Electronic Supplementary Information

Monitoring Drug-Lipid Membranes Interactions via Molecular Rotor Probe

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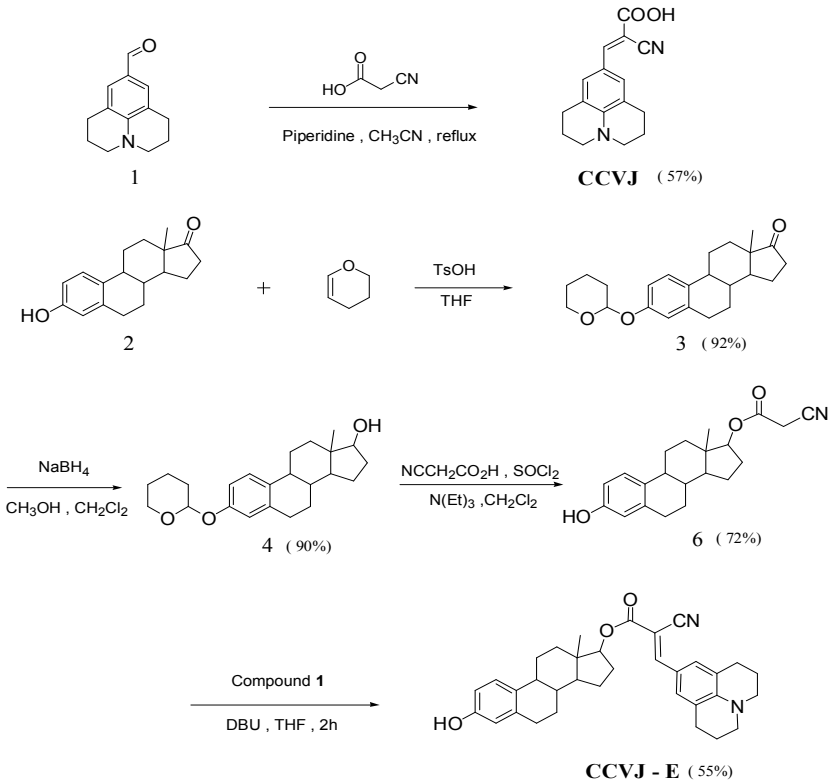
1. General methods

All commercially available reagents were used without purification unless otherwise noted. Column chromatography was performed using silica gel from Merck (100-200 mesh). Visualization of the compounds was accomplished with UV light (254 nm) and iodine. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ operating at 400 MHz and 100 MHz. Proton chemical shifts are reported relative to the residual proton signals of the deuterated solvent $CDCl_3$ (7.26 ppm) DMSO-d_6 (2.50 and 3.33 ppm) or TMS. Carbon chemical shifts were internally referenced to the deuterated solvent signals in $CDCl_3$ (77.00 ppm) or DMSO-d_6 (40.0 ppm). Data are represented as follows: chemical shift, multiplicity (bs = broad singlet, s = singlet, d = doublet, t = triplet, m = multiplet), coupling constant in Hertz (Hz), and integration. Products were identified by comparison to spectral data reported in the literature. Mass spectra at high resolution were recorded on a time-of-flight mass spectrometer with an ESI source.

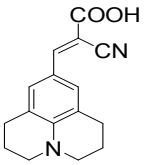
2. Synthetic routes of the probe CCVJ and CCVJ-E

The synthetic routes of the probe CCVJ and CCVJ-E were showed in Scheme 1.



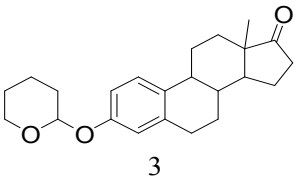
Scheme 1. Synthetic Routes of the Probe CCVJ and CCVJ-E

3. Experimental procedures and characterization data of compounds



CCVJ

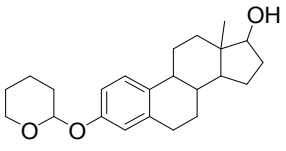
Compound **1** (142 mg, 0.7 mmol), cyanoacetic acid (119 mg, 1.4 mmol), and piperidine (59.6 mg, 0.7 mmol) was dissolved in CH_3CN. The mixture was heated to reflux for 2 h. Then the solution was cooled to rt and most solvent was evaporated under reduced pressure. The resulting acid residue was treated with water (100 mL). For the aqueous part, pH was adjusted to 1 and it was then extracted with CH_2Cl_2. The combined organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by silica gel chromatography (hexane:EtOAc = 1:1) to give the desired product **CCVJ** (107 mg, 0.4 mmol) as a orange solid in 57% yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (s, 1H), 7.50 (s, 2H), 3.33 (t, J = 5.6Hz, 4H), 2.67 (t, J = 6Hz 4H), 1.85-1.90 (m, 4H). ^{13}C NMR (100MHz, $CDCl_3$) δ 165.7, 153.9, 147.9, 131.4, 121.0, 118.8, 117.8, 91.7, 49.9, 27.5, 21.0. HRMS (ESI-TOF) m/z: calcd for $C_{16}H_{16}O_2N_2$ $[M-H]^+$ 267.1139, found 267.1141.



3

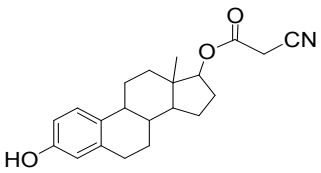
Estrone **2** (1 g, 3.7mmol) and dihydropyran (4 mL, 43.8 mmol) was dissolved in anhydrous tetrahydrofuran (30 mL). Then, 4-Methylbenzenesulfonic acid (26 mg, 0.2 mmol) was added. The reaction mixture was stirred for 1 h at 25 °C . The solution was neutralized with saturated aqueous solution of $NaHCO_3$. Then most solvent was evaporated under reduced pressure below 30 °C, The resulting solution was extracted with CH_2Cl_2 and washed with water. The combined organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by silica gel chromatography (hexane:EtOAc = 6:1) to give the desired product **3** (1.2 g, 3.4mmol) as a white solid in 92% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (d, J =

8.4 Hz, 1H), 6.88 (dd, J = 8.8, 2.4 Hz, 1H), 6.83 (d, J = 2.4 Hz, 1H), 5.42 (s, 1H), 3.98-3.92 (m, 1H), 3.64-3.59 (m, 1H), 2.92-2.90 (m, 2H), 2.56-2.40 (m, 2H), 2.30-2.25 (m, 1H), 2.21-1.96 (m, 5H), 1.89-1.85 (m, 2H), 1.72-1.43 (m, 9H), 0.93 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 220.9, 155.1, 137.7, 133.0, 126.2, 116.6, 114.1, 96.4, 62.0, 50.5, 48.0, 44.1, 38.3, 35.9, 31.6, 30.4, 29.6, 26.6, 25.9, 25.3, 21.6, 18.8, 13.9. HRMS (ESI-TOF) m/z: calcd for $C_{23}H_{30}O_3$ $[M+H]^+$ 355.2268, found 355.2272.



