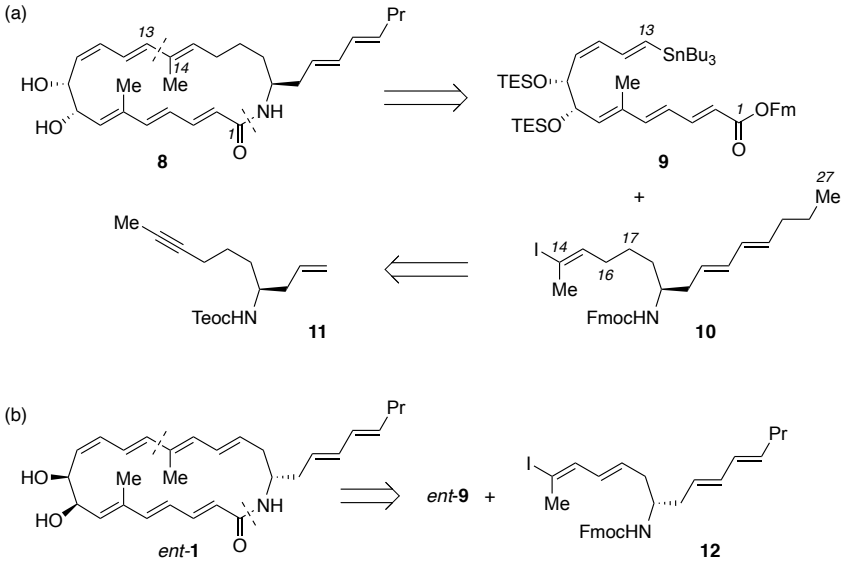


Figure 3. Most stable conformers of macrocycles of heronamide C and its 16,17-dihydro analogue. Chemical structures (a and b) and their calculated most stable conformers (c and d) of the macrocycle moiety of heronamide C (a and c) and its 6,17-dihydro analogue (b and d). Conformational search (OPLS2015, no solvent) and energy minimization was performed on Macromodel ver.11.0.

To synthesize probe **8**, C14-C27 fragment **10** was needed along with C1-C13 fragment **9**,

which could be synthesized from L-ribose (Scheme 1a).[1] Fragment **10** would be synthesized from chiral enyneamine **11** by the established protocol including a highly regioselective borylcupuration/protonation sequence.[1] *ent*-Heronamide C (*ent*-**1**) would be synthesized from *ent*-**9** [1] and C14-C27 fragment **12**, whose enantiomer was synthesized in the preceding paper[1] (Scheme 1b).

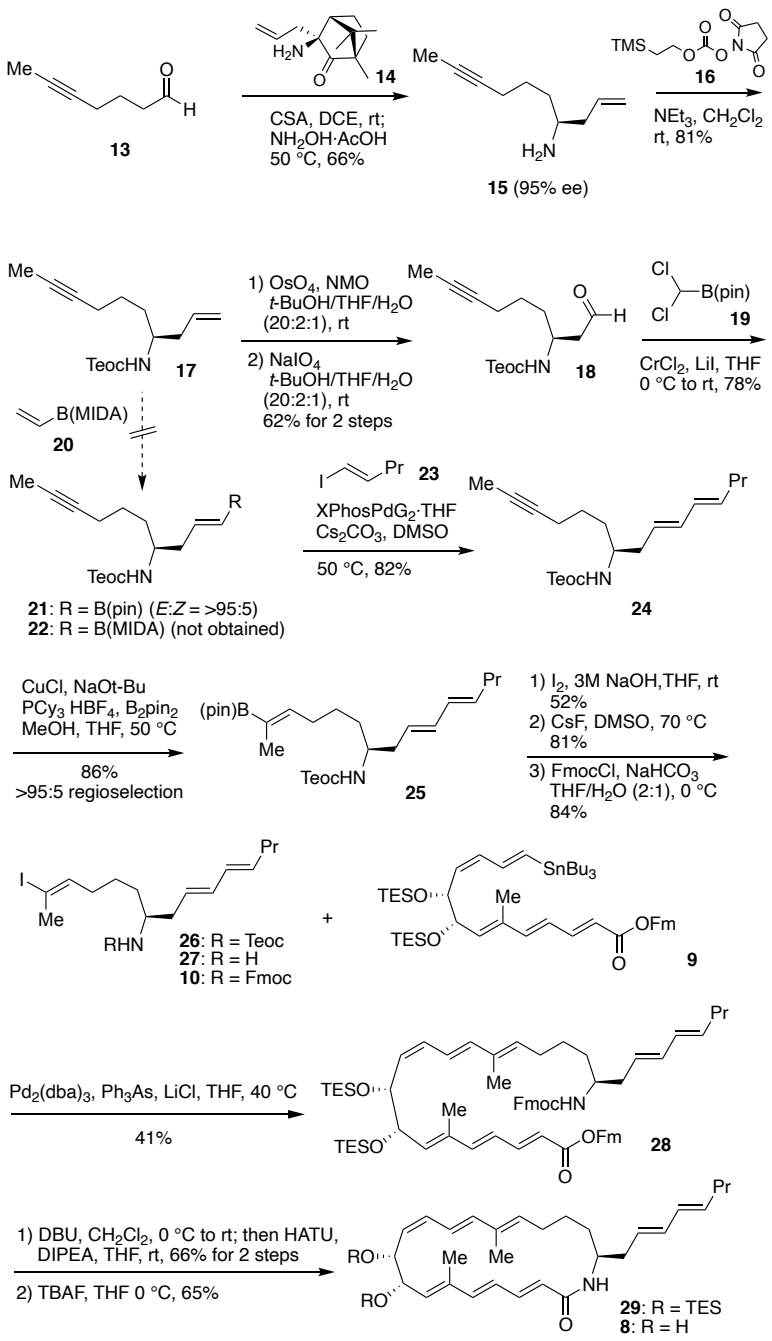


Scheme 1. Synthetic plan of 16,17-dihydroheronamide C (**8**) and *ent*-heronamide C (*ent*-**1**)

Synthesis of 16,17-dihydroheronamide C and *ent*-heronamide C

Synthesis of 16,17-dihydroheronamide C (**8**) is shown in Scheme 2. 5-Heptynal (**13**), which could be easily synthesized from 2-methyl-1,3-cyclohexanedione,[12,13] was treated under Kobayashi transfer aminoallylation reaction conditions[14] using (1*S*)-camphorquinone derivative **14** to afford homoallylamine **15** in 66% yield and 95% ee. Teoc protection of the resulting amine by using Ghosh's

procedure[15] with carbonate **16** afforded Teoc-protected enyneamine **17** in 81% yield. Dihydroxylation of the monosubstituted olefin of **17** followed by cleavage of the resulting diol provided aldehyde **18**, which was subjected to the Takai-Utimoto olefination conditions using dichloromethylboronic acid pinacol ester **19**[16] to afford (*E*)-vinylboronic acid pinacol ester **21** in 78% yield in a highly stereoselective manner (*E*:*Z* = >95:5). Direct introduction of the (*E*)-vinylboronic acid ester unit on **17** using vinylboronic acid MIDA ester **20** under olefin metathesis conditions did not work, possibly due to the intramolecular enyne metathesis of **17**.

