SUPPORTING INFORMATION

Silvestrol and Episilvestrol, Potential Anticancer Rocaglate Derivatives from *Aglaia silvestris*

Bang Yeon Hwang,[†] Bao-Ning Su,[†] Heebyung Chai,[†] Qiuwen Mi[†], Leonardus B. S. Kardono[‡], Johar J. Afriastini,[§] Soedarsono Riswan[§], Bernard D. Santarsiero,[⊥] Andrew D. Mesecar,[⊥] Robert Wild,[‖] Craig R. Fairchild,[‖] Gregory D. Vite,[‖] William C. Rose,[‖] Norman R. Farnsworth,[†] Geoffrey A. Cordell,[†] John M. Pezzuto,[†,∇] Steven M. Swanson,[†] and A. Douglas Kinghorn*[,†]

Program for Collaborative Research in the Pharmaceutical Sciences and Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, Research and Development Chemistry, Indonesian Institute of Science, Serpong, 15310 Tangerang, Indonesia, Herbarium Bogoriense, Research and Development Center for Biology, Indonesian Institute of Science, 16122 Bogor, Indonesia, Center for Pharmaceutical Biotechnology and the Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, IL 60607, and Bristol-Myers Squibb, Pharmaceutical Research Institute, P.O. Box 4000, Princeton, New Jersey 08543

List of Supporting Information:

