



BDG SYNTHESIS

Certificate of Analysis

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

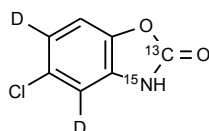
Neil Beare

Neil Beare, PhD, Director
8 January 2015

Name: Chlorzoxazone-¹³C,¹⁵N,d₂

CAS Number: 95-25-0 (unlabelled)

Structure:



Molecular Weight: C₆¹³CH₂D₂Cl¹⁵NO₂ = 173.56

Lot Number: BDG 2479

Appearance: Pale tan powder

Purity By HPLC: 99.6 %

Isotopic Purity: Under 0.5% M-4

Re-test Date: 8 January 2020

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 for H-4 and H-6 integrate for 7.4 % and 2.9 % residual hydrogen respectively. These observations show that the material contains on average 1.9 molar equivalents of deuterium.

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: the spectrum is of little value in determining isotopic purity, although the signals at the labelling sites are massively enhanced as expected.

Impurities: an unidentified signal is observed at 161 ppm. In the absence of significant impurities observed by other methods, we conclude that the signal is from a ^{13}C -labelled impurity. Given the natural abundance of ^{13}C (1 %) and similar relative peak heights of this peak and of the unlabelled carbons, we suggest that the impurity is present at about 1 mol %.

High-resolution Mass Spectrum (ESI+)

Found m/z 173.0058. $\text{C}_6^{13}\text{CH}_2\text{D}_2\text{Cl}^{15}\text{NO}_2$ $[\text{M}]^+$ requires m/z 173.0060. The deviation of 1.4 ppm is within normally accepted limits for the establishment of identity by HRMS. No signal for M-4 material was seen (detection limit about 0.5 %).

HPLC

A sharp, symmetrical peak is observed (99.6 %). 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.

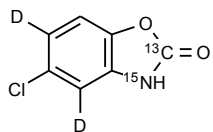
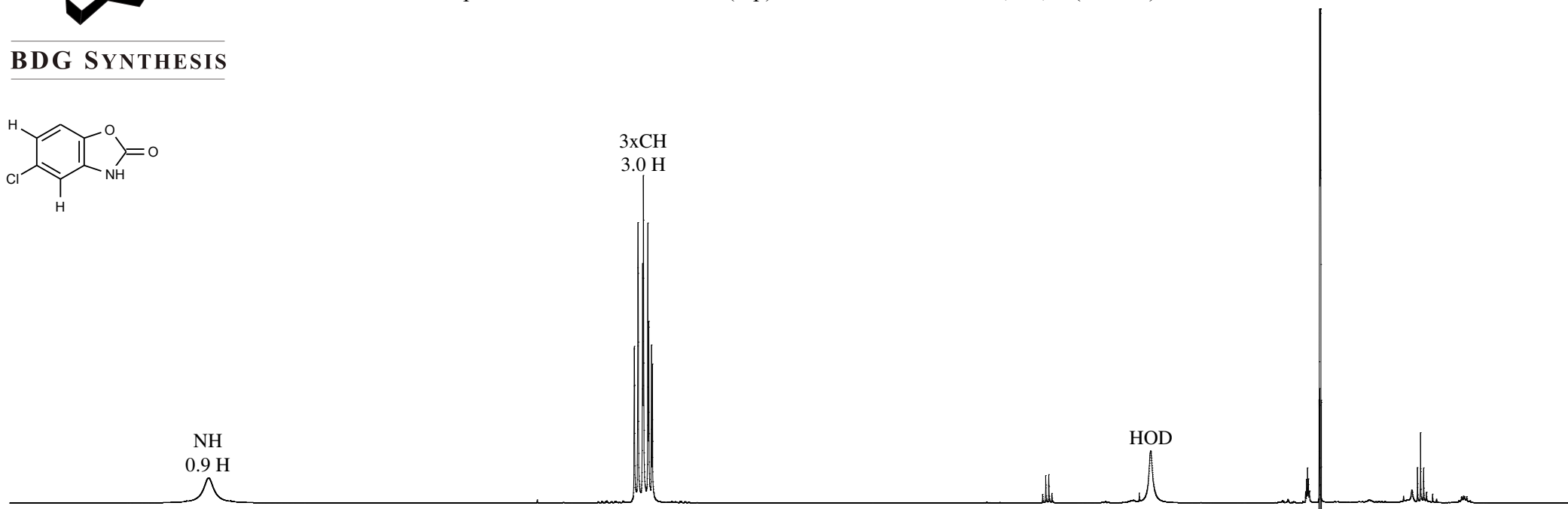
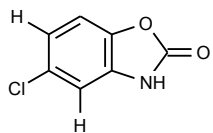
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials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

