Near Infrared Emitting Molecular Rotor Based on Merocyanine for Probing the Viscosity of Cellular Lipid Environments

Supporting Information

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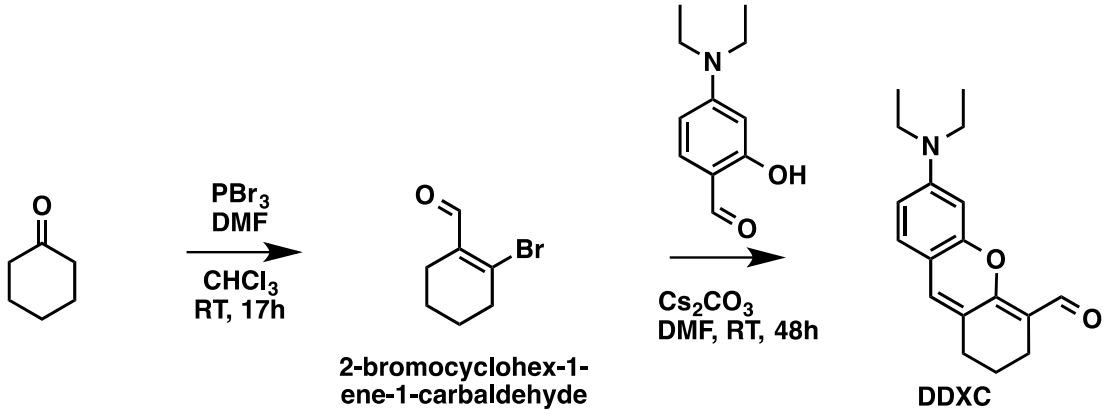
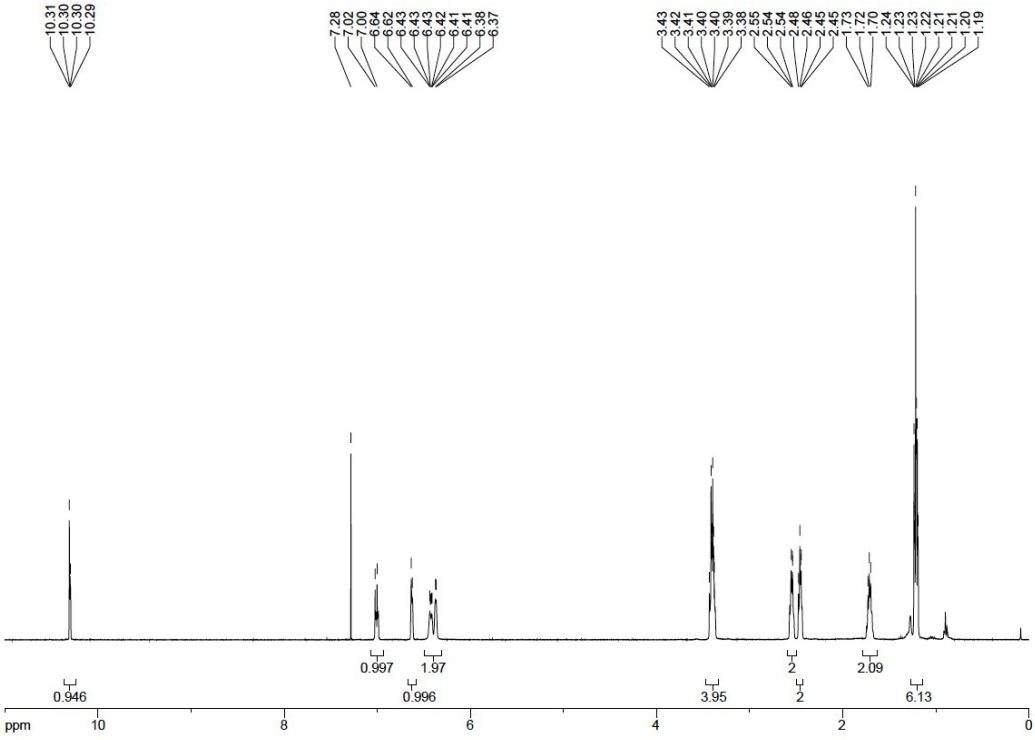
Synthesis

- Synthesis and purification of DDXC



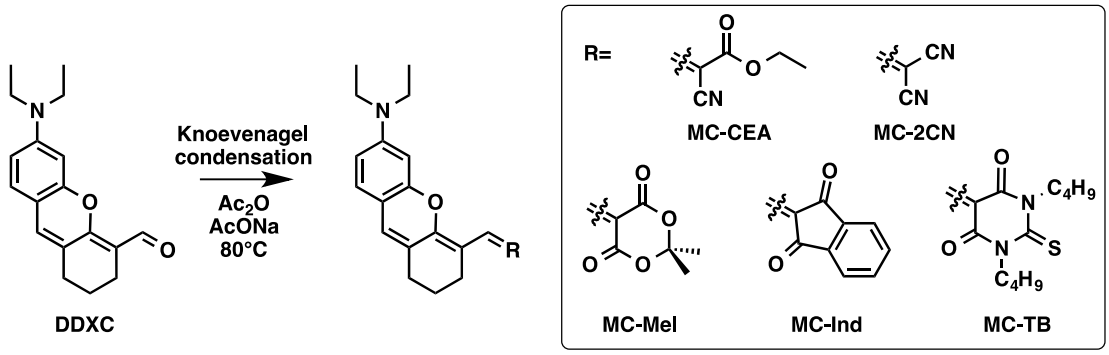
2-bromocyclohex-1-ene-1-carbaldehyde. To a mixture of DMF (12.9 mL, 167.9 mmol, 3.3 eq) and $CHCl_3$ (80 mL) was added dropwise, under stirring and at 0 °C PBr_3 (15.4 mL, 152 mmol, 3 eq). After addition the solution was allowed to stir for 1 hour. To the solution was added cyclohexanone (5.27 mL, 50.9 mmol, 1 eq) and the mixture was allowed to stir for 17 hours at room temperature. The mixture was neutralized by slow addition of water (300 mL), followed by solid $NaHCO_3$ until a pH of 7-8 was obtained. The product was washed with water and brine and was extracted with DCM. The organic phase was dried over $MgSO_4$, filtered and evaporated. The crude was purified by column chromatography on silica gel (Heptane/EtOAc: 9/1) to obtain 2.654 g of **2-bromocyclohex-1-ene-1-carbaldehyde** (28%) as a yellow oil that was directly involved in the next step.

To a solution of 4-(Diethylamino)salicylaldehyde (1.375 g, 7.05 mmol, 1 eq) in DMF (30 mL) were added **2-bromocyclohex-1-ene-1-carbaldehyde** (2.654 g, 14.11 mmol, 2 eq) and Cs_2CO_3 (6.92 g, 21.17 mmol, 3 eq). The mixture was allowed to stir for 48 hours at room temperature. To the crude was then added Et_2O and the remaining Cs_2CO_3 was filtered off before the solvents were evaporated. The product was washed with water and brine and was extracted with DCM. The organic phase was dried over $MgSO_4$, filtered and evaporated. The crude was purified by column chromatography on silica gel (Heptane/DCM/EtOAc: 7/2/1) to obtain 383 mg of **DDXC** (19%) as a deep orange solid. ^1H NMR (in $CDCl_3$) was in accordance with the literature.[1]

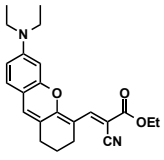


^{1}H NMR spectrum of DDXC ($CDCl_3$)

- Synthesis of MCs:

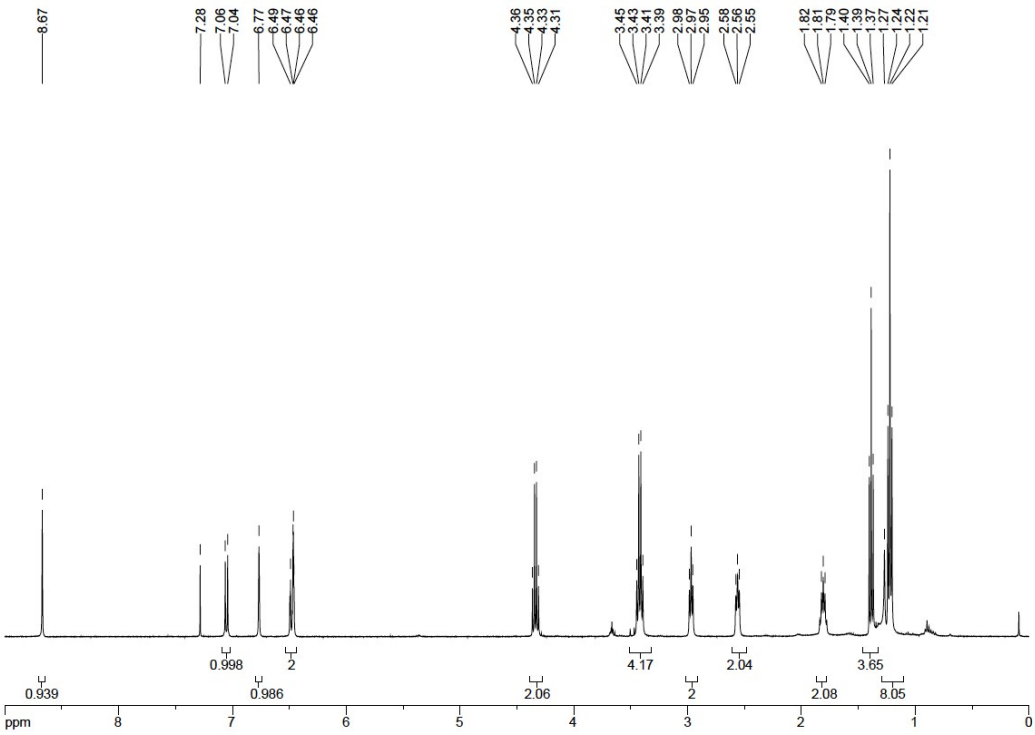


Typical procedure

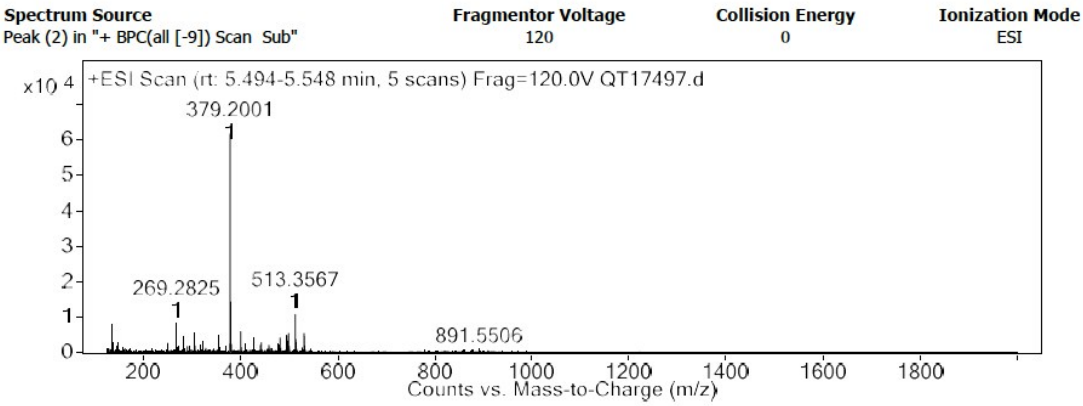


MC-CEA. To a solution of DDXC (25 mg, 0.088 mmol, 1 eq) in Ac_2O (4 mL) was added ethyl cyanoacetate (27 mg, 0.241 mmol, 2.7 eq). The mixture was stirred for 2 hours at room temperature. The solvents were evaporated and the product was washed with water, then HCl 1 M and neutralized with saturated $NaHCO_3$ and was extracted with DCM. The organic phase was dried over $MgSO_4$, filtered and evaporated. The crude was purified by column chromatography on silica gel (DCM/EtOAc: 95/5) to obtain 28 mg of MC-CEA (84%) as a dark violet solid after lyophilization. Rf=0.83 (DCM/EtOAc: 95:5). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.67 (s, 1H, HAr), 7.05 (d, *J* = 8.5 Hz, 1H, HAr), 6.77 (s, 1H, HAr), 6.49-6.46 (m, 2H, HAr), 4.34 (q, *J* = 7.1 Hz, 2H, CH_2 OEt), 3.42 (q, *J* = 8.3 Hz, 4H, CH_2

NEt), 2.97 (t, *J* = 6.1 Hz, 2H, CH_2), 2.56 (t, *J* = 5.7 Hz, 2H, CH_2), 1.81 (q, *J* = 6.1 Hz, 2H, CH_2), 1.39 (t, *J* = 7.1 Hz, 3H, CH_3 OEt), 1.23 (t, *J* = 8.3 Hz, 6H, 2 CH_3 NEt). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 166.14, 159.72, 155.12, 150.09, 147.26, 130.52, 127.76, 123.24, 119.24, 110.73, 108.73, 108.31, 97.03, 89.78, 61.35, 44.64, 29.31, 25.52, 21.00, 14.39, 12.63. HRMS (ES^+), calcd for $C_{23}H_{27}N_2O_3$ $[M+H]^+$ 379.2016, found 379.2001.

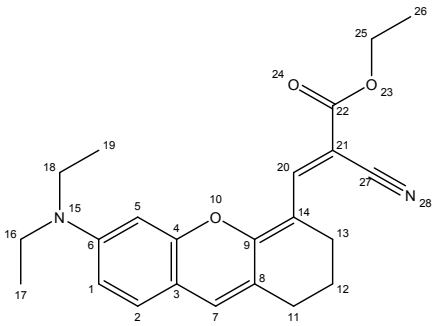


^{1}H NMR spectrum of MC-CEA ($CDCl_3$)



HRMS spectrum of MC-CEA

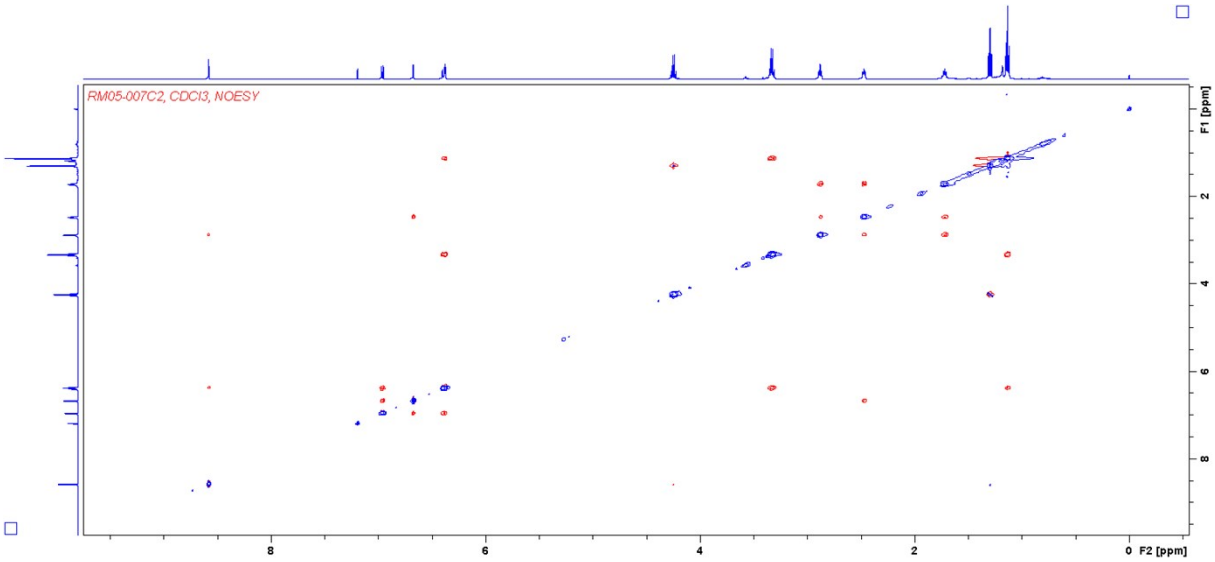
Determination of the configuration of MC-CEA by NMR studies.



