

Synthesis of ring B unsaturated estriols. Confirming the structure of a diagnostic analyte for Smith-Lemli-Opitz syndrome

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Supporting Information

Tables of ^1H and ^{13}C NMR signal assignments and ^1H coupling constants for estriols **3**, **4**, **14**, and **15**.

NOE difference results for dehydroestriols **4** and **14**,

Molecular mechanics calculations indicating conformational heterogeneity of estriols **4** and **14**.

High resolution mass spectral results for compound **7** and dehydroestriols **3**, **4**, and **14**.

^1H NMR spectra of estriols **3**, **4**, **14**, and **15**.

3: estra-1,3,5(10),7-tetraene-3,16 α ,17 β -triol

4: estra-1,3,5(10),8-tetraene-3,16 α ,17 β -triol

7: 3,16 α -dihydroxyestra-1,3,5(10),7-tetraene-17-one

14: 14 β -estra-1,3,5(10),8-tetraene-3,16 α ,17 β -triol

15: estra-1,3,5,7,9-pentaene-3,16 α ,17 β -triol

Table. ^1H NMR chemical shifts of ring B unsaturated estriols in methanol-d₄.^{a,b}

	3 Δ^7	4 Δ^8	14 $14\beta-\Delta^8$	15 $\Delta^{6,8}$
H-1	7.033	6.986	7.012	7.804
H-2	6.600	6.534	6.568	7.057
H-4	6.502	6.519 [†]	6.535	7.036
H-6 α	3.308* [†]	2.658* [†]	2.612* [†]	7.440
H-6 β	3.378* [†]	2.599* [†]	2.653* [†]	
H-7 α	5.358	2.15	2.218 [†]	7.049 [†]
H-7 β		2.11	1.970	
H-9 α	3.063			
H-11 α	2.125	2.44	2.33*	3.226 [†]
H-11 β	1.409	2.44	2.36*	3.140 [†]
H-12 α	1.529	1.532	1.779	1.750
H-12 β	1.886	1.994	1.466	2.191
H-14	2.335 [†]	2.664 [†]	2.176	3.096 [†]
H-15 α	1.414	1.675	1.290	2.055
H-15 β	2.219	1.977	2.410	2.201
H-16 β	4.043	4.119	3.999	4.228
H-17 α	3.578	3.549	3.560	3.668
H-18	0.636	0.757	0.946	0.667

^a NMR data were acquired at 500 MHz as ca. 5-10 mM solutions in methanol-d₄ at 25°C. Chemical shifts were referenced to tetramethylsilane and are corrected for effects of strong coupling. Under the specified conditions of temperature and concentration, the data are reproducible to ca. ± 0.002 ppm except for values marked by [†] (ca. ± 0.005 ppm) and values given to two decimal places (ca. ± 0.01 ppm). ^b Assignments were established from COSYDEC, HSQC, HMBC, and NOE difference experiments. Asterisks (*) indicate assignments of geminal pairs that may be interchanged.

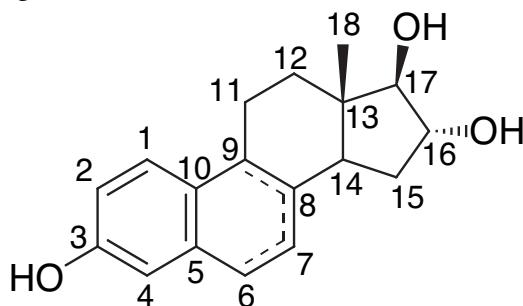


Table. ^1H NMR coupling constants of ring B unsaturated estriols in methanol-d₄.^a

