Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics

Supporting Information

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Figure S1. Response of the fluorescence intensity of FTY720 in water at different wavelength [FTY720] = 200 μM.

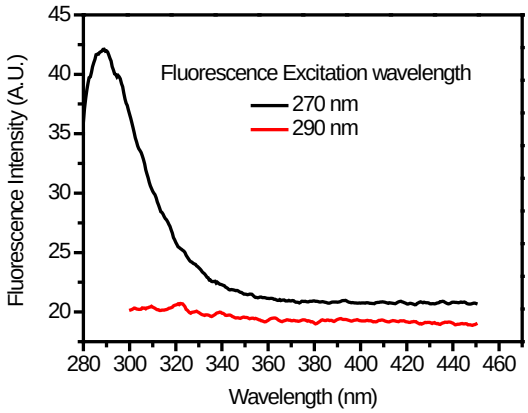


Figure S2. Response of the fluorescence intensity of 1-naphthol in water with increase in concentration of FTY720 at (A) 13 ^{o}C (B) 35 ^{o}C (λ_{ex} = 290 nm, λ_{em} = 470 nm). [1-Naphthol] = 4 μM.

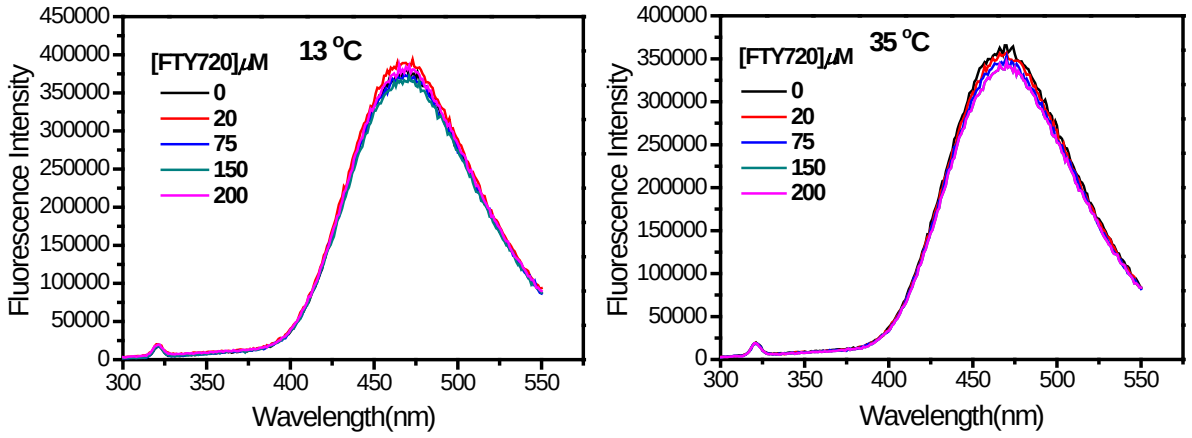


Figure S3. Response of the absorbance of 1-naphthol in water with increase in concentration of FTY720.[1-naphthol]=100μM

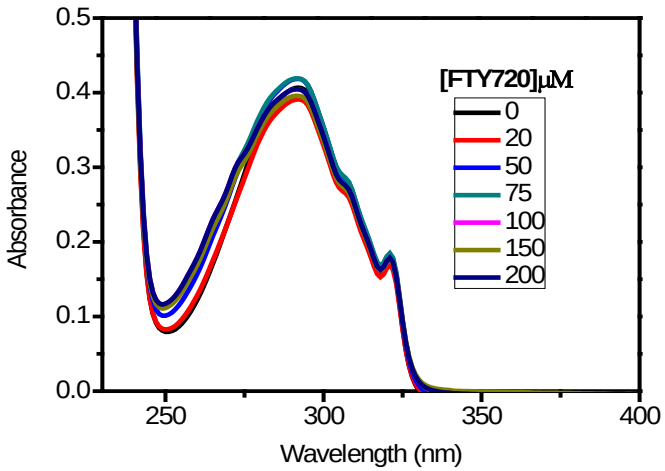


Figure S4. Response of fluorescence intensity of pyrene with increase in concentration of CPC quencher.[Pyrene]=4 μM, λ_{ex}=335 nm.

