Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.
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Electronic Supplementary Information

Two-photon absorbing 8-hydroxy-benzo[g]coumarins with giant Stokes shift: An environment insensitive dye platform for probing biomolecules

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General information

All chemicals were purchased from Sigma-Aldrich or Alfa-Aesar. Commercially available reagents were used without any further purification. All solvents were purified and dried by standard methods prior to use. Thin-layer chromatography (TLC) was performed on precoated silica gel 60F-254 glass plates. ^{1}H and ^{13}C NMR spectra were recorded on Bruker 300 MHz and 500 MHz respectively using tetramethylsilane as the internal reference. Coupling constants (*J* value) are reported in Hertz. All chemical shifts are reported in the standard notation of parts per million (ppm) using residual solvent protons as the internal standard. Mass spectral analysis was recorded with Jeol JMS 700 and was reported in units of mass to charge (*m/z*).

Spectroscopic analysis

UV-Vis absorption spectra were obtained using a HP 8453 UV-Vis spectrophotometer. Fluorescence spectra were recorded on a Photon Technical International Fluorescence System with a 3 ml quartz cell with 1–cm standard path length. 1 mM stock solutions were prepared in DMSO. All photo-physical studies were carried out in a concentration of 5 µM probe solution.

Quantum yield was calculated using the following equation (1) considering 9,10-diphenylanthracene (Φ = 0.9 in cyclohexane) as standard at a fixed excitation wavelength of 350 nm.

$$\Phi = \Phi_r \left(\frac{\eta}{\eta_r}\right)^2 \left(\frac{I}{I_r}\right)\left(\frac{A_r}{A}\right) \text{ ..... (1)}$$

Where, Φ is the quantum yield

I = measured integrated emission intensity

η = refractive index of the media

A = absorbance

The Lippert-Mataga plot was obtained by plotting Stoke's shift with orientation polarizability using equation (2).

$$\bar{\nu}_A - \bar{\nu}_F = \frac{2}{hca^3}(\mu_E - \mu_G)\Delta f + Constant \text{ ..... (2)}$$

Where, $\Delta f = \left(\frac{\epsilon-1}{2\epsilon+1}\right) - \left(\frac{\eta^2-1}{2\eta^2+1}\right)$

$(\bar{\nu}_A - \bar{\nu}_F) =$ Stokes shift in cm^{-1}

$h =$ Plank's constant

$\Delta f =$ Orientation polarisibility

$\epsilon =$ Dielectric constant

$\eta =$ Refractive index

TPACS value was calculated using equation (3) considering Rhodamine 6G in MeOH as a reference.

$$\sigma_{2new}(\lambda)\eta_{2new} = \frac{\Phi_{\text{cal}}\eta_{2\text{cal}}\sigma_{2\text{cal}}(\lambda)C_{\text{cal}}}{\Phi_{\text{new}}C_{\text{new}}}\frac{<P_{\text{cal}}(t)>^2}{<P_{\text{new}}(t)>^2}\frac{<F(t)>_{\text{new}}}{<F(t)>_{\text{cal}}}\frac{n_{\text{cal}}}{n_{\text{new}}} \text{ ..... (3)}$$

Where, σ_2 = Two Photon Absorption Cross Section

η = Quantum Efficiency

C =Fluorophore Concentration

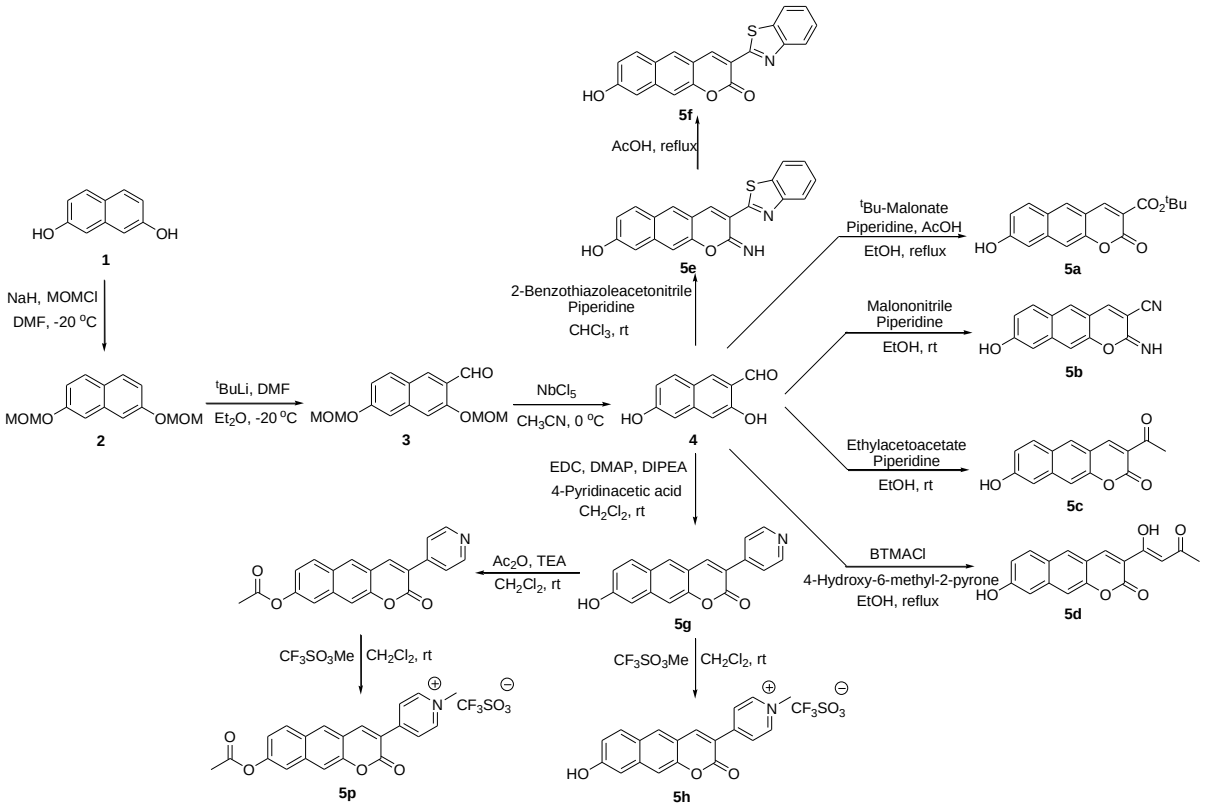
<P(t)> = Time Averaged Laser Power

<F(t)> = Time Averaged Fluorescecne Emission

n = Reflective Index of Sample

Φ = Fluorescence Collection Efficiency

Synthesis



Scheme S1 Synthesis of hydroxybenzocoumarin dyes **5a**–**5h** and esterase probe **5P** via key intermediate **4**.

2,7-Bis(methoxymethoxy)naphthalene (2). To a solution of compound **1** (3 g, 18.75 mmol) dissolved in anhydrous DMF (50 mL) was added NaH (1.87 g, 46.75 mmole) at −20 °C. The resulting mixture was stirred for 30 min, and then treated with chloromethyl methyl ether (3.56 mL, 46.75 mmol). The reaction mixture was set at room temperature and stirred until the starting material disappeared by following TLC. Then, it was quenched with saturated ammonium chloride and was subjected to an extractive work-up using ethyl acetate (three times). The combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by silica gel column chromatography using 2% ethyl acetate in hexane to afford compound **2** as a white solid (3.4 g, 73%).
^{1}H NMR (300 MHz, $CDCl_3$): δ 3.52 (s, 6H), 5.28 (s, 4H), 7.08 (dd, *J* = 9.0, 2.4 Hz, 2H), 7.31 (d, *J* = 2.4 Hz, 2H), 7.7 (d, *J* = 9.0 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 55.71, 94.18, 109.13, 116.74, 125.24, 128.94, 135.58, 155.43.

3,6-Bis(methoxymethoxy)-2-naphthaldehyde (3). Compound **2** (2 g, 8 mmol) dissolved in anhydrous diethyl ether (30 mL) was treated with *tert*-butyllithium (1.7 M in pentane, 5.93 mL, 10.1 mmol) dropwise over a period of 15 min at −20 °C. The resulting mixture was stirred at −20 °C for 30 min and then treated with anhydrous DMF (726 µL, 9.27 mmol) dropwise to give a pale brown suspension, which was stirred at the same temperature for 1 h. The reaction mixture was treated with ice water and then subjected to an extractive work-up using ethyl acetate (three times). The combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure to give a crude product, which was purified by silica gel column chromatography using 5% ethyl acetate in hexane as eluent to afford compound **3** as a white solid (1.11 g, 50%).

^{1}H NMR (300 MHz, $CDCl_3$): δ 3.52 (s, 3H), 3.56 (s, 3H), 5.3 (s, 2H), 5.39 (s, 2H), 7.1 (dd, *J* = 8.7, 2.1 Hz, 1H), 7.26 (s, 1H), 7.3 (d, *J* = 2.1 Hz, 1H), 7.38 (s, 1H), 7.79 (d, *J* = 9.0 Hz, 1H), 8.31 (s, 1H), 10.55 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 56.49, 56.69, 94.49, 94.99, 108.97, 109.51, 118.58, 124.4, 124.45, 130.71, 131.82, 139.36, 156.02, 156.06, 190.11.

3,6-Dihydroxy-2-naphthaldehyde (4). Compound **3** (600 mg, 2.17 mmol) dissolved in anhydrous acetonitrile (15 mL) was treated with $NbCl_5$ (1.23 g, 4.56 mmol) dissolved in the same solvent (10 mL) at -20 °C. The resulting mixture was stirred for 45 minutes to give a dark brown solution, which was quenched by adding a minimum volume of saturated $NaHCO_3$ solution. The mixture was extracted with ethyl acetate three times, and the combined organic phase was washed with brine, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel using 5% to 20% ethyl acetate in hexane to afford **4** as a bright yellow solid (220 mg, 54%).
^{1}H NMR (300 MHz, MeOD): δ 6.9–6.94 (m, 3H), 7.75 (d, *J* = 9.0 Hz, 1H), 8.13 (s, 1H), 10.01 (s, 1H). ^{13}C NMR (75 MHz, MeOD): δ 108.46, 110.19, 118.51, 121.99, 124.18, 132.95, 138.29, 142.15, 157.85, 160.96, 197.28.

***tert*-Butyl 8-hydroxy-2-oxo-2H-benzo[g]chromene-3-carboxylate (5a).** A solution of compound **4** (40 mg, 0.21 mmole), di-*tert*-butyl malonate (18 µL, 0.234 mmole), 10 mole% piperidine, and one drop of acetic acid in dry ethanol (3 mL) was heated to reflux for 10 h. Then the solvent was evaporated and the residue was purified by column chromatography using ethyl acetate (from 20% to 50%) in dichloromethane as eluent to afford compound **5a** as a yellow solid (50 mg, 76%).
^{1}H NMR (300 MHz, DMSO): δ 1.54 (s, 9H), 7.14 (dd, *J* = 9, 2.1 Hz, 1H), 7.19 (s, 1H), 7.63 (s, 1H), 7.93 (d, *J* = 9 Hz, 1H), 8.39 (s, 1H), 8.69 (s, 1H), 10.45 (s, 1H). ^{13}C NMR (75 MHz, DMSO): δ 27.76, 81.66, 108.08, 109.57, 115.12, 117.54, 119.43, 124.72, 131.21, 131.44, 137.76, 148.22, 150.84, 156.43, 158.63, 161.94. HRMS (EI positive): m/z: calculated for $C_{18}H_{16}O_5$ $[M]^+$ 312.0998; found 312.0996.

8-Hydroxy-2-imino-2H-benzo[g]chromene-3-carbonitrile (5b). Compound **4** (40 mg, 0.21 mmole) dispersed in dry ethanol (3 mL) was treated with malononitrile (7.7 mg, 0.234 mmole) followed by 10 mole% piperidine. The reaction mixture was stirred at room temperature for 1 h to give a turbid, orange solid. The solid was filtered under vacuum and washed with cold ethanol followed by hexane to afford compound **5b** as an orange solid (42 mg, 85%).
^{1}H NMR (300 MHz, DMSO): δ 7.07 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.13 (d, *J* = 1.8 Hz, 1H), 7.87 (d, *J* = 8.7 Hz, 1H), 8.05 (s, 1H), 8.43 (s, 1H), 8.89 (d, *J* = 1.2, 1H), 10.27 (s, 1H). ^{13}C NMR (75 MHz, DMSO): δ 102.59, 108.37, 109.1, 114.85, 115.62, 118.94, 124.25, 130.58, 131.12, 147.03, 150.08, 151.71, 158.6. HRMS (EI positive): m/z: calculated for $C_{14}H_8N_2O_2$ $[M]^+$ 236.0586; found 236.0583.

3-Acetyl-8-hydroxy-2H-benzo[*g*]chromen-2-one (5c). A solution of compound **4** (30 mg, 0.16 mmol) in dry ethanol (3 mL) was treated with diethylmalonate (30 ml, 0.24 mmol) and 10 mole% piperidine, and the resulting mixture was stirred for 4 h at room temperature. The reaction mixture was treated with hexane to provide precipitates, which were filtered under reduced pressure, and the filtrate was washed with 10% ethyl acetate in hexane to afford compound **5c** as an orange-yellow solid (37 mg, 90%).
^{1}H NMR (300 MHz, DMSO): δ 2.59 (s, 3H), 7.14 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.2 (d, *J* = 2.1 Hz, 1H), 7.66 (s, 1H), 7.94 (d, *J* = 9.0 Hz, 1H), 8.45 (s, 1H), 8.7 (s, 1H). ^{13}C NMR (75 MHz, DMSO): δ 30.18, 108.2, 109.59, 115.43, 119.57, 123.05, 124.89, 131.34, 132.42, 138, 147.63, 151.01, 158.85, 158.89, 194.95 . HRMS (EI positive): m/z: calculated for $C_{15}H_{10}O_4$ $[M]^+$ 254.0579; found 254.0577.

(*Z*)-8-Hydroxy-3-(1-hydroxy-3-oxobut-1-enyl)-2H-benzo[*g*]chromen-2-one (5d). A mixture of compound **4** (30 mg, 0.16 mmol), 4-hydroxy-6-methyl-2-pyrone (24 mg, 0.19 mmol), and benzyltrimethylammonium chloride (9 mg, 0.04 mmol) in dry ethanol (3 mL) was refluxed for 15 h. Precipitates were produced, which were filtered and washed with water and then with dichloromethane to afford compound **5d** as a yellow solid (39 mg, 85%).
^{1}H NMR (300 MHz, DMSO): δ 2.23 (d, *J* = 3.9 Hz, 3H), 6.91 (s, 1H), 7.15 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.21 (d, *J* = 2.1 Hz, 1H), 7.68 (d, *J* = 3.3 Hz, 1H), 7.94 (d, *J* = 9.0 Hz, 1H), 8.47 (s, 1H), 8.87 (s, 1H), 10.51 (s, 1H).^{13}C NMR (75 MHz, DMSO): δ 2686, 10079, 108.17, 109.5, 115.59, 118.18, 119.55, 124.87, 131.98, 146.59, 150.44, 157.72, 158.83, 173.79, 198.41.HRMS (EI positive): m/z: calculated for $C_{17}H_{12}O_5$ $[M]^+$ 296.0685; found 296.0686.