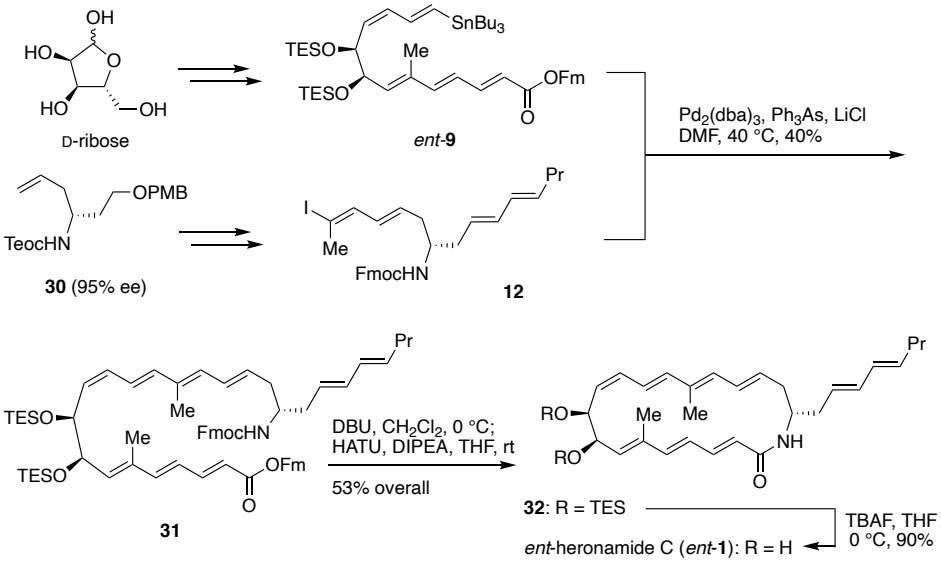


Scheme 2. Synthesis of 16,17-dihydroheronamide C (**8**)

Fragment assembly and macrolactam formation were performed as follows. Suzuki coupling of pinacol ester **21** and vinyl iodide **23** afforded dieneyne **24** in 82% yield. Borylcupration/protonation

of dienyne **24** worked well to produce triene **25** in 86% yield. It should be noted that the reaction proceeded in a highly regio- and chemoselective manner in the presence of the conjugated diene moiety. Iodination of **25** followed by Teoc deprotection and Fmoc protection gave C14-C27 unit **10** in 35% for three steps. Stille coupling of **10** with C1-C13 fragment **9** followed by the simultaneous deprotection of Fm and Fmoc groups, macrolactamization, and TES deprotection provided 16,17-dihydroheronamide C (**8**) in 18% yield for 4 steps.

Synthesis of *ent*-heronamide C (*ent*-**1**) is summarized in Scheme 3. C1-C13 Fragment *ent*-**9** and C14-C27 fragment **12** were prepared from D-ribose and homoallylamine **30** (95% ee), respectively.[1] These fragments were coupled under Stille conditions to give coupling product **31** in 40% yield, which was then treated with DBU followed by HATU to generate TES-protected *ent*-heronamide C (**32**) in 53% yield over 2 steps. Finally, by deprotection of TES groups in **32** using TBAF, *ent*-heronamide C (*ent*-**1**) was obtained in 90% yield.



Scheme 3. Synthesis of *ent*-heronamide C (*ent*-**1**).

The CD spectra of these synthetic compounds **8** and *ent*-**1**, as well as 8-deoxyheronamide C (**2**), were measured in DMSO.[5] 16,17-Dihydroheronamide C (**8**) showed a positive Cotton effect, as 8-deoxyheronamide C (**2**) did, suggesting that they have similar conformations in solution (Supporting Figure 3). Understandably, *ent*-heronamide C *(ent*-**1**) displayed a negative Cotton effect nearly symmetrical to that of 8-deoxyheronamide C.[5] In addition, 16,17-dihydroheronamide C (**8**) showed a characteristic Raman signal at ~1600 cm^{-1}, indicating the possibility of its use in the Raman imaging experiment (Supporting Figure 4).

Biological activity of 16,17-dihydroheronamide C and *ent*-heronamide C

The growth inhibition activities of 16,17-dihydroheronamide C (**8**) and *ent*-heronamide C (*ent*-**1**)

against fission yeast cells were examined[17] using 8-deoxyheronamide C (**2**) as a positive control (Figure 4). Under the experimental conditions used here, the IC_{50} values of *ent*-heronamide C (*ent*-**1**) against the wild-type strain, *erg2*Δ mutant,[18] and *erg31* Δ*erg32*Δ double mutant[18] were 0.26, 0.44, and 0.38 μM, respectively. Compared with 8-deoxyheronamide C (**2**), *ent*-heronamide C (*ent*-**1**) showed 2-fold more potent activity against the wild type cells. Since 8-deoxyheronamide C (**2**) shows 20-fold less potent activity against the wild-type cells,[5] *ent*-**1** turned out to be 10-fold less potent than heronamide C (**1**).[5] This result indicated the existence of chiral recognition between heronamide C (**1**) and cell membrane component(s).

Ent-heronamide C (*ent*-**1**) was found to be slightly active against wild type yeast cells. This sensitivity profile and cellular phenotype (data not shown) of *ent*-**1** against wild type yeast and mutants had close similarity with those of **1**,[5,6] suggesting that the mode-of-action of *ent*-**1** would be same as those of **1** and **2**. These results also indicated that the relative stereochemistries at the C8, C9, and C19 stereocenters were important not only for the activity strength but also for the selectivity profile.

