

Supplementary Information for:

Ratiometric fluorescence probes based on a Michael acceptor type of coumarins and their application for the multichannel imaging of *in vivo* glutathione

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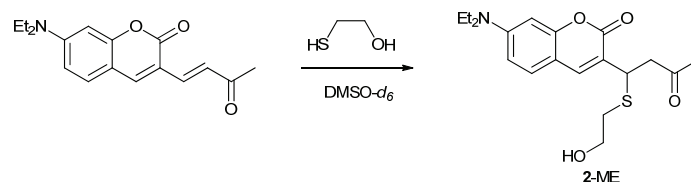
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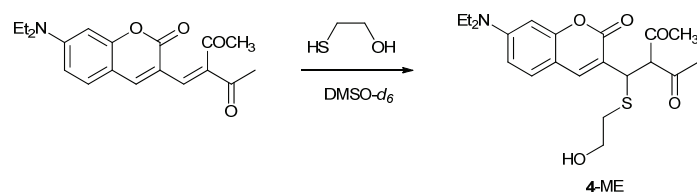
1. Preparation of probe-thiol conjugates

To a solution of probe (20 mM) and potassium carbonate (1 equiv ex) in DMSO- d_6 was added 2-mercaptoethanol(**ME**, 2.5 equiv) and time-dependent ^1H NMR spectra were measured at rt.



2-ME

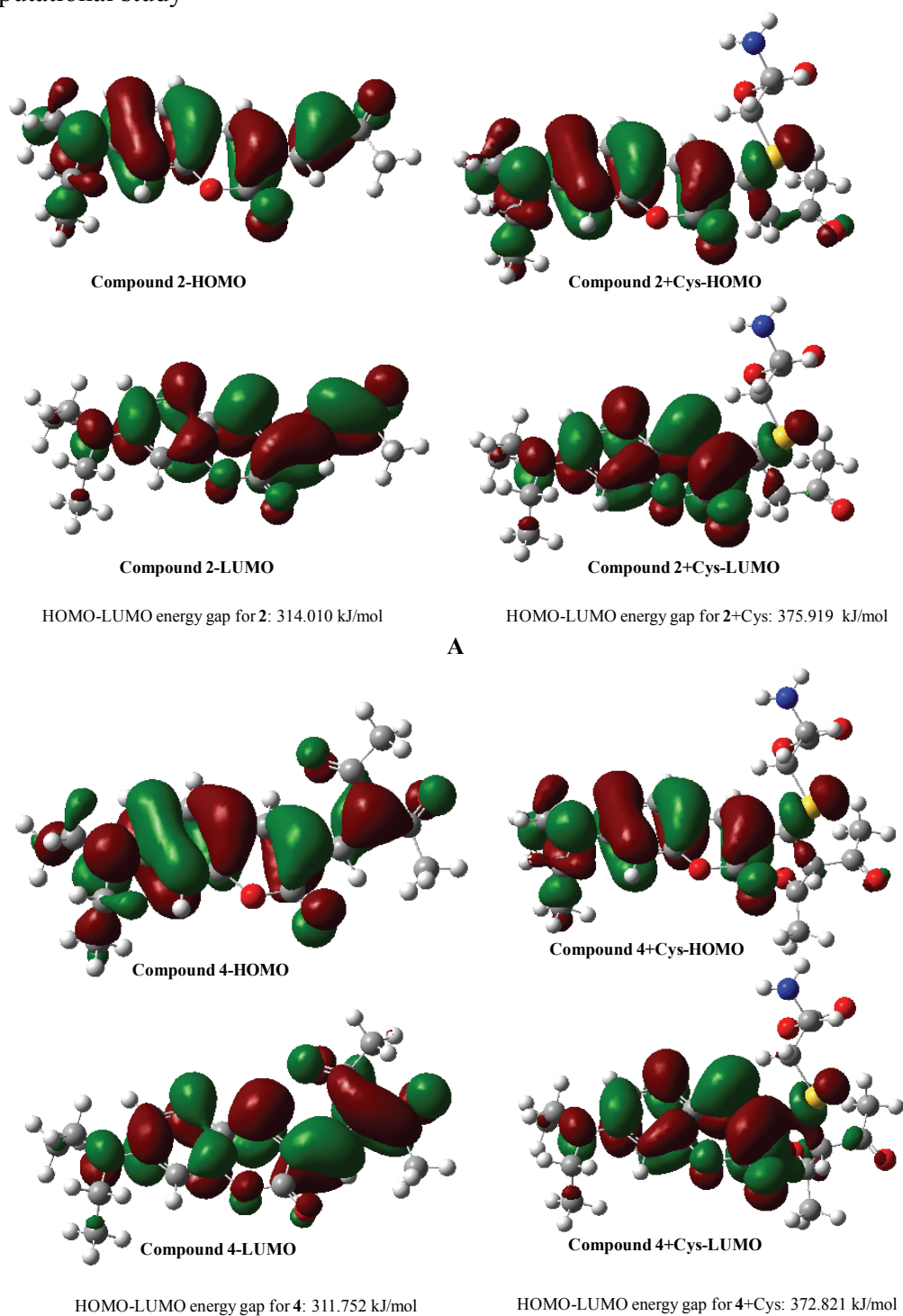
^1H NMR (200 MHz, DMSO- d_6): δ 7.80 (s, 1H), 7.40 (d, 1H, $^3J = 8.8$ Hz), 6.68 (dd, 1H, $^4J = 2.4$ Hz, $^3J = 9$ Hz), 6.51 (d, 1H, $^4J = 2.4$ Hz), 4.26 (t, 1H, $^3J = 7.4$ Hz, C^*H), 3.46 (m, 2H, $-CH_2OH$), 3.43 (q, 4H, $^3J = 6.8$ Hz, $-NCH_2CH_3$), 3.11 (dd, 2H, $^3J = 7.6$ Hz, $^3J = 7.4$ Hz C^*HCH_2), 2.53 (m, 2H, $-SCH_2CH_2OH$), 2.10 (s, 3H, $-COCH_3$), 2.36 (s, 3H, $-COCH_3$) 1.10 (t, 6H, $^3J = 6.8$ Hz, $-NCH_2CH_3$).



