

Supporting Information for

Synthesis, characterization and in vitro cytotoxicity of gallium(III)-dithiocarbamate complexes

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1. SYNTHESIS OF DITHIOCARBAMATES

1.1 Reagents

All reagents and solvents for reactions were purchased from Aldrich Chemicals (Milan, Italy) and used without further purification.

1.2 Physical measurements

Carbon, hydrogen and nitrogen analyses were performed using a Carlo Erba 1106 elemental analyzer. ^1H , $^{13}\text{C}\{^1\text{H}\}$ and two-dimensional NMR spectra were acquired in the indicated deuterated solvents at 298 K with a Bruker AMX 400 spectrometer with TopSpin 3.2 software. Chemical shifts are reported in ppm and referenced to internal residual solvent signal for ^1H (CD_2Cl_2 : 5.32 ppm, CDCl_3 : 7.26 ppm; DMSO-d_6 : 2.50 ppm; D_2O : 4.8 ppm) and to deuterated solvent signal for ^{13}C (CD_2Cl_2 : 54.00 ppm; CDCl_3 : 77.00 ppm; DMSO-d_6 : 39.5 ppm); ^{13}C NMR spectra registered in D_2O were calibrated to external tetramethylsilane. Signal assignments were confirmed by 2D experiments (^1H - ^1H COSY, ^1H - ^{13}C HSQC, ^1H - ^{13}C HMBC) where necessary. Common abbreviations for signal multiplicity were used (s = singlet, d = doublets, t = triplets, q = quartets, etc.; bs = broad singlet).

1.3 Syntheses

General procedure. In a ice-bath cooled flask, the relevant amine (3.31 mmol, 1 eq.) and NaOH (262.7 mg, 6.62 mmol, 2 eq.) were dissolved in ethanol (40 mL). Then, a solution of carbon disulphide (0.397 mL, 6.62 mmol, 2 eq.) in ethanol (10 mL) was added dropwise. The ice-bath was then removed, and the reaction mixture was stirred at room temperature for 3 h to obtain a yellow or pale-yellow solution. The solvent was removed under a dinitrogen flux and the resulting crude residue was dissolved in a minimum amount of ethanol and the dithiocarbamate was

precipitated as a powder by adding diethyl ether. The product was recrystallized from ethanol/diethyl ether to give pure white crystals.

1.3.1 Sodium 4-(4-chlorophenyl)-4-hydroxypiperidine dithiocarbamate (NaCPHPDTC).

Yield: 78.1%. Elemental Analysis: Calc. for $C_{12}H_{13}NOS_2NaCl$ (MW: 309.81 Da): C, 46.5%; H, 4.2%, N 4.5%; Found: C, 45.0%; H, 4.9%, N 4.1%. 1H NMR (CD_3OD , 400.13 MHz): δ 1.73 and 2.12 (m and m, 2H and 2H, $S_2(CNCH_2CH_2)_2$); 3.52 and 5.76 (m and m, 2H and 2H, $S_2CN(CH_2CH_2)_2$); 7.33 – 7.50 (Harom). $^{13}C\{^1H\}$ NMR (CD_3OD , 100.62 MHz): δ 35.0 ($S_2CNCH_2CH_2$); 42.8 ($S_2CNCH_2CH_2$); 69.8 ($C(OH)CH_2CH_2N$); 126.0 (C2 aromatic), 128.8 (C3 aromatic); 133.5 (C4 aromatic); 145.3 (C1 aromatic); 201.0 (CS_2).

1.3.2 Sodium 4-morpholinopiperidine dithiocarbamate (NaMPipDTC).

Yield: 80.2%. Elemental Analysis: Calc. for $C_{10}H_{17}N_2OS_2Na$ (MW: 268.38 Da): C, 44.8%; H, 6.4%, N 10.4%; Found: C, 45.3%; H, 6.8%, N 10.1%. 1H NMR (CD_3OD , 400.13 MHz): δ 2.05 (m, 2H, $CS_2NCH_2CH_2$); 2.75 (m, 4H, OCH_2CH_2N); 3.20 (m, 4H, $CS_2NCH_2CH_2$); 3.85 (m, 4H, OCH_2CH_2N); 5.71 (m, 1H, $CS_2NCH_2CH_2CH$). $^{13}C\{^1H\}$ NMR (CD_3OD , 100.62 MHz): δ 27.7 ($CS_2NCH_2CH_2$); 49.3 ($CS_2NCH_2CH_2$); 50.8 (OCH_2CH_2N); 61.4 ($CS_2NCH_2CH_2CH$); 66.6 (OCH_2CH_2N); 207.1 (CS_2).

1.3.3 Sodium hexamethyleneimine dithiocarbamate (NaAzepamDTC).

Yield: 84.3%. Elemental Analysis: Calc. for $C_{27}H_{42}N_3O_6S_6Ga$ (MW: 766.75 Da): C, 42.3%; H, 5.5%, N 5.5%; Found: C, 42.4%; H, 5.6%, N 5.4%. 1H NMR ($CDCl_3$, 400.13 MHz): δ 1.26 (t, $^3J = 7.11$ Hz, 9H, CH_3CH_2O); 1.90 (m, 6H, $NCH_2CH_2CHC(O)OEt$); 2.03 (m, 6H, $NCH_2CH_2CHC(O)OEt$); 2.55 (m, 3H, $NCH_2CH_2CHC(O)OEt$); 3.43 (m, 6H, $NCH_2CH_2CHC(O)OEt$); 4.16 (q, $^3J = 7.11$ Hz, 6H, CH_3CH_2O); 4.52 (m, 6H,

$\text{NCH}_2\text{CH}_2\text{CHC}(\text{O})\text{OEt}$). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 100.62 MHz): δ 14.2 ($\text{CH}_3\text{CH}_2\text{O}$); 27.6 ($\text{NCH}_2\text{CH}_2\text{CHC}(\text{O})\text{OEt}$); 39.1 ($\text{NCH}_2\text{CH}_2\text{CHC}(\text{O})\text{OEt}$); 50.9 ($\text{NCH}_2\text{CH}_2\text{CHC}(\text{O})\text{OEt}$); 60.8 ($\text{CH}_3\text{CH}_2\text{O}$); 173.7 (CO); 201.6 (CS_2).

1.3.4 Sodium heptamethyleneimine dithiocarbamate (NaAzocanDTC).

Yield: 76.7%. Elemental Analysis: Calc. for $\text{C}_{24}\text{H}_{36}\text{N}_3\text{O}_6\text{S}_6\text{Ga}$ (MW: 778.85 Da): C, 39.8%; H, 5.0%, N 5.8%; Found: C, 39.6%; H, 4.9%, N 5.7%. ^1H NMR (CDCl_3 , 400.13 MHz): δ 1.84 (m, 12H, $\text{NCH}_2\text{CH}_2\text{C}$); 3.80 (s, 12H, $\text{OCH}_2\text{CH}_2\text{O}$); 4.06 (m, 12H, $\text{NCH}_2\text{CH}_2\text{C}$). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 100.62 MHz): δ 34.2 ($\text{NCH}_2\text{CH}_2\text{C}$); 50.1 ($\text{NCH}_2\text{CH}_2\text{C}$); 64.5 ($\text{OCH}_2\text{CH}_2\text{O}$); 201.6 (CS_2).

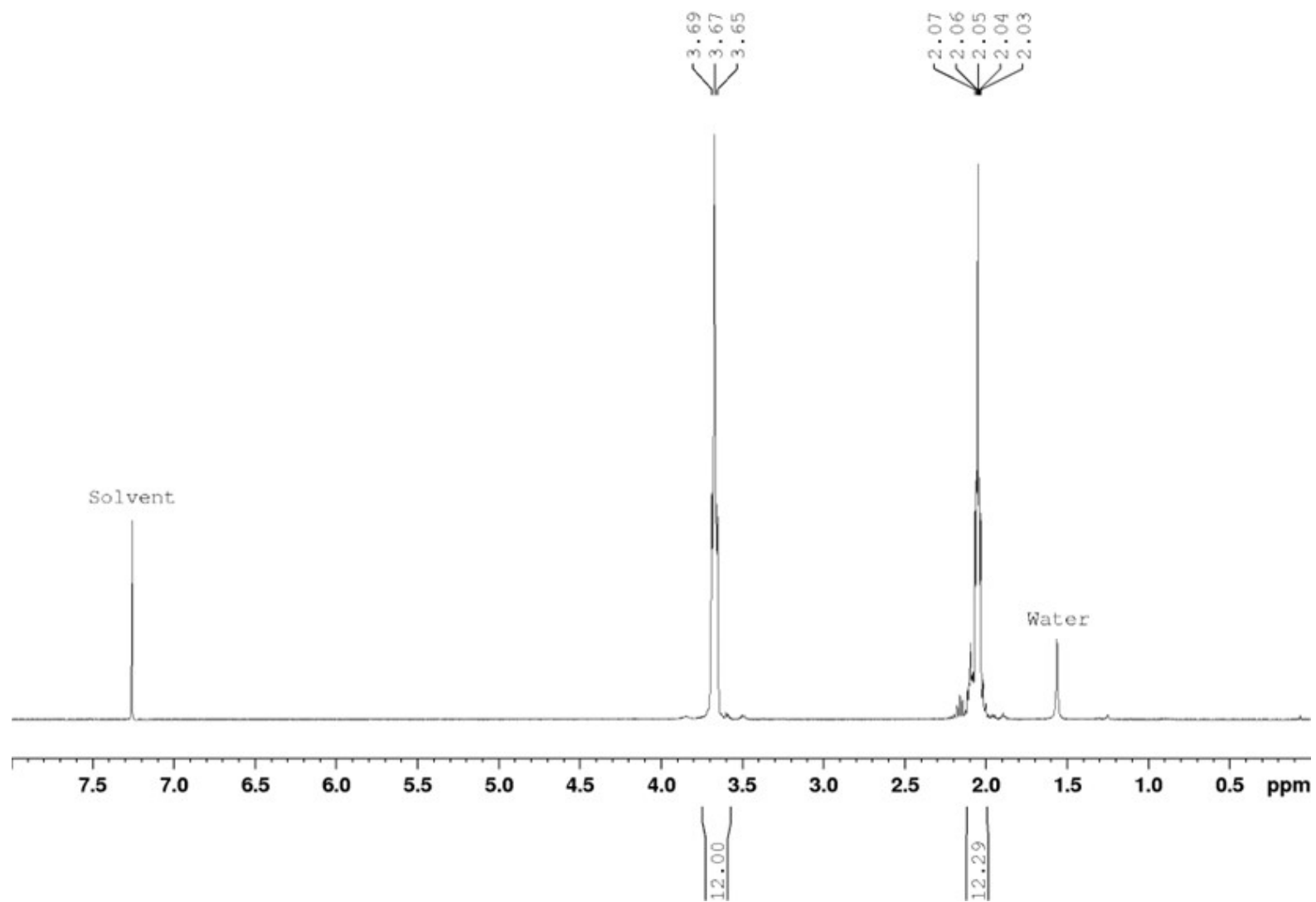
1.3.5 Sodium 4-benzylpiperidine dithiocarbamate (NaBzPipDTC).

Yield: 68.8%. Elemental Analysis: Calc. for $\text{C}_{30}\text{H}_{51}\text{N}_6\text{O}_3\text{S}_6\text{Ga}$ (MW: 805.88 Da): C, 44.71%; H, 6.38%, N 10.43%; Found: C, 41.58%; H, 6.44%, N 9.60%. ^1H NMR (DMSO-d_6 , 400.13 MHz): δ 1.57 (m, 6H, $\text{NCH}_2\text{CH}_2\text{C}$); 1.90 (m, 6H, $\text{NCH}_2\text{CH}_2\text{C}$); 2.44 (m, 12H, $\text{OCH}_2\text{CH}_2\text{N}$); 2.86 (m, 12H, $\text{NCH}_2\text{CH}_2\text{C}$); 3.85 (m, 4H, $\text{OCH}_2\text{CH}_2\text{N}$). $^{13}\text{C}\{^1\text{H}\}$ NMR (DMSO-d_6 , 100.62 MHz): δ 28.5 ($\text{CS}_2\text{NCH}_2\text{CH}_2$); 42.9 ($\text{CS}_2\text{NCH}_2\text{CH}_2$); 49.7 ($\text{OCH}_2\text{CH}_2\text{Npiperidina}$); 66.4 ($\text{OCH}_2\text{CH}_2\text{Npiperidina}$); 204.4 (CS_2).

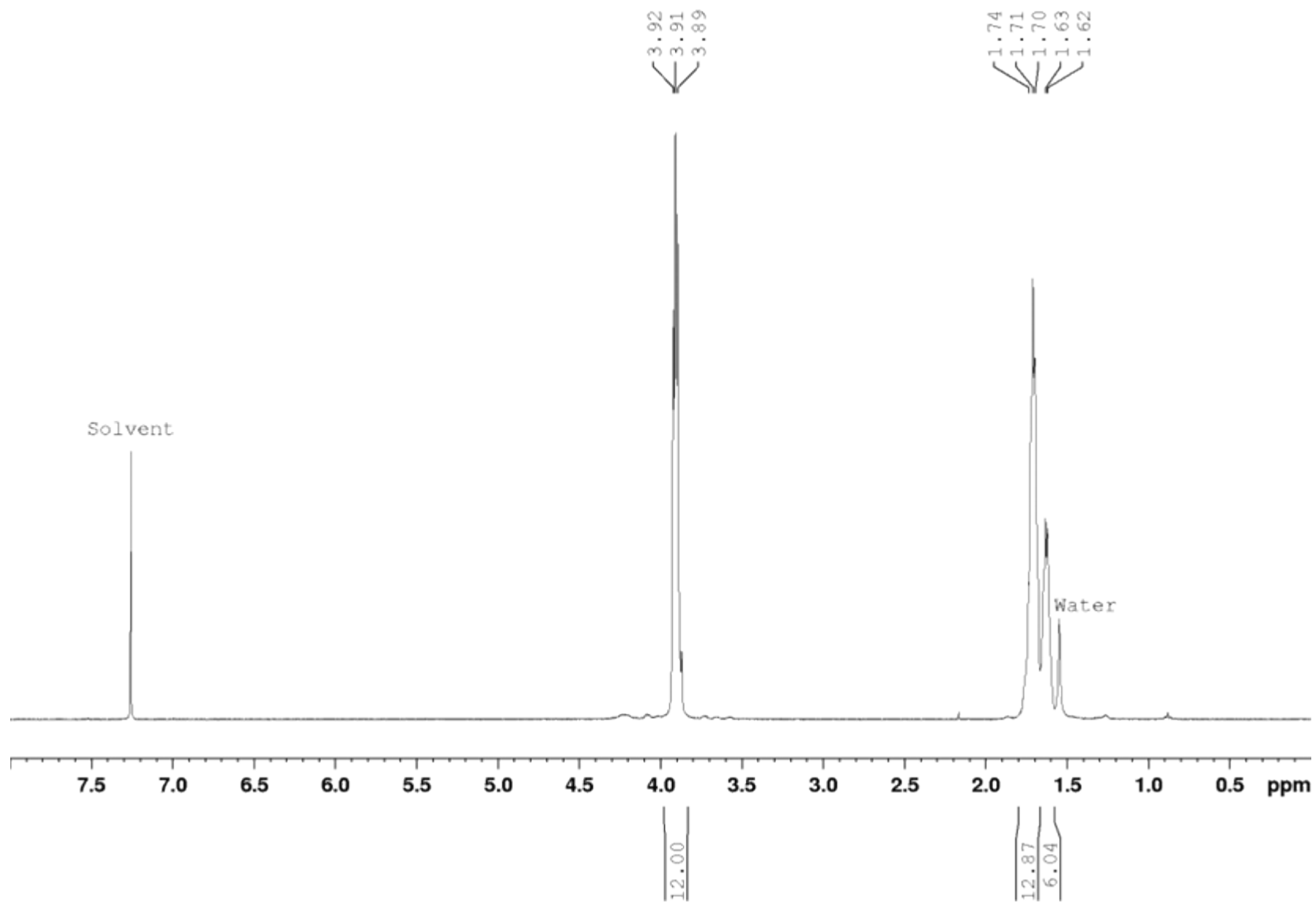
1.3.6 Sodium dipropyldithiocarbamate (NaDPDC).

