Supporting Information for

Nanostructured Self-Assembly Materials from Neat and Aqueous Solutions of C18 Lipid Pro-drug Analogues of Capecitabine – a Chemotherapy Agent. Focus on Nanoparticulate Cubosomes™ of the Oleyl Analogue.

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Electronic Supplementary Material (ESI) for Soft Matter

Amphiphile Prodrug Preparation

Materials and characterization methods

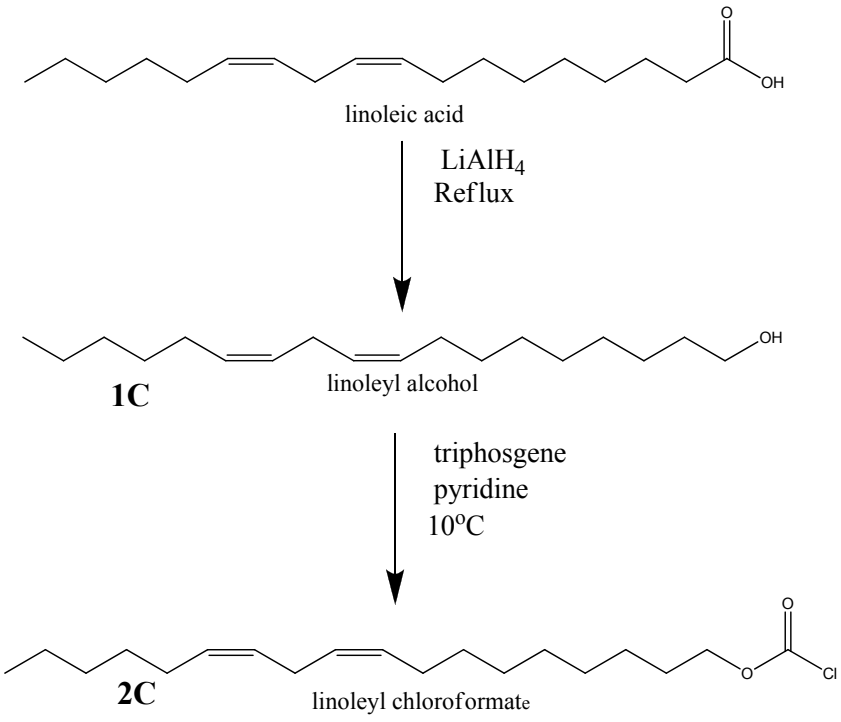
The prodrug Capecitabine was purchased from Xingcheng Chemphar Co. Ltd. (P.R.China). Oleyl alcohol, stearoyl alcohol, linoleic acid, linolenic acid and all other reagents were purchased from Sigma-Aldrich and used without further purification. All solvents were obtained from Merck and used as received.

^{1}H NMR spectra were recorded on a Bruker AC200 spectrometer in deuterated Chloroform($CDCl_3$). Tetramethylsilane ($(CH_3)_4Si$, TMS) was used as an internal standard unless otherwise stated. The spectra were analysed using MestRe-C 2.3a software. The chemical shifts (δ) were expressed in ppm and coupling constants were expressed as J values in Hertz units. Analytical HPLC was performed on a Waters HPLC equipment (Waters Corporation, Milford, MA, USA), comprising of a 600 solvent delivery system with 600 automated gradient controller and an Alltech 2000 Evaporative Light Scattering Detector (ELSD). A Phenomenex Gemini C18 column (5 micron, 150 X 4.6mm) was used for analysis of the samples. An isocratic solvent consisting of methanol/water 70/30 was used as the eluent at a flow rate of 1.00 ml/min. UPLC analysis was performed on a Waters Acquity UPLC equipped with a BEH^{TM}C18 column (1.7 µm, 50mm x 2.1mm). A water/ethanol solvent system was used at a flow rate of 0.4 ml/min, using water/ethanol 90/10 as solvent A, and 100% ethanol as solvent B. A gradient method was used for sample analysis, running from 100% A to 100% B within 2min, followed by 100% solvent B for 1 min with subsequent equilibration under the initial conditions for 2 mins. Both HPLC and UPLC results were recorded on ELSD and UV-vis (λ = 260nm) detectors. Flash column chromatography was used for the purification of most of the intermediate and final synthesized compounds. Pre-packed silica columns (40-63µm) were purchased from In Vitro Technologies. The eluting fractions were tested on thin layer chromatography (TLC) aluminium plates pre-coated with silica gel 60 containing fluorescent indicator (F_{254}). For compound visualisation, the TLC plates were dipped into a solution of 3% phosphomolybdic acid in ethanol, then heated to 600 °C using a heat gun. Mass spectra were recorded on a Thermo Finnigan LC-MS with an atmospheric pressure chemical ionization (APCI) source in the positive (+) ion mode.

