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Absolute Configuration of a Hydroxyfuranoid Acid from the Pelage of the Genus *Bos*, 18-(6*S*,9*R*,10*R*)-Bovidic Acid†

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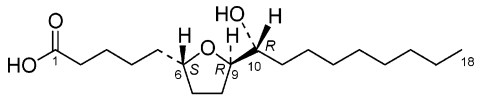
Pelage extracts of the banteng (*Bos javanicus*), the domestic cattle (*B. taurus*), the gaur (*B. frontalis*), and the yak (*B. grunniens*) were investigated by FABMS and NMR. An 18-carbon hydroxyfuranoid acid, **1** (10-hydroxy-6,9-oxidooctadecanoic acid), first reported from the wool of the domestic sheep (*Ovis aries*) was confirmed in *B. frontalis*; we suspend judgment on the occurrence of this compound in the other species we examined. The stereochemistry of **1**, determined by Mosher NMR and CD tweezer methods, was assigned as 6*S*, 9*R*, 10*R*. We propose the name *bovidic acid* for this and homologous α-hydroxylated 2,5-tetrahydrofuranoid carboxylic acids, in reference to the family Bovidae, which represents their sole known natural source.

The mammalian integument is well known as a source of unusual natural products.[1] Most comparative studies of mammalian skin chemistry are concerned with the secretory components of macroscopic glands,[2] such as the anal glands of carnivores, but novel compounds have been characterized from the skin surface as well. Equolides, giant (C_{28}−C_{36}) lactone rings of ω-hydroxyacids, for example, are known only from the pelage of the domestic horse (*Equus caballus*) and other equids.[3] These and many other mammalian skin surface chemicals are thought to arise chiefly from sebaceous glands, numerous small exocrine organs characteristically associated with hair follicles.

Early detailed investigations of pelage chemistry focused on the domestic sheep (*Ovis aries*, Bovidae), from which sterols, wax esters, hydroxyacids, and other compounds have been isolated (reviewed by Downing[4]). Comparable studies on the domestic cattle (*Bos taurus* L.), another bovid, have revealed cholesterol, free fatty acids and alcohols, steryl esters, triacylglycerols, wax esters, and phospholipids.[5] Linoleic acid (18:2) comprises approximately 10% of the total fatty acids of the skin surface lipids of this species.[6] Additionally, ceramides and glycolipids have been indicated by thin-layer chromatography (TLC) of hair extracts.[7]

A preliminary report indicated that skin extracts of the gaur (*B. frontalis* Lambert), a large (650−1000 kg) animal with a dark brown, greasy pelage ranging in forests and grassy clearings from Nepal and India to Indochina and the Malay Peninsula, contain 10-hydroxy-6,9-oxidooctadecanoic acid, with an absolute configuration of 6*R*, 9*S*, 10*S*.[8] This compound is one in a series of 14- to 18-carbon homologues first reported in the wool fat of *O. aries*.[9] We examined pelage extracts of *B. frontalis* and the following congeners for α-hydroxylated, 2,5-disubstituted tetrahydrofuranoid carboxylic acids: *B. taurus* (including Zebu cattle, considered by some taxonomists to be a separate species); the banteng (*B. javanicus* d'Alton), a dark to reddish brown animal inhabiting deciduous forests of Mayanmar, Thailand, Indochina, the Malay Peninsula, Java, and Borneo; and the yak (*B. grunniens* L.), a domesticated, dark brown animal, originating from ancestral stock in Tibet and adjacent highland regions. We also investigated the absolute configuration of this α-hydroxylated, 2,5-disubstituted tetrahydrofuranoid carboxylic acid (**1**) isolated from *B. frontalis* and discovered the absolute stereochemistry to be 6*S*, 9*R*, 10*R* (namely opposite that reported earlier[8]).