4-ME

^1H NMR (200 MHz, DMSO- d_6): δ 7.83 (s, 1H), 7.38 (d, 1H, $^3J = 9.0$ Hz), 6.70 (dd, 1H, $^4J = 2.4$ Hz, $^3J = 9$ Hz), 6.50 (d, 1H, $^4J = 2.4$ Hz), 4.90 (d, 1H, $^3J = 11.8$ Hz, C^*HCH), 4.78 (t, 1H, $^3J = 5.8$ Hz, OH), 4.40 (d, 1H, $^3J = 11.8$ Hz, C^*H), 3.48 (m, 2H, $-CH_2OH$), 3.42 (q, 4H, $^3J = 7.0$ Hz, $-NCH_2CH_3$), 2.51 (m, 2H, $-SCH_2CH_2OH$), 2.33 (s, 3H, $-COCH_3$), 2.10 (s, 3H, $-COCH_3$), 1.10 (t, 6H, $^3J = 7.0$ Hz, $-NCH_2CH_3$).

2. Computational study



B
Fig S1. DFT calculation of **2** and **4** with Cys (Spartan 2010, B3LYP, 6-31G*). Atomic color codes: C (black), N (blue), O (red), H (white). (A) **2** with Cys, (B) **4** with Cys.

3. Fluorescence spectra of **2**

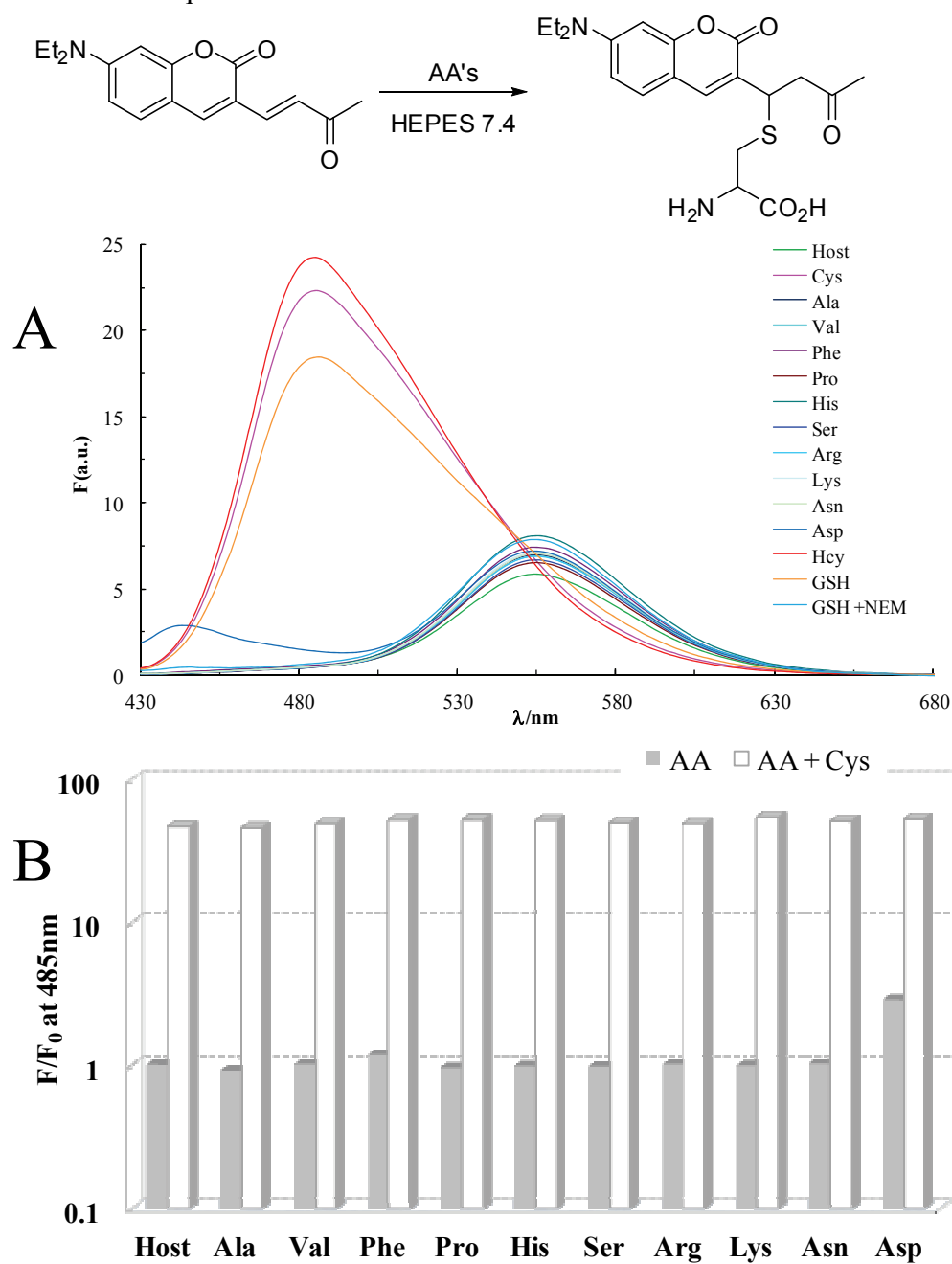


Fig S2. Fluorescence spectral changes of **2** (10 μ M) in HEPES (0.1 M HEPES, pH 7.4) upon addition of various amino acids (1000 equiv). (B) The competition graphs of GSH over various amino acids (AA, 1000 equiv).

