Design and Synthesis of a Peptide Derivative of Ametantrone Targeting the Major Groove of the d(GGCGCC)$_2$ Palindromic Sequence

Supporting Material

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Chemistry

General information. Commercially available chemicals were purchased from Sigma-Aldrich and used as received, unless otherwise stated. ^{1}H and $^{13}C\{^{1}H\}$, as 2D COSY and NOESY NMR spectra which were used for peak assignment, were recorded on a Bruker Avance III 400 MHz spectrometer and a Bruker AMX 300 MHz spectrometer. The solvent for each spectrum is given in parentheses. Chemical shifts are reported in ppm and are relative to TMS internally referenced to the residual solvent peak. Datasets were edited with Bruker TopSpin and iNMR softwares. The multiplicity of signals is reported as singlet(s), doublet (d), triplet (t), quartet (q), multiplet (m), broad (br) or a combination of any of these. High-resolution mass spectra were recorded on an Applied Biosystems Mariner ESI–TOF and on a Waters Xevo QTof. The purity profile was assayed by HPLC using a Varian Pro-Star system equipped with a Biorad 1706 UV–Vis detector (254 nm) and an Agilent C-18 column (5 μm, 4.6 × 250 mm). Purity was ≥ 97%, unless otherwise stated. An appropriate ratio of water (A) and acetonitrile (B) was used as mobile phase with an overall flow rate of 1 mL/min; the general method for the analyses is reported here: 0 min (95% A–5% B), 5 min (95% A–5% B), 15 min (5% A–95% B), 20 min (5% A–95% B), and 22 min (95% A–5% B).

*N^1-(2-Chloroethyl)ethane-1,2-diamine dihydrochloride (**15**)*

2-(2-Aminoethylamino)ethanol (3.0 g, 28.80 mmol) was dissolved in 20 mL of EtOH, then the mixture was placed in an ice bath and 7 mL of 37 % HCl were added. After evaporation, the di-hydrochloride salt of compound **2** was dissolved in 20 mL of $SOCl_2$ and the mixture was stirred under reflux for 2 h. The solid was filtered under reduced pressure and washed with hexane and toluene obtaining a white solid product (3.2 g, 57 %); ^{1}H-NMR (300 MHz, D_2O): δ 3.34 (m, 2H, CH_2), 3.42 (m, 2H, CH_2), 3.47 (t, J = 5.7 Hz, 2H, $ClCH_2C\underline{H}_2$), 3.83 (t, J

= 5.7 Hz, 2H, $ClCH_2$). HRMS (ESI) calcd for $C_4H_{12}ClN_2$ $[M+H^+]$: 123.0691, found: 123.0552.

*N^1-(2-Azidoethyl)ethane-1,2-diamine (**16**)[55]*

Compound **15** (3.20 g, 16.40 mmol) and NaN_3 (5.32 g, 81.80 mmol) were dissolved in 30 mL of deionized water, the mixture was stirred at 80 °C for 24 h then the solution was cooled down to r.t., evaporated to half volume, alkalinized with 15 mL of 40 % KOH and extracted with Et_2O (3x30 mL). The organic phase was then evaporated under reduced pressure to obtain a yellow oil (1.37 g, 65 %); ^{1}H-NMR (300 MHz, D_2O): δ 2.65 (m, 2H, $C\underline{H}_2NH_2$), 2.61-2.79 (m, 4H, $C\underline{H}_2NHC\underline{H}_2$), 3.46 (t, J = 5.8 Hz, 2H, CH_2N_3). HRMS (ESI) calcd for $C_4H_{13}N_5$ $[M+H]^+$: 130.1094, found: 130.1101.

*1,4-bis((2-((2-Azidoethyl)amino)ethyl)amino)anthracene-9,10-dione (**17**)*

Anthraquinone **9** (75 mg, 0.31 mmol) was dissolved in 0.5 mL of DMSO and the mixture was poured in a solution of compound **16** (400 mg, 3.10 mmol, 10 equiv)) in 0.5 mL of DMSO. The reaction was stirred at 35 °C for 15 h, until TLC (DMC/MeOH, 90/10) showed a complete conversion. The reaction mixture was poured in 30 mL of deionized water and extracted with a total of 160 mL of DCM. The organic phase was evaporated under reduced pressure and the crude product was purified by flash chromatography on silica gel (DCM/MeOH from 99/1 to 80/20), obtaining 63 mg of a blue ink-like solid (48 %); ^{1}H-NMR (300 MHz, Methanol-d_4): δ 2.80 (m, 2H, $C\underline{H}_2CH_2N_3$), 2.92 (t, 2H, $ArNHCH_2C\underline{H}_2$), 3.50 (t, 2H, CH_2N_3), 3.53 (t, 2H, $ArNHC\underline{H}_2$), 7.12 (s, 2H, $Ar_{2,3}H$), 7.66 (dd, J_o = 6.0 Hz, J_m = 3.3 Hz, 2H, $Ar_{5,8}H$), 8.16 (dd, 2H, $Ar_{6,7}H$, J_o = 6.0, J_m = 3.3); ^{13}C-NMR (100 MHz, Methanol-d_4): δ 41.7, 50.7, 109.1, 123.5, 125.5, 131.7, 134.1, 146.0, 181.4. HRMS (ESI) calcd for $C_{22}H_{27}N_{10}O_2$ $[M+H]^+$: 463.2320, found: 463.2341.

*N-Acetylglycine-OSu (**19**)*

N-Acetylglycine **18** (120 mg, 1.02 mmol), DCC (207 mg, 1.00 mmol) and HOSu (120 mg, 1.04 mmol) were dissolved in 3 mL of DMF and the mixture was stirred for 4 h, then the solvent was evaporated under reduced pressure providing a solid. The product was purified by flash chromatography (DCM/MeOH, 99/1 to 80/20) obtaining 210 mg (96 %) of an off-white solid; ^{1}H-NMR (300 MHz, Acetone-d_6): δ 1.99 (s, 3H, $COCH_3$), 2.88 (s, 4H, $CO(CH_2)_2CO$), 4.33 (d, J = 6.0 Hz, 2H, CH_2CO), 7.77 (br s, 1H, CONH). HRMS (ESI) calcd for $C_8H_{11}N_2O_5$ $[M+H]^+$: 215.0670, found: 215.0639.

*tert-Butyl (5-(2-acetamidoacetamido)-6-oxo-6-(prop-2-yn-1-ylamino)hexyl)carbamate (**21**)*

Boc-Lys (138 mg, 0.56 mmol) and compound **19** (220 mg, 0.56 mmol) were dissolved in 20 mL of DCM and 3 mL of DMF, and then DIPEA was added (100 µL, 0.57 mmol). The mixture was stirred for 1 h until TLC (DCM/MeOH, 90/10) showed the complete consumption of the reactants. DCC (270 mg, 1.3 mmol), HOSu (120 mg 1.0 mmol) and propargylamine (125 µL, 1.9 mmol) were then added and the mixture was stirred for 2 h, then the solvent was evaporated obtaining a solid that was purified by flash chromatography (DCM/MeOH, 99/1 to 80/20), obtaining 22 mg (10 %) of a white solid; ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.1-1.3 (m, 4H, CH_2), 1.37 (s, 9H, Boc-($C\underline{H}_3$)$_3$), 1.4-1.7 (m, 2H, CH_2), 1.86 (s, 3H, $COC\underline{H}_3$), 2.87 (m, 2H, Lys-$C\underline{H}_2$-NH), 3.09 (t, J = 2.3 Hz, 1H, C≡CH), 3.71 (d, J = 5.3 Hz, 2H, $C\underline{H}_2C{\equiv}CH$), 3.84 (d, 2H, Gly-$C\underline{H}_2NH$), 4.19 (m, 1H, Lys-$C\underline{H}CO$), 6.74 (t, 1H, CONH), 7.96 (d, J = 8.2 Hz, 1H, CONH), 8.06 (t, J = 5.3 Hz, 1H, CONH), 8.35 (t, J = 5.3 Hz, 1H, CONH); ^{13}C-NMR (100 MHz, Methanol-d_4): δ 21.0, 22.7, 27.4, 28.0, 29.1, 31.3, 39.7, 42.2, 53.1, 70.8, 78.5, 79.0, 170.3, 172.5, 172.6. HRMS (ESI) calcd for $C_{18}H_{30}N_4O_5Na$ $[M+H]^+$: 405.2140, found: 405.2140.

*N,N'-(((((((9,10-Dioxo-9,10-dihydroanthracene-1,4-diyl)bis(azanediyl))bis(ethane-2,1-diyl)) bis(azanediyl))bis(ethane-2,1-diyl))bis(1H-1,2,3-triazole-1,4-diyl))bis(methylene)) bis(2-(2-acetamidoacetamido)-6-aminohexanamide) trifluoroacetate (**1**)*

Compound **17** (4.6 mg, 0.01 mmol) and alkyne **21** (11.5 mg, 0.03 mmol) were dissolved in 1 mL of DCM. The copper catalyst was prepared separately in 1 mL of deionized water, dissolving K_2CO_3 (2.1 mg, 0.015 mmol), potassium ascorbate (1.0 mg, 0.005 mmol) and $CuSO_4.5\ H_2O$ (0.1 mg, 0.003 mmol): an orange solution was obtained that was added to the DCM solution, and the mixture was stirred for 2 h at r.t. until TLC (DCM/MeOH/TEA, 88/10/2) showed the complete conversion of the reactants giving a single product. The reaction mixture was evaporated to dryness and the crude product was purified by flash chromatography on silica gel (DCM/MeOH/TEA, 88/10/2), obtaining 8.2 mg of a blue ink-like solid (97 %). The obtained product (8.2 mg, 0.007 mmol) was dissolved in 1 mL of DCM and 1 mL of TFA was added. The mixture was stirred for 2 h at room temperature, then it was evaporated under reduced pressure obtaining 6.9 mg of compound **1** as a blue ink-like solid (100 %); ^{1}H-NMR (400 MHz, DMSO-d_6): δ 1.3-1.6 (m, 8H, CH_2), 1.85 (s, 6H, C$\underline{H}_3$), 2.75 (m, 4H, C$\underline{H}_2$-NH_2), 3.28 (m, 4H, ArNHCH_2C$\underline{H}_2$), 3.4-3.6 (m, 12H, NCH_2), 3.71 (t, J = 5.3 Hz, 4H, Gly-C$\underline{H}_2$), 3.82 (d, 4H, ArNHC$\underline{H}_2$), 4.25 (m, 2H, Lys-H_α), 4.33 (ddd, 4H, Lys-CONHC$\underline{H}_2$), 4.71 (t, 2H, CH_2N$\underline{H}$CH_2), 7.53 (s, 2H, ArH), 7.78 (br s, 6H), 7.85 (dd, 2H, ArH), 7.97 (s, 2H, H triazole), 8.02 (d, J = 8.0 Hz, 2H, Gly-CONH), 8.14 (t, J = 5.8 Hz, 2H, AcNH), 8.26 (dd, 2H, ArH), 8.44 (t, J = 5.4 Hz, 2H, Lys-CONH), 9.13 (br s, 4H, Lys-NH_2), 10.63 (t, J = 6.0 Hz, 2H, ArNH); ^{13}C-NMR (100 MHz, DMSO-d_6): δ 9.0, 11.4, 22.8 (Lys-$\underline{C}H_2$), 27.0, 30.0 (CH_3), 31.7 (Lys-CH_2), 34.7 (Lys-CONH$\underline{C}H_2$), 42.7 (Gly-CH_2), 46.2 (NCH_2), 46.8 (NCH_2), 47.0 (NCH_2), 52.7 (Lys-C_α), 110.3 (ArC), 124.0 (CH triazole), 124.6 (ArC), 126.3 (ArC), 133.4 (ArC), 134.1 (ArC), 145.6 (Cqtriazole), 145.7 (ArC-NH), 169.6 (Gly-CO), 170.4

(AcCONH), 172.0 (Lys-CO-NH), 182.2 (Ar-CO). HRMS (ESI) calcd for $C_{48}H_{71}N_{18}O_8$ $[M+H]^+$: 1027.5697, found: 1027.5671.

Mass spectrometry (MS) binding assay

Oligonucleotides were heat-denatured and folded in 150 mM ammonium acetate (pH=7) at room temperature. The oligonucleotides where diluted to final concentration of 5 μM and incubated with the tested compound at DNA:compound ratio of 1:5 overnight at 4°C. Samples were analyzed by direct infusion electrospray ionization (ESI) on a Xevo G2-XS QTof mass spectrometer (Waters, Manchester, UK) in negative ionization mode. The carrying buffer was 150 mM ammonium acetate and 10 μL samples were typically injected for each analysis. The electrospray capillary was set at 1.8 kV, the source and desolvation temperatures were 50°C and 80°C, respectively, and the sampling cone was at 120 V. All these parameters ensured minimal fragmentation of the complexes. The instrument was calibrated using a 2 mg/mL solution of sodium iodide in 50% isopropanol. Additionally, the use of the LockSpray™ during the analysis provided a typical <2 ppm mass accuracy. The internal standard LockSpray™ consisted in a solution of leuenkephalin 1 μg/ml in acetonitrile/water (50:50, v/v) containing 0.1% formic acid. Binding affinities were calculated for each experiment using the reconstructed-ion chromatogram area for each species with the following formula: $[BA= (\Sigma DNA_b/(\Sigma DNA_f + \Sigma DNA_b)) \times 100]$, where BA is the binding affinity, DNA_b is chromatogram area of bound DNA and DNA_f is the chromatogram area of free DNA. DNA_b comprises DNA with one or two bound ligands, where present. Free DNA and ammonium adducts were included in the calculation and the most intense signals were detected with the 6- charge states.

Z1 duplex was obtained by annealing 5'-ACT ATT CCC GGG TAA TGA-3' and 5'-TCA TTA CCC GGG TAA AGT-3' (monoisotopic mass: 10991.902).

Z2 duplex was obtained from 5'-ACT ATT GGC GCC TAA TGA-3' and 5'-TCA TTA GGC GCC AAT AGT-3' (monoisotopic mass: 10991.902).

R1 duplex was obtained from 5'–GTG AGA TAC CGA CAG AGG-3' and 5'-CCT CTG TCG TGA TCT CAC–3' (monoisotopic mass: 10993.893).

R2 duplex was obtained from 5'-ACT ATT TAC GTA TAA TGA-3' and 5'-TCA TTA TAC GTA AAT AGT-3' (monoisotopic mass: 10987.922). Compound **1** has a monoisotopic mass of 1026.562 ($C_{48}H_{70}N_{18}O_8$).

Spectra of described compounds.

