Supporting Information

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Rational Design and Evaluation of a Branched-Chain-Containing Glycolipid Antigen That Binds to CD1d


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A. Figures

**Figure S1.** Serum IFN-γ and IL-4 levels from mice injected (i.v.) with α-GalCer (1) or glycolipids 3–5, 7–9, 11, 12 (1 µg/mouse). Results are expressed as relative activities. Representative data of two individual experiments are shown as the means ±SD of three mice.

**Figure S2.** Superimposition of the docking conformation of 7 (green) with the crystallographic binding conformation of α-GalCer (yellow). The amino acid residues that form the central pole are shown in pink. Left, top view; right, side view.

In our docking model, the branched analogue 7 was found to be oriented similarly to α-GalCer in its crystalline complex with hCD1d (Figure S2). The galactose headgroup of glycolipid 7 was presented in nearly the same position as that of α-GalCer. The phytosphingosine moiety of 7 fitted into the F' pocket by adopting a conformation very similar to that of α-GalCer.
B. Experimental Methods

(a) Biological methods

Glycolipids were dissolved in a 0.5% tween 20 in saline as a vehicle at a concentration of 200µg/ml, and diluted with sterile PBS just before use.

Determination of the stimulating activity for NKT hybridoma cells\textsuperscript{1,2}

Mouse CD1d-transfected rat basophilic leukemia (RBL) cells were loaded with 8 ng/mL α-GalCer (1) or glycolipids 3–22. After 4 h, free glycolipids were removed by washing three times with PBS 3, and the RBL cells were incubated with DN32.D3 NKT hybridoma cells for 16 h. The level of IL-2 secreted into the supernatant was determined by Enzyme-Linked Immunosorbent Assay (ELISA).

Evaluation of the cytokine levels produced by primary splenocytes\textsuperscript{3}

Splenocytes from naïve C57BL/6 mice were cultured in the presence of 0.5 ng/mL α-GalCer or glycolipids 3–22 for 72 h. The levels of IFN-γ and IL-4 secreted into the supernatant were determined by ELISA.

Examination of the IFN-γ and IL-4 production levels In vivo\textsuperscript{4}

α-GalCer or glycolipids 3–5, 7–9, 11, and 12 (1 µg/mouse) were injected into naïve C57BL/6 mice. The serum concentrations of IFN-γ and IL-4 at each time point were determined by ELISA.
**Dimer binding assay**\textsuperscript{5}

Three-fold serially diluted \(\alpha\)-GalCer or glycolipids 7–9 were incubated overnight with the mouse CD1d dimer (BD Pharmingen, catalog No. 557599). As a negative control, vehicle was incubated with the CD1d dimer. Glycolipid-loaded CD1d dimer complexes were labeled with anti-mouse IgG1-PE (Southern Biotech) at RT and were used to stain 2x10\textsuperscript{5} DN32.D3 NKT hybridoma cells for 30 min at 4 °C. The cells were washed, fixed with 4% paraformaldehyde, and analyzed using a FACSCalibur flow cytometer (BD Pharmingen).

**Dissociation assay**\textsuperscript{6,7}

96-well plates were coated with the mouse CD1d dimer at a concentration of 1 \(\mu\)g/well in PBS. CD1d dimer was loaded with 20 \(\mu\)g/mL of \(\alpha\)-GalCer or glycolipid 7 and incubated for 24 h. After washing at the set times, DN32.D3 NKT hybridoma cells were added to each well and supernatants were collected after 24 h of culture. The IL-2 level in the supernatant was determined by ELISA.

**(b) Molecular modeling methods**

To understand the binding mode of action of the \(\alpha\)-GalCer derivatives, we performed a docking study using Surflex-Dock in Sybyl version 8.02 (Tripos Associates) operating under Red Hat Linux 4.0 on an IBM computer (Intel Pentium 4, 2.8GHz CPU, 1GB memory). Surflex-Dock automatically docks ligands into the ligand binding site of a receptor using a protomol-based method and an empirically-derived scoring function. The structures of the \(\alpha\)-GalCer analogues were drawn into the Sybyl package with
standard bond lengths and angles and were minimized using the conjugate gradient method. The Gasteiger-Huckel charge, with a distance-dependent dielectric function, was applied for the minimization process. We chose the 2PO6 (PDB code) structure from the Protein Data Bank and the structure was polished by the “Prepare protein structure” function. After running Surflex-Dock, the scores of 10 docked conformers were ranked in a molecular spread sheet. We selected the conformer with the best total score and hypothesized its detailed binding patterns in the cavity.

(c) Synthetic procedures and compound characterization

General methods

All chemicals were reagent grade and used as purchased. All reactions were performed under an inert atmosphere of dry argon or nitrogen using distilled dry solvents. Reactions were monitored by thin layer chromatography (TLC) analysis using silica gel 60 thin layer plates. Flash column chromatography was performed on silica gel grade 60 (230–400 mesh). Optical rotations were measured using sodium light (D line 589.3 nm). $^1$H NMR and $^{13}$C NMR spectra were recorded in $\delta$ units relative to deuterated solvent, which served as an internal reference, at 300 and 75 MHz, respectively. The purity of the products were > 95% based on proton NMR spectra and elemental analysis.
**Compound synthesis**

General procedure for the preparation of 24a–24t:

To a stirred solution of the amino compound 23 (50 mg, 0.046 mmol) in CH2Cl2 (2 mL) were added EDCI (18 mg, 0.092 mmol), DMAP (0.1 equiv), and fatty acids (2.0 equiv) were added. The reaction mixture was stirred in a nitrogen atmosphere for 3 h. Solvent was removed *in vacuo* and the resulting residue was purified by flash column chromatography to afford the desired products (24a–24t, 75–84%).

