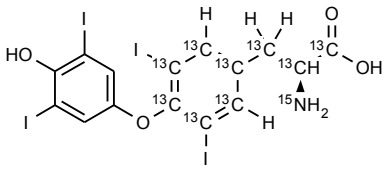
BDG SYNTHESIS

Certificate of Analysis

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

Neil Beare, PhD, Director
10 September 2016

Name:	**Thyroxine-$^{13}C_9$,^{15}N**
CAS Number:	51-48-9 (unlabelled)
Structure:	
Molecular Weight:	$C_6{}^{13}C_9H_{11}I_4{}^{15}NO_4$ = 786.80
Lot Number:	BDG 12484.3
Appearance:	White, crystalline solid
Corrected Purity:	99.0 % (HPLC) - 8.4 % (water) = 90.6 %
Isotopic Purity:	Under 0.5% M-10
Re-test Date:	10 September 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:	store in an amber vial and protect from bright light.
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 and the spectrum is of little value in determining the isotopic purity.
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* 787.7208. $C_6{}^{13}C_9H_{12}I_4{}^{15}NO_4$ $[M+H]^+$ requires *m/z* 787.7218. The deviation of 1.3 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, slightly tailing 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 21.22, H 1.89, N 1.62 %
$C_6{}^{13}C_9H_{11}I_4{}^{15}NO_4 \cdot 4.0H_2O$	Requires:	C 22.02, H 2.23, N 1.75 %, H_2O 8.39 %
$C_6{}^{13}C_9H_{11}I_4{}^{15}NO_4$	Requires:	C 24.03, H 1.41, N 1.91 %

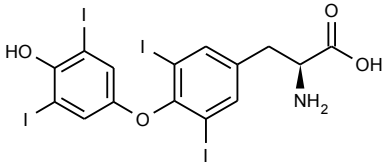
The elemental analyses fall substantially outside those expected for anhydrous material; the presence of water is reasonably expected from the method of purification and/or the type of material, and the “best-fit” hydrated molecular formula is given. In the absence of a Karl-Fischer water analysis, we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

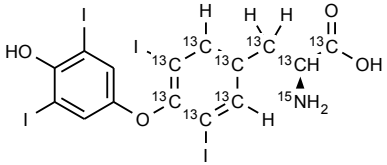
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials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





Lot Number: BDG 12484.3

4xCH
4.0 H

HOD

3xCH
3.0 H

2xCH
2.0 H

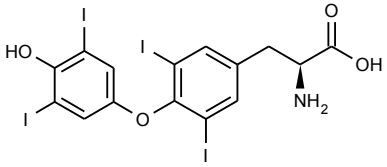
NMR
Solvent

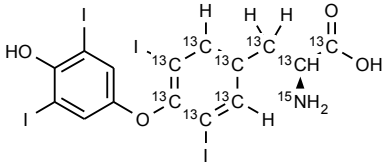
2x^{13}CH
1.9 H

3x^{13}CH+HOD

BDG SYNTHESIS

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





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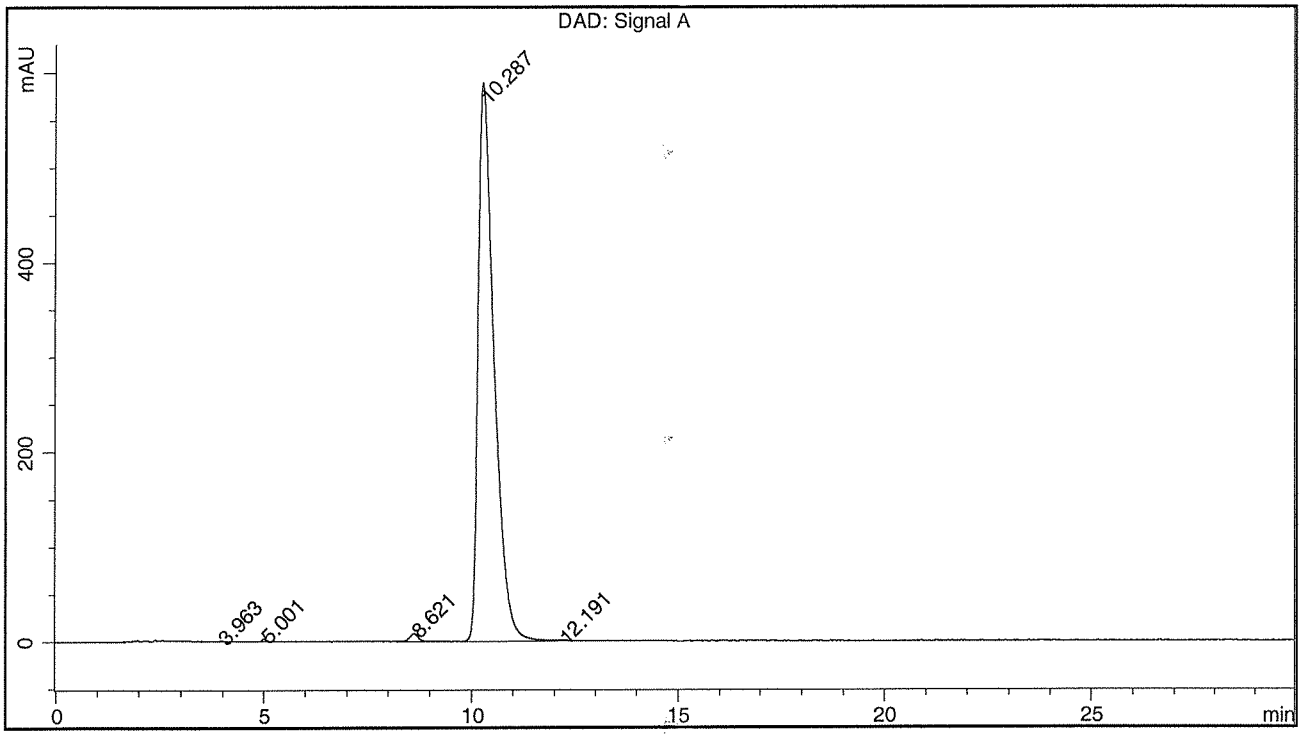
BDG - Analysis of Thyroxine-13C9,15N

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
Guard : Phenomenex SecurityGuard C18 4 x 3mm
Mobile Phase : 65:35:0.1 Water : Acetonitrile : Phosphoric Acid
Flow Rate : 1.0 mL/min
Sample Solvent : Mobile Phase
Column Temperature : 20C
Injection Volume : 10 uL
Detection : UV at 225 nm

Sample Name	BDG 12484.3	Instrument	AnalyticalLC01
Acquisition	10/09/2016, 13:47:30	Method (rev.)	LC10503a (5)
Sequence	BDG_10Sep2016a - Reprocessed	Vial Position	47
Operator	solvation010\cerityadmin	Injection	2 of 2



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	3.96 min	0.7761	6.4340	0.1147 min	0.040 %
2	5.00 min	3.0530	24.3670	0.1171 min	0.153 %
3	8.62 min	8.2116	120.4929	0.2212 min	0.755 %
4	10.29 min	589.8146	15796.8745	0.3965 min	98.959 %
5	12.19 min	0.6901	14.9566	0.2919 min	0.094 %