Yield: 66.2%. Elemental Analysis: Calc. for $\text{C}_{21}\text{H}_{36}\text{N}_3\text{S}_6\text{Ga}$ (MW: 592.64 Da): C, 42.5%; H, 6.1%, N 7.1%; Found: C, 42.4%; H, 6.0%, N 6.9%. ^1H NMR (CDCl_3 , 400.13 MHz): δ 1.61 (m, 12H, $\text{NCH}_2\text{CH}_2\text{CH}_2$); 1.84 (m, 12H, $\text{NCH}_2\text{CH}_2\text{CH}_2$); 3.90 (m, 12H, $\text{NCH}_2\text{CH}_2\text{CH}_2$). $^{13}\text{C}\{^1\text{H}\}$ NMR (CDCl_3 , 100.62 MHz): δ 27.0 ($\text{NCH}_2\text{CH}_2\text{CH}_2$); 55.4 ($\text{NCH}_2\text{CH}_2\text{CH}_2$); 201.7 (CS_2).

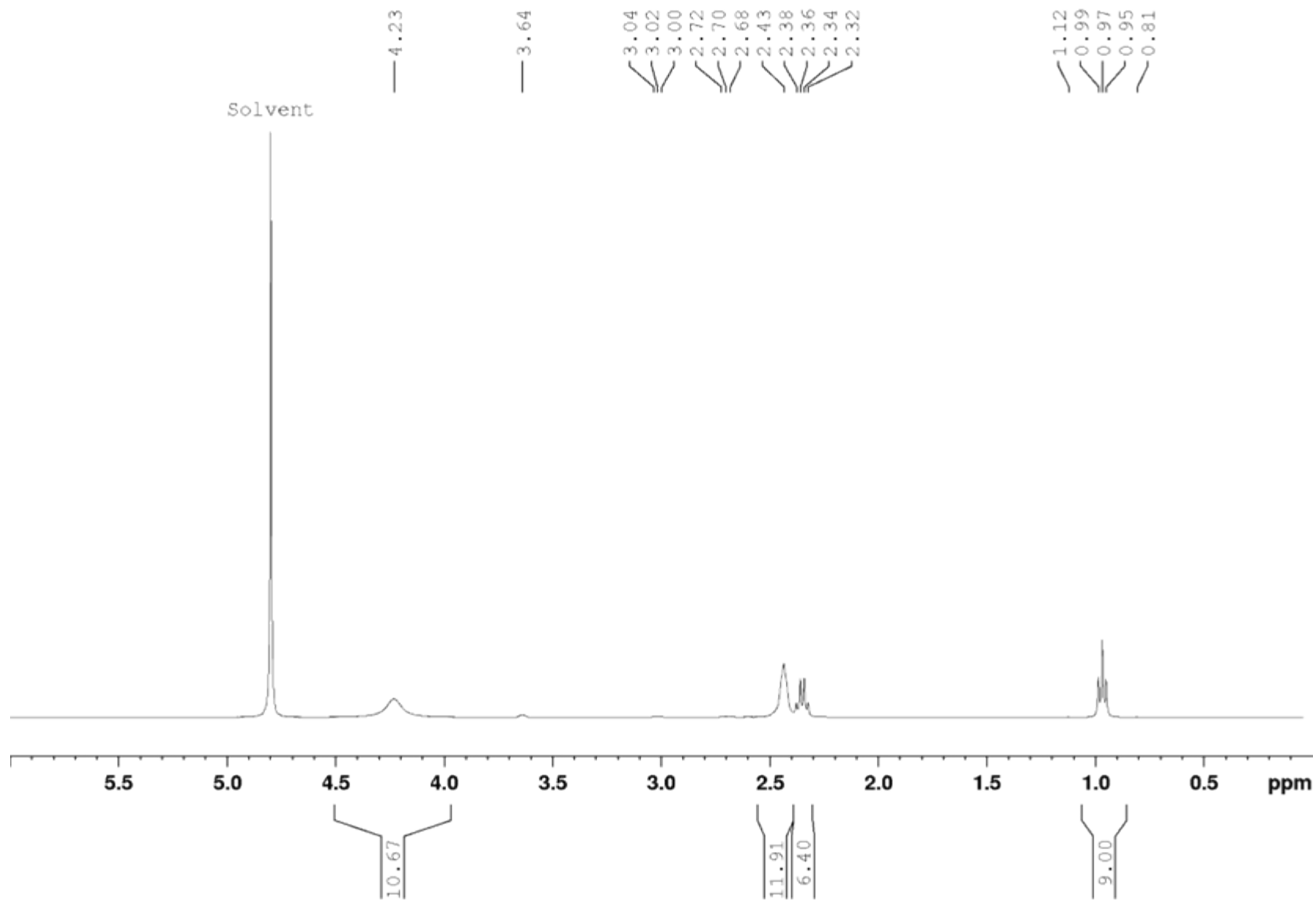
2. ^1H NMR SPECTRA OF THE GALLIUM(III) COMPLEXES



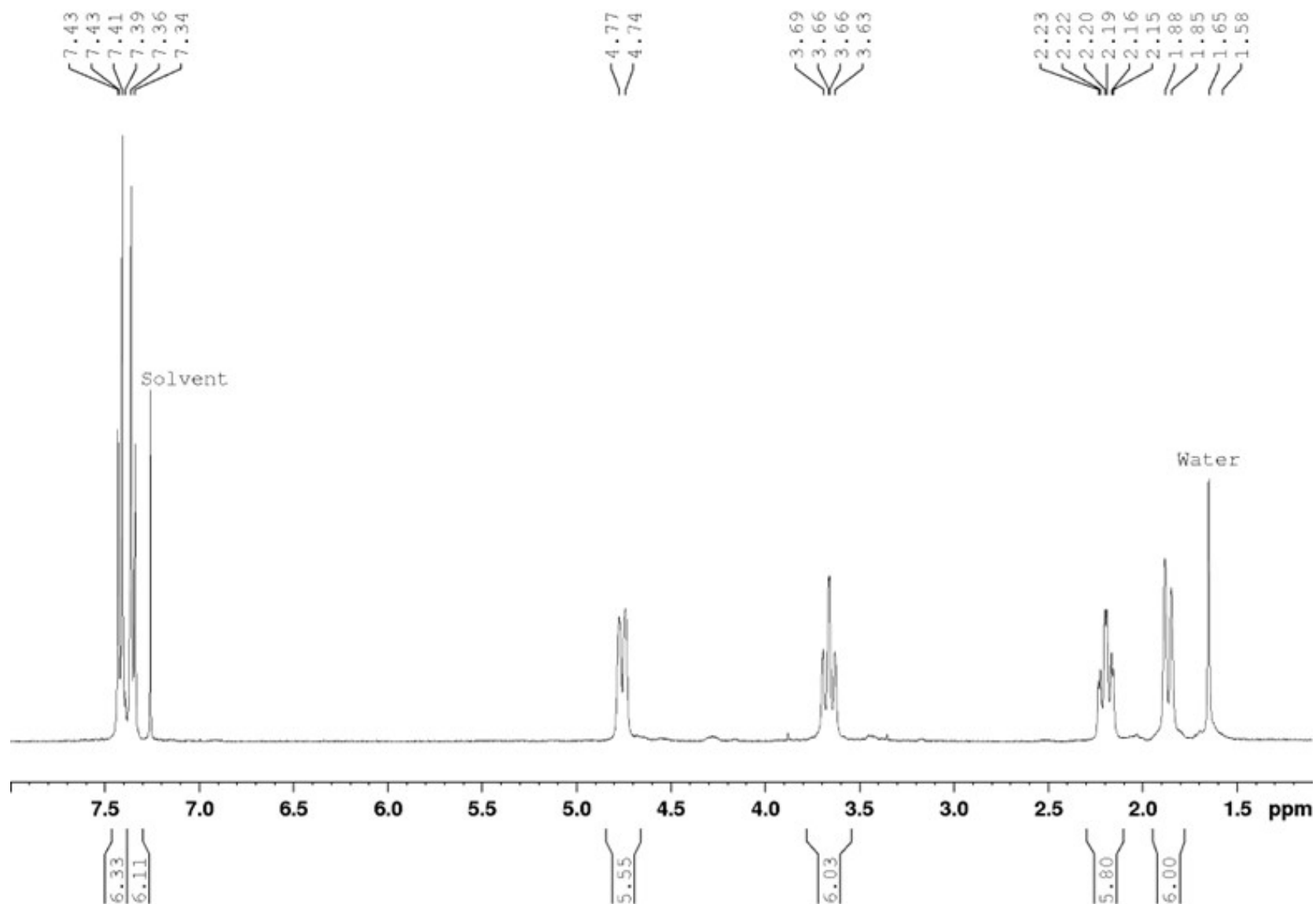
^1H NMR spectrum of **1** (298 K, CDCl_3)



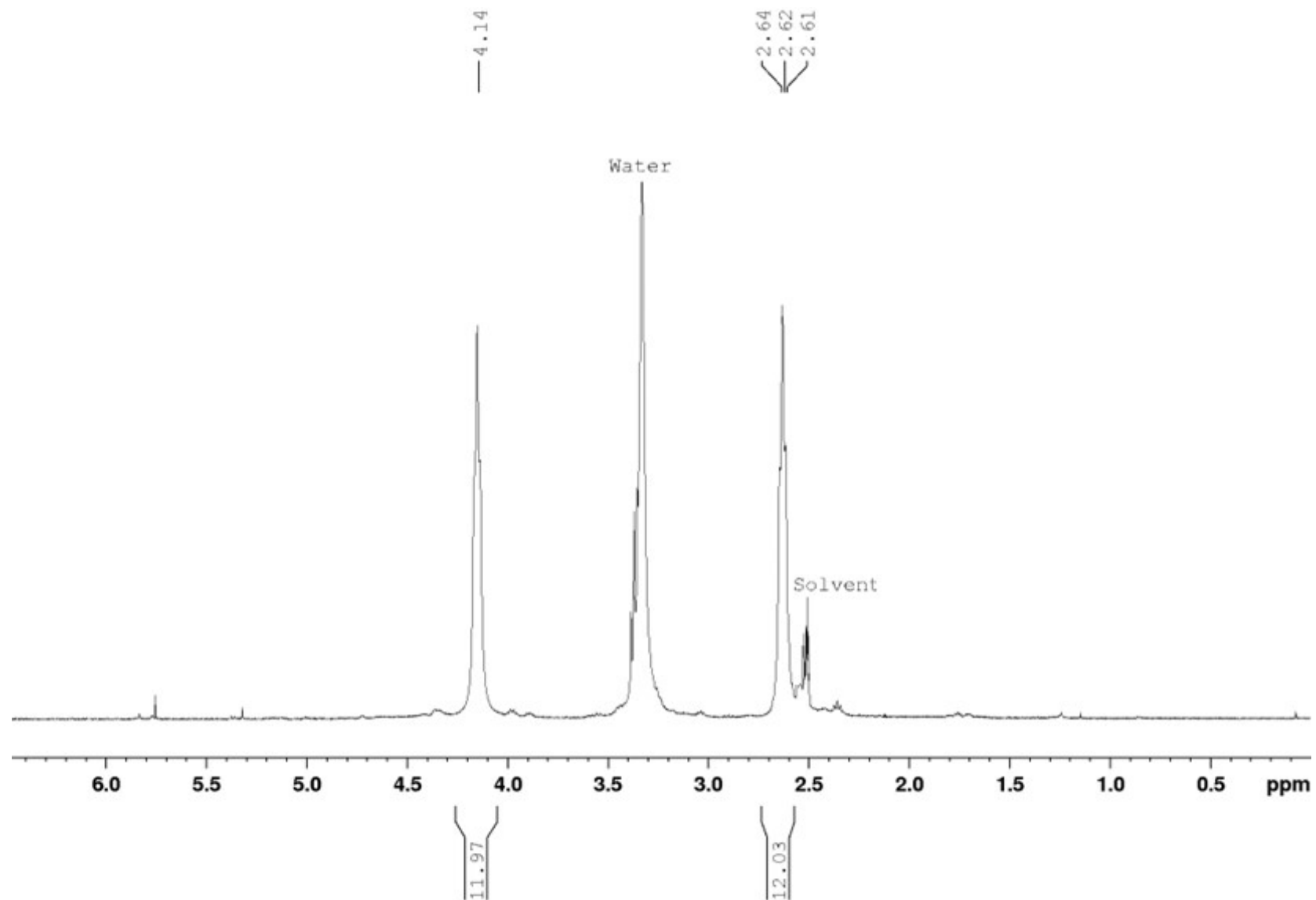
¹H NMR spectrum of **2** (298 K, CDCl₃)



