Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Stereoselective synthesis of original spirolactams displaying promising folded structures

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Supporting Information

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1. General information

All solvents were dried and freshly distilled before use. Reactions were magnetically stirred and monitored by thin layer chromatography using Merck-Kieselgel 60 F254 plates. Visualization was accomplished with UV light and exposure to a 10% solution of ninhydrin in ethanol followed by heating. Chromatography columns were performed using Merck-Kieselgel 60 (230–400 mesh). Melting points were recorded on a Buchi 510. Mass spectra were obtained on a Micromass Q-Tof mass spectrometer using electrospray ionization. The high resolution mass spectra (HRMS) were measured with a Micromass Q-Tof spectrometer equipped with electrospray source ionization (ESI), using phosphoric acid as an internal standard. HPLC analyses were performed on a Waters-Enpower Pro (column 50 x 4.6 mm Chromolith SpeedRod RP-18, UV detection). Compounds were separated using a linear gradient system comprising 0.1% aqueous formic acid (solvent A) and acetonitrile containing 0.1% formic acid (solvent B) using a constant flow rate of $3mLmin^{-1}$. Preparative HPLC were performed on a Waters Delta 4000 (column 40 x 100 nm Delta-Pack C18, UV detection at 214 nm). Compounds were separated using a linear gradient system comprising 0.1% aqueous TFA (solvent A) and acetonitrile containing 0.1% TFA (solvent B) using a constant flow rate of $50mLmin^{-1}$ with the detector set at 214 nm.

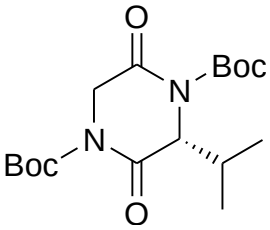
The optical rotations were obtained at 20°C on a Perkin Elmer Polarimeter with a Sodium lamp at 589 nm and reported as follows: $[\alpha]_D^{20}$ (C: gcm^{-3}, solvent), with $[\alpha]$ in $degcm^3g^{-1}mol^{-1}$. NMR spectra were recorded at ambient temperature on Bruker Avance DPX 200 MHz, Bruker Avance 300 MHz, Bruker Avance 400 MHz or Bruker Avance III 600 MHz spectrometers. Chemicals shifts (δ) are reported from tetramethylsilane with the solvent

resonance as the internal standard. Data are reported as follows: chemical shift (δ), multiplicity (s=singlet, d=doublet, t=triplet, sept=septuplet, br=broad, m=multiplet), coupling constants (*J*: Hz), integration, and assignment. The reported ^{1}H NMR signals were assigned using standard 2D-NMR techniques.

The reported ^{13}C NMR signals were assigned using DEPT-135 and HMQC experiments or by direct comparison to the ^{13}C NMR spectra of corresponding starting materials. nOe correlations were listed as s for strong (2-3 Å), m for medium (2-4 Å) and w for weak (3-5 Å). Circular dichroism analyses were performed on a Chirascan Circular Dichroism Spectrometer device (Applied Photophysics) equipped with a Xenon lamp. The standard measurements were made at 5°C on solutions of fixed concentration of 5 mmolL^{-1} of compound in a pH 7 phosphate buffer at 10 mmolL^{-1}, using cells of 0.1 mm or 0.5 mm thickness. This device was used through the structural biology platform "RIO" located at the Centre de Biochimie Structurale (CBS) in Montpellier, France.

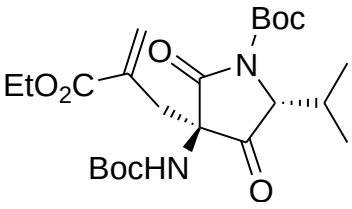
2. Experimental procedures

Preparation of the spirolactam scaffolds



Bis-Boc-*cyclo*-[Gly-(D)-Val] 1 ($C_{17}H_{28}N_2O_6$, 356.19 gmol^{-1}): To a suspension of *cyclo*-[Gly-(D)-Val] (3.02 g, 19.34 mmol, 1.0 eq.) and Boc_2O (8.86 g, 40.60 mmol, 2.1 eq.) in dry DMF (30 mL) was added DMAP (4.96 g, 40.60 mmol, 2.1 eq.). The resulting orange solution was then stirred for 1.5 h at 20°C under argon atmosphere before being diluted with AcOEt and washed 2 times with a $KHSO_4$ 1.0 N solution. The organic layer was dried on anhydrous Na_2SO_4 and solvent was removed *in vacuo*. The crude was then filtered through silica gel (CH_2Cl_2/AcOEt; 9:1), affording the protected DKP **1** with 87% yield (m=5.93 g).

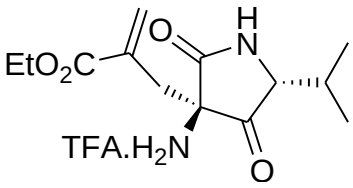
TLC R_f=0.90 (CH_2Cl_2/AcOEt; 90:10, UV); **m.p.** 130 °C; **$[\alpha]_D^{20}$**=-76.5 (C=20.4x10^{-3}, CH_2Cl_2); **^{1}H NMR** ($CDCl_3$, 300 MHz, 25°C, TMS) δ 1.04 (3H, d, *J*=6.7 Hz, $H_{CH(C\underline{H3}2)}$), 1.09 (3H, d, *J*=6.7 Hz, $H_{CH(C\underline{H3}2)}$), 1.52 (9H, s, $H_{C(C\underline{H3}3)}$), 1.53 (9H, s, $H_{C(C\underline{H3}3)}$), 2.06 (1H, m, $H_{C\underline{H}(CH3)2}$), 4.14 (1H, d, *J*=18.6 Hz, $H_{C\underline{H2}}$), 4.58 (1H, d, *J*=9.6 Hz, $H_{C\underline{H}CH(CH3)2}$), 4.73 (1H, d, *J*=18.6 Hz, $H_{C\underline{H2}}$); **^{13}C NMR** ($CDCl_3$, 75 MHz, 25°C, TMS) δ 19.5 ($C_{CH(\underline{C}H3)2}$), 19.7 ($C_{CH(\underline{C}H3)2}$), 28.0 ($C_{C(\underline{C}H3)3}$), 31.7 ($C_{\underline{C}H(CH3)2}$), 49.1 ($C_{\underline{C}H2}$), 65.5 ($C_{\underline{C}HCH(CH3)2}$), 85.0 ($C_{\underline{C}(CH3)3}$), 85.1 ($C_{\underline{C}(CH3)3}$), 149.9 ($C_{\underline{C}O\ urethane}$), 150.0 ($C_{\underline{C}O\ urethane}$), 164.9 ($C_{\underline{C}O\ lactam}$), 165.7 ($C_{\underline{C}O\ lactam}$); **HPLC r_t**=2.40 min; **ESI-MS$^+$** *m/z* 357.4; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{17}H_{28}N_2O_6$ + H$^+$] 357.2026 gmol^{-1}, found 357.2015 gmol^{-1}; Spectral data were consistent with those reported in the literature.[1]



(*3R,5R*)-*N*-Boc-3-*tert*-butoxycarbonylamino-3-(2-ethoxycarbonyl-allyl)-5-isopropyl-pyrrolidine-2,4-dione 2 ($C_{23}H_{36}N_2O_8$, 468.54 gmol^{-1}): A solution of bis Boc cyclo-[Gly-(D)-Val] **1** (2.50 g, 7.02 mmol, 1 equiv.) in anhydrous THF (35 mL) is cooled to -78°C under Argon atmosphere. A 1.0 M LiHMDS THF solution (7.72 mL, 7.72 mmol, 1.1 equiv.) is added dropwise and the reaction mixture is stirred for 45 min. Ethyl -2-(bromomethyl)acrylate is then added dropwise and the reaction is allowed to warm to room temperature. After 12 h, EtOAc is added and the organic layer is washed 5 times with 1 N aqueous HCl before being dried on $MgSO_4$ and concentrated under reduced pressure. The crude is purified by column

chromatography (petroleum ether/EtOAc 8:2) and compound **2** is obtained as colourless oil with a 38% yield (m = 1.25 g).

TLC R_f=0.49 (CH_2Cl_2/EtOAc 97:3, UV); **$[\alpha]_D^{20}$** = -8.0 (C=15.0x10^{-3}, CH_2Cl_2); **^{1}H-NMR** (400 MHz, $CDCl_3$, 25°C, TMS**)** δ 1.02 (d, 3H, *J* = 7.1 Hz, $H_{CH(CH3)2}$), 1.19 (d, 3H, , *J* = 7.1 Hz, $H_{CH(CH3)2}$), 1.29 (t, 3H, *J* = 7.1 Hz, H_{CH3CH2}), 1.33 (s, 9H, $H_{C(CH3)3}$), 1.52 (s, 9H, $H_{C(CH3)3}$), 2.41 (m, 1H, $H_{CH(CH3)2}$), 2.53 (d, 1H, *J* = 14.5 Hz, H_{CH2C*}), 2.84 (d, 1H, *J* = 14.5 Hz, H_{CH2C*}), 4.23 (q, 2H, *J* = 7.1 Hz, H_{CH3CH2}), 4.49 (d, 1H, *J* = 4.9 Hz, H_{CH*}), 5.64 (s, 1H, $H_{CH2CCO2Et}$), 6.26 (s, br, 1H, H_{NHBoc}), 6.39 (s, 1H, $H_{CH2CCO2Et}$); **^{13}C NMR** (100 Mhz, $CDCl_3$, 25°C, TMS) δ 14.2 (C_{CH3CH2}), 18.6 ($C_{CH(CH3)2}$), 19.4 ($C_{CH(CH3)2}$), 28.2 ($C_{C(CH3)3}$), 28.3 ($C_{C(CH3)3}$), 30.4 ($C_{CH(CH3)2}$), 33.5 ($C_{CH2quatC*}$), 61.7 (C_{CH2C*}), 62.0 (C_{CH3CH2}), 69.9 (C_{CH*}), 81.4 ($C_{C(CH3)3}$), 84.2 ($C_{C(CH3)3}$), 131.5 ($C_{CH2CCO2Et}$), 133.4 ($C_{CH2CCO2Et}$), 150.0 (C_{COBoc}), 155.3 (C_{COBoc}), 167.4 ($C_{COester}$), 171.0 ($C_{COlactam}$), 205.1 ($C_{COketone}$); **HPLC** r_t=2.73 min.;; **ESI-MS$^+$** *m/z* 469.3; **HRMS (TOF ES MS+)** *m/z* calculated for [$C_{23}H_{36}N_2O_8$ + H^+] 469.2550 gmol^{-1}, found 469.2540 gmol^{-1};

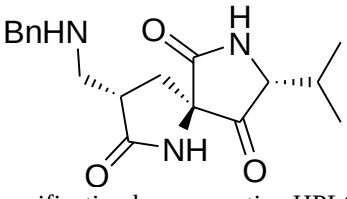


Trifluoroacetate (*3R,5R*)-3-(2-ethoxycarbonyl-allyl)-5-isopropyl-2,4-dioxo-pyrrolidin-3-yl-ammonium 3 ($C_{15}H_{20}F_3N_2O_5$, 365.32 gmol^{-1}): To a solution of **2** (1.34 g, 2.86 mmol, 1 equiv.) in CH_2Cl_2 (4v) at room temperature is added dropwise TFA (1v). The reaction mixture is stirred at room temperature for 2 h before being concentrated under reduced pressure. Excess TFA is removed by coevaporation with cyclohexane to afford the compound as a yellow oil with a quantitative yield (m = 1.09 g).

TLC R_f=0.23 (CH_2Cl_2/MeOH 96:4, UV); **$[\alpha]_D^{20}$** = +26.1 (C=23.0x10^{-3}, MeOH); **^{1}H-NMR** (400 MHz, DMSO-d_6, 25°C, TMS**)** δ 0.99 (m, 6H, $H_{CH(CH3)2}$), 1.23 (t, 3H, *J* = 7.0 Hz, H_{CH3CH2}), 1.99 (m, 1H, $H_{CH(CH3)2}$), 2.75 (d, 1H, *J* = 14.2 Hz, H_{CH2C*}), 2.90 (d, 1H, *J* = 14.2 Hz, H_{CH2C*}), 3.75 (d, 1H, *J* = 8.1 Hz, H_{CH*}), 4.09 (m, 2H, H_{CH3CH2}), 5.97 (s, 1H, $H_{CH2CCO2Et}$), 6.36 (s, 1H, $H_{CH2CCO2Et}$), 8.90 (s, br, 2H, H_{NH2}), 9.41 (s, 1H, H_{NHCO}); **^{13}C NMR** (100 Mhz, DMSO-d_6, 25°C, TMS**)** δ 14.4 (C_{CH3CH2}), 19.0 ($C_{CH(CH3)2}$), 19.7 ($C_{CH(CH3)2}$), 31.6 ($C_{CH(CH3)2}$), 33.9 ($C_{CH2quatC*}$), 59.0 (C_{CH3CH2}), 61.3 (C_{CH2C*}), 67.9 (C_{CH*}), 131.6 ($C_{CH2CCO2Et}$), 133.0 ($C_{CH2CCO2Et}$), 165.6 ($C_{COester}$), 168.9 ($C_{COlactam}$), 205.7 ($C_{COketone}$); **HPLC** r_t=1.09 min.; **ESI-MS$^+$** *m/z* 269.2; **HRMS (TOF ES MS+)** *m/z* calculated for [$C_{13}H_{20}N_2O_4$ + H^+] 269.1501 gmol^{-1}, found 269.1501 gmol^{-1}.

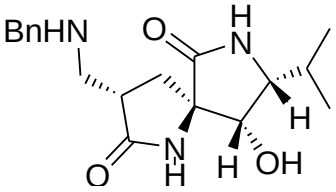
General procedure for the Michael addition/Spirocyclisation

A solution of the Boc deprotected TFA salt of **2** (332 mg, 0.87 mmol, 1.0 equiv.) and benzylamine (0.48 mL, 4.35 mmol, 5.0 equiv.) in absolute ethanol was heated using microwave irradiation at 130°C for 1 h, and then concentrated under reduced pressure. After evaporation to dryness of the reaction mixture, the resulting crude was dissolved in THF and 1.0 N HCl was added until the pH failed below pH 2. The mixture was stirred at room temperature for 1 h 30 and evaporated to dryness. The residue was triturated in EtOAc and filtered off to obtain a mixture of two diastereoisomers **3a** and **3b** with 95% yield.



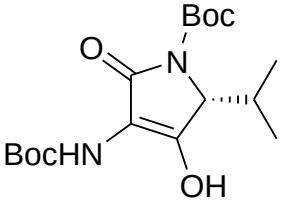
(*9S,11R,16R*)-3-((benzylamino)methyl)-8-isopropyl-1,7-diazaspiro[4,4]nonane-2,6,9-trione 3a ($C_{18}H_{23}N_3O_3$, 329.39 $gmol^{-1}$): Compound **3a** is prepared according to the general procedure for the Michael addition/Spirocyclisation with an isolated yield of 23% (m = 66 mg) after purification by preparative HPLC (linear gradient of 0-30% B over a 50 min. period).

$[\alpha]_D^{20}$ = +15.0 (C=8.0x10^{-3}, MeOH) ; **^{1}H-NMR** (300 MHz, D_2O, TMS, 25°C) δ 0.95 (d, 3H, *J* = 6.8 Hz, $H_{CH(C\underline{H}3)2}$), 1.00 (d, 3H, *J* = 6.9 Hz, $H_{CH(C\underline{H}3)2}$), 1.97 (dd, 1H, *J* = 13.2 Hz, *J* = 8.5 Hz, $H_{C\underline{H}2C*}$), 2.11 (m, 1H, $H_{C\underline{H}(CH3)2}$), 2.71 (dd, 1H, *J* = 13.5 Hz, *J* = 7.9 Hz, $H_{C\underline{H}2C*}$), 3.26-3.44 (m, 3H, $H_{C\underline{H}*CH2NHBn}$ + $H_{C\underline{H}2NHBn}$), 4.08 (d, 1H, *J* = 5.6 Hz, $H_{C\underline{H}*iPr}$), 4.33 (s, 2H, $H_{C\underline{H}2C6H5}$), 7.51 (s, 5H, $H_{CH2C6\underline{H}5}$); **^{13}C NMR** (75 Mhz, D_2O, TMS, 25°C) δ 17.2 ($C_{CH(\underline{C}H3)2}$), 17.9 ($C_{CH(\underline{C}H3)2}$), 31.0 ($C_{\underline{C}H(CH3)2}$), 34.1 ($C_{\underline{C}H2quatC*}$), 37.1 ($C_{\underline{C}H*CH2NHBn}$), 47.1 ($C_{\underline{C}H2NHBn}$), 51.4 ($C_{\underline{C}H2C6H5}$), 62.9 ($C_{quat\underline{C}*}$), 67.6 ($C_{\underline{C}H*iPr}$), 129.3-130.2 ($C_{\underline{C}6H5}$), 174.2 ($C_{\underline{C}Olactam}$), 178.9 ($C_{\underline{C}Olactam}$), 211.2 ($C_{\underline{C}Oketone}$); **HPLC** r_t=0.88 min.; **ESI-MS$^+$** *m/z* 330.2; **HRMS (TOF ES MS+)** *m/z* calculated for [$C_{18}H_{23}N_3O_3$ + H^+] 330.1818 $gmol^{-1}$, found 330.1817 $gmol^{-1}$.



(*9S,11R,16R,17S*)-3-((benzylamino)methyl)-9-hydroxy-8-isopropyl-1,7-diazaspiro[4,4]nonane-2,6-dione 4 ($C_{18}H_{25}N_3O_3$, 331.41 $gmol^{-1}$): To a solution of **3a** (100 mg, 0.30 mmol, 1.0 equiv.) in 5 mL of THF (4v) and water (1v) was added $NaBH_4$ (34 mg, 0.90 mmol, 3.0 equiv.) at 0°C. After 1 h 30 of stirring, the reaction mixture was acidified with aqueous 0.1 N HCl and evaporated to dryness. Compound **4** is obtained with an isolated yield of 90% (m = 91 mg) as a colourless oil after purification by preparative HPLC (linear gradient of 0-15% B over a 30 min. period).

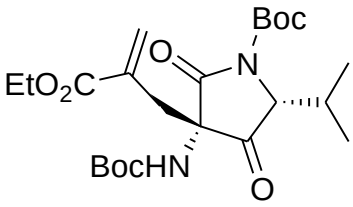
$[\alpha]_D^{20}$=+61.4 (C=5.7x10^{-3}, MeOH); **^{1}H NMR** (DMSO-d_6, 600 MHz, 25°C, TMS) δ 0.86 (d, 3H, *J*=6.7 Hz, $H_{CH(C\underline{\mathbf{H}}3)2}$), 0.91 (d, 3H, *J*=6.5 Hz, $H_{CH(C\underline{\mathbf{H}}3)2}$), 1.77 (dsept, 1H, *J*=6.7 Hz, *J*=7.8 Hz, $H_{C\underline{\mathbf{H}}(CH3)2}$), 2.04 (dd, 1H, *J*=9.7 Hz, *J*=13.1 Hz, $H_{C\underline{\mathbf{H}}2Cquat*}$), 2.17 (dd, 1H, *J*=8.5 Hz, *J*=13.1 Hz, $H_{C\underline{\mathbf{H}}2Cquat*}$), 2.93 (m, 1H, $H_{Cquat*C\underline{\mathbf{H}}2CH}$), 3.01 (m, 1H, $H_{C\underline{\mathbf{H}}*CH2NHBn}$), 3.23 (m, 1H, $H_{C\underline{\mathbf{H}}CHOH}$), 3.25 (m, 1H, $H_{Cquat*C\underline{\mathbf{H}}2CH}$), 4.04 (d, 1H, *J*=6.0 Hz, $H_{CHC\underline{\mathbf{H}}*OH}$), 4.19 (m, 2H, $H_{C\underline{\mathbf{H}}2C6H5}$), 5.61 (d, 1H, *J*=6.0 Hz, $H_{O\underline{\mathbf{H}}}$), 7.43-7.50 (m, 5H, $H_{C6\underline{\mathbf{H}}5}$), 8.12 (s, 1H, $H_{N\underline{\mathbf{H}}COCquat*}$), 8.39 (s, 1H, $H_{Cquat*N\underline{\mathbf{H}}CO}$), 8.81 (s, br, 1H, $H_{N\underline{\mathbf{H}}}$); **^{13}C NMR** (DMSO-$d_6$, 150 MHz, 25°C, TMS) δ 19.6 ($C_{CH(\underline{\mathbf{C}}H3)2}$), 20.0 ($C_{CH(\underline{\mathbf{C}}H3)2}$), 27.6 ($C_{\underline{\mathbf{C}}H(CH3)2}$), 30.0 ($C_{\underline{\mathbf{C}}H2Cquat*}$), 38.0 ($C_{\underline{\mathbf{C}}H*CH2NHBn}$), 48.6 ($C_{CH*\underline{\mathbf{C}}H2NHBn}$), 50.7 ($C_{\underline{\mathbf{C}}H2C6H5}$), 61.1 ($C_{\underline{\mathbf{C}}H*iPr}$), 65.0 ($C_{\underline{\mathbf{C}}quat*}$), 72.1 ($C_{\underline{\mathbf{C}}H*OH}$), 128.7-131.8 ($C_{\underline{\mathbf{C6}}H5}$), 175.2 ($C_{NH\underline{\mathbf{C}}OCquat*}$), 175.5 ($C_{Cquat*NH\underline{\mathbf{C}}O\ lactam}$); **HPLC $\mathbf{r_t}$**=0.76 min; **ESI-MS$^+$** *m/z* 332.2; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{18}H_{25}N_3O_3$ + H^+] 332.1974 $gmol^{-1}$, found 332.1981 $gmol^{-1}$



(*5R*)-3-*tert*-Butoxycarbonylamino-4-hydroxy-5-isopropyl-2-oxo-2,5-dihydro-pyrrole-1-carboxylic acid *tert*-butyl ester 5 ($C_{17}H_{28}N_2O_6$, 356.41 $gmol^{-1}$): To a solution of **1** (16.80 g, 47.19 mmol, 1.0 eq.) in anhydrous THF was added, at -15°C, *t*BuOK (5.83 g, 51.91 mmol, 1.1 eq.). The solution was then stirred for 1 h under argon atmosphere at -15°C. The medium was next diluted with AcOEt, washed several times with 1.0 N HCl

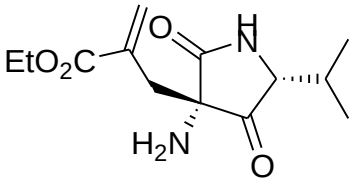
and dried on anhydrous Na_2SO_4. The solvent was removed *in vacuo* to afford the desired compound **5** as a yellow oil with a quantitative yield (m=16.80 g).

$[\alpha]_D^{20}$=-50.4 (C=11.0x10^{-3}, CH_2Cl_2); **^{1}H NMR** ($CDCl_3$, 300 MHz, 25°C, TMS) δ 0.81 (d, 3H, *J*=6.9 Hz, $H_{CH(C\underline{H3})2}$), 1.11 (d, 3H, *J*=7.2 Hz, $H_{CH(C\underline{H3})2}$), 1.45 (s, 9H, $H_{C(C\underline{H3})3}$), 1.49 (s, 9H, $H_{C(C\underline{H3})3}$), 2.45 (m, 1H, $H_{C\underline{H}(CH3)2}$), 4.26 (d, 1H, *J*=2.5 Hz, $H_{C\underline{H*}}$), 6.59 (s, br, 1H, $H_{N\underline{H}Boc}$), 11.25 (s, 1H, $H_{O\underline{H}}$); **^{13}C NMR** ($CDCl_3$, 75 MHz, 25°C, TMS) δ 15.4 ($C_{CH(\underline{C}H3)2}$), 18.4 ($C_{CH(\underline{C}H3)2}$), 27.9 ($C_{C(\underline{C}H3)3}$), 29.6 ($C_{\underline{C}H(CH3)2}$), 62.1 ($C_{\underline{C}H*iPr}$), 82.8 ($C_{\underline{C}(CH3)3}$), 83.1 ($C_{\underline{C}(CH3)3}$), 103.4 ($C_{C=\underline{C}\text{-}NHBoc}$), 149.0 ($C_{\underline{C}O\ urethane}$), 154.5 ($C_{\underline{C}O\ urethane}$), 155.7 ($C_{C=\underline{C}\text{-}OH}$), 165.3 ($C_{\underline{C}O\ lactam}$); **HPLC r_t**=2.67 min; **ESI-MS$^+$** *m/z* 357.2; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{17}H_{28}N_2O_6$ + H^+] 357.2026 gmol^{-1}, found 357.2031 gmol^{-1}. Spectral data were consistent with those reported in the literature.[1]



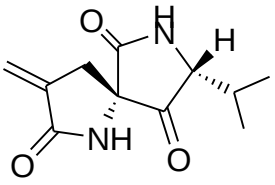
***tert*-Butyl (*3S,5R*)-1-(tert-butoxycarbonyl)-3-(2-(ethoxycarbonyl)allyl)-5-isopropyl-2,4-dioxopyrrolidin-3-ylcarbamate 6** ($C_{23}H_{36}N_2O_8$, 468.54 gmol^{-1}): To a solution of **5** (16.75 g, 47.05 mmol, 1.0 eq.) in anhydrous DMSO was added K_2CO_3 under magnetic stirring. A gentle warming was necessary for complete dissolution. The mixture became coloured, from yellow to orange. The ethyl 2-bromomethylacrylate was then added and the medium was stirred under argon atmosphere for 6 h at 20°C. Then, AcOEt was added. The organic layer was washed with 0.1 N HCl, dried on $MgSO_4$ and concentrated *in vacuo*. The crude residue was purified by column chromatography on silica gel (CH_2Cl_2/AcOEt; 99.5:0.5 → 97:3), affording the derivative **6** with 70% yield (m=14.31 g).

TLC R_f=0.51 (CH_2Cl_2/AcOEt; 97:3, UV); **$[\alpha]_D^{20}$**=-87.3 (C=15.0x10^{-3}, CH_2Cl_2); **^{1}H NMR** ($CDCl_3$, 400 MHz, 25°C, TMS) δ 1.08 (d, 3H, *J*=7.0 Hz, $H_{CH(C\underline{H3})2}$), 1.15 (d, 3H, *J*=7.2 Hz, $H_{CH(C\underline{H3})2}$), 1.31 (t, 3H, *J*=7.2 Hz, $H_{C\underline{H3}\text{-}CH2}$), 1.36 (s, 9H, $H_{C(C\underline{H3})3}$), 1.56 (s, 9H, $H_{C(C\underline{H3})3}$), 2.53 (m, 1H, $H_{C\underline{H}(CH3)2}$), 2.72 (m, 2H, $H_{C\underline{H2}Cquat*}$), 4.22 (q, 2H, *J*=7.2 Hz, $H_{CH3\text{-}C\underline{H2}}$), 4.31 (d, 1H, *J*=4.4 Hz, $H_{C\underline{H*}iPr}$), 5.65 (s, 1H, $H_{\underline{H2}CC(CO2Et)CH2\text{-}}$), 5.77 (s, br, 1H, $H_{N\underline{H}Boc}$), 6.38 (s, 1H, $H_{\underline{H2}CC(CO2Et)CH2\text{-}}$); **^{13}C NMR** ($CDCl_3$, 100 MHz, 25°C, TMS) δ 14.0 ($C_{\underline{C}H3\text{-}CH2}$), 18.6 ($C_{CH(\underline{C}H3)2}$), 19.2 ($C_{CH(\underline{C}H3)2}$), 28.0 ($C_{C(\underline{C}H3)3}$), 28.1 ($C_{C(\underline{C}H3)3}$), 30.6 ($C_{\underline{C}H(CH3)2}$), 36.3 ($C_{\underline{C}H2Cquat*}$), 61.8 ($C_{\underline{C}H2\text{-}CH3}$), 64.4 ($C_{\underline{C}quat*}$), 67.5 ($C_{\underline{C}H*}$), 80.9 ($C_{\underline{C}(CH3)3}$), 83.9 ($C_{\underline{C}(CH3)3}$), 131.3 ($C_{H2\underline{C}=C(CO2Et)CH2\text{-}}$), 133.0 ($C_{H2C=\underline{C}(CO2Et)CH2\text{-}}$), 149.9 ($C_{\underline{C}O\ urethane}$), 154.1 ($C_{\underline{C}O\ urethane}$), 166.6 ($C_{\underline{C}O\ ester}$), 169.6 ($C_{\underline{C}O\ lactam}$), 204.5 ($C_{\underline{C}O\ ketone}$); **HPLC r_t**=2.81 min; **ESI-MS$^+$** *m/z* 469.3; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{23}H_{36}N_2O_8$ + H^+] 469.2550 gmol^{-1}, found 469.2541 gmol^{-1}.



Ethyl 2-(((*3S,5R*)-3-amino-5-isopropyl-2,4-dioxopyrrolidin-3-yl)methyl)acrylate 7 ($C_{13}H_{20}N_2O_4$, 268.31 gmol^{-1}): A solution of **6** (344 mg, 0.734 mmol, 1.0 eq.) in 0.5 mL of trifluoroacetic acid was stirred at 20°C during 1 h before being co-evaporated twice with cyclohexane in order to remove the excess of trifluoroacetic acid. The resulting yellow oil was then dissolved in dichloromethane and Amberlyst A-21 was added to scavenge the remaining TFA. The mixture was stirred for 2 h, filtered and the resin was washed twice with DCM. The filtrate was then concentrated *in vacuo* and free base amine **7** was obtained with a quantitative yield (m=197 mg).

TLC $\mathbf{R_f}$=0.23 (CH_2Cl_2/MeOH; 96:4, UV); **m.p.** 109 °C; $\mathbf{[\alpha]_D^{20}}$ = +26.1 (C=23.0x10^{-3}, MeOH); **^{1}H NMR** ($CDCl_3$, 600 MHz, 25°C, TMS) δ 0.84 (d, 3H, *J*=6.8 Hz, $H_{CH(C\underline{H3})2}$), 1.04 (d, 3H, *J*=7.0 Hz, $H_{CH(C\underline{H3})2}$), 1.29 (t, 3H, *J*=7.1 Hz, $H_{C\underline{H3}\text{-}CH2}$), 1.67 (s, br, 2H, $H_{N\underline{H2}}$), 2.22 (m, 1H, $H_{C\underline{H}(CH3)2}$), 2.65 (d, 1H, *J*=13.4 Hz, $H_{C\underline{H2}Cquat*}$), 2.85 (d, 1H, *J*=13.4 Hz, $H_{C\underline{H2}Cquat*}$), 3.98 (d, 1H, *J*=3.7 Hz, $H_{C\underline{H}*iPr}$), 4.17 (m, 2H, $H_{CH3\text{-}C\underline{H2}}$), 5.75 (d, 1H, *J*=1.3 Hz, $H_{\underline{H2}C=C(CO2Et)CH2\text{-}}$), 6.35 (d, 1H, *J*=1.3 Hz, $H_{\underline{H2}C=C(CO2Et)CH2\text{-}}$), 7.14 (s, br, 1H, $H_{N\underline{H}CO}$); **^{13}C NMR** ($CDCl_3$, 150 MHz, 25°C, TMS) δ 14.2 ($C_{\underline{C}H3\text{-}CH2}$), 16.9 ($C_{CH(\underline{C}H3)2}$), 19.3 ($C_{CH(\underline{C}H3)2}$), 29.8 ($C_{\underline{C}H(CH3)2}$), 39.9 ($C_{\underline{C}H2Cquat*}$), 61.3 ($C_{\underline{C}H2\text{-}CH3}$), 61.8 ($C_{\underline{C}quat*}$), 66.1 ($C_{\underline{C}H*}$), 130.1 ($C_{H2C=\underline{C}(CO2Et)CH2\text{-}}$), 133.8 ($C_{H2\underline{C}=C(CO2Et)CH2\text{-}}$), 166.5 ($C_{\underline{C}Oester}$), 175.4 ($C_{\underline{C}Olactam}$), 210.1 ($C_{\underline{C}Oketone}$); **HPLC** $\mathbf{r_t}$=0.97 min; **ESI-MS$^+$** *m/z* 269.2; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{13}H_{20}N_2O_4$ + H^+] 269.1501 gmol^{-1}, found 269.1494 gmol^{-1}.



