

Merocyanine Dyes with Improved Photostability

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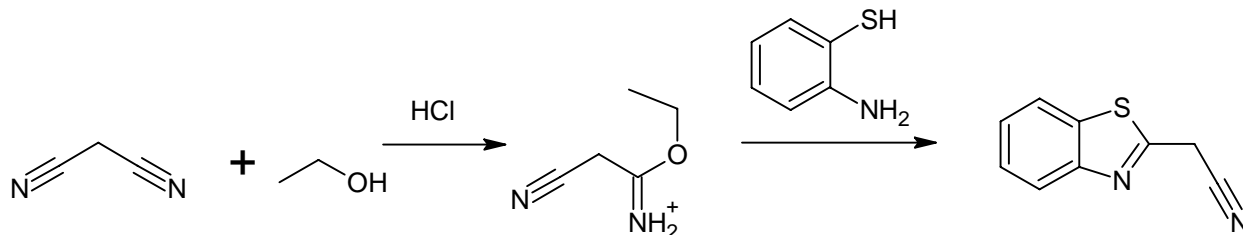
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General Aspects

Materials. Analytical grade reagents were purchased from Sigma-Aldrich Co. (2Z)-2-[(2E)-3-methoxyprop-2-en-1-ylidene]-1-benzothiophen-3(2H)-one 1,1-dioxide, (2Z)-2-[(2E,4Z)-4-(3-ethyl-1,3-benzothiazol-2(3H)-ylidene)but-2-en-1-ylidene]-1-benzothiophen-3(2H)-one 1,1-dioxide (**S-SO**), (2Z)-2-[(2E,4E)-4-(1,3,3-trimethyl-1,3-dihydro-2H-indol-2-ylidene)but-2-en-1-ylidene]-1-benzothiophen-3(2H)-one 1,1-dioxide (**I-SO**) were prepared as previously described¹.

Methods. Absorption spectra were recorded on a Hewlett-Packard 8453 diode array UV-Vis spectrophotometer, and fluorescence measurements were taken on a Spex Fluorolog 2 spectrofluorometer. Melting points are not corrected. NMR spectra were obtained on a Varian Mercury 300 MHz or on a Bruker 300 MHz DRX 300 spectrometer. Mass spectra were obtained on a Hewlett-Packard 1100 high-performance liquid

chromatograph equipped with an 1100 mass selective detector (MS-ESI). Quantum yields were measured using merocyanine 540 or Cy5 as an internal standards.



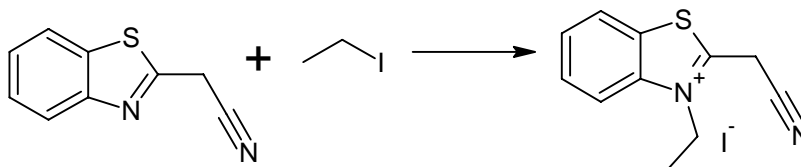
1,3-benzothiazol-2-ylacetonitrile

To a solution of malonodinitrile (13.22g, 0.200 mole) in dry chloroform (100 mL) was added ethanol (9.2 g, 0.200 mole). Hydrogen chloride (8.0 g, 0.220 mole) was passed into the solution at room temperature and the resulting suspension was stirred for an additional 16 hours at room temperature. The excess of hydrogen chloride was removed by passing dry nitrogen into the reaction mixture. A solution of o-aminothiophenol (25 g, 0.200 mole) in chloroform was added to the reaction mixture dropwise during 30 min and the reaction mixture was stirred for 1 hour at 60 C. After that the reaction mixture was cooled to room temperature, washed with water (2*100 mL) and dried with magnesium sulfate. Chloroform was removed using a rotary evaporator. The solid residue was recrystallized from ethyl alcohol. White crystals. The yield was 26.1 g (75 % of theory).

Mp = 101-103 C

^1H NMR (CDCl_3 , 300 MHz) δ 4.24 (s, 2H), 7.44 (t, $^3J_{\text{H-H}} = 7.8$ Hz, 1H), 7.53 (t, $^3J_{\text{H-H}} = 7.8$ Hz, 1H), 7.89 (d, $^3J_{\text{H-H}} = 7.8$ Hz, 1H), 8.04 (d, $^3J_{\text{H-H}} = 7.8$ Hz, 1H).

^{13}C NMR (CDCl_3 , 75 MHz) δ 22.3, 116.4, 122.2, 122.5, 125.5, 126.4, 135.0, 152.2, 160.4.

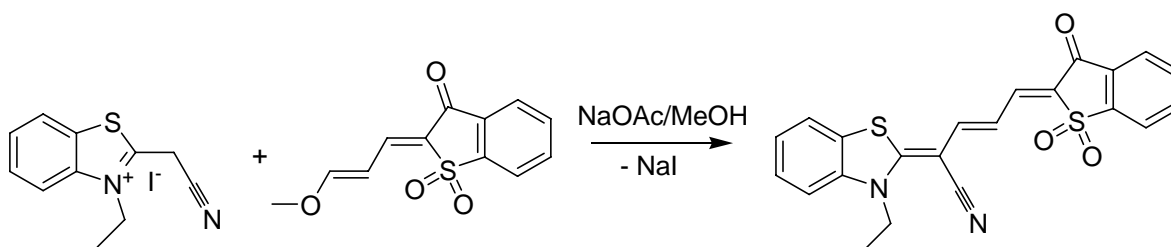


2-(cyanomethyl)-3-ethyl-1,3-benzothiazol-3-ium iodide

A solution of 1,3-benzothiazol-2-ylacetonitrile (10 g, 0.057 mole) in iodoethane (10 mL, 0.125 mole) was heated in sealed tube at 120 C for 36 hours. The precipitated solid was filtered, washed with ether (100 mL) and dried. The yield was 10 g (53 % of theory).

^1H NMR (DMSO- d_6 , 300 MHz) δ 1.31 (t, $^3J_{\text{H-H}} = 7.0$ Hz, 3H, CH_3CH_2 -), 3.93 (q, $^3J_{\text{H-H}} = 7.0$ Hz, 2H, CH_3CH_2 -), 4.76 (s, $\text{CH}_2\text{-CN}$), 7.09 (t, $^3J_{\text{H-H}} = 7.5$ Hz, 1H), 7.23 (t, $^3J_{\text{H-H}} = 7.5$ Hz, 1H), 7.31 (d, $^3J_{\text{H-H}} = 7.5$ Hz, 1H), 7.58 (d, $^3J_{\text{H-H}} = 7.5$ Hz, 1H).

^{13}C NMR (DMSO- d_6 , 75 MHz) δ 11.9, 55.1, 111.3, 122.0, 123.2, 123.9, 127.8, 142.2, 162.1.

