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

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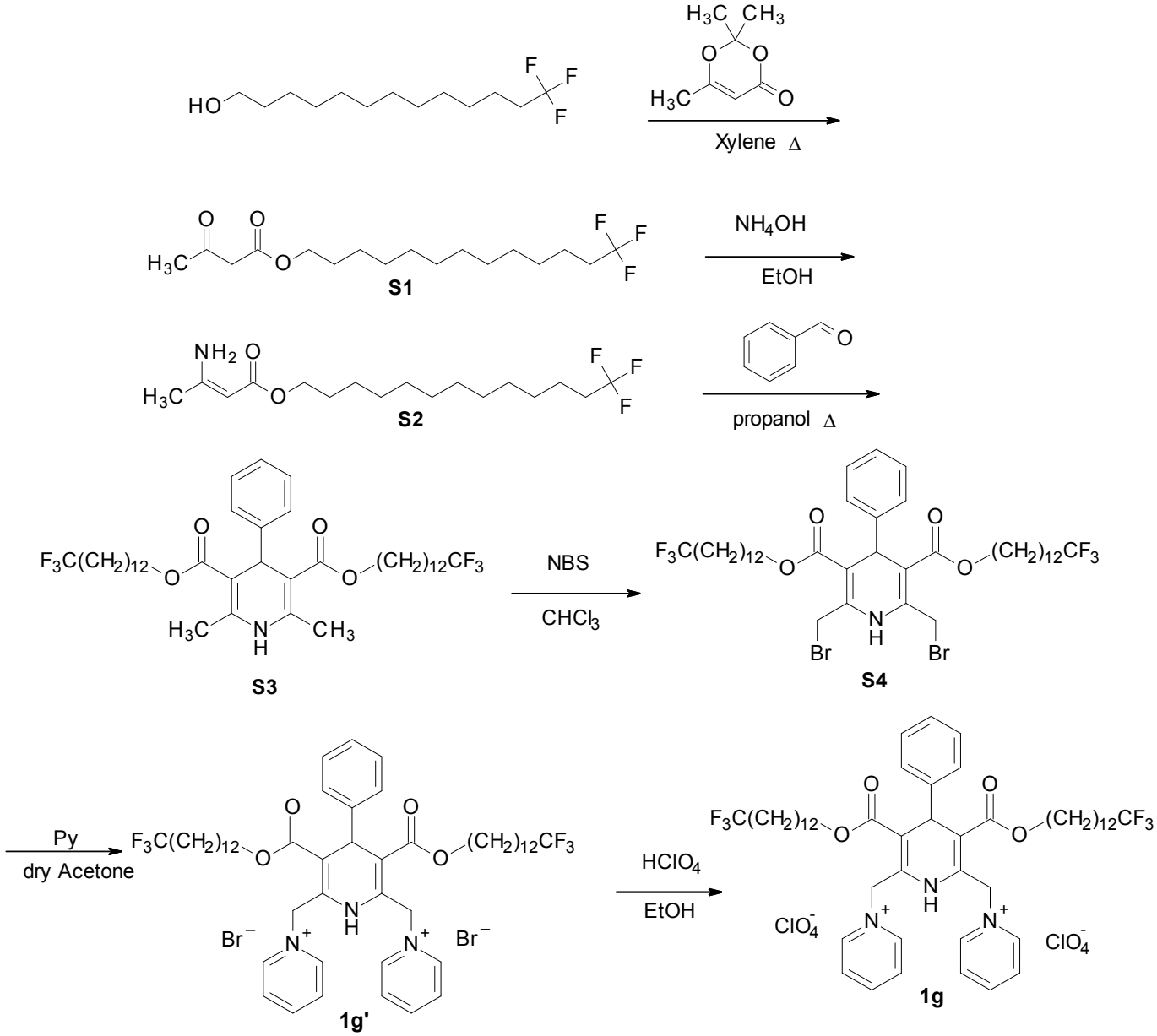


Scheme 1. Synthesis of a DHP amphiphile **1g** having esters with terminal CF_3 groups.

