Supplementary Information For

A Novel Azetidinyl γ-Lactam Based Peptide With a Preference for β-Turn Conformation

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Page No. S2-S7: Experimental procedures

Page No. S8-S18: ^{1}H NMR,^{13}C NMR, VT NMR and NOESY spectra of representative compounds

Page No S19: Energy minimized conformation of **1**

Page No S20-S28: ORTEP diagram and X-Ray Crystallographic data of compound **11**

Experimental Section

General

Melting points (m.p.) were recorded on a hot-coil stage melting point apparatus and are uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 200 MHz spectrometer unless otherwise stated. ^{2}H-chloroform was used as solvent unless otherwise mentioned. The peak at δ 7.26 was taken as reference. Chemical shifts are expressed in δ unit and ^{1}H-^{1}H coupling constants in Hz. IR spectra were recorded using KBr pellet for solids and neat for liquids. The characteristic peaks are expressed in cm^{-1}.Mass spectra were obtained from CRF, IIT-Kharagpur

.All the dry solvents used for reactions were purified according to the standard protocols. Benzene, tetrahydrofuran (THF) were distilled from sodium / benzophenone under an atmosphere of dry argon / nitrogen. Chloroform and dichloromethane were dried over phosphorous pentoxide (P_2O_5) or calcium hydride (CaH_2). Ethanol and methanol were dried first over calcium oxide (CaO) and then over magnesium turnings. Dimethyl formamide was dried by azeotropic removal of water with benzene followed by distillation over calcium hydride. Frying-dried potassium carbonate was used for alkylation reactions. All the solvents for chromatography (column and preparative layer) were distilled prior to use. In most of the column chromatographic purifications, ethyl acetate (EA) and petroleum ether (PE) of boiling range 60-80 oC were used as eluents. Columns were prepared with silica gel (60-120 mesh). For preparative layer chromatographic (PLC) purification, the layer was formed over glass plate using water gel. The silica gel-GF 254 was used for PLC plate preparation.

Synthesis of Ethyl Pyroglutamate (5)

To a solution of pyroglutamic acid (5 g, 38.76 mmol) in ethanol (25 ml) dry benzene (6 ml) and 2 drops of conc. H_2SO_4 were added. After refluxing for 5 h, benzene was evaporated and then partitioned between chloroform and water. The organic layer was washed with aq. $NaHCO_3$, then with brine and dried over Na_2SO_4. Removal of solvent afforded a crude solid from which the title compound was isolated by column chromatography (Si-gel, PE : EA = 1:1) as a white solid (79%); m.p. 46 ^{o}C; 7.00 (s, 1H),

4.22–4.18 (m, 1H), 4.16 (q, J = 7.0 Hz, 2H), δ_H 2.41-2.10 (m, 4H), 1.24 (t, J = 7.0 Hz, 3H).

Synthesis of N-Propargyl ethyl pyroglutamate (6)

To a stirring suspension of pulverized KOH (1.1 eq) and tetra-n-butyl ammonium bromide (0.2 eq) in 100 ml dry THF, a solution of propargyl bromide (1 eq) and ethyl pyroglutamate (1 eq) in 100 ml dry THF was added over an hour at room temperature. The precipitate was filtered off and the filtrate was evaporated to give an oily residue. Ether was added and the precipitated material was filtered off. The filtrate was washed with water and brine. The organic layer was dried over Na_2SO_4 and evaporated. The title compound was isolated from purified by column chromatography (Si-gel, PE : EA = 2:1) as pale brown oil (72%); δ_H 4.49 (dd, J = 2.6, 17.6 Hz, 1H), 4.32-4.26 (m, 1H), 4.12 (q, J = 7.0 Hz, 2H), 3.66 (dd, J = 2.4, 17.6 Hz, 1H), 2.38-1.97 (m, 5H), 1.19 (t, J = 7.0 Hz, 3H).

Synthesis of 5-Oxo-1-(2-oxo-1, 4-diphenyl-azetidin-3-yl methyl)-pyrrolidine-2-carboxylic acid ethyl ester (8-10)

To a solution of N-Propargyl ethyl pyroglutamate (1 mmol) in CH_3CN under argon at 0 ^oC, triethyl amine (1 eq) was added and the mixture stirred for 30 minutes. Cuprous iodide (1 eq) was added and the solution was stirred for another 5 min at 0 ^oC. A solution of the nitrone (1 eq) was added slowly over 5 min. After stirring for another 30 min at 0 ^oC, the reaction was stirred at room temperature for 4 h. The mixture was diluted with water and filtered through celite. The celite bed was washed with ethyl acetate. The combined filtrate and washings were extracted with ethyl acetate. The organic layer was washed with NH_4Cl, water and brine and dried over Na_2SO_4 and evaporated. The residue, obtained after evaporation, upon chromatography afforded three diastereomers: one *trans* and a pair of *cis* diastereomers (*trans*:combined *cis* = 1:1). The trans isomer could be easily separated from the cis pair by conventional chromatography over silica gel using hexane/ethyl acetate (4:1) as eluent; yield: 65% (overall).

5-Oxo-1-(2-oxo-1, 4(S)-diphenyl-azetidin-3(R)-ylmethyl)-pyrrolidine-2-carboxylic acid ethyl ester (8)

State: White solid; m.p. 112 ^{o}C; ν_{max} (neat) 2954, 1753, 1697, 1598, cm^{-1}; δ_H, 7.39-7.20 (m, 9H), 7.10-7.05 (m, 1H), 5.02 (d, *J* = 2.2 Hz, 1H), 4.41-4.37 (m, 1H), 4.19-4.05 (m, 3H), 3.55 (dd, *J* = 6.0, 14.9 Hz, 1H), 3.30-3.25 (m, 1H), 2.48-2.40 (m, 4H), 1.26-1.19 (m,3H); δ_C 176.5, 171.7, 165.4, 137.2, 129.0, 128.5, 125.9, 124.0, 117.1, 61.6, 60.9, 59.5, 59.2, 39.9, 29.1, 23.5, 14.1; HRMS calcd. for, $C_{23}H_{24}N_2O_4$, 392.1737; found 392.1740.

5-Oxo-1-(2-oxo-1, 4(S)-diphenyl-azetidin-3(S)-ylmethyl)-pyrrolidine-2-carboxylic acid ethyl ester (9)

State: White solid; m.p. 102 ^{o}C; ν_{max} (neat) 3272, 2927, 1742, 1689, 1669, 1549, cm^{-1}; δ_H 7.39-7.17 (m, 9H) 7.09-7.04 (1H, m), 5.21 (d, *J* = 6.06 Hz, 1H), 4.73-4.69 (m, 1H), 4.21-4.12 (m, 2H), 3.79-3.71 (m, 1H), 3.56 (dd, *J* = 4.7, 14.5 Hz, 1H), 2.67 (dd, *J* = 11.2, 14.5 Hz, 1H), 2.43-2.30 (m, 3H), 2.13-2.06 (m, 1H), 1.24 (t, *J* = 7.0 Hz, 3H), δ_C 175.1, 171.9, 166.2, 137.2, 133.7, 129.0, 128.9, 128.6, 126.6, 124.0, 117.1, 61.3, 60.0, 56.5, 52.1, 38.7, 29.2, 22.9, 14.0; HRMS calcd. for $C_{23}H_{24}N_2O_4$ 392.1737; found 392.1741.

5-Oxo-1-(2-oxo-1, 4(R)-diphenyl-azetidin-3(R)-ylmethyl)-pyrrolidine-2-carboxylic acid ethyl ester (10)

State: White solid; m.p. 110 ^{o}C; ν_{max} (neat): 2929, 1743, 1686, 1486 cm^{-1}; δ_H 7.41-7.18 (9H, m); 7.09-7.03 (m, 1H), 5.21 (d, *J* = 5.8 Hz, 1H), 4.31-4.20(m, 1H), 4.14 (q, 2H), 3.69 (dd, *J* = 7.3, 14.5 Hz, 1H), 3.22-3.17 (m, 1H), 2.97 (dd, *J* = 8.8, 14.5 Hz, 1H), 2.31-1.90 (m, 4H), 1.24 (t, *J* = 7.2 Hz, 3H); HRMS calcd. For $C_{23}H_{24}N_2O_4$ 392.1737; found 392.1741.

