Supporting Information

Membrane labeling and Immobilization via copper-free click chemistry

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1. General Considerations

Reagents were generally purchased from Acros, Aldrich or AK Scientific and used as received. Dry solvents were obtained from a Pure Solv solvent delivery system purchased from Innovative Technology, Inc. Column chromatography was performed using 230–400 mesh silica gel purchased from Sorbent Technologies. NMR spectra were obtained using a Varian Mercury 300 spectrometer. Mass spectra were obtained with JEOL DART-AccuTOF and ABI Voyager DE Pro MALDI spectrometers. Optical rotation values were obtained using a Perkin-Elmer 241 polarimeter. L-α-phosphatidylcholine (PC) and 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-(7-nitro-2-1,3-benzoxadiazol-4-yl) (ammonium salt) (NBD-PE) were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL). Liposome extruder and polycarbonate membranes were obtained from Avestin (Ottawa, Canada). Microplate-based fluorescence measurements were performed using a BioTek synergy 2 multi-detection microplate.
reader. Black and white reacti-bind streptavidin high binding capacity (HBC) coated 96-well microplates were purchased from Pierce Biotechnology (Rockford, IL).

2. Synthesis of azido-lipid 1

**S-14-Hydroxy-N-(prop-2-ynyl)-3,6,9,12-tetraoxatetradecan-1-amide (5).** Alcohol 4 (2.32 g, 8.28 mmol) was dissolved in methanol (8 mL). With stirring, 2 M sodium hydroxide (8 mL) was added and stirring was continued for 40 min. The solution was neutralized with Dowex® 50WX8-200 H⁺ ion exchange resin to pH 4. The resin was removed via filtration and the filtrate was concentrated under reduced pressure, followed by resuspension in toluene and concentration once again. At this point, the residue was dissolved in dichloromethane (50 mL), to which was added propargylamine (682 μL, 9.94 mmol), 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI, 1.9 g, 9.94 mmol), 4-dimethylaminopyridine (DMAP, 1.01 g, 8.28 mmol), and N-methylmorpholine (NMM, 2.74 mL, 24.84 mmol). This solution was then stirred at rt overnight, after which the reaction mixture was extracted with dichloromethane (2 x 50 mL) from 2 M hydrochloric acid (50 mL). The combined organic layers were then dried with magnesium sulfate, filtered, and the solvent was removed under reduced pressure. Column chromatography with silica gel and a solvent system of 7% methanol/chloroform afforded 5 as a clear oil (662 mg, 28%).

\[ ^1H \text{ NMR (300 MHz, CDCl}_3] \delta 7.61 (s, 1H), 4.11–4.09 (m, 2H), 4.03 (s, 1H), 3.76–3.60 (m, 16H), 3.05 (s, 1H), 2.26–2.24 (t, J = 2.9 Hz, 1H); \]

\[ ^13C \text{ NMR (75.5 MHz, CDCl}_3] \delta 170.0, 79.8, 72.5, 71.2, 71.1, 70.9, 70.5, 70.40, 70.38, 70.3, 70.2, 70.1, 61.4; \]


**S)-3-(4-(16-Hydroxy-3-oxo-5,8-11,14-tetraoxa-2-azahexadecyl)-1H-1,2,3-triazol-1-yl)propane-1,2-diyl distearate (7).** Alkyne-alcohol 5 (69 mg, 0.238 mmol) and (S)-3-azidopropane-1,2-diyl distearate (6, 155 mg, 0.238 mmol) were dissolved in tetrahydrofuran (1.5 mL). Copper sulfate pentahydrate (173 mg, 3 mmol) and sodium ascorbate (275 mg, 6 mmol) were then added, along with water (0.5 mL). The reaction was stirred at rt overnight, and the solvent was then removed under reduced pressure.
Column chromatography with silica gel and a solvent system of 10% methanol-chloroform afforded 7 as a white solid (131 mg, 59%).

$[\alpha]_{D}^{296K} +1.22$ (C = 5.25, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.97 (t, $J$ = 5.6 Hz, 1H), 7.65 (s, 1H), 5.39–5.36 (m, 1H), 4.64–4.54 (m, 4H), 4.32 (dd, $J$ = 4.1, 12 Hz, 1H), 4.08–4.01 (m, 3H), 3.73–3.43 (m, 17H), 2.36–2.27 (m, 4H), 1.62–1.57 (m, 4H), 1.26 (b s, 56H), 0.88 (t, $J$ = 6.3 Hz, 6H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 173.1, 172.6, 145.3, 123.6, 72.6, 70.9, 70.5, 70.4, 70.22, 70.18, 69.3, 62.0, 61.5, 50.0, 34.2, 34.0, 31.9, 29.72, 29.67, 29.5, 29.4, 29.3, 29.1, 29.0, 24.8, 24.7, 22.7, 14.1; MALDI–HRMS $[M + Na]^+$ calcd: 961.7175, found: 961.7190.

(S)-3-(4-(16-Azido-3-oxo-5,8-11,14-tetraoxa-2-azahexadecyl)-1H-1,2,3-triazol-1yl)propane-1,2-diyl distearate (1). Lipid alcohol 7 (129 mg, 0.137 mmol) was dissolved in tetrahydrofuran (3 mL) and cooled to 0 ºC under nitrogen. With stirring, triethylamine (57 $\mu$L, 0.411 mmol) and methanesulfonyl chloride (10.8 $\mu$L, 0.138 mmol) were added. Stirring was continued at 0 ºC for 1 h. The solvent was removed under reduced pressure and the residue was dissolved in N,N-dimethylformamide. Sodium azide (17.8 mg, 0.274 mmol) was added and the reaction was heated at 85 ºC overnight. The solvent was next removed under reduced pressure and column chromatography with silica gel and a solvent system of 5% methanol-chloroform afforded 1 as a tan solid (103 mg, 78%).

$[\alpha]_{D}^{296K} –0.17$ (C = 4.12, CHCl$_3$); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.72 (b s, 1H), 7.62 (s, 1H), 5.39–5.35 (m, 1H), 4.62–4.50 (m, 4H), 4.31 (dd, $J$ = 4.2, 12 Hz, 1H), 4.08–4.00 (m, 3H), 3.77–3.61 (m, 14H), 3.39 (t, $J$ = 4.8 Hz, 2H), 2.36–2.27 (m, 4H), 1.62–1.57 (m, 4H), 1.25 (b s, 56H), 0.88 (t, $J$ = 6.3 Hz, 6H); $^{13}$C NMR (75.5 MHz, CDCl$_3$) $\delta$ 173.1, 172.5, 145.2, 123.4, 71.3, 71.1, 70.7, 70.6, 70.4, 70.2, 70.0, 69.3, 62.0, 50.6, 50.0, 42.8, 34.2, 34.0, 31.9, 29.72, 29.68, 29.6, 29.5, 29.4, 29.3, 29.1, 29.0, 24.8, 24.7, 22.7, 14.1; MALDI–HRMS $[M + Na]^+$ calcd: 986.7240, found: 986.7200.
3. Liposome preparation and immobilization via copper-free click chemistry

Liposome Preparation

Stock solutions were made of synthetic azido-lipid 1, NBD-PE, and PC. Examples of each stock solution used are as follows: 1.5 mg azido-lipid 1, 1.0 mg NBD-PE, and 50 mg PC were each weighed into separate vials and dissolved in 1 mL chloroform to form stock solutions of 1.56 mM azido-lipid 1, 1.08 mM NBD-PE, and 65.8 mM PC. Using these stock solutions, 12.8 µL azido-lipid 1 stock (1%), 18.5 µL NBD-PE stock (1%), and 29.8 µL PC stock (98%) were combined in a glass vial. In addition, liposomes lacking azido-lipid 1 were prepared by combining 9.3 µL NBD-PE (1%) and 15.0 µL PC (99%). In both, the chloroform was removed under a stream of nitrogen and placed under vacuum for 1 h. The dried lipids were then hydrated by adding 500 µL of 0.5X PBS buffer, pH 7.4, and rotated on a rotary evaporator for 40 min at 40 ºC. The liposomes were then subjected to 10 freeze-thaw cycles and extruded (19 times) to uniform size and lamellarity using a 200 nm polycarbonate filter. The resulting solutions were then diluted to a total volume of 4 mL, which yielded a concentration of 500 µM total liposome suspension.

Membrane derivatization and immobilization via copper-free click chemistry

Stock solutions were made of ADIBO-TEG-biotin 2,2 and alkyne-TEG-biotin 81 (see structure below). Examples of each stock solution used are as follows: 0.8 mg ADIBO-TEG-biotin 2, and 0.4 mg alkyne-TEG-biotin 8 were each weighed into separate vials and dissolved in 1 mL chloroform to form stock solutions of 0.71 mM 2, and 0.78 mM 8. In addition, a 500 µM stock solution of D-biotin was made by dissolving 1.4 mg in 11.5 mL of 0.5X PBS buffer, pH 7.4. Using the alkyne stock solutions, 14.1 µL ADIBO-TEG-biotin 2 stock and 12.8 µL alkyne-TEG-biotin 8 stock were measured into separate glass vials, dried under a stream of nitrogen, and placed under vacuum for 1 h. To the alkyne (2 and 8) containing vials, 2 mL of 0.5X PBS, pH 7.4 buffer was added to provide 5 µM stock solutions of each. These stock solutions were then diluted into 12 solutions – 0, 0.05, 0.15, 0.3, 0.45, 0.6, 0.75, 1.0, 1.25, 1.5, 1.75, and 2.0 µM. In a 96-well
streptavidin-coated microplate, 200 µL of wash buffer (0.5X PBS, pH 7.4) was added to each row to be used. The plates were then shaken for 30 min, and the wash buffer was then removed. For the biotin preincubation control, 100 µL of 500 µM D-biotin was added to each well and incubated for 1h. This solution was removed and the wells were washed with 3 x 200 µL wash buffer. In each case, 100 µL of the respective alkyne reagent (2 or 8) was added to the appropriate well. This was immediately followed by addition of 100 µL of 500 µM of the previously described liposome solution to each well. This yielded a final liposome concentration of 250 µM in each well, and alkyne reagent concentrations of 0, 0.025, 0.075, 0.15, 0.225, 0.3, 0.375, 0.5, 0.625, 0.75, 0.875, and 1.0 µM in different wells. These solutions were incubated, with shaking, for 4 h at rt. Following incubation, the solutions were removed and each well was washed with 3 x 200 µL wash buffer. Water (100 µL) was then added to each well and fluorescence was then measured using a microplate reader with a 460 nm (± 40 nm) excitation filter and 528 nm (± 20 nm) emission filter.

![Image of chemical structure 8]
4. NMR spectra of synthetic compounds

![NMR spectra of synthetic compounds image]

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5. References Cited