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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 (GL_2E_2, 10) and tetraepoxide (GL_2E_4, 11), were synthesized from geraniol as diastereomeric mixtures. Among them, GL_2E_4 (11) was shown to exhibit ion-transport activity for Ca^{2+} ion in the test using a W-07 (liquid-membrane type) apparatus and ion-permeation activities for Ca^{2+} and K^+ ions across the human erythrocyte membrane.

Isolation of six component diastereomers of GL_2E_4 (11) [GL_2E_4-1 (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 (GL_2E_2-1, 10a and GL_2E_2-2, 10b). The relative stereostructures of these diastereomers were determined by a combination of X-ray diffraction and ^{1}H-NMR analyses. Among the six diastereomers, S_2-symmetrical GL_2E_4-4 (11f) exhibited the strongest ion-transport activity for Ca^{2+} ion while C_2-symmetrical GL_2E_4-6 (11h) exhibited the strongest ion-permeation activity for Ca^{2+} 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 (FL_1E_2, 2) and a 26-membered lactone tetraepoxide (FL_2E_4, 3), from *E*,*E*-farnesol (1) and described their ionophoretic activities for Na^+, K^+, and Ca^{2+} ions. Among those epoxides, FL_2E_4 (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 (GL_2E_2, **10**) and tetraepoxide (GL_2E_4, **11**), from geraniol (**4**) *via* a geranyl dimeric lactone (GL_2, **9**), together with the results of evaluation of their ionophoretic activities for Na^+, K^+, and Ca^{2+} ions.

Syntheses of Geranyl Dimeric Lactone Diepoxide (GL_2E_2) (10) and Tetraepoxide (GL_2E_4) (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 conditions[3] gave in 56% yield an *ω*-methoxycarbonyl derivative (**7**), which was then hydrolyzed with sodium methoxide in methanol to afford an *ω*-me-