NMR

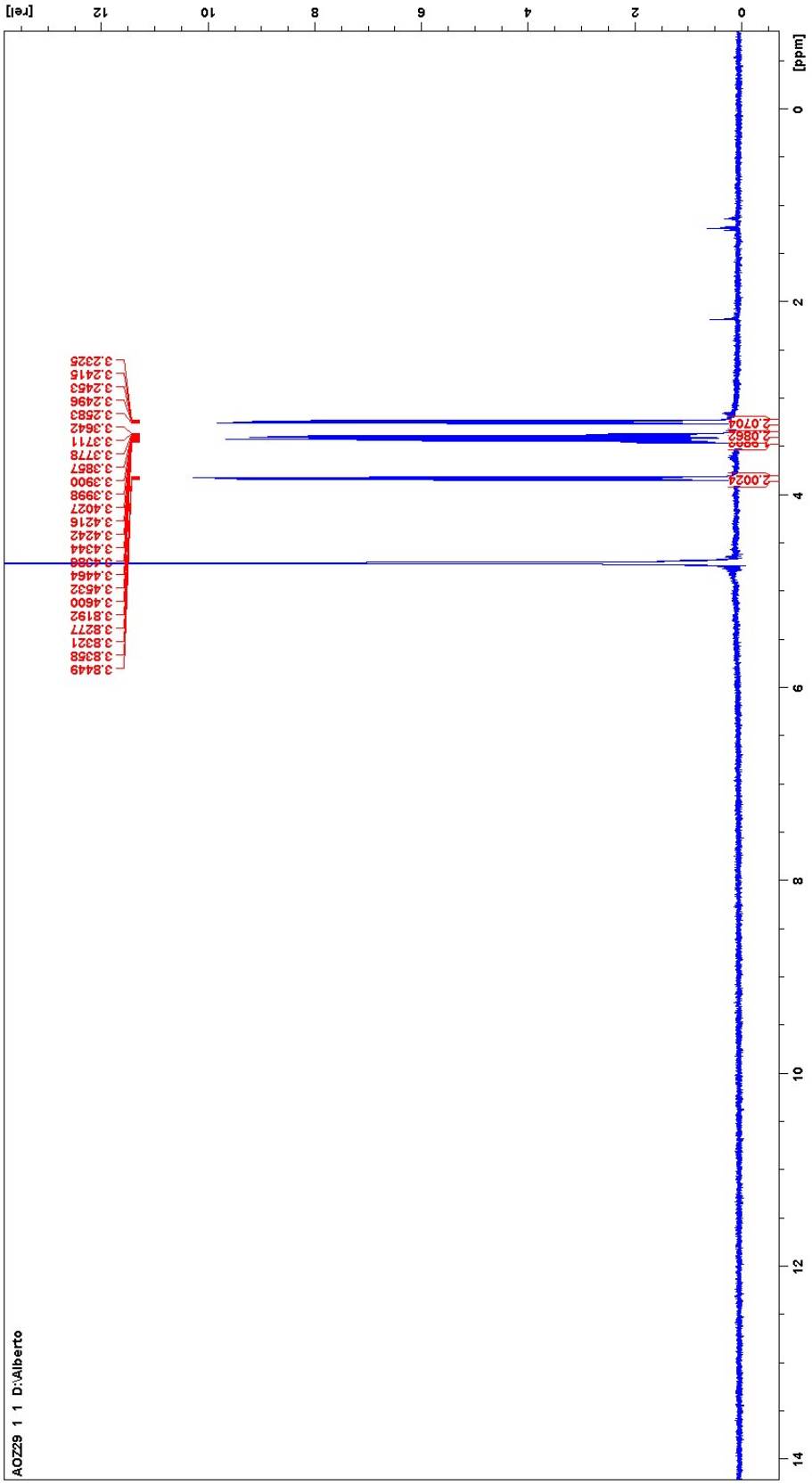

Figure S1. ^{1}H NMR of compound **15**.

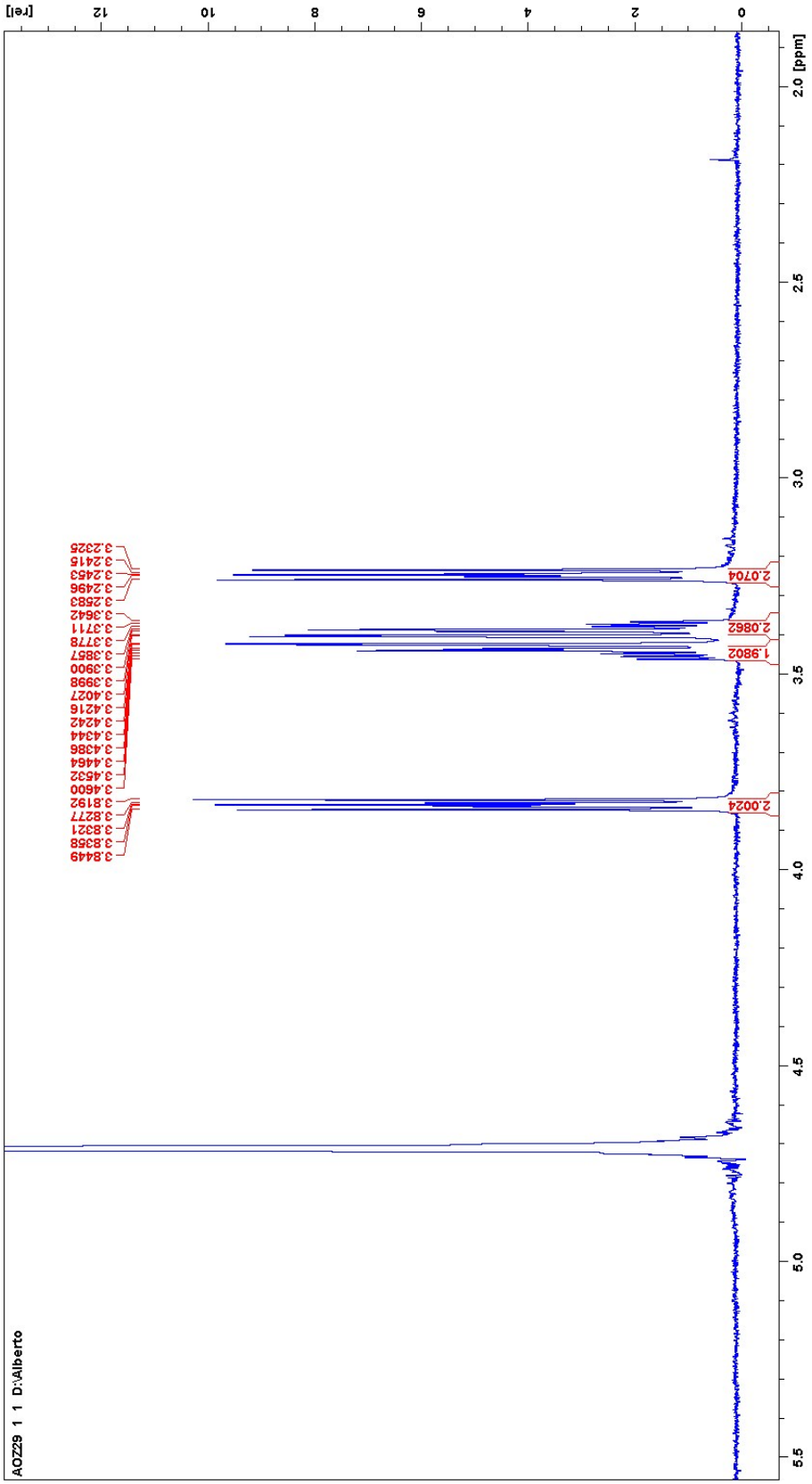

Figure S2. ^{1}H NMR of compound **15**.

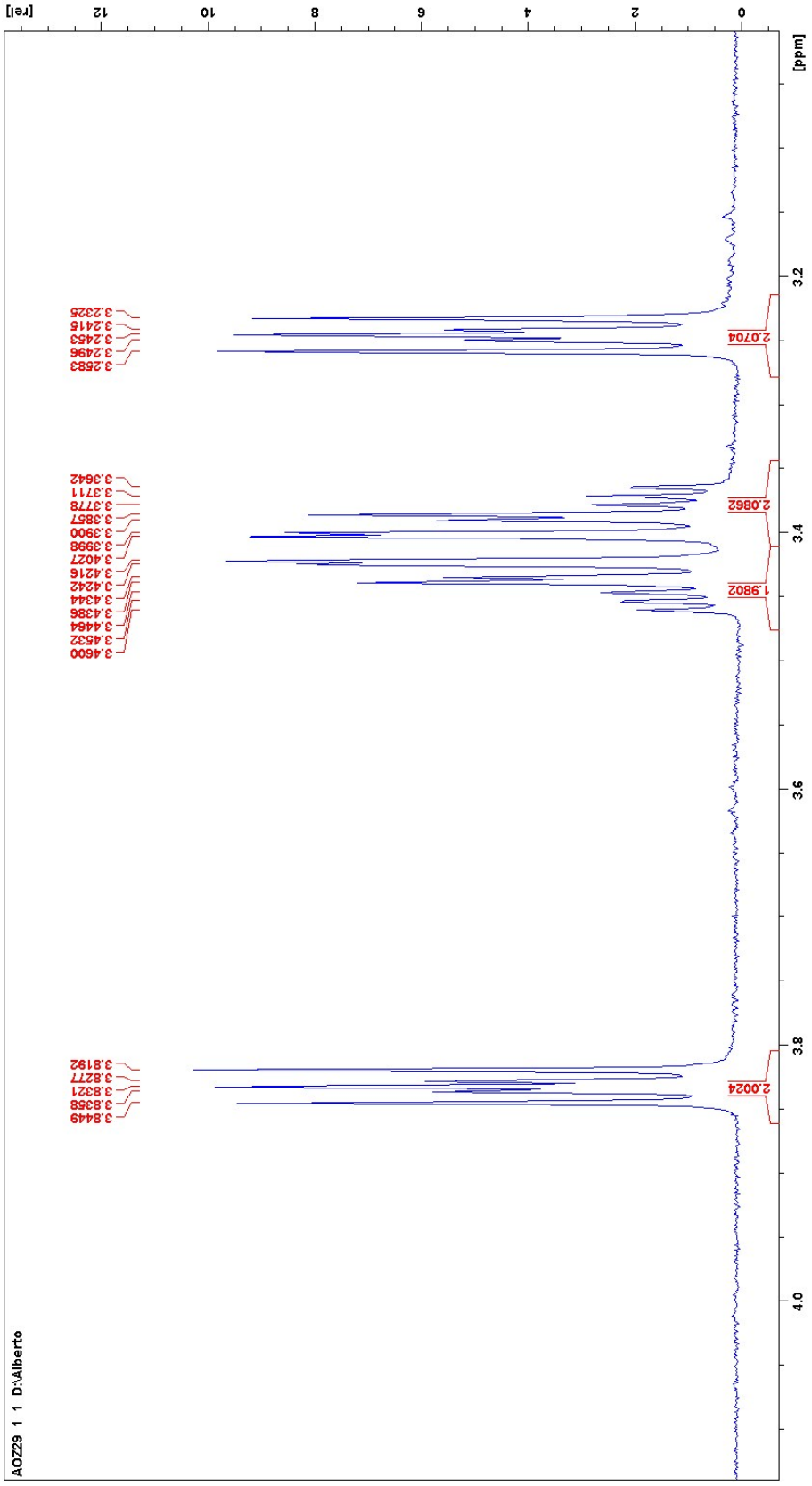

Figure S3. ^{1}H NMR of compound **15**.

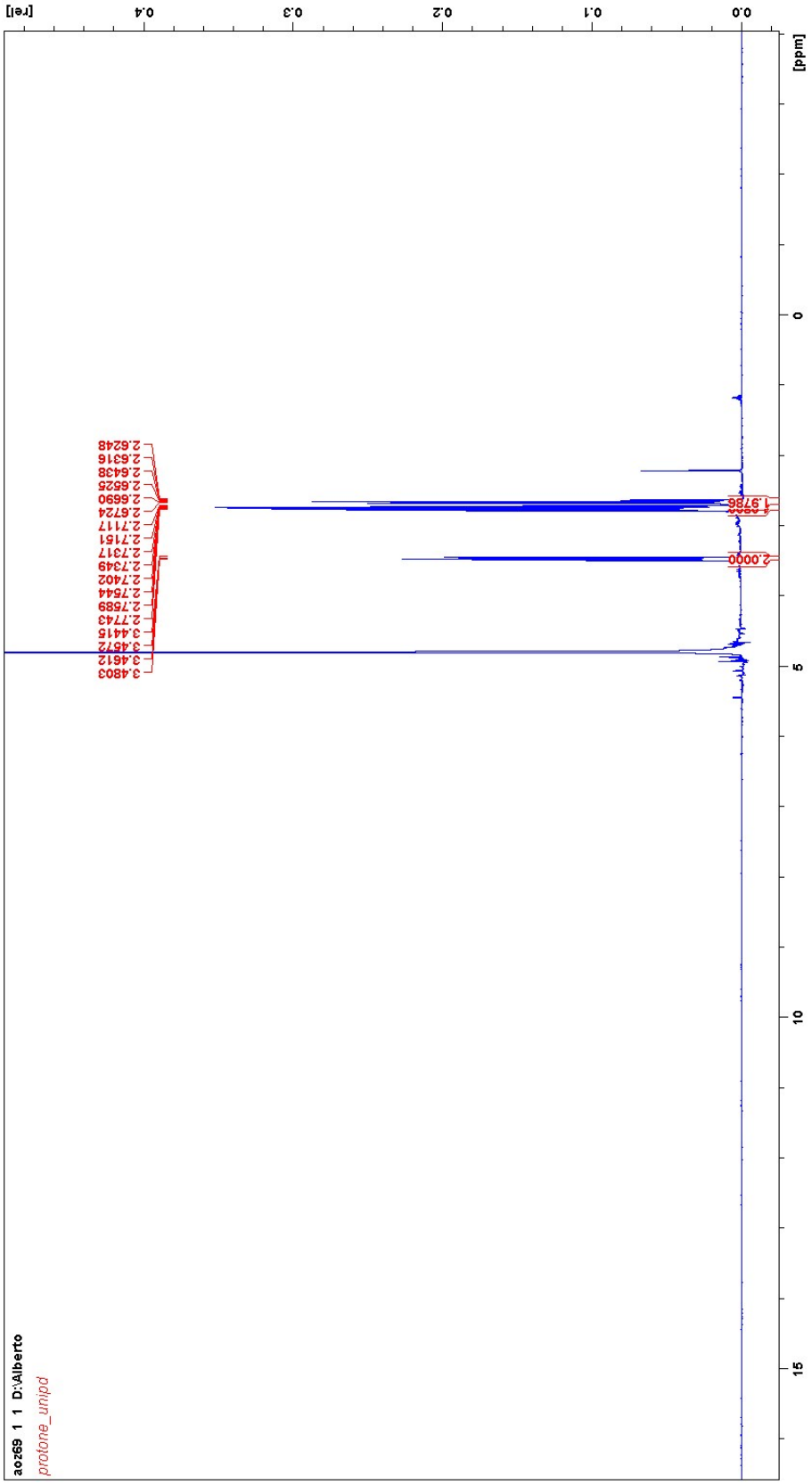

Figure S4. ^{1}H NMR of compound **16**.