4. NMR spectra

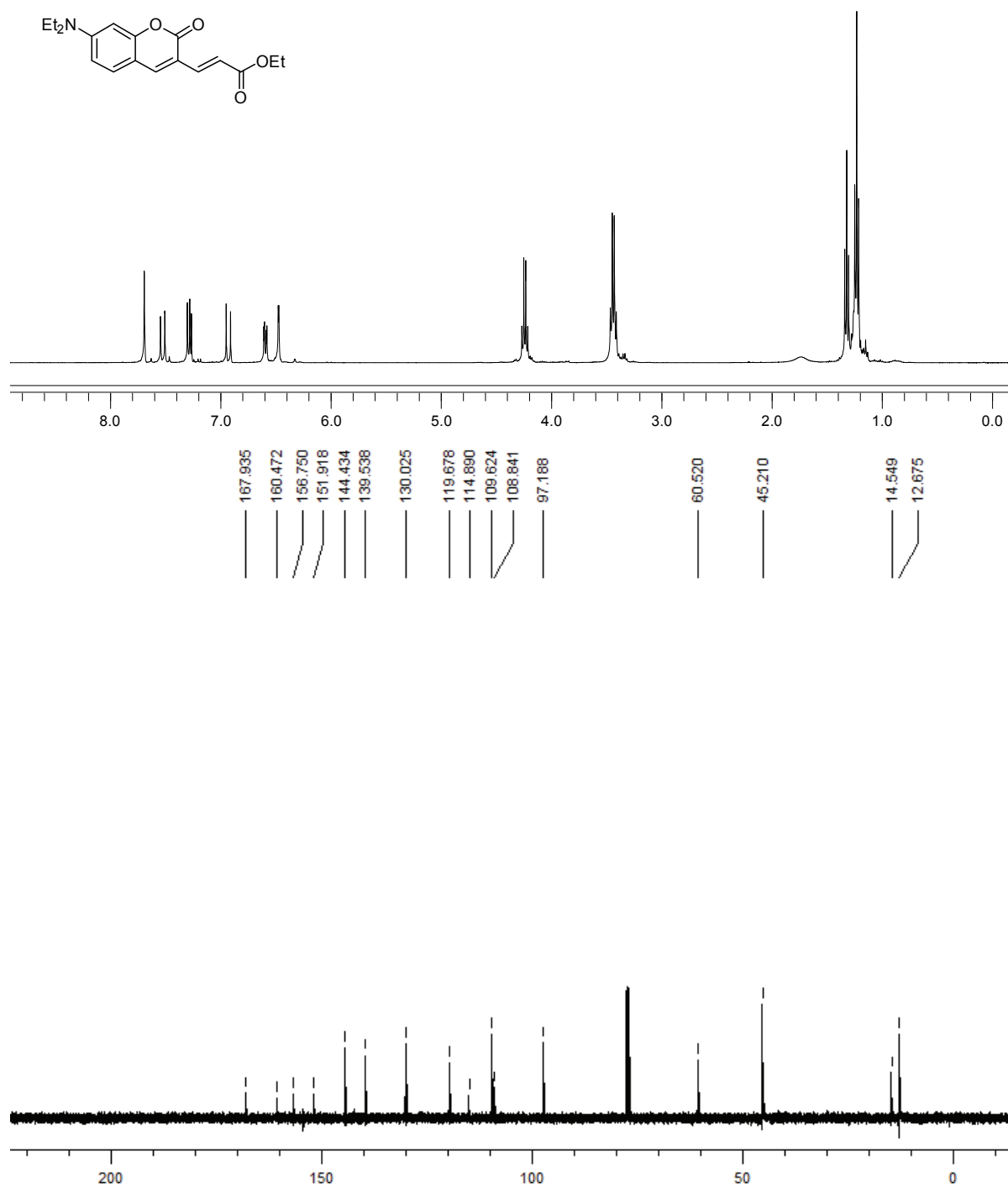


Fig. S3. ¹H and ¹³C NMR spectra of **1** in CDCl₃.

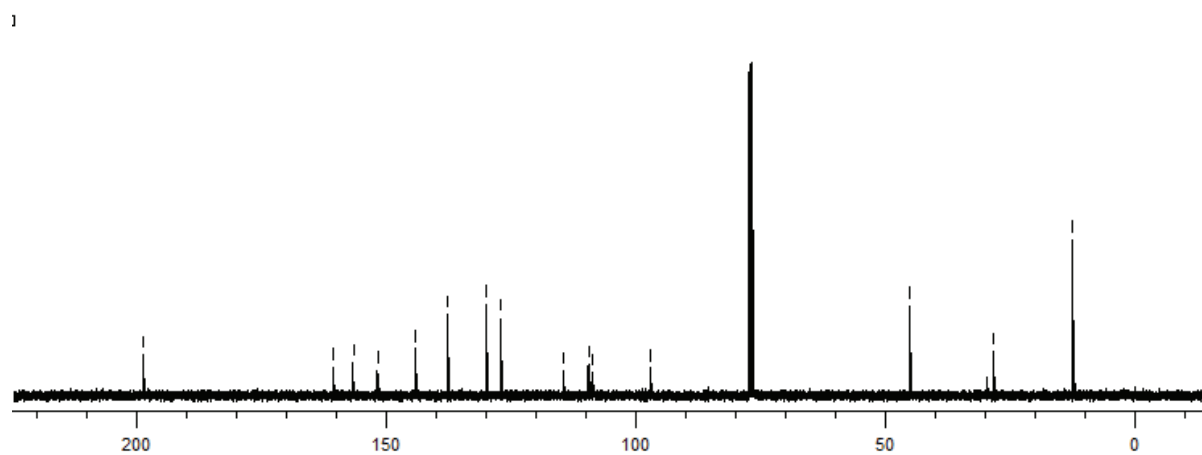
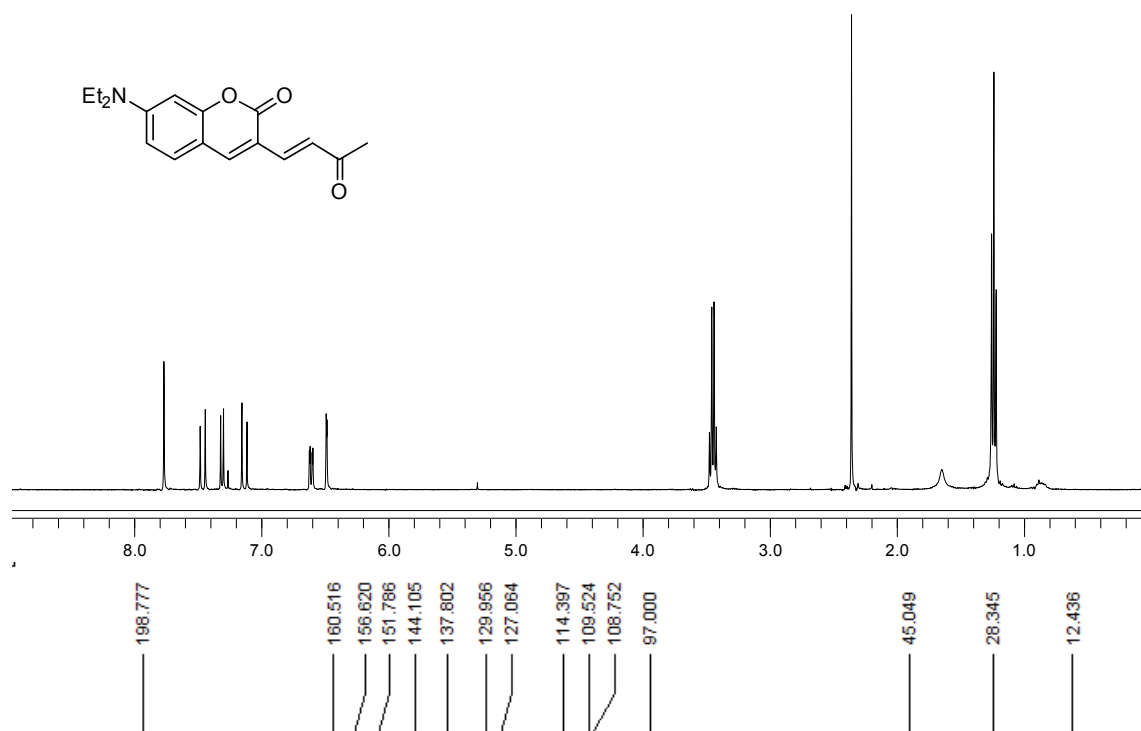