**Compound 24a.** Colorless oil (53 mg, 82%); [α]_{D}^{24} +14.3 (c 1.0, CHCl3); ¹H NMR (300 MHz, CDCl3) δ 0.88 (t, J = 6.0 Hz, 9H), 1.22-1.94 (m, 56H), 2.90 (m, 6H), 3.40-3.54 (m, 3H), 3.70-3.83 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.89-4.19 (m, 5H), 4.33-4.42 (m, 4H), 4.48 (d, J = 12.3 Hz, 1H), 4.53-4.82 (m, 7H), 4.86 (d, J = 3.3 Hz, 1H), 4.91 (d, J = 11.7 Hz, 1H), 6.23 (d, J = 7.8 Hz, 1H), 6.82 (m, 4H), 7.21-7.38 (m, 24H).
**Compound 24b.** Colorless oil (53 mg, 80%); [α]$_{24}^{D}$ +11.7 (c 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 0.88 (t, $J$ = 6.9 Hz, 9H), 1.22-1.94 (m, 60H), 2.81 (m, 6H), 3.40-3.54 (m, 3H), 3.68-3.82 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.89-4.16 (m, 5H), 4.33-4.44 (m, 4H), 4.48 (d, $J$ = 11.7 Hz, 1H), 4.53-4.82 (m, 7H), 4.86 (d, $J$ = 3.6 Hz, 1H), 4.92 (d, $J$ = 11.7 Hz, 1H), 6.16 (d, $J$ = 7.8 Hz, 1H), 6.82 (m, 4H), 7.16-7.38 (m, 24H).

**Compound 24c.** Colorless oil (53 mg, 79%); [α]$_{24}^{D}$ +18.9 (c 0.5, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 0.88 (t, $J$ = 6.9 Hz, 9H), 1.19-1.94 (m, 64H), 2.90 (m, 6H), 3.41-3.54 (m, 3H), 3.70-3.83 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.90-4.17 (m, 5H), 4.33-4.44 (m, 4H), 4.48 (d, $J$ = 12.6 Hz, 1H), 4.54-4.82 (m, 7H), 4.86 (d, $J$ = 3.6 Hz, 1H), 4.91 (d, $J$ = 11.7 Hz, 1H), 6.36 (d, $J$ = 8.4 Hz, 1H), 6.82 (m, 4H), 7.19-7.38 (m, 24H).

**Compound 24d.** Colorless oil (53 mg, 75%); [α]$_{24}^{D}$ +16.4 (c 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 0.88 (t, $J$ = 6.6 Hz, 9H), 1.23-1.92 (m, 76H), 2.88 (m, 6H), 3.44-3.54 (m, 3H), 3.70-3.82 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.90-4.16 (m, 5H), 4.33-4.58 (m, 6H), 4.64-4.82 (m, 6H), 4.86 (d, $J$ = 3.6 Hz, 1H), 4.92 (d, $J$ = 11.7 Hz, 1H), 6.22 (d, $J$ = 8.1 Hz, 1H), 6.82 (m, 4H), 7.22-7.38 (m, 24H).

**Compound 24e.** Colorless oil (55 mg, 84%); [α]$_{24}^{D}$ +11.6 (c 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 0.88 (t, $J$ = 6.6 Hz, 9H), 1.22-2.04 (m, 58H), 2.91 (m, 6H), 3.40-3.53 (m, 3H), 3.67-3.82 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.89-4.07 (m, 4H), 4.16 (m, 1H), 4.33 (d, $J$ = 7.8 Hz, 1H), 4.37 (d, $J$ = 8.7 Hz, 1H), 4.43 (d, $J$ = 10.8 Hz, 2H), 4.50 (d, $J$ = 11.7 Hz, 1H), 4.64-4.75 (m, 4H), 4.79 (d, $J$ = 11.7 Hz, 1H), 4.80 (d, $J$ = 11.4 Hz, 1H), 4.85 (d, $J$ = 3.3 Hz, 1H), 4.91 (d, $J$ = 11.4 Hz, 1H), 6.10 (d, $J$ = 9.0 Hz, 1H), 6.82
Compound 24f. Colorless oil (55 mg, 83%); [α]^{24}_D +19.6 (c 0.5, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 0.88 (t, J = 6.9 Hz, 9H), 1.23-1.96 (m, 62H), 2.38 (m, 6H), 3.40-3.55 (m, 3H), 3.70-3.82 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.87-4.16 (m, 5H), 4.32-4.53 (m, 5H), 4.57 (d, J = 11.4 Hz, 1H), 4.63-4.82 (m, 6H), 4.86 (d, J = 3.6 Hz, 1H), 4.93 (d, J = 11.7 Hz, 1H), 6.01 (d, J = 8.7 Hz, 1H), 6.82 (m, 4H), 7.21-7.39 (m, 24H).

Compound 24g. Colorless oil (52 mg, 77%); [α]^{24}_D +14.0 (c 1.0, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 0.88 (t, J = 6.8 Hz, 9H), 1.23-1.95 (m, 66H), 2.52 (m, 6H), 3.40-3.53 (m, 3H), 3.70-3.82 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.88-4.16 (m, 5H), 4.32-4.53 (m, 5H), 4.56 (d, J = 11.4 Hz, 1H), 4.63-4.82 (m, 6H), 4.86 (d, J = 3.6 Hz, 1H), 4.92 (d, J = 11.7 Hz, 1H), 6.11 (d, J = 8.4 Hz, 1H), 6.82 (m, 4H), 7.21-7.38 (m, 24H).

