



BDG SYNTHESIS

Certificate of Analysis

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

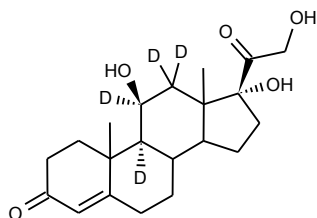
Neil Beare

Neil Beare, PhD, Director
29 October 2016

Name: Hydrocortisone-9,11,12,12-d₄

CAS Number: 73565-87-4

Structure:



Molecular Weight: C₂₁H₂₆D₄O₅ = 366.48

Lot Number: BDG 15579.3

Appearance: White, crystalline solid

Corrected Purity: 99.4 % (HPLC) - 1.1 % (ethyl acetate) = 98.3 %

Isotopic Purity: Under 0.5 % d₀

Re-test Date: 29 October 2021

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 absent, compared with the spectrum of unlabelled material, indicating clean deuteration.

Residual Solvents: a small amount of ethyl acetate (1.1 % 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 (TOF MS ES+)

Found m/z 389.2243. $C_{21}H_{26}D_4NaO_5$ $[M+Na]^+$ requires m/z 389.2242. The deviation of 0.3 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 somewhat broadened, symmetrical peak is observed (99.4 %). 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 66.88, H 7.20, D 2.21 %
$C_{21}H_{26}D_4O_5$	Requires:	C 68.82, H 7.15, D 2.20 %

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).

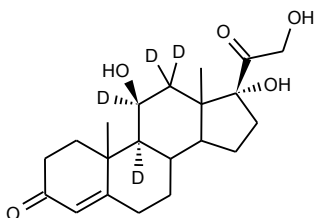
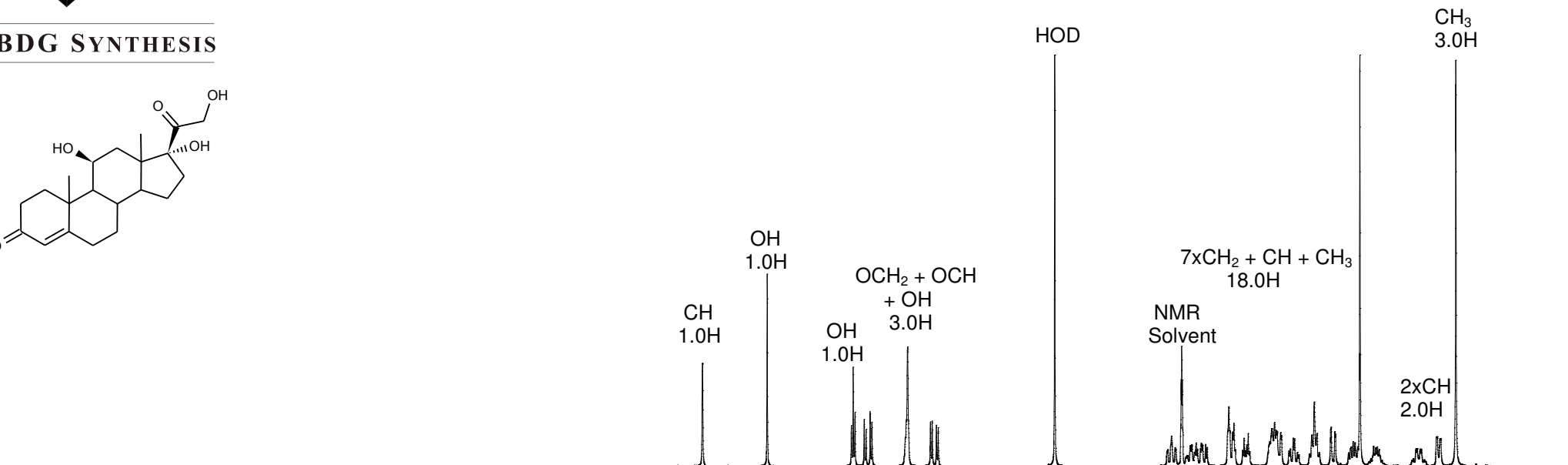
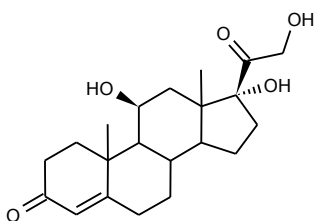
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials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