Fig. S4. ¹H and ¹³C NMR of **2** in CDCl₃.

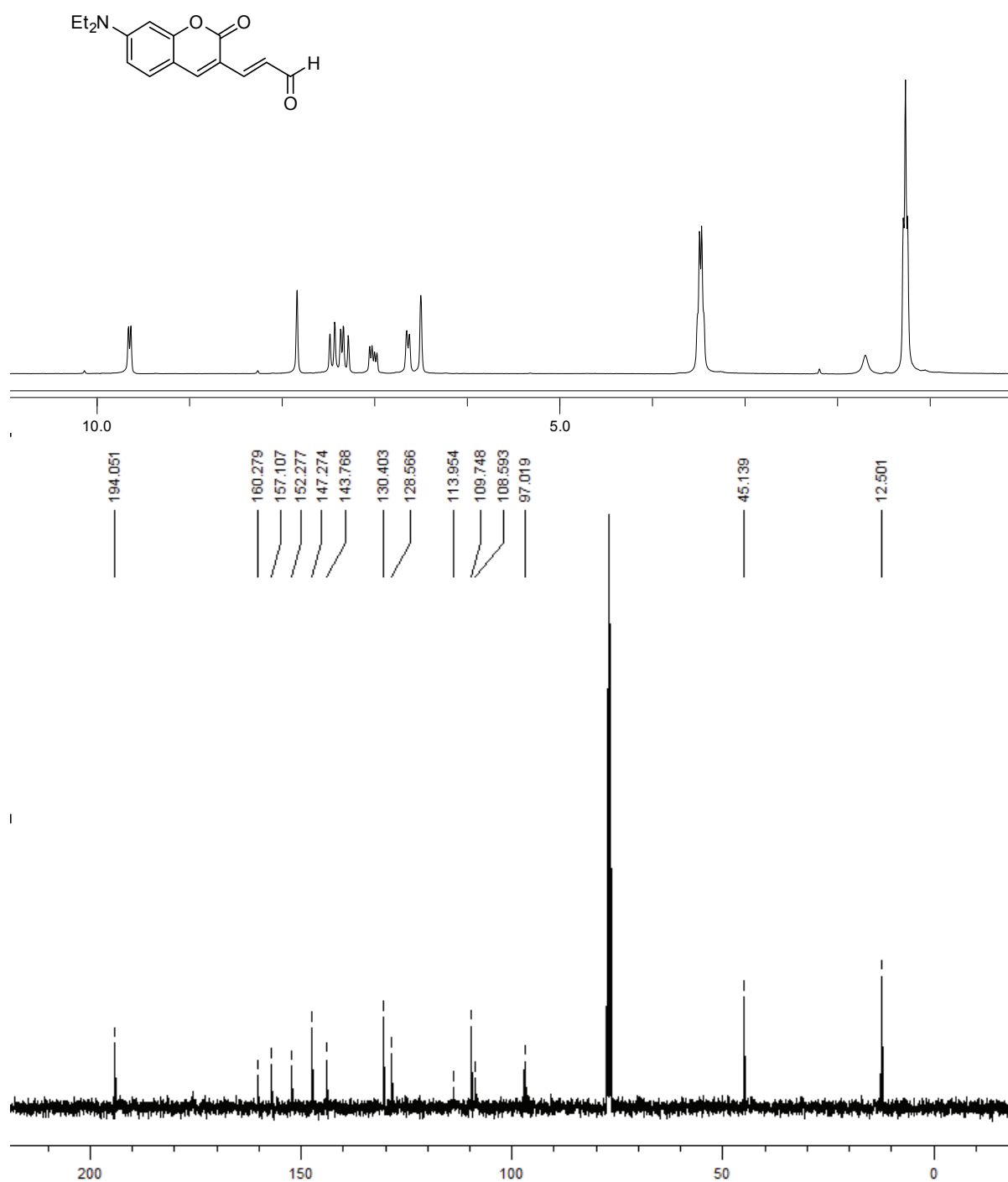


Fig. S5. ^1H and ^{13}C NMR spectra of **3** in CDCl_3 .