Compound 24h. Colorless oil (57 mg, 80%); [α]^{24}_D +10.1 (c 0.5, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 0.88 (t, J = 6.9 Hz, 9H), 1.22-1.94 (m, 78H), 2.91 (m, 6H), 3.40-3.53 (m, 3H), 3.70-3.81 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.88-4.16 (m, 5H), 4.32-4.58 (m, 6H), 4.63-4.82 (m, 6H), 4.86 (d, J = 3.6 Hz, 1H), 4.92 (d, J = 11.6 Hz, 1H), 6.17 (d, J = 8.4 Hz, 1H), 6.82 (m, 4H), 7.21-7.38 (m, 24H).

Compound 24i. Colorless oil (55 mg, 83%); [α]^{24}_D +13.1 (c 0.5, CHCl₃); ¹H NMR (300 MHz, CDCl₃) δ 0.88 (t, J = 6.0 Hz, 9H), 1.17-1.98 (m, 60H), 2.90 (m, 6H), 3.40-3.54 (m, 3H), 3.70-3.81 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.89-4.16 (m, 5H), 4.33-4.42 (m, 4H), 4.46-4.82 (m, 8H), 4.86 (d, J = 3.3 Hz, 1H), 4.91 (d, J = 11.4 Hz, 1H), 6.25 (d, J =
8.4 Hz, 1H), 6.82 (m, 4H), 7.21-7.38 (m, 24H).

**Compound 24j.** Colorless oil (53 mg, 79%); [α]$_{D}^{24}$ +14.5 ($c$ 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J = 6.3$ Hz, 9H), 1.18-1.95 (m, 64H), 2.90 (m, 6H), 3.40-3.54 (m, 3H), 3.68-3.81 (m, 2H), 3.74 (s, 3H), 3.77 (s, 3H), 3.88-4.15 (m, 5H), 4.32-4.42 (m, 4H), 4.45-4.82 (m, 8H), 4.86 (d, $J = 3.0$ Hz, 1H), 4.91 (d, $J = 11.7$ Hz, 1H), 6.20 (d, $J = 8.2$ Hz, 1H), 6.81 (m, 4H), 7.21-7.35 (m, 24H).

**Compound 24k.** Colorless oil (53 mg, 77%); [α]$_{D}^{24}$ +14.2 ($c$ 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J = 6.6$ Hz, 9H), 1.18-1.98 (m, 68H), 2.91 (m, 6H), 3.40-3.54 (m, 3H), 3.70-3.81 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.88-4.15 (m, 5H), 4.32-4.42 (m, 4H), 4.45-4.82 (m, 8H), 4.86 (d, $J = 3.6$ Hz, 1H), 4.91 (d, $J = 11.4$ Hz, 1H), 6.21 (d, $J = 8.4$ Hz, 1H), 6.81 (m, 4H), 7.21-7.38 (m, 24H).

**Compound 24l.** Colorless oil (59 mg, 81%); [α]$_{D}^{24}$ +11.8 ($c$ 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J = 6.6$ Hz, 9H), 1.18-1.95 (m, 80H), 2.91 (m, 6H), 3.40-3.54 (m, 3H), 3.70-3.81 (m, 2H), 3.74 (s, 3H), 3.77 (s, 3H), 3.87-4.16 (m, 5H), 4.32-4.42 (m, 4H), 4.45-4.81 (m, 8H), 4.86 (d, $J = 3.3$ Hz, 1H), 4.91 (d, $J = 11.4$ Hz, 1H), 6.19 (d, $J = 8.2$ Hz, 1H), 6.82 (m, 4H), 7.21-7.38 (m, 24H).

**Compound 24m.** Colorless oil (55 mg, 83%); [α]$_{D}^{24}$ +11.7 ($c$ 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.86 (t, $J = 6.5$ Hz, 9H), 1.12-1.93 (m, 62H), 2.45 (m, 6H), 3.37-3.50 (m, 3H), 3.71-3.80 (m, 2H), 3.72 (s, 3H), 3.75 (s, 3H), 3.84-4.14 (m, 5H), 4.29-4.47 (m, 4H), 4.51-4.80 (m, 8H), 4.84 (d, $J = 3.6$ Hz, 1H), 4.90 (d, $J = 11.3$ Hz, 1H),
6.12 (d, J = 8.9 Hz, 1H), 6.80 (m, 4H), 7.18-7.36 (m, 24H).

**Compound 24n.** Colorless oil (51 mg, 75%); [α]$^2_{D}$ +18.1 (c 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 0.82 (t, J = 6.6 Hz, 9H), 1.10-1.87 (m, 66H), 2.40 (m, 6H), 3.37-3.47 (m, 3H), 3.64-3.76 (m, 2H), 3.69 (s, 3H), 3.71 (s, 3H), 3.82-4.10 (m, 5H), 4.25-4.43 (m, 4H), 4.47-4.76 (m, 8H), 4.80 (d, J = 3.3 Hz, 1H), 4.86 (d, J = 11.5 Hz, 1H), 6.08 (d, J = 8.8 Hz, 1H), 6.76 (m, 4H), 7.13-7.32 (m, 24H).

**Compound 24o.** Colorless oil (53 mg, 77%); [α]$^2_{D}$ +11.0 (c 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 0.81 (t, J = 6.4 Hz, 9H), 1.10-1.86 (m, 70H), 2.37 (m, 6H), 3.35-3.42 (m, 3H), 3.61-3.76 (m, 2H), 3.68 (s, 3H), 3.70 (s, 3H), 3.80-4.07 (m, 5H), 4.24-4.39 (m, 4H), 4.42-4.75 (m, 8H), 4.78 (d, J = 3.3 Hz, 1H), 4.84 (d, J = 11.5 Hz, 1H), 6.06 (d, J = 8.6 Hz, 1H), 6.75 (m, 4H), 7.14-7.29 (m, 24H).

