Supporting Information

Chemical Methods for preparation of **AGL-597 (8)**: Column chromatography was performed on silica gel (Cica-MERCK silica gel 60, particle size 0.063-0.200 mm). TLC analyses were done on silicagel plates (MERK, art. 5554). All melting points were measured on a Yanagimoto micromelting point apparatus and are uncorrected. Mass spectra were measured on a JEOL JMS SX/SX-102 mass spectrometer. Optical rotations were measured on a JASCO DIP-140 digital polarimeter. Elemental analyses were recorded with a Perkin-Elmer 240C elemental analyzer. ^{1}H NMR spectra were obtained using a JEOL JNM-GX-500 FT NMR spectrometer; chemical shifts are expressed in δ units from tetramethylsilane (TMS) as an internal standard, and coupling constants (*J*) are reported in hertz (Hz).

(2S,3S,4R)-1-*O*-(α-D-Galactopyranosyl)-2-[*N*-12-(6-(N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)hexanoylamino)dodecanoylamino]-1,3,4-octadecanetriol (8, AGL-597): To a stirred solution of the amine described above (**3**, 187mg, 0.39mmol) in DMF (5mL) was added **4** (122 mg, 0.39 mmol) , WSC.HCl (1156 mg, 0.60 mmol) and N-hydroxybenzotriazol monohydrate (50 mg, 0.37 mmol), and the mixture was stirred at 120 °C for 1 h. The mixture was concentrated and purified by a preparative TLC using chloroform-methanol (4:1) to give glycosylceramide **5** [FDMS m/z 773 $(M+1)^+$] . This glycosylceramide (**5**) was used for the next step without further purification. To a stirred solution of **5** (126 mg, 0.16 mmol) in THF(2 ml) was added sodium hydroxide (250 mg, 6.25 mmol) in THF:methanol:H_2O =2:4:2 (ml), and the mixture was stirred at 40 °C for 1h. The reaction mixture was concentrated to half volume. The resulting prepicitate was filtered and washed with water.

This pricipitate was dried under reduced pressure to give **6** (41 mg). This was used for the next step without further purification. To a stirred solution of the amine described above (**6**, 41 mg, 0.06 mmol) in DMF (2mL) was added **7** (18 mg, 0.06 mmol), WSC.HCl (16 mg, 0.08 mmol) and the mixture was stirred at 25 °C for 15 h. The mixture was concentrated and purified by a preparative TLC using chloroform-methanol (4:1) to give NBD-α-glycosylceramide (**AGL-597**, **8**) : 20.0 mg (5.4 % from **3**); $[\alpha]_{23}^{D}$ +9.6 ° (*c* 0.1, methanol); mp 126-127 °C; FDMS *m/z* 953 $(M+H)^+$, 975 $(M+Na)^+$; ^{1}H NMR (500MHz, CD_3OD) δ 8.52 (1H, d, J = 9.0 Hz), 6.35 (1H, d, J = 9.0 Hz), 4.80 (1H, d, J = 3.4 Hz), 4.19 (1H, m), 3.87 (2H, m), 3.83 (1H, m), 3.75 (1H, m), 3.66-3.72 (3H, m), 3.60 (1H, t, J = 6.1 Hz), 3.54 (2H, m), 3.30 (2H, m), 3.13 (2H, t, J = 7.1Hz), 2.21 (2H, t, J = 7.6 Hz), 2.20 (2H, t, J = 7.3 Hz), 1.80 (2H, m), 1.68 (2H, m), 1.60 (2H, m), 1.47 (4H, m), 1.28 (14H, m) 0.89 (3H, t, J = 7.1 Hz). Anal. ($C_{48}H_{84}N_6O_{13}$) C, H, N.

Spleen cell proliferation assay: The spleens from C57BL/6 mice were dissociated in RPMI 1640 and the red cells were lysed with Tris NH_4Cl. The cells were washed three times with PBS (Nissui Pharmaceutical, Tokyo, Japan). The spleen cells (2.5 x 10^5 cells/100 µl/well) suspended in RPMI 1640 medium containing 10% FCS and graded doses (0.1, 1, 10 and 100 ng/ml) of samples (**KRN7000**, **AGL-597** and **AGL-592**) or control vehicle (0.0001, 0.001, 0.01 and 0.1% DMSO) were plated into 96-well plates (Nunc, Napervilla, IL, USA), and the plates were then cultured at 37℃ in 95% air, 5% CO_2 for 18 hours. Tritium-thymidine ([^{3}H]TdR, 0.5 µCi/well, Du Pont/ NEN Research Products, Boston, MT, USA) was added into each well and, 8 hours later, [^{3}H]TdR uptake into cells was measured by a liquid scintillation counter.