BDG SYNTHESIS

Certificate of Analysis

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

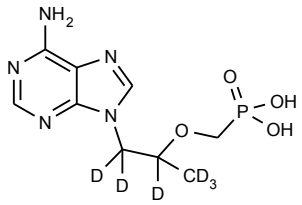
Barry Dent

Barry R. Dent, PhD, Director
29 September 2011

Name: **Tenofovir-d_6**

CAS Number: 147127-20-6 ((R) enantiomer, unlabelled)

Structure:



Molecular Weight: $C_9H_8D_6N_5O_4P$ = 293.25

Lot Number: BDG 12355.5

Appearance: White, crystalline solid

Corrected Purity: 99.0 % (HPLC) - 5.8 % (water) = 93.2 %

Isotopic Purity: Under 0.5 % d_0

Re-test Date: 29 September 2016

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.

Mailing:
BDG Synthesis Limited
PO Box 38627, Wellington Mail Centre,
Wellington 5045, New Zealand.

Shipping:
BDG Synthesis Limited
Gracefield Research Centre, Building F,
69 Gracefield Rd, Lower Hutt 5010, 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 absent, compared with the spectrum of 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* 294.1233. $C_9H_9D_6N_5O_4P$ $[M+H]^+$ requires *m/z* 294.1238. The deviation of 1.7 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 (99.0 %). 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 35.04, H 3.18, D 3.81, N 22.67 %
$C_9H_8D_6N_5O_4P{\cdot}1.0H_2O$	Requires:	C 34.73, H 3.24, D 3.88, N 22.50 %, H_2O 5.79 %
$C_9H_8D_6N_5O_4P$	Requires:	C 36.86, H 2.75, D 4.12, N 23.88 %

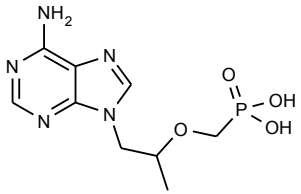
The elemental analyses fall somewhat outside those expected for anhydrous material; the presence of water is reasonably expected from the method of purification and/or the type of material, and the "best-fit" hydrated molecular formula is given. In the absence of a Karl-Fischer water analysis, we recommend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

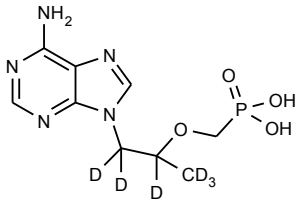
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials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

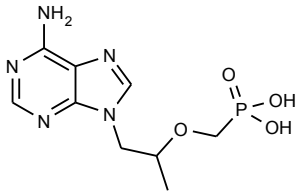


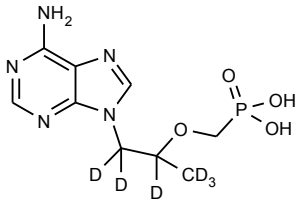


Lot Number: BDG 12355.5

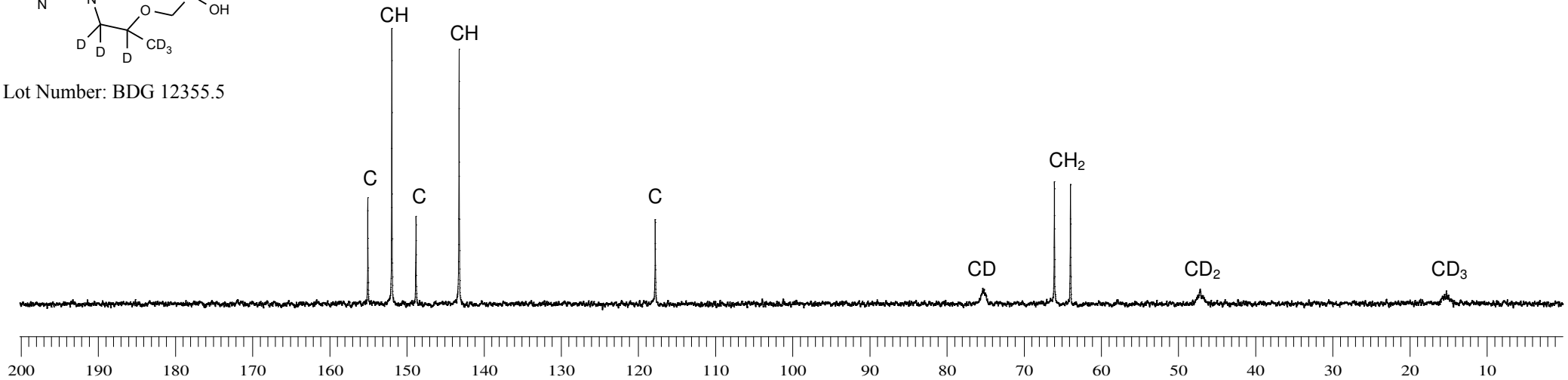
BDG SYNTHESIS

Carbon-13 NMR Spectrum of *rac*-Tenofovir (top) and Tenofovir-d_6 (bottom) in D_2O + NaOD





