

## Supplementary Material for

### Structure Elucidation and Synthesis of Hydroxylated Isatins from Streptomyces<sup>‡</sup>

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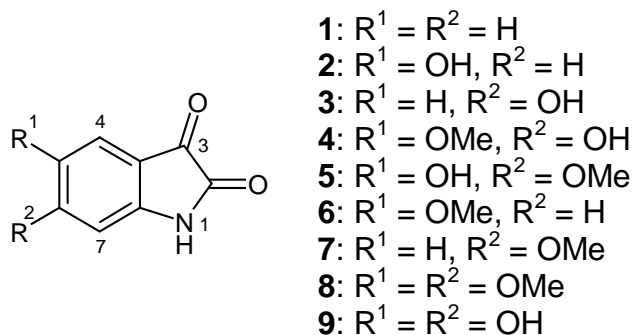


Figure S1: Chemical Structures of Compounds **1-9**

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<sup>‡</sup> Art. No. XLVIII on Marine Bacteria. Art. no. XLVII: V. Nair, I. Schuhmann, H. Anke, G. Kelter, H.-H. Fiebig, E. Helmke, H. Laatsch, Psychrotolerant bacteria from extreme Antarctic habitats as producers of rare bis- and trisindole alkaloids. *Planta Med.* **2016**, 82, 910-918.

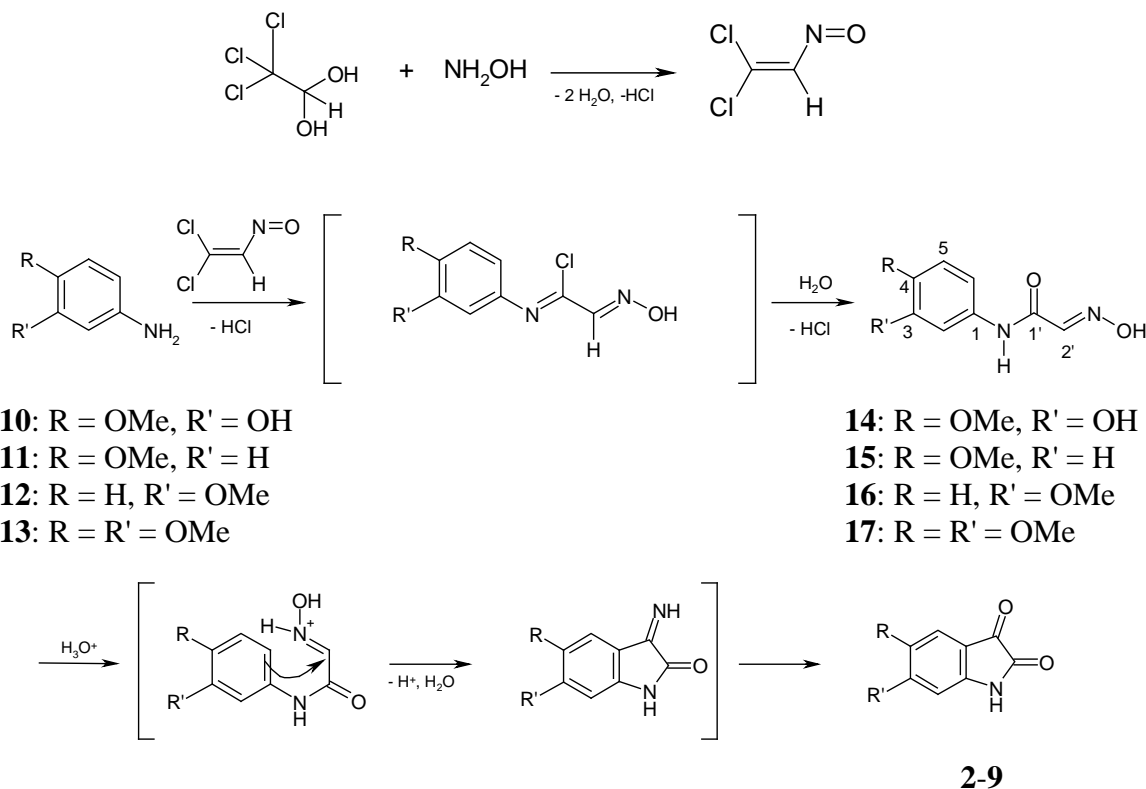
## Content

<b>Isatin syntheses .....</b>	<b>4-8</b>
<b>Additional References .....</b>	<b>9</b>
<b>Table S1. <sup>1</sup>H NMR data of oxygenated isatins; shifts as <math>\delta</math> values, coupling constants in [Hz].....</b>	<b>10</b>
<b>Table S2. <sup>13</sup>C NMR data of oxygenated isatins; shifts as <math>\delta</math> values.....</b>	<b>10</b>
<b>List of Chemical Structures and of Spectra .....</b>	<b>2-3</b>
<b>Figure S1: Chemical structures of compounds 1-9 .....</b>	<b>1</b>
<b>Figure S2: The synthesis of isatins from anilines, chloralhydrate, and hydroxylamine . .....</b>	<b>4</b>
<b>Figure S3: Crystal structure of 5-hydroxy-6-methoxyisatin (5).....</b>	<b>7</b>
<b>Figure S4: <sup>1</sup>H NMR spectrum (300 MHz) of 3,4-dimethoxyisonitrosoacetanilide (17) in DMSO-<i>d</i><sub>6</sub>.....</b>	<b>11</b>
<b>Figure S5: Exp. UV/Vis spectrum of 5-hydroxy-isatin (2) in methanol, neutral .....</b>	<b>12</b>
<b>Figure S6: Exp. UV/Vis spectrum of 5-hydroxy-isatin (2) in methanol, basic .....</b>	<b>13</b>
<b>Figure S7: UV/Vis spectra of 5-hydroxy-isatin (2).....</b>	<b>14</b>
<b>Figure S8: <sup>1</sup>H NMR spectrum (300 MHz) of 5-hydroxy-isatin (2) in methanol-<i>d</i><sub>4</sub>.....</b>	<b>15</b>
<b>Figure S9: <sup>1</sup>H NMR spectrum (300 MHz) of 5-hydroxy-isatin (2) in DMSO-<i>d</i><sub>6</sub>.....</b>	<b>16</b>
<b>Figure S10: <sup>13</sup>C NMR spectrum (125 MHz) of 5-hydroxy-isatin (2) in DMSO-<i>d</i><sub>6</sub>.....</b>	<b>17</b>
<b>Figure S11: HMBC spectrum (600 MHz) of 5-hydroxy-isatin (2) in DMSO-<i>d</i><sub>6</sub>.....</b>	<b>18</b>
<b>Figure S12: Exp. UV/Vis spectrum of 6-hydroxy-isatin (3) in methanol .....</b>	<b>19</b>
<b>Figure S13: Calculated UV/Vis spectrum of 6-hydroxy-isatin (3) .....</b>	<b>20</b>
<b>Figure S14: <sup>1</sup>H NMR spectrum (300 MHz) of 6-hydroxy-isatin (3) in DMSO-<i>d</i><sub>6</sub>.....</b>	<b>21</b>
<b>Figure S15: <sup>13</sup>C NMR spectrum (125 MHz) of 6-hydroxy-isatin (3) in DMSO-<i>d</i><sub>6</sub>.....</b>	<b>22</b>
<b>Figure S16: HMBC spectrum (300 MHz) of 6-hydroxy-isatin (3) in DMSO-<i>d</i><sub>6</sub>.....</b>	<b>23</b>
<b>Figure S17: Exp. UV/Vis spectrum of 6-hydroxy-5-methoxy-isatin (4) in methanol.....</b>	<b>24</b>
<b>Figure S18: Calculated UV/Vis spectrum of 6-hydroxy-5-methoxy-isatin (4).....</b>	<b>25</b>
<b>Figure S19: <sup>1</sup>H NMR spectrum (600 MHz) of 6-hydroxy-5-methoxy-isatin (4) in methanol-<i>d</i><sub>4</sub>.....</b>	<b>26</b>
<b>Figure S20: <sup>1</sup>H NMR spectrum (300 MHz) of 6-hydroxy-5-methoxy-isatin (4) in DMSO-<i>d</i><sub>6</sub>.....</b>	<b>27</b>

<b>Figure S21:</b> $^{13}\text{C}$ NMR spectrum (125 MHz) of 6-hydroxy-5-methoxy-isatin ( <b>4</b> ) in $\text{DMSO-}d_6$ .	28
<b>Figure S22:</b> HMBC spectrum (600 MHz) of 6-hydroxy-5-methoxy-isatin ( <b>4</b> ) in $\text{DMSO-}d_6$ .	29
<b>Figure S23:</b> $^1\text{H}$ NMR spectrum (300 MHz) of 6-hydroxy-5-methoxy-isatin ( <b>4</b> ) + ethyl-diisopropylamine.	30
<b>Figure S24:</b> Exp. UV/Vis spectrum of 5-hydroxy-6-methoxy-isatin ( <b>5</b> ) in methanol.	31
<b>Figure S25:</b> Enlarged section of the exp. UV/Vis spectrum of 5-hydroxy-6-methoxy-isatin ( <b>5</b> ) in methanol.	32
<b>Figure S26:</b> Calculated UV/Vis spectrum of 5-hydroxy-6-methoxy-isatin ( <b>5</b> ).	33
<b>Figure S27:</b> $^1\text{H}$ NMR spectrum (300 MHz) of 5-hydroxy-6-methoxy-isatin ( <b>5</b> ) in $\text{DMSO-}d_6$ .	34
<b>Figure S28:</b> $^{13}\text{C}$ NMR spectrum (75 MHz) of 5-hydroxy-6-methoxy-isatin ( <b>5</b> ) in $\text{DMSO-}d_6$ .	35
<b>Figure S29:</b> HMBC spectrum (300 MHz) of 5-hydroxy-6-methoxy-isatin ( <b>5</b> ) in $\text{DMSO-}d_6$ .	36
<b>Figure S30:</b> $^1\text{H}$ NMR spectrum (300 MHz) of 5-methoxy-isatin ( <b>6</b> ) in $\text{DMSO-}d_6$ .	37
<b>Figure S31:</b> $^{13}\text{C}$ NMR spectrum (125 MHz) of 5-methoxy-isatin ( <b>6</b> ) in $\text{DMSO-}d_6$ .	38
<b>Figure S32:</b> $^1\text{H}$ NMR spectrum (300 MHz) of 6-methoxy-isatin ( <b>7</b> ) in $\text{DMSO-}d_6$ .	39
<b>Figure S33:</b> $^{13}\text{C}$ NMR spectrum (125 MHz) of 6-methoxy-isatin ( <b>7</b> ) in $\text{DMSO-}d_6$ .	40
<b>Figure S34:</b> $^1\text{H}$ NMR spectrum (300 MHz) of 5,6-dimethoxy-isatin ( <b>8</b> ) in $\text{DMSO-}d_6$ .	41
<b>Figure S35:</b> $^{13}\text{C}$ NMR spectrum (125 MHz) of 5,6-dimethoxy-isatin ( <b>8</b> ) in $\text{DMSO-}d_6$ .	42
<b>Figure S36:</b> $^1\text{H}$ NMR spectrum of 5,6-dihydroxy-isatin ( <b>9</b> ) in methanol- $d_4$ .	43

## Isatin syntheses

For additional investigations and further confirmation of the structures, 5- and 6-hydroxyisatin (**2/3**), 5-hydroxy-6-methoxyisatin (**5**) and 6-hydroxy-5-methoxyisatin (**4**) were synthesized according to Sandmeyer's procedure [1]: Condensation of anilines with chloral hydrate and hydroxyl amine delivered the corresponding isonitrosoacetanilides, which cyclized in sulfuric acid at elevated temperature to yield the corresponding isatins. Demethylation of 5- and 6-methoxyisatin (**6** and **7**) using boron-tribromide gave the hydroxy-derivatives **2** and **3** [2], whereas 5,6-dimethoxyisatin (**8**) afforded the isomeric monoethers **5** and **4** in a ratio of 4:1. 5,6-Dihydroxyisatin (**9**) was also formed but was difficult to isolate in pure state, due to its high polarity. 6-Hydroxy-5-methoxyisatin (**4**) was obtained easier by direct cyclisation of 3-hydroxy-4-methoxyanilin (**10**). With exception of **5**, all isatins described here were obtained previously in a similar way; references are given in the following text.



**Figure S2:** The Sandmeyer synthesis of isatins from anilines, chloralhydrate, and hydroxyl-amine.

**Isonitrosoacetanilide derivatives:** A solution of chloralhydrate (8.27 g, 0.05 mole) and 130 g of sodium sulfate in 120 mL water was mixed with 0.05 mol of the aniline derivative, dissolved in 35 mL diluted HCl (1:7). Subsequently, hydroxylamine hydrochloride (10.3 g, 0.15 mol) dissolved in 50 mL water was added and the mixture was heated for 15 min under reflux. The reaction mixture was cooled in ice and the resulting precipitate was separated and washed with cold water. After drying, chromatography of samples of the brown to black crude isonitroso-acetanilides afforded colorless needles; for the cyclisation, the crude products were used.

**3-Hydroxy-4-methoxyisonitrosoacetanilide (14):** Black crystals, 0.96 g (63.6%) from 1.0 g of the aniline **10**, 1.19 g chloral hydrate and 1.48 g hydroxylamine hydrochloride.

**4-Methoxyisonitrosoacetanilide (15):** From the aniline **11**, 3.85 g (39.6%) **15** was obtained as black crystals from ethyl acetate;  $R_f = 0.57$  ( $\text{CH}_2\text{Cl}_2$ / 5%MeOH). –  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ):  $\delta = 12.03$  (s, 1 H, N-OH), 9.98 (s, 1 H, NH), 7.63 (s, 1 H, 2'-H), 7.59 (d,  $J = 8.4$  Hz, 2 H, 2-H, 6-H), 6.90 (d,  $J = 8.4$  Hz, 2 H, 3-H, 5-H), 3.73 (s, 3H, OMe).

**3-Methoxyisonitrosoacetanilide (16):** From 3.72 g aniline **12**, 3.20 g (55%) **16** were obtained as brown solid.

**3,4-Dimethoxyisonitrosoacetanilide (17)** [3]: From 7.66 g aniline **13**, 7.87 g (70.2%) **17** were obtained as brown solid. –  $^1\text{H}$  NMR (300 MHz,  $\text{DMSO}-d_6$ ; see Figure S4):  $\delta = 12.08$  (s br, 1 H, OH), 9.99 (s, 1 H, NH), 7.63 (s, 1 H, 2'-H), 7.38 (d,  $J = 2.4$  Hz, 1 H, 2-H), 7.23 (dd,  $J = 8.7, 2.4$  Hz, 1 H, 6-H), 6.90 (d,  $J = 8.7$  Hz, 1 H, 5-H), 3.73 (s, 3 H, 4-OCH<sub>3</sub>), 3.72 (s, 3 H, 3-OCH<sub>3</sub>). –  $^{13}\text{C}$  NMR (75.5 MHz,  $\text{DMSO}-d_6$ ):  $\delta = 160.5$  ( $\text{C}_q\text{-1'}$ ), 149.3 ( $\text{C}_q\text{-3}$ ), 146.1 ( $\text{C}_q\text{-4}$ ), 144.8 ( $\text{C}_q\text{-1}$ ), 132.7 (CH-2'), 112.8 (CH-6), 112.6 (CH-5), 105.9 (CH-2), 56.9 (3-OCH<sub>3</sub>), 56.2 (4-OCH<sub>3</sub>); tentative assignment according to ACD [4].

**Isatins by cyclisation of isonitrosoacetanilides:** The respective solid isonitrosoacetanilide (1-2 g) was added slowly to hot conc. sulfuric acid (6 mL, 60~70 °C). The reaction mixture was kept for further 10 min at this temperature with stirring. After cooling to room temperature, the reaction mixture was poured into 60 mL of ice-water and kept for 1 h until precipitation was complete. The red solid was filtered off, washed with cold water and dried. The resulting material was purified on silica gel with  $\text{CH}_2\text{Cl}_2$ /3% MeOH.

**5-Methoxy-isatin (6)** [5]: Orange solid (0.30 g, 15 % from 2.16 g/11 mmol **15**),  $R_f = 0.45$  ( $\text{CH}_2\text{Cl}_2$ / 5% MeOH). –  $^1\text{H}$  NMR,  $^{13}\text{C}$  NMR, see Tables S2 and S3 and Figure S30/30.

**6-Methoxy-isatin (7)** [6]: Brown solid (0.70 g, 38 % from 2.0 g/12 mmol **16**),  $R_f = 0.45$  ( $\text{CH}_2\text{Cl}_2$ / 5% MeOH), m.p. 241 °C. –  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectra, see Tables S2 and S3 and Figure S32/32.

**5,6-Dimethoxy-isatin (8)** [3]: Red solid (1.40 g, 78 % from 1.95 g/9.3 mmol **17**),  $R_f = 0.56$  ( $\text{CH}_2\text{Cl}_2$ / 5% MeOH), 217-219 °C. – UV ( $\text{CH}_3\text{OH}$ ):  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) = 210 (4.14), 265 (4.31), 321 (3.92), 465 (3.10); (MeOH/HCl): 212 (4.12), 269 (4.36), 322 (3.89), 403 (3.28), 464 nm (2.93); (MeOH/NaOH): 209 (4.15), 266 (4.34), 321 (3.92), 457 nm (3.10). –  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectra, see Tables S2 and S3 and Figure S34/34. – EI MS (70 eV):  $m/z$  (%) = 207 ( $[\text{M}]^+$ , 100), 164 ( $[\text{M}-\text{NH}-\text{C}=\text{O}]^+$ , 70), 136 ( $[\text{M}-\text{NH}-\text{C}(\text{O})-\text{C}(\text{O})]^+$ , 52); HRESI MS (70 eV):  $m/z$  = 208.06043 ( $[\text{M} + \text{H}]^+$ , calcd. 208.06044 for  $\text{C}_{10}\text{H}_{10}\text{NO}_4$ ).

**6-Hydroxy-5-methoxy-isatin (4)** [3]: 0.147 g, 36 % from 0.442 g/2.1 mmol **14**; data are given in the main part.

### Demethylation of methoxyisatins

To a solution of 2.1 mmol of the methoxyisatin in 20 mL  $\text{CH}_2\text{Cl}_2$ , 2.5 mmol boron tribromide in hexane (10 ml 0.25 M) were added dropwise at -40 °C (2 min). The reaction mixture was stirred at room temperature for 12 h, and the excess of boron tribromide was hydrolyzed by addition of 5 mL MeOH. After evaporation to dryness *i. vac.*, the crude product was purified on silica gel ( $\text{CH}_2\text{Cl}_2$ /5% MeOH).

**5-Hydroxy-isatin (2)** [7]: Orange powder (30 mg, 9% from 370 mg **6**),  $R_f = 0.62$  ( $\text{CH}_2\text{Cl}_2$ /10% MeOH), m.p. 266 °C, violet with NaOH. – UV ( $\text{CH}_3\text{OH}$ ):  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) = 210 (3.99), 255 (4.11), 311 (3.09), 437 (2.17); (MeOH/HCl): 208 (3.99), 254 (4.15), 305 (3.39), 422 nm (2.87); (MeOH/NaOH): 227 sh (4.15), 272 (4.15), 322 sh (3.38), 531 nm (3.31). –  $^1\text{H}$ ,  $^{13}\text{C}$  NMR spectra, see Tables S2 and S3 and Figure S9/10.

**6-Hydroxyisatin (3)** [8]: 15.9 mg (43%) from 37 mg **7**; data as listed in the main part.

**5-Hydroxy-6-methoxy-isatin (5), 6-hydroxy-5-methoxy-isatin (4), and 5,6-dihydroxy-isatin (9)**: From 415 mg 5,6-dimethoxy-isatin (**8**), 220 mg (57%) **4**, 120 mg (31%) **5** and 20 mg (6%) **9** were obtained. Data of **4** were described in the main part, for  $^1\text{H}$  NMR data of **9**, see Table S2 and Figure S36.

