

**ESI**

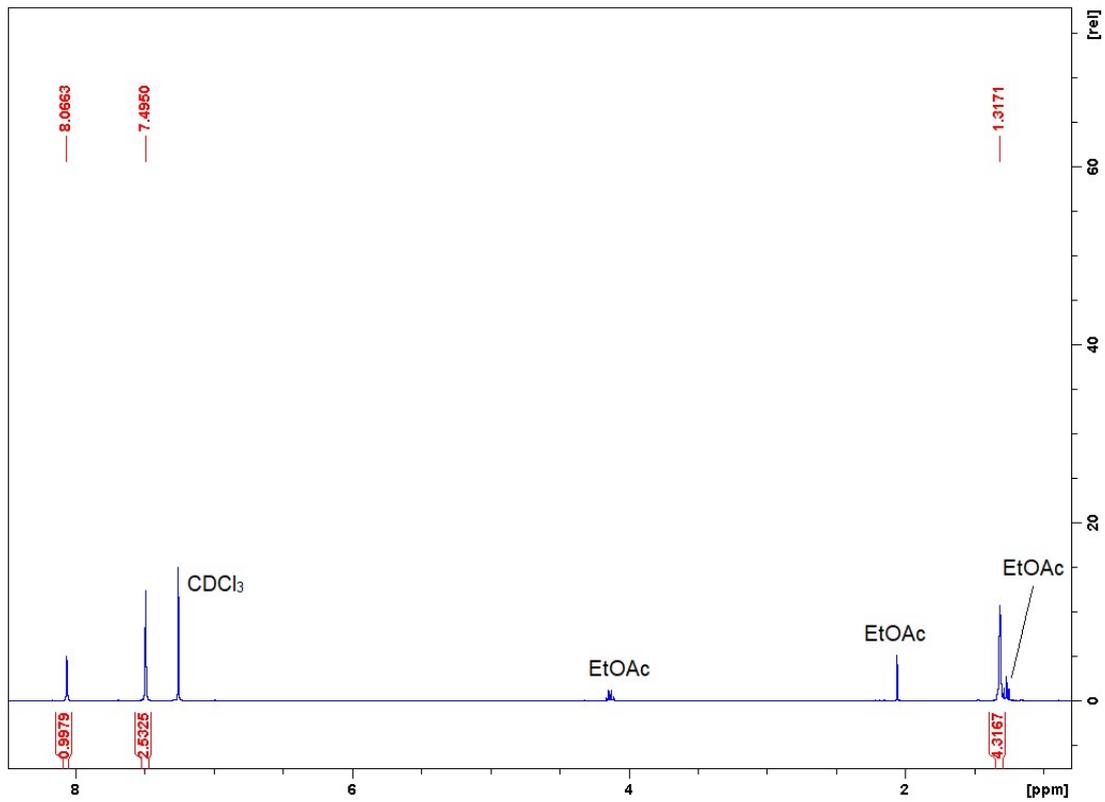
The effect of deuteration on the keto-enol equilibrium and photostability  
of the sunscreen agent avobenzone

Rhys B. Murphy, John Staton, Aditya Rawal, Tamim A. Darwish

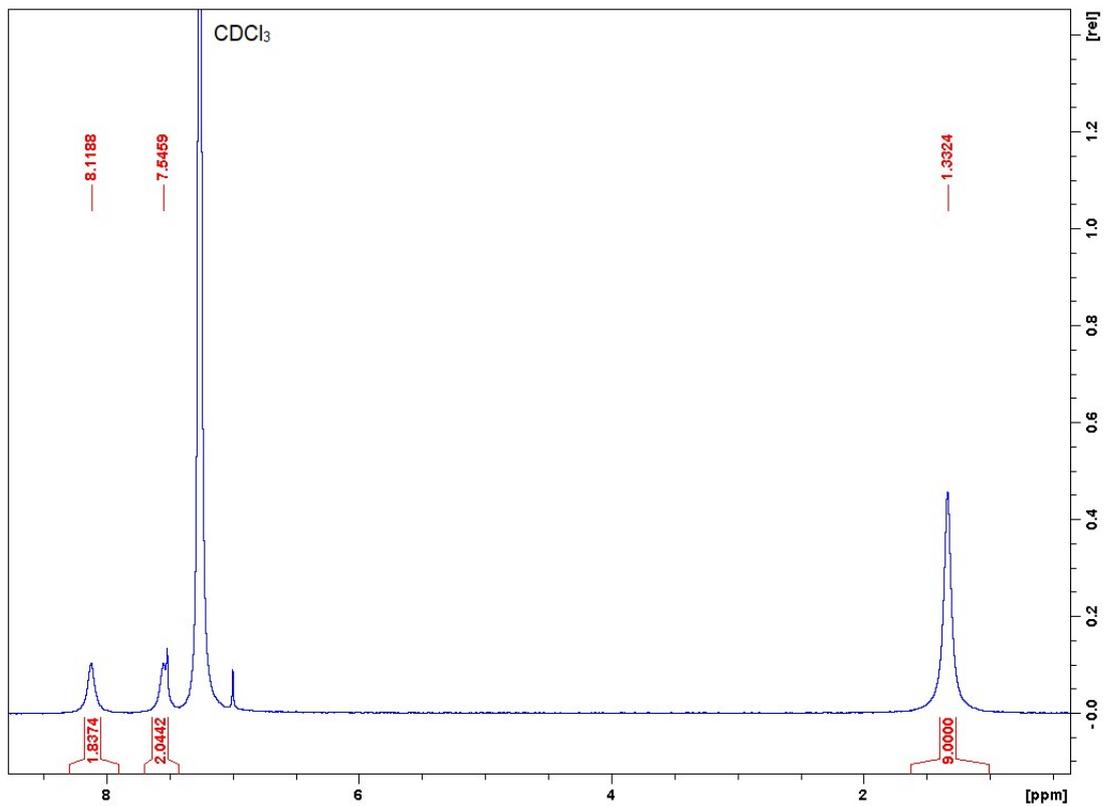
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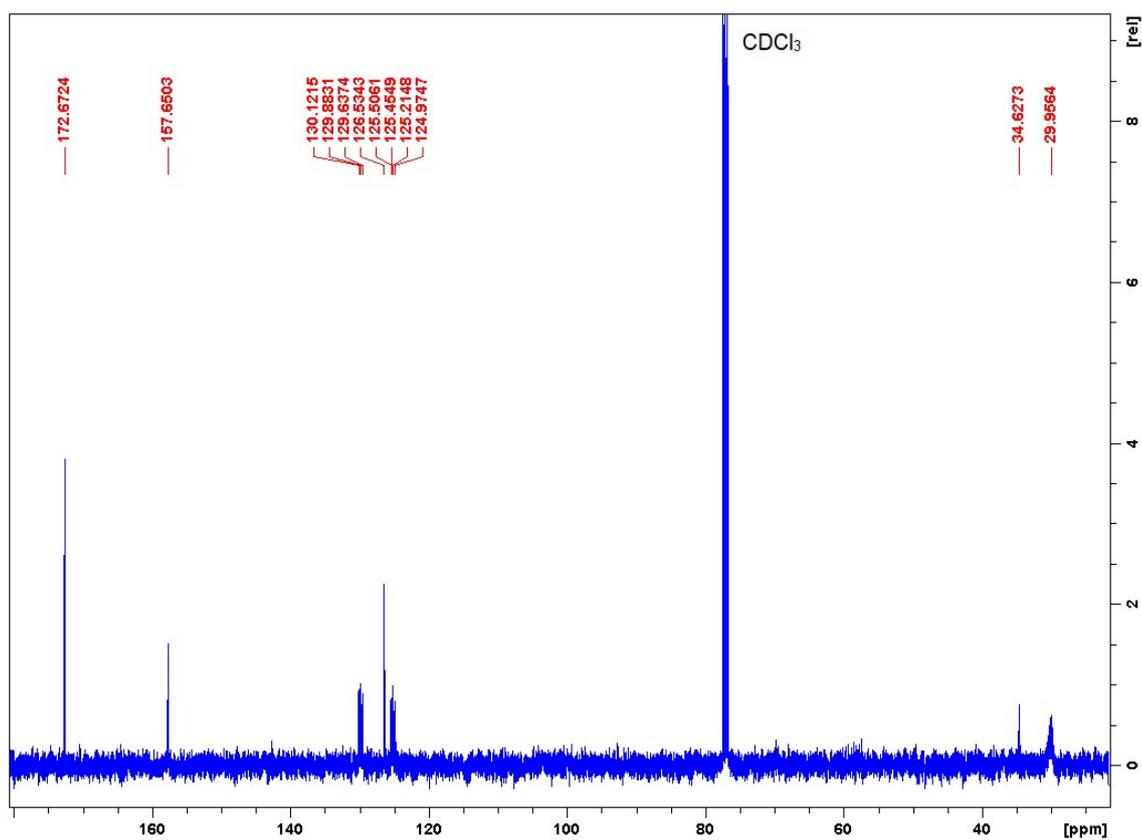
S1.  $^1\text{H}$  NMR of 4-*tert*-butylbenzoic acid- $d_{13}$ , **6**



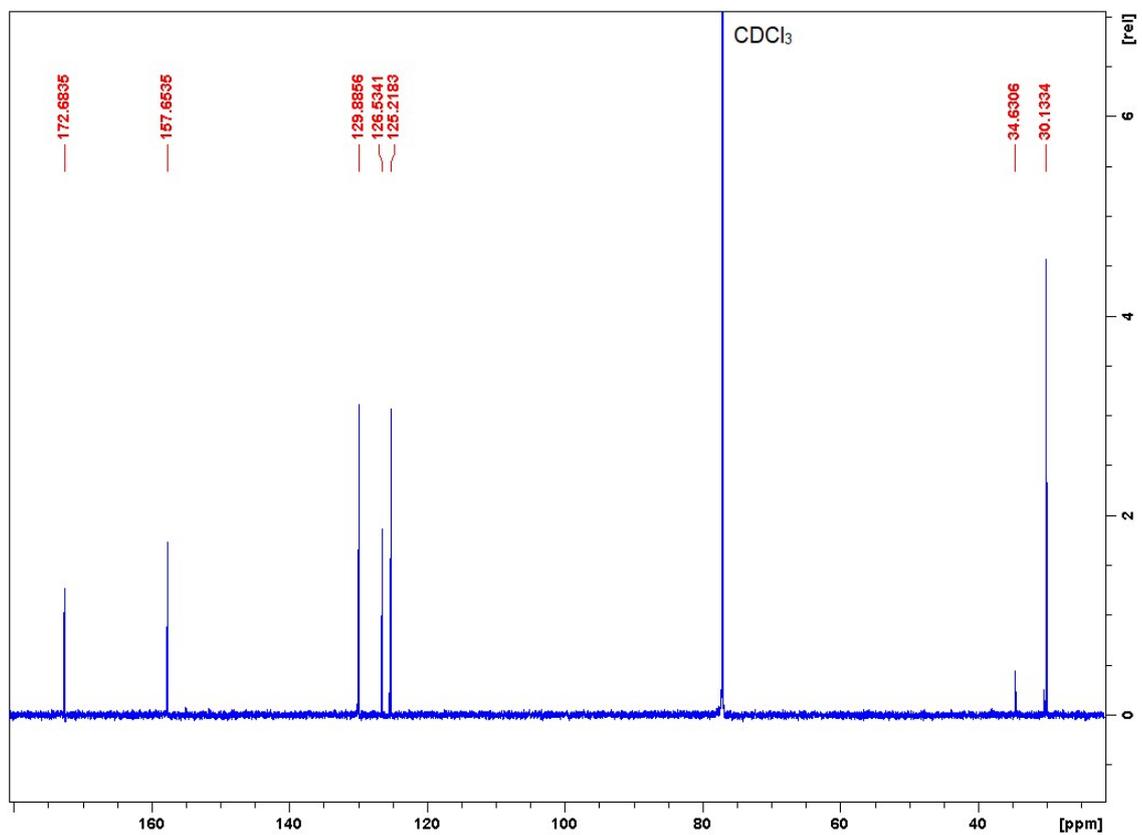
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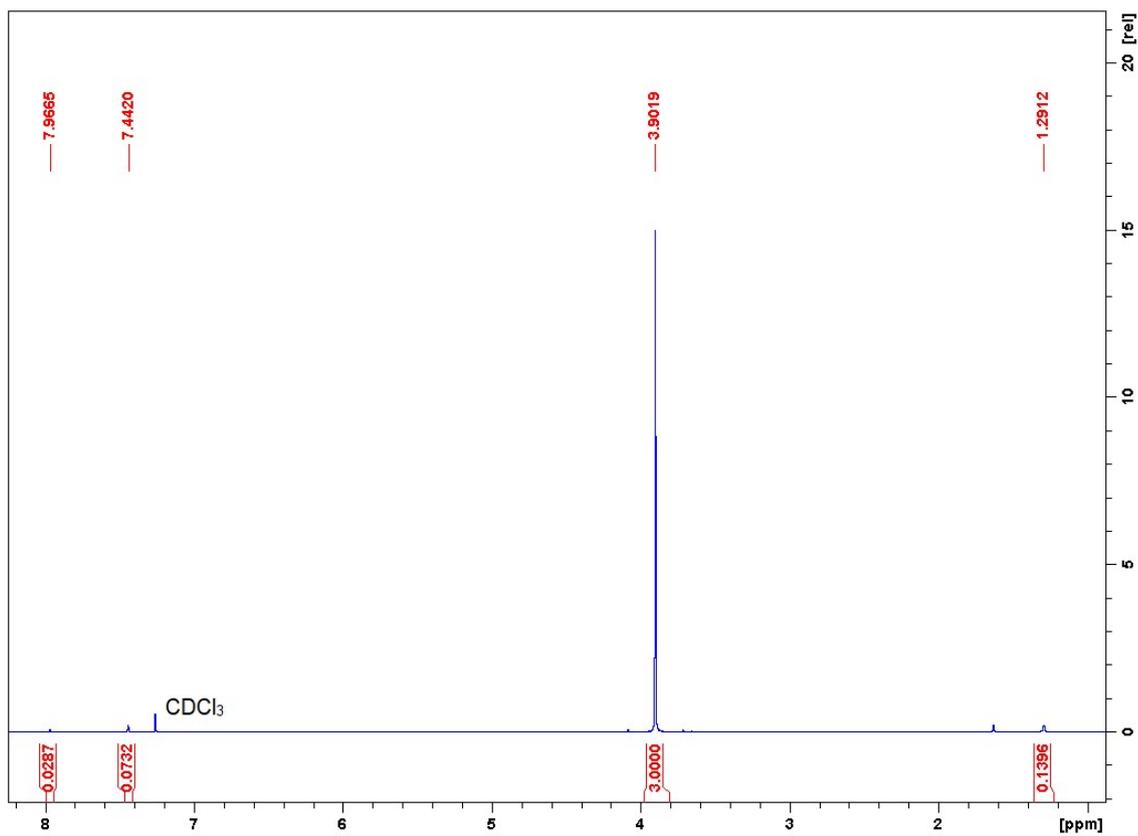
S3.  $^{13}\text{C}$  NMR ( $^1\text{H}$  power-gated decoupling) of 4-*tert*-butylbenzoic acid- $d_{13}$ , **6**



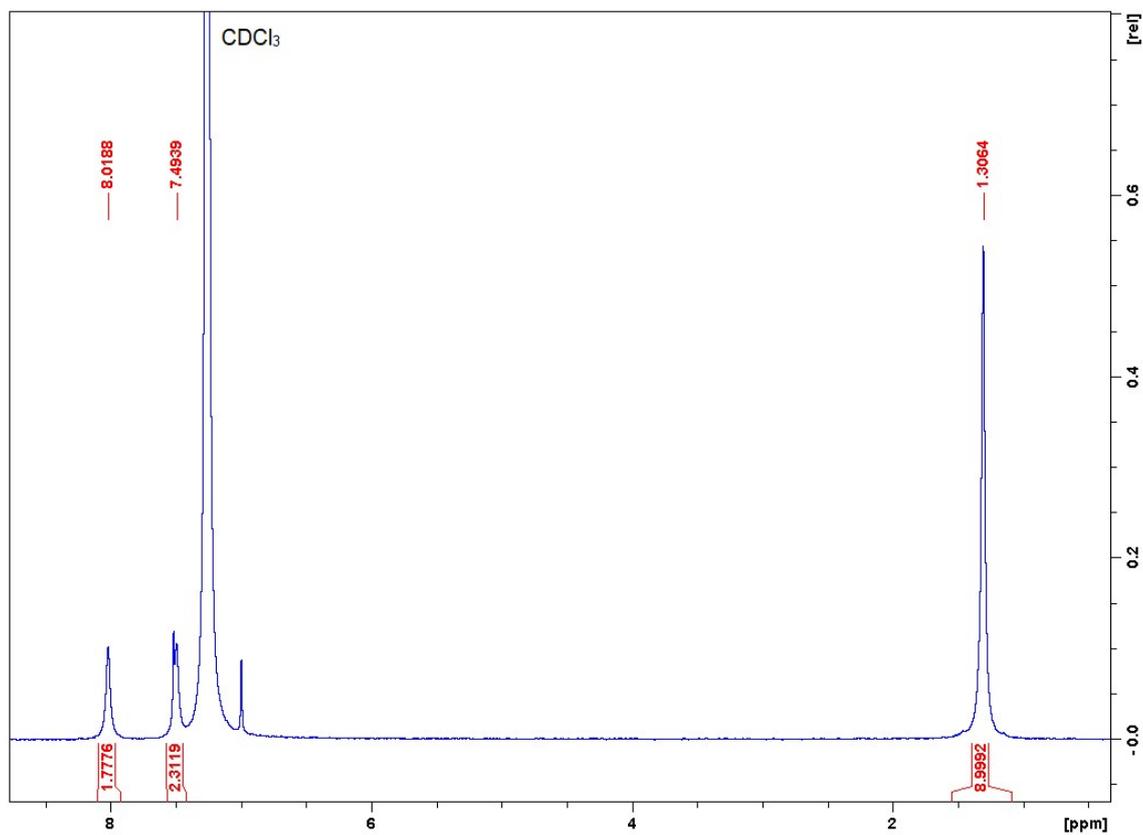
S4.  $^{13}\text{C}$  NMR ( $^1\text{H}$  and  $^2\text{H}$  gated decoupling) of 4-*tert*-butylbenzoic acid- $d_{13}$ , **6**