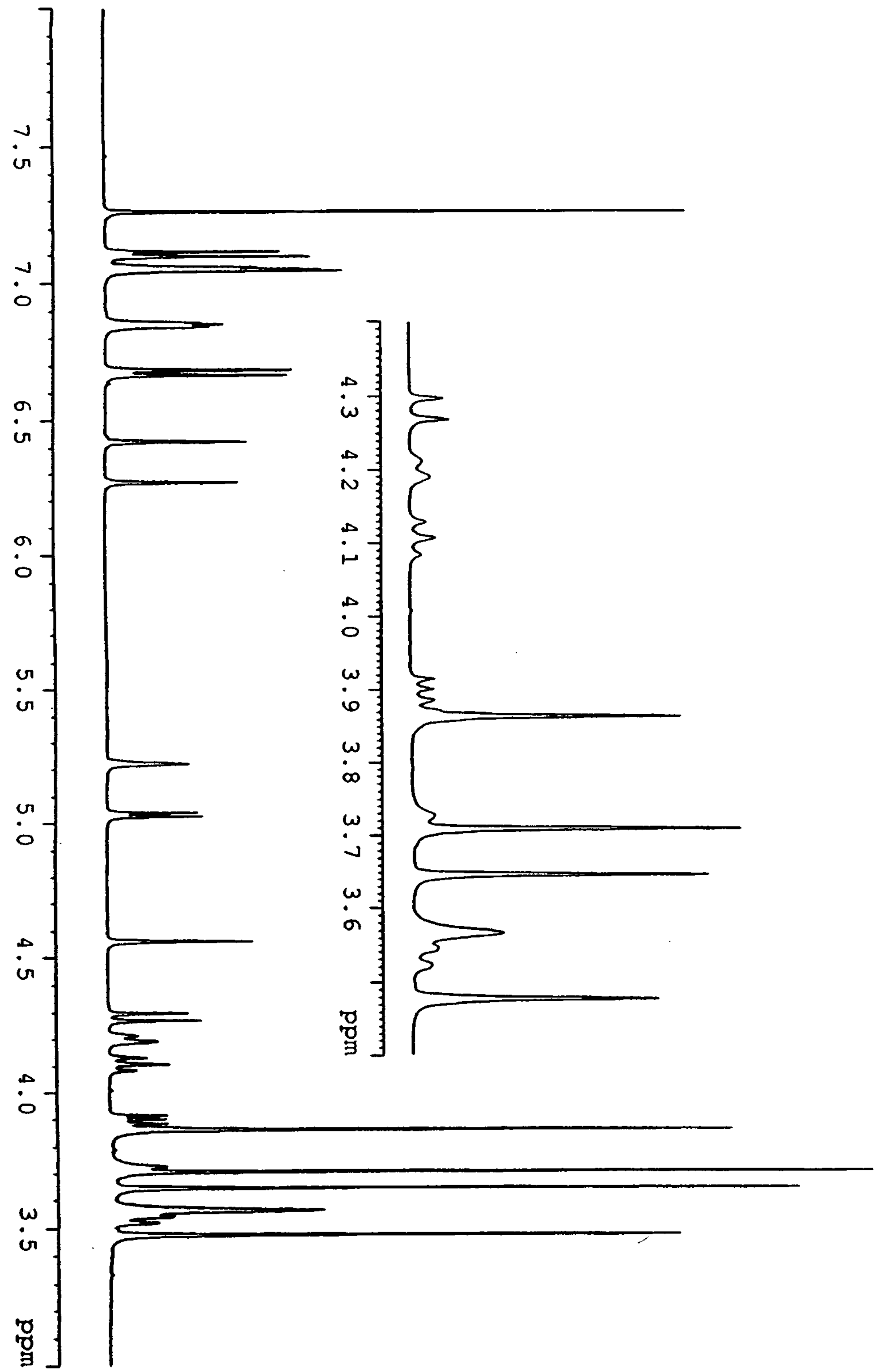


Figure S1. ^1H NMR spectrum of silvestrol (**1**) in $CDCl_3$

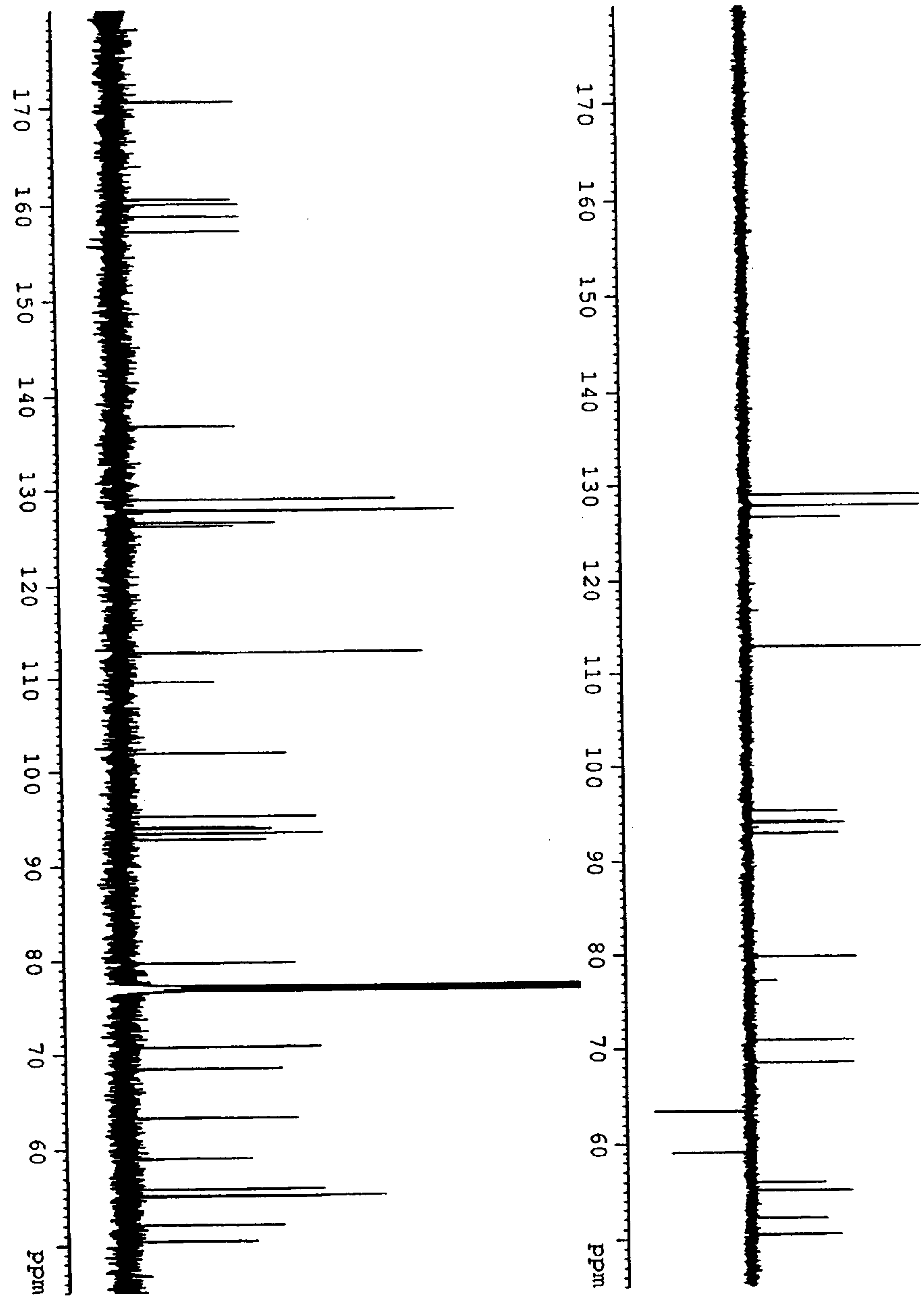


Figure S2. ^{13}C and DEPT135 NMR spectra of silvestrol (1) in $CDCl_3$

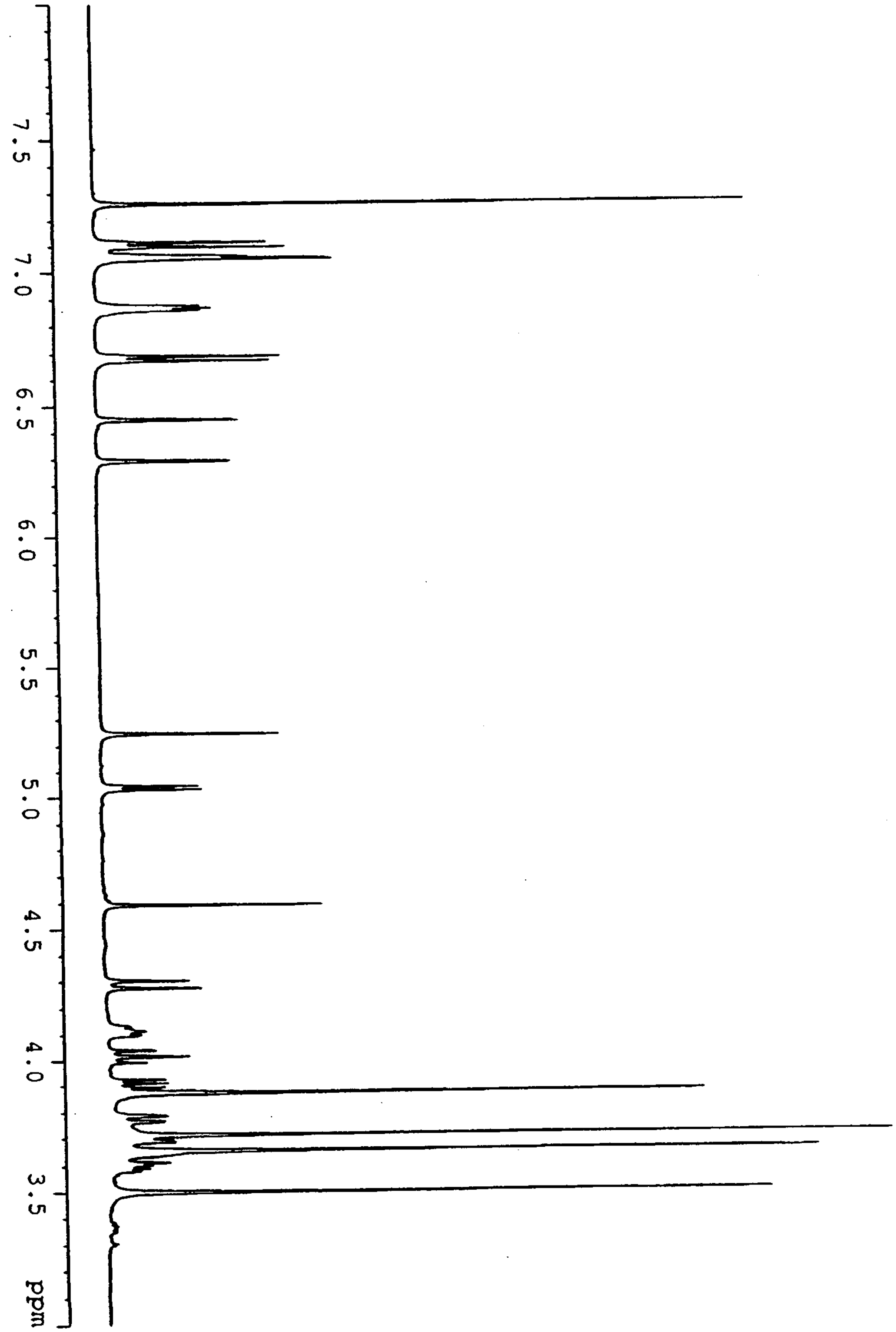


Figure S3. ^{1}H NMR spectrum of episilvestrol (**2**) in $CDCl_3$

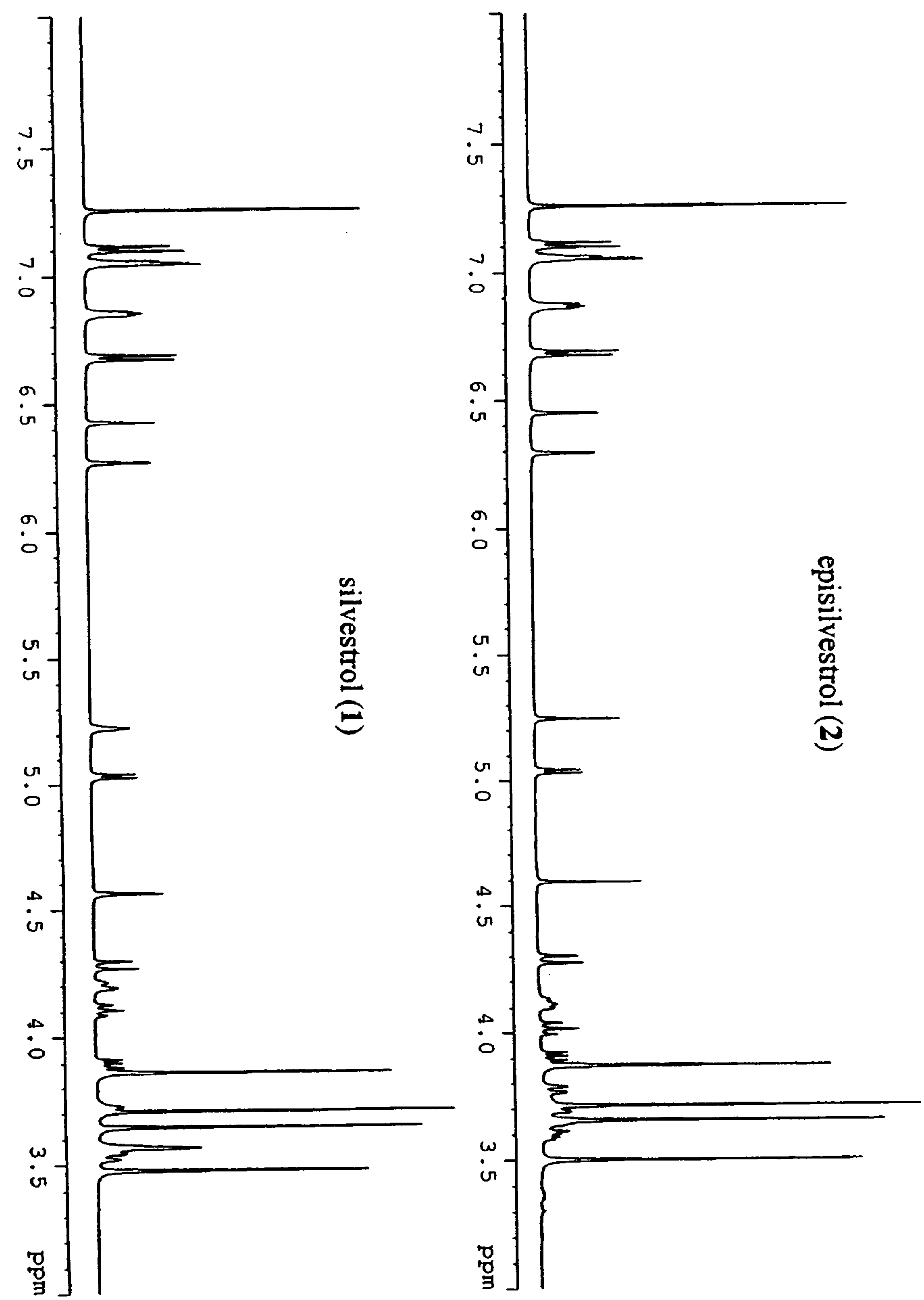


Figure S4. Comparison of the ^1H NMR spectra of silvestrol (**1**) and episilvestrol (**2**) in $CDCl_3$

