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Sphingolipids and Glycerolipids. IV.[1] Syntheses and Ionophoretic Activities of Several Analogues of Soya-cerebroside II, a Calcium Ionophoretic Sphingoglycolipid Isolated from Soybean

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For examination of the structure–activity relationship five analogues [(2′*R*)-2′-hydroxypalmitoyl (3), palmitoyl (4), (2′*S*)-2′-hydroxypalmitoyl (5), β-D-galactosyl (6), and 8,9-dihydro (7) relevancies] of soya-cerebroside II (2), which is a calcium ionophoretic sphingoglycolipid isolated from soybean, have been synthesized by using our previously reported synthetic method for sphingoglycolipids. Examinations by using a W-08 (liquid-membrane type) apparatus and by means of the human erythrocyte membrane method, have shown that the (2′*R*)-2′-hydroxypalmitoyl analogue (3) exhibits higher ion-binding and ion-permeation activities for calcium ion than soya-cerebroside II (2), which contains 3 as the major component. It has also been found that the other analogues (5, 6, 7, 8) do not show those ionophoretic activities.

An enantiomer (8) of the (2′*R*)-2′-hydroxypalmitoyl analogue (3) has been synthesized and its calcium ionophoretic activity examined. Compound 8 exhibits calcium ion-binding activity equal to that of 3, but 8 lacks the ability to support calcium ion-permeation through human erythrocyte membrane. Thus, human erythrocyte membrane precisely distinguishes the absolute configurations of 3 and 8 as regards calcium ionophoretic activity.

Keywords soya-cerebroside; calcium ionophore; sphingoglycolipid synthesis; ion-binding activity; human erythrocyte membrane; ion-permeability

In the previous papers,[1,2] we reported a versatile synthetic method for various complex lipids (*i.e.*, sphingoglycolipid, glycerophospholipid, and glyceroglycolipid), in which a chiral C4-epoxide was adopted as a common synthon. We subsequently isolated two sphingoglycolipids named soya-cerebrosides I (**1**) and II (**2**) from soybean and elucidated their chemical constituents including the compositions of the fatty acid moieties linked through amide bonds to the C-2 amino group of the sphingadienine part.[3] By examinations using a glass-cell apparatus (W-08, a liquid-membrane type)[3,4] and by employing a method using human erythrocyte membranes,[5] it has been found that soya-cerebroside II (**2**) exhibits an ion-binding activity in aqueous organic solvent and an ion-permeation activity across human erythrocyte membranes for calcium ion.

The finding of calcium ionophoretic activity of a sphingoglycolipid such as soya-cerebroside II (**2**) is unprecedented, and so we investigated the relation between structure and ionophoretic activity. In this paper, we wish to describe syntheses of six analogues of soya-cerebroside II (**2**), *i.e.*, (2′*R*)-2′-hydroxypalmitoyl (**3**) [the major constituent of soya-cerebroside II (**2**)], palmitoyl (**4**) [the minor constituent of **2**], (2′*S*)-2′-hydroxypalmitoyl (**5**), β-D-galactosyl (**6**), and 8,9-dihydro (**7**) derivatives, and an enantiomer of **3** (**8**), and to report their ionophoretic activities for calcium ion.

Our synthetic method for sphingoglycolipids[2] involves: 1) a regioselective opening of the epoxide ring of a chiral

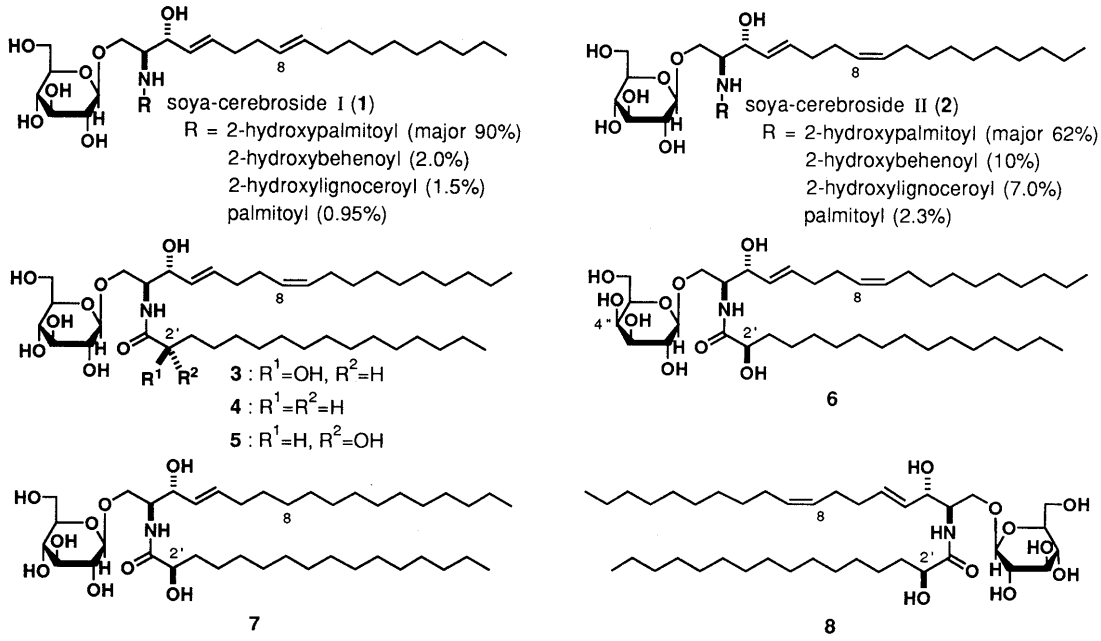


Fig. 1

C4-epoxide (**i**), which is prepared from (2*Z*)-2-butene-1,4-diol, with azide anion and subsequent reduction of the introduced azide group [step A, giving an amino-diol derivative (**ii**)], 2) oxidation of the primary hydroxyl group in **ii** and subsequent Wittig-type reaction followed by deprotection [step B, giving a sphingosine (**iii**)], and 3) successive introductions of fatty acid and sugar moieties [step C, giving sphingoglycolipid (**iv**)]. To shed light on the stereostructure-activity relationship of calcium ionophoretic activity of soya-cerebroside II (**2**),[3] we have synthesized six analogues of **2** (**3**, **4**, **5**, **6**, **7**, and **8**).

Synthesis of the (2′*R*)-2′-Hydroxypalmitoyl Analogue (3) To develop a synthetic strategy, the structure of the (2′*R*)-2′-hydroxypalmitoyl analogue (**3**), the major component (62%) of soya-cerebroside II (**2**),[3] was divided into four parts (A, B, C, and D) and four segments were designed: 1) segment A, a building block for the C1—C4 part of (4*E*, 8*Z*)-D-*erythro*-4,8-sphingadienine, may be synthesized from a chiral C4-epoxide (+)-**9**, 2) segment B, a Wittig-type reagent, may be useful for introducing the C5—C18 part, 3) segment C, *p*-nitrophenyl 2-*O*-acetyl-(2*R*)-2-hydroxypalmitate, may constitute the fatty acid moiety and 4) segment D, *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)trichloroacetimidate, may be introduced as the sugar part.

To synthesize segment A, a mixture of the 1,2-diol **10** and 1,3-diol **11** was prepared from a chiral C4-epoxide (+)-**9** (ee, 90%)[2] according to the procedure described in our previous paper.[2b] The mixture could be separated into a 1,2-diol **10** (76%) and a 1,3-diol **11** (6%) by high-performance liquid chromatography (HPLC) if necessary, but it was too labile to be separated by silica gel column chromatography. So, the mixture was immediately treated with pivaloyl chloride and pyridine to yield a mixture of monopivaloates, which was purified by silica gel column chromatography to afford the monopivaloate **12** derived from **10** as the major product. The secondary hydroxyl group was protected with a methoxymethyl (MOM) group to give the MOM ether **13**, then **13** was converted by

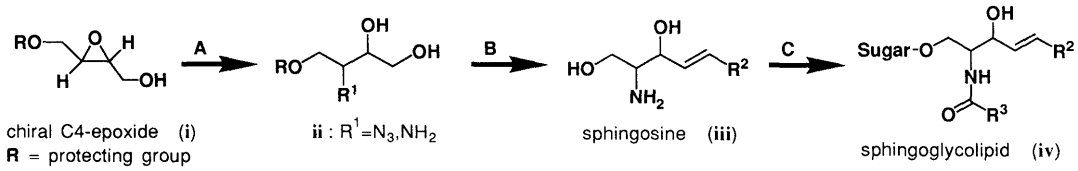


Chart 1

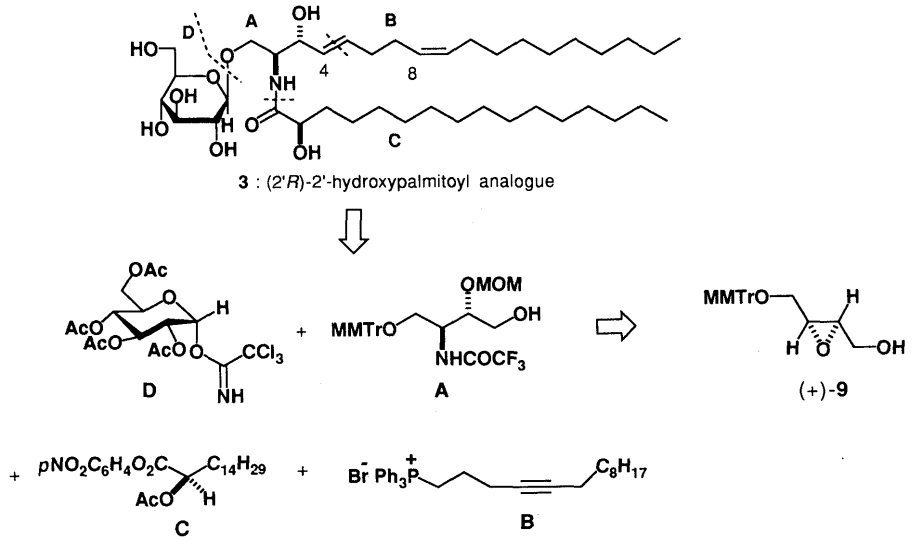


Fig. 2

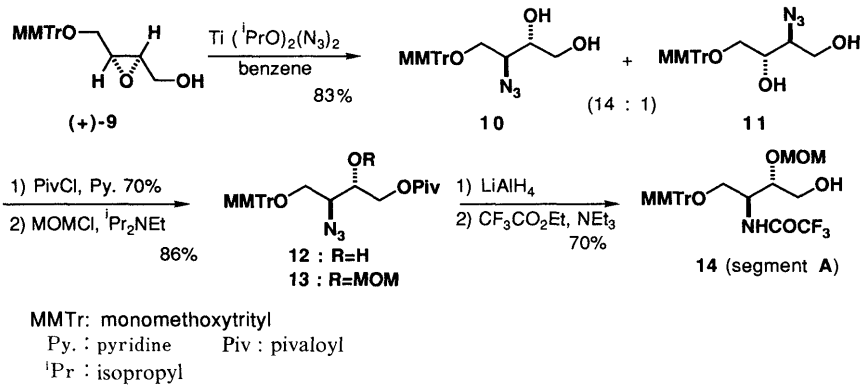


Chart 2

reduction with lithium aluminum hydride ($LiAlH_4$) and subsequent trifluoroacetylation to an amide-alcohol **14** (segment A) in a good yield.

Segment B (**17**) was synthesized through the following procedure. A dibromide **15**, which was prepared from 1-decanal by treatment with carbon tetrabromide and triphenylphosphine, was first converted to an acetylide intermediate by treatment with 2 equivalents of *n*-butyl lithium and then the acetylide was treated with 1,3-dibromopropane in hexamethylphosphoramide (HMPA) to give a tetradecynebromide **16**. Treatment of the yne-bromide **16** with triphenylphosphine at 120 °C afforded the objective phosphonium bromide **17** (segment B).

