

Supporting Information

In Situ Generation and Trapping of Thioimidates:

An Intermolecular Tandem Reaction to 4-Acylimino-4*H*-3,1-benzothiazines †

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^1H NMR and ^{13}C NMR Spectra of Ring Closure Experiments

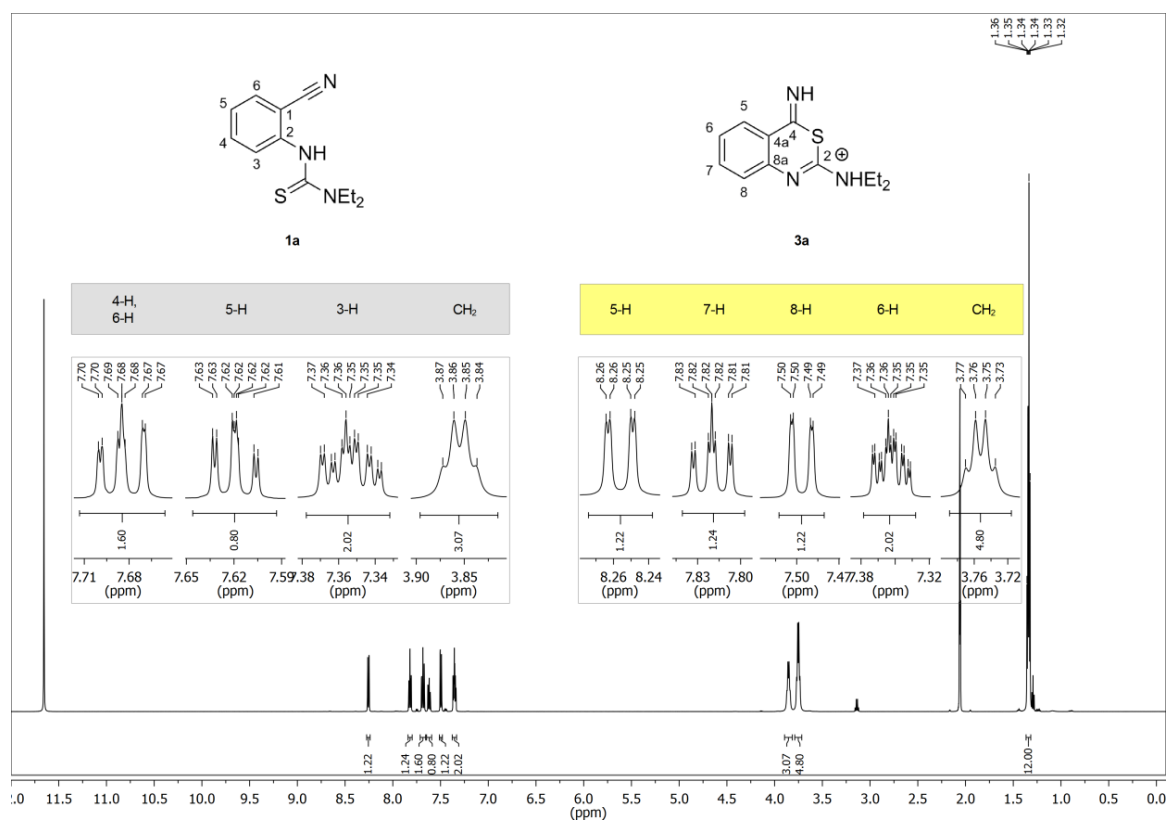


Figure S1. ^1H NMR spectrum (600 MHz, $\text{CD}_3\text{CO}_2\text{D}$) recorded of a reaction mixture of **1a** (10 mg) dissolved in $\text{CD}_3\text{CO}_2\text{D}$ (0.6 mL) after in incubation of 2 h at room temperature. The spectrum contains signals of **1a** (gray, left) and **3a** (yellow, right). Integration of the signals indicated a **3a** to **1a** ratio of 3:2.

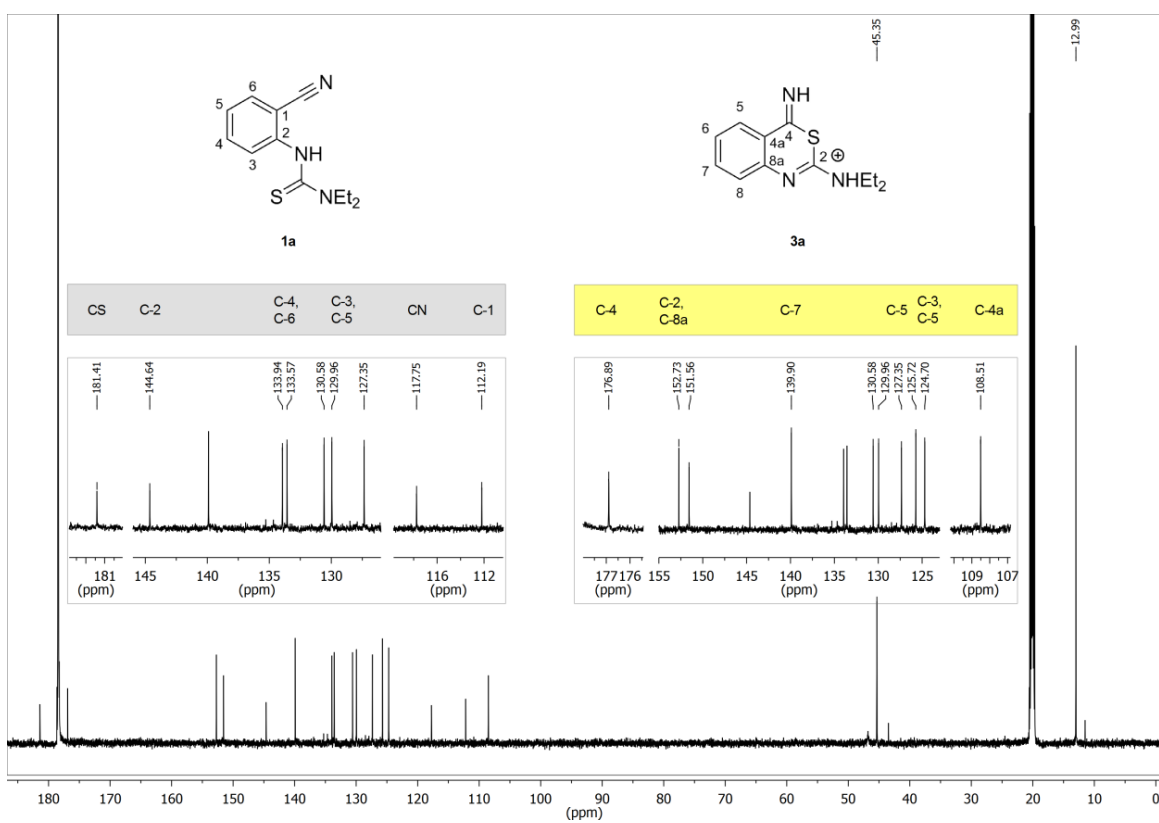


Figure S2. ¹³C NMR spectrum (151 MHz, CD₃CO₂D) recorded of a reaction mixture of **1a** (10 mg) dissolved in CD₃CO₂D (0.6 mL) after in incubation of 2 h at room temperature. The spectrum contains signals of **1a** (gray, left) and **3a** (yellow, right).

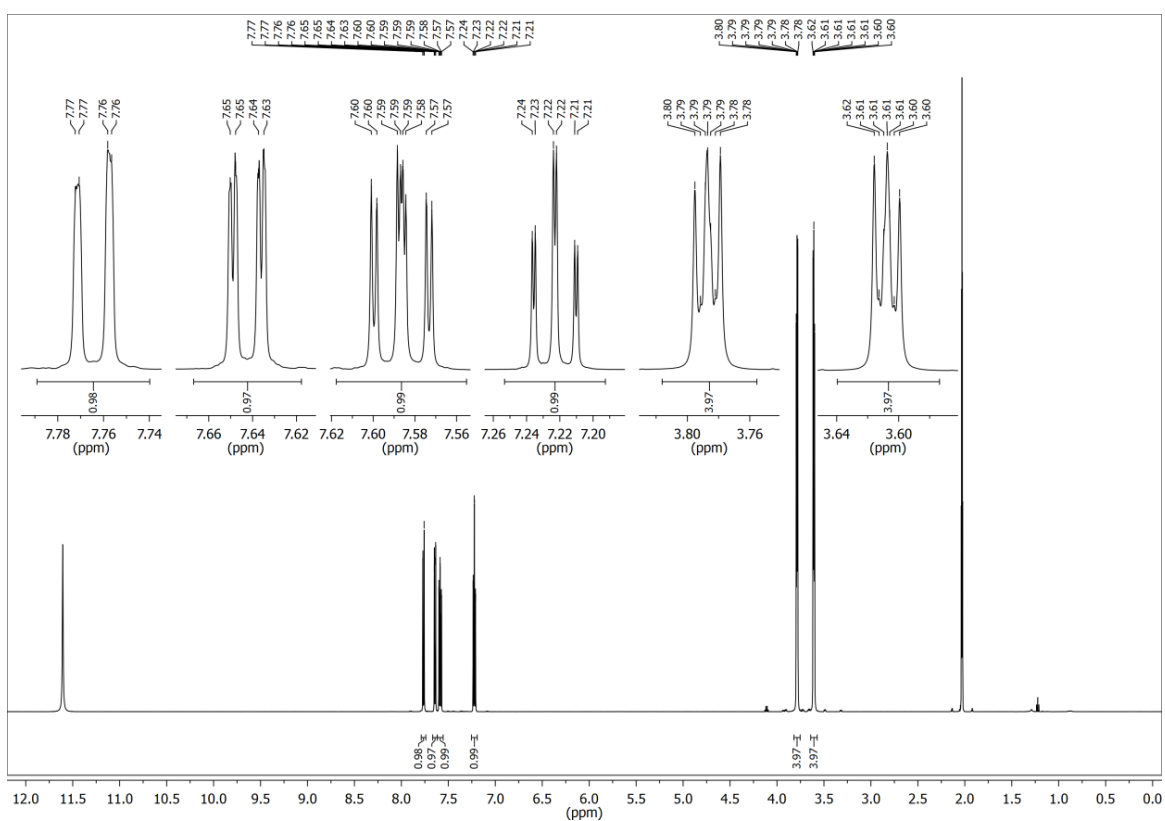


Figure S3. ¹H NMR spectrum (600 MHz, CD₃CO₂D) recorded of a reaction mixture of **2b** (10 mg) dissolved in CD₃CO₂D (0.6 mL) after in incubation of 2 h at room temperature. The spectrum contains only signals of **2b**.

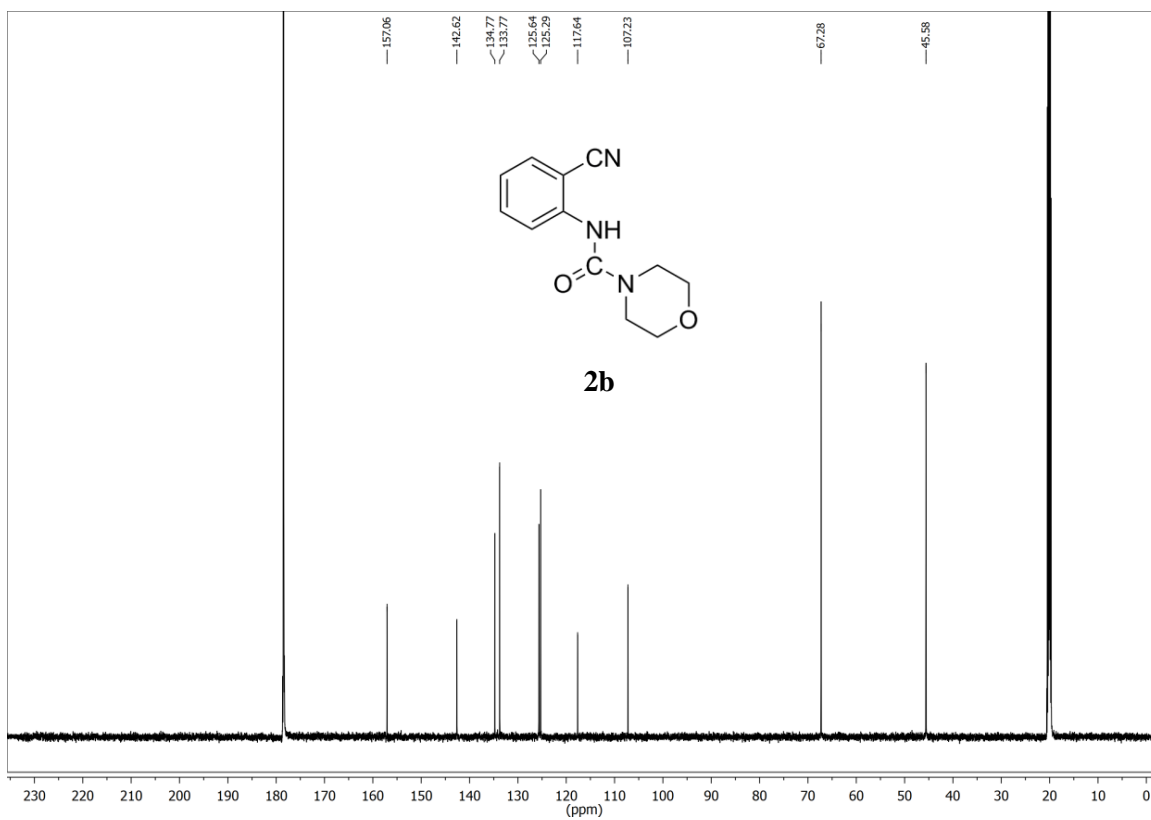


Figure S4. ¹³C NMR spectrum (151 MHz, CD₃CO₂D) recorded of a reaction mixture of **2b** (10 mg) dissolved in CD₃CO₂D (0.6 mL) after in incubation of 2 h at room temperature. The spectrum contains only signals of **2b**.

Color Change in the Course of the Formation of Acyliminium Salts

4-Acyliminothiazinium salts **9** are red colored while acylimino-4*H*-3,1-benzothiazines **11-16** gave yellow solutions. To illustrate this, compound **12a** (10 mg) was dissolved in MeCN (5 mL) in a small reaction tube containing a stirring bar (left). After addition of concd H₂SO₄ (1 drop), a red solution was formed (right).

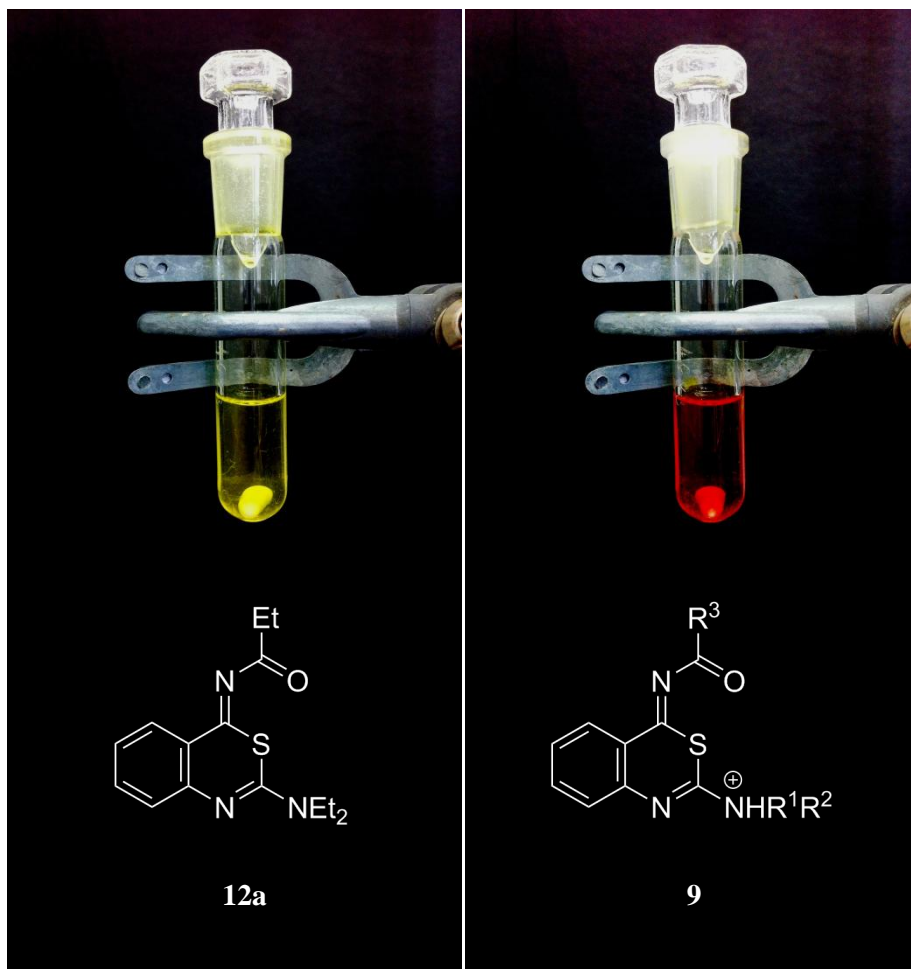


Figure S5: Bathochromic shift upon protonation of **12a**.

Calculations of Molecular Planarity and Plane Deviations

The equation $P \times x + Q \times y + R \times z - S = 0$ was used, where P, Q, R, S are constants and x, y, z are fractional coordinates (see CIF file for more detail). Least-squares solution of the equation was $P = -5.2029$, $Q = 6.7332$, $R = 0.5685$, $S = -0.9415$. Calculation was performed with PLATON for Windows (A. L. Spek, *J. Appl. Cryst.*, 2003, **36**, 7; A. L. Spek, *Acta Cryst.*, 2009, **D65**, 148).

Table S1: Plane deviation of the benzothiazine skeleton of **12a**.

Atom	Distance (Å)	x	y	z	X	Y	Z
S	-0.09205	0.5006	0.2145	0.2232	4.4314	2.7811	1.9978
N(1)	0.05970	0.3428	0.1323	0.0187	2.9966	1.2979	0.1671
C(2)	0.04935	0.3551	0.1289	0.1537	3.1296	1.7405	1.3756
C(4)	-0.01463	0.6034	0.3193	0.0595	5.3306	3.1824	0.5328
C(4a)	0.03077	0.5626	0.3063	-0.0814	4.9443	2.5670	-0.7284
C(5)	0.05823	0.6496	0.3885	-0.2100	5.7034	2.8806	-1.8798
C(6)	0.01567	0.6245	0.3742	-0.3450	5.4520	2.2731	-3.0882
C(7)	-0.05477	0.5108	0.2768	-0.3559	4.4266	1.3276	-3.1866
C(8)	-0.05627	0.4224	0.1978	-0.2329	3.6562	1.0248	-2.0846
C(8a)	0.00402	0.4442	0.2119	-0.0936	3.8806	1.6448	-0.8380

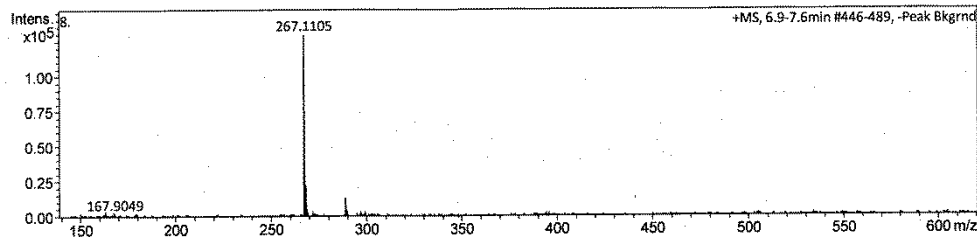
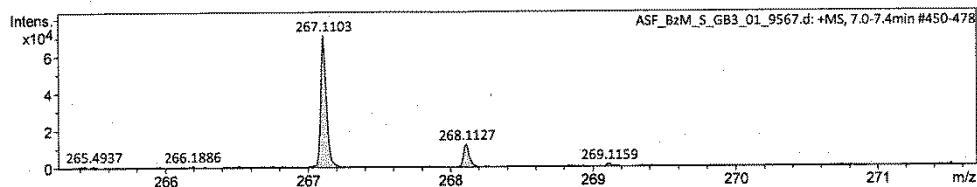
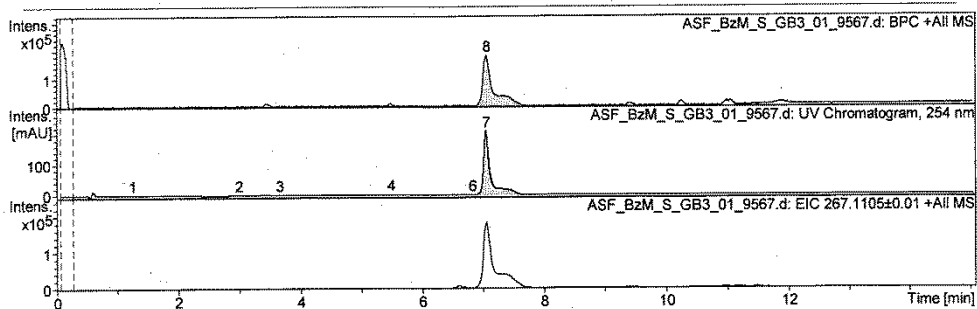
The equation $P \times x + Q \times y + R \times z - S = 0$ was used (see CIF file for more detail). Least-squares solution of the equation was $P = -5.0320$, $Q = 7.1351$, $R = 1.9429$, $S = -0.5952$.

