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J. Serb. Chem. Soc. 88 (7–8) S165–S174 (2023) *Supplementary material*

SUPPLEMENTARY MATERIAL TO

Synthesis and antiproliferative activity of (5*R*)-cleistenolide and analogues

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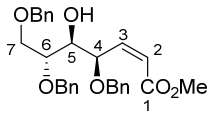
SPECTROSCOPIC DATA OF MAIN COMPOUNDS

Methyl (2Z)-4,6,7-tri-O-*benzyl-2,3-dideoxy-D*-arabino-*hept-2-enoate* (**4**)



4

IR (film): ν_{max} 3479, 1723, 1658, 1604, 1586, 1028 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.24–7.39 (*m*, 15 H, 3 × Ph), 7.41 (*dd*, 1 H, $J_{2,3}$ = 11.8, $J_{3,4}$ = 9.1 Hz, H-3), 6.02 (*d*, 1 H, $J_{2,3}$ = 11.8 Hz, H-2), 5.42 (*bd*, $J_{3,4}$ = 9.0 Hz, H-4), 4.34–4.75 (*m*, 6 H, 3 × PhCH_2), 3.87 (*dd*, 1 H, $J_{7a,7b}$ = 12.1, $J_{6,7b}$ = 4.9 Hz, H-7b), 3.73 (*m*, 3 H, H-5, H-6 and H-7a), 3.69 (*s*, 3 H, CO_2CH_3), 2.1–2.5 (*bs*, 1 H, OH).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 166.15 (CO_2CH_3), 147.32 (C-3), 138.53, 138.25, 137.95, 128.38, 128.35, 128.25, 128.14, 127.93, 127.82, 127.69, 127.60, 127.50 (3 × Ph), 122.38 (C-2), 77.85 (C-6), 74.49 (C-5), 73.54 (C-4), 73.52, 72.43, 71.27 (3 × PhCH_2), 70.74 (C-7), 51.44 (CO_2CH_3).

(+)ESI-HRMS *m/z*: calculated for [$C_{29}H_{32}O_6$ + K^+] 515.1830, observed 515.1822.

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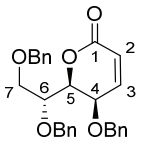
4,6,7-Tri-O-*benzyl-2,3-dideoxy-D*-arabino-*hept-2-eno-1,5-lactone* (***5***)



5

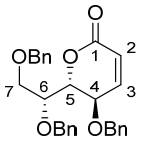
IR (film): ν_{max} 1731, 1629, 1605, 1497, 1066, 1028 cm^{-1}.

^{1}H NMR (250 MHz, $CDCl_3$, δ): 7.14–7.52 (*m*, 15 H, 3 × Ph), 6.99 (*dd*, 1 H, $J_{2,3}$ = 9.8, $J_{3,4}$ = 5.7 Hz, H-3), 6.20 (*d*, 1 H, $J_{2,3}$ = 9.8 Hz, H-2), 4.46–4.86 (*m*, 7 H, H-5 and 3 × CH_2Ph), 4.28 (*dd*, 1 H, $J_{4,5}$ = 2.5, $J_{3,4}$ = 5.6 Hz, H-4), 4.18 (*ddd*, 1 H, $J_{6,7b}$ = 2.0, $J_{6,7a}$ = 4.0, $J_{5,6}$ = 9.6 Hz, H-6), 3.96 (*dd*, 1 H, $J_{7a,7b}$ = 10.8, $J_{6,7b}$ = 2.0 Hz, H-7b), 3.82 (*dd*, 1 H, $J_{6,7a}$ = 3.9, $J_{7a,7b}$ = 10.8 Hz, H-7a).

