Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

for

Angew. Chem. Int. Ed. Z54065

69451 Weinheim, Germany

Extracting Specific Conformations Using a Carbohydrate Scaffold: Discovery of New Subtype-Selective LPA Receptor Agonists and Antagonist

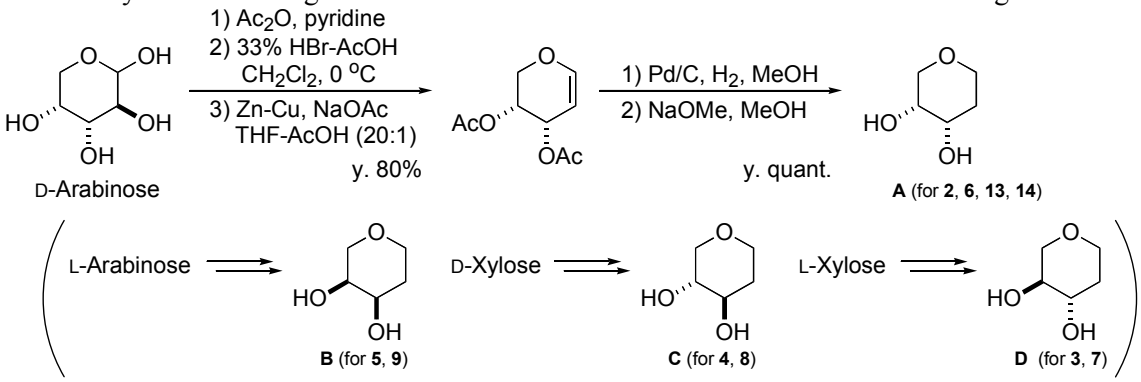
Yoko Tamaruya, Masato Suzuki, Goshu Kamura, Motomu Kanai, Kotaro Hama, Kumiko Shimizu, Junken Aoki, Hiroyuki Arai, and Masakatsu Shibasaki

General: NMR spectra were recorded on a JEOL JNM-LA500 spectrometer, operating at 500 MHz for ^{1}H NMR, 125.65 MHz for ^{13}C NMR, and 202 MHz for ^{31}P NMR. ^{1}H NMR chemical shifts were reported downfield from TMS (= 0), or using $CHCl_3$ (7.24 ppm) or CH_3OH (3.31 ppm) as an internal standard. For ^{13}C NMR, chemical shifts were reported using $CHCl_3$ (77.0 ppm) or CH_3OH (49.00 ppm) as an internal reference. ^{31}P NMR were carried out with phosphoric acid (85%) as an external standard (= 0). Optical rotations were measured on a JASCO P-1010 polarimeter. Column chromatographies were performed with silica gel Merck 60 (230-400 mesh ASTM), unless otherwise noted. Infrared (IR) spectra were recorded on JASCO FT/IR 410 Fourier transform infrared spectrophotometer. ESI mass spectra were measured on Waters-ZQ4000. HR-MS spectra were measured on JEOL JMS-700D Mstation, using Kanto Chemical PEG 600 matrix as the internal standard. In general, reactions were carried out in dry solvents under an argon atmosphere.

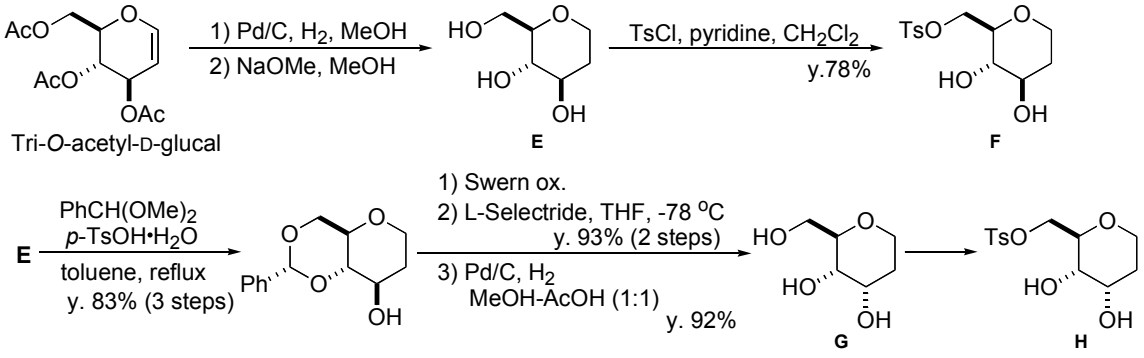
Synthetic Procedures for LPA analogues

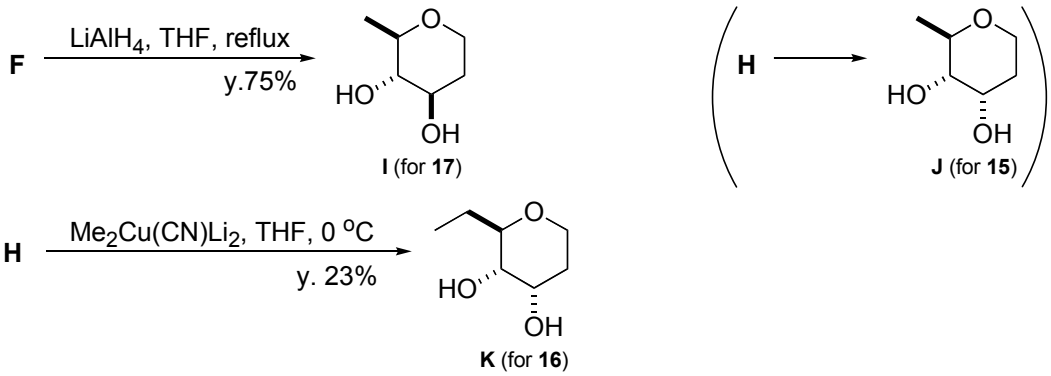
Synthesis of intermediate diols for 2-9, 13, 14

The diols were synthesized using standard chemical transformations shown in the following scheme.



Synthesis of intermediate diols for 15-17[1]

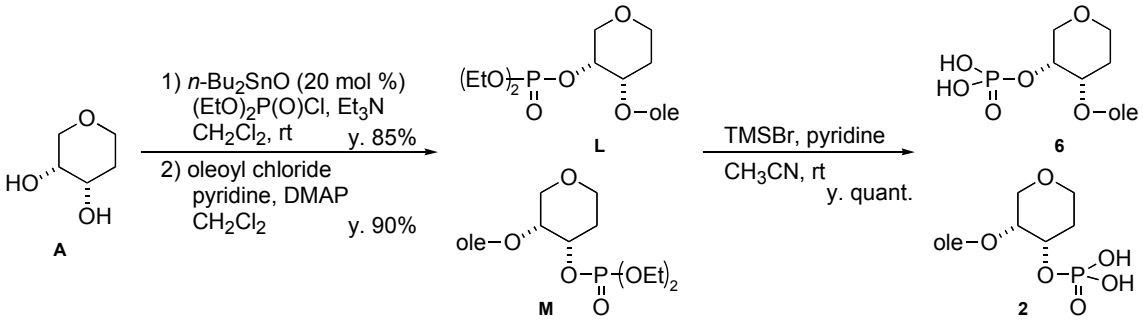




From F to I (reduction): A solution of tosylate **F** (1.0 g, 3.31 mmol) in THF (5 mL) was added to a suspension of LAH (226 mg, 5.0 mmol) in THF (8.5 mL) at 0 °C, and the mixture was stirred under reflux for 1 h. After cooling to room temperature, H_2O (0.2 mL), 15% NaOH aq. (0.2 mL), and H_2O (0.6 mL) was added successively. The resulting precipitate was filtered off, and washed with THF. Evaporation of the solvent followed by purification through silica gel column chromatography (MeOH : CH_2Cl_2 = 1: 100~1:20) afforded **I** in 75 % yield as a colorless oil.

From H to K (methylation): To a suspension of CuCN (1.36 g, 15.2 mmol) in THF (15.2 mL) were added CH_3Li (20.3 mL, 30.4 mmol, 1.5 M in Et_2O) at 0 °C. After stirring for 20 min at 0 °C, **H** (4.0 mL, 1.52 mmol, 0.38 M in THF) was added at the same temperature. The stirring was continued over night at room temperature and quenched by addition of saturated aqueous NH_4Cl. The mixture was extracted with AcOEt – THF (1:1) and the combined organic layer was dried over Na_2SO_4. Evaporation of solvent gave a crude mixture, and this mixture was purified by flash silica gel column chromatography (MeOH : CH_2Cl_2 = 1: 100) to afford **K** in 23 % yield as a colorless oil.

