

BDG SYNTHESIS

Certificate of Analysis

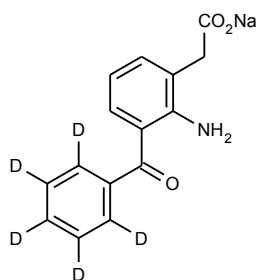
BDG Synthesis certifies that this reference material meets or exceeds the specifications stated herein.

Neil Beare

Neil Beare, PhD, Director
15 September 2014

Name: Amfenac-d₅ Sodium Salt
CAS Number: 61618-27-7 (mono-hydrate, unlabelled)

Structure:



Molecular Weight: C₁₅H₇D₅NNaO₃ = 282.28
Lot Number: BDG 9211
Appearance: Yellow, crystalline solid
Corrected Purity: 98.8 % (HPLC) - 0.1 % (ethanol) - 7.3 % (water) = 91.4 %
Isotopic Purity: Under 0.5 % d₀
Re-test Date: 15 September 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.

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: a small amount of ethanol (0.1 % 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.

High-resolution Mass Spectrum (ESI+)

Found m/z 283.1101. $C_{15}H_8D_5NNaO_3$ $[M+H]^+$ requires m/z 283.1101. The deviation of 0.2 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 (98.8 %). 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 58.10, H 3.07, D 3.07, N 4.51 %
$C_{15}H_7D_5NNaO_3 \cdot 1.5H_2O$	Requires:	C 58.25, H 3.26, D 3.26, N 4.53 %
$C_{15}H_7D_5NNaO_3$	Requires:	C 63.82, H 2.50, D 3.57, N 4.96 %

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.

Karl-Fischer Analysis

	Found:	H_2O 7.3 %
$C_{15}H_7D_5NNaO_3 \cdot 1.5H_2O$	Requires:	H_2O 8.7 %

Of necessity, only a small sample could be used and only a single or duplicate analysis performed. We are unable to state what the errors in the reported water content are, but recommend that the result be used, as the best available, when determining corrected purity.

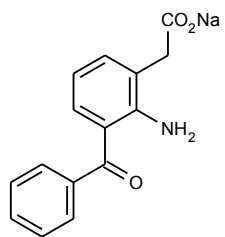
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials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

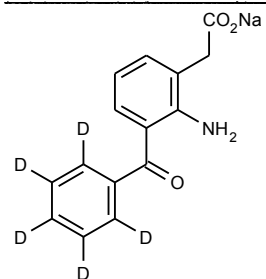


Proton NMR Spectrum of Amfenac Sodium Salt (top) and Amfenac-d₅ Sodium Salt (bottom) in Methanol-d₄