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 2h 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 11.86g (89%) of an orange oil. $C_{17}H_{29}F_3O_3$ MW 338.41. ^{1}H NMR 200 MHz (CDCl3): δ =4.12 (t, J=7.4 Hz, 2H, OCH_2), 3.44 (s, 2H, $COCH_2CO$), 2.26 (s, 3H, $-CH_3$), 2.11-1.94 (m, 2H, $-CH_2CF_3$), 1.54-1.44 (m, 4H, OCH_2CH_2 and $CH_2CH_2CF_3$), 1.31-1.18 (m, 16H $(CH_2)_8$).

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

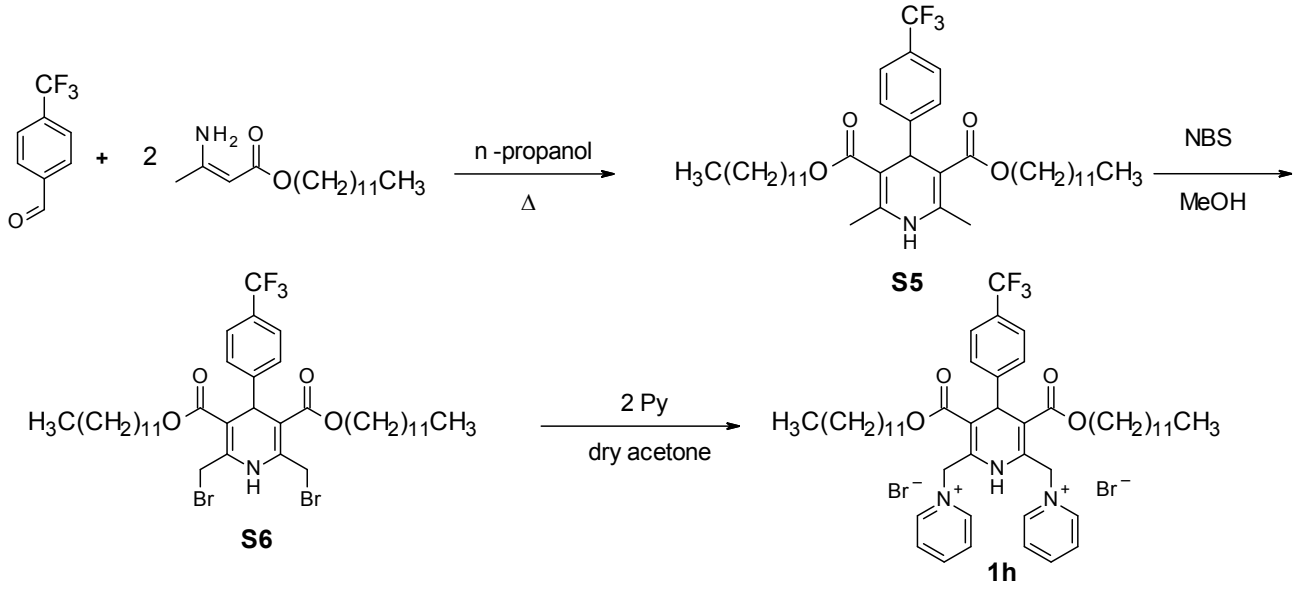
hydroxide was added. The flask was stoppered and vigorously magnetically stirred overnight. The flask was placed in the fridge to cool. The precipitated product was filtered and washed with DI water to provide a white compound 1.06 g in 77 % yield. $C_{17}H_{30}F_3NO_2$ MW 337.42. ^{1}H NMR 200 MHz ($CDCl_3$): δ =4.52 (s, 1H, C*H*=), 4.03 (t, *J*= 6.6 Hz, 2H, OCH_2), 2.13-1.94 (m, 2H, -CH_2CF_3), 1.89 (s, 3H, CH_3), 1.64-1.46 (m, 4H, OCH_2CH_2 and $CH_2CH_2CF_3$), 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. $C_{41}H_{61}F_6NO_4$ MW 745.92. ^{1}H NMR 400 MHz ($CDCl_3$): δ =7.21-7.02 (m, 5H, Ph), 5.50 (br s, 1H, NH), 4.93 (s, 1H, C_4H), 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 ($CDCl_3$): δ =167.62(COO), 147.65(C_6H_5), 143.78($C_{2,6}$), 127.88(C_6H_5), 127.83(C_6H_5), 127.21 (q, *J*=276.5 Hz, CF_3), 126.06(C_6H_5), 104.23($C_{3,5}$), 63.92 (OCH_2), 39.53(C_4), 33.71 (q, *J*=28.5 Hz, CH_2CF_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 ($CDCl_3$): δ =9.25 (br s, 1H, N*H*),7.23-7.09 (m, 5H, C_6H_5), 5.01 (s, 1H, C_4H), 4.93 (d, *J*= 10.4 Hz, 1H, CH_2Br), 4.54 (d, *J*= 10.4 Hz, 1H, CH_2Br), 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 ($CDCl_3$): δ=166.22 (COO), 145.60 (C_6H_5), 141.59 ($C_{2,6}$), 128.23 (C_6H_5), 128.03 (C_6H_5), 127.92 (C_6H_5), 127.48 (q, *J*=276 Hz, CF_3), 106.41 ($C_{3,5}$), 64.76 (OCH_2), 40.10 (C_4H), 33.75 (q, J=28.4 Hz, CH_2CF_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 ($CH_2C_{2,6}$), 21.82(kv, J=2.93Hz, CH_2) ppm. ^{19}F NMR ($CDCl_3$): δ= -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-dipyridinium 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 ($CDCl_3$): δ =10.95 (s, 1H, N*H*), 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_6H_5), 6.36 (d, *J*=13.5 Hz, 4H, 2CH_2Py), 5.93 (d, *J*=13.5 Hz, 4H, 2CH_2Py) 5.07 (s, 1H, C_4H), 4.05 (t, *J*=6.8 Hz, 4H, $2OCH_2$), 2.12-1.98 (m, 4H, 2CH_2), 1.58-1.24 (m, 40H, $2(CH_2)_{10}$). ^{13}C-NMR 100.3 MHz ($CDCl_3$): δ=166.43 (COO), 146.61(Py), 145.51(C_6H_5), 144.88 (Py), 138.01 ($C_{2,6}$), 128.86 (Py), 128.60 (C_6H_5), 128.34 (q, *J*=278 Hz, CF_3), 128.05 (C_6H_5), 127.50 (C_6H_5), 110.41 ($C_{3,5}$), 65.52 (OCH_2), 57.32 ($CH_2C_{2,6}$), 39.76 (C_4H), 33.69 (q, J=28 Hz, CH_2CF_3), 29.56(CH_2), 29.54($2CH_2$) 29.34(CH_2), 29.25(CH_2), 29.18(CH_2), 28.66(CH_2), 28.39(CH_2), 25.94(CH_2), 21.82(kv, J=2.93Hz, CH_2) ppm. ^{19}F NMR ($CDCl_3$): δ= -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**.