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FABMS exhibits peaks at *m*/*z* 315 $(M + H)^+$, 337 $(M + Na)^+$, and 353 $(M + K)^+$, while HRMS gives a molecular ion of 315.2535 corresponding to $C_{18}H_{35}O_4$ (MW 315.2535).

^{1}H NMR signals at 3.87 ppm (6-H), 3.76 ppm (9-H), and 3.36 ppm (10-H) and cross-coupled peaks in the 2D COSY suggest an α,α-disubstituted tetrahydrofuranoid ring in which one of the substituent chains carries an adjacent *sec*-hydroxyl group, leading to 10-hydroxy-6,9-oxidooctadecanoic acid (**1**) (Figure 1). The configuration of the tetrahydrofuranoid moiety was determined by comparison of its ^{1}H and ^{13}C NMR spectra with those of synthetic compounds **2**−**5**.[9] As shown in Figure 1, the ^{1}H and ^{13}C NMR chemical shifts of the oxymethyne atoms (2-H, 5-H, and 1″-H; C-6, C-9, and C-10) of compounds **3** (*trans*/*erythro)* and **5** (*cis*/*erythro*) differed from those of **1**. On the other hand, the data for compounds **2** (*trans*/*threo*) and **4** (*cis*/*threo*) are similar to those of **1**. The absence of a NOE between 6-H and 9-H in **1** indicates that 6-H and 9-H are *trans*-oriented with respect to the tetrahydrofuranoid ring. This leads to the *trans*/*threo* structure for **1**.

The absolute stereochemistry of the *sec*-hydroxyl group at C-10 was determined by the modified Mosher[11] and circular dichroism (CD) tweezer methods.[12−14] The modified Mosher method is based upon the difference between the ^{1}H NMR chemical shifts (Δδ values) of diastereomeric acid ester derivatives, such as (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic esters (MTPA),[15] (*R*)- and (*S*)-α-methoxyphenylacetic (MPA) esters,[16] and (*R*)- and (*S*)-2-naphthylmethoxyacetic acid (2-NMA) esters.[17] Although MPA is one of the most commonly used auxiliary reagents,

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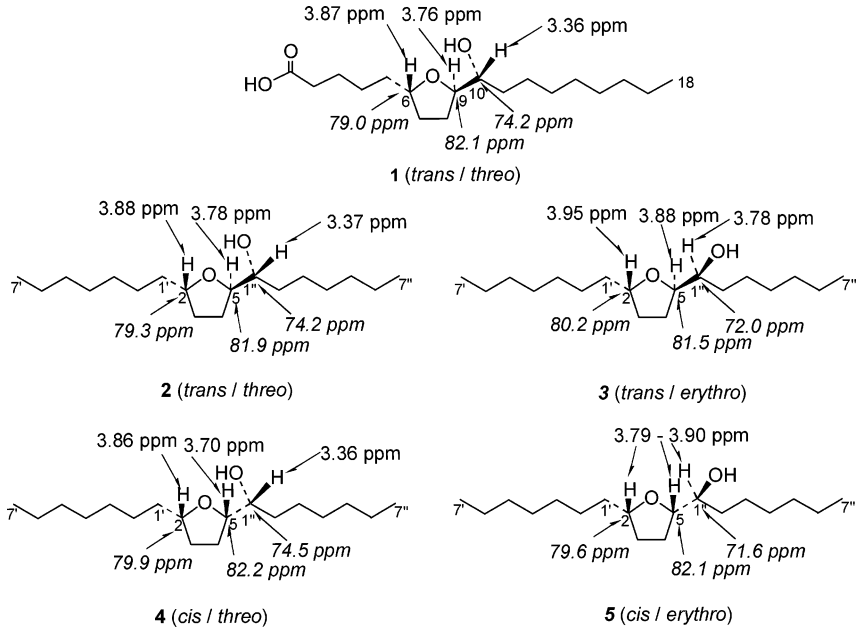
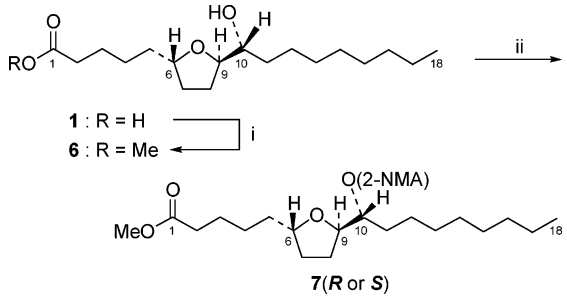


