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SUPPLEMENTARY INFORMATION

Target-selective degradation of proteins by a light-activated 2-phenylquinoline-estradiol hybrid

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Chemical synthesis.

General Procedures: 17α-ethynyl-β-estradiol (**1**) and 2-phenylquinoline-4-carboxylic acid (**6**) were purchased from Sigma Co. and Aldrich Co., respectively. Melting points were determined on a micro hot-stage (Yanako MP-S3) and were uncorrected. Optical rotations were measured on a JASCO DIP-370 photo-electric polarimeter. ^{1}H-NMR spectra were recorded on a Varian MVX-300 (300 MHz) spectrometer using trimethylsilane as internal standard unless otherwise noted. Silica gel TLC and column chromatography were performed on Merck TLC 60F-254 (0.25 mm) and Silica Gel 60 N (spherical, neutral) (Kanto Chemical Co., Inc.), respectively. Air- and/or moisture-sensitive reactions were carried out under an

atmosphere of argon using oven-dried glassware. In general, organic solvents were purified and dried using an appropriate procedure, and evaporation and concentration were carried out under reduced pressure below 30 °C, unless otherwise noted.

3-*O*-*tert*-Butyldimethylsilyl-17α-ethynyl-β-estradiol (**4**): To a stirred solution of **3** (309 mg, 1.04 mmol) in dry CH_2Cl_2 (15 mL) was added 2,6-lutidine (180 μL, 1.56 mmol) and TBSOTf (290 μL, 1.25 mmol) at 0 °C. After stirring for 2 h at 25 °C, the mixture was poured into ice-cold water. The resultant mixture was extracted with $CHCl_3$ and the extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in *vacuo*. Purification of the residue by column chromatography (40 g of silica gel, 3/1 *n*-hexane/EtOAc) gave **4** (428 mg, 100%) as white solids. *Rf* 0.80 (1/1 *n*-hexane/EtOAc); Mp. 122.5-123.5 °C; $[\alpha]^{29}_{D}$ +5.5° (*c* 1.22, $CHCl_3$); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.18 (1H, d, *J* = 8.4 Hz), 6.66 (1H, dd, *J* = 8.4 and 2.4 Hz), 6.58 (1H, d, *J* = 2.4 Hz), 2.83-2.78 (2H, m), 2.62 (1H, s), 2.38-2.12 (3H, m), 2.05-1.96 (2H, m), 1.96-1.65 (5H, m), 1.55-1.23 (4H, m), 0.96 (9H, s), 0.89 (3H, s), 0.19 (6H, s); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 153.3, 137.8, 132.9, 126.1, 119.9, 117.2, 87.5, 79.9, 74.0, 49.5, 47.1, 43.6, 39.4, 39.0, 32.8, 29.6, 27.3, 26.3, 25.7 (x 3), 22.8, 18.1, 12.7, -4.4 (x 2); Anal. Calcd for $C_{26}H_{38}O_2Si$: C, 76.04; H, 9.33. Found: C, 75.67; H, 9.36.

3-*O*-*tert*-Butyldimethylsilyl-17α-hydroxypropargyl-β-estradiol (**5**): To a stirred solution of **3** (435 mg, 1.06 mmol) in dry THF (11 mL) was added LDA (1.77 mL, 3.18 mmol) at -78 °C. The reaction mixture was stirred for 0.5 h at -78 °C, and then $(CH_2O)_n$ (95.5 mg, 3.18 mmol) was added to the mixture. After stirring for 17 h at 25 °C, the mixture was poured into ice-cold water. The resultant mixture was extracted with

EtOAc and the extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in *vacuo*. Purification of the residue by column chromatography (40 g of silica gel, 2/1 *n*-hexane/EtOAc) gave **5** (359 mg, 77%) as white solids. *Rf* 0.30 (1/1 *n*-hexane/EtOAc); Mp. 178.0-179.0 °C; $[\alpha]^{29}_{D}$ -3.3° (*c* 1.05, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 7.11 (1H, d, *J* = 8.4 Hz), 6.61 (1H, dd, *J* = 8.4 and 2.4 Hz), 6.55 (1H, d, *J* = 2.4 Hz), 4.36 (2H, s), 2.83-2.78 (2H, m), 2.36-2.18 (3H, m), 2.06-1.97 (1H, m), 1.88-1.61 (7H, m), 1.54-1.25 (4H, m) 0.97 (9H, s), 0.87 (3H, s), 0.19 (6H, s); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 153.3, 137.8, 132.9, 126.1, 119.9, 117.2, 89.3, 84.2, 79.9, 51.1, 49.6, 47.2, 43.6, 39.4, 38.9, 33.0, 29.6, 27.3, 26.3, 25.7 (x 3), 22.9, 18.1, 12.8, -4.4 (x 2);Anal. Calcd for $C_{27}H_{40}O_3Si$: C, 73.59; H, 9.15. Found: C, 73.41; H, 9.14.