Structure of MC-CEA

Atom numbering	^{1}H	^{13}C
1	6.93 ppm, d, J_{HH} = ,1H	108.8 ppm
2	6.96 ppm, dd, J_{HH} = , 1H	127.8 ppm
3	–	110.7 ppm
4	–	155.1 ppm
5	6.37 ppm, br s, 1H	97.0 ppm
6	–	150.1 ppm
7	6.67 ppm, t, J_{HH} = , 1H	130.5 ppm
8	–	123.3 ppm
9	–	159.8 ppm
11	2.47 ppm, t, J_{HH} = , 2H	29.3 ppm
12	1.72 ppm, quint., J_{HH} = , 2H	21.0 ppm
13	2.88 ppm, t, J_{HH} = , 2H	25.6 ppm
14	–	108.2 ppm
16, 18	3.33 ppm, q, J_{HH} = , 4H	44.6 ppm, 2C
17, 19	1.13 ppm, t, J_{HH} = , 6H	12.6 ppm, 2C
20	8.58 ppm, s, 1H	147.3 ppm
21	–	89.8 ppm
22	–	166.2 ppm
25	4.25 ppm, q, J_{HH} = , 2H	61.4 ppm
26	1.30 ppm, t, J_{HH} = , 3H	14.4 ppm
27	–	119.3 ppm

Assignment of proton and carbon signals of MC-CEA in $CDCl_3$, room temperature and Larmor frequency of 126 MHz.



NOESY spectrum of MC-CEA in $CDCl_3$, room temperature and Larmor frequency of 126 MHz

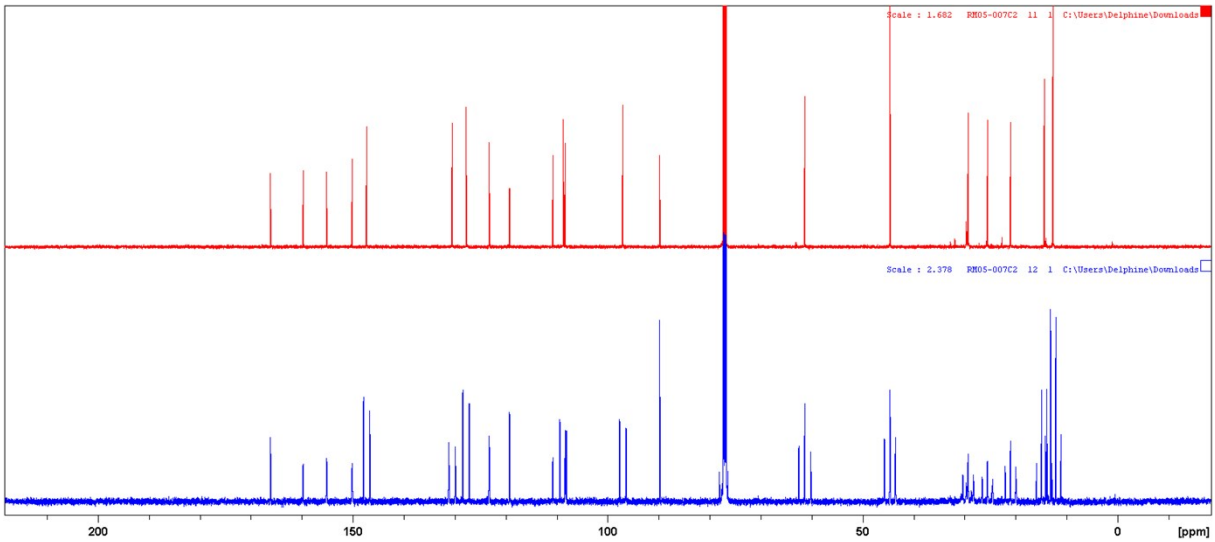
No NOE crosspeak was detected between the OEt moiety of the ester function (H_{25}/H_{26}) and the protons of the CH_2-groups of the ring (H_{12}/H_{13}). This is in line with a structure in which the alkene proton H_{20} is in *cis* of the ester group. However, one may argue that the absence of a crosspeak in a spectrum is not a definite proof, since many other parameters could also lead to this result (unfavorable dynamics or conformation, unsuitable parameters for this transition, smaller intensity NOE…).

Carbon-13 spectra are generally measured using proton decoupling methods such as CPD (composite pulse decoupling), which lead to singlets in absence of other I=1/2 nuclei. Although the spectra are easier to interpret that way (less lines, more favourable S/N ratio), some information about the coupling constants get lost.

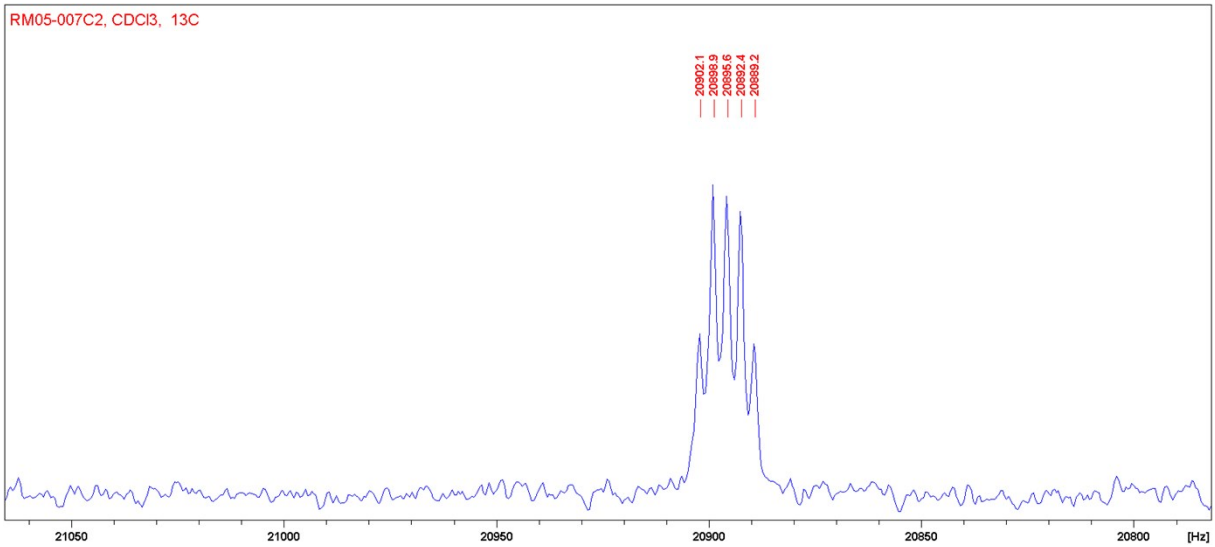
The values of the $^3J_{CH}$ coupling constants between H_{20} and the carbons attached to C_{21} strongly depend upon the geometry of the double bond. A *cis* configuration in respect to said vinylic proton should lead to smaller $^3J_{CH}$ coupling constants than a *trans* configuration.[2, 3, 4]

A ^{13}C spectrum (without proton decoupling) was recorded in $CDCl_3$ on a Bruker 500 MHz Avance III spectrometer by modifying our usual proton-decoupled *zgpg30* experiment to obtain a gated sequence (d1 = 5 s, PLW12 = 0W).

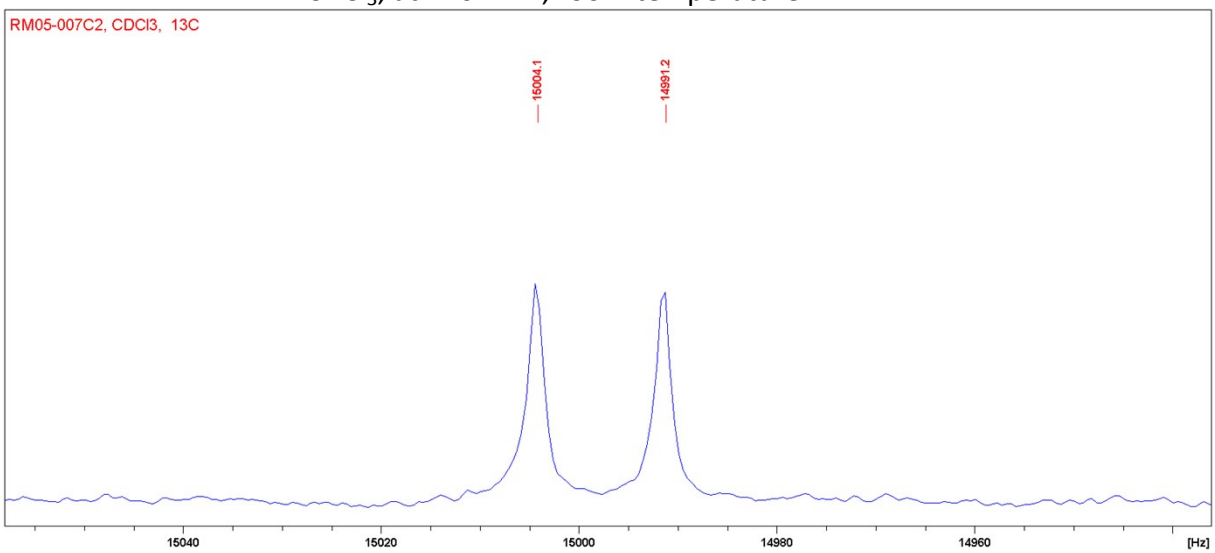
The δ = 119.3 ppm singlet corresponding to the nitrile carbon C_{27} splits into a doublet ($^3J_{CH}$ = 12.92 Hz) while C_{22} becomes a doublet of triplet ($^3J_{CH}$ = 6.60 Hz and 3.22 Hz) due to H_{20} and H_{25} respectively. Additionally, coupling constants > 12 Hz are only reported in case of *trans* configurations[1,2,3]. This confirms that the nitrile moiety stands in *trans* position in respect to the vinylic proton while the ester is positioned in *cis*. The conformation of the double bond was thus confirmed to be *E*.



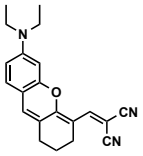
^{13}C (blue) and $^{13}C\{^{1}H\}$ (red) NMR spectra of MC-CEA, at 126 MHz, $CDCl_3$.



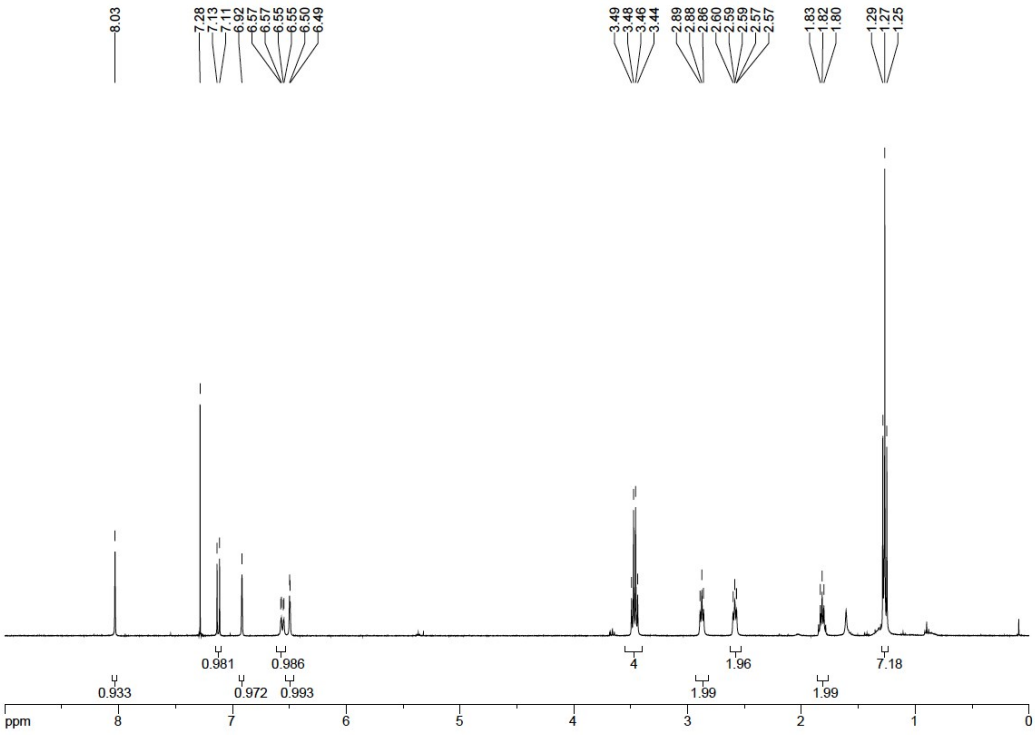
Zoom on the nitrile signal in the ^{13}C NMR spectrum of MC-CEA (δ = 119.3 ppm), in $CDCl_3$, at 126 MHz, room temperature.



