# Electronic Supplementary Information

## A Clickable NHC-Au(I)-Complex for the Preparation of Stimulus-Responsive Metallopeptide Amphiphiles

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# 1 Additional Experimental Data (Figures S1.1 – S1.8)

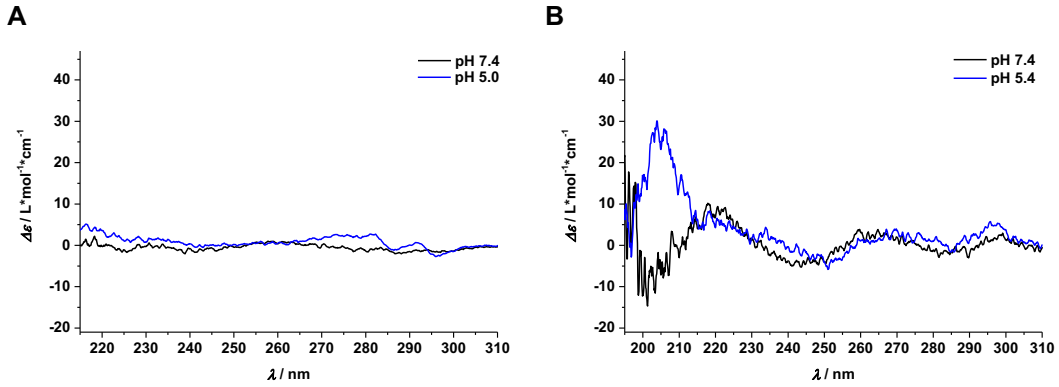



Figure S1.1 **A** CD spectra of a 300 µM solution of **1** in 10 mM phophate buffer at pH 7.4 (black) and 5.0 (blue) at 293 K. **B** CD spectra of a 100 µM solution of **3** in 10 mM phophate buffer at pH 7.4 (black) and 5.4 (blue) at 293 K.

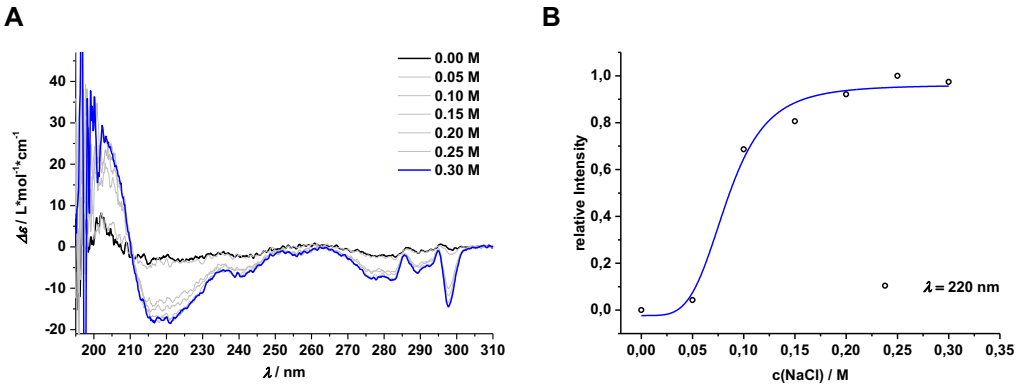



Figure S1.2 **A** CD-spectra of a 100 µM aqueous solution of **2** in 10 mM phosphate buffer (pH 7.6) at different ionic strength due to the addition of NaCl; the blue spectra represents the self-assembled state (at 0.2 to 0.3 M NaCl) and the black spectra shows the monomeric state at 0.0 M NaCl. **B** The relative CD-signal at $\lambda$ = 220 nm is given as function of the concentration of NaCl (blue line is shown as a guide for the eye).

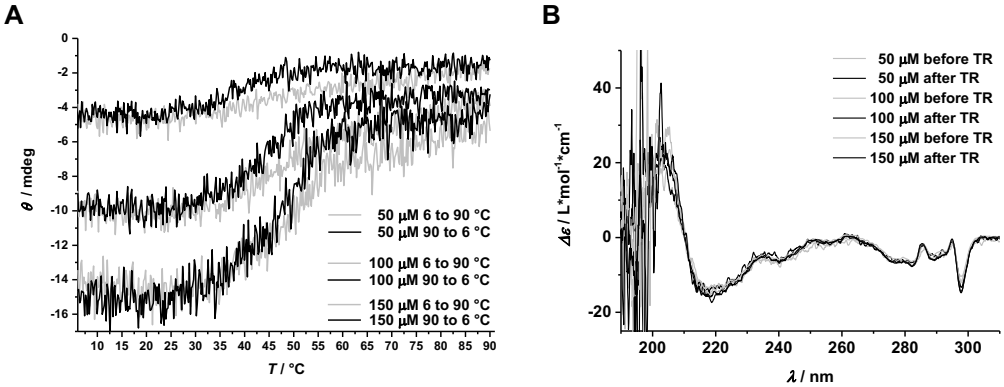



Figure S1.3 **A** Heating (6 to 90 °C, grey) and cooling (90 to 6 °C) temperature ramps of a solution of **2** in 10 mM phosphate buffer (pH 7.6) in the presence of 0.15 M NaCl at different concentration; 50 µM: two upper curves; 100 µM: two middle curves; 150 µM: two lower curves. **B** Corresponding CD-spectra of the solutions before (grey) and after (black) the temperature ramp (TR) from 6 to 90°C and back to 6 °C; 50 µM: two top curves; 100 µM: two middle curves; 150 µM: two bottom curves.

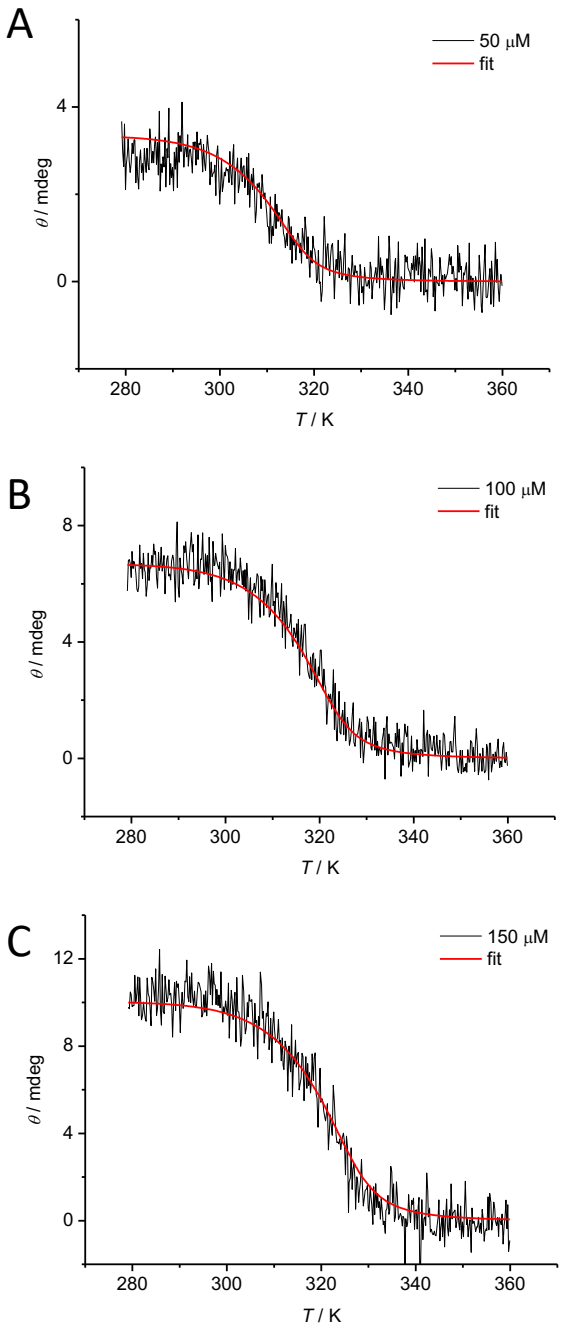



Figure S1.4 Nucleation-elongation fits of the data corresponding to the obtained cooling curves in Figure S 1.3 of a 50 μM (**A**), 100 μM (**C**) and 150 μM (**E**) solution of **2**. The cooperative polymerisation model used for the simultaneous fits is described in: H. M. M. ten Eikelder, A. J. Markvoort, T. F. A. de Greef, P. A. J. Hilbers, *J. Phys. Chem. B* **2012**, *116*, 5291-5301. Non-linear least square analysis of the three cooling curves was applied yielding the following thermodynamic parameters $\Delta H^0$ (elongation enthalpy) = -91.0 ±3.1 kJ·mol$^{-1}$, $\Delta H^0_{nucl}$ (nucleation penalty) = -83.1 ±0.6 kJ·mol$^{-1}$, $\Delta S^0$ (entropy change of monomer addition in the nucleation and elongation phase) = -0.21 ±0.01 kJ·mol$^{-1}$, $T_e$ (elongation temperature) = 326 K at 150 μM, 322 K at 100 μM and 315 K at 50 μM.

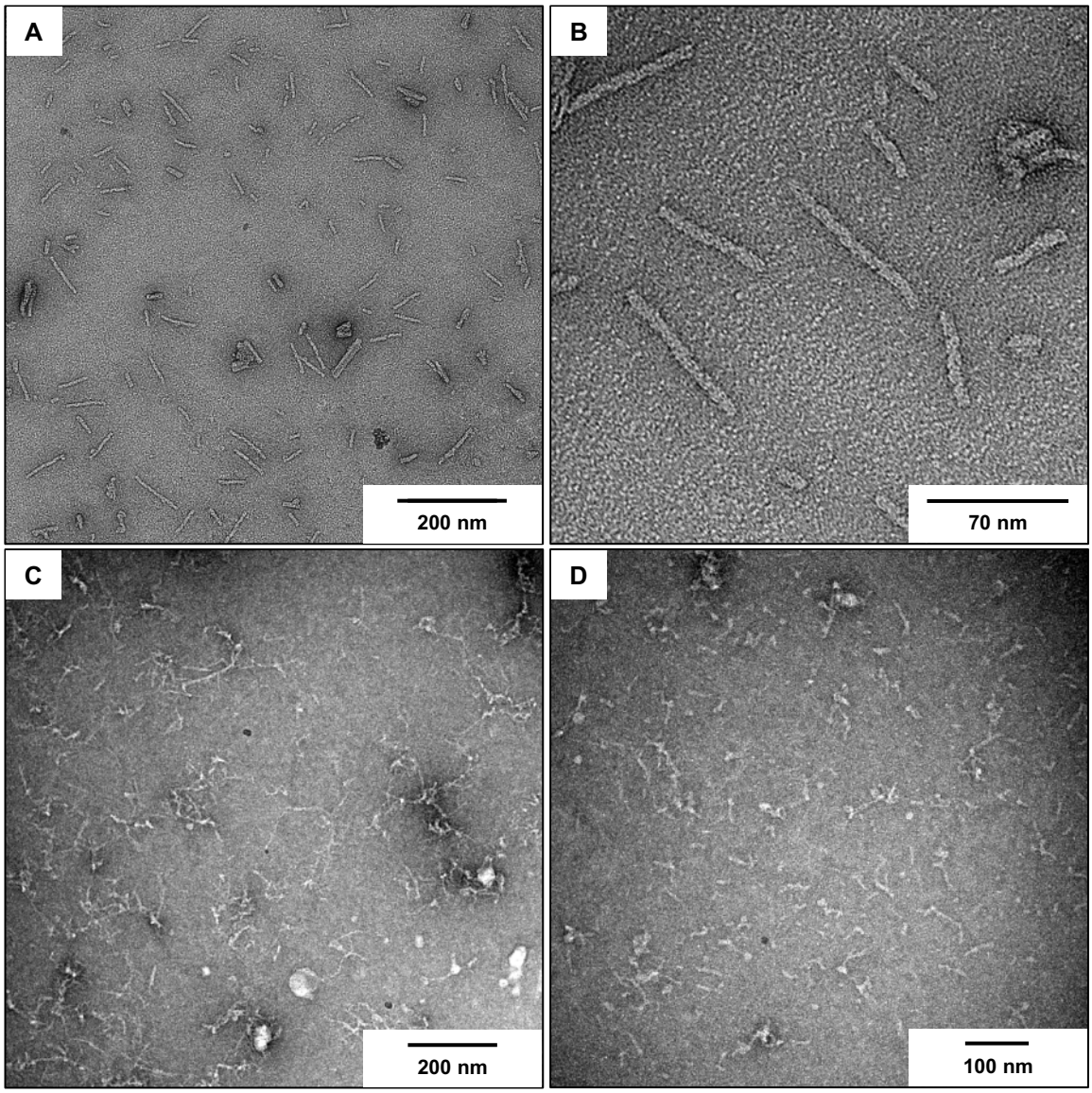



Figure S1.5 Negative stained TEM micrographs with uranyl acetate (2.0-wt%) of a 80 μM solution of **2** in TRIS buffer (10 mM) at pH 6.3 (**A** and **B**) or at pH 11.1 (**C** and **D**).

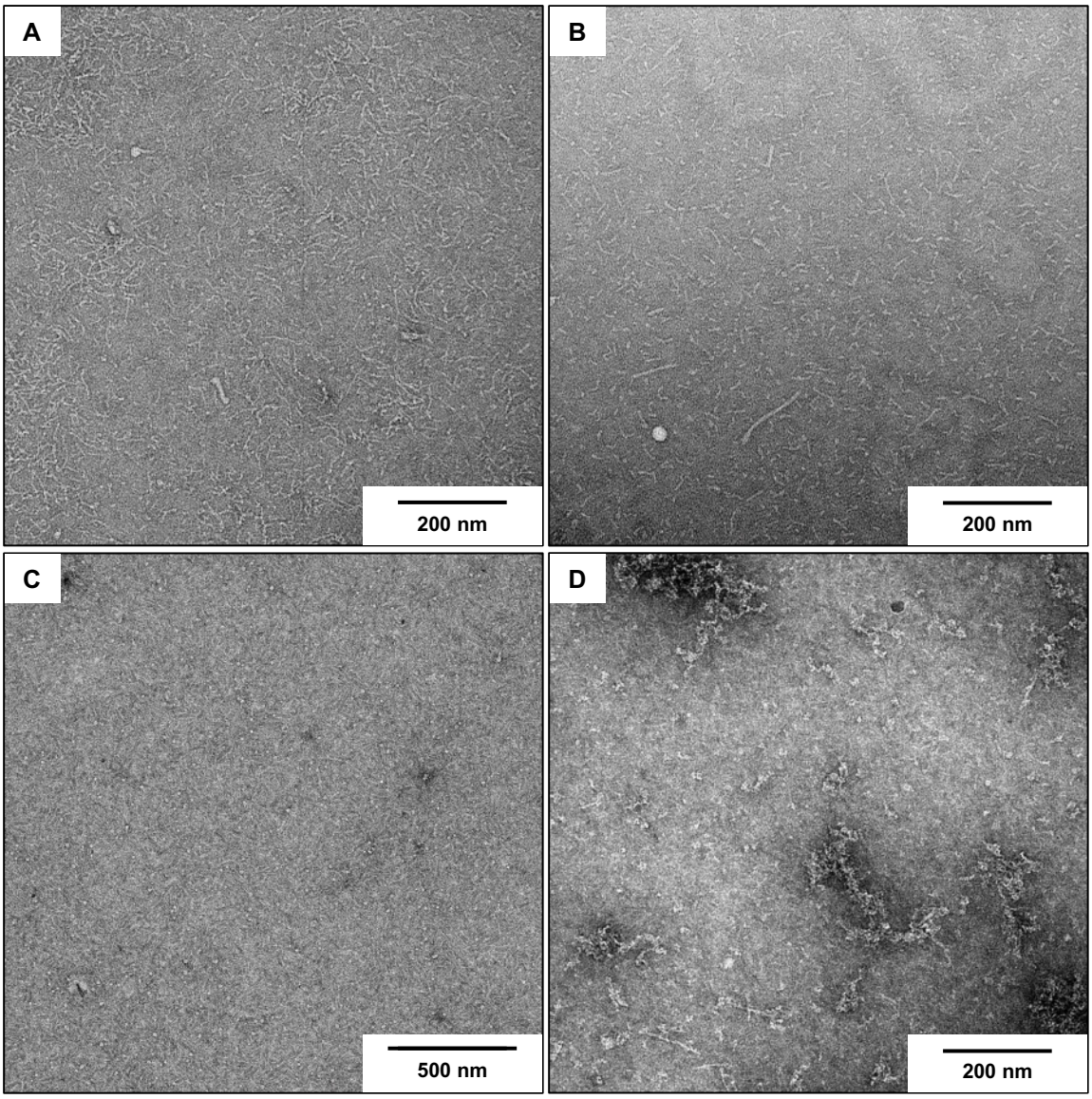



Figure S1.6 Negative stained TEM micrographs with uranyl acetate (2.0-wt%) of a 80 μM solution of **1** in TRIS buffer (10 mM) at pH 6.3 (**A** and **B**) or at pH 11.1 (**C** and **D**).

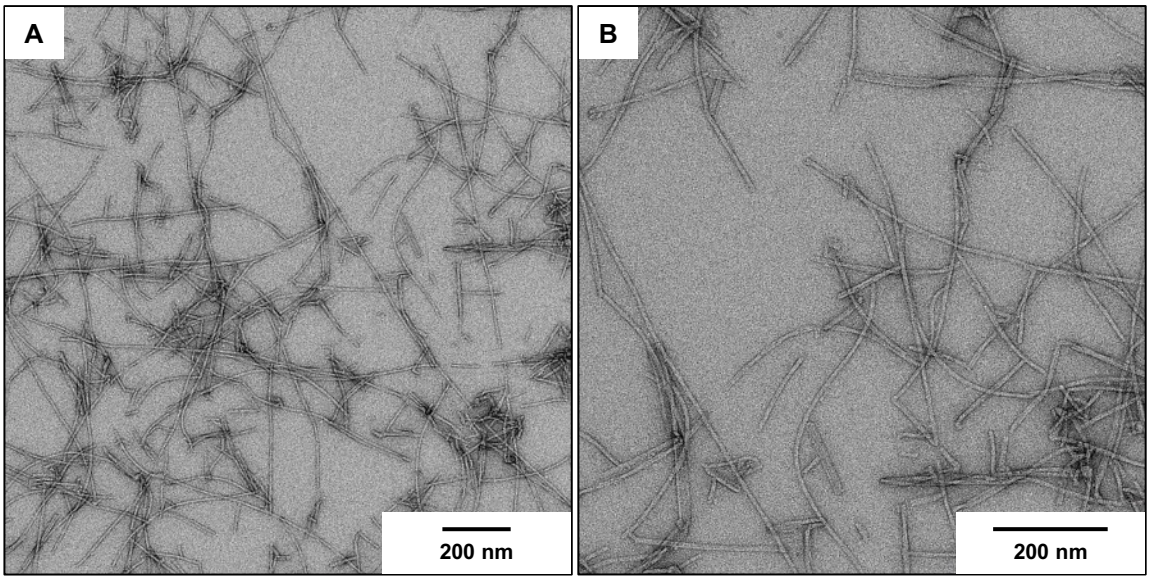



Figure S1.7 Negative stained TEM micrographs with uranyl acetate (2.0-wt%) of a 80 μM solution of **1** in TRIS buffer (10 mM) at pH 5.4 (**A** and **B**).

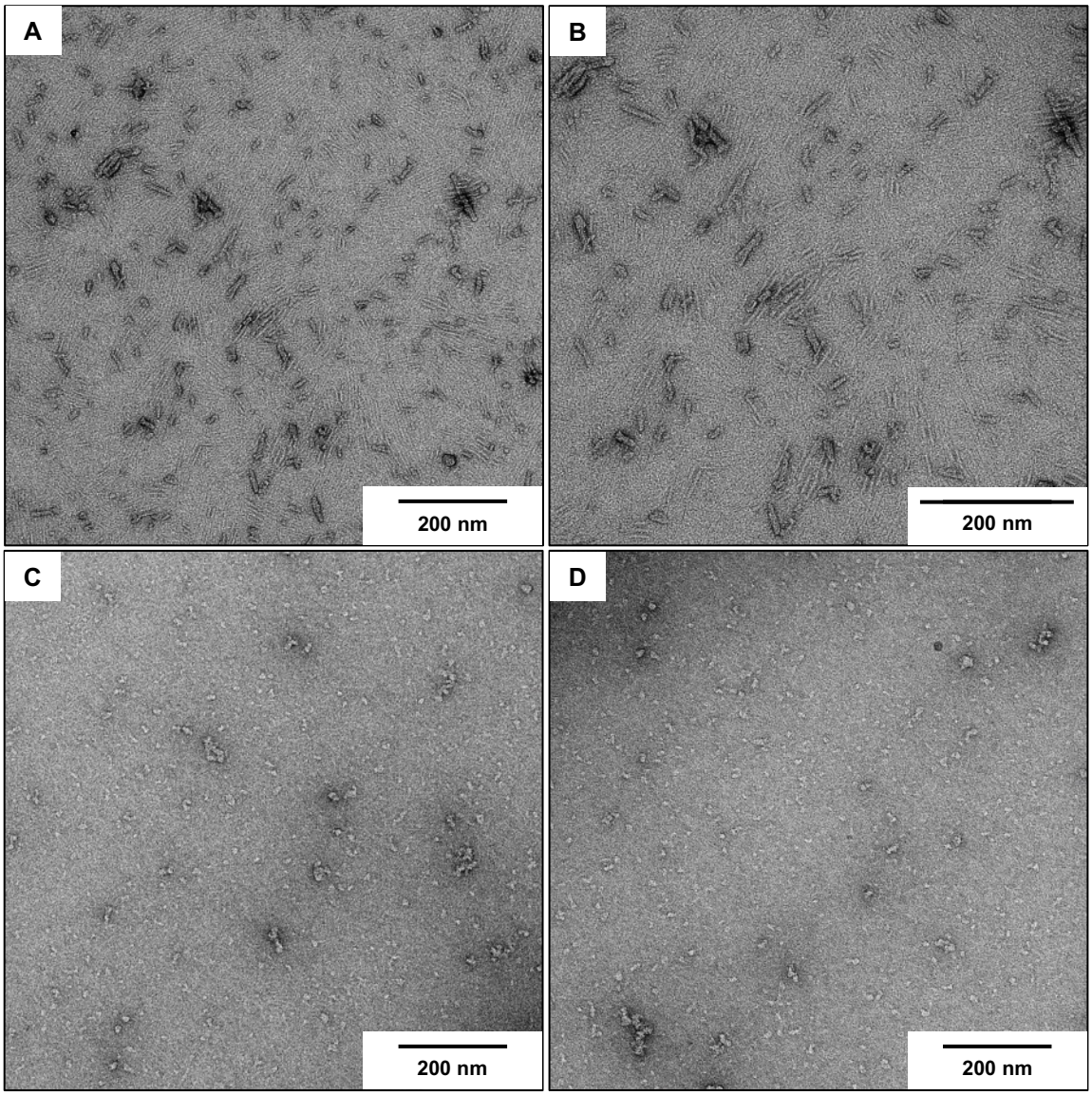



Figure S1.8 Negative stained TEM micrographs with uranyl acetate (2.0-wt%) of a 80 μM solution of **3** in TRIS buffer (10 mM) at pH 6.3 (**A** and **B**) or at pH 11.1 (**C** and **D**).

