Molecular-scale structural and functional characterization of sparsely tethered bilayer membranes

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Supplementary Information

Synthesis of 20-tetradecyloxy-3, 6, 9, 12, 15, 18, 22-heptaoxahexatricontane-1-thiol (WC14).

Silica gel (7024-2, 40 μm for Flash chromatography) was purchased from Malinckrodt, Inc. (Paris, KY); all other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI). Tetrahydrofuran (THF) and hexamethylphosphoramide (HMPA) were dried by distillation from calcium hydride, thioacetic acid was distilled under nitrogen; all other materials were of analytical reagent quality and used as received. The THF was distilled immediately before use; the HMPA was distilled and stored under nitrogen over 3Å molecular sieves. With one exception indicated below**,** all compounds were purified by chromatography (silica gel; SiO_2) to > 98% purity, determined by thin-layer chromatography (TLC) analysis (one spot by TLC). Structural assignments were made from proton (^{1}H) nuclear magnetic resonance (NMR), and, where appropriate, infrared (IR), low resolution mass spectroscopy (LRMS), and high resolution mass spectroscopy (HRMS). All reactions were carried out under nitrogen (N_2).

II: 3, 6, 9, 12, 15, 18-hexaoxaheneicos-20-en-1-ol [$HO(CH_2CH_2O)_6CH_2CH=CH_2$]. To a stirred mixture of 0.60 g (25 mmol) of sodium hydride (NaH) in 15 mL THF was added 14.12 g (50 mmol) **I** in 50 mL THF at 0°C [vigorous evolution of H_2]. The heterogeneous mixture was allowed to warm to room temperature and 3.02 g (25 mmol) allyl bromide in 35 mL THF was added dropwise. The slightly colored mixture was refluxed for 3 h. After cooling, water (1 mL) was slowly added, to remove excess NaH, and the water/THF was removed under reduced pressure and the residue was partitioned between chloroform ($CHCl_3$; 50 mL) and water (50 mL). The water further extracted with $CHCl_3$ (2 x 50 mL) and the combined $CHCl_3$ layers were washed with brine (50 mL), dried ($MgSO_4$), and concentrated. Chromatography (330 g, 55 cm x 5.5 cm; 20% methanol/ethyl acetate) afforded 4.8 g (60%) of **II** as a colorless liquid.

^{1}H NMR (270 MHz, $CDCl_3$): δ 5.92 (ddt, 1H, $CH_ACH_B{=}\mathbf{CH}_CCH_{2(D)}O$-, where H_A and H_B are *cis* and *trans* to H_C, respectively, J_{CA} = 10.4 Hz, J_{CB} = 17.1 Hz, J_{CD} = 5.7 Hz), 5.27 (dd, 1H, $CH_A\mathbf{CH}_B{=}CH_CCH_{2(D)}O$-, J_{BD} = 1.7 Hz, J_{BC} = 17.1 Hz), 5.18 (dd, 1H, $\mathbf{CH}_ACH_B{=}CH_CCH_{2(D)}O$-, J_{AD} = 1.7 Hz, J_{AC} = 10.4 Hz), 4.03 (dt, 2H, $CH_ACH_B{=}CH_C\mathbf{CH}_{2(D)}O$-, J_{DC} = 5.7 Hz, $J_{D(A\text{ and }B)}$ = 1.7 Hz), 3.75 to 3.58 [m, $(C\mathbf{H}_2C\mathbf{H}_2O)_6$].

LRMS (FAB): m/z 323 (M + H).

III: 1-(2'-tetrahydropyranyl)-3, 6, 9, 12, 15, 18-hexaoxaheneicos-20-en-1-ol [$\mathbf{RO(CH_2CH_2O)_6CH_2CH{=}CH_2, R = C_5H_9O}$]. To a mixture of 4.7151 g (14.6 mmol) **II** and 6.15 g (73.13 mmol) 3,4-dihydro-2H-pyran in 100 mL $CHCl_3$ at < 5° C was added 0.0281 g (0.148 mmol) p-toluenesulfonic acid monohydrate (p-$CH_3C_6H_4SO_3H \cdot H_2O$). After an additional 10 min, the mixture was allowed to warm to room temperature and stirred overnight. The reaction contents were then poured onto 150 mL water. The water was further extracted with $CHCl_3$ (2 x 100 mL). The combined $CHCl_3$ layers were washed with aqueous $NaHCO_3$ (150 mL), dried ($MgSO_4$), and concentrated. Chromatography (245 g SiO_2, 10% methanol/ethyl acetate) afforded 3.856 g (62%) of **III**.

