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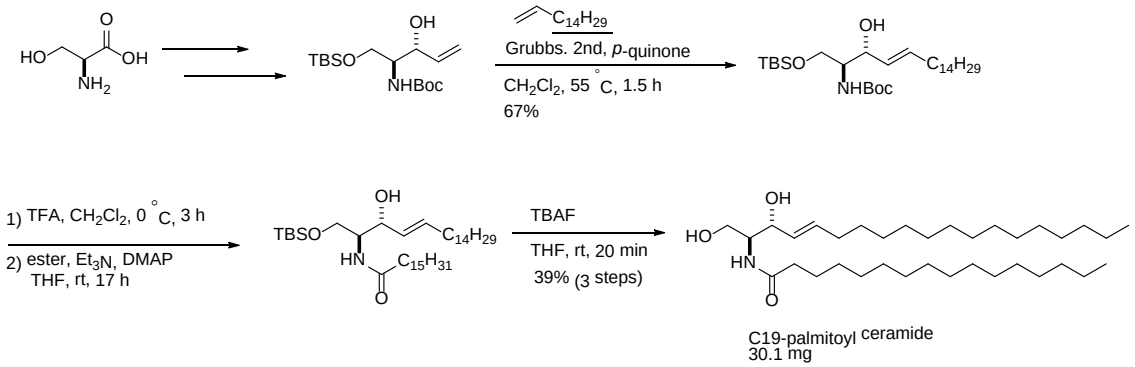
Supplemental Information

The Long-Chain Sphingoid Base of Ceramides Determines Their Propensity for Lateral Segregation

Md. Abdullah Al Sazzad, Tomokazu Yasuda, Michio Murata, and J. Peter Slotte

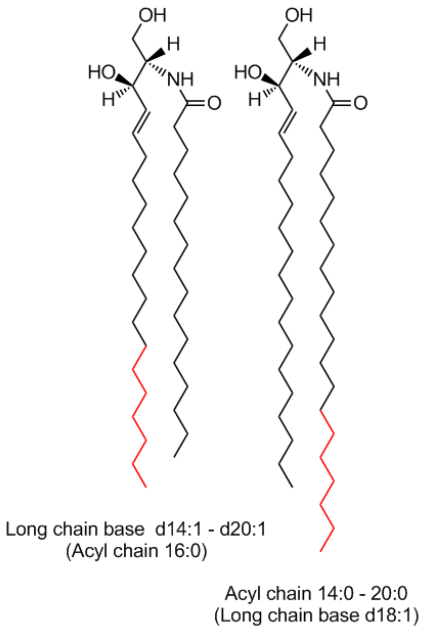
Synthesis of d19:1/16:0 ceramide



To a solution of olefin **2** in CH_2Cl_2 was added 1-hexadecene and Grubbs catalyst 2nd generation at room temperature. After the reaction mixture was stirred for 2h under reflux, solvent was removed in vacuo to give crude products. Column chromatography on silica gel gave **3** as a colorless oil.

1-hexadecene was synthesized from 1-pentadecanol by oxidation and Wittig reaction.

To a solution of **3** in CH_2Cl_2 was added trifluoroacetic acid at 0°C. After the reaction mixture was stirred for 3 h at 0°C, the reaction was quenched with Et3N, and the resulting mixture was evaporated to give the crude amine, which was subjected to the next reaction without further purification. To a solution of the crude amine in THF were added DMAP, and *p*-nitrophenyl hexadecanoate. After the reaction mixture was stirred for 17 h at room temperature, the mixture was evaporated. Purification by silica gel column chromatography afforded TBS-protected ceramide as a white solid. To a solution of the protected ceramide in THF was added TBAF at 0°C. After the reaction mixture was stirred for 20 min at room temperature, H_2O was added to the solution and the solvent was evaporated. Purification by silica gel column chromatography afforded C19-palmitoyl ceramide as a white solid. Molecular identity was verified by mass spectrometry (LQT-Orbitrap XL) and NMR (Jeol ECS 400).



SCHEME S1. Long-chain base and acyl chain length ceramide analog used in the study. The long-chain base length varied between 14 and 20 carbons (with 16:0 as *N*-linked acyl chain), and the *N*-linked acyl chain varied between 14 and 20 carbons (with d18:1 as long-chain base).