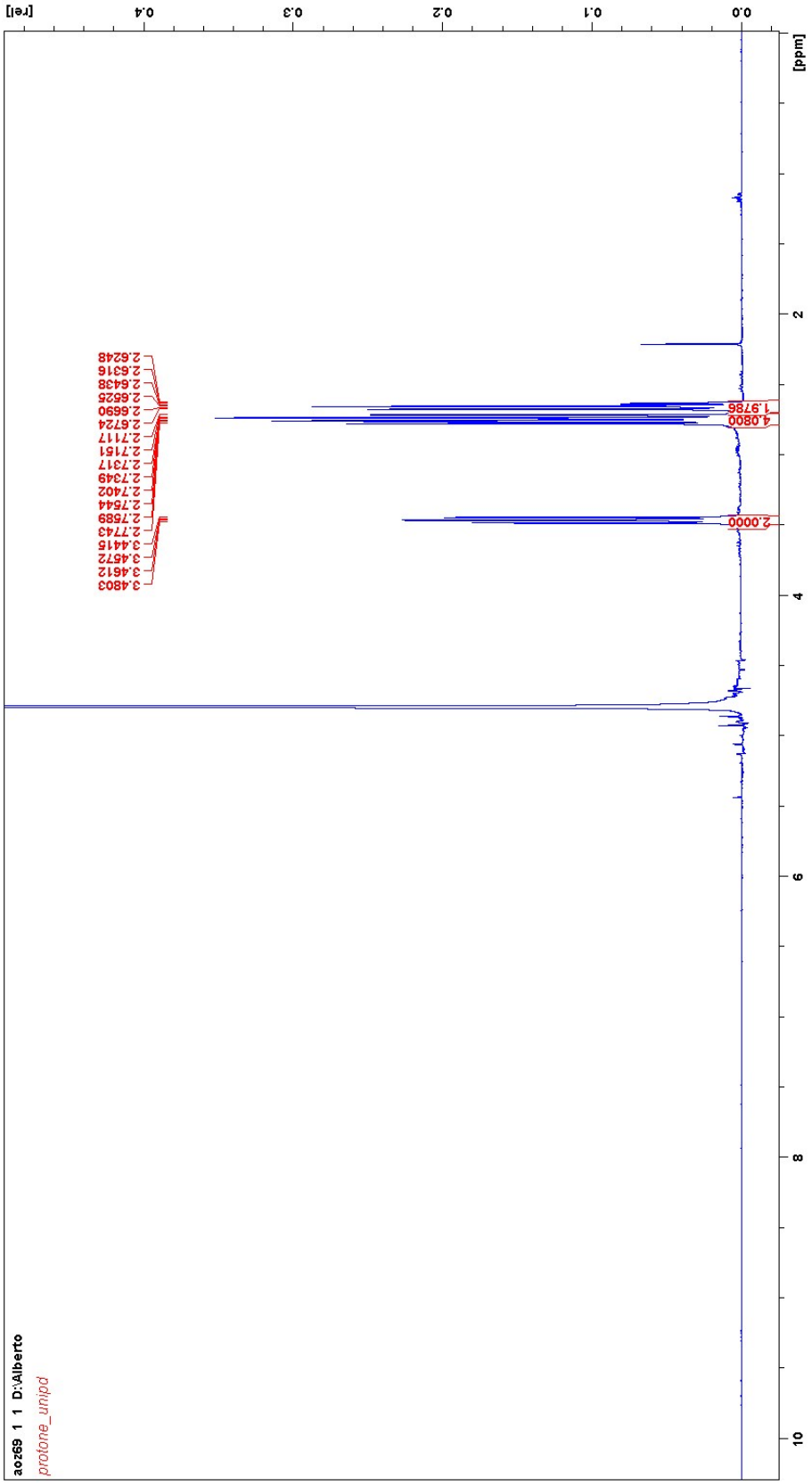

Figure S5. ^{1}H NMR of compound **16**.

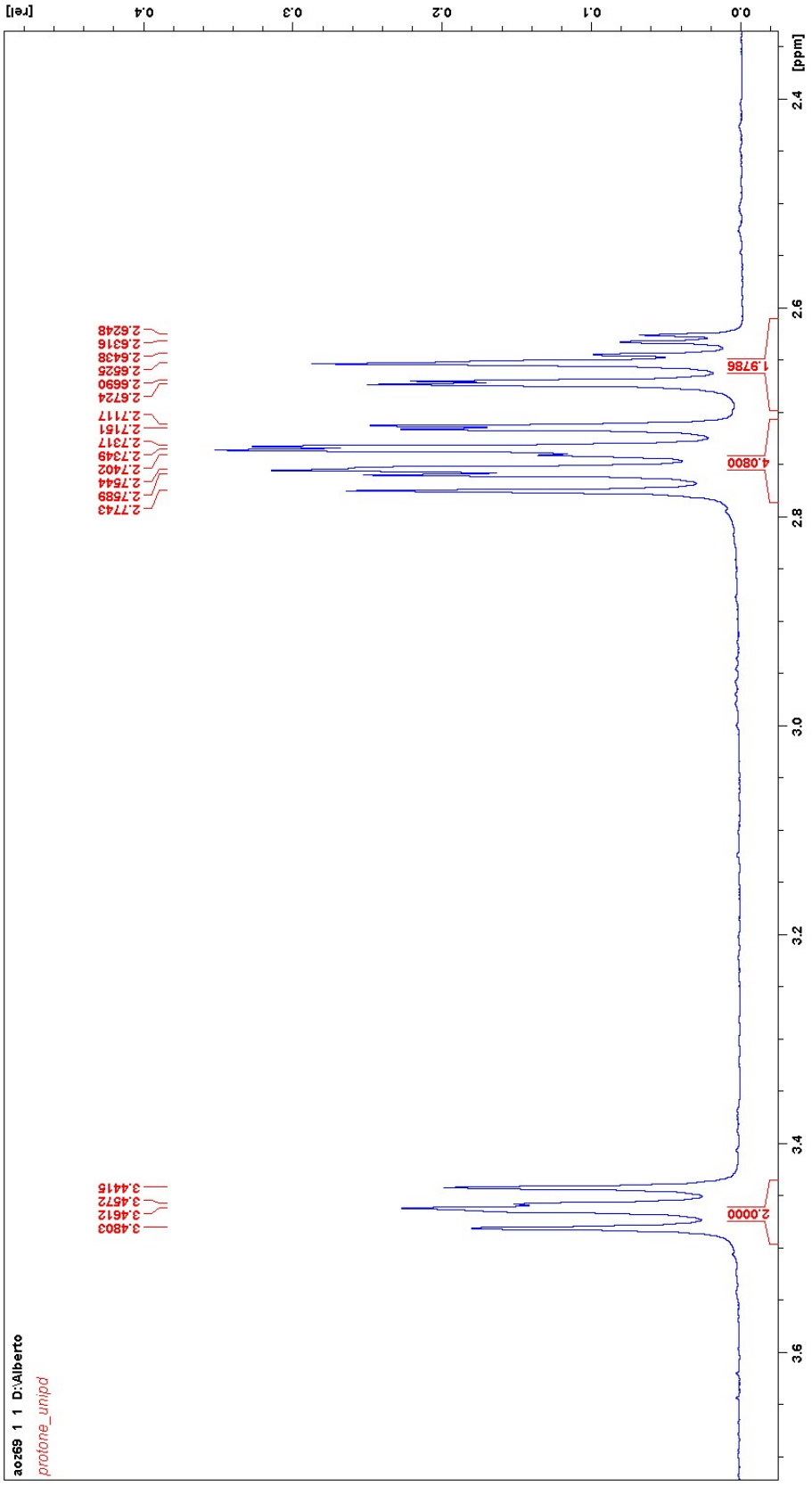

Figure S6. ^{1}H NMR of compound **16**.

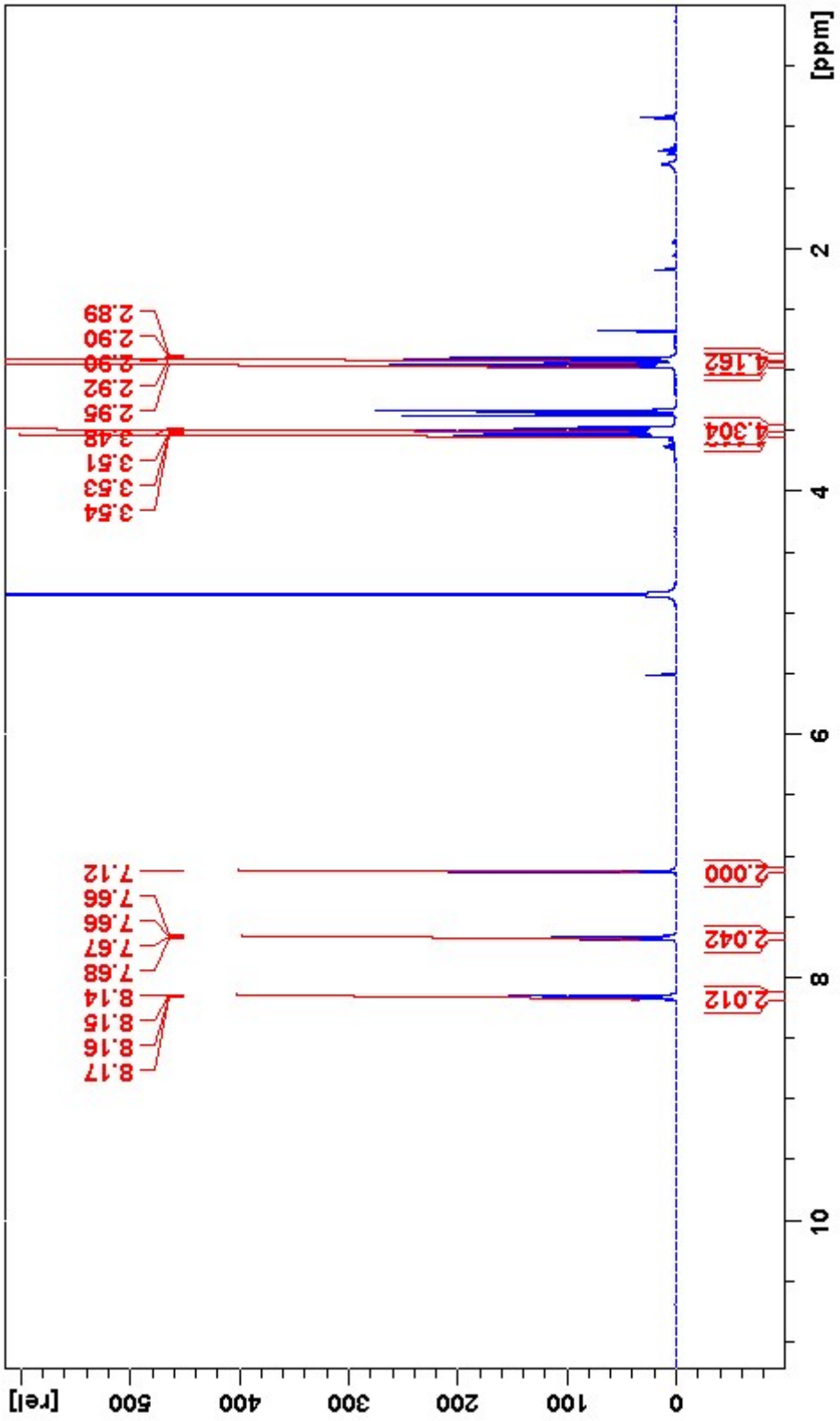

Figure S7. ^{1}H NMR of compound **17**.

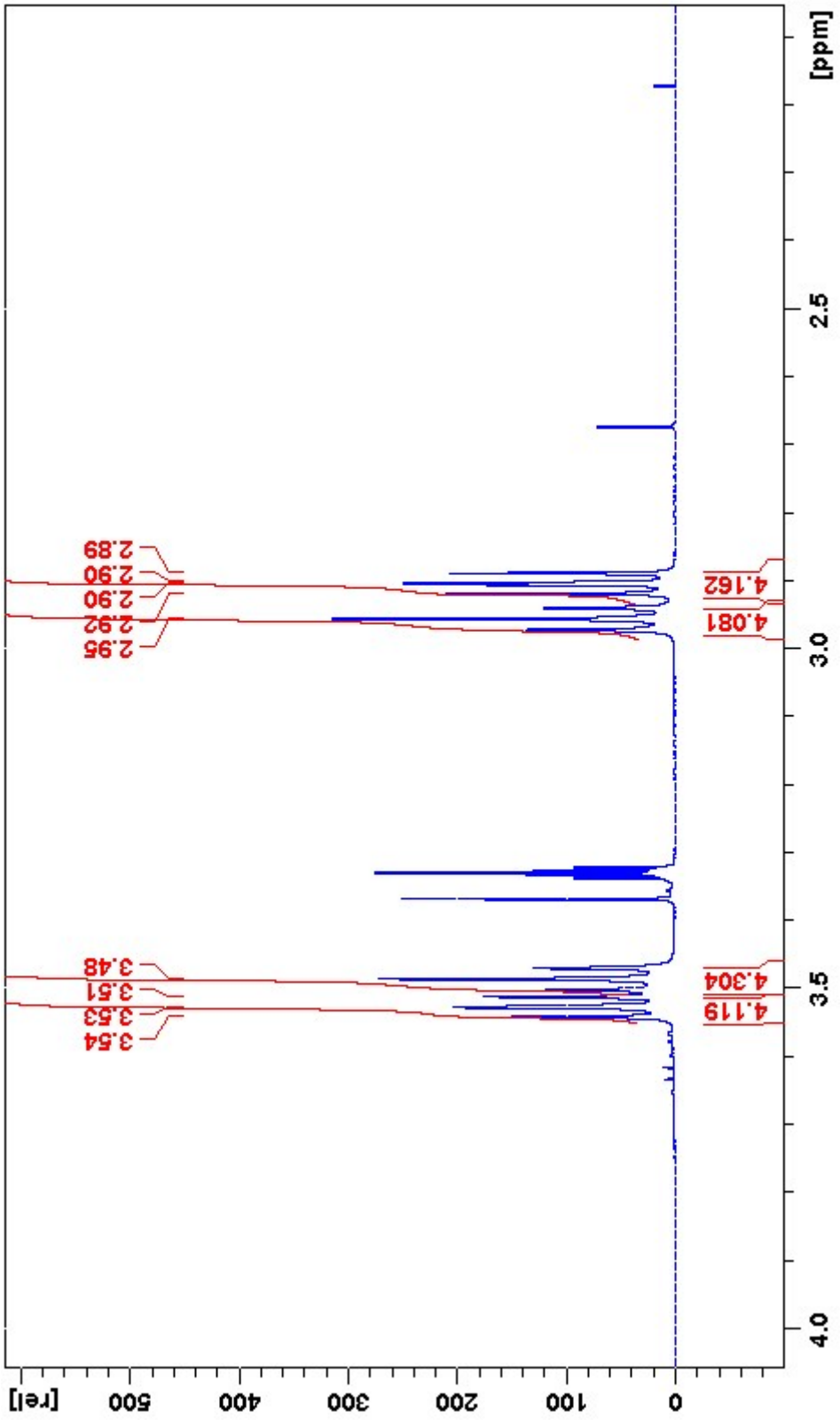

Figure S8. ^{1}H NMR of compound **17**.