8-Isopropyl-3-methylene-1,7-diaza-spiro[4.4]nonane-2,6,9-trione **9** ($C_{11}H_{14}N_2O_3$, 222.24 gmol^{-1}): A solution of **8** (116 mg, 0.43 mmol, 1.0 eq.) in 3 mL of absolute ethanol was heated using microwave irradiation at 130°C for 1 h. After evaporation to dryness of the reaction mixture, the residue was triturated in AcOEt and filtered off to afford **9** as a white solid with 90% yield (m=86 mg).

TLC $\mathbf{R_f}$=0.59 (CH_2Cl_2/MeOH; 9:1, UV); **m.p.** 219°C (decomposition); $\mathbf{[\alpha]_D^{20}}$=+45.9 (C=3.7x10^{-3}, MeOH); **^{1}H NMR** (DMSO-d_6, 600 MHz, 25°C, TMS) δ 0.81 (d, 3H, *J*=6.9 Hz, $H_{CH(C\underline{H3})2}$), 0.97 (d, 3H, *J*=6.9 Hz, $H_{CH(C\underline{H3})2}$), 2.07 (dsept, 1H, *J*=4.0 Hz, *J*=6.9 Hz, $H_{C\underline{H}(CH3)2}$), 2.77 (dt, 1H, *J*=2.6 Hz, *J*=17.4 Hz, $H_{C\underline{H2}Cquat*}$), 2.86 (dt, 1H, *J*=2.2 Hz, *J*=17.4 Hz, $H_{C\underline{H2}Cquat*}$), 4.11 (d, 1H, *J*=4.0 Hz, $H_{C\underline{H}*}$), 5.38 (s, br, 1H, $H_{C\underline{H2}=C}$), 5.77 (t, br, 1H, *J*=2.2 Hz, $H_{C\underline{H2}=C}$), 8.74 (s, br, 1H, $H_{N\underline{H}COC=CH2}$), 8.92 (s, br, 1H, $H_{N\underline{H}COCquat*}$); **^{13}C NMR** (DMSO-$d_6$, 150 MHz, 25°C, TMS) δ 17.1 ($C_{CH(\underline{C}H3)2}$), 18.8 ($C_{CH(\underline{C}H3)2}$), 29.3 ($C_{\underline{C}H(CH3)2}$), 34.7 ($C_{\underline{C}H2Cquat*}$), 60.9 ($C_{CH2\underline{C}quat*}$), 64.5 ($C_{\underline{C}H*}$), 115.6 ($C_{\underline{C}H2=C}$), 137.4 ($C_{CH2=\underline{C}}$), 169.9 ($C_{NH\underline{C}OC=CH2}$), 171.8 ($C_{NH\underline{C}OCquat*}$), 210.8 ($C_{\underline{C}Oketone}$); **HPLC** $\mathbf{r_t}$=0.94 min; **ESI-MS$^+$** *m/z* 223.1; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{11}H_{14}N_2O_3$ + H^+] 223.1083 gmol^{-1}, found 223.1083 gmol^{-1}.

As depicted below, a cautious 2D-NOESY experiment of **9** in DMSO-d_6 was performed, highlighting the absence of nOe correlations between NH(*5*) and H(*11*). This analysis proved the retention of the *R* configuration of C(*11*) during the heating step.

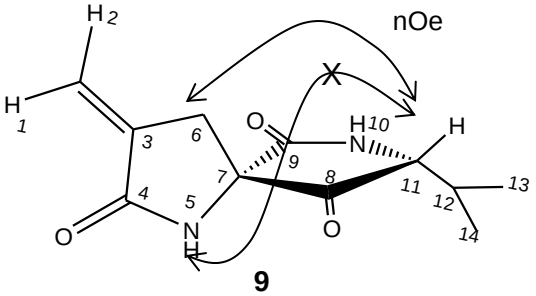


Figure S1. Atoms numbering of **9** used for NMR study.

Table S1. Chemical shifts, multiplicity, *J*-values and nOe correlations determined by NMR analysis of **9** in DMSO-d_6 (600 MHz).

Atoms	$\delta_{^{13}C}$ (ppm)	$\delta_{^{1}H}$ (ppm)	Multiplicity	*J* (Hz)	nOe correlations
C=O (*8*)	210.8	-		-	
C=O (*9*)	171.8	-		-	
C=O (*4*)	169.9	-		-	
C (*3*)	137.4	-		-	
CH (*1*)	115.6	5.77	t, br	2.2	*6* w
CH (*2*)		5.38	s, br	-	*6* w
CH (*11*)	64.5	4.11	d	4.0	*10* s / *12* s / *13-14* s / *6* m / *6'* w / *5* w
C (*7*)	60.9	-	-	-	
CH_2 (*6*)	34.7	2.86	dt	2.3 ; 17.4	*11* m / *2* m / *1* w
		2.77	dt	2.6 ; 17.4	*11* w / *2* m / *1* w
CH (*12*)	29.3	2.07	dsept	4.0 ; 6.9	*13-14* s / *11* s / *10* m
CH_3 (*13*)	18.8	0.97	d	6.9	*6* S / *11* s / *10* s / *5* m
CH_3 (*14*)	17.1	0.81	d	6.9	
NH (*10*)	-	8.92	s, br	-	*13-14* s / *11* s / *12* m / *6* w
NH (*5*)	-	8.74	s, br	-	*13-14* m / *12* w / *6* w

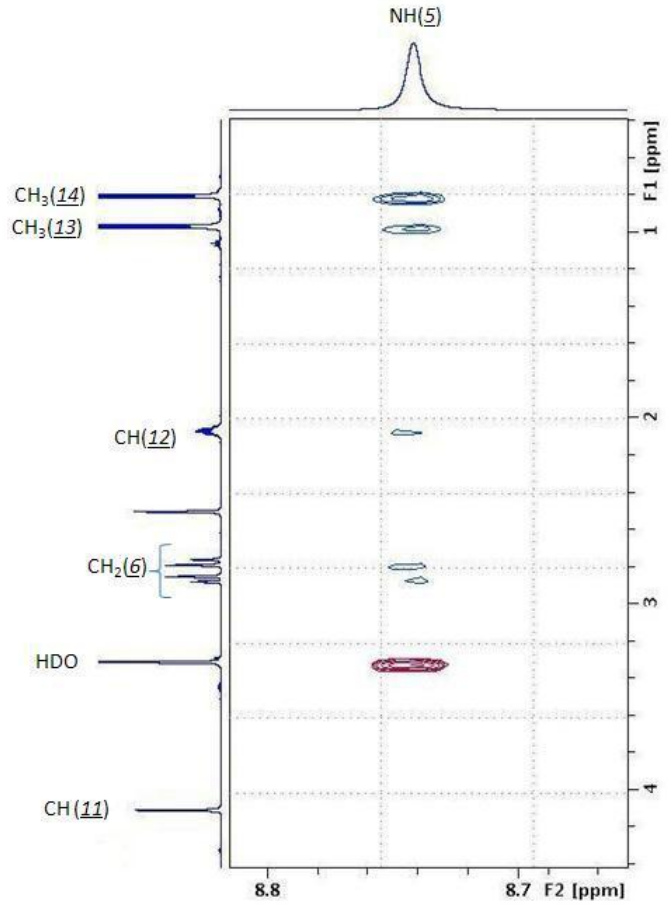
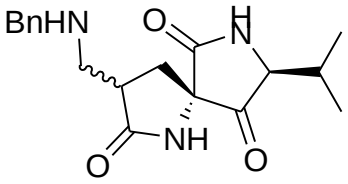


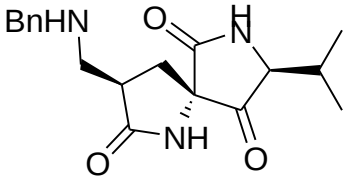
Figure S2. Relevant correlation on the NOESY spectra used for the stereospecific assignment of CH(*11*) in compound **9** in DMSO-d_6 (600 MHz).

Functionalization of the spirolactam scaffold

General procedure for the synthesis of the functionalized spirolactam.

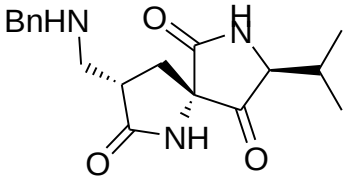


A solution of **7** (2.07 g, 5.42 mmol, 1.0 eq.) and benzylamine (5.0 eq.) in absolute ethanol was heated using microwave irradiation at 130°C for 1 h, and then concentrated *in vacuo*. After evaporation to dryness of the reaction mixture, the resulting crude was dissolved in THF and 1.0 N HCl was added until the pH fell below pH 2. The mixture was stirred at 20°C for 1.5 h and evaporated to dryness. The residue was triturated in AcOEt and filtered off to obtain a mixture of two diastereoisomers **8a** and **8b** (4:6), after removing solvent *in vacuo*, with 95% yield. Less than 5% of two other epimers were detected at this step.



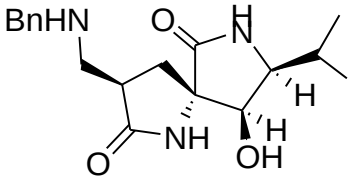
(*3R,5S,8R*)-3-(Benzylamino-methyl)-8-isopropyl-1,7-diaza-spiro[4.4]nonane-2,6,9-trione 8a ($C_{18}H_{23}N_3O_3$, 329.39 gmol^{-1}): **8a** was synthesized according to the general procedure for the synthesis of the functionalized spirolactam with 40% yield (m=588 mg) after purification by preparative HPLC (linear gradient of 0-30% B over a 1 h period) and lyophilisation.

$[\alpha]_D^{20}$=-59.5 (C=12.1x10^{-3}, MeOH); **^{1}H NMR** (D_2O, 300 MHz, 25°C, TMS) δ 0.86 (d, 3H, *J*=6.7 Hz, $H_{CH(C\underline{H3})2}$), 0.91 (d, 3H, *J*=6.9 Hz, $H_{CH(C\underline{H3})2}$), 1.88 (dd, 1H, *J*=13.4 Hz, *J*=8.7 Hz, $H_{C\underline{H2}Cquat*}$), 2.01 (m, 1H, $H_{C\underline{H}(CH3)2}$), 2.42 (dd, 1H, *J*=13.5 Hz, *J*=8.7 Hz, $H_{C\underline{H2}Cquat*}$), 3.11-3.33 (m, 3H, $H_{C\underline{H}*CH2NHBn}$ + $H_{C\underline{H2}NHBn}$), 3.99 (d, 1H, *J*=5.6 Hz, $H_{C\underline{H}*}$), 4.23 (s, 2H, $H_{C\underline{H2}C6H5}$), 7.42 (s, 5H, $H_{C6\underline{H5}}$); **^{13}C NMR** (D_2O, 75 MHz, 25°C, TMS) δ 17.1 ($C_{CH(\underline{C}H3)2}$), 17.9 ($C_{CH(\underline{C}H3)2}$), 31.0 ($C_{\underline{C}H(CH3)2}$), 34.1 ($C_{\underline{C}H2Cquat*}$), 37.1 ($C_{\underline{C}H*CH2NHBn}$), 47.1 ($C_{\underline{C}H2NHBn}$), 51.4 ($C_{\underline{C}H2C6H5}$), 62.8 ($C_{\underline{C}quat*}$), 67.5 ($C_{\underline{C}H*iPr}$), 129.3-130.1 ($C_{\underline{C6}H5}$), 174.1 ($C_{\underline{C}O\ lactam}$), 178.8 ($C_{\underline{C}O\ lactam}$), 211.1 ($C_{\underline{C}O\ ketone}$); **HPLC t_r**=1.26 min; **ESI-MS$^+$** *m/z* 330.2; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{18}H_{23}N_3O_3$ + H^+] 330.1818 gmol^{-1}, found 330.1803 gmol^{-1}.



(*3S,5S,8R*)-3-(Benzylamino-methyl)-8-isopropyl-1,7-diaza-spiro[4.4]nonane-2,6,9-trione 8b ($C_{18}H_{23}N_3O_3$, 329.39 g.mol^{-1}): **8b** was synthesized according to the general procedure for the synthesis of the functionalized spirolactam with 45% yield (m=535 mg) after purification by preparative HPLC (linear gradient of 0-30% B over a 1 h period) and lyophilisation.

$[\alpha]_D^{20}$=-3.7 (C=13.0x10^{-3}, MeOH); **^{1}H NMR** (D_2O, 300 MHz, 25°C, TMS) δ 0.86 (d, 3H, *J*=6.8 Hz, $H_{CH(C\underline{H3})2}$), 0.90 (d, 3H, *J*=6.9 Hz, $H_{CH(C\underline{H3})2}$), 2.01 (m, 2H, $H_{C\underline{H}(CH3)2}$ + $H_{C\underline{H2}Cquat*}$), 2.42 (dd, 1H, *J*=13.6 Hz, *J*=9.1 Hz, $H_{C\underline{H2}Cquat*}$), 3.02-3.30 (m, 3H, $H_{C\underline{H}*CH2NHBn}$ + $H_{C\underline{H2}NHBn}$), 3.91 (d, 1H, *J*=6.1 Hz, $H_{C\underline{H}*}$), 4.20 (s, 2H, $H_{C\underline{H2}C6H5}$), 7.39 (s, 5H, $H_{C6\underline{H5}}$); **^{13}C NMR** (D_2O, 75 MHz, 25°C, TMS) δ 17.4 ($C_{CH(\underline{C}H3)2}$), 17.9 ($C_{CH(\underline{C}H3)2}$), 31.1 ($C_{\underline{C}H(CH3)2}$), 32.7 ($C_{\underline{C}H2Cquat*}$), 36.8 ($C_{\underline{C}H*CH2NHBn}$), 47.1 ($C_{\underline{C}H2NHBn}$), 51.4 ($C_{\underline{C}H2C6H5}$), 62.7 ($C_{\underline{C}quat*}$), 67.2 ($C_{\underline{C}H*iPr}$), 129.3-130.1 ($C_{\underline{C6}H5}$), 173.4 ($C_{\underline{C}O\ lactam}$), 178.8 ($C_{\underline{C}O\ lactam}$), 210.8 ($C_{\underline{C}O\ ketone}$); **HPLC r_t**=1.20 min; **ESI-MS$^+$** *m/z* 330.2; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{18}H_{23}N_3O_3$ + H^+] 330.1818 gmol^{-1}, found 330.1798 gmol^{-1}.



(*3R,5S,8R,9R*)-3-(Benzylamino-methyl)-9-hydroxy-8-isopropyl-1,7-diaza-spiro[4.4]nonane-2,6-dione 10a ($C_{18}H_{25}N_3O_3$, 331.41 gmol^{-1}): To a solution of **8a**

(100 mg, 0.30 mmol, 1.0 eq.) in 5 mL of THF/water (4/1) was added $NaBH_4$ (34 mg, 0.90 mmol, 3.0 eq.) at 0°C under stirring. After 1.5 h, the reaction mixture was neutralised with 0.1 N HCl and evaporated to dryness. The crude was then purified by preparative HPLC (linear gradient of 0-15% B over a 30 min. period). Compound **10a** was obtained with 90% yield after lyophilisation (m=91 mg).

$[\alpha]_D^{20}$=-61.4 (C=5.7x10^{-3}, MeOH); **^{1}H NMR** (DMSO-d_6, 600 MHz, 25°C, TMS) δ 0.87 (d, 3H, *J*=6.7 Hz, $H_{CH(C\underline{H}3)2}$), 0.91 (d, 3H, *J*=6.5 Hz, $H_{CH(C\underline{H}3)2}$), 1.77 (dsept, 1H, *J*=6.7 Hz, *J*=7.8 Hz, $H_{C\underline{H}(CH3)2}$), 2.05 (dd, 1H, *J*=10.0 Hz, *J*=13.1 Hz, $H_{C\underline{H}2Cquat*}$), 2.17 (dd, 1H, *J*=8.7 Hz, *J*=13.1 Hz, $H_{C\underline{H}2Cquat*}$), 2.94 (m, 1H, $H_{Cquat*C\underline{H}2CH}$), 3.01 (tdd, 1H, *J*=5.1 Hz, *J*=8.7 Hz, *J*=10.0 Hz, $H_{C\underline{H}*CH2NHBn}$), 3.22 (dd, br, 1H, *J*=6.0 Hz, *J*=7.8 Hz, $H_{C\underline{H}CHOH}$), 3.25 (m, 1H, $H_{Cquat*C\underline{H}2CH}$), 4.05 (d, 1H, *J*=6.0 Hz, $H_{CHC\underline{H}*OH}$), 4.21 (m, 2H, $H_{C\underline{H}2C6H5}$), 5.63 (s, 1H, $H_{O\underline{H}}$), 7.43-7.50 (m, 5H, $H_{C6\underline{H}5}$), 8.11 (s, 1H, $H_{N\underline{H}COCquat*}$), 8.40 (s, 1H, $H_{Cquat*N\underline{H}CO}$), 8.79 (s, br, 1H, $H_{N\underline{H}}$); **^{13}C NMR** (DMSO-d_6, 150 MHz, 25°C, TMS) δ 19.6 ($C_{CH(\underline{C}H3)2}$), 20.0 ($C_{CH(\underline{C}H3)2}$), 27.6 ($C_{\underline{C}H(CH3)2}$), 30.0 ($C_{\underline{C}H2Cquat*}$), 38.0 ($C_{\underline{C}H*CH2NHBn}$), 48.6 ($C_{CH*\underline{C}H2NHBn}$), 50.7 ($C_{\underline{C}H2C6H5}$), 61.1 ($C_{\underline{C}H*iPr}$), 65.0 ($C_{\underline{C}quat*}$), 72.1 ($C_{\underline{C}H*OH}$), 128.7-131.8 ($C_{\underline{C}6H5}$), 175.2 ($C_{NH\underline{C}OCquat*}$), 175.5 ($C_{Cquat*NH\underline{C}O\ lactam}$); **HPLC r_t**=1.12 min; **ESI-MS$^+$** *m/z* 332.2; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{18}H_{25}N_3O_3$ + H^+] 332.1974 gmol^{-1}, found 332.1965 gmol^{-1}.

Determination of the relative configuration of **10a**

Determination of the relative configuration of **10a** was accomplished by cautious NMR analyses. Those results were confirmed by a comparison with NMR analyses of its enantiomer **4**, its absolute configuration having been solved by single crystal X-ray analysis.

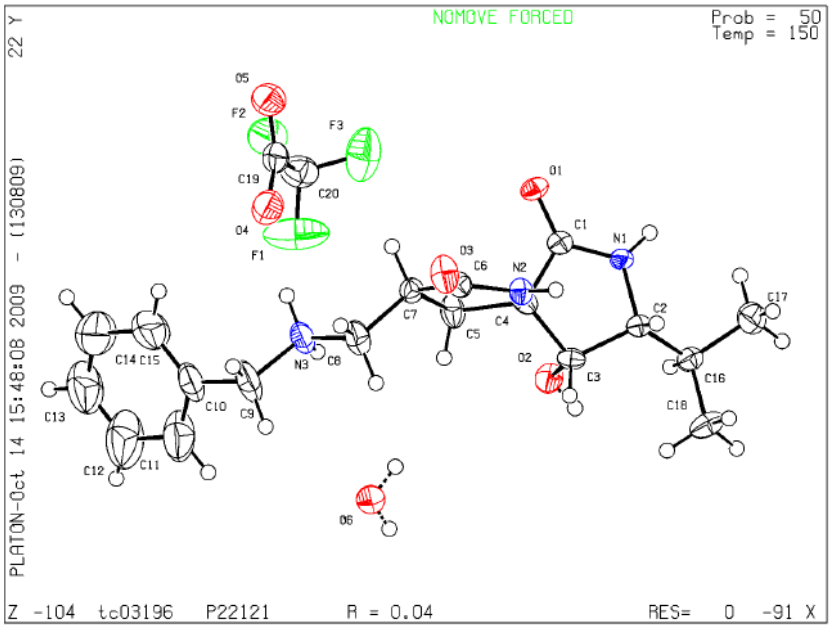


Figure S3. Single crystal of **4** (CCDC 751234 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.)

As depicted below (Figure S4 and S5), a cautious 2D-NOESY experiment of **10a** and **4** in DMSO-d_6 was performed, highlighting the inversion of the configuration of the C(*16*) bearing the *iso*-propyl moiety during the Michael addition/ Spirocyclisation tandem reaction. The presence of relevant nOe correlations between NH(*7*) and H(*17*), NH(*7*) and H(*16*), H(*16*) and H(*17*), and the absence of nOe correlations between H(*16*) and H(*10*) or H(*10'*), proved the configuration change of C(*16*) during the heating step, the *iso*-propyl group and the hydroxyl group being in a *cis* conformation. Comparison between NMR studies of **10a** and **4** confirmed their relation as enantiomers since chemical shifts, multiplicity and nOe correlations are identical. The absolute relative configuration of **10a** was then deduced from the one of **4**, which is given with absolute certainty thanks to the single crystal X-ray analysis.

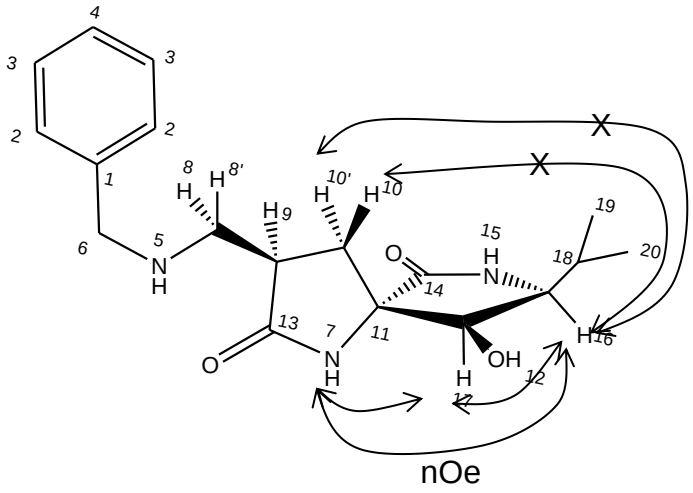


Figure S4. Atoms numbering of isomer **10a** used for NMR study. Selected 2D-NMR correlations observed, validating the configuration of the C(*16*).

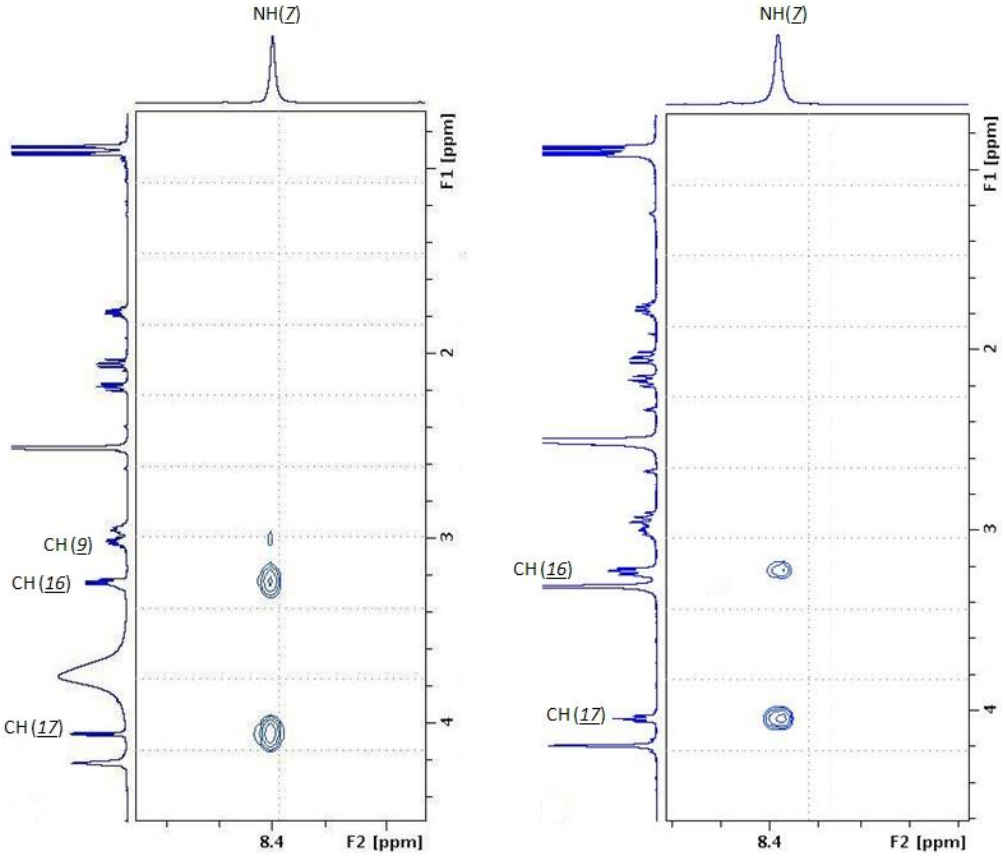


Figure S5. Relevant correlations on the NOESY spectra used for the stereospecific assignment of CH(*16*) in compound **10a** (on the left) in DMSO-d_6 (600 MHz) and in compound **4** (on the right) in DMSO-d_6 (400 MHz).

2D-NOESY experiments allowed us also to determine the relative configuration of C(*9*). Strong nOe correlations between H(*9*) and H(*10'*), and weak nOe correlations between H(*9*) and H(*10*), highlighted thus a *cis*-relation between H(*9*) and H(*10'*) and, as a result, a *trans*-relation between H(*9*) and H(*10*). Since only H(*10'*) gave a nOe correlation with H(*17*), whose configuration was previously found to be *R*, H(*10'*) was necessarily pro-*S* and C(*9*) was thus attributed with a *R* configuration. This conclusion was confirmed by the fact that C(*9*) has a *S* configuration in **4**.

Table S2. Chemical shifts, multiplicity, *J*-values and nOe correlations determined by NMR analysis of **10a** (600 MHz) and its enantiomer **4** in DMSO-d_6 (400 MHz).

Atoms	δ ^{13}C (ppm)		δ ^{1}H (ppm)		Multiplicity		Significant nOe correlations	
	4	**10a**	**4**	**10a**	**4**	**10a**	**4**	**10a**
C (*1*)	131.9[b]	131.8	-	-	-	-	-	-
CH (*2*)	129.9	130.0	7.50	7.50	m	m	*6* s / *8'* w / *19-20* w	*6* s / *8'* m / *19-20* w
CH (*3*)	128.6	128.7	7.43	7.43	m	m	-	-
CH (*4*)	128.9	129.1	7.45	7.45	m	m	-	-
NH (*5*)	-	-	8.80[a]	8.79[a]	s, br	s, br	-	-
CH_2 (*6*)	50.7	50.7	4.19	4.21	m	m	*2* s / *5* m /*8* s / *8'* s	*2* s / *5* s / *8* s / *8'* s
NH (*7*)	-	-	8.39	8.40	s	s	*16* m / *17* s	*16* s / *17* s
CH_2 (*8*)	48.6	48.6	3.24	3.24	m	m	-	-
CH_2 (*8'*)			2.93	2.94	m	m	*2* w / *6* m / *8* s / *10* w	*2* w / *6* m / *8* s / *10* m
CH (*9*)	38.0	38.0	3.01	3.01	m	tdd - 5.1; 8.7; 10.0 Hz	*6* w / *10* w / *10'* s	*6* m / *10* w / *10'* s
CH_2 (*10'*)	29.9	30.0	2.17	2.17	dd - 8.5; 13.1 Hz	dd - 8.7; 13.1 Hz	*9* m / *10* s / *18* w / *19-20* w	*9* s / *10* s / *18* w / *19-20* w
CH_2 (*10*)			2.04	2.05	dd - 9.7; 13.1 Hz	dd - 10.0; 13.1 Hz	*8'* m / *9* w / *10'* s	*8'* s / *9* w / *10'* s
C (*11*)	65.0	65.0	-	-	-	-	-	-
OH (*12*)	-	-	5.61[a]	5.63[a]	d - 6.0 Hz	s, br	-	-
C (*13*)	175.5	175.5	-	-	-	-	-	-
C (*14*)	175.2	175.2	-	-	-	-	-	-
NH (*15*)	-	-	8.12	8.11	s	s	*16* s / *17* w / *18* m / *19-20* s	*16* s / *17* w / *18* m / *19-20* s
CH (*16*)	61.0	61.1	3.23	3.22	m	dd, br - 6.0; 7.8 Hz	*6* w / *7* m / *15* m / *17* s / *18* m / *19-20* s	*7* m / *15* m / *17* s / *18* m / *19-20* s
CH (*17*)	72.1	72.1	4.04	4.05	t - 6.0 Hz	d - 6.0 Hz	*7* s / *10* w / *16* s / *18* w / *19-20* m	*7* s / *10* m / *16* s / *18* w / *19-20* m
CH (*18*)	27.5	27.6	1.77	1.77	m	dsept - 6.6; 7.8 Hz	*10'* w / *15* w / *16* m / *17* w / *19-20* s	*10'* m / *15* w / *16* m / *17* w / *19-20* s
CH_3 (*19*)	19.9	20.0	0.91	0.91	d - 6.6 Hz	d - 6.6 Hz	*12* w / *15* s / *16* s / *17* m / *18* s	*10'* w / *15* s / *16* s / *17* m / *18* s
CH_3 (*20*)	19.6	19.6	0.86	0.87	d - 6.6 Hz	d - 6.6 Hz		

[a] because of the difference of concentrations between the two samples **10a** and **4**, the amount of H_2O might vary causing differences in the resolution of those signals; [b] determined by HMBC (see Figure S6 below).

