Study of interactions of mononucleotides with 1,4-dihydropyridine vesicles by NMR and ITC techniques.

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Scheme 1. Synthesis of a DHP amphiphile 1g having esters with terminal CF₃ groups.

13,13,13-Trifluorotridecyl 3-oxobutanoate (S1). 10.00 g (0.039 mol) of 13,13,13-Trifluoro-1-tridecanol [1] was dissolved in p-xylene with warming then 2,2,6-trimethyl-4H-1,3-dioxin-4-one 5.59 g (0.039 mol) was added and the mixture was refluxed 2 h in an oil bath. The reaction mixture was cooled and the xylene removed on a rotary evaporator. The residue was purified on a silica gel chromatography column (EtOAc/hexane 1/3 eluent) providing 11.86 g (89%) of an orange oil. C₁₇H₂₉F₃O₃ MW 338.41. ¹H NMR 200 MHz (CDCl₃): δ = 4.12 (t, J=7.4 Hz, 2H, OCH₂), 3.44 (s, 2H, COCH₂CO), 2.26 (s, 3H, -CH₃), 2.11-1.94 (m, 2H, -CH₂CF₃), 1.54-1.44 (m, 16H (CH₂)₈).

13,13,13-Trifluorotridecyl 3-aminobut-2-enoate (S2). In a 10 mL RB was weighed 1.40 g (0.0041 mol) of the above compound and 2 mL EtOH was added and then 3 mL concentrated aqueous ammonium...
hydroxide was added. The flask was stoppered and vigorously magnetically stirred overnight. The flask was placed in the fridge to cool. The precipitated product was filtered and washed with DI water to provide a white compound 1.06 g in 77% yield. C$_2$H$_6$F$_3$NO$^2$ MW 337.42. $^1$H NMR 200 MHz (CDCl$_3$): $\delta$ = 4.52 (s, 1H, CH$_5$), 4.03 (t, J = 6.6 Hz, 2H, OCH$_2$), 2.13-1.94 (m, 2H, -CH$_2$CF$_3$), 1.89 (s, 3H, CH$_3$), 1.64-1.46 (m, 4H, OCH$_2$CH$_2$ and CH$_2$CH$_2$CF$_3$), 1.28-1.24 (m, 16H, (CH$_2$)$_8$).

Bis(13,13,13-Trifluorotridecyl)1,4-dihydro-2,6-dimethyl-4-phenylpyridine-3,5-dicarboxylate (S3). 0.99 g (0.0029 mol) of above compound was dissolved in 12 mL n-propanol, then 0.16 g (0.0015 mol) benzaldehyde was added and 1 mL glacial acetic acid. The reaction mixture was refluxed for 4h, the heating was stopped and left stirring overnight. The solvent was removed under reduced pressure and the residue weighed 1.17g. The compound was recrystallized twice from EtOH, filtered and washed with cold EtOH. After air drying there was obtained 0.35 g of a pale-yellow compound in 32% yield. Mp. 49-53°C. C$_{61}$H$_{61}$F$_3$NO$_4$ MW 745.92. $^1$H NMR 400 MHz (CDCl$_3$): $\delta$ = -7.21-7.02 (m, 5H, Ph), 5.50 (br s, 1H, NH), 4.93 (s, 1H, C$_4$H), 4.01-3.90 (m, 4H, OCH$_2$), 2.32 (s, 6H, CH$_3$), 2.09-1.97 (m, 4H, 2CH$_2$), 1.58-1.49 (m, 8H,4CH$_2$), 1.36-1.21 (m, 32H (CH$_2$)$_{16}$). $^{13}$C-NMR 100.3 MHz (CDCl$_3$): $\delta$ = 167.62(COO), 147.65(C$_6$H$_5$), 143.78(C$_2$H$_5$), 127.88(C$_2$H$_5$), 127.83(C$_2$H$_5$), 127.21 (q, J = 276.5 Hz, CF$_3$), 126.06(C$_6$H$_5$), 104.23(C$_3$), 63.92(OCH$_2$), 39.53(C$_4$), 33.71 (q, J = 28.5 Hz, CH$_2$CF$_3$), 29.52(CH$_2$), 29.51(CH$_2$), 29.49, (CH$_2$), 29.33, (CH$_2$), 29.26(CH$_2$), 29.18(CH$_2$), 28.68(CH$_2$), 26.06(CH$_2$), 21.81(q, J = 3.2 Hz, CH$_2$), 21.81(CH$_2$), 19.64(CH$_3$) ppm. LC/MS: MS(-ESI) m/z (rel.intensity): 744 ([M-H$^-$], 40).