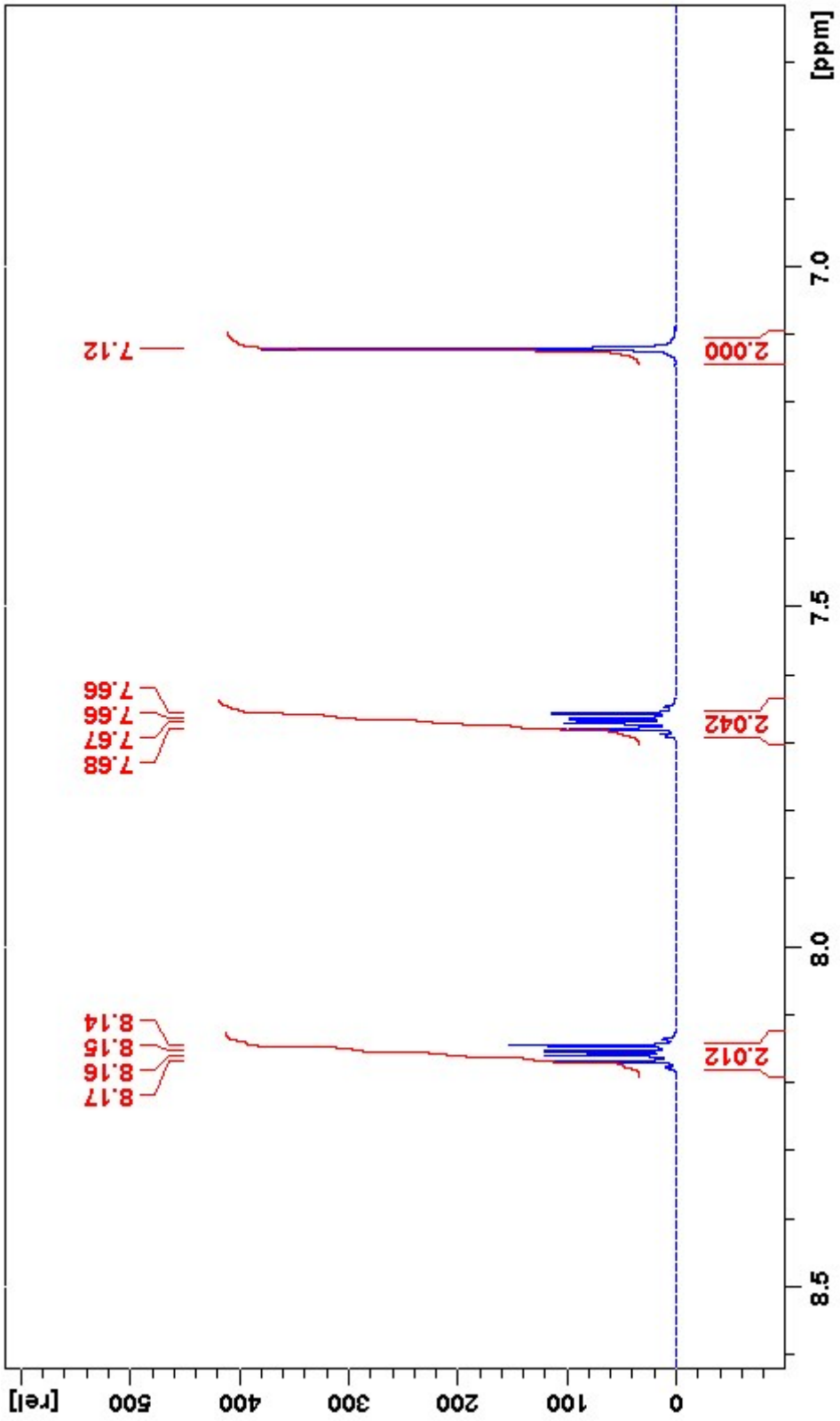

Figure S9. ^{1}H NMR of compound **17**.

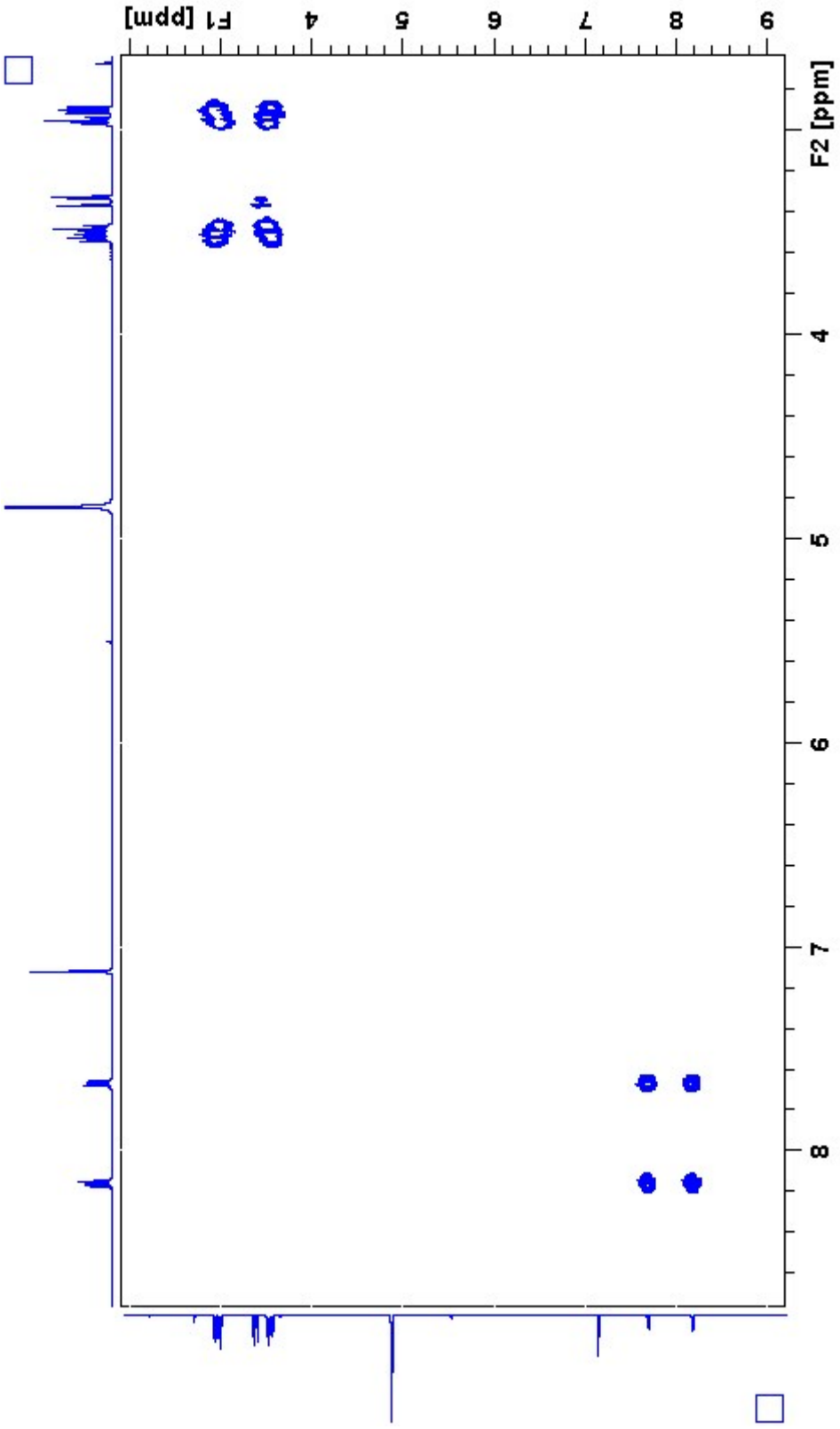

Figure S10. COSY NMR of compound **17**.

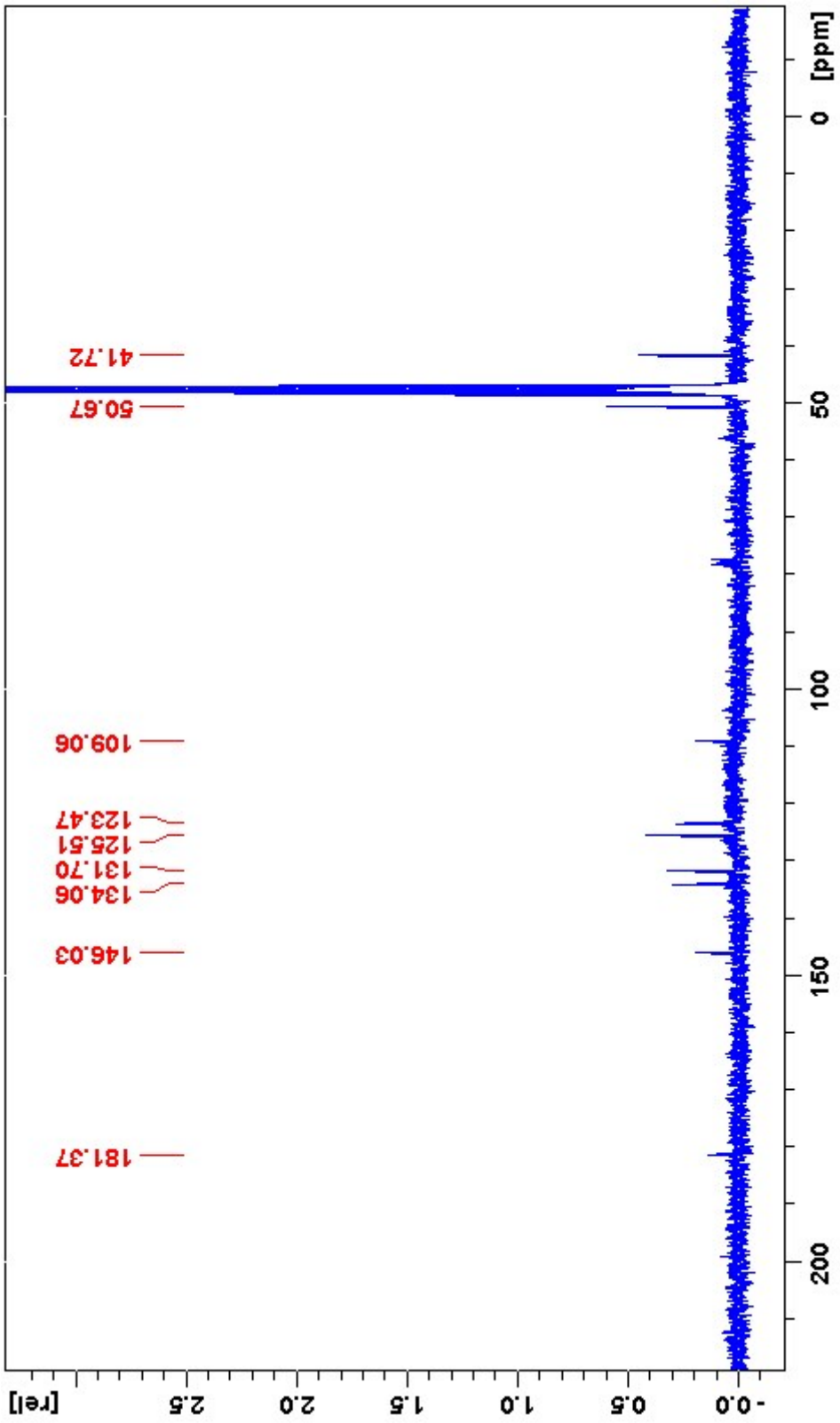

Figure S11. $^{13}C\{^{1}H\}$ NMR of compound **17**.

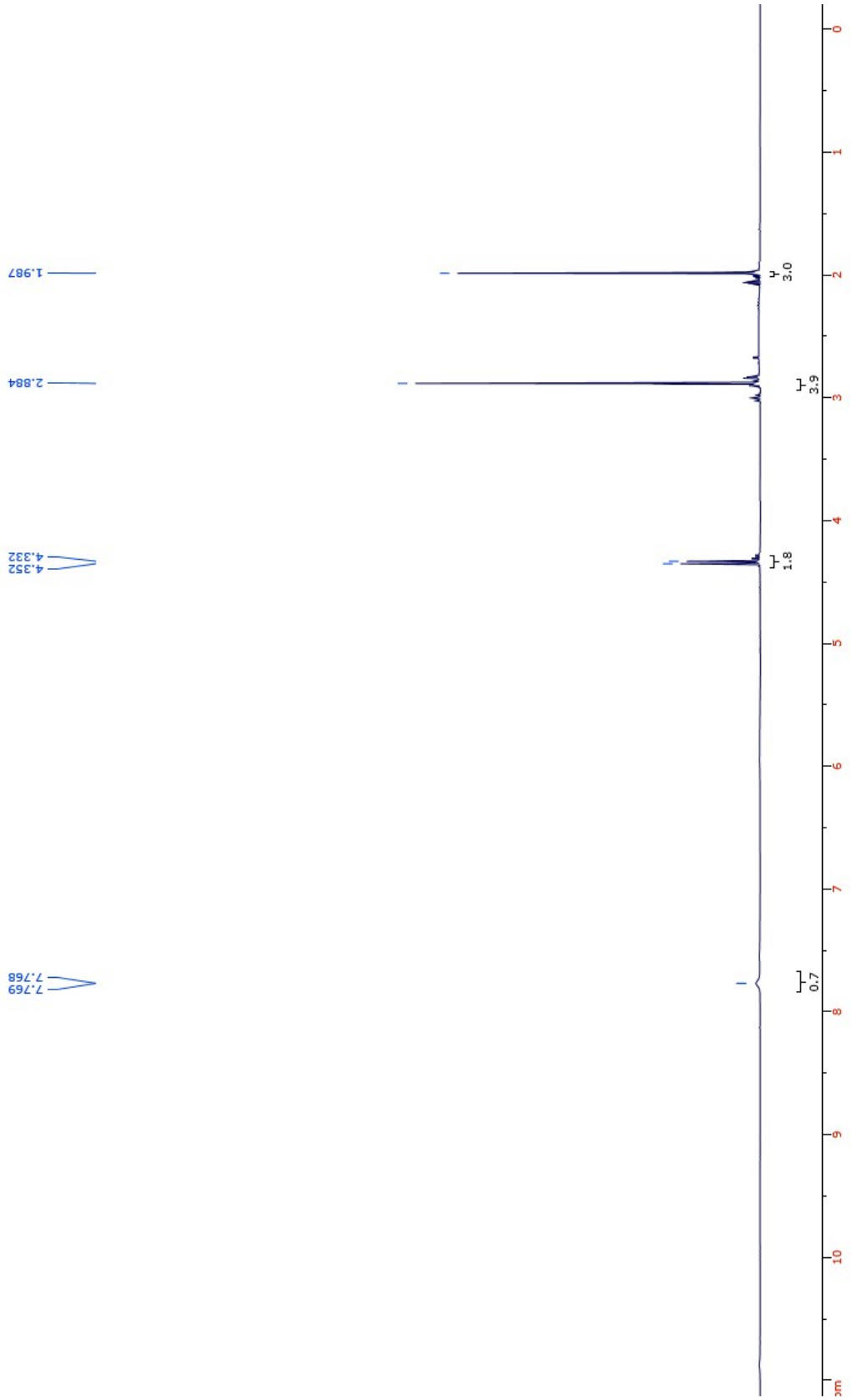

Figure S12. ^{1}H NMR of compound **19**.

