Electronic Supplementary Information

Ionic liquids with thioether motifs as synthetic cationic lipids for gene delivery

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Materials and Measurement

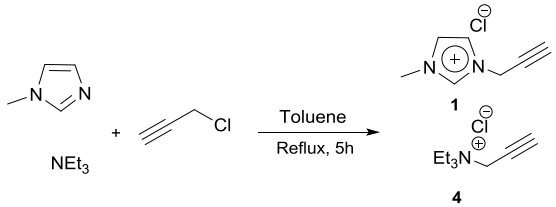
For the present study, all the thiols employed were commercially available in high purity and used without further purification. The photoinitiator (2,2-dimethoxy-2-phenylacetophenone), propargyl chloride, triethylamine and imidazole were purchased from Acros Organics and used without further purification. High purity solvents, such as methylene chloride, methanol and hexanes were purchased from Acros Organics and used without further purification.

Characterization of the products using ^{1}H and ^{13}C NMR were performed on a JEOL 400 MHz NMR with multi-nuclear capabilities using $CDCl_3$. ESI-MS analyses were performed by flow-injection on a Thermo Scientific ion trap mass spectrometer using HPLC grade acetonitrile. As our interest was in the cations of these salts, data was collected in positive ion mode. Melting points were measured using a TA Q20 differential scanning calorimeter calibrated using indium (melting point) and sapphire (heat capacity) references. All DSC traces are second heating runs and first cooling runs. Samples of 5-20 mg were analyzed in open pan under nitrogen. Melting points were determined by using a Q20 TA differential scanning calorimeter calibrated using indium as a reference with a heating and subsequent cooling rate of 10°C/min. Thermogravimetric analyses were performed on a TA instrument TGA Q50 under nitrogen flow using aluminum pan. The samples were heated at 10°C/min.

General procedure for the synthesis of *yne*-bearing ILs.

In a dry 100 ml round bottom flask, 1 equiv. of 1-methylimidazole/triethylamine (6.4mmol) was added along with 1 equiv. of propargyl chloride (6.4mmol, 0.48g) and dissolved in 10 mL of toluene. Then, the reaction mixture was vigorously stirred and refluxed for 8h. Purification was completed by washing the resulting product with toluene (3 × 30mL) and then drying under vacuum at 70°C for 8h. Products **1** and **4** were obtained as a white solid in quantitative yield (>99%).

1: ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 9.40 (s, 1H), 7.79 (dd, J = 11.4, 1.8 Hz, 2H), 5.24 (d, J = 2.3 Hz, 2H), 3.45 (s, 3 H), 2.46 (t, J = 1.8 Hz, 1H).



Scheme S1. General synthesis of the *yne*-bearing ILs **1** and **4**.

General procedure for the synthesis of ILs 2 and 3.

In a well dried 150 mL photochemical tube, 1 equiv. of 1-methyl-3-(2-propyn-1-yl)-imidazolium chloride **1** (1.5 mmol, 0.24g) was added along with 1 equiv. of 2,2-dimethoxy-2-phenylacetophenone (2.1 mmol, 0.54g) and 2 equiv. of dodecanethiol (4.2 mmol, 0.85g) or tetradacanthiol (3.0 mmol, 0.72g). 6 mL of 2:1 ratio of MeOH and $CHCl_3$ was added and the tube was capped with a septum. The septum was sealed with parafilm and stirred vigorously until a homogenous solution was obtained. The contents were then irradiated with UV for 8h at room temperature. The solvents were removed from the crude product via evaporation. The residue was then washed with hexanes (5 × 20 mL) to remove unreacted thiols and decomposed photoinitiator. For purification, the crude product was crystallized three times in MeOH and H_2O (10:1). Products **2** and **3** were obtained as a white crystalline solid in excellent yields.

2: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 10.22 (s, 1H), 7.45 (d, J = 11.4 Hz, 2H), 4.76 (d, J = 4.6 Hz, 1H), 4.51 (s, 1H), 4.11 (s, 3H), 2.95-2.83 (1H), 2.82-2.52 (m, 9H), 1.65-1.46 (m, 4H), 1.43-1.18 (m, 36H), 0.88 (t, J = 6.9 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 138.8, 122.8, 51.9, 45.9, 36.8, 35.3, 33.204, 31.9, 31.6, 29.7, 29.6, 29.4, 29.3, 29.2, 28.9, 22.7, 14.2; MS (EI): *m/z* 563.4 (M+2), 562.3 (M+1), 561.3 (M, calcd. 561.4).

3: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 10.69 (s, 1H), 7.37 (s, 1H), 7.21-7.15 (1H), 4.80 (dd, J = 14.0, 4.3 Hz, 1H), 4.49 (q, *J* = 7.2 Hz, 1H), 4.08 (s, 3H), 2.89 (dd, J = 13.5, 4.8 Hz, 1H), 2.74-2.53 (m, 5H), 1.89 (s, 7H), 1.63-1.44 (m, 4H), 1.41-1.10 (m, 48H), 0.86 (t, J = 6.6 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 139.7, 122.9, 122.1, 52.0, 45.9, 36.9, 35.4, 33.3, 32.0, 31.8, 29.8, 29.7, 29.6 29.5, 29.3, 28.9, 28.9, 22.8, 14.2; MS (EI): *m/z* 619.1 (M+2), 618.1 (M+1), 617.1 (M, calcd. 617.5).

5: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 2.67 (t, J = 7.1 Hz, 4H), 2.36 (s, 1H), 2.06 (t, J = 7.5 Hz, 6H), 1.31 (m, 56H), 0.77 (t, J = 6.5 Hz, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 39.3, 32.4, 31.8, 30.9, 29.6, 29.7, 29.4, 29.3, 29.1, 29.0, 28.6, 14.1 MS (EI): *m/z* 581.5 (M+2), 580.5 (M+1), 579.5 (M, calcd. 579.4).

6: ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 2.61 (t, J = 7.3 Hz, 4H), 2.43 (s, 1H), 1.59 (q, J = 7.5 Hz, 6H), 1.38-1.11 (m, 62H), 0.81 (t, J = 6.9 Hz, 6H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ_C 39.3, 32.2, 29.8, 29.7, 29.7, 29.6, 29.6, 29.6, 29.4, 29.3, 29.3, 29.3, 29.0, 28.6, 14.2 MS (EI): *m/z* 637.2 (M+2), 636.2 (M+1), 635.2 (M, calcd. 635.5).