Synthesis of 5-Oxo-1-(2-oxo-1, 4-diphenyl-azetidin-3-ylmethyl)-pyrrolidine-2-carboxylic acids (11-13)

To a solution of esters (1 mmol) in THF (10ml) and water (0.5 ml), LiOH powder (1 mmol) was added and stirred for 6 h at room temperature. THF was evaporated and the residue was diluted with water and treated with 2 (N) HCl to bring the pH to 2. The solution was extracted with ethyl acetate and the organic layer was washed with water, brine, dried and evaporated under vacuum and purified by column chromatography (Si-gel, CH_2Cl_2 : MeOH = 2:1) to afford the products. Yield: 75%;

5-Oxo-1-(2-oxo-1, 4(S)-diphenyl-azetidin-3(R)-ylmethyl)-pyrrolidine-2-carboxylic acid (11)

State: White solid; m.p. 119 ^{0}C; ν_{max} (neat): 3442, 2929, 1739, 1677, 1598 cm^{-1}; δ_H (CD_3OD): 7.39-7.20 (m, 9H), 7.06-7.00 (m, 1H) 5.04 (d, *J* = 2.3 Hz, 1H), 4.41-4.37 (m, 1H), 4.14–4.05 (m, 1H), 3.53 (dd, *J* = 6.6, 14.6 Hz, 1H), 3.42-3.37 (m, 1H), 2.39-2.06 (m, 4H), δ_C (CD_3OD): 179.1, 174.8, 164.2, 138.7, 138.5, 130.2, 130.1, 129.7, 127.8, 125.3, 118.3, 62.3, 60.7, 60.1, 41.2, 30.2, 24.3.

5-Oxo-1-(2-oxo-1, 4(R)-diphenyl-azetidin-3(R)-ylmethyl)-pyrrolidine-2-carboxylic acid (12) and 5-Oxo-1-(2-oxo-1,4(S)-diphenyl-azetidin-3(S)-ylmethyl)-pyrrolidine-2-carboxylic acid (13)

State: White solid; m.p. 117 ^{0}C; ν_{max} (neat): 3472, 2915,1740, 1663, 1596 cm^{-1}; δ_H (CD_3OD) 7.35-7.17 (m, 8H, for both); 7.06-6.94 (m, 2H, for both), 5.29 (d, *J* = 5.9 Hz, 1H for both), 4.56-4.51 (m, 1H major) 4.20-4.09 (m, 1H, minor), 3.83-3.75 (m, 1H, major and minor), 3.48 (dd, *J* = 6.3, 14.3 Hz, 1H, major), 3.41-3.34 (m, 1H, minor), 2.89 (dd, *J* = 9.6, 14.6 Hz, 1H, major), 2.84-2.78(m, 1H, minor), 2.35-2.0 (m, 4H, for both major and minor isomer), δ_C (CD_3OD): 178.3, 177.6, 175.8, 173.1, 167.7, 167.2, 138.5, 135.7, 135.3, 130.1, 130.0, 129.9, 129.6, 128.3, 128.0, 125.2, 118.3, 61.5, 58.6, 57.9, 52.7, 52.5, 39.6, 39.5, 30.3, 30.22, 24.2, 24.0.

Procedure for Peptide Coupling

To the solution the N- tosylato glycinyl-L-phenylalanyl benzhydryl ester[15] (**14**) (1 eq) in dry dichloromethane, acids [5-oxo-1-(2-oxo-1, 4-diphenyl-azetidin-3-ylmethyl)-pyrrolidine-2-carboxylic acid] (1eq) dissolved in CH_2Cl_2 was added followed by 1-[3-dimethyl aminopropyl]-3-ethylcarbodiimide hydrochloride (EDCI.HCl) (1 eq). The reaction mixture was cooled to 0 ^{o}C and stirred for 15 minutes, after that it was allowed to warm to room temperature, then HOBT (1.2 eq) and DMAP (1.2 eq) was added and stirred for another 6h. After partitioning between CH_2Cl_2 and water (50 ml each), the organic layer was washed brine, dried over Na_2SO_4 and evaporated. From the oily residue, the title compound was isolated pure by column chromatography.

5-Oxo-1-(2-oxo-1, 4(S)-diphenyl-azetidin-3-ylmethyl)-pyrrolidine-2-carboxylic acid benzylamide (4)

Yield 76%; State: White solid; m.p. 106 ^{o}C; ν_{max} (neat): 3374, 2926,1749, 1662, 1492 cm^{-1}; δ_H (d_6-DMSO, 500 MHz): 8.68 (t, J = 5.8Hz, 1H), 7.33-7.11 (m, 14H), 6.98 (t, J = 7.4 Hz, 1H), 4.99 (d, *J* = 2.2 Hz, 1H), 4.23-4.17 (m, 3H), 3.89 (dd, *J* = 5.8, 14.4 Hz, 1H),

3.30-3.27 (m, 1H), 3.20 (dd, *J* = 7.2, 14.4 Hz, 1H), 2.26-2.07 (m, 3H), 1.81-1.79 (m, 1H); δ_C (d_6-DMSO, 50MHz): 175.4, 170.9, 165.1, 139.0, 137.6, 137.1, 129.2, 129.0, 128.3, 127.2, 126.9, 126.1, 123.8, 116.7, 60.6, 58.5, 57.9, 42.2, 29.1, 23.3; δ_C ($CDCl_3$, 50MHz): 176.9, 170.4, 166.6, 137.7, 136.9, 136.6, 129.1, 128.7, 128.6, 127.8, 127.6, 125.9, 124.4, 117.7, 61.7, 59.3, 58.3, 43.7, 38.2, 29.5, 22.7; HRMS calcd. for $C_{28}H_{27}N_3O_3$, 453.2054; found 453.2070.

2-(2-{[5-Oxo-1-(2-oxo-1, 4(S)-diphenyl-azetidin-3(R)-ylmethyl)-pyrrolidine-2-carbonyl]-amino}-acetylamino) -3-phenyl-propionic acid benzhydryl ester (1)

Yield 64%; State: White solid; m.p. 99 ^{0}C; ν_{max} (neat): 3302, 2928, 1745, 1670, 1599 cm^{-1}; δ_H (500MHz, d_6-DMSO): 8.27 (d, *J* = 7.8 Hz, 1H); 8.21 (t, *J* = 5.8Hz, 1H.), 7.18-6.96 (m, 24H), 6.83 (t, *J* = 7.3 Hz, 1H), 6.56 (s, 1H), 4.87 (d, *J* = 1.75 Hz, 1H), 4.46 (m, 1H), 4.09 (dd, *J* = 3.4, 8.5 Hz, 1H), 3.75 (dd, *J* = 5.3, 14.0 Hz, 1H), 3.54 (d, *J* = 5.4 Hz, 1H), 3.19-3.06(m, 1H), 3.11 (dd, *J* = 7.56, 14.0 Hz, 1H), 2.88 (dd, J = 5.87, 13.9 Hz, 1H), ,), 2.73 (dd, *J* = 8.95, 13.8 Hz, 1H), 2.07-1.89 (m, 3H), 1.66-1.63 (m, 1H); δ_H (200MHz, $CDCl_3$): 7.35-7.01 (m, 24H); 6.89-6.81(m, 3H), 6.72 (d, *J* = 7.6 Hz, 1H), 4.98(q, *J* = 7.4 Hz, 1H), 4.88 (d, *J* = 2.1 Hz, 1H), 4.24-4.01(m, 2H), 3.77(dd, *J* = 4.9, 16.6 Hz, 1H), 3.24-3.03 (m, 5H), 2.55-2.35 (m, 2H), 2.15-2.04 (m, 2H); δ_C (50MHz, $CDCl_3$): 176.8, 171.2, 170.5, 168.2, 166.8, 139.2, 136.9, 136.7, 135.3, 129.2, 129.1, 128.6, 128.5, 128.2, 128.0, 127.5, 127.0, 126.8, 126.5, 125.9, 124.4, 117.2, 78.3, 61.5, 59.1, 58.3, 53.3, 42.9, 38.2, 37.3, 29.4, 22.9; MS (EI) *m/z* 735 (MH^+); HRMS calcd. for, $C_{45}H_{42}N_4O_6 + H^+$, 735.3185; found 735.3214.

