

## BDG SYNTHESIS

### Certificate of Analysis

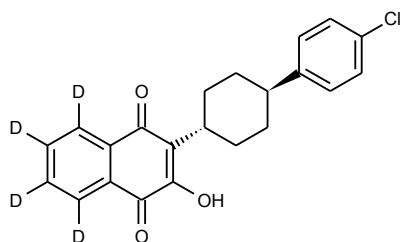
BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

*Barry Dent*

Barry R. Dent, PhD, Director  
30 October 2010

**Name:** Atovaquone-d<sub>4</sub>  
**CAS Number:** 95233-18-4 (unlabelled)

**Structure:**



**Molecular Weight:** C<sub>22</sub>H<sub>15</sub>D<sub>4</sub>ClO<sub>3</sub> = 370.86

**Lot Number:** BDG 11061.1

**Appearance:** Yellow, crystalline solid

**Purity By HPLC:** 98.4 %

**Isotopic Purity:** Under 0.5 % d<sub>0</sub>

**Re-test Date:** 30 October 2015

**Storage and Handling:**

Temperature:	refrigerate for prolonged storage; may be handled and shipped at ambient temperature.
Humidity:	not believed to be hygroscopic; may be handled in normal laboratory atmosphere.
Light:	protect from strong sunlight.
Caution:	only experienced laboratory personnel should handle the material.

## Identity and Purity

### Proton NMR Spectrum

Identity: the signals are consistent with the proposed structure and in accord with literature where available.

Isotopic Labelling: signals at the sites of deuteration are greatly diminished, compared with the spectrum of unlabelled material, indicating almost complete deuteration. Minor signals for residual protium are observed in the aromatic region.

Residual Solvents: no residual solvents are observed.

Impurities: no significant impurities are evident in the spectrum.

### Carbon-13 NMR Spectrum

Identity: the signals are consistent with the proposed structure and in accord with literature where available.

Isotopic Labelling: signals at the site of deuteration have collapsed to small multiplets compared with the spectrum of unlabelled material, indicating clean deuteration.

### High-resolution Mass Spectrum (ESI+)

Found  $m/z$  393.1170.  $C_{22}H_{15}D_4^{35}ClNaO_3$   $[M+Na]^+$  requires  $m/z$  393.1166. The deviation of 1.1 ppm is within normally accepted limits for the establishment of identity by HRMS. Small signals for M-1 and M-2 were observed indicating the presence of  $d_3$  and  $d_2$  species. No signal for  $d_0$  is observed [detection limit about 0.5 %].

### HPLC

A sharp, symmetrical peak is observed (98.4 %). Note: in the absence of reference materials for preparing calibration curves, it is assumed that all peaks have the same detector response. Where possible, the conditions of analysis follow a pharmacopeial or literature method, or have been adapted from same.

### Elemental Analysis

	Found:	C 71.45, H 4.02, D 2.14 %
$C_{22}H_{15}D_4ClO_3$	Requires:	C 71.25, H 4.08, D 2.17 %

The elemental analyses fall within generally accepted limits for establishing the molecular formula given. The results may also be taken to imply the absence of significant quantities of water or inorganic salts (which have not been elsewhere tested for because of sample size limitations).

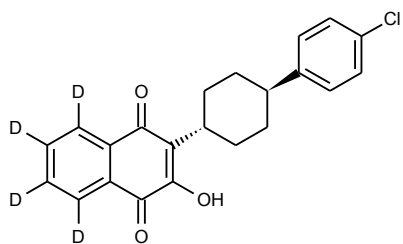
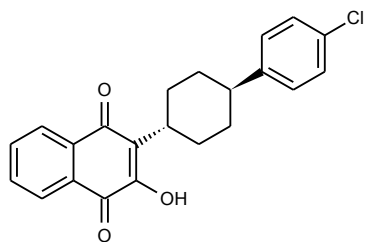
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials often contain higher levels of residual solvents and/or water, and we urge you to use the corrected purity where needed rather than the raw HPLC purity. This compound is intended for use as an analytical reference material and it is not for human administration. Structures are shown with relative stereochemistry unless otherwise specified.

