Supplementary Information for:

New fluorescent probes reveal that flippase-mediated flip-flop of phosphatidylinositol across the endoplasmic reticulum membrane does *not* depend on the stereochemistry of the lipid

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General Procedures

Solvents were purified according to standard procedures, and reagents used were of the highest purity available. NMR measurements (^{1}H, ^{13}C, ^{31}P, 2D COSY) were recorded on a 300 MHz spectrometer fitted with pulse-field gradient probe; trimethylsilane (TMS) or residual resonance of deuterated solvent were used as internal reference. For ^{31}P NMR spectra, phosphoric acid was used as external reference. ^{13}C NMR spectra were broadband ^{1}H decoupled or inverse HMQC experiments. Chemical shifts are expressed in ppm and coupling constants *J* in Hz. ^{1}H and ^{13}C assignments were made by ^{1}H-^{1}H COSY and ^{1}H-^{13}C HETCOR analysis. Electrospray ionisation mass-spectra (ESMS) and High-resolution mass-spectra (HRMS) were obtained on quadrupole and LCT-TOF (time of flight) spectrometers respectively, using acetonitrile-water (1:1) mobile

phase. Optical rotations were measured on a digital polarimeter. TLC was performed on Merck Kieselgel 60 F_{254} plates, and compounds visualized by ammonium-molybdate/ceric-sulfate developing reagent. Preparative TLC was conducted on Analtech Uniplate silica-gel plates (20 x 20 cm). Silica column chromatography was carried out with silica gel 60 (60-120 mesh). Analytical and semi-preparative HPLC purification were carried out on a Shimadzu system using Phenomenex RP-18 columns (analytical column 4.6x250 mm; semi-preparative column 10x250 mm) and an acetonitrile-water gradient system (0-100% acetonitrile in water in a total run time of 60 min using a flow rate of 1 mL/min (analytical) or 3 mL/min (semi-preparative); compounds were detected at 465 nm using an in-line photodiode array detector). Egg phosphatidylcholine (ePC) and fatty acid-free bovine serum albumin were obtained from Sigma Chemical Co. 1-Myristoyl-2-C_6-NBD-phosphatidylcholine was obtained from Avanti Polar Lipids. Salt-washed rat liver ER (SWER) was prepared as described[1] and stored frozen at -80°C at a concentration of ~20 mg/ml protein (corresponding to ~5.4 µmol/ml phospholipid). A Triton X-100 extract (TE) of SWER was prepared as described,[1] snap-frozen in aliquots and stored at -80°C. TE contained ER membrane proteins in 10 mM Hepes/NaOH, pH 7.5, 100 mM NaCl, 1% (w/v) Triton X-100.

D-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (11) and L-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (12). The two optically pure chiral antipodes were prepared by following steps.

(a) DL-2,3,4,5,6-penta-O-benzyl-myo-inositol (**8**). The starting material for this synthesis, racemic 3,4,5,6-tetra-O-benzyl-*myo*-inositol (**7**), was prepared in three steps from commercially available *myo*-inositol by a reported method.[2] In the next step, a mixture of compound **7** (27 g, 50 mmol), dibutyl-tin-oxide (13 g, 53 mmol) in toluene (200 mL) was heated to reflux for 1 h