4

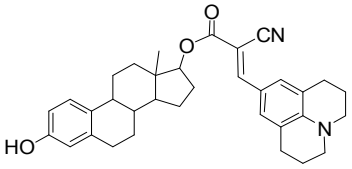
Compound **3** (1.1g, 3.1 mmol) was dissolved in a mixed solvent of anhydrous methanol (50 mL) and CH_2C1_2 (15 mL). Then, $NaBH_4$ (0.3 g, 7.9 mmol) was slowly added in 30 min at 0℃. The solution was stirred for 2 h at rt. After most solvent was evaporated under reduced pressure, the crude residue was extracted with CH_2Cl_2 and washed with water. The combined organic layer was dried with Na_2SO_4 and evaporated under reduced pressure below 30℃. The crude product was purified by silica gel chromatography (hexane:EtOAc = 4:1) to give the desired product **4** (1 g, 2.8 mmol) as a white solid in 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (d, *J* = 8.4 Hz, 1H), 6.88 (dd, *J* = 8.8, 2.0 Hz, 1H), 6.82 (s, 1H), 5.42 (s, 1H), 3.98-3.92 (m, 1H), 3.75 (t, *J* = 8.4 Hz, 1H), 3.64-3.61 (m, 1H), 2.88-2.86 (m, 2H), 2.36-2.31 (m, 1H), 2.25-1.85 (m, 7H), 1.77-1.18 (m, 13H), 0.80 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ155.0, 137.9, 133.6, 126.2, 116.6, 114.0, 96.4, 81.9, 62.0, 50.1, 44.0, 43.3, 38.8, 36.8, 30.6, 30.5, 29.8, 27.3, 26.3, 25.3, 23.1, 18.8, 11.1. HRMS (ESI-TOF) m/z:calcd for $C_{23}H_{32}O_3$ $[M+H]^+$ 357.2424, found 357.2428.



6

Cyanoacetic acid (1.2 g, 14.1 mmol) was dissolved in thionyl chloride (40 mL, 55.1

mol). The mixture was allowed to react at 100℃ for 2 h. The solution was cooled to rt and the excess thionyl chloride was removed by rotary evaporator under reduced pressure to give cyanoacetyl chloride **5** as a yellow oil, which was used without further purification. Compound **4** (0.5 g, 1.4 mmol) and triethylamine (0.6 mL, 4.2 mmol) were dissolved in anhydrous CH_2Cl_2 (30 mL). Then, a solution of Compound **5** in CH_2Cl_2 (10 mL) was slowly added at 0℃. The solution was stirred at rt overnight. The resulting solution was treated with water (100 mL) and washed with CH_2Cl_2 (100 mL). The combined organic layer was dried with Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by silica gel chromatography (hexane:EtOAc = 4:1) to give the desired product **6** (339.2 mg, 1 mmol) as a yellow solid in 72% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (d, *J* = 8.4 Hz, 1H), 6.65 (dd, *J* = 8.4, 2.0Hz, 1H), 6.59 (d, *J* = 2.4Hz, 1H), 4.96 (bs, 1H), 4.80 (t, *J* =8 Hz, 1H), 3.49 (s, 2H), 2.87-2.83 (m, 2H), 2.33-2.18 (m, 3H), 1.95-1.61 (m, 4H), 1.53-1.29 (m,6H), 0.89 (s, 3H). ^{13}C NMR (100MHz, $CDCl_3$) δ162.9, 153.5, 138.1, 132.3, 126.5, 115.3, 113.1, 112.8, 85.6, 49.6, 43.7, 43.2, 38.5, 36.8, 29.5, 27.4, 27.1, 26.1, 24.9, 23.2, 12.0. HRMS (ESI-TOF) m/z: calcd for $C_{21}H_{25}O_3N$ $[M+Na]^+$ 362.1727, found 362.1730.



CCVJ - E

Compound **1** (241.2 mg, 1.2 mmol) and Compound **6** (200 mg, 0.6 mmol) were dissolved in THF (20 mL). Then a solution DBU (136.9 mg, 0.9 mmol) in THF (10 mL) was added to the mixture. The solution was stirred at 25 °C for 2 h. Then most solvent was evaporated under reduced pressure. The crude product was purified by silica gel chromatography (hexane:EtOAc = 3:1) to give the desired product **CCVJ-E** (174 mg, 0.3 mmol) as a orange solid in 55% yield . ^{1}H NMR (400 MHz, $CDCl_3$) δ7.94 (s, 1H), 7.51 (s, 1H), 7.15 (d, *J* = 8.4Hz, 1H), 6.68 (dd, *J* = 8.4, 2.0Hz, 1H), 6.60 (d, *J* = 2.4Hz, 1H), 3.34 (t, *J* =5.6 Hz, 4H), 2.83-2.74 (m, 6H), 2.37-2.18 (m, 4H), 2.00-1.88 (m, 6H), 1.82-1.67 (m, 6H), 1.52-1.31 (m, 7H), 0.97 (s, 3H) .^{13}C NMR (100MHz, $CDCl_3$) δ164.9, 154.3, 153.8, 147.7, 138.1, 132.2, 131.7, 126.4, 120.8,

118.5, 117.9, 115.4, 112.8, 91.8, 84.2, 50.2, 49.8, 43.8, 43.4, 38.6, 36.9, 29.7, 29.6, 27.8, 27.6, 27.3, 26.3, 23.4, 21.1, 12.1. HRMS (ESI-TOF) m/z: calcd for $C_{34}H_{38}O_3N_2$ $[M+Na]^+$ 545.2775, found 545.2778.