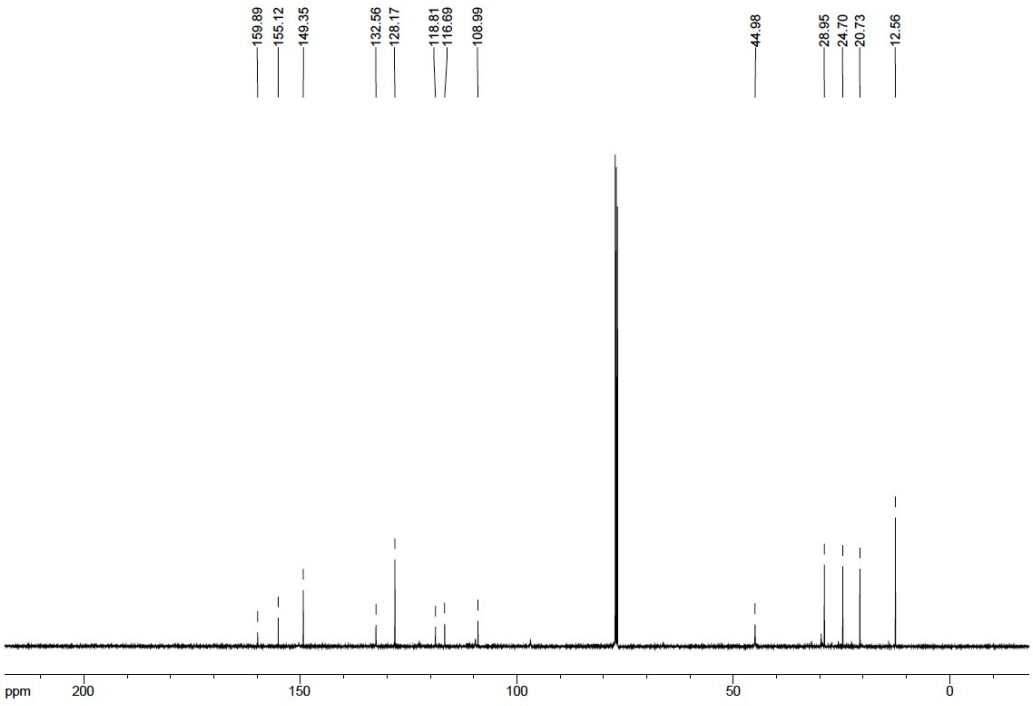
Zoom on the carbonyl signal of the ^{13}C NMR spectrum (δ = 166.2 ppm) of MC-CEA, in $CDCl_3$, at 126 MHz, room temperature



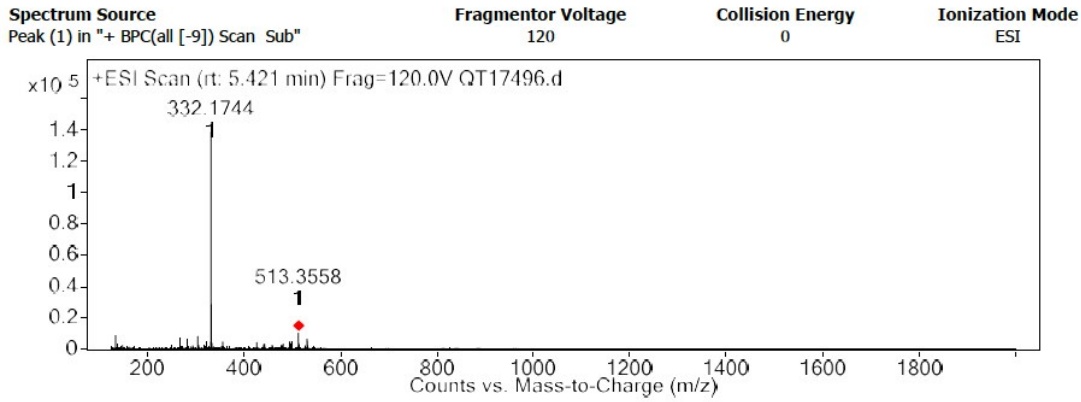
MC-2CN. Rf=0.32 (DCM/EtOAc 9/1). Conditions for column chromatography (DCM/Heptane) to obtain 18 mg of **C1** (77%) as a dark blue solid after lyophilization. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.03 (s, 1H, HAr), 7.12 (d, *J* = 8.8 Hz, 1H, HAr), 6.92 (s, 1H, HAr), 6.56 (dd, *J* = 8.7, 2.1 Hz, 1H, HAr), 6.50 (d, *J* = 2.2 Hz, 1H, HAr), 3.47 (q, *J* = 7.1 Hz, 4H, CH_2 NEt), 2.88 (t, *J* = 6.0 Hz, 2H, CH_2), 2.60-2.57 (m, 2H, CH_2), 1.82 (t, *J* = 6.1 Hz, 2H, CH_2), 1.27 (t, *J* = 7.1 Hz, 6H, CH_3 NEt). ^{13}C NMR (126 MHz, Chloroform-d) δ 159.89, 155.12, 149.36, 132.56 , 128.17 , 118.81 , 116.69 , 109.59 , 108.99 , 96.87 , 44.98 , 28.95 , 24.71 , 20.74 , 12.56. HRMS (ES$^+$), calcd for $C_{21}H_{22}N_3O$ $[M+H]^+$ 332.1757, found 332.1744.



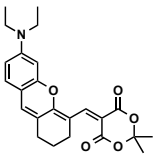
^{1}H NMR spectrum of MC-2CN ($CDCl_3$)



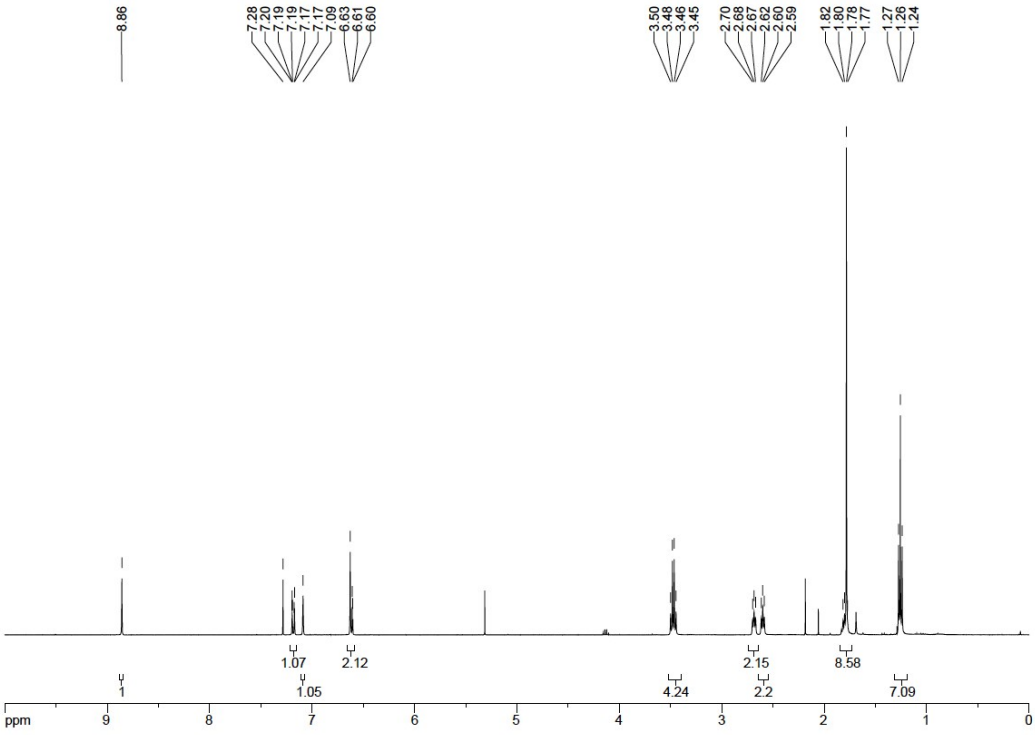
^{13}C NMR spectrum of MC-2CN ($CDCl_3$)



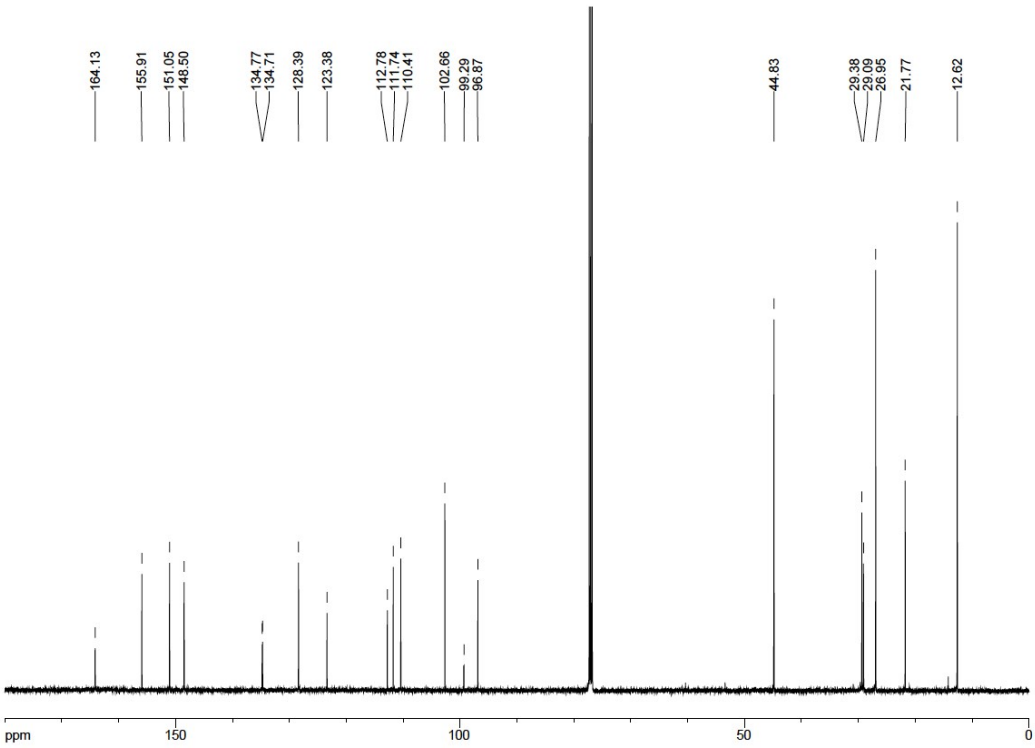
HRMS spectrum of MC-2CN



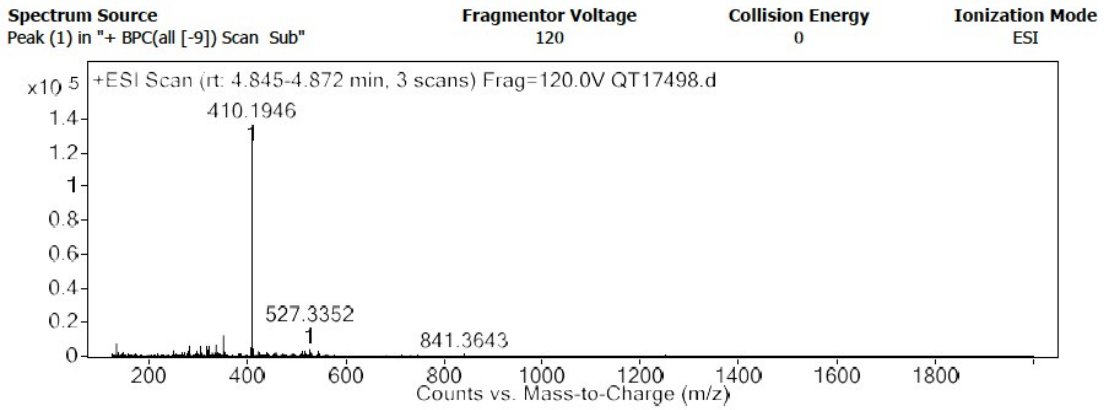
MC-Mel. Rf=0.37 (DCM/EtOAc 9/1). Conditions for column chromatography: 100% DCM to 8/2 DCM/EtOAc, yield=79%. Dark green solid after lyophilization. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.86 (s, 1H, HAr), 7.20-7.17 (m, 1H, HAr), 7.09 (s, 1H, HAr), 6.63-6.60 (m, 2H, HAr), 3.47 (q, *J* = 7.1 Hz, 4H, CH_2 NEt), 2.68 (t, *J* = 5.8 Hz, 2H, CH_2), 2.60 (t, *J* = 6.0 Hz, 2H, CH_2), 1.79 (m, 8H, CH_2, 2 CH_3), 1.26 (t, *J* = 7.1 Hz, 6H, CH_3 NEt). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 164.13, 155.91, 151.05, 148.50, 134.77, 134.71, 128.39, 123.38, 112.78, 111.74, 110.41, 102.66, 99.29, 96.87, 44.83, 29.38, 29.09, 26.95, 21.77, 12.62. HRMS (ES^+), calcd for $C_{24}H_{28}NO_5$ $[M+H]^+$ 410.1962, found 410.1946.



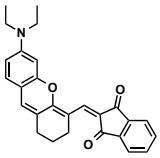
^{1}H NMR spectrum of MC-Mel ($CDCl_3$)



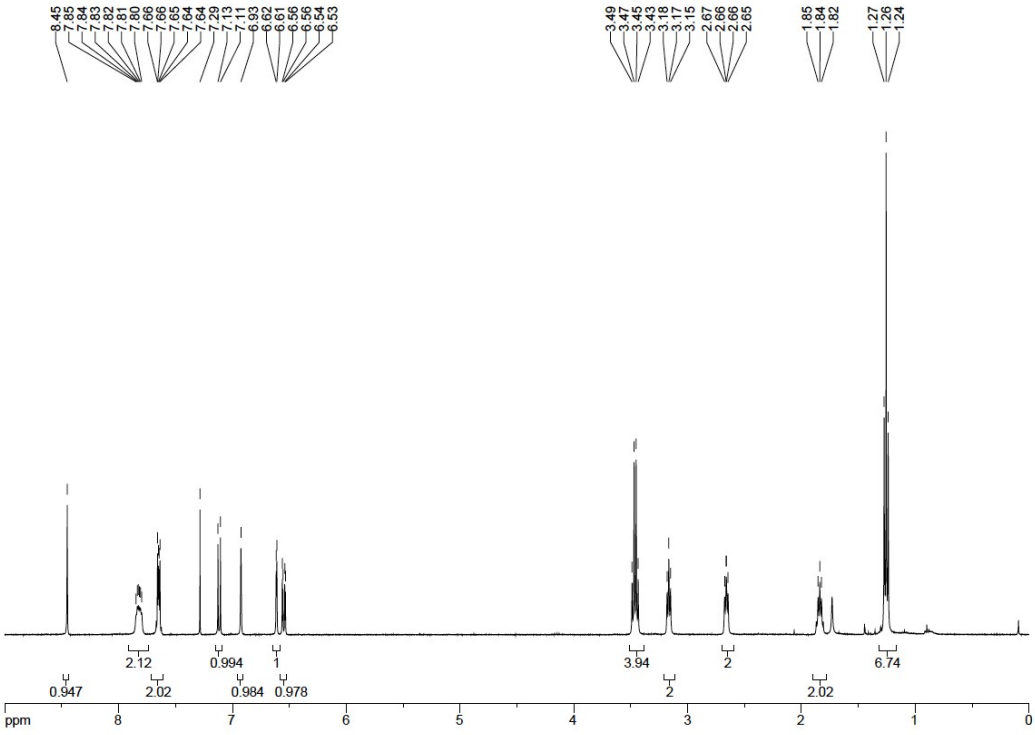
^{13}C NMR spectrum of MC-Mel ($CDCl_3$)



