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The Absolute Configuration of Gambieric Acids A–D, Potent Antifungal Polyethers, Isolated from the Marine Dinoflagellate *Gambierdiscus toxicus*

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Abstract—The absolute configurations of potent antifungal polyether compounds, gambieric acids A–D, isolated from the marine dinoflagellate *Gambierdiscus toxicus* were determined by combining the modified Mosher method, NMR analysis, and chiral fluorimetric HPLC.

A large number of polyether compounds having potent biological activities have been isolated from marine organisms.[1] Their unique and intriguing structures have been elucidated mainly by NMR spectroscopy. However, their extremely limited amounts and non-crystalline properties hampered elucidation of their stereostructures by X-ray crystallography. As the continuation of our efforts to elucidate the absolute configurations of significant polyether compounds, e.g. ciguatoxin, maitotoxin, pectenotoxin, dinophysistoxin-1, yessotoxin, and gambierol, by combining CD, NMR and synthetic methods,[2–7] we undertook a stereochemical study of gambieric acids.

Gambieric acids A–D (GA A–D, **1**–**4**) were potent antifungal compounds isolated from the culture medium of the marine dinoflagellate *Gambierdiscus toxicus* that produces ciguatoxin precursors and maitotoxin.[8] Their antifungal activities against *Aspergillus niger* by the paper disk method were 2000 times greater than that of amphotericin B. The acids **1**–**4** are endogenous growth enhancers of the dinoflagellate.[9] Therefore, GAs are fascinating compounds not only in structural features but also in their biological activities. Previously we have determined the relative stereochemistry of the segment composed of fused ether rings. For synthesis of the whole molecule and understanding of the mode of actions, however, elucidation of the absolute configuration of the whole molecule is essential. In the present study, the absolute configuration from C1 to C11 was determined by NMR analysis after introducing anisotropic reagents to the carboxyl group at C1 or 9-OH in GAB. To determine the absolute configuration at C48, we protected 49-OH in GAB, oxidatively cleaved the C46–C47 double bond, coupled the resultant carboxylic acid to a chiral fluorescent reagent, and compared the fluorescent derivative by HPLC with synthetic reference compounds. Similarly, chiral fluorescent reagents for HPLC were used to determine the stereochemistry of 3-methylglutaric acid in GAC and GAD. In this paper, we report the absolute configuration of gambieric acids A–D thus determined (see Fig. 1).

Results and Discussions

Absolute configuration at C9

The relative stereostructure of the cyclic ether segment was determined previously and found to be the same in all gambieric acids. Thus, determination of the absolute configuration at C12 or at C36 by the modified Mosher's method should lead to elucidation of all absolute stereostructure of the cyclic ether segment.[10] To avoid the possibility of having two MTPAs in vicinity, C9 and C12, **2** was chosen for esterification with (*R*)- and (*S*)-MTPA chloride. ESI MS of the main product revealed an ion at *m*/*z* 1581 $(M+pyridine)^+$ corresponding to bis-MTPA esters (**5** and **6**). Based on the ^1H NMR spectra, one MTPA resided at C9 and the other at C49. Thus, the two MTPA esters were well separated from each other to exert interaction. We first determined the absolute configuration at C9 in **2**. By analyzing the COSY, TOCSY and NOESY spectra of **5** and **6**, $\Delta\delta$

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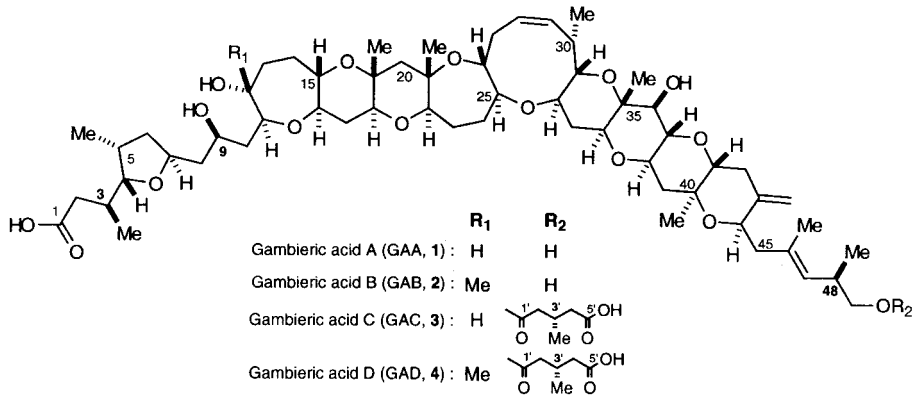


Figure 1. Structures of gambieric acids A–D.

