Supplementary Materials

Novel efficient fluorophores synthesized from citric acid

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Experimental

Citric acid, L-cysteine, o-aminothiophenol, o-aminophenol, o-phenylenediamine, trifluoroacetic acid, glutathione, 1,2-diaminopropane, N-methyloethylenediamine, ethanoloamine, 1,3-diaminopropane and 3-amino-1-propanol were supplied by Sigma-Aldrich. Cysteamine hydrochloride was supplied by Merck. Ethanol 99.8%, dichlomethane, p-xylene, hydrochloric acid, sodium hydroxide were supplied by POCH (Poland).

Synthesis and characterization of (**1b-5b)**

Citric acid was mixed with an amine source (cysteamine, L-cysteine, o-aminothiophenol, o-phenylenediamine or o-aminophenol) in molar ratio 1:1, thus the total weight of the reactants was 2 g. Then 2 ml of distilled water was added to dissolve reactants. Afterwards it was evaporated under vacuum oven at r.t. under 10 mbar. Then the mixture was heated for 1 h at 180ºC. The yellowish product of the reaction was purified by preparative high pressure liquid chromatography (Knauer HPLC set: degasser, pump K-500, detectors RI 2300 and UV/VIS A2500) at r.t. on Eurospher 100 C-18 column and eluted by 1.35x10-3 M trifluoroacetic acid in water at a flow rate 10 ml/min. The luminescent fraction of desired product was collected and freeze-dried. The chemical structure was confirmed by ^{1}H, ^{13}C and HSQC NMR experiments carried out in DMSO-d6 solution using a Varian Mercury-VX 300 MHz spectrometer. ESI-MS/MS analyses were carried out using the UPLC-MS/MS system (Waters Corporation, Milford, MA, USA). High resolution mass spectra were acquired using the MALDISynapt G2-S HDMS (Waters Corporation, Milford, MA, USA), coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadrupole). UV/VIS absorption spectra of solutions of isolated compounds were acquired on a PG Instruments Ltd P80+ spectrophotometer. The excitation and emission spectra were acquired using FLS980 spectrometer (Edinburgh Instruments); xenone lamp was used as a light source, Hamammatsu R928P photomultiplier detector with cooling system (-20ºC). The absolute quantum yield measurements of $7x10^{-3}$ g/l water solutions of achieved compounds were conducted using integrating sphere. The fluorescence intensity measurements (excitation 360 nm) were made using Ocean Optics MINI-D2 spectrofluorimeter. Fluorescence lifetime measurements were carried out at 21^{o}C on a PTI EasyLife® instrument using 325 nm LED as an excitation source. Curve fitting and calculations were performed by EasyLife X© software.

Detection of fluorophores (**6b-9b, 14b, 15b**) in products of citric acid condensation with selected amines

Citric acid was mixed with an amine source (glutathione, 1,2-diaminopropane, N-methyloethylenediamine, ethanoloamine, 1,3-diaminopropane or 3-amino-1-propanol) in molar ratio 1:1, thus the total weight of the reactants was 2 g. Then 2 ml of distilled water was added to dissolve reactants. Afterwards water was evaporated in vacuum oven at r.t. under 10mbar. Then the mixture was heated for 1h at 180ºC. The identification of fluorescent compounds was achieved by HR-ESI-MS and ESI-MS/MS fragmentation patterns of molecular ions. ESI-MS/MS analyses were carried out using the UPLC-MS/MS system (Waters Corporation, Milford, MA, USA). High resolution mass spectra were acquired using the MALDISynapt G2-S HDMS (Waters Corporation, Milford, MA, USA), coupled to a Waters TQD mass spectrometer (electrospray ionization mode ESI-tandem quadruple).

1H NMR (300 MHz, dmso)
δ 6.54 (d, J = 1.5 Hz, 1H), 6.50 (d, J = 1.5 Hz, 1H), 4.33 (t, J = 7.5 Hz, 2H), 3.49 (t, J = 7.6 Hz, 2H).
13C NMR (75 MHz, dmso)
δ 166.12, 161.15, 150.41, 142.53, 115.17, 98.24, 51.19, 28.51.

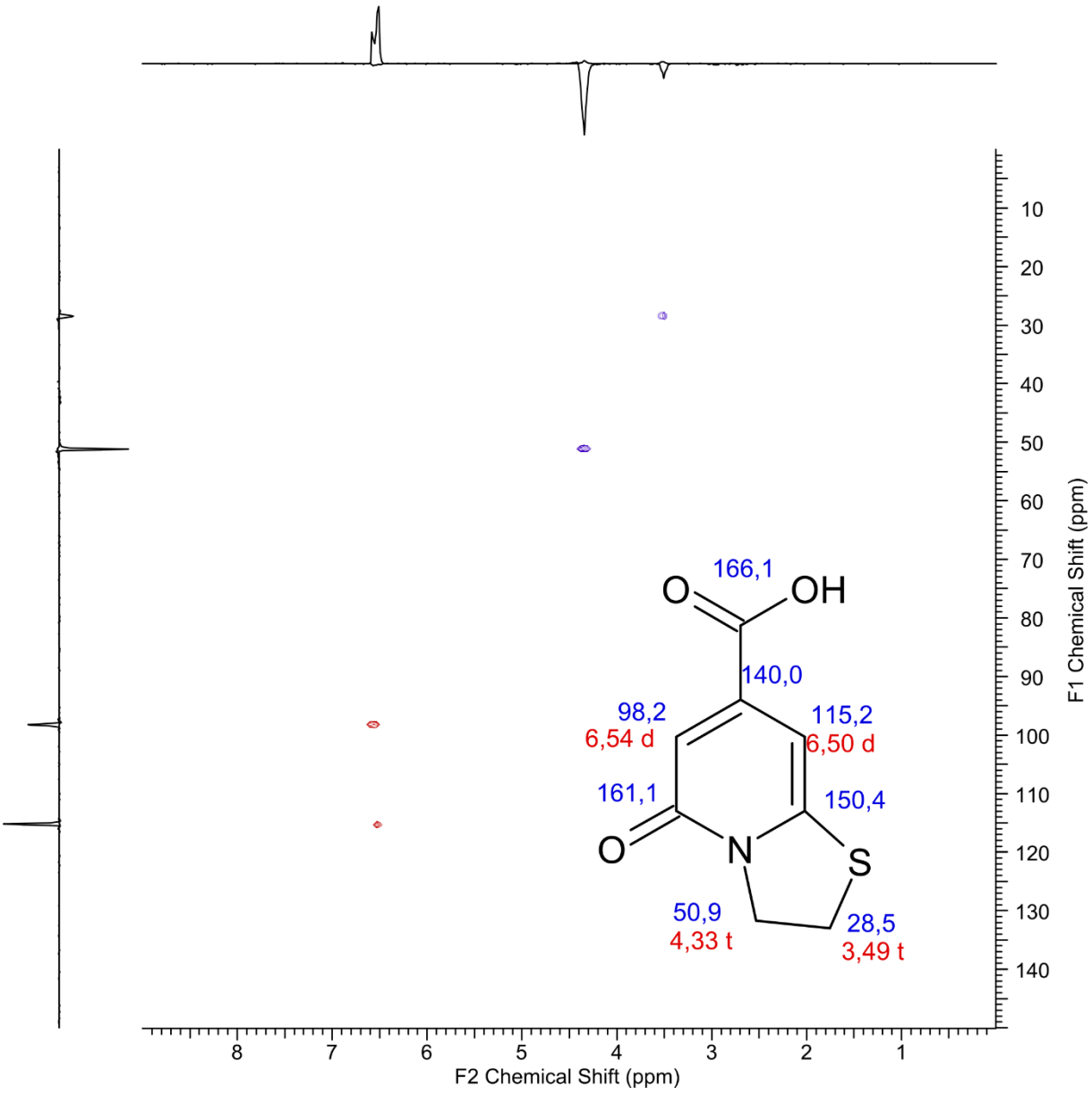

Fig. S1A HSQC spectrum and 1H, 13C NMR assignments of 1**b**.

1H NMR (300 MHz, dmso)
δ 6.60 (d, J = 1.5 Hz, 1H), 6.56 (d, J = 1.5 Hz, 1H), 5.47 (dd, J = 8.9, 1.6 Hz, 1H), 3.92 (dd, J = 11.9, 9.0 Hz, 1H), 3.61 (dd, J = 11.9, 1.7 Hz, 1H).
13C NMR (75 MHz, dmso)
δ 169.59, 166.04, 161.07, 150.40, 143.20, 115.50, 98.39, 63.06, 32.05.

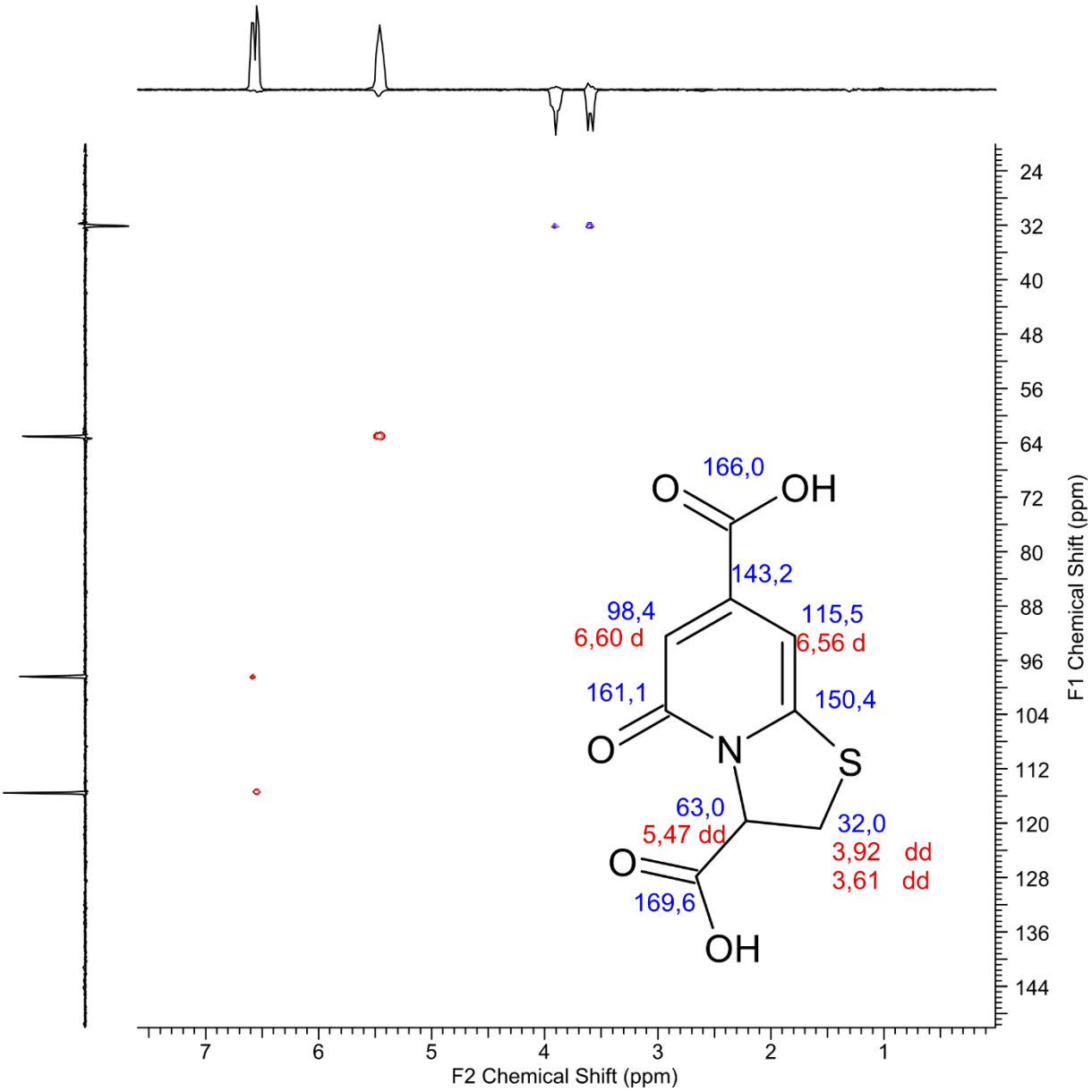

Fig. S1B HSQC spectrum and 1H, 13C NMR assignments of 2**b**.

1H NMR (300 MHz, dmso)
δ 8.41 (dd, J = 7.8, 1.0 Hz, 1H), 7.74 (d, J = 7.7 Hz, 1H), 7.54 (td, J = 7.9, 1.4 Hz, 1H), 7.46 (td, J = 7.7, 1.1 Hz, 1H), 6.74 (d, J = 1.3 Hz, 1H), 6.71 (d, J = 1.3 Hz, 1H).
13C NMR (75 MHz, dmso)
δ 166.17, 158.56, 154.03, 147.35, 142.98, 127.86, 127.18, 125.28, 116.64, 111.42, 111.31, 84.05.

