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Synthesis and Properties of a Photoactivatable Analogue of Psychosine (β -Galactosylsphingosine)

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The lysosphingolipid psychosine (shown below) is the β -galactosyl derivative of d-erythro-sphingosine.^[1] Psychosine inhibits cytokinesis and induces the formation of multinuclear globoid cells in the brains of patients afflicted with globoid cell leuko-dystrophy (Krabbe's disease), an inherited neurological disorder in which the activity of β -galactosylceramide β -galactosidase is defective.^[2] Psychosine-induced cytotoxicity is assumed to arise from the accumulation of this lipid [reviewed in Reference [2d]], but the molecular mechanisms responsible for the toxicity have not yet been elucidated. Protein-mediated events are likely to be the basis for the cellular effects of psychosine, but biophysical effects may also contribute.^[3] The receptors for psychosine have not yet been identified. TDAG8, a G protein-coupled receptor, was postulated to be a receptor for psycho-sine,^[4] but later reports indicated that TDAG8 is instead a proton-sensing receptor.^[4b,5] The identification of putative protein targets of psychosine is hindered by the lack of psycho-sine analogues that can interact covalently with the binding sites of its targets.

Photoactivatable probes have been utilized to identify lipid-binding proteins.^[6]

Benzophenones tethered covalently to various ligands are widely used to form crosslinks with receptors

because they can readily insert into C–H bonds of amino acid residues.^[7] The present study describes the synthesis of a benzophenone-linked analogue of psychosine (1). To demonstrate the potential use of this photoprobe in the identification of the psychosine receptor(s) in intact cells, we report the ability of 1 to elicit Ca^{2+} release from U937 cells at 10 and 30 μm .

As cross-metathesis reactions have been used for the preparation of the sphingosine backbone,^[8] we used this approach to prepare a glycosphingolipid conjugate bearing a terminal bromide. First, we synthesized a β -galactoside bearing a truncated sphingosine head group with a terminal alkene, as outlined in Scheme 1. Addition of vinylmagnesium bromide to commercially available (*S*)-Garner aldehyde (**2**)^[9] in THF at -78°C afforded *erythro* allylic alcohol **3** in 62% yield after separation from the *threo* by-product (10%) by chromatography. The pivaloyl group was selected for the protection of the secondary alcohol because it is less likely to undergo ester migration than other esters (e.g., acetyl or benzoyl) from the C-3 to C-1 position under basic conditions.^[10] Pivaloyl-protected compound **4** was heated in ethanolic hydrochloric acid to provide crude (*S*)-2-amino-3-*O*-pivaloyl-4-penten-1-ol, which was used in a diazotransfer reaction^[11] with triflyl azide and CuSO_4 to prepare azide **5**. With perbenzoyl α -D-galactosyl trichloroacetimidate (**6**)^[12] as the donor, 4-pentenyl aglycone **5** as the acceptor, and a catalytic amount of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ and molecular sieves (MS), we obtained β -D-galactoside **7** in 71% yield. 2-Azidosphingosine derivatives have been utilized as the acceptor in previous glycosidation reactions with protected glycosyl-1-trichloroacetimidates.^[13] Staudinger reduction of the azide^[13c-e] followed by protection of the resulting amine in situ with Boc anhydride afforded *N*-Boc derivative **8** in 79% yield.

Our initial attempts to carry out a cross metathesis reaction with an excess of 10-bromo-1-decene (prepared from 9-decen-1-ol; see Experimental Section) and azide **7** using Grubbs' second generation catalyst resulted in the formation of undesired products. However, cross metathesis of *N*-Boc derivative **8** with 10-bromo-1-decene proceeded smoothly. Metathesis product **9** was obtained with exclusive *E* selectivity (δ 5.34 ppm, $J=15.7$ Hz) after heating at reflux in dichloromethane for 16 h (Scheme 2). We found that the $\text{S}_{\text{N}}2$ reaction of bromide **9** with 4-hydroxybenzophenone in DMF using potassium carbonate as the base was sluggish even at temperatures above 100°C . When microwave conditions were used for this substitution reaction in DMF, compound **10** was obtained in 1.5 h and in 85% yield. Deprotection of the benzoyl and pivaloyl ester groups using NaOMe in methanol provided *N*-Boc compound **11**. The final step was the deprotection of the carbamate group of **11** using hydrochloric acid in acetic acid at room temperature to afford the benzophenone-linked psycho-sine derivative **1** without any by-products resulting from cleavage of the sugar under these acidic conditions.