4. Effect of TTC binding on the phase transition of DMPC bilayers

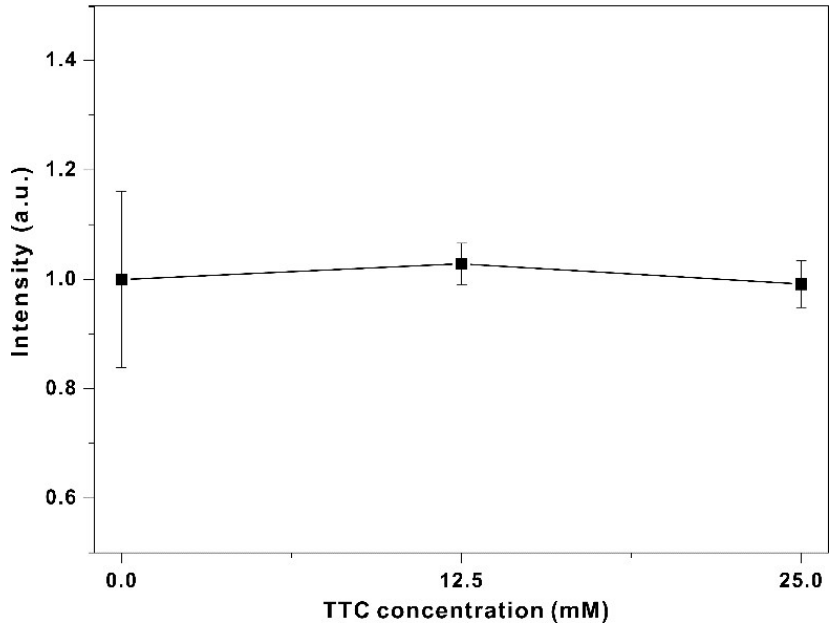


Fig. S1. The fluorescence intensities of CCVJ-E in ethylene glycol with different TTC concentration. The error bars represent the standard deviations obtained by averaging at least 3 independent measurements

5. Effect of TTC on the phase transition of cholesterol-containing DMPC Bilayers

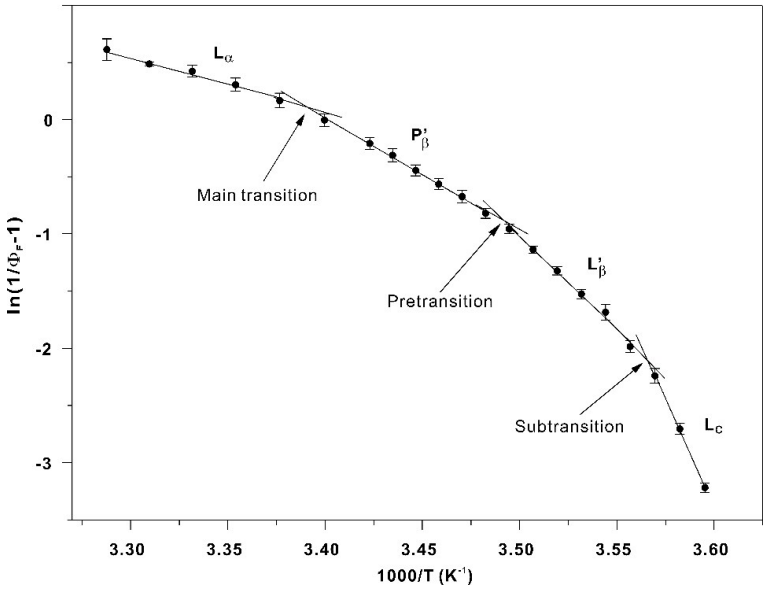


Fig. S2. Variation of the fluorescence quantum yield of CCVJ-E in containing 5 mol % cholesterol DMPC bilayers with temperature. Apparent activation energies are 317.1, 133.6, 81.7 and 40.2 kJ/mol for L_c, L_β', P_β' and L_α, respectively. The error bars represent the standard deviations obtained by averaging at least 3 independent measurements.

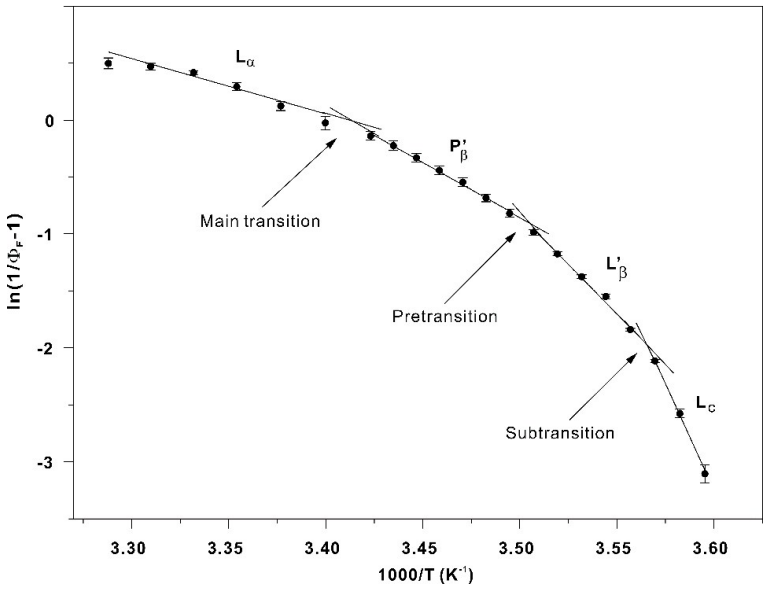


Fig. S3. Variation of the fluorescence quantum yield of CCVJ-E in containing 10 mol % cholesterol DMPC bilayers with temperature. Apparent activation energies are 321.3, 138.9, 78.8 and 40.2 kJ/mol for L_c, L_β', P_β' and L_α, respectively. The error bars represent the standard deviations obtained by averaging at least 3 independent measurements.