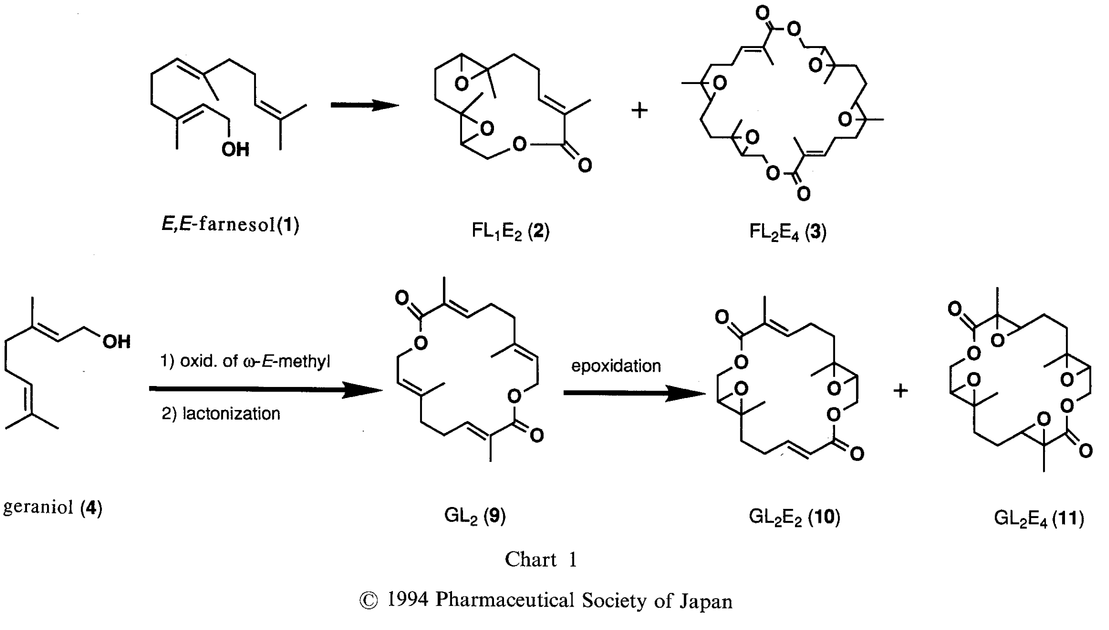


Chart 1

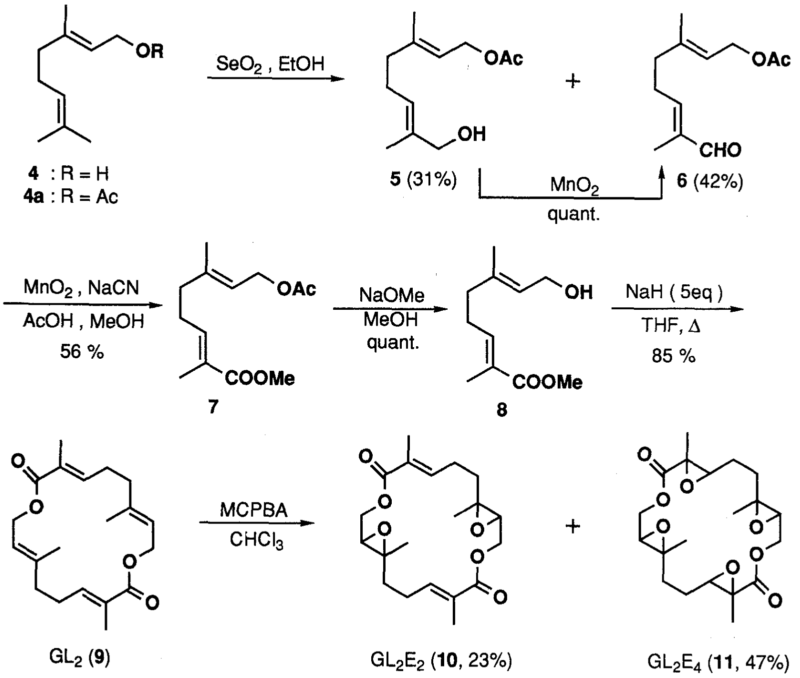


Chart 2

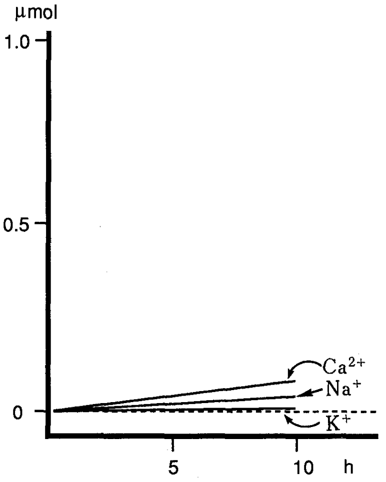


Fig. 1. Ion-Transport Activity of GL_2E_2 (**10**) for Metal Ions

Initial concentration of sample: 0.03 mol/l in $CHCl_3$.

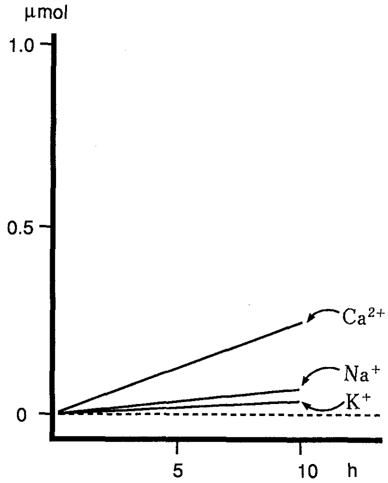


Fig. 2. Ion-Transport Activity of GL_2E_4 (**11**) for Metal Ions

Initial concentration of sample: 0.03 mol/l in $CHCl_3$. $m_{ca}=2.03\times10^{-8}$ (mol/h).

thoxycarbonylated geraniol (**8**) in quantitative yield.

Treatment of the ω-methoxycarbonylated geraniol (**8**) with sodium hydride in tetrahydrofuran (THF) under reflux[1] 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 ($m_{Ca}=2.03\times10^{-8}$ 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 μmol were 9.79 nmol/10^9 red blood cells (RBC) for K^+ ion and 2.08 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 diastereostructure-ionophoretic 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

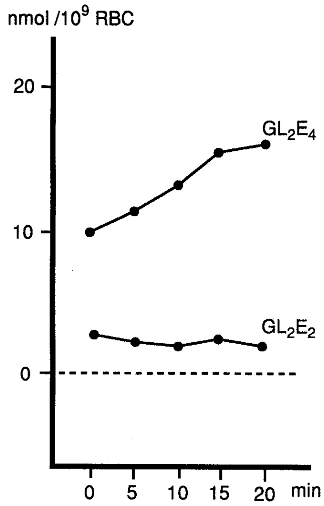


Fig. 4. Ion-Permeation Activities of GL_2E_2 (**10**) and GL_2E_4 (**11**) for K^+ Ion

Initial concentration of the sample: 0.25 μmol/10^9 RBC. Ordinary concentration of K^+ ion: 1.0—1.2 × 10^5 nmol/ml blood.

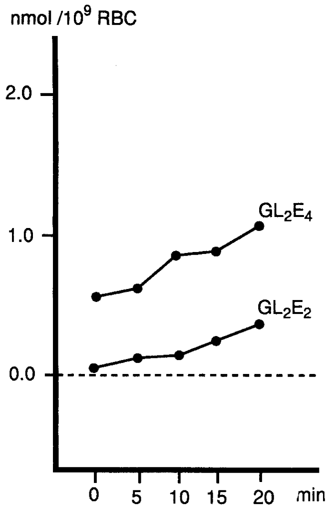


Fig. 3. Ion-Permeation Activities of GL_2E_2 (**10**) and GL_2E_4 (**11**) for Na^+ Ion

Initial concentration of the sample: 0.25 μmol/10^9 RBC. Ordinary concentration of Na^+ ion: 1.0—1.5 × 10^4 nmol/ml blood.