Representative procedure for phosphate synthesis



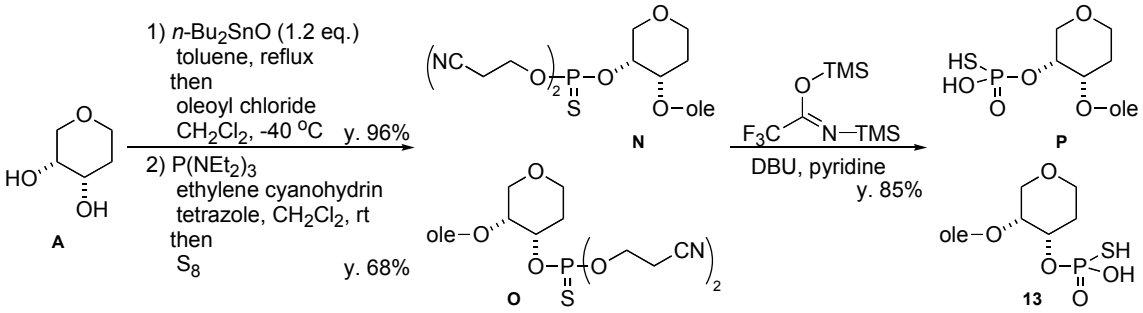
To a solution of diol **A** (177.2 mg, 1.5 mmol) and *n*Bu_2SnO (74.4 mg, 0.3 mL) in CH_2Cl_2 (3 mL) were added $(EtO)_2P(O)Cl$ (0.23 mL, 1.58 mmol) and Et_3N (0.23 mL, 1.65 mmol) at room temperature, and the reaction mixture was stirred for 4 h. After completion of the reaction, saturated aq. NH_4Cl was added for quenching. The resulting solution was extracted with AcOEt. The combined organic layer was washed with H_2O and brine, and the dried over Na_2SO_4. Solvent was removed under reduced pressure to afford the mixture of two regioisomers (~1:1) as a colorless oil. The crude product was directly used as a starting material in the next step.

The crude alcohol was dissolved in CH_2Cl_2 (3 mL). Pyridine (0.06 mL, 0.75 mmol), oleoyl chloride (0.64 mL, 1.95 mmol) and DMAP (18.3 mg, 0.15 mmol) were added successively to this solution at room temperature, and the whole mixture was stirred for 2 h. After the starting material disappeared on TLC, saturated aq. NH_4Cl was added for quenching. The resulting solution was extracted with AcOEt. The combined organic layer was washed with H_2O and brine, and the dried over Na_2SO_4. Further purification was performed by flash column chromatography on silica gel (AcOEt : hexane = 1:15) to afford **L** and **M** in

30-40 % yield as a colorless oil, respectively.
Two regioisomers are separable on TLC (AcOEt : hexane = 1:2, **L** : Rf = 0.5, **M** : Rf = 0.45). (Relative polarity of each regioisomer shows the same tendency for all stereoisomers.)

To a solution of corresponding diethylphosphate **M** (31.3 mg, 0.0603 mmol) in CH_3CN (0.30 mL) were added pyridine (0.03mL, 0.422 mmol) and TMSBr (0.04 mL, 0.302 mmol) at room temperature, and the reaction mixture was stirred for 3 h. After completion of the reaction, MeOH was added for quenching. Solvent was removed under reduced pressure, and the crude mixture was further azeotroped with MeOH. Further purification was performed by open column on Dowex 50WX8 50-100 (H^+ form, MeOH) to afford **2** in quantitative yield.

Representative procedure for thiophosphate synthesis



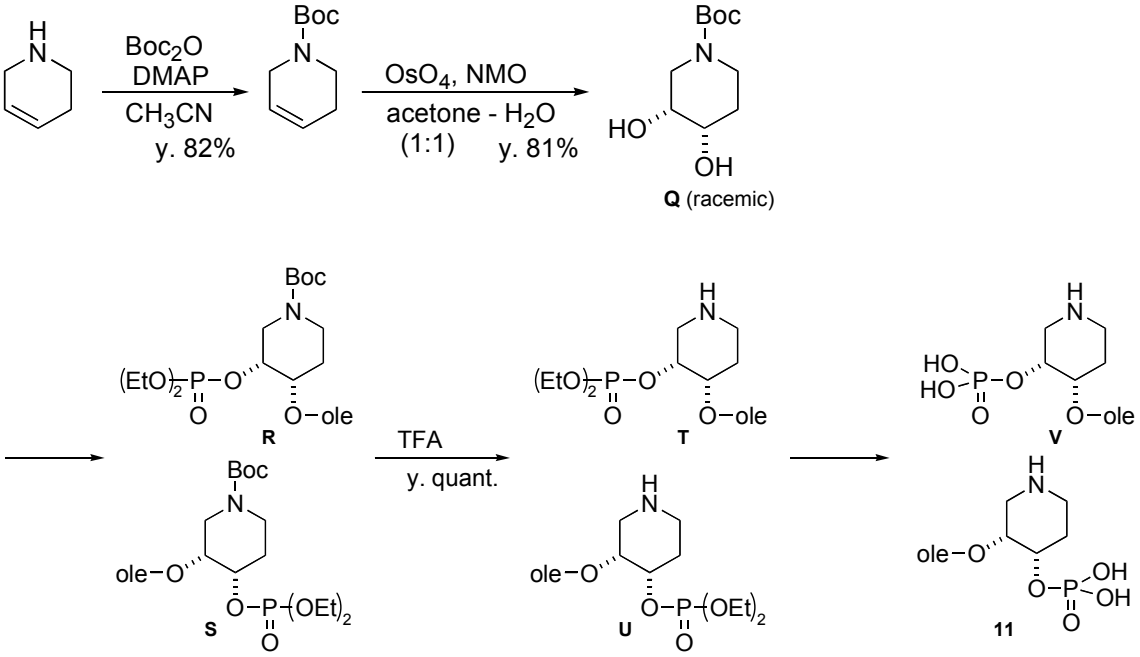
To a solution of **A** (500 mg, 4.23 mmol) in toluene (50 mL) was added nBu$_2$SnO (1.16 g, 4.66 mmol). After refluxing the reaction mixture for 2 h, solvent was removed under reduced pressure. Resulting suspension in remaining toluene (5 mL) was cooled down to –40 °C, then CH_2Cl_2 (25 mL) was added. Oleoyl chloride (1.47 mL, 4.44 mmol) was added to this suspension, and the reaction mixture was allowed to stir for 2 h at the same temperature. After the starting material disappeared on TLC, H_2O was added for quenching. The resulting mixture was extracted with CH_2Cl_2, and the combined organic layer was washed with H_2O and brine, and then dried over Na_2SO_4. After removal of solvent, a flash column chromatography on silica gel (AcOEt : hexane = 1:8) was carried out to afford a mixture of two regioisomers (ca. 1:1) in 96 % yield as a colorless oil.

The resulting mixture of two regioisomers (172 mg, 0.449 mmol) was dissolved in CH_2Cl_2 (1.8 mL). $P(NEt_2)_3$ (0.13 mL, 0.458 mmol) and 1H-tetrazole (37.8 mg, 0.539 mmol) were added to this solution at room temperature. After the starting material disappeared on TLC in 30 min, 3-hydroxypropionitrile (0.07 mL, 0.962 mmol) and 1H-tetrazole (62.9 mg, 0.898 mmol) were added successively at the same temperature. After the reaction completed, S_8 (18.8 mg, 0.584 mmol) was added, and the whole mixture was allowed to stir for 2 h. H_2O was added for quenching and the resulting mixture was extracted with AcOEt. The combined organic layer was washed with H_2O and brine, and dried over Na_2SO_4. Further purification was performed by flash column chromatography on silica gel (AcOEt : hexane = 1:4) to afford each regioisomer in 35 % yield as a colorless oil.
Two regioisomers are separable on TLC (AcOEt : CH_2Cl_2 = 1:10, **N** : Rf = 0.4, **O** : Rf = 0.45).

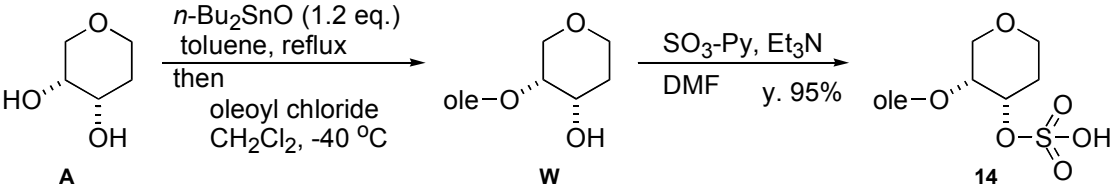
To a solution of thiophosphate **O** (57.6 mg, 0.0985 mmol) in pyridine (3.5 mL) were added bis(trimethylsilyl)trifluoroacetamide (1.6 mL, 5.91 mmol) and DBU (0.13 mL, 0.887 mmol). After stirring for 30 min, solvent was removed under reduced pressure, and the crude mixture was further azeotroped with MeOH. H_2O was added to the crude mixture, and washed with Et_2O. Further purification was performed by open column on Dowex 50WX8 50-100 (H^+ form, MeOH) to afford **13** in 80 % yield.

Synthesis of 11

Synthesis of **11** was performed basically using the same procedures reported above (see the following scheme).

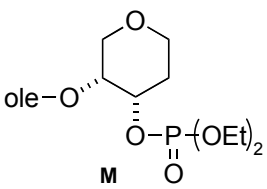


Synthesis of 14

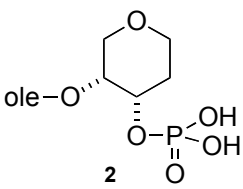


To a solution of **W** (49 mg, 0.128 mmol) in DMF (1.5 mL) was added SO_3-Py (120 mg, 0.754 mmol). After stirring for 3 h, Et_3N was added for quenching. After evaporation of solvent, the crude mixture was further azeotroped with toluene. The crude mixture was purified by flash silica gel column chromatography (AcOEt : hexane = 1 : 2 – MeOH : $CHCl_3$ = 1 : 10) to afford **14** in 95 % yield.