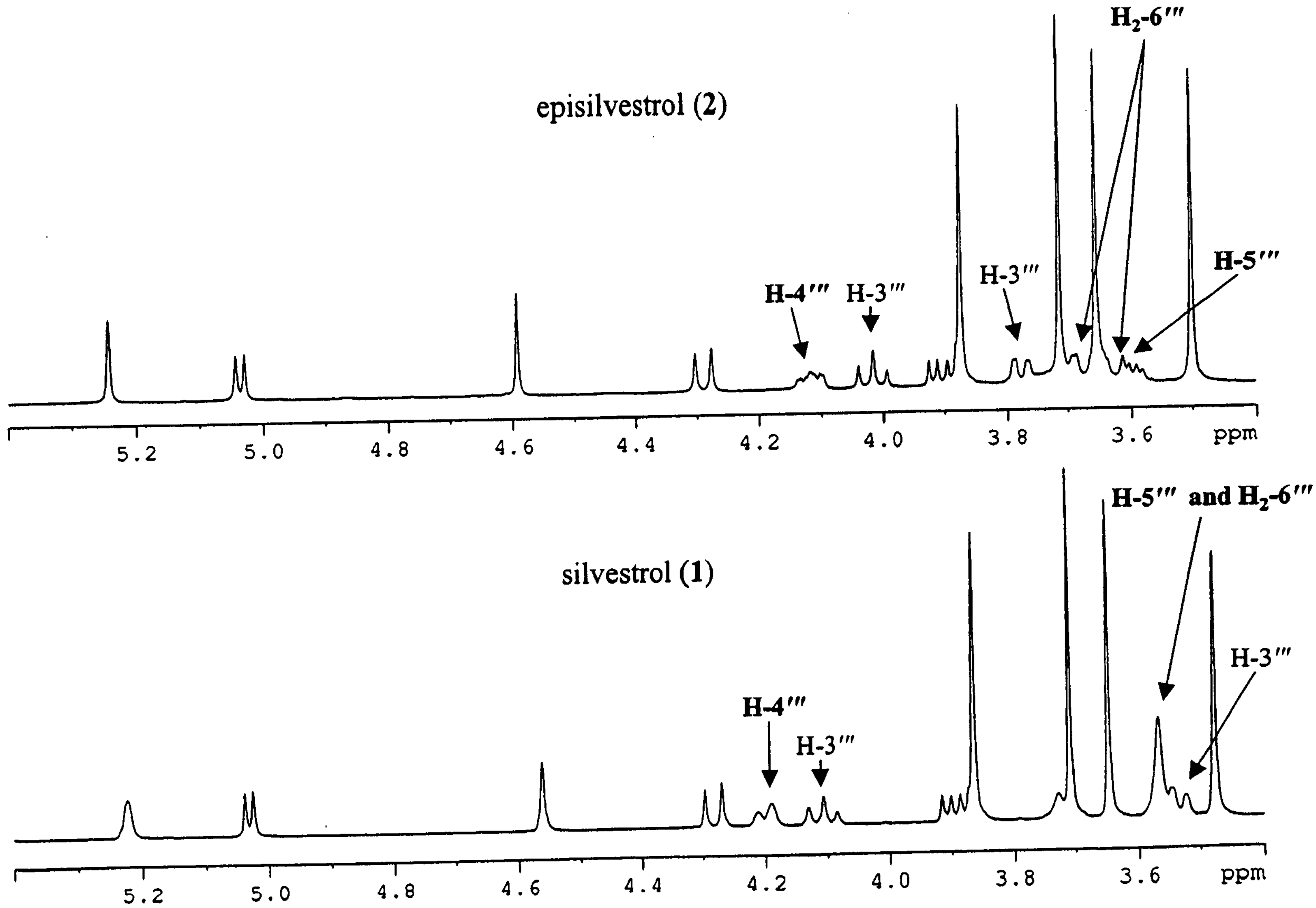


Figure S5. Comparison of the partial ^{1}H NMR spectra of silvestrol (**1**) and episilvestrol (**2**) in $CDCl_3$