with continuous azeotropic removal of water. The solvent was evaporated and the residue was suspended in anhyd DMF (100 mL) and allyl bromide (7 mL, 81 mmol) was added. The reaction was heated at 80°C for 4 h, after which it was concentrated and the residue was dissolved in diethyl ether (100 mL). The ether layer was washed with saturated aqueous $NaHCO_3$ (100 mL) and brine (100 mL). The organic layer was filtered through celite and the filtrate was concentrated. The residue was subjected to flash column chromatography (hexane-CH_2Cl_2; 4:10) to obtain recemic 1-*O*-allyl-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol (12.5 g) and un-reacted starting material (10 g). In the next step, this intermediate (11 g, 18 mmol) was dissolved in anhyd DMF (100 mL) and was treated with NaH (1.66 g, 38 mmol) and benzyl bromide (4.5 mL, 38 mmol) at 0°C and the reaction mixture was stirred overnight at room temperature. The excess of NaH was destroyed by treatment with MeOH (5 mL), diluted with water (200 mL) and the product was extracted into ethyl acetate (3 x 100 mL). The organic extract was dried (Na_2SO_4), concentrated, and product purified by silica column to give racemic-1-*O*-allyl-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (13 g). This compound (13 g) was dissolved in ethanol (250 mL) and 5% palladium-charcoal (1.6 g), p-toluene sulfonic acid (1.3 g), water (12 mL) were added. The reaction mixture was refluxed for two hours and then quenched with $NaHCO_3$ (1 g). The mixture was cooled, filtered through celite and solvent evaporated. The residue was crystallized from hexane to provide racemic 2,3,4,5,6-penta-*O*-benzyl-myo-inositol **8** (11.5 g, 74%). δ_H (300 MHz, $CDCl_3$): 2.21 (1 H, d, *J* 6.3, OH), 3.44 (3 H, m, H-1, 3, 5), 3.81 (2 H, dd, *J* 9.5, H-4), 4.03 (1 H, dd, *J* 2, H-2), 4.06 (1 H, dd, J 10, H-6), 5.02-4.69 (10 H, m, 5 x O*CH*$_2$Ph), 7.24-7.34 (25 H, m, 5 x Ph); δ_C (75 MHz, $CDCl_3$): 71.29, 71.51, 74.02, 74.13, 74.52, 74.62, 78.27, 80.09, 81.37, 81.95, 82.73, 127.08-128.15 (m), 138.69, 138.87, 139.22, 139.41; MS (positive ion ESMS, M+Na^+) calcd for $C_{41}H_{42}O_6Na$ 653.2879, found 653.2885.

(b) D-1-O-[(1S)-(-)-camphanoyl]-2,3,4,5,6-penta-O-benzyl-myo-inositol (**9**) *and L - 1-O-[(1S)-(-)-camphanoyl]-2,3,4,5,6-penta-O-benzyl-myo-inositol* (**10**). To a cooled solution of racemic 2,3,4,5,6-penta-O-benzyl-myo-inositol **8** (8 g, 12.68 mmol) in anyd pyridine (64 mL) was added (1S)-(-)-camphanic chloride (3.45 g, 16 mmol). After stirring for 16 h at 20°C, the reaction was quenched with MeOH (4 mL) and the mixture was concentrated under reduced pressure. The residue was taken in CH_2Cl_2 (100 mL), washed with water (25 mL), 1M $NaHCO_3$ (25 mL) and water (25 mL). The organic layer was dried, concentrated and the residue was purified on a flash silica column (hexane-CH_2Cl_2; 10:90 to 0:100) to provide 4.0 g (36% yield) of optically pure D-1-*O*-[(1S)-(-)-camphanoyl]-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**9**). TLC, Rf = 0.23 (hexane-ether, 1:1); $[\alpha]^{20}_D$ = -18 (c = 1, $CHCl_3$). δ_H (300 MHz, $CDCl_3$): 0.83 (3 H, s, camph CH_3), 0.96 (3 H, s, camph CH_3), 1.07 (3 H, s, camph CH_3), 1.61-1.70 (2 H, m, camph CH_2), 1.80-1.95 (2 H, m, camph CH_2), 2.23-2.32 (1 H, m, camph CH), 3.57 (1 H, dd, J 9.5, H-3, 5), 4.11 (1 H, dd, J 9.5, H-4), 4.17 (1 H, dd, J 6.1, H-6), 4.21 (1 H, m, H-2), 4.64-4.87 (10 H, m, 5 x *CH*$_2$Ph), 4.90 (1 H, m, H-1), 7.25-7.39 (25 H, m, 5 x Ph); δ_C (75 MHz, $CDCl_3$): 9.52, 16.47, 28.79, 30.81, 53.95, 54.63, 72.94, 74.58, 75.74, 75.13, 78.81, 80.85, 81.29, 83.42, 90.69, 127.08-128.30, 137.88, 138.17, 138.26, 138.50, 167.29, 177.71; MS (positive ion ESMS, $M+Na^+$) calcd for $C_{51}H_{54}O_9Na$ 833.3666, found 833.3675, and the L-diastereoisomer, L-1-*O*-[(1S)-(-)-camphanoyl]-2,3,4,5,6-penta-*O*-benzyl-*myo*-inositol (**10**), TLC, Rf = 0.24 (hexane-ether, 1:1); $[\alpha]^{20}_D$ = +11.8 (c = 1, $CHCl_3$). δ_H (300 MHz, $CDCl_3$): 0.90 (3 H, s, camph CH_3), 1.00 (3 H, s, camph CH_3), 1.08 (3 H, s, camph CH_3), 1.60-1.67 (1 H, m, camph CH), 1.76-1.90 (2 H, m, camph CH_2), 2.24-2.33 (1 H, m), 3.57 (2 H, dd, J 9.5, H-3, 5), 4.10 (1 H, dd, J 9.5, H-4), 4.13 (1 H, dd, J 2.4, H-2), 4.17 (1 H, m, H-6), 4.64-4.94 (10 H, m, 5 *CH*$_2$Ph), 4.98 (1 H, dd, H-1), 7.19-7.40 (25 H, m, 5 x Ph); δ_C (75 MHz, $CDCl_3$): 9.46, 16.38, 16.53, 28.62, 30.52, 53.96, 54.52,