To assess the biological activity of **1**, we carried out intracellular calcium concentration determinations in living cells with a fluorescent reporter assay. We chose the myelomonocyte cell line U937 because of its reported high sensitivity to psychosine.^[2a] We recorded the endogenous receptor-mediated responses in U937 cells to psychosine and **1**, applied at several concentrations between 1 and $30\text{ }\mu\text{M}$, as well as to 1 mM ATP for comparison. Both psychosine and analogue **1** elicited robust Ca^{2+} -dependent fluorescence intensity increases consistent with the activation of endogenous receptors. In Ca^{2+} -free buffer, the threshold concentrations for intracellular Ca^{2+} mobilization of psychosine and analogue **1** were 3 and $10\text{ }\mu\text{M}$, respectively, while in Ca^{2+} -containing buffer the corresponding values were 1 and $3\text{ }\mu\text{M}$, respectively. Overall, the responses in both conditions were similar, except that in the presence of Ca^{2+} the fluorescence increases proceeded more rapidly and were more robust. Figure 1 shows that the intracellular Ca^{2+} responses, measured in Ca^{2+} -free buffer, were very similar for $10\text{ }\mu\text{M}$ psychosine and $30\text{ }\mu\text{M}$ **1**, except that the initial rise proceeded a little slower. These results indicate that analogue **1** exhibits only approximately a threefold lower potency compared with psychosine. Therefore, analogue **1** may be a useful tool in identifying its cognate receptor.^[14]

In conclusion, the first synthesis of a photoreactive analogue of the lipid mediator psychosine has been accomplished. An *E*-selective olefin cross-metathesis reaction between

10-bromo-1-decene and protected pentenyl- β -D-galactoside **8** and a microwave-assisted displacement of the terminal bromide in meta-thesis product **9** with 4-hydroxybenzophenone were the key steps in the synthesis of the photoreactive psychosine derivative **1**. Measurements of Ca^{2+} flux in U937 cells treated with psychosine or analogue **1** revealed a similar potency of receptor activation, since the photoreactive derivative was only approximately threefold less active than psychosine. Thus this photoactivatable psychosine analogue may be useful for identifying the molecular target of psychosine after photoactivation and crosslinking.

Experimental Section

Chemistry

General methods—The solvents were dried as follows: Et_3N , CH_2Cl_2 and DMF were distilled over CaH_2 ; THF was refluxed over sodium benzophenone ketyl; MeOH was refluxed over Mg. Silica gel 60 F254 aluminum TLC plates of 0.2 mm thickness (Dynamic Adsorbents, Norcross, USA) were used to monitor the reactions; TLC plates were visualized by short wavelength ultraviolet light or by charring with 15% sulfuric acid. Flash chromatography was carried out with silica gel 60 (230–400 ASTM mesh; SiliCycle, Quebec, Canada). ^1H NMR spectra were recorded at 400 and 500 MHz and ^{13}C NMR spectra were recorded at 100 MHz on Bruker Avance I and III spectrometers; the chemical shifts (δ) are given in parts per million (ppm). HRMS was performed on an Agilent Technologies G6520A Q-TOF mass spectrometer using electrospray ionization (ESI).

(*S*)-tert-Butyl-4-((*R*)-1-hydroxyallyl)-2,2-dimethyloxazolidine-3-carboxylate (3**):** A solution of (*S*)-Garner aldehyde (**2**, 7.0 g, 30.5 mmol) in dry THF (50 mL) under N_2 at 78°C was slowly treated via cannula with $\text{CH}_2=\text{CHMgBr}$ (92 mL, 92 mmol, 1M in THF). After the cloudy yellow solution was stirred for 2 h at -78°C , the reaction was warmed to 0°C and quenched with saturated aq NH_4Cl (60 mL). The product was extracted with Et_2O (2×60 mL), and the combined organic layers were washed with brine (60 mL), dried (Na_2SO_4), filtered and concentrated in vacuo. The *erythro* and *threo* diastereomers were separated by gravity column chromatography (hexane/ EtOAc , 5:1) to afford **3** (4.8 g, 62%) and its C3-epimer (0.8 g, 10.2%) as colorless liquids. Compound **3**: $[\alpha]_D^{20} = -37.7$ ($c = 1.8$ in CHCl_3); $R_f = 0.26$ ($\text{EtOAc}/\text{hexane}$, 1:3); ^1H NMR (CDCl_3): $\delta = 1.48$ (s, 3H), 1.50 (s, 9H), 1.58 (s, 3H), 3.91 (m, 2H), 3.98 (m, 1H), 4.25 (m, 1H), 4.39 (m, 1H), 5.24 (m, 1H), 5.38 (m, 1H), 5.85 ppm (m, 1H); ^{13}C NMR (CDCl_3): $\delta = 24.2, 26.3, 28.3, 61.8, 64.4, 73.3, 81.4, 94.4, 116.1, 117.8, 137.6, 155.0$ ppm; HRMS: m/z [$M + \text{Na}$] $^+$ calcd for $\text{C}_{13}\text{H}_{23}\text{NO}_4\text{Na}$: 280.1525, found: 280.1519.