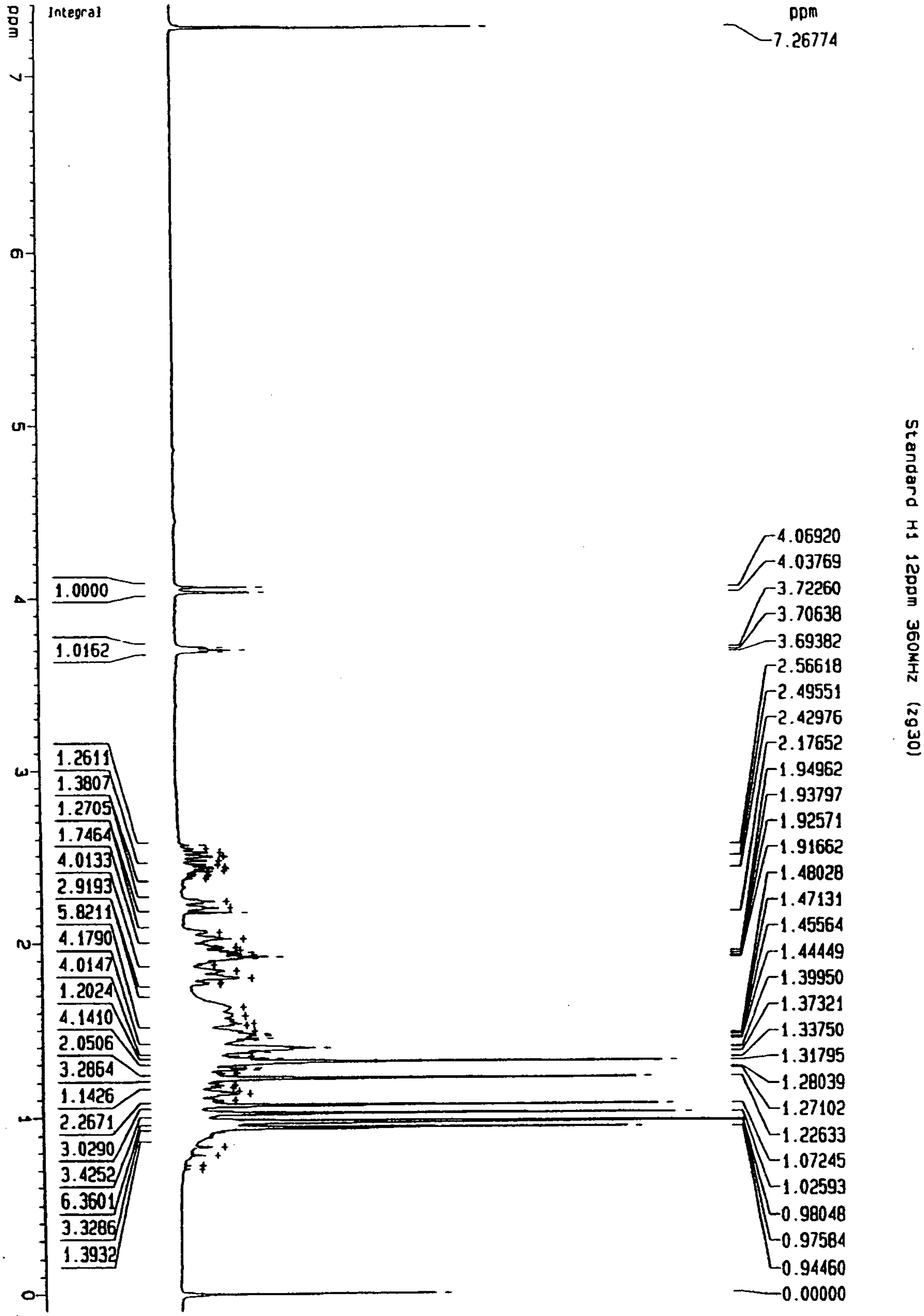


Figure S6. ^{1}H NMR spectrum of compound **4** in $CDCl_3$

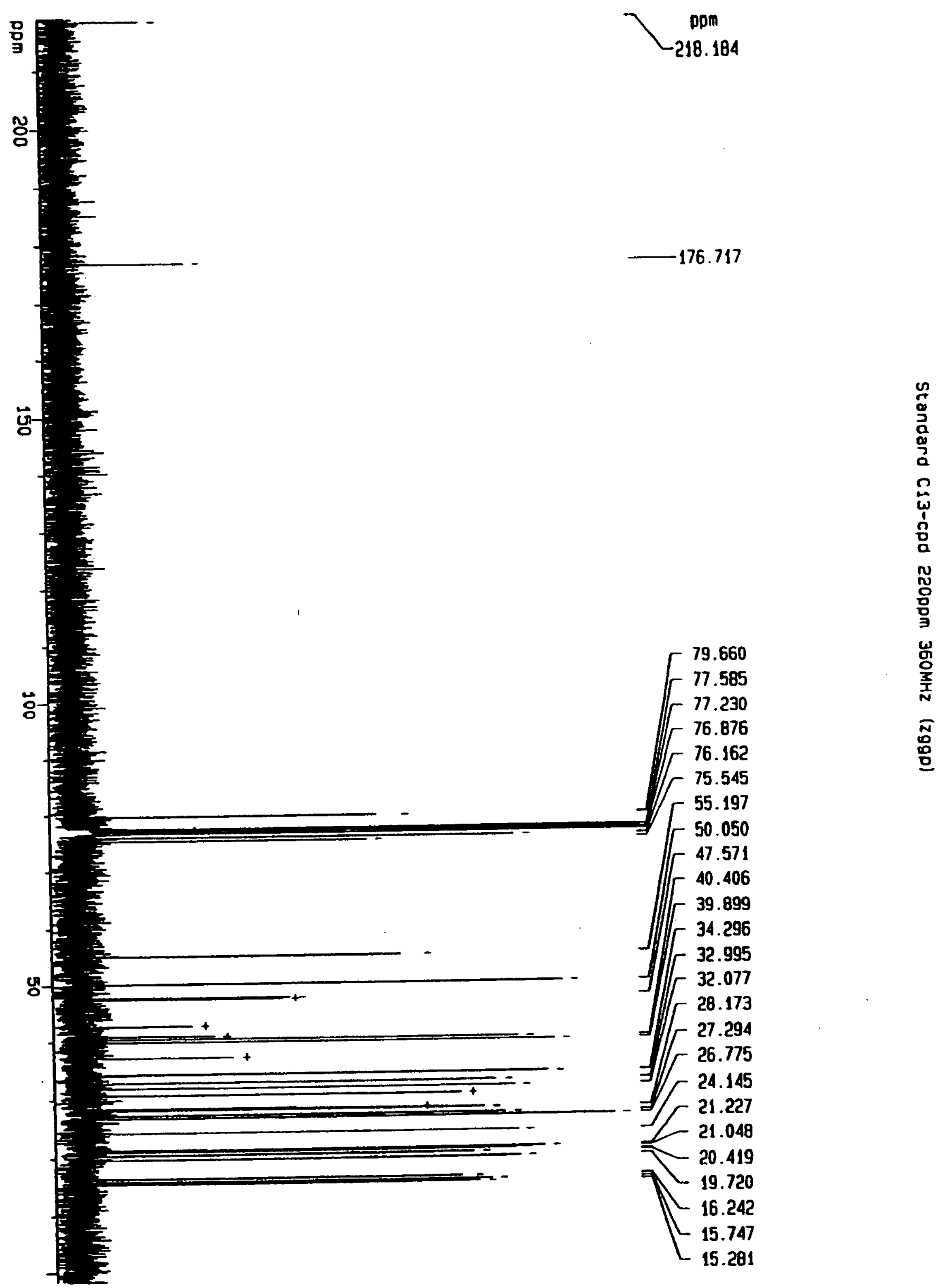


Figure S7. ^{13}C NMR spectrum of compound **4** in $CDCl_3$

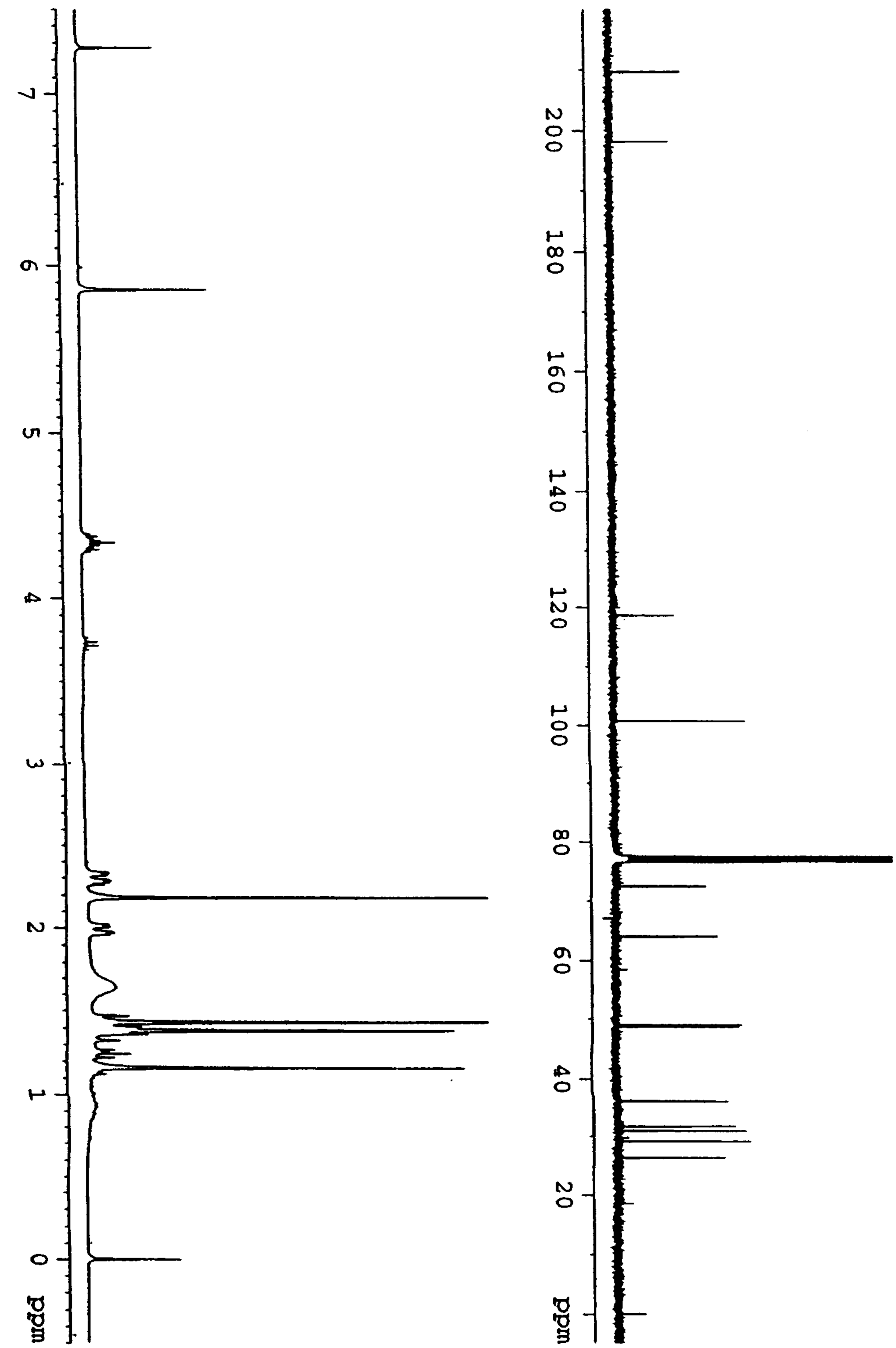


Figure S8. ^{1}H and ^{13}C NMR spectra of compound **7** in $CDCl_3$

