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Structural Characterization of a 4-Hydroxy-2-alkenal-derived Fluorophore that Contributes to Lipoperoxidation-dependent Protein Crosslinking in Aging and Degenerative Disease

Guozhang Xu and Lawrence M. Sayre*

Supplementary Material

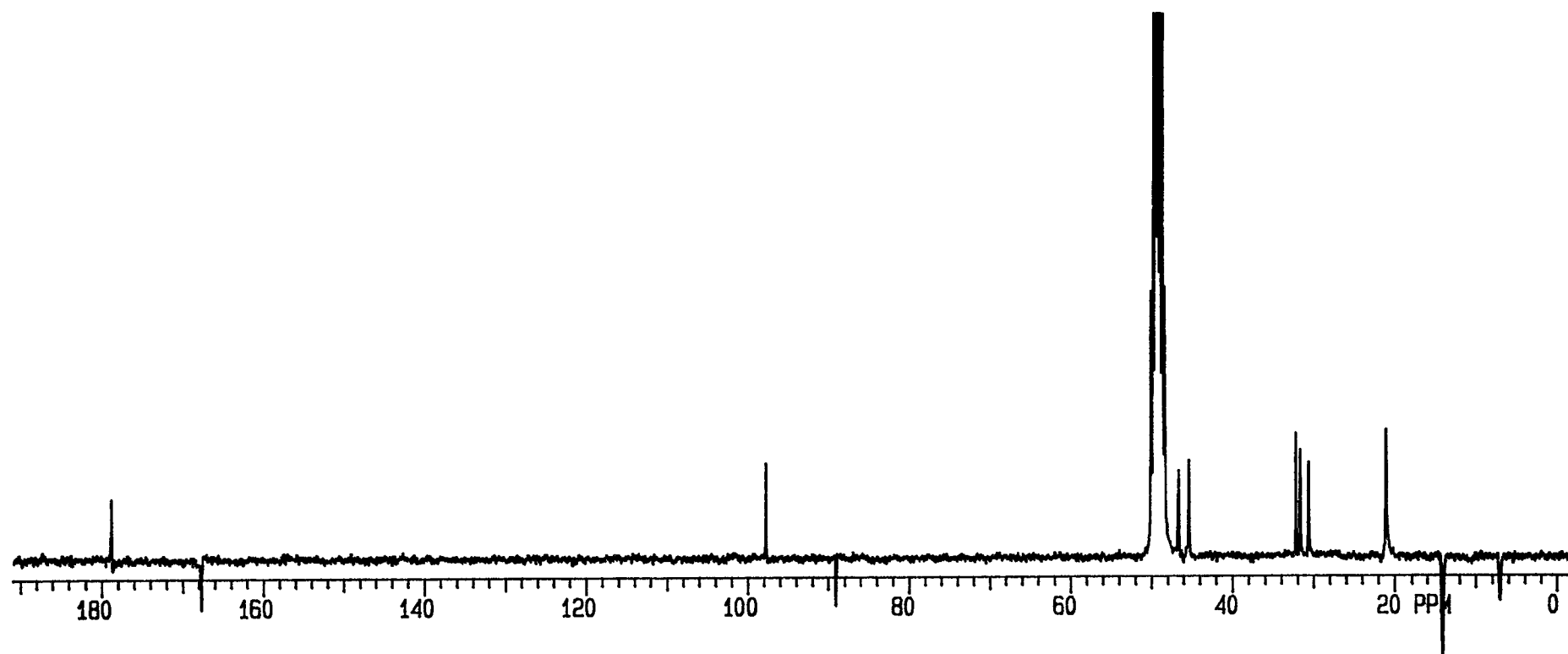
Preparation of (*E*)-4-oxo-2-nonenal (4). A solution of HNE dimethyl acetal (35) (0.5 g, 2.45 mmol) in 20 mL dry CH₂Cl₂ was added dropwise to a well-stirred solution of pyridinium chlorochromate (1.06 g, 4.90 mmol) in dry CH₂Cl₂ containing 1.5 g NaOAc. After 6 h the solution was concentrated, and the dark residue was diluted with 250 mL of dry ether and filtered through cotton gauze. Upon evaporation of solvent, the residue was purified by flash chromatography (EtOAc as eluent) to yield 460 mg (96%) of (*E*)-1,1-dimethoxy-2-nonen-4-one: ¹H NMR (CDCl₃) δ 0.85 (t, J = 6.81 Hz, 3H), 1.20-1.32 (m, 4H), 1.53-1.63 (m, 2H), 2.53 (t, J = 7.41 