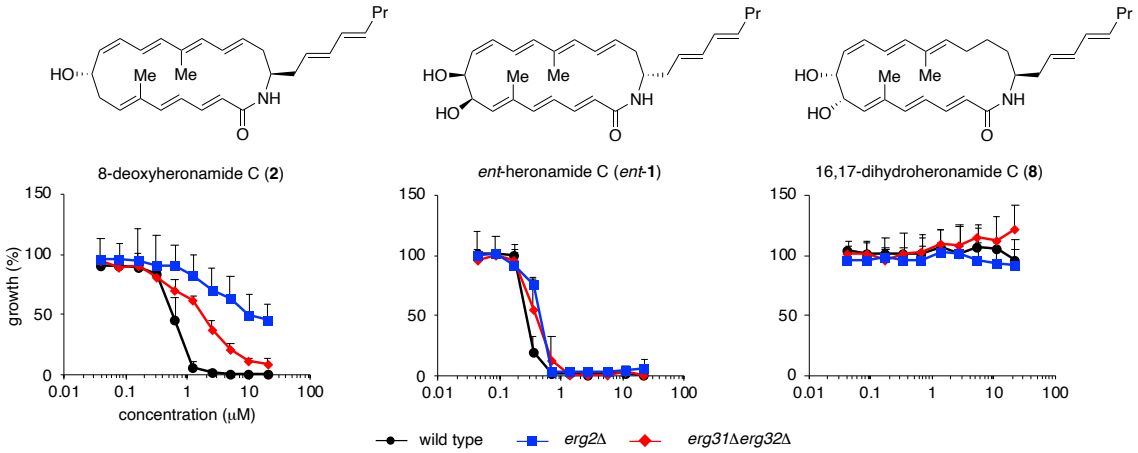


Figure 4. Growth inhibition activity of heronamides toward fission yeast cells

To our surprise, 16,17-dihydroheronamide C (**8**) did not show any growth inhibition up to a concentration of 50 μM. This result indicated that the C16-C17 double bond is crucial for the antifungal activity of **1**. Possible explanations for this result would be as follows: (1) recognition of heronamide C (**1**) by the cell membrane component(s) is quite susceptible to the conformation of **1** and/or the environment around the C16-C17 double bond,[19] or (2) the susceptibilities of heronamide C toward the thermal/aerobic and photochemical reactions and/or the reaction products (or intermediates) are essential for the biological activity. Although we can not conclude which explanation is correct at this stage, *ent*-heronamide C (*ent*-**1**) and 16,17-dihydroheronamide C (**8**) would be useful probes in a future study to understand the cellular activity of heronamide C-type natural products.

Summary

In this study, we designed and synthesized 16,17-dihydroheronamide C (**8**) and *ent*-heronamide C (*ent*-**1**) as probes for the mode-of-action studies of heronamides. A highly modular strategy developed in the preceding paper[1] was successfully applied for the synthesis of these probes. Examination of the antifungal activity of *ent*-heronamide C (*ent*-**1**) and 16,17-dihydroheronamide C (**8**) revealed (1) the presence of chiral recognition between heronamide C (**1**) and cellular components, which increases the potency of the biological activity but is not essential for that, and (2) an unexpected significance of the C16-C17 double bond for the biological activity of heronamide C (**1**). Detailed analyses of mode-of-action studies using *ent*-heronamide C (*ent*-**1**) and 16,17-dihydroheronamide C (**8**) as positive and negative probes, respectively, including live-cell Raman imaging studies, are now in progress and will be reported in due course.

Experimental Section

General Remarks

All reactions were carried out under an argon atmosphere with dehydrated solvents under anhydrous conditions, unless otherwise noted. Dehydrated THF and CH_2Cl_2 were purchased from Kanto Chemical Co., Inc. Other solvents were dehydrated and distilled according to standard protocols. Reagents were obtained from commercial suppliers and used without further purification, unless otherwise noted. Reactions were monitored by thin-layer chromatography (TLC) carried out on silica gel plates (Merck Kieselgel 60 F254). Column chromatography was performed on Silica gel 60N (Kanto Chemical Co., Inc.; spherical, neutral, 63–210 μm), and flash column chromatography was performed on Silica gel 60N (Kanto Chemical Co., Inc.; spherical, neutral, 40–50 μm). Optical rotations were measured on a JASCO P-2200 Digital Polarimeter at rt, using the sodium D line. CD spectra were measured on a JASCO J-720 spectropolarimeter. IR spectra were recorded on a JASCO FT/IR-410 Fourier Transform Infrared Spectrophotometer or Travel-IR™. ^{1}H-NMR (400 and 600 MHz) and ^{13}C-NMR spectra (100 and 150 MHz) were recorded on a JEOL JNM-AL-400, and JEOL JNM-ECA-600 or JEOL ECZ600 spectrometers, respectively. For ^{1}H-NMR spectra, chemical shifts (δ) are given from TMS (0.00 ppm) in $CDCl_3$ or a C2 proton (8.71 ppm) of deuteriopyridine in pyridine-d_5 as internal standards. For ^{13}C-NMR spectra, chemical shifts (δ) are given from $CDCl_3$ (77.0 ppm) or a C2 carbon of pyridine-d_5 (149.2 ppm) as internal standards. The following

abbreviations were used to explain the multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, sext = sextet, sept = septet, m = multiplet, br = broad. Mass spectra were recorded on JEOL JMS-DX303, JEOL JNM-AL500, JEOL JMS-700, and Thermo Scientific Exactive mass spectrometers.

Amine 15

To a solution of ketoamine **14**[14] (2.01 g, 8.69 mmol) and aldehyde **13**[20] (1.05 g, 9.54 mmol) in 1,2-dichloroethane (20 mL) was added CSA (22.2 mg, 0.95 mmol) at 0 °C. The mixture was allowed to warm to room temperature and stirred for 23.5 h. Then, a solution of $HONH_2$·AcOH (0.5 M in methanol, 40 mL) was added to the reaction mixture. After being stirred at 50 °C for 3 h, the mixture was allowed to cool to room temperature. The mixture was acidified with 6 *N* aq. HCl to pH ~1 and washed with CH_2Cl_2 (30 mL). The resulting aqueous layer was basified to pH ~10 with 6 *N* aq. NaOH and extracted with CH_2Cl_2 (50 mL x 5). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$ = 1/10) to give amine **15** (950 mg, 6.28 mmol, 66%) as a colorless oil.