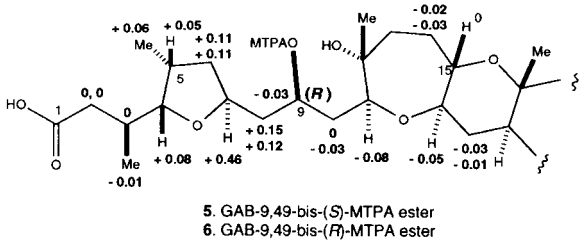


Figure 2. The partial structure of **5** and **6** indicating the $\Delta\delta$ ($\delta_S-\delta_R$) values (ppm).

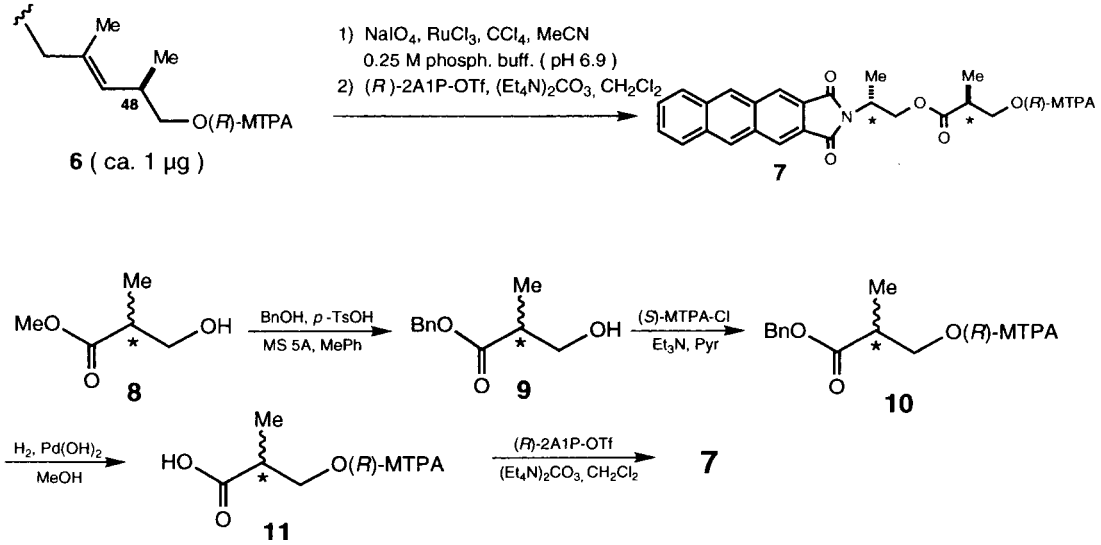
($\delta_S-\delta_R$) values of the protons around C9 were obtained, as depicted in Fig. 2. The signs of $\Delta\delta$ values were clearly distributed symmetrically around C9. The signs of $\Delta\delta$ values for protons from H4 to H_2-8 were positive, while those for protons from H_2-10 to H16 were negative. The distribution of the signs of protons well reflected the anisotropic effects of the MTPA ester at C9 and allowed us to assign *R* configuration at C9 in **2**.

Absolute configuration at C48

The absolute configuration at C48 in **2** was determined by HPLC by using (*R*)-2A1P-OTf [(*R*)-2-(anthracene-2,3-dicarboximide)-1-propyl trifluoromethanesulfonate] as a chiral fluorigenic reagent.[11] As shown in Scheme 1, 1 μg of the bis-(*R*)-MTPA ester (**6**) was cleaved at the C46–C47 double bond with $NaIO_4/RuCl_3$, and the resultant carboxylic acid was esterified to give a fluorescent (*R*)-2A1P derivative (**7**). Reference 48*S*- and 48*R*- 2A1P-esters (**7**) were prepared from 3-hydroxy-2(*R* or *S*)-methylpropionate (**8**) as shown in Scheme 1, and their structures were confirmed by ESI MS (MH^+, *m/z* 608) and NMR spectra. The two reference diastereomers showed clearly different retention times (*R*: 14.72, *S*: 16.36 min) on a chiral HPLC column (Chiracel OJ-R, 4.6×250 mm) with 65% MeCN. The retention time of the derivative prepared from **2** agreed well with that of the reference 2A1P-ester having 2*S* configuration. Consequently, C48 configuration in **2** before C46–C47 bond cleavage was unambiguously determined to be *R*.

Absolute configurations at C3 and C4

The absolute configuration at C3 was determined by the modified Mosher's method using a chiral anisotropic reagent, phenylglycine methyl ester (PGME), originally developed to identify the absolute configuration of α chiral carboxylic acids.[12] GAB (**2**) was condensed, respectively, with (*S*)- and (*R*)-PGME in DMF to give GAB (*S*)-PGME



Scheme 1.