# 2 Materials and General Methods

## 2.1 General Considerations

Reactions involving air or moisture sensitive reagents or intermediates were performed under argon atmosphere using SCHLENK techniques. The glassware used had been oven dried at 120 °C or heatgun dried under high vacuum. For the addition of reagents or solvents through septa disposable syringes and needles that had been flushed with argon repeatedly were utilized. Solids were added in an argon counterflow or as a solution. Degassing of solvents was achieved by performing multiple freeze-pump-thaw.

All used Materials were obtained from commercial sources at the highest purity available and used without further purification. Chloro(tetrahydrothiophene)gold(I) [Au$^{I}$Cl(tht)] was purchased from UMICORE (UMICORE AG & Co. KG, Hanau-Wolfgang, Germany). Compound **10** was synthesized as published before.[1] Water was demineralized prior to use. Solvents used for air or moisture sensitive reactions were purchased anhydrous or dried prior to use according to common drying techniques or the solvent purification system *MB-SPS-800* (MBRAUN). Tetrahydrofurane (THF) was used containing no stabilizing additives. Purification *via* preparative flash column chromatography (FC) was carried out using silica gel with an average grain size of 15–40 µm (ACROS ORGANICS). TLC-Analysis of the collected fractions was carried out with silica coated aluminum sheets (60 Å $F_{254}$, MACHEREY-NAGEL). Size exclusion chromatography (SEC) was carried out using Sephadex™ LH-20 beads (GE HEALTHCARE BIO-SCIENCES, Uppsala, Sweden) as stationary phase. Solvents used for FC or SEC were purchased either in technical grade and distilled before use or obtained in p.a. quality and used without further purification.

## 2.2 Nuclear Magnetic Resonance (NMR) Spectroscopy

The NMR spectra for the $^{1}$H-NMR and $^{13}$C-NMR spectroscopy were measured on the spectrometers Avance III HD 300 (BRUKER), Avance II 400 (BRUKER), Avance III HD 400 (BRUKER) and Avance III 600 (BRUKER) at the *Institute of Organic Chemistry*, *Johannes Gutenberg-University Mainz* using $CDCl_3$, MeOD-$d_4$, DMSO-$d_6$ and DMF-$d_7$ as deuterated solvents. The chemical shifts ($\delta$) are reported relative to the residual solvent protons ($^{1}$H-NMR) or the deuterium coupled $^{13}$C solvent signal ($^{13}$C{$^{1}$H} NMR). For the declaration of the spin multiplicities the following abbreviations were used: s (singlet), d (doublet), t (triplet) and m (multiplet), as well as appropriate combinations of these. Coupling constants of protons ($J$) are stated in Hertz (Hz). The NMR spectra were analyzed using the software *MestReNova v.10.0.2-15465* (Mestrelab Research S.L.).

## 2.3 Mass Spectrometry

High resolution mass spectra (HRMS) were recorded on the electrospray ionization (ESI) devices *Micromass QTof Ultima 3* (WATERS) or *LC/MS 6545 QToF* (AGILANT) by the massspectrometric department of the *Institute of Organic Chemistry*, *Johannes Gutenberg University Mainz*.

## 2.4 Circular Dichroism (CD) Spectroscopy

CD measurements were carried out on a J-815 spectrometer (JASCO Labor- und Datentechnik GmbH, Gross-Umstadt, Germany) using the software *Spectra Manager v. 2.12.00*. All spectra were recorded under the conditions indicated using precision quartz cells with a path length of 2 mm (HELLMA GmbH & Co. KG,

Müllheim, Germany). Data was processed using *OriginPro v. 9.1.0* (OriginLab Corporation, Northampton, USA). All Spectra were averaged over three replicates and corrected by subtraction of the background (cuvette).

### 2.4.1 Temperature-Dependent CD Spectroscopy: Linear Temperature Ramp

After dissolution of **2** in phosphate buffer (10 mM, pH 7.4) and addition of 0.15 M NaCl by using a standard solution of 5 M NaCl, a solution of **2** (50 µM, 100 µM, 150 µM) was placed in a pre-cooled cell holder (6 °C) and heated linearly to 90 °C and cooled back linearly to 6 °C (0.5 °C/min). The assembly processes were followed at $\lambda$ = 220 nm.

## 2.5 Transmission Electron Microscopy (TEM)

TEM investigations were accomplished on a *FEI Tecnai™ T12* transmission electron microscope equipped with a BioTWIN lens and a $LaB_6$ cathode operated at 120 kV. Thereto sample droplets were adsorbed on freshly glow-discharged copper grids (Electron Microscopy Sciences, CF300-Cu, 300 mesh) covered by a 3 - 4 nm carbon layer followed by negative staining using a 2% aqueous solution of uranyl acetate (Polysciences). Digital electron micrographs were recorded with a 4k × 4k CMOS camera (TVIPS) or 1k × 1k CCD camera (MegaSYS).

### 2.5.1 Length Histograms

The lengths distribution of the observed rod-like filaments were measured using the software *ImageJ 1.50e* (Wayne Rasband National Institutes of Health, USA). The histograms were prepared using *OriginPro v. 9.1.0* (OriginLab Corporation, Northampton, USA) and *Excel 2013 v. 15.0.4981.1000* (Microsoft Corporation, Redmond, USA). The PDI, values of $L_n$ and $L_w$ and statistic parameters were calculated as previously reported:[2-3]

$$L_n = \frac{\sum_{i=1}^{n} n_i L_i}{\sum_{i=1}^{n} n_i} \qquad L_w = \frac{\sum_{i=1}^{n} n_i {L_i}^2}{\sum_{i=1}^{n} n_i L_i} \qquad PDI = \frac{L_w}{L_n}$$

with $L_n$ = number average rod length [nm], $L_w$ = weighted average rod length [nm], $D$ = average micelle diameter [nm,] $n$ = sample size, $\sigma$ = standard deviation and PDI = polydispersity index.

## 2.6 Solid Phase Peptide Synthesis (SPPS)

SPPS was carried out on an automated *CS 136XT* batch peptide synthesizer (CS Bio Co.) using SPPS-grade solvents and 2-chlorotrityl chloride resin (resin loading capacity of 1.6 mmol/g, IRIS Biotech GmbH) for Fmoc amino acid loading.

# 3 Synthesis

## 3.1 Standard operating procedures (SOP)

### 3.1.1 Synthesis of the peptides *via* SPPS (SOP 1)

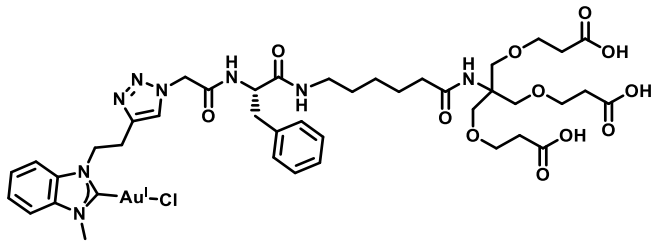
The loading of the resin was performed in a MERRIFIELD apparatus according to a procedure described in literature.[4-6] An excess of the Fmoc-protected phenylalanine (2.0 eq. relative to resin loading capacity) was dissolved in a mixture of dry DCM and a small amount of dry DMF to help solvation (DCM/DMF 9:1-v%, 10 mL/g resin). The dissolved amino acid was added to the 2-chlorotrityl chloride resin, DIPEA (2.0 eq.) was added and it was agitated for 5 min at room temperature. Additional DIPEA (3.0 eq.) was added and the reaction mixture was shaken for 1 h at room temperature. Remaining reactive 2-chlorotrityl groups were quenched with MeOH (1 mL/g resin), followed by agitation for 15 min at room temperature. The vessel was drained and the resin washed consecutively three times with DCM, DMF, DCM and MeOH. Afterwards the beads were dried *in vacuo* overnight and the beads were transferred for the subsequent synthesis to a reaction vessel of the peptide synthesizer.

The step-wise chain elongation was performed according to a procedure described in literature.[7-8] The dried resin was swollen in DCM for 10 min while shaking the reaction vessel. After draining of the solution, piperidine (20% in DMF) was added and the vessel was shaken for 20 min. After draining of the solution, the beads were washed four times with DMF and twice with DCM. The resin was treated with a solution of the corresponding protected amino acid (4.0 eq.), HBTU (4.0 eq.), HOBt (4.0 eq.) and DIPEA (6.0 eq.) in DMF. After shaking for 1 h, the solution was removed and the resin washed five times with DMF. This procedure was repeated with the corresponding amino acid for every coupling process, starting with the Fmoc deprotection on the resin. Finally the resin was washed with DCM.

The cleavage of resin-bound peptides was carried out according to a procedure described in the literature.[8] The resin beads were transferred to a MERRIFIELD glass reactor, treated with a mixture of TFA/DCM 1:1-v% (5 mL/g resin) and shaken for 40 min at room temperature. The solution was drained and the beads were washed at least two times with a small amount of DCM. The combined solutions were concentrated under reduced pressure, repeatedly co-distilled with toluene to remove the trifluoroacetic acid under reduced pressure and precipitated into a cold solution of CH/$Et_2O$ 2:1-v% or water. Isolation *via* centrifugation and drying afforded the desired peptide. The cleaving procedure was repeated until no more precipitate was formed.

### 3.2 Synthesis of Compounds 1 to 16

#### 3.2.1 Compound 1



$CuSO_4 \cdot 5H_2O$ (0.9 mg, 3.6 µmol, 0.2 eq.) and TBTA (1.9 mg, 3.6 µmol, 0.2 eq.) were added as solution in THF/$H_2O$ 1:1-v% to **5** (7.4 mg, 18 µmol, 1.0 eq.) and **11** (12 mg, 18 µmol, 1.0 eq.) in THF/$H_2O$ 1:1-v% with an overall volume of 750 µL. NaAsc (1.4 mg, 7.1 µmol, 0.4 eq.) was added and it was stirred for 24 h at 45 °C under the exclusion of light. The slightly purple reaction mixture was purified as follows: the solution was concentrated and the aqueous residue brought to pH ≈ 10 - 11 by the addition of 1M NaOH solution. It was washed with $CHCl_3$ (2 x 250 µL) and the product was precipitated by the addition of 1M HCl to pH ≈ 2.5 - 3. The product was obtained by centrifugation and lyophilization.

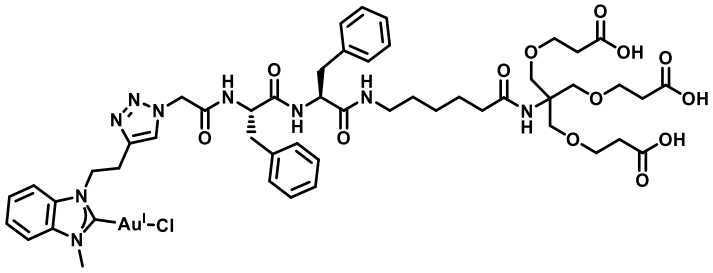
**Yield**: 12 mg (11 µmol, 62%); white solid.

**ESI-HRMS (MeOH) (*m/z*)**: Calculated for $[C_{84}H_{110}Au_2Cl_2N_{16}O_{252}\text{-}2Cl\text{-}2H\text{+}3K\text{+}H_2O]^{3+}$: 751,8737, found 751,8799; Calculated for $[C_{84}H_{110}Au_2Cl_2N_{16}O_{252}\text{-}2Cl\text{-}3H\text{+}3K\text{+}H_2O]^{2+}$: 1127,3069, found 1127,3144.

**$^1$H-NMR (600 MHz, DMSO-$d_6$, 318 K, COSY, HSQC, HMBC)**: $\delta$ / ppm = 12.07 (bs, 3H, COO*H*), 8.03 (bs, 1H, N*H*$^{Phe}$), 7.92 – 7.85 (m, 2H, C*H*$^{BzIm}$, N*H*C$H_2^{Ahx}$), 7.72 (dd, *J* = 7.7, 1.2 Hz, 1H, C*H*$^{BzIm}$), 7.56 – 7.46 (m, 2H, C*H*$^{BzIm}$), 7.16 – 6.99 (m, 5H, C*H*$^{Phe}$), 6.81 (s, 1H, $C_q$N*H*), 5.06 – 4.87 (m, 4H, C$H_2^{Gly}$, NC$H_2^{BzIm}$), 4.49 (td, *J* = 8.1, 6.0 Hz, 1H, α-C*H*$^{Phe}$), 3.85 (s, 3H, NC$H_3^{BzIm}$), 3.60 – 3.49 (m, 12H, C$H_2^{Dnd}$), 3.39 (t, *J* = 7.1 Hz, 2H, NCH$_2$C$H_2^{BzIm}$), 3.03 – 2.96 (m, 1H, NHC$H_2^{Ahx}$), 2.91 – 2.83 (m, 2H, NHC$H_2^{Ahx}$, β-C$H_2^{Phe}$), 2.78 (dd, *J* = 13.6, 8.0 Hz, 1H, β-C$H_2^{Phe}$), 2.41 (t, *J* = 6.4 Hz, 6H, COC$H_2^{Dnd}$), 2.01 (t, *J* = 7.5 Hz, 2H. NH[CH$_2$]$_4$C$H_2^{Ahx}$), 1.41 - 1.35 (m, 2H, NH[CH$_2$]$_3$C$H_2^{Ahx}$), 1.30 – 1.23 (m, 2H, NHCH$_2$C$H_2^{Ahx}$), 1.15 - 1.08 (m, 2H, NH[CH$_2$]$_2$C$H_2^{Ahx}$).

**$^{13}$C-NMR (151 MHz, DMSO-$d_6$, 318 K, HSQC, HMBC)**: $\delta$ / ppm = 194.9 (N$_2$*C*Au), 172.4 (*C*OO$^{Dnd}$), 172.2 (*C*O$^{Ahx}$), 169.9 (*C*O$^{Phe}$), 166.0 (*C*O$^{Gly}$), 163.6 ($C_q^{Triazole}$), 147.8 (*C*H$^{Triazole}$), 137.1 ($C_q^{Phe}$), 133.6 ($C_q^{BzIm}$), 132.54 ($C_q^{BzIm}$), 129.0 (*C*$^{Phe}$), 127.8 (*C*$^{Phe}$), 126.0 (*C*$^{Phe}$), 124.1 (*C*$^{BzIm}$), 124.0 (*C*$^{BzIm}$), 111.8 (*C*$^{BzIm}$), 68.2 (*C*$^{Dnd}$), 66.6 (*C*$^{Dnd}$), 59.4 ($C_q^{Dnd}$), 53.8 (α-*C*$^{Phe}$), 53.3 (*C*H$_2^{Gly}$), 48.2 (N*C*H$_2^{BzIm}$), 38.3 (N*C*$^{Ahx}$), 38.2 (β-*C*$^{Phe}$), 35.7 (NCCCC*C*$^{Ahx}$), 34.5 (CO*C*$^{Dnd}$), 34.3 (N*C*H$_3^{BzIm}$), 28.6 (NC*C*$^{Ahx}$), 25.8 (NCC*C*$^{Ahx}$), 24.8 (NCCC*C*$^{Ahx}$).

### 3.2.2 Compound 2



$CuSO_4 \cdot 5H_2O$ (6.0 mg, 24 µmol, 0.2 eq.) and TBTA (13 mg, 24 µmol, 0.2 eq.) were added as solution in THF/$H_2O$ 1:1-v% to **5** (50.3 mg, 121 µmol, 1.0 eq.) and **12** (100 mg, 121 µmol, 1.0 eq.) in THF/$H_2O$ 1:1-v% with an overall volume of 6 mL. NaAsc (9.6 mg, 48 µmol, 0.4 eq.) was added and it was stirred for 24 h at 45 °C under the exclusion of light. The slightly purple reaction mixture was purified as follows: the solution was concentrated and the aqueous residue brought to pH ≈ 10 - 11 by the addition of 1M NaOH solution. It was washed with $CHCl_3$ (2 x 1 mL) and the product was precipitated by the addition of 1M HCl at pH ≈ 2.5 - 3. The product was obtained by centrifugation and lyophilization.

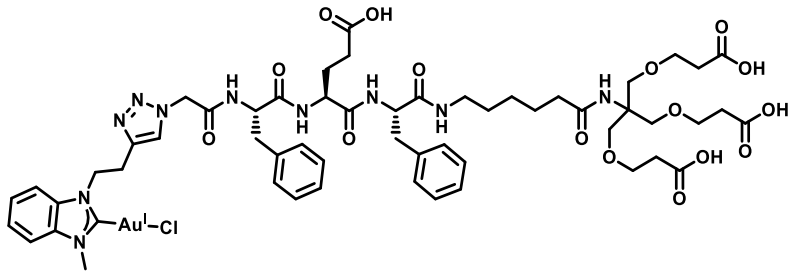
**Yield**: 120 mg (96 µmol, 80%); white solid.

**ESI-HRMS (MeOH) (*m/z*)**: Calculated for $[C_{102}H_{130}Au_2Cl_2N_{18}O_{26}\text{-}2Cl]^{2+}$: 1208.9379, found 1208.9371.

**$^1$H-NMR (600 MHz, DMSO-*d*$_6$, 318 K, COSY, HSQC, HMBC)**: $\delta$ / ppm = 12.06 (bs, 3H, COO*H*), 8.17 (d, *J* = 8.2 Hz, 1H, N*H*$^{Phe}$), 7.83 (d, *J* = 7.9 Hz, 1H, C*H*$^{BzIm}$), 7.75 (d, *J* = 8.2 Hz, 1H, N*H*$^{Phe}$), 7.67 – 7.64 (m, 2H, C*H*$^{BzIm}$, N*H*CH$_2^{Ahx}$), 7.54 – 7.46 (dt, *J* = 19.7, 7.4 Hz, 2H, C*H*$^{BzIm}$), 7.21 – 6.92 (m, 10H, C*H*$^{Phe}$), 6.81 (s, 1H, C$_q$N*H*), 4.98 – 4.89 (m, 2H, C*H*$_2^{Gly}$), 4.90 – 4.84 (m, 2H, NC*H*$_2^{BzIm}$), 4.56 (td, *J* = 8.1, 4.9 Hz, 1H, α-C*H*$^{Phe}$), 4.41 (td, *J* = 8.2, 6.0 Hz, α-C*H*$^{Phe}$), 3.76 (s, 3H, NC*H*$_3^{BzIm}$), 3.59 – 3.54 (m, 12H, C*H*$_2^{Dnd}$), 3.37 (t, *J* = 7.4 Hz, 2H, NCH$_2$C*H*$_2^{BzIm}$), 3.04 – 3.97 (m, 1H, NHC*H*$_2^{Ahx}$), 2.94 – 2.86 (m, 3H, NHC*H*$_2^{Ahx}$, β-C*H*$_2^{Phe}$), 2.79 (dd, *J* = 13.7, 8.3 Hz, 1H, β-C*H*$_2^{Phe}$), 2.73 (dd, *J* = 13.8, 8.3 Hz, 1H, β-C*H*$_2^{Phe}$), 2.41 (t, *J* = 6.4 Hz, 6H, COC*H*$_2^{Dnd}$), 2.02 (t, *J* = 7.5 Hz, 2H, NH[CH$_2$]$_4$C*H*$_2^{Ahx}$), 1.44 - 1.37 (m, 2H, NH[CH$_2$]$_3$C*H*$_2^{Ahx}$), 1.31 - 1.22 (m, 2H, NHCH$_2$C*H*$_2^{Ahx}$), 1.13 (m, 2H, NH[CH$_2$]$_2$C*H*$_2^{Ahx}$).