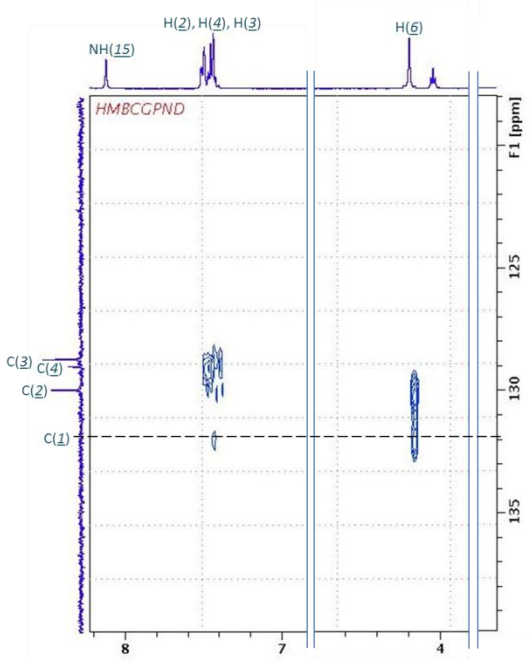
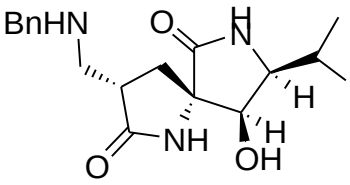
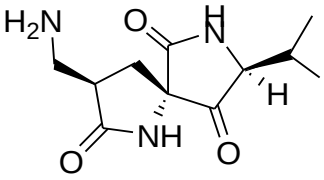


Figure S6. HMBC correlations allowing determining the chemical shift of C (*1*).



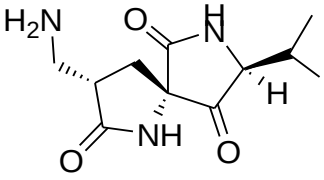
(*3S,5S,8R,9R*)-3-(Benzylamino-methyl)-9-hydroxy-8-isopropyl-1,7-diaza-spiro[4.4]nonane-2,6-dione 10b ($C_{18}H_{25}N_3O_3$, 331.41 gmol^{-1}): To a solution of **8b** (38 mg, 0.12 mmol, 1.0 eq.) in 5 mL of THF/water (4/1) was added $NaBH_4$ (14 mg, 0.36 mmol, 3.0 eq.) at 0°C under stirring. After 1.5 h, the reaction mixture was neutralised with 0.1 N HCl and evaporated to dryness. The crude was then purified by preparative HPLC (linear gradient of 0-15% B over a 30 min. period). Compound **10b** was obtained with 90% yield after lyophilisation (m=32 mg).

[α]$_D^{20}$=- 42.2 (C=18.0x10^{-3}, MeOH); **^{1}H NMR** (D_2O, 300 MHz, 25°C, TMS) δ 0.84 (d, 3H, *J*=4.6 Hz, $H_{CH(C\underline{H3})2}$), 0.86 (d, 3H, *J*=4.5 Hz, $H_{CH(C\underline{H3})2}$), 1.74 (m, 1H, $H_{C\underline{H}(CH3)2}$), 1.81 (dd, 1H, *J*=13.7 Hz, *J*=8.2 Hz, $H_{C\underline{H2}Cquat*}$), 2.62 (dd, 1H, *J*=13.8 Hz, *J*=9.5 Hz, $H_{C\underline{H2}Cquat*}$), 2.98 (m, 1H, $H_{C\underline{H}*CH2NHBn}$), 3.17-3.33 (m, 3H, $H_{C\underline{H*}iPr}$ + $H_{C\underline{H2}NHBn}$), 4.20 (s, 2H, $H_{C\underline{H2}C6H5}$), 4.25 (d, 1H, *J*=5.6 Hz, $H_{C\underline{H}*OH}$), 7.38 (s, 5H, $H_{C6\underline{H5}}$); **^{13}C NMR** (D_2O, 75 MHz, 25°C, TMS) δ 18.9 ($C_{CH(\underline{C}H3)2}$), 27.3 ($C_{\underline{C}H(CH3)2}$), 27.5 ($C_{\underline{C}H2Cquat*}$), 37.4 ($C_{\underline{C}H*CH2NHBn}$), 47.5 ($C_{\underline{C}H2NHBn}$), 51.4 ($C_{\underline{C}H2C6H5}$), 62.1 ($C_{\underline{C}H*iPr}$), 67.0 ($C_{\underline{C}quat*}$), 73.2 ($C_{\underline{C}H*OH}$), 129.3-130.2 ($C_{\underline{C6}H5}$), 176.0 ($C_{\underline{C}O\ lactam}$), 178.2 ($C_{\underline{C}O\ lactam}$); **HPLC r_t**=1.07 min; **ESI-MS$^+$** *m/z* 332.2; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{18}H_{25}N_3O_3$ + H$^+$] 332.1974 gmol^{-1}, found 332.1962 gmol^{-1}.



(*3R,5S,8R*)-3-Aminomethyl-8-isopropyl-1,7-diaza-spiro[4.4]nonane-2,6,9-trione 11a ($C_{11}H_{17}N_3O_3$, 239.27 gmol^{-1}): A mixture of *N*-benzylated compound **8a** (60 mg, 0.18 mmol, 1.0 eq.) and 6 mg (10 wt%) of 10% Pd/C in a 3.0 M HCl/EtOH (1: 9) solution was kept under 1 atm of H_2 at 20°C for 2 days (no starting material left according to HPLC analysis). The mixture was then filtered through Celite and evaporated to dryness. The crude was then purified by preparative HPLC (linear gradient of 0-15% B over a 30 min. period). Compound **11a** was obtained with 93% yield after lyophilisation (m=41 mg).

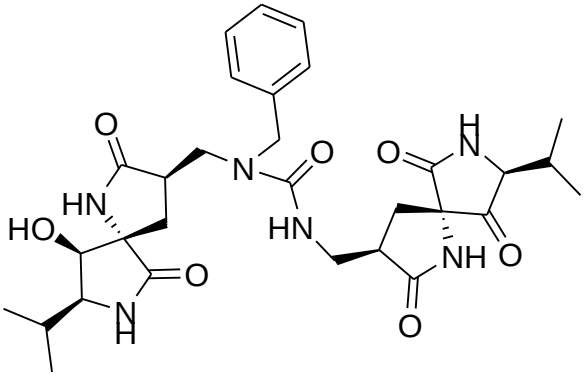
[α]$_D^{20}$=-13.3 (C=24.0x10^{-3}, MeOH); **^{1}H NMR** (D_2O, 300 MHz, 25°C, TMS) δ 0.87 (d, 3H, *J*=6.8 Hz, $H_{CH(C\underline{H3})2}$), 0.92 (d, 3H, *J*=6.9 Hz, $H_{CH(C\underline{H3})2}$), 1.93 (dd, 1H, *J*=13.1 Hz, *J*=8.4 Hz, $H_{C\underline{H2}Cquat*}$), 2.05 (m, 1H, $H_{C\underline{H}(CH3)2}$), 2.66 (dd, 1H, *J*=13.0 Hz, *J*=8.2 Hz, $H_{C\underline{H2}Cquat*}$), 3.06-3.32 (m, 3H, $H_{C\underline{H}*CH2NHBn}$ + $H_{C\underline{H2}NHBn}$), 4.00 (d, 1H, *J*=5.7 Hz, $H_{C\underline{H*}}$); **^{13}C NMR** (D_2O, 75 MHz, 25°C, TMS) δ 16.3 ($C_{CH(\underline{C}H3)2}$), 17.1 ($C_{CH(\underline{C}H3)2}$), 30.1 ($C_{\underline{C}H(CH3)2}$), 33.0 ($C_{\underline{C}H2Cquat*}$), 36.9 ($C_{\underline{C}H*CH2NH2}$), 39.1 ($C_{\underline{C}H2NHBn}$), 62.0 ($C_{\underline{C}quat*}$), 66.7 ($C_{\underline{C}H*iPr}$), 173.4 ($C_{\underline{C}O\ lactam}$), 178.4 ($C_{\underline{C}O\ lactam}$), 210.4 ($C_{\underline{C}O\ ketone}$); **HPLC r_t**=0.63 min; **ESI-MS$^+$** *m/z* 240.1; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{11}H_{17}N_3O_3$ + H$^+$] 240.1348 gmol^{-1}, found 240.1342 gmol^{-1}.



(*3S,5S,8R*)-3-Aminomethyl-8-isopropyl-1,7-diaza-spiro[4.4]nonane-2,6,9-trione 11b ($C_{11}H_{17}N_3O_3$, 239.27 gmol^{-1}): A mixture of *N*-benzylated compound **8b** (38 mg, 0.12 mmol, 1.0 eq.) and 3.8 mg (10 wt%) of 10% Pd/C in a 3.0 M HCl/EtOH (1: 9) solution was kept under 1 atm of H_2 at 20°C for 2 days (no starting material left according to HPLC analysis). The mixture was then filtered through Celite and evaporated to dryness. The crude was then purified by preparative HPLC (linear gradient of 0-15% B over a 30 min. period). Compound **11b** was obtained with 95% yield after lyophilisation (m=25 mg).

$[\alpha]_D^{20}$=- 6.3 (C=24.0x10^{-3}, MeOH); **^{1}H NMR** (D_2O, 300 MHz, 25°C, TMS) δ 0.84 (d, 3H, *J*=6.8 Hz, $H_{CH(C\underline{\mathbf{H3}})2}$), 0.88 (d, 3H, *J*=6.9 Hz, $H_{CH(C\underline{\mathbf{H3}})2}$), 1.97 (m, 1H, $H_{C\underline{\mathbf{H}}(CH3)2}$), 2.04 (dd, 1H, *J*=13.8 Hz, *J*=8.5 Hz, $H_{C\underline{\mathbf{H2}}Cquat*}$), 2.45 (dd, 1H, *J*=13.7 Hz, *J*=9.2 Hz, $H_{C\underline{\mathbf{H2}}Cquat*}$), 2.95-3.25 (m, 3H, $H_{C\underline{\mathbf{H}}*CH2NHBn}$ + $H_{C\underline{\mathbf{H2}}NHBn}$), 3.90 (d, 1H, *J*=6.1 Hz, $H_{C\underline{\mathbf{H*}}}$); **^{13}C NMR** ($D_2O$, 75 MHz, 25°C, TMS) δ 17.5 ($C_{CH(\underline{\mathbf{C}}H3)2}$), 17.9 ($C_{CH(\underline{\mathbf{C}}H3)2}$), 31.1 ($C_{\underline{\mathbf{C}}H(CH3)2}$), 32.6 ($C_{\underline{\mathbf{C}}H2Cquat*}$), 37.6 ($C_{\underline{\mathbf{C}}H*CH2NH2}$), 40.0 ($C_{\underline{\mathbf{C}}H2NHBn}$), 62.8 ($C_{\underline{\mathbf{C}}quat*}$), 67.3 ($C_{\underline{\mathbf{C}}H*iPr}$), 173.7 ($C_{\underline{\mathbf{C}}O\ lactam}$), 179.3 ($C_{\underline{\mathbf{C}}O\ lactam}$), 211.0 ($C_{\underline{\mathbf{C}}O\ ketone}$); **HPLC r_t**=0.67 min; **ESI-MS$^+$** *m/z* 240.1; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{11}H_{17}N_3O_3$ + H^+] 240.1348 $gmol^{-1}$, found 240.1349 $gmol^{-1}$.

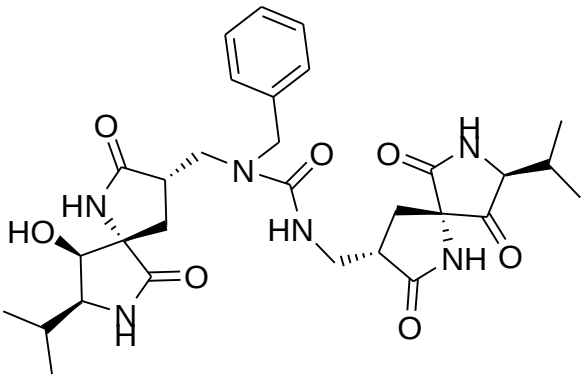
Access to dimeric structures



(*3R,5S,8R,9R*)-3-((Benzylamino)methyl)-8-isopropyl-2,6-dioxo-1,7-diazaspiro[4.4]nonan-9-yl ((*3R,5S,8R*)-8-isopropyl-2,6,9-trioxo-1,7-diazaspiro[4.4]nonan-3-yl)methylcarbamate 12a ($C_{30}H_{40}N_6O_7$, 596.67 $gmol^{-1}$): To compound **11a** (25 mg, 0.10 mmol, 1.0 eq.) in 3 mL of anhydrous THF at 0°C under a flow of argon was added a solution of diisopropylethylamine (40 µL, 0.23 mmol, 2.2 eq.) in 1 mL of anhydrous THF. After 5 min stirring, a solution of triphosgene (11 mg, 0.037 mmol, 0.36 eq.) in 1 mL of anhydrous THF was added drop-wise. The reaction was stirred at 0°C for 1 h before adding **10a** (42 mg, 0.13 mmol, 1.2 eq.) in solution with diisopropylethylamine (42 µL, 0.24 mmol, 2.2 eq.) in 4 mL of anhydrous THF. The reaction mixture was then stirred at 30°C for 1 h before being quenched with water and concentrated to dryness. The crude was then purified by preparative HPLC (linear gradient of 0-40% B over a 1 h period). After lyophilisation, **12a** was obtained with 90% yield (m=54 mg).

Detailed NMR data could be found in the next section entitled "Complete NMR analysis of Dimer **12a** and **12b**".

$[\alpha]_D^{20}$=-75.0 (C=6.0x10^{-3}, MeOH); **HPLC r_t**=1.59 min; **ESI-MS$^+$** *m/z* 597.3; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{30}H_{40}N_6O_7$ + H^+] 597.3037 $gmol^{-1}$, found 597.3033 $gmol^{-1}$; **Circular dichroism**: λ_{min}=222 nm; λ_{max}=241 nm**.**



(*3S,5S,8R,9R*)-3-((Benzylamino)methyl)-8-isopropyl-2,6-dioxo-1,7-diazaspiro[4.4]nonan-9-yl ((*3S,5S,8R*)-8-isopropyl-2,6,9-trioxo-1,7-diazaspiro[4.4]nonan-3-yl)methylcarbamate 12b ($C_{30}H_{40}N_6O_7$, 596.67 $gmol^{-1}$): To compound **11b** (29 mg, 0.12 mmol, 1.0 eq.) in 3 mL of anhydrous THF at 0°C under argon atmosphere was added a solution of diisopropylethylamine (47 µL, 0.27 mmol, 2.2 eq.) in 1 mL of anhydrous THF. After 5 min stirring, a solution of triphosgene (13 mg, 0.044 mmol, 0.36 eq.) in 1 mL of anhydrous THF was added drop-wise. The reaction was stirred at 0°C for 1 h before adding **10b** (49 mg, 0.15 mmol, 1.2 eq.) in solution with diisopropylethylamine (49 µL, 0.28 mmol, 2.2 eq.) in 4 mL of anhydrous THF. The reaction mixture was then stirred at 30°C for 1 h before being quenched with water and concentrated to dryness. The crude was then purified by preparative HPLC (linear gradient of 0-40% B over a 1 h period). After lyophilisation, **12b** was obtained with 87% yield (m=62 mg).

Detailed NMR data could be found in the next section entitled "Complete NMR analysis of Dimer **12a** and **12b** ".

$[\alpha]_D^{20}$=-35.0 (C=6.0x10^{-3}, MeOH); **HPLC r_t**=1.49 min; **ESI-MS$^+$** *m/z* 597.3; **HRMS** (TOF ES MS+) *m/z* calculated for [$C_{30}H_{40}N_6O_7$ + H^+] 597.3037 $gmol^{-1}$, found 597.3033 $gmol^{-1}$; **Circular dichroism**: λ_{min}=208 nm; λ_{max}=227 nm.

Complete NMR analysis of Dimers 12a and 12b

Experimental procedure

The following NMR experiments were carried out on Bruker Avance III 600 MHz spectrometer (Bruker Biospin, France), equipped with 5 mm z-gradient probehead. Analyses in DMSO-d_6 (99.8%, purchased from Sigma-Aldrich) were performed using 10 mg of product diluted into the organic solvent. ^{1}H and ^{13}C chemical shifts were internally referenced from the DMSO-d_6 residual peaks. Analyses in D_2O (99.97%, purchased from euriso-top) were performed using 10 mg of product diluted into the aqueous solvent. ^{1}H chemical shifts were internally referenced from the HDO residual peak. Additional analyses in water were performed in a H_2O:D_2O 9: 1 mixture, pH being adjusting to pH 4 by dilution with 0.01 mL of a 0.1 M solution of aqueous HCl in 10 mL of a H_2O/D_2O 9: 1 solution. Analysis in water pH4 was performed using 2-3 mg of product diluted into the aqueous solvent in 2.5 mm diameter NMR tubes for Bruker MATCH holder. All spectra were recorded using a preliminary H_2O presaturation.

To assign the ^{1}H and ^{13}C spins, series of conventional one- and two-dimensional experiments were performed at 300 K. Homonuclear ^{1}H-^{1}H dqf-COSY and ^{1}H-^{1}H ROESY (mixing time 300 ms) experiments were recorded using time domain sizes of 2048 (t2) * 256 (t1) complex points and 16 transients per t1 increment for COSY - 32 transients per t1 increment for ROESY. For the ^{1}H-^{13}C ge-HSQC experiments, a delay of 3.4 ms (corresponding to a $^{1}J_{1H\text{-}13C}$ coupling constant of 145 Hz) was used to detect the directly bounded atoms. A 50 ms delay (corresponding to a $^{2}J_{1H\text{-}13C}$ coupling constant of 10 Hz) was applied in the ^{1}H-^{13}C ge-HMBC spectra in order to optimize the ^{1}H-^{13}C magnetization transfer through long-range heteronuclear coupling constants.

Heteronuclear ^{1}H-^{13}C ge-HSQC experiments were realized with time domain size of 1024 complex points in t1 and 256 complex points in t2, with 16 transients per t1 increment. For the ^{1}H-^{13}C ge-HMBC, the time domain size in t1 only changed, compared to the HSQC, with a value of 2048.

The data were acquired and processed with the Topspin 3.0 software (Bruker). During the process, zero filling and apodization functions were applied to the FIDs (sine-bell and squared sine-bell, unshifted or $\pi/2$ shifted) in the both dimensions. A polynomial baseline correction was also used in order to further improve the 2D spectra.

NMR analysis of results

Due to the complex structure of **12**, we have conducted a complete and cautious NMR assignment procedure. We chose first to record NMR data in DMSO-d_6, a polar solvent where both isomers gave well resolved sharp signals. As expected, 1D NMR ^{1}H spectra of both isomers showed very close patterns, including five exchangeable signals in the low field region, which matched to the five amides protons of **12**. The signal of the hydroxyl group was detected around 5.5 ppm, the chemical exchange being sufficiently attenuated in DMSO-d_6 to allow its observation.

To fully characterize each isomer, and to extract the distance constraints, the complete assignments of both ^{1}H and ^{13}C atoms were performed by analysis of the 2D homo- and heteronuclear experiments. For example, the atoms numbering of **12a** is given on Figure S6, the whole set of NMR data being given thereafter. The cautious analysis of 2D homonuclear ROESY map allowed us to identify parts of the spin systems. The four connection pathways, NH(*31*)-CH(*32*)-CH(*33*)-CH_3(*34*/*35*), OH(*12*)-CH(*17*)-CH(*16*)-CH(*18*)-CH_3(*19*/*20*), CH_2(*8*)-CH(*9*)-CH_2(*10*) and NH(*22*)-CH_2(*23*)-CH(*24*)-CH_2(*28*) led us to assign elements of each spirolactam unit (Figure S6). On the basis of the heteronuclear $^{1}J_{H\text{-}C}$ correlations in the ^{1}H-^{13}C HSQC map, the corresponding carbons signals were elucidated.

Then, non-coupled H(*7*) and H(*26*) amides protons were identified using their respective long range correlations to the neighbouring carbons in the ^{1}H-^{13}C HMBC spectrum. Indeed, each of these amides protons gave both intracyclic connections and connections to the carbons of the adjacent cycle. Finally, the ^{3}J coupling of the C(*21*) with the (*6*/*6*'), (*8*/*8'*) and (*23*/*23'*) methylene protons has confirmed the arrangement of the three fragments formed by the two spirolactam cycles and the benzylic group.

The absolute configurations of the stereocenters already present in the monomeric moiety, C(*16*), C(*17*), C(*32*), were validated, so as the configurations of C(*9*) and C(*24*), using the spatial interactions given by the intensity of the cross correlations on the ROESY spectra.

Dimer 12a
Assignments

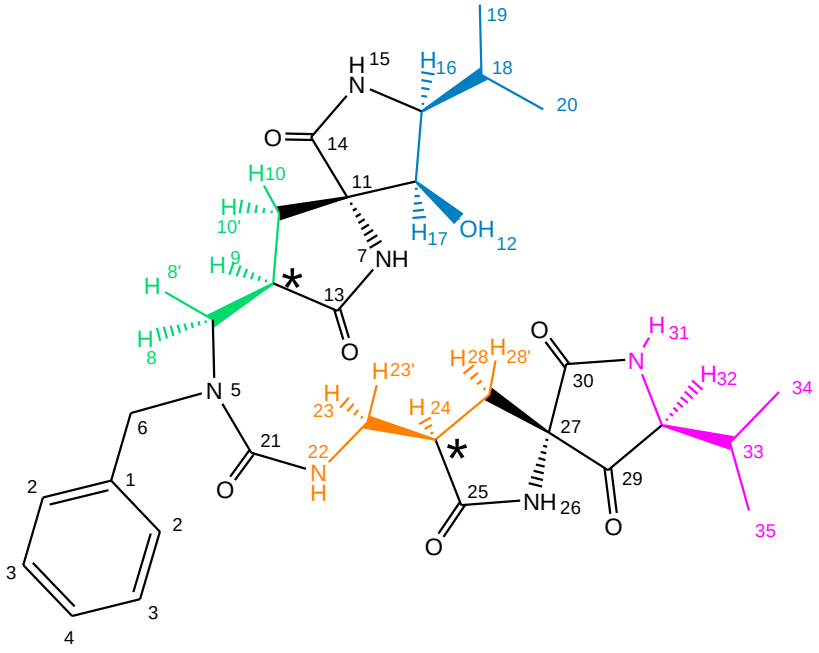


Figure S7. Atoms numbering of isomer **12a** used for NMR study.

Table S3. ^{1}H Chemical shifts, multiplicities and *J* values of **12a** obtained in DMSO-d_6, D_2O and water pH 4 (600 MHz).

Atoms	**DMSO-d_6**			**D_2O**			**Water pH 4**		
	δ (ppm)	Multiplicity	*J* (Hz)	δ (ppm)	Multiplicity	*J* (Hz)	δ (ppm)	Multiplicity	*J* (Hz)
NH (*31*)	8.87	s	-	-	-	-	8.94	s	-
NH (*7*)	8.19	s	-	-	-	-	8.28	s	-
NH (*15*)	8.01	s	-	-	-	-	8.11	s	-
NH (*26*)	7.73	s	-	-	-	-	7.88	s	-
H_{Ar} (*3*)	7.30	t	7.5	7.45	t	7.6	7.45	t	7.3
H_{Ar} (*4*)	7.22	t	7.5	7.39	t	7.6	7.39	t	7.3
H_{Ar} (*2*)	7.16	d	7.5	7.31	d	7.6	7.31	d	7.3
NH (*22*)	6.65	dd	4.7; 6.1	-	-	-	6.4	br	-
OH (*12*)	5.47	d	5.8	-	-	-	-	-	-
H (*6*)	4.46	d	16.6	4.66	d	16.9	4.65	d	16.9
H (*6'*)	4.43	d	16.6	4.55	d	16.9	4.55	d	16.9
H (*17*)	3.93	t	5.2	4.35	d	5.8	4.35	d	5.7
H (*32*)	3.79	d	4.8	4.07	d	5.8	4.07	d	5.7
H (*23*)	3.46	dt	13.5; 4.8	3.67	dd	4.2; 14.2	3.67	m	-
H (*8*)	3.33	dd	7.5; 14.7	3.80	dd	5.2; 15.2	3.80	m	-
H (*8'*)	~3.34[a]	-	-	3.63	dd	7.5; 15.2	3.62	m	-
H (*16*)	3.21	dd	5.4; 8.8	3.45	dd	5.8; 9.0	3.45	dd	5.8; 8.4
H (*23'*)	3.01	ddd	6.1; 9.5; 13.5	3.39	dd	6.7; 14.2	3.39	dd	6.0; 13.4
H (*24*)	2.76	dd	4.7; 9.0	3.00	m	-	3.00	m	-
H (*9*)	2.72	m	-	3.06	m	-	3.07	m	-
H (*10*)	2.00	dd	8.8; 13.3	2.23	dd	8.1; 13.6	2.23	dd	7.8; 13.5
H (*28*)	2.14	dd	9.2; 12.8	2.38	dd	9.4; 13.4	2.38	dd	9.4; 13.2
H (*28'*)	1.69	dd	8.8; 12.8	1.96	dd	8.9; 13.4	1.95	dd	8.9; 13.2
H (*33*)	1.94	m	-	2.10	m	-	2.10	m	-
H (*10'*)	1.94	dd	6.2; 13.3	2.15	dd	9.6; 13.6	2.15	dd	9.5; 13.5
H (*18*)	1.75	dsept	8.8; 6.7	1.83	m		1.84	m	-
H (*34*)	0.94	d	6.8	1.04	d	7.0	1.04	d	7.0
H (*19*)	0.90	d	6.7	1.02	d	6.6	1.02	d	6.6
H (*20*)	0.88	d	6.7	0.98	d	6.6	0.98	d	6.6
H (*35*)	0.85	d	6.7	1.00	d	6.8	1.00	d	6.8

[a] Overlap with the water signal.

Table S4. ^{13}C Chemical shifts of **12a** obtained in DMSO-d_6, D_2O and water pH 4 (600 MHz).

Atoms	**DMSO-*d*$_6$**	**D_2O**	**Water pH 4**
	δ (ppm)	δ (ppm)	δ (ppm)
C (*29*)	211.4	211.5	211.5
C (*13*)	177.2[a]	180.5	180.5
C (*25*)	177.2[a]	180.9	180.9
C (*14*)	175.4	177.0	177.0
C (*30*)	173.1	174.9	174.9
C (*21*)	157.9	160.0	160.0
C (*1*)	138.5	137.4	137.4
C (*3*)	128.3	129.0	129.0
C (*2*)	126.9	127.7	127.6
C (*4*)	126.7	126.9	126.9
C (*17*)	72.8	72.8	72.9
C (*32*)	66.2	67.6	67.7
C (*11*)	65.2	66.6	66.7
C (*16*)	61.3	62.0	62.1
C (*27*)	61.3	63.0	63.1
C (*6*)	49.1	51.1	51.0
C (*8*)	46.4	48.3	48.3
C (*23*)	42.0	40.2	40.3
C (*24*)	40.8	41.8	41.7
C (*9*)	40.6	41.0	41.0
C (*28*)	34.3	33.7	33.7
C (*33*)	30.8	31.3	31.2
C (*18*)	27.3	27.4	27.4
C (*10*)	28.1	28.0	28.0
C (*19*)	19.8	19.4	19.4
C (*20*)	19.6	19.0	19.0
C (*34*)	18.6	18.2	18.2
C (*35*)	17.6	17.6	17.6

[a] May be reversed.

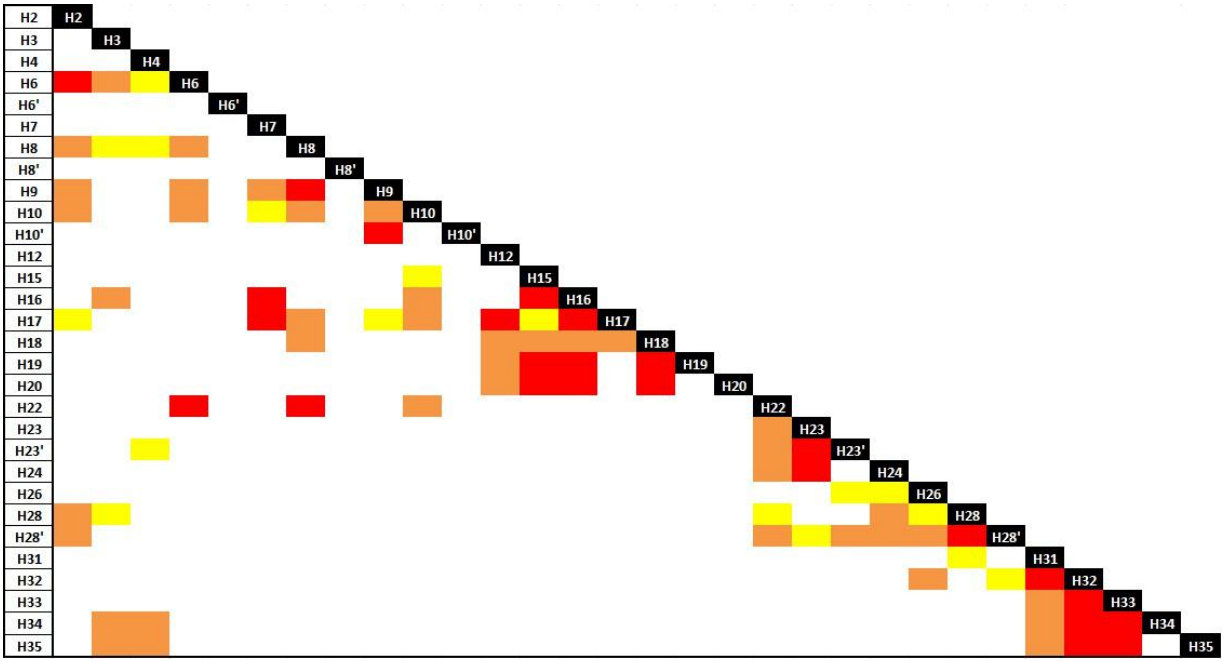


Figure S8. nOe correlations of **12a** in DMSO-d_6 (Red for *strong*, orange for *medium* and yellow for *weak*)

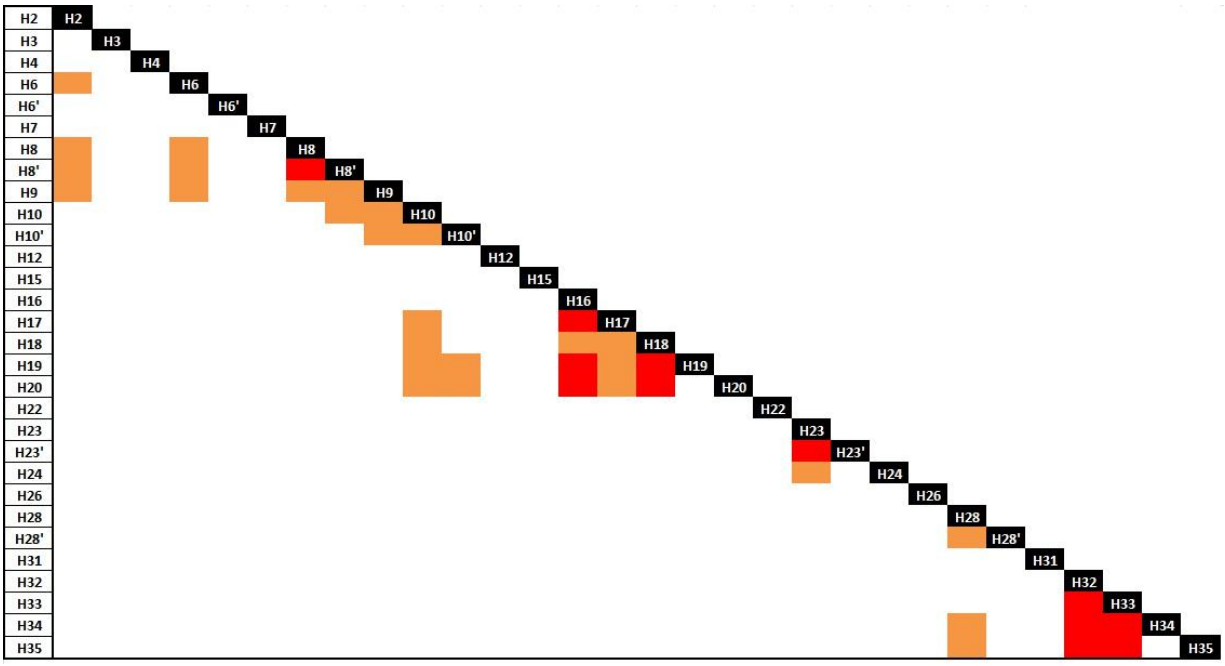


Figure S9. nOe correlations of **12a** in D_2O (Red for *strong*, orange for *medium* and yellow for *weak*).