Hz, 2H), 3.31 (s, 6H), 4.90 (dd, J = 3.84 and 1.08 Hz, 1H), 6.33 (dd, J = 16.89 and 1.08 Hz, 1H), 6.55 (dd, J = 16.09 and 3.96 Hz, 1H); ¹³C NMR (CDCl₃) δ 13.94 (-), 22.48 (+), 23.72 (+), 31.43 (+), 40.68 (+), 52.98 (-), 101.08 (-), 132.12 (-), 139.91 (-), 200.47 (+); HRMS calcd for C₁₁H₂₀O₃ *m/z* 200.1413, found 200.1412. The latter compound (460 mg, 2.3 mol) was added to 30 mL of 2N HCl/acetone (1:2 v/v), and after stirring for 4 h, the reaction mixture was concentrated and extracted with CH₂Cl₂. The combined organic layer was dried (Na₂SO₄), and concentrated to afford 350 mg (100%) of 4: ¹H NMR (CDCl₃) δ 0.89 (t, J = 6.65 Hz, 3H), 1.27-1.36 (m, 4H), 1.60-1.70 (m, 2H), 2.68 (t, J = 7.35 Hz, 2H), 6.76 (dd, J = 16.59 and 6.42 Hz, 1H), 6.87 (d, J = 16.17 Hz, 1H), 9.77 (t, J = 6.72 Hz, 1H); ¹³C NMR (CDCl₃) δ 13.89 (-), 22.43 (+), 23.37 (+), 31.26 (+), 41.21 (+), 137.33 (-), 144.96 (-), 193.45 (-), 200.17 (+); HRMS calcd for C₉H₁₄O₂ *m/z* 154.0994, found 154.0995.

