Supplementary Information

Electrospinning of pyrazole-isothiazole derivatives: nanofibers from small molecules

Silvia Locarno,[a] Aitziber Eleta-Lopez,[b] Maria Giovanna Lupo,[c] Maria Luisa Gelmi,[a] Francesca Clerici*[a] and Alexander M. Bittner*[b,d]

[a] Department of Pharmaceutical Sciences, General and Organic Chemistry Section "A. Marchesini", University of Milan, Via Venezian 21, 20133 Milano Italy; [b] CIC nanoGUNE, Tolosa Hiribidea 76, 20018 Donostia-San Sebastián, Spain; [c] Department of Pharmaceutical and Pharmacological Sciences, Via Marzolo 5, 35131 Padua, Italy; [d] Ikerbasque, Basque Foundation for Science, Ma Díaz de Haro 3, E-48013 Bilbao, Spain.

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Experimental

General Information

All chemicals were purchased from Sigma-Aldrich, Iris Biotech or Fluka. All solvents were of ACS grade or higher and were obtained from Sigma-Aldrich. Eagle's minimum essential medium (MEM) was purchased from Sigma, while trypsin-EDTA, penicillin, streptomycin, sodium pyruvate, non-essential amino acid solution, fetal calf serum (FCS), plates and Petri dishes were purchased from EuroClone. NMR spectra were recorded on Varian Gemini 300 using appropriate deuterated solvents. Mass spectra were acquired on Fisons MD800 spectrometer and electrospray ion trap on a Finnigan LCQ ADVANTAGE Thermo-spectrometer. FTIR spectra were recorded in KBr pellet in PerkinElmer FTIR spectrometer. We report peak positions rounded to 5 cm^{-1}. Melting points were measured on SMP3 apparatus (Stuart Scientific). Specific rotation: $[\alpha]_D$ were measured on PerkinElmer model 343 plus polarimeter. $[\alpha]_D$ values were given in 10^{-1} deg cm^2 g^{-1}. Raman spectra were recorded on Alpha300, WITec confocal Raman spectrometer in specular reflection. The sample was deposited on a clean silicon wafer and excited with a frequency-doubled Nd:YAG laser at 532 nm (ν_1/c=18797 cm^{-1}, green), focused by a 100x objective with a 0.9 NA lens. Raman emission was collected through the same optics.

Synthesis. Compound **4** was already known and was prepared according to the literature.[1]

Electrospinning. We prepared solutions of the compounds with concentrations of 30% wt/wt in hexafluoroisopropanol (HFIP), which required short shaking. They were electrospun either on the <5 μl scale with the microliter electrospinning technique, or on the 0,5 ml scale with a conventional setup (flow rate 0.5 ml/h). In the first case, the high voltage is applied to a vertically oriented platinum (Pt) wire of 0.25 mm of diameter, placed 15 cm above the collector. The solution is directly placed from a micropipette on the tip of the Pt wire, in this way it is possible to test the electrospinnability of substances using μg amounts. In the second case, the high voltage is applied to a steel needle connected to a standard syringe pump to control the flow rate of solution, placed at various distances above the collector. In all experiments, the collector was either an aluminium foil, or a glass slide, or a pre-cleaned silicon wafer. A positive voltage15 kV (HP030R, Applied Kilovolts) was applied at the tip; other values gave less satisfactory results. The setup is situated in a large chamber (>500 litres), where the temperature and the humidity are controlled with a hot air gun placed inside, in order to achieve values around 30% and 27°C, respectively. The chamber is fitted with a low suction vacuum in order to remove toxic gases.

Optical microscopy. Optical micrographs were acquired on BX50, Olympus equipped with a digital camera (D300, Nikon) with a magnification up to 1000X, depositing the fiber on a glass substrate.

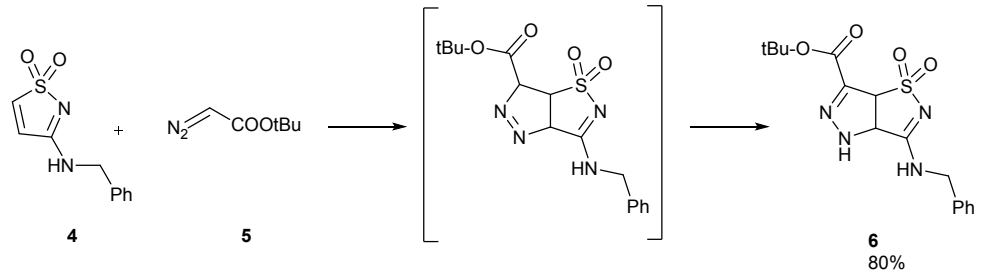
Scanning Electron Microscopy (SEM). SEM micrographs were acquired on QuantaTM 250FEG (FEI) in high vacuum (voltage 2-10 KV), depositing the fibers on clean silicon wafers. The beam current was limited by

selecting a small aperture, thus limiting beam damage. The average fiber diameter was obtained by using Image-J software.

Atomic Force Microscopy (AFM). AFM micrographs were acquired in air on AFM 5500 keysight in oscillating (AC) mode from samples deposited on clean silicon wafers. Silicon tips (with aluminum reflective back side, RTESPA from Bruker) were used with fundamental frequency of 175 kHz and a spring constant of 6 N/m. The scan speed was between 0.2-1.2 ln/s and the images have 512 x 512 points/lines. The data were processed with Gwyddion 2.3.

Silicon wafers (1 x 1 cm^2, Silicon Valley, SV) were cleaned by sonication with isopropanol, acetone and rinsed with Milli-Q water. Finally the substrates were treated with oxygen plasma (Diener electronics) for 8 minutes with an oxygen flow of 10 sccm and 98% of power and 0.1mbar. Then, the fibers were electrospun directly on the cleaned silicon substrates.

Synthetic Procedures



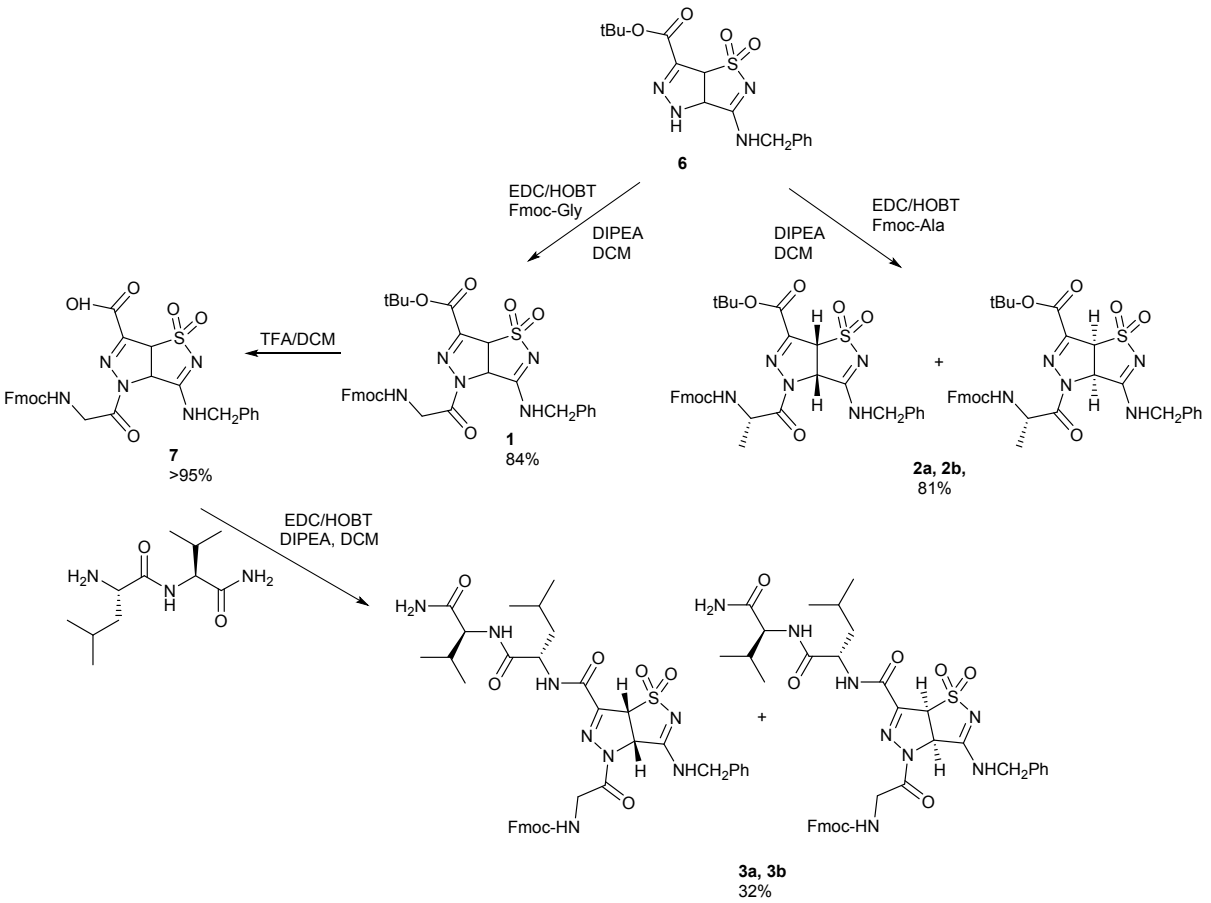
Scheme ESI1 Synthesis of compound **6**

Cycloaddition reaction

3-Benzylamino isothiazole 1,1 dioxide **4** (0,5 g, 2,2 mmol) is dissolved in tetrahydrofuran (5 ml/mmol) and heated to 65°C. Then, tButyldiazoacetate **5** (1 eq) was added and the mixture is refluxed for 48-72 hours. The reaction is monitored by TLC (ethyl acetate/hexane 8:2) and if it is necessary, an excess of diazoacetate is added. The solvent was removed under reduced pressure and the residue crystallized from methanol-ether to afford the pure product.

