Supporting Information

Squaryl group modified phosphoglycolipid analogs as potential modulators of GPR55

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1. Biological experiments (S2-S6)
   1-1. Materials and methods
   1-2. Turning assay

2. Quantum mechanics calculations (S7)

3. Chemical experiments (S8-S48)
   3-1. General procedures
   3-2. Synthesis and compound characterization
1. Biological experiments

1-1. Materials and methods

**Animals**

All experimental protocols using animals were approved by the Wako Animal Experiments Committee at RIKEN. Fertilized Boris Brown chicken eggs were obtained from a local supplier (Inoue Poultry Farm, Sagamihara, Japan) and stored in a 38 °C rocking incubator until the embryos had developed to Hamburger and Hamilton Stage 36 (*Hamburger and Hamilton*, 1951).

**Cell culture**

Dorsal root ganglia (DRGs) were dissected from Stage 36 chick embryos and DRG sensory neurons were dissociated into single cells by trypsinization and manual trituration. Dissociated neurons were then suspended in Leibowitz’s L15 medium (Life Technologies, CA) with N2 Supplement (Life Technologies), 20 ng/ml nerve growth factor (NGF; Promega, WI) and 750 μg/ml bovine serum albumin (Life Technologies), and seeded onto 3 cm glass-based dishes that had been coated with 9 μg/cm² mouse laminin (Life Technologies). Seeded dishes were placed in a 37 °C incubator with 5% CO₂ for 1 – 4 hours before use in the turning assay. As described previously (*Guy et al.*, 2015), these neuronal cultures allowed us to selectively analyse NGF-responsive nociceptive axons. Test compounds were dissolved in a vehicle of 0.001% (v/v) DMSO/PBS and used at an in-pipette concentration of 10 or 20 μM. Where used, specific GPR55 inhibitor ML193 (*Kotsikorou et al.*, 2013; Tocris Bioscience, Bristol, England) was dissolved in DMSO and added to the cell culture medium to give a final bath concentration of 10 μM, at least 20 minutes before start of the turning assay. For control experiments, an equivalent volume of DMSO (0.001% (v/v)) was added to the cell culture medium 20 minutes before the assay.

**In vitro axon turning assay**

Axon turning assays (*Lohof et al.*, 1992) were performed as described previously (*Guy et al.*, 2015) with minor modifications. On an Olympus IX51 inverted microscope (Olympus Corporation, Tokyo, Japan), a growth cone, the tip of single extending axons, was introduced to a microscopic concentration gradient of DFQ test compound and imaged for 45 minutes using a QICAM Fast 1394 (QImaging, Burnaby, Canada) CCD digital camera controlled by Metavue software (version 7.8.2.0, Molecular Devices, CA). The microscopic concentration gradient was produced by repeated pulsatile ejection of DFQ solution through a glass micropipette (borosilicate glass with filament,
catalog # BF100-50-10, Sutter Instrument, CA) the tip of which was placed 100 μm away from the growth cone. Micropipettes were fashioned using a P-97 Flaming/Brown micropipette puller (Sutter Instrument), designed to have a steep shoulder and an approximate tip diameter of 1 – 2 μm.

Positive nitrogen gas pressure of 1 – 4 psi was applied to the test solution within the pipette by connection to a PV820 Picopump (World Precision Instruments, FL) electrically gated pressure control system. The frequency (2 Hz) and duration (20 ms) of positive pressure was controlled by a pulse generator (model AWG-50, ELMOS, Osaka, Japan). This pulsatile ejection of test solution from the pipette tip induces and maintains a microscopic concentration gradient of test compound in the culture medium for the duration of the assay (Lohof et al, 1992, Zheng et al, 1994). At a distance of 100 μm from the pipette tip, test compound concentration is approximately 1000 times lower than the in-pipette concentration (Lohof et al, 1992, Ming et al, 1997). At the end of the assay, the turning angle (the angle between the growth cone’s start position and its final position, measured relative to its axon) and axon extension (in microns) were calculated using Metavue software (Figure S-1). Growth cones that bifurcated, collapsed, retracted or failed to extend more than 10 μm during the 45 minutes of the assay were discounted from the study. All turning assays in the initial exploratory experiment (Figure 2b) were carried out with the experimenter blind to the identity of the compound being tested.

Statistics

Statistical analyses were carried out using GraphPad Prism (version 6.0c, GraphPad Software Inc, CA). Experimental datasets were tested for normality by D’Agostino and Pearson omnibus test, and for homoskedasticity using Bartlett’s test. Datasets that failed normality or equal variance tests (Figure 2c, Compound 1A turning angle) were analyzed using Kruskal-Wallis test, with Dunn’s multiple comparisons post-test. All three datasets shown in Figure S-2 were found to have normal distribution and equal variances, and were analyzed by 1-way ANOVA with Tukey’s multiple comparisons post-test. Detailed statistical test data can be provided on request.
Figure S-1. Method of calculation of turning angle and axon extension.  A: Representative images of start (left panel) and end (right panel) of chemorepulsive axon turning observed during turning assay.

Numbers in each panel show the minutes after onset of concentration gradient, arrows indicate the source of the gradient.  Scale bar, 10 µm.  B: the same axonal growth cone as shown in A, with a white line with arrowhead superimposed on the image to show the initial direction of growth (left panel). A second straight line was traced from the center of the growth cone at the beginning of the assay to the center of the growth cone at the end of the assay (right panel).  Turning angle ($\theta$) between this line and the original direction of growth was calculated using Metavue software.  Axon extension ($e$) was measured as the length of the second straight line in microns, using the same program.
Figure S-2. Axon growth is unaffected by compound 1A or treatment with ML193. Bars show mean axon extension in microns ± SEM, numbers in parentheses indicate the number of axons tested. No significant difference in axon extension was observed.

Figure S-3. Compounds 1D and 8A were not active in axon turning assays. Compared to compound 1A which displayed a dose-dependent increase in chemorepulsive activity, 1D and 8A did not show significant chemotrophic activity even when tested at the higher in-pipette concentration of 20 µM. Bars show mean axon turning angle ± SEM, numbers in parentheses indicate the number of axons tested. Data for 8A, 1D, 1A and 20 µM 1A bars are the same experimental data as shown in Figure 2b and 2d.
References


2. Quantum mechanics calculations

Quantum mechanics simulations were performed utilizing the Gaussian 09 program on the RIKEN internal HOKUSAI cluster. All geometries were optimized at the MP2/6-31+G(d) level followed by single point energy calculations. The dihedral angle of the anomeric linkage is referred to as \( \phi \), measured between O(ring)-C(ring)-O(ester)-P and O(ring)-C(ring)-N-C(double bond) in compound 2 and 3 respectively. The inner dihedral angle of the phosphate on the carbohydrate side in 2 is referred to as \( \psi \) composed of C(ring)-O(ester)-P-O(ester), while the second inner phosphate dihedral is referred to as \( \omega \), measured between O(ester)-P-O(ester)-C(methyl). Potential energy scans of 3 were conducted with all three defined dihedrals \( \phi \), \( \psi \) and \( \omega \) constrained. At each set of \( \phi \) and \( \psi \) three different positions of \( \omega \), namely gauche\(^+\), gauche\(^-\) and anti, were evaluated and the lowest energy conformation was selected to generate the plot in figure 1a and the corresponding supporting figure S4. In case of compound 3, \( \psi \) is defined as C(ring)-N-C(double bond)-C(carbonyl). During potential energy scans of 3 only \( \phi \) and \( \psi \) were restrained and utilized to generate figure 1b. All potential energy plots were prepared utilizing the GNUplot 4.6 software package.