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5xCH
5.0 H



Lot Number: BDG 9211

2xCH
2.0 H

CH
1.0 H

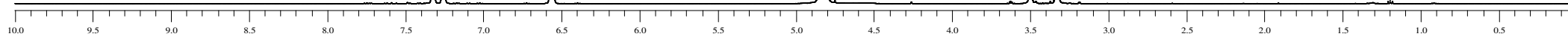
HOD

CH₂
2.0 H

NMR
Solvent

ethanol

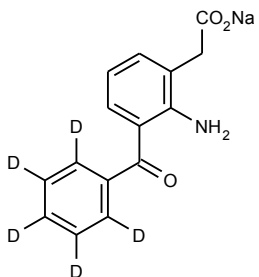
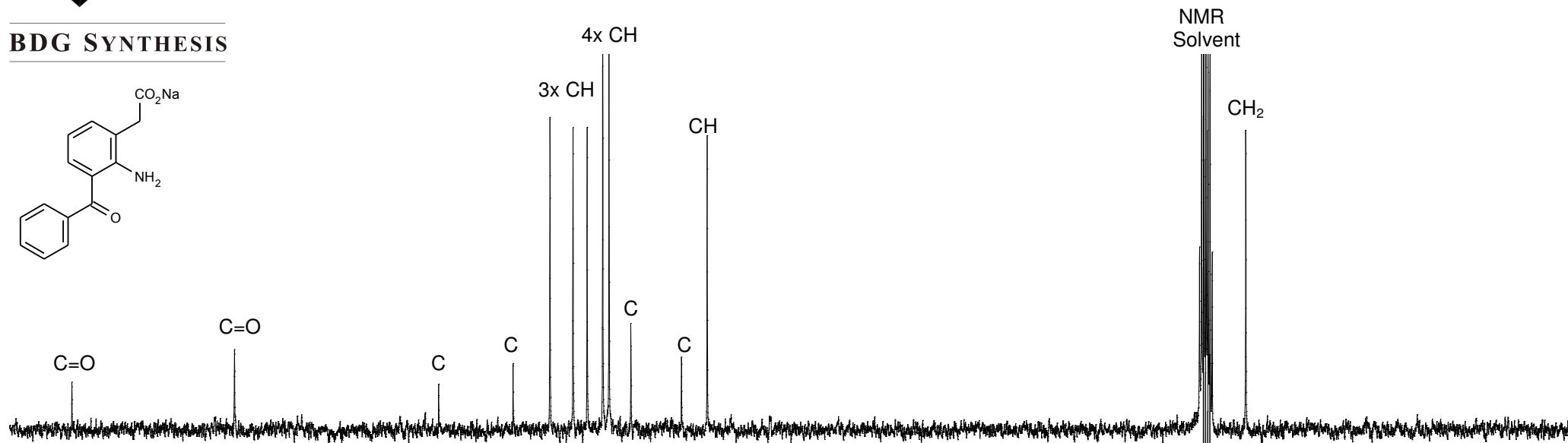
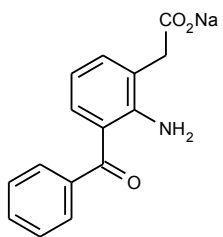
ethanol



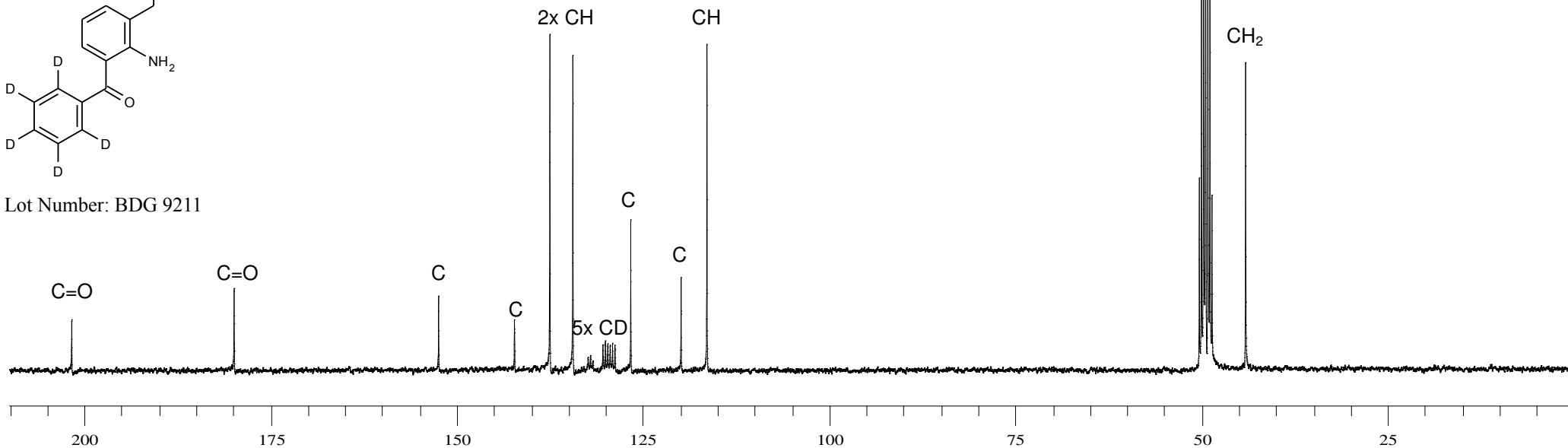


Carbon-13 NMR Spectrum of Amfenac Sodium Salt (top) and Amfenac-d₅ Sodium Salt (bottom) in Methanol-d₄