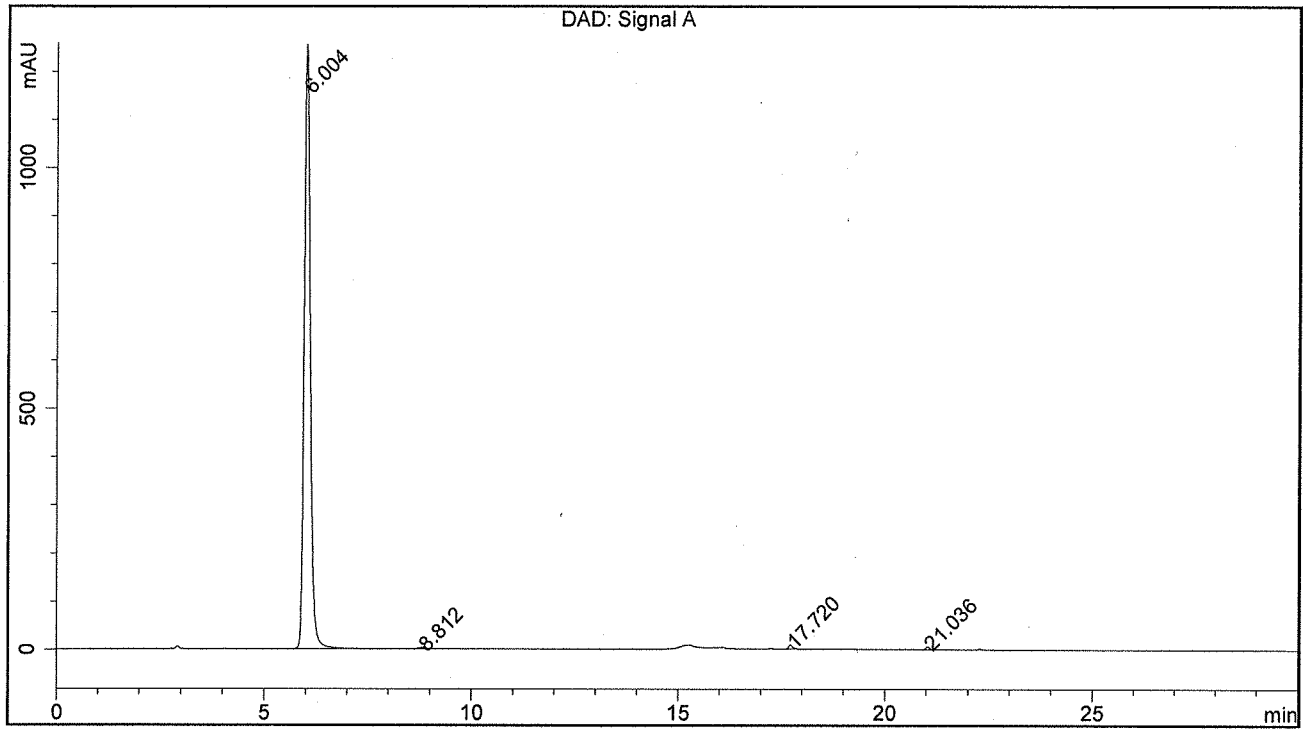
Lot Number: BDG 12355.5



BDG - Analysis of Tenofovir-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 : 50 mM diPotassium Hydrogen Phosphate pH = 7.0
Mobile Phase B : Acetonitrile
Gradient (A:B) : T0=95:5, T10=95:5, T25=40:60, T30=40:60, T32=95:5, T35=95:5
Flow Rate : 1 mL/min
Sample Solvent : Initial Mobile Phase
Column Temperature : 20C
Injection Volume : 10 uL
Detection : UV at 260 nm

Sample Name	BDG 12355.5	**Instrument**	AnalyticalLC01
Acquisition	29/09/2011, 18:35:39	**Method (rev.)**	LC10458d (13)
Sequence	BDG_29Sep2011a - Reprocessed	**Vial Position**	11
Operator	solvation010\cerityadmin	**Injection**	2 of 2



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	6.00 min	1253.6604	12840.7020	0.1575 min	98.980 %
2	8.81 min	2.8157	37.9717	0.2053 min	0.293 %
3	17.72 min	8.6184	58.6346	0.1014 min	0.452 %
4	21.04 min	6.0568	35.6778	0.0907 min	0.275 %