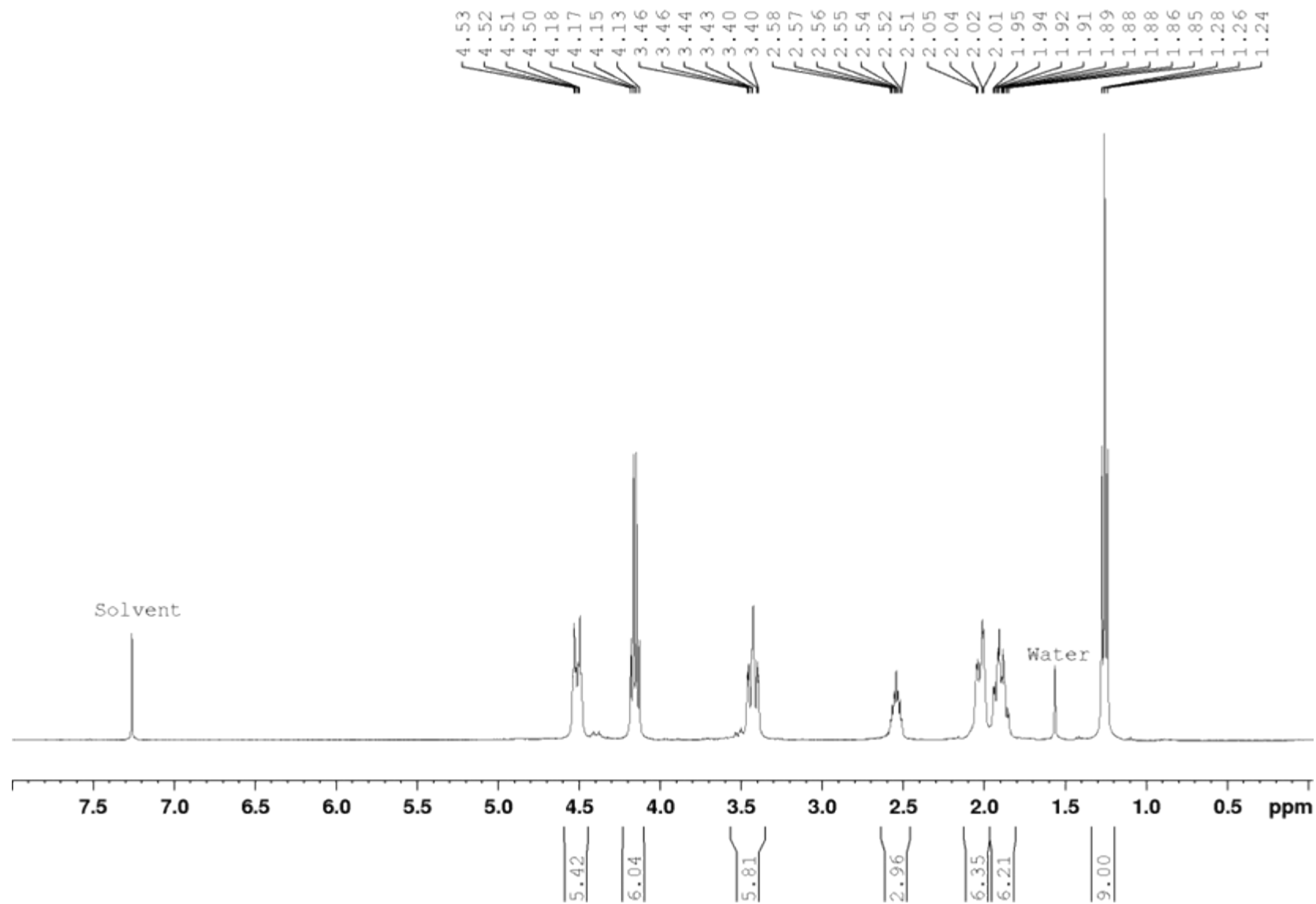
^1H NMR spectrum of **3** (298 K, $\text{D}_2\text{O}/\text{DCI}$)



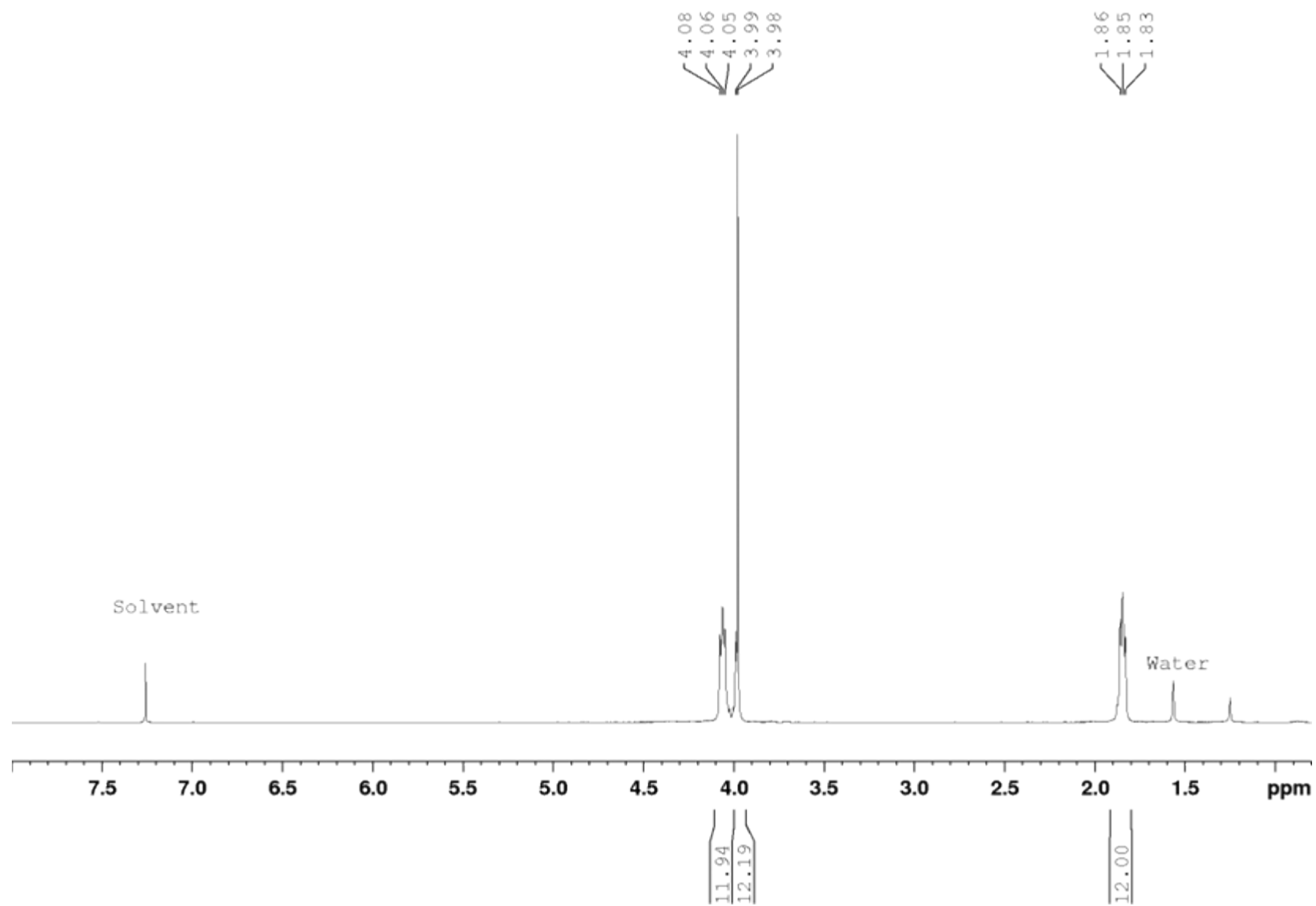
¹H NMR spectrum of 4 (298 K, CDCl₃)



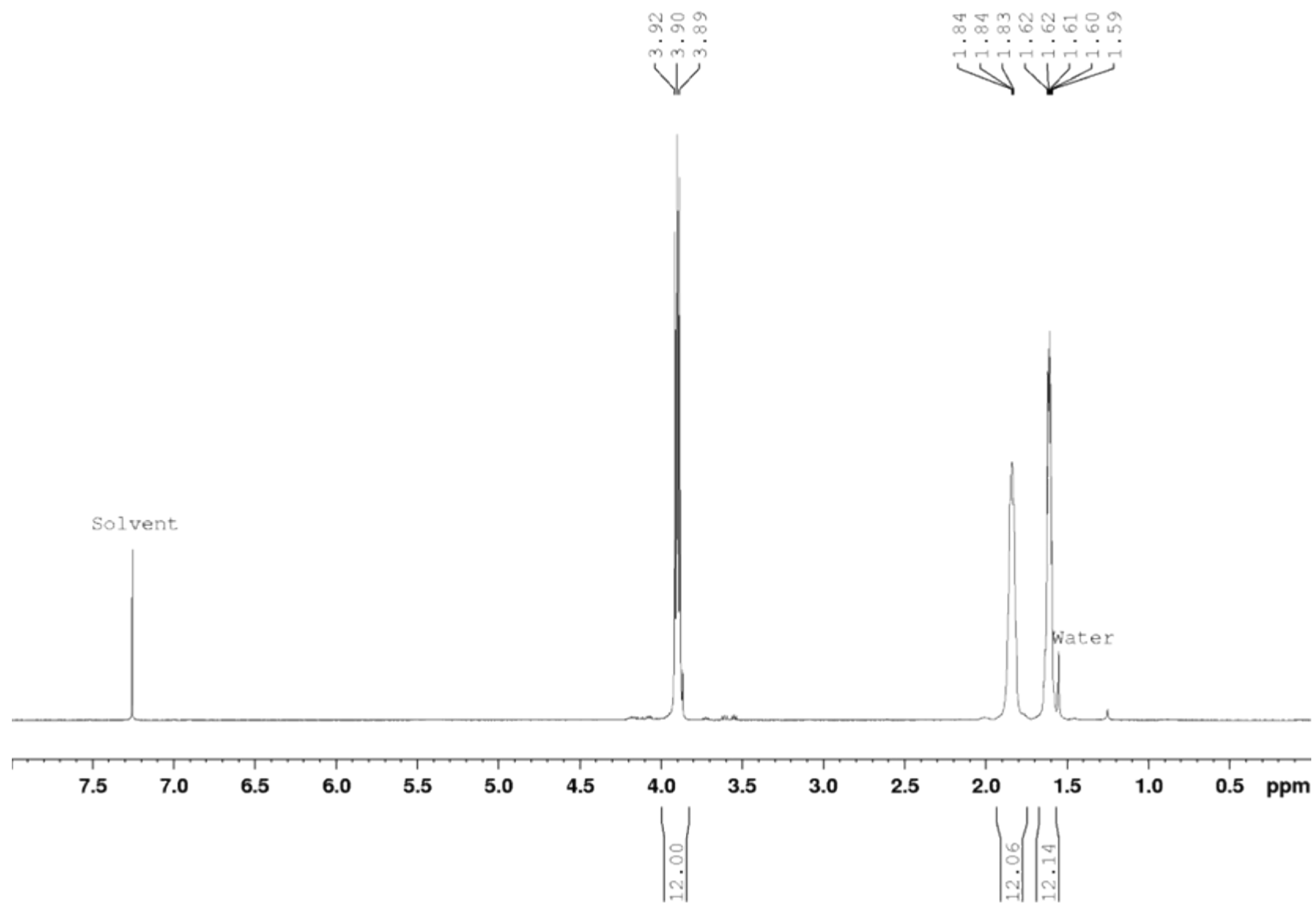
¹H NMR spectrum of **5** (298 K, DMSO-*d*₆)



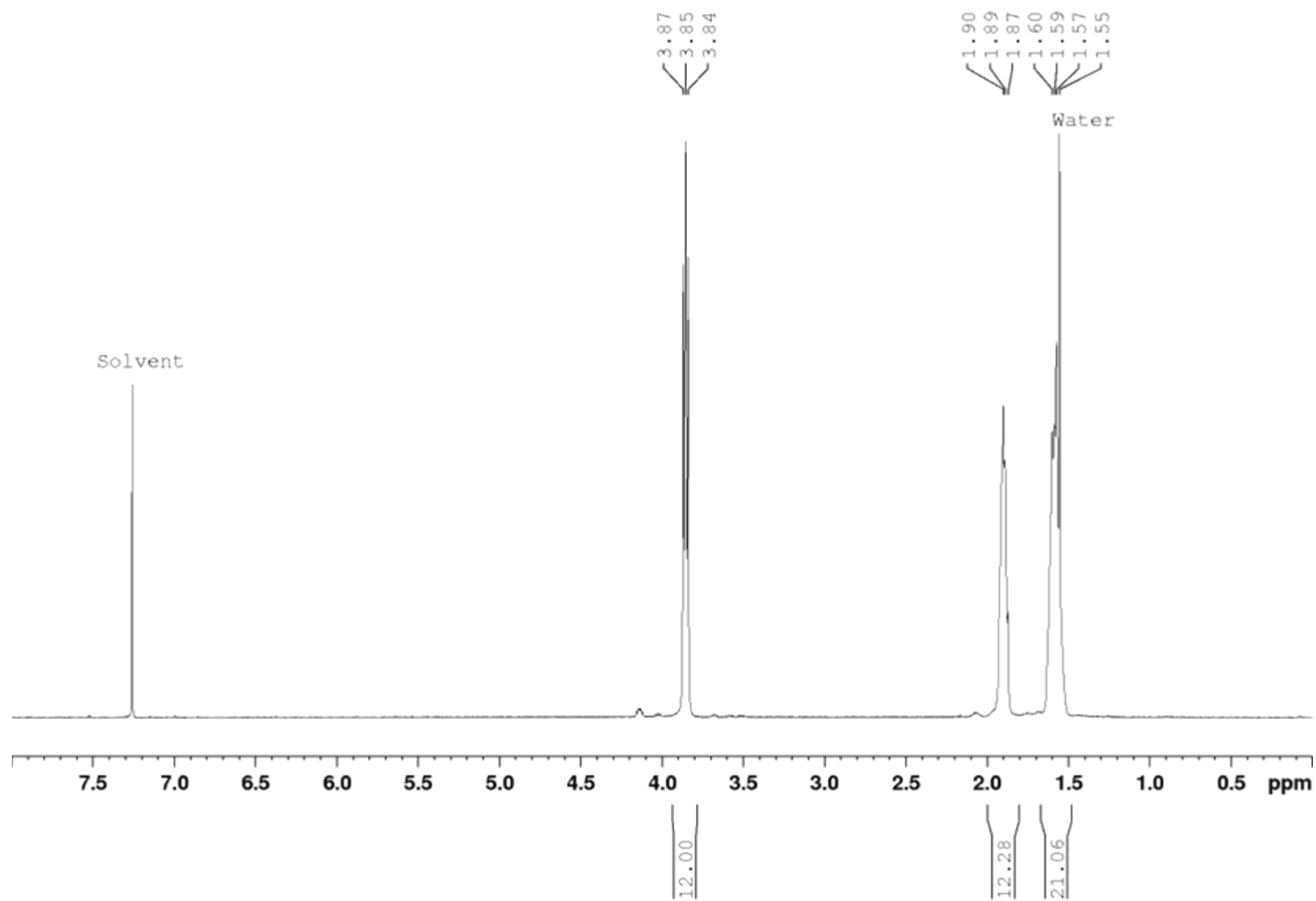
^1H NMR spectrum of **6** (298 K, CDCl_3)