3-*O*-*tert*-Butyldimethylsilyl-17α-hydroxypropargyl-β-estradiolyl 2-phenylquinoline-4-carboxylate (**7**): To a stirred solution of **5** (66.0 mg, 0.150 mmol) and **6** (45.0 mg, 0.181 mmol) in dry CH_2Cl_2 (1.5 mL) was added EDC (57.0 mg, 0.300 mmol) and DMAP (9.0 mg, 0.0749 mmol) at 0 °C. After stirring for 2 h, the reaction mixture was poured into ice-cold water. The resultant mixture was extracted with $CHCl_3$ and the extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in *vacuo*. Purification of the residue by column chromatography (30 g of silica gel, 3/1 *n*-hexane/EtOAc) gave **7** (95.6 mg, 95%) as white solids. *Rf* 0.80 (1/1 *n*-hexane/EtOAc); Mp. 86.0-87.0 °C; $[\alpha]^{27}_{D}$ -5.1° (*c* 0.91, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 8.75 (1H, dd, *J* = 8.4 and 1.0 Hz), 8.43 (1H, s), 8.26-8.13 (3H, m), 7.81-7.43 (5H, m), 7.04 (1H, d, *J* = 8.4 Hz), 6.60 (1H, dd, *J* = 8.4 and 2.4 Hz), 6.54 (1H, d, *J* = 2.4 Hz), 5.16 (2H, s), 2.78 (2H, m), 2.43-2.13 (3H, m), 2.11-1.91 (2H, m), 1.91-1.64 (5H, m),

1.55-1.22 (4H, m), 0.98 (9H, s), 0.89 (3H, s), 0.19 (6H, s); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 165.6, 156.7, 153.3, 149.3, 138.7, 137.7, 135.0, 132.8, 130.4, 130.0, 129.8, 128.9 (x 2), 127.9, 127.4 (x 2), 126.1, 125.2, 123.9, 120.5, 119.9, 117.2, 91.3, 80.0, 79.5, 53.7, 49.7, 47.5, 43.6, 39.4, 38.9, 33.0, 29.6, 27.3, 26.3, 25.7 (x 3), 22.9, 18.2, 12.8, -4.4 (x 2); Anal. Calcd for $C_{43}H_{49}NO_4Si$: C, 76.86; H, 7.35; N, 2.08. Found: C, 76.70; H, 7.63; N, 1.94.

17α-hydroxypropagyl-β-estradiolyl 2-phenylquinoline-4-carboxylate (**2**): To a stirred solution of **7** (84.0 mg, 0.125 mmol) in dry THF (1.3 mL) was added AcOH (7.0 μL, 0.150 mmol) and TBAF (150 μL, 0.150 mmol) at 0 °C. After stirring for 1 h, the reaction mixture was poured into ice-cold water. The resultant mixture was extracted with EtOAc and the extracts were washed with brine, dried over anhydrous Na_2SO_4 and concentrated in *vacuo*. Purification of the residue by column chromatography (30 g of silica gel, 2/1 *n*-hexane/EtOAc) gave **2** (69.7 mg, 100%) as white solids. *Rf* 0.50 (1/1 *n*-hexane/EtOAc); Mp. 102.0-103.0 °C; $[\alpha]^{27}_{D}$ -6.5° (*c* 0.41, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 8.75 (1H, dd, *J* = 8.4 and 1.0 Hz), 8.43 (1H, s), 8.27-8.13 (3H, m), 7.82-7.43 (5H, m), 7.07 (1H, d, *J* = 8.4 Hz), 6.61 (1H, dd, *J* = 8.4 and 2.4 Hz), 6.55 (1H, d, *J* = 2.4 Hz), 5.16 (2H, s), 4.57 (1H, s), 2.80 (2H, m), 2.44-2.12 (3H, m), 2.11-1.91 (2H, m), 1.91-1.64 (5H, m), 1.50-1.30 (4H, m), 0.89 (3H, s, Me-13); ^{13}C-NMR (75 MHz, $CDCl_3$) δ 165.7, 156.8, 153.5, 149.1, 138.6, 138.0, 135.1, 132.1, 130.2, 130.0, 129.8, 128.9 (x 2), 127.9, 127.5 (x 2), 126.4, 125.2, 123.8, 120.6, 115.3, 112.7, 91.3, 80.0, 79.5, 53.8, 49.6, 47.5, 43.5, 39.4, 38.8, 32.9, 29.5, 27.2, 26.3, 22.9, 12.8; Anal. Calcd for $C_{37}H_{35}NO_4$: C, 79.69; H, 6.33; N, 2.51. Found: C, 79.72; H, 6.54; N, 2.77.