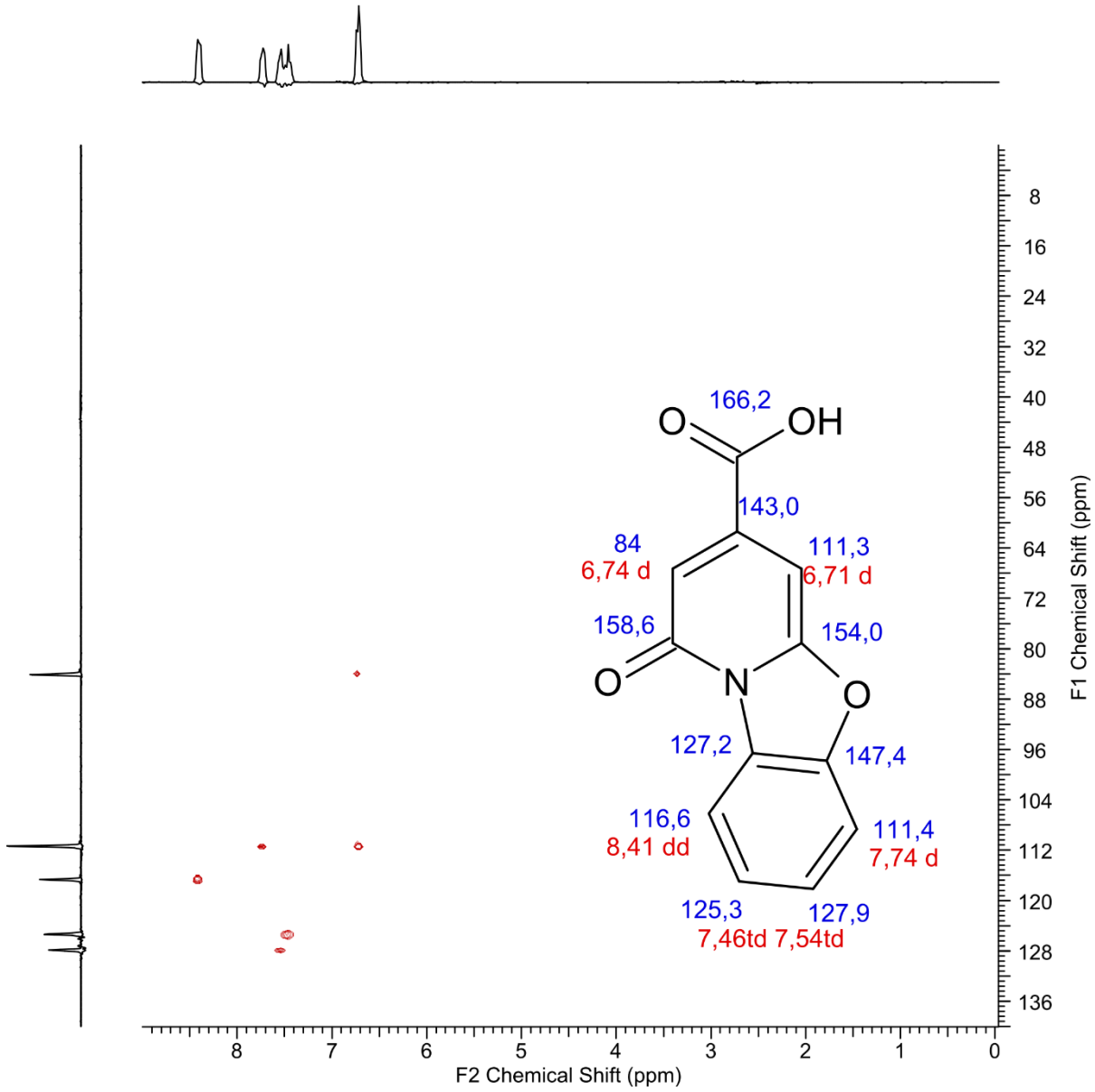

Fig. S1C HSQC spectrum and 1H, 13C NMR assignments of 3**b**.

1H NMR (300 MHz, dmso)
δ 9.15 – 9.08 (m, 1H), 8.03 – 7.95 (m, 1H), 7.53 – 7.48 (m, 2H), 7.34 (d, J = 1.6 Hz, 1H), 6.76 (d, J = 1.6 Hz, 1H).
13C NMR (75 MHz, dmso)
δ 166.16, 161.88, 147.71, 140.05, 138.01, 127.60, 127.24, 126.67, 123.01, 119.83, 113.34, 99.43.

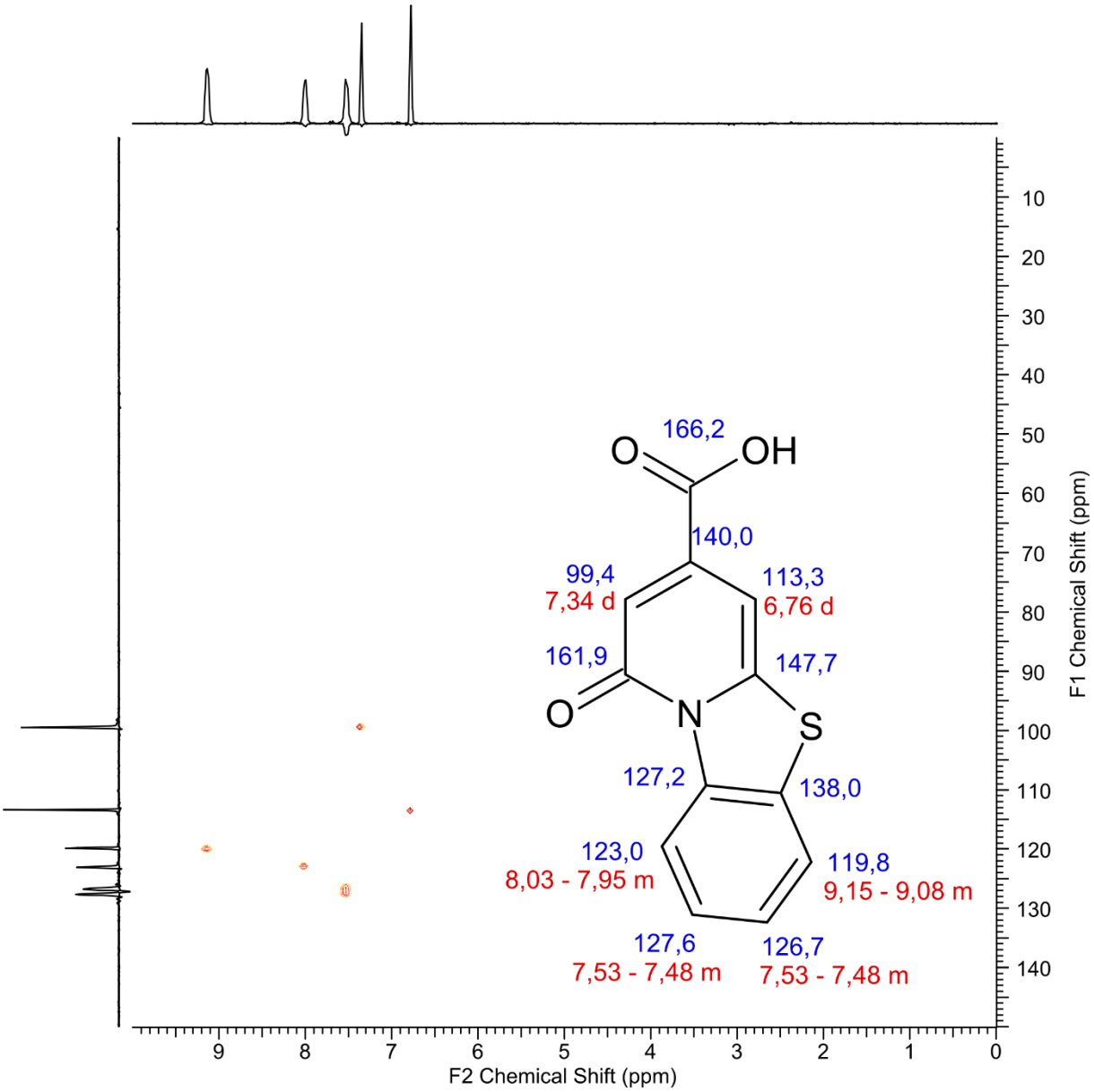

Fig. S1D HSQC spectrum and 1H, 13C NMR assignments of 4**b**.

1H NMR (300 MHz, dmso)
δ 8.70 (d, J = 8.1 Hz, 1H), 7.55 – 7.45 (m, 2H), 7.35 – 7.26 (m, 1H), 6.60 (d, J = 1.5 Hz, 1H), 6.36 (d, J = 1.5 Hz, 1H).
13C NMR (75 MHz, dmso)
δ 167.25, 159.38, 144.88, 141.08, 132.63, 127.93, 127.13, 121.69, 117.04, 111.08, 102.34, 84.01.

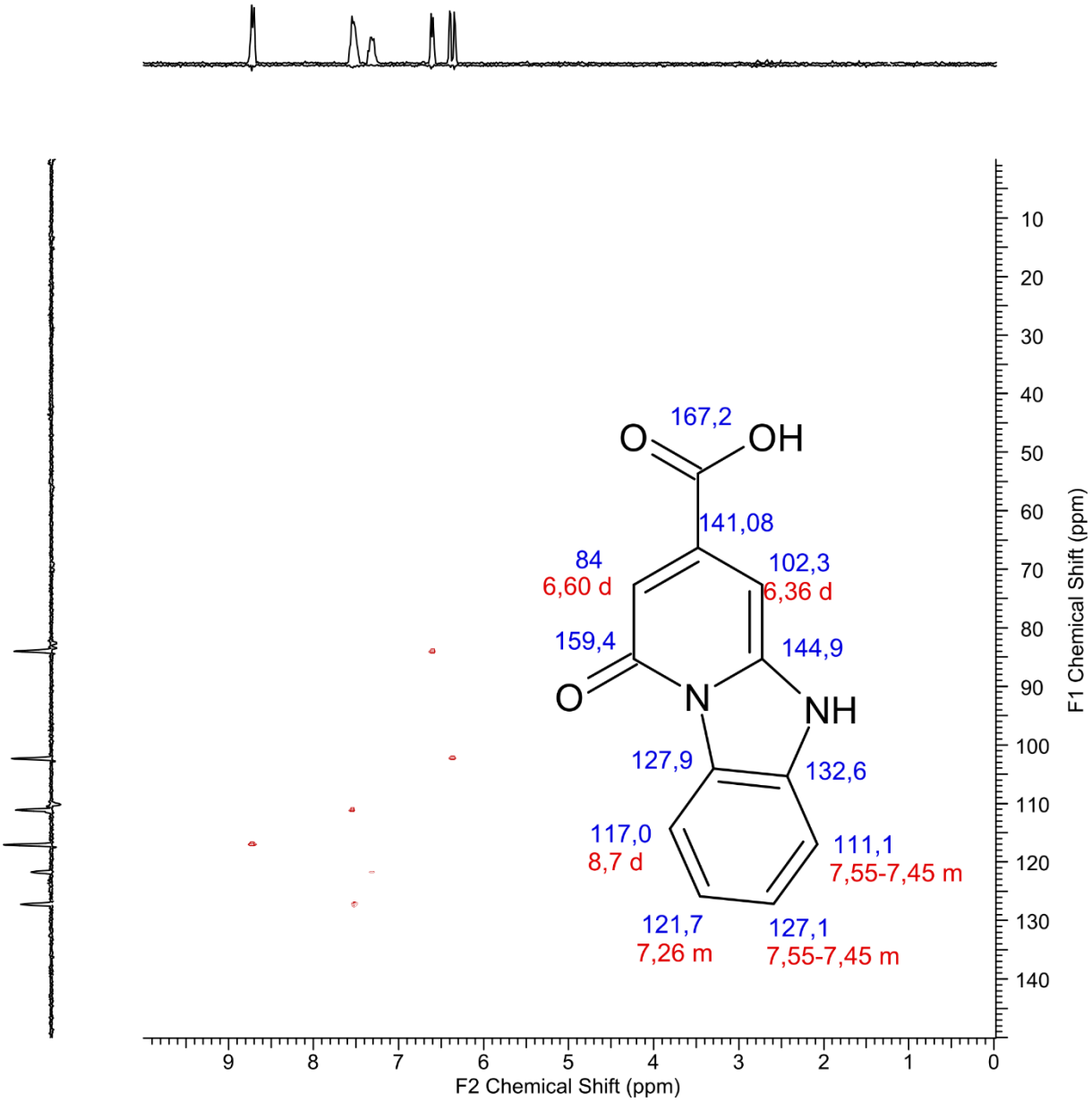

Fig. S1E HSQC spectrum and 1H, 13C NMR assignments of 5**b**.

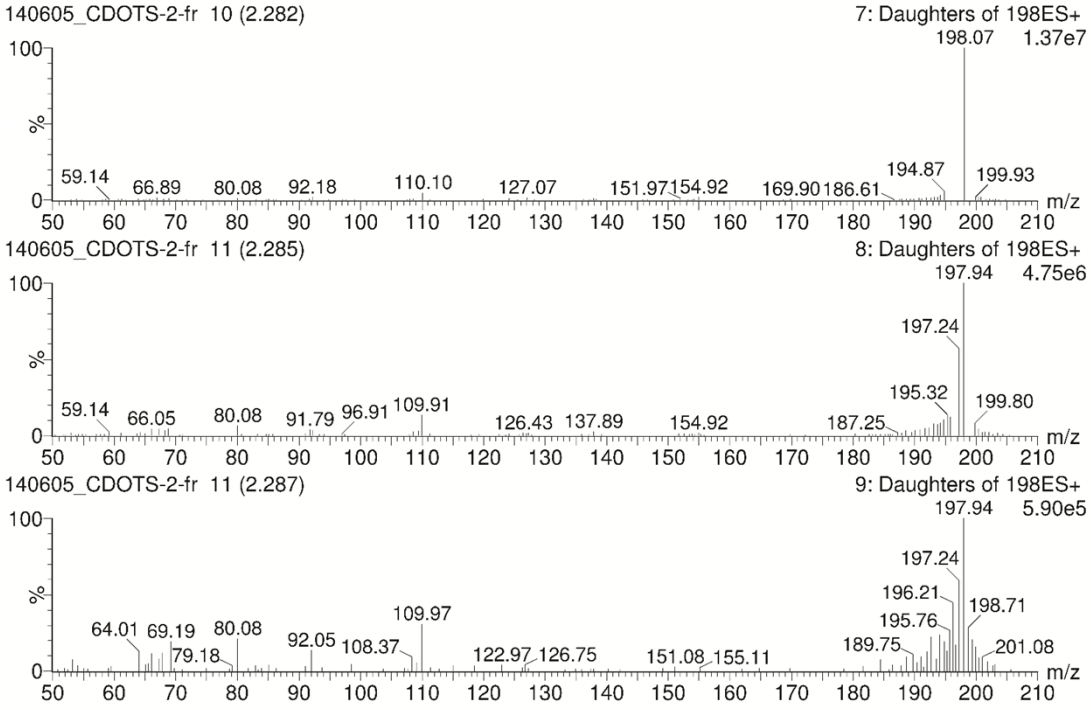

Fig. S2A ESI-MS/MS fragmentation spectra of **1b**.