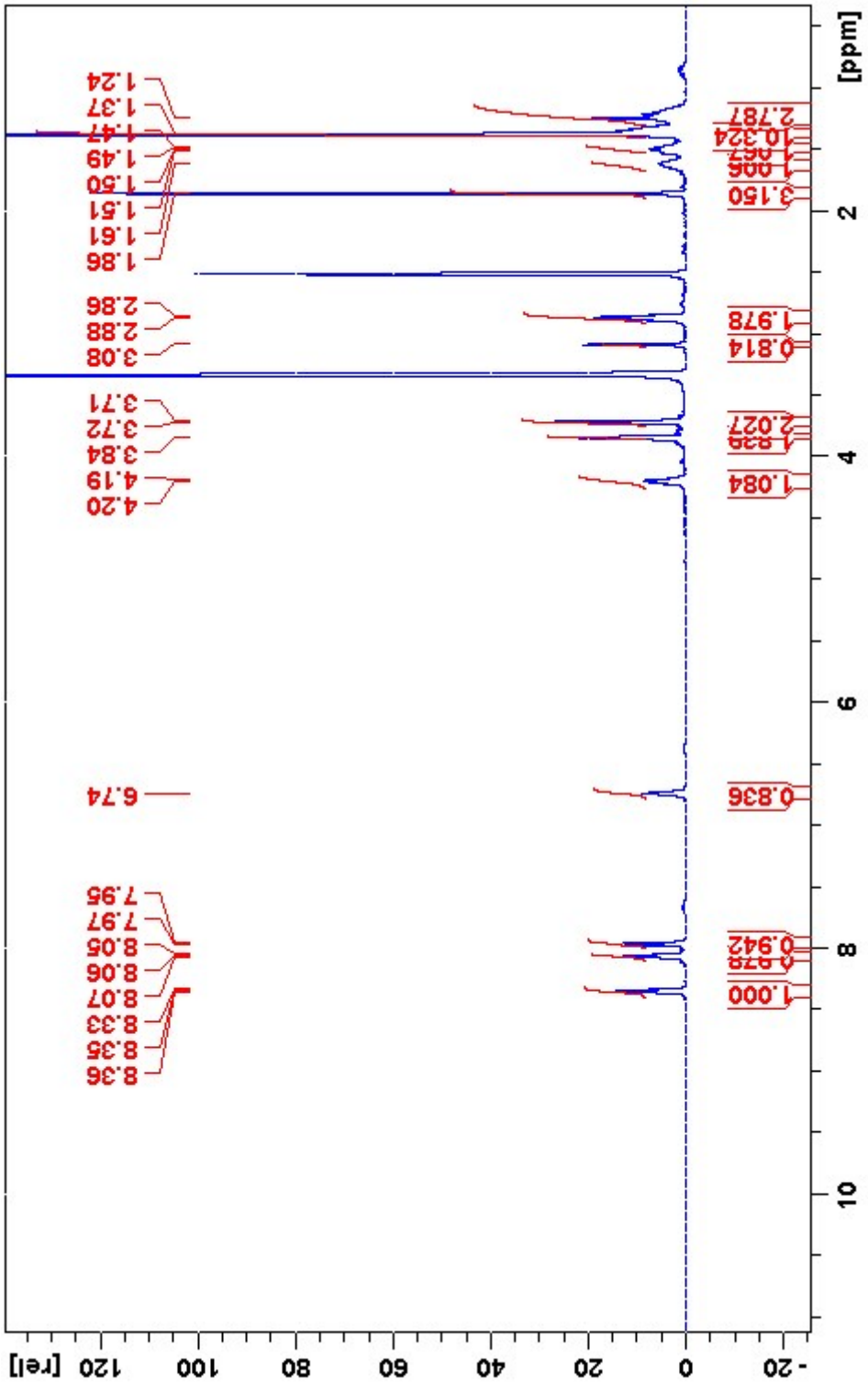

Figure S13. ^{1}H NMR of compound **21**.

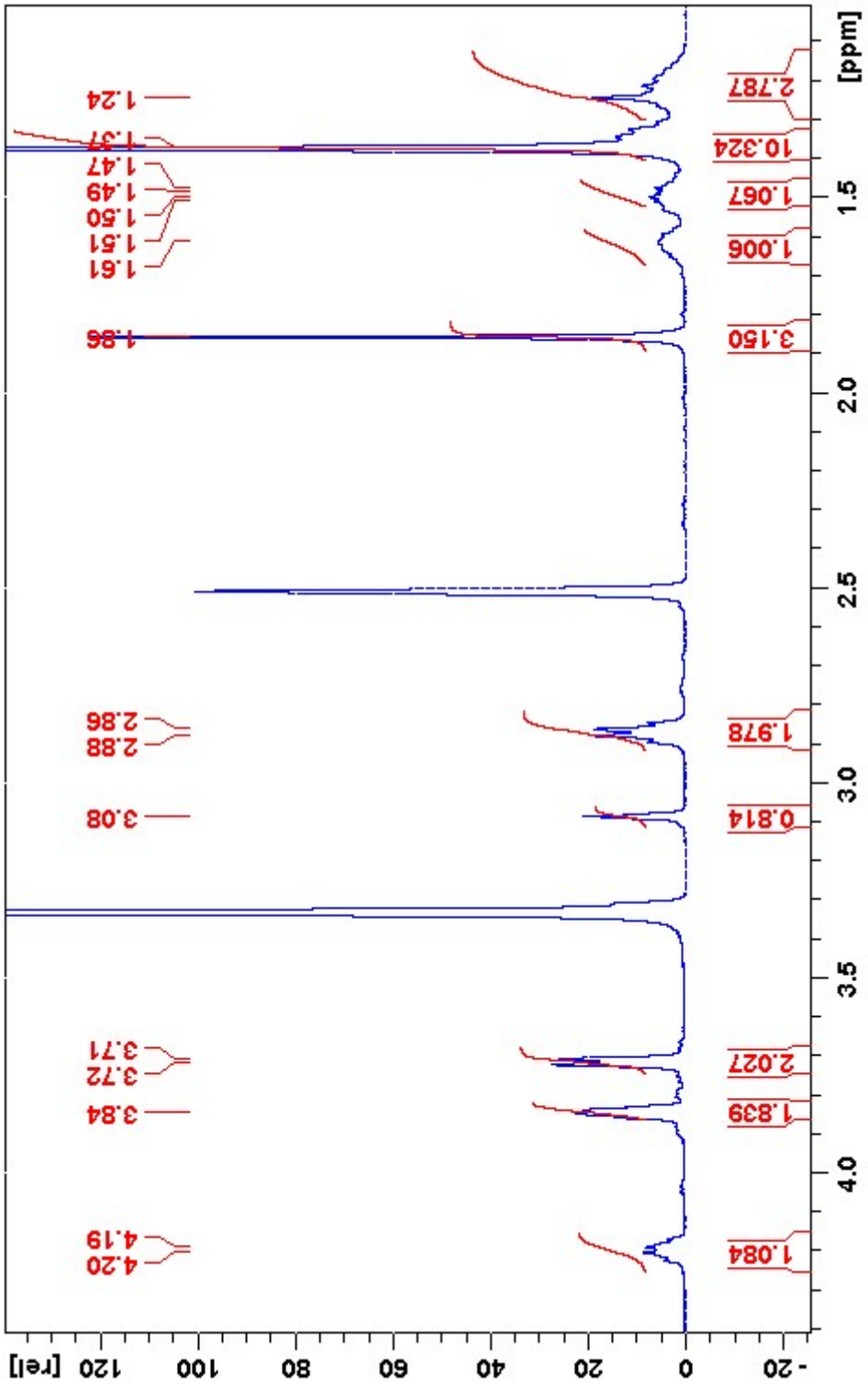

Figure S14. ^{1}H NMR of compound **21**.

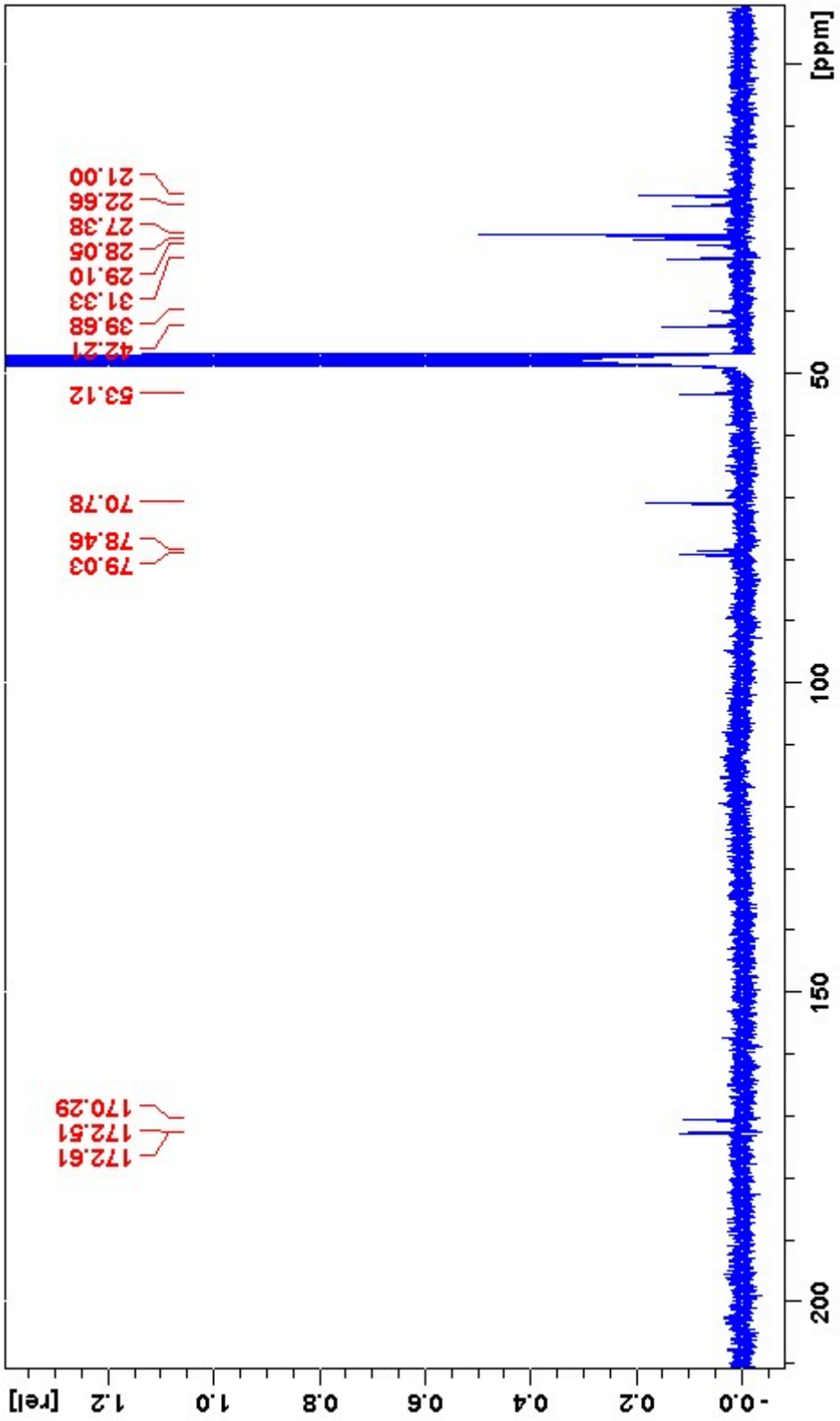

Figure S15. $^{13}C\{^{1}H\}$ NMR of compound **21**.

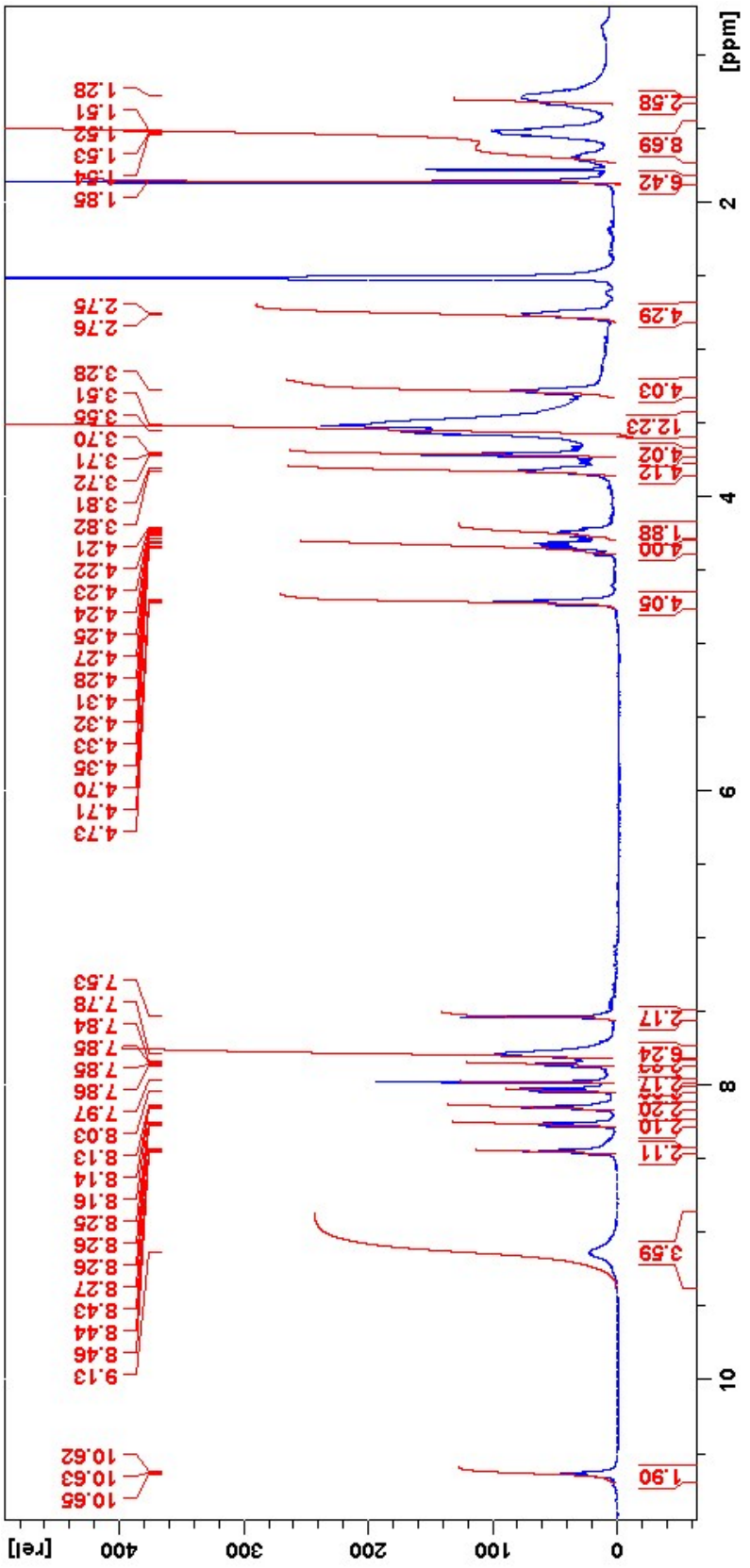

Figure S16. ^{1}H NMR of compound **1**.