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 dodecyl 3-aminobut-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. $C_{40}H_{62}F_3NO_4$ MW 677.92 Mp. 64-67°C. ^{1}H NMR 400 MHz ($CDCl_3$): δ =7.44 (d, *J*=8.4 Hz, 2H, C_6H_4), 7.36 (d, *J*=8.4 Hz, 2H, C_6H_4), 5.58 (br s, 1H, N*H*), 5.04 (s, 1H, C_4H), 4.06-3.98 (m, 4H, 2OCH_2), 2.34 (s, 6H, 2CH_3), 1.58-1.54 (m, 4H 2OCH$_2$CH_2), 1.32-1.23 (m, 36H, 2(CH_2)$_{10}$), 0.88 (t, *J*=6.8 Hz, 6H, 2CH_3). ^{13}C-NMR 100.3 MHz ($CDCl_3$): δ =167.28(*C*OO), 151.49 (C_6H_5), 144.21($C_{2,6}$), 128.19 (C_6H_5), 128.27 (kv, *J*=32.3 Hz, C_6H_5), 126.56 (kv, *J*=270.3 Hz, CF_3), 124.83 (kv, *J*=4 Hz, C_6H_5), 103.71 ($C_{3,5}$), 64.09(OCH2), 39.68 (C_4H), 31.88(CH_2), 29.62(2CH_2), 29.61(CH_2), 29.53(CH_2), 29.33(CH_2), 29.28(CH_2), 28.70, (CH_2), 26.08(CH_2), 22.68(CH_2), 19.68(CH_3), 14.10(CH_3) ppm. 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 MeOH was removed on a rotary evaporator and the residue was triturated with carbon tetrachloride to precipitate succinimide. The solids were filtered and the solvent removed to give the crude dibromide as an orange oil 1.05 g in quantitative yield. $C_{40}H_{60}Br_2F_3NO_4$ MW 835.71. ^{1}H NMR 200 MHz ($CDCl_3$): δ =7.49 (d, *J*=9.0 Hz, 2H, C_6H_4), 7.37 (d, *J*=9.0 Hz, 2H, C_6H_4), 6.57 (br s, 1H, NH), 5.08 (s, 1H, CH), 4.86 (d, *J*=11.4 Hz, 1H, CH_2), 4.66 (d, *J*=11.4 Hz, 1H, CH_2), 4.07 (t, *J*=6.8 Hz, OCH_2), 1.58-1.54 (m, 4H OCH_2CH_2), 1.32-1.23 (m, 36H, $(CH_2)_9CH_3$), 0.87 (t, *J*=6.8 Hz, 6H, CH_3). ^{13}C-NMR 100.3 MHz ($CDCl_3$): δ = 165.88 (COO), 149.44 (C_6H_4), 142.21 ($C_{2,6}$), 129.04 (kv, *J*=32.0 Hz, C_6H_4), 128.25 (C_6H_4), 126.78 (kv, *J*=266Hz, CF_3), 125.24 (kv, *J*=4.1, C_6H_4), 105.34 ($C_{3,5}$), 64.96 (OCH_2), 40.12 (C_4), 31.89 (CH_2), 29.63 (CH_2), 29.62 (CH_2), 29.59(CH_2), 29.53(CH_2), 29.34(CH_2), 29.29(CH_2), 28.55 (CH_2), 27.06 (CH_2-$C_{2,6}$), 26.03 (CH_2), 22.67 (CH_2), 14.09 (CH_3) ppm. ^{19}F NMR ($CDCl_3$): δ= -62.41.

