Aplaminal: A Novel Cytotoxic Aminal Isolated from the Sea Hare *Aplysia kurodai*

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List of Supporting Information

Experimental Procedures

Material. The sea hare *Aplysia kurodai* was collected at the reef of Azurihama, Mie, Japan.

Extraction and isolation. Approximately 18 kg (wet weight) of sea hare was extracted with methanol (36 L) for 1 week. The extract was filtered, and the filtrate was concentrated. The residue was partitioned between ethyl acetate (3 × 1 L) and water (1 L). The ethyl acetate layer was washed with water (500 mL) and concentrated. The residue (21.9 g) was partitioned between 90% methanol (1 L) and hexane (2× 1 L). The 90% methanol layer was concentrated and partitioned between 60% methanol (1 L) and dichloromethane (2 × 1 L). The dichloromethane layer was concentrated. The residue (11.8 g) was loaded on a silica gel column (200 g) and eluted with benzene-ethyl acetate = 1:1 → ethyl acetate → ethyl acetate-methanol = 4:1 → 1:1 → methanol (1.2 L each). The concentrated fraction (0.7 g) eluted with ethyl acetate-methanol = 1:1 was loaded on an aluminum oxide column (0.7 g) and eluted with ethyl acetate → ethyl acetate-methanol = 19:1 → 9:1. The concentrated fraction (9.7 mg) eluted with ethyl acetate – methanol = 19:1 was chromatographed by reversed-phase HPLC [1. Develosil ODS-HG-5, ϕ 20 × 250 mm, acetonitrile-0.02 M ammonium acetate = 65:35, 5 mL/min, t_R = 5 min; 2. Develosil ODS-HG-5, ϕ 20 × 250 mm, methanol-water = 50:50, 5 mL/min, t_R = 21 min] to give **1** (2.0 mg, 0.00001% based on wet wt).

^{1}H NMR Data for aplaminal (1; 270 MHz, acetone-d_6) δ 7.76 (d, *J* = 9.1 Hz, 2H), 6.85 (d, *J* = 9.1 Hz, 2H), 6.62 (br s, 1H), 4.26 (ddd, *J* = 1.4, 5.7, 9.2 Hz, 1H), 3.85-3.75 (m, 1H), 3.79 (s, 3H), 3.78 (s, 3H), 3.66 (m, 1H), 3.36 (d, *J* = 9.2 Hz, 1H), 3.23 (m, 1H), 2.46 (s, 3H).