The re-test date is assigned from experience gained with the material in the laboratory and/or on storage. It is not possible to perform formal storage studies because of the small amount of material available.

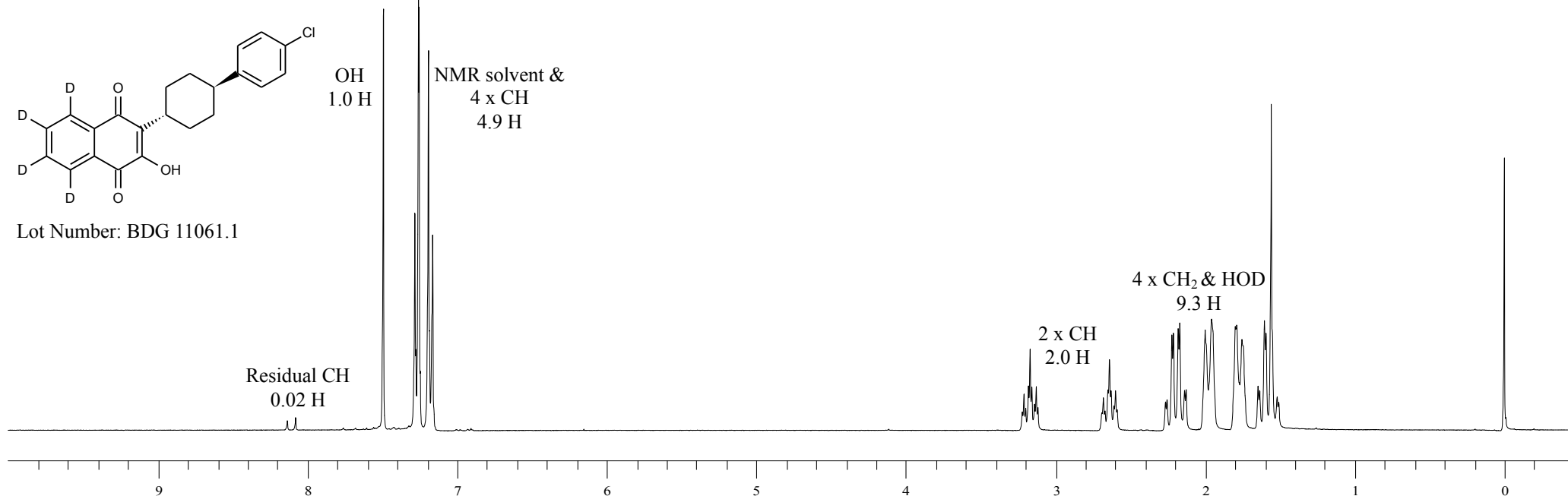


Proton NMR Spectrum of Atovaquone (top) and Atovaquone-d<sub>4</sub> (bottom) in CDCl<sub>3</sub>