Segment C (**21a**) was synthesized by employing an optical resolution during the synthetic procedure from methyl palmitate (**18**). Thus, successive treatment of **18** with lithium diisopropylamide (LDA) and MoO_5·pyridine·HMPA complex[6] provided racemic methyl α-hydroxypalmitate (**19**). The diastereomeric mixture, prepared by treatment of **19** with *O*-acetyl-L-mandelyl chloride[7] and pyridine, was readily separated by silica gel column chromatography to afford the (2*R*)-ester **20a** and the (2*S*)-ester **20b**, each of which was hydrolyzed under alkaline conditions to yield (2*R*)-2-hydroxypalmitic acid and (2*S*)-2-hydroxypalmitic acid, respectively. After acetylation, these acids were treated with *p*-nitrophenyl trifluoroacetate to provide the desired segment C (**21a**) and the enantiomer (**21b**). The absolute configurations of **21a** and **21b** were ascertained from the values of their optical rotations, $[\alpha]_D$ of **21a**: +15.2° in $CHCl_3$ and $[\alpha]_D$ of **21b**: −15.3° in $CHCl_3$.[8]

Segment D (**22**), which is *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)trichloroacetimidate, was prepared from D-glucose through the Schmidt's procedure.[9]

Since the four segments (Fig. 2) were available, we next examined combination of these building blocks. Thus, Wittig reaction in the presence of methanol[10] of an aldehydic derivative, which was prepared from segment A (**13**) by pyridinium dichromate (PDC) oxidation, with segment B (**17**) furnished an 4*E*/4*Z* mixture of the C18-compound **23** in a ratio of 12:1. Without separation, the mixture **23** was subjected to Lindlar hydrogenation and subsequent acidic hydrolysis to afford a (4*E*,8*Z*)-D-*erythro*-4,8-sphingadienine derivative (**24**, in 75% yield) and a (4*Z*,8*Z*)-D-*erythro*-4,8-sphingadienine derivative (**24a**, in 6% yield). Geometries at the 4,5 double bond of **24** and **24a** were substantiated by their carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra. Thus, the carbon signals due to C-3 (δ_C 78.4) and C-6 (δ_C 32.3) of **24** were observed at lower field than those of **24a** (C-3: δ_C 73.3 and C-6: δ_C 27.8). The enantiomeric excess of both **24** and **24a** is

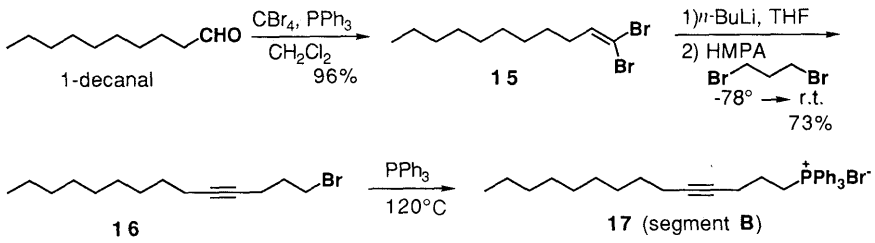


Chart 3

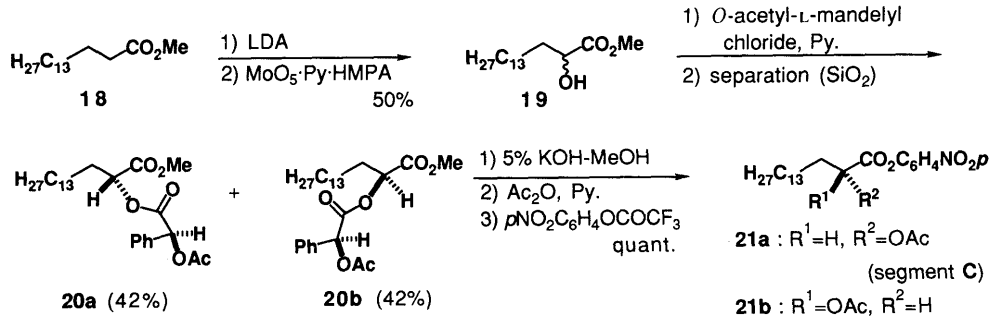


Chart 4

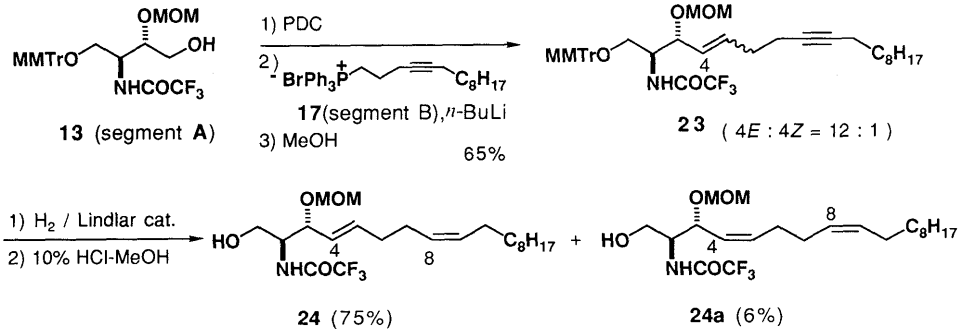
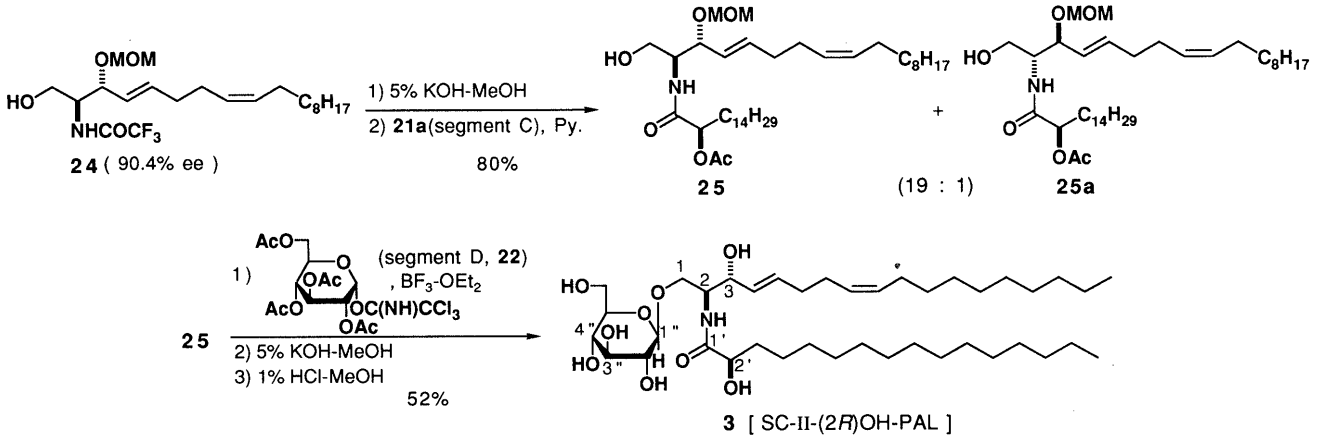


Chart 5



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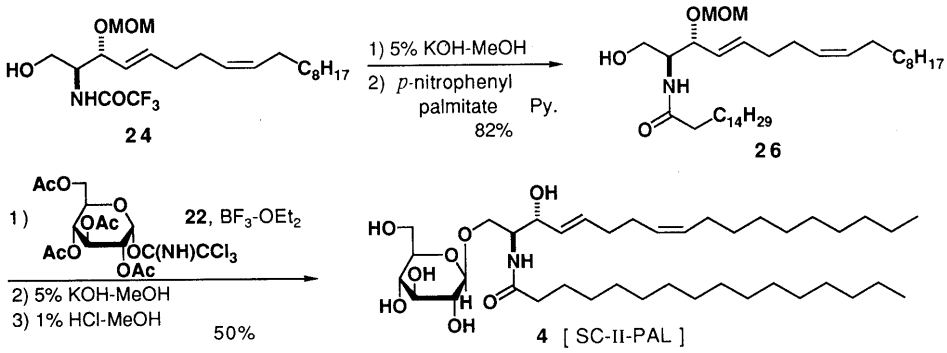

Chart 7

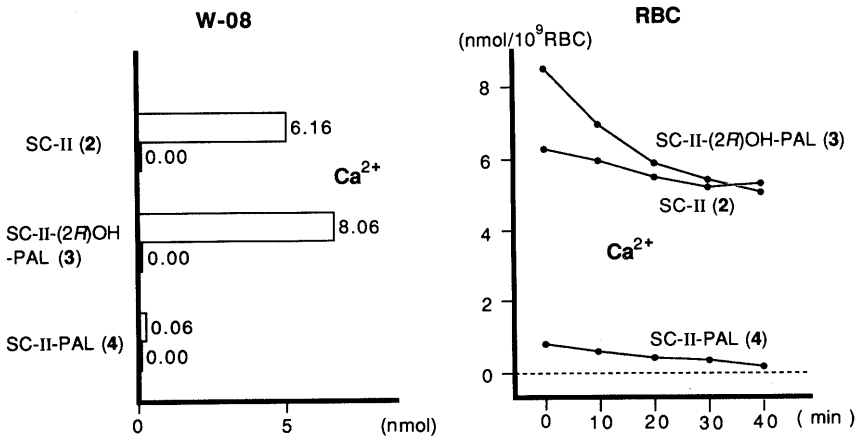

Fig. 3. Calcium Ionophoretic Activities of SC-II-(2*R*)OH-PAL (**3**) and SC-II-PAL (**4**), Compared with That of SC-II (**2**) by Using the W-08 Apparatus (Initial Concentration of Sample: 0.01 M in 1-Decanol) and the Human Erythrocyte Membrane Method

Initial concentration of sample: 0.10 μmol/10^9 red blood cells (RBC). □, ion-binding: ■, ion-transport (after 10 h).

considered to be approximately 90%, the same as that of the starting (+)-**9**.[2)]

After removal of the trifluoroacetyl group by alkaline hydrolysis, the (4*E*,8*Z*)-D-*erythro*-4,8-sphingadienine derivative **24** was condensed with segment C (**21a**) to afford a D-*erythro*-type ceramide **25** (in 76% yield) and an L-*erythro*-counterpart **25a** (in 4% yield), the latter being derived from the enantiomeric contaminant in (+)-**9** (90% ee). Glucosylation of the desired D-*erythro*-ceramide **25** with *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)trichloroacetimidate (segment D, **22**) gave an acetylated β-glucoside, which was then hydrolyzed consecutively under alkaline and acidic conditions to furnish the desired (2′*R*)-2′-hydroxypalmitoyl analogue (**3**) [SC-II-(2*R*)OH-PAL] of soya-cerebroside II (**2**). The infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR), and ^{13}C-NMR spectra of **3** were quite similar to those of **2**.

Synthesis of the Palmitoyl Analogue (4) In order to examine the effect of the presence of the 2′-hydroxyl function on the calcium ionophoretic activity of soya-cerebroside II (**2**), we next prepared a palmitoyl analogue (**4**) of **2**.

For this purpose, the above-mentioned (4*E*,8*Z*)-D-*erythro*-4,8-sphingadienine derivative **24** was taken as the starting material. Alkaline hydrolysis and subsequent

treatment with *p*-nitrophenyl palmitate of **24** afforded a ceramide **26** in 82% yield. Condensation of the ceramide **26** with segment D (**22**) under the same reaction conditions as for the synthesis of **3** from **25**, furnished a palmitoyl analogue **4** [SC-II-PAL] of soya-cerebroside II (**2**) in moderate yield. The L-*erythro*-type impurity expected to be derived from the L-*erythro* contaminant in the starting **24** was presumably removed during the purification of **4** by silica gel column chromatography.

Calcium Ionophoretic Activities of (2′*R*)-2′-Hydroxypalmitoyl and Palmitoyl Analogues (3, 4) By using the apparatus W-08,[3] which was designed for measuring both ion-transport and ion-binding activities, the above-prepared (2′*R*)-2′-hydroxypalmitoyl analogue (**3**) [SC-II-(2*R*)OH-PAL] and the palmitoyl analogue (**4**) [SC-II-PAL] were examined. It was found that **3** (0.01 M in 1-decanol) exhibited higher calcium ion-binding activity (8.07 mmol after 10 h) than that of soya-cerebroside II (**2**) [SC-II] (0.01 M in 1-decanol, 6.16 mmol after 10 h), while **4** did not exhibit the activity. As for the calcium ion-transport activity, neither of the analogues (**3**, **4**) exhibited any activity.