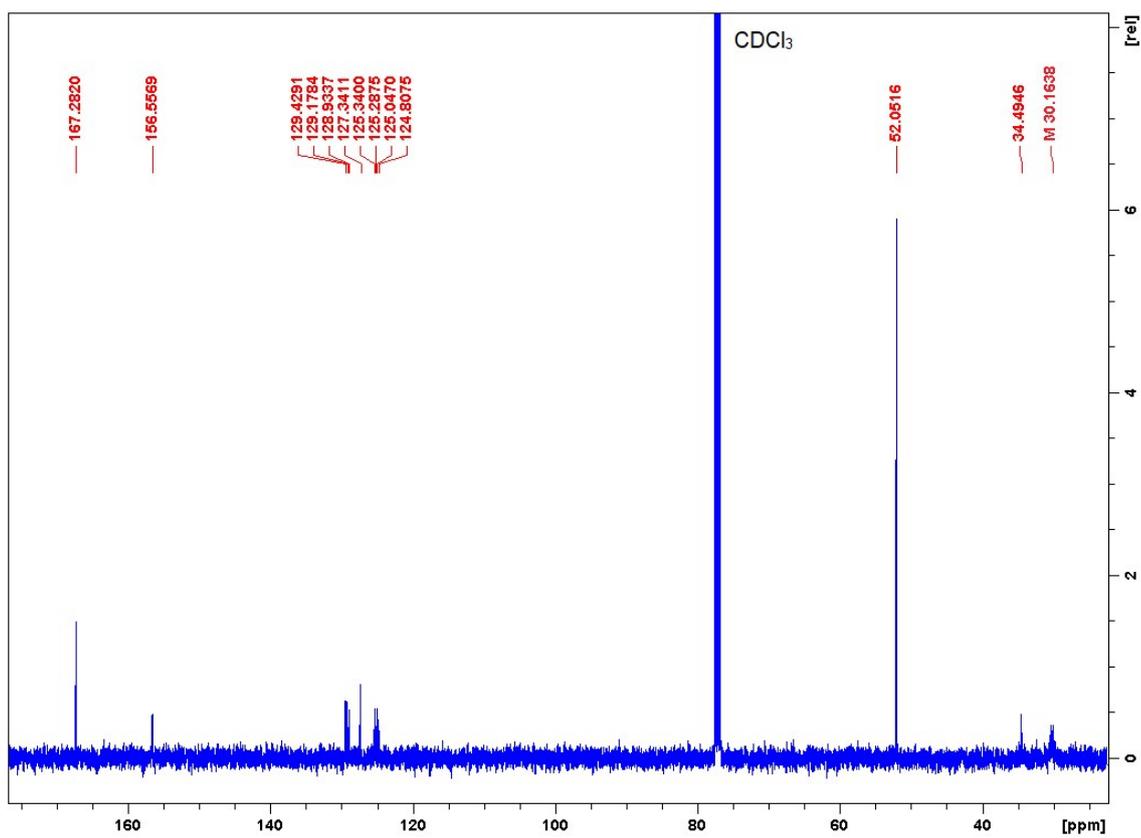
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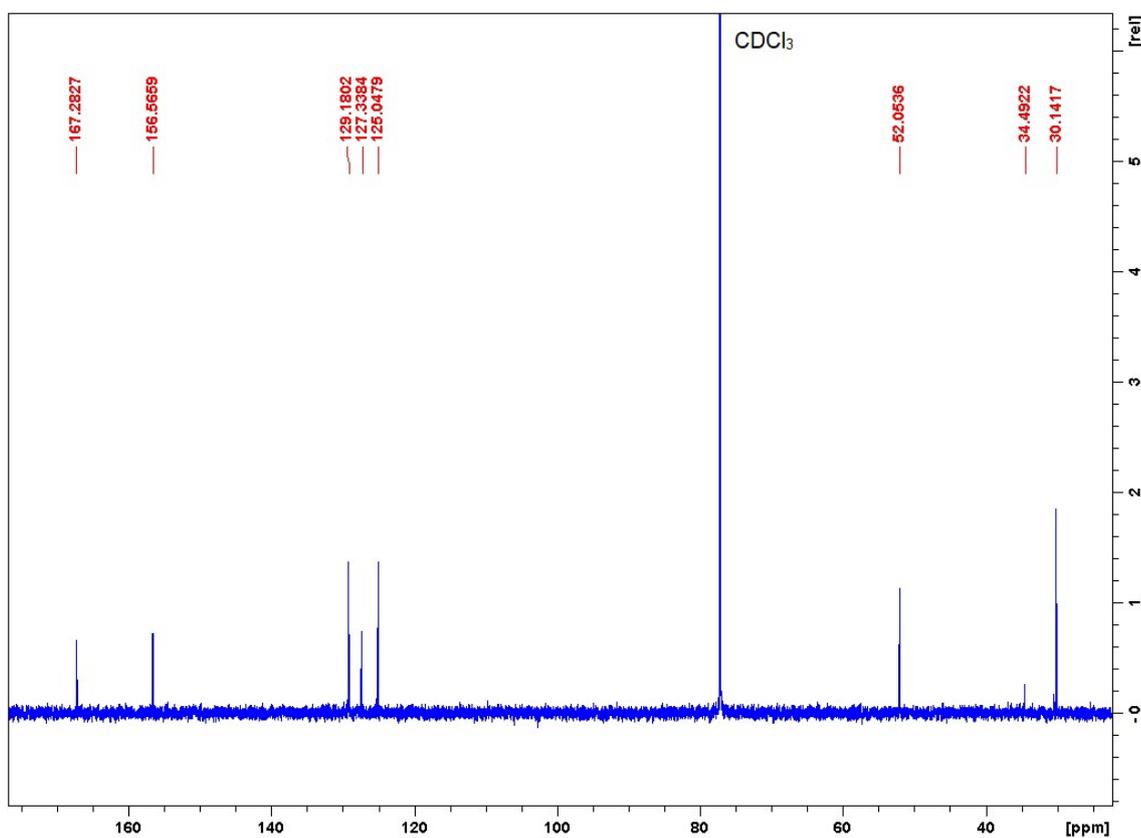
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S7.  $^{13}\text{C}$  NMR ( $^1\text{H}$  power-gated decoupling) of 4-*tert*-butylbenzoic acid methyl ester- $d_{13}$ , **7**



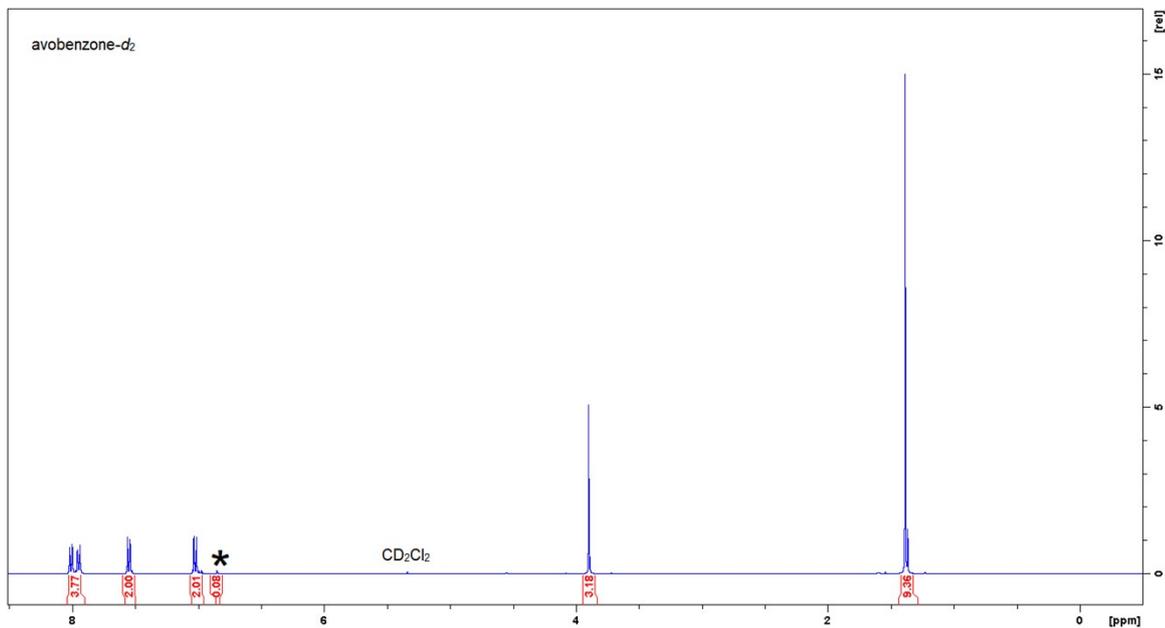
S8.  $^{13}\text{C}$  NMR ( $^1\text{H}$  and  $^2\text{H}$  gated decoupling) of 4-*tert*-butylbenzoic acid methyl ester- $d_{13}$ , **7**



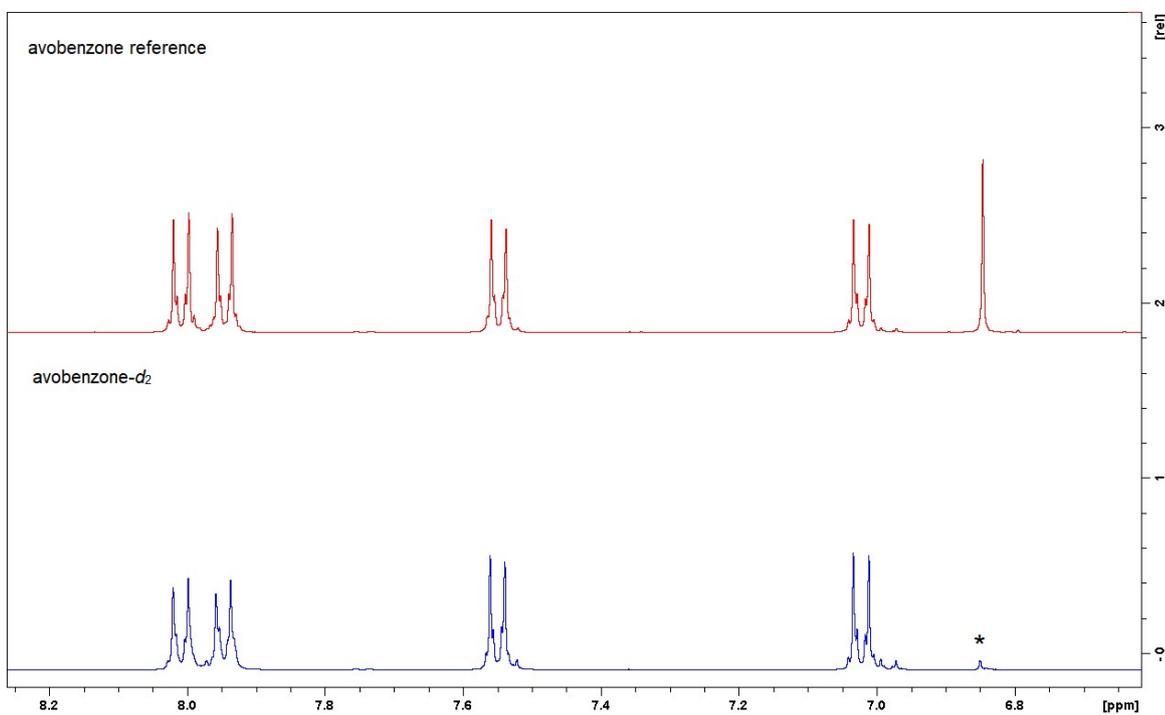
### S9. $^1\text{H}$ NMR of avobenzene- $d_2$ , 1

in  $\text{CD}_2\text{Cl}_2$  (a) full spectrum showing >92 %D for the keto-enol  $d_2$  based on residual integration of resonance at 6.84 ppm (which has an integration of 1 for the non-deuterated reference), (b,c) expanded regions, relative to non-deuterated reference, \* indicates deuteration

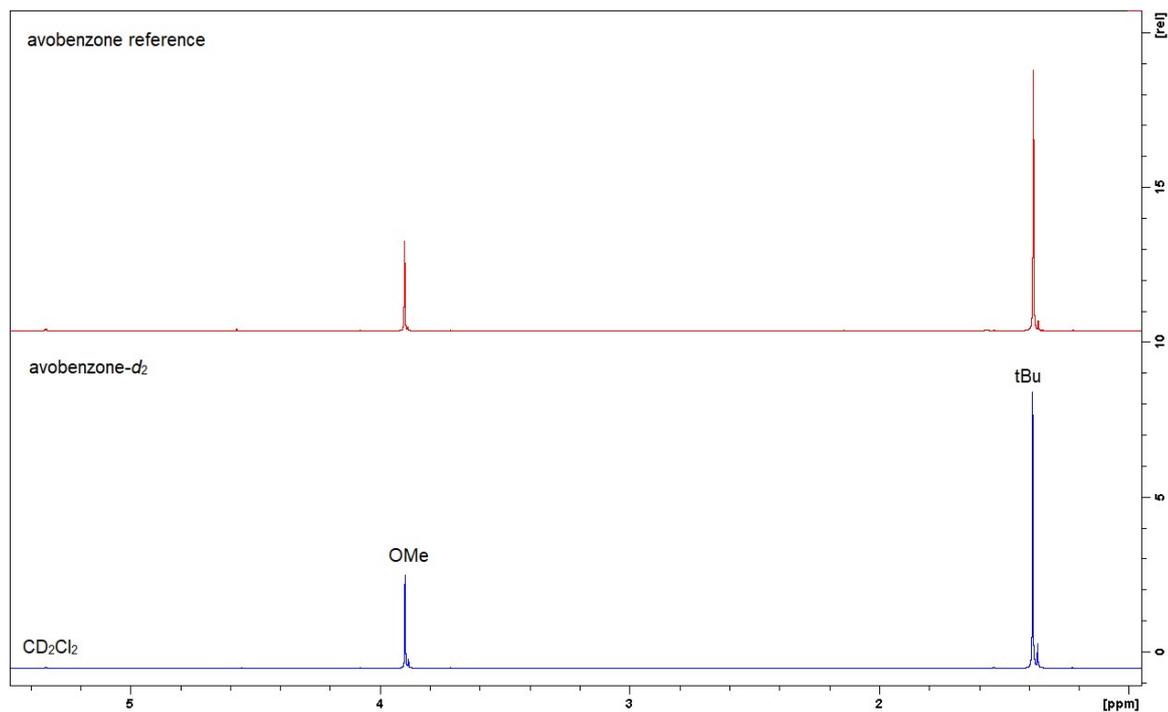
(a)



(b)

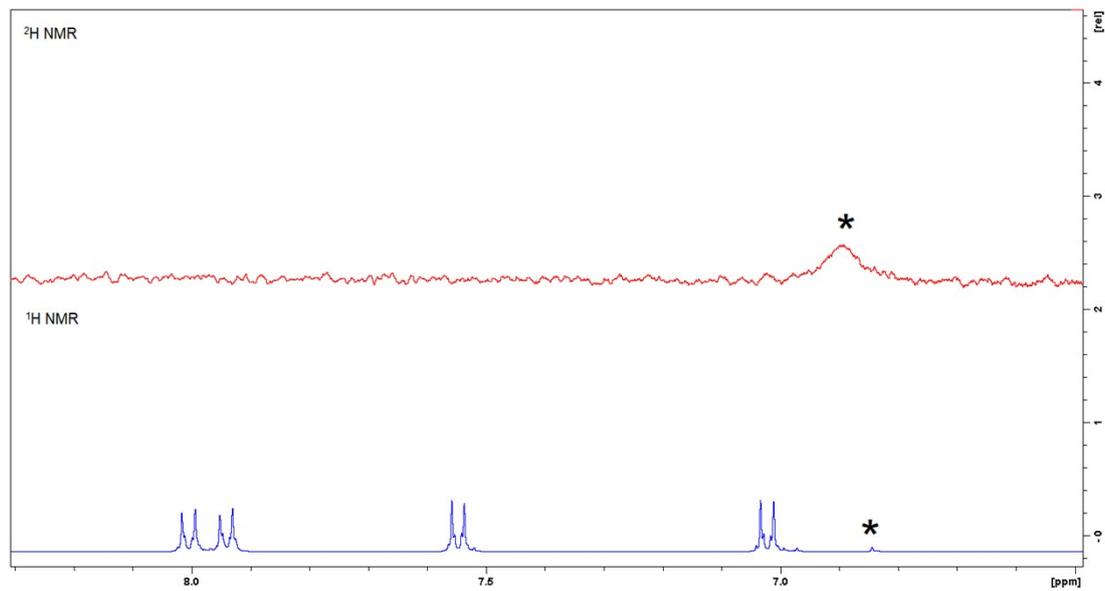


(c)



