BDG SYNTHESIS

Certificate of Analysis

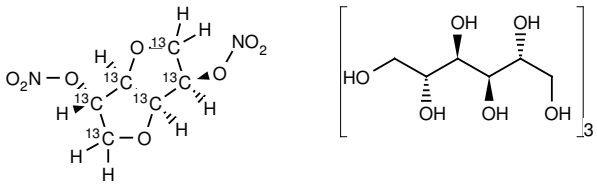
BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

Neil Beare, PhD, Director
2 December 2015

Name: **Isosorbide-$^{13}C_6$ Dinitrate : D-Mannitol 1:3 *w/w***

CAS Number: none

Structure:



Molecular Weight: $3C_6H_{14}O_6 \cdot {}^{13}C_6H_8N_2O_8 = 788.61$

Lot Number: BDG 16592.1

Appearance: White, crystalline solid

Corrected Purity: 100.0 % (HPLC) - 0.5 % (ethanol) = 99.5 %

Isotopic Purity: Under 0.5% $^{13}C_0$

Re-test Date: 2 December 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:	the material is shock and heat sensitive if not diluted with Mannitol. Only experienced laboratory personnel should handle the material. **Note: All analyses were obtained prior to dilution of the product (BDG 17235.4) with D-Mannitol.**

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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: the spectrum is of little value in determining isotopic purity. All proton signals are split by ^{13}C-^{1}H coupling as expected.
Residual Solvents: a small amount of ethanol (0.5 % *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: the spectrum is of little value in determining isotopic purity although all signals are massively enhanced and contain ^{13}C-^{13}C coupling as expected.

High-resolution Mass Spectrum (ESI+)

Found *m/z* 265.0376. $^{13}C_6H_8N_2NaO_8$ $[M+Na]^+$ requires *m/z* 265.0380. The deviation of 1.5 ppm is within normally accepted limits for the establishment of identity by HRMS. No signal for $^{13}C_0$ material was seen (detection limit about 0.5 %).

HPLC

A sharp, symmetrical peak is observed (100.0 %). 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 32.45, H 3.23, N 11.38 %
$^{13}C_6H_8N_2O_8$	Requires:	C 32.23, H 3.33, N 11.57 %

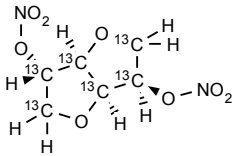
The elemental analyses fall within generally accepted limits for establishing the molecular formula given. The results may also be taken to imply the absence of significant quantities of water or inorganic salts (which have not been elsewhere tested for because of sample size limitations).

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.

BDG SYNTHESIS

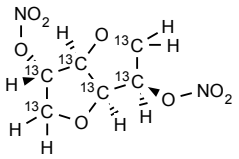
Proton NMR Spectrum of Isosorbide-$^{13}C_6$ Dinitrate in $CDCl_3$



Lot Number: BDG 17235.4

BDG SYNTHESIS

Carbon-13 NMR Spectrum of Isosorbide-$^{13}C_6$ Dinitrate in $CDCl_3$

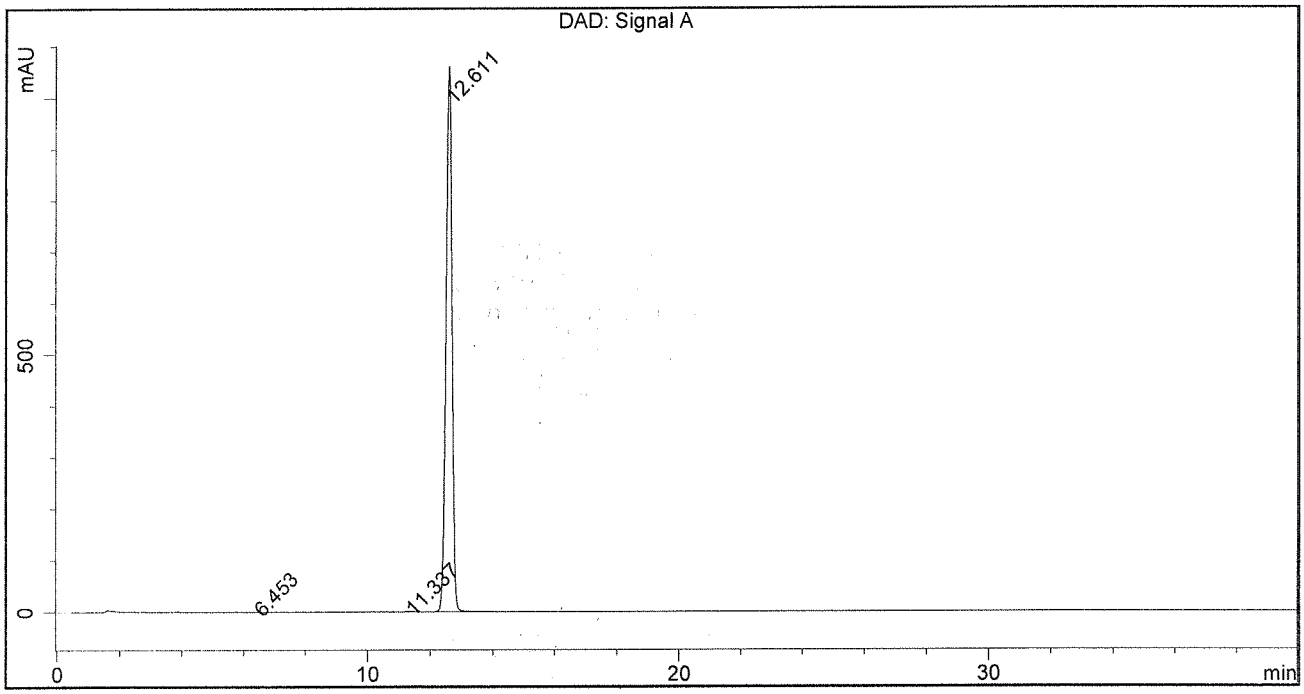


Lot Number: BDG 17235.4

BDG - Analysis of isosorbide-13C6-dinitrate

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
Mobile Phase : 60:40 10 mM Ammonia solution : Acetonitrile
Flow Rate : 1 mL/min
Sample Solvent : Mobile Phase
Column Temperature : 20C
Injection Volume : 10 uL
Detection : UV at 215 nm

Sample Name	BDG 17235.4	Instrument	AnalyticalLC01
Acquisition	02/12/2015, 16:12:03	Method (rev.)	LC10075a (8)
Sequence	BDG_02Dec2015a - Reprocessed	Vial Position	12
Operator	solvation010\cerityadmin	Injection	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	6.45 min	0.1377	2.6100	0.2352 min	0.018 %
2	11.34 min	0.1399	2.3417	0.2313 min	0.017 %
3	12.61 min	1060.1689	14130.8193	0.2075 min	99.965 %