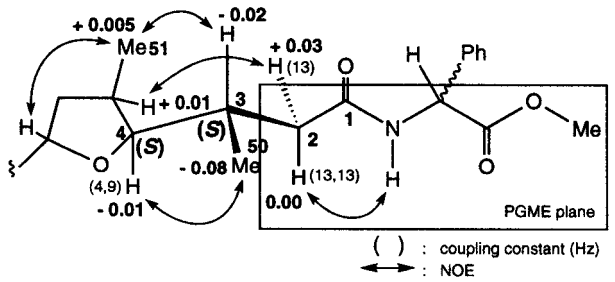


Figure 3. Presumable conformation and the $\Delta\delta$ ($\delta_S-\delta_R$) values (ppm) of C1–C7 portion of **12** and **13**.

amide (**12**) and GAB (*R*)-PGME amide (**13**). The $\Delta\delta$ values ($\delta_S-\delta_R$) are indicated in Fig. 3. To apply the PGME method to a β chiral carboxylic acid, conformation analysis is essential. The $\Delta\delta$ value of the high-field proton at C2 showed little shift, while that of the low-field proton showed positive sign. The NOE correlation from the amide proton was observed only to the high-field proton at C2. Thus, the high-field proton was positioned on the PGME plane and the low-field proton had *pro-R* position (Fig. 3). Moreover, the $\Delta\delta$ values of H3, Me50, and H4 showed negative signs, while those of H5 and Me51 showed positive signs. In 1D-HOHAHA spectra irradiated at Me50, the high-field proton at C2 was observed as a triplet having 13 Hz coupling, the low-field proton as a doublet having 13 Hz coupling, and H4 as a double-doublet having 9 and 4 Hz couplings. These results indicated that H3 was *anti* to both the high-field proton at C2 and H4, and was *gauche* to the low-field proton at C2. Observed NOE correlations Me50/H4, the low-field proton H2/H5, and H3/Me51 indicated that Me50 and C5 were in *anti* conformation. These $\Delta\delta$ values, coupling constants, and NOE correlations unanimously supported the conformation from PGME to C7 as shown in Fig. 3. From the conformation and the $\Delta\delta$ values, the configurations at both C3 and C4 were determined to be *S*.

Configuration at C11 and C12

The absolute configuration at C11 was determined by elucidating the conformation between C7 and C11 based on $^3J_{H,H}$ and NOE data.[13] The same signal shapes of H7, H9 and H11 in **2**, **12** and **13** indicated that the conformation from C7 to C11 in **2** was kept unchanged by amidation. The amide **12** was chosen for analysis, because signals for H7, H9 and

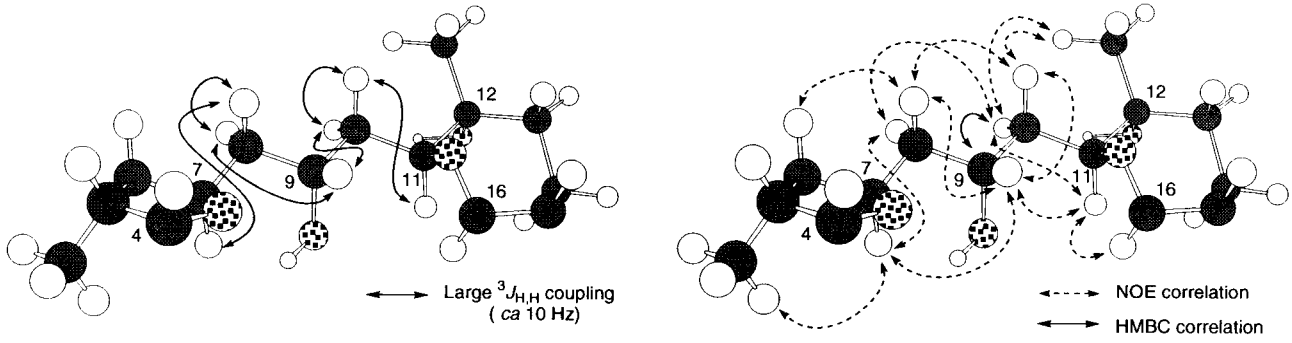
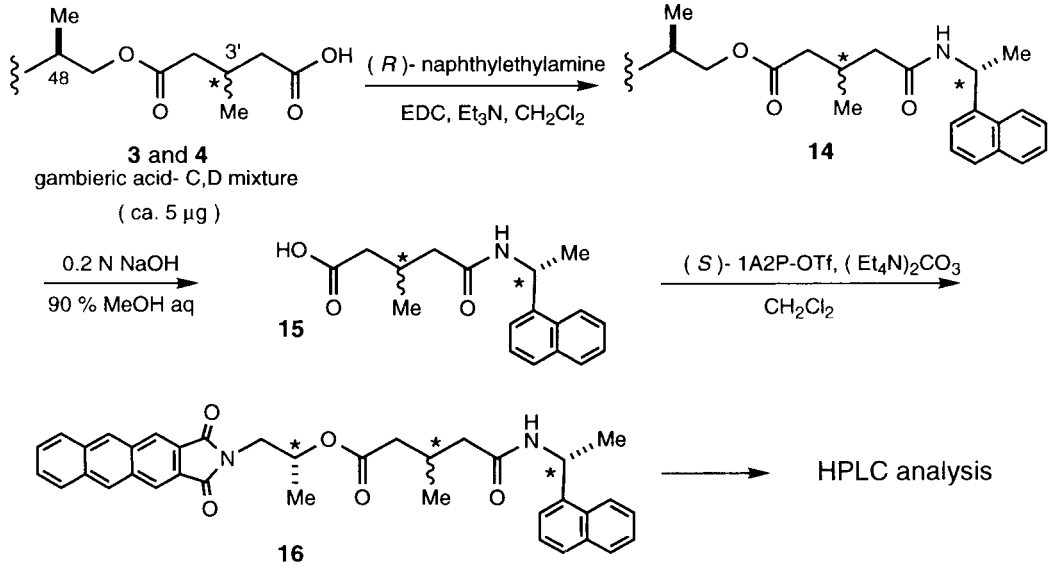