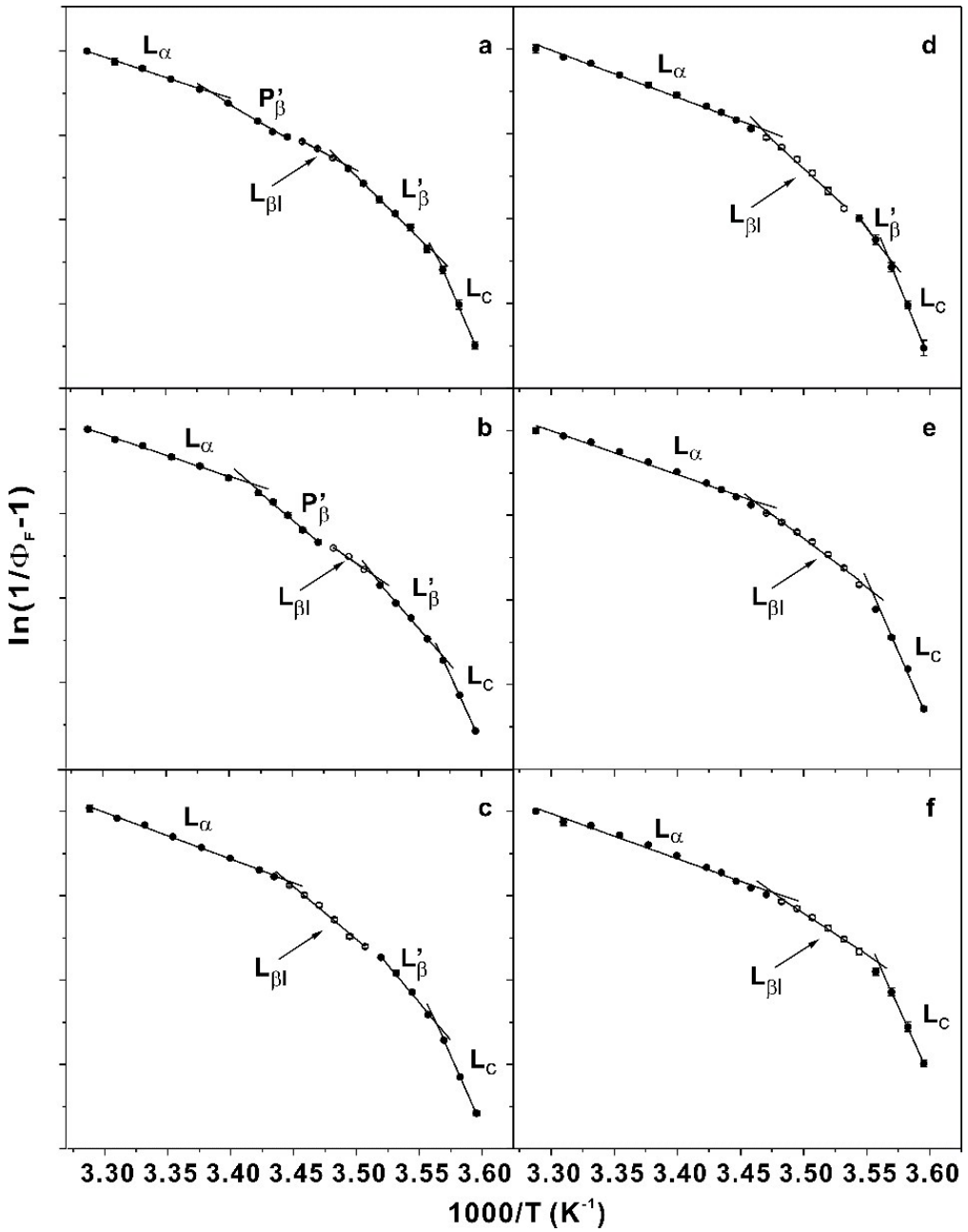


Fig. S4. Phase transition behavior of containing 5 mol % cholesterol DMPC SUVs including TTC of various concentrations: (a) 2.5, (b) 5, (c) 8.5, (d) 12.5, (e) 17 and (f) 25 mM. The error bars represent the standard deviations obtained by averaging at least 3 independent measurements.

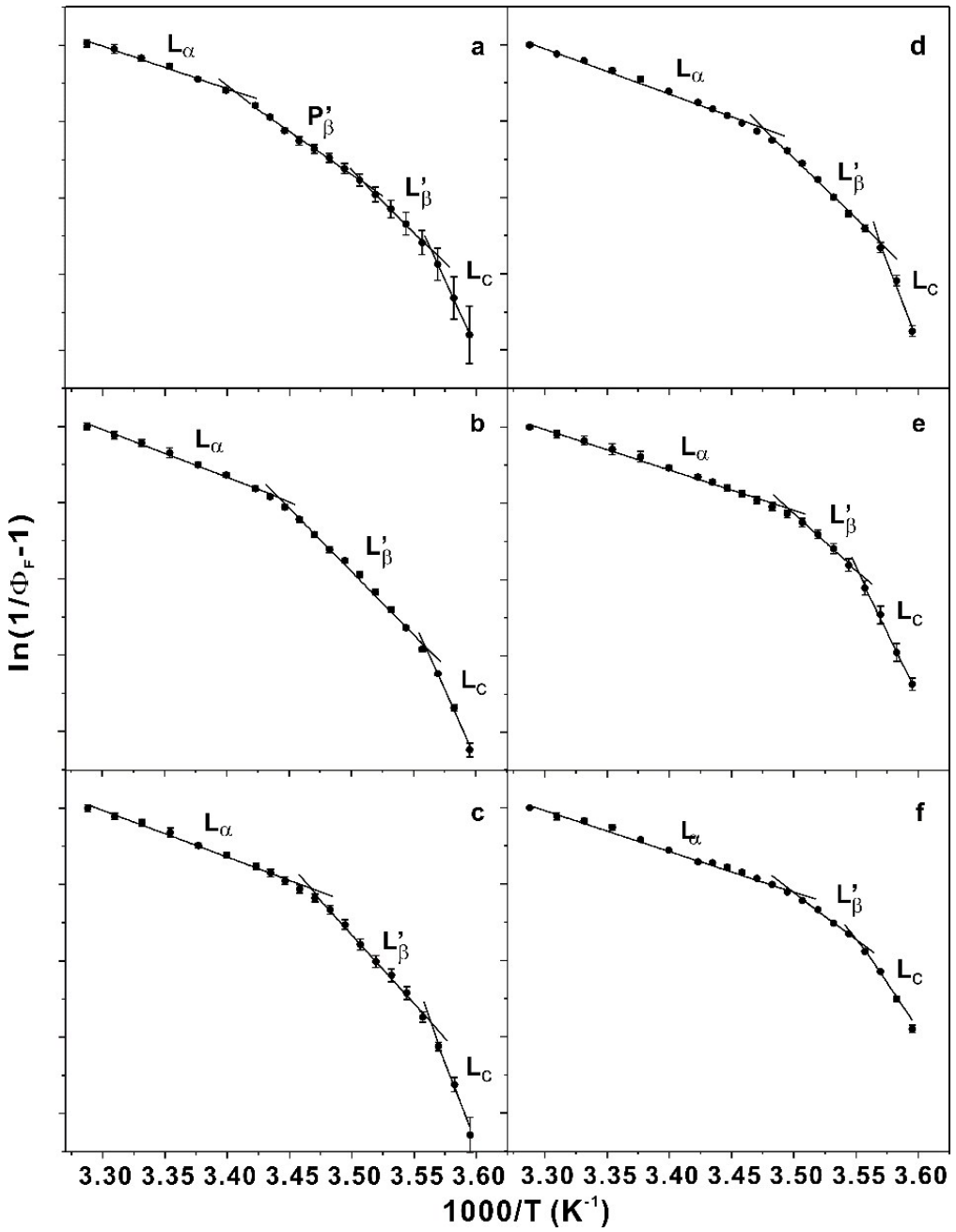


Fig. S5. Phase transition behavior of containing 10 mol % cholesterol DMPC SUVs including TTC of various concentrations: (a) 2.5, (b) 5, (c) 8.5, (d) 12.5, (e) 17 and (f) 25 mM. The error bars represent the standard deviations obtained by averaging at least 3 independent measurements.