tert-Butyl-(2*S*,3*R*)-1-hydroxy-3-(pivaloyl)pent-4-en-2-yl-carbamate (4**):** A solution of alcohol **3** (2.7 g, 10.5 mmol) in CH_2Cl_2 (25 mL) was treated with Et_3N (4.4 mL, 31.5 mmol), PivCl (2 mL, 15.8 mmol) and DMAP (250 mg, 2.1 mmol) at 0°C . After 10 min, the cooling bath was removed and the reaction mixture was stirred overnight at RT. The reaction was diluted with saturated aq NH_4Cl (60 mL) and extracted with CH_2Cl_2 (2×50 mL). The combined organic extracts were dried (Na_2SO_4), filtered and concentrated in vacuo. The product was purified by vacuum distillation; the fraction boiling at $120\text{--}125^\circ\text{C}$ (4 mm of Hg) was collected to afford pivaloyl-protected alcohol **4** as a colorless liquid (3.35 g, 93%); $R_f = 0.45$ ($\text{EtOAc}/\text{hexane}$, 1:2); ^1H NMR (CDCl_3): $\delta = 1.23$ (s, 9H), 1.46–1.58 (m, 15H), 3.96 (m, 2H), 4.14 (m, 1H), 5.20 (m, 1H), 5.28 (m, 1H), 5.57 (m, 1H), 5.75 ppm (m, 1H); ^{13}C NMR (CDCl_3): $\delta = 26.4, 27.2, 28.3, 38.8, 59.3, 63.6, 73.2, 80.3, 93.9, 117.4, 134.0, 151.8, 177.2, 185.0$ ppm; HRMS: m/z [$M + \text{Na}$] $^+$ calcd for $\text{C}_{18}\text{H}_{31}\text{NO}_5\text{Na}$: 364.2100, found: 364.2101.

(2S,3R,4E)-2-Azido-3-O-pivaloyl-pent-4-ene (5): Azido compound **5** was prepared by first treating pivalate **4** (760 mg, 2.22 mmol) with 2M HCl/EtOH (1:4, 10 mL) at 70°C to hydrolyze the acetonide and *N*-Boc groups. After 3 h, the solution was concentrated and H₂O (10 mL) was added. The amine hydrochloride salt was extracted with CHCl₃/MeOH (7:1), dried (Na₂SO₄), filtered and concentrated in vacuo. The crude product was used in the next step (diazo transfer reaction) without purification. Triflyl azide (TfN₃) was prepared just prior to the reaction.^[11] A solution of the amine hydrochloride salt in H₂O (1.5 mL) was treated with K₂CO₃ (16.5 mg, 0.12 mmol) and CuSO₄·H₂O (0.3 mg, 1.2 μmol). After TfN₃ in CH₂Cl₂ (1.5 mL) was added to the reaction mixture, MeOH (5 mL) was added slowly along the walls of the reaction flask. After 5 h, when TLC indicated completion of the reaction, the solution was concentrated and purified by chromatography (EtOAc/hexane, 1:3), affording azide **5** as a white solid (352 mg, 70%): $[\alpha]_D^{20} = -29.4$ ($c=3.7$ in CHCl₃); $R_f = 0.31$ (EtOAc/hexane, 1:3); ¹H NMR (CDCl₃): δ=1.23 (s, 9H), 3.05 (br s, 1H), 3.57 (m, 1H), 3.71 (m, 2H), 5.31–5.42 (m, 3H), 5.86 ppm (m, 1H); ¹³C NMR (CDCl₃): δ=27.0, 39.0, 61.4, 65.6, 73.4, 119.2, 131.8, 177.4 ppm; HRMS: m/z [$M+Na$]⁺ calcd for C₁₀H₁₇N₃O₃Na: 250.1168, found: 250.1161.

