BDG SYNTHESIS

# Certificate of Analysis

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

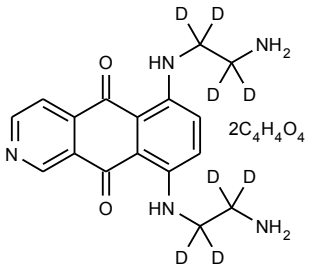
Barry Dent

Barry R. Dent, PhD, Director
5 April 2012

**Name:** **Pixantrone-$d_8$ Dimaleate**

**CAS Number:** 144675-97-8 (unlabelled)

**Structure:**




**Molecular Weight:** $C_{17}H_{11}D_8N_5O_2 \cdot 2C_4H_4O_4 = 565.56$

**Lot Number:** BDG 9160.1

**Appearance:** Blue, crystalline solid

**Corrected Purity:** 98.5 % (HPLC) - 0.5 % (acetic acid) - 4.4 % (water) = 93.6 %

**Isotopic Purity:** Under 0.5 % $d_0$

**Re-test Date:** 5 April 2017

**Storage and Handling:**

| | |
|---|---|
| Temperature: | refrigerate for prolonged storage; may be handled and shipped at ambient temperature. |
| Humidity: | may be hygroscopic; store desiccated; recommended to determine water content periodically. |
| 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: signals at the sites of deuteration are absent, compared with the spectrum of unlabelled material, indicating clean deuteration.
Residual Solvents: a small amount of acetic acid (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: 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* 334.2114. $C_{17}H_{12}D_8N_5O_2$ $[M+H]^+$ requires *m/z* 334.2119. The deviation of 1.5 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 (98.5 %). 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 50.74, H 3.52, D 2.96, N 11.69 % |
| $C_{17}H_{11}D_8N_5O_2{\cdot}2C_4H_4O_4{\cdot}1.5H_2O$ | Requires: | C 50.67, H 3.74, D 2.72, N 11.82 % |
| $C_{17}H_{11}D_8N_5O_2{\cdot}2C_4H_4O_4$ | Requires: | C 53.09, H 3.39, D 2.85, N 12.38 % |

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: | $H_2O$ 4.4 % |
| $C_{17}H_{11}D_8N_5O_2{\cdot}2C_4H_4O_4{\cdot}1.5H_2O$ | Requires: | $H_2O$ 4.6 % |

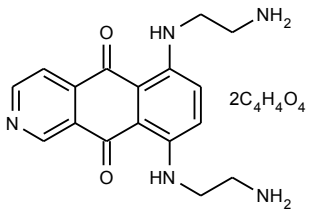
Of necessity, only a small sample could be used and only a single or duplicate analysis performed. We are unable to state what the errors in the reported water content are, but recommend that the result be used, as the best available, when determining corrected purity.

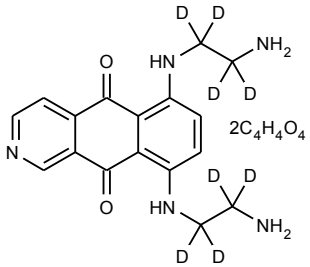
The available quantity of custom-synthesised material is always small, and this limits the extent and type of analytical data which can be obtained. This Certificate is presented in descriptive format for use by analytical chemists who are trained in the use of custom-synthesised materials. Custom materials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

BDG SYNTHESIS

Proton NMR Spectrum of Pixantrone Dimaleate (top) and Pixantrone-$d_8$ Dimaleate (bottom) in DMSO-$d_6$

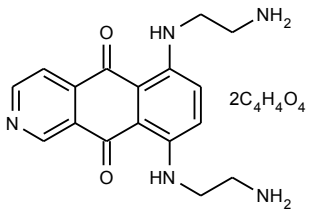


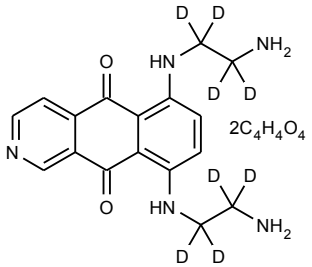




Lot Number: BDG 9160.1

BDG SYNTHESIS

Carbon-13 NMR Spectrum of Pixantrone Dimaleate (top) and Pixantrone-$d_8$ Dimaleate (bottom) in DMSO-$d_6$



