

Chemical Transformation of Terpenoids. X.¹⁾ Ionophoretic Activities of Macrocyclic Lactone Epoxides Synthesized from Geraniol

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Two coronand-type 18-membered lactone epoxides, *i.e.*, geranyl dimeric lactone diepoxide (GL₂E₂, 10) and tetraepoxide (GL₂E₄, 11), were synthesized from geraniol as diastereomeric mixtures. Among them, GL₂E₄ (11) was shown to exhibit ion-transport activity for Ca²⁺ ion in the test using a W-07 (liquid-membrane type) apparatus and ion-permeation activities for Ca²⁺ and K⁺ ions across the human erythrocyte membrane.

Isolation of six component diastereomers of GL₂E₄ (11) [GL₂E₄-1 (11c), -2 (11d), -3 (11e), -4 (11f), -5 (11g), -6 (11h)], was effected by HPLC separation of two diastereomeric tetraepoxides (11a, 11b) which were prepared from two diepoxides (GL₂E₂-1, 10a and GL₂E₂-2, 10b). The relative stereostructures of these diastereomers were determined by a combination of X-ray diffraction and ¹H-NMR analyses. Among the six diastereomers, S₂-symmetrical GL₂E₄-4 (11f) exhibited the strongest ion-transport activity for Ca²⁺ ion while C₂-symmetrical GL₂E₄-6 (11h) exhibited the strongest ion-permeation activity for Ca²⁺ ion across the human erythrocyte membrane.

Keywords geraniol; lactone epoxide macrocyclic; ionophoretic activity; ion-transport activity; ion-permeation activity; Ca ionophore

In a previous paper,¹⁾ we reported syntheses of two diastereomeric coronand-type macrocyclic lactone epoxides, *i.e.*, a 13-membered lactone diepoxide (FL₁E₂, 2) and a 26-membered lactone tetraepoxide (FL₂E₄, 3), from *E,E*-farnesol (1) and described their ionophoretic activities for Na⁺, K⁺, and Ca²⁺ ions. Among those epoxides, FL₂E₄ (3) was shown to exhibit ion-transport activity for K⁺ ion in the test using a W-07 (liquid-membrane type) apparatus and ion-permeation activity for K⁺ ion across the human erythrocyte membrane. Furthermore, those ionophoretic activities were discussed in relation to the configurations of the epoxide moieties.

We now wish to report syntheses of two coronand-type 18-membered lactone epoxides, *i.e.* diastereomeric mixtures of geranyl dimeric lactone diepoxide (GL₂E₂, 10)

and tetraepoxide (GL₂E₄, 11), from geraniol (4) *via* a geranyl dimeric lactone (GL₂, 9), together with the results of evaluation of their ionophoretic activities for Na⁺, K⁺, and Ca²⁺ ions.

Syntheses of Geranyl Dimeric Lactone Diepoxide (GL₂E₂) (10) and Tetraepoxide (GL₂E₄) (11) Selenium dioxide oxidation^{1,2)} of geranyl acetate (4a) furnished an *ω*-*E*-methyl hydroxylated derivative (5) and an *ω*-*E*-formylated derivative (6) in 31% and 42% yields, respectively. The *ω*-hydroxylated derivative (5) was quantitatively converted to the *ω*-*E*-aldehyde (6) by treatment with manganese dioxide. Oxidation of 6 under Corey's conditions³⁾ gave in 56% yield an *ω*-methoxycarbonyl derivative (7), which was then hydrolyzed with sodium methoxide in methanol to afford an *ω*-me-