15: $[\alpha]_D^{29}$ –4.4 (*c* 0.224, $CHCl_3$); IR (neat): 3075, 2920, 2859, 1639, 1575, 1438, 996, 915 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.86-5.73 (m, 1H), 5.11 (d, *J* = 5.8 Hz, 1H), 5.08 (s, 1H), 2.85-2.74 (m, 1H), 2.30-2.19 (m, 1H), 2.19-2.10 (m, 1H), 2.06-1.92 (m, 1H), 1.78 (t, *J* = 2.4 Hz, 3H), 1.66-1.42 (m, 3H), 1.43-1.33 (m, 1H), 1.25 (brs, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 135.8, 117.3, 79.0, 75.7, 50.2,

42.6, 36.9, 25.8, 18.8, 3.4; HRMS (FAB): calcd for $C_{10}H1_{18}N$ ($[M+H]^+$) 152.1439, found 152.1407.

Teoc-protected amine 17

To a solution of amine **15** (30 mg, 0.20 mmol) and carbonate **16**[21] (67 mg, 0.26 mmol) in CH_2Cl_2 (1.3 mL) was added NEt_3 (0.030 mL, 0.30 mmol) at room temperature. After being stirred for 45 min, the mixture was concentrated *in vacuo*. The residue was diluted with sat. aq. $NaHCO_3$ (2 mL) and extracted with EtOAc (5 mL x 3). The combined layers were washed with brine (2 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/15) to give Teoc-protected amine **17** (51 mg, 0.17 mmol, 86%) as a colorless oil.

17: $[\alpha]_D^{25}$ –10.7 (*c* 0.214, $CHCl_3$); IR (neat): 2952, 1691, 1534, 1251, 1063, 837 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 5.81-5.74 (m, 1H), 5.10 (s, 1H), 5.07 (s, 1H), 4.39 (brs, 1H), 4.14 (t, *J* = 8.2 Hz, 2H), 3.70 (brs, 1H), 2.29-2.15 (m, 1H), 2.15-2.04 (m, 2H), 1.77 (t, *J* = 2.4 Hz, 3H), 1.68-1.37 (m, 4H), 0.97 (t, *J* = 8.2 Hz, 2H), 0.04 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 156.3, 134.2, 117.8, 78.7, 75.9, 62.8, 50.1, 39.6, 33.9, 25.4, 18.6, 17.7, 3.4, –1.5; HRMS (FAB): calcd for $C_{16}H_{30}NO_2Si$ ($[M+H]^+$) 296.2046, found 296.2041.

Aldehyde 18

Teoc-protected amine **17** (100 mg, 0.338 mmol) was dissolved in a mixture of *t*-BuOH/THF/H_2O (10:2:1, 4 mL). To the solution was added 4-methylmorpholine *N*-oxide (158 mg, 1.35 mmol) and OsO_4 (0.10 M in H_2O, 16.9 μmol) at room temperature. The reaction mixture was stirred at the same temperature for 4 h and then quenched with sat. aq. $Na_2S_2O_3$ (5 mL). The separated aqueous layer was extracted with EtOAc (5 mL x 3). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was dissolved in a mixture of *t*-BuOH/THF/H_2O (20:2:1, 1 mL) and $NaIO_4$ (218 mg, 1.01 mmol) was added at room temperature. After stirring for 9.5 h, sat. aq. $Na_2S_2O_3$ (5 mL) was added. The separated aqueous layer was extracted with EtOAc (5 mL x 3). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (AcOEt/hexane = 1/3) to give aldehyde **18** (63.8 mg, 0.214 mmol, 64%) as a colorless oil.

18: $[\alpha]_D^{26}$ –20.4 (*c* 0.200, $CHCl_3$); IR (neat): 3330, 2952, 1723, 1529, 1249, 1069, 838 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 9.77 (s, 1H), 4.76 (d, *J* = 7.3 Hz, 1H), 4.13 (t, *J* = 8.7 Hz, 2H), 4.08 (d, *J* = 7.3 Hz, 1H), 2.65 (brs, 2H), 2.23-2.15 (m, 2H), 1.77 (t, *J* = 2.4 Hz, 3H), 1.69-1.45 (m, 4H), 0.96 (t, *J* = 8.7 Hz, 2H), 0.03 (s, 9H): ^{13}C-NMR (100 MHz, $CDCl_3$): δ 200.9, 156.2, 118.2, 78.4, 76.2, 63.1, 49.0, 34.0, 25.5, 18.4, 17.7, 3.4, -1.5; HRMS (FAB): calcd for $C_{15}H_{28}NO_3Si$ ($[M+H]^+$) 298.1838, found 298.1851.

Pinacol ester 21

To a solution of flame-dried $CrCl_2$ (1.19 g, 9.68 mmol) in THF (20 mL) was added $Cl_2CHB(pin)$[16] (0.370 mL, 2.42 mmol) *via* syringe. Then, a solution of aldehyde **18** (361 mg, 1.21 mmol) and LiI (649 mg, 4.84 mmol) in THF (4 mL) was added *via* cannula at 0 °C. After being stirred for 5 min, the reaction mixture was allowed to warm to room temperature. After being stirred for 7 h, the reaction was quenched with H_2O (40 mL), and the mixture was extracted with EtOAc (50 mL x 3). The combined organic layers were washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/10) to give pinacol ester **21** (398 mg, 0.944 mmol, 78%, *E*/*Z* = >95:5) as a colorless oil.

21: $[\alpha]_D^{23}$ –33.5 (*c* 0.237, $CHCl_3$); IR (neat): 3334, 2952, 1696, 1639, 1529, 1363, 1250, 1146, 838 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.55 (dt, *J* = 18.3 Hz, 6.8 Hz, 1H), 5.50 (d, 18.3 Hz, 1H), 4.44 (d, *J* = 8.7 Hz, 1H), 4.12 (t, *J* = 8.3 Hz, 2H), 3.74 (brs, 1H), 2.35 (d, *J* = 4.8 Hz, 2H), 2.13 (d, *J* = 2.7 Hz, 2H), 1.77 (t, *J* = 2.7 Hz, 3H), 1.67-1.39 (m, 4H), 1.26 (s, 12H), 0.97 (t, *J* = 8.3 Hz, 2H), 0.04 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 156.2, 149.1, 83.1, 78.7, 76.7, 62.9, 49.9, 41.5, 33.7, 24.8, 24.7, 18.6, 17.7, 3.4, -1.5; HRMS (FAB): calcd for $C_{22}H_{40}NO_4SiBK$ ($[M+K]^+$) 460.2457, found 460.2434.