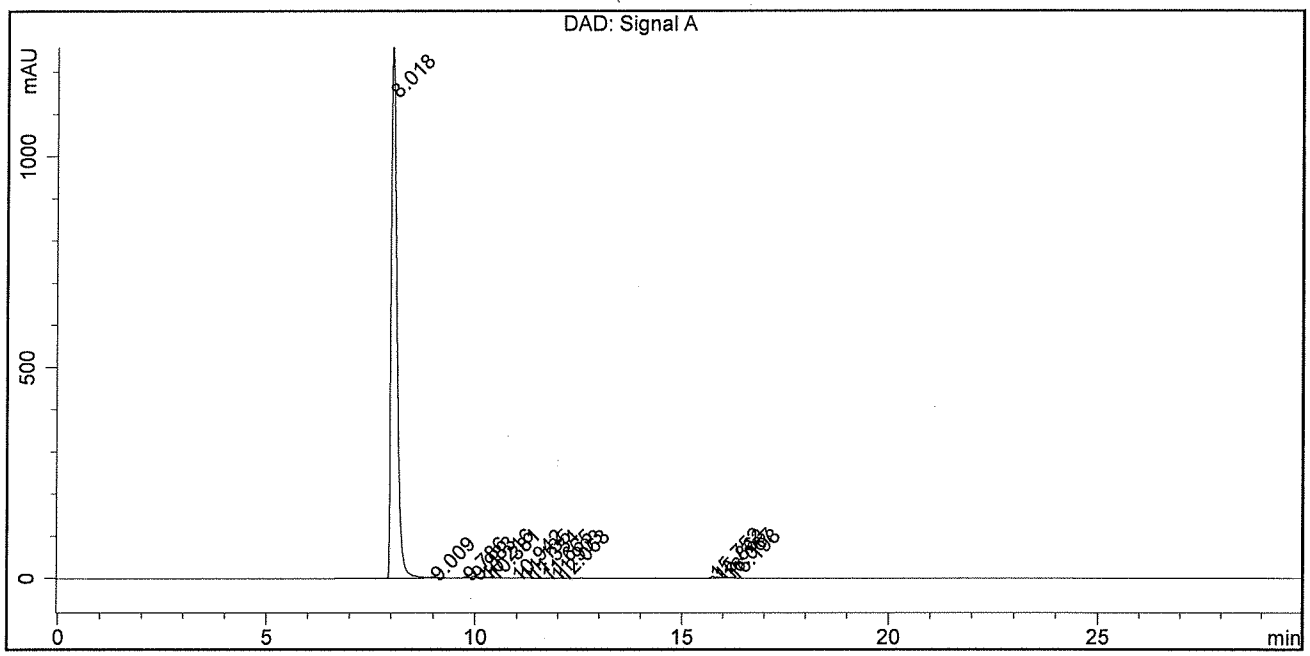




Lot Number: BDG 9160.1

BDG - Analysis of Pixantrone-d8 Dimaleate

Column : Phenomenex Luna C18(2) 5um 250 x 4.6 mm
Guard : Phenomenex Security Guard C18 RP 4 x 3 mm
Mobile Phase A : 95:5 20 mM Potassium diHydrogen Phosphate pH=3.0 : Acetonitrile
Mobile Phase B : 50:50 20 mM Potassium diHydrogen Phosphate pH=3.0 : Acetonitrile
Gradient ( A:B ) : T0=100:0, T20=0:100, T24=0:100, T27=100:0, T30=100:0
Flow Rate : 1.0 mL/min . . . . . Sample Solvent : Water . . . . . Column Temperature : 20C
Injection Volume : 10 uL . . . . . Detection : Vis at 640 nm

| Sample Name | BDG 9160.1 | Instrument | AnalyticalLC01 |
|---|---|---|---|
| Acquisition | 05/04/2012, 16:48:37 | Method (rev.) | LC10501a ( 11) |
| Sequence | BDG_05Apr2012i - Reprocessed | Vial Position | 1 |
| Operator | solvation010\cerityadmin | Injection | 1 of 2 |




**Area Percent Report**

| Peak# | RT | Peak Height | Peak Area | Width | Area % |
|---|---|---|---|---|---|
| 1 | 8.02 min | 1289.9164 | 12154.1728 | 0.1459 min | 98.517 % |
| 2 | 9.01 min | 1.3195 | 7.6533 | 0.0937 min | 0.062 % |
| 3 | 9.79 min | 2.4649 | 17.4015 | 0.1084 min | 0.141 % |
| 4 | 9.98 min | 4.1969 | 40.1176 | 0.1415 min | 0.325 % |
| 5 | 10.22 min | 2.3408 | 19.9539 | 0.1255 min | 0.162 % |
| 6 | 10.38 min | 2.0508 | 16.5715 | 0.1143 min | 0.134 % |
| 7 | 10.94 min | 0.4048 | 1.8739 | 0.0720 min | 0.015 % |
| 8 | 11.14 min | 1.4167 | 7.2016 | 0.0793 min | 0.058 % |
| 9 | 11.33 min | 0.6474 | 3.2579 | 0.0787 min | 0.026 % |
| 10 | 11.67 min | 0.2180 | 1.6404 | 0.1058 min | 0.013 % |
| 11 | 11.90 min | 0.2755 | 2.4550 | 0.1220 min | 0.020 % |
| 12 | 12.06 min | 0.2477 | 1.9906 | 0.1158 min | 0.016 % |
| 13 | 15.75 min | 4.1208 | 22.5661 | 0.0839 min | 0.183 % |
| 14 | 15.86 min | 2.2175 | 11.3498 | 0.0797 min | 0.092 % |
| 15 | 16.07 min | 3.4039 | 18.1695 | 0.0823 min | 0.147 % |
| 16 | 16.20 min | 1.8635 | 10.7955 | 0.0876 min | 0.088 % |