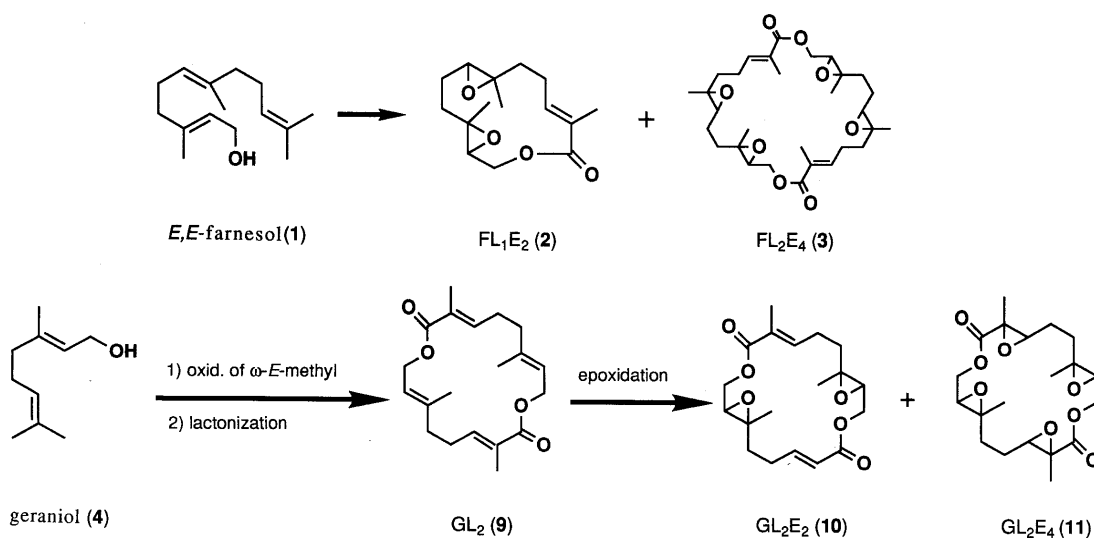


Chart 1

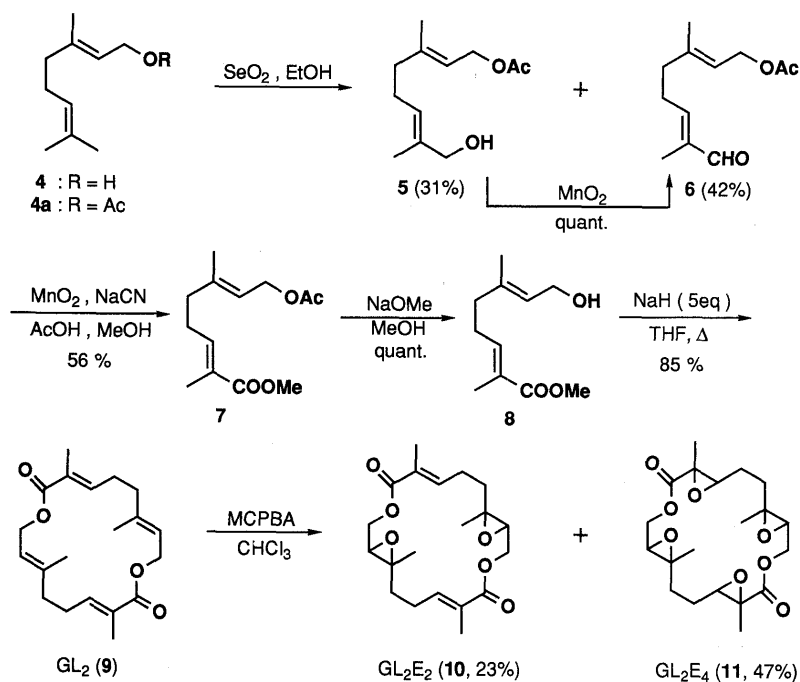


Chart 2

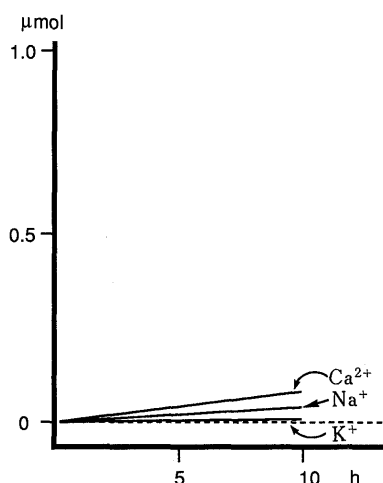


Fig. 1. Ion-Transport Activity of GL_2E_2 (10) for Metal Ions
Initial concentration of sample: 0.03 mol/l in CHCl_3 .

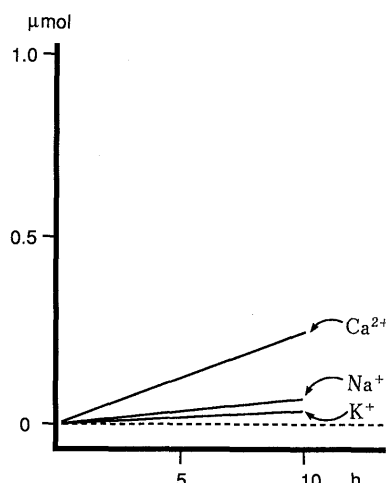


Fig. 2. Ion-Transport Activity of GL_2E_4 (11) for Metal Ions
Initial concentration of sample: 0.03 mol/l in CHCl_3 , $m_{\text{Ca}} = 2.03 \times 10^{-8}$ (mol/h).

thoxycarbonylated geraniol (8) in quantitative yield.

Treatment of the ω -methoxycarbonylated geraniol (8) with sodium hydride in tetrahydrofuran (THF) under reflux¹⁾ afforded geranyl dimeric lactone (GL_2 , 9) as a single product in 85% yield. Subsequent oxidation of GL_2 (9) with *m*-chloroperbenzoic acid (MCPBA) provided two 18-membered dilactone epoxides, *i.e.* geranyl dimeric lactone diepoxide (GL_2E_2 , 10) and tetraepoxide (GL_2E_4 , 11), in 23% and 47% yields, respectively. The dilactone epoxides GL_2E_2 (10) and GL_2E_4 (11) thus obtained, were mixtures of three and six diastereomers, respectively.

Ionophoretic Activities of GL_2E_2 (10) and GL_2E_4 (11) The ion-transport activities of GL_2E_2 (10) and GL_2E_4 (11) for Na^+ , K^+ and Ca^{2+} ions were examined by using a W-07 apparatus.⁴⁾ It was found that GL_2E_2 (10) did not show marked ion-transport activities for the three ions (Fig. 1), while GL_2E_4 (11) exhibited moderate

ion-transport activity only for Ca^{2+} ion ($m_{\text{Ca}} = 2.03 \times 10^{-8}$ mol/h with an initial concentration of 0.03 mol/l in CHCl_3 ⁴⁾).

Next, the ion-permeation activities of GL_2E_2 (10) and GL_2E_4 (11) were examined by means of a method using human erythrocyte membrane.⁵⁾ It was found that GL_2E_4 (11) increased the concentrations of K^+ and Ca^{2+} ions inside erythrocytes (Figs. 3—5), while GL_2E_2 (10) did not show any notable activity for any of the three ions. The permeated ions just after administration of $0.25 \mu\text{mol}$ were $9.79 \text{ nmol}/10^9$ red blood cells (RBC) for K^+ ion and $2.08 \text{ nmol}/10^9$ RBC for Ca^{2+} ion.

In consequence, it has been demonstrated that GL_2E_4 (11) exhibits ion-transport and ion-permeation activities for Ca^{2+} ion. As mentioned above, 11 is a mixture of six diastereomers. In order to examine the diastereoisomer-activity relationship of 11, we next tried to

separate the six diastereomers of **11** so that we could submit them to ion-transport and ion-permeation activity tests for Ca^{2+} ion.

After several attempts at separation of diastereomers of GL_2E_4 (**11**) by means of high-performance liquid chromatography (HPLC) under various conditions, it was clear that the direct HPLC separation of **11** was rather troublesome. However, it was fortunately found that GL_2E_2 (**10**), a precursory diepoxide for **11**, could be separated by HPLC with an ordinary-phase adsorbent to afford the two diastereomers, GL_2E_2 -1 (**10a**) and GL_2E_2 -2 (**10b**), in a ratio of 7:4. The relative stereostructures of **10a** and **10b** were not clarified at this stage.

The two diastereomers, **10a** and **10b**, were then oxidized with MCPBA in chloroform to afford tetraepoxides, **11a** and **11b**, respectively, in moderate yields. The tetraepoxide **11a** prepared from GL_2E_2 -1 (**10a**) was further separated by ordinary-phase HPLC to provide three diastereomers, GL_2E_4 -1 (**11c**), GL_2E_4 -2 (**11d**), and GL_2E_4 -6 (**11h**), in 2:8:3 ratio. Among these three diastereomers, the relative

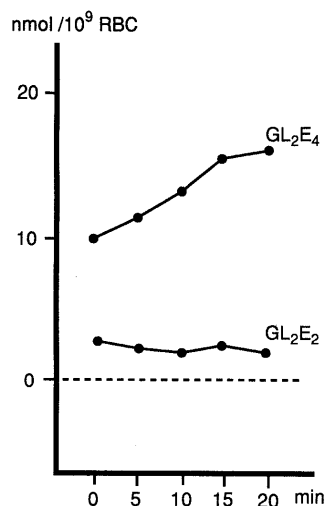


Fig. 4. Ion-Permeation Activities of GL_2E_2 (**10**) and GL_2E_4 (**11**) for K^+ Ion

Initial concentration of the sample: $0.25 \mu\text{mol}/10^9$ RBC. Ordinary concentration of K^+ ion: $1.0\text{--}1.2 \times 10^5$ nmol/ml blood.