1,1`-[(3,5-Bis(dodecyloxycarbonyl)-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. $C_{50}H_{70}Br_2F_3N_3O_4$ MW 993.91. ^{1}H NMR 400 MHz ($CDCl_3$): δ =11.04 (br s, 1H, N*H*), 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_6H_4), 7.46 (d, *J*=8.4 Hz, 2H, C_6H_4), 6.39 (d, *J*=13.8 Hz, 4H, 2C*H*$_2$Py), 5.92 (d, *J*=13.8 Hz, 4H, 2C*H*$_2$Py), 5.17 (s, 1H, C_4H), 4.05 (t, *J*=6.8 Hz, 4H, 2OC*H*$_2$), 1.60-1.53 (m, 4H, 2C*H*$_2$), 1.32-1.19 (m, 36H, 2(C*H*$_2$)$_{10}$), 0.87 (t, *J*=6.8 Hz, 6H, 2C*H*$_3$). ^{13}C-NMR 100.3 MHz ($CDCl_3$): δ = 166.08 (COO), 149.30 (C_6H_4), 145.08 (Py), 143.33 (Py), 138.43 ($C_{2,6}$), 129.58 (kv, *J*=32.0 Hz, C_6H_4), 128.76 (C_6H_4), 128.53 (Py), 125.53 (kv, *J*=4.1, C_6H_4), 125.38 (kv, *J*=272.3Hz, CF_3), 109.91 ($C_{3,5}$), 65.65 (OCH_2), 57.35 (CH_2-$C_{2,6}$), 40.02 (C_4), 31.89 (CH_2), 29.63 (CH_2), 29.62 (CH_2CH_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 ($CDCl_3$): δ= -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}.