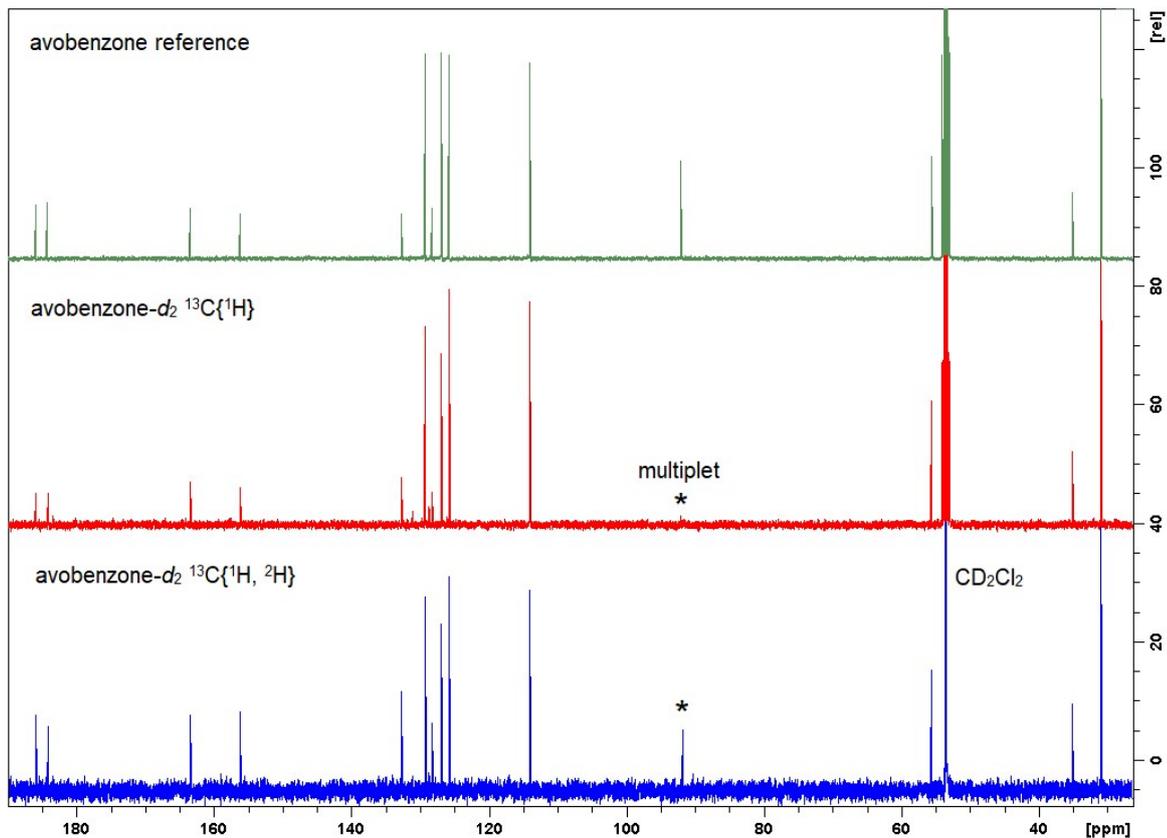
S10. <sup>2</sup>H NMR of avobenzene-d<sub>2</sub>, **1**

in CD<sub>2</sub>Cl<sub>2</sub>, including comparison to <sup>1</sup>H NMR of avobenzene-d<sub>2</sub>, \* indicates deuteration



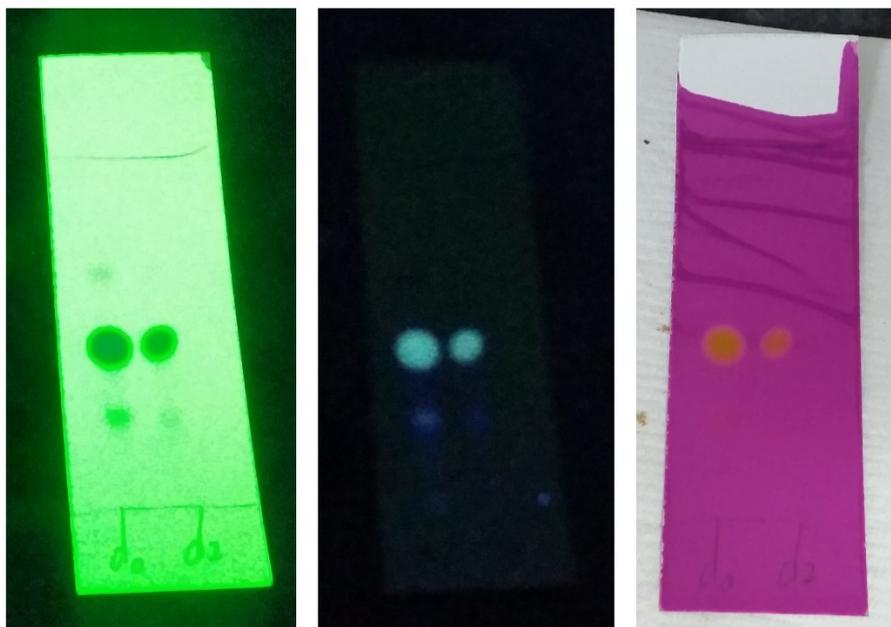
S11.  $^{13}\text{C}$  NMR of avobenzene- $d_2$ , **1**

in  $\text{CD}_2\text{Cl}_2$ , decoupled from  $^1\text{H}$  only, and both  $^1\text{H}$  and  $^2\text{H}$ , relative to avobenzene reference, \* indicates deuteration



S12. TLC of avobenzene- $d_2$  and non-deuterated avobenzene (Sigma-Aldrich PHR1073)

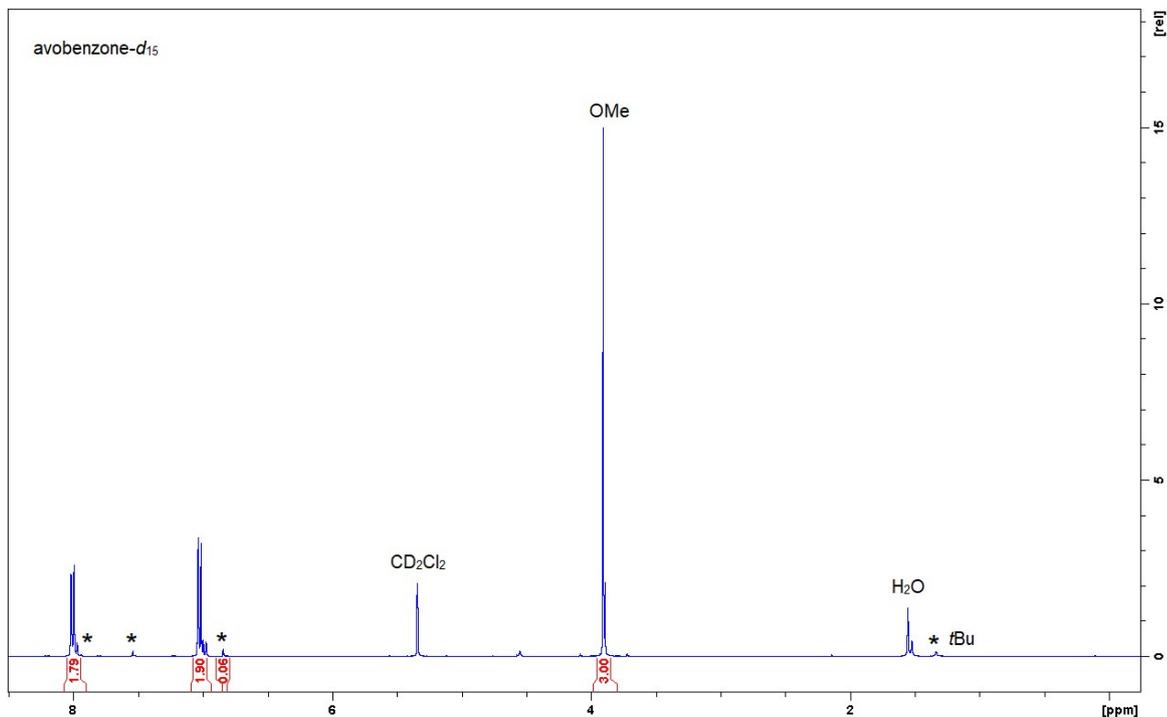
silica, 25% ethyl acetate in  $n$ -hexane, visualised using a UV lamp (short wave 254 nm and long wave 365 nm) and  $\text{KMnO}_4$  dip stain. Each contains a small amount of a UV-active impurity.



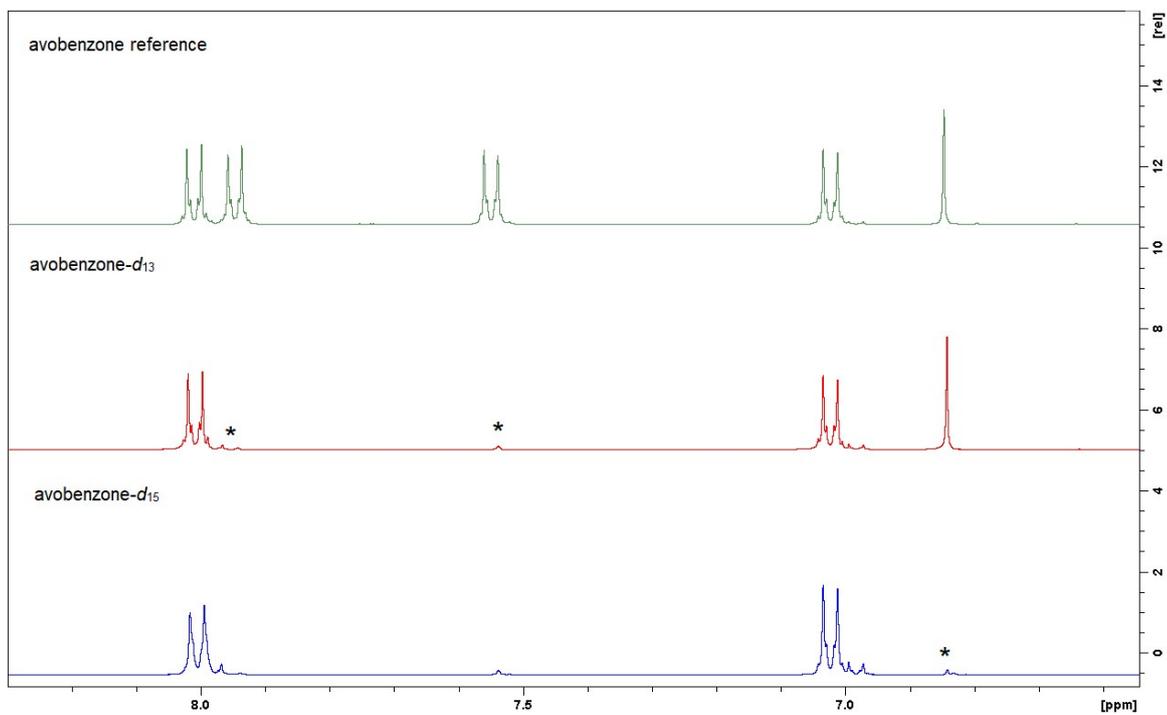
S13.  $^1\text{H}$  NMR of avobenzene- $d_{15}$ , **2**

in  $\text{CD}_2\text{Cl}_2$  (a) full spectrum showing >94 %D for the keto-enol  $d_2$  based on residual integration of resonance at 6.84 ppm (which has an integration of 1 for the non-deuterated reference), (b,c) expanded regions, relative to avobenzene- $d_{13}$  precursor and non-deuterated reference, \* indicates deuteration

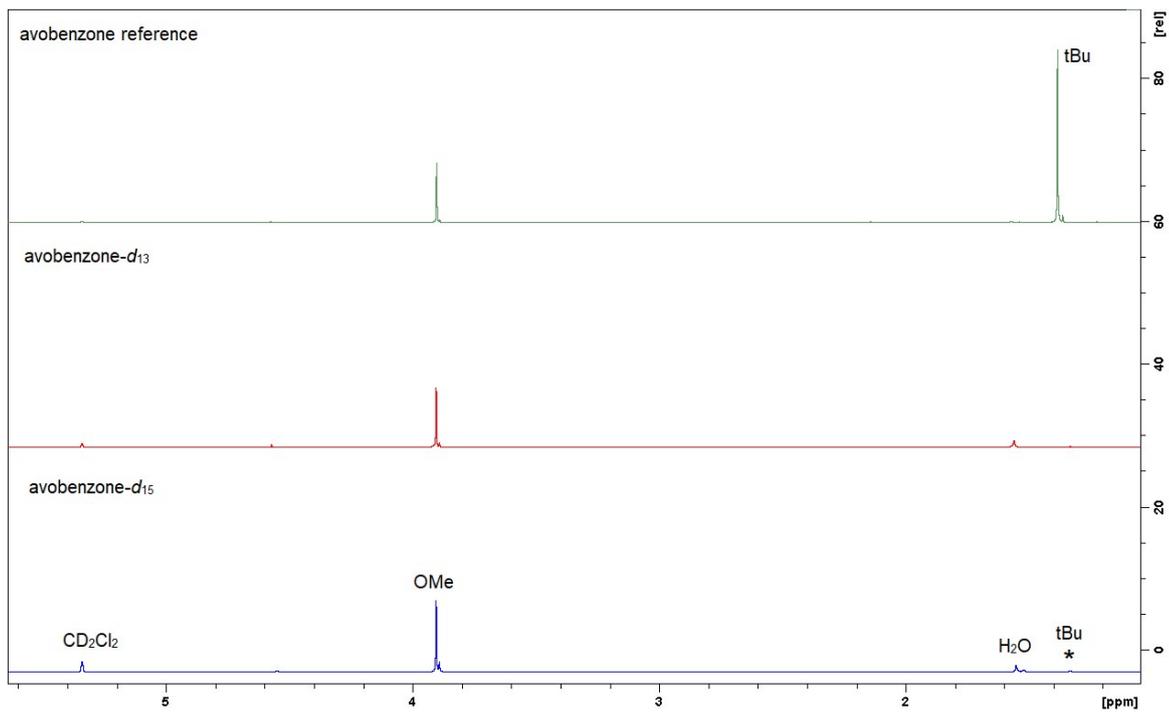
(a)



(b)

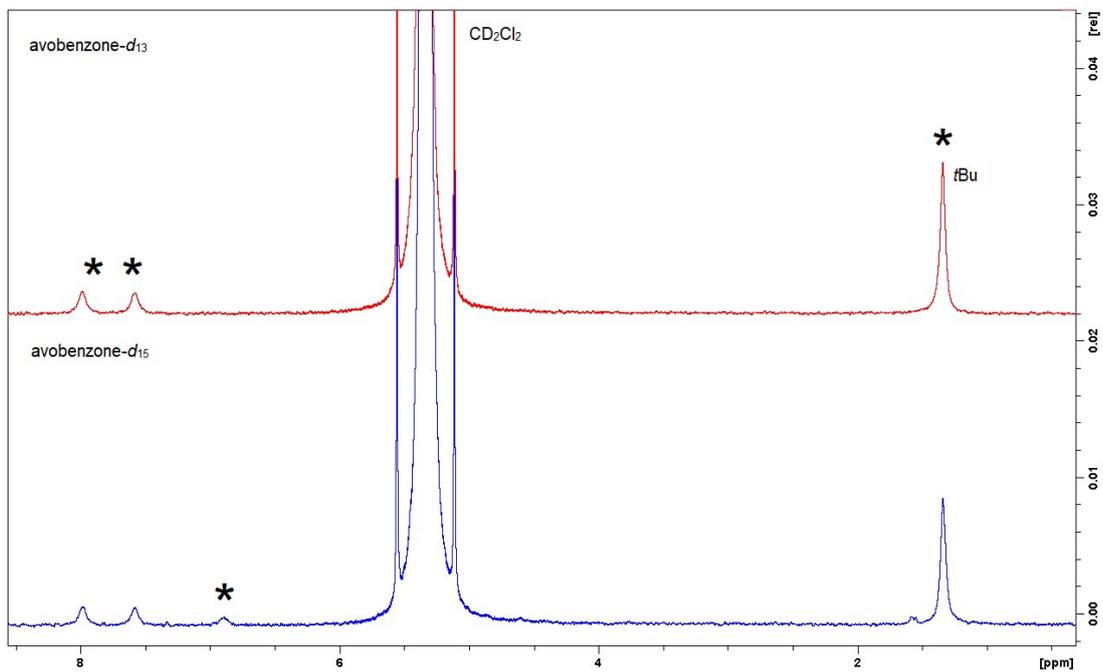


(c)



