



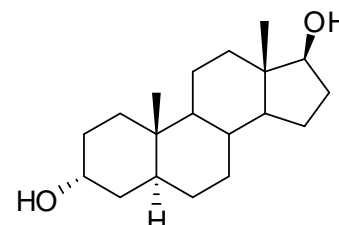
# CERTIFIED REFERENCE MATERIAL CERTIFICATE OF ANALYSIS

## NMIA D634b: 5 $\alpha$ -Androstane-3 $\alpha$ , 17 $\beta$ -diol

Report ID: D634b.2018.03 (Ampouled 180920)

Chemical Formula: C<sub>19</sub>H<sub>32</sub>O<sub>2</sub>

Molecular Weight: 292.5 g/mol



### Certified value

Batch No.	CAS No.	Mass per ampoule
10-S-03	1852-53-5	997 $\pm$ 15 $\mu$ g

The uncertainty has been calculated according to ISO Guide 35 and is stated at the 95% confidence limit ( $k = 2$ ).

**IUPAC:** (3 $\alpha$ ,5 $\alpha$ ,17 $\beta$ )-Androstane-3,17-diol

**Expiration of certification:** The property values are valid till 10 October 2021, i.e. three years from the date of re-certification provided the **unopened** material is handled and stored in accordance with the recommendations below. The material as issued in the unopened container and stored as recommended below should be suitable for use beyond this date, subject to confirmation of batch stability from the issuing body. The expiry date/shelf life does not apply to sample bottles that have been opened. In such cases it is recommended that the end-user conduct their own in-house stability trials.

**Description:** The compound is supplied as a dried aliquot in a sealed ampoule under an atmosphere of argon. The CRM is intended for a single use to prepare a standard solution containing D634b. This material was prepared by synthesis, and certified for identity and purity by NMIA.

**Intended use:** This certified reference material is suitable for use as a primary calibrator.

**Instructions for use:** Open the ampoule and carefully rinse the interior at least three times with a suitable organic solvent (e.g. chloroform). This will transfer 997  $\pm$  15  $\mu$ g of anhydrous 5 $\alpha$ -androstane-3 $\alpha$ , 17 $\beta$ -diol. The mass of analyte in each ampoule is calculated from the assigned purity of the bulk and the concentration of bulk material in a stock solution used to prepare the ampoules.

**Recommended storage:** When not in use this material should be stored at or below 4  $^{\circ}$ C in a closed container in a dry, dark area.

**Metrological traceability:** The certified purity value is traceable to the SI unit for mass (kg) through Australian national standards via balance calibration. In the mass balance approach all impurities are quantified as a mass fraction and subtracted from 100%. Quantitative NMR provides an independent direct measure of the mass fraction of the analyte of interest, calibrated with an internal standard certified for purity (mass fraction).

**Stability:** In the absence of stability data the measurement uncertainty at the 95% coverage interval has been expanded to accommodate any potential change in the property value. The stability component has been estimated from stability trials conducted on similar materials by NMI Australia over the last 10 years. The long-term stability of the compound in solution has not been examined.

**Homogeneity assessment:** The homogeneity of the material was assessed using purity assay by GC-FID on seven randomly selected ampoules of the material. The material was judged to be sufficiently homogeneous at this level of sampling as the variation in analysis results between samples was not significantly different at a 95% confidence level from that observed on repeat analysis of the same sample.

**Safety:** Treat as hazardous substance. Use appropriate work practices when handling to avoid skin or eye contact, ingestion or inhalation of dust. Refer to the provided safety data sheet.

S. R. Davies

Dr Stephen R. Davies,  
Team Leader,  
Chemical Reference Materials, NMI.  
31 January 2020

This report supersedes any issued prior to 31 January 2020

**NATA logo notice:** Accredited for compliance with ISO 17034. Accreditation No. 198 / Corporate Site No. 20844. The results of the tests, calibrations and/or measurements included in this document are traceable to Australian/national standards.

**Legal notice:** Neither NMIA as a representative of the Commonwealth of Australia, nor any person acting on NMIA's behalf, assumes any liability with respect to the use of, or for damages resulting from the use of, this reference material or the information contained in this document.