Tert-butyl 3-(benzylamino)-3a,6a-dihydro-4H-pyrazolo[3,4-d]isothiazole-6-carboxylate 1,1-dioxide (6) (0.64 g, 80%). White solid. mp 142-146°C. Elemental Analysis: Found: C, 52.6; H, 5.6; N, 15.2; Calcd. For $C_{16}H_{20}N_4O_4S$: C, 52.7; H, 5.5; N, 15.4; ν_{max}/cm^{-1} 3355 (NH), 1687 (CO), 1615. ^1H NMR (300 MHz, $(CD_3)_2CO$) δ 1.53 (9H, s, CH_3), 4.58 (2H, d, J=5.5 Hz, CH_2), 5.00 (1H, d, J=12 Hz, CH-6a), 5.80 (1H, dd, J=12 Hz, CH-3a), 7.23-7.42 (5H, m, ArylH), 8.23 (1H, br s, NH), 8.37 (1H, br s NH). ^{13}C NMR (300 MHz, $(CD_3)_2CO$) δ 27.60 (CH_3), 47.35 (CH_2), 67.82, 70.90 (CH-3a + CH-6a), 81.22 (C) 127.85, 128.14, 128.82 (ArCH), 136.94, 137.31 (C-6 + ArC), 160.21 (C-3), 164.43 (CO). m/z 363.27 $[M-H]^-$

Coupling Reactions



Scheme ESI2 Synthetic scheme of compounds **1**, **2a**/**2a** and **3a**/**3b.** The absolute configuration of the diastereoisomers **2a/2b** and **3a**/**3b** are arbitrarily assigned.

3aS*, 6aR*- Tert-butyl 4-((((9H-fluoren-9-yl)methoxy)carbonyl)glycyl)-3-(benzylamino)-3a,6a-dihydro-4H-pyrazolo[3,4-d]isothiazole-6-carboxylate 1,1-dioxide (1). Fmoc-Gly-OH (0.1 g, 0.33 mmol) is dissolved in dichloromethane (DCM, 10 ml/mmol) under nitrogen. The mixture is cooled at 0 °C before adding 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 1.1 eq) and hydroxybenzotriazole (HOBt, 1.1 eq) and is stirred for 1 hour. Then, compound **6** (0.13 g, 0,36 mmol) and N,N-diisopropylethylamine (DIPEA) are added until pH=7-8. The mixture is allowed to react overnight at rt. The solvent is removed under reduced pressure. The residue is dissolved in DCM and the organic phase is washed with 5% w/v aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine. The organic layer is dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The reaction is monitored by TLC (ethyl acetate/hexane 2:1) and the pure product is afforded by crystallization from methanol-ether.

1 (0.18 g, 84%). White solid. mp 164-169°C. Elemental Analysis: Found: C, 61.8; H, 5.1; N, 10.7; Calcd. For $C_{33}H_{33}N_5O_7S$: C, 61.6; H, 5.2; N, 10.9; ν_{max}/cm^{-1} 3412, 3319 (NH), 1727, 1683, 1622 (CO). ^1H NMR (300 MHz, $CDCl_3$) δ 1.61 (9H, s, CH_3), 4.22 (1H, t, J=6.7 Hz, CH), 4.34-4.66 (6H, m, CH_2Ph + $CH_{2(G)}$ + CH2), 5.15 (1H, d, J=10.9

Hz, CH-6a), 5.26 (1H, m, NH), 5.88 (1H, d, J=10.9, CH-3a), 7.23-7.43 (9H, m, ArylH), 7.57-7.66 (3H, m, ArylH + NH), 7.76 (2H, d, J=7.5 Hz, ArylH). ^{13}C NMR (300 MHz, $CDCl_3$) δ 27.88 (CH_3), 43.77 (CH_2), 47.08 (CH), 48.29 (CH_2Ph), 66.92, 67.07 (CH-3a + CH-6a), 67.30 ($CH_{2(G)}$), 85.39 (C), 120.04, 125.05, 127.10, 127.78, 127.92, 128.33, 129.04 (ArCH), 135.32, 141.30, 143.70 (ArC), 145.67 (C-6), 156.42 (COO), 157.67 (COO), 160.43 (C-3), 171.13 (CO). m/z 642.86 $[M-H]^-$, 666.13 $[M+Na]^+$

(3aS*,6aR*)-Tert-butyl 4-((((9H-fluoren-9-yl)methoxy)carbonyl)-(S)-alanyl)-3-(benzylamino)-3a,6a-dihydro-4H-pyrazolo[3,4-d]isothiazole-6-carboxylate 1,1-dioxide (2a and 2b). Fmoc-L-Ala-OH (0.1 g, 0.32 mmol) is dissolved in DCM (10 ml/mmol) under nitrogen. The mixture is cooled at 0 °C before adding EDC (1.1 eq) and HOBt (1.1 eq) and is stirred for 1 hour. Then, compound **6** (0.1 g, 0,35 mmol) and DIPEA are added until pH=7-8. The mixture is allowed to react overnight at rt. The solvent is removed under reduced pressure. The residue is dissolved in DCM and the organic phase is washed with 5% w/v aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine. The organic layer is dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The reaction is monitored by TLC (ethyl acetate/hexane 2:1) and the two diasteroisomers were separated by silica gel column chromatography (hexane/ethyl acetate 2:1).

2a (0.09 g, 44%). White solid. Rf 0.3 in AcOEt. mp 112°C. $[\alpha]_D$ = -60 (0.002 g/ml in CH_3OH). Elemental Analysis: Found: C, 61.8; H, 5.3; N, 10.3; Calcd. For $C_{34}H_{35}N_5O_7S$: C, 62.1; H, 5.4; N, 10.6; ν_{max} /cm^{-1} 3332 (NH), 1720, 1682, 1624 (CO). ^{1}H NMR (300 MHz, $CDCl^3$) δ 1.42 (3H, d, J=6.3 Hz, CH_3), 1.63 (9H, s, CH_3), 4.21 (1H, t, J=6.3 Hz, CH), 4.39 (2H, d, J=6.2 Hz, CH_2), 4.56-4.61 (2H, m, CH_2Ph), 5.07 (1H, t, J=6.6, CH_α), 5.17 (1H, d, J=9.0, CH-6a), 5.30 (1H, d, J=6.9, NH), 5.95 (1H, d, J=8.9 Hz, CH-3a), 7.24-7.43 (9H, m, ArylH), 7.58 (2H, m, ArylH), 7.72 (1H, s, NH), 7.77 (2H, d, J=7.5, ArylH). ^{13}C NMR (300 MHz, $CDCl_3$) δ 17.91 (CH_3) 27.87 (CH_3), 47.08 (CH), 48.11 (CH_2Ph), 48.96 (CH_α), 66.90 (CH-3a + CH-6a), 67.22 (CH_2), 85.29 (C), 120.01, 124.99, 127.09, 127.71, 127.77, 128.33, 128.62, 129.03 (ArCH), 135.57, 141.30, 143.70 (ArC), 145.86 (C-6), 155.81 (COO), 157.54 (COO), 160.73 (C-3), 175.12 (CO). m/z 680.32 $[M+Na]^+$

2b (0.078 g, 37%). White solid. Rf 0.19 in AcOEt. mp 191°C. $[\alpha]_D$ = +62 (0.002 g/ml in CH_3OH). Elemental Analysis: Found: C, 61.7; H, 5.2; N, 10.2; Calcd. For $C_{34}H_{35}N_5O_7S$: C, 62.1; H, 5.4; N, 10.6; ν_{max}/cm^{-1} 3393, 3319 (NH), 1747, 1720-1684, 1627 (CO). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (3H, d, J=6.3 Hz, CH_3), 1.63 (9H, s, CH_3), 4.21 (1H, m, CH), 4.40 (2H, m, CH_2), 4.55 (2H, m, CH_2Ph), 5.12 (1H, m, CH_α), 5.20 (1H, d, J=11 Hz, CH-6a), 5.31 (1H, m, NH), 5.92 (1H, d, J=11 Hz, CH-3a), 7.25-7.46 (9H, m, ArylH), 7.60 (2H, m, ArylH), 7.71 (1H, br s, NH), 7.80 (2H, d, J=7.4 Hz, ArylH). ^{13}C NMR (300 MHz, $CDCl_3$) δ 17.84 (CH_3), 27.89 (CH_3), 47.11 (CH), 48.30 (CH_2Ph), 49.49 (CH_α), 66.73, 66.81 (CH-3a + CH-6a), 67.14 (CH_2), 85.40 (C), 120.03, 124.98, 127.08, 127.75, 128.21, 128.94 (ArCH), 135.22, 141.32, 143.73 (ArC), 145.53 (C-6), 155.35 (COO), 157.52 (COO), 160.50 (C-3), 174.66 (CO). m/z 656.45 $[M-H]^-$, 680.52 $[M+Na]^+$

(3aS*,6aR*)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)glycyl)-3-(benzylamino)-3a,6a-dihydro-4H-pyrazolo[3,4-d]isothiazole-6-carboxylic acid 1,1-dioxide (7). Compound **1** (0.2 g, 0.31 mmol) is dissolved in DCM (10

ml/mmol) and cooled to 0°C before adding trifluoroacetic acid (10 ml/mmol) dropwise. The mixture is stirred overnight at rt, then the solvent is evaporated under reduced pressure and the residue is washed three times with ethyl ether. The deprotected product is used in the following step without further purification.

