Discovery and Total Synthesis of a New Estrogen Receptor Heterodimerizing Actinopolymorphol A from *Actinopolymorpha rutilus*

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Supporting Information

Table of Contents

General experimental procedures. Optical rotations were measured in CH_3OH on a Perkin-Elmer 241 polarimeter at the sodium D line (589 nm). ^1H and ^{13}C NMR spectra were recorded at 25°C on a Varian Unity Inova 500 instrument operating at 500 MHz for ^1H and 125 MHz for ^{13}C nuclei. ^1H and ^{13}C NMR chemical shifts were referenced to residual solvent signals. ^1H-^1H COSY, HMQC (J_{CH}=140 Hz), and gHMBC ($^nJ_{XH}$ =8.0 Hz) were performed using standard VARIAN pulse sequences. Electrospray ionization-mass (ESI-MS) spectra were acquired on an IonSpec HiResMALDI FT–Mass spectrometer with a 7 tesla superconducting magnet. Semipreparative HPLC was performed on a Varian liquid chromatograph system with an Atima-C18, 10 mm ×25 cm column. Silica gel chromatography applied to purification of specified synthetic compounds used 230-400 mesh size silica from Natland Int. Corp. Other chromatographic matrices included Lichroprep RP-18 gel (40–63 μm, Merck, Dramstadt, Germany) or Sephadex LH-20 (pharmacia) where indicated. Reactions were routinely run in flame-dried roundbottom flasks and under an atmosphere of Ar. All chemical reagents, unless otherwise noted, were obtained from Sigma-Aldrich and used as obtained directly from the manufacturer.

Fermentation and isolation. The strain *Actinopolymorpha rutilus* was obtained from Yunnan Institute of Microbiology, Yunnan University, Kunming, 650091, P. R. China. The inoculum was prepared by introducing the periphery of 10-day-old petri dish cultures of *A. rutilus* into 250 ml flasks containing 50 mL of the ISP2 broth, followed by shaking (250 rpm) continuously for 5 days at 28 ± 0.5°C. The follow-up fermentation was accomplished by adding the inoculum (50 mL) into 2-L flasks containing 500 mL of the culture broth [Sucrose (100 g), glucose (10.0 g), casamino acids (0.1 g), yeast extract (5.0 g), MOPS (21.0 g), trace elements (1 mL), K_2SO_4 (0.25g), $MgCl_2$ -6H_2O (10.0 g), in a final volume of 1.0-L H_2O, pH 7.0], and then shaking for 7 days in the same condition. Solid phase extraction of the broth using resin (HP-20), filtration through cheesecloth, and elution of the resin with acetone afforded, after solvent removal under vacuum, a gummy extract was obtained. The extract (8.5 g) was chromatographed on silica gel column using $CHCl_3$–MeOH (100:0, 50:1, 20:1, 10:1, 5:1, 1:1 and 0:100, 1.50 L each) as the mobile phase to get seven fractions A-G. Fractions B and C (50:1 and 20:1, total 1.1g) were further chromatographed over RP-18 column eluted with a gradient (10% methanol, 30% methanol, 60% methanol, 90% methanol, 100% methanol) to yield five subfractions B1-B5. Subfractions B3 and B4 were combined and then subjected to Sephadex LH-20 column and eluted with methanol to yield three subfractions. These three subfractions were finally purified by semi-preparative HPLC to afford compounds **1** (1.5 mg), **2** (0.8 mg), and **3** (0.7 mg).

Physico-chemical properties of 1, 2, and 3

Actinopolymorphol A (1): Colorless oil; $[\alpha]_D^{23}$ +20.9 (*c* 0.15, CH_3OH) for natural product; $[\alpha]_D^{23}$ +23.5 (*c* 0.28, CH_3OH) for synthesized; HR-ESIMS (positive ion) *m/z* 287.12567 (calcd for $C_{15}H_{20}O_4Na$ $[M+Na]^+$, 287.12538); ^1H NMR and ^{13}C NMR, see Table S1.

Actinopolymorphol B (2): Colorless oil; $[\alpha]_D^{23}$ +7.8 (*c* 0.10, CH_3OH); HR-ESIMS (positive ion) *m/z* 226.08290 (calcd for $C_{12}H_{13}NO_2Na$ $[M+Na]^+$, 226.08380); ^1H NMR and ^{13}C NMR, see Table S1.

Actinopolymorphol C (3): Colorless oil; HR-ESIMS (positive ion) *m/z* 315.11250 (calcd for $C_{18}H_{16}$ N_2O_2Na $[M+Na]^+$, 315.11039); ^1H NMR and ^{13}C NMR, see Table S1.

Synthesis of 1 (*S*)-2-hydroxy-3-(4-hydroxyphenyl)-propionic acid (4)

(*S*)-3-(4-Hydroxyphenyl)-2-hydroxypropionyl methyl ester (5). (*S*)-3-(4-Hydroxyphenyl)-2-hydroxypropionic acid (1 g, 5.5 mmol) from Astatech, Inc. (Bristol, PA) was dissolved in 120 mL ACS grade MeOH at rt. The methanolic solution was then subjected to a constant stream of HCl gas (generated via dropwise addition of H_2SO_4 to solid NaCl) for a period of 2 h at rt followed by solvent removal *in vacuo* at 50ºC. The resulting faint yellowish gum was then subjected to column chromatography over silica (2:1:0.4 Hex:EtOAc:MeOH) affording 1.08 g of methyl ester **5** as a thick transparent oil. All spectral data matched those previously reported.[1]

(*S*)-3-(4-*O*-tert-butyldimethylsiloxyphenyl)-2-tert-butyldimethylsiloxy propionyl methyl ester (6). (*S*)-3-(4-Hydroxyphenyl)-2-hydroxypropionyl methyl ester (**5**) (4 g, 20.4 mmol) was dissolved in 50 mL anhydrous DMF and the solution chilled for 10 min to 0ºC followed by addition of imidazole (4.16 g, 61.2 mmol). The solution was stirred at 0ºC for 10 min and then TBSCl (9.22 g, 61.2 mmol) was added and the resulting mixture stirred at 0ºC for 15 min. The reaction was then allowed to warm from 0ºC to rt over the course of 4 h, and TLC (2:1:0.4 Hex:EtOAc:MeOH) revealed complete loss of starting material and generation of one UV active fast mobility spot. The reaction was then chilled back to 0ºC and quenched by addition of cold dilute NH_4Cl (100 mL) and ~20 g NaCl. After 5 min the reaction was partitioned into an equal volume of EtOAc. Organic layer was separated and remaining aqueous phase extracted with EtOAc (3 x 50 mL). Organic layers were combined and washed with satd NH_4Cl (3 x 50) followed by washing with brine (2 x 100 mL). Organic layer was then dried over anhydrous Na_2SO_4 for 10 min. Solids were filtered off and solvent removed *in vacuo* to render 9.17 g crude **6**. Column chromatography over silica (2:1:0.4 Hex:EtOAc:MeOH) afforded 7.8 g pure **6** (90% yield). Colorless oil; HR-ESIMS (positive ion) *m/z* 447.23734 (calcd for $C_{22}H_{40}$ O_4Si_2Na $[M+Na]^+$, 447.23573); ^1H NMR in $CDCl_3$: δ 6.75 (d, *J* = 8.4 Hz), 7.07 (d, *J* = 8.4 Hz), 3.01 (dd, *J* = 3.6, 12.6 Hz), 2.80 (dd, *J* = 9.2, 12.6 Hz), 4.28 (dd, *J* = 3.6, 9.2 Hz), 3.72 (OMe, s), -0.20 (s), -0.13 (s), 0.17 (s), 0.80 (s), and 0.97 (s); ^{13}C NMR: δ_c 130.8 (s, C-1), 131.2 (d, C-2, 6), 120.5 (d, C-3, 5), 154.9 (s, C-4), 41.4 (t, C-7), 74.6 (d, C-8), 174.3 (s, C-9), 52.3 (q, OMe), 26.2, 26.1, 18.8, -3.9, -5.1.

(*S*)-3-(4-*O*-tert-butyldimethylsiloxyphenyl)-2-tert-butyldimethylsiloxy N-methoxy, N-methyl propionamide (7). To methyl ester **6** (390 mg, 0.92 mmol) was added 3 mL anhydrous THF in a flame dried roundbottom flask under Ar atmosphere. The solution was chilled to 0ºC for 10 min followed by the addition of *N*,*O*-dimethylhydroxylamine hydrochloride (140 mg, 1.44 mmol). The slurry was then chilled to -20ºC and 1.38 mL (2.8 mmol) of $(CH_3)_2CHMgCl$ (2M in THF) added dropwise. The mixture was stirred at -20ºC for 1 h followed by quenching via addition of satd NH_4Cl (10 mL) and partitioning into an equal volume of EtOAc. Aqueous layer was extracted with EtOAc (3 x 50 mL) and then organic fractions pooled and washed with saturated NH_4Cl (3 x 50 mL) and then brine (2 x 50 mL). Organic layer was dried by addition of anhydrous Na_2SO_4 for 10 min. Solids were then filtered off and solvent removed *in vacuo*. The resulting gum was subjected to column chromatography (10:1:1 Hex:EtOAc:MeOH) to afford 340 mg of **7** (81% yield). Colorless oil; HR-ESIMS (positive ion) *m/z* 476.26342 (calcd for $C_{23}H_{43}$ NO_4Si_2Na $[M+Na]^+$, 476.26228); ^1H NMR in $CDCl_3$: δ_H 6.74 (d, *J* = 8.4 Hz), 7.07 (d, *J* = 8.4 Hz), 2.95 (dd, *J* = 4.4, 13.2 Hz), 2.74 (dd, *J* = 8.8, 13.2 Hz), 4.61 (dd, *J* = 4.4, 8.8 Hz), 3.61 (OMe, s), 3.29 (NMe), -0.16 (s), -0.13 (s), 0.15 (s), 0.78 (s), and 0.97 (s); ^{13}C NMR: δ_c 131.2 (s, C-1), 131.0 (d, C-2, 6), 120.2 (d, C-3, 5), 154.6 (s, C-4), 32.8 (t, C-7), 72.1 (d, C-8), 174.3 (s, C-9), 61.4 (q, OMe), 40.7 (q, NMe), 26.0, 26.1, 18.6, -4.8.