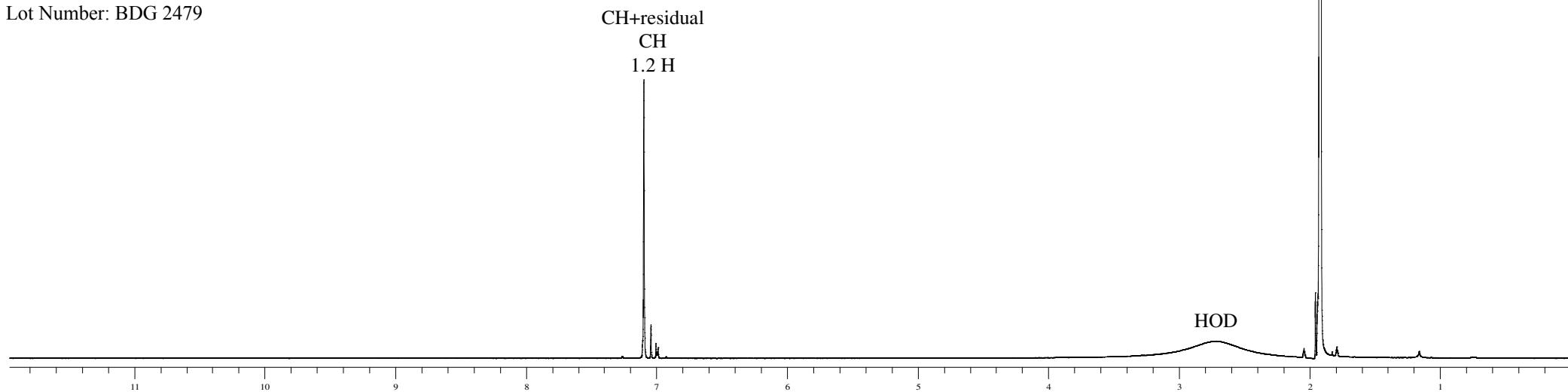


Proton NMR Spectrum of Chlorzoxazone (top) and Chlorzoxazone-¹³C,¹⁵N,d₂ (bottom) in Acetone-d₆

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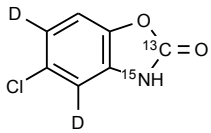
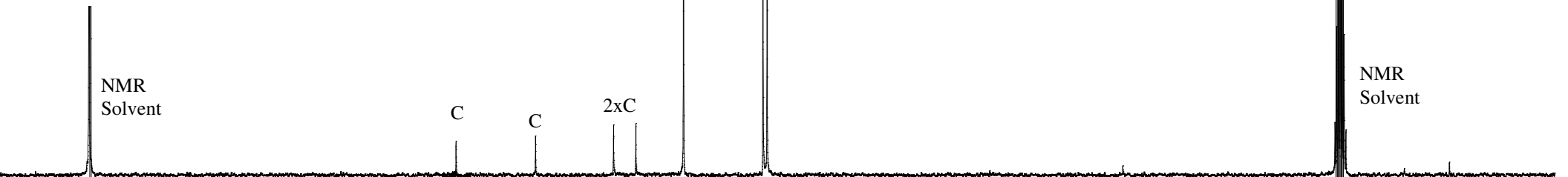
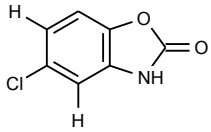
Lot Number: BDG 2479



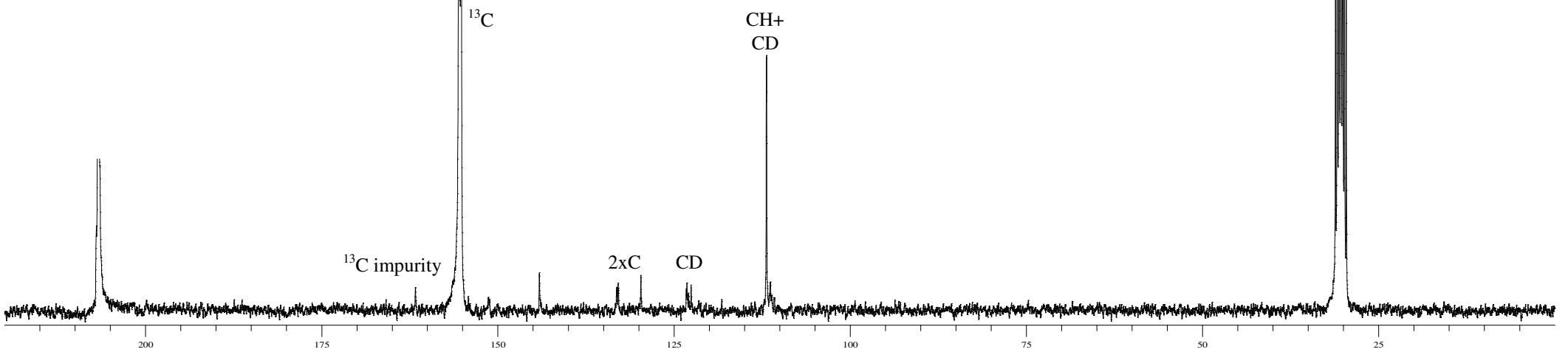


Carbon-13 NMR Spectrum of Chlorzoxazone (top) and Chlorzoxazone-¹³C,¹⁵N,²D (bottom) in Acetone-d₆