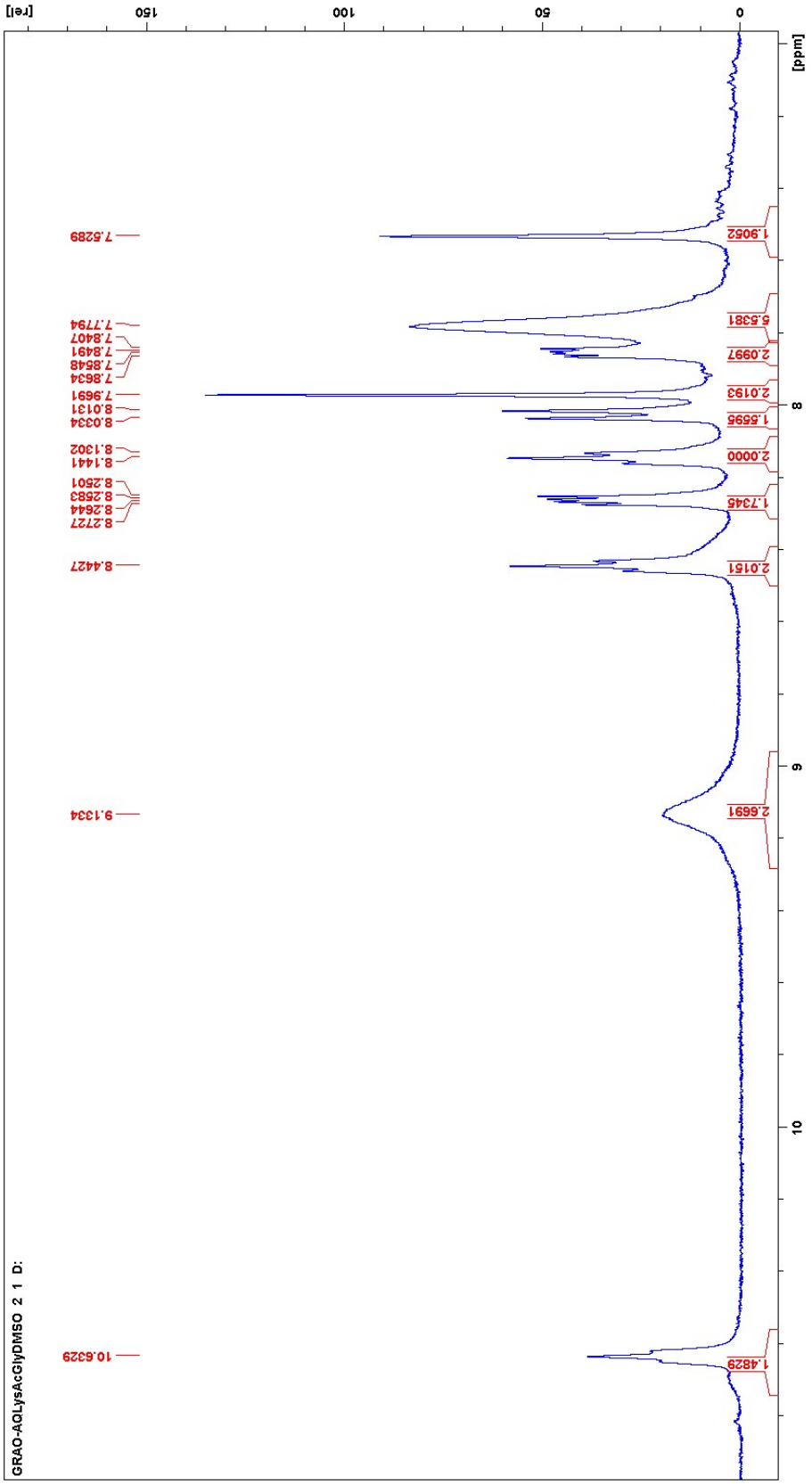

Figure S17. ^{1}H NMR of compound **1**.

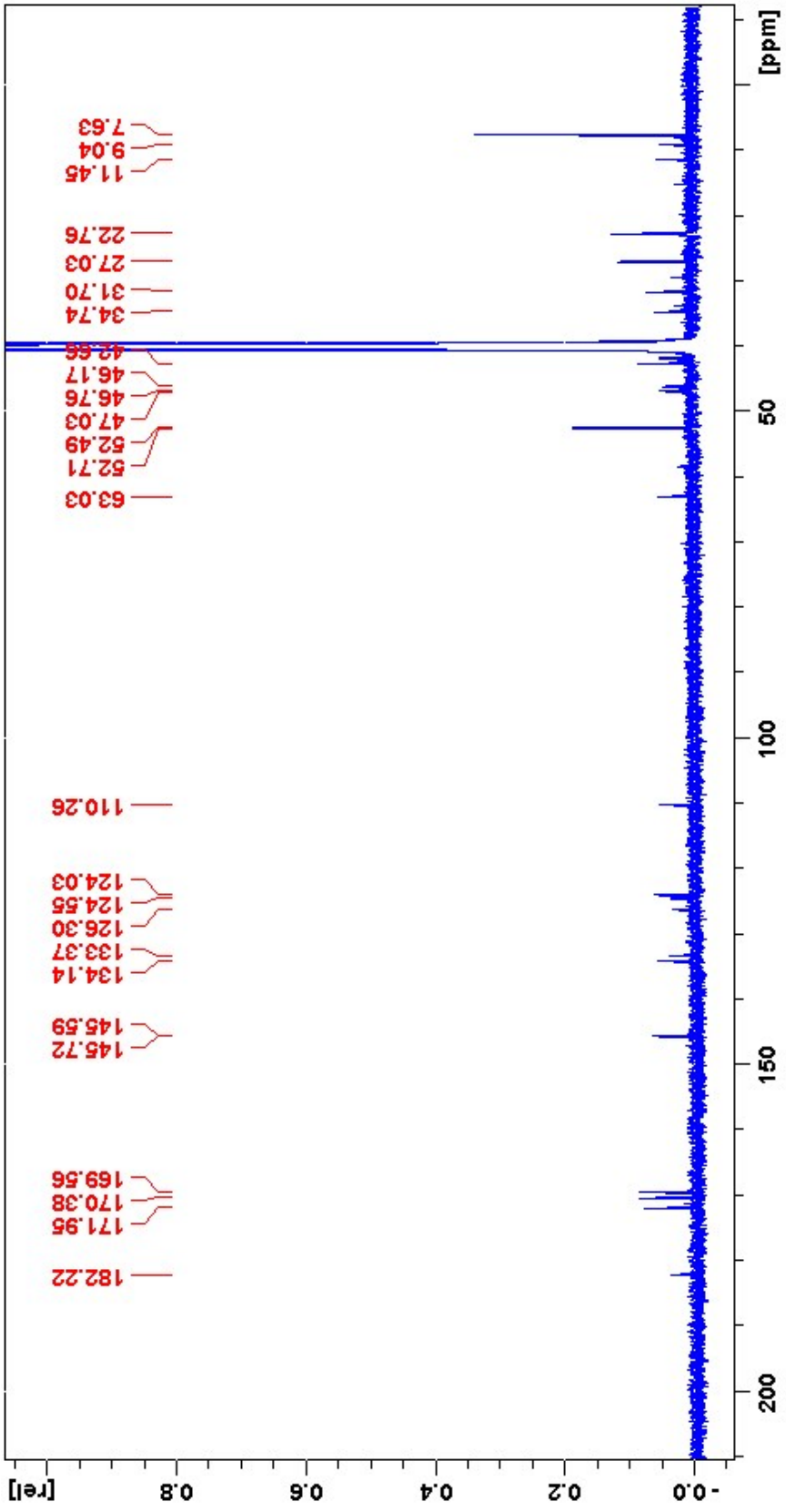

Figure S18. $^{13}C\{^{1}H\}$ NMR of compound **1**.

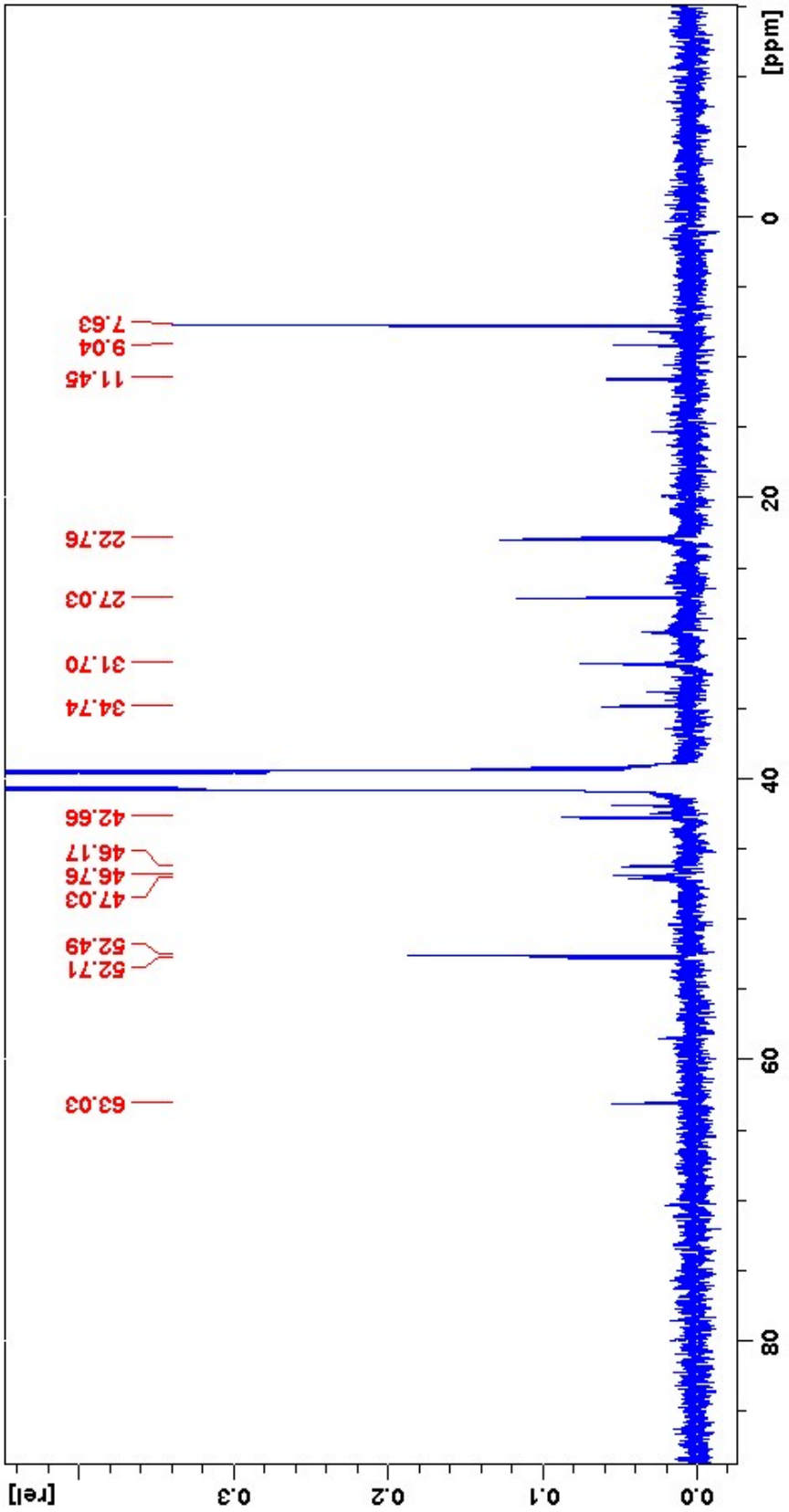

Figure S19. $^{13}C\{^{1}H\}$ NMR of compound **1**.

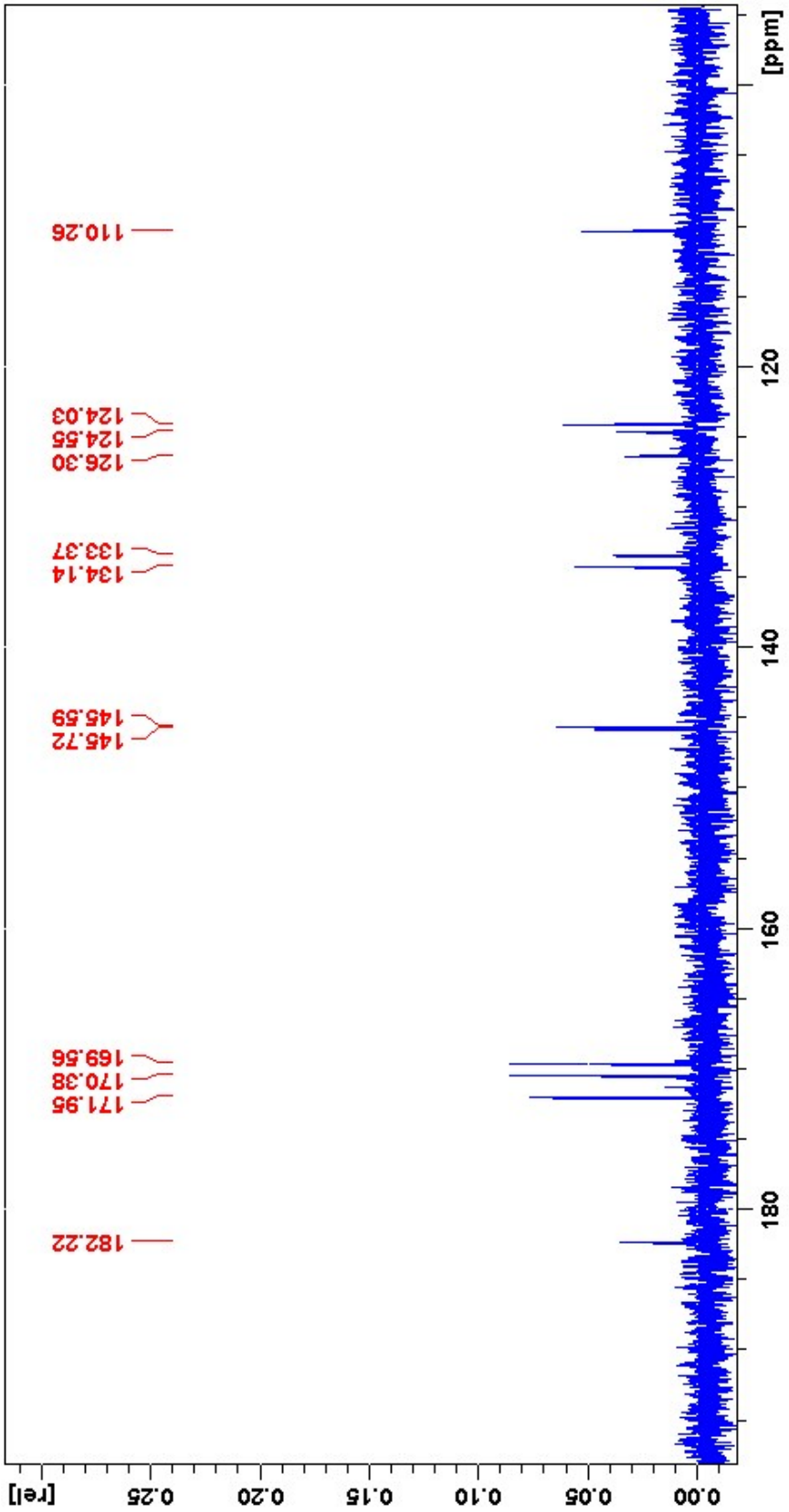

Figure S20. $^{13}C\{^{1}H\}$ NMR of compound **1**.