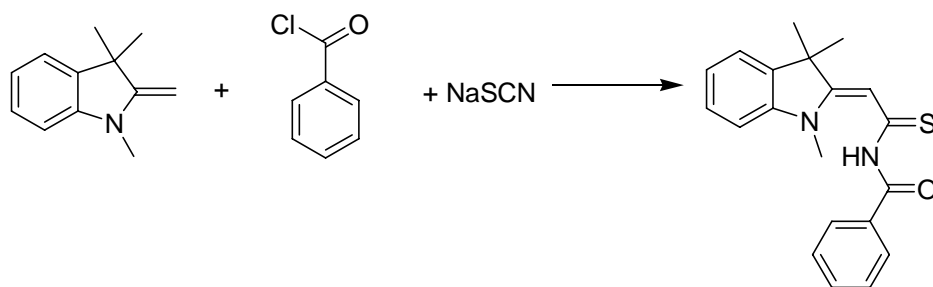


(2E,3E,5Z)-5-(1,1-dioxido-3-oxo-1-benzothiophen-2(3H)-ylidene)-2-(3-ethyl-1,3-benzothiazol-2(3H)-ylidene)pent-3-enenitrile (S-SO-CN).

In a one-necked round bottom 100 mL flask equipped with magnetic stir bar and reflux condenser were added 2-(cyanomethyl)-3-ethyl-1,3-benzothiazol-3-ium iodide (1.00 g, 3.03 mmol), (2Z)-2-[(2E)-3-methoxyprop-2-en-1-ylidene]-1-benzothiophen-3(2H)-one 1,1-dioxide (1.00 g, 4.00 mmol) and CHCl_3 -MeOH mixture (1:1, 20 mL). The flask was heated on an oil bath with stirring until boiling began, and a solution of sodium acetate (320 mg, 4.00 mmol) in 10 ml of methanol was added to the flask. The stirring was continued under reflux for an additional 30 min. The flask was cooled to room temperature and the precipitated solid was filtered, washed with methanol and dried. Green crystals. The yield was 730 mg (57% of theory).

^1H NMR (CDCl_3 , 300 MHz) δ 1.49 (t, $^3J_{\text{H-H}} = 7.2$ Hz, 3H), 4.71 (q, $^3J_{\text{H-H}} = 7.2$ Hz, 2H), 6.78 (t, $^3J_{\text{H-H}} = 12.9$ Hz, 1H), 7.47 (t, $^3J_{\text{H-H}} = 7.5$ Hz, 1H), 7.61 (t, $^3J_{\text{H-H}} = 7.5$ Hz, 1H), 7.8-8.2 (m, 8H).

ESI-MS: 421 (MH⁺).



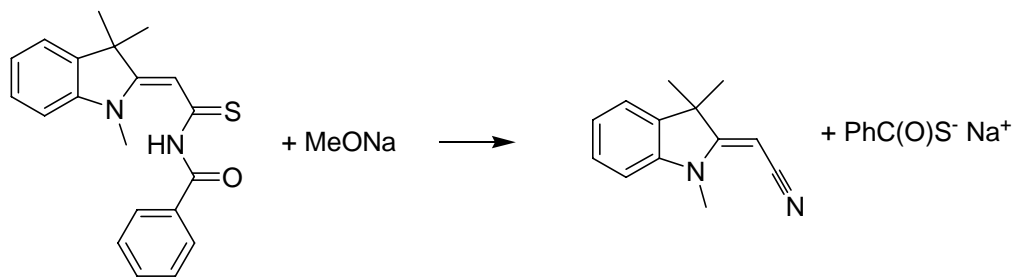
***N*-[(2*Z*)-2-(1,3,3-trimethyl-1,3-dihydro-2*H*-indol-2-ylidene)ethanethioyl]benzamide**

A two-necked round bottom 500 mL flask was equipped with a magnetic stir bar, additional funnel and a reflux condenser. To the flask were added sodium thiocyanate (8.1 g, 0.10 mole) and dry acetone (100 mL). The stirring was started and benzoyl chloride (14.1 g, 0.1 mole) was added to the flask by drops. When the addition of benzoyl chloride was complete the flask was heated on an oil bath with stirring until boiling began and the flask contents were stirred under reflux for an additional 30 minutes. The oil bath was removed and 3,3-trimethyl-2-methyleneindoline (15.8 g, 0.10 mole) was added to the flask by drops. After completion of the addition the flask was heated again on an oil bath for an additional 30 min at reflux. The flask was cooled to room temperature and the reaction mixture was poured in 1 L beaker containing 500 mL of water. The precipitated solid was filtered, washed with water and dried. The product was purified by recrystallization from methanol. The yield was 19.1 g (57 % of theory).

Mp = 108-110 C

¹H NMR (CDCl₃, 300 MHz) δ 1.76 (s, 6H), 3.75 (s, 3H), 7.19 (d, ³J_{H-H} = 7.8 Hz, 1H), 7.31 (t, ³J_{H-H} = 7.2 Hz, 1H), 7.40-7.46 (m, 2H), 7.60-7.80 (m, 4H), 8.02 (d, ³J_{H-H} = 6.9 Hz, 2H), 9.31 (s, 1H, NH).

¹³C NMR (CDCl₃, 75 MHz) δ 25.8, 37.2, 49.5, 99.2, 110.0, 122.0, 124.1, 127.2, 127.9, 128.7, 132.3, 133.9, 139.8, 144.6, 163.7, 177.4, 185.2.



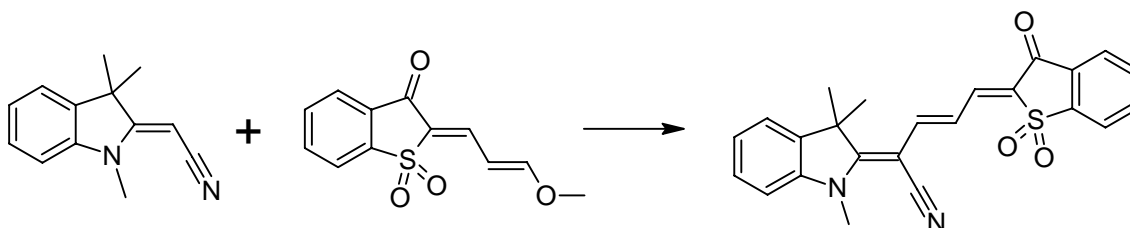
(1,3,3-trimethyl-2,3-dihydro-1H-indol-2-yl)acetonitrile

A 500 mL one-necked flask was equipped with a magnetic stir bar and a reflux condenser. 200 mL of methanol were added to the flask, sodium (3.0 g, 0.13 mole) was added to the methanol as small pieces (~ 0.5 g). After the sodium dissolved, N-[(2Z)-2-(1,3,3-trimethyl-1,3-dihydro-2H-indol-2-ylidene)ethanethioyl]benzamide (19.0 g, 0.056 mole) was added to the flask and the reaction mixture was heated at reflux with stirring for 2 hours. The reaction mixture was cooled to room temperature and the methanol was evaporated using a rotary evaporator. The residue was treated with 200 mL of water and extracted with dichloromethane (3*100 mL). Combined organic extracts were dried over magnesium sulfate and solvent was evaporated on a rotary evaporator. The red solid was recrystallized from methanol-water (7:3) to give slightly red crystals. The yield was 4.5 g (40 % of theory).