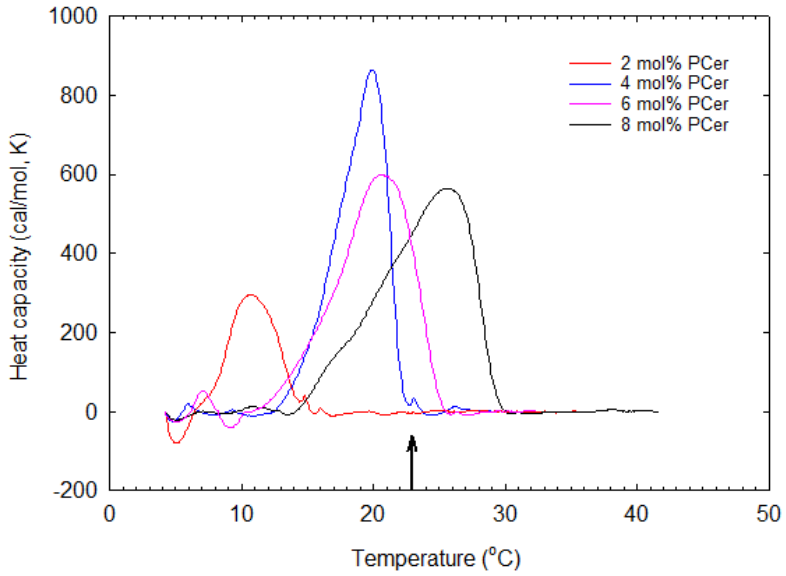


FIGURE S1. DSC analysis of ceramide gel phase melting as a function of ceramide concentration. The heating scan rate was 1 °C/min, and only upscan data are shown. The arrow indicate 23 °C, the temperature of tPA fluorescence lifetime analysis in Fig 1, allowing for easy comparison of DSC data with tPA fluorescence lifetime data.

TABLE S1. Main transition temperature (T_m) of some hydrated ceramide analogs. T_m values given are for heating scans (1 °C /min).

Ceramide	T_m (°C)
d18:1/12:0	85.7[a]
d18:1/16:0	91.5[a]
d18:1/18:0	91.1[a]
d18:1/20:0	92.6
d15:1/16:0	82.4
d16:1/16:0	84.8
d17:1/16:0	87.8
d18:1/16:0	91.5
d20:1/16:0	96.4

Values marked [a] are taken from (12)

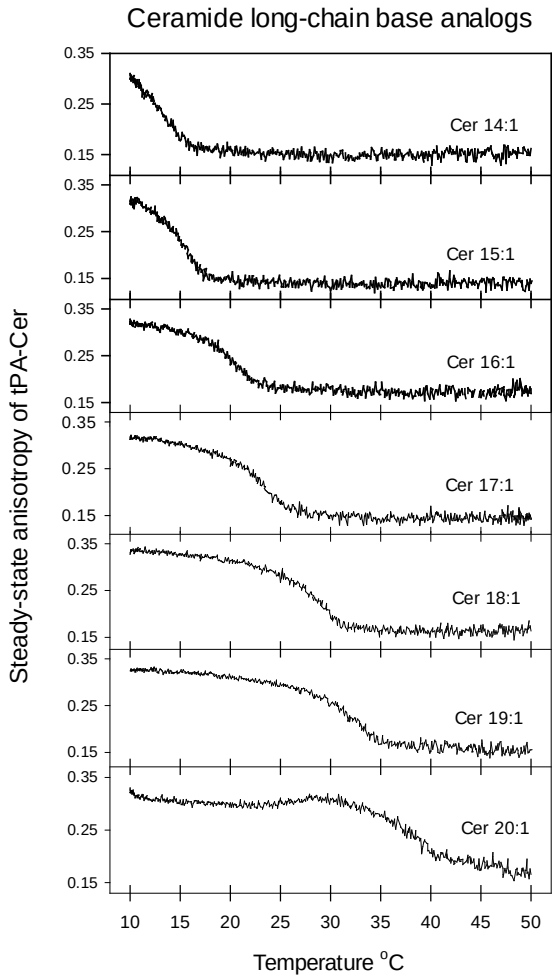


FIGURE S2. tPA-Cer anisotropy in bilayers containing ceramide long-chain base sanalogs. The temperature scan rate was 5 °C/min, and curves are representative of 2-3 replicates.

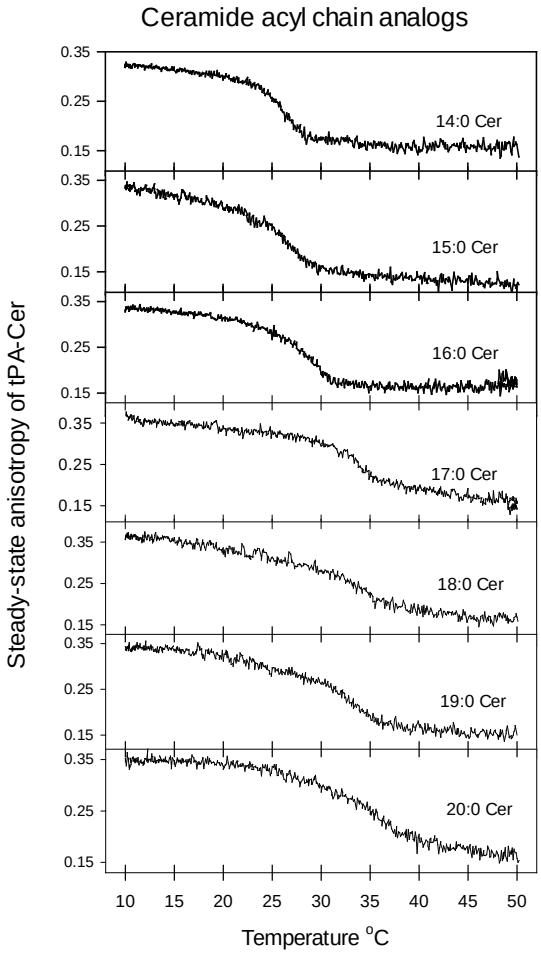


FIGURE S3. tPA-Cer anisotropy in bilayers containing ceramide acyl-chain analogs. The temperature scan rate was 5 °C/min, and curves are representative of 2-3 replicates.

