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Total Synthesis of (−)-Thallusin: Utilization of Enzymatic Hydrolysis Resolution

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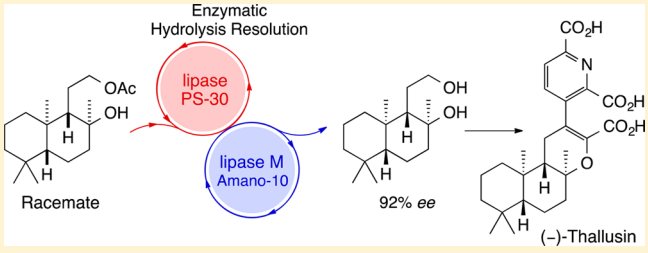
Supporting Information

ABSTRACT: (−)-Thallusin, isolated from a marine bacterium, is the only known natural product to act as an algal morphogenesis inducer. Because (−)-thallusin can only be obtained in exceedingly limited amounts from microbial cultivation, a synthetic supply of this compound is highly desirable. Here, we describe a novel synthetic pathway to (±)-thallusin and the first asymmetric synthesis of (−)-thallusin utilizing the enzymatic hydrolysis resolution with the combination of lipase PS-30 and lipase M Amano-10.



INTRODUCTION

In 2005, Matsuo and co-workers reported the isolation of an algal growth factor (named thallusin, Figure 1) from the marine

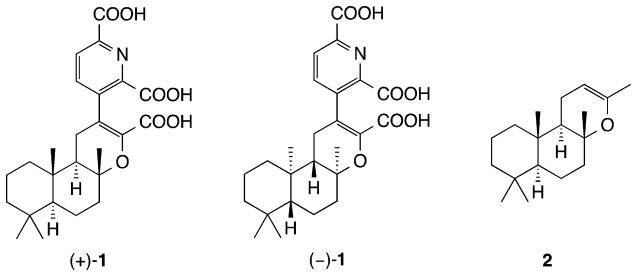


Figure 1. Structures of (+)-thallusin and (−)-thallusin.

bacterium strain YM2-23 belonging to the Cytophaga-Flavobacterium-Bacteroides (CFB) group (that adheres to a green alga *Monostroma* sp.).[1] Thallusin is indispensable for forming the foliaceous morphology of green macroalgae and strongly induces cell-differentiation at the exceptionally low concentration of between 1 fg/mL and 1 ag/mL.

The molecular structure of thallusin was established mainly using 2D NMR techniques and single-crystal X-ray diffraction analysis, although the absolute configuration was not assigned. Thus, Snider and co-workers accomplished the first synthesis of (+)-**1** from sclareol oxide **2**.[2] However, (+)-**1** did not display morphogenesis-inducing activity, and so the absolute structure of the natural product was determined to be antipodal (−)-**1**. Later, two racemic syntheses of thallusin were achieved independently by Snider's group[3] and our group[4] on the basis of a common synthetic strategy that featured a tandem cyclization of (*E*,*E*)-farnesol derivatives. The morphogenesis-inducing activity of the synthesized (±)-thallusin was examined for *Monostroma oxyspermum*. It was found that (±)-thallusin showed comparable potency to natural thallusin, although the efficacy was reduced by 50%.[4]

Recently, there has been a decrease in the number and variety of marine plants in the oceans of the world.[5−10] Although the exact mechanism for this reduction in biodiversity is unclear, the biological investigation of thallusin, which controls the growth of marine plants, may help limit any further decline in their number.[1] However, natural thallusin can only be obtained in exceedingly limited amounts from microbial cultivation (i.e., 0.14 mg from 180 L of culture fluid of the YM2-23 strain). Moreover, our previous synthesis of (±)-thallusin consisted of a large number of steps (22 steps), which included several low-yielding reactions (2.1% overall yield).[4] Thus, we planned on developing an efficient synthetic procedure in order to provide a practical supply of material for further biological studies.

RESULTS AND DISCUSSION

The key steps of our novel synthetic plan are shown in Scheme 1. Because the dipicolinic acid moiety is high polarity, it must be formed in the latter stages of total synthesis. Thus, triflate derivative **3** was designed as a key intermediate in Suzuki−Miyaura coupling[11] with a pyridylboronic acid derivative. Compound **3** was expected to be constructed through the intramolecular O−H insertion reaction[12] of **4** with $Rh_2(OAc)_4$