Table S2: Plane deviation of the atoms S, C(4), N(3), C(13) and O of **12a**.

Atom	Distance (Å)	x	y	z	X	Y	Z
S	0.04018	0.5006	0.2145	0.2232	4.4314	2.7811	1.9978
O	-0.05528	0.7245	0.3328	0.3193	6.4252	4.2207	2.8588
N(3)	0.00604	0.7167	0.4062	0.0614	6.3443	3.9981	0.5497
C(4)	-0.04760	0.6034	0.3193	0.0595	5.3306	3.1824	0.5328
C(13)	0.05665	0.7689	0.4136	0.1951	6.8219	4.5372	1.7465

HRMS-ESI of the Product of the Treatment of 2c with Ac₂O/H₂SO₄ and Workup with H₂O

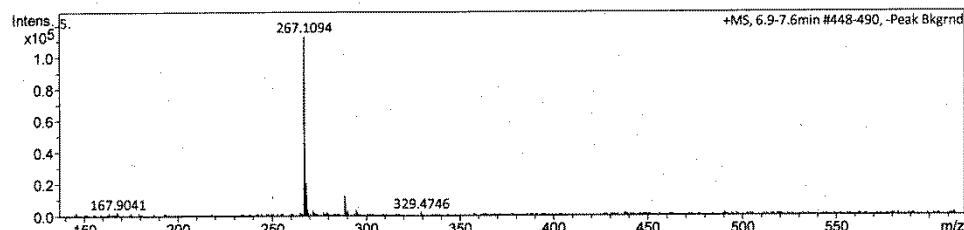
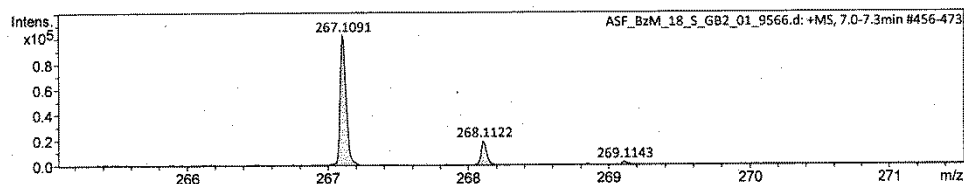
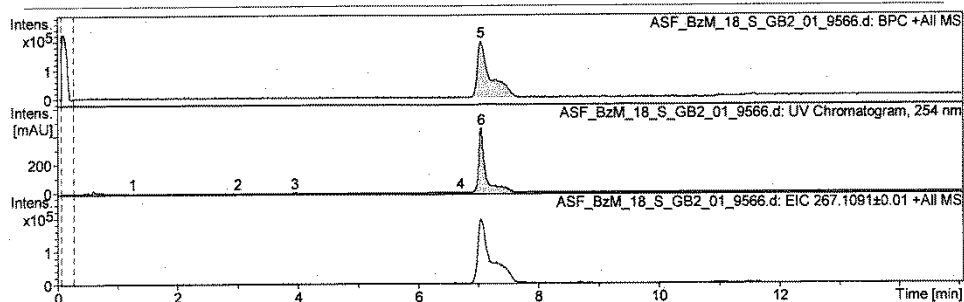
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 Method standard-smallmolecules_acetat_posuv220.m Instrument micrOTOF-Q III
 Sample Name ASF_BzM_S
 Comment



#	RT [min]	Area	Int. Type	I	S/N	Chromatogram	Max. m/z	FWHM [min]	Area Frac. %
1	1.2	2.8512	Manual	0	1532.8	UV Chromatogram, 254 nm	0.0000		0.17430
2	3.0	2.6415	Manual	0	1683.6	UV Chromatogram, 254 nm	0.0000		0.16148
3	3.7	2.9989	Manual	1	2567.9	UV Chromatogram, 254 nm	0.0000		0.18332
4	5.5	7.6722	Manual	2	7525.7	UV Chromatogram, 254 nm	0.0000		0.46901
5	6.7	1.2527	Manual	1	1576.9	UV Chromatogram, 254 nm	0.0000		0.07658
6	6.8	0.1325	Manual	1	1084.4	UV Chromatogram, 254 nm	0.0000		0.00810
7	7.0	1618.2816	Manual	209	1047610.4	UV Chromatogram, 254 nm	0.0000		98.92722
8	7.1	2167797.2500	Manual	182201	85.6	BPC +All MS	267.1105		100.00000

HRMS-ESI of the Product of the Treatment of 2c with Ac₂O/H₂SO₄ and Workup with H₂¹⁸O

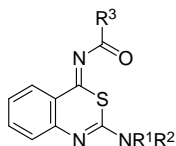
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 Comment:
 Acquisition Date: 10/7/2015 7:17:16 PM
 Operator: BDAL@DE
 Instrument: microTOF-Q III



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1	1.2	1.9549	Manual	0	1320.9	UV Chromatogram, 254 nm	0.0000	0.05637	
2	3.0	3.9733	Manual	0	2598.6	UV Chromatogram, 254 nm	0.0000	0.11458	
3	3.9	2.0620	Manual	1	1906.0	UV Chromatogram, 254 nm	0.0000	0.05946	
4	6.7	1.3353	Manual	1	1504.1	UV Chromatogram, 254 nm	0.0000	0.03851	
5	7.0	2794776.0000	Manual	196199	106.7	BPC +All MS	267.1094	100.00000	
6	7.0	3458.3660	Manual	451	2264035.3	UV Chromatogram, 254 nm	0.0000	99.73107	

Enzymatic Studies

Table S3. Protease inhibition by 4-acylimino-2-amino-4*H*-3,1-benzothiazines.

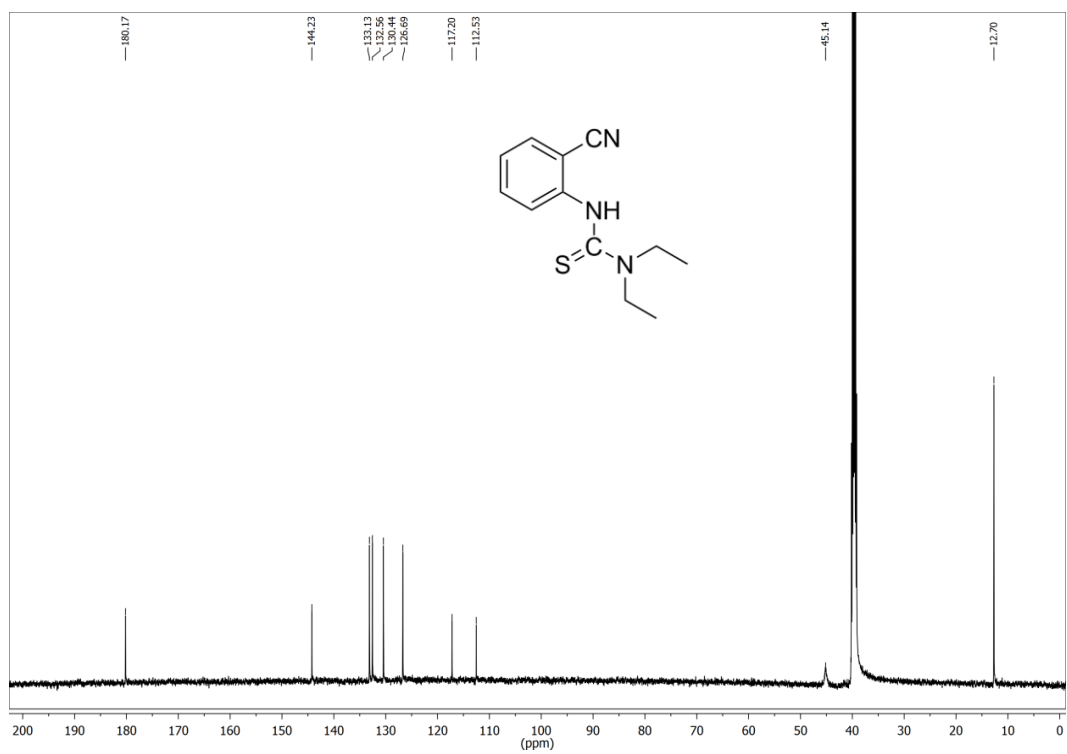
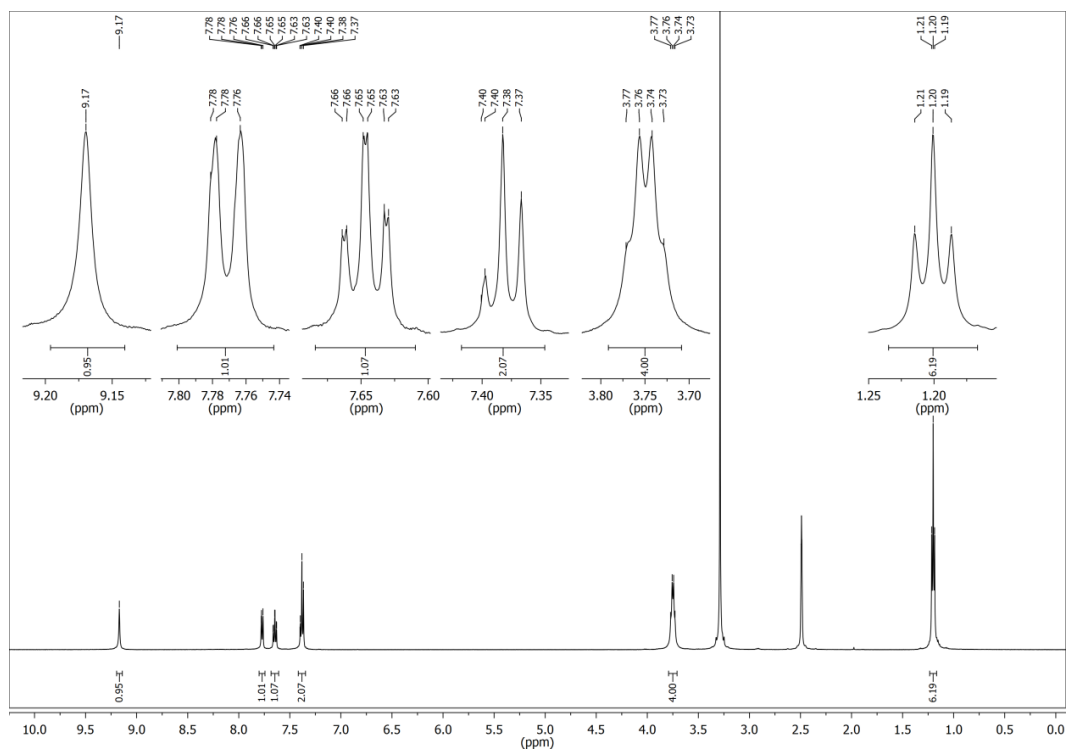


Compd	NR ¹ R ²	R ³	IC ₅₀ (μM) ^a			
			HLE	chymotrypsin	cathepsin B	cathepsin L
11a	N(Et) ₂	Me	>100	>100	>20	>20
11b	morpholino	Me	>100	>100	>20	>20
11c	N(Me)Bn	Me	>20	>20	>20	>20
12a	N(Et) ₂	Et	>100	>100	>20	>20
12b	morpholino	Et	>100	>100	>20	>20
12c	N(Me)Bn	Et	4.16 ± 1.52 ^b	>100	>20	>20
13a	N(Et) ₂	<i>i</i> -Pr	>20	>20	>20	>100
13b	morpholino	<i>i</i> -Pr	>100	>100	>20	>20
13c	N(Me)Bn	<i>i</i> -Pr	6.45 ± 0.50 ^b	10.4 ± 0.2 ^b	>100	>100
14a	N(Et) ₂	<i>i</i> -Bu	6.85 ± 2.08 ^b	>20	>20	>100
14b	morpholino	<i>i</i> -Bu	>20	>100	>20	>20
14c	N(Me)Bn	<i>i</i> -Bu	3.88 ± 0.78 ^b	>20	>20	>100
15a	N(Et) ₂	CH ₂ Cl	>20	>20	>20	>100
15b	morpholino	CH ₂ Cl	>20	>20	>20	>100
15c	N(Me)Bn	CH ₂ Cl	>20	>100	>20	>100
16a	N(Et) ₂	OBn	5.50 ± 1.92 ^b	>20	>100	>100
16b	morpholino	OBn	15.5 ± 2.4 ^b	>20	>100	>100
16c	N(Me)Bn	OBn	3.17 ± 1.63 ^b	>20	>100	>100

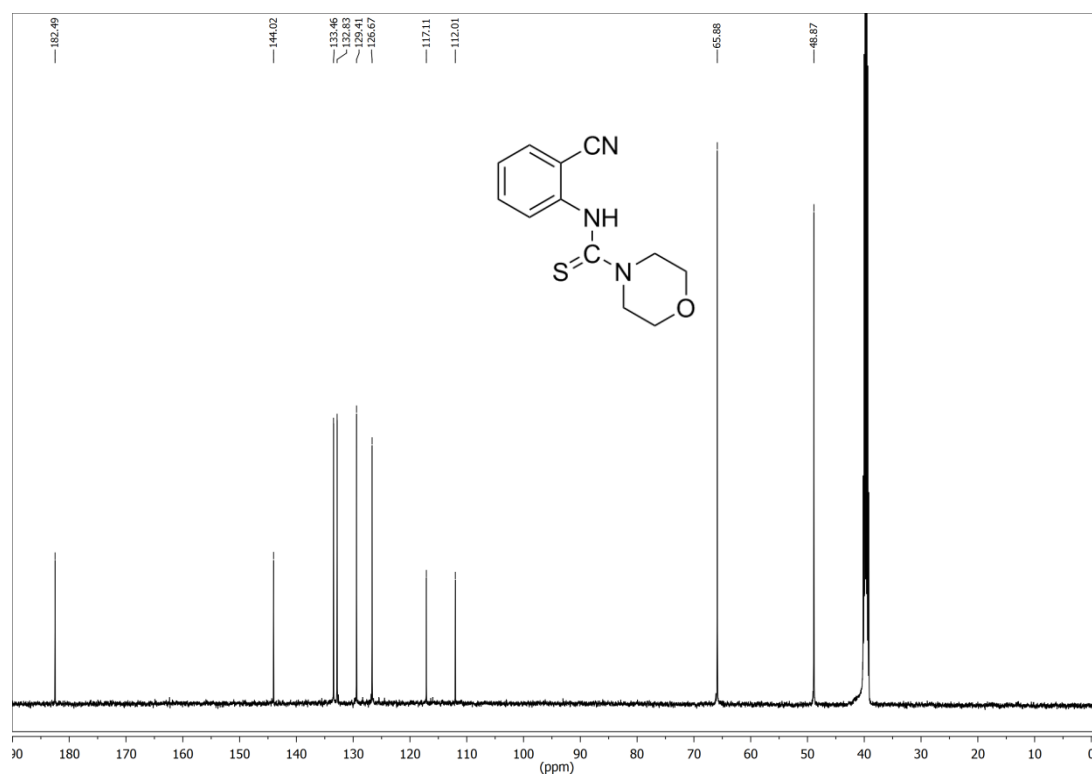
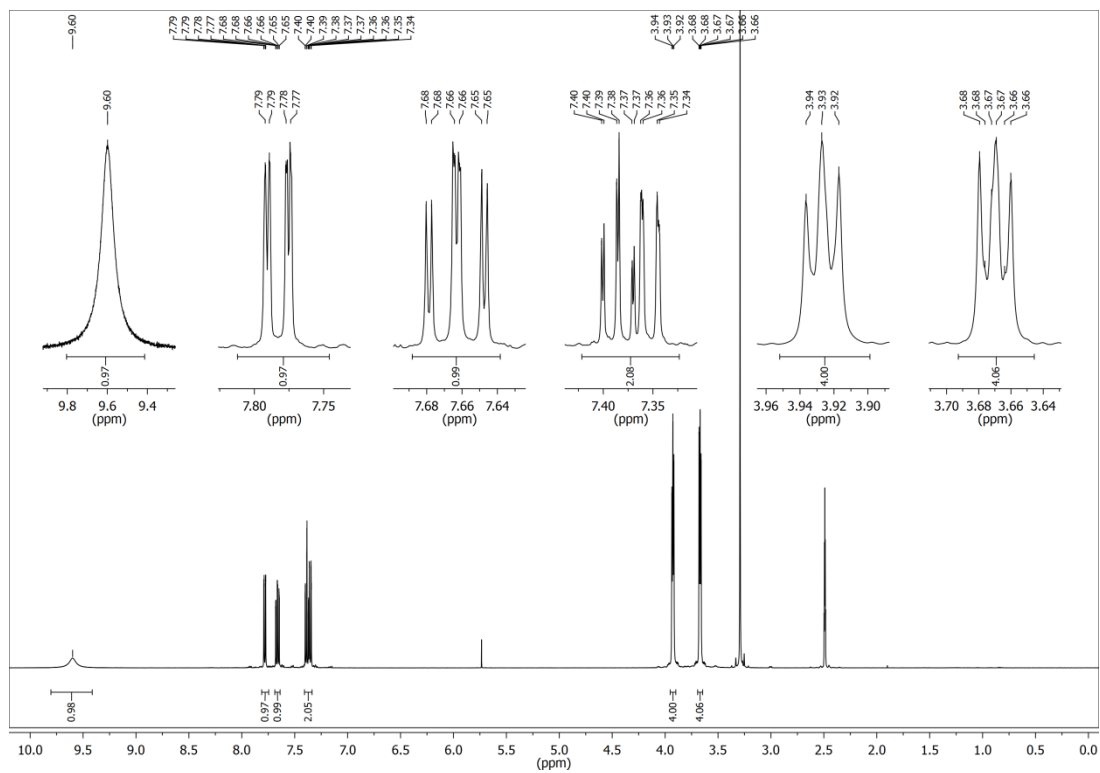
^a IC₅₀ values obtained from duplicate measurements at a single inhibitor concentration.

^b IC₅₀ values ± standard errors obtained from duplicate measurements with five different inhibitor concentrations (0.5-10 μM).

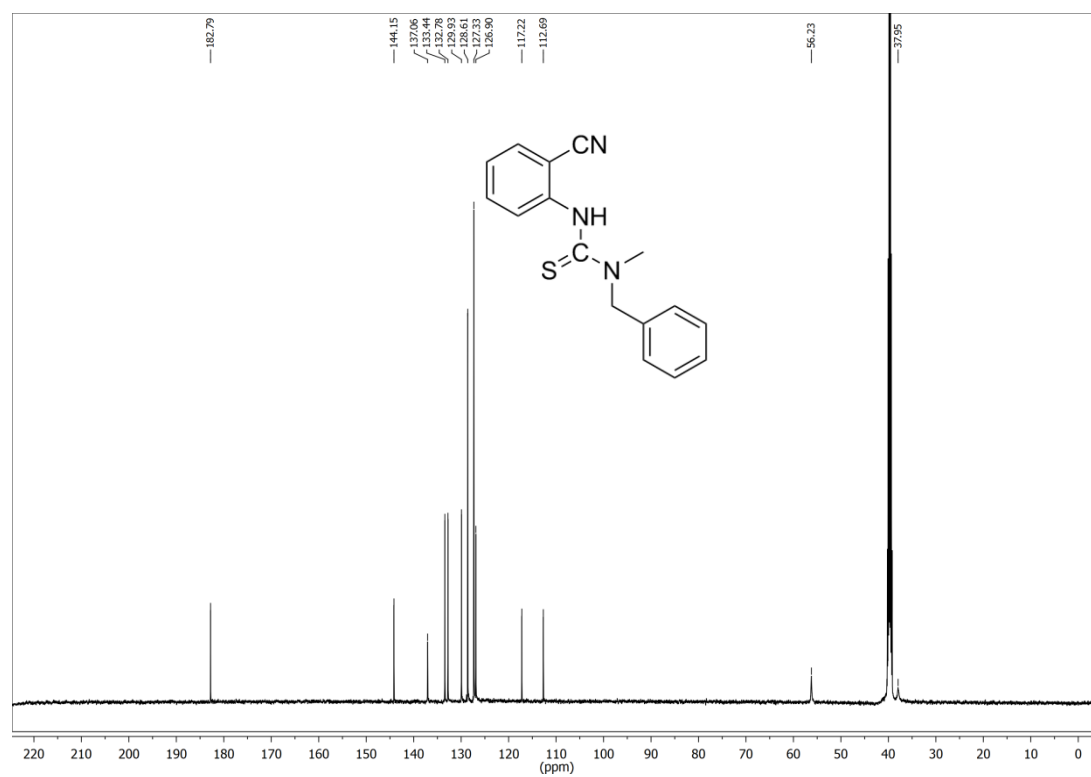
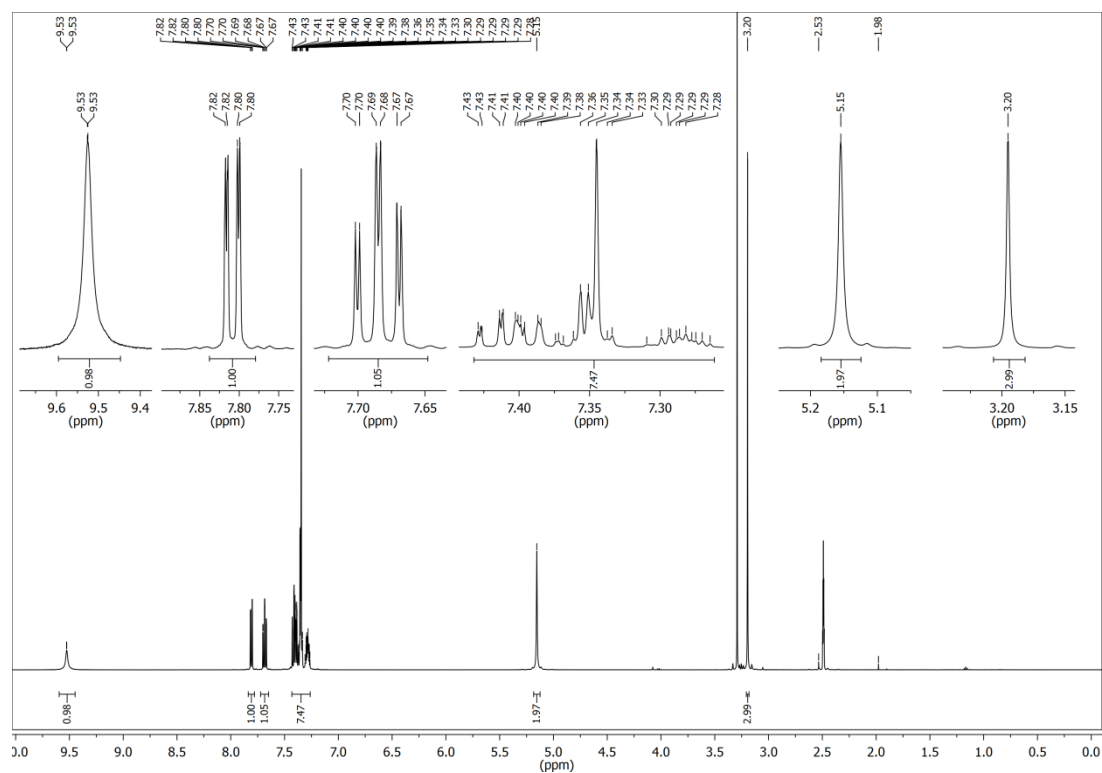
¹H NMR and ¹³C NMR Spectra of All Compounds
2-(3-Diethylthioureido)benzonitrile (1a), DMSO-d₆