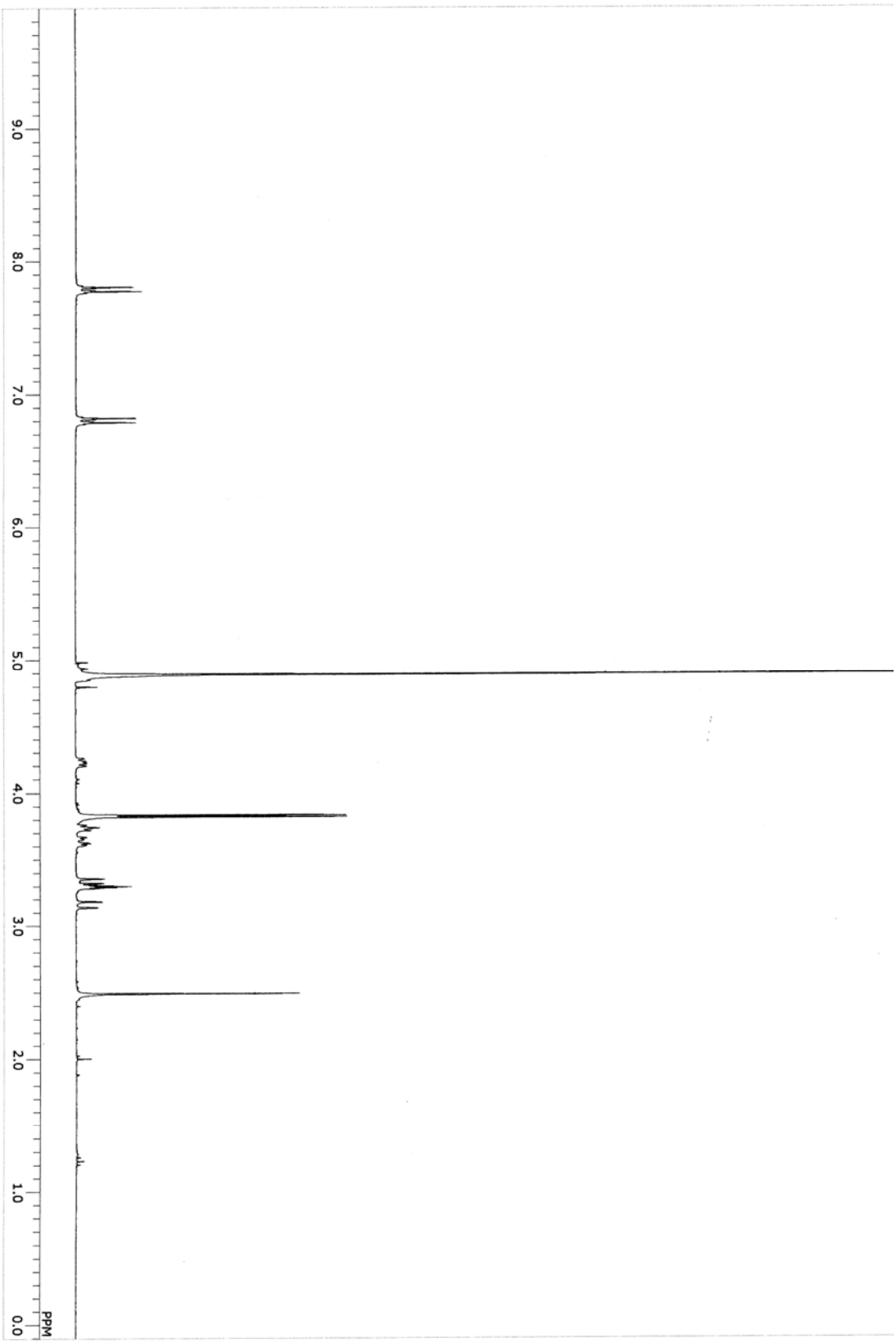

Chart 1. ^{1}H NMR spectrum of **1** [270 MHz, CD_3OD].