Spectroscopic data for the representative compounds

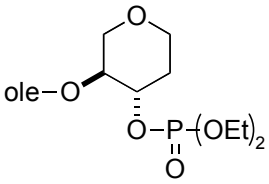


(3*R*,4*S*)-3-Oleoyloxy-4-(diethyloxyphosphoryloxy)tetrahydropyran (M). Colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.15-1.40 (m, 26H), 1.50-1.70 (m, 2H), 1.88-2.05 (m, 5H), 2.10-2.18 (m, 1H), 2.36 (t, *J* = 7.3 Hz, 2H), 3.56 (ddd, *J* = 11.6. 8.0 3.6 Hz, 1H), 3.60 (dd, *J* = 12.2, 2.5 Hz, 1H), 3.86 (dd, *J* = 12.2, 5.7 Hz, 1H), 3.87-3.92 (m, 1H), 4.03-4.15 (m, 4H), 4.66-4.73 (m, 1H), 5.02-5.07 (m, 1H), 5.30-5.38 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 14.0, 16.0, 16.1, 22.6, 24.8, 27.1, 27.2, 29.01, 29.05, 29.1, 29.3, 29.4, 29.6, 29.7, 29.9, 31.8, 34.2, 63.81, 63.86, 64.2, 66.5, 68.3, 72.6, 129.6, 129.9, 172.9; ^{31}P NMR ($CDCl_3$): δ -1.36; ; IR (neat, cm^{-1}): 2925, 2854, 1739; ESI-MS *m/z* 541 (M^++Na); FAB-HRMS Calcd for $C_{27}H_{52}O_7P$ (M^++H) : 519.3451, Found : 519.3448; $[\alpha]^{25}_D$-11.2 (c = 0.345, $CHCl_3$).

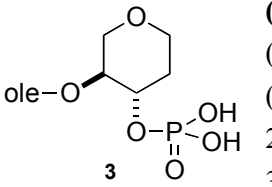


(3*R*,4*S*)-3-Oleoyloxy-4-phosphoryloxytetrahydropyran (2). Colorless oil; ^{1}H NMR (CD_3OD): δ 0.89 (t, *J* = 7.0 Hz, 3H), 1.21-1.40 (m, 20H), 1.57-1.65 (m, 2H), 1.92 (ddd, *J* = 13.5, 6.0, 3.2 Hz, 1H), 2.00-2.14 (m, 3H), 2.37 (t, *J* = 7.7 Hz, 2H), 3.57 (ddd, *J* = 11.6, 8.3, 3.8 Hz, 1H), 3.60 (dd, *J* = 9.5 2.8 Hz, 1H), 3.79-3.90 (m, 2H), 4.56-4.63 (m, 1H), 4.95-5.10 (m, 1H), 5.30-5.40 (m, 2H); ^{13}C NMR (CD_3OD): δ 14.4, 23.7, 25.9, 28.1, 30.1, 30.2, 30.31, 30.34, 30.4, 30.6, 30.82, 30.85, 30.9, 33.1, 35.1, 65.3, 67.5, 70.3, 73.0, 130.8, 130.9,

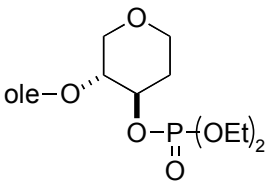
174.7; ^{31}P NMR (CD_3OD): δ -0.65; IR (neat, cm^{-1}): 2924, 2854, 1735; ESI-MS *m/z* 485 (M^++Na); FAB-HRMS Calcd for $C_{23}H_{44}O_7P$ (M^++H): 463.2825, Found : 463.2835; $[\alpha]^{23}_D$-28.0 (c = 0.11, MeOH).



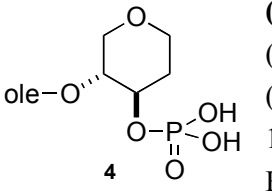
(3*S*,4*S*)-3-Oleoyloxy-4-(diethyloxyphosphoryloxy)tetrahydropyran. Colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.88 (t, *J* = 5.0 Hz, 3H), 1.27-1.36 (m, 26H), 1.61-1.62 (m, 2H), 1.85-1.87 (m, 1H), 2.00-2.01 (m, 4H), 2.20-2.22 (m, 1H), 2.34 (t, *J* = 8.5 Hz, 2H), 3.38 (dd, *J* = 12.0, 7.5 Hz, 1H), 3.52 (m, 1H), 3.86-3.89 (m, 1H), 3.97 (dd, *J* = 11.0, 3.5 Hz, 1H), 4.08-4.13 (m, 4H), 4.46-4.52 (m, 1H), 4.83-4.87 (m, 1H), 5.36 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 14.0, 16.0, 16.1, 22.6, 24.8, 27.2, 29.0, 29.1, 29.3, 29.5, 29.6, 29.7, 30.9, 31.8, 34.1, 53.4, 63.9, 64.3, 66.7, 69.6, 129.6, 130.0, 172.7; ^{31}P NMR ($CDCl_3$): δ -2.37; IR (neat, cm^{-1}): 2925, 2854, 1743; ESI-MS *m/z* 541 (M^++Na); FAB-HRMS Calcd for $C_{27}H_{52}O_7P$ (M^++H) : 519.3451, Found : 519.3450; $[\alpha]^{25}_D$+24.4 (c = 1.34, $CHCl_3$).



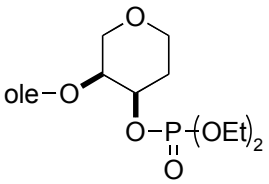
(3*S*,4*S*)-3-Oleoyloxy-4-phosphoryloxytetrahydropyran (3). Colorless oil; ^{1}H NMR (CD_3OD): δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.24-1.50 (m, 20H), 1.58-1.69 (m, 2H), 1.77-1.86 (m, 1H), 1.99-2.12 (m, 4H), 2.19 (dddd, *J* = 13.6, 6.3, 3.3, 3.2 Hz, 1H), 2.37 (dt, *J* = 2.6, 7.3 Hz, 2H), 3.41 (dd, *J* = 11.9 7.0 Hz, 1H), 3.54 (ddd, *J* = 11.7, 8.3, 3.3 Hz, 1H), 3.87 (ddd, *J* = 11.7, 6.3, 4.3 Hz, 1H), 3.92 (dd, *J* = 11.9, 4.0 Hz, 1H), 4.35-4.45 (m, 1H), 4.76-4.81 (m, 1H), 5.35 (t, *J* = 4.8 Hz, 2H); ^{13}C NMR (CD_3OD): δ 14.4, 23.7, 25.9, 28.1, 30.15, 30.17, 30.26, 30.33, 30.4, 30.6, 30.79, 30.83, 31.9, 33.0, 35.0, 65.3, 67.5, 71.2, 74.0, 130.8, 130.9, 174.5; ^{31}P NMR (CD_3OD): δ -0.89; IR (neat, cm^{-1}): 2924, 2854, 1740; ESI-MS *m/z* 485 (M^++Na); FAB-HRMS Calcd for $C_{23}H_{44}O_7P$ (M^++H) : 463.2825, Found : 463.2825; $[\alpha]^{23}_D$+18.7 (c = 0.761, MeOH).



(3*R*,4*R*)-3-Oleoyloxy-4-(diethyloxyphosphoryloxy)tetrahydropyran. Colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.88 (t, *J* = 7.0 Hz, 3H), 1.26-1.36 (m, 26H), 1.61-1.64 (m, 2H), 1.83-1.90 (m, 1H), 1.99-2.03 (m, 4H), 2.18-2.24 (m, 1H), 2.34 (t, *J* = 7.6 Hz, 2H), 3.38 (dd, *J* = 11.6, 7.0 Hz, 1H), 3.53 (ddd, *J* = 11.6, 8.5, 3.1 Hz 1H), 3.86-3.91 (m, 1H), 3.98 (dd, *J* = 11.6, 4.3 Hz , 1H), 4.08-4.15 (m, 4H), 4.49(ddd, *J* = 15.3, 7.3, 4.3 Hz , 1H), 4.85 (td, *J* = 7.0, 4.0 Hz, 1H), 5.30-5.38 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 14.1, 16.05, 16.09, 22.6, 24.8, 27.1, 29.05, 29.12, 29.3, 29.5, 29.6, 29.7, 30.9, 31.9, 34.1, 63.8, 63.9, 66.7, 69.6, 73.8, 129.7, 130.0, 172.7; ^{31}P NMR ($CDCl_3$): δ -1.89; IR (neat, cm^{-1}): 2925, 2854, 1743; ESI-MS *m/z* 541 (M^++Na); FAB-HRMS Calcd for $C_{27}H_{52}O_7P$ (M^++H) : 519.3451, Found : 519.3450; $[\alpha]^{25}_D$-0.137 (c = 1.03, $CHCl_3$).