Figure 1. Relevant ^{1}H and ^{13}C NMR chemical shifts of compounds **1**−**5** for relative configurational assignment.

Scheme 1. Preparation of (*R*)- and (*S*)-2-NMA Esters of **1**[a]



[a] (i) MeI, Na_2CO_3, DMF, rt; (ii) (*R*)- or (*S*)-2-NMA, EDC, DAP, CH_2Cl_2, rt.

in the present case reliable $\Delta\delta$ values ($\delta_R - \delta_S$) could not be obtained from the (*R*)- and (*S*)-MPA esters of **1** due to overlap of the aliphatic proton signals. Therefore, (*R*)- and (*S*)-2-NMA, which exert improved long-range anisotropic effects, were employed. The derivatives were prepared by treatment of **1** with (*R*)- and (*S*)-2-NMA, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), and 4-(dimethylamino)pyridine (DMAP) in dichloromethane (CH_2Cl_2) (Scheme 1). The $\Delta\delta$ values obtained from these 2-NMA esters are depicted in Figure 2. The $\Delta\delta$ values of the protons attached to the wing with the carboxylic acid group (L_2) are all negative, while those located on the hydroxy-alkyl wing (L_1) are positive. The absolute configuration at C-10 is thus *R*.

The *R*-absolute configuration at C-10 also was assigned by application of the recently developed microscale chiroptical protocol based on host/guest complexation between chiral substrate (guest) and achiral dimeric zinc tetraarylporphyrin tweezer (host).[12−14] In this method, the chiral substrate, e.g., monoalcohols, monoamines, and α-substituted carboxylic acids, is linked in two steps to the trifunctional bidentate carrier molecule (3-(aminopropyl)-amino)acetic acid (see Scheme 2); the resulting conjugate is then treated with a porphyrin tweezer to yield 1:1 host/guest sandwich complexes. The complexation leads to stereodifferentiation dictated by the relative steric sizes of substituents at the stereogenic center so that complexes with P-1/P-2 porphyrin helicity, where the larger group L at the stereogenic center protrudes outside the binding pocket with the medium group M inside, are formed preferentially. This stereocontrolled complexation leads to characteristic CD exciton couplets diagnostic for the resulting preferred porphyrin helicity, and hence for the absolute configurational assignment of the chiral substrate, provided the steric distinction between large (L) and medium (M) substituents has been made in a clear-cut manner. It was found that in cases where the substituent steric size cannot be determined on the basis of conformational energy difference for the cyclohexane model, the NMR data for the porphyrin ring current-induced ^{1}H chemical shifts of the complex or molecular modeling studies (MMFFs) can provide additional helpful information.[12,14]

The host/guest complex of compound **1** was prepared using established methodology, which is outlined in Scheme 2. Briefly, acid **1** was protected as a methyl ester to give **6**, and the free hydroxyl group was subsequently esterified using α-bromoacetyl bromide to provide bromoacetate **8**. Base-catalyzed bromide displacement with propylenediamine gave conjugate **9**. Equimolar host/guest complexation produced **10**, the species evaluated in this chiral recognition experiment. The CD spectrum of host/guest complex **10** in methylcyclohexane exhibits an intense negative CD couplet within the Soret region with an amplitude $A_{CD} = -271$ (Figure 3a).