Figure 4. The plausible conformation of C7–C11 segment of **12** and **13** based on NMR experiments data. For clarity, C3, C17, and the ether oxygen at C15 were omitted in the models.



Scheme 2.

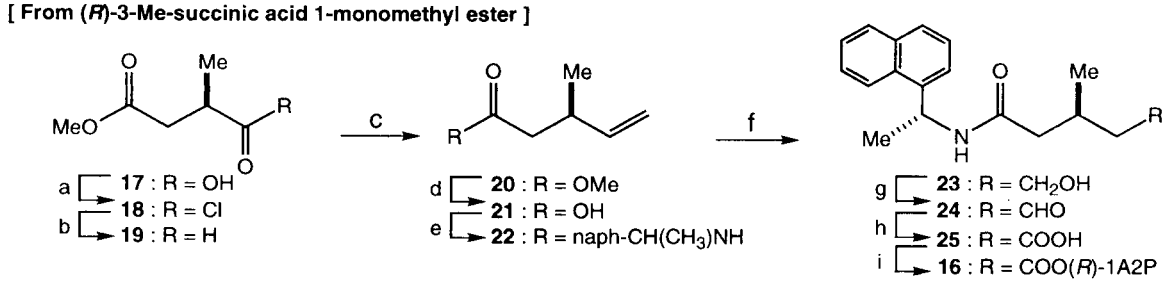
H11 were well isolated in the 1D ^{1}H NMR spectrum. Decoupling difference spectra irradiated at H7, H9 and H11 in **12** were measured to elucidate coupling constants between these oxymethine protons and interposed methylenes. In the spectra the low-field proton (H8-β) at C8 had a large coupling constant (10 Hz) and thus was *anti* to H7. The high-field proton (H8-α) at C8 was deduced to be *gauche* to H7 and anti to H9. Similarly, the low-field proton (H10-α) at C10 having a large coupling constant (10 Hz) was deduced to be *anti* to H9, while a high-field proton (H10-β) at C10 was *gauche* to H9 and *anti* to H11. NOE correlations were observed between H8-β/H6-β, H8-β/H10-β, H8-α/H6-α, H8-α/H10-α, H9/H7, H9/H11, H10-α/Me on C12, H10-α/H11, and H10-β/Me on C12. Based on the dependence of $^2J_{C,H}$ on the dihedral angle of an oxygen function, the HMBC correlation from H10-α to C9 suggested that H10-α was *gauche* to C9-OH.[14] These data pointed to the conformation from C7 to C11 as shown in Fig. 4. As the absolute configuration at C9 had been already determined as *R*, the absolute configuration at C11 was determined to be *S*. As the relative configurations of other asymmetric centers had been established in previous experiment,[8] the present result led to the absolute configuration of GAB as shown in **2**. The *R* configuration at C12 was supported by the absence of NOE correlation H11/Me on C12 in the ROESY spectrum.