**Compound 24p.** Colorless oil (58 mg, 80%); [α]$^2_{D}$ +13.0 (c 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 0.86 (t, J = 6.6 Hz, 9H), 1.13-1.91 (m, 82H), 2.42 (m, 6H), 3.39-3.47 (m, 3H), 3.66-3.80 (m, 2H), 3.72 (s, 3H), 3.75 (s, 3H), 3.82-4.13 (m, 5H), 4.29-4.43 (m, 4H), 4.47-4.80 (m, 8H), 4.83 (d, J = 3.5 Hz, 1H), 4.90 (d, J = 11.3 Hz, 1H), 6.12 (d, J = 8.6 Hz, 1H), 6.80 (m, 4H), 7.19-7.34 (m, 24H).

**Compound 24q.** Colorless oil (56 mg, 84%); [α]$^2_{D}$ +12.3 (c 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) δ 0.88 (t, J = 6.8 Hz, 9H), 1.15-1.97 (m, 64H), 2.48 (m, 6H), 3.41-3.53 (m, 3H), 3.70-3.83 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.87-4.07 (m, 5H), 4.32-4.50 (m, 4H), 4.53-4.82 (m, 8H), 4.85 (d, J = 3.6 Hz, 1H), 4.93 (d, J = 11.4 Hz, 1H),
6.08 (d, $J = 8.4$ Hz, 1H), 6.82 (m, 4H), 7.21-7.39 (m, 24H).

**Compound 24r.** Colorless oil (53 mg, 77%); $[\alpha]_{D}^{24} +8.6$ (c 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J = 6.6$ Hz, 9H), 1.18-1.97 (m, 68H), 2.47 (m, 6H), 3.40-3.53 (m, 3H), 3.67-3.82 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.87-4.07 (m, 5H), 4.32-4.50 (m, 4H), 4.53-4.81 (m, 8H), 4.85 (d, $J = 3.6$ Hz, 1H), 4.92 (d, $J = 11.7$ Hz, 1H), 6.09 (d, $J = 8.7$ Hz, 1H), 6.82 (m, 4H), 7.21-7.38 (m, 24H).

**Compound 24s.** Colorless oil (55 mg, 79%); $[\alpha]_{D}^{24} +11.7$ (c 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J = 6.6$ Hz, 9H), 1.19-1.96 (m, 72H), 2.56 (m, 6H), 3.40-3.53 (m, 3H), 3.70-3.83 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.87-4.14 (m, 5H), 4.32-4.50 (m, 4H), 4.53-4.82 (m, 8H), 4.85 (d, $J = 3.6$ Hz, 1H), 4.92 (d, $J = 11.4$ Hz, 1H), 6.11 (d, $J = 8.7$ Hz, 1H), 6.82 (m, 4H), 7.21-7.39 (m, 24H).

**Compound 24t.** Colorless oil (59 mg, 80%); $[\alpha]_{D}^{24} +10.6$ (c 1.0, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 0.88 (t, $J = 6.6$ Hz, 9H), 1.20-1.96 (m, 84H), 2.46 (m, 6H), 3.40-3.53 (m, 3H), 3.67-3.83 (m, 2H), 3.75 (s, 3H), 3.77 (s, 3H), 3.87-4.15 (m, 5H), 4.32-4.50 (m, 4H), 4.53-4.82 (m, 8H), 4.85 (d, $J = 3.6$ Hz, 1H), 4.92 (d, $J = 11.4$ Hz, 1H), 6.09 (d, $J = 9.0$ Hz, 1H), 6.82 (m, 4H), 7.21-7.39 (m, 24H).
General procedure for the preparation of 3–22:

The amide compounds 24 (1.0 equiv) and Pd(OH)$_2$ (50% w/w) was suspended in a solution of EtOH/CH$_2$Cl$_2$ (3:1, 0.01–0.1 M). The reaction mixture was hydrogenated (1 atm) at RT. After 12 h, the palladium catalyst was removed by filtration through a syringe filter (PTFE, 0.50 µm, hydrophobic) and rinsed with EtOH/CH$_2$Cl$_2$ (3:1). The filtrate was concentrated in vacuo and purified with flash column chromatography to afford 3–22 (73–82 %).

**Compound 3.** White waxy solid (24 mg, 80%); [α]$^25_D$ +46.5 (c 0.5, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) δ 0.79-0.87 (m, 9H), 1.19-1.38 (m, 42H), 1.65 (m, 2H), 1.85 (m, 8H), 2.10 (m, 1H), 2.27 (m, 1H), 2.45 (t, $J = 6.9$ Hz, 2H), 3.02 (m, 6H), 4.29-4.68 (m, 10H), 5.21 (m, 1H), 5.56 (d, $J = 3.6$ Hz, 1H), 8.60 (d, $J = 8.4$ Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) δ 14.2, 14.3, 22.4, 22.8, 22.9, 27.1, 27.2, 28.1, 29.1, 29.4, 29.6, 29.7, 30.0, 31.5, 31.9, 32.1, 64.2, 68.8, 70.9, 76.1, 76.8, 101.8, 173.1; HRMS (FAB) calcd for C$_{45}$H$_{91}$O$_9$N$_2$ 803.6725 ([M+H]$^+$), found 803.6752.
**Compound 4.** White waxy solid (24 mg, 78%); $\left[\alpha\right]_{D}^{25} +47.8$ (c 0.3, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) δ 0.81-0.87 (m, 9H), 1.19-1.38 (m, 46H), 1.64 (m, 2H), 1.85 (m, 8H), 2.11 (m, 1H), 2.27 (m, 1H), 2.44 (t, $J$ = 6.9 Hz, 2H), 3.01 (m, 6H), 4.29-4.68 (m, 10H), 5.19 (m, 1H), 5.56 (d, $J$ = 3.6 Hz, 1H), 8.56 (d, $J$ = 8.4 Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) δ 14.7, 23.3, 23.4, 27.0, 27.8, 29.9, 30.1, 30.4, 30.6, 30.9, 32.4, 32.6, 34.9, 36.9, 52.0, 63.1, 70.8, 71.4, 72.1, 72.9, 73.5, 77.3, 101.9, 173.6; HRMS (FAB) calcd for C$_{47}$H$_{95}$O$_9$N$_2$ 831.7038 ([M+H$^+$]), found 831.7040.