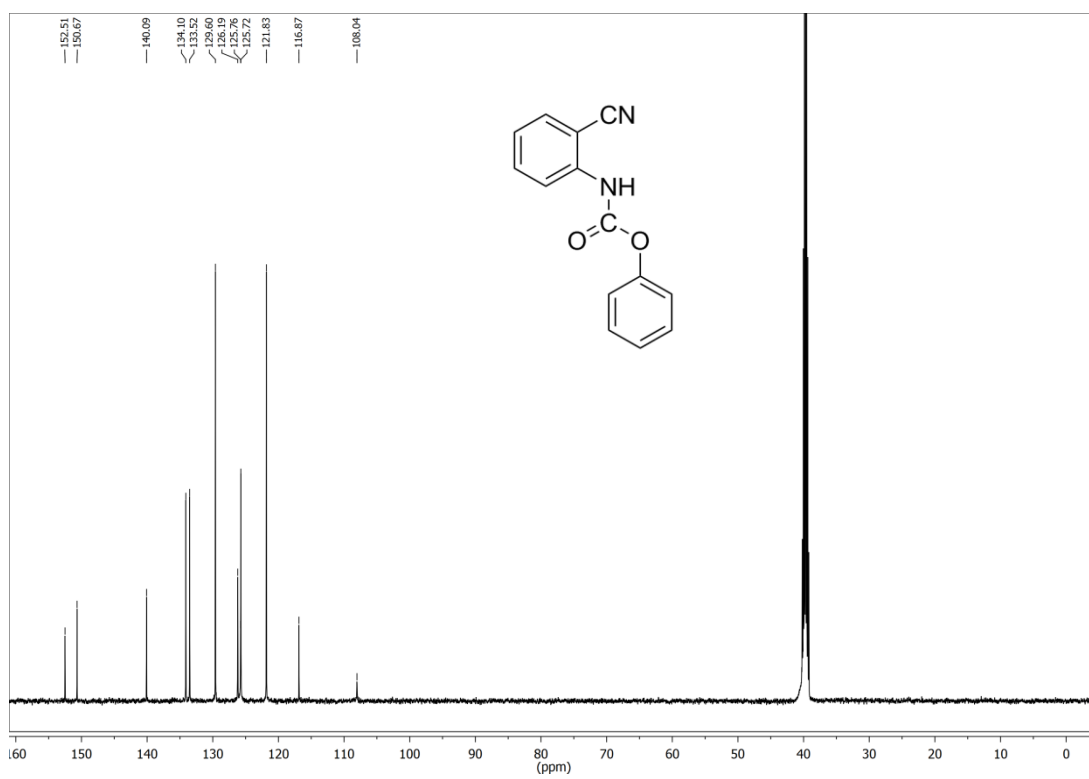
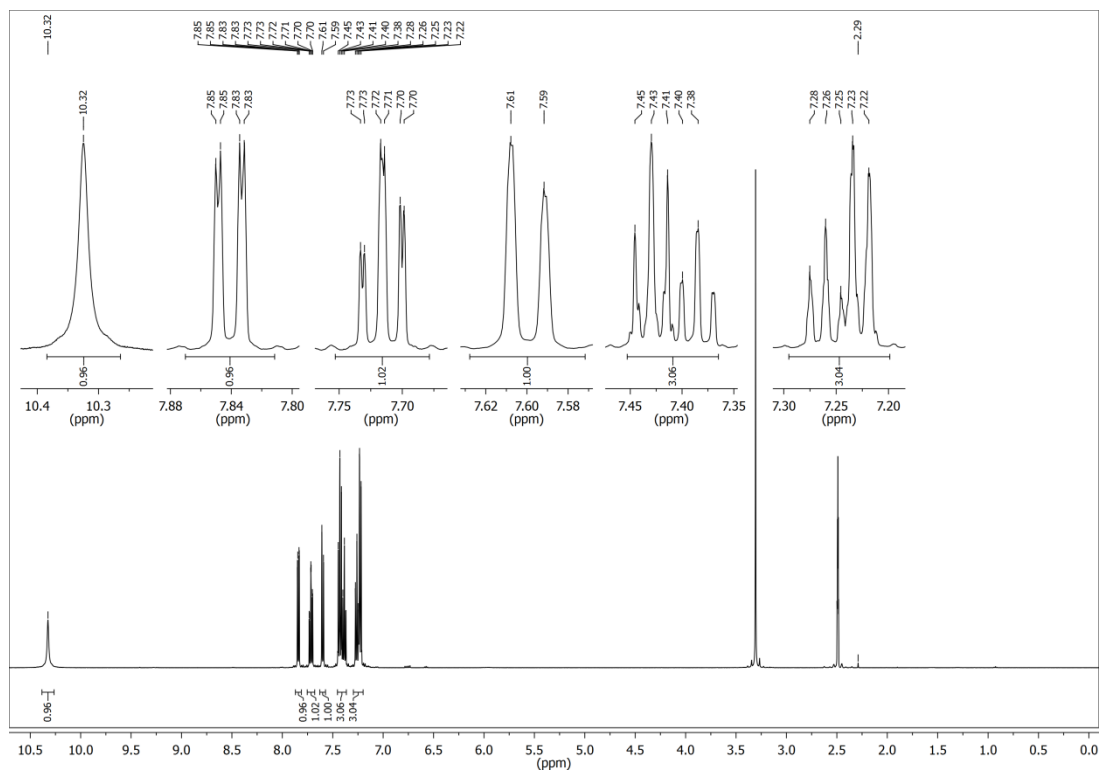
2-(Morpholinothiocabonylamino)benzonitrile (1b), DMSO-*d*₆



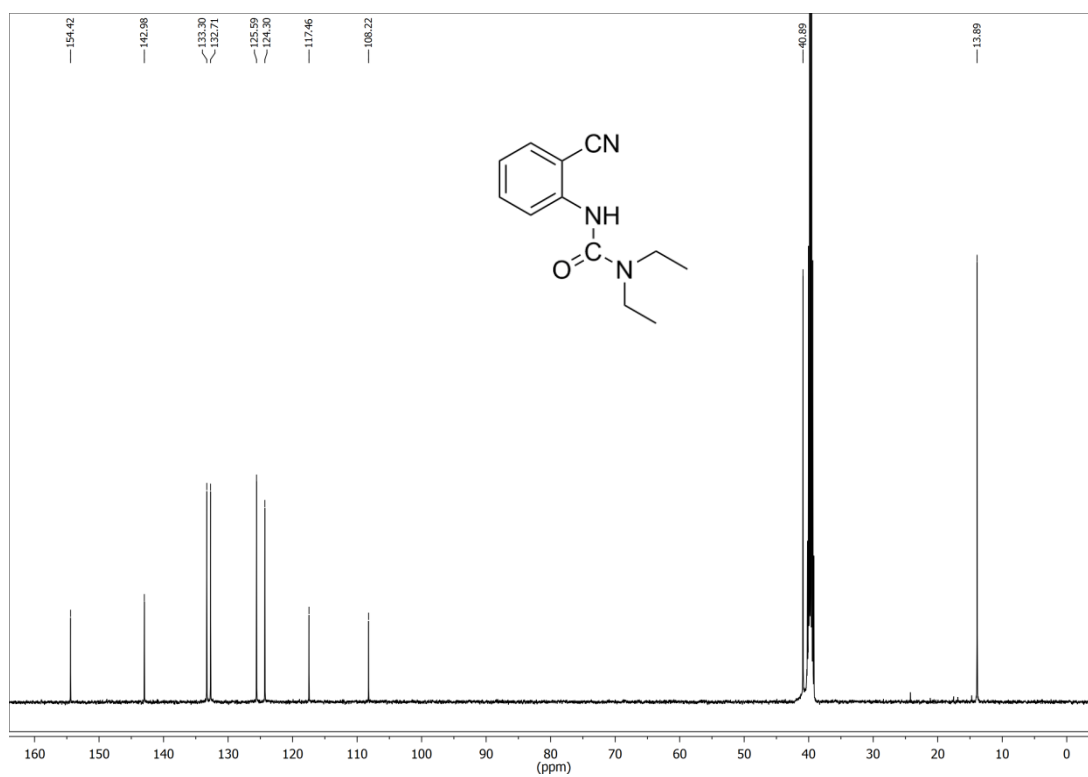
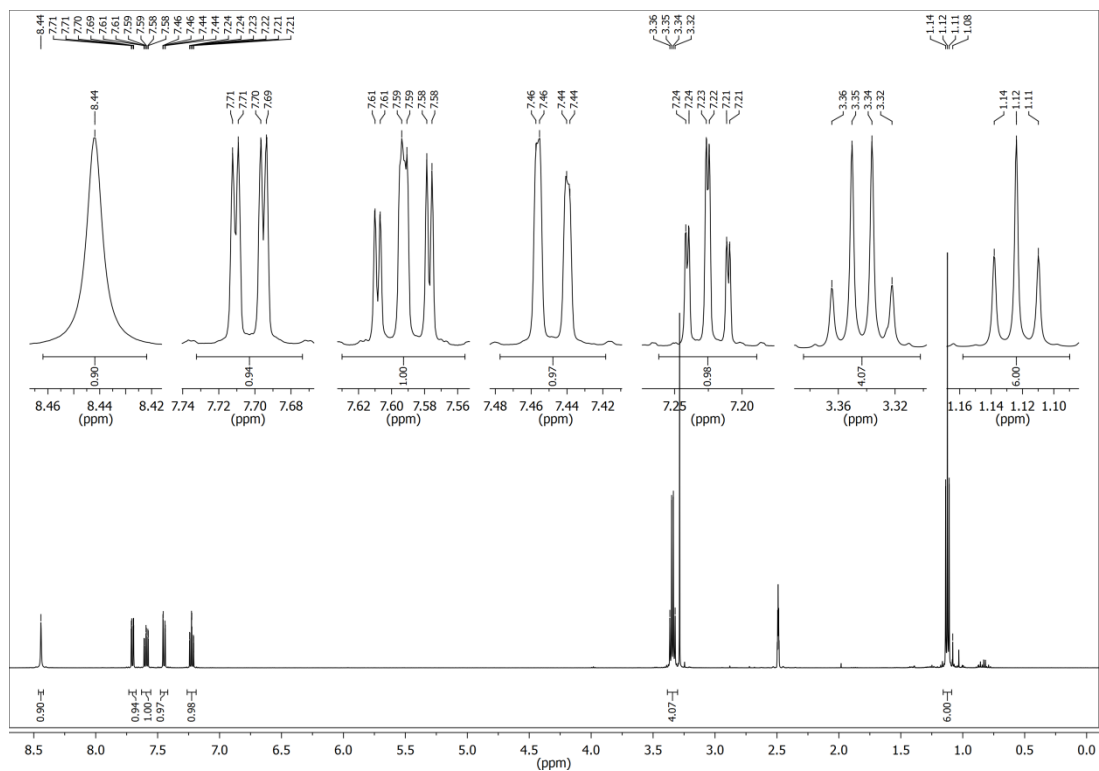
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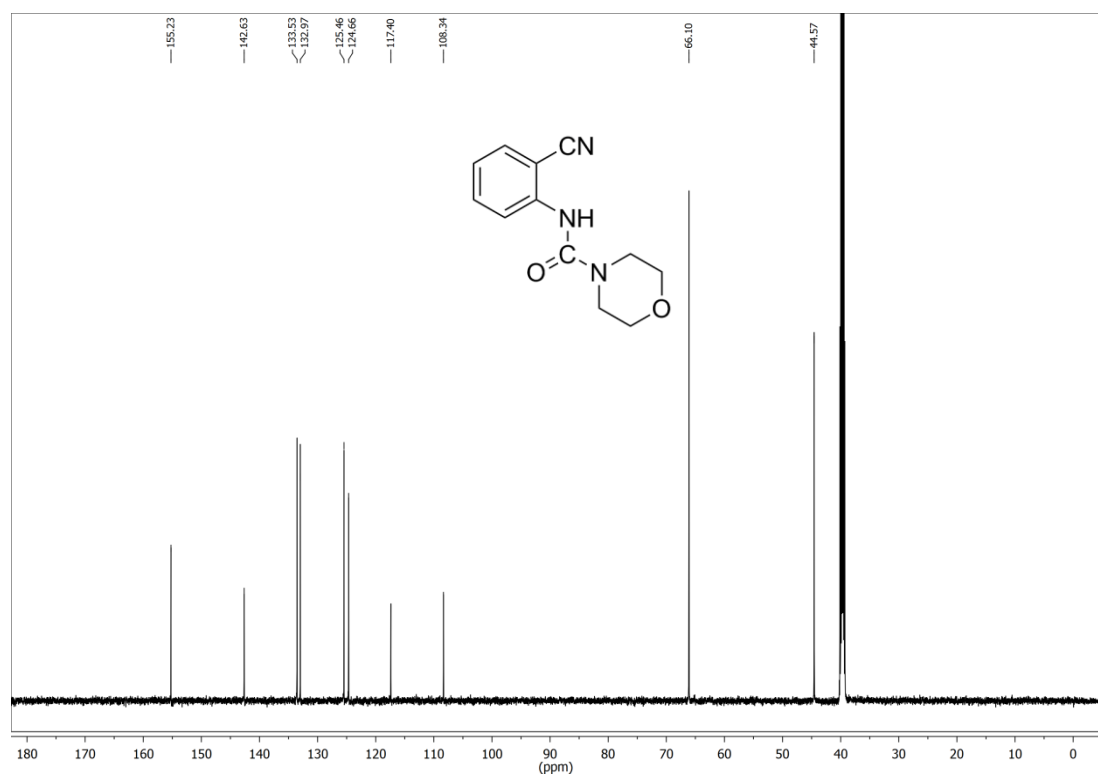
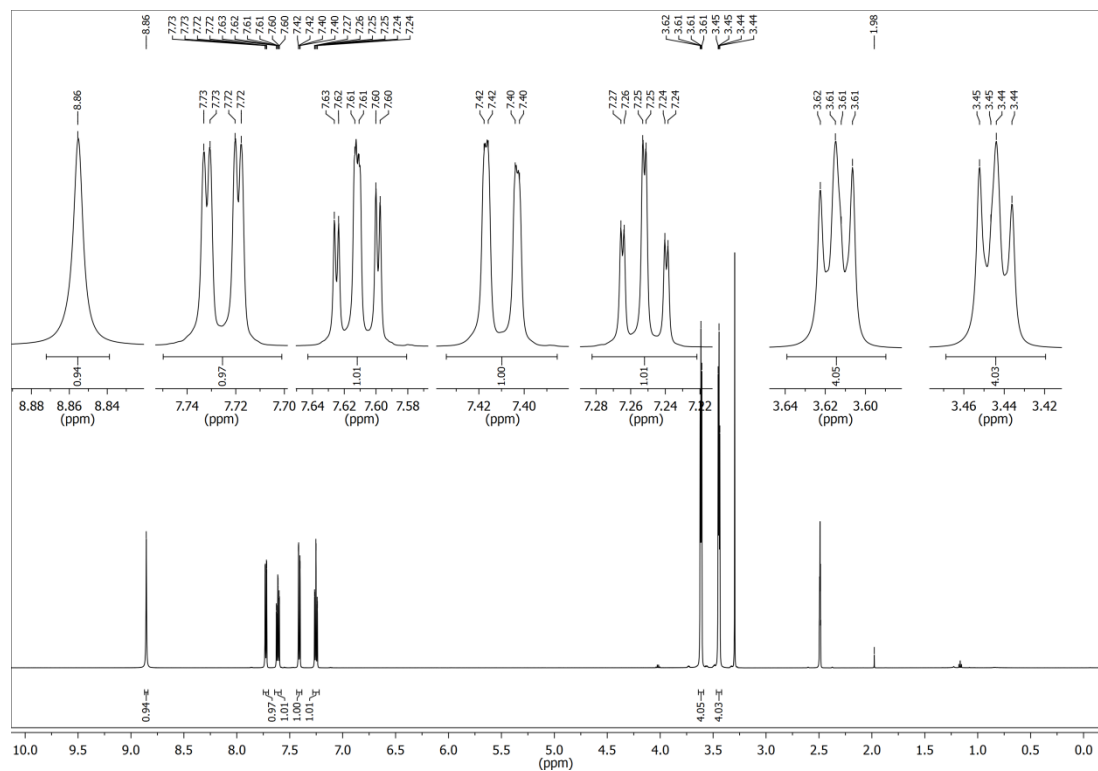
***N*-(2-Cyanophenyl)phenylcarbamate, DMSO-*d*₆**



