BDG SYNTHESIS

Certificate of Analysis

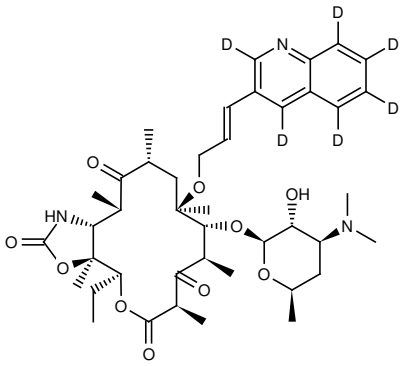
BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

Neil Beare, PhD, Director
21 January 2017

Name: **Cethromycin-d_6**

CAS Number: 205110-48-1 (unlabelled)

Structure:



Molecular Weight: $C_{42}H_{53}D_6N_3O_{10}$ = 771.97

Lot Number: BDG 10922.1

Appearance: Off-white, crystalline solid

Corrected Purity: 96.3 % (HPLC) - 2.6 % (heptane) - 2.3 % (water) = 91.4 %

Isotopic Purity: Under 0.5 % d_0

Re-test Date: 21 January 2022

Storage and Handling:

Temperature:	freeze (-20°C) 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:	store in an amber vial and protect from bright light.
Caution:	only experienced laboratory personnel should handle the material. Solutions of this compound in aqueous methanol or acetonitrile are light sensitive.

Mailing:
BDG Synthesis
PO Box 38 627, Wellington Mail Centre,
Wellington, New Zealand.

Shipping:
BDG Synthesis
Gracefield Research Centre, Building F,
Gracefield Road, Lower Hutt, New Zealand.

Phone: + 64 4 569 0520
Fax: + 64 4 569 0521

info@bdg.co.nz
www.bdg.co.nz

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 clean deuteration.
Residual Solvents: a small amount of heptane (2.6 % *w/w*) is 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* 772.4658. $C_{42}H_{54}D_6N_3O_{10}$ $[M+H]^+$ requires *m/z* 772.4655. The deviation of 0.4 ppm is within normally accepted limits for the establishment of identity by HRMS. No signal for d_0 material was seen (detection limit about 0.5 %).

HPLC

A sharp, symmetrical peak is observed (96.3 %). 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 63.85, H 7.19, D 1.63, N 5.16 %
$C_{42}H_{53}D_6N_3O_{10}\cdot 1.0H_2O$	Requires:	C 63.86, H 7.02, D 1.53, N 5.32 %, H_2O 2.28 %
$C_{42}H_{53}D_6N_3O_{10}$	Requires:	C 65.35, H 6.92, D 1.57, N 5.44 %

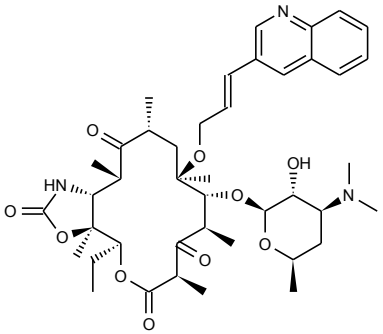
The elemental analyses fall somewhat outside those expected for anhydrous material; the presence of water is reasonably expected from the method of purification and/or the type of material, and the “best-fit” hydrated molecular formula is given. In the absence of a Karl-Fischer water analysis, we recommend that the “best-fit” water content be used when determining corrected purity.

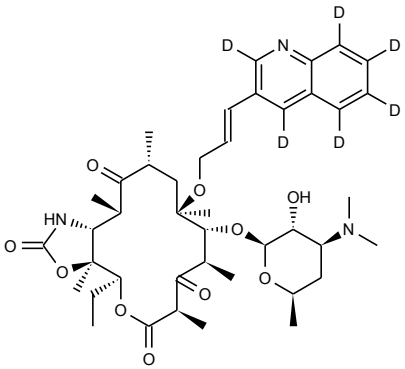
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials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

BDG SYNTHESIS

Proton NMR Spectrum of Cethromycin (top) and Cethromycin-d_6 (bottom) in $CDCl_3$ + TMS

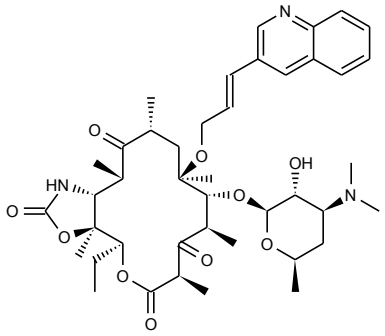




Lot Number: BDG 10922.1

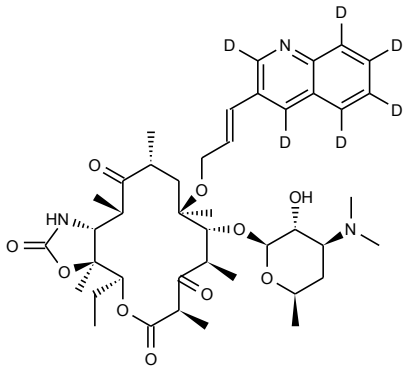
BDG SYNTHESIS

Carbon-13 NMR Spectrum of Cethromycin (top) and Cethromycin-d_6 (bottom) in $CDCl_3$ + TMS