Cell Culture

Human cervical HeLa cells (ATCC CCL-2, American Type Culture Collection, Manassas, VA) were used to perform cytotoxicity tests. Human epithelial kidney HEK 293T cells (ATCC CRL-11268) were used for transfection experiments. Both cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 2 mM Glutamax, 100 U ml^{-1} penicillin G, 100 µg ml^{-1} streptomycin and 0.25 µg ml^{-1} amphotericin B at 37 °C with 5% (v/v) CO_2. All cell culture reagents were purchased from Thermo Fisher Scientific, Waltham, MA unless otherwise noted.

Cell transfection

HEK 293T cells were plated such that 12 well tissue-culture treated plates were about 20-40% confluent on the day of transfection. $pCMV_{ie}eGFP$, a DNA plasmid expressing green fluorescent protein with enhanced promotor for mammalian cell expression, was employed throughout all transfection experiments. For each IL concentration transfected experimentally, 2 µg $pCMV_{ie}eGFP$ was combined with Poly-L-lysine Hydrobromide (PLL, MW 70000g/mol) (MP Biomedicals, LLC., Solon, OH) and phosphate buffered saline, pH 7.4 (PBS) and incubated at room temperature for 30 min in order to condense plasmid DNA. In a separate tube, ILs were diluted in PBS. Condensed $pCMV_{ie}eGFP$ and IL dilutions were then combined and incubated at room temperature for another 30 min. Serum free DMEM was added to mixtures to increase the volume to 0.1ml to allow for easier distribution to wells.

Mixtures were added dropwise to cells in 2ml of complete DMEM and incubated for 48 hours at 37 °C with 5% (v/v) CO_2. For quantitation, plates were scanned on a Typhoon Trio Variable Mode Imager (Amersham Biosciences Corp, Sunnyvale, CA) using an excitation of 488nm and emission of 526nm. ImageQuant Version 5.1 (Amersham Biosciences Corp, Sunnyvale, CA) was used to quantitate and compare relative expression of fluorescence per well to the maximum fluorescence observed per individual IL.

Cytotoxicity

HeLa cells were plated in 96 well tissue-culture treated plates and seeded at a density of 1.1×10^5 cells/mL approximately 24 hours prior to experiments. ILs were diluted using complete DMEM, as described previously, and then incubated at 56°C for 15-30 min to create a homogenous solution. Culture media was removed from cells, IL dilutions were added to wells in triplicate, and then cells were incubated with treatment at 37 °C with 5% (v/v) CO_2 for 24 hours. Cells were observed after incubation and then treated according to the TACS MTT Cell Proliferation and Viability Assay kit (Trevigen, Inc., Gaithersburg, MD). Absorbance was measured at 560nm using a TECAN Genios Pro 96/384 Multifunction Microplate Reader (Tecan Group, Ltd., Männedorf, Switzerland).

Confocal Microscopy

HEK 293T cells were plated in 6 well plates onto glass slide cover slips such that they were about 20-40% confluent at the time of transfection. After cells were transfected and incubated for 48 hours, they were observed microscopically for green fluorescence. DRAQ5 was used as a non-specific nuclear counter stain (Thermo Fisher Scientific, Waltham, MA) at a final concentration of 7µM 30min prior to imaging. Cells were imaged using an Olympus IX81 inverted confocal microscope (Olympus America Inc., Center Valley, PA).

Representative $^{1}H/^{13}C$ NMR Spectra and DSC/TGA thermograms

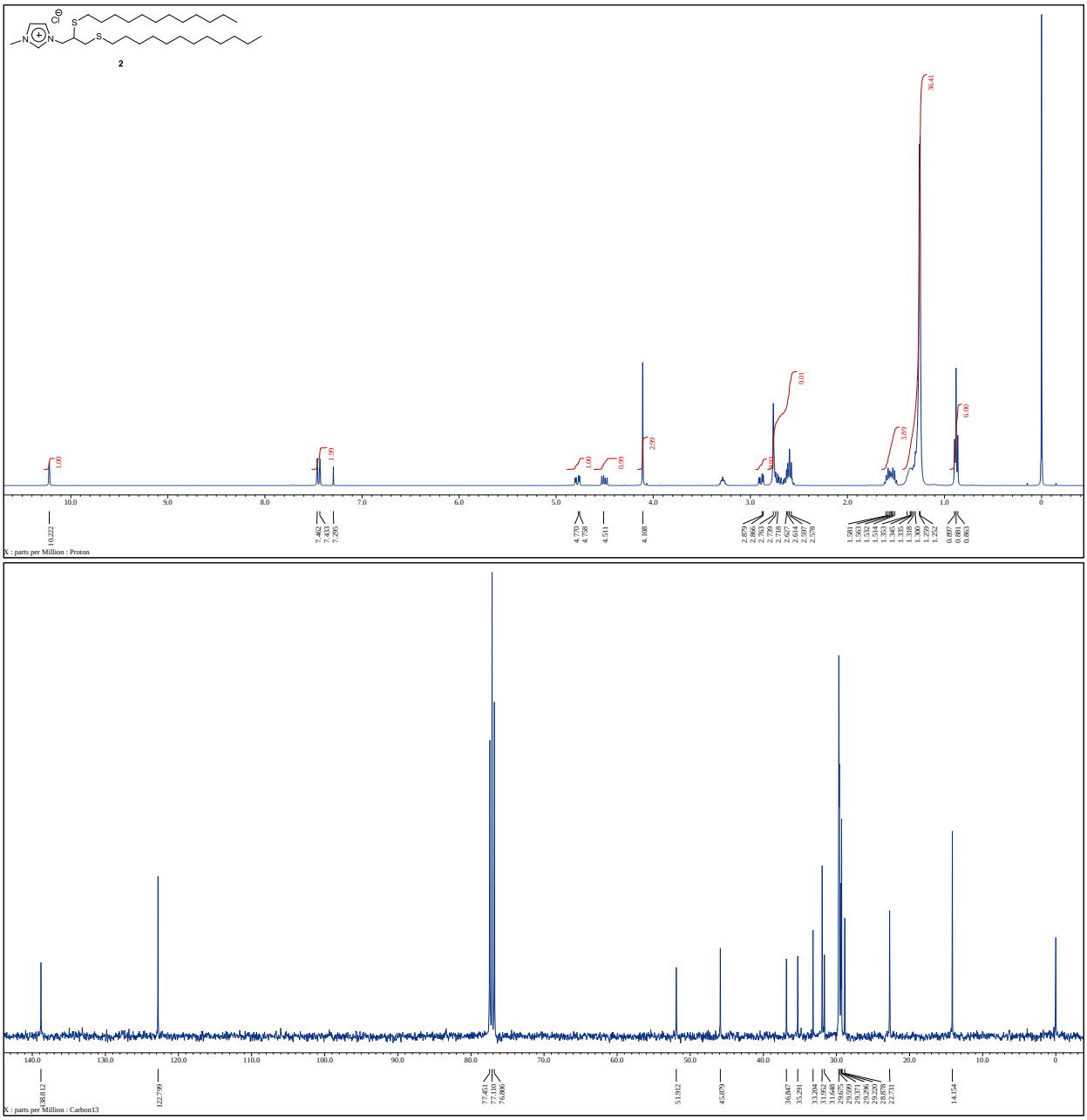


Figure S1. ^{1}H and ^{13}C NMR Spectra of **2**.