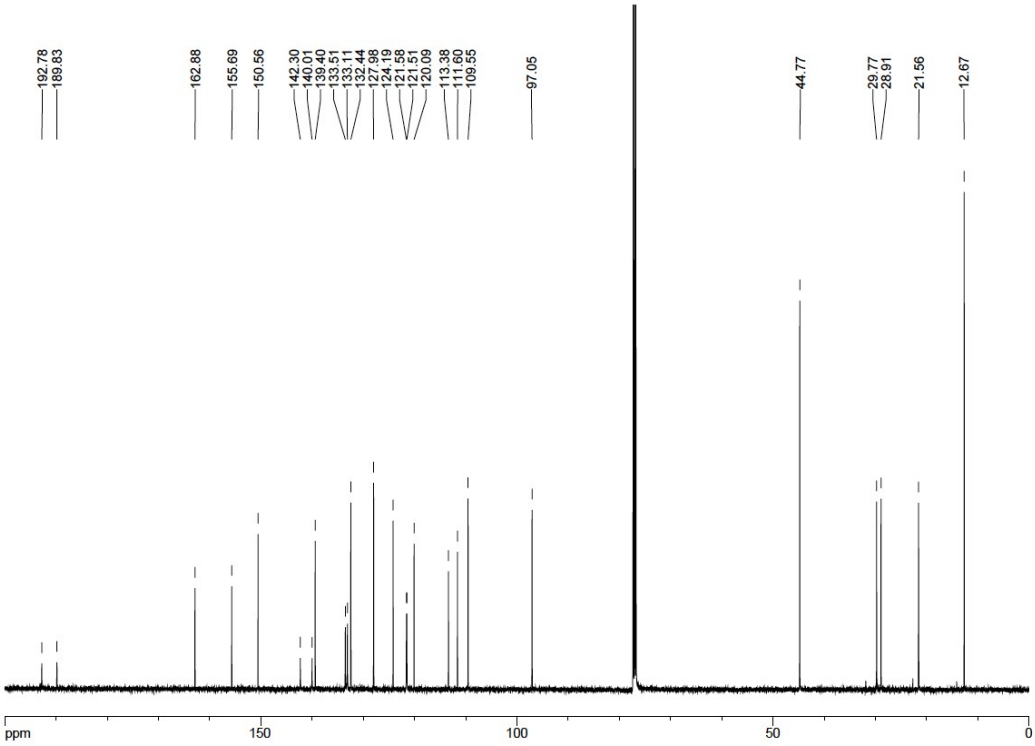
HRMS spectrum of MC-Mel



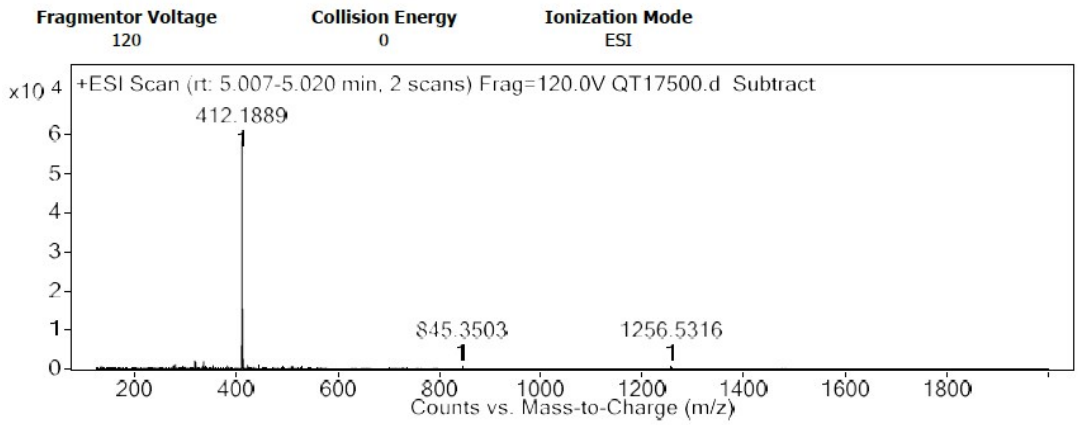
MC-Ind. Rf=0.34 (Heptane/EtOAc 6:4). Conditions for column chromatography: 9/1 to 6/4 Heptane:EtOAc, yield=58%. Dark green powder after lyophilization. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.45 (s, 1H, HAr), 7.82 (td, *J* = 7.8, 4.2 Hz, 2H, HAr), 7.66-7.64 (m, 2H, HAr), 7.12 (d, *J* = 8.8 Hz, 1H, HAr), 6.93 (s, 1H, HAr), 6.61 (d, *J* = 2.2 Hz, 1H, HAr), 6.55 (dd, *J* = 8.8, 2.5 Hz, 1H, HAr), 3.46 (q, *J* = 7.1 Hz, 4H, CH_2 NEt), 3.17 (t, *J* = 6.0 Hz, 2H, CH_2), 2.66 (dd, *J* = 5.8, 5.6 Hz, 2H, CH_2), 1.84 (t, *J* = 6.0 Hz, 2H, CH_2), 1.26 (t, *J* = 7.1 Hz, 6H, CH_3 NEt). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 192.78, 189.83, 162.88, 155.69, 150.56, 142.30, 140.01, 139.40, 133.51, 133.11, 132.44, 127.98, 124.19, 121.58, 121.51, 120.09, 113.38, 111.60, 109.55, 97.05, 44.77, 29.77, 28.91, 21.56, 12.67. HRMS (ES^+), calcd for $C_{27}H_{26}NO_3$ $[M+H]^+$ 412.1907, found 412.1889.



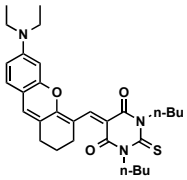
^{1}H NMR spectrum of MC-Ind ($CDCl_3$)



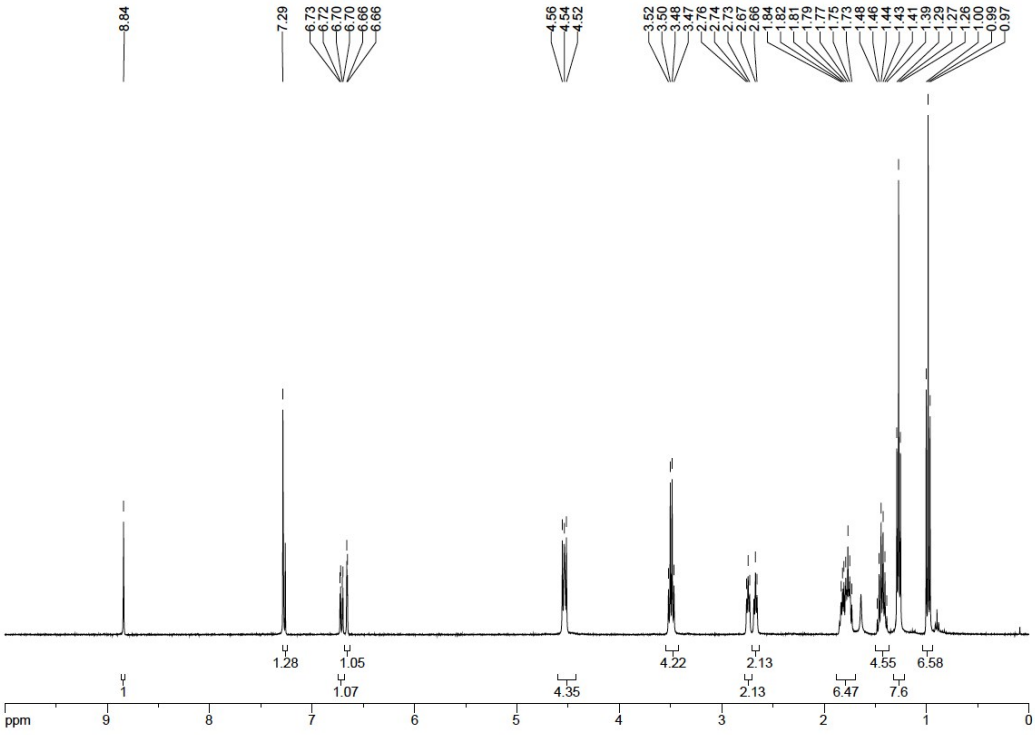
^{13}C NMR spectrum of MC-Ind ($CDCl_3$)



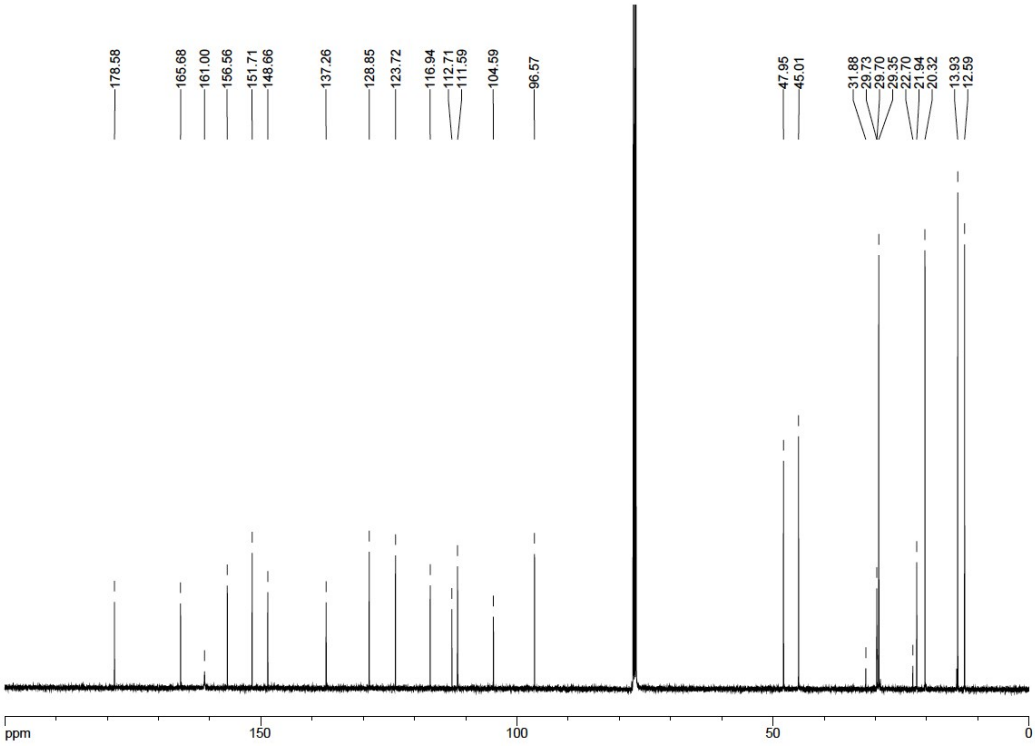
HRMS spectrum of MC-Ind



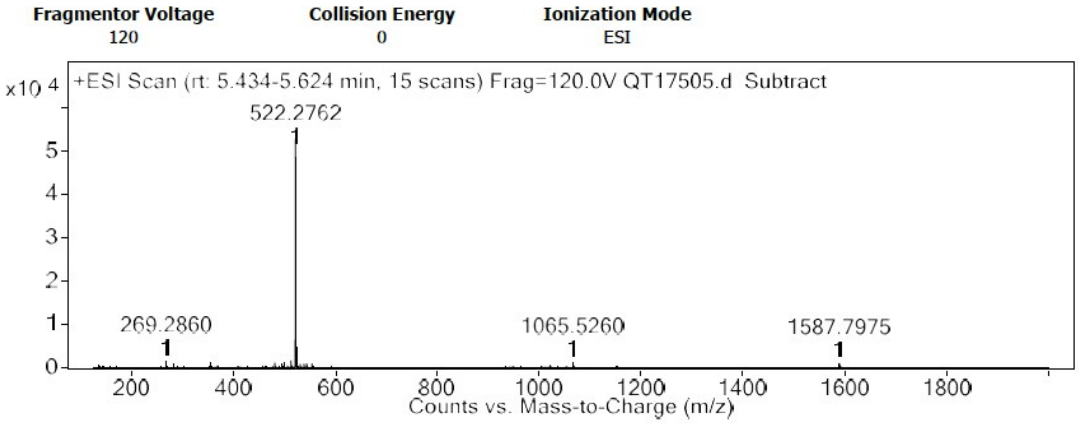
MC-TB. Rf=0.14 (heptane/EtOAc 8/2). Conditions for column chromatography: 9/1 to 6/4 heptane/EtOAc, yield=39%. Dark green powder after lyophilization. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.84 (s, 1H, HAr), 7.28 (d, *J* = 9.5 Hz, 2H, HAr), 6.71 (dd, *J* = 8.9, 2.5 Hz, 1H, HAr), 6.66 (d, *J* = 2.3 Hz, 1H, HAr), 4.56-4.52 (m, 4H, 2 NCH_2 NBu), 3.49 (q, *J* = 7.1 Hz, 4H, CH_2 NEt), 2.74 (t, *J* = 6.0 Hz, 2H, CH_2), 2.66 (d, *J* = 5.9 Hz, 2H, CH_2), 1.79 (m, 6H, CH_2, 2 CH_2 nBu), 1.44 (sextet, *J* = 7.6 Hz, 4H, CH_2), 1.27 (t, *J* = 7.1 Hz, 6H, CH_3 NEt), 0.99 (t, *J* = 7.4 Hz, 6H, CH_3 nBu). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 178.58, 165.68, 161.00, 156.56, 151.71, 148.66, 137.26, 128.85, 123.72, 116.94, 112.71, 111.59, 104.59, 96.57, 47.95, 45.01, 31.88, 29.73, 29.70, 29.35, 22.70, 21.94, 20.32, 13.93, 12.59. HRMS (ES^+), calcd for $C_{30}H_{40}N_3O_3S$ $[M+H]^+$ 522.2785, found 522.2762.