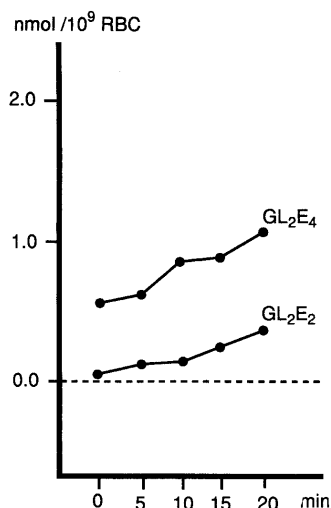


Fig. 3. Ion-Permeation Activities of GL_2E_2 (**10**) and GL_2E_4 (**11**) for Na^+ Ion

Initial concentration of the sample: $0.25 \mu\text{mol}/10^9$ RBC. Ordinary concentration of Na^+ ion: $1.0\text{--}1.5 \times 10^4$ nmol/ml blood.

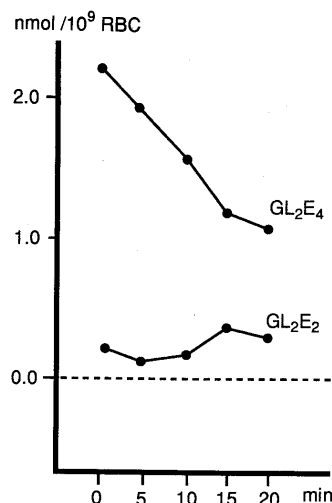
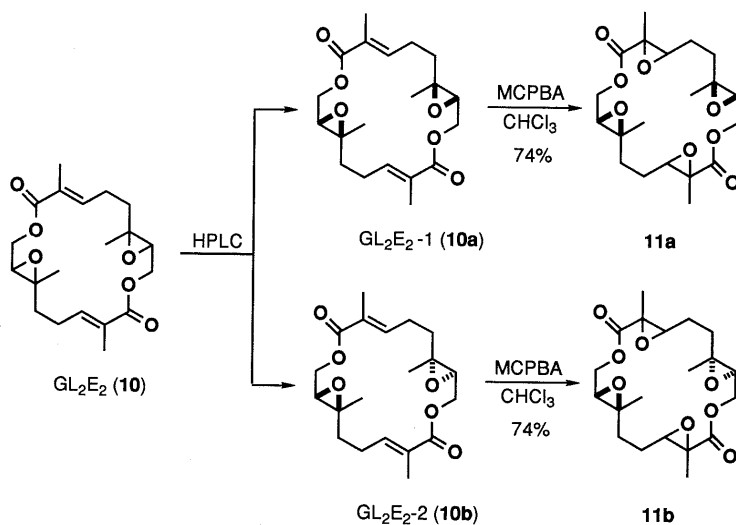


Fig. 5. Ion-Permeation Activities of GL_2E_2 (**10**) and GL_2E_4 (**11**) for Ca^{2+} Ion

Initial concentration of the sample: $0.25 \mu\text{mol}/10^9$ RBC. Ordinary concentration of Ca^{2+} ion: 2 nmol/ml blood.



stereostructures of GL₂E₄-2 and GL₂E₄-6 were determined by X-ray crystallographic analysis to be **11d** and **11h** (Fig. 6), respectively. From the spectroscopic properties, it was clear that the remaining diastereomer GL₂E₄-1 was a *syn*-type diepoxidized derivative of GL₂E₂-1 (**10a**), so the relative stereostructure of GL₂E₄-1 was assigned as **11c**.

On the other hand, another tetraepoxide **11b** obtained by MCPBA oxidation of GL₂E₂-2 (**10b**) was separated by ordinary-phase HPLC to provide three diastereomers, GL₂E₄-3 (**11e**), GL₂E₄-4 (**11f**), and GL₂E₄-5 (**11g**), in 8:7:5 ratio. Among them, GL₂E₄-4, obtained as colorless needles, was subjected to X-ray crystallographic analysis to determine the relative stereostructure as **11f** (Fig. 6). In the proton nuclear magnetic resonance (¹H-NMR) spectrum, one diastereomer, GL₂E₄-5, showed two dimethyl signals (each 6H) at δ 1.32 and 1.45, while another diastereomer, GL₂E₄-3, showed four methyl signals (each 3H) at δ 1.27, 1.31, 1.46 and 1.48. These findings have shown that GL₂E₄-5 possesses a symmetrical structure (**11g**), whereas GL₂E₄-3 possesses an asymmetrical structure (**11e**).

Ionophoretic Activities of Six Diastereomers of GL₂E₄ (11) To shed light on the relationship between ionophoretic activities and stereostructures, we next carried out ion-transport and ion-permeation activity tests of six

diastereomers (**11c**, **11d**, **11e**, **11f**, **11g**, and **11h**) of GL₂E₄ (**11**) for Ca²⁺ ion by using a W-07 apparatus⁴⁾ and by employing a human erythrocyte membrane method.⁵⁾