The lowest energy conformation of 2 at each of the three identified potential energy minima was screened against all conformations of 3. To rank the degree of similarity, first the heavy atoms of the tetrahydropyrene ring of each structure were superimposed. Next the shortest distance between either of the phosphate carbonyl oxygen of 2 with either of the carbonyl oxygen of 3 was determined, as well as the distance between the methyl group carbon of the 2 and 3. The top 8 structures of 3 with the smallest distance difference compared to the respective conformation of 2 were plotted on the energy landscape of 3 to identify the corresponding conformational space. The structures were visualized with the VMD [Humphrey, A. Dalke, K. Schulten; J. Molec. Graphics, 1996, 14, 33-38] software package and initially ranked utilizing an in-house script.
Figure S4 (A) Heatmap of second inner dihedral phosphate angle of compound 2; green, anti; yellow, gauche', red, gauche'; (B) overlay with conformational energy, dark areas indicate regions of low energy.

Figure S5: Lowest energy conformation of compound 2 in potential energy minima II (A) and III (B) is superimposed with close matching conformation of compound 3.
3. Chemical experiments and compound characterization

3-1. General procedures

All reactions sensitive to air and/or moisture were carried out under nitrogen or argon atmosphere with anhydrous solvents. Column chromatography was performed on silica gel 60 (EM Science, 40–100 mesh). Reactions were monitored by thin-layer chromatography (TLC) on Kieselgel 60 F254 (EM Science), and compounds were detected by examination under UV light and by charring with 10% sulfuric acid in MeOH. Solvents were removed under reduced pressure at <40 °C. All reactions were carried out under an argon atmosphere. $^1$H NMR and $^{13}$C NMR spectra were recorded on a Bruker Advance 500 spectrometer with a cryoprobe and on a JEOL ECX 400 spectrometer. $^1$H NMR spectra were recorded in CDCl$_3$ referenced to TMS at 0.00 ppm or to CHCl$_3$ at 7.24 ppm and to H$_2$O at 4.79 ppm, and $^{13}$C NMR spectra were referenced to CDCl$_3$ at 77.0 ppm or native scale. Assignments were made by standard 2D experiments. MALDI-TOF mass spectra were recorded on a Bruker Autoflex Speed spectrometer and a SHIMADZU Kompact MALDI AXIMA-CFR spectrometer with 2-hydroxy-5-methoxybenzoic acid as the matrix. HRMS determinations were made the use of a JEOL AccuTOF JMS-T700LCK mass spectrometer with CF$_3$CO$_2$Na as the internal standard. All other reagents were purchased from Kanto Chemicals Co. Inc., Tokyo Chemical Industries Co., Ltd., Wako Pure Chemical Industries Ltd., and Aldrich Chemical Co.
3-2. Synthesis and compound characterization

**Scheme S1.** Synthesis of 3-(octadecylthio)propan-1-amine (6G):

2-(3-chloropropyl)-1H-isooindole-1,3(2H)-dione (S1):\(^1\)

Phthalic anhydride (2.96 g, 20 mmol) and 3-chloropropylamine hydrochloric salt (2.86 g, 22 mmol) were heated in an oil bath at 160 °C. The reaction mixture was maintained at the same temperature for 15 min. Subsequently, it was cooled to room temperature, and 30 ml H\(_2\)O were added before solidification, to form slurry. The product was filtered, washed twice with H\(_2\)O, and purified by flash chromatography on silica gel (elucent, n-hexane: EtOAc = 10:1 to 6:1) afforded the title compound S1 (3.30 g, 74% yield) as a white solid.\(^1\)H NMR (CDCl\(_3\), 400 MHz): δ 7.79–7.65 (m, 4H), 3.78 (t, \(J = 6.88\) Hz, 2H), 3.52 (t, \(J = 6.44\) Hz, 2H), 2.15–2.08 (m, 2H); \(^13\)C NMR (CDCl\(_3\), 100 MHz): δ 168.29, 134.13, 132.05, 123.35, 42.14, 35.68, 31.51; HRMS (ESI) m/z [M+Na]\(^+\) calculated for C\(_{11}\)H\(_{10}\)ClNO\(_2\)Na 246.0298, found 246.0294.

S-(3-(1,3-Dioxoisoindolin-2-yl)propyl)ethanethioate (S2):\(^2\)

To a stirred solution of S1 (2.23 g, 11 mmol) in THF (120 mL) was added potassium thioacetate (33 mmol), and the mixture was refluxed for 4 h at 85 °C. The solvent was evaporated to dryness in vacuo, and then added water (100 mL), and the organic materials were extracted with EtOAc (3 × 30 mL). The combined extracts were washed with water and brine, dried over anhydrous MgSO\(_4\), and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (elucent, n-hexane: EtOAc = 10:1 to 5:1) to give compound S2 as white powder (2.49 g, 86% yield).\(^1\)H NMR (CDCl\(_3\), 400 MHz): δ 7.78–7.64 (m, 4H), 3.68 (t, \(J = 6.88\) Hz, 2H), 2.82 (t, \(J = 7.32\) Hz, 2H), 2.25 (s, 3H), 1.93–1.86 (m, 2H); \(^13\)C NMR (CDCl\(_3\), 100 MHz): δ 168.29, 134.13, 132.05, 123.35, 42.14, 35.68, 31.51; HRMS (ESI) m/z [M+Na]\(^+\) calculated for C\(_{13}\)H\(_{13}\)NO\(_3\)SNa 286.0514, found 286.0523.

2-(3-Mercaptopropyl)isoindoline-1,3-dione (S3):

A solution of S2 (2 g, 7.6 mmol) in anhydrous methanol (23 mL) was first degassed and refilled with N\(_2\). Concentrated HCl (3 mL) was added to the solution, and the resulting mixture was refluxed for 5 h under N\(_2\) atmosphere until no more starting material could be detected by TLC. The reaction mixture was quenched with water (20 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was dried over anhydrous Na\(_2\)SO\(_4\) and concentrated in vacuo to give crude product, which was purified by flash chromatography on silica gel (elucent, n-hexane: EtOAc = 10:1 to 5:1) to give compound S3 as white powder (1.41 g, 84% yield).\(^1\)H NMR (CDCl\(_3\), 400 MHz): δ 7.81–7.66 (m, 4H), 3.76 (t, \(J = 6.88\) Hz, 2H), 2.82 (t, \(J = 7.32\) Hz, 2H), 2.25 (s, 3H), 1.98–1.91 (m, 2H); \(^13\)C NMR (CDCl\(_3\), 100 MHz): δ 168.45, 134.09, 132.06, 123.34, 36.46, 32.78, 21.96; HRMS (ESI) m/z [M+Na]\(^+\) calculated for C\(_{11}\)H\(_{11}\)NO\(_2\)SNa 244.0408, found 244.0402.

2-(3-(octadecylthio)propyl)isoindoline-1,3-dione (S4):

1-Bromooctadecane (2.12 g, 6.36 mmol) was added to a mixture containing sodium hydride (424
mg, 10.6 mmol) and compound S3 (1.17 g, 5.3 mmol) in dry THF (15 mL). The mixture was stirred at room temperature for 2 h. The reaction mixture was cooled to 0 °C and water (20 mL) was added. The solution was extracted with EtOAc (3 × 30 mL). The organic layer was separated, dried with anhydrous Na₂SO₄, and evaporated under vacuum. The crude product was purified using flash chromatography on silica gel (eluent, n-hexane: EtOAc = 10:1 to 5:1) to give compound S3 as white powder (2.3 g, 92% yield).

