SUPPORTING INFORMATION

Helical foldamers incorporating photoswitchable residues for light-mediated modulation of conformational preference

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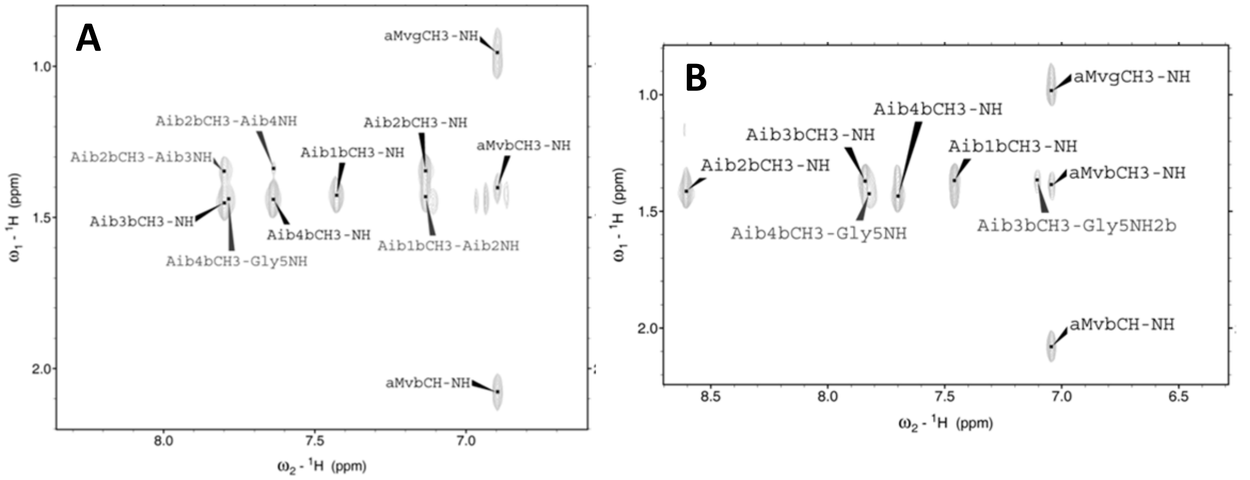


Figure S1. $\beta CH_3 \rightarrow \alpha NH$ region of the NOESY spectra (500 MHz, CD_3CN) of peptides **2a** (**A**) and **3a** (**B**). Sequential ($i \rightarrow i+1$) and medium-range ($i \rightarrow i+2$), diagnostic of the presence of a 3_{10}-helical structure are visible in both spectra.

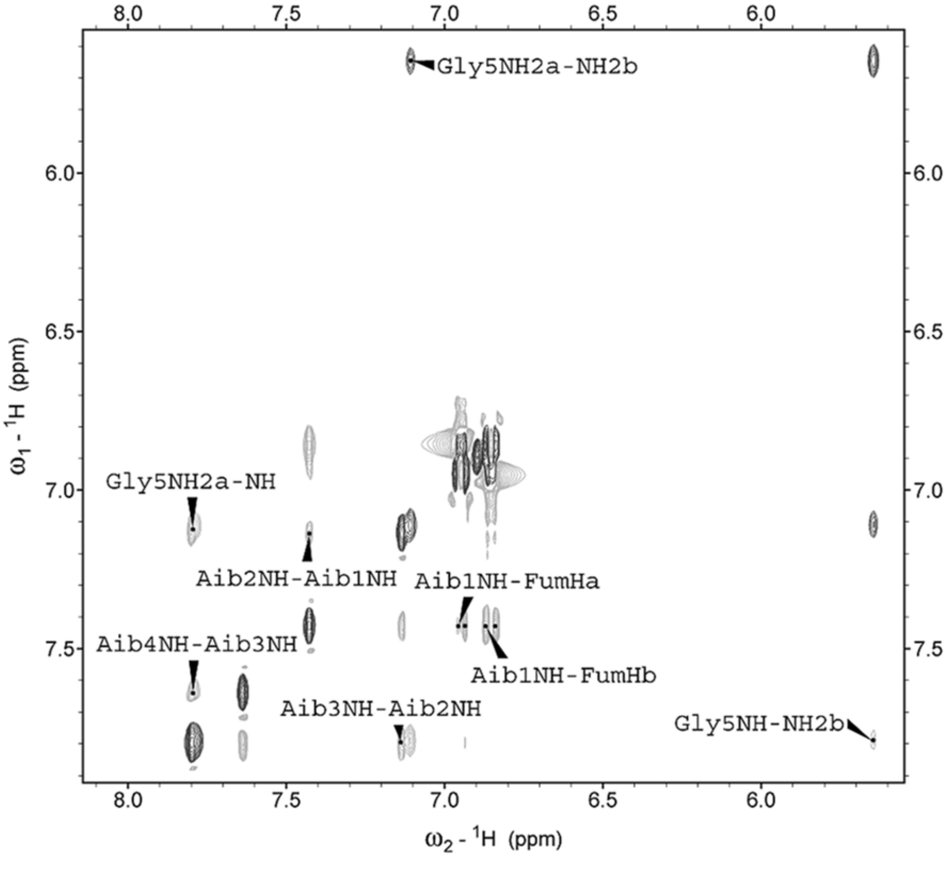


Figure S2. Amide region of the NOESY spectrum (500 MHz, CD_3CN) of **2a**. Sequential $\alpha NH(i) \rightarrow \alpha NH(i+1)$ are assigned in the spectrum.

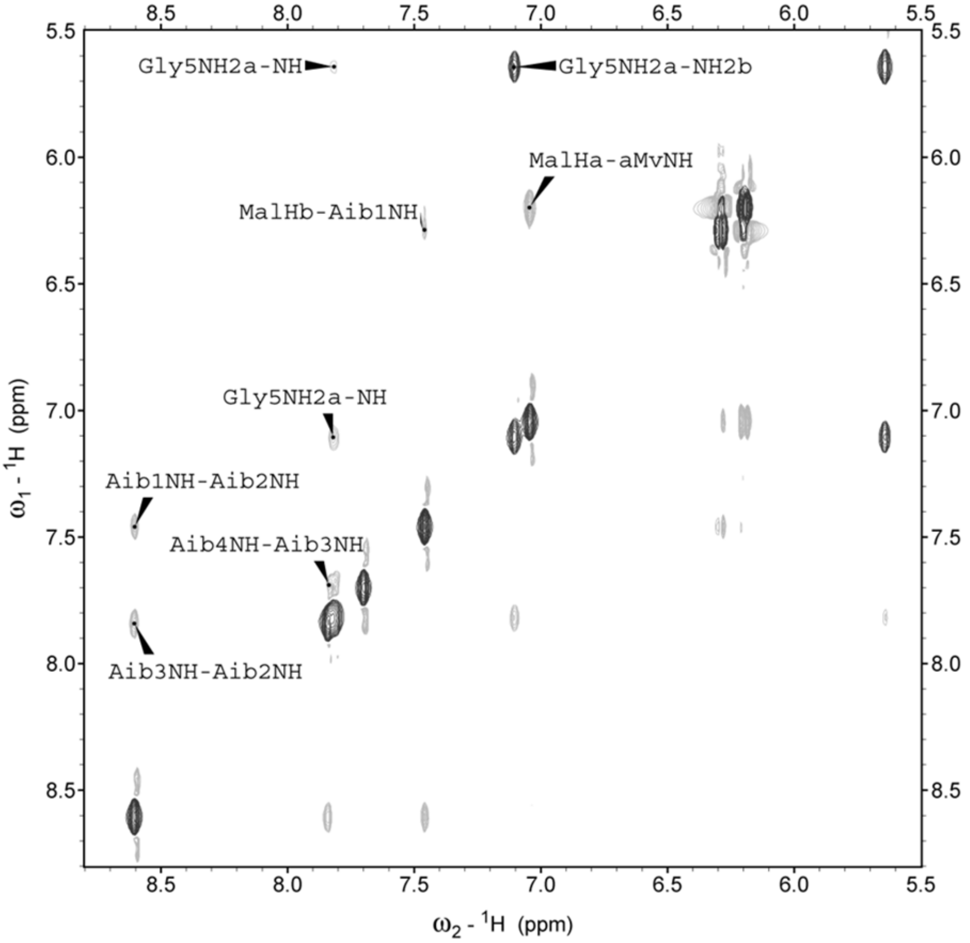


Figure S3. Amide region of the NOESY spectrum (500 MHz, CD_3CN) of **3a**. Sequential αNH(*i*)→αNH(*i*+1) are assigned in the spectrum.

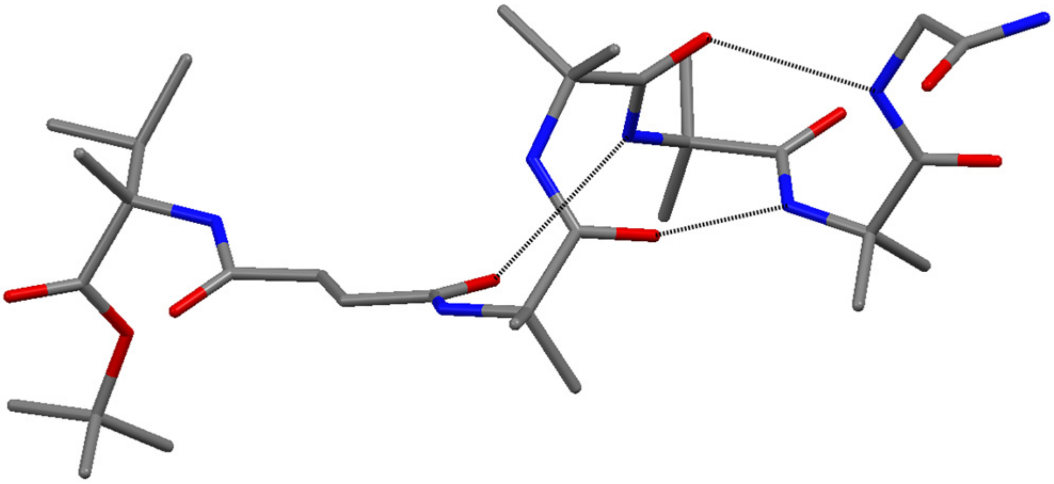


Figure S4. Model of **2a** with the -(Aib)$_4$- helical segment in the left-handed screw sense.

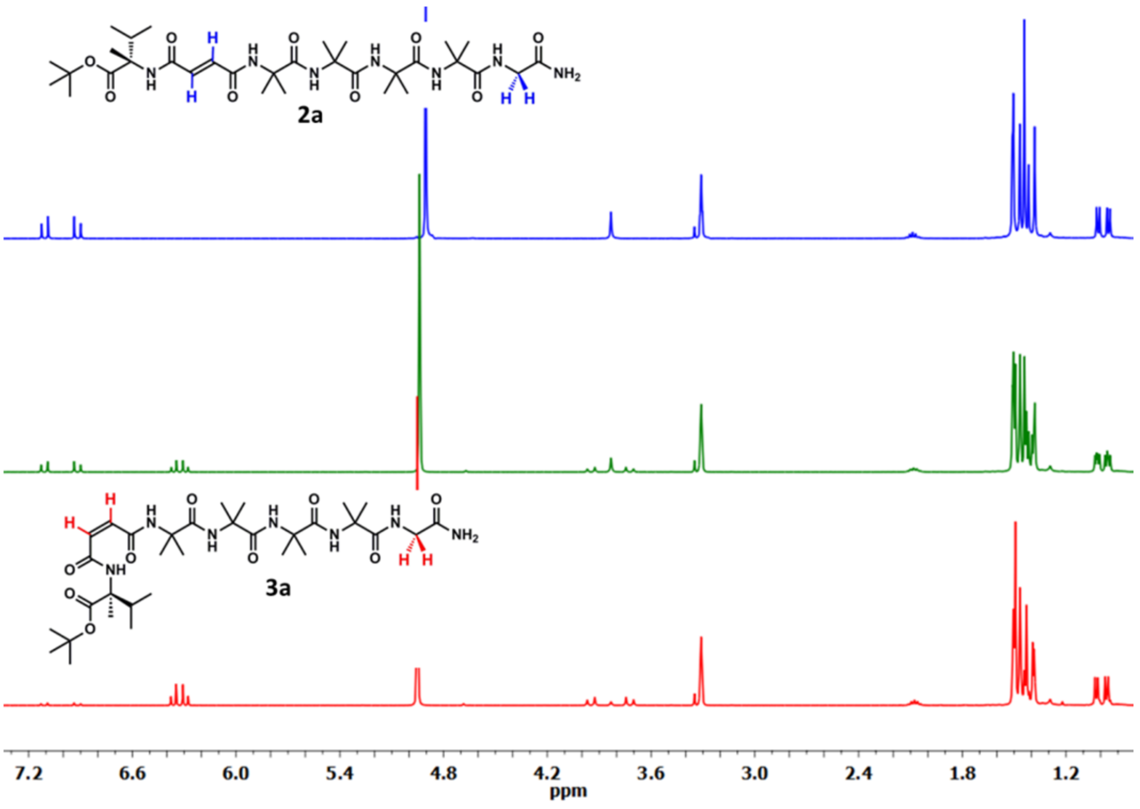


Figure S5. ^{1}H NMR spectra (500 MHz in CD_3OD) of **2a** before irradiation (blue line) and after irradiation at 254 nm for different times (30 min, green line and 1h, red line).

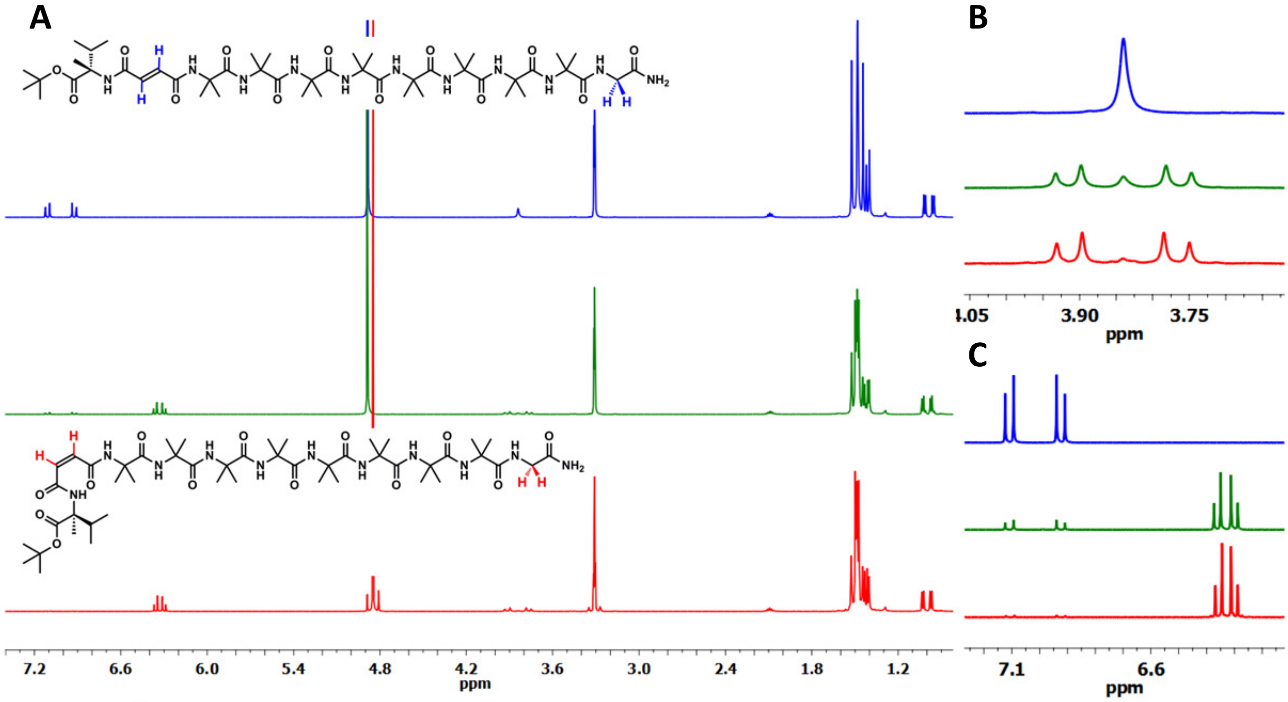


Figure S6. ^{1}H NMR spectra (500 MHz in CD_3OD) of **2b** before irradiation (blue line) and after irradiation at 254 nm for different times (30 min, green line and 1h, red line) (**A**). Details of the ^{1}H NMR spectra showing the glycinamide signals (**B**) and the olefininic protons (**C**).

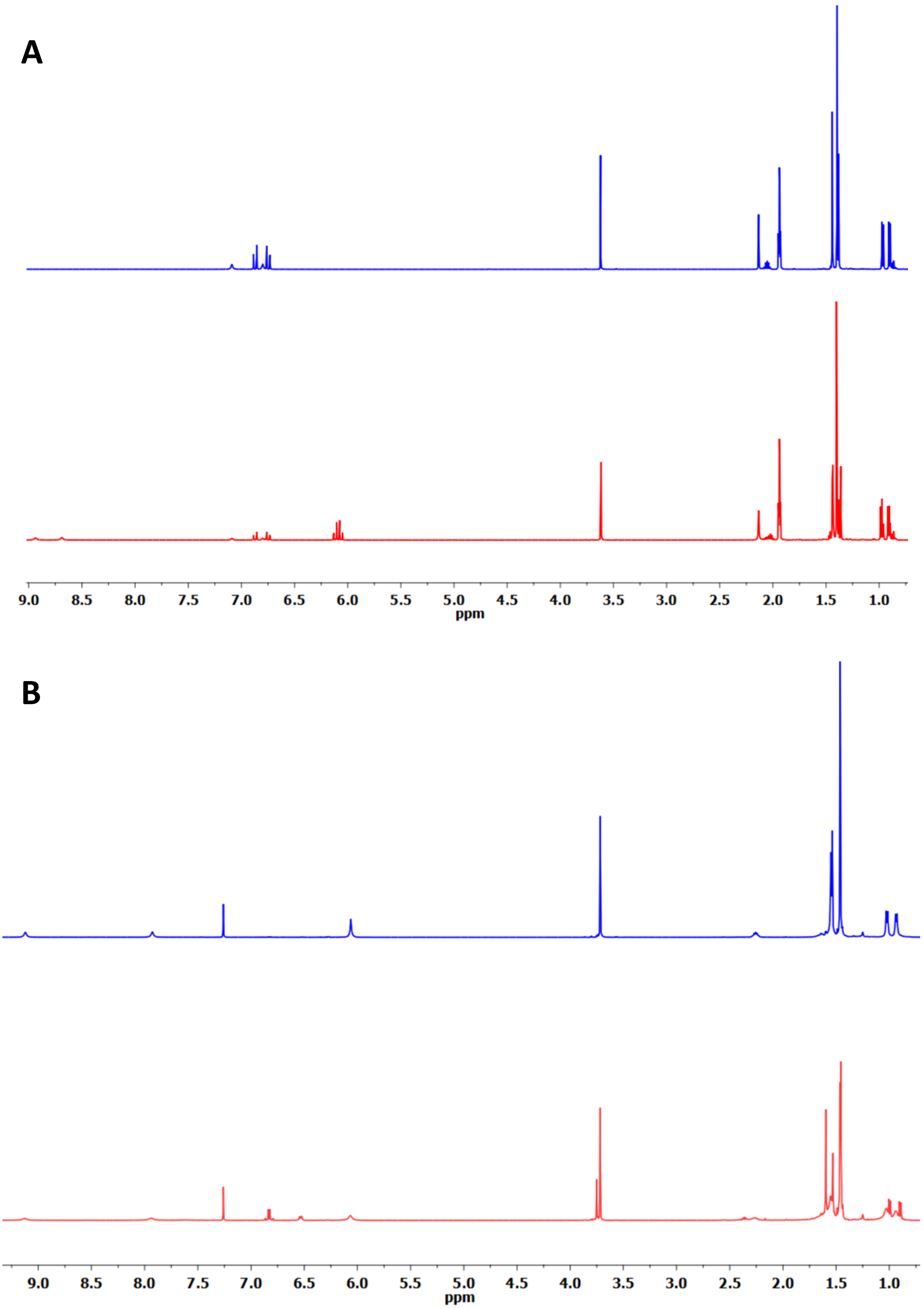


Figure S7. (**A**) ^{1}H NMR spectra (500 MHz in CD_3CN) of **2c** before irradiation (blue line) and after irradiation at 254 nm for 2h. (**B**) ^{1}H NMR spectra (500 MHz in $CDCl_3$) of **3c** before irradiation (blue line) and after irradiation at 312 nm for 2h.