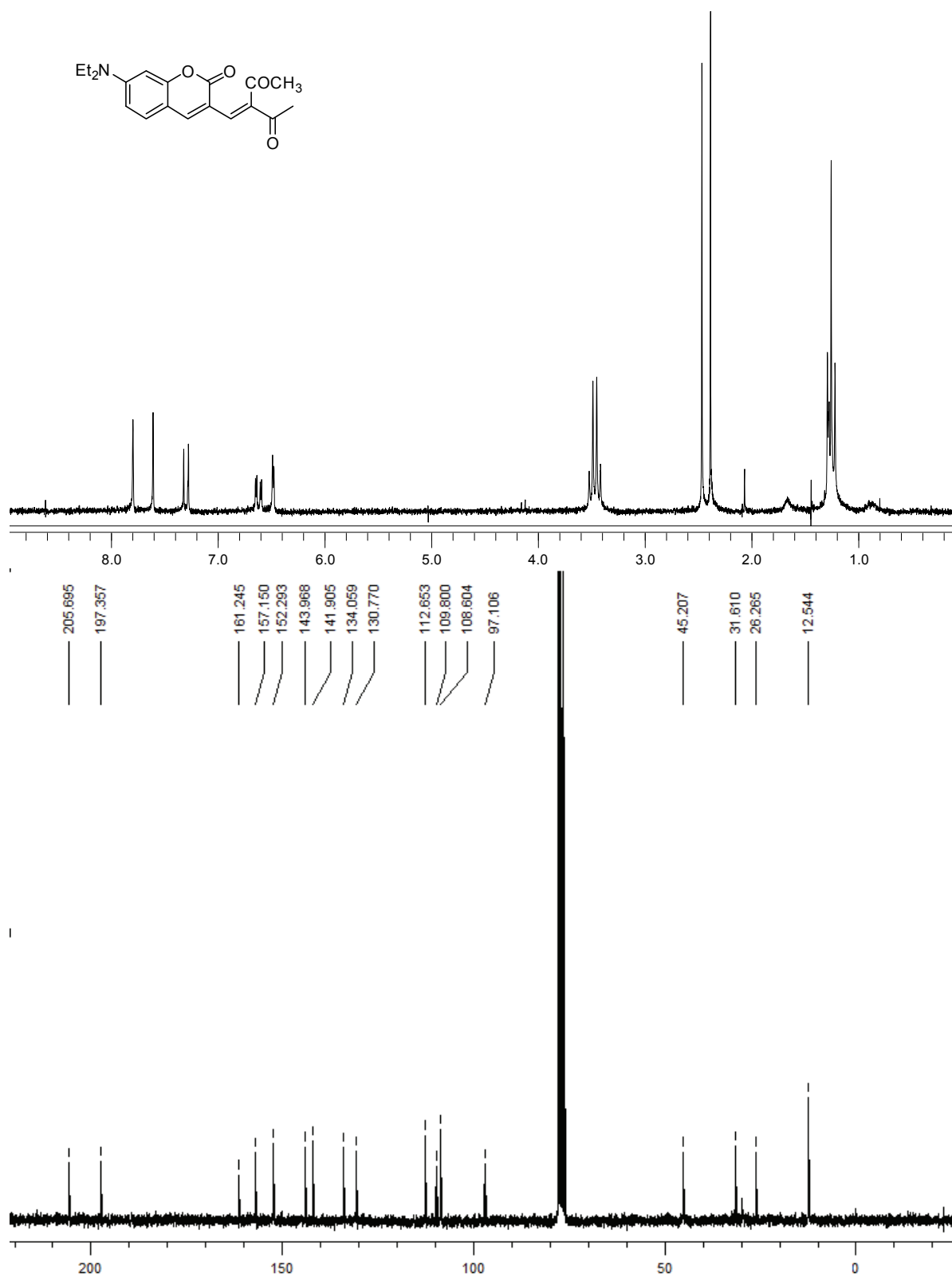


Fig. S6. ¹H and ¹³C NMR spectra of **4** in CDCl₃.

