

## BDG SYNTHESIS

### Certificate of Analysis

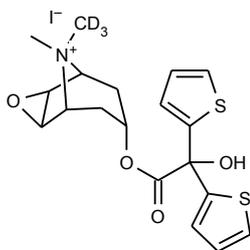
BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

*Neil Beare*

Neil Beare, PhD, Director  
9 October 2014

**Name:** Tiotropium-d<sub>3</sub> Iodide  
**CAS Number:** 136310-93-5 (bromide salt, unlabelled)

**Structure:**



**Molecular Weight:** C<sub>19</sub>H<sub>19</sub>D<sub>3</sub>NO<sub>4</sub>S<sub>2</sub>·I = 522.44  
**Lot Number:** BDG 5264.3  
**Appearance:** Off-white powder  
**Purity By HPLC:** 99.9 %  
**Isotopic Purity:** Under 0.5 % d<sub>0</sub>  
**Re-test Date:** 9 October 2019  
**Storage and Handling:** Temperature: refrigerate for prolonged storage; may be handled and shipped at ambient temperature.  
Humidity: may be hygroscopic; store desiccated; recommended to determine water content periodically.  
Light: store in an amber vial and protect from bright light.  
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: the first NCH<sub>3</sub> signal is greatly diminished (0.2 H) and the second NCH<sub>3</sub> signal is slightly diminished (2.8 H) with respect to unlabelled material, indicating clean deuteration.  
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 sites of deuteration have collapsed to small multiplets compared with the spectrum of unlabelled material, indicating clean deuteration.

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

Found  $m/z$  395.1175. C<sub>19</sub>H<sub>19</sub>D<sub>3</sub>NO<sub>4</sub>S<sub>2</sub> [M-I]<sup>+</sup> requires  $m/z$  395.1173. The deviation of 0.5 ppm is within normally accepted limits for the establishment of identity by HRMS. No signal for d<sub>0</sub> material was seen (detection limit about 0.5 %).

### HPLC

A somewhat broadened, slightly tailing peak is observed (99.9 %). The peak at 2.5 min has been identified as the iodide counterion. 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 43.52, H 3.77, D 1.19, N 2.65 %
C <sub>19</sub> H <sub>19</sub> D <sub>3</sub> NO <sub>4</sub> S <sub>2</sub> ·I	Requires:	C 43.68, H 3.67, D 1.16, N 2.68 %

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).

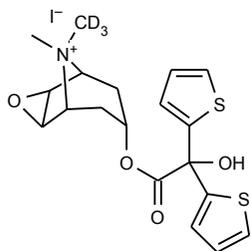
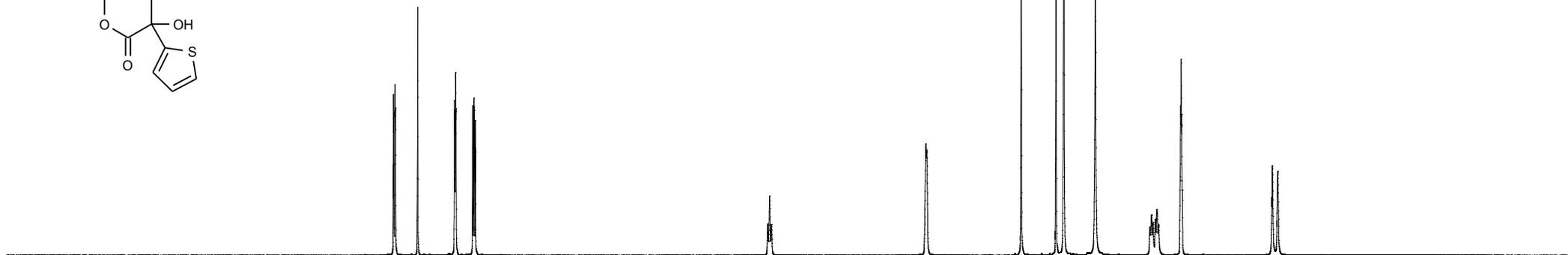
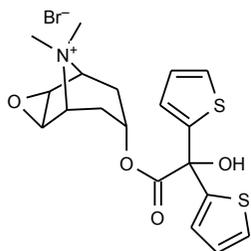
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials 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.