On the other hand, by employing the human erythrocyte membrane method,[5] it was found that the (2′*R*)-2′-hydroxypalmitoyl analogue (**3**) exhibited higher calcium ion-permeation activity than **2**, while the activity of the palmitoyl analogue (**4**) was very low. It is noteworthy that the ion-permeation activity of **3** was significantly higher than that of **2** at the early stage of examination.

These findings indicated that the presence of a hydroxyl function at the C-2′ position of the (2′*R*)-2′-hydroxypalmitoyl analogue (**3**) is essential for calcium ion-binding and ion-permeation activities.

We next analyzed the ^{1}H-NMR spectrum of **3** in the presence of calcium ion. Thus, the ^{1}H-NMR spectra of **3** were taken in several deuteriated solvents with or without several calcium salts. In the spectrum of **3** taken in dimethyl sulfoxide-d_6 (d_6-DMSO) containing 1 mol eq of calcium chloride, the signals due to the hydroxyl protons at C-2′, C-3″, and C-4″ were observed at lower fields (0.06 ppm for 2′-OH, 0.08 ppm each for 3″-OH and 4″-OH) than the corresponding signals observed without calcium ion (Fig. 4). Consequently, the hydroxyl functions at C-2′, C-3″, and C-4″ may be involved in calcium ion-binding of soya-cerebroside II (**2**) or the (2′*R*)-2′-hydroxypalmitoyl analogue (**3**).

In order to substantiate further the participations of these C-2′*R* and C-4″ hydroxyl functions in the calcium ionophoretic activity of **3**, we synthesized the (2′*S*)-2′-

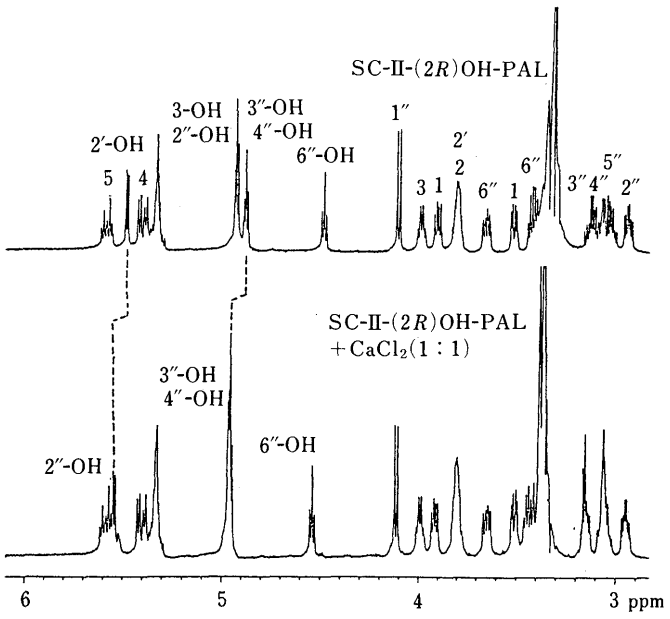

Fig. 4. ^{1}H-NMR Spectra (from 3 to 6 ppm) of **3** [SC-II-(2*R*)OH-PAL] Taken in DMSO-d_6 without (Upper) or with (Lower) $CaCl_2$ (500 MHz)

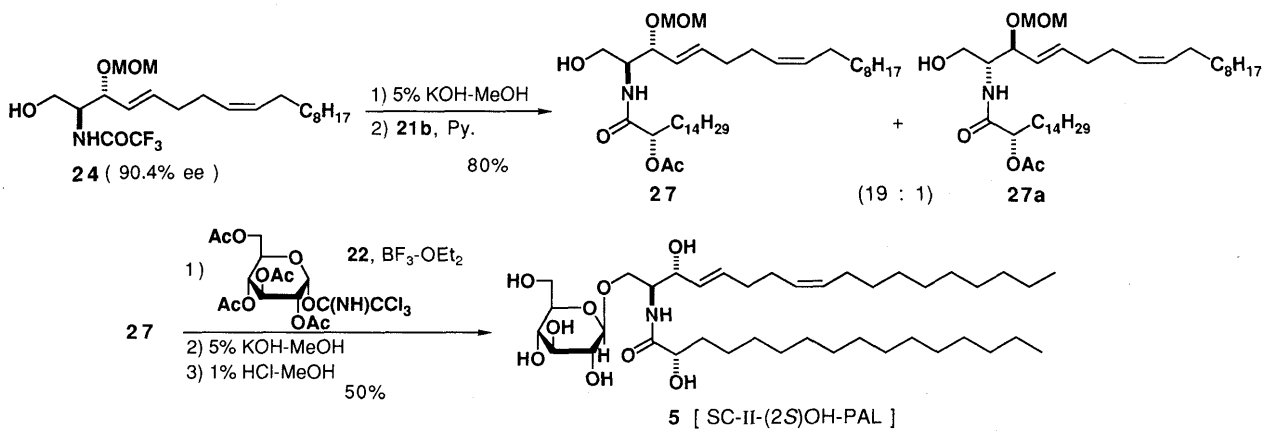

Chart 8

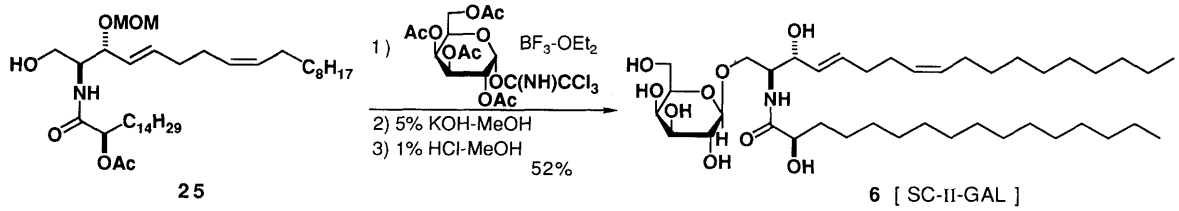

Chart 9

hydroxypalmitoyl analogue (**5**), the 2′-epimer of **3** and the β-D-galactosyl analogue (**6**, the 4″-epimer of **3**).

Syntheses and Ionophoretic Activities of (2′*S*)-2′-Hydroxypalmitoyl and β-D-Galactosyl Analogues (5, 6) Alkaline hydrolysis of the above-mentioned sphingadienine derivative (**24**) and subsequent reaction with *p*-nitrophenyl (2*S*)-2-acetoxypalmitate (**21b**), provided a D-*erythro*-type ceramide (**27**) and an L-*erythro* counterpart (**27a**) in 76% and 4% yields, respectively. Glucosylation [with segment D (**22**)] followed by deprotection of **27** furnished the (2′*S*)-2′-hydroxypalmitoyl analogue (**5**) [SC-II-(2*S*)OH-PAL] of soya-cerebroside II (**2**).

On the other hand, the D-*erythro*-type ceramide (**25**), an intermediate for the synthesis of the (2′*R*)-2′-hydroxypalmitoyl analogue (**3**), was condensed with *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)trifluoroacetimidate[9] and the product was subsequently subjected to deprotections under alkaline and acidic conditions to furnish the β-D-galactosyl analogue (**6**) [SC-II-GAL] of soya-cerebroside II (**2**). The configuration at the anomeric position of **6** was confirmed to be β by consideration of the coupling constant ($J=7.6$ Hz) of a doublet due to 1″-H in the ^{1}H-NMR spectrum.

The two analogues (**5**, **6**) thus prepared were then subjected to the tests for calcium ionophoretic activity mentioned above.[3,5] It has been found that both the (2′*S*)-2′-hydroxypalmitoyl and the β-D-galactosyl analogues (**5**, **6**) exhibit very low activities of calcium ion-binding and calcium ion-permeation. These findings demonstrate again that the 4″α-hydroxyl function (as in the glucosyl moiety) and the 2′*R*-hydroxyl function in the fatty acid moiety of soya-cerebroside II (**2**) are essential for exhibiting the ionophoretic activity for calcium ion.

Synthesis and Ionophoretic Activity of the 8,9-Dihydro Analogue (7) In order to clarify the effect of the C-8,9 double bond of **2** on the calcium ionophoretic activity, an 8,9-dihydro analogue (**7**) [2H-SC-II-(2*R*)OH-PAL] was next synthesized from segment A (**13**). Thus, the aldehydic derivative prepared from segment A (**13**) by PDC oxidation was treated with a C14-phosphorane reagent in the presence

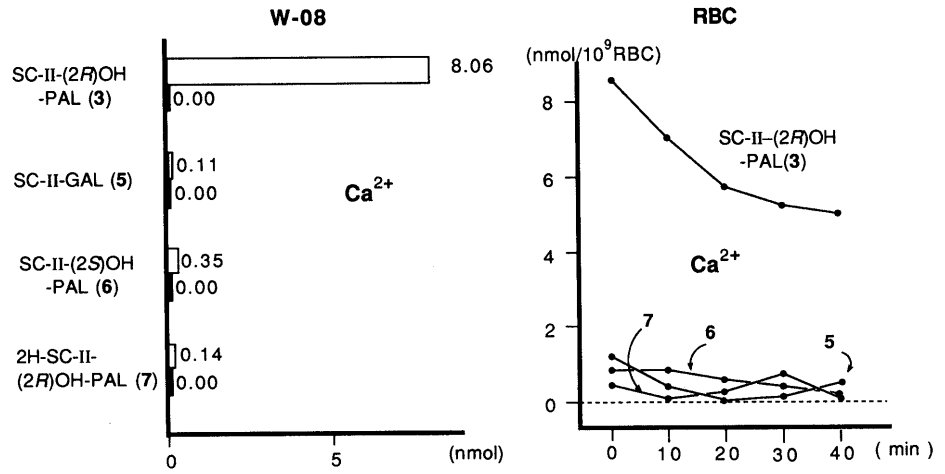

Fig. 5. Calcium Ionophoretic Activities of SC-II-(2*S*)OH-PAL (**5**), SC-II-GAL (**6**) and 2H-SC-II-(2*R*)OH-PAL (**7**), Compared with That of SC-II-(2*R*)OH-PAL (**3**) by Using the W-08 Apparatus (Initial Concentration of Sample: 0.01 M in 1-Decanol) and the Human Erythrocyte Membrane Method

Initial concentration of sample: 0.10 μmol/10⁹ red blood cells (RBC). □, ion-binding; ■, ion-transport (after 10 h).

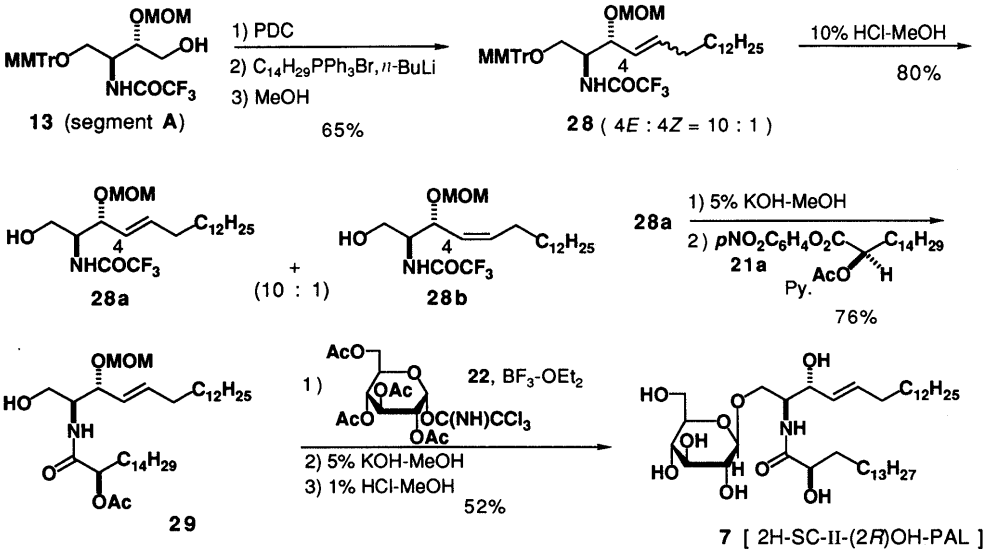

Chart 10

of methanol to yield a 10:1 mixture of 4*E* and 4*Z* C18-sphingosine derivatives (**28**). The mixture was then hydrolyzed with 10% hydrogen chloride in methanol to remove the monomethoxytrityl (MMTr) group, providing a 4*E*-D-*erythro*-C18-sphingosine derivative (**28a**, 73%) and its 4*Z* isomer (**28b**, 7%). After removal of the protecting trifluoroacetyl group, the major 4*E* compound (from **28a**) was condensed with *p*-nitrophenyl (2*R*)-2-acetoxypalmitate (**21a**) to furnish a 4*E*-ceramide (**29**). Glucosylation with **22** and subsequent removal of the protecting groups of **29** furnished the desired 8,9-dihydro analogue (**7**) [2H-SC-II-(2*R*)OH-PAL] of soya-cerebroside II (**2**).