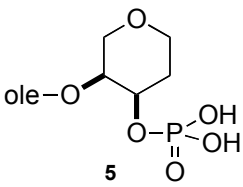


(3*R*,4*R*)-3-Oleoyloxy-4-phosphoryloxytetrahydropyran (4). Colorless oil; ^{1}H NMR (CD_3OD): δ 0.89 (t, *J* = 6.9 Hz, 3H), 1.25-1.38 (m, 20H), 1.56-1.65 (m, 2H), 1.76-1.84 (m, 1H), 1.99-2.07 (m, 4H), 2.18 (m, 1H), 2.36 (td, *J* = 7.6, 3.0 Hz, 2H), 3.39 (dd, *J* = 12.0, 7.0 Hz, 1H), 3.53 (ddd, *J* = 11.6, 8.3, 3.8 Hz, 1H), 3.86 (ddd, *J* = 11.6, 5.8, 3.8 Hz, 1H), 3.91 (dd, *J* = 12.4, 4.0 Hz, 1H), 4.35-4.42 (m, 1H), 4.75-4.80 (m, 1H), 5.34 (t, *J* = 4.6 Hz, 2H); ^{13}C NMR (CD_3OD): δ 14.4, 23.7, 25.9, 28.1, 30.15, 30.18, 30.28, 30.34, 30.4, 30.6, 30.80, 30.84, 32.0, 33.1, 35.0, 65.3, 67.5, 71.2, 74.1, 130.8, 130.9, 174.4; ^{31}P NMR (CD_3OD): δ -0.89; IR (neat, cm^{-1}): 2924, 2853, 1740; ESI-MS *m/z* 485 (M^++Na); FAB-HRMS Calcd for $C_{23}H_{44}O_7P$ (M^++H) : 463.2825, Found : 463.2821; $[\alpha]^{22}_D$-19.8 (c = 0.89, MeOH).



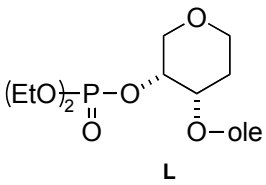
(3*S*,4*R*)-3-Oleoyloxy-4-(diethyloxyphosphoryloxy)tetrahydropyran. Colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.87 (t, *J* = 6.9 Hz, 3H), 1.24-1.35 (m, 26H), 1.60-1.64 (m, 2H), 1.91-1.95 (m, 1H), 1.98-2.02 (m, 4H), 2.11-2.14 (m, 1H), 2.34-2.37 (m, 2H), 3.54-3.61 (m, 2H), 3.84-3.90 (m, 2H), 4.06-4.13 (m, 4H), 4.68-4.69 (m, 1H), 5.03-5.04 (m, 1H), 5.32-5.35 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 14.0, 16.0, 16.1, 22.6, 24.8, 27.1, 27.2, 29.02, 29.05, 29.1, 29.3, 29.5, 29.6, 29.7, 29.9, 31.8, 34.2, 63.84, 63.89, 64.2, 66.5, 68.3, 72.6, 129.6, 129.9, 172.9; ^{31}P NMR ($CDCl_3$): δ –1.55; IR (neat, cm^{-1}): 2925, 2854, 1737, 1642; ESI-MS *m/z*

541 (M^++Na); FAB-HRMS Calcd for $C_{27}H_{52}O_7P$ (M^++H) : 519.3451, Found : 519.3456; $[\alpha]^{24}_D$+8.3 (c = 0.53, $CHCl_3$).



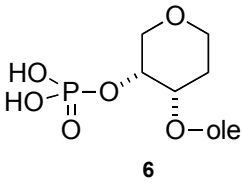
5

(3*S*,4*R*)-3-Oleoyloxy-4-phosphoryloxytetrahydropyran (5). Colorless oil; ^{1}H NMR (CD_3OD): δ 0.89 (t, *J* = 6.9 Hz, 3H), 1.20-1.40 (m, 20H), 1.58-1.67 (m, 2H), 1.87-1.95 (m, 1H), 1.99-2.13 (m, 5H), 2.37 (t, *J* = 7.5 Hz, 2H), 3.51-3.65 (m, 2H), 3.80-3.95 (m, 2H), 4.52-4.60 (m, 1H), 4.95-5.02 (m, 1H), 5.30-5.40 (m, 2H); ^{13}C NMR (CD_3OD): δ 14.4, 23.7, 25.9, 28.1, 30.15, 30.19, 30.31, 30.33, 30.4, 30.6, 30.77, 30.82, 30.84, 31.0, 33.1, 35.1, 65.5, 67.6, 70.6, 72.7, 130.8, 130.9, 174.8; ^{31}P NMR (CD_3OD): δ -0.86; IR (neat, cm^{-1}): 2923, 2853, 1733; ESI-MS *m/z* 485 (M^++Na); FAB-HRMS Calcd for $C_{23}H_{44}O_7P$ (M^++H) : 463.2825, Found : 463.2835; $[\alpha]^{22}_D$+25.0 (c = 0.12, MeOH).



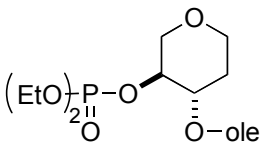
L

(3*R*,4*S*)-4-Oleoyloxy-3-(diethyloxyphosphoryloxy)tetrahydropyran (L). Colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.81 (t, *J* = 6.7 Hz, 3H), 1.13-1.31 (m, 26H), 1.53-1.61 (m, 2H), 1.69-1.76 (m, 1H), 1.90-2.00 (m, 5H), 2.28 (dt, *J* = 2.8, 7.7 Hz, 2H), 3.50 (ddd, *J* = 3.2, 8.7, 11.8 Hz, 1H), 3.60 (dd, *J* = 1.9, 12.1 Hz, 1H), 3.79 (ddd, *J* = 5.3, 5.3, 11.8 Hz, 1H), 3.90 (dd, *J* = 5.4 12.1 Hz, 1H), 4.01-4.12 (m, 4H), 4.46-4.51 (m, 1H), 5.01-5.07 (m, 1H), 5.24-5.34 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 14.3, 16.2, 16.3, 22.8, 25.0, 27.3, 27.4, 28.1, 29.3, 29.4, 29.48, 29.49, 26.7, 29.88, 29.94, 32.1, 34.5, 64.1, 64.9, 68.1, 68.9, 72.3, 129.9, 130.2, 173.1; ^{31}P NMR ($CDCl_3$): δ -0.96; IR (neat, cm^{-1}): 2925, 2854, 1739; ESI-MS *m/z* 541 (M^++Na); FAB-HRMS Calcd for $C_{27}H_{52}O_7P$ (M^++H) : 519.3451, Found : 519.3447; $[\alpha]^{23}_D$-22.1 (c = 0.49, $CHCl_3$).

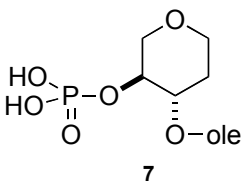


6

(3*R*,4*S*)-4-Oleoyloxy-3-phosphoryloxytetrahydropyran (6). Colorless oil; ^{1}H NMR (CD_3OD): δ 0.89 (t, *J* = 7.0 Hz, 3H), 1.20-1.40 (m, 20H), 1.55-1.66 (m, 2H), 1.71-1.81 (m, 1H), 1.98-2.07 (m,5H), 2.36 (td, *J* = 7.5, 1.7 Hz, 2H), 3.58 (ddd, *J* = 11.8, 8.4, 1.7 Hz, 1H), 3.68 (dd, *J* = 12.2, 2.6 Hz, 1H), 3.78-3.84 (m, 1H), 3.91 (dd, *J* = 12.2, 5.5 Hz, 1H), 4.40-4.47 (m, 1H), 5.04-5.11 (m, 1H), 5.30-5.40 (m, 2H); ^{13}C NMR (CD_3OD): δ 14.4, 23.7, 28.1, 28.9, 30.18, 30.29, 30.33, 30.4, 30.6, 30.75, 30.80, 30.83, 33.0, 35.1, 65.6, 68.8, 70.3, 72.5, 130.8, 130.9, 174.7; ^{31}P NMR (CD_3OD): δ -0.40; IR (neat, cm^{-1}): 2925, 2854, 1733; ESI-MS *m/z* 485 (M^++Na); FAB-HRMS Calcd for $C_{23}H_{44}O_7P$ (M^++H): 463.2825, Found : 463.2835; $[\alpha]^{23}_D$-28.0 (c = 0.11, MeOH).



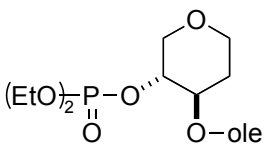
(3*S*,4*S*)-4-Oleoyloxy-3-(diethyloxyphosphoryloxy)tetrahydropyran. Colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.88 (t, *J* = 7.1 Hz, 3H), 1.27-1.35 (m, 26H), 1.62-1.71 (m, 3H), 1.99-2.03 (m, 4H), 2.10-2.15(m, 1H), 2.34 (t, *J* = 7.7 Hz, 2H), 3.46 (dd, *J* = 11.6, 8.0 Hz, 1H), 3.51-3.53 (m, 1H), 3.84-3.89 (dt, *J* = 11.6, 4.6 Hz, 1H), 4.07-4.14 (m, 5H), 4.31(ddd, *J* = 15.6, 8.0, 4.3 Hz, 1H), 4.97 (td, *J* = 8.6, 4.6 Hz, 1H), 5.34 (dd, *J* = 8.3, 5.8 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 14.0, 16.0, 16.1, 22.6, 24.8, 27.2, 29.0, 29.1, 29.3, 29.5, 29.6, 29.7, 30.9, 31.8, 34.1, 53.4, 63.9, 64.3, 66.7, 69.6, 129.6, 130.0, 172.7; ^{31}P NMR ($CDCl_3$): δ 1.73; IR (neat, cm^{-1}): 2925, 2854, 1740; ESI-MS *m/z* 541 (M^++Na); FAB-HRMS Calcd for $C_{27}H_{52}O_7P$ (M^++H) : 519.3451, Found : 519.3451; $[\alpha]^{25}_D$+20.8 (c = 2.62 $CHCl_3$).