1H NMR (CDCl₃, 400 MHz): δ 7.85–7.70 (m, 4H), 3.79 (t, J = 7.32 Hz, 2H), 2.56–2.48 (m, 2H), 1.99–1.92 (m, 2H), 1.58–1.51 (m, 2H), 1.34–1.24 (m, 32H), 0.87 (t, J = 6.40 Hz, 3H);

13C NMR (CDCl₃, 100 MHz): δ 168.46, 134.04, 132.19, 123.34, 37.31, 32.16, 32.06, 29.79, 29.71, 29.66, 29.63, 29.46, 29.34, 29.00, 28.55, 22.79, 14.23; HRMS (ESI) m/z [M+Na]⁺ calculated for C₂₉H₄₇NO₂SNa 496.3225, found 496.3234.

3-(octadecylthio)propan-1-amine (S5):
N-Cinnamylphthalimide S4 (473 mg, 1 mmol) was mixed with hydrazine hydrate monohydrate (250 mg, 5 mmol) and MeOH (10 mL), and the mixture was stirred at 70 °C for 4 h. After the solvent was removed by a rotary evaporator, the solid residue was treated with 1 N NaOH (15 mL), and the resulting mixture was extracted with EtOAc (3 × 30 mL) and dried over anhydrous Na₂SO₄. Evaporation of the solvent under reduced pressure afforded the crude product S5, which were used for next step without purification.

Scheme S2. Synthesis of compounds 6A, 6B and 6C:

(2S)-3-Bromo-2-hydroxypropan-1-ammonium bromide (9s).³ Triphenyl phosphate (6.88 g, 26.25 mmol) was added to a stirred solution of phthalimide (3.86 g, 47 mmol) in THF (40 mL) at 0 °C, followed by (S)-(2)-glycidol (2.24 g, 30.3 mmol). Di-tert-butyl azodicarboxylate (6.04 g, 26.25 mmol) was added dropwise and the solution stirred for 5 h at room temperature. The solvent was removed under reduced pressure, dissolved in methanol (20 mL) and cooled to 0 °C. Hydrobromic acid (48% aq., 8.35 mL) was added slowly to the rapidly stirred solution until no further effervescence was observed and a white precipitate formed. The solution was diluted with water (200 mL) and filtered. The aqueous layer extracted with CH₂Cl₂ (3 × 30 mL) and the solvent was removed under reduced pressure. The residue was purified using flash chromatography on silica gel (eluent, n-hexane: EtOAc = 10:1 to 5:1) to give compound 9s as white powder (5.96 g, 80% yield for two steps).
\[ 1^H \text{NMR (CDCl}_3, \text{ 400 MHz): } \delta 7.85–7.70 (m, 4H), 3.79 (t, J = 7.32 Hz, 2H), 2.56–2.48 (m, 2H), 1.99–1.92 (m, 2H), 1.34–1.24 (m, 32H), 0.87 (t, J = 6.40 Hz, 3H); \]

\[ ^{13}C \text{ NMR (CDCl}_3, \text{ 100 MHz): } \delta 168.46, 134.04, 132.19, 123.34, 37.31, 32.16, 32.06, 29.79, 29.71, 29.66, 29.63, 29.46, 29.34, 29.00, 28.55, 22.79, 14.23; \]

HRMS (ESI) m/z [M+Na]^+ calculated for C\text{\textsubscript{11}}\text{H}_\text{10}\text{BrNO}_\text{3}Na 305.9742, found 305.9733.

(S)-2-(3-bromo-2-((tert-butyldimethylsilyl)oxy)propyl)isoindoline-1,3-dione (10s).

To a solution of compound 9s (3.90 g, 13.8 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (20 mL) containing imidazole (2.34 g, 34.50 mmol) was added TBSCl (3.10 g, 20.60 mmol) dropwise under N\textsubscript{2} at room temperature. The solution was stirred at room temperature for 8 h, concentrated in vacuo and purified using flash chromatography on silica gel (eluent, n-hexane: EtOAc = 20:1 to 10:1) to give compound 10s as white powder (5.14 g, 94% yield).

\[ 1^H \text{NMR (CDCl}_3, \text{ 400 MHz): } \delta 7.87–7.70 (m, 4H), 4.25–4.19 (m, 1H), 3.855 (dd, J = 2.28, 6.88 Hz, 2H), 3.37 (d, J = 5.04 Hz, 3H), 0.82 (s, 9H), 0.08 (s, 3H), -0.08 (s, 3H); \]

\[ ^{13}C \text{ NMR (CDCl}_3, \text{ 100 MHz): } \delta 169.29, 134.22, 132.06, 123.41, 69.38, 42.70, 35.41, 25.66, 17.91, -4.66, -4.86; \]

HRMS (ESI) m/z [M+Na]^+ calculated for C\text{\textsubscript{17}}\text{H}_\text{24}\text{BrNO}_\text{3}SiNa 420.0607, found 420.0602.

(S)-S-(2-((tert-butyldimethylsilyl)oxy)-3-(1,3-dioxoisoindolin-2-yl)propyl)ethanethioate (11s):

To a stirred solution of 10s (4.08 g, 10.25 mmol) in THF (60 mL) was added potassium thioacetate (3.51 g, 30.75 mmol), and the mixture was refluxed for 8 h at 85 °C. The solvent was evaporated to dryness in vacuo, and then added water (100 mL), and the organic materials were extracted with EtOAc (3 × 30 mL). The combined extracts were washed with water and brine, dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (eluent, n-hexane: EtOAc = 10:1 to 5:1) to give compound 11s as white powder (3.71 g, 92% yield).

\[ 1^H \text{NMR (CDCl}_3, \text{ 400 MHz): } \delta 7.84–7.68 (m, 4H), 4.16–4.10 (m, 1H), 3.78–3.67 (m, 2H), 3.08–2.96 (m, 2H), 2.32 (s, 3H), 0.80 (s, 9H), 0.05 (s, 3H), -0.11 (s, 3H); \]

\[ ^{13}C \text{ NMR (CDCl}_3, \text{ 100 MHz): } \delta 195.28, 168.32, 134.12, 132.10, 123.34, 68.79, 42.81, 34.19, 30.62, 25.67, 17.86, -4.72, -4.89; \]

HRMS (ESI) m/z [M+Na]^+ calculated for C\text{\textsubscript{19}}\text{H}_\text{27}NO\text{\textsubscript{4}}SSiNa 416.1328, found 416.1322.

(S)-2-(2-((tert-butyldimethylsilyl)oxy)-3-mercaptopropyl)isoindoline-1,3-dione (12s):

A solution of 11s (3.7 g, 9.41 mmol) in anhydrous methanol (35 mL) was first degassed and refilled with N\textsubscript{2}. NaOMe (2.5 g, 47 mmol) was added to the solution, and the resulting mixture was stirred at room temperature for 0.5 h until no more starting material could be detected by TLC. The reaction mixture was quenched with Dowex 50WX2 hydrogen form and filtered through celite. The organic layer was dried over anhydrous Na\textsubscript{2}SO\textsubscript{4}, and concentrated in vacuo to give crude product, which was purified by flash chromatography on silica gel (eluent, n-hexane: EtOAc = 10:1 to 5:1) to give compound 12s as white powder (3.10 g, 94% yield).

\[ 1^H \text{NMR (CDCl}_3, \text{ 400 MHz): } \delta 7.86–7.69 (m, 4H), 4.14–4.09 (m, 1H), 3.91–3.79 (m, 2H), 2.70–2.55 (m, 2H), 0.83 (s, 9H), 0.06 (s, 3H), -0.05 (s, 3H); \]

\[ ^{13}C \text{ NMR (CDCl}_3, \text{ 100 MHz): } \delta 168.40, 134.15, 132.10, 123.37, 70.40, 42.21, 29.88, 25.76, 17.99, -4.63, -4.75; \]

HRMS (ESI) m/z [M+Na]^+ calculated for C\text{\textsubscript{17}}\text{H}_\text{25}NO\text{\textsubscript{3}}SSiNa 374.1222, found 374.1216.