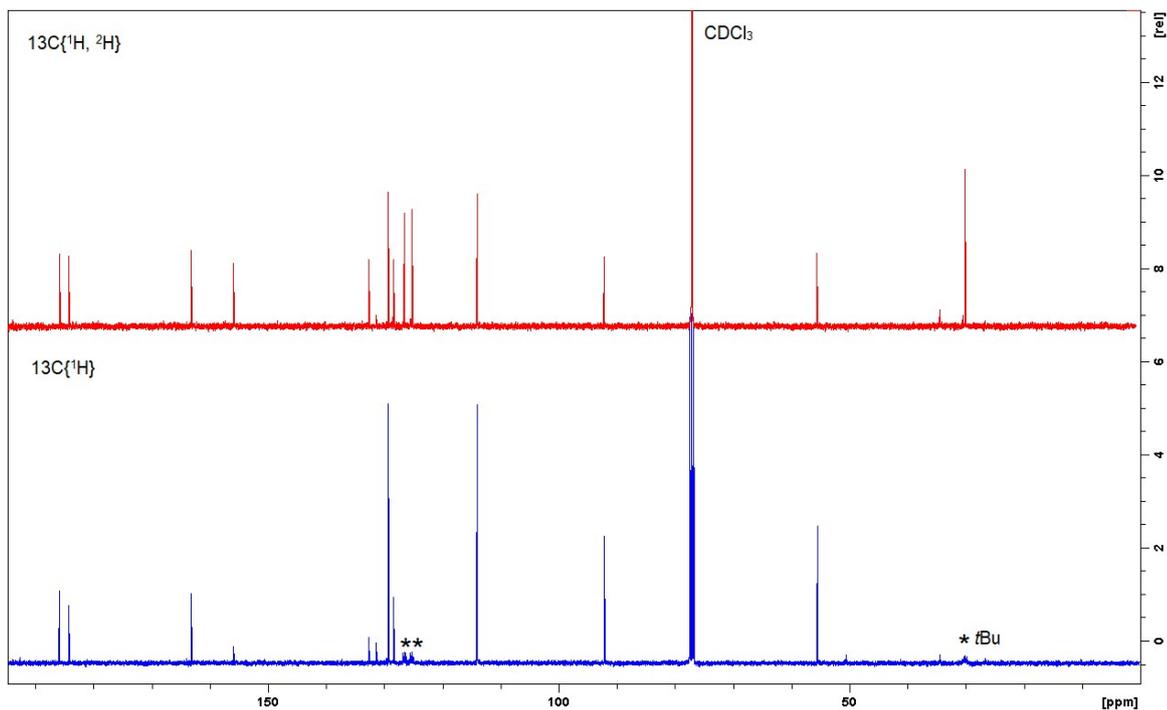
S14. <sup>2</sup>H NMR of avobenzene-d<sub>15</sub>, **2**

in CD<sub>2</sub>Cl<sub>2</sub>, relative to avobenzene-d<sub>13</sub> precursor, \* indicates deuteration



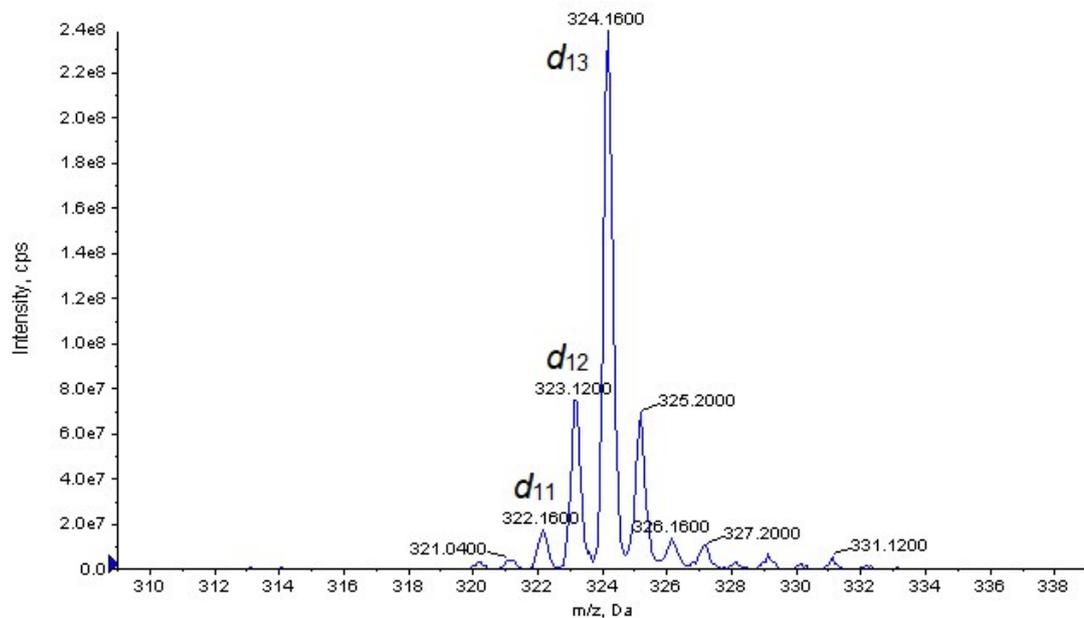
S15.  $^{13}\text{C}$  NMR of avobenzene- $d_{13}$ , **8**

in  $\text{CDCl}_3$ ,  $\{^1\text{H}\}$  relative to  $\{^1\text{H}, ^2\text{H}\}$ , \* indicates deuteration



S16. MS of avobenzene- $d_{13}$  precursor, **8**

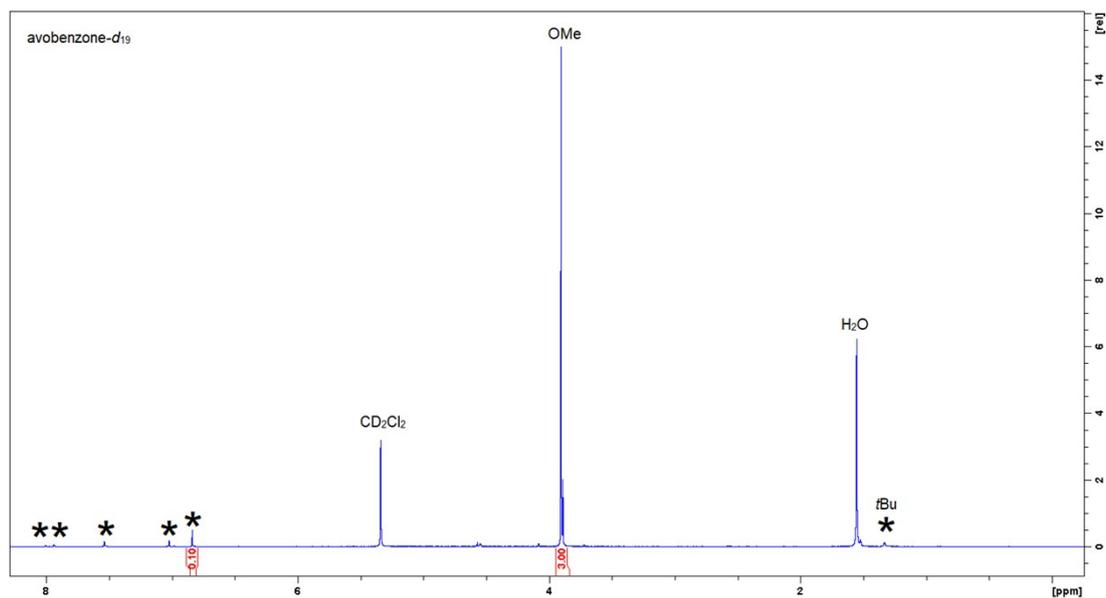
$[\text{M}+\text{H}]^+ \text{C}_{20}\text{H}_{10}\text{D}_{13}\text{O}_3^+$ : Calc 324.25. Found 324.16.



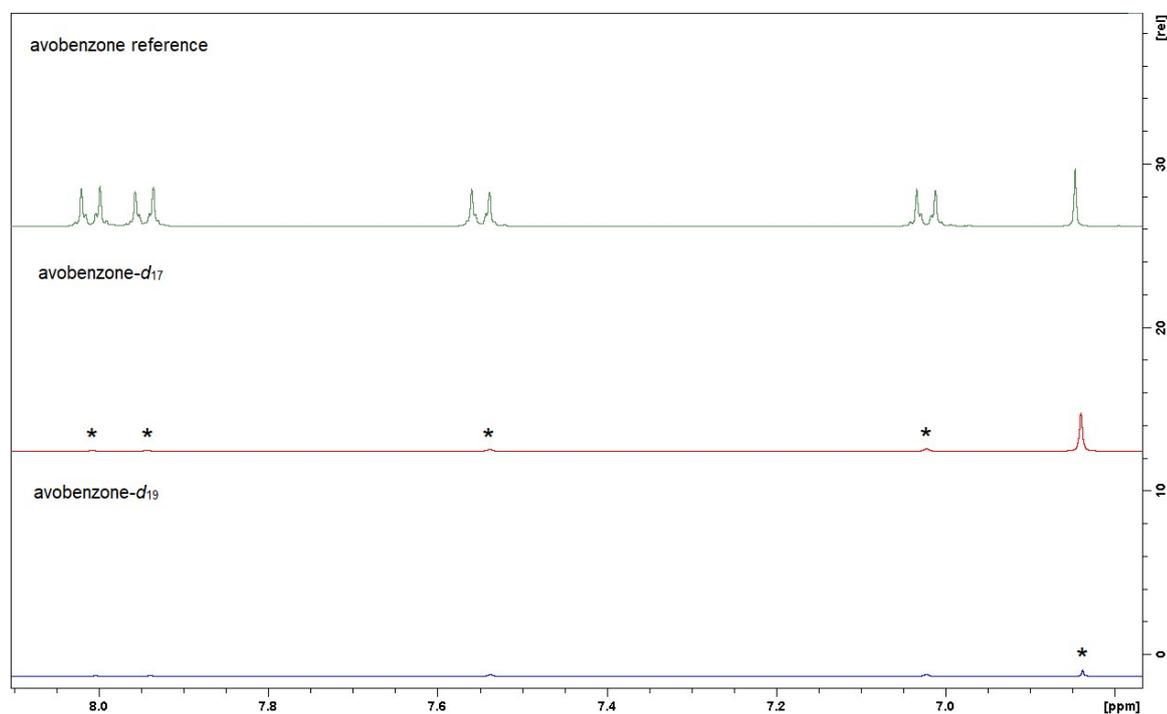
### S17. $^1\text{H}$ NMR of avobenzene- $d_{19}$ , **3**

in  $\text{CD}_2\text{Cl}_2$  (a) full spectrum showing  $>90\%$  D for the keto-enol  $d_2$  based on residual integration of resonance at 6.84 ppm (which has an integration of 1 for the non-deuterated reference), (b,c) expanded regions, relative to avobenzene- $d_{17}$  precursor and non-deuterated reference, \* indicates deuteration

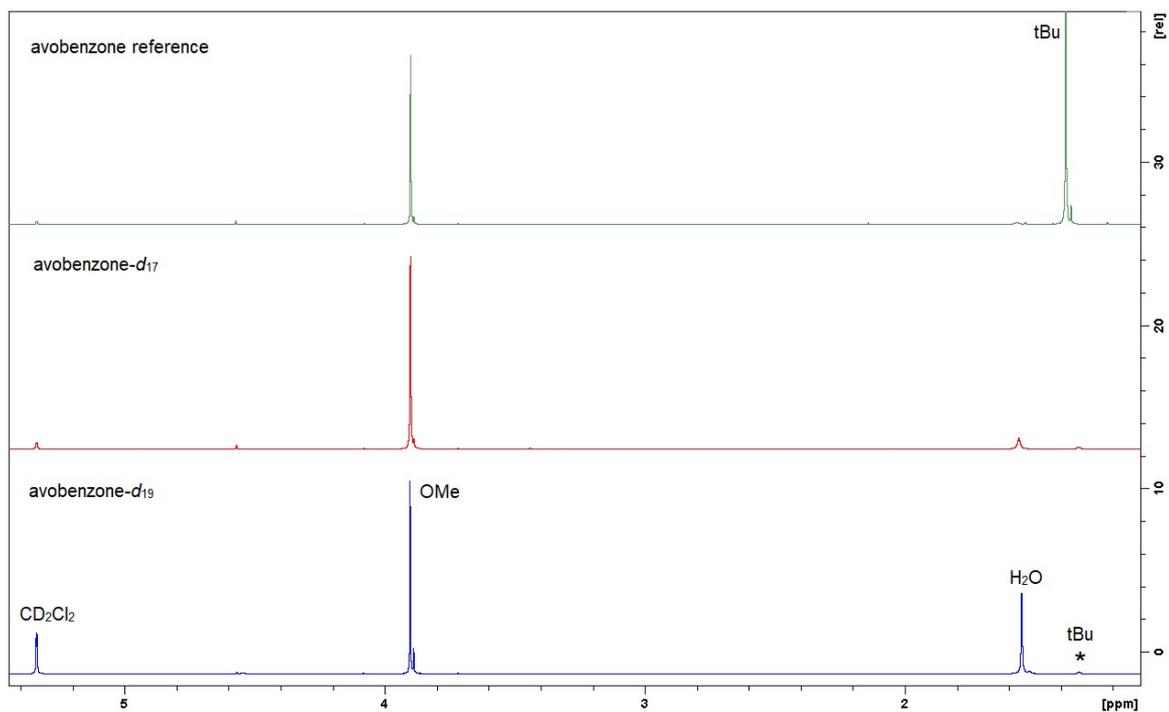
(a)



(b)

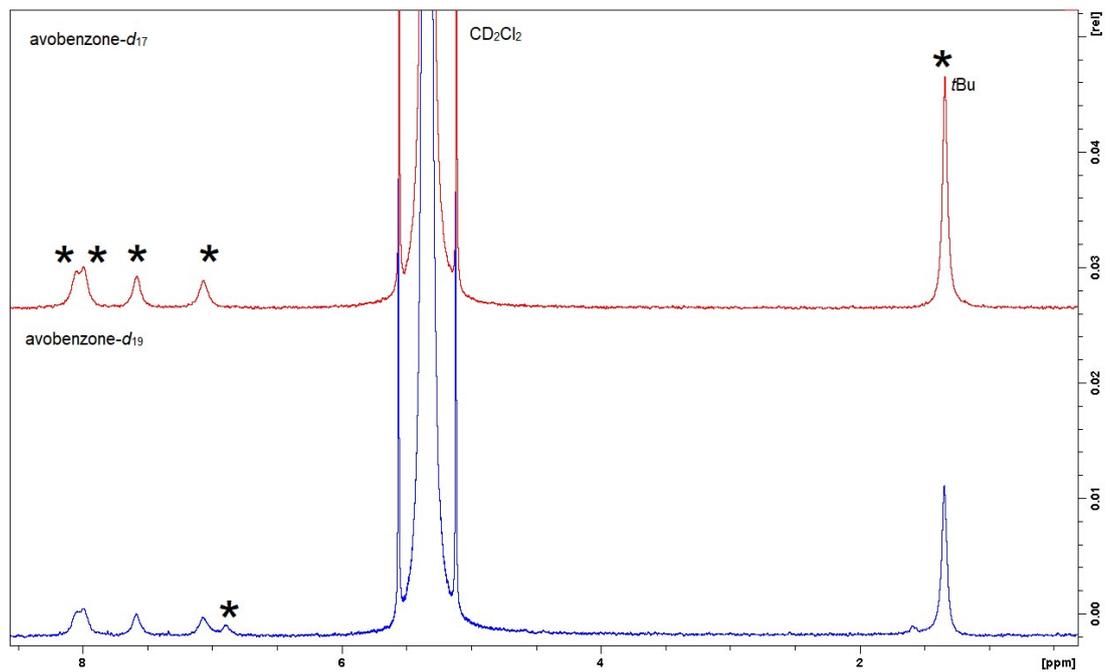


(c)