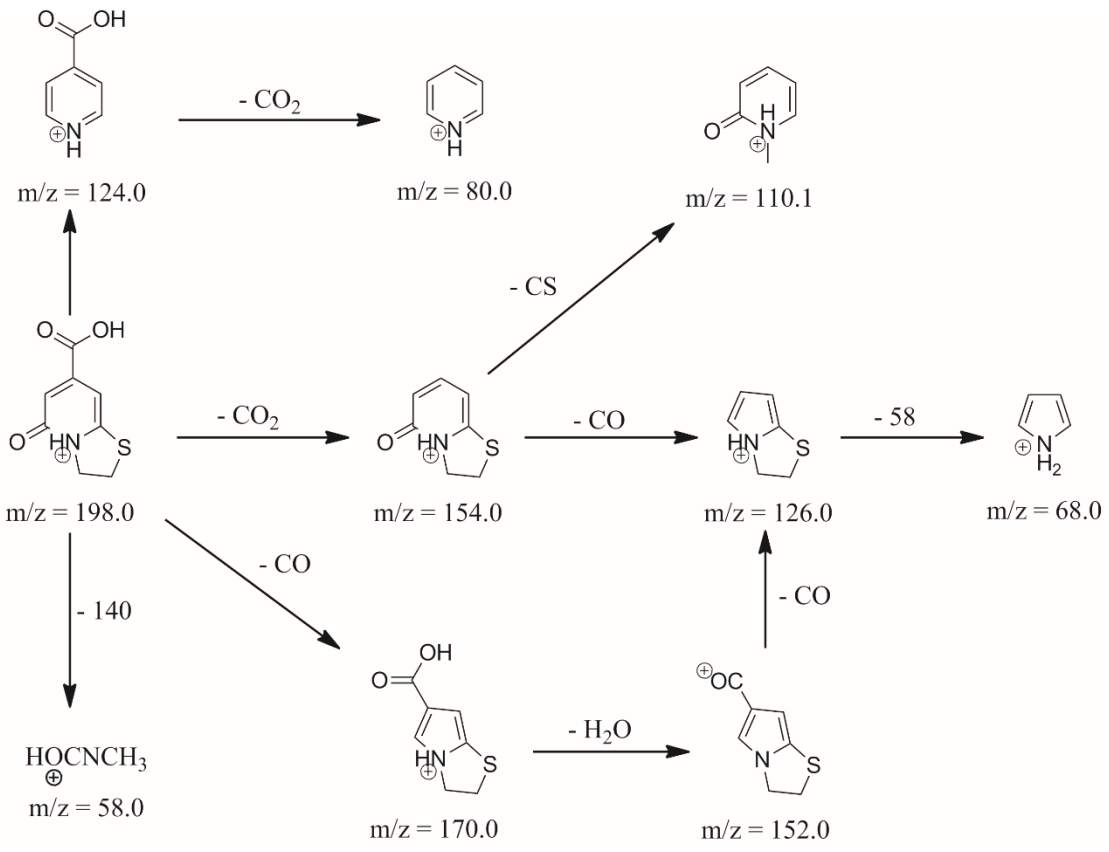

Fig. S2B ESI-MS/MS fragmentation pattern of **1b**.

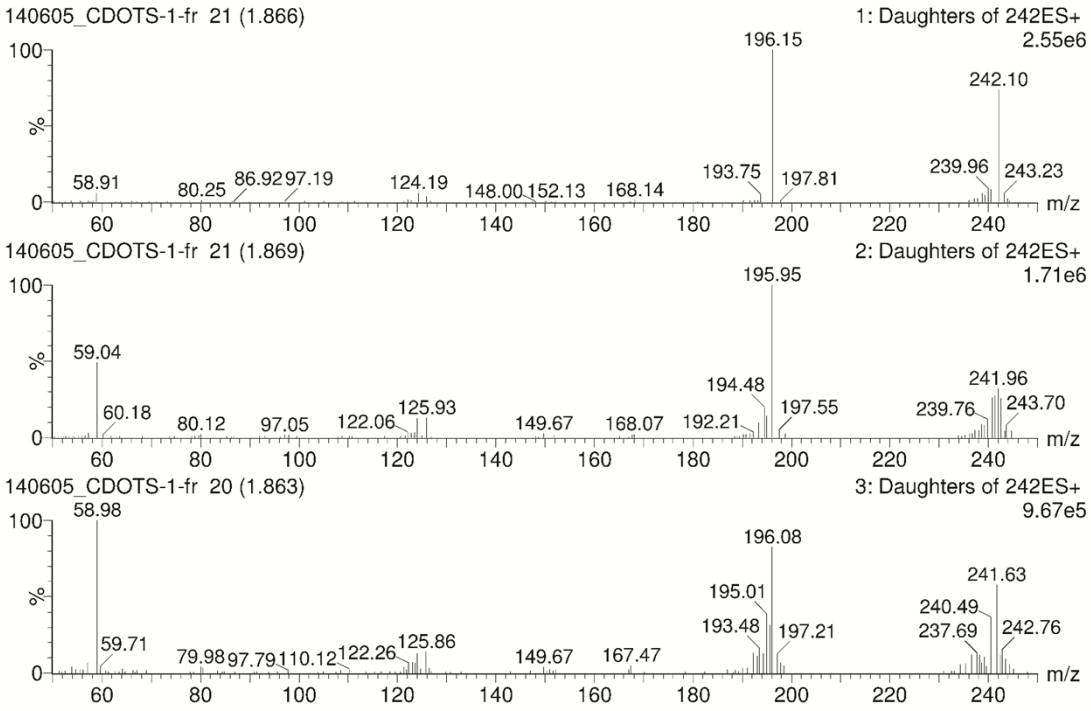

Fig. S2C ESI-MS/MS fragmentation spectra of **2b**.

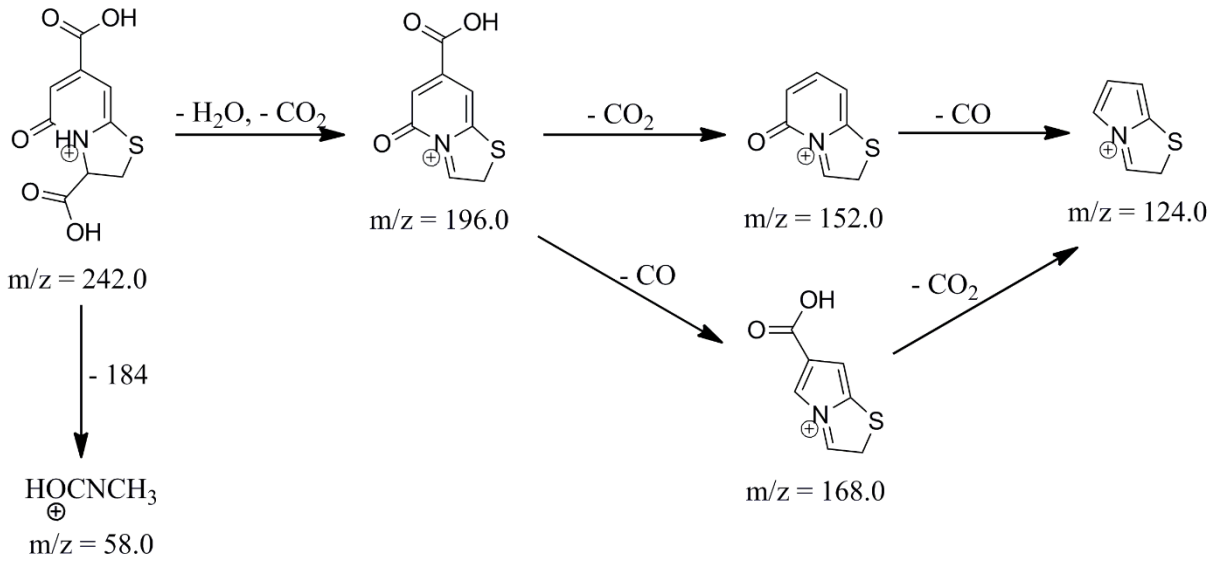

Fig. S2D ESI-MS/MS fragmentation pattern of **2b**.

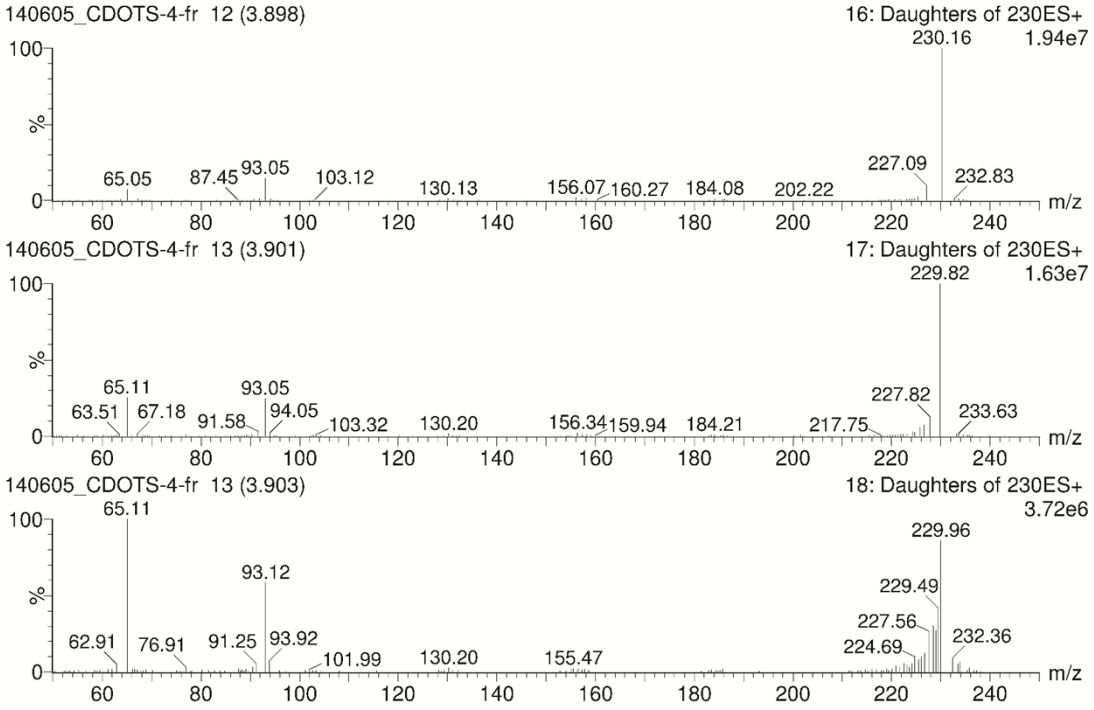

Fig. S2E ESI-MS/MS fragmentation spectra of **3b**.

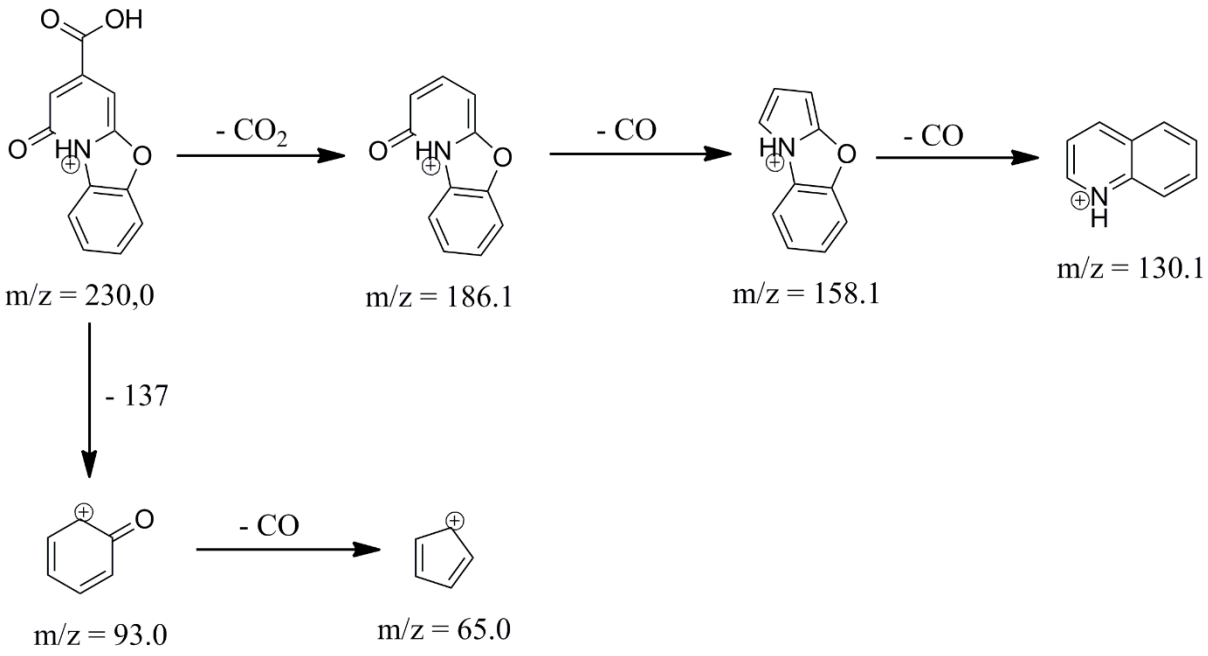

Fig. S2F ESI-MS/MS fragmentation pattern of **3b**.