The observed negative CD exciton couplet was interpreted as evidence for the *R*-configuration at C-10 in **6** and of the free acid **1**. In the present case, the tetrahydrofuranoid moiety was assigned as the large substituent L and the alkyl chain as the medium substituent M, on the basis of conformational energy *A*-values described by Winstein and Holness[18] for the cyclohexane model.

This assignment L/M in **6** also is supported by the preliminary conformational modeling calculations on the complex of the truncated model conjugate **12**. We applied the MMFFs method recently for predicting the porphyrin helicity of numerous other tweezer host/guest complexes.[12] The MMFFs method predicted a negative CD exciton chirality for the tweezer complex of **12** when *R*-absolute configuration was chosen for the calculations. Namely, in the conformation with a preferred negative porphyrin helicity, the tetrahydrofuranoid group is the larger group L that protrudes outside of the binding pocket.[19]

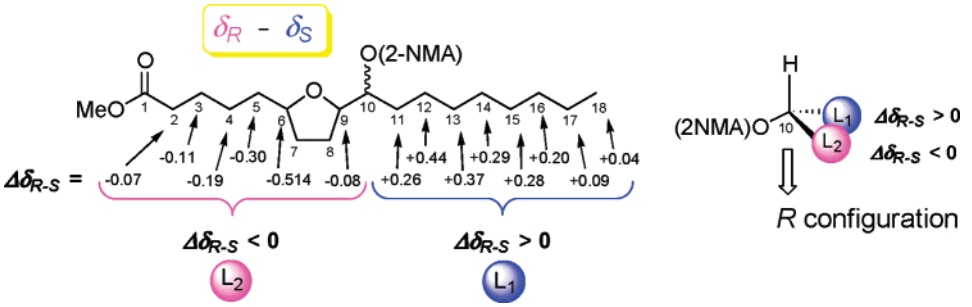
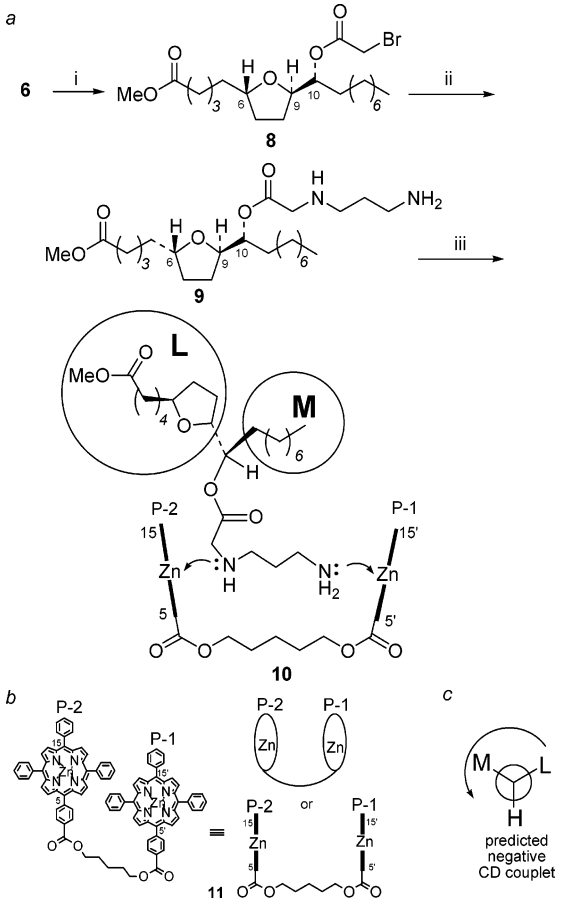


Figure 2. Δδ values for 2-NMA derivatives of compound **1** for modified Mosher method determination of absolute configuration.