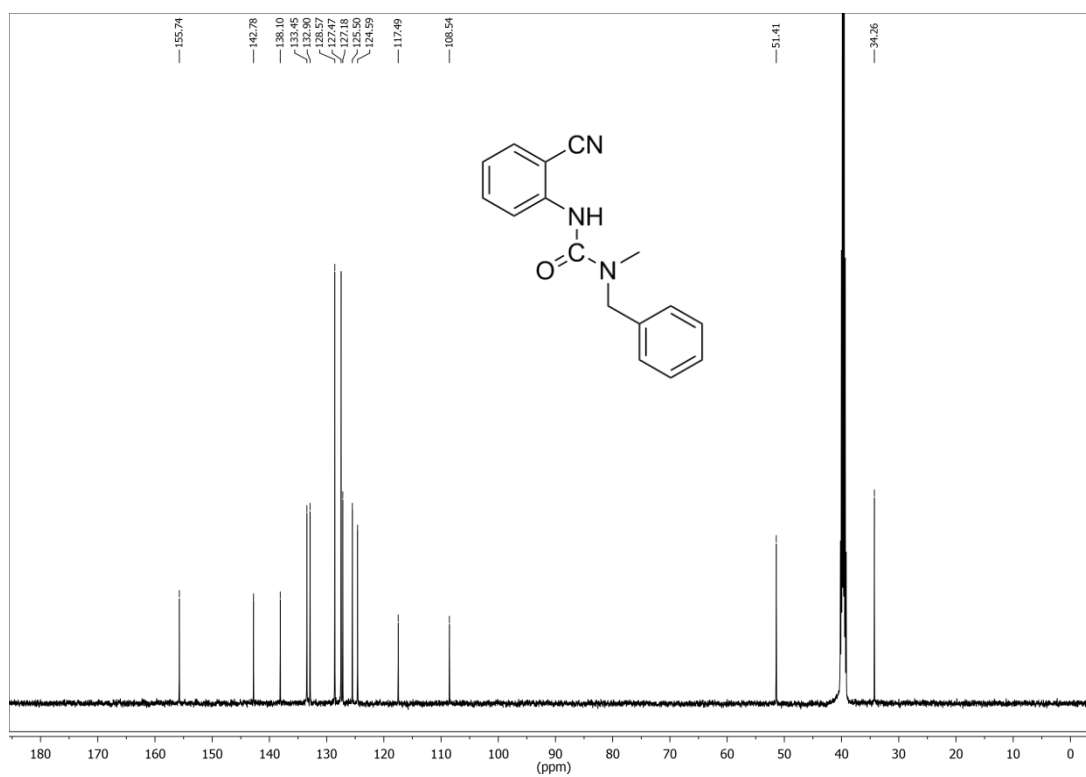
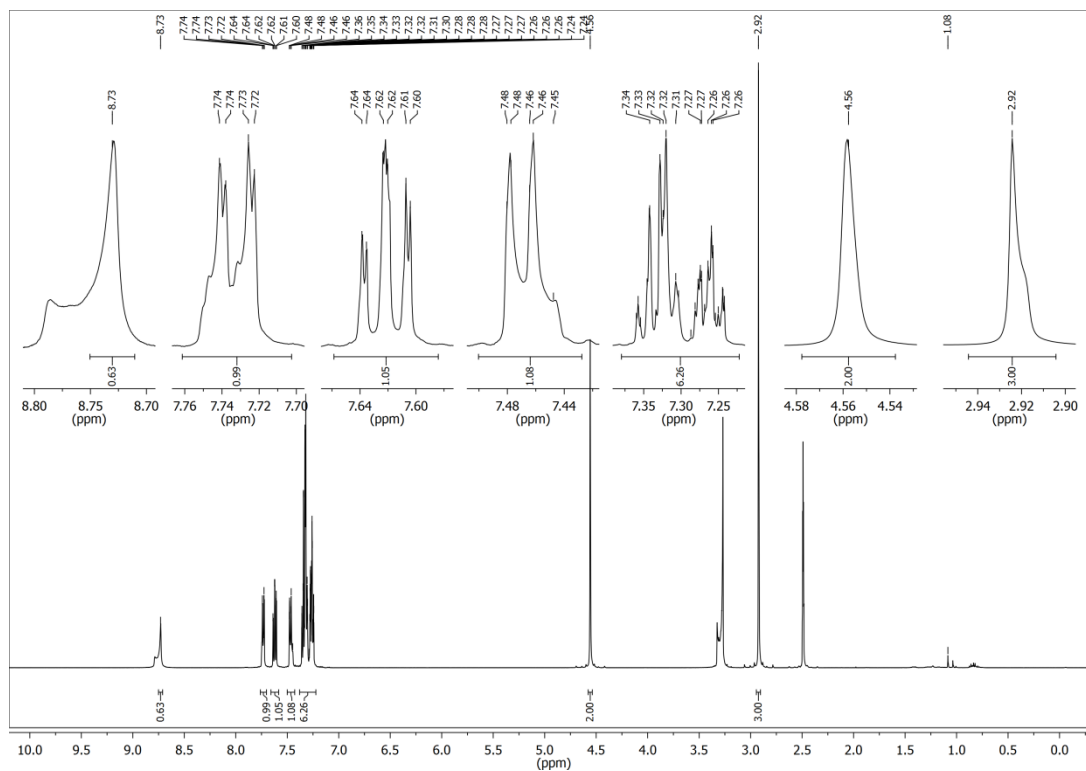
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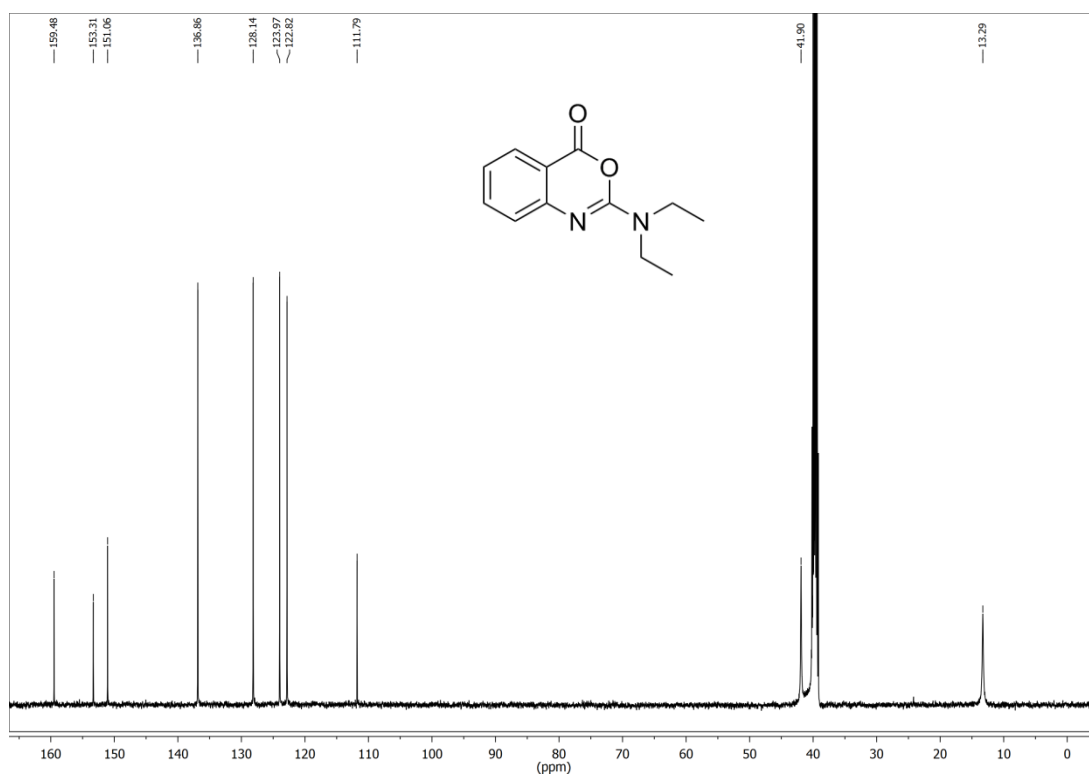
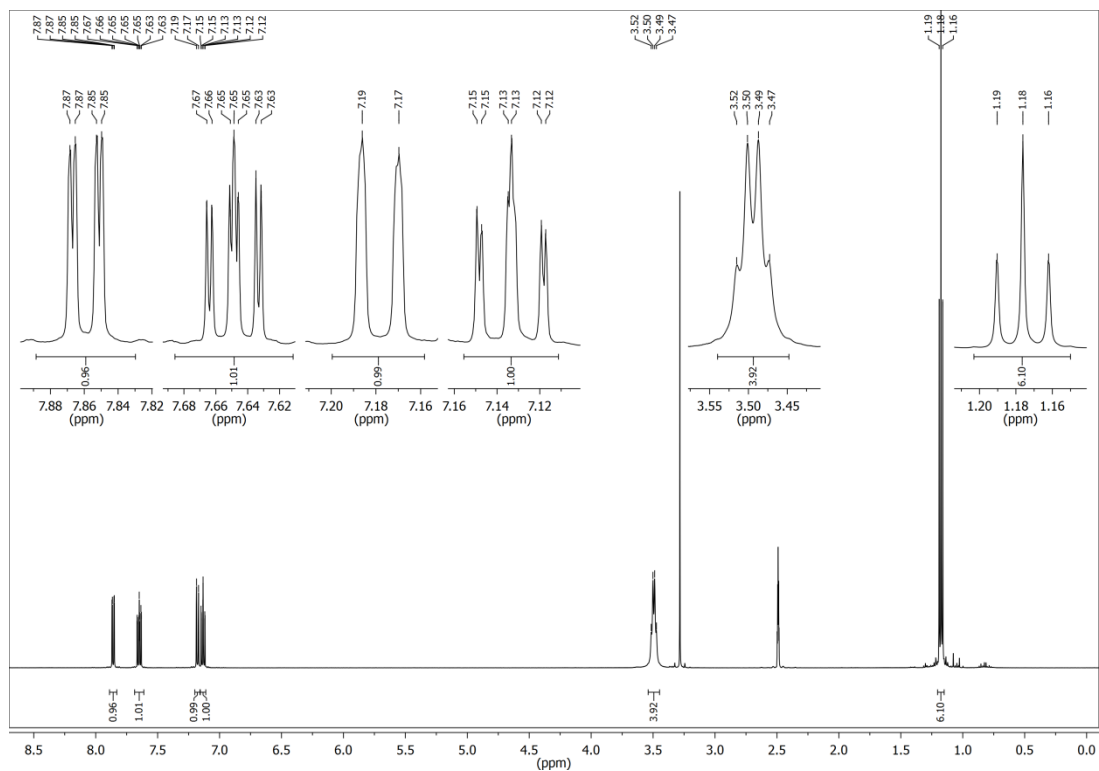
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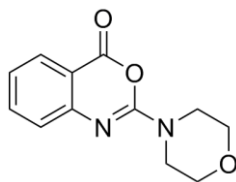
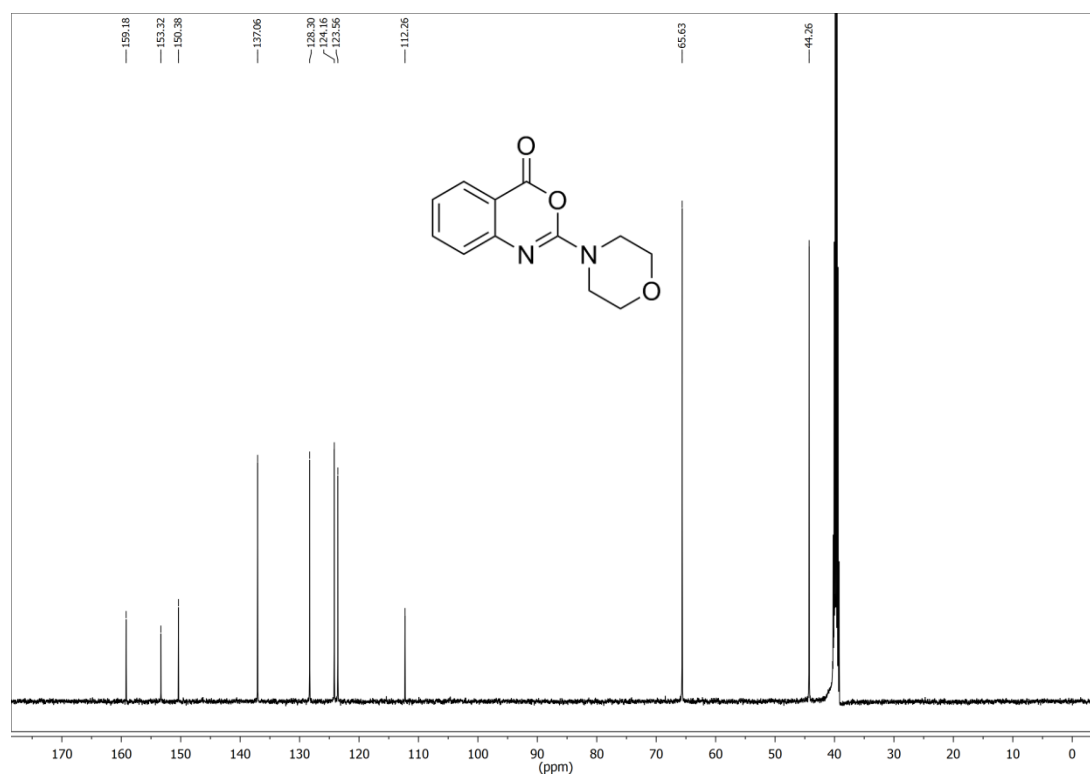
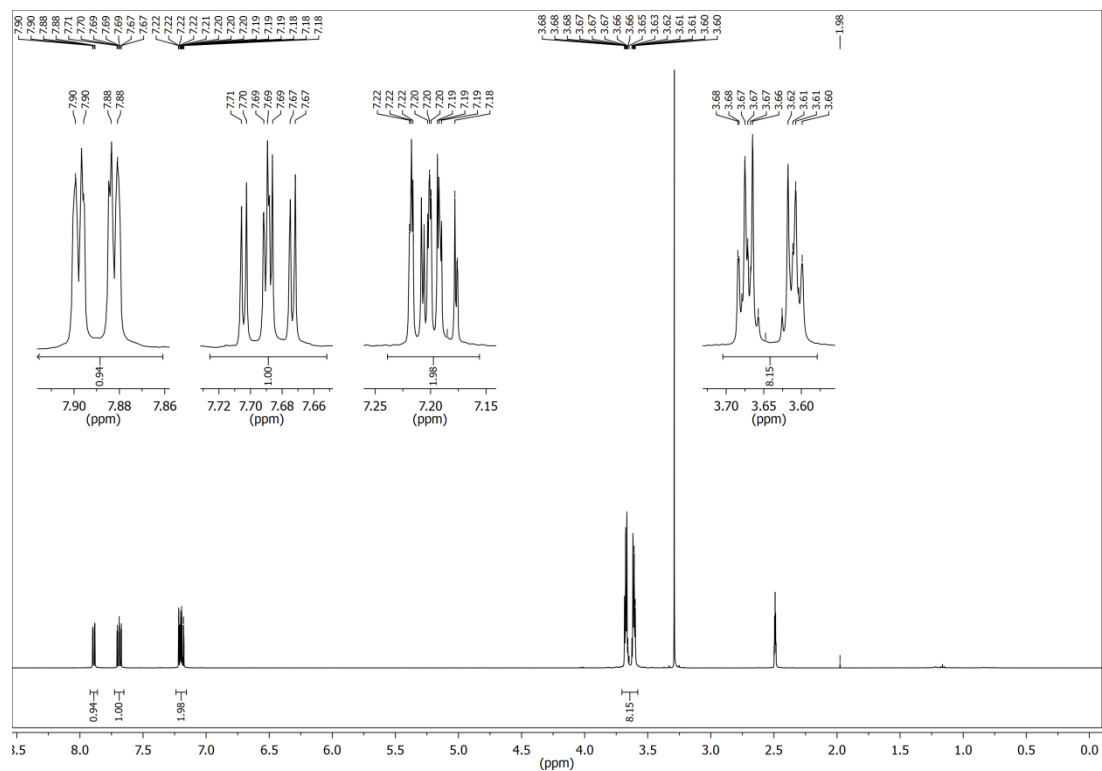
2-(3-Benzyl-3-methylureido)benzonitrile (2c), DMSO-*d*₆