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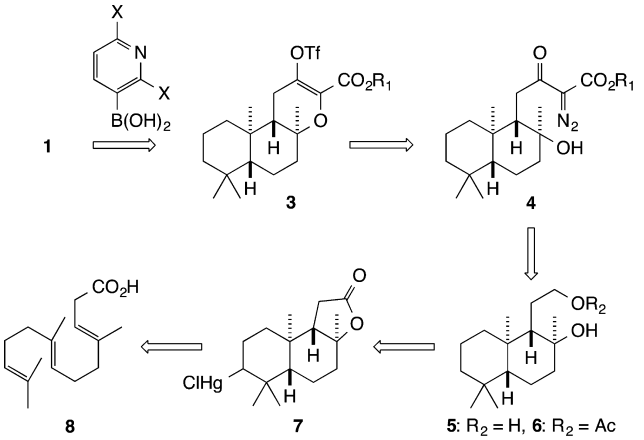
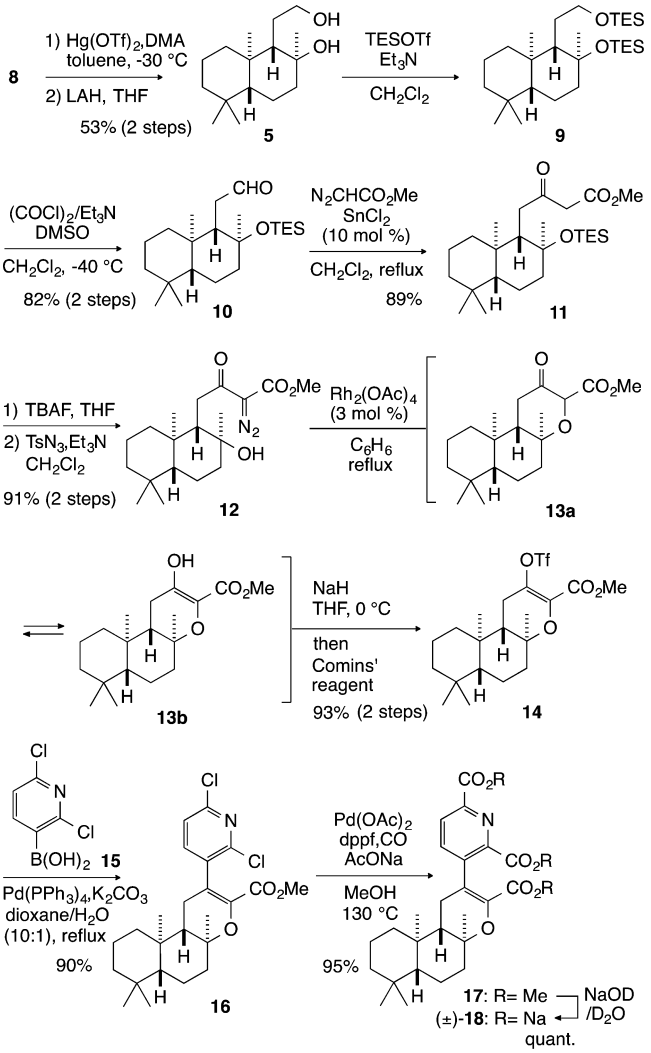
Scheme 1. Retrosynthetic Analysis of Thallusin



Scheme 2. Novel Synthetic Pathway to (±)-Thallusin



after construction of α-diazo β-keto ester from **5**. According to our knowledge,[13] **5** could be prepared by $Hg(OTf)_2$-induced olefin cyclization of trienecarboxylic acid **8** via the tricyclic ring product **7** with the same relative stereochemistry as that of thallusin. Therefore, **8** was selected as the starting material. In addition, we anticipated that the asymmetric synthesis of thallusin would be achieved by including the enzymatic hydrolysis resolution of **6**, which could be readily constructed from **5**, into the synthetic route.

The established racemic route is illustrated in Scheme 2. Starting material **8** was readily prepared from commercially available (*E*,*E*)-farnesol via hydrolysis of the cyano derivative.[14] Using a combination of Nishizawa reagent[15] and *N*,*N*-dimethylaniline (DMA) in CH_3NO_2 at −30 °C, the desired cyclization of **8** proceeded smoothly to give **7** as a major product.[16] The crude extract was directly treated with LAH to obtain the bicyclic diol **5** in 53% yield. The hydroxyl groups of **5** were converted to TES ethers, and then Swern oxidation of **9** was carried out at −40 °C in CH_2Cl_2 according to Spur's report.[17] Selective cleavage of the TES group on the primary moiety was performed, followed by oxidation of the resulting alcohol, which proceeded to give the desired **10** in 82% yield. This aldehyde was converted to β-keto ester **11** by the addition of methyl diazoacetate in the presence of a catalytic amount of $SnCl_2$ at reflux.[18] The TES group of **11** was deprotected with TBAF to generate an acid-sensitive β-keto ester, which without isolation, was immediately treated with Et_3N and TsN_3 to give a diazo alcohol **12** in 91% yield. The addition of 3 mol % of $Rh_2(OAc)_4$ to **12** in toluene upon reflux allowed the desired cyclization to proceed instantly giving **13** as a keto−enol mixture. The mixture was treated with NaH at 0 °C, and the resulting sodium enolate was reacted with Comins' reagent[19] at room temperature to obtain a single enol triflate **14**. With the requisite **14** in hand, we then attempted Suzuki−Miyaura coupling with **15**. Although the coupling with **15**[20] was accomplished by the combination of $Pd(PPh_3)_4$, $Ba(OH)_2$, and LiCl in the previous synthesis of (±)-thallusin, unfortunately, the desired coupling of **14** with **15** did not proceed under the same conditions. After an extensive search for alternative conditions,[21] we found that the combination of the $Pd(PPh_3)_4$ catalyst and K_2CO_3 in a 10:1 mixture of dioxane and H_2O without LiCl was suitable for the reaction, giving **16** in 90% yield within 15 min. The coupling product **16** was converted to **17** via the carbonylation with $Pd(OAc)_2$·dppf and NaOAc in MeOH under a CO atmosphere (30 atm),[22] and the trimethyl ester of **17** was finally alkaline-hydrolyzed to give the trisodium salt isomer (±)-**18** of (±)-thallusin.[23] The ^1H and ^{13}C NMR spectral data of (±)-**18** correspond well to those previously reported in the literature.[1,2]

Building on the successful synthesis of (±)-**18**, we explored methods suitable for the preparation of optically active **5** to accomplish the asymmetric synthesis of thallusin. Additionally, the paucity of chemical approaches suitable for the construction of the terpene skeleton with the same absolute stereochemistry as natural thallusin motivated our chemical investigations aimed at securing optically pure thallusin by synthetic means.[24] In fact, no asymmetric synthesis of (−)-thallusin had been reported in the literature.