**5-Hydroxy-6-methoxy-isatin (5)** [3]: Orange solid,  $R_f = 0.56$  ( $\text{CH}_2\text{Cl}_2$ / 5% MeOH), m.p. 236 °C, solution in MeOH orange-red, violet with NaOH. – UV ( $\text{CH}_3\text{OH}$ ):  $\lambda_{\text{max}}$  ( $\log \epsilon$ ) = 206

(4.57), 270 (2.62), 332 (4.24), 370 (3.11), 396 (3.91), 478 nm (3.86); (CH<sub>3</sub>OH/HCl):  $\lambda_{\text{max}}$  (log  $\epsilon$ ) = 268 (4.00), 320 (3.52), 400 (3.03), 515 sh (2.76), 550 sh, 570 sh; (CH<sub>3</sub>OH/NaOH):  $\lambda_{\text{max}}$  (log  $\epsilon$ ) = 282 (3.99), 335 (3.54), 516 (2.89), 550 (2.91) very broad. – <sup>1</sup>H NMR spectrum, see Tables S2 and S3 and Figure S27/27. – (+)-HRESI MS:  $m/z$  = 216.0270 ([M+Na]<sup>+</sup>, calcd. 216.02728 for C<sub>9</sub>H<sub>7</sub>NO<sub>4</sub>Na).

**Crystal structure determination of 5-hydroxy-6-methoxy-isatin (5):** Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Centre: CCDC 1414741 contains the supplementary crystallographic data. These data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax +44-(0)1223-336033 or email: deposit@ccdc.cam.ac.uk).

**Table S1:** Crystal structure data of 5-hydroxy-6-methoxy-isatin (**5**)

Formula	C <sub>9</sub> H <sub>7</sub> N O <sub>4</sub> , C <sub>2</sub> H <sub>6</sub> O S
$M_r$	271.28
Cryst. size, mm <sup>3</sup>	0.02 × 0.045 × 0.047
Crystal system	monoclinic
Space group	$P2_1/c$
$a$ , Å	13.2311(3)
$b$ , Å	13.4539(3)
$c$ , Å	6.7455(1)
$\alpha$ , deg	90
$\beta$ , deg	97.2469(9)
$\gamma$ , deg	90
$V$ , Å <sup>3</sup>	1191.17(4)
$Z$	4
$D_{\text{calcd}}$ , g cm <sup>-3</sup>	1.513
$\mu(\text{MoK}\alpha)$ , mm <sup>-1</sup>	2.572
$F(000)$ , e	568
$hkl$ range	e.g. -15, 9; -15, 13; $\pm 7$
$((\sin\theta)/\lambda)_{\text{max}}$ , Å <sup>-1</sup>	0.61
Refl. measured	10529
Refl. unique	1947 [1938 with $I > 2\sigma(I)$ ]
$R_{\text{int}}$	0.023
Param. refined	172
$R(F) / wR(F^2)^a$ (all reflexions)	0.0484, 0.1147
GoF ( $F^2$ ) <sup>b</sup>	1.15
$\Delta\rho_{\text{fin}}$ (max / min), e Å <sup>-3</sup>	0.80, -0.31

<sup>a</sup>  $R1 = \|F_o\| - \|F_c\| / \sum \|F_o\|$ ,  $wR2 = [\sum w(F_o^2 - F_c^2)^2 / \sum w(F_o^2)^2]^{1/2}$ ,  $w = [\sigma^2(F_o^2) + (0.0136P)^2 + 4.0427P]^{-1}$ , where  $P = (\text{Max}(F_o^2, 0) + 2F_c^2) / 3$ ; <sup>b</sup> GoF =  $[\sum w(F_o^2 - F_c^2)^2 / (n_{\text{obs}} - n_{\text{param}})]^{1/2}$



## Additional references

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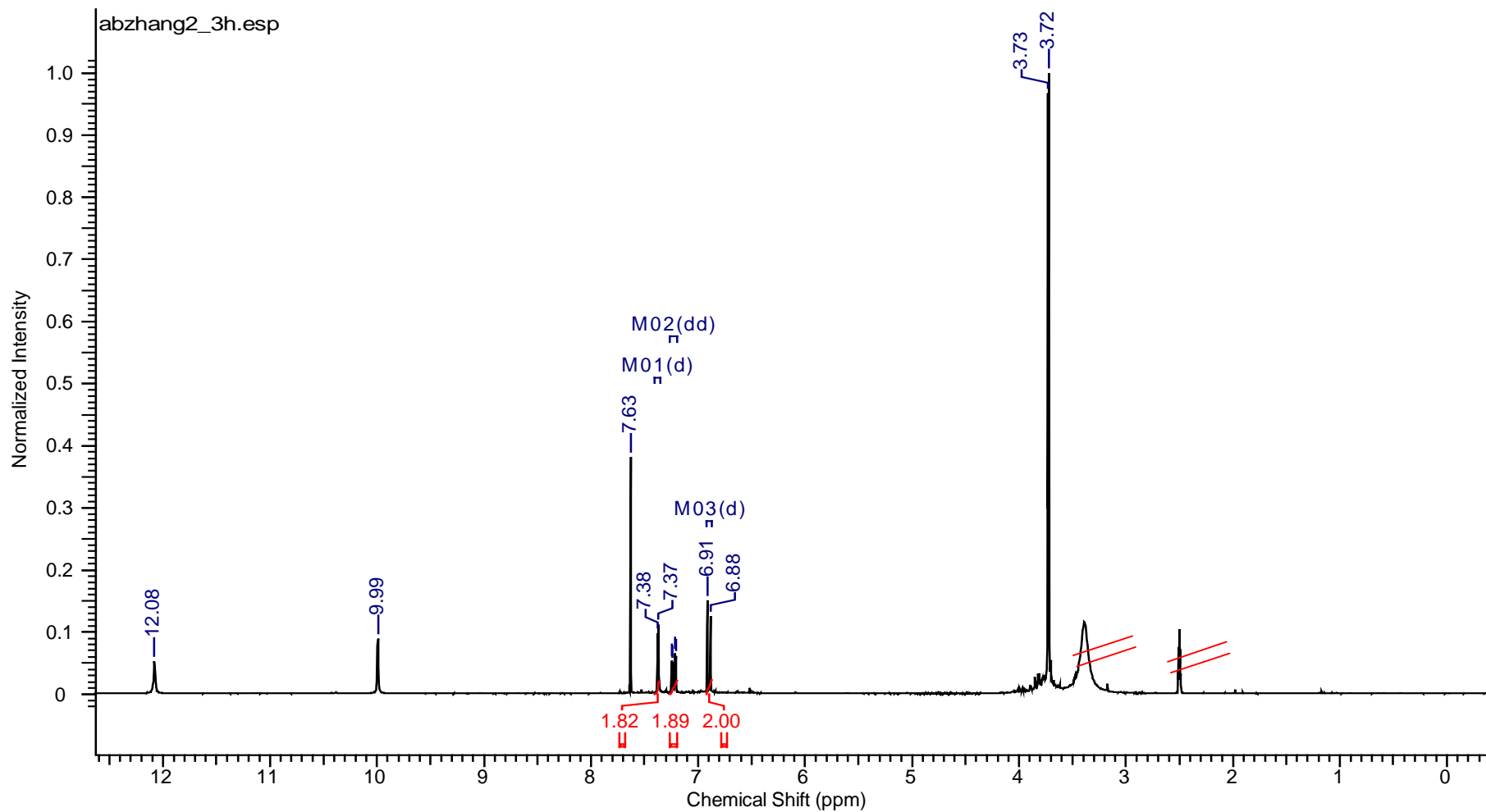
**Table S2.** <sup>1</sup>H NMR data of oxygenated isatins; shifts as  $\delta$  values, coupling constants in [Hz]

Formula	2	2	3	3	4	4	4	5	5	6	7	8	9
solvent/	DMSO	MeOD	DMSO	MeOD	DMSO	DMSO	MeOD	DMSO	MeOD	DMSO	DMSO	MeOD	MeOD
Freq [MHz]	300	300	300	300	300	300	600	300	300	300	300	300	300
H-4	6.83 dd	6.93 dd	7.39 d	7.42 d	6.55 s	6.93 s	7.07 s	6.85 s	6.92 s	7.07 d	7.47 d	7.09 s	8.54 s
	2.7, 0.5	2.7, 0.4	8.3	8.5			6.71 s			2.7	8.5		
H-5	-	-	6.40 dd	6.43 dd	-	-	-	-	-	-	6.58 dd	-	-
			8.3, 2.4	8.3, 2.0							8.5, 2.1		
H-6	7.00 ddd	7.02 dd			-	-	-	-	-	7.19 dd	-	-	-
	8.4, 2.7, 0.7	8.3, 2.0								8.4, 2.7			
H-7	6.75 dd	6.77 dd	6.29 d	6.29 d	5.68 s	6.25 s	6.32 s	6.45 s	6.53 s	6.85 d	6.39 d	6.55 s	5.93 s
	8.4, 0.5	8.4, 0.4	2.4	2.0			5.84 s			8.4	2.1		
5-OMe	-	-	-	-	3.58 s	3.71 s	3.82 s	-	-	3.75 s	-	3.78 s	-
							3.70 s						
6-OMe	-	-	-	-	-	-	-	3.92 s	3.97 s	-	3.87 s	3.94 s	-
OH/NH	10.10 s	-	10.79 s, 11.2 s	-	9.77 s	10.38 s	-	-	-	-	10.92 s	-	-

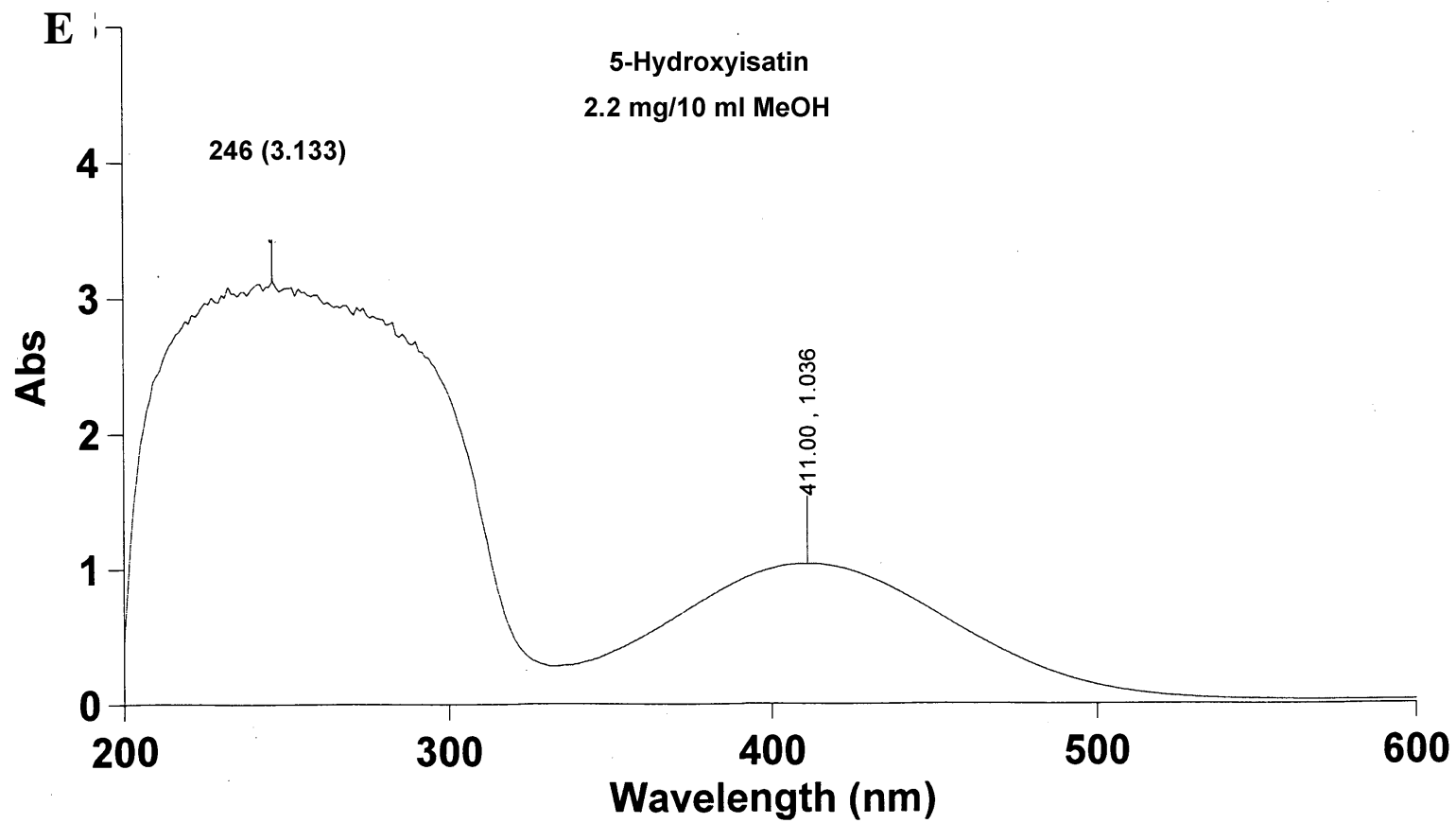
**Table S3.** <sup>13</sup>C NMR data of oxygenated isatins; shifts as  $\delta$  values

Formula	2	2	3	3	4	4	5	6	7	8
solvent Freq [MHz]	DMSO	MeOD	DMSO	MeOD	DMSO	MeOD	MeOD	DMSO	DMSO	DMSO
	125	125	125	125	125	150	125	125	125	125
C-2	159.2	161.7	160.6	163.2	161.8	(163.7)	162.6	159.3	167.7	160.6
C-3	184.6	186.0	180.6	182.4	179.8	175.1	183.2	184.3	181.5	181.5
C-3a	118.0	119.9	109.8	111.6	106.7	103.4	111.0	117.9	108.8	107.3
C-4	110.3	112.0	127.5	129.1	107.8	105.7	111.5	108.6	107.8	108.6
C-5	153.0	155.3	110.1	111.8	144.8	150.2	148.7	155.1	144.8	148.7
C-6	124.9	126.3	167.0	169.6	160.7	178.1	158.9	124.7	161.8	158.3
C-7	112.9	114.3	98.9	100.6	99.9	102.8	97.3	113.0	99.9	96.4
C-7a	142.9	144.6	153.5	155.3	149.1	154.4	144.2	144.4	149.1	145.0
5-OMe	-	-	-	-	56.0	56.0	-	55.7	56.0	56.3*
6-OMe	-	-	-	-	-	-	57.0	-	-	56.0*

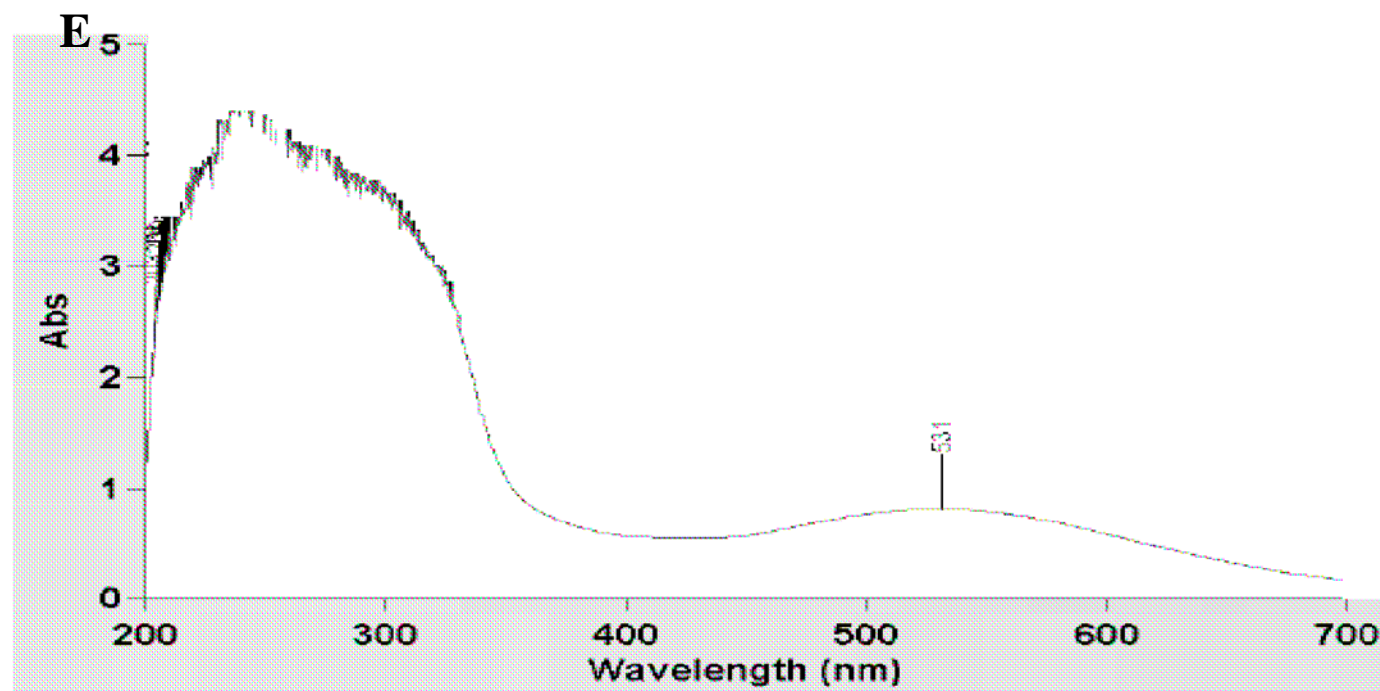
\* signals may be exchanged.



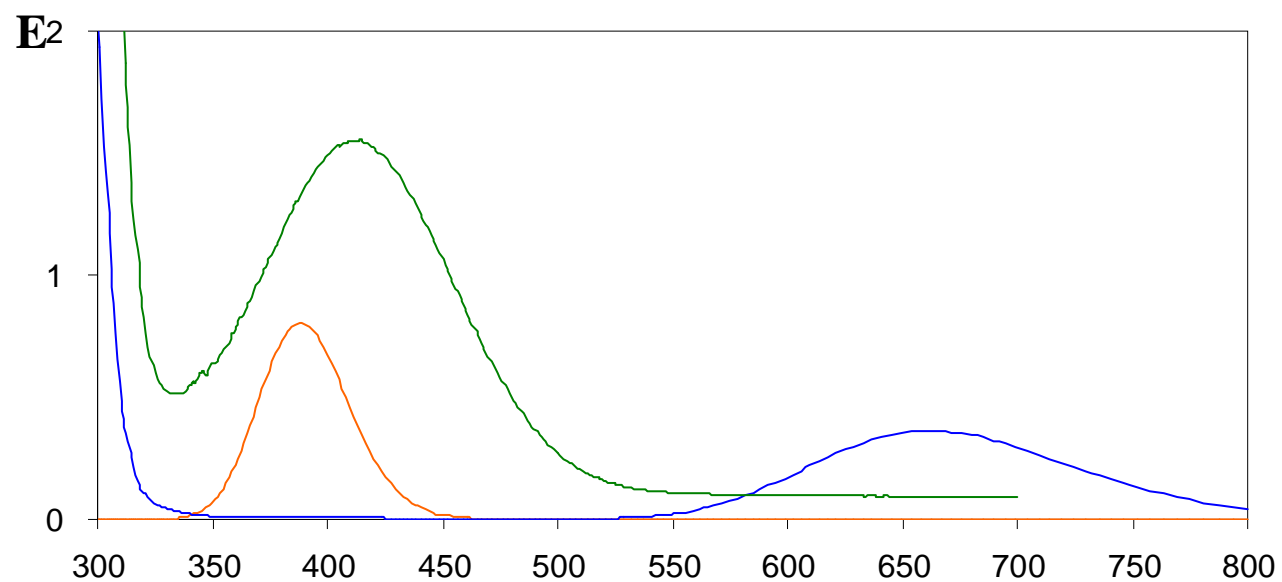
**Figure S4:**  $^1\text{H}$  NMR spectrum (300 MHz) of 3,4-dimethoxyisonitrosoacetanilide (**17**) in  $\text{DMSO}-d_6$ ; DMSO and water signals are crossed out.