**Compound 5.** White waxy solid (25 mg, 81%); $\left[\alpha\right]_{D}^{25} +44.4$ (c 0.5, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) δ 0.82-0.86 (m, 9H), 1.08-1.38 (m, 50H), 1.66 (m, 2H), 1.88 (m, 8H), 2.10 (m, 1H), 2.27 (m, 1H), 2.44 (t, $J$ = 5.7 Hz, 2H), 3.06 (m, 6H), 4.29-4.68 (m, 10H), 5.24 (m, 1H), 5.56 (d, $J$ = 3.6 Hz, 1H), 8.60 (d, $J$ = 8.4 Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) δ 14.7, 23.3, 23.4, 27.0, 27.3, 27.7, 29.2, 29.9, 30.1, 30.2, 30.4, 30.5, 30.6, 30.9, 32.5, 32.6, 35.0, 52.1, 53.3, 53.6, 63.1, 68.9, 70.8, 71.4, 72.1, 72.9, 73.5, 77.3, 101.9, 173.6; HRMS (FAB) calcd for C$_{49}$H$_{99}$O$_9$N$_2$ 859.7351 ([M+H$^+$]), found 859.7385.

**Compound 6.** White waxy solid (25 mg, 77%); $\left[\alpha\right]_{D}^{25} +46.7$ (c 0.5, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) δ 0.83-0.87 (m, 9H), 1.08-1.42 (m, 62H), 1.66 (m, 2H), 1.77-1.98 (m, 8H), 2.10 (m, 1H), 2.27 (m, 1H), 2.45 (t, $J$ = 6.9 Hz, 2H), 3.08 (m, 6H), 4.30-4.68 (m, 10H), 5.23 (m, 1H), 5.56 (d, $J$ = 3.9 Hz, 1H), 8.61 (d, $J$ = 8.4 Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) δ 14.3, 20.8, 22.9, 27.4, 29.6, 29.9, 31.5, 32.1, 34.2, 63.2, 68.3, 69.5, 71.8, 72.4, 77.4, 101.2, 172.7; HRMS (FAB) calcd for C$_{55}$H$_{111}$O$_9$N$_2$ 943.8290 ([M+H$^+$]), found 943.8311.
**Compound 7.** White waxy solid (25 mg, 79%); $[\alpha]^{25}_D$ +44.9 (c 0.3, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) $\delta$ 0.79-0.88 (m, 9H), 1.17-1.45 (m, 44H), 1.63 (m, 2H), 1.77 (m, 2H), 1.93 (m, 7H), 2.27 (m, 1H), 2.45 (m, 2H), 3.14 (m, 6H), 4.29-4.68 (m, 10H), 5.24 (m, 1H), 5.56 (d, $J = 3.9$ Hz, 1H), 8.67 (d, $J = 8.7$ Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) $\delta$ 14.2, 14.3, 22.7, 22.9, 23.7, 23.8, 26.0, 26.5, 26.8, 27.1, 28.9, 29.0, 29.6, 29.9, 30.0, 30.1, 30.4, 31.8, 32.1, 34.5, 51.5, 52.5, 52.8, 62.4, 70.3, 70.7, 71.6, 72.5, 72.9, 76.6, 101.2, 173.6; HRMS (FAB) calcd for C$_{46}$H$_{93}$O$_9$N$_2$ 817.6882 ([M+H]$^+$), found 817.6910.

**Compound 8.** White waxy solid (25 mg, 77%); $[\alpha]^{25}_D$ +47.8 (c 0.5, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) $\delta$ 0.80-0.86 (m, 9H), 1.18-1.45 (m, 48H), 1.63 (m, 2H), 1.75-1.88 (m, 9H), 2.25 (m, 1H), 2.48 (m, 2H), 3.09 (m, 6H), 4.28-4.67 (m, 10H), 5.23 (m, 1H), 5.55 (d, $J = 3.7$ Hz, 1H), 8.81 (d, $J = 8.4$ Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) $\delta$ 14.2, 14.3, 22.8, 22.9, 24.1, 26.1, 26.5, 26.9, 27.2, 29.0, 29.2, 29.4, 29.6, 29.9, 30.0, 30.1, 30.3, 31.9, 32.1, 34.5, 36.6, 51.5, 52.7, 53.0, 62.4, 68.4, 70.3, 70.8, 71.6, 72.4, 72.9, 76.6, 101.3, 173.5; HRMS (FAB) calcd for C$_{48}$H$_{97}$O$_9$N$_2$ 845.7195 ([M+H]$^+$), found 845.7177.