^{13}C NMR (62.5 MHz, $CDCl_3$, δ): 162.68 (C-1), 143.13 (C-3), 138.25, 138.17 137.69, 128.52, 128.42, 128.13, 128.05, 127.90, 127.75, 127.71, 127.66 (3 × Ph), 124.31 (C-2), 77.91 (C-5), 75.35 (C-6), 73.54, 72.36, 71.38 (3 × CH_2Ph), 67.92 (C-7), 65.46 (C-4).

(+)ESI-HRMS *m/z*: calculated for [$C_{28}H_{28}O_5$ + K^+] 483.1568, observed 483.1564.

4,6,7-Tri-O-*benzyl-2,3-dideoxy-D*-lyxo-*hept-2-eno-1,5-lactone* (***6***)



6

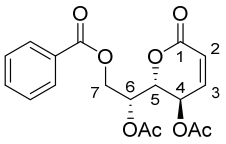
IR (film): ν_{max} 3020, 1731, 1497, 1101, 1027 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.25–7.43 (*m*, 15 H, 3 × Ph), 6.85 (*dd*, 1 H, $J_{2,3}$ = 10.0, $J_{3,4}$=2.2 Hz, H-3), 5.99 (*dd*, 1 H, $J_{2,3}$ = 10.0, $J_{2,4}$ = 1.8 Hz, H-2), 4.34–4.84 (*m*, 8 H, 3 × CH_2Ph, H-4 and H-5), 4.01 (*td*, 1 H, $J_{6,7a}$ = 6.2, $J_{6,7b}$ = 6.0, $J_{5,6}$ = 1.9 Hz, H-6), 3.88 (*dd*, 1 H, $J_{6,7b}$ = 5.8, $J_{7a,7b}$ = 9.8 Hz, H-7b), 3.84 (*dd*, 1 H, $J_{6,7a}$ = 6.4, $J_{7a,7b}$ = 9.8 Hz, H-7a).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 162.43 (C-1), 146.07 (C-3), 137.94, 137.78, 136.91, 128.51, 128.36, 128.31, 128.13, 127.94, 127.87, 127.78, 127.68, 127.63 (3 × Ph), 120.24 (C-2), 80.08 (C-5), 74.68 (C-6), 73.49, 72.64 and 71.6 (3 × CH_2Ph), 69.03 (C-7), 68.80 (C-4).

(+)ESI-HRMS *m/z*: calculated for [$C_{28}H_{28}O_5$ + Na^+] 467.1834, observed 467.1827.

*(5*R*)-Cleistenolide (**2**)*



(5*R*)-Cleistenolide (**2**)

IR (film): ν_{max} 1744, 1604, 1176 cm^{-1}.

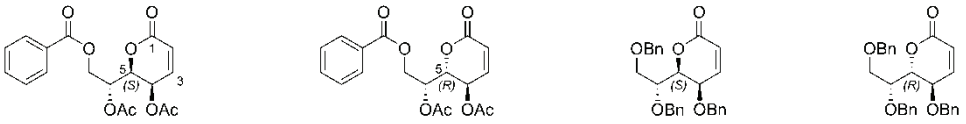
^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.40–8.05 (*m*, 5 H, Ph), 6.77 (*dd*, 1 H, $J_{2,3}$ = 10.0, $J_{3,4}$ = 2.7 Hz, H-3), 6.10 (*dd*, 1 H, $J_{2,4}$ = 1.9, $J_{2,3}$ = 10.0 Hz, H-2), 5.57 (*ddd*, 1 H, $J_{2,4}$ = 1.9, $J_{3,4}$ = 2.6, $J_{4,5}$ = 8.5 Hz, H-4), 5.50 (*ddd*, 1 H, $J_{5,6}$ = 2.2, $J_{6,7b}$ = 5.3, $J_{6,7a}$ = 7.3 Hz, H-6), 4.74 (*dd*, 1 H, $J_{5,6}$ = 2.2, $J_{4,5}$ = 8.5 Hz, H-5), 4.61 (*dd*, 1 H, $J_{6,7b}$ = 5.3, $J_{7a,7b}$ = 11.7 Hz, H-7b), 4.56 (*dd*, 1 H, $J_{6,7a}$ = 7.3, $J_{7a,7b}$ = 11.7 Hz, H-7a), 2.10 and 2.13 (2 × *s*, 3 H each, 2 × COCH_3).