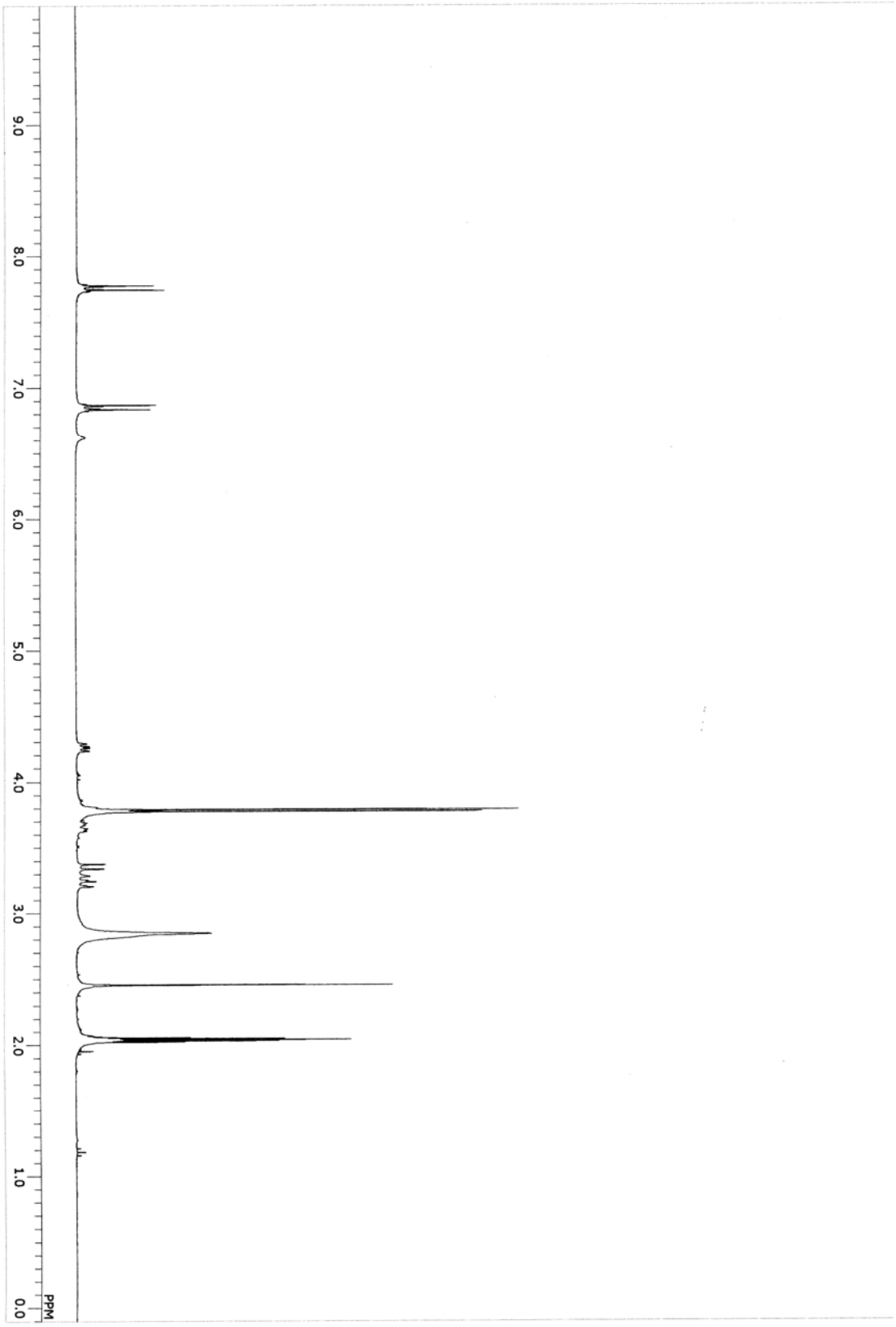

Chart 2. ^{1}H NMR spectrum of **1** [270 MHz, acetone-d_6].