7 (0.18 g, >98%). White solid. mp 208°C dec. Elemental Analysis: Found: C, 59.2; H, 4.2; N, 11.6; Calcd. For $C_{29}H_{25}N_5O_7S$: C, 59.3; H, 4.3; N, 11.9; ν_{max}/cm^{-1} 3357, 3305 (NH), 1721, 1683, 1625 (CO). ^{1}H NMR (300 MHz, DMSO-*d6*) δ 3.32 (1H, br s, OH), 4.13 (1H, d, J=6.1 Hz, CH), 4.20-4.32 (6H, m, CH_2Ph + $CH_{2(G)}$), 4.46 (2H, m, CH_2), 5.23 (1H, d, J=10.6 Hz, CH-6a), 6.12 (1H, d, J=10.6 Hz, CH-3a), 7.17-7.43 (9H, m, ArylH), 7.70 (2H, d, J=7.5 Hz, ArylH), 7.80 (1H, m, NH), 7.88 (2H, d, J=7.5 Hz, ArylH), 8.35 (1H, t, J=6.0 Hz, NH). ^{13}C NMR (300 MHz, DMSO-*d6*) δ 43.38 (CH_2), 45.97 (CH), 47.21 (CH_2Ph), 66.31 ($CH_{2(G)}$), 67.91 (CH-6a), 68.90 (CH-3a), 120.56, 125.66, 127.52, 127.60, 128.08, 128.88 (ArCH), 137.32, 141.17 (ArC), 144.25 (C-6), 156.86 (COO), 161.30 (COO), 161.85 (C-3), 171.22 (CO). m/z 542.35 $[M\text{-}COOH]^-$.

(3aS*,6aR*)-(9H-fluoren-9-yl)methyl(2-(6-(((S)-1-(((S)-1-amino-3-methyl-1-oxobutan-2-yl)amino)-4-methyl-1-oxopentan-2-yl)carbamoyl)-3-(benzylamino)-1,1-dioxido-3a,6a-dihydro-4H-pyrazolo[3,4-d]isothiazol-4-yl)-2-oxoethyl)carbamate (3a and 3b). Intermediate **7** (0.18 g, 0.3 mmol) is dissolved in DCM (10 ml/mmol) under nitrogen. The mixture is cooled at 0 °C before adding EDC (1.1 eq) and HOBt (1.1 eq) and is stirred for 1 hour. Then, NH_2-Leu-Va-$CONH_2$ (0.1 g, 0,33 mmol) and DIPEA are added until pH=7-8. The mixture is allowed to react overnight at rt. The solvent is removed under reduced pressure. The residue is dissolved in DCM and the organic phase is washed with 5% w/v aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine. The organic layer is dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The reaction is monitored by TLC (ethyl acetate/hexane 2:1) and the two diasteroisomers were separated by silica gel column chromatography (from hexane/ethyl acetate 2:1 to ethyl acetate).

3a (0.038 g, 16%). White solid. Rf 0.18 in AcOEt. mp 185°C. $[\alpha]_D$ = -4.8 (0.002 g/ml in CH_3OH). Elemental Analysis: Found C, 60.4; H, 5.6; N, 13.8; Calcd. For $C_{40}H_{46}N_8O_8S$: C, 60.1; H, 5.8; N, 14.0; ν_{max}/cm^{-1} 3430 (NH), 1627 (CO). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.94 (12H, m, CH_3), 1.73-1.74 (3H, m, $CH_{(L)}$ + CH_2), 2.21 (1H, m, $CH_{(V)}$), 4.25 (2H, m, CH + $CH_{\alpha(V)}$), 4.40 (4H, m, $CH_{2(G)}$ + CH_2), 4.49 (1H, m, $CH_{\alpha(L)}$), 4.47-4.57 (2H, m, CH_2Ph), 5.44 (1H, d, J=10.6 Hz, CH-6a), 5.91 (1H, m, NH), 6.0 (2H, m, CH-3a + NH), 6.48 (1H, s, NH), 7.00 (1H, m, $NH_{(V)}$), 7.25-7.29 (7H, m, ArylH), 7.40 (2H, m, ArylH), 7.52 (1H, m, $NH_{(L)}$), 7.62 (2H, d, J=7.2 Hz, ArylH), 7.77 (2H, d, J=7.2 Hz, ArylH), 7.84 (1H, m, NH). ^{13}C NMR (300 MHz, $CDCl_3$) δ 18.18, 19.32, 21.91. 22.84 (CH_3), 24.99 ($CH_{(L)}$), 29.92 ($CH_{(V)}$), 40.83 ($CH2_{(L)}$), 43.49 (CH_2), 47.06 ($CH_{(V)}$), 48.19 (CH_2Ph), 53.60 ($CH_{\alpha(L)}$), 58.96 ($CH_{\alpha(V)}$), 66.69 (CH-6a), 67.36 ($CH_{2(G)}$), 67.41 (CH-3a), 120.00, 125.14 127.11, 127.79, 128.16, 128.94 (ArCH), 135.36, 141.28, 143.70-143-77 (ArC), 146.37 (C-6), 156.79 (COO), 159.66 (CONH), 161.20 (C-3), 170.99 (CO), 171,52 ($CONH_2$), 173.84 (CONH). m/z 797.55 $[M\text{-}H]^-$, 821.87 $[M+Na]^+$

3b (0.031 g, 13%). White solid. Rf = 0.07 in AcOEt. mp 216°C. $[\alpha]_D$ = +7.2 (0.002 g/ml in CH_3OH). Elemental Analysis: Found C, 60.3; H, 5.5; N, 13.7; Calcd. For $C_{40}H_{46}N_8O_8S$: C, 60.1; H, 5.8; N, 14.0; ν_{max}/cm^{-1} 3412 (NH), 1668, 1624.37 (CO). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.93 (12H, s, CH_3), 1.72 (3H, m, $CH_{(L)}$ + CH_2), 2.11 (1H, m, $CH_{(V)}$),

4.22-4.23 (2H, m, CH + $CH_{\alpha(V)}$), 4.39 (2H, m, CH_2), 4.42-4.57 (4H, m, $CH_{2(G)}$ + CH_2Ph), 4.65 (1H, m, $CH_{\alpha(L)}$), 5.45 (1H, d, J=10.2 Hz, CH-6a), 5.73 (1H, m, NH), 5.96 (1H, d, J=10.2 Hz, CH-3a), 6.27 (1H, s, NH), 6.62 (1H, s, NH), 7.26-7.30 (7H, m, ArylH), 7.40 (2H, m, ArylH), 7.60 (4H, m, $NH_{(L)}$ + $NH_{(v)}$ + ArylH), 7.75 (3H, m, ArylH + NH). ^{13}C NMR (300 MHz, $CDCl_3$) δ 18.41, 19.30, 21.84. 22.85 (CH_3), 24.81 ($CH_{(L)}$), 30.49 ($CH_{(V)}$), 40.88 ($CH2_{(L)}$), 43.46 (CH_2), 47.04 ($CH_{(V)}$), 48.16 (CH_2Ph), 53.22 ($CH_{\alpha(L)}$), 58.97 ($CH_{\alpha(V)}$), 66.77 (CH-6a), 67.37 ($CH2_{(G)}$), 67.49 (CH-3a), 120.01, 125.13, 127.12, 127.79, 128.16, 128.95 (ArCH), 135.37, 141.28, 143.73-143.77 (ArC), 146.62 (C-6), 156.66 (COO), 159.17 (CONH), 161.04 (C-3), 170.99 (CO), 172,28 ($CONH_2$), 174.07 (CONH). m/z 797.25 $[M-H]^-$, 821.65 $[M+Na]^+$

NOE effects

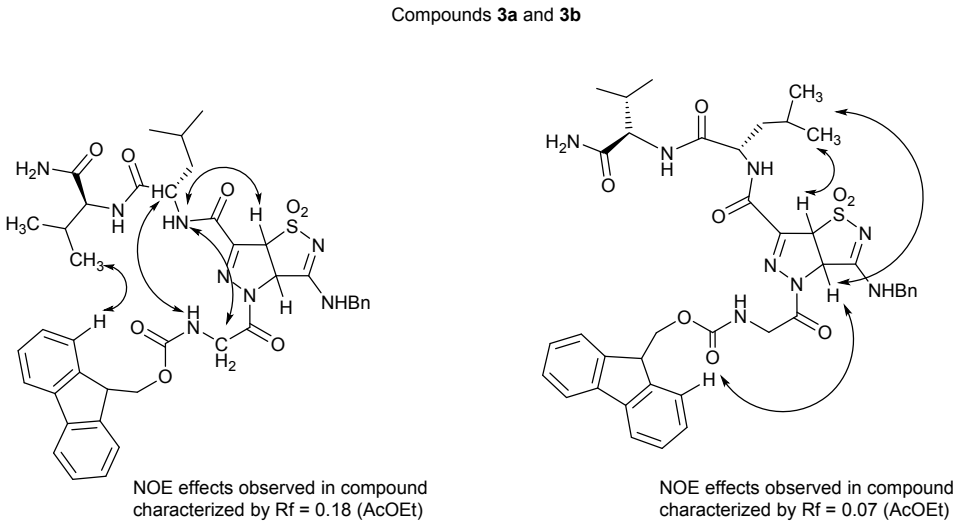
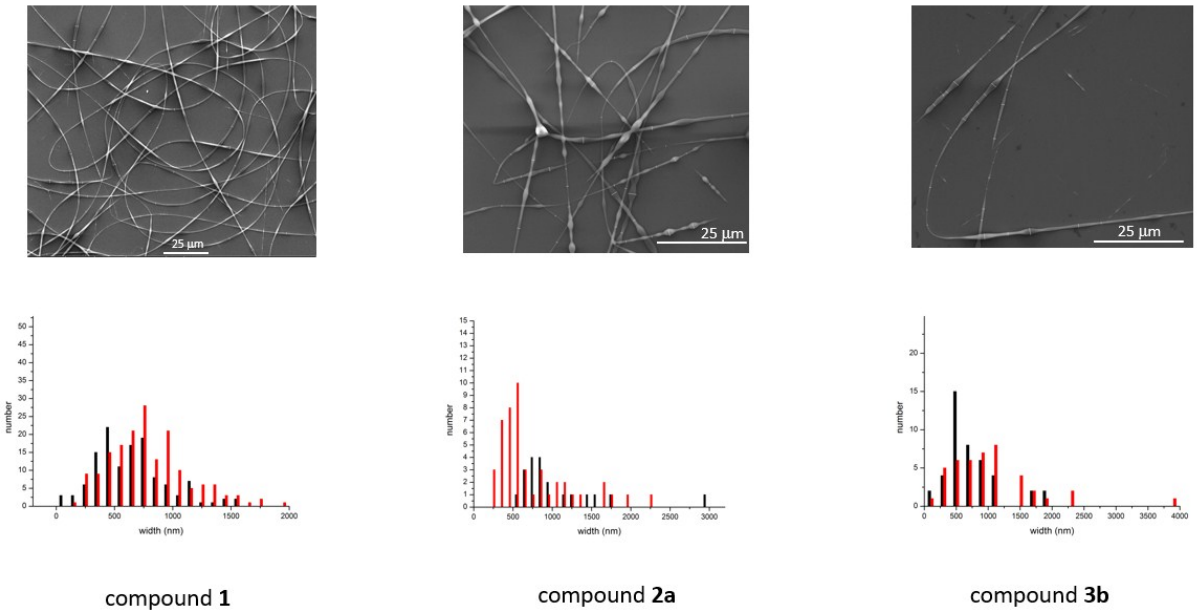


Figure ESI1. Significant NOE effects (300 MHz, $CDCl_3$, 300 ms).