In 1996, Oritani and co-worker reported that the hydrolysis resolution of (±)-**6**[25] proceeded using lipase PS-30 at 30 °C to give optically enriched (−)-**5** in ≥98% ee (Scheme 3).[26,27] This method is considered to be pioneering work and a practical procedure for the preparation of optically active **5**. However, the reaction with lipase PS-30 is unsuitable for the procurement of enantiomeric (+)-**5** that could be advanced to (−)-thallusin by the established 10-step sequence. In addition, the optical purity of the recovered (−)-**6**, which was unreacted in the hydrolysis resolution, was moderate (up to 51% ee). Therefore, our study focused on the further enzymatic hydrolysis of the

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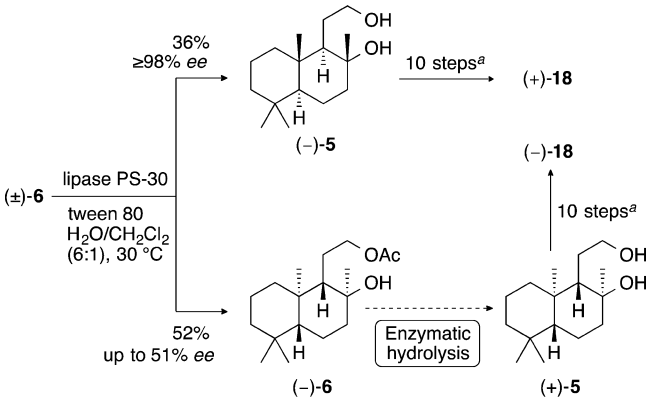
Scheme 3. Synthetic Strategy of (+)-18 and (−)-18



[a]This 10-step sequence is identical to that described in Scheme 2.

recovered acetate (−)-**6** to obtain optically pure (+)-**5**. Initially, we carried out the hydrolysis resolution of racemic substrate (±)-**6** with a wide variety of commercially available lipases including G Amano 50 (source; *Penicillium camemberti*), AYS Amano (*Candida rugosa*), F-AP Amano 15 (*Rhizopus oryzae*), AS Amano (*Aspergillus niger*), and M Amamo-10 (*Mucor javanicus*) to screen for the most suitable enzyme (entries 1−5, Table 1). These investigations identified lipase M Amamo-10 as

Table 1. Screen of Lipases Using the Hydrolysis Resolution of (±)-6

entry[a]	lipase	substrate	time (days)	product	yield (%)	ee (%)
1	G Amano 50	(±)-**6**	3	n.d.	0 (99)[b]	nd
2	AYS Amano	(±)-**6**	3	(+)-**5**	79 (0)[b]	4
3	F-AP Amano 15	(±)-**6**	3	(+)-**5**	20 (68)[b]	60
4	AS Amano	(±)-**6**	3	(−)-**5**	12 (86)[b]	49
5	M Amano 10	(±)-**6**	3	(+)-**5**	32 (60)[b]	68
6	M Amano 10	(−)-**6**[c]	3	(+)-**5**	46 (41)[b]	77
7	M Amano 10	(−)-**6**[c]	1	(+)-**5**	29 (63)[b]	92

[a]A suspension of (±)-**6** (75 mg, 0.25 mmol), 0.2% aqueous Tween 80 solution (0.1 mL), and lipase (50 mg) in 0.1 M phosphate buffer (3 mL) and CH_2Cl_2 (0.5 mL) was stirred at 35 °C. [b]Recovery (%) of nonhydrolyzed **6**. [c](−)-**6** with 48% ee was used as a substrate.

suitable for obtaining the desired (+)-**5**, although the optical purity was moderate (68% ee) (entry 5). Thus, we attempted the hydrolysis resolution with optically enriched substrate (−)-**6**, which was recovered from the reaction with lipase PS-30, in the presence of lipase M Amamo-10 with the aim of enhancing the enantiomeric excess (entries 6 and 7). Using (−)-**6** with 48% ee, the optical purity of the product (+)-**5** was increased to 77% ee at 35 °C for 3 days (entry 6). Moreover, 92% ee was achieved under the same conditions when the reaction was stopped within 1 day (entry 7).

With a concise asymmetric pathway to (+)-**5** established (Scheme 3), the first synthetic sample of (−)-**18** as well as (+)-**18** could be synthesized on a scale of hundreds of milligrams (the overall yields of (−)-**18** and (+)-**18** were 8% and 19% from (±)-**6**, respectively). Our attention then shifted to exploring the potential morphogenesis-inducing properties of these compounds for *Monostroma oxyspermum*.

When (−)-, (±)-, and (+)-**18** were independently added to the unicellular system of *M. oxyspermum* at a concentration of 1 fmol/mL to 100 nmol/mL (10^8 fmol/mL) under light conditions, (−)-**18** and (±)-**18** were observed to induce cellular-differentiation and then morphogenesis at 1.5 and 3.0 fmol/mL after 7 days of cultivation, respectively (Table 2).

Table 2. Morphogenesis Inducing Activity for *M. oxyspermum* after 7 Days of Cultivation

compd	MEC[a] (fmol/mL)	OEC[b] (fmol/mL)
(−)-**18**	1.5	>12.2[c]
(±)-**18**	3.0	>24.4[c]
(+)-**18**	≥25000	nd

[a]Minimum effective concentration. [b]Optimal effective concentration. [c]Effective concentration is in a range of not higher than 10 nmol/mL.