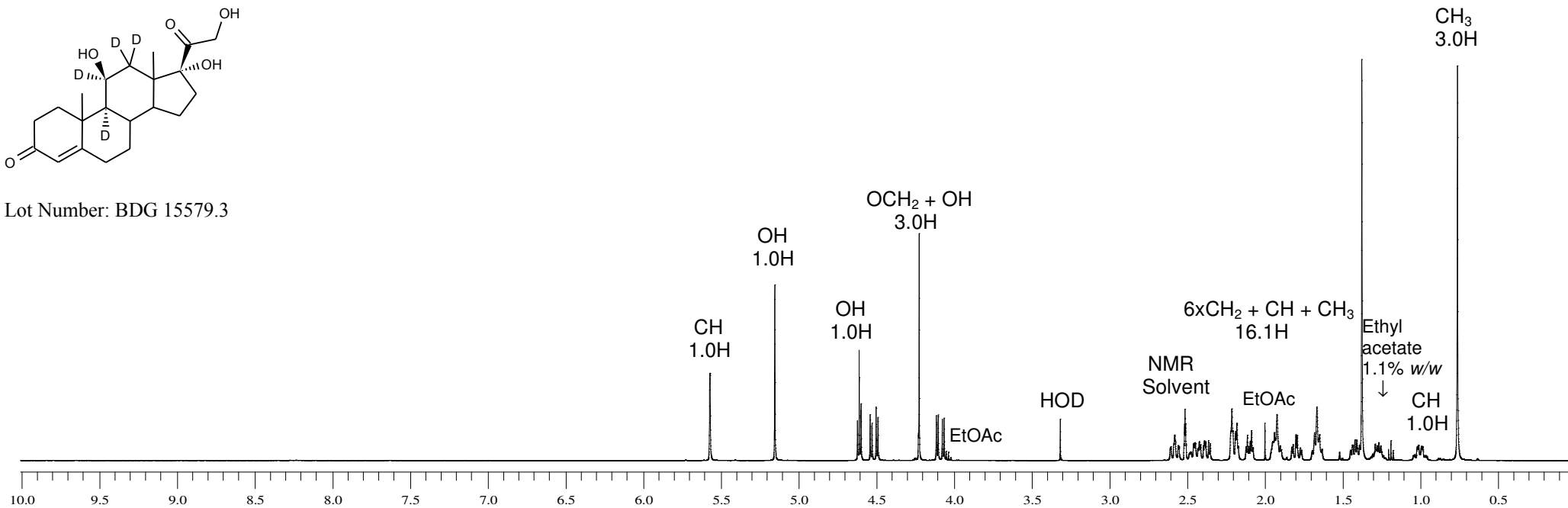


Proton NMR Spectrum of Hydrocortisone (top) and Hydrocortisone-9,11,12,12-d₄ (bottom) in DMSO-d₆

BDG SYNTHESIS



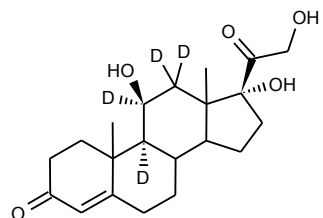
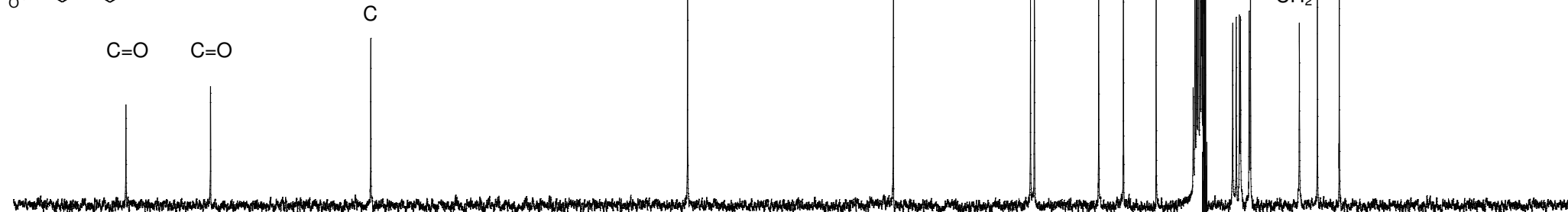
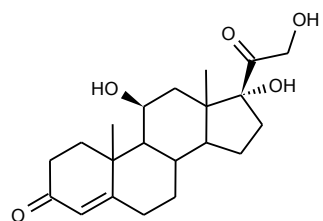
Lot Number: BDG 15579.3



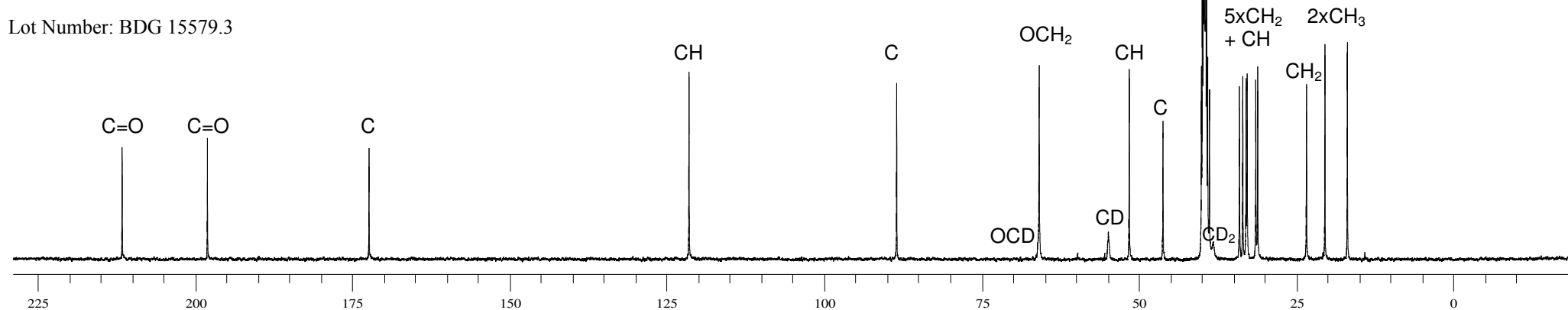


Carbon-13 NMR Spectrum of Hydrocortisone (top) and Hydrocortisone-9,11,12,12-d₄ (bottom) in DMSO-d₆