2-(2-{[5-Oxo-1-(2-oxo-1, 4(S)-diphenyl-azetidin-3(S)-ylmethyl)-pyrrolidine-2-carbonyl]-amino}-acetylamino) -3-phenyl-propionic acid benzhydryl ester

Major Isomer (2)

Yield 62% (major + minor); State: White solid; m.p. 76 ^{o}C; ν_{max} (neat): 3374, 2930,1748, 1666, 1586 cm^{-1}; δ_H (d_6-DMSO, 500MHz): 8.42 (d, *J* = 7.8 Hz, 1H); 8.33 (t, *J* = 5.8Hz, 1H), 7.39-7.10 (m, 24H), 7.05 (t, J=7.32 Hz, 1H), 6.75 (s, 1H), 5.39 (d, *J* = 6.0 Hz, 1H), 4.69-4.65 (m,1H), 4.39 (dd, J = 2.5, 8.7 Hz, 1H), 3.84-3.80(m, 1H), 3.74 (dd, *J* = 5.75, 15.70 Hz, 1H), 3.70-3.68 (m,1H), 3.24 (dd, J = 6.3, 14.2 Hz, 1H), 3.08 (dd, J = 5.9, 13.7 Hz, 1H), 2.93(dd, J = 8.6, 13.7 Hz, 1H), 2.63(dd, J = 9.6, 14.2 Hz, 1H), 2.15-2.04 (m,

3H), 1.85-1.79 (m, 1H); MS (EI) *m/z* 735 (MH^+); HRMS calcd. for, $C_{45}H_{42}N_4O_6$+ H^+, 735.3185; found 735.3269 (MH^+).

2-(2-{[5-Oxo-1-(2-oxo-1, 4(R)-diphenyl-azetidin-3(R)-ylmethyl)-pyrrolidine-2-carbonyl]-amino}-acetylamino) -3-phenyl-propionic acid benzhydryl ester

Minor Isomer (3)

State: White solid; m.p. 76 ^oC; ν_{max} (neat): 3374, 2930, 1748, 1666, 1586 cm^{-1}; δ_H (500MHz, d_6-DMSO): 8.46 (d, *J* = 7.8 Hz, 1H). 8.18(t, *J* = 5.8 Hz, 1H), 7.39-7.10 (m, 24H), 7.05 (t, *J* = 7.32 Hz, 1H), 6.75 (s, 1H), 5.36 (d, *J* = 6.0 Hz, 1H), 4.69-4.65 (m, 1H), 4.04 (dd, *J* = 7.8,15.4 Hz, 1H), 3.84-3.78 (m, 2H), 3.70-3.67 (m, 1H), 3.53 (m, 1H), 3.10-3.06 (m, 1H), 2.95-2.90 (m, 1H), 2.46(m, 1H), 2.15-2.04 (m, 3H), 1.85-1.79 (m, 1H);

Mixture δ_C ($CDCl_3$, 50MHz): 175.6, 172.2, 170.4, 167.9, 167.0, 139.3, 139.2, 137.0, 135.2, 133.3, 129.3, 129.0, 128.7, 128.6, 128.5, 128.2, 128.0, 127.5, 127.0, 126.8, 124.3, 117.4, 78.2, 61.4, 57.1, 52.3, 52.4, 42.9, 38.3, 37.6, 31.5, 29.5, 22.6, 22.1; MS (EI) *m/z* 735 (MH^+); HRMS calcd. for, $C_{45}H_{42}N_4O_6 + H^+$, 735.3185; found 735.3255 .