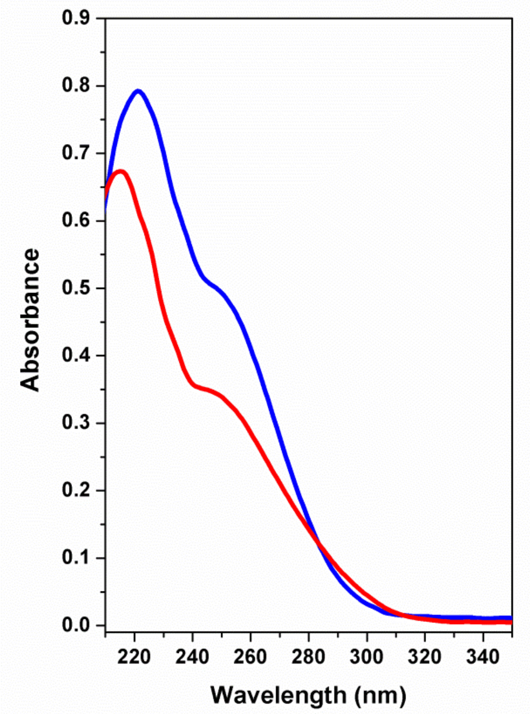


Figure S8. UV-Vis absorption spectra of **2c** (blue line) and **3c** (red line) in MeOH solution.

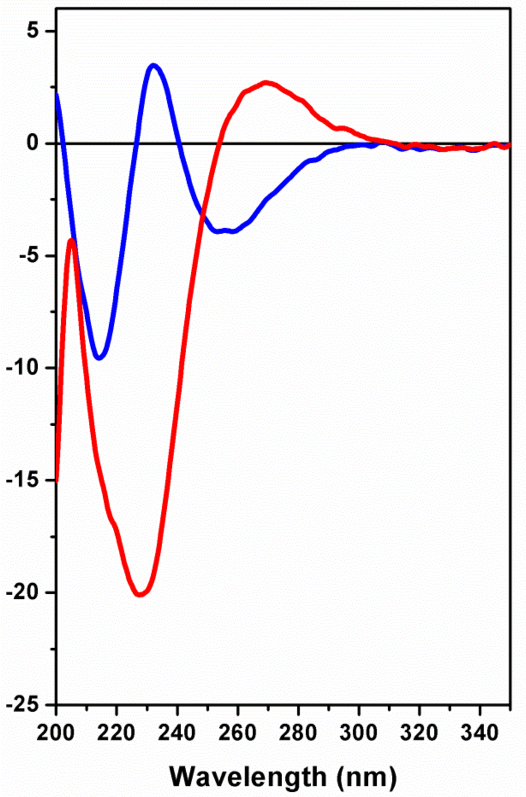


Figure S9. CD spectra of **2f** in MeOH before irradiation (blue line) and after irradiation (red line) at 254 nm (0.2 mM).

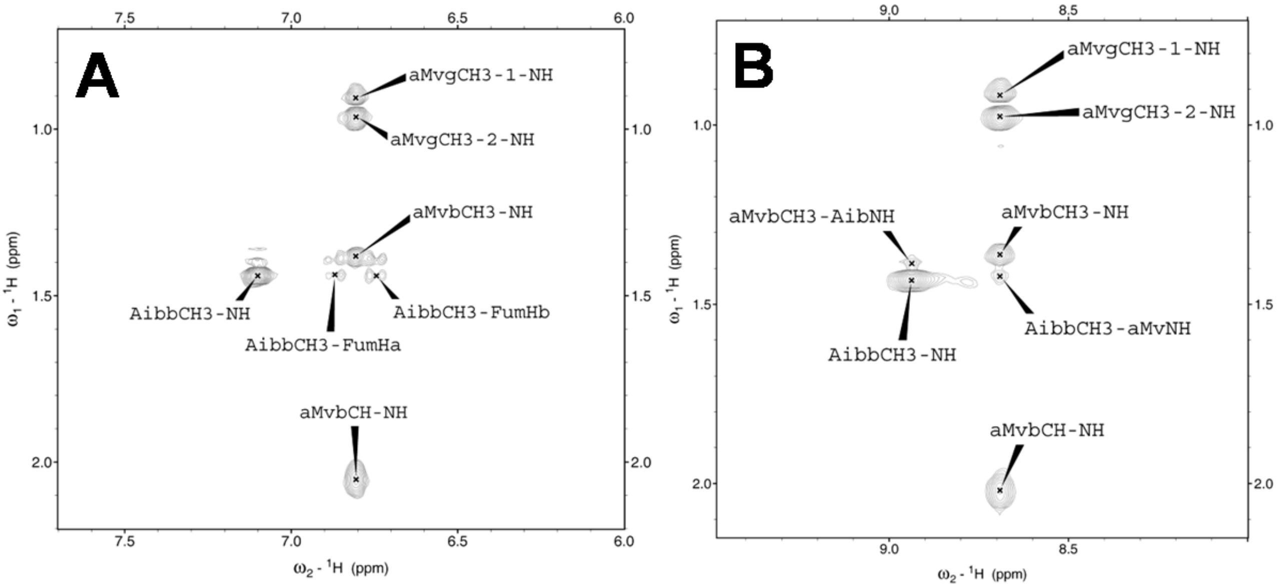


Figure S10. $\beta CH_3 \rightarrow \alpha NH$ region of the NOESY spectra (400 MHz, CD_3CN) of peptides **2c** (**A**) and **3c** (**B**).

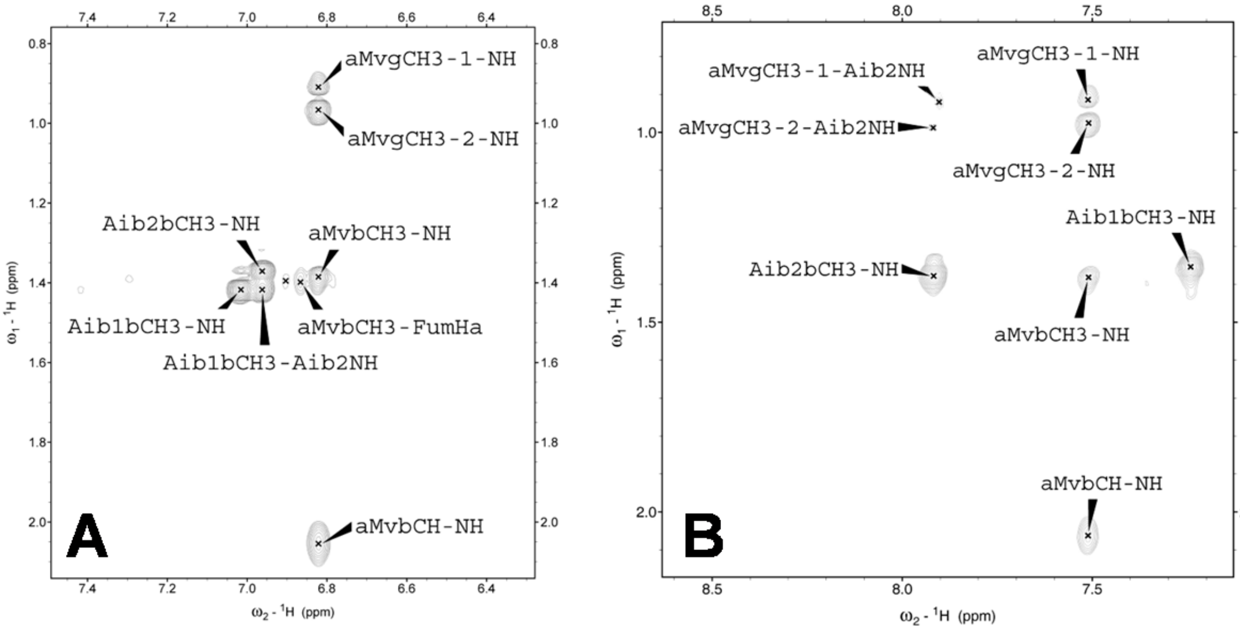


Figure S11. $\beta CH_3 \rightarrow \alpha NH$ region of the NOESY spectra (400 MHz, CD_3CN) of peptides **2d** (**A**) and **3d** (**B**).

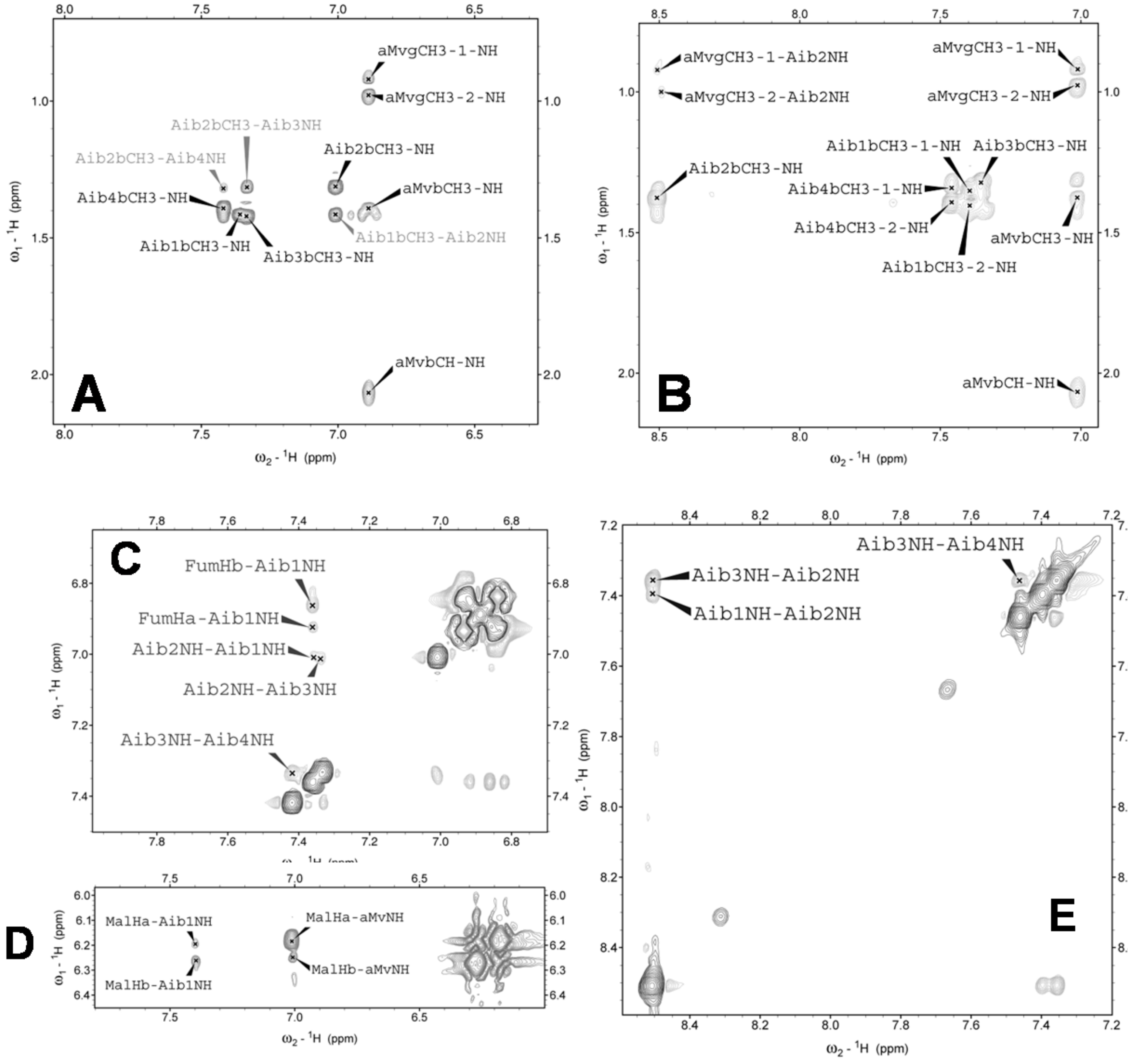


Figure S12. $\beta CH_3 \rightarrow \alpha NH$ region of the NOESY spectra (400 MHz, CD_3CN) of peptides **2e** (**A**) and **3e** (**B**). NH → NH region of the NOESY spectra (400 MHz, CD_3CN) of peptides **2e** (**C**) and **3e** (**D**,**E**).

General Methods

High-Performance Liquid Chromatography. The HPLC measurements were performed using an Agilent 1200 apparatus (Palo Alto, CA), equipped with a UV detector at 226 nm and a column Agilent Extend-C_{18} (stationary phase). Eluants: A= 9:1 H_2O/CH_3CN, 0.05 % TFA; B= 1:9 H_2O/CH_3CN, 0.05 % TFA.

Nuclear Magnetic Resonance. ^1H NMR and 2D-NMR spectra (DQF-COSY, TOCSY and NOESY experiments) were recorded at 25, 45 and 65 °C on a Bruker Avance 400, 500 or 600 MHz instruments. ^1H and ^{13}C spectra were referenced relative to the solvent residual peaks and chemical shifts (δ) reported in ppm downfield of tetramethylsilane ($CDCl_3$ δ H: 7.26 ppm, δ C: 77.16 ppm; CD_3OD δ H: 3.31 ppm, δ C 49.05 ppm, CD_3CN δ H: 1.94 ppm, δ C: 118.26 ppm). The multiplicity of a signal is indicated as br, broad; s, singlet; d, doublet; t, triplet; m, multiplet. Where ^1H NMR spectra were run in MeOD exchangeable protons (NH, OH) are reported only where observed.

Mass Spectrometry. High-resolution (HR) mass spectra by electrospray ionization (ESI), collected in the positive mode, were performed on two different instruments:

i) Perseptive Biosystem Mariner ESI-ToF5220 spectrometer (Foster City, CA);

ii) Thermo Finnigan MAT95XP (data were recorded by staff at the University of Manchester and are accurate to ± 0.001Da).

Circular Dichroism. CD measurements were carried using a Jasco J-715 spectropolarimeter at different temperatures (20, 40 and 60°C) and a thermostatic system to control the temperature of the sample. Fused quartz cells of 0.2-mm and 1-mm path length (Hellma, Müllheim, Germany) were used. The value are expressed in terms of $[\theta]_T$, the total molar ellipticity (deg x cm^2 x $dmol^{-1}$).

Fourier Transform-Infrared Spectroscopy. FT-IR absorption spectra were recorded with a ATi Perkin Elmer Spectrum RX1 FT-IR spectrometer. The $\bar{\upsilon}$ maxima for the main absorption bands are given.

Melting point. Mps were determined on a GallenKamp apparatus and are uncorrected.

UV lamp. A handheld UV Lamp (mineralight lamp, Model UVG-54) with wavelength of 254 nm (6W) was used in the photoisomerization experiments.

UV-Vis Absorption. The UV-Vis absorption spectra were recorded using a Shimadzu model UV-2501 PC spectrophotometer. A 1-cm path length quartz cell was used.

Photoisomerization experiments

The sample was dissolved in deuterated solvent (CD_3CN) and placed in a quartz NMR tube (Norrell S-500-QTZ). The sealed NMR tube was directly irradiated under the UV lamp without protective filter at a distance of about 4 cm from the light bulb. The NMR spectra were recorded before and after different irradiation times. Typically, 1-2 h of irradiation were sufficient to achieve *E* to *Z* conversion in 80-95% yield (without decomposition according to NMR and HPLC analyses). Formation of byproducts (< 5%) was detected by NMR only after long irradiation times (>18 h).

Diastereoselection experiments

Reactions of 5(4*H*)oxazolones with H-D,L-Val-OMe were performed in CH_3CN or CH_2Cl_2 at controlled temperature using a thermostatic oil bath (20, 35, 45 and 70 °C).

The carboxylic acid (**10a-b** or **11a-b**) (0.05 mmol) was suspended in 5 mL of the appropriate solvent and EDC·HCl (0.06 mmol) was added, and the solution stirred for 10 min at r.t. The quantitative formation of the oxazolone (**12a-b** or **13a-b**) was controlled by HPLC.

Separately H-D,L-Val-OMe·HCl (0.31 mmol) and DIPEA (0.31 mmol) were suspended in 1 mL of the appropriate solvent.

In a typical experiment, the oxazolone solution (500 μL, 1 equiv.) and the racemate solution (125 μL, 8 equiv.) were mixed and the resulting solution was maintained under stirring in a thermostatic oil bath. The reaction was monitored by HPLC, by following the disappearance of the oxazolone accompanied by the formation of the two diastereomeric products. The formation of the two resulting peptides **15a-d** (*Z* isomer) can be quantified directly by HPLC. Whereas in the case of **14a-d** (*E* isomer), after the disappearance of the oxazolone reactant, the product mixture was irradiated at 254 nm and successively analyzed by HPLC. The independent preparation of each diastereomer was performed by reaction of the oxazolone with either D or L H-Val-OMe.

X-Ray diffraction

Crystals of **2a** and **3c** were grown by slow evaporation from CH_3CN and MeOH solutions, respectively. X-Ray diffraction data were collected with a Gemini E four-circle kappa diffractometer (Agilent Technologies) equipped with a 92 mm EOS CCD detector, using graphite monochromated Cu Kα radiation (λ = 1.54178 Å). Data collection and reduction were performed with the CrysAlisPro software (version 1.171.36.28, Agilent Technologies). A semi-empirical absorption correction based on the multi-scan technique using spherical harmonics, implemented in the SCALE3 ABSPACK scaling algorithm, was applied. For **3c**, diffraction data were collected up to theta = 51.48°, as the crystal did not diffract significantly beyond 1.0 Å resolution, in all probability as a result of the combination of the small crystal size (minimum dimension 0.05 mm) with the relatively large asymmetric unit (five independent molecules, shown in Figure S13, for a total of 135 non-H atoms).

Both structures were solved by ab initio procedures of the SIR 2014 program,[1] and refined by full-matrix least-squares on F^2, using all data, by application of the SHELXL-2014 program,[2] with anisotropic displacement parameters for all of the non-H atoms. H-Atoms were calculated at idealized positions and refined using a riding model. In the refinement of **3c**, restraints were applied to the anisotropic displacement parameters of the non-H atoms (RIGU command in SHELX-2014). Relevant crystal data and structure refinement parameters, selected torsion angles, and intra-and intermolecular H-bond parameters are listed in Tables S1-S3 for **2a**, and in Tables S4-S6 for **3c**.