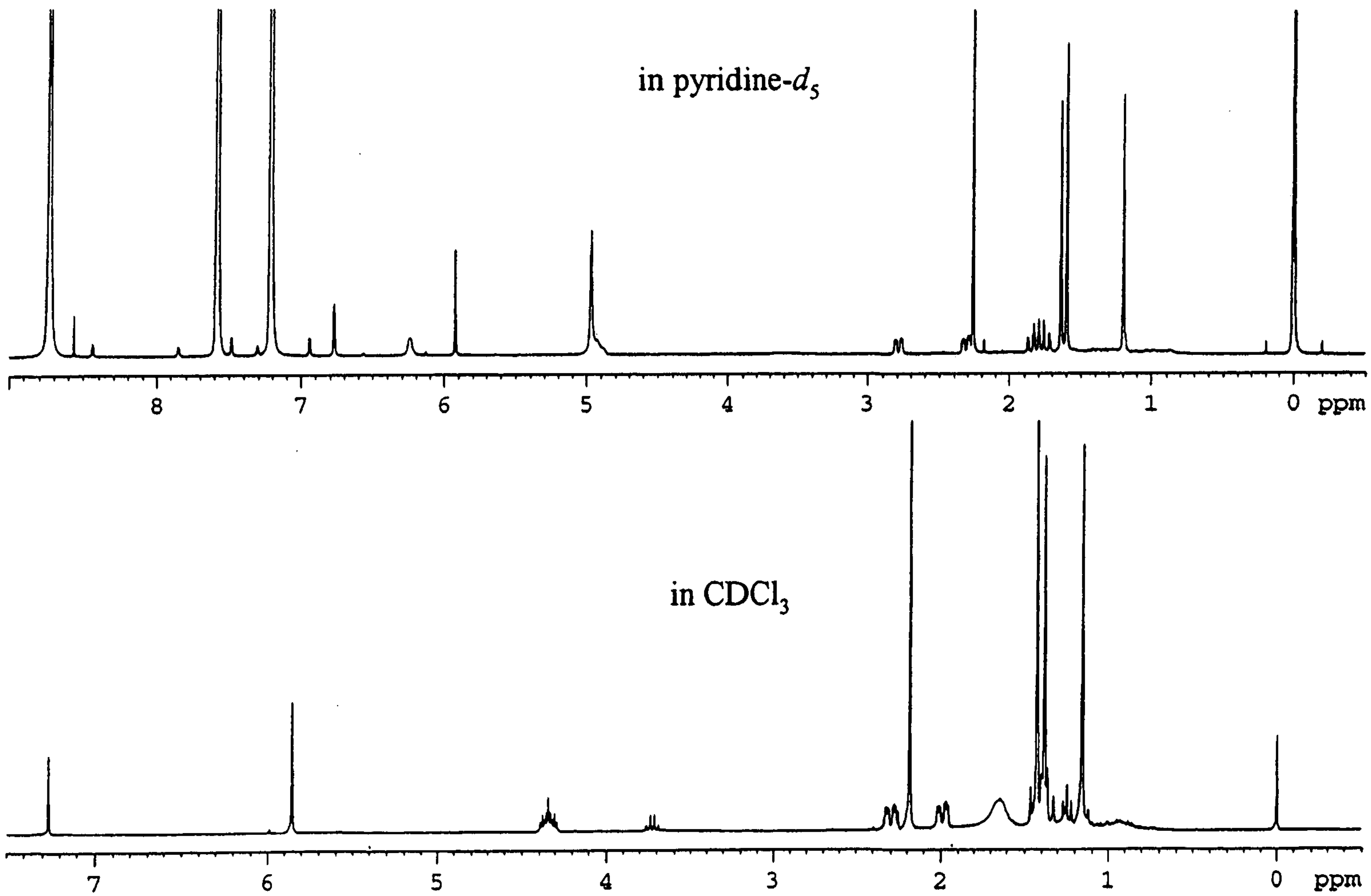


Figure S9. Comparison of the ^{1}H NMR spectra of compound 7 obtained in $CDCl_3$ and pyridine-d_5

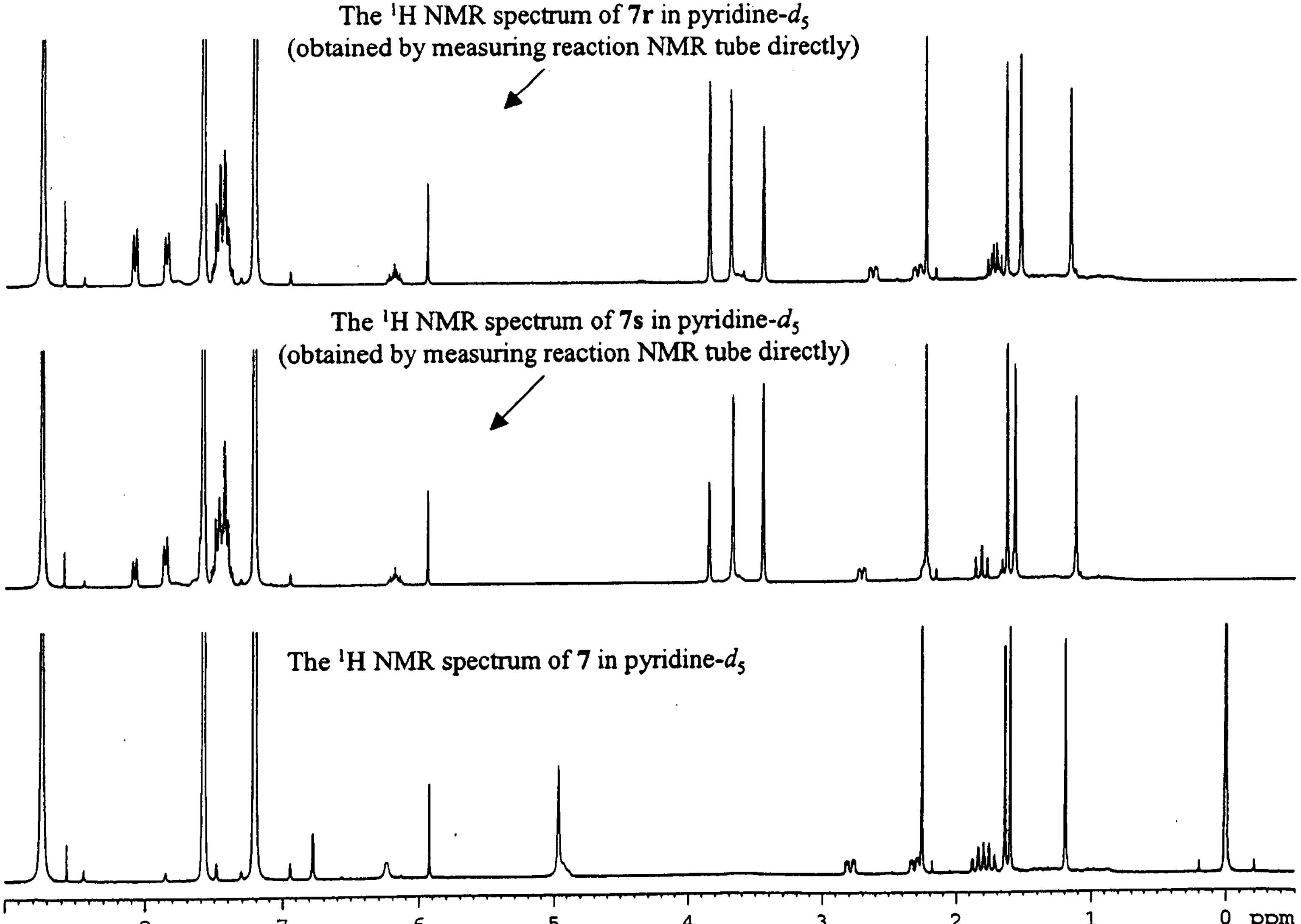


Figure S10. ^{1}H NMR spectra of **7r** and **7s** obtained by measurement in reaction tubes directly