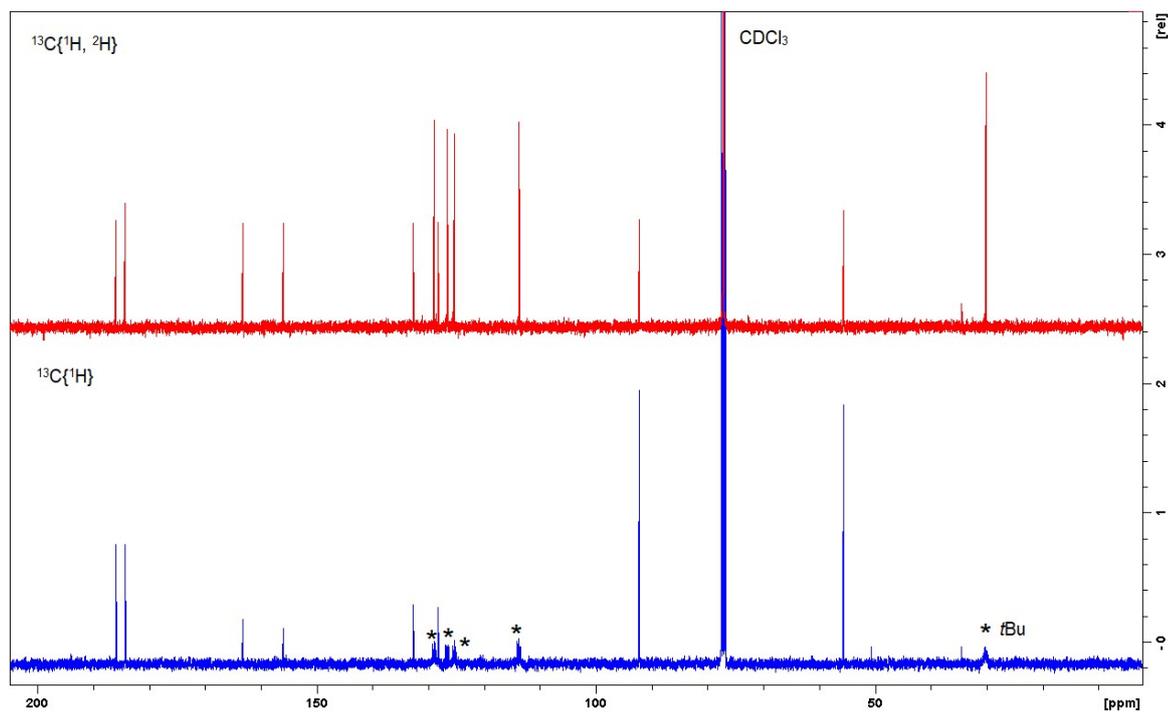
S18.  $^2\text{H}$  NMR of avobenzene- $d_{19}$ , **3**

in CD<sub>2</sub>Cl<sub>2</sub>, relative to avobenzene- $d_{17}$  precursor, \* indicates deuteration



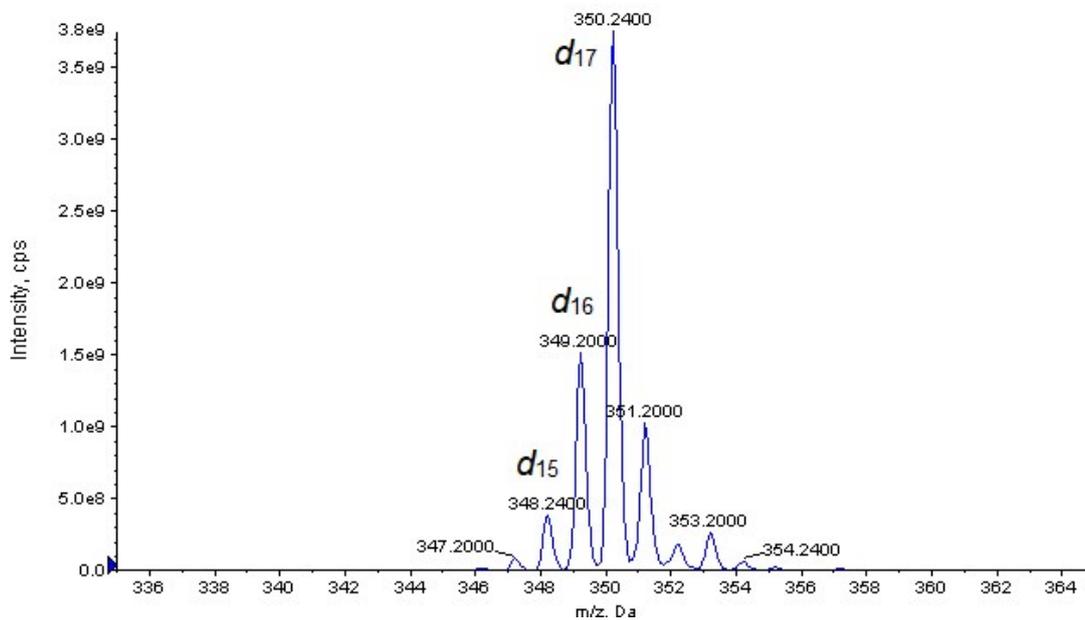
S19.  $^{13}\text{C}$  NMR of avobenzene- $d_{17}$ , **9**

in  $\text{CDCl}_3$ ,  $\{^1\text{H}\}$  relative to  $\{^1\text{H}, ^2\text{H}\}$ , \* indicates deuteration



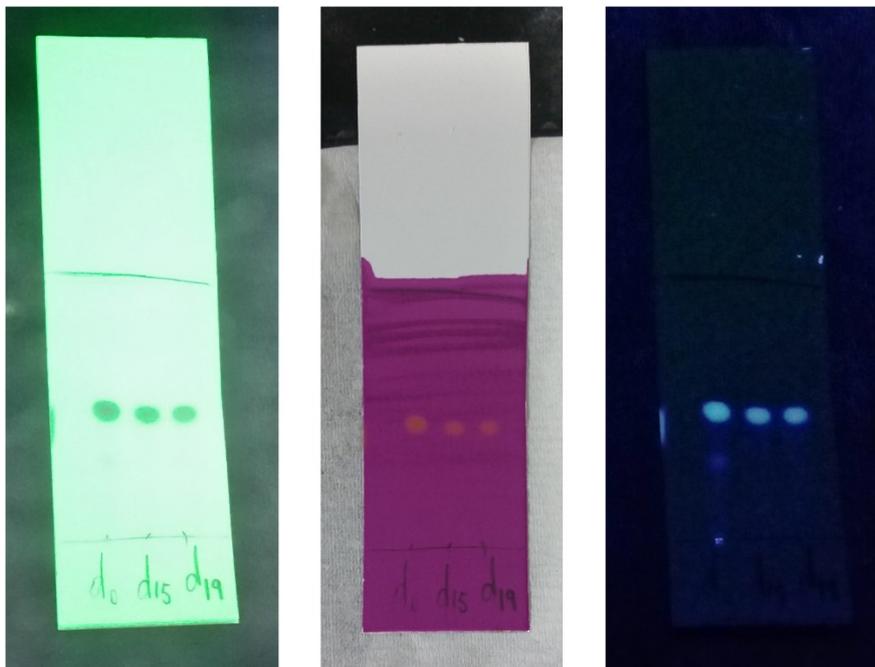
S20. MS of avobenzene- $d_{17}$  precursor, **9**

$[\text{M}+\text{Na}]^+ \text{C}_{20}\text{H}_5\text{D}_{17}\text{O}_3\text{Na}^+$ : Calc 350.25. Found 350.24.



S21. TLC of avobenzene- $d_{15}$ , avobenzene- $d_{19}$  and non-deuterated avobenzene (TCI Chemicals B3382)

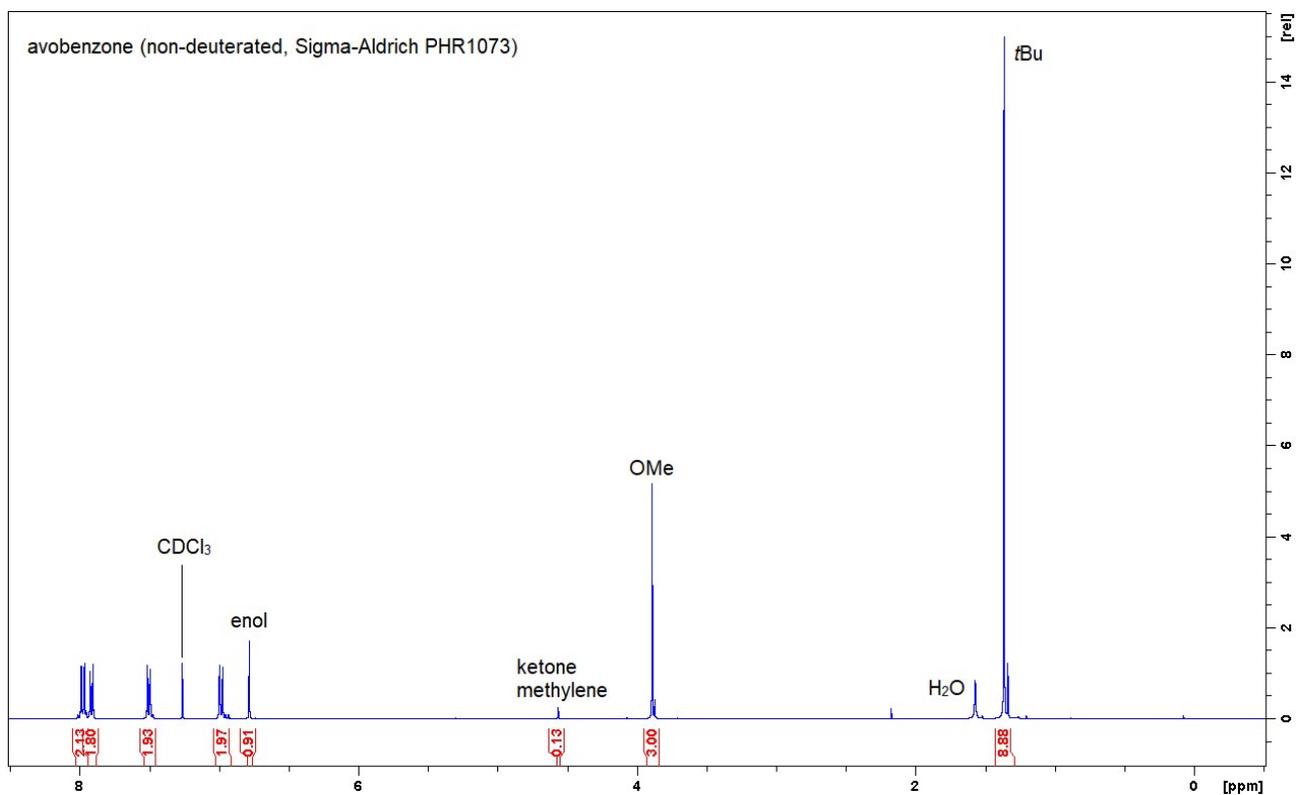
silica, 25% ethyl acetate in *n*-hexane, visualised using a UV lamp (short wave 254 nm and long wave 365 nm) and  $KMnO_4$  dip stain. The reference non-deuterated avobenzene contains a UV-active impurity.



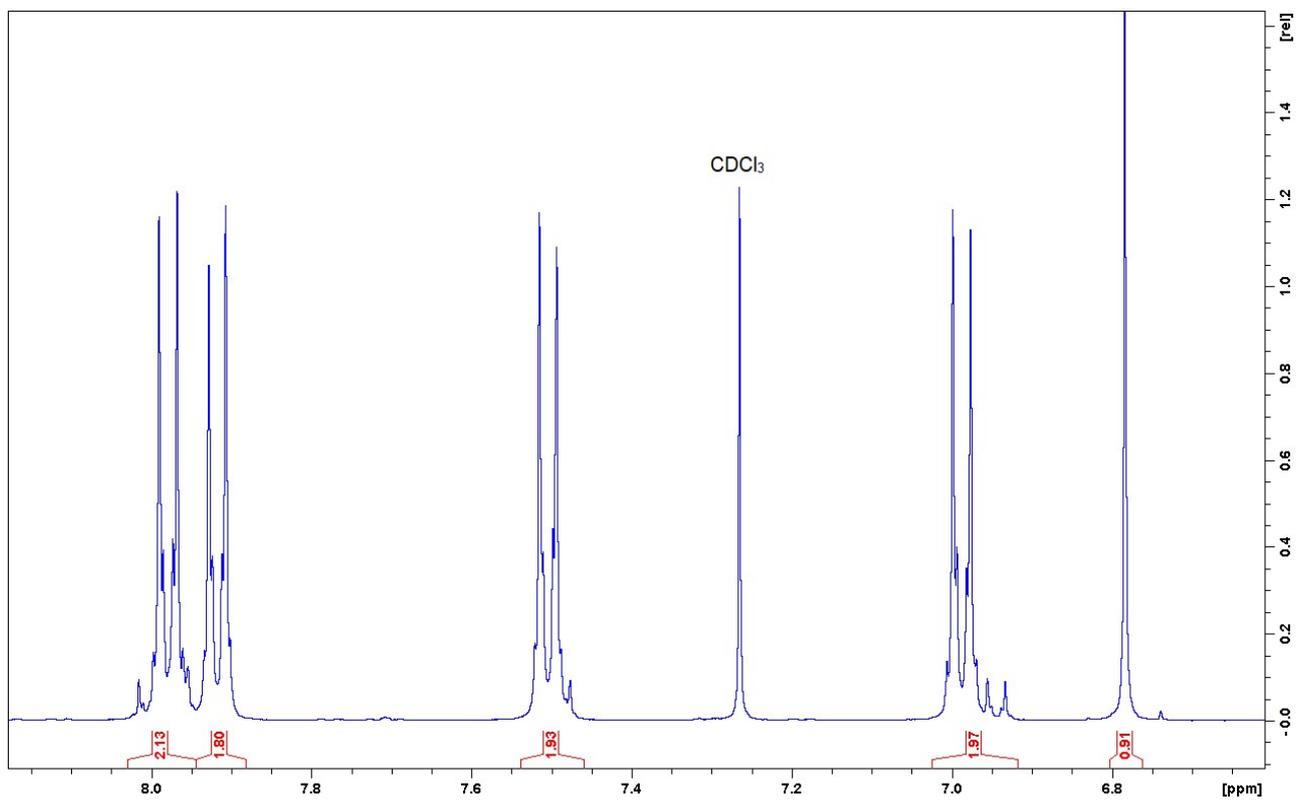
S22. <sup>1</sup>H NMR of avobenzene (non-deuterated, Sigma reference standard PHR1073)

in CDCl<sub>3</sub> (a) full spectrum showing, (b,c) expanded regions

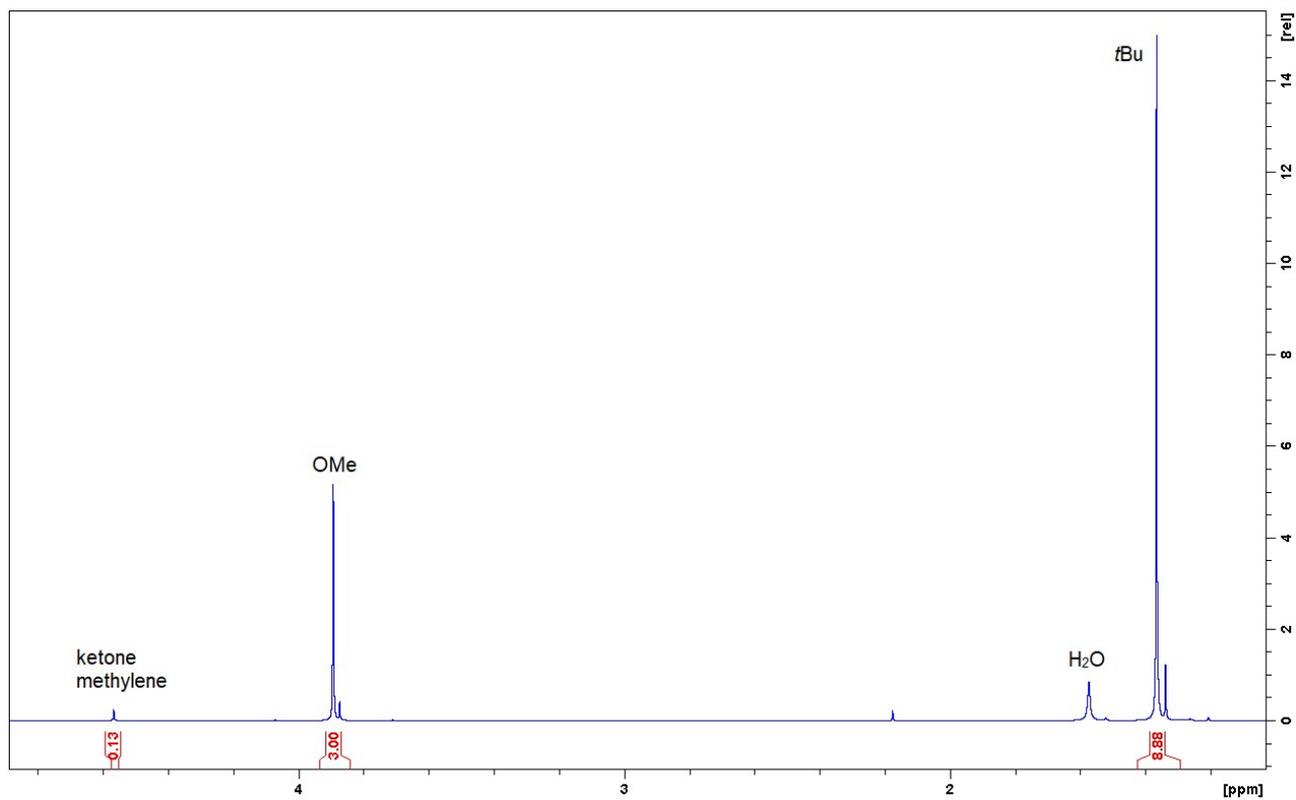
(a)



(b)