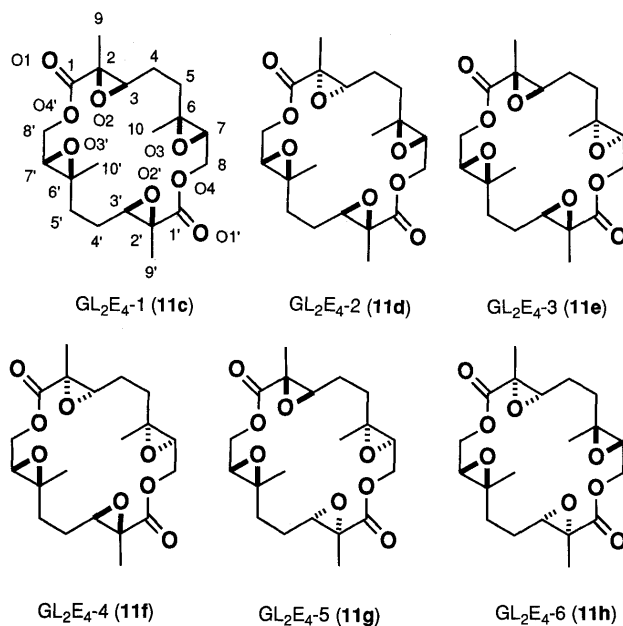
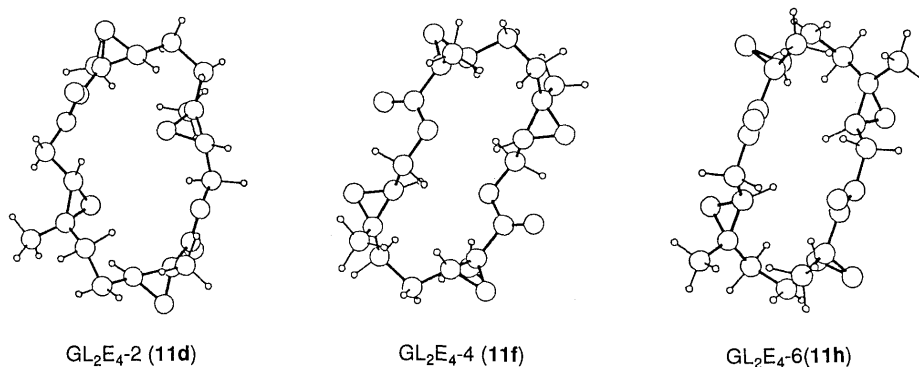
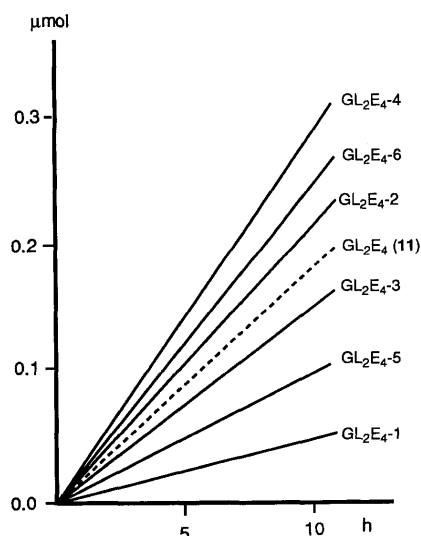
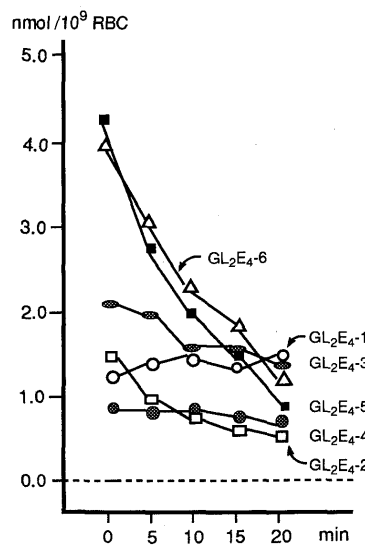


Chart 4

Fig. 6. ORTEP Drawings of GL₂E₄-2 (**11d**), GL₂E₄-4 (**11f**), and GL₂E₄-6 (**11h**)Fig. 7. Ca²⁺ Ion-Transport Activities of GL₂E₄-1—6
Initial concentration of samples: 0.03 M in CHCl₃.Fig. 8. Ca²⁺ Ion-permeation Activities of GL₂E₄-1—6
Initial concentration of samples: 0.25 mmol/10⁹ RBC.

The ion-transport activities of the six diastereomers were in the order of GL₂E₄-4 (**11f**) > -6 (**11h**) > -2 (**11d**) > **11** (the parent diastereomeric mixture) > -3 (**11e**) > -5 (**11g**) > -1 (**11c**). One of the structural features of GL₂E₄-4 (**11f**), -6 (**11h**), and -2 (**11d**), which showed greater activity than the parent mixture (**11**), is that these diastereomers have one or two pair(s) of mutually *anti*-faced epoxide rings on either side of the ester-bond.

In regard to the ion-permeation activity, the order was GL₂E₄-6 (**11h**), -5 (**11g**) > -3 (**11e**) > -1 (**11c**) > -4 (**11f**), -2 (**11d**). In this case, the structural feature of GL₂E₄-6 (**11h**) and GL₂E₄-5 (**11g**), which exhibited much stronger ion-permeation activity for Ca²⁺ ion across the human erythrocyte membrane, is that both diastereomers possess two pairs of *anti*-faced epoxide rings beyond a dimethylene bond (*i.e.*, C₄-C₅ and C₄-C₅) in one component of the dilactone structure, respectively. Contrary to our expectation, GL₂E₄-1 (**11c**), with four epoxide rings facing the same side, exhibited weak activity in both tests.

Moreover, it is noteworthy that, as is shown in the ORTEP drawing (Fig. 6), GL₂E₄-6 (**11h**), the most active diastereomer in both ion-transport and ion-permeation tests, has all four epoxide rings facing outside the molecule in its crystalline structure. Therefore, we hypothesize that GL₂E₄-6 (**11h**), when acting as a Ca²⁺ ionophore, may change its conformation to one favorable for trapping Ca²⁺ ion. This will be the subject of a future investigation.

Experimental

The instruments used to obtain physical data and the experimental conditions for chromatography were the same as described in our previous paper.¹⁾

Selenium Dioxide Oxidation of Geranyl Acetate (4a) A solution of geranyl acetate (**4a**, 58.5 g, 0.30 mol) in 95% aqueous EtOH (450 ml) was treated with 95% SeO₂ (49.1 g, 0.42 mol, 1.4 eq) at room temperature with vigorous stirring. The whole mixture was then heated under reflux for 1.5 h. After cooling, the reaction mixture was poured into ice-water and the whole was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaHCO₃ and brine, then dried over MgSO₄. Removal of the solvent under reduced pressure gave a product (60 g), which was purified by silica gel column chromatography (SiO₂ 2.5 kg, *n*-hexane:EtOAc=7:1) to afford **5** (21.2 g, 0.10 mol, 31%) and **6** (25.9 g, 0.12 mol, 42%).

5: A colorless oil. IR (CCl₄) cm⁻¹: 3600–3100 (br), 1740, 1665. ¹H-NMR (90 MHz, CDCl₃) δ: 1.61, 1.70 (3H each, both brs, vinyl methyl × 2), 1.98 (3H, s, -OCOCH₃), 3.86 (2H, brs, -CH₂-OH), 4.49 (2H, d, *J*=7 Hz, -CH₂-OCOCH₃), 5.2–5.5 (2H, m, two olefinic protons). EI-MS *m/z* (%): 152 (M⁺ - AcOH, 5), 43 (100). *Anal.* Calcd for C₁₂H₂₀O₃: C, 67.89; H, 9.50. Found: C, 67.97; H, 9.53.

6: A colorless oil. IR (film) cm⁻¹: 1734, 1691, 1640. UV (MeOH) nm (ε): 229 (20000). ¹H-NMR (90 MHz, CDCl₃) δ: 1.75 (6H, brs, vinyl methyl × 2), 2.06 (3H, s, -OCOCH₃), 4.60 (2H, d, *J*=7 Hz, -CH₂-OH), 5.40 (1H, t, *J*=7 Hz, olefinic proton), 6.46 (1H, t, *J*=7 Hz, olefinic proton), 9.39 (1H, s, -CHO). EI-MS *m/z* (%): 150 (M⁺ - AcOH, 47), 84 (100). *Anal.* Calcd for C₁₂H₁₈O₃: C, 68.54; H, 8.63. Found: C, 68.54; H, 8.73.