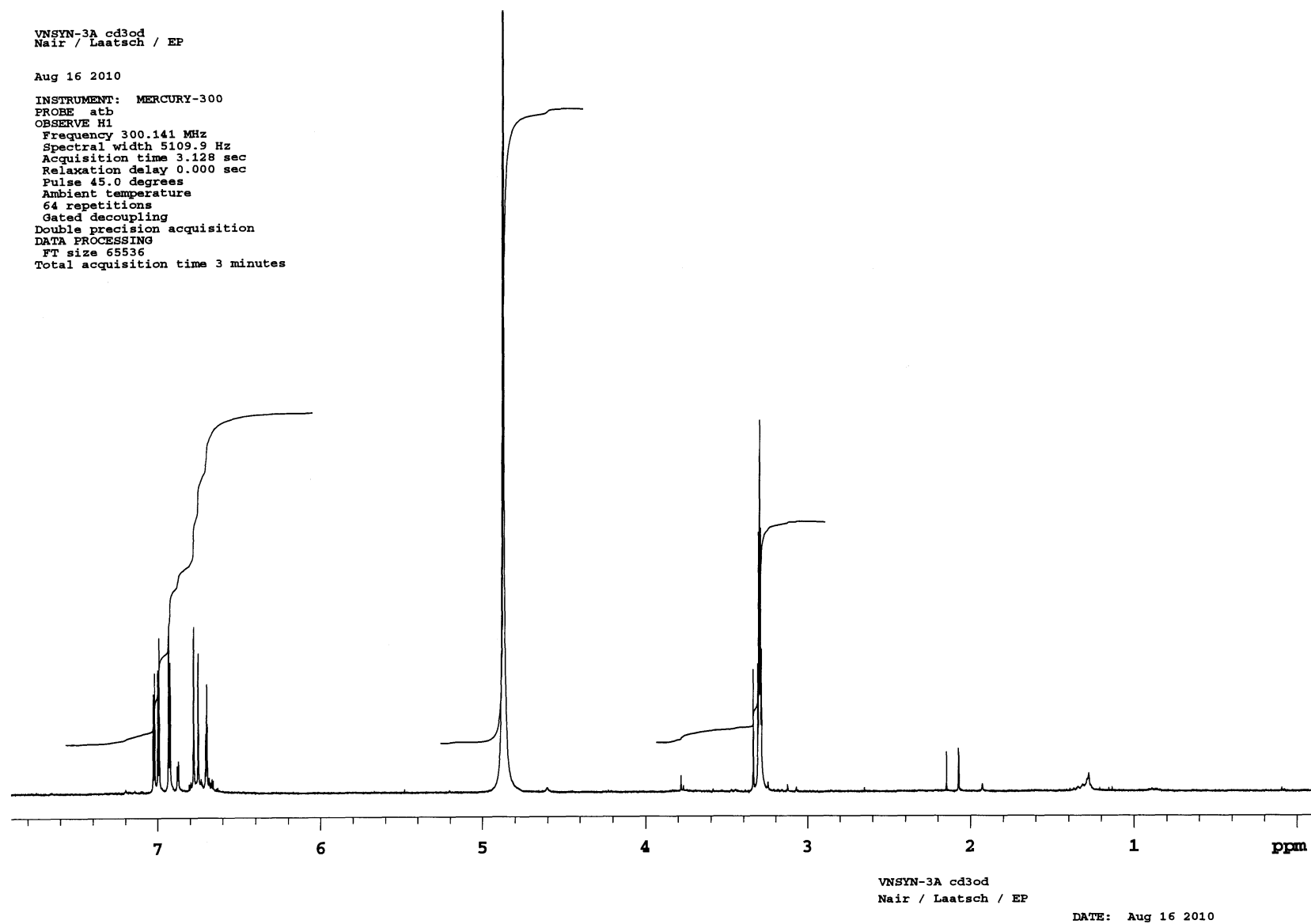
**Figure S5:** Exp. UV/Vis spectrum of 5-hydroxy-isatin (**2**) in methanol, neutral.



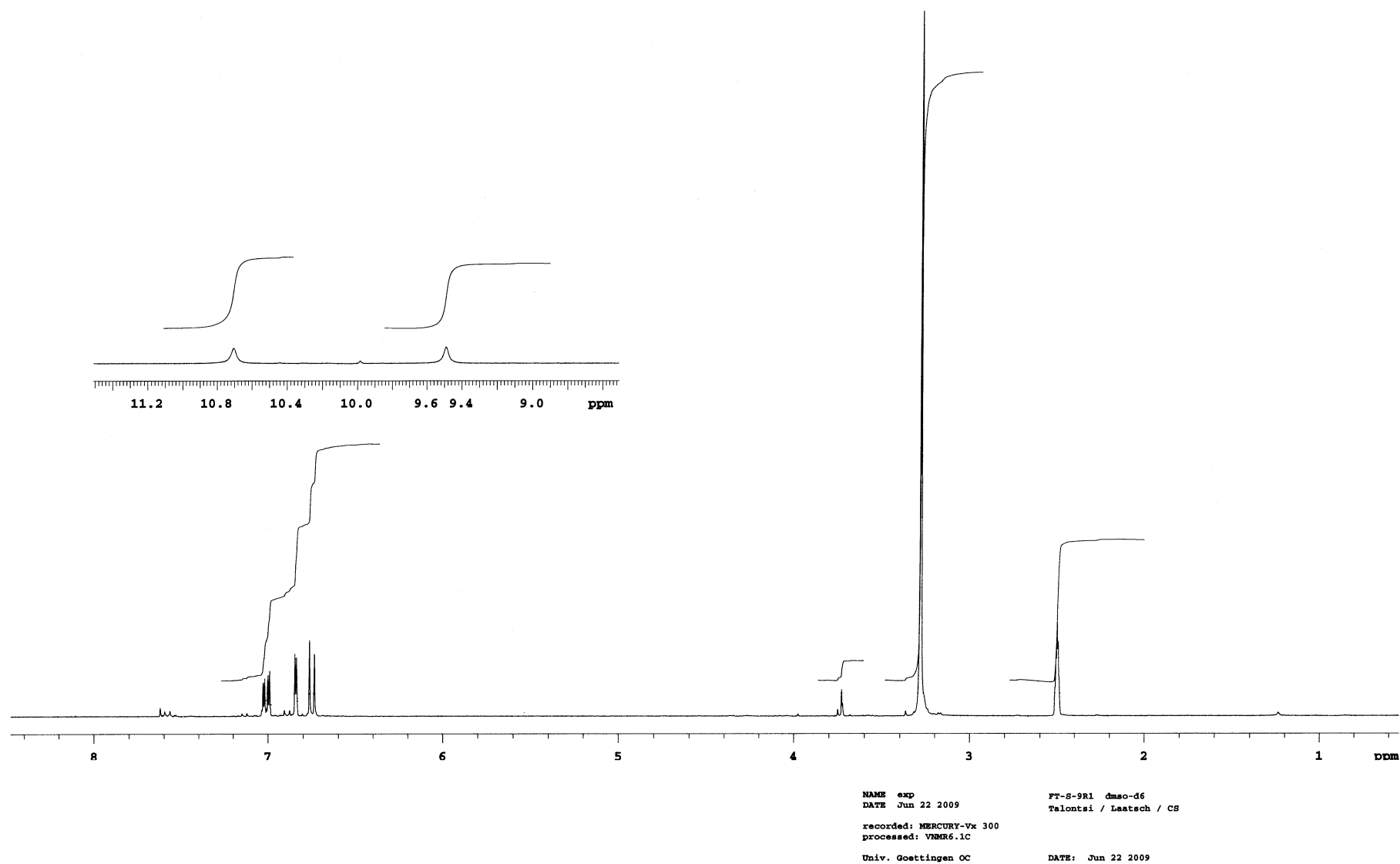
**Figure S6:** Exp. UV/Vis spectrum of 5-hydroxy-isatin (**2**) in methanol, basic



**Figure S7:** UV/Vis spectra of 5-hydroxy-isatin (**2**); green = experimental, MeOH; orange = calculated, neutral; blue = calculated, basic.



**Figure S8:**  $^1\text{H}$  NMR spectrum (300 MHz) of 5-hydroxy-isatin (**2**) in methanol- $d_4$



**Figure S9:**  $^1\text{H}$  NMR spectrum (300 MHz) of 5-hydroxy-isatin (**2**) in  $\text{DMSO}-d_6$



VNSYN-3 dmsc-d6  
 Nair / Laatsch  
 Feb 6 2010

INSTRUMENT INOVA-500  
 PROBE coldc 3mm  
 OBSERVE C13  
 Frequency 125.707 MHz  
 Spectral width 30165.9 Hz  
 Acquisition time 2.122 sec  
 Relaxation delay 0.000 sec  
 Pulse width 36.0 degrees  
 Temperature 35.0 deg. C / 308.1 K  
 No. repetitions 640  
 DECOUPLE H1  
 Frequency 499.879 MHz  
 Power 32 dB  
 Decoupler continuously on  
 WALTZ-16 modulated  
 Double precision acquisition  
 DATA PROCESSING  
 Line broadening 1.0 Hz  
 FT size 262144  
 Total acquisition time 22 minutes

VNSYN-3 dmsc-d6  
 Nair / Laatsch

SPECTRAL LINES FOR TH= 5.3  
 FROM -2.5 PPM TO 197.5 PPM  
 RFL= 6972.8 RFP= 4964.9

INDEX	FREQ	PPM	HEIGHT
1	23206.4	184.63	15.4
2	20016.1	159.24	19.5
3	19236.8	153.04	25.2
4	17962.3	142.90	24.4
5	15702.0	124.92	46.9
6	14832.5	118.00	23.9
7	14192.2	112.91	46.1
8	13868.2	110.33	45.6
9	5038.8	40.09	7.2
10	5027.8	40.00	70.0
11	5018.1	39.92	16.2
12	5006.8	39.83	212.0
13	4997.1	39.76	28.2
14	4985.9	39.67	423.7
15	4976.2	39.59	26.4
16	4964.9	39.50	500.0
17	4944.0	39.33	426.1
18	4923.0	39.17	212.5
19	4901.9	39.00	69.8

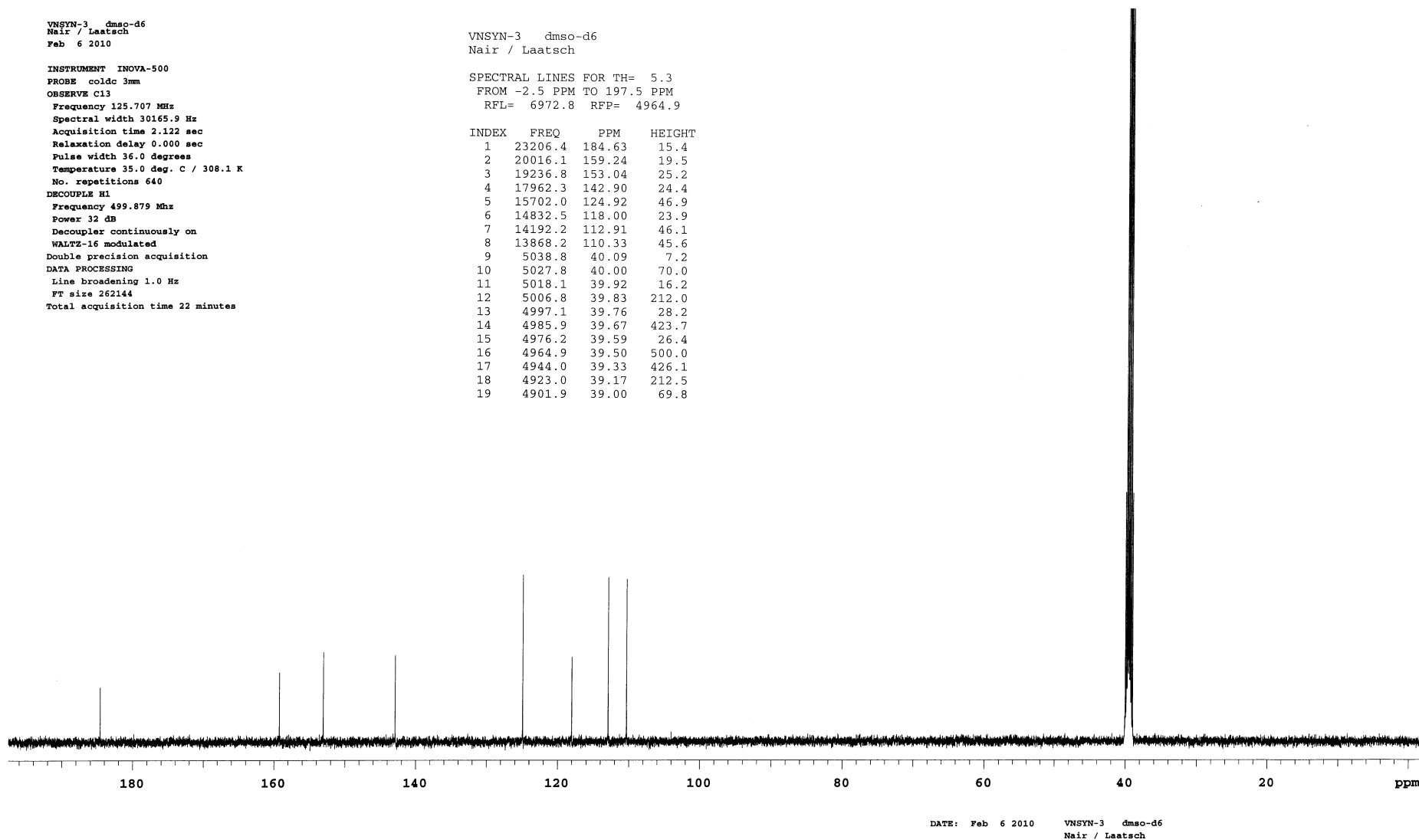
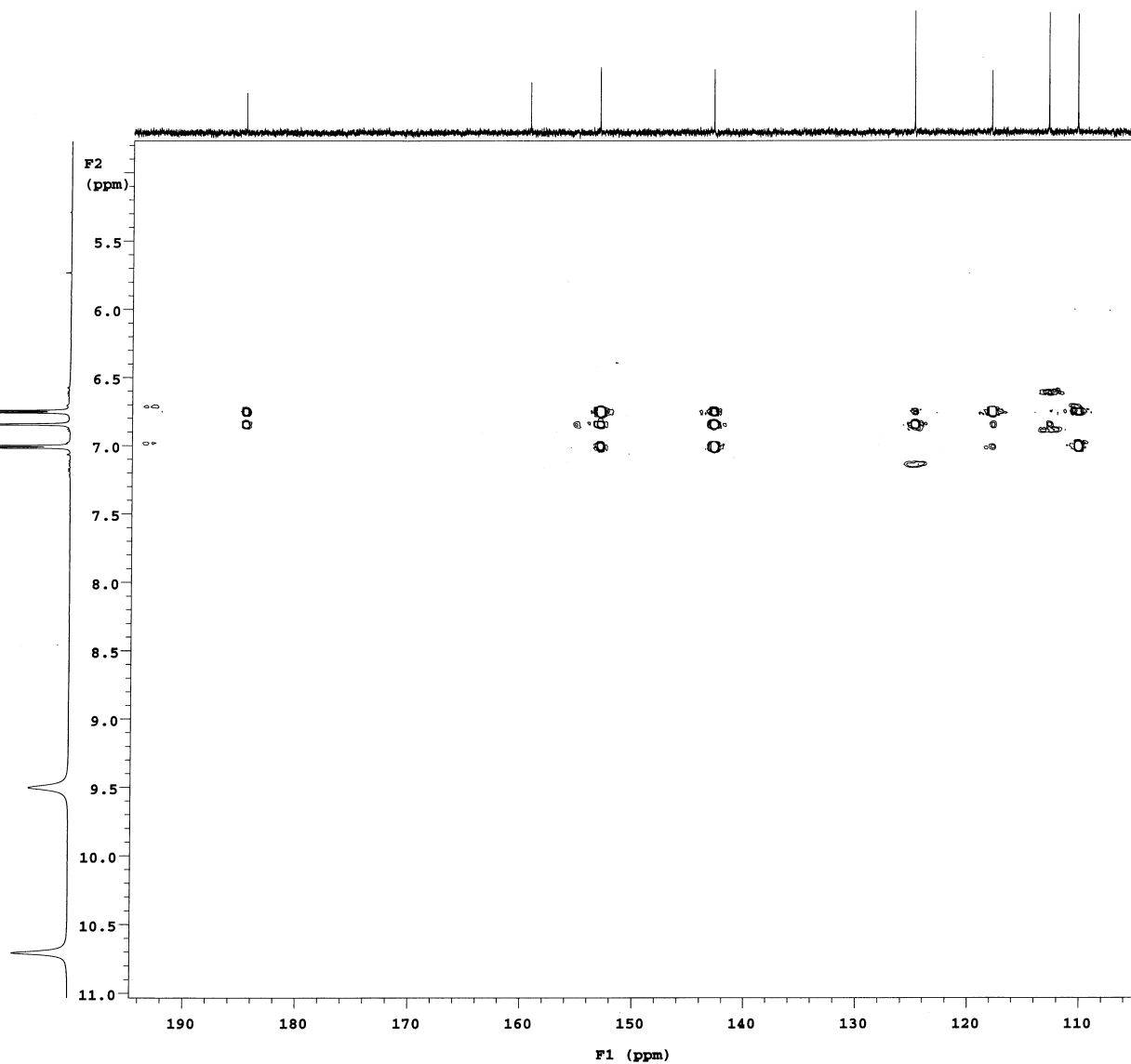
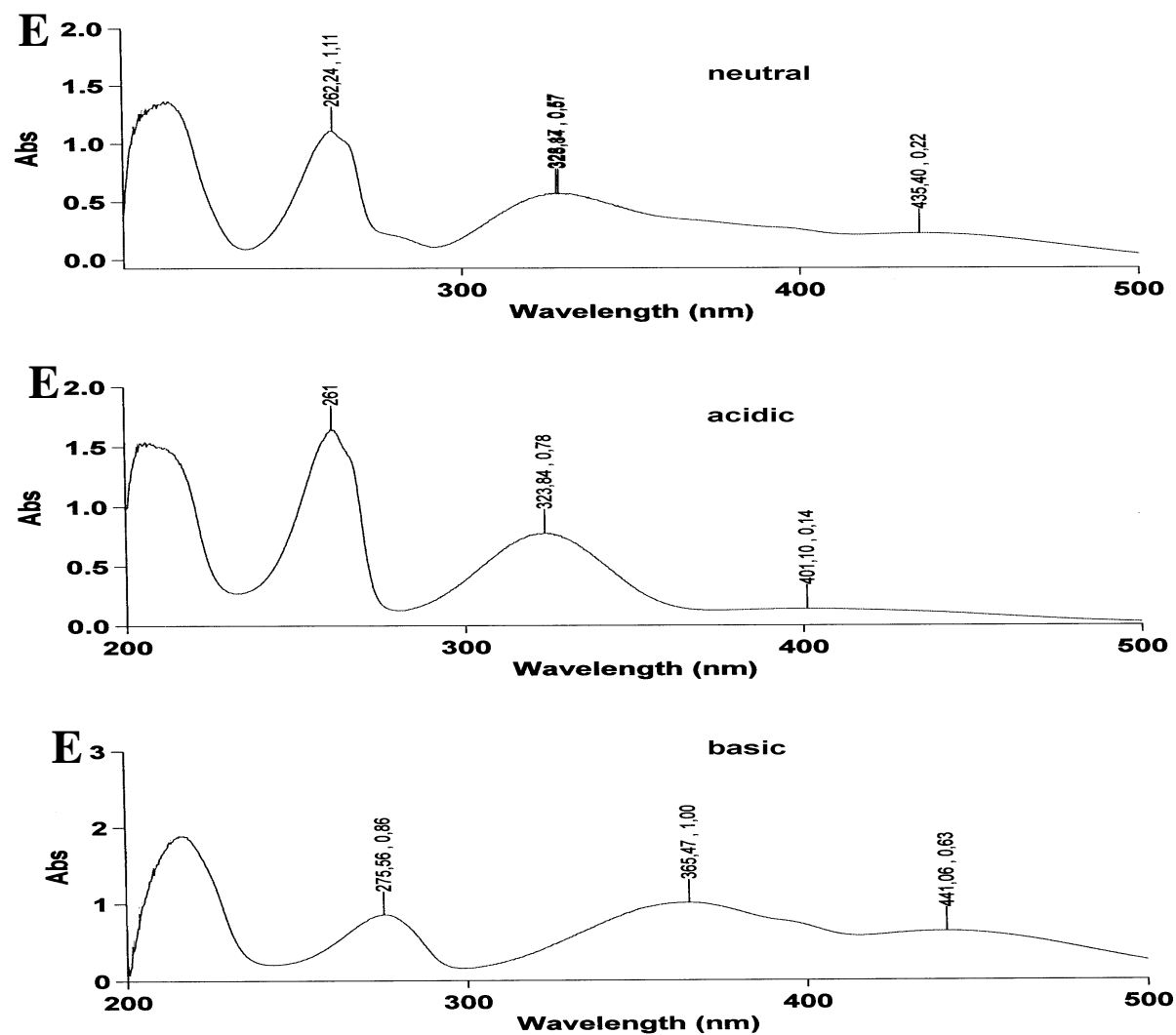