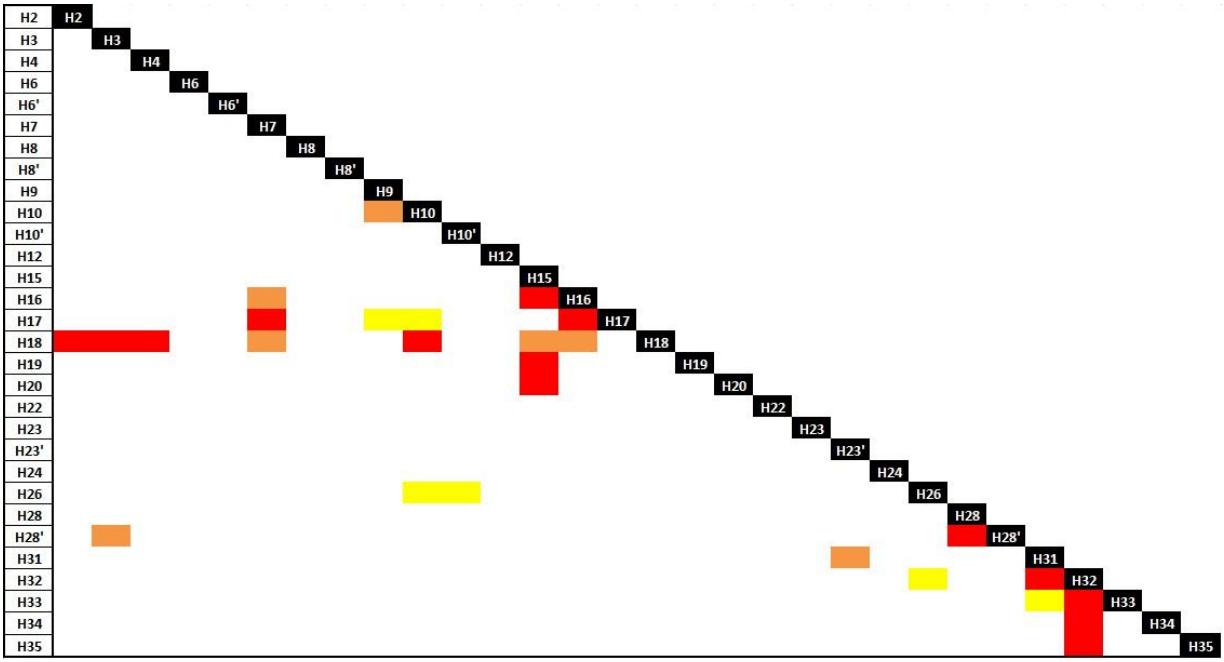


Figure S10. nOe correlations of **12a** in water pH 4 (Red for *strong*, orange for *medium* and yellow for *weak*).

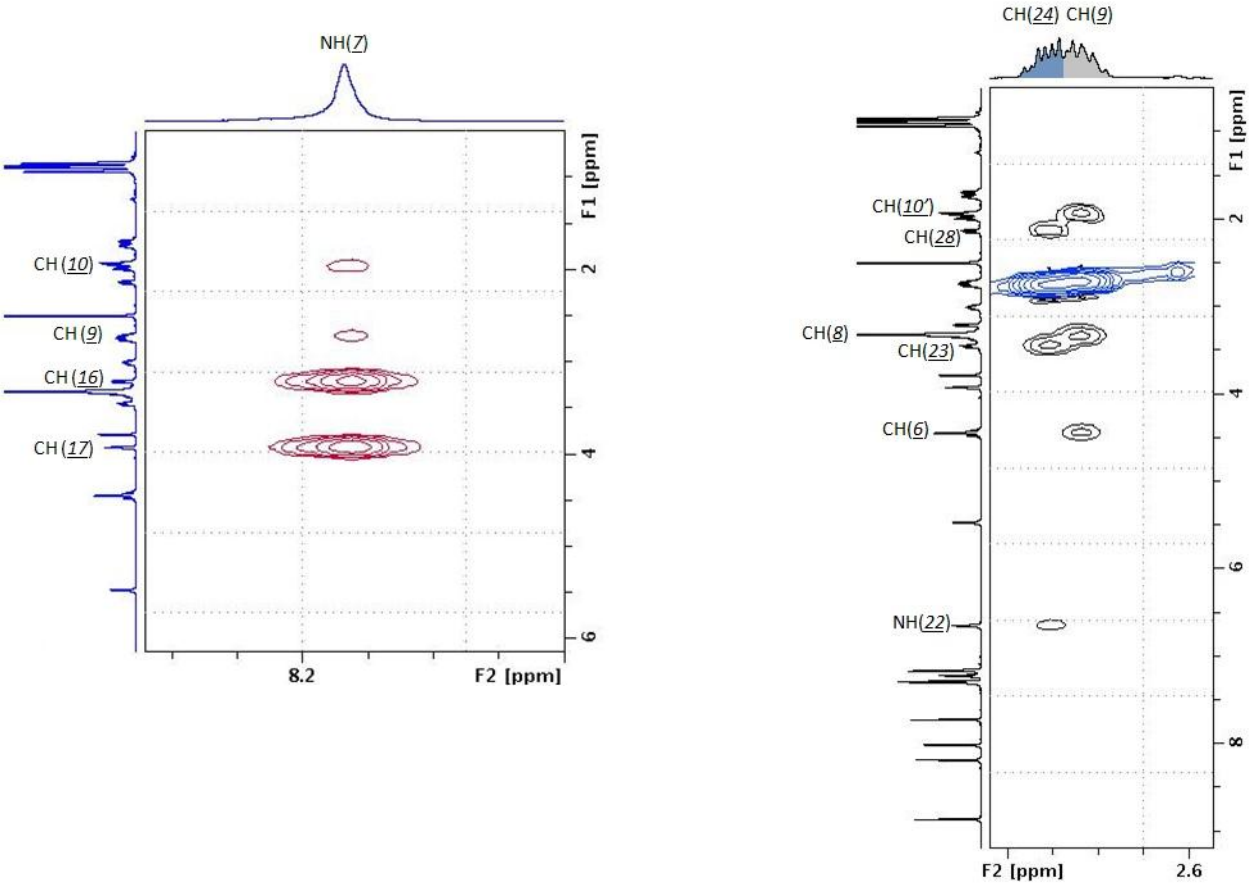


Figure S11. Main relevant nOe correlations on dimer **12a** in DMSO-d_6 (600 MHz), confirming the configuration of stereogenic centres previously described for monomer **10a.**

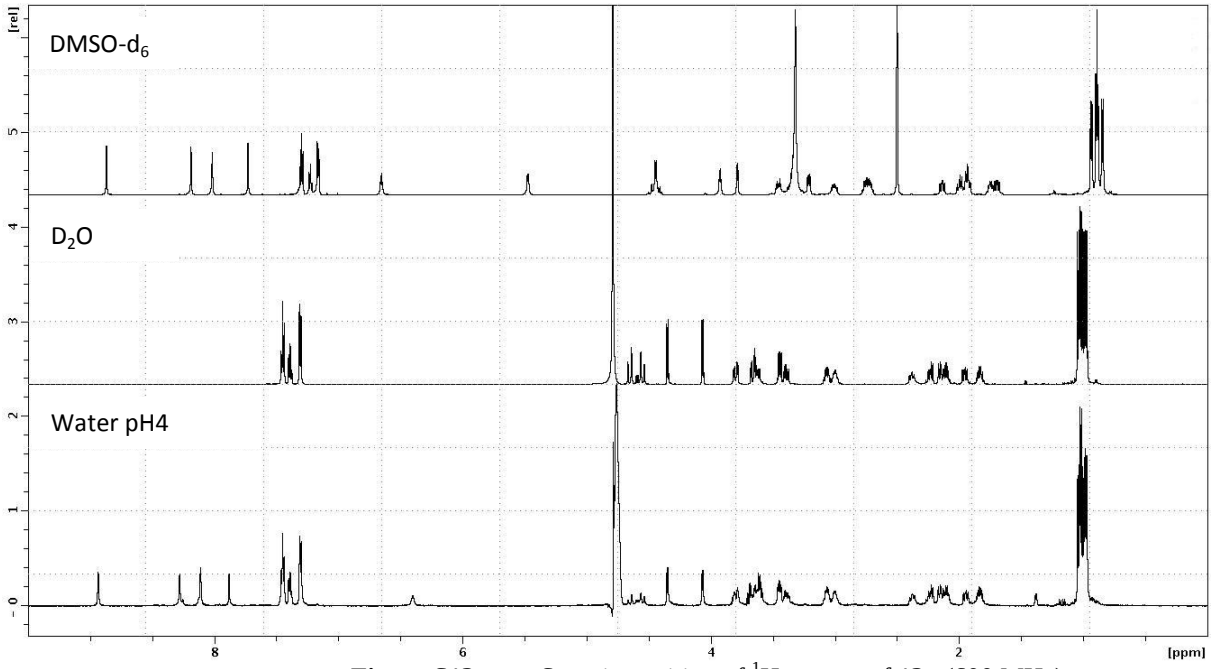


Figure S12. Superimposition of ^{1}H spectra of **12a** (600 MHz).

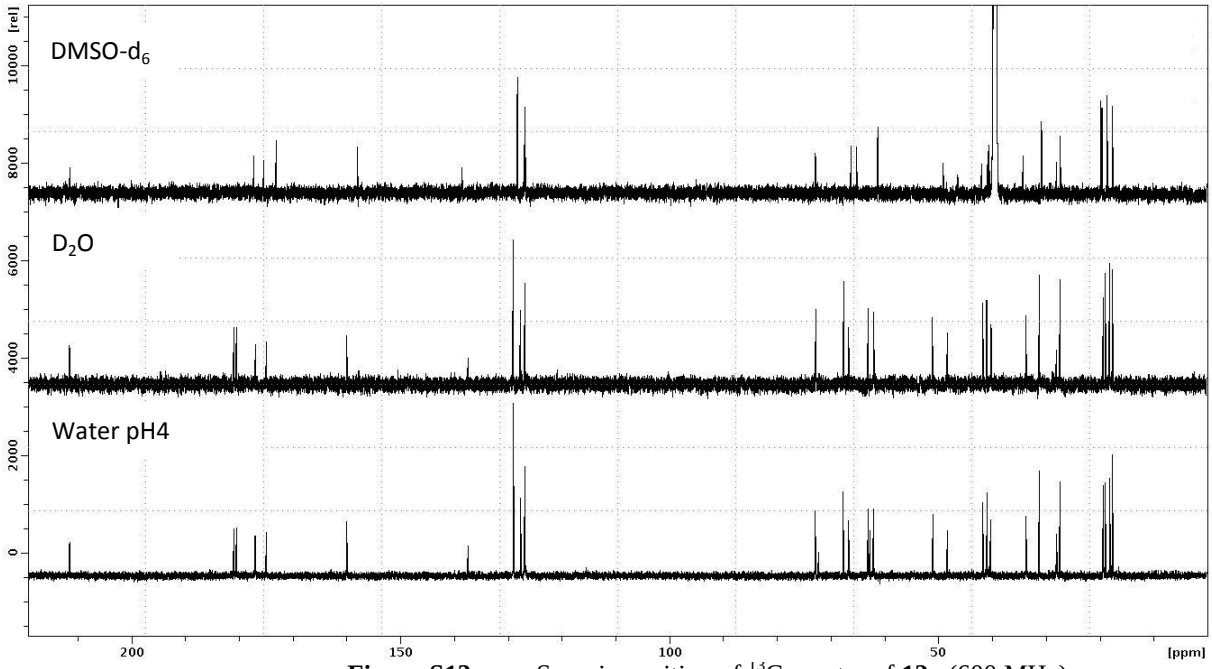


Figure S13. Superimposition of ^{13}C spectra of **12a** (600 MHz).

Dimer 12b
Assignments

A similar strategy used for **12a** was performed for the assignment of dimer **12b**.

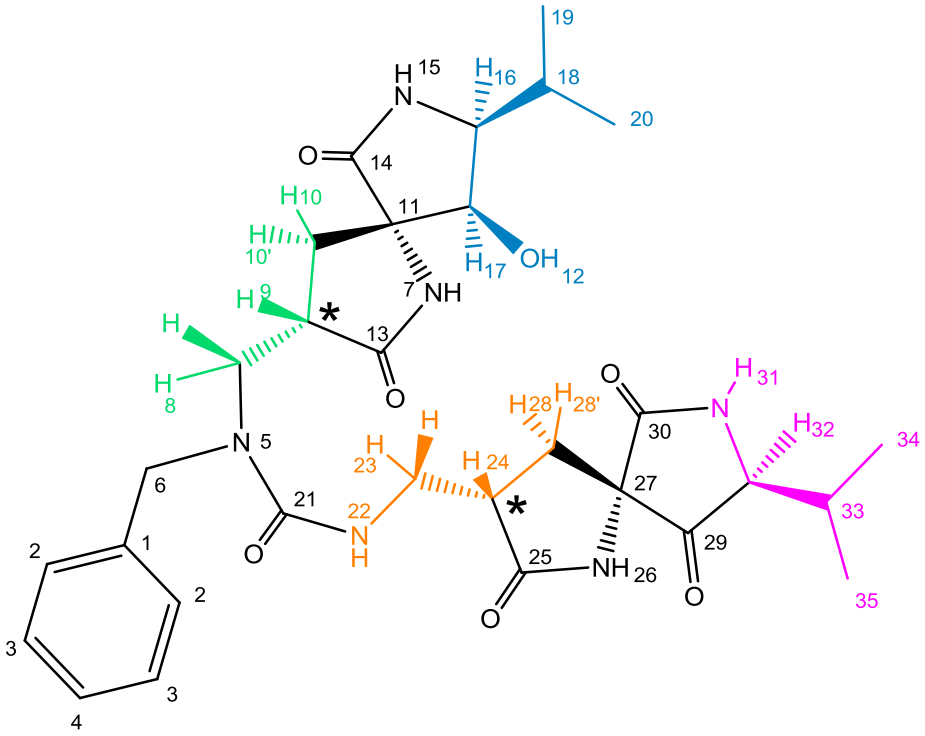


Figure S14. Atoms numbering of isomer **12b** used for NMR study.

Table S5. ^{1}H Chemical shifts multiplicities and *J* values of **12b** in DMSO-d_6 and D_2O (600 MHz).

Atoms	**DMSO-d_6**			**D_2O**		
	δ (ppm)	Multi	*J* (Hz)	δ (ppm)	Multi	*J* (Hz)
NH (*31*)	8.93	s	-	-	-	-
NH (*7*)	8.21	s	-	-	-	-
NH (*15*)	8.16	s	-	-	-	-
NH (*26*)	7.79	s	-	-	-	-
H_{Ar} (*3*)	7.29	t	7.2	7.45	t	7.4
H_{Ar} (*4*)	7.22	t	7.2	7.38	t	7.4
H_{Ar} (*2*)	7.16	d	7.2	7.30	d	7.4
NH (*22*)	6.73	t	-	-	-	-
OH (*12*)	5.50	d	5.9	-	-	-
H (*6*)	4.51	d	16.5	4.63	d	17.0
H (*6'*)	4.42	d	16.5	4.58	d	17.0
H (*17*)	3.88	t	5.2	4.39	d	5.9
H (*32*)	3.76	d	5.2	4.02	d	6.5
H (*23*)	3.46	m	-	3.64	dd	5.0; 14.0
H (*8*)	3.38	m	-	3.84	dd	5.5; 15.0
H (*8'*)	3.38	-	-	3.64	dd	8.2; 15.0
H (*16*)	3.20	dd	5.2; 8.7	3.46	dd	5.9; 9.0
H (*23'*)	3.07	m	-	3.39	dd	8.0; 14.0
H (*24*)	2.68	m	-	-	-	-
H (*9*)	2.63	m	-	-	-	-
H (*10*)	2.28	dd	9.4; 12.6	2.56	dd	9.4; 13.6
H (*28*)	1.96	m	-	2.24	dd	9.4; 13.4
H (*28'*)	1.96	m	-	2.04	dd	8.9; 13.4
H (*33*)	1.93	m	-	2.10	hex	6.7
H (*10'*)	1.76	m	-	1.92	dd	7.6; 13.6
H (*18*)	1.76	m	-	1.89	dsep	9.0; 6.7
H (*34*)	0.94	d	6.6	1.07	d	6.9
H (*19*)	0.90	d	6.6	1.03	d	6.7
H (*20*)	0.89	d	6.6	0.99	d	6.7
H (*35*)	0.87	d	6.6	1.04	d	6.9

Table S6. ^{13}C chemical shifts of **12b** in DMSO-d_6 and D_2O (600 MHz).

Atoms	**DMSO-d_6**	**D_2O**
	δ (ppm)	δ (ppm)
C (*29*)	211.8	213.0
C (*13*)	176.8	181.6
C (*25*)	177.2	182.3
C (*14*)	175.2	177.8
C (*30*)	172.4	175.3
C (*21*)	157.7	161.0
C (*1*)	138.8	138.7
C (*3*)	128.2	130.2
C (*2*)	126.9	128.0
C (*4*)	126.7	128.8
C (*17*)	73.2	74.7
C (*32*)	65.9	68.5
C (*11*)	65.9	68.1
C (*16*)	61.5	63.1
C (*27*)	61.3	64.3
C (*6*)	49.0	52.1
C (*8*)	47.2	50.0
C (*23*)	42.3	42.1 a
C (*24*)	40.6	42.2 a
C (*9*)	40.0	42.0 a
C (*28*)	33.1	34.1
C (*33*)	30.9	32.7
C (*18*)	27.3	29.7
C (*10*)	27.2	29.1
C (*19*)	19.9	20.5
C (*20*)	19.4	20.3
C (*34*)	18.6	19.3
C (*35*)	17.9	19.1

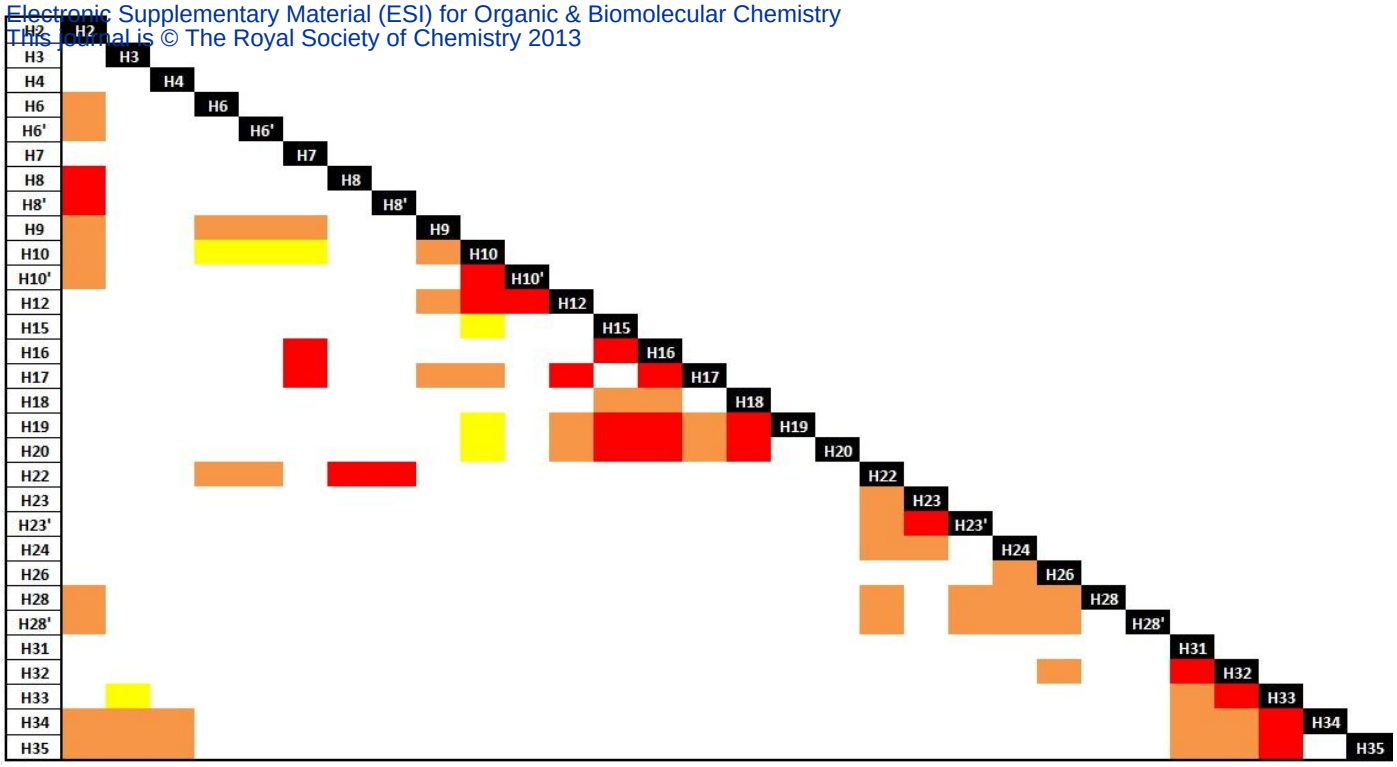


Figure S15. nOe correlations of **12b** in DMSO-d_6 (Red for *strong*, orange for *medium* and yellow for *weak*).

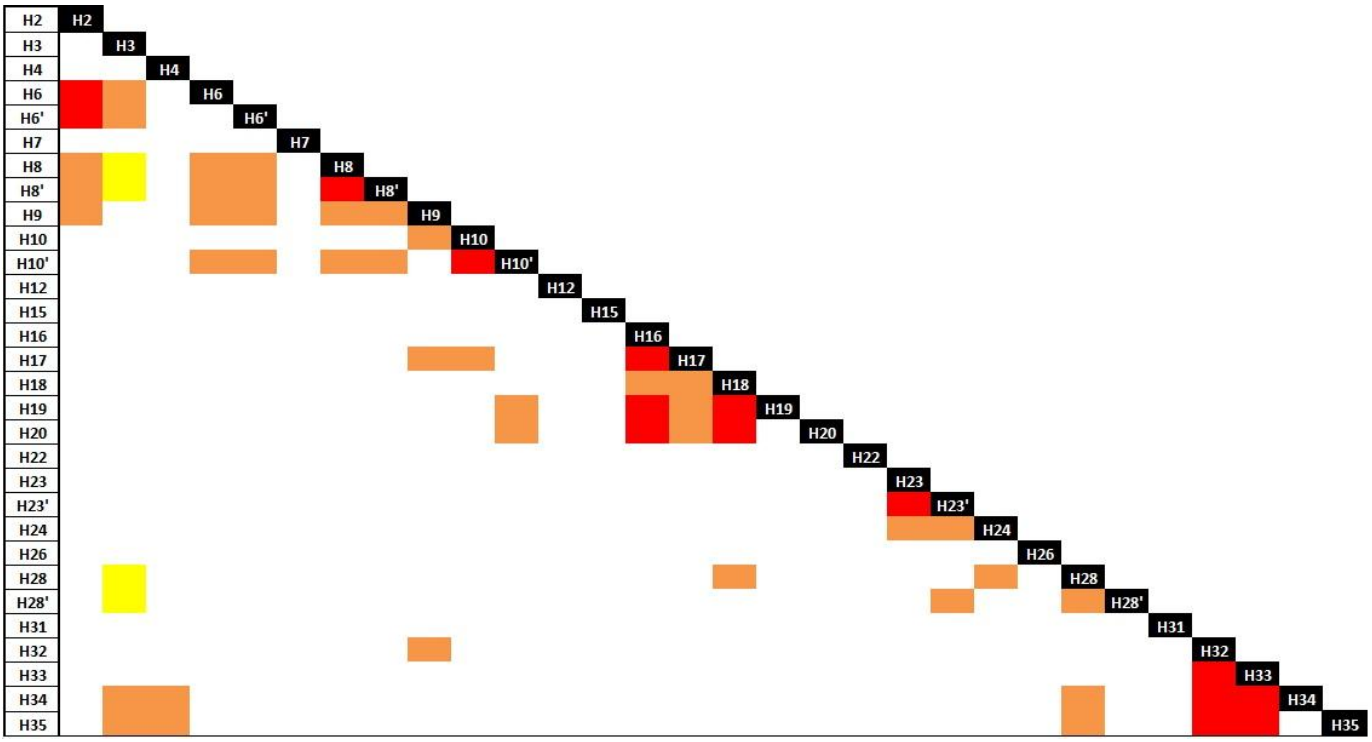


Figure S16. nOe correlations of **12b** in D_2O (Red for *strong*, orange for *medium* and yellow for *weak*).

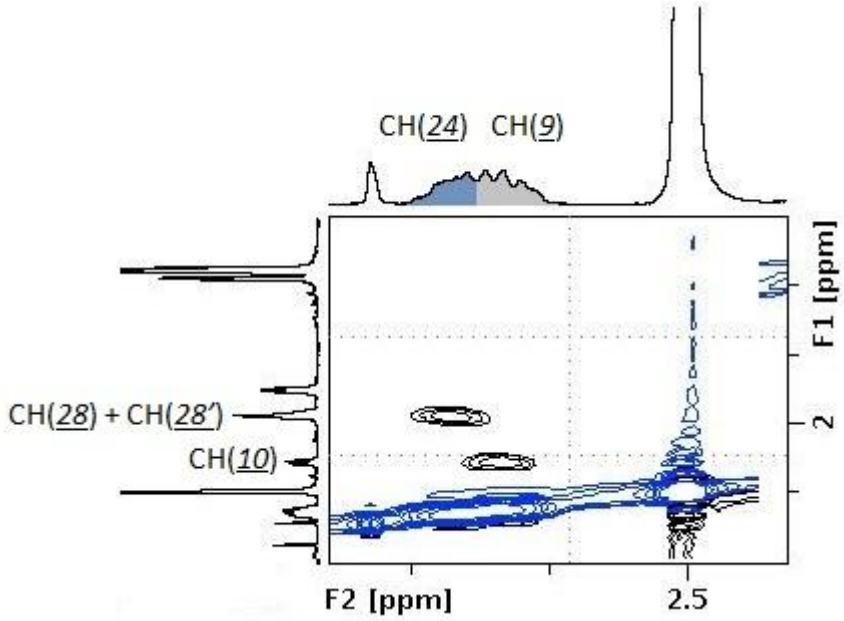


Figure S17. Main relevant nOe correlations on dimer **12b** in DMSO-d_6 (600 MHz), confirming a different configuration on C(9).

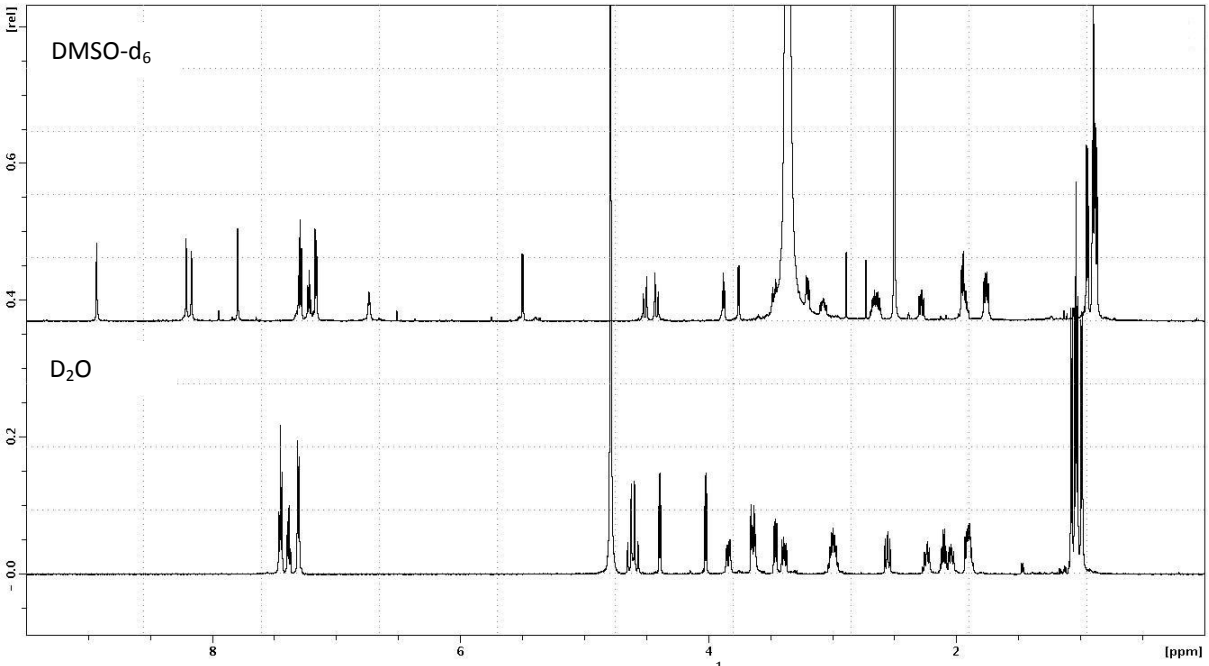


Figure S18. Superimposition of ^{1}H spectra of **12b** (600 MHz).