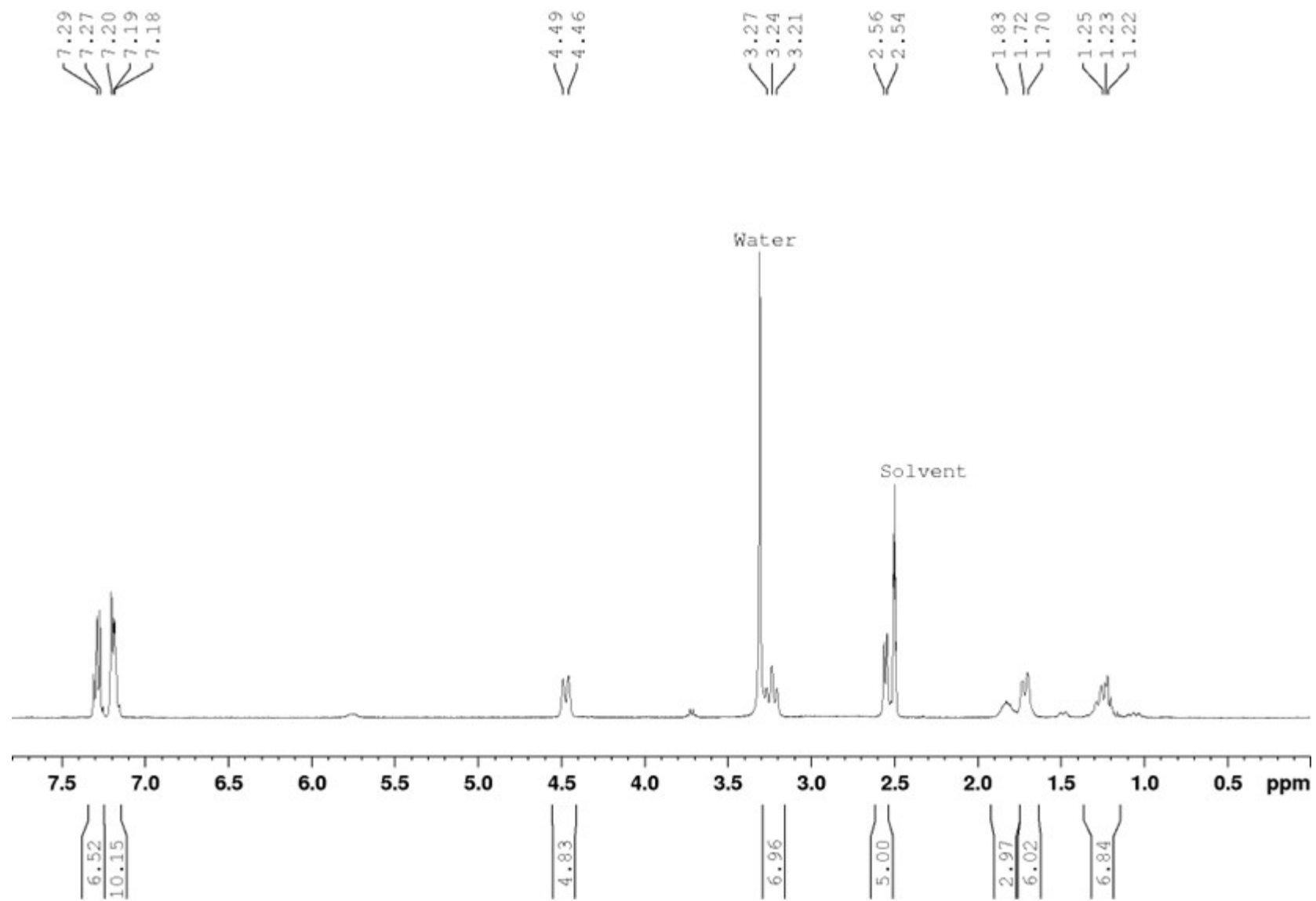
¹H NMR spectrum of 7 (298 K, CDCl₃)



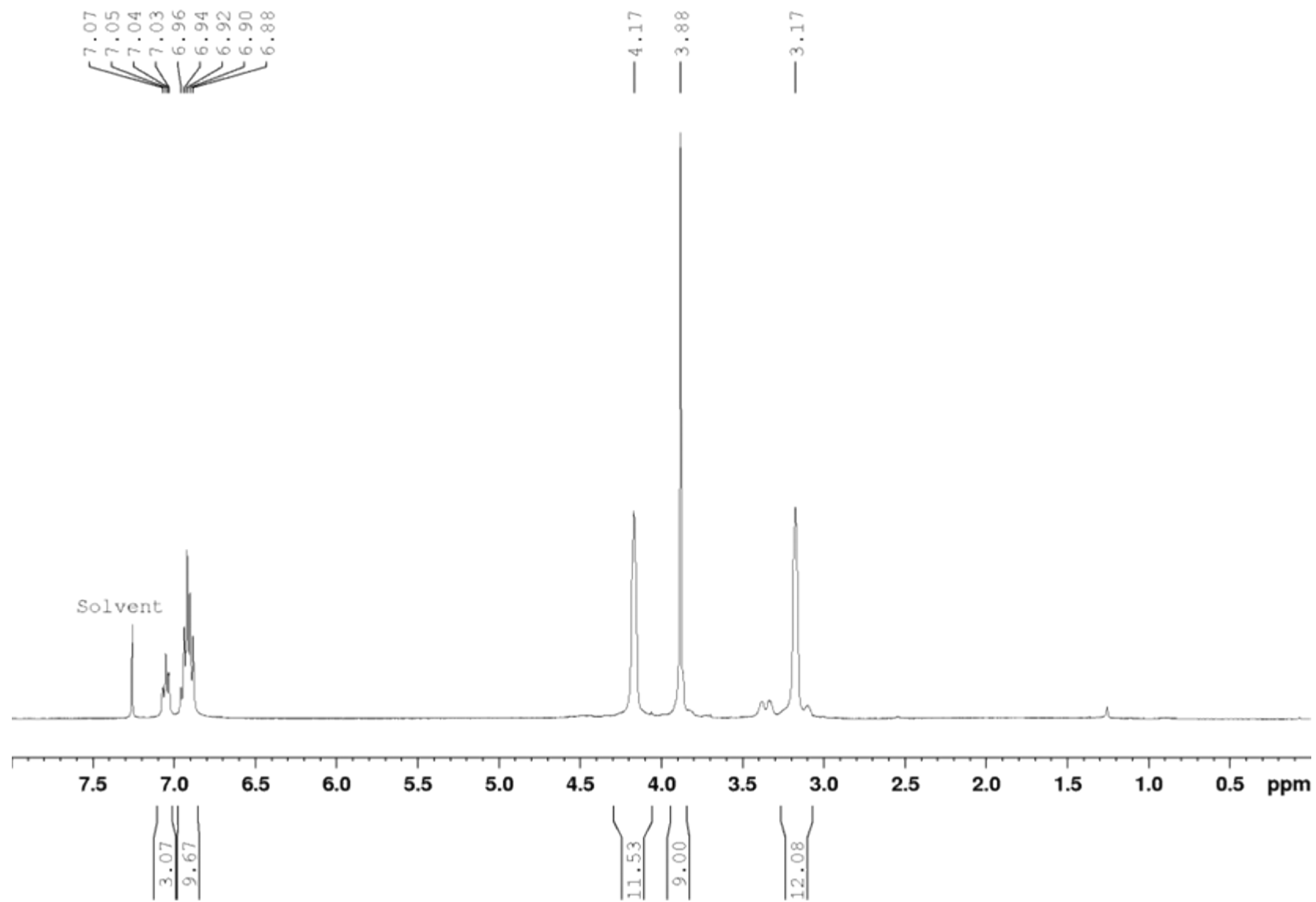
¹H NMR spectrum of **9** (298 K, CDCl₃)



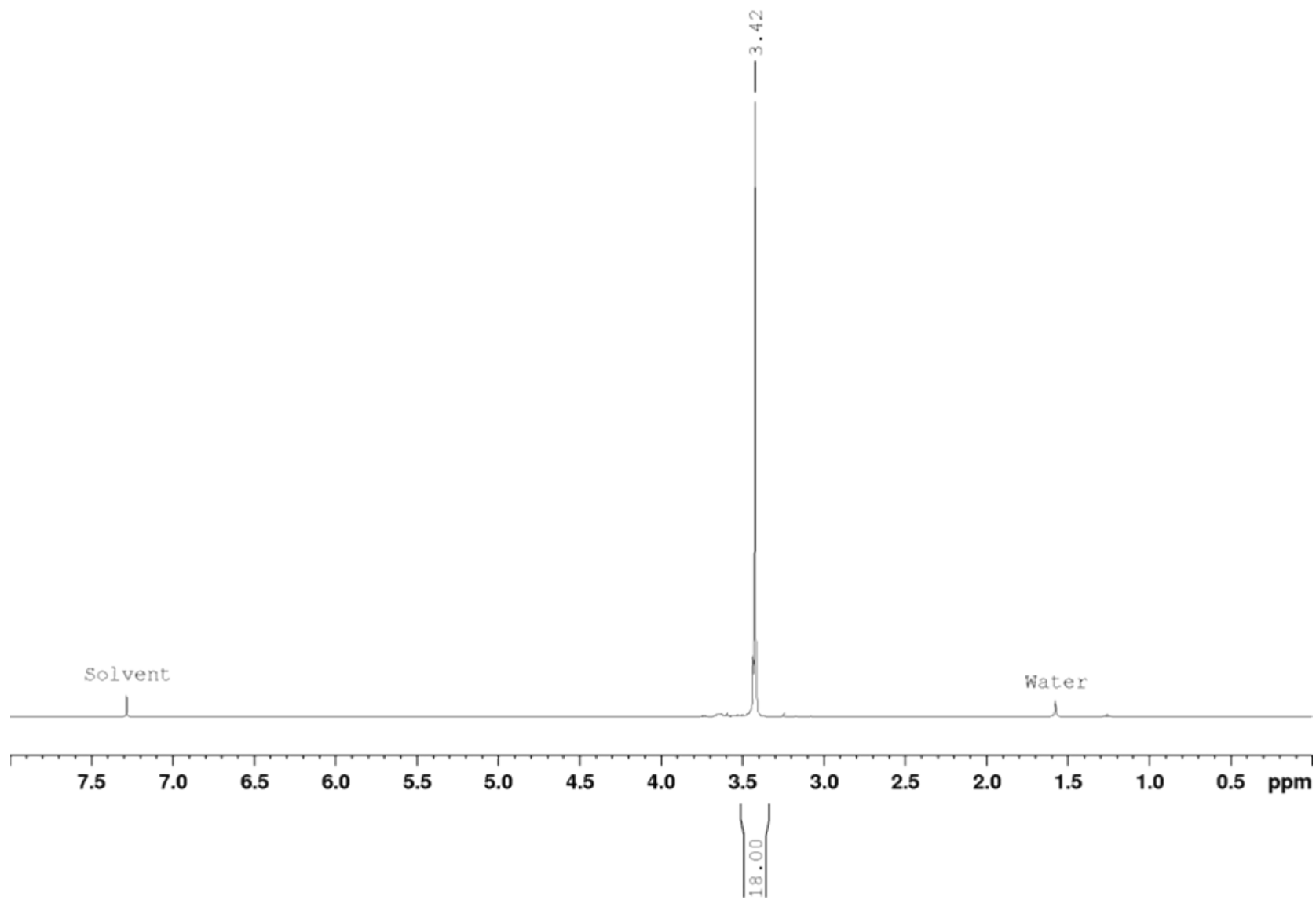
¹H NMR spectrum of **10** (298 K, CDCl₃)