BDG SYNTHESIS



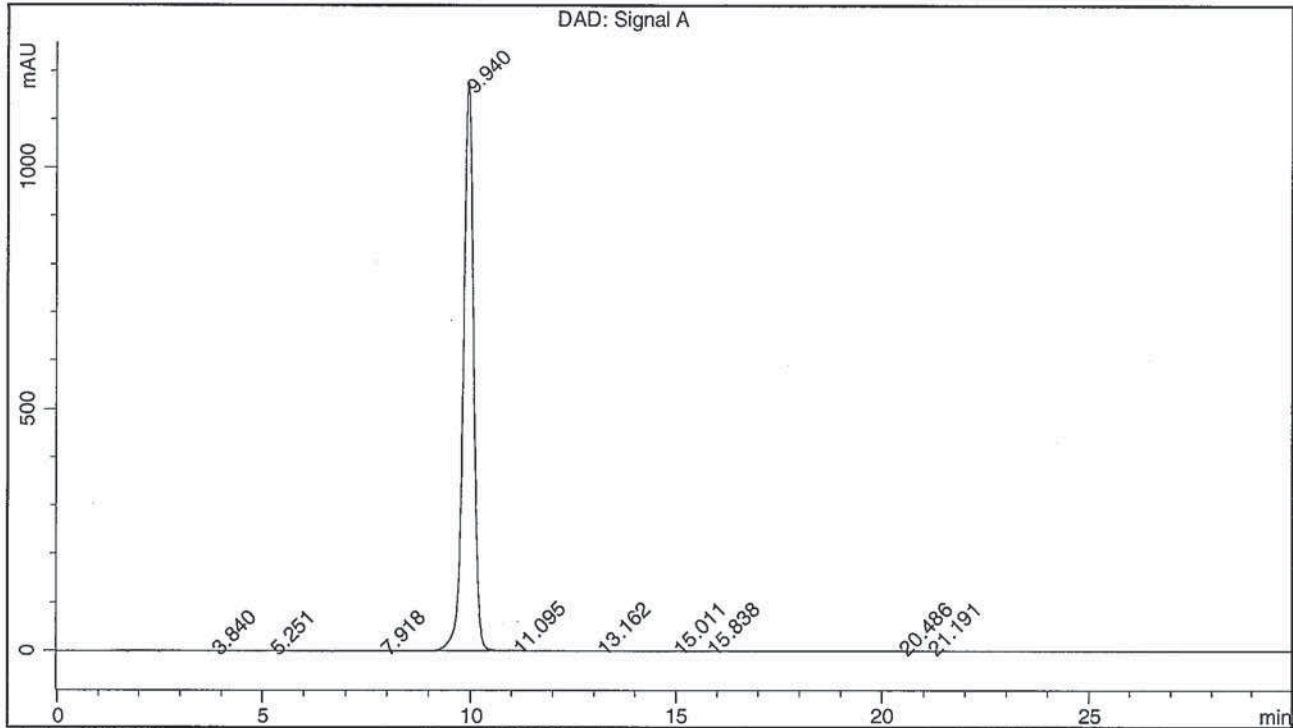
Lot Number: BDG 15579.3



BDG - Analysis of Hydrocortisone-9,11,12,12-d4

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
 Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
 Mobile Phase : 40:60 Water : Methanol
 Column Temperature : 20 C Flow Rate : 1.0 mL/min Injection Volume : 10 uL
 Sample Solvent : Mobile Phase Detection : UV at 254 nm

Sample Name	BDG 15579.3	Instrument	AnalyticalLC01
Acquisition	29/10/2016, 12:54:05	Method (rev.)	LC10111a (9)
Sequence	BDG_29Oct2016a - Reprocessed	Vial Position	5
Operator	solvation010\cerityadmin	Injection	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	3.84 min	0.3937	3.5393	0.1289 min	0.017 %
2	5.25 min	1.0736	10.2937	0.1439 min	0.050 %
3	7.92 min	0.7563	12.8256	0.2138 min	0.063 %
4	9.94 min	1182.2595	20380.3326	0.2651 min	99.449 %
5	11.09 min	1.1904	22.1947	0.2335 min	0.108 %
6	13.16 min	0.4338	10.3300	0.3098 min	0.050 %
7	15.01 min	0.6542	17.0790	0.3246 min	0.083 %
8	15.84 min	0.6736	17.8170	0.3326 min	0.087 %
9	20.49 min	0.4284	11.0481	0.3169 min	0.054 %
10	21.19 min	0.2827	7.7287	0.3350 min	0.038 %