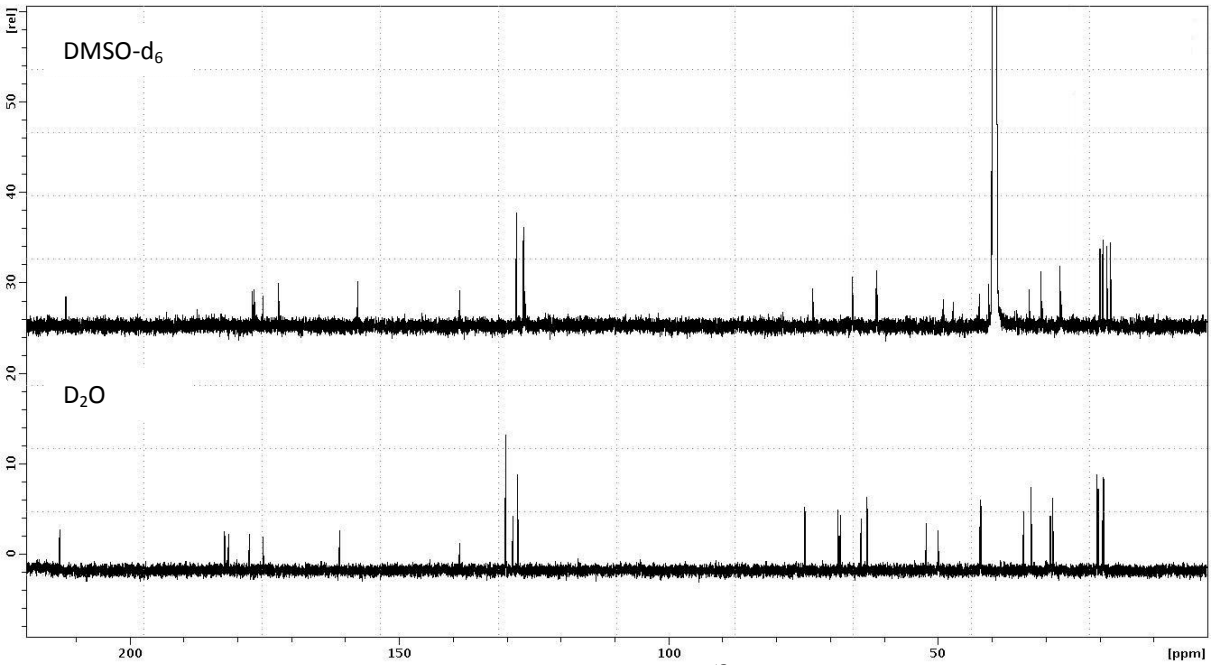


Figure S19. Superimposition of ^{13}C spectra of **12b** (600 MHz).

Circular dichroism. Additional results.

Secondary structural analysis of monomers by CD

As expected, CD spectra of monomers alone revealed a behaviour which was not in favour of a PPII folded state. While CD spectra of monomers **10** unambiguously showed a lack of conformation, spectra of monomers **11** exhibited more well-defined signals. Analyses of CD spectra of monomers **10a** and **11a** showed a negative band around 222 nm and 212 nm respectively, and a weakly to moderately positive band around 254 nm and 235 nm respectively. We can note here, by comparison of the shifts of the λ_{min} and the λ_{max} between those two monomers, that we observe the exact bathochromic effect displayed by the dimer **12a** compared to the natural PPII spectra which could be correlated to the presence of the benzyl moiety.

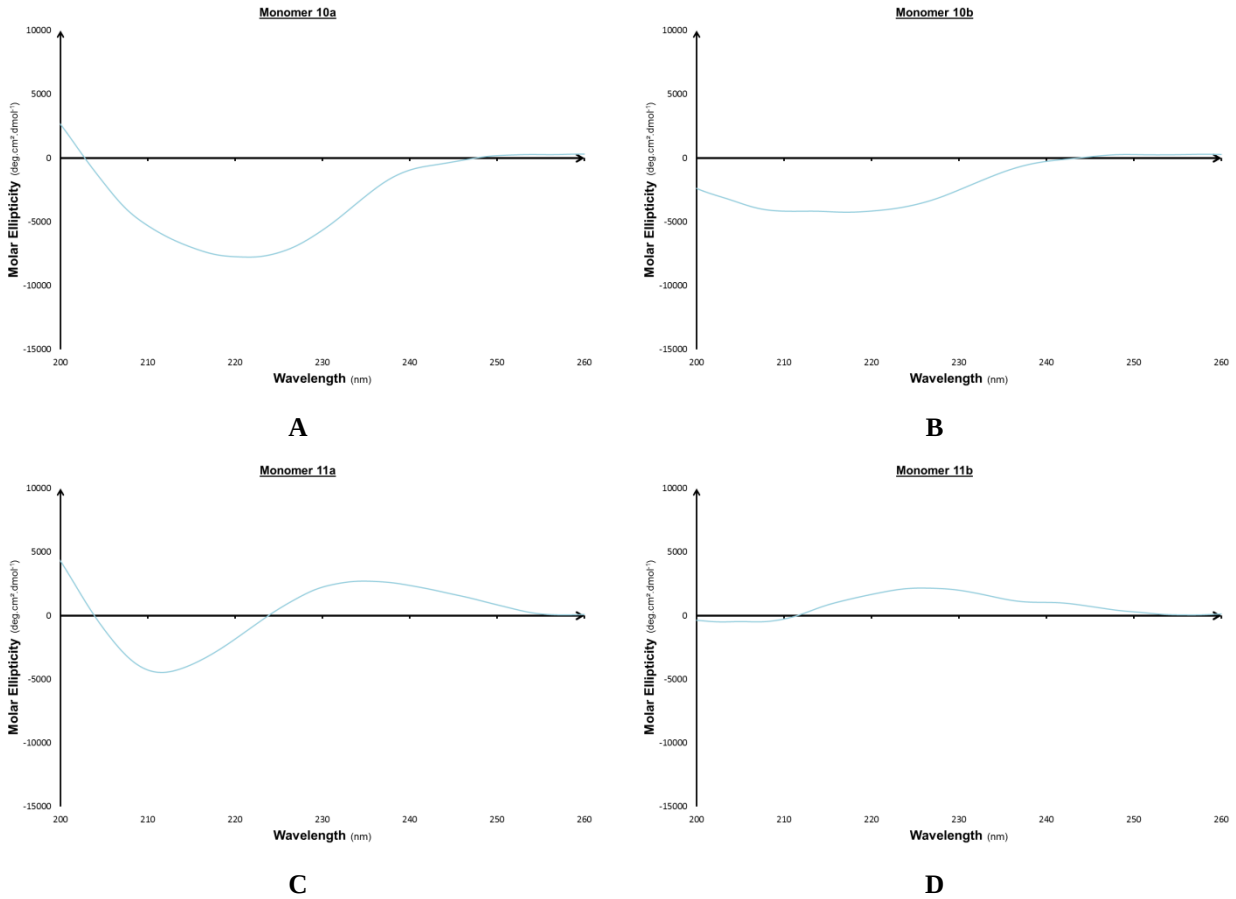


Figure S20. CD spectra of monomer **10a** (**A**), **10b** (**B**), **11a** (**C**) and **11b** (**D**) in phosphate buffer at 5°C.

To understand the influence of the carbamide linker in the folding of dimers **12** and see whether CD spectra of dimers were not only resulting from the sum of the spectra of each monomer, we recorded CD spectra of equimolar mixtures of monomers under the same conditions. To prove that the mixture of monomers did not form organized aggregates in solution which could give a typical CD signal,[2] we calculated a spectrum by summation of

the spectra of the individual monomers[3] and subtracted this spectrum to the experimental one. As shown in Figure S21 (**E** and **F**), we obtained a baseline with no molar ellipticity, confirming hence that an equimolar mixture of monomers did not form organized aggregates. By comparison of the spectra presented in Figure S21 with the spectra of dimers **12**, we already can note that the carbamide linker has a great influence since the signal obtained for dimer **12b** is completely different than the one recorded from the equimolar mixture of **10b** and **11b**. The differences between spectra of **12a** and **10a**+**11a** are however less noticeable even though we can remark a global decrease in molar ellipticity ($[\theta]_{max}$ around 3300 deg.cm^2.dmol^{-1} for dimer **12a** against 1650 deg.cm^2.dmol^{-1} for the mixture).

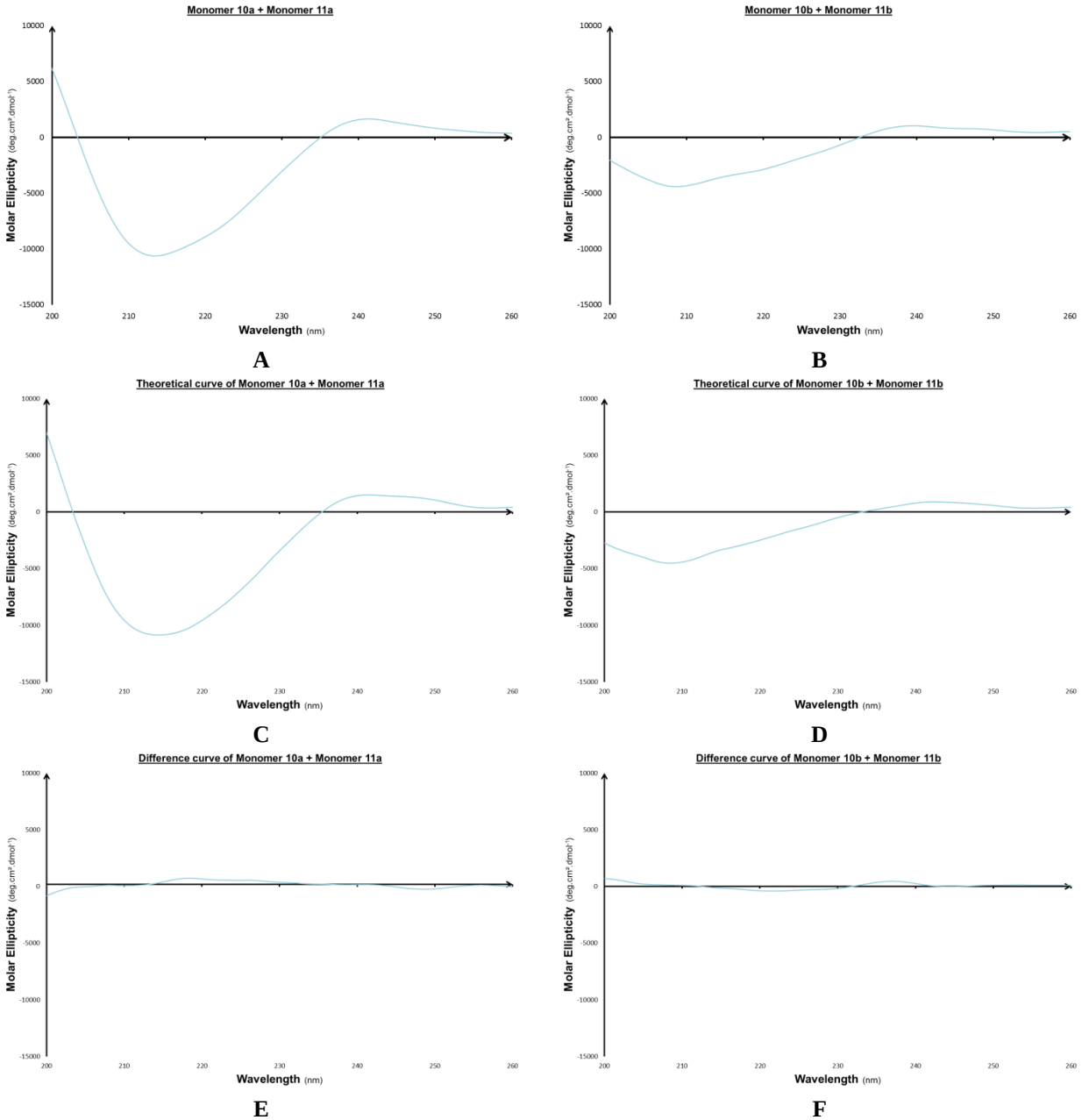


Figure S21. CD spectra of mixture of monomers **10** and **11** in phosphate buffer at 5°C (**A** and **B**); Calculated spectra of mixture of monomers **10** and **11** (**C** and **D**); Subtraction of the spectra of individual monomers **10** and **11** from CD spectra of mixtures (**E** and **F**).

To see if dimers got a particular behaviour which cannot be related to the behaviour of each monomer only, we decided to perform on both monomers **11** and mixture of monomers **10** and **11** the same temperature and guanidinium chloride experiments we performed on dimers **12**. When increasing concentrations of guanidinium chloride were added to monomer **11a**, the positive band of the CD spectrum decreased very slowly with an average loss of 300 deg.cm^2.dmol^{-1}. Those results indicated that, even though monomers are not greatly affected by the presence of a chaotropic agent, they are not also behaving as a PPII structure like **12a** is and for which an extreme stability with a typical intensification of the molar ellipticity was observed in the same conditions ($[\theta]_{max}$ value around 6400 deg.cm^2.dmol^{-1} in presence of a 3M solution of guanidinium chloride). Same conclusions can be made with the mixture of **10a** and **11a**, with an average loss of 600 deg.cm^2.dmol^{-1} however. Concerning monomers **10b** and **11b**, we can confirm here again that the carbamide linker has an enormous influence on the behaviour of those compounds: while a 1.5M solution of guanidinium chloride caused a brutal decrease of **12b** molar ellipticity (almost 4000 deg.cm^2.dmol^{-1} loss), neither monomer **11b** nor the mixture seem to be really affected by the chaotropic agent.

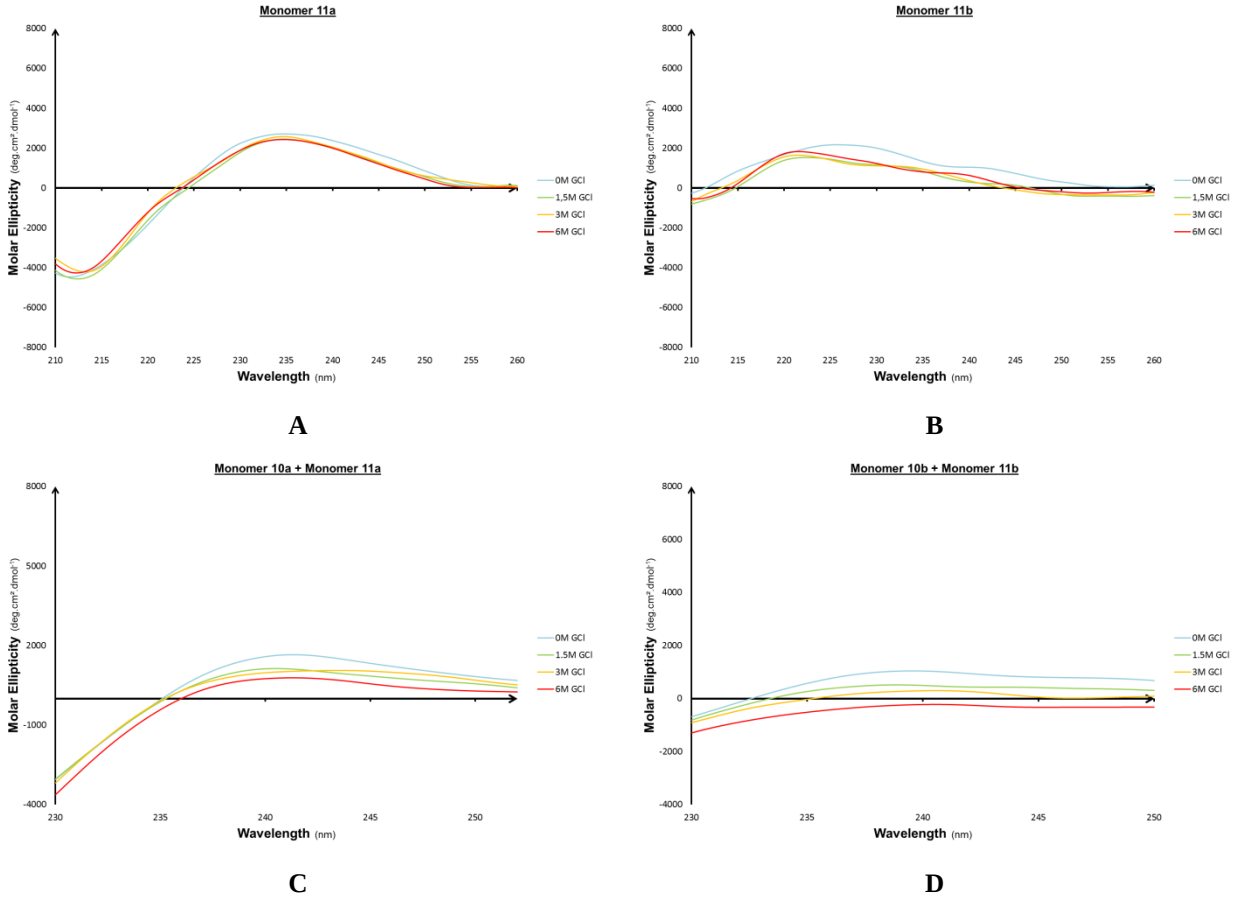


Figure S22. CD spectra of monomers **11** (**A** and **B**) and of the mixture of monomers **10** and **11** (**C** and **D**) in the presence of increasing concentrations of guanidinium chloride (— 0M — 1.5 M, — 3.0 M, — 6.0 M).

Similarly, neither monomers nor the mixture displayed the same behaviour than the dimers in the presence of increasing temperatures.

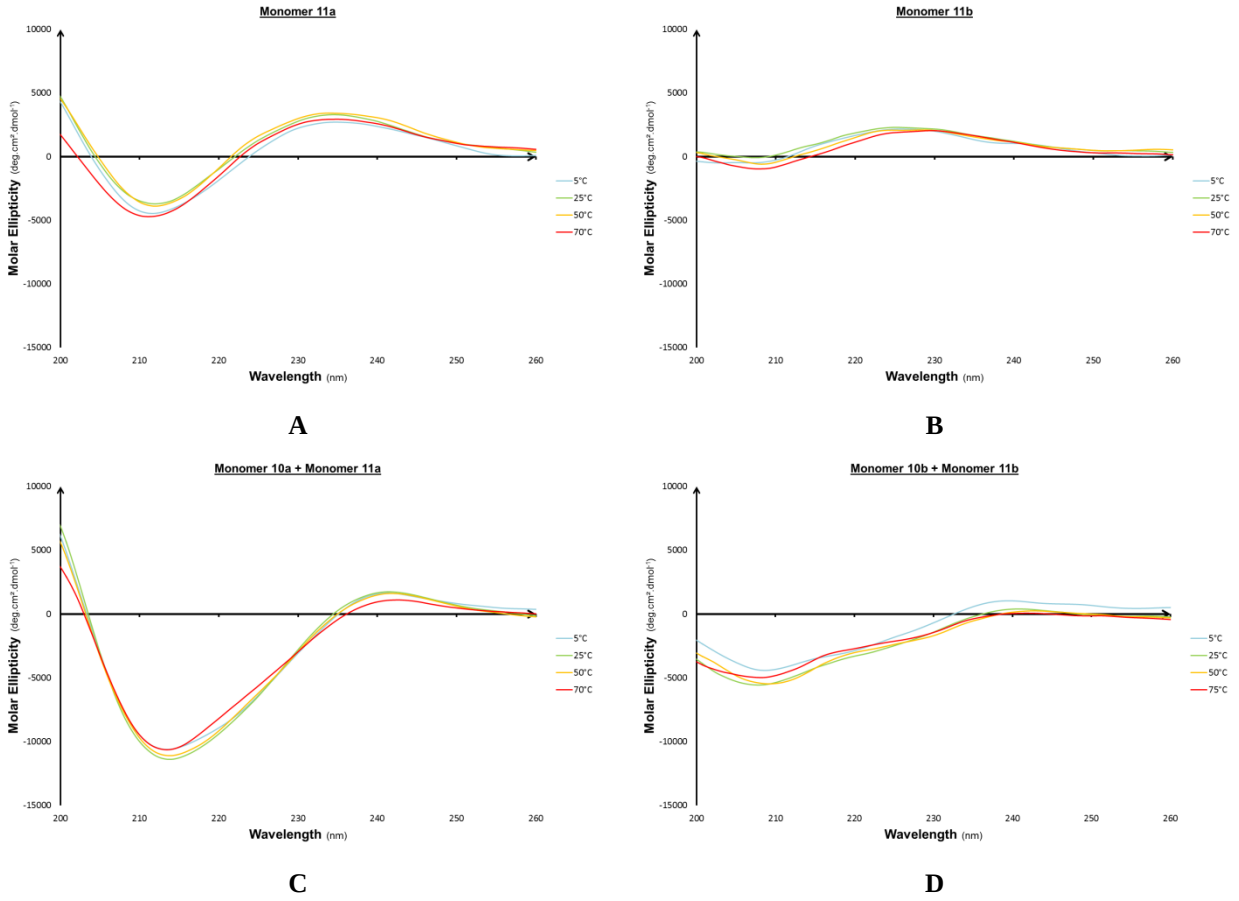


Figure S23. CD spectra of monomers **11** (**A** and **B**) and of the mixture of monomers **10** and **11** (**C** and **D**) in the presence of increasing temperatures (— 5°C, — 25°C, — 50°C, — 70°C).

Since compounds **10** and **11** have completely different properties if not linked together as a dimer unit, we demonstrated the importance of such link. Moreover, no monomer presented the same characteristics as their related dimers, which is particularly obvious for compounds **10a**, **11a** and **12a**. This last observation could not allow us to conclude whether monomers are capable of folding, but we can affirm they do not behave as PPII-like structures like dimer **12a**.

Additional results in secondary structural analysis of 12a by CD

CD spectra of **12a** obtained during the temperature experiment were compared with the ones of a hexaproline, H-$(Pro)_6$-NH_2, recorded in the same conditions

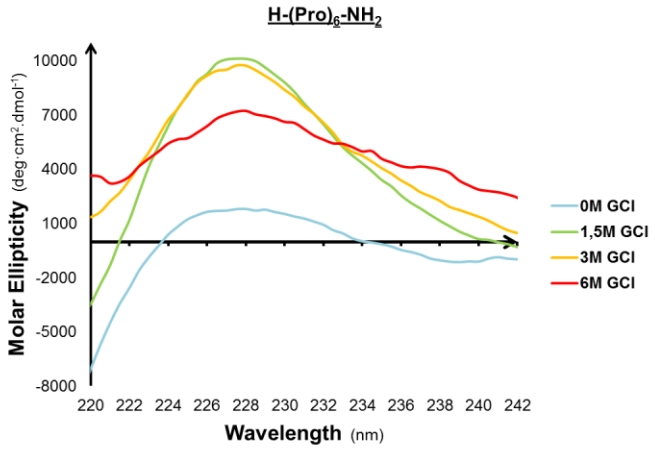


Figure S24. CD spectra of a polyproline model in phosphate buffer at 5°C, in the presence of increasing concentrations of guanidine hydrochloride (— 0M — 1.5 M, — 3.0 M, — 6.0 M).

Because the nature of the solvent could influence the propensity of an oligomer to adopt a folded structure, CD studies were also performed in less polar solvent such as TFE. Comparable results with PBS were obtained.

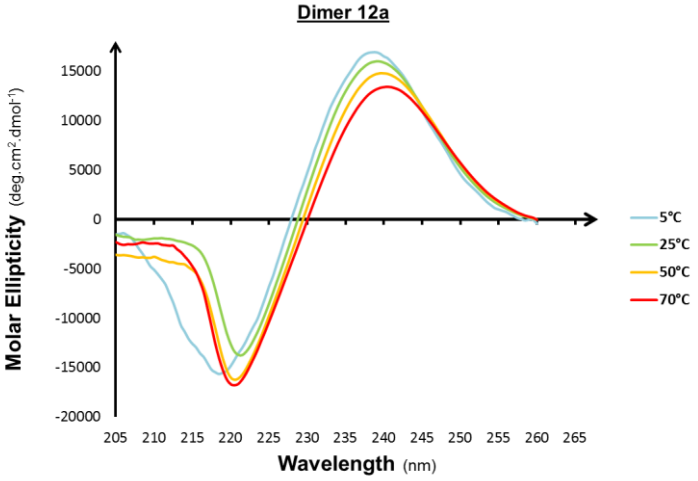


Figure S25. CD spectra of **12a** in TFE (— 5°C, — 25°C, — 50°C, — 70°C).

Additional results in secondary structural analysis of 12b by CD

CD studies of **12b** were also performed in TFE and comparable results with PBS were obtained.

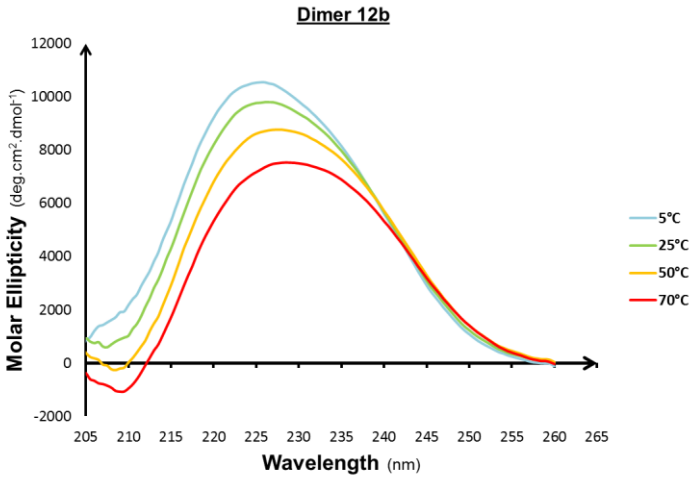


Figure S26. CD spectra of dimer **12b** in TFE (— 5°C, — 25°C, — 50°C, — 70°C).

Molecular modelling - Docking

In order to evaluate the potentiality to get 'PPII-like' conformations in solutions for compound **12a**, we applied an original methodology consisting in modelling the spirocompound bound to a physiological 'PPII-binding protein', *i.e.* the SH3 domain from protein kinases.

To achieve this goal, we applied a fully flexible docking methodology using Surflex-Dock algorithm[2] as implemented in the 1.2 release of Sybyl-X Tripos package (St. Louis, USA). The SH3 domains used for the docking procedures were extracted from two X-Ray structures (PDB code 1AVZ[3] and 1EFN[4]). A small polyproline helix (pentaprolines) aligned to the proline-rich region of the protein Nef (residues 72-76) was used to define the Surflex protomol with bloat and threshold parameters set to 0.99 and 0 respectively. Initial models of **12a** were built in the mol2 format using Maestro (http://www.schrodinger.com/products/). The 3D conformation used as a starting configuration for docking compound **12a** onto the Fyn SH3 domain was then generated by molecular dynamics simulation with CHARMM under constraints during a 5ns MD run performed *in vacuo*, using Charmm general force field for organic molecules, with an integration step of 1 fs, in the NPT ensemble (300 K, 1 atm.).[5] The resulting docked conformations generated by Surflex (Surflex with Geom-X option) were then rescored using Sybyl-X CScore module[6] and ranked using its PMF scoring function implementation.[7] The 10 best compounds (highest scores) were then assessed for their capacity to fulfil the experimentally observed NMR nOes. Theoretical nOes restraints of the generated models were then compared to the experimentally observed nOes (back calculations) and are listed in Fig S22. For the back calculations, interatomic distances between all hydrogens were calculated using an in-house tcl script in VMD[8] (http://www.ks.uiuc.edu/Research/vmd/). Theoretical nOes were classified as strong, medium or weak for hydrogens within distances of 2.5Å, 3.7Å and 5.0Å respectively. The final model is presented Figure 4 and 5. This model is fully compatible with the NMR observed nOes (magnetically equivalent hydrogens must be taken into account when comparing both experimental and theoretical nOes) and was ranked as model #2 by surflex-dock, in presence of the SH3 domain extracted from the Fyn kinase protein.