CCDC 1473792-1473793 contain the supplementary crystallographic data for this paper. These data can be obtained from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif

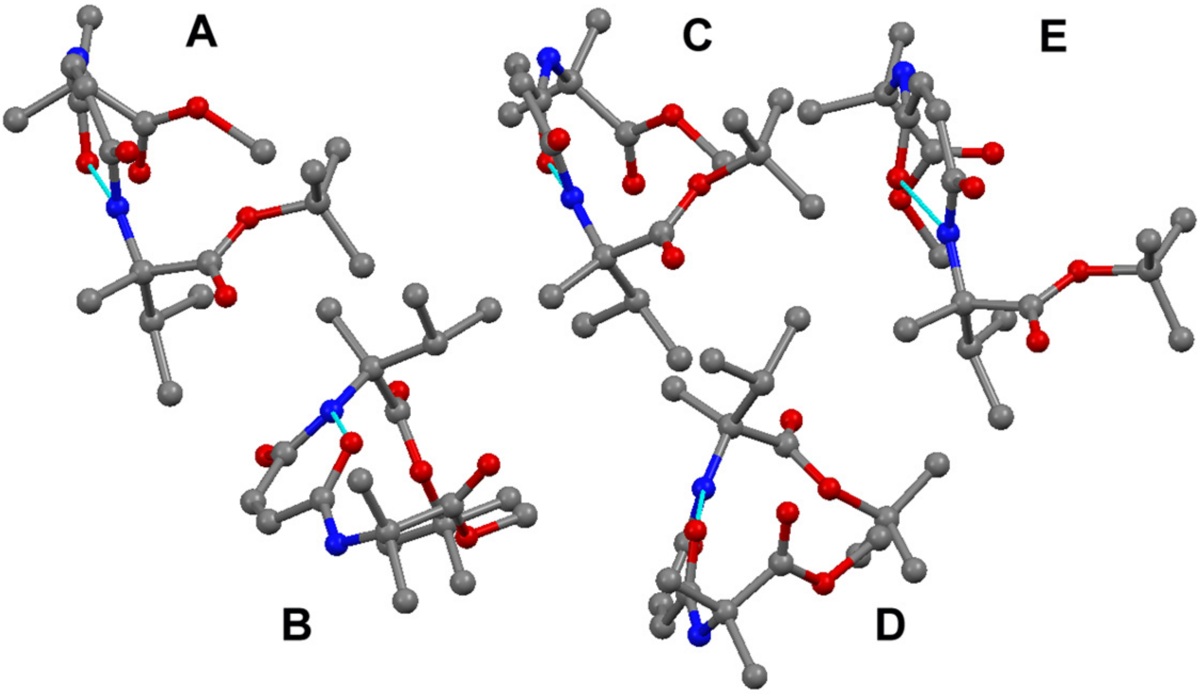


Figure S13. Perspective view of the five independent molecules (**A**-**E**) composing the asymmetric unit in the X-ray diffraction structure of **3c**. In each molecule, the intramolecular H-bond between the L-(αMe)Val N-H and the maleamide carbonyl oxygen next to the Aib residue is represented by a dashed line.

Table S1. Crystal data and structure refinement for **2a**.

Identification code	mc262b	
Empirical formula	$C_{32} H_{55} N_7 O_9$	
Formula weight	681.83	
Temperature	293(2) K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	$P\ 2_1\ 2_1\ 2_1$	
Unit cell dimensions	a = 9.01420(14) Å	α= 90°.
	b = 10.94112(14) Å	β= 90°.
	c = 38.4416(6) Å	γ = 90°.
Volume	3791.32(10) $Å^3$	
Z	4	
Density (calculated)	1.195 Mg/m^3	
Absorption coefficient	0.724 mm^{-1}	
F(000)	1472	
Crystal size	0.25 × 0.20 × 0.05 mm^3	
Theta range for data collection	2.299 to 70.959°.	
Index ranges	$-11 \le h \le 10, -13 \le k \le 12, -47 \le l \le 41$	
Reflections collected	33257	
Independent reflections	7272 [R(int) = 0.0326]	
Completeness to theta = 67.679°	100.0 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1.00000 and 0.22453	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	7272 / 0 / 433	
Goodness-of-fit on F^2	1.031	
Final R indices [I>2sigma(I)]	$R_1 = 0.0406$, $wR_2 = 0.1098$	
R indices (all data)	$R_1 = 0.0438$, $wR_2 = 0.1126$	
Absolute structure parameter	0.01(6)	
Largest diff. peak and hole	0.269 and -0.225 e.$Å^{-3}$	

Table S2. Selected torsion angles [°] for **2a**.

C1F-N6-C6A-C6	-58.4(3)
N6-C6A-C6B1-C6G1	174.5(2)
N6-C6A-C6B1-C6G2	-61.3(3)
CT1-OT-C6-C6A	178.9(2)
N6-C6A-C6-OT	-47.9(3)
C6A-N6-C1F-C2F	-171.0(2)
N6-C1F-C2F-C3F	159.6(3)
C1F-C2F-C3F-C4F	-174.6(3)
C2F-C3F-C4F-N1	169.1(3)
C3F-C4F-N1-C1A	-176.2(2)
C4F-N1-C1A-C1	-54.3(3)
N1-C1A-C1-N2	-31.4(3)
C1A-C1-N2-C2A	-172.9(2)
C1-N2-C2A-C2	-48.6(3)
N2-C2A-C2-N3	-33.5(3)
C2A-C2-N3-C3A	-176.9(2)
C2-N3-C3A-C3	-51.1(3)
N3-C3A-C3-N4	-40.4(3)
C3A-C3-N4-C4A	-172.8(2)
C3-N4-C4A-C4	-66.1(3)
N4-C4A-C4-N5	-23.3(3)
C4A-C4-N5-C5A	178.2(2)
C4-N5-C5A-C5	66.2(4)
N5-C5A-C5-NT	-135.8(3)

Table S3. Hydrogen bonds for **2a** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N3-H3...O2F	0.86	2.45	3.292(3)	168
N4-H4...O1	0.86	2.15	2.947(3)	155
N5-H5...O2	0.86	2.29	3.069(3)	151
N6-H6...O6#1	0.86	2.15	2.989(3)	164
N1-H1...O3#2	0.86	2.32	3.142(2)	161
N2-H2...O5#3	0.86	2.29	2.906(3)	128
NT-HT1...O3#4	0.86	2.37	3.197(4)	160
NT-HT2...O2#4	0.86	2.50	3.061(4)	124

Symmetry transformations used to generate equivalent atoms:

#1 -x+2, y-1/2, -z+1/2; #2 x, y+1, z; #3 x+1/2, -y+1/2, -z; #4 x-1/2, -y-1/2, -z

Table S4. Crystal data and structure refinement for **3c**.

Identification code	mc265f	
Empirical formula	$C_{19} H_{32} N_2 O_6$	
Formula weight	384.46	
Temperature	293(2) K	
Wavelength	1.54178 Å	
Crystal system	Orthorhombic	
Space group	$P\ 2_1\ 2_1\ 2_1$	
Unit cell dimensions	a = 12.78876(14) Å	α= 90°.
	b = 21.3074(2) Å	β= 90°.
	c = 42.2055(6) Å	γ = 90°.
Volume	11500.8(2) $Å^3$	
Z	20	
Density (calculated)	1.110 Mg/m^3	
Absorption coefficient	0.678 mm^{-1}	
F(000)	4160	
Crystal size	0.40 × 0.20 × 0.05 mm^3	
Theta range for data collection	2.323 to 51.482°.	
Index ranges	$-12 \le h \le 12$, $-21 \le k \le 21$, $-42 \le l \le 42$	
Reflections collected	50259	
Independent reflections	12491 [R(int) = 0.0263]	
Completeness to theta = 51.482°	99.5 %	
Absorption correction	Semi-empirical from equivalents	
Max. and min. transmission	1.00000 and 0.77746	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	12491 / 975 / 1216	
Goodness-of-fit on F^2	1.044	
Final R indices [I>2sigma(I)]	$R_1 = 0.0525$, $wR_2 = 0.1481$	
R indices (all data)	$R_1 = 0.0601$, $wR_2 = 0.1558$	
Absolute structure parameter	0.04(5)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.683 and -0.276 e.$Å^{-3}$	

Table S5. Selected torsion angles [°] for **3c**.

C1M-N1-C1A-C1	-51.7(6)
N1-C1A-C1B1-C1G1	59.8(6)
N1-C1A-C1B1-C1G2	-173.3(6)
N1-C1A-C1-OTA	-37.7(6)
C1A-C1-OTA-CT1A	-176.6(7)
C1-OTA-CT1A-CT4A	180.0(9)
C1-OTA-CT1A-CT3A	61.5(11)
C1-OTA-CT1A-CT2A	-62.4(11)
C1A-N1-C1M-C2M	-179.1(5)
O1M-C1M-C2M-C3M	168.4(7)
N1-C1M-C2M-C3M	-12.3(11)
C1M-C2M-C3M-C4M	1.8(13)
C2M-C3M-C4M-O2M	15.1(11)
C2M-C3M-C4M-N2	-166.5(7)
C3M-C4M-N2-C2A	-176.6(6)
C4M-N2-C2A-C2	-55.0(8)
N2-C2A-C2-OTB	-34.5(9)
C2A-C2-OTB-CTB	166.0(13)
C11M-N3-C3A-C3	-46.2(7)
N3-C3A-C3B1-C3G2	-65.3(7)
N3-C3A-C3B1-C3G1	168.1(6)
N3-C3A-C3-OTC	-43.8(6)
C3A-C3-OTC-CT1C	176.3(5)
C3-OTC-CT1C-CT2C	-59.7(11)
C3-OTC-CT1C-CT3C	67.6(10)
C3-OTC-CT1C-CT4C	-177.4(7)
C3A-N3-C11M-C12M	171.3(5)
O11M-C11M-C12M-C13M	165.8(6)
N3-C11M-C12M-C13M	-12.5(10)
C11M-C12M-C13M-C14M	3.7(12)
C12M-C13M-C14M-O12M	22.4(11)
C12M-C13M-C14M-N4	-159.3(7)
C13M-C14M-N4-C4A	-179.8(7)
C14M-N4-C4A-C4	-46.7(10)
N4-C4A-C4-OTD	-40.4(9)
C4A-C4-OTD-CTD	177.0(8)
C21M-N5-C5A-C5	-48.9(6)

N5-C5A-C5B1-C5G2	-60.7(7)
N5-C5A-C5B1-C5G1	172.8(6)
N5-C5A-C5-OTE	-43.3(6)
C5A-C5-OTE-CT1E	179.6(5)
C5-OTE-CT1E-CT3E	-177.0(6)
C5-OTE-CT1E-CT2E	-58.2(8)
C5-OTE-CT1E-CT4E	63.0(8)
C5A-N5-C21M-C22M	179.0(5)
O21M-C21M-C22M-C23M	166.8(7)
N5-C21M-C22M-C23M	-13.6(11)
C21M-C22M-C23M-C24M	4.8(12)
C22M-C23M-C24M-O22M	21.8(11)
C22M-C23M-C24M-N6	-158.0(7)
C23M-C24M-N6-C6A	-179.6(5)
C24M-N6-C6A-C6	-44.4(8)
N6-C6A-C6-OTF	-41.8(7)
C6A-C6-OTF-CTF	173.0(6)
C31M-N7-C7A-C7	-47.3(7)
N7-C7A-C7B1-C7G2	-61.8(7)
N7-C7A-C7B1-C7G1	174.5(6)
N7-C7A-C7-OTG	-47.1(6)
C7A-C7-OTG-CT1G	176.5(5)
C7-OTG-CT1G-CT3G	-173.1(7)
C7-OTG-CT1G-CT4G	64.7(9)
C7-OTG-CT1G-CT2G	-57.4(9)
C7A-N7-C31M-C32M	179.2(5)
O31M-C31M-C32M-C33M	163.7(6)
N7-C31M-C32M-C33M	-16.5(10)
C31M-C32M-C33M-C34M	3.3(12)
C32M-C33M-C34M-O32M	22.5(11)
C32M-C33M-C34M-N8	-161.7(7)
C33M-C34M-N8-C8A	179.9(7)
C34M-N8-C8A-C8	-43.8(11)
N8-C8A-C8-OTH	-44.3(10)
C8A-C8-OTH-CTH	177.5(8)
C41M-N9-C9A-C9	-46.3(7)
N9-C9A-C9B1-C9G1	41.0(9)
N9-C9A-C9B1-C9G2	167.1(8)
N9-C9A-C9-OTI	-45.0(6)

C9A-C9-OTI-CT1I	-177.6(5)
C9-OTI-CT1I-CT4I	63.8(8)
C9-OTI-CT1I-CT3I	-60.4(8)
C9-OTI-CT1I-CT2I	-177.6(6)
C9A-N9-C41M-C42M	179.9(5)
O41M-C41M-C42M-C43M	173.9(6)
N9-C41M-C42M-C43M	-5.2(10)
C41M-C42M-C43M-C44M	5.0(12)
C42M-C43M-C44M-O42M	12.9(10)
C42M-C43M-C44M-N10	-168.8(6)
C43M-C44M-N10-C10A	-176.0(5)
C44M-N10-C10A-C10	-52.9(7)
N10-C10A-C10-OTL	140.6(5)
C10A-C10-OTL-CTL	-180.0(7)

Table S6. Hydrogen bonds for **3c** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N1-H1...O2M	0.86	1.89	2.708(5)	157.5
N3-H3...O12M	0.86	1.91	2.715(6)	155.2
N5-H5...O22M	0.86	1.93	2.746(6)	157.8
N7-H7...O32M	0.86	1.91	2.723(6)	156.0
N9-H9...O42M	0.86	1.89	2.706(5)	158.8
N2-H2...O31M#1	0.86	2.06	2.911(6)	168.8
N4-H4...O21M#2	0.86	2.04	2.875(6)	163.6
N6-H6...O11M#1	0.86	2.13	2.970(6)	166.6
N8-H8...O1M#2	0.86	2.04	2.883(6)	165.6
N10-H10...O41M#3	0.86	2.04	2.877(5)	164.0

Symmetry transformations used to generate equivalent atoms:

#1 -x,y+1/2,-z+1/2 #2 -x+1,y-1/2,-z+1/2 #3 x-1/2,-y+1/2,-z

Synthesis and characterization of compounds

Materials

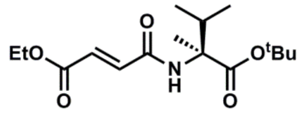
N,N-diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), *mono*ethyl fumarate, LiOH, triethylamine (TEA), H-L-Val-O*t*Bu·HCl, *tert*-butyl *α*-bromoisobutyrate, α-bromo*iso*butyric acid, Pd/C catalyst (10% wt. loading), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) were obtained from Sigma-Aldrich. 1-hydroxy-7-aza-1,2,3-benzotriazole (HOAt) was purchased from GL Biochem (Shanghai).

H-L-(αMe)Val-OH, H-Aib-OMe·HCl, H-L-Val-OMe and H-D,L-Val-OMe were obtained from Bachem.The deuterated solvents DMSO-d_6, $CDCl_3$, MeOH-d_3 and MeOD-d_4 were purchased from Euriso-Top (France).

Methods for the synthesis of H-L-(αMe)Val-O^{t}Bu,[3] H-Aib_4-Gly-NH_2,[4] N_3-Aib_4-OMe[5] and N_3-Aib_8-OMe[6] have been reported previously.

a) Synthesis of fumaric acid derivative 1

Synthesis of 14

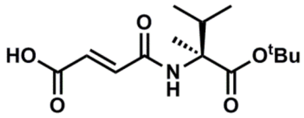


*Mono*ethyl fumarate (390 mg, 2.7 mmol) and HOAt (365 mg, 2.7 mmol) were dissolved in CH_2Cl_2. The suspension was cooled to 0 °C and EDC·HCl (500 mg, 2.7 mmol) was added. After complete dissolution H-(αMe)Val-O*t*Bu (350 mg, 1.87 mmol) and TEA (400 μL, 2.9 mmol) were added and the reaction mixture stirred overnight at r.t. The solvent was removed under reduced pressure and the residue dissolved in EtOAc. The organic phase was washed with $KHSO_{4(aq)}$ 5%, $NaHCO_{3(aq)}$ 5%, brine, dried over $MgSO_4$, filtered and concentrated. The crude was purified *via* flash chromatography (eluant: petroleum ether/EtOAc increasing the solvent mixture polarity from 9:1 to 8:2). The product was obtained as a colorless oil (500 mg, 85 % yield).

HRMS (ES$^+$, MeOH) *m/z* calcd. for $C_{16}H_{27}NO_5Na$ ([M+Na]$^+$) 336.1787, found 336.1783. $[\alpha]_D^{20}$ = -36.3 (c 1, MeOH). FT-IR $\bar{\upsilon}_{max}$ 3351, 2977, 1727, 1682, 1259, 1368, 1296, 1272, 1150 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.91 (d, *J* = 15.3 Hz, 1H, CH Fum), 6.75 (d, *J* = 15.3 Hz, 1H, CH Fum), 6.60 (s, 1H, NH), 4.24 (q, *J* = 7.1 Hz, 2H, CH_2 Et), 2.42 (hept, *J* = 6.9 Hz, 1H, βCH), 1.63 (s, 3H, βCH_3), 1.47 (s, 9H, O*t*Bu), 1.31 (t, *J* = 7.1 Hz, 3H, CH_3 Et), 1.01 (d, *J* = 7.0 Hz, 3H, γCH_3), 0.90 (d,

J = 6.9 Hz, 3H, γCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.57, 165.74, 162.55, 137.31, 130.19, 82.56, 64.33, 61.29, 34.17, 28.08, 19.28, 17.75, 17.67, 14.29.

Synthesis of 1

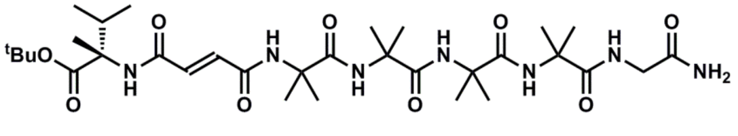


14 (500 mg, 1.6 mmol) was dissolved in 30 mL of THF and a solution of LiOH (260 mg, 10.8 mmol) in 10 mL of H_2O was added. The solution was stirred at r.t. until TLC indicated complete consumption of the starting material. The organic solvent was removed under reduced pressure and the aqueous residue was diluted with 10 mL of H_2O. The aqueous solution was acidified with HCl 1M and extracted with EtOAc (3v). The combined organic phases were washed with $KHSO_{4(aq)}$ 5%, brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The compound was recovered as a white solid (400 mg, 88 % yield).

HRMS (ES$^+$, MeOH) *m/z* calcd. for $C_{14}H_{23}NO_5Na$ ([M+Na]$^+$) 308.1474, found 308.1477. $[\alpha]_D^{20}$ = -38.4 (c 1, MeOH). Mp 213-215 °C. FT-IR $\bar{\upsilon}_{max}$ 3297, 2975, 1727, 1640, 1534, 1368, 1276, 1147 cm^{-1}. ^{1}H NMR (500 MHz, MeOD) δ 8.42 (s, 1H, NH), 7.11 (d, J = 15.5 Hz, 1H, CH Fum), 6.64 (d, J = 15.5 Hz, 1H, CH Fum), 2.13-2.03 (m, 1H, βCH), 1.44 (s, 9H, O*t*Bu), 1.42 (s, 3H, βCH_3), 1.01 (d, J = 6.9 Hz, 3H, γCH_3), 0.95 (d, J = 6.9 Hz, 3H, γCH_3). ^{13}C NMR (101 MHz, MeOD) δ 173.48, 168.52, 165.68, 137.85, 131.35, 82.35, 64.55, 35.87, 28.20, 17.71, 17.53, 17.50.

b) Synthesis of fumaramides 2a-f

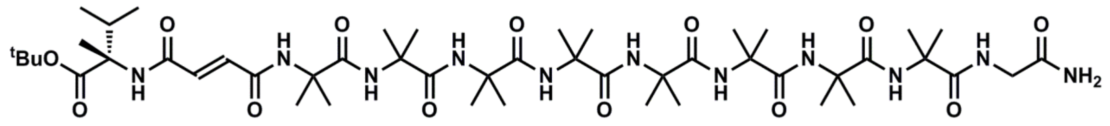
Synthesis of 2a



1 (140 mg, 0.49 mmol) and HOAt (70 mg, 0.51 mmol) were dissolved in CH_2Cl_2. The suspension was cooled to 0 °C and EDC·HCl (94 mg, 0.49 mmol) was added. After complete dissolution H-Aib$_4$-Gly-NH$_2$ (150 mg, 0.36 mmol) and DIPEA (100 μL, 0.57 mmol) were added and the reaction mixture stirred overnight at r.t. The solvent was removed under reduced pressure and the residue dissolved in EtOAc. The organic phase was washed with $KHSO_{4(aq)}$ 5%, $NaHCO_{3(aq)}$ 5%, brine, dried over $MgSO_4$, filtered and concentrated. The crude was purified *via* flash chromatography (eluant: CH_2Cl_2/MeOH 92:8). The product was obtained as a white solid (190 mg, 77% yield).