	3 Δ^7	4 Δ^8	14 $14\beta\text{-}\Delta^8$	15 $\Delta^{6,8}$
H-1	8.4, 0.7	8.2	8.4	9.0
H-2	8.4, 2.7	8.2, 2.7	8.3, 2.6	9.0, 2.6
H-4	2.7	2.7	2.5	2.5
H-6 α	21.8 ($\Sigma\text{J}=33$ Hz) ^b	m ^c	15.1, 8.8, 6.3	8.4
H-6 β	22.0 ($\Sigma\text{J}=31$ Hz) ^b	15, 7, 7 † ^c	15.4, 9.6, 6.5	
H-7 α	3.4, 1.7 \times 4	m	m	8.3 †
H-7 β		m	15, 8, 8 †	
H-9 α	12, 5 \times 3 †			
H-11 α	13.2, 5.2, 3.9, 2.6	m	m	18.0, 7.7
H-11 β	13.3, 13.3, 12.0, 3.6	m	m	18.2, 11.2, 7.6 †
H-12 α	12.8, 12.8, 4.0, 0.7	m	13.1, 7.3, 5.7	12.5, 11.4, 7.8, 0.6
H-12 β	12.6, 3.8, 2.6	m	13.1, 6.5, 5.8	12.5, 7.5 †
H-14	12.8, 6.5, 1.8 \times 3 †	12, 8 ($\Sigma\text{J}=25$ Hz) $^{\dagger b}$	8.6, 8.6	12.5, 7.8 †
H-15 α	13.3, 6.7, 1.9	13.2, 7.6, 1.8	12.9, 8.9, 8.9	13.3, 7.8, 2.1
H-15 β	13.1, 13.1, 9.2	13.2, 13.2, 9.0, 0.6	12.9, 8.0, 8.0	13.1, 13.1, 9.2
H-16 β	9.1, 5.4, 2.0	9.0, 5.3, 1.8, 0.6	8.8, 7.9, 6.8	9.1, 5.6, 2.1
H-17 α	5.4	5.3	6.9	5.6
H-18	0.6	0.7	s	0.6

^a Coupling constants (absolute values, in units of Hz) were measured from resolution-enhanced spectra acquired in methanol-d₄. Couplings are generally accurate to ± 0.2 Hz except for coupling sets marked by † and multiple couplings of the same nominal value. Aside from multiplets (m), resonances are described by their first-order splittings (d, dd, ddd, dddd, or ddddd); the multiplier sign (\times) is used to describe three or four couplings of essentially the same value. ^b These coupling patterns include several unresolved small couplings; ΣJ indicates the sum of all couplings. ^c Stereochemical assignments for the geminal protons were not established; the multiplet (m) corresponds to the downfield proton.

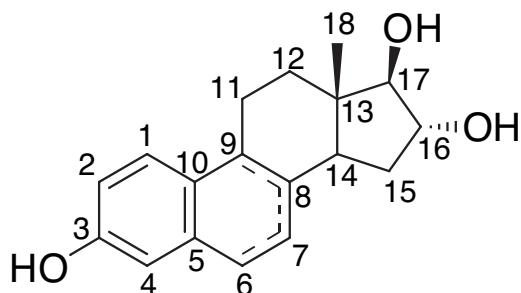
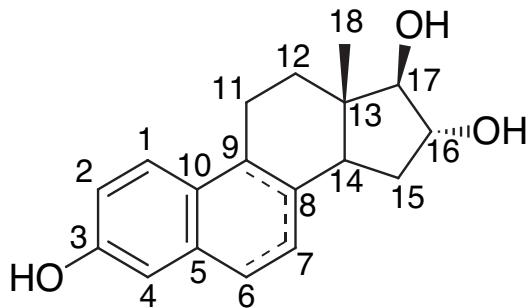


Table. ^{13}C NMR chemical shifts of ring B unsaturated estriols in methanol-d₄.^a

	3 Δ^7	4 Δ^8	14 $14\beta\text{-}\Delta^8$	15 $\Delta^{6,8}$
C-1	129.78	124.03	124.20	125.64
C-2	115.10	114.07	113.71	119.74
C-3	156.70	157.96	156.77	156.57
C-4	115.30	116.04	115.39	111.09
C-5	135.41	138.29	138.34	135.45
C-6	30.86	29.82	30.01	125.90
C-7	115.37	26.15	29.04	125.70
C-8	138.01	131.67	134.38	133.76
C-9	42.04	126.96	126.61	131.36
C-10	130.14	128.77	129.86	128.13
C-11	33.50	24.79	23.18	24.98
C-12	38.97	35.23	32.93	35.53
C-13	46.98	44.79	41.71	44.69
C-14	49.38	46.05	47.90	45.53
C-15	32.67	34.69	37.52	35.59
C-16	78.76	79.51	78.52	79.56
C-17	91.24	89.95	84.44	90.12
C-18	13.11	12.52	22.05	11.99

NMR data were acquired at 125 MHz as ca. 5-20 mM solutions in methanol-d₄ at 25°C. Chemical shifts were referenced to the CD₃OD signal at δ 49.15.