Mp = 74-76 C.

^1H NMR (dmso- d_6 , 300 MHz) δ 1.55 (s, 6H, 2*CH₃), 3.13 (s, 3H, CH₃), 4.48 (s, 1H, CH-CN), 6.85-7.0 (m, 2H), 7.21 (t, $^3J_{\text{H-H}} = 7.7$ Hz, 1H), 7.31 (d, $^3J_{\text{H-H}} = 6.9$ Hz, 1H).

^{13}C NMR (dmso- d_6 , 75 MHz) δ 25.3, 28.9, 46.3, 57.6, 107.4, 120.2, 121.3, 121.7, 137.1, 143.6, 171.9.



(2Z,3E,5Z)-5-(1,1-dioxido-3-oxo-1-benzothiophen-2(3H)-ylidene)-2-(1,3,3-trimethyl-1,3-dihydro-2H-indol-2-ylidene)pent-3-enenitrile (I-SO-CN).

A mixture of (1,3,3-trimethyl-2,3-dihydro-1*H*-indol-2-yl)acetonitrile (397 mg, 2.00 mmol) and (2*Z*)-2-[(2*E*)-3-methoxyprop-2-en-1-ylidene]-1-benzothiophen-3(2*H*)-one 1,1-dioxide (501 mg, 2.00 mmol) were placed in a 10 mL one-necked round bottom flask. The flask was heated in an oil bath at 210 C for 2 hours and then cooled to room temperature. The reaction mixture was extracted with dichloromethane (100 mL). The organic solution was passed through a short silica column, the dichloromethane was evaporated and the pure dye was obtained by recrystallization from methanol. The yield was 511 mg of green crystals (63% of theory).

¹H NMR (CDCl₃, 300 MHz) δ 1.68 (s, 6H, 2*CH₃), 3.86 (s, 3H, CH₃), 6.89 (t, ³J_{H-H} = 13.1 Hz, 1H), 7.1-8.4(m, 10 H).

ESI-MS: 431 (MH⁺).

Photobleaching experiments.

15 mL of 0.01 mM solution of dye in methanol were placed in a 20 mL Borosilicate Glass Scintillation Vial (Fisher catalog # 03-337-15). The vial was illuminated by filtering through glass the light of a 90W halogen reflector lamp (120 V, 1280 Lumens, 12 Degree Beam Spread). The distance between the vial and the lamp was 11.0 cm. A fan (120 V, 150 mm)) was used to cool the vial. At time intervals a sample was removed and the sample absorbance spectra was obtained on a Hewlett-Packard 8453 diode array spectrophotometer. Rate constants of dye photobleaching were obtained from a plot of $\ln(A)$ vs. time.

Table 1. Photobleaching of **S-SO** and **S-SO-CN** dyes.

Dye	Time (seconds)	A_t/A_0^a	$-\ln(A_t/A_0)$
S-SO	0	1.0000	0.0000
	66900	0.7409	0.3000
	154200	0.4485	0.8018
	258300	0.2880	1.2448
	349800	0.1560	1.8579
S-SO-CN	0	1.0000	0.0000
	66900	0.9851	0.0150
	154200	0.9790	0.0212
	258300	0.9613	0.0395
	349800	0.9530	0.0481

^a Absorbances were measured at wavelengths equal to λ_{\max} (597 nm for **S-SO** and 550 nm for **S-SO-CN**)

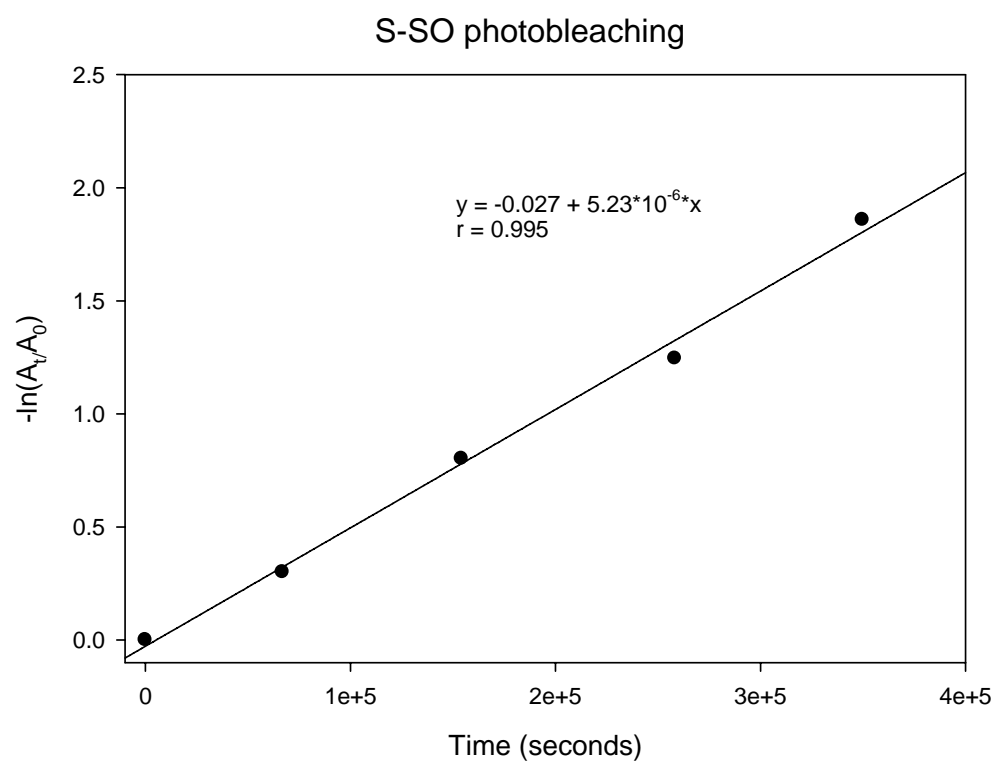


Figure 1. Photobleaching of **S-SO** dye.