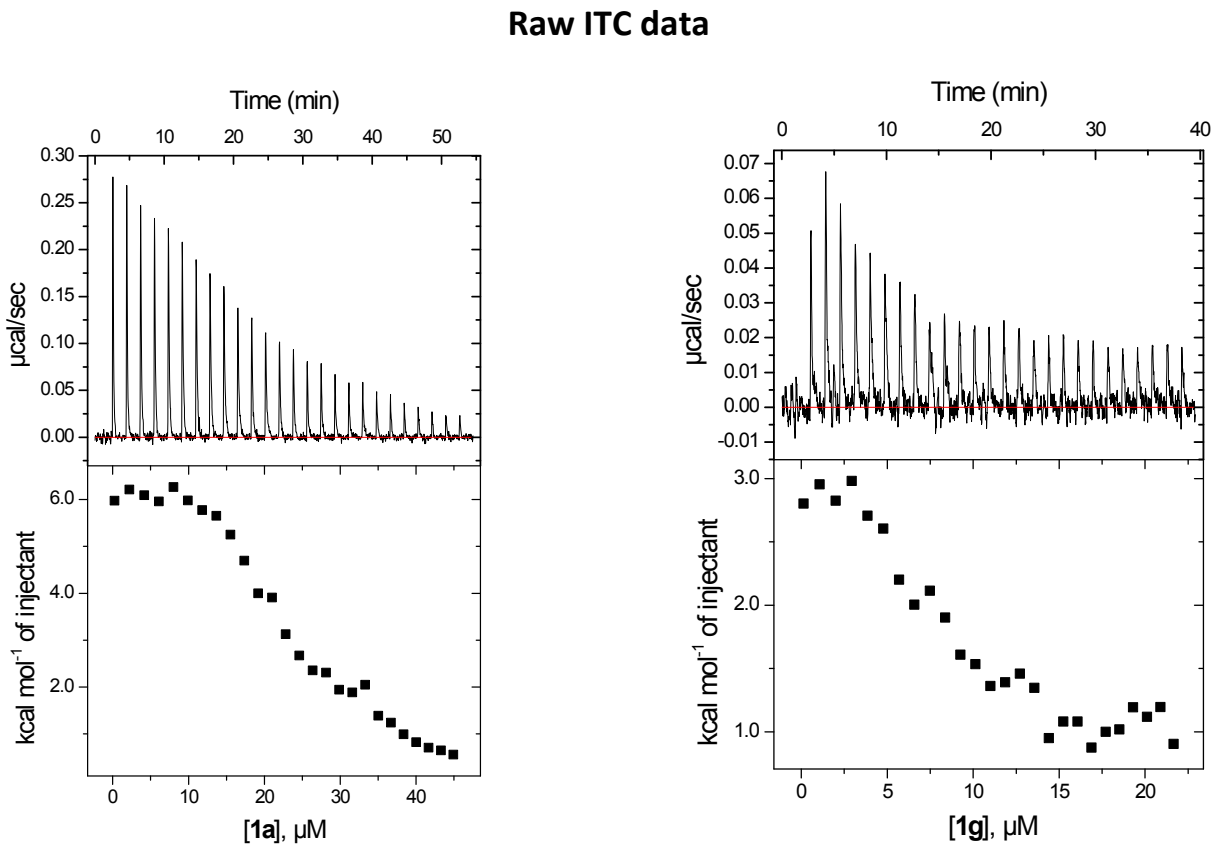


Figure 1. Raw ITC titration data for **1a** and **1g** (Figure 1 in the main article). Concentrations for 1,4-DHPs in syringe: for **1a** is 0.270 mM and for **1g** is 0.130 mM.