Figure S10:  $^{13}\text{C}$  NMR spectrum (125 MHz) of 5-hydroxy-isatin (**2**) in  $\text{DMSO-}d_6$

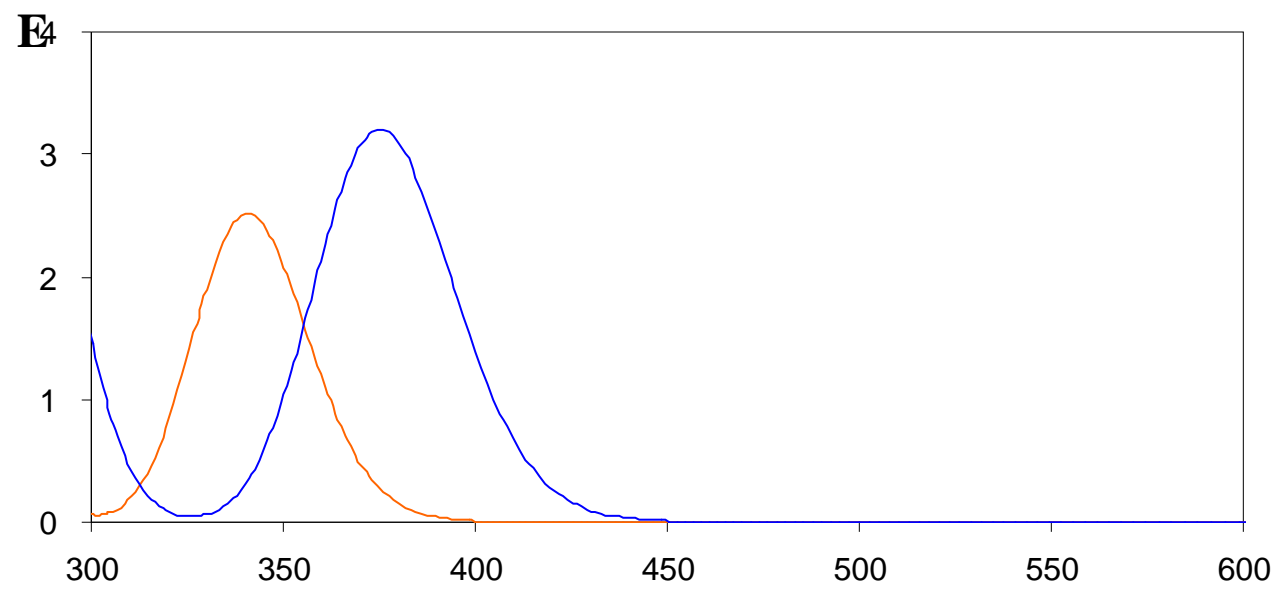
vnsyn-3a d6-dmsc  
 Nair/Laatsch  
 Jan 5 2011  
 INSTRUMENT INOVA-600  
 Pulse sequence gHMQCAD  
 OBSERVE M1  
 Frequency 599.744 MHz  
 Spectral width 7072.1 Hz  
 2D Spectral width 25632.8 Hz  
 Acquisition time 0.150 sec  
 Relaxation delay 1.000 sec  
 Temperature 35.0 deg. C / 308.1 K  
 No. repetitions 56  
 No. increments 256 X2  
 Double precision acquisition  
 DATA PROCESSING  
 Sine bell squared 0.075 sec  
 FT size 2048  
 F1 DATA PROCESSING  
 Sine bell square 0.020 sec  
 Shifted by -0.020 sec  
 FT size 2048  
 Total acquisition time 9:12 hours



**Figure S11:** HMBC spectrum (600 MHz) of 5-hydroxy-isatin (**2**) in DMSO- $d_6$



**Figure S12:** Exp. UV/Vis spectrum of 6-hydroxy-isatin (**3**) in methanol; green = neutral, blue = basic.



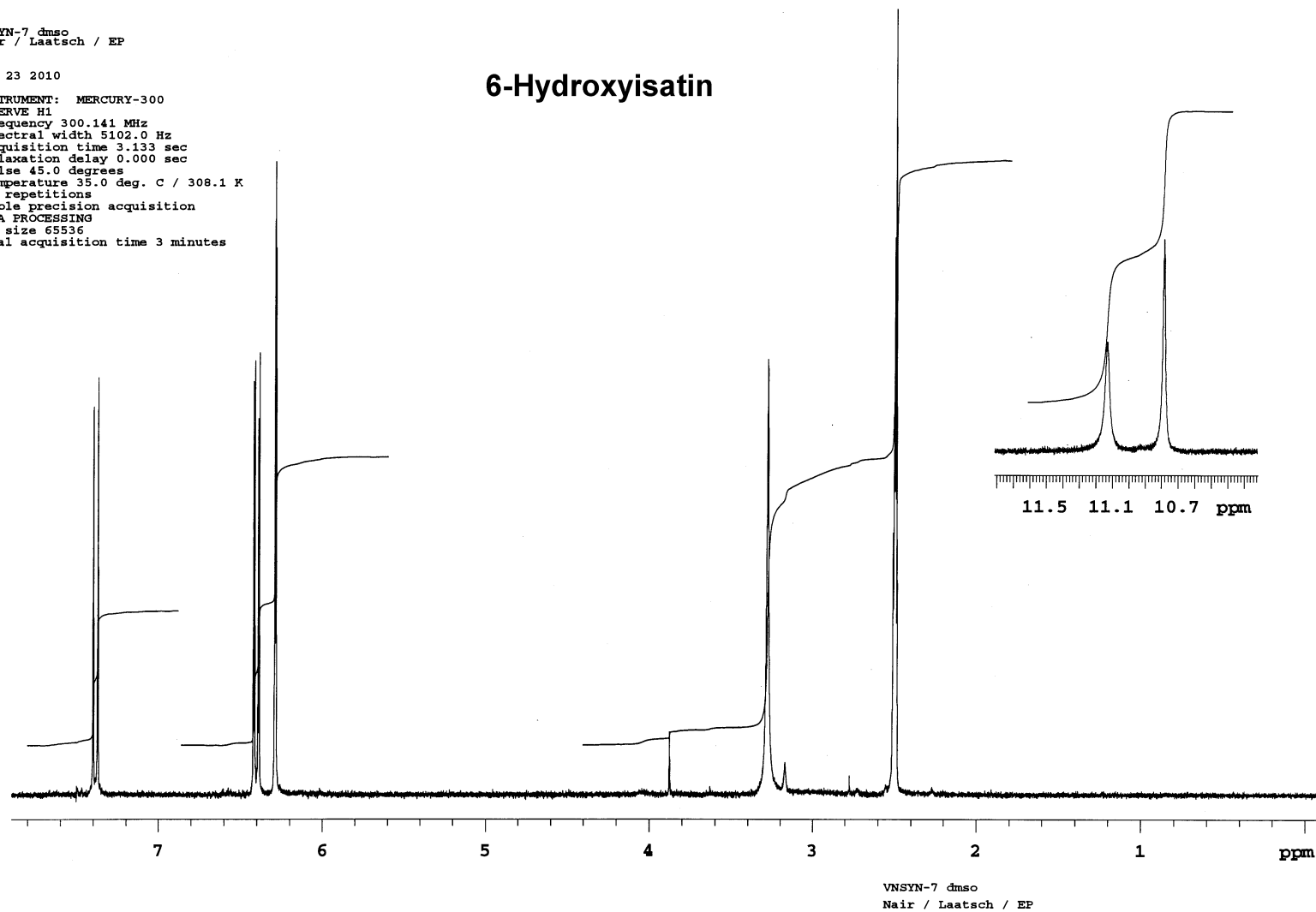
**Figure S13:** Calculated UV/Vis spectrum of 6-hydroxy-isatin (**3**); orange = neutral, blue = basic.

VNSYN-7 dmsc  
Nair / Laatsch / EP

Feb 23 2010

INSTRUMENT: MERCURY-300  
OBSERVE H1  
Frequency 300.141 MHz  
Spectral width 5102.0 Hz  
Acquisition time 3.133 sec  
Relaxation delay 0.000 sec  
Pulse 45.0 degrees  
Temperature 35.0 deg. C / 308.1 K  
64 repetitions  
Double precision acquisition  
DATA PROCESSING  
FT size 65536  
Total acquisition time 3 minutes

## 6-Hydroxyisatin



**Figure S14:**  $^1\text{H}$  NMR spectrum (300 MHz) of 6-hydroxy-isatin (**3**) in  $\text{DMSO}-d_6$ .

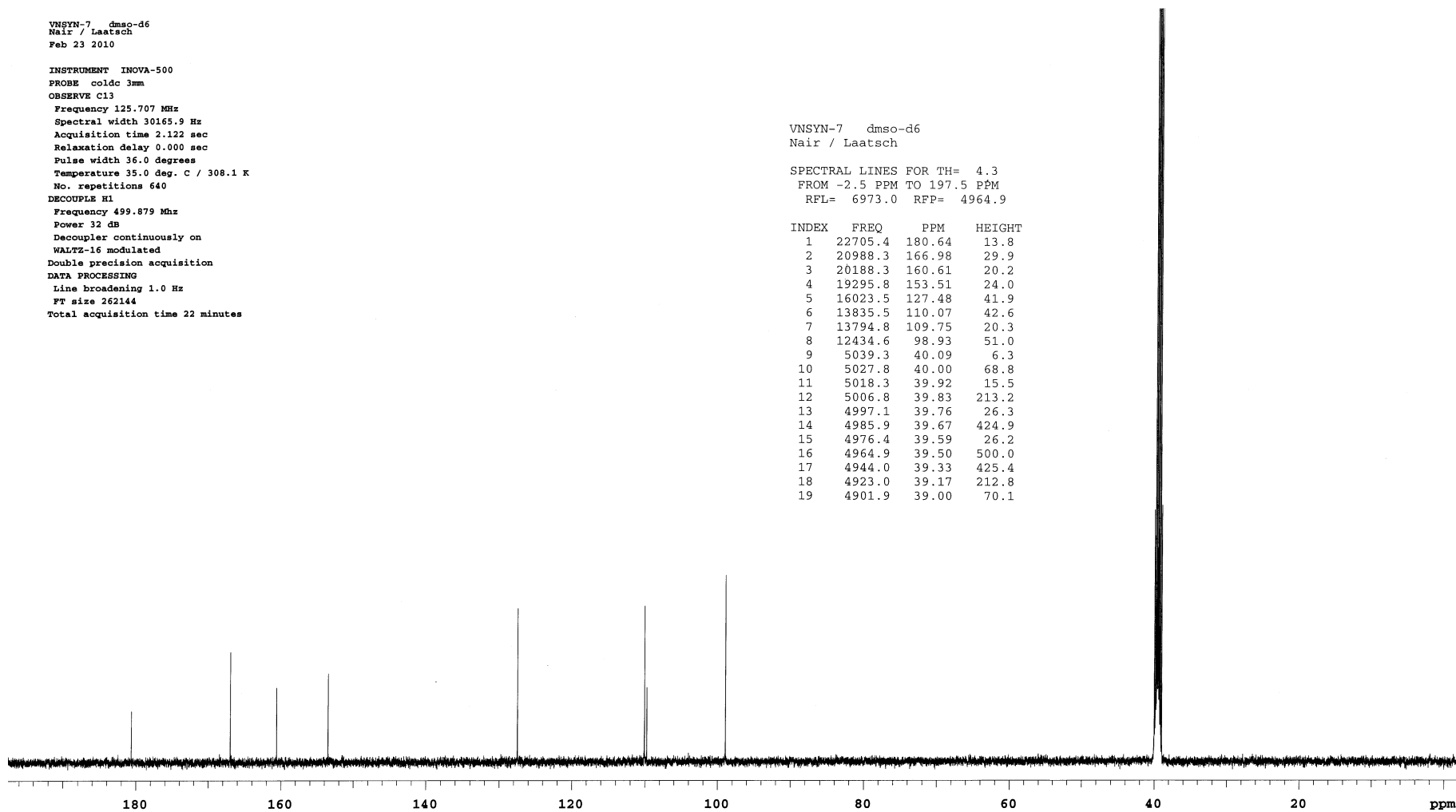
VNSYN-7 dmsc-d6  
Nair / Laatsch  
Feb 23 2010

INSTRUMENT INOVA-500  
PROBE coldc 3mm  
OBSERVE C13  
Frequency 125.707 MHz  
Spectral width 30165.9 Hz  
Acquisition time 2.122 sec  
Relaxation delay 0.000 sec  
Pulse width 36.0 degrees  
Temperature 35.0 deg. C / 308.1 K  
No. repetitions 640  
DECOUPLE H1  
Frequency 499.879 MHz  
Power 32 dB  
Decoupler continuously on  
WALTZ-16 modulated  
Double precision acquisition  
DATA PROCESSING  
Line broadening 1.0 Hz  
FT size 262144  
Total acquisition time 22 minutes

VNSYN-7 dmsc-d6  
Nair / Laatsch

SPECTRAL LINES FOR TH= 4.3  
FROM -2.5 PPM TO 197.5 PPM  
RFL= 6973.0 RFP= 4964.9

INDEX	FREQ	PPM	HEIGHT
1	22705.4	180.64	13.8
2	20988.3	166.98	29.9
3	20188.3	160.61	20.2
4	19295.8	153.51	24.0
5	16023.5	127.48	41.9
6	13835.5	110.07	42.6
7	13794.8	109.75	20.3
8	12434.6	98.93	51.0
9	5039.3	40.09	6.3
10	5027.8	40.00	68.8
11	5018.3	39.92	15.5
12	5006.8	39.83	213.2
13	4997.1	39.76	26.3
14	4985.9	39.67	424.9
15	4976.4	39.59	26.2
16	4964.9	39.50	500.0
17	4944.0	39.33	425.4
18	4923.0	39.17	212.8
19	4901.9	39.00	70.1

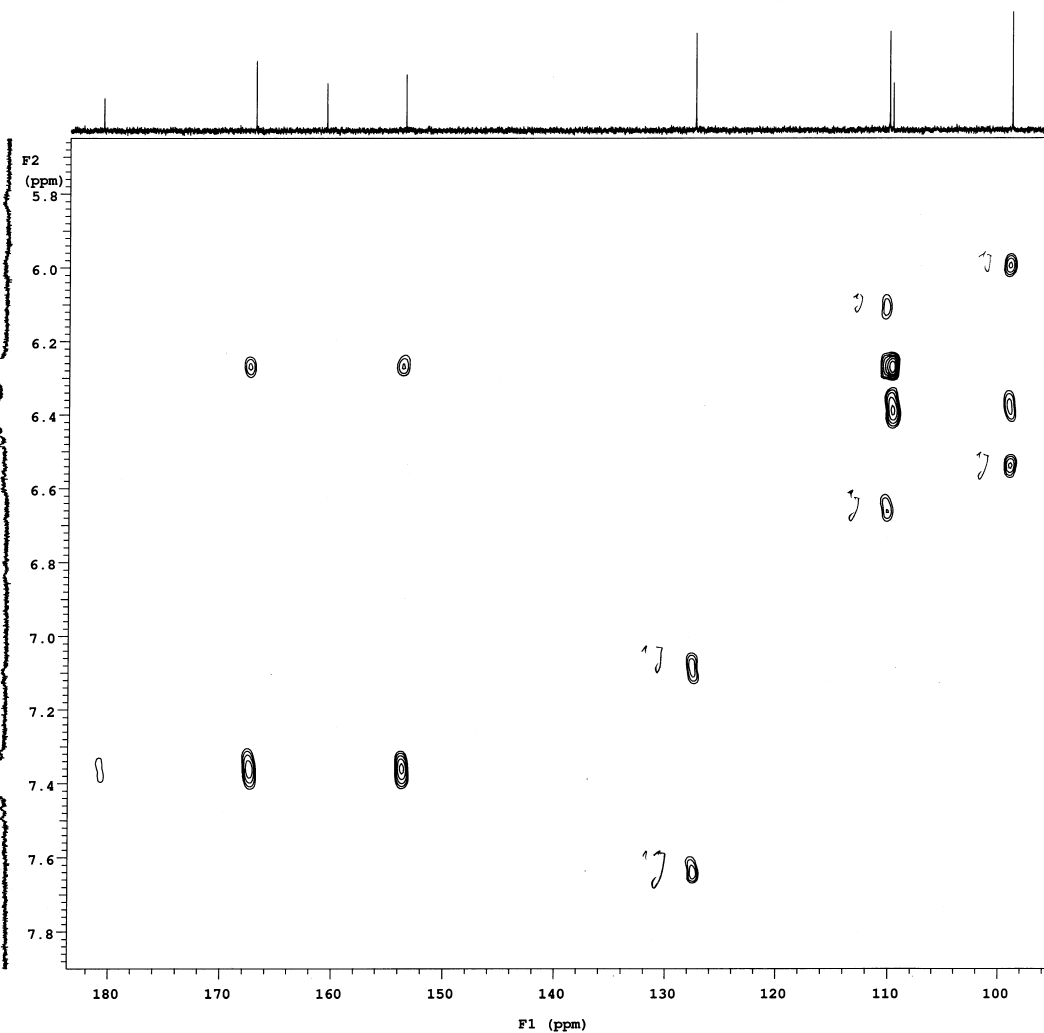


VNSYN-7 dmsc-d6  
Nair / Laatsch

Figure S15: <sup>13</sup>C NMR spectrum (125 MHz) of 6-hydroxy-isatin (**3**) in DMSO-*d*<sub>6</sub>.

VNSYN-7 dms-d6  
 Nair / Laatsch  
 Jan 6 2011  
 INSTRUMENT VNMR-300  
 Pulse sequence ghmhbc  
 OBSERVE H1  
 Frequency 300.537 MHz  
 Spectral width 3551.1 Hz  
 2D Spectral width 12091.9 Hz  
 Acquisition time 0.128 sec  
 Relaxation delay 1.000 sec  
 Mixing time 0.080 sec  
 Temperature 35.0 deg. C / 308.1 K  
 No. repetitions 64  
 No. increments 256  
 Double precision acquisition  
 DATA PROCESSING  
 Sine bell 0.064 sec  
 FT size 2048  
 F1 DATA PROCESSING  
 Sine bell 0.021 sec  
 FT size 2048  
 Total acquisition time 5:33 hours