Preparation of 3,4-dioxononanal (5). A solution of 2.5 M *n*-butyllithium in hexane (8 mL, 20 mmol) was added to a stirred solution of 1-heptyne (1.92 g, 20 mmol) and HMPA (10 mL, 57.5 mmol) in 40 mL of THF under nitrogen at -78 °C. The temperature was raised to -45 °C for 45 min, re-lowered to -78 °C, and a solution of bromoacetaldehyde diethyl acetal (3.94 g, 20 mmol) in 10 mL of THF was added dropwise. After stirring to room temperature over 20 h, the reaction mixture was concentrated, diluted into 30 mL of aqueous NH₄Cl, and extracted with ether (120 mL). The organic extracts were washed with brine and water, dried (Na₂SO₄), and concentrated to afford a

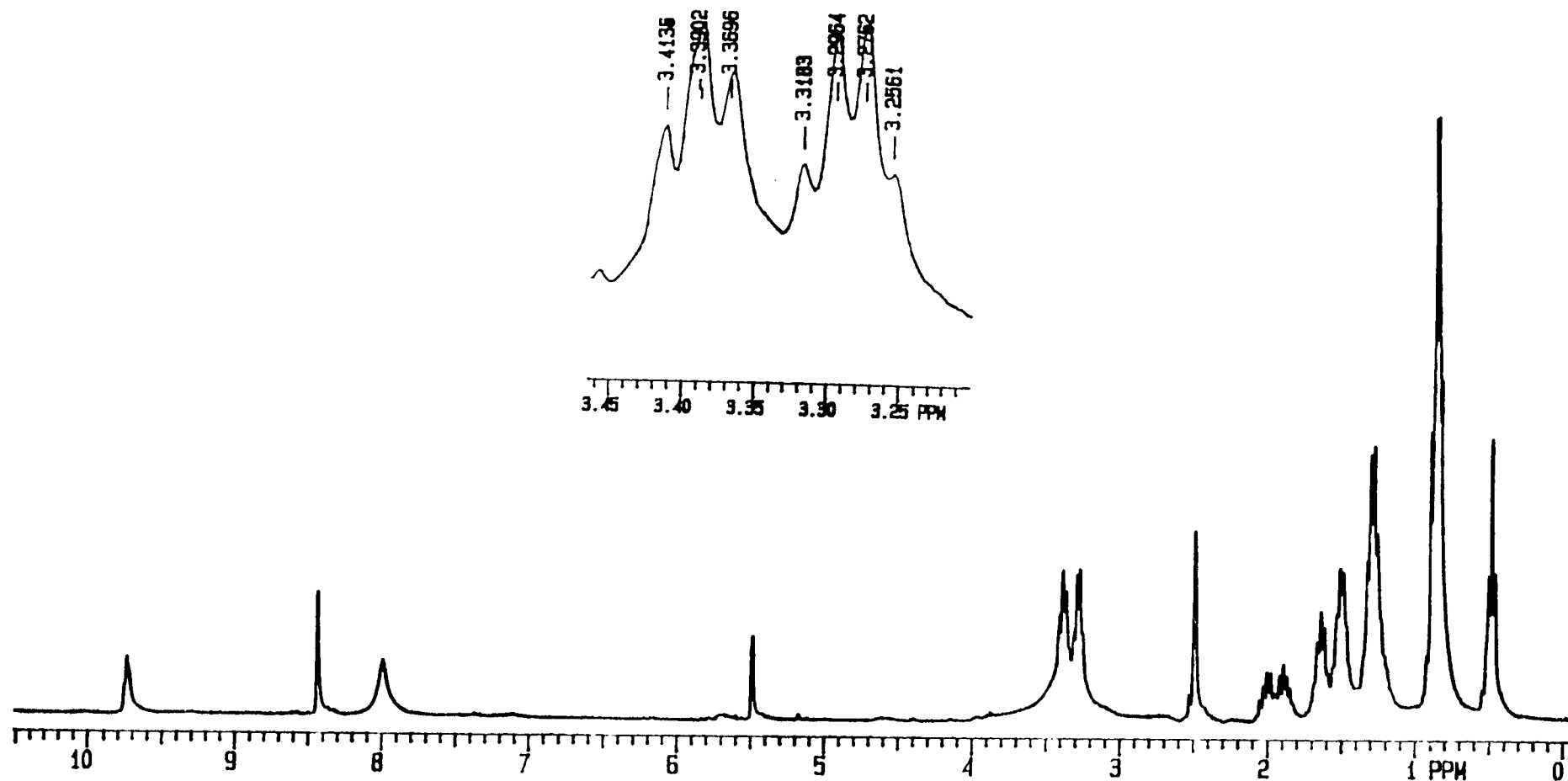
brown residue which was purified by flash chromatography on silica gel with 1:1 EtOAc-hexane to afford 5.68 g (96%) of 1,1-diethoxy-3-nonyne as yellow oil: ^1H NMR (CDCl_3) δ 0.86 (t, J = 6.87 Hz, 3H), 1.19 (t, J = 7.05 Hz, 6H), 1.26-1.38 (m, 4H), 1.39-1.50 (m, 2H), 2.12 (t, J = 6.99 Hz, 2H), 2.46 (app d, J = 5.61 Hz, 2H), 3.53 (dq, J = 8.2 and 7.1 Hz, 2H), 3.65 (dq, J = 8.2 and 7.2 Hz, 2H), 4.58 (t, J = 5.76 Hz, 1H); ^{13}C NMR (CDCl_3) δ 13.89 (-), 15.17 (-), 18.70 (+), 22.18 (+), 25.02 (+), 28.60 (+), 30.99 (+), 61.62 (+), 75.14 (+), 81.80 (+), 101.32 (-); HRMS calcd for ($\text{C}_{13}\text{H}_{24}\text{O}_2 - \text{C}_2\text{H}_5\text{O}$) m/z 167.1437, found 167.1436. Ozonized oxygen was bubbled through a solution of 2.16 g (10 mmol) of the latter alkyne in 50 mL of CHCl_3 at -78°C using a mechanical stirrer. When