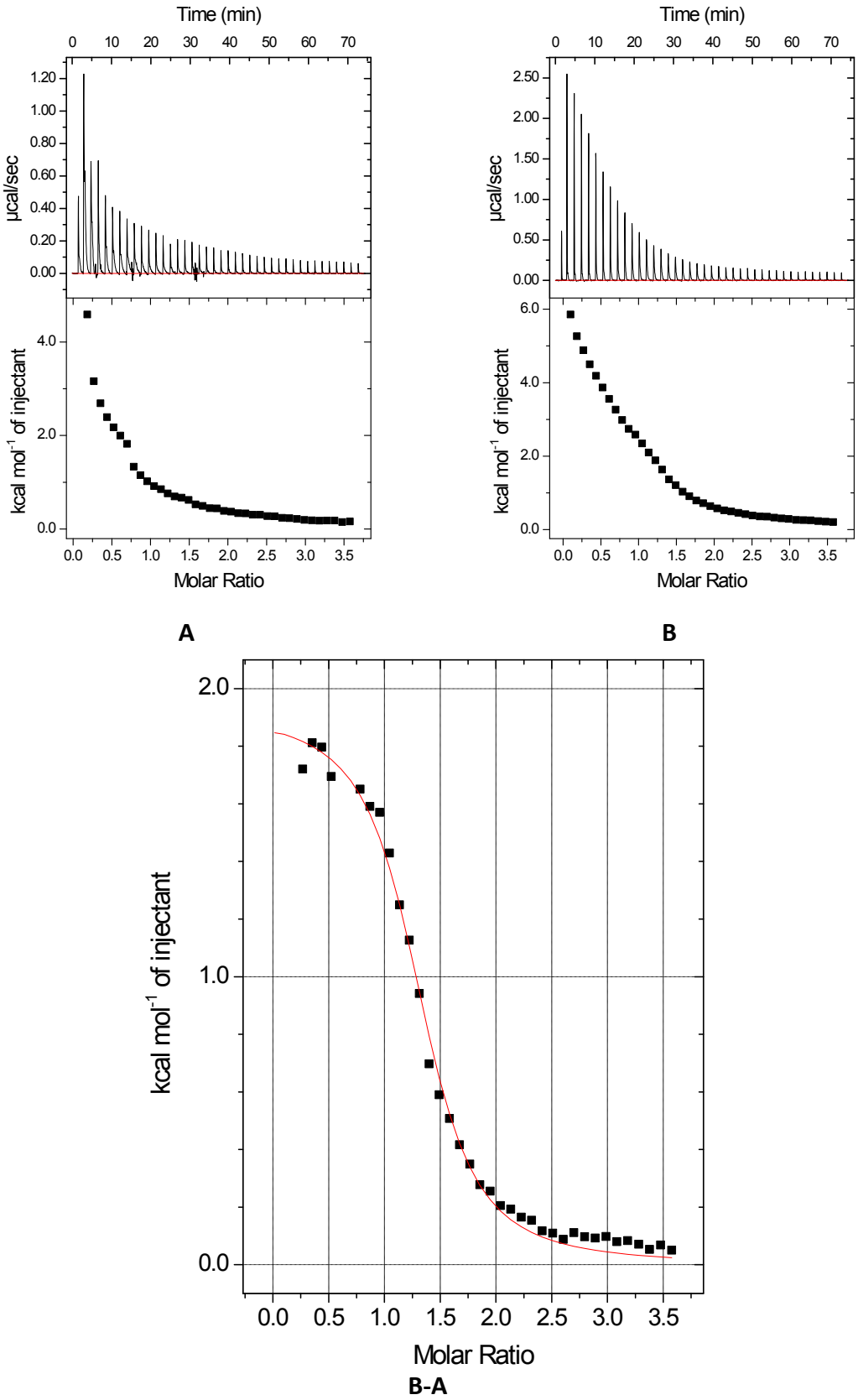


Figure 2. There are tree images (**A**, **B**, **B-A**).

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.