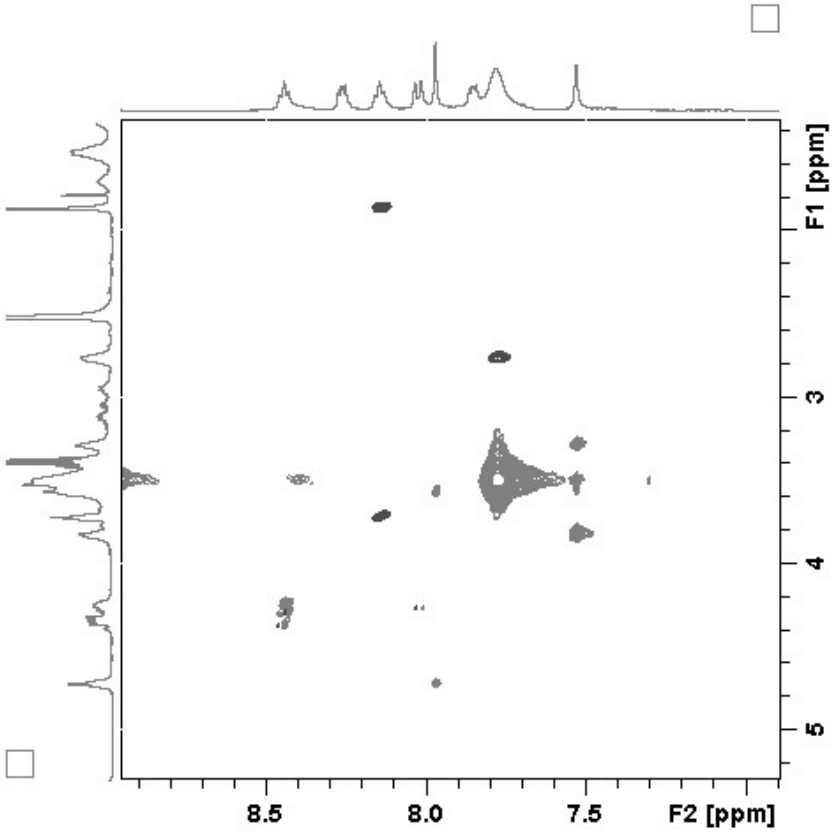

Figure S21. NOESY of compound **1**.

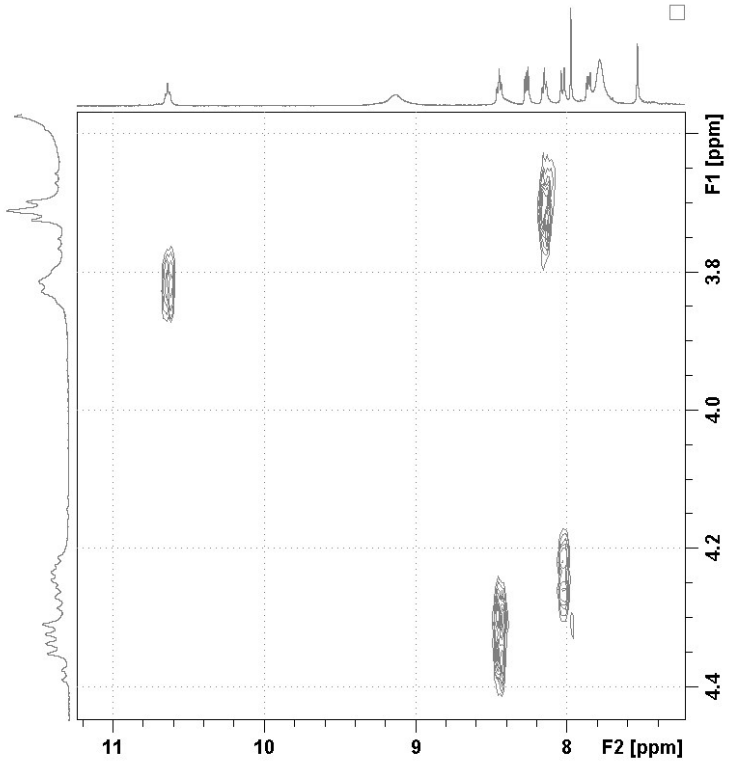

Figure S22. COSY of compound **1**.

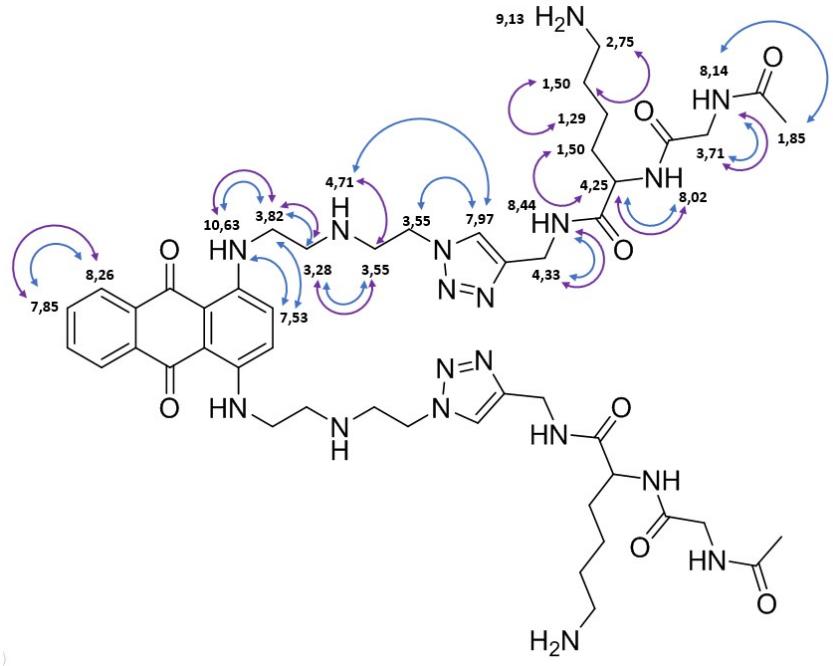

Figure S23. ^{1}H NMR chemical shifts assignment for compound **1**. NOESY interactions are represented in blue and COSY interactions in violet.

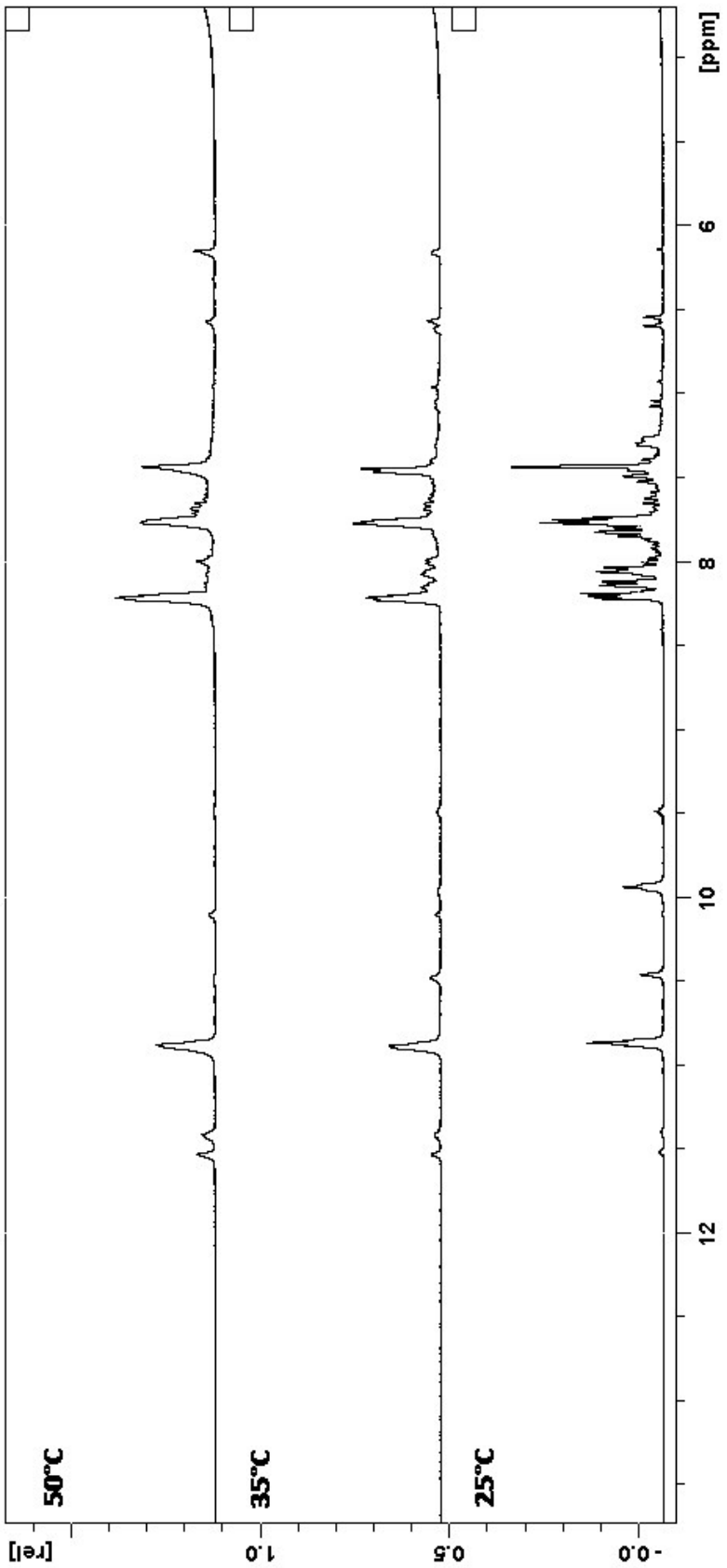

Figure S24. Optimization of the reaction conditions for the formation of compound **17**. Comparison of the NMR experiments on the reactions carried out at 25, 35 and 50°C.

HPLC

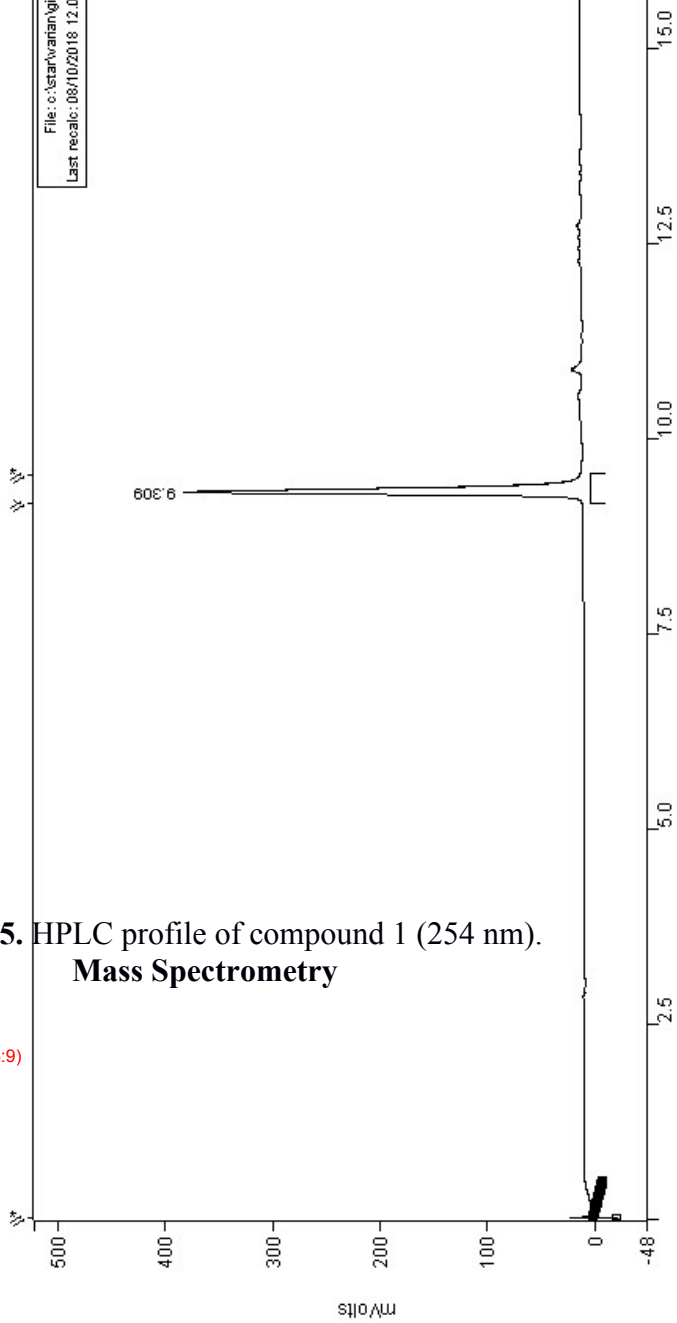

Figure S25. HPLC profile of compound 1 (254 nm).