^{1}H NMR (270 MHz, $CDCl_3$): δ all the characteristics of **II** plus 4.63 (m, 1 H, 2'**H** of C_5H_9O), 3.95 to 3.80 (m, 2H, -C(6')$\mathbf{H}_2$ of C_5H_9O), and 1.9 to 1.4 (br. m, 6H, C(3', 4', and 5')$\mathbf{H}_2$ of C_5H_9O).

IV: 1-(2'-tetrahydropyranyl)-3, 6, 9, 12, 15, 18-hexaoxaheneicosane-1, 20, 21-triol [$\mathbf{RO(CH_2CH_2O)_6CH_2CH(OH)CH_2OH, R = C_5H_9O}$]. To a mixture of 3.673 g (9.03 mmol) of

III and 2.1171 g (18.05 mmol) of 4-methylmorpholine-N-oxide in 120 mL of water/acetone (1/8, v/v) was added 0.0258 g (0.09 mmol) osmium tetroxide (OsO_4). After 17 h, 30 mL of a saturated aqueous sodium bisulfite ($NaHSO_3$) solution was added followed by removal of the acetone under reduced pressure. The aqueous residue was continually extracted with ethyl acetate for 22 h. The ethyl acetate was washed with brine (100 mL), dried ($MgSO_4$), and concentrated to give 3.98 g (82%) crude **IV**, which was used without further purification.

^{1}**H NMR** (270 MHz, $CDCl_3$): δ 4.63 (m, 1 H, 2'**H** of C_5H_9O), 3.95 to 3.80 (m, 2H, $C(6')\mathbf{H}_2$ of C_5H_9O), 3.75 to 3.58 [m, $(\mathbf{CH}_2\mathbf{CH}_2O)_6$], 1.9 to 1.4 (br. m, 6H, $C(3',4'$, and $5')\mathbf{H}_2$ of C_5H_9O).

V: 1-(2'-tetrahydropyranyl), 20-tetradecyloxy-3, 6, 9, 12, 15, 18, 22-heptaaoxahexatricontan-1-ol [$\mathbf{RO(CH_2CH_2O)_6CH_2CH(OC_{14}H_{29})CH_2OC_{14}H_{29}}$, R = $\mathbf{C_5H_9O}$]. A mixture of 0.9116 g (2.07 mmol) **IV** and 1.26 g (22.5 mmol) KOH were refluxed in 100 mL benzene in a flask equipped with a Dean-Stark trap for 5 h. After cooling, 1.5379 g (5.26 mmol) of tetradecyl mesylate ($C_{14}H_{29}OSO_2CHJ_3$, M.p. 41.5° – 44° C), prepared from the reaction of tetradecanol and mesyl chloride, was added and the mixture refluxed for 9 h. TLC analysis of the cooled reaction mixture indicated the complete absence of **IV.** Water (50 mL) was then added. The water was extracted with benzene (2 x 30 mL), the benzene layers were combined, washed with brine (30 mL), dried ($MgSO_4$), and concentrated. Chromatography (92 g SiO_2, 33 x 3 cm) afforded 1.10 g (64%) of **V** as a liquid.

^{1}**H NMR** (270 MHz, $CDCl_3$): δ all the characteristics of **IV** plus 1.26 (br. s, $OCH_2CH_2(\mathbf{CH}_2)_{11}CH_3$, 0.87 (br. t, $OC_{13}H_{26}\mathbf{CH}_3$).

VI: 20-tetradecyloxy-3, 6, 9, 12, 15, 18, 22-heptaaoxahexatricontan-1-ol [$\mathbf{HO(CH_2CH_2O)_6CH_2CH(OC_{14}H_{29})CH_2OC_{14}H_{29}}$]. A solution of 1.10 g of **V** in 28 mL of acetic acid/THF/water (4/2/1, v/v/v) was stirred at room temperature for 2 h. Chromatography (95 g SiO_2, 34 x 3 cm, 5% methanol/ethyl acetate), afforded 0.918 g (94%) **VI**.