Dieneyne 24

To a solution of pinacol ester **21** (20.0 mg, 0.0480 mmol) and (*E*)-1-iodo-1-pentene[22] (310 μL, 0.333 mmol) in degassed DMSO (0.48 mL) were added Cs_2CO_3 (92.9 mg, 0.285 mmol) and 2nd generation XphosPd cycle (3.74 mg, 4.75 μmol) at room temperature. After stirring for 10 min, the reaction mixture was warmed to 50 °C with stirring for 1.5 h. The reaction was allowed to cool to room temperature and quenched with sat. NH_4Cl (1 mL). The mixture was extracted with EtOAc (2 mL x 3). The combined organic layers were washed with brine (1 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/20) to give dieneyne **24** (14.1 mg, 0.0388 mmol 82%) as a white amorphous solid.

24: $[\alpha]_D^{27}$ –26.3 (*c* 0.291, $CHCl_3$); IR (neat): 3329, 2954, 1692, 1531, 1250, 1062, 988, 860, 838 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.02 (dd, *J* = 14.5, 11.6Hz, 2H), 5.60 (dt, 14.5, 7.2 Hz, 1H), 5.50 (dt, *J* = 14.5, 7.2 Hz, 1H), 4.14 (t, *J* = 8.2 Hz, 2H), 3.68 (brs, 1H), 2.25 (dd, *J* = 7.8, 7.2 Hz, 2H), 2.14 (d, *J* = 2.8 Hz, 2H), 2.03 (dt, 7.2, 7.2 Hz, 2H), 1.77 (t, *J* = 2.8 Hz, 3H), 1.63-1.45 (m, 4H), 1.45-1.34 (m, 2H), 0.96 (t, *J* = 8.2 Hz, 2H), 0.90 (t, *J* = 7.2 Hz, 3H), 0.03 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 156.3, 133.6, 133.4, 130.1, 126.7, 78.8, 75.8, 62.8, 50.5, 38.4, 34.6, 33.8, 25.4, 22.5, 18.6, 17.7, 13.7, 3.4, –1.5; HRMS (EI): calcd for $C_{21}H_{37}NO_2Si$ (M^+) 363.2594, found 363.2586.

Pinacol ester 25

To a solution of dieneyne **24** (88.5 mg, 0.243 mmol) in THF (2.4 mL) were added CuCl (7.22 mg,

0.0729 mmol), NaO*t*-Bu (18.9 mg, 0.197 mmol), $PCy_3 \cdot HBF_4$ (40.3 mg, 0.109 mmol), B_2pin_2 (92.5 mg, 0.365 mmol) and MeOH (0.098 mL, 2.43 mmol) at room temperature. After stirring for 5 min, the reaction mixture was warmed to 40 °C with stirring for 1.5 h. The reaction was quenched with H_2O (2 mL). The mixture was extracted with EtOAc (5 mL x 3). The combined organic extracts were washed with brine (3 mL), dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/15) to give pinacol ester (**25**) (102 mg, 0.208 mmol, 86%) as a colorless oil.

25: $[\alpha]_D^{17}$ –21.2 (*c* 0.179, $CHCl_3$); IR (neat): 3330, 2954, 1692, 1531, 1370 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.28 (t, *J* = 6.8 Hz, 1H), 6.01 (dd, *J* = 15.2, 10.8 Hz, 2H), 5.60 (dt, *J* = 15.2, 6.8 Hz, 1H), 5.49 (dt, *J* = 15.2, 7.2 Hz, 1H), 4.37 (d, *J* = 7.9 Hz, 1H), 4.19 (t, *J* = 8.7 Hz, 2H), 3.66 (brs, 1H), 2.26-2.12 (m, 2H), 2.06 (brs, 2H), 2.03 (td, 7.3, 6.8 Hz, 2H), 1.66 (s, 3H), 1.60-1.36 (m, 4H), 1.46-1.37 (m, 2H), 1.26 (s, 12H), 0.97 (t, *J* = 8.7 Hz, 2H), 0.90 (t, *J* = 7.3 Hz, 3H), 0.03 (s, 9H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 156.3, 145.8, 140.7, 134.3, 117.7, 83.1, 83.0, 62.7, 50.3, 39.6, 34.5, 28.4, 27.7, 26.0, 25.2, 24.6, 17.7, 14.2, 13.9, -1.5; HRMS (FAB): calcd for $C_{27}H_{51}NO_4SiB$ ($[M+H]^+$) 492.3686, found 492.3665.

Vinyl iodide 26

To a solution of pinacol ester **25** (19.0 mg, 0.0386 mmol) in THF (0.39 mL) was added 2 M aq. NaOH

(0.116 mL, 0.232 mmol) at room temperature. After stirring for 1 h, iodine (14.7 mg, 0.116 mmol) was added to the reaction mixture. After stirring for 2.5 h, the reaction was quenched with sat. aq. $Na_2S_2O_3$. The mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/30) to give vinyl iodide **26** (8.70 mg, 0.0177 mmol, 46%) as a colorless oil.

26: $[\alpha]_D^{22}$ –20.6 (*c* 0.095, $CHCl_3$); IR (neat): 3325, 2953, 1689, 1532, 1250, 1061, 987, 859, 837 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.13 (t, *J* = 7.3 Hz, 1H), 6.08-5.96 (m, 2H), 5.65-5.57 (m, 1H), 5.52-5.43 (m, 1H), 4.37 (d, *J* = 7.7 Hz, 1H), 4.13 (t, *J* = 7.8 Hz, 1H), 3.67 (brs, 1H), 2.36 (s, 3H), 2.24-2.18 (m, 2H), 2.07-2.01 (m, 4H), 1.62-1.27 (m, 4H), 0.97 (t, *J* = 7.8 Hz, 2H), 0.90 (t, *J* = 7.3 Hz, 3H), 0.04 (s, 12H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 156.3, 140.8, 133.7, 133.6, 130.0, 126.5, 93.9, 62.9, 50.5, 38.3, 37.5, 34.1, 30.3, 27.5, 25.2, 22.5, 17.8, 13.7, -1.5; HRMS (FAB): calcd for $C_{21}H_{38}NO_2SiKI$ ($[M+K]^+$) 530.1354, found 530.1328.