VS= 1783  
 TH= 2



FILE=nmrdat: laatsch/vnsyn-7\_3ghr

**Figure S16:** HMBC spectrum (300 MHz) of 6-hydroxy-isatin (**3**) in DMSO-*d*<sub>6</sub>.

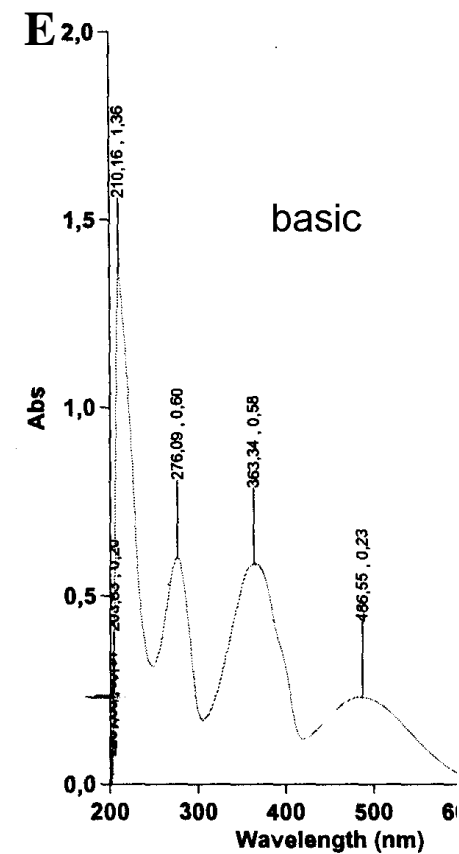
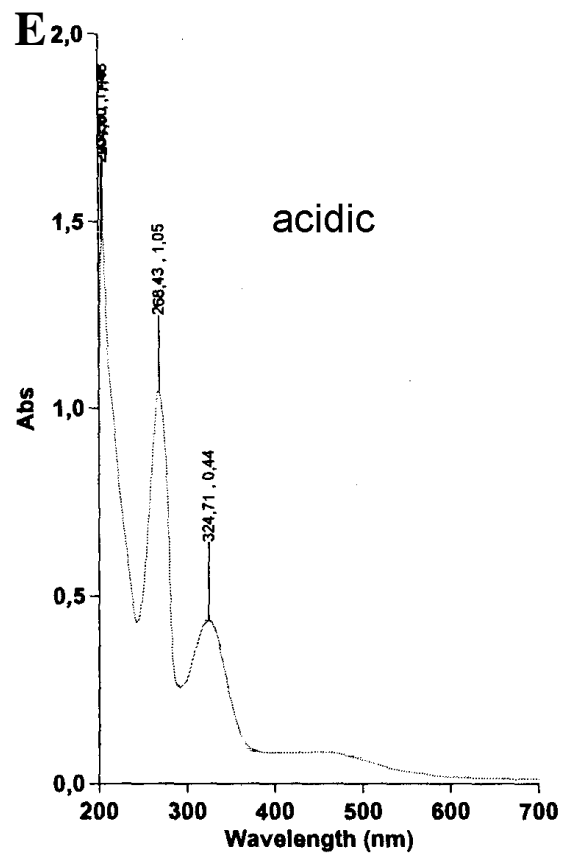
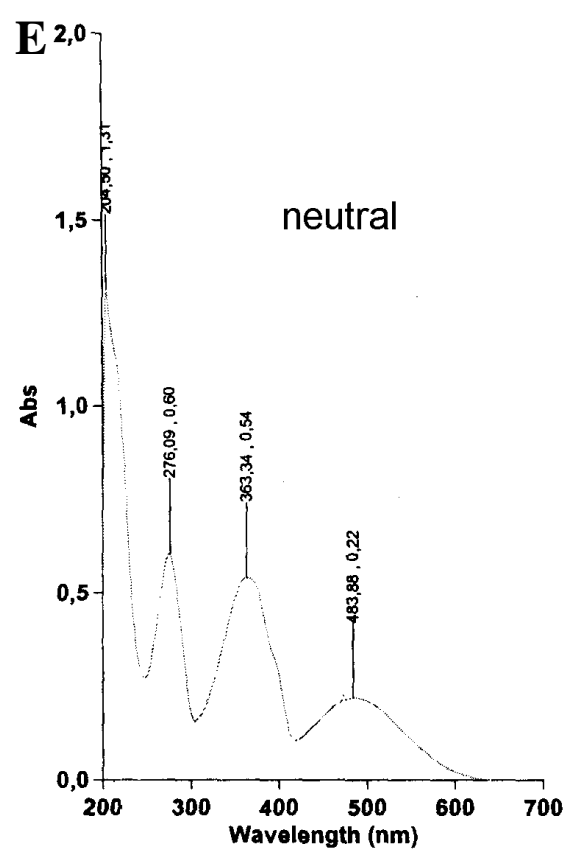
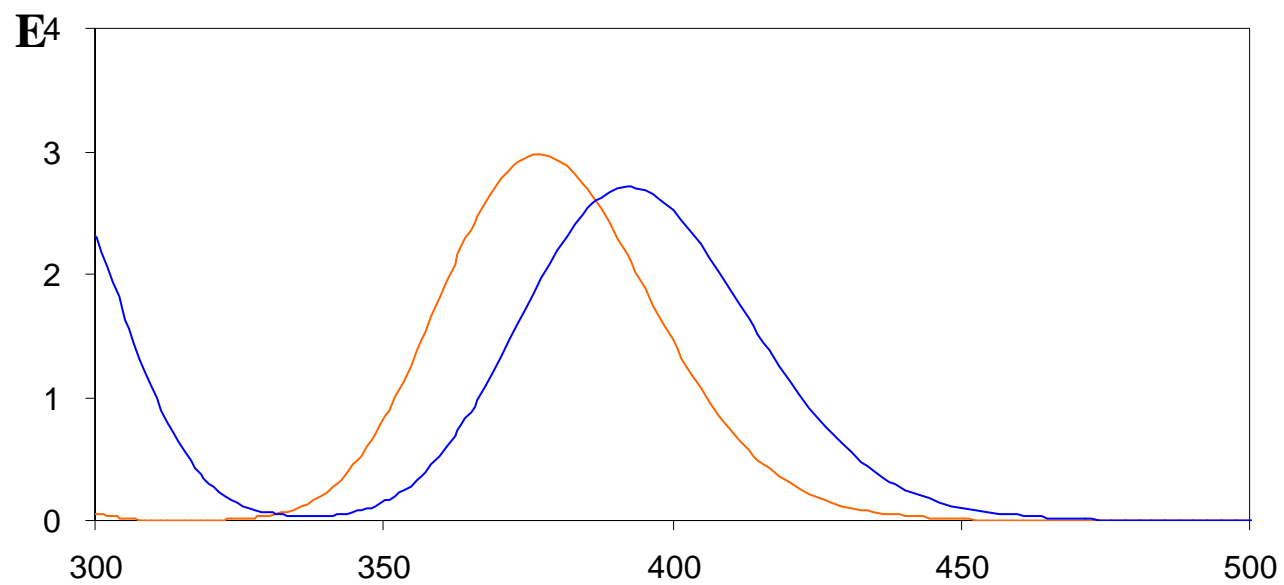
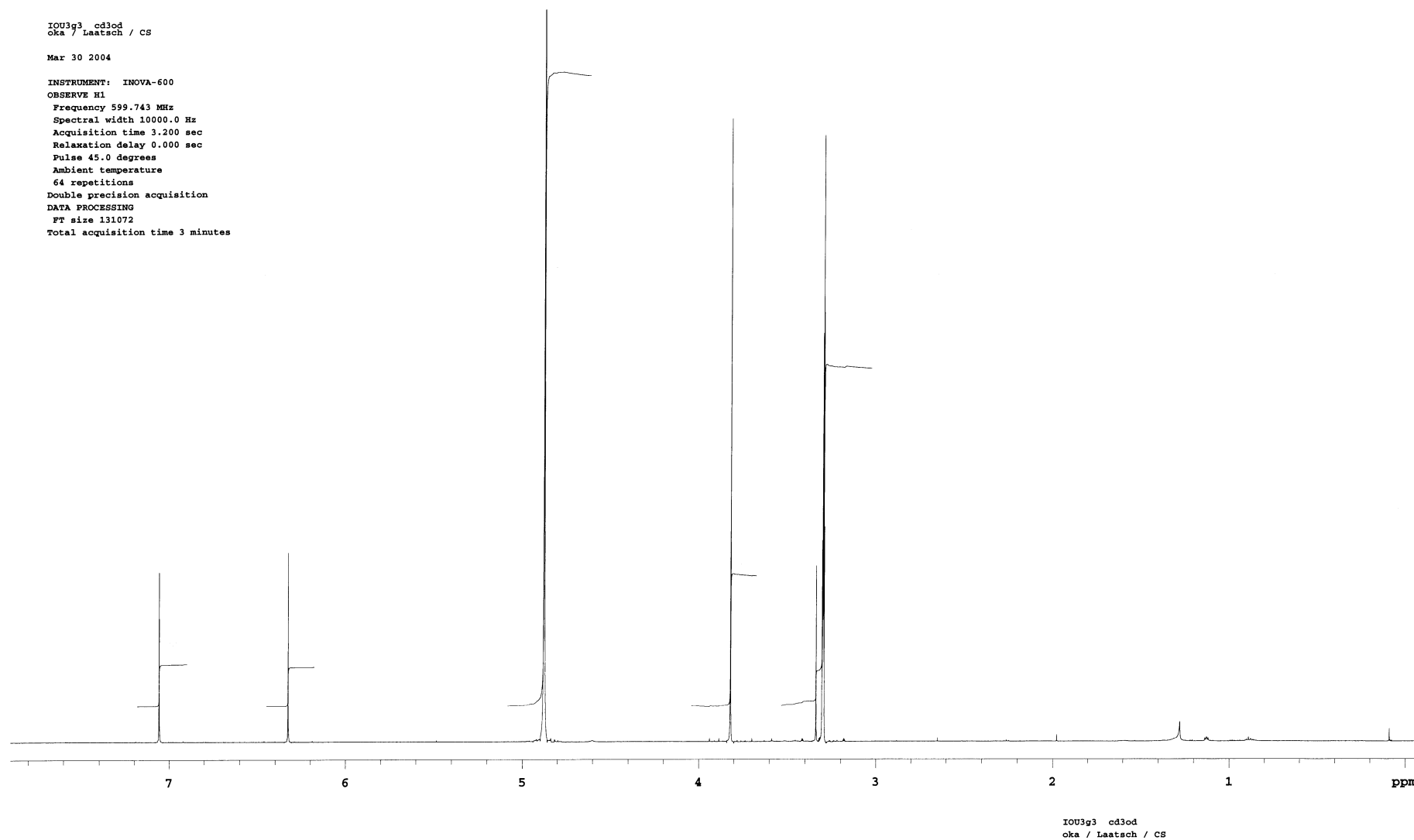


Figure S17: Exp. UV/Vis spectrum of 6-hydroxy-5-methoxy-isatin (4) in methanol.

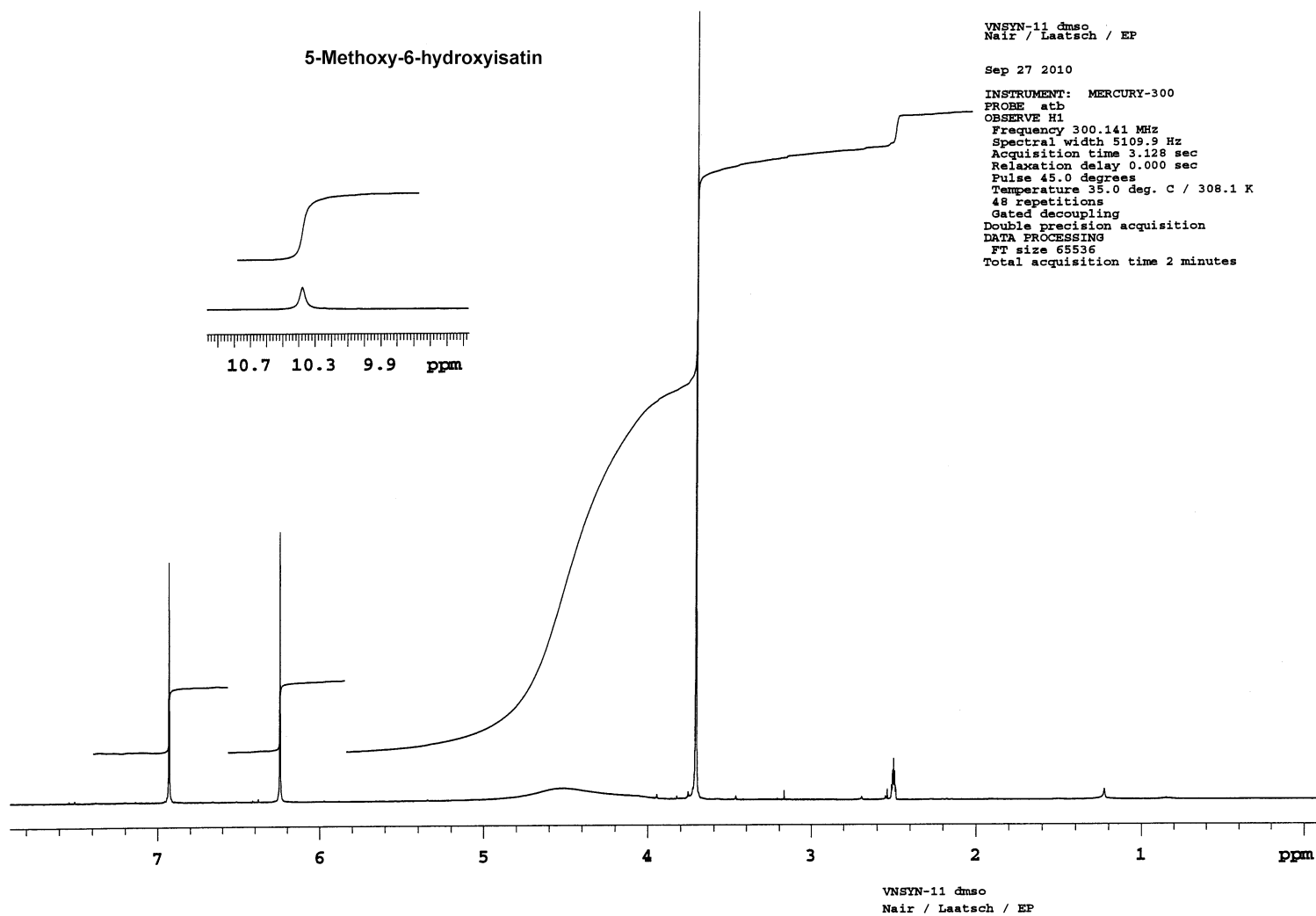




**Figure S18:** Calculated UV/Vis spectrum of 6-hydroxy-5-methoxy-isatin (**4**); orange = neutral, blue = basic.



**Figure S19:**  $^1\text{H}$  NMR spectrum (600 MHz) of 6-hydroxy-5-methoxy-isatin (**4**) in methanol- $d_4$ ; also 2D data are available in methanol



**Figure S20:**  $^1\text{H}$  NMR spectrum (300 MHz) of 6-hydroxy-5-methoxy-isatin (**4**) in  $\text{DMSO}-d_6$ .

5-Methoxy-6-hydroxyisatin

vnsyn-11 d6-dmsc  
Nair/Laatsch  
Sep 28 2010

INSTRUMENT INOVA-500  
PROBE coldc 3mm  
OBSERVE C13  
Frequency 125.707 MHz  
Spectral width 30120.5 Hz  
Acquisition time 2.125 sec  
Relaxation delay 0.000 sec  
Pulse width 36.0 degrees  
Temperature 35.0 deg. C / 308.1 K  
No. repetitions 640  
DECOUPLE H1  
Frequency 499.879 MHz  
Power 32 dB  
Decoupler continuously on  
WALTZ-16 modulated  
Double precision acquisition  
DATA PROCESSING  
Line broadening 1.0 Hz  
FT size 262144  
Total acquisition time 22 minutes

vnsyn-11 d6-dmsc  
Nair/Laatsch  
SPECTRAL LINES FOR TH= 4.9  
FROM -2.5 PPM TO 197.5 PPM  
RFL= 6897.9 RFP= 4964.9

INDEX	FREQ	PPM	HEIGHT
1	22585.1	179.68	24.5
2	20334.9	161.78	38.7
3	20203.0	160.73	13.8
4	18736.2	149.06	37.2
5	18205.6	144.84	33.4
6	13544.8	107.76	75.2
7	13416.5	106.74	26.1
8	12555.7	99.89	67.9
9	7034.7	55.97	130.1
10	5039.1	40.09	5.0
11	5027.9	40.00	42.9
12	5018.0	39.92	11.8
13	5007.0	39.83	129.1
14	4996.6	39.75	20.4
15	4986.1	39.67	255.2
16	4964.9	39.50	300.0
17	4944.0	39.33	254.7
18	4923.1	39.17	126.8
19	4902.0	39.00	41.2

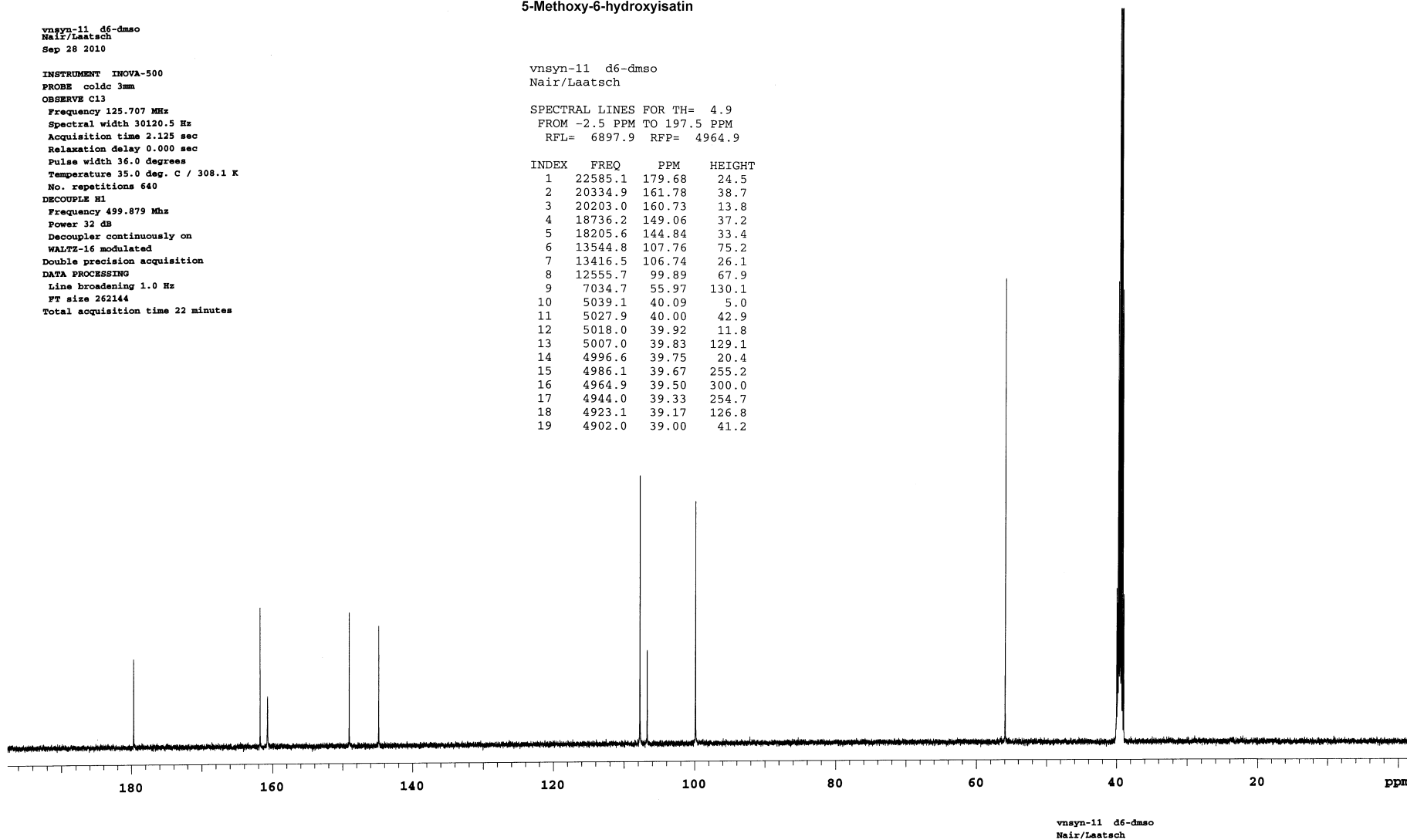


Figure S21:  $^{13}\text{C}$  NMR spectrum (125 MHz) of 6-hydroxy-5-methoxy-isatin (4) in  $\text{DMSO}-d_6$ .