Preparation of 7 from 5 via 6 A solution of **5** (10.6 g, 0.050 mol) in *n*-hexane-CHCl₃ (10:1, 1100 ml) was treated with MnO₂ (90 g, 0.14 mol, *ca.* 20 eq) at room temperature with vigorous stirring for 5 h. The reaction mixture was filtered to remove solids and the filtrate was evaporated under reduced pressure to give **6** (10.5 g, 0.050 mol, quantitative yield). A solution of **6** (10.5 g) in dry MeOH (1000 ml) was treated with 95% NaCN (5.93 g, 0.115 mol, 2.3 eq), MnO₂ (130 g, 1.50 mol, 30 eq), and freshly distilled AcOH (7.90 ml, 0.138 mol, 2.8 eq) at room temperature with vigorous stirring for 24 h. After removal of solids by filtration, the filtrate was poured into ice-water and the whole mixture was extracted with EtOAc. The EtOAc extract was washed with aqueous saturated NaHCO₃ and brine, then dried over MgSO₄. Removal of the solvent

under reduced pressure gave a product (9.2 g), which was purified by silica gel column chromatography (SiO₂ 1 kg, *n*-hexane:EtOAc=7:1 → 2:1) to afford **7** (5.88 g, 28.0 mmol, 56%).

7: A colorless oil. IR (film) cm⁻¹: 1726 (br), 1652. UV (MeOH) nm (ε): 217 (13000). ¹H-NMR (90 MHz, CDCl₃) δ: 1.72, 1.83 (3H each, both brs, vinyl methyl × 2), 2.05 (3H, s, -OCOCH₃), 3.73 (3H, s, -COOCH₃), 4.58 (2H, d, *J*=7 Hz, -CH₂-OCOCH₃), 5.37 (1H, t, *J*=7 Hz, olefinic proton), 6.71 (1H, t, *J*=7 Hz, olefinic proton). EI-MS *m/z* (%): 180 (M⁺ - AcOH, 24), 43 (100). *Anal.* Calcd for C₁₃H₂₀O₄: C, 64.98; H, 8.39. Found: C, 64.99; H, 8.55.

Alkaline Hydrolysis of 7 Giving 8 A solution of **7** (3.41 g, 14.2 mmol) in MeOH (35 ml) was treated with 10% KOH-MeOH (35 ml) at room temperature for 30 min. The reaction mixture was poured into ice-water and the whole mixture was extracted with EtOAc. The EtOAc extract was washed with brine and dried over MgSO₄. Removal of the solvent under reduced pressure furnished **8** (2.56 g, 14.2 mmol, quantitative yield).

8: A colorless oil. IR (film) cm⁻¹: 3600–3100, 1704, 1645. UV (MeOH) nm (ε): 217 (17000). ¹H-NMR (90 MHz, CDCl₃) δ: 1.70, 1.84 (3H, each, both brs, vinyl methyl × 2), 3.73 (3H, s, -COOCH₃), 4.17 (2H, d, *J*=7 Hz, -CH₂-OH), 5.44 (1H, t, *J*=7 Hz, olefinic proton), 6.74 (1H, t, *J*=7 Hz, olefinic proton). EI-MS *m/z* (%): 180 (M⁺ - H₂O, 21.5%), 43 (100). *Anal.* Calcd for C₁₁H₁₈O₃: C, 66.64; H, 9.15. Found: C, 66.42; H, 9.33.

Lactonization of 8 Giving GL₂ (9) A solution of **8** (1.80 g, 9.10 mmol) in dry THF (910 ml) was treated with 60% NaH (1.82 g, 45.5 mmol, 5.0 eq) and the whole mixture was heated under reflux for 2 h. After cooling, the reaction mixture was treated with aqueous saturated NH₄Cl and the whole mixture was extracted with EtOAc. The EtOAc extract was washed with brine and dried over MgSO₄. Removal of the solvent gave a product (2.1 g). Purification of the product by silica gel column chromatography (SiO₂ 100 g, *n*-hexane:EtOAc=10:1) afforded GL₂ (**9**, 1.24 g, 3.86 mmol, 85%).

GL₂(**9**): A white powder. IR (CHCl₃) cm⁻¹: 1697. UV (MeOH) nm (ε): 212 (18000). ¹H-NMR (90 MHz, CDCl₃) δ: 1.70, 1.84 (3H each, both brs, vinyl methyl × 2), 4.34 (4H, d, *J*=8 Hz, -CH₂-OCO- × 2), 5.37 (2H, t, *J*=8 Hz, olefinic proton), 6.69 (1H, t, *J*=6 Hz, olefinic proton). EI-MS *m/z* (%): 332 (M⁺, 0.6), 82 (100). High-resolution (HR) EI-MS *m/z*: Calcd for C₂₀H₂₈O₄: 332.199. Found: 332.198 (M⁺).

Epoxidation of GL₂ (9) Giving GL₂E₂ (10) and GL₂E₄ (11) A solution of GL₂ (**9**, 480 mg, 1.45 mmol) in CHCl₃ (100 ml) was treated with 70% *m*-chloroperbenzoic acid (1.43 g, 5.79 mmol, 4.0 eq) at room temperature with stirring for 4 h. The reaction mixture was then treated with aqueous saturated Na₂SO₃ (25 ml) and extracted with CHCl₃. The CHCl₃ extract was washed with aqueous saturated NaHCO₃ and brine, then dried over MgSO₄. Evaporation of the solvent from the CHCl₃ extract gave a product (580 mg), which was purified by silica gel column chromatography (SiO₂ 30 g, *n*-hexane:EtOAc=4:1 → 3:2) to afford GL₂E₂ (**10**, 121 mg, 0.33 mmol, 23%) and GL₂E₄ (**11**, 269 mg, 0.68 mmol, 47%).

GL₂E₂ (**10**): A white powder. IR (CHCl₃) cm⁻¹: 1709, 1645. UV (MeOH) nm (ε): 214 (3700). ¹H-NMR (90 MHz, CDCl₃) δ: 1.41 (3H, s, methyl), 1.39, 1.58 (totally 3H, both s, methyl), 1.85 (6H, brs, vinyl

methyl × 2), 2.8–3.1 (2H, m, >C-CH-CH₂- × 2), 4.0–4.4 (4H, m, -CH₂-OCO- × 2), 6.6–6.8 (2H, m, two olefinic protons). EI-MS *m/z* (%): 364 (M⁺, 2), 95 (100). HR-EI-MS *m/z*: Calcd for C₂₀H₂₈O₆: 364.188. Found: 364.186 (M⁺).