the persistence of blue color signaled complete reaction, the reaction solution was flushed with N_2 for 10 min, and Me_2S (1.47 mL, 20 mmol) was added (37). After 4 h, the reaction mixture was partitioned between CHCl_3 and water, the organic layer was concentrated, and the residue was purified by flash chromatography (EtOAc/hexane 1:2 v/v) to afford 2.24 g (92%) of 1,1-diethoxy-3,4-nonanedione: ^1H NMR (CDCl_3) δ 0.86 (t, J = 6.75 Hz, 3H), 1.14 (t, J = 7.11 Hz, 6H), 1.24-1.32 (m, 4H), 1.50-1.59 (m, 2H), 2.68 (t, J = 7.31 Hz, 2H), 3.03 (d, J = 5.94 Hz, 2H), 3.48 (dq, J = 8.2 and 7.1 Hz, 2H), 3.61 (dq, J = 8.2 and 7.0 Hz, 2H), 4.93 (t, J = 5.79 Hz, 1H). ^{13}C NMR (CDCl_3): δ 13.80 (-), 15.10 (-), 22.22 (+), 22.60 (+), 31.26 (+), 35.83 (+), 41.27 (+), 61.78 (+), 99.16 (-), 196.81 (+), 199.82 (+). HRMS calcd for $\text{C}_{13}\text{H}_{24}\text{O}_4$ 244.1675, $\text{C}_{11}\text{H}_{19}\text{O}_3$ ($\text{M}^+ - \text{C}_2\text{H}_5\text{O}$) 199.1335, found 199.1338. The latter acetal (1.0 g) was quantitatively deprotected in 30 mL of 2N HCl/acetone (1:2 v/v) to afford **5**, which exists exclusively in its enol form in both CHCl_3 and water: ^1H NMR (CDCl_3) δ 0.83 (t, J = 6.78 Hz, 3H), 1.21-1.29 (m, 4H), 1.52-1.62 (m, 2H), 2.75 (t, J = 7.31 Hz, 2H), 6.18 (d, J = 3.72 Hz, 1H), 8.61 (d, J = 3.18 Hz, 1H); ^{13}C NMR (CDCl_3) δ 13.89 (-), 22.41 (+), 22.87 (+), 31.27 (+), 36.92 (+), 98.63 (-), 184.28 (-), 196.70 (+), 199.79 (+); HRMS calcd for $\text{C}_9\text{H}_{14}\text{O}_3$ m/z 170.0943, found 170.0939.