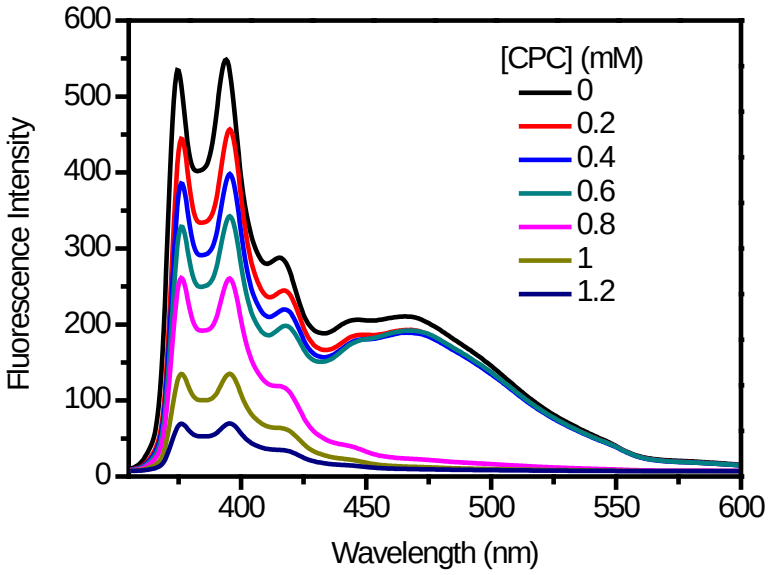


Table S1. Temperature dependence fluorescence lifetime data of NpOH* (A) absence of FTY720 and (B) presence of FTY720 ([FTY720] =150 μM), (λ_{ex}= 295 nm, λ_{em}= 370 nm) in DMPC liposome. [DMPC] =0.4 mM, [1-Naphthol] =4 μM. (Error = ± 5 %)

A)

Temp. (^{o}C)	τ_s (ns) (α_1)	τ_l (ns) (α_2)	τ_{avg} (ns)	χ^2
13	2.99 (0.27)	6.99 (0.73)	6.44	1.13
16	2.94 (0.28)	6.91 (0.72)	6.34	1.43
19	3.05 (0.30)	6.84 (0.70)	6.23	1.22
21	3.07 (0.31)	6.73 (0.69)	6.10	1.14
22	3.08 (0.32)	6.63 (0.68)	5.99	1.22
23	3.07 (0.33)	6.45 (0.67)	5.80	1.28
25	3.02 (0.35)	6.25 (0.65)	5.58	1.07
27	2.88 (0.36)	6.14 (0.64)	5.45	1.24
30	2.89 (0.40)	6.07 (0.60)	5.30	1.21
33	2.79 (0.41)	5.90 (0.59)	5.12	1.22

B)

Temp. (^{o}C)	τ_s (ns) (α_1)	τ_l (ns) (α_2)	τ_{avg} (ns)	χ^2
13	2.69 (0.30)	6.62 (0.70)	6.03	1.38
16	2.73 (0.30)	6.57 (0.70)	5.98	1.21
19	2.88 (0.31)	6.38 (0.69)	5.78	1.27
21	3.02 (0.32)	6.11 (0.68)	5.52	1.20
22	3.06 (0.36)	6.09 (0.64)	5.42	1.24
23	3.03 (0.37)	6.00 (0.63)	5.31	1.14
25	3.03 (0.41)	5.91 (0.59)	5.15	1.24
27	2.93 (0.41)	5.79 (0.59)	5.04	1.10
30	2.52 (0.42)	5.27 (0.58)	4.56	1.22
33	2.57 (0.43)	5.33 (0.57)	4.59	1.23