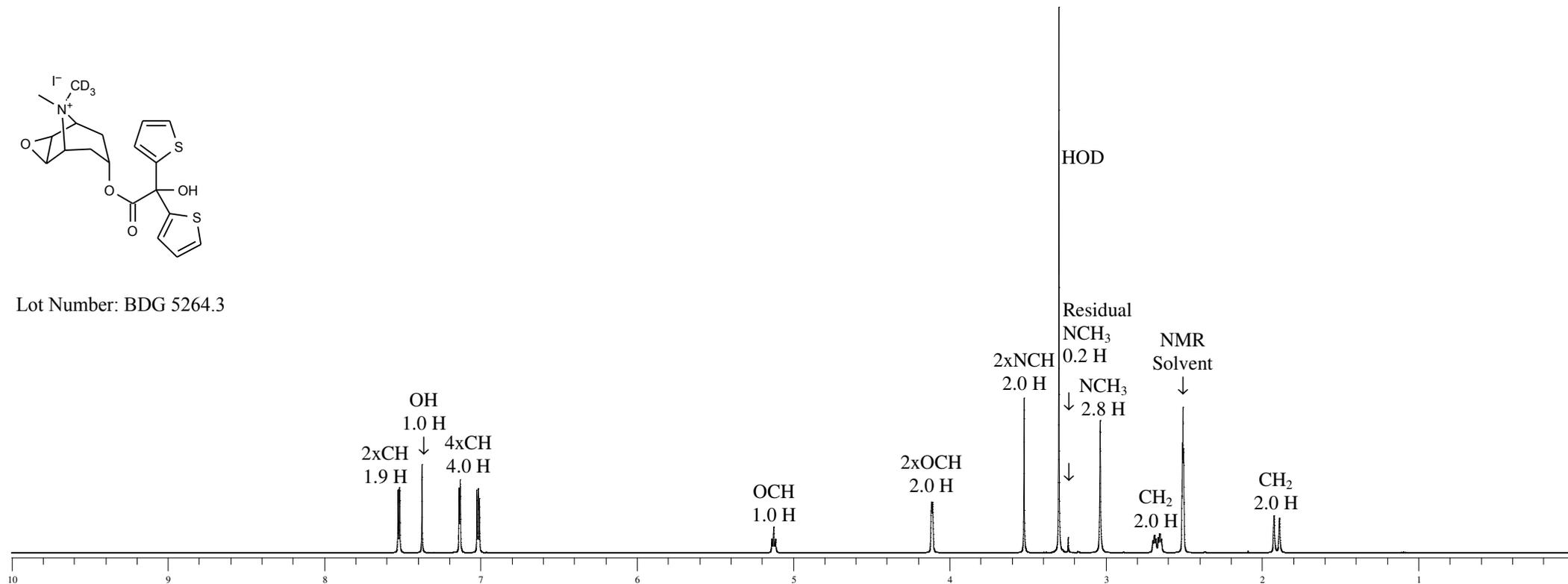


Proton NMR Spectrum of Tiotropium Bromide (top) and Tiotropium-d<sub>3</sub> Iodide (bottom) in DMSO-d<sub>6</sub>