**BDG SYNTHESIS**



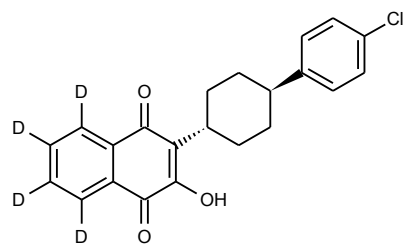
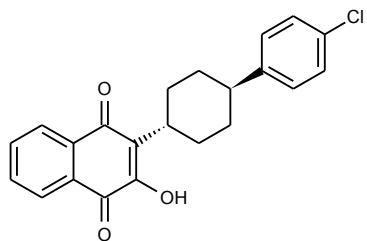
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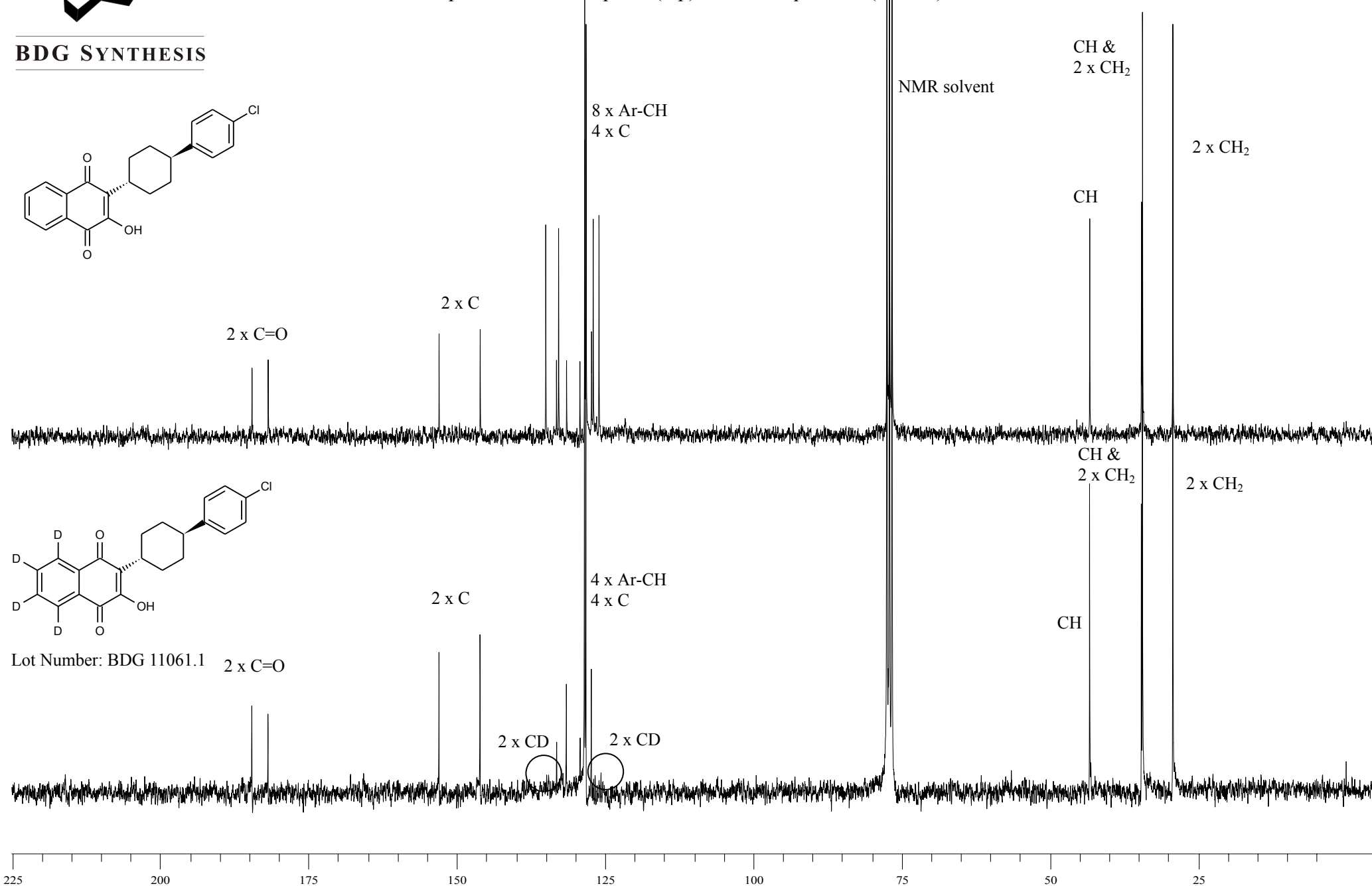


**BDG SYNTHESIS**

Carbon-13 NMR Spectrum of Atovaquone (top) and Atovaquone-d<sub>4</sub> (bottom) in CDCl<sub>3</sub>