Table S2. Fluorescence lifetime data of NpO^{-}* in water (λ_{ex}=295 nm, λ_{em}=470 nm) with the increase in FTY720 at 13^{o}C. [1-Naphthol]=4 μM. (Error = ± 5 %)

[FTY720]µM	τ (ns)	χ^2
0	8.41	1.20
50	8.32	1.20
150	8.27	1.24

Table S3. Temperature dependence fluorescence lifetime data of NpO^{-}* (A) absence of FTY720 and (B) presence of FTY720 ([FTY720] =150 μM), (λ_{ex}= 295 nm, λ_{em}= 460 nm) in DMPC liposome. [DMPC] =0.4 mM, [1-Naphthol] =4 μM. (Error = ± 5 %)

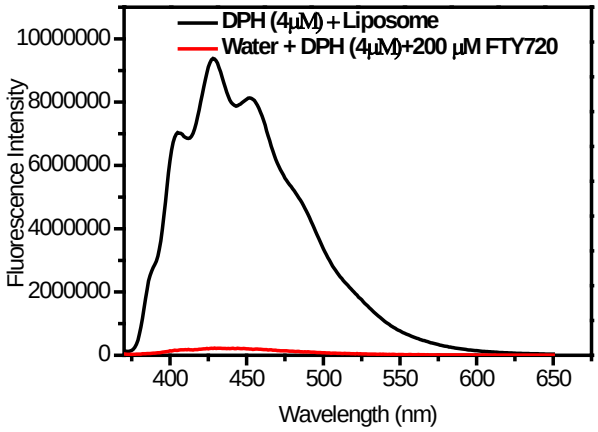
A)

Temp. (^{o}C)	τ_s (ns) (α_1)	τ_l (ns) (α_2)	τ_{avg} (ns)	χ^2
13	8.30 (0.61)	21.43 (0.31)	16.47	1.21
16	8.26 (0.60)	21.16 (0.40)	16.39	1.21
19	8.27 (0.58)	20.74 (0.42)	16.31	1.21
21	8.37 (0.56)	20.66 (0.44)	16.47	1.21
22	8.29 (0.53)	20.34 (0.47)	16.54	1.21
23	8.59 (0.41)	19.22 (0.59)	16.70	1.28
25	8.94 (0.41)	19.48 (0.59)	16.93	1.13
27	9.00 (0.42)	19.16 (0.58)	16.66	1.21
30	8.96 (0.44)	18.91 (0.56)	16.21	1.08
33	8.79 (0.46)	18.67 (0.54)	15.81	1.25

B)

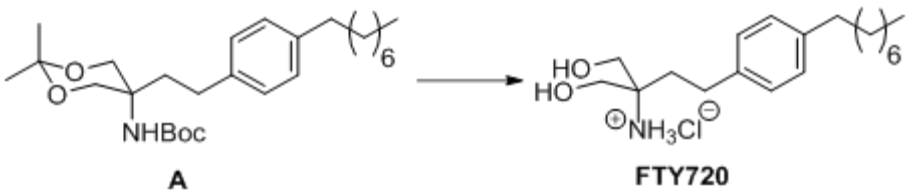
Temp. (^{o}C)	τ_s(ns) (α_1)	τ_l (ns) (α_2)	τ_{avg} (ns)	χ^2
13	7.90 (0.74)	19.06 (0.26)	13.01	1.26
16	7.75 (0.70)	18.22 (0.30)	13.00	1.35
19	7.68 (0.65)	17.68 (0.35)	13.21	1.22
21	8.25 (0.52)	17.91 (0.48)	14.69	1.11
22	8.65 (0.53)	17.98 (0.47)	14.69	1.10
23	8.69 (0.53)	17.75 (0.47)	14.52	1.19
25	9.03 (0.56)	17.86 (0.44)	14.56	1.21
27	8.99 (0.56)	17.29 (0.44)	13.98	1.22
30	8.55 (0.58)	16.70 (0.42)	12.90	1.29
33	8.78 (0.63)	16.69 (0.36)	12.89	1.21

Figure S5: Response of the fluorescence intensity of DPH in water with 200 μM of FTY720 and in DMPC liposome media. (λ_{ex} = 370 nm, λ_{em} = 428 nm). [DMPC]=0.4mM, [DPH] = 4 μM.