Ptotein photo-degradation.

Human estrogen receptor-α (hER-α), bovine serum albumin (BSA) and hen egg lysozyme (Lyso) were purchased from Sigma Co. A UV lamp (365 nm, 100 W, Blak-ray (B-100A), UVP. Inc.) was used for the photo-irradiation. All the protein degradation experiments were performed with hER-α, BSA or Lyso (1.0 μM) in a volume of 10 μL containing 20% acetonitrile in 50 mM Tris-HCl buffer (pH 8.0) at 25 °C for 2 h under irradiation of the UV lamp placed at 10 cm from the mixture. The protein-sample levels were varied as indicated in the figure captions.

Electrophoresis.

SDS/polyacrylamide gel electrophoresis (SDS-PAGE) experiments were performed as reported.[1] After addition of a 4.8 μL solution containing SDS (5%, wt/vol), glycerol (27%, vol/vol), DTT (0.5%, wt/vol) and bromophenol blue (0.007%, wt/vol) to the photoirradiated samples. Gels (8% for BSA and 12% for hER-α and Lyso) were run by applying 110 V for 1.5 h for BSA or 2.5 h for hER-α and Lyso. The gels were stained with SYPRO Ruby Protein Gel Stain (Bio-Rad Lab. Inc.) for 3 h, destained in acetic acid (7%, vol/vol) and methanol (10%, vol/vol) for 0.5 h, and then washed with water. The gels were scanned with a Molecular Imager FX (Bio-Rad Lab. Inc.) and images were processed using Adobe Photoshop software. Molecular weight markers were used in each gel for calibration.

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ESR Spectrometry.

ESR spectrum[2] was recorded using a Bruker BioSpin EMX EPR operating at 9.5 GHz with 100 kHz modulation. A mixture of **2** (4 mM) and DMPO (100 mM) in a volume of 1.0 mL containing 50% acetonitrile Tris-HCl buffer (pH 8.0, 50 mM) was placed in a quartz flat cell and irradiated directly inside the microwave cavity of the spectrometer using a UV lamp (365 nm, 100 W, Blak-ray (B-100A), UVP. Inc.).

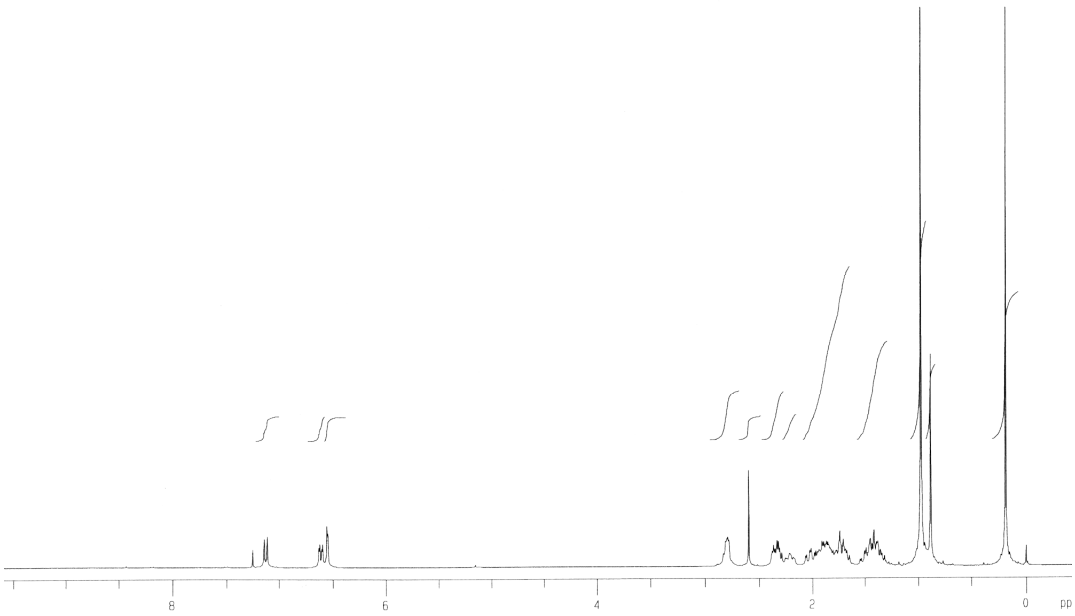
SI-Fig. S1. ESR spectra obtained a) by treatment of DMPO with **2** without photo-irradiation or b) by photo-irradiation of DMPO in the absence of **2**. Compound **2** (4 mM) and DMPO (100 mM) were used in 50% acetonitrile/Tris-HCl buffer (pH 8.0, 50 mM).