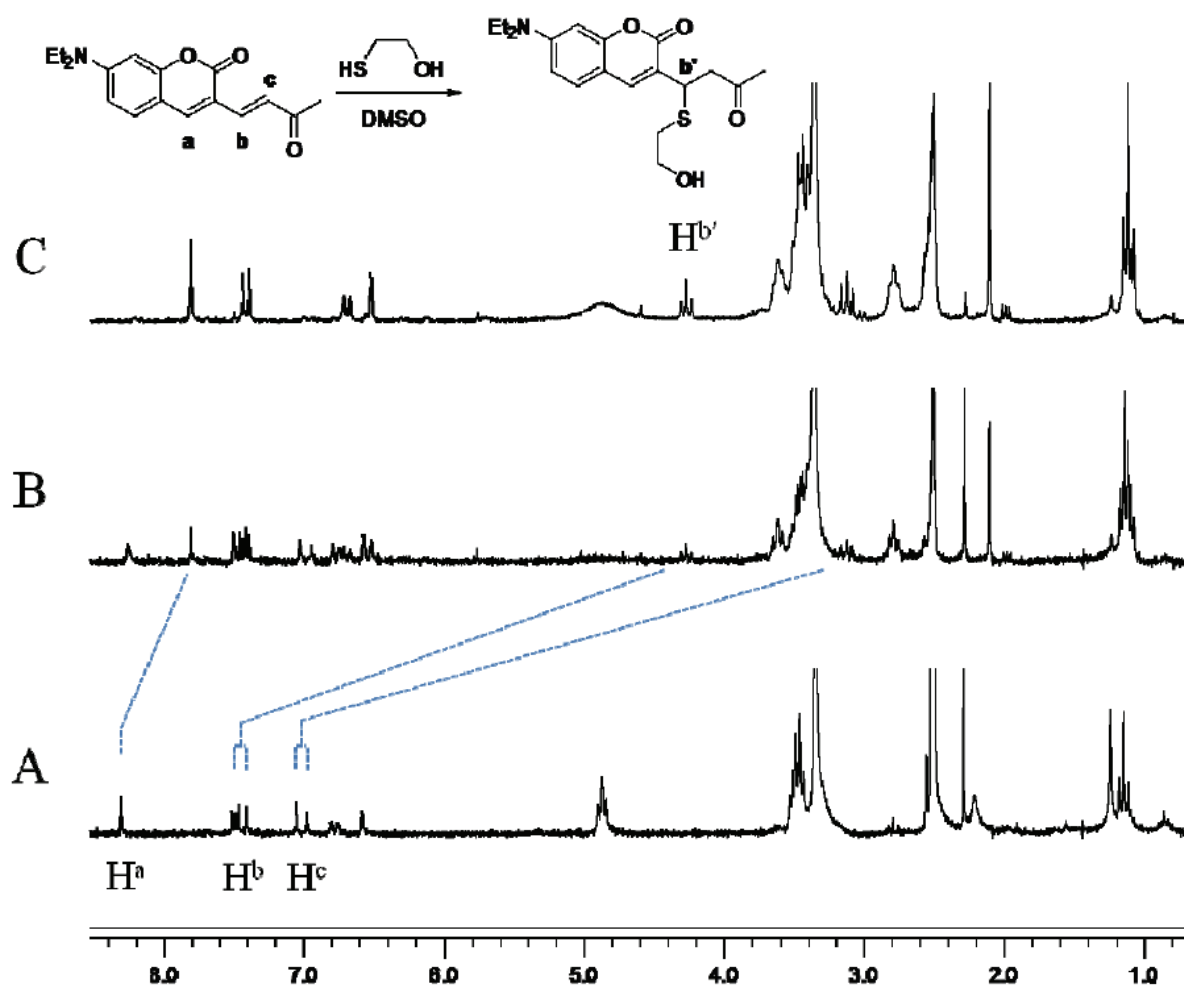


Fig. S7. Full ¹H NMR spectra of **2** (20 mM) in DMSO-*d*₆ upon the addition of ME (2.5 equiv). (A) 0 min, (B) 10 min, (C) 1 h 10 min.

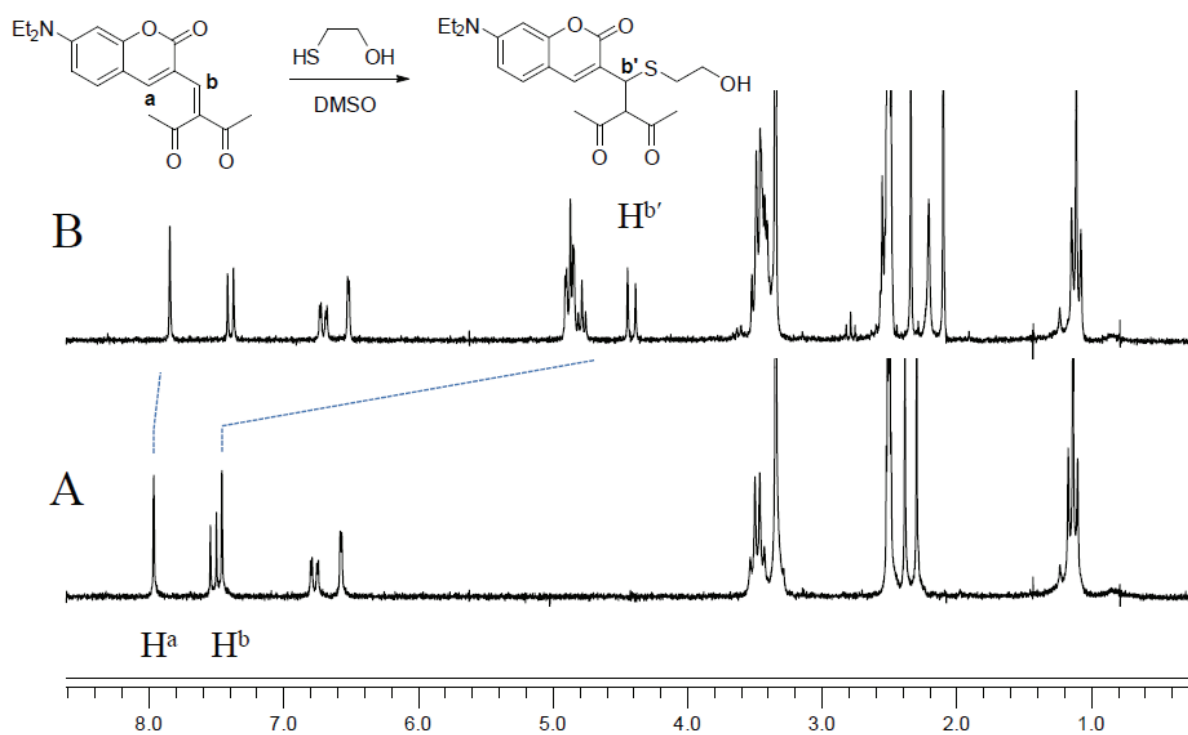
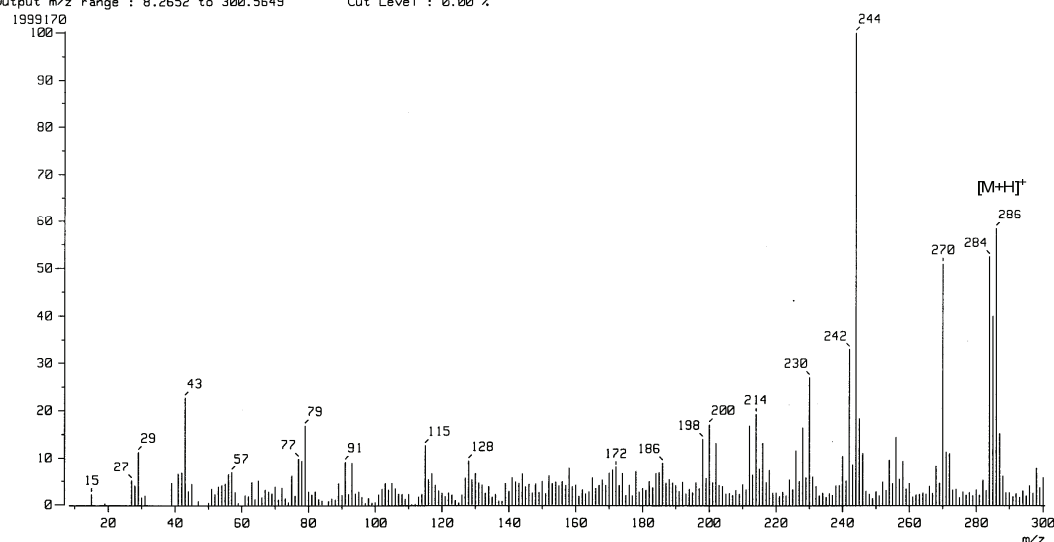
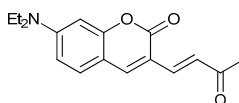