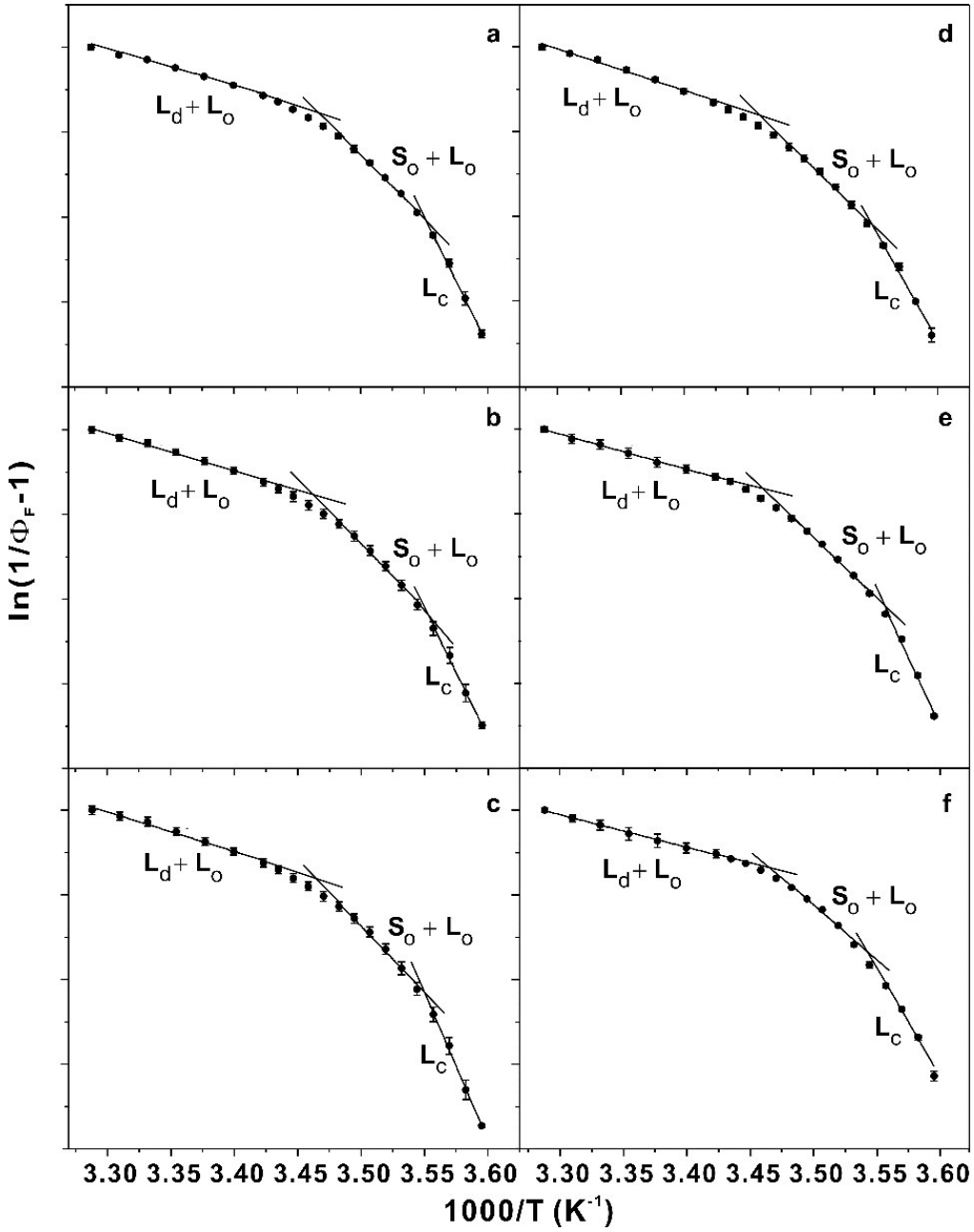
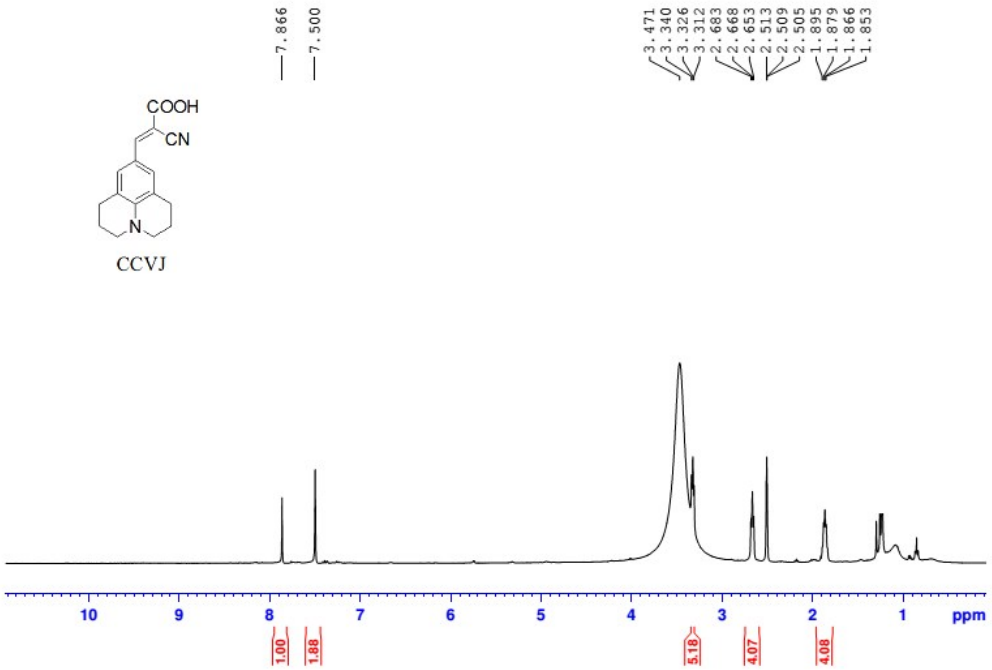