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## Characterisation Report:

GC-FID: Instrument: Varian CP-3800 / Agilent 7890A  
 Column: HP-5, 30 m x 0.32 mm I.D. x 0.25 μm  
 Program: 230 °C (0.2 min), 5 °C/min to 265 °C, 30 °C/min to 280 °C (10 min)  
 Injector: 250 °C  
 Detector Temp: 320 °C  
 Carrier: Helium  
 Split ratio: 20/1  
 Relative mass fraction of the main component as the *bis*-TMS derivative:  
 Initial analysis: Mean = 99.5%, s = 0.007% (7 ampoules in duplicate, October 2018)

### The following analytical data was obtained on the bulk material subsequently used in the preparation of the ampoules.

The identity was confirmed by a range of spectroscopic techniques, NMR, IR and MS. The certified purity value was obtained by mass balance from a combination of traditional analytical techniques, including GC-FID, thermogravimetric analysis, Karl Fischer analysis and <sup>1</sup>H NMR spectroscopy. The purity value is calculated as per Equation 1.

$$\text{Purity} = (100 \% - I_{\text{ORG}}) \times (100 \% - I_{\text{VOL}} - I_{\text{NVR}}) \quad \text{Equation 1}$$

$I_{\text{ORG}}$  = Organic impurities of related structure,  $I_{\text{VOL}}$  = volatile impurities,  $I_{\text{NVR}}$  = non-volatile residue.

Supporting evidence is provided by quantitative NMR, qualitative headspace GC-MS analysis of occluded solvents and elemental microanalysis. The purity value by qNMR was measured against a certified internal standard of potassium hydrogen maleate.

GC-FID: Instrument: Varian CP-3800  
 Column: HP-1, 30 m x 0.32 mm I.D. x 0.25 μm  
 Program: 230 °C (0.2 min), 5 °C/min to 265 °C, 30 °C/min to 280 °C (10 min)  
 Injector: 250 °C  
 Detector Temp: 320 °C  
 Carrier: Helium  
 Split ratio: 20/1  
 Relative mass fraction of the main component as the *bis*-TMS derivative:  
 Initial analysis: Mean = 99.5%, s = 0.03% (10 sub samples in duplicate, May 2010)  
 Initial analysis: Mean = 99.5%, s = 0.02% (5 sub samples in duplicate, May 2010)  
 Re-analysis: Mean = 99.5%, s = 0.02% (5 sub samples in duplicate, April 2015)

GC-FID: Instrument: Varian CP-3800  
 Column: VF-1MS, 30 m x 0.32 mm I.D. x 0.25 μm  
 Program: 180 °C (0.2 min), 7 °C/min to 230 °C, 20 °C/min to 280 °C (3 min), 10 °C/min to 265 °C (2 min), 30 °C/min to 300 °C (3min)  
 Injector: 280 °C  
 Detector Temp: 320 °C  
 Carrier: Helium  
 Split ratio: 20/1  
 Relative mass fraction of the main component as the *bis*-TMS derivative:  
 Initial analysis: 99.5%, s = 0.01% (5 sub samples in duplicate, May 2010)

Thermogravimetric analysis: Volatile content 0.2% and non-volatile residue < 0.2% mass fraction (July 2010)

Karl Fischer analysis: Moisture content 0.3% mass fraction (May 2010 and April 2015)  
 Moisture content 6.1% (4 sub samples, March 2008 and 2 sub samples, October 2013)  
 Moisture content 6.0% (2 sub samples, November 2016)

qNMR: Instrument: Bruker Avance III-400  
 Field strength: 400 MHz  
 Solvent: MeOH-*d*<sub>4</sub> (3.31 ppm)  
 Internal standard: Potassium hydrogen maleate (98.8% mass fraction)  
 Initial analysis: Mean = 98.6%, s = 0.4% (5 sub samples in duplicate, May 2010)