3-(Benzo[*d*]thiazol-2-yl)-2-imino-2H-benzo[*g*]chromen-8-ol (5e).A solution of compound **4** (40 mg, 0.21 mmol), benzothiazole-2-acetonitrile (48 mg, 0.27 mmol), and 10 mole% piperidine in dry ethanol (3 mL) was stirred for 5 h at room temperature. Precipitates resulted, which were filtered under vacuum and washed with hexane and then with chloroform to afford compound **5e** as a yellowish-brown solid (64 mg, 87%).
^{1}H NMR (300 MHz, DMSO): *δ* 7.08 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.15 (d, *J* = 1.8 Hz, 1H), 7.41–7.57 (m, 3H), 7.84 (d, *J* = 9.0 Hz, 1H), 8.07 (d, *J* = 7.8 Hz, 1H), 8.13 (d, *J* = 7.8 Hz, 1H), 8.29 (s, 1H), 8.81 (s, 1H), 9.17 (d, *J* = 1.8 Hz, 1H), 10.24 (s, 1H). ^{13}C NMR (75 MHz, DMSO): *δ* 108.31, 108.42, 116.47, 118.6, 120.5, 121.91, 122.33, 124.95, 126.29, 130.59, 135.71, 136.87, 136.9, 150.08, 151.76, 153.37, 157.88, 161.04. HRMS (FAB positive): m/z: calculated for $C_{20}H_{13}N_2O_2S$ $[M+1]^+$ 345.0692; found 345.0695.

3-(Benzo[*d*]thiazol-2-yl)-8-hydroxy-2H-benzo[*g*]chromen-2-one (5f). Compound **5f** (40 mg, 0.116 mmol) in glacial acetic acid (3 mL) was refluxed for 3 h. The reaction mixture was concentrated, and the residue was treated with ethyl acetate and hexane (1:1) to give precipitates, which were filtered and washed with hexane to afford compound **5f** as a saffron solid (32 mg, 80%).
^{1}H NMR (300 MHz, DMSO): *δ* 7.17 (dd, *J* = 9.0, 2.4 Hz, 1H), 7.24 (d, *J* = 2.1 Hz, 1H), 7.45–7.51 (m, 2H), 7.56 (d, *J* = 1.2 Hz, 1H), 7.6 (dd, *J* = 7.2, 1.2 Hz, 1H), 7.77–8.56 (m, 2H), 9.28 (s, 1H), 10.48 (s, 1H). ^{13}C NMR (75 MHz, DMSO): *δ* 108.23, 109.88, 115.82, 117.82, 122.18, 119.59, 122.18, 122.43, 125.06, 125.62, 126.62, 131.1, 131.38, 135.88, 137.43, 142.42, 150.06, 151.96, 158.55, 159.74, 160.05. HRMS (EI positive): m/z: calculated for $C_{20}H_{11}NO_3S$ $[M]^+$ 345.0460; found 345.0456.

8-Hydroxy-3-(pyridin-4-yl)-2H-benzo[*g*]chromen-2-one (5g). To a mixture of compound **4** (100 mg, 0.53 mmol) and 4-pyridineacetic acid hydrochloride (138 mg, 0.8 mmol) suspended in dichloromethane (30 ml), was added triethylamine (370 µL, 2.65 mmol) followed by 4-dimethylaminopyridine (6.5 mg, 0.053 mmol) and EDC (153 mg, 0.8 mmol), and the mixture was stirred at room temperature overnight. Yellow precipitates formed were filtered under reduced pressure, and the filtrate was washed thoroughly with water and then with ethyl acetate to afford compound **5g** as a yellow solid (130 mg, 87%).
^{1}H NMR (300 MHz, DMSO): *δ* 7.14 (dd, *J* = 9.0, 2.1 Hz, 1H), 7.21 (s, 1H), 7.69 (s, 1H), 7.78 (d, *J* = 6.0 Hz, 2H), 7.95 (d, *J* = 9 Hz, 1H), 8.27 (s, 1H), 8.53 (s, 1H), 8.67 (d, *J* = 6 Hz, 2H), 10.33 (s, 1H). ^{13}C NMR (75 MHz, DMSO): δ 108.05, 109.54, 116.35, 119.3, 122.75, 124.79, 129.59, 130.72, 136.76, 142.21, 142.25, 142.76, 149.67, 150.26, 157.93, 159.28. HRMS (EI positive): m/z: calculated for $C_{18}H_{11}NO_3$ $[M]^+$ 289.0739; found 289.074.

8-Hydroxy-3-(*N*-methyl-pyridinum-4-yl)-2*H*-benzo[*g*]chromen-2-one trifluorosulfonate (5h). To a solution of compound **5g** (100 mg, 0.346 mmol) in dichloromethane, was added methyl trifluoromethanesulfonate (80 ml, 0.7 mmol) dropwise. The reaction mixture was allowed to stir for 7 h at room temperature.

Precipitates formed were filtered under reduced pressure, and the filtrate was washed with ethyl acetate and then with hexane to afford compound **5h** as an orange solid (130 mg, 87%).
^{1}H NMR (300 MHz, MeOD): δ 4.4 (s, 3H), 7.13–7.18 (m, 2H), 7.59 (s, 1H), 7.93 (d, *J* = 9 Hz, 1H), 8.3 (s, 1H), 8.57 (s, 1H), 8.6 (s, 1H), 8.82 (s, 1H), 8.87 (s, 1H), 8.89 (s, 1H). ^{13}C NMR (75 MHz, DMSO): δ 47.38, 108.24, 109.76, 109.87, 116.01, 119.46, 119.66, 124.93, 125.61, 131.25, 131.37, 137.78, 145.12, 146.53, 150.07, 150.41, 158.83. HRMS (FAB positive): m/z: calculated for $C_{19}H_{14}NO_3$ (cationic part only) $[M]^+$ 304.0974; found 304.336.

4-(8-Acetoxy-2-oxo-2*H*-benzo[*g*]chromen-3-yl)-1-methylpyridinium trifluoromethanesulfonate (5P). A suspension of compound **5g** (40 mg, 0.14 mmol) and triethylamine (58 µL, 0.42 mmol) in dichloromethane was treated with acetic anhydride (16 µL, 0.17 mmol), and the resulting mixture was allowed to stir for 5 h at room temperature. Finally, the solvent was evaporated to give white precipitates, which were dissolved in dichloromethane and then treated with methyl trifluoromethanesulfonate (80 µL, 0.7 mmol). The mixture was stirred at room temperature for 6 h to give precipitates, which were filtered under reduced pressure and washed with ethyl acetate and then with dichloromethane to afford compound **5P** as a bright greenish-yellow solid (50 mg, 72%).
^{1}H NMR (300 MHz, DMSO): δ 2.36 (s, 3H), 4.36 (s, 3H), 7.43 (dd, *J* = 9, 2.4 Hz, 1H), 7.84 (d, *J* = 2.1 Hz, 1H), 8.02 (s, 1H), 8.24 (d, *J* = 9.0 Hz, 1H), 8.52 (s, 1H), 8.54 (s, 2H), 8.95 (s, 1H), 9.05 (s, 1H), 9.07 (s, 1H). ^{13}C NMR (75 MHz, DMSO): δ 20.92, 47.5, 111.82, 118.32, 118.61, 121.92, 122.28, 126.02, 127.86, 130.82, 130.98, 135.81, 145.71, 149.79, 150.27, 150.78, 158.47, 169.22. HRMS (FAB positive): m/z: calculated for $C_{21}H_{16}NO_4$ (cationic part only) $[M]^+$ 346.1079; found 346.301.

UV-Vis absorption spectra of 5a–5h

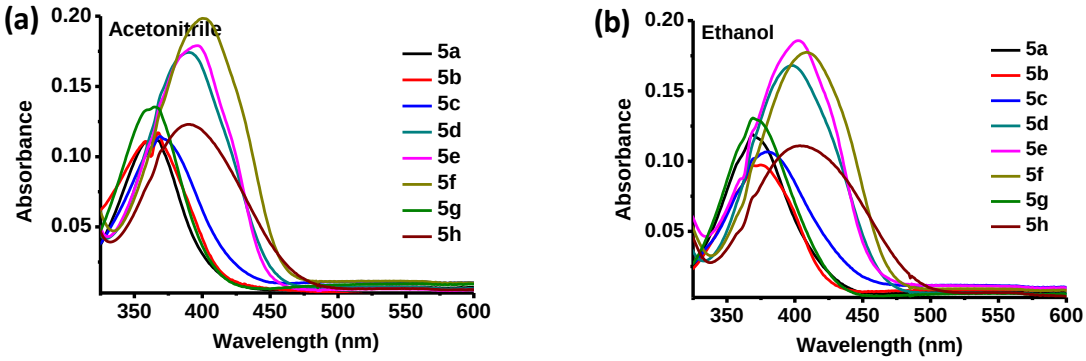


Fig. S1 UV-Vis absorption spectra of compounds **5a–5h** in a) acetonitrile and in b) ethanol. All the measurements were conducted at 5 µM concentration of each compound in a cuvette with 1-cm path length.

Fluorescence emission spectra of 5a–5h

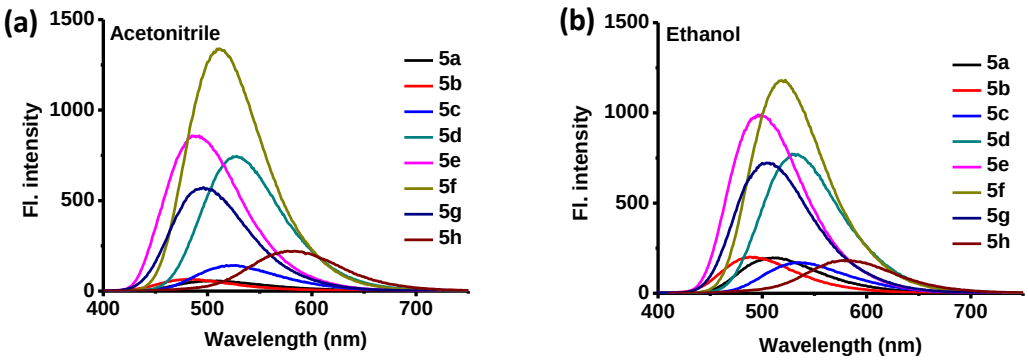


Fig. S2 Fluorescence emission spectra of compounds **5a–5h** in a) acetonitrile and in b) ethanol upon excitation at their respective maximum absorption wavelength. All the measurements were conducted at 5 µM concentration of each compound in a cuvette with 1-cm path length and the slit was fixed at 2 nm.

Lippert-Mataga plot of 5a–5h

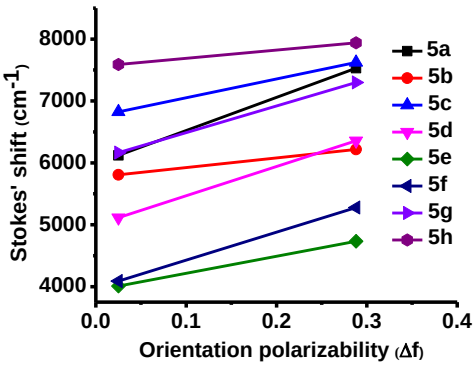


Fig. S3 Lippert-Mataga plot for **5a–5h** constructed from the Stokes shift data obtained in 1,4-dioxane and acetonitrile.

Aqueous solubility data of 5h

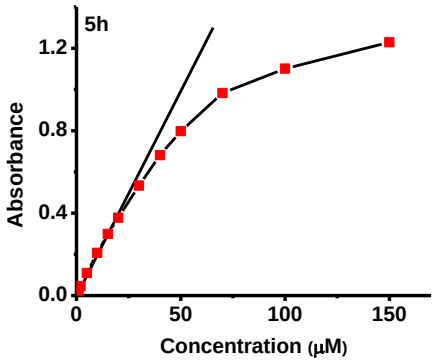


Fig. S4 Solubility of **5h** in PBS 7.4 buffer.

Photostability of 5a–5h

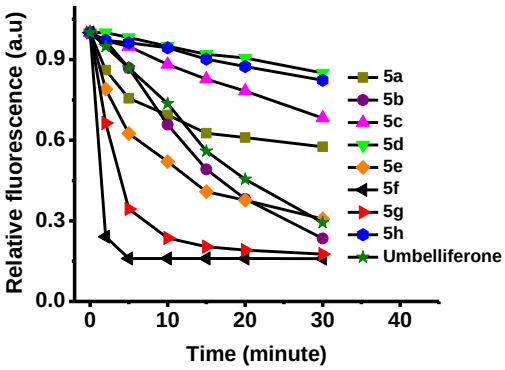


Fig. S5 Photostability of **5a–5h** in PBS 7.4 in comparison to Umbelliferone. Dyes were irradiated with 365 nm UV light in a WUV-L10 chamber up to 30 minutes.

Table S1 Molar absorptivity of **5a–5h** in different solvents.

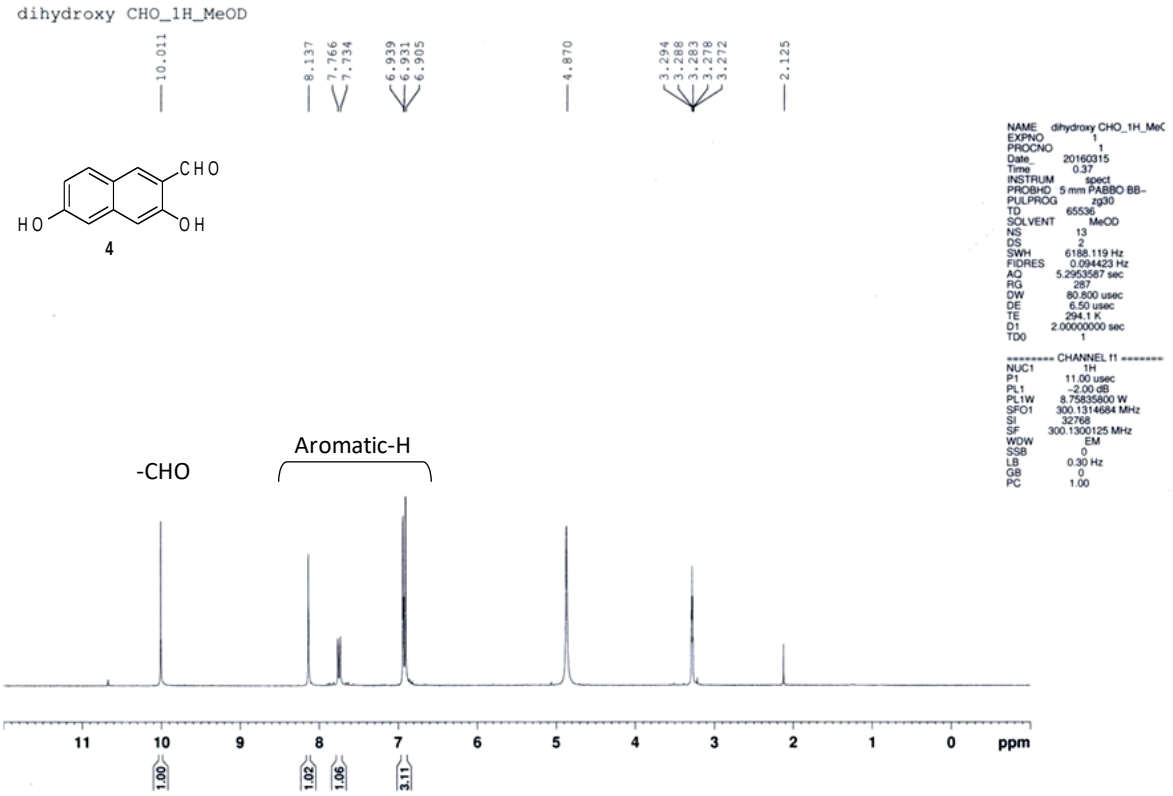
		5a	5b	5c	5d	5e	5f	5g	5h
Molar absorptivity (ε) (L Mol^{-1} cm^{-1})	1,4-Dioxane	22200	19720	21600	35600	36800	37200	28400	26200
	Acetonitrile	22200	23400	22800	34800	35800	39600	27000	24600
	Ethanol	23800	19400	21200	33600	37000	35400	26000	22200
	PBS 7.4	15900	10100	10700	13400	13500	11300	4800	17200