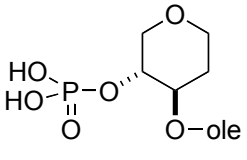
7

(3*S*,4*S*)-4-Oleoyloxy-3-phosphoryloxytetrahydropyran (7). Colorless oil; ^{1}H NMR (CD_3OD): δ 0.90 (t, *J* = 7.0 Hz, 3H), 1.25-1.40 (m, 20H), 1.60-1.70 (m, 3H), 2.00-2.12 (m, 5H), 2.37 (td, *J* = 7.5, 2.4 Hz, 2H), 3.45 (dd, *J* = 11.8 8.1 Hz, 1H), 3.52 (ddd, *J* = 12.0, 9.4, 2.7 Hz, 1H), 3.84 (ddd, *J* = 12.0 4.5, 4.5 Hz, 1H), 4.05 (dd, *J* = 11.8, 4.3 Hz, 1H), 4.15-4.22 (m, 1H), 4.90-5.00 (m,, 1H), 5.34 (t, *J* = 4.6 Hz, 2H); ^{13}C NMR (CD_3OD): δ 14.5, 23.7, 25.9, 28.1, 30.19, 30.29, 30.33, 30.4, 30.6, 30.80, 30.84, 33.1, 35.1, 65.9, 69.4 (d, *J* = 3.1 Hz), 72.3, 73.8, 130.8, 130.9, 174.5; ^{31}P NMR (CD_3OD): δ -0.73; IR (neat, cm^{-1}): 2924, 2854, 1740; ESI-MS *m/z* 485 (M^++Na); FAB-HRMS Calcd for $C_{23}H_{44}O_7P$ (M^++H) : 463.2825, Found : 463.2835;

$[\alpha]^{22}_D$+13.7 (c = 0.11, MeOH).

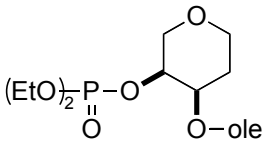


(3*R*,4*R*)-4-Oleoyloxy-3-(diethyloxyphosphoryloxy)tetrahydropyran. Colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.88 (t, *J* = 7.1 Hz, 3H), 1.26-1.35 (m, 26H), 1.60-1.71 (m, 3H), 1.99-2.03 (m, 4H), 2.10-2.15 (m, 1H), 2.34 (t, *J* = 8.0 Hz, 2H), 3.44-3.53 (m, 2H), 3.86 (dt, *J* = 11.6, 4.6 Hz, 1H), 4.07-4.14 (m, 5H), 4.28-4.34 (m, 1H), 4.97 (td, *J* = 8.6, 4.6 Hz, 1H), 5.30-5.36 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 172.8, 130.0, 129.6, 73.0, 70.8, 68.2, 65.0, 64.1, 63.9, 34.3, 31.8, 29.7, 29.6, 29.7, 29.5, 29.3, 29.12, 29.06, 27.2, 27.1, 24.8, 22.6, 16.1, 16.0, 14.0; ^{31}P NMR ($CDCl_3$): δ –1.75; IR (neat, cm^{-1}): 2925, 2854, 1740; ESI-MS *m/z* 541 (M^++Na); FAB-HRMS Calcd for $C_{27}H_{52}O_7P$ (M^++H) : 519.3451, Found : 519.3450; $[\alpha]^{25}_D$-23.1 (c = 0.56 $CHCl_3$).

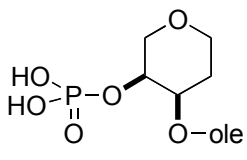


8

(3*R*,4*R*)-4-Oleoyloxy-3-phosphoryloxytetrahydropyran (8). Colorless oil; ^{1}H NMR (CD_3OD): δ 0.80 (t, *J* = 7.0 Hz, 3H), 1.15-1.30 (m, 20H), 1.48-1.60 (m, 3H), 1.85-2.05 (m, 5H), 2.27 (td, *J* = 7.5, 2.9 Hz, 2H), 3.31-3.39 (m, 1H), 3.42 (ddd, *J* = 11.9, 9.4, 2.8 Hz, 1H), 3.73 (dt, *J* = 11.9, 4.6 Hz, 1H), 3.92-3.99 (m, 1H), 4.02-4.12 (m, 1H), 4.83-4.90 (m, 1H), 5.24 (t, *J* = 3.7 Hz, 2H); ^{13}C NMR (CD_3OD): δ 14.4, 23.7, 25.9, 28.1, 30.19, 30.29, 30.33, 30.4, 30.6, 30.80, 30.84, 33.1, 35.1, 65.9, 69.5, 72.4, 73.5, 130.8, 130.9, 174.6; ^{31}P NMR (CD_3OD): δ -0.25; IR (neat, cm^{-1}): 2924, 2853, 1739; ESI-MS *m/z* 485 (M^++Na); FAB-HRMS Calcd for $C_{23}H_{44}O_7P$ (M^++H) : 463.2825, Found : 463.2835; $[\alpha]^{22}_D$-7.7 (c = 0.36, MeOH).

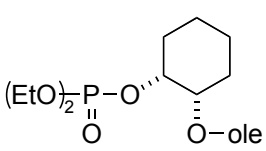


(3*S*,4*R*)-4-Oleoyloxy-3-(diethyloxyphosphoryloxy)tetrahydropyran. Colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.25-1.43 (m, 26H), 1.60-1.65 (m, 2H), 1.77-1.81 (m, 1H), 1.99-2.05 (m, 5H), 2.33-2.36 (m, 2H), 3.57 (ddd, *J* = 3.4, 8.6, 11.6 Hz, 1H), 3.66 (dd, *J* = 2.3, 12.4 Hz, 1H), 3.85-3.88 (m, 1H), 3.97 (dd, *J* = 5.5, 12.2 Hz, 1H), 4.04-4.16 (m, 4H), 4.54-4.56 (m, 1H), 5.09-5.11 (m, 1H), 5.32-5.35 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 14.1, 16.06, 16.09, 22.6, 24.8, 27.1, 27.2, 27.8, 29.1, 29.2, 29.3, 29.5, 29.67, 29.72, 31.9, 34.3, 63.9, 64.7, 67.9, 68.7, 72.1, 129.7, 130.0, 172.9; ^{31}P NMR ($CDCl_3$): δ -1.42; IR (neat, cm^{-1}): 2925, 2854, 1738; ESI-MS *m/z* 541 (M^++Na); FAB-HRMS Calcd for $C_{27}H_{52}O_7P$ (M^++H) : 519.3451, Found : 519.3456; $[\alpha]^{25}_D$+19.5 (c = 0.77, $CHCl_3$).

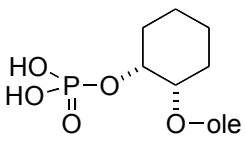


9

(3*S*,4*R*)-4-Oleoyloxy-3-phosphoryloxytetrahydropyran (9). Colorless oil; ^{1}H NMR (CD_3OD): δ 0.89 (t, *J* = 6.9 Hz, 3H), 1.28-1.32 (m, 20H), 1.59-1.62 (m, 2H), 1.75-1.78 (m, 1H), 2.00-2.02 (m, 5H), 2.36 (td, *J* = 7.5, 1.6 Hz, 2H), 3.58 (ddd, *J* = 11.6, 8.3, 3.4 Hz, 1H), 3.69 (dd, *J* = 12.0, 2.2 Hz, 1H), 3.77-3.84 (m, 1H), 3.90 (dd, *J* = 12.0, 5.4 Hz, 1H), 4.40-4.46 (m, 1H), 5.07-5.12 (m, 1H), 5.34 (m, 2H); ^{13}C NMR (CD_3OD): δ 14.4, 23.7, 25.9, 26.0, 28.1, 29.0, 30.2, 30.3, 30.4, 30.6, 30.75, 30.82, 30.85, 33.1, 35.1, 65.5, 68.8, 70.5, 72.3, 130.8, 130.9, 178.1; ^{31}P NMR (CD_3OD): δ -0.35; IR (neat, cm^{-1}): 2924, 2853, 1737; ESI-MS *m/z* 485 (M^++Na); FAB-HRMS Calcd for $C_{23}H_{44}O_7P$ (M^++H) : 463.2825, Found : 463.2826; $[\alpha]^{22}_D$+16.9 (c = 0.52, MeOH).



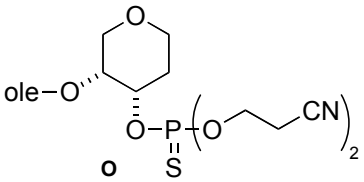
***cis*-2-Oleoyloxy-1-(diethyloxyphosphoryloxy)cyclohexane.** Colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.20-1.45 (m, 22H), 1.53-1.74 (m, 8H), 1.81-1.89 (m, 1H), 1.94-2.05 (m, 5H), 2.32 (dt, *J* = 1.4, 7.5 Hz, 2H), 4.05-4.15 (m, 4H), 4.53-4.60 (m, 1H), 4.93-5.00 (m, 1H), 5.26-5.40 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 14.0, 16.0, 22.6, 24.8, 27.1, 29.0, 29.1, 29.2, 29.3, 29.4, 29.6, 29.7, 31.8, 34.4, 63.5, 63.6, 71.4, 75.5, 129.6, 129.9, 173.0; ^{31}P NMR ($CDCl_3$): δ -1.05.