Absolute configuration at C3′

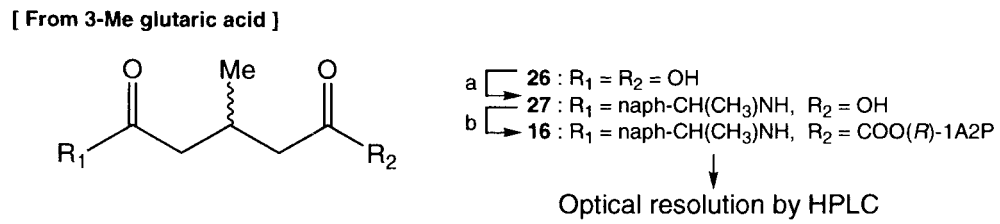
The absolute configuration at C3′ in **3** and **4** was determined by a chiral HPLC method as shown in Scheme 2. As chromatographic separation of **3** and **4** was impossible, 5 μg of a mixture containing **3** and **4** in a ratio 3:2 was used for experiments. The carboxylic moiety in **3** and **4** was protected with (*R*)-naphthylethylamine to generate amides **14**. The ester bond in **14** was then hydrolyzed with 0.2 N NaOH in 90% MeOH. The resultant carboxylic acid **15** was reacted with a chiral fluorigenic reagent (*S*)-1A2P-OTf [(*S*)-1-(anthracen-2,3-dicarboximide)-2-propyl trifluoromethanesulfonate] to produce a fluorescent derivative **16**.

References for **16** in HPLC analysis were prepared from (*R*)-3-methylsuccinic acid 1-monomethyl ester **17** according to Scheme 3. The carboxylic acid **17** was reduced to aldehyde **19** (2 steps). Wittig reaction of the aldehyde **19** with $(Ph_3PMe)Br$ produced olefin **20**. After hydrolysis of **20** under alkaline condition, **21** was reacted with (*R*)-naphthylethylamine to generate amide **22**. Hydroboration of **22** with 9-BBN and H_2O_2 produced alcohol **23**. The resultant alcohol **23** was oxidized to carboxylic acid **25**, followed by esterification with (*S*)-1A2P-OTf to produce an HPLC standard (*R*)-**16**. A diastereomixture of (*R*)-**16** and (*S*)-**16** was prepared from 3-methylglutaric acid **26** by amidation with (*R*)-naphthylethylamine and esterification with (*S*)-1A2P-OH. These diastereomers were separated on a reversed phase column (Cosmosil 5C18 AR, 4.6×250 mm) with 60% MeCN. The retention time of the fluorescent derivative **16** prepared from **3** and **4** agreed with that of (3′*S*) standard (*S*)-**16**. Further confirmation was accomplished by LC/MS analysis by selected ion monitoring at *m/z* 587. The derivative having 3′*R* configuration was detected in a ratio of *S* to *R* in 10:1. However, based on the predominance of *S* configuration at C3′ in the products, we assigned *S* configuration to C3′. The absolute configuration of entire molecule of gambieric acids A–D was thus elucidated by using extremely small amounts of samples.

The stereochemical information obtained in this study is expected to enhance synthetic efforts toward these intriguing molecules.



Reagents and conditions : (a) $(COCl)_2$, DMF, MeCN, THF, CH_2Cl_2, -30°C; (b) $LiAlH(OBu\text{-}t)_3$, THF, -78°C ; (c) $MePPh_3Br$, NaHMDS, THF, 0°C ; (d) NaOH, MeOH aq, 15%(4 steps) ; (e) (*R*)-naphthylethylamine, EDC, Et_3N, CH_2Cl_2, 71% ; (f) 9-BBN, THF, then H_2O_2, NaOH ; (g) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2, ; (h) $NaClO_2$, NaH_2PO_4, 2-Me-2-butene, t-BuOH, H_2O, 80% (3 steps) ; (i) (*S*)-1A2P-OTf, $(Et_4N)_2CO_3$, CH_2Cl_2, 50%.



Reagents and conditions : (a) (*R*)-naphthylethylamine, EDC, Et_3N, CH_2Cl_2, 70% ; (b) (*R*)-1A2P-OH, EDC, Et_3N, CH_2Cl_2, 34%.

Scheme 3.

Experimental

ESI MS and NMR spectra measurements

ESI MS spectra were measured with a Finnigan mat TSQ 700 spectrometer. NMR spectra of GAB-MTPA esters and GAB-PGME amides were measured with a Varian Unity INOVA 600 spectrometer in pyridine-d_5 at 20°C.

Preparation of GAB-9,49-bis-MTPA esters (5 and 6)

To 200 μg of **2** dissolved in pyridine were added DMAP (500 μg), TEA and (*R*)- or (*S*)-MTPA chloride. The mixture was kept under stirring at rt for 5 h. The reaction was stopped by adding MeOH and the residue extracted with $CHCl_3$ was purified on a Capcell Pak C8 UG120 column with linear gradient elution from 65% MeCN to 100% MeCN.

GAB-9,49-bis-(*S*)-MTPA esters (5). δ_H (ppm): 2 (2.22, 2.37), 3 (2.51), Me50 (1.30), 4 (3.68), 5 (2.16), Me51 (0.92), 6 (1.59, 1.65), 7 (4.48), 8 (2.05, 2.15), 9 (6.01), 10 (2.23, 2.52), 11 (3.87), 14 (2.03, 2.16), 15 (3.75), 16 (3.60), 17 (1.92, 2.54).