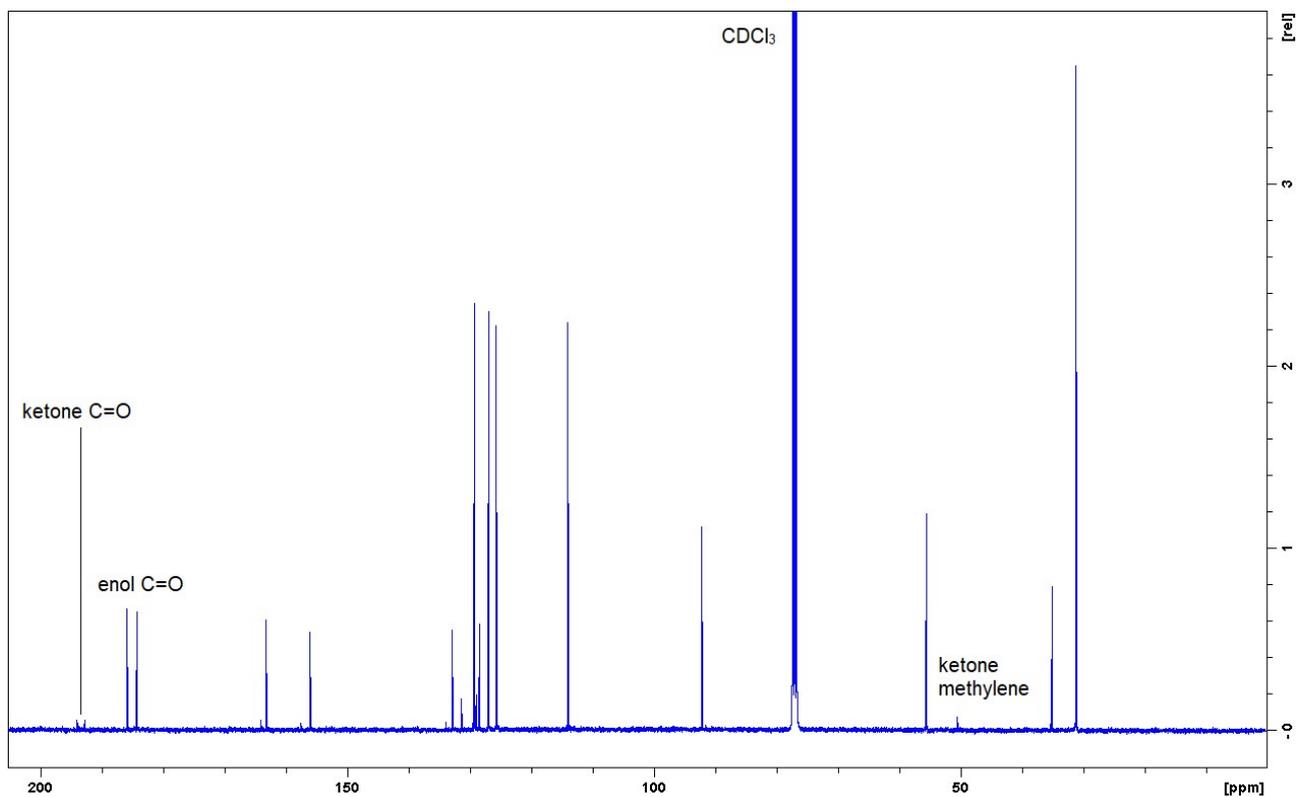
(c)



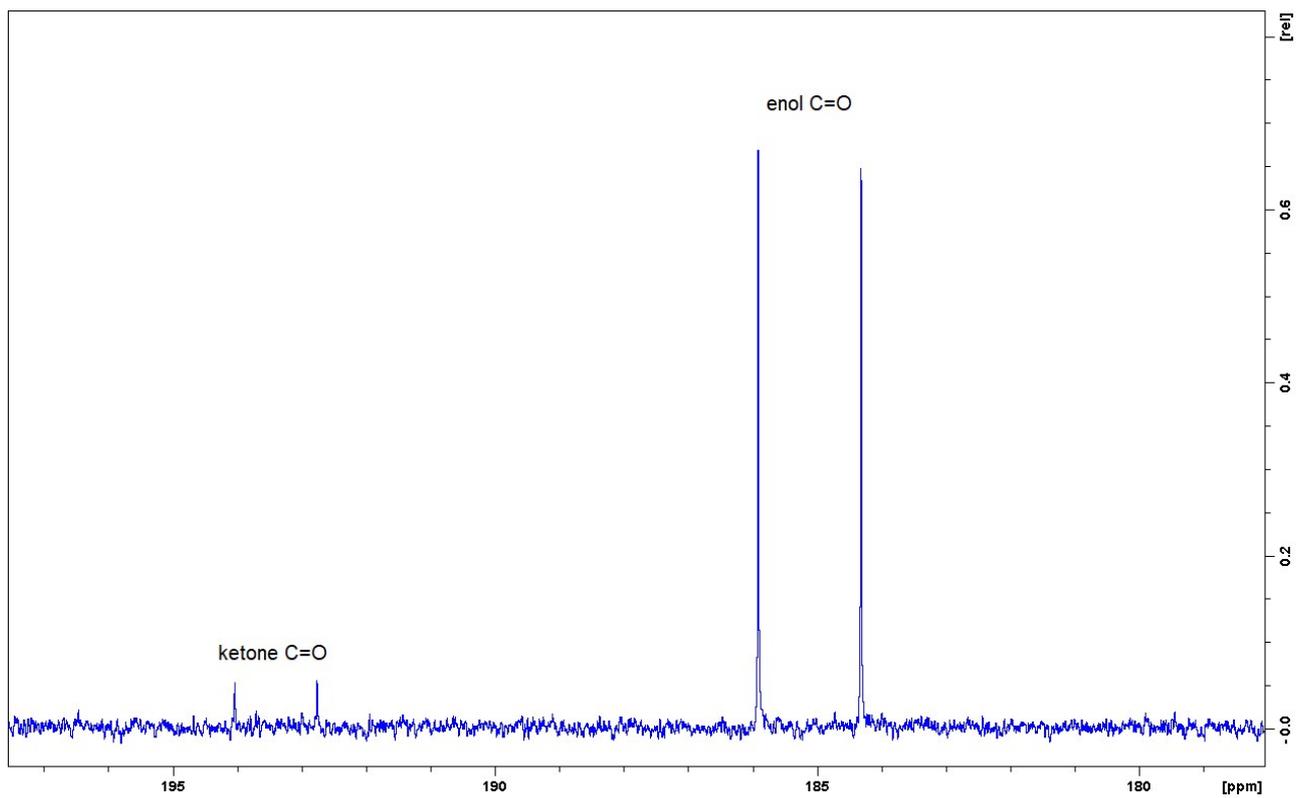
S23. <sup>13</sup>C NMR of avobenzone (non-deuterated, Sigma reference standard PHR1073)

in CDCl<sub>3</sub> (a) full spectrum showing, (b,c) expanded regions

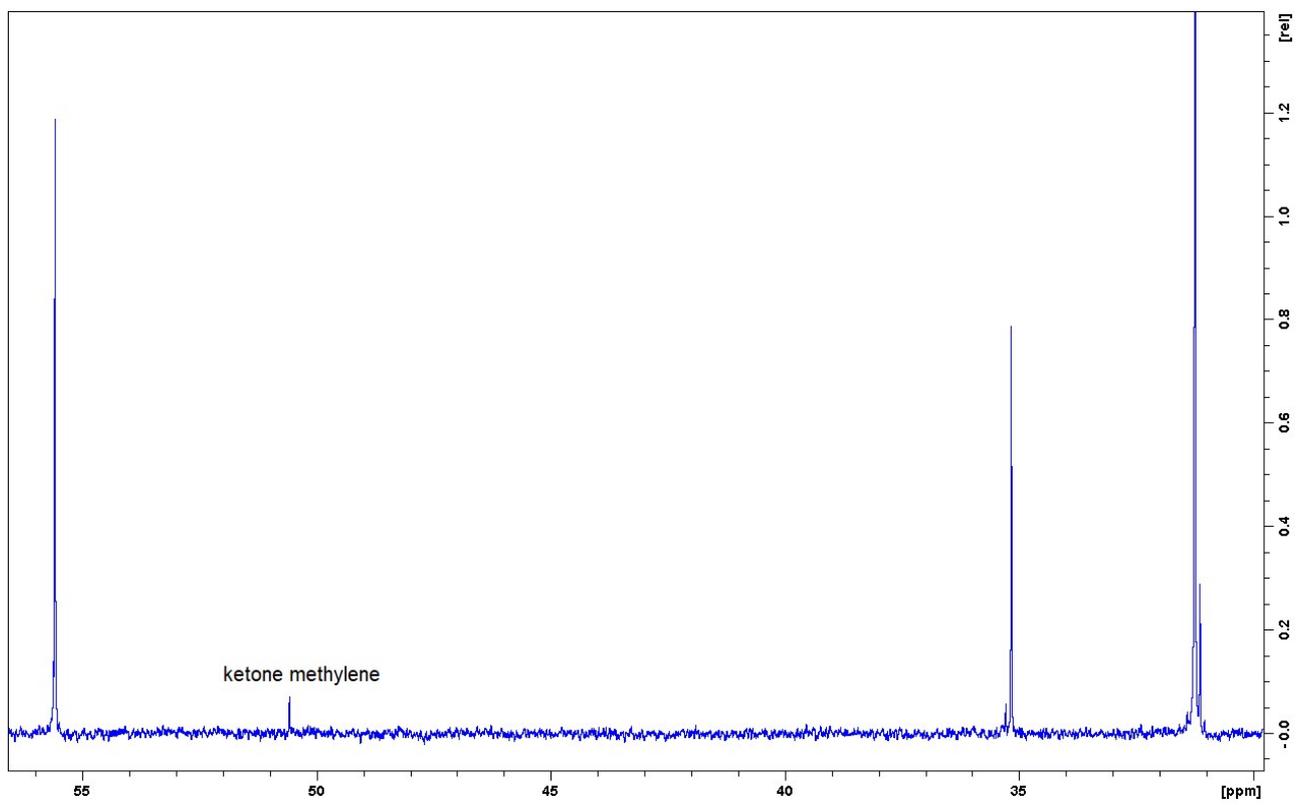
(a)



(b)

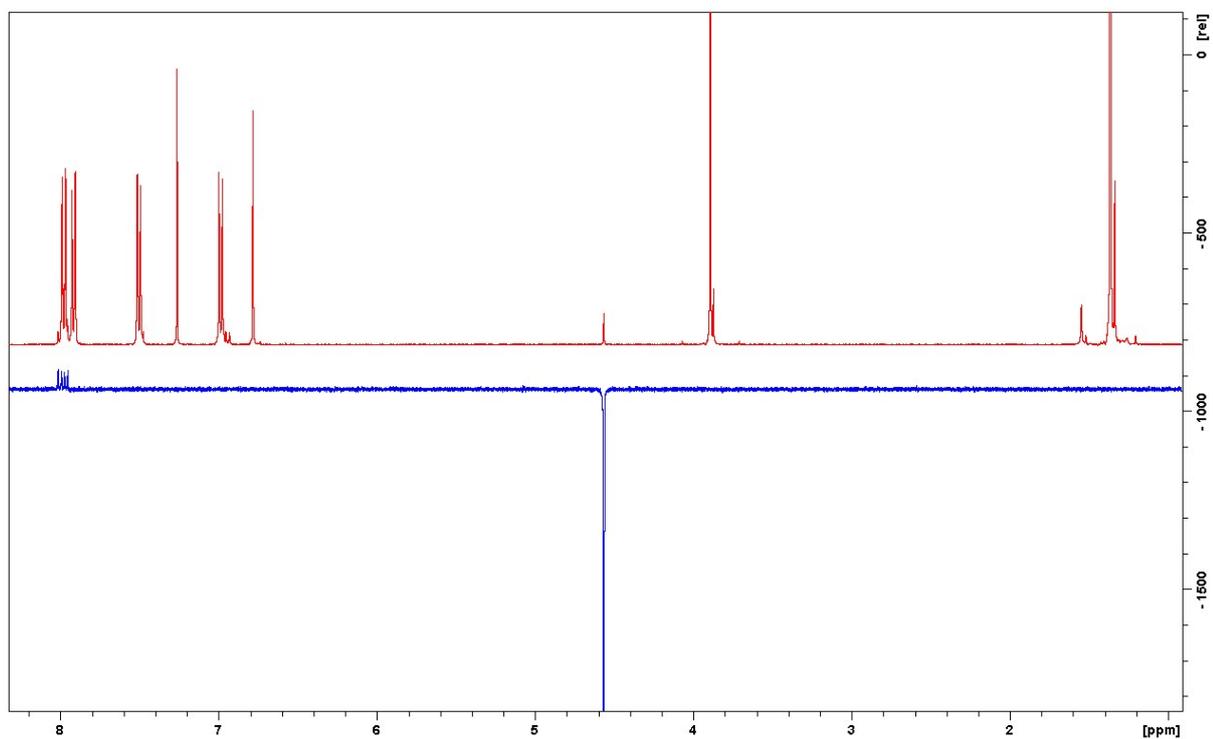


(c)



S24. Selective irradiation NOE of avobenzene (non-deuterated, Sigma reference standard PHR1073)

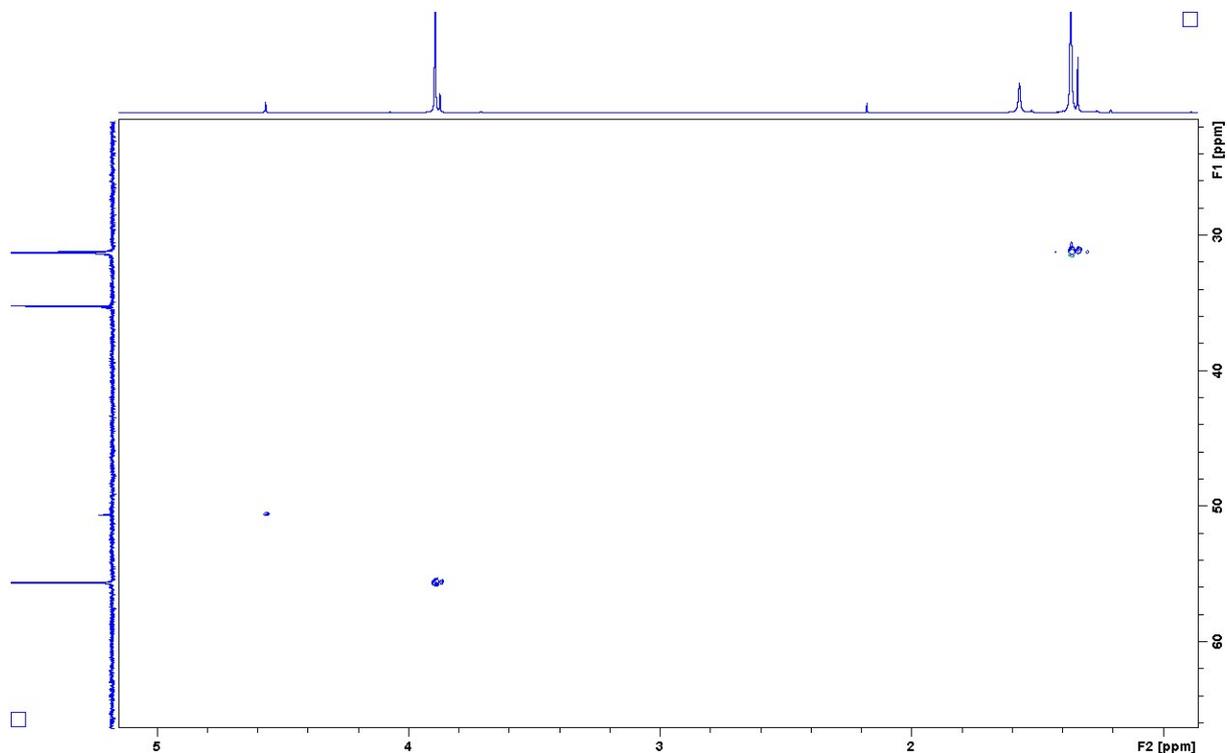
in  $\text{CDCl}_3$ , after one day of equilibration, the diketone methylene resonance at 4.57 ppm shows NOE into small aromatic peaks at 8.04-7.94 ppm.