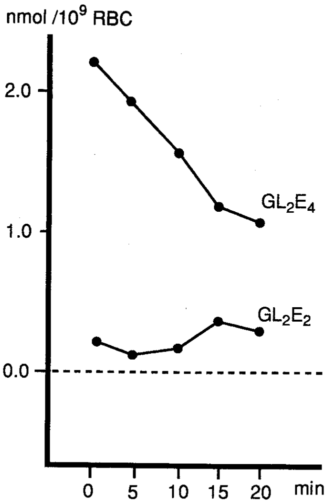


Fig. 5. Ion-Permeation Activities of GL_2E_2 (**10**) and GL_2E_4 (**11**) for Ca^{2+} Ion

Initial concentration of the sample: 0.25 μmol/10^9 RBC. Ordinary concentration of Ca^{2+} ion: 2 nmol/ml blood.

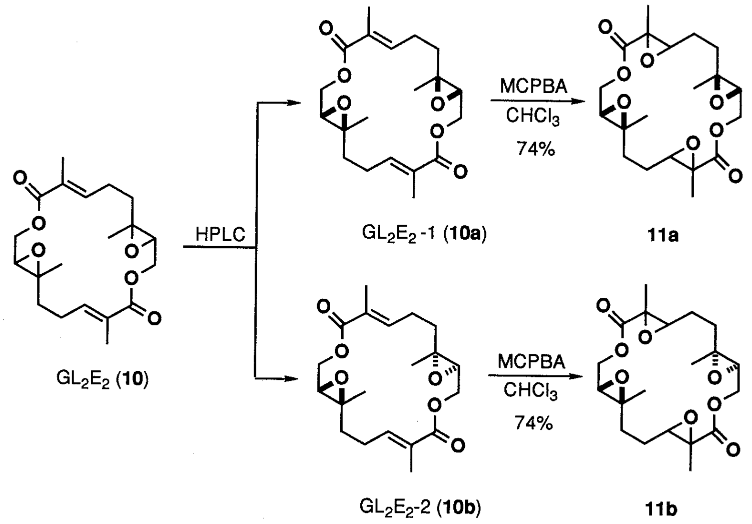
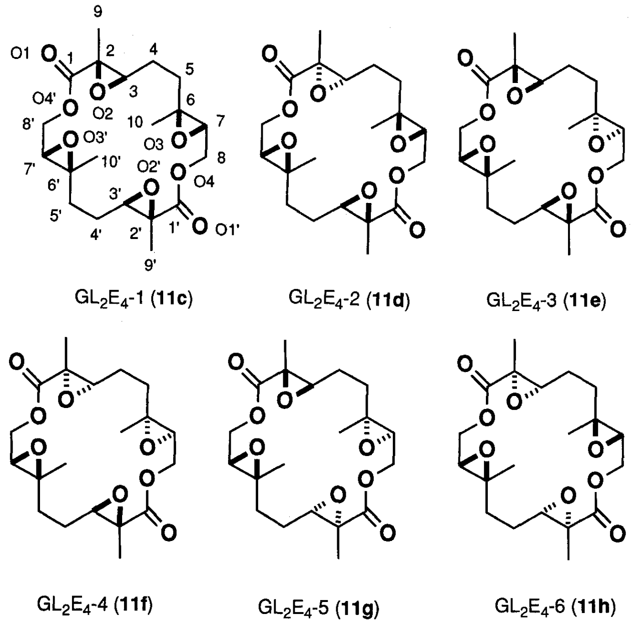


Chart 3

stereostructures of GL_2E_4-2 and GL_2E_4-6 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 GL_2E_4-1 was a *syn*-type diepoxidized derivative of GL_2E_2-1 (**10a**), so the relative stereostructure of GL_2E_4-1 was assigned as **11c**.

On the other hand, another tetraepoxide **11b** obtained by MCPBA oxidation of GL_2E_2-2 (**10b**) was separated by ordinary-phase HPLC to provide three diastereomers, GL_2E_4-3 (**11e**), GL_2E_4-4 (**11f**), and GL_2E_4-5 (**11g**), in 8 : 7 : 5 ratio. Among them, GL_2E_4-4, 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 (^{1}H-NMR) spectrum, one diastereomer, GL_2E_4-5, showed two dimethyl signals (each 6H) at δ 1.32 and 1.45, while another diastereomer, GL_2E_4-3, showed four methyl signals (each 3H) at δ 1.27, 1.31, 1.46 and 1.48. These findings have shown that GL_2E_4-5 possesses a symmetrical structure (**11g**), whereas GL_2E_4-3 possesses an asymmetrical structure (**11e**).