GL₂E₄ (**11**): A white powder. IR (CHCl₃) cm⁻¹: 1733. ¹H-NMR (90 MHz, CDCl₃) δ: 1.3–1.4 (6H, m), 1.5–1.6 (6H, m) (methyl × 4),

2.8–3.4 (4H, m, >C-CH-CH₂- × 4), 3.9–4.5 (4H, m, -CH₂-OCO-). EI-MS *m/z* (%): 396 (M⁺, 0.7), 149 (100). HR-EI-MS *m/z*: Calcd for C₂₀H₂₈O₈: 396.178. Found: 396.179 (M⁺).

Separation of Two Diastereomers of GL₂E₂ (10) GL₂E₂ (**10**, 145 mg) was submitted to HPLC (YMC 043-10, *n*-hexane:EtOAc=8:1) to afford GL₂E₂-1 (**10a**, 93 mg) and GL₂E₂-2 (**10b**, 52 mg).

Epoxidation of GL₂E₂-1 (10a) A solution of GL₂E₂-1 (**10a**, 93 mg, 0.255 mmol) in CHCl₃ (20 ml) was treated with 70% MCPBA (189 mg, 0.765 mmol, 3.0 eq) and the whole mixture was heated under reflux for 5 h. After cooling, the reaction mixture was treated with aqueous saturated Na₂SO₃ (10 ml) and extracted with CHCl₃. The CHCl₃ extract was washed with aqueous saturated NaHCO₃ and brine, then dried over MgSO₄. Removal of the solvent gave a product (152 mg), which was purified by silica gel column chromatography (SiO₂ 15 g, *n*-hexane:

EtOAc=3:2) to afford **11a** (75 mg, 0.189 mmol, 74%).

Epoxidation of GL₂E₂-2 (10b) 11b (42 mg, 0.106 mmol, 74%) was obtained from GL₂E₂-2 (**10b**, 52 mg, 0.143 mmol) through a procedure similar to that employed for the preparation of **11a** from GL₂E₂-1 (**10a**).

HPLC Separation of 11a **11a** (75 mg) was submitted to HPLC (YMC 043-10, *n*-hexane:EtOAc=2:1) to afford GL₂E₄-1 (**11c**, 12 mg), GL₂E₄-2 (**11d**, 46 mg), and GL₂E₄-6 (**11h**, 17 mg).

GL₂E₄-1 (**11c**): A white powder. IR (CHCl₃) cm⁻¹: 1730. ¹H-NMR (500 MHz, CDCl₃) δ: 1.28, 1.49 (6H each, both s, CH₃ × 4), 1.38–1.61 (4H, m), 1.81–2.02 (4H, m) (4-H₂, 5-H₂, 4'-H₂, 5'-H₂), 2.93 (2H, dd, *J*=4.2, 6.4 Hz), 3.06 (2H, dd, *J*=5.5, 7.3 Hz) (3-H, 7-H, 3'-H, 7'-H), 4.02 (2H, dd, *J*=6.4, 12.2 Hz), 4.29 (2H, dd, *J*=4.2, 12.2 Hz) (8-H_a, 8-H_b, 8'-H_a, 8'-H_b). EI-MS *m/z* (%): 396 (M⁺, 0.1), 109 (100). HR-EI-MS *m/z*: Calcd for C₂₀H₂₈O₈: 396.178. Found: 396.179 (M⁺).

GL₂E₄-2 (**11d**): Colorless needles, mp 195–196 °C (EtOAc). IR (CHCl₃) cm⁻¹: 1733. ¹H-NMR (500 MHz, CDCl₃) δ: 1.47, 1.46, 1.28, 1.25 (3H each, all s, CH₃ × 4), 1.46–1.73 (4H, m), 2.01–2.13 (4H, m) (4-H₂, 5-H₂, 4'-H₂, 5'-H₂), 2.98 (1H, t, *J*=4.8 Hz), 3.07 (1H, t, *J*=5.8 Hz), 3.09 (1H, dd, *J*=4.8, 5.7 Hz), 3.23 (1H, t, *J*=6.1 Hz) (3-H, 7-H, 3'-H, 7'-H), 4.05 (1H, dd, *J*=5.8, 11.9 Hz), 4.12 (1H, dd, *J*=4.8, 12.2 Hz), 4.21 (1H, dd, *J*=5.7, 12.2 Hz), 4.31 (1H, dd, *J*=5.8, 11.9 Hz) (8-H_a, 8-H_b, 8'-H_a, 8'-H_b). EI-MS *m/z* (%): 396 (M⁺, 1), 109 (100). HR-EI-MS *m/z*: Calcd for C₂₀H₂₈O₈: 396.178. Found: 396.178 (M⁺).

TABLE I. Experimental Data for the X-Ray Diffraction Studies of GL₂E₄-2(**11d**), -4(**11f**), and -6(**11h**)

	GL ₂ E ₄ -2(11d)	GL ₂ E ₄ -4(11f)	GL ₂ E ₄ -6(11h)
Formula	C ₂₀ H ₂₈ O ₈	C ₂₀ H ₂₈ O ₈	C ₂₀ H ₂₈ O ₈
FW	396.178	396.178	396.178
<i>F</i> (000)	1104	222	1696
System	Orthorhombic	Triclinic	Orthorhombic
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁	<i>P</i> 1	<i>P</i> _{bca}
<i>a</i> (Å)	6.372 (1)	10.649 (4)	8.669 (2)
<i>b</i> (Å)	10.206 (2)	8.559 (4)	20.847 (4)
<i>c</i> (Å)	31.085 (5)	5.734 (2)	21.746 (4)
α (°)	90.0	107.77 (3)	90.0
β (°)	90.0	91.41 (3)	90.0
γ (°)	90.0	100.57 (3)	90.0
<i>V</i> (Å ³)	2021.6 (5)	487.6 (3)	3930 (1)
<i>Z</i>	4	1	8
<i>F</i> _o used	3657	1696	2420
No. of param.	252	252	344
<i>R</i>	0.0671	0.0692	0.0869
<i>R</i> _w	0.0747	0.0806	0.0965

TABLE II. Fractional Coordinates of Non-H Atoms and Equivalent Isotropic Temperature Factors with e.s.d.'s in Parentheses

Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> _{eq} / <i>B</i> _{iso} (Å ²)	Atom	<i>x</i>	<i>y</i>	<i>z</i>	<i>B</i> _{eq} / <i>B</i> _{iso} (Å ²)
GL ₂ E ₄ -2(11d)					GL ₂ E ₄ -4(11f)				
C1	0.0094 (8)	0.5571 (5)	0.6922 (1)	3.7 (2)	C5'	0.5268 (4)	-0.0821 (6)	1.114 (1)	3.9 (2)
C2	-0.1342 (8)	0.6176 (4)	0.6596 (1)	3.7 (2)	C6'	0.6389 (3)	-0.1695 (5)	1.0316 (7)	2.6 (1)
C3	-0.0206 (9)	0.6817 (4)	0.6243 (1)	3.9 (2)	C7'	0.7591 (4)	-0.0726 (5)	0.9712 (7)	2.8 (1)
C4	-0.111 (1)	0.6964 (5)	0.5796 (2)	4.7 (2)	C8'	0.8805 (4)	-0.1258 (5)	1.0081 (8)	3.4 (1)
C5	-0.0927 (9)	0.5665 (5)	0.5548 (1)	4.3 (2)	C9'	0.5423 (4)	0.3480 (5)	1.429 (1)	3.9 (2)
C6	0.1332 (8)	0.5251 (4)	0.5464 (1)	3.6 (2)	C10'	0.6388 (5)	-0.3257 (5)	1.1036 (9)	3.9 (2)
C7	0.1852 (8)	0.3852 (5)	0.5527 (1)	3.9 (2)	O1	0.9424 (3)	-0.0469 (5)	0.6014 (6)	4.4 (1)
C8	0.3580 (9)	0.3183 (5)	0.5286 (2)	4.6 (2)	O2	1.1759 (3)	0.1679 (4)	0.5772 (6)	4.2 (1)
C9	-0.3530 (9)	0.5640 (6)	0.6556 (2)	5.0 (2)	O3	1.1287 (3)	0.5788 (4)	1.5407 (5)	3.7 (1)
C10	0.254 (1)	0.6109 (6)	0.5165 (2)	5.7 (3)	O4	0.8035 (3)	0.3940 (4)	1.3243 (5)	3.33 (9)
C1'	0.3703 (8)	0.1052 (5)	0.5604 (1)	3.9 (2)	O1'	0.8577 (3)	0.4343 (5)	1.7186 (6)	4.8 (1)
C2'	0.4812 (8)	0.0222 (5)	0.5929 (2)	3.8 (2)	O2'	0.6155 (3)	0.2262 (4)	1.7395 (6)	3.8 (1)
C3'	0.3376 (8)	-0.0500 (4)	0.6219 (2)	3.8 (2)	O3'	0.6693 (3)	-0.1857 (4)	0.7767 (5)	3.5 (1)
C4'	0.3914 (9)	-0.0925 (5)	0.6670 (2)	4.5 (2)	O4'	0.9937 (3)	-0.0024 (4)	0.9942 (6)	3.9 (1)
C5'	0.2616 (9)	-0.0148 (5)	0.7001 (2)	4.1 (2)	GL ₂ E ₄ -6 (11h)				
C6'	0.2999 (7)	0.1304 (5)	0.6990 (1)	3.2 (2)	C1	-0.1326 (5)	0.0368 (2)	0.3422 (2)	3.2 (2)
C7'	0.1191 (8)	0.2170 (5)	0.7013 (2)	3.9 (2)	C2	-0.0362 (6)	-0.0209 (2)	0.3595 (2)	3.2 (2)
C8'	0.1293 (9)	0.3530 (5)	0.7187 (2)	4.5 (2)	C3	0.0292 (5)	0.0238 (2)	0.4221 (2)	3.1 (2)
C9'	0.7041 (9)	0.0550 (6)	0.6038 (2)	5.2 (3)	C4	0.1730 (6)	-0.0599 (2)	0.4396 (2)	3.6 (2)
C10'	0.5162 (9)	0.1753 (6)	0.7132 (2)	5.5 (3)	C5	0.3002 (6)	-0.0135 (2)	0.4589 (2)	3.6 (2)
O1	0.1439 (7)	0.6126 (4)	0.7112 (1)	5.8 (2)	C6	0.3546 (5)	0.0301 (2)	0.4083 (2)	3.2 (2)
O2	-0.1160 (7)	0.7585 (3)	0.6586 (1)	4.8 (2)	C7	0.2717 (5)	0.0901 (2)	0.3977 (2)	3.3 (2)
O3	0.2430 (7)	0.4806 (4)	0.5841 (1)	4.7 (2)	C8	0.2624 (7)	0.1231 (2)	0.3363 (2)	3.9 (2)
O4	0.4593 (6)	0.2230 (3)	0.5571 (1)	4.4 (1)	C9	0.0283 (8)	-0.0572 (3)	0.3058 (2)	5.5 (3)
O1'	0.2149 (7)	0.0724 (4)	0.5410 (1)	5.3 (2)	C10	0.4491 (7)	-0.0009 (3)	0.3583 (3)	4.6 (2)
O2'	0.4389 (7)	-0.1160 (3)	0.5867 (1)	5.2 (2)	C1'	0.1047 (6)	0.2097 (2)	0.3060 (2)	3.3 (2)
O3'	0.2214 (6)	0.1957 (3)	0.6614 (1)	4.1 (1)	C2'	0.0324 (6)	0.2732 (2)	0.3234 (2)	3.4 (2)
O4'	-0.0250 (6)	0.4279 (3)	0.6947 (1)	4.4 (1)	C3'	0.0386 (6)	0.2940 (2)	0.3882 (2)	3.6 (2)
GL ₂ E ₄ -4(11f)					C4'	-0.0793 (6)	0.3360 (2)	0.4183 (2)	4.0 (2)
C1	1.0137 (4)	0.0156 (5)	0.7758 (7)	2.5 (1)	C5'	-0.1812 (6)	0.2982 (2)	0.4622 (2)	3.9 (2)
C2	1.1481 (4)	0.1145 (5)	0.7900 (7)	2.7 (1)	C6'	-0.2709 (6)	0.2440 (2)	0.4335 (2)	3.2 (2)
C3	1.1635 (4)	0.2885 (5)	0.8041 (7)	2.9 (1)	C7'	-0.1940 (5)	0.1823 (2)	0.4245 (2)	3.1 (2)
C4	1.2803 (4)	0.4176 (5)	0.9370 (8)	3.2 (1)	C8'	-0.2410 (6)	0.1357 (2)	0.3746 (2)	3.2 (2)
C5	1.2716 (4)	0.4829 (5)	1.2222 (8)	2.9 (1)	C9'	-0.0908 (7)	0.2943 (3)	0.2794 (2)	4.9 (3)
C6	1.1599 (4)	0.5646 (5)	1.2925 (7)	2.7 (1)	C10'	-0.4103 (6)	0.2632 (2)	0.3967 (3)	4.3 (2)
C7	1.0419 (4)	0.4679 (5)	1.3379 (7)	2.8 (1)	O1	-0.1827 (6)	0.0446 (2)	0.2916 (2)	6.2 (2)
C8	0.9130 (4)	0.5123 (6)	1.3015 (8)	3.4 (1)	O2	-0.1075 (4)	-0.0591 (2)	0.4071 (2)	4.9 (2)
C9	1.2479 (4)	0.0398 (6)	0.887 (1)	4.2 (2)	O3	0.4155 (4)	0.0910 (2)	0.4297 (2)	4.4 (2)
C10	1.1515 (4)	0.7126 (5)	1.2054 (9)	3.4 (1)	O4	0.1995 (4)	0.1869 (1)	0.3479 (2)	4.3 (2)
C1'	0.7787 (4)	0.3790 (5)	1.5401 (8)	3.1 (1)	O1'	0.0727 (6)	0.1827 (2)	0.2597 (2)	6.9 (2)
C2'	0.6450 (4)	0.2797 (5)	1.5324 (7)	3.1 (1)	O2'	0.1407 (5)	0.3216 (2)	0.3426 (2)	5.3 (2)
C3'	0.6316 (4)	0.1002 (5)	1.5101 (8)	3.3 (1)	O3'	-0.2982 (4)	0.1902 (2)	0.4752 (1)	4.2 (2)
C4'	0.5145 (4)	-0.0277 (5)	1.3819 (9)	3.4 (1)	O4'	-0.1545 (4)	0.0768 (1)	0.3881 (1)	3.7 (1)