S-SO-CN Photobleaching

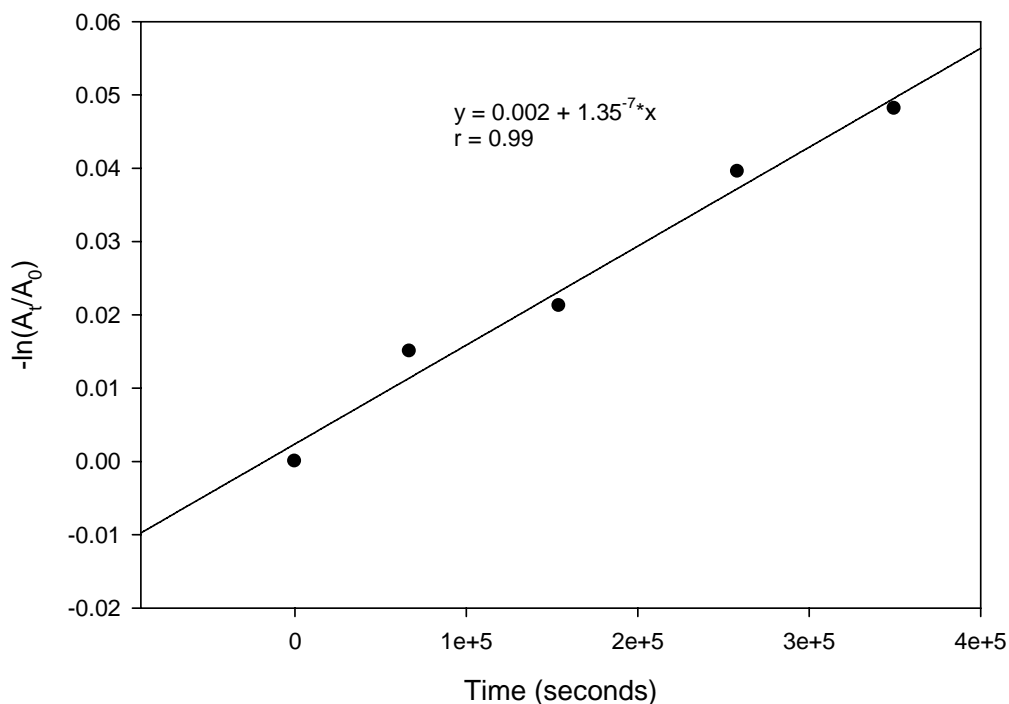


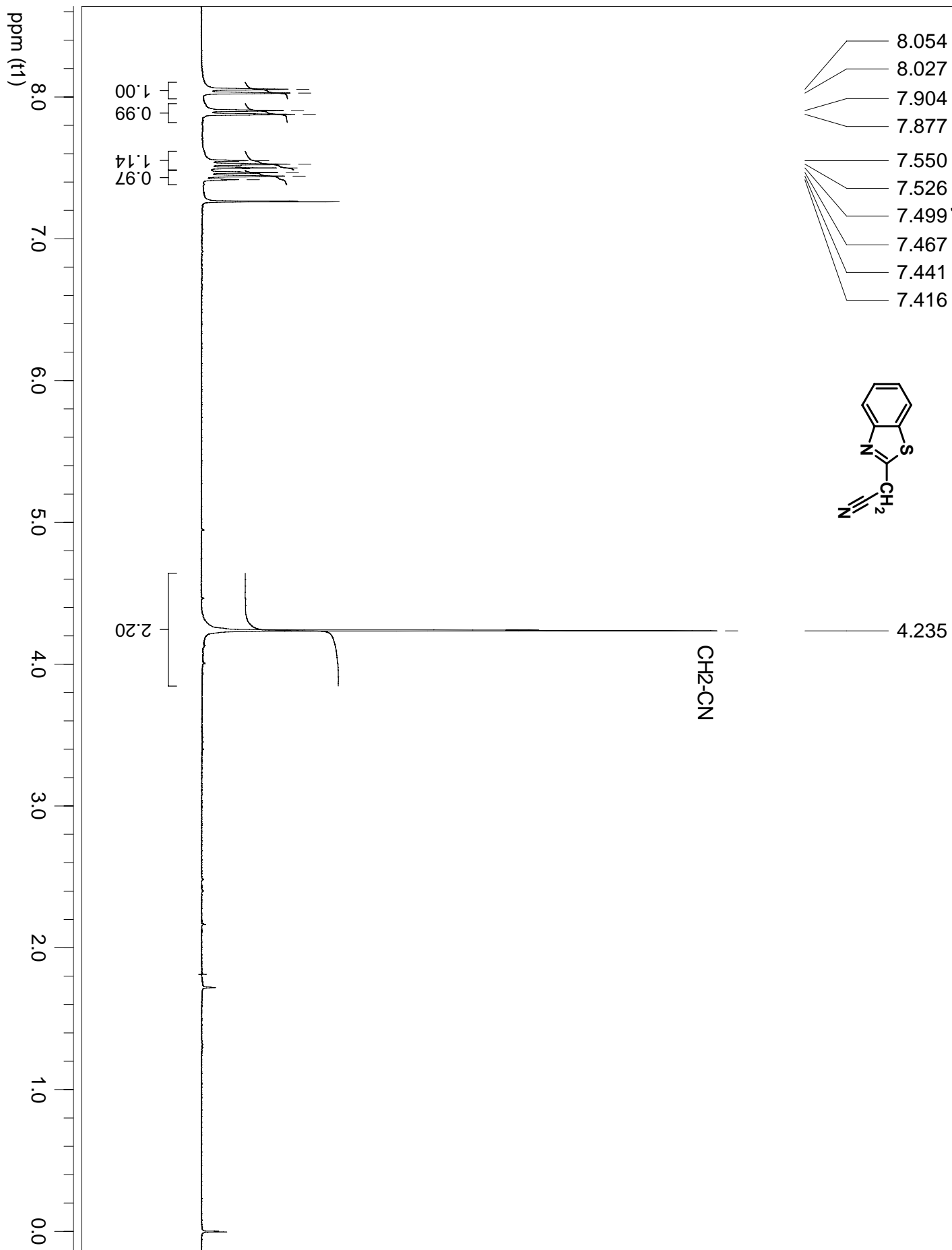
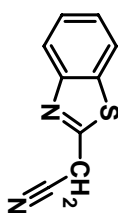
Figure 2. Photobleaching of **S-SO-CN** dye.

Photobleaching of the S-SO, I-SO, S-SO-CN, I-SO-CN dyes in the presense of photosensitizer

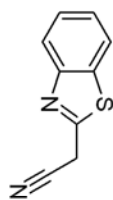
(Methylene Blue)

15 mL of a methanol solution containing dye (3.33 μM) and Methylene Blue (3.33 μM) were placed in a 20 mL Borosilicate Glass Scintillation Vial (Fisher catalog # 03-337-15). The vial was illuminated with the light of a 90W halogen reflector lamp (120 V, 1280 Lumens, 12 Degree Beam Spread). To avoid direct excitation of the dye, light was filtered through a methanol solution containing 6.67 μM of each of the dyes: **I-SO, S-SO, I-SO-CN and S-SO-CN**, the length of the filtering solution was 1.0 cm. The distance between the vial and the lamp was 11.0 cm. A fan (120 V, 150 mm)) was used to cool the vial. After 40 min, illumination was stopped and absorbance spectra of the sample were obtained on a Hewlett-Packard 8453 diode array spectrophotometer.