SEM diameters of the electrospun fibers

In addition to the data presented in Fig. 3 (and in Fig. 4) we carried out a statistical analysis of SEM images from all spun fibers. We selected two images (color codes black and red) and measured various diameters at randomly chosen points (white bars inside the images). The distributions are rather wide (typical for peptide fibers), but monomodal (typical for most electrospinning modes). They compare well with the AFM heights (compound **1**, 70 to 800 nm), but they provide more reliable widths than AFM (where the tip convolution enhances the width). This proves that the fibers are not completely flattened out into stripes/bands (which might form by evaporation of absorbed solvent, and from hollow tubes).

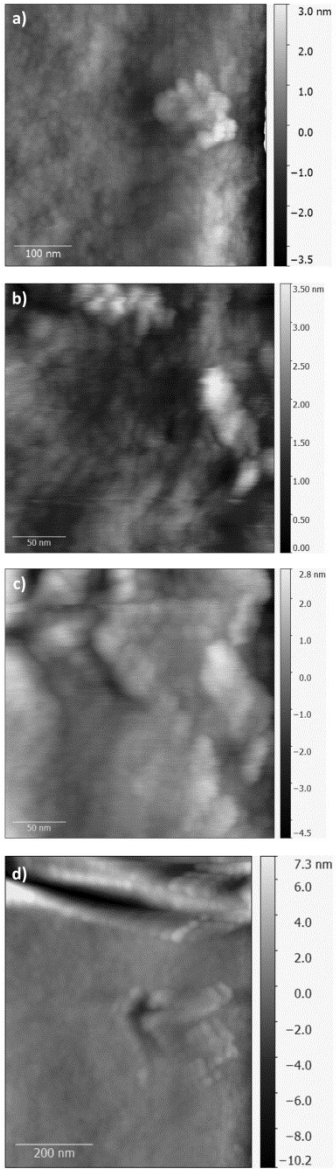


compound **1** compound **2a** compound **3b**

Figure ESI2. SEM micrographs of the electrospun compounds and corresponding diameter statistics. The red and black bars refer to two different images, of which only one is shown.

AFM data of electrospun fibers from compound 1

Because roughness scales with image size, we tested various areas (scans, height histograms, and roughness rms values).



rms small areas (nm):
0.329
0.254
0.388
0.497
0.451
0.781
0.771

Rms total image: 0.72-0.88 nm

rms small areas (nm):
0.407
0.612
0.33
0.39
0.439
0.395

Rms total image: 0.480 nm

rms small areas (nm):
0.547
0.488
0.259
1.020
0.944
0.412

Rms total image: 0.89 nm

rms small areas (nm):
0.65
0.33
0.46
0.37
0.98
3.22
0.41

Rms total image: 1.87 nm

Figure ESI3. a-d) AFM scans from Figure 4 (main text)

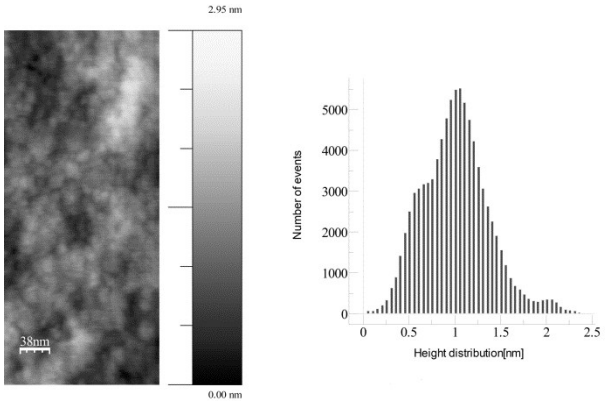


Figure ESI4. Left part of the Figure 4c (main text). The height histogram differs slightly from that presented in the main text because this part of the image is flatter. Rms value 0.36 nm.

X-ray diffraction

The peptides do not easily form crystals, but in electrospun fiber form this might be different. Hence we recorded powder diffractograms in reflection mode in an X-ray diffractometer (X-Pert, Panalytical) with CuKα radiation (45 kV, 40 mA) on a "zero background" Si holder. The material was scraped off the glass slides used as electrospinning collectors. The very small amount translated into long recording times (>1 h), which revealed various features that are all from the holder (black trace in Fig. ESI5, all peaks marked with "x"). The most intense feature, apart from the broad peak at ca. 7º, is the main Si peak at 28.5º, which we employed also for calibrating the angle. Compound **3b** (blue trace) shows no evidence of crystalline material at all, while compound **1** (red) gave three peaks, which are again not due to the peptide: 26.6º is assigned to quartz (oxidized holder and/or dust), 24.2º to $KHCO_3$ traces from preparation and purification, and the very clear feature at 33.3º to hydrated NaCl, from the brine washing procedure (the other main peaks from hydrohalite, 30.0º and 35.6º, are masked by our artefacts). While the X-ray data, due to the small amount of material, cannot provide clear proof, but good indications that the two compounds are amorphous also in fiber form.

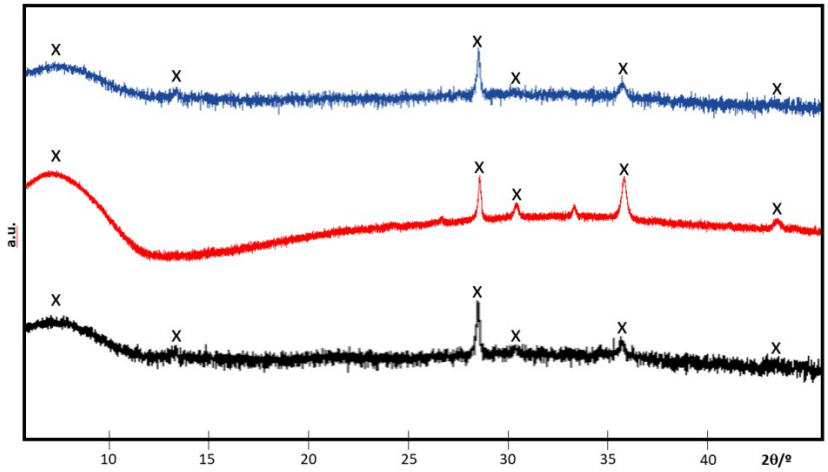


Figure ESI5. Powder X-ray diffraction of electrospun fibers of compounds **1** (red) and **3b** (blue) on a Si "zero background" holder. The black trace is from the bare holder.

IR spectral comparison powder/fibers of compound 1

A more detailed interpretation of figure 5a (main text) is provided here. The “amide A” N-H stretching band is located at 3300-3350, and features free (not H-bonded) N-H. The peak at 2940 cm^{-1}, attributed to some of the

three CH groups, shows a large redshift (from 2940 to 2910 cm^{-1}). The redshift would mean that the bicyclic system interacts with neighboring molecules more in the fiber than in the powder. The ether (incl. carbamate) groups have a band at 1730. The "amide I" C=O stretching band is located around 1685. Finally, the phenyl signal at 1075 cm^{-1} , interpreted as C-H in pane bending in the phenyl ring, is clearly weaker in the fiber than in the powder, so the phenyl ring should be oriented differently.

Raman hyperspectral imaging of compound 1 electrospun fibers

We here present an extension of figure 5b (main text).

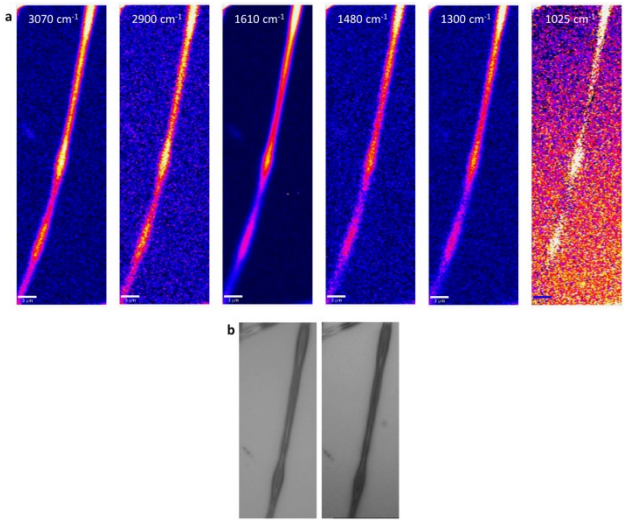


Figure ESI6 a) Raman hyperspectral micrographs of electrospun **1**. Each pixel contains a complete Raman spectrum; the images are produced by selecting bands at 3070 cm^{-1}, 2900 cm^{-1} etc. Note that each band has a different signal/noise ratio. The intensities are shown in false color. The scale-bar is 3 µm. The three beads show enhanced intensity because more material is present. b) Grey scale images: corresponding optical micrographs of the upper two beads of the fiber before (left) and after (right) recording the Raman images, proving that even long-term irradiation with the 532 nm CW laser is not damaging, as long as the intensity is carefully controlled.