BDG SYNTHESIS



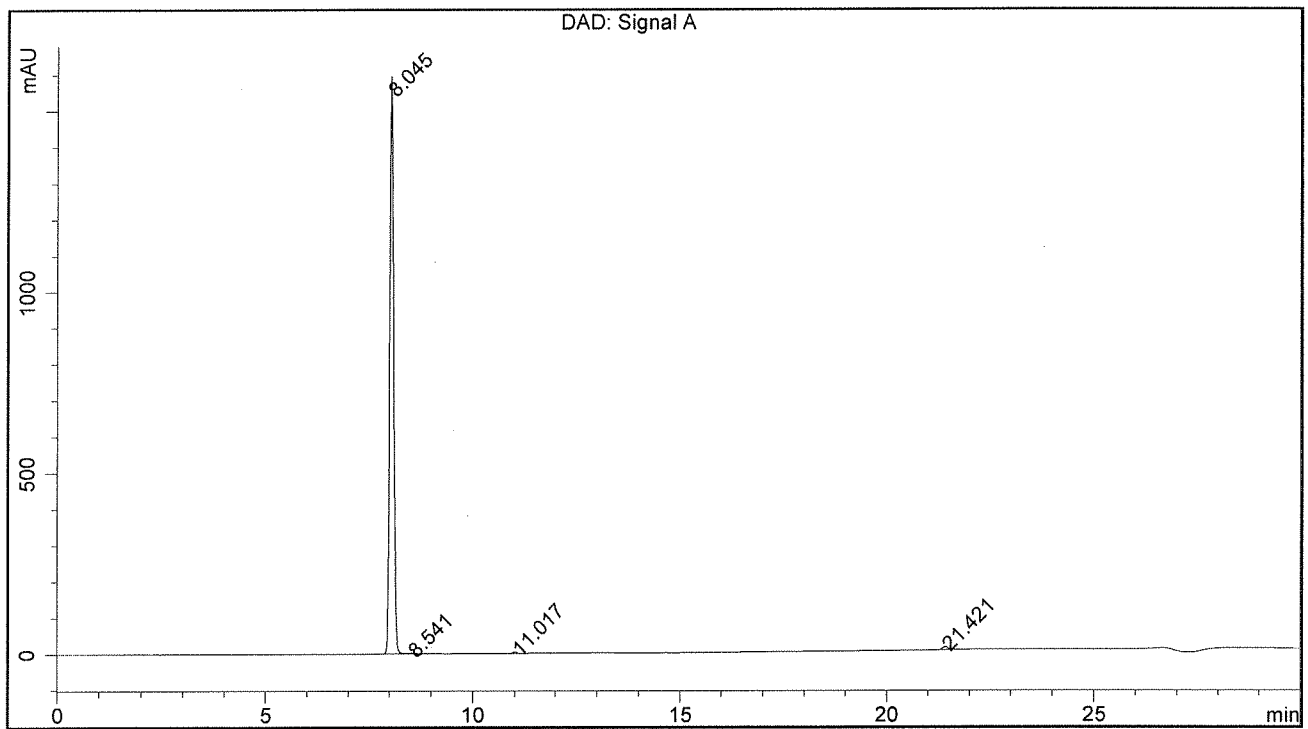
Lot Number: BDG 9211



BDG - Analysis of Amfenac-d5 Sodium Salt

Column : Phenomenex Luna C18 5um 250 x 4.6 mm
 Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
 Mobile Phase A : 75:25 50 mM Phosphate Buffer pH=7.0 : Acetonitrile
 Mobile Phase B : 35:65 50 mM Phosphate Buffer pH=7.0 : Acetonitrile
 Gradient : T0=100:0, T20=0:100, T27=0:100, T30=100:0, T35=100:0
 Flow Rate : 1.0 mL/min
 Column Temperature : 20C
 Sample Solvent : 2:1 Water : Acetonitrile
 Detection : UV 245nm

Sample Name	BDG 9211	Instrument	AnalyticalLC01
Acquisition	15/09/2014, 12:16:29	Method (rev.)	LC10176b (12)
Sequence	BDG_15Sep2014a - Reprocessed	Vial Position	14
Operator	solvation010\cerityadmin	Injection	2 of 2



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	8.05 min	1596.0403	10214.9074	0.0987 min	98.790 %
2	8.54 min	0.8132	12.4130	0.2061 min	0.120 %
3	11.02 min	4.1698	26.7611	0.1029 min	0.259 %
4	21.42 min	8.6861	85.9063	0.1514 min	0.831 %