Scheme 2. (a) Preparation of Guest **9** and Conjugate **10**, Which Is Formed by Complexation with Tweezer **11**; (b) Schematic Representation of 5,15 and 5′,15″ Directions; (c) Newman Projection of Conjugate **9**[a]



[a] (i) $BrCOCH_2Br$, Na_2CO_3, DMF, rt; (ii) $NH_2CH_2CH_2CH_2NH_2$, Na_2CO_3, DMF, rt; (iii) tweezer **11**, methylcyclohexane, rt.

Figure 3b presents the sterically favored conformation **I** of complex **10** between zinc porphyrin tweezer **11** (host) and conjugate molecule **9** (guest) where the larger group L protrudes outside the P-1/P-2 binding pocket, as well as the unfavored conformation **II** when this large group L is inside. Thus, the absolute configuration of the stereogenic center C-10 carrying a hydroxyl group can be correlated with the sign of the CD exciton couplet by following the simple rule, introduced earlier in studies of other secondary alcohol conjugates:[13] if a negative exciton-coupled CD spectrum is observed, the L, M, and H groups are arranged in a counterclockwise fashion, with the hydroxyl group in the rear of the Newman projection, and vice versa. In conclusion, the application of the porphyrin tweezer method leads to the *R*-configurational assignment at C-10, in full agreement with the result of the Mosher method.

Our results confirm the general structure of 10-hydroxy-6,9-oxidooctadecanoic acid for compound **1** in *B. frontalis*.[8] This compound is one of five 14- to 18-carbon α-hydroxylated, 2,5-disubstituted tetrahydrofuranoid carboxylic acids first reported from the wool of *O. aries*.[9] It is worth noting that similarities between the skin surface lipids of *Bos* and *Ovis* (and among other bovids) were suggested by a TLC survey of 45 species (20 families) of mammals.[20] Because *Bos* (Bovinae) and *Ovis* (Caprinae) are distantly related within the Bovidae,[21] we infer that **1**, and possibly homologues of it, is widespread within this family. Thus, we propose the name *bovidic acid* to denote these compounds, where the carbon-chain length is indicated by a numeric prefix, e.g., 18-bovidic acid. LC-MS analyses of *B. grunniens*, *B. javanicus*, and *B. taurus*, however, reveal only faint signals and retention time consistent with 18-bovidic acid. Thus, we suspend judgment on its occurrence in these species.

Ito et al.[9] proposed a 6*R*, 9*S*, 10*S* stereoconfiguration for 16-bovidic acid from *O. aries* and, by inference, the other acids in the 14- to 18-carbon series observed in this species. Our results and recent stereospecific synthetic studies[22] indicate an enantiomeric 6*S*, 9*R*, 10*R* stereoconfiguration for 18-bovidic acid **1** in *B. frontalis*, the sole such compound we observe. In *O. aries*, 16-bovidic acid predominates (85%), and the C-18 homologue is a minor component (4.4%). Further comparative studies of bovidic acids in these and other taxa are needed.

Experimental Section

General Experimental Procedures. FABMS was performed on a JMS-110/110 tandem mass specrometer (Tokyo, Japan) using a *m*-nitrobenzyl alcohol (*m*-NBA) matrix. ^1H NMR spectra were recorded on a Bruker DPX-300 (300 MHz), DMX-400 (400 MHz), or DMX-500 (500 MHz) spectrometer and are reported in ppm from $CHCl_3$ internal standards, 7.24 ppm. Data are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, dd = doublet of doublets, dt = double of triplets; coupling constants in Hz; integration. The CD spectra, which were recorded on a JASCO-810 spectrophotometer, were converted into $\Delta\epsilon_{max}$ [L $mol^{-1}cm^{-1}$]/λ [nm] units. The zinc tetraarylporphyrin tweezer (**11**) is commercially available from TCI (Portland, OR, cat. no. P1364).