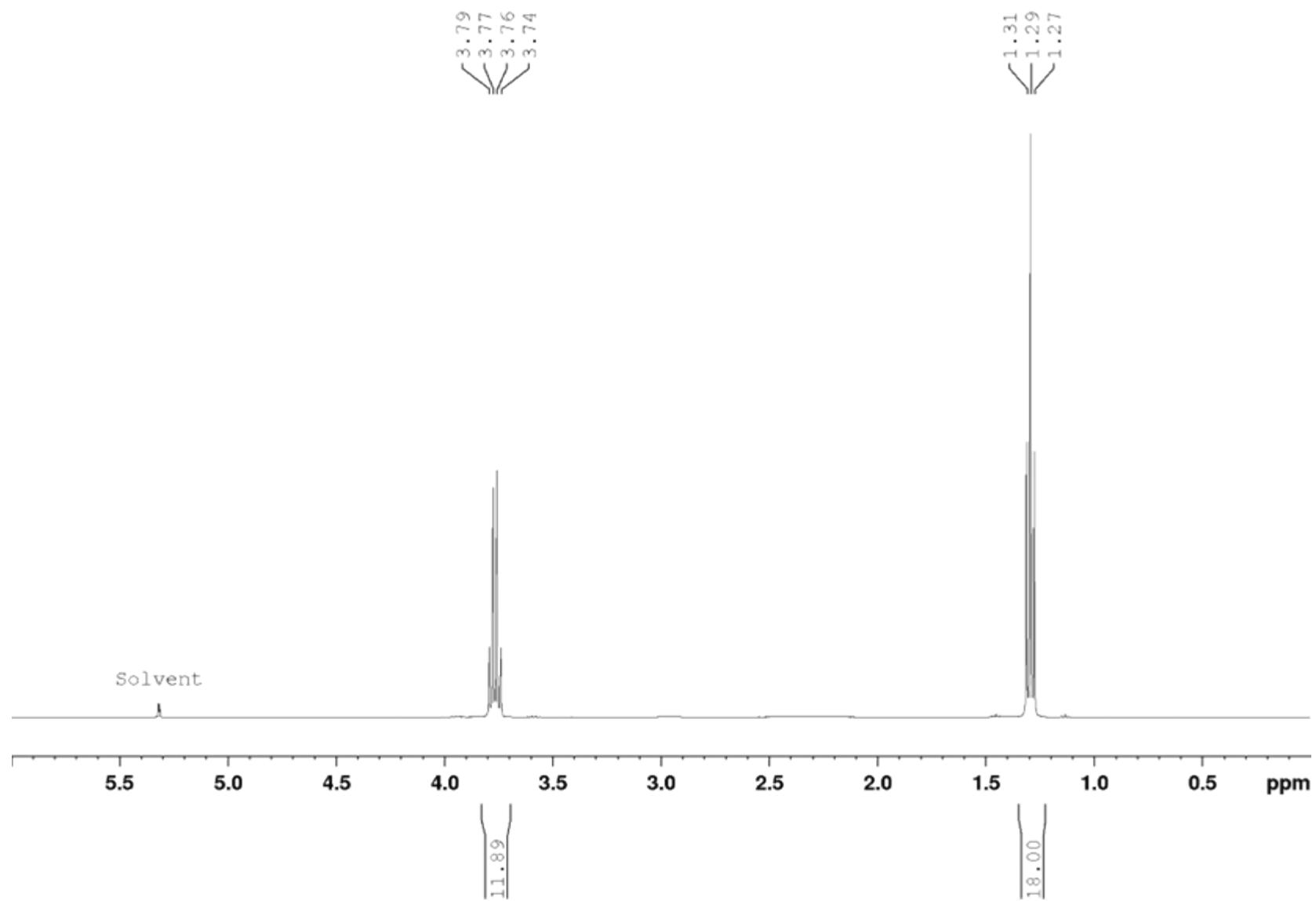
¹H NMR spectrum of **11** (298 K, DMSO-*d*₆)



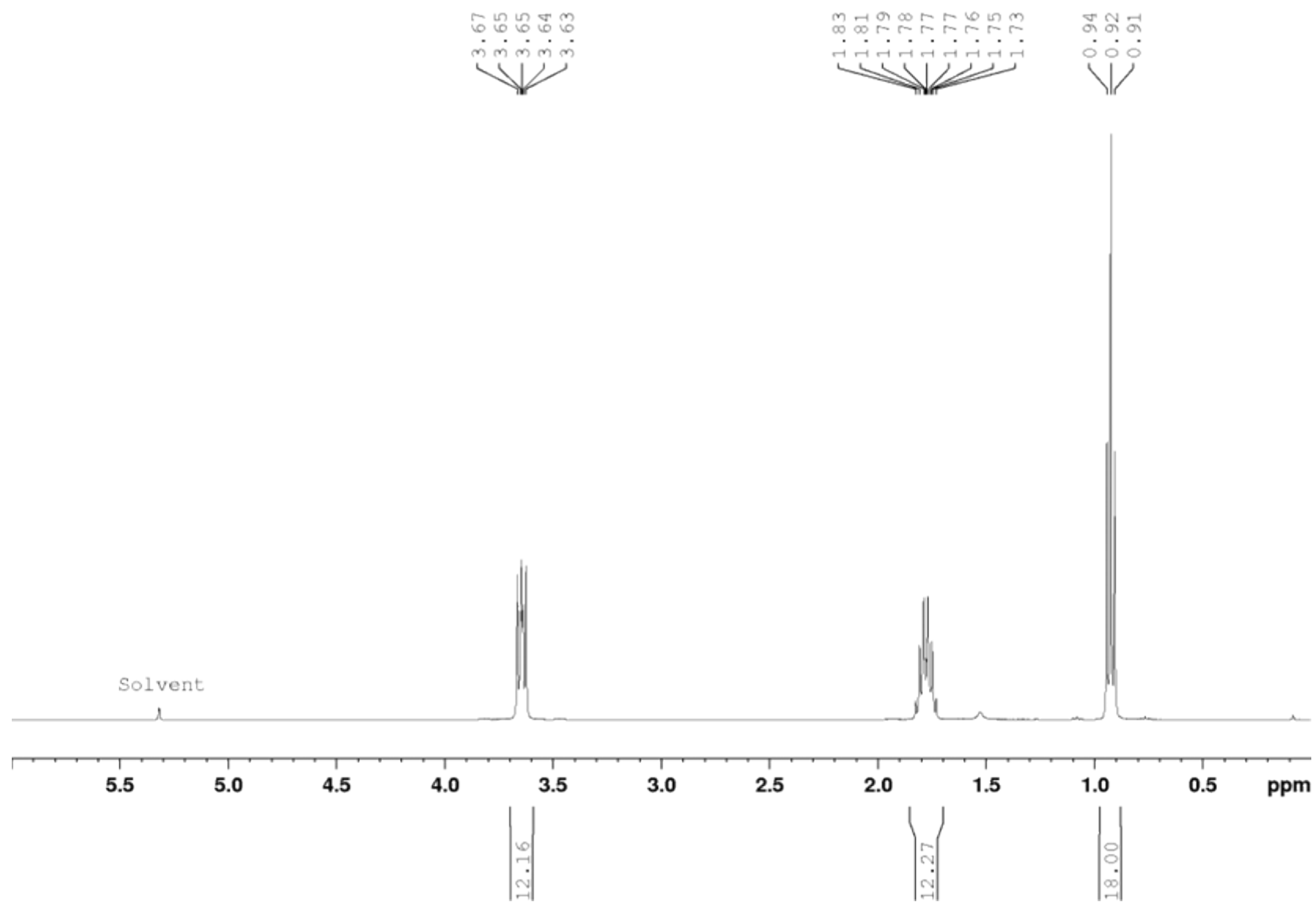
^1H NMR spectrum of **12** (298 K, CDCl_3)



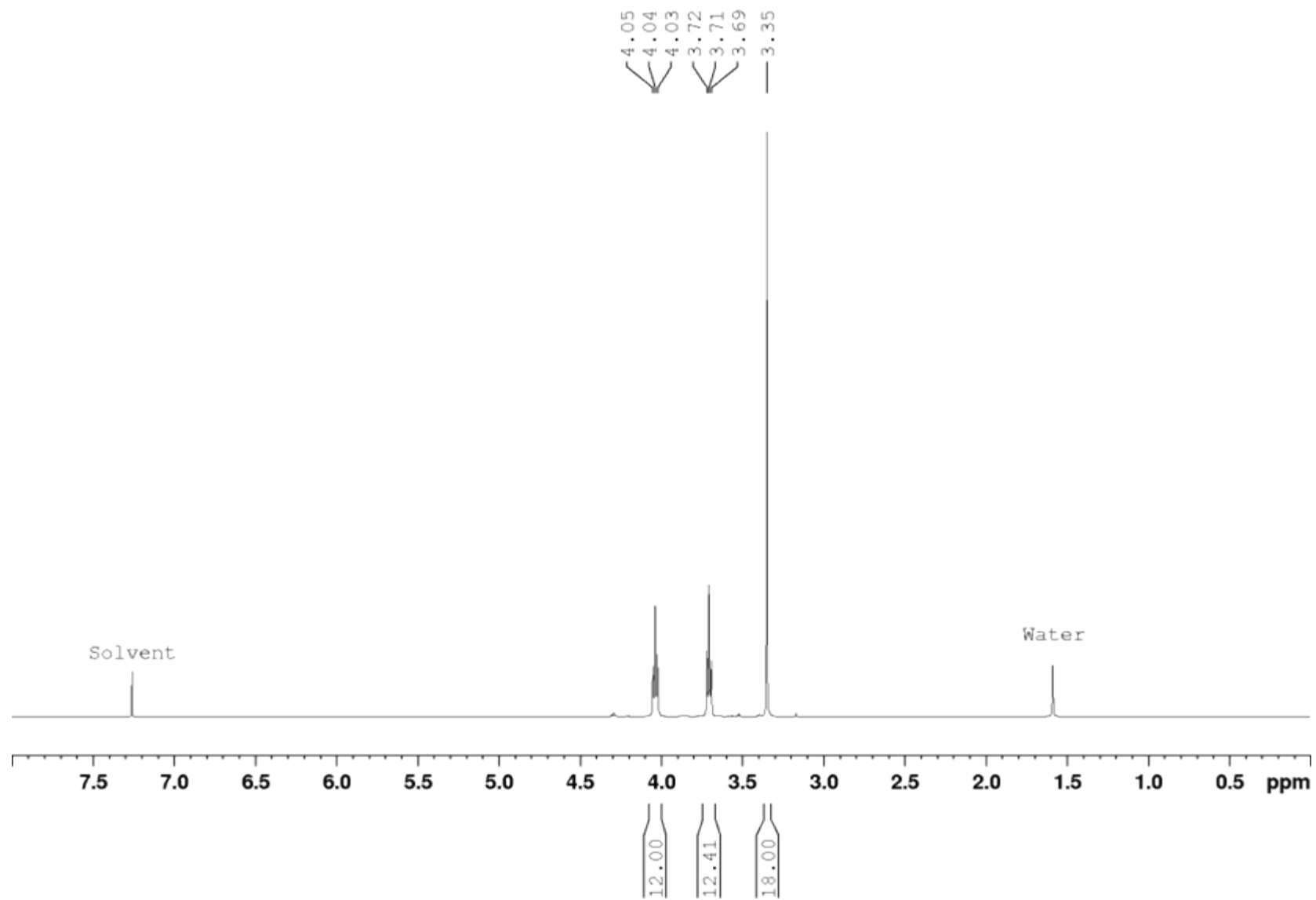
^1H NMR spectrum of **13** (298 K, CDCl_3)



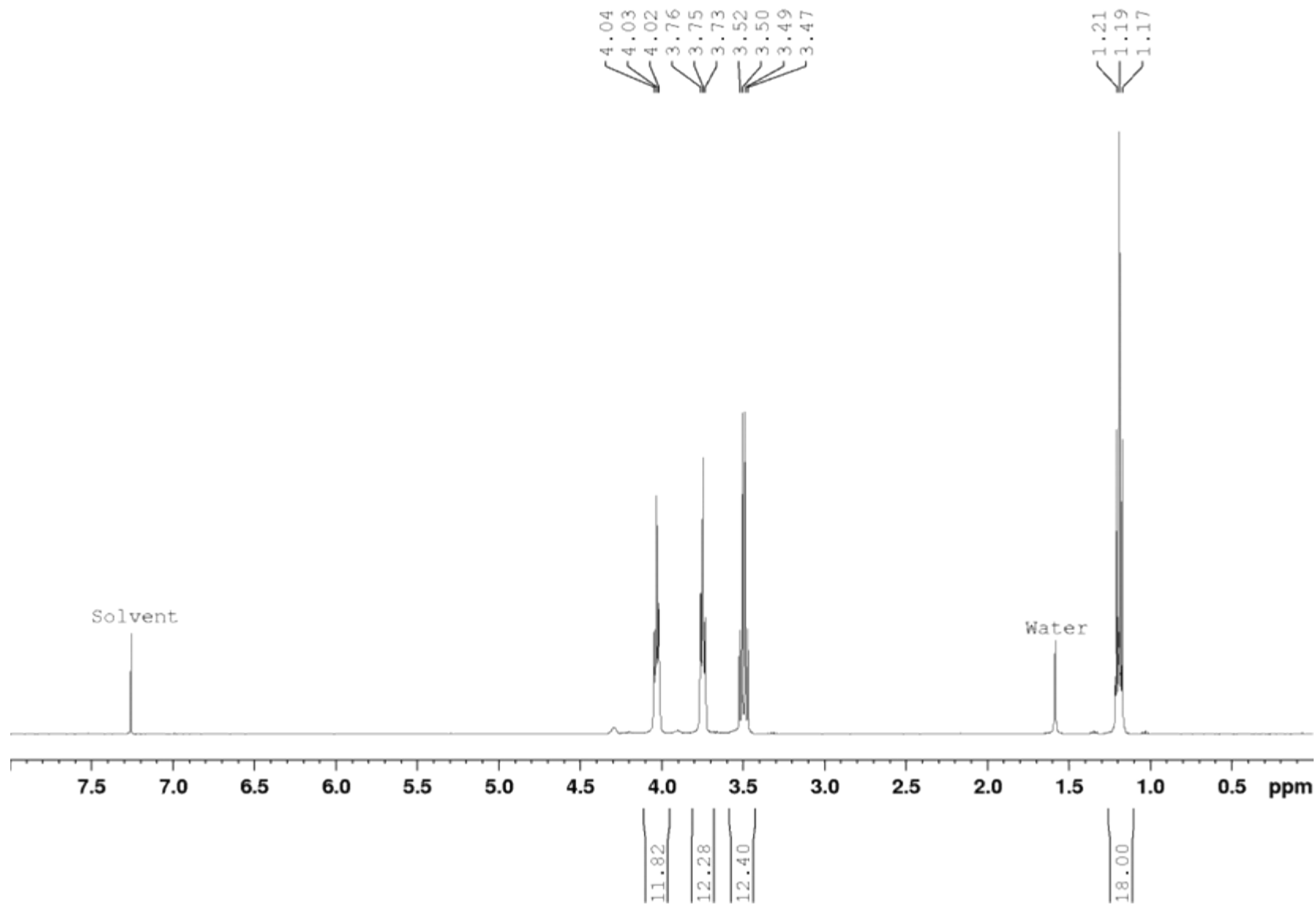
^1H NMR spectrum of **14** (298 K, CD_2Cl_2)