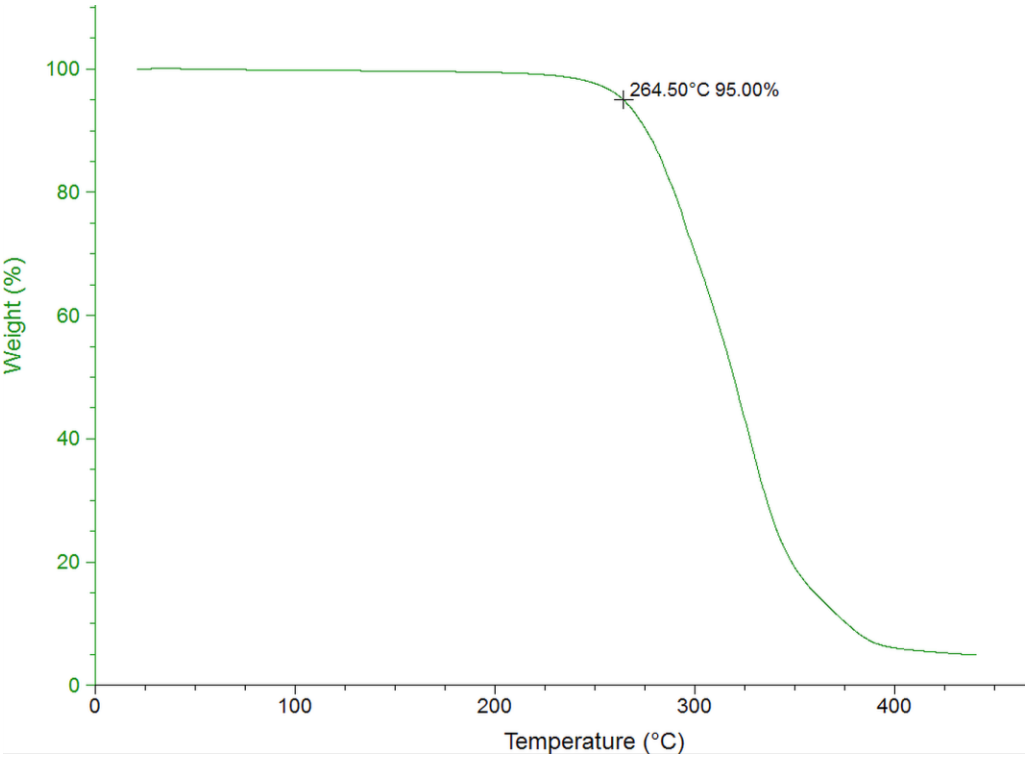


Figure S2. TGA thermogram of **2**. 5% weight loss at 264.5^{o}C due to the degradation process.

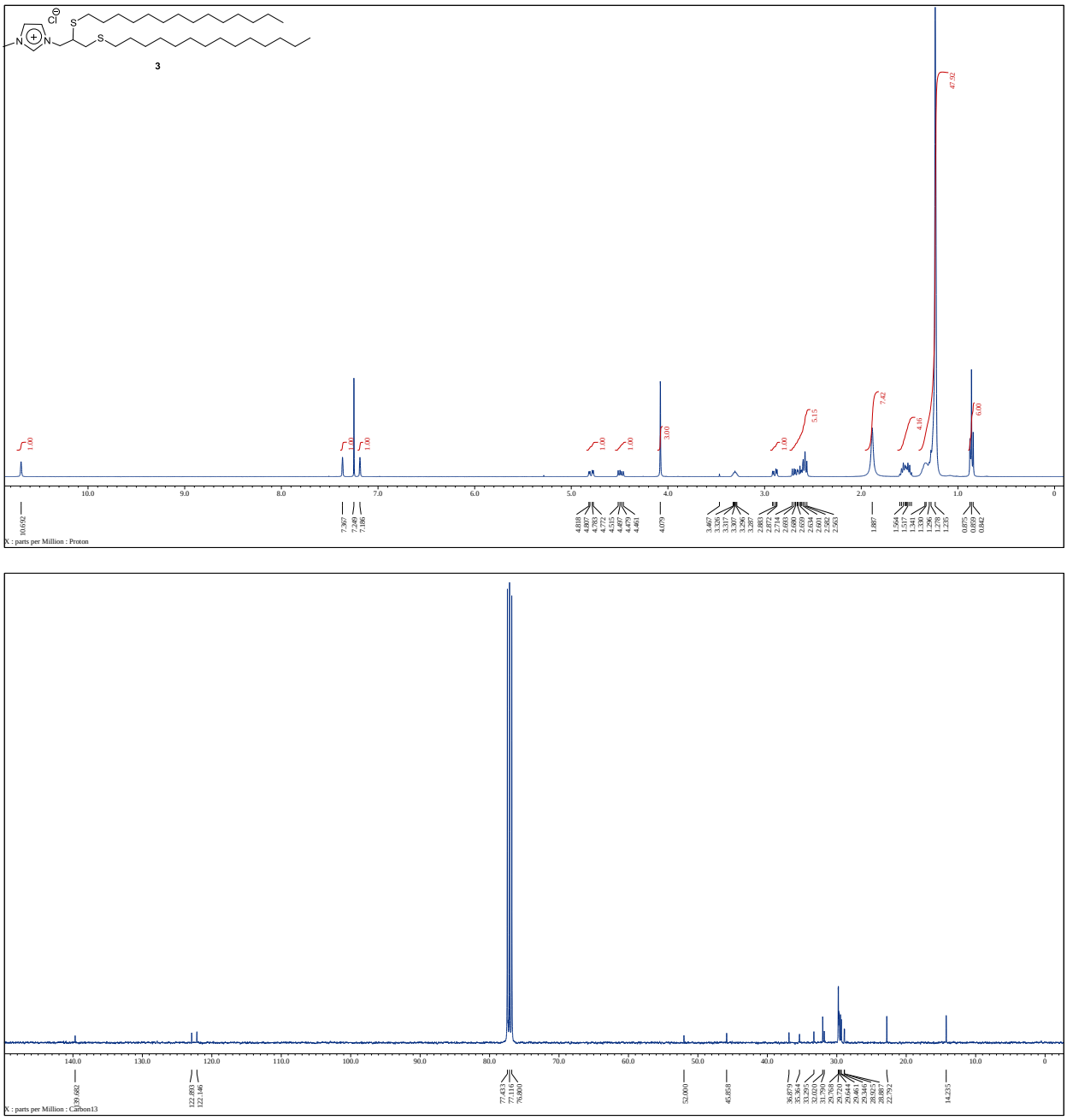


Figure S3. ^{1}H and ^{13}C NMR Spectra of **3**.