72.77, 74.78, 74.99, 75.69, 75.74, 78.90, 80.65, 81.23, 83.19, 90.58, 127.02-128.19, 137.85, 138.09, 138.17, 138.38, 167.05, 177.68; MS (positive ion ESMS, $M+Na^+$) calcd for $C_{51}H_{54}O_9Na$ 833.3666, found 833. 3677.

(c) D-2,3,4,5,6-penta-O-benzyl-myo-inositol (**11**). The D-camphanate **9** (560 mg, 0.68 mmol) was treated with 2% NaOH solution in MeOH (100 mL) at 60-65°C for 30 min. The mixture was cooled, methanol evaporated and the residue dissolved in CH_2Cl_2 (100 mL), washed with water. The organic layer was dried (Na_2SO_4), concentrated and product was purified by silica chromatography to give optically pure **11** (430 mg, 99%). Rf = 0.27 (hexane-ether, 1:1) $[\alpha]^{20}_D$ = -9 (c = 1 $CHCl_3$). δ_H (300 MHz, $CDCl_3$): 2.20 (1 H, d, OH), 3.46 (1 H, dd, J 9.5, H-3), 3.48 (2 H, m, H-1, 5), 3.80 (1 H, dd, J 9.5, H-6), 4.02 (1 H, dd, J 2.5, H-2), 4.06 (1 H, dd, J 9.5, H-4), 4.69-5.01 (10 H, m, 5 x CH_2Ph), 7.24-7.36 (25 H, m, 5 x Ph); δ_C (75 MHz, $CDCl_3$): 72.33, 72.88, 74.66, 75.42, 75.66, 75.77, 77.06, 81.03, 81.82, 82.08, 83.54, 127.52-128.48, 138.17, 138.58, 138.67. MS (positive ion ESMS, $M+Na^+$) calcd for $C_{41}H_{42}O_6Na$ 653.2879, found 653.2777.