GAB-9,49-bis-(*R*)-MTPA esters (6). δ_H (ppm): 2 (2.22, 2.37), 3 (2.51), Me50 (1.31), 4 (3.60), 5 (2.11), Me51 (0.86), 6 (1.48, 1.54), 7 (4.02), 8 (1.90, 2.03), 9 (6.04), 10 (2.23, 2.55), 11 (3.95), 14 (2.05, 2.19), 15 (3.75), 16 (3.65), 17 (1.95, 2.55).

Preparation of fluorescent derivative (7) from GAB-9,49-bis-(*R*)-MTPA ester (6)

To the ester **6** (1 μg) in CCl_4/MeCN (1:1), $NaIO_4$ and $RuCl_3$ in 0.25 M phosphate buffer (pH 6.9) were added and the solution was stirred for 1 h at rt. After adding $NaHCO_3$ the solution was washed with CH_2Cl_2. The aqueous layer was acidified with 6N HCl, extracted with CH_2Cl_2, and the organic layer was dried over $MgSO_4$. The extract was reacted with (*R*)-2A1P-OTf and $(Et_4N)CO_3$ in CH_2Cl_2 by stirring for 7 h at 40°C. The reaction mixture was purified on a Chiracel OJ-R column with 65% MeCN to give **7**.

Preparation of fluorescent reference compounds (7) from methyl 3-hydroxy-2(*R* and *S*)-methylpropionates (8)

A mixture of **8** (26.5 mg), benzyl alcohol (70 μl), *p*-toluene sulfonic acid (13 mg), and molecular sieves in toluene (500 μl) was stirred for 10 h at 80°C. The reaction mixture was diluted with EtOAc, washed with $NaHCO_3$ solution, dried over $MgSO_4$, and purified on silica gel with hexane/EtOAc (3:2) to give benzyl ester **9** (*R* 21 mg, *S* 24 mg). The ester **9** (5 mg) was reacted with (*S*)-MTPA chloride in pyridine (90 μl) and TEA (10 μl). The reaction mixture was diluted with EtOAc (4 ml), washed with $NaHCO_3$ and NH_4Cl solutions, dried over $MgSO_4$ and purified on silica gel with hexane/EtOAc (6:1) to give (*R*)-MTPA ester **10**. The (*R*)-MTPA ester **10** in MeOH was reduced with 10% $Pd(OH)_2$ under an H_2 atmosphere by stirring for 1 h at rt. After removal of the catalyst by filtration, the product **11** was concentrated in vacuo. The carboxylic acid **11** was reacted with (*R*)-2A1P-OTf in the presence of $(Et_4N)_2CO_3$ in CH_2Cl_2. The reaction mixture was chromatographed on silica gel with hexane/EtOAc (5:2) to give the fluorescent reference **7**.

(***R***)-**7**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.12 (3H, d), 1.55 (3H, d), 2.78 (1H, m), 3.42 (3H, S), 4.27(1H, dd), 4.40 (1H,), 4.43 (1H, dd), 4.51(1H, t), 4.70 (1H, m), 7.29, 7.39, 7.61, 8.07, 8.48, 8.61 (phe and anthracene); HR-FAB MS $[M+H]^+$ 608.1899; calcd for $C_{33}H_{29}F_3NO_7$ 608.1897.

(***S***)-**7**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.06 (3H, d), 1.55 (3H, d), 2.80 (1H, m), 3.44 (3H, s), 4.28 (1H, dd), 4.33 (1H, dd), 4.40 (1H, dd), 4.62 (1H, t), 4.69 (1H, m), 7.35, 7.42, 7.61, 8.09, 8.50, 8.63 (phe and anthracene); HR-FAB MS $[M+H]^+$ 608.1895.

Fluorimetric HPLC analysis for 7

The fluorimetric HPLC analysis was performed under the following conditions: column, Chiracel OJ-R (4.6×250 mm, Daicel Kogyo); solvent, 65% MeCN; flow, 1.2 ml/min; detection, excitation at 298 nm and emission at 462 nm. The retention time was 14.72 min for *R*- and 16.36 min for *S*-ester.

Preparation of GAB-(*S*)- and (*R*)-PGME amides (12 and 13)

To GAB (200 μg) and (*S*)- or (*R*)-PGME-HCl in DMF cooled to 0°C were added TEA, PyBOP and HOBt. After stirring for 17 h at rt, the mixture was added with $CHCl_3$ and washed with H_2O. The organic layer was chromatographed on a Capcell pak C8 (4.6×150 mm, Shiseido) with 50% MeCN to give **10** and **11** (*m/z* 1219.6 for $[M+H]^+$).