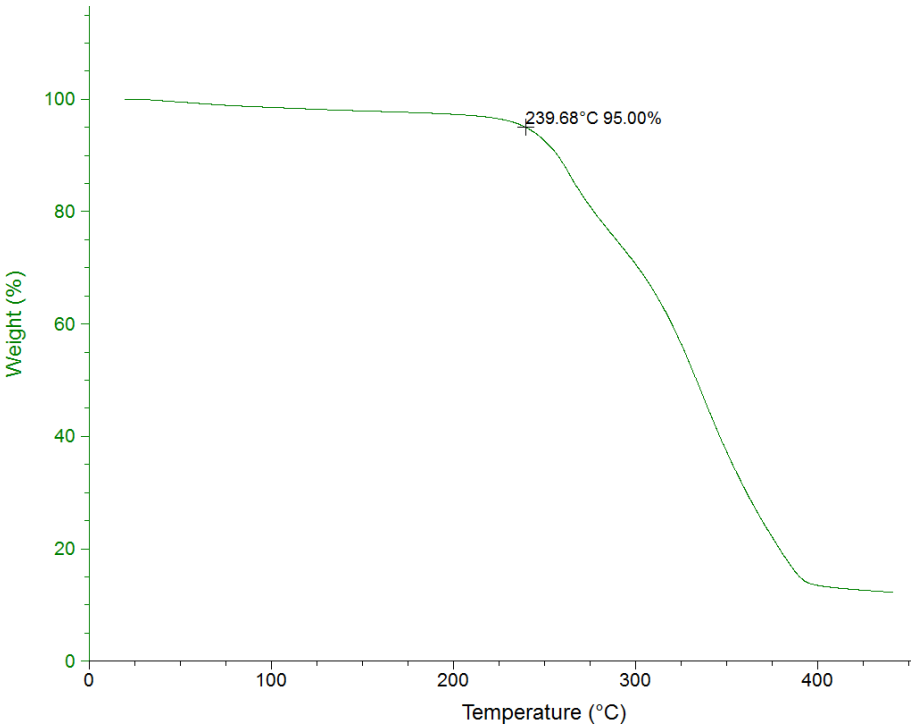
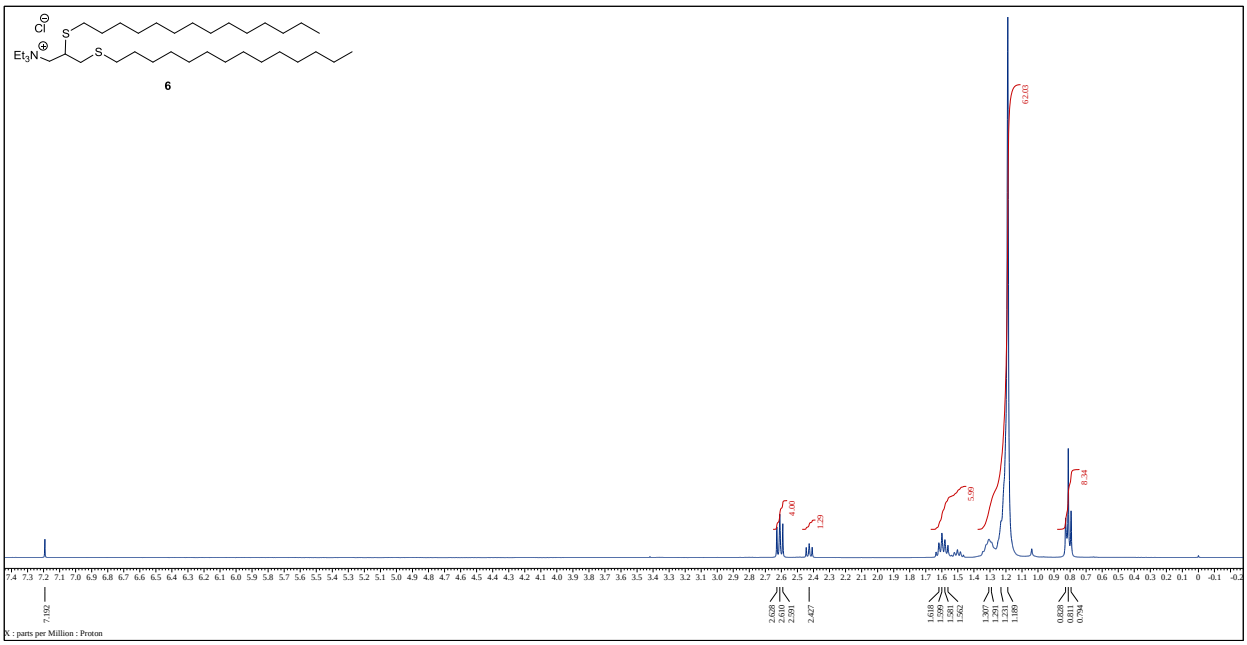


Figure S4. TGA thermogram of **3**. 5% weight loss at 239.68°C due to the degradation process.