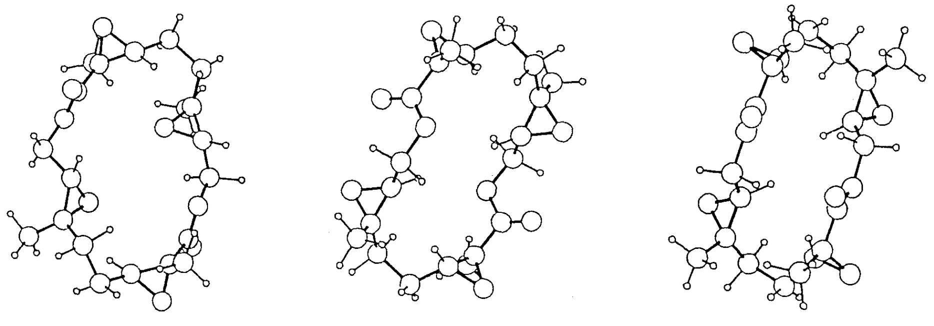
Ionophoretic Activities of Six Diastereomers of GL_2E_4 (11) To shed light on the relationship between ionophoretic activities and stereostructures, we next carried out ion-transport and ion-permeation activity tests of six diastereomers (**11c**, **11d**, **11e**, **11f**, **11g**, and **11h**) of GL_2E_4 (**11**) for Ca^{2+} ion by using a W-07 apparatus[4] and by employing a human erythrocyte membrane method.[5]



GL_2E_4-1 (**11c**) GL_2E_4-2 (**11d**) GL_2E_4-3 (**11e**)

GL_2E_4-4 (**11f**) GL_2E_4-5 (**11g**) GL_2E_4-6 (**11h**)

Chart 4



GL_2E_4-2 (**11d**) GL_2E_4-4 (**11f**) GL_2E_4-6(**11h**)

Fig. 6. ORTEP Drawings of GL_2E_4-2 (**11d**), GL_2E_4-4 (**11f**), and GL_2E_4-6 (**11h**)

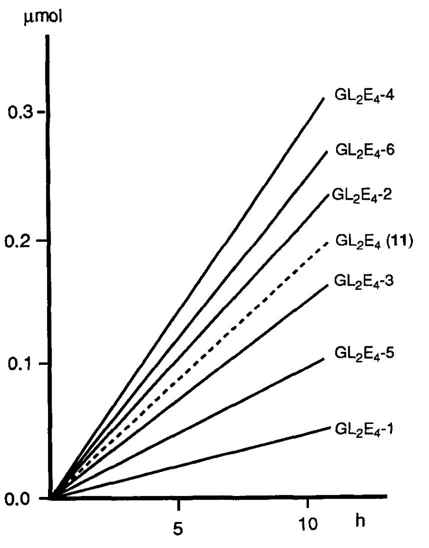


Fig. 7. Ca^{2+} Ion-Transport Activities of GL_2E_4-1—6

Initial concentration of samples: 0.03 M in $CHCl_3$.

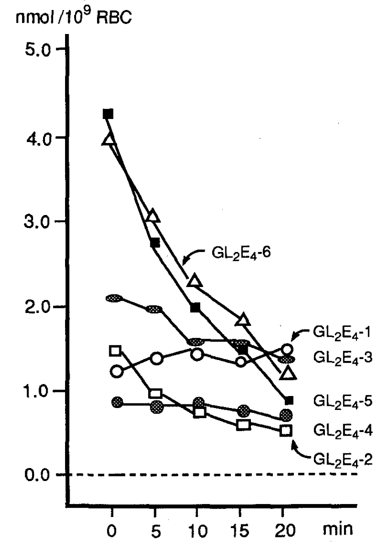


Fig. 8. Ca^{2+} Ion-permeation Activities of GL_2E_4-1—6

Initial concentration of samples: 0.25 mmol/10^9 RBC.