1,3-benzothiazol-2-ylacetonitrile, 03/03/2006, CDCl₃, 300 MHz



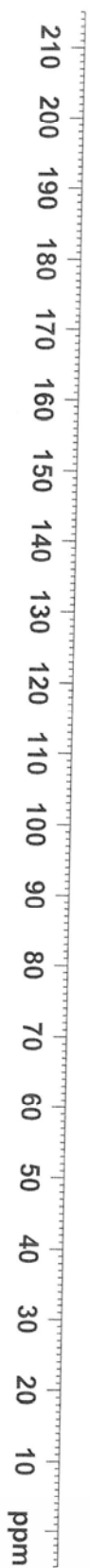
1,3-benzothiazol-2-ylacetonitrile, 300 MHz, dmso-d₆



160.35
152.15
134.97
128.37
128.42
122.49
122.16
116.40

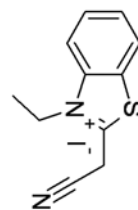
40.26
39.99
39.71
39.43
39.15
38.87
38.59

22.32



2-(cyanomethyl)-3-ethyl-1,3-benzothiazol-3-ium iodide, 300 MHz, dmso-d₆

7.579
7.337
7.311
7.286
7.257
7.241
7.214
7.110
7.085
7.060
6.917

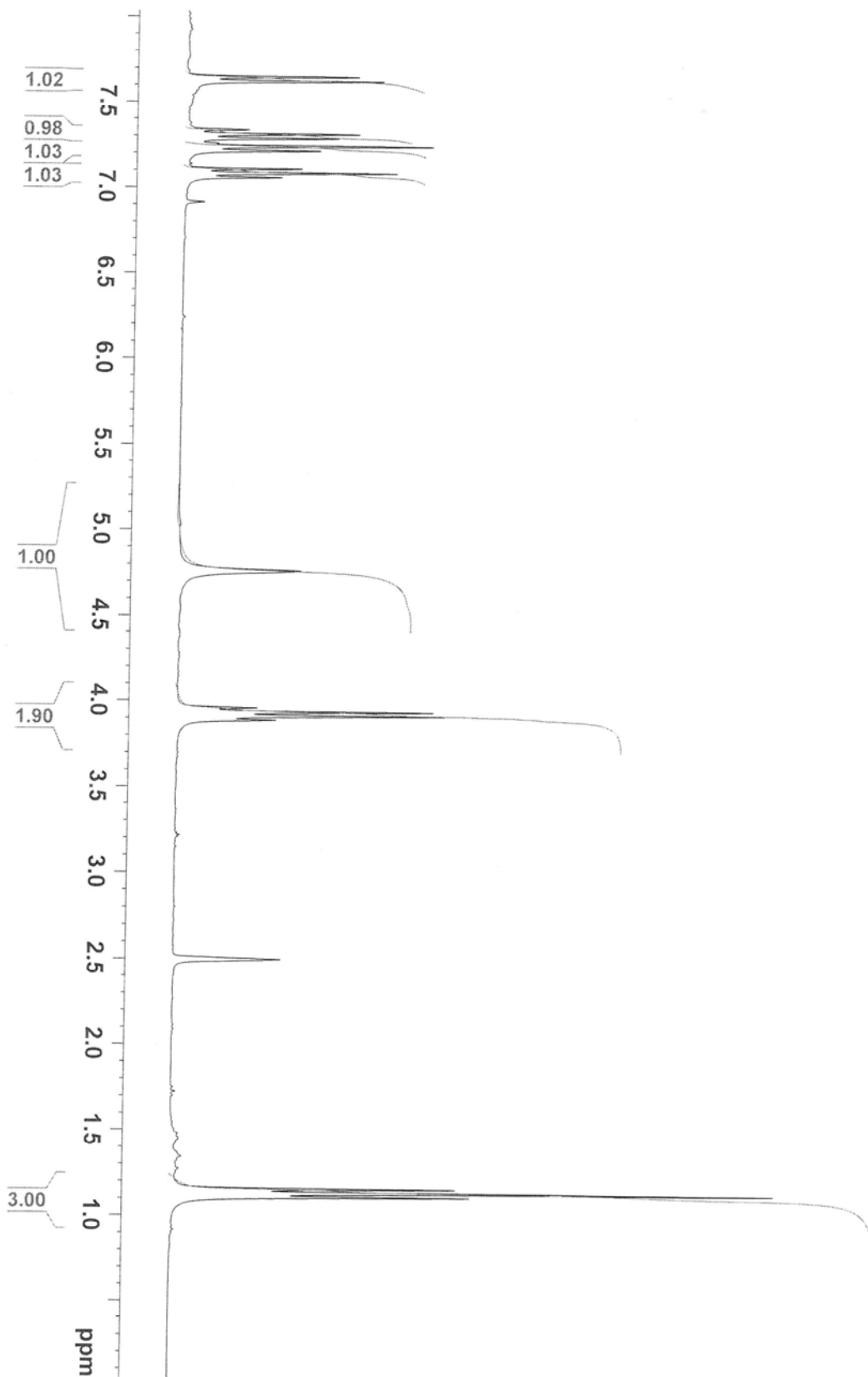


4.762

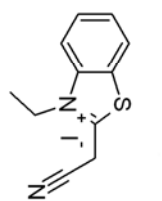
3.961
3.937
3.914
3.890

2.500

1.155
1.131
1.108



2-(cyanomethyl)-3-ethyl-1,3-benzothiazol-3-ium iodide, 300 MHz, dmsd-d6