(d) L-2,3,4,5,6-penta-O-benzyl-myo-inositol (**12**). The L-camphanate **10** (560 mg, 0.68 mmol) was treated with 2% NaOH solution in MeOH (100 mL) at 60-65°C for 30 min. The mixture was cooled, methanol evaporated and the residue dissolved in CH_2Cl_2 (100 mL), washed with water. The organic layer was dried (Na_2SO_4), concentrated and product was purified by silica chromatography to give optically pure **12** (430 mg, 99%). Rf = 0.28 (hexane-ether, 1:1) $[\alpha]^{20}_D$ = +9.2 (c = 1 $CHCl_3$). δ_H (300 MHz, $CDCl_3$): 2.22 (1 H, d, OH), 3.46 (1 H, dd, J 9.5, H-3), 3.48 (2 H, m, H-1, 5), 3.81 (1 H, dd, J 9.5, H-6), 4.03 (1 H, dd, J 2.5, H-2), 4.06 (1 H, dd, J 9.5, H-4), 4.69-5.01 (10 H, m, 5 x CH_2Ph), 7.24-7.36 (25 H, m, 5 x Ph); δ_C (75 MHz, $CDCl_3$): 72.33, 72.88, 74.66, 75.42, 75.66, 75.77, 77.06, 81.03, 81.82, 82.08, 83.54, 127.52-128.48, 138.17, 138.58, 138.67. MS (positive ion ESMS, $M+Na^+$) calcd for $C_{41}H_{42}O_6Na$ 653.2879, found 653.2890.

2-*O*-octadecanoyl-1-*O*-[6-(N-carbobenzyloxyamino)-hexanoyl]-sn-glyceryl-H-phosphonate (17). This lipid intermediate was prepared in 4 steps.

(a) *3-(p-methoxybenzyl)-sn-glycerol* (**14**). The commercially available chiral D-1,2-*O*-isopropylidene-*sn*-glycerol (**13**, 1S-(+)-2,2-dimethyl-1,3-dioxolane-4-methanol, 2 g, 15.2 mmol), $[\alpha]^{20}_D$ = +13.5 (neat), dissolved in anhyd DMF (50 mL) was treated with NaH (910 mg, 22 mmol) and PMBCl (2.36 g, 18 mmol) and the mixture was stirred for 1h. The reaction was quenched with MeOH (2 mL) and diluted with CH_2Cl_2 (100 mL). The organic layer was washed with brine, dried (Na_2SO_4) and concentrated. The residue was taken in MeOH (40 mL) and treated with pTSA (160 mg) and stirred for 2 h at room tempearure. The reaction was neutralized by the addition of solid $NaHCO_3$ (200 mg), solvent evaporated and residue purified by a silica column (50% to 80% EtOAc-hexane) to give desired compound **14** (2.4 g); $[\alpha]^{20}_D$ = -0.92 (c = 5, MeOH). The ^1H and ^{13}C NMR spectra were identical to the reported data.[3] δ_H (300 MHz, $CDCl_3$): 7.23 (2 H, d, PMB), 6.88 (2 H, d, PMB), 4.48 (2 H, s, O*CH*$_2$Ph), 3.85 (1 H, brs, H-2), 3.80 (3 H, s, OCH_3), 3.64-3.45 (4 H, m, H_2-1, H_2-3); δ_C (75 MHz, $CDCl_3$): 129.5, 113.8, 73.18, 71.45, 70.45, 64.03, 55.18. ESMS: 212.30 (M^+) for $C_{11}H_{16}O_4$.

(b) *1-O-[6-(N-carbobenzyloxyamino)-hexanoyl]-2-O-octadecanoyl-3-O-(p-methoxybenzyl)-sn-glycerol* (**15**). To a solution of 3-*O*-(p-methoxybenzyl)-*sn*-glycerol (1.54 g, 7.28 mmol) and 6-(*N*-carbobenzyloxyamino)-hexanoic acid (1.8 g, 7 mmol) in anhyd CH_2Cl_2 (100 mL) at 0°C was added DCC (1.8 g, 8.6 mmol) and DMAP (1 g). The reaction mixture was stirred at 0°C for 24 h. Now the temperature was raised to 30°C and additional DCC (400 mg) was added followed by addition of stearic acid (octadecanoic acid, 1.2 g, 4.4 mmol). The reaction was stirred further overnight at 30°C, concentrated and redissolved in anhyd EtOAc. The dicyclohexylurea (DCU) byproduct was removed by filtration and solution concentrated. The residue was purified on a

