Supporting information for

Singular interaction between an anti-metastatic agent and the lipid bilayer: the Ohmline case.

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Supporting Information
Synthesis of deuterated Ohmline at the sn2-position; NMR spectra in solution of the intermediates and CD3-Ohmline; MD starting conformation and parameters developing.

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1. Synthesis of CD₃-Ohmline

General

The Solvents were dried with a solvent purification system (MBraun-SPS). All compounds were fully characterized by liquid state NMR: ¹H (500.13 or 400.133 or 300.135 MHz; number of scan: 16; Pulse: 7.25 μs; P1: 1 db; recycling delay: 2s; Pulse: 21.40 μs; Channel f2 (proton decoupling): Waltz16; PCPD2: 80 μs) and ¹³C (125.773 or 75.480 MHz; JMOD sequence NS: X to Y; channel f1: p1 10.7 μs; P2 21.40 μs; Channel f2 (proton decoupling): Waltz16; PCPD2: 80 μs) with Bruker spectrometers (AC 300, Avance DRX 400 or Avance DRX 500). Coupling constants J are given in Hertz. Abbreviations used: s for singlet, d doublet, t triplet, q quadruplet, qt quintuplet, m for multiplet and dt for doublet of triplets. ¹H homonuclear COSY (500.132 MHz; number of scan: 32; P16: 100 ms; P0: 7.30 μs; P1: 7.50 μs; recycling delay: 1.50 s), ¹³C heteronuclear HMQC (125.769 MHz; number of scan: 48; P16: 100 ms; channel f1 P1: 7.50 μs; P2: 15.00 μs; channel f2: P3: 12.85 μs; PCPD2: 70.00 μs; recycling delay: 2s) and HMBC (125.774 MHz; number of scan: 96; P16: 100 ms; channel f1 P1: 7.50 μs; P2: 15.00 μs; channel f2: P3: 12.85 μs; recycling delay: 2s) were used to unambiguously establish molecular structures when needed.

Compounds 4¹ and 5² were prepared according to reported procedures.

Synthesis of Lactose hepta-O-acetyl trichloroacetimidate 1

To a stirred solution of 4 (6.27 g, 9.85 mmol, 1.0 eq.) in dry CH₂Cl₂ (100 mL) is added trichloroacetonitrile (5.43 g, 54.2 mmol, 5.5 eq.) and potassium carbonate (1.5 g, 10.8 mmol, 1.1 eq.). The mixture is stirred overnight at room temperature, then filtrated over Celite and concentrated to give the crude compound 1. The product is purified on chromatography on silica gel (Petroleum spirit/Ethyl acetate (1: 1)) to give the pure 1 (31 % yield).

Rf (Petroleum spirit/ Ethyl acetate (1: 1)): 0.43

NMR ¹H (CDCl₃, 399.972): 8.64 (s, 1 H, NH); 6.44 (d, 1 H, ³JHH = 3.6 Hz, H₃); 5.51 (t, 1 H, ³JHH = 8.8 Hz, H₂); 5.32-5.30 (m, 2 H, H₅+H₆); 5.27-5.22 (m, 1 H, H₂); 5.15-5.01 (m, 3 H); 4.93-4.91 (dd, 1 H, ³JHH = 10.6 ³JHH = 3.2, H₃); 4.14-4.46 (m, 2 H, H₅+H₆); 4.09-4.06 (m, 3 H, J); 3.88-3.87 (J); 2.11-1.92 (m, 21 H, 7 OCH₃)

Synthesis of 1-O-hexadecyl-2-O-methyl-d₅-3-O-trityl-glycerol 6
To a stirred solution of NaH (88 mg, 3.68 mmol, 1.4 eq.) in dry THF (7 mL) is added 5 (1.47 g, 2.63 mmol, 1.0 eq.) in solution in dry THF (3 mL) dropwise. The mixture is stirred 1 hour and CD3I (245 µL, 3.95 mmol, 1.5 eq.) in solution in dry THF (3 mL) is added dropwise. The reaction mixture is stirred at reflux 1h30 then at room temperature overnight. The reaction is quenched by addition of water (few mL) and the solvent is removed. The oil is dissolved in diethyl ether (20 mL). The organic layer is washed three times with an aqueous saturated NaCl solution (3 x 5 mL), dried over MgSO4, filtered and concentrated to give the crude compound 6 which is used without purification.