## Spectroscopic and other characterisation data

GC-MS:	Parent compound:	
	Instrument:	Agilent 6890/5973
	Column:	HP-1MS, 30 m x 0.32 mm I.D. x 0.25 $\mu$ m
	Program:	180 $^{\circ}$ C (1 min), 20 $^{\circ}$ C/min to 240 $^{\circ}$ C (8 min), 30 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
	Injector:	250 $^{\circ}$ C
	Transfer line temp:	300 $^{\circ}$ C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	<i>Bis</i> -TMS derivative:	
	Instrument:	Agilent 6890/5973
	Column:	HP-1MS, 30 m x 0.32 mm I.D. x 0.25 $\mu$ m
	Program:	180 $^{\circ}$ C (1 min), 20 $^{\circ}$ C/min to 240 $^{\circ}$ C (8 min), 30 $^{\circ}$ C/min to 300 $^{\circ}$ C (3 min)
	Injector:	250 $^{\circ}$ C
	Transfer line temp:	300 $^{\circ}$ C
	Carrier:	Helium, 1.0 mL/min
	Split ratio:	20/1
	The retention times of the parent compound and <i>bis</i> -TMS derivative are reported with the major peaks in the mass spectra. The latter are reported as mass/charge ratios and (in brackets) as a percentage relative to the base peak.	
	Parent (7.3 min):	292 ( $M^+$ , 50), 277 (39), 274 (24), 259 (28), 233 (59), 215 (100), 165 (68), 148 (45), 133 (35), 121 (31), 107 (64), 92 (62), 79 (53), 67 (45), 55 (39), 41 (26) <i>m/z</i>
	<i>Bis</i> -TMS (8.2 min):	436 ( $M^+$ , 20), 421 (18), 346 (30), 331 (32), 256 (55), 241 (100), 215 (53), 129 (93), 75 (93) <i>m/z</i>
HS-GC-MS:	Instrument:	Agilent 6890/5973/G1888
	Column:	DB-624, 30 m x 0.25 mm I.D. x 1.4 $\mu$ m
	Program:	50 $^{\circ}$ C (5 min), 7 $^{\circ}$ C/min to 120 $^{\circ}$ C, 15 $^{\circ}$ C/min to 220 $^{\circ}$ C (8.3 min)
	Injector:	150 $^{\circ}$ C
	Transfer line temp:	280 $^{\circ}$ C
	Carrier:	Helium, 1.2 mL/min
	Split ratio:	50/1
	Solvents detected:	Chloroform
TLC:	Conditions:	Kieselgel 60F <sub>254</sub> . Dichloromethane/ethyl acetate (4/1) Single spot observed, $R_f$ = 0.29. Visualisation with vanillin stain
IR:	Instrument:	Biorad FTS300MX FT-IR
	Range:	4000-400 $cm^{-1}$ , KBr powder
	Peaks:	3392, 2967, 2922, 2848, 2681, 1442, 1362, 1266, 1166, 1133, 1048, 1005, 671 $cm^{-1}$
$^1H$ NMR:	Instrument:	Bruker Avance III-400
	Field strength:	400 MHz
	Solvent:	MeOH- <i>d</i> <sub>4</sub> (3.31 ppm)
	Spectral data:	$\delta$ 0.73 (3H, s), 0.72-0.79 (1H, m), 0.83 (3H, s), 0.86-1.08 (3H, m), 1.16-1.74 (16H, m), 1.82 (1H, ddd, $J$ = 3.5, 3.5, 12.0 Hz), 1.96 (1H, m), 3.56 (1H, dd, $J$ = 2.6, 8.6 Hz), 3.96 (1H, m) ppm Methanol and chloroform were estimated at 0.1% mass fraction using $^1H$ NMR
$^{13}C$ NMR:	Instrument:	Bruker Avance III-400
	Field strength:	100 MHz
	Solvent:	DMSO- <i>d</i> <sub>6</sub> (39.5 ppm)
	Spectral data:	$\delta$ 11.1, 11.3, 20.0, 23.0, 28.2, 28.6, 29.8, 31.4, 32.0, 35.2, 35.68, 35.74, 36.6, 38.6, 42.5, 50.7, 54.2, 64.1, 80.1 ppm
Melting point:		223-224 $^{\circ}$ C
Microanalysis:	Found:	C = 77.5%; H = 11.2% (July 2010)
	Calculated:	C = 78.0%; H = 11.0% (Calculated for C <sub>19</sub> H <sub>32</sub> O <sub>2</sub> )