The 8,9-dihydro analogue (**7**) was found to exhibit neither ion-binding nor ion-permeation activity for calcium ion, as assayed with the W-08 apparatus[3] and by the human erythrocyte membrane method (Fig. 5).[5] It should be noted that soya-cerebroside I (**1**) (Fig. 1), possessing the 4*E*,8*E*-type sphingosine base, exhibited very low ion-binding and ion-permeation activities for calcium ion. Consequently, it has become evident that the 8*Z*-double bond in the sphingosine base of soya-cerebroside II (**2**) is essential for exhibiting significant ionophoretic activity. The 8*Z*-double bond may stabilize the calcium complex, which presumably involves the 2'- and 4''-hydroxyl functions of soya-cerebroside II (**2**).

Synthesis and Ionophoretic Activity of the Enantiomer (8) of the (2'*R*)-2'-Hydroxypalmitoyl Analogue (3) As described above, the (2'*R*)-2'-hydroxypalmitoyl analogue (**3**) of soya-cerebroside II (**2**) exhibits higher ion-binding and ion-permeation activities for calcium ion than **2**. To investigate the influence of chirality on the ionophoretic activity, the enantiomer (**8**) of **3** was synthesized in an analogous manner to that used for the synthesis of **3**, namely starting from the C4-epoxide (−)-**9** which is antipodal to (+)-**9**, the starting C4-epoxide for the synthesis of the (2'*R*)-2'-hydroxypalmitoyl analogue (**3**).

The C4-epoxide (−)-**9** was converted to a (4*E*,8*Z*)-L-*erythro*-4,8-sphingadienine derivative **30** through the same procedure as used for the preparation of the (4*E*,8*Z*)-D-*erythro*-4,8-sphingadienine derivative (**24**) from (+)-**9**. After alkaline hydrolysis, **30** was condensed with *p*-nitrophenyl (2'*S*)-2'-acetoxypalmitate (**21b**) to provide an L-*erythro*-type ceramide **31** and a D-*erythro* counterpart **31a** in 76% and 4% yields, respectively. The major ceramide

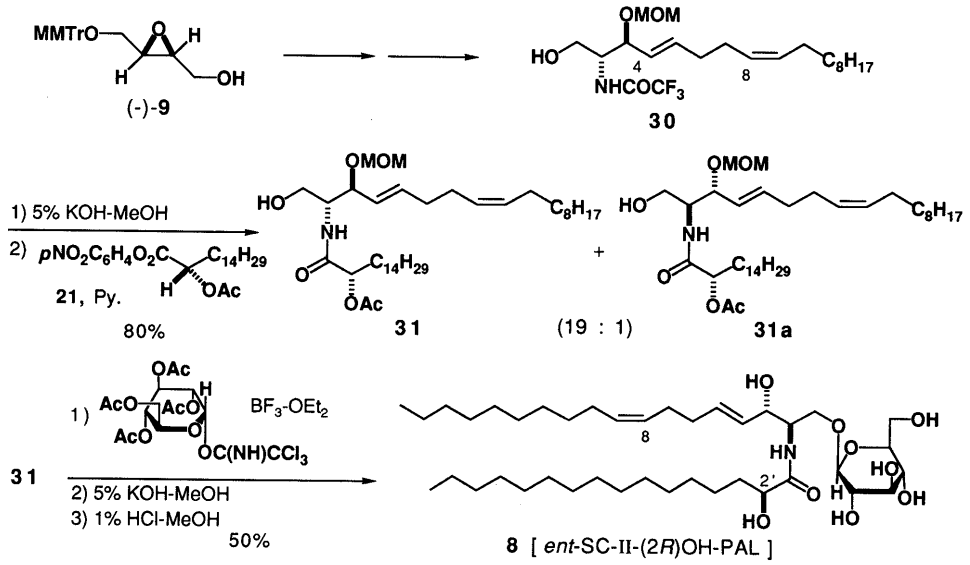

Chart 11

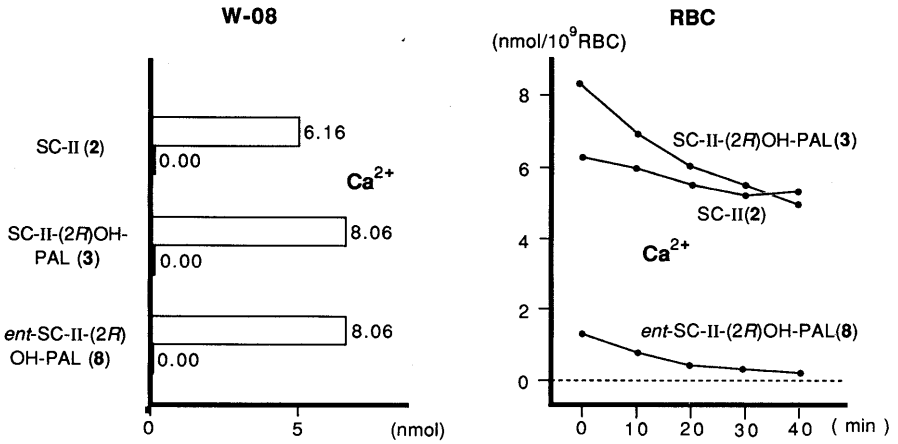

Fig. 6. Calcium Ionophoretic Activities of *ent*-SC-II-(2*R*)OH-PAL (**8**) Compared with Those of SC-II (**2**) and SC-II-(2*R*)OH-PAL (**3**) by Using the W-08 Apparatus (Initial Concentration of Sample: 0.01 M in 1-Decanol) and the Human Erythrocyte Membrane Method

Initial concentration of sample: 0.10 μmol/10^9 red blood cells (RBC). □, ion-binding; ■, ion-transport (after 10 h).

31 was then glucosylated with *O*-(2,3,4,6-tetra-*O*-acetyl-α-L-glucopyranosyl)trichloroacetimidate (the enantiomer of **22**) and the protecting groups of the product were removed under alkaline and acidic conditions finally to furnish the enantiomer (**8**) [*ent*-SC-II-(2*R*)OH-PAL] of (2′*R*)-2′-hydroxypalmitoyl analogue (**3**) [SC-II-(2*R*)OH-PAL].

Interestingly, the enantiomer **8** was shown with the W-08 apparatus[3] (initially 0.01 M in 1-decanol, 8.06 mmol after 10 h) to exhibit calcium ion-binding activity as potent as that of the (2′*R*)-2′-hydroxypalmitoyl analogue (**3**). However, the assay employing the human erythrocyte membrane method[5] showed that the calcium ion-permeation activity of the enantiomer **8** was extremely low as compared with the activity of **3** (Fig. 6). It follows that the human erythrocyte membrane, a biomembrane, can discriminate precisely the difference of absolute configurations of the two enantiomeric ionophores (**3**, **8**).

Experimental

Melting points were determined on a Yanagimoto micromelting point apparatus recorded as observed. Optical rotations were measured in a 0.5 dm tube with a JASCO DIP-370 polarimeter. Electron impact mass spectra (EI-MS) were taken on a JEOL JMS-D300 spectrometer. Fast atom bombardment (FAB)-MS were taken on a JEOL JMS-SX102 spectrometer. Infrared (IR) spectra were taken on a Hitachi 260-30 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on JEOL EX-270 (270 MHz) and GX-500 (500 MHz) spectrometers with tetramethylsilane (TMS) as a standard. Chemical shifts are given on the δ scale (ppm). The following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet and br = broad. Coupling constants (*J* value) are given in hertz (Hz). HPLC was carried out on Shimadzu LC-5A and LC-6A chromatographs. Column chromatography was performed on Kieselgel 60 (Merck, 70—230 mesh). Thin-layer chromatography (TLC) was carried out with pre-coated Kieselgel 60F_{254} plates (Merck). All reactions were carried out under a nitrogen or an argon atmosphere unless otherwise specified.

The Monopivaloate (12) A solution of the mixture (10 g, 23.9 mmol) of the 1,2-diol (**10**) and 1,3-diol (**11**) in pyridine (40 ml) was treated with pivaloyl chloride (3.2 ml, 26.3 mmol, 1.1 eq) with stirring in an ice-water bath for 30 min. The reaction mixture was poured into ice-water and extracted with EtOAc. The EtOAc extract was washed with 5% aqueous HCl, aqueous saturated $NaHCO_3$, and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product, which was purified by silica gel column chromatography (SiO_2 500 g, *n*-hexane : EtOAc = 6 : 1) to afford the monopivaloate **12** (8.4 g, 16.7 mmol, 70%).

12: A colorless oil. $[\alpha]_D$ +16.4° (*c* = 1.3 in $CHCl_3$ at 23 °C). IR (film): 3500, 2950, 2094, 1724, 1616, 1518 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.19 (9H, s), 3.3—3.5 (4H, m), 3.80 (3H, s), 4.1—4.2 (2H, m), 6.6—7.5 (14H, m). EI-MS *m/z* (%): 503 (M^+, 4.3), 273 (MMTr, 100). High-resolution EI-MS *m/z*: Calcd for $C_{29}H_{33}N_3O_5$: 503.2403. Found: 503.2410 (M^+).

Methoxymethylation of 12 Giving 13 A solution of **12** (7.1 g, 14.0 mmol) in CH_2Cl_2 (70 ml) was treated with diisopropylethylamine (17.1 ml, 98.0 mmol, 7 eq) and chloromethyl methyl ether (4.3 ml, 56.0 mmol, 4 eq). The whole mixture was heated under reflux for 4 h. After cooling, the reaction mixture was poured into ice-water and extracted with EtOAc. The EtOAc extract was washed with 5% aqueous HCl, aqueous saturated $NaHCO_3$, and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product, which was purified by silica gel column chromatography (SiO_2 280 g, *n*-hexane : EtOAc = 7 : 1) to afford the methoxymethyl ether derivative **13** (6.6 g, 12.0 mmol, 86%).

13: A colorless oil. $[\alpha]_D$ +4.5° (*c* = 2.0 in $CHCl_3$ at 23 °C). IR (film): 2900, 2095, 1727, 1607, 1502 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (9H, s), 3.20 (3H, s), 3.3—3.8 (4H, m), 3.80 (3H, s), 4.2—4.3 (2H, m), 4.51, 4.57 (2H, ABq, *J* = 6.9 Hz), 6.8—7.5 (14H, m). EI-MS *m/z* (%): 547 (M^+, 2.6), 273 (MMTr, 100). High-resolution EI-MS *m/z*: Calcd for $C_{31}H_{37}N_3O_6$: 547.2668. Found: 547.2671 (M^+).

Preparation of 14 (Segment A) from 13 A solution of **13** (6.5 g, 11.9 mmol) in dry tetrahydrofuran (THF) (10 ml) was added to a suspension of lithium aluminum hydride (677 mg, 17.9 mmol, 1.5 eq) in dry THF (60 ml), and the whole mixture was stirred at room temperature for 30 min. After quenching of the reaction with aqueous ether, the whole mixture was filtered and the filtrate was dried over Na_2SO_4. Removal of the solvent under reduced pressure gave an amino-alcohol (6.2 g). A solution of the amino-alcohol in MeOH (20 ml) was treated with ethyl trifluoroacetate (2.83 ml, 23.8 mmol) and triethylamine (1.99 ml, 23.8 mmol) at room temperature for 10 min. The reaction mixture was extracted with EtOAc. The EtOAc extract was worked up in the usual manner to give a product, which was purified by silica gel column chromatography (SiO_2 310 g, *n*-hexane : EtOAc = 2 : 1) to afford **14** (segment A, 4.44 g, 8.33 mmol, 70%).