Characterisation and purity of Fingolimod:

Analytically pure **A** (the ultimate precursor of FTY720) prepared according to our reported procedure[1] was used for the preparation of FTY720.



A **FTY720**

A solution of **A** (0.580 g, 1.42 mmol) in CH_2Cl_2 (6 mL) and TFA (1.1 mL), was stirred at room temperature for 12 h. The reaction mixture was quenched with sat. aq $NaHCO_3$ soln (5 mL) and then extracted with EtOAc (60 mL). The organic layer was washed with brine (40 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue obtained was then dissolved in dry THF (10 mL). To this solution ethereal HCl solution (2 mL) was added at 0 °C and the resulting mixture was warmed to room temperature and stirred for 3 h. The solution was concentrated under reduced pressure to afford the desired product as solid. This solid was thoroughly washed with dry ether (3 x 10 mL) and dried under high vacuum to afford **FTY720** (0.412 g, 90% over two steps). The material was directly used for the studies, after recording ^{1}H and ^{13}C NMR spectrum.

***tert*-butyl 2,2-dimethyl-5-(4-octylphenethyl)-1,3-dioxan-5-ylcarbamate (A) :**

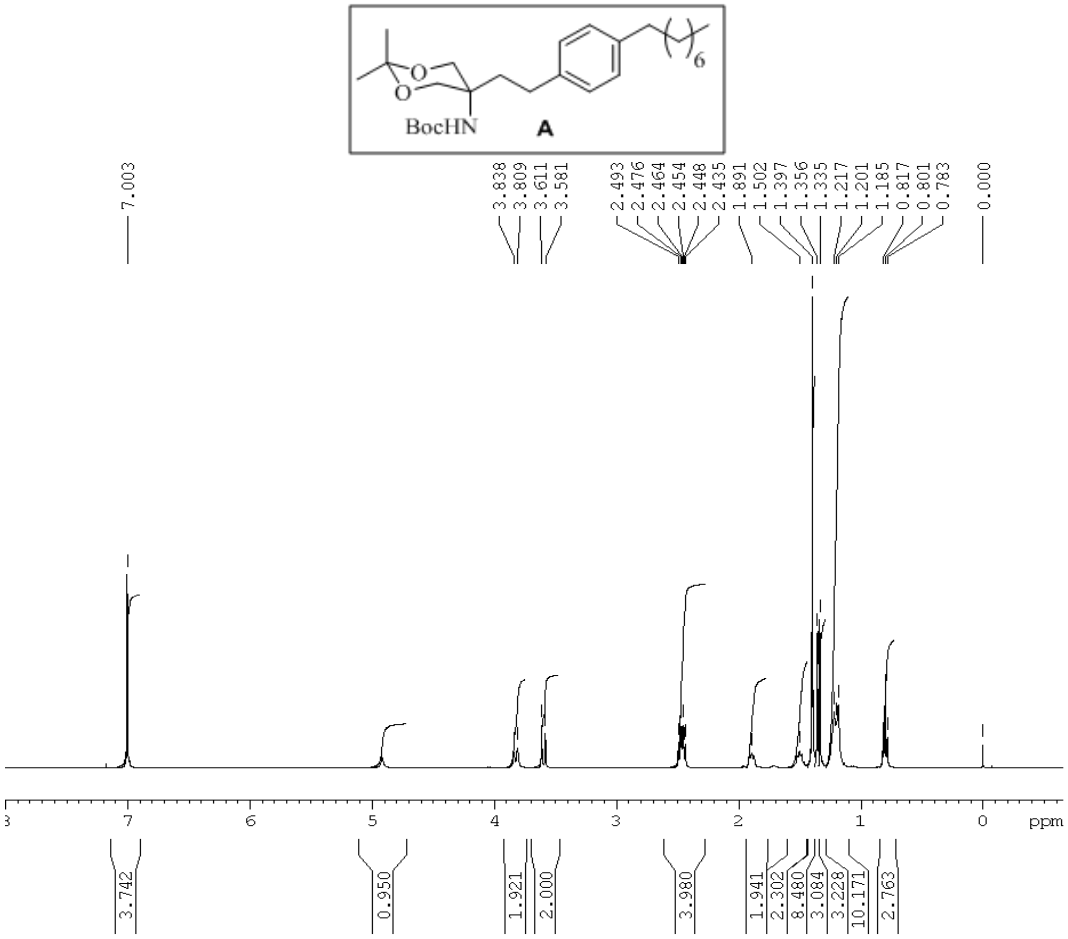
Colorless solid, m.p.: 62-64 ^{o}C.; R_f 0.6 (hexanes: ethyl acetate, 7:3); IR ($CHCl_3$): 2921, 2851, 1720, 1495, 1166 cm^{-1}; ^{1}H NMR [$CDCl_3$, 400 MHz] *δ:* 0.80 (t, 3H, *J* = 6.8 Hz, -C*H*$_3$), 1.18-1.22 (m, 10H, 5 x C*H*$_2$), 1.34 (s, 3H, -CC*H*$_3$), 1.36 (s, 3H, -CC*H*$_3$), 1.40 (s, 9H, -C(C*H*$_3$)$_3$), 1.49-1.52 (m, 2H, -C*H*$_2$), 1.88-1.92 (m, 2H, -CC*H*$_2$), 2.43-2.49 (m, 4H, 2 x ArC*H*$_2$), 3.60 (d, 2H, *J* = 11.6 Hz, -OC*H*$_2$), 3.82 (d, 2H, *J* = 11.6 Hz, -OC*H*$_2$), 4.91 (bs, 1H, -N*H*), 7.00 (s, 4H, Ar*H*). ^{13}C NMR [$CDCl_3$, 100 MHz] *δ:* 13.1, 18.9, 21.6, 26.3, 27.4, 27.7, 28.3, 28.5, 30.5, 30.9, 32.8, 34.5, 50.8, 65.4, 78.3, 97.4, 127.0, 127.2, 137.8, 139.3, 153.7.; HRMS (ESI): Calcd for $C_{27}H_{46}NO_4Na$ ($[M+Na]^+$): 470.3246. Found: 470.3248.

2-amino-2-(4-octylphenethyl)propane-1,3-diol hydrochloride (FTY720)