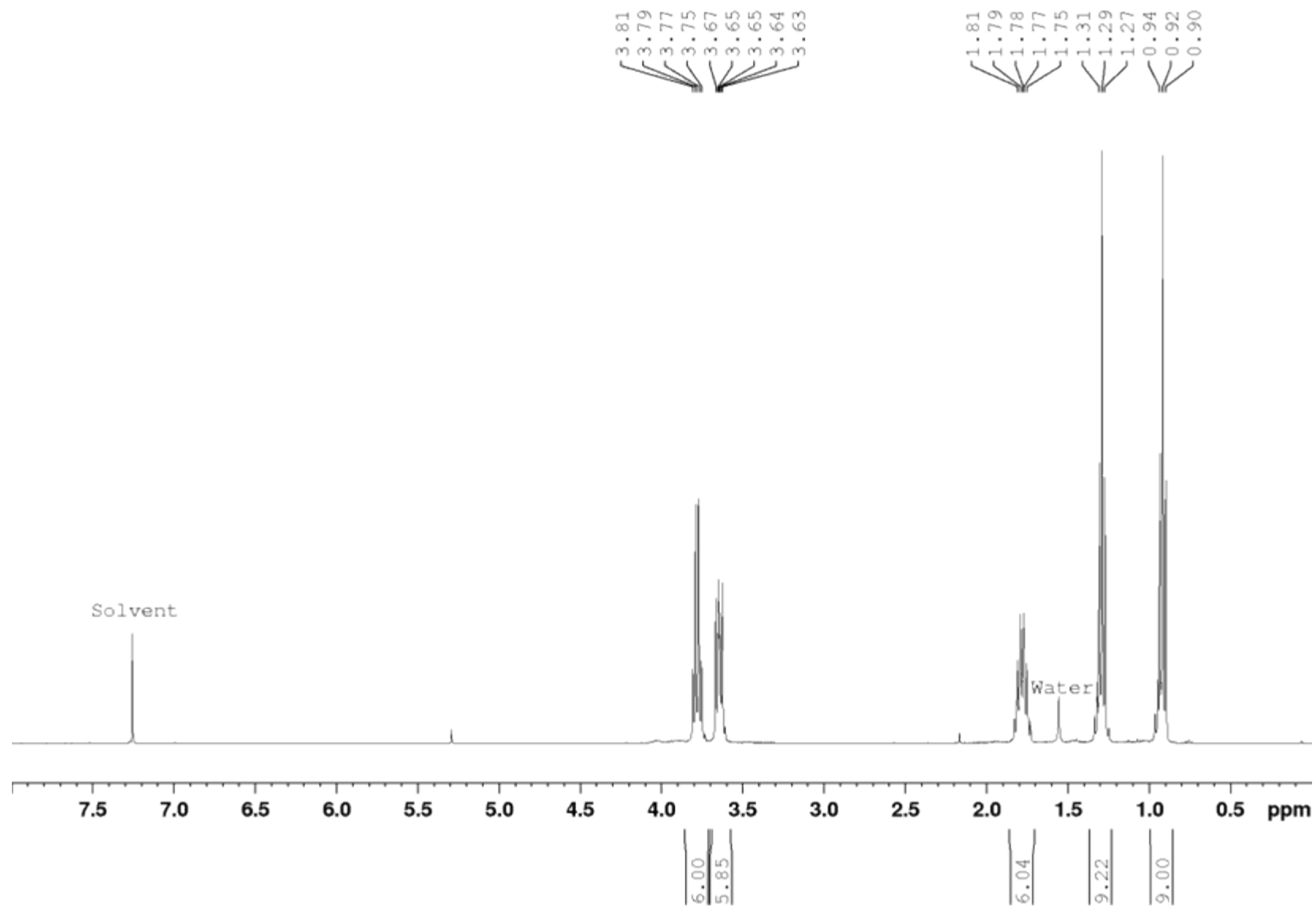
¹H NMR spectrum of **15** (298 K, CD₂Cl₂)



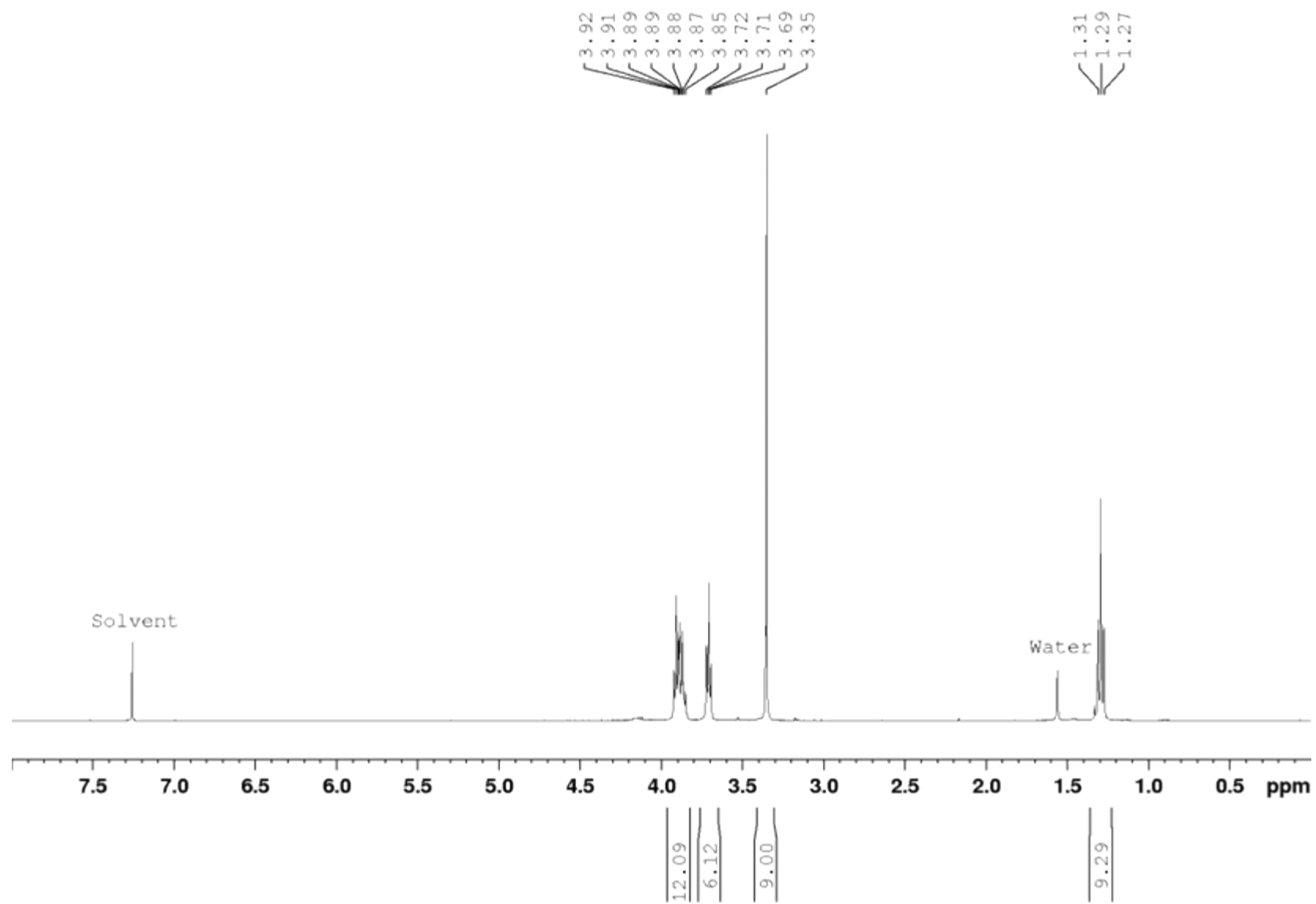
¹H NMR spectrum of **16** (298 K, CDCl₃)



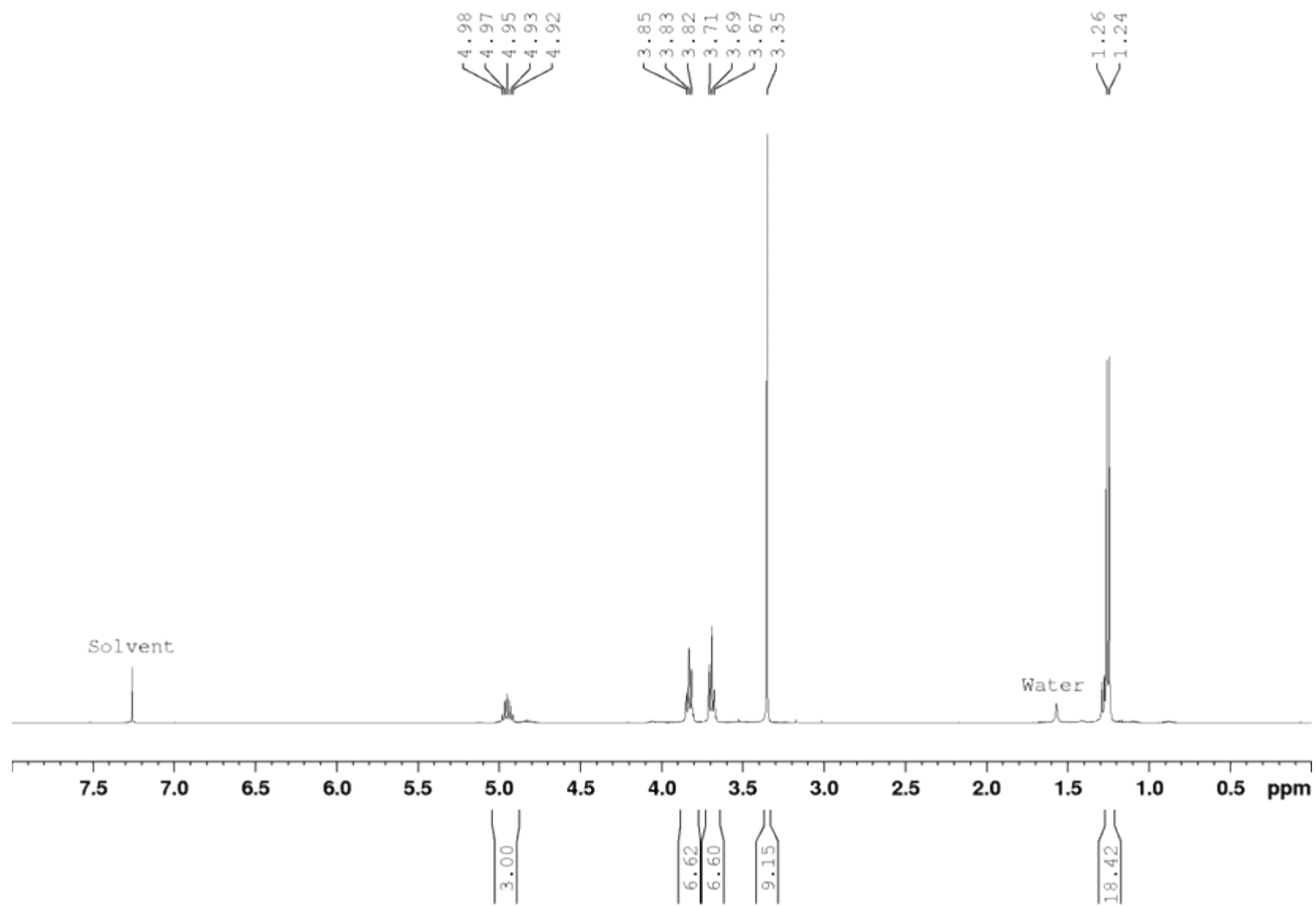
¹H NMR spectrum of 17 (298 K, CDCl₃)



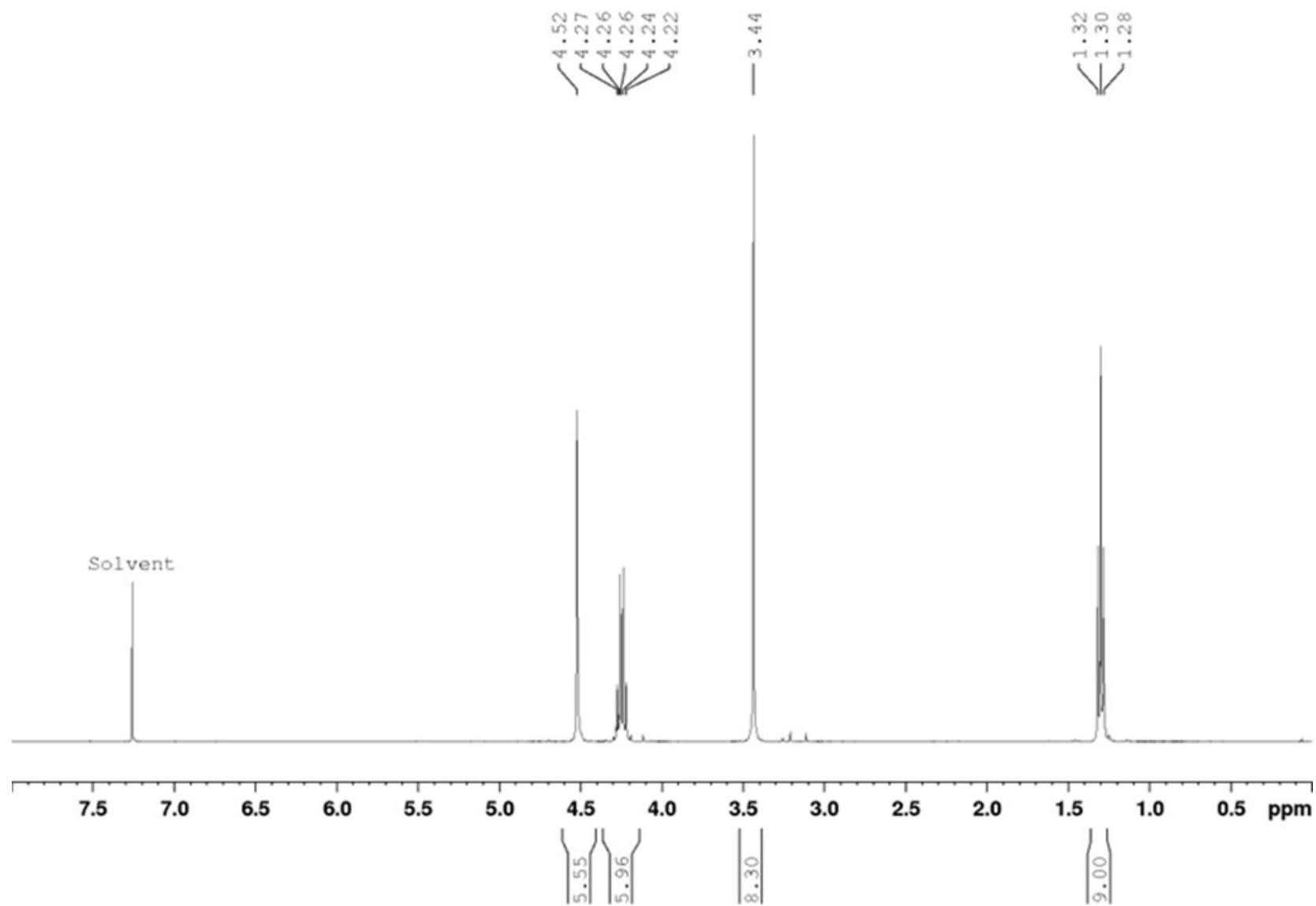
^1H NMR spectrum of **18** (298 K, CDCl_3)