***(S)*-8-(*O*-tert-butyldimethylsiloxy)-4-*O*-tert-butyldimethylsiloxysattabacin (8).** To Weinreb amide **7** (270 mg, 0.6 mmol) was added 4 mL anhydrous THF in a flame dried roundbottom flask under Ar atmosphere. The solution was chilled to 0ºC for 10 min followed by the addition of 0.7 mL 2M $(CH_3)_2CHCH_2MgCl$ in THF (1.4 mmol) at 0ºC. Reaction was stirred at 0ºC for 2 h and then allowed to warm to rt; reaction was stirred at rt for 24h. Following incubation at rt, the reaction was chilled back to 0ºC and then quenched via addition of cold 2N HCl (pH ~ 3) (15 mL) and partitioned into an equal volume of EtOAc. Aqueous layer was extracted with EtOAc (3 x 25 mL) and then organic fractions pooled and washed with saturated NH_4Cl (3 x 50 mL) and then brine (2 x 50 mL). Organic layer was dried by addition of anhydrous Na_2SO_4 for 10 min. Solids were then filtered off and solvent removed *in vacuo*. The resulting gum (310 mg crude) was subjected to column chromatography (15:1 Hex:EtOAc) to afford 180 mg of **8** (67% yield). Colorless oil; HR-ESIMS (positive ion) *m/z* 473.28975 (calcd for $C_{25}H_{46}$ O_3Si_2Na $[M+Na]^+$, 473.28777); ^1H NMR in $CDCl_3$: δ_H 6.76 (d, *J* = 8.4 Hz), 7.05 (d, *J* = 8.4 Hz), 2.84 (dd, *J* = 4.4, 13.6 Hz), 2.69 (dd, *J* = 8.8, 13.6 Hz), 4.10 (dd, *J* = 4.4, 8.8 Hz), 2.31-2.45 (m), 2,14 (m), 0.90 (d, *J* = 6.8 Hz), 0.88 (d, *J* = 6.8 Hz), -0.30 (s), -0.11 (s), 0.17 (s), 0.85 (s), 0.89 (s); ^{13}C NMR: δ_c 130.4 (s, C-1), 131.3 (d, C-2, 6), 120.3 (d, C-3, 5), 154.8 (s, C-4), 40.9 (t, C-7), 80.8 (d, C-8), 213.1 (s, C-9), 47.0 (t, C-10), 23.7 (d, C-11), 23.1 (q, C-12), 23.1 (q, C-13), 26.2, 26.1, 18.6, -5.1.

***(S)*-4-hydroxysattabacin (9).** Diprotected ketone **8** (350 mg, 0.78 mmol) was dissolved in 8.5 mL anhydrous THF at 0ºC. After 10 min 2.3 mL of tetrabutylammonium fluoride (TBAF) (1M in THF) (2.33 mmol) was added dropwise at 0ºC. Reaction was stirred at 0ºC for 45 min at which time TLC analysis (15:1 Hex:EtOAc) revealed surprisingly little change to smarting material. The reaction was then allowed to warm to rt over the course of 1.5 h after which time it was apparent by TLC that starting material was consumed. The reaction was chilled back to 0ºC and quenched via addition of cold 2N HCl (pH ~3) (15 mL) and partitioned into an equal volume of EtOAc. Aqueous layer was extracted with EtOAc (3 x 25 mL) and then organic fractions pooled and washed with satd NH_4Cl (3 x 50 mL) and then brine (2 x 50 mL). Organic layer was dried by addition of anhydrous Na_2SO_4 for 10 min. Solids were then filtered off and solvent removed *in vacuo*. The resulting 150 mg of crude diol was subjected to column chromatography (3:1 Hex:EtOAc) to afford 140 mg of **9** (81% yield). Colorless oil; ^{1}H NMR in $CDCl_3$: δ_H 6.66 (d, J = 8.4 Hz), 7.03 (d, J = 8.4 Hz), 3.06 (dd, J = 4.4, 14.4 Hz), 2.74 (dd, J = 7.6, 14.4 Hz), 4.36 (m), 2.39 (2H, d, J = 7.2 Hz), 2.18 (m), 0.92 (d, J = 6.8 Hz), 0.92 (d, J = 6.8 Hz), 6.18 (OH), 3.67 (OH); ^{13}C NMR: δ_c 128.2 (s, C-1), 130.6 (d, C-2, 6), 115.7 (d, C-3, 5), 155.0 (s, C-4), 39.3 (t, C-7), 77.9 (d, C-8), 211.9 (s, C-9), 47.7 (t, C-10), 24.9 (d, C-11), 22.9 (q, C-12), 22.8 (q, C-13).

***(S)*-4-acetoxy-8-acetylsattabacin (10).** Diol **9** (110 mg, 0.5 mmol) was dissolved in 2 mL anhydrous CH_2Cl_2 and chilled to 0ºC for 10 min. To the chilled solution of **9** was added 500 mL of a premixed 1:1 combination of acetic anhydride and pyridine dropwise over a 5 min duration. The reaction was then allowed to warm to rt and to stir overnight. The reaction was quenched via addition of 3 mL MeOH and 3 mL EtOAc at 0ºC. The solvents were removed *in vacuo* with gentle heating (~45ºC) and the crude syrup subjected to immediate column chromatography (3:1 Hex:EtOAc) to afford 110 mg of diacetate **10** (72% yield). Colorless oil; $[\alpha]_D^{23}$ +25.1 (*c* 0.18, CH_3OH); HR-ESIMS (positive ion) *m/z* 329.13618 (calcd for $C_{17}H_{22}O_5Na$ $[M+Na]^+$, 329.13595); ^{1}H NMR in $CDCl_3$: δ_H 7.00 (d, J = 8.4 Hz), 7.19 (d, J = 8.4 Hz), 3.05 (dd, J = 4.4, 14.0 Hz), 2.93 (dd, J = 8.8, 14.0 Hz), 5.13 (dd, J = 4.4, 8.8), 2.32 (dd, J = 6.4, 17.2 Hz), 2.17 (dd, J = 6.8, 17.2 Hz), 2.11 (m), 0.85 (d, J = 6.5 Hz), 0.86 (d, J = 6.5 Hz), 2.25 (OAc), 2.05 (OAc); ^{13}C NMR: δ_c 133.9 (s, C-1), 130.6 (d, C-2, 6), 121.9 (d, C-3, 5), 149.5 (s, C-4), 36.2 (t, C-7), 79.1 (d, C-8), 206.8 (s, C-9), 48.4 (t, C-10), 24.0 (d, C-11), 22.8 (q, C-12), 22.8 (q, C-13), 170.6 (OAc), 169.7 (OAc), 21.4 (OAc), 20.9 (OAc).

Actinopolymorphol A (1). Diacetate **10** (70 mg, 0.23 mmol) was dissolved in 1 mL pyrrolidine at rt and stirred for 1 min. The reaction was then diluted with 2 mL cold EtOAc and transferred immediately to a rapidly stirring solution of 10 mL 1:1 2N HCl:satd NH_4Cl at 0ºC. The mixture was then partitioned into 25 mL EtOAc. The layers were separated and remaining aqueous layer was extracted with EtOAc (3 x 25 mL) and then organic fractions pooled and washed with satd NH_4Cl (3 x 50 mL) and then brine (2 x 50 mL). Organic layer was dried by addition of anhydrous Na_2SO_4 for 10 min. Solids were then filtered off and solvent removed *in vacuo* to afford 40 mg of monoacetate **1** in > 95 % purity based on ^{1}H-NMR (66% yield). Colorless oil; $[\alpha]_D^{23}$ +20.9 (*c* 0.15, CH_3OH); HR-ESIMS (positive ion) *m/z* 287.12567 (calcd for $C_{15}H_{20}O_4Na$ $[M+Na]^+$, 287.12538); ^{1}H NMR and ^{13}C NMR data see Table S1. See Figure 1B in manuscript for key HMBC and COSY correlations.

Table S1. Summary of NMR data for compounds **1**, **2**, and **3** in methanol-d_4.

	1		**2**		**3**	
No.	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)	^{13}C	^{1}H (*J* in Hz)
1	126.9				129.8	
1a			137.2			
2	130.3	7.02 (d, 8.4)	123.8	7.14 (s)	130.5	7.06 (d, 9.0)
3	115.1	6.68 (d, 8.4)	117.4		116.0	6.67 (d, 9.0)
4	156.4		118.6	7.60 (d, 9.0)	156.5	
4a			128.1			
5	115.1	6.68 (d, 8.4)	118.9	7.03 (dt, 0.8, 9.0)	116.0	6.67 (d, 9.0)
6	130.3	7.02 (d, 8.4)	121.5	7.11 (dt, 0.8, 9.0)	130.5	7.06 (d, 9.0)
7	35.7	2.98 (dd, 5.0, 14.4)	111.4	7.35 (d, 9.0)	40.2	3.96 (s)
8	79.6	5.08 (dd, 5.0, 8.2)	30.0	3.21 (dd, 5.5, 15.0)	154.7	
				3.09 (dd, 7.0, 15.0)		
9	207.8		77.9	4.39 (dd, 5.5, 7.0)	143.9	8.44 (s)
10	47.8	2.35 (dd, 6.6, 17.4)	212.7			
		2.16 (dd, 6.6, 17.4)				
11	43.7	2.03 (m)	25.5	2.14 (s)		
12	21.7	0.82 (d, 6.8)				
13	21.7	0.84 (d, 6.8)				
OAc	170.9					
OAc	19.3	2.04 (s)				

References:

Andrus, M. B.; Hicken, E. J.; Stephens, J.C.; Bedke, D. K. *J. Org. Chem.* **2006**, *71*, 8651-8654.