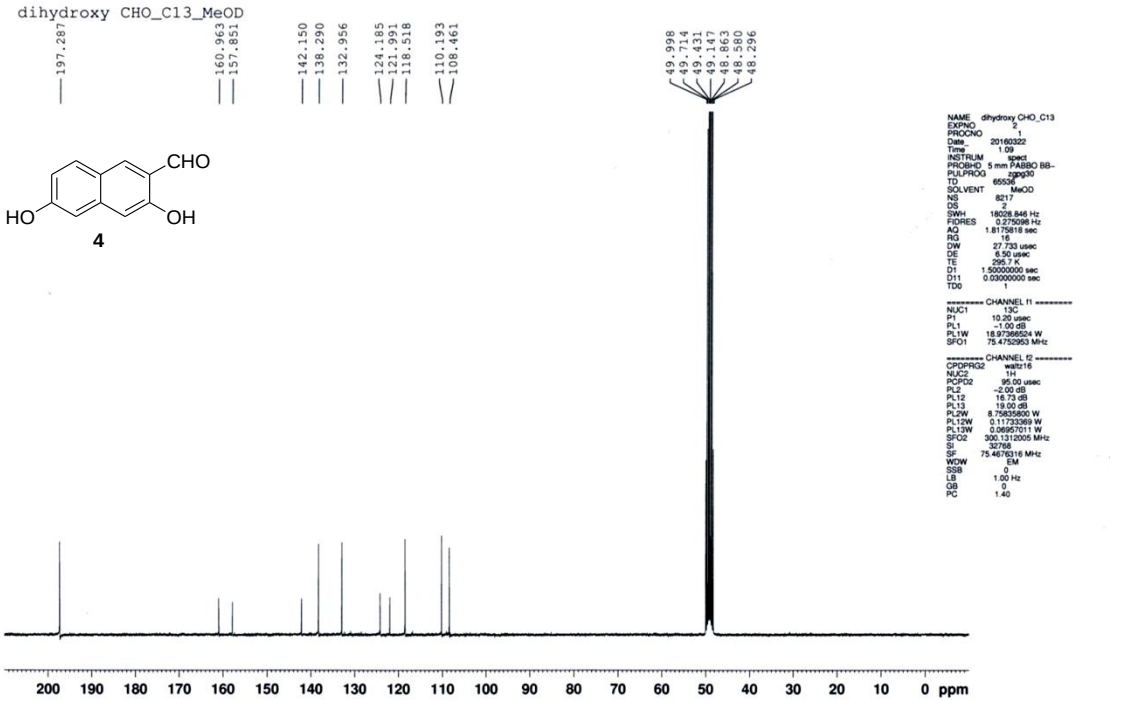
Table S2 Two-photon absorption cross section (TPACS) values in acetonitrile[a]

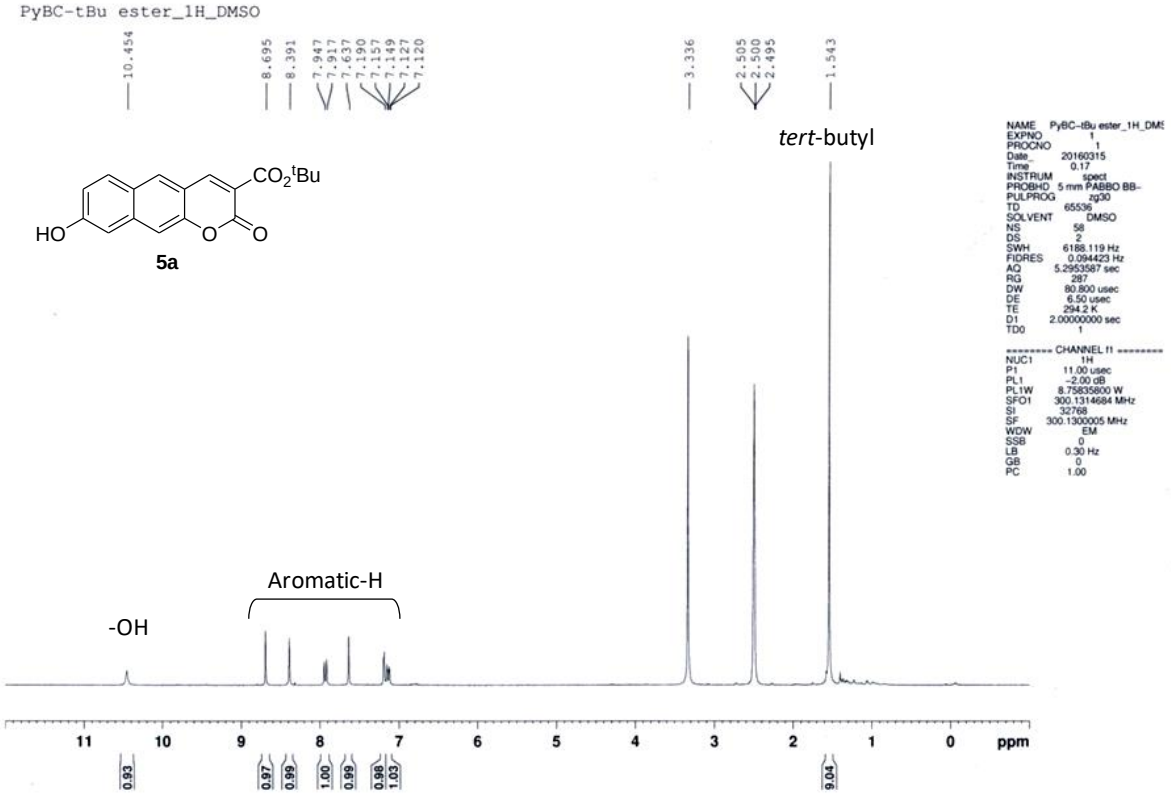
		780 nm	800 nm	820 nm
TPACS value (in GM)	**5f**	48	77	75
	5h	56	92	85

[a]Calculated considering Rhodamine 6G in methanol as reference.

^{1}H & ^{13}C NMR data



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7.120
3.336
2.505
2.500
2.495
1.543
tert-butyl
CO2tBu
HO
O
O
5a
Aromatic-H
-OH
11
10
9
8
7
6
5
4
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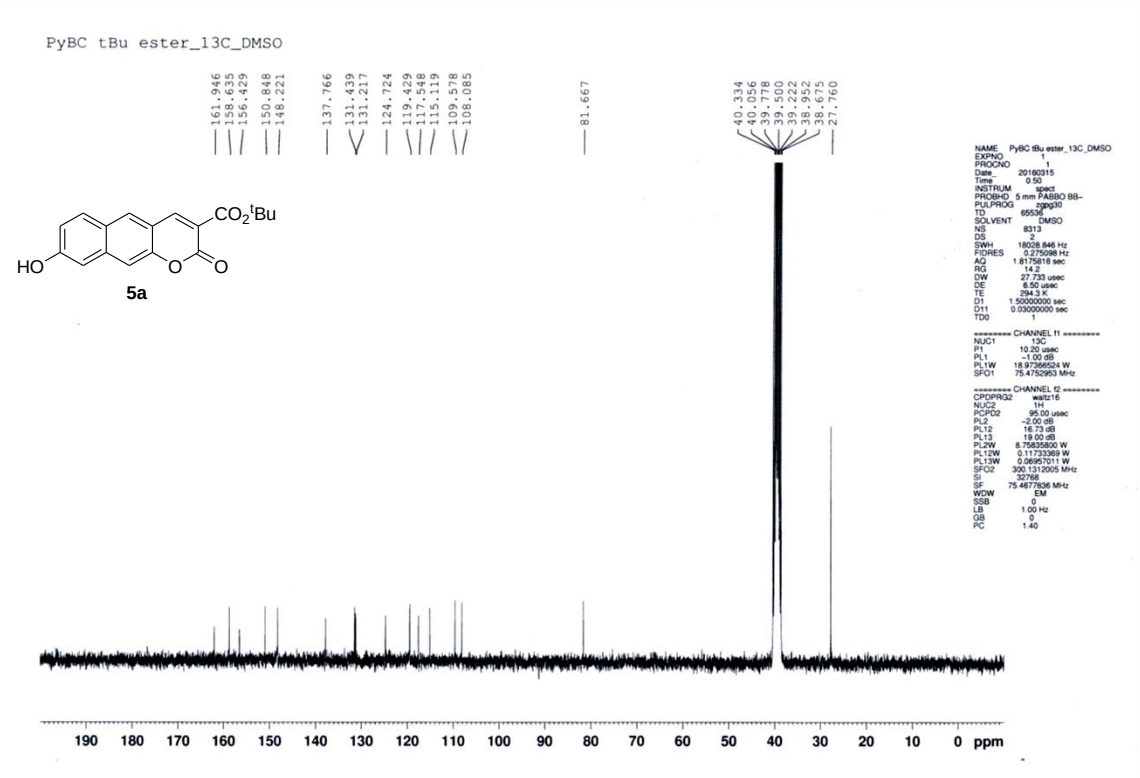
0.99
0.98
1.03
9.04