(S)-2-(2-((tert-butyldimethylsilyl)oxy)-3-(octadecylthio)propyl)isoindoline-1,3-dione (13s):

1-Bromooctadecane (366 mg, 0.91 mmol) was added to a mixture containing sodium hydride (73 mg, 10.6 mmol) and compound 12s (320 mg, 1.1 mmol) in dry THF (3 mL). The mixture was stirred at room temperature for 2 h. The reaction mixture was cooled to 0 °C and water (20 mL) was added. The solution was extracted with EtOAc (3 × 30 mL). The organic layer was separated, dried with
anhydrous Na₂SO₄, and evaporated under vacuum. The crude product was purified using flash chromatography on silica gel (eluent, n-hexane: EtOAc = 20:1 to 10:1) to give compound 13s as white powder (493 mg, 90% yield).

1H NMR (CDCl₃, 400 MHz): δ 7.86–7.68 (m, 4H), 4.19–4.13 (m, 1H), 3.93–3.75 (m, 2H), 2.68–2.53 (m, 4H), 1.59–1.52 (m, 2H), 1.36–1.18 (m, 32H), 0.86 (t, J = 6.88 Hz, 3H), 0.79 (s, 9H), 0.04 (s, 3H), -0.13 (s, 3H); 13C NMR (CDCl₃, 100 MHz): δ 168.40, 134.03, 132.20, 123.26, 69.30, 43.24, 37.57, 33.12, 32.02, 29.79, 29.69, 29.62, 29.46, 29.34, 28.96, 25.72, 22.78, 17.88, 14.22, -4.56, -4.87; HRMS (ESI) m/z [M+Na]+ calculated for C₃₅H₆₁NO₃SSiNa 626.4039, found 626.4041.

(S)-2-(2-hydroxy-3-(octadecylthio)propyl)isoindoline-1,3-dione (13s₁):
To a solution of compound 13s (90 mg, 0.15 mmol) in THF (4 mL) was added a 1 M solution of tetrabutylammonium fluoride in THF (225 μL, 0.225 mmol) and AcOH (17 μL) at room temperature. The solution was stirred for overnight and diluted with 10 mL of EtOAc solution. The organic layer was separated and washed with saturated NaHCO₃ (3 × 10 mL). The water extract was washed with EtOAc solution (2 × 10 mL), and the organic layers were combined and dried over Na₂SO₄. The solvent was evaporated in vacuo, and the residue was chromatographed over silica gel (eluent, n-hexane: EtOAc = 10:1 to 5:1) to give 13s₁ as white powder (493 mg, 85% yield).

1H NMR (CDCl₃, 400 MHz): δ 7.87–7.72 (m, 4H), 4.06–3.99 (m, 1H), 3.89–3.82 (m, 2H), 2.85–2.34 (m, 4H), 1.96 (br, 1H), 1.67–1.53 (m, 2H), 1.43–1.11 (m, 32H), 0.89 (t, J = 6.88 Hz, 3H); 13C NMR (CDCl₃, 100 MHz): δ 168.72, 134.23, 132.04, 123.56, 68.34, 43.05, 37.31, 32.03, 29.78, 29.62, 29.47, 29.31, 28.90, 22.79, 14.23; HRMS (ESI) m/z [M+Na]+ calculated for C₂₉H₄₇NO₃SNa 512.3174, found 512.3170.

(S)-1-amino-3-(octadecylthio)propan-2-ol (6A):
N-Cinnamylphthalimide 13s₁ (473 mg, 1 mmol) was mixed with hydrazine hydrate monohydrate (250 mg, 5 mmol) and MeOH (10 mL), and the mixture was stirred at 70 °C for 4 h. After the solvent was removed by a rotary evaporator, the solid residue was treated with 1 N NaOH (15 mL), and the resulting mixture was extracted with EtOAc (3 × 30 mL) and dried over anhydrous Na₂SO₄. Evaporation of the solvent under reduced pressure afforded the crude product 6A, which were used for next step without purification.

(S)-2-(2-((tert-butyldimethylsilyl)oxy)-3-(octadecylsulfonyl)propyl)isoindoline-1,3-dione (14s) and 2-((2S)-2-((tert-butyldimethylsilyl)oxy)-3-(octadecylsulfanyl)propyl)isoindoline-1,3-dione (15s):
To 13s (416 mg, 0.69 mmol) in DCM (20 mL) at room temperature was added OXONE (1.27 g, 1.04 mmol). The resulting cloudy white reaction mixture was stirred vigorously at room temperature for 8 h and then poured onto brine and the layers separated. The aqueous phase was extracted with EtOAc and the combined organic extracts dried over Na₂SO₄. The solvent was evaporated in vacuo, and the residue was chromatographed over silica gel (eluent, n-hexane: EtOAc = 10:1 to 5:1) to give 14s (280 mg, 64% yield) as white powder and 15s (128 mg, 30% yield) as white powder. Data for sulfone 14s: 1H NMR (CDCl₃, 400 MHz): δ 7.85–7.70 (m, 4H), 4.57–4.51 (m, 1H), 3.81 (d, J = 6.84 Hz, 2H), 3.24–2.99 (m, 4H), 1.84–1.76 (m, 2H), 1.38–1.23 (m, 30H), 0.88 (s, 9H), 0.86 (t, J = 6.88 Hz, 3H), 0.18 (s, 3H), 0.16 (s, 3H); 13C NMR (CDCl₃, 100 MHz): δ 168.20, 134.40, 131.88, 123.58, 65.67, 58.08, 54.97, 43.09, 32.01, 29.79, 29.67, 29.59, 29.45, 29.35, 29.19, 28.53, 25.80, 22.78, 21.68, 17.89, 14.22, -4.59, -4.70; HRMS (ESI) m/z [M+Na]+ calculated for C₃₅H₆₁NO₃SSiNa 658.3937, found 658.3932.
Data for sulfoxide 15s: \(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) 7.86–7.68 (m, 4H), 4.48–4.42 (m, 1H), 3.94–3.78 (m, 2H), 2.95–2.65 (m, 4H), 1.76–1.71 (m, 2H), 1.41–1.23 (m, 32H), 0.90–0.83 (m, 12H), 0.20 (s, 3H), 0.19 (s, 3H); \(^1^\)C NMR (CDCl\(_3\), 100 MHz): \(\delta\) 168.23, 134.33, 131.97, 123.52, 65.16, 59.53, 53.31, 43.41, 32.01, 29.79, 29.61, 29.45, 29.25, 28.88, 25.82, 25.72, 22.86, 22.78, 18.10, 14.22, -4.42, -4.86; HRMS (ESI) m/z [M+Na]^+ calculated for C\(_{35}\)H\(_{61}\)NO\(_4\)SSiNa 642.3988, found 642.3981.

\((S\!-\!2\!-(2\!\text{-hydroxy-3-(octadecylsulfonyl)propyl})isoindoline-1,3-dione (14s_1):\)

To a solution of compound 14s (230 mg, 0.362 mmol) in THF (7 mL) was added a 1 M solution of tetrabutylammonium fluoride in THF (1.1 mL, 0.225 mmol) and AcOH (84 \(\mu\)L) at room temperature. The solution was stirred for overnight and diluted with 10 mL of EtOAc solution. The organic layer was separated and washed with saturated NaHCO\(_3\) (3 \(\times\) 10 mL). The water extract was washed with EtOAc solution (2 \(\times\) 10 mL), and the organic layers were combined and dried over Na\(_2\)SO\(_4\). The solvent was evaporated in vacuo, and the residue was chromatographed over silica gel (eluent, n-hexane: EtOAc = 5:1 to 2:1) to give 14s_1 as white powder (166 mg, 88% yield).  