**Compound 9.** White waxy solid (24 mg, 79%); $[\alpha]^{25}_D$ +50.0 (c 1.0, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) $\delta$ 0.81-0.85 (m, 9H), 1.17-1.42 (m, 52H), 1.63 (m, 2H), 1.74-1.95 (m, 9H), 2.25 (m, 1H), 2.52 (m, 2H), 3.18 (m, 6H), 4.28-4.67 (m, 10H), 5.24 (m, 1H), 5.54 (d, $J = 3.6$ Hz, 1H), 8.94 (d, $J = 8.4$ Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) $\delta$ 14.3, 22.9, 23.0, 24.5, 26.1, 26.5, 27.0, 27.3, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 29.9, 30.0, 30.1, 30.4, 32.0, 32.1, 34.4, 36.6, 49.6, 51.5, 52.9, 53.1, 62.5, 68.5, 70.3, 70.9, 71.6,
Compound 10. White waxy solid (26 mg, 74%); $[\alpha]^{25}_D +43.4$ (c 0.3, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) $\delta$ 0.83-0.87 (m, 9H), 1.17-1.43 (m, 64H), 1.62 (m, 2H), 1.75-1.96 (m, 9H), 2.26 (m, 1H), 2.48 (m, 2H), 3.16 (m, 6H), 4.29-4.68 (m, 10H), 5.25 (m, 1H), 5.55 (d, $J = 3.6$ Hz, 1H), 8.83 (d, $J = 8.4$ Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) $\delta$ 14.3, 22.9, 26.2, 26.5, 27.5, 29.4, 29.6, 29.7, 29.9, 30.0, 30.1, 30.4, 32.1, 34.4, 36.7, 51.4, 62.6, 70.3, 70.9, 71.6, 72.4, 73.0, 76.7, 101.5, 173.2; HRMS (FAB) calcd for C$_{32}$H$_{62}$O$_8$N$_3$ 957.8447 ([M+H]$^+$), found 957.8463.

Compound 11. White waxy solid (25 mg, 77%); $[\alpha]^{25}_D +49.5$ (c 0.5, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) $\delta$ 0.78-0.87 (m, 9H), 1.14-1.42 (m, 46H), 1.65 (m, 2H), 1.77 (m, 2H), 1.89 (m, 6H), 2.10 (m, 1H), 2.26 (m, 1H), 2.45 (t, $J = 7.2$ Hz, 2H), 3.08 (m, 6H), 4.29-4.68 (m, 10H), 5.23 (m, 1H), 5.56 (d, $J = 3.6$ Hz, 1H), 8.54 (d, $J = 8.7$ Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) $\delta$ 14.2, 14.3, 22.8, 22.9, 26.1, 26.4, 27.2, 29.1, 29.6, 29.9, 30.0, 31.3, 31.9, 32.1, 34.7, 63.2, 71.6, 73.0, 77.2, 101.5, 173.2; HRMS (FAB) calcd for C$_{47}$H$_{95}$O$_9$N$_2$ 831.7038 ([M+H]$^+$), found 831.7028.

Compound 12. White waxy solid (25 mg, 80%); $[\alpha]^{25}_D +47.9$ (c 0.5, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) $\delta$ 0.80-0.87 (m, 9H), 1.15-1.43 (m, 50H), 1.66 (m, 2H), 1.78 (m, 2H), 1.89 (m, 6H), 2.10 (m, 1H), 2.26 (m, 1H), 2.45 (t, $J = 7.2$ Hz, 2H), 3.08 (m, 6H), 4.29-4.68 (m, 10H), 5.23 (m, 1H), 5.52 (d, $J = 3.6$ Hz, 1H), 8.54 (d, $J = 8.7$ Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) $\delta$ 14.7, 23.3, 23.4, 26.6, 27.0, 27.7, 29.5, 29.7, 29.8,
30.1, 30.4, 30.5, 30.6, 30.9, 32.6, 34.9, 37.1, 52.0, 53.2, 53.5, 63.1, 69.0, 70.8, 71.4, 72.1, 72.9, 73.5, 75.8, 77.3, 102.0, 173.7; HRMS (FAB) calcd for C_{49}H_{98}O_{9}N_{2} 859.7351 ([M+H]^+), found 859.7343.

**Compound 13.** White waxy solid (24 mg, 75%); \([\alpha]_{25}^{25}D +46.9\) (c 0.5, pyridine); \(^1\)H NMR (300 MHz, C\(_5\)D\(_5\)N) \(\delta\) 0.81-0.86 (m, 9H), 1.16-1.42 (m, 54H), 1.66 (m, 2H), 1.76 (m, 2H), 1.92 (m, 6H), 2.10 (m, 1H), 2.26 (m, 1H), 2.44 (t, \(J = 7.2\) Hz, 2H), 3.18 (m, 6H), 4.29-4.68 (m, 10H), 5.23 (m, 1H), 5.56 (d, \(J = 3.6\) Hz, 1H), 8.53 (d, \(J = 8.7\) Hz, 1H); \(^{13}\)C NMR (75 MHz, C\(_5\)D\(_5\)N) \(\delta\) 14.3, 22.9, 23.0, 23.9, 26.1, 26.5, 27.2, 29.2, 29.4, 29.6, 29.7, 29.9, 30.0, 32.0, 32.1, 34.5, 68.5, 70.3, 70.9, 71.6, 72.4, 76.8, 101.5, 173.2; HRMS (FAB) calcd for C\(_{51}\)H\(_{103}\)O\(_9\)N\(_2\) 887.7664 ([M+H]^+), found 887.7630.