PyBC tBu ester_13C_DMSO
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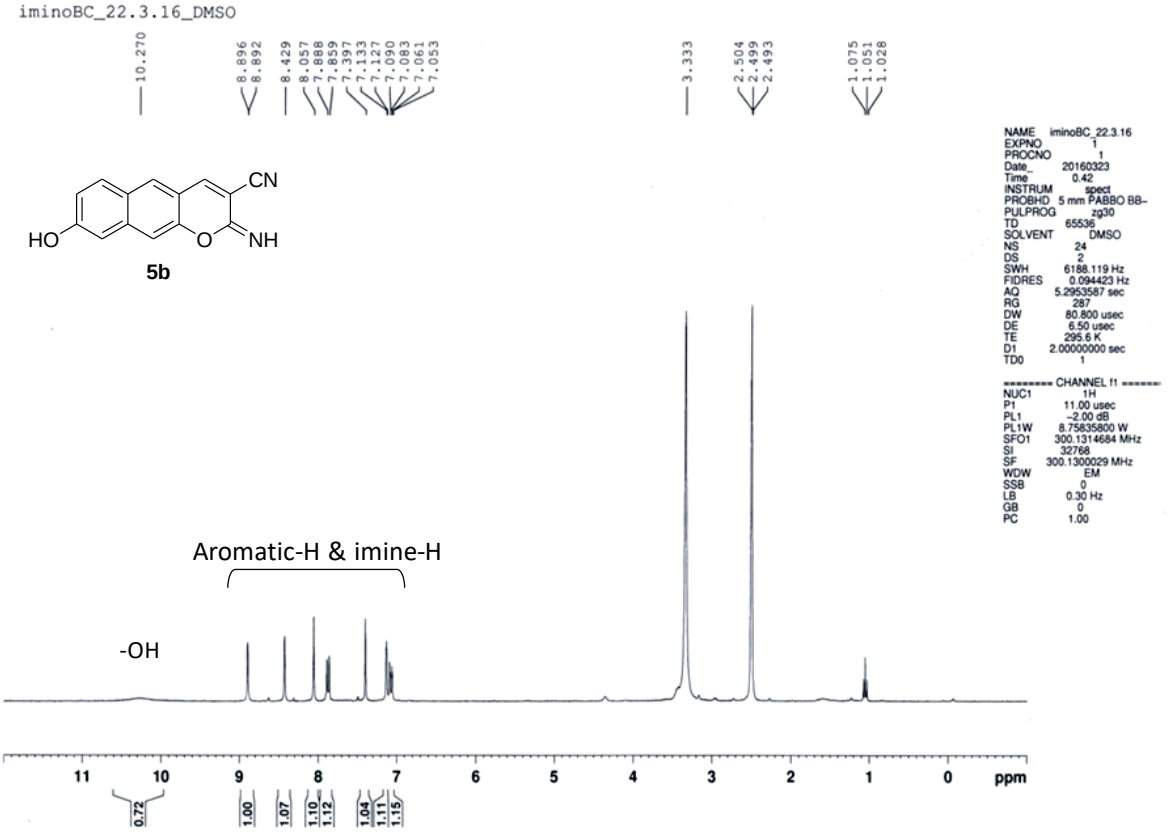
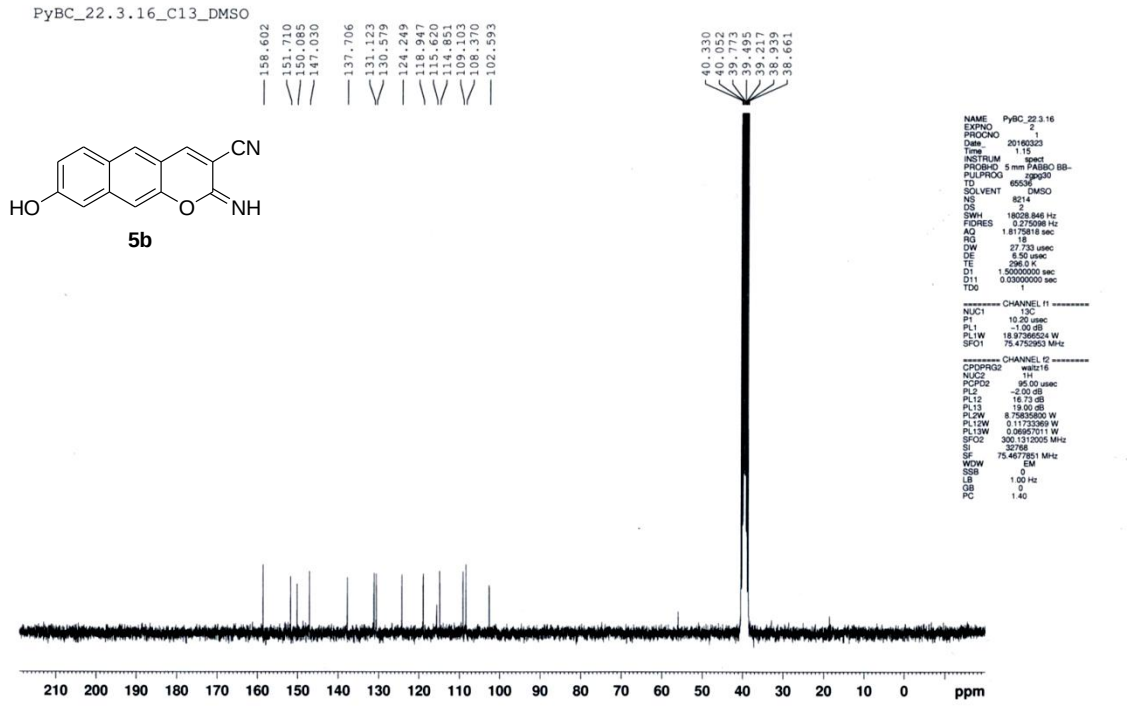
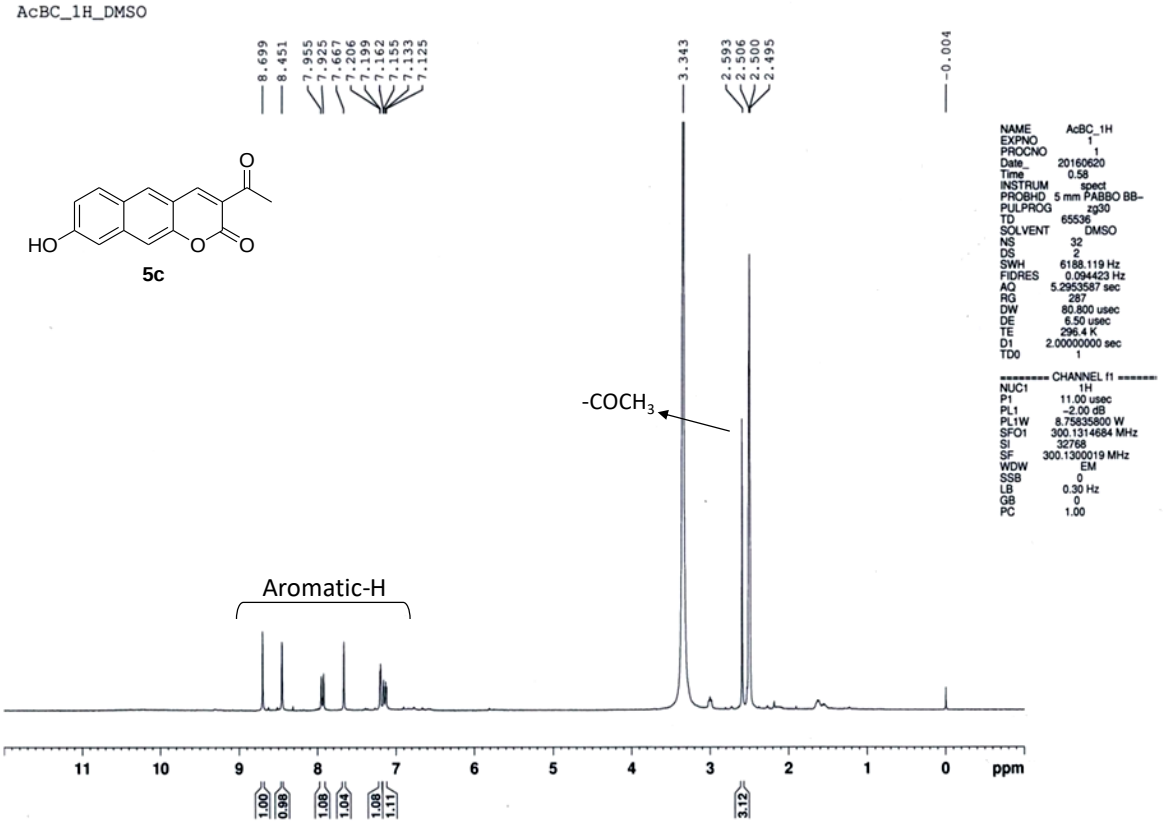
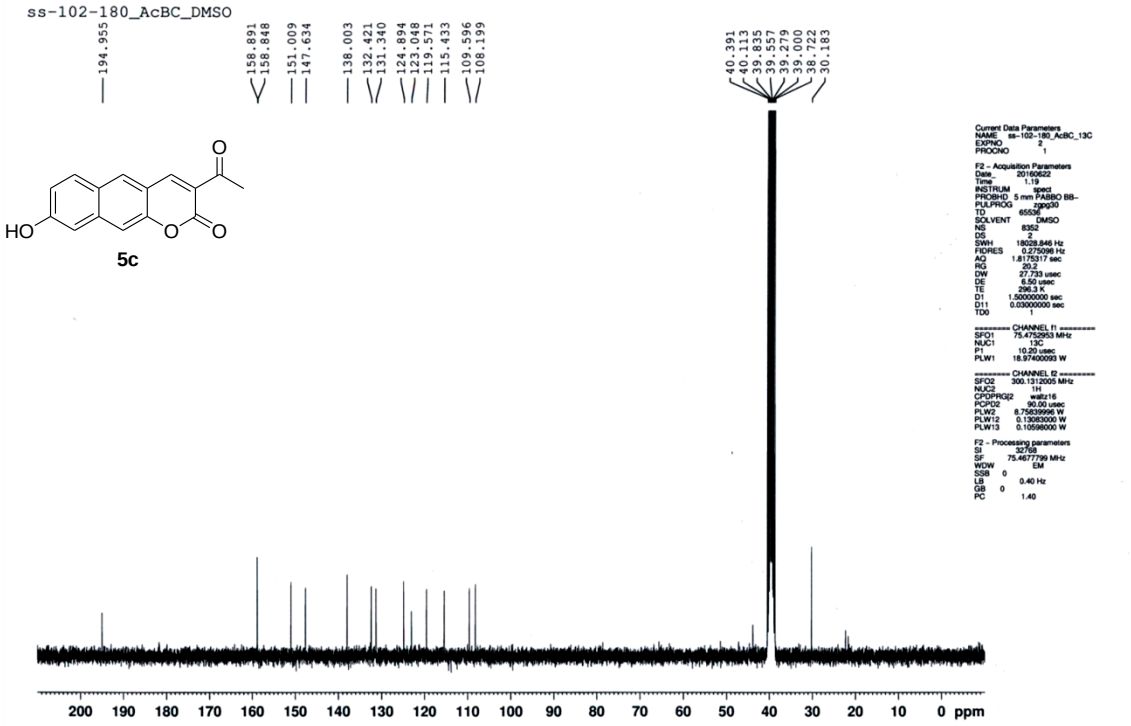
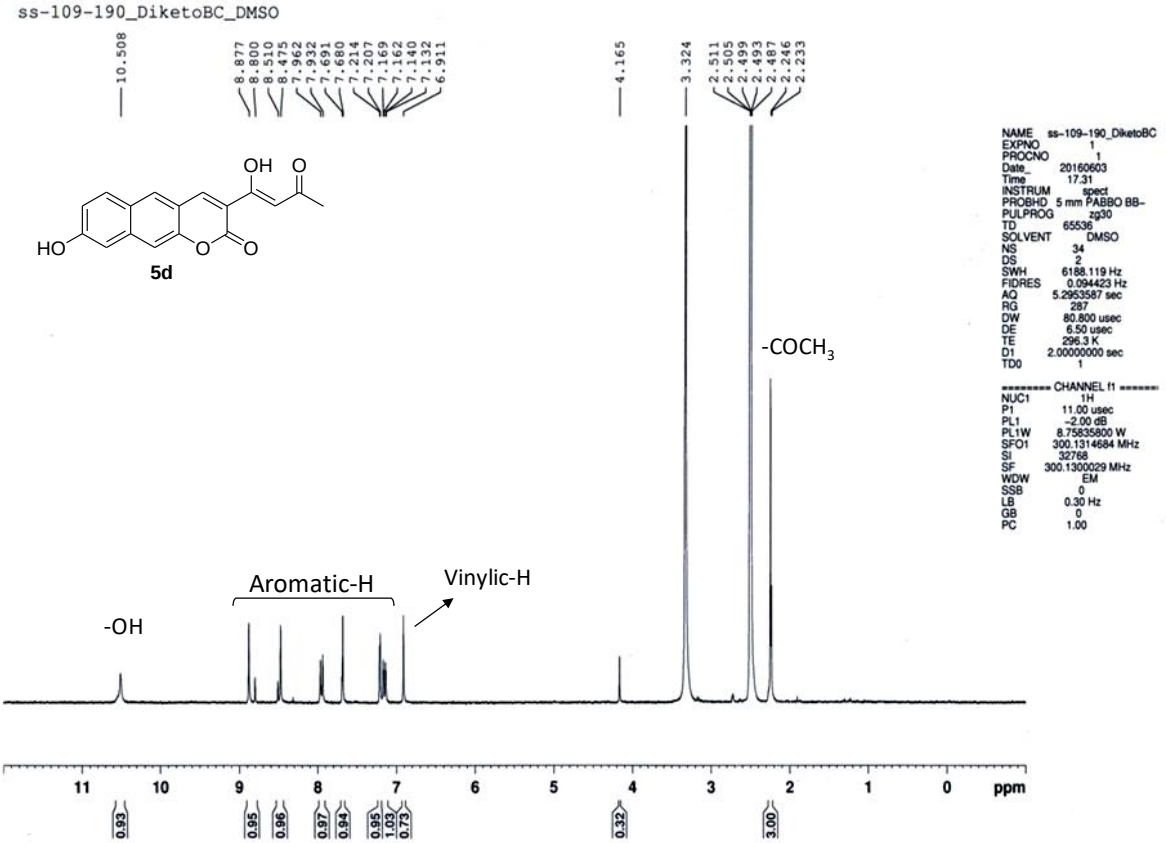
131.439
131.217
124.724
119.429
117.548
115.119
109.578
108.085
81.667
40.334
40.056
39.778
39.500
39.222
38.952
38.675
27.760
CO2tBu
HO
O
O
5a
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

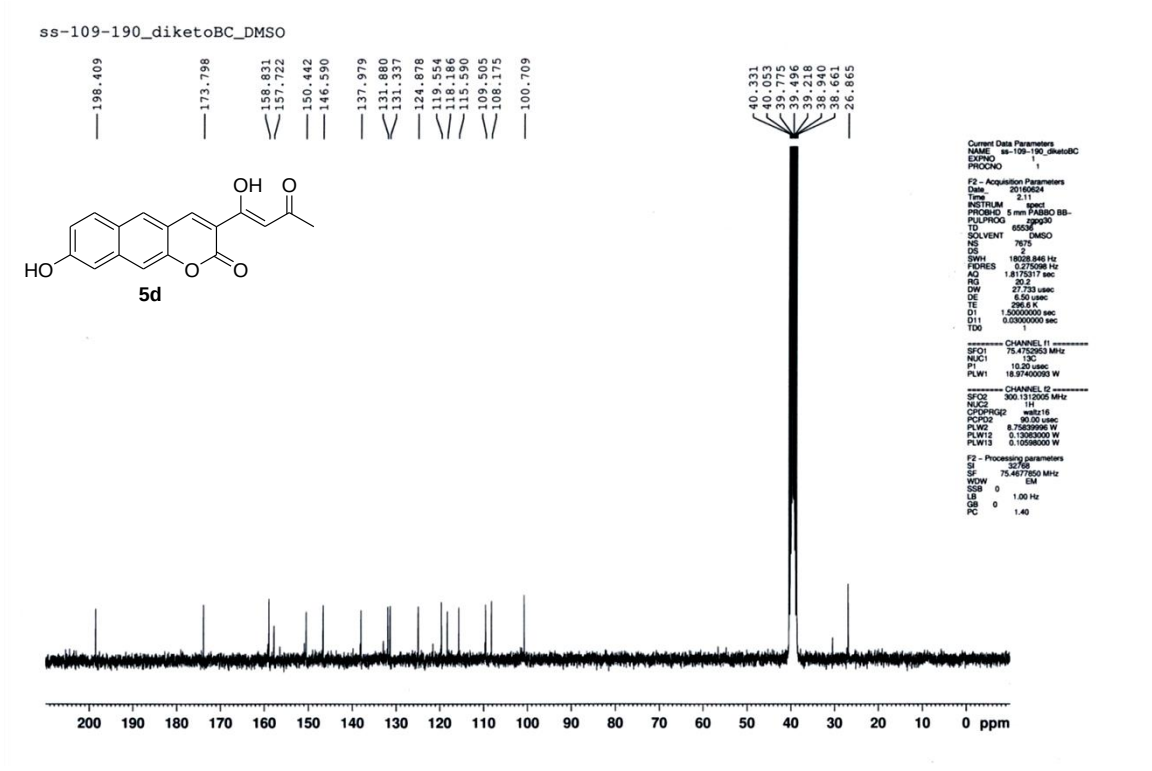

iminoBC_22.3.16_DMSO
10.270
8.896
8.892
8.429
8.057
7.888
7.859
7.397
7.133
7.127
7.090
7.083
7.061
7.053
3.333
2.504
2.499
2.493
1.075
1.051
1.028
CN
HO
O
NH
5b
NAME iminoBC_22.3.16
EXPNO 1
PROCNO 1
Date_ 20160323
Time 0.42
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 24
DS 2
SWH 6188.119 Hz
FIDRES 0.094423 Hz
AQ 5.2953587 sec
RG 287
DW 80.800 usec
DE 6.50 usec
TE 295.6 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 11.00 usec
PL1 -2.00 dB
PL1W 8.75835800 W
SFO1 300.1314684 MHz
SI 32768
SF 300.1300029 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Aromatic-H & imine-H
-OH
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.72
1.00
1.07
1.10
1.12
1.04
1.11
1.15

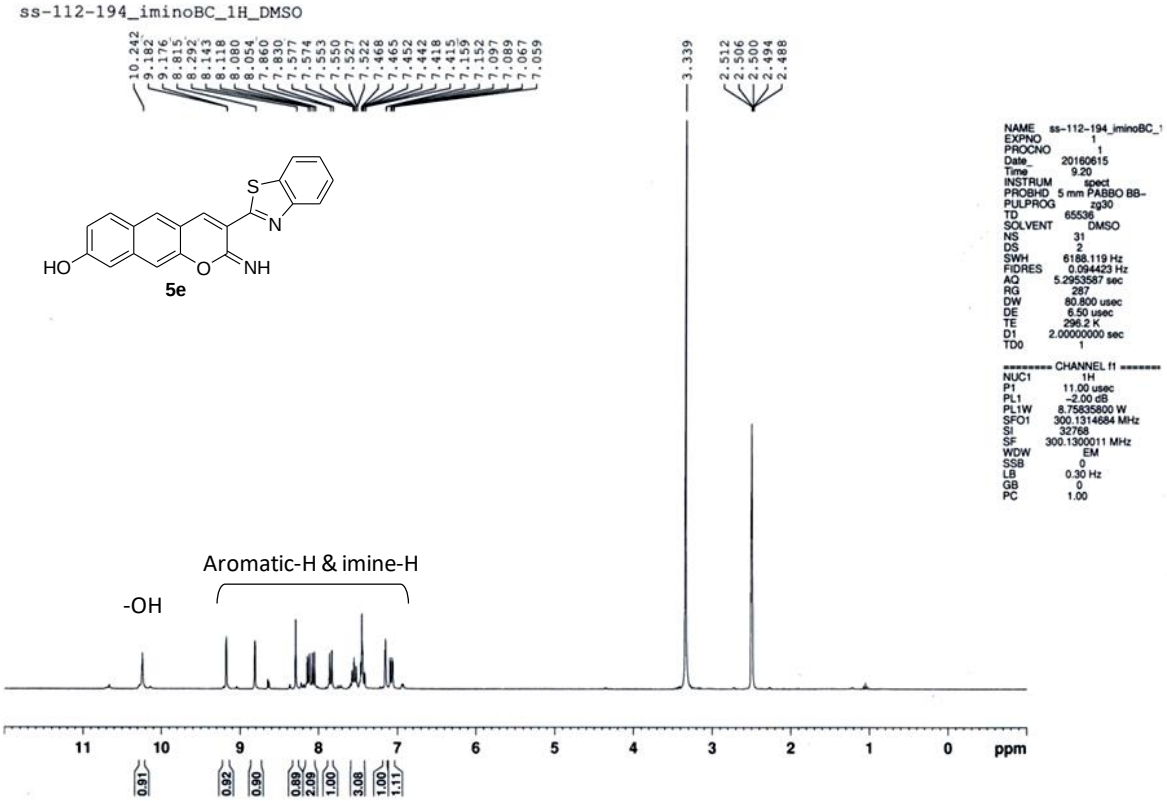

PyBC_22.3.16_C13_DMSO
158.602
151.710
150.085
147.030
137.706
131.123
130.579
124.249
118.947
115.620
114.851
109.103
108.370
102.593
40.330
40.052
39.773
39.495
39.217
38.939
38.661
CN
HO
O
NH
5b
NAME PyBC_22.3.16
EXPNO 2
PROCNO 1
Date_ 20160323
Time 1.15
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 8214
DS 2
SWH 18028.846 Hz
FIDRES 0.275098 Hz
AQ 1.8175818 sec
RG 18
DW 27.733 usec
DE 6.50 usec
TE 296.0 K
D1 1.50000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 10.20 usec
PL1 -1.00 dB
PL1W 18.97366524 W
SFO1 75.4752953 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 95.00 usec
PL2 -2.00 dB
PL12 16.73 dB
PL13 19.00 dB
PL2W 8.75835800 W
PL12W 0.11733369 W
PL13W 0.06957011 W
SFO2 300.1312005 MHz
SI 32768
SF 75.4677851 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