iou3g3 cd3od  
Oka/Laatsch

Apr 29 2004

INSTRUMENT: INOVA-600

Pulse sequence gHMQC

OBSERVE H1

Frequency 599.742 MHz

Spectral width 4501.7 Hz

2D Spectral width 36199.1 Hz

Acquisition time 0.150 sec

Relaxation delay 1.000 sec

Ambient temperature

16 repetitions

2 x 160 increments

Double precision acquisition

DATA PROCESSING

Sine bell squared 0.057 sec

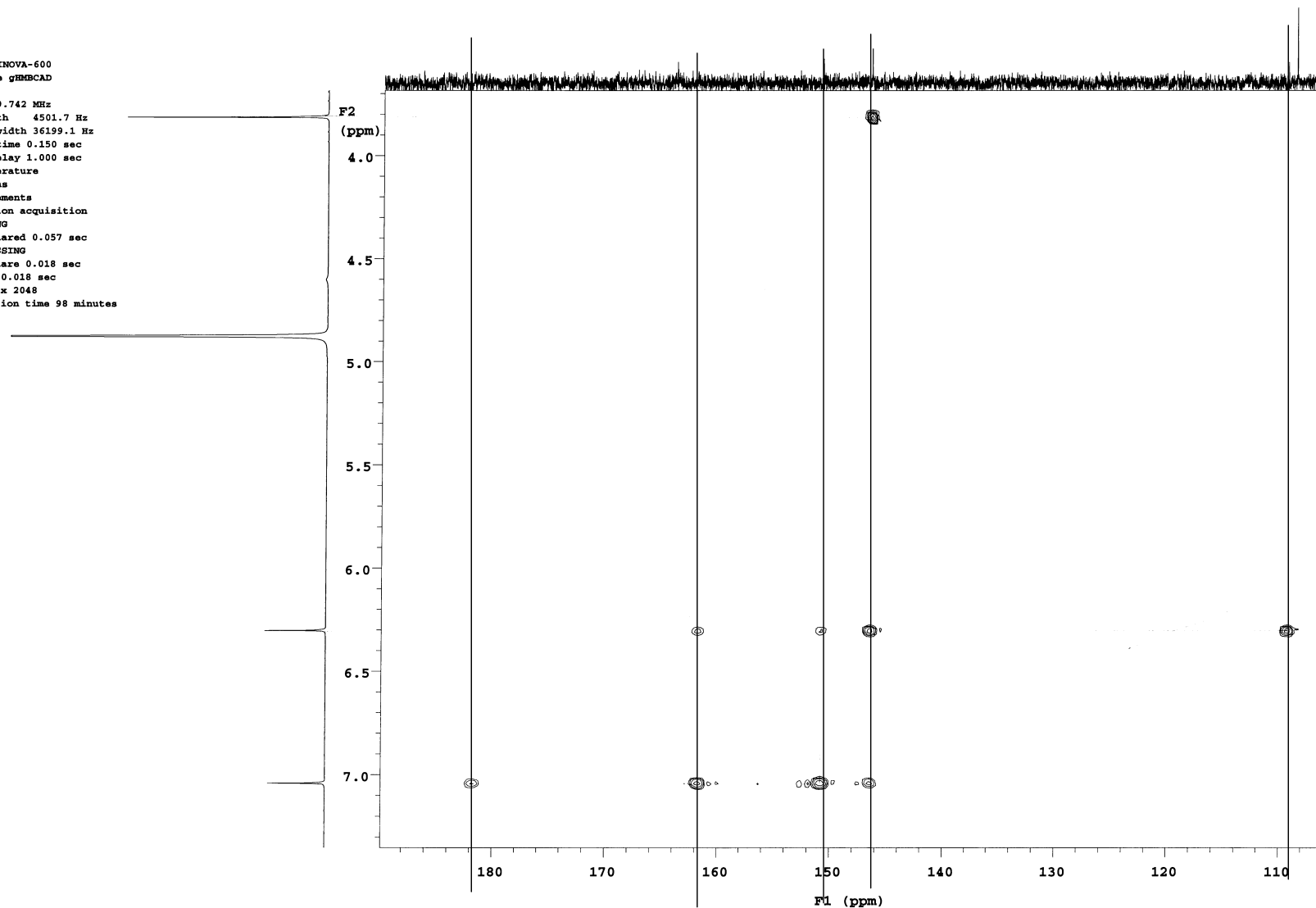
F1 DATA PROCESSING

Sine bell square 0.018 sec

Shifted by -0.018 sec

FT size 1024 x 2048

Total acquisition time 98 minutes



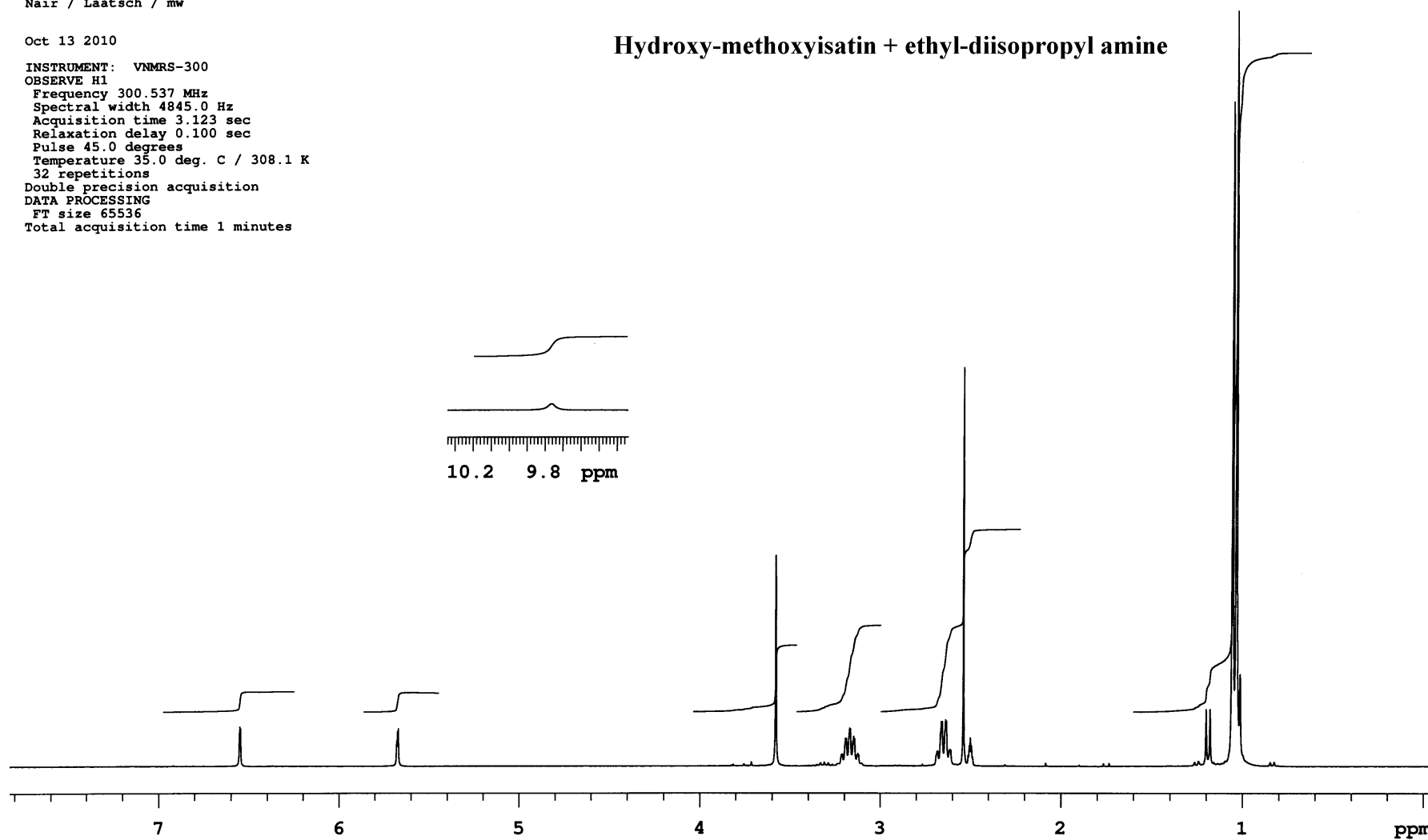
**Figure S22:** HMBC spectrum (600 MHz) of 6-hydroxy-5-methoxy-isatin (**4**) in DMSO- $d_6$ .

VNSYN-11+DIPEA dms0-d6  
Nair / Laatsch / mw

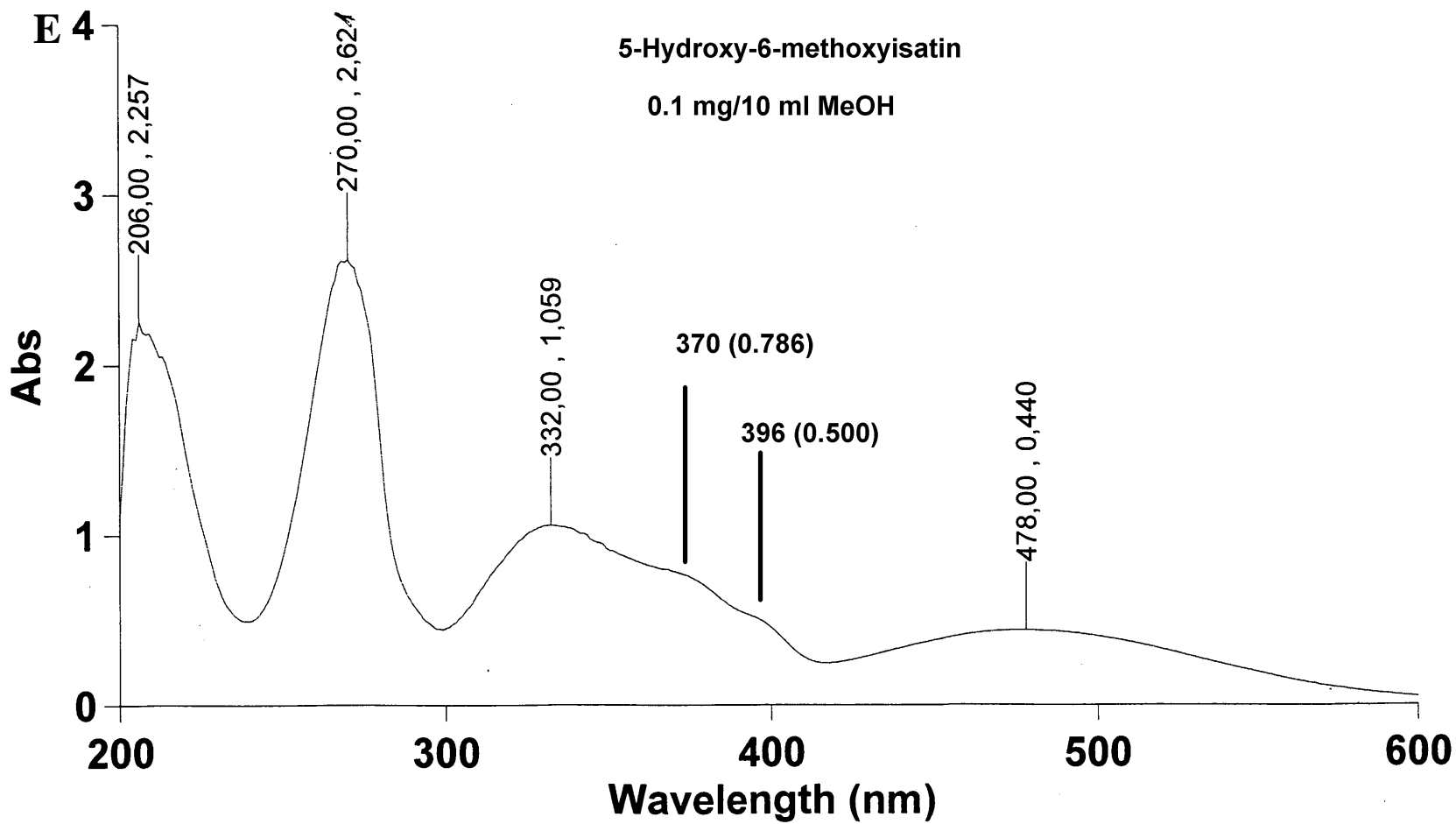
Oct 13 2010

INSTRUMENT: VNMRS-300  
OBSERVE H1  
Frequency 300.537 MHz  
Spectral width 4845.0 Hz  
Acquisition time 3.123 sec  
Relaxation delay 0.100 sec  
Pulse 45.0 degrees  
Temperature 35.0 deg. C / 308.1 K  
32 repetitions  
Double precision acquisition  
DATA PROCESSING  
FT size 65536  
Total acquisition time 1 minutes

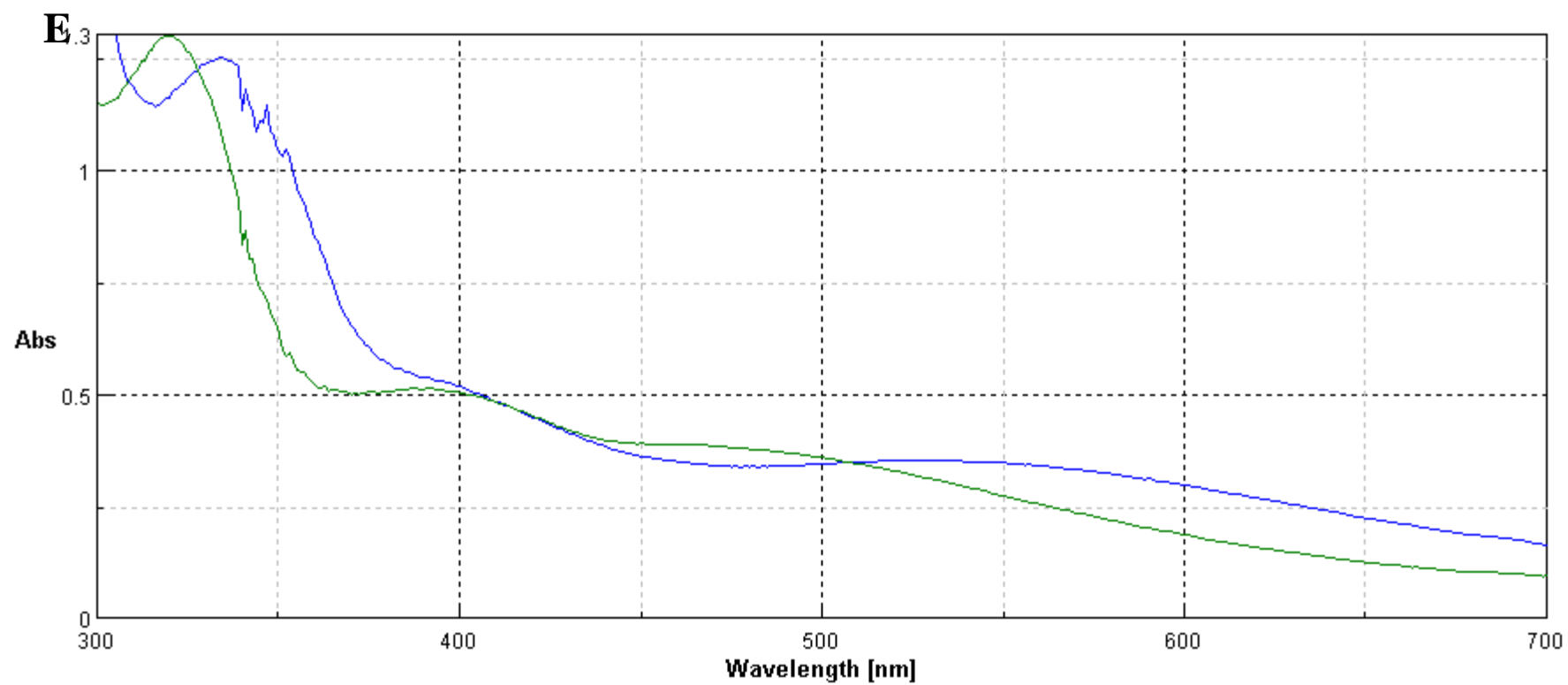
# Hydroxy-methoxyisatin + ethyl-diisopropyl amine



**Figure S23:** <sup>1</sup>H NMR spectrum (300 MHz) of 6-hydroxy-5-methoxy-isatin (**4**) in DMSO-*d*<sub>6</sub> + ethyl-diisopropylamine.

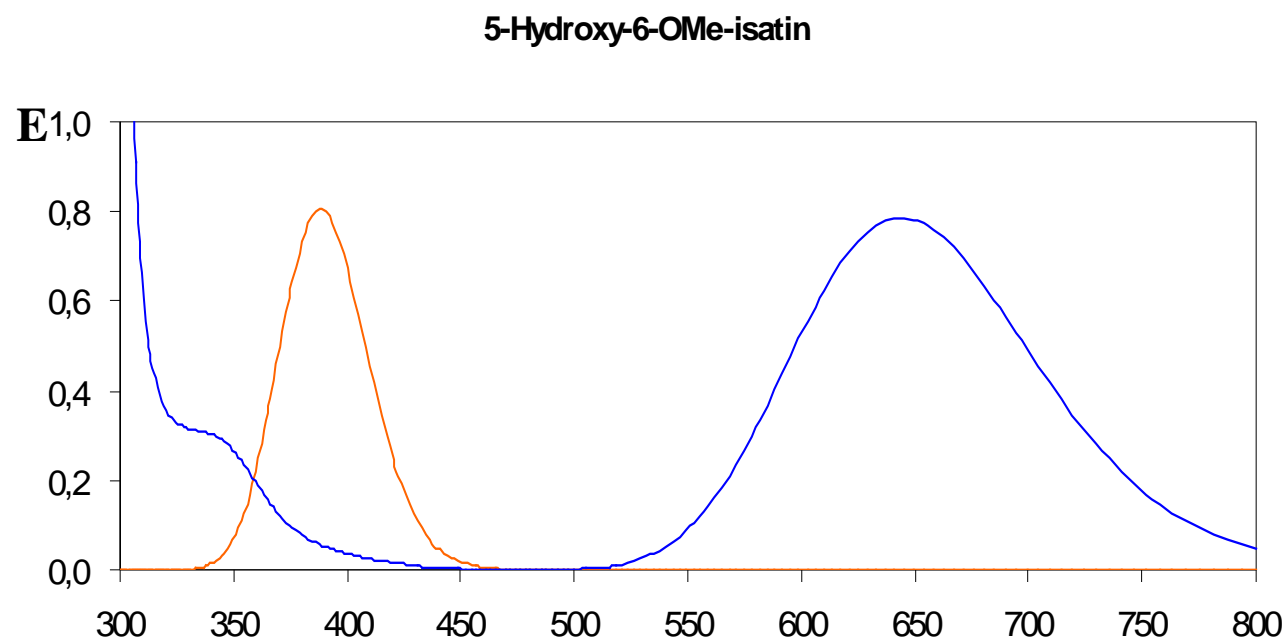


**Figure S24:** Exp. UV/Vis spectrum of 5-hydroxy-6-methoxy-isatin (**5**) in methanol (neutral)

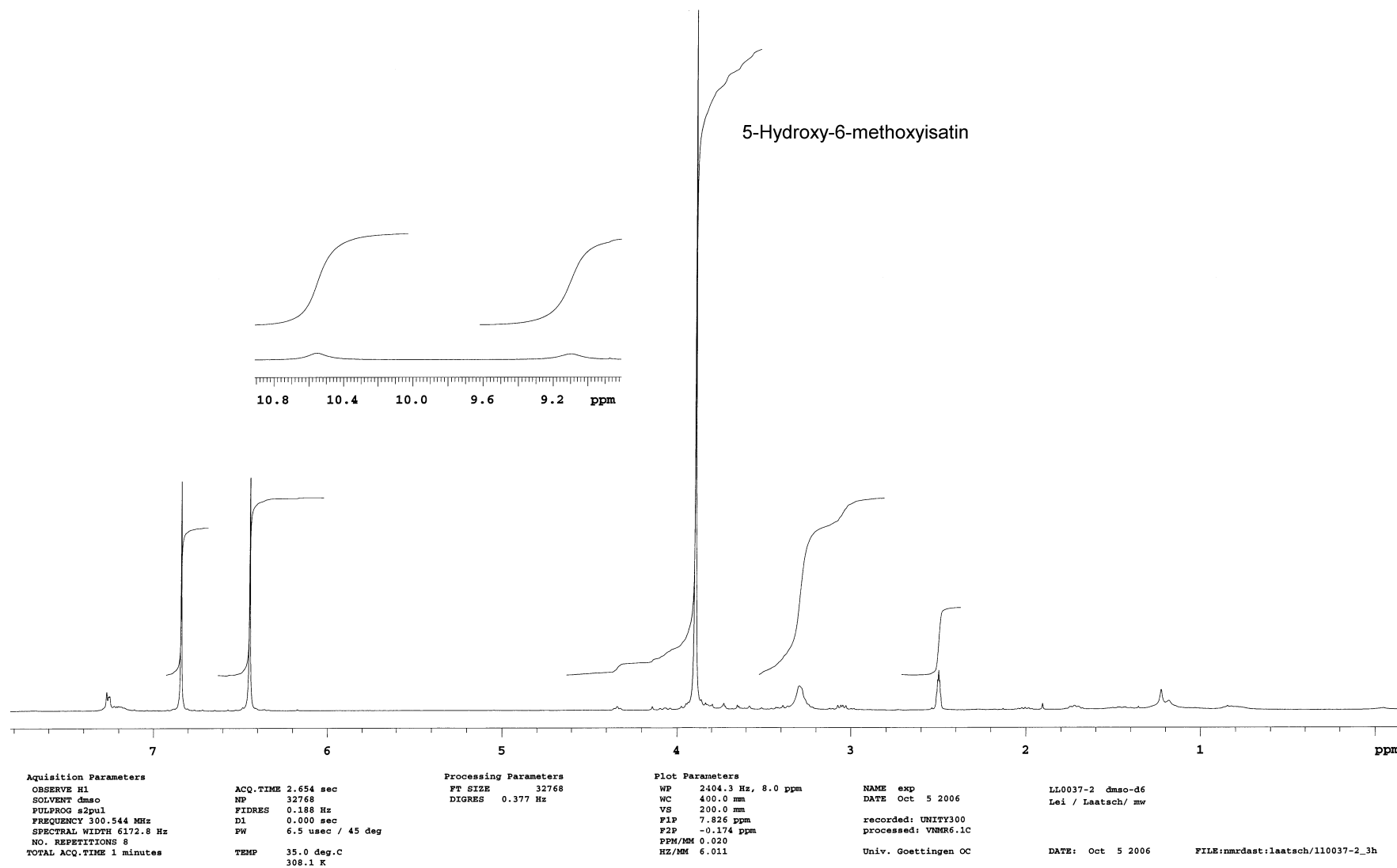


**Figure S25:** Enlarged section of the exp. UV/Vis spectrum of 5-hydroxy-6-methoxy-isatin (**5**) in methanol; green = neutral, blue = basic.