Mass Spectrometry

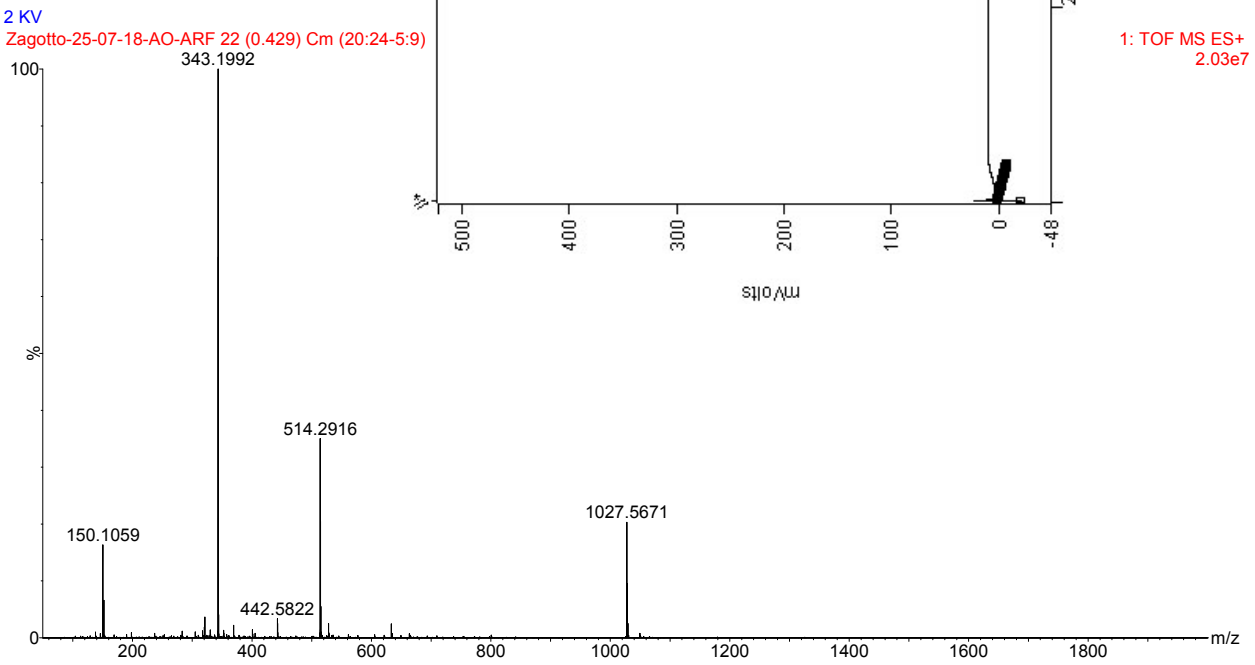

Figure S26. Mass spectrum of compound **1**.

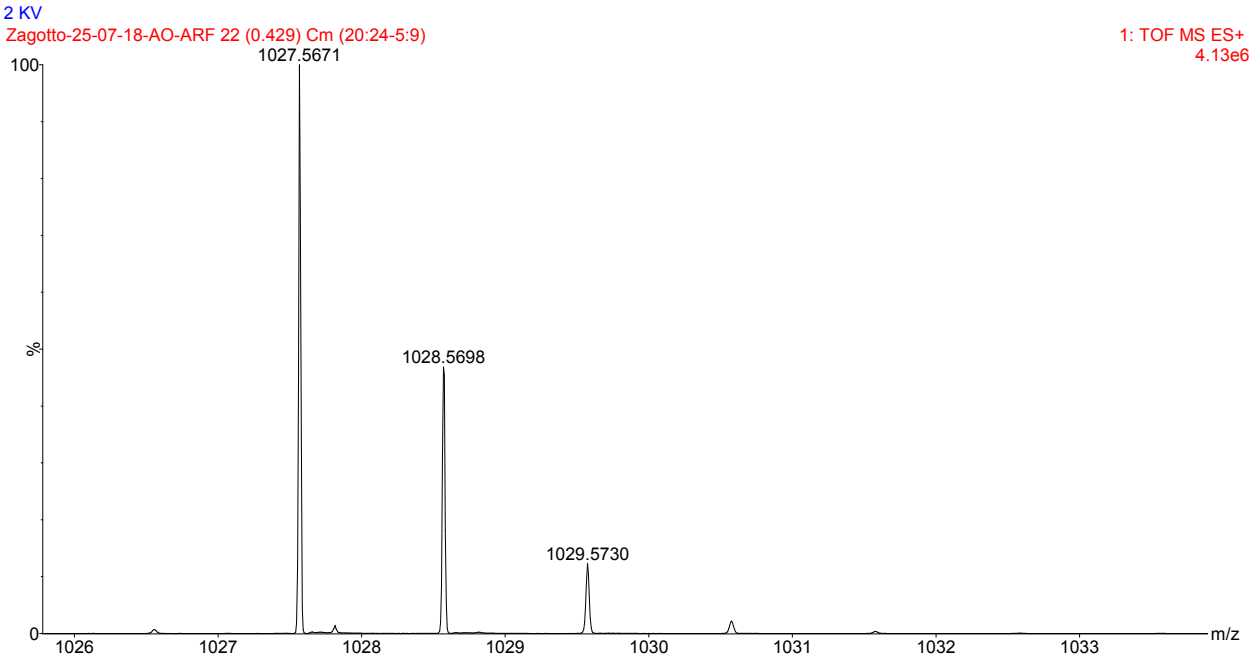

Figure S27. Mass spectrum of compound **1** (z = 1).

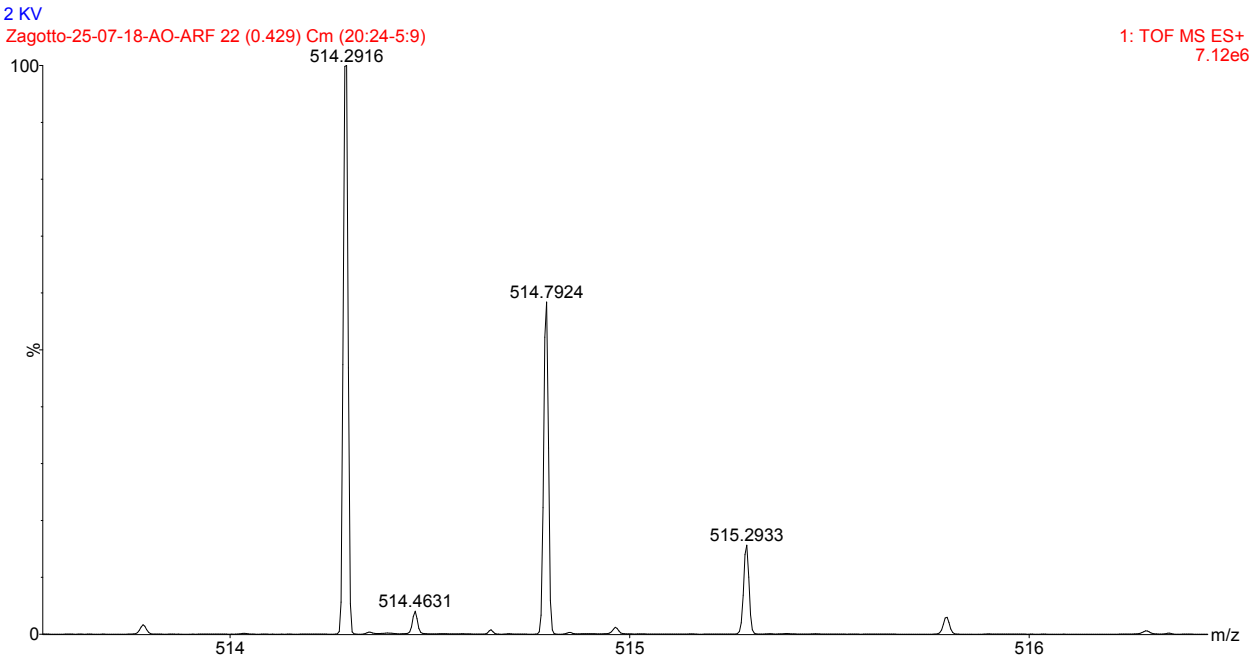

Figure S28. Mass spectrum of compound **1** (z = 2).

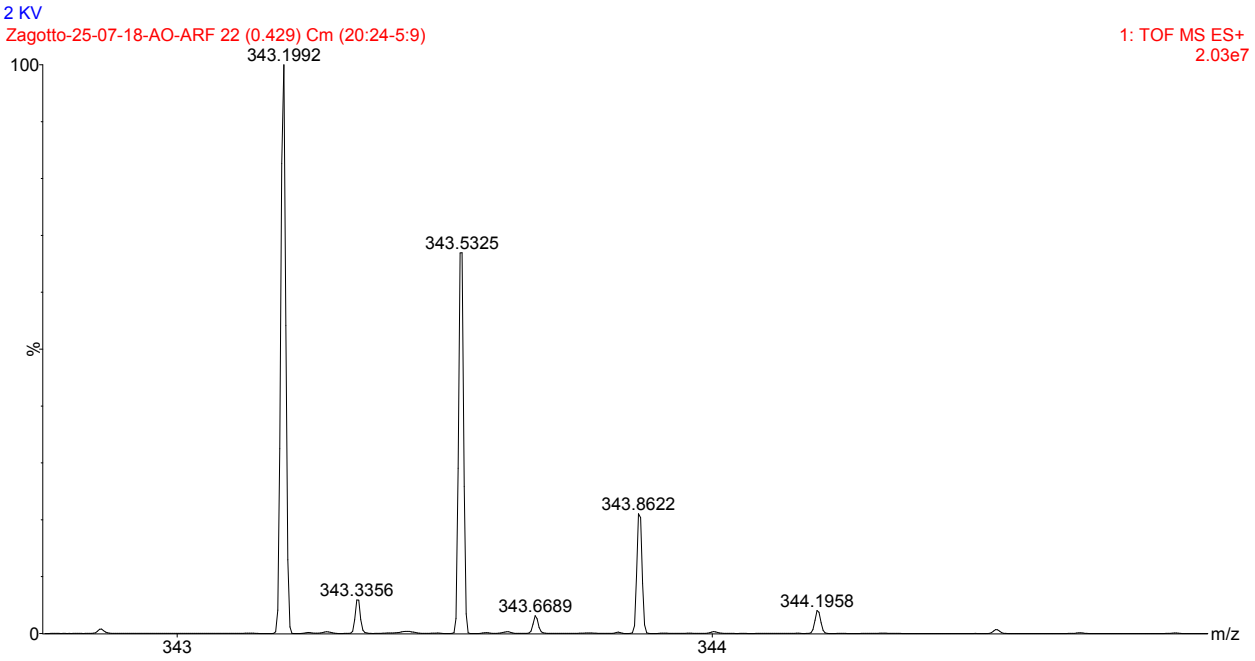

Figure S29. Mass spectrum of compound **1** (z = 3).

UV

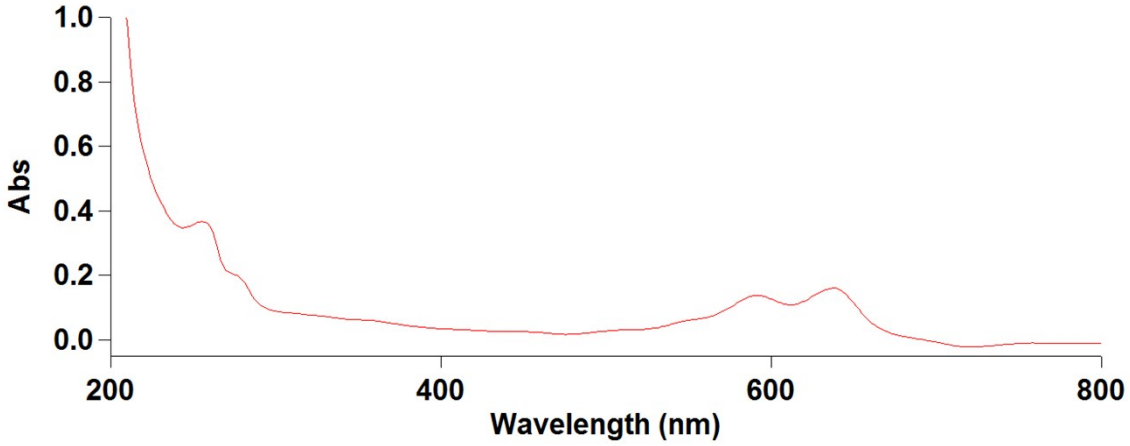

Figure S30. UV spectrum of compound **1.**

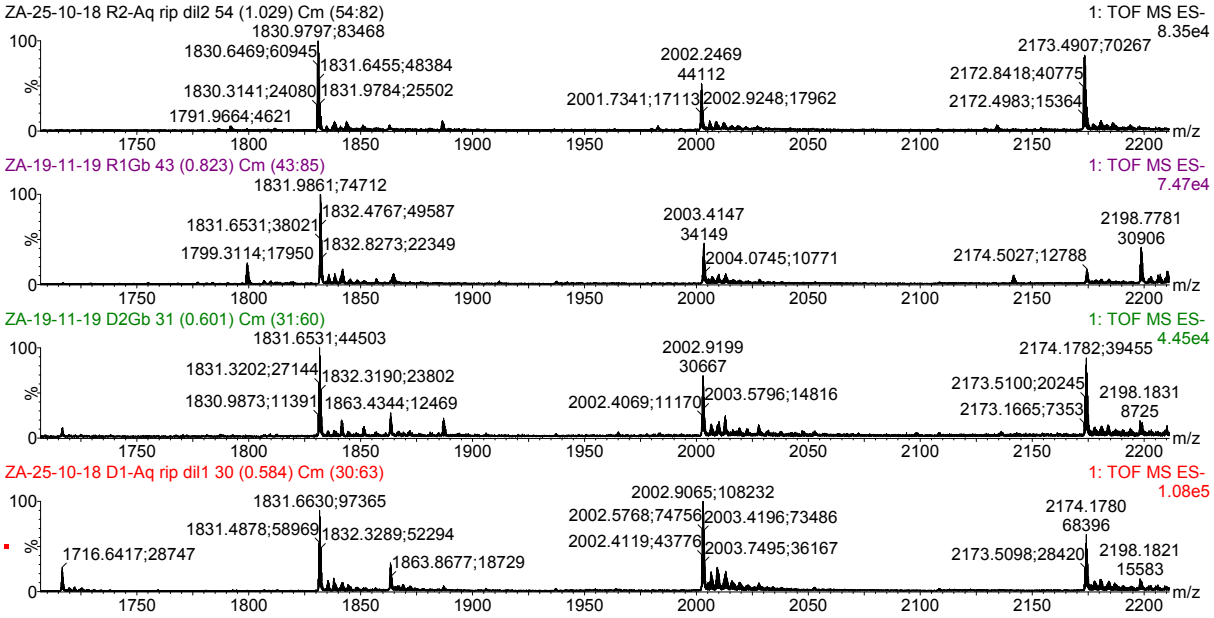

Figure S31. ESI-MS binding experiment performed to evaluate the binding affinity of compound **1** towards sequence Z1, Z2, R1 and R2 (from bottom to top). Signal intensities, included in the binding affinity calculation, are reported below the corresponding m/z values.