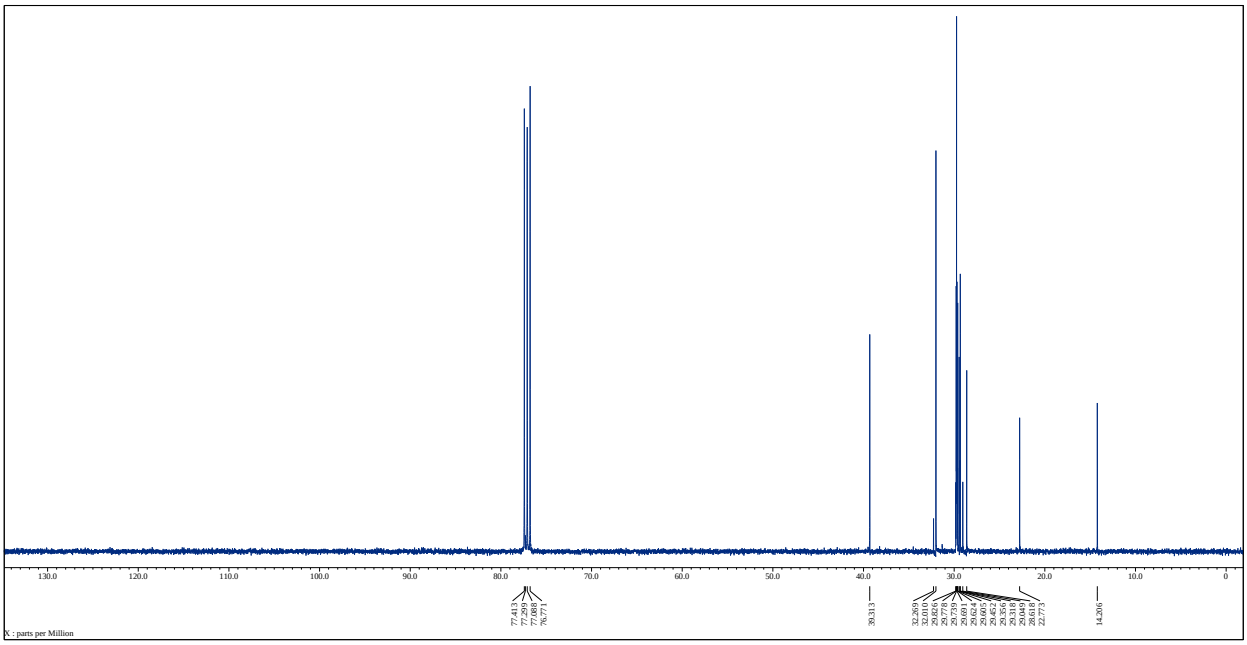


Figure S5. ^{1}H and ^{13}C NMR Spectra of **6**.

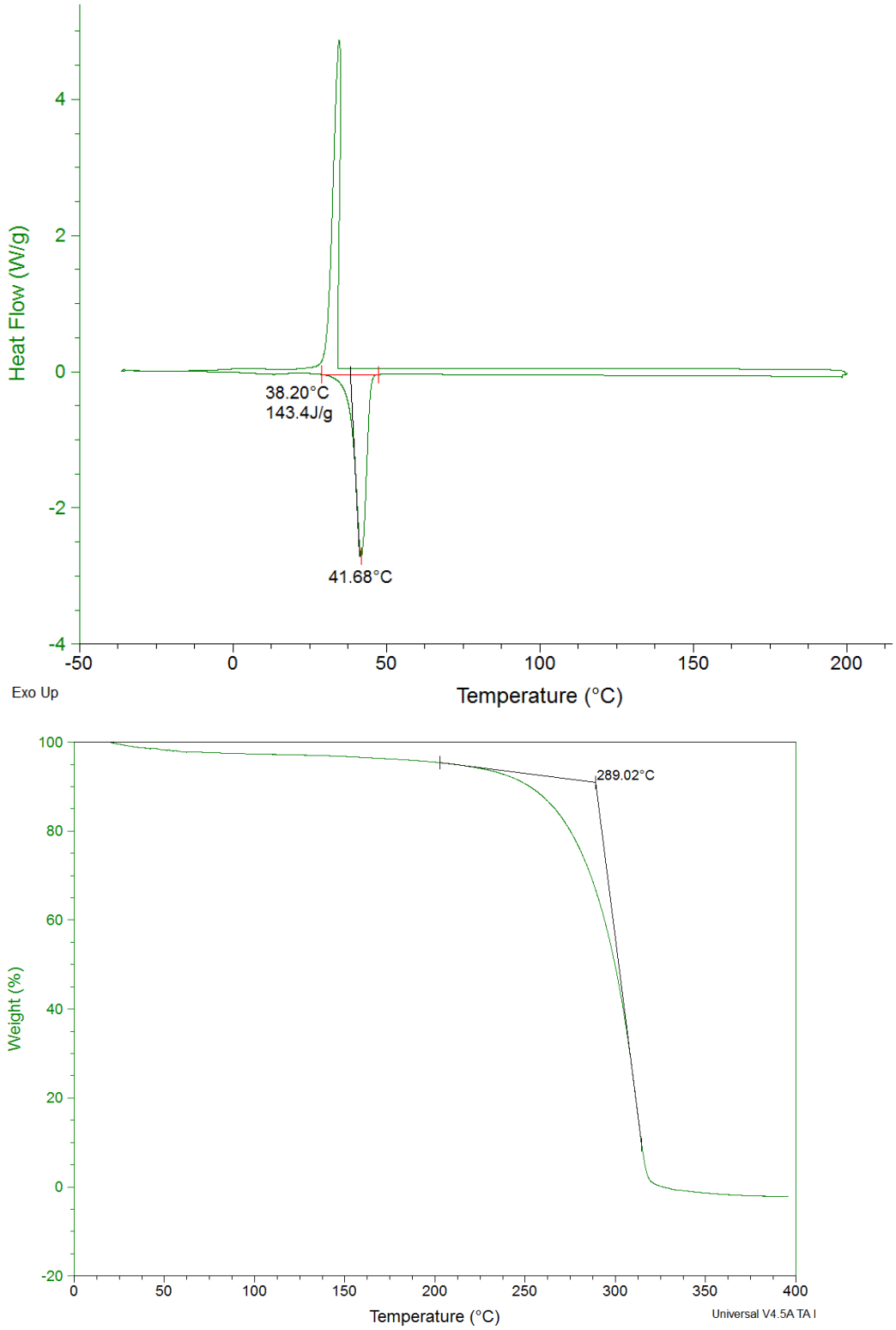


Figure S6. DSC (top) and TGA thermogram (bottom) of **6** (10°C/min). It exhibits transition from crystalline solid to mesophase at 57.30°C.5%. Weight loss at 289.02°C due to the degradation process.

Transfection efficiency of ILs 5 and 6, employing plasmid DNA (pCMV$_{ie}$eGFP) in 293T cells.