CH

7xCH+2xC



Lot Number: BDG 10922.1

NMR Solvent+ 2xOCH

$N(CH_3)_2$

2xOCH +NCH

OCH_2

NCH+ 3xCH

CH_2 CH

$2xCH_2$

$3xCH_3$

$5xCH_3$

4xCD+2xCH +2xC

OCH

2xC-O

2xC=O

C=O

C=O

C

CD

CD

NMR Solvent

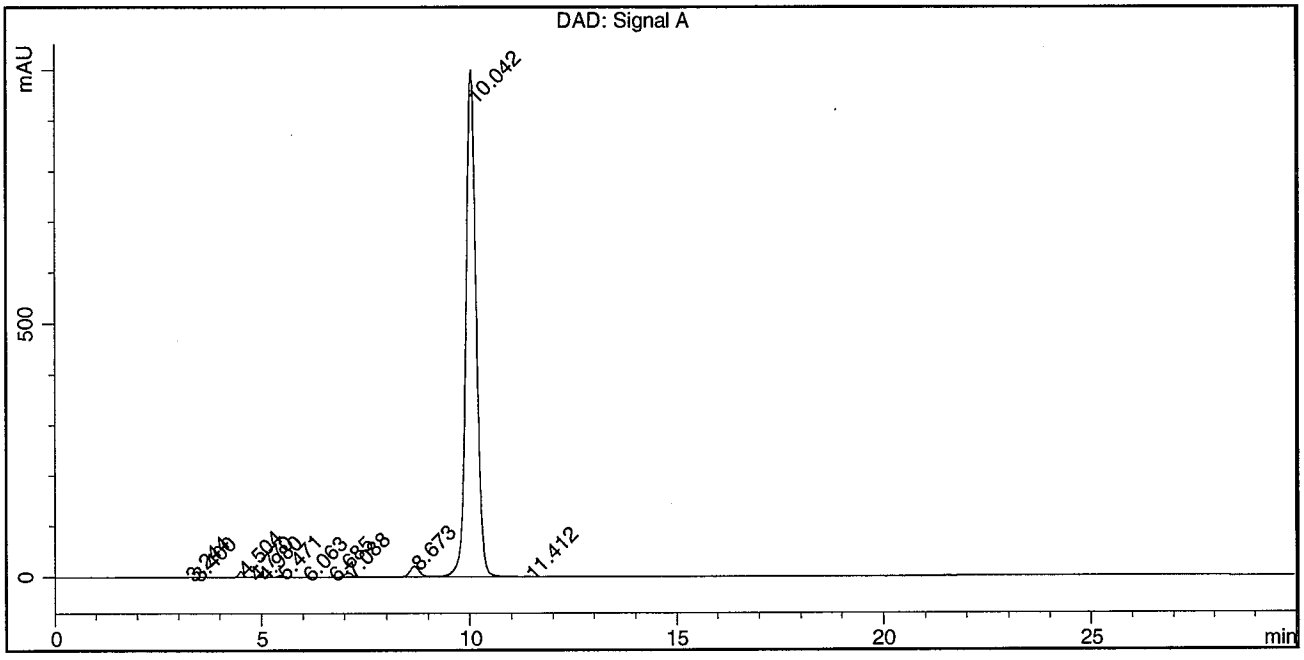
heptane

200 175 150 125 100 75 50 25

BDG - Analysis of Cethromycin-d6

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
Guard : Phenomenex Security Guard C18 4 x 3 mm
Mobile Phase A : 20:80 10 mM diPotassium Hydrogen Phosphate pH=7.0 : Methanol
Mobile Phase C : Methanol
Gradient (A:C) : T0=100:0, T16=100:0, T18=90:10, T24=90:10, T26=100:0, T30=100:0
Flow Rate : 1.0 mL/min . . . . . Sample Solvent : Initial Mobile Phase
Column Temperature : 40 C . . . . . Injection Volume : 10 uL . . . . . Detection : UV at 250 nm

Sample Name	BDG 10922.1	**Instrument**	AnalyticalLC01
Acquisition	21/01/2017, 13:55:58	**Method (rev.)**	LC10400o (3)
Sequence	BDG_21Jan2017b - Reprocessed	**Vial Position**	72
Operator	solvation010\cerityadmin	**Injection**	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	3.24 min	0.9505	4.7093	0.0818 min	0.027 %
2	3.40 min	0.4763	2.9586	0.1005 min	0.017 %
3	4.50 min	11.3704	90.9194	0.1213 min	0.513 %
4	4.77 min	0.8448	6.4514	0.1131 min	0.036 %
5	4.98 min	1.3106	12.1847	0.1405 min	0.069 %
6	5.47 min	3.4223	37.0223	0.1742 min	0.209 %
7	6.06 min	0.5885	10.8480	0.2271 min	0.061 %
8	6.68 min	0.6466	6.8729	0.1480 min	0.039 %
9	7.09 min	8.4477	111.4492	0.2019 min	0.629 %
10	8.67 min	21.1682	353.4542	0.2588 min	1.994 %
11	10.04 min	998.9423	17078.3466	0.2635 min	96.339 %
12	11.41 min	0.6991	12.0729	0.2135 min	0.068 %