**$^{13}$C-NMR (151 MHz, DMSO-*d*$_6$, 318 K, HSQC, HMBC)**: $\delta$ / ppm = 195.0 (N$_2$*C*Au), 172.4 (*C*OO$^{Dnd}$), 172.2 (*C*O$^{Ahx}$), 170.04 (*C*O$^{Phe}$), 169.99 (*C*O$^{Phe}$), 166.5 (*C*O$^{Gly}$), 162.9 (*C*$_q^{Triazole}$), 148.4 (*C*H$^{Triazole}$), 137.42 (C$_q^{Phe}$), 137.0 (C$_q^{Phe}$), 133.6 (C$_q^{BzIm}$), 132.6 (C$_q^{BzIm}$), 129.03 (*C*$^{Phe}$), 128.97 (*C*$^{Phe}$), 127.9 (*C*$^{Phe}$), 127.7 (*C*$^{Phe}$), 126.1 (*C*$^{Phe}$), 125.9 (*C*$^{Phe}$), 124.0 (*C*$^{BzIm}$), 123.9 (*C*$^{BzIm}$), 111.72 (*C*$^{BzIm}$), 111.65 (*C*$^{BzIm}$), 68.2 (*C*$^{Dnd}$), 66.6 (*C*$^{Dnd}$), 59.4 (*C*$_q^{Dnd}$), 54.0 (α-*C*$^{Phe}$), 53.9 (*C*H$_2^{Gly}$), 53.4 (α-*C*$^{Phe}$), 48.3 (NCH$_2^{BzIm}$), 38.4 (N*C*$^{Ahx}$), 37.8 (β-*C*$^{Phe}$), 37.5 (β-*C*$^{Phe}$), 35.7 (NCCCC*C*$^{Ahx}$), 34.5 (CO*C*$^{Dnd}$), 34.2 (N*C*H$_3^{BzIm}$), 28.6 (NC*C*$^{Ahx}$), 28.3 (NC*C*$^{BzIm}$), 25.80 (NCC*C*$^{Ahx}$), 24.81 (NCCC*C*$^{Ahx}$)

### 3.2.3 Compound 3



$CuSO_4 \cdot 5H_2O$ (0.70 mg, 3.0 µmol, 0.2 eq.) and TBTA (1.5 mg, 3.0 µmol, 0.2 eq.) were added as solution in THF/$H_2O$ 1:1-v% to **5** (5.9 mg, 14 µmol, 1.0 eq.) and **13** (13 mg, 14 µmol, 1.0 eq.) in THF/$H_2O$ 1:1-v% with an overall volume of 750 µL. NaAsc (1.1 mg, 5.6 µmol, 0.4 eq.) was added and it was stirred for 24 h at 45 °C under the exclusion of light. The slightly purple reaction mixture was purified as follows: the solution was concentrated and the aqueous residue brought to pH ≈ 10 - 11 by the addition of 1M NaOH solution. It was washed with $CHCl_3$ (2 x 250 µL) and the product was precipitated by the addition of 1M HCl at pH ≈ 2.5 - 3. The product was obtained by centrifugation and lyophilization.

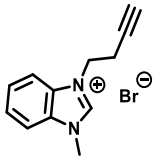
**Yield**: 11 mg (8.2 µmol, 58%); white solid.

**ESI-HRMS (MeOH) (*m/z*)**: Calculated for $[C_{56}H_{72}AuClN_{10}O_{16}\text{-}Cl\text{-}H+NH_4]^+$: 1354,5053; found: 1353,3122.

**$^1$H-NMR (600 MHz, DMSO-*d*$_6$, 318 K, COSY, HSQC, HMBC)**: $\delta$ / ppm = 12.03 (bs, 4H, COO*H*), 8.19 (d, *J* = 7.8 Hz, 1H, N*H*$^{Glu}$), 7.89 – 7.73 (m, 4H, N*H*$^{Phe}$, C*H*$^{BzIm}$, N*H*CH$_2^{Ahx}$), 7.68 (d, *J* = 8.0 Hz, 1H, C*H*$^{BzIm}$), 7.52 – 7.44 (m, 2H, C*H*$^{BzIm}$), 7.36 – 6.90 (m, 10H, C*H*$^{Phe}$), 6.80 (s, 2H, C$_q$N*H*), 4.97 – 4.89 (m, 2H, C*H*$_2^{Gly}$), 4.89 – 4.80 (m, 2H, NC*H*$_2$), 4.66 – 4.54 (m, 1H, α-C*H*$^{Phe}$), 4.48 – 4.41 (m, 1H, α-C*H*$^{Phe}$), 4.25 – 4.19 (m, 1H, α-C*H*$^{Glu}$), 3.81 (s, 3H, NC*H*$_3$), 3.61 – 3.52 (m, 12H, C*H*$_2^{Dnd}$), 3.39 – 3.33 (m, 2H, NCH$_2$C*H*$_2$), 3.07 – 3.01 (m, 1H, NHC*H*$_2^{Ahx}$), 2.96 – 2.90 (m, 3H, NHC*H*$_2^{Ahx}$, β-C*H*$_2^{Phe}$), 2.85 – 2.78 (m, 1H, β-C*H*$_2^{Phe}$), 2.74 – 2.68 (m, 1H, β-C*H*$_2^{Phe}$), 2.42 (t, *J* = 6.4 Hz, 6H, COC*H*$_2^{Dnd}$), 2.19 – 2.13 (m, 2H, γ-C*H*$_2^{Glu}$), 2.02 (t, *J* = 7.5 Hz, 2H, NH[CH$_2$]$_4$C*H*$_2^{Ahx}$), 1.88 – 1.79 (m, 1H, β-C*H*$_2^{Glu}$), 1.76 – 1.67 (m, 1H, β-C*H*$_2^{Glu}$), 1.44 - 1.37 (m, 2H, NH[CH$_2$]$_3$C*H*$_2^{Ahx}$), 1.34 - 1.26 (m, 2H, NHCH$_2$C*H*$_2^{Ahx}$), 1.19 - 1.10 (m, 2H, NH[CH$_2$]$_2$C*H*$_2^{Ahx}$).

**$^{13}$C-NMR (151 MHz, DMSO-*d*$_6$, 318 K, HSQC, HMBC)**: $\delta$ / ppm = 195.0 (N$_2$*C*Au), 173.7 (*C*OO$^{Glu}$), 172.4 (*C*O$^{Ahx}$), 172.3 (*C*OO$^{DNd}$), 170.6 (*C*O$^{Glu}$), 170.4 (*C*O$^{Phe}$), 170.1 (*C*O$^{Phe}$), 166.4 (*C*O$^{Gly}$), 137.4 (*C*$_q^{Phe}$), 137.1 (*C*$_q^{Phe}$), 133.6 (*C*$_q^{BzIm}$), 132.6 (*C*$_q^{BzIm}$), 128.99 (*C*$^{Phe}$), 128.97 (*C*$^{Phe}$), 128.92 (*C*$^{Phe}$), 128.6 (*C*$^{Phe}$), 127.8 (*C*$^{Phe}$), 127.70 (*C*$^{Phe}$), 127.66 (*C*$^{Phe}$), 126.1 (*C*$^{Phe}$), 125.9 (*C*$^{BzIm}$), 124.0 (*C*$^{BzIm}$), 111.7 (*C*$^{BzIm}$), 109.4 (*C*$^{BzIm}$), 68.2 (*C*$^{Dnd}$), 66.6 (*C*$^{Dnd}$), 59.4 (*C*$_q^{Dnd}$), 53.8 (α-*C*$^{Phe}$), 53.8 (*C*H$_2^{Gly}$), 53.4 (α-*C*$^{Phe}$), 51.3 (α-*C*$^{Phe}$), 48.8 (N*C*H$_2^{BzIm}$), 38.4 (N*C*$^{Ahx}$), 37.8 (β-*C*$^{Phe}$), 37.6 (β-*C*$^{Phe}$), 35.7 (NCCCC*C*$^{Ahx}$), 34.5 (CO*C*$^{Dnd}$), 34.2 (N*C*H$_3^{BzIm}$), 30.0 (γ-*C*$^{Glu}$), 28.6 (NC*C*$^{Ahx}$), 27.3 (NC*C*$^{BzIm}$), 25.8 (NCC*C*$^{Ahx}$), 24.8 (NCCCC*C*$^{Ahx}$).

### 3.2.4 Compound 4[10]



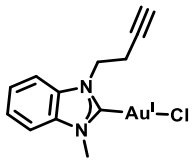
1-Methyl-1*H*-benzo[*d*]-imidazole (317 mg, 2.4 mmol, 1.0 eq.) and 4-bromo-but-1-yne (478 mg, 3.60 mmol, 1.5 eq.) were stirred for 45 h at 120 °C in toluene (3 mL). The resulting precipitation was centrifuged and washed with diethylether (3 times).

**Yield**: 554 mg (2.10 mmol, 87%); grey powder.

**ESI-HRMS (MeOH) (*m*/*z*)**: Calculated for $[C_{12}H_{13}N_2Br\text{-}Br]^+$: 185.1073, found 185.1075.

**$^1$H-NMR (400 MHz, MeOD, 296 K)**: $\delta$ / ppm = 11.37 (s, 1H, NC*H*N), 7.87 – 7.80 (m, 1H, C$H^{BzIm}$), 7.75 – 7.64 (m, 3H, C$H^{BzIm}$), 4.85 (t, $J$ = 6.5 Hz, 2H, NC$H_2$), 4.30 (s, 3H, NC$H_3$), 3.05 (td, $J$ = 6.4, 2.7 Hz, 2H, $NCH_2CH_2$), 2.05 (t, $J$ = 2.6 Hz, 1H, C$H^{alkyne}$).

### 3.2.5 Compound 5[9]




The following procedure was carried out in the dark under argon SCHLENK-technique. In dried and degased DCM (9 mL), **4** (300 mg, 1.13 mmol, 1.0 eq.) and $Ag_2O$ (157 mg, 679 µmol, 0.6 eq.) were stirred for 48 h at room temperature. [Au$^{I}$Cl(tht)] (363 mg, 1.13 mmol, 1.0 eq.) and NaCl (99.0 mg, 1.70 mmol, 1.5 eq.) were added, the resulting mixture was stirred for 2 h and the obtained crude mixture was filtered over Kieselguhr. The concentrated solution was precipitated into cold THF, the obtained precipitation was filtered off and washed with THF (2 times).

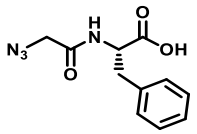
**Yield**: 363 mg (0.867 mmol, 77%); off-white solid.

**ESI-HRMS (MeOH) (*m/z*)**: Calculated for $[C_{24}H_{24}Au_2N_4Cl_2\text{-}Au\text{-}Cl_2]^+$: 565.17, found: 565.21

**$^1$H-NMR (400 MHz, $CDCl_3$, 296 K)**: $\delta$ / ppm = 7.63 – 7.59 (m, 1H, C$H^{BzIm}$), 7.52 – 7.45 (m, 3H, C$H^{BzIm}$), 4.65 (t, $J$ = 6.9 Hz, 2H, NC$H_2$), 4.09 (s, 3H, NC$H_3$), 2.94 – 2.85 (m, 2H, $NCH_2CH_2$), 2.03 (t, $J$ = 2.0 Hz, 1H, C$H^{alkyne}$).

**$^{13}$C-NMR (151 MHz, $CDCl_3$, 296 K, COSY, HSQC)**: $\delta$ / ppm = 178.8 ($N_2CAu$), 133.8 ($C_q^{BzIm}$), 133.2 ($C_q^{BzIm}$), 124.9 ($C^{BzIm}$), 124.8 ($C^{BzIm}$), 111.7 ($C^{BzIm}$), 111.5 ($C^{BzIm}$), 79.7 ($C_q^{alkyne}$), 72.3 ($CH^{alkyne}$), 47.03 ($NCH_2$), 35.5 ($NCH_3$), 20.6 (NCC).

### 3.2.6 Compound 7



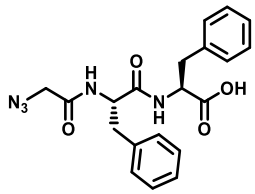
The synthesis was carried out according to **SOP1**.

**Yield**: 350 mg (1.41 mmol, 98%); white solid.

**ESI-HRMS (MeOH) (*m/z*)**: Calculated for $[C_{11}H_{12}N_4O_3\text{-}H]^-$: 247.0837, found 247.0832.

**$^1$H-NMR (400 MHz, DMSO-$d_6$, 296 K)**: $\delta$ / ppm = 12.88 (s, 1H, COO*H*), 8.44 (d, $J$ = 8.1 Hz, 1H, N*H*), 7.32 – 7.17 (m, 5H, C*H*$^{Phe}$), 4.50 – 4.41 (m, 1H, α-C*H*$^{Phe}$), 3.88 – 3.72 (m, 2H, C$H_2^{Gly}$), 3.08 (dd, $J$ = 13.8, 4.9 Hz, 1H, β-C$H_2^{Phe}$), 2.89 (dd, $J$ = 13.9, 9.3 Hz, 1H, β-C$H_2^{Phe}$).

### 3.2.7 Compound 8



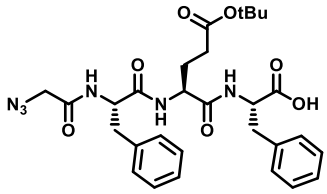
The synthesis was carried out according to **SOP1**.

**Yield**: 554 mg (1.40 mmol, 92%); white solid.

**ESI-HRMS (MeOH) (*m*/*z*)**: Calculated for $[C_{20}H_{21}N_5O_4+Na]^+$: 418.1484, found 418.1491.

**$^1$H-NMR (400 MHz, DMSO-*d*$_6$, 296 K)**: $\delta$ / ppm = 12.77 (s, 1H, COO*H*), 8.46 (d, *J* = 7.9 Hz, 1H, N*H*), 8.26 (d, *J* = 8.6 Hz, 1H, N*H*), 7.32 – 7.13 (m, 10H, C*H*$^{Phe}$), 4.64 – 4.54 (m, 1H, α-C*H*$^{Phe}$), 4.54 – 4.39 (m, 1H, α-C*H*$^{Phe}$), 3.80 – 3.63 (m, 2H, C*H*$_2^{Gly}$), 3.08 (dd, *J* = 13.9, 5.2 Hz, 1H, β-C*H*$_2^{Phe}$), 3.00 (dd, *J* = 13.9, 4.2 Hz, 1H, β-C*H*$_2^{Phe}$), 2.92 (dd, *J* = 13.9, 9.0 Hz, 1H, β-C*H*$_2^{Phe}$), 2.70 (dd, *J* = 13.8, 9.8 Hz, 1H, β-C*H*$_2^{Phe}$).

### 3.2.8 Compound 9



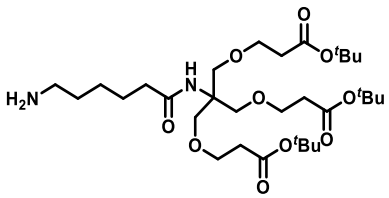
The synthesis was carried out according to **SOP1**.

**Yield**: 412.5 mg (710 µmol, 52%); white solid.

**ESI-HRMS (MeOH) (*m*/*z*)**: Calculated for $[C_{29}H_{36}N_6O_7+Na]^+$: 603.2538, found 603.2541.

**$^1$H-NMR (400 MHz, DMSO-*d*$_6$, 296 K)**: $\delta$ / ppm = 12.79 (s, 1H, COO*H*), 8.30 (d, *J* = 8.3 Hz, 1H, N*H*$^{Phe}$), 8.23 (d, *J* = 8.1 Hz, 1H, N*H*$^{Glu}$), 8.14 (d, *J* = 7.7 Hz, 1H, N*H*$^{Phe}$), 7.40 – 7.05 (m, 10H, C*H*$^{Phe}$), 4.58 (ddd, *J* = 9.9, 8.3, 4.1 Hz, 1H, α-C*H*), 4.48 – 4.39 (m, 1H, α-C*H*), 4.29 (td, *J* = 8.1, 5.4 Hz, 1H, α-C*H*), 3.83 – 3.64 (m, 2H, C*H*$_2^{Gly}$), 3.07 (dd, *J* = 14.0, 5.1 Hz, 1H, β-CH$_2^{Phe}$), 3.00 – 2.85 (m, 2H, CH$_2^{Phe}$), 2.75 – 2.65 (m, 1H, β-C*H*$_2^{Phe}$), 2.23 – 2.14 (m, 2H, γ-C*H*$_2^{Glu}$), 1.91 – 1.79 (m, 1H, β-C*H*$_2^{Glu}$), 1.77 – 1.65 (m, 1H, β-C*H*$_2^{Glu}$), 1.39 (d, *J* = 1.1 Hz, 9H).

### 3.2.9 Compound 10[1]



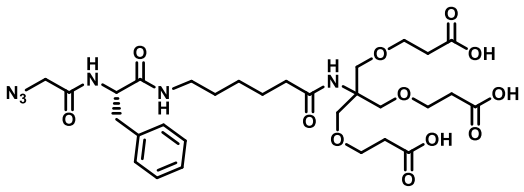
The Cbz-protected derivative (298 mg, 396 µmol, 1 eq.) was dissolved in MeOH (5 mL). Pd/C (10-wt%, 30 mg), was added and the flask was filled with $H_2$. After 24 h of stirring, the suspension was filtered over Kieselguhr and concentrated under reduced pressure.

**Yield**: 238 mg (385 µmol, 97%); colourless oil.

**ESI-HRMS (MeOH) (*m/z*)**: Calculated for $[C_{31}H_{58}N_2O_{10}+H]^+$: 619.4164, found 619.36.

**$^1$H-NMR (400 MHz, $CDCl_3$, 296 K)**: $\delta$ / ppm = 6.10 (s, 1H, $C_qNH$), 3.69 (s, 6H, $CH_2^{Dnd}$), 3.63 (t, $J$ = 6.3 Hz, 6H, $CH_2^{Dnd}$), 2.72 (t, $J$ = 7.0 Hz, 2H, $NH_2CH_2^{Ahx}$), 2.44 (t, $J$ = 6.3 Hz, 6H, $COCH_2^{Dnd}$), 2.16 (t, $J$ = 7.5 Hz, 2H, $NH[CH_2]_4CH_2^{Ahx}$), 1.66 – 1.54 (m, 2H, $CH_2^{Ahx}$), 1.53 – 1.46 (m, 2H, $CH_2^{Ahx}$), 1.44 (s, 27H, $CH_3^{tBu}$), 1.38 – 1.24 (m, 2H, $CH_2^{Ahx}$).

### 3.2.10 Compound 11



**14** (52 mg, 61 µmol, 1.0 eq.) was treated with 4.4 mL TFA in DCM 1:1-v%. The solution was stirred 40 min at room temperature, concentrated under reduced pressure and another 4.4 mL TFA in DCM 1:1-v% were added. The concentrated reaction mixture was co-distilled with toluene 5 times and the product was obtained without further purification.

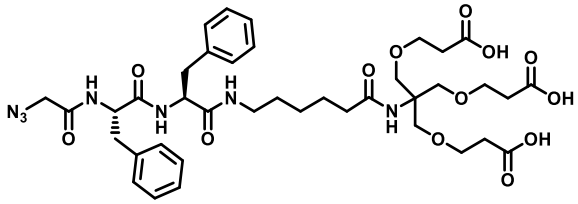
**Yield**: 42 mg (61 µmol, quant.); white solid.

**ESI-HRMS (MeOH) (*m/z*)**: Calculated for $[C_{30}H_{44}N_6O_{12}+Na]^+$: 703.2909, found 703.2906.

**$^1$H-NMR (400 MHz, DMSO-*d*$_6$, 296 K, COSY, HSQC, HMBC)**: $\delta$ / ppm = 8.35 (d, *J* = 8.4 Hz, 1H, N*H*$^{Phe}$), 8.04 (t, *J* = 5.6 Hz, 1H, N*H*CH$_2^{Ahx}$), 7.29 – 7.16 (m, 5H, C*H*$^{Phe}$), 6.93 (s, 1H, C$_q$N*H*), 4.57 (td, *J* = 8.7, 5.5 Hz, 1H, α-C*H*$^{Phe}$), 3.85 – 3.66 (m, 2H, C*H*$_2^{Gly}$), 3.58 – 3.50 (m, 12H, C*H*$_2^{Dnd}$), 3.10 – 3.00 (m, 1H, NHC*H*$_2^{Ahx}$), 2.99 – 2.89 (m, 2H, NHC*H*$_2^{Ahx}$, β-C*H*$_2^{Phe}$), 2.77 (dd, *J* = 13.6, 9.0 Hz, 1H, β-C*H*$_2^{Phe}$), 2.41 (t, *J* = 6.3 Hz, 6H, COC*H*$_2^{Dnd}$), 2.03 (t, *J* = 7.5 Hz, 2H, NH[CH$_2$]$_4$C*H*$_2^{Ahx}$), 1.46 - 1.36 (m, 2H, NH[CH$_2$]$_3$C*H*$_2^{Ahx}$), 1.36 - 1.26 (m, 2H, NHCH$_2$C*H*$_2^{Ahx}$), 1.20 - 1.09 (m, 2H, NH[CH$_2$]$_2$C*H*$_2^{Ahx}$).

**$^{13}$C-NMR (101 MHz, DMSO-*d*$_6$, 296 K, HSQC, HMBC)**: $\delta$ / ppm = 172.7 (*C*OO), 172.5 (*C*O$^{Ahx}$), 170.3 (*C*O$^{Phe}$), 167.1 (*C*O$^{Gly}$), 137.6 (*C*$_q^{Phe}$), 129.2 (*C*$^{Phe}$), 128. 1 (*C*$^{Phe}$), 126.4 (*C*$^{Phe}$), 68.2 (*C*$^{Dnd}$), 66.7 (*C*$^{Dnd}$), 59.5 (*C*$_q^{Dnd}$), 54.1 (α-*C*$^{Phe}$), 50.5 (*C*H$_2^{Gly}$), 38.5 (N*C*$^{Ahx}$), 38.1 (β-*C*$^{Phe}$), 35.8 (NCCCC*C*$^{Ahx}$), 34.6 (*C*COO), 28.8 (NC*C*$^{Ahx}$), 26.0 (NCC*C*$^{Ahx}$), 25.0 (NCCC*C*$^{Ahx}$).

### 3.2.11 Compound 12



**15** (458 mg, 460 µmol, 1.0 eq.) was treated with 33 mL TFA in DCM 1:1-v%. The solution was stirred for 40 min at room temperature, concentrated under reduced pressure and another 33 mL TFA in DCM 1:1-v% were added. The concentrated reaction mixture was co-distilled with toluene 5 times and the product was obtained without further purification.

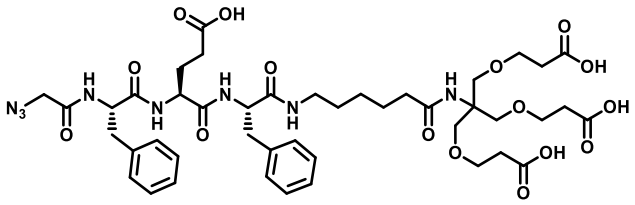
**Yield**: 325 mg (393 µmol, 85%); white solid.