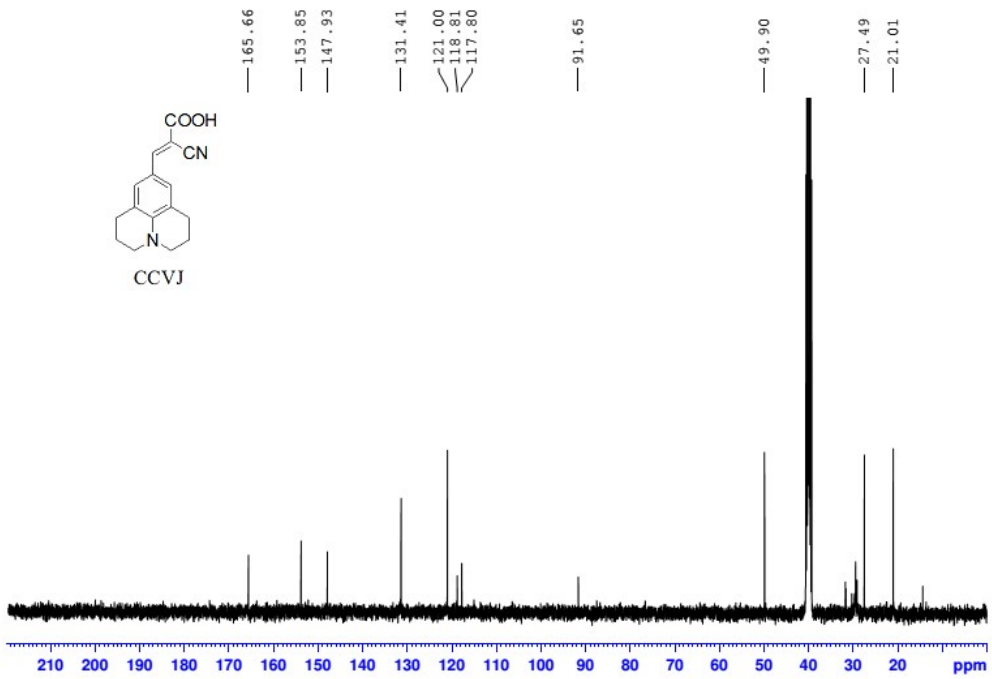
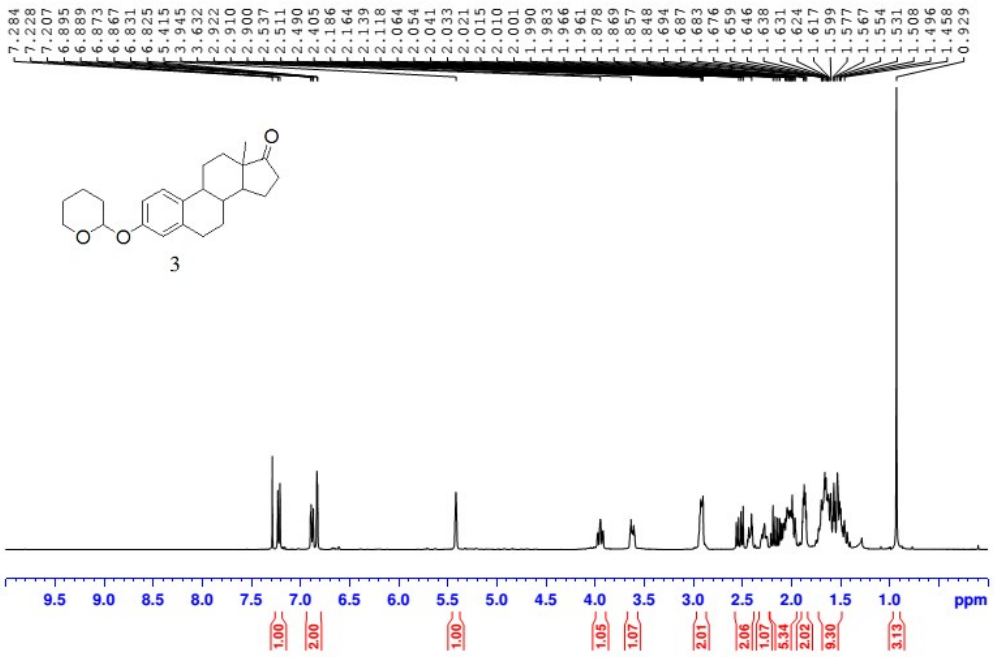