**BDG SYNTHESIS**



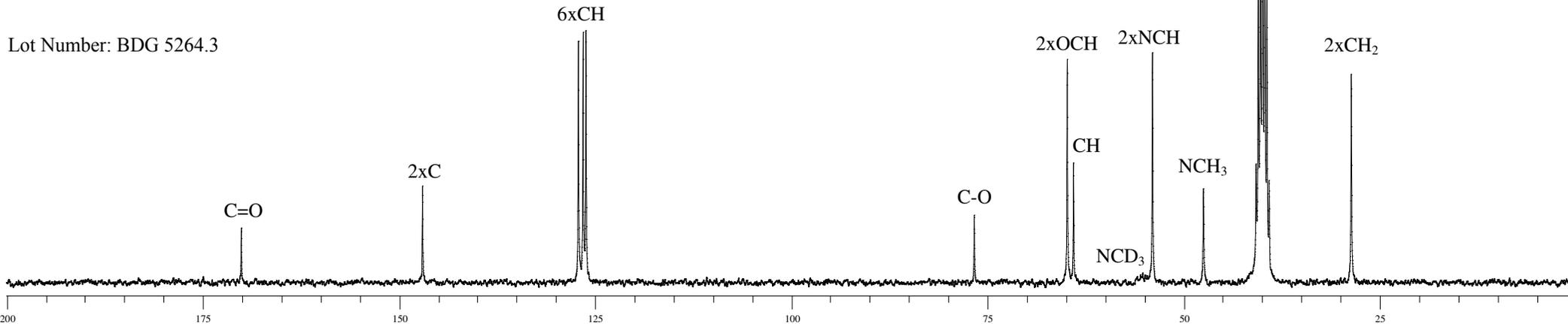
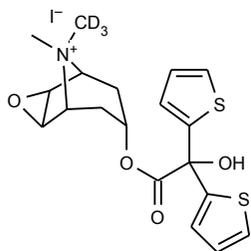
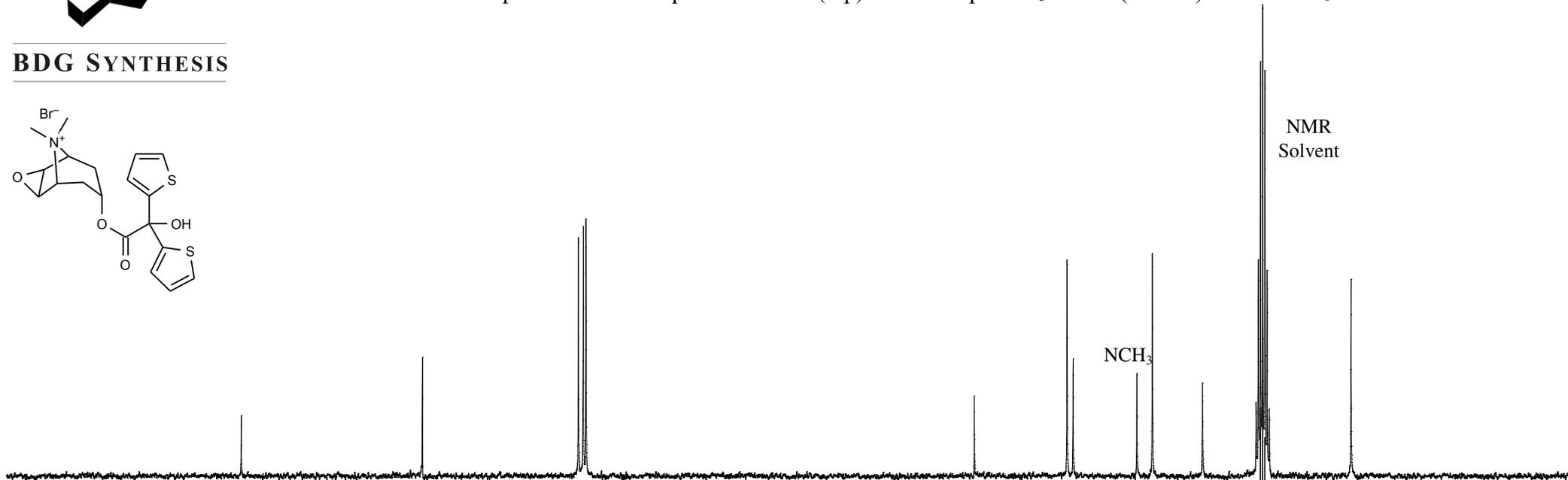
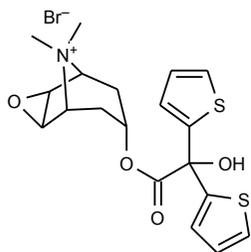
Lot Number: BDG 5264.3





Carbon-13 NMR Spectrum of Tiotropium Bromide (top) and Tiotropium-d<sub>3</sub> Iodide (bottom) in DMSO-d<sub>6</sub>