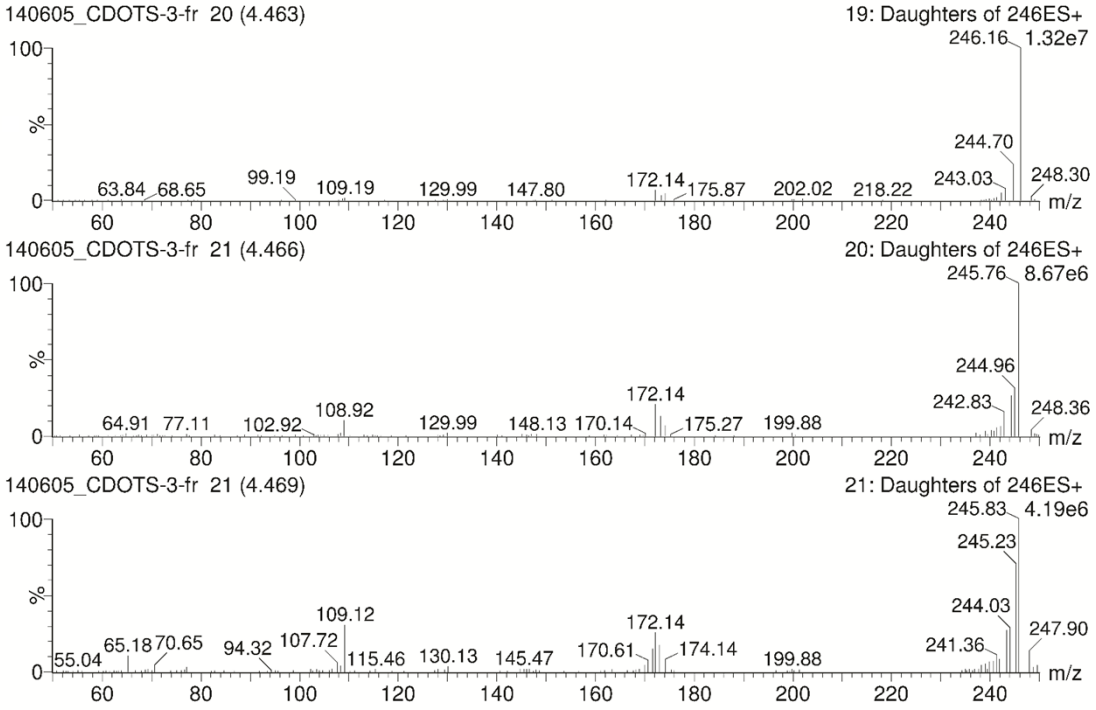

Fig. S2G ESI-MS/MS fragmentation spectra of **4b**.

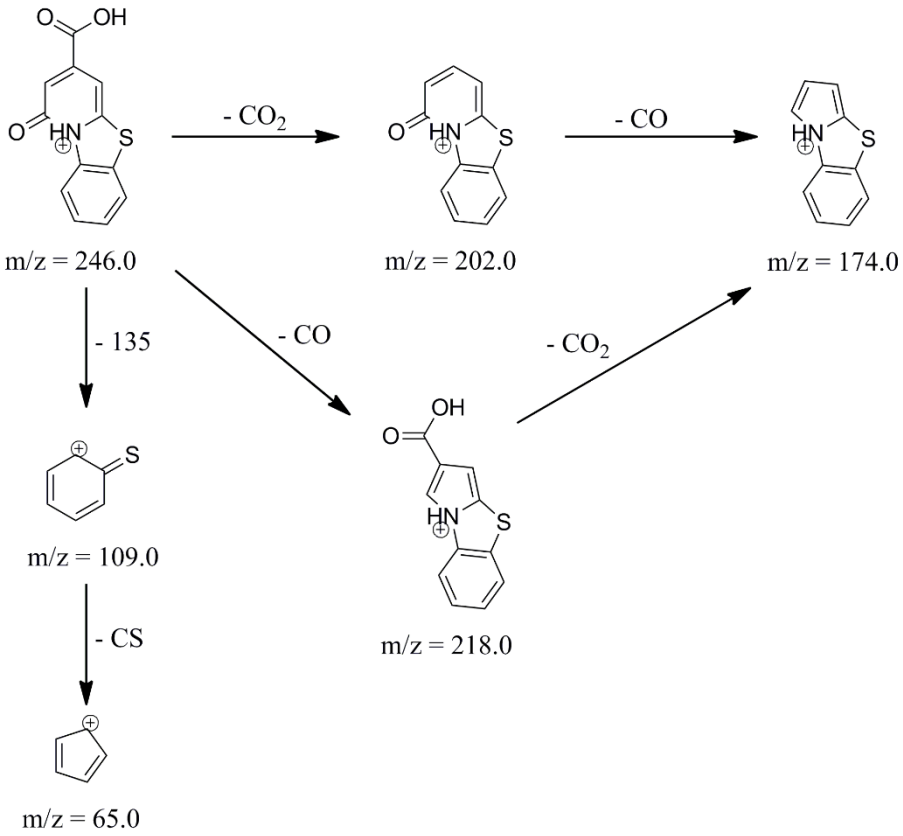

Fig. S2H ESI-MS/MS fragmentation pattern of **4b**.

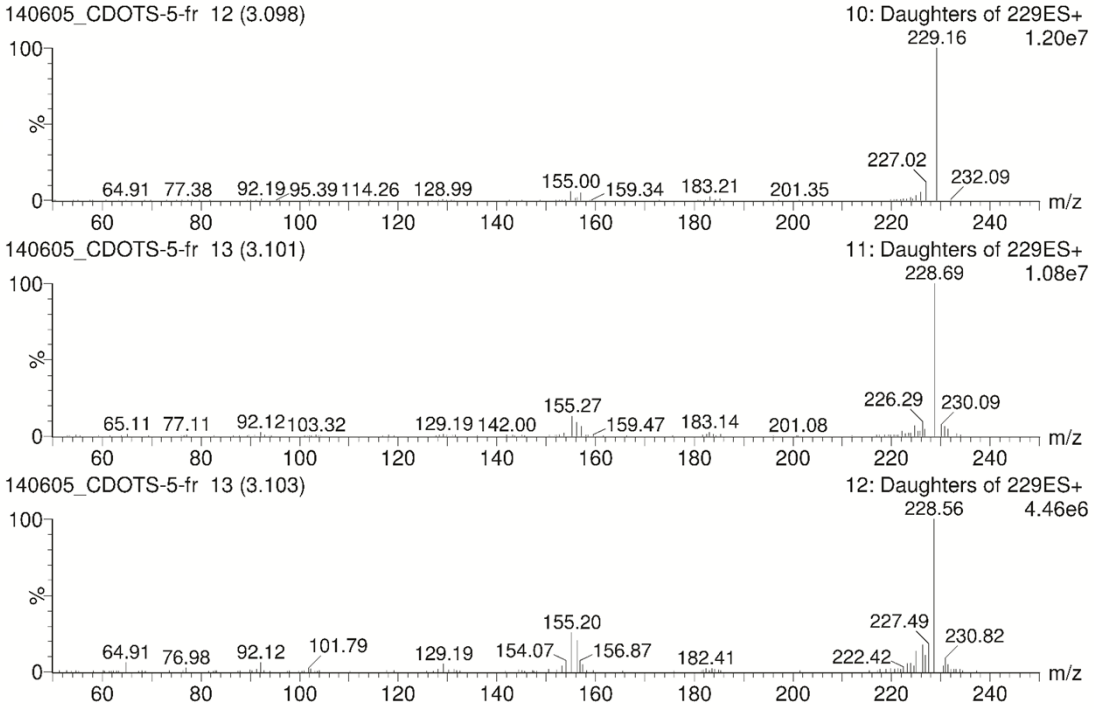

Fig. S2I ESI-MS/MS fragmentation spectra of **5b**.

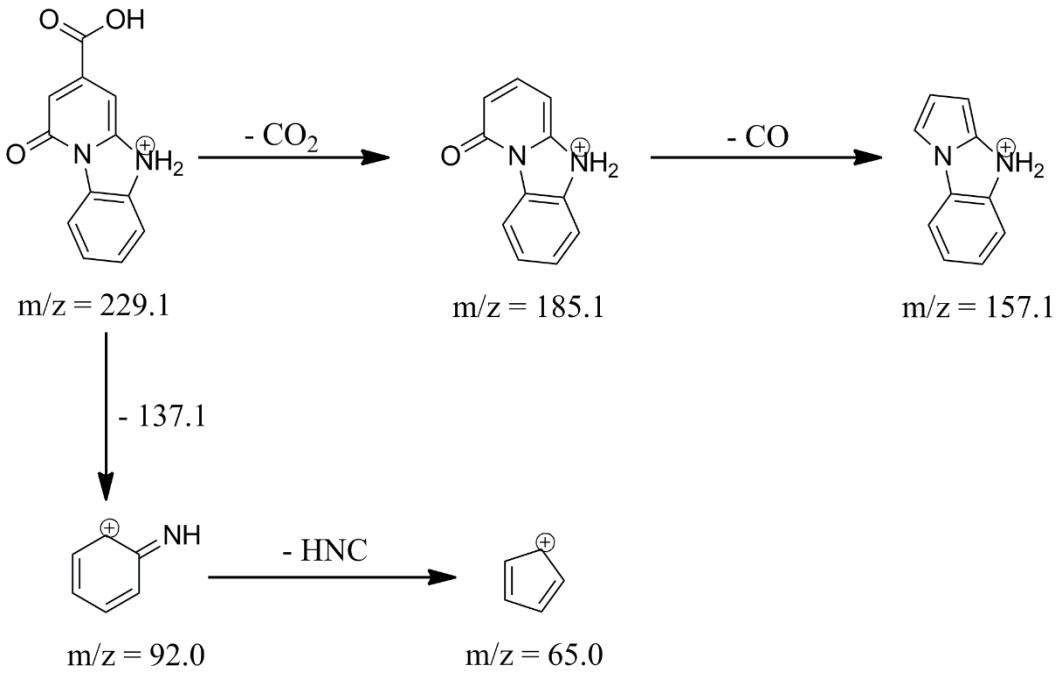

Fig. S2J ESI-MS/MS fragmentation pattern of **5b**.

Table S1 Identification of the empirical formulae of compounds 1b, 2b, 3b, 4b and 5b using elemental analysis and HR-ESI-MS.

Product	Empirical formula calculated from elemental analysis	HR-ESI-MS			
		Calculated mass (M+1)	Observed mass (M+1)	Empirical formula	Fit confidence
(1b)	$C_8H_7NO_3S$	198.0225	198.0220	$C_8H_8NO_3S$	100 %
(2b)	$C_9H_7NO_5S$	242.0123	242.0124	$C_9H_8NO_5S$	100 %
(3b)	$C_{12}H_7NO_4$	230.0453	230.0452	$C_{12}H_8NO_4$	100 %
(4b)	$C_{12}H_7NO_3S$	246.0225	246.0228	$C_{12}H_8NO_3S$	99.99 %
(5b)	$C_{12}H_8N_2O_3$	229.0613	229.0612	$C_{12}H_9N_2O_3$	100 %

Table S2 Fluorescence properties of compounds **1b**, **2b**, **3b**, **4b** and **5b**.

Compound	QY, %	Fluorescence lifetime, ns	Stokes shift, cm^{-1}
(1b)	61	$\tau = 7.49$, $\chi^2 = 1.067$	5390
(2b)	70	$T = 8.24$, $\chi^2 = 1.073$	5007
(3b)	79	$\tau = 7.99$, $\chi^2 = 1.058$	3023
(4b)	74	$\tau = 4.49$, $\chi^2 = 1.245$	4016
(5b)	63	$\tau_1 = 0.20$, $\tau_2 = 7.28$, $\chi^2 = 1.077$	3372

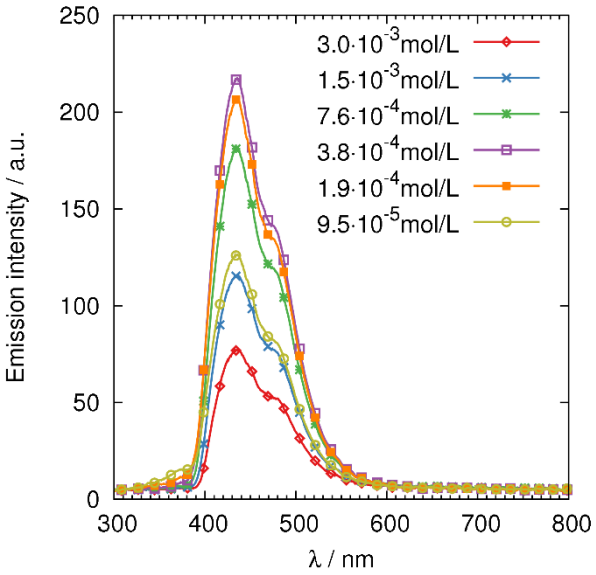

Fig. S3A Dependence of the fluorescence intensity on concentration of **1b** water solutions.

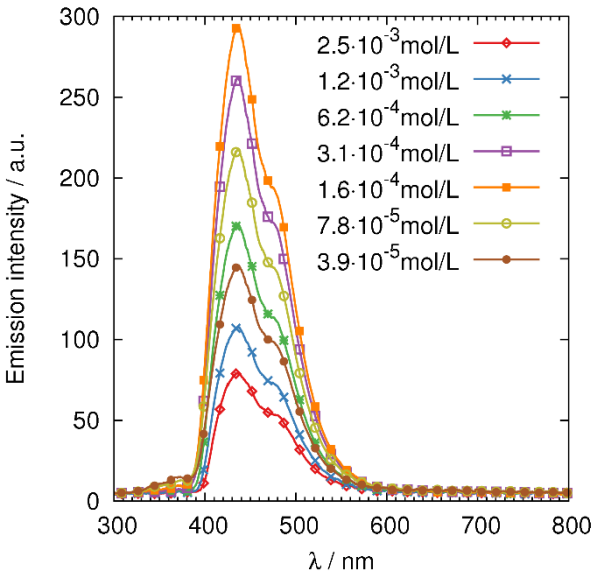

Fig. S3B Dependence of the fluorescence intensity on concentration of **2b** water solutions.