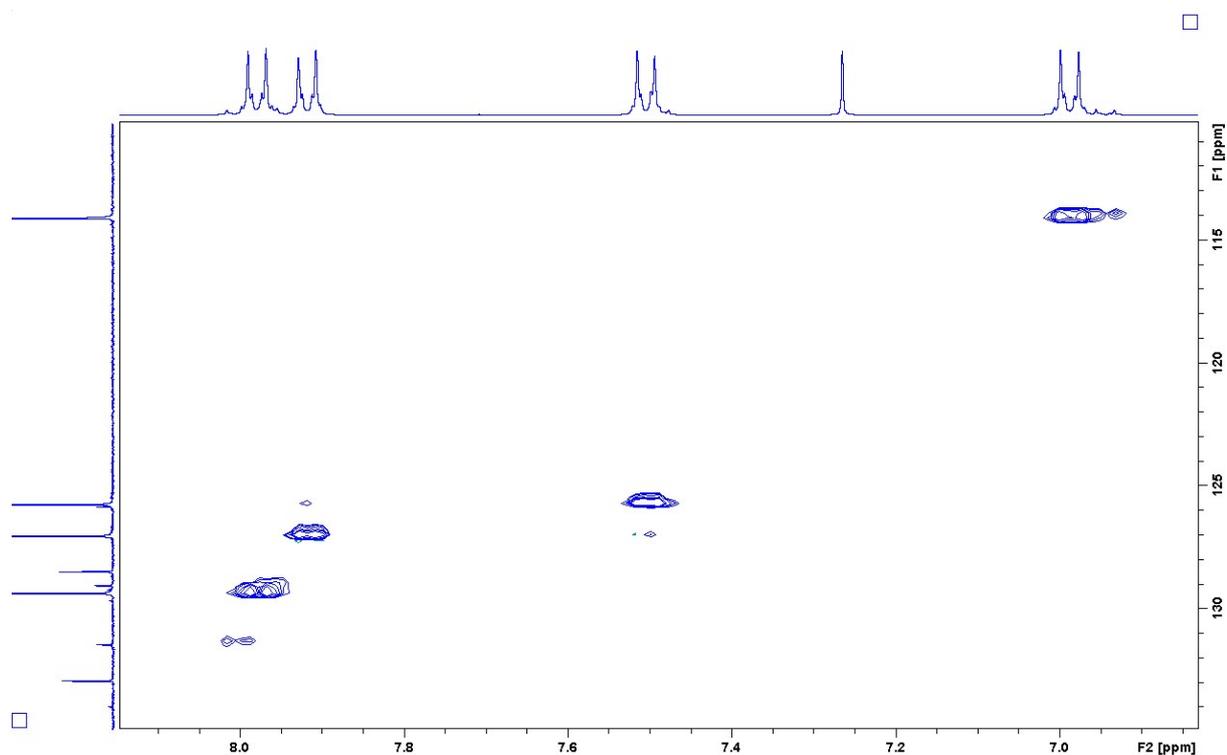
S25. HSQC of avobenzone (non-deuterated, Sigma reference standard PHR1073)

in CDCl<sub>3</sub>, zoomed, showing the short range <sup>1</sup>H-<sup>13</sup>C correlations between (a) the small peaks for the methoxy and *tert*-butyl groups, as well as the diketone methylene <sup>1</sup>H resonance at 4.57 ppm and its <sup>13</sup>C resonance at 50.5 ppm, (b) aromatic region.

(a)



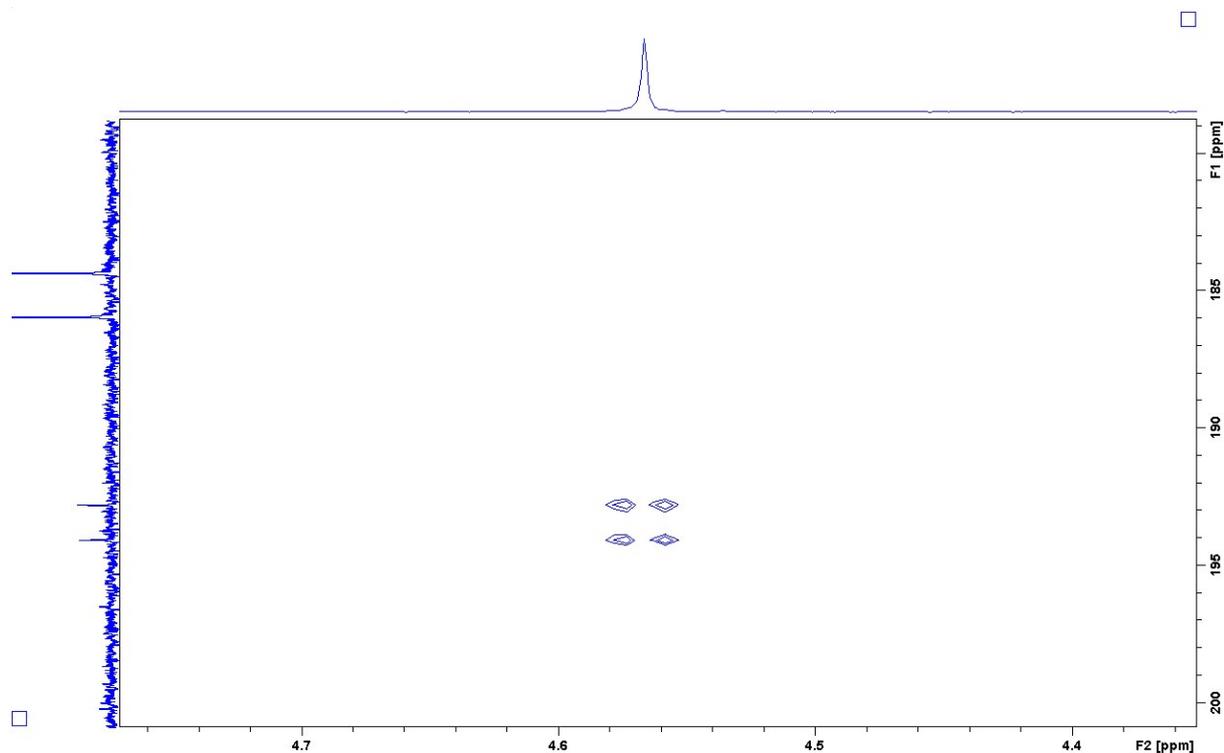
(b)



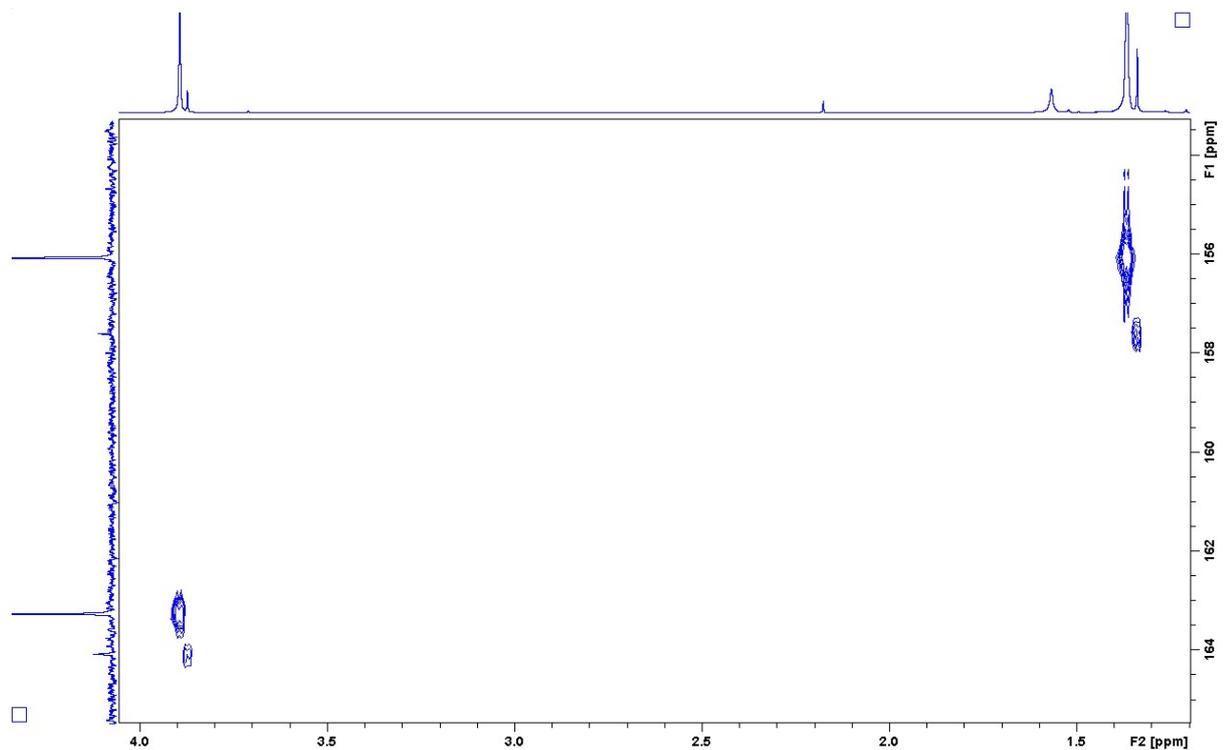
S26. HMBC of avobenzone (non-deuterated, Sigma reference standard PHR1073)

in  $\text{CDCl}_3$ , zoomed, showing the long range  $^1\text{H}$ - $^{13}\text{C}$  correlations between the small peaks for the (a) diketone methylene  $^1\text{H}$  resonance at 4.57 ppm into the diketone  $^{13}\text{C}$  pair of resonances at 192.8 and 194.0 ppm, (b) methoxy and *tert*-butyl groups into the aromatic region.

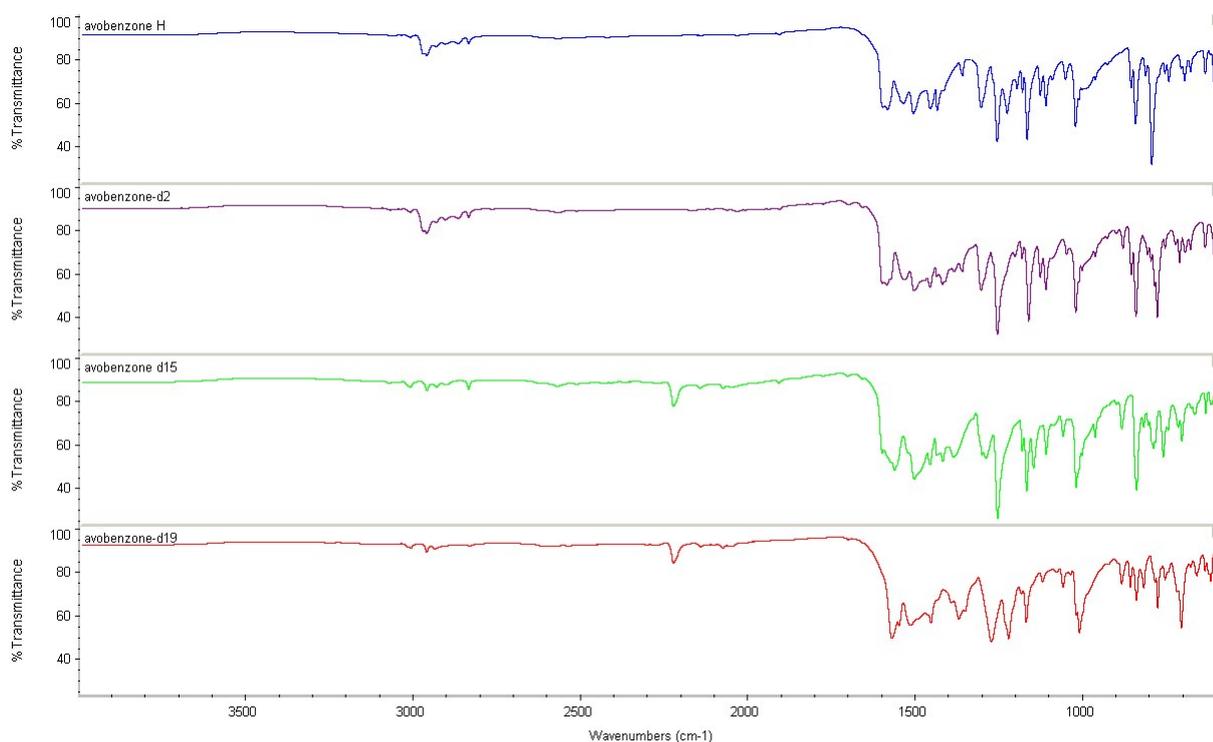
(a)



(b)



## S27. ATR-FT-IR of the avobenzene isotopologues 1-3.

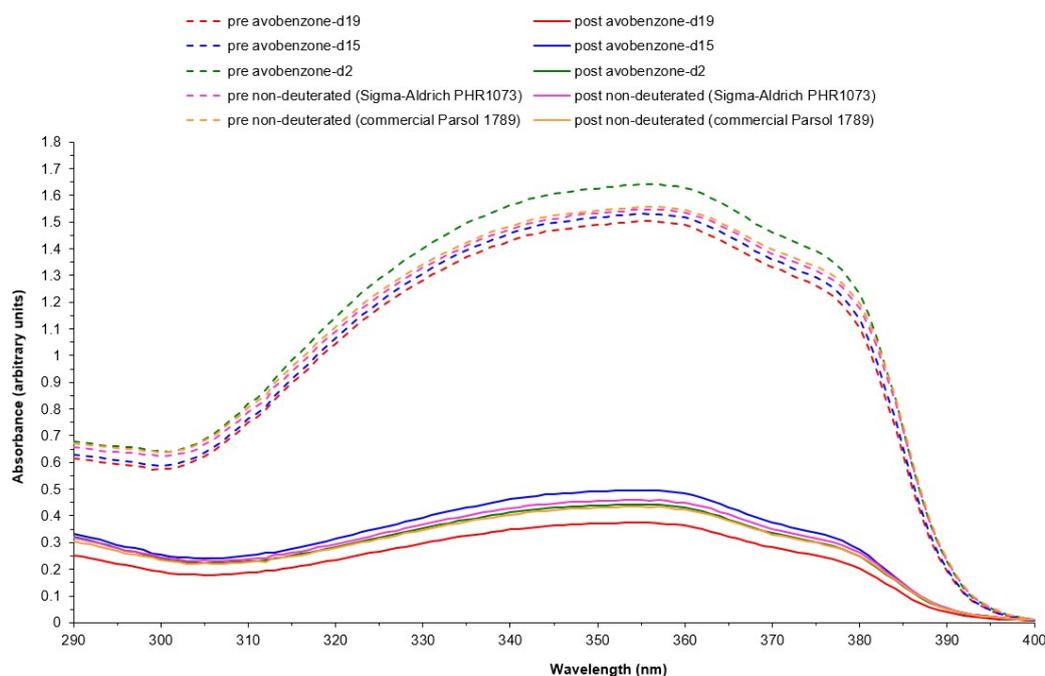


Phenyl ring deuteration (including the 4-*tert*-butyl group) led to a reduction in the peaks at 2800-3000 cm<sup>-1</sup> and the appearance of peaks at 2000-2200 cm<sup>-1</sup> (e.g. going from avobenzene-d<sub>2</sub> or non-deuterated to avobenzene-d<sub>15</sub> or avobenzene-d<sub>19</sub>), typical of deuterated alkyl and phenyl moieties [1-4].

There are differences between 700-1600 cm<sup>-1</sup> for avobenzene-d<sub>15</sub> and avobenzene-d<sub>19</sub> relative to non-deuterated (and much less so for avobenzene-d<sub>2</sub>, only in the 1200-1300 cm<sup>-1</sup> region).

- [1] Jones, R.N., *THE INFRARED ABSORPTION SPECTRA OF DEUTERATED ESTERS: III. METHYL LAURATE*. Canadian Journal of Chemistry, 1962. **40**(2): p. 301-320.
- [2] Nolin, B. and R.N. Jones, *THE INFRARED ABSORPTION SPECTRA OF DEUTERATED ESTERS: II. ETHYL ACETATE*. Canadian Journal of Chemistry, 1956. **34**(10): p. 1392-1404.
- [3] Nolin, B. and R.N. Jones, *THE INFRARED ABSORPTION SPECTRA OF DEUTERATED ESTERS: I. METHYL ACETATE*. Canadian Journal of Chemistry, 1956. **34**(10): p. 1382-1391.
- [4]. Hudgins, D.M., J.C.W. Bauschlicher, and S.A. Sandford, *The Impact of Deuteration on the Infrared Spectra of Interstellar Polycyclic Aromatic Hydrocarbons*. The Astrophysical Journal, 2004. **614**(2): p. 770-780.

The UV-Vis absorbance of avobenzone isotopologues, pre- and post- UV irradiation.



% Absorbance decrease for the deuterated isotopologues of avobenzone synthesised, measured according to ISO 24443.

Sample	Absorbance before UV irradiation $\pm$ SD <sup>a</sup>	% RSD of absorbance before UV irradiation	Absorbance post UV irradiation $\pm$ SD <sup>a</sup>	% RSD of absorbance post UV irradiation	% Decrease in absorbance at 357 nm after irradiation <sup>a</sup>
Avobenzone <sup>b</sup>	1.56 $\pm$ 0.12	7.9 %	0.44 $\pm$ 0.06	14.5 %	72.1
Avobenzone <sup>c</sup>	1.55 $\pm$ 0.11	7.1 %	0.46 $\pm$ 0.10	21.0 %	70.3
Avobenzone- <i>d</i> <sub>2</sub>	1.64 $\pm$ 0.14	8.8 %	0.44 $\pm$ 0.08	17.8 %	73.1
Avobenzone- <i>d</i> <sub>15</sub>	1.53 $\pm$ 0.13	8.5 %	0.49 $\pm$ 0.06	12.0 %	67.7
Avobenzone- <i>d</i> <sub>19</sub>	1.50 $\pm$ 0.13	8.6 %	0.37 $\pm$ 0.04	13.3 %	75.2

<sup>a</sup> average of nine surface locations.