HRMS (ES^+, MeOH) *m/z* calcd. for $C_{32}H_{55}N_7O_9Na$ ($[M+Na]^+$) 704.3959, found 704.3976. $[\alpha]_D^{20}$ = -8.5 (c 1, MeOH). Mp 263-265 °C. FT-IR $\bar{\upsilon}_{max}$ 3293, 2930, 1654, 1537, 1468, 1383, 1364 cm^{-1}. ^1H NMR (500 MHz, CD_3CN) δ 7.80 (s, 1H, NH Aib^3), 7.79 (t, *J* = 6.5 Hz, 1H, NH Gly), 7.64 (s, 1H, NH Aib^4), 7.43 (s, 1H, NH Aib^1), 7.14 (s, 1H, NH Aib^2), 7.11 (s br, 1H, NHa $GlyNH_2$), 6.95 (d, *J* = 15.0 Hz, 1H, CH Fum), 6.90 (s, 1H, NH αMeVal), 6.85 (d, *J* = 15.0 Hz, 1H, CH Fum), 5.65 (s, 1H, NHb $GlyNH_2$), 3.67 (d, *J* = 6.4 Hz, 2H, CH_2 Gly), 2.10-2.06 (m, 1H, βCH αMeVal), 1.45 (s, 6H, 2x βCH_3 Aib), 1.44 (s, 6H, 2x βCH_3 Aib), 1.43 (s, 6H, 2x βCH_3 Aib), 1.41 (s, 9H, O*t*Bu), 1.40 (s, 3H, βCH_3 αMeVal), 1.35 (s, 5H, 2x βCH_3 Aib), 0.99 (d, *J* = 6.9 Hz, 3H, γCH_3 αMeVal), 0.92 (d, *J* = 6.9 Hz, 3H, γCH_3 αMeVal). ^{13}C NMR (101 MHz, CD_3CN) δ 177.07 (CO Aib^3), 176.60 (CO Aib^2), 176.27 (CO Aib^4), 175.30 (CO Aib^1), 173.24 (CO Gly), 172.59 (CO αMeVal), 165.62 (C^bO Fum), 164.18 (C^aO Fum), 134.74 (C^a Fum), 132.98 (C^b Fum), 81.41 (C O*t*Bu), 64.04 (αC αMeVal), 57.72 (αC Aib), 57.54 (αC Aib), 57.51 (αC Aib), 57.48 (αC Aib), 43.60 (αC Gly), 35.49 (βCH αMeVal), 28.07 (CH_3 O*t*Bu), 25.54 (βC Aib^4), 25.21 (βC Aib^3), 25.10 (βC Aib^2), 24.91 (βC Aib^1), 17.67 (βCH_3 αMeVal), 17.55 (γC αMeVal), 17.44 (γC αMeVal).

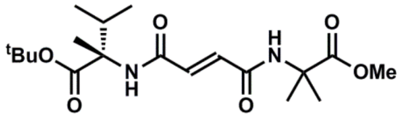
Synthesis of 2b



OtBu-(αMe)Val-Mal-Aib_4-OH (100 mg, 0.16 mmol) was dissolved in 4 mL of dry CH_2Cl_2 and EDC·HCl (38 mg, 0.2 mmol) was added. The solution was stirred at r.t. for 1 h. The solvent was removed under reduced pressure and the residue dissolved in EtOAc. The organic solution was washed with $KHSO_4$, brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure The crude oxazolone was then placed under high vacuum before being dissolved in CH_3CN. Then H-Aib_4-Gly-NH_2 (100 mg, 0.24 mmol) was added. The reaction was stirred under reflux for 5 d. The solution was diluted with CH_2Cl_2 and the precipitate was recovered by filtration. After purification by flash chromatography (eluant: CH_2Cl_2/MeOH 95:5), the pure product was recovered as a white solid (55 mg, 33 % yield).

HRMS (ES$^+$ MeOH) *m/z* calcd. for $C_{48}H_{83}N_{11}O_{13}Na$ ([M+Na]$^+$) 1044.6064, found 1044.6060. $[\alpha]_D^{20}$ = -5.3 (c 1, MeOH). Mp decompose >270 °C. FT-IR $\bar{\upsilon}_{max}$ 3289, 2981, 1657, 1539, 1384, 1363, 1228 cm^{-1}. ^{1}H NMR (500 MHz, CD_3CN) δ 7.84 (m, 2H, 2x NH), 7.81 (s, 1H, NH), 7.79 (m, 3H, NH), 7.66 (s, 1H, NH), 7.51 (s, 1H, NH), 7.20 (s, 1H, NH), 7.12 (s, 1H, NH), 6.96 (d, *J* = 15.0 Hz, 1H, CH Fum), 6.91 (s, 1H, NH), 6.87 (d, *J* = 15.0 Hz, 1H, CH Fum), 5.65 (s, 1H, NH), 3.67 (d, *J* = 5.7 Hz, 2H, CH_2 Gly), 2.08 (m, 1H, βCH αMeVal), 1.48 (s, 6H, 2x βCH_3 Aib), 1.45 (s, 6H, 2x βCH_3 Aib), 1.44 (s, 18H, 6x βCH_3 Aib), 1.43 (s, 6H, 2x βCH_3 Aib), 1.42 (s, 6H, 2x βCH_3 Aib), 1.41 (s, 9H, O*t*Bu), 1.40 (s, 3H, βCH_3 αMeVal), 1.36 (s, 6H, 2x βCH_3 Aib), 0.99 (d, *J* = 6.9 Hz, 3H, γCH_3 αMeVal), 0.92 (d, *J* = 6.9 Hz, 3H, γCH_3 αMeVal). ^{13}C NMR (101 MHz, CD_3CN) δ 177.32, 177.09, 176.98, 176.95, 176.94, 176.30, 175.39, 173.22, 172.56, 165.66, 164.18, 134.75, 132.99, 81.44, 64.07, 57.72, 57.57, 57.48, 57.43, 57.33, 57.30, 57.27, 43.61, 35.47, 28.06, 25.19, 17.76, 17.53, 17.42.

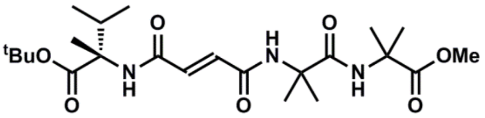
Synthesis of 2c



1 (35 mg, 0.12 mmol) and HOAt (17 mg, 0.12 mmol) were dissolved in dry CH_2Cl_2. The suspension was cooled to 0 °C and EDC·HCl (23 mg, 0.12 mmol) was added. After complete dissolution H-Aib-OMe·HCl (37 mg, 0.24 mmol) and DIPEA (65 µL, 0.37 mmol) were added and the reaction mixture stirred overnight at r.t. The solvent was removed under reduced pressure and the residue dissolved in EtOAc. The organic phase was washed with $KHSO_{4(aq)}$ 5%, $NaHCO_{3(aq)}$ 5%, brine, dried over $MgSO_4$, filtered and concentrated. The product was obtained as a white solid after precipitation from EtOAc/petroleum ether (40 mg, 85% yield).

HRMS (ES$^+$ MeOH) *m/z* calcd. for $C_{19}H_{33}N_2O_6$ ([M+H]$^+$) 385.2333, found 385.2403. Mp 149-152 °C. FT-IR $\bar{\upsilon}_{max}$ 3351, 2978, 1730, 1649, 1533, 1367, 1335, 1283, 1152 cm^{-1}.
^{1}H NMR (500 MHz, CD_3CN) δ 7.09 (s, 1H, NH Aib), 6.87 (d, *J* = 15.1 Hz, 1H, CH Fum), 6.80 (s, 1H, NH αMeVal), 6.75 (d, *J* = 15.1 Hz, 1H, CH Fum), 3.62 (s, 3H, OMe), 2.05 (dt, *J* = 13.8, 6.9 Hz, 1H, βCH αMeVal), 1.44 (s, 6H, 2x βCH_3 Aib), 1.40 (s, 9H, OtBu), 1.38 (s, 3H, βCH_3 αMeVal), 0.97 (d, *J* = 6.9 Hz, 3H, γCH_3 αMeVal), 0.90 (d, *J* = 6.9 Hz, 3H, γCH_3 αMeVal). ^{13}C NMR (101 MHz, CD_3CN) δ 175.24, 172.67, 164.38, 134.40, 132.99, 81.43, 64.06, 56.93, 52.78, 35.51, 28.15, 25.21, 17.87, 17.60, 17.47.

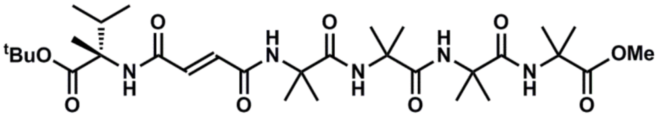
Synthesis of 2d



1 (35 mg, 0.12 mmol) and HOAt (17 mg, 0.12 mmol) were dissolved in dry CH_2Cl_2. The suspension was cooled to 0 °C and EDC·HCl (23 mg, 0.12 mmol) was added. After complete dissolution H-Aib$_2$-OMe (48 mg, 0.24 mmol) and DIPEA (22 µL, 0.12 mmol) were added and the reaction mixture stirred overnight at r.t. The solvent was removed under reduced pressure and the residue dissolved in EtOAc. The organic phase was washed with $KHSO_{4(aq)}$ 5%, $NaHCO_{3(aq)}$ 5%, brine, dried over $MgSO_4$, filtered and concentrated. The product was obtained as a white solid after precipitation from EtOAc/petroleum ether (45 mg, 80% yield).

HRMS (ES$^+$ MeOH) *m/z* calcd. for $C_{23}H_{40}N_3O_7$ ([M+H]$^+$) 470.2871, found 470.2853. Mp 89-90 °C. FT-IR $\tilde{\upsilon}_{max}$ 3356, 2980, 1733, 1652, 1532, 1367, 1277, 1151 cm^{-1}. ^{1}H NMR (500 MHz, CD_3CN) δ 7.02 (s, 1H, NH Aib^1), 6.96 (s, 1H, NH Aib^2), 6.88 (d, *J* = 15.0 Hz, 1H, CH Fum), 6.81 (d, *J* = 15.1 Hz, 2H, CH Fum and NH αMeVal), 3.60 (s, 3H, OMe), 2.05 (dt, *J* = 13.7, 6.8 Hz, 1H, βCH αMeVal), 1.42 (s, 6H, 2x βCH_3 Aib), 1.40 (s, 9H, OtBu), 1.39 (s, 3H, βCH_3 αMeVal), 1.37 (s, 6H, 2x βCH_3 Aib), 0.97 (d, *J* = 6.9 Hz, 3H, γCH_3 αMeVal), 0.91 (d, *J* = 6.8 Hz, 3H, γCH_3 αMeVal). ^{13}C NMR (101 MHz, CD_3CN) δ 175.78, 174.25, 172.67, 164.49, 164.46, 133.95, 133.89, 81.45, 64.07, 57.88, 56.77, 52.59, 35.54, 28.16, 25.23, 25.21, 17.88, 17.60, 17.47.

Synthesis of 2e

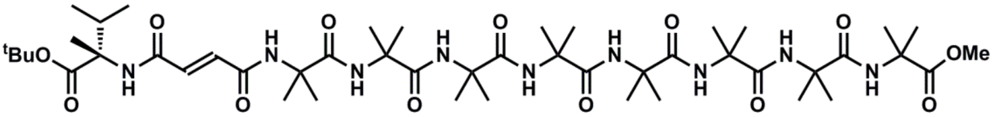


1 (230 mg, 0.81 mmol) and HOAt (110 mg, 0.81 mmol) were dissolved in dry CH_2Cl_2. The suspension was cooled to 0 °C and EDC·HCl (155 mg, 0.81 mmol) was added. After complete dissolution H-Aib$_4$-OMe (200 mg, 0.54 mmol, prepared by the quantitative hydrogenolysis of N_3Aib$_4$-OMe) and DIPEA (140 µL, 0.81 mmol) were added and the reaction mixture stirred overnight at r. t. The solvent was removed under reduced pressure and the residue dissolved in EtOAc. The organic phase was washed with $KHSO_{4(aq)}$ 5%, $NaHCO_{3(aq)}$ 5%, brine, dried over $MgSO_4$, filtered and concentrated. The crude was purified *via* flash chromatography using as eluent 94:6 CH_2Cl_2/MeOH. The product was obtained as a white solid (280 mg, 81% yield).

HRMS (ES^+, MeOH) *m/z* calcd. for $C_{31}H_{54}N_5O_9$ ($[M+H]^+$) 640.3916, found 640.3922. $[\alpha]_D^{20}$ = -8.1 (c 1, MeOH). Mp 136-137 °C. FT-IR $\bar{\upsilon}_{max}$ 3309, 2983, 1728, 1645, 1530, 1457, 1385, 1365, 1273, 1222, 1151, 732 cm^{-1}.

^{1}H NMR (500 MHz, CD_3CN) δ 7.42 (s, 1H, NH Aib^4), 7.37 (s, 1H, NH Aib^1), 7.34 (s, 1H, NH Aib^3), 7.01 (s, 1H, NH Aib^2), 6.93 (d, *J* = 15.0 Hz, 1H, CH Fum), 6.89 (s, 1H, NH αMeVal), 6.84 (d, *J* = 15.0 Hz, 1H, CH Fum), 3.58 (s, 3H, OMe), 2.06 (dq, *J* = 13.7, 6.9 Hz, 1H, βCH αMeVal), 1.42 (s, 6H, 2x βCH_3 Aib^3), 1.42 (s, 6H, 2x βCH_3 Aib^1), 1.40 (s, 9H, OtBu), 1.39 (s, 9H, 2x βCH_3 Aib^4 and βCH_3 αMeVal), 1.31 (s, 6H, 2x βCH_3 Aib^2), 0.98 (d, *J* = 6.9 Hz, 3H, γCH_3 αMeVal), 0.92 (d, *J* = 6.9 Hz, 3H, γCH_3 αMeVal). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.85, 174.80, 173.66, 173.02, 172.43, 164.97, 163.10, 135.26, 131.82, 82.14, 64.12, 57.48, 57.03, 56.92, 55.98, 52.32, 34.37, 29.85, 28.12, 25.65, 25.39, 25.05, 24.98, 18.65, 17.70, 17.67.

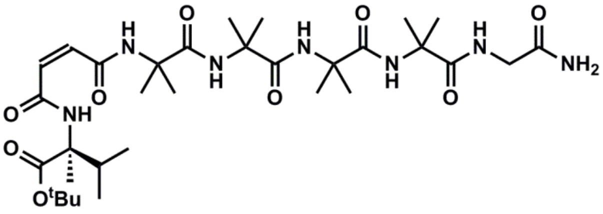
Synthesis of 2f



1 (100 mg, 0.35 mmol) and HOAt (50 mg, 0.37 mmol) were dissolved in dry CH_2Cl_2. The suspension was cooled to 0 °C and EDC·HCl (70 mg, 0.36 mmol) was added. After complete dissolution, H-Aib_8-OMe (140 mg, 0.2 mmol, prepared by the quantitative hydrogenolysis of N_3Aib_8-OMe) and DIPEA (60 μL, 0.36 mmol) were added and the reaction mixture stirred overnight at r.t. The solvent was removed under reduced pressure and the residue dissolved in EtOAc. The organic phase was washed with $KHSO_{4(aq)}$ 5%, $NaHCO_{3(aq)}$ 5%, brine, dried over $MgSO_4$, filtered and concentrated. The product was obtained as a white solid after precipitation from EtOAc/petroleum ether (150 mg, 77% yield).

HRMS (ES^+ MeOH) *m/z* calcd. for $C_{47}H_{82}N_9O_{13}$ ($[M+H]^+$) 980.6027, found 980.6179. Mp 172-175 °C. FT-IR $\bar{\upsilon}_{max}$ 3310, 2986, 2942, 1733, 1659, 1535, 1467, 1458, 1385, 1364, 1229, 1152 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (s, 1H, NH), 7.85 (s, 1H, NH), 7.80 (s, 1H, NH), 7.75 (s, 2H, 2x NH), 7.72 (s, 1H, NH), 7.49 (s, 1H, NH), 7.27 (s, 1H, NH), 7.08 (s, 1H, NH), 6.95-6.73 (m, 2H, AB system CH_2 Fum), 3.69 (s, 3H, OMe), 2.34 (dt, *J* = 14.1, 6.9 Hz, 1H, βCH αMeVal), 1.78 (s, 12H, 4x βCH_3 Aib), 1.57 (s, 3H, βCH_3 αMeVal), 1.52 (s, 12H, 4x βCH_3 Aib), 1.49 (s, 12H, 4x βCH_3 Aib), 1.47 (s, 15H, OtBu and 2x βCH_3 Aib), 1.41 (s, 6H, 2x βCH_3 Aib), 1.02 (d, *J* = 6.8 Hz, 3H, γCH_3 αMeVal), 0.93 (d, *J* = 6.8 Hz, 3H, γCH_3 αMeVal). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.49, 176.18, 176.09, 175.72, 175.22, 175.19, 174.69, 172.47, 165.44, 81.97, 64.11, 57.41, 56.99, 56.87, 56.73, 56.70, 56.26, 52.42, 34.47, 28.16, 25.12, 24.93, 18.83, 17.76.

c) Synthesis of 3a

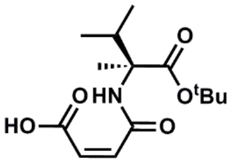


A solution of **6a** (30 mg, 0.044 mmol) in 3 mL of MeOH was irradiated in a quartz cuvette at 254 nm for 2 h. The solvent was removed under reduced pressure obtaining the crude product. Pure compound **7a** was obtained by semipreparative HPLC, after freeze-drying as a white solid.