2,3,4,6-Tetra-O-benzoyl-α-D-galactopyranosyl trichloroacetimidate (6): A solution of D-galactose (1.8 g, 10.0 mmol) in pyridine (10 mL) was treated with BzCl (6.2 mL, 53 mmol) and stirred overnight at RT. The reaction was quenched with H₂O (30 mL), extracted with CH₂Cl₂ (2×30 mL), dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/hexane, 1:2) to afford perbenzoyl D-galactose as a crystalline solid (85%): ¹H NMR (CDCl₃): δ=4.67–4.85 (m, 3H), 5.82–5.92 (m, 2H), 6.31 (m, 1H), 6.86 (d, 1H, $J=4.8$ Hz), 7.10–8.18 ppm (m, 25H). A solution of the protected D-galactose (6.2 g, 8.8 mmol) in CH₂Cl₂ (3 mL) was treated with HBr (57.2 mmol, 10 mL, 33% in HOAc) and Ac₂O (0.2 mL, 2.2 mmol). After the reaction mixture was stirred for 2 h at RT, the mixture was diluted with CH₂Cl₂ (30 mL) and cold H₂O (20 mL). Saturated aq NaHCO₃ (30 mL) was added slowly and the product was extracted with CH₂Cl₂ (2×30 mL), dried (Na₂SO₄), filtered and concentrated in vacuo. A solution of the crude perbenzoyl-β-D-galactosyl bromide in acetone/H₂O (4:1, 20 mL) was treated with Ag₂CO₃ (7.4 g, 27 mmol) and stirred for 3 h at RT. The mixture was filtered through a Celite pad and the filtrate was concentrated in vacuo. A solution of the resulting hemiacetal (3.0 g, 5.0 mmol) and Cl₃CCN (3.5 mL, 35 mmol) in CH₂Cl₂ (15 mL) at 0°C was treated with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.9 mL, 6.0 mmol) under N₂, and stirring was continued at this temperature for 3 h. The mixture was concentrated and purified by flash chromatography (CHCl₃) to provide **6** (2.8 g, 76%) as a crystalline solid: $R_f=0.28$ (EtOAc/hexane, 1:2). The NMR spectra are in agreement with the reported spectra.^[12]

(2S,3R,4E)-2-Azido-3-O-pivaloyl-1-O'-(2',3',4',6'-tetra-O-benzoyl-β-D-galactopyranosyl)-4-pentene (7): A solution of azide **5** (285 mg, 1.25 mmol) and galactosyl donor **6** (1.40 g, 1.87 mmol) in dry CH₂Cl₂ (5 mL) was stirred for 1 h at RT in the presence of 4 Å molecular sieves. A solution of BF₃·Et₂O (0.1M, 0.23 mL, 0.023 mmol) in dry CH₂Cl₂ was added slowly to the reaction mixture. After 4 h, TLC indicated the completion of reaction. The reaction was quenched with saturated aq NaHCO₃ (15 mL) and the product was extracted with CH₂Cl₂ (2×15 mL). The combined organic layers were washed with brine (10 mL), dried (Na₂SO₄), filtered and concentrated in vacuo. The residue was purified by flash chromatography (EtOAc/hexane, 1:4) to afford **7** as a white solid (720 mg, 71%): $[\alpha]_D^{20} = +77.2$ ($c=0.86$ in CHCl₃); $R_f=0.19$ (EtOAc/hexane, 1:3); ¹H NMR (CDCl₃): δ=1.19 (s, 9H), 3.70 (dd, 1H, $J=5.5, 10.4$ Hz), 3.82 (m, 1H), 4.00 (dd, 1H, $J=7.3, 10.4$ Hz), 4.34 (m, 1H), 4.43 (m, 1H), 4.68 (dd, 1H, $J=6.6, 11.2$ Hz), 4.88 (d, 1H, $J=7.8$ Hz), 5.19 (m, 2H), 5.35 (m, 1H), 5.60 (dd, 1H, $J=3.7, 10.6$ Hz), 5.73 (m, 1H), 5.81 (dd, 1H, $J=8.0, 10.4$ Hz), 6.00 (d, 1H, $J=3.5$ Hz), 7.21–8.03 ppm (m, 20H); ¹³C NMR (CDCl₃): δ=27.1,

38.9, 61.9, 63.1, 67.9, 69.5, 71.6, 73.1, 73.7, 101.2, 119.1, 119.9, 128.3, 130.0, 131.5, 132.5–133.6, 165.1–166.0, 176.8 ppm; HRMS: m/z [$M+Na$]⁺ calcd for C₄₄H₄₃N₃O₁₂Na: 828.2744, found: 828.2743.