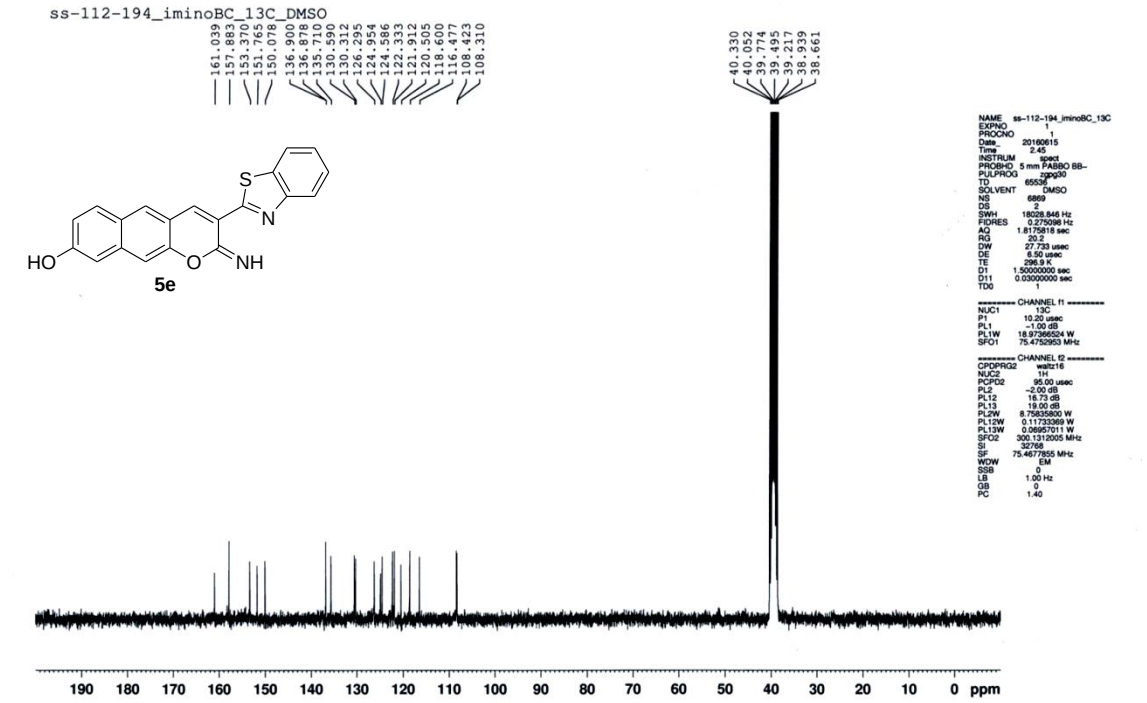

AcBC_1H_DMSO
8.699
8.451
7.955
7.925
7.667
7.206
7.199
7.162
7.155
7.133
7.125
3.343
2.593
2.506
2.500
2.495
-0.004
HO
O
O
O
5c
-COCH3
Aromatic-H
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
0.98
1.08
1.04
1.08
1.11
3.12

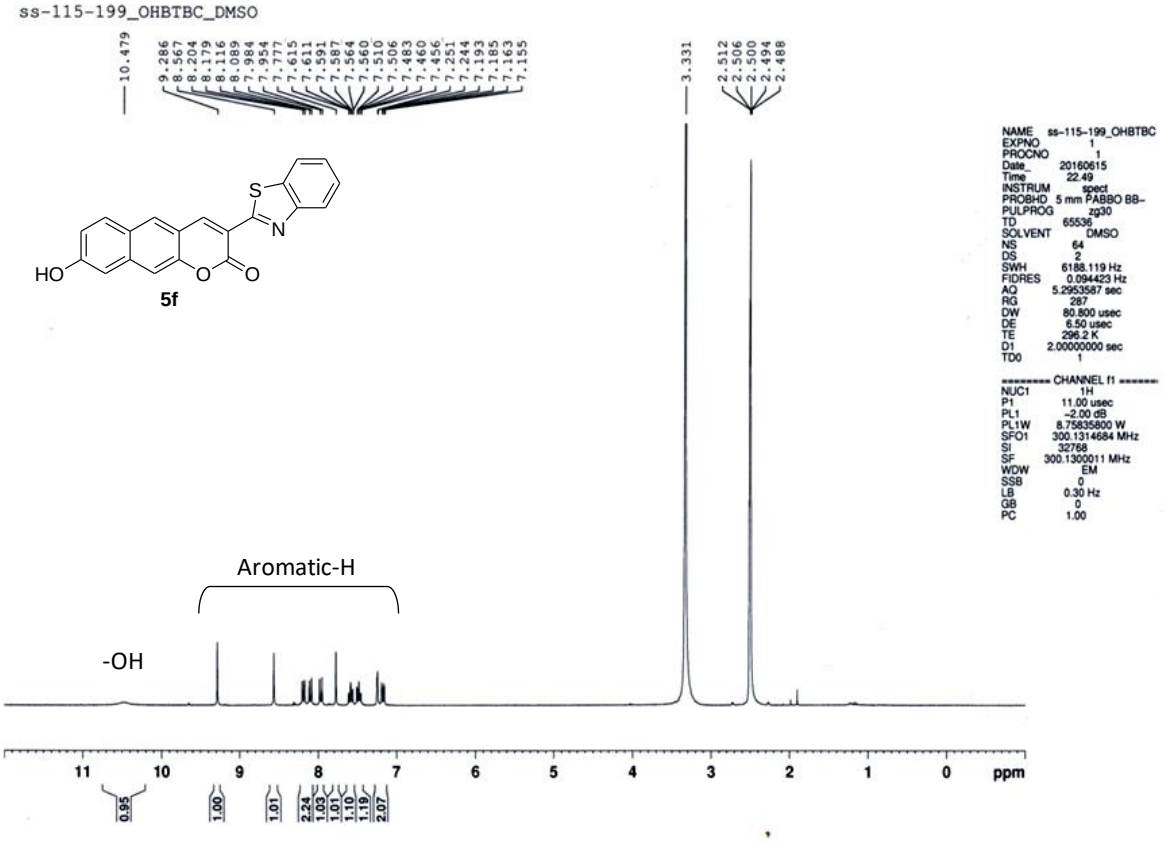

ss-102-180_AcBC_DMSO
194.955
158.891
158.848
151.009
147.634
138.003
132.421
131.340
124.894
123.048
119.571
115.433
109.596
108.199
40.391
40.113
39.835
39.557
39.279
39.000
38.722
30.183
HO
O
O
O
5c
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

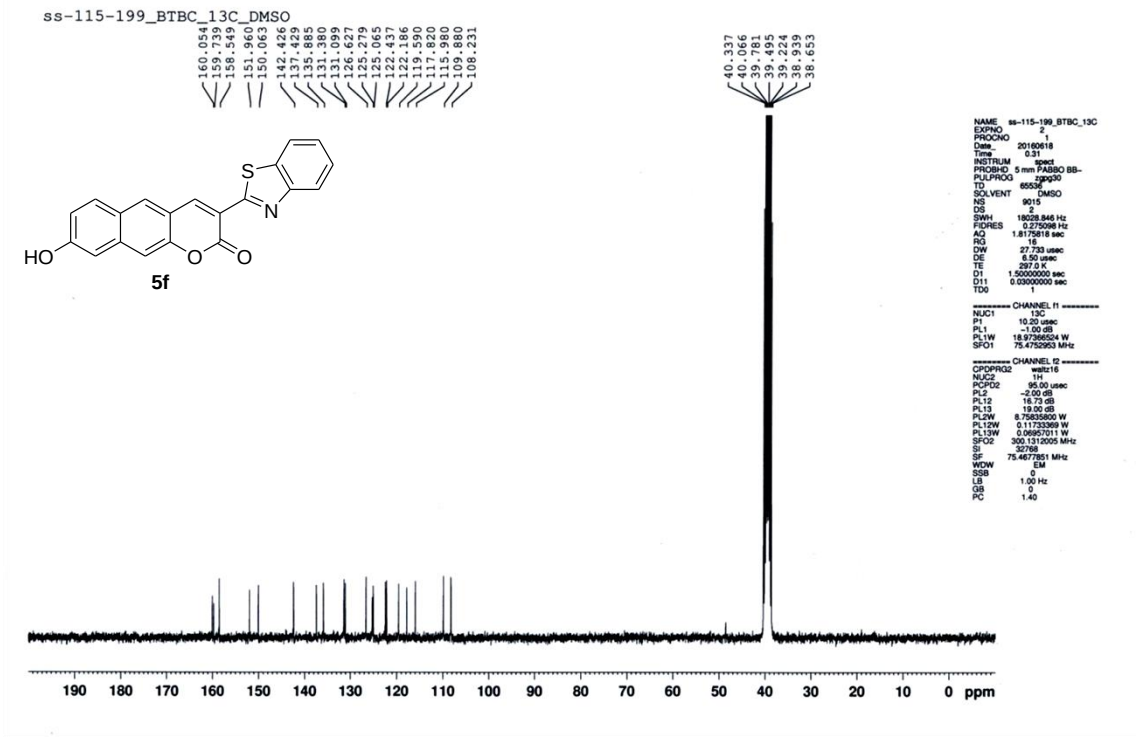

ss-109-190_DiketoBC_DMSO
10.508
8.877
8.800
8.510
8.475
7.962
7.932
7.691
7.680
7.214
7.207
7.169
7.162
7.140
7.132
6.911
4.165
3.324
2.511
2.505
2.499
2.493
2.487
2.246
2.233
OH
O
HO
O
O
5d
-COCH3
-OH
Aromatic-H
Vinylic-H
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.93
0.95
0.96
0.97
0.94
0.95
1.03
0.73
0.32
3.00

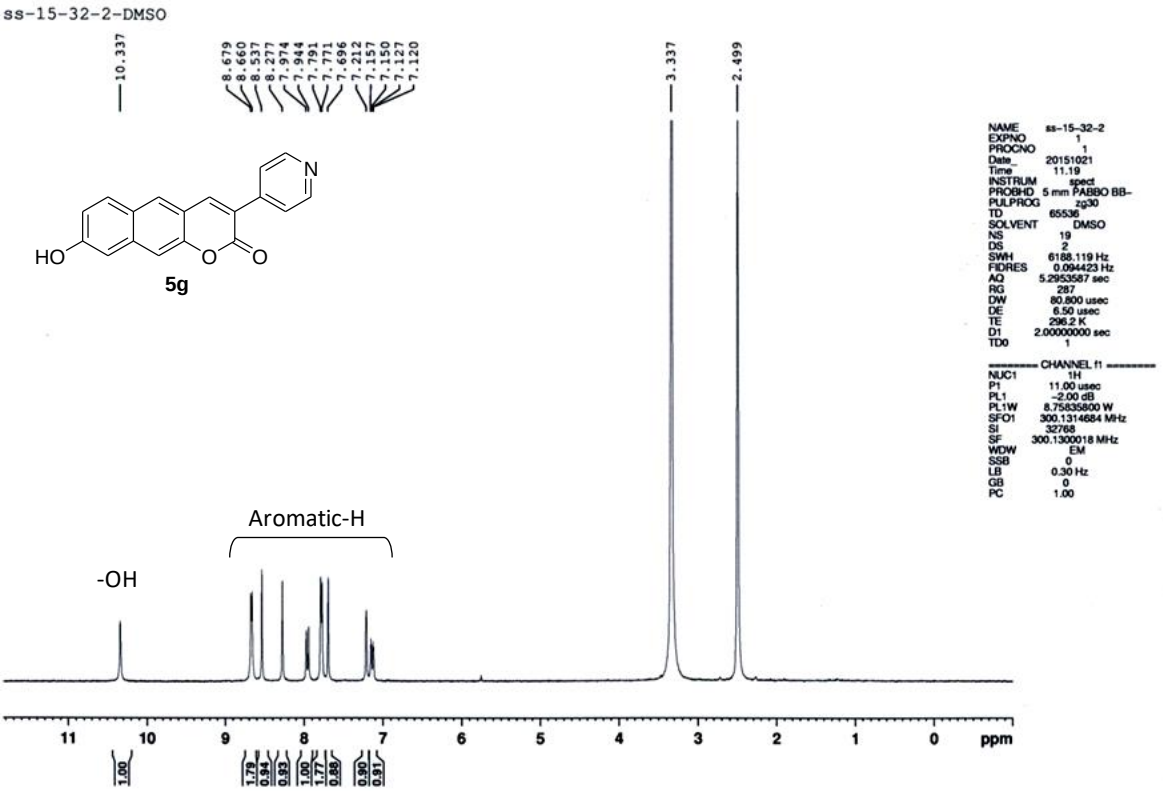

ss-109-190_diketoBC_DMSO
198.409
173.798
158.831
157.722
150.442
146.590
137.979
131.880
131.337
124.878
119.554
118.186
115.590
109.505
108.175
100.709
40.331
40.053
39.775
39.496
39.218
38.940
38.661
26.865
OH
O
HO
O
O
5d
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

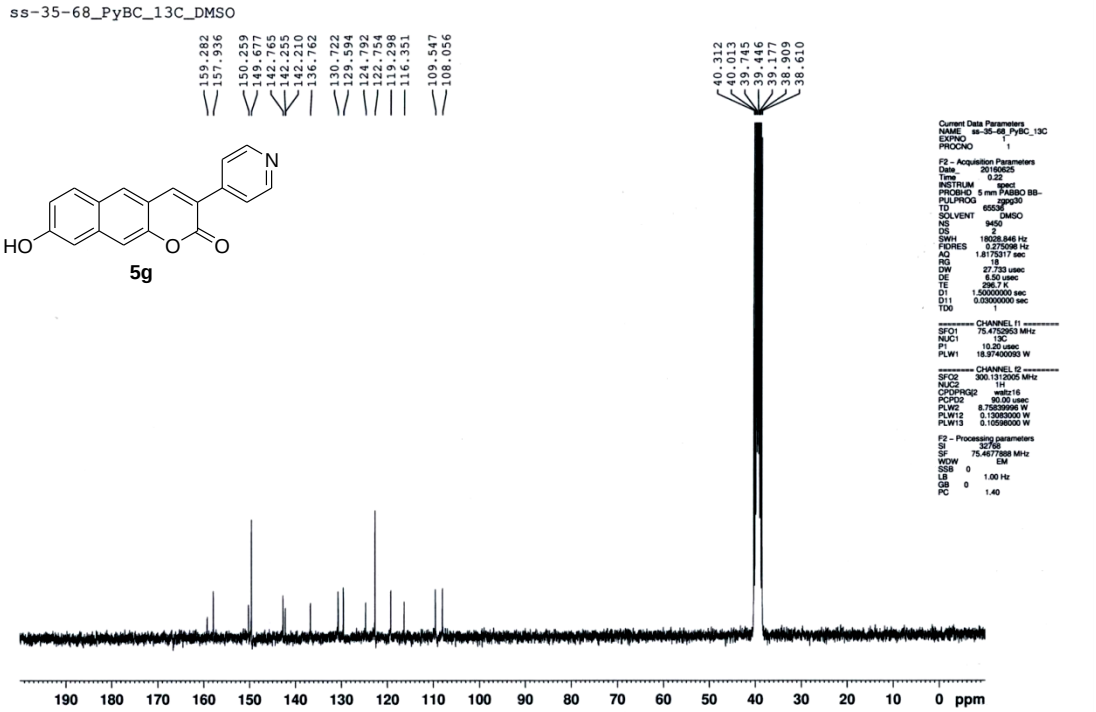

ss-112-194_iminoBC_1H_DMSO
10.242
9.182
9.176
8.815
8.292
8.143
8.118
8.080
8.054
7.860
7.830
7.577
7.574
7.553
7.550
7.527
7.522
7.468
7.465
7.452
7.442
7.418
7.415
7.159
7.152
7.097
7.089
7.067
7.059
3.339
2.512
2.506
2.500
2.494
2.488
S
N
HO
O
NH
5e
NAME ss-112-194_iminoBC_1
EXPNO 1
PROCNO 1
Date_ 20160615
Time 9.20
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 31
DS 2
SWH 6188.119 Hz
FIDRES 0.094423 Hz
AQ 5.29530587 sec
RG 287
DW 80.800 usec
DE 6.50 usec
TE 296.2 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 =======
NUC1 1H
P1 11.00 usec
PL1 -2.00 dB
PL1W 8.75835800 W
SFO1 300.1314684 MHz
SI 32768
SF 300.1300011 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Aromatic-H & imine-H
-OH
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.91
0.92
0.90
0.89
2.09
1.00
3.08
1.00
1.11