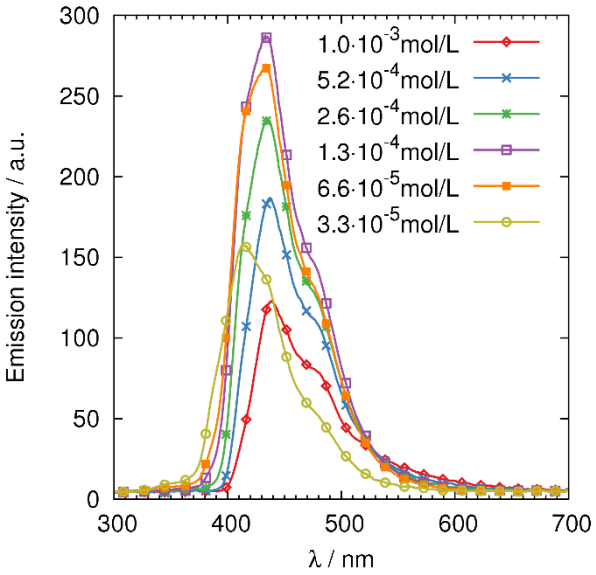

Fig. S3C Dependence of the fluorescence intensity on concentration of **3b** water solutions.

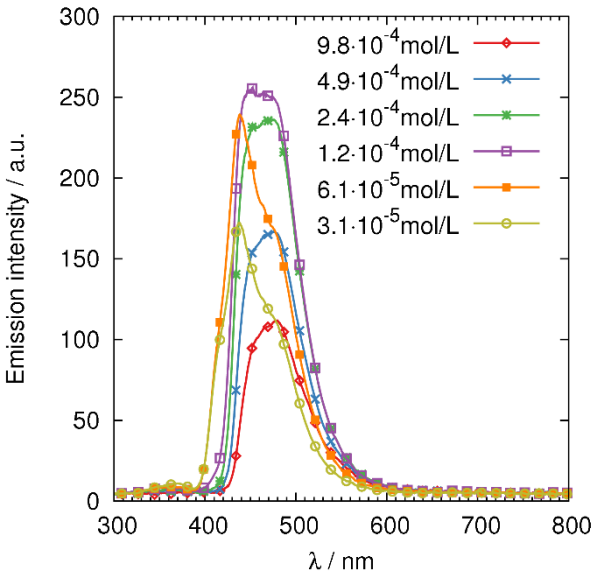

Fig. S3D Dependence of the fluorescence intensity on concentration of **4b** water solutions.

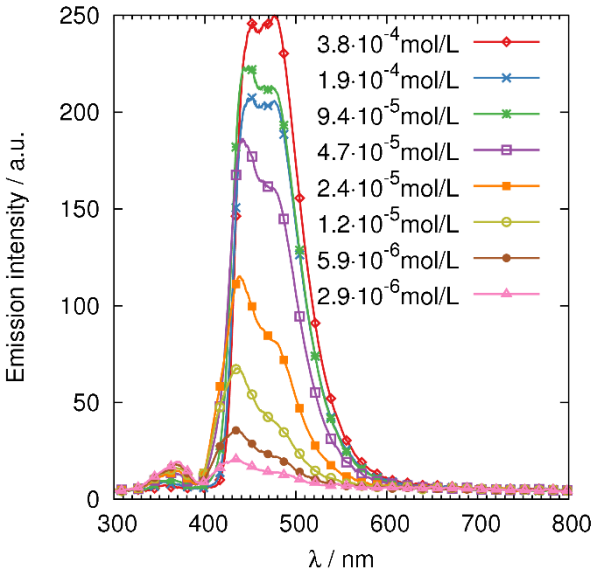

Fig. S3E Dependence of the fluorescence intensity on concentration of **5b** water solutions.

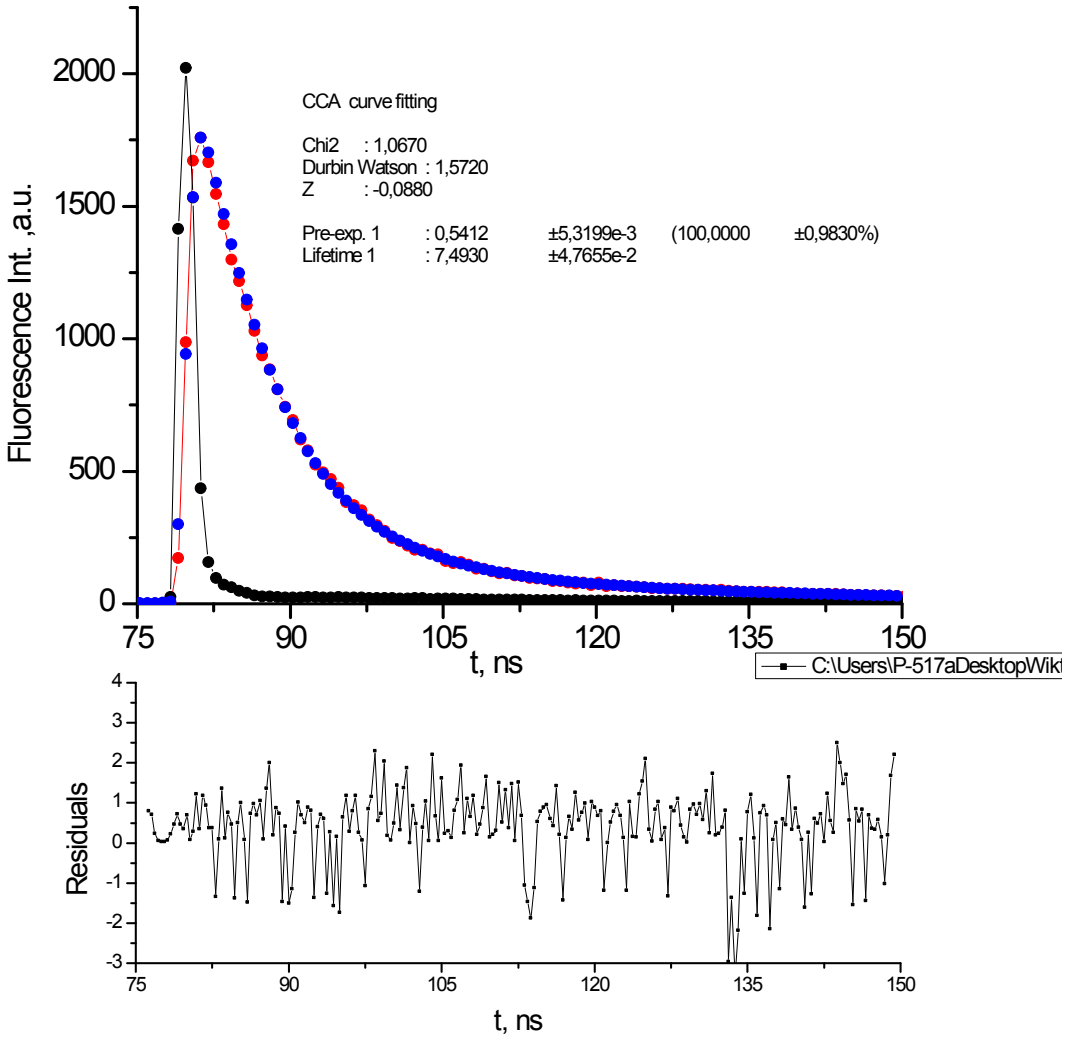

Fig. S4A Fluorescence decay curves of **1b** (325 nm LED as an excitation source was used).

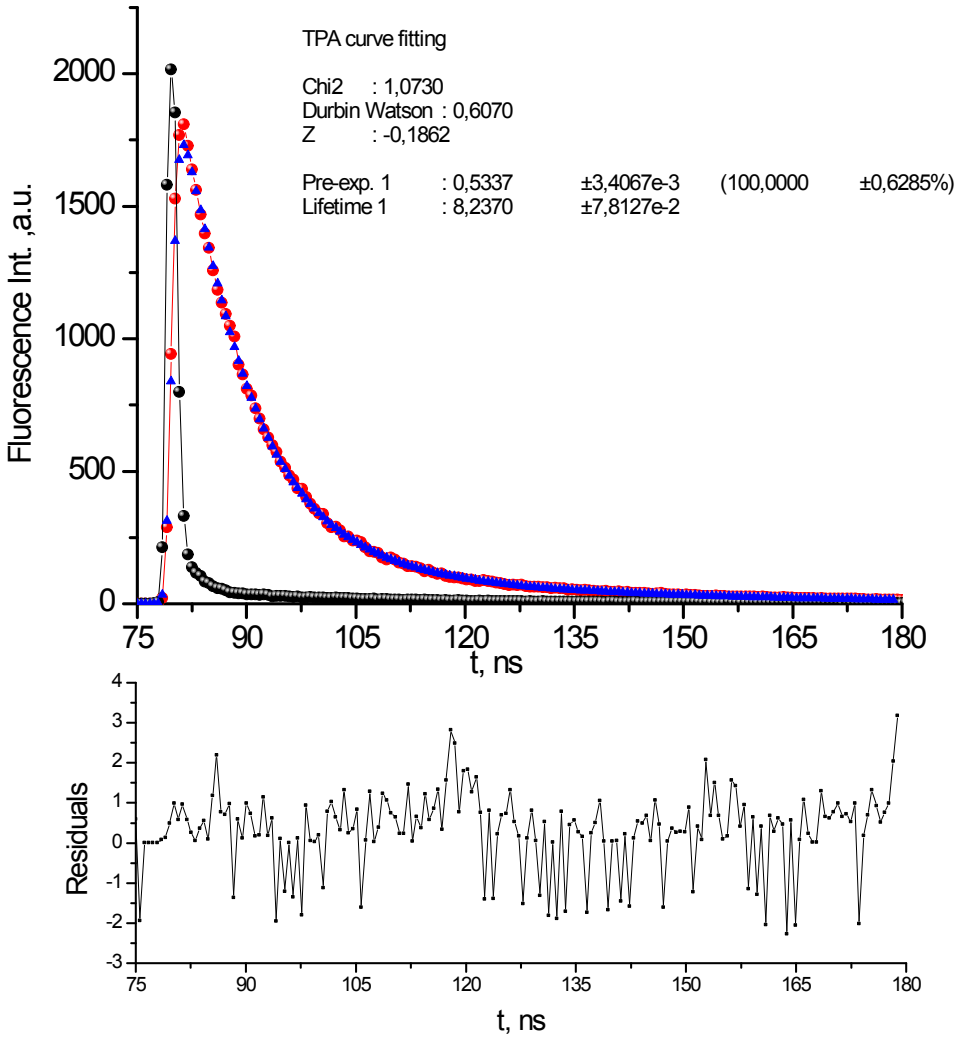

Fig. S4B Fluorescence decay curves of **2b** (325 nm LED as an excitation source was used).

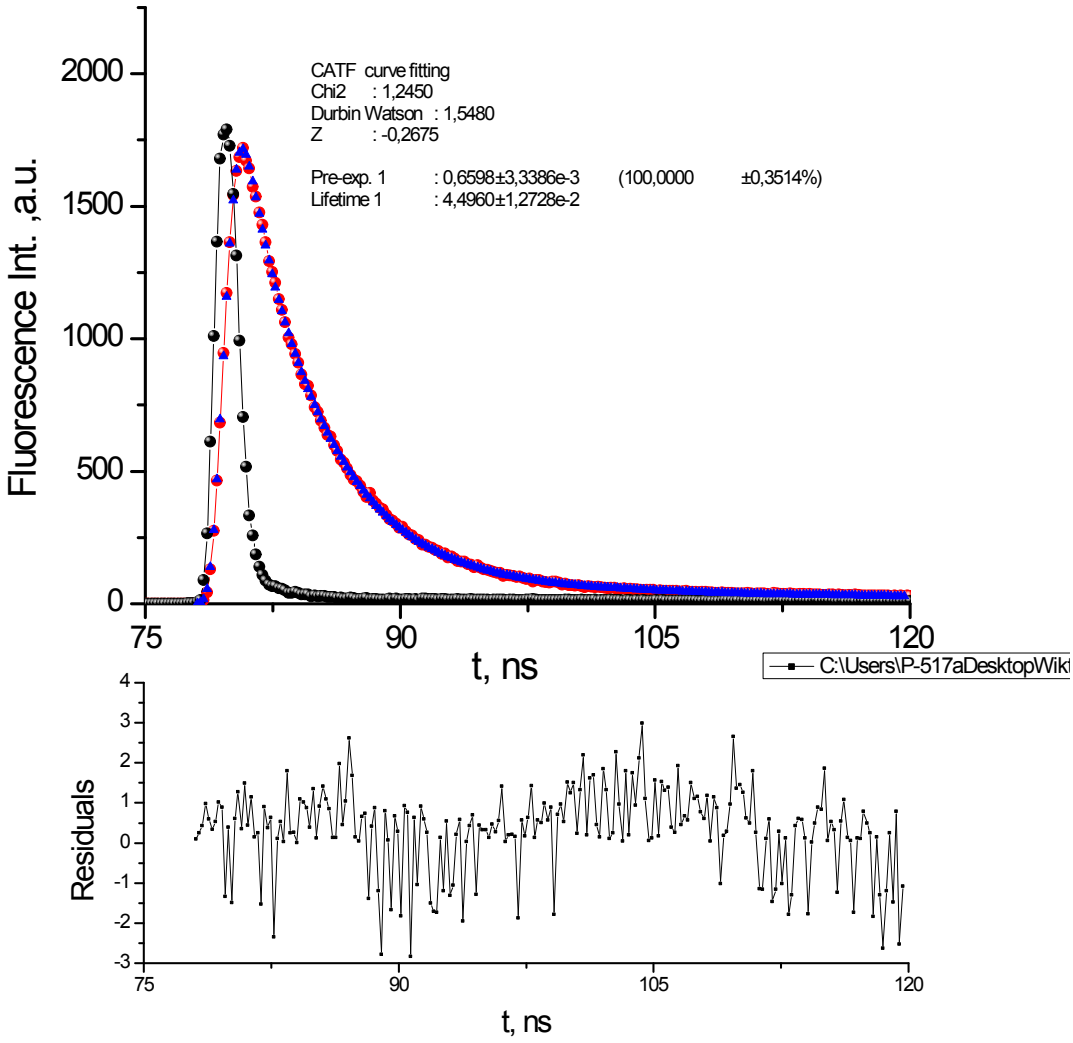

Fig. S4C Fluorescence decay curves of **3b** (325 nm LED as an excitation source was used).