Amine 27

To a solution of vinyl iodide **27** (80.6 mg, 0.164 mmol) in DMSO (1.64 mL) was added CsF (175 mg, 1.15 mmol) at room temperature. After stirring at 70 °C for 2 h, the reaction was quenched with saturated aqueous NH_4Cl (2 mL). The reaction mixture was extracted with EtOAc (5 mL x 3). The

combined organic layers were washed with brine (2 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$ = 1/80 to 1/10) to give amine **27** (46.3 mg, 0.133 mmol, 81%) as a colorless oil.

27: $[\alpha]_D^{27}$ +5.9 (*c* 0.065, $CHCl_3$); IR (neat): 2926, 1456, 988 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 6.16-6.00 (m, 3H), 5.63 (dt, *J* = 14.5, 7.3 Hz, 1H), 5.52 (dt, *J* = 14.5, 7.3 Hz, 1H), 5.29 (brs, 2H), 2.98 (brs, 1H), 2.36 (s, 3H), 2.43-2.05 (m, 2H), 2.04 (q, *J* = 2.3 Hz, 2H), 1.64-1.36 (m, 6H), 0.90 (t, *J* = 7.3 Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 140.5, 134.4, 134.1, 130.0, 125.8, 94.1, 51.5, 38.5, 34.6, 34.1, 30.3, 27.5, 25.1, 22.4, 13.7; HRMS (FAB): calcd for $C_{15}H_{27}NI$ ($[M+H]^+$) 348.1188, found 348.1180.

Fmoc-protected amine 10

To a solution of amine **27** (46.3 mg, 0.133 mmol) in THF (0.9 mL) and H_2O (0.4 mL) were added $NaHCO_3$ (12.3 mg, 0.146 mmol) and FmocCl (42.3 mg, 0.146 mmol) at 0 °C. After stirring for 40 min at the same temperature, the mixture was quenched with sat. aq. NH_4Cl (2 mL), and extracted with EtOAc (5 mL x 3). The combined organic layers were washed with brine (2 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/15) to give Fmoc-amine **10** (67.0 mg, 0.112 mmol, 84%) as a white solid.

10: $[\alpha]_D^{24}$ –11.3 (*c* 0.148, $CHCl_3$); IR (neat): 3318, 2946, 1686, 1541, 1254, 986, 736 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.77 (d, *J* = 7.7 Hz, 2H), 7.58 (d, *J* = 7.3 Hz, 2H), 7.40 (dd, *J* = 7.7, 7.2 Hz, 2H), 7.33 (dd, *J* = 7.3, 7.2 Hz, 2H), 6.16 (t, *J* = 6.3 Hz, 1H), 6.06-5.94 (m, 2H), 5.69-5.56 (m, 1H), 5.55-5.34 (m, 1H), 4.53 (d, *J* = 6.8 Hz, 1H), 4.50-4.33 (m, 2H), 4.20 (t, *J* = 6.8 Hz, 1H), 3.68 (brs, 1H), 2.35 (s, 3H), 2.28-2.10 (m, 2H), 2.08-1.97 (m, 4H), 1.46-1.30 (m, 6H), 0.89 (t, *J* = 7.2 Hz, 3H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 155.9, 143.93, 143.88, 141.3, 140.8, 133.7, 129.9, 127.6, 127.0, 126.4, 125.0, 119.9, 93.9, 66.3, 50.7, 47.3, 38.3, 34.6, 33.9, 30.3, 27.5, 25.2, 22.4, 13.7; HRMS (FAB): calcd for $C_{30}H_{36}INO_2$ ($[M+H]^+$) 570.1869, found 570.1883.

Octanene 28

To a solution of vinyl iodide **10** (14.0 mg, 0.0246 mmol) and vinyl stannane **9** (36.5 mg, 0.0386 mmol), which was prepared from (4*R*,5*S*)-4-{[(4-methoxybenzyl)oxy]methyl}-2,2-dimethyl-5-(prop-1-yn-1-yl)-1,3-dioxolane as described in the preceding paper,[1] in DMF (0.5 mL) were added LiCl (2.1 mg, 0.0492 mmol) and Ph_3As (7.5 mg, 0.0246 mmol), $Pd_2(dba)_3$ (6.4 mg, 6.15 μmol) at room temperature. After stirring for 5 min, the mixture was warmed to 40 °C and stirred for 2 h. The reaction was quenched with pH 6.8 phosphate buffer (1 mL) and H_2O (1 mL), and the resulting mixture was extracted with EtOAc (5 mL x 3). The combined organic layers were washed with brine (1 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel

column chromatography (EtOAc/hexane = 1/10) to give octaene **28** (11.0 mg, 0.0102 mmol, 41%) as a colorless oil.

28: $[\alpha]_D^{22}$ –14.7 (*c* 0.166, $CHCl_3$); IR (neat): 3337, 2953, 1790, 1744, 1715, 1616, 1523, 1450, 1234 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.76 (dd, *J* = 8.3, 7.5 Hz, 4H), 7.59 (dd, *J* = 18.6, 7.5 Hz, 4H), 7.43-7.37 (m, 5H), 7.32-7.28 (m, 4H), 6.61 (d, *J* = 15.8 Hz, 1H), 6.36 (d, *J* = 11.0 Hz, 1H), 6.33 (d, *J* = 11.0 Hz, 1H), 6.23 (d, *J* = 15.1 Hz, 1H), 6.07-5.99 (m, 5H), 5.71 (d, *J* = 9.8 Hz, 1H), 4.50-4.48 (m, 1H), 4.45-4.37 (m, 3H), 4.28 (t, *J* = 7.5 Hz, 1H), 4.21 (t, *J* = 6.5 Hz, 1H), 3.72 (brs, 1H), 2.23-2.11 (m, 2H), 2.05-1.99 (m, 2H), 1.83 (s, 3H), 1.74 (s, 3H), 1.66-1.61 (m, 3H), 1.43-1.25 (m, 5H), 0.94-0.87 (m, 21H), 0.57-0.52 (m, 12H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 168.7, 167.1, 145.9, 145.8, 144.0, 141.3, 138.9, 134.0, 133.7, 133.68, 133.66, 131.1, 130.4, 130.0, 127.8, 127.6, 127.1, 127.0, 125.2, 125.1, 125.0, 122.0, 120.0, 119.94, 119.86, 73.2, 72.9, 70.6, 66.4, 50.9, 47.4, 46.9, 40.8, 38.3, 34.7, 29.1, 25.9, 25.5, 22.5, 17.5, 13.7, 13.2, 12.4, 6.8, 4.9, 0.0, –1.6; HRMS (FAB): calcd for $C_{70}H_{91}NO_6Si_2K$ ($[M+K]^+$) 1136.6022, found 1136.6050.