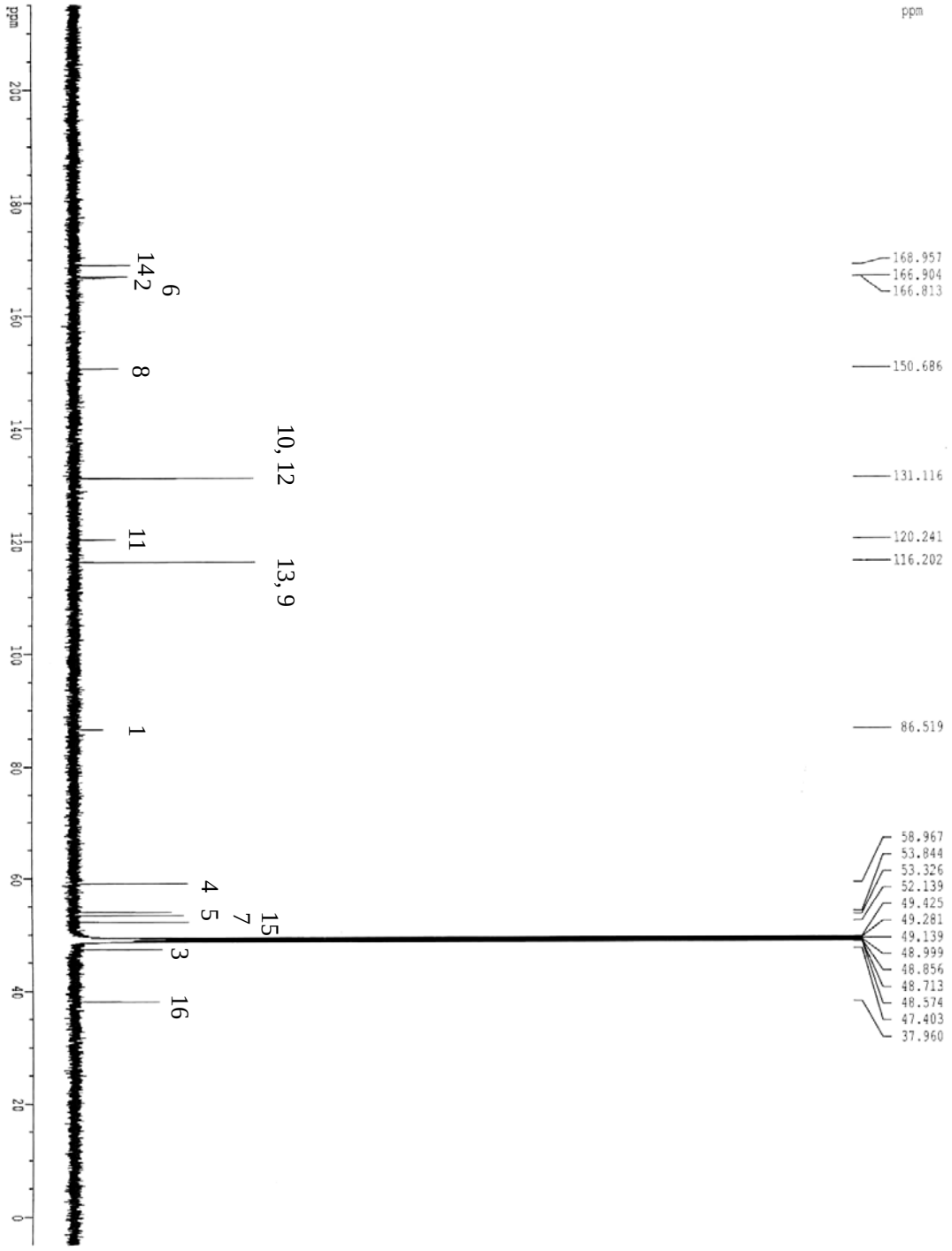

Chart 3. ^{13}C NMR spectrum of **1** [151 MHz, CD_3OD].