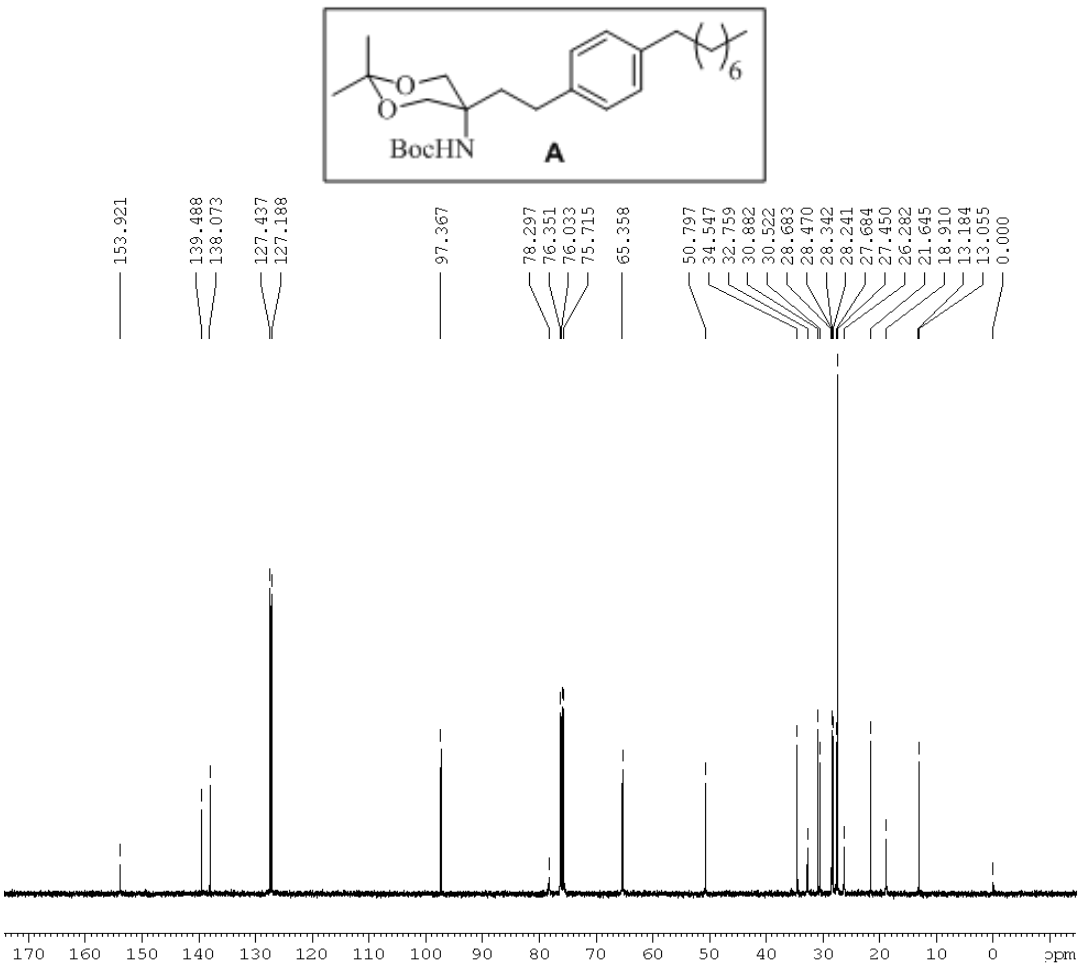
Colorless solid; 90% (over two steps); m.p.: 103-105 ^{o}C; R_f 0.4 (methanol: chloroform, 1:9); IR ($CHCl_3$): 2921, 2851, 1720, 1495, 1166 cm^{-1}; ^{1}H NMR [DMSO-d_6, 400 MHz] *δ:* 0.85 (t, 3H, *J* = 6.8 Hz, -C*H*$_3$), 1.23-1.26 (m, 10H, 5 x C*H*$_2$), 1.53-1.54 (m, 2H, -C*H*$_2$), 1.76-1.80 (m, 2H, -CC*H*$_2$), 2.54-2.59 (m, 4H, 2 x ArC*H*$_2$), 3.52 (d, 4H, *J* = 4.8 Hz, -OC*H*$_2$), 5.39 (d, 2H, *J* = 4.8 Hz, 2 x -O*H*), 7.10 (s, 4H, Ar*H*), 7.89 (bs, 3H, 3 x -N*H*). ^{13}C NMR [DMSO-d_6, 125 MHz] *δ:* 13.9, 22.1, 27.9, 28.7, 28.8, 31.1, 31.3, 33.2, 34.7, 60.3, 61.0, 128.0, 128.2, 138.8, 139.8.; MS (ESI): 308 ([M-Cl]$^{+}$).

[1. Sivaraman, B.; Senthilmurugan A.; Aidhen, I. S. *Synlett* **2007**, 2841-2846.]