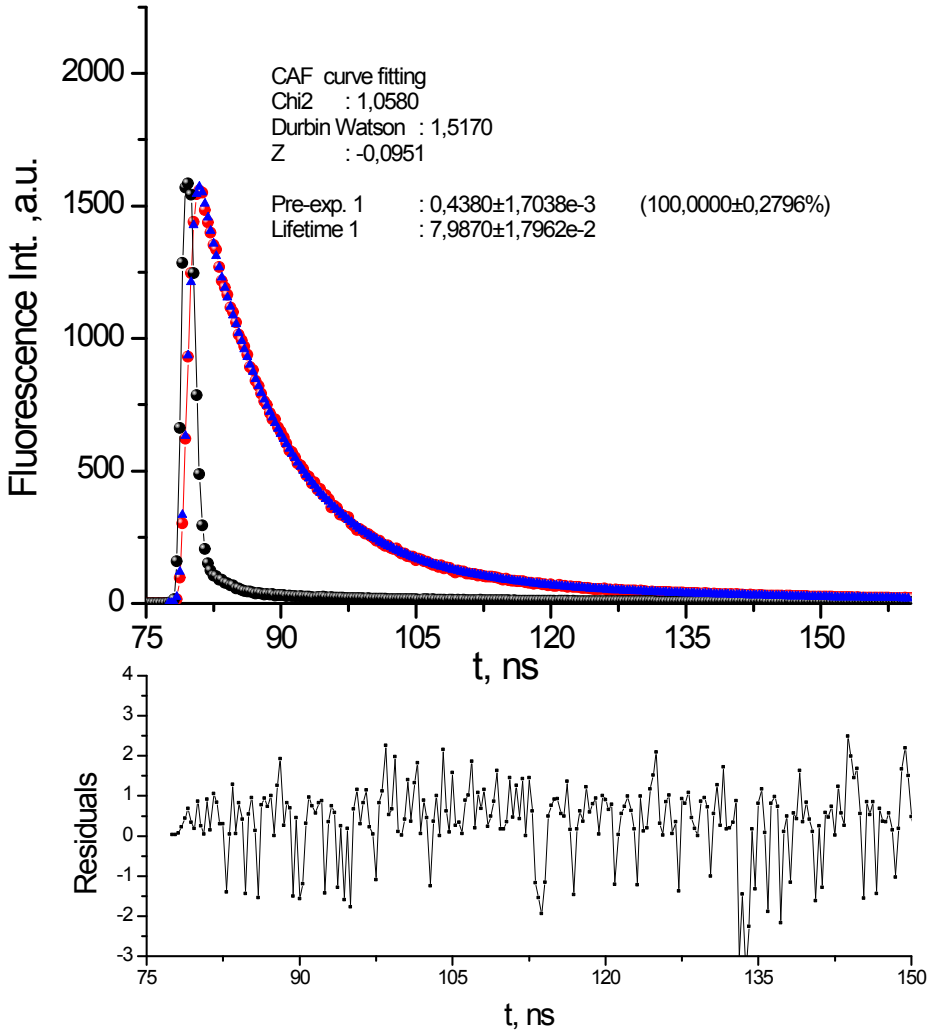

Fig. S4D Fluorescence decay curves of **4b** (325 nm LED as an excitation source was used).

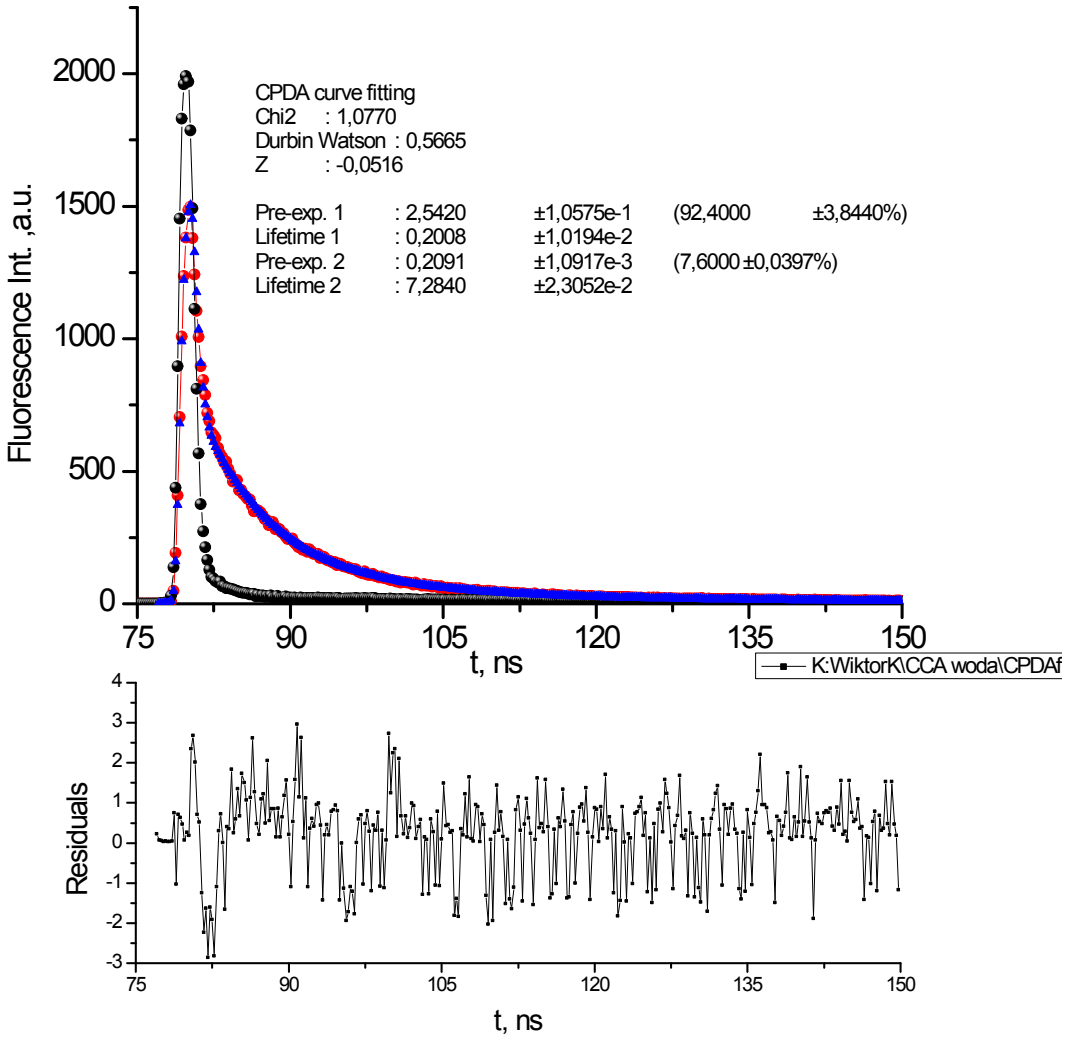

Fig. S4E Fluorescence decay curves of **5b** (325 nm LED as an excitation source was used).

Tab. S3 HR-ESI-MS data indicating the presence of compounds **6b**, **7b**, **8b**, **9b**, **14b** and **15b** in reaction mixtures.

Compound	HR-ESI-MS			
	Calculated mass (M+1)	Observed mass (M+1)	Empirical formula	Fit confidence
3-[(carboxymethyl)carbamoyl]-5-oxo-2,3-dihydro-5*H*-[1,3]thiazolo[3,2-*a*]pyridine-7-carboxylic acid **(6b)**	299.0338	299.0333	$C_{11}H_{11}N_2O_6S$	100%
1-methyl-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-*a*]pyridine-7-carboxylic acid **(7b)**	195.0770	195.0769	$C_9H_{11}N_2O_3$	100%
2-methyl-5-oxo-1,2,3,5-tetrahydroimidazo[1,2-*a*]pyridine-7-carboxylic acid **(8b)**	195.0770	195.0767	$C_9H_{11}N_2O_3$	100%
5-oxo-2,3-dihydro-5*H*-[1,3]oxazolo[3,2-*a*]pyridine-7-carboxylic acid **(14b)**	182.0453	182.0451	$C_8H_8NO_4$	100%
6-oxo-1,3,4,6-tetrahydro-2*H*-pyrido[1,2-*a*]pyrimidine-8-carboxylic acid **(9b)**	195.0770	195.0768	$C_9H_{11}N_2O_3$	100%
6-oxo-3,4-dihydro-2H,6H-pyrido[2,1-*b*][1,3]oxazine-8-carboxylic acid **(15b)**	196.0610	196.0610	$C_9H_{10}NO_4$	100%

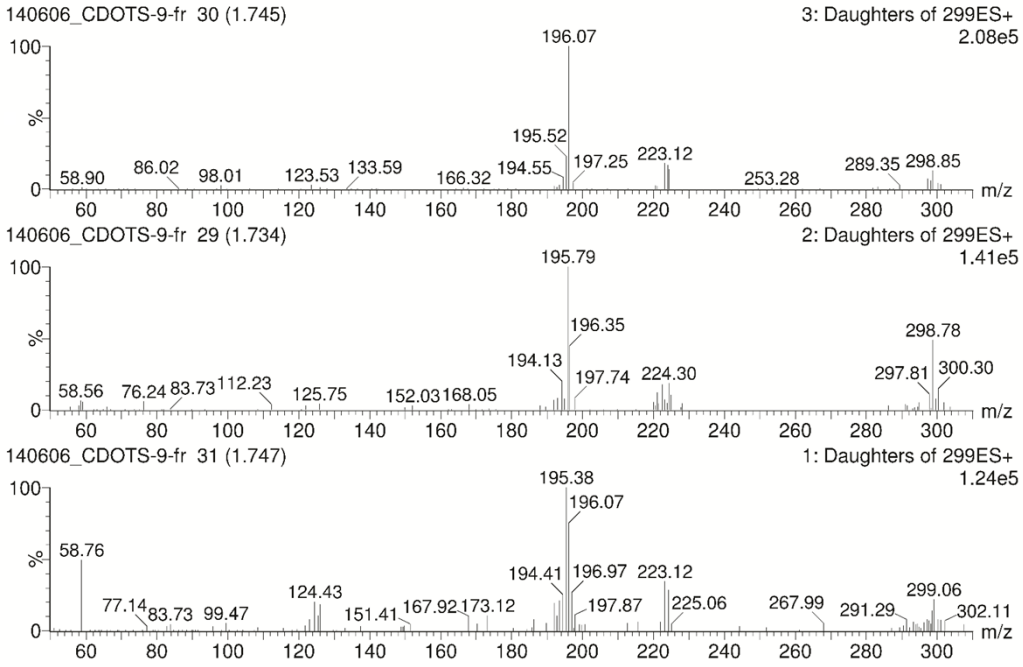

Fig. S5A ESI-MS/MS fragmentation spectra of **6b**.

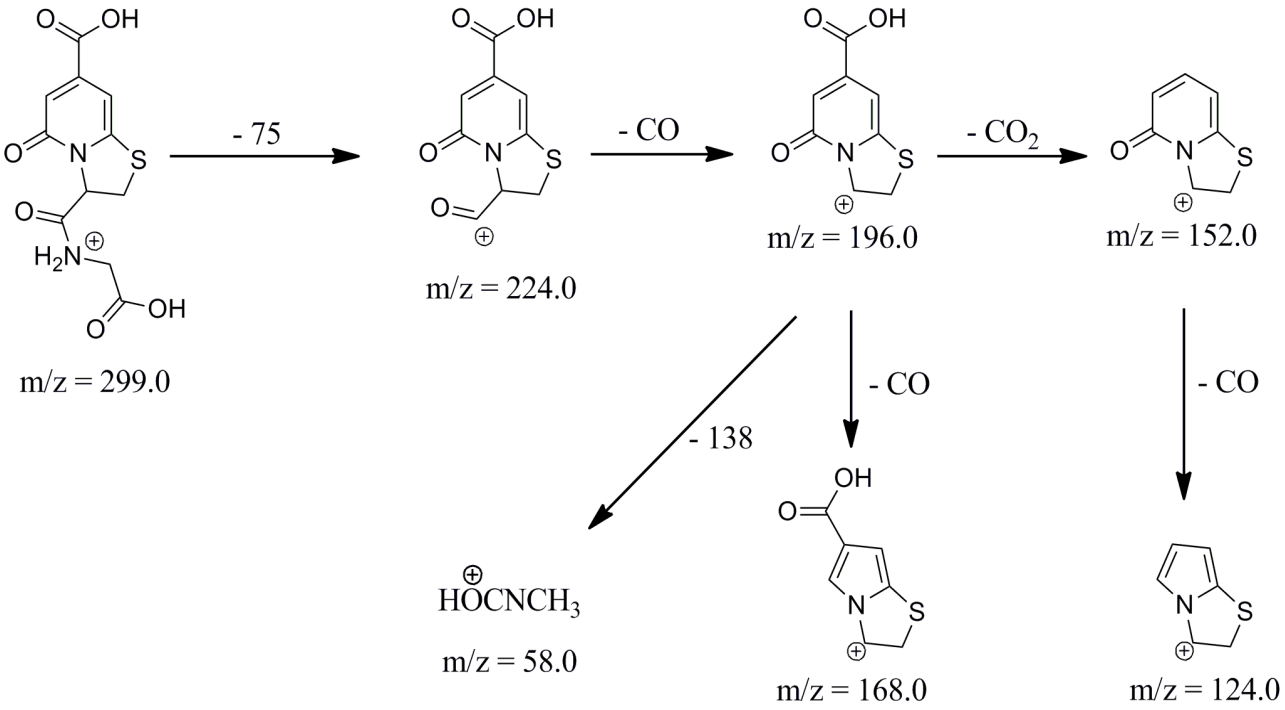

Fig. S5B ESI-MS/MS fragmentation pattern of **6b**.