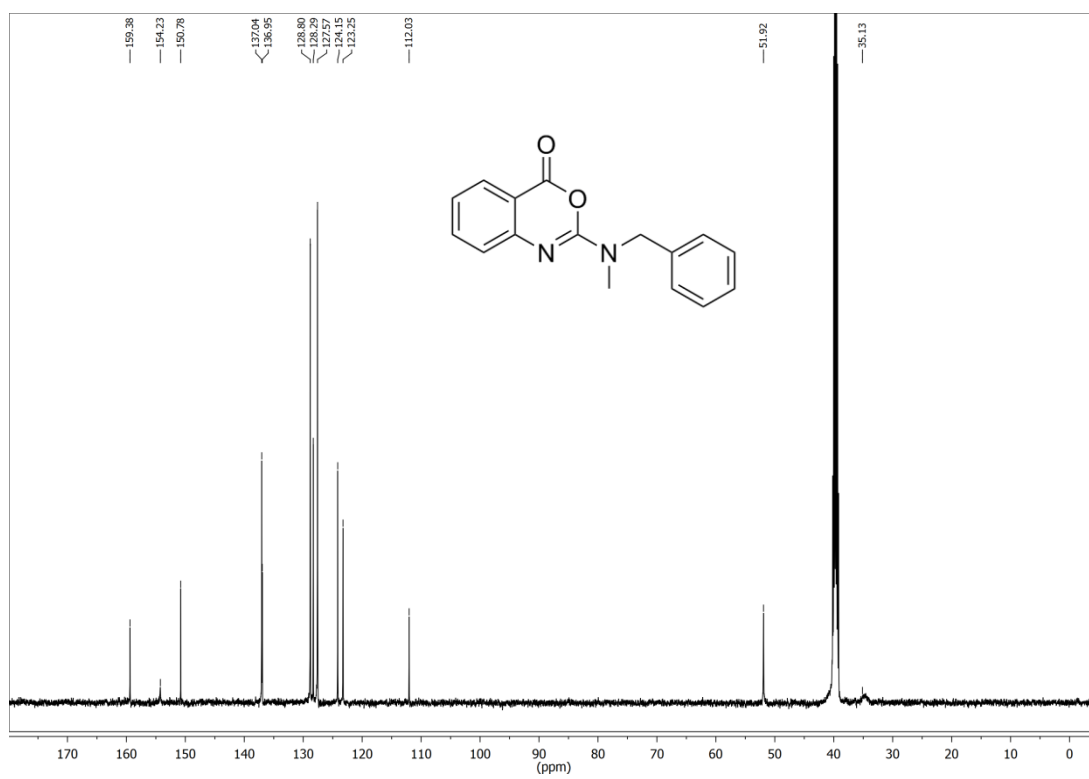
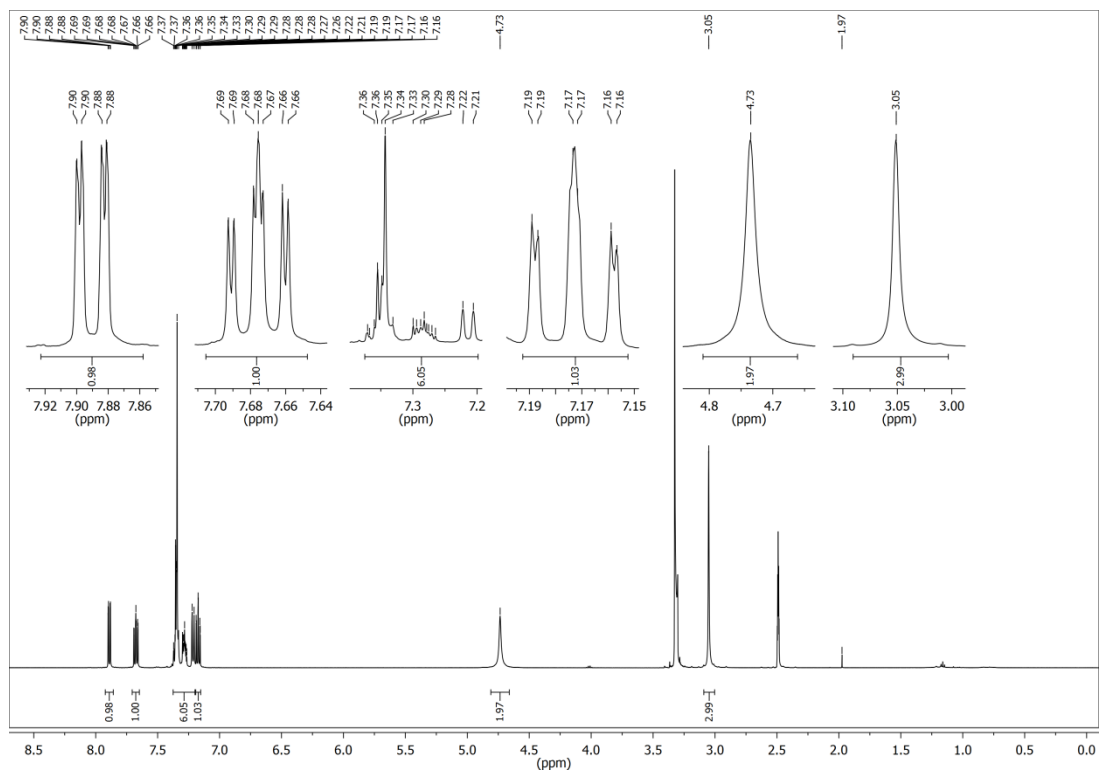
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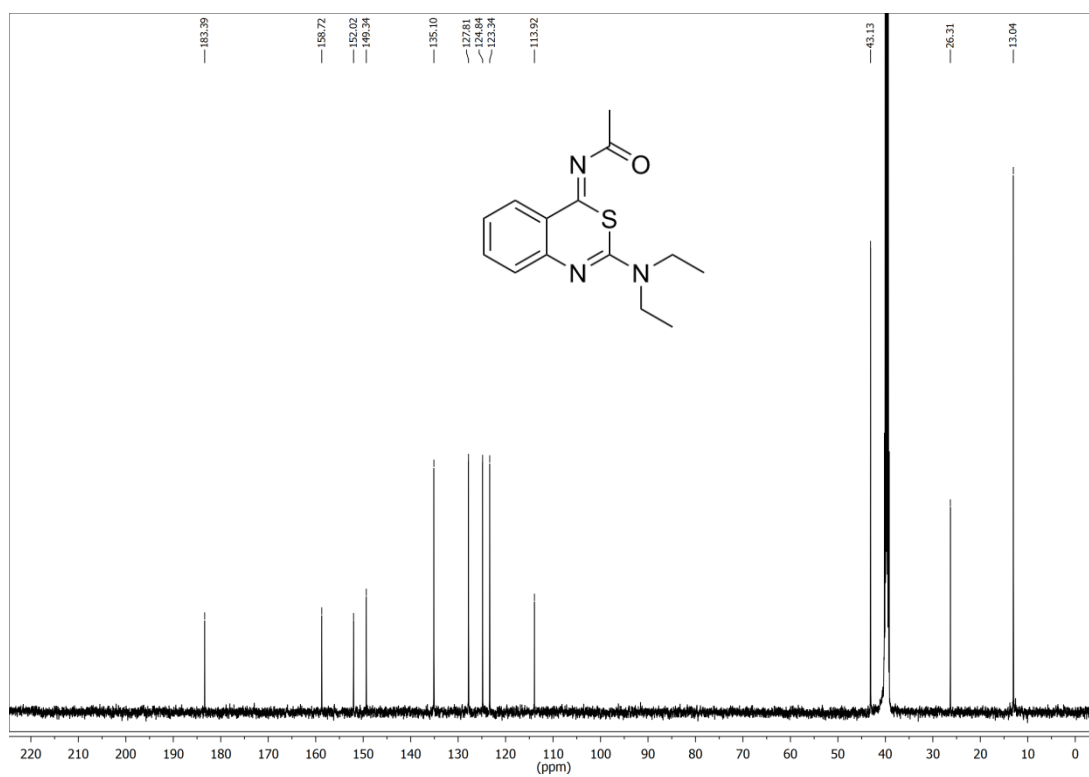
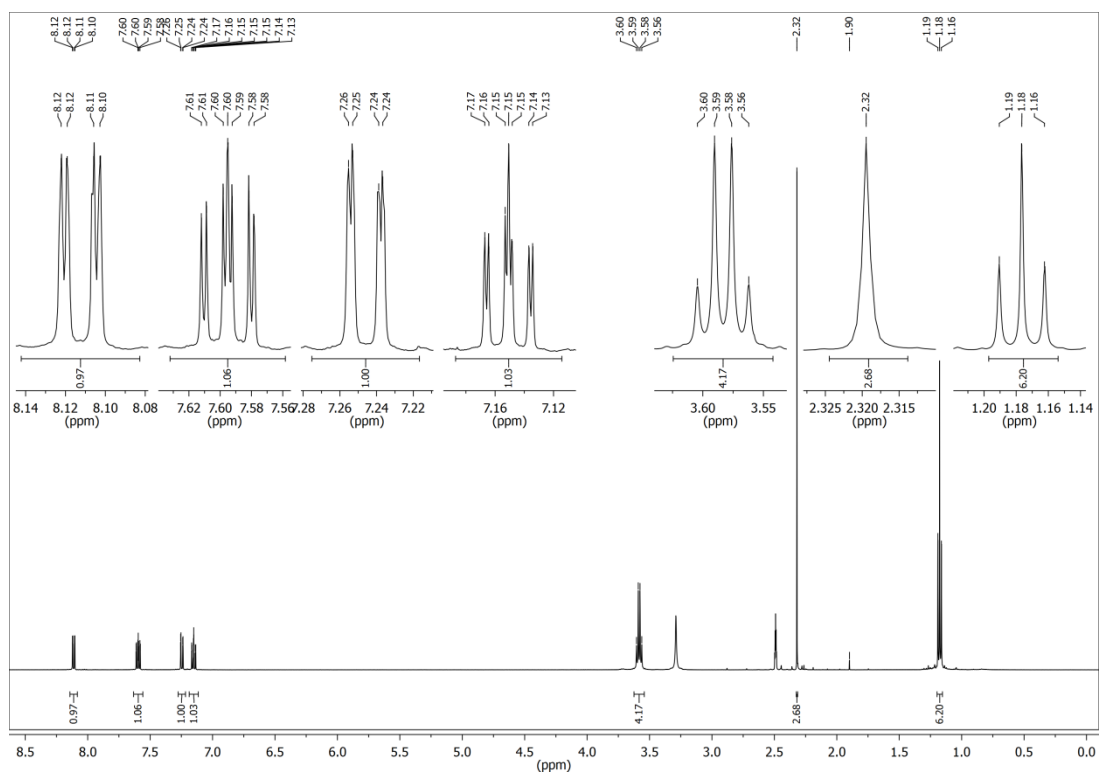
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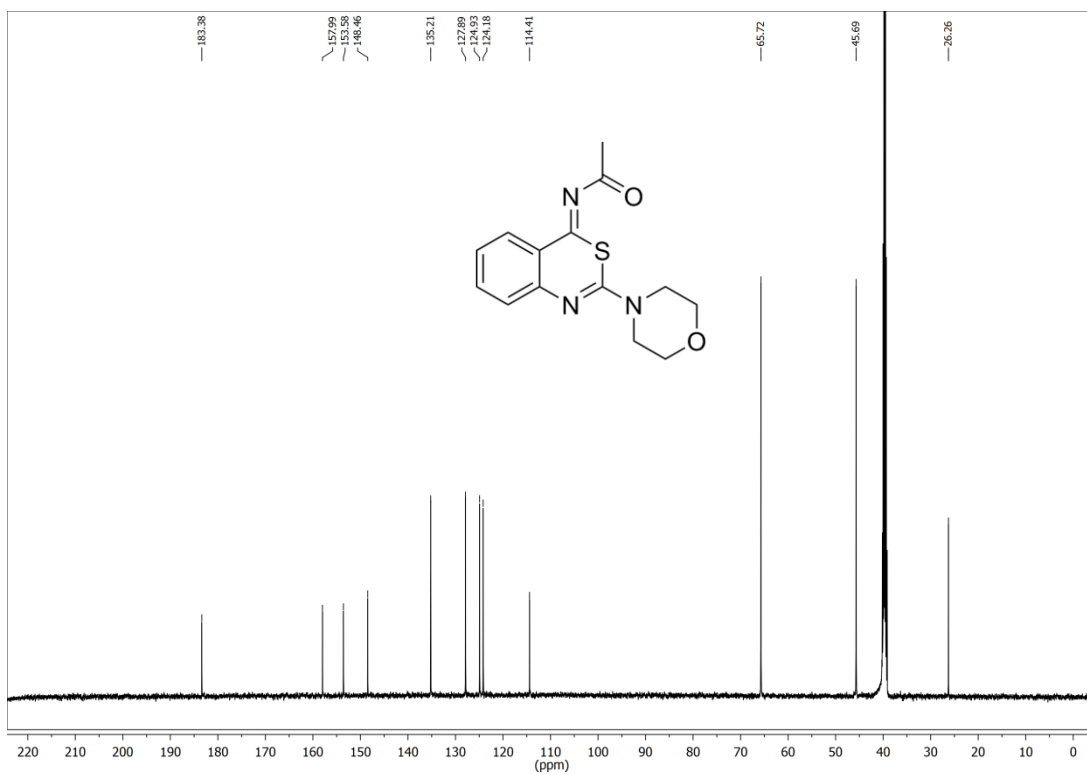
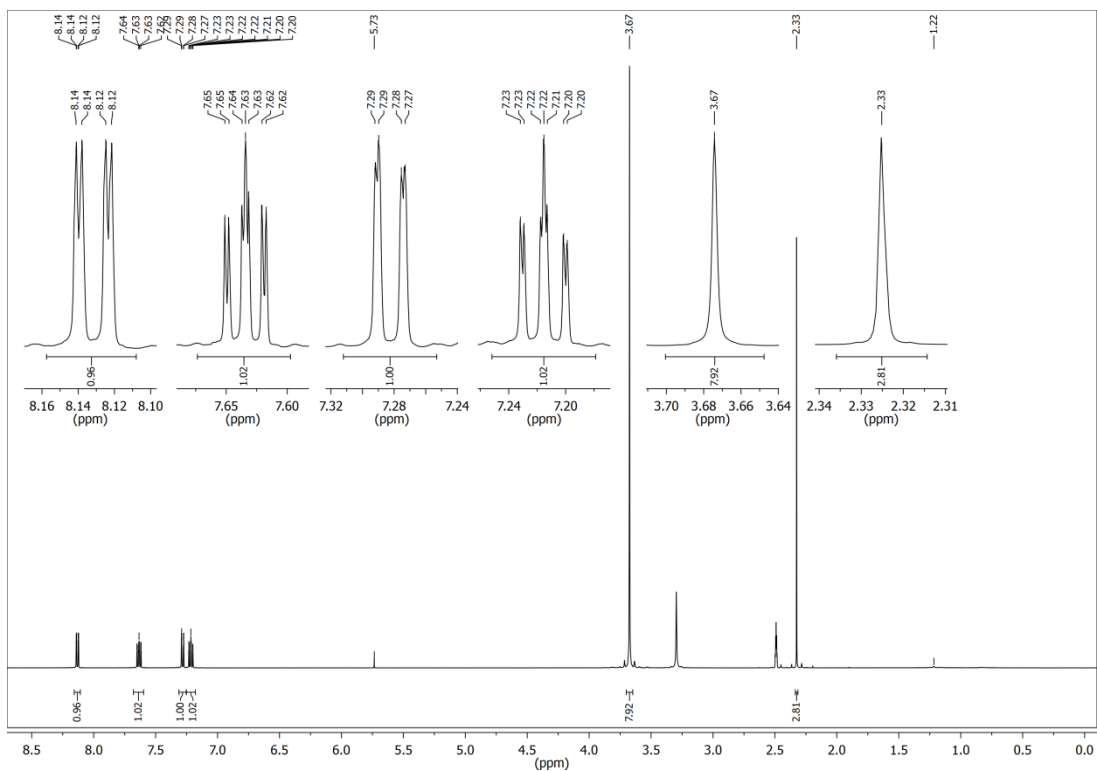
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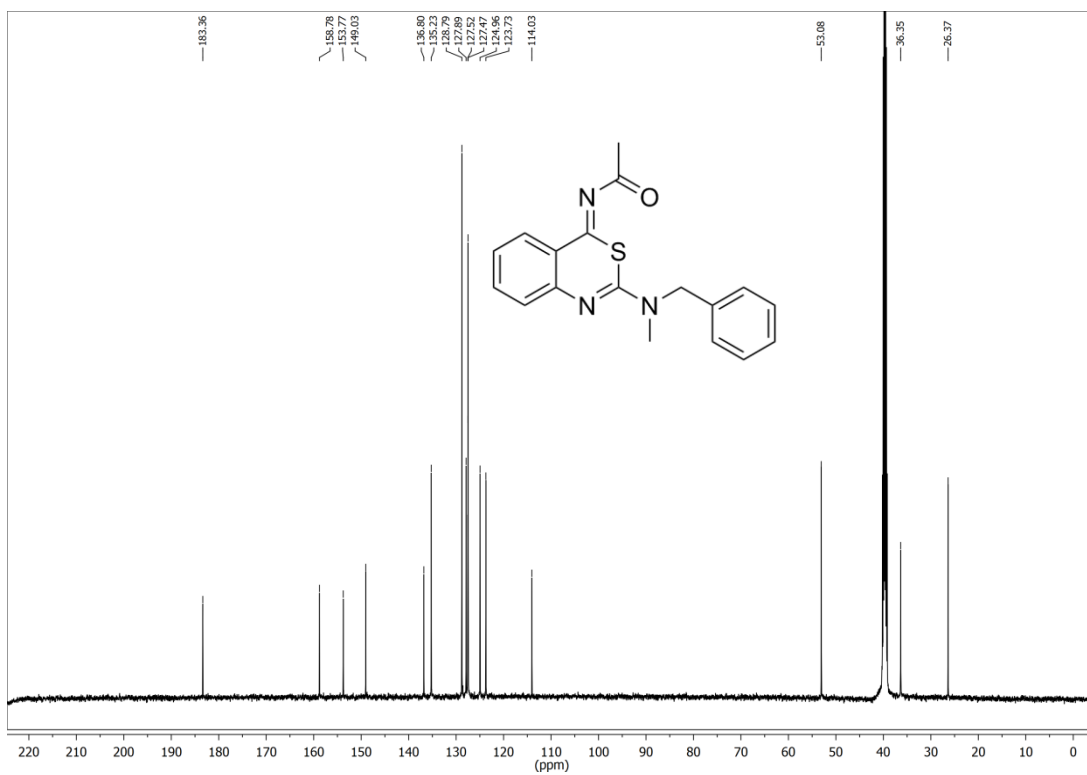
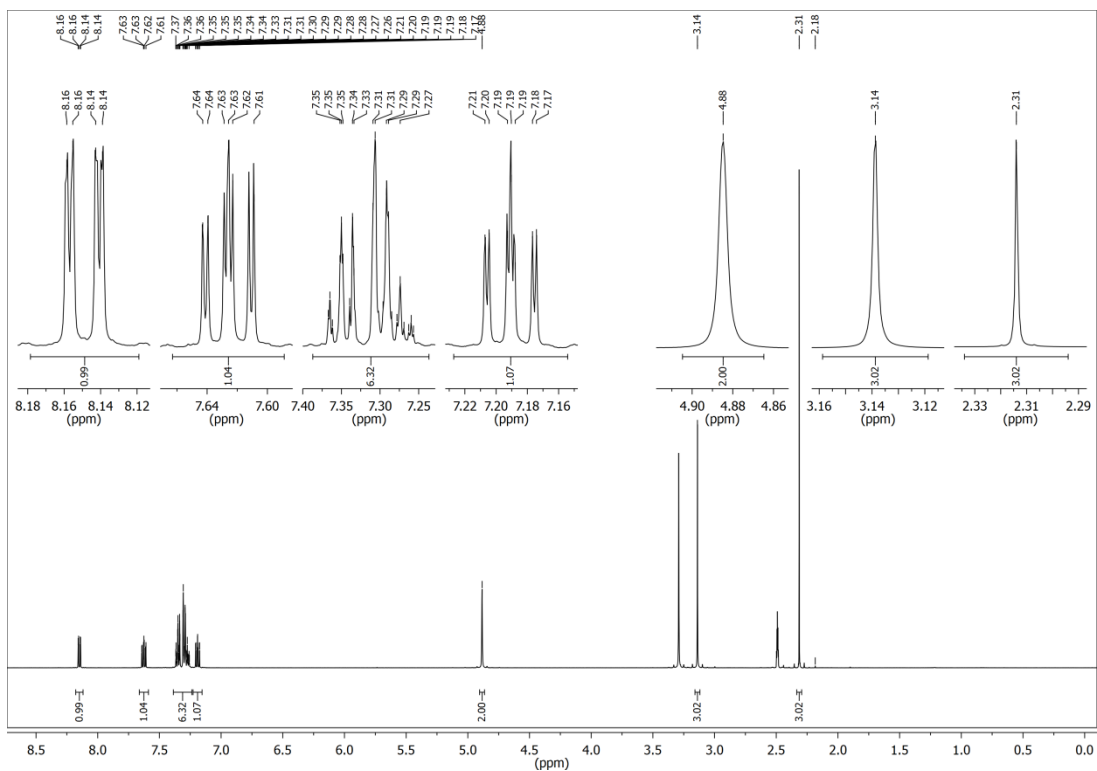
4-Acetylimino-2-diethylamino-4H-3,1-benzothiazine (11a), DMSO-*d*₆



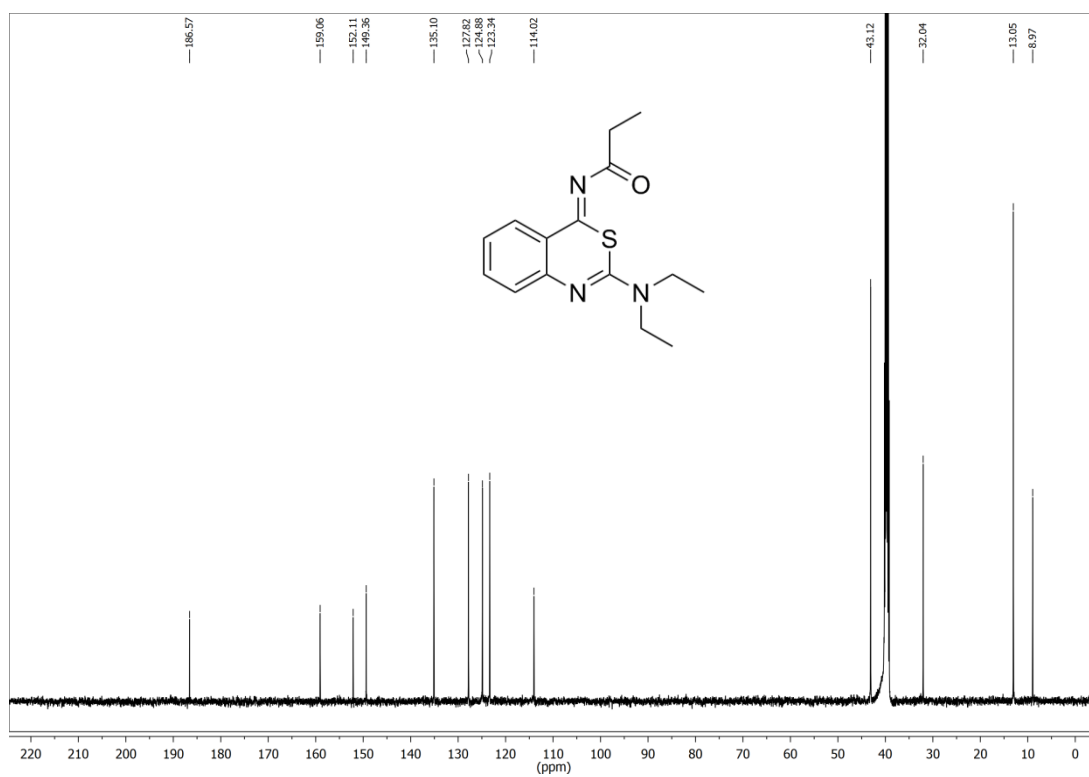
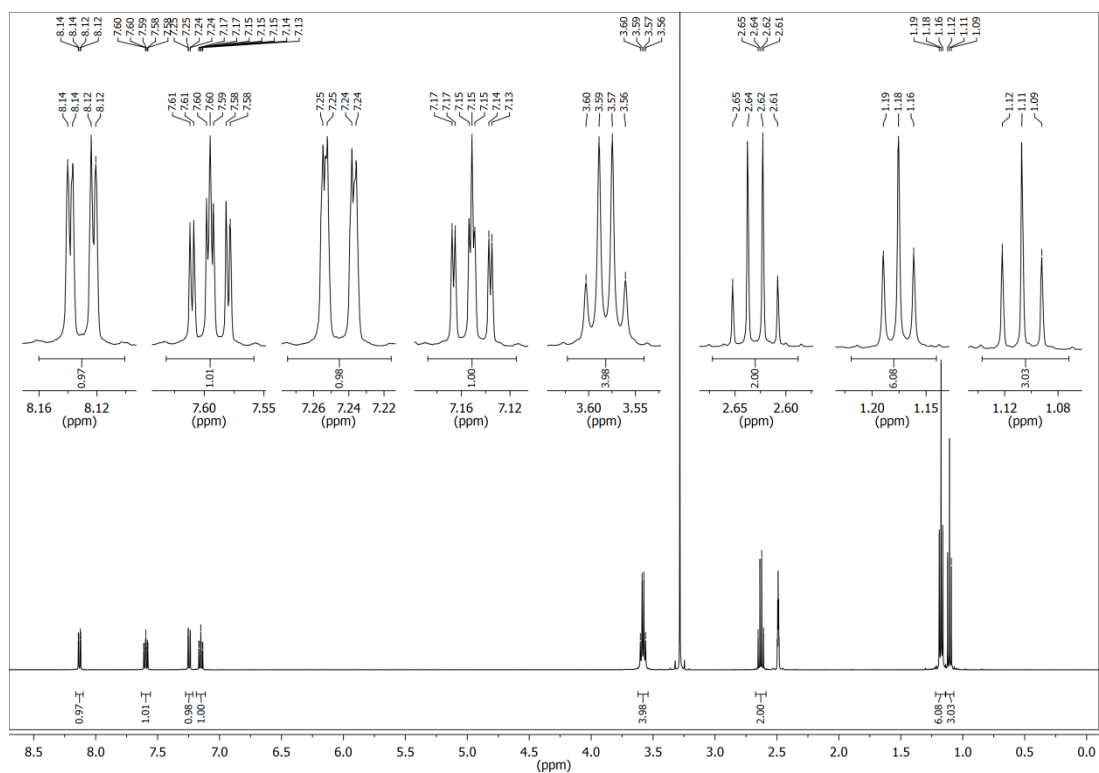
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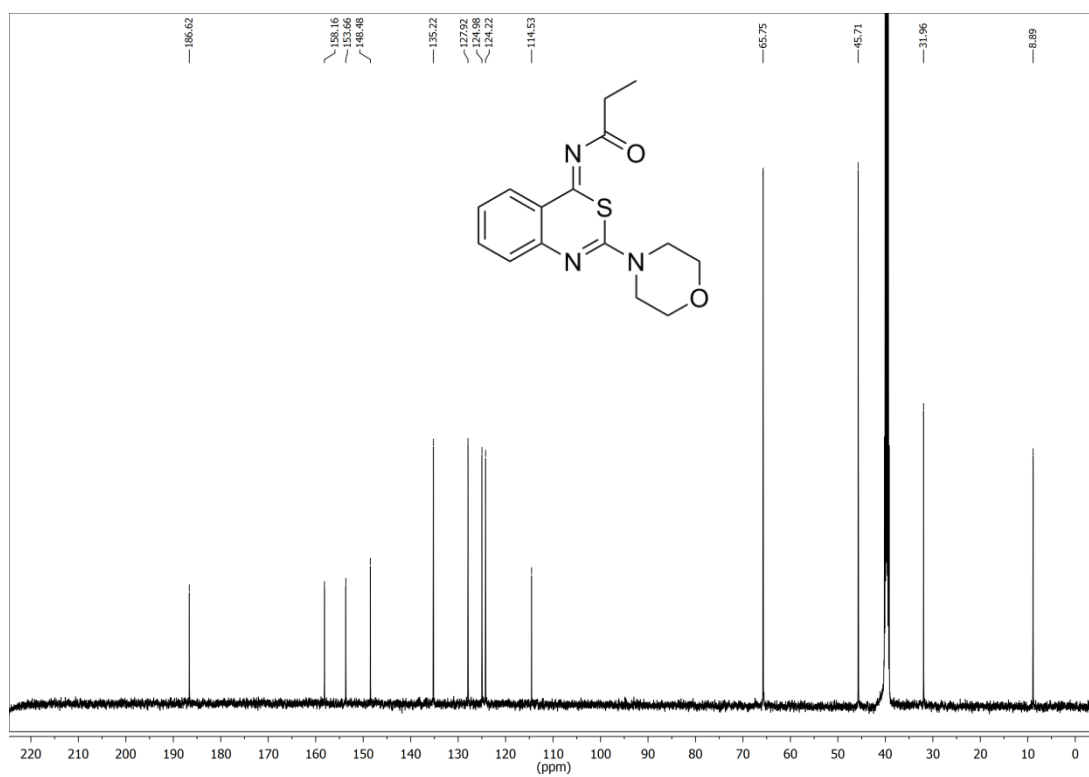
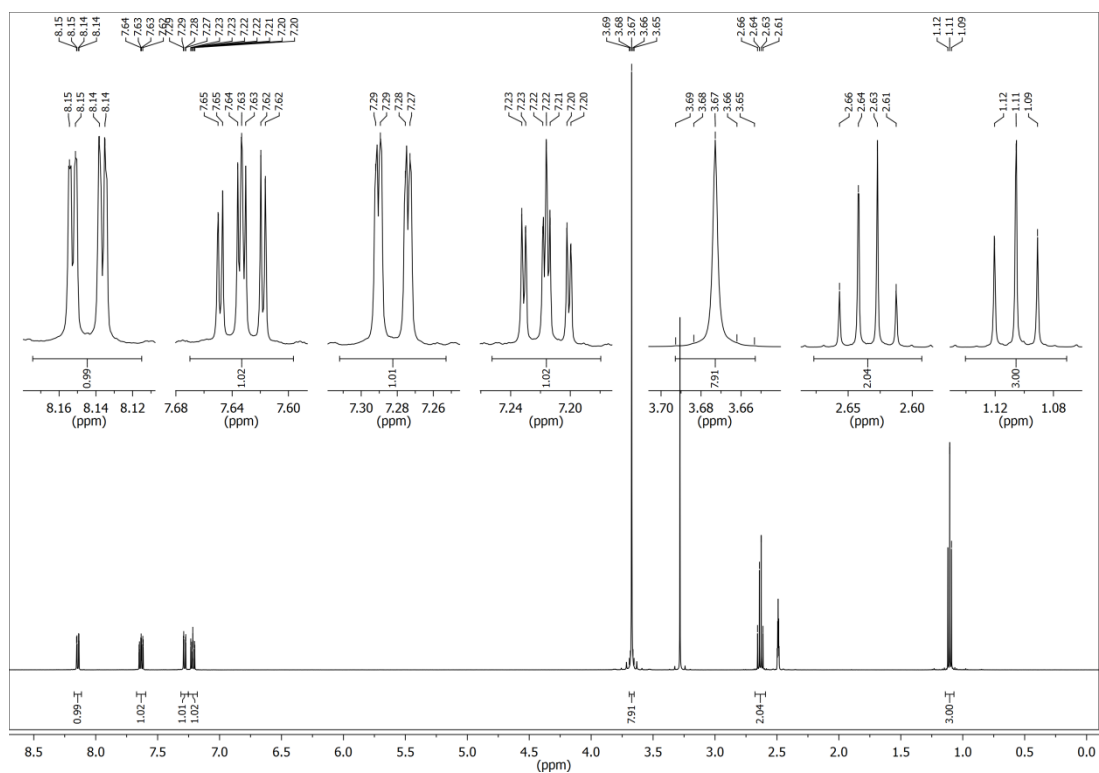
4-Acetylimino-2-benzylmethylamino-4H-3,1-benzothiazine (11c), DMSO-*d*₆