182.13

140.13

127.83

127.87

127.29

125.24

121.85

111.30

55.12

41.56

41.01

40.73

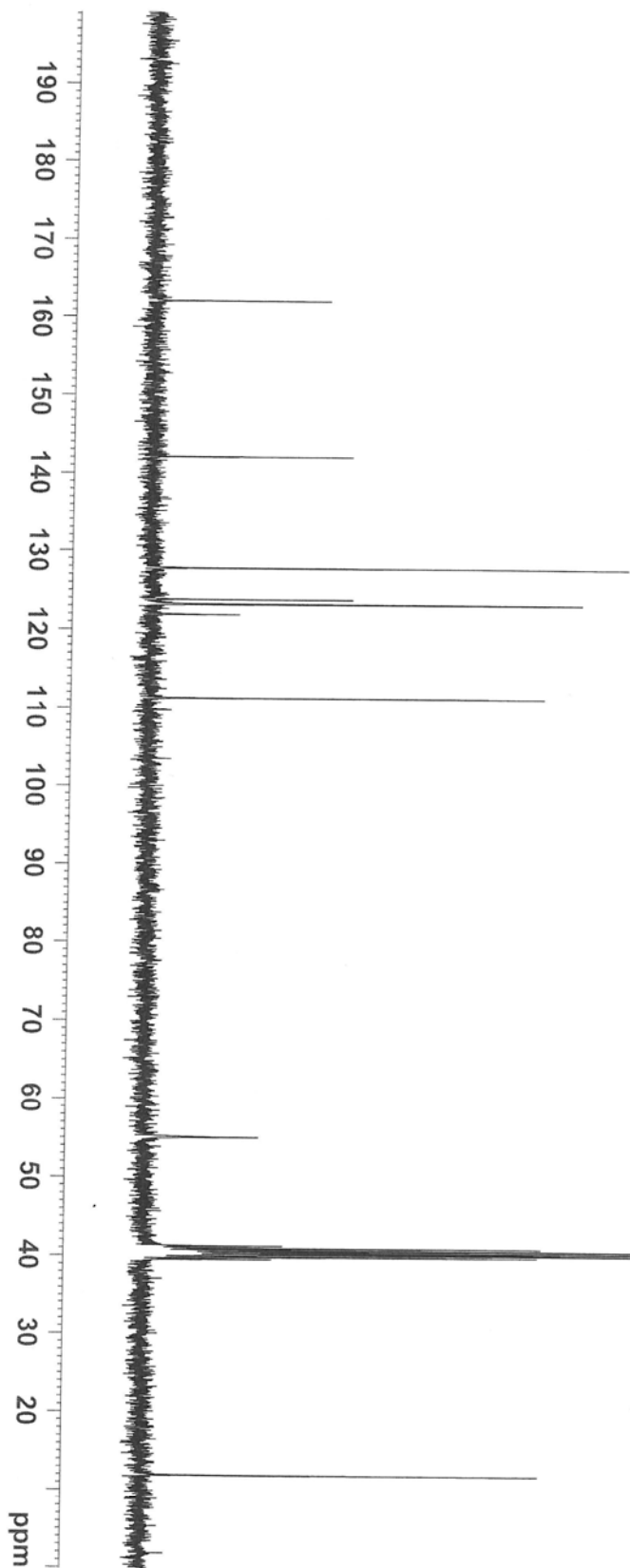
40.45

40.17

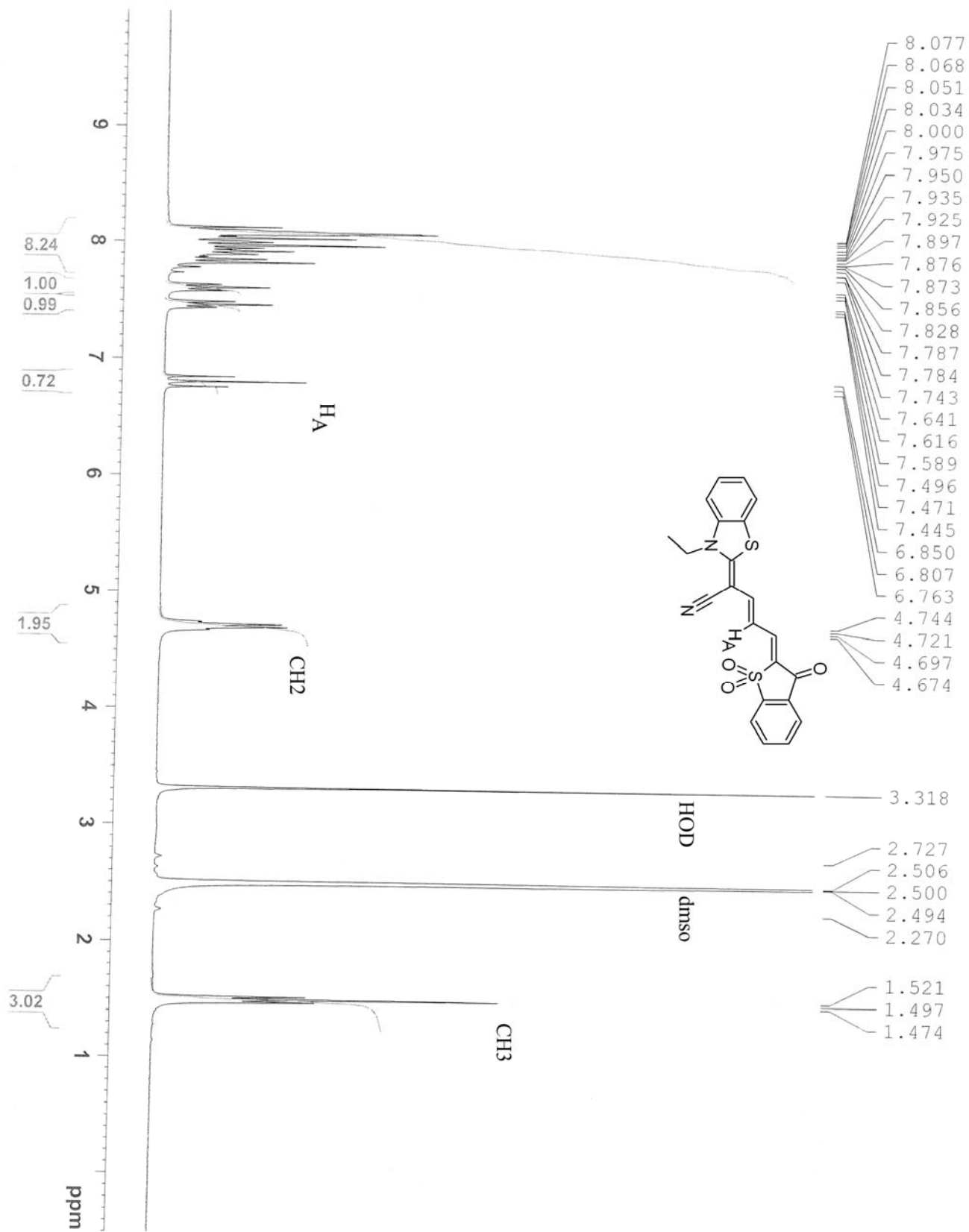
39.66

39.61

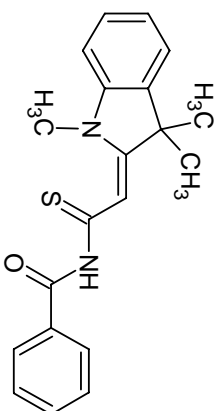
11.83



(2E,3E,5Z)-5-(1,1-dioxido-3-oxo-1-benzothiophen-2(3H)-ylidene)-2-(3-ethyl-1,3-benzothiazol-2(3H)-ylidene)pent-3-enenitrile, 300 MHz, dmso-d₆



N-[(2Z)-2-(1,3,3-trimethyl-1,3-dihydro-2H-indol-2-ylidene)ethanethioyl]benzamide, 11-28-2005, CDCl₃, 300 MHz



9.313
8.033
8.010
8.004
7.700
7.677
7.676
7.643
7.621
7.617
7.593
7.583
7.441
7.429
7.427
7.425
7.416
7.407
7.405
7.403
7.309
7.285
7.202
7.176

