Supporting Information

Ionic Liquid-Controlled Conformational Bias of Tetracycline

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Materials and Methods

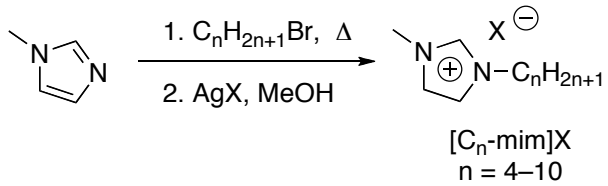
All reagents and solvents were from commercial sources (Sigma-Aldrich, Acros, Alfa Aesar) and were used as received. Tetracycline was purchased from Sigma-Aldrich. Stock solution of tetracycline in DMSO was prepared fresh prior to experiments, and used within 12 h. TC stock solutions were prepared in DMSO at 2 mM concentration for fluorescence measurements and at 20 mM concentration for absorbance and CD measurements. Ionic liquids were synthesized and purified according to literature procedures.[1-3] **NMR spectra** were recorded on a Varian (300 MHz) spectrometer. The chemical shifts are reported in ppm (δ) downfield from tetramethylsilane in $CDCl_3$, residual DMSO in DMSO-d_6 or residual acetone in acetone-d_6. **IR spectra** were recorded as neat films: ionic liquid between NaCl discs.

All solutions of tetracycline in ionic liquids were prepared by addition of the DMSO stock solution to the ionic liquid, followed by vortexing at 3000 rpm for 10-30 seconds. **Absorbance measurements** were performed on an Agilent 8453 UV-visible instrument with a resolution of 1 nm using 0.01 cm quartz cells (demountable cell from NSG Precision Cells, Inc). **Fluorescence measurements** were performed using a Shimadzu RF-5301PC; the measurements were carried out as follows: λ_{ex} = 400 nm; emission collected from 410 to 750nm; excitation and emission slit widths were 3 nm and 3 nm; sensitivity – high; 1.0 cm quartz cells. All spectra were background subtracted using appropriate blanks, and subsequently smoothed using manufacturer provided software. **Resonance light scattering measurements** were carried out on a Shimadzu RF-5301PC spectrofluorimeter employing a synchronous scanning mode in which the emission and excitation monochromators were preset to identical wavelengths. The resonance light scattering spectrum was recorded from 250 to 800 nm with both excitation and emission slit widths set at 1.5 nm and intensity set to low. **CD spectra** were acquired on a Jasco J-815 using 0.01 cm quartz cells (demountable cell from NSG Precision Cells, Inc); spectra were recorded at

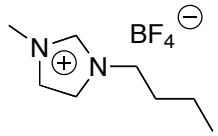
room temperature and 1 nm resolution with a scan rate of 100 nm/min; two or four scans were acquired and averaged for each sample; raw data were manipulated by subtraction of appropriate background spectra, followed by smoothing using manufacturer provided software.

Synthesis of Ionic Liquids

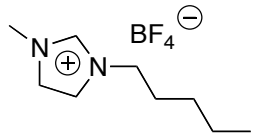
All ionic liquids were prepared according to literature procedures or modified literature procedures following the general sequences shown in Scheme S1.[1-3] All ionic liquids were purified as follows: ionic liquids were dissolved in CH_2Cl_2, followed by filtration to get rid of inorganic impurities. Next, ionic liquids were repeatedly treated with charcoal in EtOH at elevated temperatures followed by filtration and removal of EtOH in vacuo (for an azeotropic removal of residual water). Finally, the ionic liquids were dried under vacuum for 8-12 hours. All sample preparations and spectroscopic measurements that involved tetracycline were conducted immediately after removing the ionic liquids from the vacuum with special care to minimize the exposure to moisture.



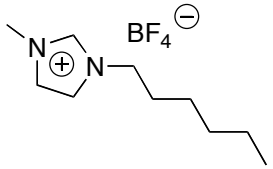
Scheme S1. Synthesis of ionic liquids



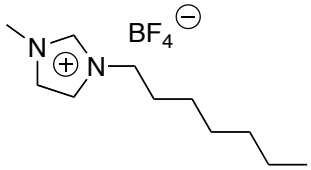
[C_4-mim]BF_4:[2] ^{1}H NMR (300MHz, acetone-d_6): δ = 8.91 (1H, s), 7.72 (t, *J* = 1.7 Hz, 1H), 7.66 (t, *J* = 1.7 Hz, 1H), 4.31 (t, *J* = 7.4 Hz, 2H), 4.0 (s, 3H), 1.89 (pent, *J* = 7.5 Hz, 2H) 1.37 (sext, *J* = 7.5 Hz, 4H), 0.92 (3H, t, *J* = 7.4 Hz); ^{19}F NMR (282 MHz, acetone-d_6): δ = –150.96 (m); ^{13}C NMR (75 MHz, acetone-d_6): δ = 136.9, 124.0, 122.7, 49.4, 35.8, 32.1, 19.3, 13.0; IR (neat): 3164, 3122, 2964, 2983, 2877, 1574, 1466, 1171, 1064 cm^{-1}.



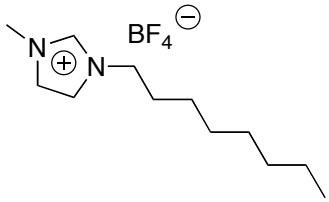
[C_5-mim]BF_4:[2] ^{1}H NMR (300 MHz, acetone-d_6): δ = 8.99 (s, 1H), 7.75 (t, *J* = 1.8 Hz, 1H), 7.69 (t, *J* = 1.8 Hz, 1H), 4.34 (t, *J*=7.4 Hz, 2 H), 4.03 (3H, s), 1.94 (pent, *J* = 7.4 Hz, 2H), 1.34 (m, 4H), 0.88 (t, *J* = 6.9 Hz, 3H); ^{19}F NMR (282 MHz, acetone-d_6): δ = –151.44 (m); ^{13}C NMR (75 MHz, acetone-d_6): δ = 136.9, 124.0, 122.7, 49.7, 35.8, 29.8, 22.0, 13.5 IR (neat): 3166, 3125, 2961, 2875, 1175, 1471, 1171, 1063 cm^{-1}.



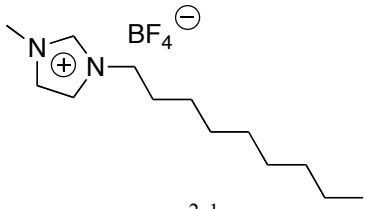
[C_6-mim]BF_4:[2] ^{1}H NMR (300 MHz, acetone-d_6): δ = 8.96 (s, 1H), 7.74 (s, 1H), 7.68 (s, 1H), 4.32 (t, *J* = 7.5 Hz, 2H), 4.02 (s, 3H), 1.92 (pent, *J* = 7.4 Hz, 2H), 1.32 (m, 6H,), 0.86 (t, *J* = 7.1 Hz, 3H); ^{19}F NMR (282 MHz, acetone-d_6): δ= –151.16 (m); ^{13}C NMR (75 MHz, acetone-d_6): δ = 136.9, 124.1, 122.7, 49.7, 35.8, 31.2, 30.1, 25.8, 22.4, 15.6; IR (neat): 3161, 3122, 2957, 2933, 2861, 1575, 1468, 1170, 1060 cm^{-1}.



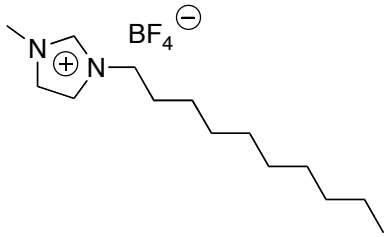
[C_7-mim]BF_4:[2] ^{1}H NMR (300 MHz, acetone-d_6): δ = 9.03 (s, 1H), 7.78 (s, 1H), 7.72 (s, 1H), 4.36 (t, *J* = 7.4 Hz, 2H), 4.05 (s, 3H), 1.95 (m, 2H), 1.34 (m, 8H), 0.87 (t, *J* = 6.8 Hz, 3H); ^{19}F NMR (282 MHz, acetone-d_6): δ= –151.25 (m); ^{13}C NMR (75 MHz, acetone-d_6): δ = 135.9, 124.1, 122.7, 49.7, 35.9, 31.7, 30.2, 28.7, 26.1, 22.5, 13.7; IR (neat): 3166, 3122, 2930, 3859, 1575, 1471, 1170, 1059 cm^{-1}.



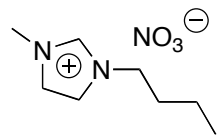
[C_8-mim]BF_4:[2] ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.07 (s, 1H), 7.75 (s, 1H), 7.68 (s, 1H), 4.12 (t, *J* = 7.5 Hz, 2H), 3.82 (s, 3H), 1.75 (pent, *J* = 7.1 Hz, 2H), 1.23 (m, 10H) 0.84 (t, *J* = 6.6 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6): δ= –148.61 (m); ^{13}C NMR (75 MHz, acetone-d_6): δ = 136.9, 124.1, 122.7, 49.7, 35.9, 31.8, 30.2, 29.5, 29.1, 26.1, 22.6, 13.7; IR (neat): 3161, 3220, 2954, 2859, 1574, 1467, 1172, 1056 cm^{-1}.



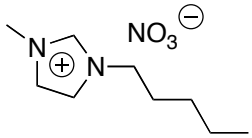
[C_9-mim]BF_4:[2] ^{1}H NMR (acetone-d_6): δ = 9.00 (s, 1H), 7.76 (t, *J* = 1.8 Hz, 1H), 7.70 (t, *J* = 1.7 Hz, 1H), 4.34 (t, *J* = 7.35, 2H), 4.03 (s, 3H), 1.94 (pent, *J* = 7.4 Hz, 2H), 1.34 (m, 12H), 0.86 (t, *J* = 6.6 Hz, 3H); ^{19}F NMR (282 MHz, acetone-d_6): δ= –151.47 (m); ^{13}C NMR (75 MHz, acetone-d_6): δ = 136.9, 124.0, 122.7, 49.7, 35.8, 31.9, 30.2, 29.5, 29.3, 29.1, 26.1, 22.7, 13.8; IR (neat): 3163, 3122, 2955, 2927, 2856, 1575, 1467, 1172, 1064 cm^{-1}.



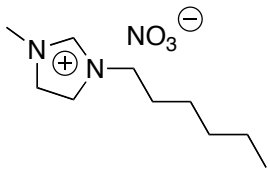
[C_{10}-mim]BF_4:[2] ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.01 (s, 1H), 7.75 (s, 1H), 7.68 (s, 1H), 4.12 (t, *J* = 6.2 Hz, 2H), 3.82 (s, 3H), 1.75 (pent, *J* = 7.1 Hz, 2H), 1.22 (m, 14H), 0.84 (t, *J* = 6.5 Hz, 3H); ^{19}F NMR (282 MHz, DMSO-d_6): δ = –148.62 (m); ^{13}C NMR (75 MHz, acetone-d_6): δ = 136.9, 124.1, 122.7, 49.7, 35.9, 31.9, 30.2, 29.6, 28.5, 29.4, 29.1, 26.1, 22.7, 13.7; IR (neat): 3166, 3122, 3954, 3928, 2856, 1575, 1560, 1467, 1172, 1056 cm^{-1}.



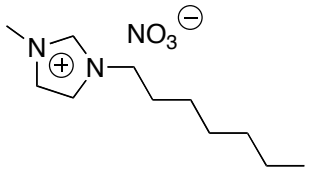
[C_4-mim]NO_3: [3,4] ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.18 (s, 1H), 7.78 (s, 1H), 7.70 (s, 1H), 4.15 (t, *J* = 7.2 Hz, 2H), 3.83 (s, 3H), 1.74 (pent, *J* = 7.4 Hz, 2H), 1.23 (sext, *J* = 7.4 Hz, 2H), 0.87 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, acetone-d_6): δ = 138.0, 124.0, 122.7, 49.3, 35.7, 32.2, 19.3, 13.1; IR (neat): 3147, 3103, 2962, 2936, 2875, 1575, 1475, 1344, 1169 cm^{-1}.



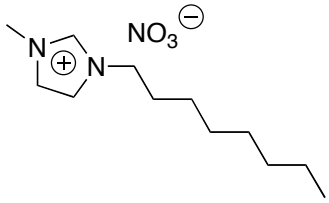
[C_5-mim]NO_3:[5] ^{1}H NMR (300 MHz, acetone-d_6): δ = 9.72 (s, 1H), 7.85 (t, *J* = 1.5 Hz, 1H), 7.78 (t, *J* = 1.6 Hz, 1H), 4.36 (t, *J* = 7.4 Hz, 2H), 4.04 (s, 3H), 1.93 (pent, *J* = 7.2 Hz, 2H), 1.33 (m, 4H), 0.88 (t, *J* = 6.9 Hz, 3H). ^{13}C NMR (75 MHz, acetone-d_6): δ = 137.6, 124.1, 122.8, 49.58, 35.78, 29.93, 28.24, 22.06, 13.56; IR (neat): 3146, 3100, 2959, 2933, 2872, 1574, 1467, 1341, 1169 cm^{-1}.



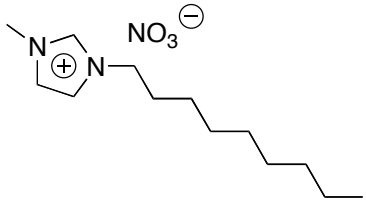
[C_6-mim]NO_3:[3,4] ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.10 (s, 1H), 7.76 (s, 1H), 7.69 (s, 1H), 4.13 (t, *J* = 7.1 Hz, 2H), 3.82 (s, 3H), 1.74 (pent, *J* = 6.9 Hz, 2H), 1.24 (m, 6H), 0.84 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (acetone-d_6): δ = 138.1, 124.0, 122.7, 49.6, 35.7, 31.2, 30.2, 25.8, 22.4, 13.6; IR (neat): 3144, 3100, 2956, 2931, 2859, 1575, 1468, 1340, 1168 cm^{-1}.



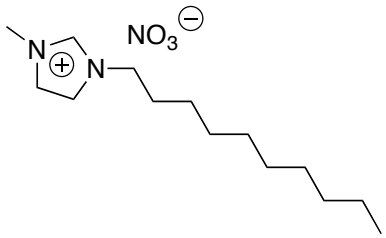
[C_7-mim]NO_3: ^{1}H NMR (300 MHz, acetone-d_6): δ = 9.72 (s, 1H), 9.61 (s, 1H), 7.80 (s, 1H), 7.74 (s, 1H), 4.37 (t, *J* = 7.4 Hz, 2H), 4.05 (s, 3H), 1.94 (m, 2H), 1.33 (m, 8H), 0.87 (t, *J* = 6.8 Hz, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ = 138.1, 124.0, 122.8, 49.6, 35.7, 31.7, 30.3, 28.7, 26.1, 22.5, 13.7; IR (neat): 3147, 3103, 2955, 2859, 1175, 1467, 1347, 1168 cm^{-1}.



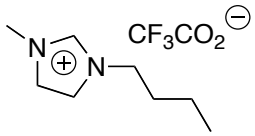
[C_8-mim]NO_3:[3,5] ^{1}H NMR (300 MHz, DMSO-d_6): δ = 9.09 (s, 1H), 7.75 (s, 1H), 7.68 (s, 1H), 4.12 (t, *J* = 7.1 Hz, 2H), 3.82 (s, 3H), 1.75 (m, 2H), 1.23 (m, 10H), 0.84 (t, *J* = 6.5 Hz, 3H); ^{13}C NMR (75 MHz, acetone-d_6): δ = 137.9, 124.0, 122.7, 49.6, 35.8, 31.8, 30.3, 29.2, 29.0, 26.2, 22.6, 13.7; IR (neat): 3147, 3100, 2956, 2929, 2857, 1575, 1466, 1341, 1168 cm^{-1}.



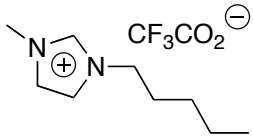
[C_9-mim]NO_3: ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.72 (s, 1H), 7.86 (s, 1H), 7.79 (s, 1H), 4.36 (t, *J* = 7.2 Hz, 2H), 4.04 (s, 3H), 1.93 (pent, *J* = 6.9 Hz, 2H), 1.26 (m, 12H), 0.86 (t, *J* = 6.6 Hz, 3H); ^{13}C NMR (75 MHz, acetone-d_6) δ = 137.7, 124.1, 122.8, 49.6, 35.8, 31.9, 30.3, 29.5, 29.3, 29.1, 26.2, 22.7, 13.8; IR (neat): 3148, 3100, 2954, 2925, 2855, 1575, 1466, 1347, 1169 cm^{-1}.



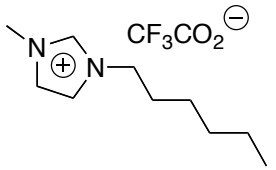
[C_{10}-mim]NO_3:[3] ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.66 (s, 1H), 7.81 (s, 1H), 7.75 (s, 1H), 4.36 (t, *J* = 7.4 Hz, 2H), 4.05 (s, 3H), 1.94 (m, 2H), 1.27 (m, 14H), 0.87 (t, *J* = 6.0 Hz, 3H); ^{13}C NMR (acetone-d_6): δ = 138.1, 124.0, 122.7, 49.6, 35.7, 31.9, 30.3, 29.6, 29.5, 29.4, 29.1, 26.2, 22.7, 13.7; IR (neat): 3150, 3103, 2925, 2855, 1575, 1466, 1346, 1169 cm^{-1}.



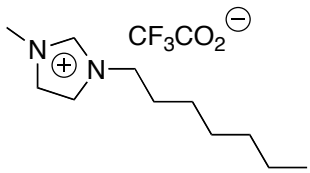
[C_4-mim]CF_3CO_2:[6] ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.93 (s, 1H), 7.89 (t, *J* = 1.65 Hz, 1H), 7.82 (t, *J* = 1.8 Hz, 1H), 4.37 (t, *J* = 7.2 Hz, 2H), 4.05 (s, 3H), 1.89 (pent, *J* = 7.35, 2H), 1.36 (sext, *J* = 7.44, 2H), 0.92 (t, *J* = 7.35, 3H); ^{19}F NMR (282 MHz, acetone-d_6) δ = –75.53 (s); ^{13}C NMR (75 MHz, acetone-d_6): δ = 159.9 (q, *J* = 31.3 Hz), 138.2, 124.0, 122.7, 118.0 (q, *J* = 296.7 Hz), 49.2, 35.7, 32.2, 19.3, 13.0; IR (neat): 3147, 2097, 2965, 2877, 1687, 1648, 1575, 1468, 1199, 1169, 1123 cm^{-1}.



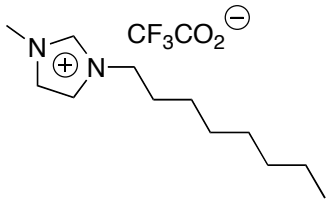
[C_5-mim]CF_3CO_2: ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.73 (s, 1H), 7.90 (t, *J* = 1.5 Hz, 1H), 7.83 (t, *J* = 1.5, 1H), 4.35 (t, *J* = 7.35 Hz, 2H), 4.04 (s, 3H), 1.90 (pent, *J* = 7.28 Hz, 2H), 1.31 (m, 4H), 0.85 (t, *J* = 6.6 Hz, 3H); ^{19}F NMR (282 MHz, acetone-d_6) δ = –75.52 (s); ^{13}C NMR (75 MHz, acetone-d_6): δ = 159.8 (q, *J* = 31.3 Hz), 137.9, 124.1, 122.7, 118.0 (q, *J* = 296.7 Hz), 49.5, 35.7, 29.9, 28.2, 22.0, 13.5; IR (neat): 3150, 2097, 2964, 2870, 1689, 1648, 1570, 1471, 1194, 1169, 1123 cm^{-1}.



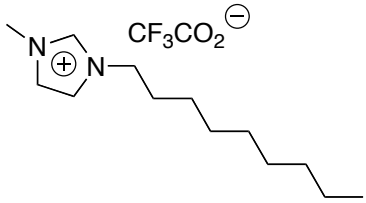
[C_6-mim]CF_3CO_2: ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.77 (s, 1H), 7.90 (t, *J* = 1.8, 1H), 7.83 (t, *J* = 1.65 Hz, 1H), 4.35 (t, *J* = 7.35 Hz, 2H), 4.04 (s, 3H), 1.90 (pent, *J* = 7.2 Hz, 2H), 1.29 (m, 6H), 0.84 (t, *J* = 6.9, 3H); ^{19}F NMR (282 MHz, acetone-d_6) δ = –75.50 (s); ^{13}C NMR (75 MHz, acetone-d_6): δ = 160.0 (q, *J* = 31.9 Hz) 137.9, 124.0, 122.7, 120.0 (q, *J* = 296.9 Hz), 49.5, 35.74, 31.2, 30.2, 25.8, 22.4, 13.6; IR (neat): 3149, 3091, 2965, 2865, 1691, 1573, 1407, 1199, 1125 cm^{-1}.



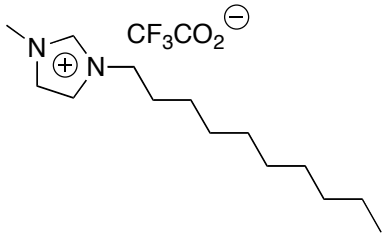
[C_7-mim]CF_3CO_2: ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.96 (s, 1H), 7.82 (s, 1H), 7.76 (s, 1H), 4.37 (t, *J* = 7.35 Hz, 2H), 4.06 (s, 3H), 1.93 (m, 2H), 1.38 (m, 8H), 0.86 (t, *J* = 6.75 Hz, 3H); ^{19}F NMR (282 MHz, acetone-d_6) δ = –75.38 (s); ^{13}C NMR (75 MHz, acetone-d_6): 160.1 (q, *J* = 30.6 Hz), 137.8, 124.1, 122.8, 120.0 (q, *J* = 298.3 Hz), 49.5, 35.7, 31.7, 30.3, 28.7, 26.1, 22.5, 13.7; IR (neat): 3149, 3086, 2934, 2861, 1693, 1572, 1470, 1407, 1196, 1125 cm^{-1}.



[C_8-mim]CF_3CO_2: ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.93 (s, 1H), 7.88 (t, *J* = 1.8 Hz, 1H), 7.81 (t, *J* = 1.8 Hz, 1H), 4.37 (t, *J* = 7.35, 2H), 4.05 (s, 3H), 1.92 (m, 2H), 1.32 (m, 10 H), 0.86 (t, J= 6.75, 3H); ^{19}F NMR (282 MHz, acetone-d_6) δ = –75.45 (s); ^{13}C NMR (75 MHz, acetone-d_6): 159.5 (q, *J* = 31.0 Hz), 138.2, 124.0, 122.7, 119.1 (q, *J* = 297.7 Hz), 49.5, 35.7, 31.8, 30.3, 29.1, 29.0, 26.1, 22.6, 13.7; IR (neat): 3148, 3094, 2931, 2861, 1694, 1572, 1467, 1407, 1193, 1125 cm^{-1}.



[C_9-mim]CF_3CO_2: ^{1}H NMR (300 MHz, acetone-d_6) δ = 9.95 (s, 1H), 7.82 (t, *J* = 1.8 Hz, 1H), 7.76 (t, *J* = 1.8, 1H), 4.37 (t, *J* = 7.35, 2H), 4.06 (s, 3H), 1.93 (m, 2H), 1.34 (m, 12H), 0.87 (t, *J* = 6.6, 3H); ^{19}F NMR (282 MHz, acetone-d_6) δ = –75.36 (s); ^{13}C NMR (75 MHz, acetone-d_6): 160.0 (q, *J* = 31.2Hz), 138.0, 124.1, 122.8, 120.0 (q, *J* = 297.1 Hz), 49.5, 35.7, 31.9, 30.4, 29.8, 29.1, 26.2, 22.7, 13.8; IR (neat): 3147, 3090, 2949, 2860, 1694, 1572, 1466, 1407, 1337, 1171, 1132 cm^{-1}.



[C_{10}-mim]CF_3CO_2:[7] ^{1}H NMR (300 MHz, acetone-d_6): δ = 9.91 (s, 1H), 7.80 (t, *J* = 1.7 Hz, 1H), 7.74 (t, *J* = 1.8 Hz, 1H), 4.38 (t, *J* = 7.35 Hz, 2H), 4.06 (s, 3H), 1.94 (m, 2H), 1.27 (m, 14H), 0.87 (t, *J* = 6.8 Hz, 3H); ^{19}F NMR (282 MHz, acetone-d_6) δ = –75.36 (s); ^{13}C NMR (75 MHz, acetone-d_6): δ = 159.5 (q, *J* = 31.1 Hz), 138.3, 123.9, 122.6, 118.1 (q, *J* = 297.7 Hz), 49.5, 35.7, 31.9, 30.3, 29.6, 29.5, 29.5, 29.1, 26.1, 22.7, 13.7. IR (neat): 3150, 3097, 1928, 2856, 1966, 1695, 1649, 1575, 1467, 1201, 1172, 1127 cm^{-1}.

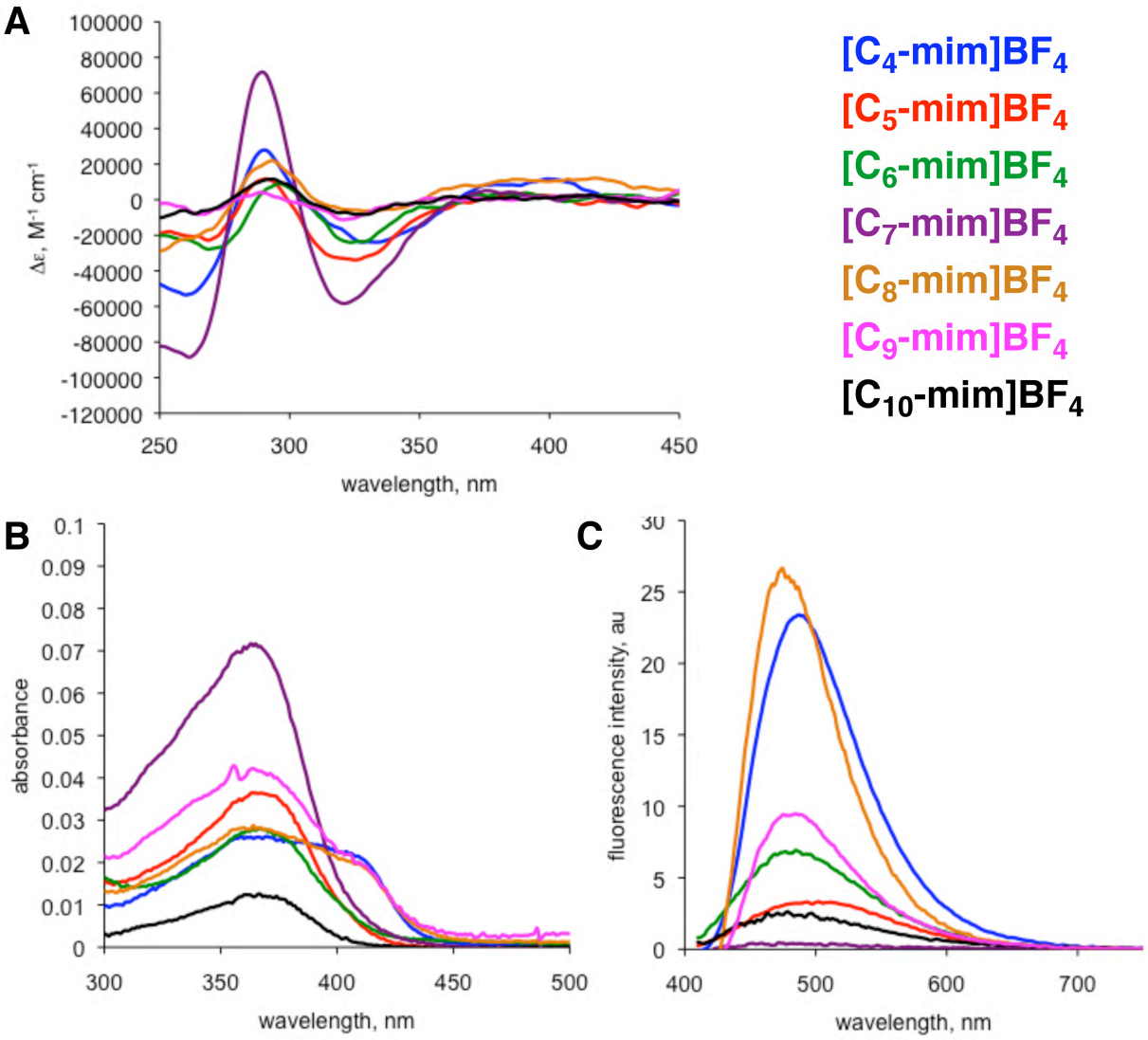


Figure S1: CD (**A**), absorbance (**B**) and emission (**C**) spectra of TC in [C_n-mim]BF_4 ionic liquids (1% DMSO, v/v).

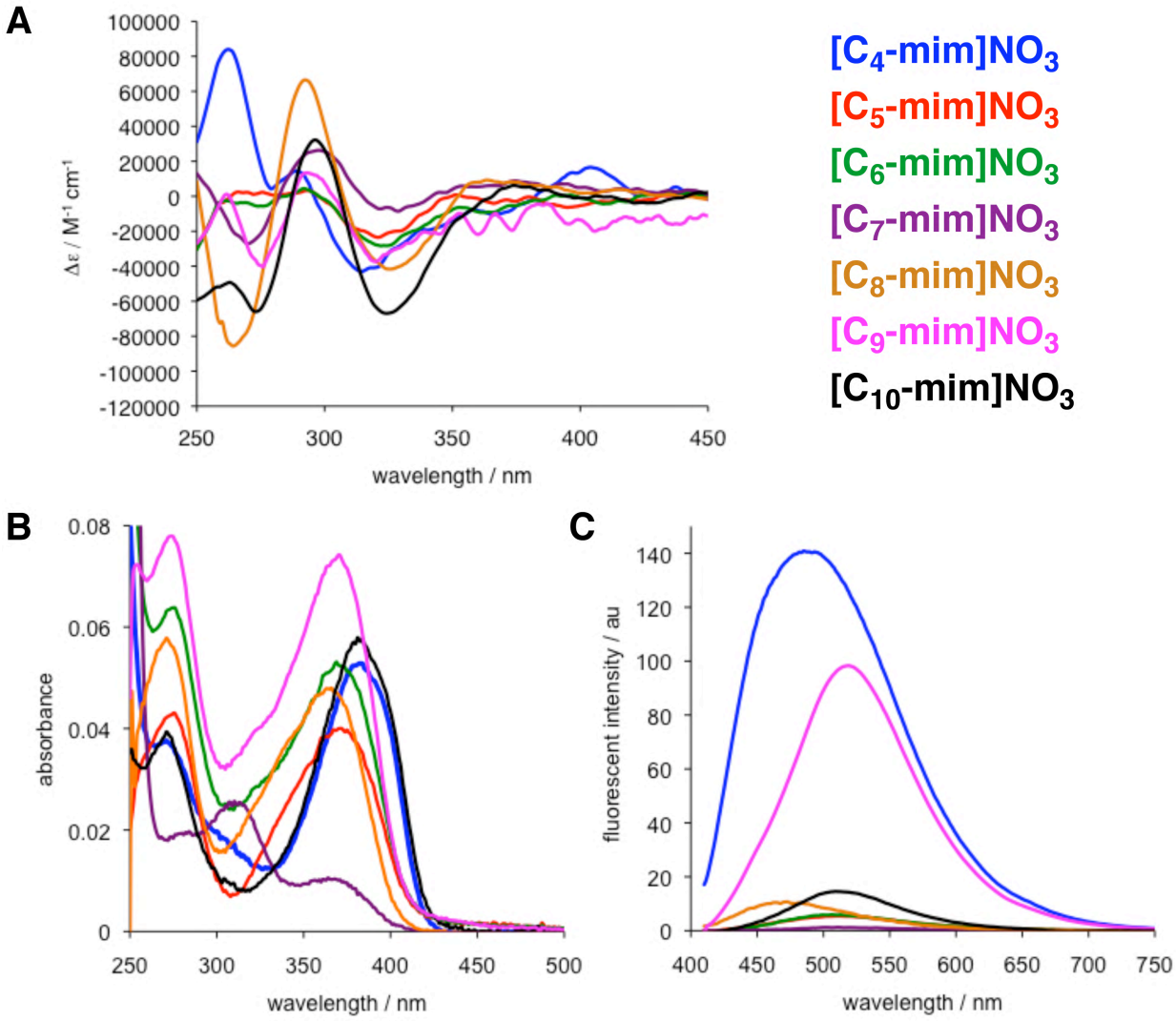


Figure S2: CD (**A**), absorbance (**B**) and emission (**C**) spectra of TC in $[C_n\text{-mim}]NO_3$ ionic liquids (1% DMSO, v/v).

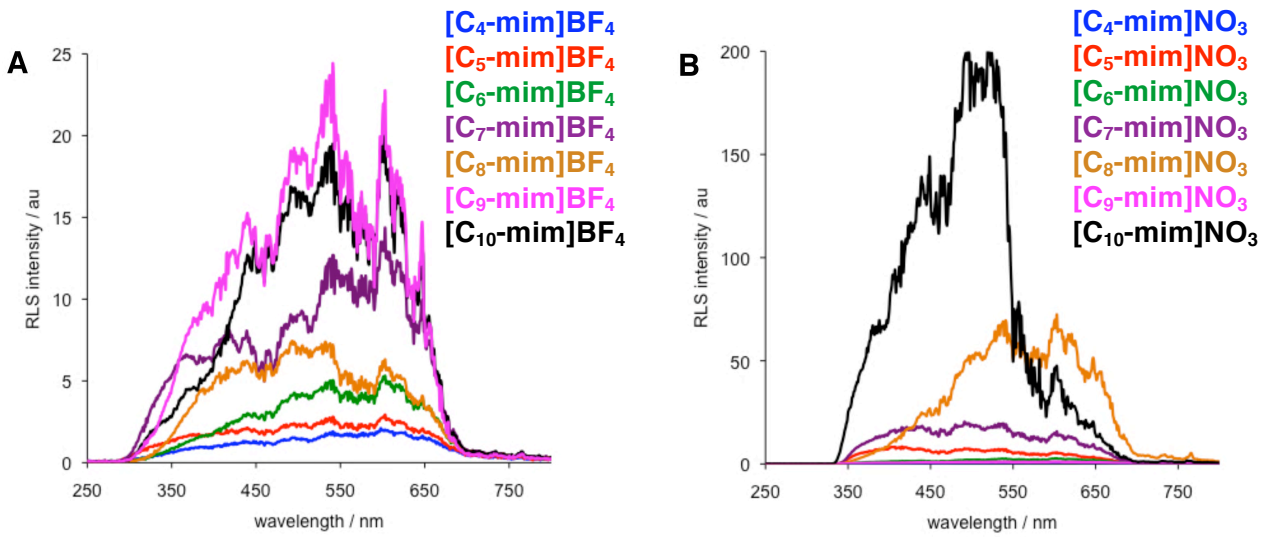


Figure S3: RLS data for $[C_n\text{-mim}]BF_4$ (**A**) and $[C_n\text{-mim}]NO_3$ (**B**) ionic liquids (1% DMSO, v/v).

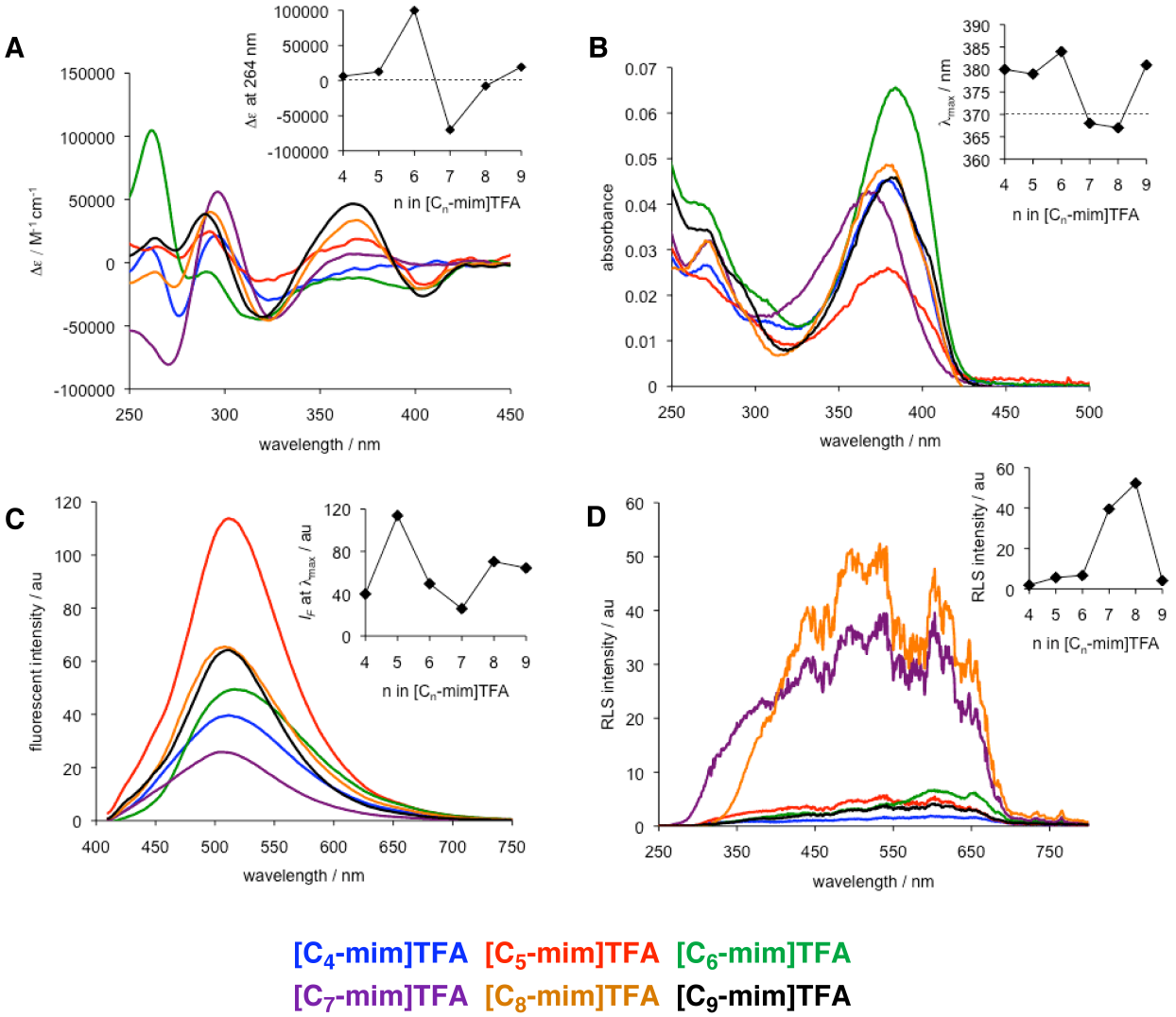


Figure S4: CD (**A**), absorbance (**B**), emission (**C**) and RLS (**D**) spectra of TC in [C_n-mim]TFA ionic liquids (1% DMSO, v/v).

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