However, by observing the degree of growth of the formed fronds, optimal effective concentrations of (−)-**18** and (±)-**18** were found to be more than 12.2 fmol/mL and 24.4 fmol/mL, respectively. (+)-**18** also acted as a morphogenesis inducer at high concentration (25000 fmol/mL) in this assay because a minuscule amount of (−)-**18** was present in the synthesized (+)-**18**. It is conceivable that pure (+)-**18** is completely inactive in accordance with previous reports.[1,2] The most interesting finding from our observations was that (−)-**18** and (±)-**18** showed growth-suppressing activity at high concentration. Growth suppression of fronds was clearly observed for (−)-**18** or (±)-**18** at a concentration range of 10 to 100 nmol/mL.[28] This activity may have been overlooked in previous studies due to the relatively high dose of compound needed. Although more detailed investigation is required for full elucidation of the effect, the hidden property of thallusin could be investigated using this assay.

■ CONCLUSION

The synthesis of (±)-thallusin has been achieved on the basis of $Hg(OTf)_2$−DMA complex induced tandem cyclization of **8** as a key step. This novel procedure (28% overall yield in 12 steps from **8**) is a significant improvement by comparison to our previous approach,[4] which required 22 steps (longest linear sequence) and proceeded in only 2.1% overall yield. Moreover, this new strategy was applied to the asymmetric synthesis of (−)-**18** and (+)-**18**, which facilitated investigations into the biological properties of thallusin.

■ EXPERIMENTAL SECTION

(2*S,4a*R**,8a*R**)-1-(2-Hydroxyethyl)-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (5).** Initially, $Hg(OTf)_2$ was prepared by the following procedure; to a suspension of HgO (5.41 g, 25 mmol) in CH_3CN (50 mL) was added dropwise Tf_2O (7.05 g, 25 mmol) at 0 °C, and the mixture was stirred at 0 °C until the yellow color disappeared. The resulting colorless solution was transferred to 250 mL of volumetric flask and diluted with anhydrous CH_3CN to give a 0.1 M solution. A 0.1 M CH_3CN solution of $Hg(OTf)_2$ (145 mL, 14.5 mmol) was added to a mixture of **8** (3.03 g, 12.1 mmol) and *N*,*N*-dimethylaniline (1.76 g, 14.5 mmol) in CH_3NO_2 (55 mL) at −30 °C. The mixture was stirred for 1 h at the same temperature (−30 °C) and then quenched with saturated NaCl solution (150 mL) at room temperature. The organic layer was partitioned with AcOEt (100 mL), and the aqueous layer was extracted with AcOEt (2 × 50 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was successively added to a suspension of $LiAlH_4$ (1.84 g, 48.4 mmol) in THF (120 mL) at 0 °C, and the mixture was stirred for 1 h at the same temperature (0 °C). Aqueous NH_3 solution (28%, 20 mL) and CH_2Cl_2 (400 mL) were dropwise added to the mixture at room temperature until the gray color disappeared. The resulting white

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suspension was filtrated through a Celite pad, and the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc, 2:1) to give **5** (1.63 g, 53%) as a white solid: FT IR (neat) 3280, 2995, 2949, 2866, 2843 cm^{-1}; ^1H NMR (400 MHz in $CDCl_3$) δ 0.79 (6H, s), 0.88 (3H, s), 0.95 (2H, dd, J = 12.0, 2.0 Hz), 1.16 (1H, dd, J = 13.2, 4.4 Hz), 1.20 (3H, s), 1.28 (2H, m), 1.35−1.60 (3H, m), 1.61−1.65 (5H, m), 1.90 (1H, dt, J = 12.4, 3.2 Hz), 3.46 (1H, m), 3.78 (1H, dt, J = 10.4, 4.0 Hz); ^{13}C NMR (100 MHz in $CDCl_3$) δ 15.3, 18.4, 20.5, 21.5, 24.7, 27.9, 33.3, 33.4, 39.0, 39.3, 41.9, 44.3, 56.0, 59.1, 64.2, 73.1; MS (Cl) m/z 254 (M^+); HRMS (CI^+) m/z calcd for $C_{16}H_{30}O_2$ (M^+) 254.2246, found 254.2267.

Triethylsilane Derivative of (2*S,4a*R**,8a*R**)-1-(2-Hydroxyethyl)-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol (9).** Et_3N (3.93 g, 38.5 mmol) and then TESOTf (6.00 g, 19.2 mmol) were added to a solution of **5** (826 mg, 1.49 mmol) in CH_2Cl_2 (13 mL) at 0 °C. The mixture was stirred for 20 min at 0 °C and quenched with distilled water (10 mL) at room temperature. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc, 5:1) to give **9** (3.08 g, quant) as a colorless syrup: FT IR (neat) 2954, 2911, 2876 cm^{-1}; ^1H NMR (500 MHz in $CDCl_3$) δ 0.49−0.62 (12H, m), 0.77 (3H, s), 0.78 (3H, s), 0.85 (3H, s), 0.88 (2H, br d, J = 13.5 Hz), 0.93 (9H, t, J = 8.0 Hz), 0.96 (9H, t, J = 8.0 Hz), 1.10−1.26 (2H, m), 1.17 (3H, s), 1.33−1.50 (4H, m), 1.52−1.77 (5H, m), 1.88 (1H, br d, J = 12.0 Hz), 3.45 (1H, dt, J = 10.0, 5.5 Hz), 3.67 (1H, dt, J = 10.0, 6.0 Hz); ^{13}C NMR (125 MHz in $CDCl_3$) δ 4.4, 6.4, 6.8, 6.8, 7.1, 7.3, 15.6, 18.5, 20.6, 21.5, 24.8, 29.5, 33.2, 33.4, 39.7, 42.0, 44.4, 56.1, 58.5, 65.5; MS (Cl) m/z 483 (M^+ + H); HRMS (CI^+) m/z calcd for $C_{28}H_{59}O_2Si_2$ (M^+ + H) 483.4054, found 483.4043.