**DI-13,13,13-Trifluorotridecyl 2,6-bis(bromomethyl)-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (S4).** 0.45 g (0.60 mmol) of the above compound was dissolved in 3 mL chloroform and 2 mL MeOH. The solution was cooled in an ice bath and during 10 min. 0.21g (1.20 mmol) of NBS was added in small portions. After all the NBS was added the reaction was stirred for 4h at RT and put in the fridge overnight. The solvent was removed on the rotary evaporator and to the residue was added hexane to precipitate the succinimide. The mixture was filtered and the hexane was distilled off under reduced pressure to give the dibromide 0.54g in 99% yield which was not purified further. $^1$H NMR 200 MHz (CDCl$_3$): $\delta$ = 9.25 (br s, 1H, NH), 6.20-7.09 (m, 5H, C$_6$H$_5$), 5.01 (s, 1H, C$_4$H), 4.93 (d, J = 10.4 Hz, 1H, CH$_2$Br), 4.54 (d, J = 10.4 Hz, 1H, CH$_2$Br), 4.06 (t, J = 6.8 Hz, 4H, OCH$_2$), 2.10-1.96 (m, 4H, CH$_2$), 1.59-1.44 (m, 8H, 4CH$_2$), 1.31-1.18 (m, 32H (CH$_2$)$_{16}$). $^{13}$C-NMR 100.3 MHz (CDCl$_3$): $\delta$ = 166.22 (COO), 145.60 (C$_6$H$_5$), 141.59 (C$_3$), 128.23 (C$_6$H$_5$), 128.03 (C$_6$H$_5$), 127.92 (C$_6$H$_5$), 127.48 (q, J = 276 Hz, CF$_3$), 106.41 (C$_3$), 64.76 (OCH$_2$), 40.10 (C$_4$H), 33.75 (q, J = 28.4 Hz, CH$_2$CF$_3$), 29.53(CH$_2$), 29.52(CH$_2$), 29.50(CH$_2$), 29.35(CH$_2$), 29.24(CH$_2$), 29.18(CH$_2$), 28.68(CH$_2$), 28.55(CH$_2$), 27.34(CH$_2$), 26.01 (CH$_2$C$_2$H$_5$), 21.82(kv, J=2.93Hz, CH$_3$) ppm. $^{19}$F NMR (CDCl$_3$): $\delta$ = -66.42 (t, J=11.6 Hz, 3F, CF$_3$).