14: A colorless oil. $[\alpha]_D$ +4.3° (*c* = 1.0 in $CHCl_3$ at 23 °C). IR (film): 3300, 3059, 2935, 1720, 1607, 1509 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.2—3.8 (4H, m), 3.27 (3H, s), 3.72 (3H, s), 4.3—4.4 (2H, m), 4.57, 4.62 (2H, ABq, *J* = 6.9 Hz), 6.8—7.4 (14H, m), 7.74 (1H, br d). ^{13}C-NMR ($CDCl_3$) δ_C: 50.7 (C-2), 54.7 55.3 (totally 2C, $-OCH_2O\underline{C}H_3$, $\underline{C}H_3O$–Ar), 61.0, 62.0 (totally 2C, C-1, C-4), 78.6 (C-3), 86.5 ($-O\underline{C}Ar_3$), 96.9 ($-O\underline{C}H_2OCH_3$), 113.0—114.0 (totally 18C), 156.7 ($-NHCO\underline{C}F_3$), 158.3 ($-NH\underline{C}OCF_3$). EI-MS *m/z* (%): 533 (M^+, 2.5), 273 (MMTr, 100). High-resolution EI-MS *m/z*: Calcd for $C_{28}H_{30}NO_6F_3$: 533.2018. Found: 533.2020 (M^+).

Preparation of 15 from 1-Decanal A solution of 1-decanal (20 g, 0.128 mol) in CH_2Cl_2 (100 ml) was treated with a reagent [prepared from a solution of triphenylphosphine (134.3 g, 0.512 mol) in CH_2Cl_2 (300 ml) and carbon tetrabromide (84.9 g, 0.256 mol)] with stirring in 0 °C for 15 min. The reaction mixture was poured into ice-water and extracted with CH_2Cl_2. The combined organic phase was concentrated under reduced pressure, and the residue was washed with *n*-hexane to remove triphenylphosphine oxide. Removal of the solvent under reduced pressure gave a product, which was purified by silica gel column chromatography (SiO_2 1 kg, *n*-hexane) to afford **15** (38.3 g, 0.123 mol, 96%).

15: A colorless oil. IR (film): 2940, 2860, 1728, 1620 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t-like), 1.2—1.5 (17H, m), 2.0—2.1 (2H, m), 6.37 (1H, t, *J* = 7.3 Hz). EI-MS *m/z* (%): 314 ($C_{11}H_{20}{}^{81}Br_2$, 0.1), 312 ($C_{11}H_{20}{}^{79}Br^{81}Br$, 0.2), 310 ($C_{11}H_{20}{}^{79}Br_2$, 0.1), 57 (100). High-resolution EI-MS *m/z*: Calcd for $C_{11}H_{20}{}^{81}Br_2$: 313.9610; for $C_{11}H_{20}{}^{79}Br^{81}Br$: 311.9438; for $C_{11}H_{20}{}^{79}Br_2$: 309.9714. Found: 313.9579 ($C_{11}H_{20}{}^{81}Br_2$); 311.9386 ($C_{11}H_{20}{}^{79}Br^{81}Br$); 309.9690 ($C_{11}H_{20}{}^{79}Br_2$).

Preparation of 16 from 15 A solution of **15** (15.15 g, 48.6 mmol) in dry THF (70 ml) was treated with *n*-butyl lithium (1.6 M in *n*-hexane, 60.75 ml, 97.2 mmol) while stirring at −78 °C for 30 min and further at room temperature for 1 h. Hexamethylphosphoramide (10 ml) and 1,3-dibromopropane (12.3 ml, 121.5 mmol) were successively added to the reaction mixture at −78 °C. After warming to room temperature, the whole was stirred for 2 h. Work-up of the reaction mixture in the usual manner gave a product, which was purified by silica gel column chromatography (SiO_2 1 kg, petroleum ether) to afford **16** (9.71 g, 35.6 mmol, 73%).

16: A colorless oil. IR (film): 2950, 2824, 1342 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t-like), 1.27 (10H, br s), 1.4—1.5 (2H, m), 1.9—2.1 (2H, m), 2.1—2.2 (2H, m), 2.3—2.4 (2H, m), 3.53 (2H, t, *J* = 6.6 Hz). ^{13}C-NMR ($CDCl_3$) δ_C: 14.1 (C-14), 17.5, 18.7 (each 1C), 22.8 (C-2), 28.8—29.5 (totally 5C), 31.9 (C-3, C-6), 32.6 (C-1), 77.8, 81.6 (C-4, C-5). EI-MS *m/z* (%): 274 ($C_{14}H_{25}{}^{81}Br$, 0.2), 272 ($C_{14}H_{25}{}^{79}Br$, 0.2), 81 (100). High-resolution EI-MS *m/z*: Calcd for $C_{14}H_{25}{}^{81}Br$: 274.1120; for $C_{14}H_{25}{}^{79}Br$: 272.1139. Found: 274.2240 ($C_{14}H_{25}{}^{81}Br$); 272.0674 ($C_{14}H_{25}{}^{79}Br$).

Preparation of 17 (Segment B) from 16 See below under the preparation of **23** from segment A (**13**) and segment B (**17**).

Preparation of 19 from 18 A solution of methyl palmitate (**18**, 5.88 g, 21.78 mmol) in dry THF (5 ml) was added at −78 °C to a lithium diisopropylamide reagent [prepared from diisopropylamine (4.6 ml, 32.67 mmol) and *n*-butyl lithium (1.6 M in *n*-hexane, 16.3 ml, 26.14 mmol) in THF (30 ml)], and the whole mixture was stirred at the same temperature for 30 min. After warming to room temperature, the reaction mixture was added at −78 °C to a suspension of MoO_5·pyridine·HMPA complex (27.6 g, 28.31 mmol) in dry THF (250 ml), and the whole was stirred for 1 h. After addition of aqueous saturated Na_2SO_3, the reaction mixture was worked up in the usual manner to give a product. Purification of the product by silica gel column chromatography (SiO_2 150 g, *n*-hexane : ether = 4 : 1) afforded **19** (3.13 g, 10.89 mmol, 50%).

19: A white powder. IR ($CHCl_3$): 3527, 2930, 2844, 1725 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t-like), 1.26 (24H, br s), 1.6—1.8 (2H, m), 3.79 (3H, s), 4.19 (1H, dd, *J* = 10.2, 7.3 Hz). EI-MS *m/z* (%): 287 (M^+, 27), 83 (100). High-resolution EI-MS *m/z*: Calcd for $C_{17}H_{34}O_3$: 286.2506. Found: 286.2503 (M^+).

Optical Resolution of 19 Giving 20a and 20b A solution of **19** (2.55 g, 8.87 mmol) in pyridine (40 ml) was treated with *O*-acetyl-L-mandelyl chloride (4 ml) at room temperature for 40 min. After dilution with ether, the whole mixture was washed successively with 5% aqueous HCl, aqueous

saturated $NaHCO_3$, and water, then dried over Na_2SO_4. Removal of the solvent under reduced pressure gave a product, which was purified by silica gel column chromatography (SiO_2 1.2 kg, benzene : ether = 100 : 3) to afford **20a** (1.72 g, 3.37 mmol, 42%) and **20b** (1.72 g, 3.73 mmol, 42%).

20a: A colorless oil. $[\alpha]_D$ +37.2° (c = 1.5 in $CHCl_3$ at 24 °C). IR (film) 2931, 2851, 1743 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t-like), 1.25 (14H, br s), 1.7—1.8 (2H, m), 2.19 (3H, s), 3.72 (3H, s), 5.01 (1H, t, J = 6.6 Hz), 6.06 (1H, s), 7.2—7.6 (5H, m). EI-MS m/z (%): 462 (M^+, 0.2), 176 (100). High-resolution EI-MS m/z: Calcd for $C_{27}H_{42}O_6$: 462.2982. Found: 462.2985 (M^+).

20b: A colorless oil. $[\alpha]_D$ +24.0° (c = 1.5 in $CHCl_3$ at 24°C). IR (film): 2930, 2850, 1738 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t-like), 1.25 (14H, br s), 1.7—1.9 (2H, m), 2.20 (3H, s), 3.56 (3H, s), 5.09 (1H, dd, J = 7.6, 4.5 Hz), 6.00 (1H, s), 7.2—7.6 (5H, m). EI-MS m/z (%): 462 (M^+, 0.4), 176 (100). High-resolution EI-MS m/z: Calcd for $C_{27}H_{42}O_6$: 462.2988. Found: 462.2982 (M^+).

Preparation of 21a (Segment C) from 20a A solution of **20a** (1.66 g, 3.58 mmol) in 5% KOH–MeOH (10 ml) was stirred at room temperature for 1 h, and poured into ice-water. The whole mixture was then acidified with 5% aqueous HCl and extracted with ether, and the ether extract was dried over Na_2SO_4. Removal of the solvent from the ether extract under reduced pressure gave (2*R*)-2-hydroxypalmitic acid, which was dissolved in pyridine (7 ml) and treated with acetic anhydride (7 ml) at room temperature for 9 h. The whole mixture was concentrated under reduced pressure to give a product, which was then treated with *p*-nitrophenyl trifluoroacetate (2.53 g, 10.7 mmol) in pyridine (4 ml) at room temperature for 1 h. The reaction mixture was evaporated to leave a product, which was purified by silica gel column chromatography (SiO_2 120 g, *n*-hexane : EtOAc = 3 : 1) to afford **21a** (segment C, 1.5 g, 3.57 mmol, 99%).

21a: A white powder, $[\alpha]_D$ +15.2° (c = 1.1 in $CHCl_3$ at 24 °C). IR (CCl_4): 2924, 2844, 1776, 1743, 1549, 1346 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t-like), 1.26 (22H, br s), 1.4—1.6 (2H, m), 2.0—2.1 (2H, m), 2.19 (3H, s), 5.13 (1H, t, J = 6.3 Hz), 7.27 (2H, d, J = 8.9 Hz), 8.27 (2H, d, J = 8.9 Hz). EI-MS m/z (%): 297 (54), 97 (100). *Anal.* Calcd for $C_{24}H_{37}NO_6$: C, 66.20; H, 8.51; N, 3.22. Found: C, 66.24; H, 8.51; N, 3.03.

Preparation of 21b from 20b The other acetate **21b** (1.5 g, 3.57 mmol, 99%) was obtained from **20b** (1.66 g, 3.58 mmol) through a procedure similar to that used for the preparation of **21a** from **20a**.

21b: A white powder, $[\alpha]_D$ −15.3° (c = 1.0 in $CHCl_3$ at 24 °C). IR (CCl_4): 2924, 2844, 1776, 1743, 1549, 1346 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t-like), 1.26 (22H, br s), 1.4—1.6 (2H, m), 2.0—2.1 (2H, m), 2.19 (3H, s), 5.13 (1H, t, J = 6.3 Hz), 7.27 (2H, d, J = 8.9 Hz), 8.27 (2H, d, J = 8.9 Hz). EI-MS m/z (%): 297 (54), 97 (100). *Anal.* Calcd for $C_{24}H_{37}NO_6$: C, 66.20; H, 8.51; N, 3.22. Found: C, 65.92; H, 8.42; N, 3.09.