Extraction and Chromatographic Isolation. Hair was cut or combed from the pelage of adult (number of individuals)-*B. frontalis* (5), *B. grunniens* (>10), *B. javanicus* (3), and *B. taurus* (>15). Samples (0.5−19.1 g) were extracted with up to 800 mL of acetone, filtered at room temperature, and stored at −10 °C. Cold extracts were filtered and then rinsed with chilled acetone to remove a white precipitate. Extraction yields from hair samples following cold filtration ranged from 0.6% (for *B. grunniens*) to 10.2% (for *B. frontalis*). Dried residues (30−600 mg) were dissolved in dichloromethane (CH_2Cl_2) and applied to silica gel columns (250 × 23 mm and 285 × 23 mm) and then eluted with *n*-hexane (120 mL), *n*-hexane−ethyl

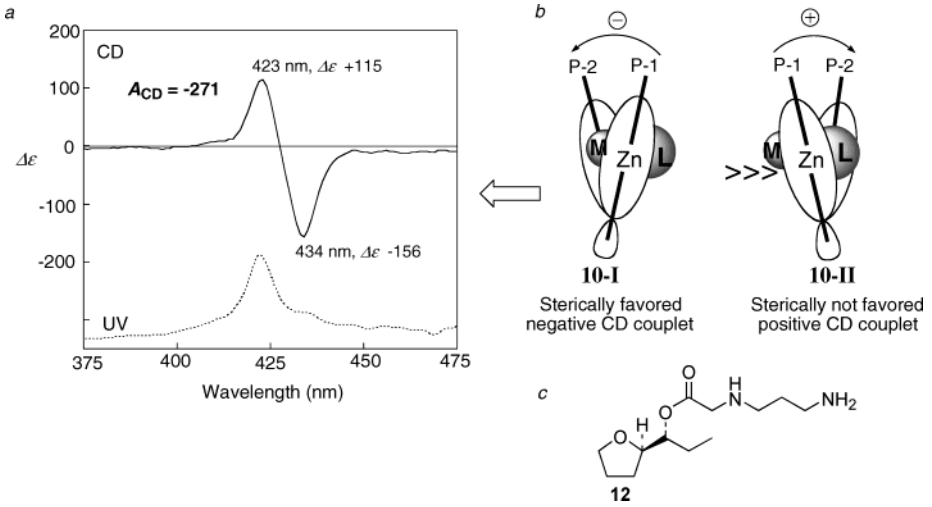


Figure 3. (a) CD and UV spectra of complex **10** in MCH. (b) The favored intra-porphyrin twist according to the relative size of substituents. (c) A truncated model conjugate of **9**.

acetate (39:1, 19:1, 9:1, 3:1, 1:1) (100 mL each), ethyl acetate (100 mL), and ethyl acetate−ethanol (1:1) (100−150 mL). Extracts and extract fractions eluted from silica gel columns with ethyl acetate and ethyl acetate−ethanol were subjected to TLC in diethyl ether−ethanol or ethyl acetate−ethanol (19:1) and/or HPLC. HPLC purification was performed using an Agilent 1100 HPLC fitted with a Supelcosil LC-18 (150 × 2.1 mm) column. This was eluted with water−acetonitrile (both containing 0.1% formic acid), using a 50:50 to 10:90 gradient over 30 min, at 100 μL/min flow rate. Column, TLC (R_f 0.5), and HPLC (retention time, 18.30 min) eluants were examined by FABMS and ^{1}H NMR.

(6*S*,9*R*,10*R*)-10-Hydroxy-6,9-oxidooctadecanoic acid (1). A cold-filtered extract (360 mg) of a female *B. frontalis* fractionated on a silica gel column (170−400 mesh, 250 × 23 mm) yielded a beige, penultimate band following elution with 50 mL of ethyl acetate−ethanol that contained primarily compound **1** (1.82 mg, 0.5%) as a beige semisolid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.87 (m, 1H), 3.76 (q, *J* = 7.4 Hz, 1H), 3.36 (m, 1H), 2.32 (t, *J* = 7.5 Hz, 2H), 2.01−1.91 (m, 2H), 1.67−1.55 (m, 2H), 1.53−1.44 (m, 4H), 1.43−1.39 (m, 3H), 1.38−1.31 (m, 3H), 1.24 (s, 12H), 0.86 (t, *J* = Hz, 3H); ^{13}C NMR (500 MHz, $CDCl_3$) δ 82.1, 79.0, 74.2, 35.1, 11.7, 33.3, 31.9, 29.7, 29.5, 29.3, 28.4, 25.6, 25.6, 24.7, 22.7, 14.1; HRFABMS *m/z* 315.2535 $[M + H]^+$, calcd for $C_{18}H_{35}O_4$, 315.2535.