^{13}C NMR (100 MHz, $CDCl_3$, δ): 169.91 and 169.64 (2 × *C*OCH_3), 165.83 (*C*OPh), 160.89 (C-1), 144.09 (C-3), 133.25, 129.91, 129.31, 128.45 (Ph), 121.82 (C-2), 77.80 (C-5), 67.80 (C-6), 63.20 (C-4), 62.40 (C-7), 20.60 (2 × CO*C*H_3).

(+)ESI-LRMS *m/z*: 363 [M + H^+].

Combustion analysis for $C_{18}H_{18}O_8$: Calculated: C 59.67, H 5.01; found: C 59.49, H 4.89.

SAR ANALYSIS



(−)-Cleistenolide (**1**) (5*R*)-Cleistenolide (**2**) **7** **8**

Fig. S-1. Structures of compounds used for SAR analysis

TABLE S-I. *In vitro* cytotoxicities used for SAR analysis.

Compounds	IC_{50} (μM)							
	K562	HL-60	Jurkat	Raji	MCF-7	MDA-MB 231	HeLa	A549
1	7.65	1.21	14.22	36.94	26.07	2.25	7.32	16.34
2	0.21	7.31	19.41	2.47	21.28	7.66	6.45	9.38
5	0.34	12.55	9.24	29.66	1.39	0.09	3.58	1.85
6	0.33	8.27	17.03	1.05	20.06	7.04	5.90	17.21

The structure-activity relationships were accessed as follows: the IC_{50} values of two compounds were compared, and the Δ log IC_{50} was calculated (Δ log IC_{50} is a difference between the log IC_{50} values of an analogue and the corresponding control compound). Positive Δ log IC_{50} values show a decrease of

antiproliferative activity, whereas negative values indicate an increase in the activity upon the structural modification being considered. The results are presented in Fig. S2.