The ion-transport activities of the six diastereomers were in the order of GL_2E_4-4 (**11f**) > -6 (**11h**) > -2 (**11d**) > **11** (the parent diastereomeric mixture) > -3 (**11e**) > -5 (**11g**) > -1 (**11c**). One of the structural features of GL_2E_4-4 (**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 GL_2E_4-6 (**11h**), -5 (**11g**) > -3 (**11e**) > -1 (**11c**) > -4 (**11f**), -2 (**11d**). In this case, the structural feature of GL_2E_4-6 (**11h**) and GL_2E_4-5 (**11g**), which exhibited much stronger ion-permeation activity for Ca^{2+} ion across the human erythrocyte membrane, is that both diastereomers possess two pairs of *anti*-faced epoxide rings beyond a dimethylene bond (*i.e.*, C_4–C_5 and $C_{4'}$–$C_{5'}$) in one component of the dilactone structure, respectively. Contrary to our expectation, GL_2E_4-1 (**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), GL_2E_4-6 (**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 hypothesis that GL_2E_4-6 (**11h**), when acting as a Ca^{2+} ionophore, may change its conformation to one favorable for trapping Ca^{2+} 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% SeO_2 (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 $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product (60 g), which was purified by silica gel column chromatography (SiO_2 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 (CCl_4) cm^{-1}: 3600—3100 (br), 1740, 1665. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.61, 1.70 (3H each, both br s, vinyl methyl ×2), 1.98 (3H, s, $-OCOCH_3$), 3.86 (2H, br s, $-C\underline{H}_2-OH$), 4.49 (2H, d, $J = 7$ Hz, $-C\underline{H}_2-OCOCH_3$), 5.2—5.5 (2H, m, two olefinic protons). EI-MS *m/z* (%): 152 ($M^+ - AcOH$, 5), 43 (100). *Anal.* Calcd for $C_{12}H_{20}O_3$: 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). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.75 (6H, br s, vinyl methyl ×2), 2.06 (3H, s, $-OCOCH_3$), 4.60 (2H, d, $J = 7$ Hz, $-C\underline{H}_2-OH$), 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 $C_{12}H_{18}O_3$: 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–$CHCl_3$ (10 : 1, 1100 ml) was treated with MnO_2 (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), MnO_2 (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 $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent under reduced pressure gave a product (9.2 g), which was purified by silica gel column chromatography (SiO_2 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). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.72, 1.83 (3H each, both br s, vinyl methyl ×2), 2.05 (3H, s, $-OCOCH_3$), 3.73 (3H, s, $-COOCH_3$), 4.58 (2H, d, $J = 7$ Hz, $-C\underline{H}_2-OCOCH_3$), 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 $C_{13}H_{20}O_4$: 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 $MgSO_4$. 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). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.70, 1.84 (3H, each, both br s, vinyl methyl ×2), 3.73 (3H, s, $-COOCH_3$), 4.17 (2H, d, $J = 7$ Hz, $-C\underline{H}_2-OH$), 5.44 (1H, t, $J = 7$ Hz, olefinic proton), 6.74 (1H, t, $J = 7$ Hz, olefinic proton). EI-MS *m/z* (%): 180 ($M^+ - H_2O$, 21.5%), 43 (100). *Anal.* Calcd for $C_{11}H_{18}O_3$: C, 66.64; H, 9.15. Found: C, 66.42; H, 9.33.

Lactonization of 8 Giving GL_2 (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 NH_4Cl and the whole mixture was extracted with EtOAc. The EtOAc extract was washed with brine and dried over $MgSO_4$. Removal of the solvent gave a product (2.1 g). Purification of the product by silica gel column chromatography (SiO_2 100 g, *n*-hexane : EtOAc = 10 : 1) afforded GL_2 (**9**, 1.24 g, 3.86 mmol, 85%).

GL_2(**9**): A white powder. IR ($CHCl_3$) cm^{-1}: 1697. UV (MeOH) nm (ε): 212 (18000). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.70, 1.84 (3H each, both br s, vinyl methyl ×2), 4.34 (4H, d, $J = 8$ Hz, $-C\underline{H}_2-OCO-$ ×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 $C_{20}H_{28}O_4$: 332.199. Found: 332.198 (M^+).

Epoxidation of GL_2 (9) Giving GL_2E_2 (10) and GL_2E_4 (11) A solution of GL $_2$ (**9**, 480 mg, 1.45 mmol) in $CHCl_3$ (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 Na_2SO_3 (25 ml) and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Evaporation of the solvent from the $CHCl_3$ extract gave a product (580 mg), which was purified by silica gel column chromatography (SiO_2 30 g, *n*-hexane : EtOAc = 4 : 1 → 3 : 2) to afford GL_2E_2 (**10**, 121 mg, 0.33 mmol, 23%) and GL_2E_4 (**11**, 269 mg, 0.68 mmol, 47%).

GL_2E_2 (**10**): A white powder. IR ($CHCl_3$) cm^{-1}: 1709, 1645. UV (MeOH) nm (ε): 214 (3700). ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.41 (3H, s, methyl), 1.39, 1.58 (totally 3H, both s, methyl), 1.85 (6H, br s, vinyl methyl ×2), 2.8—3.1 (2H, m, $>\overset{O}{C-C}\underline{H}-CH_2-$ ×2), 4.0—4.4 (4H, m, $-CH_2-OCO-$ ×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 $C_{20}H_{28}O_6$: 364.188. Found: 364.186 (M^+).