Methylester Derivative of 1. To a solution of compound **1** (6.7 mg, 21 μmol) in dimethyl formamide (DMF) (1.0 mL) under argon at room temperature was added sodium carbonate (4.6 mg, 43 μmol). After the resultant mixture was stirred for 5 min, methyl iodide (2.0 μL, 32 μmol) was added and stirring continued for 3 h at room temperature. The reaction mixture was concentrated in vacuo to give the crude product. Further purification was performed by TLC on silica gel (250 μm, 20 × 20 cm) with *n*-hexane−ethyl acetate (2:1) to give **3** (5.4 mg, 79%) as a colorless oil: $[\alpha]^{23.1}_D$ +15.4° (*c* 0.01, MCH); ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.89 (m, 1H), 3.78 (ddd, *J* = 7.0, 7.0, 7.0 Hz, 1H), 3.67 (s, 3H), 3.36 (m, 1H), 2.39 (d, *J* = 3.6 Hz, 1H), 2.32 (t, *J* = 7.5 Hz, 2H), 1.99 (m, 2H), 1.64 (m, 2H), 1.54−1.25 (m, 20H), 0.88 (t, *J* = 7.1 Hz, 3H); HRFABMS *m/z* 329.2706 $[M + H]^+$, calcd for $C_{19}H_{37}O_4$, 329.2692 (Δ +1.4 mmu).

(R)*- and *(S)*-2-NMA Derivative of 6.** To a solution of compound **3** (1.0 mg, 3.0 μmol) in CH_2Cl_2 (1.0 mL) under argon at room temperature were added (*R*)-2-naphthylmethoxyacetic acid (3.6 mg, 17 μmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (6.6 mg, 34 μmol), and 4-(dimethylamino)pyridine (DMAP) (3.2 mg, 26 μmol), and the resultant mixture was stirred for 24 h. The reaction mixture was concentrated in vacuo to give the crude product. Further purification was performed by TLC on silica gel (250 μm, 20 × 20 cm) with *n*-hexane−ethyl acetate (2:1) to give **7*R (0.9 mg, 57%) as a colorless oil. The (*S*)-2-NMA ester **7*S*** was prepared in the same way using (*S*)-2-naphthylmethoxyacetic acid. **7*R***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (brs, 1H), 7.84−7.81 (m, 3H), 7.57 (dd, *J* = 1.6, 6.9 Hz, 1H), 7.48 (m, 2H), 4.95 (s, 1H), 4.90 (m, 1H), 3.87 (m, 1H), 3.68 (s, 3H), 3.47 (s, 3H), 3.33(m, 1H), 2.21 (t, *J* = 7.4 Hz, 2H), 1.66 (m, 1H), 1.63 (m, 2H), 1.51 (m, 2H), 1.25 (m, 13H), 1.07 (m, 6H), 0.87 (t, *J* = 7.1 Hz, 3H); HRFABMS *m/z* 526.3291 $[M]^+$, calcd for $C_{32}H_{46}O_6$, 526.3294 (Δ +0.3 mmu). **7*S***: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (brs, 1H), 7.84−7.81 (m, 3H), 7.57 (dd, *J* = 1.6, 6.9 Hz, 1H), 7.47 (m, 2H), 4.97 (s, 1H), 4.88 (m, 1H), 3.96 (dd, *J* = 6.6, 6.6 Hz, 1H), 3.84 (m, 1H), 3.67 (s, 3H), 3.48 (s, 3H), 2.28 (t, *J* = 7.4 Hz, 1H), 1.92 (m, 2H), 1.62 (m, 2H), 1.53 (m, 1H), 1.40 (m, 1H), 1.37 (m, 4H), 1.26 (m, 2H), 1.16 (m, 2H), 1.05 (m, 2H), 0.96 (m, 2H), 0.88 (m, 2H), 0.85 (m, 2H), 0.83 (t, *J* = 7.2 Hz, 3H), 0.81 (m, 2H); HRFABMS *m/z* 526.3303 $[M]^+$, calcd for $C_{32}H_{46}O_6$, 526.3294 (Δ +0.9 mmu).