References.

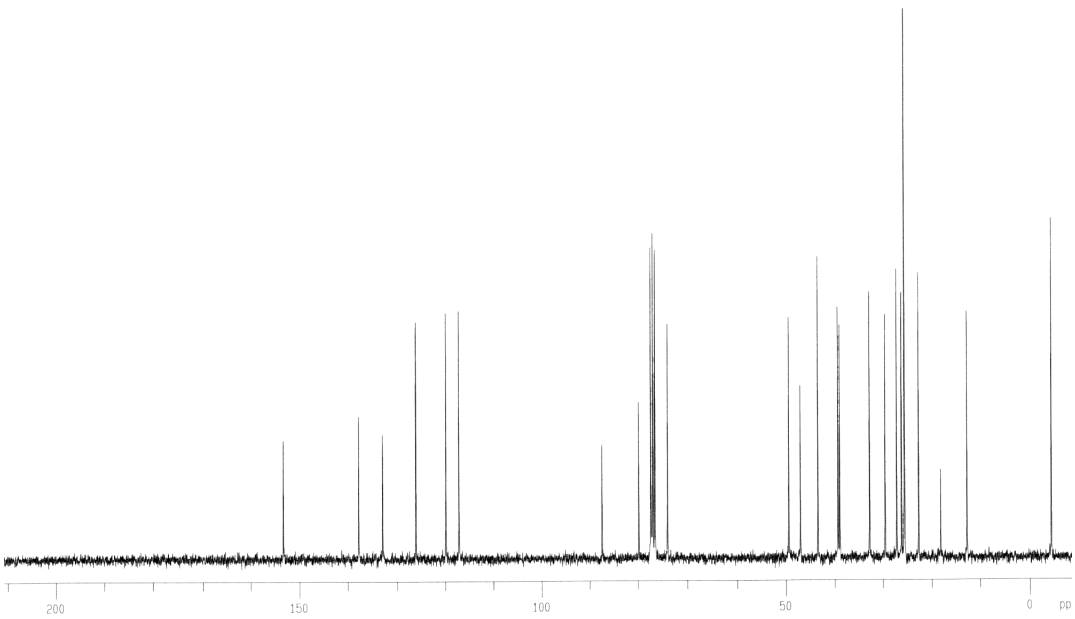
1 H. Schägger and G. von Jagow, *Anal. Biochem*., 1987, **166**, 368.

2 B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford, 1985; K. J. A. Davies, *J. Biol. Chem*., 1987, **262**, 9895.