**Compound 14.** White waxy solid (27 mg, 73%); \([\alpha]_{25}^{25}D +45.1\) (c 0.5, pyridine); \(^1\)H NMR (300 MHz, C\(_5\)D\(_5\)N) \(\delta\) 0.83-0.87 (m, 9H), 1.18-1.43 (m, 66H), 1.66 (m, 2H), 1.77 (m, 2H), 1.91 (m, 6H), 2.10 (m, 1H), 2.26 (m, 1H), 2.44 (t, \(J = 6.9\) Hz, 2H), 3.10 (m, 6H), 4.29-4.68 (m, 10H), 5.23 (m, 1H), 5.56 (d, \(J = 3.6\) Hz, 1H), 8.51 (d, \(J = 8.4\) Hz, 1H); \(^{13}\)C NMR (75 MHz, C\(_5\)D\(_5\)N) \(\delta\) 14.3, 22.9, 26.5, 27.3, 29.6, 29.8, 30.0, 31.4, 32.1, 34.2, 52.9, 70.9, 73.2, 101.5, 173.2; HRMS (FAB) calcd for C\(_{57}\)H\(_{115}\)O\(_9\)N\(_2\) 971.8603 ([M+H]^+), found 971.8629.

**Compound 15.** White waxy solid (24 mg, 75%); \([\alpha]_{25}^{25}D +46.1\) (c 0.5, pyridine); \(^1\)H NMR (300 MHz, C\(_5\)D\(_5\)N) \(\delta\) 0.78-0.84 (m, 9H), 1.18-1.45 (m, 48H), 1.66 (m, 2H), 1.79 (m, 2H), 1.85 (m, 6H), 2.10 (m, 1H), 2.29 (m, 1H), 2.44 (t, \(J = 7.4\) Hz, 2H), 3.04 (m, 6H), 4.29-4.70 (m, 10H), 5.29 (m, 1H), 5.59 (d, \(J = 3.7\) Hz, 1H), 8.57 (d, \(J = 8.1\) Hz,
1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) $\delta$ 14.5, 14.6, 23.1, 23.3, 27.5, 27.9, 29.4, 29.7, 29.9, 30.2, 30.3, 30.5, 30.7, 31.0, 32.2, 32.4, 33.7, 34.8, 51.8, 53.2, 53.5, 63.0, 66.0, 70.6, 71.3, 72.0, 72.8, 73.4, 77.1, 101.9, 173.5; HRMS (FAB) calcd for C$_{48}$H$_{96}$O$_9$N$_2$Na $\delta$ 867.7116 ([M+Na]$^+$), found 867.7042.

**Compound 16.** White waxy solid (25 mg, 82%); $[\alpha]_{D}^{25} +45.3$ (c 0.2, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) $\delta$ 0.80-0.84 (m, 9H), 1.14-1.44 (m, 52H), 1.62 (m, 2H), 1.78 (m, 2H), 1.85 (m, 6H), 2.10 (m, 1H), 2.27 (m, 1H), 2.42 (t, $J$ = 7.3 Hz, 2H), 3.06 (m, 6H), 4.30-4.71 (m, 10H), 5.27 (m, 1H), 5.58 (d, $J$ = 3.7 Hz, 1H), 8.55 (d, $J$ = 8.6 Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) $\delta$ 14.2, 22.4, 22.8, 25.8, 27.4, 29.9, 30.0, 30.6, 31.9, 34.7, 62.7, 63.2, 70.3, 73.4, 74.2, 77.6, 101.3, 173.2; HRMS (FAB) calcd for C$_{50}$H$_{101}$O$_9$N$_2$ 873.7508 ([M+H]$^+$), found 873.7523.

**Compound 17.** White waxy solid (24 mg, 74%); $[\alpha]_{D}^{25} +46.5$ (c 0.1, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) $\delta$ 0.81-0.86 (m, 9H), 1.12-1.40 (m, 56H), 1.58 (m, 2H), 1.78 (m, 2H), 1.87 (m, 6H), 2.10 (m, 1H), 2.27 (m, 1H), 2.42 (t, $J$ = 7.5 Hz, 2H), 3.03 (m, 6H), 4.30-4.67 (m, 10H), 5.26 (m, 1H), 5.58 (d, $J$ = 4.2 Hz, 1H), 8.55 (d, $J$ = 8.6 Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) $\delta$ 14.3, 22.9, 25.9, 29.5, 29.6, 30.0, 30.2, 32.0, 34.4, 52.1, 61.0, 63.2, 71.6, 72.4, 74.6, 77.2, 101.5, 173.2; HRMS (FAB) calcd for C$_{52}$H$_{105}$O$_9$N$_2$ 901.7821 ([M+H]$^+$), found 901.7820.

**Compound 18.** White waxy solid (26 mg, 73%); $[\alpha]_{D}^{25} +47.2$ (c 0.2, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) $\delta$ 0.83-0.86 (m, 9H), 1.13-1.45 (m, 68H), 1.54 (m, 2H), 1.78 (m, 2H), 1.90 (m, 6H), 2.10 (m, 1H), 2.27 (m, 1H), 2.44 (t, $J$ = 7.3 Hz, 2H), 3.09 (m,
6H), 4.30-4.67 (m, 10H), 5.27 (m, 1H), 5.58 (d, J = 3.6 Hz, 1H), 8.52 (d, J = 8.4 Hz, 1H); \(^{13}\)C NMR (75 MHz, C\(_5\)D\(_5\)N) \(\delta\) 14.3, 22.9, 27.8, 29.6, 29.9, 31.9, 32.1, 34.9, 63.7, 68.8, 70.8, 72.3, 72.6, 77.1, 101.3, 173.3; HRMS (FAB) calcd for C\(_{58}\)H\(_{117}\)O\(_9\)N\(_2\) 985.8760 ([M+H]\(^+\)), found 985.8783.