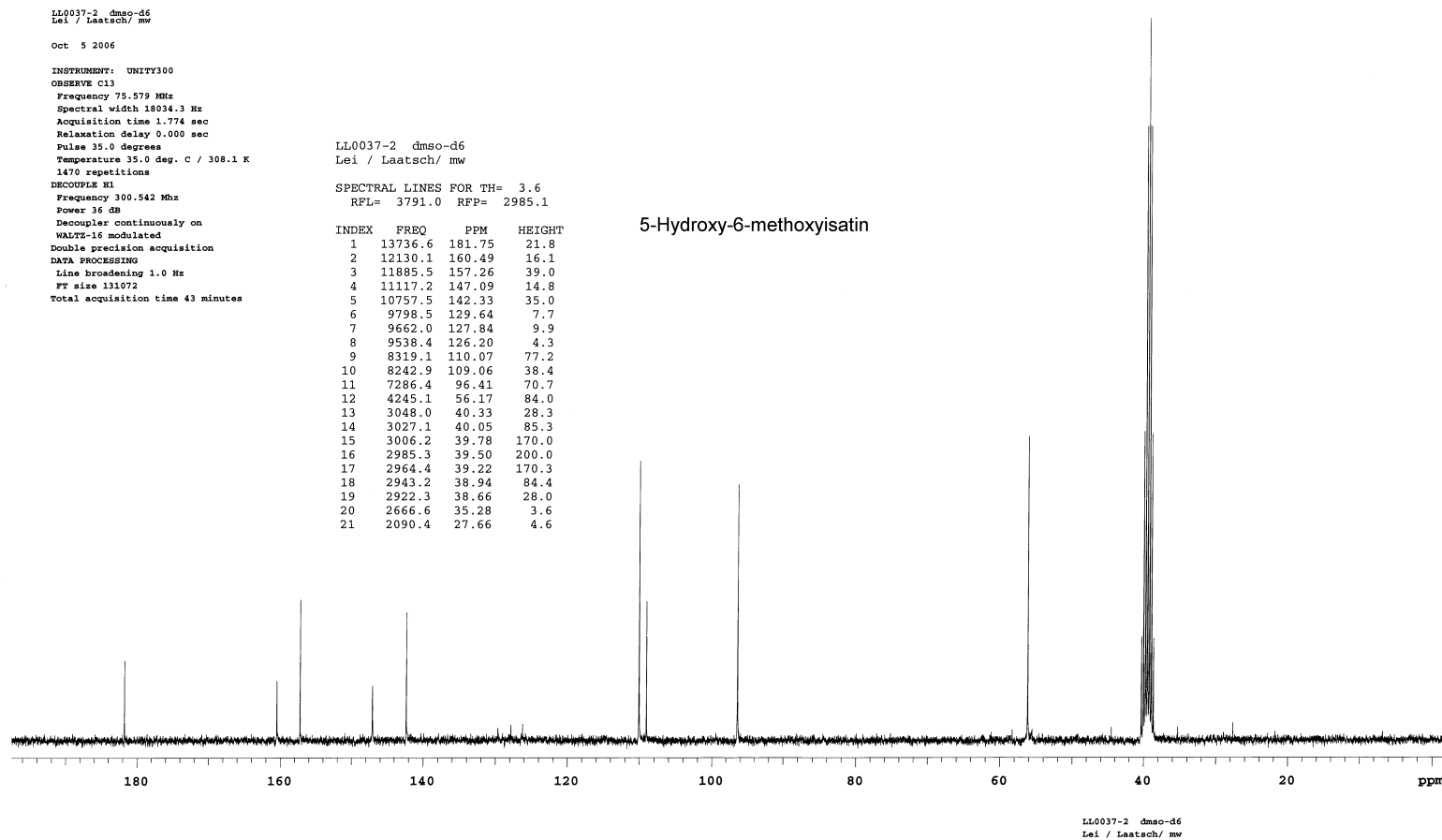




**Figure S26:** Calculated UV/Vis spectrum of 5-hydroxy-6-methoxy-isatin (**5**); orange = neutral, blue = basic.



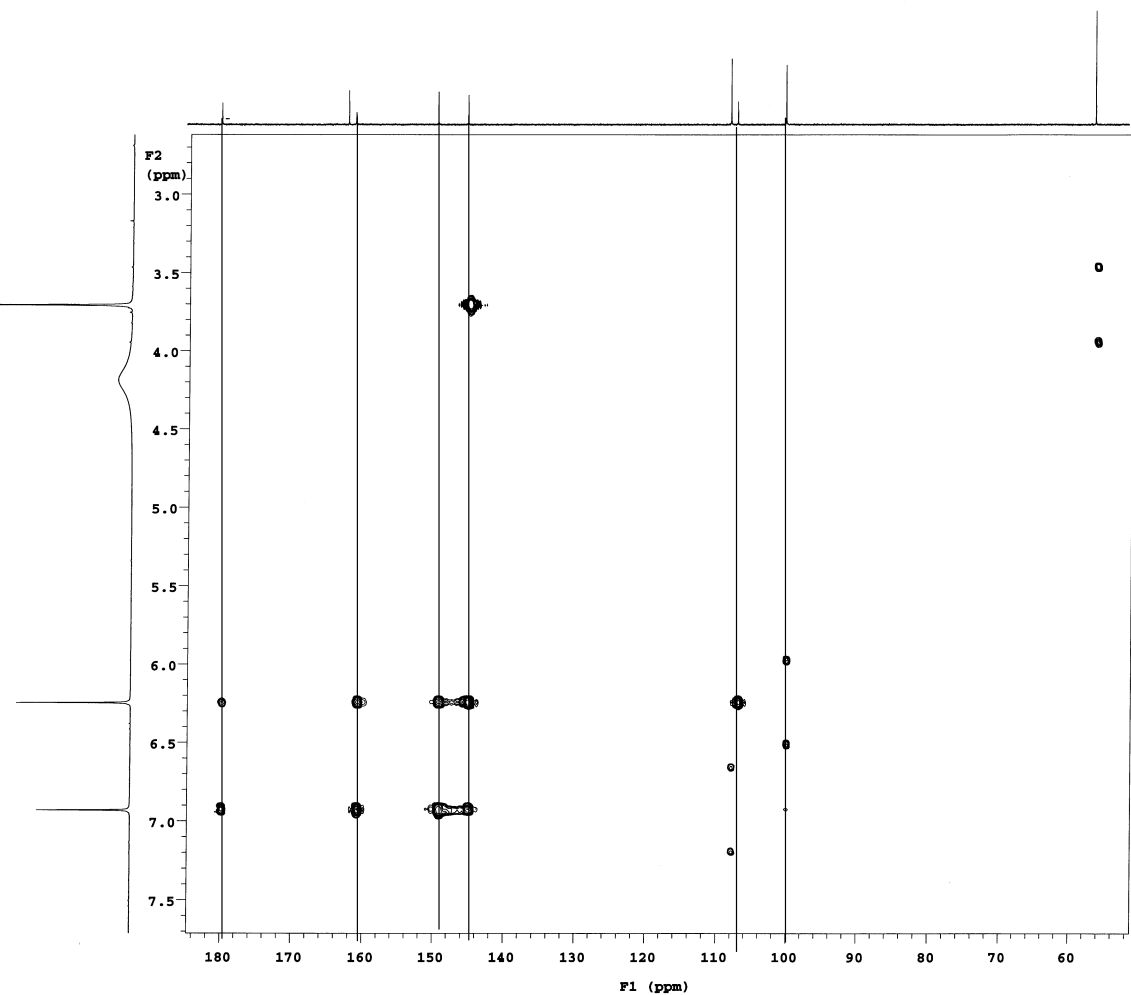
**Figure S27:**  $^1\text{H}$  NMR spectrum (300 MHz) of 5-hydroxy-6-methoxy-isatin (**5**) in  $\text{DMSO}-d_6$ .



**Figure S28:**  $^{13}\text{C}$  NMR spectrum (75 MHz) of 5-hydroxy-6-methoxy-isatin (**5**) in  $\text{DMSO-}d_6$ .

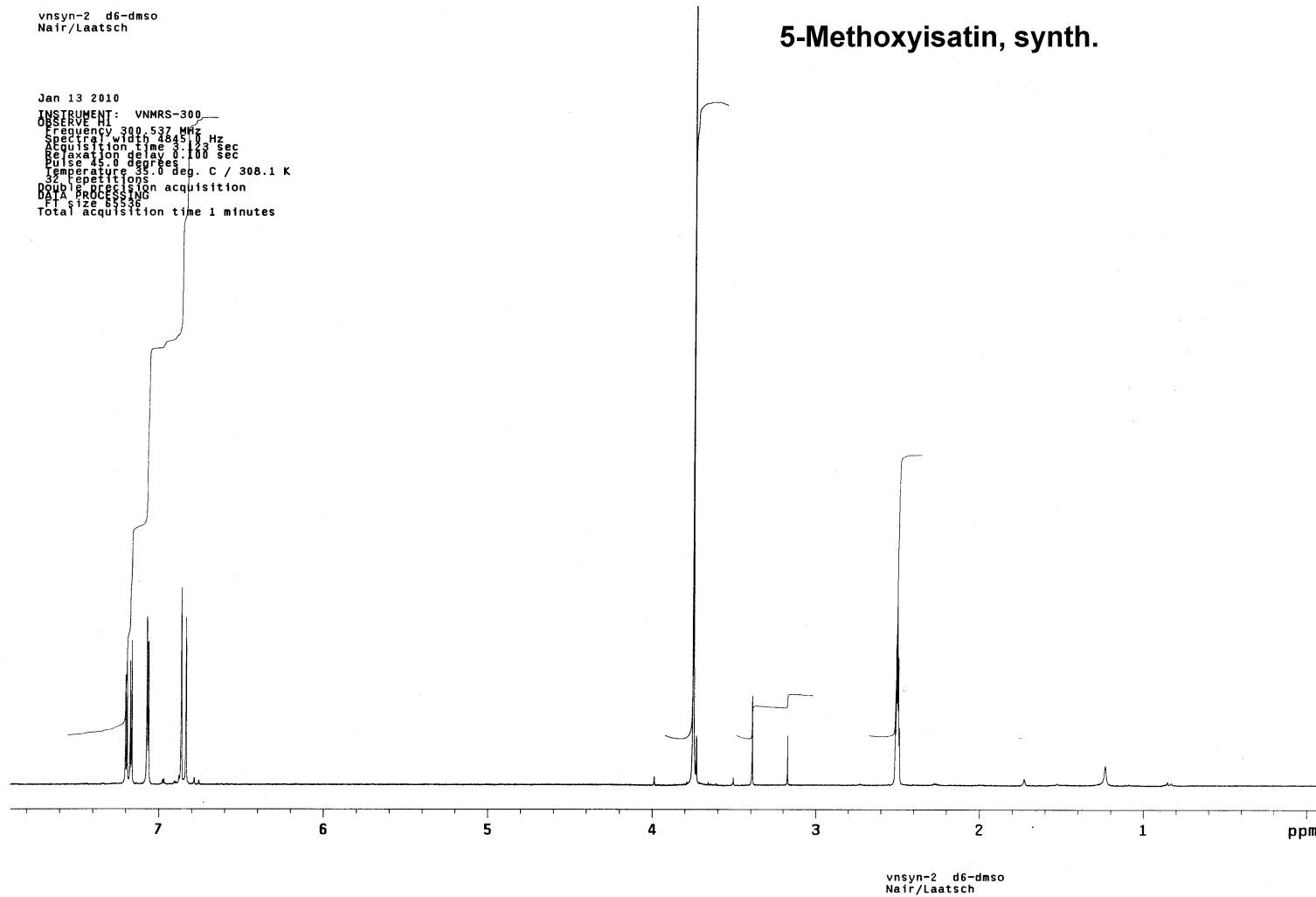
VN1SYN-11 6mas-d6  
 Nair / Laatsch / mw  
 Oct 2 2010  
 INSTRUMENT MERCURY-300  
 PROBE atb  
 Pulse sequence ghmec  
 OBSERVE H1  
 Frequency 300.141 MHz  
 Spectral width 3273.3 Hz  
 2D Spectral width 12837.0 Hz  
 Acquisition time 0.150 sec  
 Relaxation delay 1.000 sec  
 Temperature 35.0 deg. C / 308.1 K  
 No. repetitions 96  
 No. increments 256  
 Double precision acquisition  
 DATA PROCESSING  
 Sine bell 0.075 sec  
 FT size 1024  
 F1 DATA PROCESSING  
 Sine bell 0.020 sec  
 FT size 2048  
 Total acquisition time 7:55 hours

VS= 288  
 TH= 2

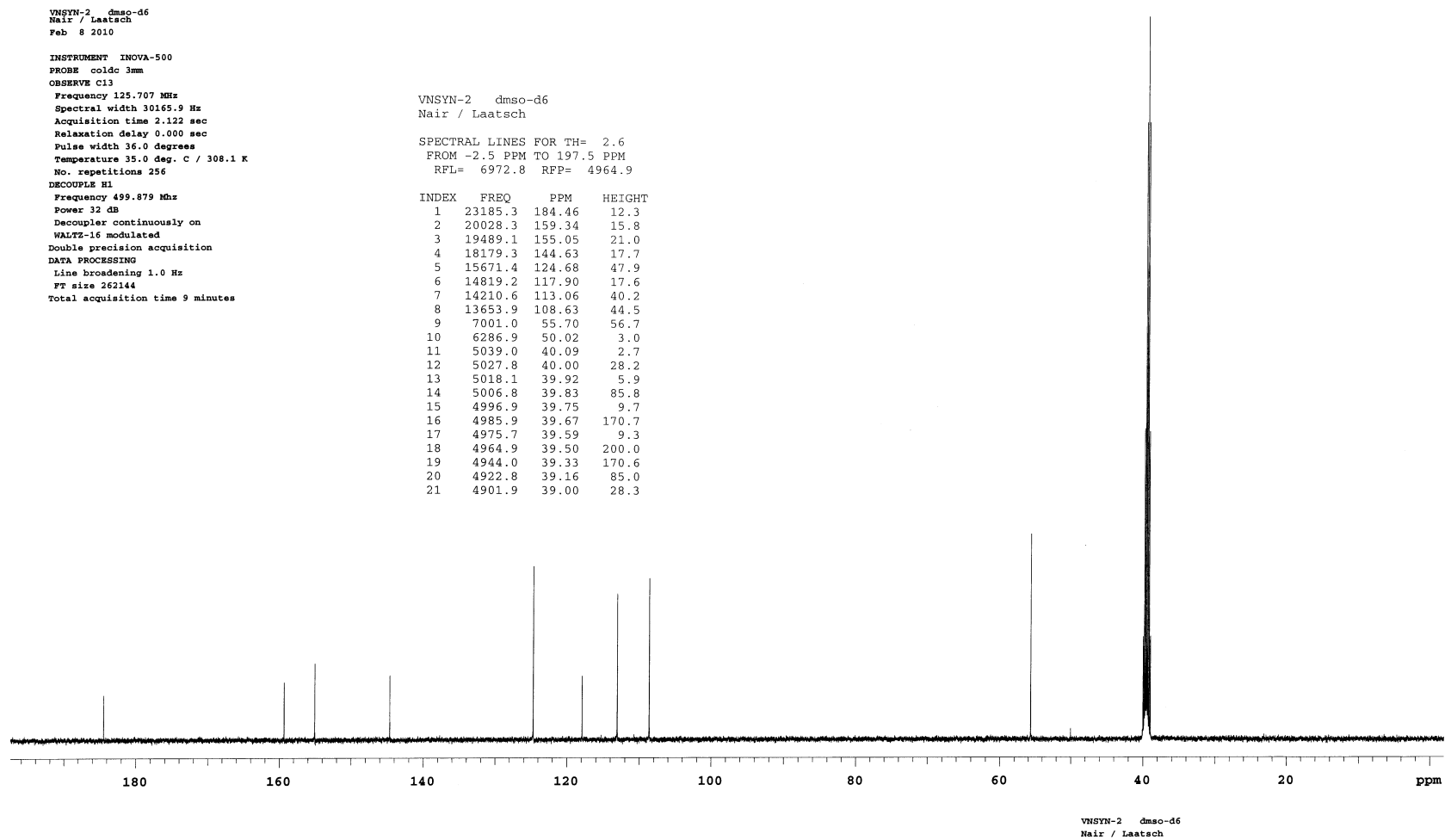


FILE=msdset: laatsch/vn1syn-11\_3ghmbo

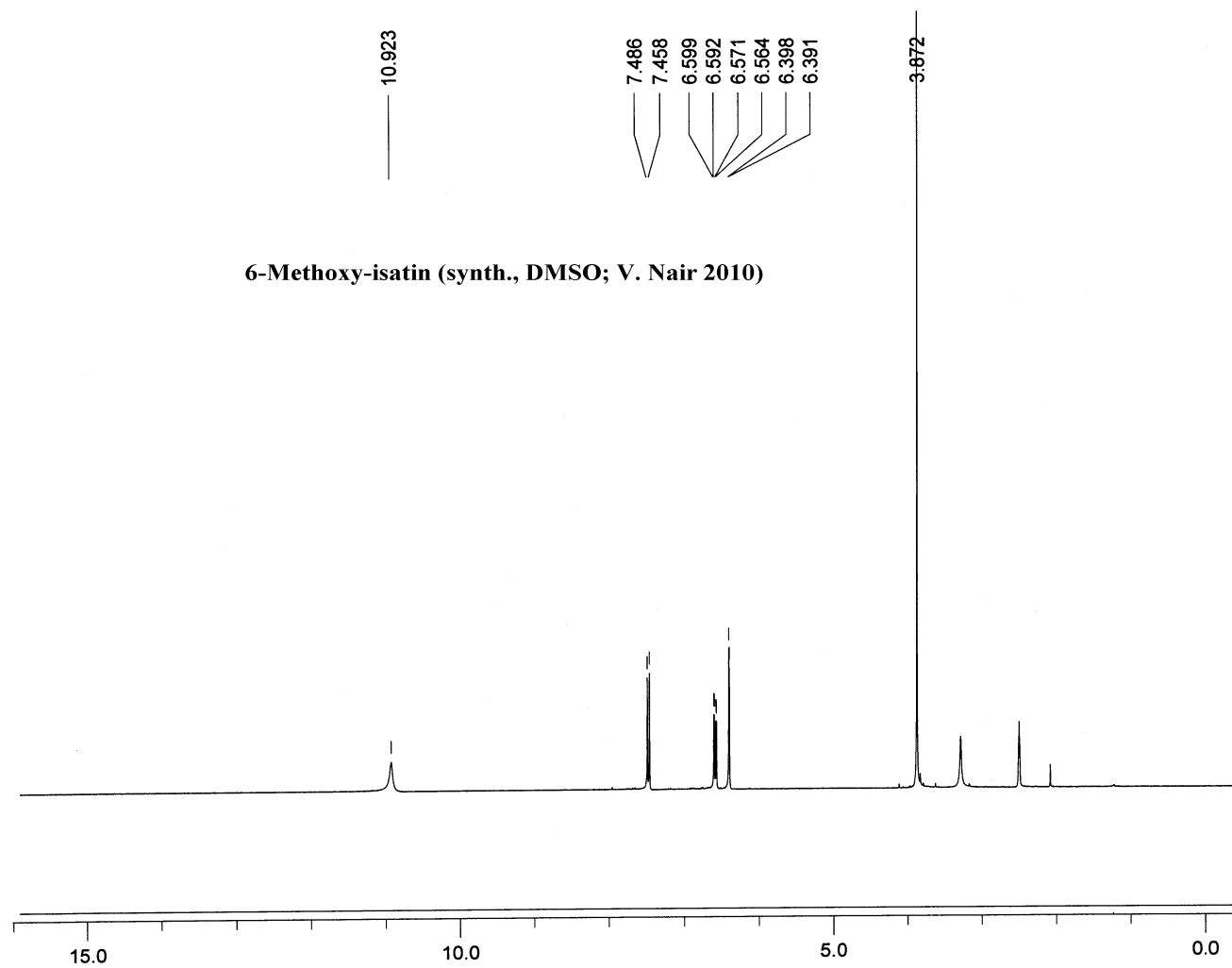
**Figure S29:** HMBC spectrum (300 MHz) of 5-hydroxy-6-methoxy-isatin (**5**) in DMSO-*d*<sub>6</sub>.