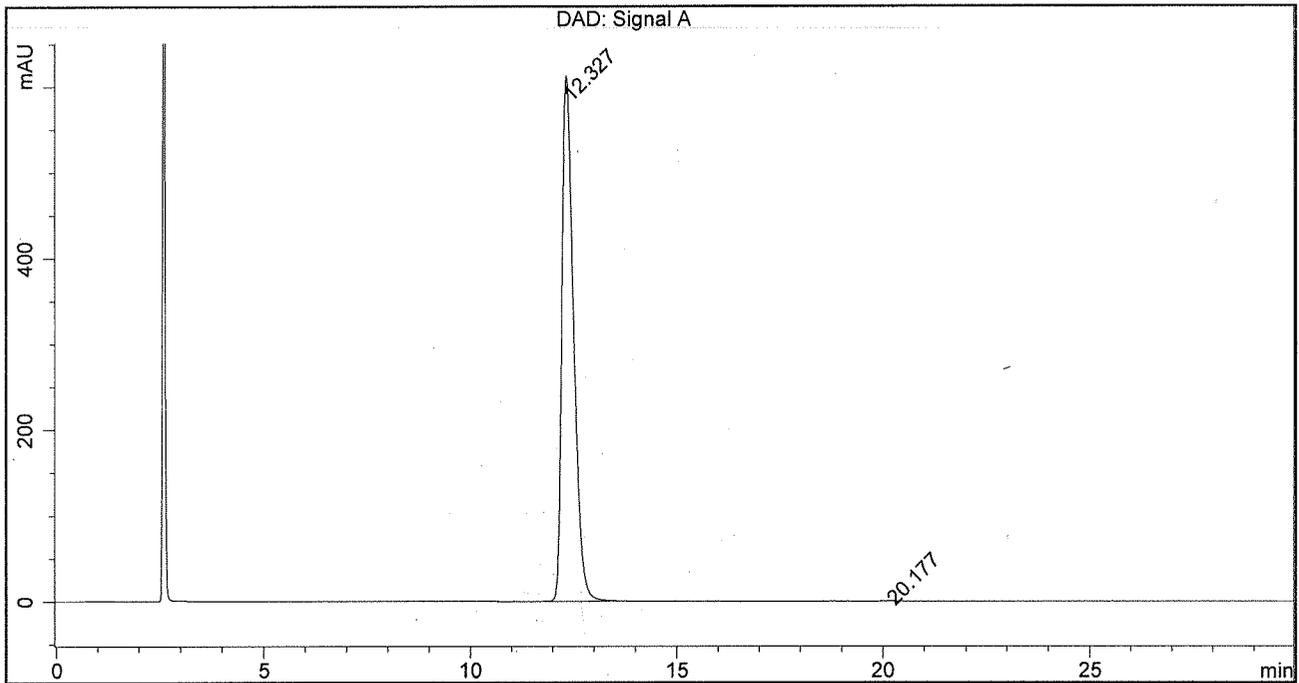
**BDG SYNTHESIS**



BDG - Analysis of Tiotropium-d3 Iodide

Column : Phenomenex Luna C18(2) 5 um 250 x 4.6 mm  
 Guard : Phenomenex Security Guard C18 RP 4 x 3 mm  
 Mobile Phase : 70:30 50 mM Potassium diHydrogen Phosphate + 10 mM Tetrapropylammonium Bromide  
 pH=5.50 ( 1 M diPotassium Hydrogen Phosphate ) : Methanol  
 Flow Rate : 1.5 mL/min . . . . . Column Temperature : 30 C . . . . . Detection: UV 238 nm  
 Sample Solvent : Mobile Phase . . . . . Injection Volume : 10 uL

<b>Sample Name</b>	BDG 5264.3	<b>Instrument</b>	AnalyticalLC01
<b>Acquisition</b>	09/10/2014, 15:12:22	<b>Method (rev.)</b>	LC10013c ( 6)
<b>Sequence</b>	BDG_09Oct2014b - Reprocessed	<b>Vial Position</b>	24
<b>Operator</b>	solvation010\cerityadmin	<b>Injection</b>	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	12.33 min	612.3933	11921.3955	0.2991 min	99.928 %
2	20.18 min	0.3233	8.6202	0.3231 min	0.072 %