silica column (25% EtOAc-CH_2Cl_2) to give desired product (1.5 g, 55% yield). δ_H (300 MHz, $CDCl_3$): 7.29 (5 H, m, Ph), 7.19, 7.15, 6.82, 6.79 (4 H, 4s, PMB), 5.15 (1 H, m, H-2), 5.02 (2 H, s, O*CH*$_2$Ph), 4.83 (1 H, brs, NH), 4.40 (2 H, dd, J 11.8, OCH_2), 4.26 (1 H, dd, J 3.7 and 11.8, H-1), 4.12 (1 H, m, H-1), 3.73 (3 H, s, OCH_3), 3.49 (2 H, d, J 5, H-3), 3.12 (2 H, m, CH_2N), 2.28 (4 H, m, 2 x CH_2CO), 1.70-1.10 (36 H, m, aliphatic), 0.84 (3 H, t, 6.2, CH_3); δ_C (75 MHz, $CDCl_3$): 173.1, 173.1, 159.3, 156.4, 136.6, 129.7, 129.3, 128.5, 128.0, 113.8, 72.9, 70.0, 67.8, 66.5, 62.8, 55.2, 40.8, 33.94, 29.5, 29.3, 29.0, 26.1, 24.9, 24.4, 22.7, 14.1; MS (positive-ion ESMS, $M+Na^+$) calcd $C_{43}H_{67}O_8NNa$ (M+Na) 748.4764, found 748.4734.

(c) *1-O-[6-(N-carbobenzyloxyamino)-hexanoyl]-2-O-octadecanoyl-sn-glycerol* (**16**). The above PMB protected glycerol intermediate (1.15 g, 1.59 mmol) was dissolved in CH_2Cl_2-H_2O (50 mL, 99:1) and treated with DDQ (750 mg) and the mixture stirred at room temperature overnight, diluted with CH_2Cl_2 (100 mL), washed three times with 10% $NaHCO_3$ solution. The organic layer was concentrated and the residue purified on a silica column to provide desired compound (900 mg, 93%). δ_H (300 MHz, $CDCl_3$): 7.30 (5 H, m, Ph), 5.23-5.10 (3 H, m, H-2 and CH_2Ph), 4.86 (1 H, brs, NH), 4.25 (1 H, dd, J 3.7 and 11.8, H-1), 4.17 (1 H, dd, J 3.7 and 11.8, H-1), 3.65 (2 H, dd, J 5.6 and 5.7, H-3), 3.14 (2 H, q, J 6.5, CH_2N), 2.30-2.25 (4 H, m, 2 x CH_2CO), 1.70-1.10 (36 H, m, aliphatic), 0.83 (3 H, t, J 6.2, CH_3); δ_C (75 MHz, $CDCl_3$): 173.4, 156.4, 156.4, 136.5, 128.5, 128.5, 128.1, 72.0, 66.6, 62.1, 61.4, 40.8, 33.9, 29.5, 29.3, 29.0, 26.1, 24.9, 24.4, 22.7, 14.1; MS (ESMS, $M+H^+$) calcd for $C_{35}H_{60}NO_7$ (M+H) 606.4370, found 606.4340.

(d) *2-O-octadecanoyl-1-O-[6-(N-carbobenzyloxyamino)-hexanoyl]-sn-glyceryl-H-phosphonate* (**17**). To a stirred solution of imidazole (42.5 mg, 0.61 mmol, dried through toluene evaporation) in anhyd toluene (0.5 mL) at 0°C was added PCl_3 (12 μL, 0.135 mmol dissolved in