**Compound 19.** White waxy solid (27 mg, 81%); \([\alpha]^{25}_D +44.9\) (c 2.0, pyridine); \(^1\)H NMR (300 MHz, C\(_5\)D\(_5\)N) \(\delta\) 0.78-0.87 (m, 9H), 1.15-1.45 (m, 50H), 1.60 (m, 2H), 1.77 (m, 2H), 1.82 (m, 6H), 1.99 (m, 1H), 2.23 (m, 1H), 2.53 (t, J = 6.9 Hz, 2H), 3.17 (m, 6H), 4.29-4.67 (m, 10H), 5.26 (m, 1H), 5.54 (d, J = 3.6 Hz, 1H), 8.92 (d, J = 8.4 Hz, 1H); \(^{13}\)C NMR (75 MHz, C\(_5\)D\(_5\)N) \(\delta\) 14.2, 22.8, 22.9, 24.1, 26.3, 26.5, 27.1, 27.2, 29.3, 29.5, 29.6, 29.9, 30.0, 30.4, 31.9, 32.1, 34.4, 36.8, 49.7, 51.5, 52.8, 62.6, 68.6, 70.3, 70.9, 71.6, 72.4, 73.0, 76.8, 101.5, 122.8, 173.2; HRMS (FAB) calcd for C\(_{49}\)H\(_{98}\)O\(_9\)N\(_2\)Na 881.7272 ([M+Na]\(^+\)), found 881.7175.

**Compound 20.** White waxy solid (25 mg, 79%); \([\alpha]^{25}_D +53.0\) (c 1.0, pyridine); \(^1\)H NMR (300 MHz, C\(_5\)D\(_5\)N) \(\delta\) 0.77-0.90 (m, 9H), 1.12-1.48 (m, 54H), 1.62 (m, 2H), 1.80 (m, 2H), 1.98 (m, 7H), 2.26 (m, 1H), 2.53 (t, J = 7.2 Hz, 2H), 3.18 (m, 6H), 4.29-4.66 (m, 10H), 5.26 (m, 1H), 5.55 (d, J = 3.7 Hz, 1H), 8.90 (d, J = 8.7 Hz, 1H); \(^{13}\)C NMR (75 MHz, C\(_5\)D\(_5\)N) \(\delta\) 14.2, 22.8, 22.9, 24.1, 26.3, 26.5, 27.1, 27.2, 29.3, 29.5, 29.6, 29.9, 30.0, 31.9, 32.1, 34.4, 36.7, 49.6, 51.4, 52.7, 52.9, 62.4, 68.4, 70.2, 70.8, 71.5, 72.4, 72.8, 76.6, 101.3, 173.5; HRMS (FAB) calcd for C\(_{51}\)H\(_{103}\)O\(_9\)N\(_2\) 887.7664 ([M+H]\(^+\)), found 887.7693.

**Compound 21.** White waxy solid (24 mg, 73%); \([\alpha]^{25}_D +46.0\) (c 1.0, pyridine); \(^1\)H
NMR (300 MHz, C$_5$D$_5$N) $\delta$ 0.80-0.86 (m, 9H), 1.12-1.44 (m, 58H), 1.62 (m, 2H), 1.80 (m, 2H), 1.98 (m, 7H), 2.27 (m, 1H), 2.49 (t, $J = 7.5$ Hz, 2H), 3.14 (m, 6H), 4.30-4.69 (m, 10H), 5.26 (m, 1H), 5.57 (d, $J = 3.6$ Hz, 1H), 8.42 (d, $J = 8.6$ Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) $\delta$ 14.2, 14.3, 22.9, 26.3, 26.5, 27.3, 29.4, 29.5, 29.6, 29.7, 29.9, 30.0, 30.1, 30.4, 32.0, 32.1, 34.4, 36.7, 51.4, 53.0, 53.1, 62.6, 68.5, 70.3, 70.9, 71.6, 72.4, 73.0, 76.7, 101.5, 173.2; HRMS (FAB) calcd for C$_{53}$H$_{107}$O$_9$N$_2$ 915.7977 ([M+H]$^+$), found 915.7949.

**Compound 22.** White waxy solid (28 mg, 76%); $\left[\alpha\right]_{D}^{25}$ $^{+}$42.4 (c 1.0, pyridine); $^1$H NMR (300 MHz, C$_5$D$_5$N) $\delta$ 0.83-0.87 (m, 9H), 1.17-1.42 (m, 64H), 1.61 (m, 2H), 1.78 (m, 2H), 1.99 (m, 7H), 2.24 (m, 1H), 2.53 (t, $J = 6.9$ Hz, 2H), 3.20 (m, 6H), 4.29-4.68 (m, 10H), 5.25 (m, 1H), 5.54 (d, $J = 3.9$ Hz, 1H), 8.92 (d, $J = 8.7$ Hz, 1H); $^{13}$C NMR (75 MHz, C$_5$D$_5$N) $\delta$ 14.3, 22.9, 24.2, 24.3, 26.7, 26.5, 27.2, 27.3, 29.3, 29.4, 29.5, 29.6, 29.7, 29.8, 29.9, 30.0, 30.1, 30.4, 32.1, 34.4, 36.7, 49.6, 51.4, 52.7, 53.0, 62.5, 68.5, 70.3, 70.9, 71.6, 72.4, 73.0, 76.7, 101.5, 173.2; HRMS (FAB) calcd for C$_{59}$H$_{118}$O$_9$N$_2$ 999.8916 ([M+H]$^+$), found 999.8951.
C. $^1$H and $^{13}$C NMR Spectra of Selected Compounds
D. References of Supporting Information