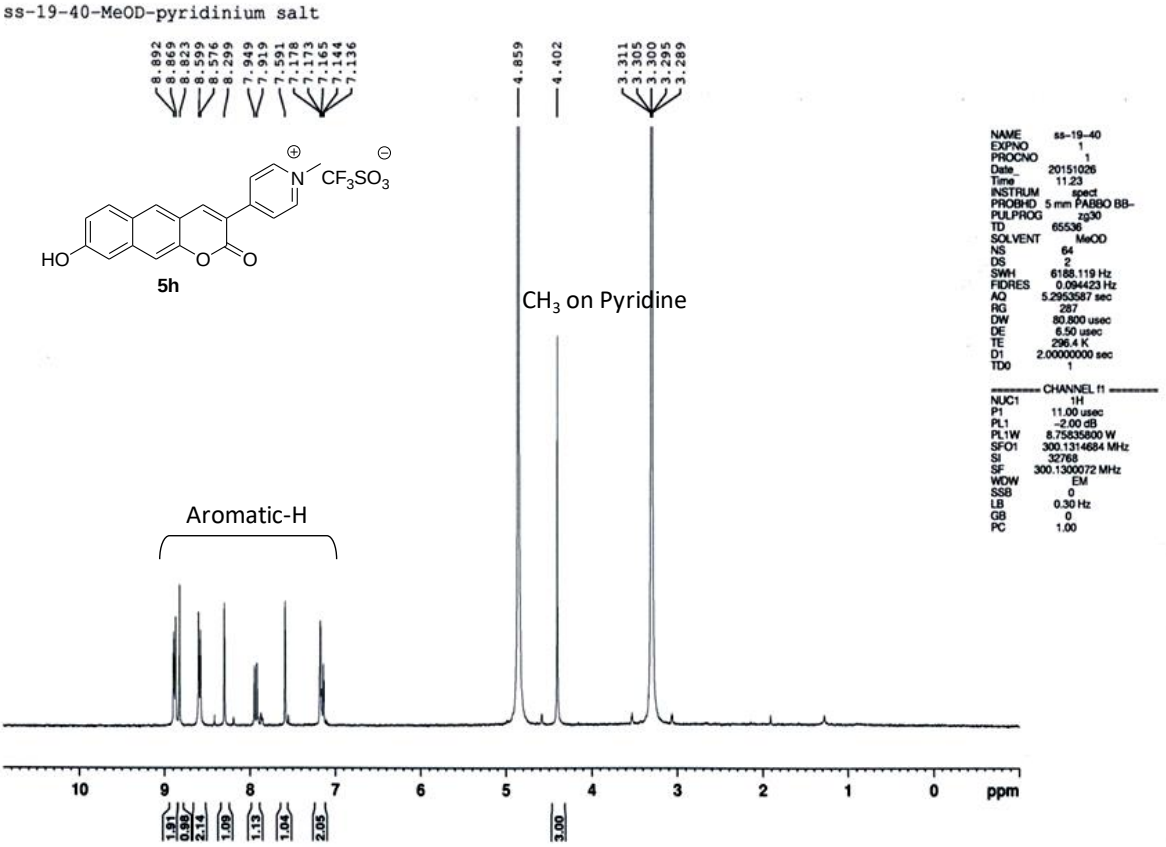

ss-112-194_iminoBC_13C_DMSO
161.039
157.883
153.370
151.765
150.078
136.900
136.878
135.710
130.590
130.312
126.295
124.954
124.586
122.333
121.912
120.505
118.600
116.477
109.423
108.310
40.330
40.052
39.774
39.495
39.217
38.939
38.661
S
N
HO
O
NH
5e
NAME ss-112-194_iminoBC_13C
EXPNO 1
PROCNO 1
Date_ 20160615
Time 2.45
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 6869
DS 2
SWH 18028.846 Hz
FIDRES 0.275098 Hz
AQ 1.81758818 sec
RG 20.2
DW 27.733 usec
DE 6.50 usec
TE 298.9 K
D1 1.50000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 =======
NUC1 13C
P1 10.20 usec
PL1 -1.00 dB
PL1W 18.97366524 W
SFO1 75.4752953 MHz
======== CHANNEL f2 =======
CPDPRG2 waltz16
NUC2 1H
PCPD2 95.00 usec
PL2 -2.00 dB
PL12 16.73 dB
PL13 19.00 dB
PL2W 8.75835800 W
PL12W 0.11733369 W
PL13W 0.08957011 W
SFO2 300.1312005 MHz
SI 32768
SF 75.4677855 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

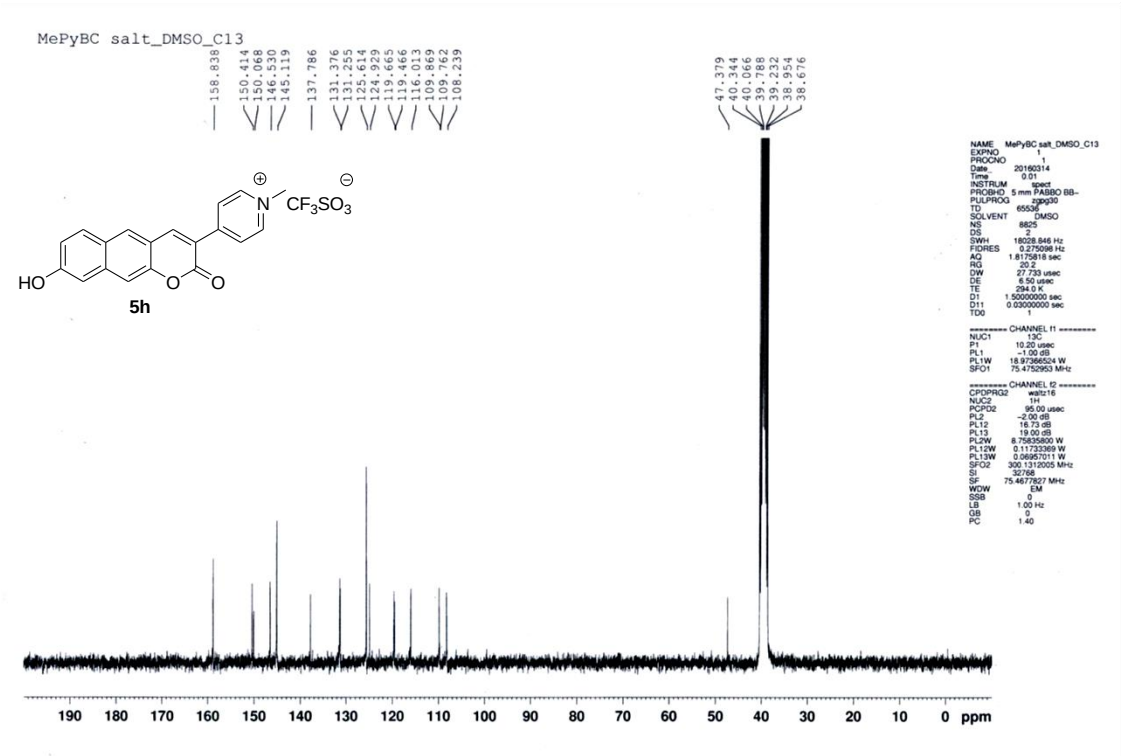

ss-115-199_OHBTBC_DMSO
10.479
9.286
8.567
8.204
8.179
8.116
8.089
7.984
7.954
7.777
7.615
7.611
7.591
7.587
7.564
7.560
7.510
7.506
7.483
7.460
7.456
7.251
7.244
7.193
7.185
7.163
7.155
3.331
2.512
2.506
2.500
2.494
2.488
HO
S
N
O
O
5f
NAME ss-115-199_OHBTBC
EXPNO 1
PROCNO 1
Date_ 20160615
Time 22.49
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 64
DS 2
SWH 6188.119 Hz
FIDRES 0.094423 Hz
AQ 5.2953587 sec
RG 287
DW 80.800 usec
DE 6.50 usec
TE 296.2 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 1H
P1 11.00 usec
PL1 -2.00 dB
PL1W 8.75835800 W
SFO1 300.1314684 MHz
SI 32768
SF 300.1300011 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
Aromatic-H
-OH
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.95
1.00
1.01
2.24
1.03
1.01
1.10
1.19
2.07

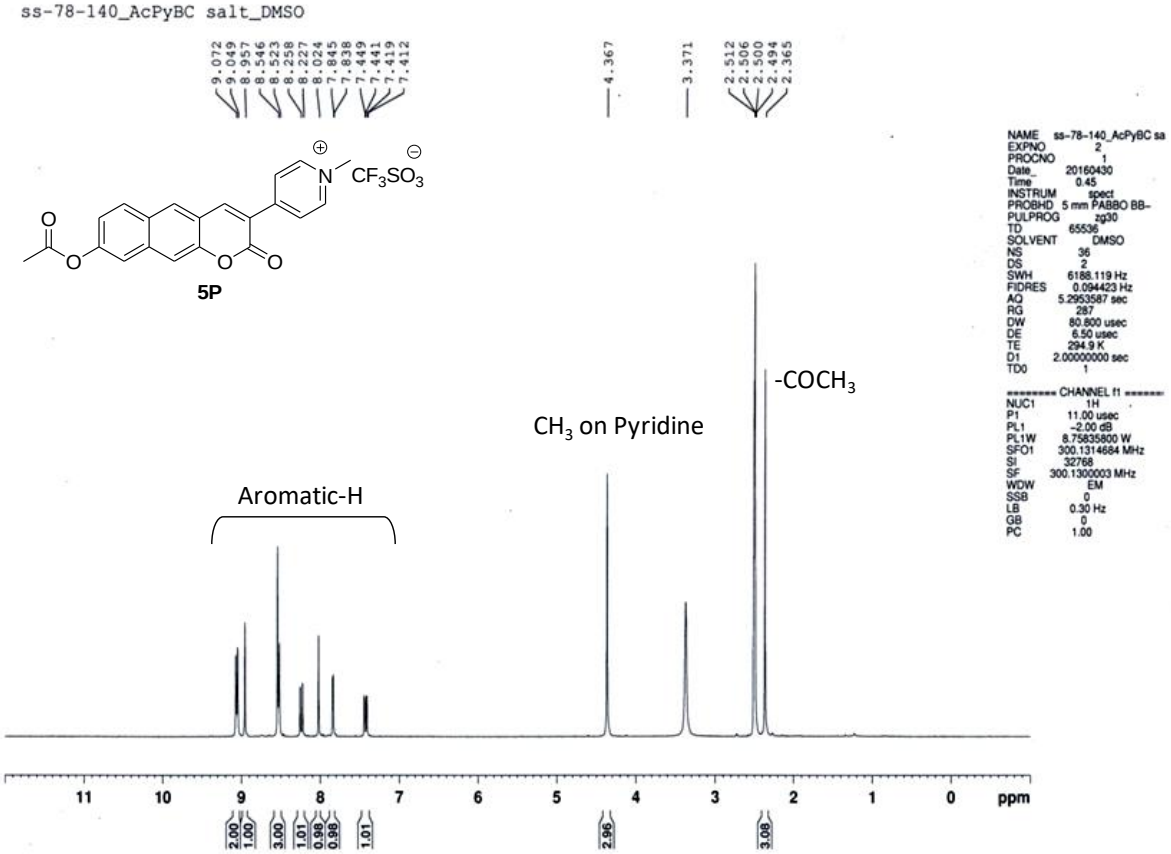

ss-115-199_BTBC_13C_DMSO
160.054
159.739
158.549
151.960
150.063
142.426
137.429
135.885
131.380
131.099
131.059
126.627
125.279
123.065
122.437
122.186
119.590
117.820
115.980
109.880
108.231
40.337
40.066
39.781
39.495
39.224
38.929
38.653
HO
S
N
O
O
5f
NAME ss-115-199_BTBC_13C
EXPNO 2
PROCNO 1
Date_ 20160618
Time 0.31
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 9015
DS 2
SWH 18028.846 Hz
FIDRES 0.275098 Hz
AQ 1.8175818 sec
RG 16
DW 27.733 usec
DE 6.50 usec
TE 297.0 K
D1 1.50000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 10.20 usec
PL1 -1.00 dB
PL1W 18.97366524 W
SFO1 75.4752953 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 95.00 usec
PL2 -2.00 dB
PL12 16.73 dB
PL13 19.00 dB
PL2W 8.75835800 W
PL12W 0.11733369 W
PL13W 0.06957011 W
SFO2 300.1312005 MHz
SI 32768
SF 75.4677851 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

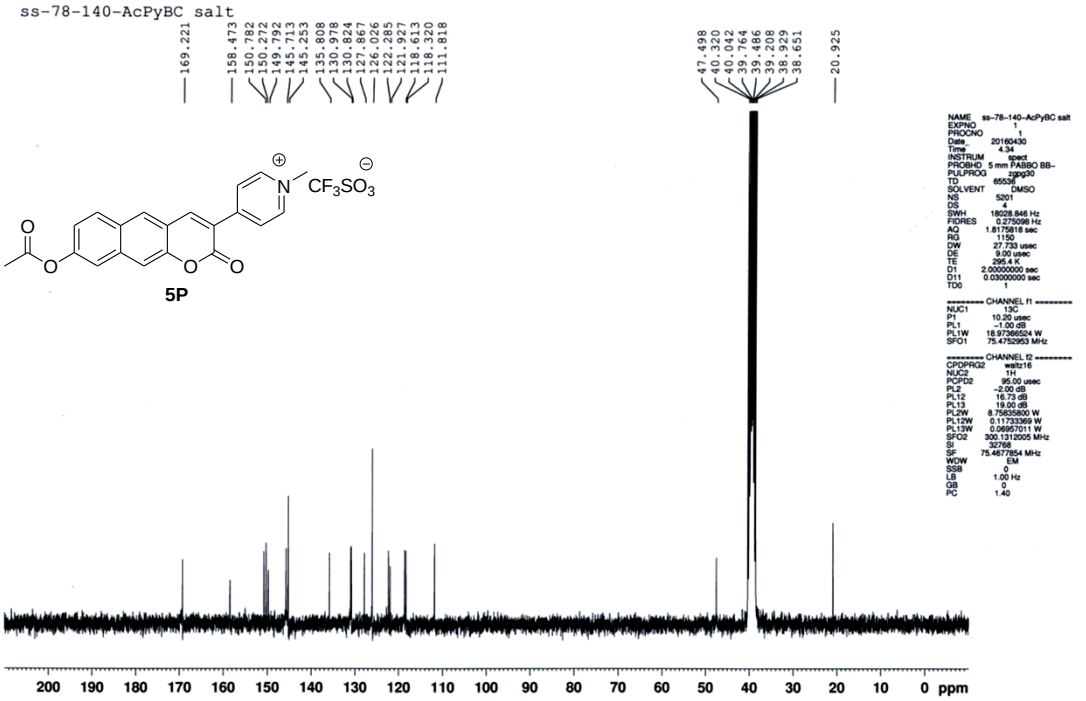

ss-15-32-2-DMSO
10.337
8.679
8.660
8.537
8.277
7.974
7.944
7.791
7.771
7.696
7.212
7.157
7.150
7.127
7.120
3.337
2.499
HO
N
O
O
5g
Aromatic-H
-OH
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.00
1.79
0.94
0.93
1.00
1.77
0.88
0.90
0.91


ss-35-68_PyBC_13C_DMSO
159.282
157.936
150.259
149.677
142.765
142.255
142.210
136.762
130.722
129.594
124.792
122.754
119.298
116.351
109.547
108.056
40.312
40.013
39.745
39.446
39.177
38.909
38.610
HO
N
O
O
5g
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