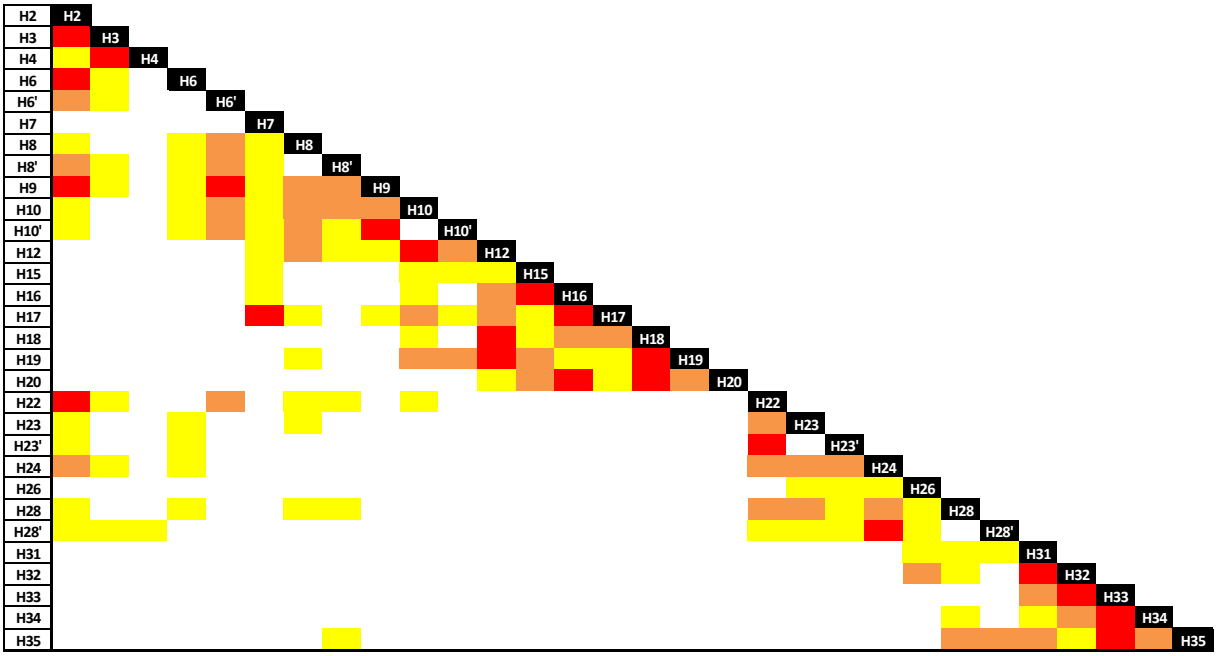
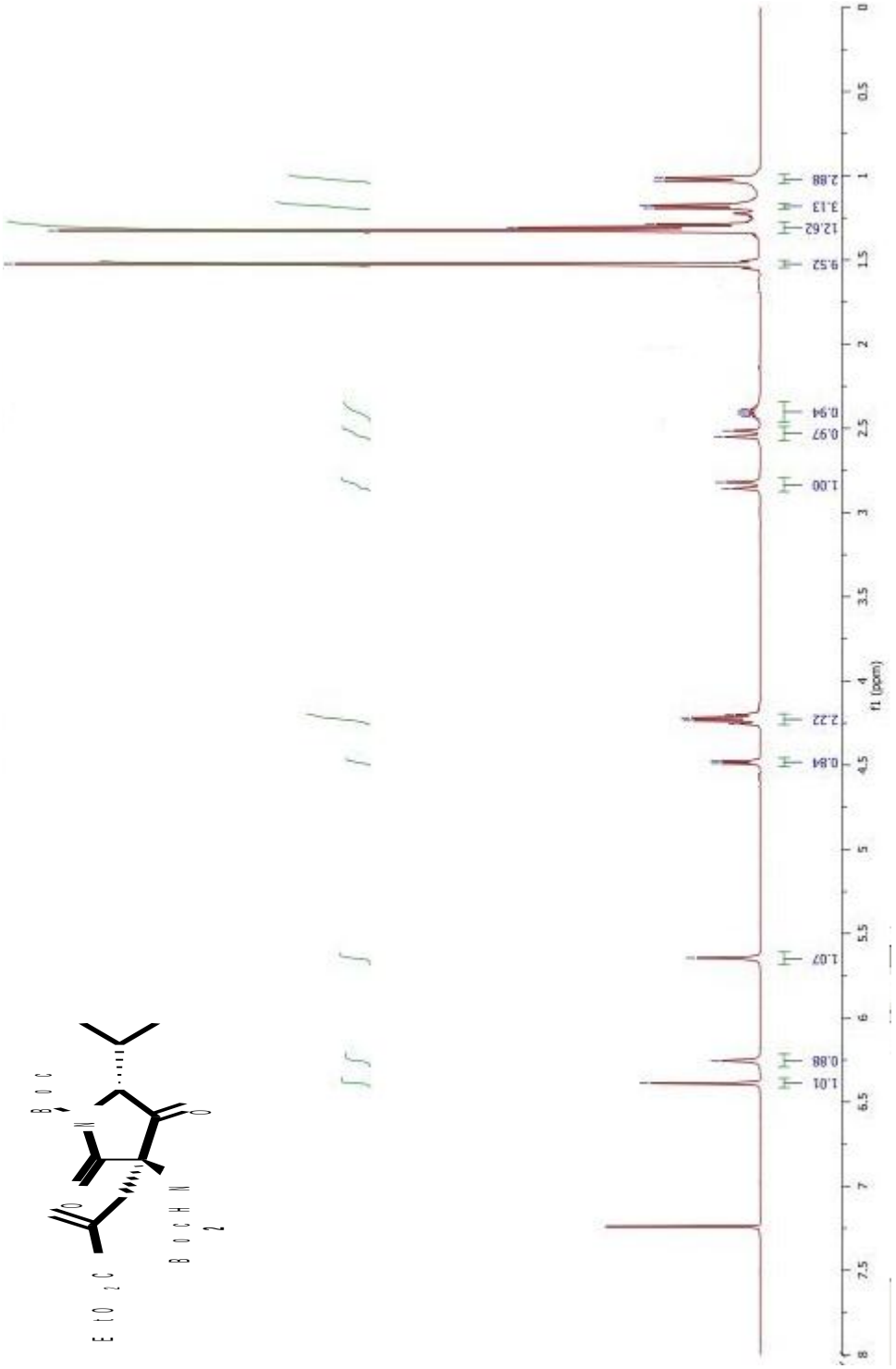


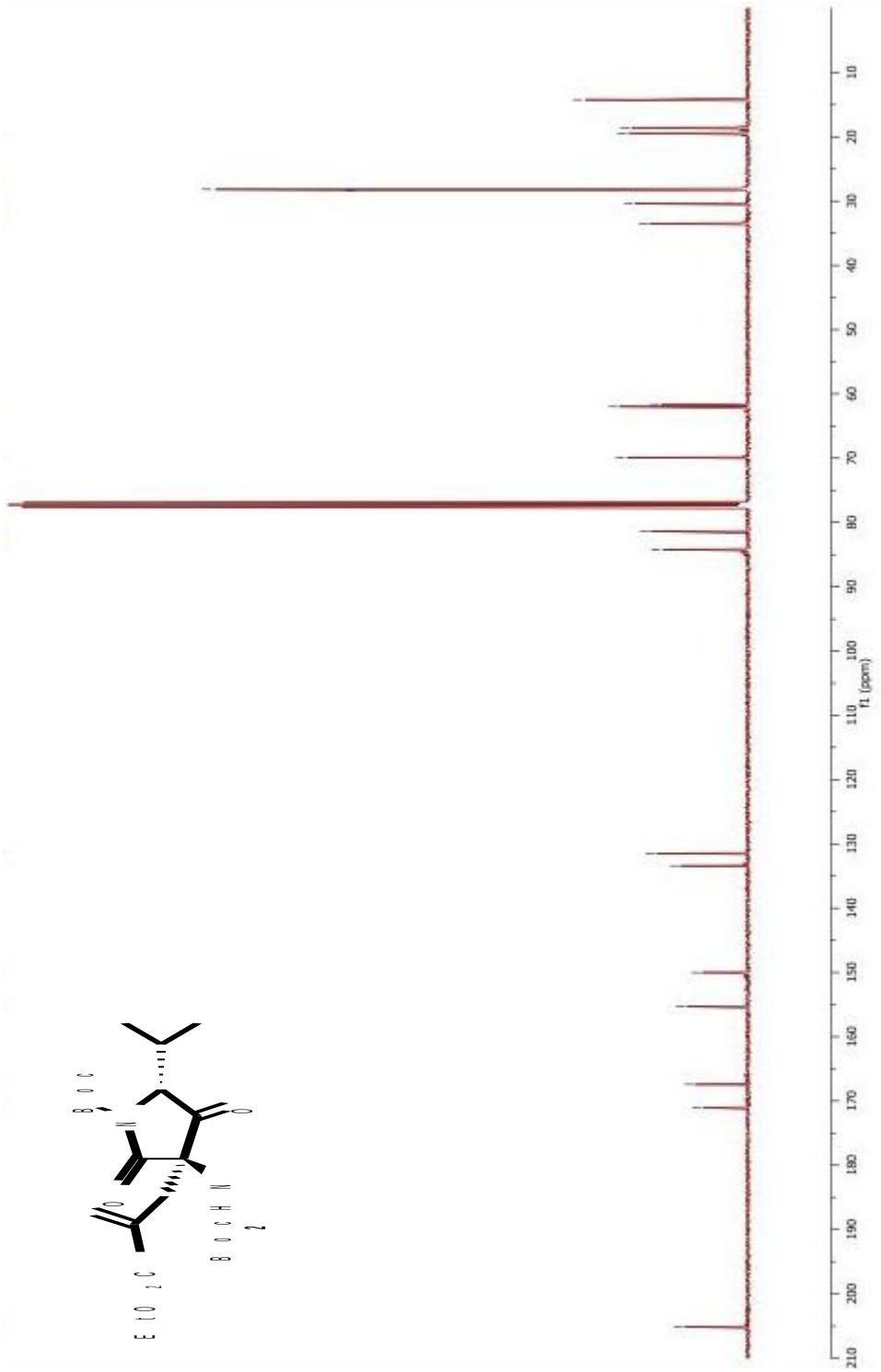
Figure S27. Theoretical nOe correlations of **12a** docking model (Red for *strong*, orange for *medium* and yellow for *weak*). Whereas several nOe correlations are theoretically observed but not experimentally, 100% of experimental observed nOe correlations in **12a** are present in the docking model (Figure S8).

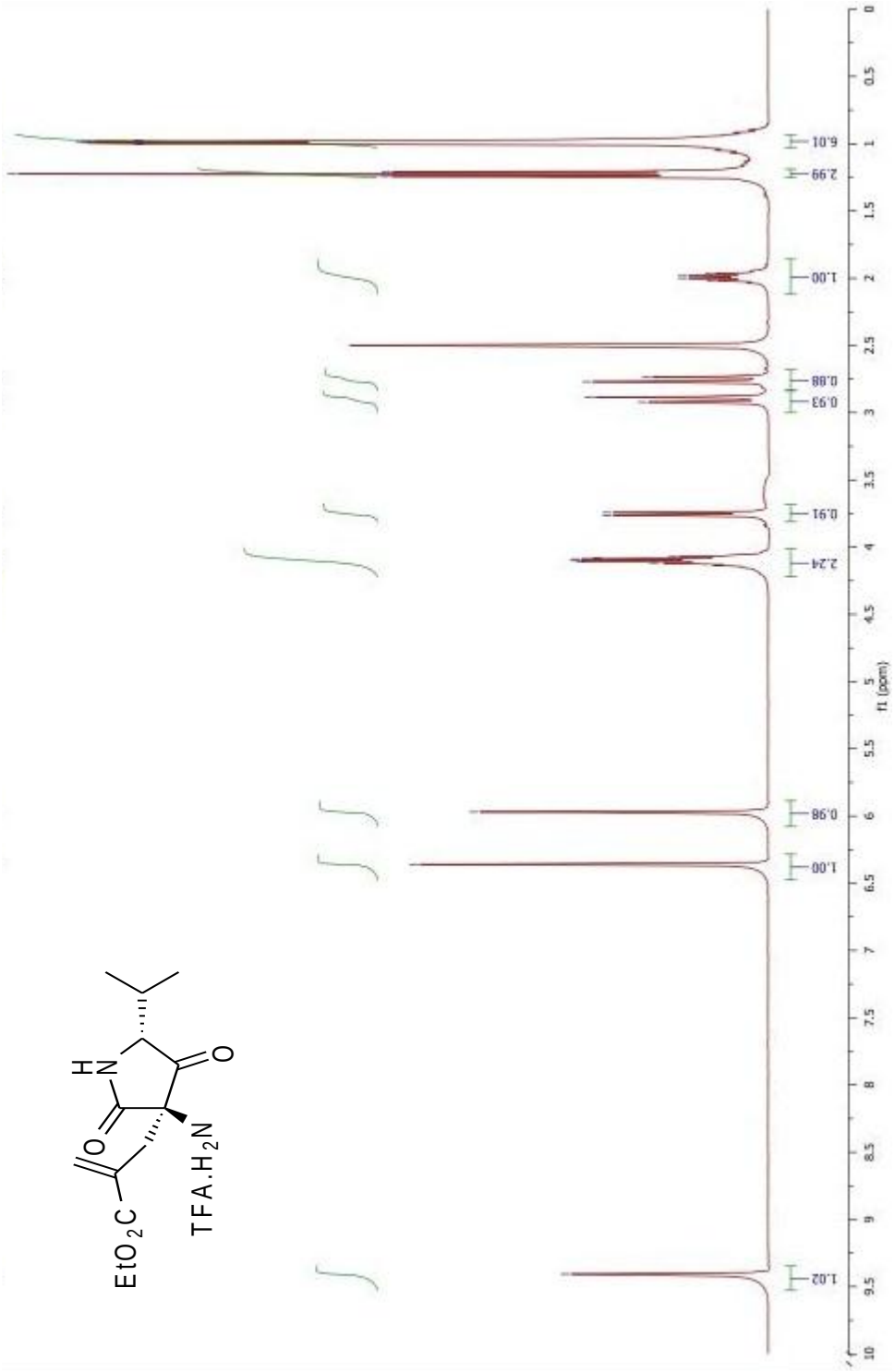
Scanning Electron Microscopy

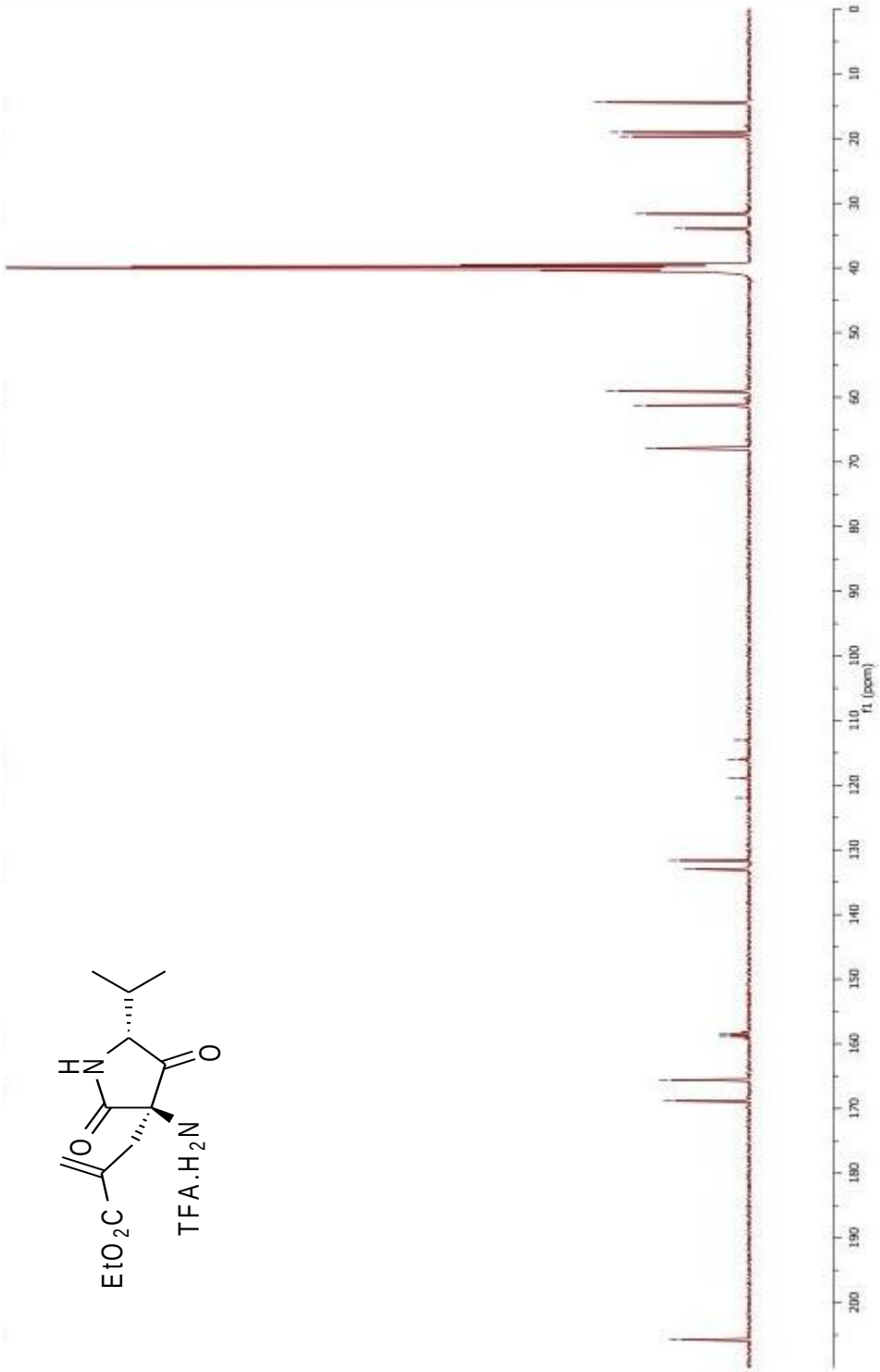
SEM experiments were taken on a FEI Quanta FEG 200. The compound in solid form was deposited directly on an adhesive pad for observation.

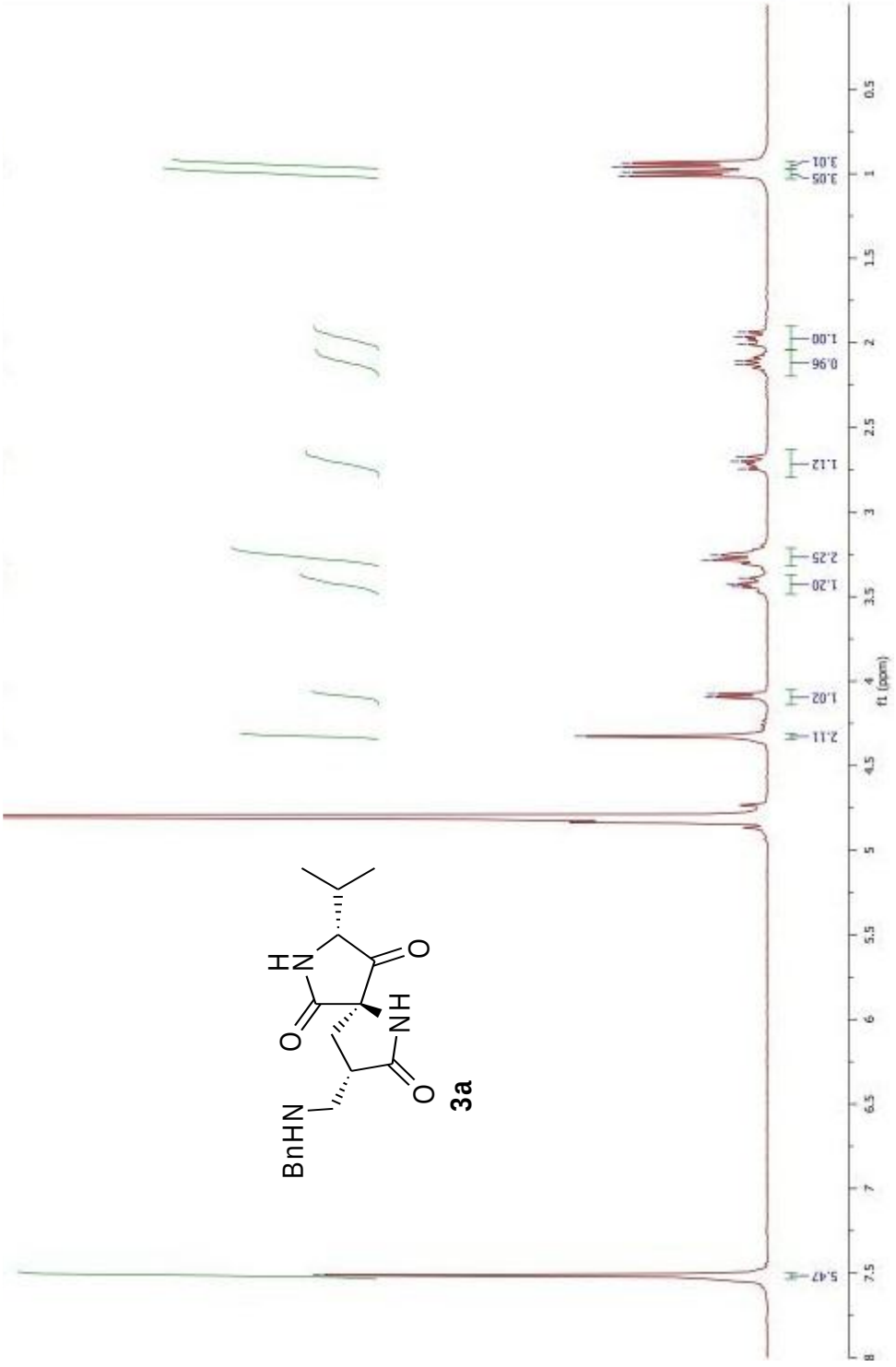
NMR Spectra for Compounds 2 to 11b

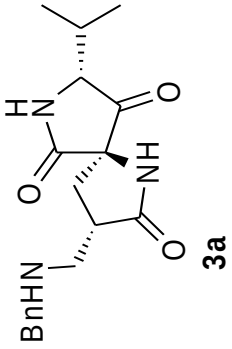



f1 (ppm)

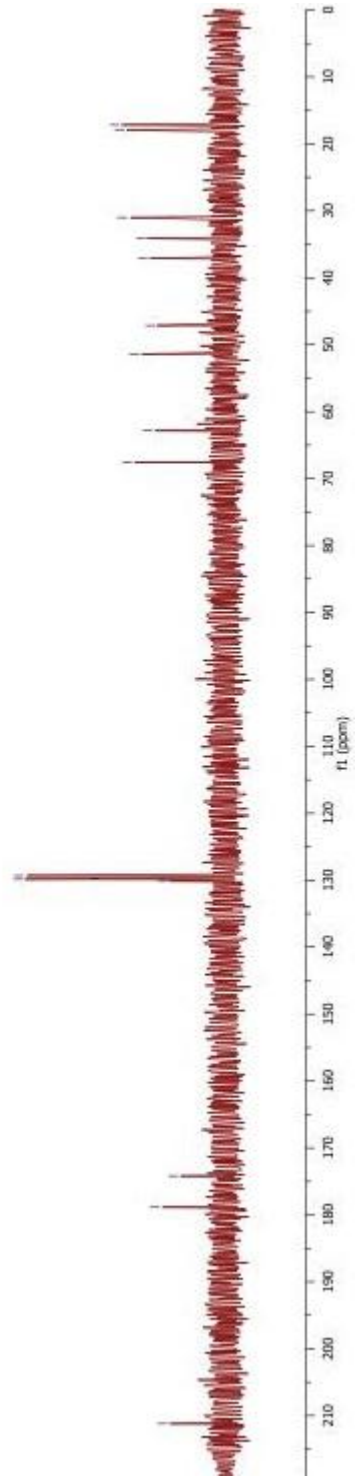


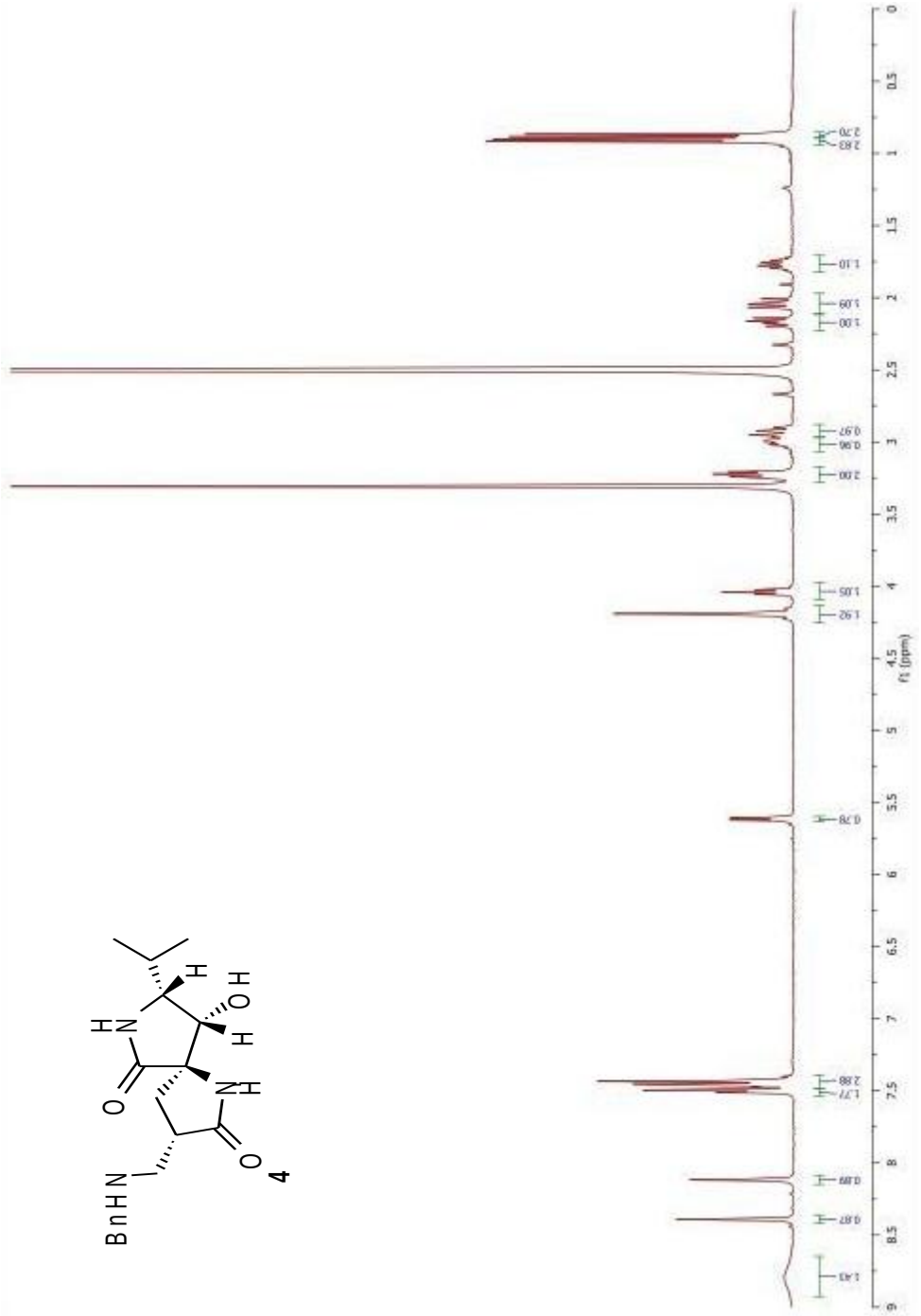


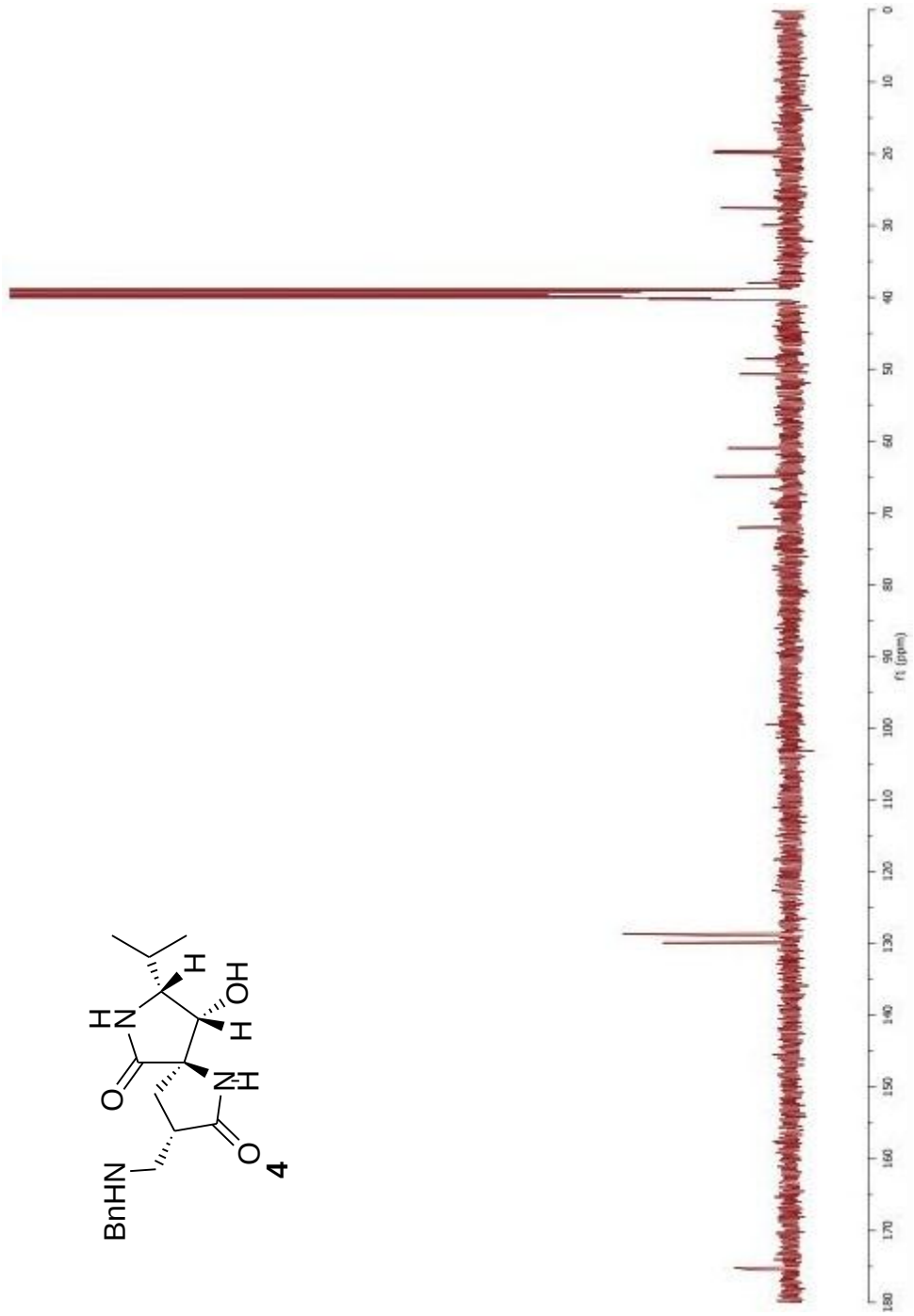


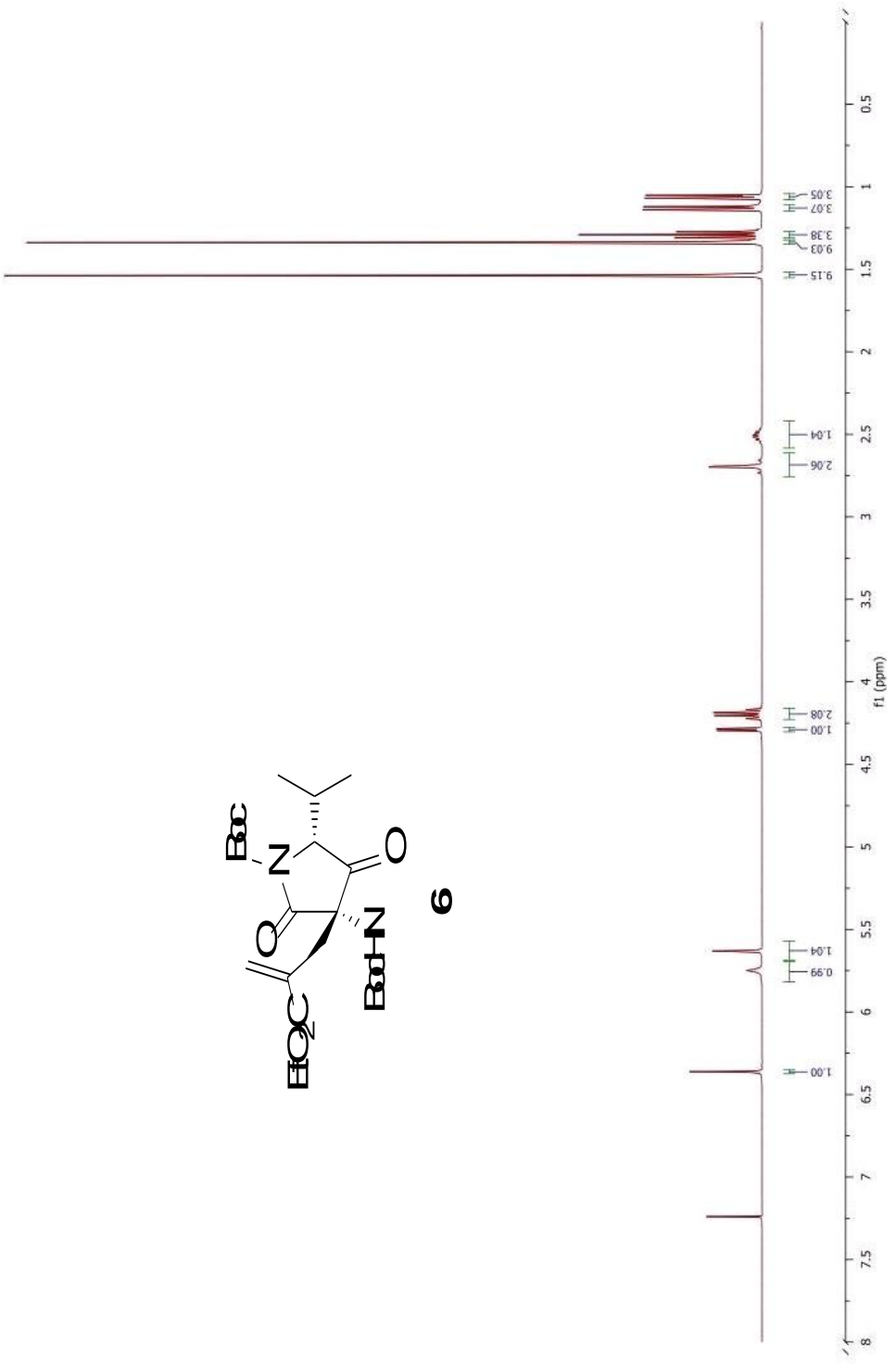


3a









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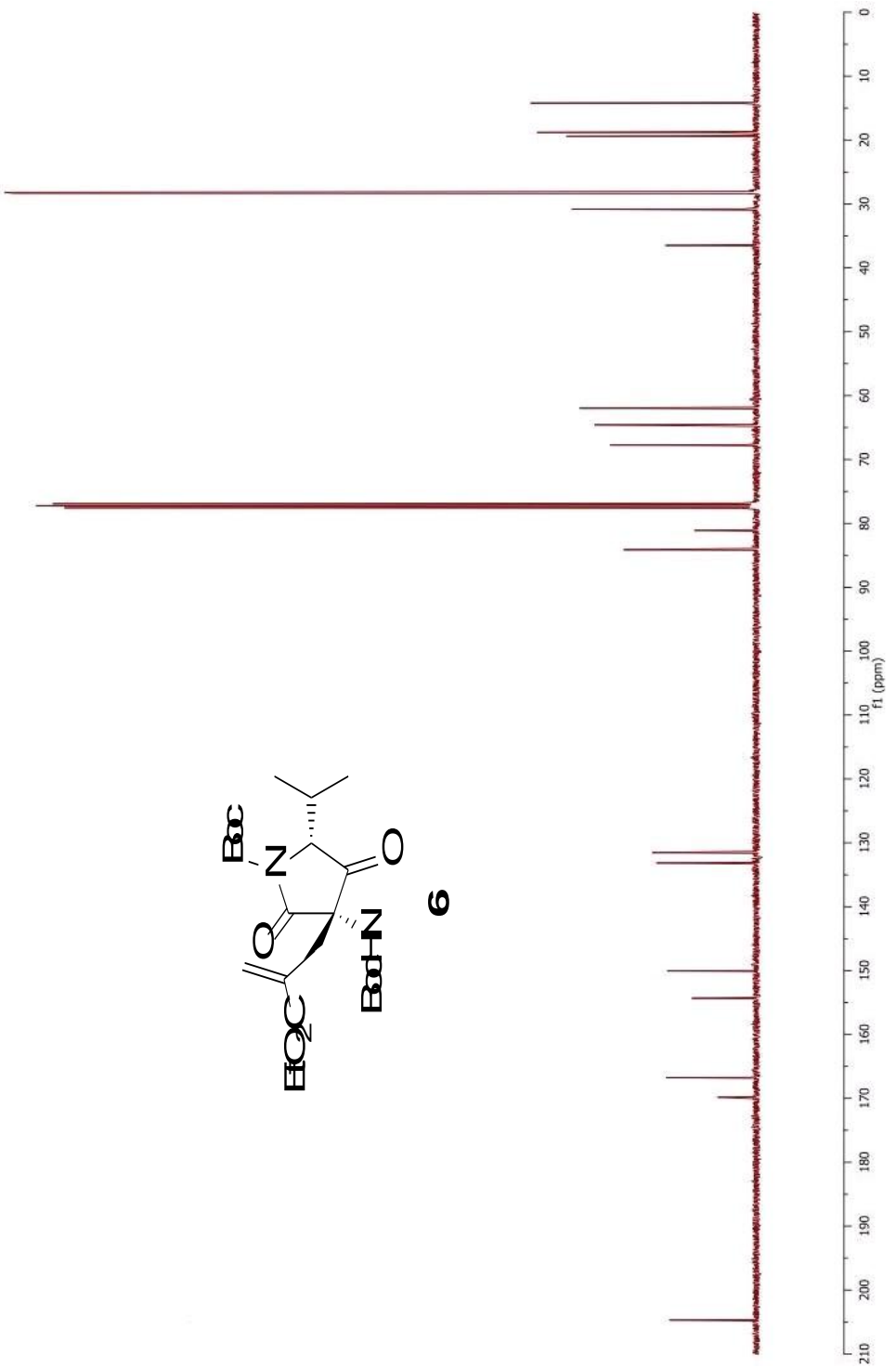