Bis(13,13,13-trifluorotridecyl)1,4-dihydro-2,6-dimethyl-4-phenylpyridine-3,5-dicarboxylate-2,6-dipryridinium bromide (1g'). In a 10 mL RB was weighed 0.54g (0.60 mmol) of the above DHP and dissolved in 1.5 mL dry acetone. Pyridine 0.09 g (1.20 mmol) was added and the reaction mixture was left stirring overnight. The reaction mixture was triturated with diethyl ether to precipitate the product. The precipitate was filtered and washed with diethyl ether to give 0.33g of a pale-yellow compound in 52% yield. $^1$H NMR 400 MHz (CDCl$_3$): $\delta$ = 10.95 (s, 1H, NH), 9.33 (d, J=6.2 Hz, 4H, Py), 8.62 (t, J=7.8 Hz, 2H, Py), 8.20 (m, 4H, Py), 7.26 (m, 5H, C$_6$H$_5$), 6.36 (d, J =13.5 Hz, 4H, 2CH$_2$Py), 5.93 (d, J =13.5 Hz, 4H, 2CH$_2$Py) 5.07 (s, 1H, C$_4$H), 4.05 (t, J =6.8 Hz, 4H, 2CH$_2$), 2.12-1.98 (m, 4H, 2CH$_2$), 1.58-1.24 (m, 40H, 2CH$_2$)$_{10}$). $^{13}$C-NMR 100.3 MHz (CDCl$_3$): $\delta$ = 166.43 (COO), 146.61(Py), 145.51(C$_6$H$_5$), 144.88 (Py), 138.01 (C$_3$), 128.86 (Py), 128.60 (C$_6$H$_5$), 128.34 (q, J=278 Hz, CF$_3$), 128.05 (C$_6$H$_5$), 127.50 (C$_6$H$_5$), 110.41 (C$_3$), 65.52 (OCH$_2$), 57.32 (CH$_2$C$_2$H$_5$), 39.76 (C$_4$H), 33.69 (q, J=28 Hz, CH$_2$CF$_3$), 29.56(CH$_2$), 29.54(2CH$_2$) 29.34(CH$_2$), 29.25(CH$_3$), 29.18(CH$_2$), 28.66(CH$_2$), 28.39(CH$_2$), 25.94(CH$_2$), 21.82(kv, J=2.93Hz, CH$_3$) ppm. $^{19}$F NMR (CDCl$_3$): $\delta$ = -66.42 (t, J=11.6 Hz, 3F, CF$_3$). IR (disks): 3285.8 (NH), 2926.0, 2855.6 (CH), 1742.7, 1692.1 (CO) cm$^{-1}$. The 1,4-DHP pyridinium dibromide was dissolved in ethanol and concentrated perchloric acid was added drop by drop
until no more precipitate of the diperchlorates formed. The salt was filtered and washed with diethyl ether and dried providing compound 1g.

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\begin{align*}
\text{CF}_3 & \quad + \quad 2 \quad \text{NH}_2 \quad \text{O(OCH}_2)_1\text{H}_3 \quad \xrightarrow{n\text{-propanol} \Delta} \quad \text{H}_3\text{C(CH}_2)_1\text{O} \\
\text{S5} & \quad \xrightarrow{2 \text{ Py} \quad \text{dry acetone}} \quad \text{H}_3\text{C(CH}_2)_1\text{O} \\
\end{align*}
\]