Preparation of 23 from Segment A (13) and Segment B (17) A mixture of **16** (2.69 g, 9.84 mmol) and triphenylphosphine (2.58 g, 9.84 mmol) was heated at 120 °C for 6 h. After cooling, the resulting solid was washed repeatedly with ether and dried *in vacuo* at 100 °C to give segment B (**17**, 5.2 g). On the other hand, a solution of **13** (segment A, 2.28 g, 4.28 mmol) in CH_2Cl_2 (10 ml) was treated with molecular sieves 4A (2.9 g) and pyridinium dichromate (2.42 g, 4.28 mmol) with vigorous stirring at room temperature for 1.5 h. The reaction mixture was diluted with ether (300 ml) and the whole was passed through a Florisil column (200 g). The filtrate was evaporated to provide an aldehydic derivative. A suspension of segment B (**17**, 5.02 g, 9.37 mmol, 2.2 eq) in THF (28 ml) was treated with *n*-butyl lithium (1.6 M in *n*-hexane, 5.62 ml, 8.99 mmol, 2.1 eq) and the whole was stirred at room temperature for 30 min, then a solution of the above aldehydic derivative in THF (4 ml) was added at −78 °C. The reaction mixture was stirred at the same temperature for 1 h, and then treated with dry MeOH (10 ml) with stirring at −40 °C for 4 h. After addition of water (10 ml) to the reaction mixture, the whole was stirred at room temperature for 1 h. The resulting mixture was worked up in the usual manner to give a product, which was purified by silica gel column chromatography (SiO_2 190 g, *n*-hexane : EtOAc = 4 : 1) to afford the mixture **23** (1.96 g, 65%).

Preparation of 24 and 24a from 23 A solution of **23** (1.0 g, 1.42 mmol) in *n*-hexane (15 ml) was treated with 5% Pd-$CaCO_3$ (3 g) and quinoline (1 ml) and the whole mixture was stirred vigorously under an H_2 atmosphere at room temperature for 2 h. After removal of the catalyst by filtration, the solvent was evaporated off to yield a product. Purification of the product by silica gel column chromatography (SiO_2 50 g, *n*-hexane : EtOAc = 5 : 1) afforded an 8*Z*-compound, which was dissolved in 10% HCl–MeOH (10 ml). The whole mixture was stirred in an ice-water bath for 30 min. After neutralization of the reaction mixture with Ag_2CO_3, the precipitate was removed by filtration. The filtrate was evaporated to yield a product, which was purified by silica gel column chromatography (SiO_2 30 g, *n*-hexane : EtOAc = 4 : 1) to afford **24** (465 mg, 78%) and **24a** (37 mg, 6.3%).

24: A colorless oil. $[\alpha]_D$ −55.6° (c = 0.6 in $CHCl_3$ at 24 °C). IR (film): 3426, 3286, 2926, 2846, 1712 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.85 (3H, t-like), 1.27 (14H, br s), 1.9—2.2 (6H, m), 3.38 (3H, s), 3.6—4.3 (4H, m), 4.56, 4.65 (2H, ABq, J = 6.6 Hz), 5.2—5.5 (3H, m), 5.7—5.9 (1H, m), 7.24 (1H, br. d). ^{13}C-NMR ($CDCl_3$) δ_C: 14.1 (C-18), 22.6 (C-17), 26.5 (C-7), 27.3 (C-10), 29.2—29.6 (totally 5C), 31.9 (C-16), 32.3 (C-6), 53.9 (C-2), 55.7 (–$OCH_2O\underline{C}H_3$), 60.7 (C-1), 78.4 (C-3), 94.4 (–$O\underline{C}H_2OCH_3$), 125.5, 128.2 (C-8, C-9), 130.9 (C-4), 136.9 (C-5), 156.9 (–$NHCO\underline{C}F_3$), 158.7 (–$NH\underline{C}OCF_3$). EI-MS m/z (%): 437 (M^+, 0.5), 81 (100). High-resolution EI-MS m/z: Calcd for $C_{22}H_{38}F_3NO_4$: 437.2588. Found: 437.2571 (M^+).

24a: A colorless oil. $[\alpha]_D$ −82.3° (c = 0.5 in $CHCl_3$ at 24 °C). IR (film): 3435, 3306, 2920, 2851, 1708 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t-like), 1.26 (14H, br s), 1.9—2.3 (6H, m), 3.37 (3H, s), 3.6—4.8 (4H, m), 4.53, 4.64 (2H, ABq, J = 6.6 Hz), 5.3—5.5 (3H, m), 5.7—5.8 (1H, m), 7.20 (1H, br d). ^{13}C-NMR ($CDCl_3$) δ_C: 14.1 (C-18), 22.7 (C-17), 26.8 (C-7), 27.3 (C-10), 27.8 (C-6), 29.3—29.7 (totally 5C), 31.9 (C-16), 53.9 (C-2), 55.7 (–$OCH_2O\underline{C}H_3$), 60.9 (C-1), 73.2 (C-3), 94.2 (–$O\underline{C}H_2OCH_3$), 125.4, 128.0 (C-8, C-9), 131.3 (C-4), 136.7 (C-5), 156.9 (–$NHCO\underline{C}F_3$), 158.7 (–$NH\underline{C}OCF_3$). EI-MS m/z (%): 437 (M^+, 0.5), 81 (100). High-resolution EI-MS m/z: Calcd for $C_{22}H_{38}F_3NO_4$: 437.2750. Found: 437.2745 (M^+).

Preparation of 25 and 25a from 24 and 21a A solution of **24** (89 mg, 0.204 mmol) in MeOH (2 ml) was treated with 10% KOH–MeOH (1 ml) with stirring at room temperature for 5 h. The reaction mixture was poured into ice-water and the whole was extracted with $CHCl_3$. The $CHCl_3$ extract was dried over Na_2SO_4. Removal of the solvent under reduced pressure gave a crude amino-alcohol. A solution of **21a** (114 mg, 0.27 mmol) in pyridine (0.5 ml) was added to a solution of the above crude amino-alcohol in pyridine (1 ml), and the mixture was stirred at 50 °C for 2 h. The reaction mixture was concentrated under reduced pressure to give a product, which was purified by silica gel column chromatography (SiO_2 9g, CH_2Cl_2 : ether = 7 : 1) to afford **25** (99 mg, 0.16 mmol, 76%) and **25a** (5.2 mg, 0.082 mmol, 4%).

25: A white powder, $[\alpha]_D$ −21.1° (c = 0.6 in $CHCl_3$ at 24 °C). IR ($CHCl_3$): 3442, 2931, 2846, 1739, 1672 cm^{-1}. ^{1}H-NMR (d_5-pyridine) δ: 0.85 (6H, t-like, 18-H_3, 16′-H_3), 1.26 (40H, br s, $CH_2 \times 20$), 1.4—1.6 (2H, m, 3′-H_2), 2.04 (3H, s, –OCOC$\underline{H}_3$), 2.0—2.2 (6H, m, 6-H_2, 7-H_2, 10-H_2), 3.41 (3H, s, –$OCH_2OC\underline{H}_3$), 4.1—4.7 (5H, m, 1-H_2, 2-H, 3-H, 2′-H), 4.70, 4.88 (2H, ABq, J = 6.6 Hz, –$OC\underline{H}_2OCH_3$), 5.4—5.5 (3H, m, 4-H, 8-H, 9-H), 5.6—5.8 (1H, m, 5-H), 8.34 (1H, br d, –NHCO–). ^{13}C-NMR (d_5-pyridine) δ_C: 14.3 (totally 2C, C-18, C-16′), 20.8, 22.9, 25.5, (totally 4C), 27.1 (C-7), 27.6 (C-10), 29.5—29.9 (totally 15C), 32.1 (totally 2C, C-16, C-14′), 32.7 (C-6), 55.0 (C-2), 55.5 (–$OCH_2O\underline{C}H_3$), 61.0 (C-1), 74.8, 76.8 (C-3, C-2′), 94.0 (–$O\underline{C}H_2OCH_3$), 128.3 (totally 2C, C-8, C-9), 129.2 (C-4), 130.8 (C-5), 170.2, 170.3 (C-1′, –$O\underline{C}OCH_3$). EI-MS m/z (%): 637 (M^+, 0.4), 60 (100). High-resolution EI-MS m/z: Calcd for $C_{38}H_{71}NO_6$: 637.5332. Found: 637.5337 (M^+).

25a: A white powder, $[\alpha]_D$ −22.7° (c = 0.4 in $CHCl_3$ at 24 °C). IR ($CHCl_3$): 3434, 2922, 2848, 1736, 1671 cm^{-1}. ^{1}H-NMR (d_5-pyridine) δ: 0.85 (6H, t-like, 18-H_3, 16′-H_3), 1.26 (40H, br s, $CH_2 \times 20$), 1.4—1.6 (2H, m, 3′-H_2), 2.04 (3H, s, –OCOC$\underline{H}_3$), 2.0—2.2 (6H, m, 6-H_2, 7-H_2, 10-H_2), 3.41 (3H, s, –$OCH_2OC\underline{H}_3$), 4.1–4.7 (5H, m, 1-H_2, 2-H, 3-H, 2′-H), 4.70, 4.88 (2H, ABq, J = 6.6 Hz, –$OC\underline{H}_2OCH_3$), 5.4—5.5 (3H, m, 4-H, 8-H, 9-H), 5.6—5.8 (1H, m, 5-H), 8.34 (1H, br d, –NHCO–). ^{13}C-NMR (d_5-pyridine) δ_C: 14.3 (totally 2C, C-18, C-16′), 20.8, 22.9, 25.5 (totally 4C), 27.1 (C-7), 27.6 (C-10), 29.5—29.9 (totally 15C), 32.1 (totally 2C, C-16, C-14′), 32.7 (C-6), 55.0 (C-2), 55.5 (–$OCH_2O\underline{C}H_3$), 61.0 (C-1), 74.8, 76.8 (C-3, C-2′), 94.0 (–$O\underline{C}H_2CH_3$), 128.3 (totally 2C, C-8, C-9), 129.2 (C-4), 130.8 (C-5), 170.2, 170.3 (C-1′, –$O\underline{C}OCH_3$). EI-MS m/z (%): 637 (M^+, 0.8), 60 (100). High-resolution EI-MS m/z: Calcd for $C_{38}H_{71}NO_6$: 637.5372. Found: 637.5382 (M^+).

Glucosylation of 25 Followed by Deprotection, Giving the (2′*R*)-2′-Hydroxypalmitoyl Analogue (3) A solution of *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)trichloroacetimidate (114 mg, 0.236 mmol, 2.5 eq) in CH_2Cl_2 (1.5 ml) was treated with boron trifluoride etherate (47% in ether, 14.7 ml, 0.048 mmol, 0.5 eq). The mixture was then added to a suspension of **25** (61 mg, 0.095 mmol) and molecular sieves 4A (40 mg) in CH_2Cl_2 (3 ml), and the whole was stirred at −30 °C for 1 h. The reaction mixture was poured into ice-water and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product, which was dissolved in MeOH (2 ml) and treated with 5% KOH–MeOH (2 ml). The whole mixture was stirred at room temperature for 30 min. After neutralization of the reaction mixture with Dowex 50W × 8 (H^+

form), the resin was removed by filtration. The filtrate was evaporated to yield a product, which was purified by silica gel column chromatography (SiO_2 5 g, $CHCl_3$: MeOH = 10 : 1) to afford a white powder (62 mg). A solution of the white powder (42 mg) in 1% HCl–MeOH (1 ml) was stirred at room temperature for 3 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated under reduced pressure to give a product, which was purified by silica gel column chromatography (SiO_2 3.5 g, $CHCl_3$: MeOH = 10 : 1) to afford the (2′*R*)-2′-hydroxypalmitoyl analogue (**3**, 29 mg, 63%) of soya-cerebroside II (**2**).