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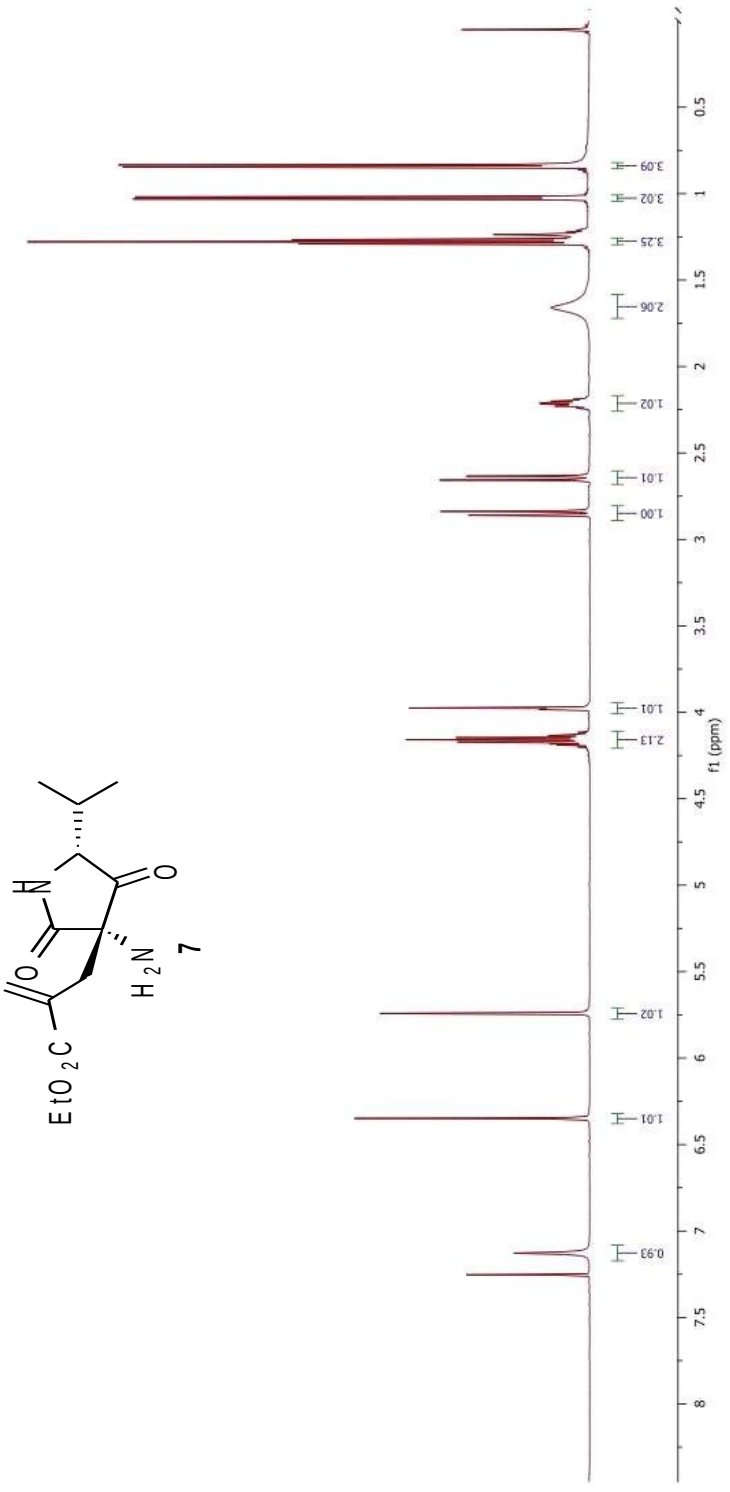

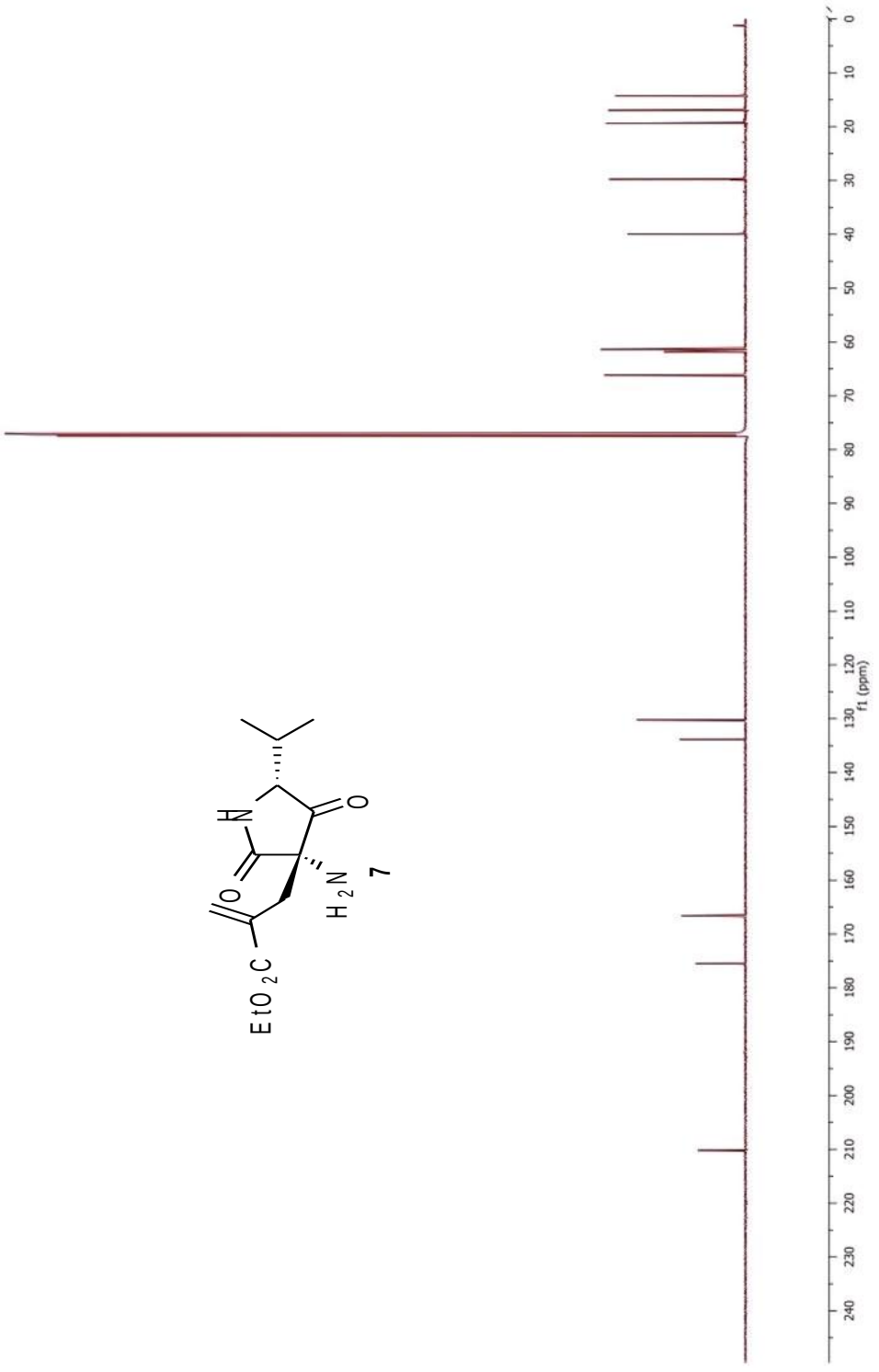

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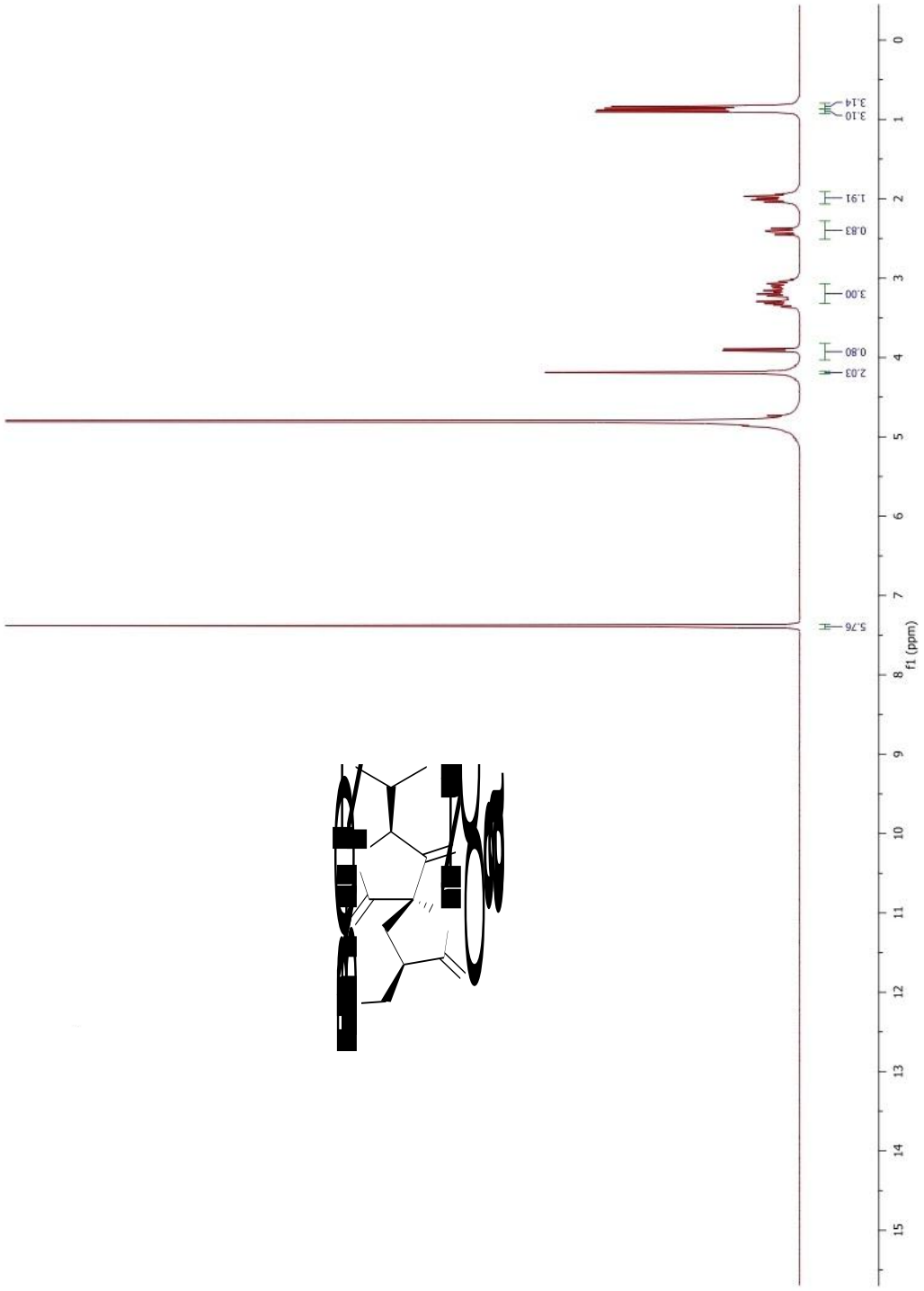

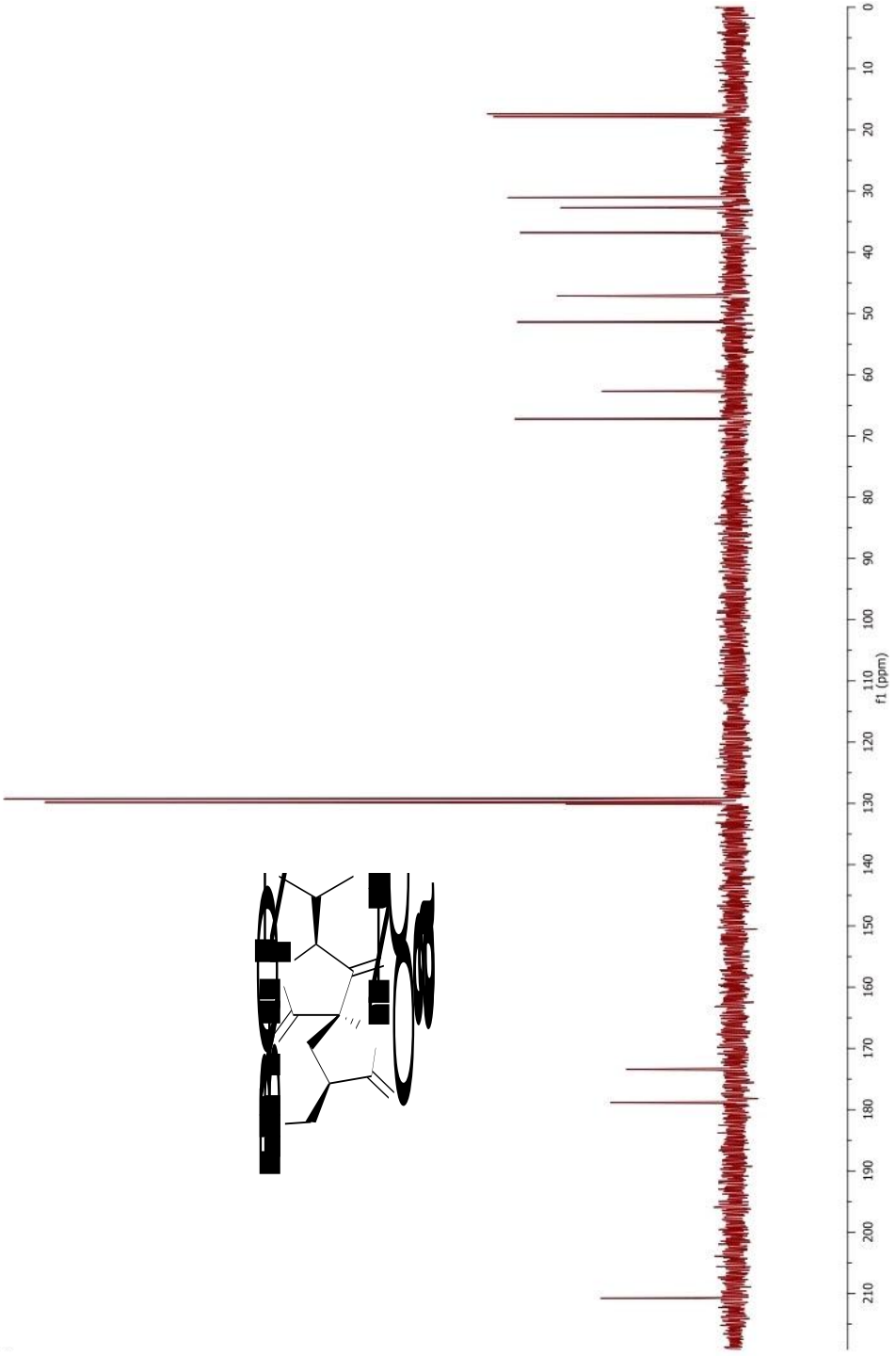

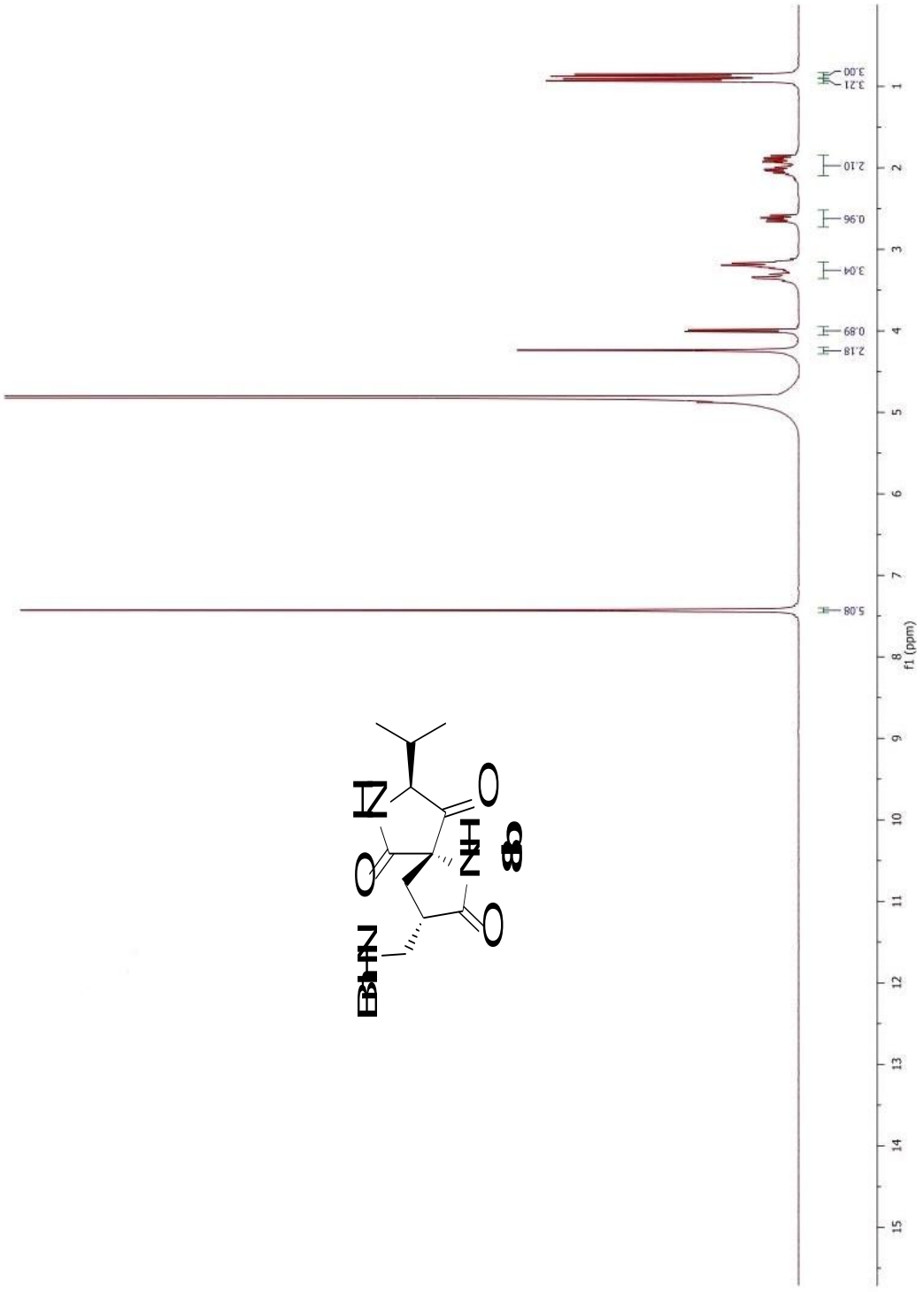

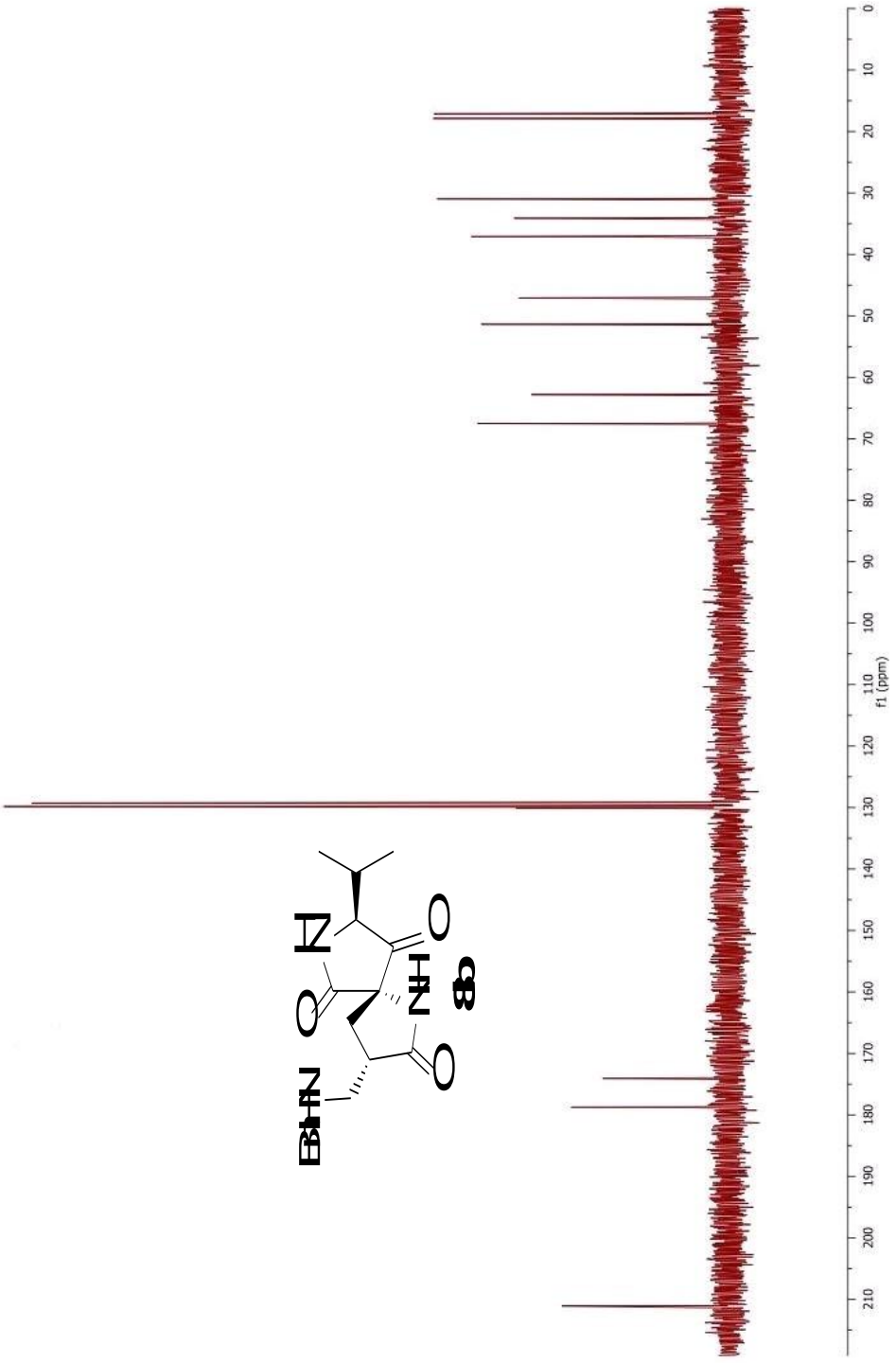

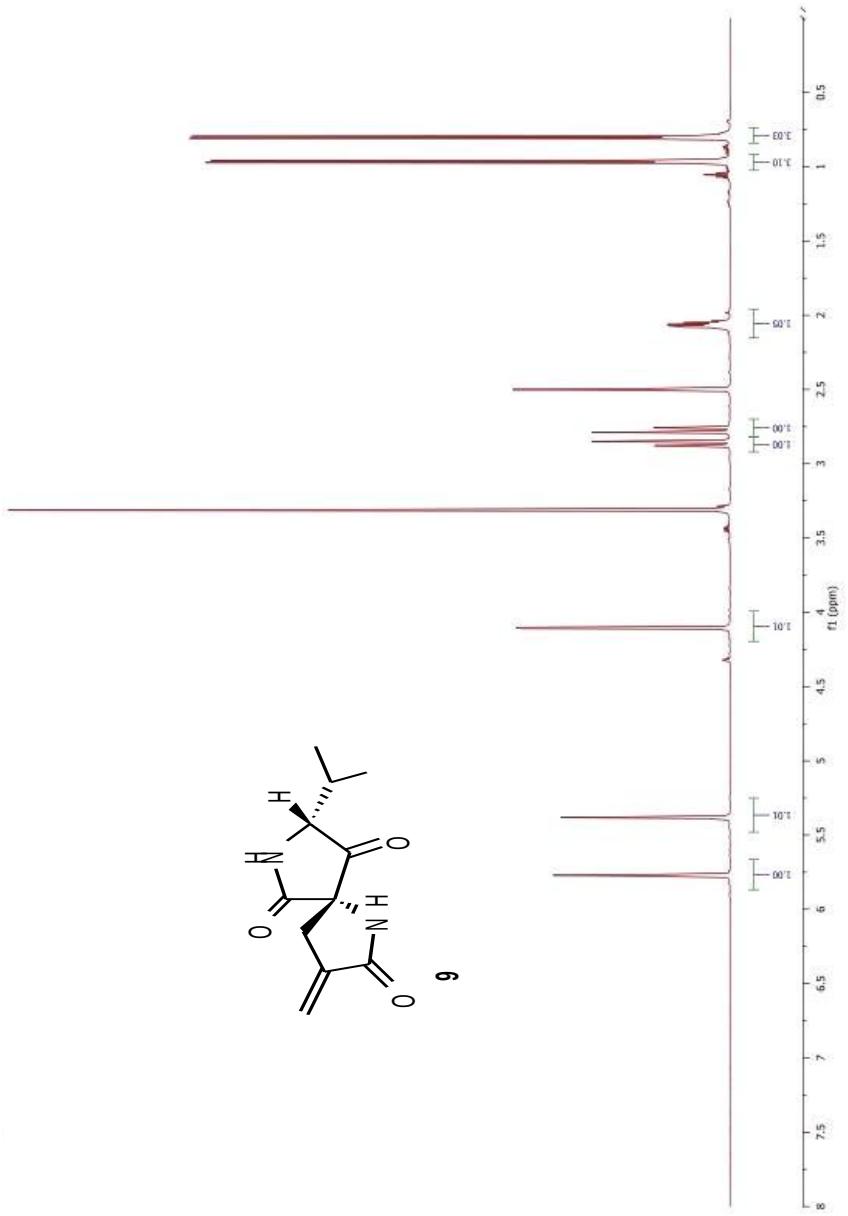

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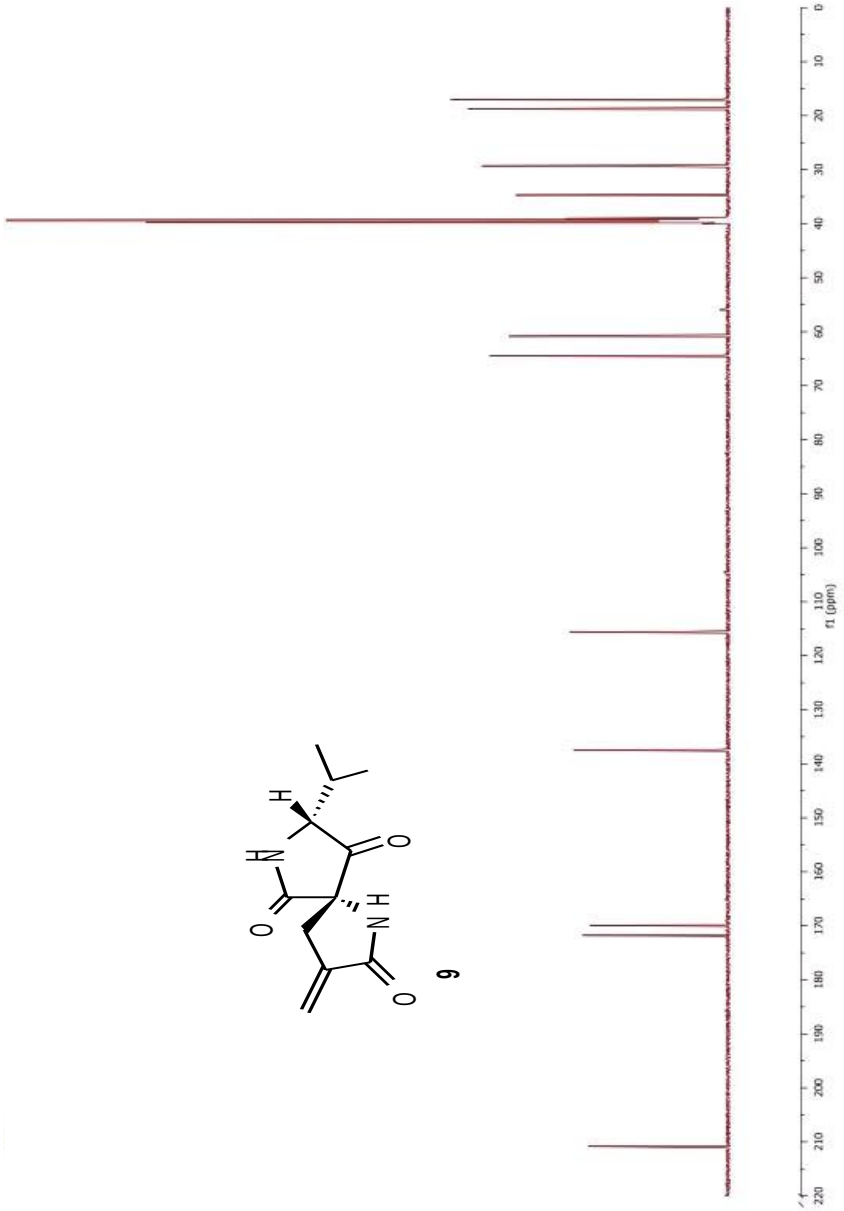