^{1}H NMR spectrum of MC-TB ($CDCl_3$)

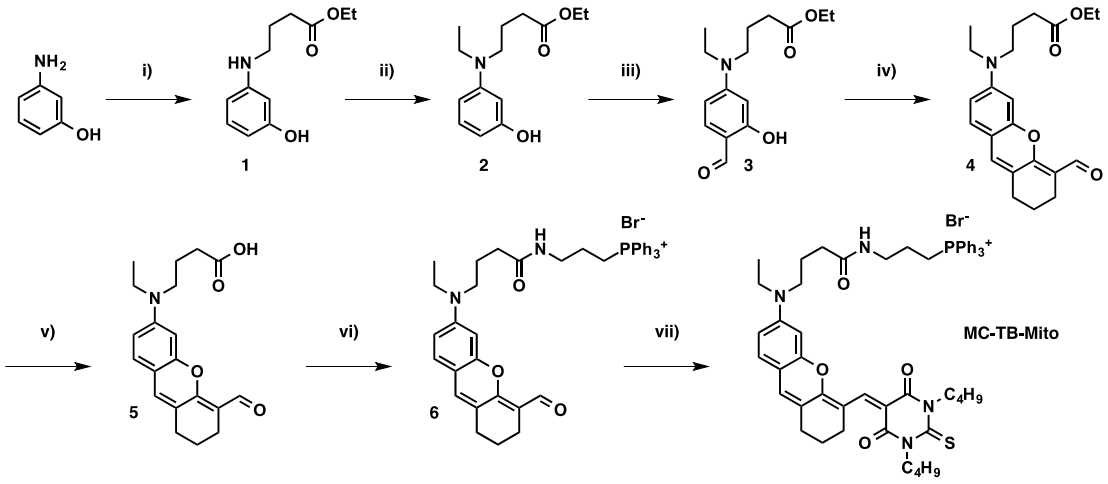


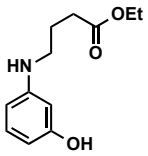
^{13}C NMR spectrum of MC-TB ($CDCl_3$)



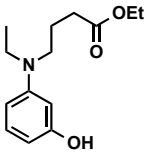
HRMS spectrum of MC-TB

- Synthesis of MC-TB-Mito

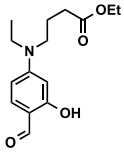




1. was synthesized according to a described procedure.[5]

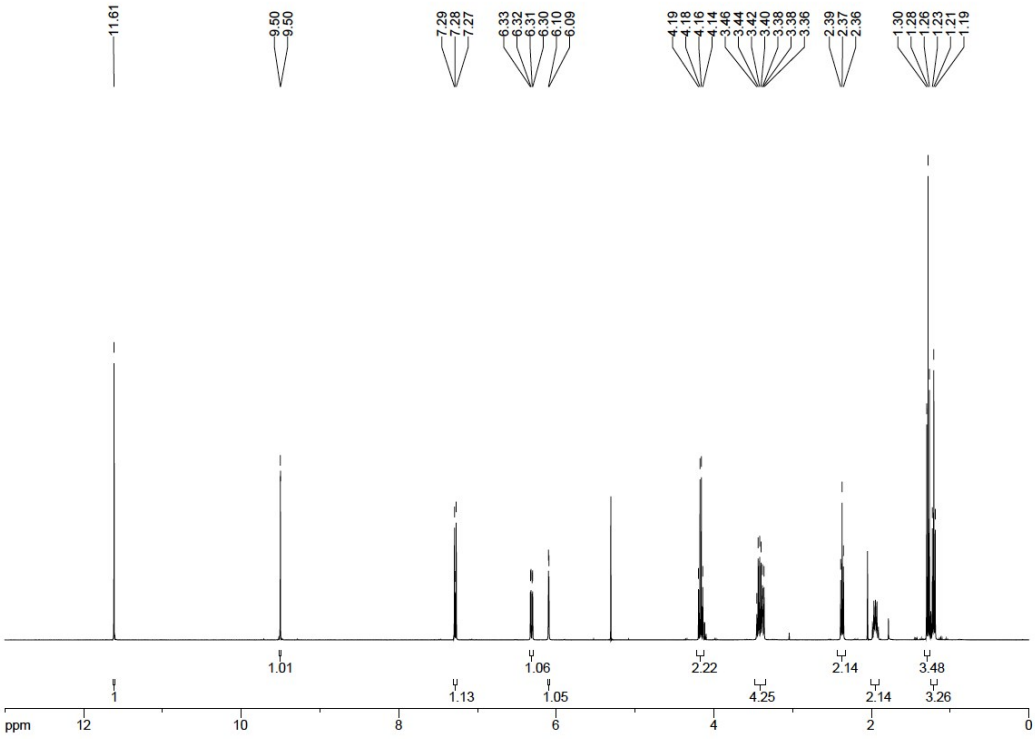


2. was synthesized according to a described procedure.[6]

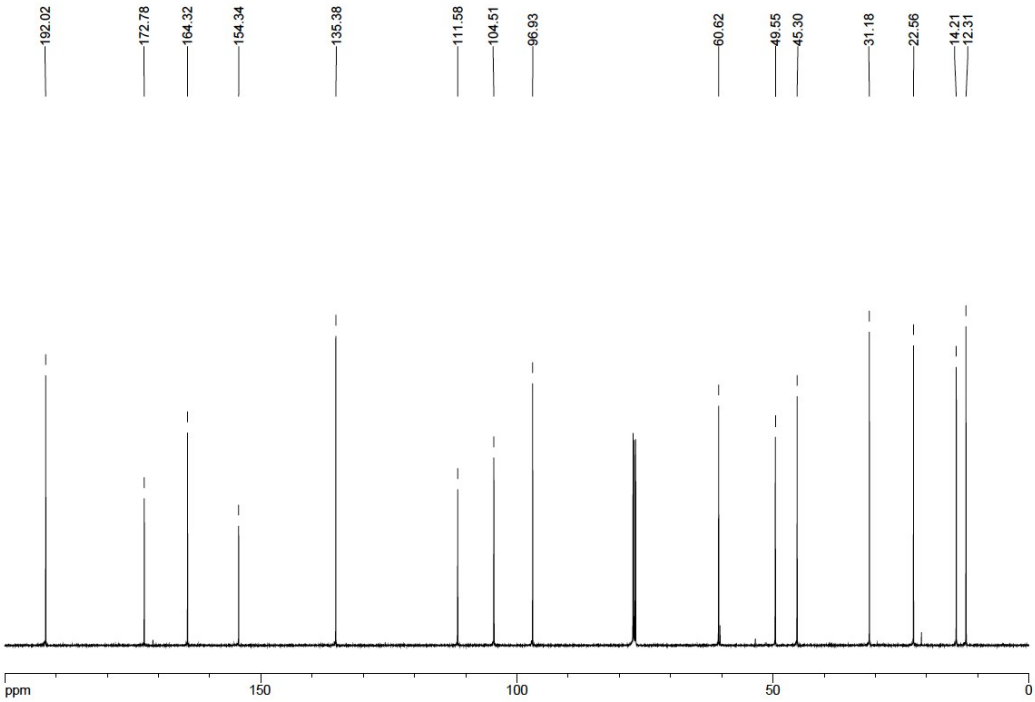


3. To a solution of DMF (5 mL), $POCl_3$ (1.8 mL, 19.24 mmol, 3 eq) was added dropwise at 0°C under stirred, and was allowed to react for 10 min under argon atmosphere. To the solution was added **2** (1.611 g, 6.414 mmol, 1 eq) previously solubilized in 5 mL DMF and the mixture was heated up to 65°C and stirred for 2 hours. Without evaporating the solvents, water was slowly added (200 mL), and then it was neutralized with $NaHCO_3$. The product was washed with water and brine and extracted with DCM. The organic phase was dried over $MgSO_4$, filtered and evaporated. The crude was purified by column chromatography on silica gel (Heptane/EtOAc 9/1 to 7/3) to obtain 1.351 g of **3** (75%) as a clear oil. Rf=0.38 (Heptane/EtOAc 7/3). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 11.61 (s, 1H, CHO), 9.50 (d, *J* = 0.5 Hz, 1H, HAr), 7.29-7.27 (m, 1H, HAr), 6.31 (dd, *J* = 8.9, 2.5 Hz, 1H, HAr), 6.10 (d, *J* = 2.4 Hz, 1H, HAr), 4.17 (q, *J* = 7.1 Hz, 2H, CH_2 OEt), 3.46-3.36 (m, 4H, CH_2 NEt), 2.37 (t, *J* = 7.0 Hz, 2H, CH_2), 1.99-1.91 (m, 2H, CH_2), 1.28 (t, *J* = 7.1 Hz, 3H, CH_3 OEt), 1.21 (t, *J* = 7.1 Hz, 3H, CH_3 NEt). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 192.02, 172.78, 164.32, 154.34, 135.38, 111.58, 104.51, 96.93, 60.62, 49.55, 45.30, 31.18, 22.56, 14.21, 12.31. HRMS (ES^+), calcd for $C_{15}H_{22}NO_4$ $[M+H]^+$ 280.1543, found

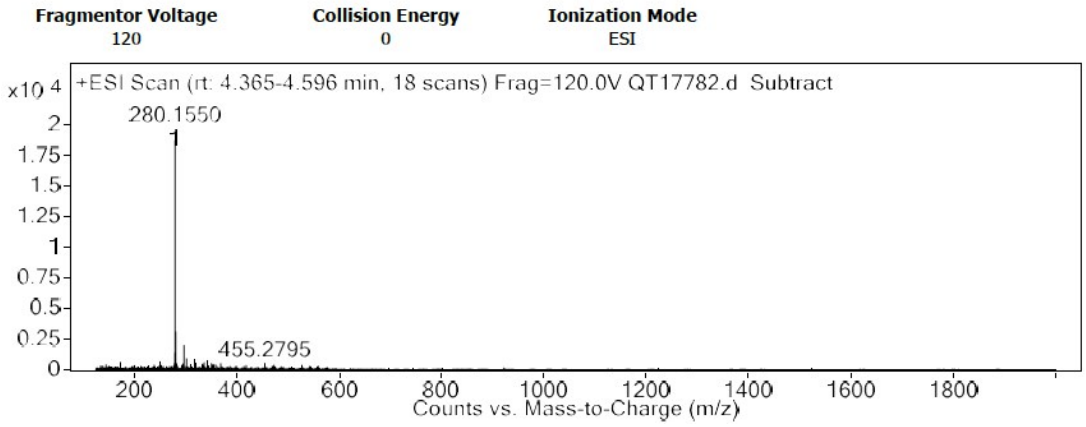
280.1550.



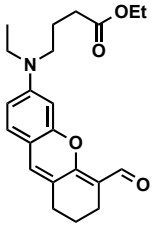
1H NMR spectrum of **3** (CDCl3)



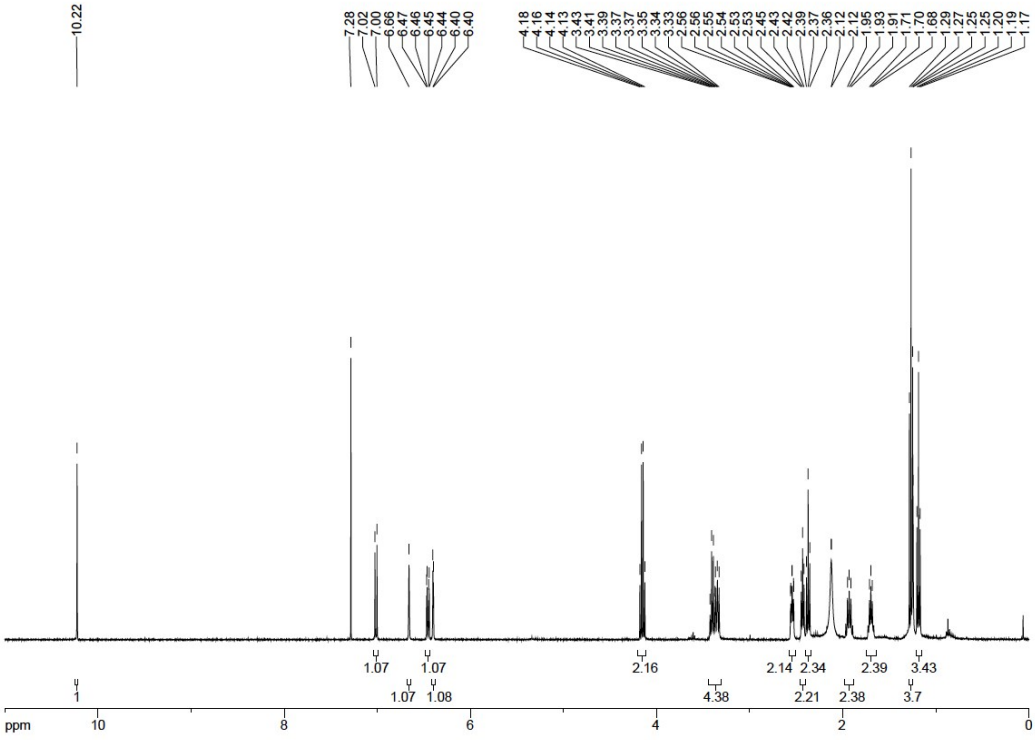
13C NMR spectrum of **3** (CDCl3)