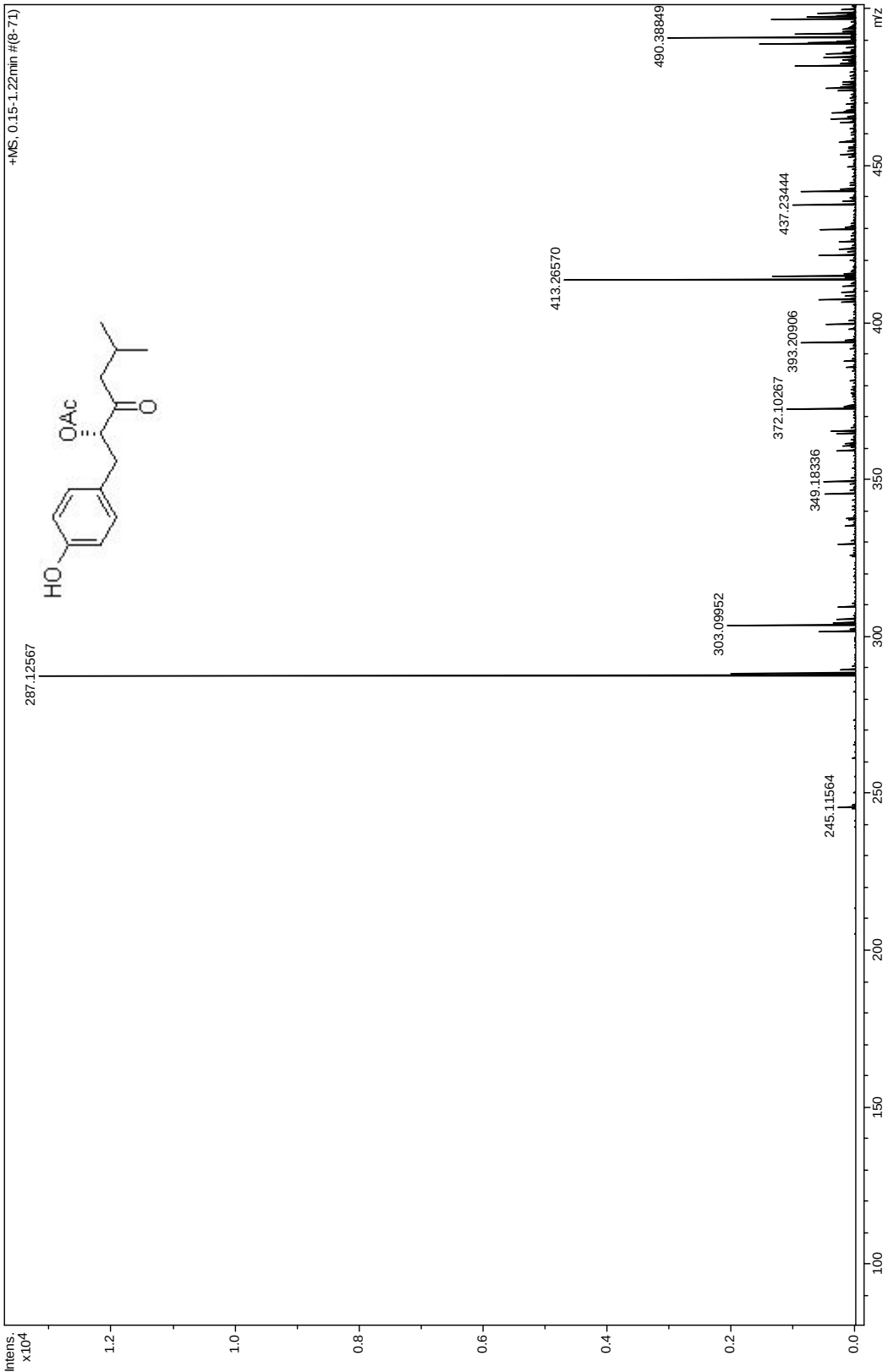

Figure S1. HRESIMS of **1**

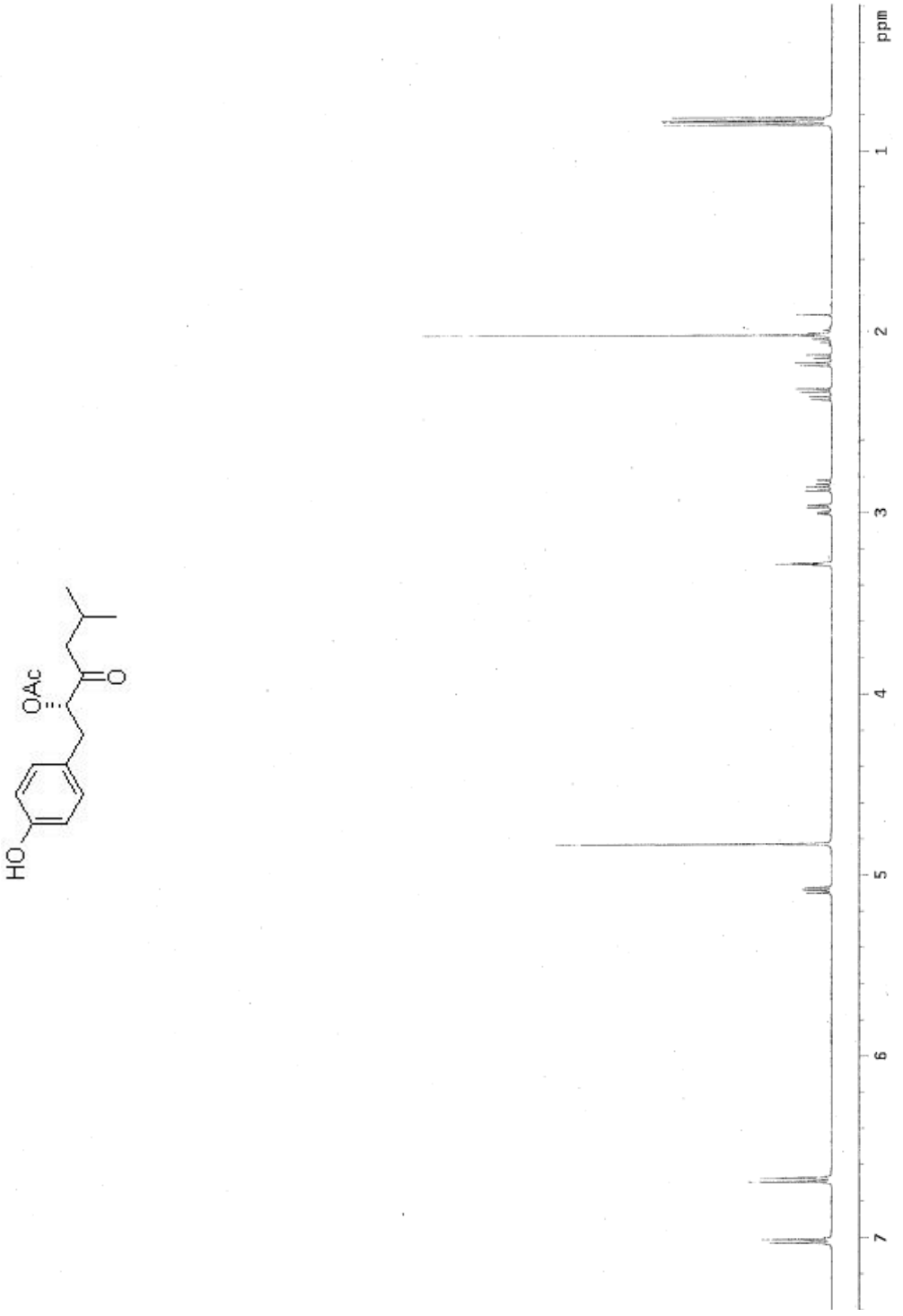

Figure S2. ^{1}H NMR spectrum of **1**

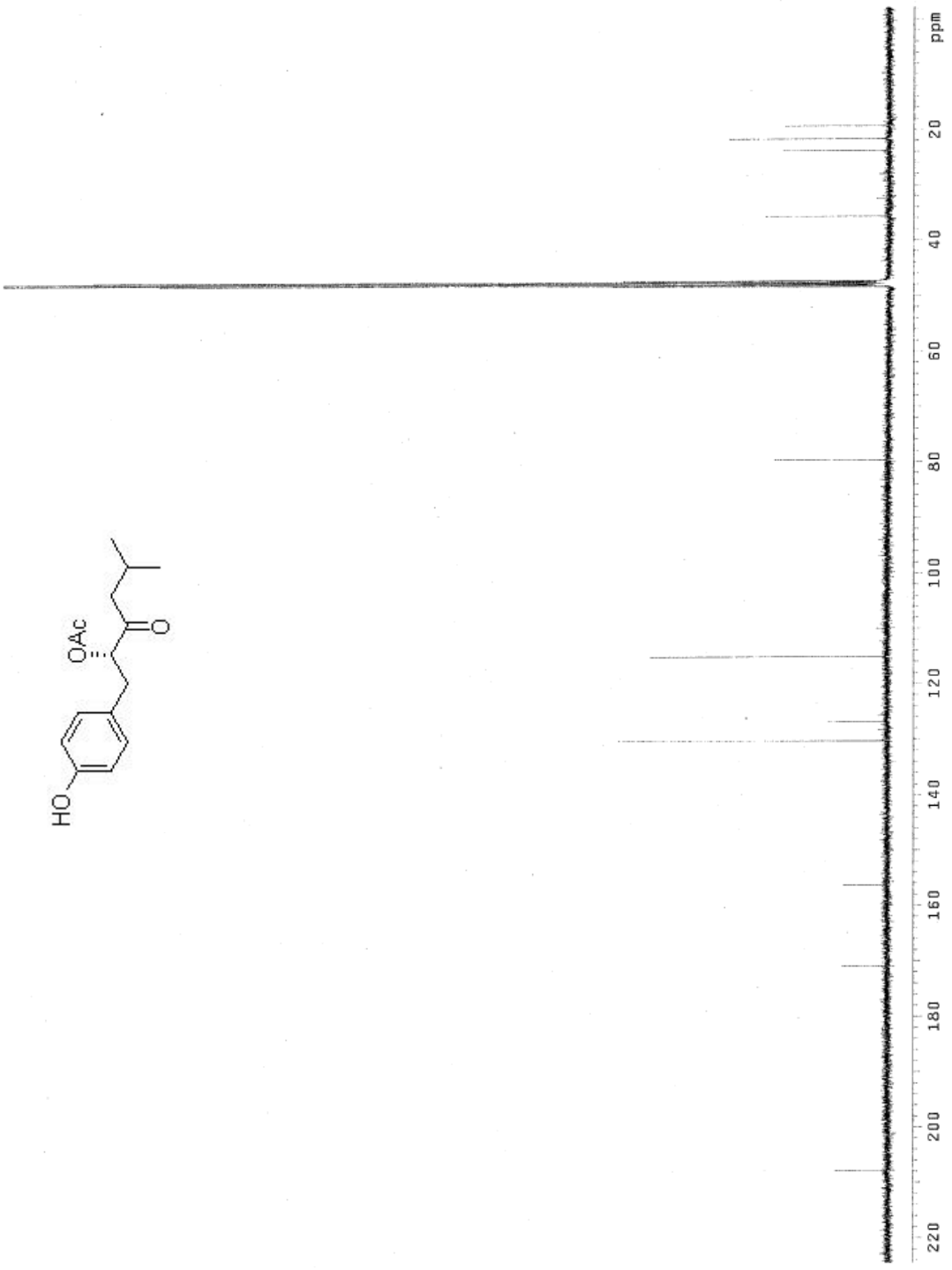

Figure S3. ^{13}C NMR spectrum of **1**