Bromoacetate Derivative of 6. To a solution of compound **6** (4.0 mg, 12 μmol) in DMF (1.0 mL) under argon at room temperature was added sodium carbonate (5.0 mg, 47 μmol). After the resultant mixture was stirred for 5 min, methyl iodide (2.0 μL, 32 μmol) was added and stirring continued for 24 h at room temperature. The reaction mixture was concentrated in vacuo to give the crude product. Further purification was performed by TLC on silica gel (250 μm, 20 × 20 cm) with *n*-hexane−ethyl acetate (3:1) to give (3.5 mg, 63%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.88 (m, 1H), 3.99 (dd, *J* = 6.7, 6.7 Hz, 1H), 3.98 (m, 1H), 3.85 (s, 2H), 3.65 (S, 3H), 2.30 (t, *J* = 7.5 Hz, 2H), 1.99 (m, 2H), 1.64 (m, 2H), 1.55 (m, 4H), 1.44 (m, 2H), 1.33−1.22 (m, 14H), 0.87 (t, *J* = 6.6 Hz, 3H); HRFABMS *m/z* 447.1769 $[M - H]^+$, calcd for $C_{21}H_{36}O_5{}^{79}Br_1$, 447.1746 (Δ +2.3 mmu).

1,3-Diaminopropane Derivative of 8. To a solution of compound **8** (2.8 mg, 6.2 μmol) in tetrahydrofuran (THF) (1.0 mL) under argon at room temperature were added sodium carbonate (1.0 mg, 9.4 μmol) and 1,3-diaminopropane (50 μL, 500 μmol). After the resultant mixture was stirred for 5 min, methyl iodide (2.0 μL, 32 μmol) was added, and stirring continued for 18 h at room temperature. The reaction mixture was concentrated in vacuo to give the crude product. Then, the reaction mixture was diluted with CH_2Cl_2 (20 mL), washed with brine (5 mL), dried with sodium sulfate, and evaporated to give **9** (2.3 mg, 84%) as a pale yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.90 (m, 1H), 3.96 (dd, *J* = 6.6, 6.6 Hz, 1H), 3.86 (m, 1H), 3.65 (m, 2H), 3.43 (m, 2H), 2.82 (m, 2H), 2.70 (m, 2H), 2.30 (t, *J* = 7.5 Hz, 2H), 1.98 (m, 2H), 1.78−1.25 (m, 25H), 0.87 (t, *J* = 6.6 Hz, 3H); FABMS *m/z* 443 $[M + H]^+$.

CD Measurement. Porphyrin tweezer **11** solution, 0.1 mM in anhydrous CH_2Cl_2 (10 μL), was added to methylcyclohexane (MCH) (1 mL). To this solution, 3 mM **9** in anhydrous CH_2Cl_2 (10 μL) was added. This solution was shaken, and then UV and CD were recorded at room temperature and corrected by

background subtraction. The CD spectra were measured in millidegrees and normalized into $\Delta\epsilon_{max}$ [L mol^{-1} cm^{-1}]/λ [nm] units.

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