HRMS spectrum of **3**



4. To a solution of **3** (1.351 g, 4.84 mmol, 1 eq) in DMF (15 mL) was added 2-bromocyclohex-1-ene-1-carbaldehyde (1.82 g, 9.68 mmol, 2 eq) and Cs_2CO_3 (4.73 g, 14.52, 3 eq). The mixture was stirred for 24 hours at room temperature before the solvents were evaporated. The product was washed with water and brine and extracted with DCM. The organic phase was dried over $MgSO_4$, filtered and evaporated. The crude was purified by column chromatography on silica gel (DCM/EtOAc 9/1 to 7/3) to obtain 258 mg of **4** (14%) as a bright orange solid. Rf=0.57 (DCM/EtOAc 8/2). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 10.22 (s, 1H, CHO), 7.01 (d, *J* = 8.6 Hz, 1H, HAr), 6.66 (s, 1H, HAr), 6.46 (dd, *J* = 8.6, 2.5 Hz, 1H, HAr), 6.40 (d, *J* = 2.3 Hz, 1H, HAr), 4.15 (q, *J* = 7.1 Hz, 2H, CH_2 OEt), 3.43-3.33 (m, 4H, CH_2 NEt), 2.54 (td, *J* = 6.2, 1.0 Hz, 2H, CH_2), 2.43 (t, *J* = 6.1 Hz, 2H, CH_2), 2.37 (t, *J* = 7.1 Hz, 2H, CH_2), 1.93 (t, *J* = 7.6 Hz, 2H, CH_2), 1.70 (t, *J* = 6.1 Hz, 2H, CH_2), 1.27 (t, *J* = 7.1 Hz, 3H, CH_3 OEt), 1.19 (t, *J* = 7.1 Hz, 3H, CH_3 NEt). ^{13}C-NMR (126 MHz, $CDCl_3$): δ 187.36, 173.16, 162.40, 154.12, 149.74, 128.39, 127.65, 123.32, 111.34, 110.56, 108.06, 97.44, 60.63, 49.50, 45.17, 31.32, 29.78, 22.61, 21.54, 20.60, 14.18, 12.21. HRMS (ES^+), calcd for $C_{22}H_{28}NO_4$ $[M+H]^+$ 370.2013, found 370.2003.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7.00
6.66
6.47
6.46
6.45
6.44
6.40
6.40
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4.14
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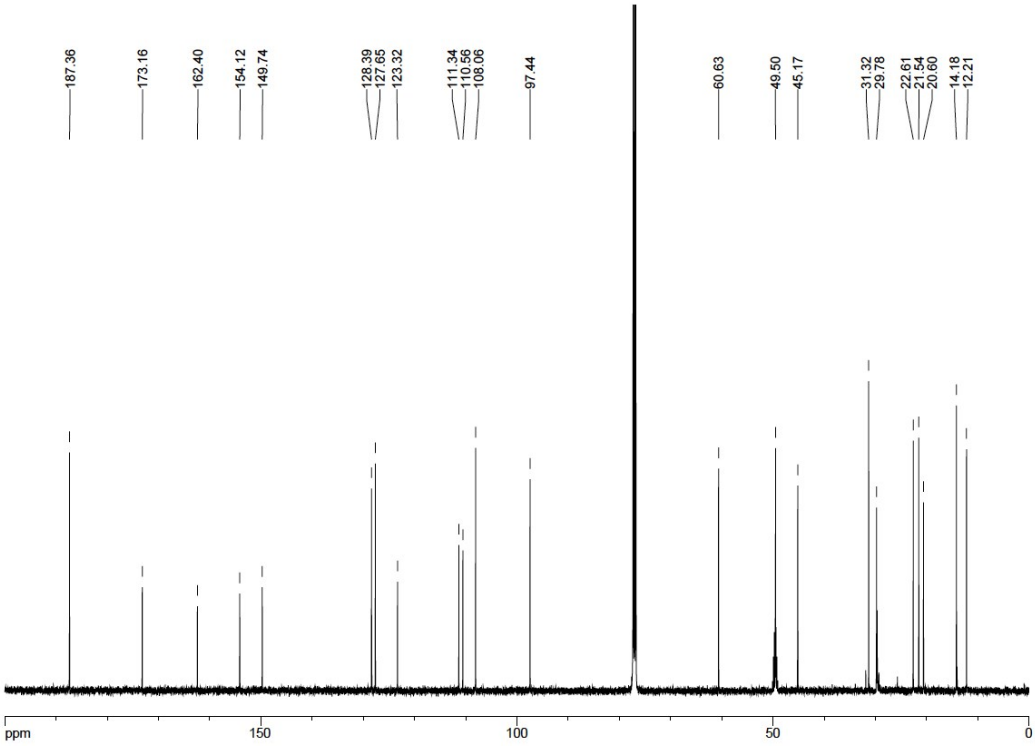
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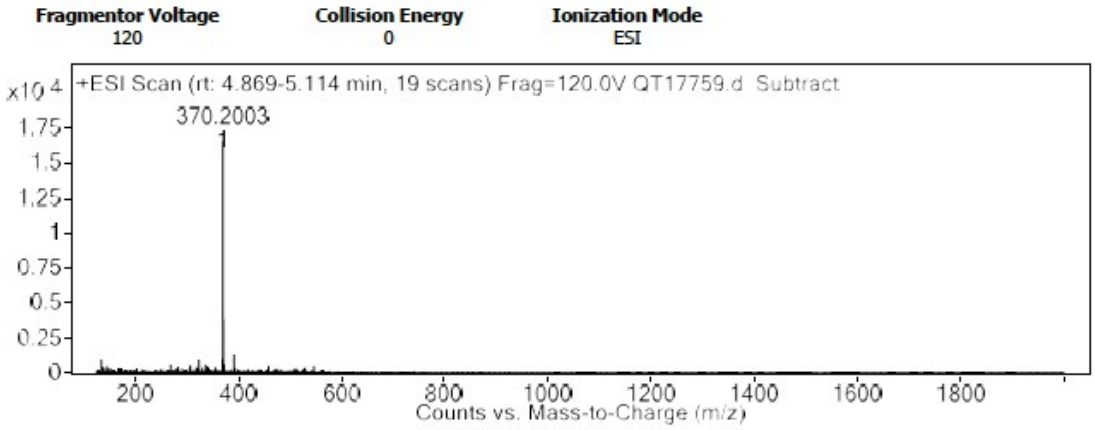
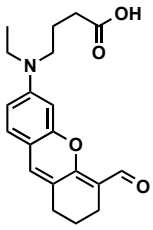
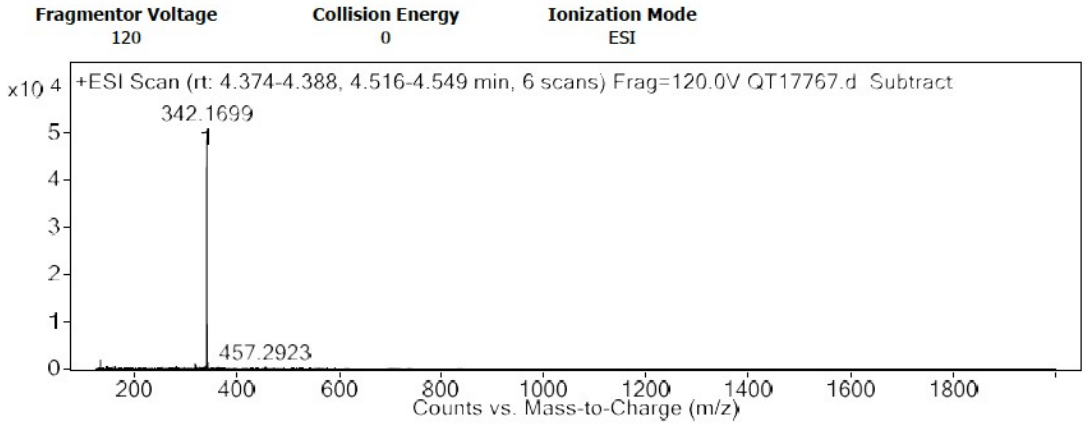
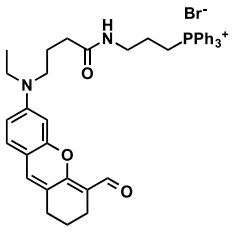




5. To a solution of **4** (258 mg, 0.7 mmol, 1 eq) in methanol/water (5:3, 8 mL) was added NaOH (168 mg, 4.2 mmol. 6 eq). The mixture was stirred for 30 minutes at room temperature. Without evaporating the solvents, the product was washed with citric acid 10% and extracted with DCM. The solvent was evaporated and the crude was used directly in the next step. 100 mg (42%) as an orange/red solid. Rf=0.73 (DCM/MeOH 95/5). HRMS (ES^+), calcd for $C_{20}H_{24}NO_4$ $[M+H]^+$ 342.1700, found 342.1699.

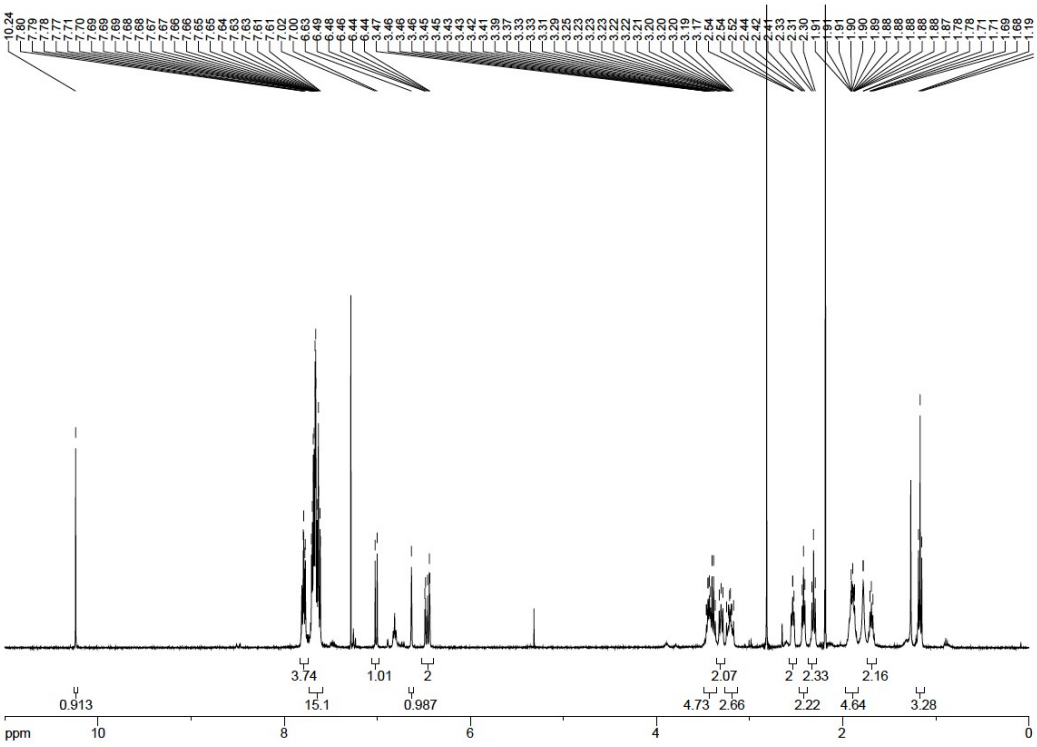


HRMS spectra of **5**

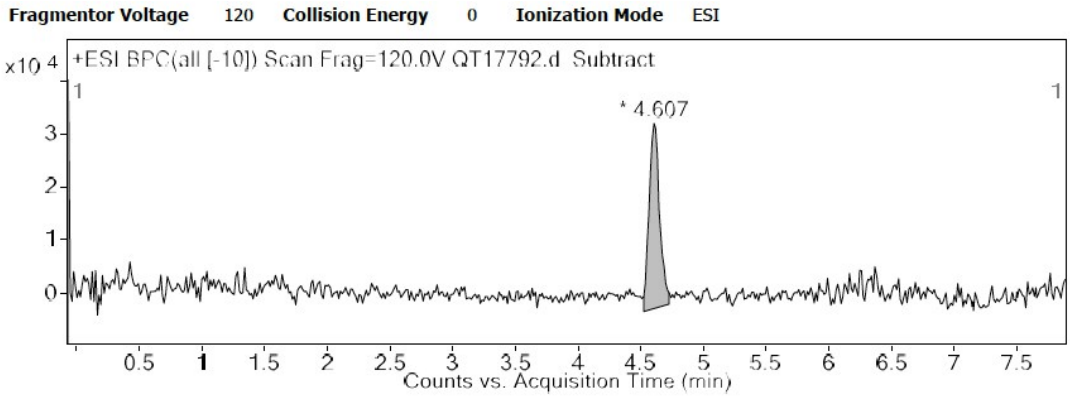


6. To a solution of **5** (100 mg, 0.29 mmol, 1 eq) in DMF (5 mL) was added (3-ammoniopropyl) triphenylphosphonium di bromide[7] (180 mg, 0.377 mmol, 1.3 eq), HATU (133 mg, 0.350 mmol, 1.2 eq) and DIEA (0.5 mL, 2.94 mmol, 10 eq). The mixture was allowed to stir for 5 hours at room temperature before the solvents were evaporated. The product was washed with water and brine and extracted with DCM. The organic phase was dried over $MgSO_4$, filtered and evaporated. The crude was purified by column chromatography on silica gel (DCM/MeOH 9/1 to 8/2) to obtain 30 mg of **6** (14%) as orange oil. Rf=0.76 (DCM/MeOH 8/2). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 10.24 (s, 1H, CHO), 7.78 (dd, *J* = 7.2, 2.2 Hz, 4H), 7.71-7.61 (m, 15H), 7.01 (d, *J* = 8.6 Hz, 1H), 6.63 (s, 1H), 6.49-6.44 (m,

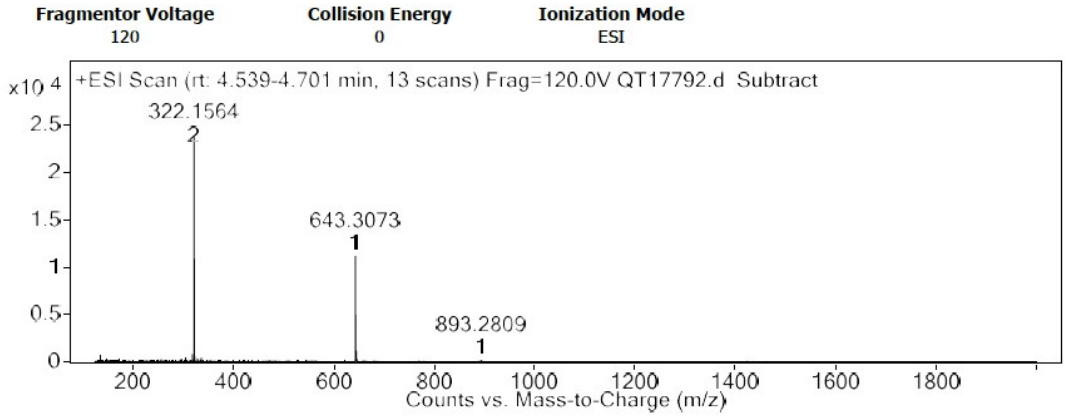
2H), 3.47-3.37 (m, 5H), 3.33-3.29 (m, 2H), 3.25-3.17 (m, 3H), 2.54-2.52 (m, 2H), 2.42 (t, *J* = 6.0 Hz, 2H), 2.31 (t, *J* = 7.2 Hz, 2H), 1.91-1.87 (m, 5H), 1.71-1.68 (m, 2H), 1.17 (t, *J* = 7.1 Hz, 3H). HRMS (ES$^+$), calcd for $C_{41}H_{44}BrN_2O_3P$ $[M]^+$ 643.3084, found 643.3073.