^{1}H NMR (270 MHz, $CDCl_3$): δ 3.8 to 3.4 [$(C\mathbf{H}_2C\mathbf{H}_2O)_6$ + $2(OC\mathbf{H}_2C_{13}H_{27})$], 2.75 (br. s, 0.7H, **H**O-), 1.54 (m, 4H, $-OCH_2C\mathbf{H}_2C_{12}H_{25}$), 1.25 (br. s, $-OCH_2CH_2(C\mathbf{H}_2)_{11}CH_3$), 0.88 (br. t, 6H, $-OC_{13}H_{26}C\mathbf{H}_3$).

LRMS (FAB): m/z 871 (M + K), 855 (M + Na).

VII: 1-bromo-20-tetradecyloxy-3, 6, 9, 12, 15, 18, 22-heptaaoxahexatricontane [$BrCH_2CH_2(CH_2CH_2O)_5CH_2CH(OC_{14}H_{29})CH_2OC_{14}H_{29}$]. A mixture of 0.918 g (1.26 mmol) **VI** and 0.49 g (1.88 mmol) trifluoroacetic anhydride was stirred in THF for 1 h. The volatiles were removed under reduced pressure and then *in vacuo* to remove all traces of trifluoroacetic acid. The residue was then dissolved in 50 mL THF/HMPA (1/1, v/v) and 0.6239 g (7.18 mmol) dry lithium bromide (LiBr) added. The mixture was refluxed overnight then partitioned between hexanes and water. The hexanes solubles were chromatography (90 g SiO_2, 33 cm x 3 cm, 50% hexanes/ethyl acetate) to afford 0.87 g (86%) **VII**.

^{1}H NMR (270 MHz, $CDCl_3$): δ all the characteristics of **VI** plus 3.81 (t, 2H, $BrC\mathbf{H}_2CH_2$-, J = 7.2 Hz).

<u>WC14</u> 20-tetradecyloxy-3, 6, 9, 12, 15, 18, 22-heptaoxahexatricontane-1-thiol [$HSCH_2CH_2(CH_2CH_2O)_5CH_2CH(OC_{14}H_{29})CH_2OC_{14}H_{29}$]: A solution of 0.874 g (1.08 mmol) **VII** and 27 mL of 0.118 mol/L sodium thiolacetate ($CH_3COS^-NA^+$), prepared from the addition of thioacetic acid to a sodium methoxide/methanol solution, was deoxygenated (evacuated then flushed with nitrogen three times) then refluxed overnight. After cooling, the solvent was removed under reduced pressure and 5 mL 5% HCl added. The acidic aqueous layer was extracted with ethyl acetate (3 x 50 mL), which were combined, dried ($MgSO_4$), and concentrated to give 0.676 g crude **WC14**. Chromatography (90 g, 33 cm x 3 cm, 25% hexanes/ethyl acetate) afforded 0.488 g (82%) **WC14**.

^{1}H NMR (270 MHz, $CDCl_3$): δ 3.8 to 3.5 [m, 37H, $(C\mathbf{H}_2C\mathbf{H}_2O)_5 + 2(OC\mathbf{H}_2C_{13}H_{27})$], 3.42 (t, 2H, $H_ASCH_{2(B)}C\mathbf{H}_{2(C)}O$- , J_{CB} = 6.4 Hz), 2.70 (dt, 2H, $H_ASC\mathbf{H}_{2(B)}CH_{2(C)}O$- , J_{BA} = 6.4 Hz, J_{BC} = 8.3 Hz), 1.59 (t, $\mathbf{H}_ASCH_{2(B)}CH_{2(C)}O$- J_{AB} = 8.3 Hz) not resolved from 1.56 (m, 1.55 (m, $-OCH_2C\mathbf{H}_2C_{12}H_{25}$) total integration from 1.65 to 1.45 = 5 H, 1.25 (br. s, $-OCH_2CH_2(C\mathbf{H}_2)_{11}CH_3$), 0.88 (br. t, 6H, $-OC_{13}H_{26}C\mathbf{H}_3$).

LRMS (FAB): m/z (M + Na) 787.5. **HRMS (FAB): m/z** (M + H) 765.6235. Calcd. for $C_{43}H_{89}O_8S$ 765.6278.