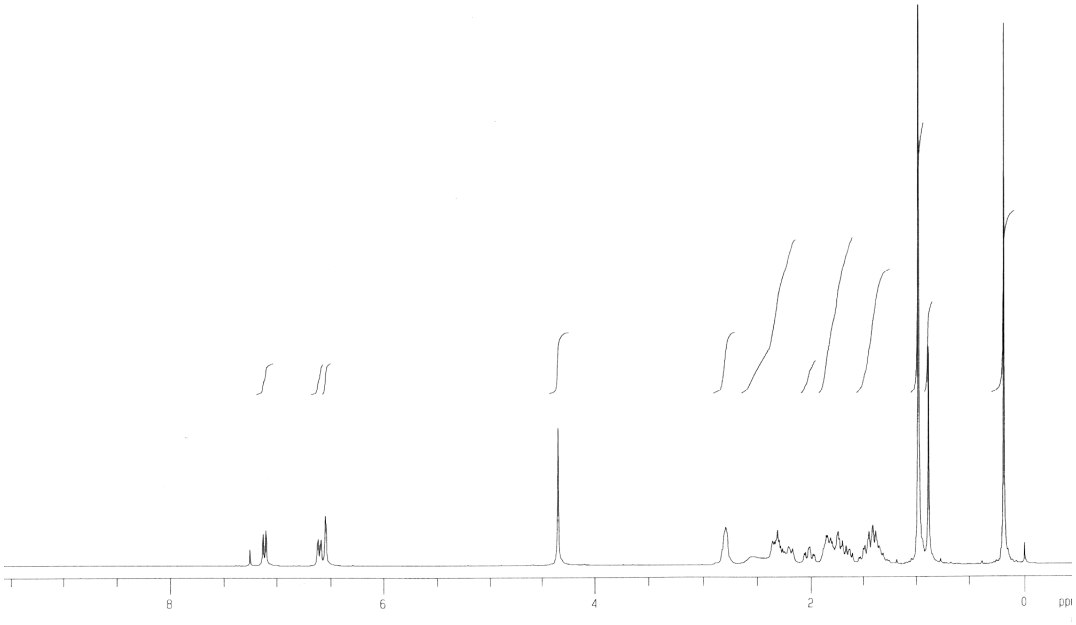
^{1}H NMR spectrum of **4**.



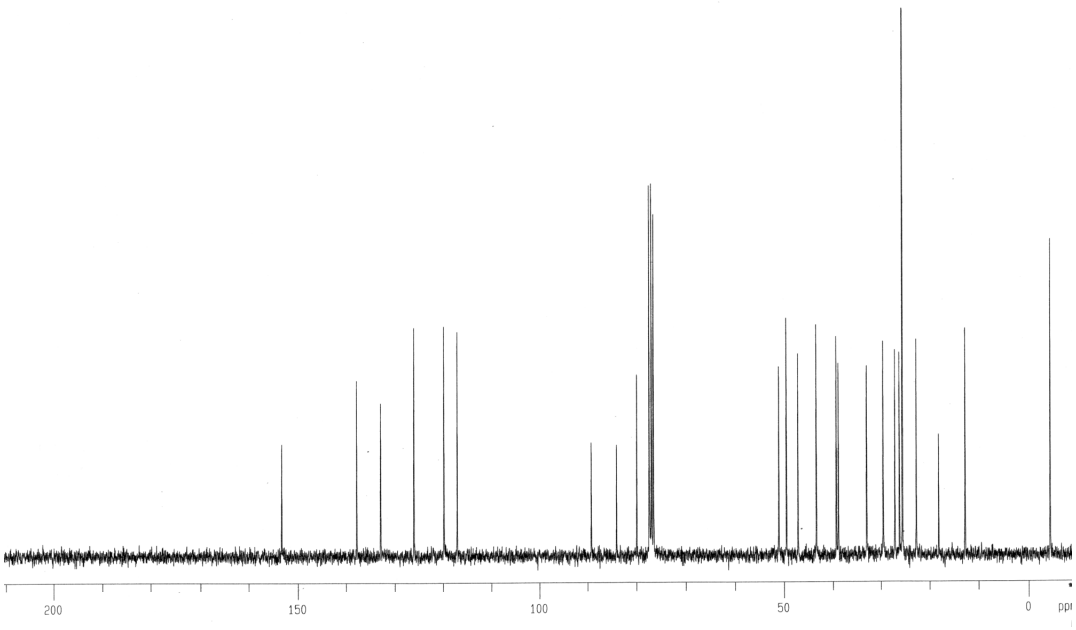
^{13}C NMR spectrum of **4**.