GL₂E₄-6 (**11h**): Colorless needles, mp 160–161 °C (EtOAc). IR (CHCl₃) cm⁻¹: 1732. ¹H-NMR (500 MHz, CDCl₃) δ: 1.30, 1.45 (6H each, both s, CH₃ × 4), 1.50–1.78 (4H, m), 1.83–1.97 (4H, m) (4-H₂, 5-H₂, 4'-H₂, 5'-H₂), 3.06 (2H, t, *J* = 6.1 Hz), 3.24 (2H, t, *J* = 5.8 Hz) (3-H, 7-H, 3'-H, 7'-H), 4.17 (2H, dd, *J* = 6.1, 11.9 Hz), 4.25 (2H, dd, *J* = 6.1, 11.9 Hz) (8-H_a, 8-H_b, 8'-H_a, 8'-H_b). EI-MS *m/z* (%): 396 (M⁺, 0.2), 109 (100). HR-EI-MS *m/z*: Calcd for C₂₀H₂₈O₈: 396.178. Found: 396.176 (M⁺).

HPLC Separation of 11b **11b** (42 mg) was submitted to HPLC (Zorbax SIL 5SL, *n*-hexane:EtOAc = 2:1) to afford GL₂E₄-3 (**11e**, 17 mg), GL₂E₄-4 (**11f**, 15 mg), and GL₂E₄-5 (**11g**, 10 mg).

GL₂E₄-3 (**11e**): A white powder. IR (CHCl₃) cm⁻¹: 1733. ¹H-NMR (500 MHz, CDCl₃) δ: 1.27, 1.31, 1.46, 1.48 (3H each, all s, CH₃ × 4), 1.51–1.81 (4H, m), 1.90–2.07 (4H, m) (4-H₂, 5-H₂, 4'-H₂, 5'-H₂), 2.89 (1H, dd, *J* = 4.2, 6.2 Hz), 3.02 (1H, t, *J* = 5.9 Hz), 3.13 (1H, t, *J* = 5.9 Hz), 3.21 (1H, t, *J* = 6.2 Hz) (3-H, 7-H, 3'-H, 7'-H), 4.10 (1H, dd, *J* = 6.2, 12.1 Hz), 4.12 (2H, d, *J* = 5.9 Hz), 4.21 (1H, dd, *J* = 4.2, 12.1 Hz) (8-H_a, 8-H_b, 8'-H_a, 8'-H_b). EI-MS *m/z* (%): 396 (M⁺, 1), 95 (100). HR-EI-MS *m/z*: Calcd for C₂₀H₂₈O₈: 396.178. Found: 396.177 (M⁺).

GL₂E₄-4 (**11f**): Colorless needles, mp 169–170 °C (EtOAc). IR (CHCl₃) cm⁻¹: 1731. ¹H-NMR (500 MHz, CDCl₃) δ: 1.29, 1.48 (6H each, both s, CH₃ × 4), 1.51–1.79 (4H, m), 1.93–1.98 (4H, m) (4-H₂, 5-H₂, 4'-H₂, 5'-H₂), 2.93 (2H, t, *J* = 5.2 Hz), 3.12 (2H, t, *J* = 6.1 Hz) (3-H, 7-H, 3'-H, 7'-H), 4.16 (2H, dd, *J* = 5.2, 11.9 Hz), 4.25 (2H, dd, *J* = 5.2, 11.9 Hz) (8-H_a, 8-H_b, 8'-H_a, 8'-H_b). EI-MS *m/z* (%): 396 (M⁺,

0.1), 95 (100). HR-EI-MS *m/z*: Calcd for C₂₀H₂₈O₈: 396.178. Found: 396.178 (M⁺).

GL₂E₄-5 (**11g**): A white powder. IR (CHCl₃) cm⁻¹: 1735. ¹H-NMR (500 MHz, CDCl₃) δ: 1.32, 1.45 (6H each, both s, CH₃ × 4), 1.50–1.80 (4H, m), 1.94–2.05 (4H, m) (4-H₂, 5-H₂, 4'-H₂, 5'-H₂), 3.07 (2H, dd, *J* = 4.8, 7.5 Hz), 3.18 (2H, dd, *J* = 4.8, 6.6 Hz) (3-H, 7-H, 3'-H, 7'-H), 4.16 (2H, dd, *J* = 6.6, 12.0 Hz), 4.25 (2H, dd, *J* = 4.8, 12.0 Hz) (8-H_a, 8-H_b, 8'-H_a, 8'-H_b). EI-MS *m/z* (%): 396 (M⁺, 0.1), 109 (100). HR-EI-MS *m/z*: Calcd for C₂₀H₂₈O₈: 396.178. Found: 396.180 (M⁺).

Ion-Transport and Ion-Permeation Activity Test Ion-transport tests using a W-07 (liquid-membrane type) apparatus were carried out according to the reported method,⁴⁾ and ion-permeation activity was assayed with human erythrocyte membrane by means of the procedure described in the literature.⁵⁾

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