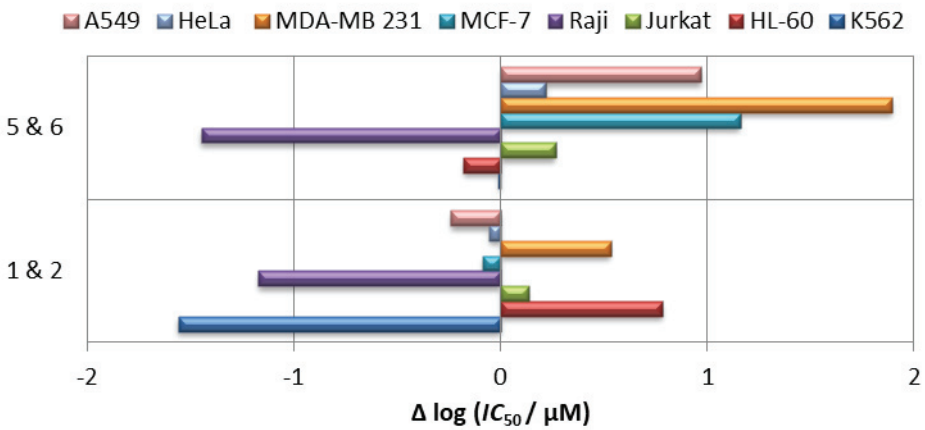
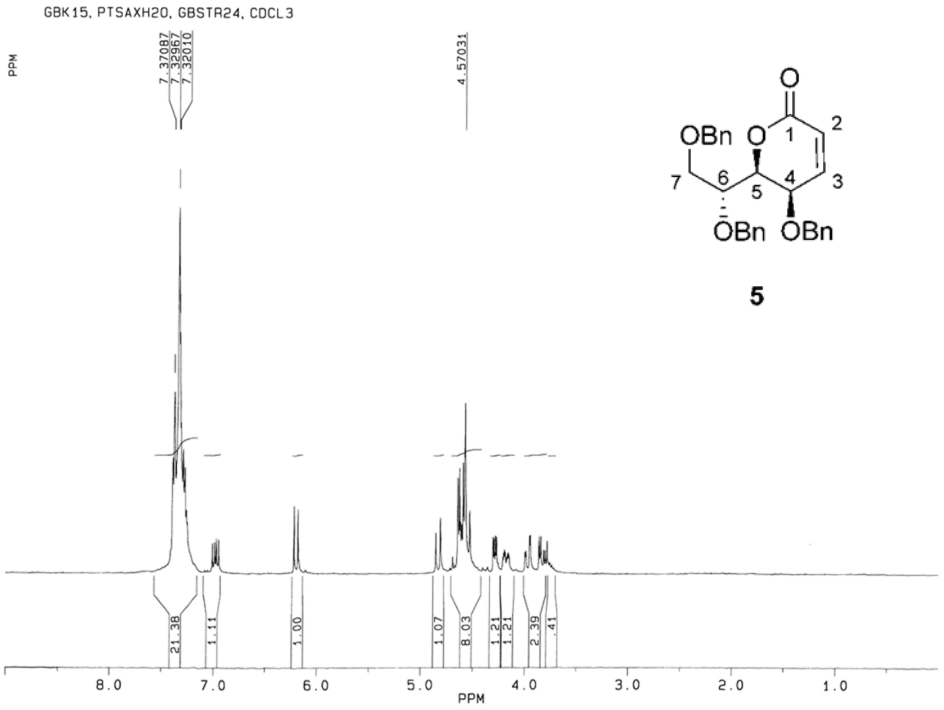


Fig. S-2. The effect of stereochemistry at the C-5 position on the cytotoxicity of stereoisomers.