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) 7.86–7.75 (m, 4H), 4.60–4.57 (m, 1H), 3.88 (t, \(J = 5.48\) Hz, 2H), 3.26–3.05 (m, 4H), 1.82–1.72 (m, 2H), 1.47–1.24 (m, 30H), 0.86 (t, \(J = 6.88\) Hz, 3H); \(^1^\)C NMR (CDCl\(_3\), 100 MHz): \(\delta\) 168.68, 134.52, 131.80, 123.76, 67.07, 65.27, 54.87, 54.19, 43.56, 43.19, 32.02, 29.79, 29.69, 29.60, 29.46, 29.37, 29.14, 28.51, 22.78, 21.81, 14.23; HRMS (ESI) m/z [M+Na]^+ calculated for C\(_{29}\)H\(_{47}\)NO\(_5\)SSNa 544.3073, found 544.3067.

\((S\!-\!1\!-\text{amino-3-(octadecylsulfonyl)propan-2-ol (6B):}\)

N-Cinnamylphthalimide 14s_1 (125 mg, 1 mmol) was mixed with hydrazine hydrate monohydrate (72 \(\mu\)L) and EtOH (5 mL), and the mixture was stirred at 80 °C for 4 h. After the solvent was removed by a rotary evaporator, the solid residue was treated with 1 N NaOH (15 mL), and the resulting mixture was extracted with EtOAc (3 \(\times\) 30 mL) and dried over anhydrous Na\(_2\)SO\(_4\). Evaporation of the solvent under reduced pressure afforded the crude product 6B, which were used for next step without purification.

2-((2\(S\!\))-2-hydroxy-3-(octadecylsulfinyl)propyl)isoindoline-1,3-dione (15s_1):

To a solution of compound 15s (130 mg, 0.21 mmol) in THF (4 mL) was added a 1 M solution of tetrabutylammonium fluoride in THF (630 \(\mu\)L, 0.63 mmol) and AcOH (97 \(\mu\)L) at room temperature. The solution was stirred for overnight and diluted with 10 mL of EtOAc solution. The organic layer was separated and washed with saturated NaHCO\(_3\) (3 \(\times\) 10 mL). The water extract was washed with EtOAc solution (2 \(\times\) 10 mL), and the organic layers were combined and dried over Na\(_2\)SO\(_4\). The solvent was evaporated in vacuo, and the residue was chromatographed over silica gel (eluent, n-hexane: EtOAc = 5:1 to 2:1) to give 15s_1 as white powder (90 mg, 85% yield).

\(^1\)H NMR (CDCl\(_3\), 400 MHz): \(\delta\) 7.86–7.71 (m, 4H), 4.63–4.57 (m, 1H), 3.88 (t, \(J = 5.48\) Hz, 2H), 3.26–3.05 (m, 4H), 1.82–1.72 (m, 2H), 1.47–1.24 (m, 30H), 0.86 (t, \(J = 6.88\) Hz, 3H); \(^1^\)C NMR (CDCl\(_3\), 100 MHz): \(\delta\) 168.66, 134.52, 131.80, 123.76, 65.61, 57.01, 54.87, 43.56, 43.30, 32.02, 29.79, 29.62, 29.44, 29.25, 28.85, 22.77, 22.43, 14.21; HRMS (ESI) m/z [M+Na]^+ calculated for C\(_{29}\)H\(_{47}\)NO\(_5\)SSNa 528.3123, found 528.3129.

\((2\!S\!)-1\!-\text{amino-3-(octadecylsulfinyl)propan-2-ol (6C):}\)

N-Cinnamylphthalimide 15s_1 (75 mg, 0.15 mmol) was mixed with hydrazine hydrate monohydrate (90\(\mu\)L) and EtOH (10 mL), and the mixture was stirred at 80 °C for overnight. After the solvent was
removed by a rotary evaporator, the solid residue was treated with 1 N NaOH (15 mL), and the resulting mixture was extracted with EtOAc (3 × 30 mL) and dried over anhydrous Na₂SO₄. Evaporation of the solvent under reduced pressure afforded the crude product 6C, which were used for next step without purification.

**Scheme S3.** Synthesis of compounds 6D, 6E and 6F:

In a similar manner, compounds 6D, 6E and 6F were prepared from (R)-glycidol as starting material.

**Scheme S4.** Synthesis of sugar squaramides:

3-ethoxy-4-(glucosylamino)cyclobut-3-ene-1,2-dione 5.

To an aqueous solution of D-glucose (2.5 g, 13.9 mmol) containing NH₄HCO₃ (1.1 g), commercial aq. NH₃ (68 mL) was added. This solution was heated during 36 h at 42 °C, concentrated to half volume, and then freeze-dried, which were used for next step without purification.

DIPEA (1.23 mL, 6.95 mmol) was added dropwise to a vigorously stirred solution of diethyl squarate (DES; 3.07 mL, 20.85 mmol) and crude glycosylamine 5a in EtOH (30 mL). Then the reaction mixture was stirred at room temperature for 12 hours. Evaporation of the solvent under reduced pressure afforded the crude product, which was purified by flash chromatography on silica gel (eluent, CHCl₃: MeOH = 10:1 to 5:1) to give compound 5 as yellow powder (3.5 g, 83% yield for two steps).

¹H NMR (CD₃OD, 400 MHz): δ 4.75–4.70 (m, 2H), 3.84–3.62 (m, 2H), 3.40–3.33 (m, 5H), 1.44 (t, J = 7.36 Hz, 3H); ¹³C NMR (CD₃OD, 100 MHz): δ 193.5, 188.95, 178.03, 174.04, 78.64, 77.28, 73.09, 69.83, 69.75, 61.23, 58.57, 14.78; HRMS (ESI) m/z [M+Na]⁺ calculated for C₁₂H₁₇NO₈Na 326.0852, found 326.0848.
3-ethoxy-4-(xylosylamino)cyclobut-3-ene-1,2-dione 7.

In a similar manner, compound 7 was prepared from d-xylose as starting material (63% yield for two steps).

$^1$H NMR (CD$_3$OD, 400 MHz): $\delta$ 4.78–4.70 (m, 2H), 3.85 (dd, $J = 11.44$, 5.48 Hz, 1H), 3.53–3.47 (m, 1H), 3.36–3.26 (m, 4H), 1.44 (t, $J = 6.88$ Hz, 3H); $^{13}$C NMR (CD$_3$OD, 100 MHz): $\delta$ 188.14, 184.69, 178.07, 173.97, 85.21, 84.49, 77.30, 72.97, 69.79, 69.53, 67.38, 14.78; HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{11}$H$_{15}$NO$_7$Na 296.0746, found 296.0747.

$(2R,3R,4S,5R)$-2-(N-(2-ethoxy-3,4-dioxocyclobut-1-en-1-yl)acetamido)tetrahydro-2H-pyran-3,4,5-triy triacetate 7b

To further confirm the conformation of compound 7, fully protected acetyl group of compound 7b was prepared: Ac$_2$O (0.5 mL) was added dropwise to a vigorously stirred solution of compound 7 (100 mg, 0.36 mmol) in pyridine (1 mL). Then the reaction mixture was stirred at room temperature for 12 hours. Evaporation of the solvent under reduced pressure afforded the crude product, which was purified by flash chromatography on silica gel (eluent, n-hexane: EtOAc = 5:1 to 2:1) to give compound 7b as yellow powder (118 mg, 73% yield).