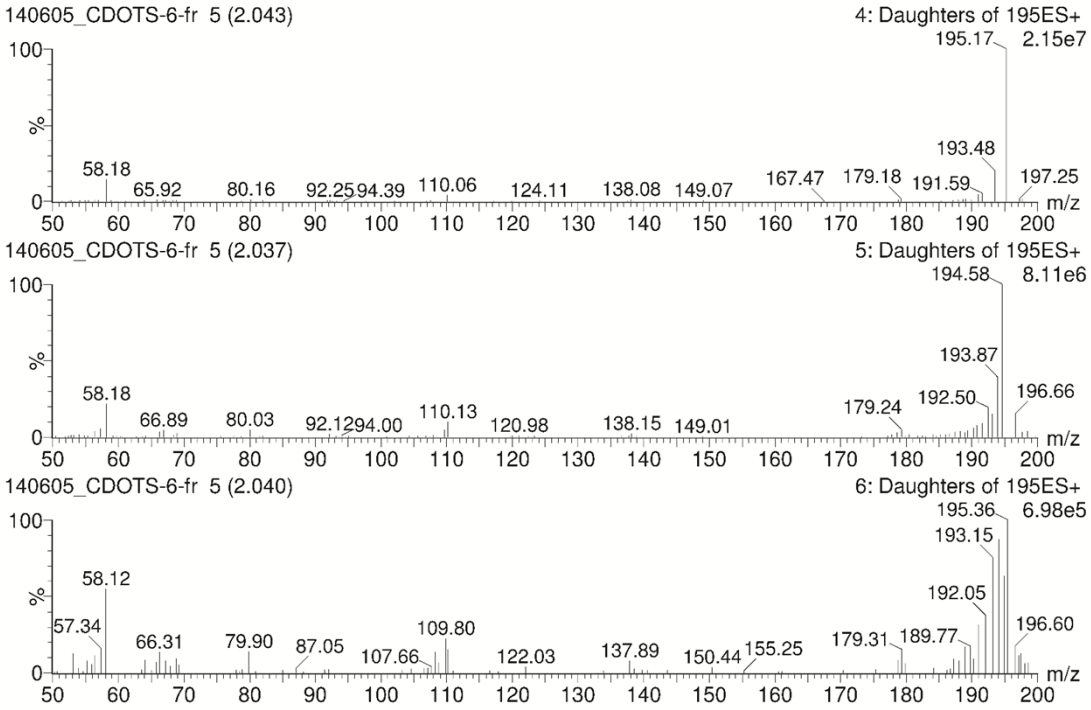

Fig. S6A ESI-MS/MS fragmentation spectra of **7b**.

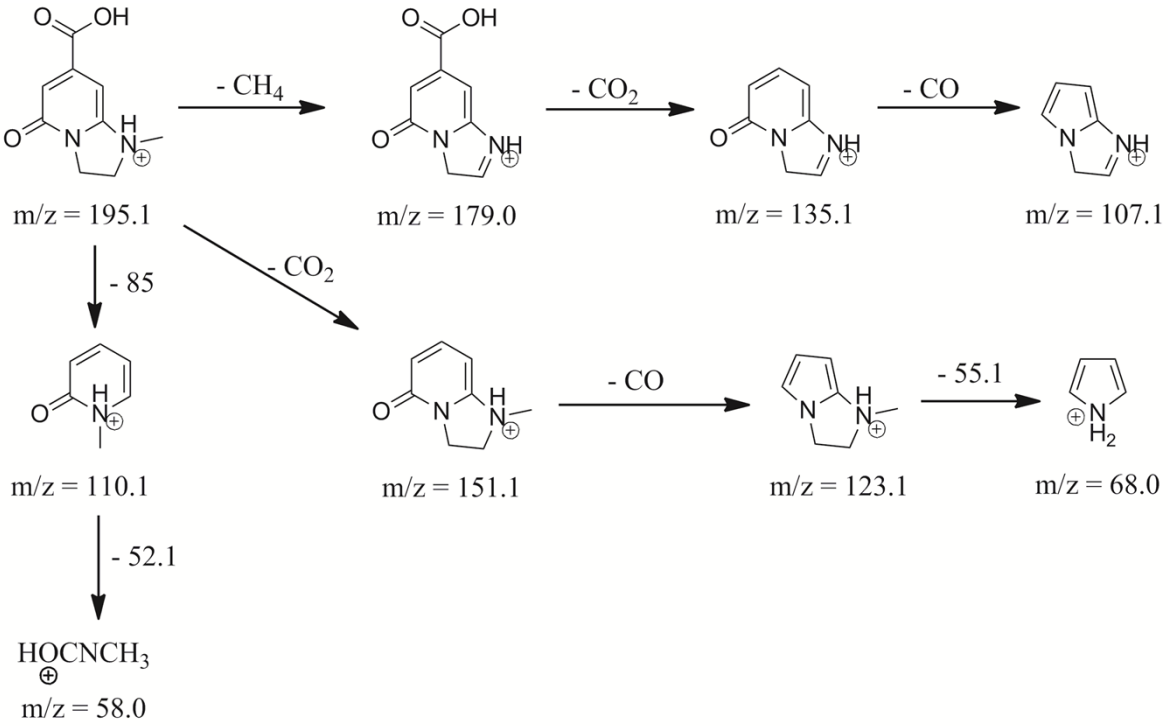

Fig. S6B ESI-MS/MS fragmentation pattern of 7**b**.

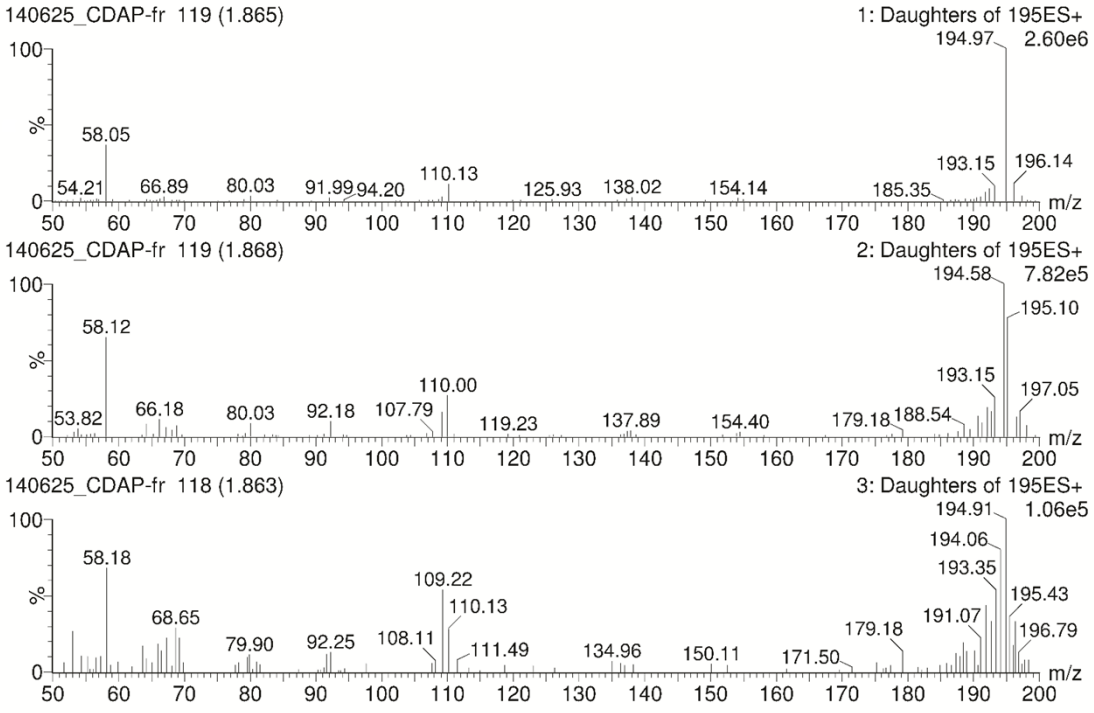

Fig. S7A ESI-MS/MS fragmentation spectra of **8b**.

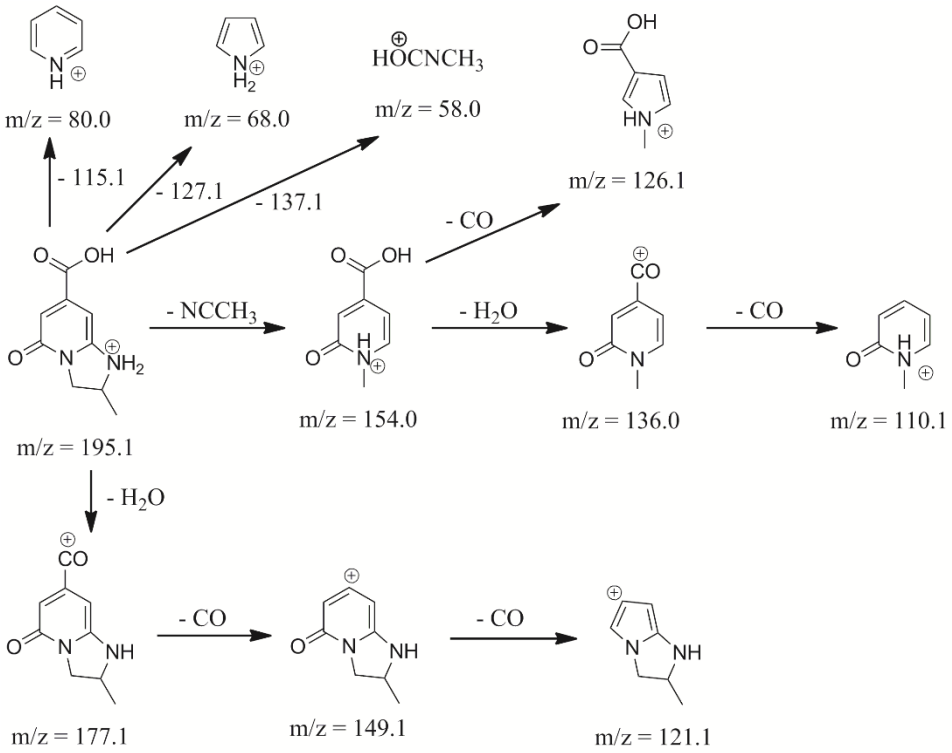

Fig. S7B ESI-MS/MS fragmentation pattern of **8b**.

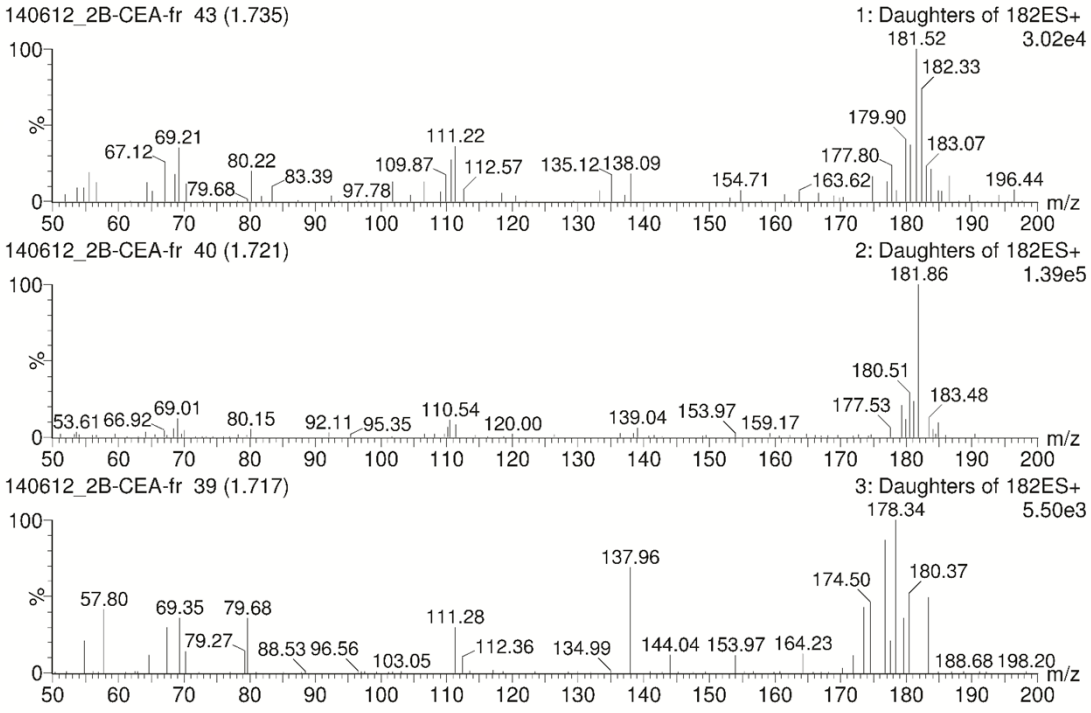

Fig. S8A ESI-MS/MS fragmentation spectra of **14b**.

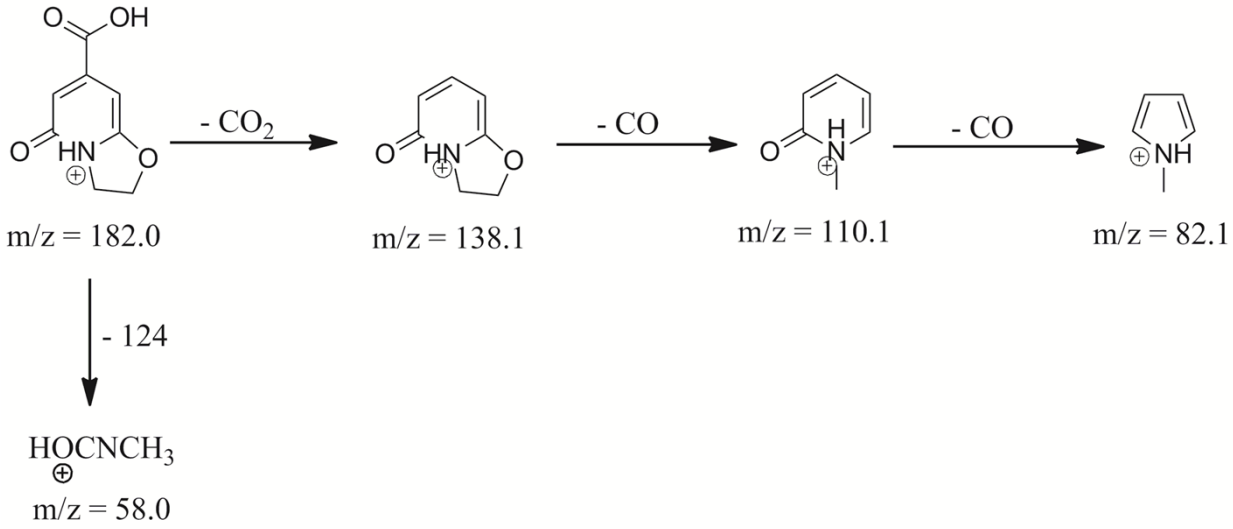

Fig. S8B ESI-MS/MS fragmentation pattern of **14b**.