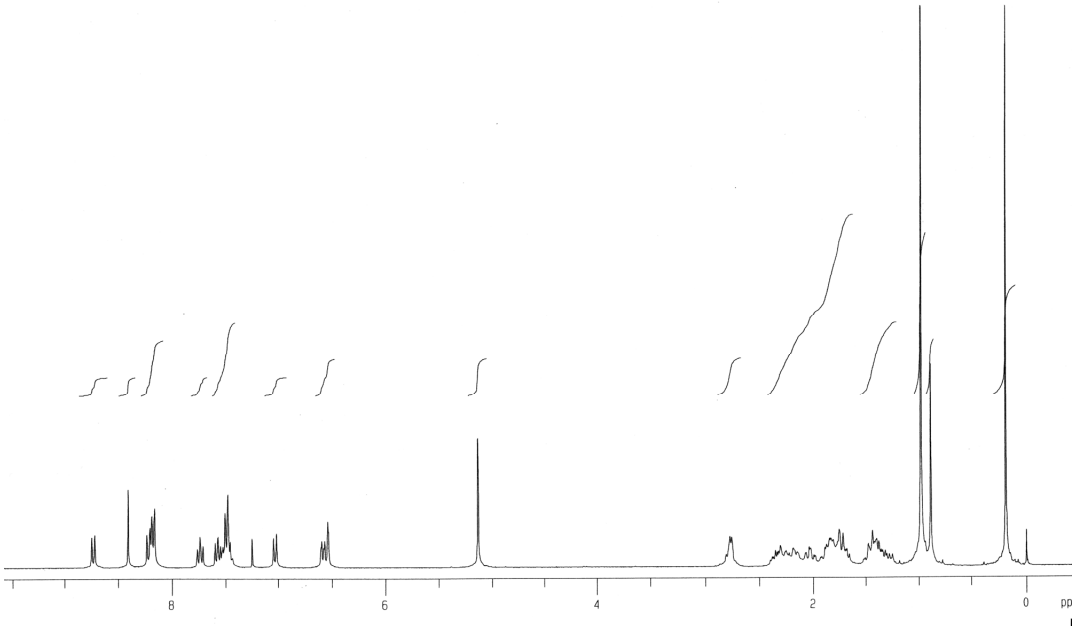
^{1}H NMR spectrum of **5**.



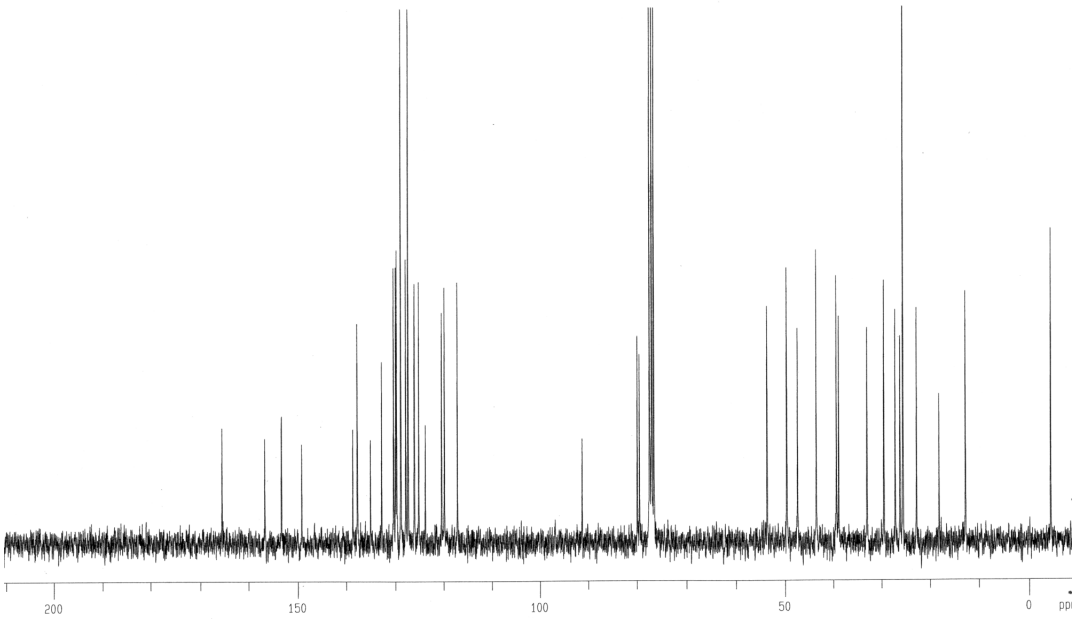
^{13}C NMR spectrum of **5**.