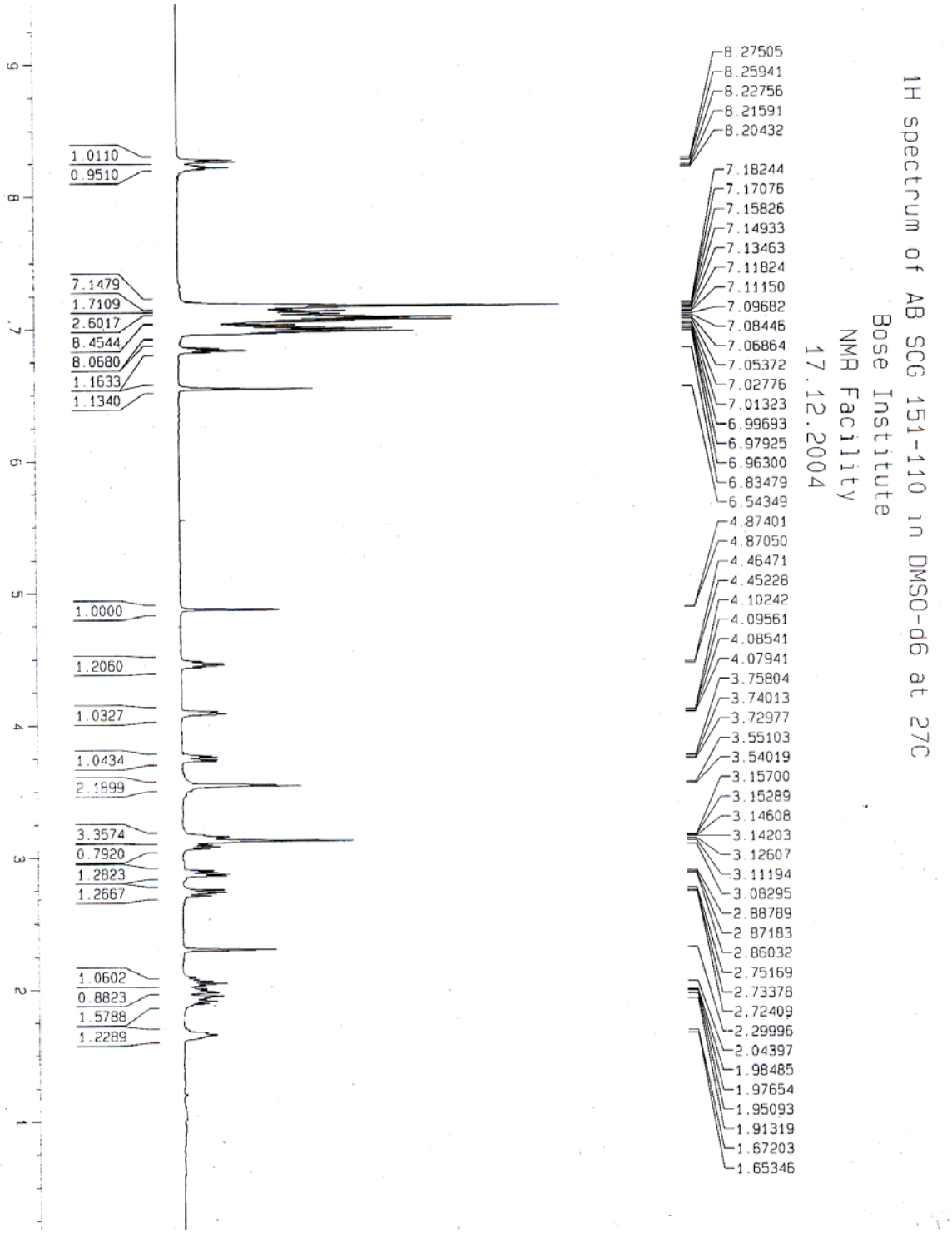

Figure S1: ^{1}H NMR for compound **1** in d_6DMSO

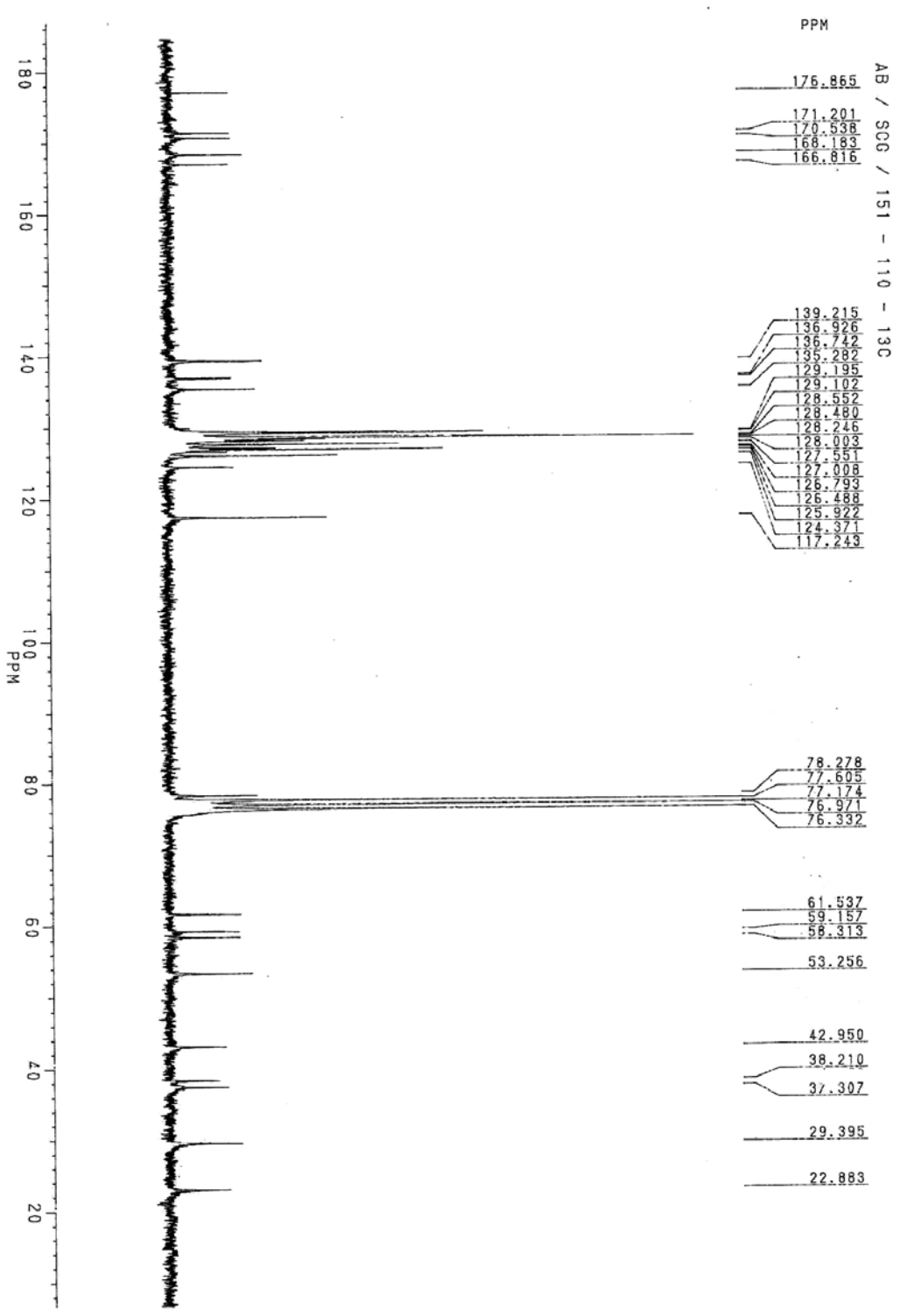

Figure S2: ^{13}C NMR for compound **1** in $CDCl_3$

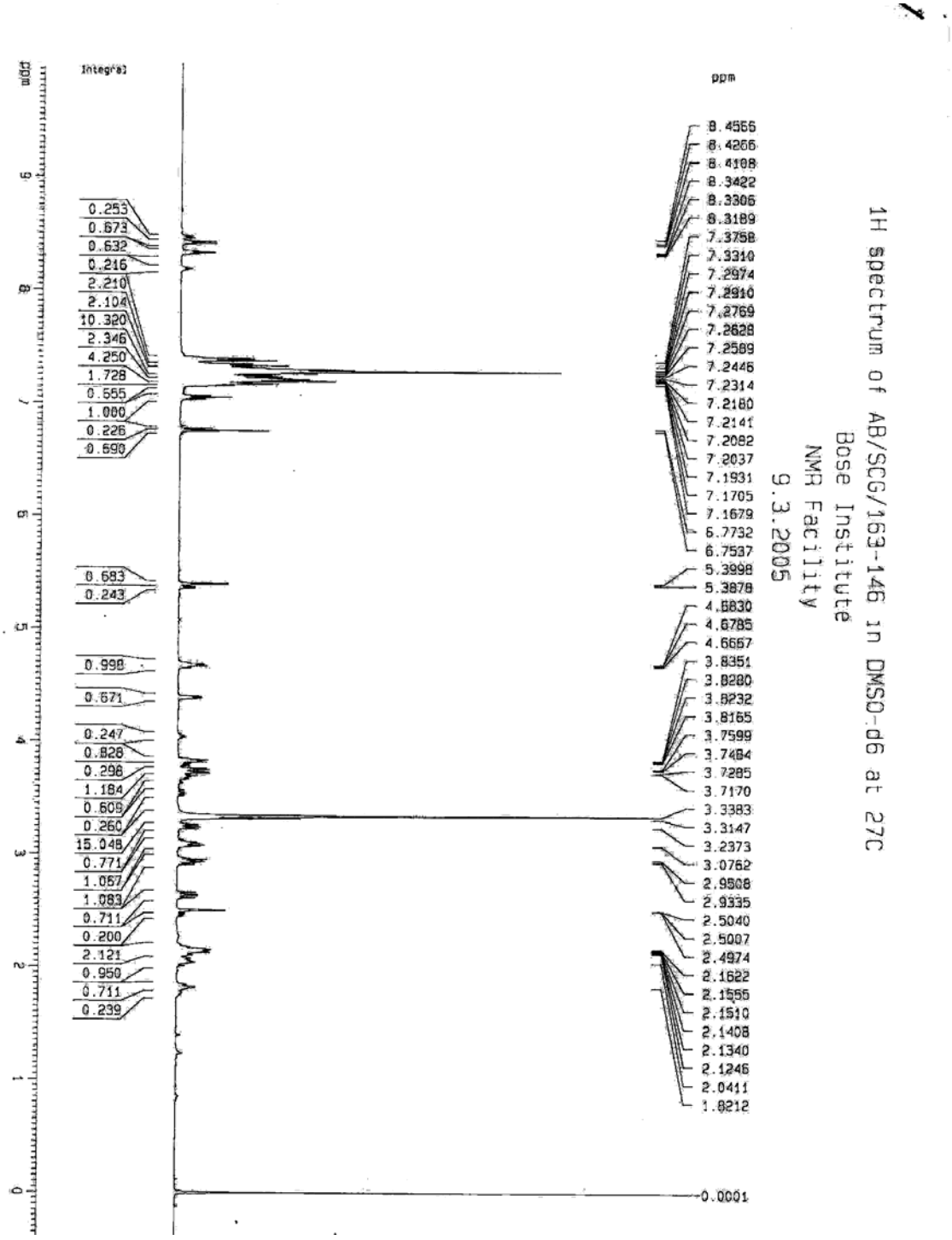

Figure S3: ^{1}H NMR for compound **2 & 3** in d_6DMSO