**ESI-HRMS (MeOH) (*m/z*)**: Calculated for $[C_{39}H_{53}N_7O_{13}+Na]^+$: 850.3594, found 850.3583.

**$^1$H-NMR (400 MHz, DMSO-*d*$_6$, 296 K, COSY, HSQC, HMBC)**: $\delta$ / ppm = 8.28 (d, *J* = 8.3 Hz, 1H, N*H*$^{Phe}$), 8.24 (d, *J* = 8.3 Hz, 1H, N*H*$^{Phe}$), 7.81 (t, *J* = 5.6 Hz, 1H, N*H*CH$_2^{Ahx}$), 7.29 – 7.10 (m, 10H, C*H*$^{Phe}$), 6.93 (s, 1H, C$_q$N*H*), 4.55 (td, *J* = 8.8, 4.7 Hz, 1H, α-C*H*$^{Phe}$), 4.44 (td, *J* = 8.5, 5.8 Hz, 1H, α-C*H*$^{Phe}$), 3.79 – 3.67 (m, 2H, C*H*$_2^{Gly}$), 3.58 – 3.53 (m, 12H, C*H*$_2^{Dnd}$), 3.02 (p, *J* = 6.8 Hz, 1H, NHC*H*$_2^{Ahx}$), 2.99 - 2.90 (m, 3H, NHC*H*$_2^{Ahx}$, C*H*$_2^{Phe}$), 2.81 (dd, *J* = 13.6, 8.7 Hz, 1H, β-C*H*$_2^{Phe}$), 2.71 (dd, *J* = 13.8, 9.4 Hz, 1H, β-C*H*$_2^{Phe}$), 2.42 (t, *J* = 6.3 Hz, 6H, COC*H*$_2^{Dnd}$), 2.03 (t, *J* = 7.5 Hz, 2H, N[CH$_2$]$_4$C*H*$_2^{Ahx}$), 1.45 - 1.36 (m, 2H, N[CH$_2$]$_3$C*H*$_2^{Ahx}$), 1.34 - 1.26 (m, 2H, NCH$_2$C*H*$_2^{Ahx}$), 1.20 - 1.10 (m, 2H, N[CH$_2$]$_2$C*H*$_2^{Ahx}$).

**$^{13}$C-NMR (101 MHz, DMSO-*d*$_6$, 296 K, HSQC, HMBC)**: $\delta$ / ppm = 172.7 (*C*OO$^{Dnd}$), 172.4 (*C*O$^{Ahx}$), 170.4 (*C*O$^{Phe}$), 170.3 (*C*O$^{Phe}$), 167.11 (*C*O$^{Gly}$), 137.65 (*C*$_q^{Phe}$), 137.46 (*C*$_q^{Phe}$), 129.19 (*C*$^{Phe}$), 128.91 (*C*$^{Phe}$), 128.22 (*C*$^{Phe}$), 128.05 (*C*$^{Phe}$), 126.29 (*C*$^{Phe}$), 68.14 (*C*$^{Dnd}$), 66.68 (*C*$^{Dnd}$), 59.51 (*C*$_q^{Dnd}$), 54.13 (α-*C*), 53.76 (α-*C*), 50.44 (*C*H$_2^{Gly}$), 38.46 (N*C*$^{Ahx}$), 37.92 (β-*C*$^{Phe}$), 37.58 (β-*C*$^{Phe}$), 35.80 (NCCCC*C*$^{Ahx}$), 34.60 (*C*COO), 28.79 (NC*C*$^{Ahx}$), 25.93 (NCC*C*$^{Ahx}$), 24.98 (NCCC*C*$^{Ahx}$).

### 3.2.12 Compound 13



**16** (160 mg, 118 µmol, 1.0 eq.) was treated with 16 mL TFA in DCM 1:1-v%. The solution was stirred 40 min at room temperature, concentrated under reduced pressure and another 16 mL TFA in DCM 1:1-v% were added. The concentrated reaction mixture was co-distilled with toluene 5 times and the product was obtained without further purification.

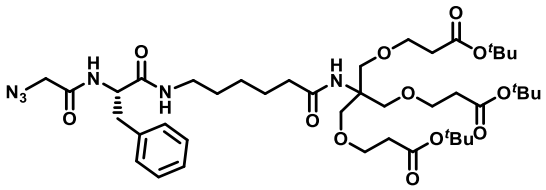
**Yield**: 100 mg (104 µmol, 89%); colourless solid.

**ESI-HRMS (MeOH) (*m/z*)**: Calculated for $[C_{44}H_{60}N_8O_{16}+Na]^+$: 979.4019, found 979.4022.

**$^1$H-NMR (600 MHz, DMSO-*d*$_6$, 296 K, COSY, HSQC, HMBC)**: $\delta$ / ppm = 12.13 (s, 4H, COO*H*), 8.34 (d, *J* = 8.3 Hz, 1H, N*H*$^{Phe}$), 8.28 (d, *J* = 7.8 Hz, 1H, N*H*$^{Glu}$), 7.96 (d, *J* = 8.1 Hz, 1H, N*H*$^{Phe}$), 7.91 (t, *J* = 5.7 Hz, 1H, N*H*CH$_2^{Ahx}$), 7.27 – 7.11 (m, 10H, C*H*$^{Phe}$), 6.95 (s, 1H, C$_q$N*H*), 4.59 – 4.53 (m, 1H, α-C*H*$^{Phe}$), 4.46 – 4.41 (m, 1H, α-C*H*$^{Phe}$), 4.25 – 4.19 (m, 1H, α-C*H*$^{Glu}$), 3.81 – 3.69 (m, 2H, C*H*$_2^{Gly}$), 3.58 – 3.50 (m, 12H, C*H*$_2^{Dnd}$), 3.08 – 3.01 (m, 1H, NHC*H*$_2^{Ahx}$), 2.99 – 2.88 (m, 3H, NHC*H*$_2^{Ahx}$, β-C*H*$_2^{Phe}$), 2.81 (dd, *J* = 13.7, 8.5 Hz, 1H, β-C*H*$_2^{Phe}$), 2.71 (dd, *J* = 13.9, 10.1 Hz, 1H, β-C*H*$_2^{Phe}$), 2.41 (t, *J* = 6.3 Hz, 6H, COC*H*$_2^{Dnd}$), 2.18 (dt, *J* = 9.7, 6.0 Hz, 2H, γ-C*H*$_2^{Glu}$), 2.02 (t, *J* = 7.5 Hz, 2H, N[CH$_2$]$_4$C*H*$_2^{Ahx}$), 1.84 (m, 1H, β-C*H*$_2^{Glu}$), 1.76 – 1.67 (m, 1H, β-C*H*$_2^{Glu}$), 1.44 – 1.36 (m, 2H, N[CH$_2$]$_3$C*H*$_2^{Ahx}$), 1.33 – 1.26 (m, 2H, NCH$_2$C*H*$_2^{Ahx}$), 1.17 – 1.09 (m, 2H, N[CH$_2$]$_2$C*H*$_2^{Ahx}$).

**$^{13}$C-NMR (101 MHz, DMSO-*d*$_6$, 296 K, HSQC, HMBC)**: $\delta$ / ppm = 174.0 (*C*OO$^{Glu}$), 172.7 (*C*OO$^{Dnd}$), 172.5 (*C*O$^{Ahx}$), 170.9 (*C*O$^{Phe}$), 170.6 (*C*O$^{Glu}$), 170.3 (*C*O$^{Phe}$), 167.3 (*C*O$^{Gly}$), 137.63 (*C*$_q^{Phe}$), 137.57 (*C*$_q^{Phe}$), 129.2 (*C*$^{Phe}$), 128.08 (*C*$^{Phe}$), 128.07 (*C*$^{Phe}$), 126.33 (*C*$^{Phe}$), 126.31 (*C*$^{Phe}$), 68.1 (*C*$^{Dnd}$), 66.7 (*C*$^{Dnd}$), 59.5 (*C*$_q^{Dnd}$), 53.9 (α-C$^{Phe}$), 53.8 (α-*C*$^{Phe}$), 52.1 (α-*C*$^{Glu}$), 38.5 (N*C*$^{Ahx}$), 37.9 (β-*C*$^{Phe}$), 37.5 (β-*C*$^{Phe}$), 35.8 (NCCCC*C*$^{Ahx}$), 34.6 (CO*C*$^{Dnd}$), 30.1 (γ-*C*$^{Glu}$), 28.8 (NC*C*$^{Ahx}$), 27.4 (β-C$^{Glu}$), 26.0 (NCC*C*$^{Ahx}$), 25.0 (NCCC*C*$^{Ahx}$).

### 3.2.13 Compound 14



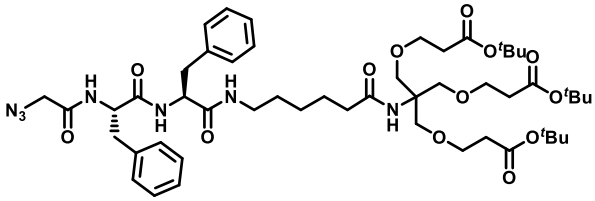
DIPEA (75.5 µL, 434 µmol, 1.1 eq.) was added to a stirred solution of **7** (127 mg, 512 µmol, 1.3 eq.), **10** (244 mg, 394 µmol, 1.0 eq.), PyBOP (287 mg, 552 µmol, 1.4 eq.) and HOAt (53.7 mg, 394 µmol, 1.0 eq.) in peptide grade DMF at 0 °C. The reaction mixture was stirred overnight at room temperature and concentrated *in vacuo*. The crude product was purified *via* flash chromatography on $SiO_2$ (EtOAc 100%; $R_F$ = 0.57).

**Yield**: 176 mg (207 µmol, 53%); colourless solid.

**ESI-HRMS (MeOH) (*m/z*)**: Calculated for $[C_{42}H_{68}N_6O_{12}+Na]^+$: 871.4787, found 871.4765.

**$^1$H-NMR (400 MHz, DMSO-*d*$_6$, 296 K)**: $\delta$ / ppm = 8.35 (d, *J* = 8.4 Hz, 1H, N*H*$^{Phe}$), 8.04 (t, *J* = 5.6 Hz, 1H, N*H*CH$_2^{Ahx}$), 7.30 – 7.14 (m, 5H, C*H*$^{Phe}$), 6.90 (s, 1H, C$_q$N*H*), 4.58 (td, *J* = 8.7, 5.5 Hz, 1H, α-C*H*$^{Phe}$), 3.86 – 3.68 (m, 2H, C*H*$_2^{Gly}$), 3.59 – 3.48 (m, 12H, C*H*$_2^{Dnd}$), 3.03 (td, *J* = 12.6, 12.1, 6.0 Hz, 1H, NHC*H*$_2^{Ahx}$), 2.94 (dd, *J* = 13.8, 5.5 Hz, 2H, NHC*H*$_2^{Ahx}$, β-C*H*$_2^{Phe}$), 2.76 (dd, *J* = 13.6, 9.0 Hz, 1H, β-C*H*$_2^{Phe}$), 2.38 (t, *J* = 6.1 Hz, 6H, COC*H*$_2^{Dnd}$), 2.02 (t, *J* = 7.5 Hz, 2H, NH[CH$_2$]$_4$C*H*$_2^{Ahx}$), 1.45 – 1.35 (m, 2H, NH[CH$_2$]$_3$C*H*$_2^{Ahx}$), 1.39 (s, 27H, CH$_3^{tBu}$), 1.35 - 1.26 (m, 2H, NHCH$_2$C*H*$_2^{Ahx}$), 1.121 - 1.08 (m, 2H, NH[CH$_2$]$_2$C*H*$_2^{Ahx}$).

### 3.2.14 Compound 15



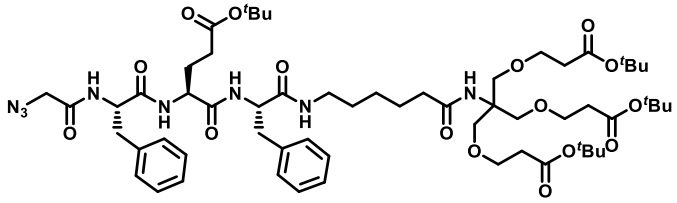
DIPEA (86.7 µL, 498 µmol, 1.1 eq.) was added to a stirred solution of **8** (233 mg, 588 µmol, 1.3 eq.), **10** (280 mg, 453 µmol, 1.0 eq.), PyBOP (330 mg, 634 µmol, 1.4 eq.) and HOAt (62.6 mg, 453 µmol, 1.0 eq.) in peptide grade DMF at 0 °C. The reaction mixture was stirred overnight at room temperature and concentrated *in vacuo*. The crude product was purified *via* flash chromatography on $SiO_2$ (EtOAc/CH 9:1; $R_F$ = 0.35).

**Yield**: 464 mg (465 µmol, quant.); colourless solid.

**ESI-HRMS (MeOH) (*m*/*z*)**: Calculated for $[C_{51}H_{77}N_7O_{13}+H]^+$: 996.5658, found 996.5677.

**$^1$H-NMR (400 MHz, DMSO-*d*$_6$, 296 K)**: $\delta$ / ppm = 8.28 (d, $J$ = 8.3 Hz, 1H, N*H*$^{Phe}$), 8.24 (d, $J$ = 8.3 Hz, 1H, N*H*$^{Phe}$), 7.80 (t, $J$ = 5.6 Hz, 1H, N*H*CH$_2^{Ahx}$), 7.28 – 7.12 (m, 10H, C*H*$^{Phe}$), 6.90 (s, 1H, C$_q$N*H*), 4.55 (td, $J$ = 8.8, 4.7 Hz, 1H, α-C*H*), 4.44 (q, $J$ = 7.8 Hz, 1H, α-C*H*), 3.78 – 3.66 (m, 2H, C*H*$_2^{Gly}$), 3.58 – 3.46 (m, 12H, C*H*$_2^{Dnd}$), 3.07 – 2.99 (m, 1H, NHC*H*$_2^{Ahx}$), 2.98 – 2.91 (m, 3H, NHC*H*$_2^{Ahx}$, β-C*H*$_2^{Phe}$), 2.81 (dd, $J$ = 13.6, 8.7 Hz, 1H, β-C*H*$_2^{Phe}$), 2.71 (dd, $J$ = 13.8, 9.4 Hz, 1H, β-C*H*$_2^{Phe}$), 2.38 (t, $J$ = 6.1 Hz, 6H, COC*H*$_2^{Dnd}$), 2.02 (t, $J$ = 7.5 Hz, 2H, N[CH$_2$]$_4$C*H*$_2^{Ahx}$), 1.47 – 1.10 (m, 6H, NCH$_2$C*H*$_2^{Ahx}$, N[CH$_2$]$_2$C*H*$_2^{Ahx}$, N[CH$_2$]$_3$C*H*$_2^{Ahx}$), 1.39 (s, 27H, C*H*$_3^{t\text{Bu}}$).

### 3.2.15 Compound 16



DIPEA (48.7 µL, 279 µmol, 1.1 eq.) was added to a stirred solution of **9** (192 mg, 330 µmol, 1.3 eq.), **10** (157 mg, 254 µmol, 1.0 eq.), PyBOP (185 mg, 356 µmol, 1.4 eq.) and HOAt (34.6 mg, 254 µmol, 1.0 eq.) in peptide grade DMF at 0 °C. The reaction mixture was stirred overnight at room temperature and concentrated *in vacuo*. The crude product was purified *via* flash chromatography on $SiO_2$ (EtOAc/CH 9:1; $R_F$ = 0.48).

**Yield**: 140 mg (119 µmol, 46%); colourless solid.

**ESI-HRMS (MeOH) (*m/z*)**: Calculated for $[C_{60}H_{92}N_8O_{16}+Na]^+$: 1203.6524, found 1203.6524.

**$^1$H-NMR (400 MHz, DMSO-*d*$_6$, 296 K)**: $\delta$ / ppm = 8.34 (d, $J$ = 8.2 Hz, 1H, N$H^{Phe}$), 8.28 (d, $J$ = 7.8 Hz, 1H, N$H^{Glu}$), 7.94 (d, $J$ = 8.2 Hz, 1H, N$H^{Phe}$), 7.90 (t, $J$ = 5.6 Hz, 1H, N$H$C$H_2^{Ahx}$), 7.26 – 7.13 (m, 10H, C$H^{Phe}$), 6.93 (s, 1H, $C_q$N$H$), 4.56 (td, $J$ = 9.2, 4.0 Hz, 1H, α-C$H^{Phe}$), 4.44 (td, $J$ = 8.2, 5.8 Hz, 1H, α-C$H^{Phe}$), 4.24 – 4.17 (m, 1H, α-C$H^{Glu}$), 3.80 – 3.68 (m, 2H, C$H_2^{Gly}$), 3.53 – 3.51 (m, 12H, C$H_2^{Dnd}$), 3.04 (dq, $J$ = 13.0, 6.7 Hz, 1H, NHC$H_2^{Ahx}$), 2.99 – 2.90 (m, 3H, NHC$H_2^{Ahx}$, β-C$H_2^{Phe}$), 2.81 (dd, $J$ = 13.7, 8.5 Hz, 1H, β-C$H_2^{Phe}$), 2.75 – 2.68 (m, 1H, β-C$H_2^{Phe}$), 2.38 (t, $J$ = 6.1 Hz, 6H, COC$H_2^{Dnd}$), 2.19 – 2.09 (m, 2H, γ-C$H_2^{Glu}$), 2.02 (t, $J$ = 7.5 Hz, 2H, N[CH$_2$]$_4$C$H_2$), 1.85 – 1.77 (m, 1H, β-C$H_2^{Glu}$), 1.73 – 1.65 (m, 1H, β-C$H_2^{Glu}$), 1.43 – 1.37 (m, 2H, N[CH$_2$]$_3$C$H_2$), 1.39 (s, 9H, C$H_3^{tBu}$), 1.32 – 1.27 (m, 2H, NCH$_2$C$H_2$), 1.16 – 1.09 (m, 2H, N[CH$_2$]$_2$C$H_2$).

# 4 NMR Spectra

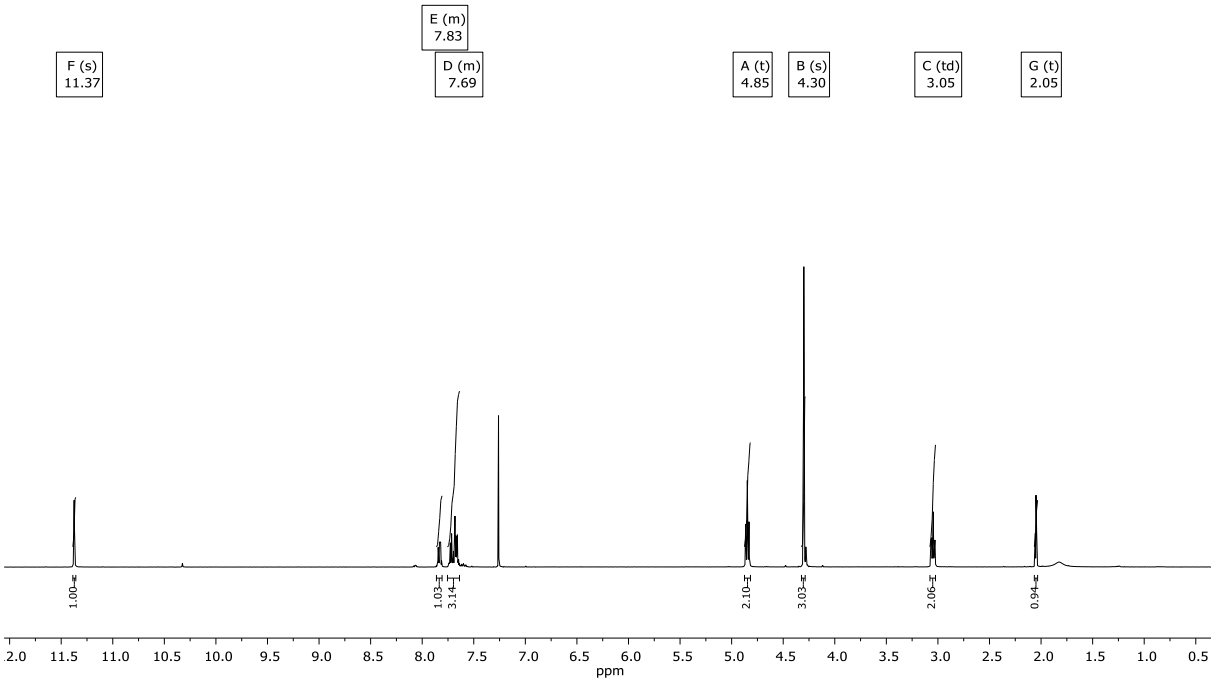



Figure S2. $^{1}H$ NMR spectrum of compound **4** in $CDCl_3$.