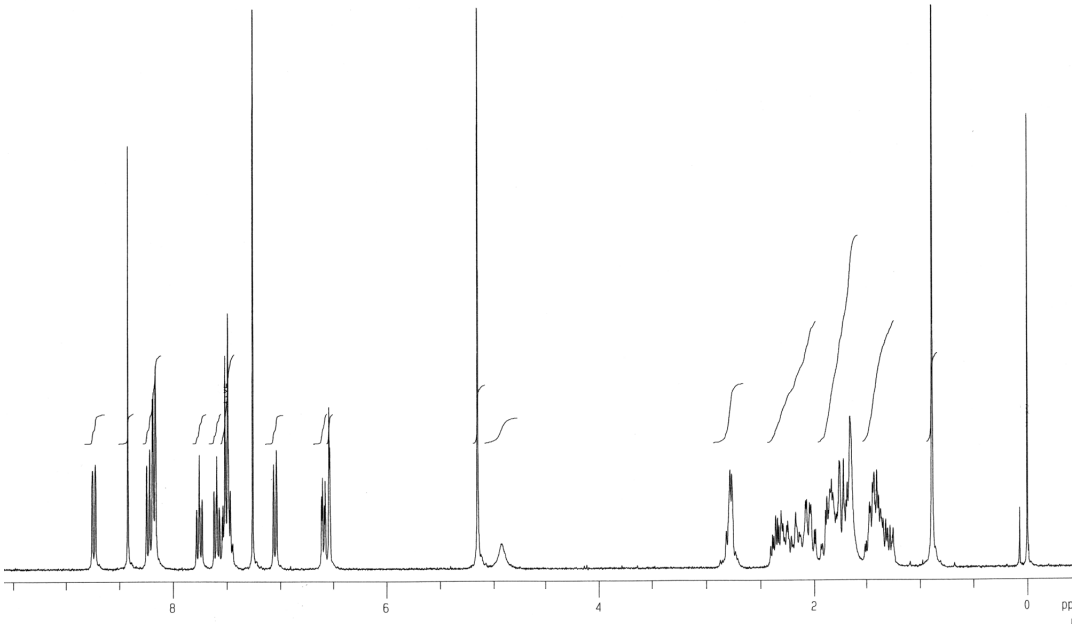
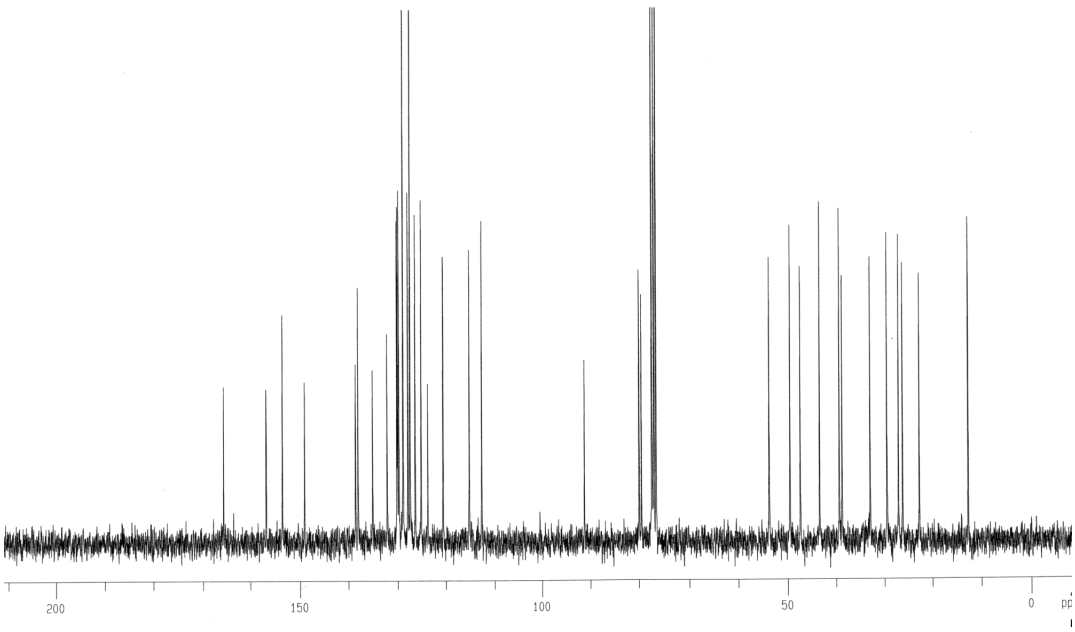
^{1}H NMR spectrum of **7**.



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



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



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