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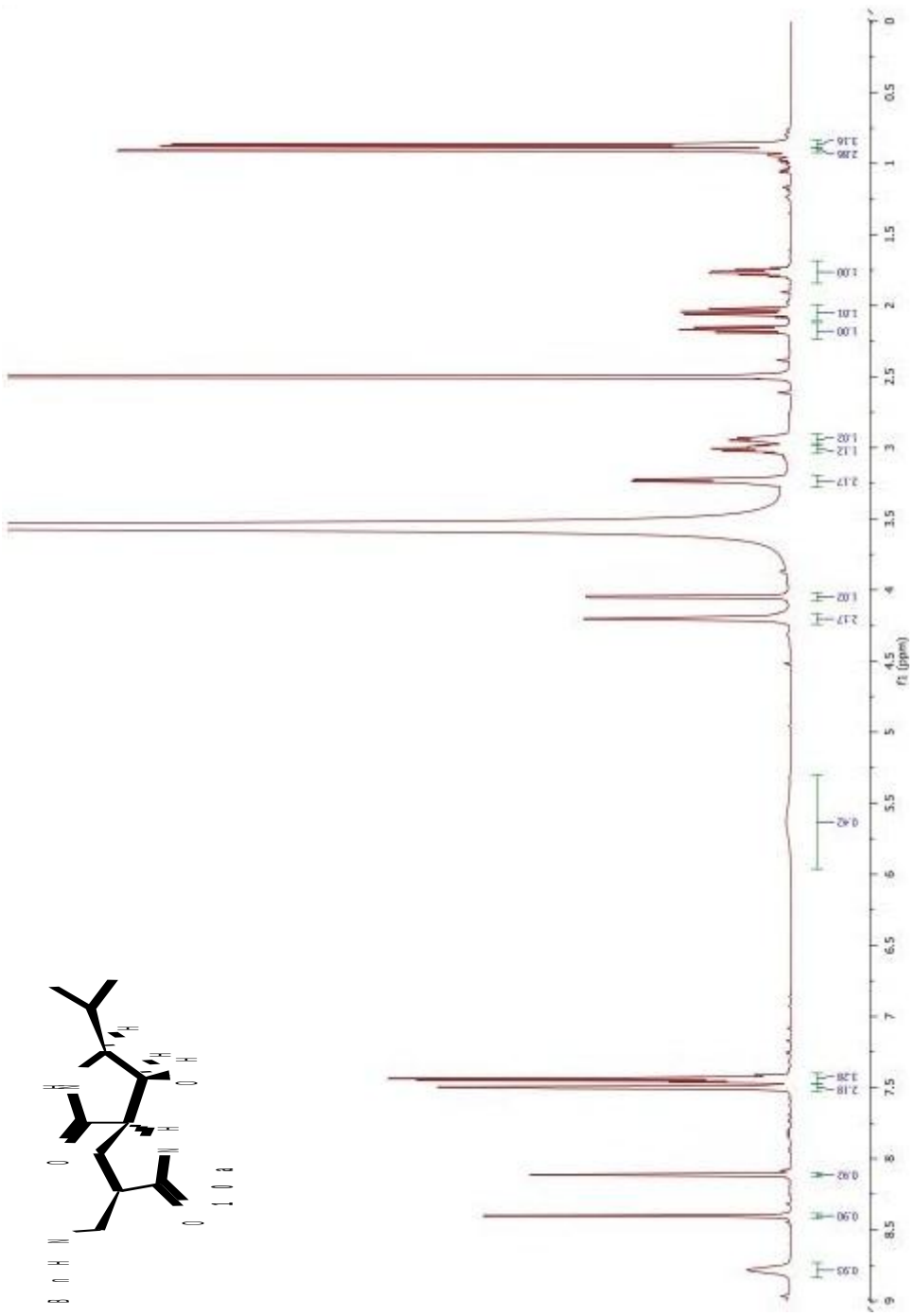

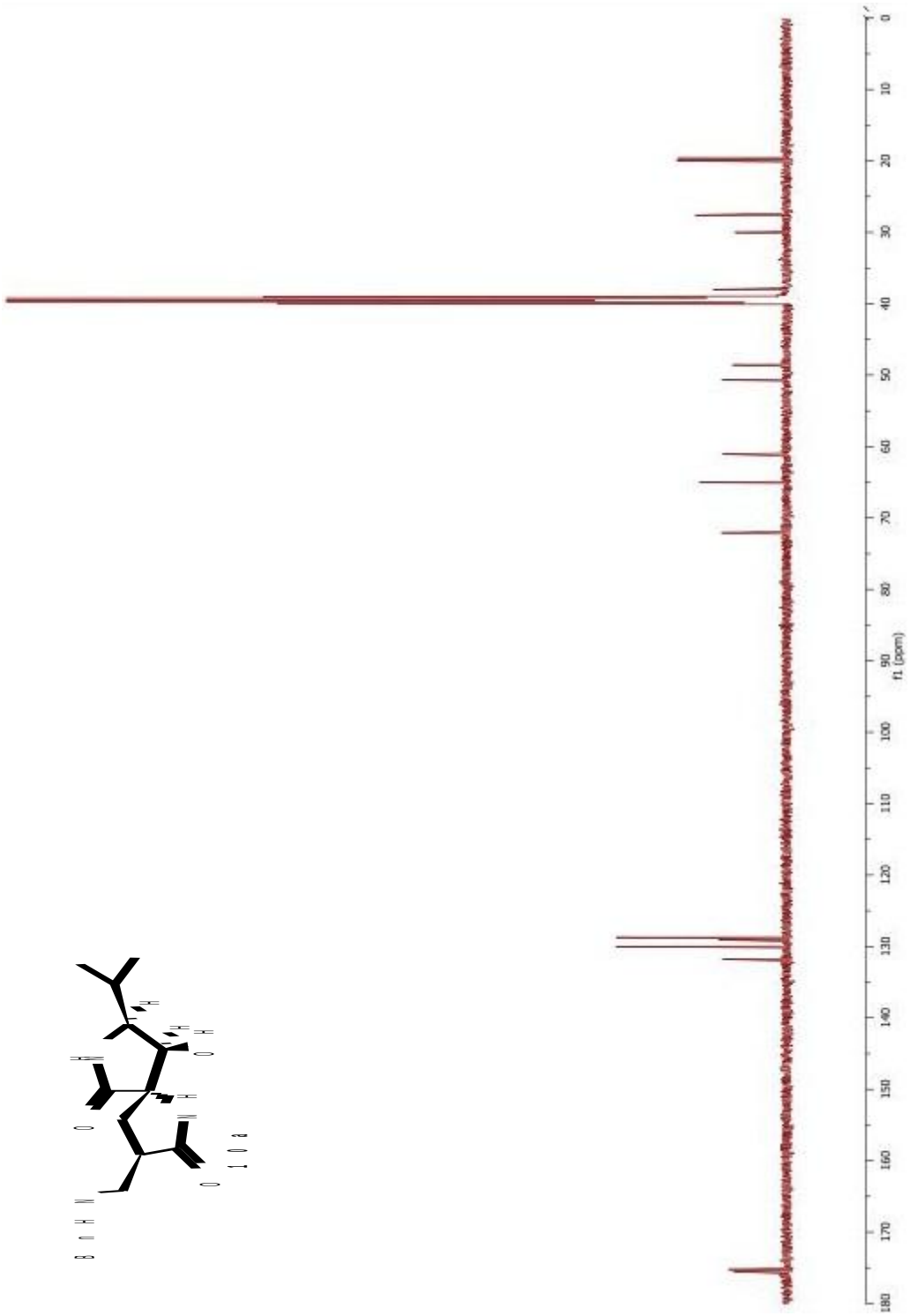

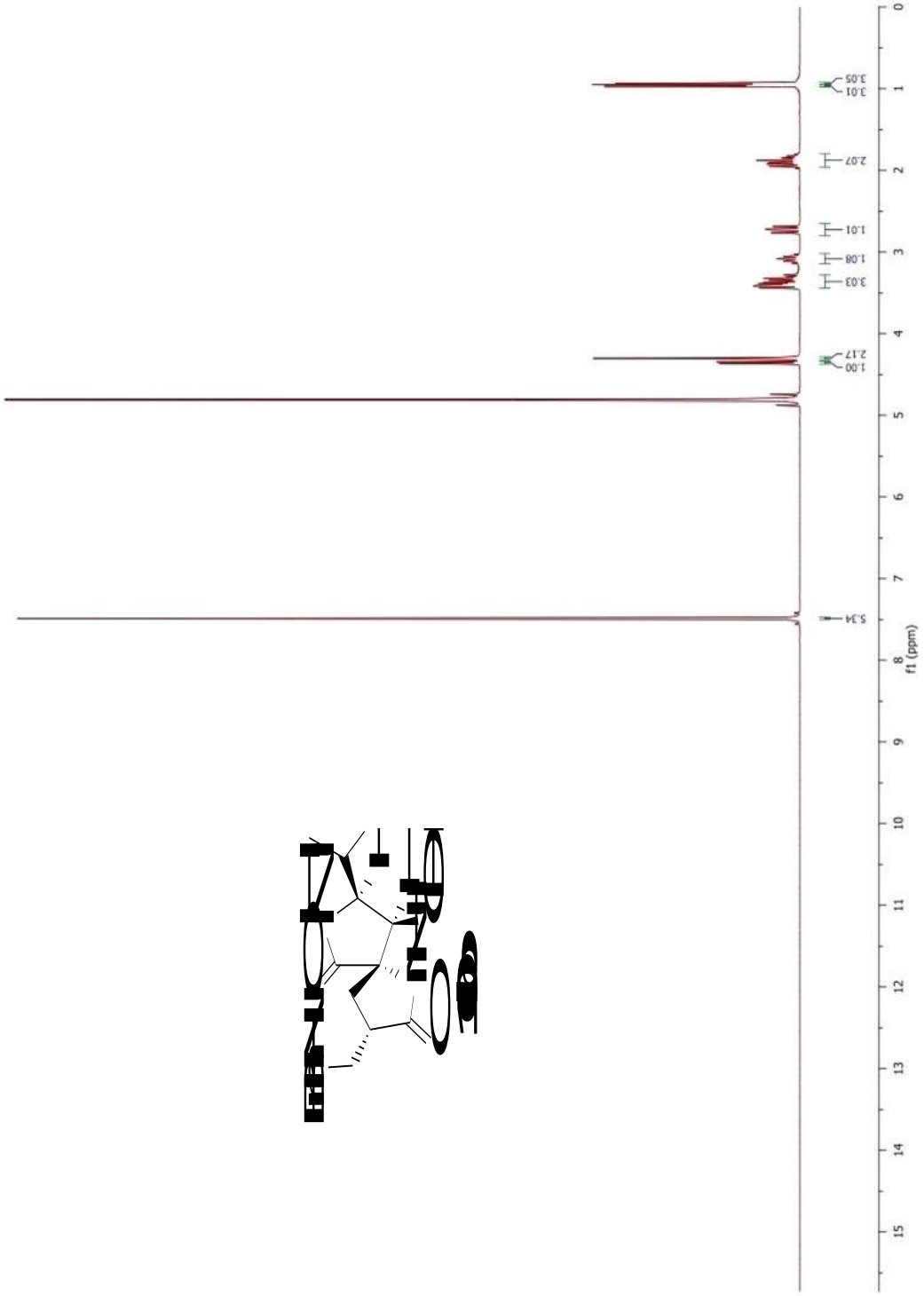

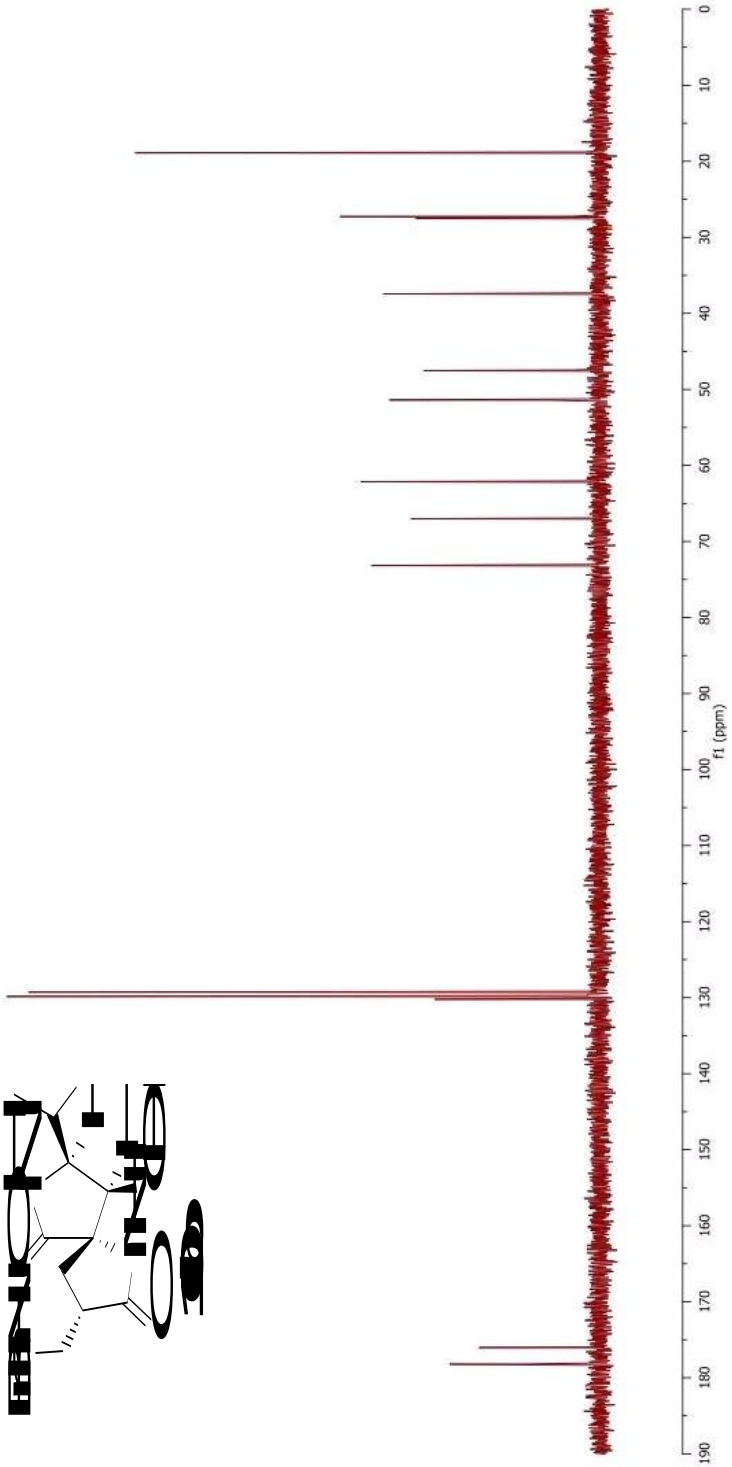

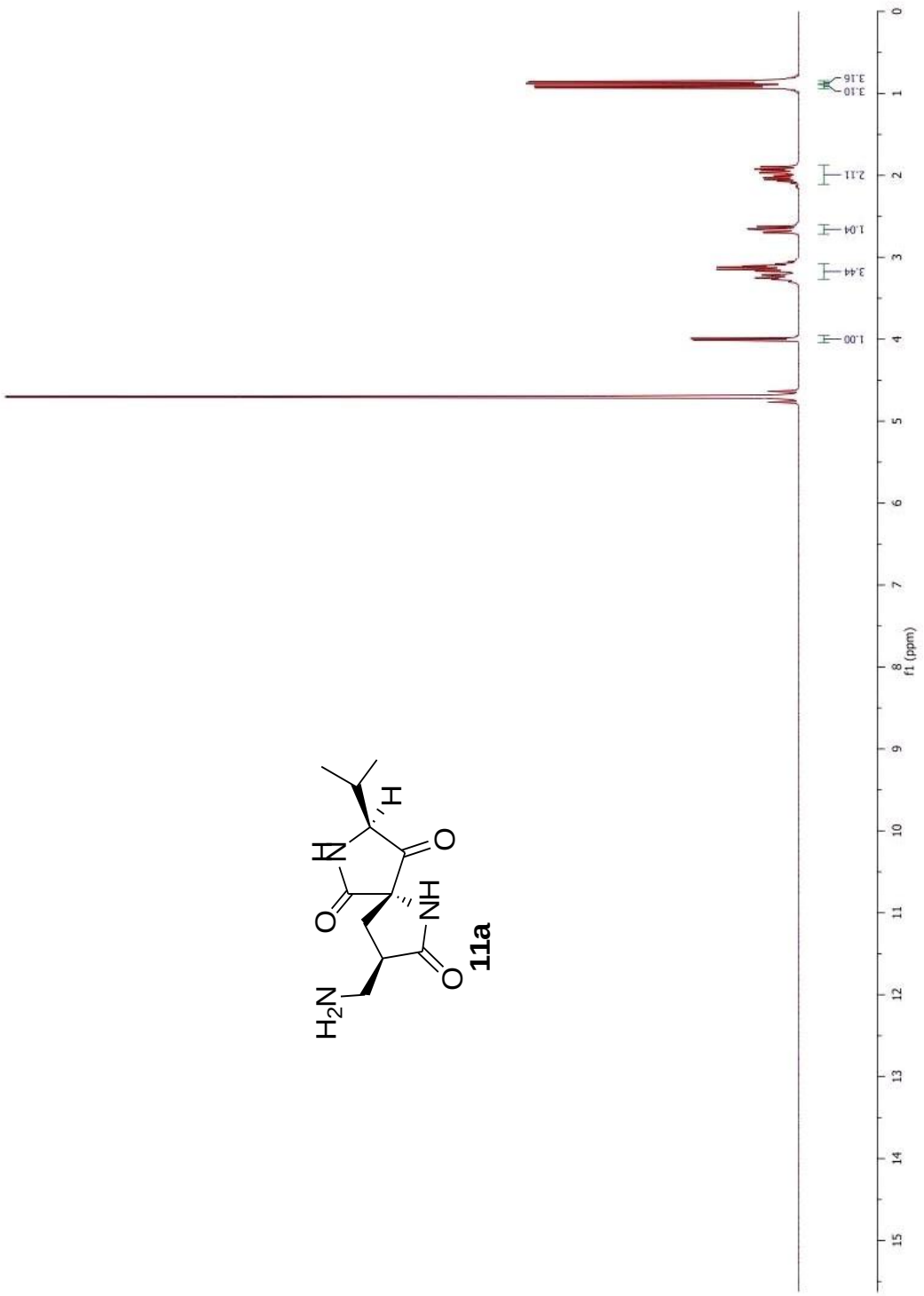

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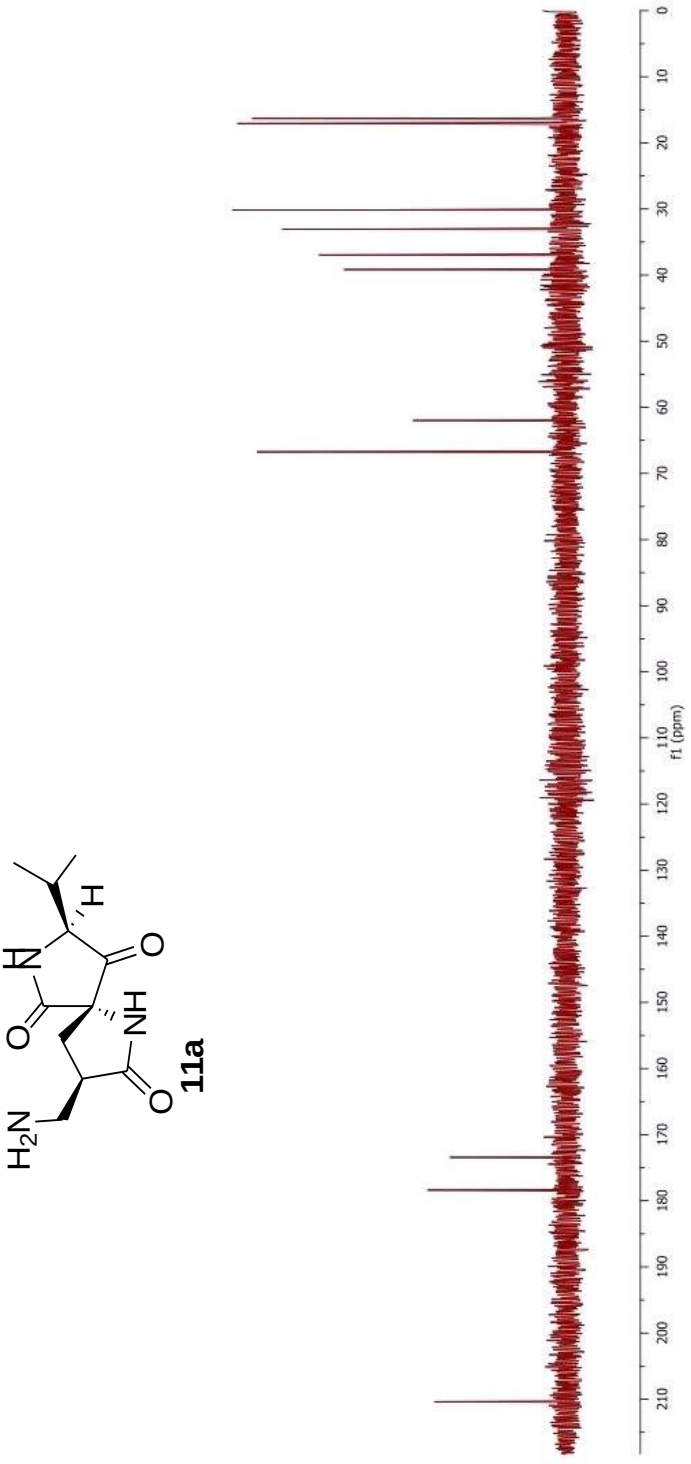

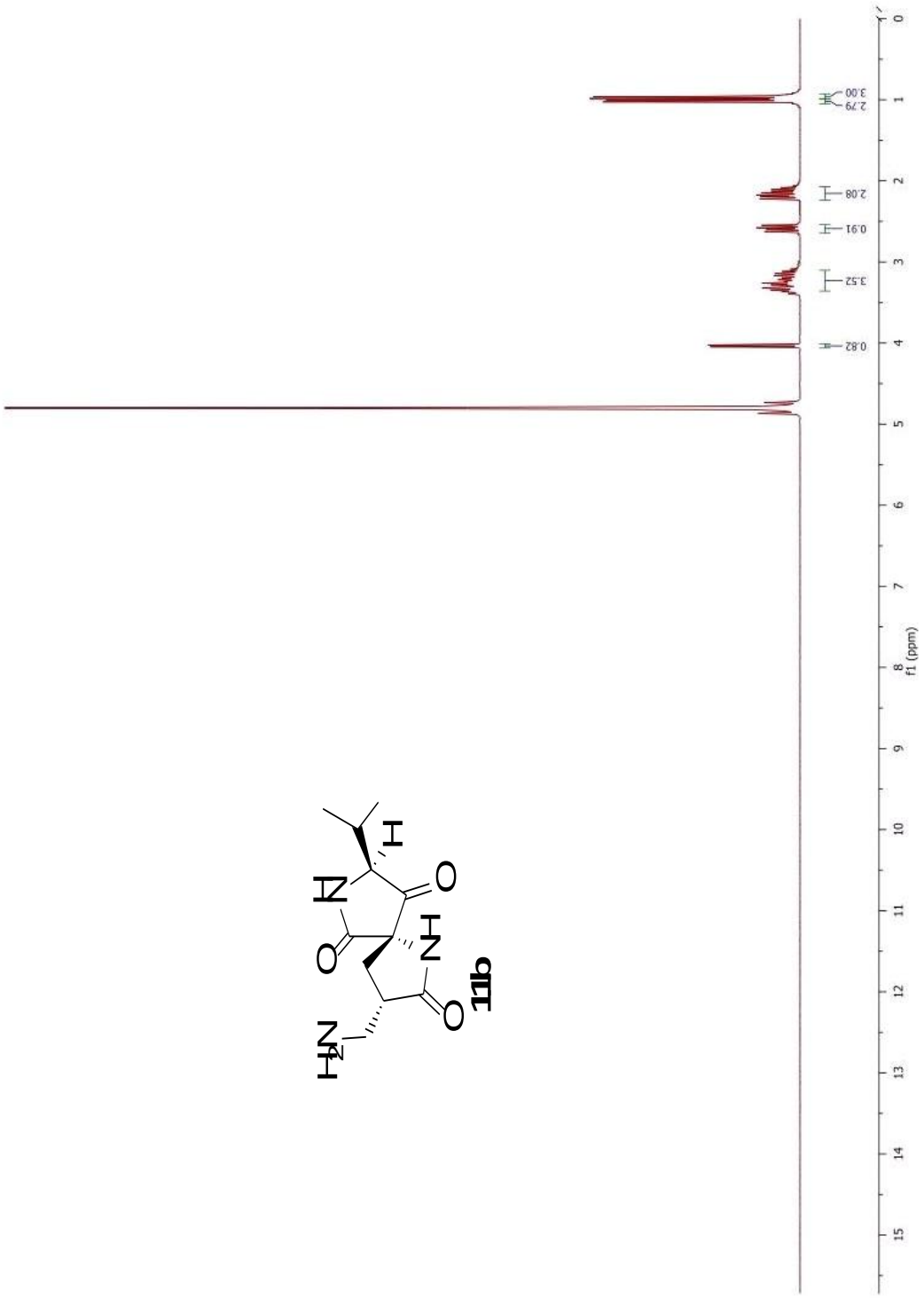

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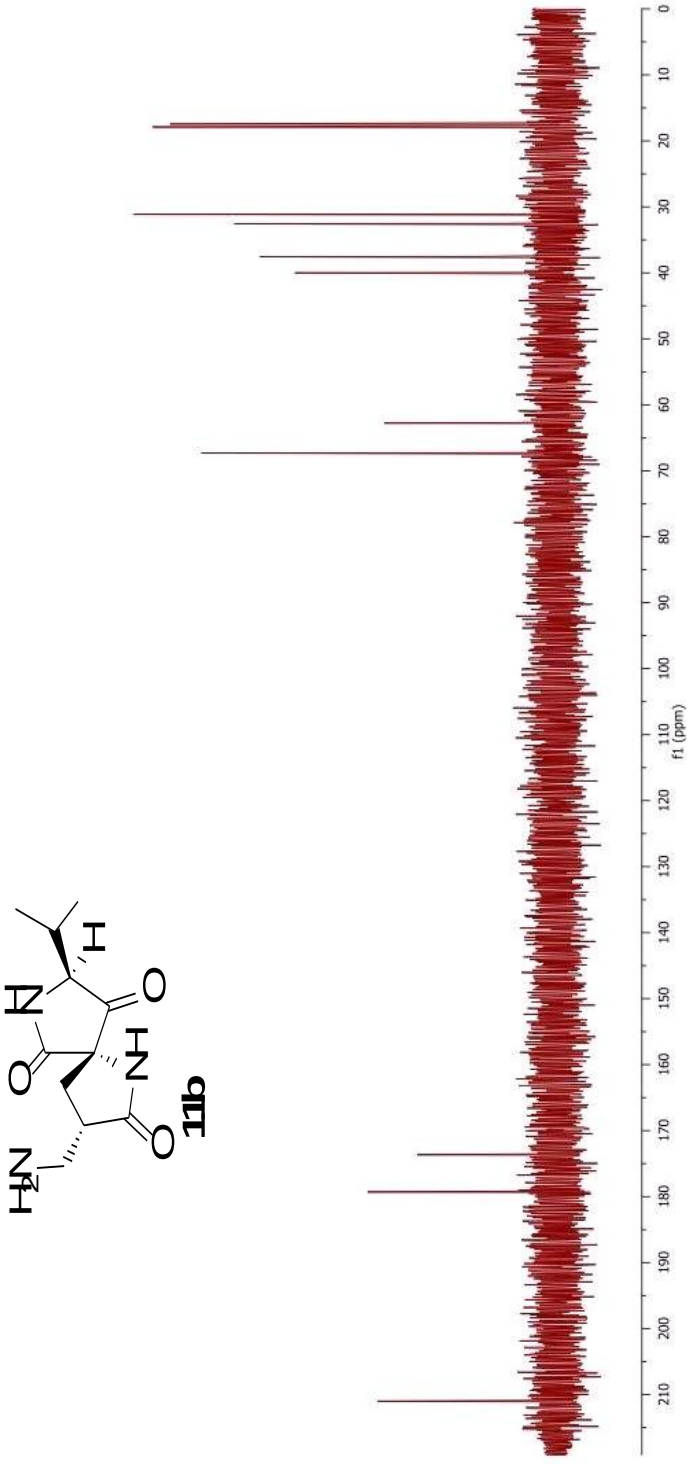

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