Supp. 6, pp. 1-7

^{13}C NMR spectrum (75 MHz) of **1** in CD_3OD

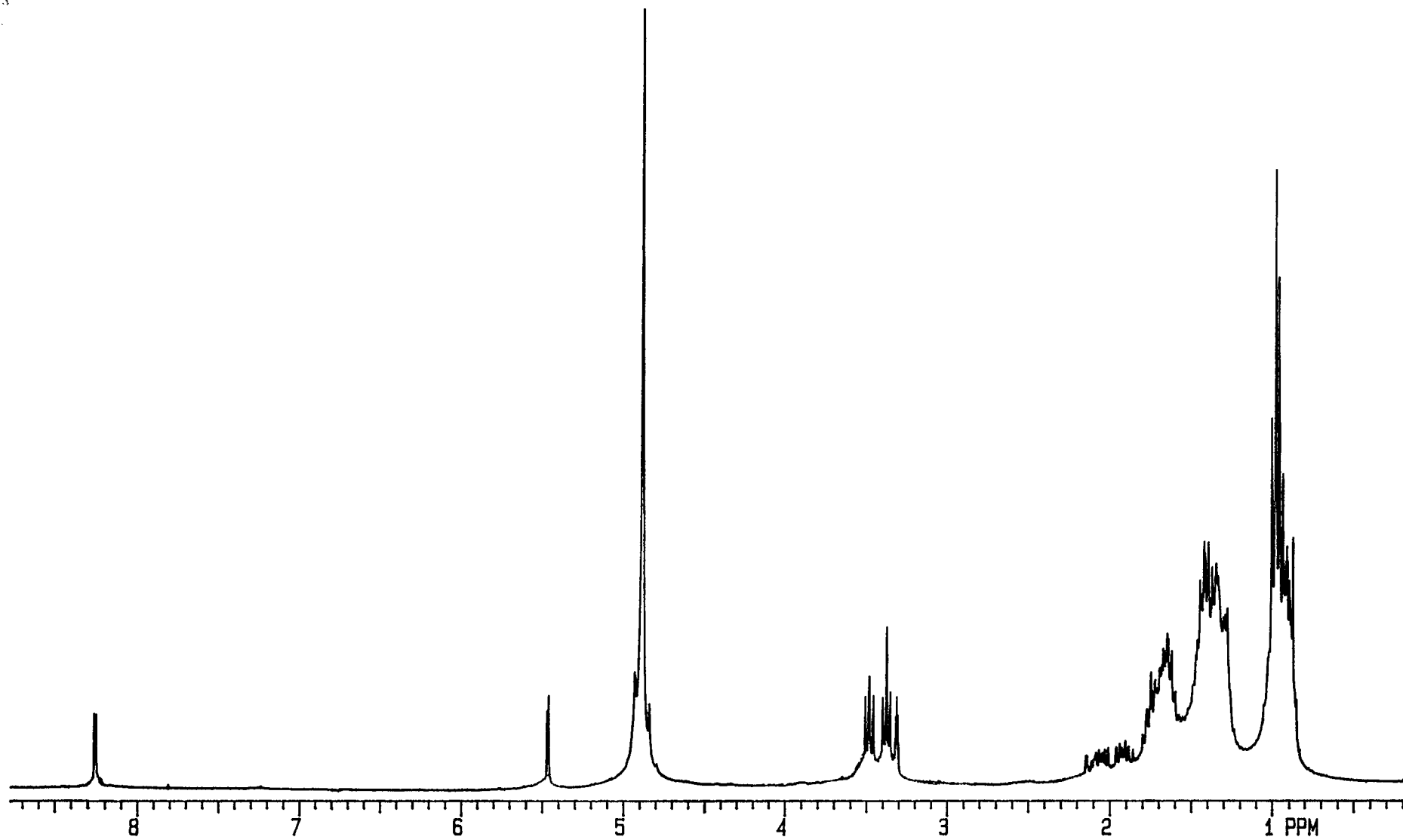


¹H NMR spectrum (300 MHz) of **1** in DMSO-*d*₆



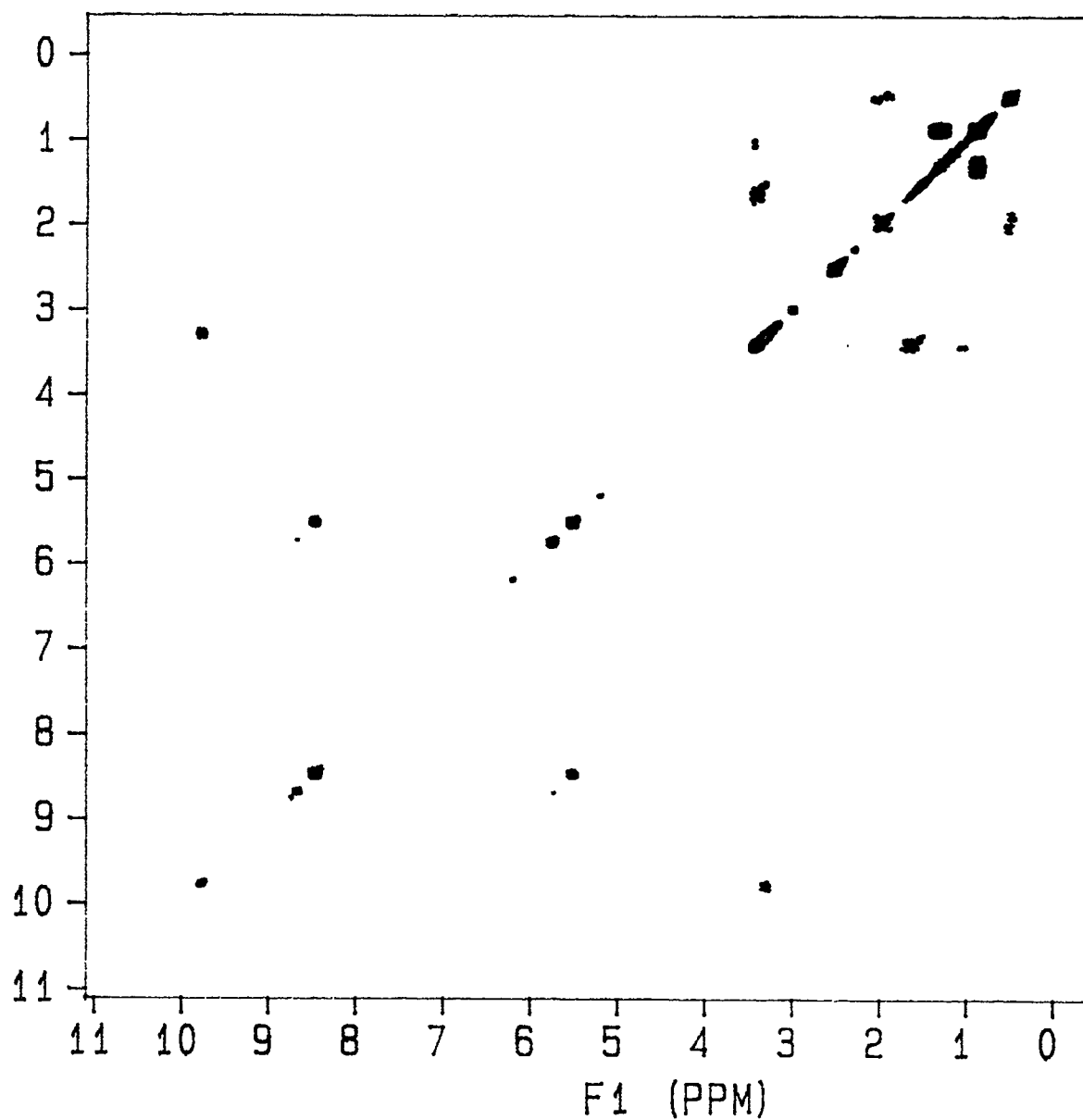
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^1H NMR spectrum (300 MHz) of **3** in CD_3OD



^1H - ^1H COSY spectrum (300 MHz) of **1** in DMSO- d_6

F2 (PPM)



Suppl. A17

HPLC chromatogram of the reaction mixture between HHE and *n*-butylamine after 2 days. UV detection was carried out at (A) 245-295 nm and (B) 355-365 nm using a Hewlett-Packard diode array detector (HP 1050). The reaction and HPLC conditions are described in the Experimental Section. Elution of the HHE-derived fluorophore **1** is indicated.

