BDG SYNTHESIS

Certificate of Analysis

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

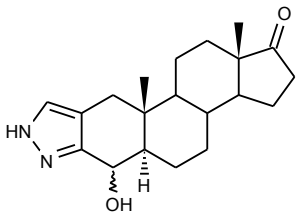
Barry Dent

Barry R. Dent, PhD, Director
5 May 2012

Name: **4α/β-Hydroxy-2'H-5α-androst-2-eno[3,2-c]pyrazol-17-one**

CAS Number: none

Structure:



Molecular Weight: $C_{20}H_{28}N_2O_2$ = 328.45

Lot Number: BDG 13420.9

Appearance: White, crystalline solid

Corrected Purity: 99.9 % (HPLC) - 2.4 % (THF) = 97.5 %

Re-test Date: 5 May 2013

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. The spectrum shows that the two epimeric alcohols are present in approximately a 2:1 ratio.
Residual Solvents: a small amount of THF (2.4 % *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. The complexity of the spectrum indicates that tautomers of the two epimeric alcohols are present in solution.

High-resolution Mass Spectrum (ESI+)

Found *m/z* 329.2233. $C_{20}H_{29}N_2O_2$ $[M+H]^+$ requires *m/z* 329.2229. The deviation of 1.2 ppm is within normally accepted limits for the establishment of identity by HRMS.

HPLC

Two sharp, symmetrical peaks are observed at 8.7 mins (37.0 %) and 9.4 mins (62.9 %), and these signals are assigned to the 4α and 4β hydroxysteroids respectively through NMR and HPLC experiments. 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 72.88, H 8.81, N 8.30 %
$C_{20}H_{28}N_2O_2$	Requires:	C 73.14, H 8.59, N 8.53 %

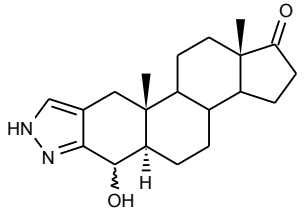
The elemental analyses fall within generally accepted limits for establishing the molecular formula given. The results may also be taken to imply the absence of significant quantities of water or inorganic salts (which have not been elsewhere tested for because of sample size limitations).

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.

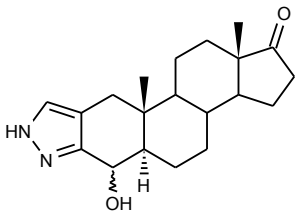
BDG SYNTHESIS

Proton NMR Spectrum of 4α/β-Hydroxy-2'H-5α-androst-2-eno[3,2-c]pyrazol-17-one in DMSO-d_6



Lot Number: BDG 13420.9

BDG SYNTHESIS



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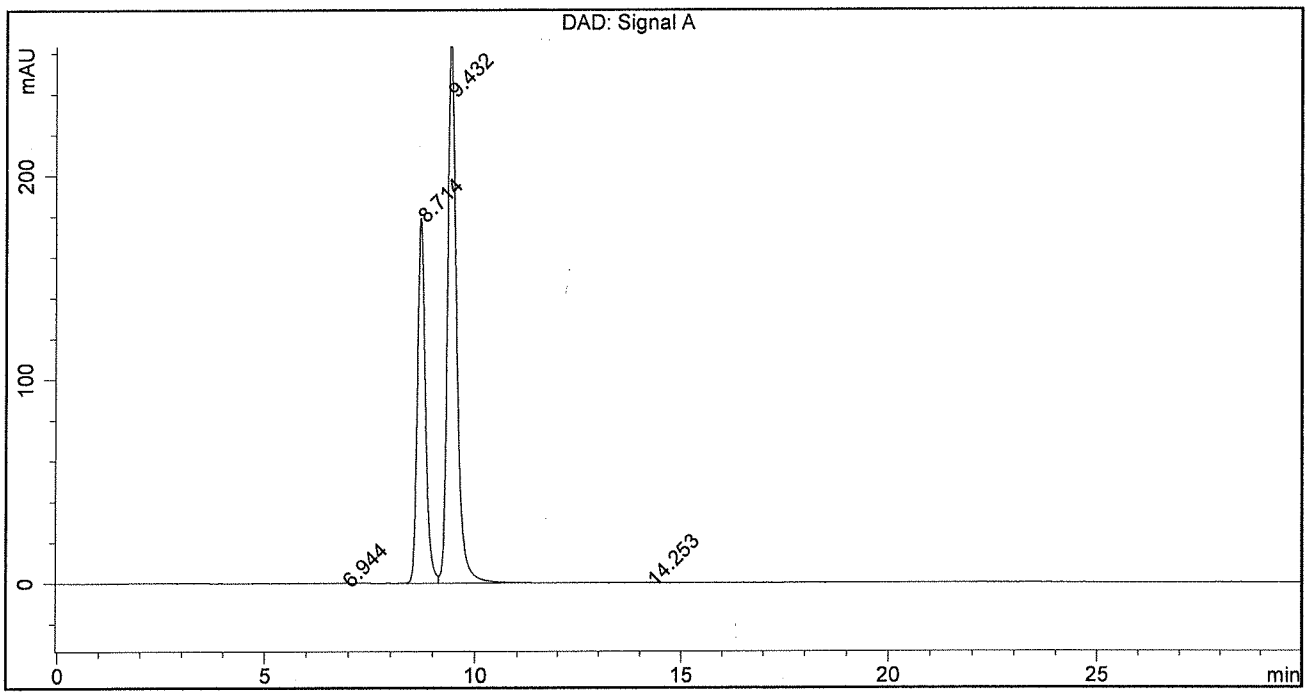
Carbon-13 NMR Spectrum of 4α/β-Hydroxy-2'H-5α-androst-2-eno[3,2-c]pyrazol-17-one in DMSO-d_6

BDG - Analysis of 4alpha/beta-Hydroxy-2'H-5alpha-androst-2-eno(3,2-c)pyrazol-17-one

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 Water : Acetonitrile
Flow Rate : 1.0 mL/min
Sample Solvent : 50:50 Water : Acetonitrile
Column Temperature : 20C
Injection Volume : 10 uL
Detection : UV at 224 nm

Sample Name	BDG 13420.9	Instrument	AnalyticalLC01
Acquisition	04/05/2012, 19:14:00	Method (rev.)	LC10504b (6)
Sequence	BDG_04May2012c	Vial Position	51
Operator	solvation010\cerityadmin	Injection	1 of 1



Area Percent Report

Peak#	RT	Peak Height	Peak Area	Width	Area %
1	6.94 min	0.1102	1.1260	0.1352 min	0.018 %
2	8.71 min	178.9918	2363.1095	0.2000 min	36.993 %
3	9.43 min	267.5779	4018.9449	0.2252 min	62.914 %
4	14.25 min	0.2511	4.8050	0.2352 min	0.075 %