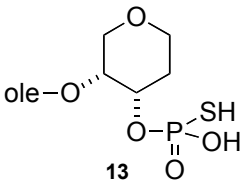
10

***cis*-2-Oleoyloxy-1-phosphoryloxycyclohexane (10).** Colorless oil; ^{1}H NMR (CD_3OD): δ 0.90 (t, *J* = 7.0 Hz, 3H), 1.20-1.50 (m, 22H), 1.55-1.80 (m, 6H), 1.84-1.93 (m, 1H), 1.95-2.10 (m, 5H), 2.35 (t, *J* = 7.5 Hz, 2H), 4.43-4.52 (m, 1H), 4.91-4.99 (m, 1H); ^{13}C NMR (CD_3OD): δ 14.5, 22.2, 22.9, 23.7, 26.0, 28.2, 30.19,

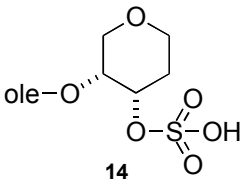
30.26, 30.31, 30.34, 30.5, 30.6, 30.81, 30.85, 33.1, 35.3, 73.3, 75.7, 130.8, 130.9, 175.0 ^{31}P NMR (CD_3OD): δ -0.55.



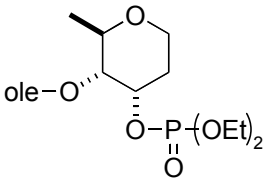
(3*R*,4*S*)-3-Oleoyloxy-4-(bis(2-cyanoethoxy)thiophosphoryloxy) tetrahydropyran (O). Colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.88 (t, *J* = 6.9 Hz, 3H), 1.20-1.37 (m, 20H), 1.60-1.68 (m, 2H), 1.90-1.98 (m, 1H), 1.99-2.05 (m, 4H), 2.12-2.20 (m, 1H), 2.39 (ddd, *J* = 2.9, 7.5, 7.5 Hz, 2H), 2.77 (t, *J* = 6.3 Hz, 4H), 3.55-3.65 (m, 2H), 3.85 (dd, *J* = 5.2, 12.2 Hz, 1H), 3.89-3.95 (m, 1H), 4.23-4.35 (m, 4H), 4.80-4.87 (m, 1H), 5.08-5.13 (m, 1H), 5.29-5.39 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 14.0, 19.3, 22.5, 24.8, 27.1, 28.4, 28.9, 29.3, 29.5, 29.6, 29.7, 31.8, 34.2, 62.5, 64.4, 65.2, 66.9, 67.9, 116.3, 129.6, 129.9, 172.9; ^{31}P NMR ($CDCl_3$): δ 66.45; IR (neat, cm^{-1}): 2925, 2854, 1736; ESI-MS *m/z* 607 (M^++Na); FAB-HRMS Calcd for $C_{29}H_{50}O_6N_2PS$ (M^++H) : 585.3127, Found : 585.3136; $[\alpha]^{22}_D$-21.3 (c = 0.8, $CHCl_3$).



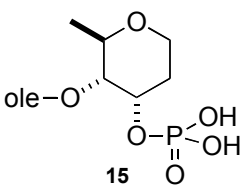
(3*R*,4*S*)-3-Oleoyloxy-4-thiophosphoryloxytetrahydropyran (13). Colorless oil; ^{1}H NMR (CD_3OD): δ 0.90 (t, *J* = 7.1 Hz, 3H), 1.29-1.34 (m, 20H), 1.56-1.70 (m, 2H), 1.87-1.95 (m, 1H), 2.00-2.08 (m, 2H), 2.09-2.17 (m, 1H), 2.32-2.43 (m, 2H), 3.55-3.64 (m, 2H), 3.81-3.91 (m, 2H), 4.73-4.80 (m, 1H), 4.96-5.02 (m, 1H), 5.30-5.40 (m, 2H); ^{13}C NMR (CD_3OD): δ 14.6, 23.9, 26.1, 28.3, 30.3, 30.5, 30.6, 30.8, 31.0, 33.2, 35.3, 65.7, 67.8, 70.6, 73.3, 131.0, 175.0; ^{31}P NMR (CD_3OD): δ 61.81; IR (neat, cm^{-1}): 2924, 2853, 2358, 1716; ESI-MS *m/z* 501 (M^++Na); FAB-HRMS Calcd for $C_{23}H_{44}O_6PS$ (M^++H) : 479.2596, Found : 479.2591; $[\alpha]^{21}_D$6.25 (c = 0.16, MeOH).



(3*R*,4*S*)-3-Oleoyloxy-4-(hydroxysulfonyloxy)tetrahydropyran (14). Colorless oil; ^{1}H NMR (CD_3OD): δ 0.89 (t, *J* = 7.0 Hz, 3H), 1.29-1.32 (m, 16H), 1.59-1.65 (m, 2H), 1.89-1.94 (m, 1H), 2.00-2.04 (m, 4H), 2.10-2.18 (m 1H), 2.37 (td, *J* = 7.4, 3.0 Hz, 2H), 3.30 (q, *J* = 1.5 Hz, 2H), 3.53-3.59 (m, 2H), 3.85-3.91 (m, 2H), 4.61 (td, *J* = 7.3, 4.0 Hz, 1H), 5.04-5.05 (m, 1H), 5.32-5.34 (m, 2H) ^{13}C NMR (CD_3OD): δ 9.2, 14.4, 23.7, 25.9, 28.1, 30.1, 30.2, 30.3, 30.4, 30.6, 30.8, 33.1, 35.1, 48.0, 66.1, 68.1, 70.5, 74.0, 130.83, 130.85, 174.9; IR (neat, cm^{-1}): 2926, 2854, 1730, 1646; ESI-MS *m/z* 485 (M^++Na); FAB-HRMS Calcd for $C_{23}H_{43}O_7S$ (M^++H) : 463.2729, Found : 463.2718; $[\alpha]^{25}_D$-25.2 (c = 1.68, MeOH).



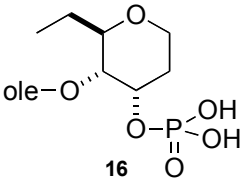
(2*R*,3*R*,4*S*)-2-Methyl-3-oleoyloxy-4-(diethyloxyphosphoryloxy)tetrahydro-pyran. Colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.86 (t, *J* = 6.9 Hz, 3H), 1.14 (d, *J* = 6.3 Hz, 3H), 1.20-1.35 (m, 26H), 1.51-1.65 (m, 2H), 1.93-2.05 (m, 6H), 2.32 (dt, *J* = 2.1, 7.4 Hz, 2H), 3.72-3.78 (m, 1H), 3.82 (dq, *J* = 6.3, 9.8 Hz, 1H), 4.03-4.14 (m, 4H), 4.47-4.51 (m, 1H), 4.83-4.88 (m, 1H), 5.28-5.42 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 14.2, 16.2, 16.3, 18.0, 22.8, 24.9, 27.27, 27.32, 29.2, 29.27, 29.32, 29.4, 29.6, 29.8, 29.9, 31.8, 32.0, 34.4, 59.5, 61.8, 63.9, 64.0, 69.8, 72.2, 73.4, 129.8, 130.1, 173.0; ^{31}P NMR ($CDCl_3$): δ -1.33; IR (neat, cm^{-1}): 2925, 2854, 1743; ESI-MS *m/z* 555 (M^++Na); FAB-HRMS Calcd for $C_{27}H_{52}O_7P$ (M^++H) : 533.3607, Found : 533.3602; $[\alpha]^{23}_D$+55.7 (c = 0.24, $CHCl_3$).



(2*R*,3*R*,4*S*)-2-Methyl-3-oleoyloxy-4-phosphoryloxytetrahydropyran (15). Colorless oil; ^{1}H NMR (CD_3OD): δ 0.90 (t, *J* = 7.0 Hz, 3H), 1.01 (d, *J* = 6.4 Hz, 3H), 1.25-1.39 (m, 20H), 1.58-1.66 (m, 2H), 1.94-2.09 (m, 6H), 2.37 (t, *J* = 7.5 Hz, 2H), 3.73 (ddd, *J* = 11.6, 5.2, 3.1 Hz, 1H), 3.81 (td, *J* = 11.6, 2.8 Hz, 1H), 3.87 (m, 1H), 4.43 (dt, *J* = 9.8, 2.4 Hz, 1H), 4.70-4.80 (m, 1H), 5.34 (t, *J* = 4.6 Hz, 2H); ^{13}C NMR (CD_3OD): δ 14.4, 18.3, 23.7, 25.8, 28.1, 30.18, 30.20, 30.29, 30.33, 30.4, 30.6, 30.78, 30.83, 32.7, 33.0, 35.0, 62.7, 70.9, 72.1, 75.1, 130.8, 130.9, 174.6; ^{31}P NMR (CD_3OD): δ -0.53; IR (neat, cm^{-1}): 2924, 2853, 1742; ESI-MS *m/z* 499 (M^++Na); FAB-HRMS Calcd for $C_{24}H_{46}O_7P$ (M^++H) : 477.2981, Found : 477.2977;

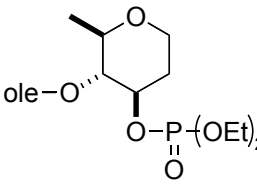
$[\alpha]^{22}_{D}$+54.4 (c = 0.99, MeOH).