GL_2E_4 (**11**): A white powder. IR ($CHCl_3$) cm^{-1}: 1733. ^{1}H-NMR (90 MHz, $CDCl_3$) δ: 1.3—1.4 (6H, m), 1.5—1.6 (6H, m) (methyl ×4), 2.8—3.4 (4H, m, $>\overset{O}{C-C}\underline{H}-$ ×4), 3.9—4.5 (4H, m, $-C\underline{H}_2-OCO-$). EI-MS *m/z* (%): 396 (M^+, 0.7), 149 (100). HR-EI-MS *m/z*: Calcd for $C_{20}H_{28}O_8$: 396.178. Found: 396.179 (M^+).

Separation of Two Diastereomers of GL_2E_2 (10) GL_2E_2 (**10**, 145 mg) was submitted to HPLC (YMC 043-10, *n*-hexane : EtOAc = 8 : 1) to afford GL_2E_2-1 (**10a**, 93 mg) and GL_2E_2-2 (**10b**, 52 mg).

Epoxidation of GL_2E_2-1 (10a) A solution of GL_2E_2-1 (**10a**, 93 mg, 0.255 mmol) in $CHCl_3$ (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 Na_2SO_3 (10 ml) and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with aqueous saturated $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the solvent gave a product (152 mg), which was purified by silica gel column chromatography (SiO_2 15 g, *n*-hexane:

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

Epoxidation of GL_2E_2-2 (10b) **11b** (42 mg, 0.106 mmol, 74%) was obtained from GL_2E_2-2 (**10b**, 52 mg, 0.143 mmol) through a procedure similar to that employed for the preparation of **11a** from GL_2E_2-1 (**10a**).

HPLC Separation of 11a **11a** (75 mg) was submitted to HPLC (YMC 043-10, *n*-hexane : EtOAc=2 : 1) to afford GL_2E_4-1 (**11c**, 12 mg), GL_2E_4-2 (**11d**, 46 mg), and GL_2E_4-6 (**11h**, 17 mg).

GL_2E_4-1 (**11c**): A white powder. IR ($CHCl_3$) cm^{-1}: 1730. ^1H-NMR (500 MHz, $CDCl_3$) δ: 1.28, 1.49 (6H each, both s, CH_3 ×4), 1.38—1.61 (4H, m), 1.81—2.02 (4H, m) (4-H_2, 5-H_2, 4′-H_2, 5′-H_2), 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 $C_{20}H_{28}O_8$: 396.178. Found: 396.179 (M^+).

GL_2E_4-2 (**11d**): Colorless needles, mp 195—196 °C (EtOAc). IR ($CHCl_3$) cm^{-1}: 1733. ^1H-NMR (500 MHz, $CDCl_3$) δ: 1.47, 1.46, 1.28, 1.25 (3H each, all s, CH_3 ×4), 1.46—1.73 (4H, m), 2.01—2.13 (4H, m) (4-H_2, 5-H_2, 4′-H_2, 5′-H_2), 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 $C_{20}H_{28}O_8$: 396.178. Found: 396.178 (M^+).

TABLE I. Experimental Data for the X-Ray Diffraction Studies of GL_2E_4-2(**11d**), -4(**11f**), and -6(**11h**)