FT-IR $\bar{\upsilon}_{max}$ 3289, 2922, 1650, 1539, 1468, 1383, 1364 cm^{-1}. ^{1}H NMR (500 MHz, CD_3CN) δ 8.61 (s, 1H, NH Aib^2), 7.84 (s, 1H, NH Aib^3), 7.82 (t, *J* = 6.2 Hz, 1H, NH Gly), 7.70 (s, 1H, NH Aib^4), 7.46 (s, 1H, NH Aib^1), 7.10 (s br, 1H, GlyNH$_2$), 7.05 (s, 1H, NH αMeVal), 6.29 (d, *J* = 12.0 Hz, 1H, CH Mal), 6.20 (d, *J* = 12.0 Hz, 1H, CH Mal), 5.64 (s, 1H, GlyNH$_2$), 2.11-2.06 (m, 1H, βCH αMeVal), 1.44 (s, 12H, O*t*Bu and βCH$_3$ Aib), 1.43 (s, 3H, βCH$_3$ Aib), 1.42 (s, 3H, βCH$_3$ Aib), 1.41 (s, 6H, 2x βCH$_3$ Aib), 1.38 (s, 3H, βCH$_3$ αMeVal), 1.36 (s, 6H, 2x βCH$_3$ Aib), 1.35 (s, 3H, βCH$_3$ Aib), 0.99 (d, *J* = 6.9 Hz, 3H, γCH$_3$ αMeVal), 0.92 (d, *J* = 6.9 Hz, 3H, γCH$_3$ αMeVal). ^{13}C NMR (101 MHz, CD_3CN) δ 177.35 (CO Aib^2), 176.93 (CO Aib^3), 176.30 (CO Aib^4), 175.88 (CO Aib^1), 173.26 (CO Gly), 172.31 (CO αMeVal), 168.69 (C^{b}O Mal), 164.53 (C^{a}O Mal), 136.05 (C^b Mal), 126.97 (C^a Mal), 81.63 (C O*t*Bu), 64.05 (αC αMeVal), 57.71 (αC Aib), 57.57 (αC Aib x2), 57.33 (αC Aib), 43.60 (αC Gly), 35.46 (βC αMeVal), 28.19 (CH$_3$ O*t*Bu), 26.43 (βC Aib^4), 26.23 (βC Aib^2), 26.17 (βC Aib^3), 26.06 (βC Aib^1), 24.66 (βC Aib^4), 24.46 (βC Aib^1), 24.33 (βC Aib^2), 24.26 (βC Aib^3), 17.52 (βCH$_3$ αMeVal), 17.40 (γC αMeVal), 17.37 (γC αMeVal).

d) Synthesis of 3c

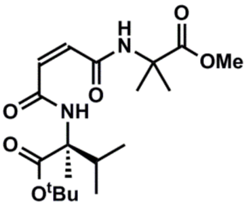
Synthesis of 15



H-(αMe)Val-O*t*Bu (150 mg, 0.8 mmol) was dissolved in 2 mL of dry CH_3CN and TEA (100 µL, 0.71 mmol) were added. Maleic anhydride (70 mg, 0.71 mmol) was dissolved in 1 mL of dry CH_3CN and added to the solution of the amino acid. The reaction mixture stirred overnight at r.t. The solvent was removed under reduced pressure and the residue dissolved in EtOAc. The organic phase was washed with $KHSO_{4(aq)}$ 5% and brine, dried over Na_2SO_4, filtered and concentrated. The product was obtained as a white solid (170 mg, 74% yield).

HRMS (ES^+, MeOH) *m/z* calcd. for $C_{14}H_{24}NO_5$ ($[M+H]^+$) 286.1649, found 286.1703. Mp 156-159 °C. FT-IR ῡ$_{max}$ 3306, 3002, 2980, 1727, 1712, 1632, 1593, 1558, 1490, 1368, 1147, 858 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ 7.28 (s, 1H, NH), 6.36 (d, *J* = 12.8 Hz, 1H, CH Mal), 6.29 (d, *J* = 12.9 Hz, 1H, CH Mal), 2.50-2.37 (m, 1H, βCH), 1.65 (s, 3H, βCH_3), 1.49 (s, 9H, OtBu), 1.04 (d, *J* = 7.0 Hz, 3H, γCH_3), 0.92 (d, *J* = 6.9 Hz, 3H, γCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.71, 165.22, 164.68, 136.95, 131.52, 83.40, 65.32, 33.84, 27.88, 18.82, 17.53.

Synthesis of 3c



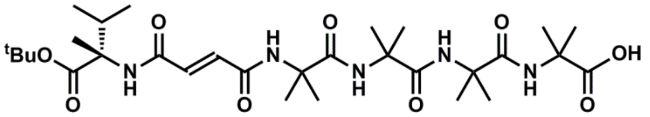
15 (50 mg, 0.17 mmol) and HOAt (23 mg, 0.17 mmol) were dissolved in dry CH_2Cl_2. The suspension was cooled to 0 °C and EDC·HCl (33 mg, 0.17 mmol) was added. After complete dissolution H-Aib-OMe·HCl (54 mg, 0.35 mmol) and DIPEA (90 µL, 0.52 mmol) were added and the reaction mixture stirred overnight at r.t. The solvent was removed under reduced pressure and the residue dissolved in EtOAc. The organic phase was washed with $KHSO_{4(aq)}$ 5%, $NaHCO_{3(aq)}$ 5%, brine, dried over $MgSO_4$, filtered and concentrated. The product was obtained as an oil (50 mg, 75% yield).

HRMS (ES^+, MeOH) *m/z* calcd. for $C_{19}H_{33}N_2O_6$ ($[M+H]^+$) 385.2333, found 385.2435. FT-IR ῡ$_{max}$ 3280, 2978, 1748, 1729, 1671, 1620, 1578, 1548, 1368, 1286, 1263, 1149, 854 cm^{-1}.

^{1}H NMR (500 MHz, CD_3CN) δ 8.94 (s, 1H, NH Aib), 8.70 (s, 1H, NH αMeVal), 6.12 (d, *J* = 13.4 Hz, 1H, CH Mal), 6.06 (d, *J* = 13.4 Hz, 1H, CH Mal), 3.62 (s, 3H, OMe), 2.02 (dt, *J* = 13.7, 6.9 Hz, 1H, βCH αMeVal), 1.44 (s, 3H, $βCH_3$ Aib), 1.43 (s, 3H, $βCH_3$ Aib), 1.40 (s, 9H, OtBu), 1.36 (s, 3H, $βCH_3$ αMeVal), 0.98 (d, *J* = 6.9 Hz, 3H, $γCH_3$ αMeVal), 0.91 (d, *J* = 6.9 Hz, 3H, $γCH_3$ αMeVal). ^{13}C NMR (101 MHz, CD_3CN) δ 175.15, 165.20, 134.14, 132.84, 81.36, 64.09, 56.86, 52.76, 35.73, 28.15, 25.16, 25.08, 17.67, 17.61, 17.43.

e) Synthesis of carboxylic acids 4a-b

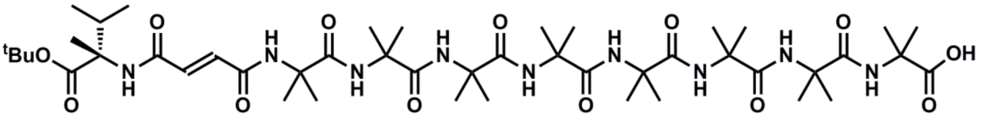
Synthesis of 4a



2e (320 mg, 0.54 mmol) was dissolved in 15 mL of THF and a solution of LiOH (85 mg, 3.5 mmol) in 5 mL of water was added. The solution was stirred at 40°C for 24 h. The organic solvent was removed under reduced pressure and the aqueous phase was acidified with HCl 1M. The compound was extracted using EtOAc (3v). The organic phase was washed with $KHSO_{4(aq)}$ 5% and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The product was obtained as a white solid (230 mg, 73% yield).

HRMS (ES$^+$, MeOH) *m/z* calcd. for $C_{30}H_{51}N_5O_9Na$ ($[M+Na]^+$) 648.3579, found 648.3584. $[\alpha]_D^{20}$ = -8 (c 1, MeOH). Mp 152-154 °C. FT-IR $\tilde{\upsilon}_{max}$ 3305, 2982, 1728, 1650, 1534, 1458, 1385, 1260, 1225, 1151 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.01 (s, 1H, NH), 7.89 (s, 1H, NH), 7.87 (s, 1H, NH), 7.23 (s, 1H, NH), 6.97 (s, 1H, NH), 6.87 (m, 2H, AB system CH_2 Fum), 2.40 (m, 1H, βCH αMeVal), 1.59 (s, 3H, $βCH_3$ αMeVal), 1.56 (s, 6H, 2x $βCH_3$ Aib), 1.52 (s, 6H, 2x $βCH_3$ Aib), 1.50 (s, 6H, 2x $βCH_3$ Aib), 1.47 (s, 9H, OtBu), 1.43 (s, 6H, 2x $βCH_3$ Aib), 1.01 (d, *J* = 6.7 Hz, 3H, $γCH_3$ αMeVal), 0.91 (d, *J* = 6.7 Hz, 3H, $γCH_3$ αMeVal). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.78, 175.75, 175.56, 174.53, 172.66, 165.39, 163.86, 133.74, 133.07, 82.43, 64.30, 57.33, 56.97, 56.91, 34.21, 29.85, 28.11, 25.19, 24.85, 18.98, 17.78, 17.73.

Synthesis of 4b



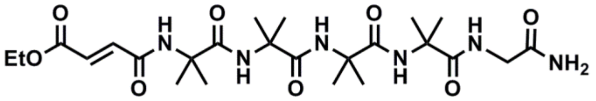
2f (120 mg, 0.12 mmol) was dissolved in 10 mL of THF and a solution of LiOH (20 mg, 0.86 mmol) in 5 mL of water was added. The solution was stirred at 60 °C for 48 h, The solution was diluted with CH_2Cl_2 and the aqueous phase was acidified with HCl 1M. The compound was extracted using CH_2Cl_2 (3v). The organic phase was washed with $KHSO_{4(aq)}$ 5% and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The crude was purified *via* flash chromatography (eluant: CH_2Cl_2/MeOH, 93:7→8:2). The product was obtained as a white solid (80 mg, 68% yield).

HRMS (ES$^+$ MeOH) *m/z* calcd. for $C_{46}H_{80}N_9O_{13}$ ([M+H]$^+$) 966.5870, found 966.6056. Mp 190-193 °C. FT-IR $\bar{\upsilon}_{max}$ 3309, 2985, 2939, 1728, 1659, 1535, 1385, 1365, 1228 cm^{-1}.

^{1}H NMR (400 MHz, MeOD) δ 7.10 (d, *J* = 15.1 Hz, 1H, CH Fum), 6.92 (d, *J* = 15.1 Hz, 1H, CH Fum), 2.09 (dt, *J* = 13.6, 6.7 Hz, 1H, βCH αMeVal), 1.52 (s, 6H, 2x βCH$_3$ Aib), 1.51 (s, 6H, 2x βCH$_3$ Aib), 1.49 (s, 6H, 2x βCH$_3$ Aib), 1.48 (s br, 24H, 4x βCH$_3$ Aib), 1.44 (s, 9H, O*t*Bu), 1.42 (s, 3H, βCH$_3$ αMeVal), 1.40 (s, 6H, 2x βCH$_3$ Aib), 1.01 (d, *J* = 6.7 Hz, 3H, γCH$_3$ αMeVal), 0.96 (d, *J* = 6.7 Hz, 3H, γCH$_3$ αMeVal). ^{13}C NMR (101 MHz, MeOD) δ 177.63, 177.48, 177.42, 177.15, 177.01, 176.74, 176.21, 173.54, 166.31, 166.02, 134.67, 133.75, 82.33, 64.57, 57.95, 57.89, 57.83, 57.79, 57.73, 35.86, 28.24, 25.49, 25.14, 17.72, 17.69, 17.56.

f) Synthesis of 12

Synthesis of 16

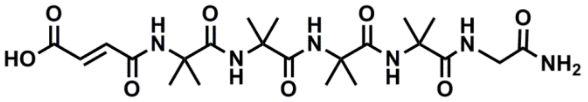


*Mono*ethyl fumarate (90 mg, 0.62 mmol) and HOAt (85 mg, 0.62 mmol) were dissolved in dry CH_2Cl_2. The suspension was cooled to 0 °C and EDC·HCl (120 mg, 0.62 mmol) was added. After complete dissolution, H-Aib$_4$-Gly-NH$_2$ (130 mg, 0.31 mmol) and TEA (90 µL, 0.65 mmol) were added and the reaction mixture stirred for 48 h at r.t. The precipitate collected and washed with CH_2Cl_2. The product was recovered after precipitation from MeOH/Et_2O obtaining a white solid (100 mg, 60% yield).

HRMS (ES$^+$, MeOH) *m/z* calcd. for $C_{24}H_{40}N_6O_8Na$ ([M+Na]$^+$) 563.2805, found 563.2794. Mp 274-275 °C. FT-IR $\tilde{\upsilon}_{max}$ 3264, 2985, 1720, 1652, 1533, 1455, 1386, 1363, 1281, 1223, 1172 cm^{-1}.

^{1}H NMR (400 MHz, MeOD) δ 7.10 (d, *J* = 15.4 Hz, 1H, CH Fum), 6.74 (d, *J* = 15.5 Hz, 1H, CH Fum), 4.26 (q, *J* = 7.1 Hz, 2H, CH_2 Et), 3.83 (s, 2H, CH_2 Gly), 1.50 (s, 6H, 2x βCH_3 Aib), 1.49 (s, 6H, 2x βCH_3 Aib), 1.47 (s, 6H, 2x βCH_3 Aib), 1.39 (s, 6H, 2x βCH_3 Aib), 1.31 (t, *J* = 7.1 Hz, 3H, CH_3 Et). ^{13}C NMR (101 MHz, MeOD) δ 178.09, 178.05, 177.81, 176.09, 175.40, 166.79, 165.65, 137.58, 131.26, 62.32, 58.14, 58.03, 57.88, 57.79, 43.68, 25.58, 25.27, 14.45.

Synthesis of 10

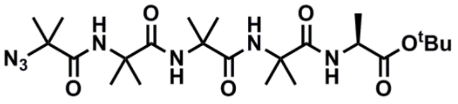


16 (70 mg, 0.13 mmol) was dissolved in 6 mL of THF and a solution of LiOH (22 mg, 0.92 mmol) in 4 mL of water was added. The solution was stirred at r.t. for 1 h. The organic solvent was removed under reduced pressure and the aqueous phase was acidified with HCl 1M. After 24 h, the compound precipitated from the solution was recovered by filtration. The product was obtained as a white solid (50 mg, 73% yield).

HRMS (ES$^+$, MeOH) *m/z* calcd. for $C_{22}H_{36}N_6O_8Na$ ([M+Na]$^+$) 535.2492, found 535.2493. Mp 274-275 °C. FT-IR $\tilde{\upsilon}_{max}$ 3300, 2986, 1659, 1537, 1386, 1224 cm^{-1}.

^{1}H NMR (400 MHz, MeOD) δ 7.07 (d, *J* = 15.4 Hz, 1H, CH Fum), 6.71 (d, *J* = 15.4 Hz, 1H, CH Fum), 3.83 (s, 2H, CH_2 Gly), 1.50 (s, 6H, 2x βCH_3 Aib), 1.49 (s, 6H, 2x βCH_3 Aib), 1.47 (s, 6H, 2x βCH_3 Aib), 1.39 (s, 6H, 2x βCH_3 Aib). ^{13}C NMR (101 MHz, MeOD) δ 178.17, 178.12, 178.11, 178.09, 177.92, 176.14, 175.41, 168.38, 165.86, 137.55, 131.85, 58.23, 58.14, 57.85, 57.81, 43.68, 25.58, 25.30, 25.08.

Synthesis of N_3-Aib$_4$-Ala-OtBu (17)

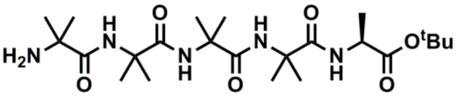


N_3-Aib$_4$-OH (230 mg, 0.6 mmol) and HOAt (106 mg, 0.77 mmol) were dissolved in dry CH_2Cl_2. After cooling to 0 °C EDC·HCl (120 mg, 0.6 mmol) was added. Then H-Ala-OtBu·HCl (217 mg, 1.2 mmol) and TEA (250 µL, 1.8 mmol) were added and the reaction mixture stirred for 48 h at r.t. The reaction mixture was diluted with CH_2Cl_2 and washed with $KHSO_{4(aq)}$ 5%, $NaHCO_{3(aq)}$ 5%, brine. The organic phase was dried over $MgSO_4$, filtered and concentrated. The crude was purified *via* flash chromatography (eluant: CH_2Cl_2/MeOH 93:7), yielding the product as a white solid (260 mg, 85 % yield).

HRMS (ES$^+$, MeOH) *m/z* calcd. for $C_{23}H_{41}N_7O_6Na$ ([M+Na]$^+$) 534.3016, found 534.3008. $[\alpha]_D^{20}$ = -53.1 (c 1, MeOH). Mp 164-166 °C. FT-IR $\bar{\upsilon}_{max}$ 3325, 2982, 2112, 1732, 1655, 1519, 1457, 1382, 1365, 1223, 1152 cm^{-1}.

^{1}H NMR (400 MHz, MeOD) δ 4.19 (q, *J* = 7.3 Hz, 1H, αCH Ala), 1.53 (s, 3H; βCH_3 Aib), 1.52 (s, 6H, 2x βCH_3 Aib), 1.49 (s, 3H, βCH_3 Aib), 1.44 (s, 6H, 2x βCH_3 Aib), 1.44 (s, 9H, OtBu), 1.42 (d, *J* = 7.4 Hz, 3H, βCH_3 Ala), 1.39 (s, 3H, βCH_3 Aib), 1.35 (s, 3H, βCH_3 Aib). ^{13}C NMR (101 MHz, MeOD) δ 177.21, 176.35, 176.25, 174.61, 173.64, 82.13, 64.75, 57.97, 57.94, 57.80, 50.77, 28.22, 27.41, 26.74, 25.52, 24.53, 24.46, 24.17, 24.12, 23.91, 17.10.

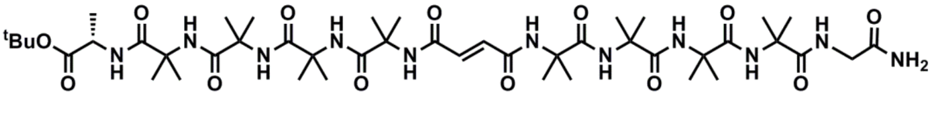
Synthesis of H-Aib$_4$-Ala-OtBu (11)



N_3-Aib$_4$-Ala-OtBu (210 mg, 0.41 mmol) was dissolved in 8 mL of EtOH under a nitrogen atmosphere. Pd/C catalyst (30 mg) was carefully added and the reaction mixture stirred under H_2 atmosphere for 24 h. The catalyst was removed by filtration through a pad of Celite and the filtrate concentrated under reduced pressure to yield the product as a white solid (170 mg, 85% yield).

HRMS (ES$^+$, MeOH) *m/z* calcd. for $C_{23}H_{43}N_5O_6Na$ ([M+Na]$^+$) 508.3111, found 508.3114. $[\alpha]_D^{20}$ = -52.3 (c 1, MeOH). Mp 179-181 °C. FT-IR $\bar{\upsilon}_{max}$ 3308, 2981, 1730, 1653, 1525, 1456, 1382, 1363, 1226, 1165 cm^{-1}. ^{1}H NMR (400 MHz, MeOD) δ 4.20 (q, *J* = 7.3 Hz, 1H, αCH Ala), 1.52 (s, 3H, βCH_3 Aib), 1.50 (s, 3H, βCH_3 Aib), 1.44 (s, 15H, O*t*Bu and 2x βCH_3 Aib), 1.42 (d, *J* = 7.4 Hz, 3H, βCH_3 Ala), 1.40 (s, 3H, βCH_3 Aib), 1.36 (s, 3H, βCH_3 Aib), 1.33 (s, 3H, βCH_3 Aib), 1.32 (s, 3H, βCH_3 Aib). ^{13}C NMR (101 MHz, MeOD) δ 179.61, 177.26, 176.62, 176.49, 173.66, 82.13, 57.96, 57.74, 57.40, 55.67, 50.75, 28.54, 28.23, 28.08, 27.43, 26.83, 25.70, 24.20, 24.17, 24.00, 17.12.