125 μL toluene) followed by anhyd triethyamine (48 μL, 0.35 mmol dissolved in 125 μL toluene). The stirring was continued for 10 min at 0°C and the temperature was lowered to –5°C and then a solution of 1-*O*-[6-*(N*-carbobenzyloxyamino)-hexanoyl]-2-*O*-octadecanoyl-sn-glycerol (27 mg, 44 μmol dissolved in 0.5 mL toluene) was added dropwise. The reaction was stirred at –5°C for 2 h and then quenched with pyridine-water (2.5 mL, 4:1) and stirred further for 30 min. This was followed by addition of $CHCl_3$ (7.5 mL), washing with H_2O (2.5 mL x 3). The $CHCl_3$ layer was dried (Na_2SO_4), concentrated and the residue purified through a silica column (1% MeOH-CH_2Cl_2 to 10% MeOH-CH_2Cl_2 with 1% TEA) to provide the desired lipid H-phosphonate donor **17** (25 mg, 92%). δ_H (300 MHz, $CDCl_3$): 7.25-7.01 (5 H, m, Ph), 6.80 (1 H, d, J_{PH} 628, P-H), 5.15-4.98 (3 H, m, H-2, CH_2Ph), 4.26 (1 H, dd, J 3.6 and 11.7, H-1), 4.00 (1 H, m, H-1), 3.88 (2 H, dd, J 5.1 and 7.8, H_2-3), 3.49 (2 H, m, CH_2N), 2.18 (4 H, t, J 7.2, 2 x CH_2CO), 1.50-1.10 (36 H, m, aliphatic), 0.76 (3 H, t, J 6.2, CH_3); MS (negative ion ESMS, $M\text{-}H^-$) calcd for $C_{35}H_{59}O_9NP$ (M-H) 668.3927, found 668.3412.

2-O-octadecanoyl-3-O-[6-(N-carbobenzyloxyamino)-hexanoyl]-sn-glyceryl-H-phosphonate (**22**). This compound was prepared (Scheme-4b) from non-natural glycerol intermediate chiral L-2,3-*O*-isopropylidene-*sn*-glycerol (**18**, 1R-(-)2,2-dimethyl-1,3-dioxolane-4-methanol), $[\alpha]^{20}_D$ = -13.7 (neat), by a similar synthetic sequence as described above for preparation of H-phosphonate **17**. The spectral data of compound **22**: δ_H (300 MHz, $CDCl_3$): 7.25-7.01 (5 H, m, Ph), 6.80 (1 H, d, J_{PH} 628, P-H), 5.15-4.98 (3 H, m, H-2, CH_2Ph), 4.28 (1 H, dd, J 3.6 and 11.7, H-1), 4.02 (1 H, m, H-1), 3.86 (2 H, dd, J 5.1 and 7.8, H_2-3), 3.50 (2 H, m,, NCH_2), 2.18 (4 H, m, 2 x CH_2CO), 1.50-1.10 (36 H, m, aliphatic), 0.76 (3 H, t, J 6.2, CH_3); MS (negative ion ESMS, $M\text{-}H^-$) calcd for $C_{35}H_{59}O_9NP$ (M-H) 668.3927, found 668.3415.

1-*O*-myristoyl-2-*O*-[6-(*N*-carbobenzyloxyamino)-hexanoyl]-sn-glyceryl-H-phosphonate (**27**). This lipid intermediate was prepared in 4 steps as described below