Macrolactam 29

To a solution of octaene **28** (11.0 mg, 0.0102 mmol) in CH_2Cl_2 (0.2 mL) was added DBU (6.1μL, 0.0408 mmol) at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 1 h. Then, THF (10 mL), HATU (38.7 mg, 0.102 mmol) and DIEPA (21 μL, 0.122 mmol) were added

to the reaction mixture. After stirring at room temperature for 16 h, the reaction was quenched with pH 6.8 phosphate buffer (5 mL). The mixture was extracted with EtOAc (5 mL x 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/6) to give macrolactam **29** (4.6 mg, 66% overall) as a colorless oil.

29: ^{1}H-NMR (400 MHz, pyridine-d_5) δ 8.10 (d, J = 9.6 Hz, 1H), 7.53 (dd, J = 15.0, 10.5 Hz, 1H), 6.49-6.42 (m, 3H), 6.35-6.29 (m, 2H), 6.25-6.15 (m, 2H), 6.06 (dd, J = 14.5, 10.3 Hz, 1H), 5.84 (t, J = 10.1 Hz, 1H), 5.80-5.76 (m, 1H), 5.63-5.59 (m, 3H), 4.62 (d, J = 8.9 Hz, 1H), 4.60 (brs, 1H), 2.39-2.35 (m, 2H), 2.03-1.97 (m, 3H), 1.86-1.83 (m, 1H), 1.73 (s, 3H), 1.62-1.58 (m, 1H), 1.49-1.42 (m, 1H), 1.37-1.30 (m, 2H), 1.28-1.23 (m, 2H), 1.10-1.05 (m, 18H), 0.84 (t, J = 7.2 Hz, 3H), 0.83-0.69 (m, 12H); ^{13}C-NMR (150 MHz, pyridine-d_5): δ 169.0, 143.6, 141.5, 139.4, 139.1, 134.7, 134.6, 133.4, 133.2, 131.5, 130.8, 130.1, 129.4, 126.8, 125.7, 122.4, 75.9, 72.5, 51.3, 39.7, 37.4, 35.3, 30.8, 27.3, 23.2, 14.2, 13.1, 12.4, 7.55, 7.52, 5.74, 5.67; HRMS (EI): calcd for $C_{41}H_{69}NO_3Si_2$ (M^+) 679.4816, found 679.4811.

16,17-Dihydroheronamide C (8)

To a solution of macrolactam **29** (4.6 mg, 6.76 μmol) in THF (0.7 mL) was added TBAF (27 μL, 0.0271 mmol) at 0 °C. After stirring for 20 min, the reaction was quenched with pH 6.8 phosphate

buffer (1.0 mL). The mixture was extracted with EtOAc (5 mL x 3) and the combined organic layers were washed with brine (2 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$ = 1/20) to give macrolactam **8** (2.0 mg, 4.43 μmol, 65%) as a white solid.

8: See Supporting Figure 3 for the CD spectrum; ^{1}H NMR (600 MHz, pyridine-d_5) δ 8.07 (d, J = 10.1 Hz, 1H), 7.51 (dd, J = 14.9, 10.9 Hz, 1H), 6.45 (m, 2H), 6.38 (m, 2H), 6.28 (s, 2H), 6.21 (m, 2H), 6.05 (dd, J = 15.0, 10.5 Hz, 1H), 5.95 (d, J = 7.9 Hz, 1H), 5.77 (m, 1H), 5.61 (m, 1H), 5.56 (dd, J = 8.9, 5.4 Hz, 1H), 5.38 (d, J = 7.8 Hz, 1H), 5.01 (d, J = 8.9 Hz, 1H), 4.61 (s, 1H), 2.35 (m, 2H), 2.27 (m, 1H), 1.99 (m, 2H), 1.83 (s, 3H), 1.68 (s, 3H), 1.59 (m, 1H), 1.43 (m, 1H), 1.32 (m, 2H), 1.23 (m, 2H), 0.82 (t, J = 7.4 Hz, 3H); ^{13}C NMR (150 MHz, pyridine-d_5) δ 168.7, 143.7, 141.2, 139.6, 138.4, 134.4, 133.7, 132.94, 132.92, 132.91, 131.8, 131.1, 129.8, 129.0, 125.8, 124.8, 122.8, 73.8, 71.6, 50.9, 39.3, 36.9, 34.9, 30.4, 26.9, 22.7, 13.8, 12.7, 11.9; HRMS (ESI) calcd for $C_{29}H_{42}NO_3$ $[M + H]^+$ 452.3165, found 452.3189.

Nonaene 31

To a solution of vinyl iodide **12** (14.2 mg, 25.0 μmol) and vinyl stannane *ent*-**9** (15.0 mg, 15.9 μmol) in DMF (0.32 mL) were added LiCl (1.35 mg, 31.8 μmol), Ph_3As (4.87 mg, 15.9 μmol), and $Pd_2(dba)_3$ ·$CHCl_3$ (4.10 mg, 3.98 μmol) at room temperature. After stirring for 5 min, the mixture was warmed

to 40 °C, and stirred further for 2 h. The reaction was quenched by an addition of pH 6.8 phosphate buffer (1 mL) and H_2O (1 mL), and the resultant mixture was extracted with EtOAc (5 mL x 3). The combined organic layers were washed with brine (1 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/10) to give nonaene **31** (6.87 mg, 6.27 μmol, 40%) as a colorless oil.

