BDG SYNTHESIS

Certificate of Analysis

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

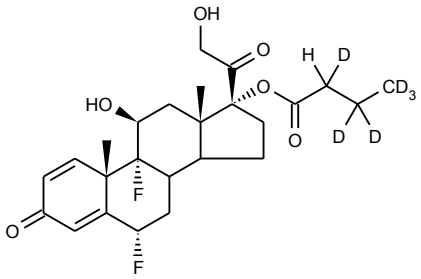
Barry Dent

Barry R. Dent, PhD, Director
19 August 2014

Name: **6α,9α-Difluoroprednisolone-17-butyrate-d_6**

CAS Number: 23640-96-2 (unlabelled)

Structure:



Molecular Weight: $C_{25}H_{26}D_6F_2O_6$ = 472.55

Lot Number: BDG 9235.1

Appearance: White, crystalline solid

Corrected Purity: 98.7 % (HPLC) - 0.2 % (acetone) - 3.0 % (water) = 95.5 %

Isotopic Purity: Under 0.5 % d_0

Re-test Date: 19 August 2019

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. Solutions of this compound undergo 17,21 transesterification when heated.

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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, with the exception of the methylene signal adjacent to the ester group, where significant H/D exchange is observed.
Residual Solvents: a small amount of acetone (0.2 % *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, with the exception of the methylene signal adjacent to the ester group, where significant H/D exchange is observed.

High-resolution Mass Spectrum (ESI+)

Found *m/z* 495.2401. $C_{25}H_{26}D_6F_2NaO_6$ $[M+Na]^+$ requires *m/z* 495.2435. The deviation of 6.8 ppm is somewhat outside normally accepted limits for the establishment of identity by HRMS, and the mass spectral data should be considered in conjunction with other identity criteria. No signal for d_0 material was seen (detection limit about 0.5 %). Substantial signals are observed for d_5 and d_7 material.

HPLC

A sharp, symmetrical peak is observed (98.7 %). 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 61.60, H 5.80, D 2.67 %
$C_{25}H_{26}D_6F_2O_6{\cdot}0.8H_2O$	Requires:	C 61.66, H 5.71, D 2.48 %, H_2O 2.96 %
$C_{25}H_{26}D_6F_2O_6$	Requires:	C 63.54, H 5.55, D 2.56 %

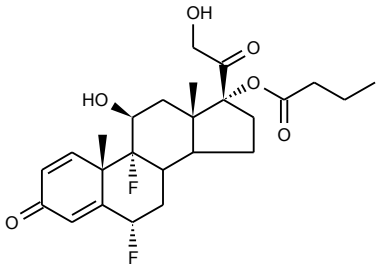
The elemental analyses fall somewhat outside those expected for anhydrous material; the presence of water is reasonably expected from the method of purification and/or the type of material, and the “best-fit” hydrated molecular formula is given. In the absence of a Karl-Fischer water analysis, we recommend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

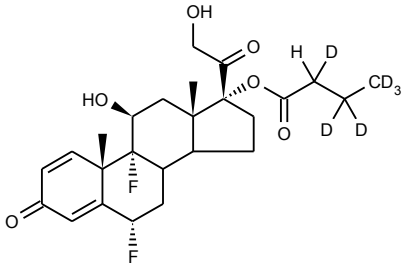
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials 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 6α,9α-Difluoroprednisolone-17-butyrate (top) and 6α,9α-Difluoroprednisolone-17-butyrate-d_6 (bottom) in DMSO-d_6

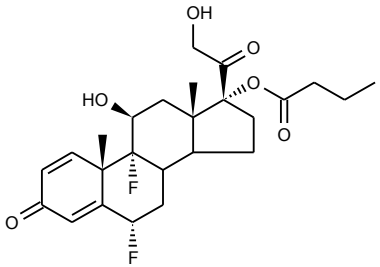


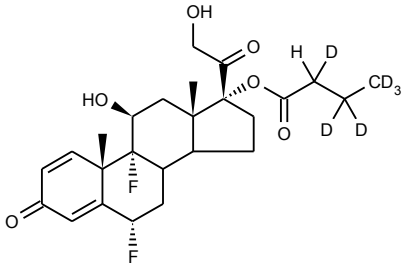


Lot Number: BDG 9235.1

BDG SYNTHESIS

Carbon-13 NMR Spectrum of 6α,9α-Difluoroprednisolone-17-butyrate (top) and 6α,9α-Difluoroprednisolone-17-butyrate-d_6 (bottom) in DMSO-d_6