(2*S*,3*R*,4*E*)-2-*tert*-Butoxycarbonylamino-3-*O*-pivaloyl-1-*O*-(2',3',4',6'-tetra-*O*-benzoyl-β-*D*-galactopyranosyl)-4-pentene (8): A solution of azide **7** (290 mg, 0.36 mmol) in 10 mL of benzene with H₂O (1 drop) was treated with Ph₃P (236 mg, 0.90 mmol) and stirred overnight at RT. After TLC indicated the complete conversion of the azide to the amine, the reaction mixture was concentrated in vacuo. The residue was redissolved in CH₂Cl₂ (5 mL), treated with Boc₂O (196 mg, 0.90 mmol) and Et₃N (150 μL, 1.0 mmol), and stirred overnight at RT. The reaction was quenched with saturated aq NH₄Cl (10 mL) and the product was extracted with CH₂Cl₂ (2×10 mL). The combined organic layers were dried (Na₂SO₄), filtered and concentrated in vacuo. Purification by chromatography (hexane/EtOAc, 6:1) gave *N*-Boc-protected compound **8** as a white solid (250 mg, 79%): [α]_D²⁰ = +62.4 (c =1.14 in CHCl₃); R_f =0.18 (EtOAc/hexane, 1:3); ¹H NMR (CDCl₃): δ=1.20 (s, 9H), 1.32 (s, 9H), 3.67 (dd, 1H, J =4.3, 9.8 Hz), 4.00 (m, 1H), 4.13 (dd, 1H, J =3.3, 9.8 Hz), 4.34 (m, 1H), 4.41 (dd, 1H, J =6.7, 11.4 Hz), 4.63 (dd, 1H, J =6.6, 11.2 Hz), 4.80 (m, 1H), 4.83 (d, 1H, J =7.8 Hz), 5.20 (m, 2H), 5.35 (t, 1H, J =6.2 Hz), 5.63 (dd, 1H, J =3.4, 10.4 Hz), 5.76 (m, 2H), 6.00 (d, 1H, J =3.4 Hz), 7.21–8.12 ppm (m, 20H); ¹³C NMR (CDCl₃): δ=27.1, 28.3, 38.8, 52.2, 62.0, 67.9, 69.9, 71.5, 73.4, 79.4, 101.4, 118.3, 128.3–130.0, 133.3, 133.6, 155.3, 165.2–166.0, 176.9 ppm; HRMS: m/z [$M+Na$]⁺ calcd for C₄₉H₅₃NO₁₄Na: 902.3364, found: 902.3354.

10-Bromo-1-decene:^[15] A solution of 9-decen-1-ol (5.0 g, 32.4 mmol) and Ph₃P (11.0 g, 42.1 mmol) in CH₂Cl₂ (50 mL) was cooled to 0°C and treated in small portions with *N*-bromosuccinimide (7.5 g, 42.1 mmol) over a period of 1 h at 0°C. The mixture was filtered through a silica gel pad, washed with CH₂Cl₂ (50 mL), concentrated, and purified by vacuum distillation. The product boiling at 30–35°C (2 mm Hg) was collected, providing 10-bromo-1-decene as a colorless liquid (5.8 g, 83%): R_f =0.71 (EtOAc/hexane, 1:5); ¹H NMR (CDCl₃): δ=1.30 (m, 10H), 1.85 (p, 2H, J =7.2 Hz), 2.03 (q, 2H, J =7.0 Hz), 3.40 (t, 2H, J =7.0 Hz), 4.96 (m, 2H), 5.81 ppm (m, 1H); ¹³C NMR (CDCl₃): δ=28.2, 28.8, 28.9, 29.0, 29.2, 29.3, 32.8, 33.6, 33.8, 34.0, 114.2, 138.9 ppm.