$^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 5.78 (d, $J = 9.64$ Hz, 1H, H-1), 5.45 (t, $J = 9.64$ Hz, 1H, H-2), 5.31 (t, $J = 9.60$ Hz, 1H, H-3), 5.08–4.97 (m, 3H, H-4 and CH$_2$), 4.14 (dd, $J = 11.48$, 5.52 Hz, 1H, H-5a), 3.44 (t, $J = 11.00$ Hz, 1H, H-5e), 2.36 (s, 3H, NAc), 2.04 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.96 (s, 3H, OAc), 1.57 (t, $J = 7.32$ Hz, 3H); $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 189.52, 187.10, 186.40, 170.30, 170.01, 169.91, 169.13, 82.37, 72.47, 72.00, 70.11, 68.22, 65.29, 24.06, 20.72, 20.40, 15.88; HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{19}$H$_{23}$NO$_{11}$Na 464.1169, found 464.11687.

Scheme S5. Synthesis of squaramide analogues:

Scheme S6. Synthesis of squaramide analogues:
General synthetic procedure for the synthesis of squaramide analogues 1A-1G.

DIPEA (0.5 equiv.) was added dropwise to a vigorously stirred solution of glycosylamine (1 equiv.) and the appropriate amine (1.2 equiv.) in EtOH (20 mL/mmol). Then the reaction mixture was stirred at 40 °C for 12-36 hours. Evaporation of the solvent under reduced pressure afforded the crude product, which was purified by flash chromatography on silica gel (eluent, CHCl₃: MeOH = 10:1 to 5:1) to give title compounds 1A-1G as white powder.

3-(((S)-2-hydroxy-3-(octadecylthio)propyl)amino)-4-(glucosylamino)cyclobut-3-ene-1,2-dione 1A:
Following the general procedure, starting with 3 (15.0 mg, 0.05 mmol), 6A (22.0 mg, 0.06 mmol) and DIPEA (5 μL, 0.025 mmol) in 1 mL of dry ethanol (reaction time: 24 h), compound 1A was obtained as white powder (23 mg, 76% yield).

1H NMR (pyridine-d₅, 400 MHz): δ 4.42–3.88 (m, 7H), 3.62–3.55 (m, 1H), 2.86 (d, J = 6.44 Hz, 2H), 2.55 (t, J = 7.36 Hz, 2H), 1.51–1.44 (m, 2H), 1.29–1.06 (m, 32H), 0.76 (t, J = 6.40 Hz, 3H);
13C NMR (pyridine-d₅, 100 MHz): δ 185.82, 183.95, 170.08, 169.35, 85.67, 80.08, 78.52, 70.93, 70.59, 61.64, 49.37, 37.23, 32.79, 32.92, 29.97, 29.87, 29.71, 29.68, 29.51, 29.40, 29.37, 29.29, 22.84, 14.19; HRMS (ESI) m/z [M+Na]+ calculated for C₃₁H₅₆N₂O₈SNa 639.3655, found 639.3663.

3-(((S)-2-hydroxy-3-(octadecylsulfonyl)propyl)amino)-4-(glucosylamino)cyclobut-3-ene-1,2-dione 1B:
Following the general procedure, starting with 3 (10.0 mg, 0.033 mmol), 6B (16.0 mg, 0.04 mmol) and DIPEA (3 μL, 0.017 mmol) in 1 mL of dry ethanol (reaction time: 36 h), compound 1B was obtained as white powder (15 mg, 71% yield).

1H NMR (pyridine-d₅, 400 MHz): δ 4.40–4.01 (m, 7H), 3.62–3.55 (m, 1H), 2.86 (d, J = 6.44 Hz, 2H), 2.55 (t, J = 7.36 Hz, 2H), 1.38–1.06 (m, 32H), 0.76 (t, J = 6.40 Hz, 3H);
13C NMR (pyridine-d₅, 100 MHz): δ 185.44, 183.80, 169.69, 168.82, 85.69, 80.03, 78.16, 75.11, 74.11, 70.49, 66.76, 57.42, 54.90, 49.44, 31.90, 29.78, 29.70, 29.61, 29.39, 29.20, 28.54, 22.73, 21.96, 14.10; HRMS (ESI) m/z [M+Na]+ calculated for C₃₁H₅₆N₂O₁₀SNa 671.3553, found 671.3546.

3-(((S)-2-hydroxy-3-(octadecylsulfinyl)propyl)amino)-4-(glucosylamino)cyclobut-3-ene-1,2-dione 1C:
Following the general procedure, starting with 3 (10.0 mg, 0.033 mmol), 6C (15.0 mg, 0.04 mmol) and DIPEA (3 μL, 0.017 mmol) in 1 mL of dry ethanol (reaction time: 36 h), compound 1C was obtained as white powder (12 mg, 61% yield).

1H NMR (pyridine-d₅, 400 MHz): δ 4.71–4.78 (m, 1H), 4.42–4.06 (m, 7H), 3.31–4.06 (m, 1H), 2.94–2.77 (m, 2H), 1.62–1.74 (m, 2H), 1.25–1.09 (m, 32H), 0.76 (t, J = 6.88 Hz, 3H); 13C NMR
3-(((R)-2-hydroxy-3-(octadecylthio)propylamino)-4-(glucosylamino)cyclobut-3-ene-1,2-dione 1D:
Following the general procedure, starting with 3 (15.0 mg, 0.05 mmol), 6D (22.0 mg, 0.06 mmol) and DIPEA (5 μL, 0.025 mmol) in 1 mL of dry ethanol (reaction time: 24 h), compound 1D was obtained as white powder (22 mg, 72% yield).

1H NMR (pyridine-d$_5$, 400 MHz): δ 4.38–3.88 (m, 7H), 3.70–3.37 (m, 1H), 2.95–2.48 (m, 4H), 1.53–1.41 (m, 2H), 1.29–1.06 (m, 32H), 0.79 (t, J = 6.88 Hz, 3H);

13C NMR (pyridine-d$_5$, 100 MHz): δ 185.91, 184.24, 170.17, 168.97, 85.97, 80.29, 78.66, 75.37, 71.04, 70.61, 49.46, 37.36, 32.89, 31.95, 29.83, 29.76, 29.72, 29.44, 29.40, 29.00, 22.77, 22.06, 14.12; HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{31}$H$_{56}$N$_2$O$_9$SNa 655.3604, found 655.3605.

3-(((R)-2-hydroxy-3-(octadecylsulfonyl)propylamino)-4-(glucosylamino)cyclobut-3-ene-1,2-dione 1E:
Following the general procedure, starting with 3 (10.0 mg, 0.033 mmol), 6E (16.0 mg, 0.04 mmol) and DIPEA (3 μL, 0.017 mmol) in 1 mL of dry ethanol (reaction time: 36 h), compound 1E was obtained as white powder (16 mg, 76% yield).

1H NMR (pyridine-d$_5$, 400 MHz): δ 4.46–4.04 (m, 7H), 3.75–3.69 (m, 1H), 3.60–3.42 (m, 4H), 1.92–1.88 (m, 2H), 1.29–1.09 (m, 32H), 0.80 (t, J = 7.32 Hz, 3H);

13C NMR (pyridine-d$_5$, 100 MHz): δ 186.03, 184.05, 169.97, 169.09, 86.03, 80.30, 78.57, 75.38, 70.64, 66.91, 61.36, 56.74, 54.96, 49.64, 31.94, 29.82, 29.74, 29.65, 29.43, 29.23, 22.76, 22.06, 14.10; HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{31}$H$_{56}$N$_2$O$_{10}$SNa 671.3553, found 671.3568.