(2*R*,3*R*,4*S*)-2-Ethyl-3-oleoyloxy-4-(diethyloxyphosphoryloxy)tetrahydro-pyran. Colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.88 (t, *J* = 6.9 Hz, 3H), 0.95 (d, *J* = 7.5 Hz, 3H), 1.27-1.40 (m, 20H), 1.64 (m, 4H), 2.00 (m, 6H), 2.34 (dt, J = 2.8, 7.8 Hz, 2H), 3.64 (m, 1H), 3.78 (m, 2H), 4.11 (m, 4H), 4.59 (m, 1H), 4.87 (m, 1H), 5.34 (m, 2H).

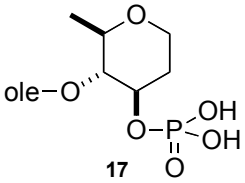


16

(2*R*,3*R*,4*S*)-2-Methyl-3-oleoyloxy-4-phosphoryloxytetrahydropyran (16). Colorless oil; ^{1}H NMR (CD_3OD): δ 0.89 (t, *J* = 7.0 Hz, 3H), 0.93 (d, *J* = 7.6 Hz, 3H), 1.25-1.37 (m, 20H), 1.61 (m, 2H), 2.02 (m, 4H), 2.36 (t, J = 7.3 Hz, 2H), 3.65 (m, 1H), 3.76 (m, 2H), 4.53 (m, 1H), 4.77 (m, 1H), 5.34 (m, 2H).



(2*R*,3*R*,4*R*)-2-Methyl-3-oleoyloxy-4-(diethyloxyphosphoryloxy)tetrahydro-pyran. Colorless oil; ^{1}H NMR ($CDCl_3$): δ 0.89 (t, *J* = 7.1 Hz, 3H), 1.12 (d, *J* = 6.1 Hz, 3H), 1.22-1.40 (m, 20H), 1.55-1.65 (m, 2H), 1.82 (dddd, *J* = 5.2, 12.8, 12.8, 12.8 Hz, 1H), 1.95-2.08 (m, 4H), 2.20 (m, 1H), 2.31-2.42 (m, 2H), 3.38 (dq, *J* = 6.1, 9.5 Hz, 1H), 3.46 (ddd, *J* = 1.7, 12.2, 12.8 Hz, 1H), 3.90 (ddd, *J* = 1.6, 5.2, 12.2 Hz, 1H), 4.32 (m, 1H), 4.62 (dd, *J* = 9.4, 9.4 Hz, 1H), 5.33 (t, *J* = 4.6 Hz, 2H); ^{13}C NMR ($CDCl_3$): δ 14.0, 17.8, 22.6, 24.7, 27.07, 27.13, 29.0, 29.1, 29.2, 29.4, 29.6, 29.7, 31.8, 33.0, 34.2, 63.7, 63.8, 64.9, 74.6, 74.8, 76.4, 129.6, 129.9, 172.8; ^{31}P NMR ($CDCl_3$): δ -0.93; IR (neat, cm^{-1}): 2926, 2854, 1746; ESI-MS *m/z* 555 (M^++Na); FAB-HRMS Calcd for $C_{27}H_{52}O_7P$ (M^++H) : 533.3607, Found : 533.3602; $[\alpha]^{24}_{D}$+3.6 (c = 1.24, $CHCl_3$).



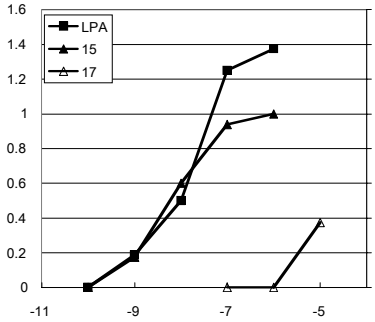
17

(2*R*,3*R*,4*R*)-2-Methyl-3-oleoyloxy-4-phosphoryloxytetrahydropyran (17). Colorless oil; ^{1}H NMR (CD_3OD): δ 0.89 (t, *J* = 6.9 Hz, 3H), 1.20-1.48 (m, 22H), 1.52-1.74 (m, 8H), 1.81-1.89 (m, 1H), 1.93-2.05 (m, 5H), 2.32 (dt, *J* = 1.4, 7.5 Hz, 2H), 4.05-4.15 (m, 4H), 4.52-4.63 (m, 1H), 4.90-5.00 (m, 2H); ^{13}C NMR (CD_3OD): δ 1.45, 18.4, 23.7, 25.8, 28.09, 28.12, 30.19, 30.25, 30.30, 30.34, 30.4, 30.6, 30.81, 30.84, 33.1, 34.4, 35.1, 75.9, 76.7, 77.0, 130.8, 130.9, 174.7; ^{31}P NMR (CD_3OD): δ -1.05; IR (neat, cm^{-1}): 2925, 2854, 1744; ESI-MS *m/z* 499 (M^++Na); FAB-HRMS Calcd for $C_{24}H_{46}O_7P$ (M^++H) : 477.2981, Found : 477.2974; $[\alpha]^{22}_{D}$+25.0 (c = 0.12, MeOH).

Biologic Assays

Assays were performed at least three times, and either representative data or data with error bars were shown.

Ca^{2+} Assay (for LPA_2 and LPA_3 agonist activity).[2] Sf9 cells were harvested for 2 days after baculovirus infection, washed gently with an HBS buffer (20 mM Hepes, pH 7.4, containing 120 mM NaCl, 4.7 mM KCl, 1.2 mM $MgCl_2$, 1.25 mM $CaCl_2$, 1.2 mM KH_2PO_4, and 10 mM glucose), and loaded with 2 μM Fura-2 acetoxymethyl ester (Fura-2 AM; Molecular Probes Inc., Eugene, OR) for 1 h. Free Fura-2 AM was washed out, and the cells were resuspended in the HBS buffer to produce a concentration of 10^6 cells/ml. Agonist-induced Fura-2 AM fluorescence of samples in quartz cuvettes kept at 27 °C was monitored at excitation wavelengths of 340 nm and 380 nm and an emission wavelength of 300 nm using a CAF-110 spectrofluorimeter (Japan Spectroscopy, Inc., Tokyo, Japan). Fluorescence was recorded before and after addition of LPA and selected compounds, which were dissolved in phosphate-buffered saline with 0.01% (w/v) of fatty acid-free bovine serum albumin (Sigma). LPA_3



agonist activities of **2**–**9** and **13** are reported in Figure 2 in the text. LPA_3 agonist activities of **10**–**12** and **14**–**17** are described here (Figure 1). No response was observed till 10^4 nM for compounds **10**–**12**, **14**, and **16** (not included in Figure 1). All the compounds did not have any LPA_2 agonist activities. Wild-type Sf9 cells did not show any Ca^{2+} responses to 1-oleoyl LPA and the agonists, which eliminates the effect of unknown receptors.

Migration Assay (for LPA_1 agonist activity).[3] MDA cells were maintained in RPMI 1640 with 5% heat-inactivated FBS at 27 °C at 5% CO_2. Polycarbonate filters with 8-μm pores (Neuro Probe, Inc.) were coated with 13.3 mg/ml fibronectin (Sigma-Aldrich) in PBS for 60 min. A dry coated filter was placed on a 96-blind well chamber (Neuro Prove, Inc.) containing the indicated amounts of LPA (18:1; Avanti Polar Lipids Inc.) or selected compounds, and cells (200 μL 8x10^4 per well) were added to the top wells. The ligand solution and cell suspension were prepared in the same buffer (serum-free RPMI 1640 medium containing 0.1 % BSA). The cells on the filter were fixed with methanol and stained with Diff-Quick staining kit (International Reagents Corp.). The top side of the filter was scraped free of cells. The number of cells that migrated to the bottom side was determined by measuring optical densities at 590 nm using a 96-well microplate reader (model 3550; Bio-Rad Laboratories). When LPA or selected compounds were added to the cells, they were suspended in serum-free media containing 0.1% BSA. LPA_1-agonist activities of **2**–**8** and **15** using MDA cells are shown in Figure 2(a). Compound **15** had ca, 10-times stronger agonist activity than 1-oleoyl LPA when PC3 cells were used (Figure 2(b)). The estimated EC50 values were summarized in Table 1.