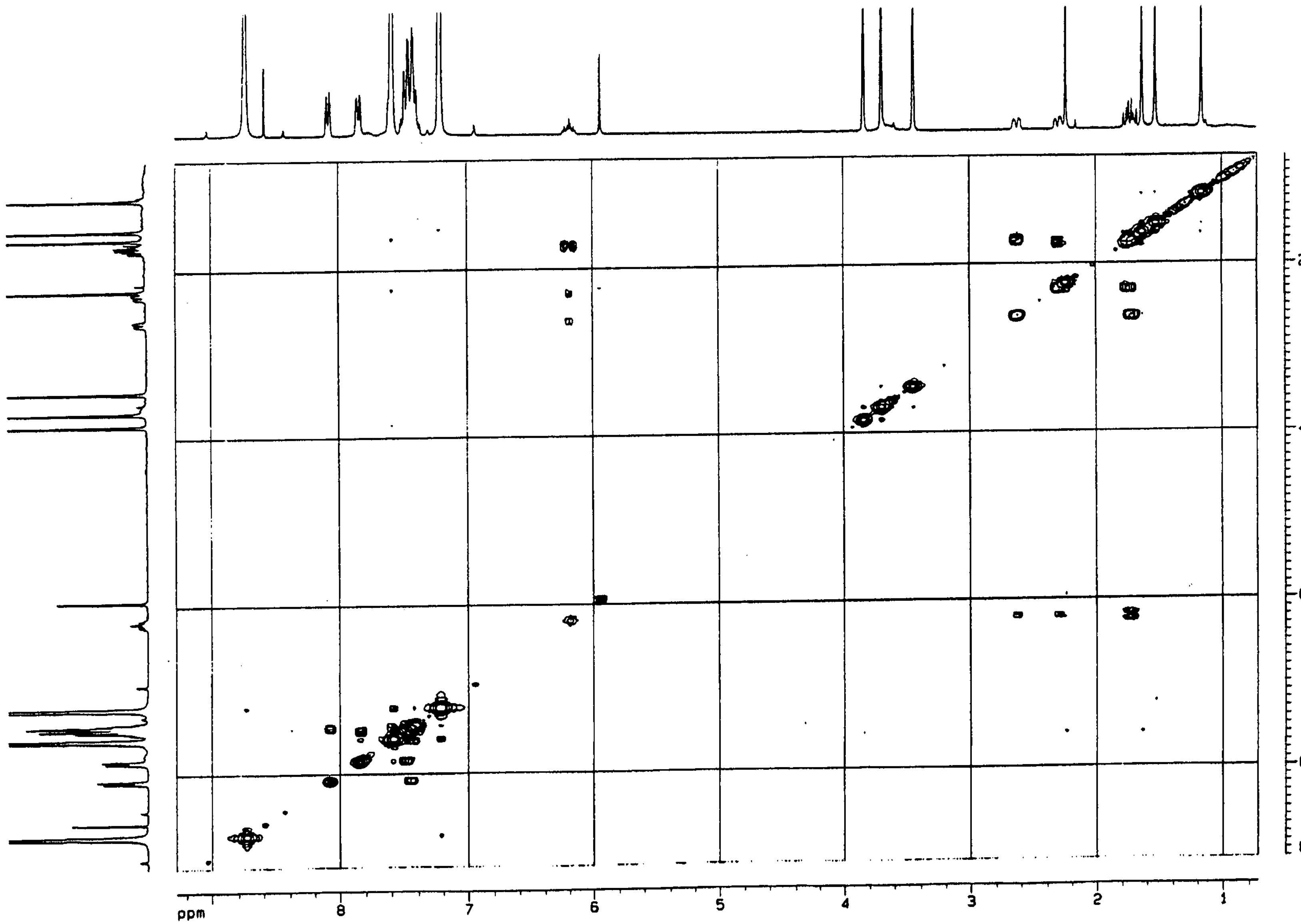


Figure S11. ^1H-^1H COSY spectrum of **7r** obtained by measurement in reaction tube directly

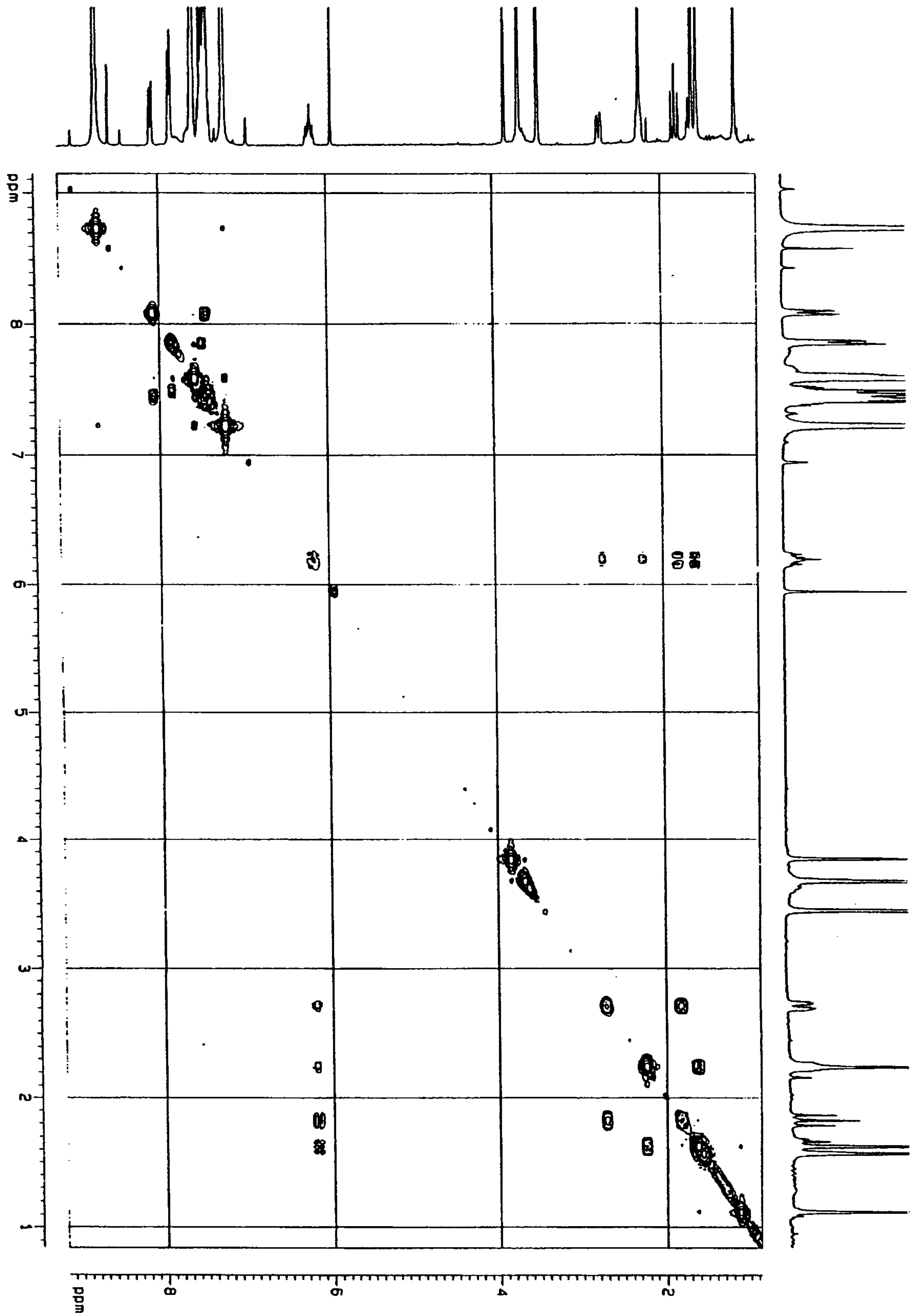


Figure S12. ^{1}H-^{1}H COSY spectrum of **7s** obtained by measurement in reaction tube directly

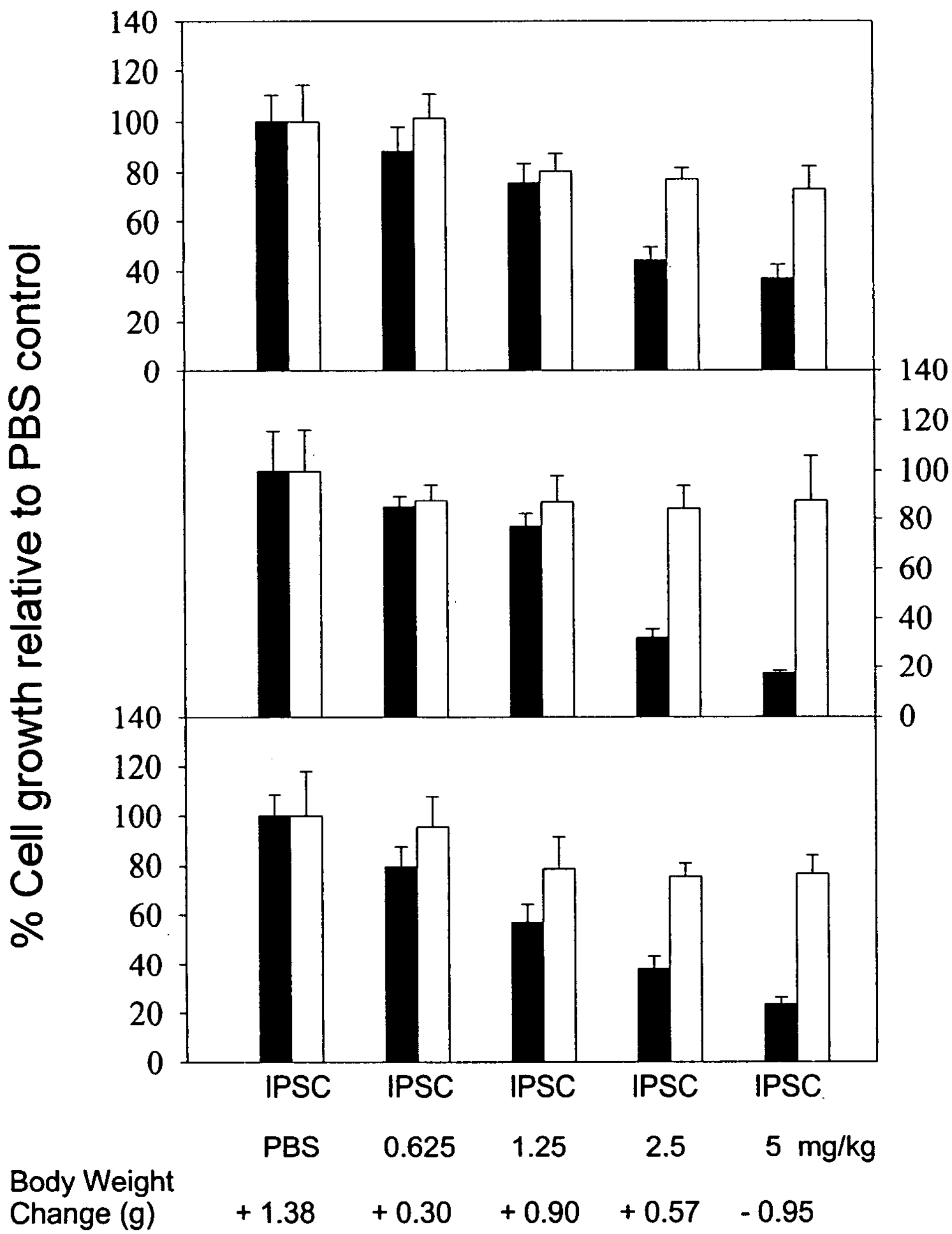


Figure S13. Effect of silvestrol (**1**) on the growth of KB, LNCaP, and Col2 cells implanted at the ip (solid column) and the sc (open column) compartments of NCr *nu/nu* mice in the *in vivo* hollow fiber assay.