3-(((2R)-2-hydroxy-3-(octadecylsulfinyl)propylamino)-4-(glucosylamino)cyclobut-3-ene-1,2-dione 1F:
Following the general procedure, starting with 3 (10.0 mg, 0.033 mmol), 6F (15.0 mg, 0.04 mmol) and DIPEA (3 μL, 0.017 mmol) in 1 mL of dry ethanol (reaction time: 36 h), compound 1F was obtained as white powder (13 mg, 63% yield).

1H NMR (pyridine-d$_5$, 400 MHz): δ 4.80–4.74 (m, 1H), 4.40–4.04 (m, 7H), 3.32–3.09 (m, 2H), 2.96–2.68 (m, 2H), 1.68–1.56 (m, 2H), 1.38–1.05 (m, 32H), 0.76 (t, J = 6.88 Hz, 3H);

13C NMR (pyridine-d$_5$, 100 MHz): δ 185.74, 183.80, 169.89, 168.57, 85.51, 79.87, 78.33, 74.97, 70.67, 66.57, 66.51, 66.57, 57.93, 52.92, 52.41, 49.90, 49.16, 31.90, 31.90, 29.79, 29.70, 29.51, 29.39, 29.86, 23.06, 22.97, 22.72, 14.10; HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{31}$H$_{56}$N$_2$O$_9$SNa 655.3604, found 655.3596.

3-((3-(octadecylthio)propylamino)-4-(glucosylamino)cyclobut-3-ene-1,2-dione 1G:
Following the general procedure, starting with 3 (61.0 mg, 0.2 mmol), 6G (82.0 mg, 0.24 mmol) and DIPEA (18 μL, 0.1 mmol) in 4 mL of dry ethanol (reaction time: 18 h), compound 1G was obtained as white powder (99 mg, 83% yield).

1H NMR (pyridine-d$_5$, 400 MHz): δ 4.38–3.79 (m, 7H), 2.90–2.88 (m, 1H), 2.58–2.33 (m, 4H), 1.95–1.80 (m, 2H), 1.53–1.42 (m, 2H), 1.29–1.06 (m, 32H), 0.76 (t, J = 6.88 Hz, 3H); HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{31}$H$_{56}$N$_2$O$_7$SNa 623.3706, found 623.3698.

3-((S)-2-hydroxy-3-(octadeeylthio)propylamino)-4-(xylosylamino)cyclobut-3-ene-1,2-dione 8A:
Following the general procedure, starting with 7 (13.7 mg, 0.05 mmol), 6A (22.0 mg, 0.06 mmol) and DIPEA (5 μL, 0.025 mmol) in 1 mL of dry ethanol (reaction time: 24 h), compound 8A was obtained as white powder (22 mg, 76% yield).

$^1$H NMR (pyridine-d$_5$, 400 MHz): $\delta$ 4.27–3.79 (m, 6H), 2.90–2.83 (m, 2H), 2.58–2.46 (m, 3H), 1.50–1.44 (m, 2H), 1.29–1.06 (m, 32H), 0.76 (t, $J = 6.88$ Hz, 3H); $^{13}$C NMR (pyridine-d$_5$, 100 MHz): $\delta$ 185.26, 183.59, 169.73, 169.01, 85.89, 78.54, 74.53, 70.62, 70.56, 68.51, 49.25, 40.50, 36.86, 32.80, 31.90, 29.79, 29.71, 29.65, 29.39, 29.36, 28.96, 22.73, 14.10; HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{30}$H$_{54}$N$_2$O$_7$SNa 609.3549, found 609.3544.

3-(((S)-2-hydroxy-3-(octadecylsulfonyl)propyl)amino)-4-(xylosylamino)cyclobut-3-ene-1,2-dione 8B:

Following the general procedure, starting with 7 (9.0 mg, 0.033 mmol), 6B (16.0 mg, 0.04 mmol) and DIPEA (3 μL, 0.017 mmol) in 1 mL of dry ethanol (reaction time: 36 h), compound 8B was obtained as white powder (15 mg, 75% yield).

$^1$H NMR (pyridine-d$_5$, 400 MHz): $\delta$ 4.73–4.84 (m, 1H), 4.23–4.31 (m, 1H), 4.18–3.85 (m, 5H), 3.81–3.67 (m, 2H), 3.56–3.51 (m, 1H), 3.44–3.39 (m, 2H), 1.96–1.88 (m, 2H), 1.35–1.06 (m, 32H), 0.78 (t, $J = 7.32$ Hz, 3H); $^{13}$C NMR (pyridine-d$_5$, 100 MHz): $\delta$ 184.96, 183.82, 169.74, 169.52, 85.99, 78.60, 74.00, 70.67, 68.55, 66.47, 57.21, 54.94, 49.68, 40.25, 31.92, 29.80, 29.72, 29.63, 29.41, 29.21, 28.57, 22.74, 22.01, 14.09; HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{30}$H$_{54}$N$_2$O$_9$SNa 641.3448, found 641.3435.

3-((3-(octadecylthio)propyl)amino)-4-(xylosylamino)cyclobut-3-ene-1,2-dione 8G:

Following the general procedure, starting with 7 (55.0 mg, 0.2 mmol), 6G (82.0 mg, 0.24 mmol) and DIPEA (18 μL, 0.1 mmol) in 4 mL of dry ethanol (reaction time: 24 h), compound 8G was obtained as white powder (91 mg, 80% yield).

$^1$H NMR (pyridine-d$_5$, 400 MHz): $\delta$ 4.28–3.71 (m, 6H), 2.51 (t, $J = 7.32$ Hz, 2H), 2.38 (t, $J = 7.76$ Hz, 2H), 1.88–1.81 (m, 2H), 1.48–1.41 (m, 2H), 1.27–1.08 (m, 32H), 0.76 (t, $J = 6.88$ Hz, 3H); $^{13}$C NMR (pyridine-d$_5$, 100 MHz): $\delta$ 185.43, 183.98, 169.56, 169.08, 86.25, 78.78, 74.72, 70.78, 68.61, 43.25, 31.95, 31.90, 31.46, 29.83, 29.76, 29.71, 29.44, 29.38, 28.99, 22.80, 22.77, 14.11; HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{30}$H$_{54}$N$_2$O$_6$SNa 593.3600, found 593.3589.

3-(((R)-2-hydroxy-3-(octadecylthio)propyl)amino)-4-(xylosylamino)cyclobut-3-ene-1,2-dione 8D:

Following the general procedure, starting with 7 (13.7 mg, 0.05 mmol), 6D (22.0 mg, 0.06 mmol) and DIPEA (5 μL, 0.025 mmol) in 1 mL of dry ethanol (reaction time: 24 h), compound 8D was obtained as white powder (23 mg, 78% yield).

$^1$H NMR (pyridine-d$_5$, 400 MHz): $\delta$ 4.29–4.04 (m, 6H), 3.84–3.82 (m, 2H), 2.86–2.78 (m, 2H), 2.55–2.51 (m, 2H), 1.51–1.44 (m, 2H), 1.29–1.06 (m, 32H), 0.76 (t, $J = 6.88$ Hz, 3H); $^{13}$C NMR (pyridine-d$_5$, 100 MHz): $\delta$ 185.26, 183.61, 169.56, 168.96, 85.87, 78.56, 74.63, 70.60, 70.48, 68.53, 49.18, 36.88, 32.79, 31.90, 29.79, 29.71, 29.66, 29.39, 29.35, 28.96, 22.73, 14.10; HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{30}$H$_{54}$N$_2$O$_7$SNa 609.3549, found 609.3549.