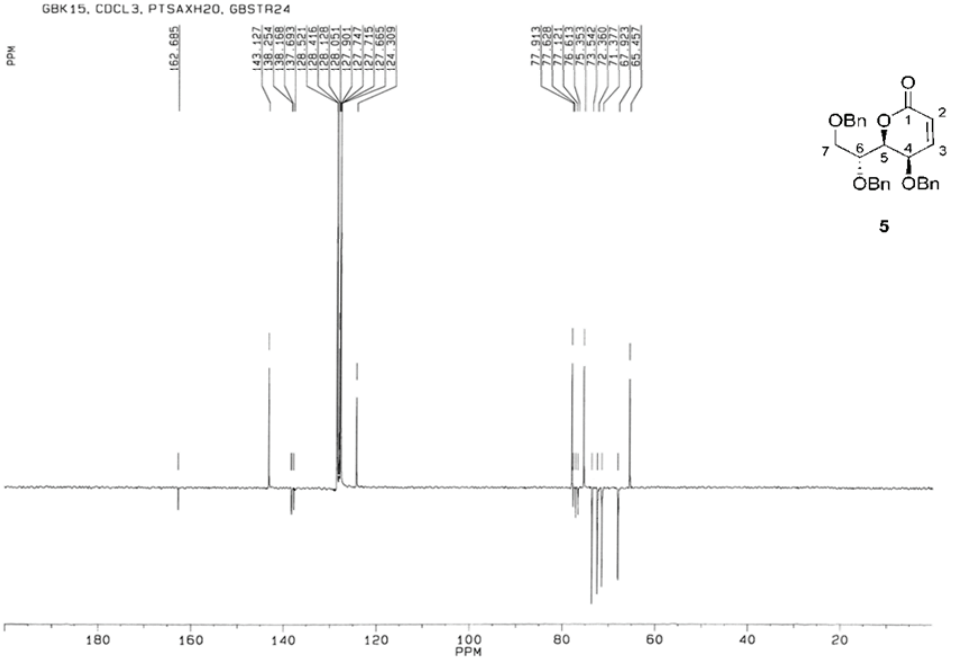
NMR SPECTRA OF MAIN COMPOUNDS

250 MHz ^{1}H NMR Spectrum of compound **5** ($CDCl_3$)

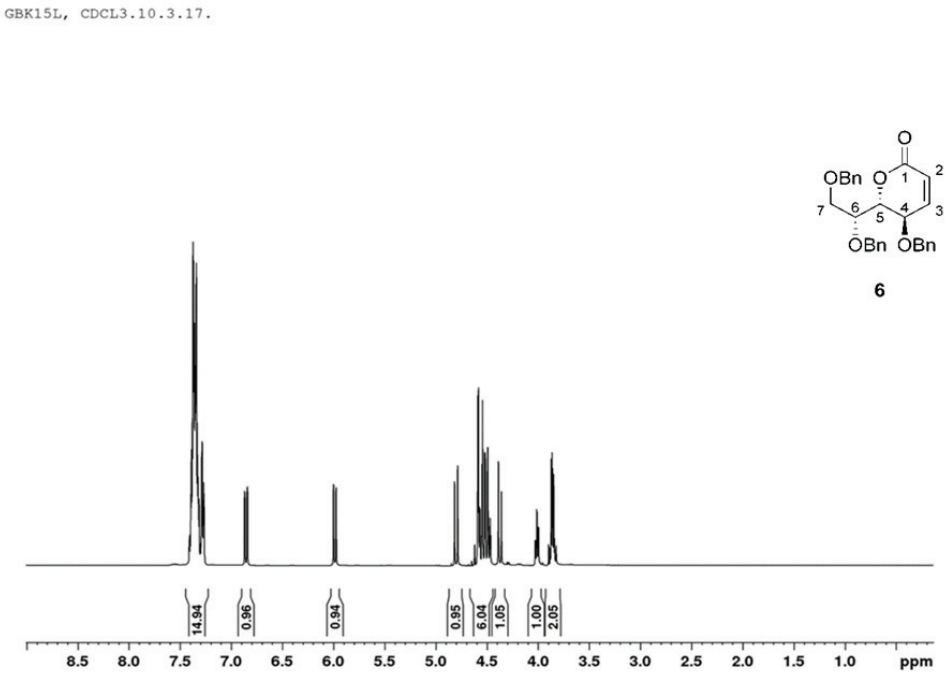


5

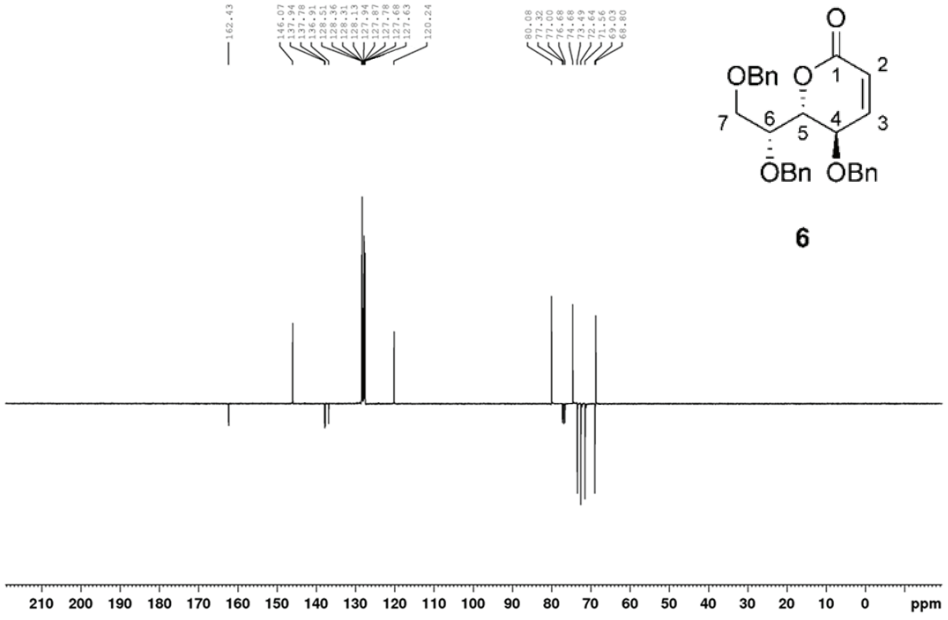
62.9 MHz ^{13}C NMR Spectrum of compound **5** ($CDCl_3$)