Fig. S8. Full ¹H NMR spectra of **4** (20 mM) in DMSO-*d*₆ upon the addition of ME (2.5 equiv). (A) 0 min, (B) 5 min.

5. Mass spectra

[Mass Spectrum]
 Date : 20-Oct-2010 17:07
 Sample: R-H
 Note : Glycerol + DMSO
 Inlet : Direct
 Spectrum Type : Normal Ion [MF-Linear]
 RT : 1.17 min Scan# : (3,14)
 BP : m/z 244.0000 Int. : 190.11
 Output m/z range : 8.2652 to 300.5649 Cut Level : 0.00 %



[Elemental Composition]

Date : 22-Oct-2010 14:45
 Sample: R-H
 Note : m-NBA
 Inlet : Direct
 RT : 1.18 min
 Elements : C 100/0, H 100/0, N 10/0, O 10/0
 Mass Tolerance : 1000ppm, 3mmu if m/z < 3, 5mmu if m/z > 5
 Unsaturation (U.S.) : 0.0 - 100.0

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Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
286.1440	100.0	-10.6 / -3.0	13.0	C 20 H 18 N 2
		+12.8 / +3.7	10.0	C 11 H 14 N 10
		+8.1 / +2.3	9.5	C 13 H 16 N 7 O
		+3.4 / +1.0	9.0	C 15 H 18 N 4 O 2
		-1.3 / -0.4	8.5	C 17 H 20 N O 3
		+17.5 / +5.0	5.0	C 10 H 18 N 6 O 4
		+12.8 / +3.7	4.5	C 12 H 20 N 3 O 5
		-7.7 / -2.2	1.0	C 4 H 18 N 10 O 5

[Theoretical Ion Distribution]

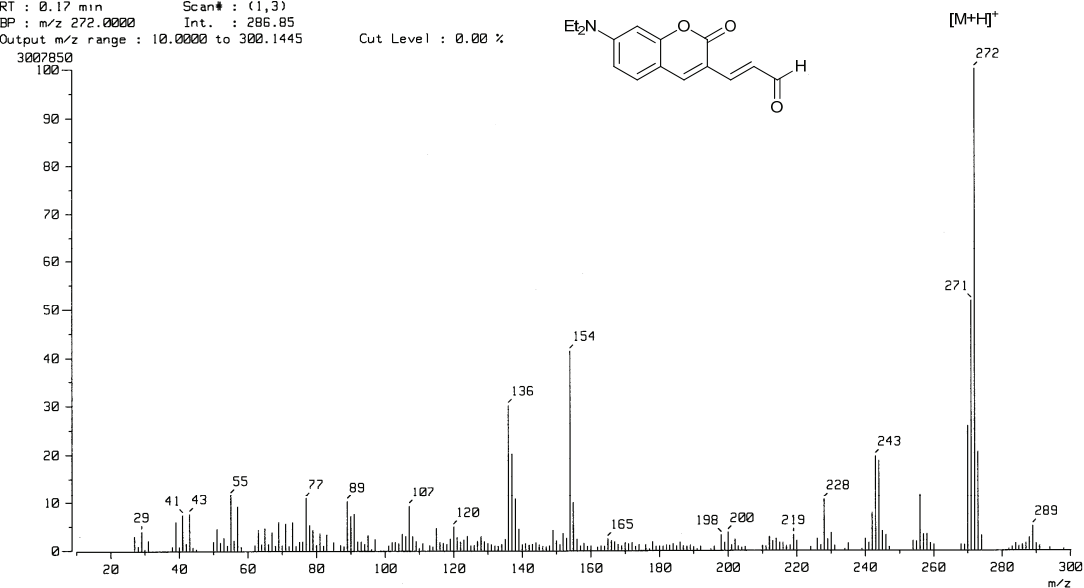
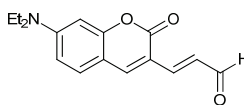
Molecular Formula : C17 H20 N O3
 (m/z 286.1443, MW 286.3507, U.S. 8.5)
 Base Peak : 286.1443, Averaged MW : 286.3488(a), 286.3495(w)

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m/z	INT.	
286.1443	100.0000	*****
287.1476	19.3896	*****
288.1502	2.3754	*
289.1528	0.2181	
290.1554	0.0159	
291.1580	0.0010	

Fig. S9. Mass spectra of **2**.