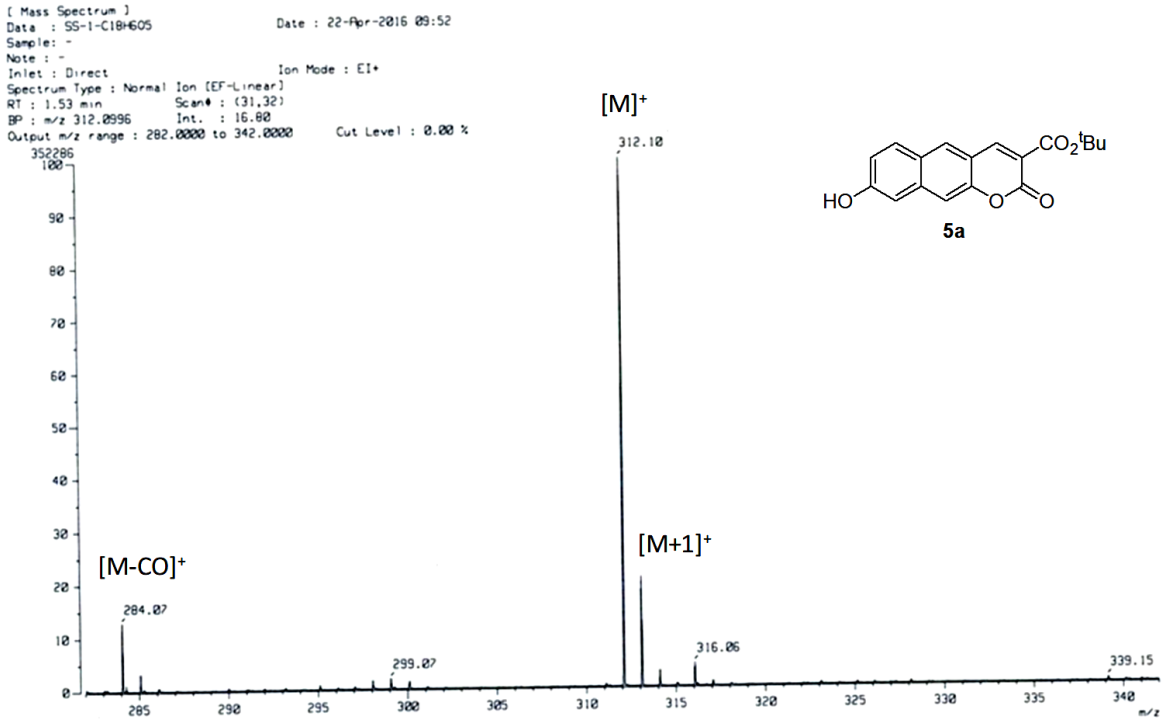

ss-19-40-MeOD-pyridinium salt
8.892
8.869
8.823
8.599
8.576
8.299
7.949
7.919
7.591
7.178
7.173
7.165
7.144
7.136
4.859
4.402
3.311
3.305
3.300
3.295
3.289
N
CF3SO3
HO
O
O
5h
CH3 on Pyridine
Aromatic-H
NAME ss-19-40
EXPNO 1
PROCNO 1
Date_ 20151026
Time 11.23
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT MeOD
NS 64
DS 2
SWH 6188.119 Hz
FIDRES 0.094423 Hz
AQ 5.2953587 sec
RG 287
DW 80.800 usec
DE 6.50 usec
TE 296.4 K
D1 2.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
P1 11.00 usec
PL1 -2.00 dB
PL1W 8.75835800 W
SFO1 300.1314684 MHz
SI 32768
SF 300.1300072 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
10
9
8
7
6
5
4
3
2
1
0
ppm
1.91
0.98
2.14
1.09
1.13
1.04
2.05
3.00

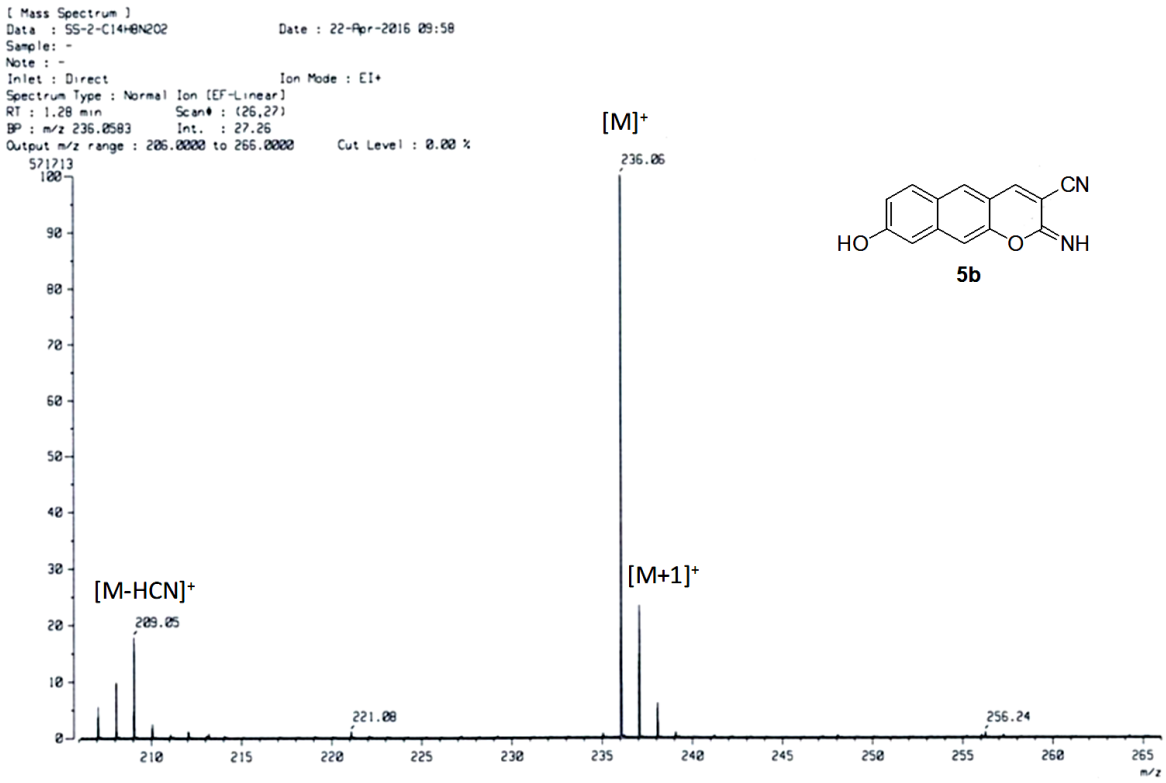

MePyBC salt_DMSO_C13
158.838
150.414
150.068
146.530
145.119
137.786
131.376
131.255
125.614
124.929
119.665
119.466
116.013
109.869
109.762
108.239
47.379
40.344
40.166
39.788
39.232
38.954
38.676
N
CF3SO3
HO
O
O
5h
NAME MePyBC salt_DMSO_C13
EXPNO 1
PROCNO 1
Date_ 20160314
Time 0.01
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 8825
DS 2
SWH 18028.846 Hz
FIDRES 0.275098 Hz
AQ 1.8175818 sec
RG 20.2
DW 27.733 usec
DE 6.50 usec
TE 294.0 K
D1 1.50000000 sec
D11 0.03000000 sec
TD0 1
CHANNEL f1
NUC1 13C
P1 10.20 usec
PL1 -1.00 dB
PL1W 18.97366524 W
SFO1 75.4752953 MHz
CHANNEL f2
CPDPRG2 waltz16
NUC2 1H
PCPD2 95.00 usec
PL2 -2.00 dB
PL12 16.73 dB
PL13 19.00 dB
PL2W 8.75835800 W
PL12W 0.11733369 W
PL13W 0.06957011 W
SFO2 300.1312005 MHz
SI 32768
SF 75.4677827 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm

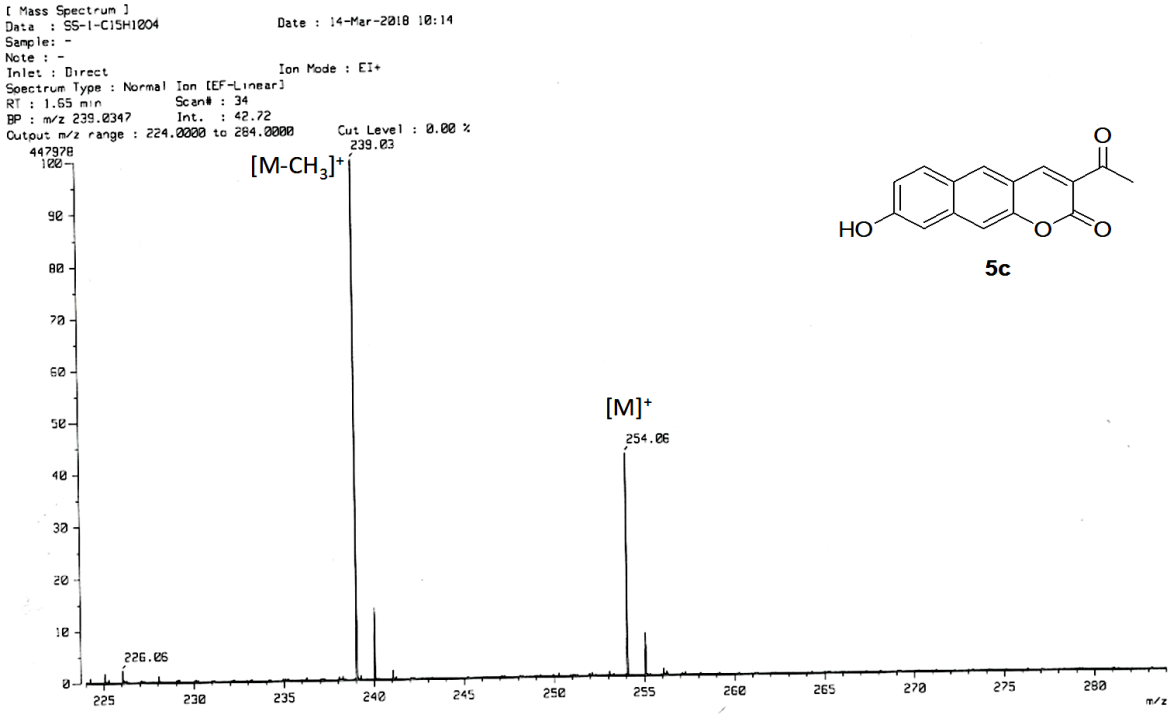

ss-78-140_AcPyBC salt_DMSO
9.072
9.049
8.957
8.546
8.523
8.258
8.227
8.024
7.845
7.838
7.449
7.441
7.419
7.412
4.367
3.371
2.512
2.506
2.500
2.494
2.365
CF3SO3
5P
-COCH3
CH3 on Pyridine
Aromatic-H
NAME ss-78-140_AcPyBC sa
EXPNO 2
PROCNO 1
Date_ 20160430
Time 0.45
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zg30
TD 65536
SOLVENT DMSO
NS 36
DS 2
SWH 6188.119 Hz
FIDRES 0.094423 Hz
AQ 5.2953587 sec
RG 287
DW 80.800 usec
DE 6.50 usec
TE 294.9 K
D1 2.00000000 sec
TD0 1
======== CHANNEL f1 ======
NUC1 1H
P1 11.00 usec
PL1 -2.00 dB
PL1W 8.75835800 W
SFO1 300.1314684 MHz
SI 32768
SF 300.1300003 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
11 10 9 8 7 6 5 4 3 2 1 0 ppm
2.00
1.00
3.00
1.01
0.98
0.98
1.01
2.96
3.08

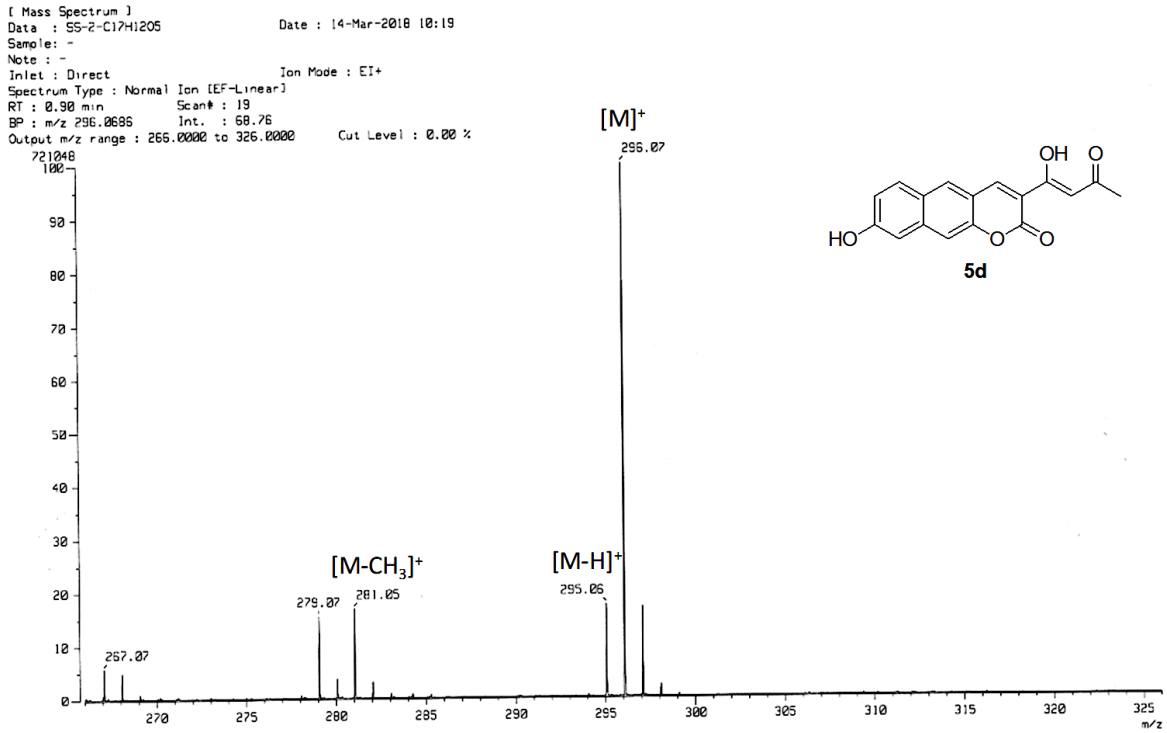

ss-78-140-AcPyBC salt
169.221
158.473
150.782
150.272
149.792
145.713
145.253
135.808
130.978
130.824
127.867
126.026
122.285
121.927
118.613
118.320
111.818
47.498
40.320
40.042
39.764
39.486
39.208
38.929
38.651
20.925
CF3SO3
5P
NAME ss-78-140-AcPyBC salt
EXPNO 1
PROCNO 1
Date_ 20160430
Time 4.34
INSTRUM spect
PROBHD 5 mm PABBO BB-
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 5201
DS 4
SWH 18028.846 Hz
FIDRES 0.275098 Hz
AQ 1.8175818 sec
RG 1150
DW 27.733 usec
DE 9.00 usec
TE 295.4 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 10.20 usec
PL1 -1.00 dB
PL1W 18.97366524 W
SFO1 75.4752953 MHz
======== CHANNEL f2 ========
CPDPRG2 waltz16
NUC2 1H
PCPD2 95.00 usec
PL2 -2.00 dB
PL12 16.73 dB
PL13 19.00 dB
PL2W 8.75835800 W
PL12W 0.11733369 W
PL13W 0.06957011 W
SFO2 300.1312005 MHz
SI 32768
SF 75.4677854 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm

HRMS data

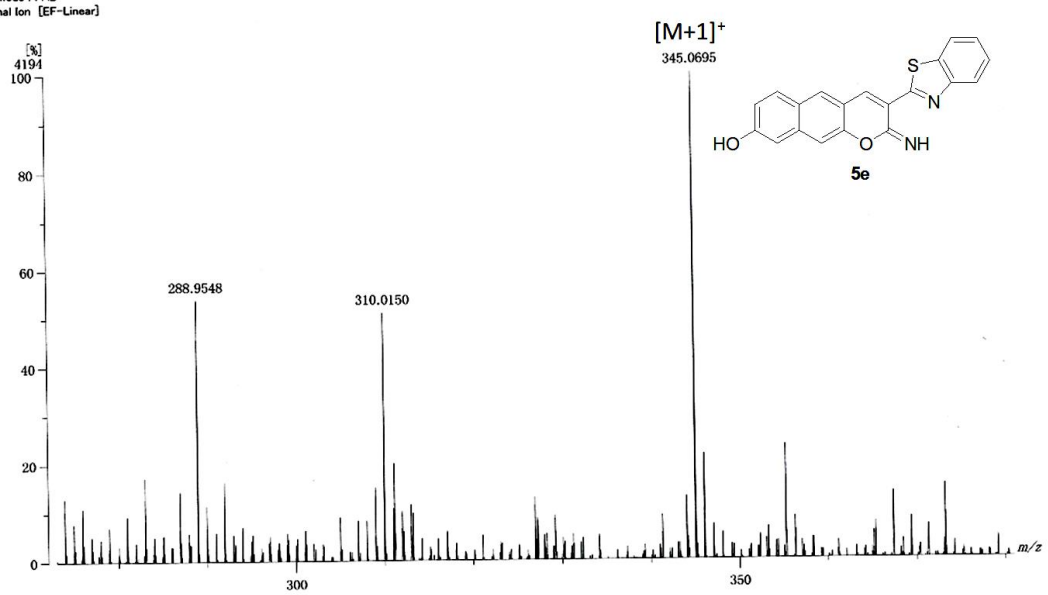

[Mass Spectrum]
Data : SS-1-C18H6O5
Date : 22-Apr-2016 09:52
Sample: -
Note : -
Inlet : Direct
Ion Mode : EI+
Spectrum Type : Normal Ion [EF-Linear]
RT : 1.53 min
Scan# : (31,32)
BP : m/z 312.0996
Int. : 16.80
Output m/z range : 282.0000 to 342.0000
Cut Level : 0.00 %
352286
[M]+
312.10
[M+1]+
[M-CO]+
284.07
299.07
316.06
339.15
m/z
CO2tBu
HO
O
O
5a


[Mass Spectrum]
Data : SS-2-C14H8N2O2
Date : 22-Apr-2016 09:58
Sample: -
Note : -
Inlet : Direct
Ion Mode : EI+
Spectrum Type : Normal Ion [EF-Linear]
RT : 1.28 min
Scan# : (26,27)
BP : m/z 236.0583
Int. : 27.26
Output m/z range : 206.0000 to 266.0000
Cut Level : 0.00 %
571713
[M]+
236.06
[M+1]+
[M-HCN]+
209.05
221.08
256.24
m/z
CN
HO
O
NH
5b

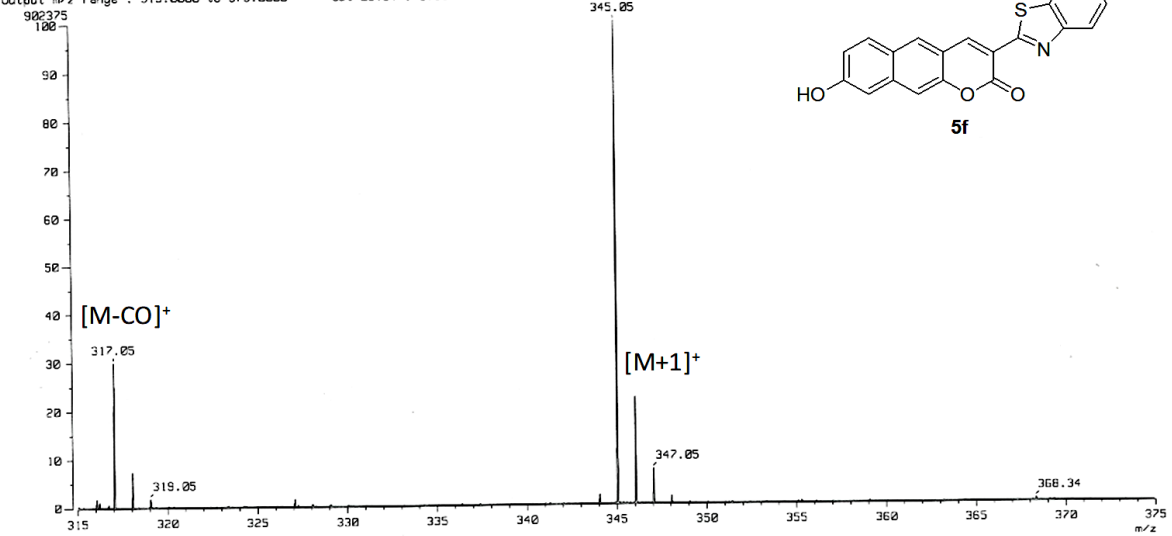

[Mass Spectrum]
Data : SS-1-C15H1004
Date : 14-Mar-2018 10:14
Sample: -
Note : -
Inlet : Direct
Ion Mode : EI+
Spectrum Type : Normal Ion [EF-Linear]
RT : 1.65 min
Scan# : 34
BP : m/z 239.0347
Int. : 42.72
Output m/z range : 224.0000 to 284.0000
Cut Level : 0.00 %
447978
[M-CH3]+
239.03
[M]+
254.06
226.06
m/z
5c

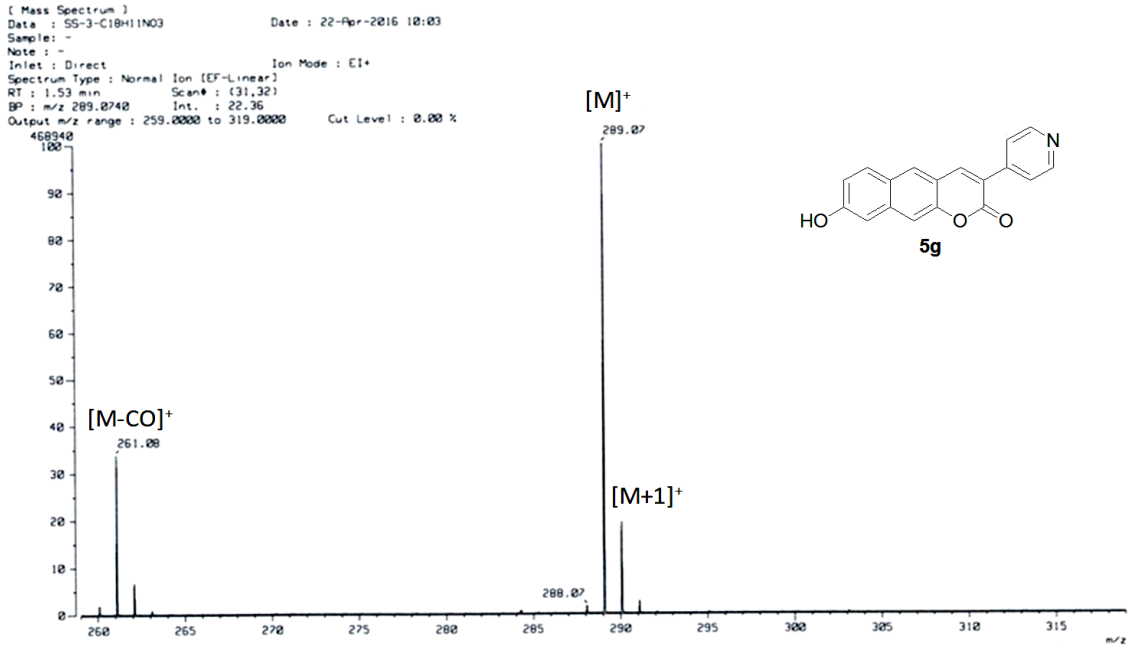

[Mass Spectrum]
Data : SS-2-C17H1205
Date : 14-Mar-2018 10:19
Sample: -
Note : -
Inlet : Direct
Ion Mode : EI+
Spectrum Type : Normal Ion [EF-Linear]
RT : 0.90 min
Scan# : 19
BP : m/z 296.0686
Int. : 68.76
Output m/z range : 266.0000 to 326.0000
Cut Level : 0.00 %
721048
[M]+
296.07
[M-CH3]+
279.07
281.05
[M-H]+
295.06
267.07
m/z
5d

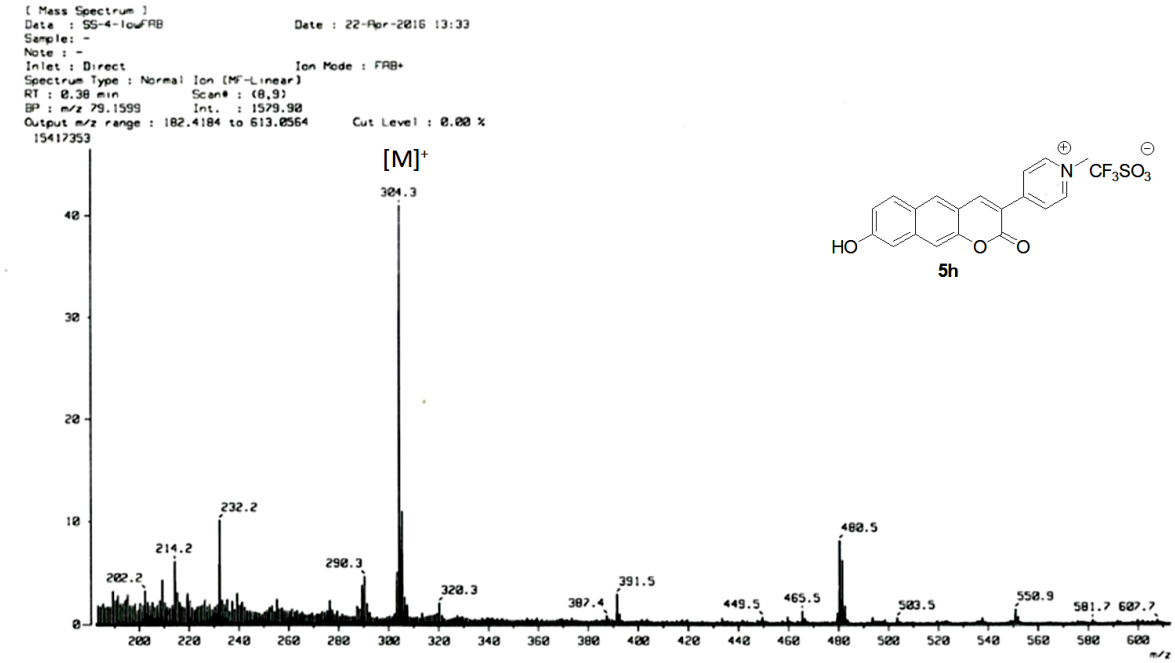
[Mass Spectrum]
Data : SS-3-C20H12N2O2S-HRFAB Date : 14-Mar-2018 10:18
Instrument : MStation
Sample : -
Inlet : Direct Ion Mode : FAB+
Spectrum Type : Normal Ion [EF-Linear]

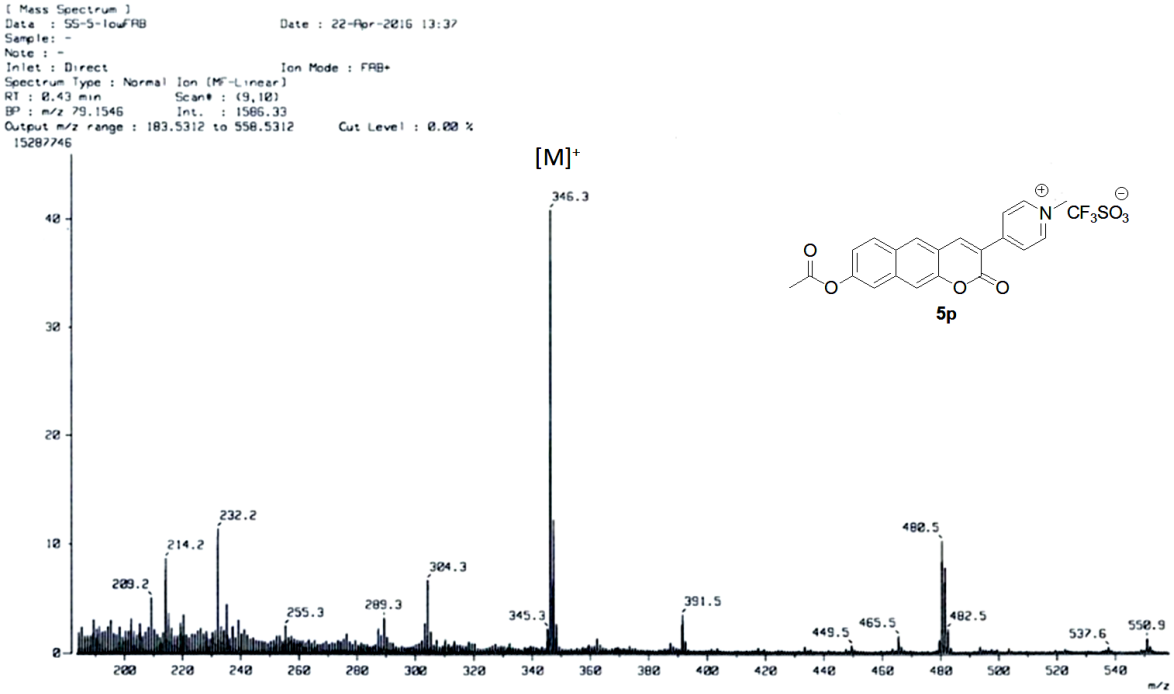


[Mass Spectrum]
Data : SS-4-C20H11NO3S Date : 14-Mar-2018 10:28
Sample: -
Note : -
Inlet : Direct Ion Mode : EI+
Spectrum Type : Normal Ion [EF-Linear]
RT : 1.35 min Scan# : 28
BP : m/z 345.0456 Int. : 86.06
Output m/z range : 315.0000 to 375.0000 Cut Level : 0.00 %




[Mass Spectrum]
Data : SS-3-C18H11NO3
Date : 22-Apr-2016 10:03
Sample: -
Note : -
Inlet : Direct
Ion Mode : EI+
Spectrum Type : Normal Ion (EF-Linear)
RT : 1.53 min
Scan# : (31,32)
BP : m/z 289.0740
Int. : 22.36
Output m/z range : 259.0000 to 319.0000
Cut Level : 0.00 %
468940
[M]+
289.07
[M-CO]+
261.08
[M+1]+
288.07
m/z
5g


[Mass Spectrum]
Data : SS-4-lowFAB
Date : 22-Apr-2016 13:33
Sample: -
Note : -
Inlet : Direct
Ion Mode : FAB+
Spectrum Type : Normal Ion (MF-Linear)
RT : 0.38 min
Scan# : (8,9)
BP : m/z 79.1599
Int. : 1579.90
Output m/z range : 182.4184 to 613.0564
Cut Level : 0.00 %
15417353
[M]+
304.3
232.2
214.2
202.2
290.3
320.3
387.4
391.5
449.5
465.5
480.5
503.5
550.9
581.7
607.7
m/z
5h


[Mass Spectrum]
Data : SS-S-lowFAB
Date : 22-Apr-2016 13:37
Sample: -
Note : -
Inlet : Direct
Ion Mode : FAB+
Spectrum Type : Normal Ion (MF-Linear)
RT : 0.43 min
Scan# : (9,10)
BP : m/z 79.1546
Int. : 1586.33
Output m/z range : 183.5312 to 558.5312
Cut Level : 0.00 %
15287746
[M]+
346.3
232.2
214.2
209.2
255.3
289.3
304.3
345.3
391.5
449.5
465.5
480.5
482.5
537.6
550.9
m/z
5p