IR spectra of compounds 3a and 3b

The spectra shown in figure 7b (main text) were interpreted in more detail. The bands at 3430 and 3410 should be (more or less) free amide A, the most likely candidate would be the primary amide CO-NH_2, and or the CONH next to it. This vibration is stronger in **3a**, but the difference is not extreme, and the broad nature of this band will not allow to elucidate more details. 3330 for bound amides appear as a shoulder (with quite a lot of intensity) in both **3a** and **3b**. 3060 is stronger in **3b**. This peak stems from aromatic C-H stretches. Possibly the aromatics in **3a** have a lot more interaction with other hydrophobic groups than in **3b**. This would support a

more open structure for **3b**. 2915 (CH_2) is identical, but 2950 much stronger in **3b**. 2950 can be from CH_3, but also from CH_2 next to electron-withdrawing groups like CO or O or N. The 1720 esters form similar shoulders. Amide I at 1668 is very much stronger in **3b**. The explanation could be an unusual and (for the amide groups) nearly point-symmetric structure of **3a**. 1627 is from the aromatics, esp. Fmoc, and is equal for both molecules. The next big difference is 1170, nearly absent in **3a**. This peak can be from esters, but is much stronger from SO_2-N.Accidently, this is also one of the strongest Raman peaks, as it should be, but present for both. Another Raman band is 880, much stronger for **3b**. Assignment is very difficult here; the pyrazoline group would fit well.

Cytotoxicity tests

We employed a human colorectal adenocarcinoma cell line (Caco-2) in standard medium, with various concentrations of compound **1** (0, 12.5, 25, 50, 100, 200 µM). The cell viability has been determined by sulphorhodamine B (SRB) assay after 48 h of incubation with compounds, and the effects of the compounds vs control were analysed by two-tailed Student's t test for unpaired data. [2] (Fig. ESI7)

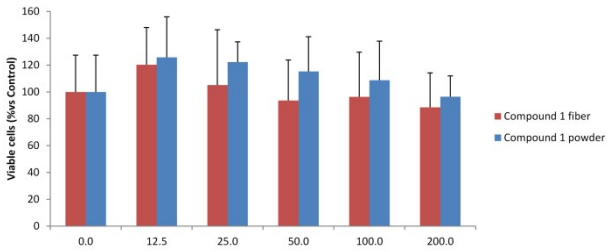


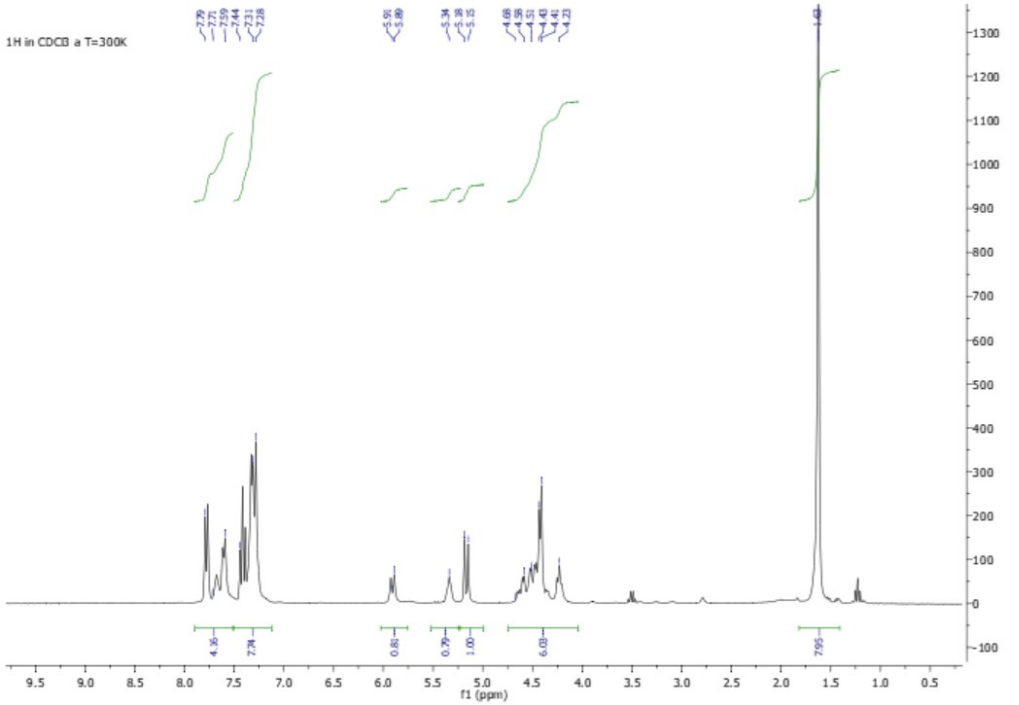
Figure ESI7. Cytotoxicity test of powder (blue) and fiber (red) of compound **1**

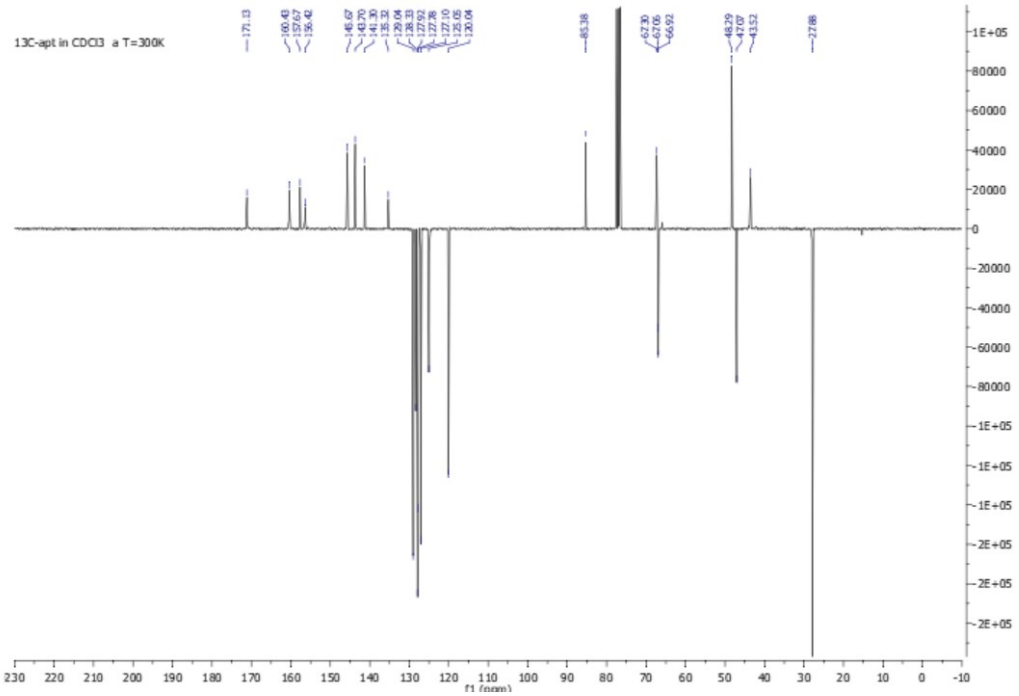
The compounds were dissolved in dimethyl sulfoxide (DMSO) before performing each experiment. The concentrations utilized ranged from 200µM to 25µM. The same volume of solvent was added to control conditions and did not exceed 0.5% v/v. Human colorectal adenocarcinoma cell line (Caco-2) was cultured in MEM supplemented with 10% FCS, L-glutamine, sodium-pyruvate and non-essential amino acids, penicillin/streptomycin at 37°C in a humidified atmosphere of 5% CO_2 and 95% air and passed every three days to maintain it in subconfluent conditions.

Cell viability was determined by (SRB) assay. Cells were seeded in a 96-well tray (8 x 10^3 cells/well) in octuple replicas and after 24h treated with compounds at aforementioned concentrations. SRB assay was then performed after 48h incubation. Experimental data are expressed as mean ± S.D. The effects of the compounds vs control were analyzed by two-tailed Student's t test for unpaired data. The concentration of compounds required to reduce cell viability by 50% (IC50) was calculated by nonlinear regression curve (GraphPad Prism, Version 5.01).