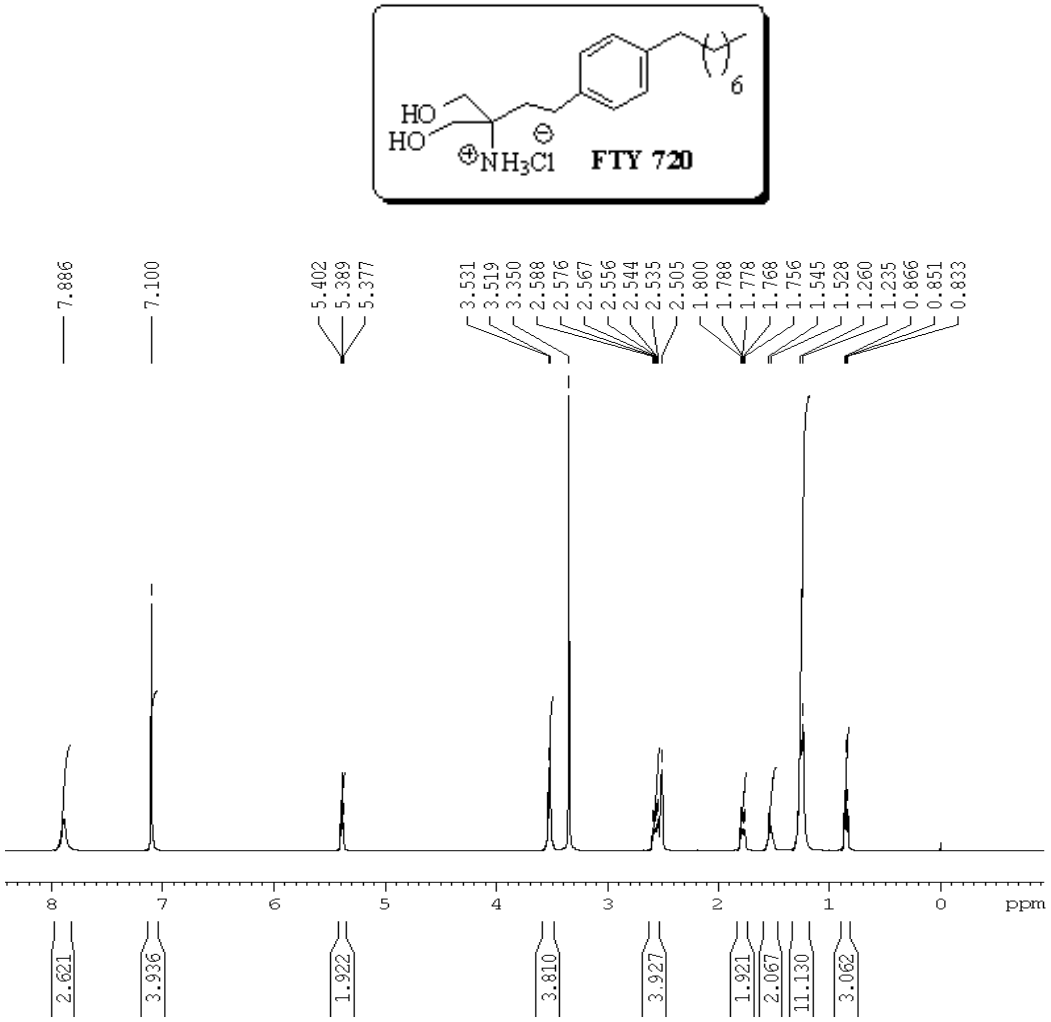
^{1}H NMR (400 MHz) of *tert*-butyl 2,2-dimethyl-5-(4-octylphenethyl)-1,3-dioxan-5-ylcarbamate (A)



^{13}C NMR (100 MHz) of *tert*-butyl 2,2-dimethyl-5-(4-octylphenethyl)-1,3-dioxan-5-ylcarbamate (A)



^{1}H NMR (400 MHz) of 2-amino-2-(4-octylphenethyl)propane-1,3-diol hydrochloride (FTY720)



^{13}C NMR (125 MHz) of 2-amino-2-(4-octylphenethyl)propane-1,3-diol hydrochloride (FTY720)