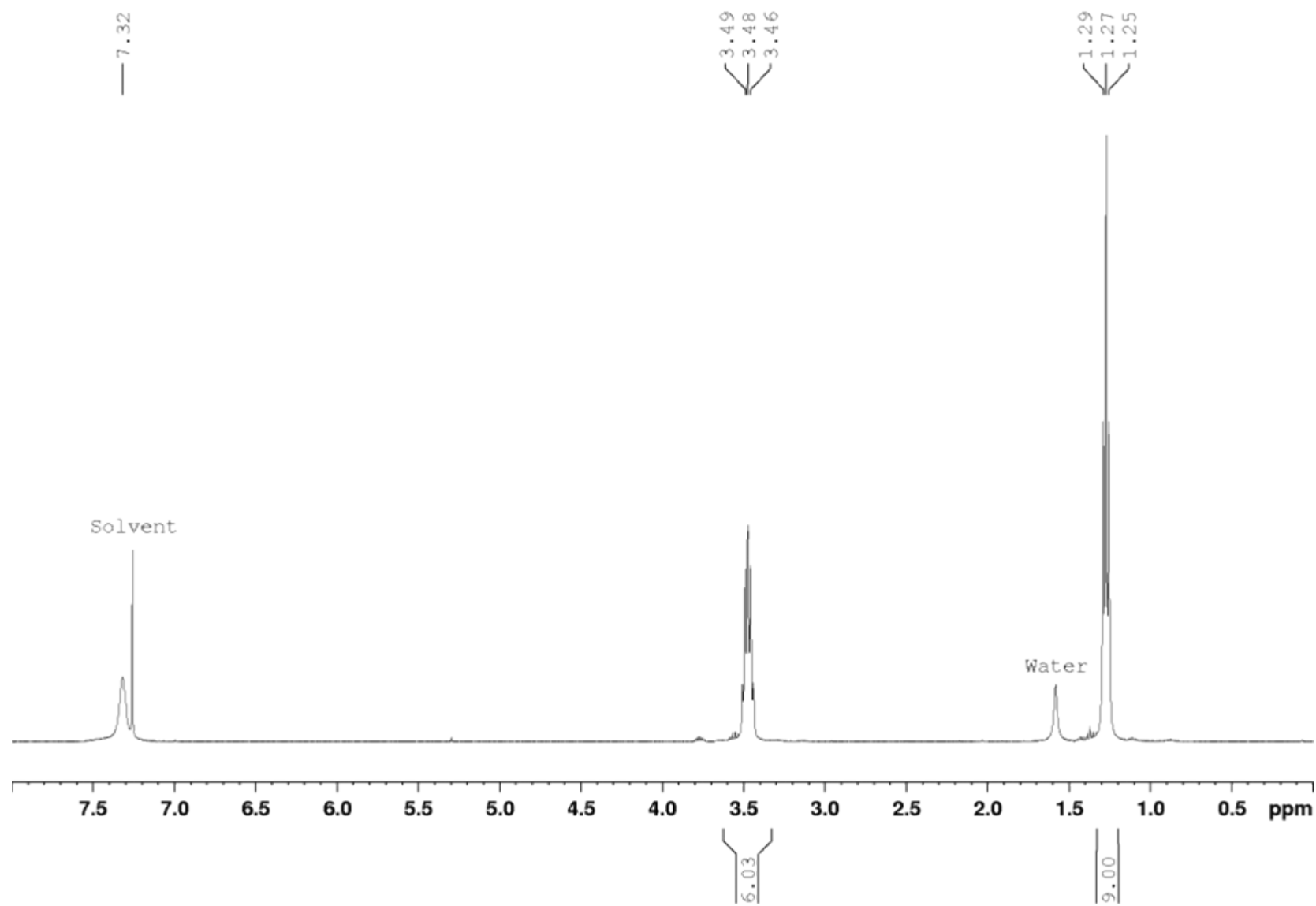
¹H NMR spectrum of **19** (298 K, CDCl₃)



¹H NMR spectrum of **20** (298 K, CDCl₃)



¹H NMR spectrum of **21** (298 K, CDCl₃)



¹H NMR spectrum of **22** (298 K, CDCl₃)

3. Crystal data of complex 22

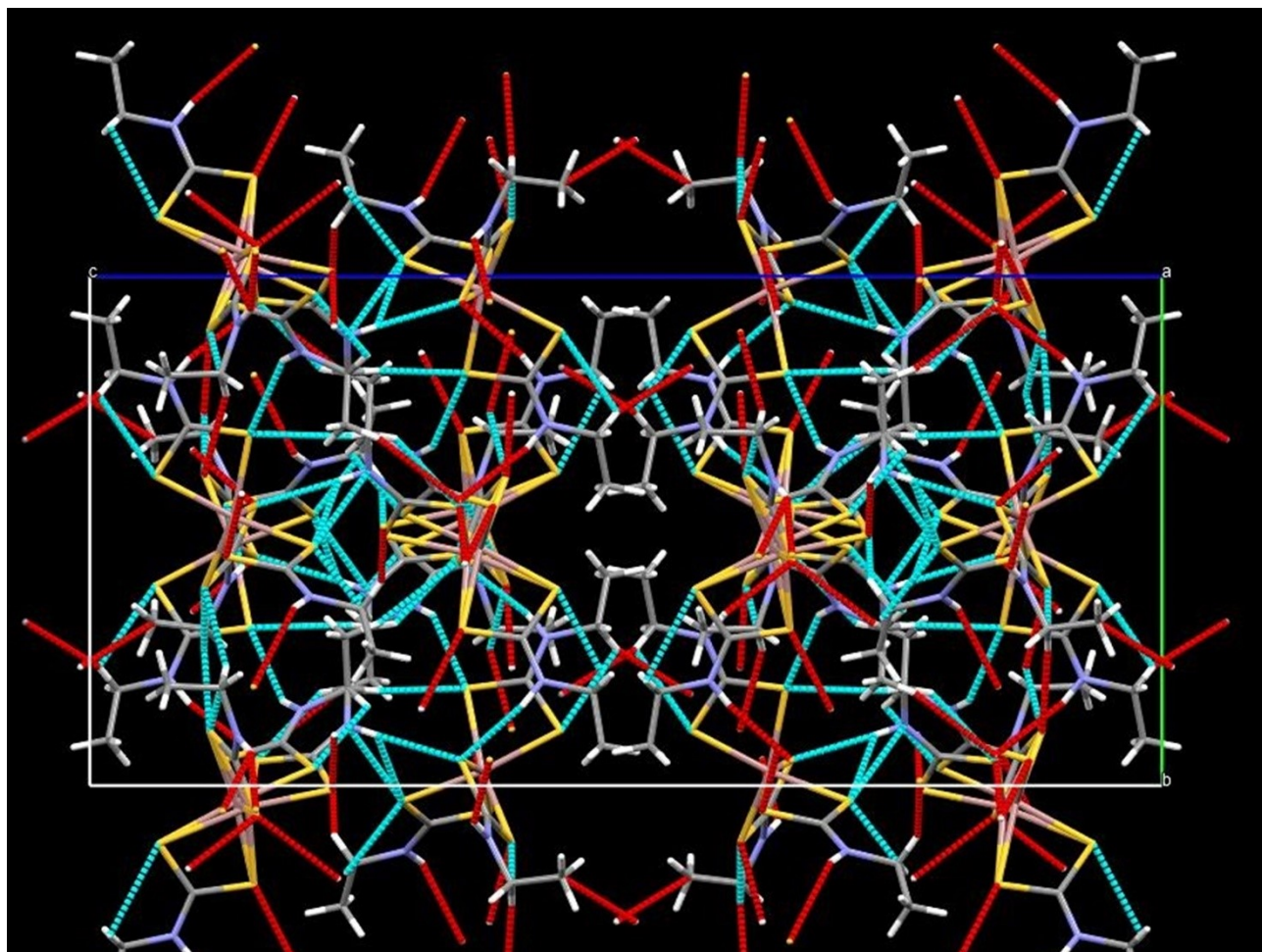


Figure S1. Molecular packing diagram showing hydrogen bonds (HB, dashed in cyan) and other short nonbonding contacts (dashed in red). The cell is seen looking down the crystallographic a axis.

Table S1. Tightest HB and non-bonding interactions of complex **22**

Acceptor (A) atom	Donor (D)atom	Parent (P) atom ^a	Distance A...D (Å)	Angle A-D-P (°) ^b	Symmetry
S7	H3A	N3A	2.56	173.9	$1 - x, 1/2 + y, 1/2 - z$
S11	H8BC	C8	2.57	163.6	$1 - x, 1/2 + y, 1/2 - z$
S10	H4	N4	2.60	142.5	$-1/2 + x, 1 - y, z$
S5	H1	N1	2.62	137.8	$1/2 + x, 2 - y, z$
S7	H2	N2	2.62	137.6	x, y, z
S5A	H1	N1	2.65	158.4	$1/2 + x, 2 - y, z$

^a Atom bound to Donor atom responsible for HB contact; ^b angle between acceptor-donor-parent atoms.

4. IN SOLUTION STABILITY OF 6 AND 21 COMPLEXES

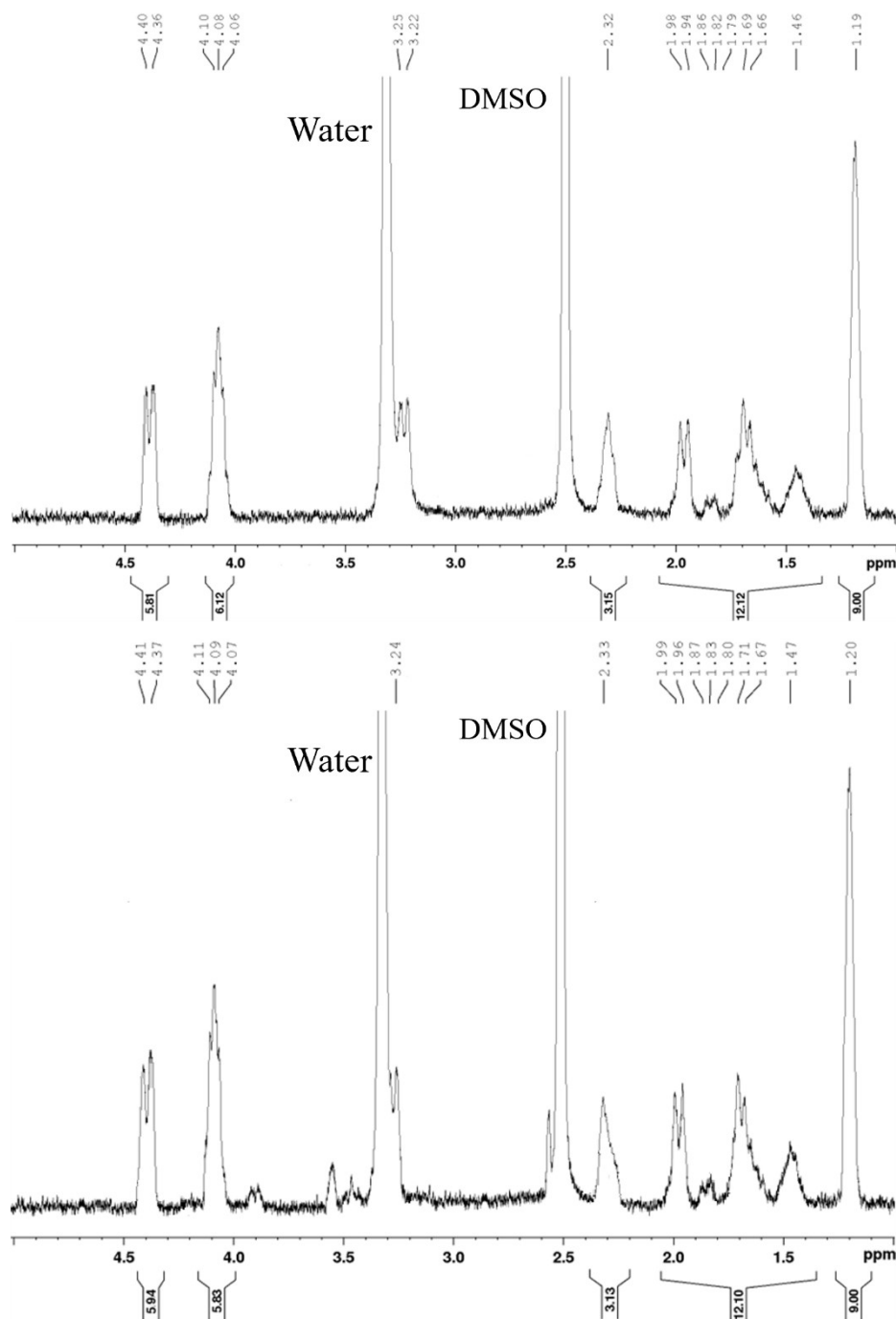


Figure S2. ¹H NMR spectrum of complex **6** in DMSO-*d*₆/deuterated physiologic saline solution (conc. 2 mg/mL). Top: 0 min; bottom: 72 hours. The sample was prepared as indicated in the main text. ¹H NMR: δ_{H} (DMSO-*d*₆/deuterated physiologic saline solution, 400.13 MHz) 1.19 (bt, 9H, CH₃CH₂O); 1.47-1.98 (series of m, 12H total, NCH₂CH₂CHC(O)OEt); 2.32 (m, 3H, NCH₂CH₂CHC(O)OEt); 3.23 (m, partially overlapped with residual H₂O, \approx 6H, NCH₂CH₂CHC(O)OEt); 4.08 (bq, 6H, CH₃CH₂O); 4.38 (m, 6H, NCH₂CH₂CHC(O)OEt).