Synthesis of OtBu-Ala-Aib$_4$-Fum-Aib$_4$-Gly-NH$_2$ (12)



10 (33 mg, 0.062 mmol) and HOAt (10 mg, 0.073 mmol) were dissolved in dry CH_2Cl_2. After cooling to 0 °C EDC·HCl (12 mg, 0.062 mmol) was added. Then **11** (35 mg, 0.072 mmol) and DIPEA (20 µL, 0.11 mmol) were added and the reaction mixture stirred for 48 h at r.t. The solid was recovered and washed with CH_2Cl_2. The crude product was purified *via* flash chromatography (eluant: CH_2Cl_2/MeOH 90:15). The product was recovered as a white solid (20 mg, 33 % yield). HRMS (ES$^+$, MeOH) *m/z* calcd. for $C_{45}H_{78}N_{11}O_{13}$ ($[M+H]^+$) 980.5781, found 980.5770. $[\alpha]_D^{20}$ = +11 (c 0.1, MeOH). Mp >267 °C decompose. FT-IR ῡ$_{max}$ 3295, 2985, 1656, 1537, 1384, 1364, 1226 cm^{-1}. ^{1}H NMR (500 MHz, MeOD) δ 7.08 (s, 2H, CH_2 Fum), 4.22 (q, *J* = 7.3 Hz, 1H, αCH Ala), 3.83 (s, 2H, CH_2 Gly), 1.53 (s, 3H, βCH_3 Aib), 1.52 (s, 3H, βCH_3 Aib), 1.51 (s, 12H, 4x βCH_3 Aib), 1.50 (s, 3H, βCH_3 Aib), 1.47 (s, 3H, βCH_3 Aib), 1.47 (s, 6H, 2x βCH_3 Aib), 1.46 (s, 6H, 2x βCH_3 Aib), 1.45 (s, 9H, OtBu), 1.44 (s, 3H, βCH_3 Aib), 1.43 (d, *J* = 7.4 Hz, 3H, βCH_3 Ala), 1.38 (s, 3H, βCH_3 Aib), 1.37 (s, 3H, βCH_3 Aib), 1.34 (s, 3H, βCH_3 Aib).

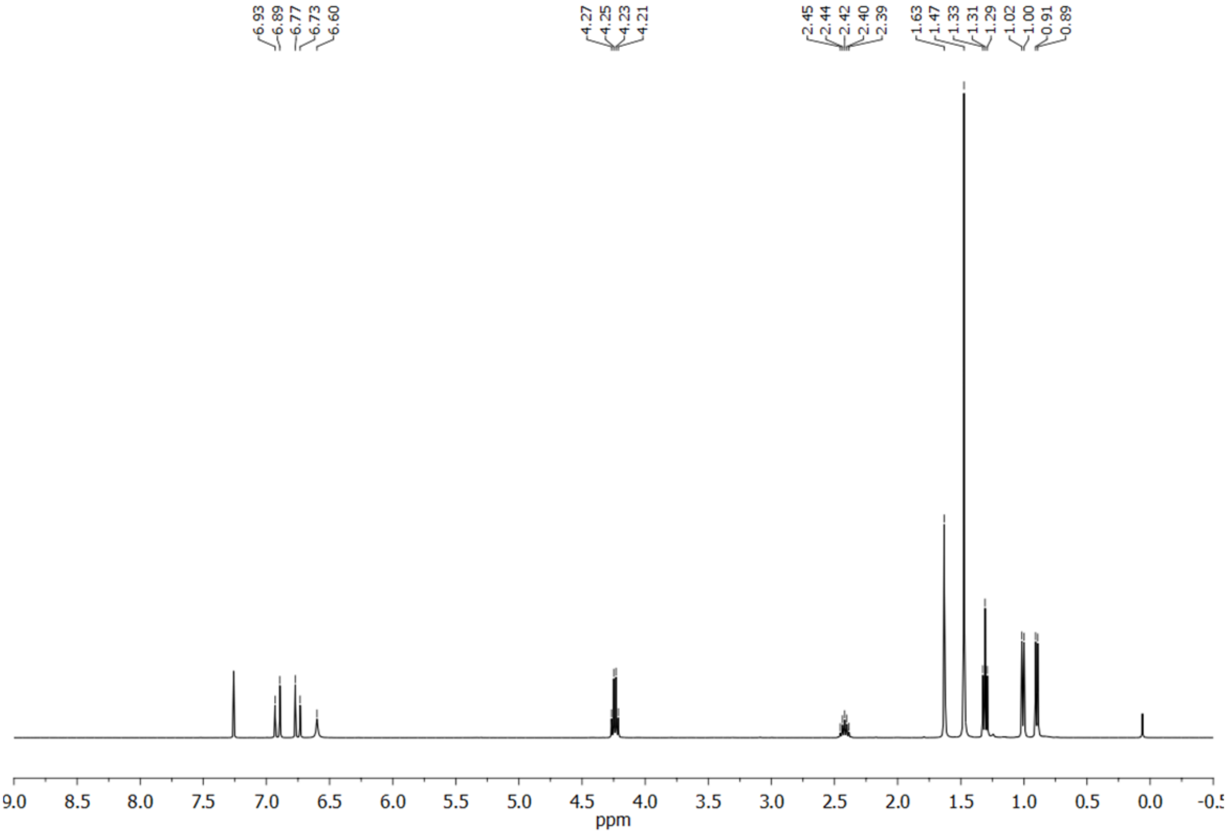


Figure S14. ^{1}H NMR (400 MHz) spectra of **14** in $CDCl_3$.

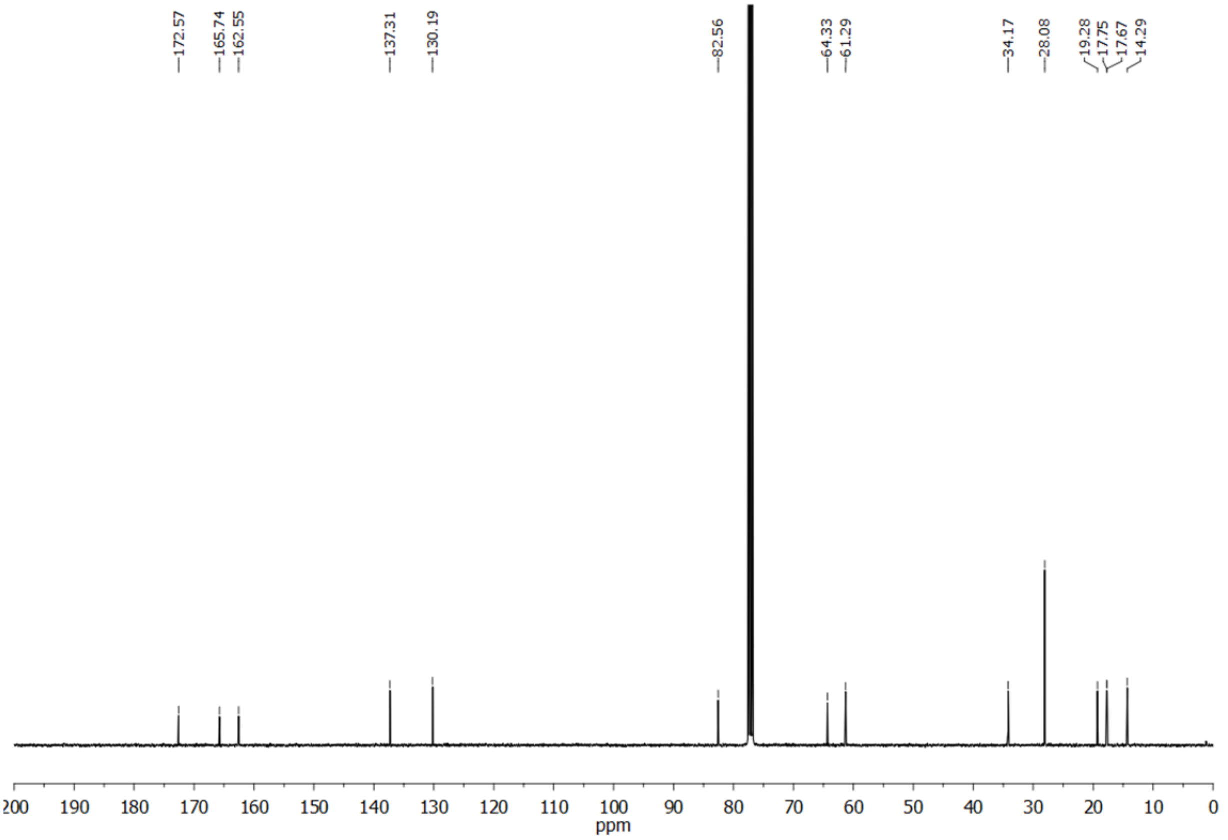


Figure S15. ^{13}C NMR (101 MHz) spectra of **14** in $CDCl_3$.

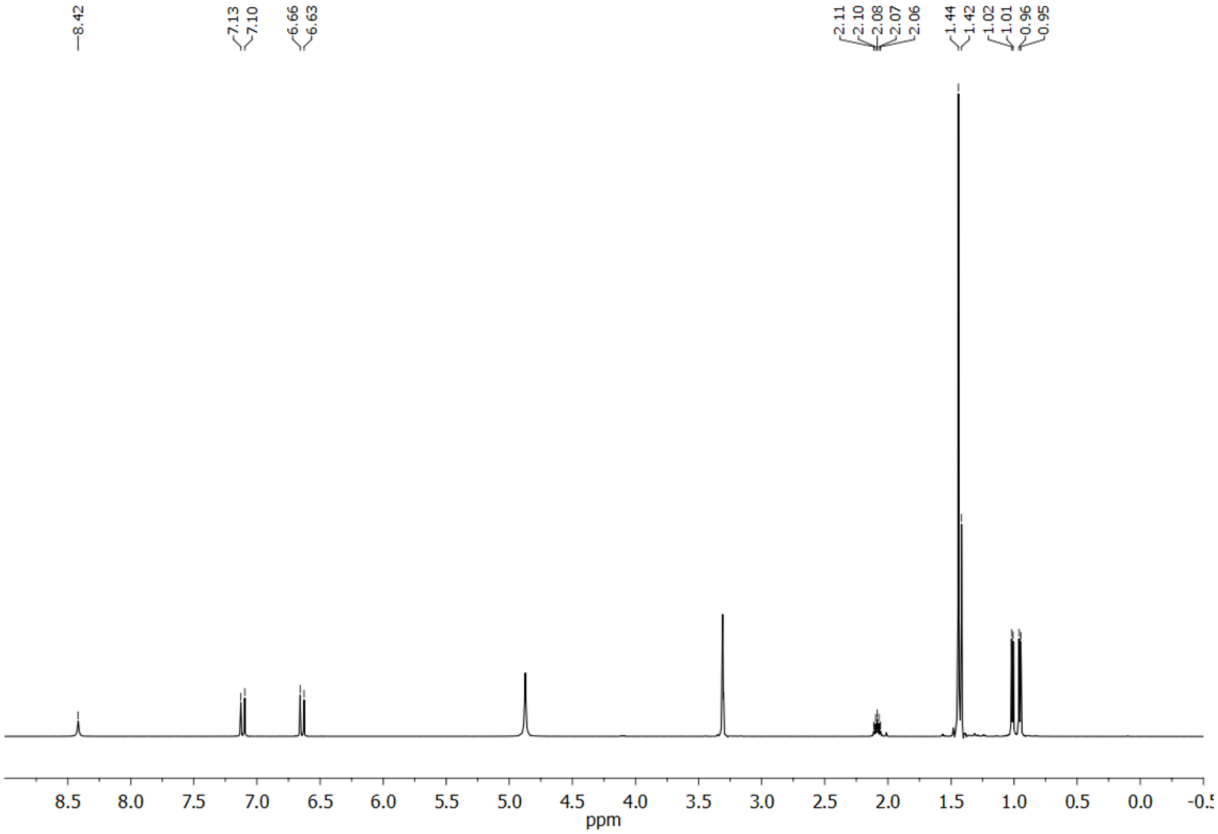


Figure S16. ^{1}H NMR (500 MHz) spectra of **1** in MeOD.

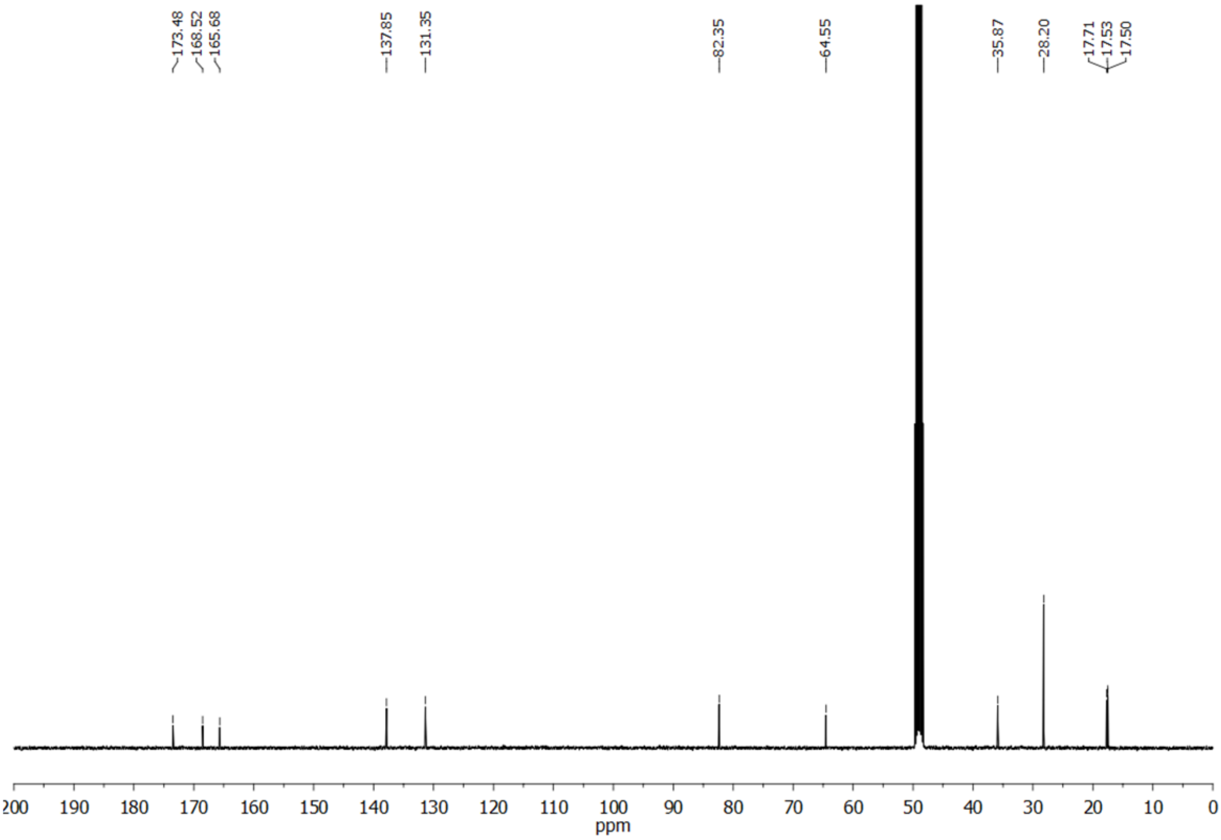


Figure S17. ^{13}C NMR (101 MHz) spectra of **1** in MeOD.

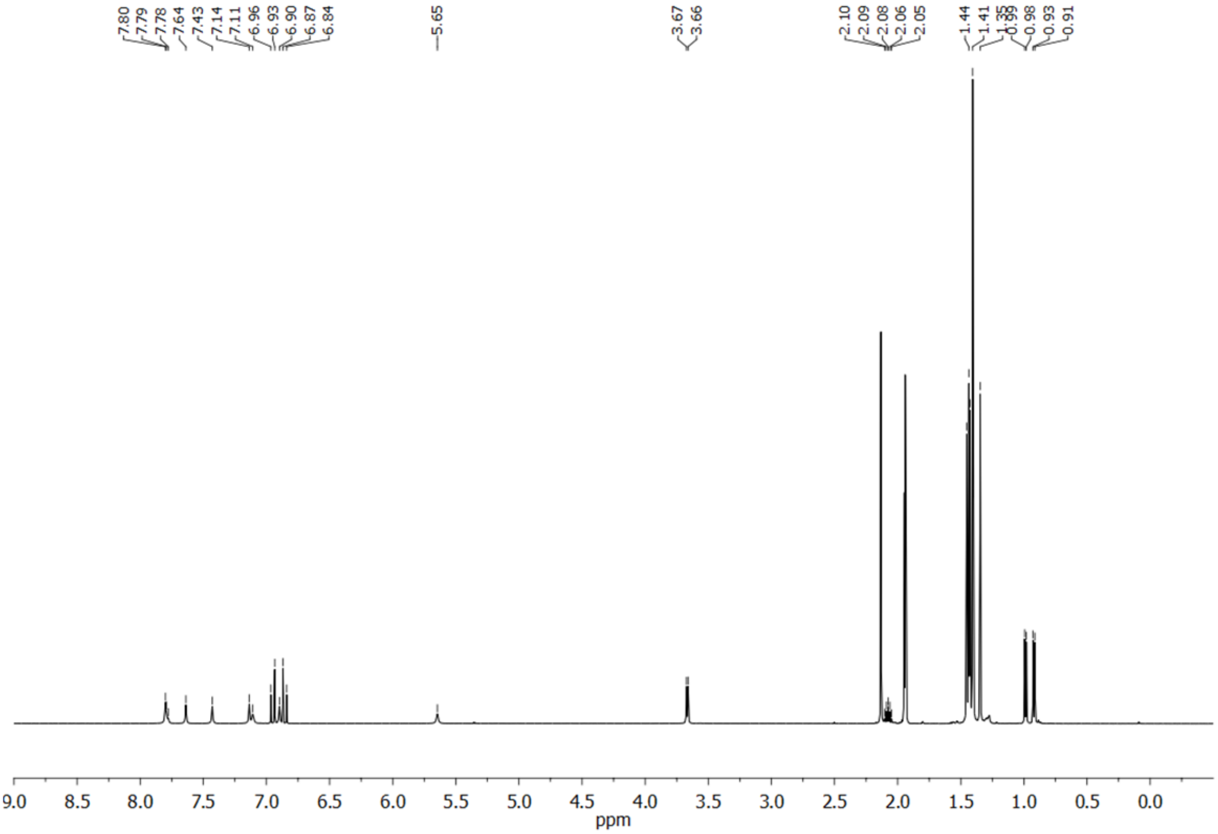


Figure S18. ^{1}H NMR (500 MHz) spectra of **2a** in CD_3CN.