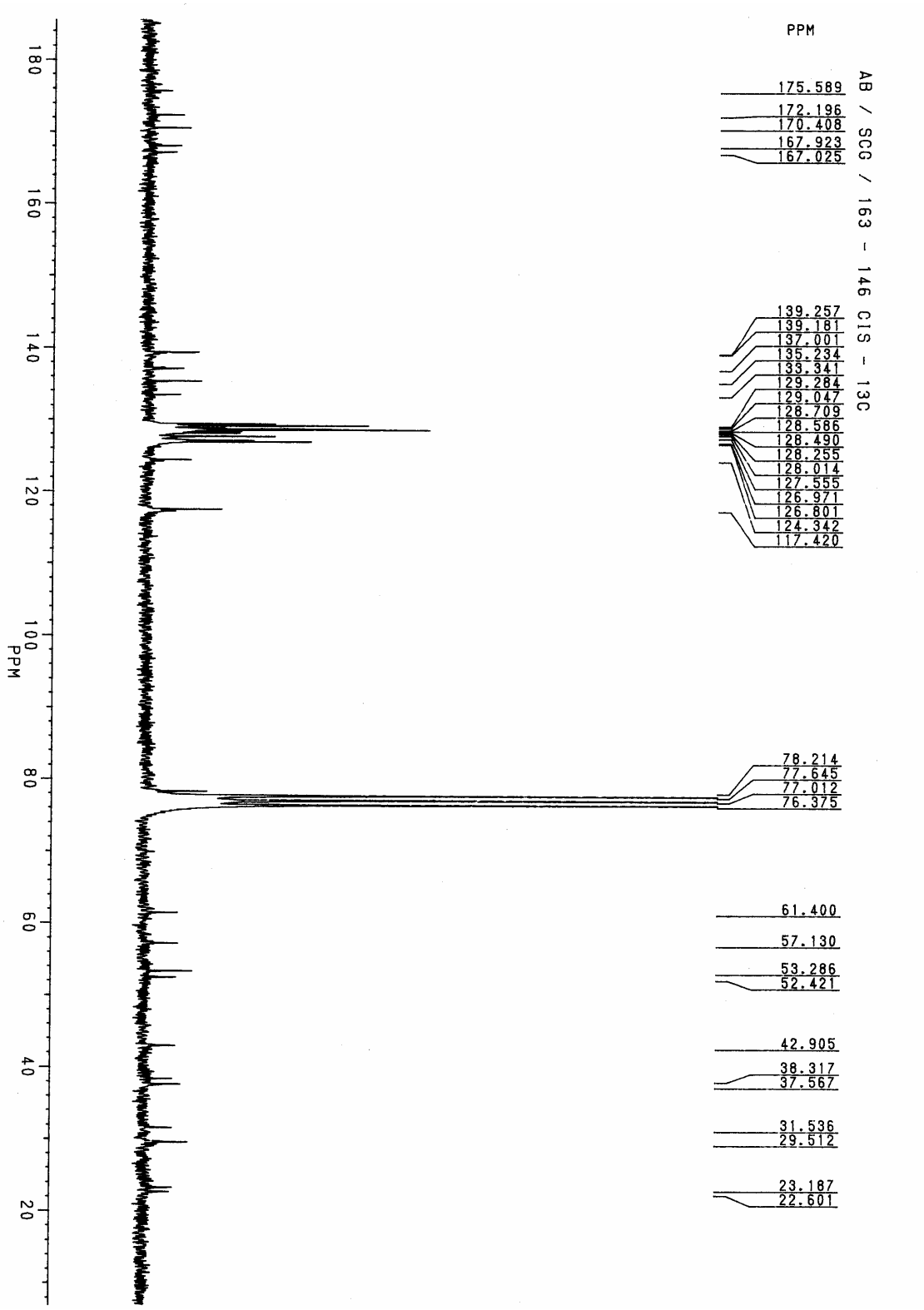

Figure S4: ^{13}C NMR for compound **2 & 3** in $CDCl_3$

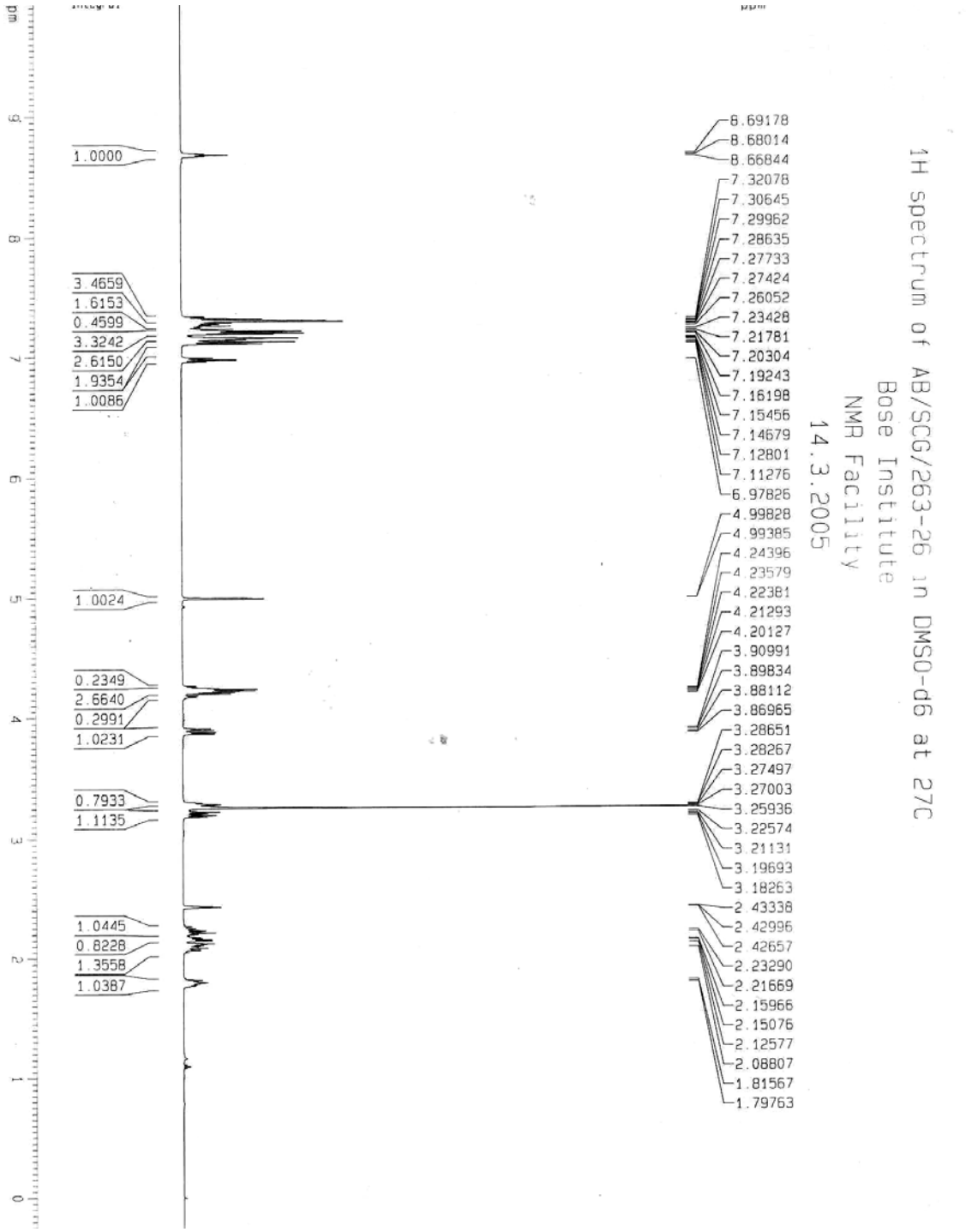

Figure S5: ^{1}H NMR for compound **4** in d_6DMSO

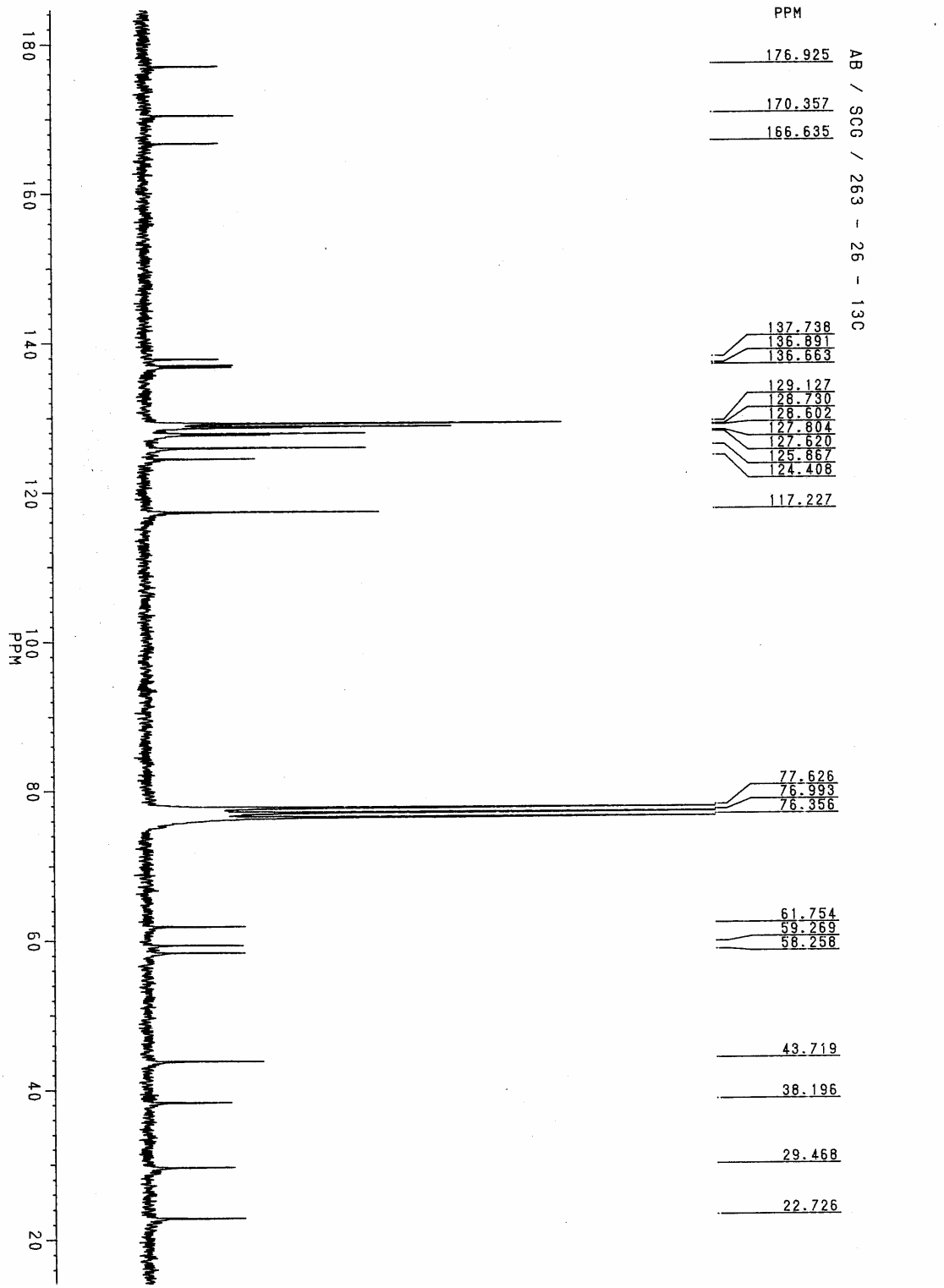