(a) *1-O-myristoyl-2-O-[6-(N-carbobenzyloxyamino)-hexanoyl]-3-O-(p-methoxybenzyl)-sn-glycerol* (**25**). To a stirred solution of 3-*O*-(4-methoxybenzyl)-*sn*-glycerol (**14**, 2.358 mmol, 500 mg), myristic acid (1 eq., 2.358 mmol, 537 mg) and DMAP (331.6 mg) in dry DCM (26 ml) at 0 ^{0}C was added drop-wise solution of DCC (560 mg) in dry DCM (6.5 ml). The reaction mixture was stirred at 0 ^{0}C for 16hours and concentrated. The residue was dissolved in dry ether (20 ml), filtered, concentrated and column purified (25% ethyl-acetate in hexane to give 1-*O*-myristoyl-3-*O*-(p-methoxybenzyl)-*sn*-glycerol (640 mg, 64%). TLC (40 % ethylacetate-hexane): Rf = 0.25. δ_H (300 MHz, $CDCl_3$): 0.81 (3 H, t, J 6.3, CH_3), 1.88 (23 H, m, aliphatic), 1.49-1.68 (3 H, m, aliphatic), 2.24 (4H, t, J 7.5, 2 x CH_2CO), 3.36-3.49 (2 H, m, H_2-3), 3.73 (3 H, s, OCH_3), 4.08 (1 H, m, H-2), 4.41 (2 H, m, CH_2Ph), 6.82 (2 H, d, J 6.6, PMB), 7.16 (2 H, d, J 9 Hz, PMB), 8.14. δ_C (75 MHz, $CDCl_3$): 13.98, 22.56, 24.57, 24.81, 25.35, 29.02, 29.14, 29.23, 29.34, 29.48, 29.52, 31.80, 33.84, 34.06, 34.81, 38.90, 55.13, 65.32, 68.79, 70.54, 73.07, 106.48, 113.78, 129.29, 149.39, 159.29, 173.83; ESMS (positive-ion): 445.02 $(M+Na)^+$. To a solution of above compound (1.516 mmol, 640 mg), DMAP (93 mg) and 6-Cbz-aminocaproic acid (2 eq., 3.033 mmol, 803.7 mg) in dry DCM (20 ml) was added a solution of DCC (1.82 mmol, 375mg) in dry DCM (10 ml). The solution was stirred at rt overnight and concentrated. The residue was dissolved in dry ether (20 ml), filtered, concentrated and column purified (20 % ethyl-acetate in hexane) to give product **25** (820 mg, 81%). TLC (40%EA/H, Rf = O.29) δ_H (300 MHz, $CDCl_3$): 0.81 (3 H, t, J 6.9, CH_3), 1.19 (24 H, m, aliphatic), 1.51 (8 H, m, aliphatic), 2.18-2.25 (6 H, m, 3 x $COCH_2$), 3.09-3.12 (2 H, q, J 6.3, NCH_2), 3.47-3.49 (2 H, d, J 5.4), 3.73 (3 H, s,OCH_3), 4.06-4.12 (1 H, dd, J 6.3), 4.24-4.29 (1 H, dd, J 3.9), 4.38-4.40 (2 H, d, J 3.9), 5.02 (2

H, m, OCH_2Ph), 5.13-5.16 (1 H, m, H-2), 6.79-6.82 (2 H, d, J 8.7, PMB), 7.14-7.29 (7 H, m, PMB and Ph). δ_C (75 MHz, $CDCl_3$): 13.98, 22.57, 24.36, 24.77, 25.99, 29.01, 29.16, 29.23, 29.37, 29.50, 29.54, 29.56, 31.80, 33.97, 40.73, 55.15, 62.50, 66.48, 67.80, 70.07, 72.84, 113.74, 127.96, 128.39, 129.20, 136.57, 159.24, 172.67, 173.29. ESMS (positive-ion): 670 $(M+H)^+$, 692 $(M+Na)^+$.

(b) *1-O-myristoyl-2-O-[6-(N-carbobenzyloxyamino)-hexanoyl]-sn-glycerol* (**26**). To a solution of above compound **25** (1.223 mmol, 820 mg) in wet DCM (39 ml) was added DDQ (577 mg). The reaction mixture was stirred overnight at rt, diluted with DCM and DCM layer washed with 10% aq. $NaHCO_3$, dried, concentrated and column purified (30% ethyl-acetate in hexane) to give product **26** (590 mg, 88 %). TLC (40%EA/H, Rf = 0.12). δ_H (300 MHz, $CDCl_3$): 0.81 (3 H, t, J 6.9, CH_3), 1.19 (28 H, m, aliphatic), 1.44-1.59 (10 H, m, aliphatic), 2.22-2.31 (4 H, m, 2 x $COCH_2$), 3.10-3.16 (2 H, q, J 6.6, CH_2), 3.67 (2 H, m), 4.12-4.18 (2 H, m), 4.23-4.25 (1 H, dd), 5.00-5.03 (3 H, m, H-2 and OCH_2Ph), 7.19-7.30 (5 H, m, Ph) δ_C (75 MHz, $CDCl_3$): 13.98, 22.56, 24.32, 24.77, 25.89, 29.00, 29.14, 29.23, 29.34, 29.49, 29.52, 29.55, 31.80, 33.93, 33.98, 40.69, 60.01, 61.41, 64.04, 65.01, 65.44, 72.15, 127.99, 128.41, 136.52, 140.42, 172.97, 173.62. ESMS (positive-ion): 553 $(M+H)^+$, 575 $(M+Na)^+$.