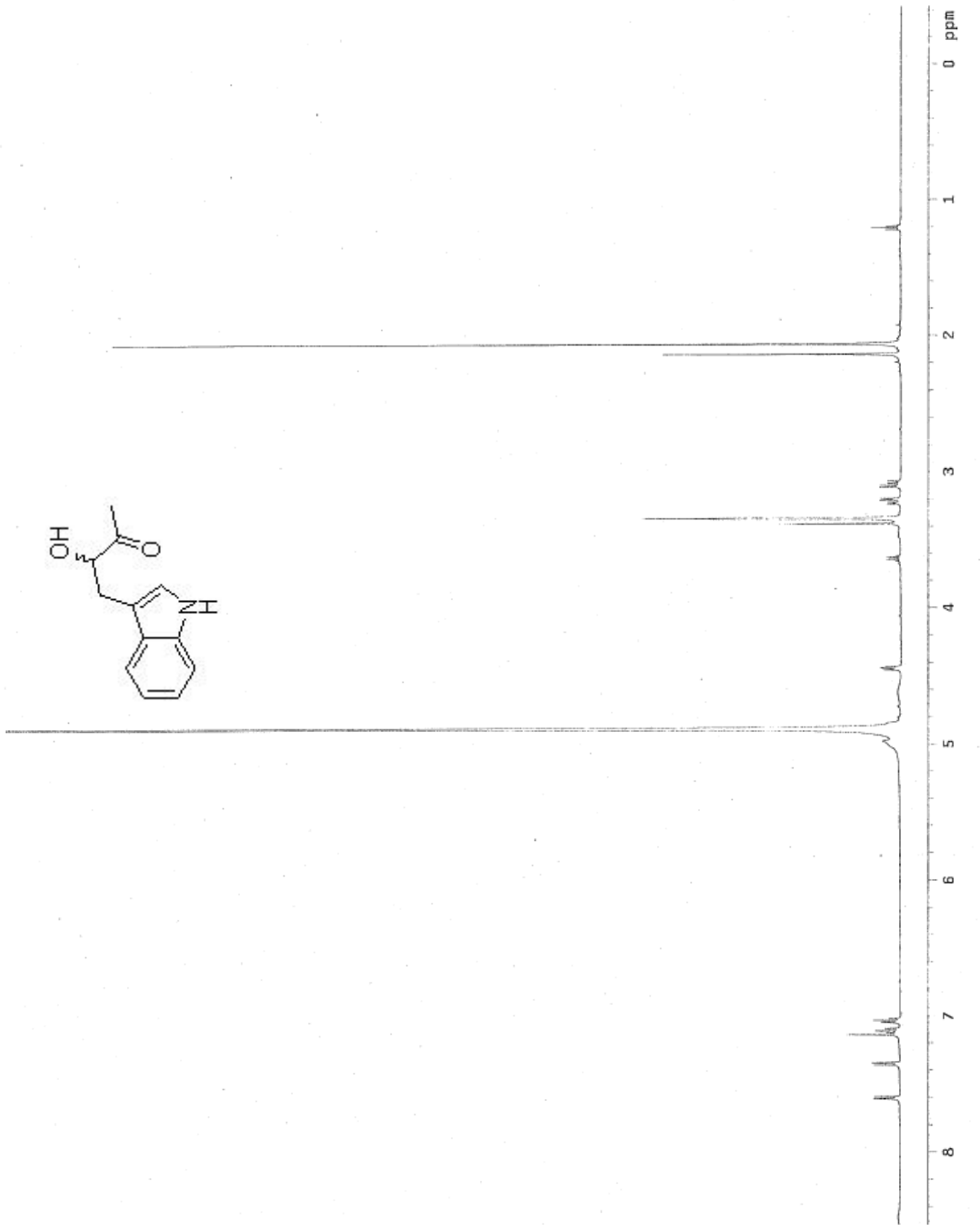

Figure S4. ^{1}H NMR spectrum of **2**

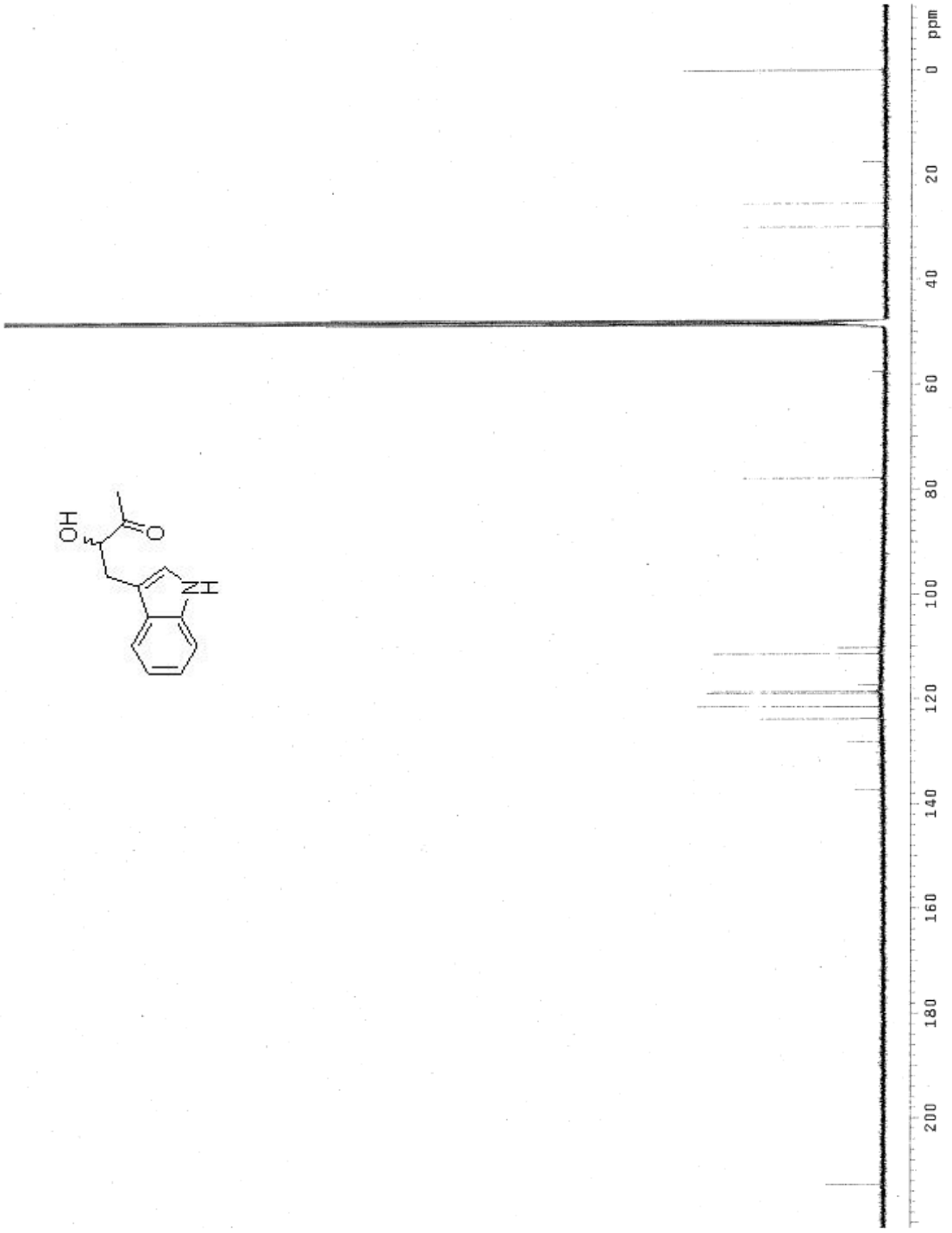

Figure S5. ^{13}C NMR spectrum of **2**

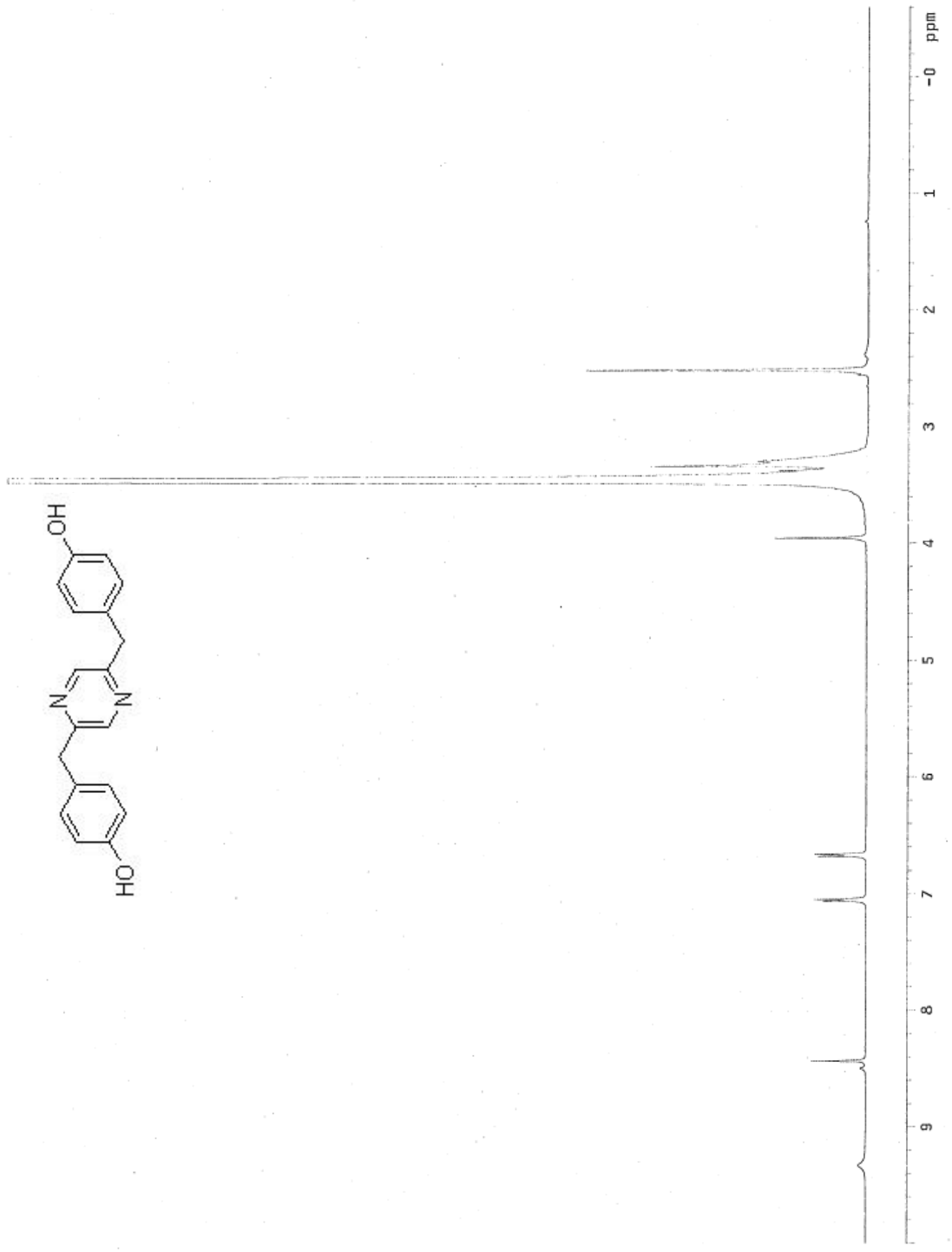

Figure S6. ^{1}H NMR spectrum of **3**