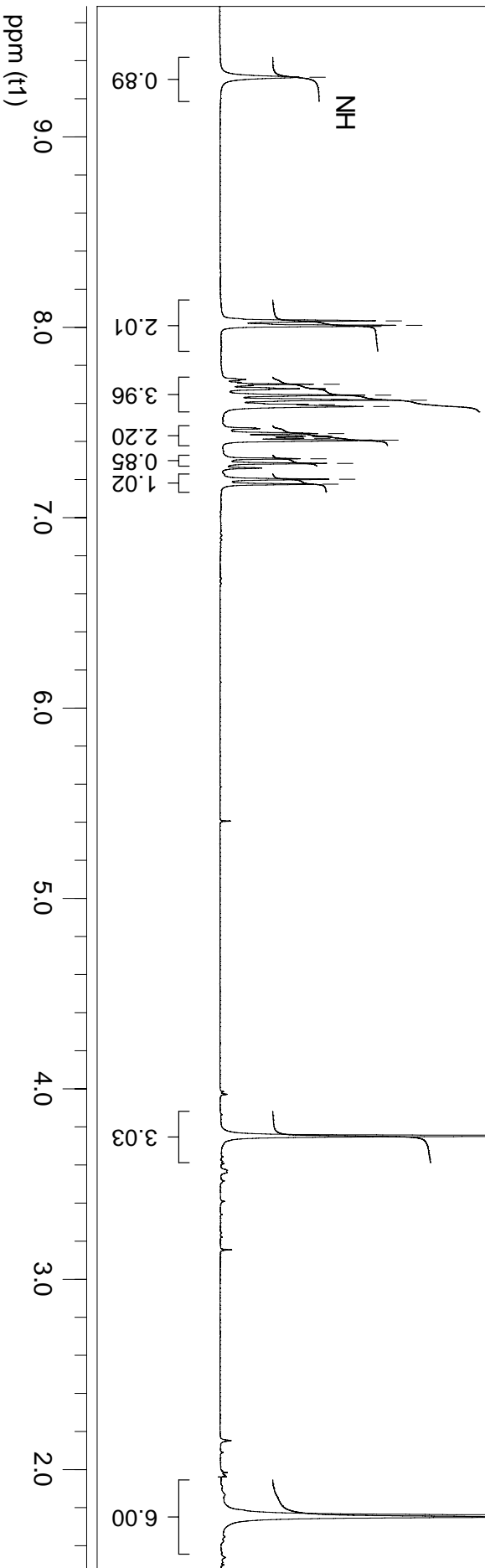
3.752

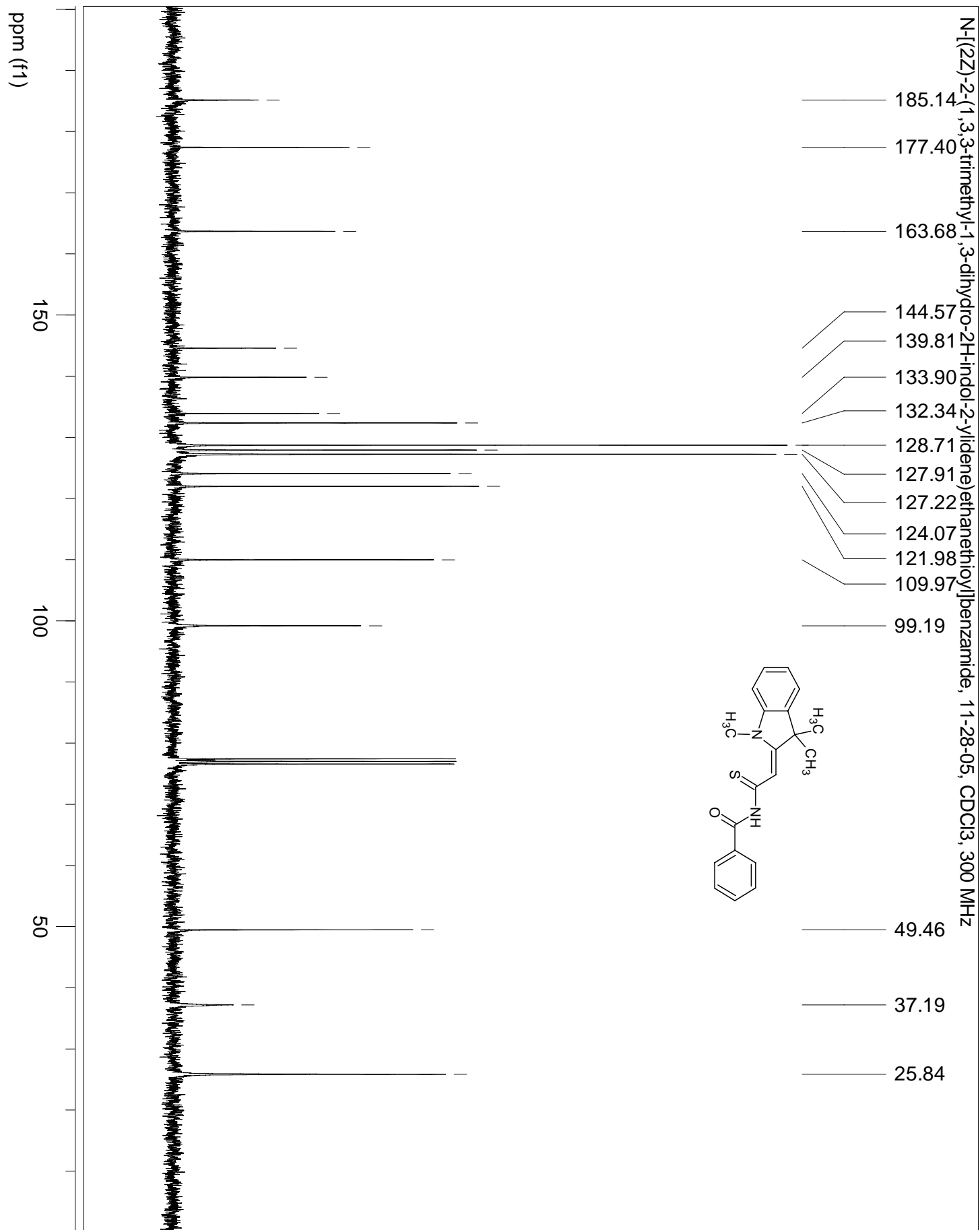
2*CH₃

1.756

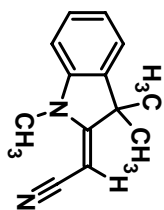
CH₃

S15





(2Z)-(1,3,3-trimethyl-1,3-dihydro-2H-indol-2-ylidene)acetonitrile, 04/05/2006, DMSO-d₆, 300 MHz