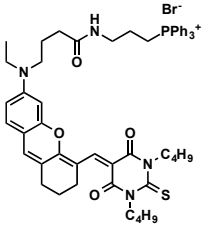
^{1}H NMR spectrum of **6**



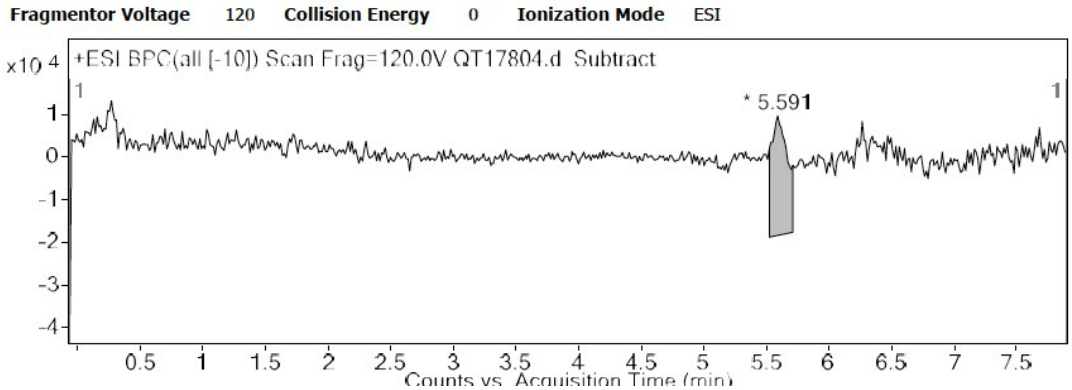
HPLC trace of **6**



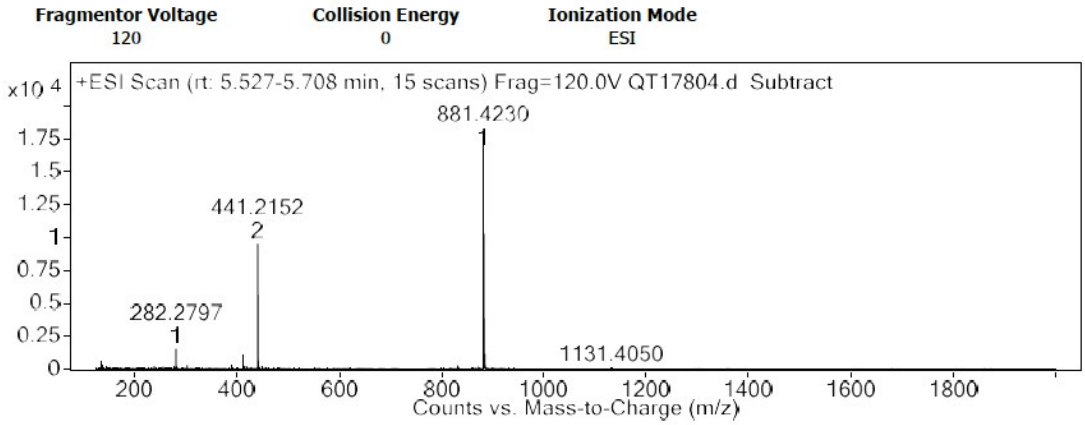
HRMS spectrum of **6**



MC-TB-Mito. To a solution of 6 (29 mg, 0.040 mmol) in acetic anhydride (4 mL) was added 1,3-Di-*N*-butyl-2-thiobarbituric acid (24 mg, 0.094 mmol, 2.3 eq) and sodium acetate (9 mg, 0,109 mmol, 2.7 eq). The mixture was allowed to stir at room temperature for 20 min before being evaporated. To the mixture was neutralized by slow addition of of water (300 mL), followed by solid $NaHCO_3$ until a pH of 7-8 was obtained. The product was extracted with DCM and washed with water and brine. The organic phase was dried over $MgSO_4$, filtered and evaporated. The crude was purified by column chromatography on silica gel (DCM/MeOH: 9/1) to obtain 7 mg of **MC-TB-Mito** (17%) as a dark green solid after lyophilization.



HPLC trace of **MC-TB-Mito**



^{1}H NMR spectrum of **MC-TB-Mito**

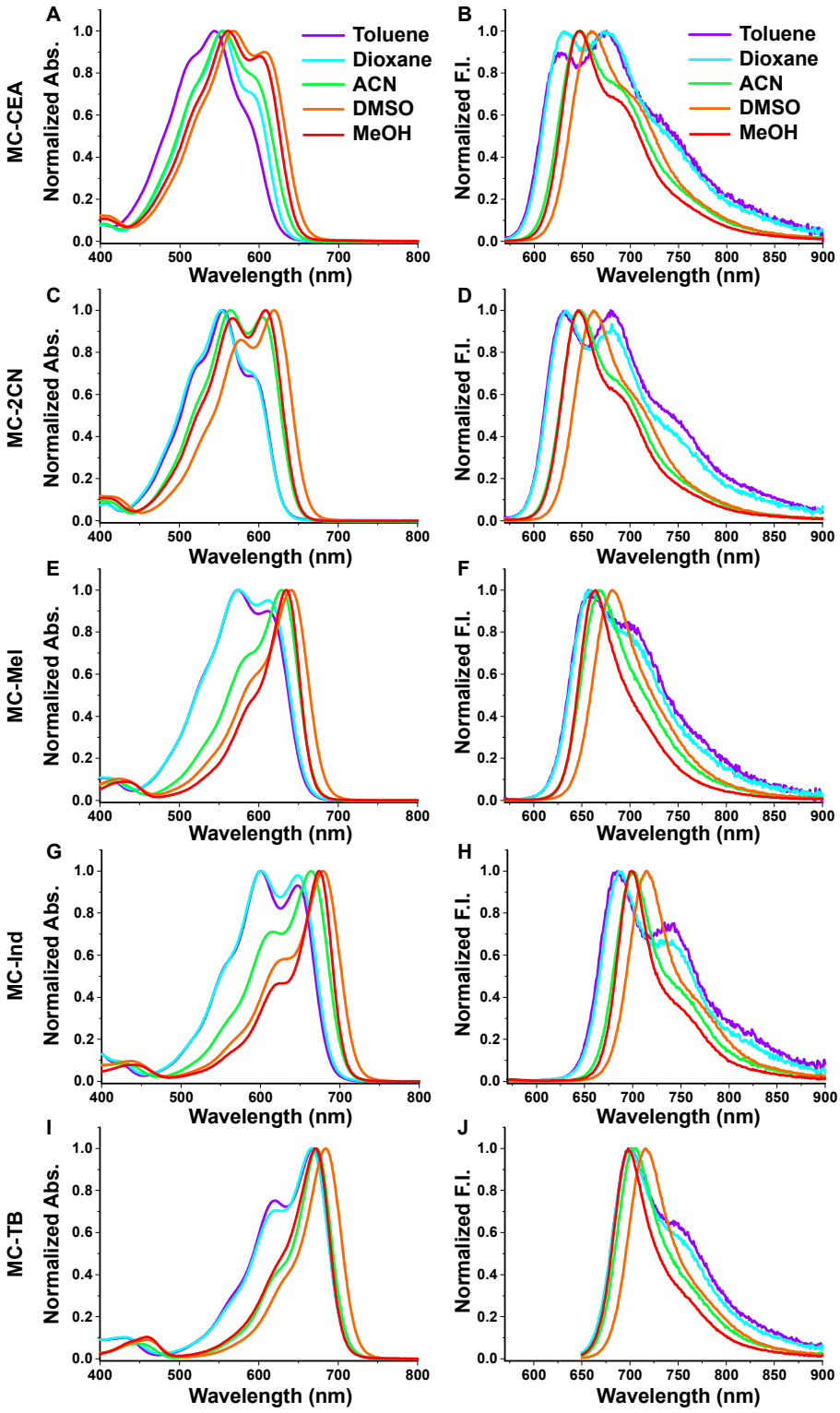


Figure S1. Normalized absorption (A, C, E, G, I) and emission (B, D, F, H, J) spectra of the merocyanines in various solvents with increasing polarity. Concentration was 5 μM. Excitation was 560 nm expect for MC-TB (640 nm).

Table S1. Photophysical properties of MC-CEA in various solvents

Solvent	λ_{Abs} max (nm)	ε ($M^{-1}.cm^{-1}$)	$FWHM_{abs}$ (nm)	λ_{Em} max (nm)	$FWHM_{Em}$ (nm)	Stoke Shift (nm)	ϕ
toluene	544	32,600	115	675	138	131	0.007
dioxane	543	34,000	116	673	131	130	0.008
ACN	555	39,600	119	646	92	91	0.03
DMSO	567	36,600	121	659	89	92	0.08
MeOH	591	37,500	120	648	83	57	0.07

Table S2. Photophysical properties of MC-2CN in various solvents

Solvent	λ_{Abs} max (nm)	ε ($M^{-1}.cm^{-1}$)	$FWHM_{abs}$ (nm)	λ_{Em} max (nm)	$FWHM_{Em}$ (nm)	Stoke Shift (nm)	ϕ
toluene	555	42,800	108	681	130	126	0.004
dioxane	553	35,800	109	633	111	80	0.006
ACN	564	42,800	111	648	83	84	0.03
DMSO	619	47,600	101	662	77	43	0.07
MeOH	609	57,800	109	646	76	37	0.03

Table S3. Photophysical properties of MC-Mel in various solvents

Solvent	λ_{Abs} max (nm)	ε ($M^{-1}.cm^{-1}$)	$FWHM_{abs}$ (nm)	λ_{Em} max (nm)	$FWHM_{Em}$ (nm)	Stoke Shift (nm)	ϕ
toluene	574	40,000	106	657	102	83	0.003
dioxane	575	47,400	119	659	99	84	0.003
ACN	629	66,200	90	668	68	39	0.008
DMSO	641	61,400	84	682	64	41	0.03
MeOH	635	86,400	57	664	54	29	0.05

Table S4. Photophysical properties of MC-Ind in various solvents

Solvent	λ_{Abs} max (nm)	ε ($M^{-1}.cm^{-1}$)	$FWHM_{abs}$ (nm)	λ_{Em} max (nm)	$FWHM_{Em}$ (nm)	Stoke Shift (nm)	ϕ
toluene	600	48,800	119	684	104	84	0.006
dioxane	601	54,600	122	686	97	85	0.005
ACN	664	66,200	100	700	54	36	0.01
DMSO	679	80,000	91	714	54	35	0.05
MeOH	674	106,600	52	699	42	25	0.08

Table S5. Photophysical properties of MC-TB in various solvents

Solvent	λ_{Abs} max (nm)	ε ($M^{-1}.cm^{-1}$)	$FWHM_{abs}$ (nm)	λ_{Em} max (nm)	$FWHM_{Em}$ (nm)	Stoke Shift (nm)	ϕ
toluene	668	74,600	97	701	90	33	0.005
dioxane	667	74,400	94	700	85	33	0.005
ACN	673	104,200	54	705	57	32	0.001
DMSO	684	101,000	56	716	53	32	0.003
MeOH	672	106,000	59	698	49	26	0.01

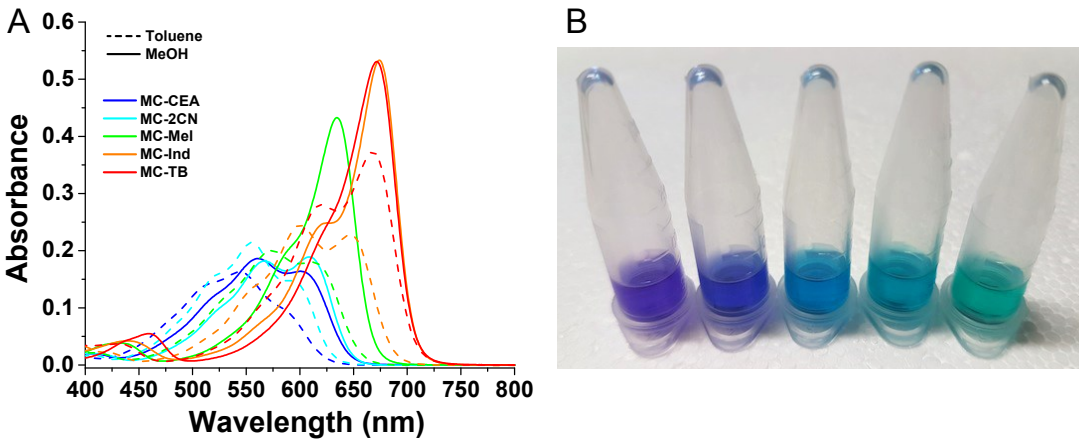


Figure S2. (A) Absorption spectra of MC dyes (5 μM) in toluene and methanol. Solutions of MCs (10 μM) in methanol.

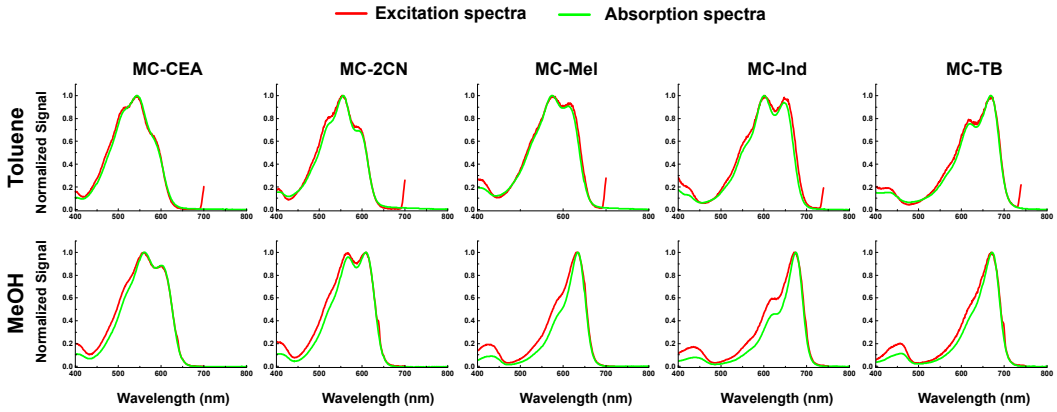


Figure S3. Normalized absorption and excitation spectra of the merocyanines in toluene (top line) and MeOH (bottom line). Concentration was 5 μM.

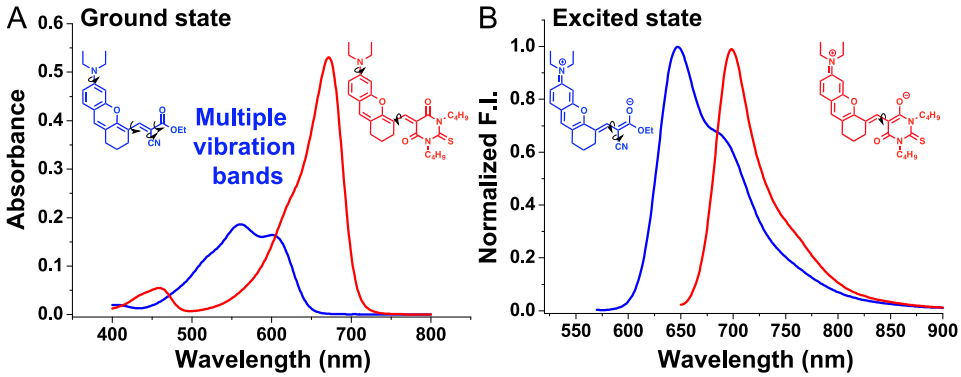


Figure S4. Difference between MCs with open and cyclic substituents illustrated by representative examples: MC-CEA (in blue) and MC-TB (in red). Absorption (A) and emission (B) spectra in MeOH depicting the various vibration bands at the steady state and the excited state respectively. Black arrows depict the possible rotation around the σ bounds.