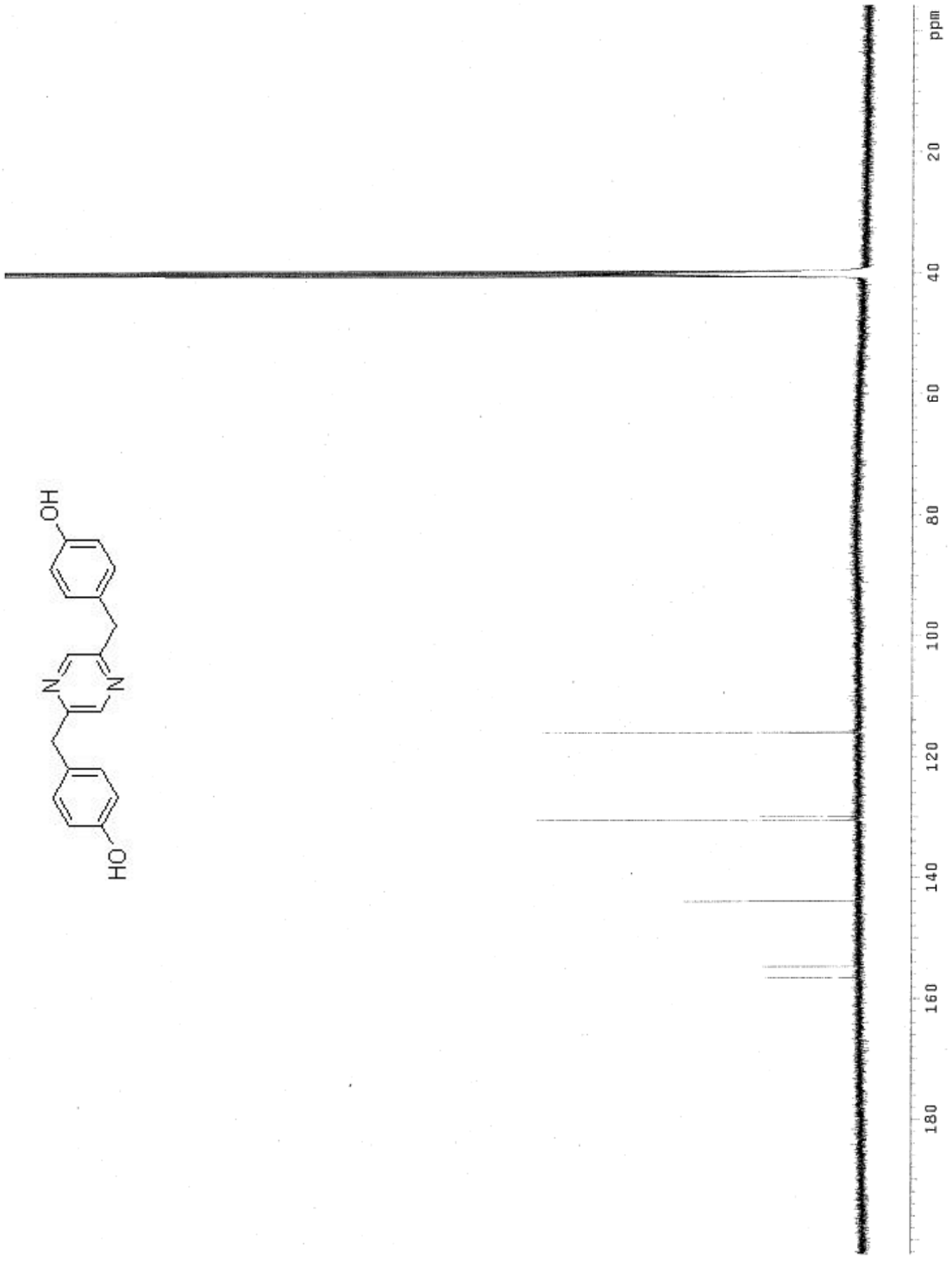

Figure S7. ^{13}C NMR spectrum of **3**

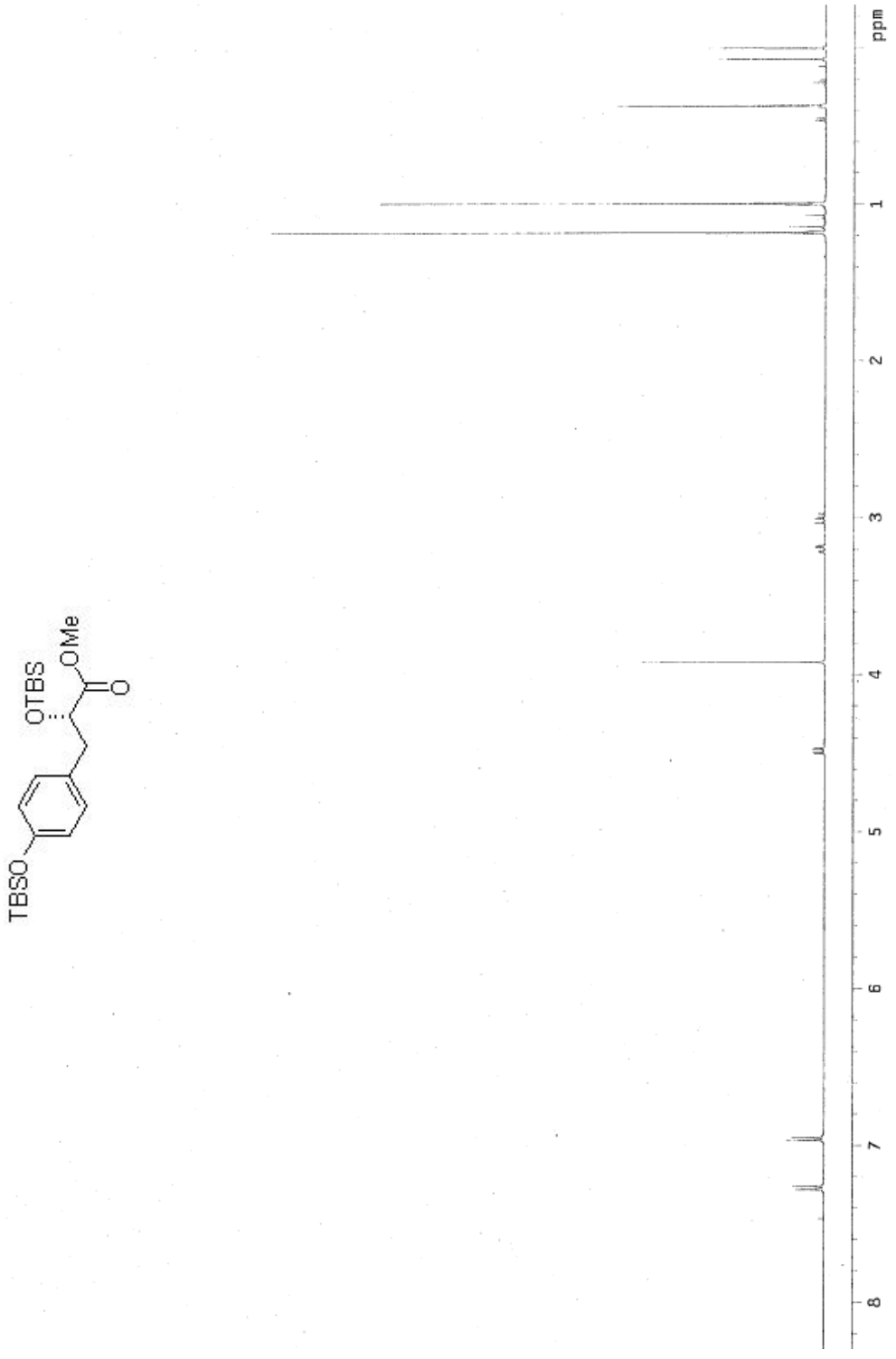

Figure S8. ^{1}H NMR spectrum of **6**

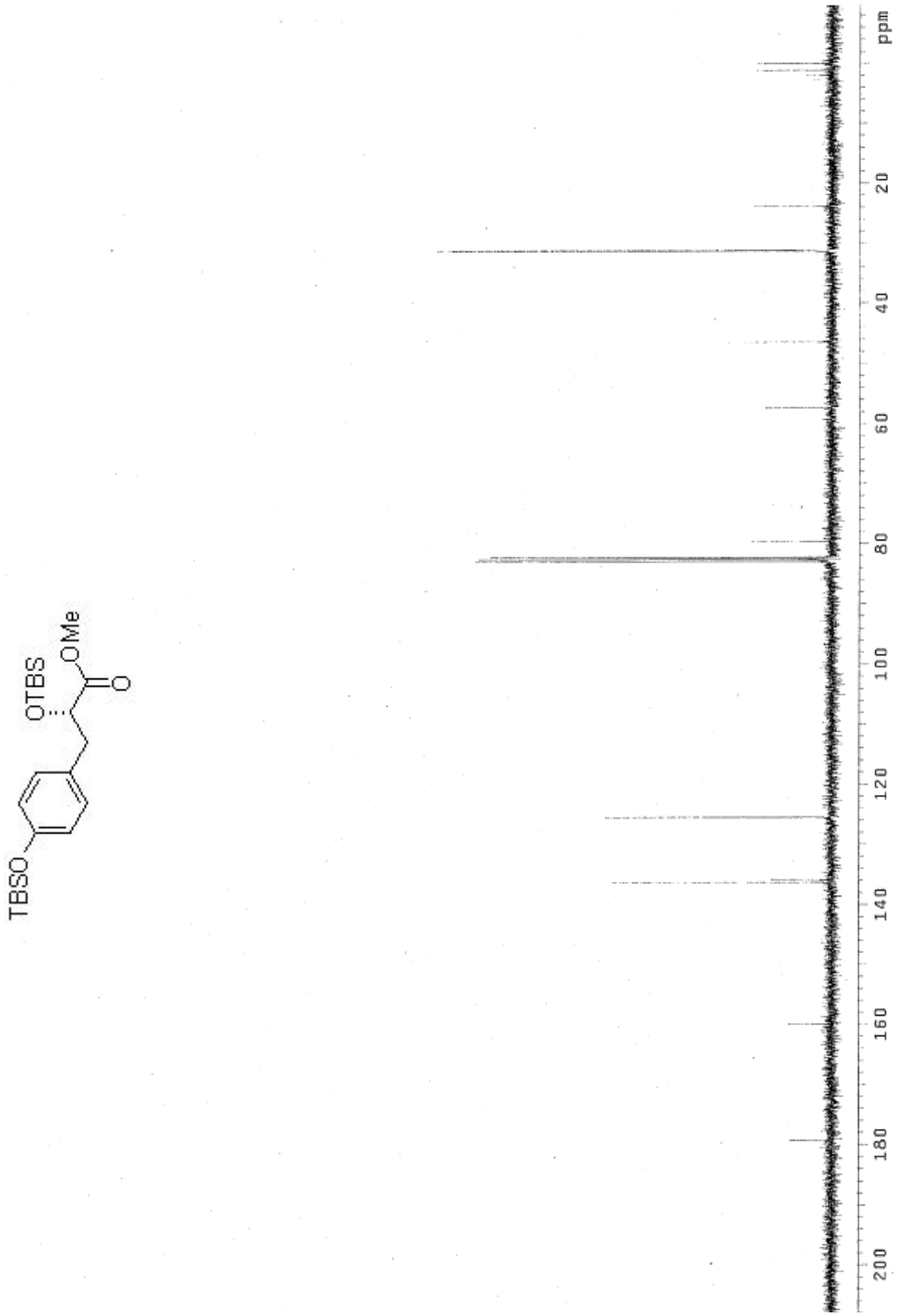