Figure S6: ^{13}C NMR for compound **4** in $CDCl_3$

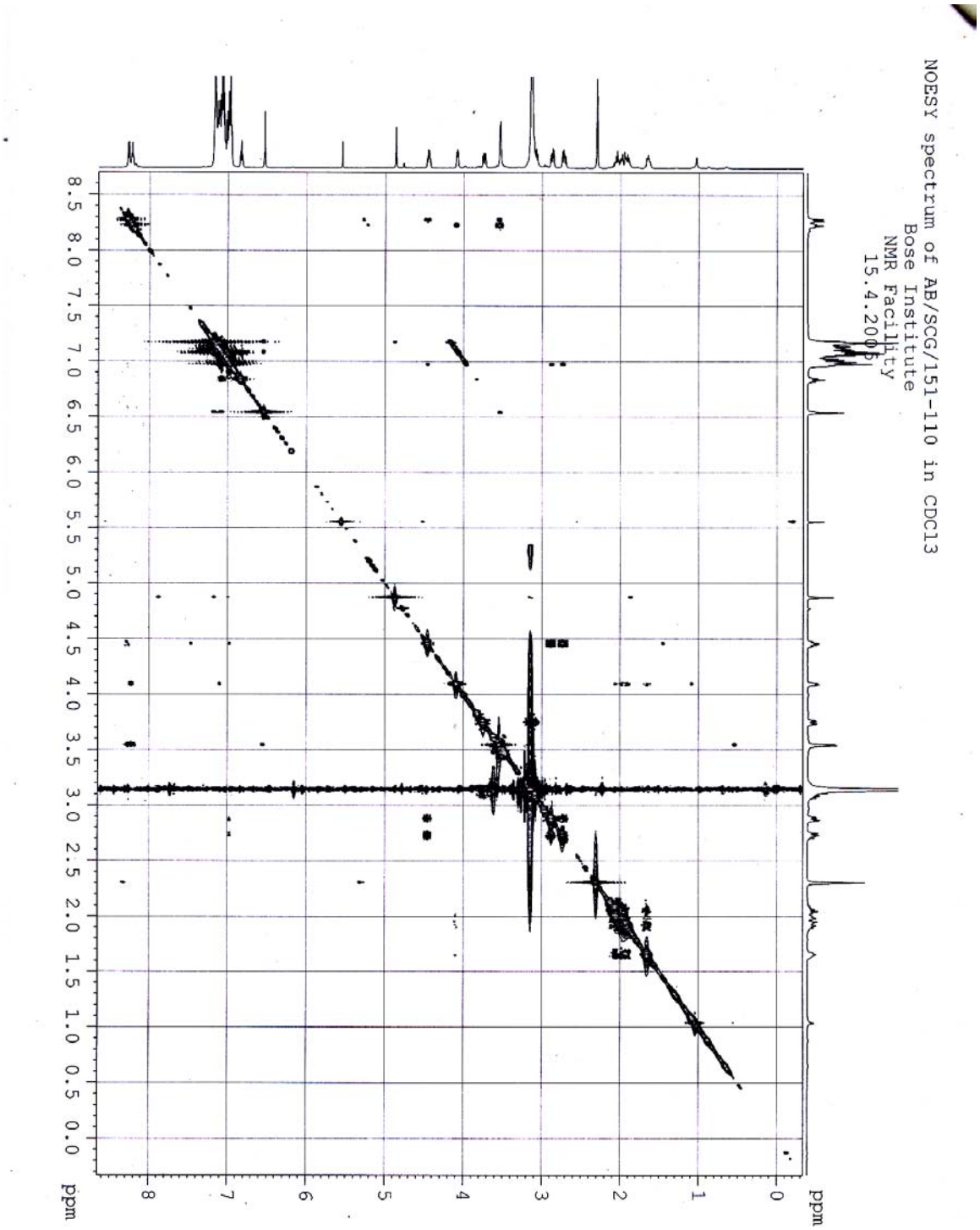

Figure S7: NOESY spectra for compound **1** in d_6DMSO

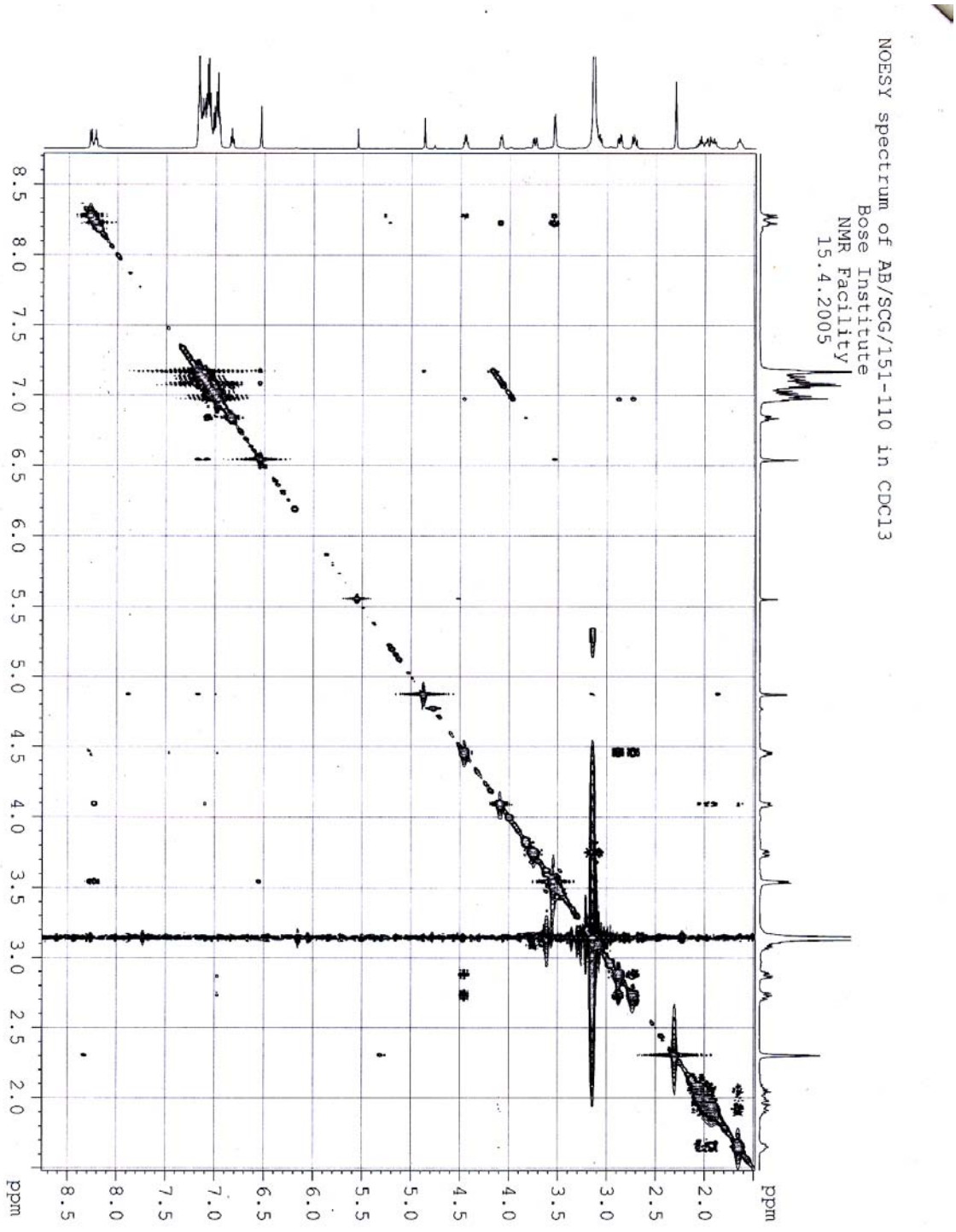

Figure S8: NOESY spectra for compound **1** in d_6DMSO