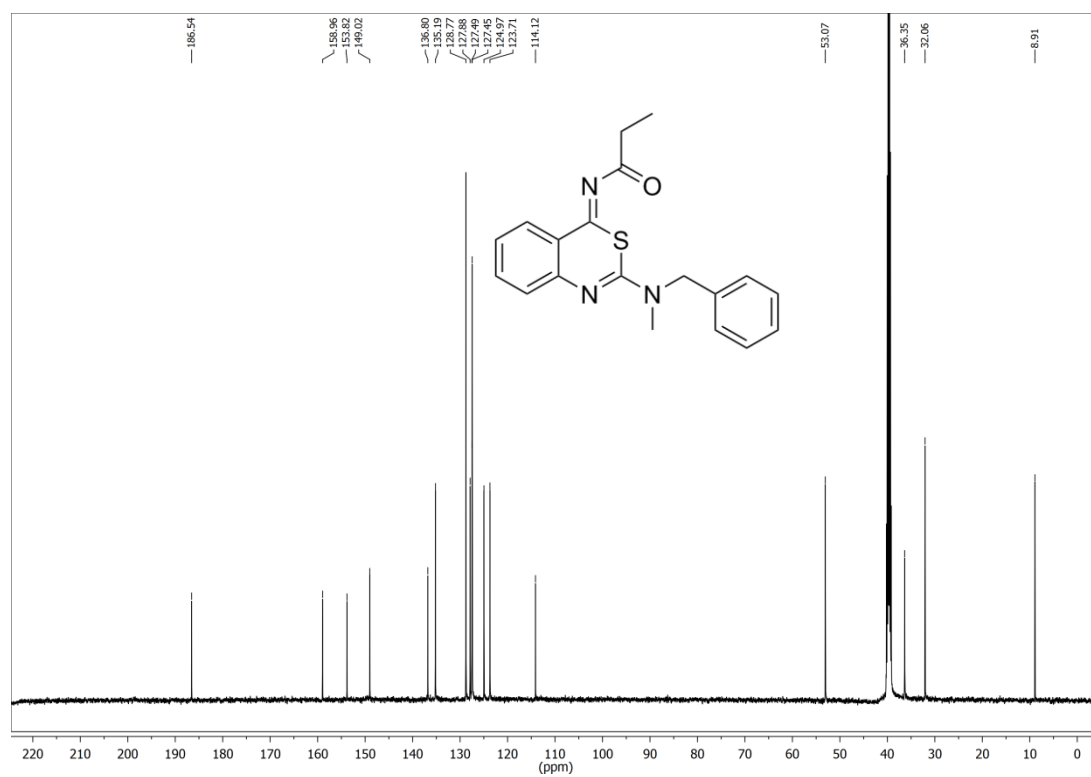
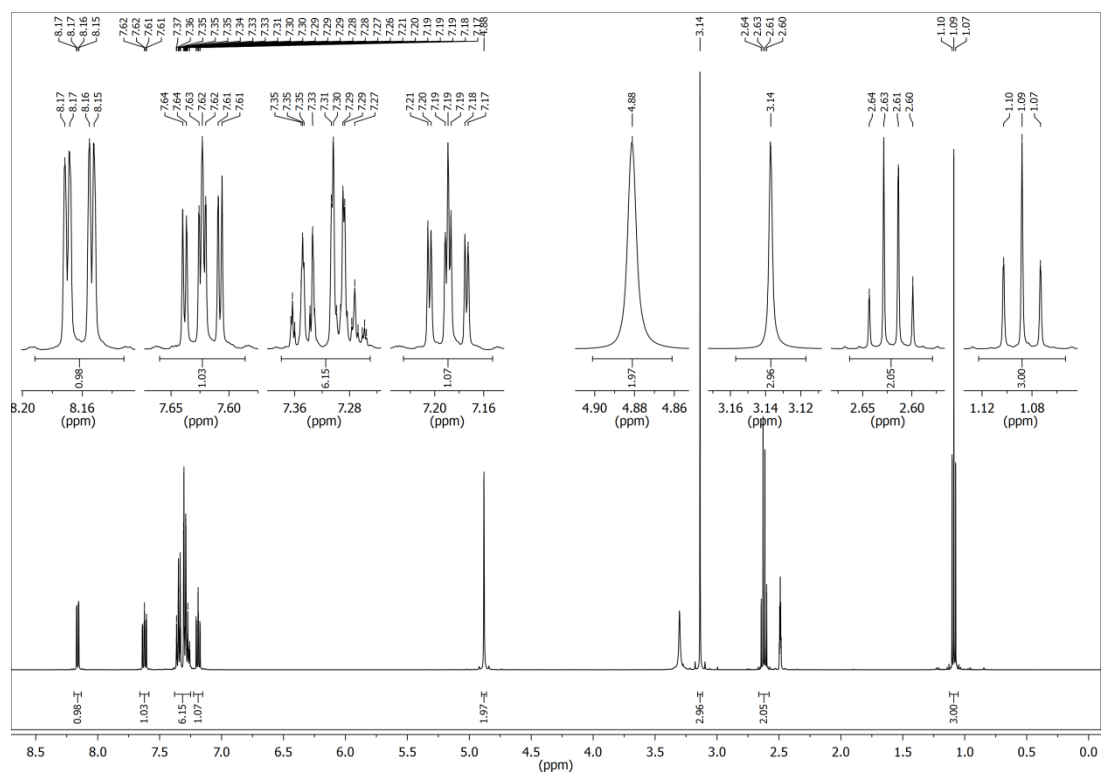
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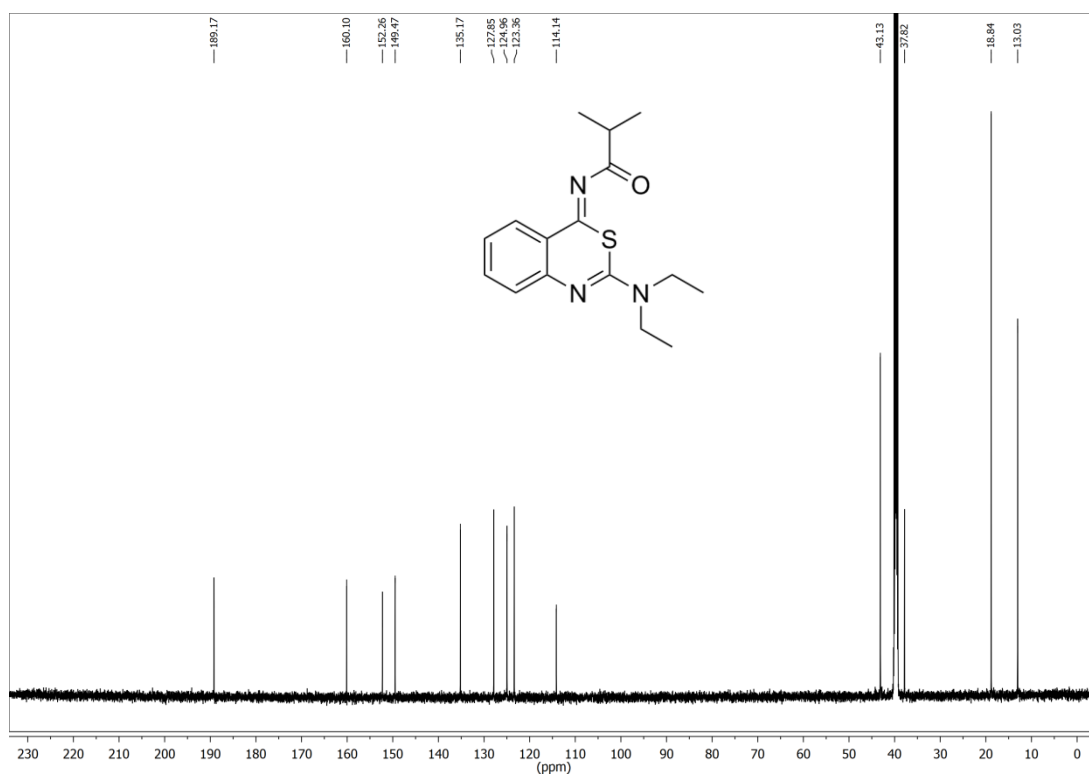
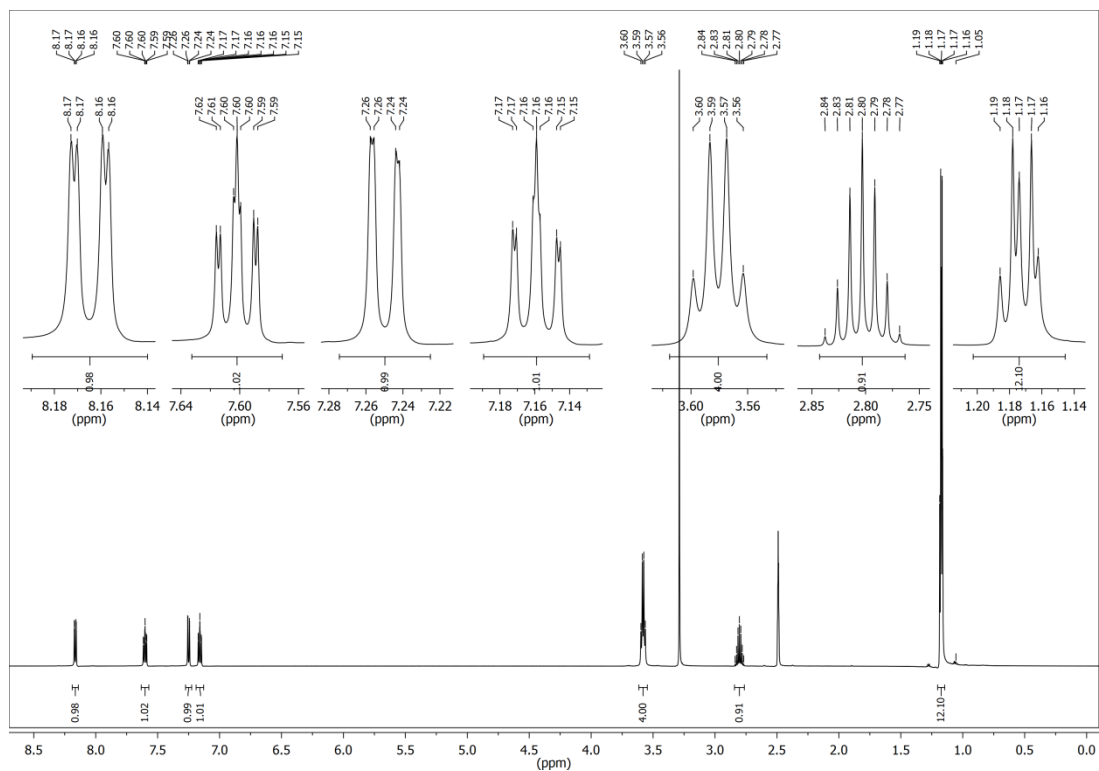
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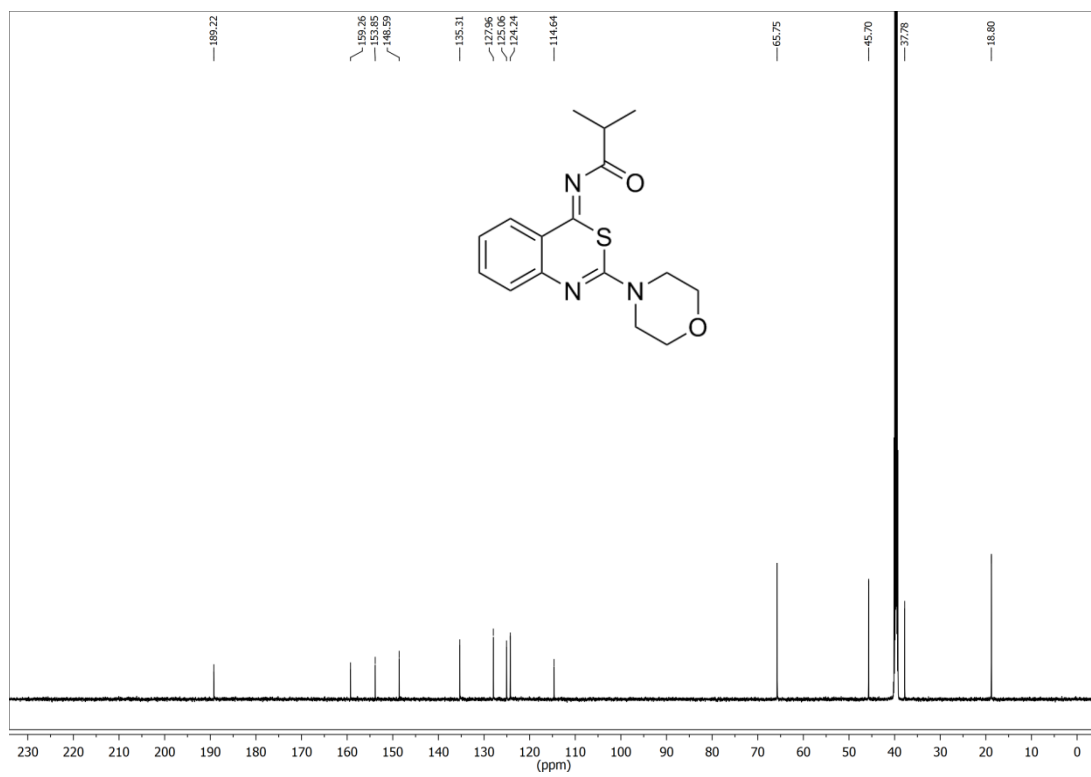
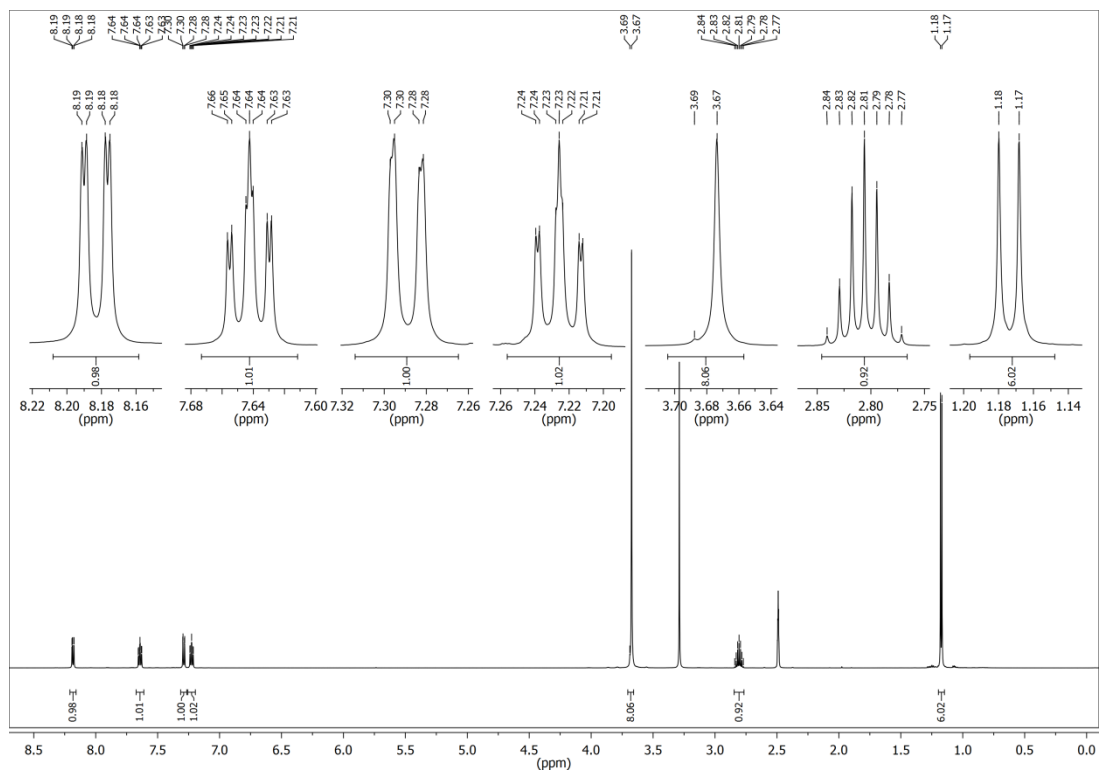
2-Benzylmethylamino-4-propionylimino-4H-3,1-benzothiazine (12c), DMSO-*d*₆