Figure S9. ^{13}C NMR spectrum of **6**

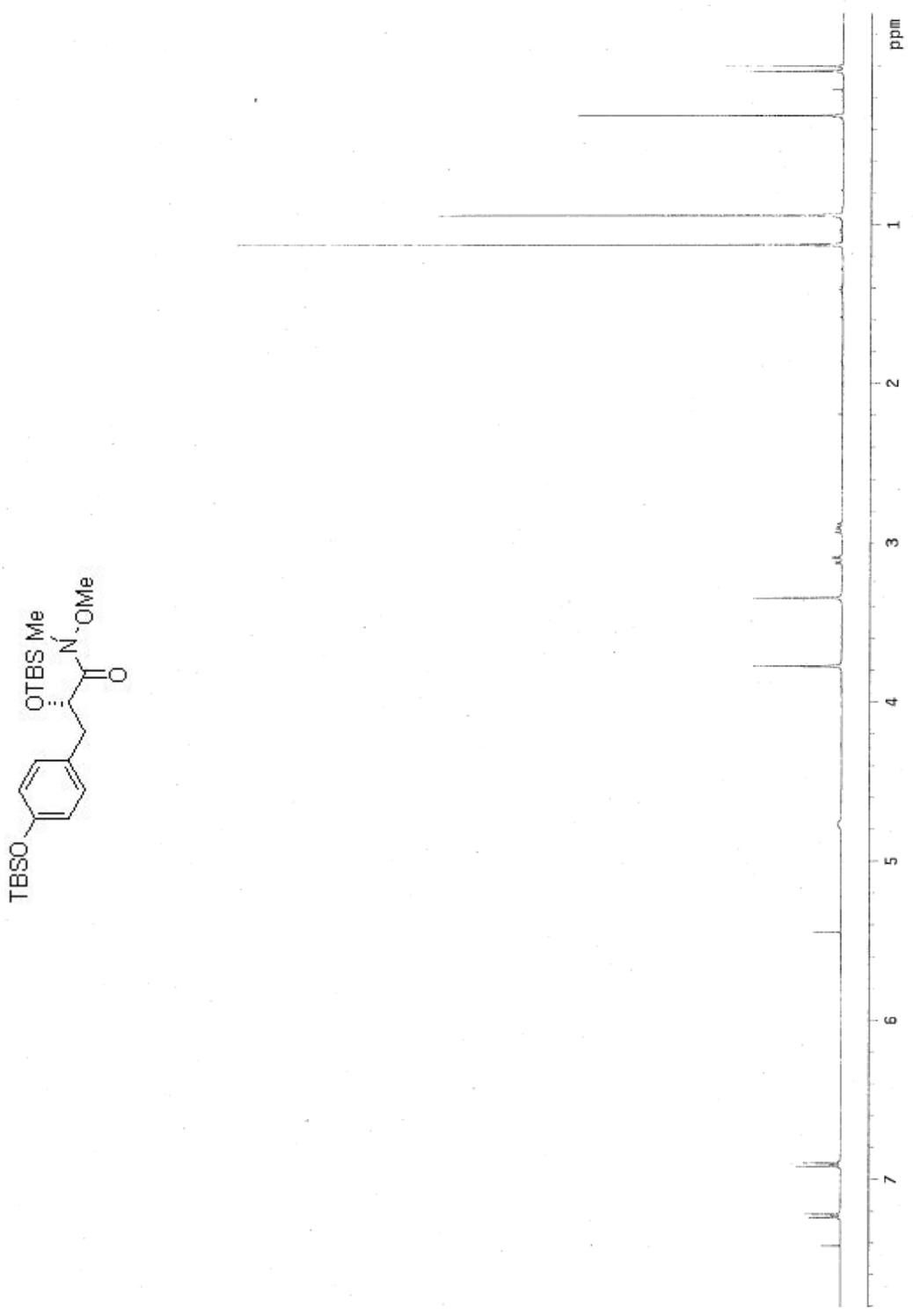

Figure S10. ^{1}H NMR spectrum of **7**

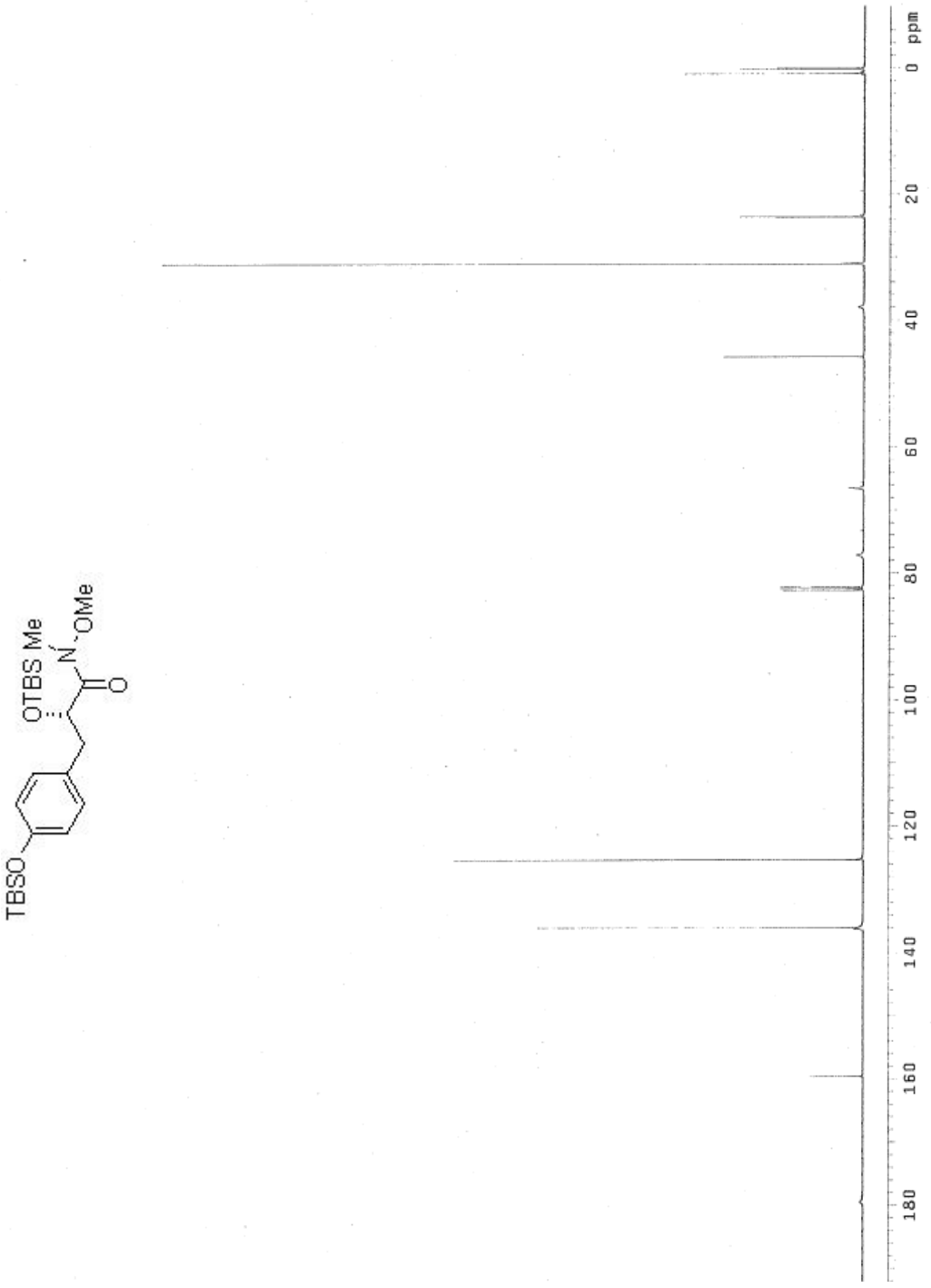

Figure S11. ^{13}C NMR spectrum of **7**

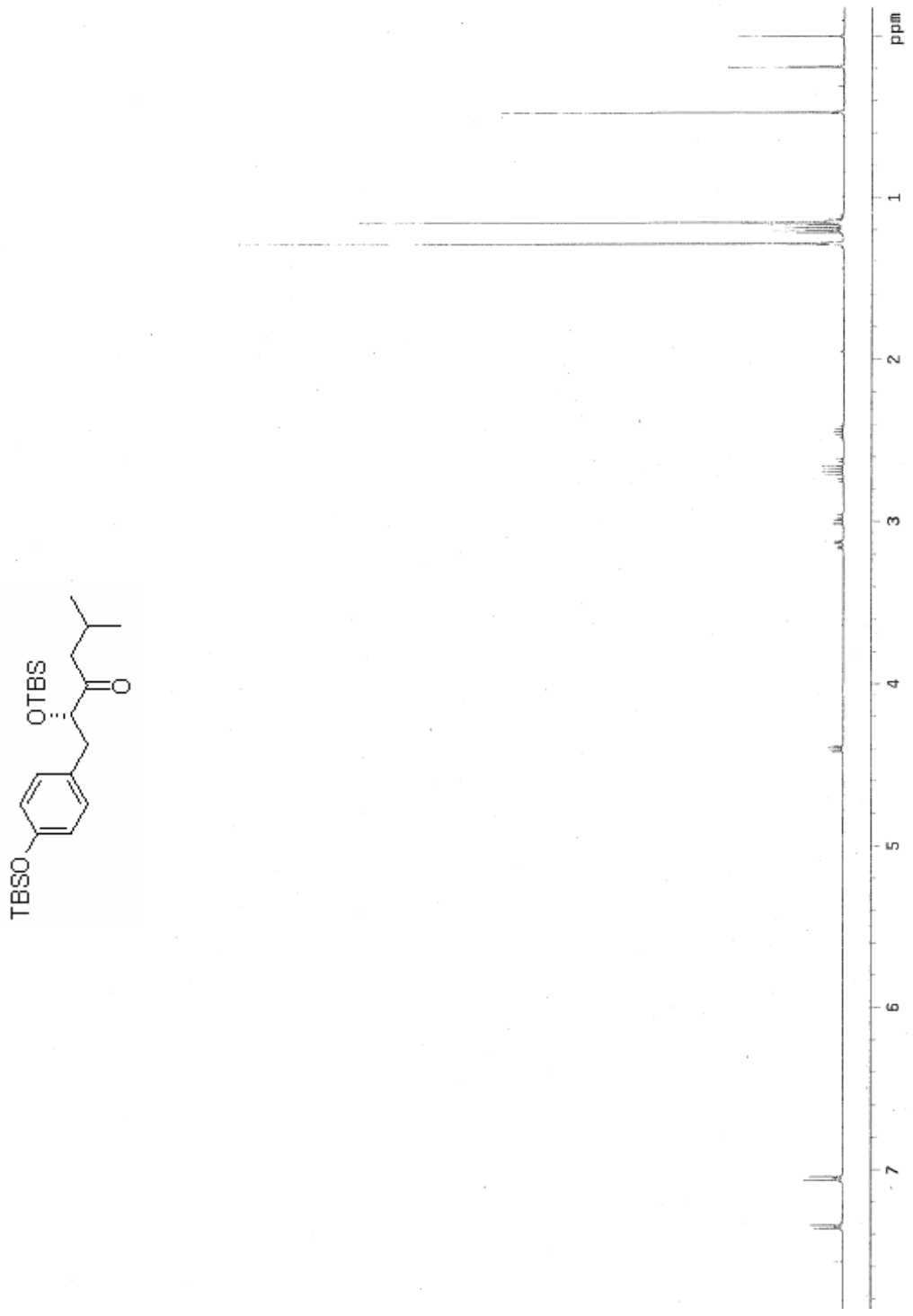