7.320
7.297
7.238
7.214
7.187
6.968
6.944
6.919

4.482

3.133

1.551

2*CH₃

CH₃

CH-CN

1.08
1.09
2.20

0.97

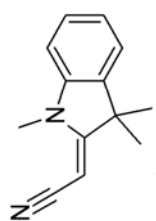
3.09

6.00

S17

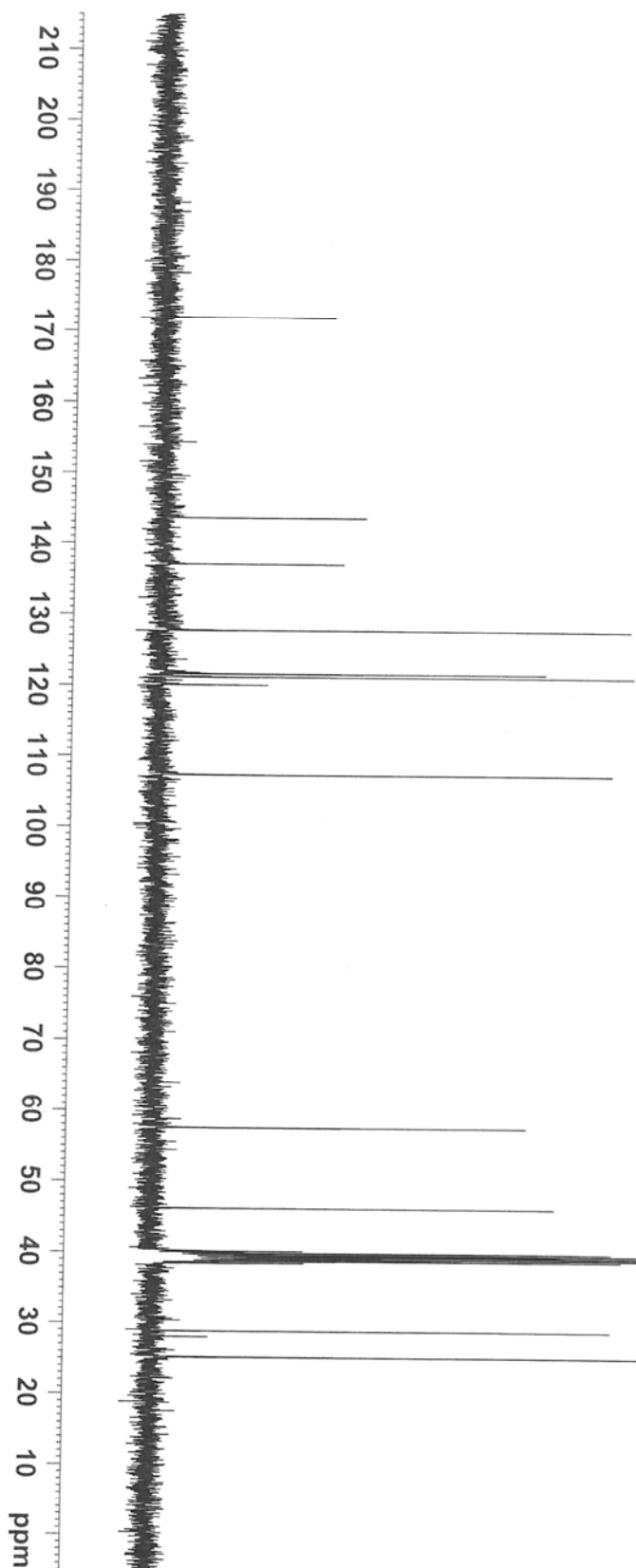
ppm (t1)

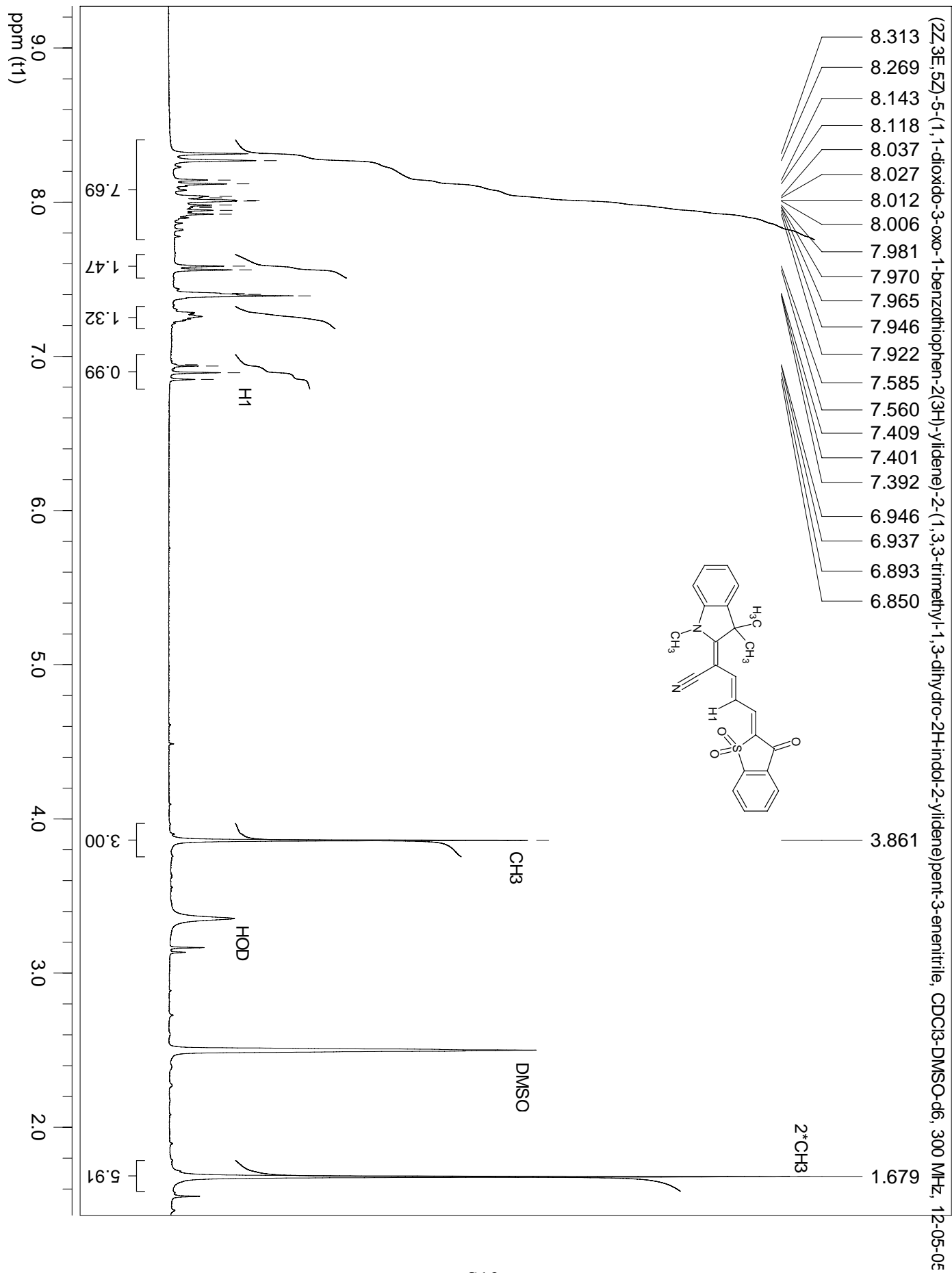
(2Z)-(1,3,3-trimethyl-1,3-dihydro-2H-indol-2-ylidene)acetonitrile, 300 MHz, dmso-d₆



171.94
143.41
137.12
127.78
121.69
121.19
120.14
107.39

57.58
44.25
40.27
39.88
39.71
39.43
38.13
38.00
38.00
28.51
28.35





Literature.

1. Touthkine, A.; Kraynov, V.; Hahn, K. Solvent-Sensitive Dyes to Report Protein Conformational Changes in Living Cells. *J. Am. Chem. Soc.* **2003**, 125, 4132-4145.