Fig. S6. Phase transition behavior of containing 25 mol % cholesterol DMPC SUVs including TTC of various concentrations: (a) 2.5, (b) 3.75, (c) 5, (d) 8.5, (e) 17 and (f) 25 mM. The error bars represent the standard deviations obtained by averaging at least 3 independent measurements.

6. Copies of ^{1}H NMR and ^{13}C NMR Spectra



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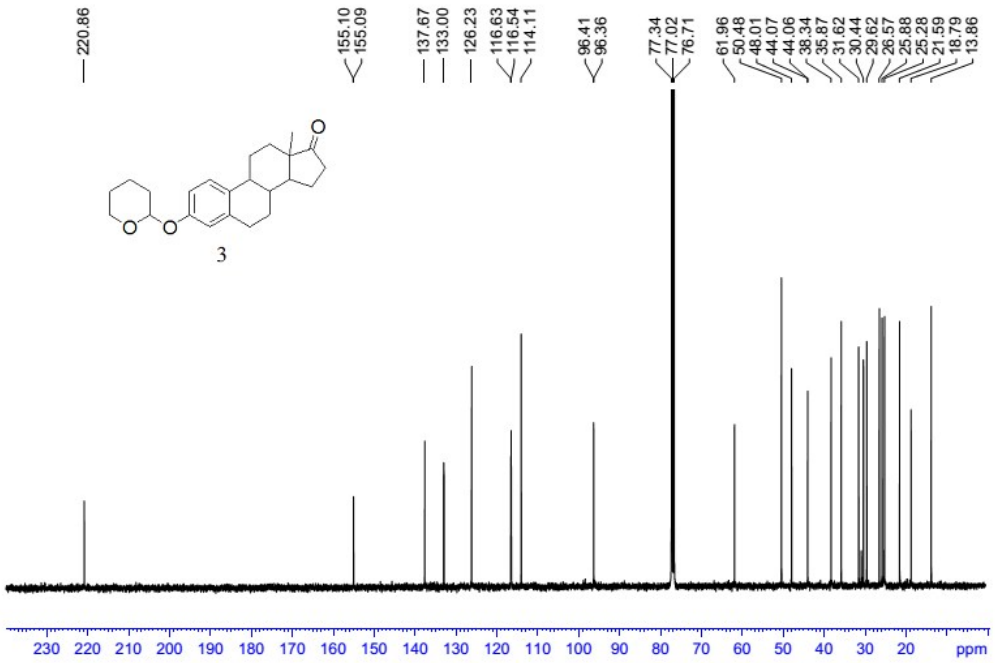
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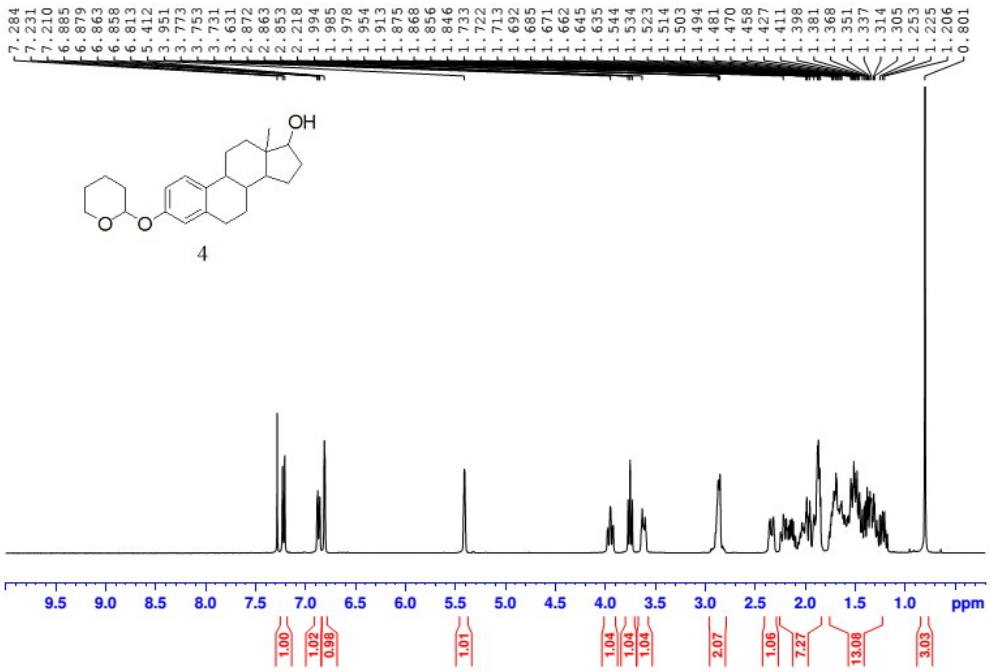
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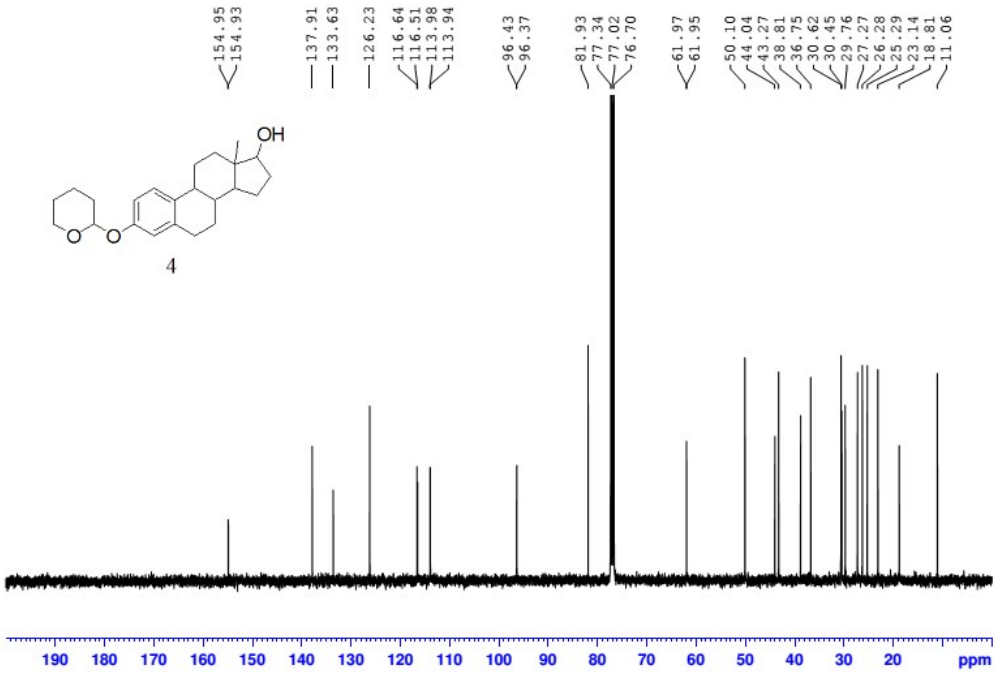