2-[(2*S,4a*R**,8a*R**)-2,5,5,8a-Tetramethyl-2-[(triethylsilyl)oxy]decahydronaphthalen-1-yl]acetaldehyde (10).** $(COCl)_2$ (3.88 g, 30.5 mmol) was added to a solution of DMSO (4.46 g, 57.1 mmol) in CH_2Cl_2 (50 mL) at −78 °C. After 15 min, a solution of **9** (3.08 g, 6.38 mmol) in CH_2Cl_2 (50 mL) was dropwise added to the mixture at the same temperature (−78 °C). The reaction mixture was allowed to warm to −40 °C and stirred for 1 h. The reaction was quenched with Et_3N (10 mL) at −78 °C, and the resulting mixture was allowed to room temperature. The organic layer was washed with distilled water, and the aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc, 8:1) to give **10** (1.93 g, 82%) as a white solid: FT IR (neat) 2951, 2935, 2874, 2711, 1726 cm^{-1}; ^1H NMR (400 MHz in $CDCl_3$) δ 0.56 (6H, q, J = 8.0 Hz), 0.79 (3H, s), 0.80 (3H, s), 0.89 (3H, s), 0.92 (9H, t, J = 8.0 Hz), 1.00 (1H, dd, J = 12.0, 2.4 Hz), 1.09−1.30 (3H, m), 1.17 (3H, s), 1.35−1.63 (5H, m), 1.68 (1H, m), 1.92 (1H, dd, J = 7.6, 4.8 Hz), 1.96 (1H, dt, J = 12.4, 3.2 Hz), 2.25 (1H, ddd, J = 16.0, 4.8, 2.0 Hz), 2.47 (1H, ddd, J = 16.0, 7.6, 3.2 Hz), 9.60 (1H, dd, J = 3.2, 2.0 Hz) ^{13}C NMR (75 MHz in $CDCl_3$) δ 6.8, 7.2, 15.5, 18.5, 20.4, 21.4, 24.8, 33.2, 33.6, 38.1, 40.1, 40.2, 41.7, 44.0, 56.0, 57.3, 76.4, 203.4; MS (Cl^+) m/z 366 (M^+); HRMS (CI^+) m/z calcd for $C_{22}H_{42}O_2$ Si (M^+) 366.2954, found 366.2931.

Methyl 3-Oxo-4-[(2*S,4a*R**,8a*R**)-2,5,5,8a-tetramethyl-2-[(triethylsilyl)oxy]-decahydronaphthalen-1-yl]butanoate (11).** Methyl diazoacetate (920 mg, 9.19 mmol) and then $SnCl_2$ (70.2 mg, 0.37 mmol) were added to a solution of **10** (1.35 g, 3.68 mmol) in CH_2Cl_2 (37 mL) at room temperature. The mixture was stirred for 1.5 h under reflux and quenched with saturated $NaHCO_3$ at room temperature. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc, 30:1) to give **11** (1.44 g, 89%) as a white solid: FT IR (neat) 2952, 2874, 1752, 1722 cm^{-1}; ^1H NMR (400 MHz in $CDCl_3$) δ 0.55 (6H, q, J = 8.0 Hz), 0.77 (6H, s), 0.87 (3H, s), 0.91 (9H, t, J = 8.0 Hz), 1.01 (1H, dd, J = 12.0, 2.0 Hz), 1.14 (3H, s), 1.17 (1H, m), 1.22 (1H, dd, J = 12.4, 3.2 Hz), 1.26−1.43 (3H, m), 1.51 (1H, dt, J = 13.6, 4.0 Hz), 1.55−1.69 (3H, m), 1.92 (1H, dt, J = 12.0, 3.2 Hz), 2.02 (1H, br t, J = 5.6 Hz), 2.42 (1H, dd, J = 16.8, 5.6 Hz), 2.65 (1H, dd, J = 16.8, 4.4 Hz), 3.48 (1H, d, J = 15.2 Hz), 3.52 (1H, d, J = 15.2 Hz), 3.72 (3H, s); ^{13}C NMR (100 MHz in $CDCl_3$) δ 6.8, 7.2, 15.6, 18.3, 20.5, 21.4, 24.9, 33.2, 33.3, 38.3, 39.5, 39.8, 41.7, 43.8, 48.7, 52.2, 55.7, 56.5, 76.3, 168.1, 202.7; MS (Cl) m/z 438 (M^+); HRMS (CI^+) m/z calcd for $C_{25}H_{46}O_4Si$ (M^+) 438.3166, found 438.3158.