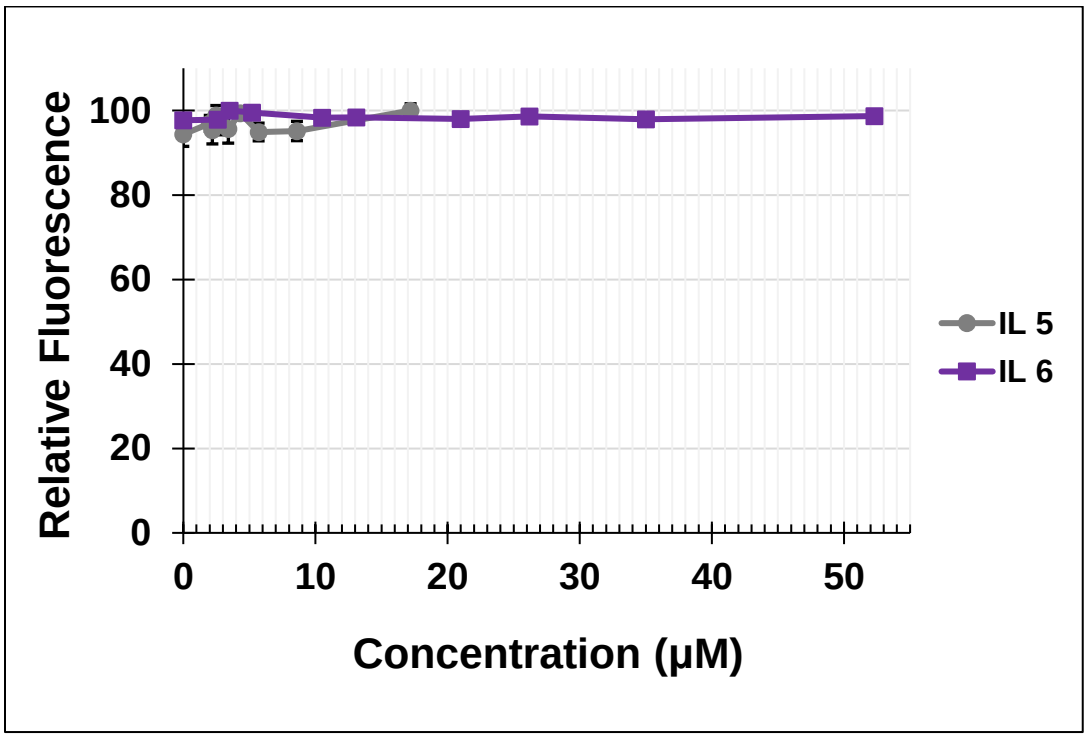


Figure S7. Transfection efficacy of **5** and **6** with GFP DNA in the 293T cell line. ILs show no difference in transfection efficiency, compared to the negative control.

Cytotoxicity of ILs 5 and 6 measured via MTT assay in HeLa cells.

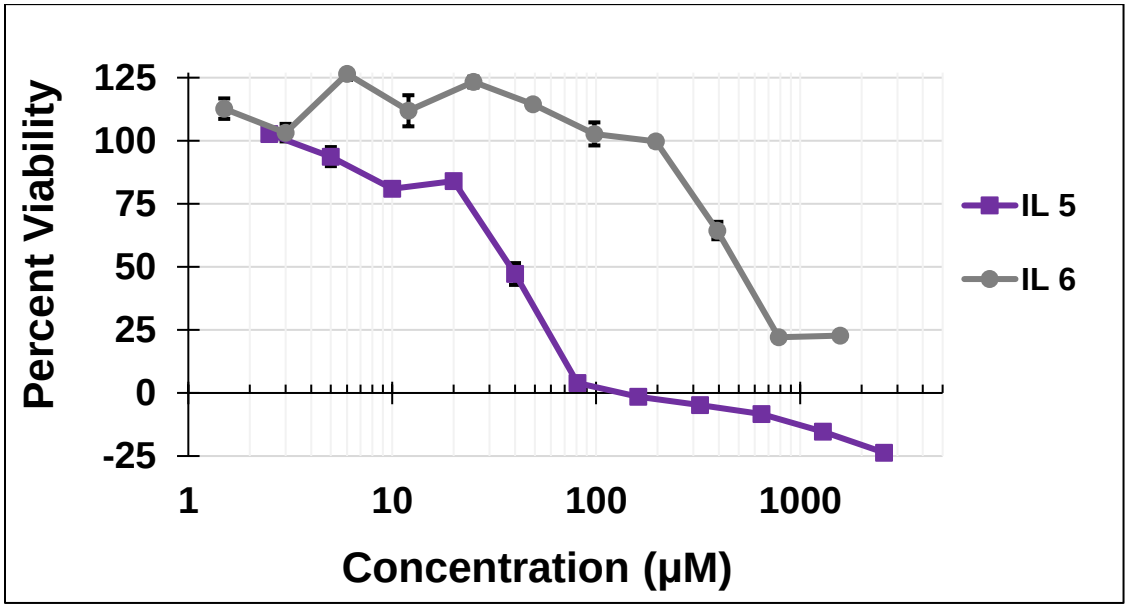
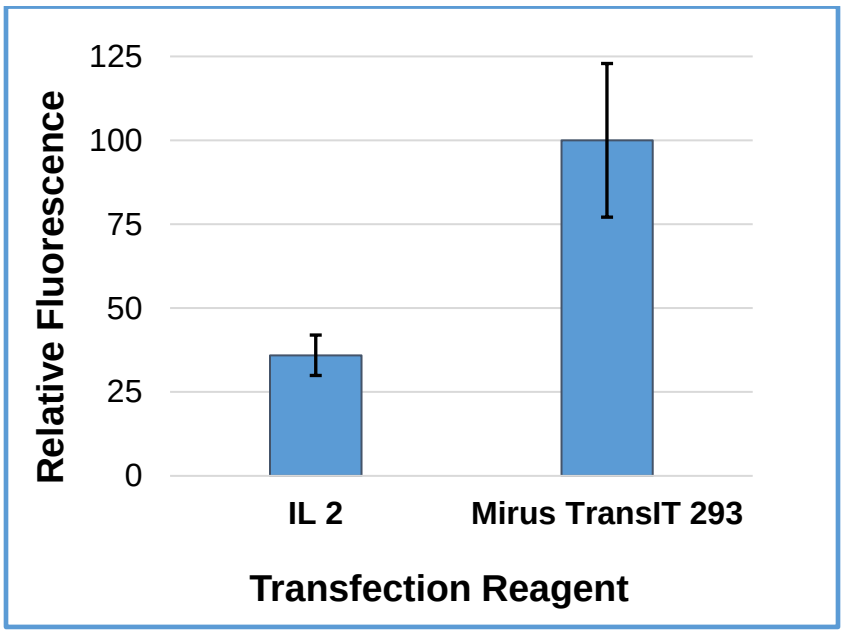


Figure S8. Cellular toxicity of IL **5** and **6** in HeLa cells. Cell viability is presented as IL-treated cell absorbance normalized to the absorbance of non-treated cell control. Absorbance measured at 560nm.