3: A white powder, $[\alpha]_D$ +5.2° (c = 0.1 in MeOH at 24 °C). IR (KBr): 3380, 2913, 2843, 1628 cm^{-1}. ^{1}H-NMR (d_5-pyridine) δ: 0.90 (6H, t-like, 18-H_3, 16′-H_3), 1.28 (38H, br s, $CH_2 \times 19$), 1.4—2.1 (8H, m, 6-H_2, 7-H_2, 10-H_2, 3′-H_2), 3.8—4.8 (11H, m, 1-H_2, 2-H, 3-H, 2′-H, 2″-H, 3″-H, 4″-H, 5″-H, 6″-H_2), 4.89 (1H, d, J = 7.6 Hz, 1″-H), 5.4—5.5 (3H, m, 4-H, 8-H, 9-H), 5.8—6.1 (1H, m, 5-H), 8.36 (1H, br d, –NHCO–); (d_6-DMSO) δ: 0.85 (6H, t-like, 18-H_3, 16′-H_3), 1.23 (38H, br s, $CH_2 \times 19$), 1.3—1.4 (1H, m, 3′-H_a), 1.5—1.6 (1H, m, 3′-H_b), 2.0—2.1 (6H, m, 6-H_2, 7-H_2, 10-H_2), 2.95 (1H, m, 2″-H), 3.0—3.2 (3H, m, 3″-H, 4″-H, 5″-H), 3.4—3.5 (1H, m, 6″-H_a), 3.53 (1H, dd, J = 10.5, 4.0 Hz, 1-H_a), 3.6—3.7 (1H, m, 6″-H_b), 3.80 (2H, m, 2-H, 2′-H), 3.91 (1H, dd, J = 10.5, 6.0 Hz, 1-H_b), 4.00 (1H, dd, J = 12.5, 6.5 Hz, 3-H), 4.12 (1H, d, J = 7.6 Hz, 1″-H), 4.49 (1H, t-like, 6″-OH), 4.88 (2H, m, 3″-OH, 4″-OH), 4.93 (2H, m, 3-OH, 2″-OH), 5.3—5.4 (2H, m, 8-H, 9-H), 5.40 (1H, dd, J = 15.0, 6.5 Hz, 4-H), 5.49 (1H, d, J = 5.0 Hz, 2′-OH), 5.59 (1H, dt, J = 15.0, 6.5 Hz, 5-H); [(d_6-DMSO + $CaCl_2$ (1 eq)] δ: 0.85 (6H, t-like, 18-H_3, 16′-H_3), 1.23 (38H, br s, $CH_2 \times 19$), 1.3—1.4 (1H, m, 3′-H_a), 1.5—1.6 (1H, m, 3′-H_b), 2.0—2.1 (6H, m, 6-H_2, 7-H_2, 10-H_2), 2.95 (1H, m, 2″-H), 3.0—3.2 (3H, m, 3″-H, 4″-H, 5″-H), 3.4—3.5 (1H, m, 6″-H_a), 3.53 (1H, dd, J = 10.5, 4.0 Hz, 1-H_a), 3.6—3.7 (1H, m, 6″-H_b), 3.80 (2H, m, 2-H, 2′-H), 3.91 (1H, dd, J = 10.5, 6.0 Hz, 1-H_b), 4.00 (1H, dd, J = 12.5, 6.5 Hz, 3-H), 4.12 (1H, d, J = 7.6 Hz, 1″-H), 4.53 (1H, t-like, 6″-OH), 4.96 (4H, m, 3-OH, 2″-OH, 3″-OH, 4″-OH), 5.3—5.4 (2H, m, 8-H, 9-H), 5.40 (1H, dd, J = 15.0, 6.5 Hz, 4-H), 5.55 (1H, d, J = 5.0 Hz, 2′-OH), 5.59 (1H, dt, J = 15.0, 6.5 Hz, 5-H). ^{13}C-NMR (d_5-pyridine) δ_C: 14.2 (totally 2C, C-18, C-16′), 22.9 (totally 2C, C-17, C-15′), 25.9 (totally 1C), 27.3 (C-7), 27.5 (C-10), 29.6—30.0 (totally 14C), 32.1 (totally 2C, C-16, C-14′), 32.9 (C-6), 35.6 (C-3′), 54.5 (C-2), 62.6 (C-6″), 70.0 (C-1), 71.4 (C-4″), 72.2 (C-3), 72.4 (C-2′), 75.0 (C-2″), 78.3 (C-3″), 78.4 (C-5″), 105.5 (C-1″), 129.4, 130.6 (C-8, C-9), 132.0 (C-4), 132.1 (C-5), 175.6 (C-1′). FAB-MS m/z: 736 $(M+Na)^+$. High-resolution FAB-MS m/z: Calcd for $C_{40}H_{75}NO_9$ + Na: 736.5297. Found: 736.5293 $(M+Na)^+$.

Preparation of the Palmitoyl Analogue (4) from 24 *via* 26 A solution of **24** (80 mg, 0.183 mmol) in MeOH (2 ml) was treated with 10% KOH–MeOH (1 ml) with stirring at room temperature for 5 h. The reaction mixture was poured into ice-water and the whole was extracted with $CHCl_3$. The $CHCl_3$ extract was dried over Na_2SO_4. Romoval of the solvent under reduced pressure gave a crude amino-alcohol. *p*-Nitrophenyl palmitate (104 mg, 0.28 mmol) in pyridine (0.5 ml) was added to a solution of the above crude amino-alcohol in pyridine (1 ml), and the mixture was stirred at 50 °C for 2 h, then concentrated under reduced pressure. Purification of the product by silica gel column chromatography (SiO_2 9 g, *n*-hexane : EtOAc = 2 : 1) afforded a ceramide (**26**, 88 mg, 0.15 mmol, 82%). The ceramide (**26**, 40 mg, 0.069 mmol) was then converted into the palmitoyl analogue (**4**, 24 mg, 0.0345 mmol, 50%) through the same procedure as used for the preparation of the (2′*R*)-2′-hydroxypalmitoyl analogue (**3**) from **25**.

4: A white powder, $[\alpha]_D$ −11.8° (c = 0.2 in MeOH at 24 °C). IR (KBr): 3400, 2924, 2848, 1633 cm^{-1}. ^{1}H-NMR (d_5-pyridine) δ: 0.88 (6H, t-like, 18-H_3, 16′-H_3), 1.27 (40H, br s, $CH_2 \times 20$), 1.7—2.2 (6H, m, 6-H_2, 7-H_2, 10-H_2), 2.43 (2H, t, J = 7.3 Hz, 2′-H_2), 3.9—4.8 (10H, m, 1-H_2, 2-H, 3-H, 2″-H, 3″-H, 4″-H, 5″-H, 6″-H_2), 4.92 (1H, d, J = 7.6 Hz, 1″-H), 5.4—5.5 (3H, m, 4-H, 8-H, 9-H), 5.8—6.1 (1H, m, 5-H), 8.36 (1H, br d, –NHCO–). ^{13}C-NMR (d_5-pyridine) δ_C: 14.2 (totally 2C, C-18, C-16′), 22.9 (totally 2C, C-17, C-15′), 26.3 (C-3′), 27.3 (C-7), 27.5 (C-10), 29.5—29.9 (totally 15C), 32.1 (totally 2C, C-16, C-14′), 32.8 (C-6), 36.8 (C-2′), 54.9 (C-2), 62.6 (C-6″), 70.4 (C-1), 71.5 (C-4″), 72.5 (C-3), 75.1 (C-2″), 78.4 (totally 2C, C-3″, C-5″), 105.7 (C-1″), 129.3, 130.6 (C-8, C-9), 131.8 (C-4), 132.5 (C-5), 175.6 (C-1′). FAB-MS m/z: 720 $(M+Na)^+$. High-resolution FAB-MS m/z: Calcd for $C_{40}H_{75}NO_8$ + Na: 720.5365. Found: 720.5367 $(M+Na)^+$.

Preparation of the (2′*S*)-2′-Hydroxypalmitoyl Analogue (5) from 24 and 21b *via* 27 A solution of *p*-nitrophenyl (2′*S*)-2′-acetoxypalmitate (**21b**, 86.5 mg 0.21 mmol) in pyridine (0.5 ml) was added to a solution of an amino-alcohol [prepared from **24** (60 mg, 0.137 mmol) by treatment with 10% KOH–MeOH (1 ml)] in pyridine (1 ml). The mixture was stirred at 50 °C for 2 h, and the solvent was evaporated off to yield a product. Purification of the product by silica gel column chromatography (SiO_2 7 g, CH_2Cl_2: ether = 7 : 1) afforded a D-*erythro*-type ceramide (**27**, 66 mg, 0.104 mmol) and an L-*erythro*-type isomer (**27a**, 3.5 mg, 0.0055 mmol). The D-*erythro*-type ceramide (**27**, 52 mg, 0.081 mmol) was then converted to the (2′*S*)-2′-hydroxypalmitoyl analogue (**5**, 29 mg, 0.041 mmol, 38% from **24**) through a procedure similar to that used for the preparation of the (2′*R*)-2′-hydroxypalmitoyl analogue (**3**) from **25**.

5: A white powder, $[\alpha]_D$ −12.6° (c = 0.2 in MeOH at 24 °C). IR (KBr): 3350, 2922, 2844, 1642 cm^{-1}. ^{1}H-NMR (d_5-pyridine) δ: 0.87 (6H, t-like, 18-H_3, 16′-H_3), 1.24 (38H, br s, $CH_2 \times 19$), 1.7—2.2 (8H, m, 6-H_2, 7-H_2, 10-H_2, 3′-H_2), 3.9—4.9 (11H, m, 1-H_2, 2-H, 3-H, 2′-H, 2″-H, 3″-H, 4″-H, 5″-H, 6″-H_2), 4.91 (1H, d, J = 7.6 Hz, 1″-H), 5.3—5.5 (3H, m, 4-H, 8-H, 9-H), 5.8—6.1 (1H, m, 5-H), 8.34 (1H, br d, –NHCO–). ^{13}C NMR (d_5-pyridine) δ_C: 14.2 (totally 2C, C-18, C-16′), 22.9 (totally 2C, C-17, C-15′), 25.8 (totally 1C), 27.3 (C-7), 27.5 (C-10), 29.6—30.0 (totally 14C), 32.1 (totally 2C, C-16, C-14′), 32.8 (C-6), 35.6 (C-3′), 54.8 (C-2), 62.5 (C-6″), 69.9 (C-1), 71.4 (C-4″), 72.1 (C-3), 72.5 (C-2′), 75.0 (C-2″), 78.4 (C-3″), 78.5 (C-5″), 105.6 (C-1″), 129.3 (totally 2C, C-8, C-9), 130.6 (C-4), 131.9 (C-5), 175.6 (C-1′). FAB-MS m/z: 736 $(M+Na)^+$. High-resolution FAB-MS m/z: Calcd for $C_{40}H_{75}NO_9$ + Na: 736.5339. Found: 736.5361 $(M+Na)^+$.

Preparation of the Galactosyl Analogue (6) from 25 A solution of *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl)trichloroacetimidate (87 mg, 0.18 mmol, 2.5 eq) in CH_2Cl_2 (1.5 ml) and boron trifluoride etherate (47% in ether, 14.7 ml, 0.048 mmol, 0.5 eq) were added to a suspension of **25** (50 mg, 0.072 mmol) and molecular sieves 4A (40 mg) in CH_2Cl_2 (3 ml). The reaction mixture was stirred at −30 °C for 1 h, poured into ice-water and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product, which was dissolved in MeOH (2 ml) and treated with 5% KOH–MeOH (2 ml). The whole mixture was stirred at room temperature for 30 min. After neutralization of the reaction mixture with Dowex 50W × 8 (H^+ form), the resin was removed by filtration. The filtrate was evaporated to yield a product, which was purified by silica gel column chromatography (SiO_2 5 g, $CHCl_3$: MeOH = 10 : 1) to afford a while powder (62 mg). A solution of the white powder (42 mg) in 1% HCl–MeOH (1 ml) was stirred at room temperature for 3 h, then neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated under reduced pressure to give a product, which was purified by silica gel column chromatography (SiO_2 3.5 g, $CHCl_3$: MeOH = 10 : 1) to afford the galactosyl analogue (**6**, 26.4 mg, 52%) of soya-cerebroside II (**2**).