Methyl 2-Diazo-4-[(2*S,4a*R**,8a*R**)-2-hydroxy-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl]-3-oxobutanoate (12).** A 1.0 M THF solution of TBAF (7.0 mL, 7.0 mmol) was added to a solution of **11** (1.44 g, 3.28 mmol) in THF (33 mL) at room temperature. The mixture was stirred for 30 min at the same temperature and then quenched with distilled water. The organic layer was partitioned with Et_2O (30 mL), and the aqueous layer was extracted with Et_2O (2 × 15 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was successively dissolved in CH_2Cl_2 (33 mL), and then Et_3N (1.32 g, 13.1 mmol) and TsN_3 (1.29 g, 6.56 mmol) were added to the mixture at room temperature. The reaction mixture was stirred for 4 h at the same temperature and quenched with saturated NH_4Cl. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc, 3:1) to give **12** (1.05 g, 91%) as a white solid: FT IR (neat) 3500, 2990, 2925, 2853, 2132, 1726, 1658 cm^{-1}; ^1H NMR (400 MHz in $CDCl_3$) δ 0.79 (3H, s), 0.84 (3H, s), 0.88 (3H, s), 0.94 (1H, dd, 13.2, 4.0 Hz), 1.02 (1H, dd, J = 12.0, 2.4 Hz), 1.14 (1H, m), 1.15 (3H, s), 1.23−1.47 (5H, m), 1.52−1.63 (2H, m), 1.69 (1H, ddd, J = 13.2, 6.0, 3.6 Hz), 1.94 (1H, dt, J = 12.4, 3.2 Hz), 2.09 (1H, dd, J = 6.0, 4.0 Hz), 2.94 (1H, dd, J = 18.0, 6.0 Hz), 3.04 (1H, dd, J = 18.0, 4.0 Hz), 3.85 (3H, s); ^{13}C NMR (100 MHz in $CDCl_3$) δ 15.8, 18.4, 20.6, 21.4, 23.2, 33.3, 33.4, 35.4, 38.6, 39.3, 41.8, 44.7, 52.2, 55.9, 56.2, 73.3, 162.0, 194.3; MS (Cl) m/z 351 (M^+ + H); HRMS (CI^+) m/z calcd for $C_{19}H_{31}O_4N_2$ (M^+ + H) 351.2284, found 351.2260.

Methyl (4a*S,6a*R**,10a*R**)-4a,7,7,10a-Tetramethyl-2-[[(trifluoromethyl)sulfonyl]oxy]-4a,5,6,6a,7,8,9,10,10a,10b-decahydro-1*H*-benzo[*f*]chromene-3-carboxylate (14).** $Rh_2(OAc)_4$ (37.8 mg, 8.55 μmol) was added to a solution of **12** (1.00 g, 2.85 mmol) in benzene (14 mL) at room temperature. The mixture was stirred for 5 min under reflux and cooled to room temperature. The resulting mixture was filtrated through a silica gel pad, and the filtrate was concentrated under reduced pressure. The residue (keto−enol mixture of **13a** and **13b**) was successively dissolved in THF (14 mL), and NaH (60% in oil, 171 mg, 4.28 mmol) was added to the solution at 0 °C. After 10 min, Comins reagent (2-[*N*,*N*-bis(trifluoromethanesulfonyl)amino-5-chloropyridine) (1.34 g, 3.42 mmol) was added to the mixture at the same temperature (0 °C). The mixture was stirred for 30 min at 0 °C and quenched with saturated NH_4Cl. The organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc, 10:1) to give **14** (1.20 g, 93%) as a white solid: FT IR (neat) 3000, 2981, 2950, 2872, 2847, 1747, 1660 cm^{-1}; ^1H NMR (400 MHz in $CDCl_3$) δ 0.82 (3H, s), 0.84 (3H, s), 0.90 (3H, s), 0.96 (2H, dd, J = 12.0, 2.4 Hz), 1.16 (1H, dt, J = 13.2, 3.6 Hz), 1.24 (3H, s), 1.32 (1H, ddt, J = 13.6, 12.4, 3.6 Hz), 1.40−1.51 (2H, m), 1.55−1.69 (4H, m), 1.77 (1H, m), 2.12 (1H, dt, J = 12.8, 3.2 Hz), 2.31 (1H, d, J = 10.4 Hz), 2.31 (1H, d, J = 8.0 Hz), 3.84 (3H, s); ^{13}C NMR (100 MHz in $CDCl_3$) δ 14.8, 18.3, 19.6, 21.5, 23.5, 33.1, 33.3, 36.9, 39.1, 39.8, 41.6, 51.6, 52.5, 55.6, 79.4, 136.5, 137.9, 161.3; MS (Cl) m/z 455 (M^++H); HRMS (CI^+) m/z calcd for $C_{20}H_{30}O_6F_3$ S (M^+ + H) 455.1715, found 455.1713.

Methyl (4a*S,6a*R**,10a*R**)-2-(2,6-Dichloropyridin-3-yl)-4a,7,7,10a-tetramethyl-4a,5,6,6a,7,8,9,10,10a,10b-decahydro-1*H*-benzo[*f*]chromene-3-carboxylate (16).** $Pd(PPh_3)_4$ (381 mg, 330 μmol) was added to a suspension of K_2CO_3 (1.09 g, 7.92 mmol), **14** (600 mg, 1.32 mmol), and (2,6-dichloropyridin-3-yl)boronic acid (**15**) (1.27 g, 6.60 mmol) in 10% aqueous dioxane solution (11 mL) at room temperature. The mixture was stirred for 15 min under reflux and cooled at room temperature. The reaction was quenched with saturated NH_4Cl at room temperature. The organic layer was dried