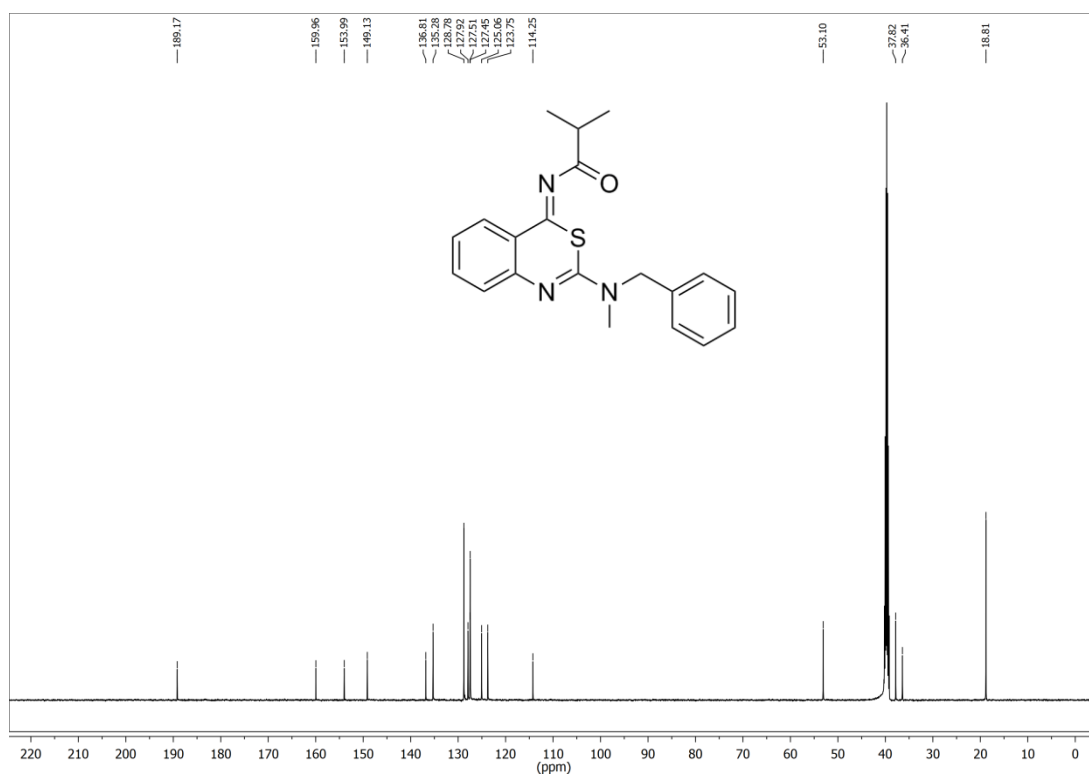
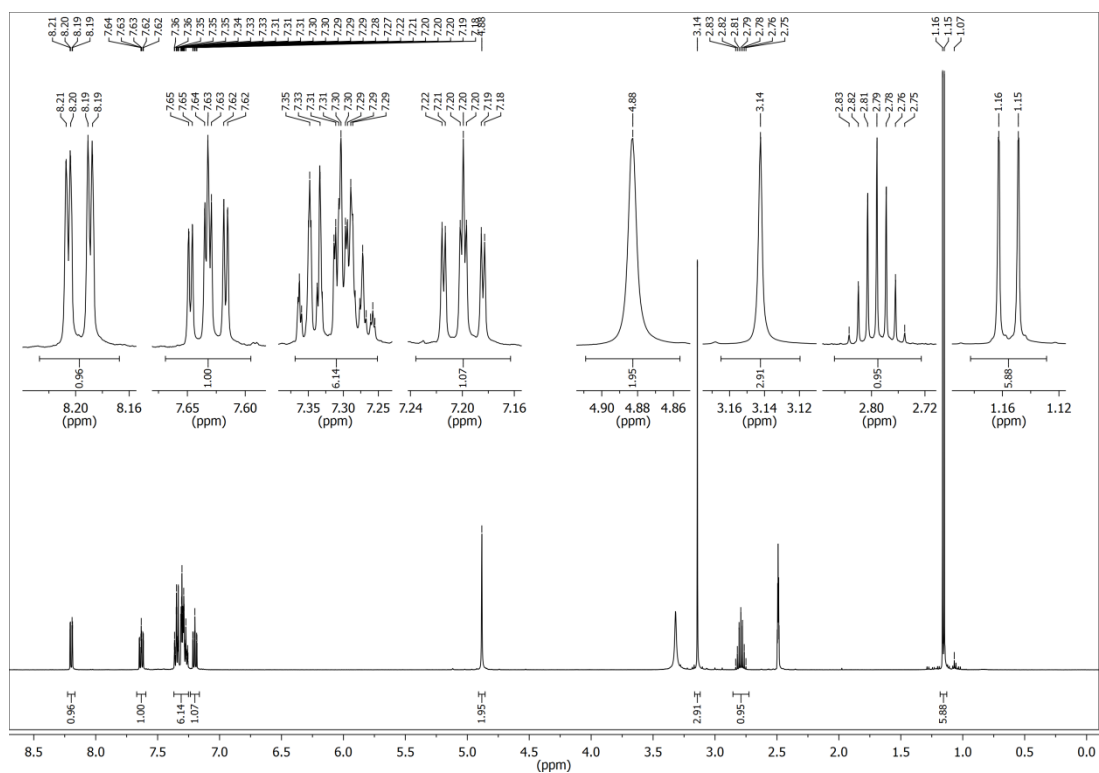
2-Diethylamino-4-isobutyrylimino-4H-3,1-benzothiazine (13a), DMSO-*d*₆



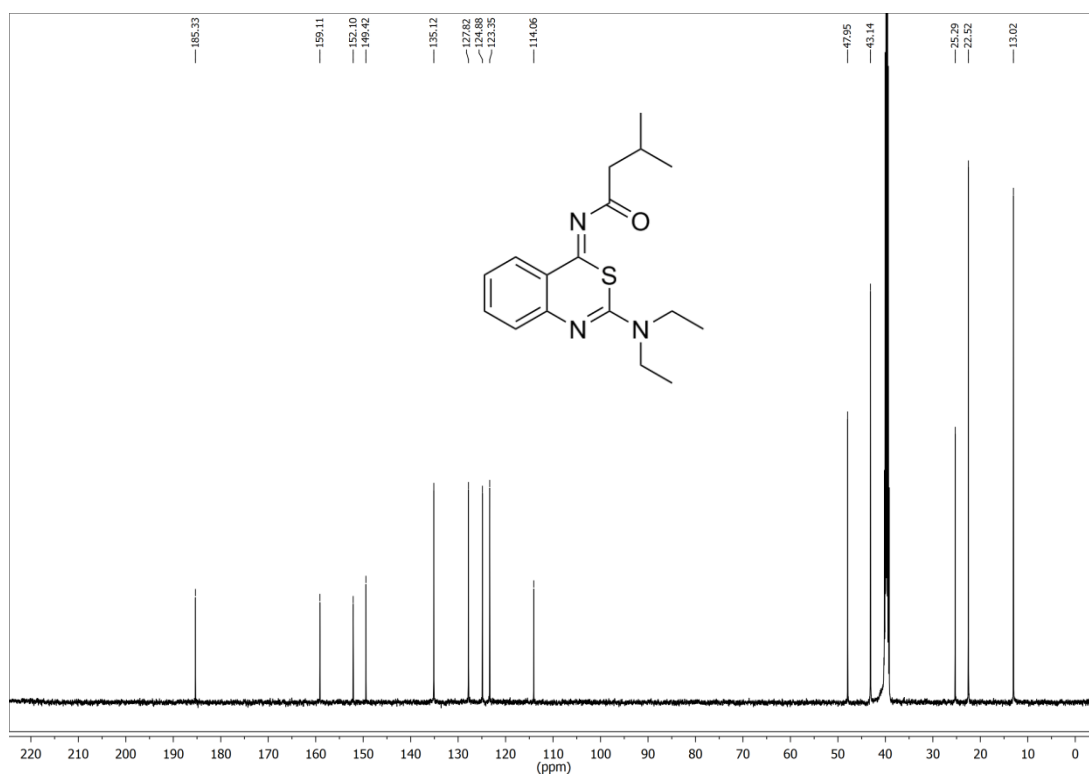
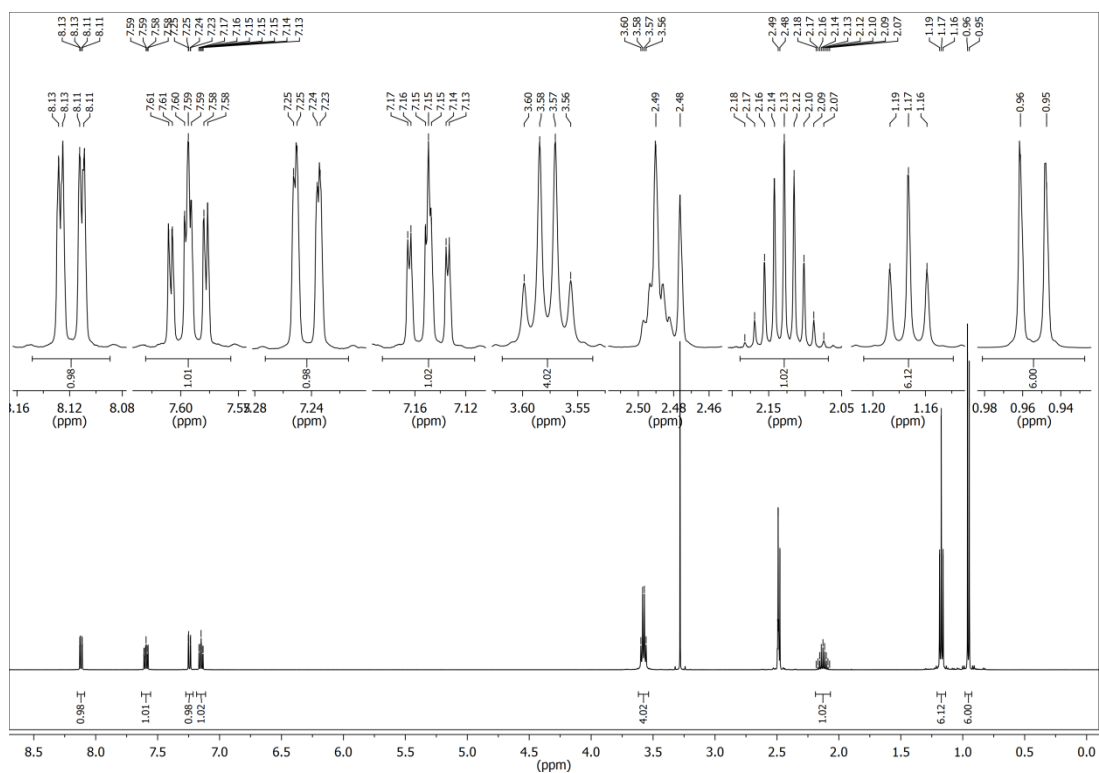
4-Isobutyrylimino-2-morpholino-4*H*-3,1-benzothiazine (13b), DMSO-*d*₆



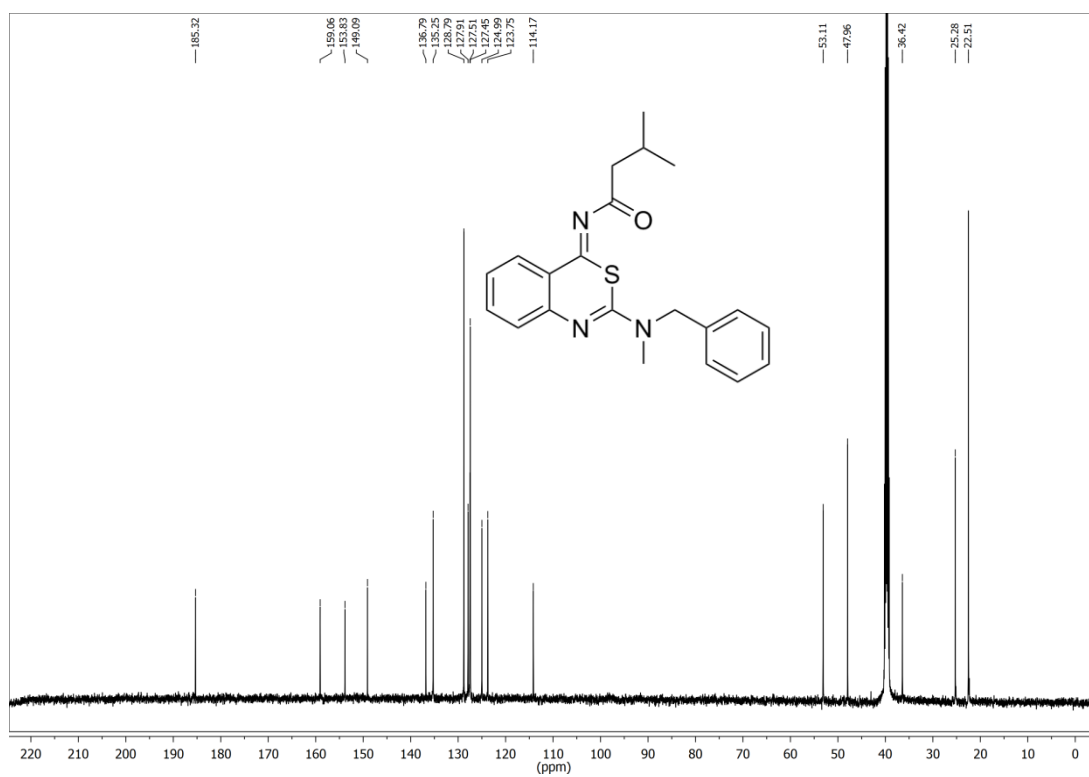
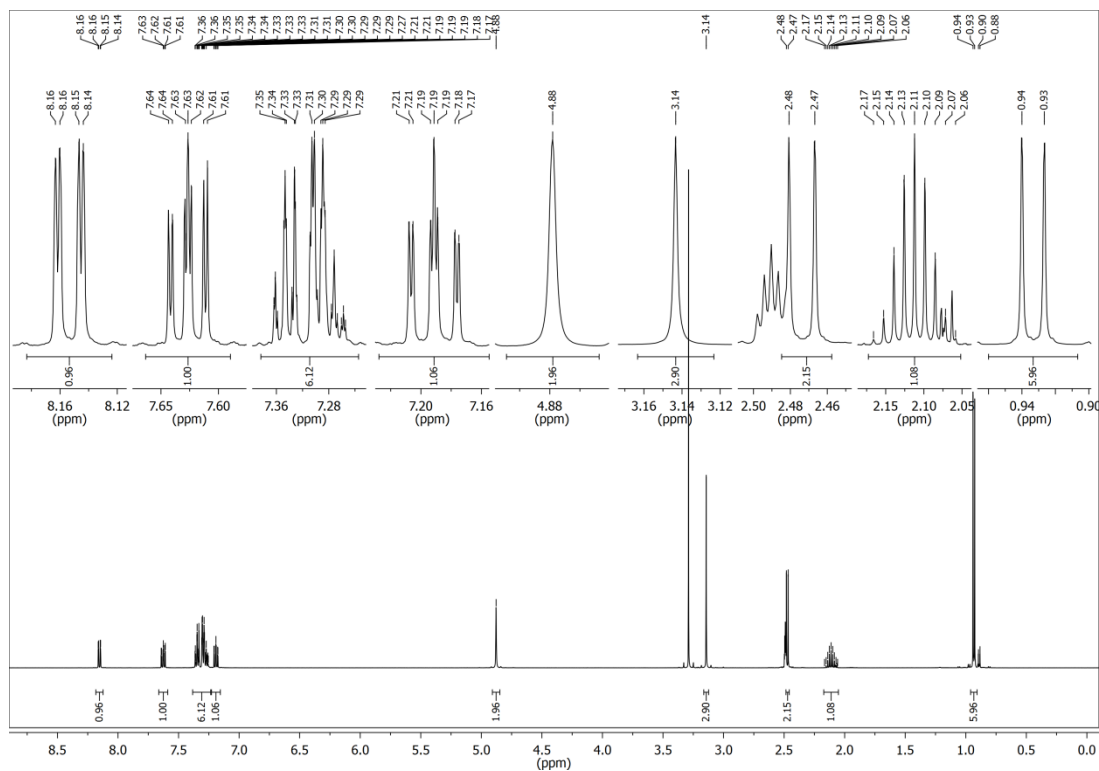
2-Benzylmethylamino-4-isobutyrylimino-4H-3,1-benzothiazine (13c), DMSO-*d*₆



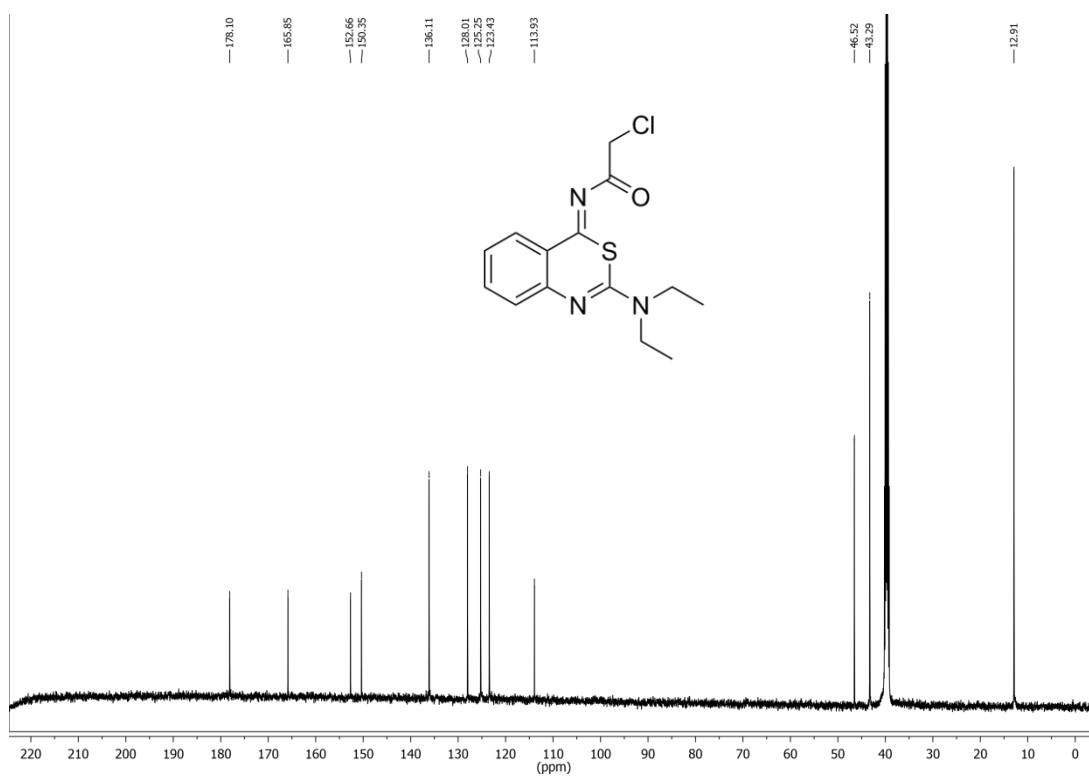
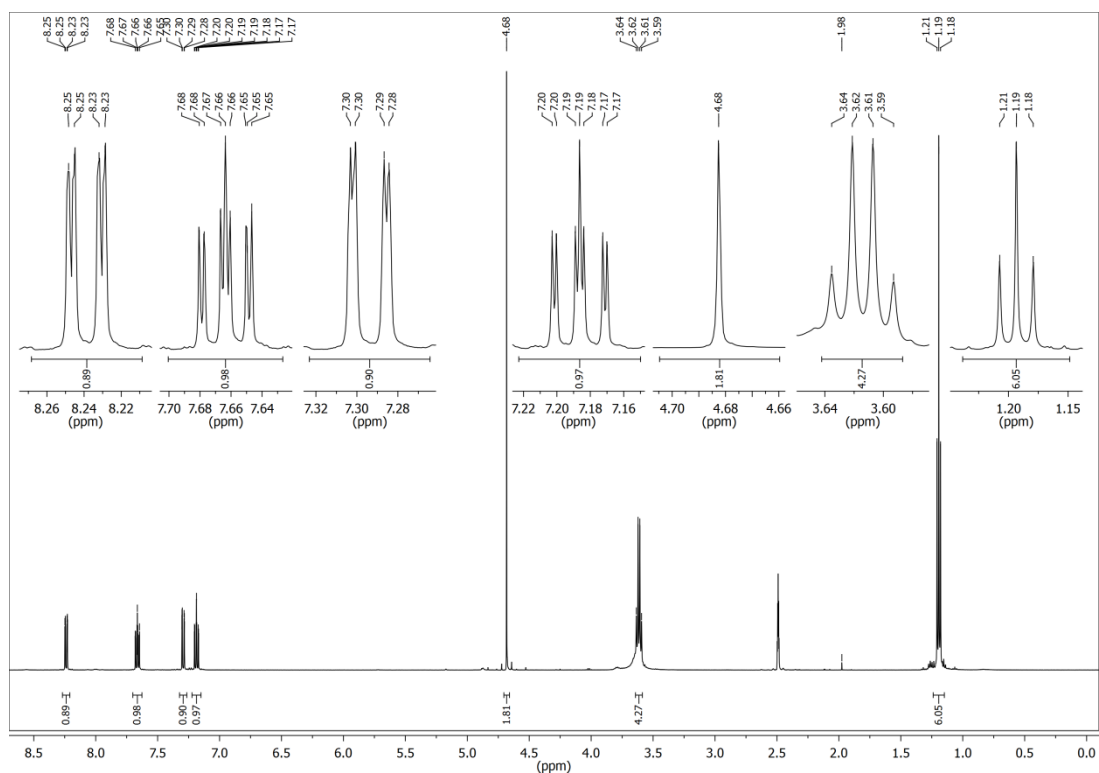
2-Diethylamino-4-(3-methylbutyrylimino)-4H-3,1-benzothiazine (14a), DMSO-*d*₆