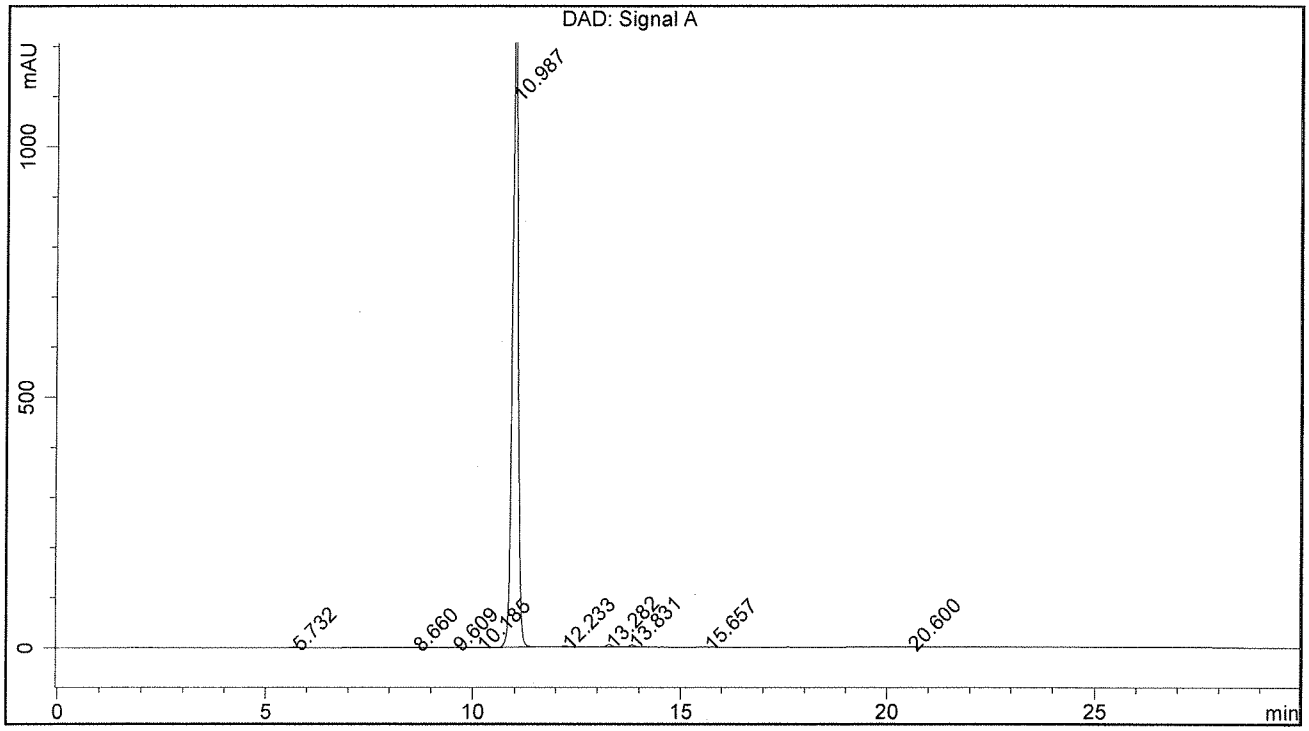


Lot Number: BDG 9235.1

BDG - Analysis of 6a,9a-Difluoroprednisolone-17-butyrate-d6

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
Mobile Phase A : 10 mM diPotassium Hydrogen Phosphate pH = 7.0
Mobile Phase B : Acetonitrile
Gradient (A:B) : T0=60:40, T20=25:75, T24=25:75, T27=25:75, T30=60:40
Flow Rate : 1.0 mL/min . . . . . Sample Solvent : 1:1 Water : Acetonitrile
Column Temperature : 20C . . . . . Injection Volume : 10 uL . . . . . Detection : UV at 240 nm

Sample Name	BDG 9235.1	**Instrument**	AnalyticalLC01
Acquisition	19/08/2014, 17:51:34	**Method (rev.)**	LC10413b (6)
Sequence	BDG_19Aug2014c	**Vial Position**	2
Operator	solvation010\cerityadmin	**Injection**	2 of 2



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	5.73 min	1.7974	20.6127	0.1778 min	0.166 %
2	8.66 min	0.2677	3.0270	0.1599 min	0.024 %
3	9.61 min	0.6020	5.3524	0.1337 min	0.043 %
4	10.18 min	0.7256	6.7302	0.1402 min	0.054 %
5	10.99 min	1262.2896	12252.7122	0.1492 min	98.681 %
6	12.23 min	2.1203	21.7718	0.1578 min	0.175 %
7	13.28 min	4.8942	50.0104	0.1532 min	0.403 %
8	13.83 min	4.4941	41.8376	0.1426 min	0.337 %
9	15.66 min	0.3882	3.3283	0.1380 min	0.027 %
10	20.60 min	1.1171	11.1073	0.1520 min	0.089 %