Samples were dissolved in LC-MS grade methanol. Elemental analysis of the final compounds was carried out at the Campbell Microanalytical Laboratory, University of Otago, New Zealand.

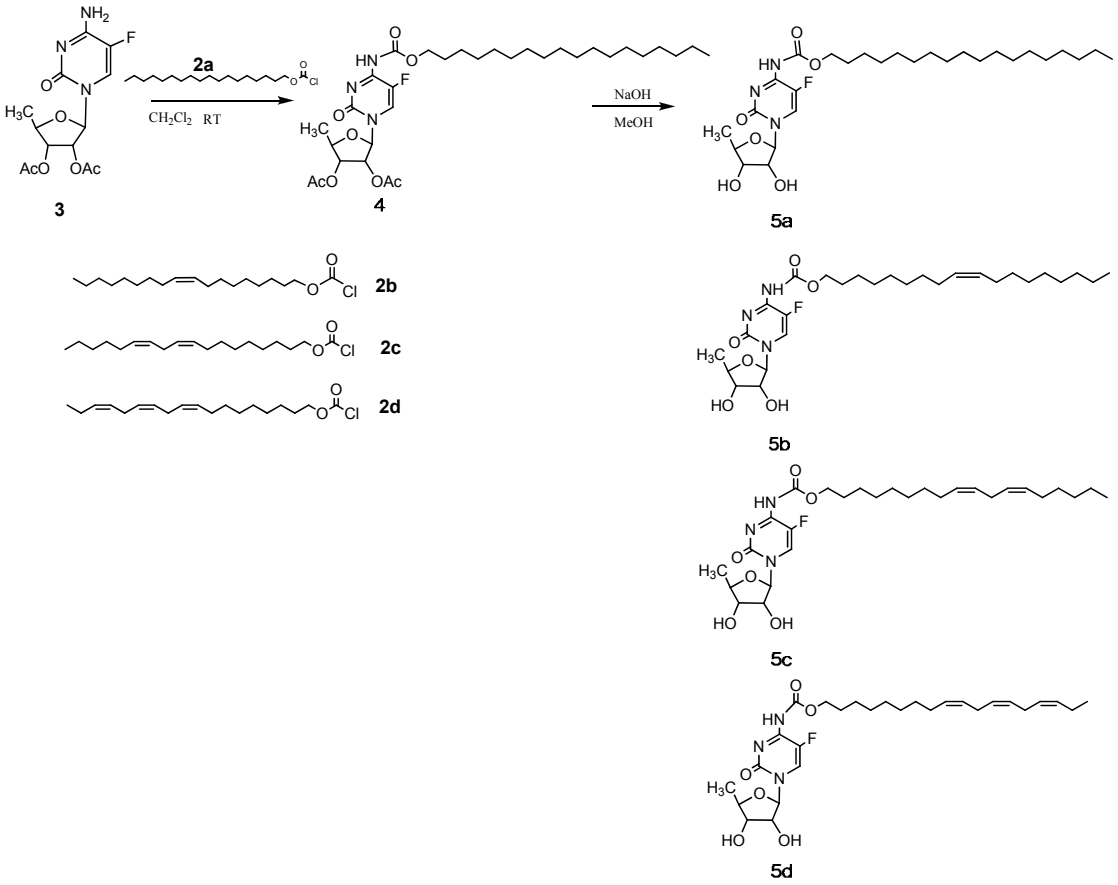
Synthesis

5-FC prodrug amphiphiles with various degrees of unsaturation were synthesized by the same synthesis route as described for 5-FCPal[1] and 5-FCPhy.[2] Fatty alcohols, when not available, were prepared by reduction of the corresponding acids using Lithium aluminium hydride ($LiAlH_4$). The fatty alcohols were subsequently transformed to the corresponding chloroformate using triphosgene, following a modified procedure of Ding and Fritz.[3]



Scheme 1: A typical synthetic route for the preparation of the fatty alcohol and chloroformate conjugates of linoleyl chloroformate.

The synthesis route for the prodrug amphiphiles is shown in scheme 2, using compound 3, synthesised as previously reported.[1]



Scheme2: The synthetic route for the preparation of 5-FC-prodrug amphiphiles.

Linoleyl alcohol ***(1c)***

A solution of Linoleic acid (1g, 3.57 mmol) in 10 ml diethyl ether was added dropwise to $LiAlH_4$ (0.17 g, 4.5 mmol), suspended in 20 ml dry diethyl ether, creating a gentle reflux, and stirred overnight. The reaction vessel was protected from light to prevent degradation of the sample. The reaction mixture was then placed in an ice