1H (CDCl3, 399.972): 7.48-7.24 (m, 15 H, CHPhenyl); 3.61-3.21 (m, 5 H, CH2sn-2 + CH2sn-1 + CH2sn-3); 3.23 (t, 2 H, 3JHH = 4.8 Hz, CH2α fatty chain); 1.60-1.55 (m, 2 H, CH2β fatty chain); 1.38-1.23 (m, 26 H, CH2 fatty chain); 0.92 (t, 3 H, 3JHH = 6.8 Hz, CH3 fatty chain)

Synthesis of 1-O-hexadecyl-2-O-methyl-glycerol 2

To a stirred solution of MeOH/CHCl3 (1: 1, 30 mL) is added HCl conconcentrated (200 µL, 2.41 mmol, 1.0 eq.). At 0°C 6 (1.38 g, 2.41 mmol, 1.0 eq.) in solution in MeOH/CHCl3 (1: 1, 30 mL) is added dropwise. The mixture is stirred 15 hours at 0°C. An aqueous saturated NaHCO3 solution (15 mL) is added and the mixture is stirred 15 minutes at room temperature. The aqueous layer is extracted twice with CHCl3 (2 x 30 mL) and the combined organic layers are washed three time with an aqueous saturated NaCl solution (3 x 20 mL). The organic layer is dried upon MgSO4, filtered and concentrated to give the crude compound 2. The product is purified on chromatography on silica gel (Petroleum spirit/Ethyl acetate (8:2)) to give the pure 2 (39 % overall yield).

Rf (Petroleum spirit/Ethyl acetate (8:2)): 0.44

1H (CDCl3, 399.972): 3.72 (ABX, part A, dd, 1 H, 2J[Ha-Hb]sn-3= 11.4 3J[Ha-Ha sn-3-H sn-2]= 4.2, H2 CH2 sn-3); 3.61 (ABX, part B, dd, 1 H, 2J[Ha-Hb]sn-3= 11.5 3J[Ha-Ha sn-3-H sn-2]= 5.2, H2 CH2 sn-3); 3.55-3.47 (m, 2 H, CH2 sn-1) 3.44-3.39 (m, 3 H, CH sn-2 + CH2 α fatty chain); 2.25 (brs, 1 H, OH); 1.58-1.51 (qt, 2 H, 3JHH = 6.9, CH2 β fatty chain); 1.32-1.20 (m, 26 H, CH2 fatty chain); 0.86 (t, 3 H, 3JHH = 7.0 Hz, CH3 fatty chain)

13C (CDCl3, 75.475): 79.9 (CH sn-2); 72.0 (CH2 α fatty chain); 70.7 (CH2 sn-1); 62.7 (CH2 sn-3); 32.0 (CH2 fatty chain); 29.8 (CH2 fatty chain); 29.7 (CH2 fatty chain); 29.5 (CH2 fatty chain); 29.4 (CH2 fatty chain); 26.1 (CH2 fatty chain); 22.8 (CH2 fatty chain); 14.2 (CH3 fatty chain)