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over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc, 30:1) to give **16** (537 mg, 90%) as a white solid: FT IR (neat) 2981, 2949, 2927, 2868, 2846, 1731, 1645, 1573, 1539 cm^{-1}; ^1H NMR (300 MHz in $CDCl_3$ at 20 °C) δ 0.83 (3H, s), 0.84 (3H, s), 0.91 (3H, s), 1.01 (2H, m), 1.17 (1H, dt, J = 9.9, 2.7 Hz), 1.26−1.49 (4H, m), 1.56 (4H, m), 1.70 (2H, m), 1.78 (1H, br d, J = 10.2 Hz), 2.00 (1H, br d, J = 12.0 Hz), 2.15 (1H, dt, J = 9.3, 2.4 Hz), 2.34 (1H, br d, J = 12.0 Hz), 3.60 (3H, s), 7.25 (1H, br d, J = 6.0 Hz), 7.42 (1H, br s); ^{13}C NMR (75 MHz in $CDCl_3$ at 20 °C) δ 14.8, 18.4, 19.7, 20.4 (br), 21.5, 23.1 (br), 33.2, 33.4, 36.8, 39.2, 40.5, 41.7, 51.7 (br), 52.2, 56.1 (br), 118.1, 122.9 (br), 123.2, 135.4 (br), 140.9 (br), 142.0, 148.3, 163.1; ^1H NMR (400 MHz in DMSO at 25 °C) δ 0.79 (3H, s), 0.80 (3H, s), 0.88 (3H, s), 0.99 (2H, m), 1.12 (1H, m), 1.27 (3H, s), 1.37 (3H, m), 1.41−1.66 (4H, m), 1.64 (1H, br d, J = 13.2 Hz), 2.02 (1H, br d, J = 13.2 Hz), 2.14 (2H, m), 3.34 (3H, s), 7.55 (1H, br s), 7.82 (1H, br s); ^{13}C NMR (100 MHz in DMSO at 25 °C) δ 14.6, 18.1, 19.4, 20.3, 21.5, 25.4 (br), 25.9 (br), 33.0, 33.3, 36.4, 38.6, 41.5, 51.2 (br), 51.5 (br), 51.9, 55.1, 55.5 (br), 77.8, 118.0 (br), 123.5, 123.6, 124.2, 135.6 (br), 138.9, 139.5, 142.2, 142.5, 146.9, 147.8 (br), 162.6; ^1H NMR (400 MHz in DMSO at 55 °C) δ 0.82 (6H, s), 0.89 (3H, s), 0.97 (1H, m), 1.03 (1H, br d, J = 11.7 Hz), 1.15 (1H, br t, J = 6.0 Hz), 1.29 (3H, s), 1.37 (3H, m), 1.51−1.64 (4H, m), 1.71 (1H, br d, J = 13.6 Hz), 2.02 (1H, br d, J = 12.5 Hz), 2.15 (2H, m), 3.50 (3H, s), 7.51 (1H, d, J = 7.8 Hz), 7.78 (1H, d, J = 7.8 Hz); ^{13}C NMR (100 MHz in DMSO at 55 °C) δ 14.3, 17.9, 19.2, 20.1, 21.3, 25.4, 32.7, 33.1, 36.3, 38.5, 41.3, 51.3, 51.5, 55.3, 77.7, 117.5, 123.2, 123.8, 135.4, 139.3 (br), 142.1, 146.7, 147.7, 162.4; MS (Cl) m/z 452 (M^+ + H); HRMS (CI^+) m/z calcd for $C_{24}H_{32}Cl_2NO_3$ (M^+ + H) 452.1759, found 452.1760.

Dimethyl 3-[(4a*S,6a*R**,10a*R**)-3-(Methoxycarbonyl)-4a,7,7,10a-tetramethyl-4a,5,6,6a,7,8,9,10,10a,10b-decahydro-1*H*-benzo[*f*]chromen-2-yl]pyridine-2,6-dicarboxylate (17).** Compound **16** (530 mg, 1.17 mmol), AcONa (384 mg, 4.68 mmol), $Pd(OAc)_2$ (52.5 mg, 234 μmol), and 1,1′-bis-(diphenylphosphino)ferrocene (324 mg, 585 μmol) were dissolved in deoxygenated MeOH (6 mL) under argon atomosphere. The mixture was stirred for 36 h under CO (30 kgf/cm^2) using a TAIATSU SU316 microautoclave at 130 °C and cooled to room temperature. The resulting suspension was filtrated through a Celite pad, and the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc, 3:1) to give **17** (555 mg, 95%) as a white solid. **17**: white solid; FT IR (neat) 2990, 2948, 2926, 2865, 1726, 1636, 1557 cm^{-1}; ^1H NMR (400 MHz in $CDCl_3$ at 20 °C) δ 0.83 (3H, s), 0.85 (3H, s), 0.91 (3H, s), 1.00 (1H, br d, J = 14.0 Hz), 1.05 (1H, br d, J = 11.3 Hz), 1.17 (1H, ddd, J = 13.5, 12.8, 3.7 Hz), 1.36 (3H, s), 1.33−1.81 (8H, m), 2.14 (1H, dt, J = 12.6, 3.3 Hz), 2.18−2.28 (2H, m), 3.50 (3H, s), 3.91 (3H, s), 4.02 (3H, s), 7.67 (1H, d, J = 8.0 Hz), 8.23 (1H, d, J = 8.0 Hz); ^{13}C NMR (100 MHz in $CDCl_3$ at 20 °C) δ 14.9, 14.9, 18.4, 19.7, 20.4 (br), 21.5, 27.4, 33.1, 33.4, 36.7, 39.2, 40.5, 41.7, 51.7, 51.9, 52.0, 53.0, 53.1, 56.0, 78.2, 119.7 (br), 126.55, 126.64, 138.9, 141.3 (br), 145.7, 147.7 (br), 163.2, 165.0, 165.6 (br); MS (CI) m/z 500 (M^+ + H); HRMS (CI^+) m/z calcd for $C_{28}H_{38}O_7N$ (M^+ + H) 500.2648, found 500.2645.