(2*S*,3*R*,4*E*)-2-*tert*-Butoxycarbonylamino-3-*O*-pivaloyl-13-bromo-1-*O*-(2',3',4',6'-tetra-*O*-benzoyl-β-*D*-galactopyranosyl)-4-tridecene (9): 10-Bromo-1-decene (183 mg, 0.84 mmol) was added to a solution of alkene **8** (185 mg, 0.21 mmol) in dry CH₂Cl₂ (8 mL) and the solution was flushed twice with N₂ (10 min each time). The reaction mixture was treated with Grubbs' second generation catalyst (53.6 mg, 0.063 mmol) at RT under N₂ and stirred for 16 h at reflux. The reaction was cooled to RT and concentrated in vacuo. Purification by chromatography (hexane/EtOAc, 4:1) gave **9** as a white solid (142 mg, 63%): [α]_D²⁰ = +41.5 (c =0.67 in CHCl₃); R_f =0.68 (EtOAc/hexane, 1:2); ¹H NMR (CDCl₃): δ=1.17–1.39 (m, 28H), 1.83 (p, 2H, J =7.0 Hz), 1.94 (q, 2H, J =6.9 Hz), 3.39 (t, 2H, J =6.9 Hz), 3.67 (dd, 1H, J =3.9, 9.8 Hz), 3.97 (m, 1H), 4.13 (dd, 1H, J =3.3, 10.0 Hz), 4.34 (m, 1H), 4.39 (dd, 1H, J =6.8, 11.0 Hz), 4.63 (dd, 1H, J =6.2, 11.0 Hz), 4.76 (m, 1H), 4.80 (d, 1H, J =8.0 Hz), 5.25 (m, 1H), 5.34 (dd, 1H, J =7.5, 15.7 Hz), 5.62 (dd, 1H, J =3.6, 10.4 Hz), 5.68 (m, 1H), 5.74 (dd, 1H, J =8.0, 10.5 Hz), 5.98 (d, 1H, J =2.8 Hz), 7.21–8.12 ppm (m, 20H); ¹³C NMR (CDCl₃): δ=27.1, 28.2, 28.3, 28.7, 28.8, 29.0, 29.2, 32.8, 34.0, 38.8, 52.2, 61.9, 67.9, 69.9, 71.5, 73.3, 79.3, 101.4, 124.9, 128.3–130.0, 133.3, 133.6, 136.5, 155.3, 165.3–166.0, 176.9 ppm; HRMS: m/z [$M+Na$]⁺ calcd for C₅₇H₆₈BrNO₁₄Na: 1092.3721, found: 1092.3723.

(2*S*,3*R*,4*E*)-2-*tert*-Butoxycarbonylamino-3-*O*-pivaloyl-13-*O*-(4''-benzoylphenyl)-1-*O*-(2',3',4',6'-tetra-*O*-benzoyl-β-*D*-galactopyranosyl)-4-tridecene (10): A solution of

bromide **9** (35 mg, 0.033 mmol), 4-hydroxybenzophenone (10 mg, 0.049 mmol) and K_2CO_3 (7.0 mg, 0.049 mmol) in dry DMF (3.5 mL) was heated in a Smith Creator microwave reactor at 120°C for 1.5 h. The reaction mixture was quenched with saturated aq NH_4Cl (10 mL) and extracted with EtOAc (2×10 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated in vacuo. Purification by chromatography (hexane/EtOAc, 4:1) afforded benzophenone-linked product **10** as a white solid (33 mg, 85%): $[\alpha]_D^{20} = +46.71$ ($c=1.55$ in $CHCl_3$); $R_f=0.48$ (EtOAc/hexane, 1:2); 1H NMR ($CDCl_3$): $\delta=1.16-1.48$ (m, 28H), 1.81 (m, 2H), 1.97 (q, 2H, $J=7.2$ Hz), 3.68 (dd, 1H, $J=4.0, 9.5$ Hz), 4.00 (m, 1H), 4.04 (t, 2H, $J=7.2$ Hz), 4.14 (m, 1H), 4.35 (t, 1H, $J=6.8$ Hz), 4.42 (dd, 1H, $J=6.8, 11.2$ Hz), 4.66 (dd, 1H, $J=7.5, 12.3$ Hz), 4.80 (m, 1H), 4.83 (d, 1H, $J=8.0$ Hz), 5.28 (m, 1H), 5.38 (dd, 1H, $J=7.5, 15.0$ Hz), 5.65 (dd, 1H, $J=3.4, 10.2$ Hz), 5.72 (m, 1H), 5.78 (dd, 1H, $J=7.8, 10.2$ Hz), 6.01 (d, 1H, $J=3.0$ Hz), 6.97 (d, 2H, $J=9.2$ Hz), 7.25–8.13 ppm (m, 27H); ^{13}C NMR ($CDCl_3$): $\delta=26.0, 27.1, 28.3, 28.3, 28.8, 29.0, 29.1, 29.3, 29.4, 29.6, 32.2, 38.8, 52.3, 61.9, 64.8, 68.0, 68.3, 69.9, 71.3, 71.6, 73.4, 79.3, 101.4, 114.0, 126.2, 127.9-133.6, 136.6, 138.4, 155.3, 162.9, 165.3-166.0, 176.9, 195.6$ ppm; HRMS: m/z [$M+Na$] $^+$ calcd for $C_{70}H_{77}NO_{16}Na$: 1210.5140, found: 1210.5141.