6:A white powder, $[\alpha]_D$ +6.1° (c = 0.2 in MeOH at 24 °C). IR (KBr): 3400, 2924, 2850, 1639 cm^{-1}. ^{1}H-NMR (d_5-pyridine) δ: 0.88 (6H, t-like, 18-H_3, 16′-H_3), 1.28 (38H, br s, $CH_2 \times 19$), 1.7—2.2 (8H, m, 6-H_2, 7-H_2, 10-H_2, 3′-H_2), 3.8—4.8 (11H, m, 1-H_2, 2-H, 3-H, 2′-H, 2″-H, 3″-H, 4″-H, 5″-H, 6″-H_2), 4.84 (1H, d, J = 7.6 Hz, 1″-H), 5.4—5.5 (3H, m, 4-H, 8-H, 9-H), 5.8—6.1 (1H, m, 5-H), 8.36 (1H, br d, –NHCO–). ^{13}C-NMR (d_5-pyridine) δ_C: 14.2 (totally 2C, C-18, C-16′), 22.9 (totally 2C, C-17, C-15′), 25.9 (totally 1C), 27.3 (C-7), 27.6 (C-10), 29.6—30.0 (totally 14C), 32.1 (totally 2C, C-16, C-14′), 32.8 (C-6), 35.6 (C-3′), 54.5 (C-2), 62.4 (C-6″), 70.2 (C-1), 72.3 (C-3), 72.5 (totally 2C, C-2′, C-2″), 75.2 (C-3″), 77.1 (C-5″), 106.2 (C-1″), 129.4, 130.6 (C-8, C-9), 132.0 (C-4), 132.2 (C-5), 175.6 (C-1′). FAB-MS m/z: 736 $(M+Na)^+$. High-resolution FAB-MS m/z: Calcd for $C_{40}H_{75}NO_9$: 736.5339. Found: 736.5347 $(M+Na)^+$.

Preparation of 28a and 28b from Segment A (13) A solution of segment A (**13**, 140 g, 2.63 mmol) in CH_2Cl_2 (20 ml) was stirred with pyridinium dichromate (2.42 g, 6.42 mmol) and molecular sieves 4A (2.2 g) at room temperature for 1 h. After addition of ether (500 ml) to the reaction mixture, the whole was passed through a Florisil column (100 g). The eluate was concentrated under reduced pressure to yield a product. A solution of the product in THF (3 ml) was added at −78 °C to a triphenyltetradecyl phosphorane reagent [prepared from triphenylphosphine tetradecyl bromide (3.55 g, 6.58 mmol) in THF (9 ml) and *n*-BuLi (1.6 M in *n*-hexane, 3.61 ml, 5.79 mmol)]. The mixture was stirred at −78 °C for 1 h, then dry MeOH (5 ml) was added to the reaction mixture and the whole was stirred at −30°—−50 °C for 4 h. The reaction was quenched with water (5 ml), and the mixture was worked up in the usual manner to give a product. Purification of the product by silica gel column chromatography (SiO_2 20 g, *n*-hexane : ether = 6 : 1) afforded **28** (1.21 g, 65%). A solution of **28** (500 mg) in MeOH (5 ml) was treated with 10% HCl–MeOH (5 ml) at 0 °C for 30 min. The reaction mixture was neutralized with Ag_2CO_3 powder, and the precipitate was removed by filtration. The solvent was evaporated

from the filtrate under reduced pressure to give a product, which was purified by silica gel column chromatography (SiO_2 25 g, *n*-hexane : EtOAc = 4 : 1) to afford **28a** (226 mg, 73%) and **28b** (23 mg, 7%).

28a: A colorless oil, $[\alpha]_D$ −22.3° (c = 0.5 in $CHCl_3$ at 24 °C). IR (film): 3427, 3316, 2926, 2855, 1713 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t-like), 1.26 (22H, br s), 2.0—2.7 (2H, m), 3.37 (3H, s), 3.6—4.3 (4H, m), 4.53, 4.64 (2H, ABq, J = 6.6 Hz), 5.37 (1H, dd, J = 15.5, 7.9 Hz), 5.80 (1H, dt, J = 15.5, 6.5 Hz), 7.20 (1H, br d). ^{13}C-NMR ($CDCl_3$) δ_C: 14.1 (C-18), 22.7 (C-17), 29.5—29.7 (totally 9C), 32.0 (C-16), 32.5 (C-6), 54.5 (C-2), 56.0 (–$OCH_2\underline{C}H_3$), 62.0 (C-1), 78.9 (C-3), 94.5 (–$O\underline{C}H_2OCH_3$), 125.5 (C-4), 138.0 (C-5), 156.0 (–NH$\underline{C}OCF_3$), 158.7 (–NH$\underline{C}OCF_3$). EI-MS m/z (%): 439 (M^+, 0.5), 81 (100). High-resolution EI-MS m/z: Calcd for $C_{22}H_{40}F_3NO_4$: 439.2750. Found: 439.2742 (M^+).

28b: A colorless oil, $[\alpha]_D$ −75.2° (c = 0.5 in $CHCl_3$ at 24 °C). IR (film): 3428, 3302, 2924, 2855, 1714 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t-like), 1.26 (22H, br s), 2.0—2.7 (2H, m), 3.36 (3H, s), 3.6—4.3 (4H, m), 4.52, 4.63 (2H, ABq, J = 6.6 Hz), 5.5—5.9 (2H, m), 7.20 (1H, br d). ^{13}C-NMR ($CDCl_3$) δ_C: 14.1 (C-18), 22.6 (C-17), 28.9 (C-6), 29.5—29.7 (totally 9C), 32.3 (C-16), 54.5 (C-2), 55.0 (–$OCH_2\underline{C}H_3$), 60.5 (C-1), 72.9 (C-3), 93.4 (–$O\underline{C}H_2OCH_3$), 126.9 (C-4), 137.8 (C-5), 156.0 (–NH$\underline{C}OCF_3$), 158.7 (–NH$\underline{C}OCF_3$). EI-MS m/z (%): 439 (M^+, 0.5), 81 (100). High-resolution EI-MS m/z: Calcd for $C_{22}H_{40}F_3NO_4$: 439.1650. Found: 439.1632 (M^+).

Preparation of the 8,9-Dihydro Analogue (7) from 28a A solution of **28a** (72.1 mg, 0.164 mmol) in MeOH (1 ml) was treated with 10% KOH–MeOH (0.5 ml) at room temperature. The reaction mixture was poured into ice-water and extracted with $CHCl_3$. The $CHCl_3$ extract was dried over Na_2SO_4. Removal of the solvent from the extract under reduced pressure gave a product (a crude amino-alcohol). A solution of **21a** (103.3 mg, 0.246 mmol) in pyridine (0.5 ml) was added to a solution of the product in pyridine (1 ml), and the mixture was stirred at 50 °C for 2 h. After cooling, the reaction mixture was evaporated under reduced pressure. Purification of the product by silica gel column chromatography (SiO_2 9 g, CH_2Cl_2 : ether = 7 : 1) afforded D-*erythro*-type 4*E*-ceramide (**29**, 80 mg, 0.125 mmol, 76%) and its L-*erythro*-type isomer (4.2 mg, 4%). The 4*E*-ceramide (**29**, 45 mg, 0.070 mmol) was then converted into the 8,9-dihydro analogue (**7**, 17 mg, 0.024 mmol, 63%) of soya-cerebroside II (**2**) through a procedure similar to that used for the preparation of the (2′*R*)-2′-hydroxypalmitoyl analogue (**3**) from the D-*erythro*-ceramide (**25**).

7: A white powder, $[\alpha]_D$ −2.2° (c = 0.3 in MeOH at 24 °C). IR (KBr): 3350, 2916, 2847, 1641 cm^{-1}. ^{1}H-NMR (d_5-pyridine) δ: 0.88 (6H, t-like, 18-H_3, 16′-H_3), 1.27 (46H, br s, CH_2 × 23), 1.7—2.3 (4H, m, 6-H_2, 3′-H_2), 3.8—4.8 (11H, m, 1-H_2, 2-H, 3-H, 2′-H, 2″-H, 3″-H, 4″-H, 5″-H, 6″-H_2), 4.89 (1H, d, J = 7.9 Hz, 1-H″), 5.8—6.0 (2H, m, 4-H, 5-H), 8.32 (1H, br d, –NHCO–). ^{13}C-NMR (d_5-pyridine) δ_C: 14.2 (totally 2C, C-18, C-16′), 22.9 (totally, 2C, C-15′, C-17), 25.9 (totally 1C), 29.6—30.0 (totally 18C), 32.1 (totally 2C, C-16, C-14′), 32.6 (C-6), 35.6 (C-3′), 54.5 (C-2), 62.6 (C-6″), 70.1 (C-1), 71.4 (C-4″), 72.3 (C-3), 72.4 (C-2′), 75.0 (C-2″), 78.3 (C-3″), 78.4 (C-5″), 105.6 (C-1″), 132.7, 131.6, (C-4, C-5), 175.6 (C-1′). FAB-MS m/z: 738 ($M+Na)^+$. High-resolution FAB-MS m/z: Calcd for $C_{40}H_{77}NO_9$ + Na: 738.5496. Found: 738.5453 ($M+Na)^+$.

Preparation of 30 from the C4-Epoxide [(−)-9] A (4*E*, 8*Z*)-L-*erythro*-4,8-sphingadienine derivative (**30**) was obtained in 19% yield from (−)-**9** through a procedure similar to that used for the preparation of the (4*E*,8*Z*)-D-*erythro*-4,8-sphingadienine derivative (**24**) from (+)-**9**.

30: A colorless oil. $[\alpha]_D$ +54.8° (c = 0.5 in $CHCl_3$ at 25 °C). High-resolution EI-MS m/z: Calcd for $C_{22}H_{38}F_3NO_4$: 437.2588. Found: 437.2576 (M^+).

Preparation of 31 and 31a from 30 The ceramides **31** (75%) and **31a** (4%) were obtained from **30** through a precedure similar to that used for the preparation of **25** and **25a** from **24**.

31: A white powder, $[\alpha]_D$ +20.8° (c = 0.5 in $CHCl_3$ at 25 °C). High-resolution EI-MS m/z: Calcd for $C_{38}H_{71}NO_6$: 637.5372. Found: 637.5334 (M^+). IR, ^{1}H-NMR, ^{13}C-NMR, and EI-MS data for **31** were identical with those for **25**.

31a: A white powder, $[\alpha]_D$ +22.2° (c = 0.5 in $CHCl_3$ at 25 °C). High-resolution EI-MS m/z: Calcd for $C_{38}H_{71}NO_6$: 637.5332. Found: 637.5382 (M^+). IR, ^{1}H-NMR, ^{13}C-NMR, and EI-MS data for **31a** were identical with those for **25a**.

Preparation of the Enantiomer (8) of the (2′*R*)-2′-Hydroxypalmitoyl Analogue (3) from 31 A solution of *O*-(2,3,4,6-tetra-*O*-acetyl-α-L-glucopyranosyl)trichloroacetimidate (114 mg, 0.236 mmol, 2.5 eq) in CH_2Cl_2 (1.5 ml) and boron trifluoride etherate (47% in ether, 14.7 ml, 0.048 mmol, 0.5 eq) was added to a suspension of **31** (61 mg, 0.095 mmol) and molecular sieves 4A (40 mg) in CH_2Cl_2 (3 ml), and the mixture was stirred at −30 °C for 1 h. The reaction mixture was poured into ice-water and extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed with aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product, which was dissolved in MeOH (2 ml) and treated with 5% KOH–MeOH (2 ml). The mixture was stirred at room temperature for 30 min. After neutralization of the reaction mixture with Dowex 50W × 8 (H^+ form), the resin was removed by filtration. The filtrate was evaporated under reduced pressure to yield a product, which was purified by silica gel column chromatography (SiO_2 5 g, $CHCl_3$: MeOH = 10 : 1) to afford a white powder (62 mg). A solution of the white powder (42 mg) in 1% HCl–MeOH (1 ml) was stirred at room temperature for 3h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was evaporated under reduced pressure to give a product, which was purified by silica gel column chromatography (SiO_2 3.5 g, $CHCl_3$: MeOH = 10 : 1) to afford the enantiomer (**8**, 29 mg, 63%) of the (2′*R*)-2′-hydroxypalmitoyl analogue (**3**) of soya-cerebroside II (**2**).

8: A white powder, $[\alpha]_D$ −5.1° (c = 0.3 in MeOH at 24 °C). High-resolution FAB-MS m/z: Calcd for $C_{40}H_{75}NO_9$ + Na: 736.5297. Found: 736.5293 $(M+Na)^+$. IR, ^{1}H-NMR, ^{13}C-NMR, and FAB-MS data for **8** were identical with those for **3**.

Testing of Ion-Binding and Ion-Permeation Activities Ion-binding tests using the W-08 apparatus were carried out according to the reported method,[3] and ion-permeation activity was assayed with human erythrocyte membrane by means of the procedure described in the literature.[5]

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