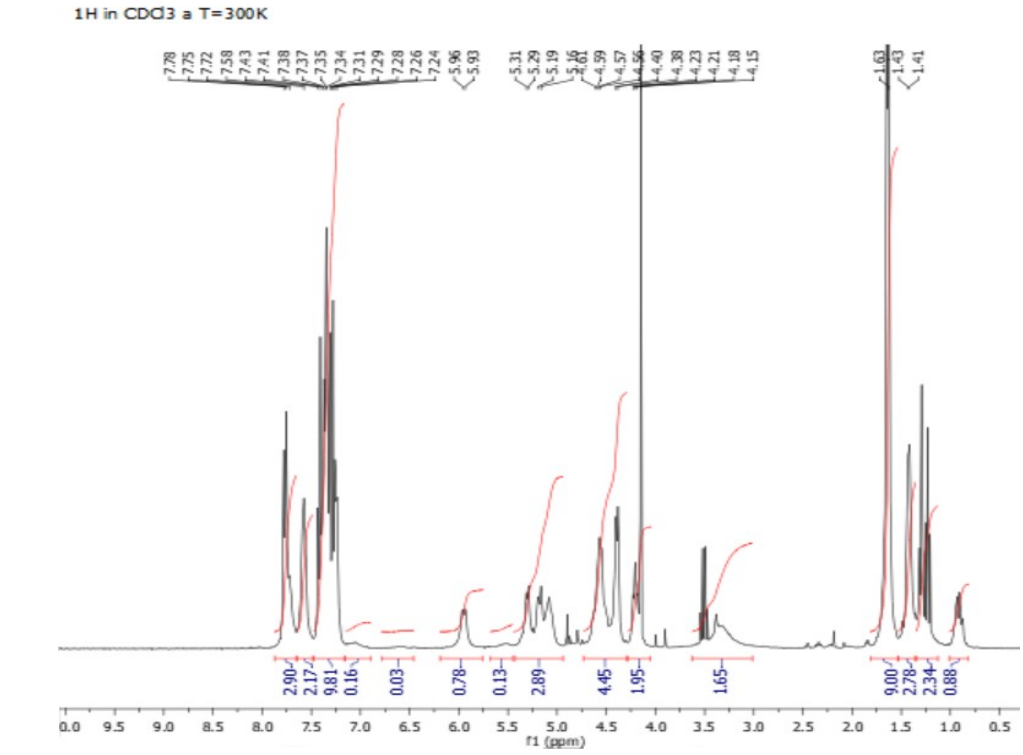
Fid ^{1}H-NMR, ^{13}C-NMR

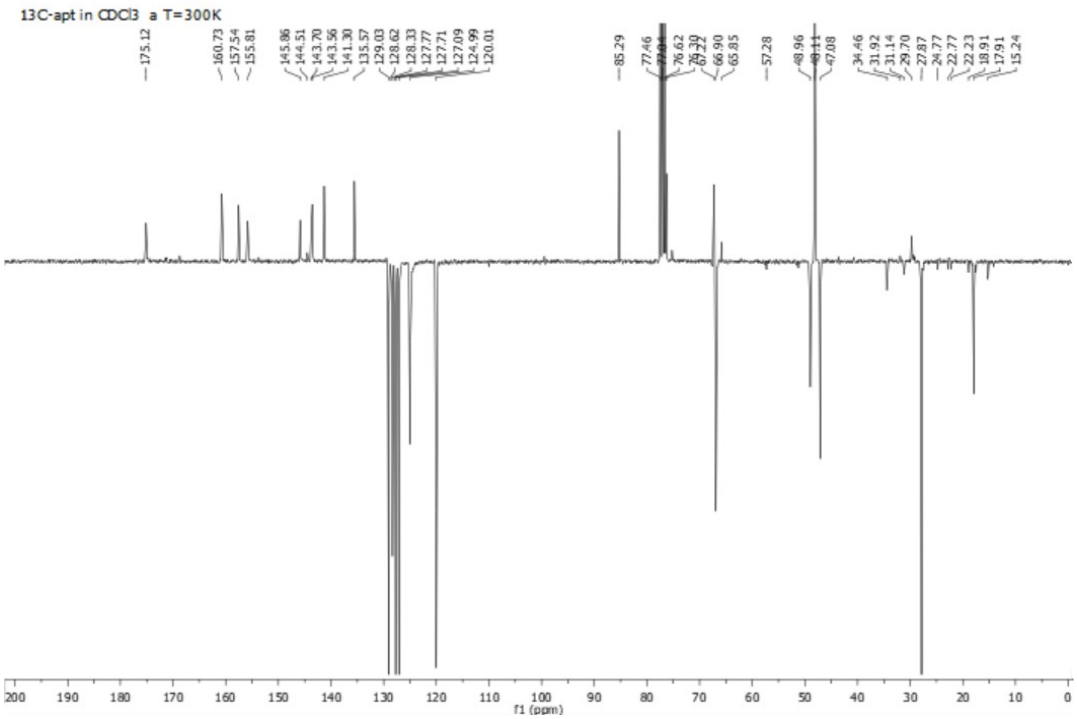
Compound 1



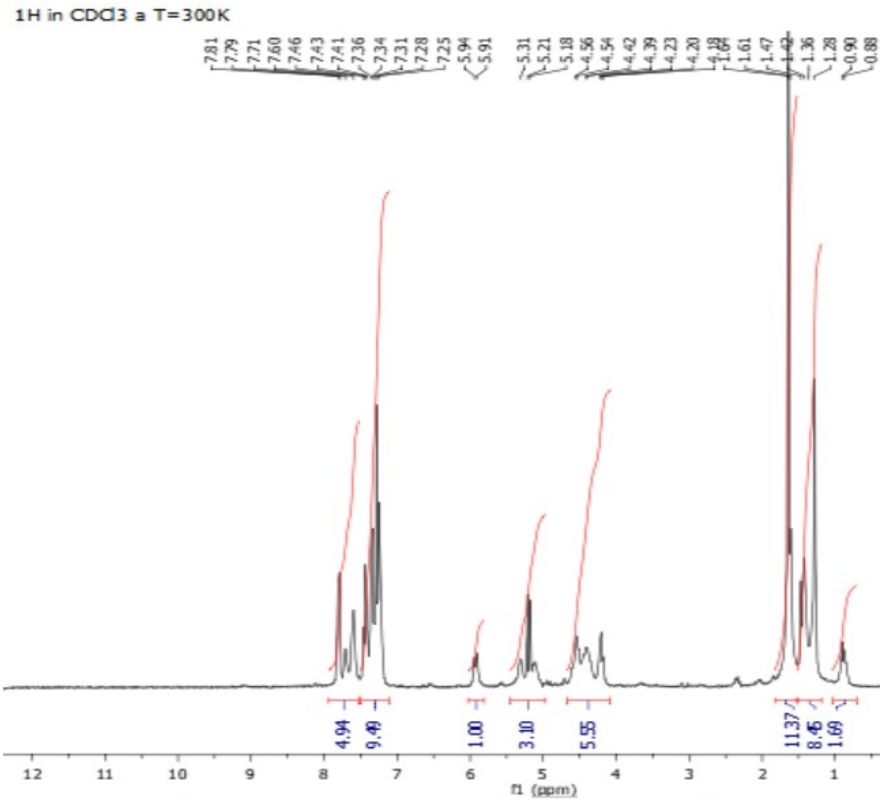


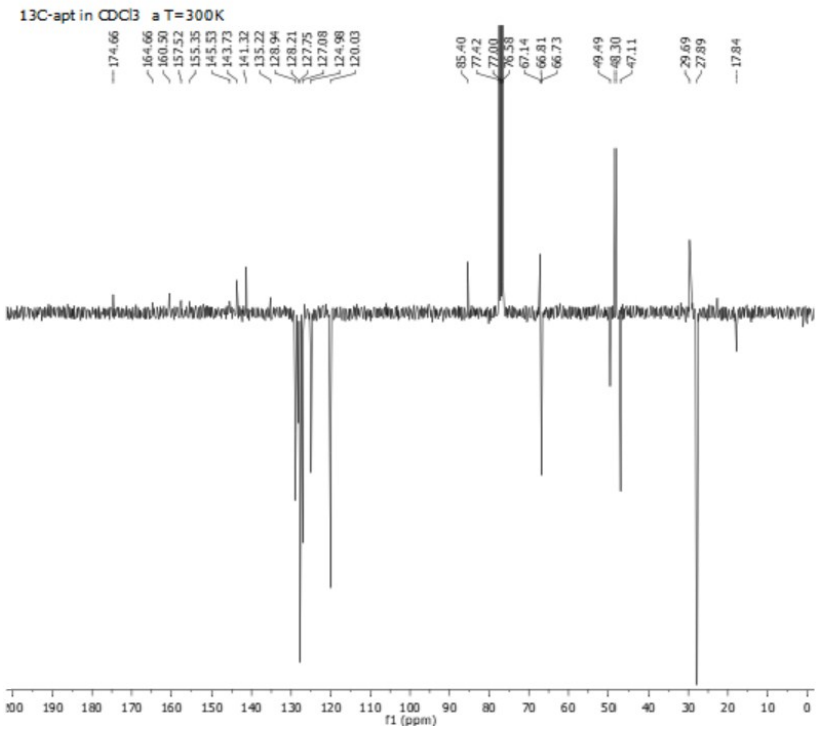
Compound 2a



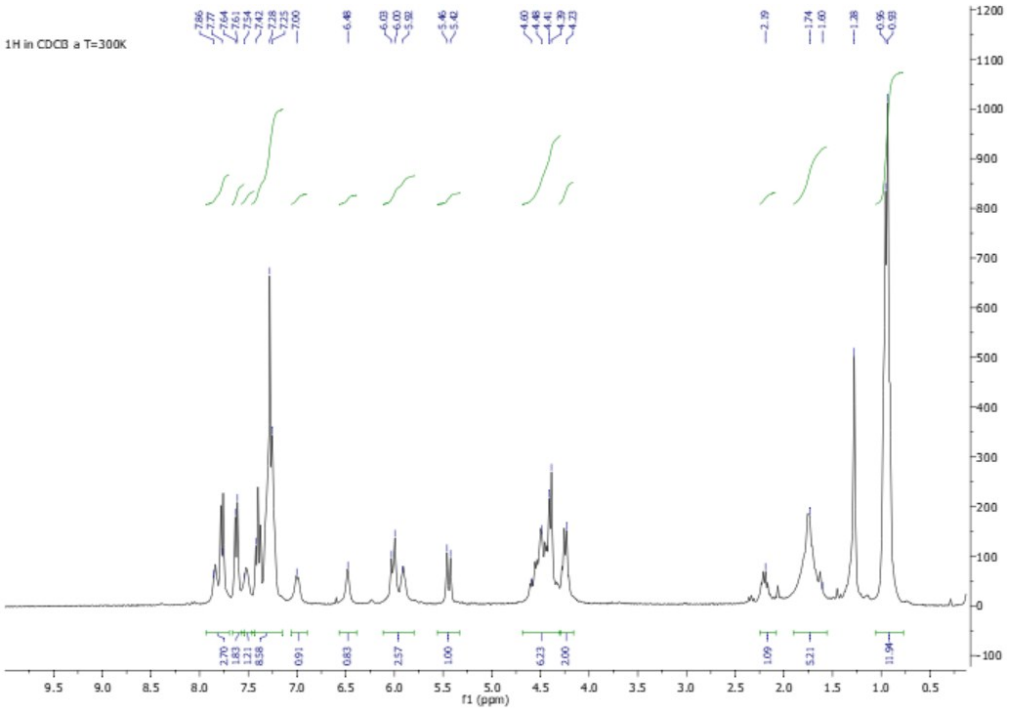


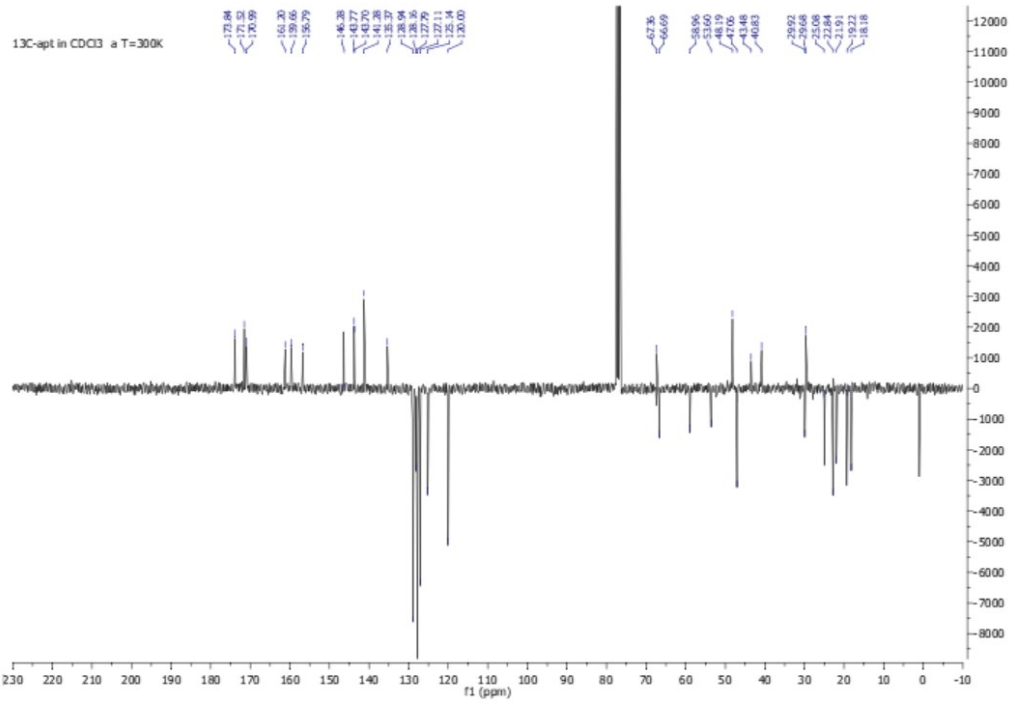
Compound 2b


1H in CDCl3 a T=300K
7.81
7.79
7.71
7.60
7.46
7.43
7.41
7.36
7.34
7.31
7.28
7.25
5.94
5.91
5.31
5.21
5.18
4.56
4.54