(c) *1-O-myristoyl-2-O-[6-(N-carbobenzyloxyamino)-hexanoyl]-sn-glyceryl-H-phosphonate* (**27**). To a stirred solution of imidazole (42.5 mg, 0.61 mmol, dried through toluene evaporation) in anhyd toluene (0.5 mL) at 0 ^oC was added PCl_3 (12 μL, 0.135 mmol dissolved in 125 μL toluene) followed by anhyd triethyamine (48 μL, 0.35 mmol dissolved in 125 μL toluene). The stirring was continued for 10 min at 0 ^oC and the tempearature was lowered to –5°C and then a solution of 1-*O*-myristoyl-2-*O*-[6-(*N*-carbobenzyloxyamino)-hexanoyl]-*sn*-glycerol **26** (27 mg, 44 μmol dissolved in 0.5 mL toluene) was added dropwise. The reaction was stirred at –5°C for

2 h and then quenched with pyridine-water (2.5 mL, 4:1) and stirred further for 30 min. This was followed by addition of $CHCl_3$ (7.5 mL), washing with H_2O (2.5 mL x 3). The $CHCl_3$ layer was dried (Na_2SO_4), concentrated and the residue purified through a silica column (1% MeOH-CH_2Cl_2 to 10% MeOH-CH_2Cl_2 with 1% TEA) to provide the desired lipid H-phosphonate donor **27** (25 mg, 92%). δ_H (300 MHz, $CDCl_3$): 7.25-7.01 (5 H, m, Ph), 6.80 (1 H, d, J_{PH} 628, H-P), 5.15-4.98 (3 H, m, H-2 and OCH_2Ph), 4.26 (1 H, dd, J 3.6 11.7,), 4.00 (1 H, m), 3.88 (2 H, dd, J 5.1 and 7.8), 3.49 (2 H, q, J 6.7, NCH_2), 2.18 (4 Ht, J 7.2, 2 x $COCH_2$), 1.50-1.10 (28 H, m, aliphatic), 0.76 (3 H, t, J 6.2, CH_3); MS (negative ion ESMS, M-H^-) calcd for $C_{31}H_{51}O_9NP$ (M-H) 612.3301, found 612.3451.

3-*O*-myristoyl-2-*O*-[6-(N-carbobenzyloxyamino)-hexanoyl]-*sn*-glyceryl-H-phosphonate (**30**). This was prepared (Scheme-7b) from the non-natural glycerol intermediate chiral 2,3-isopropylidene-sn-glycerol (**18**, 1R-2,2-dimethyl-1,3-dioxolane-4-methanol) by similar synthetic sequence as described above for the H-phosphonate **27**. The spectral data of compound **30**: δ_H (300 MHz, $CDCl_3$): 7.25-7.01 (5 H, m, Ph), 6.80 (1 H, d, J_{PH} 628, H-P), 5.16-4.99 (3 H, m, H-2 and CH_2Ph), 4.27 (1 H, dd, J 3.6 and 11.7), 4.00 (1 H, m), 3.90 (2 H, dd, J 5.1, 7.8), 3.49 (2 H, q, J 67.2), 2.18 (4 H, t, J 7.2), 1.50-1.10 (28 H, m, aliphatic), 0.76 (3 H, t, 6.2, CH_3); MS (negative ion ESMS, M-H^-) calcd for $C_{31}H_{51}O_9NP$ (M-H) 612.3301, found 612.3460.

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