GAB-(*S*)-PGME amide (**12**). δ_H (ppm): 2 (2.36, 2.58), 3 (2.46), Me50 (1.35), 4 (3.61), 5 (2.18), Me51 (0.873).

GAB-(*R*)-PGME amide (**13**). δ_H (ppm): 2 (2.36, 2.55), 3 (2.48), Me50 (1.43), 4 (3.62), 5 (2.17), Me51 (0.868).

Preparation of fluorescent derivative 16 from GAC (3) and GAD (4) mixture

A mixture of **3** and **4** (3:2, 5 μg), EDC-HCl (170 μg) and (*R*)-naphthylethylamine (810 μg) were dissolved in CH_2Cl_2 (100 μl) and stirred for 13 h. The reaction mixture was diluted with CH_2Cl_2 (4 ml), washed with NH_4Cl, and dried over $MgSO_4$. The amide **14** was hydrolyzed in 0.2N NaOH/90% MeOH (200 μl) for 6 h. The reaction mixture was diluted with 0.1N NaOH (2 ml) and washed with EtOAc (2 ml). The aqueous layer acidified with HCl was extracted with EtOAc (2 ml), and the organic layer was dried over $MgSO_4$. The resultant glutaric acid derivative **15** was reacted with (*S*)-1A2P-OTf (300 μg) in CH_2Cl_2 (100 μl) in the presence of $(Et_4N)_2CO_3$ (300 μg) for 15 h at 40°C. The reaction mixture was developed on a silica gel TLC plate with hexane/EtOAc (1:1) and the fluorescent derivative **16** was extracted from the plate.

Synthesis of references for the fluorescent derivative 16

To a solution of DMF (550 μl) in CH_2Cl_2 (8.6 ml) was added $(COCl)_2$ (900 μl) at 0°C. After stirring for 1 h at 0°C, MeCN (7.7 ml) and THF (13.7 ml) were added, and the solution was cooled to −30°C. (*R*)-3-Methylsuccinic acid 1-monomethyl ester **17** (1 g in 580 μl pyridine and 13.7 ml THF) was added dropwise for 30 min and the solution was stirred for 1 h at −30°C and for 30 min at −20°C. The resultant acid chloride **18** was reduced by adding dropwise a THF solution of $LiAlH(O\text{-}t\text{-}Bu)_3$ (2 g in 10 ml) in 30 min at −78°C. The solution was further stirred for 30 min at −78°C, acidified with 2 M HCl (9.6 ml) brought to rt, and washed with H_2O (20 ml). The aqueous layer was further extracted with diethylether (10 ml×2). The combined organic layer was washed with saturated solution of $NaHCO_3$ and NH_4Cl, and dried over $MgSO_4$ to give aldehyde **19**. A mixture of $(Ph_3PMe)Br$ (13 g) in THF (50 ml) $(Me_3Si)_2NNa$ in THF/H_2O (4:6, 15 ml) was stirred for 15 min at 0°C, and aldehyde **19** in THF (5 ml) was added. The solution was further stirred for 30 min and washed with saturated NH_4Cl solution. The organic layer was dried over $MgSO_4$ to give olefin **20**. Unpurified olefin **20** was hydrolyzed in 1N NaOH/90% MeOH (50 ml) for 7 h. The hydrolyzate was diluted with H_2O (50 ml) and extracted first with CH_2Cl_2 (100 ml×2) and, after acidification with HCl (20 ml), with $CHCl_3$ (50 ml×2) and diethylether (50 ml). The combined organic layer was dried over $MgSO_4$ and purified on silica gel with $CHCl_3$/MeOH (5:1) to give carboxylic acid **21** (135 mg, *m/z* 127.2 for $[M-H]^-$). Carboxylic acid **21** (135 mg) and EDC-HCl (240 mg) were dissolved in CH_2Cl_2 (2 ml) and mixed with (*R*)-naphthylethylamine (180 mg) for reaction under stirring for 11 h. Purification of the reaction mixture over silica gel with hexane/EtOAc (3:1) yielded amide **22** (225 mg, *m/z* 268.0 for $[M+H]^+$). To a solution of amide **22** (100 mg, in THF 5 ml) was added 0.5 M 9-BBN in THF (1.5 ml) at 0°C. The temperature was brought to rt and the solution was stirred for 3.5 h at rt. The second addition of 9-BBN was made and the solution was stirred for 1.5 h. The hydroborate adduct was oxidized by adding 3N NaOH (1 ml) and H_2O_2 (1 ml) at 0°C and by stirring the mixture for 10 h at rt. The reaction mixture was diluted with H_2O (20 ml) and extracted with EtOAc (20 ml×2). The combined organic layer was washed with saturated NaCl solution (10 ml), dried over $MgSO_4$ and the residue was purified over silica gel with hexane/EtOAc (1:10) to give alcohol **23** (*m/z* 286.0 for $[M+H]^+$). Solution of $(COCl)_2$ (300 μl in 3 ml CH_2Cl_2) and DMSO (300 μl) were mixed at −78°C and stirred for 10 min. Alcohol **23** in CH_2Cl_2 (2 ml) was added and stirring continued for another 15 min. Triethylamine was added and the solution was brought to rt under stirring. A saturated solution of NH_4Cl (20 ml) was added and the reaction mixture was extracted with diethyl ether (20 ml). The organic layer was dried over $MgSO_4$ to give aldehyde **24** (*m/z* 284.0 $[M+H]^+$). The aldehyde **24** was oxidized with $NaClO_2$ (100 mg) in a solution containing 2-methyl-2-butene (200 μl) and NaH_2PO_4 (60 mg) in 10 ml of *t*-BuOH/H_2O (7:3). After 3 h, the reaction mixture was acidified with dil. HCl, extracted with $CHCl_3$ (10 ml), and dried over $MgSO_4$. Purification of the residue on silica gel with $CHCl_3$/MeOH (5:1) yielded carboxylic acid **25** (90 mg) {*m/z* 298.0 $[M+H]^+$}. The carboxylic acid **25** was reacted with (*S*)-1A2P-OTf (8.8 mg) in a $(Et_4N)_2CO_3$ (9.6 mg) in CH_2Cl_2 (1 ml) for 4 h under stirring at rt. The fluorescent ester **16** was obtained by purification on silica gel with $CHCl_3$/MeOH (50:1).