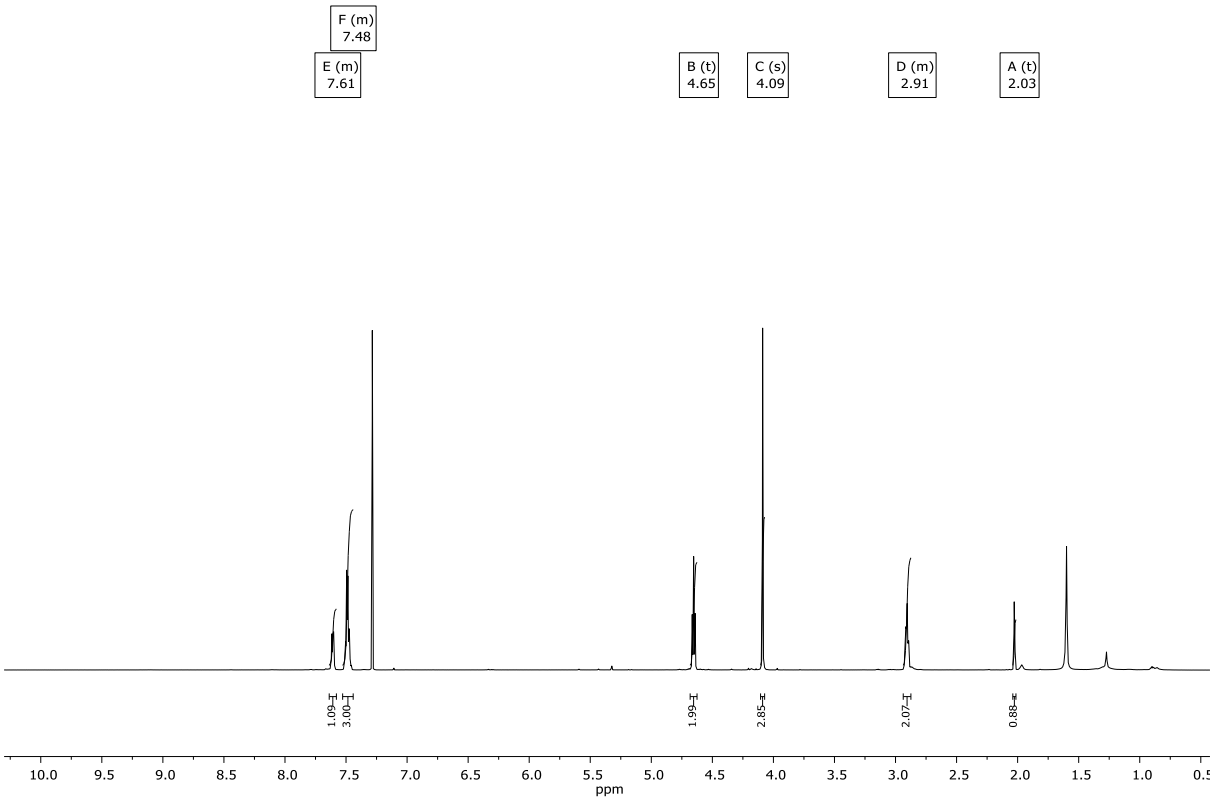



Figure S3. $^1$H NMR spectrum of compound **5** in $CDCl_3$.

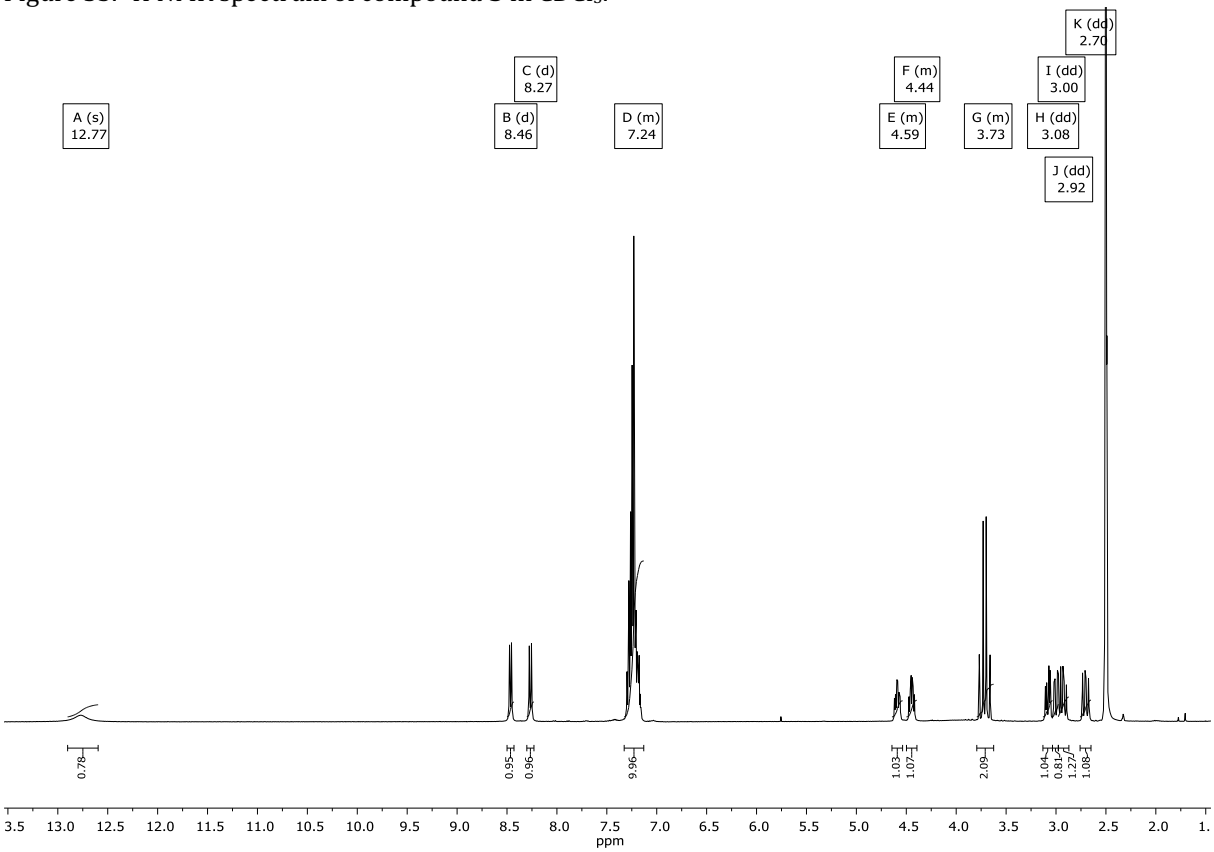



Figure S4. $^1$H NMR spectrum of compound **8** in DMSO-$d_6$.

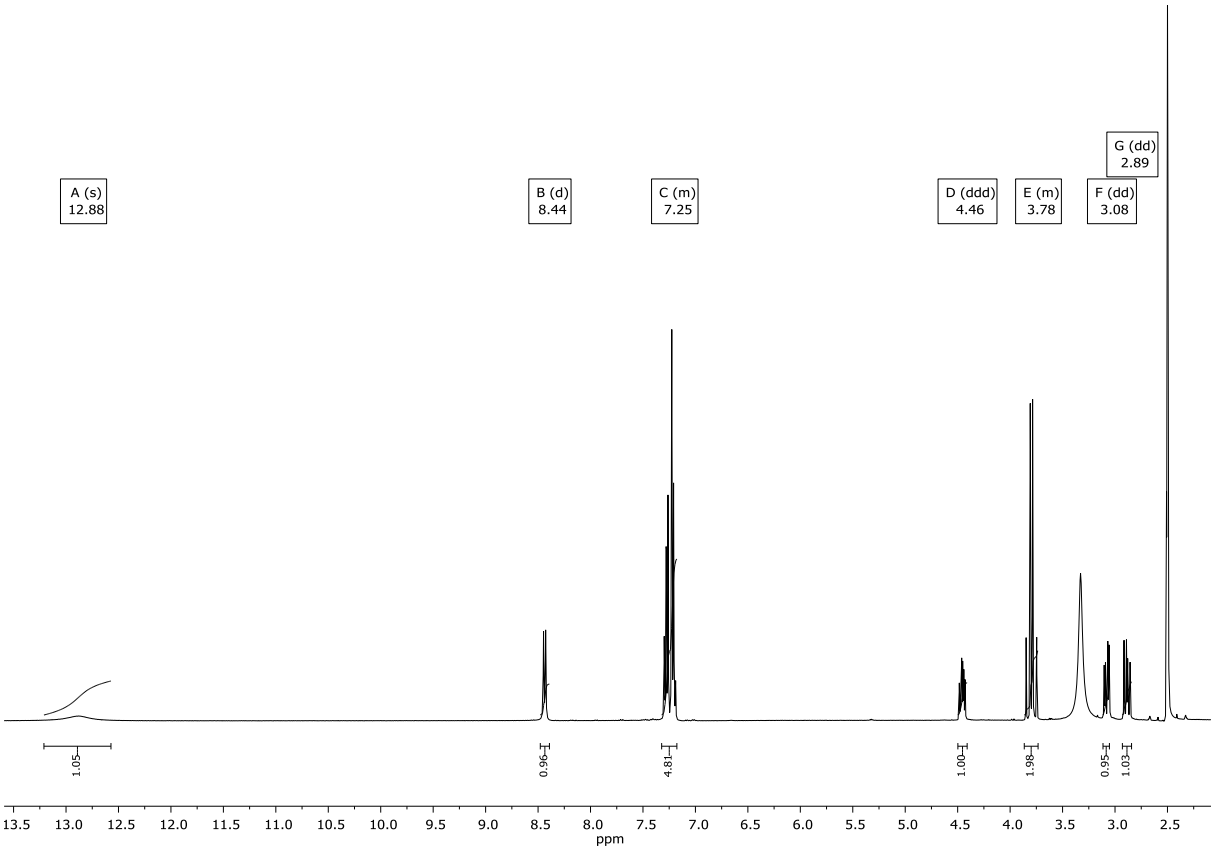



Figure S5. $^{1}$H NMR spectrum of compound **7** in DMSO-$d_6$.

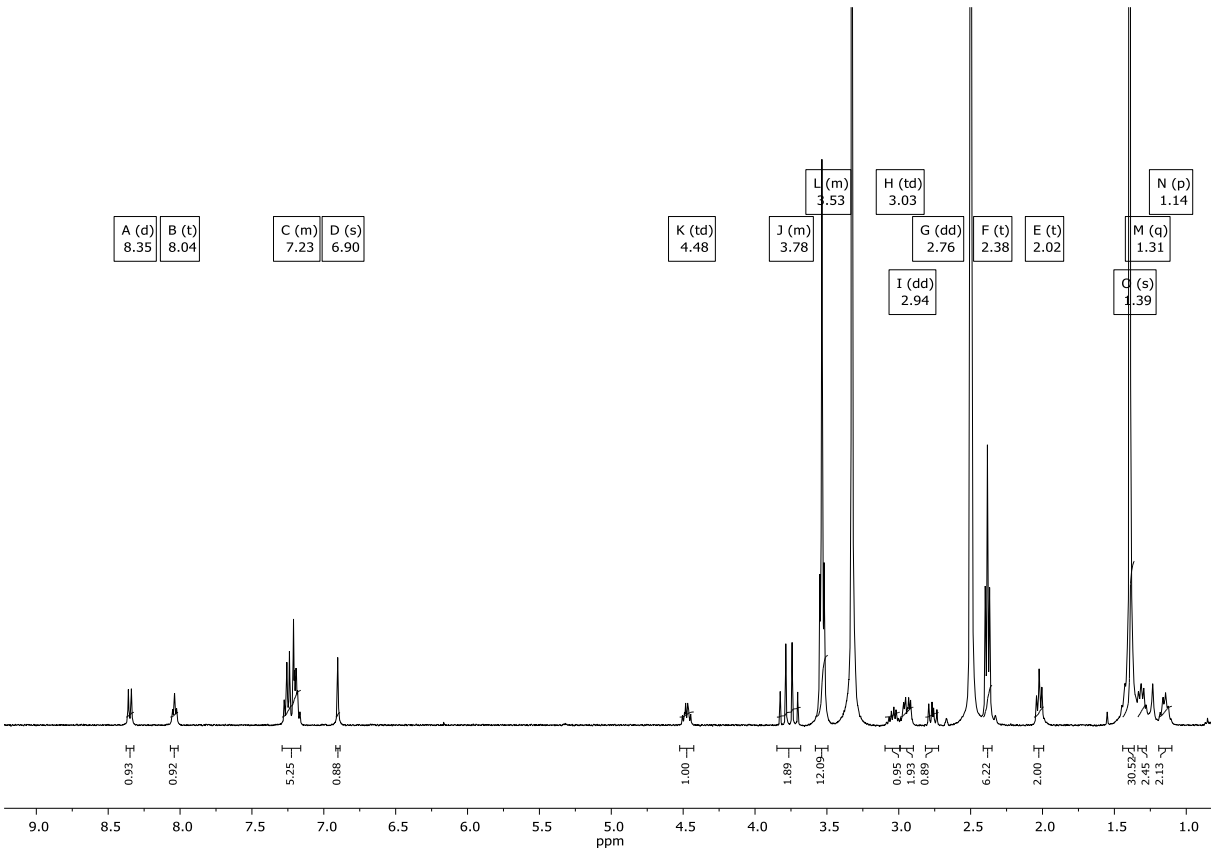



Figure S6. $^{1}$H NMR spectrum of compound **9** in DMSO-$d_6$.

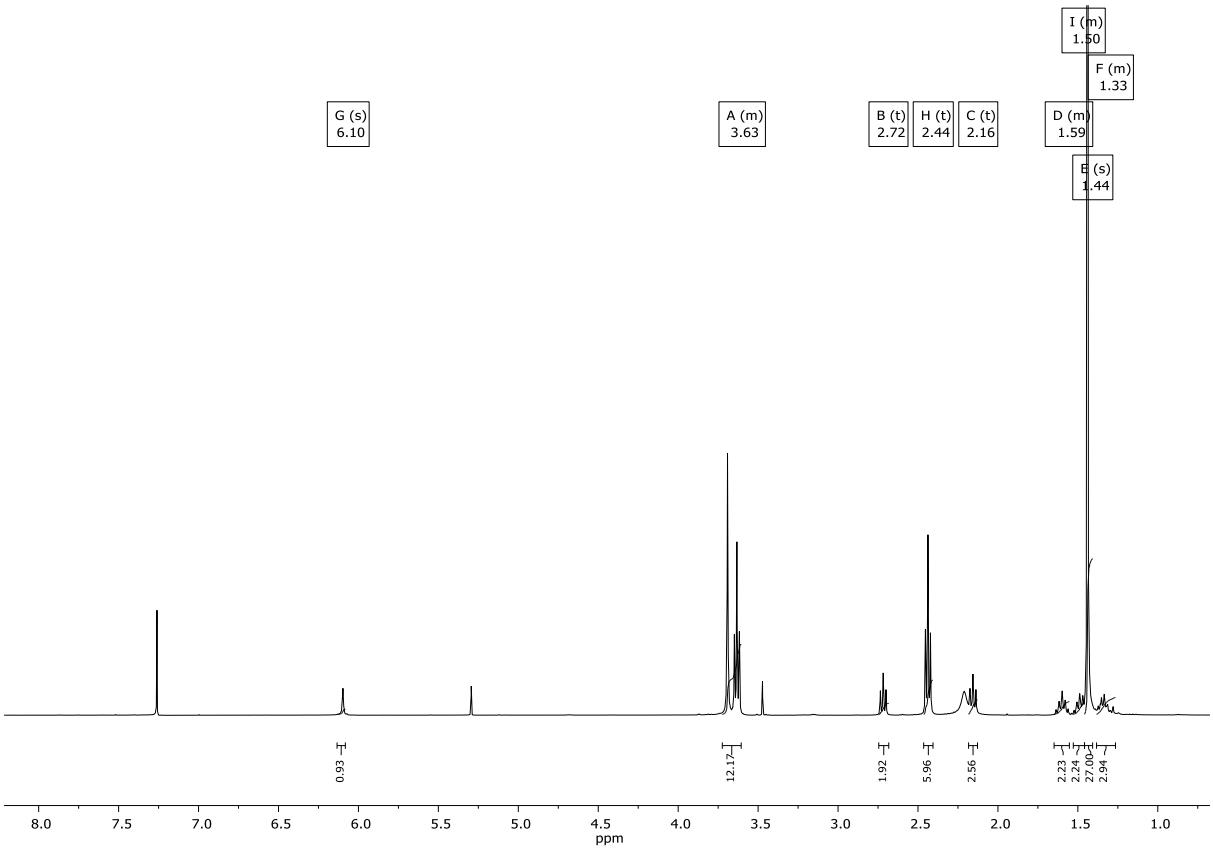



Figure S7. $^{1}$H NMR spectrum of compound **10** in $CDCl_3$.

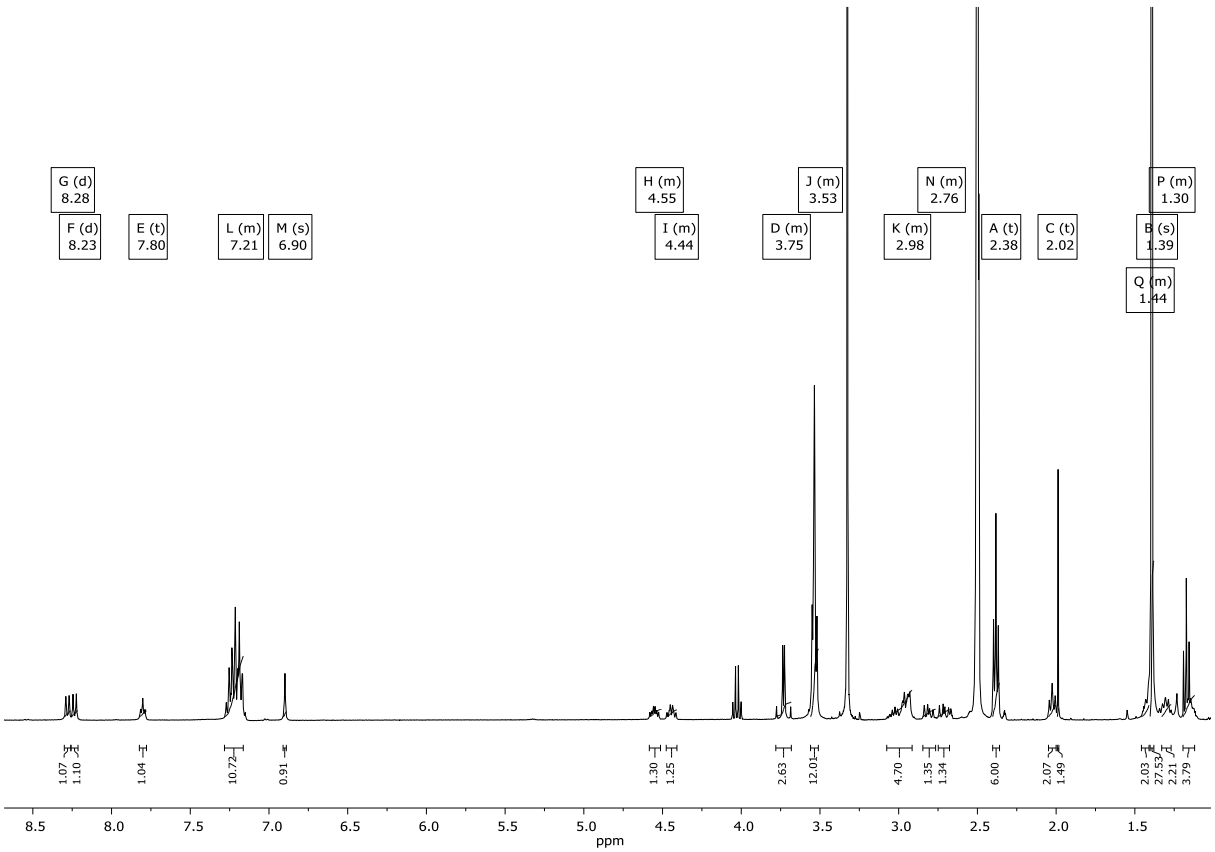



Figure S8. $^{1}$H NMR spectrum of compound **15** in DMSO-$d_6$.

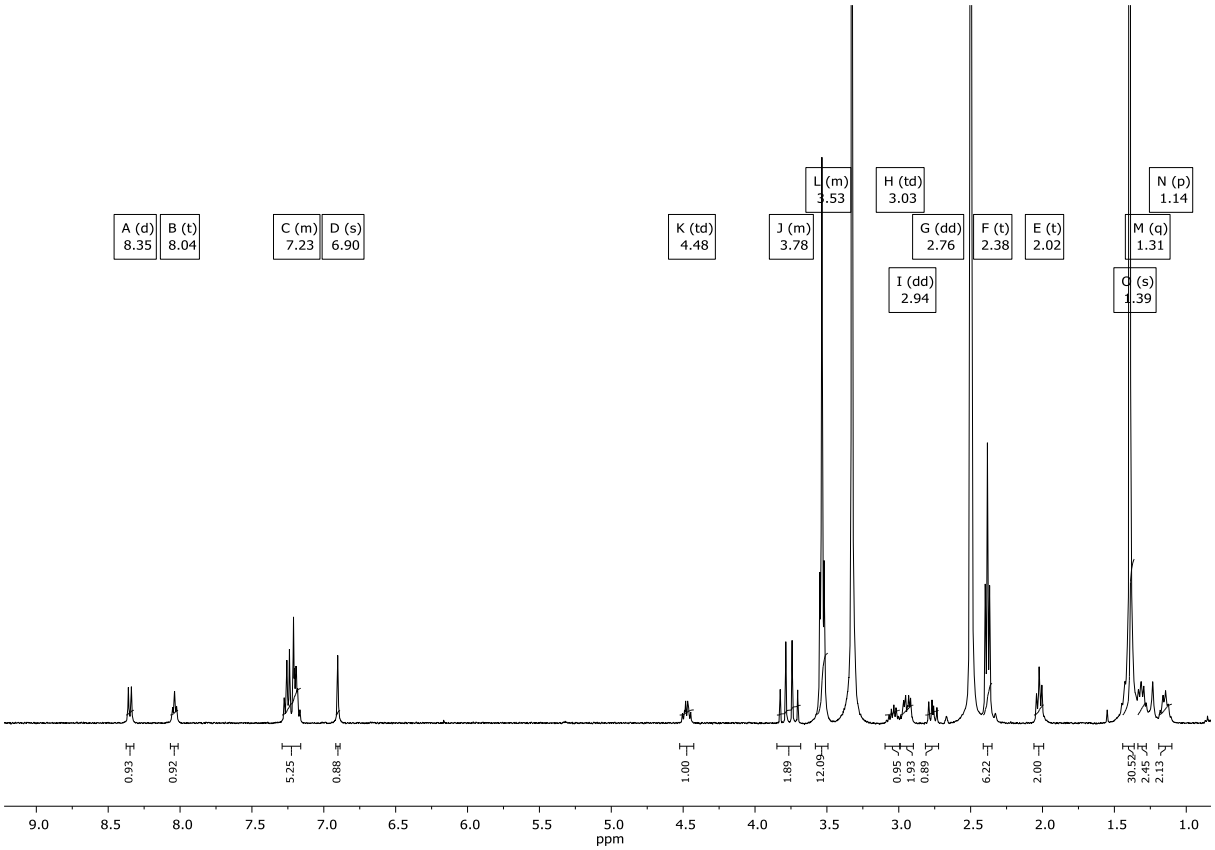



Figure S9. $^{1}$H NMR spectrum of compound **14** in DMSO-$d_6$.