(2S,3R,4E)-2-tert-Butoxycarbonylamino-3-hydroxy-13-O-(4'-benzoylphenyl)-1-O- β -D-galactopyranosyl-4-tridecene (11): $Na(s)$ (5 mg) was dissolved in dry MeOH (3 mL) and added to a solution of **10** in dry MeOH/THF (5:1, 6 mL). The reaction mixture was stirred for 2 d at RT. Dowex 50W-X8 resin (prewashed thoroughly with MeOH) was added to neutralize the reaction mixture, which was then filtered and concentrated. Purification by chromatography ($CHCl_3$ /MeOH, 10:1) gave **11** as a white solid (15.6 mg, 90%): $[\alpha]_D^{20} = -1.05$ ($c=0.76$ in $CHCl_3$ /MeOH, 3:1); $R_f=0.4$ ($CHCl_3$ /MeOH, 3:1); 1H NMR ($CDCl_3$ /CD $_3$ OD, 20:1): $\delta=1.27-1.47$ (m, 19H), 1.80 (p, 2H, $J=6.6$ Hz), 2.01 (q, 2H, $J=6.4$ Hz), 3.55 (m, 1H), 3.62 (m, 2H), 3.70 (m, 2H), 3.82 (m, 2H), 3.91 (m, 1H), 4.02 (t, 2H, $J=6.4$ Hz), 4.05 (m, 1H), 4.16 (m, 1H), 4.28 (d, 1H, $J=7.8$ Hz), 5.48 (dd, 1H, $J=6.9, 15.2$ Hz), 5.73 (dt, 1H, $J=6.7, 14.0$ Hz), 6.94 (d, 2H, $J=8.8$ Hz), 7.37–8.07 ppm (m, 7H); ^{13}C NMR ($CDCl_3$ /CD $_3$ OD 20:1): $\delta=26.0, 28.4, 29.1, 29.2, 29.3, 29.4, 32.4, 54.6, 61.3, 64.8, 67.9, 68.2, 68.9, 69.4, 71.0, 72.5, 73.3, 74.6, 79.6, 103.8, 114.0, 126.2, 127.5-132.6, 138.2, 156.3, 162.9, 195.8$ ppm; HRMS: m/z [$M+Na$] $^+$ calcd for $C_{37}H_{53}NO_{11}Na$: 710.3516, found: 710.3515.

(2S,3R,4E)-2-Amino-3-hydroxy-13-O-(4'-benzoylphenyl)-1-O- β -D-galactopyranosyl-4-tridecene (1): A mixture of compound **11** (12 mg, 0.017 mmol) and 1M HCl in 90% aqueous HOAc (50 μ L) was stirred at RT in CH_2Cl_2 (10 mL) for 35 min. After concentration, the residue was diluted with MeOH (5 mL) and again concentrated to afford the hydrochloride salt of **1** as a white solid (10.2 mg, 94%): $[\alpha]_D^{20} = -6.22$ ($c=0.45$ in $CHCl_3$ /MeOH, 4:1); $R_f=0.08$ ($CHCl_3$ /MeOH, 3:1); 1H NMR ($CDCl_3$ /CD $_3$ OD, 20:1): $\delta=1.24-1.39$ (m, 10H), 1.72 (p, 2H, $J=6.6$ Hz), 2.01 (q, 2H, $J=6.9$ Hz), 3.27 (m, 1H), 3.35 (m, 1H), 3.48 (m, 2H), 3.66 (m, 2H), 3.74 (m, 1H), 3.81 (m, 1H), 3.87 (m, 1H), 3.95 (t, 2H, $J=6.6$ Hz), 4.21 (m, 1H), 4.37 (m, 1H), 5.34 (dd, 1H, $J=5.6, 15.3$ Hz), 5.73 (dt, 1H, $J=6.6, 14.3$ Hz), 6.86 (d, 2H, $J=8.8$ Hz), 7.35–7.96 ppm (m, 7H); ^{13}C NMR ($CDCl_3$ /CD $_3$ OD 20:1): $\delta=21.8, 29.8, 32.8, 32.9, 33.1, 33.2, 33.5, 36.2, 61.6, 65.5, 69.5, 72.2, 73.0, 73.4, 74.9, 76.9, 78.9, 106.9, 117.9, 130.2, 132.1, 133.5, 133.6, 135.9, 136.6, 139.4, 142.0, 166.9, 200.3$ ppm; HRMS: m/z [$M+Na$] $^+$ calcd for $C_{32}H_{45}NO_9Na$: 610.2992, found: 610.2997.