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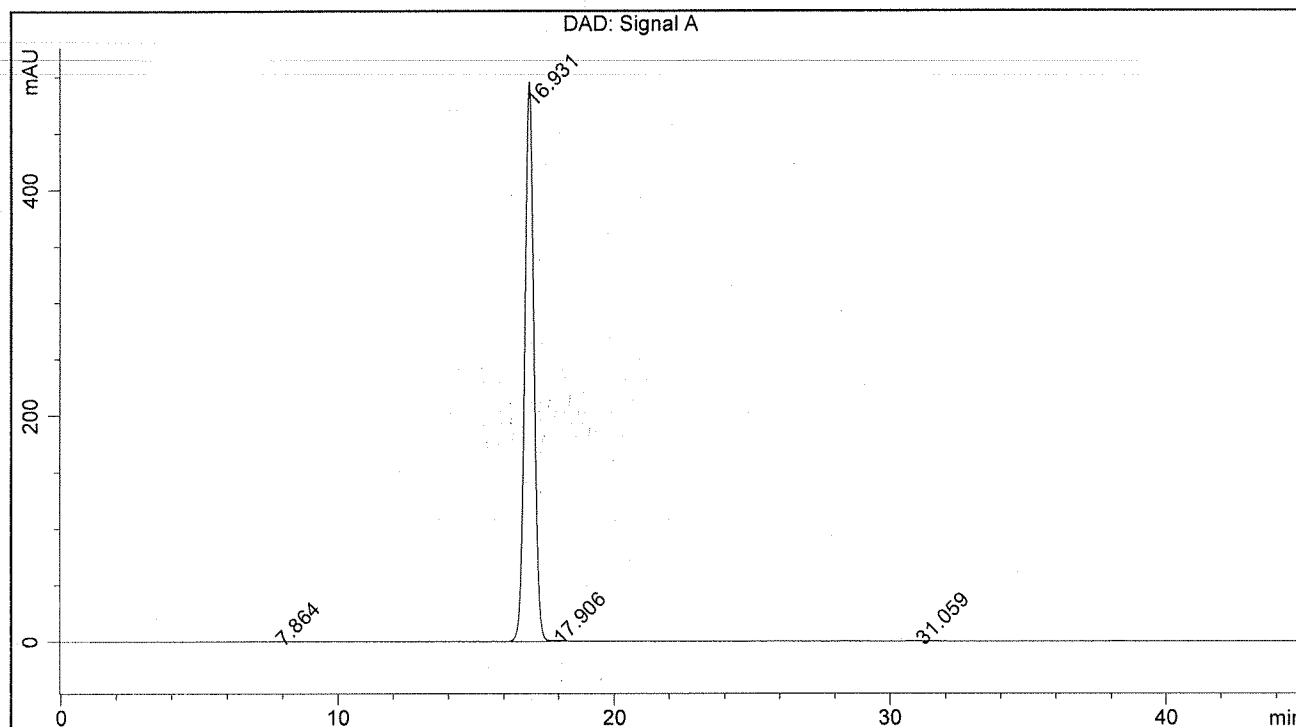
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BDG - Analysis of Chlorzoxazone-13C,15N,d2

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 Ammonium Acetate pH=4.25 (Acetic Acid) : Acetonitrile
 Flow Rate : 1.0 mL/min Column Temperature : 20 C Detection: UV 82 nm
 Sample Solvent : 70:30 Water Acetonitrile Injection Volume : 10 uL

Sample Name	BDG 2479	Instrument	AnalyticalLC01
Acquisition	08/01/2015, 18:25:24	Method (rev.)	LC10645a (4)
Sequence	BDG_08Jan2015c - Reprocessed	Vial Position	1
Operator	solvation010\cerityadmin	Injection	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	7.86 min	0.1757	2.9265	0.2503 min	0.025 %
2	16.93 min	494.8042	11500.9275	0.3592 min	99.558 %
3	17.91 min	1.0758	38.5992	0.4706 min	0.334 %
4	31.06 min	0.2177	9.5042	0.5454 min	0.082 %