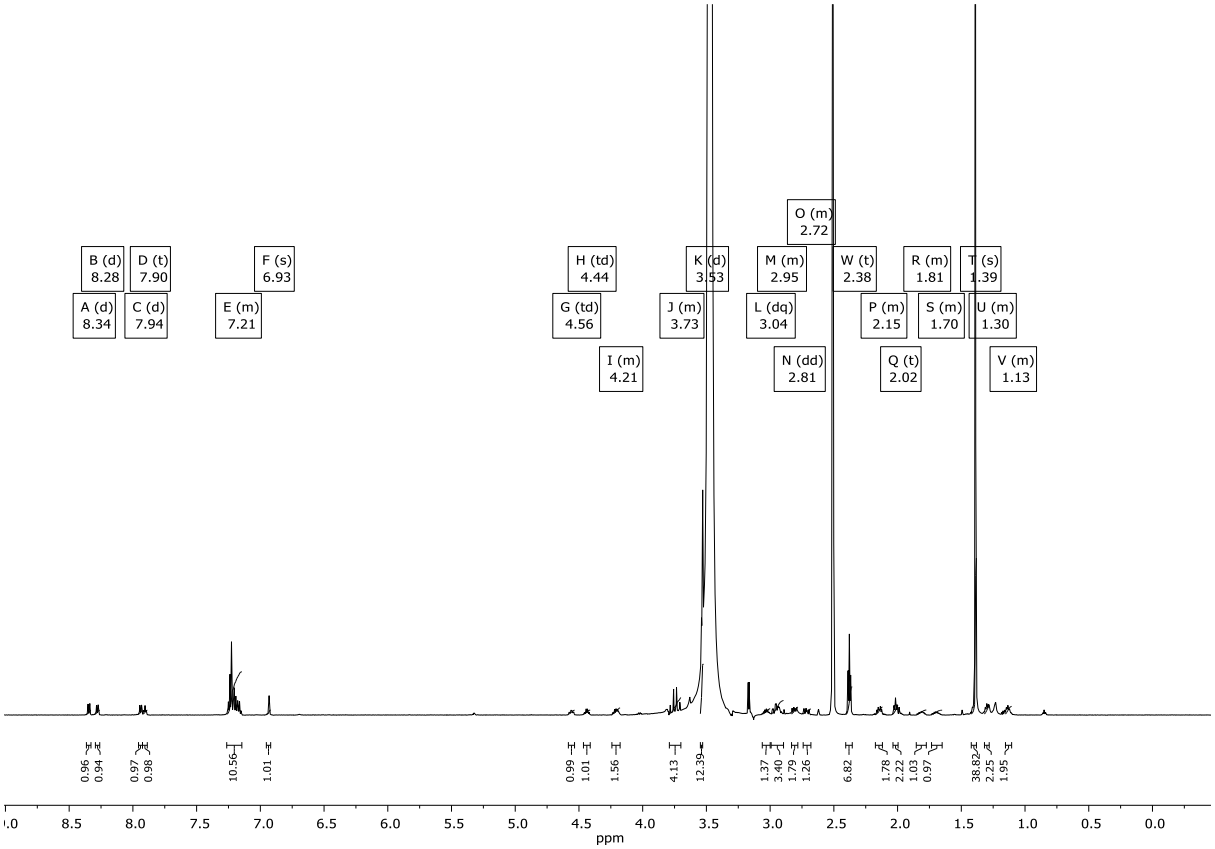



Figure S10. $^{1}$H NMR spectrum of compound **16** in DMSO-$d_6$.

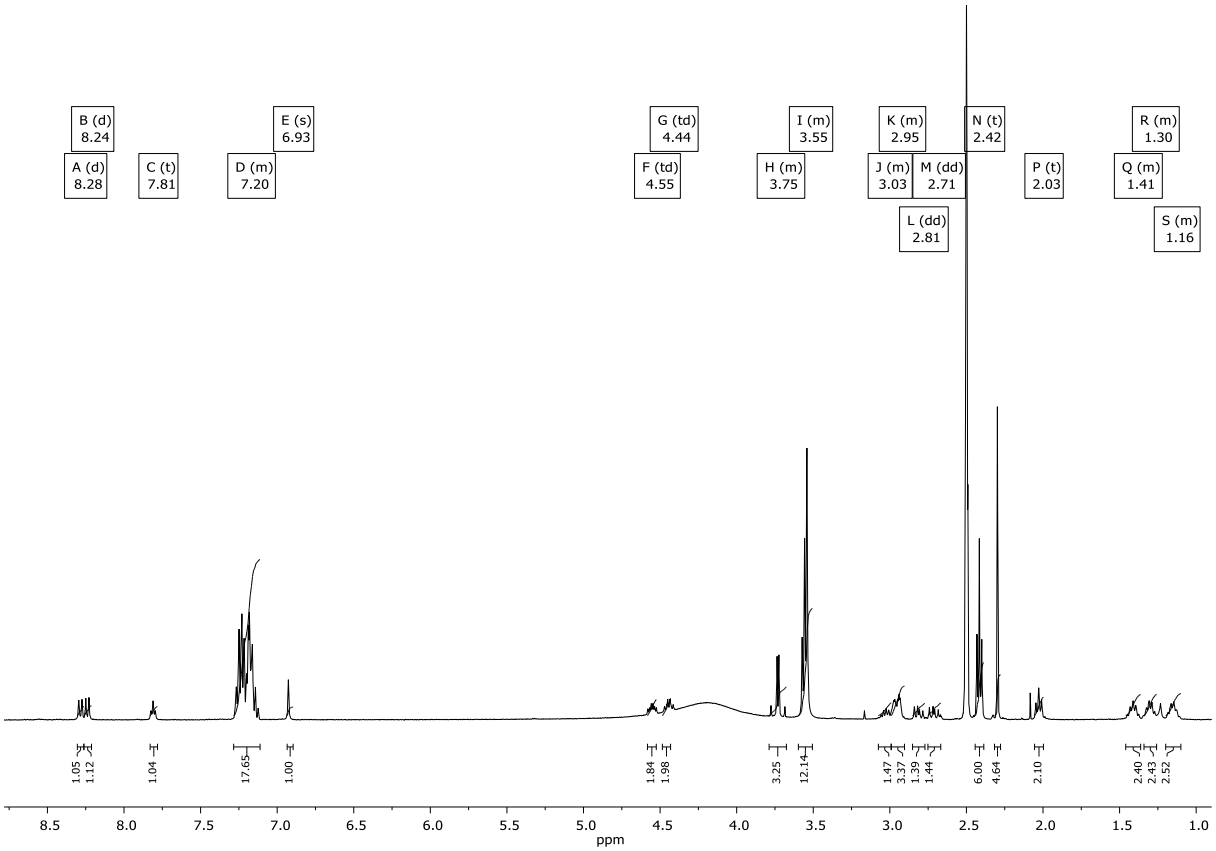



Figure S11. $^{1}$H NMR spectrum of compound **12** in DMSO-$d_6$.

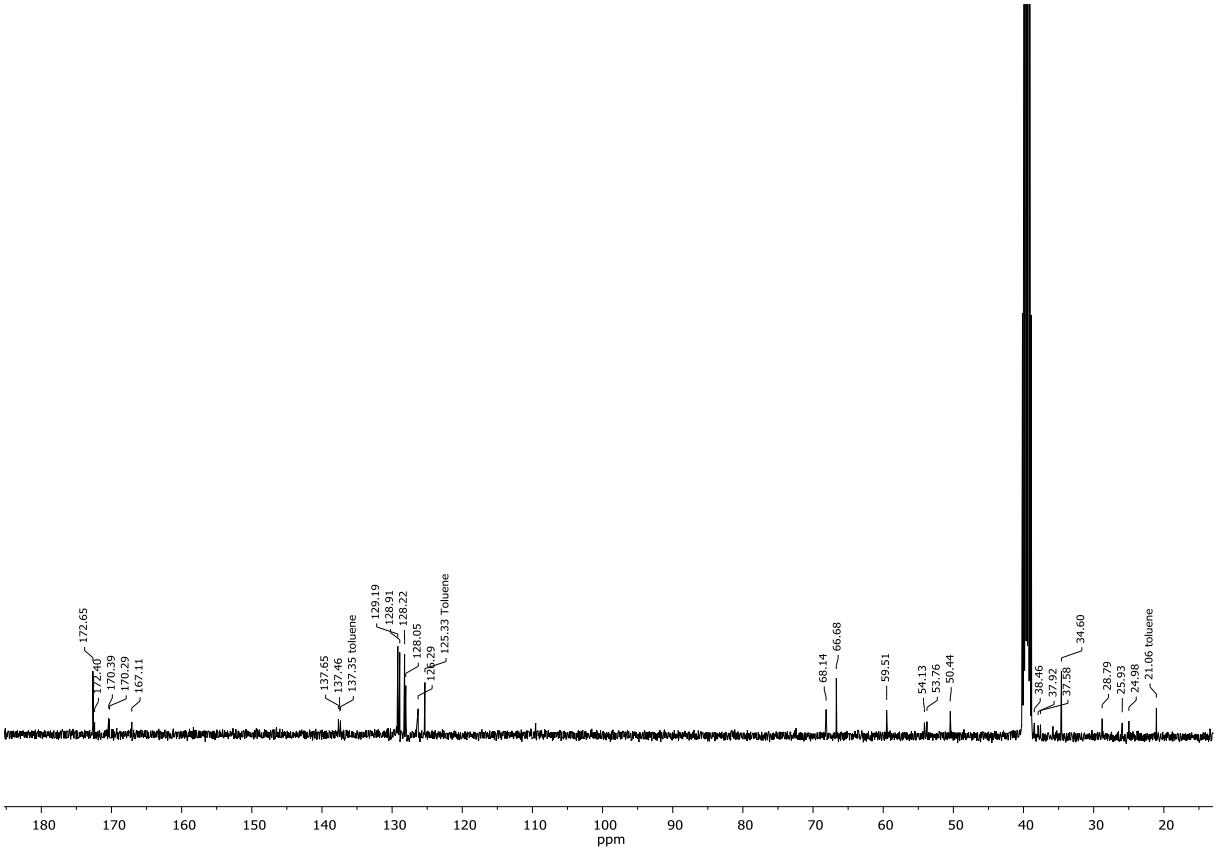



Figure S12. $^{13}$C NMR spectrum of compound **12** in DMSO-$d_6$.

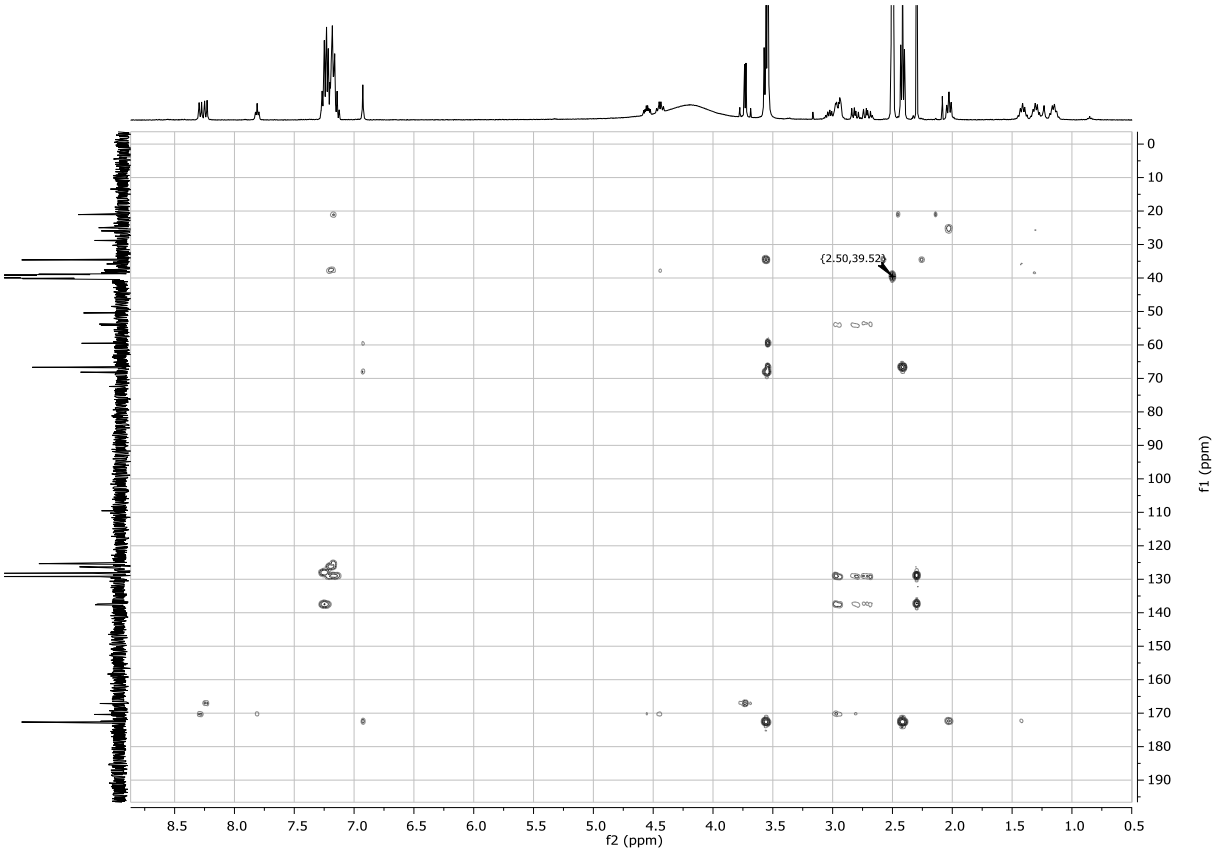



Figure S13. 2D-HMBC NMR spectrum of compound **12** in DMSO-$d_6$.

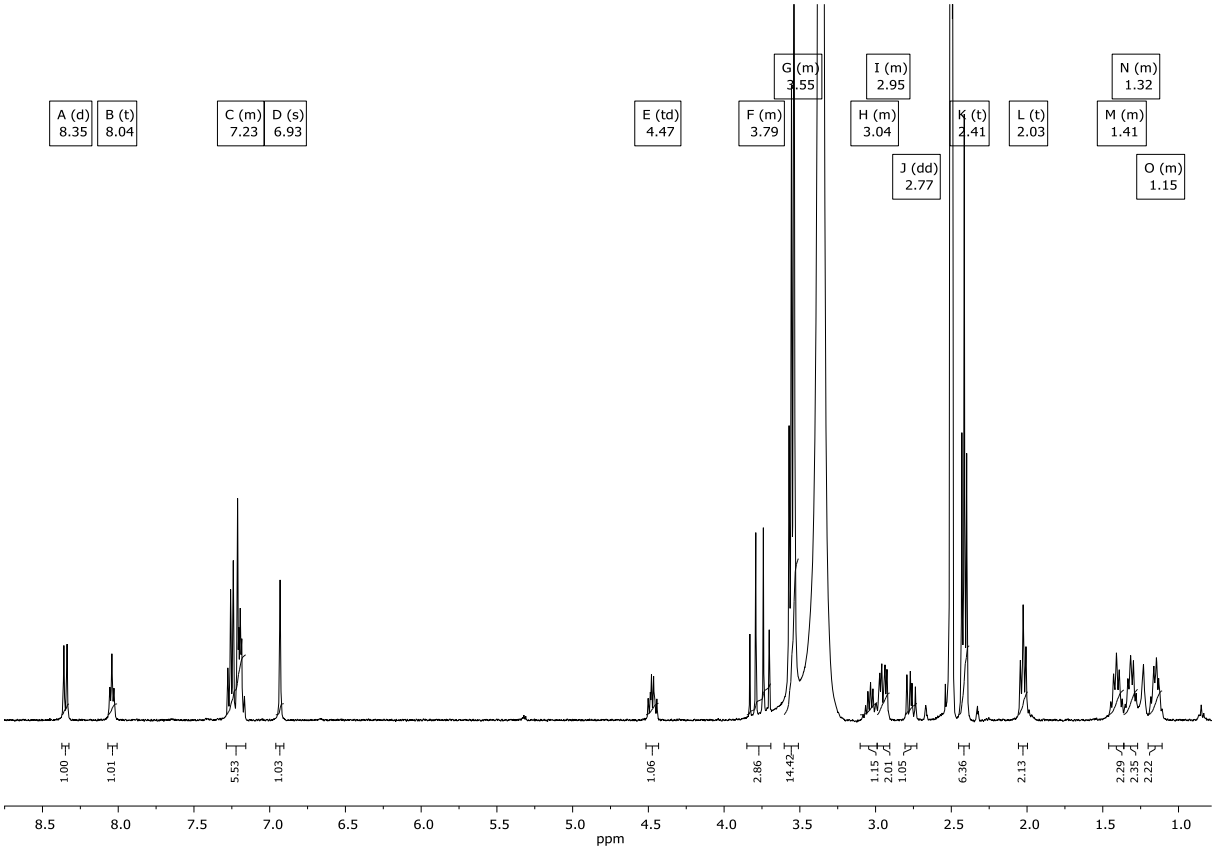



Figure S14. $^1$H NMR spectrum of compound **11** in DMSO-$d_6$.

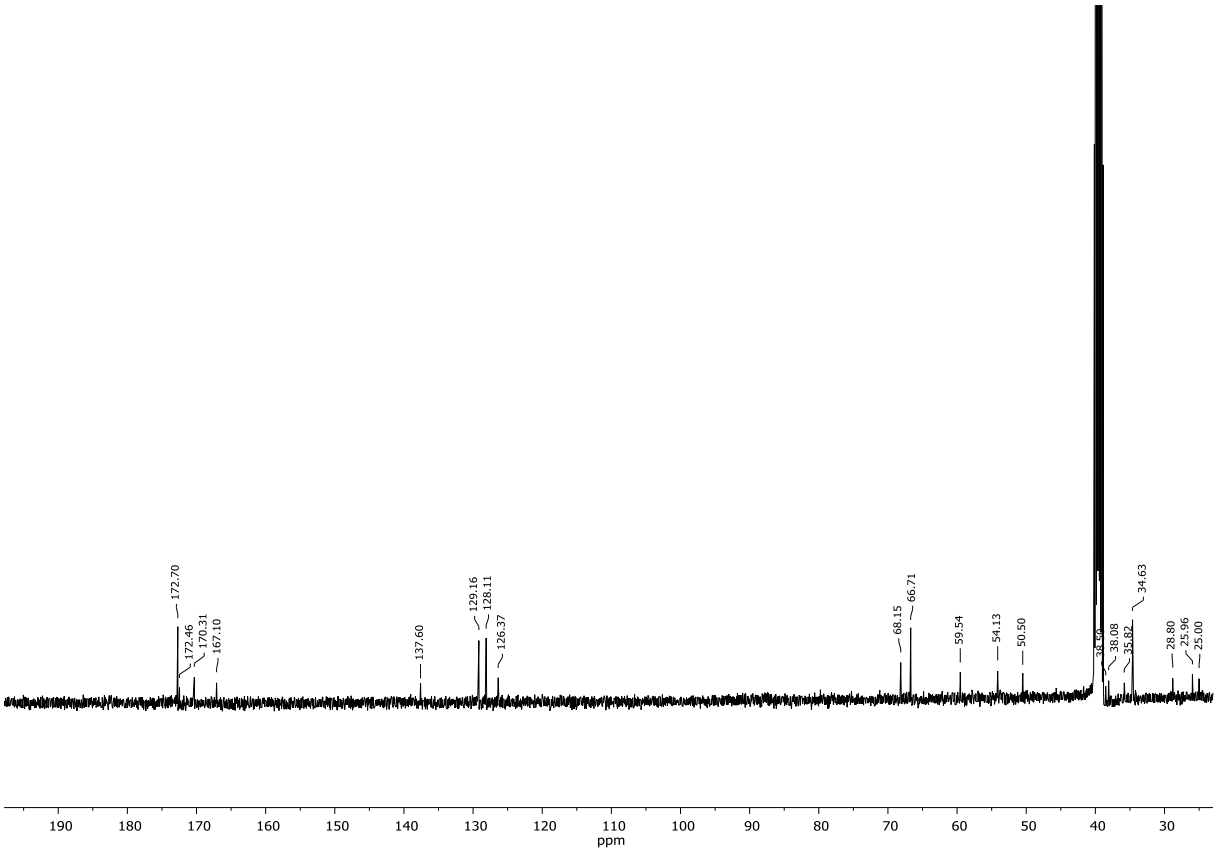



Figure S15. $^{13}C$ NMR spectrum of compound **11** in DMSO-$d_6$.