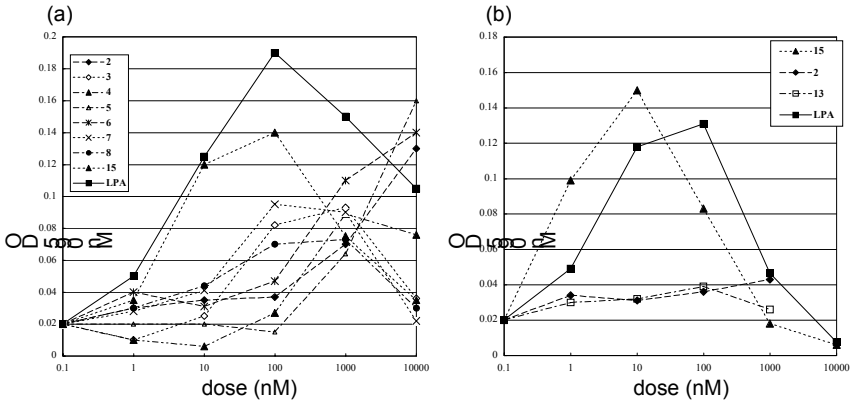
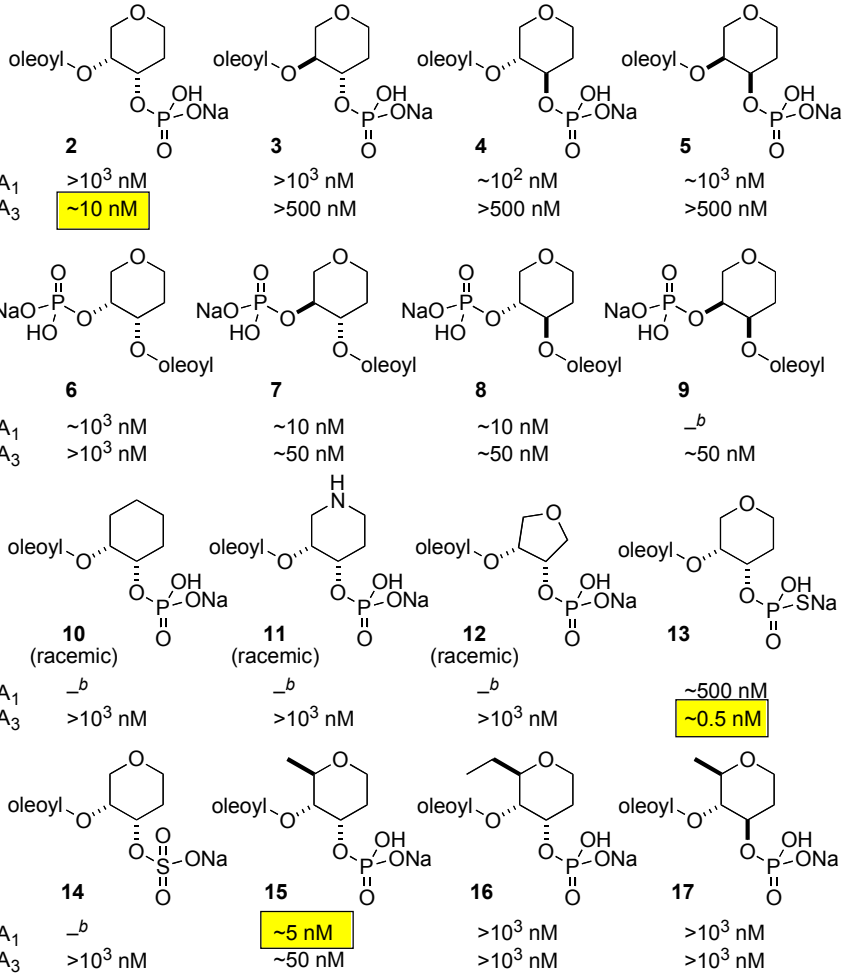


Figure 2. LPA_1 agonist activities. (a) Using MDA cells. Results of other compounds are reported in the text. (b) Using PC3 cells.

Table. Estimated EC_{50} values[a]



	2	**3**	**4**	**5**
LPA_1	>10^3 nM	>10^3 nM	~10^2 nM	~10^3 nM
LPA_3	~10 nM	>500 nM	>500 nM	>500 nM

	6	**7**	**8**	**9**
LPA_1	~10^3 nM	~10 nM	~10 nM	–[b]
LPA_3	>10^3 nM	~50 nM	~50 nM	~50 nM

	10 (racemic)	**11** (racemic)	**12** (racemic)	**13**
LPA_1	–[b]	–[b]	–[b]	~500 nM
LPA_3	>10^3 nM	>10^3 nM	>10^3 nM	~0.5 nM

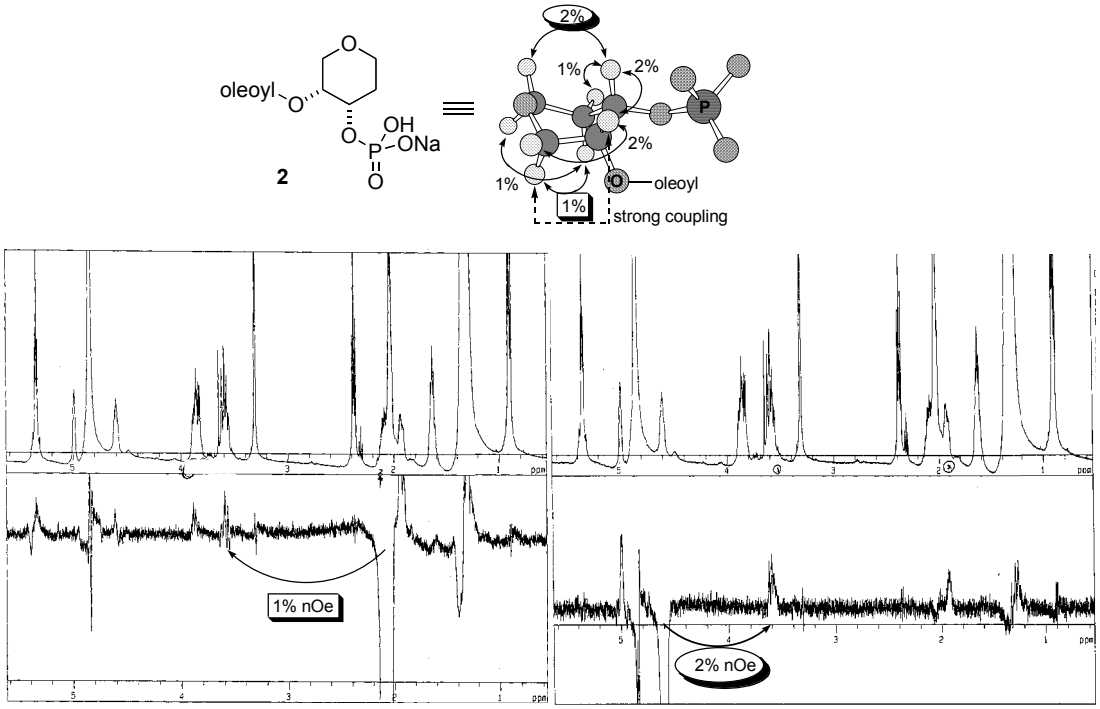
	14	**15**	**16**	**17**
LPA_1	–[b]	~5 nM	>10^3 nM	>10^3 nM
LPA_3	>10^3 nM	~50 nM	>10^3 nM	>10^3 nM

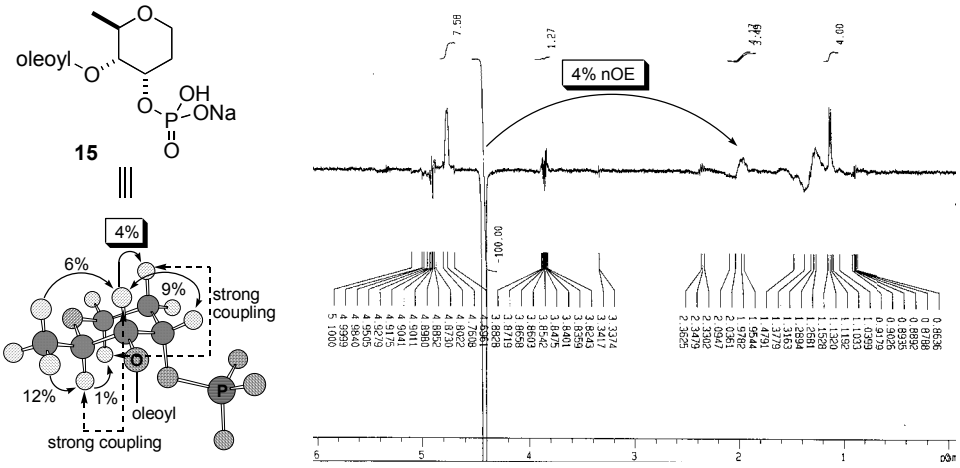
[a] 1-Oleoyl LPA (standard) showed EC_{50} ~ 10 nM to LPA_1, and EC_{50} ~ 100 nM to LPA_3.
[b] Not investigated.

Inhibitory assay (for LPA_3, data shown in the text). Inhibitory activity was assessed basically using the same procedures as described in Ca^{2+} assay for LPA_3 agonist activity, except that the inhibitor **14** (10 μM) was prescribed prior to the addition of the activators (1-oleoyl LPA: 1 μM and **13**: 10 nM).

Determination of the Ring Conformation of 2 and 15

The conformations of **2** and **15** were determined based on nOe experiments (1D and 2D) and coupling constants observed in 1D and 2D (COSY) NMR. The key nOe data are shown below.





References:

1. Masumoto, S.; Yabu, K.; Kanai, M.; Shibasaki, M. *Tetrahedron Lett*. **2002**, *43*, 2919.

2. Bandoh, K.; Aoki, J.; Hosono, H.; Kobayashi, S.; Kobayashi, T.; Murakami-Murofushi, K.; Tsujimoto, M.; Arai, H.; Inoue, K. *J. Biol. Chem.* **1999**, *274*, 27776.

3. Umezu-Goto, M.; Kishi, Y.; Taira, A.; Hama, K.; Dohmae, N.; Takio, K.; Yamori, T.; Mills, G. B.; Inoue, K.; Aoki, J.; Arai, H. *J. Cell Biol.* **2002**, *158*, 227.