Assignments were established from DEPT, HSQC, and HMBC experiments.



NOE difference results for the 14α and 14β epimers **4** and **14**.

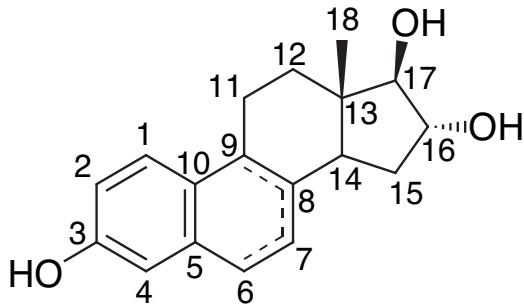
^1H NMR spectra for NOE experiments were acquired nonspinning at 500 MHz as ca. 5 mM solutions in methanol-d₄ at 25°C. Experiments consisted of a data set for each irradiated resonance and a control spectrum (irradiation at -2 ppm). A single line of each target multiplet was irradiated at low power for 2 s, followed by a 90° pulse and acquisition of the FID. Approximately 100 blocks of 16 scans were collected for each data set (total ca. 1600 scans). The NOE difference spectra were obtained by Fourier transformation with 0.5 Hz exponential line broadening, followed by subtraction of the control spectrum. Enhanced signals in the difference spectra were integrated after calibration of the large negative irradiated signal to -100%. The results below indicate the positive integrals for each enhanced signal that is not coupled to the irradiated resonance.

NOE results for **4** (estra-1,3,5(10),8-tetraene-3,16 α ,17 β -triol):

Irradiation of H-18 (δ 0.757):	H-16 β (2.1%), H-17 α (0.3%), H-14 α (0.2%), H-11 α/β (1.1%), H-15 β plus H-12 β (1.9%).
Irradiation of H-12 α (δ 1.532):	H-17 α (5.9%), H-14 α (5.0%).
Irradiation of H-4 (δ 6.519):	H-6, upfield signal (5.5%).
Irradiation of H-1 (δ 6.986):	H-11 α/β (8.5%).

NOE results for **14** (14 β -estra-1,3,5(10),8-tetraene-3,16 α ,17 β -triol):

Irradiation of H-18 (δ 0.946):	H-16 β (1.6%), H-17 α (0.4%), H-11 α/β (1.1%), H-14 β (2.6%), H-12 α (0.4%), H-12 β (1.1%).
Irradiation of H-15 α (δ 1.290):	H-17 α (1.2%), H-7 α (3.0%), H-12 α (1.8%).
Irradiation of H-12 β (δ 1.466):	H-17 α (1.8%), H-18 (3.3%).
Irradiation of H-12 α (δ 1.779):	H-17 α (5.2%), H-15 α (2.3%), H-18 (1.3%).



Molecular mechanics calculations indicating conformational heterogeneity of estriols **4** and **14**.

Coupling constants and NOE difference data for estriols **4** and **14** suggested conformational heterogeneity in rings B and C. As shown in the Table below, molecular mechanics calculations with the MM3 and MMX force fields revealed multiple conformers of similar energies. In the 14β estriol **14**, both the 12α - and 12β -sofas (shown below) appear to be significantly populated. Similarly, ring B of **4** and **14** exists as a mixture of $6\alpha,7\beta$ - and $6\beta,7\alpha$ -half chairs. This conformational heterogeneity is qualitatively compatible with the observed coupling constants and NOE difference results.