2. NMR Spectra in solution
Figure S1: $^1$H NMR in CDCl$_3$ of compound 1
Figure S2: $^1$H NMR in CDCl$_3$ of compound 6
Figure S3: $^1$H NMR in CDCl$_3$ of compound 2
Figure S4: $^1$H NMR in CDCl$_3$ of compound 3
Figure S5: $^{13}\text{C}$ NMR in CDCl$_3$ of compound 3
Figure S6: $^1\text{H} \text{NMR}$ in DMSO-$d_6$ of compound D3-Ohmline
Figure S7: $^2$D NMR with D2O probe of compound D3-Ohmline
Figure 8: $^{13}$C NMR in DMSO-<i>d</i>6 of compound D3-Ohmline
Figure S9: 2D NMR COSY in DMSO-d6 of compound D3-Ohmline
Figure S10: 2D NMR HMQC in DMSO-d6 of compound D3-Ohmline
Figure S11: 2D NMR HMBC in DMSO-d6 of compound D3-Ohmline
3. MD parameter development

The parameters used for OHM were developed from scratch since there are not yet published any for this molecule.

As it was previously described, the OHM molecule contains a sugar head (β-lactose) connected to the glycerol group linked to a methyl group and a palmitoyl-acyl chain (the SN2 and SN1 substituents respectively). Therefore the parameters used for the OHM force field were taken from two recognized force fields for sugars and lipids. There many sugar force fields developed for explicit solvent simulation of hexopyranose-based carbohydrates, many of those being improvements of previous versions of the same force field. The force field used in this work for the sugar head was the GROMOS 56A(CARBO), equivalent to the 53A6 for non-carbohydrate systems, this force field is an improvement of the 45A4 and a new version was recently released, with corrections that stabilizes the regular (4)C1 chair for α-anomers (not the case of the OHM molecule head).

The lipid forced field used for the lipid tail of the molecule was the same as the force field used for the membrane lipids (GROMOS 43A1-S3 force field). In particular this force field had been shown to reproduce quite well some experimental data and is as good as many others to reproduce different membrane properties.

The charge groups were defined according to the functional groups involved. Atomic charges for the sugar head were extracted from GROMOS 56A (CARBO) force field (lactose molecule) while the charges of the lipid tail were extracted from the GROMOS 43A1-S3 force field. The bonded parameters for almost all atoms were obtained also from both force fields, while the bonded parameter for the linking atoms between the sugar head and the lipid tail were taken from similar atoms in both force fields.

Finally, it should be notice that the set of parameters obtained for the OMH molecule is completely compatible with both force fields GROMOS 56A (CARBO) and GROMOS 43A1-S3 since new atom types from the first one were added into the last one in order to obtain a new complete force field that is a combination of both.

Starting conformations:

For the membrane preparation (with the OHM), the final structure of the previous simulations (system without the OHM molecule) were used and the water molecules were removed. The OHM molecule preparation consist in the random location of the desired number molecules (OHM) into both monolayers, having the correct orientation and an approximately position in the Z direction of the membrane. Next, the lipid position of the membrane only were scale using a scaling factor of 5 followed by a series of compression steps. Each step scales the lipid position with a scaling factor of 0.95; slowly bringing the system back to more natural dimensions. After each scaling step the system is energy-minimized to eliminate clashes. Finally, when the systems arrive to the desired dimensions, the water molecules were added back and the systems were subjected to 100ns of equilibration using molecular dynamics simulations. Followed by 200 or 400 ns of production MD simulation.
Figure S12. A) Density profile of lipid components. Color code: DSPC (black), SGML (red), cholesterol (green) and water (blue). In all cases, full lines (bilayer control) and dotted lines (bilayer with OHM). Bottom row: Number densities for the OHM molecule (black) and its specific moieties (LEFT: sugar in red, tail in blue; RIGHT: sugar in pale red, tail in pale blue). B) Scheme of lipid bilayer after incorporation of OHM, top view (top) lateral view (bottom). The DSPC molecules are shown in blue, the SGML in green and the CHOL molecules in red. The OHM molecules are represented in magenta with its O and H atoms in red and white respectively.

Figure S13. Acyl chains order parameter SCD for the sn-1 (black) and sn-2 (red) chain as a function of the tail carbon number; control (full line) and OHM (dotted line)
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