3-(((R)-2-hydroxy-3-(octadecylsulfonyl)propyl)amino)-4-(xylosylamino)cyclobut-3-ene-1,2-dione 8E:

Following the general procedure, starting with 7 (9.0 mg, 0.033 mmol), 6E (16.0 mg, 0.04 mmol) and DIPEA (3 μL, 0.017 mmol) in 1 mL of dry ethanol (reaction time: 36 h), compound 8E was obtained as white powder (14 mg, 70% yield).

$^1$H NMR (pyridine-d$_5$, 400 MHz): $\delta$ 4.78–4.84 (m, 1H), 4.27–3.85 (m, 5H), 3.81–3.72 (m, 2H), 1.93–1.87 (m, 2H), 1.35–1.06 (m, 32H), 0.78 (t, $J = 7.32$ Hz, 3H); $^{13}$C NMR (pyridine-d$_5$, 100 MHz): $\delta$ 184.96, 183.82, 169.74, 169.52, 85.99, 78.60, 74.00, 70.67, 68.55, 66.47, 57.21, 54.94, 49.68, 40.25, 31.92, 29.80, 29.72, 29.63, 29.41, 29.21, 28.57, 22.74, 22.01, 14.09; HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{30}$H$_{54}$N$_2$O$_9$SNa 641.3448, found 641.3435.
Hz, 3H); $^{13}$C NMR (pyridine-d$_5$, 100 MHz): $\delta$ 185.43, 183.05, 169.46, 169.08, 85.88, 78.50, 74.54, 70.60, 68.48, 66.50, 57.24, 54.94, 49.64, 31.90, 29.77, 29.69, 29.61, 29.38, 29.19, 28.55, 22.72, 21.93, 14.08; HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{30}$H$_{54}$N$_2$O$_9$SNa 641.3448, found 641.3459.

References:
NMR spectra of all new compounds

$^1$H NMR, 400 MHz, CDCl$_3$

$^{13}$C NMR, 100 MHz, CDCl$_3$
$^1$H NMR, 400 MHz, CDCl$_3$

S22
$^1$H NMR, 400 MHz, CDCl$_3$

$^{13}$C NMR, 100 MHz, CDCl$_3$
$^{13}$C NMR, 100 MHz, CDCl$_3$

$^1$H NMR, 400 MHz, CDCl$_3$
$^1$H NMR, 400 MHz, CDCl$_3$

$^{13}$C NMR, 100 MHz, CDCl$_3$
$^1$H NMR, 400 MHz, CDCl$_3$

$^13$C NMR, 100 MHz, CDCl$_3$
$^1$H NMR, 400 MHz, CDCl$_3$

$^{13}$C NMR, 100 MHz, CDCl$_3$
$^1$H NMR, 400 MHz, CDCl$_3$

$^{13}$C NMR, 100 MHz, CDCl$_3$
$^1$H NMR, 400 MHz, CDCl$_3$

$^{13}$C NMR, 100 MHz, CDCl$_3$
$^1$H NMR, 400 MHz, CDCl$_3$

$^1$H NMR spectrum

$^13$C NMR, 100 MHz, CDCl$_3$

$^13$C NMR spectrum
$^{1}H$ NMR, 400 MHz, CDCl$_3$

$^{13}C$ NMR, 100 MHz, CDCl$_3$
\(^1\)H NMR, 400 MHz, CDCl\(_3\)

\(^{13}\)C NMR, 100 MHz, CDCl\(_3\)
$^1$H NMR, 400 MHz, CDCl$_3$

$^{13}$C NMR, 100 MHz, CDCl$_3$
$^1$H NMR, 400 MHz, CD$_3$OD

$^{13}$C NMR, 100 MHz, CD$_3$OD
$^1$H NMR, 400 MHz, CD$_3$OD

$^{13}$C NMR, 100 MHz, CD$_3$OD
$^1$H NMR, 400 MHz, CDCl$_3$

$^{13}$C NMR, 100 MHz, CDCl$_3$

cosy, 400 MHz, CDCl$_3$
HMBC, 100 MHz, CDCl₃

HMQC, 400 MHz, CDCl₃
$^1$H NMR, 400 MHz, pyridine-d5

![NMR spectrum](image1)

$^{13}$C NMR, 100 MHz, pyridine-d5

![NMR spectrum](image2)

HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{31}$H$_{56}$N$_2$O$_7$SNa 623.3706, found 623.3698.
$^1$H NMR, 400 MHz, pyridine-d$_5$

$^{13}$C NMR, 100 MHz, pyridine-d$_5$

HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{31}$H$_{56}$N$_2$O$_8$SNa 639.3655, found 639.3663.
$^1$H NMR, 400 MHz, pyridine-d$_5$

$^{13}$C NMR, 100 MHz, pyridine-d$_5$

HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{31}$H$_{56}$N$_2$O$_{10}$SNa 671.3553, found 671.3546.
$^1$H NMR, 400 MHz, pyridine-d$_5$ 

\[
\text{\includegraphics[width=0.5\textwidth]{h_nmr.png}}
\]

$^{13}$C NMR, 100 MHz, pyridine-d$_5$ 

\[
\text{\includegraphics[width=0.5\textwidth]{c_nmr.png}}
\]

HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{31}$H$_{56}$N$_2$O$_9$Na 655.3604, found 655.3605.
$^1$H NMR, 400 MHz, pyridine-d$_5$

HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{31}$H$_{56}$N$_2$O$_8$SNa 639.3655, found 639.3658.

$^{13}$C NMR, 100 MHz, pyridine-d$_5$
$^1$H NMR, 400 MHz, pyridine-d$_5$

\[ \text{HRMS (ESI) m/z [M+Na]$^+$ calculated for C}_{31}\text{H}_{56}\text{N}_2\text{O}_{16}\text{SNa 671.3553, found 671.3568.} \]
\(^1\)H NMR, 400 MHz, pyridine-d5

\[^{13}\text{C}\] NMR, 100 MHz, pyridine-d5

HRMS (ESI) m/z [M+Na]\(^+\) calculated for C\(_{31}\)H\(_{56}\)N\(_2\)O\(_9\)SNa 655.3604, found 655.3596.
\(^1\)H NMR, 400 MHz, pyridine-d\(_5\)

\(^{13}\)C NMR, 100 MHz, pyridine-d\(_5\)

HRMS (ESI) m/z [M+Na]^+ calculated for C\(_{30}\)H\(_{54}\)N\(_2\)O\(_6\)SNa 593.3600, found 593.3589.
$^1$H NMR, 400 MHz, pyridine-d$_5$

$^{13}$C NMR, 100 MHz, pyridine-d$_5$

HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{30}$H$_{54}$N$_2$O$_7$SNa 609.3549, found 609.3544.
$^1$H NMR, 400 MHz, pyridine-d$_5$

\[ \text{HRMS (ESI) } m/z [M+Na]^+ \text{ calculated for } C_{30}H_{54}N_2O_9SNa 641.3448, \text{ found } 641.3435. \]
$^1$H NMR, 400 MHz, pyridine-d$_5$

![H NMR spectrum](image1)

$^{13}$C NMR, 100 MHz, pyridine-d$_5$

![C NMR spectrum](image2)

HRMS (ESI) m/z [M+Na]$^+$ calculated for C$_{30}$H$_{54}$N$_2$O$_7$Na 609.3549, found 609.3549

![HRMS spectrum](image3)
\(^1\)H NMR, 400 MHz, pyridine-d5

\(^1\)C NMR, 100 MHz, pyridine-d5

HRMS (ESI) m/z [M+Na]\(^+\) calculated for C\(_{30}\)H\(_{32}\)N\(_2\)O\(_9\)Na 641.3448, found 641.3459.