**Scheme 2.** Synthesis of DHP amphiphile 1h with 4-p-CF3 benzene group.

**Didodecyl 2,6-dimethyl-4-[4-(trifluoromethyl)phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (S5).** p-Trifluoromethylbenzaldehyde 1.75g (0.01 mol) was weighed in a 100 mL RB then didodecyl 3-amino-2-enoate 5.39 g (0.02 mol) was added and 3 drops of glacial acetic acid with 25 mL n-propanol. The reaction mixture was refluxed 6 hours and the heat turned off. The reaction mixture was left stirring at RT overnight and poured in ice water. The precipitated product was filtered and recrystallized from EtOH to yield a white powder 2.76 g in 41% yield. C_{29}H_{54}N_O MW 677.92 Mp. 64-67°C. \(^1\)H NMR 400 MHz (CDCl_3): \(\delta =7.44 \ (d, J=8.4 \ Hz, 2H, \text{C}_6\text{H}_4), 7.36 \ (d, J=8.4 \ Hz, 2H, \text{C}_6\text{H}_4), 5.58 \ (br \ s, 1H, NH), 5.04 \ (s, 1H, \text{C}_6\text{H}_4), 4.06-3.98 \ (m, 4H, 2OCH_2), 2.34 \ (s, 6H, 2CH_3), 1.58-1.54 \ (m, 4H, 2CH_2), 1.32-1.23 \ (m, 36H, 2(C_\text{C}(CH_2)_1\text{H}_3)), 0.88 \ (t, J=6.8 \ Hz, 6H, 2CH_3). \(^13\)C-NMR 100.3 MHz (CDCl_3): \(\delta =167.28(\text{COO}), 151.49 \ (C_6\text{H}_3), 144.21(C_2\text{,CH}), 128.19 \ (C_6\text{H}_4), 128.27 \ (kv, J=32.3 \ Hz, \text{C}_6\text{H}_3), 126.56 \ (kv, J=270.3 \ Hz, \text{CF}_3), 124.83 \ (kv, J=4 \ Hz, \text{C}_6\text{H}_3), 103.71 \ (C_3\text{,CH}), 64.09(\text{OCH}_2), 39.68 \ (\text{C}_6\text{H}_4), 31.88(\text{CH}_2), 29.62(2\text{CH}_2), 29.61(\text{CH}_2), 29.53(\text{CH}_2), 29.33(\text{CH}_2), 29.28(\text{CH}_2), 28.70(\text{CH}_2), 26.08(\text{CH}_2), 22.68(\text{CH}_2), 19.68(\text{CH}_3), 14.10(\text{CH}_3) \ ppm. \text{LC/MS: MS(-ESI) m/z (rel. intensity): 676 ([M-H]^+, 100).}

**Didodecyl 2,6-bis(bromomethyl)-4-[4-(trifluoromethyl)phenyl]-1,4-dihydropyridine-3,5-dicarboxylate (S6).** In a 10 mL RB was weighed 0.68 g (0.001 mol) of the above compound and dissolved in 5 mL MeOH. NBS 0.36 g (0.002 mol) was added and the reaction mixture was sonicated in an ultrasonic bath for 14 min. The solids were filtered and the solvent removed to give the crude dibromide as an orange oil 1.05 g in quantitative yield. C_{40}H_{56}Br_2F_3NO_4 MW 835.71. \(^1\)H NMR 200 MHz (CDCl_3): \(\delta =7.49 \ (d, J=9.0 \ Hz, 2H, \text{C}_6\text{H}_4), 7.37 \ (d, J=9.0 \ Hz, 2H, \text{C}_6\text{H}_4), 6.57 \ (br \ s, 1H, NH), 5.08 \ (s, 1H, CH), 4.86 \ (d, J=11.4 \ Hz, 1H, CH), 4.66 \ (d, J=11.4 \ Hz, 1H, CH), 4.07 \ (t, J=6.8 \ Hz, OCH_2), 1.58-1.54 \ (m, 4H OCH_2CH_2), 1.32-1.23 \ (m, 36H, 2(C_\text{C}(CH_2)_1\text{H}_3)), 0.87 \ (t, J=6.8 \ Hz, 6H, CH_3). \(^13\)C-NMR 100.3 MHz (CDCl_3): \(\delta =165.88 \ (\text{COO}), 149.44 \ (C_6\text{H}_4), 142.21(C_2\text{,CH}), 129.04 \ (kv, J=32.0 \ Hz, \text{C}_6\text{H}_4), 128.25(\text{C}_6\text{H}_4), 126.78 \ (kv, J=266Hz, \text{CF}_3), 125.24 \ (kv, J=4.1 \ Hz, C_6\text{H}_3), 105.34 \ (C_3\text{,CH}), 64.96(\text{OCH}_2), 40.12 \ (C_4), 31.89(\text{CH}_2), 29.63(\text{CH}_2), 29.62(\text{CH}_2), 29.59(\text{CH}_2), 29.53(\text{CH}_2), 29.34(\text{CH}_2), 29.29(\text{CH}_2), 28.55(\text{CH}_2), 27.06 \ (C_2\text{-C_2\text{,CH}}), 26.03(\text{CH}_2), 22.67(\text{CH}_2), 14.09(\text{CH}_3) \ ppm. \(^19\)F NMR (CDCl_3): \(\delta =-62.41.