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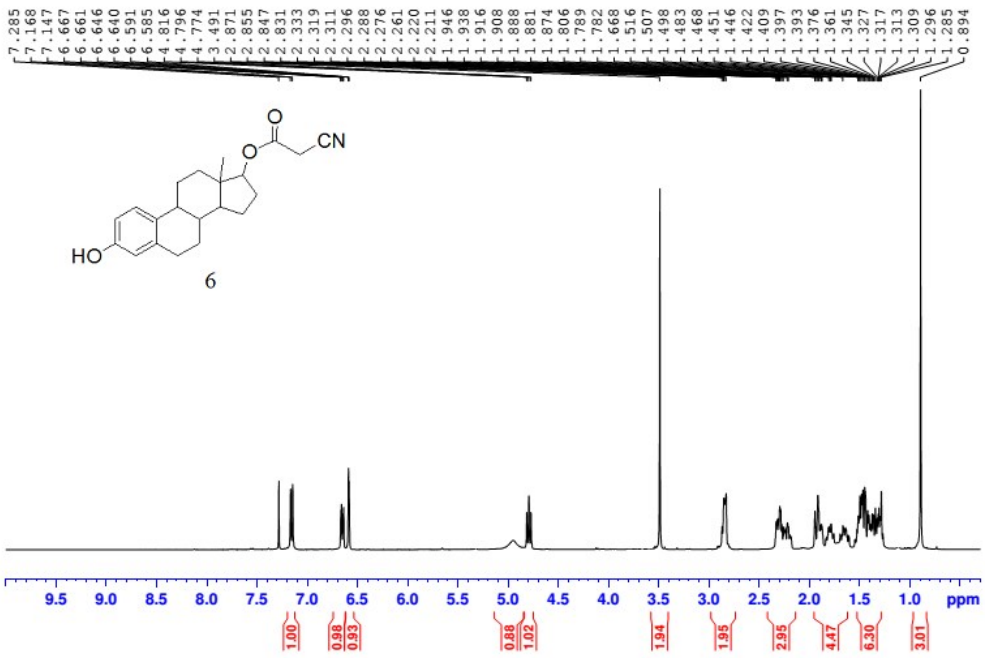
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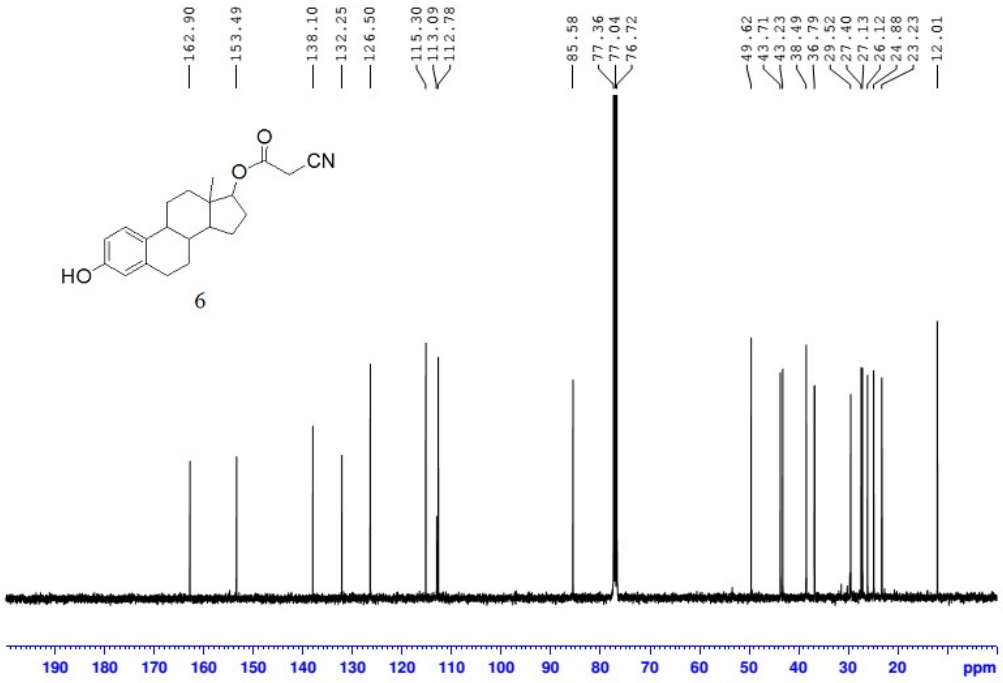
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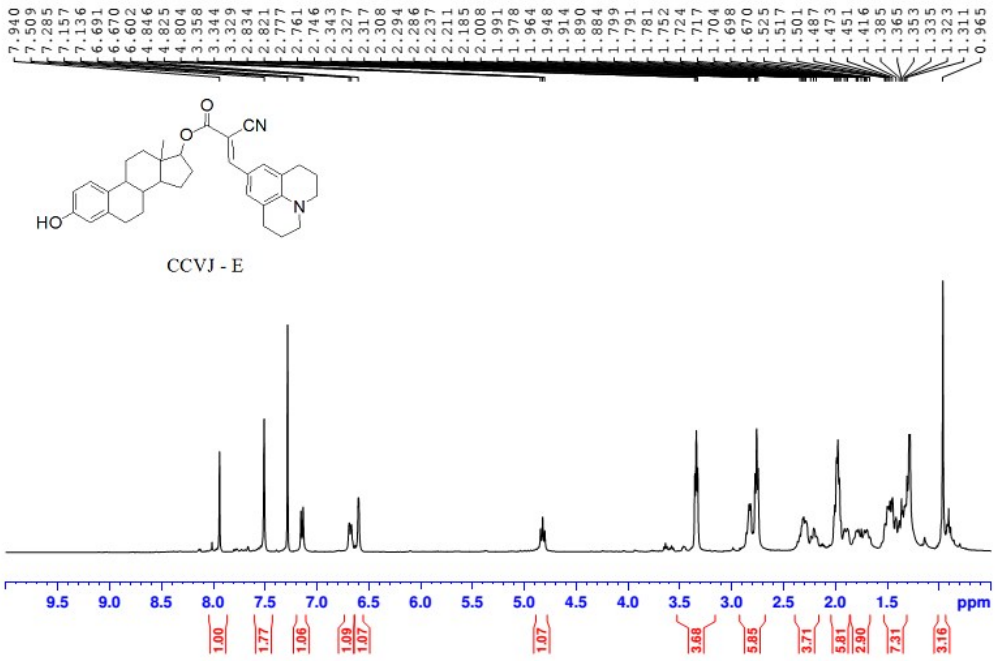
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2.761
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2.343
2.327
2.317
2.308
2.294
2.286
2.237
2.211
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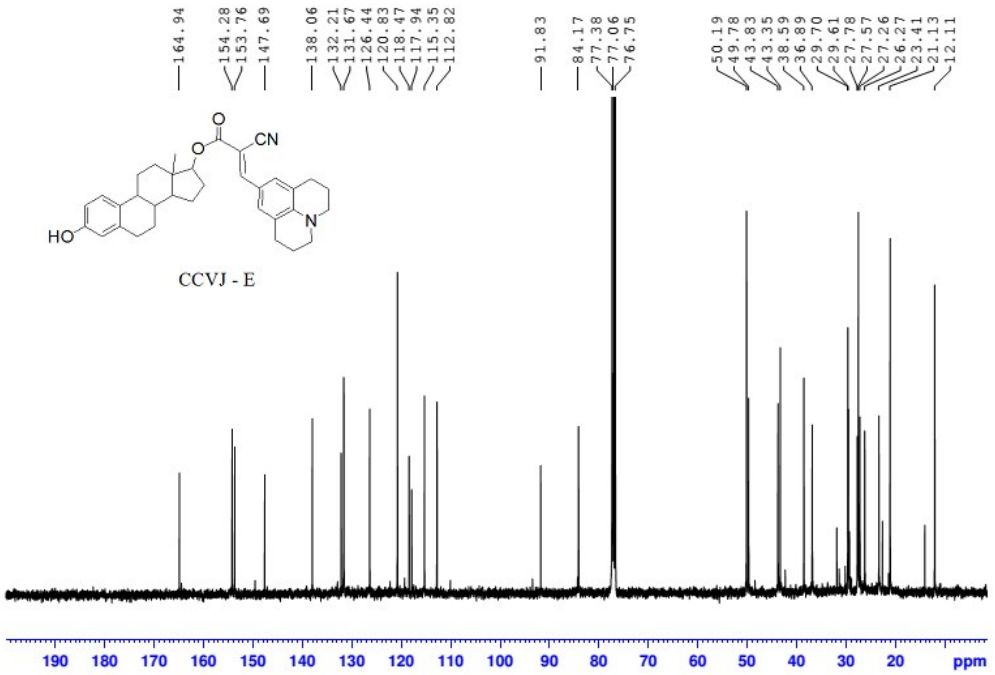
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