water bath and 4 ml of 10 % sulphuric acid added with care. The organic layer was separated, and the aqueous suspension washed with ether (20 ml x3). The pooled ether phase was washed with water twice and dried over Na_2SO_4. Following filtration, the solvent was evaporated to dryness to obtain linoleyl alcohol (**1c**) as a white-yellowish wax (85 % yield).

Linolenyl alcohol (**1d**) was synthesised from linolenic acid in a similar manner.

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*Stearyl Chloroformate **(2a)***

To a solution of stearyl alcohol (2.70 g, 10 mmol) in 10 ml dichloromethane (DCM), triphosgene (0.99 g, 6.7 mmol) dissolved in 20 ml DCM was added. The reaction mixture was cooled to 10– 15 ^{o}C. Pyridine (0.8 g, 20 mmol) was then added dropwise over a 1 h period. The reaction mixture was stirred for an additional 1 h, and subsequently the solvent was evaporated under reduced pressure. The residue was redissolved in DCM, washed three times with cold water, and dried over Na_2SO_4, giving 1.88 g (83% yield) of stearyl chloroformate (**2a**).

*Oleyl chloroforamte **(2b)***

Oleyl chloroforamte **(2b)** was synthesised as above with 84.3% yield. ^{1}H NMR in $CDCl_3$: δ, 0.88 (t, 3H, *J*=6.3Hz, CH_3), 1.29 (d, 22H, *J*=7.7Hz, CH_2), 1.73 (t, 2H, *J*=6.6Hz, βCH_2), 2.02 (d, 4H, *J*=5.4Hz, -CH_2-CH=CH-CH_2-), 4.31 (t, 2H, *J*=6.6Hz, αCH_2), 5.35 (m, 2H, -CH=CH).

Linoleyl and linolenyl chloroformates (**2c** and **2d**) were synthesized in a similar manner to oleyl chloroforamte with yields of 87% and 79 % respectively.