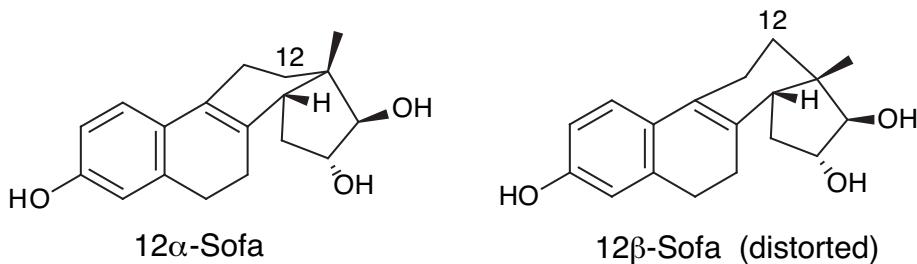


Table. Heats of formation for estriols **4** and **14** as calculated by PCMODEL.^{a,b}

Conformer	Heat of formation (kcal/mol)	
	MMX	MM3
4 (estra-1,3,5(10),8-tetraene-3,16 α ,17 β -triol):		
12 α -sofa	6 $\beta,7\alpha$ -half chair	10.93
12 α -sofa	6 $\alpha,7\beta$ -half chair	10.74
14 (14 β -estra-1,3,5(10),8-tetraene-3,16 α ,17 β -triol):		
12 α -sofa	6 $\beta,7\alpha$ -half chair	8.63
12 β -sofa	6 $\beta,7\alpha$ -half chair	6.98
12 α -sofa	6 $\alpha,7\beta$ -half chair	7.22
12 β -sofa	6 $\alpha,7\beta$ -half chair	7.87

^a Molecular mechanics calculations were carried out with PCMODEL 7.0 (Linux version; Serena Software; Bloomington, IN). ^b The half chairs and 12β -sofas are intermediate forms, not pure sofas or half chairs.

High resolution mass spectral results for compound **7** and dehydroestriols **3**, **4**, and **14**.

High resolution mass spectra were measured on a ZAB-HF mass spectrometer. The instrument was tuned 10,000 resolution (10% valley) and operated in the EI+ mode at 70 eV. The estriol samples were introduced into the ion source by direct probe. Masses were calibrated against a perfluorokerosene standard introduced through the direct inlet.

Compound	Formula	Calcd. Mass	Obsd. Mass ^a	Error (mmu) ^b	S.D. (mmu) ^a
7	C ₁₈ H ₂₀ O ₃	284.1412	284.1417	-0.5	2.3
3	C ₁₈ H ₂₂ O ₃	286.1569	286.1556	1.3	1.5
4	C ₁₈ H ₂₂ O ₃	286.1569	286.1583	-1.4	3.6
14	C ₁₈ H ₂₂ O ₃	286.1569	286.1571	-0.2	1.8

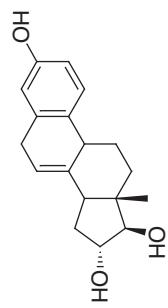
^a The observed mass (Obsd. Mass) represents the mean of multiple measurements; S.D. denotes the standard deviation of these measurements. ^b Difference between calculated and observed mass in millimass units.

We thank Jihai Pang of Rice University for the acquisition and processing of the mass spectral data.