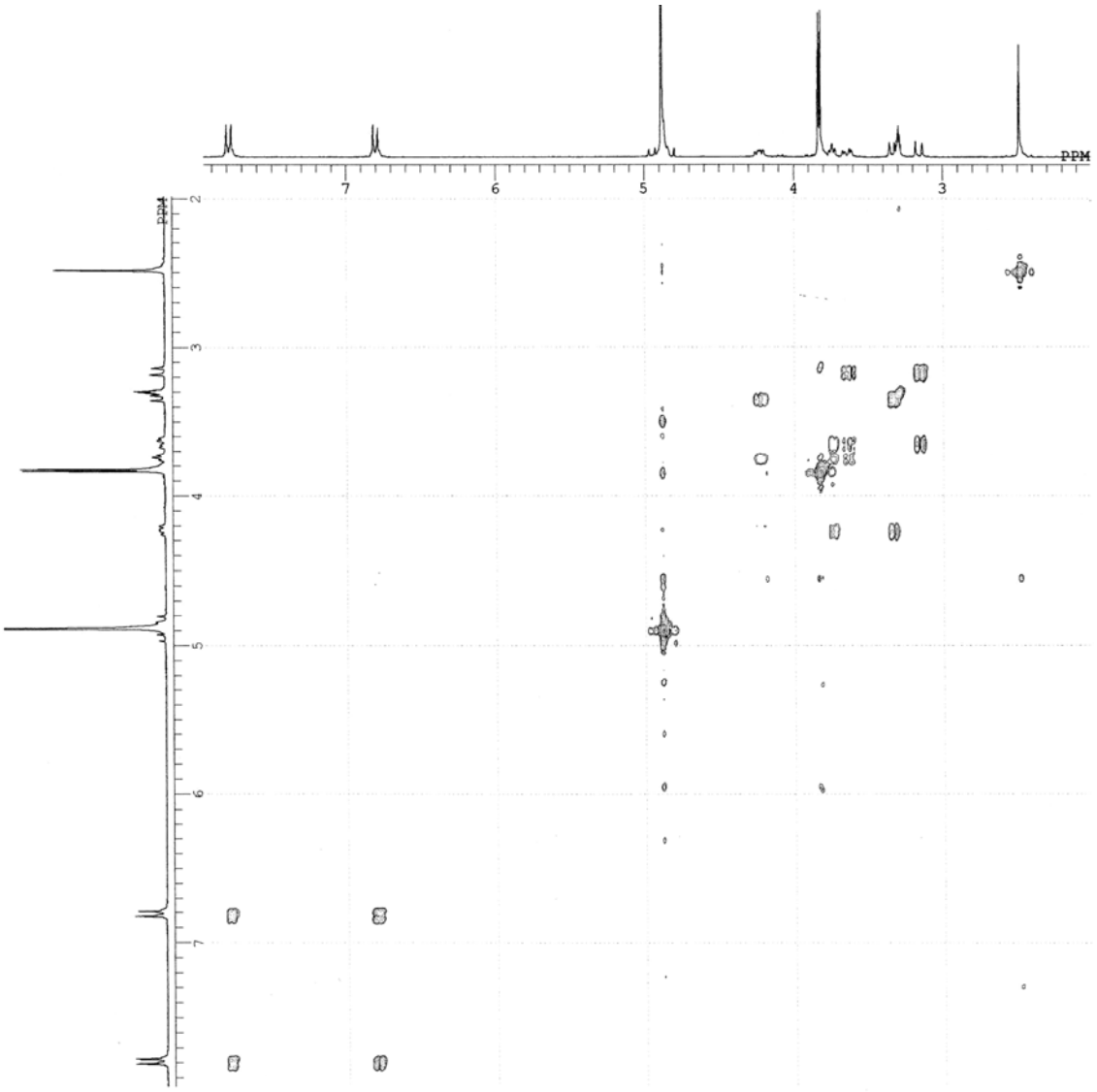

Chart 4. ^{1}H-^{1}H COSY spectrum of **1** [270 MHz, CD_3OD].

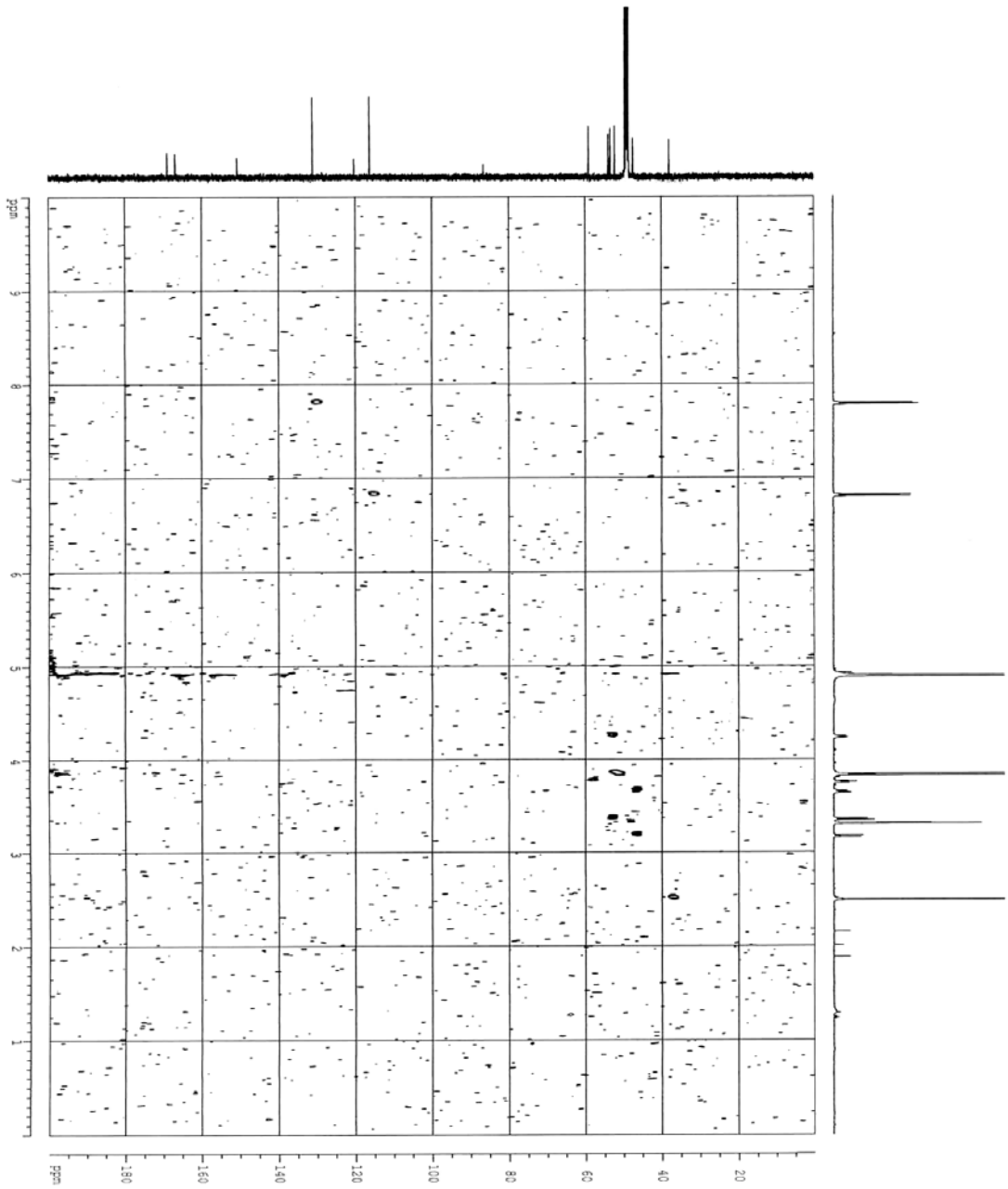

Chart 5. HMQC spectrum of **1** [600 MHz, CD_3OD].