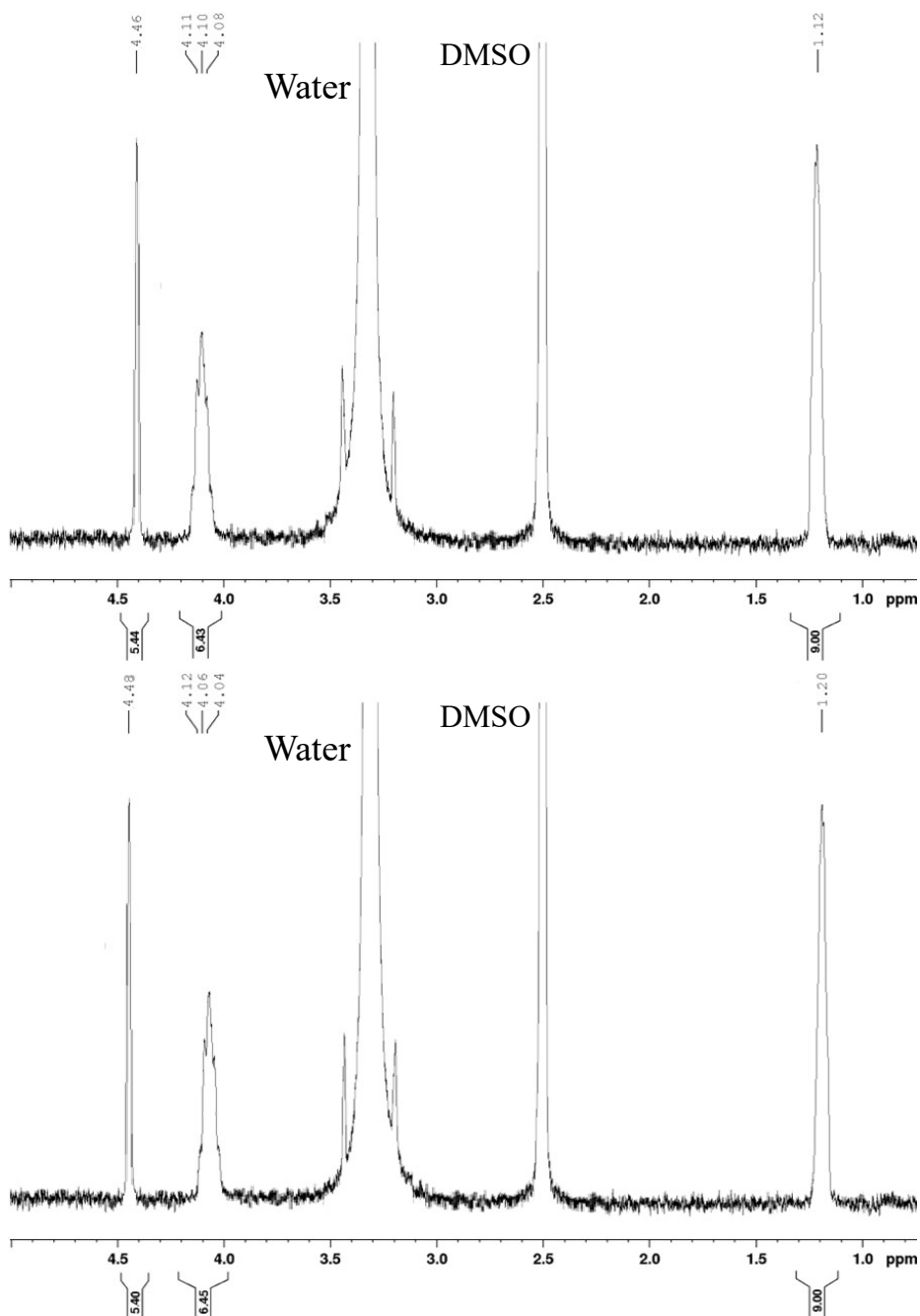


Figure S3. ^1H NMR spectrum of complex 21 in $\text{DMSO-}d_6$ /deuterated physiologic saline solution (conc. 2 mg/mL). Top: 0 min; bottom: 72 hours. The sample was prepared as indicated in the main text. ^1H NMR: δ_{H} ($\text{DMSO-}d_6$ /deuterated physiologic saline solution, 400.13 MHz) 1.12 (bt, 9H, OCH_2CH_3); ~ 3.33 (overlapped with residual H_2O , NCH_3); 4.10 (bq, 6H, OCH_2CH_3); 4.46 (s, 6H, $\text{NCH}_2\text{COOCH}_2\text{CH}_3$).

Brief description of the concepts “soluble, insoluble , less soluble” referred in the main text.

Soluble, slightly soluble, and insoluble are here intended as qualitative determinations. When we indicate a compound as “soluble” in a solvent, we mean that the compound completely dissolves in the given solvent at all the concentration used for every analysis we performed. On the contrary, “insoluble” means that the compound does not dissolve in the given solvent, at any concentration. “Slightly soluble” means that it dissolves in the given solvent at a concentration required for a test but does not dissolve at a higher concentration required for another analysis. As an example, compounds **3, 4, 7, 10, 14-16** and **18-19** were found suitably soluble in DMSO (1 mg/mL) for biological testing whereas they were not as much as soluble at concentrations required for NMR analysis (10-20 mg/mL); **8, 22** and **23** were totally insoluble in DMSO, thus precluding biological testing.

5. FIGURES RELATED TO BIOLOGICAL TESTS

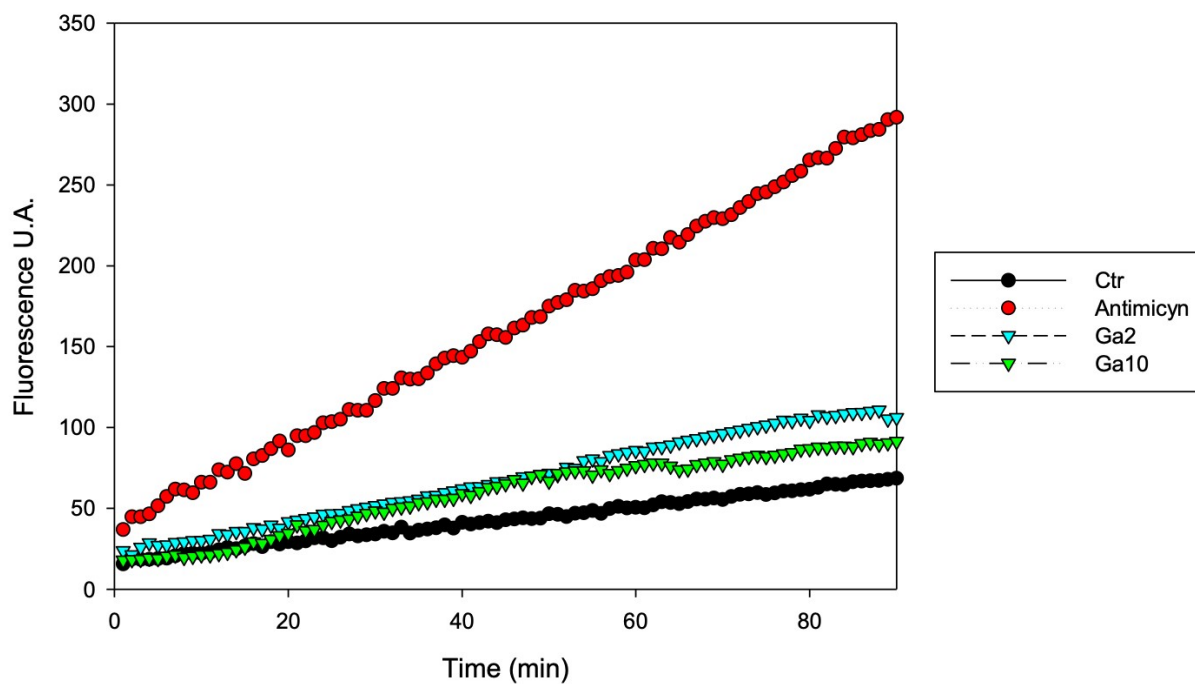


Figure S4. Effect of Ga-DTC complexes on hydrogen peroxide formation in HCT-15 cells. HCT-15 cells were pre-incubated in PBS/10 mM glucose medium for 20 min at 37 °C in presence of 10 μ M CM-DCFDA and then treated with 10 μ M of tested compounds.

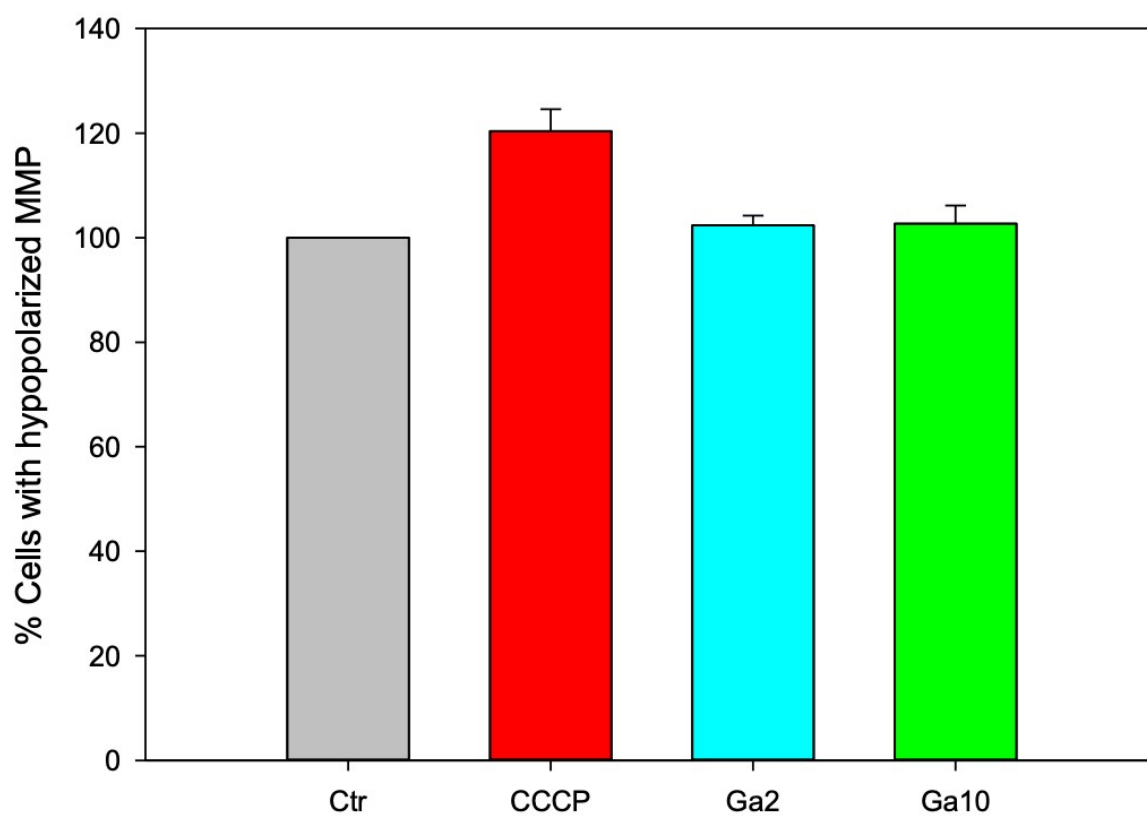


Figure S5. HCT-15 cells were treated for 24 h with IC50 concentrations of tested complexes or CCCP (3 μ M). The mitochondrial membrane potential was determined by Mito-ID® Membrane Potential Kit. Data are the means of three independent experiments. Error bars indicate S.D. $P < 0.1$, $**P < 0.01$ compared with control.

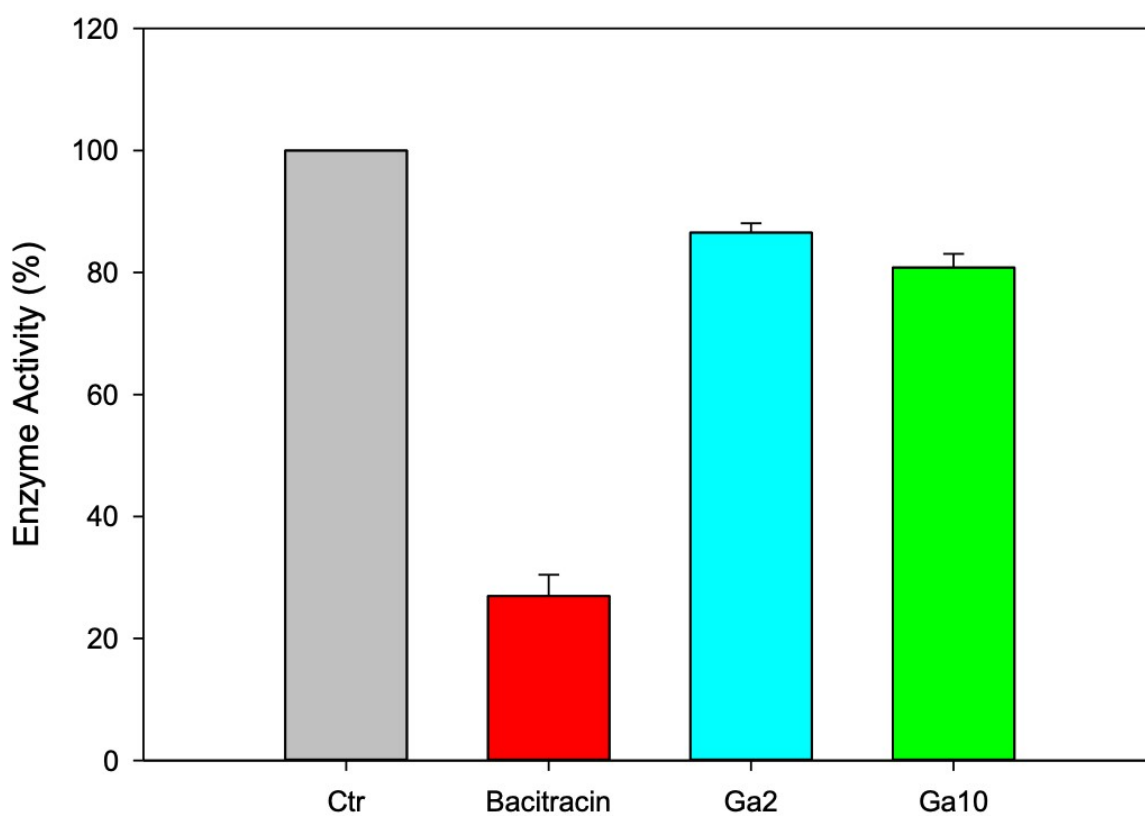


Figure S6. PDI inhibition induced by tested compounds was measured by Proteostat PDI assay kit. The PDI inhibitor Bacitracine (0.5 mM) was used as a positive control. Error bars indicate S.D. $**p < 0.01$ compared with control.