Acetylation of Silvestrol (1a). Silvestrol (**1**) (7.0 mg) was acetylated with acetic anhydride (0.5 mL) and pyridine (0.5 mL) at room temperature overnight. The reaction product was purified by preparative TLC using *n*-hexane-EtOAc (1:1) to give silvestrol 5‴,6‴-diacetate (**1a**, 6.0 mg). **1a**: mp 106-109 °C; $[\alpha]^{20}_{D}$ −155.0° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 228 (4.32), 273 (3.45) nm; IR (film) ν_{max} 3493, 1744, 1611, 1514, 1453, 1372, 1221, 1183, 1063, 1032, 755 cm^{-1}; FABMS *m/z* 761 $[M + Na]^+$ (50), 329 (30), 176 (100), 154 (26), 136 (25), 92 (18); HRFABMS *m/z* 761.2416 $[M + Na]^+$ (calcd for $C_{38}H_{42}O_{15}Na$, 761.2421).

Benzoylation of Silvestrol (1b). Compound **1** (10.0 mg) was treated with benzoic anhydride (20 mg) and DMAP (16 mg) in pyridine (1 mL) at room temperature for 48 h. The reaction product was purified by preparative TLC using *n*-hexane-EtOAc (1:1) to give silvestrol 5‴,6‴-dibenzoate (**1b**, 7.0 mg). **1b**: mp 120-124 °C; $[\alpha]^{20}_{D}$ −87.0° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 232 (4.41), 273 (3.55) nm; IR (film) ν_{max} 3497, 1724, 1612, 1508, 1457, 1257, 1128, 1064, 1019, 757 cm^{-1}; FABMS *m/z* 885 $[M + Na]^+$ (100), 743 (10), 670 (15), 479 (10), 385 (10), 329 (95), 176 (100), 154 (95), 136 (93), 105 (98); HRFABMS *m/z* 885.2728 $[M + Na]^+$ (calcd for $C_{48}H_{46}O_{15}Na$, 885.2734).

***p*-Bromobenzoylation of Silvestrol (1c-1e).** Silvestrol (**1**, 10.0 mg) was treated with *p*-bromobenzoyl chloride (15 mg), 4-(dimethylamino)pyridine (DMAP, 10 mg), and triethylamine (80 μL) in CH_2Cl_2 (1.0 mL) at room temperature for 48 h. The reaction product was purified by preparative TLC using *n*-hexane-EtOAc (1:1) to give silvestrol 5‴,6‴-di-*p*-bromobenzoate (**1c**, 7.0 mg), 1,5‴,6‴-tri-*p*-bromobenzoate (**1d**, 3.0 mg), and 1,5‴,6‴,8b-tetra-*p*-bromobenzoate (**1e**, 1.2 mg). **1c**: mp 215-217 °C; $[\alpha]^{20}_{D}$ −15.0° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.43), 235 (4.42), 274 (3.52) nm; IR (film) ν_{max} 3499, 1726, 1599, 1507, 1455, 1392, 1262, 1128, 1065, 1020, 756 cm^{-1}; FABMS *m/z* 1041 $[M + Na]^+$ (2), 545 (2), 436 (3), 326 (12), 283 (20), 176 (100), 133 (98), 92 (25); HRFABMS *m/z* 1041.0959 $[M + Na]^+$ (calcd for $C_{48}H_{44}O_{15}{}^{79}Br_2Na$, 1041.0945). **1d**: mp 137-141 °C; $[\alpha]^{20}_{D}$ −35.0° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 215 (4.50), 242 (4.55), 275 (3.64) nm; IR (film) ν_{max} 3476, 1728, 1597, 1508, 1455, 1268, 1174, 1127, 1018, 756 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; ESIMS *m/z* 1227 $[M + Na]^+$; HRTOFMS *m/z* 1227.0295 $[M + Na]^+$ (calcd for $C_{55}H_{47}O_{16}{}^{79}Br^{81}Br_2Na$, 1227.0271).

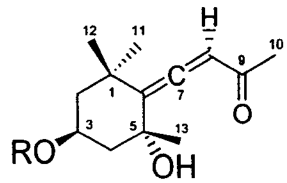
1e: mp 292-296 °C; $[\alpha]^{20}_{D}$ −47.0° (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 216 (4.48), 236 (4.47), 276 (3.77) nm; IR (film) ν_{max} 3002, 1727, 1625, 1510, 1459, 1265, 1128, 1020, 756 cm^{-1}; ESIMS *m/z* 1408 $[M + Na]^+$, 1424 $[M + K]^+$; HRTOFMS *m/z* 1408.9674 $[M + Na]^+$ (calcd for $C_{62}H_{50}O_{17}{}^{79}Br_2{}^{81}Br_2Na$, 1408.9638).

Hydrolysis of Silvestrol. Compound **1** (6.0 mg) was dissolved in 2 mL MeOH in a 25 mL round-bottomed flask, and aqueous LiOH (1 N, 1 mL) was added to the flask. The mixture was stirred for 8 h at 50 °C, and then the pH value was adjusted to 7 using 1 N HCl. After evaporating the solvent under reduced pressure, the crude product was passed through a small silica gel column (0.5×8 cm) with $CHCl_3$-MeOH (7:1) as eluent, and yielded a hydrolysis product of silvestrol, silvestric acid (**1f**, 5.3 mg). **1f**: $[\alpha]^{20}_{D}$ −133.3° (*c* 0.42, $CHCl_3$); ESIMS *m/z* 663 $[M + Na]^+$ (100), 647 $[M + Li]^+$ (15), 619 (15), 591 (5), 535 (5), 437 (7), 381 (12), 353 (5); HRESIMS *m/z* 663.2081 $[M + Na]^+$ (calcd for $C_{33}H_{36}O_{13}Na$, 663.2054).

Data for 17,24-Epoxy-25-hydroxy-3-oxobaccharan-21-oic Acid Methyl Ester (4a): Compound **4** (5 mg) was treated with excess fresh CH_2N_2 (in ethyl ether solution) at room temperature overnight, to give its methyl ester (**4a**): colorless oil; $[\alpha]^{20}_{D}$ +28.5° (*c* 0.10, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 212 (3.21) nm; IR (film) ν_{max} 3472, 1718, 1698, 1125, 1047 cm^{-1}; ^1H and ^{13}C NMR data, see Table 3; FABMS *m/z* 525 $[M + Na]^+$.

X-ray Crystallography of 1c. A colorless crystal, 0.10×0.20×0.23 mm, obtained from a $CHCl_3$-MeOH (1:1) mixture, was selected for data collection. It was immersed in Fluorolube oil and cooled to 150 K to minimize crystal degradation and X-ray radiation damage. Cell parameters: a = 17.7406 (5) Å; b = 14.925 (5) Å; c = 17.8418 (7) Å; β = 111.3770 (10)°, space group $P2_1$, Z = 4, D_{calc} = 1.541 mg/cm^3, λ = 0.71073 Å, μ = 1. 915 mm^{-1}, F(000) = 2088, T = 150(2) K. Diffraction data were collected on a Enraf-Nonius Kappa CCD area detector equipped with a rotating anode X-ray generator and MoKα radiation. Data collection yielded 10486 reflections of which all were considered unique. Full-matrix least-squares refinement led to final R, R (all), and GOF values of 0.0468, 0.0644, and 1.044. Crystallographic data (excluding structure factors) for the structure of this compound have been deposited with the Cambridge Crystallographic Data Center as supplementary publication number 210938. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Rd., Cambridge CB2 1EZ, U.K. [fax +44 (0) 1223 336033 or e-mail deposit@ccdc.cam.ac.uk].