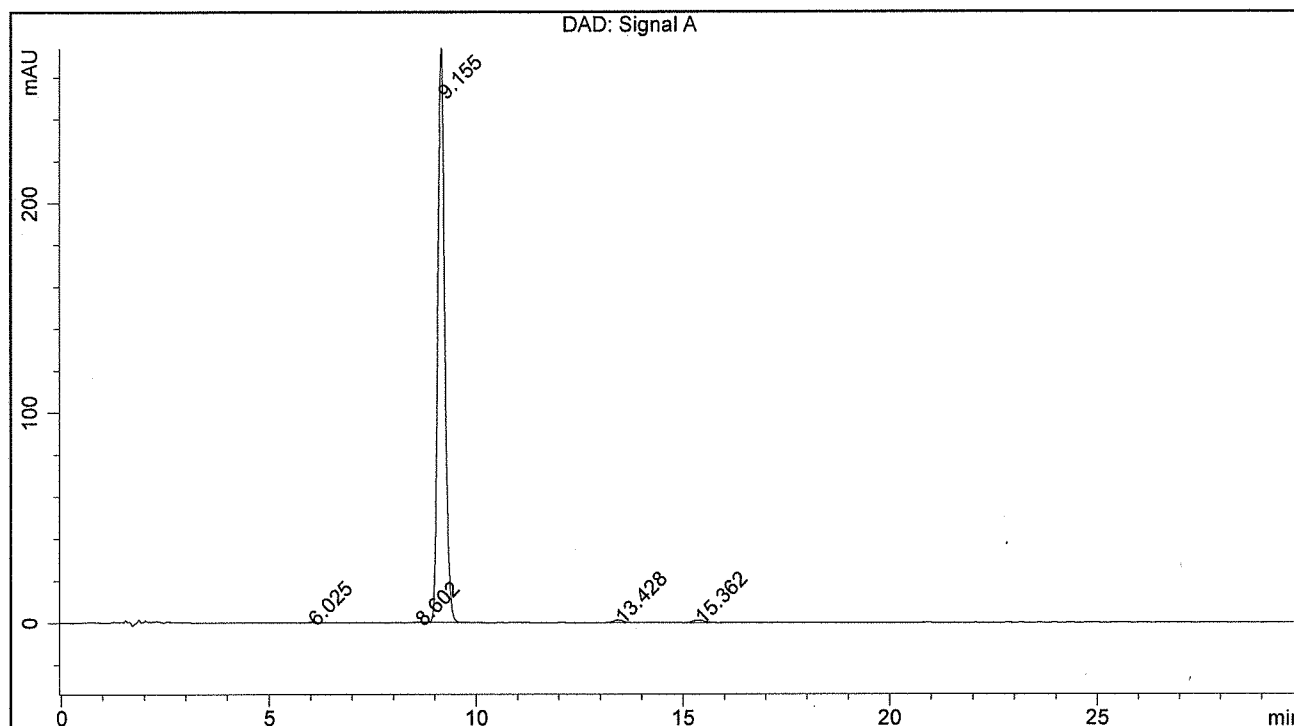
Lot Number: BDG 11061.1



BDG - Analysis of Atovaquone-d4

Column : Phenomenex Luna C18 (2) 5um 250 x 4.6 mm  
 Guard : Phenomenex Security Guard C18 RP 4 x 3 mm  
 Mobile Phase : 63:16:21:0.5 Acetonitrile : Water : Methanol : Phosphoric Acid  
 Flow Rate : 1.5 mL/min  
 Column Temperature : 25C  
 Sample Solvent : 20:80:0.5 Water : Acetonitrile : Phosphoric Acid  
 Injection Volume : 10 uL  
 Detection : UV at 220 nm

<b>Sample Name</b>	BDG 11061.1	<b>Instrument</b>	AnalyticalLC01
<b>Acquisition</b>	30/10/2010, 15:06:40	<b>Method (rev.)</b>	LC10371a ( 9)
<b>Sequence</b>	BDG_29Oct2010a - Reprocessed	<b>Vial Position</b>	11
<b>Operator</b>	solvation010\cerityadmin	<b>Injection</b>	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	6.02 min	0.4271	3.8974	0.1385 min	0.119 %
2	8.60 min	0.3248	3.3927	0.1539 min	0.104 %
3	9.16 min	275.4048	3215.8231	0.1802 min	98.431 %
4	13.43 min	1.2385	20.6534	0.2586 min	0.632 %
5	15.36 min	1.2154	23.3113	0.2958 min	0.714 %