Figure S12. ^{1}H NMR spectrum of **8**

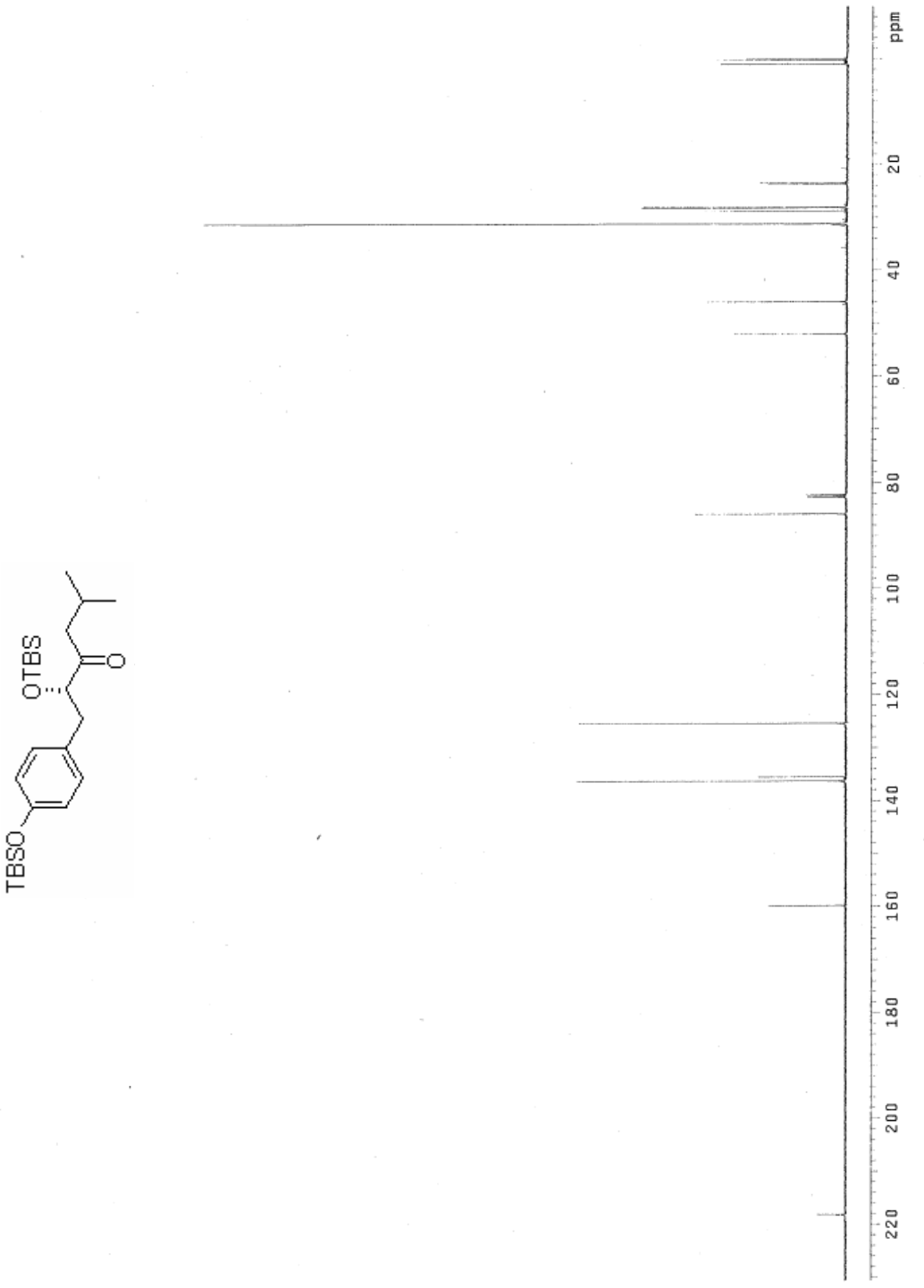

Figure S13. ^{13}C NMR spectrum of **8**

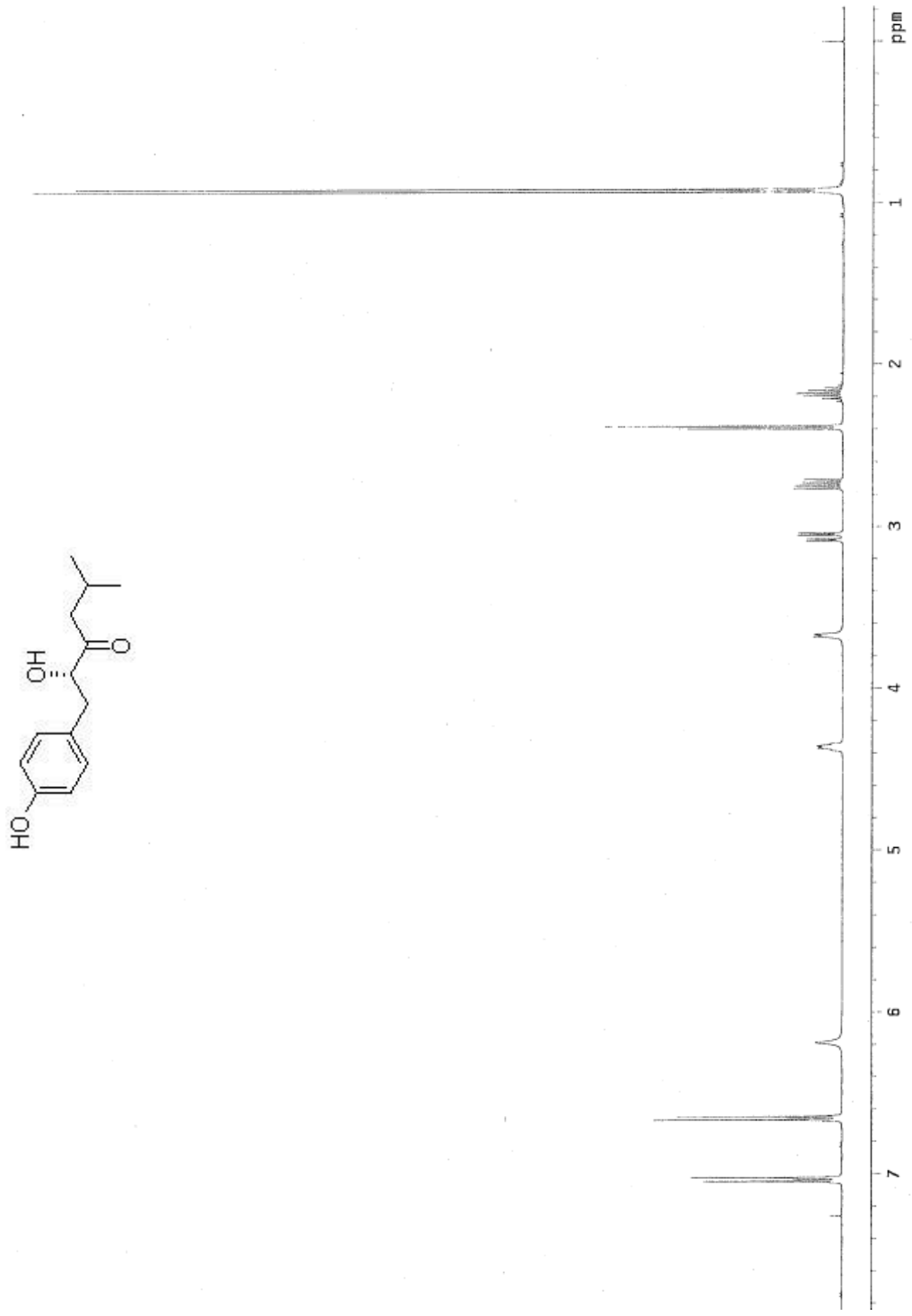

Figure S14. ^{1}H NMR spectrum of **9**