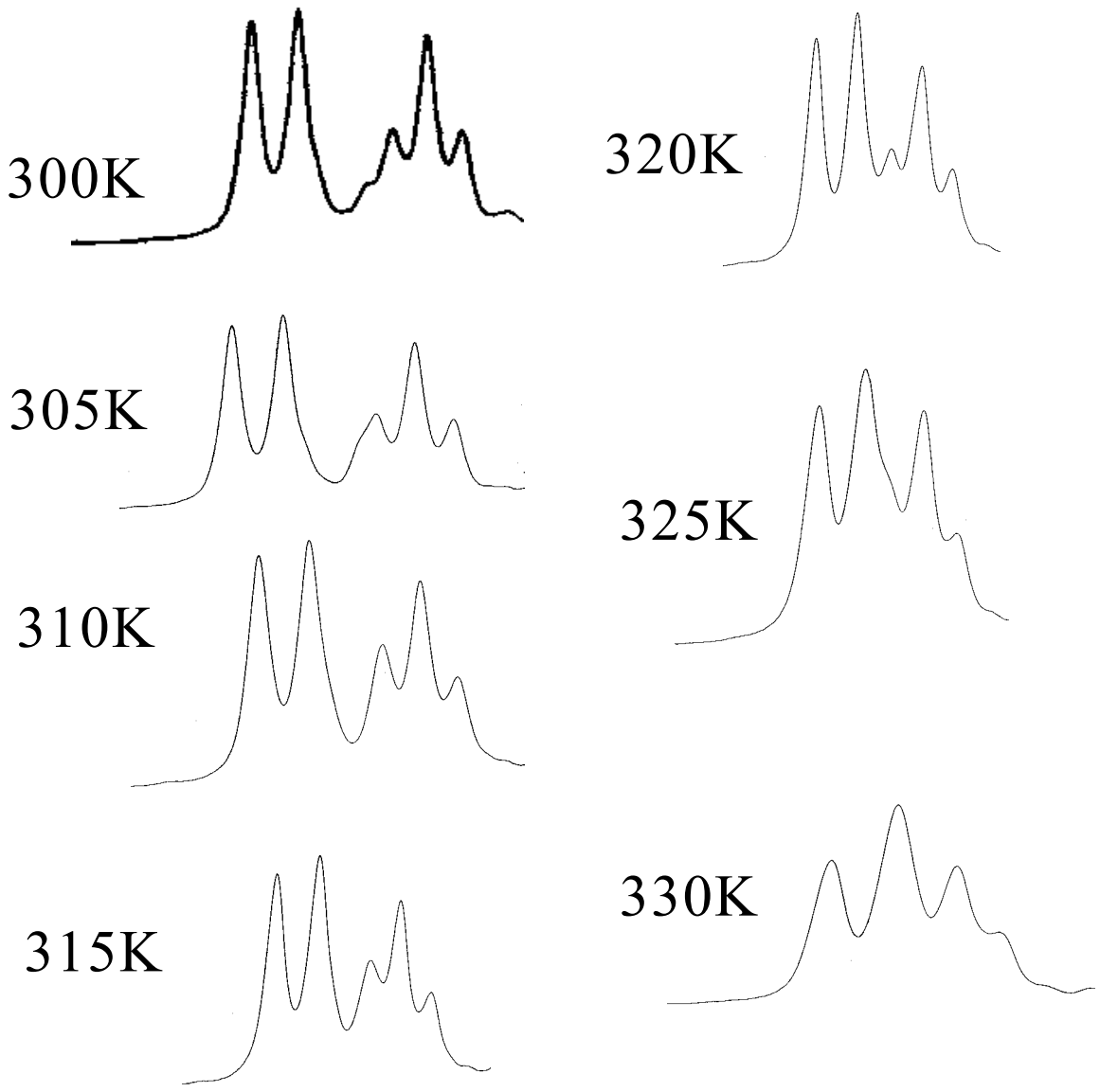

Figure S9: Variable Temperature (VT) spectra for compound **1** in d_6DMSO

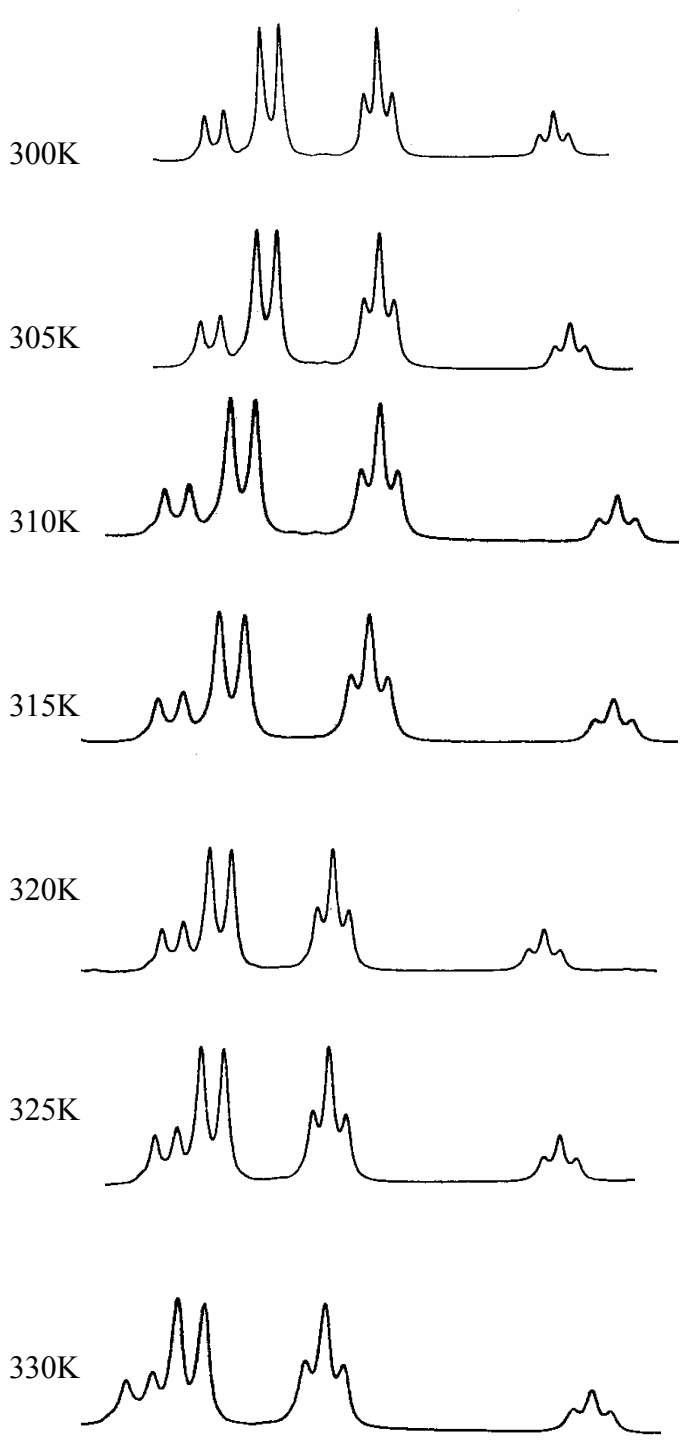

Figure S10: Variable Temperature (VT) spectra for compound **2 & 3** in d_6DMSO

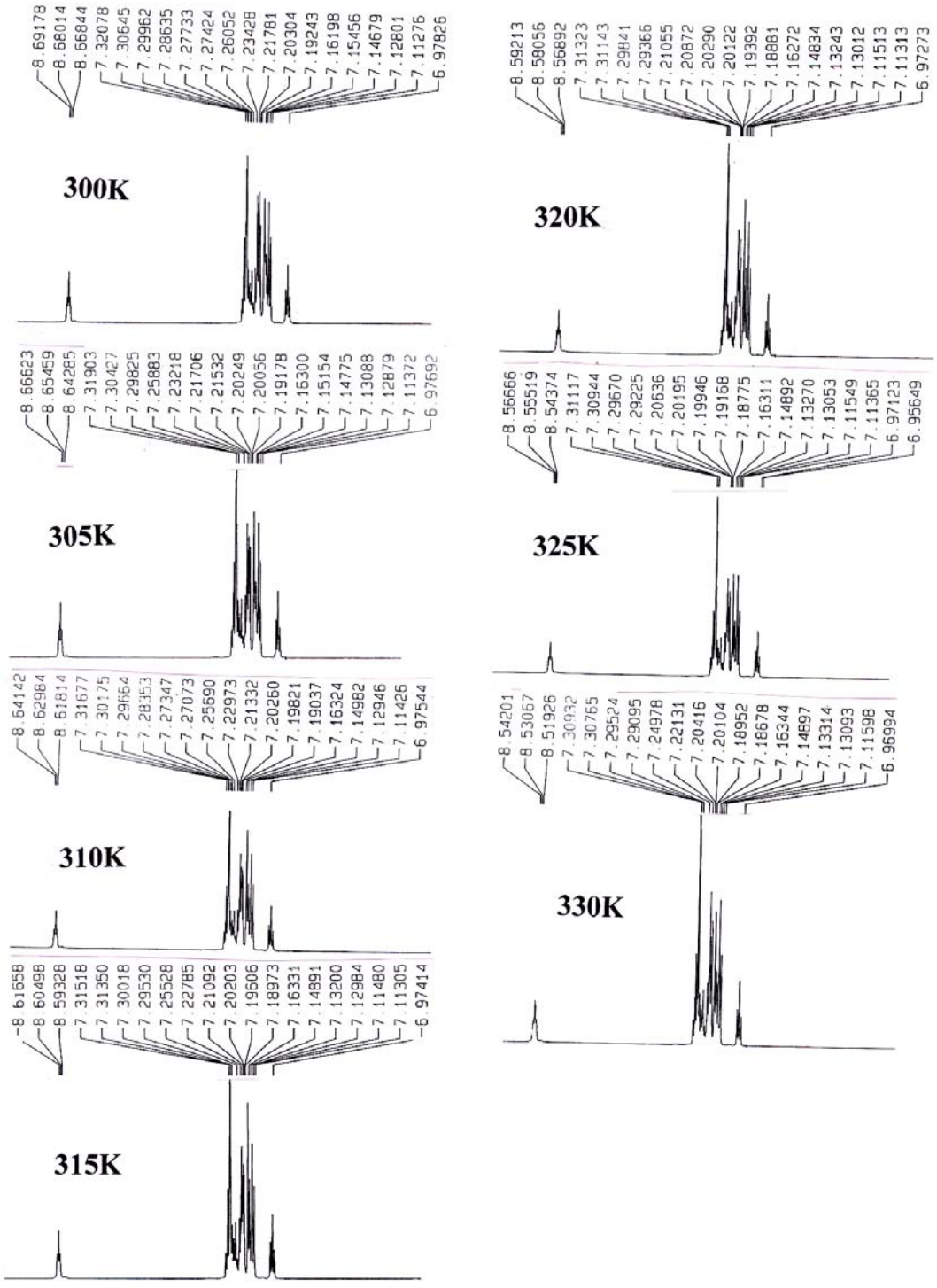