[Mass Spectrum]
 Date : 31-May-2011 16:05
 Sample: enol
 Note : m-NBA
 Inlet : Direct Ion Mode : FAB+
 Spectrum Type : Normal Ion [MF-Linear]
 RT : 0.17 min Scan# : (1,3)
 BP : m/z 272.0000 Int. : 286.85
 Output m/z range : 10.0000 to 300.1445 Cut Level : 0.00 %



[Elemental Composition]

Data : FAB-D294 Date : 09-Jun-2011 14:40
 Sample: enol
 Note : m-NBA
 Inlet : Direct Ion Mode : FAB+
 RT : 0.14 min Scan# : (1,13)
 Elements : C 100/0, H 150/0, N 10/0, O 10/0
 Mass Tolerance : 1000ppm, 3mmu if m/z < 3, 5mmu if m/z > 5
 Unsaturation (U.S.) : 0.0 - 100.0

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Observed m/z	Int%	Err[ppm / mmu]	U.S.	Composition
272.1283	100.0	-11.2 / -3.1	13.0	C 19 H 16 N 2
		+13.4 / +3.7	10.0	C 10 H 12 N 10
		+8.5 / +2.3	9.5	C 12 H 14 N 7 O
		+3.6 / +1.0	9.0	C 14 H 16 N 4 O 2
		-1.4 / -0.4	8.5	C 16 H 18 N O 3
		+18.3 / +5.0	5.0	C 9 H 16 N 6 O 4
		+13.4 / +3.7	4.5	C 11 H 18 N 3 O 5
		-8.2 / -2.2	1.0	C 3 H 16 N 10 O 5
		+8.5 / +2.3	4.0	C 13 H 20 O 6
		-13.1 / -3.6	0.5	C 5 H 18 N 7 O 6
		-18.0 / -4.9	0.0	C 7 H 20 N 4 O 7

[Theoretical Ion Distribution]

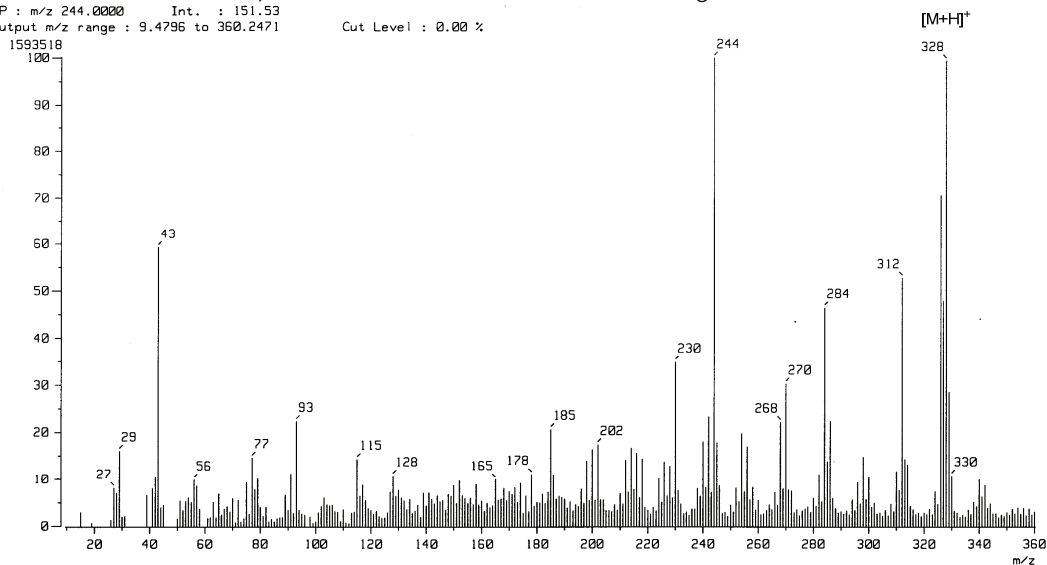
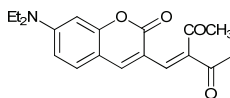
Molecular Formula : C16 H18 N O3
 (m/z 272.1287, MW 272.3238, U.S. 8.5)
 Base Peak : 272.1287, Averaged MW : 272.3221(a), 272.3228(w)

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m/z	INT.	
272.1287	100.0000	*****
273.1319	18.2774	*****
274.1345	2.1721	*
275.1371	0.1940	
276.1397	0.0138	
277.1422	0.0008	

Fig. S10. Mass spectra of 3.

[Mass Spectrum]
 Date : 20-Oct-2010 17:03
 Sample: R-COCH3
 Note : Glycerol + DMSO
 Inlet : Direct
 Spectrum Type : Normal Ion [MF-Linear]
 RT : 2.00 min Scan# : (13,14)
 BP : m/z 244.0000 Int. : 151.53
 Output m/z range : 9.4796 to 360.2471 Cut Level : 0.00 %



[Elemental Composition]

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Date : 22-Oct-2010 14:25
 Sample: R-COCH3
 Note : m-NBA
 Inlet : Direct
 RT : 3.01 min
 Elements : C 100/0, H 100/0, N 10/0, O 10/0
 Mass Tolerance : 1000ppm, 3mmu if m/z < 3, 5mmu if m/z > 5
 Unsaturation (U.S.) : 0.0 - 100.0

Observed m/z	Int%	Err [ppm / mmu]	U.S.	Composition
328.1544	100.0	-5.7 / -1.9	14.5	C 20 H 18 N 5
		-9.8 / -3.2	14.0	C 22 H 20 N 2 O
		+10.7 / +3.5	11.0	C 13 H 16 N 10 O
		+6.6 / +2.2	10.5	C 15 H 18 N 7 O 2
		+2.5 / +0.8	10.0	C 17 H 20 N 4 O 3
		-1.6 / -0.5	9.5	C 19 H 22 N O 4
		+14.7 / +4.8	6.0	C 12 H 20 N 6 O 5

[Theoretical Ion Distribution]

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Molecular Formula : C19 H22 N O4
 (m/z 328.1549, MW 328.3880, U.S. 9.5)
 Base Peak : 328.1549, Averaged MW : 328.3858(a), 328.3865(w)

m/z	INT.
328.1549	100.0000
329.1581	21.6522
330.1608	3.0278
331.1634	0.3178
332.1660	0.0268
333.1685	0.0019
334.1711	0.0001

Fig. S11. Mass spectra of 4.