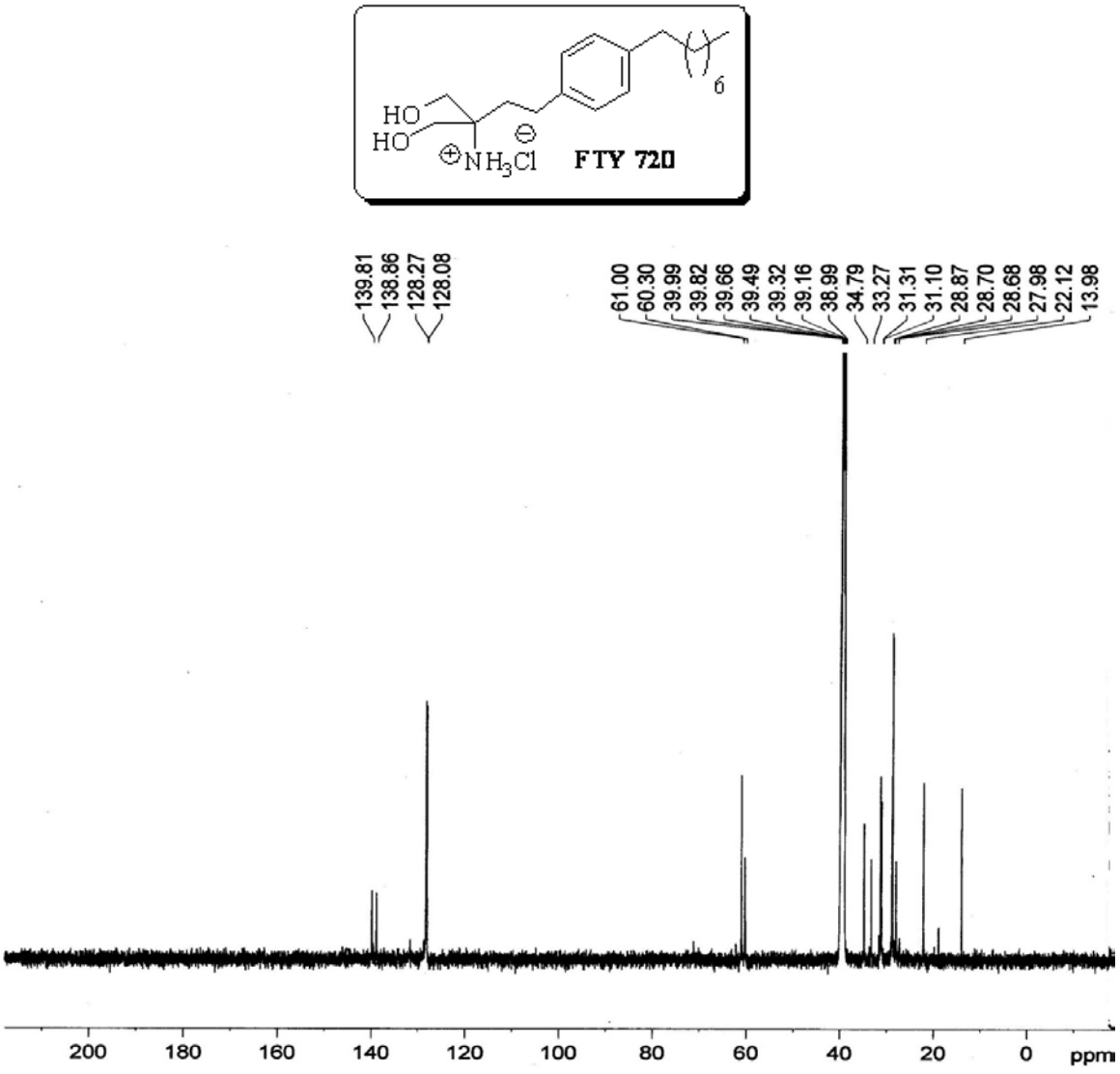


Figure S6. Schematic representation of interaction of FTY720 with DMPC lipid bilayer: