Electronic Supplementary Information

Effective Chemiluminogenic Systems Based on Acridinium Esters Bearing Substituents of Various Electronic and Steric Properties

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Synthesis of substituted acridinium esters (XAEs)

Substituted 9-[(phenoxy)carbonyl]-10-methylacridinium triflates (XAEs, Scheme 1) were synthesized in three steps. Reflux of the solution of dehydrated acridine-9-carboxylic acid in thionyl chloride (C = 0.4 M, 4 h) yielded 9-(chlorocarbonyl)acridinium chloride quantitatively. After evaporation of the excess of $SOCl_2$, the crude product was washed with dry benzene and stirred with slight excess (5−10 mol%) of appropriate phenol (distilled) in dry dichloromethane. The esterifications were carried out in dry conditions in the presence of N,N,N-triethylamine (4-fold molar excess) and catalytic amounts of N,N-(dimethyl)-4-aminopyridine (DMAP) (room temperature, 25−50 hours). After the completion, the reaction mixtures were diluted with CH_2Cl_2, filtered and applied to a gravitational LC columns (stationary phase: SiO_2, mobile phase: 2/1 v/v cyclohexane/ethyl acetate), giving chromatographically pure products (one spot on TLC plates under 254/360 nm UV lamp) − 9-[(phenoxy)carbonyl]acridines (yields of 70−90%). The analyses are listed in the following manner: retention factor (R_F) (TLC SiO_2 plate, cyclohexane/ethyl acetate = 3/2 v/v, detection: UV lamp, 254 nm/360 nm), melting point (m.p., K, *Stuart Melting Point SMP30*, Bibby Scientific Ltd., UK), elemental analysis (% calculated/found, *Eager 200 CHN*, Carlo Erba Instuments, Italy; *Vario EL Cube CHNS*, Elementar Analysensysteme GmbH, Germany). For 9-[(2-fluorophenoxy)carbonyl]acridine: R_F = 0.59; m.p. = 436–437 K (Differential Scanning Calorimetry (DSC): 437 K[32]), C = 75.70/75.46, H = 3.81/3.98, N = 4.41/4.26; for 9-[(2-chlorophenoxy)carbonyl]acridine: R_F = 0.61; m.p. = 412–413 K, C = 71.9/70.1, H = 3.62/3.50, N = 4.20/4.33; for 9-[(2-bromophenoxy)carbonyl]acridine: R_F = 0.61; m.p. = 424−426 K, C = 63.5/62.8, H 3.20/3.21, N = 3.70/3.3; for 9-[(2-iodophenoxy)carbonyl]acridine: m.p. = 438–439 K (438 K[32]), R_F = 0.61; C = 56.5/56.4, H = 2.84/2.90, N = 3.29/2.99; for 9-{[(2-trifluoromethyl)phenoxy]carbonyl}acridine: m.p. = 408.0−409.0 K, C = 68.7/68.6, H = 3.29/3.37, N: 3.81/3.77; for 9-[(2-nitrophenoxy)carbonyl]acridine: R_F = 0.52, m.p. = 421.5−424 K (DSC: 422 K[32]), C = 69.7/69.7, H = 3.51/3.46, N = 8.14/8.10; for 9-[(2-methoxyphenoxy)carbonyl]acridine: R_F = 0.56, m.p. = 459−461 K (DSC: 462 K[32]) , C = 76.6/76.4, H = 4.59/4.55, N = 4.25/4.22; for 9-[(2,6-difluorophenoxy)carbonyl]acridine: R_F = 0.61, m.p. = 467–468 K (DSC: 467 K[32]), C = 71.6/71.5, H = 3.31/3.27, N = 4.18/4.12; for 9-[(2,6-dichlorophenoxy)carbonyl]acridine: R_F = 0.66, m.p. = 417.5–418.5 K (DSC: 418 K[32]), C = 65.2/65.5, H = 3.01/3.20, N = 3.80/3.68; for 9-[(2,6-dibromorophenoxy)carbonyl]-acridine: R_F = 0.67, m.p. = 431.5–432.5 K (DSC: 432 K[32]), C = 52.5/51.9, H = 2.43/2,30, N = 3.06/2.95;

for 9-[(2,6-diiodophenoxy)carbonyl]acridine: m.p. = 450.5–452 K (DSC: 453 K[32]), C = 43.59/44.21, H = 2.01/1.86, N = 2.54/2.45; for 9-[(3-fluorophenoxy)carbonyl]acridine: R_F = 0.61, m.p. = 436–438 K, C = 75.70/75.97, H = 3.81/4.03, N = 4.41/4.27; for 9-[(4-fluorophenoxy)carbonyl]acridine: R_F = 0.61, m.p. = 472–474 K, C = 75.70/74.60, H = 3.81/3.93, N = 4.41/4.29; for 9-[(4-chlorophenoxy)carbonyl]acridine: R_F = 0.66, m.p. = 441.5–443   K,   C = 71.90/70.20,   H = 3.62/3.67,   N   =   4.20/3.96;   for 9-[(4-bromophenoxy)carbonyl]acridine: R_F = 0.64; m.p. = 461−463 K, C = 63.51/62.30, H = 3.20/3.33, N = 3.70/3.27.

The above esters were subjected to N-methylation, using 5–10 molar excess of methyl trifluoromethanesulphonate ($CF_3OSO_2CH_3$, *Sigma-Aldrich*) in purified chloroform (Ar atmosphere, room temperature, 3–4 hours) in the presence of a catalytic amount of polymer-bound 2,6-di-*tert*-butylpyridine (*Sigma-Aldrich*). The crude products were purified by dilution the reaction mixture with small amount of acetonitrile, filtration and precipitation the salts with an excess of dry diethyl ether. XAEs were isolated by filtration, washed with ether and dried under vacuum (yields: 72–84%). Their purity, assessed with the aid of the RP-HPLC technique fall in the range of 92.0–100% (% of areas under the main signal with detection wavelength set at 254 nm and 367 nm, n = 3). Analyses of XAEs are listed in the following manner: retention time (R_T) (stationary phase: Waters *Symmetry* C-8 column (3×150 mm), mobile phase: acetonitrile / 0.1 M TFA = 40/60 v/v, 1 ml/min.; melting point (m.p.), elemental analysis (% calculated/found, *Carlo Erba* NCS, Italy). Analyses for **2-F** ($C_{22}H_{15}F_4NO_5S$): R_T = 3.72 min., m.p. = 498−500 K (dec.) (DSC: 503 K[32]), C = 54.9/55.3, H = 3.14/3.23, N = 2.91/2.82, S = 6.66/6.55; for **2-Cl** ($C_{22}H_{15}ClF_3NO_5S$): R_T = 5.38 min., m.p. = 490–491 K (dec.), C = 53.1/53.0, H = 3.04/3.12, N = 2.81/2.72, S = 6.44/6.27; for **2-Br** ($C_{22}H_{15}BrF_3NO_5S$): R_T = 6.12 min., m.p. = 504–505.5 K (dec.), C = 48.7/49.7, H = 2.79/2.80, N = 2.58/2.52, S = 5.91/5.72; for **2-I** ($C_{22}H_{15}F_3INO_5S$): R_T = 6.35 min., m.p. = 483–484 K (dec.) (DSC: 489 K[32]), C = 44.8/44.6, H = 2.57/2.59, N = 2.38/2.36; S = 5.44/5.34; for **2-CF₃** ($C_{23}H_{15}F_6NO_5S$): R_T = 6.34 min., m.p. = 495.5–497 K (dec.), C = 56.0/55.8, H = 3.68/3.71, N = 2.84/2.77, S = 6.50/6.56; for **2-NO₂** ($C_{22}H_{15}F_3N_2O_7S$): R_T = 3.79   min.,   m.p. = 501.5–503   K   (dec.)   (DSC: 506,   509   K[32]),   C = 52.0/52.9, H = 2.97/3.16, N = 5.51/5.36, S = 6.31/6.15; for **2-OCH₃** ($C_{23}H_{18}F_3NO_6S$): R_T = 4.20 min., m.p. = 515–516 K (dec.) (DSC: 516 K[32]), C = 52.0/51.6, H = 2.85/2.92, N = 2.64/2.64, S = 6.06/6.06; for **2,6-diF** ($C_{22}H_{14}F_5NO_5S$): R_T = 4.21 min., m.p. = 516–517.5 K (dec.) (DSC: 517 K[32]), C = 52.9/53.0, H = 2.83/2.90, N = 2.80/2.80, S = 6.42/6.50; for **2,6-diCl**

($C_{22}H_{14}Cl_2F_3NO_5S$): R_T = 7.10 min., m.p. = 522.5–524.5 K (dec.) (DSC: 527 K[32]), C = 49.6/50.5, H = 2.65/2.59, N = 2.63/2.57, S = 6.02/5.90; for **2,6-diBr** ($C_{22}H_{14}Br_2F_3NO_5S$): R_T = 7.60 min., m.p. = 519–521.5 K (dec.) (DSC: 520 K[32]), C = 42.5/43.4, H = 2.27/2.24, N = 2.25/2.16, S = 5.16/4.90, for **2,6-diI** ($C_{22}H_{14}F_3I_2NO_5S$): m.p. = 463, 481 K (DSC[31]), C = 36.91/36.94, H = 1.95/1.97, N = 1.90/1.96, S = 4.57/4.48; for **3-F**: R_T = 5.53 min., m.p. = 501–502 K (dec.); for **4-F** ($C_{22}H_{15}F_4NO_5S$): R_t = 4.58 min., m.p. = 474–475 K (dec.), C = 54.9/55.1, H = 3.14/3.13, N = 2.91/2.90; S = 6.66/6.66; for **4-Cl** ($C_{22}H_{15}ClF_3NO_5S$): R_t = 7.28 min., m.p. = 495.5–497.5 K (dec.), C = 53.1/53.3, H = 3.04/3.02, N = 2.81/2.70; S = 6.44/6.56; for **4-Br** ($C_{22}H_{15}BrF_3NO_5S$): R_t = 7.99 min., m.p. = 505–506 K (dec.), C = 48.7/49.0, H = 2.79/2.77, N = 2.58/2.58, S = 5.91/5.90.

Calculations of limits of detection and limits of quantification (*LOD, LOQ*)

The limits of detection (*LOD*) and limits of quantification (*LOQ*) were calculated using described procedures, assuming *t*-Student parameter t = 95% prediction interval of the regression graphs: Area under CL profile (A, RLU^2) = $f(c_{XAE}$ [M]) (Figure S5, lower graph). The equations used for *LOD* and *LOQ* calculations are presented below.[46]

$$LOD = \frac{2 \cdot t \cdot S_{yx}}{a} \cdot \sqrt{1 + \frac{1}{n} + \frac{(b + y_c - \bar{y})^2}{a^2 \cdot Q_{xx}}} \qquad \text{(eq. S1)}$$

where: $y_c = t \cdot S_{yx} \cdot \sqrt{1 + \frac{1}{n} + \frac{\bar{x}^2}{Q_{xx}}}$

$$LOQ = \frac{y_h - b + t \cdot S_{yx}}{a} \cdot \sqrt{1 + \frac{1}{n} + \frac{(b + y_h - \bar{y})^2}{a^2 \cdot Q_{xx}}} \qquad \text{(eq. S2)}$$

where: $y_h = b + 2 \cdot t \cdot S_{yx} \cdot \sqrt{1 + \frac{1}{n} + \frac{(y_c/a - \bar{x})^2}{Q_{xx}}}$

$\bar{x}$ − denotes the average value of the variable x ($\bar{x} = \frac{\sum x_i}{n}$); $\bar{y}$ − the average value of the variable y ($\bar{y} = \frac{\sum y_i}{n}$); Q_{xx} − the sum of the squares $Q_{xx} = \sum(x_i - \bar{x})^2$; S_{yx} − standard error of estimate of y on x, residual standard deviation:

$$S_{yx} = \sqrt{\frac{\sum(y_i - y_{t_i})^2}{n - 2}} = \sqrt{\frac{\sum y_i^2 - b\sum y_i - a\sum x_i y_i}{n - 2}} \qquad \text{(eq. S3)}$$

Table S1 ^{1}H NMR (room temperature) and MALDI-TOF MS data for 9-[(phenoxy)carbonyl)]-10-methylacridinium triflates (XAEs) substituted in the benzene ring. Structures, names and codes of the compounds with the numbering of atoms are given in Scheme 1.

compound code (Scheme 1)	^{1}H NMR*										Main cations detected** (MALDI-TOF MS)		
	H1/H8	H2/H7	H3/H6	H4/H5	H19	H20	H21	H22	H23	H24	M^+, $(M \pm 1)^+$	$MeAcr^+$, $(MeAcr \pm 1)^+$	Others
2-F CD3OD	8.96 (9.3) d, 2H	8.21 (7.8) t, 2H	8.55 (8.8) m, 2H	8.57 (9.3) m, 2H		7.96 (7.8) t, 1H	7.49 (7.6) t, 1H	7.56 (5.6) m, 1H	7.64 (10) t, 1H	4.96 s, 3H	332 (100), 333 (33)	193 (79)	164 (11)
2-Cl CD3OD	8.96 (9.3) d, 2H	8.22 (7.8) t, 2H	8.56 (7.8) t, 2H	8.67 (8.8) d, 2H		8.07 (7.8) d, 1H	7.55 (7.6) t, 1H	7.66 (7.6) t, 1H	7.81 (7.8) d, 1H	4.96 s, 3H	348 (100), 349 (75)	193 (40)	269 (11)
2-Br DMSO-d6	8.96 (9.3) d, 2H	8.22 (7.3) t, 2H	8.57 (8.3) t, 2H	8.72 (8.8) d, 2H		8.08 (8.3) d, 1H	7.47 (7.6) t, 1H	7.69 (7.6) t, 1H	7.94 (7.8) d, 1H	4.96 s, 3H	394 (100), 393 (75)	193 (96)	139 (27), 165 (24)
2-I DMSO-d6	8.98 (9.3) d, 2H	8.21 (7.8) t, 2H	8.59 (8.0) t, 2H	8.79 (8.3) d, 2H		8.10 (7.1) d, 1H	7.30 (7.6) t, 1H	7.70 (7.8) t, 1H	8.07 (7.8) d, 1H	4.98 s, 3H	440 (100), 441 (39)	193 (44)	284 (14)
2-CF₃ DMSO-d6	8.99 (9.3) d, 2H	8.21 (7.6) t, 2H	8.58 (6.8) m, 4H (+H4/H5)	8.58 (6.8) m, 4H (+H3/H6)		8.37 (7.8) d, 2H	7.73 (7.3) t, 1H	8.01 (7.8) d, 1H	8.05 (7.3) d, 1H	4.98 s, 3H	382 (36), 383 (41)	193 (44), 194 (100)	415 (57), 532 (41)
2-NO₂ DMSO-d6	8.97 (9.6) d, 2H	8.24 (9.0) t, 2H	8.57 (7.9) sxt, 2H	8.68 (8.4) d, 2H		8.35 (8.2, 1.6) q, 1H	8.07 (7.9) sxt, 1H	7.78 (7.9) t, 1H	8.20 (6.8) d, 1H	4.99 s, 3H	359 (13), 358 (18)	193 (100)	238 (28), 509 (4)
2-OCH₃ DMSO-d6	8.94 (9.2) d, 2H	8.23 (7.6) t, 2H	8.56 (7.9) m, 2H	8.67 (8.0) d, 2H		7.39 (7.4) q, 1H	7.17 (7.6) sxt, 1H	7.47 (7.8) m, 1H	7.70 (8.0) q, 1H	4.96 s, 3H	344 (63), 345 (8)	193 (100), 194 (32)	421 (100), 494 (10)
2,6-diF CD3OD	9.00 (9.3) d, 2H	8.27 (7.8) t, 2H	8.60 (7.8) t, 2H	8.44 (8.3) d, 2H		7.58 (8.8) m, 2H (+H22)	7.64 (8.3) m, 1H	7.58 (8.8) m, 2H (+H20)		4.99 s, 3H	350 (24), 351 (32)	193 (100), 192 (86)	151 (83), 500 (42)
2,6-diCl CD3OD	9.00 (9.3) d, 2H	8.28 (7.8) t, 2H	8.60 (7.8) t, 2H	8.78 (8.3) d, 2H		7.88 (8.3) d, 2H (+H22)	7.73 (8.1) t, 1H	7.88 (8.3) d, 2H (+H20)		4.99 s, 3H	383 (100)	193 (100)	529 (21)
2,6-diBr CD3OD	9.00 (9.3) d, 2H	8.25 (7.6) t, 2H	8.59 (7.8) t, 2H	8.96 (8.8) d, 2H		8.00 (8.3) d, 2H (+H22)	7.44 (8.1) t, 1H	8.00 (8.3) d, 2H (+H20)		4.99 s, 3H	472 (25), 471 (33)	193 (100)	440 (100), 622 (10)
2,6-diI CD3OD	9.16 (8.8) d, 2H	8.21 (7.8) t, 2H	8.58 (7.8) t, 2H	8.99 (9.3) d, 2H		8.12 (7.8) d, 2H (+H22)	7.02 (7.8) t, 1H	8.12 (7.8) d, 2H (+H20)		5.00 s, 3H	565 (100); 566 (76)		376 (44), 575 (38)
3-F DMSO-d6	8.96 (9.3) d, 2H	8.18 (7.8) t, 2H	8.57 (8.0) t, 2H	8.68 (8.8) d, 2H	7.89 (9.3) d, 1H		7.40 (7.4) m, 1H	7.64 (9.3) t, 1H	7.71 (8.3) q, 1H	4.97 s, 3H	332 (83)	192 (100), 193 (21)	165 (16), 220 (11)
4-F CD3OD	8.90 (9.3) d, 2H	8.20 (7.6) t, 2H	8.57 (8.1) t, 2H	8.65 (8.8) d, 2H	7.70 (6.8) m, 2H (+H23)	7.37 (8.8) t, 2H (+H22)		7.37 (8.8) t, 2H (+H20)	7.69 (6.8) m, 2H(+H19)	5.05 s, 3H	332 (78), 333 (46)	192 (90), 193 (100)	221 (14), 482 (32)
4-Cl CD3OD	8.96 / (9.3) d, 2H	8.18 (8.3) t, 2H	8.57 (7.3) t, 2H	8.66 (8.8) d, 2H	7.74 (8.8) d, 2H (+H23)	7.86 (8.8) d, 2H (+H22)		7.86 (8.8) d, 2H(+H20)	7.74 (8.8) d, 2H (+H19)	4.97 s, 3H	349 (35)	192 (100), 193 (26)	151 (36)
4-Br CD3OD	8.90 (9.3) d, 2H	8.20 (7.8) t, 2H	8.57 (8.3) t, 2H	8.65 (8.3) d, 2H	7.62 (8.8) d, 2H (+H23)	7.79 (8.8) d, 2H (+H22)		7.79 (8.8) d, 2H(+H20)	7.62 (8.8) d, 2H (+H19)	5.05 s, 3H	394 (30), 393 (22)	193 (100), 194 (34)	151 (12), 165 (13)

Abbreviations and notes:

s – singlet, d – dublet, t – triplet, q – quartet, sxt – sextet, m – multiplet; nH – relative area under signal (H integration).

* values denote chemical shifts according to TMS (in ppm), values in parentheses denote coupling constants (in Hz); **numbers denote relative mass of the ion (m/z), numbers in parentheses denote its relative abundance (in %); M^+ denotes the parent cation of XAE, $MeAcr^+$ denotes 10-metylacridinium cation

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Table S2 ^{13}C NMR (room temperature) data for 9-(phenoxycarbonyl)-10-methylacridinium trifluoromethanesulfonates (XAEs) substituted in the benzene ring. Structures, names and codes of the compounds with the numbering of atoms are given in Scheme 1.

compound code (Scheme 1)	^{13}C NMR*															
	C1/C8	C2/C7	C3/C6	C4/C5	C9	C11/C14	C12/C13	C15	C18	C19	C20	C21	C22	C23	C24	R
2-F CD3OD	129.3	133.8	139.3	123.1	142.4	119.1	147.4	162.3	147.7	129.3	128.9	123.8	127.9	114.9	38.9	
2-Cl CD3OD	126.1	130.7	139.3	123.1	142.4	123.8	146.0	162.4	147.7	129.3	128.7	127.7	128.7	119.1	38.8	
2-Br CD3CN	124.7	134.7	140.3	123.7	142.9	119.9	147.8	163.5	148.0	134.0	130.2	128.5	130.0	115.7	40.3	
2-I CD3CN	128.8	140.9	142.9	124.0	144.6	123.7	148.0	164.1	151.1	140.3	130.9	130.0	130.2	119.9	40.3	
2-CF₃ CD3CN	128.3	130.4	135.0	125.0	143.0	123.6	147.4	163.5	147.5	140.2	128.7	128.3	128.4	120.0	40.4	128.1
2-NO₂ CD3CN	125.9	130.4	136.5	123.9	142.4	123.0	142.9	163.3	147.0	140.2	129.5	127.1	128.4	119.9	40.4	
2-OCH₃ CD3OD	122.2	129.1	138.6	121.1	142.3	119.9	149.1	162.6	150.9	139.3	128.4	123.1	128.1	112.8	38.6	55.3
2,6-diF CD3OD	127.1	139.4	142.4	123.1	146.9	119.3	153.9	161.7	155.9	112.7	129.6	125.6	128.7	112.6	38.9	
2,6-diCl CD3OD	129.2	139.3	139.3	127.9	142.4	123.4	142.8	161.5	146.8	119.2	129.6	128.2	129.3	119.2	39.0	
2,6-diBr CD3OD	133.4	139.3	139.3	123.5	142.4	119.1	145.5	161.3	146.5	116.9	130.0	128.4	129.1	116.9	39.1	
2,6-diI CD3OD	139.2	140.5	142.4	123.5	146.3	121.7	148.0	161.2	152.0	119.0	129.2	128.9	128.9	119.1	39.2	
3-F CD3CN	132.0	132.1	140.3	123.5	142.9	119.9	148.3	163.9	151.0	115.2	110.4	130.4	128.4	117.9	40.2	
4-F CD3CN	128.5	130.3	140.2	123.5	142.9	123.0	146.3	162.5	148.5	120.5	124.1	117.3	124.1	119.8	40.2	
4-Cl CD3OD	129.8	132.6	139.3	127.7	142.4	127.7	148.1	163.0	148.6	122.9	129.4	119.1	129.4	122.9	38.8	
4-Br CD3CN	128.4	130.4	133.8	124.3	140.2	123.5	142.9	163.9	149.6	120.7	128.4	119.8	128.4	120.7	40.2	

Abbreviations and notes:

* values denote chemical shifts according to TMS (in ppm)

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Table S3 An exemplary ^{1}H NMR data for **4-F** (Scheme 1) and its unmethylated precursor, 9-[(4-fluorophenoxy)carbonyl]acridine obtained with the assistance of ACD/HNMR database[55]. Chemical shifts, assayed with the coefficient of variation (CV) in the range of 0.10–4.1%, are averaged for each group of protons.

No. of ^{1}H nuclei (Scheme 1)	chem. shift (ppm)	std. dev. (ppm)	relative intensity (integration)	coupling constants (^nJ, Hz)
9-[(4-Fluorophenoxy)carbonyl]acridine				
1/8	8.60	0.50	2H	$^3J_{1,2} = 7.61$; $^4J_{1,3} = 1.39$; $^5J_{1,4} = 0.65$
2/7	7.74	0.24	2H	$^3J_{7,8} = 7.61$; $^3J_{2,3} = 7.14$; $^4J_{2,4} = 1.32$
3/6	7.72	0.03	2H	$^3J_{3,4} = 8.08$; $^3J_{6,7} = 7.14$; $^4J_{6,8} = 1.39$
4/5	8.47	0.03	2H	$^3J_{5,6} = 8.08$; $^4J_{5,7} = 1.32$; $^5J_{5,8} = 0.65$
19/23	7.25	0.13	2H	$^4J_{19,23} = 3.35$
20/22	7.12	0.12	2H	$^3J_{20,22;21F} = 6.32$
9-[(4-Fluorophenoxy)carbonyl]-10-methylacridinium triflate (**4-F**)				
1/8	8.95	0.01	2H	$^3J_{8,7} = 8.85$; $^4J_{8,6} = 1.72$; $^5J_{8,5} = 0.94$; $^5J_{1,4} = 0.50$
2/7	7.46	0.31	2H	$^3J_{2,1} = 8.85$; $^3J_{7,6} = 6.57$
3/6	7.95	0.22	2H	$^3J_{3,4} = 8.84$; $^4J_{3,1} = 1.72$
4/5	8.47	0.35	2H	$^3J_{5,6} = 8.84$; $^4J_{5,7} = 1.46$
19/23	7.25	0.13	2H	$^3J_{19,20} = 9.17$; $^4J_{19,21F} = {}^4J_{23,21F} = 6.32$; $^4J_{19,23} = 3.35$; $^5J_{19,22} = 0.35$
20/22	7.12	0.12	2H	$^3J_{22,23} = 9.17$; $^3J_{20,21F} = {}^3J_{22,21F} = 6.62$; $^4J_{20,22} = 2.85$; $^5J_{20,23} = 0.35$
24	4.22	0.04	3H	$^2J_{24,24} = 14.6$

Table S4 Chemiluminogenic properties of substituted 9-[(phenoxy)carbonyl)]-10-methylacridinium triflates (XAEs, Scheme 1) in various environments; k_{CL} denotes the pseudo-first order constants of CL decay (in s^{-1}), $RCLE$ − relative CL efficiency, normalized to the highest value, QY − quantum yield (in %). The vales were obtained basing on five runs for each concentration of XAE, CV = 2−8%.

compound code	k_{CL}^1	k_{CL}^2	k_{CL}^3	k_{CL}^4	$RCLE^1$	$RCLE^2$	$RCLE^3$	$RCLE^4$	QY^5	$RCLE$ in buffered solutions[6] pH			stability[7]
										12.0	10.0	8.0	
2-F	4.44	5.19	2.91	6.77	0.808	0.880	0.544	0.410	2.31	83.5	46.7	18.4	51.5
2-Cl	4.29	4.24	2.88	6.77	0.729	0.774	0.489	0.417	2.08	80.5	65.8	17.8	42.1
2-Br	5.12	4.63	3.02	7.03	0.770	0.845	0.539	0.460	2.02	85.0	47.8	16.9	44.7
2-I	3.92	5.17	3.14	6.17	0.782	0.977	0.574	0.495	2.05	93.1	82.6	11.1	46.0
2-CF₃	3.95	4.06	2.77	7.54	0.662	0.704	0.444	0.287	1.74	73.0	64.4	40.5	44.6
2-NO₂	3.75	4.39	3.22	7.43	0.687	0.692	0.421	0.360	1.80	76.1	59.8	21.4	26.3
2-OCH₃	0.42	0.91	0.29	0.21	0.635	0.765	0.475	0.513	1.67	70.1	19.3	0.474	71.9
2.6-diF	3.98	4.24	3.07	8.19	0.759	0.796	0.527	0.407	1.99	85.5	71.3	37.6	44.7
2.6-diCl	3.90	4.18	3.21	8.50	0.747	0.775	0.488	0.295	1.96	72.8	28.5	19.0	50.3
2.6-diBr	3.83	3.89	3.46	7.87	0.709	0.656	0.461	0.271	1.86	55.8	16.3	13.0	70.4
3-F	4.14	4.35	2.93	7.39	0.906	**1.00**	0.661	0.464	2.38	100	97.4	17.0	46.0
4-F	2.95	3.35	2.02	3.46	0.662	0.882	0.542	0.500	1.74	73.1	70.2	1.14	54.8
4-Cl	4.24	4.00	2.65	7.39	0.704	0.917	0.593	0.463	1.85	77.7	73.7	4.98	50.2
4-Br	3.97	4.19	3.23	7.23	0.762	0.920	0.612	0.505	2.00	84.1	80.4	5.03	51.8

Notes:

Final concentrations of reagents (plate):

[1]c_{XAE} = 2.0×10^{-5} M in 1 mM HCl; triggers: c_{H2O2} = 4.5 mM in 10 mM HNO₃; c_{NaOH} = 50 mM in H₂O (standard triggering mixture)

[2]c_{XAE} = 2.0×10^{-5} M in 1 mM HCl; triggers: c_{H2O2} = 4.5 mM in 10 mM HNO₃; c_{TBAOH} = 50 mM in H₂O

[3]c_{XAE} = 2.0×10^{-5} M in 1 mM HCl; triggers: c_{H2O2} = 4.5 mM in 10 mM HNO₃; c_{DBU} = 50 mM in H₂O

[4]c_{XAE} = 2.0×10^{-5} M in CH₃OH; triggers: c_{H2O2} = 4.5 mM in CH₃OH; c_{TBAOH} = 50 mM in CH₃OH

[5]Quantum yields (QY) were calculated for standard triggering system (see note 1) using luminol as standard.[22]

[6]Standard phosphate buffers were used (*Aldrich*); triggering system: see note 1.

[7]Samples of XAEs were incubated in Britton-Robinson phosphate buffer: c = 2.0×10^{-5} M, pH = 8.0, T = 298 K; t = 30 min.; triggering system: see note 1.
Stabilities were calculated as % of the initial activity: 100% × (integral CL (t = 30 min.)/integral CL (t = 0 min.)).

For details see Experimental section.

Entity no. (Scheme 2)	compound (Scheme 1)				
	2-F	**2-CF₃**	**2-NO₂**	**2-OCH₃**	**2,6-diBr**
XAE					
a					
b					
c					

9
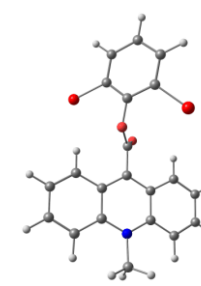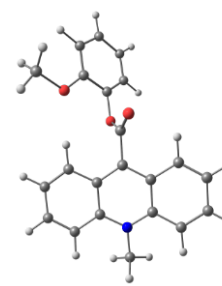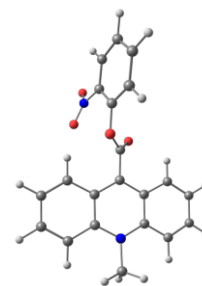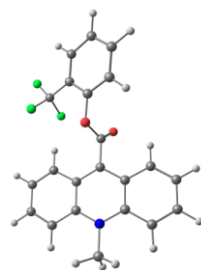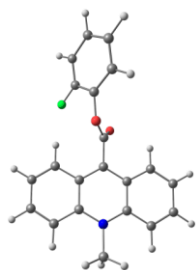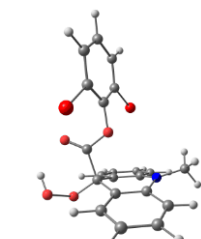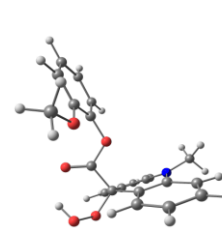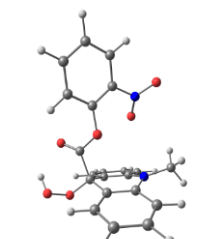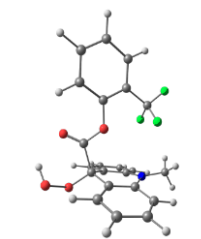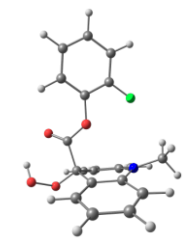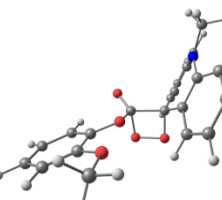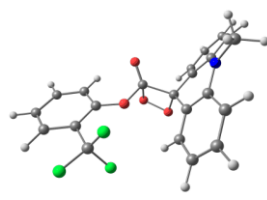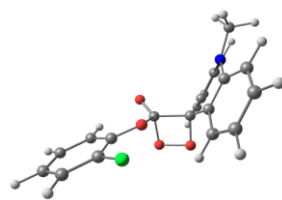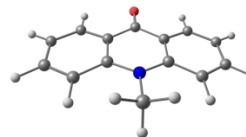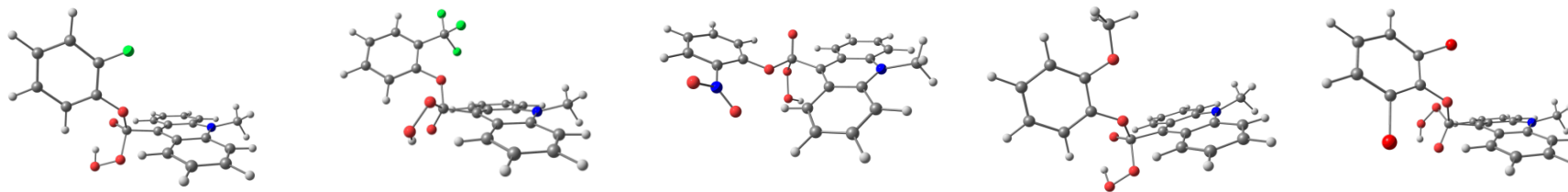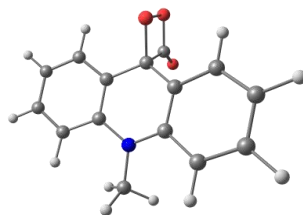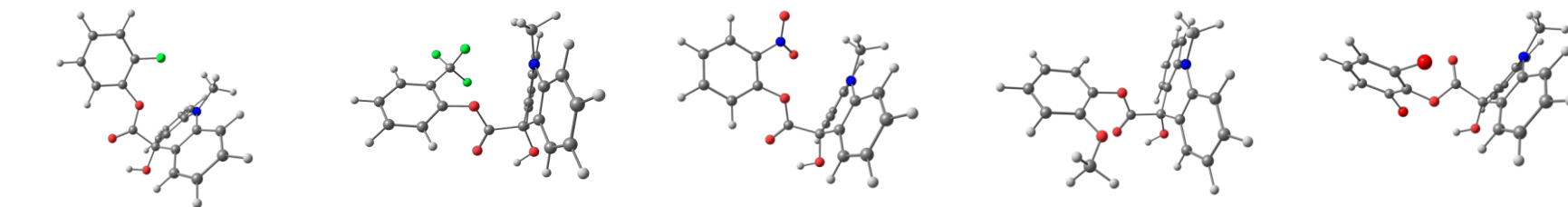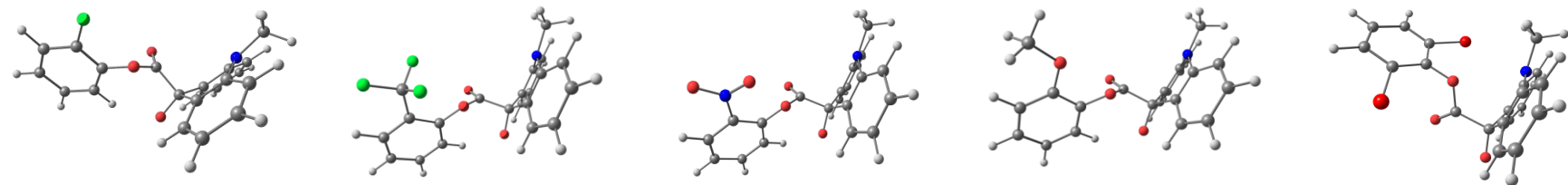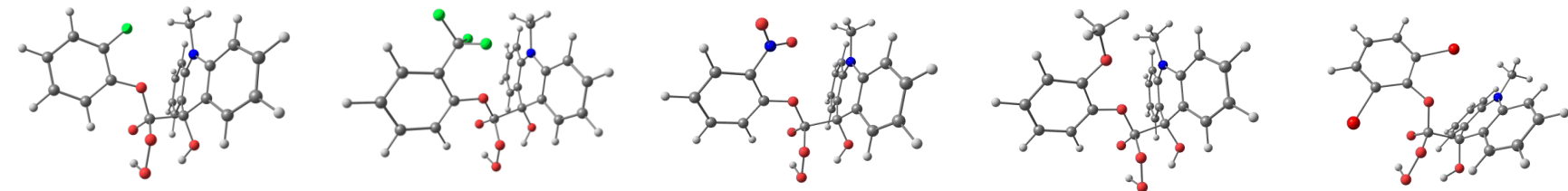
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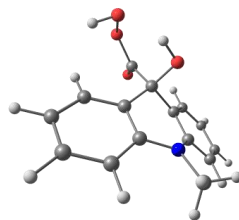
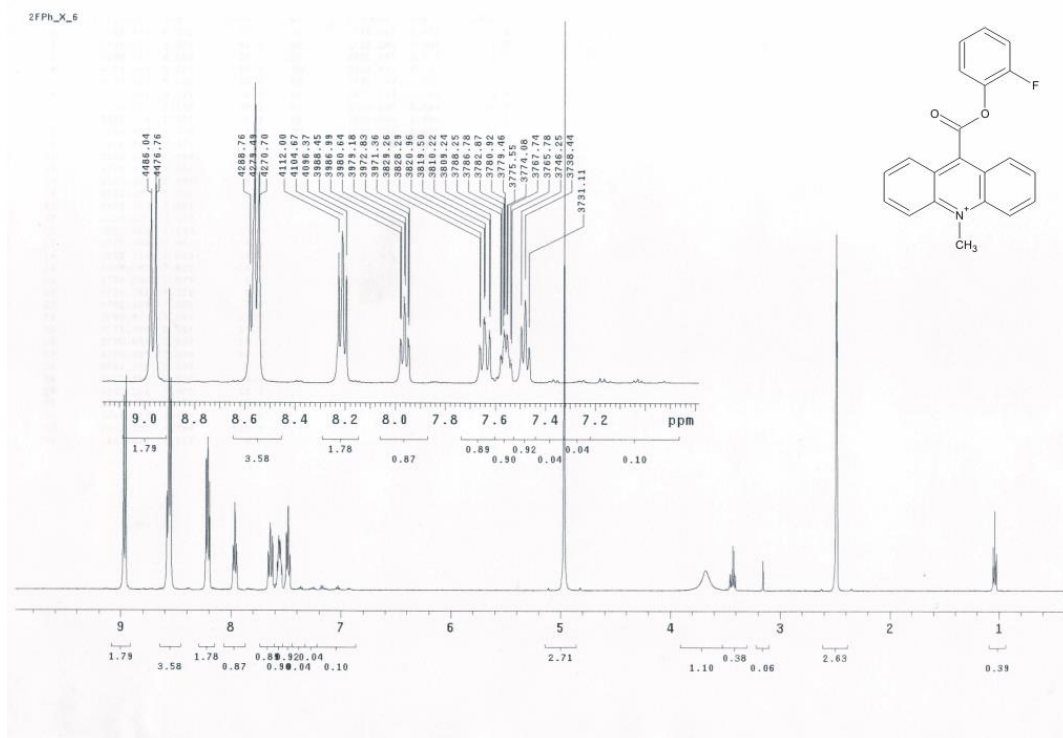**j**
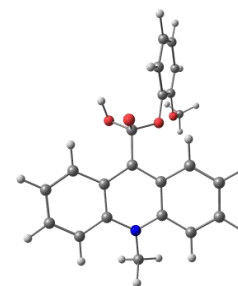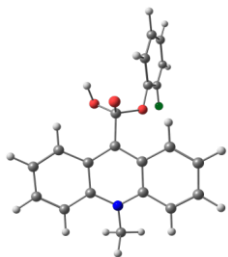
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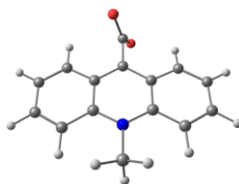
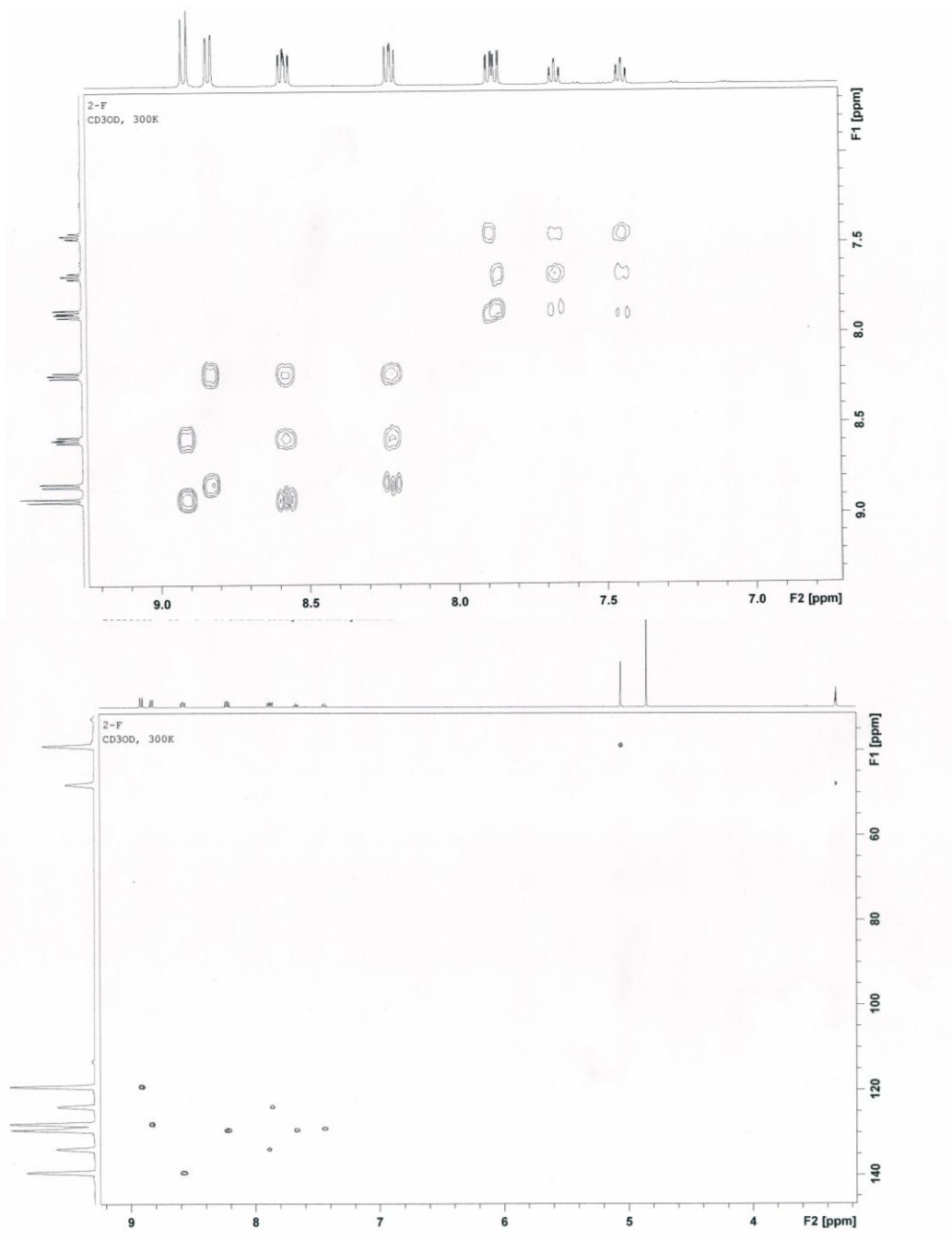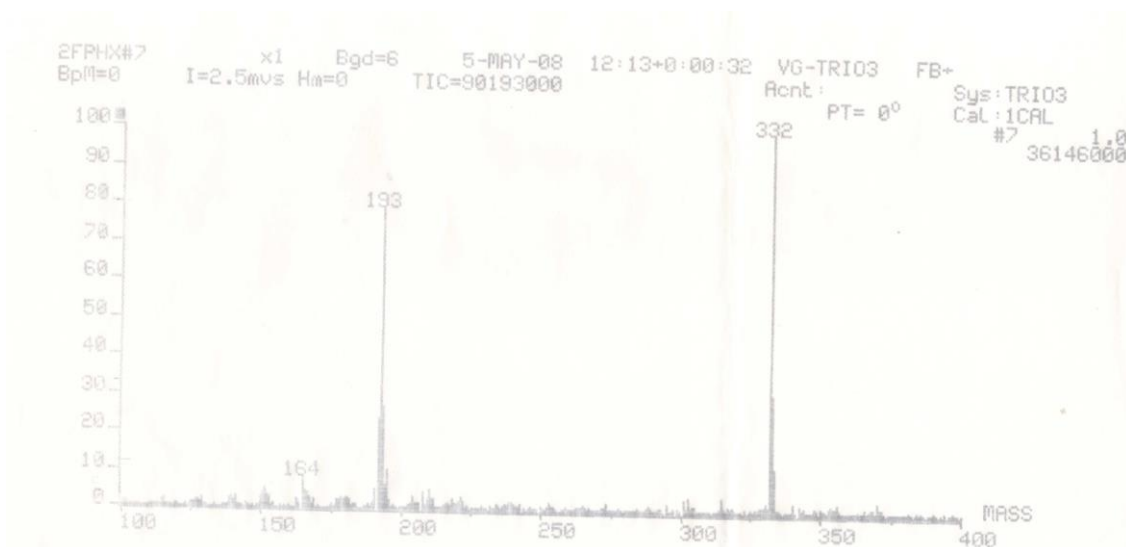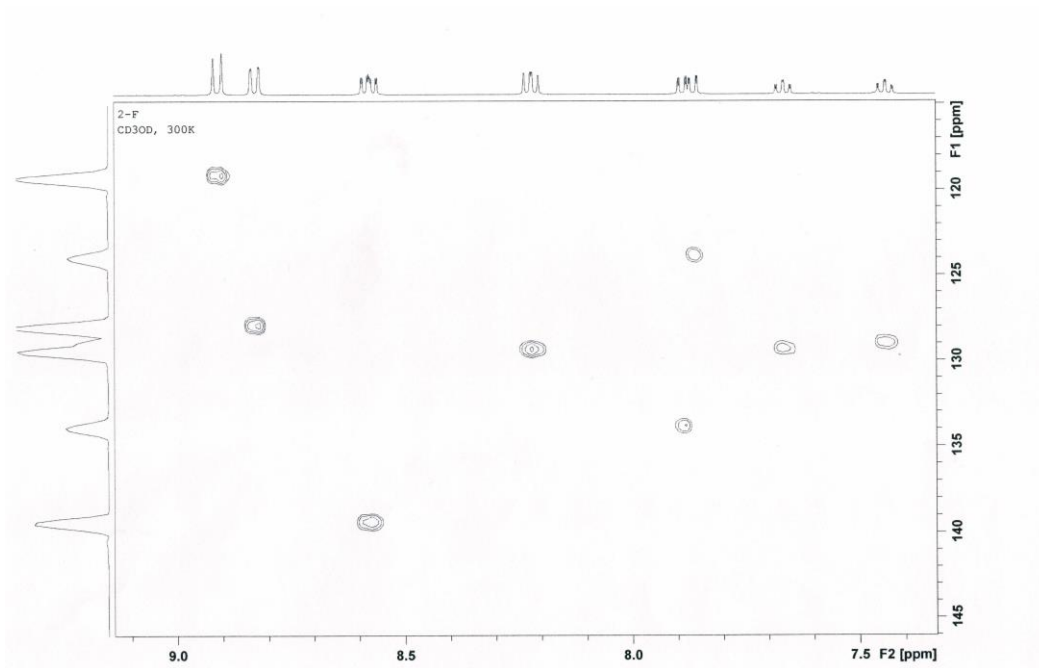**l**
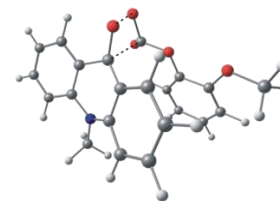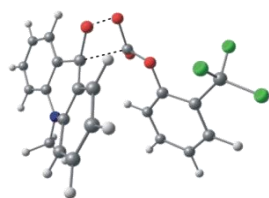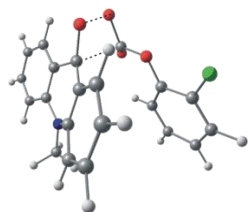
TSVII

TSX
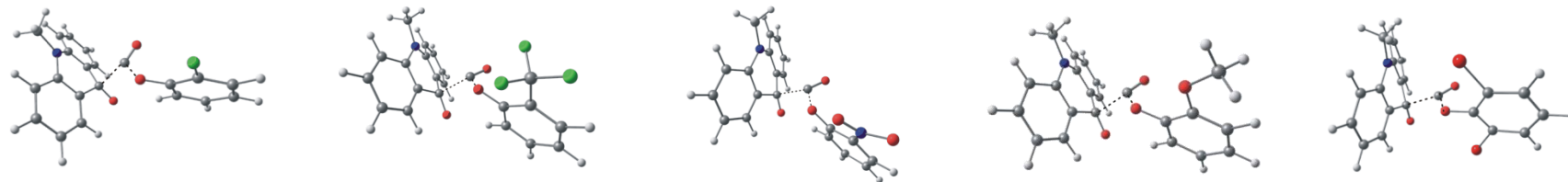
TSXIII
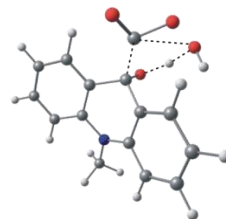
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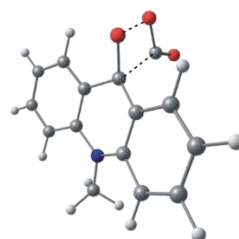
Fig. S1 DFT (B3LYP/6–31G**) optimized geometries of selected entities occurring on the reaction pathways of XAE's with OOH^- and OH^- (**TSVII**, **TSX**, **TSXIII**, **TSXVIII** represent transition state geometries; the dotted lines denote the bonds formed and broken during the course of the reaction).

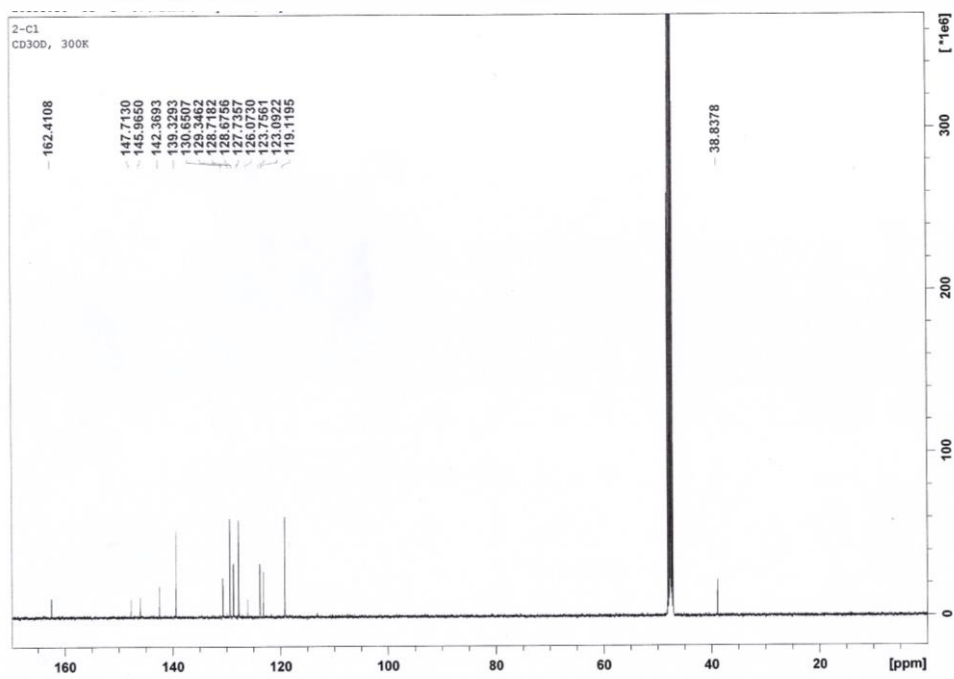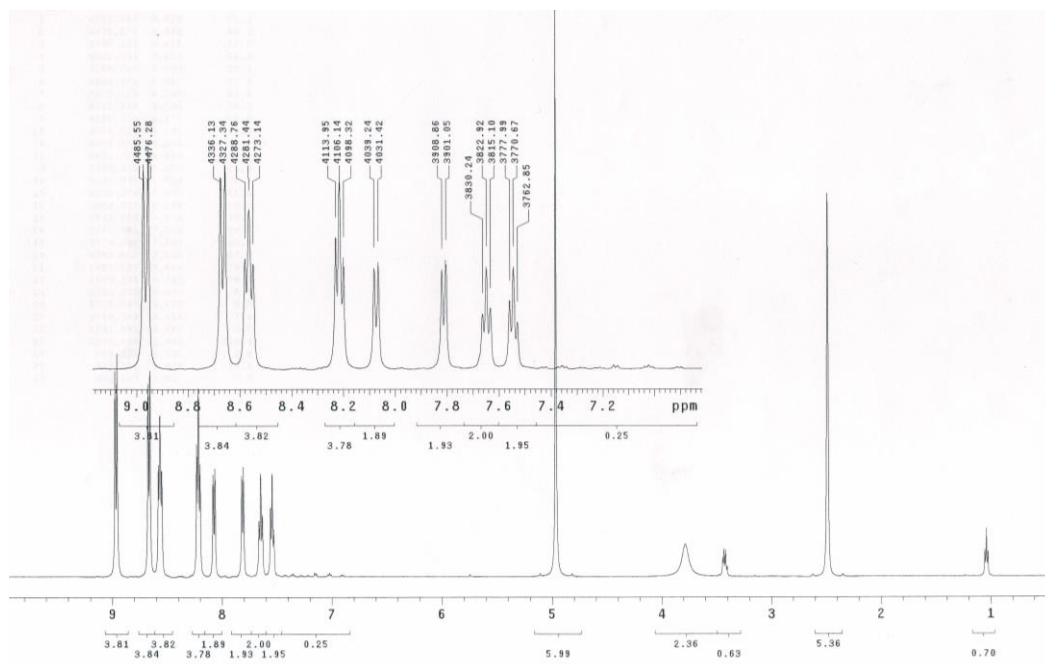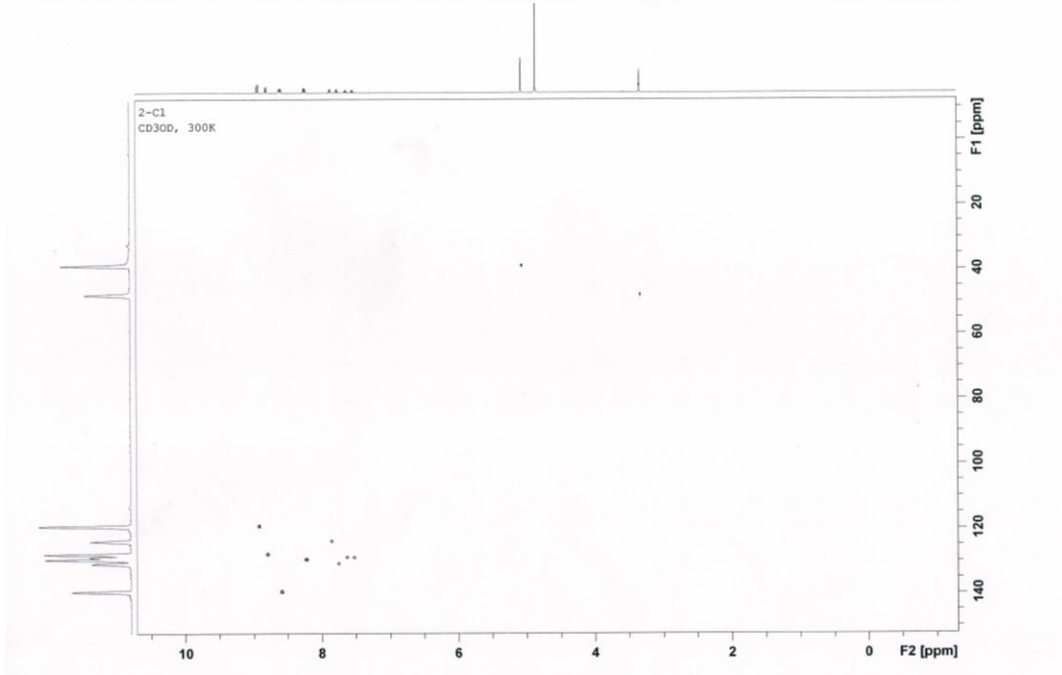9-[(2-fluorophenoxy)carbonyl]-10-methylacridinium (**2-F**)

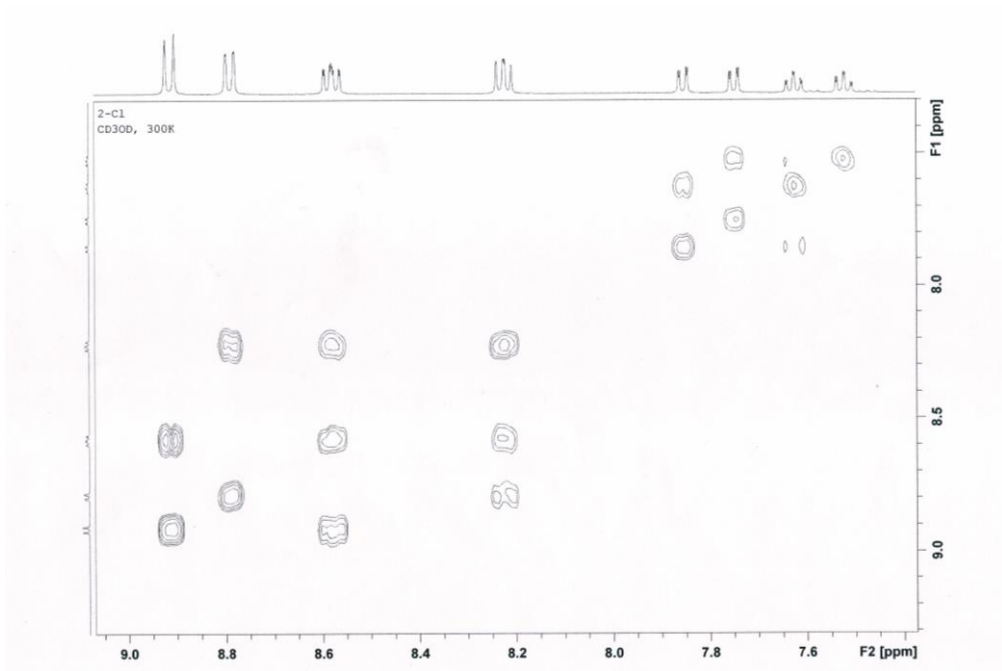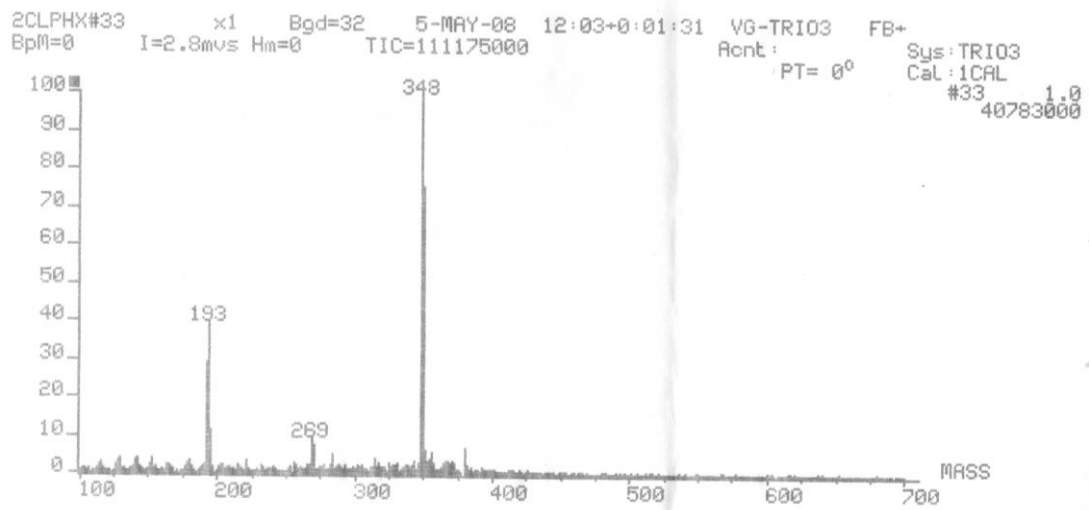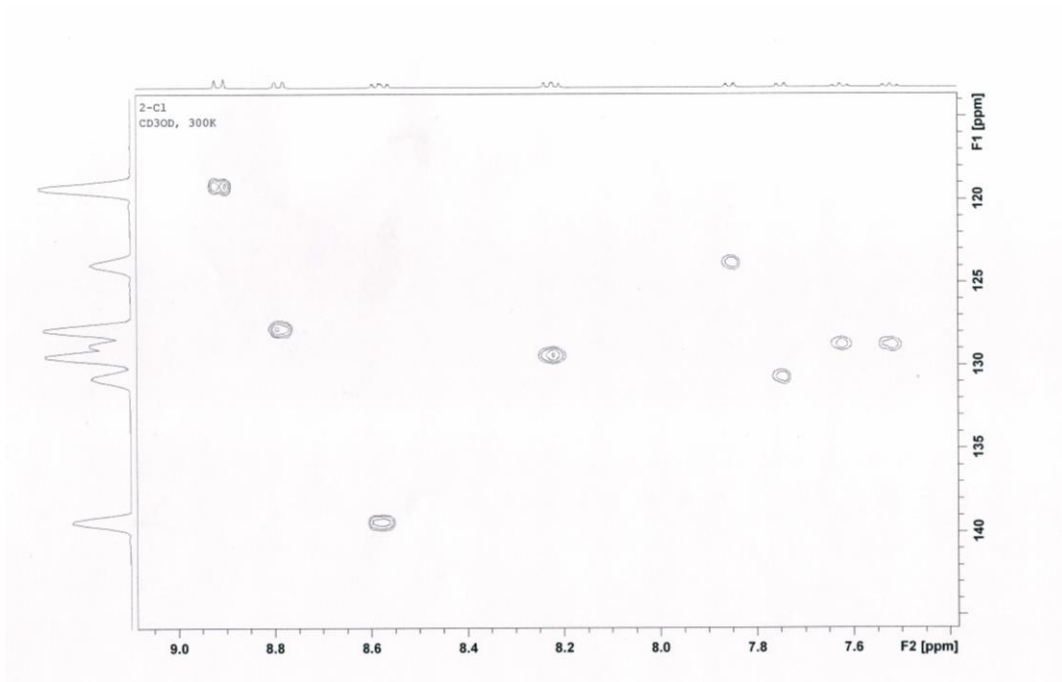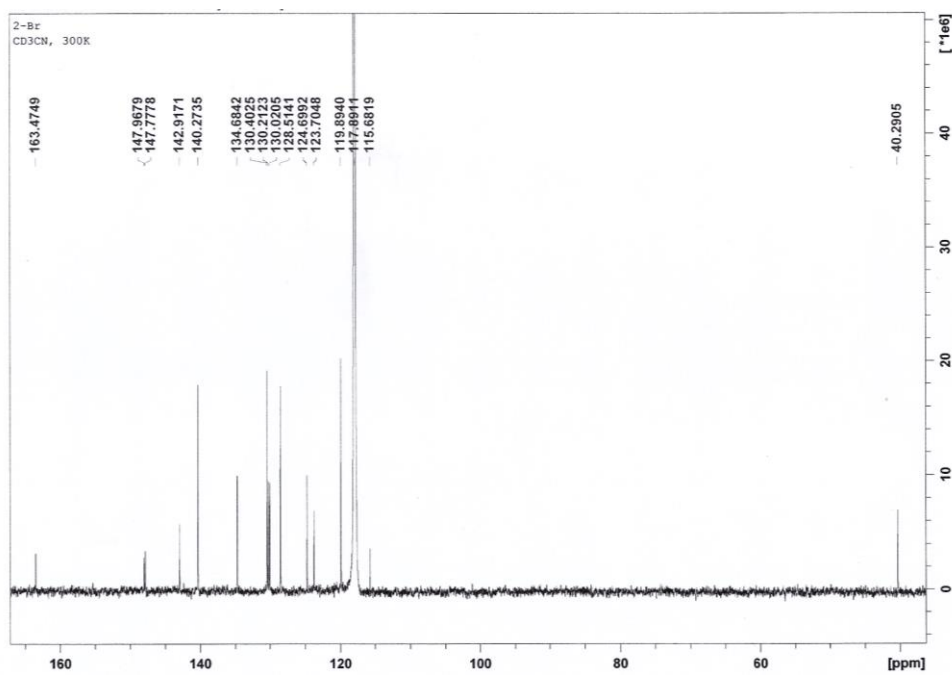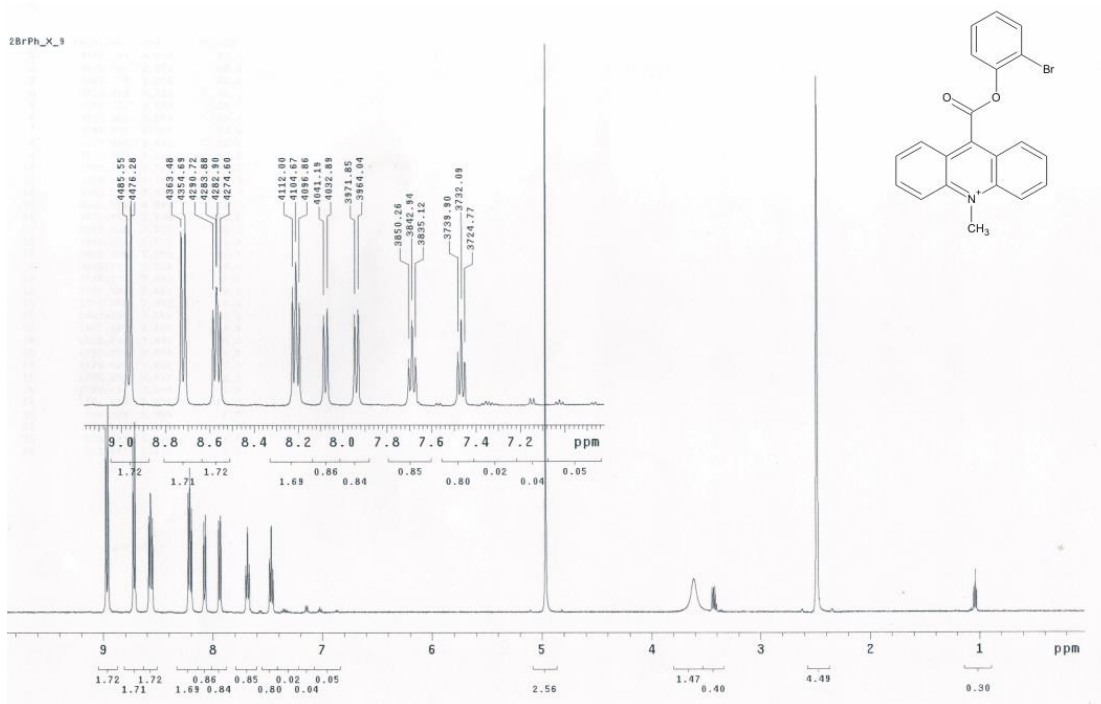9-[(2-chlorophenoxy)carbonyl]-10-methylacridinium (**2-Cl**)

2-Cl
CD3OD, 300K

1H NMR peak labels (ppm region):
485.55
476.28
436.13
432.34
428.76
424.44
423.14
413.95
406.14
409.32
403.24
403.42
398.86
391.05
3830.24
382.92
385.10
377.99
377.95
370.67
3762.85

Integration values: 3.81, 3.82, 3.84, 3.82, 1.89, 3.78, 1.89, 1.93, 2.00, 1.95, 0.25

Lower axis integrations: 3.81, 3.82, 3.84, 3.78, 1.89, 1.93, 2.00, 1.95, 0.25, 5.99, 2.36, 0.63, 5.36, 0.70

13C NMR peak labels:
162.4108
147.7130
145.9650
142.3693
139.3293
130.6507
129.3462
128.7182
128.6756
127.7357
126.0730
123.7661
123.0922
119.1195
38.8378

2-C1
CD3OD, 300K

2-C1
CD3OD, 300K

2-C1
CD3OD, 300K

2CLPHX#33   x1   Bgd=32   5-MAY-08   12:03+0:01:31   VG-TRIO3   FB+
BpM=0   I=2.8mvs Hm=0   TIC=111175000   Acnt: Sys:TRIO3
                                                           PT= 0°   Cal:1CAL
                                                                      #33   1.0
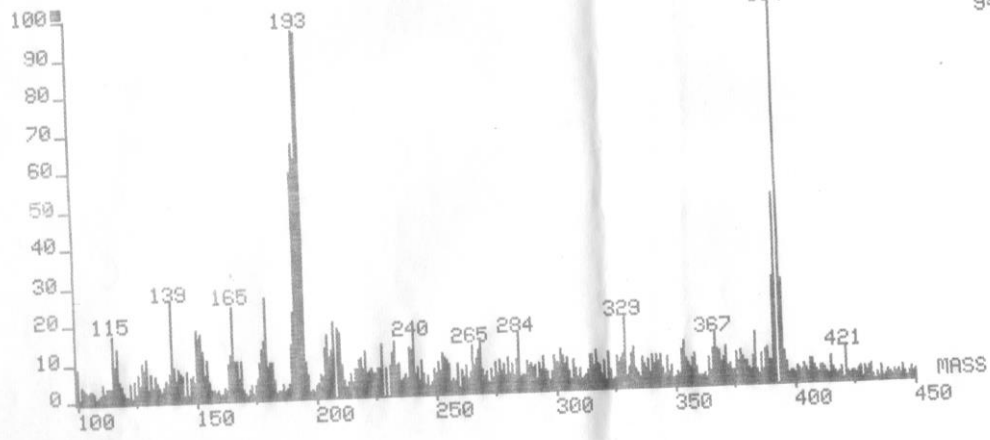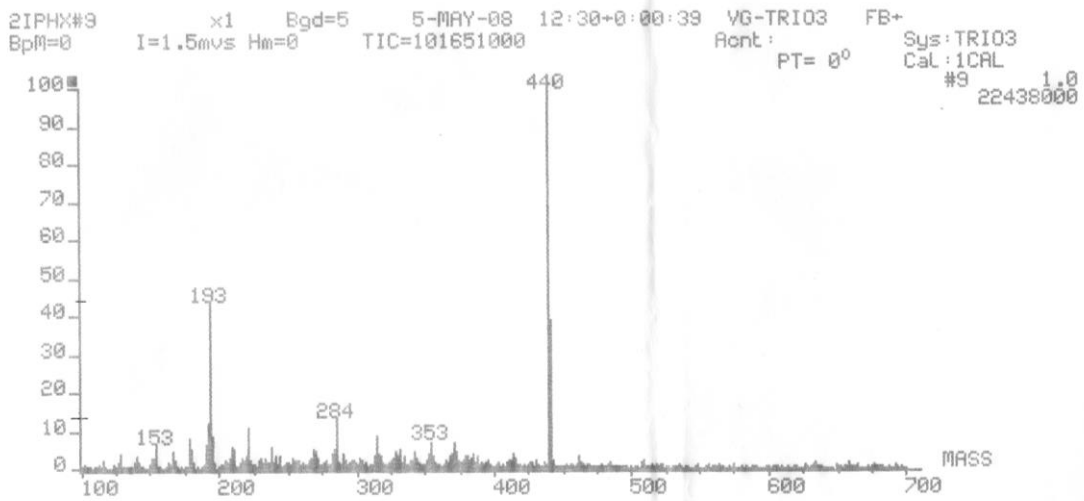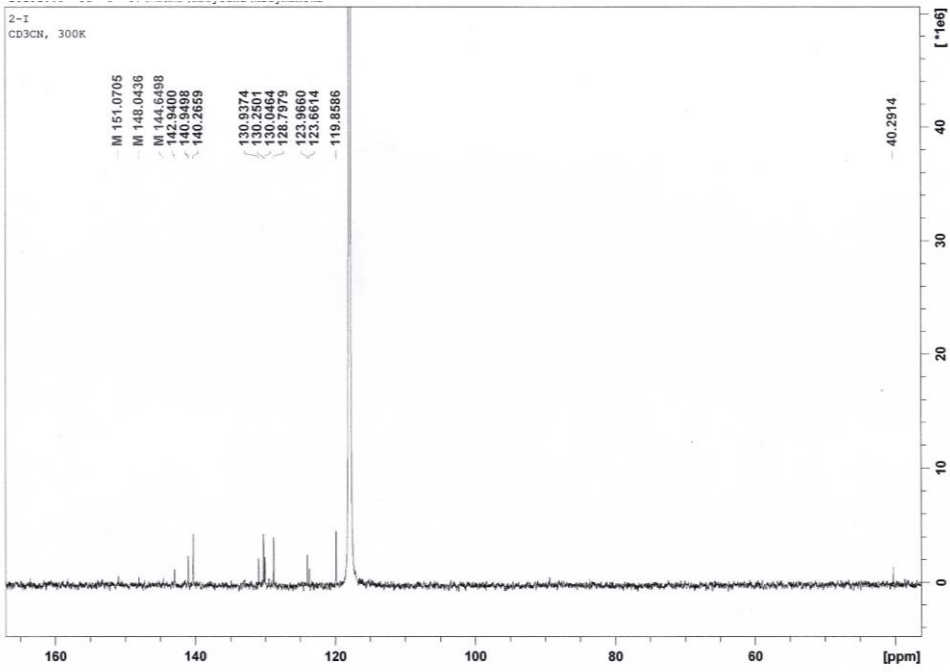   40783000
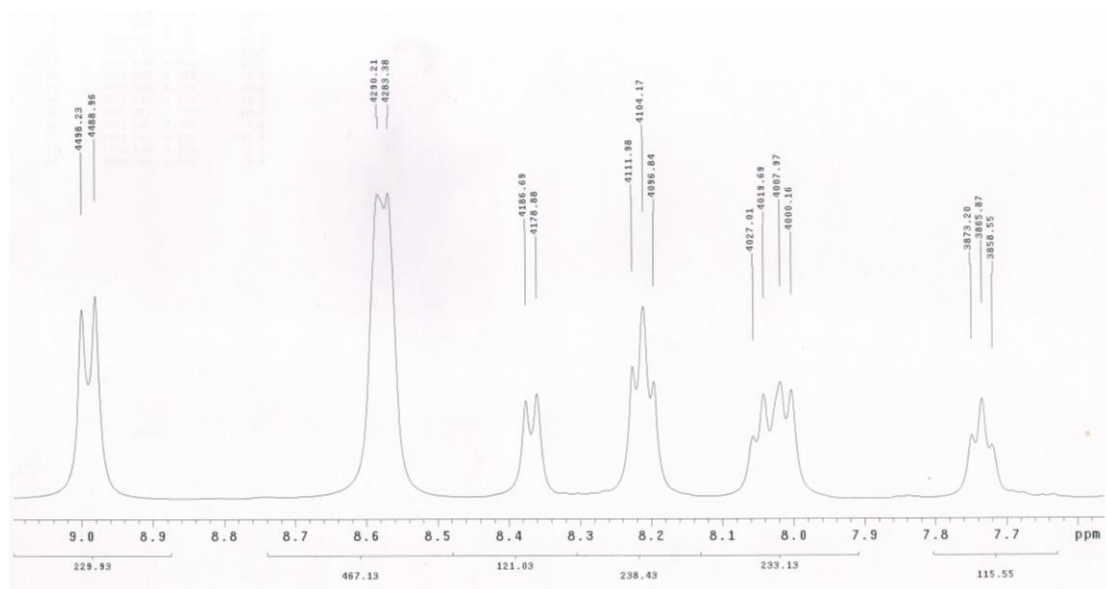
9-[(2-bromophenoxy)carbonyl]-10-methylacridinium (**2-Br**)

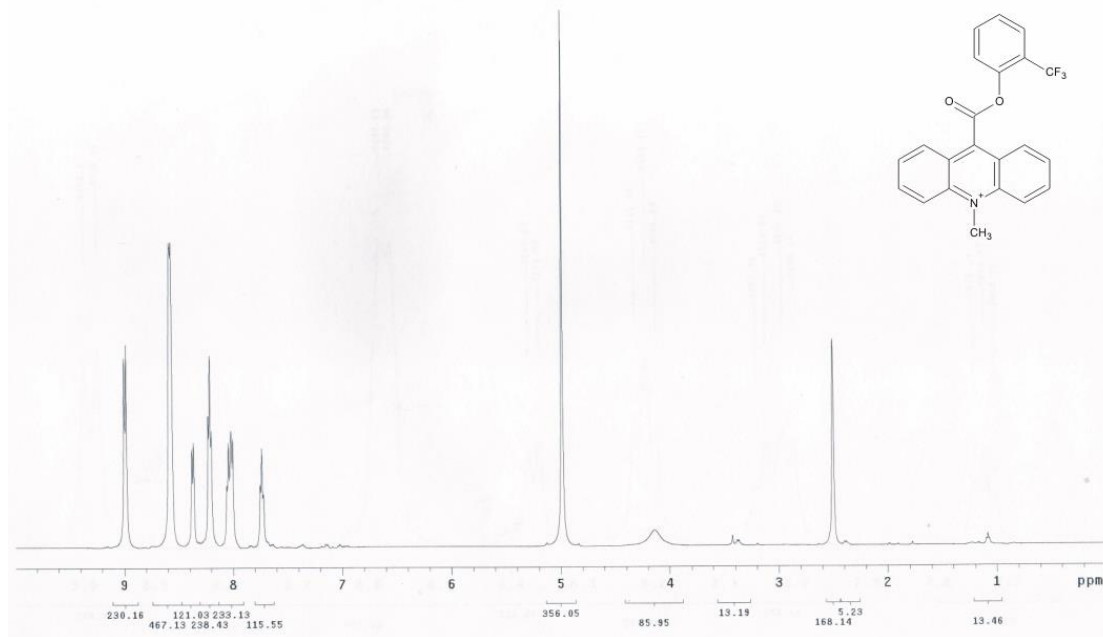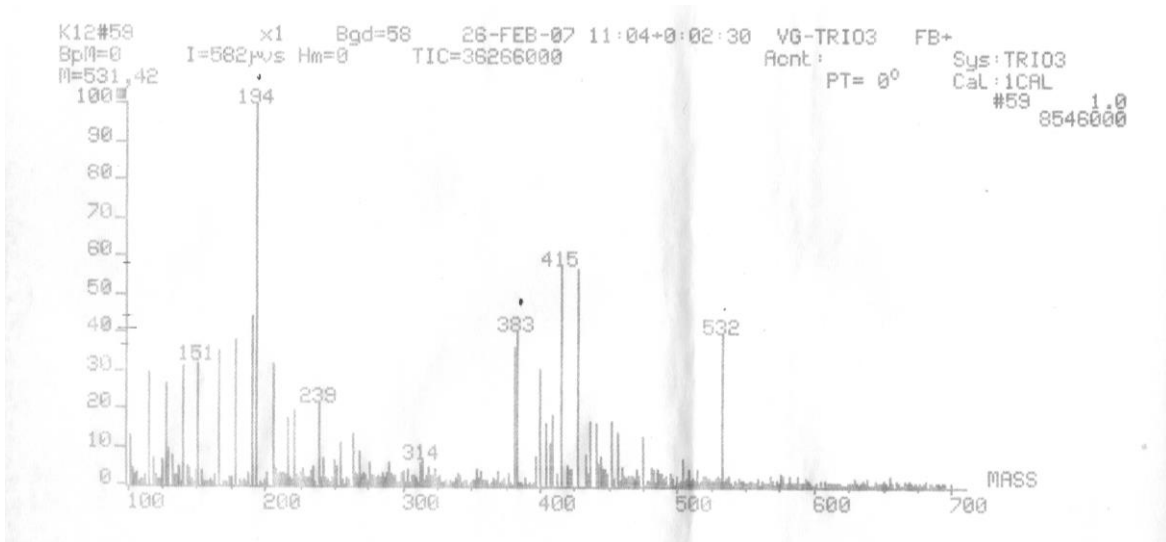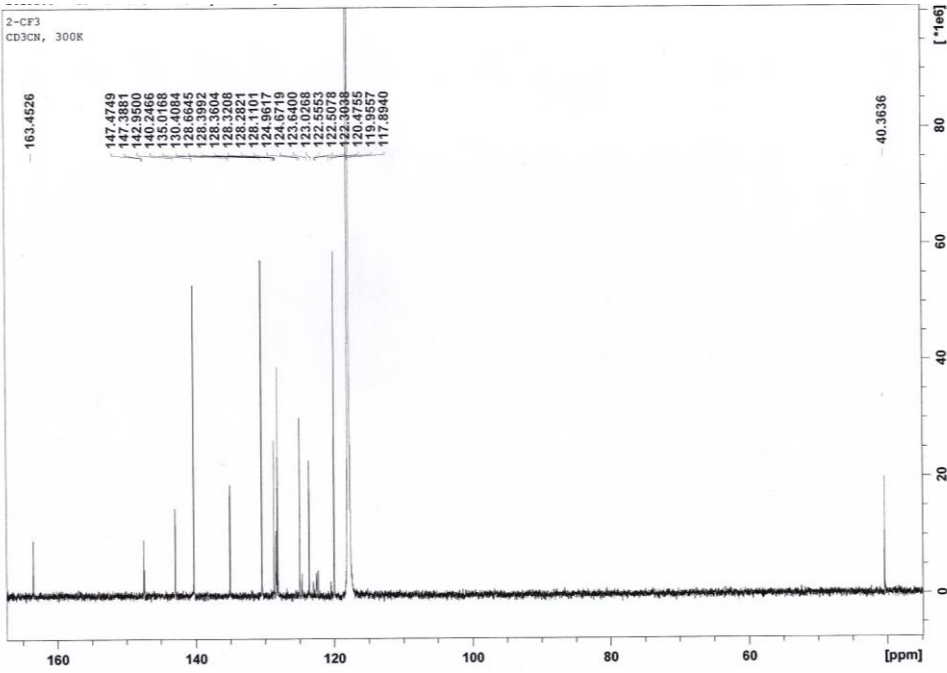2BrPh_X_9

2-Br
CD3CN, 300K

2BRPHX#12   x1   Bgd=10   5-MAY-08   11:55+0:00:45   VG-TRIO3   FB+
BpM=0   I=644μvs Hm=0   TIC=124185000   Acnt: Sys:TRIO3
                                                                        PT= 0°   Cal:1CAL
                                                                        394   #12   1.0
                                                                                          9467000

9-[(2-iodophenoxy)carbonyl]-10-methylacridinium (**2-I**)

2-I
CD3CN, 300K

M 151.0705
M 148.0436
M 144.6498
142.9400
140.9498
140.2659
130.9374
130.2501
130.0464
128.7979
123.9660
123.6614
119.8586
40.2914

[*1e6]

40

30

20

10

0

160   140   120   100   80   60   [ppm]

2IPHX#9   x1   Bgd=5   5-MAY-08   12:30+0:00:39   VG-TRI03   FB+
BpM=0   I=1.5mvs Hm=0   TIC=101651000   Acnt: Sys:TRI03
                                                      PT= 0°   Cal:1CAL
                                                                    #9   1.0
100■   440   22438000
90
80
70
60
50
40   193
30
20   284
10   153   353
0   MASS
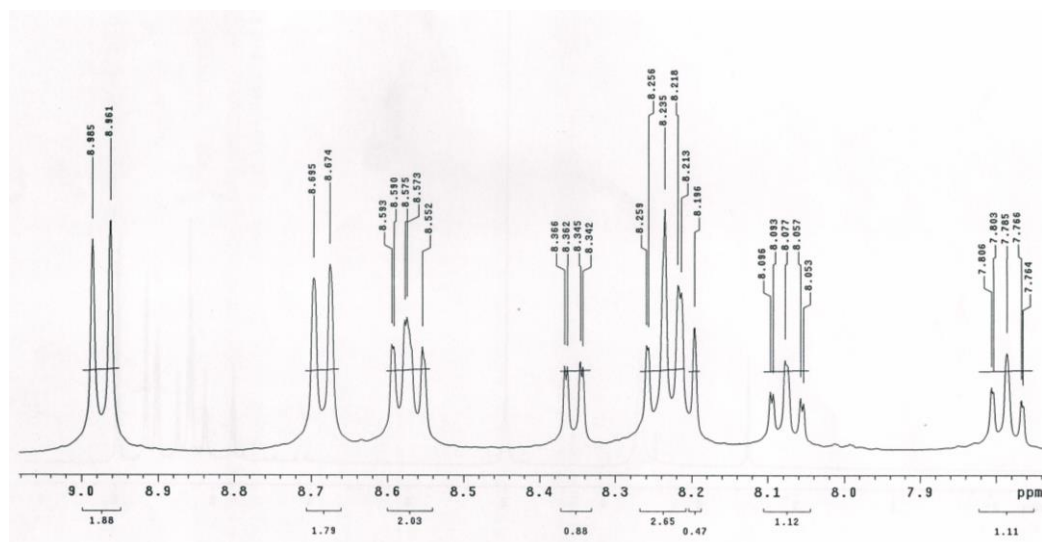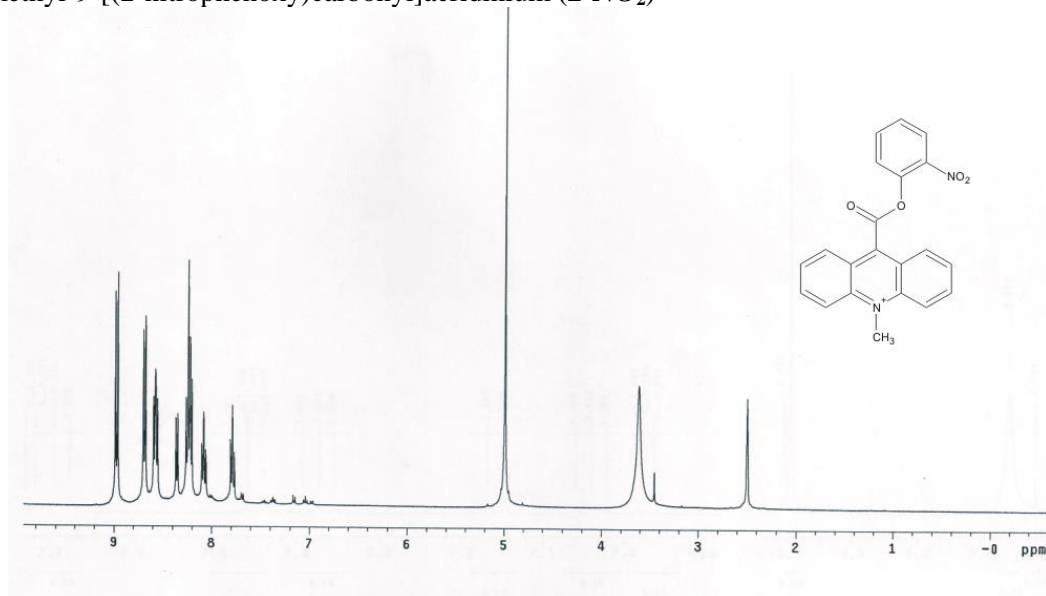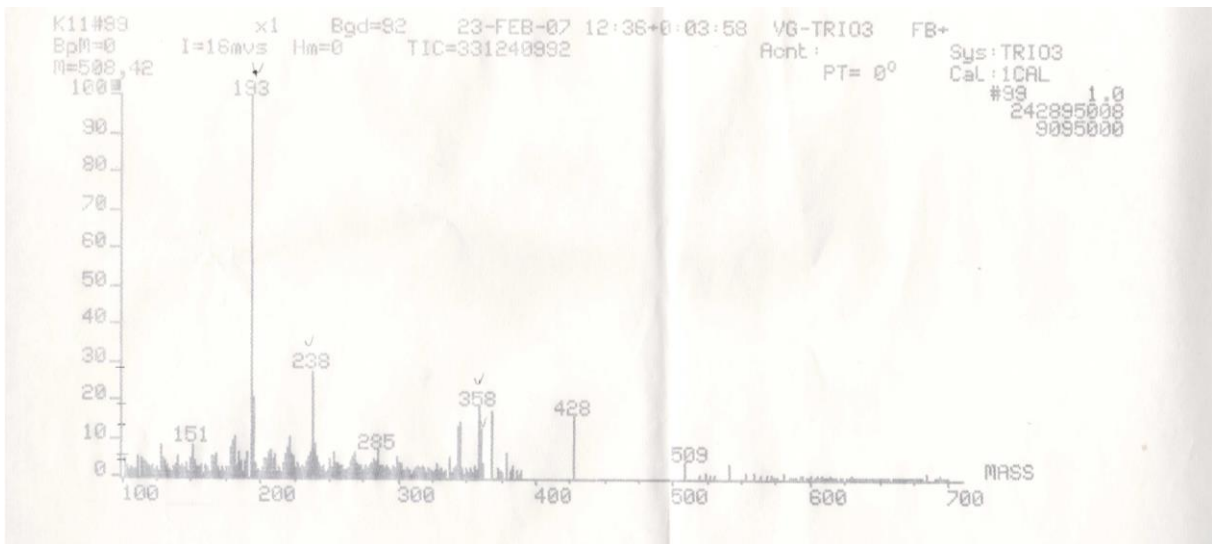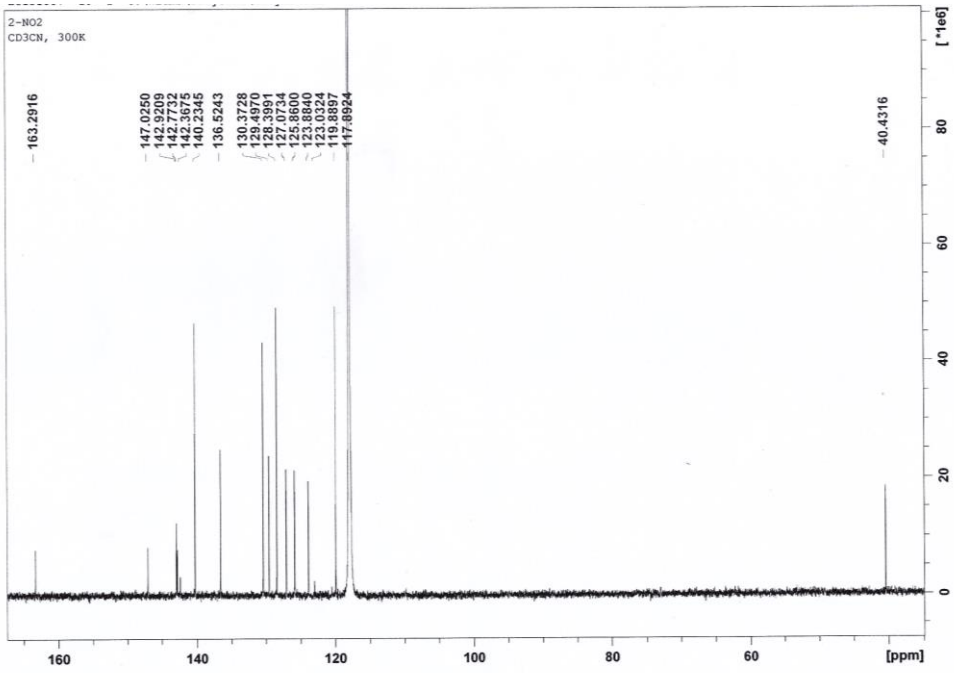   100   200   300   400   500   600   700

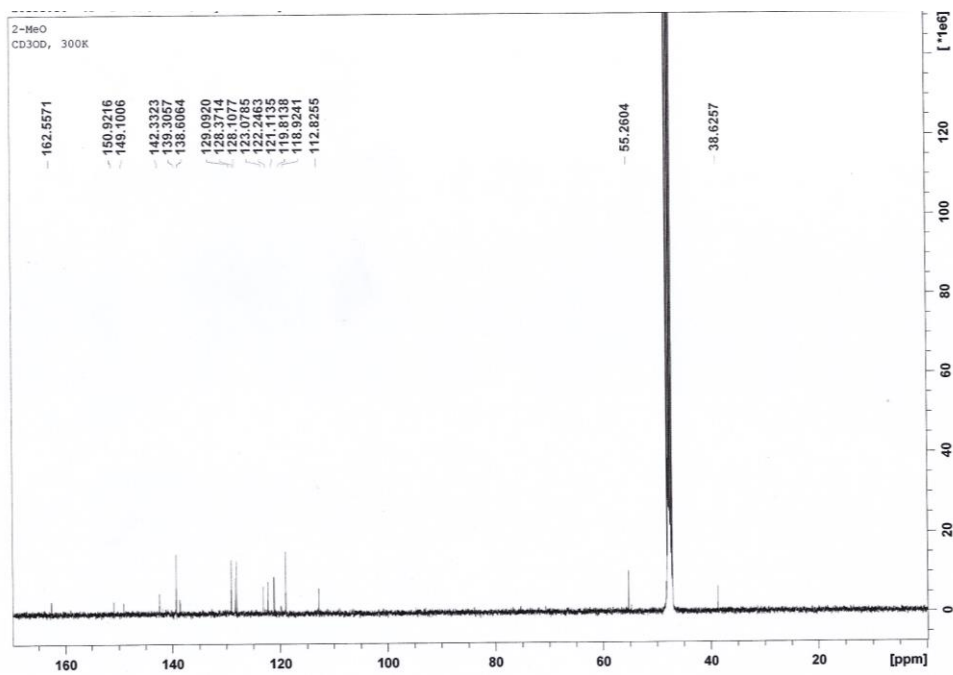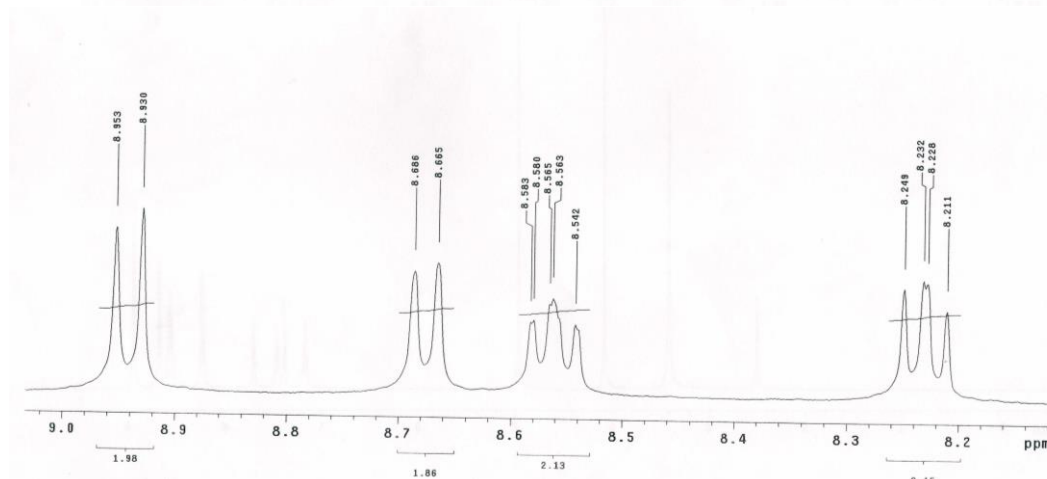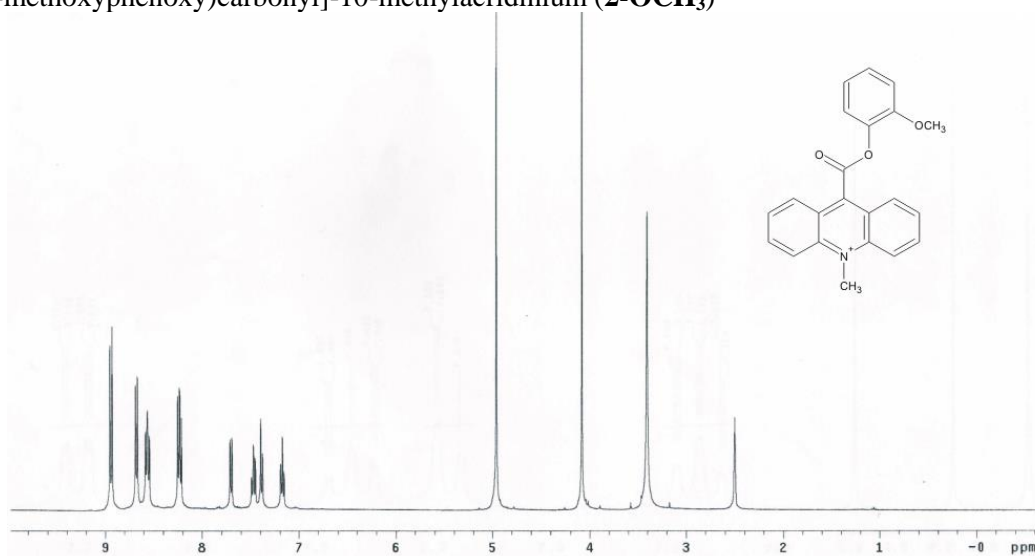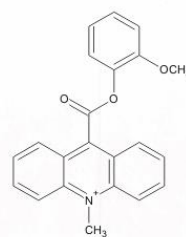10-methyl-9-{[2-(trifluoromethyl)phenoxy]carbonyl}acridinium (**2-CF₃**)

2-CF3
CD3CN, 300K

163.4526
147.4749
147.3881
142.9500
140.2466
135.0168
130.4084
128.6645
128.3992
128.3604
128.3208
128.2821
128.1101
124.9617
124.6719
123.6400
123.0268
122.5553
122.5078
122.3038
120.4755
119.9557
117.8940
40.3636

K12#59   ×1   Bgd=58   26-FEB-07 11:04+0:02:30   VG-TRIO3   FB+
BpM=0   I=582µvs Hm=0   TIC=36266000   Acnt: Sys:TRIO3
M=531,42   PT= 0°   Cal:1CAL
100   194   #59   1.0
90   8546000
80
70
60   415
50
40   383   532
151
30
20   238
10   314
0   MASS
      100   200   300   400   500   600   700
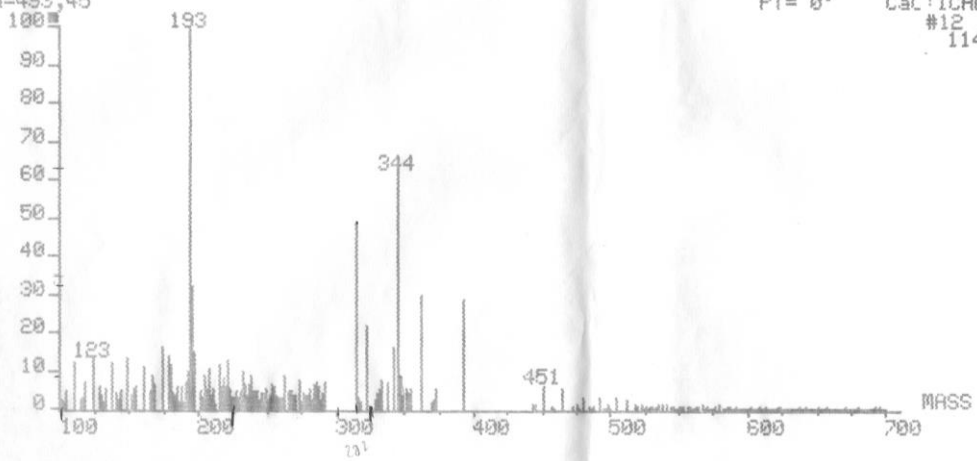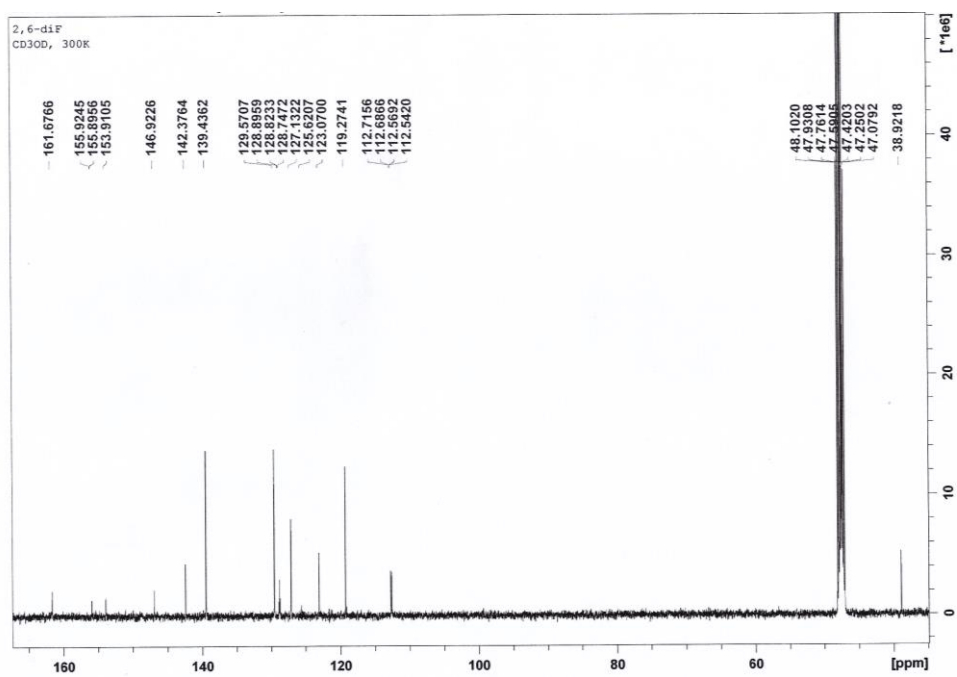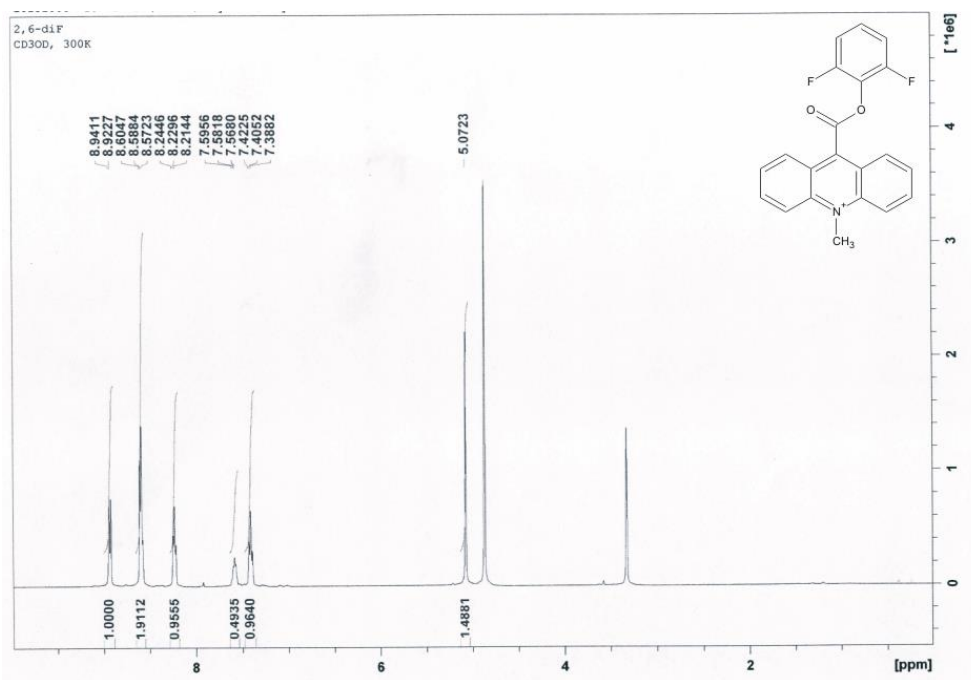
10-methyl-9-[(2-nitrophenoxy)carbonyl]acridinium (**2-NO₂**)

2-NO2
CD3CN, 300K

163.2916
147.0250
142.9209
142.7732
142.3675
140.2345
136.5243
130.3728
129.4970
128.3991
127.0734
125.8600
123.8840
123.0324
119.8897
117.8924
40.4316

160   140   120   100   80   60   [ppm]

K11#99   x1   Bgd=92   23-FEB-07 12:36+0:03:58 VG-TRIO3   FB+
BpM=0   I=16mvs Hm=0   TIC=331240992   Aont: Sys:TRIO3
M=508,42   PT= 0°   Cal:1CAL
100   193   #99   1.0
                                                                242895008
90   9095000
80
70
60
50
40
30   238
20   358
                                        428
10   151   285
0   509   MASS
  100   200   300   400   500   600   700

9-[(2-methoxyphenoxy)carbonyl]-10-methylacridinium (**2-OCH₃**)

9-[(2,6-difluorophenoxy)carbonyl]-10-methylacridinium trifluoromethanesulfonate (**2,6-diF**)
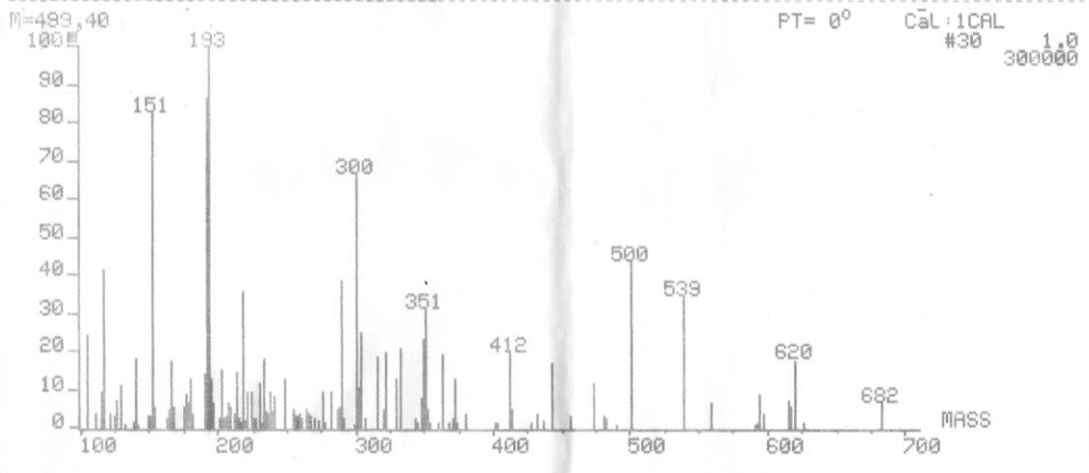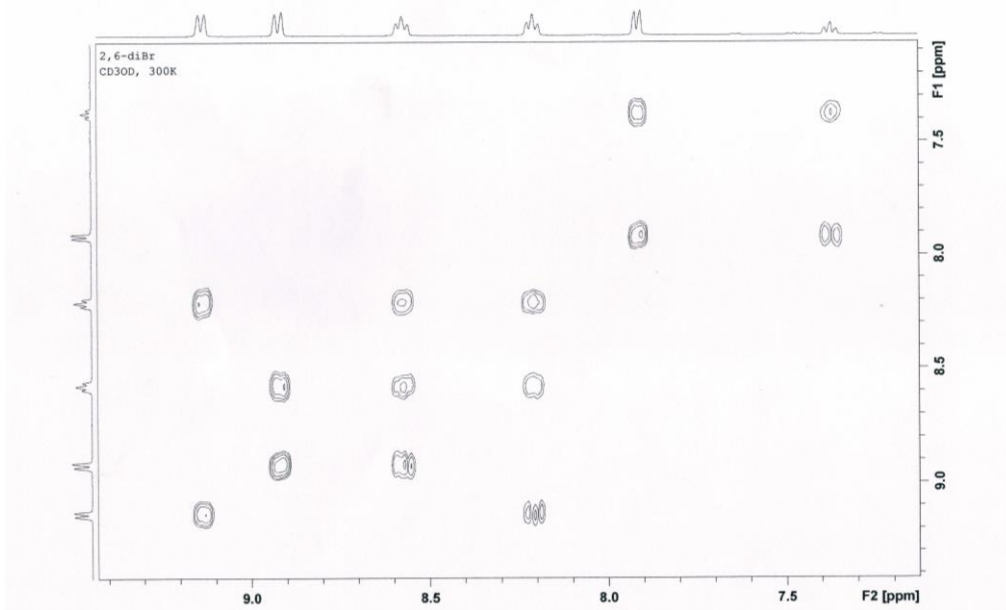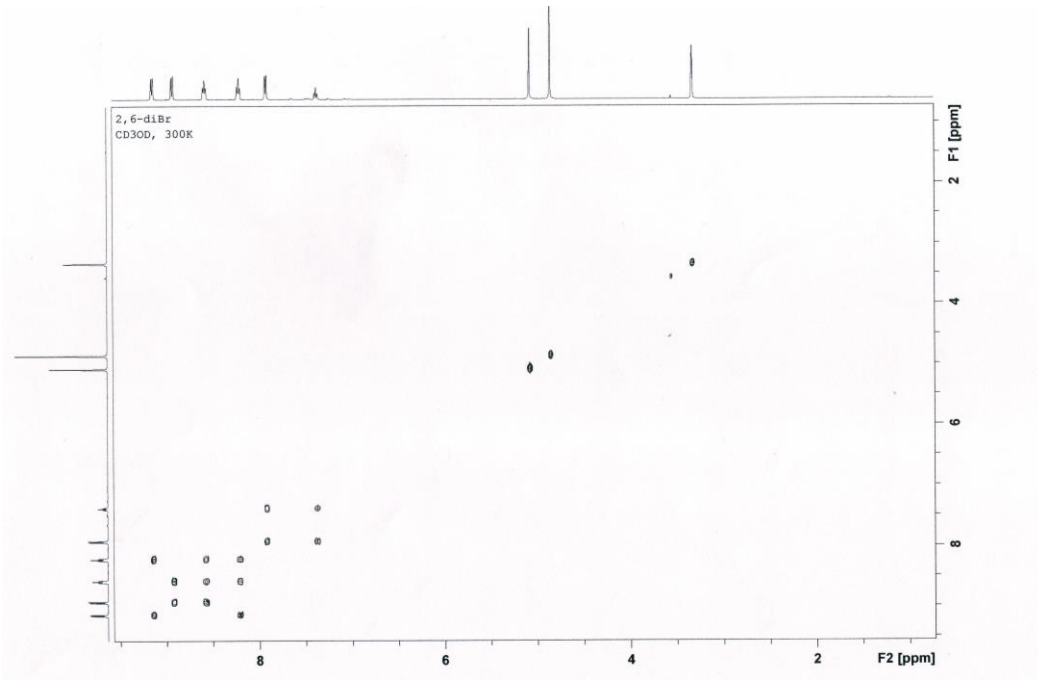
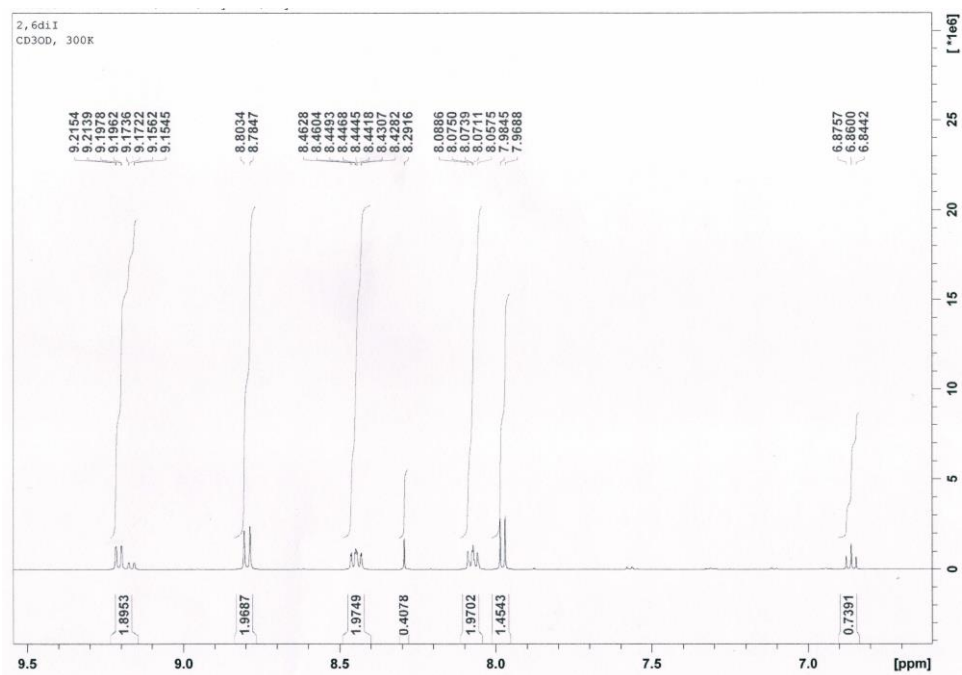9-[(2,6-dichlorophenoxy)carbonyl]-10-methylacridinium trifluoromethanesulfonate (**2,6-diCl**)
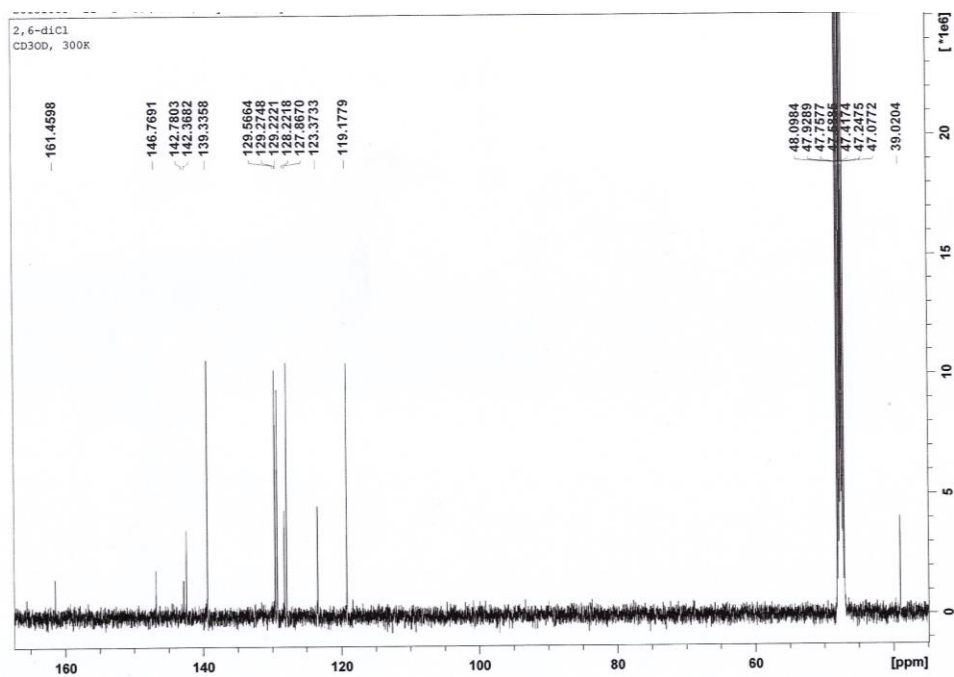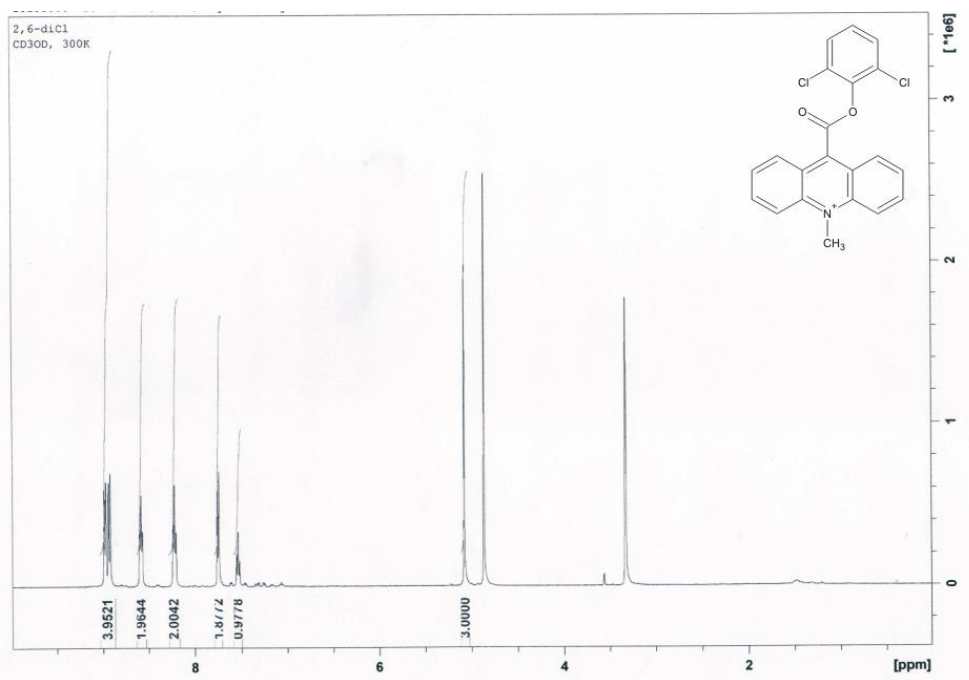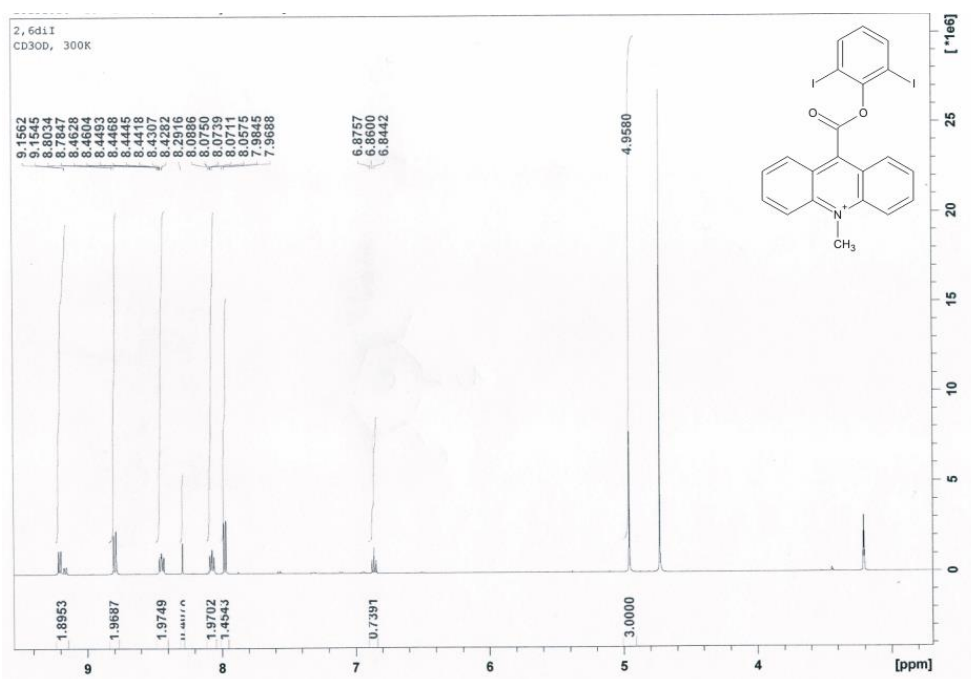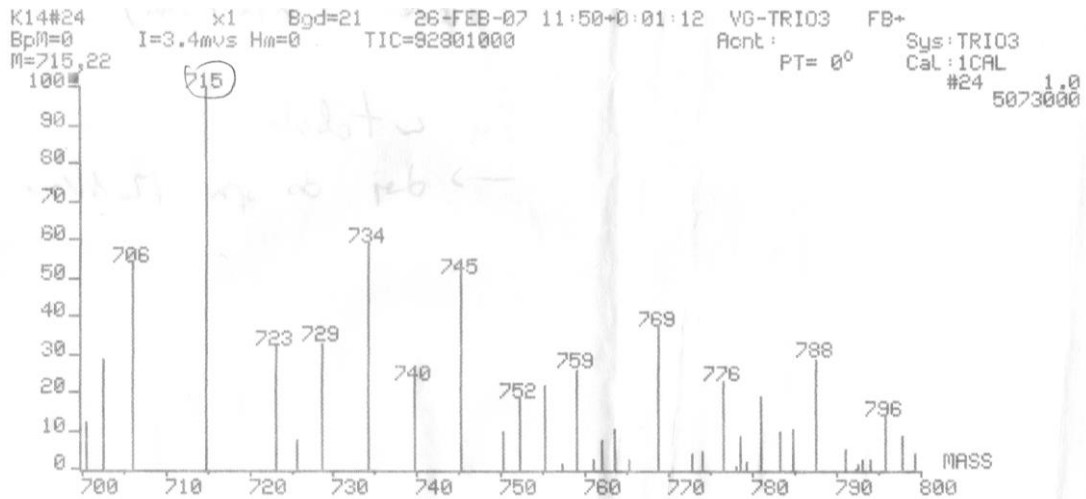
9-[(2,6-dibromophenoxy)carbonyl]-10-methylacridinium trifluoromethanesulfonate (**2,6-diBr**)

2,6-diBr
CD3OD, 300K

9.1438
9.1264
8.9283
8.9098
8.5889
8.5740
8.5570
8.2226
8.2081
8.1917
7.9211
7.9050
7.3874
7.3713
7.3551

5.0734

1.0000
1.0334
1.0279
1.0282
1.0303
0.5178
1.5705

2,6-diBr
CD3OD, 300K

161.3393

146.5215
145.4675
142.3863
139.2834
133.3679
130.0334
129.0718
128.3644
123.4642
119.1007
116.8951

48.1019
47.9305
47.7612
47.5903
47.4198
47.2502
47.0789
39.1016

2,6-diBr
CD3OD, 300K

2,6-diBr
CD3OD, 300K

2,6-diBr
CD3OD, 300K

K6#29   x1   Bgd=3   23-FEB-07 12:01+0:01:25 VG-TRIO3   FB+
BpM=0   I=272µvs Hn=0   TIC=15493000   Acnt: Sys:TRIO3
M=621.21   PT= 0°   CaL:1CAL
                                                                 #29   1.0
                                                                      251000
                                                                       25000

440
505
470
532
587
609
564
622
640
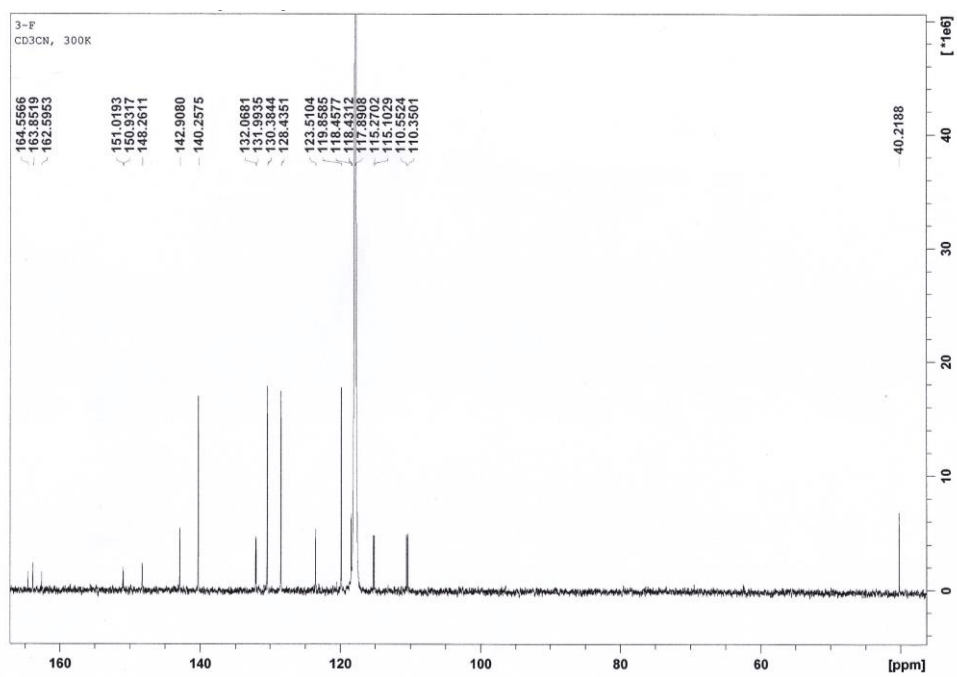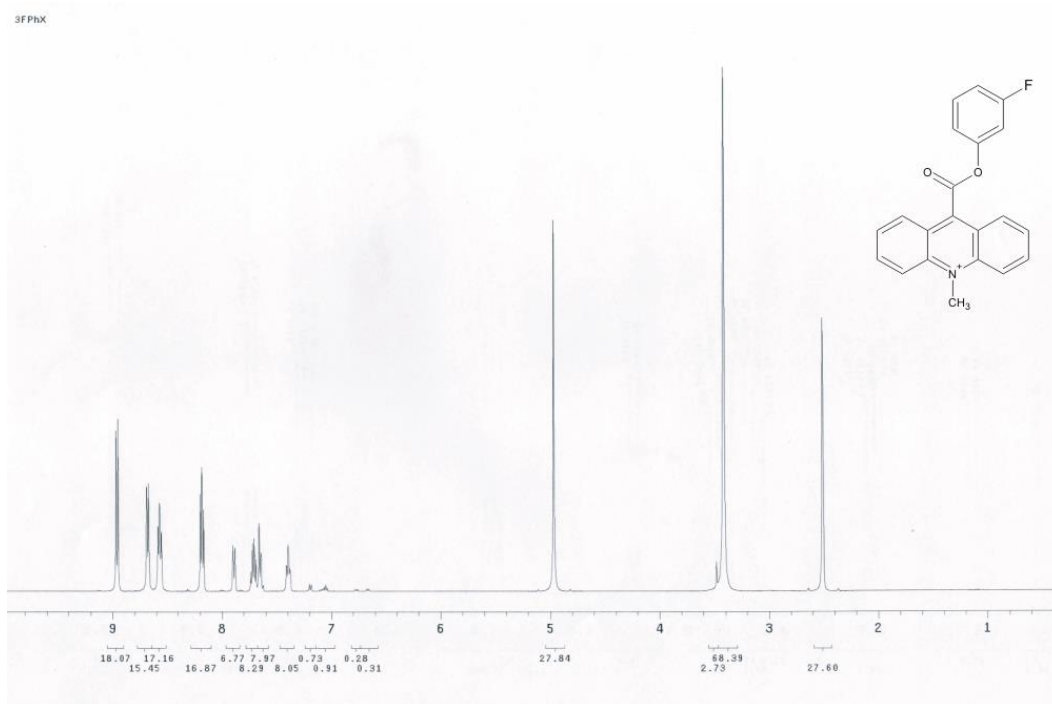MASS

9-[(2,6-diiodophenoxy)carbonyl]-10-methylacridinium (**2,6-diI**)

2,6diI
CD3OD, 300K

161.3083
161.1464
152.1555
151.8440
148.0448
146.3274
142.4460
140.5475
139.2199
139.3967
130.3967
129.2318
129.1087
128.9283
128.8556
123.5325
121.6544
119.1207
119.0737
119.0412
93.1043
91.1263
89.7319
39.2342

160   150   100   50   40   20   [ppm]

K14#24   x1   Bgd=21   26-FEB-07 11:50+0:01:12 VG-TRIO3   FB+
BpM=0   I=3.4mVs Hm=0   TIC=92801000   Acnt: Sys:TRIO3
M=715,22   PT= 0°   CaL:1CAL
100   #24   1.0
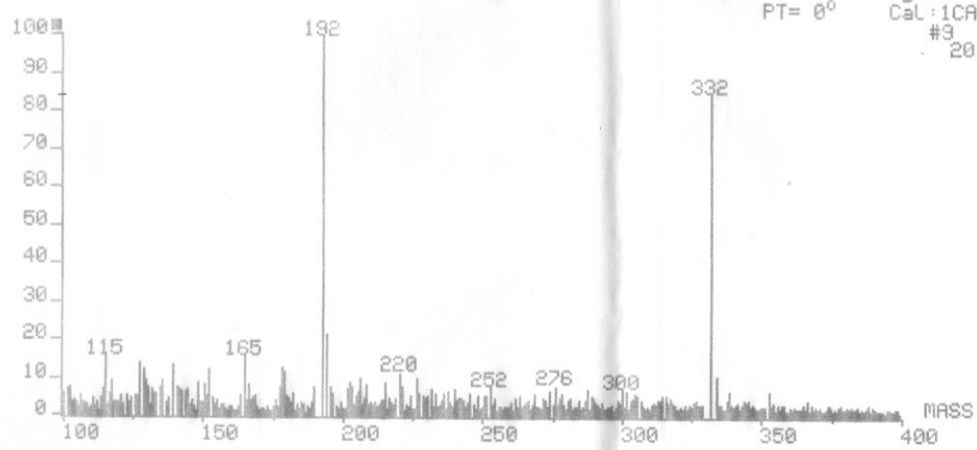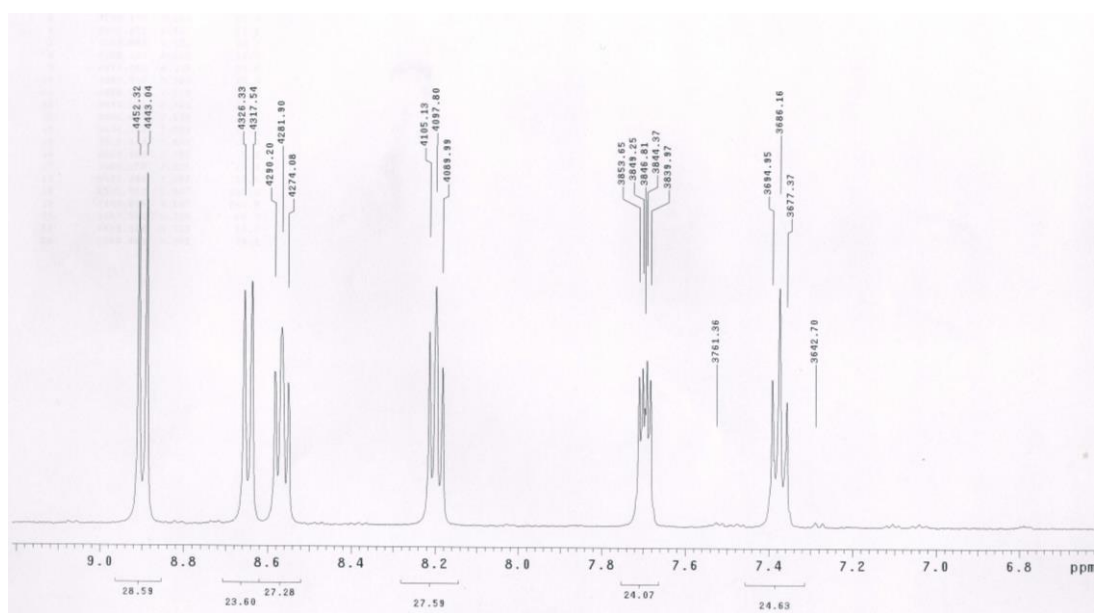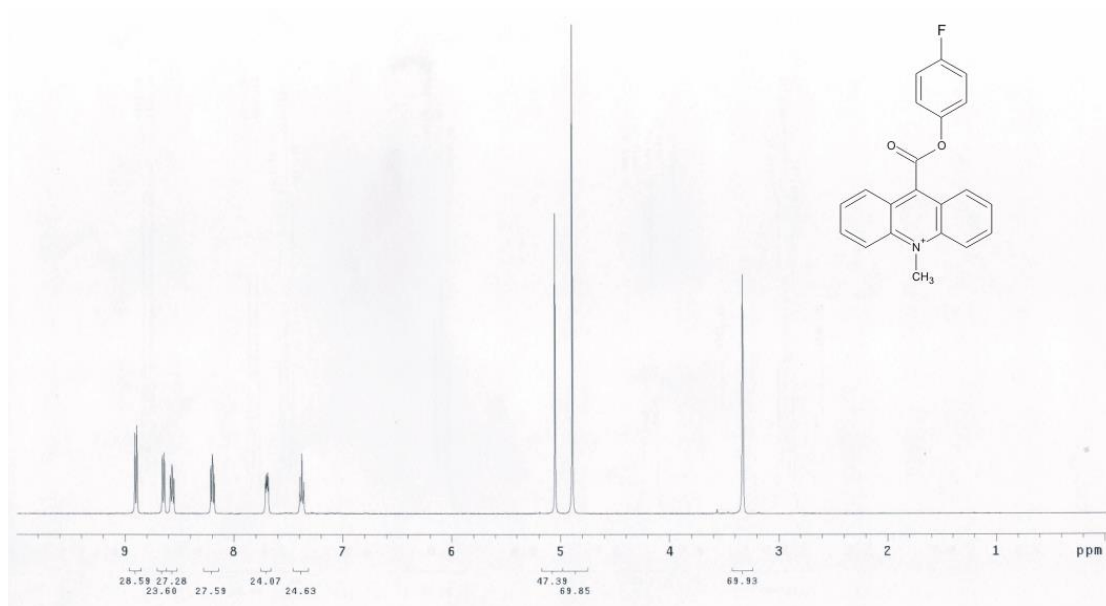   5073000

MASS

9-[(3-fluorophenoxy)carbonyl]-10-methylacridinium (**3-F**)
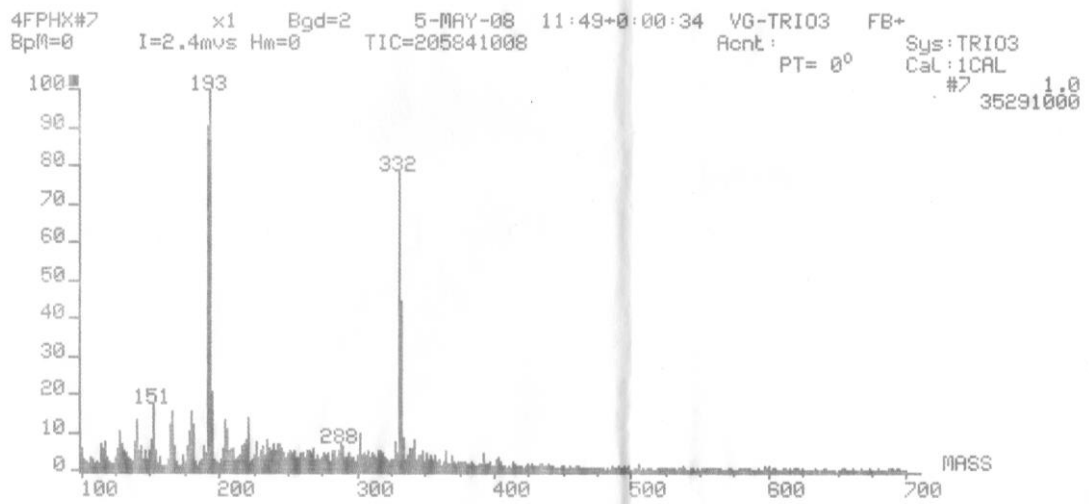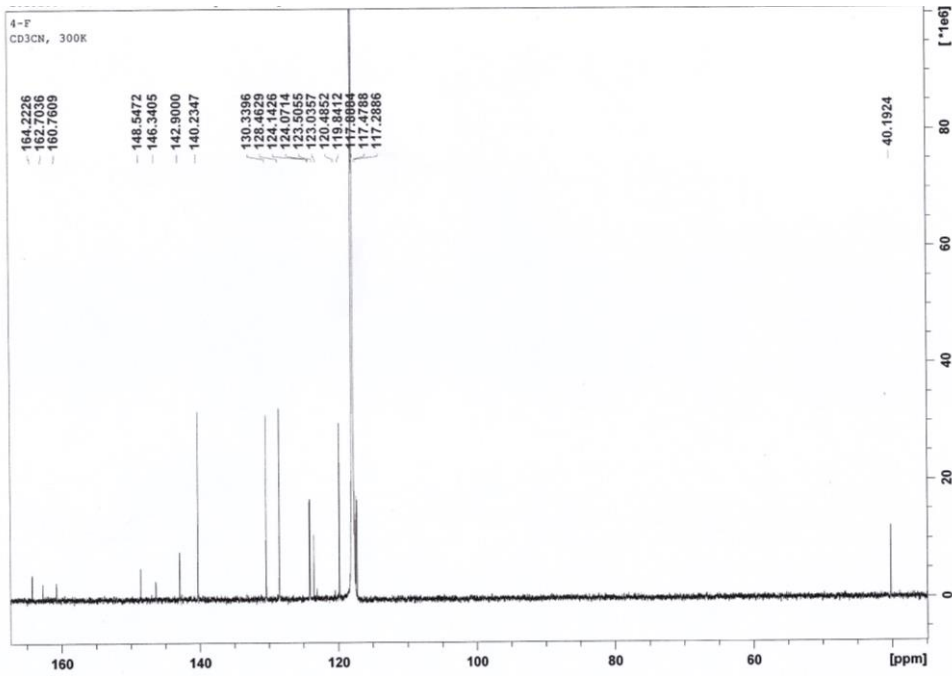
9-[(4-fluorophenoxy)carbonyl]-10-methylacridinium (**4-F**)

4-F
CD3CN, 300K

164.2226
162.7036
160.7609
148.5472
146.3405
142.9000
140.2347
130.3396
128.4629
124.1426
124.0714
123.5055
123.0357
120.4852
119.8412
117.8004
117.4788
117.2886
40.1924

[*1e6]

160   140   120   100   80   60   [ppm]

4FPHX#7   x1   Bgd=2   5-MAY-08 11:49+0:00:34 VG-TRIO3   FB+
BpM=0   I=2.4mvs Hm=0   TIC=205841008   Acnt: Sys:TRIO3
                                                       PT= 0°   Cal:1CAL
                                                                    #7   1.0
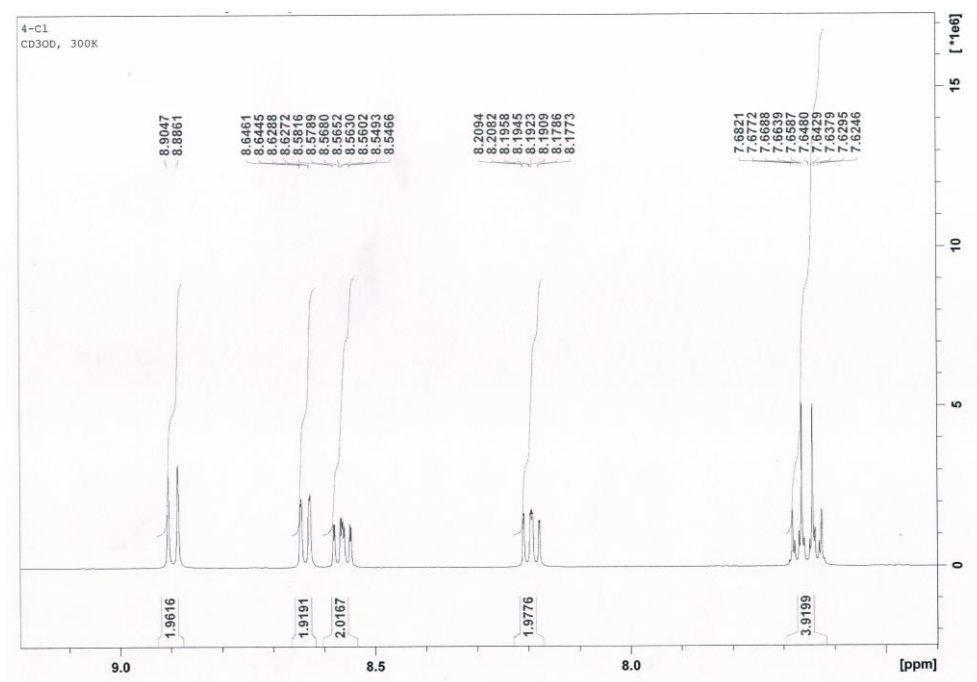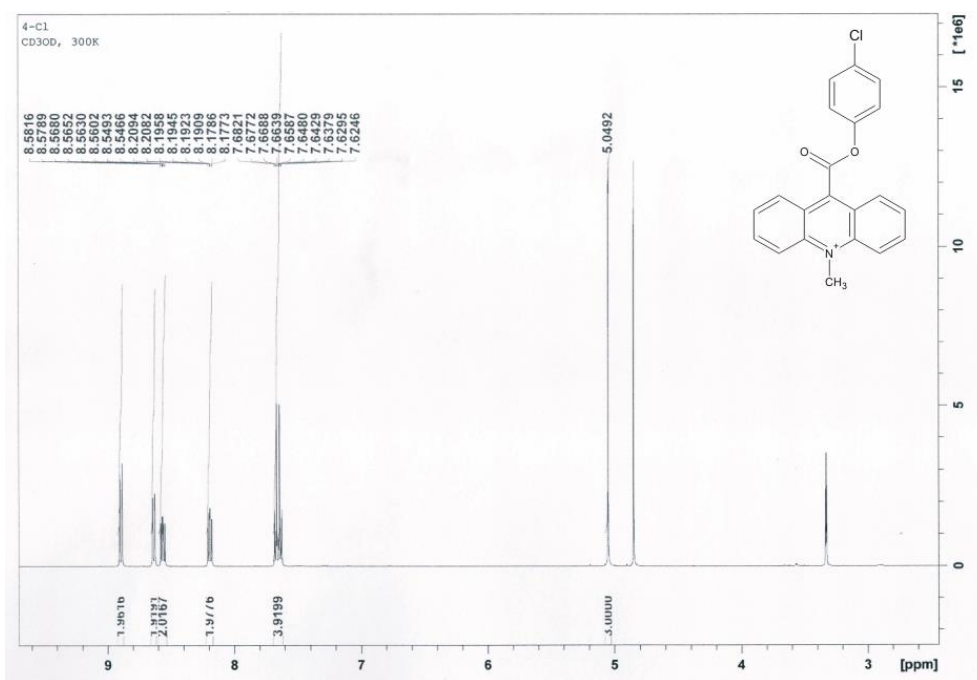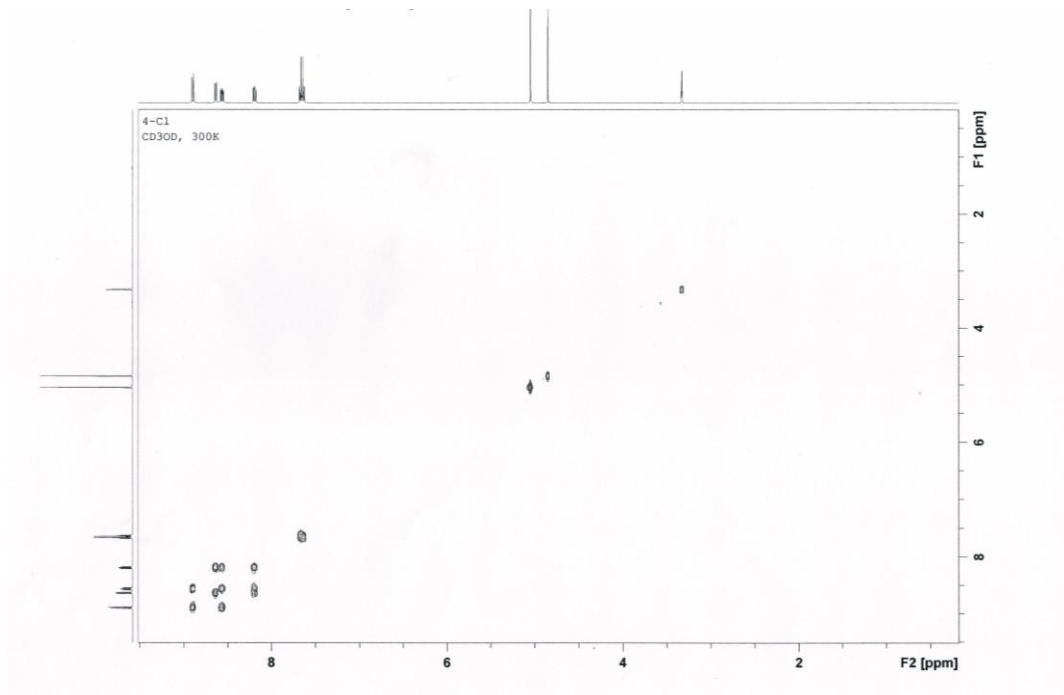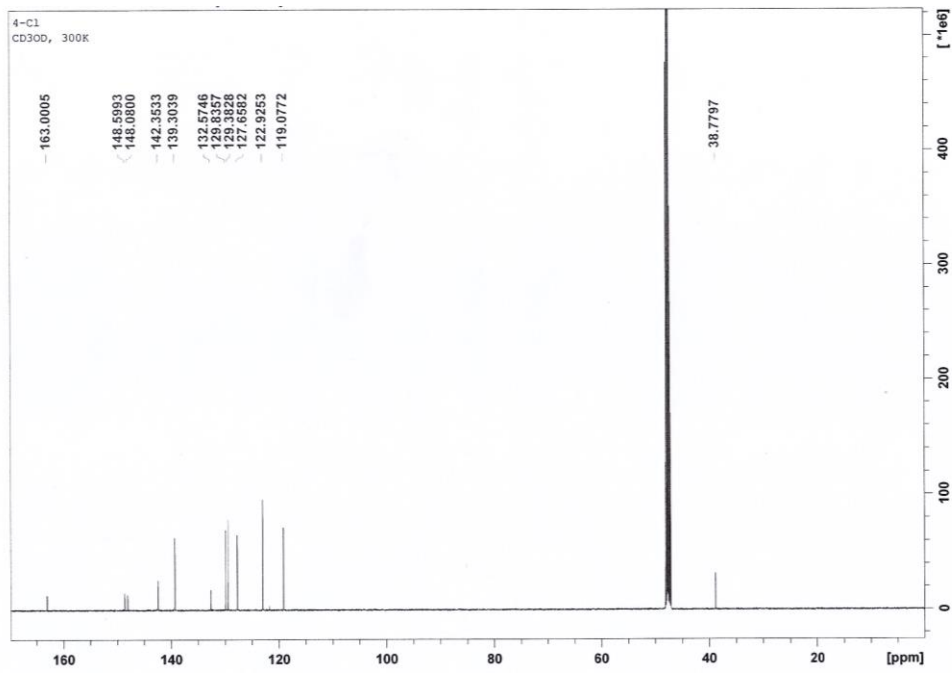   35291000

100
90
80   193
70
60   332
50
40
30
20   151
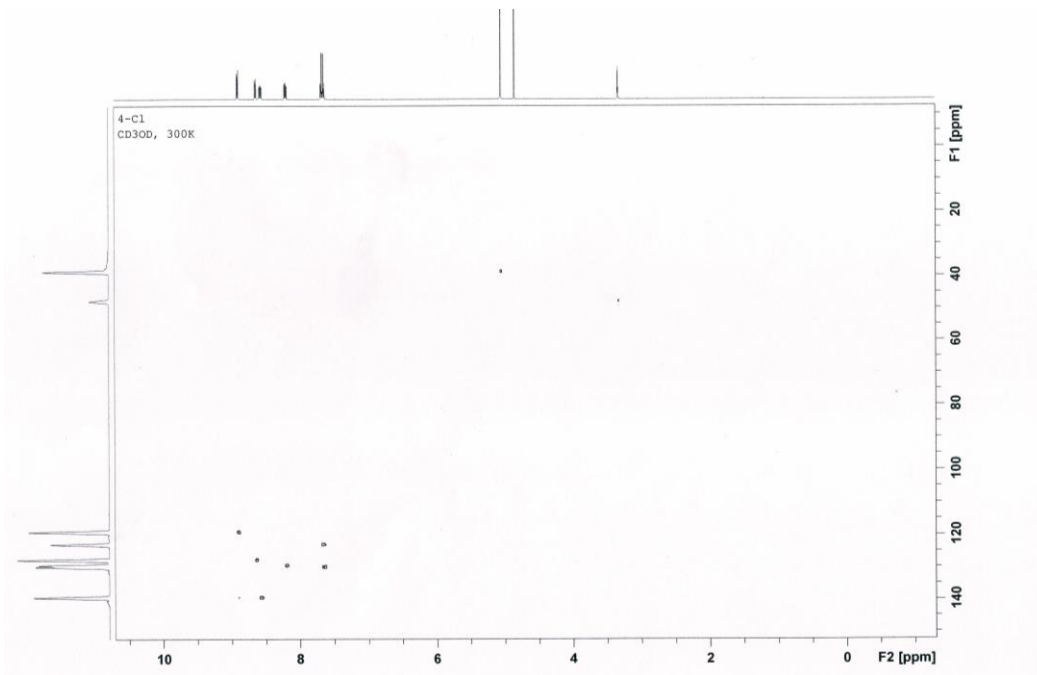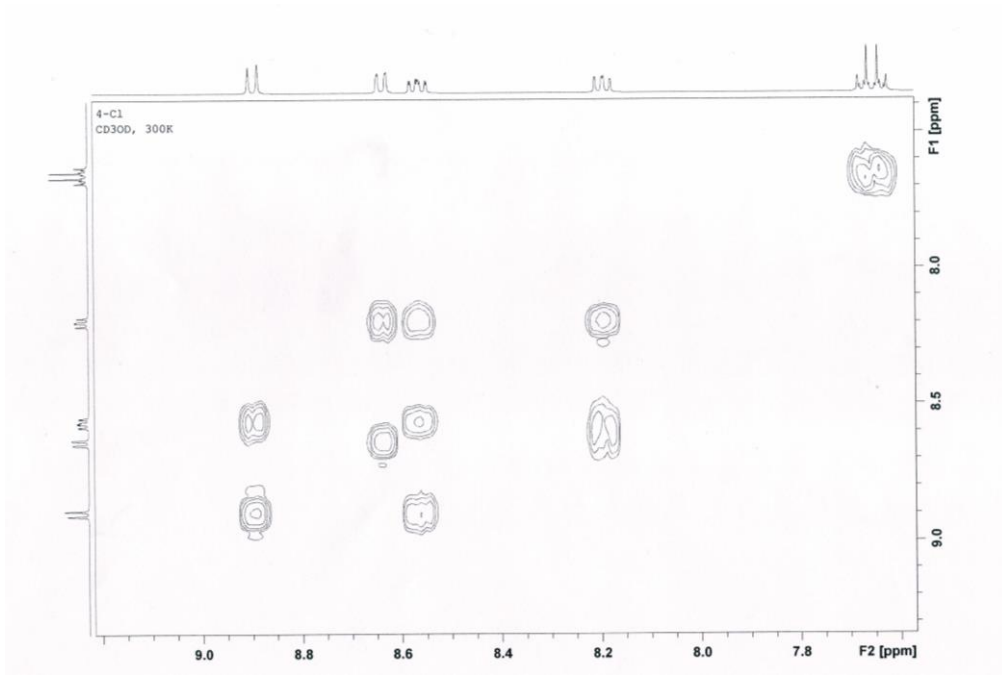10   288
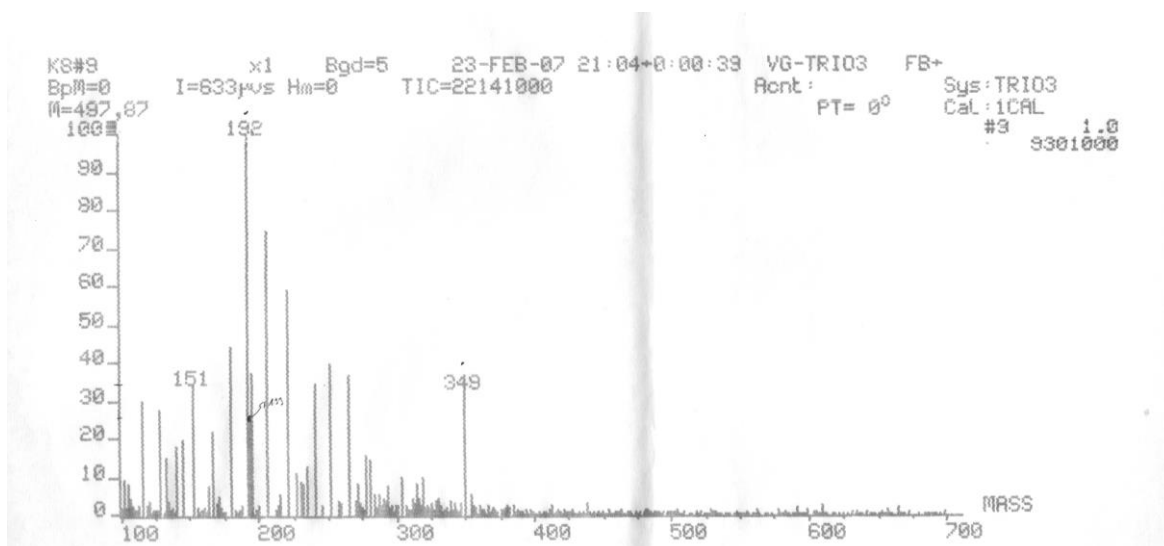0
    100   200   300   400   500   600   700   MASS

9-[(4-chlorophenoxy)carbonyl]-10-methylacridinium (**4-Cl**)

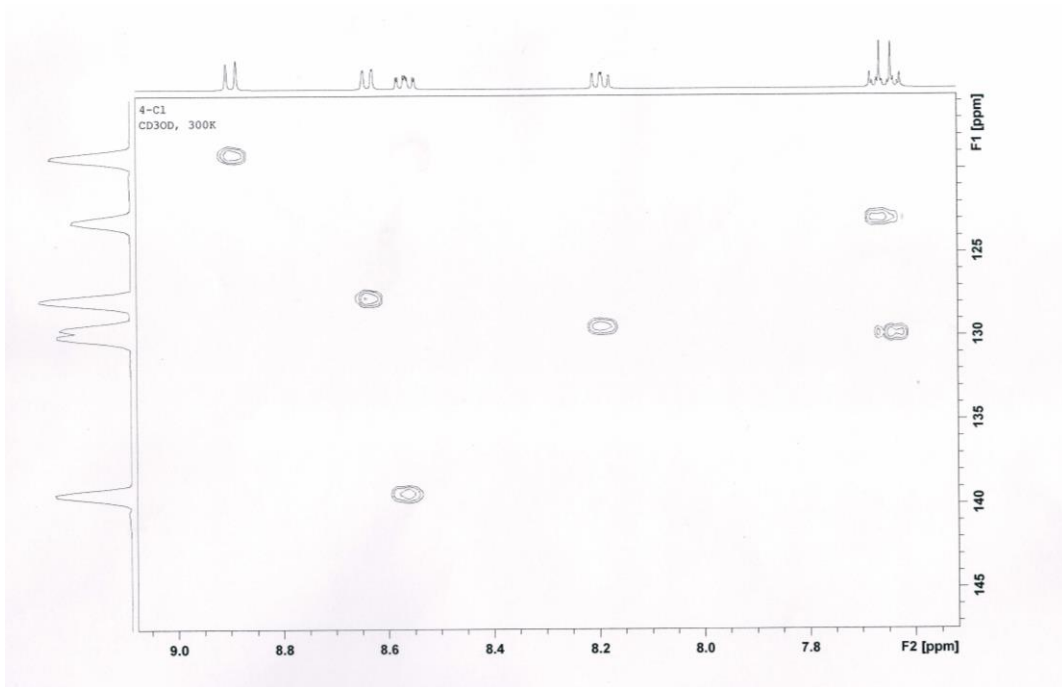4-Cl
CD3OD, 300K

163.0005
148.5993
148.0800
142.3533
139.3039
132.5746
129.8357
129.3828
127.6582
122.9253
119.0772
38.7797

4-Cl
CD3OD, 300K

4-C1
CD3OD, 300K

4-C1
CD3OD, 300K

4-Cl
CD3OD, 300K

K8#9   x1   Bgd=5   23-FEB-07 21:04+0:00:39   VG-TRIO3   FB+
BpM=0   I=633μvs Hm=0   TIC=22141000   Acnt: Sys:TRIO3
M=487,87   PT= 0⁰   CaL:1CAL
100   #3   1.0
                                                                            9301000
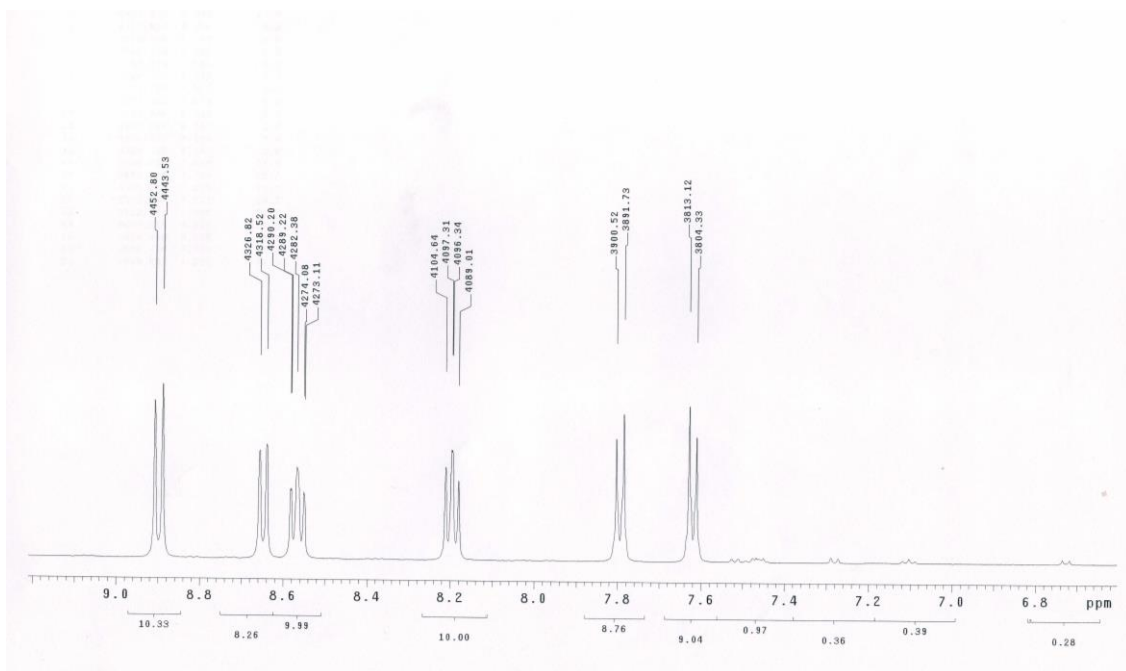
192

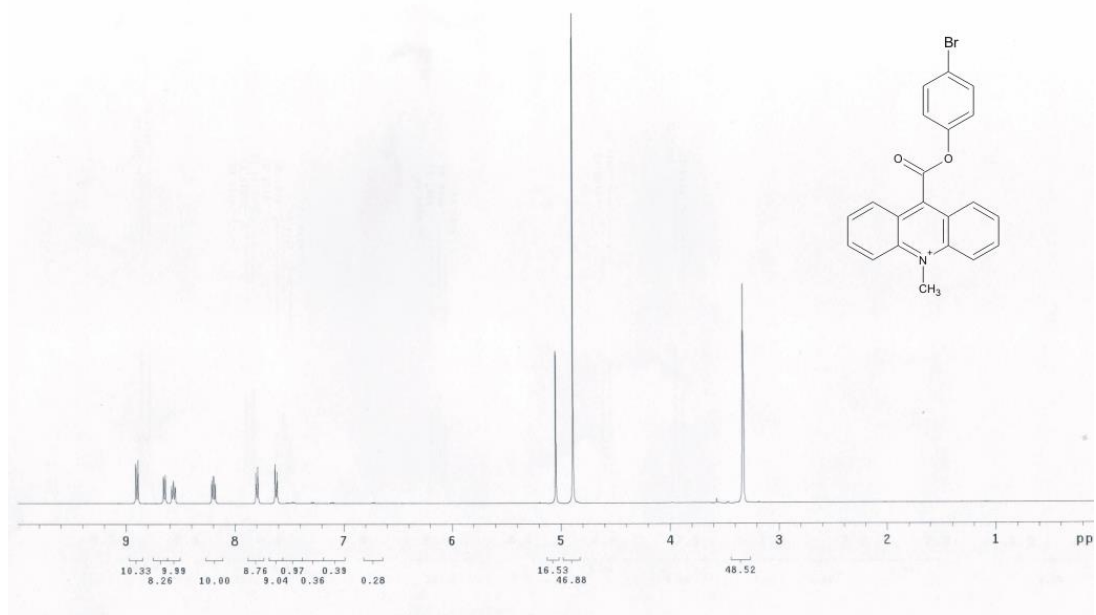151   349

MASS

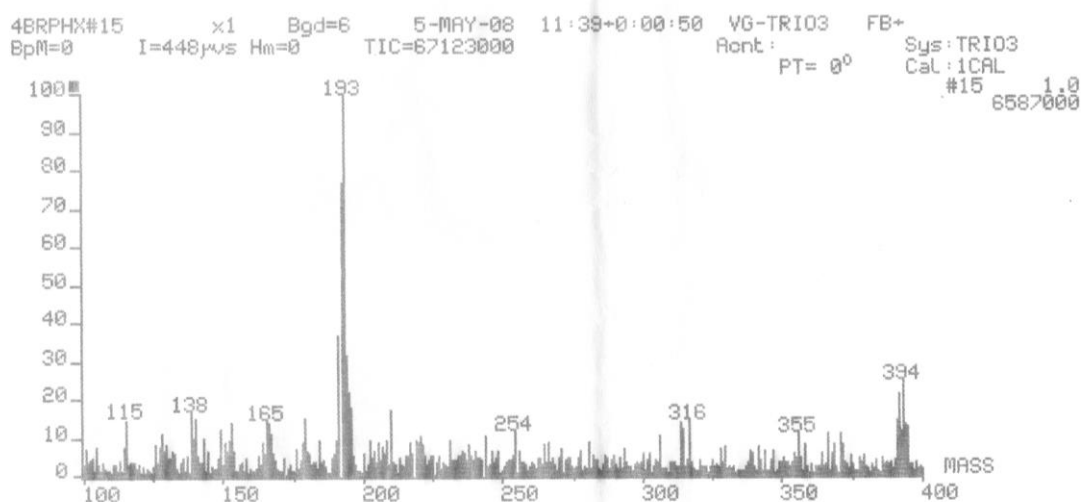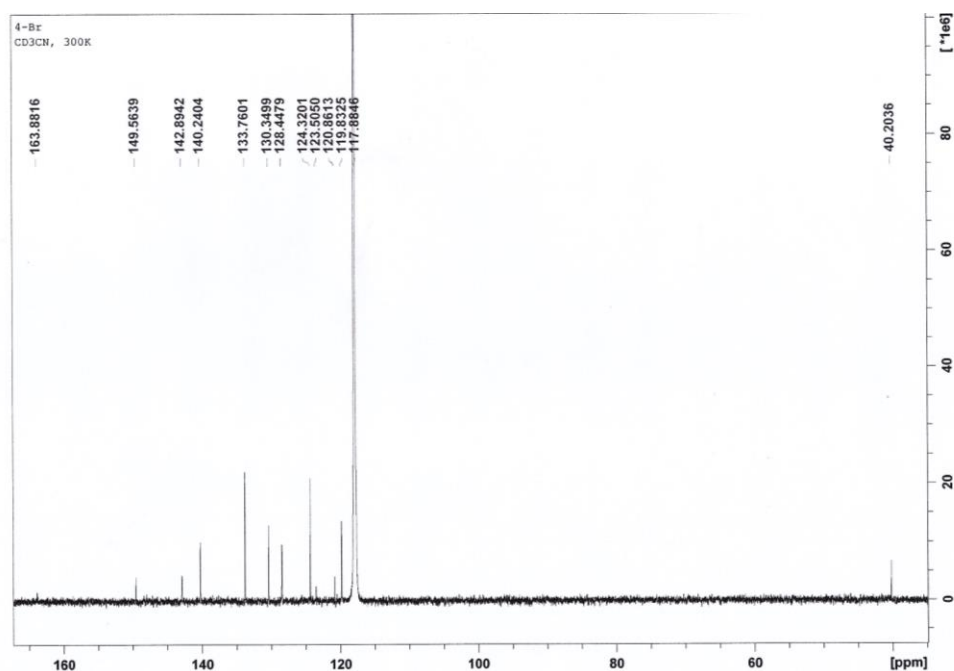9-[(4-bromophenoxy)carbonyl]-10-methylacridinium (**4-Br**)

Fig. S2 ^{1}H NMR, ^{13}C NMR, [1]H−^{1}H COSY and [1]H−^{13}C NMR HETCOR (HSQC) spectra (500 MHz, 100 MHz, respectively, room temperature (~300 K)) and MALDI-TOF MS spectra of XAE's. The aromatic region is enlarged above the general view of spectrum. For ^{1}H NMR and MALDI-TOF MS spectra see also Table S1†; for ^{13}C NMR spectra see also Table S2†. Details of experiments are described in Experimental section.
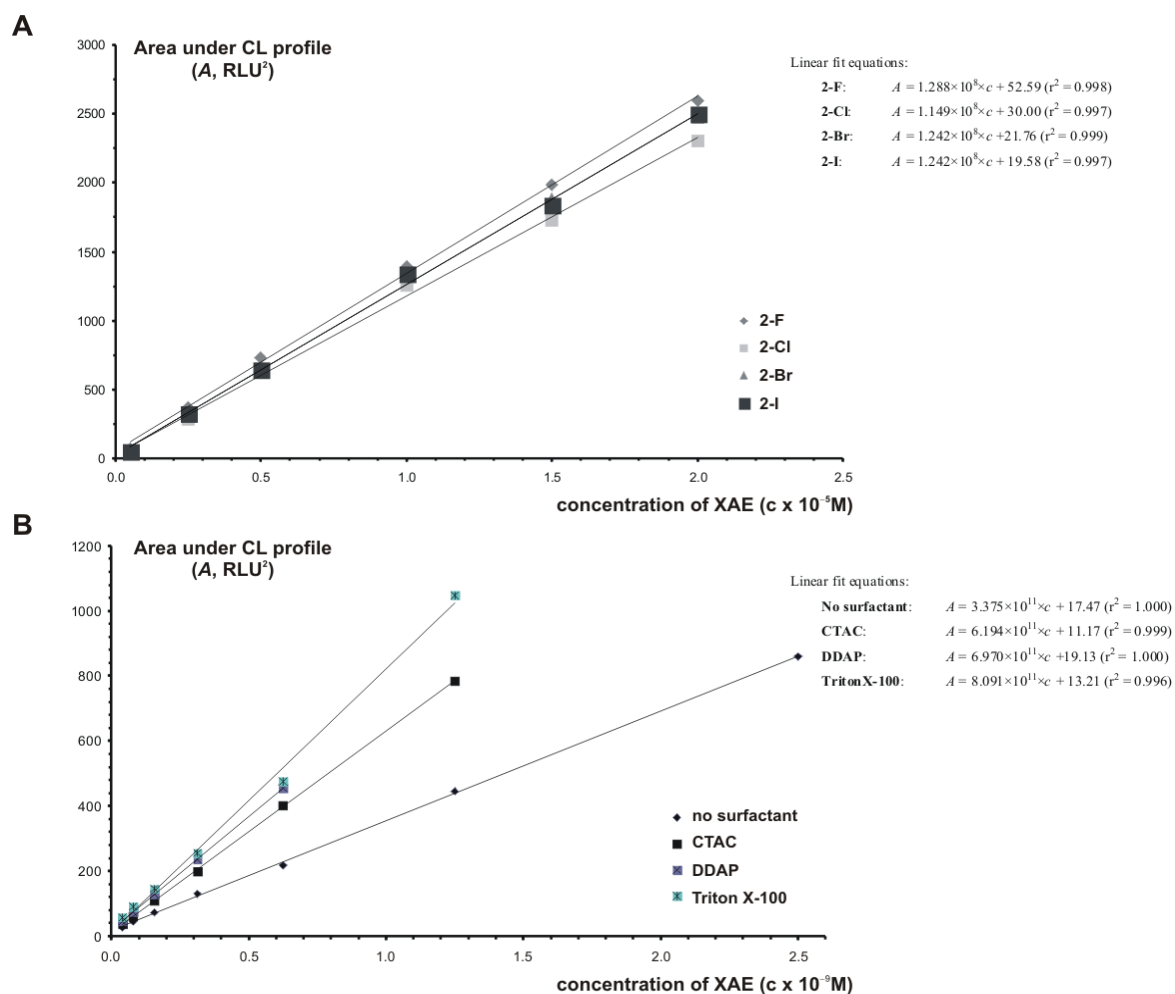
A

Linear fit equations:

2-F:	$A = 1.288 \times 10^8 \times c + 52.59$ ($r^2 = 0.998$)
2-Cl:	$A = 1.149 \times 10^8 \times c + 30.00$ ($r^2 = 0.997$)
2-Br:	$A = 1.242 \times 10^8 \times c + 21.76$ ($r^2 = 0.999$)
2-I:	$A = 1.242 \times 10^8 \times c + 19.58$ ($r^2 = 0.997$)

B

Linear fit equations:

No surfactant:	$A = 3.375 \times 10^{11} \times c + 17.47$ ($r^2 = 1.000$)
CTAC:	$A = 6.194 \times 10^{11} \times c + 11.17$ ($r^2 = 0.999$)
DDAP:	$A = 6.970 \times 10^{11} \times c + 19.13$ ($r^2 = 1.000$)
Triton X-100:	$A = 8.091 \times 10^{11} \times c + 13.21$ ($r^2 = 0.996$)

Fig. S3 Calibration graphs for exemplary XAEs (Scheme 1) used for estimating relative CL efficiencies (*RCLE*) summarized in Table S3† (graph A) and Δ*RCLE*, *LOD* and *LOQ* values given in Table 3 (graph B). *RCLE*s were calculated as the slopes of the above relationships ($R^2 > 0.99$ in all cases) and normalized to the highest value. For details see Experimental section.
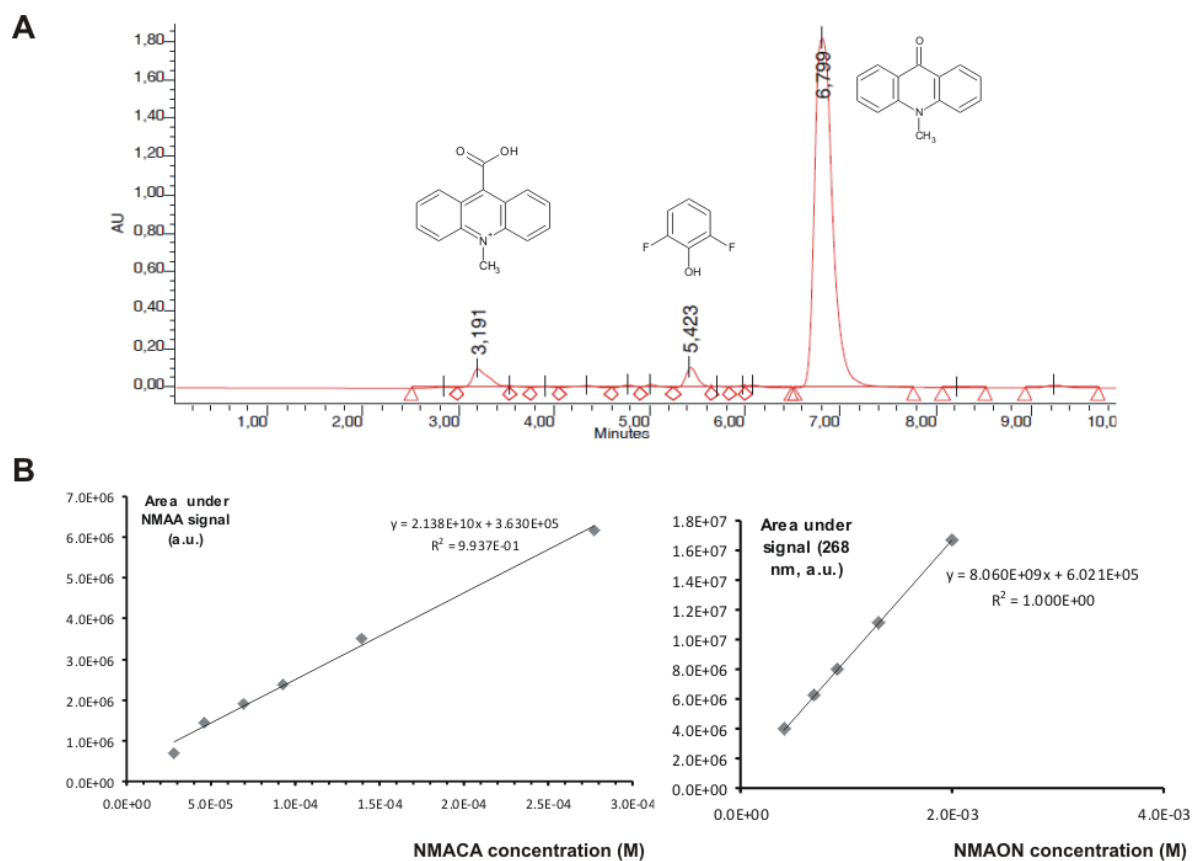
Fig. S4 Graph A: exemplary RP-HPLC analysis for post-CL reaction mixture obtained with the participation of **2,6-diF** (Scheme 1) and standard triggering reagents (see note 1 under the Table S3†). Graph B: calibration chart for 10-methylacridinium-9-carboxylic acid (NMACA, left) and 10-methyl-9-acridinone (NMAON, right). For details see Experimental section.
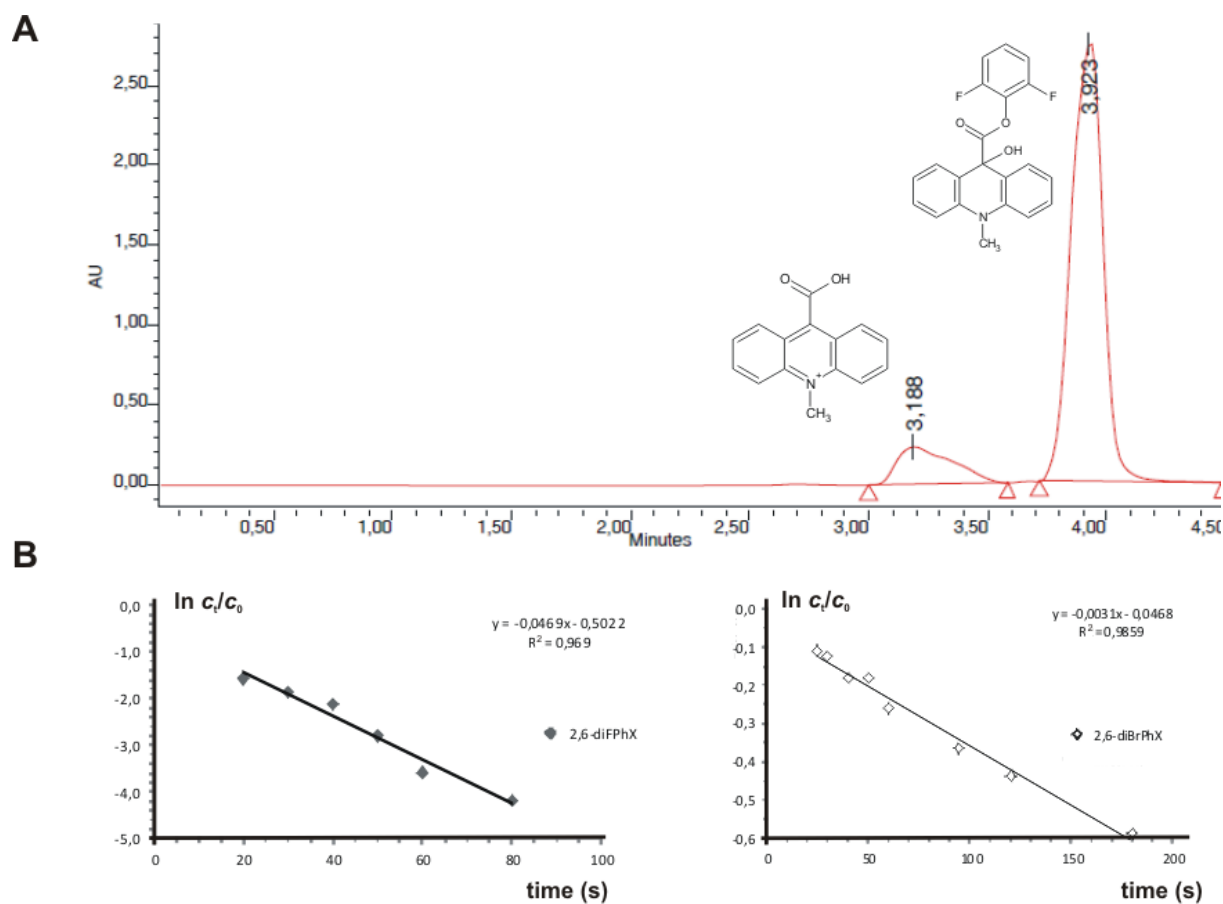
Fig. S5 Graph A: hydrolysis of **2,6-diF** in an alkaline environment (pH = 12.0) monitored chromatographically (RP-HPLC). Graph B: Linear charts used for estimation of the pseudo-first order hydrolysis constants (k_{OH}, Table 4) for exemplary XAEs (Scheme 1): **2,6-diF** (left) and **2,6-diBr** (right). For details see Experimental section.