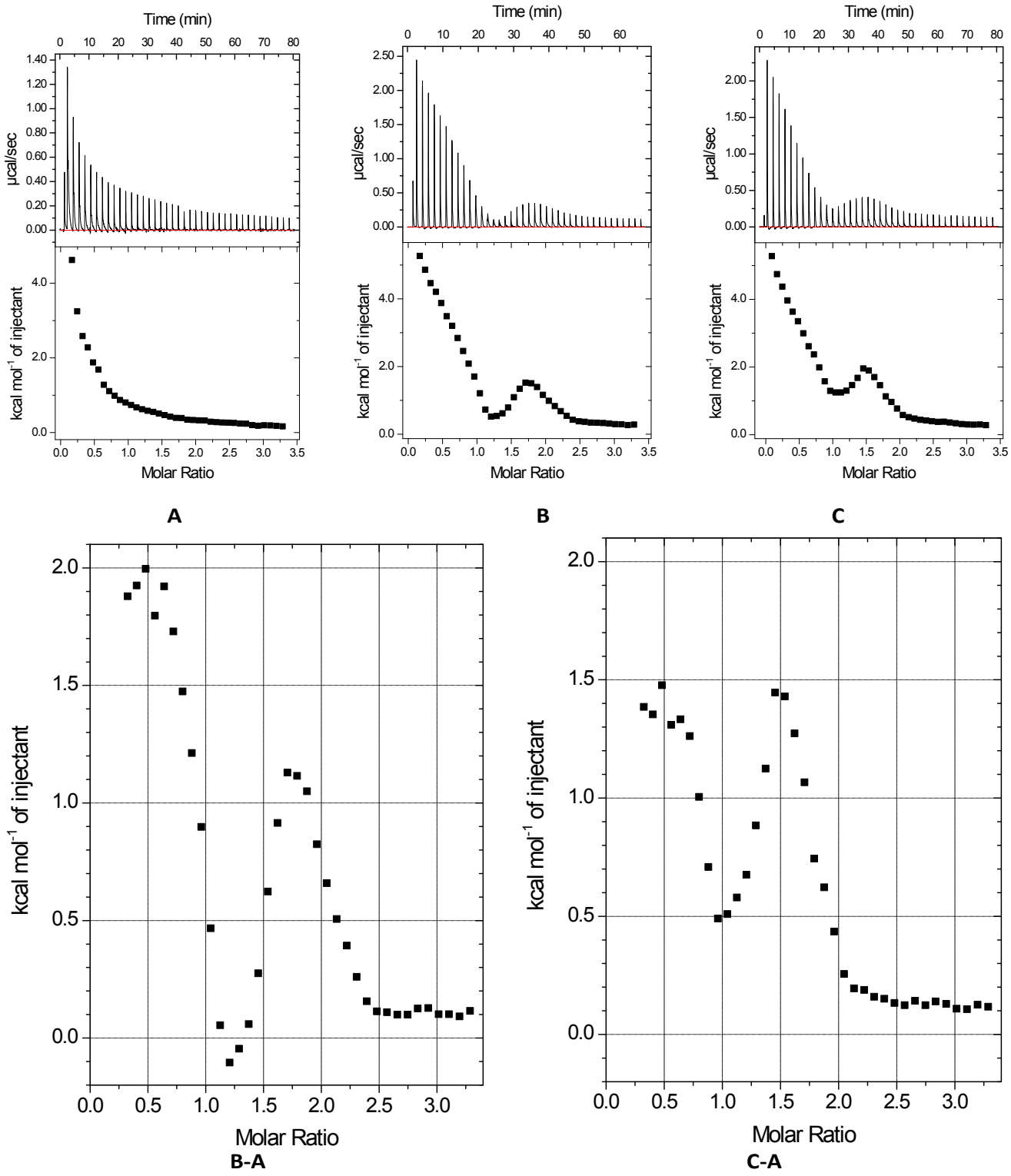


Figure 3. ITC raw data with delta H obtained from integrals (from the Figure 6. In article). Two different experiments under the same conditions (**1a** is 3.27 mM as a guest (syringe), **2c** is 0.207 mM as a host (cell). (3.27).

References

1. Graupe, M.; Koini, T.; Wang, V. Y.; Nassif, G. M.; Colorado, R.; Villazana, R. J.; Dong, H.; Miura, Y. F.; Shmakova, O. E.; Lee, T. R. Terminally perfluorinated long-chain alkanethiols. *J. Fluor. Chem.* **1999**, *93*, 107–115.