	GL_2E_4-2(**11d**)	GL_2E_4-4(**11f**)	GL_2E_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
F (000)	1104	222	1696
System	Orthorhombic	Triclinic	Orthorhombic
Space group	$P2_12_12_1$	$P1$	P_{bca}
a (Å)	6.372 (1)	10.649 (4)	8.669 (2)
b (Å)	10.206 (2)	8.559 (4)	20.847 (4)
c (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
V (Å)	2021.6 (5)	487.6 (3)	3930 (1)
Z	4	1	8
F_o used	3657	1696	2420
No. of param.	252	252	344
R	0.0671	0.0692	0.0869
R_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	*x*	*y*	*z*	B_{eq}/B_{iso} (Å^2)
GL_2E_4-2(**11d**)				
C1	0.0094 (8)	0.5571 (5)	0.6922 (1)	3.7 (2)
C2	−0.1342 (8)	0.6176 (4)	0.6596 (1)	3.7 (2)
C3	−0.0206 (9)	0.6817 (4)	0.6243 (1)	3.9 (2)
C4	−0.111 (1)	0.6964 (5)	0.5796 (2)	4.7 (2)
C5	−0.0927 (9)	0.5665 (5)	0.5548 (1)	4.3 (2)
C6	0.1332 (8)	0.5251 (4)	0.5464 (1)	3.6 (2)
C7	0.1852 (8)	0.3852 (5)	0.5527 (1)	3.9 (2)
C8	0.3580 (9)	0.3183 (5)	0.5286 (2)	4.6 (2)
C9	−0.3530 (9)	0.5640 (6)	0.6556 (2)	5.0 (2)
C10	0.254 (1)	0.6109 (6)	0.5165 (2)	5.7 (3)
C1′	0.3703 (8)	0.1052 (5)	0.5604 (1)	3.9 (2)
C2′	0.4812 (8)	0.0222 (5)	0.5929 (2)	3.8 (2)
C3′	0.3376 (8)	−0.0500 (4)	0.6219 (2)	3.8 (2)
C4′	0.3914 (9)	−0.0925 (5)	0.6670 (2)	4.5 (2)
C5′	0.2616 (9)	−0.0148 (5)	0.7001 (2)	4.1 (2)
C6′	0.2999 (7)	0.1304 (5)	0.6990 (1)	3.2 (2)
C7′	0.1191 (8)	0.2170 (5)	0.7013 (2)	3.9 (2)
C8′	0.1293 (9)	0.3530 (5)	0.7187 (2)	4.5 (2)
C9′	0.7041 (9)	0.0550 (6)	0.6038 (2)	5.2 (3)
C10′	0.5162 (9)	0.1753 (6)	0.7132 (2)	5.5 (3)
O1	0.1439 (7)	0.6126 (4)	0.7112 (1)	5.8 (2)
O2	−0.1160 (7)	0.7585 (3)	0.6586 (1)	4.8 (2)
O3	0.2430 (7)	0.4806 (4)	0.5841 (1)	4.7 (2)
O4	0.4593 (6)	0.2230 (3)	0.5571 (1)	4.4 (1)
O1′	0.2149 (7)	0.0724 (4)	0.5410 (1)	5.3 (2)
O2′	0.4389 (7)	−0.1160 (3)	0.5867 (1)	5.2 (2)
O3′	0.2214 (6)	0.1957 (3)	0.6614 (1)	4.1 (1)
O4′	−0.0250 (6)	0.4279 (3)	0.6947 (1)	4.4 (1)
GL_2E_4-4(**11f**)				
C1	1.0137 (4)	0.0156 (5)	0.7758 (7)	2.5 (1)
C2	1.1481 (4)	0.1145 (5)	0.7900 (7)	2.7 (1)
C3	1.1635 (4)	0.2885 (5)	0.8041 (7)	2.9 (1)
C4	1.2803 (4)	0.4176 (5)	0.9370 (8)	3.2 (1)
C5	1.2716 (4)	0.4829 (5)	1.2222 (8)	2.9 (1)
C6	1.1599 (4)	0.5646 (5)	1.2925 (7)	2.7 (1)
C7	1.0419 (4)	0.4679 (5)	1.3379 (7)	2.8 (1)
C8	0.9130 (4)	0.5123 (6)	1.3015 (8)	3.4 (1)
C9	1.2479 (4)	0.0398 (6)	0.887 (1)	4.2 (2)
C10	1.1515 (4)	0.7126 (5)	1.2054 (9)	3.4 (1)
C1′	0.7787 (4)	0.3790 (5)	1.5401 (8)	3.1 (1)
C2′	0.6450 (4)	0.2797 (5)	1.5324 (7)	3.1 (1)
C3′	0.6316 (4)	0.1002 (5)	1.5101 (8)	3.3 (1)
C4′	0.5145 (4)	−0.0277 (5)	1.3819 (9)	3.4 (1)
C5′	0.5268 (4)	−0.0821 (6)	1.114 (1)	3.9 (2)
C6′	0.6389 (3)	−0.1695 (5)	1.0316 (7)	2.6 (1)
C7′	0.7591 (4)	−0.0726 (5)	0.9712 (7)	2.8 (1)
C8′	0.8805 (4)	−0.1258 (5)	1.0081 (8)	3.4 (1)
C9′	0.5423 (4)	0.3480 (5)	1.429 (1)	3.9 (2)
C10′	0.6388 (5)	−0.3257 (5)	1.1036 (9)	3.9 (2)
O1	0.9424 (3)	−0.0469 (5)	0.6014 (6)	4.4 (1)
O2	1.1759 (3)	0.1679 (4)	0.5772 (6)	4.2 (1)
O3	1.1287 (3)	0.5788 (4)	1.5407 (5)	3.7 (1)
O4	0.8035 (3)	0.3940 (4)	1.3243 (5)	3.33 (9)
O1′	0.8577 (3)	0.4343 (5)	1.7186 (6)	4.8 (1)
O2′	0.6155 (3)	0.2262 (4)	1.7395 (6)	3.8 (1)
O3′	0.6693 (3)	−0.1857 (4)	0.7767 (5)	3.5 (1)
O4′	0.9937 (3)	−0.0024 (4)	0.9942 (6)	3.9 (1)
GL_2E_4-6 (**11h**)				
C1	−0.1326 (5)	0.0368 (2)	0.3422 (2)	3.2 (2)
C2	−0.0362 (6)	−0.0209 (2)	0.3595 (2)	3.2 (2)
C3	0.0292 (5)	0.0238 (2)	0.4221 (2)	3.1 (2)
C4	0.1730 (6)	−0.0599 (2)	0.4396 (2)	3.6 (2)
C5	0.3002 (6)	−0.0135 (2)	0.4589 (2)	3.6 (2)
C6	0.3546 (5)	0.0301 (2)	0.4083 (2)	3.2 (2)
C7	0.2717 (5)	0.0901 (2)	0.3977 (2)	3.3 (2)
C8	0.2624 (7)	0.1231 (2)	0.3363 (2)	3.9 (2)
C9	0.0283 (8)	−0.0572 (3)	0.3058 (2)	5.5 (3)
C10	0.4491 (7)	−0.0009 (3)	0.3583 (3)	4.6 (2)
C1′	0.1047 (6)	0.2097 (2)	0.3060 (2)	3.3 (2)
C2′	0.0324 (6)	0.2732 (2)	0.3234 (2)	3.4 (2)
C3′	0.0386 (6)	0.2940 (2)	0.3882 (2)	3.6 (2)
C4′	−0.0793 (6)	0.3360 (2)	0.4183 (2)	4.0 (2)
C5′	−0.1812 (6)	0.2982 (2)	0.4622 (2)	3.9 (2)
C6′	−0.2709 (6)	0.2440 (2)	0.4335 (2)	3.2 (2)
C7′	−0.1940 (5)	0.1823 (2)	0.4245 (2)	3.1 (2)
C8′	−0.2410 (6)	0.1357 (2)	0.3746 (2)	3.2 (2)
C9′	−0.0908 (7)	0.2943 (3)	0.2794 (2)	4.9 (3)
C10′	−0.4103 (6)	0.2632 (2)	0.3967 (3)	4.3 (2)
O1	−0.1827 (6)	0.0446 (2)	0.2916 (2)	6.2 (2)
O2	−0.1075 (4)	−0.0591 (2)	0.4071 (2)	4.9 (2)
O3	0.4155 (4)	0.0910 (2)	0.4297 (2)	4.4 (2)
O4	0.1995 (4)	0.1869 (1)	0.3479 (2)	4.3 (2)
O1′	0.0727 (6)	0.1827 (2)	0.2597 (2)	6.9 (2)
O2′	0.1407 (5)	0.3216 (2)	0.3426 (2)	5.3 (2)
O3′	−0.2982 (4)	0.1902 (2)	0.4752 (1)	4.2 (2)
O4′	−0.1545 (4)	0.0768 (1)	0.3881 (1)	3.7 (1)