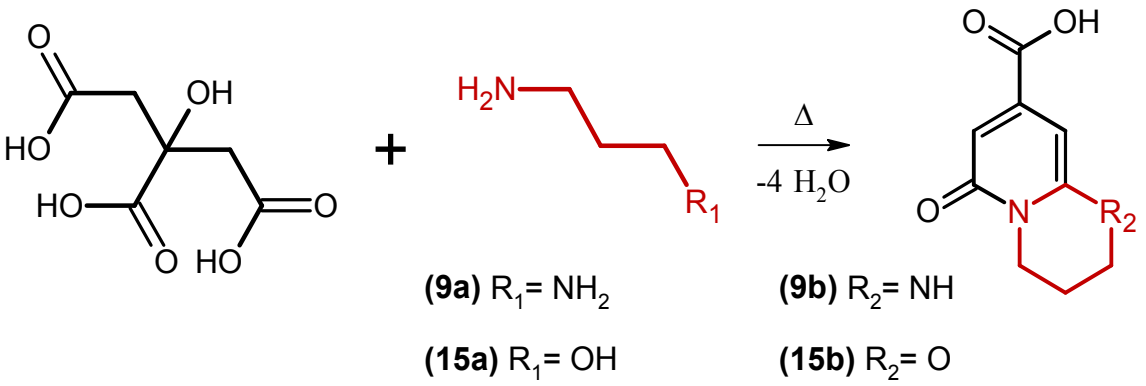

Fig. S9 Formation of fluorescent 2-pyridones from citric acid and selected γ-substituted amines.

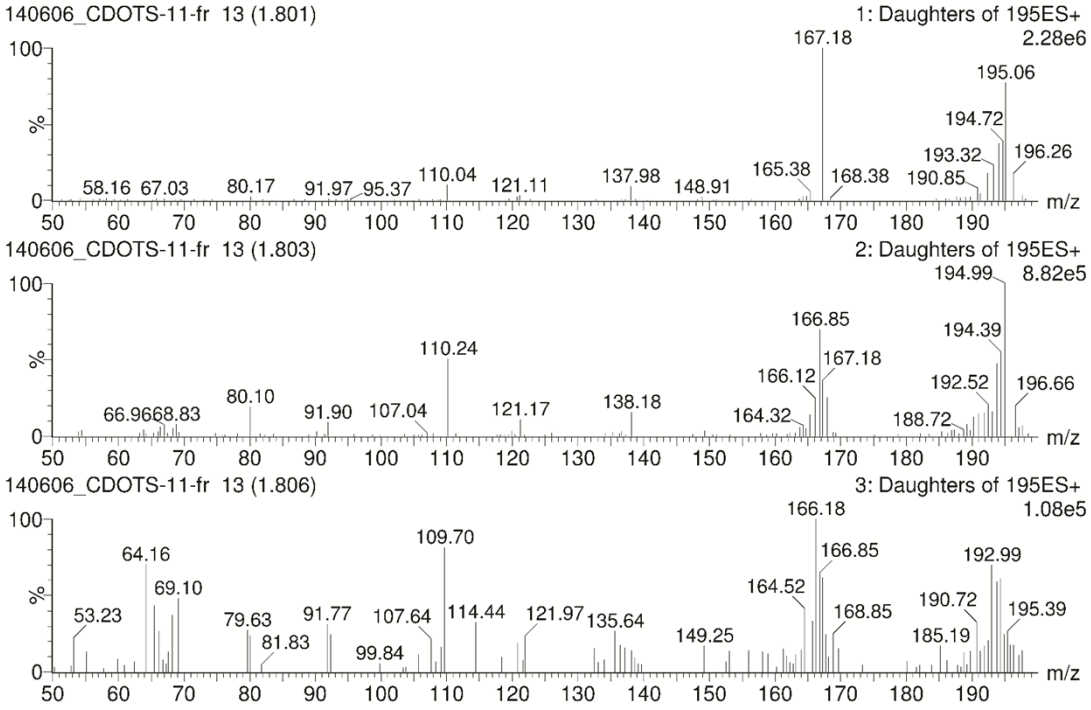

Fig. S10A ESI-MS/MS fragmentation spectra of **9b**.

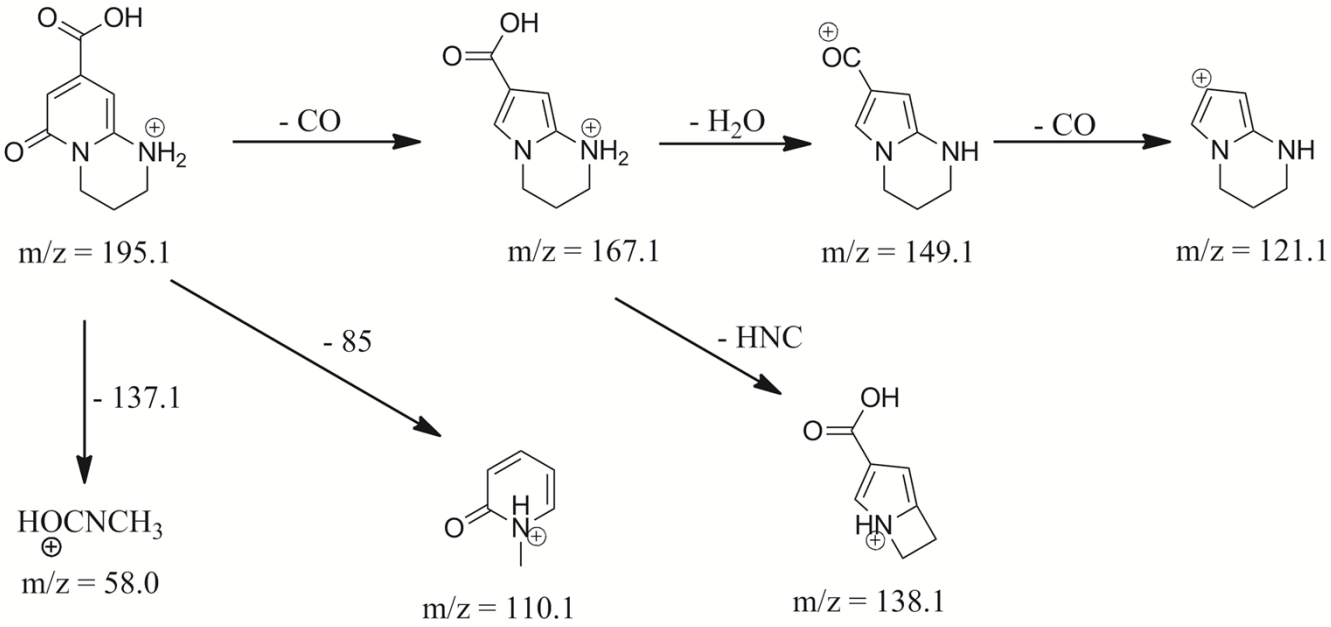

Fig. S10B ESI-MS/MS fragmentation pattern of **9b**.

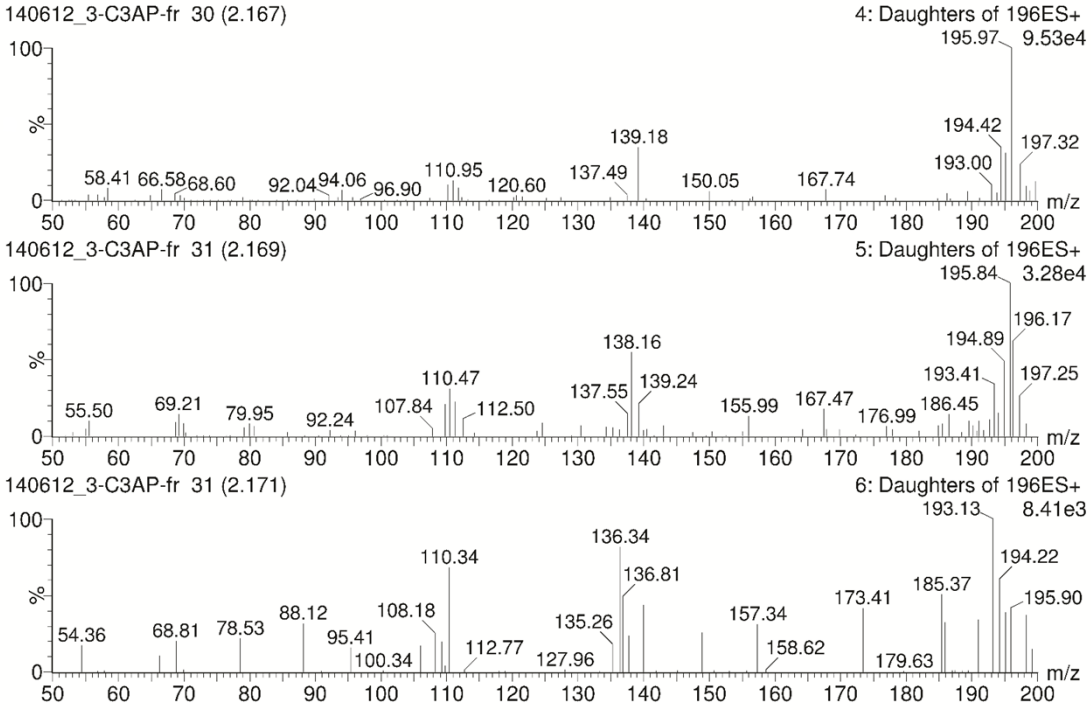

Fig. S11A ESI-MS/MS fragmentation spectra of **15b**.

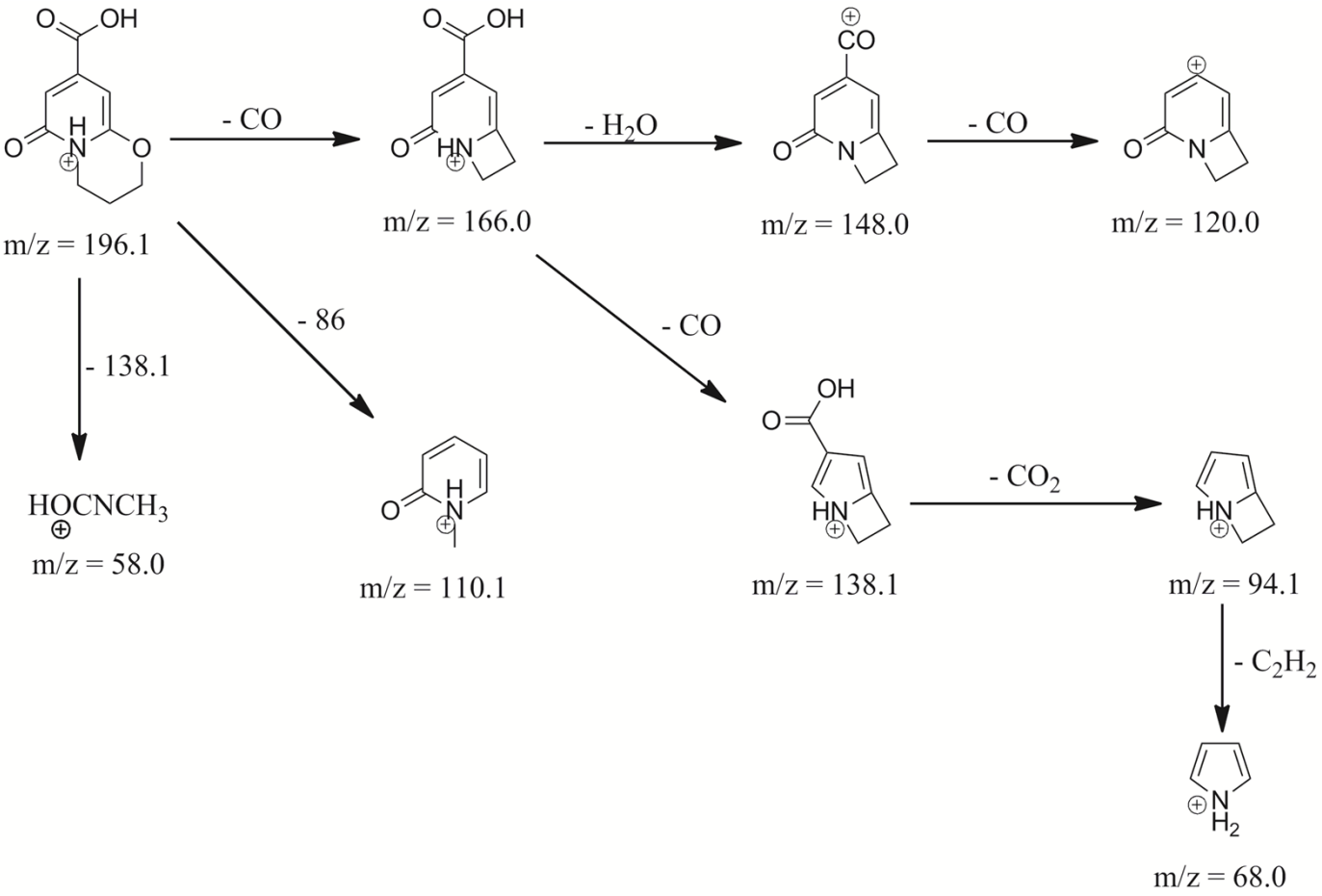

Fig. S11B ESI-MS/MS fragmentation pattern of **15b**.