31: $[\alpha]_D^{22}$ +14.7 (*c* 0.166, $CHCl_3$); IR (neat): 3337, 2953, 1790, 1744, 1715, 1616, 1523, 1450, 1234 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.76 (dd, *J* = 9.0, 7.8 Hz, 4H), 7.62 (d, *J* = 7.2 Hz, 2H), 7.57 (d, *J* = 7.8 Hz, 2H), 7.42-7.38 (m, 5H), 7.33-7.28 (m, 4H), 6.61 (d, *J* = 15.0 Hz, 1H), 6.50-6.42 (m, 2H), 6.35 (dd, *J* = 15.0, 11.4 Hz, 1H), 6.26 (d, *J* = 15.0 Hz, 1H), 6.13-5.99 (m, 5H), 5.70 (d, *J* = 9.0 Hz, 2H), 5.60 (dt, *J* = 15.0, 14.4 Hz, 1H), 5.52-5.46 (m, 1H), 5.35 (t, *J* = 16.2 Hz, 1H), 4.66 (d, *J* = 9.0 Hz, 1H), 4.49 (dd, *J* = 8.4, 4.8 Hz, 1H), 4.44 (d, *J* = 7.8 Hz, 2H), 4.39-4.37 (m, 3H), 4.28 (t, *J* = 7.2 Hz, 1H), 4.21 (t, *J* = 6.6 Hz, 1H), 3.88-3.77 (m, 1H), 2.40-2.20 (m, 4H), 2.03 (q, *J* = 6.6 Hz, 2H), 1.85 (s, 3H), 1.84 (s, 3H), 1.39 (sext, *J* = 7.2 Hz, 2H), 0.93-0.89 (m, 21H), 0.57-0.52 (m, 12H); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 167.1, 150.8, 145.8, 145.7, 144.0, 143.9, 141.3, 138.8, 138.6, 135.4, 134.6, 134.2, 134.1, 133.9, 133.8, 131.8, 131.5, 130.4, 130.05, 129.96, 129.0, 128.4, 128.3, 128.2, 127.8, 127.6, 127.1, 127.0, 125.14, 125.10, 125.07, 120.01, 119.96, 119.9, 73.1, 72.9, 66.5, 66.3, 47.3, 46.9, 37.5, 34.7, 29.7, 22.4, 13.8, 13.7, 13.2, 12.6, 6.8, 4.8; HRMS (ESI): calced for $C_{70}H_{89}O_6NNaSi_2$ $[M+Na]^+$ 1118.6121, found 1118.6115.

Macrolactam 32

To a solution of nonaene **31** (6.87 mg, 6.27 μmol) in CH_2Cl_2 (0.13 mL) was added DBU (3.75 μL, 25.1 μmol) at 0 °C. After stirring for 1 h, THF (6.3 mL), HATU (23.8 mg, 0.627 mmol) and DIEPA (13.1 μL, 0.752 mmol) were added to the mixture. After stirring at room temperature for 16 h, the reaction was quenched with an addition of pH 6.8 phosphate buffer (5 mL). The mixture was extracted with EtOAc (5 mL x 3). The combined organic layers were washed with brine (5 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/hexane = 1/6) to give macrolactam **32** (2.25 mg, 3.32 μmol, 53% overall) as a colorless oil.

32: ^{1}H-NMR (600 MHz, pyridine-d_5): δ 7.85 (d, J = 10.2 Hz, 1H), 7.41 (dd, J = 15.6, 7.8 Hz, 1H), 6.45 (dd, J = 14.4, 12.0 Hz, 1H), 6.37-6.25 (m, 5H), 6.21-6.16 (m, 1H), 6.12 (d, J = 11.4 Hz, 1H), 6.02 (dd, J = 15.0, 10.2 Hz, 1H), 5.87-5.80 (m, 3H), 5.75 (dt, J = 15.0, 14.4 Hz, 1H), 5.59 (dt, J = 15.0, 14.4 Hz, 1H). 5.54 (d, J = 7.8 Hz, 1H), 4.65 (d, J = 8.4 Hz, 1H), 4.58-4.54 (m, 1H), 3.69-3.55 (m, 1H), 2.53-2.51 (m, 2H), 2.44-2.34 (m, 2H), 1.96 (q, J = 6.6 Hz, 2H), 1.86 (s, 3H), 1.77 (s, 3H), 1.31 (sext, J = 7.8 Hz, 2H), 1.08-1.03 (m, 18H), 0.81 (t, J = 7.8 Hz, 3H), 0.75-0.68 (m, 12H); ^{13}C-NMR (150 MHz, pyridine-d_5): δ 162.1, 142.9, 140.7, 138.8, 137.2, 134.5, 132.5, 131.7, 131.1, 130.9, 130.6, 130.5, 130.4, 130.3, 129.1, 128.3, 125.3, 124.1, 74.6, 71.3, 49.7, 41.0, 38.4, 34.3, 29.4, 22.1, 13.2, 12.0, 6.6,

6.5, 4.73, 4.68; HRMS (ESI): calced for $C_{41}H_{68}O_3NSi_2$ $[M+H]^+$ 678.4732 found 678.4719.

***ent*-Heronamide C (*ent*-1)**

To a cooled (0 °C) solution of macrolactam **32** (2.25 mg, 3.32 μmol) in THF (0.33 mL) was added TBAF (1.0 M solution of in THF, 13.3 μL, 13.3 μmol). The reaction was stirred at 0 °C for 30 min. The reaction was quenched with pH 6.8 phosphate buffer (0.5 mL). The resultant mixture was extracted with EtOAc (5 mL x 3). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (MeOH/$CHCl_3$ = 1/20) to *ent*-heronamide C (*ent*-**1**) (1.34 mg, 2.98 μmol, 90%) as a colorless oil. Spectroscopic data for *ent*-**1** except for CD data (see Supporting Figure 3) were matched with those reported.

Biological tests

The growth inhibition and morphological changes by synthetic 8-deoxyheronamide C (**2**), *ent*-heronamide C (*ent*-**1**), and 16,17-dihydroheronamide C (**8**) were tested as described previously[17] using the following fission yeast strains: JY1 (h^-) and *erg* mutants[18] (*h^- ura4-C190T leu1-32 erg2::ura4$^+$*; *h^- ura4-C190T leu1-32 erg31::ura4-FOA^R erg32::ura4$^+$*).

Supporting Information

MD-simulated atom distribution and tilt angle of heronamides A–C, 8-deoxyheronamide C, 16,17-

dehydroheronamide C, and cholesterol in DMPC lipid bilayer; methods for the MD simulations; Raman spectra of heronamide C and 16,17-dehydroheronamide C; methods for the Raman experiments; CD spectra of 8-deoxyheronamide C, *ent*-heronamide C, and 16,17-dehydroheronamide C; copies of the NMR spectra of synthesized compounds (PDF).

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