	Fluor Intensity per Well			Avg	StDev	Fluor Intesity per Well %			Avg %	StDev %
IL 2	24	31	23	26	4.4	33.2	42.9	31.8	35.9	6.0
Mirus TransIT 293	55	88	74	72.3	16.6	76.0	121.7	102.3	100.0	22.9



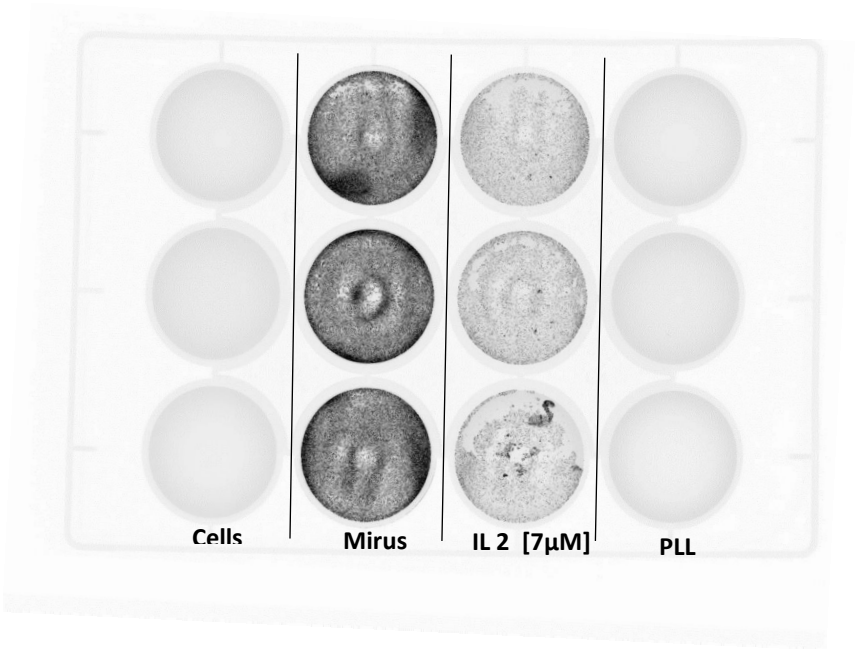
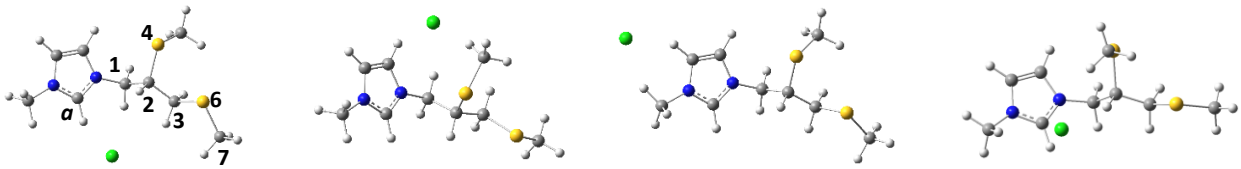


Figure S9. Quantitative fluorescence intensity showing transfection efficiency of IL **2** at 7µM compared to cells only (no plasmid), commercially available Mirus TransIT 293 reagent, and PLL condensed plasmid.

Computational studies



(a) 0.0 cal/mol **(b)** 6.95 kcal/mol **(c)** 12.74 kcal/mol **(d)** 1.41 kcal/mol

Figure S10: Four optimized IL complexes at the B3LYP/6-311++G(d,p) level.

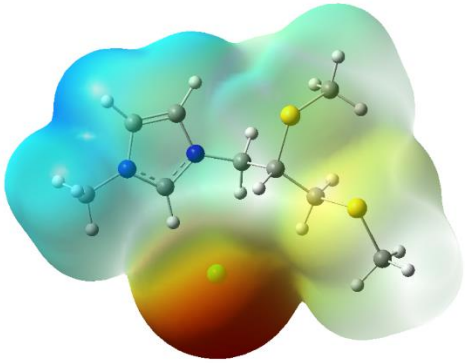
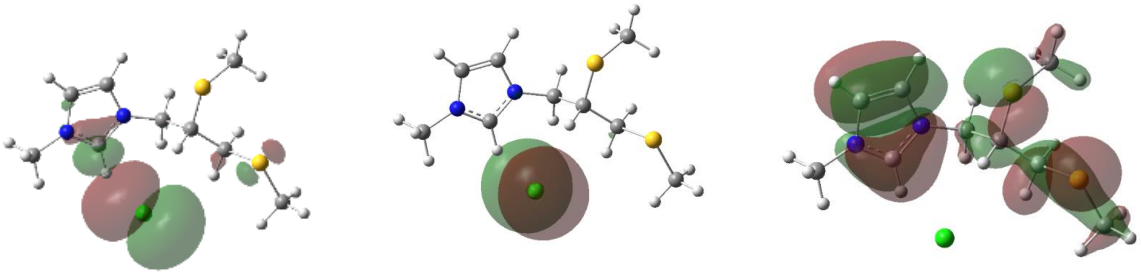
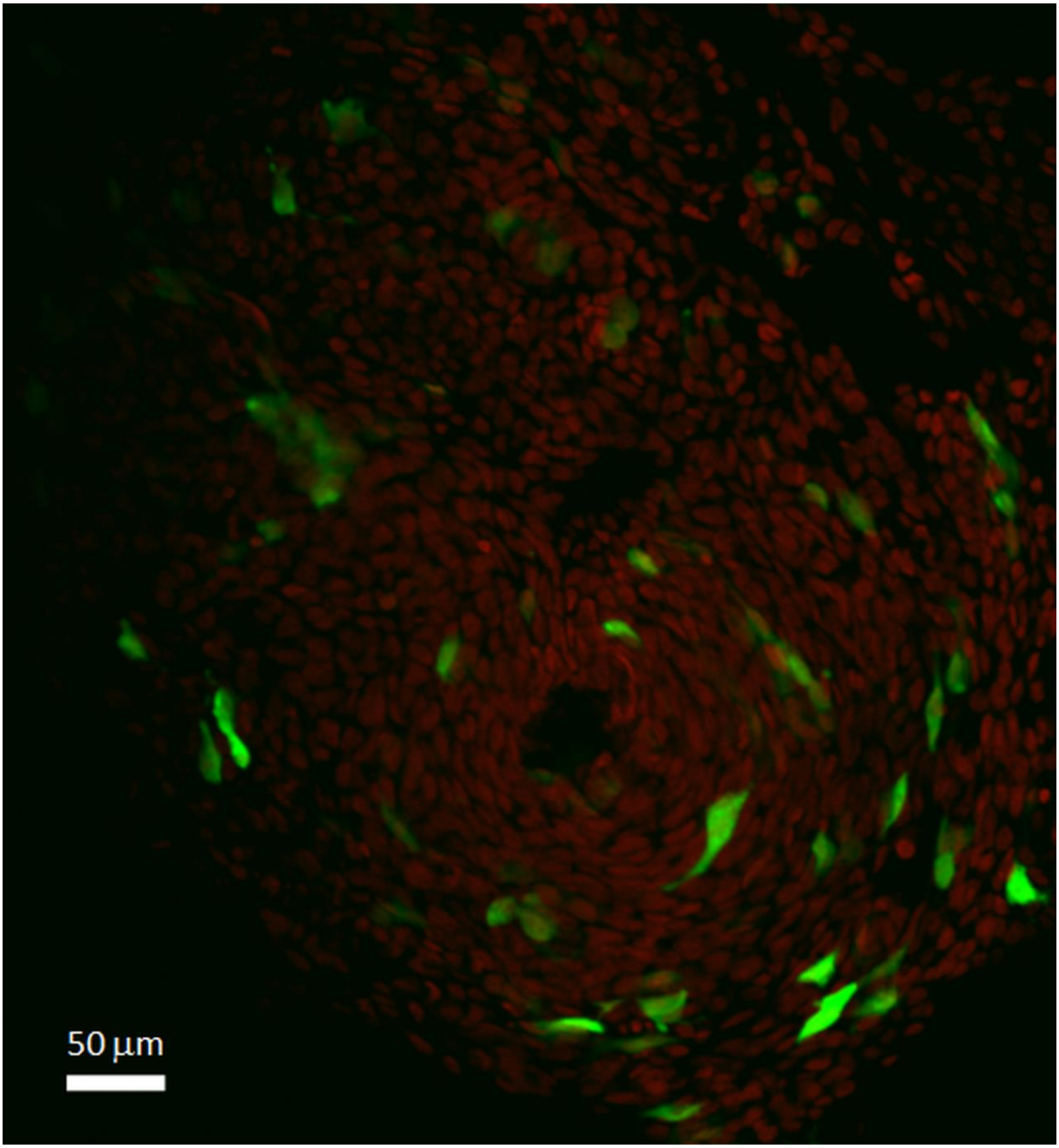