<sup>b</sup> commercial sample (Parsol 1789).

<sup>c</sup> pharmaceutical secondary standard and certified reference material (Sigma-Aldrich, PHR1073).

In the UV-Vis, the enol has an absorbance wavelength maxima typically around 350-363 nm<sup>1-6</sup>, thought to be the chelated *Z*- (*cis*-) enol. There are conflicting reports of where the non-chelated *E*- (*trans*-) enol absorbs, either contributing as a shoulder to the main band at 370 nm<sup>1,7</sup> or as a transient species at 300 nm<sup>4</sup>. The diketone is reportedly observed as a smaller shoulder in the UV-Vis spectrum at approximately 260-280 nm<sup>1,2,4-6</sup>.

The avobenzone isotopologues were examined by using ISO 24443 for the determination of sunscreen UVA photoprotection *in vitro*<sup>8</sup>. The method (described below) is equivalent to that for an SPF 50 + sunscreen after a full day of exposure, which avobenzone (normally chemically stabilised) survives.

The % decrease in UV-Vis absorbance of the chelated enol (357 nm) after UV irradiation was determined (refer to table, above), where avobenzone was deuterated instead of being chemically stabilised. No clear trend in the photostability data was observed for the isotopologues compared to the non-deuterated reference standards. The reasons for this could be several-fold; (1) the oil (avobenzone in decyl oleate) is coated by finger onto the PMMA surface and the film is likely to migrate during irradiation at 34 °C, and a non-uniform film thickness would lead to the large % RSD for the absorbance observed at the randomly selected locations, (2) the selected surface locations for measurement are different pre- and post- UV irradiation, (3) the presence of trace water in the decyl oleate solvent could lead to

back-exchange of OD to OH as previously discussed for other solvents, and (4) the method uses irradiation of a known dose for approximately 80 minutes to simulate UV exposure and so small differences in photostability due to deuteration may not be observed using this technique.

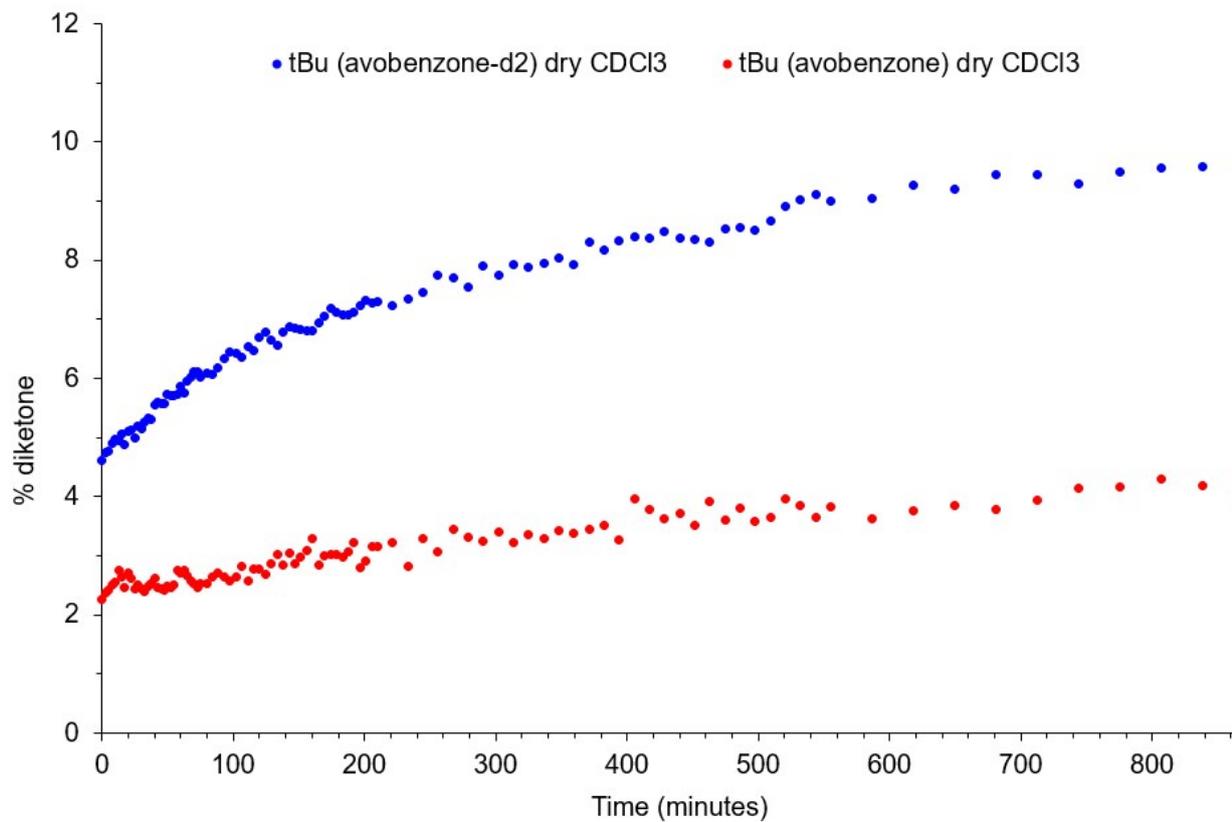
## Method

Photostability was tested to ISO 24443 specifications for the determination of sunscreen UVA photoprotection *in vitro*<sup>8</sup>, according to the following method<sup>9</sup>. Avobenzone (3% w/v) was dissolved in decyl oleate solvent, and coated onto a PMMA slide by finger at an application of 1.3 mg.cm<sup>-2</sup>. The slide was irradiated with 88 standard erythemal doses over 82 minutes at 34 °C in a solar simulator (Labsphere 2000, 7 mW.cm<sup>-2</sup> UVA), and the UV-Vis absorbance measured pre- and post- irradiation at nine locations over the 25 cm<sup>2</sup> area. The average % decrease in absorbance was calculated at 357 nm.

## References

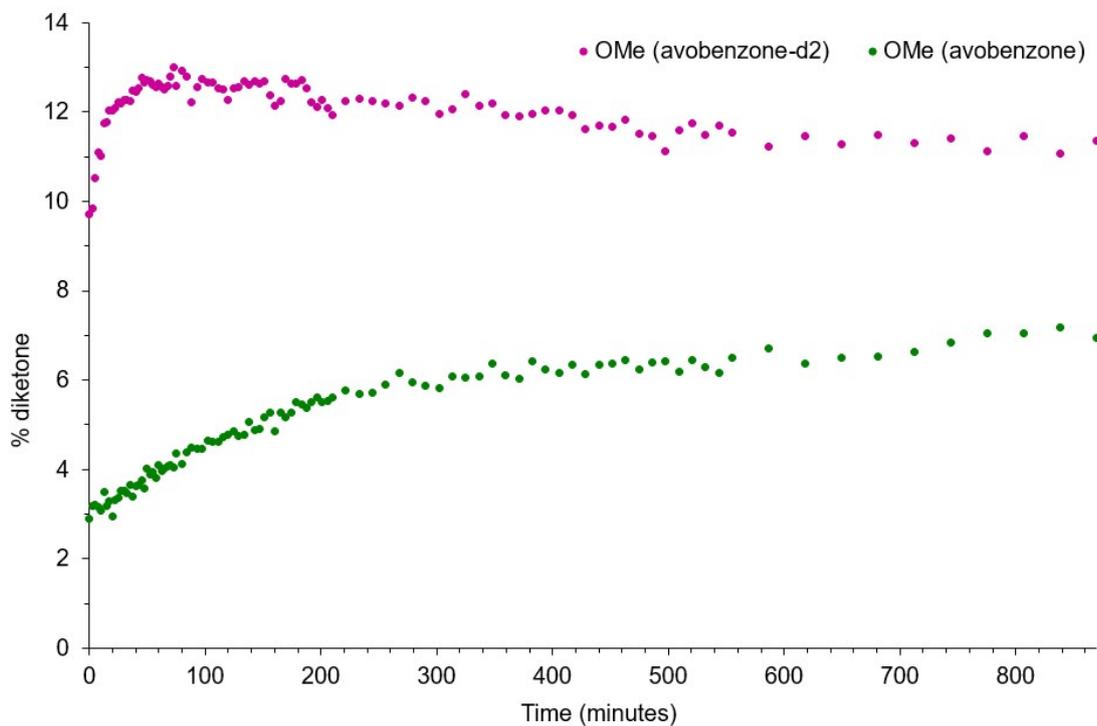
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S29. Equilibration to diketone from enol with time for avobenzene- $d_2$  and non-deuterated in anhydrous  $CDCl_3$ , using the relative integration of the pair of *tert*-butyl resonances.

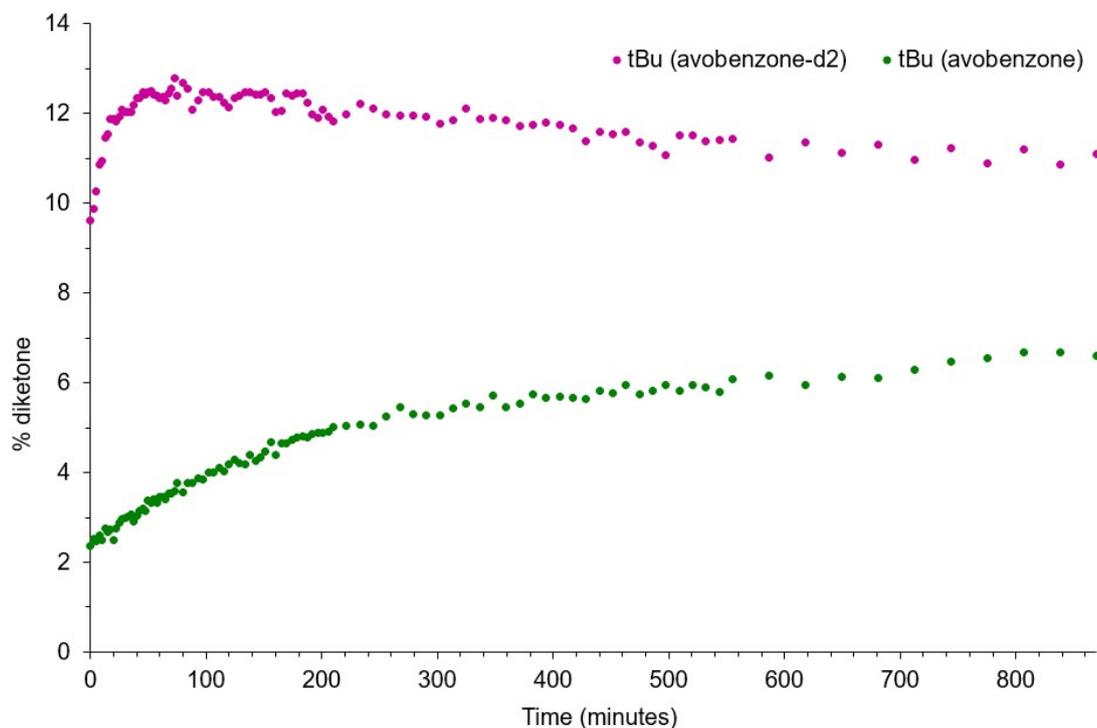


S30. Equilibration to diketone from enol with time for avobenzene- $d_2$  and non-deuterated in a typical (not anhydrous) sample of  $CDCl_3$ , using the relative integration of the pair of (a) methoxy and (b) *tert*-butyl resonances.

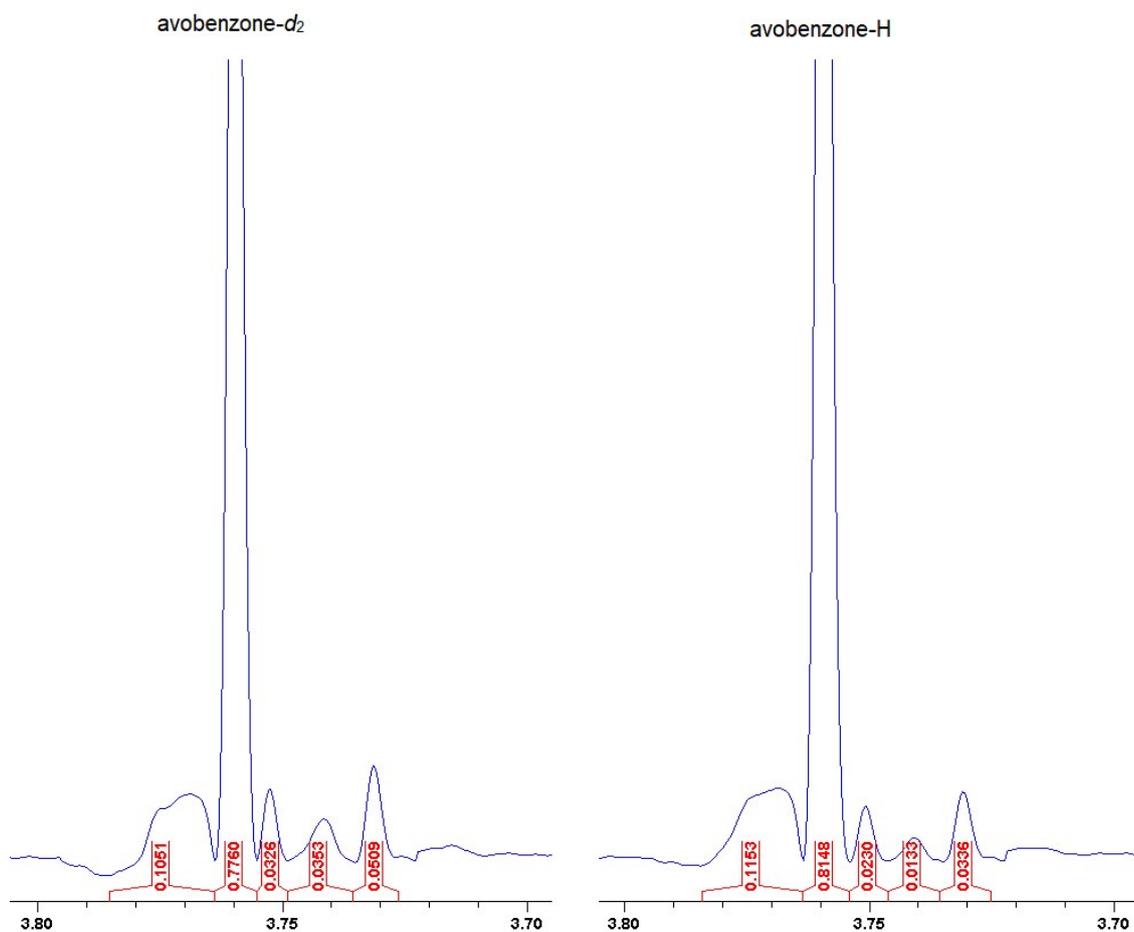
(a)



(b)



S31. <sup>1</sup>H NMR (methoxy resonance) of avobenzene (approx. 3.2 mM in C<sub>6</sub>D<sub>12</sub>) with *ex situ* UV irradiation for 9 hours, processed by lb = -1, gb = 0.05, gfp, manual baseline correction.



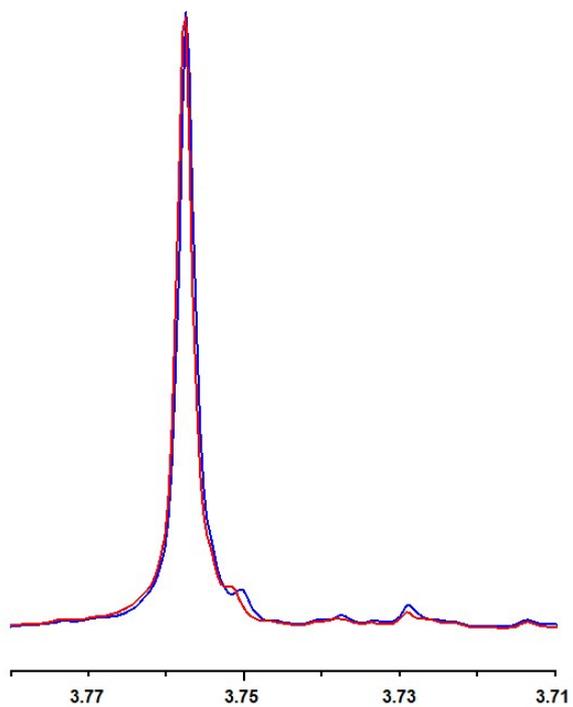
$$\% \text{ photodegradation} = 100 - 100 \cdot \left( \frac{\text{peak area of diketone and cis - enol peaks}}{\text{peak area of all peaks}} \right)$$

% photodegradation for avobenzene-*d*<sub>2</sub> = 17.30

% photodegradation for avobenzene-H = 15.16

Δ (% photodegradation) = 2.14, but significant variability depending on manual baseline correction.

S32.  $^1\text{H}$  NMR (methoxy resonance) of avobenzene- $d_2$  and non-deuterated (approx. 5.4 mM in  $\text{C}_6\text{D}_{12}$ ) with *in situ* UV LED irradiation for 18 hours.



S33. Zoom of  $^{13}\text{C}$  solid state NMR

Scaled to the same intensity for

