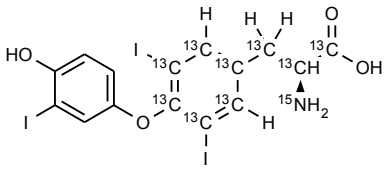
BDG SYNTHESIS

Certificate of Analysis

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

Barry Dent

Barry R. Dent, PhD, Director
12 February 2012

Name:	**Liothyronine-$^{13}C_9$,^{15}N**
CAS Number:	6893-02-3 (unlabelled)
Structure:	
Molecular Weight:	$C_6{}^{13}C_9H_{12}I_3{}^{15}NO_4$ = 660.90
Lot Number:	BDG 12412.4
Appearance:	Off-white, crystalline solid
Purity By HPLC:	99.7 %
Isotopic Purity:	Under 0.5% M-10
Re-test Date:	12 February 2017
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.

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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: large coupling constants are observed for protons which are attached to ^{13}C when compared to the spectrum of the unlabelled analog.
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: the labelled carbons from the tyrosine portion of the molecule appear as very large multiplets due to coupling to adjacent labelled carbons.

High-resolution Mass Spectrum (ESI+)

Found *m/z* 661.8256. $C_6{}^{13}C_9H_{13}I_3{}^{15}NO_4$ $[M+H]^+$ requires *m/z* 661.8251. The deviation of 0.8 ppm is within normally accepted limits for the establishment of identity by HRMS. No signal for M-10 material was seen (detection limit about 0.5 %).

HPLC

A sharp, symmetrical peak is observed (99.7 %). 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 28.32, H 1.95, N 2.30 %
$C_6{}^{13}C_9H_{12}I_3{}^{15}NO_4$	Requires:	C 28.61, H 1.83, N 2.27 %

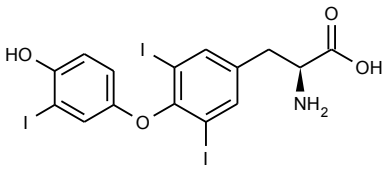
The elemental analyses fall within generally accepted limits for establishing the molecular formula given. The results may also be taken to imply the absence of significant quantities of water or inorganic salts (which have not been elsewhere tested for because of sample size limitations).

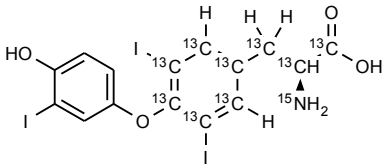
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials 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 Liothyronine (top) and Liothyronine-$^{13}C_9$,^{15}N (bottom) in DMSO-d_6

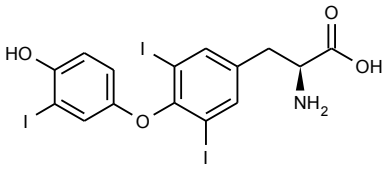


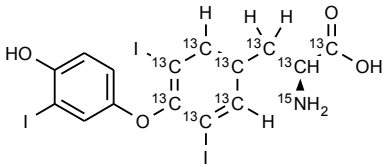


Lot Number: BDG 12412.4

BDG SYNTHESIS

Carbon-13 NMR Spectrum of Liothyronine (top) and Liothyronine-$^{13}C_9$,^{15}N (bottom) in DMSO-d_6





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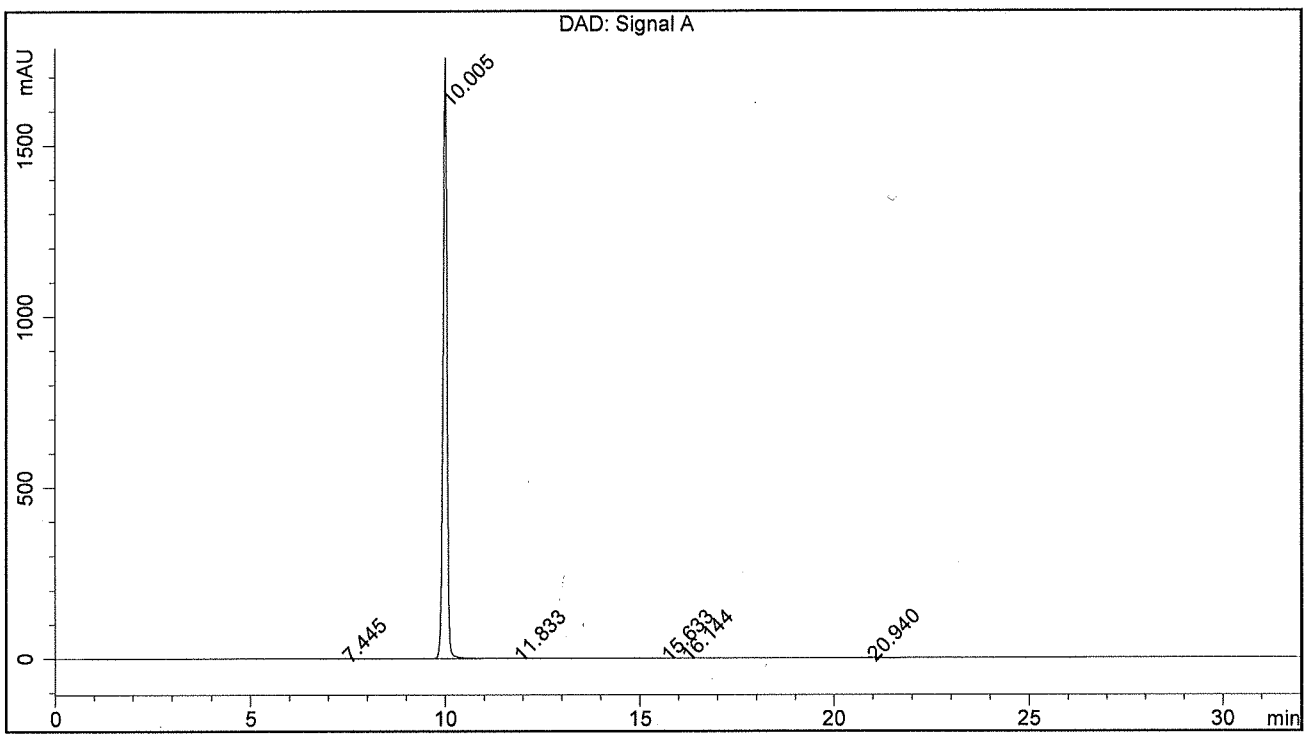
BDG - Analysis of Liothyronine-13C9,15N

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
Mobile Phase A : 75:25 Water : Acetonitrile
Mobile Phase B : Acetonitrile
Gradient (A:B) : T0=100:0, T25=20:80, T30=20:80, T32=100:0, T35=100:0
Flow Rate : 1.0 mL/min
Sample Solvent : Intial Mobile Phase
Column Temperature : 30C
Injection Volume : 10 uL
Detection : UV at 230 nm

Sample Name	BDG 12412.4	Instrument	AnalyticalLC01
Acquisition	12/02/2012, 12:52:45	Method (rev.)	LC10022e (4)
Sequence	BDG_12Feb2012a - Reprocessed	Vial Position	1
Operator	solvation010\cerityadmin	Injection	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	7.45 min	0.2922	2.1303	0.1050 min	0.017 %
2	10.01 min	1757.2342	12310.0256	0.1057 min	99.725 %
3	11.83 min	0.1820	2.0926	0.1561 min	0.017 %
4	15.63 min	0.5011	6.8675	0.1919 min	0.056 %
5	16.14 min	0.3899	6.0996	0.2185 min	0.049 %
6	20.94 min	1.0610	16.7116	0.2258 min	0.135 %