(***R***)-**16**: ^1H NMR (300 MHz, $CDCl_3$): δ 0.92 (3H, d), 1.23 (3H, d), 1.68 (3H, d), 2.04 (1H, dd), 2.23 (3H, m), 2.35 (1H, dd), 3.82 (2H, d), 5.27 (1H, dd), 5.99 (1H, m), 6.48 (amide, d), 7.42, 7.51, 7.54, 7.62, 7.78, 7.83, 8.09, 8.43, 8.60 (naph and anthracene), ESIMS; 587.2 $[M+H]^+$.

Preparation of a diastereomixture standard from 3-methylglutaric acid (26)

3-Methylglutaric acid **26** (365 mg) was subjected to reaction with (*R*)-naphthylethylamine (970 mg) and EDC-HCl (479 mg) in CH_2Cl_2 (4 ml) for 10 h under stirring. The reaction mixture was diluted with $CHCl_3$ (30 ml) and washed with 1N HCl (10 ml). The organic layer was dried over $MgSO_4$, and the residue was chromatographed on silica gel with $CHCl_3$/MeOH (10:1) to give half amide **27** (522 mg). The amide **27** (180 mg), EDC-HCl (120 mg), and (*R*)-1A2P-OH (61 mg) were dissolved in CH_2Cl_2 (4 ml) and stirred for 10 h. The reaction mixture was diluted with EtOAc (50 ml) and washed successively with 1N HCl (30 ml), H_2O (30 ml), and $NaHCO_3$ (30 ml). The organic layer was dried over $MgSO_4$ and chromatographed on silica gel with $CHCl_3$/MeOH (50:1). The diastereomers of **16** were separated on a Wakosil-II 5SIL-100 column with $CHCl_3$.

(***S***)-**16**: ^1H NMR (300 MHz, $CDCl_3$): δ 0.90 (3H, d), 1.30 (3H, d), 1.63 (3H, d), 1.97 (1H, dd), 2.23 (3H, m), 2.37 (1H, dd), 3.86 (2H, d), 5.27 (1H, dd), 5.92 (1H, m), 6.15 (amide, d), 7.39, 7.52, 7.63,7.83, 8.07, 8.44, 8.57 (naph and anthracene), ESIMS; 587.3 $[M+H]^+$.

Fluorimetric HPLC analysis of 16

The fluorimetric HPLC analysis was performed under the following conditions: column, Cosmosil 5C18 (4.6× 250 mm, Nacalai tesque); solvent, 60% MeCN; flow, 1.6 ml/min; detection, excitation at 298 nm and emission at 462 nm. The retention time for (*S*)-**16** was 18.30 min and for (*R*)-**16** 19.67 min.

LC/MS analysis of 16

The LC/MS analysis was carried out under the following conditions: column, Capcell pak (2.0×150 mm, Shiseido); solvent, 60% MeCN; flow, 0.4 ml/min; mass spectrometer, Finnigan mat TSQ-700; ion mode, positive; capillary celsius, 250°C; electron multiplier, 1200 V; sheath gas, 60 psi; auxiliary gas, 20 flow-units; dwell time, 2.0 s. The retention time 24.34 min for (*S*)-**16** and 26.27 min for (*R*)-**16**.

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