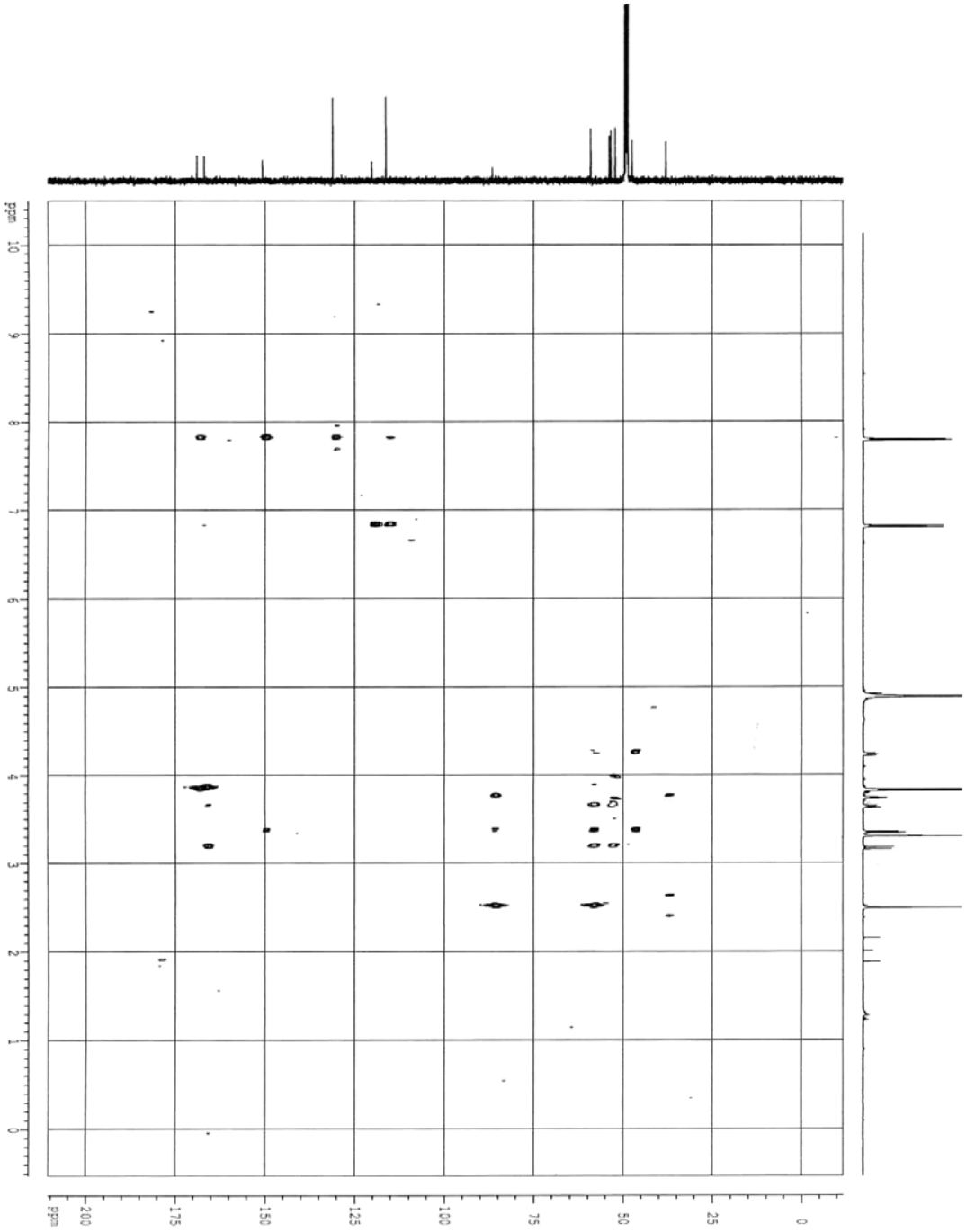

Chart 6. HMBC spectrum of **1** [600 MHz, CD_3OD].