**1,1’-[(3,5-Bis(dodecroyloxy carbonyl)-4-[4-(trifluoromethyl)phenyl]-1,4-dihydropyridin-2,6-diyl)] bis(methylene)] bis(pyridinium) bromide (1h).** The above crude dibromide 1.05 g (0.001 mol) was dissolved in diethyl ether and 0.17 g (0.002 mol) pyridine was added. The mixture was stirred overnight.
and the precipitated product was filtered through a glass fritted funnel, washed with diethyl ether and dried to give 0.61 g of a white powder in 73% yield. Mp 174-186°C. C$_{50}$H$_{70}$Br$_3$N$_3$O$_4$ MW 993.91. $^1$H NMR 400 MHz (CDCl$_3$): $\delta$ =11.04 (br s, 1H, NH), 9.38 (d, 4H, J=6.2 Hz, 4H, Py), 8.56 (t, J=8.1 Hz, 2H, Py), 8.19 (dd, J=8.1, J=6.2 Hz, 4H, Py), 7.55 (d, J=8.4 Hz, 2H, C$_6$H$_4$), 7.46 (d, J=8.4 Hz, 2H, C$_6$H$_4$), 6.39 (d, J=13.8 Hz, 4H, 2CH$_2$Py), 5.92 (d, J=13.8 Hz, 4H, 2CH$_2$Py), 5.17 (s, 1H, C$_6$H), 4.05 (t, J=6.8 Hz, 4H, 2OC$_2$H$_2$), 1.60-1.53 (m, 4H, 2CH$_2$), 1.32-1.19 (m, 36H, 2(C$_2$H$_5$)$_{10}$), 0.87 (t, J=6.8 Hz, 6H, 2C$_3$H$_3$) ppm.

$^{13}$C-NMR 100.3 MHz (CDCl$_3$): $\delta$ = 166.08 (COO), 149.30 (C$_6$H$_4$), 145.08 (Py), 143.33 (Py), 138.43 (C$_2$H$_2$), 129.58 (C$_6$H$_4$), 128.76 (C$_6$H$_4$), 128.53 (Py), 125.53 (kv, J=4.1, C$_6$H$_4$), 125.38 (kv, J=272.3Hz, CF$_3$), 109.91 (C$_3$), 65.65 (OCH$_2$), 57.35 (CH$_2$-C$_2$, 40.02 (C$_4$), 31.89 (CH$_2$), 29.63 (CH$_2$), 28.62 (CH$_2$), 29.56(CH$_2$), 29.34(CH$_2$), 29.26(CH$_2$), 28.39 (CH$_2$), 25.98 (CH$_2$), 22.66 (CH$_2$), 14.09 (CH$_3$) ppm. $^{19}$F NMR (CDCl$_3$): $\delta$= -62.41. LC/MS: MS(+ESI) m/z (rel. intensity): 834 ([M-2Br]$^+$, 50). IR (disks): 3421.3 (NH), 2924.1, 2953.7 (CH), 1695.0, 1630.8 (CO) cm$^{-1}$.

**Raw ITC data**

![Figure 1](image_url)

**Figure 1.** Raw ITC titration data for 1a and 1g (Figure 1 in the main article). Concentrations for 1,4-DHPs in syringe: for 1a is 0.270 mM and for 1g is 0.130 mM.
Figure 2. There are three images (A, B, B-A).
A- presents the reference ITC data.
B- the ITC titration data for 1a and 2a;
B-A - experimental data minus reference data the same as Figure 5 in the manuscript.
Initial concentrations of 1a is 0.183 mM and 2a is 3.143 mM.
Figure 3. ITC raw data with delta H obtained from integrals (from the Figure 6. In article). Two different experiments under the same conditions (**A** is 3.27 mM as a guest (syringe), **C** is 0.207 mM as a host (cell). (3.27).

References