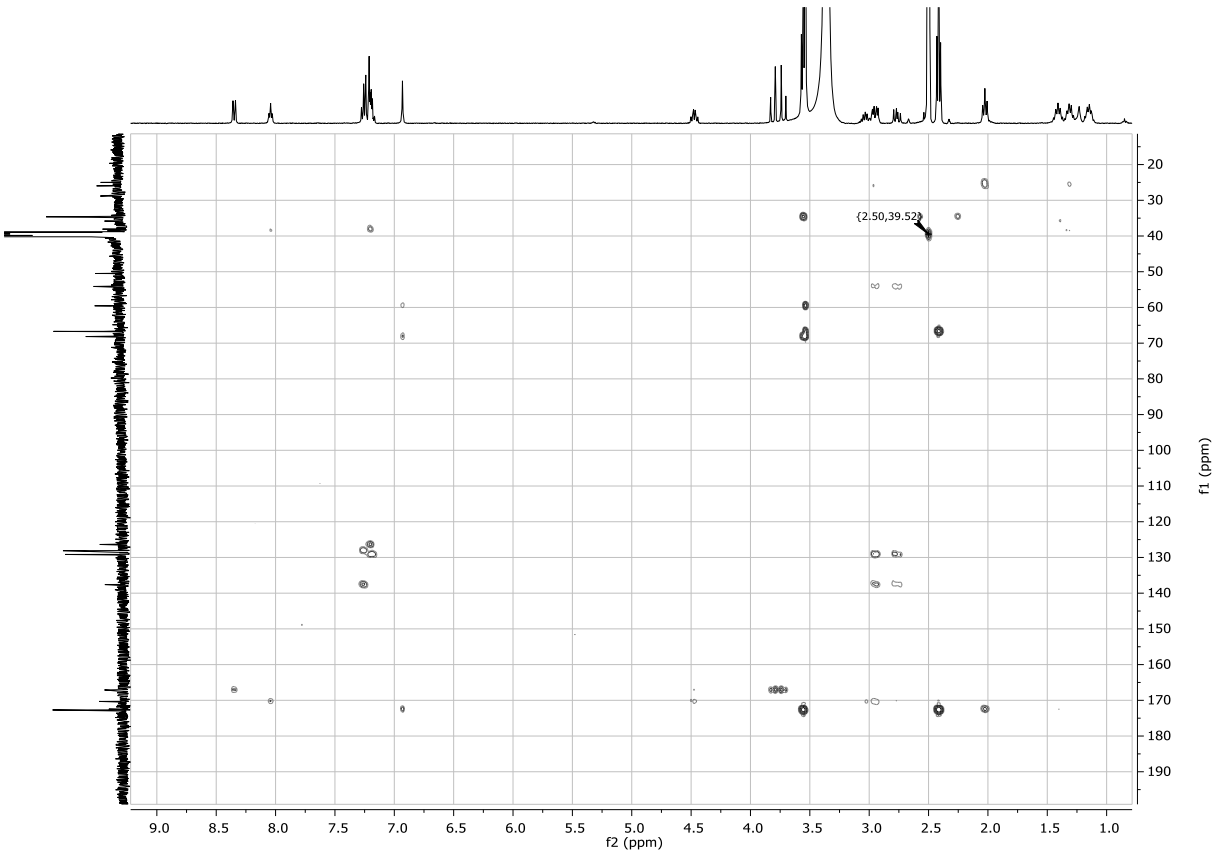



Figure S16. 2D-HMBC NMR spectrum of compound **11** in DMSO-$d_6$.

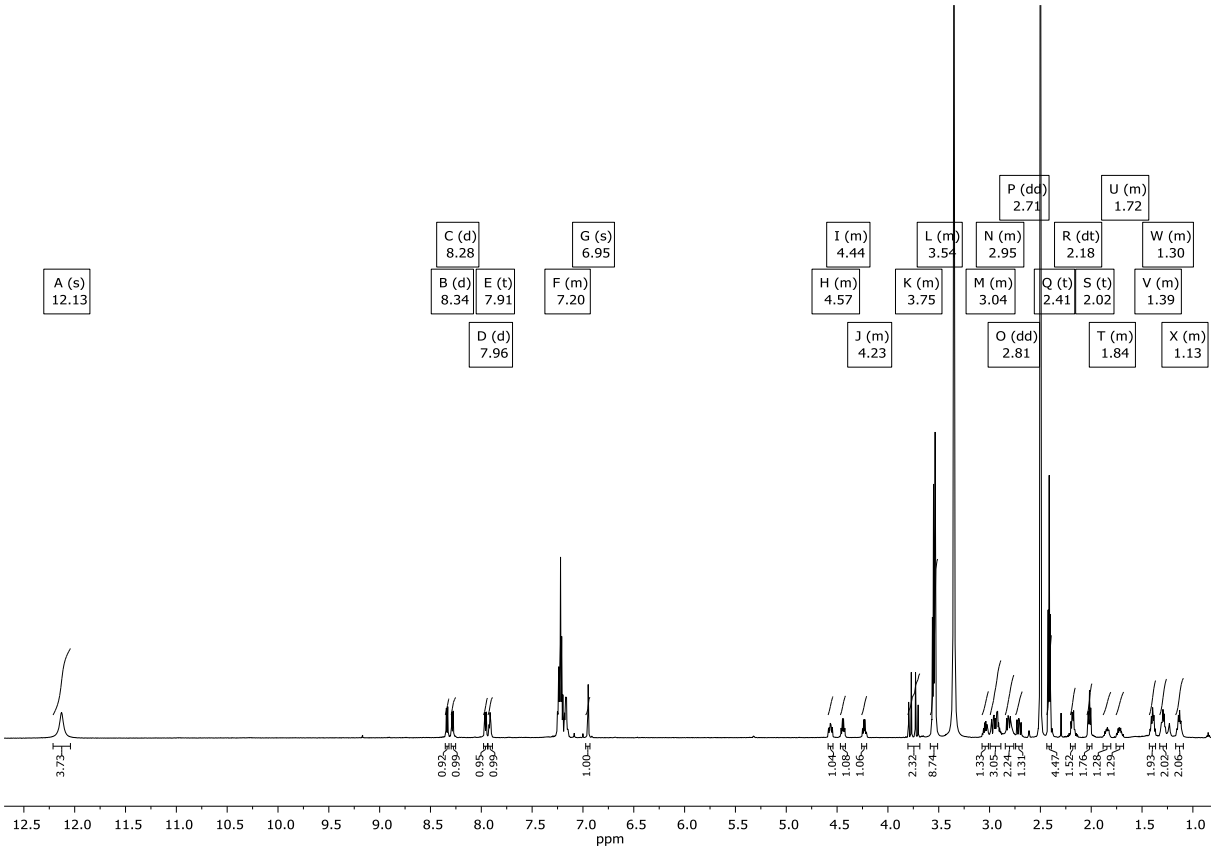



Figure S17. $^{1}$H NMR spectrum of compound **13** in DMSO-$d_6$.

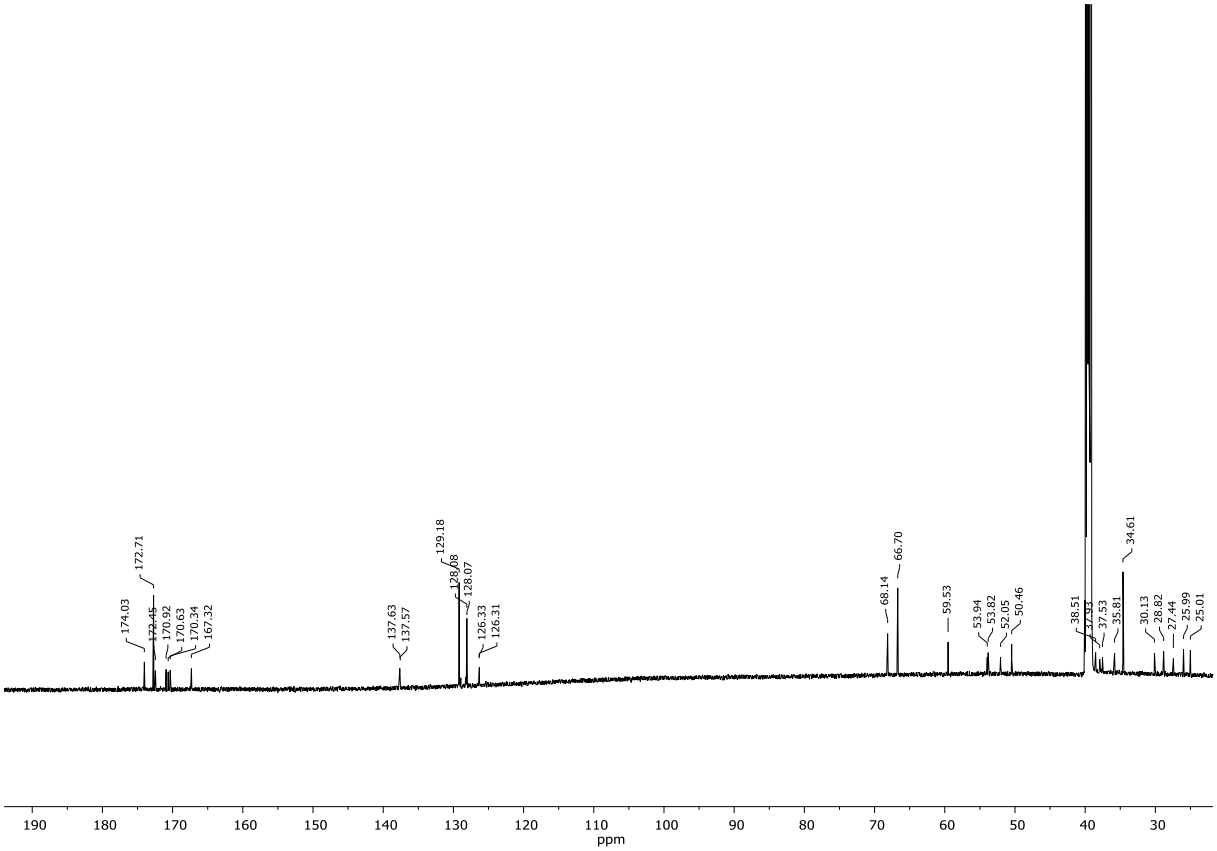



Figure S18. $^{13}$C NMR spectrum of compound **13** in DMSO-$d_6$.

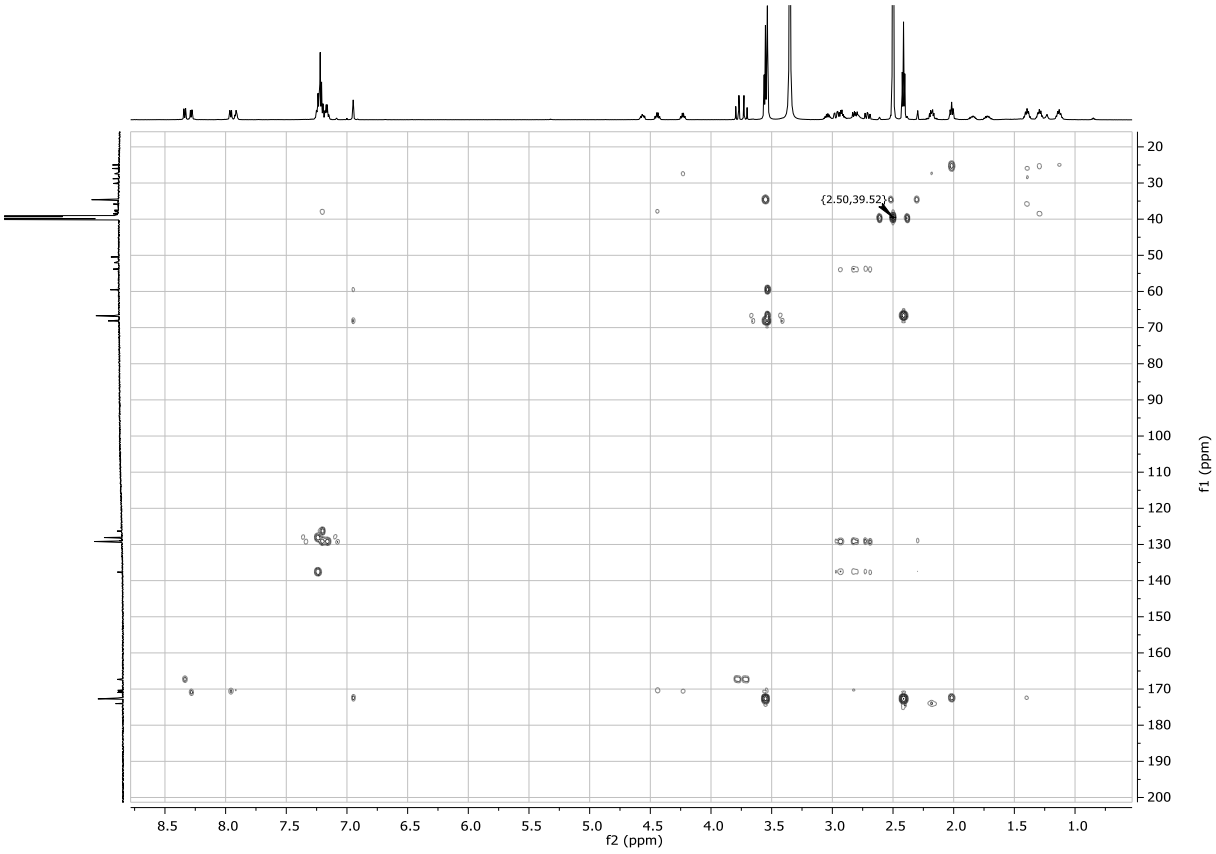



Figure S19. 2D-HMBC NMR spectrum of compound **13** in DMSO-$d_6$.

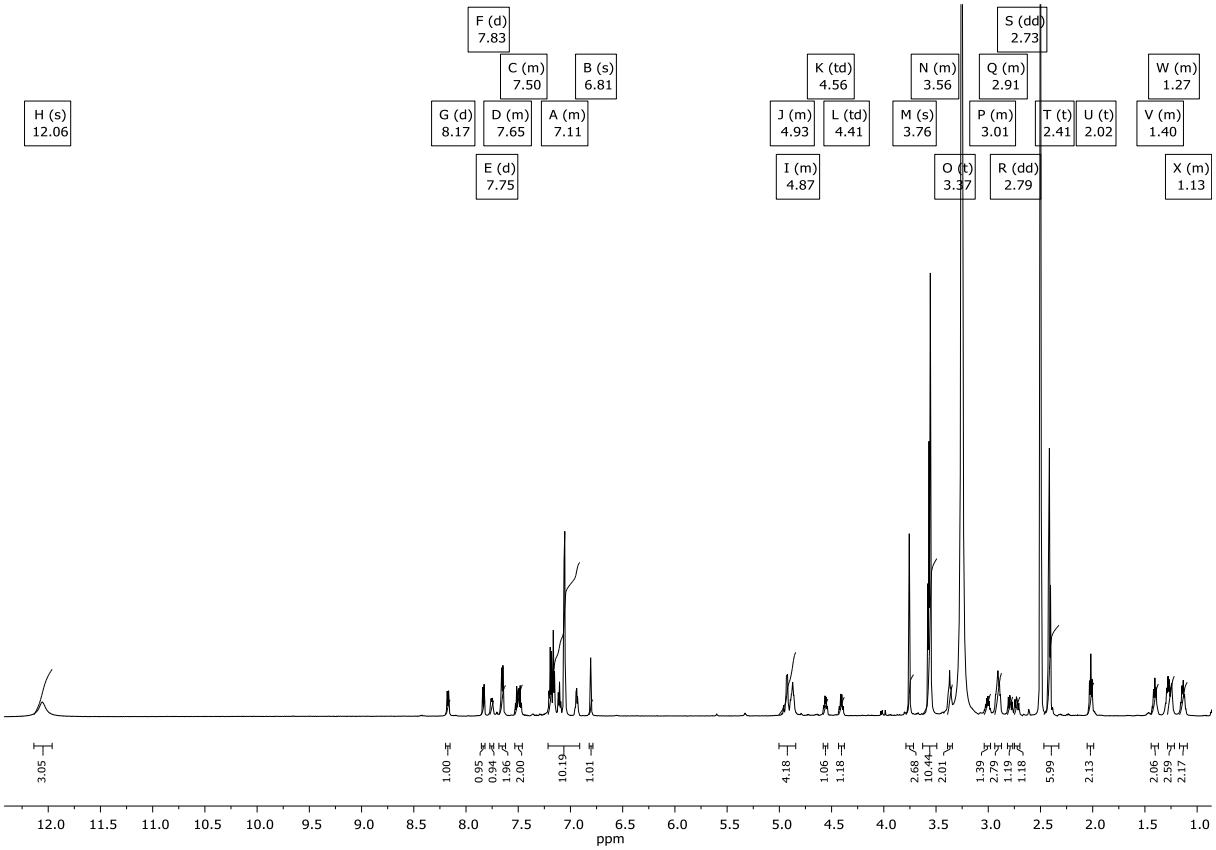



Figure S20. $^1$H NMR spectrum of compound **2** in DMSO-$d_6$.

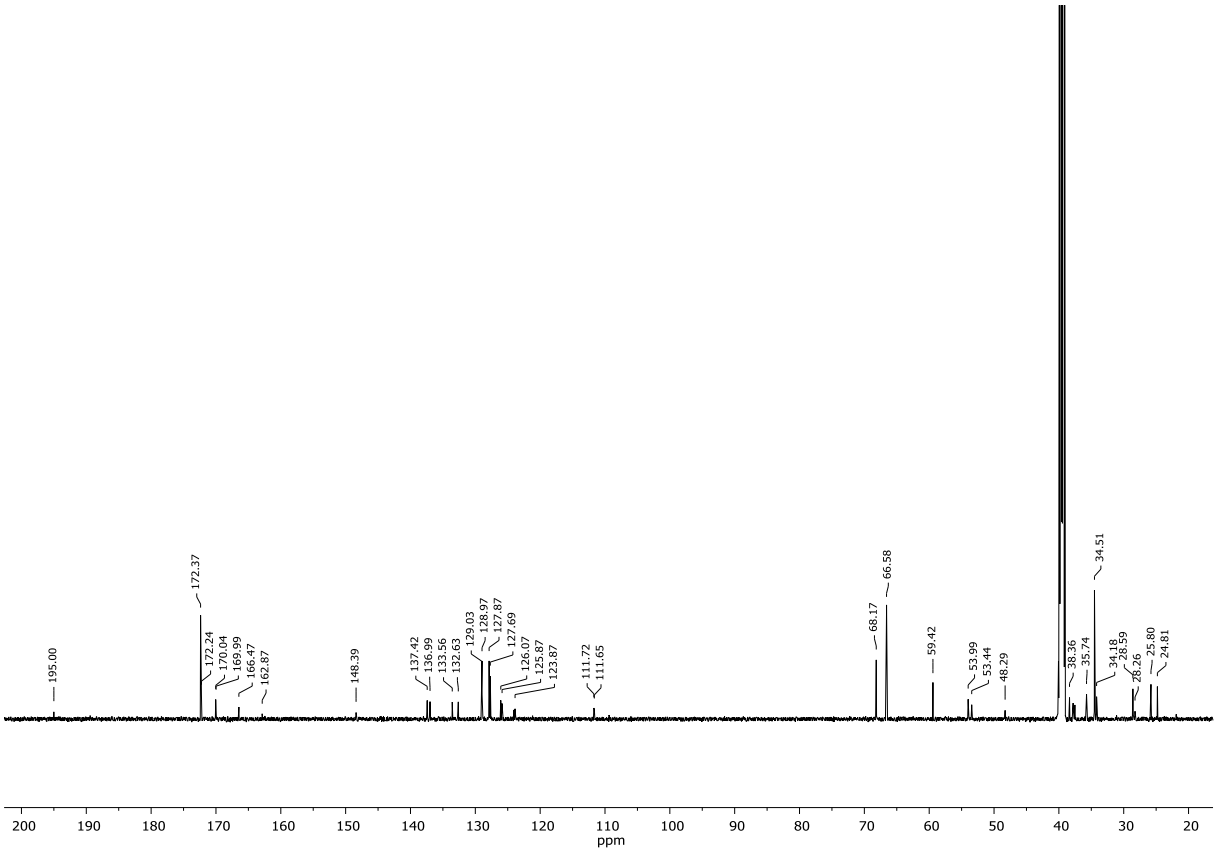



Figure S21. $^{13}C$ NMR spectrum of compound **2** in DMSO-$d_6$.

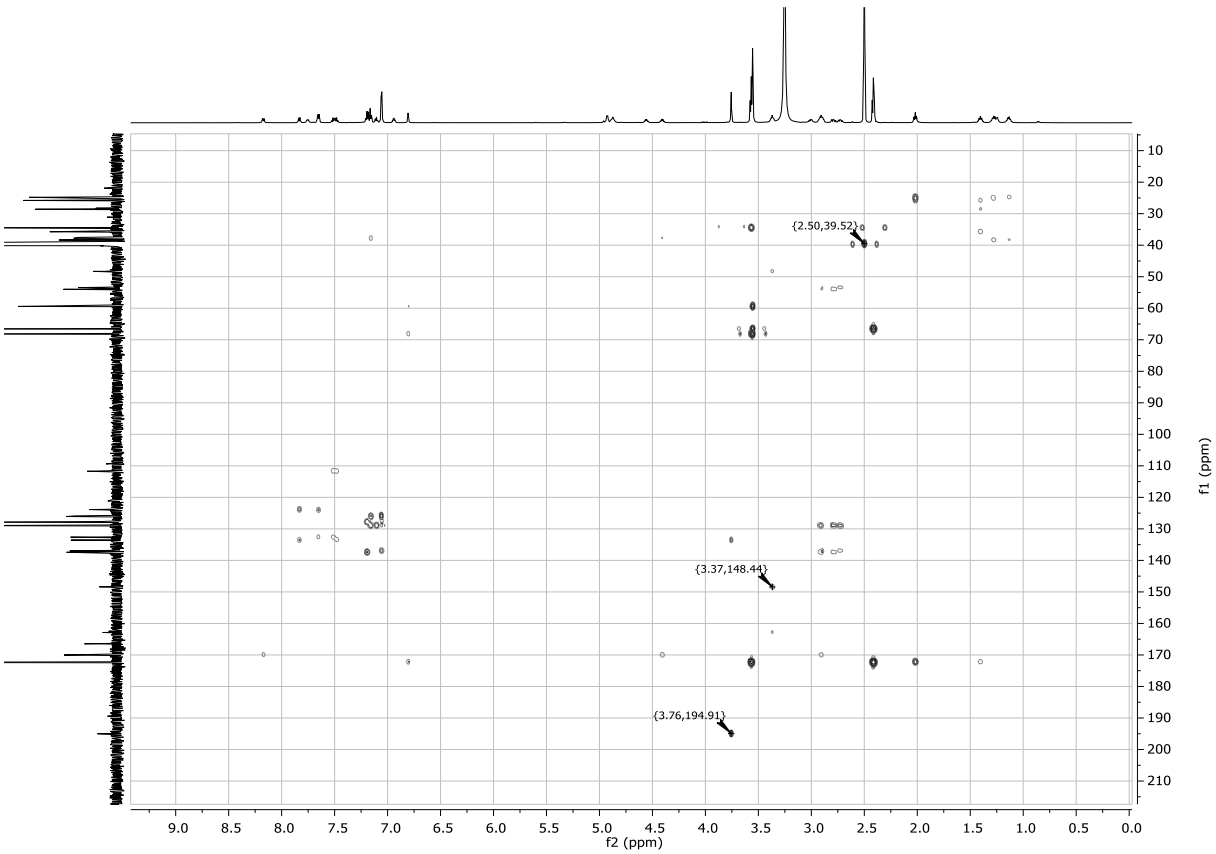



Figure S22. 2D-HMBC NMR spectrum of compound **2** in DMSO-$d_6$.