4.42
4.39
4.23
4.20
1.61
1.47
1.42
1.36
1.28
0.90
0.88
4.94
9.49
1.00
3.10
5.55
11.37
8.45
1.69
12
11
10
9
8
7
6
5
4
3
2
1
f1 (ppm)

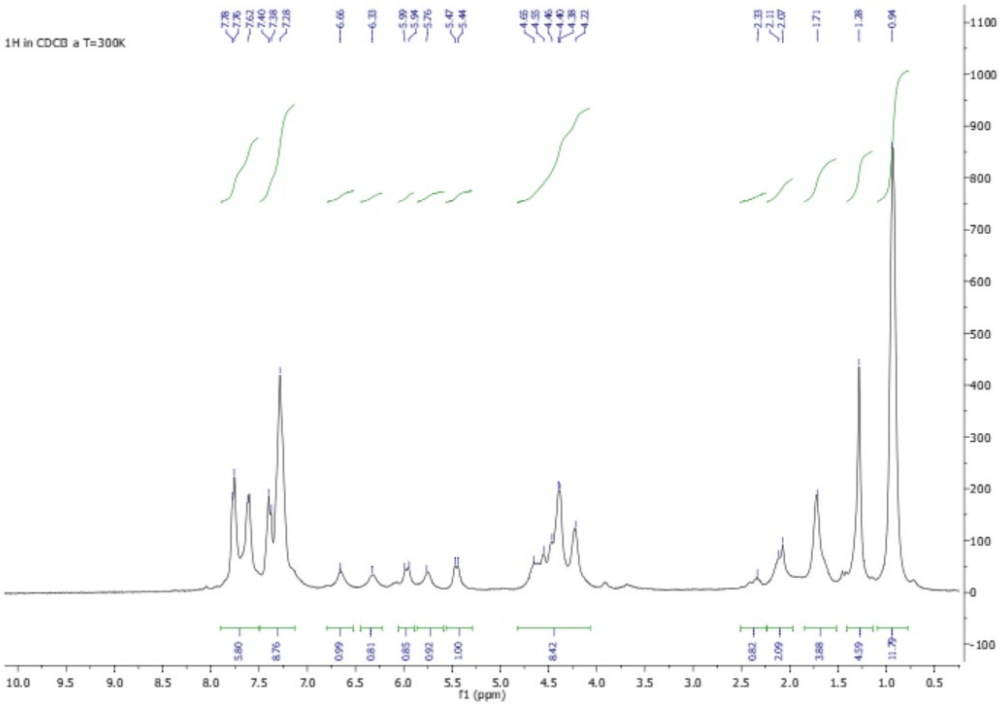

13C-apt in CDCl3 a T=300K
174.66
164.66
160.90
157.52
155.35
145.53
143.73
141.32
135.22
128.94
128.21
127.75
127.08
124.98
120.03
85.40
77.42
77.00
76.58
67.14
66.81
66.73
49.49
48.30
47.11
29.69
27.89
17.84
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