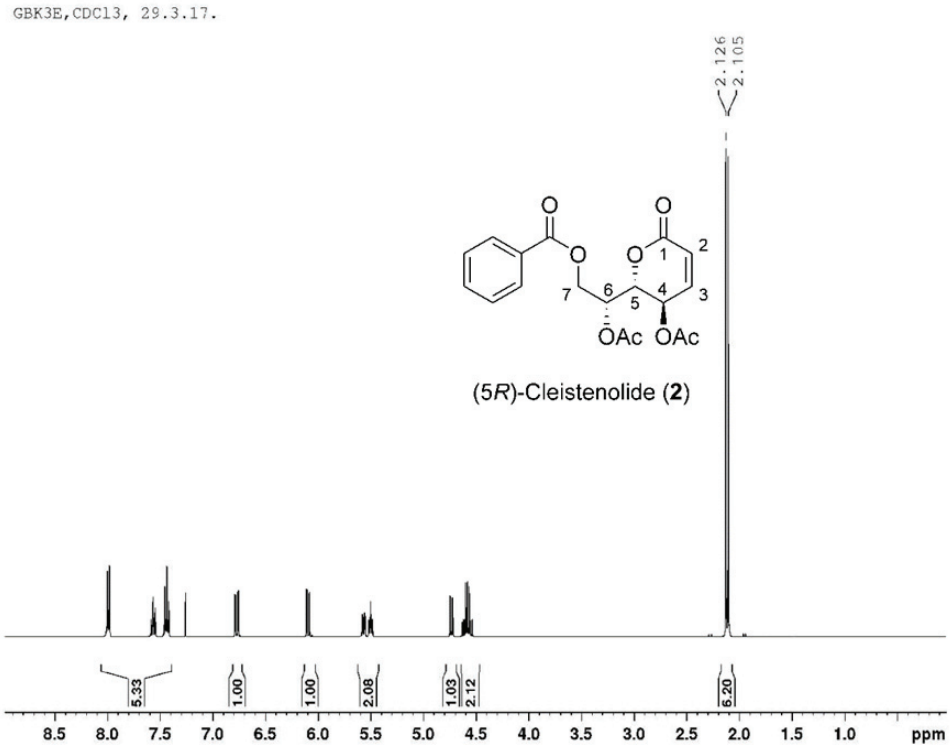
400 MHz ^{1}H NMR Spectrum of compound **6** ($CDCl_3$)