Biology

Intracellular Ca^{2+} mobilization measurements—The permanent human leukemia cell line U937 was cultured in RPMI 1640 medium with HEPES containing 10% fetal bovine serum (FBS), 2 mM glutamate, and a penicillin-streptomycin solution (Sigma, St. Louis, USA) in a humidified air/ CO_2 (19:1) atmosphere. Cells were diluted to 5×10^5 mL $^{-1}$ density,

and on the next day the cells were washed with phosphate-buffered saline (PBS; 137 mM NaCl, 16 mM Na₂HPO₄, 6.4 mM KH₂PO₄, pH 7.2). Cells were loaded with 2.5 μM Fluo-4 AM (Invitrogen, Carlsbad, USA) mixed with 0.02% pluronic acid (final concentrations) for 45 min at 37°C/5% CO₂, and then washed with either Ca²⁺-free Krebs ringer solution (120 mM NaCl, 5 mM KCl, 0.62 mM MgSO₄, 10 mM HEPES, 6 mM glucose, 50 μM EDTA, pH 7.4) or Ca²⁺-containing Krebs ringer solution (120 mM NaCl, 5 mM KCl, 0.62 mM MgSO₄, 1.8 mM CaCl₂, 10 mM HEPES, 6 mM glucose, pH 7.4). The dye-loaded cells were kept in the dark. Fluo-4 fluorescence was detected in a Jobin Yvon FluoroMax-3 spectrofluorometer with excitation at 485 nm and emission at 525 nm in a thermo-stated cuvette at 37°C. After the cell density was adjusted to 2.5×10⁶ mL⁻¹, the baseline was recorded for 30 s, and psychosine or **1** was added from 1 mM stock solutions in methanol. After gentle mixing, the signal was recorded for 5 min. In agreement with published data,^[2a] neither psychosine nor **1** was directly cytotoxic to U937 cells up to 30 μM, the highest concentration applied.

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Figure 1.

Intracellular Ca^{2+} mobilization by **1** and psychosine in U937 human leukemia cells. a) Time course of Ca^{2+} mobilization by **1** (—) and psychosine (---) measured in Ca^{2+} -free buffer. Cells were loaded with Fluo-4 AM, washed, and the fluorescence intensity was recorded in a cuvette. The compounds were added to the cell suspensions at the time indicated by the arrow. For comparison, the response to ATP (---••) by endogenous purinergic receptors was also examined. b) The average response of U937 cells to **1** (30 μM) and psychosine (10 μM) at 60 s after addition of the compounds. Fluo-4 fluorescence intensities were read from similar traces shown in panel a. Data represent the mean $\pm$ SEM of three independent determinations.

Scheme 1.

Synthesis of pentenyl- β -D-galactosides **7** and **8**. *Reagents and conditions*: a) $\text{CH}_2=\text{CHMgBr}$, THF, -78°C , 2 h, 62%; b) $t\text{BuC}(\text{O})\text{Cl}$, cat DMAP/ Et_3N , CH_2Cl_2 , $0^\circ\text{C} \rightarrow \text{RT}$, 93 %; c) 1. HCl (2_{M})/ EtOH (1:4), $70-80^\circ\text{C}$, 3 h; 2. TfN_3 in CH_2Cl_2 , K_2CO_3 , $\text{CuSO}_4 \cdot \text{H}_2\text{O}$ (0.01 equiv), MeOH , H_2O , 5 h, 70% (two steps); d) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, dry CH_2Cl_2 , 4 Å MS, RT, 4 h, 71%; e) 1. Ph_3P , C_6H_6 , H_2O (1 drop), overnight; 2. $(\text{Boc})_2\text{O}$, Et_3N , CH_2Cl_2 , RT, overnight, 79% (two steps). Abbreviations: Piv, pivaloyl; DMAP, (4-dimethylamino)pyridine; Boc, *tert*-butyloxycarbonyl; TfN_3 , trifyl azide; MS, molecular sieves; $(\text{Boc})_2\text{O}$, di-*tert*-butyl dicarbonate.

Scheme 2.

Synthesis of psychosine analogue **1** by olefin metathesis. *Reagents and conditions:* a) 10-bromo-1-decene (4 equiv), Grubbs' second generation catalyst (0.3 equiv), CH₂Cl₂, reflux, 16 h, 63%; b) 4-hydroxybenzophenone, K₂CO₃, DMF, microwave, 120 °C, 1.5 h, 85 %; c) NaOMe in MeOH, THF, RT, 2 d, 90%; d) HCl (1M) in 90 % HOAc, CH₂Cl₂, RT, 35 min, 94%. Abbreviations: O-BP, 4-*O*-benzophenone.