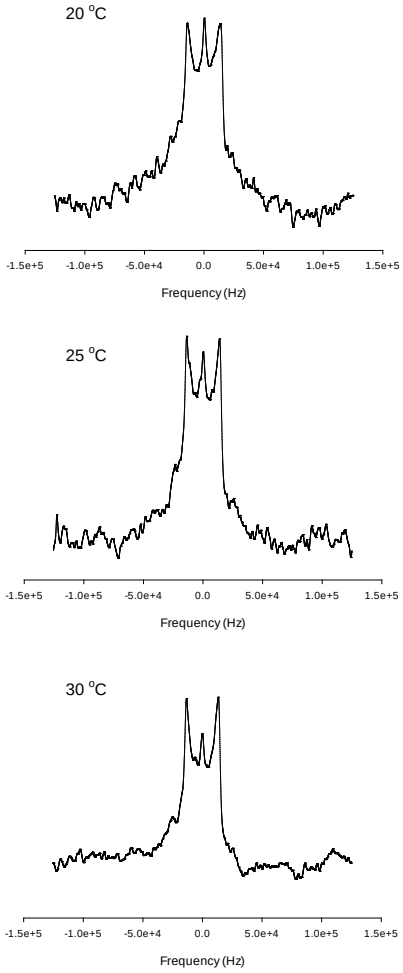


FIGURE S4. ^{2}H NMR spectra of long-chain base deuterated (d2) stearoyl ceramide. The composition was 10 mol% stearoyl ceramide in POPC, and the temperature 20, 25 and 30 °C. The ceramide was deuterated (d2) at the 10 position of the long-chain base.

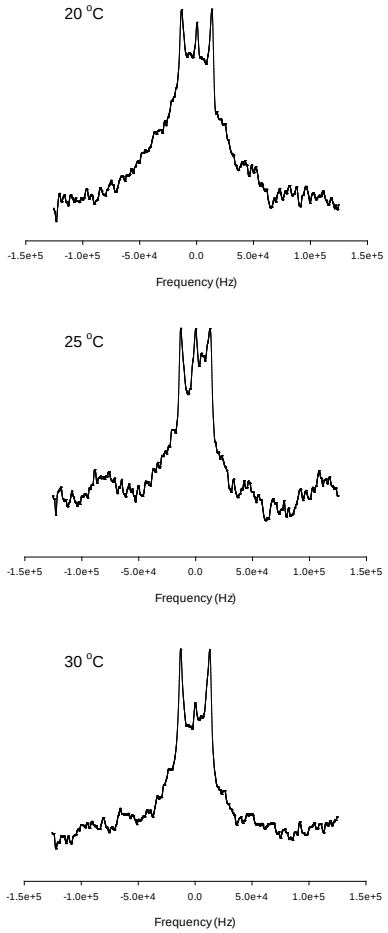


FIGURE S5. ^{2}H NMR spectra of acyl chain deuterated (d2) stearoyl ceramide. The composition was 10 mol% stearoyl ceramide in POPC, and the temperature 20, 25 and 30 °C. The ceramide was deuterated (d2) at the 12 position of the acyl chain.

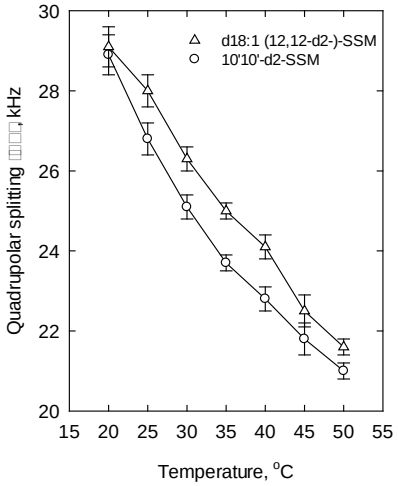


FIGURE S6. Quadrupolar splitting of site-deuterated long-chain base or acyl chain in stearoyl sphingomyelin (SSM, 10 mol%) in POPC (90 mol%) bilayers. The sphingosine long-chain base was site-deuterated (d2) at carbon 10, or the stearoyl acyl chain at carbon 12. The $\Delta\nu$ was determined as a function of temperature for the two SSM analogs.

Supplemental reference

12. Maula, T., M. A. Al Sazzad, and J. P. Slotte. 2015. Influence of Hydroxylation, Chain Length, and Chain Unsaturation on Bilayer Properties of Ceramides. Biophys J 109: 1639-1651.