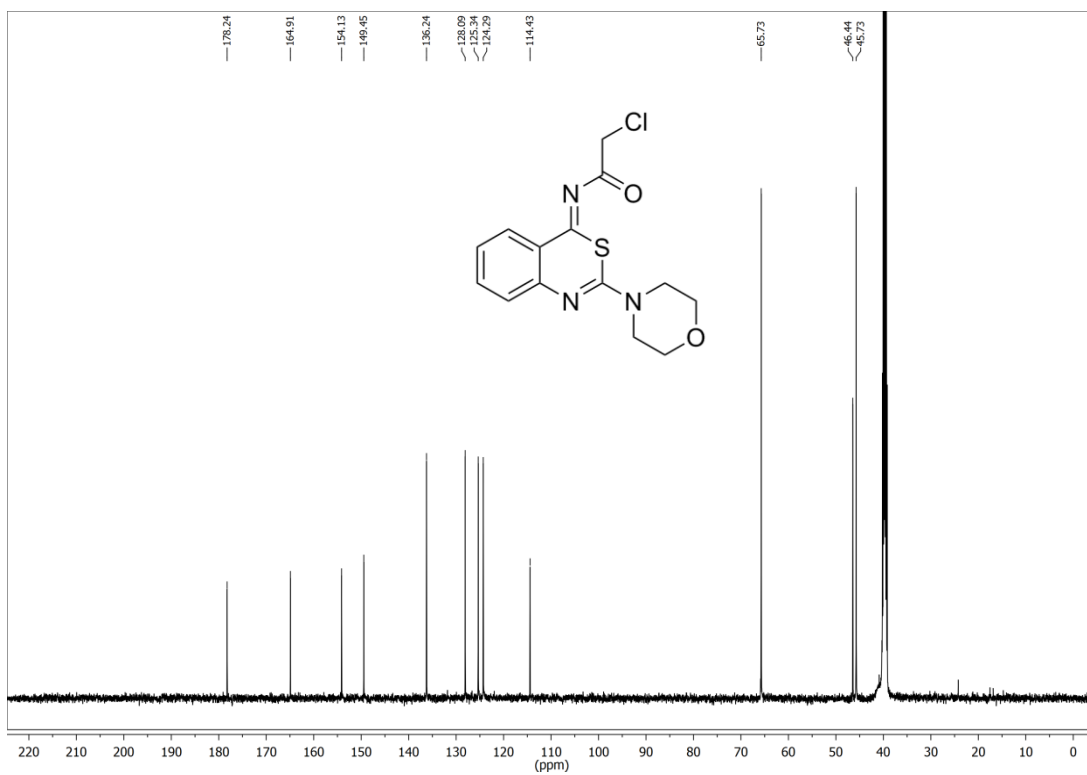
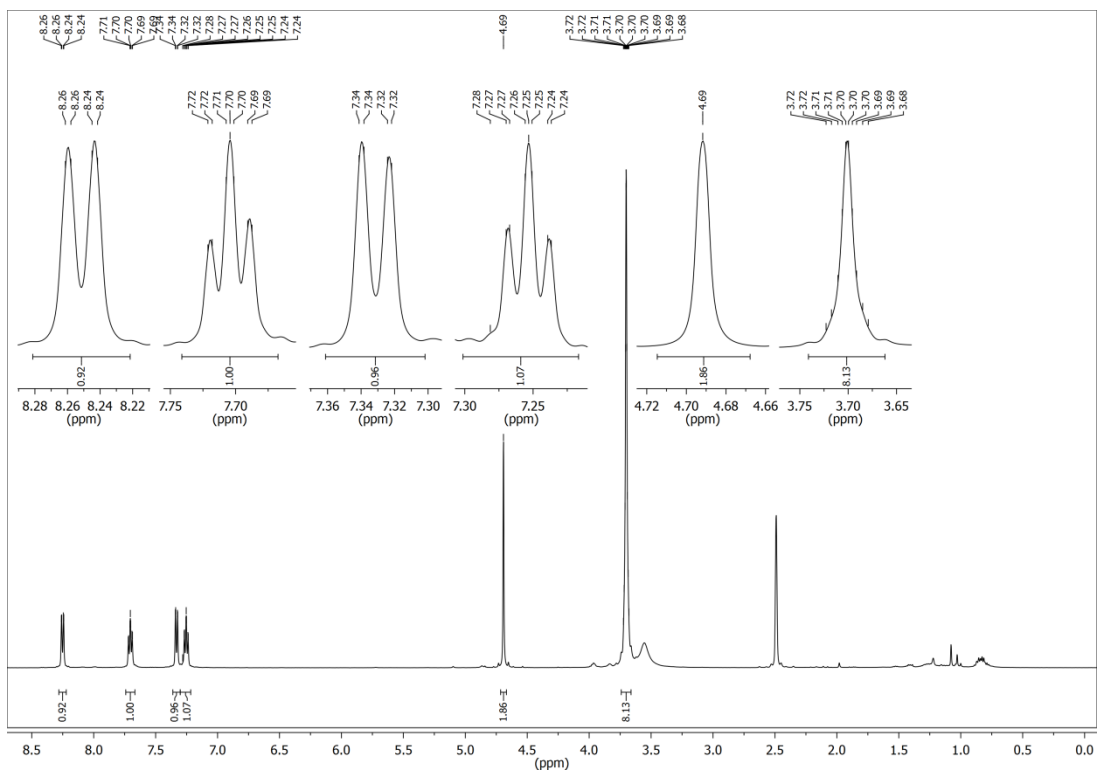
2-Benzylmethylamino-4-(3-methylbutyrylimino)-4H-3,1-benzothiazine (14c), DMSO-*d*₆



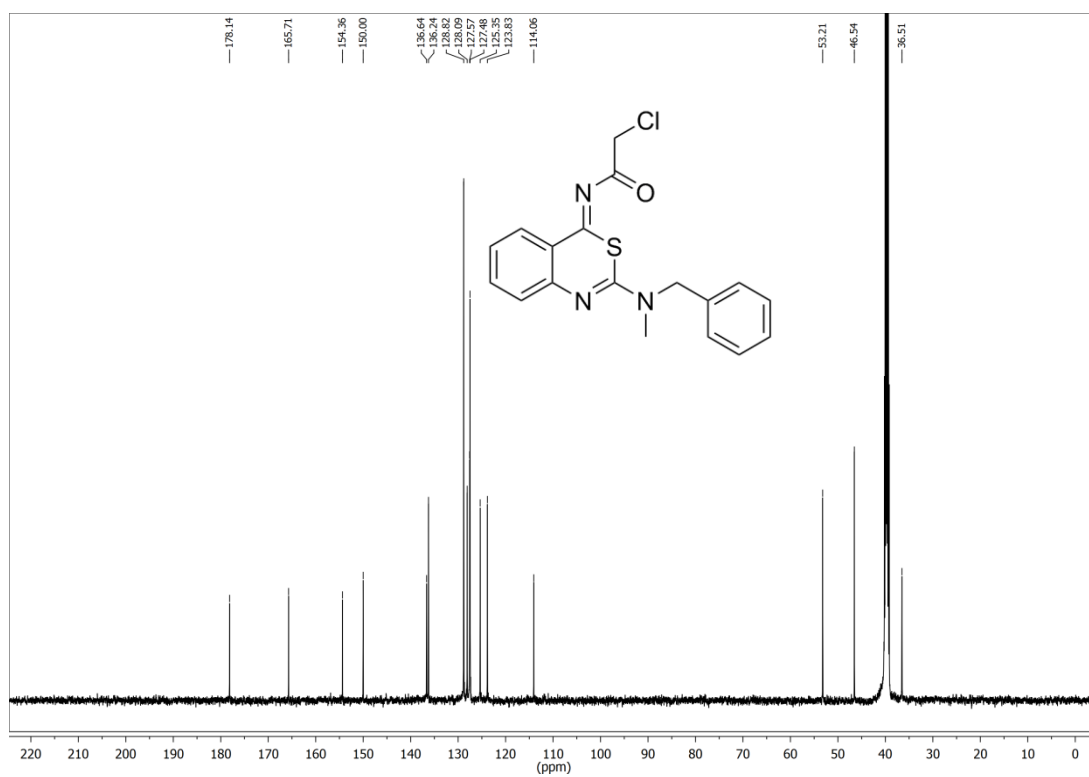
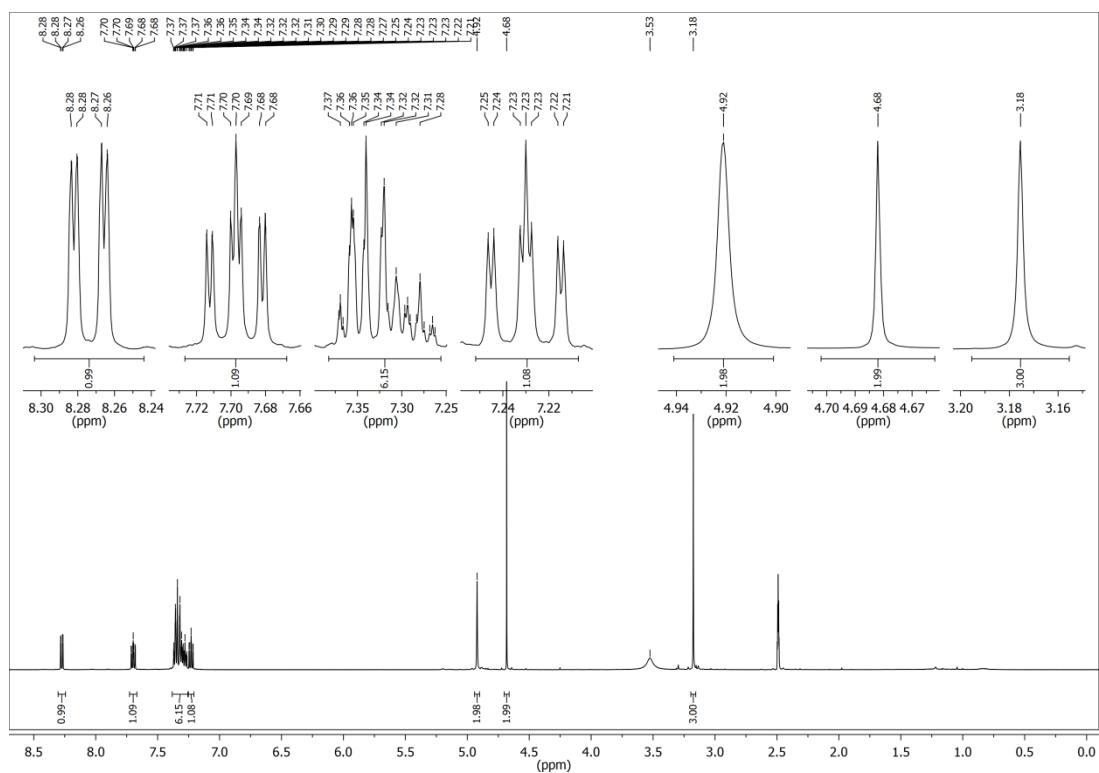
4-Chloroacetylimino-2-diethylamino-4H-3,1-benzothiazine (15a), DMSO-*d*₆



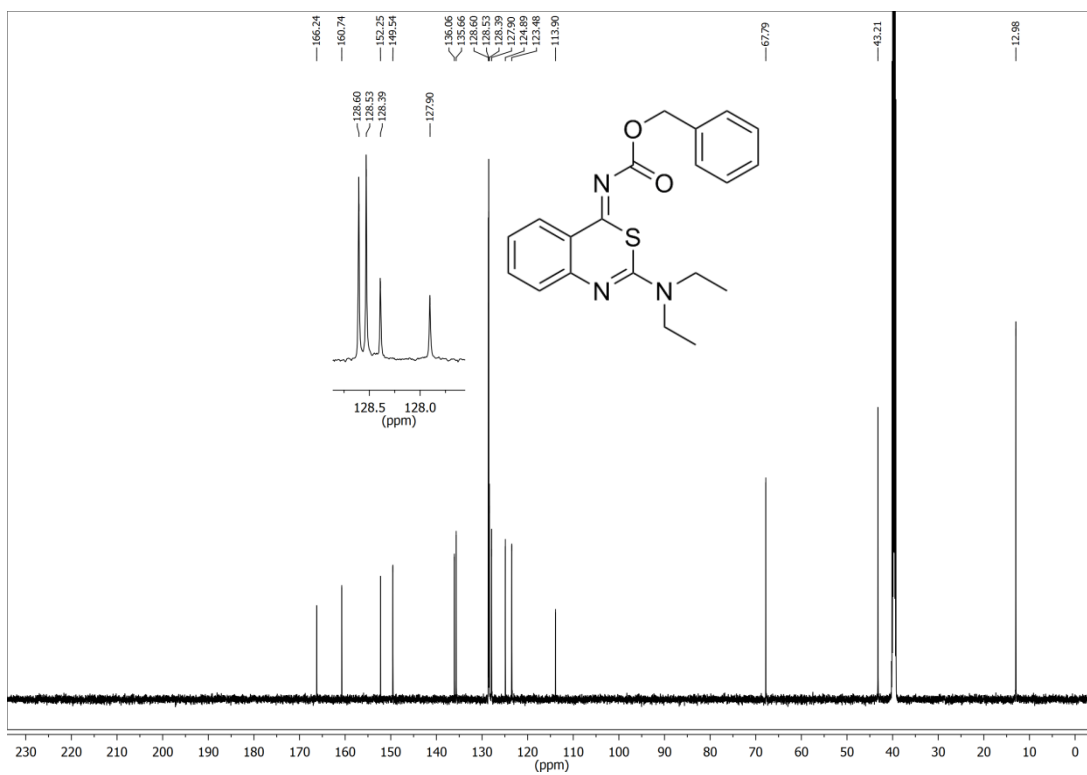
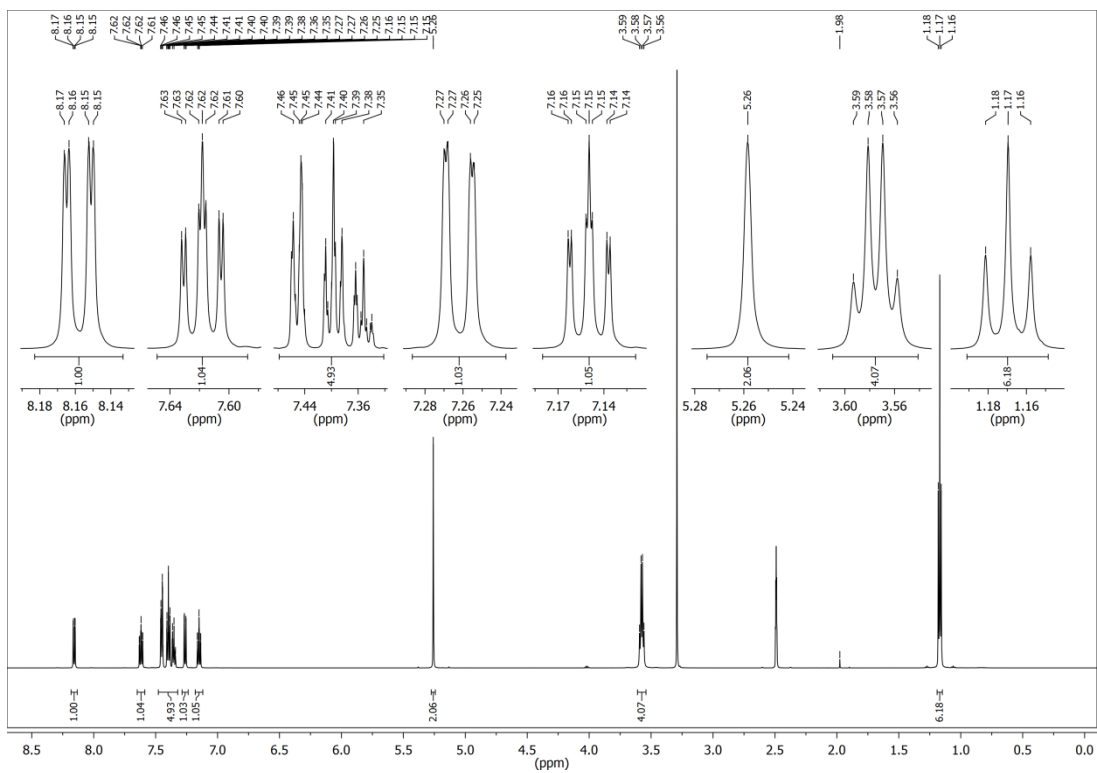
4-Chloroacetylimino-2-morpholino-4H-3,1-benzothiazine (15b), DMSO-*d*₆



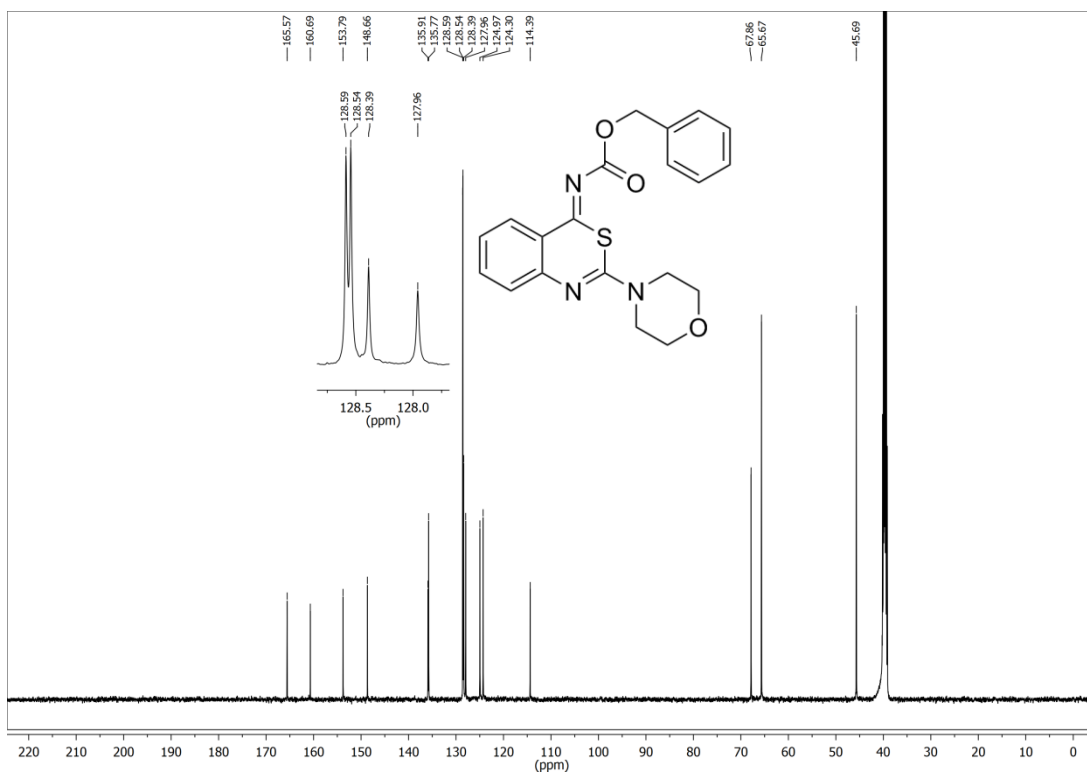
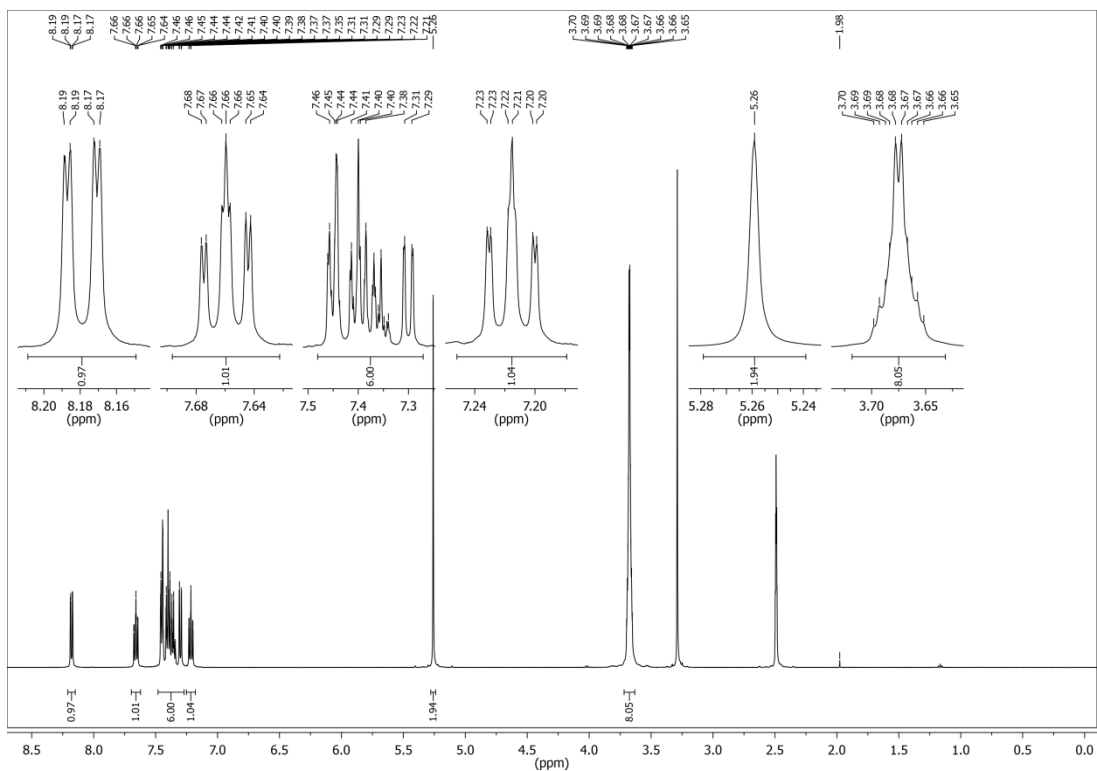
2-Benzylmethylamino-4-chloroacetylmino-4H-3,1-benzothiazine (15c), DMSO-*d*₆



4-Benzyloxycarbonylimino-2-diethylamino-4H-3,1-benzothiazine (16a), DMSO-*d*₆



4-Benzoyloxycarbonylimino-2-morpholino-4*H*-3,1-benzothiazine (16b), DMSO-*d*₆



2-Benzylmethylamino-4-benzoyloxycarbonylimino-4H-3,1-benzothiazine (16c), DMSO-*d*₆