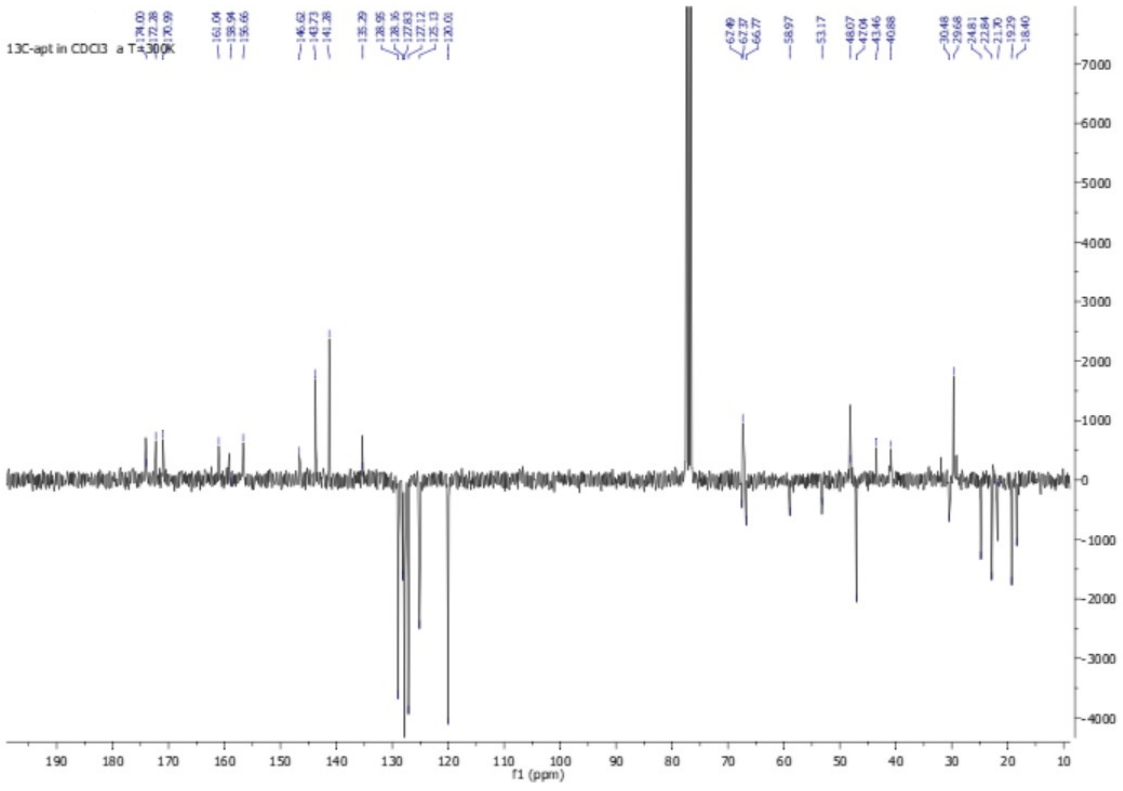
Compound 3a



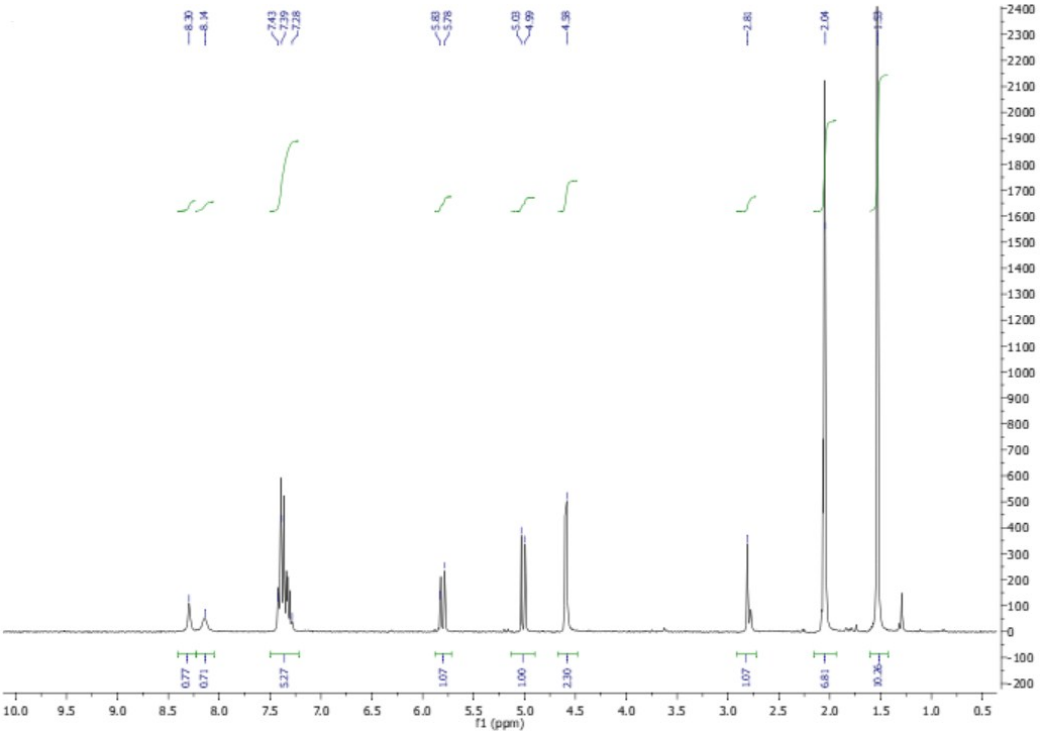


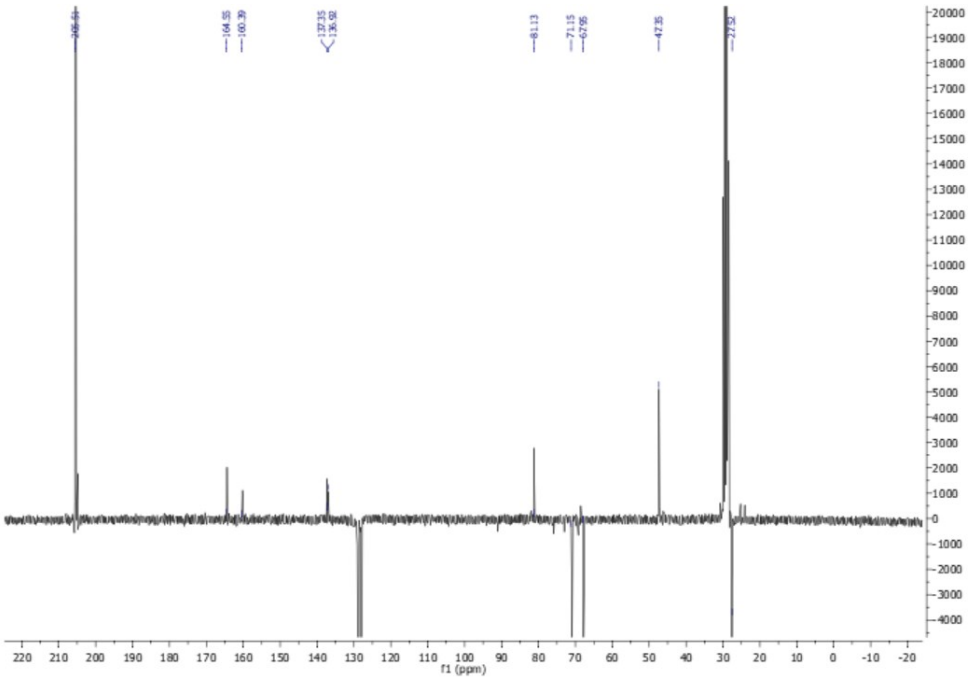
Compound 3b



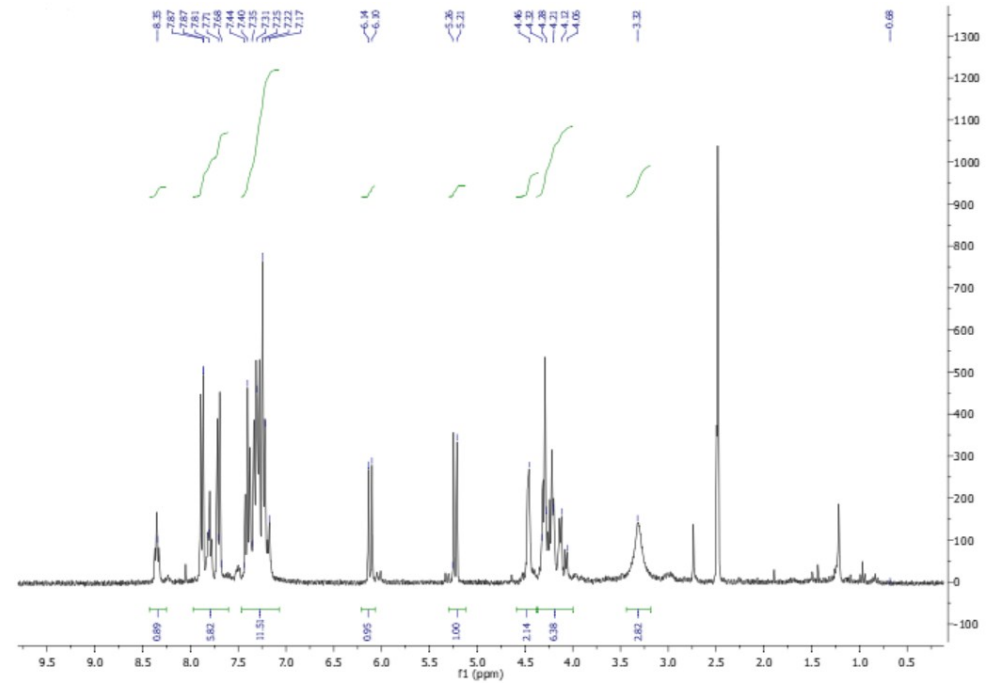


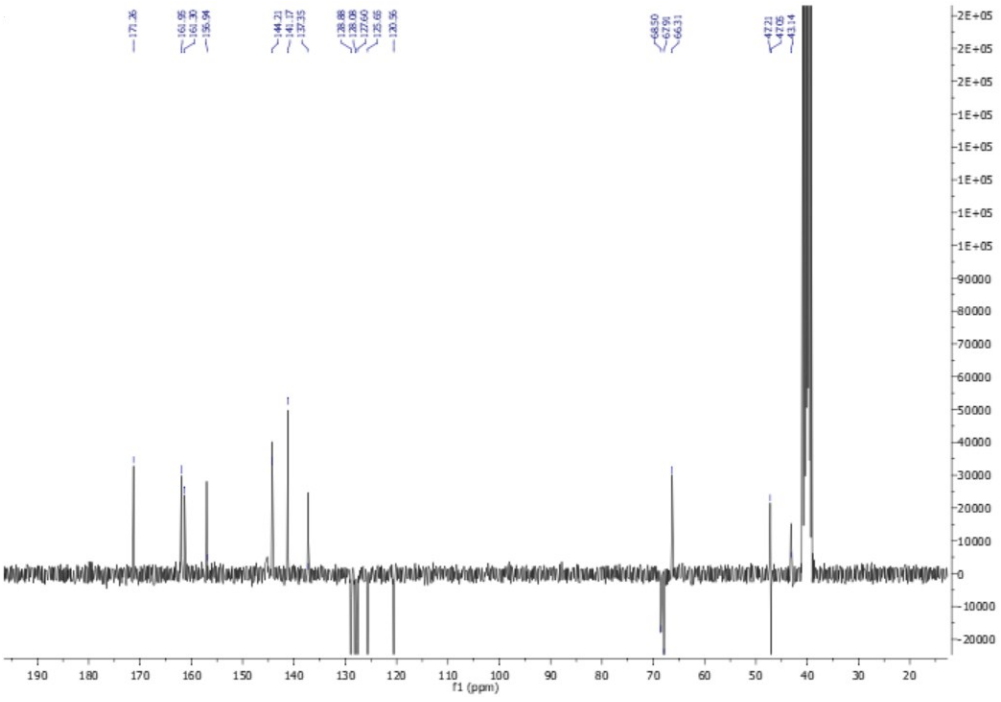
Compound 6


8.30
8.14
7.43
7.39
7.28
5.83
5.78
5.03
4.99
4.98
2.81
2.04
1.59
0.77
0.71
5.27
1.07
1.00
2.30
1.07
6.81
10.26
f1 (ppm)


206.69
164.55
160.39
137.35
136.98
81.13
71.15
67.96
47.26
27.52
f1 (ppm)

Compound 7


8.35
7.87
7.87
7.81
7.71
7.68
7.44
7.40
7.35
7.31
7.25
7.22
7.17
6.34
6.30
5.26
5.21
4.46
4.32
4.28
4.21
4.12
4.06
3.32
0.68
0.89
5.82
11.51
0.95
1.00
2.14
6.38
2.82
f1 (ppm)


171.26
160.95
161.30
156.94
144.21
141.17
137.35
128.88
128.08
127.60
125.66
120.36
68.50
67.96
66.31
47.21
47.05
43.14
f1 (ppm)

References

1. F. Clerici, M. L. Gelmi, C. Monzani, D. Pocar and A. Sala, *Het. Chem*, 2006, **43**.
2. I. Rimoldi, G. Facchetti, G. Lucchini, E. Castiglioni, S. Marchianò, N. Ferri, *Bioorganic & medicinal chemistry,* 2017, 25, 1907