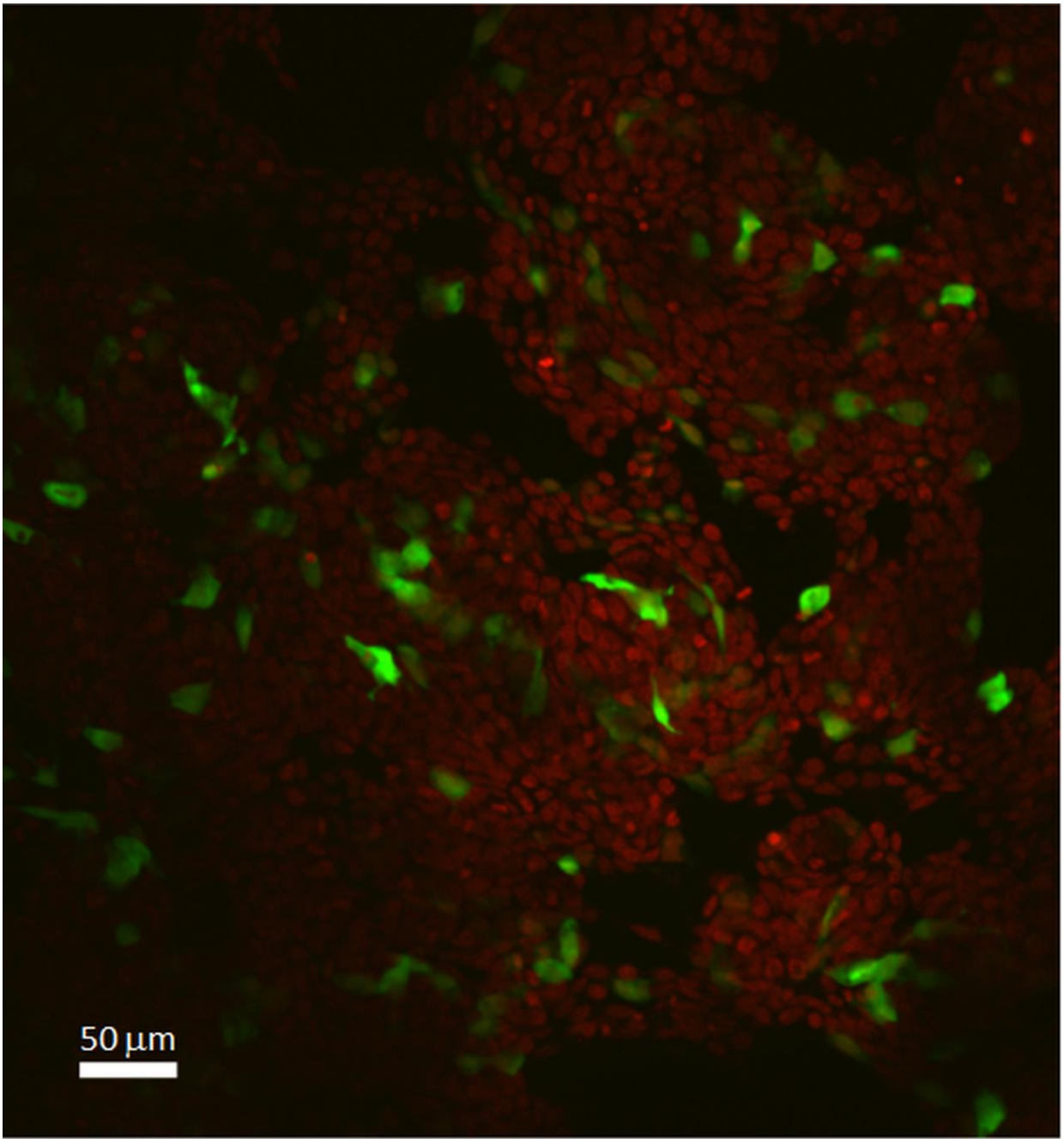
Figure S11. The electrostatic potential energy map of imidazolium IL. The electrostatic potential energy map of the imidazolium IL demonstrates a strong interaction between the chloride (in red) and the positively charged imidazolium ring (blue).



HOMO-2 -0.21006 **HOMO** -0.20172 **LUMO** -0.04006

Figure S12. Frontier molecular orbitals of an imidazolium IL. The cation-anion interaction can be depicted visually by the contour plots of the frontier orbitals. The HOMO-2 has a σ-type symmetry and exhibits lone pair LP(Cl^-)σ*(H_{C2}) interaction, which could be crucial to stabilize ion pair.


50 µm



Confocal microscopy images of 293T cells transfected with $pCMV_{ie}eGFP$ using **2** (top) and **3** (bottom). Plasmid expression in the 293T cell line was observed as green fluorescence. Cell nuclei were counterstained with a far-red dye (646/697nm ex/em wavelength) DRAQ5 Fluorescent Probe and false colored red to contrast GFP expression