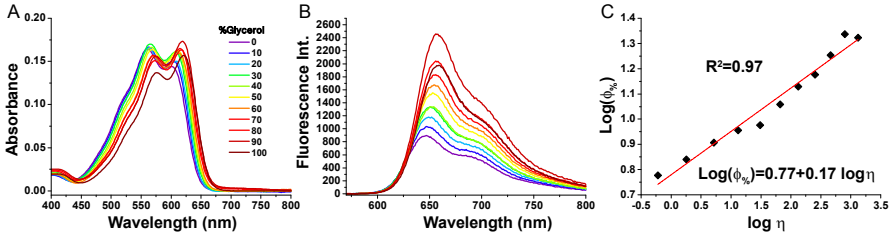


Figure S5. Absorption (A) and emission (B) spectra of MC-CEA in various solutions of Glycerol/MeOH mixture. (C) Correlation of the fluorescence quantum yield with the viscosity of the medium according to the displayed Förster–Hoffmann equation. Concentration of the dye was 1 μM. Temperature was 20°C.

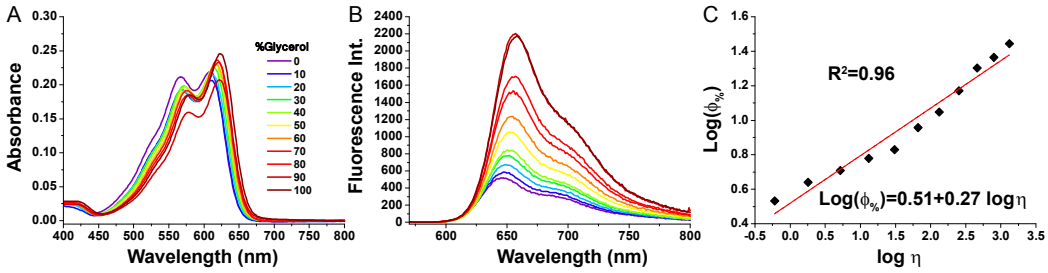


Figure S6. Absorption (A) and emission (B) spectra of MC-2CN in various solutions of Glycerol/MeOH mixture. (C) Correlation of the fluorescence quantum yield with the viscosity of the medium according to the displayed Förster–Hoffmann equation. Concentration of the dye was 5 μM. Temperature was 20°C.

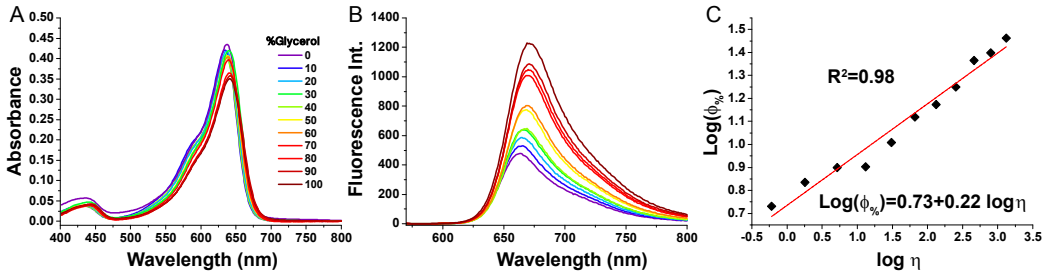


Figure S7. Absorption (A) and emission (B) spectra of MC-Mel in various solutions of Glycerol /MeOH mixture. (C) Correlation of the fluorescence quantum yield with the viscosity of the medium according to the displayed Förster–Hoffmann equation. Concentration of the dye was 5 μM. Temperature was 20°C.

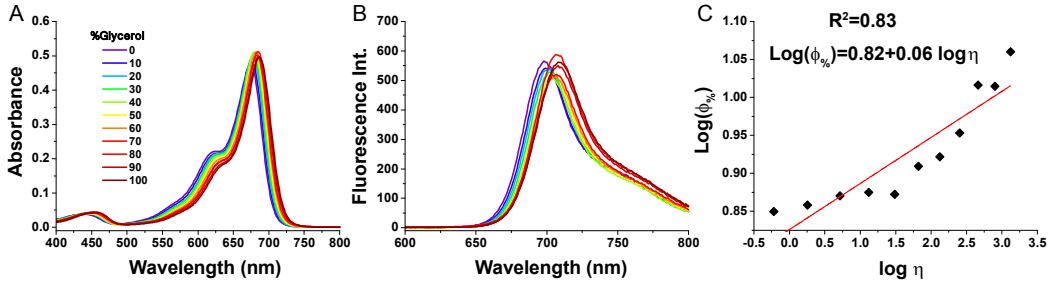


Figure S8. Absorption (A) and emission (B) spectra of MC-Ind in various solutions of Glycerol/MeOH mixture. (C) Correlation of the fluorescence quantum yield with the viscosity of the medium according to the displayed Förster–Hoffmann equation. Concentration of the dye was 5 μM. Temperature was 20°C.

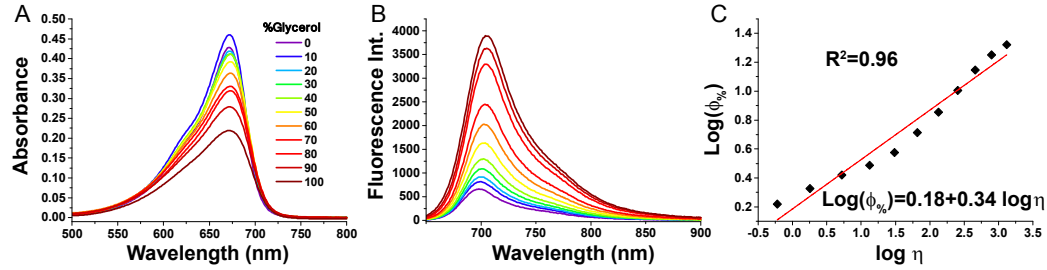


Figure S9. Absorption (A) and emission (B) spectra of MC-TB in various solutions of Glycerol/MeOH mixture. (C) Correlation of the fluorescence quantum yield with the viscosity of the medium according to the displayed Förster–Hoffmann equation. Concentration of the dye was 5 μM. Temperature was 20°C.

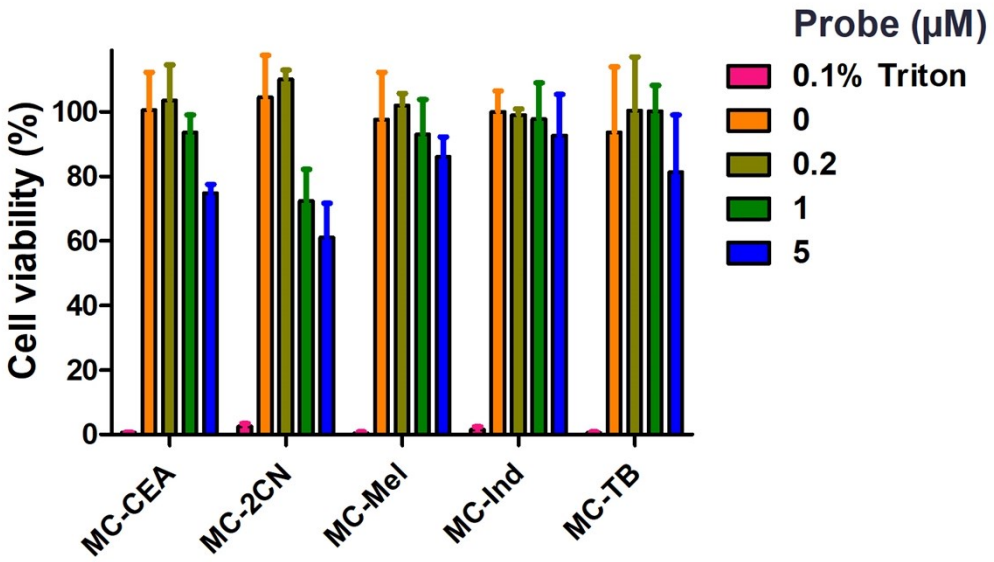


Figure S10. Assessment of MCs's cytotoxicity at various concentrations using the MTT test.

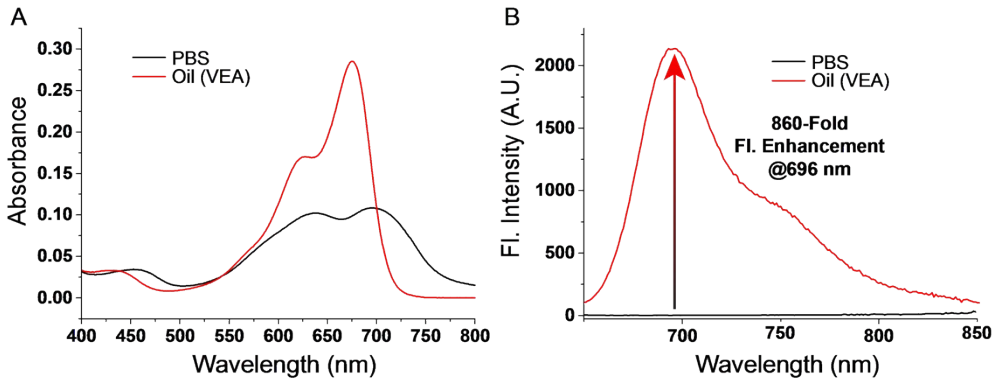


Figure S11. (A) Absorption and (B) emission spectra of MC-TB in PBS (black lines) and vitamine E acetate, VEA (red lines).

Table S6. Measured lifetime values of MCTB in various Glycerol/Methanol mixtures.

% glycerol	Mean Tau (ns)	τ1 (ns)	% τ1	t2 (ns)	% τ2
0	0.16	0.16	1.00	-	-
20	0.26	0.26	1.00	-	-
40	0.42	1.02	0.01	0.42	0.99
60	0.76	0.69	0.90	1.41	0.10
80	1.25	0.91	0.65	1.88	0.35
100	1.97	1.65	0.69	2.68	0.31
Rhod800 (ref)	1.98	1.98	1.00	-	-

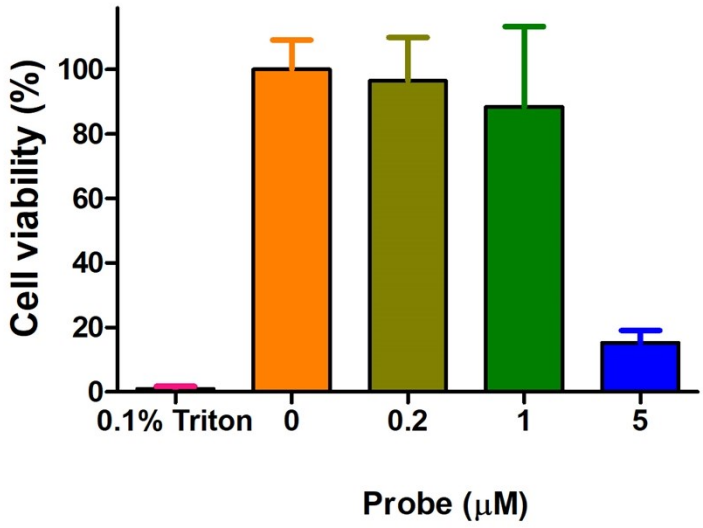


Figure S12. Assessment of MC-TB-Mito's cytotoxicity at various concentrations using the MTT test.

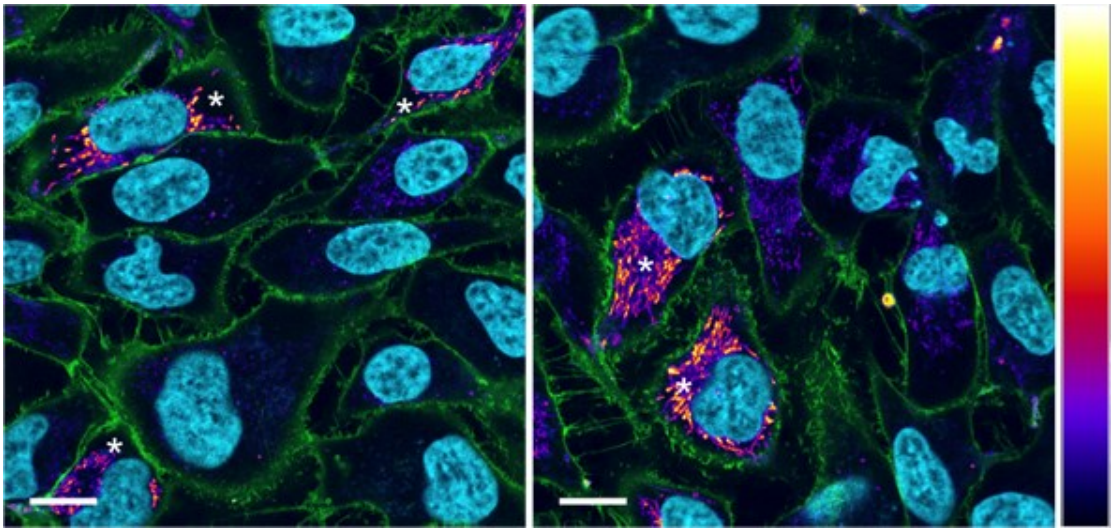


Figure S13. Laser scanning confocal images of HeLa cells incubated with MC-TB-Mito (1 μM), displaying heterogeneous intensity within the cell population. The white stars indicate the cells displaying higher signals. Scale bar is 15 μm.

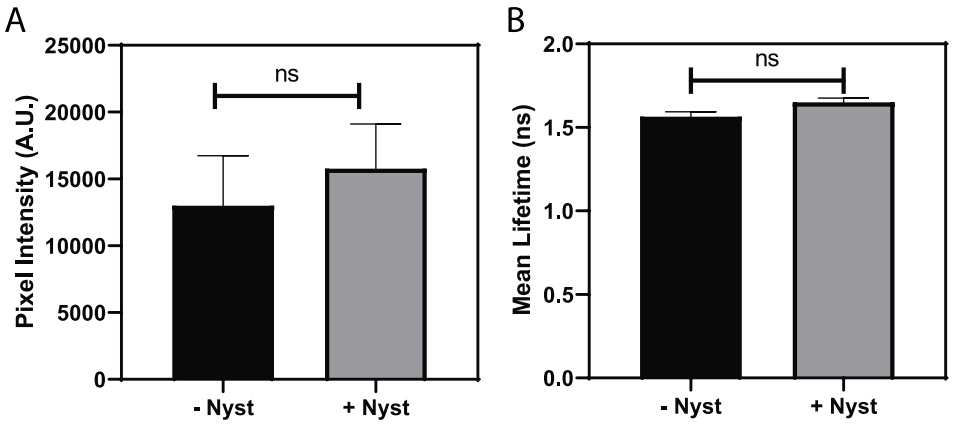


Figure S14. FLIM imaging analysis. (A) Mean fluorescence intensity and (B) mean lifetime of cells in both + an - nystatin conditions, showing that the lifetime of non-fluorescent cells could not be measured due to low fluorescence intensity of cells during FLIM imaging. 'ns' on the graph signifies non-significant difference.

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