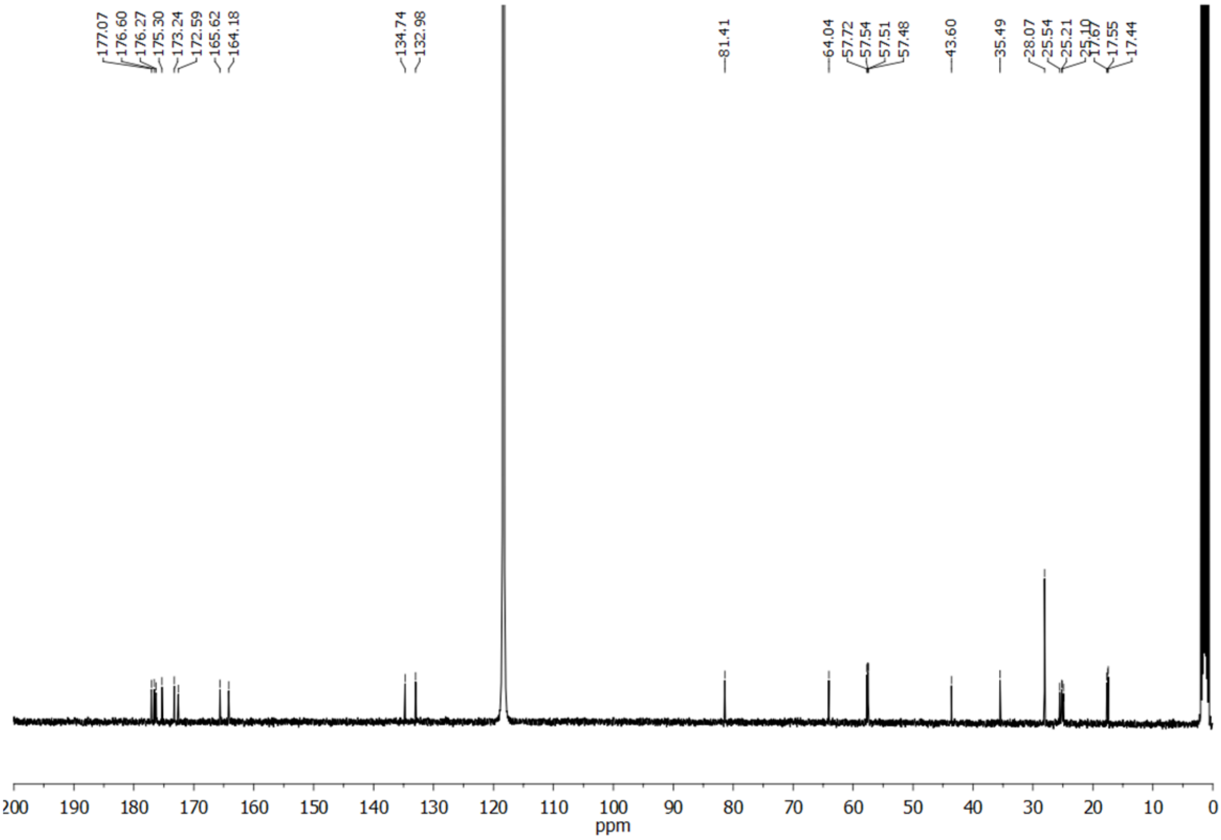


Figure S19. ^{13}C NMR (101 MHz) spectra of **2a** in CD_3CN.

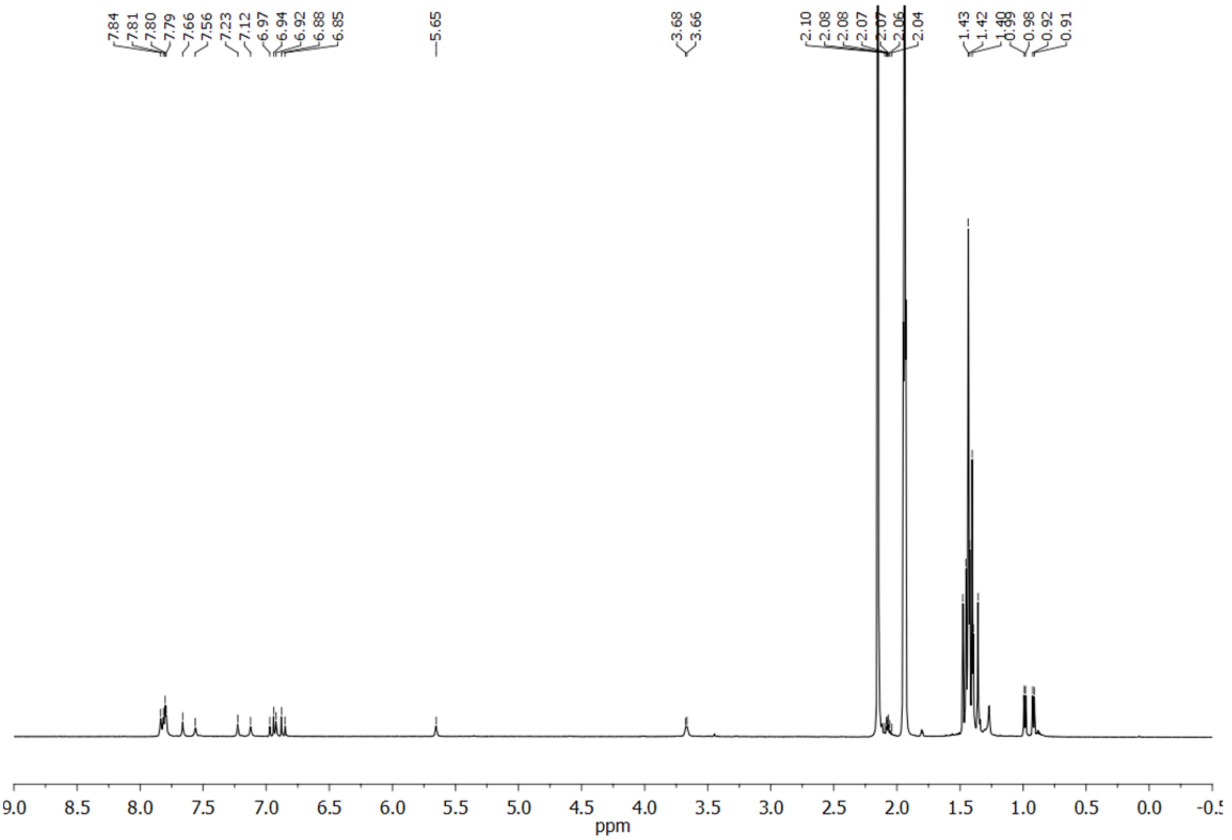


Figure S20. ^{1}H NMR (500 MHz) spectra of **2b** in CD$_3$CN.

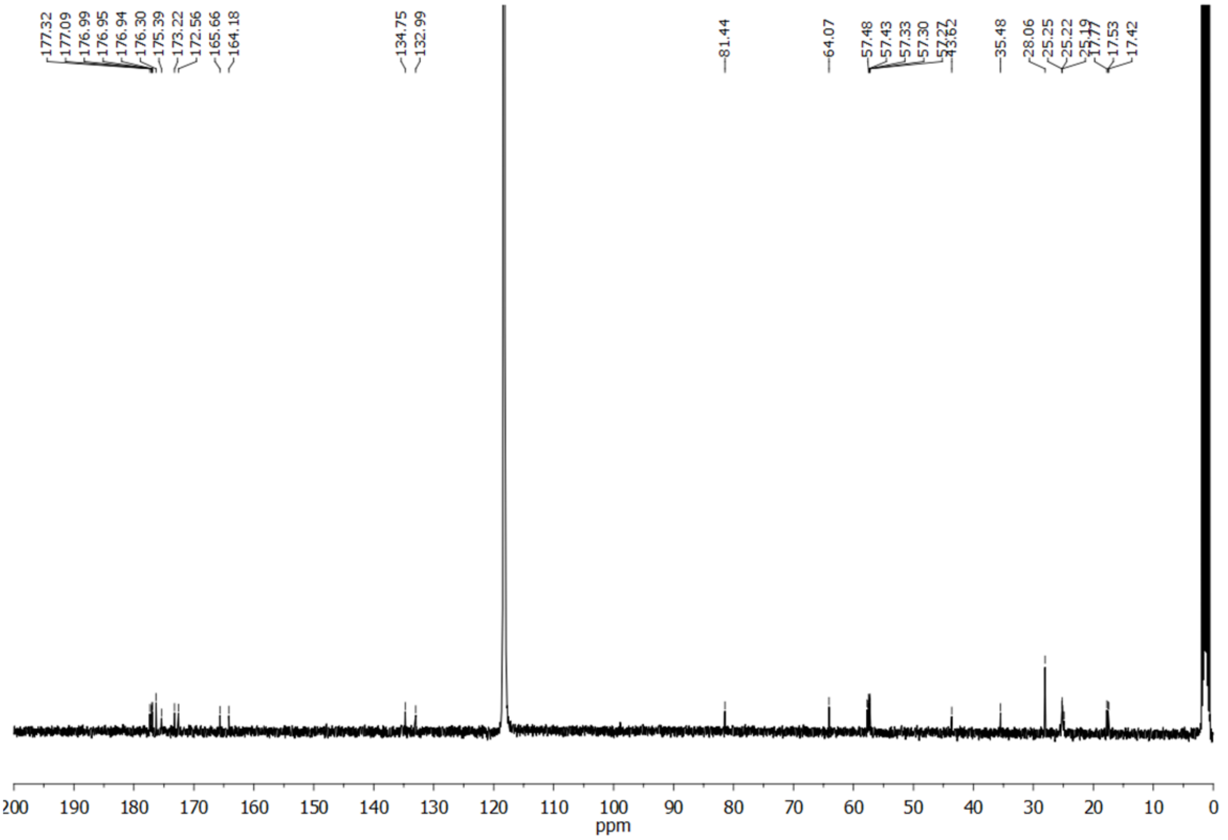


Figure S21. ^{13}C NMR (101 MHz) spectra of **2b** in CD$_3$CN.

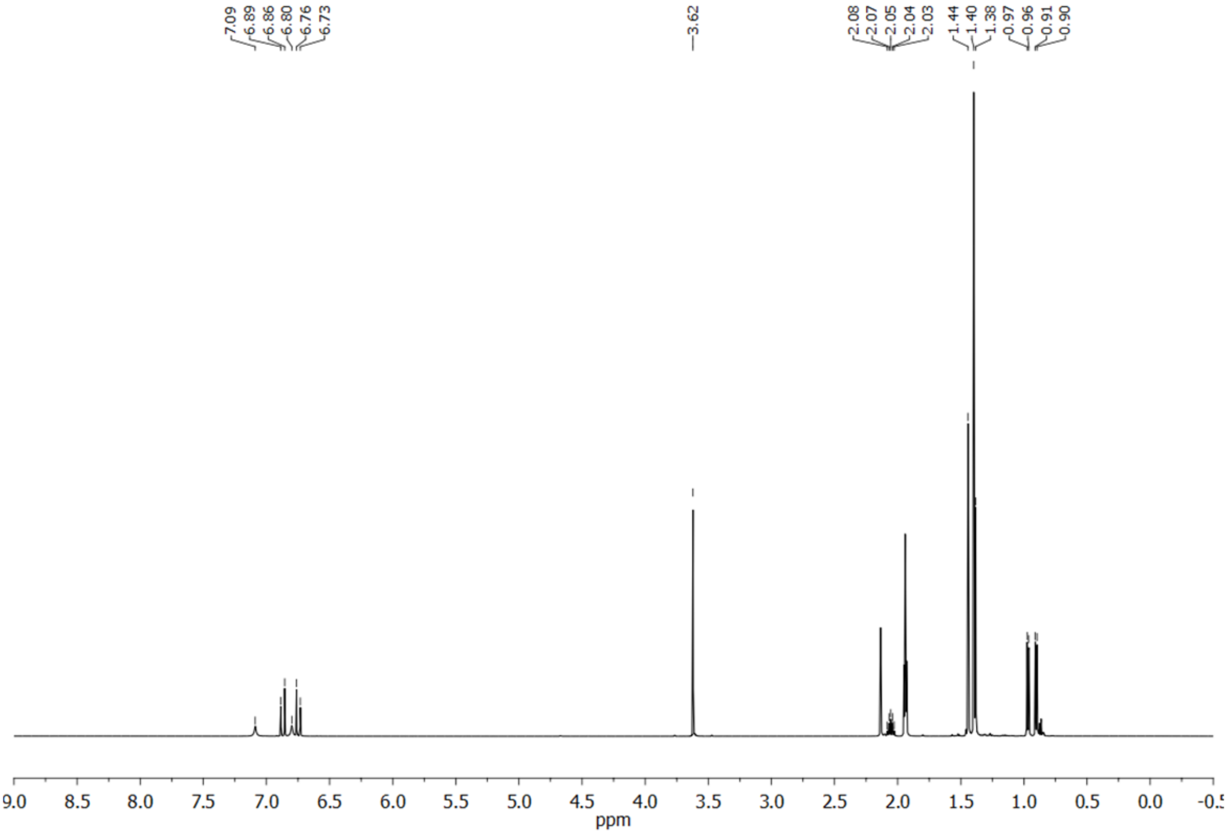


Figure S22. ^{1}H NMR (500 MHz) spectra of **2c** in CD_3CN.

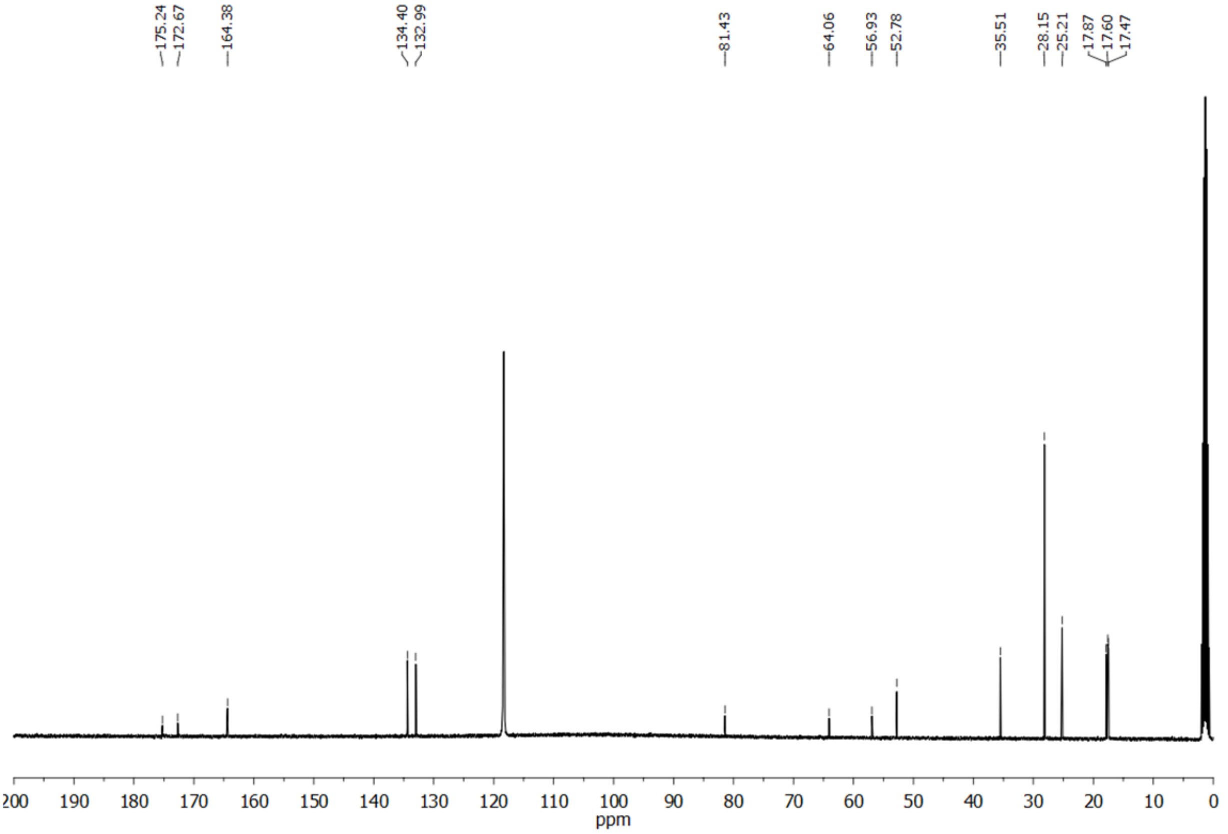


Figure S23. ^{13}C NMR (101 MHz) spectra of **2c** in CD_3CN.

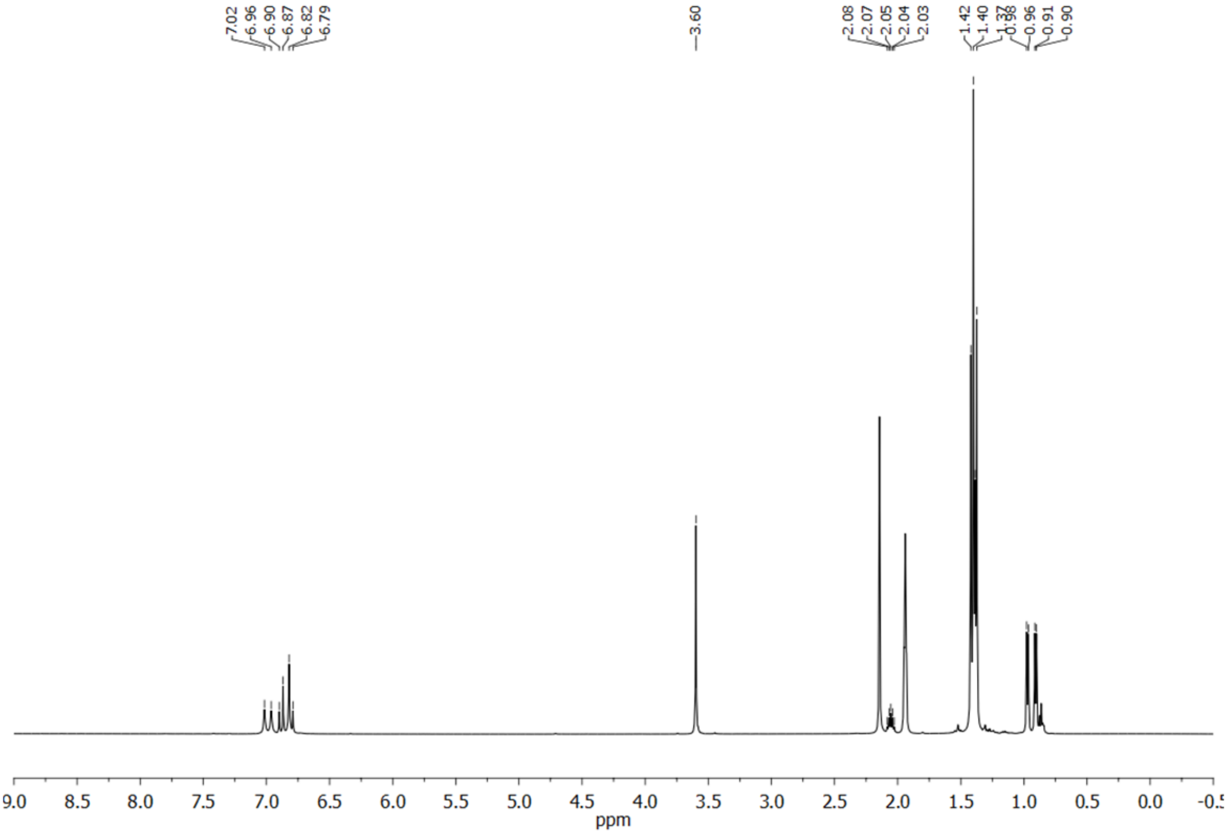


Figure S24. ^{1}H NMR (500 MHz) spectra of **2d** in CD_3CN.