7-Dehydroestradiol (**3**), ca. 10 mM in CD_3OD , ^1H NMR, 500 MHz, 25°C

TMS

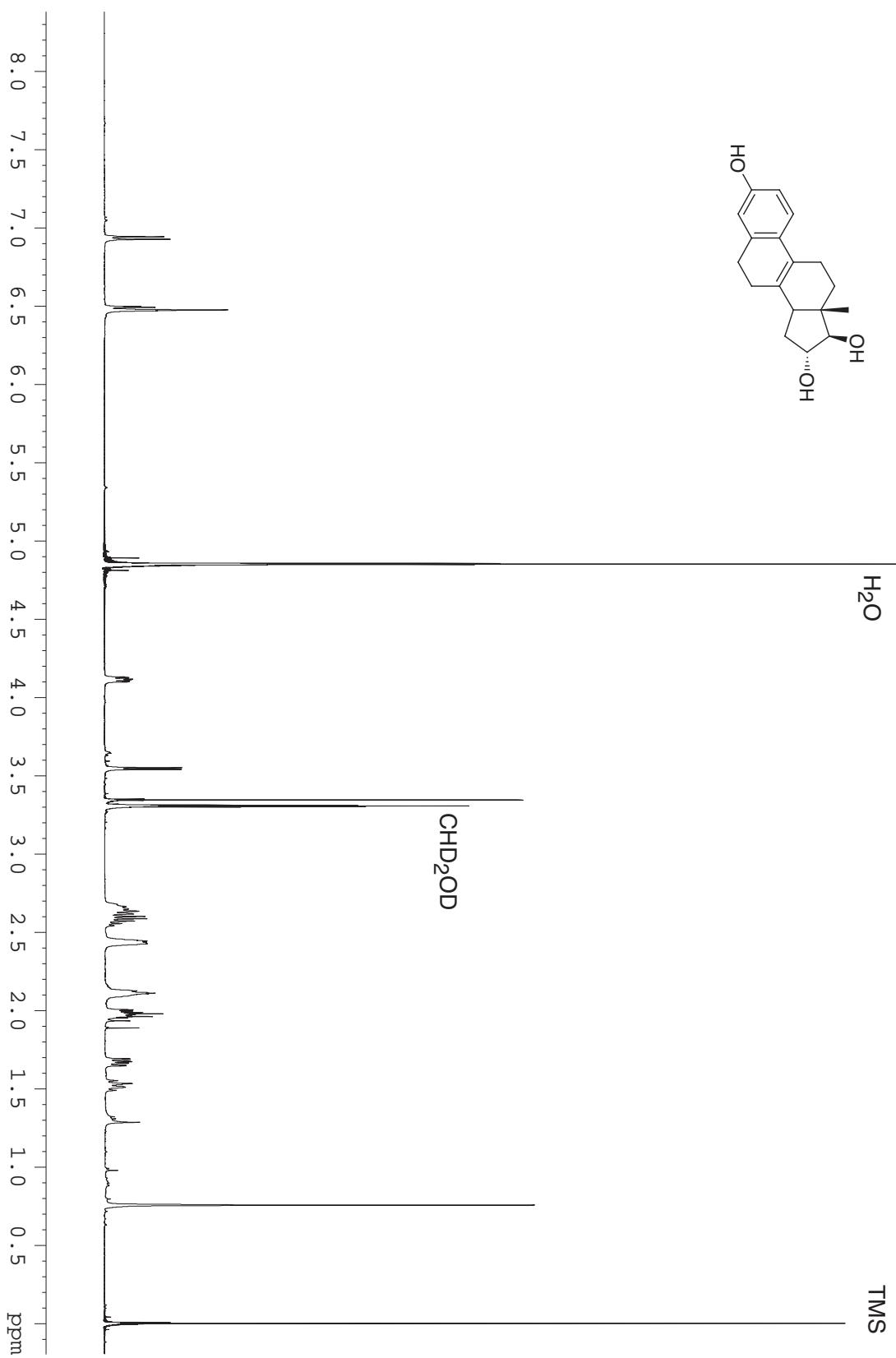
H_2O



CHD_2OD

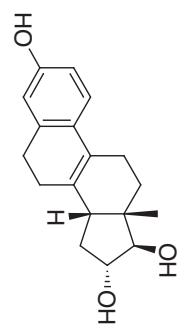
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

8-Dehydroestriol (**4**),
ca. 10 mM in CD₃OD, ¹H NMR, 500 MHz, 25°C



14 β -H-8-Dehydroestriol (14**),**

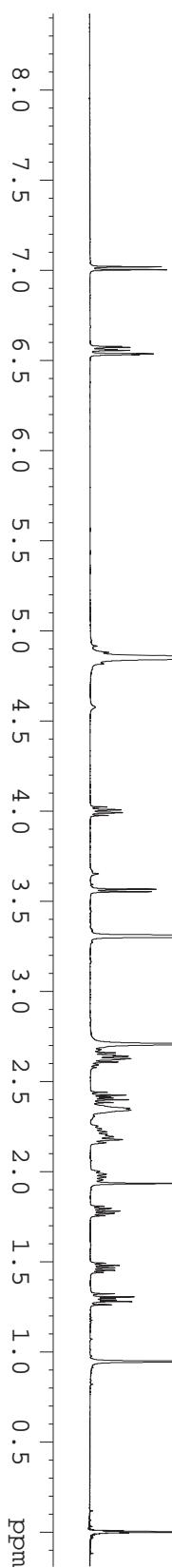
ca. 10 mM in CD₃OD, ¹H NMR, 500 MHz, 25°C



H₂O

TMS

CD₃OD



6,8-Didehydroestriol (**15**) containing 15% 7-dihydroestriol (**3**), ca. 10 mM in CD_3OD , ^1H NMR, 500 MHz, 25°C