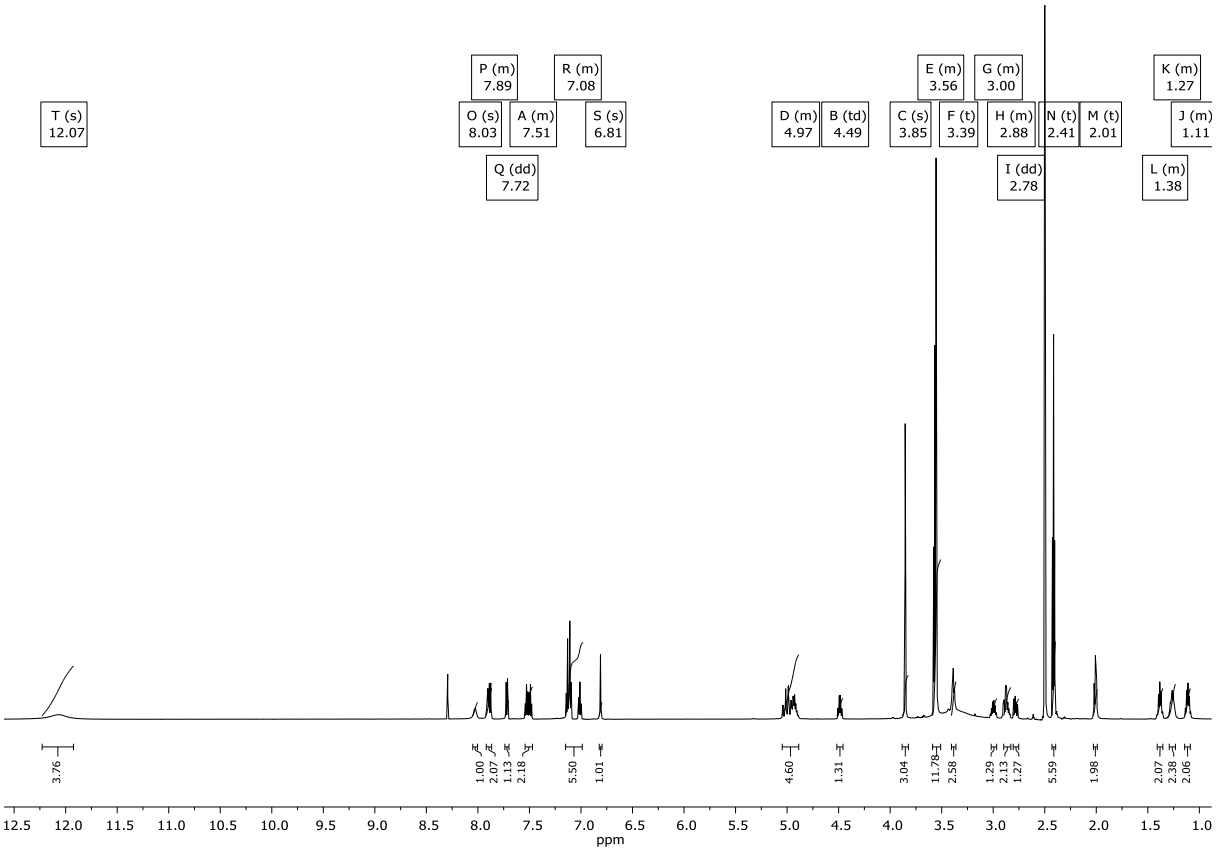



Figure S23. $^{1}$H NMR spectrum of compound **1** in DMSO-$d_6$.

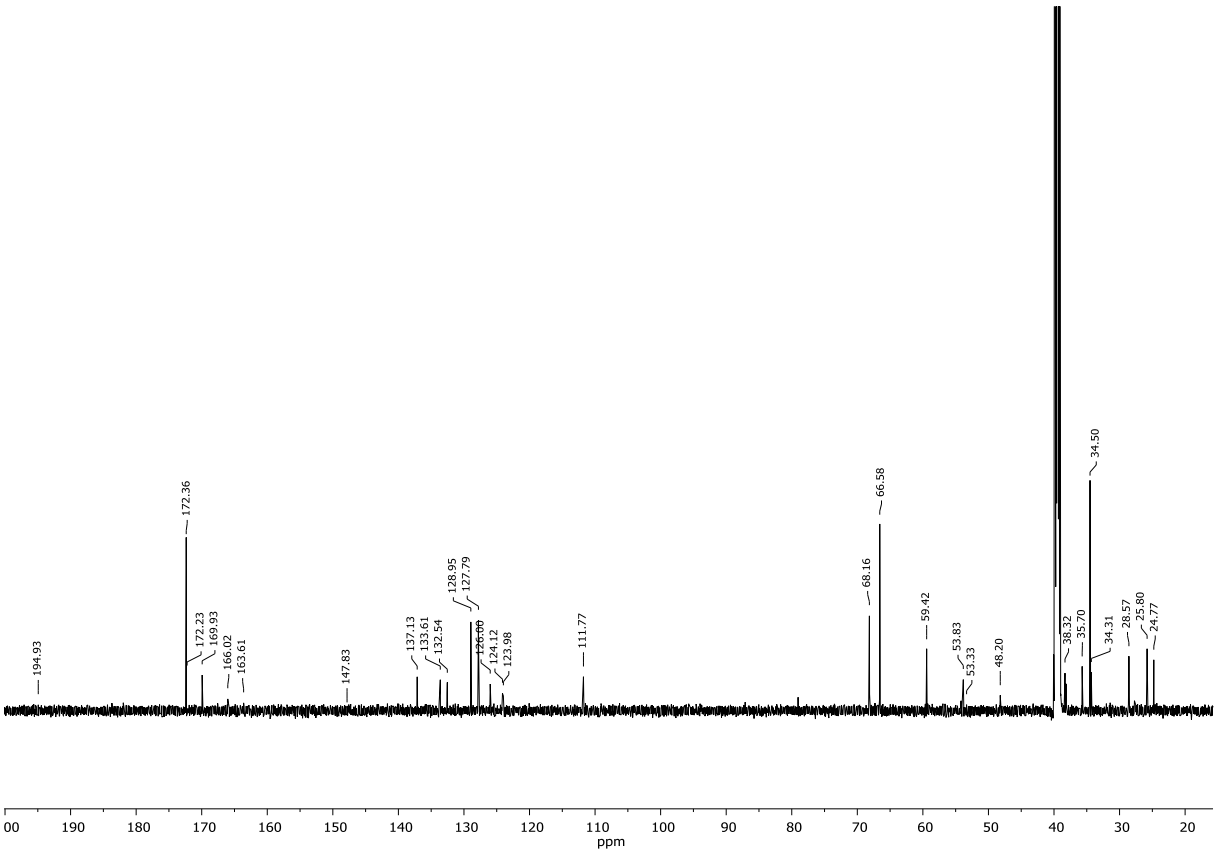



Figure S24. $^{13}$C NMR spectrum of compound **1** in DMSO-$d_6$.

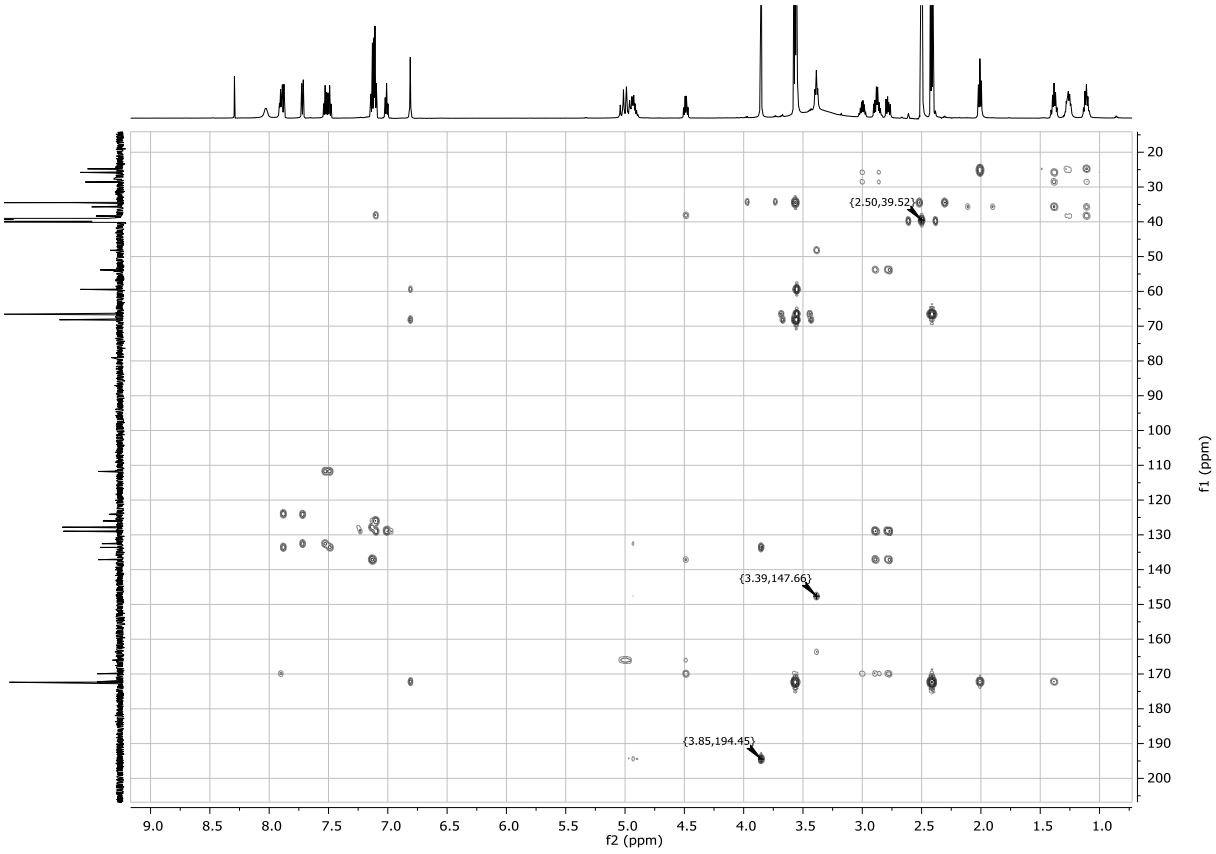



Figure S25. 2D-HMBC NMR spectrum of compound **1** in DMSO-$d_6$.

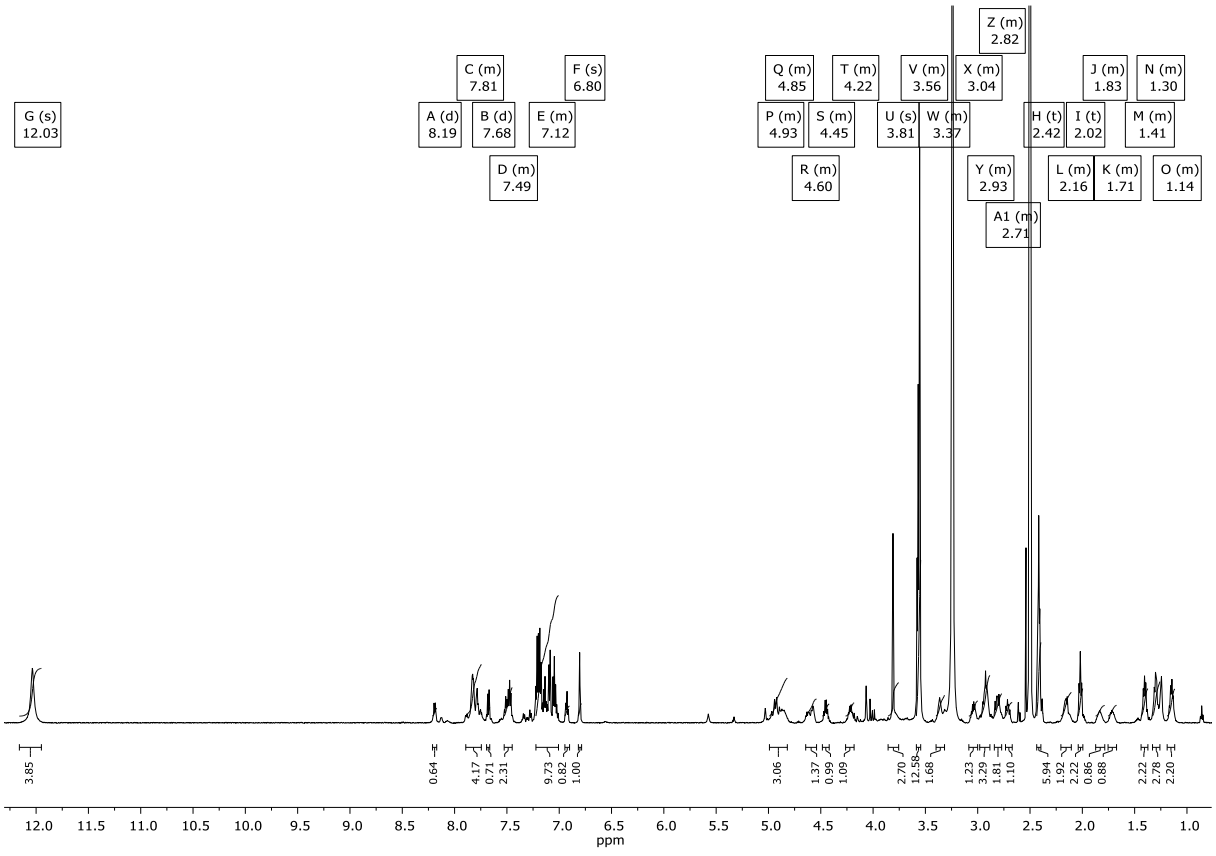



Figure S26. $^1$H NMR spectrum of compound **3** in DMSO-$d_6$.

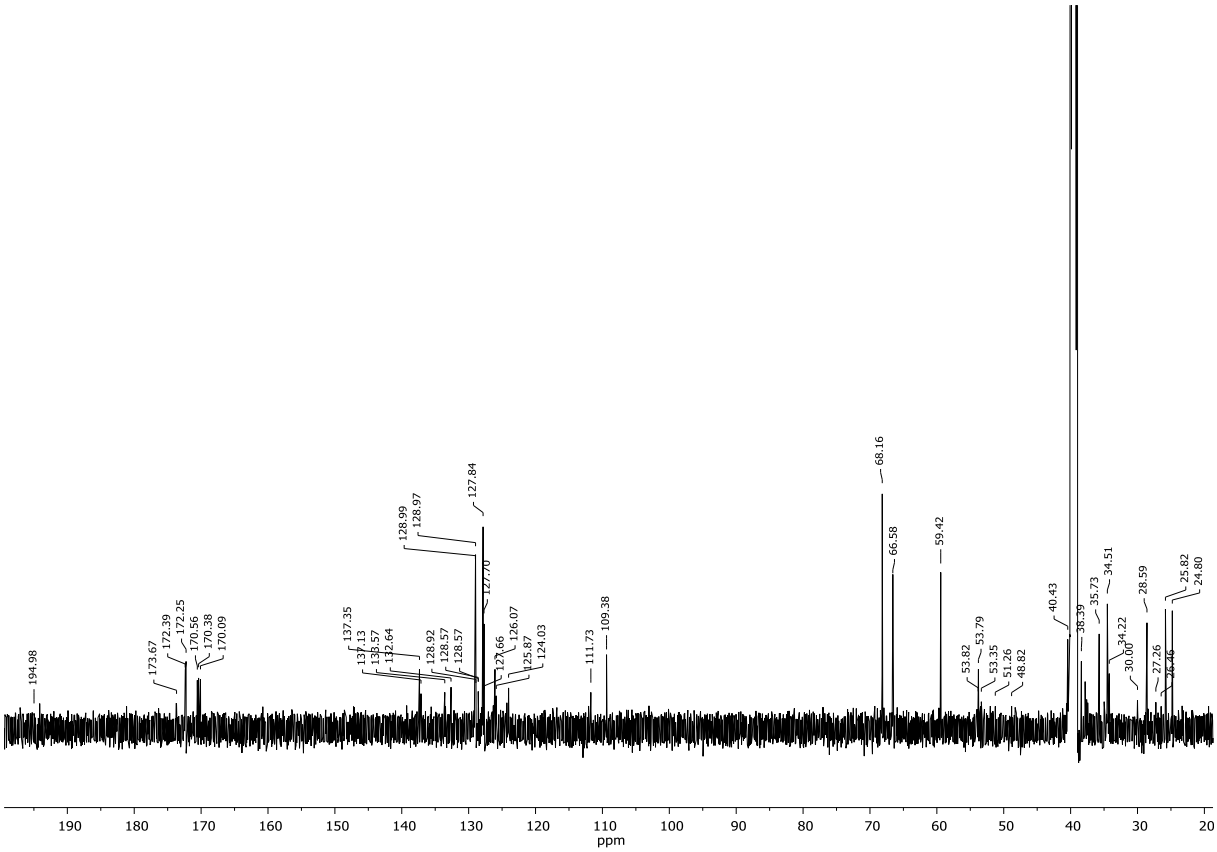



Figure S27. $^{13}C$ NMR spectrum of compound **3** in DMSO-$d_6$.

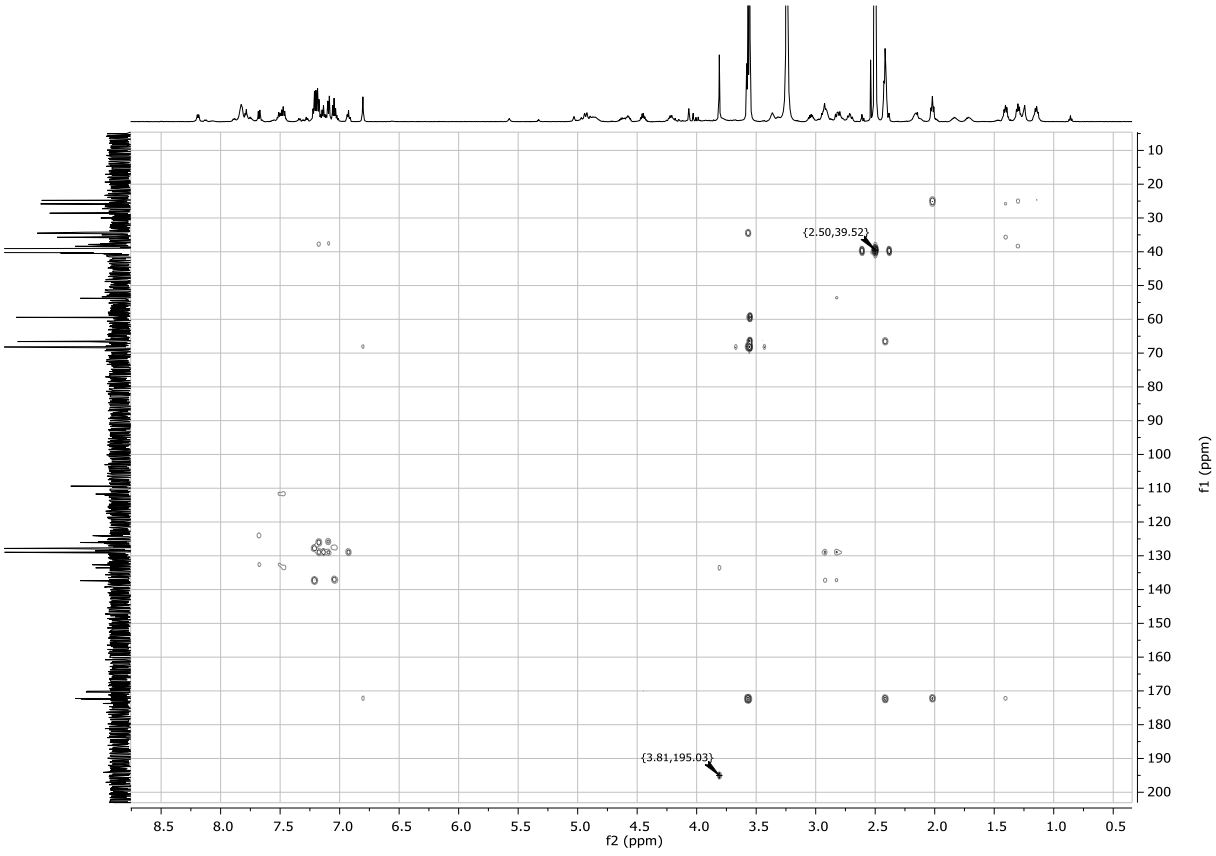



Figure S28. 2D-HMBC NMR spectrum of compound **3** in DMSO-$d_6$.

# 5 References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