GL_2E_4-6 (**11h**): Colorless needles, mp 160—161 °C (EtOAc). IR ($CHCl_3$) cm^{-1}: 1732. ^1H-NMR (500 MHz, $CDCl_3$) δ: 1.30, 1.45 (6H each, both s, CH_3 ×4), 1.50—1.78 (4H, m), 1.83—1.97 (4H, m) (4-H_2, 5-H_2, 4'-H_2, 5'-H_2), 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 $C_{20}H_{28}O_8$: 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 GL_2E_4-3 (**11e**, 17 mg), GL_2E_4-4 (**11f**, 15 mg), and GL_2E_4-5 (**11g**, 10 mg).

GL_2E_4-3 (**11e**): A white powder. IR ($CHCl_3$) cm^{-1}: 1733. ^1H-NMR (500 MHz, $CDCl_3$) δ: 1.27, 1.31, 1.46, 1.48 (3H each, all s, CH_3 ×4), 1.51—1.81 (4H, m), 1.90—2.07 (4H, m) (4-H_2, 5-H_2, 4'-H_2, 5'-H_2), 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 $C_{20}H_{28}O_8$: 396.178. Found: 396.177 (M^+).

GL_2E_4-4 (**11f**): Colorless needles, mp 169—170 °C (EtOAc). IR ($CHCl_3$) cm^{-1}: 1731. ^1H-NMR (500 MHz, $CDCl_3$) δ: 1.29, 1.48 (6H each, both s, CH_3 ×4), 1.51—1.79 (4H, m), 1.93—1.98 (4H, m) (4-H_2, 5-H_2, 4'-H_2, 5'-H_2), 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 $C_{20}H_{28}O_8$: 396.178. Found: 396.178 (M^+).

GL_2E_4-5 (**11g**): A white powder. IR ($CHCl_3$) cm^{-1}: 1735. ^1H-NMR (500 MHz, $CDCl_3$) δ: 1.32, 1.45 (6H each, both s, CH_3 ×4), 1.50—1.80 (4H, m), 1.94—2.05 (4H, m) (4-H_2, 5-H_2, 4'-H_2, 5'-H_2), 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 $C_{20}H_{28}O_8$: 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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