Trisodium Salt of 3-[(4a*R,6a*S**,10a*S**)-3-Carboxy-4a,7,7,10a-tetramethyl-4a,5,6,6a,7,8,9,10,10a,10b-decahydro-1*H*-benzo[*f*]chromen-2-yl]pyridine-2,6-dicarboxylic Acid ((±)-18).** A 40% aqueous NaOD solution (58 μL, 5.00 mmol) was added to a solution of **17** (500 mg, 1.00 mmol) in D_2O (3 mL). The mixture was stirred at 100 °C for 30 min and cooled at room temperature. Removal of the solvent gave a white residue, which was purified by ODS column chromatography (H_2O/MeOH, 1:4) to give (±)-**18** (523 mg, quant) as a white solid: FT IR (neat) 3382, 2988, 2920, 2865, 1596 cm^{-1}; ^1H NMR (400 MHz in D_2O) δ 0.75 (3H, s), 0.77 (3H, s), 0.81 (3H, s), 0.88 (1H, br dd, J = 14.0, 12.2 Hz), 0.98 (1H, br d, J = 12.4 Hz), 1.09 (1H, br dd, J = 13.4, 12.8 Hz), 1.23 (3H, s), 1.25−1.39 (3H, m), 1.43−1.59 (4H, m), 1.69 (1H, br d, J = 13.2 Hz), 1.95 (1H, br d, J = 12.0 Hz), 2.10 (1H, dd, J = 17.6, 4.4 Hz), 2.24 (1H, dd, J = 17.6, 12.4 Hz), 7.59 (1H, d, J = 8.0 Hz), 7.72 (1H, d, J = 8.0 Hz); ^{13}C NMR (100 MHz in D_2O) δ 15.0, 18.8, 20.0, 20.6, 21.6, 25.6, 33.1, 33.4, 37.0, 39.3, 42.1, 52.5, 56.1, 79.0, 113.6, 123.7, 136.6, 140.0, 144.5, 150.9, 154.8, 172.2, 173.8, 176.0; MS (FAB^+) m/z 524 (M^+ + H); HRMS (FAB^+) m/z calcd for $C_{25}H_{29}O_7NNa_3$(M^+ + H) 524.1657, found 524.1614.

2-[(2*S,4a*R**,8a*R**)-2-Hydroxy-2,5,5,8a-tetramethyldecahydronaphthalen-1-yl]ethyl Acetate ((±)-6).** Ac_2O (2.54 g, 24.9 mmol) was added to a solution of (±)-**5** (4.23 g, 16.6 mmol) in dehydrated pyridine (17 mL) at 0 °C. The mixture was stirred for 10 min at the same temperature (0 °C). HCl (1 N, 40 mL) was added to the mixture, which was extracted with AcOEt (3 × 40 mL). The organic layer was washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc, 4:1) to give (±)-**6** (4.71 g, 96%) as a colorless syrup: FT IR (neat) 3470, 2995, 2941, 2868, 1741 cm^{-1}; ^1H NMR (500 MHz in $CDCl_3$) δ 0.79 (6H, s), 0.84 (3H, s), 0.92 (2H, m), 1.14 (2H, m), 1.16 (3H, s), 1.28 (1H, ddd, J = 13.5, 12.5, 3.5 Hz), 1.36−1.46 (4H, m), 1.54−1.78 (4H, m), 1.89 (1H, dt, J = 12.5, 3.5 Hz), 2.05 (3H, s), 4.12 (2H, m); ^{13}C NMR (100 MHz in $CDCl_3$) δ 15.3, 18.4, 20.5, 21.1, 21.5, 23.9, 24.4, 33.3, 33.4, 38.7, 39.6, 41.9, 44.3, 56.0, 58.0, 66.6, 73.5, 171.2; MS (Cl) m/z 296 (M^+); HRMS (CI^+) m/z calcd for $C_{18}H_{32}O_3$ (M^+) 296.2351, found 296.2356.

(1*R*,2*R*,4a*S*,8a*S*)-1-(2-Hydroxyethyl)-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol ((−)-5). A suspension of (±)-**6** (3.75 g, 12.6 mmol), 0.2% aqueous Tween 80 solution (5.0 mL), and lipase PS-30 (source: *Burkholderia cepacia*) (2.50 g) in 0.1 M phosphate buffer (150 mL) and CH_2Cl_2 (25 mL) was stirred at 30 °C for 1 day. After the mixture was filtered through a Celite pad, the filtrate was extracted with $CHCl_3$ (100 mL). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc, 2:1) to give the diol product (−)-**5** (1.15 g, 36%, ≥98% ee) and nonhydrolyzed (−)-**6** (1.94 g, 52%, 48% ee). The enantiomeric purity of the hydrolyzed product (−)-**5** was determined by HPLC analyses in comparison with (±)-**5** (see the Supporting Information). The enantiomeric purity of nonhydrolyzed product (−)-**6** was determined in accordance with the reported procedure.[27] (−)-**5** with ≥98% ee: $[\alpha]^{22}_D$ = −17.5 (c = 1.0).

(1*S*,2*S*,4a*R*,8a*R*)-1-(2-Hydroxyethyl)-2,5,5,8a-tetramethyldecahydronaphthalen-2-ol ((+)-5). A suspension of (−)-**6** with 48% ee (1.94 g, 6.54 mmol), 0.2% aqueous Tween 80 solution (2.6 mL), and lipase M Amano-10 (source: *Mucor javanicus*) (1.30 g) in 0.1 M phosphate buffer (78 mL) and CH_2Cl_2 (13 mL) was stirred at 35 °C for 1 day. After the mixture was filtered through a Celite pad, the filtrate was extracted with $CHCl_3$ (50 mL). The organic layer was dried over $MgSO_4$ and evaporated under reduced pressure. The residue was purified by flash column chromatography (silica gel, hexane/EtOAc, 2:1) to give diol (+)-**5** (482 mg, 29%, 92% ee). The enantiomeric purity of the hydrolyzed product (+)-**5** was determined by HPLC analyses in comparison with (±)-**5** (see the Supporting Information). (+)-**5** with 92% ee: $[\alpha]^{21}_D$ = 17.0 (c = 1.3).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of ^1H and ^{13}C NMR spectra and a description of biological tests. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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(28) Details of these results are provided in the Supporting Information.