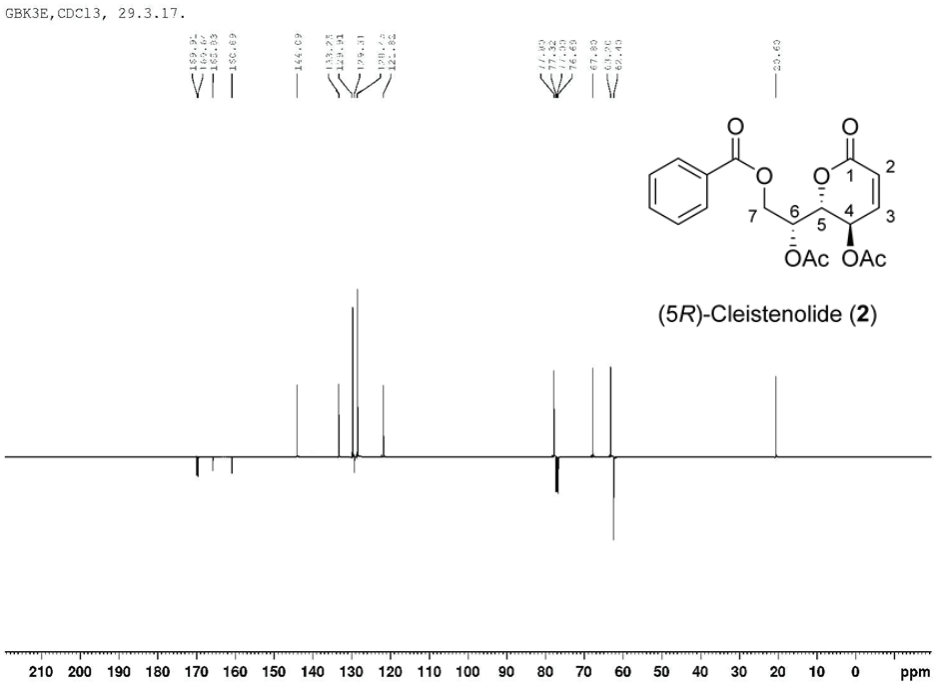
100 MHz ^{13}C NMR Spectrum of compound **6** (CDC_{13})



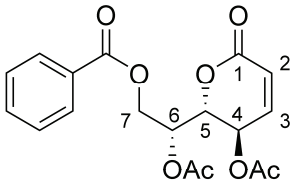
400 MHz ^{1}H NMR Spectrum of compound **2** ($CDCl_3$)



100 MHz ^{13}C NMR Spectrum of compound **2** ($CDCl_3$)



COMPARISON OF NMR DATA OF FINAL PRODUCT **2** WITH PUBLISHED VALUES



(5*R*)-Cleistenolide (**2**)

TABLE S-II. Comparison of NMR data of final product **2** with published values ($CDCl_3$)

C/H	δ_H (*J*, Hz)		δ_C	
	This work	Ref. 1	This work	Ref. 1
1	—		160.9	160.9
2	6.10 *dd* (1.9, 10.0)	6.12 *dd* (1.7, 10.0)	121.8	121.9
3	6.77 *dd* (10.0, 2.7)	6.79 *dd* (10.0, 2.8)	144.1	144.1
4	5.57 *ddd* (1.9, 2.6, 8.5)	5.59 *dt* (2.0, 2.0, 6.3)	63.2	63.3
5	4.74 *dd* (2.2, 8.5)	4.75 *dd* (2.0, 8.5)	77.8	78.0

TABLE S-II. Continued

C/H	δ_H (*J*, Hz)		δ_C	
	This work	Ref. 1	This work	Ref. 1
6	5.50 *ddd* (2.2, 5.3, 7.3)	5.52 *ddd* (1.9, 5.3, 7.0)	67.8	67.9
7a	4.56 *dd* (7.3, 11.7)	4.58 *dd* (7.3, 11.5)	62.4	62.4
7b	4.61 *dd* (5.3, 11.7)	4.63 *dd* (5.3, 11.5)		
Me	2.10 and 2.13 (2 × *s*)	2.13 and 2.15 (2 × *s*)	20.6	20.7
Me*C*O	—	—	169.6 and 169.9	169.7 and 170.0
Ph	7.40–8.05 *m*	7.46–8.01*m*	128.4, 129.3, 129.9, 133.2	128.5, 129.4, 129.7, 133.4
Ph*C*O	—	—	166.0	165.9

REFERENCES

1. P. S. Mahajan, R. G. Gonnade, S. B. Mhaske, *Eur. J. Org. Chem*. **2014** (2014) 8049 (https://dx.doi.org/10.1002/ejoc.201403123).