Figure S11: Variable Temperature (VT) spectra for compound **4** in d_6DMSO

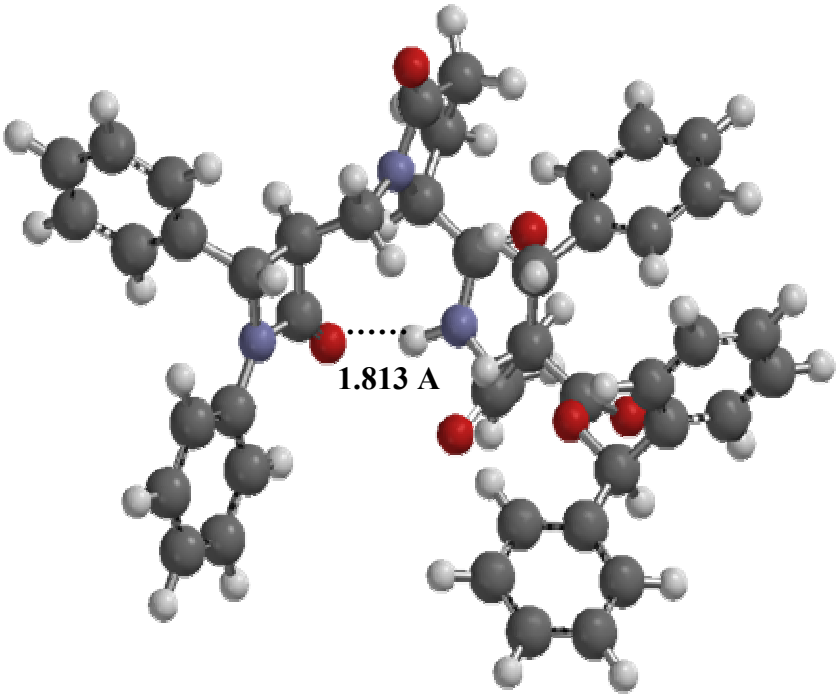

Figure S12: Energy minimized conformation of **1**

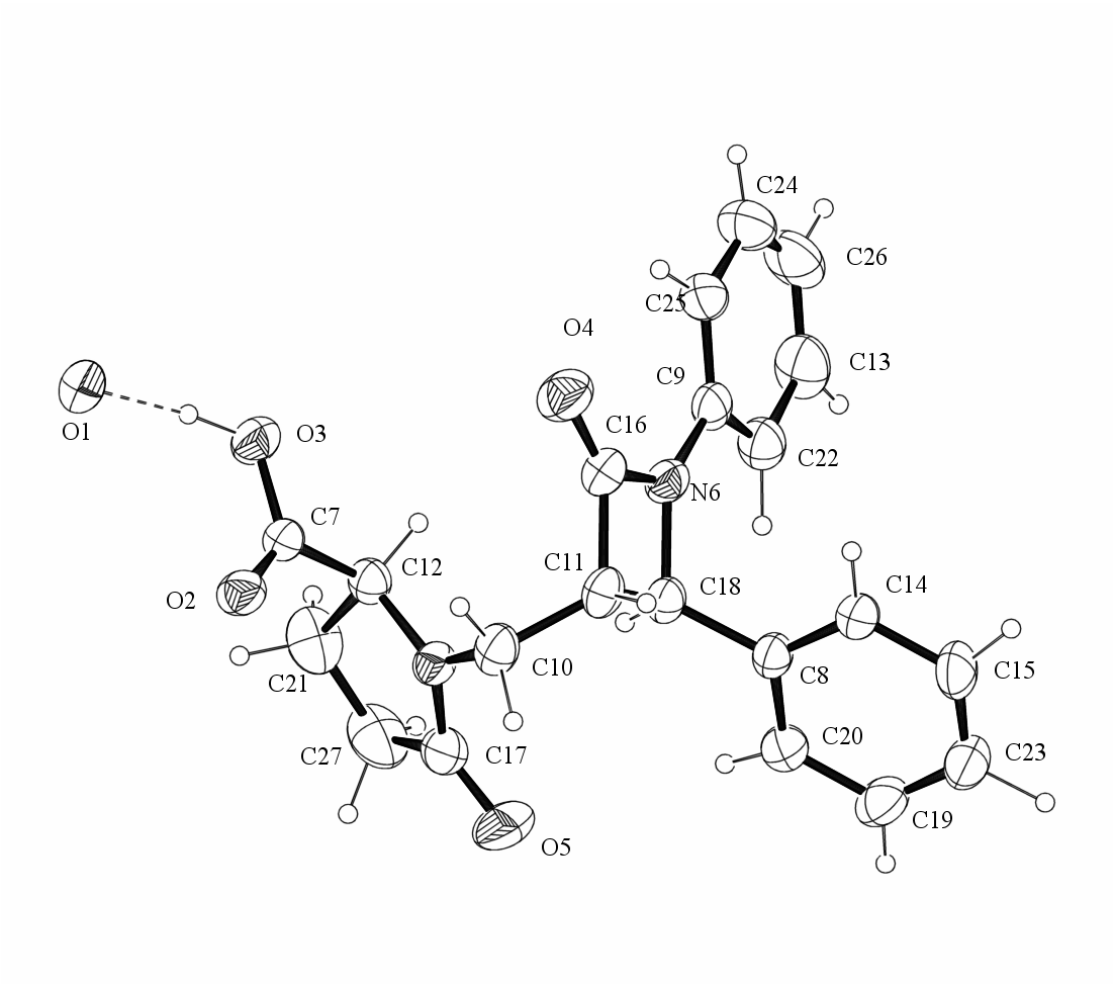

Figure S12: ORTEP diagram of compound **11**