*5'-deoxy-5-fluoro-N^4-(stearyloxycarbonyl) cytidine **(5a)***

Compound **3** (1.5 g, 4.6 mmol) was dissolved in 20 ml dry DCM and 1 ml anhydrous pyridine, and the flask placed in an ice bath. Stearyl chloroformate (**2a**) (1.82 g, 5.5 mmol) was added dropwise to the ice-cooled solution, and stirred overnight at room temperature. 250 µl of methanol was then added to the mixture. The reaction mixture was evaporated to dryness and subsequently redissolved in diethyl ether. The precipitate was removed by filtration and washed with diethyl ether. The filtrate and the wash were collected, dried over Na_2SO_4 and evaporated to dryness to give compound **4a.**

Compound **4a** was dissolved in methanol (20 ml) and cooled on an ice bath. Following addition of 10 ml of 8M NaOH dropwise, the reaction was quickly neutralised with 4M HCl. The reaction mixture was then extracted by DCM and water. After a water wash and drying over sodium sulphate, the organic layer was evaporated to yield compound **5a** as a white solid. The crude compound was then purified using normal silica column chromatography with a DCM and methanol solvent system (gradually increasing the volume ratio from 100:0 to 90:10). This yielded 1.97 g (78 %) of the pure title compound.

^{1}H NMR($CDCl_3$): δ, 0.9 (t, 3H, *J*=6.4 Hz, -CH_3), 1.25 (m, 28H, CH_2), 1.39 (d, 3H, *J*=6.6 Hz, ribose-CH_3 (H-5)), 1.7 (t, 2H, *J*=7. βCH_2), 3.9 (dd, 1H, *J*=3.7,5.1, H-4), 4.17-4.27 (m, 1H, H-2), 4.20 (t, 2H, *J*=6.4Hz, α-CH_2), 4.29-4.40 (m, 1H, H-3), 5.64 (d, 1H, *J*=4 Hz, H-1), 7.8 (b.s., 1H, N-CH=C-F).

5'-deoxy-5-fluoro-N^4-(oleyloxycarbonyl) cytidine ***(5b)***

The procedure used to synthesise compound **5a** was also used for compound **5b**, and the pure title compound was obtained (59.8% yield).

^{1}H NMR ($CDCl_3$) : δ, 0.88 (t, 3H, *J*=6.0 Hz, CH_3), 1.28 (m, 22H, *J*=4.0 Hz, CH_2), 1.42 (d, 3H, *J*=6.5 Hz, ribose-CH3 (H-5)), 1.58-1.77 (m, 2H, βCH_2), 2.01 (d, 4H, *J*=5.1 Hz, -CH_2-CH=CH-CH_2-), 3.73 (br s, 1H, H-4), 3.87 (t, 1H, *J*=4.7 Hz, H-4), 4.18 (t, 3H, *J*=6.4 Hz, α-CH_2, and m, H-2), 4.27 (d, 1H, *J*=4.5 Hz, H-3), 5.35 (t, 2H, *J*=5.7 Hz, -CH=CH-), 5.71 (d, 1H, *J*=2.9 Hz, H-1), 7.79 (br s, 1H, N-CH=C-F).

5'-deoxy-5-fluoro-N^4-(linoleyloxycarbonyl) cytidine ***(5c)***

This compound was synthesised in a similar manner to that presented above and the pure title compound obtained in 87% yield.

^{1}H NMR ($CDCl_3$) : δ, 0.88 (t, 3H, *J*=6.2 Hz, CH_3), 1.20-1.4 (m, 14H, CH_2), 1.39 (d, 3H, *J*=6.4 Hz, ribose-CH3 (H-5)), 1.63-1.73 (m, 2H, βCH_2), 2.01 (d, 4H, *J*=5.1 Hz, -CH_2-CH=CH-CH_2-), 2.77(t, 2H, *J*=5.52 Hz, =CH-CH2-CH=), 3.9 (t, 1H, H-4), 4.17-4.24 (t, 2H, α-CH_2), 4.20 (m, 1H, H-2), 4.33-4.38 (m, H-3), 5.34 (m, 4H, =CH), 5.71(d, 1H, *J*=4Hz, H-1), 7.8 (br s, 1H, N-CH=C-F).

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