**Figure S30:**  $^1\text{H}$  NMR spectrum (300 MHz) of 5-methoxy-isatin (**6**) in  $\text{DMSO}-d_6$ .



**Figure S31:**  $^{13}\text{C}$  NMR spectrum (125 MHz) of 5-methoxy-isatin (**6**) in  $\text{DMSO}-d_6$ .



**Figure S32:**  $^1\text{H}$  NMR spectrum (300 MHz) of 6-methoxy-isatin (**7**) in  $\text{DMSO}-d_6$ .

VNSYN-6 dms0-d6  
Nair / Laatsch / mw  
Jan 7 2011

INSTRUMENT INOVA-500  
PROBE coldc 3mm  
OBSERVE C13  
Frequency 125.707 MHz  
Spectral width 30120.5 Hz  
Acquisition time 2.125 sec  
Relaxation delay 0.000 sec  
Pulse width 36.0 degrees  
Temperature 35.0 deg. C / 308.1 K  
No. repetitions 640  
DECOUPLE H1  
Frequency 499.879 MHz  
Power 32 db  
Decoupler continuously on  
WALTZ-16 modulated  
Double precision acquisition  
DATA PROCESSING  
Line broadening 1.0 Hz  
FT size 262144  
Total acquisition time 22 minutes

VNSYN-6 dms0-d6  
Nair / Laatsch / mw

SPECTRAL LINES FOR TH= 3.6  
FROM -2.5 PPM TO 197.5 PPM  
RFL= 6900.0 RFP= 4964.9

INDEX	FREQ	PPM	HEIGHT
1	22808.3	181.46	15.3
2	21079.5	167.70	24.6
3	20177.5	160.53	20.2
4	19296.5	153.52	24.1
5	15989.9	127.21	47.6
6	13966.2	111.11	21.5
7	13671.2	108.77	51.3
8	12287.8	97.76	59.1
9	7047.4	56.07	57.5
10	5027.9	40.00	29.1
11	5018.0	39.92	8.4
12	5007.0	39.83	86.3
13	4996.9	39.75	14.5
14	4985.8	39.67	170.7
15	4964.9	39.50	200.0
16	4944.0	39.33	169.9
17	4922.9	39.17	85.1
18	4902.0	39.00	28.0

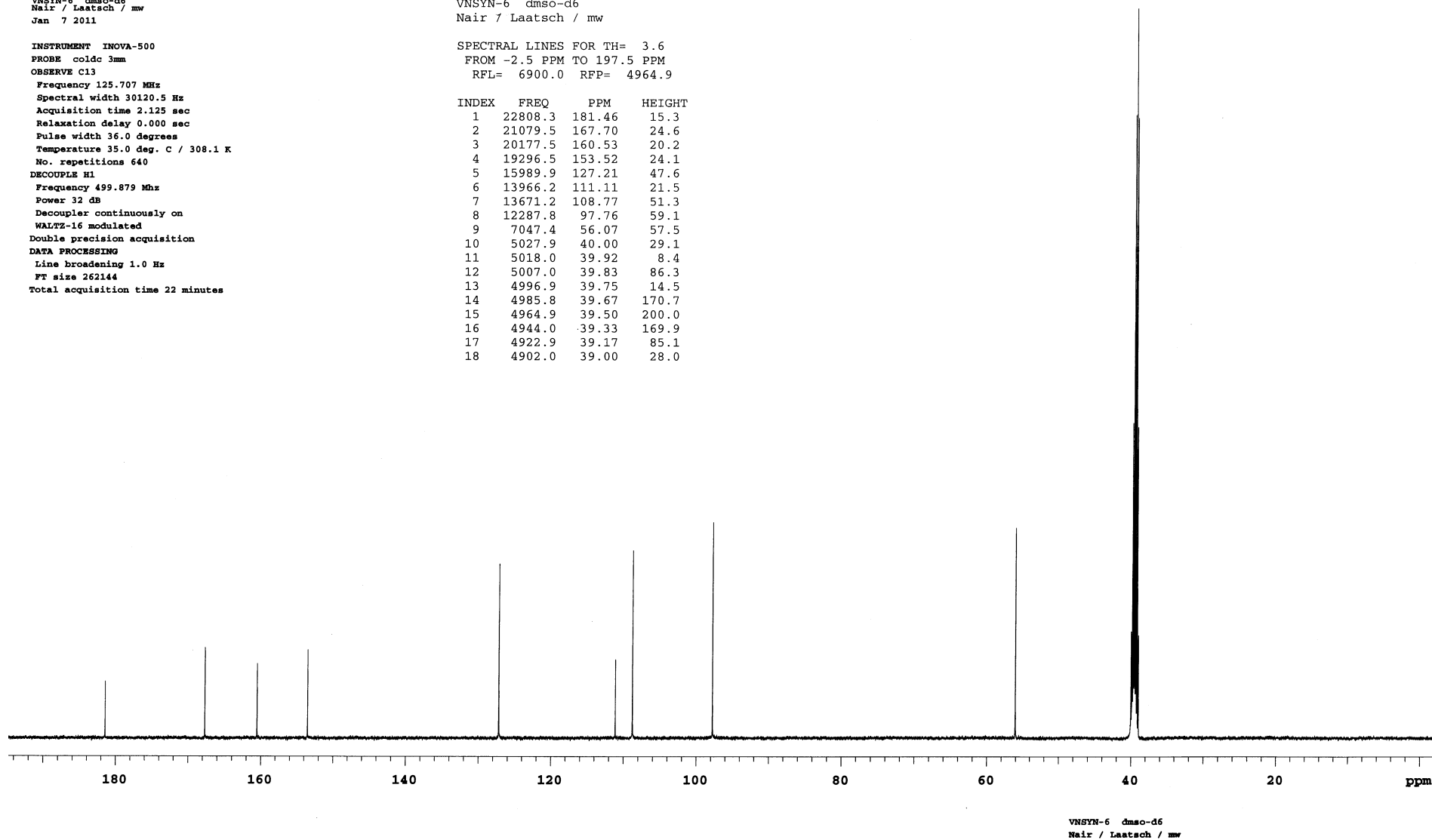


Figure S33:  $^{13}\text{C}$  NMR spectrum (125 MHz) of 6-methoxy-isatin (**7**) in  $\text{DMSO-}d_6$ .



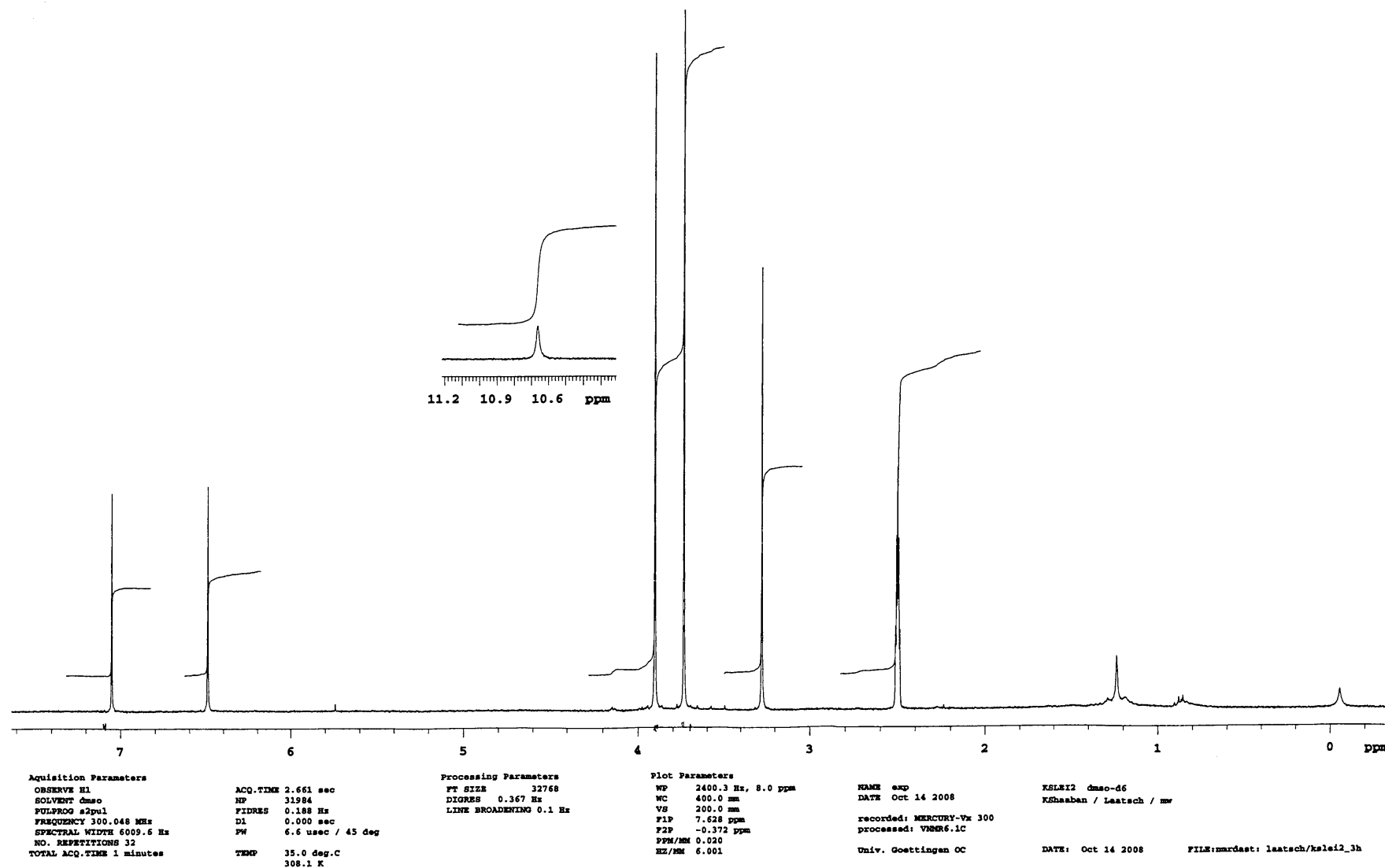


Figure S34:  $^1\text{H}$  NMR spectrum (300 MHz) of 5,6-dimethoxy-isatin (**8**) in  $\text{DMSO}-d_6$ .

VNSYN-9 dmsc-d6  
Nair / Laatsch  
Jan 6 2011

INSTRUMENT INOVA-500  
PROBE coldc 3mm  
OBSERVE C13

Frequency 125.707 MHz  
Spectral width 30120.5 Hz  
Acquisition time 2.125 sec  
Relaxation delay 0.000 sec  
Pulse width 36.0 degrees  
Temperature 35.0 deg. C / 308.1 K  
No. repetitions 64

DECOUPLE H1  
Frequency 499.879 MHz  
Power 32 dB  
Decoupler continuously on  
WALTZ-16 modulated  
Double precision acquisition

DATA PROCESSING  
Line broadening 1.0 Hz  
FT size 262144  
Total acquisition time 2 minutes

VNSYN-9 dmsc-d6  
Nair / Laatsch

SPECTRAL LINES FOR TH= 5.9  
FROM -2.5 PPM TO 197.5 PPM  
RFL= 6900.0 RFP= 4964.9

INDEX	FREQ	PPM	HEIGHT
1	22814.7	181.51	35.7
2	20186.5	160.60	47.2
3	19896.5	158.29	49.2
4	18690.0	148.69	56.8
5	18223.3	144.98	52.8
6	13649.6	108.59	52.5
7	13483.6	107.27	81.8
8	12116.6	96.40	96.1
9	7076.1	56.30	102.5
10	7040.5	56.01	110.8
11	5028.1	40.00	27.8
12	5007.0	39.83	84.2
13	4986.1	39.67	170.2
14	4976.6	39.59	10.1
15	4964.9	39.50	200.0
16	4944.0	39.33	171.8
17	4923.1	39.17	85.8
18	4902.0	39.00	28.2

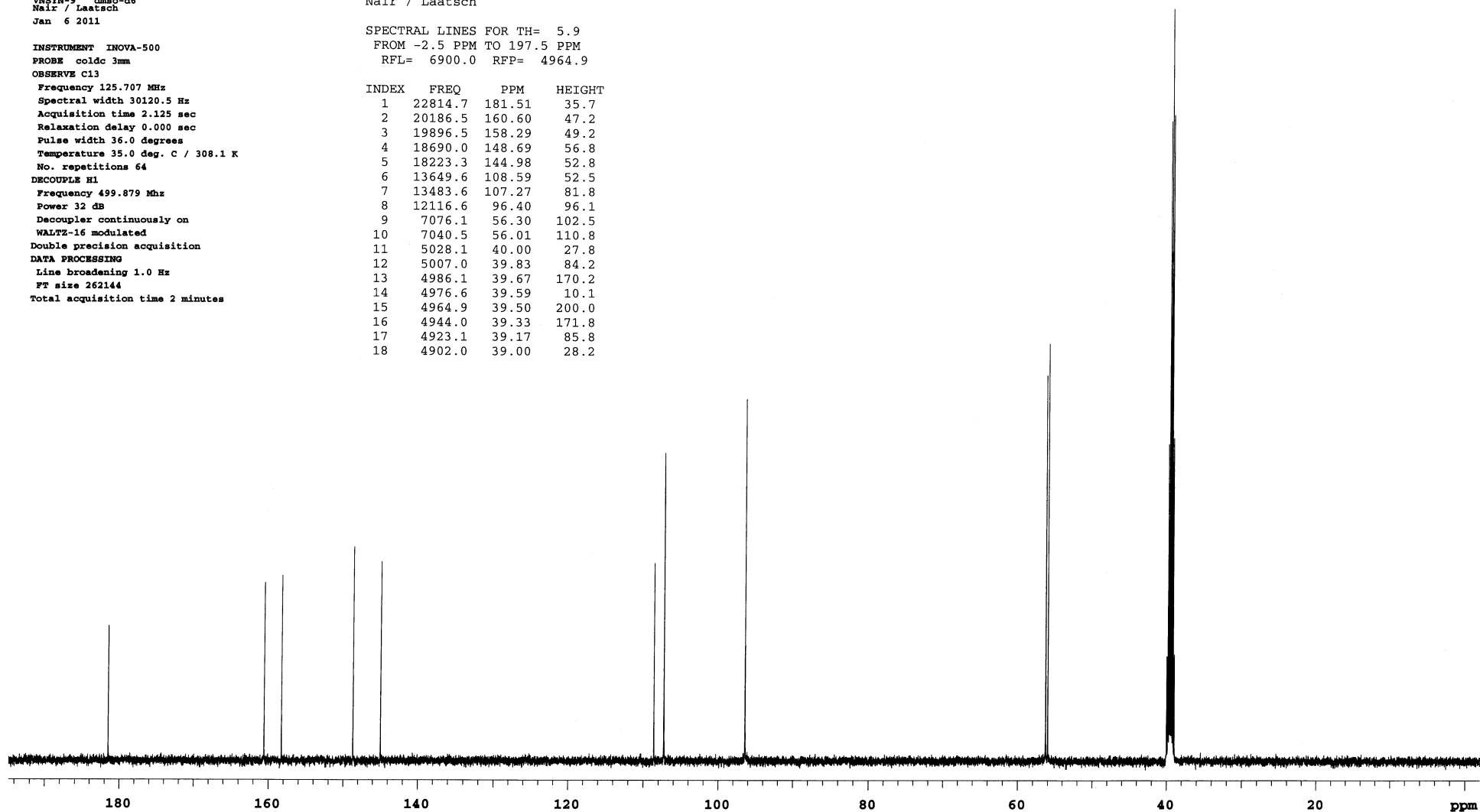
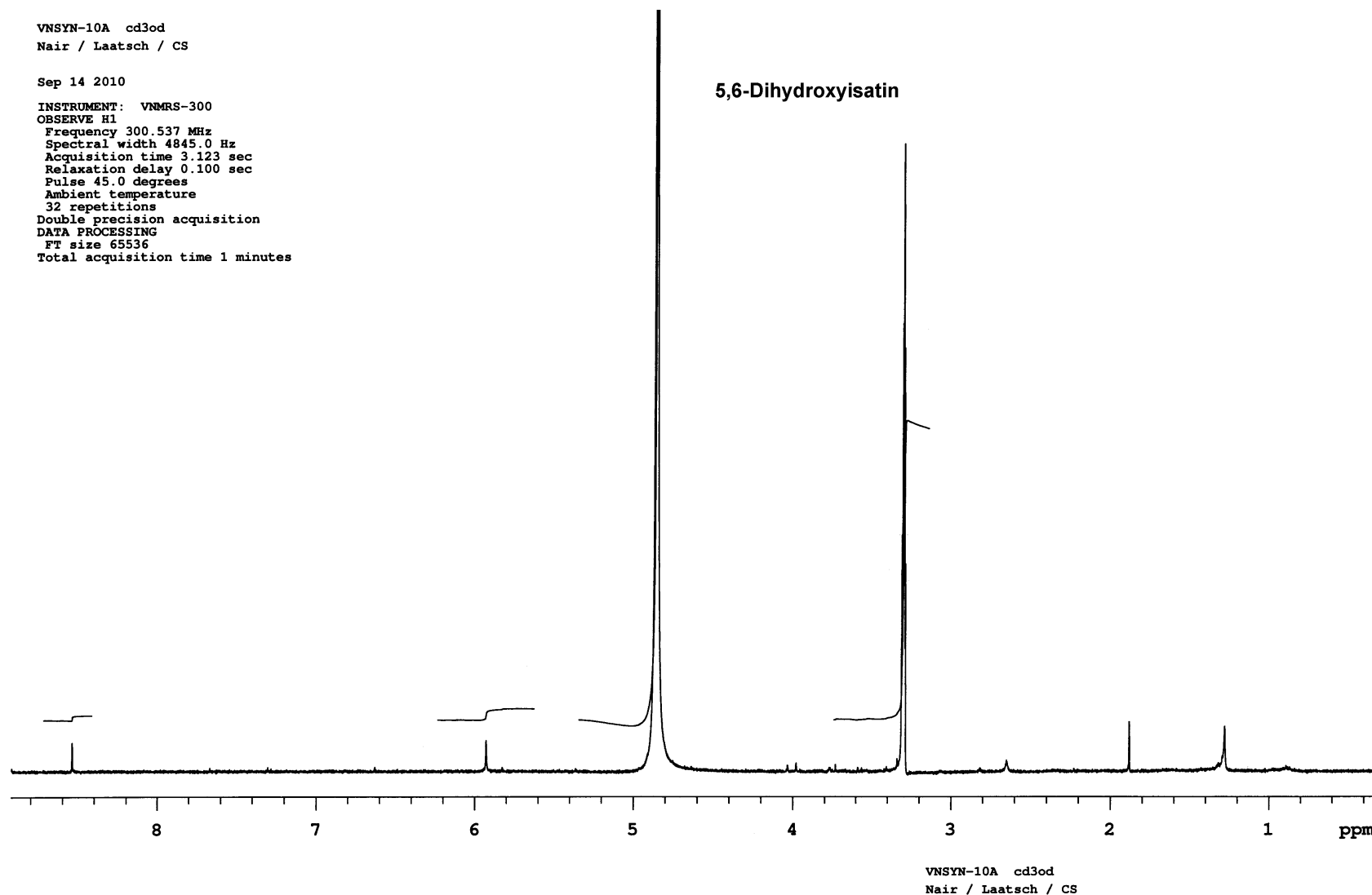


Figure S35:  $^{13}\text{C}$  NMR spectrum (125 MHz) of 5,6-dimethoxy-isatin (**8**) in  $\text{DMSO}-d_6$ .

VNSYN-10A cd3od  
Nair / Laatsch / CS

Sep 14 2010

INSTRUMENT: VNMRS-300  
OBSERVE H1  
Frequency 300.537 MHz  
Spectral width 4845.0 Hz  
Acquisition time 3.123 sec  
Relaxation delay 0.100 sec  
Pulse 45.0 degrees  
Ambient temperature  
32 repetitions  
Double precision acquisition  
DATA PROCESSING  
FT size 65536  
Total acquisition time 1 minutes



**Figure S36:**  $^1\text{H}$  NMR spectrum of 5,6-dihydroxy-isatin (**9**) in methanol- $d_4$ .