X-ray Crystallography of Compound 3. Crystal size, 0.15×0.40×0.45 mm, obtained from $CHCl_3$-MeOH (4:1). It was immersed in fluorolube oil and cooled to 150 K to minimize crystal degradation and X-ray radiation damage. Cell parameters: a = 8.1676 (4) Å; b = 10.8642 (5) Å; c = 15.1032 (8) Å; V = 47341324.82 (11) Å^3, space group $P2_1$, Z = 2, D_{calc} = 1.150 g/cm^3, λ = 0.71073 Å, μ = 0.072 mm^{-1}, F(000) = 508, T = 150(2) K. Data collection yielded 5392 reflections of which all were considered unique. Full-matrix least-squares refinement led to a final R, R (all), and GOF values of 0.0563, 0.0657, and 1.168. Crystallographic data (excluding structure factors) for the structure of this compound have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication number CCDC 210937. Copies of the data can be obtained as mentioned for compound **1c**.



7 R = H
7r R = (*R*)-MTPA
7s R = (*S*)-MTPA

Data for Grasshopper Ketone (7): colorless oil; $[\alpha]^{20}_{D}$ –36.4° (*c* 0.55, $CHCl_3$); ^{1}H NMR data ($CDCl_3$, 300 MHz, TMS) δ 5.85 (1H, s, H-8), 4.34 (1H, m, H-3), 2.29 (1H, ddd, *J* = 12.9, 4.0, 2.1 Hz, H-4a), 2.18 (3H, s, CH_3-10), 1.99 (1H, ddd, *J* = 12.7, 4.2, 2.1 Hz, H-2a), 1.43 (3H, s, CH_3-13), 1.38 (3H, s, CH_3-11), 1.38-1.47 (1H, m, H-4b), 1.32-1.37 (1H, m, H-2b), 1.16 (3H, s, CH_3-12); ^{13}C NMR data ($CDCl_3$, 75 MHz, TMS) δ 209.6 (s, C-7), 198.3 (s, C-9), 118.7 (s, C-6), 100.8 (d, C-8), 72.3 (s, C-5), 63.9 (d, C-3), 49.0 (t, C-2), 48.7 (t, C-4), 36.1 (s, C-1), 31.7 (q, CH_3-12), 30.9 (q, CH_3-13), 29.1 (q, CH_3-11), 26.4 (q, CH_3-10); ESIMS *m/z* 247 $[M+Na]^+$ (100), 207 (8), 189 (14), 171 (5), 151 (5); EIMS *m/z* 224 $[M]^+$ (18), 209 (50), 191 (15), 177 (16), 163 (65), 149 (30), 133 (45), 123 (100), 89 (75); HRESIMS *m/z* 247.1320 $[M+Na]^+$ (calcd for $C_{13}H_{20}O_3Na$, 247.1310).

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of 7 by a Convenient Mosher Ester Procedure. Two portions (each 1.0 mg) of **7** were treated with (*S*)-(+)-α- and (*R*)-(-)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (3 μL) in deuterated pyridine (0.5 mL) directly in separate NMR tubes at room temperature, which afforded the (*R*)- and (*S*)-MTPA ester derivatives (**7r** and **7s**, respectively) of **7**.[25] The ^{1}H NMR spectra of **7r** and **7s** were obtained by measuring the reaction NMR tubes without prior purification. **7r**: ^{1}H NMR data (pyridine-d_5, 300 MHz) δ 6.192 (1H, m, H-3), 5.940 (1H, s, H-8), 2.624 (1H, ddd, *J* = 12.5, 4.0, 2.1 Hz, H-4a), 2.300 (1H, ddd, *J* = 12.8, 4.1, 2.0 Hz, H-2a), 2.237 (3H, s, CH_3-10), 1.673-1.777 (2H, m, H-2b, H-4b), 1.629 (3H, s, CH_3-11), 1.525 (3H, s, CH_3-13), 1.159 (3H, s, CH_3-12); ESIMS *m/z* 463 $[M+Na]^+$ (100), 447 $[M+Li]^+$ (14). **7s**: ^{1}H NMR data (pyridine-d_5, 300 MHz) δ 6.185 (1H, m, H-3), 5.936 (1H, s, H-8), 2.713 (1H, ddd, *J* = 12.4, 4.0, 2.1 Hz, H-4a), 2.235 (4H, m, H-2a and CH_3-10), 1.820 (1H, t *J* = 11.9 Hz, H-4b), 1.621 (3H, s, CH_3-13), 1.565 (3H, s, CH_3-11), 1.119 (3H, s, CH_3-12); ESIMS *m/z* 463 $[M+Na]^+$ (100), 447 $[M+Li]^+$ (15).

General Procedures. Melting points are uncorrected. NMR spectral data were recorded at room temperature on a 300 or 500 MHz spectrometer with tetramethylsilane (TMS) as internal standard. Standard pulse sequences were employed to obtain ^{1}H-^{1}H COSY, HMQS, HMBC, and NOESY spectra. The SIR-92 direct methods package (Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A. *J. Appl. Cryst.* **1993**, *26*, 343-350) was used to locate the non-hydrogen atoms, and the WinGX package (Farrugia, L. J. *J. Appl. Cryst.* **1999**, *32*, 837-838) was used for completing the structure determination, with PLUTON (Spek, A. L. *Acta Crystallogr.* **1990**, *A46*, C34) and ORTEP (Johnson, C. K. Report ORNL-3794, Oak Ridge National Laboratory, Oak Ridge, TN, 1965) used in Figures 3 and 4, respectively. Column chromatography was carried out with Si gel G (230-400 mesh). Analytical thin-layer chromatography (TLC) was performed on precoated 250 μm thick Merck Si gel 60 F_{254} aluminum plates, while preparative thin-layer chromatography was performed on precoated 500 or 1000 μm thick Si gel 60 F_{254} glass plates.

COMPOUND CHARACTERIZATION CHECKLIST

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Corresponding Author: A. Douglas Kinghorn

Title: Silvestrol and Episilvestrol, Novel Rocaglate Derivatives from Aglaia silvestris with Potential Anticancer Activity

TO DISPLAY INSTRUCTIONS: Double-click HERE (Formula Bar must be visible: Select View>Formula Bar) TO CLOSE: Enter/Return

Compound or structure number (with substituent/isomer identifier if needed)	IDENTITY: Known compound [citation given]	IR	UV-Vis	1H NMR	13C NMR	Other NMR	MS	High resolution MS	X-ray [ORTEP and CIF in SI*]	Optical rotation/ORD/CD	er/dr [NMR or chromatography]		PURITY: Copy of 1H/13C NMR in SI*	Quant. GC, HPLC, or electrophoresis	Elemental analysis				COMPUTATIONAL DATA in SI*: Cartesian coordinates or Z-matrix	# of imaginary frequencies	Total energy
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Compound 1a		y	y	y	y	2D	y	y	n	y			y	n	n				n	n	n
Compound 1b		y	y	y	y	2D	y	y	n	y			n	n	n				n	n	n
Compound 1c		y	y	y	y	2D	y	y	ORTEP	y			n	n	n				n	n	n
Compound 1d		y	y	y	y	2D	y	y	n	y			n	n	n				n	n	n
Compound 1e		y	y	y	y	2D	y	y	n	y			n	n	n				n	n	n

* SI = Supporting Information

Compound or structure number (with substituent/ isomer identifier if needed)	IDENTITY												PURITY						COMPUTATIONAL DATA in SI*		
	Known compound [citation given]	IR	UV-Vis	1H NMR	13C NMR	Other NMR	MS	High resolution MS	X-ray [ORTEP and CIF in SI*]	Optical rotation/ORD/CD	er/dr [NMR or chromatography]		Copy of 1H/13C NMR in SI*	Quant. GC, HPLC, or electrophoresis	Elemental analysis				Cartesian coordinates or Z-matrix	# of imaginary frequencies	Total energy
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Compound 2		y	y	y	y	2D	y	y	n	y			y	n	n				n	n	n
Compound 3		y	y	y	y	2D	y	y	OR TE P	y			n	n	n				n	n	n
Compound 4		y	y	y	y	2D	y	y	n	y			y	n	n				n	n	n
Compound 4a		y	y	y	y	2D	y	y	n	y			n	n	n				n	n	n
Compound 7		y	y	y	y	2D	y	y	n	y			y	n	n				n	n	n
Compound 7r		n	n	y	n	2D	y	n	n	n			y	n	n				n	n	n

* SI = Supporting Information

Compound or structure number (with substituent/ isomer identifier if needed)	IDENTITY: Known compound [citation given]	IR	UV-Vis	1H NMR	13C NMR	Other NMR	MS	High resolution MS	X-ray [ORTEP and CIF in SI*]	Optical rotation/ORD/CD	er/dr [NMR or chromatography]		PURITY: Copy of 1H/13C NMR in SI*	Quant. GC, HPLC, or electrophoresis	Elemental analysis				COMPUTATIONAL DATA in SI*: Cartesian coordinates or Z-matrix	# of imaginary frequencies	Total energy
Compound 7s		n	n	y	n	2D	y	n	n	n			y	n	n				n	n	n

* SI = Supporting Information