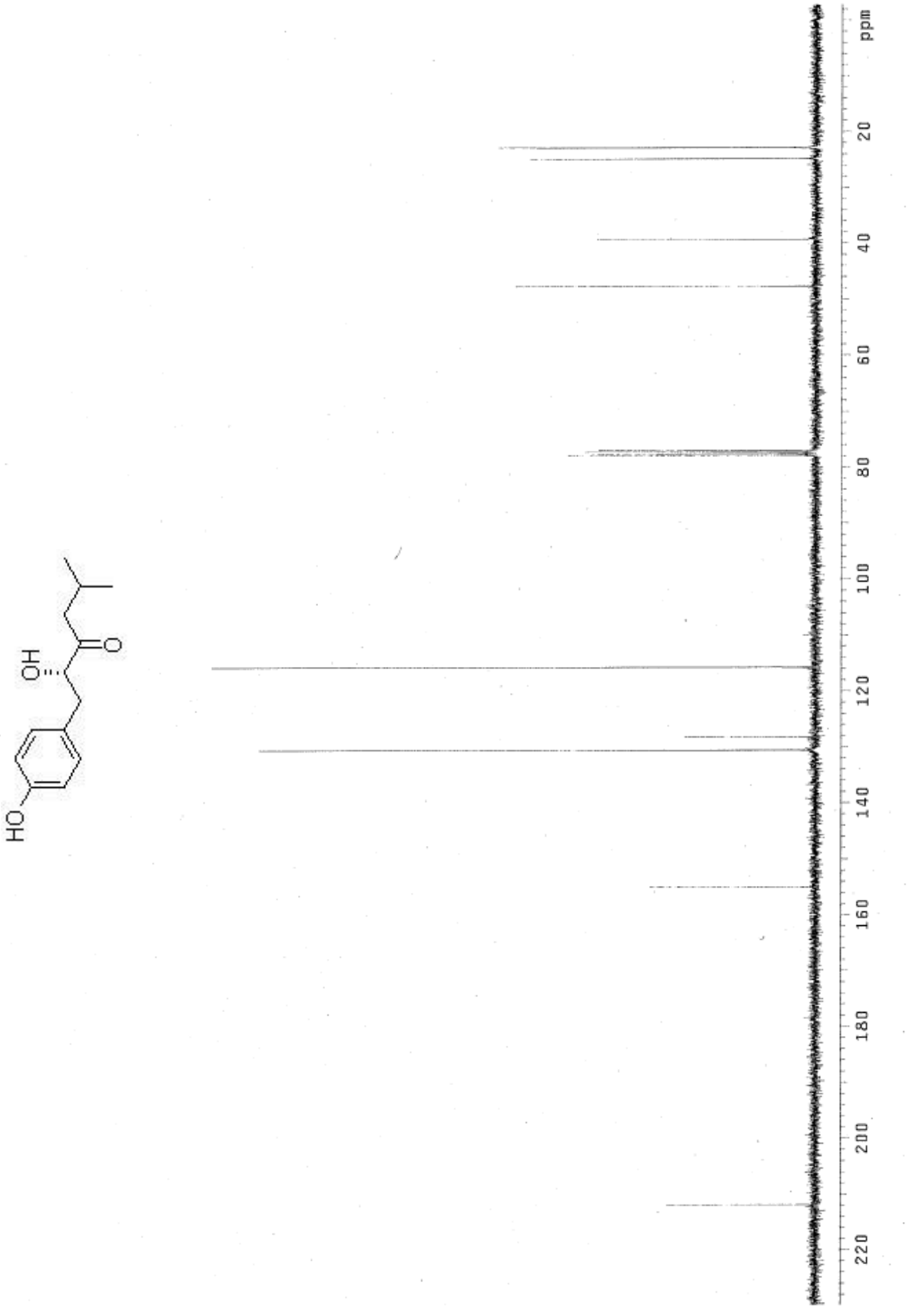

Figure S15. ^{13}C NMR spectrum of **9**

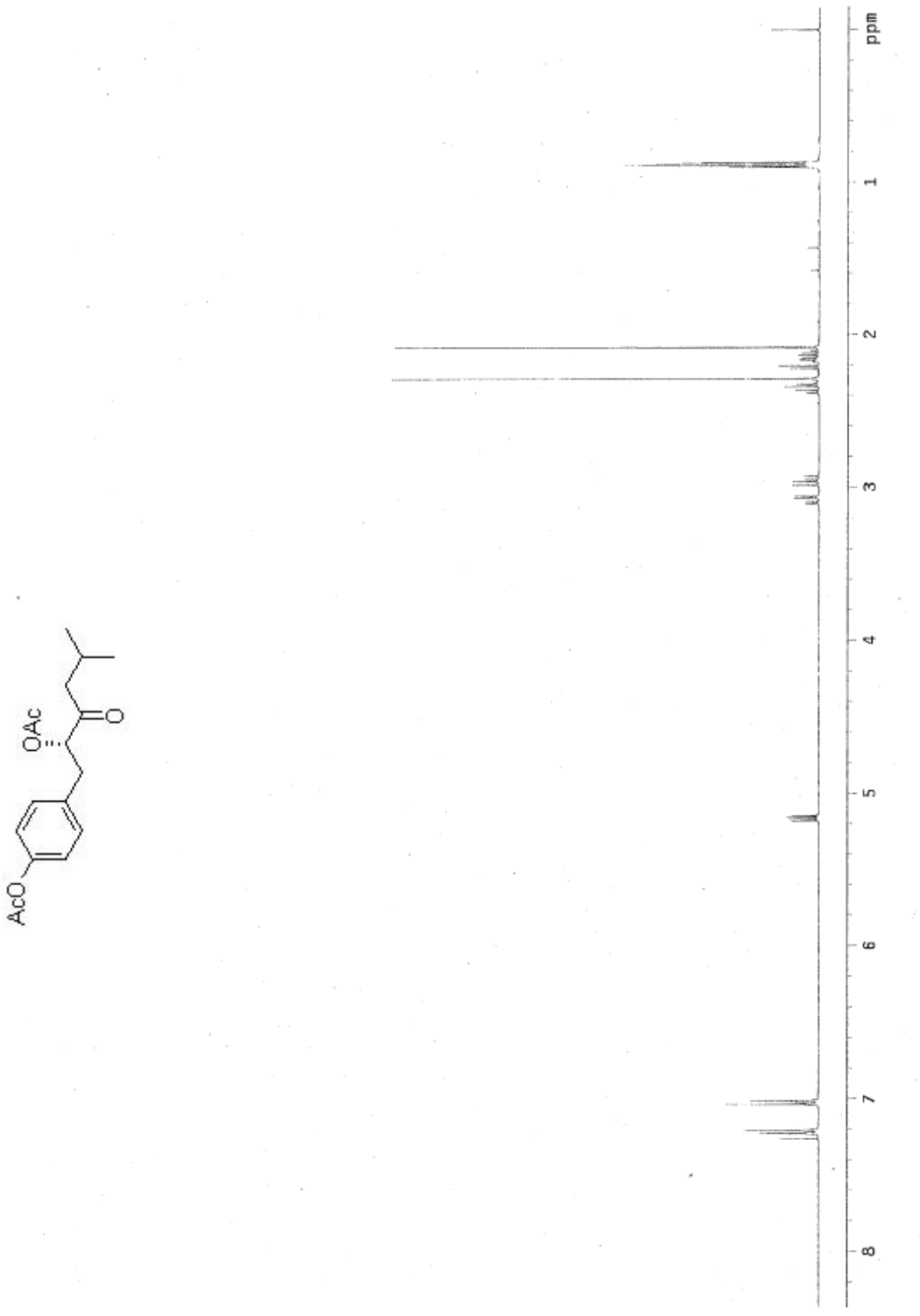

Figure S16. ^{1}H NMR spectrum of **10**

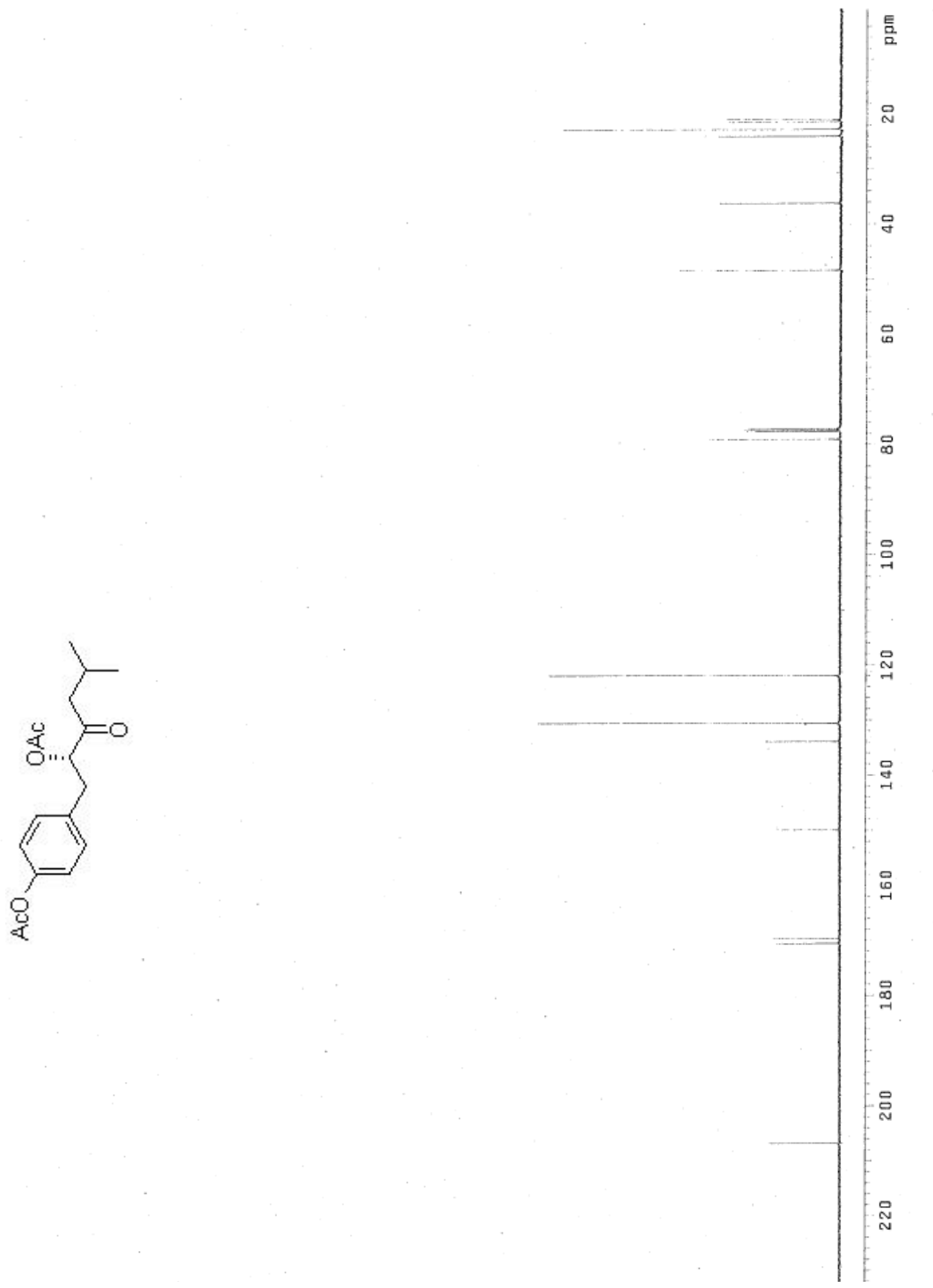

Figure S17. ^{13}C NMR spectrum of **10**