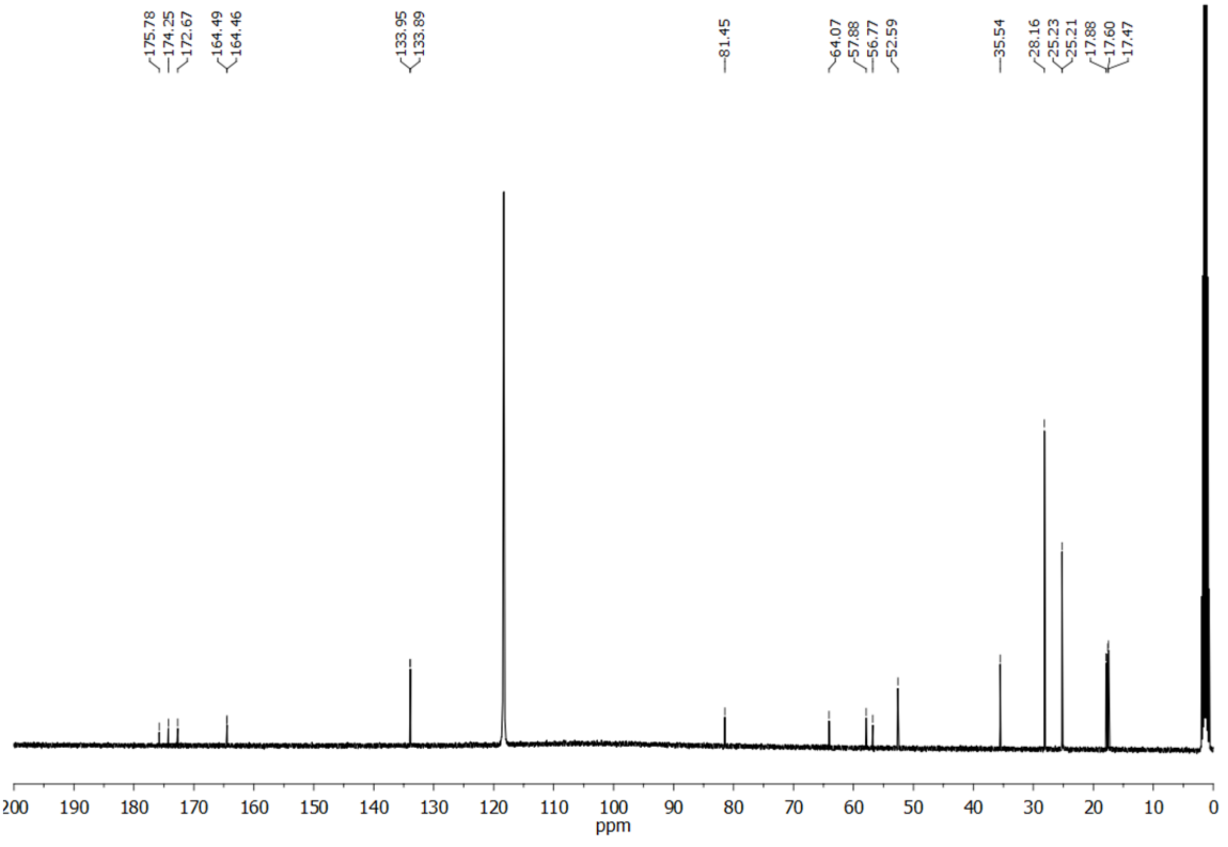


Figure S25. ^{13}C NMR (101 MHz) spectra of **2d** in CD_3CN.

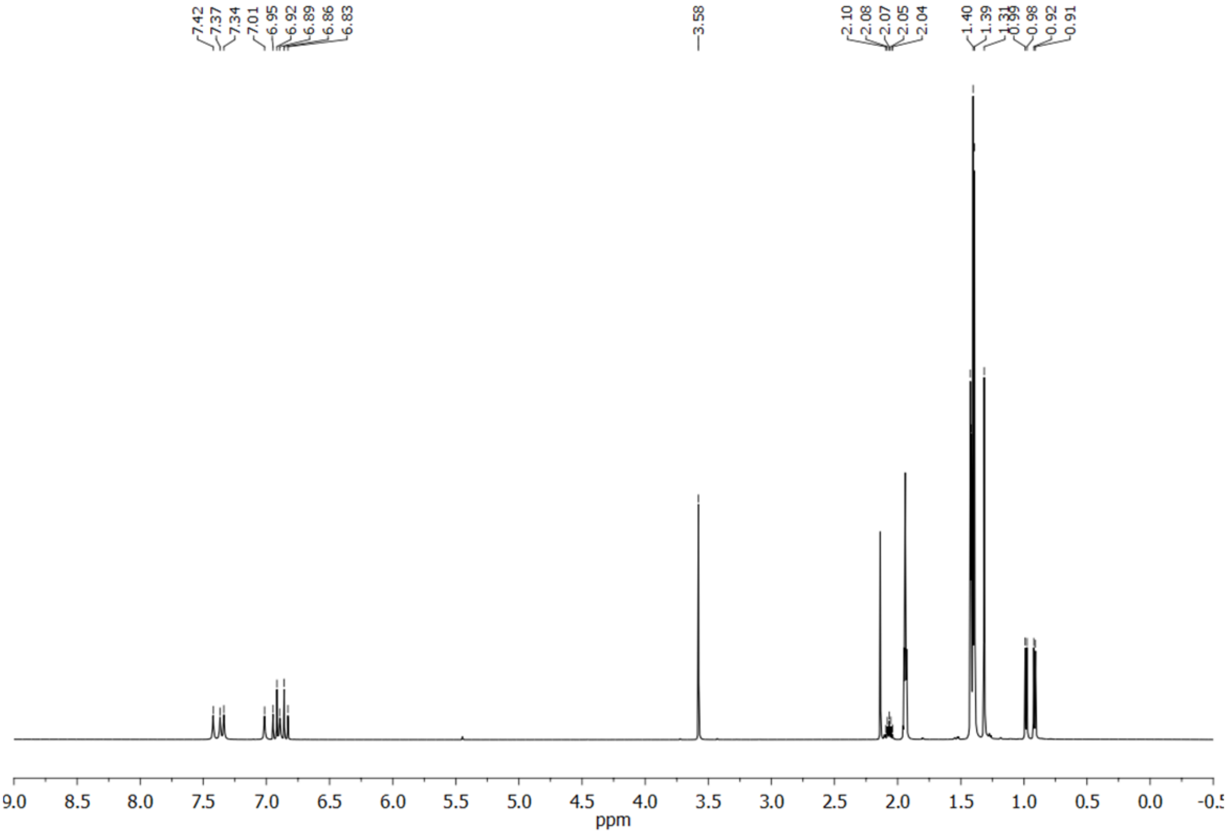


Figure S26. ^{1}H NMR (500 MHz) spectra of **2e** in CD_3CN.

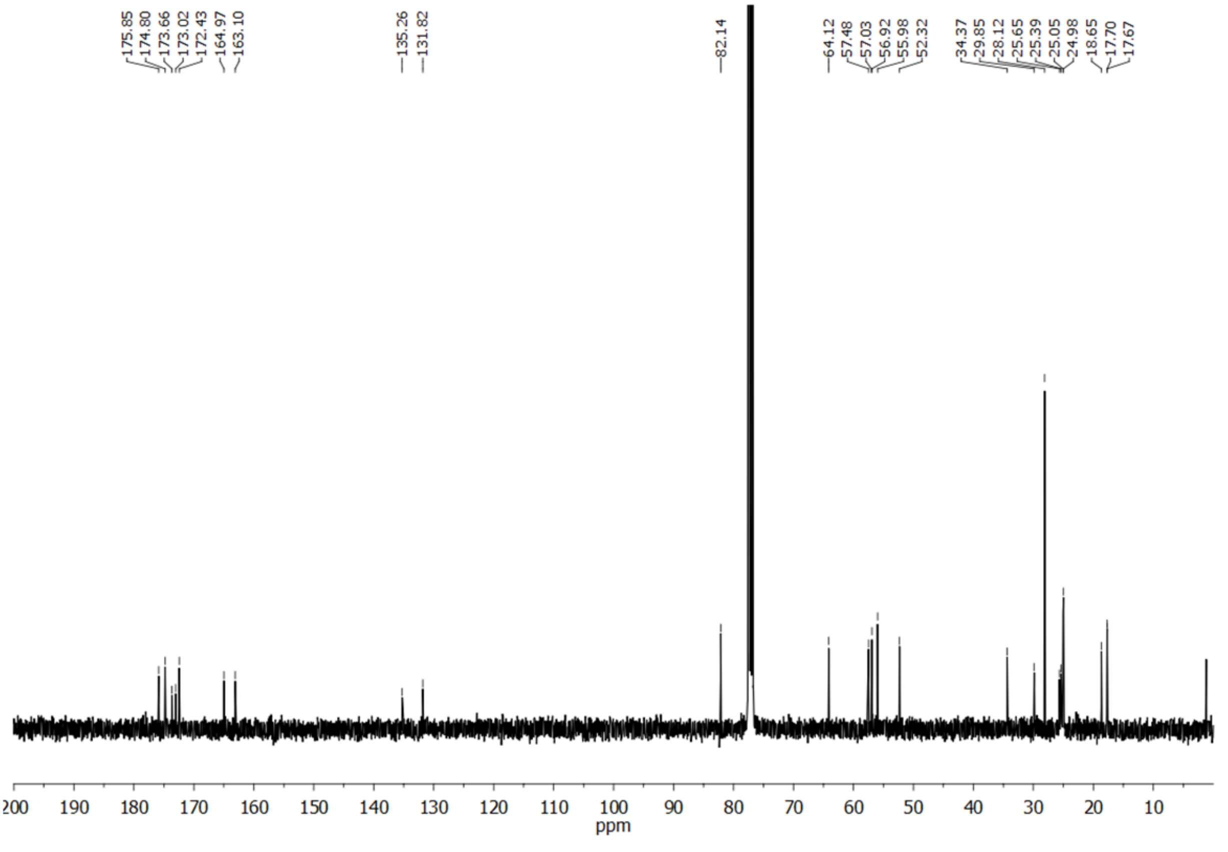


Figure S27. ^{13}C NMR (101 MHz) spectra of **2e** in $CDCl_3$.

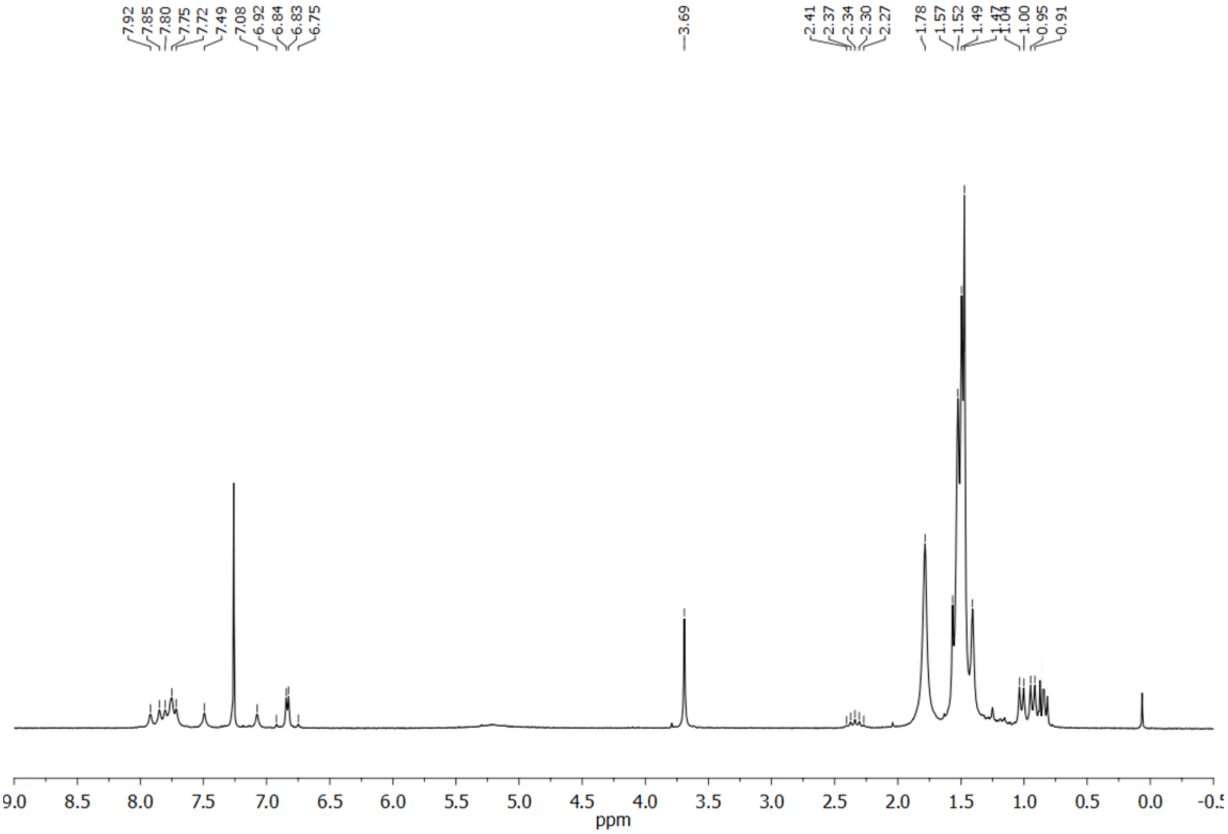


Figure S28. ^{1}H NMR (400 MHz) spectra of **2f** in CDCl$_3$.

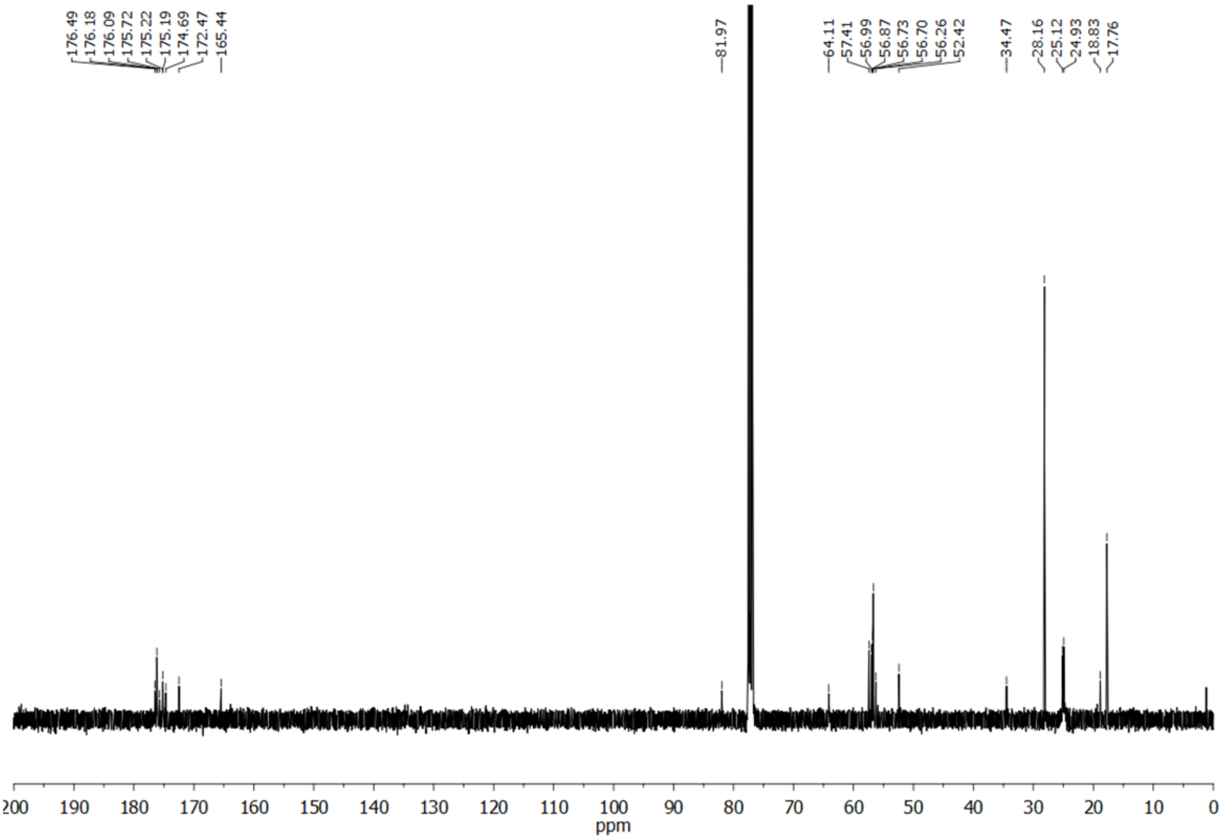


Figure S29. ^{13}C NMR (101 MHz) spectra of **2f** in CDCl$_3$.

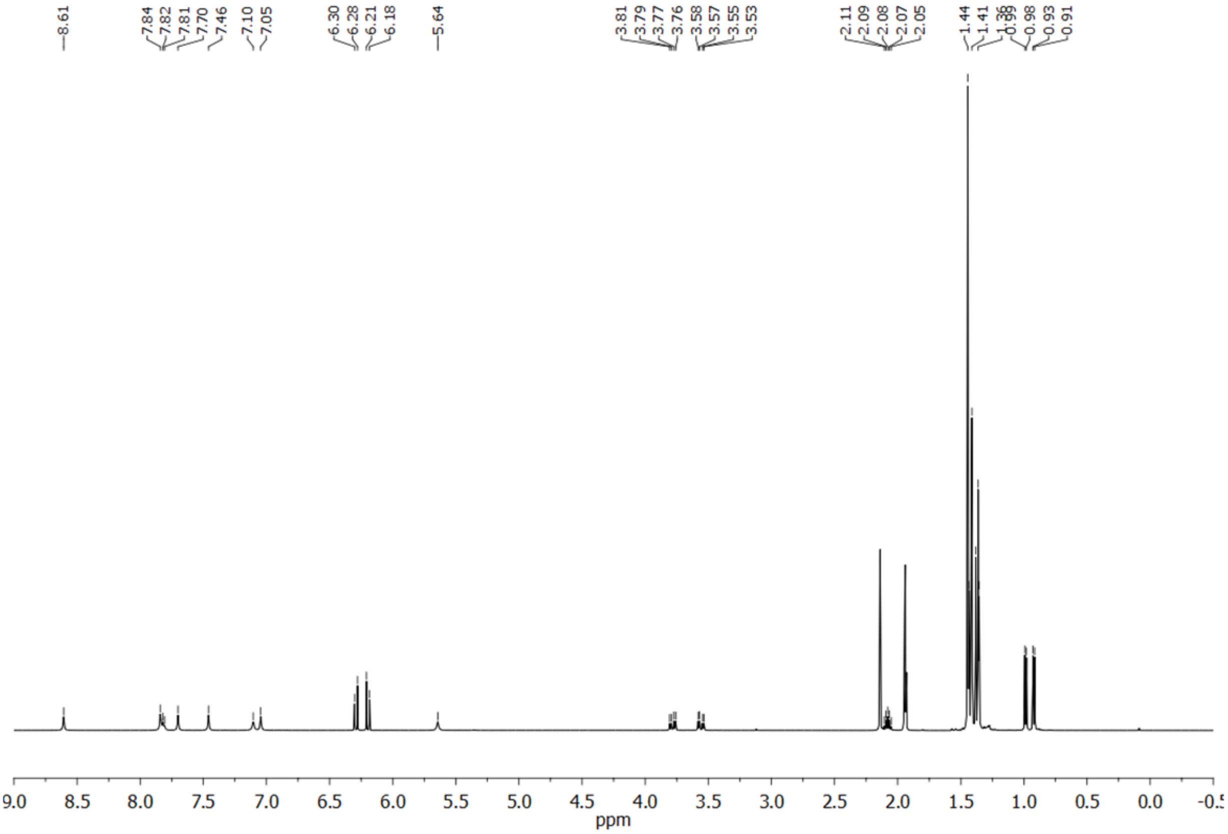


Figure S30. ^{1}H NMR (500 MHz) spectra of **3a** in CD_3CN.

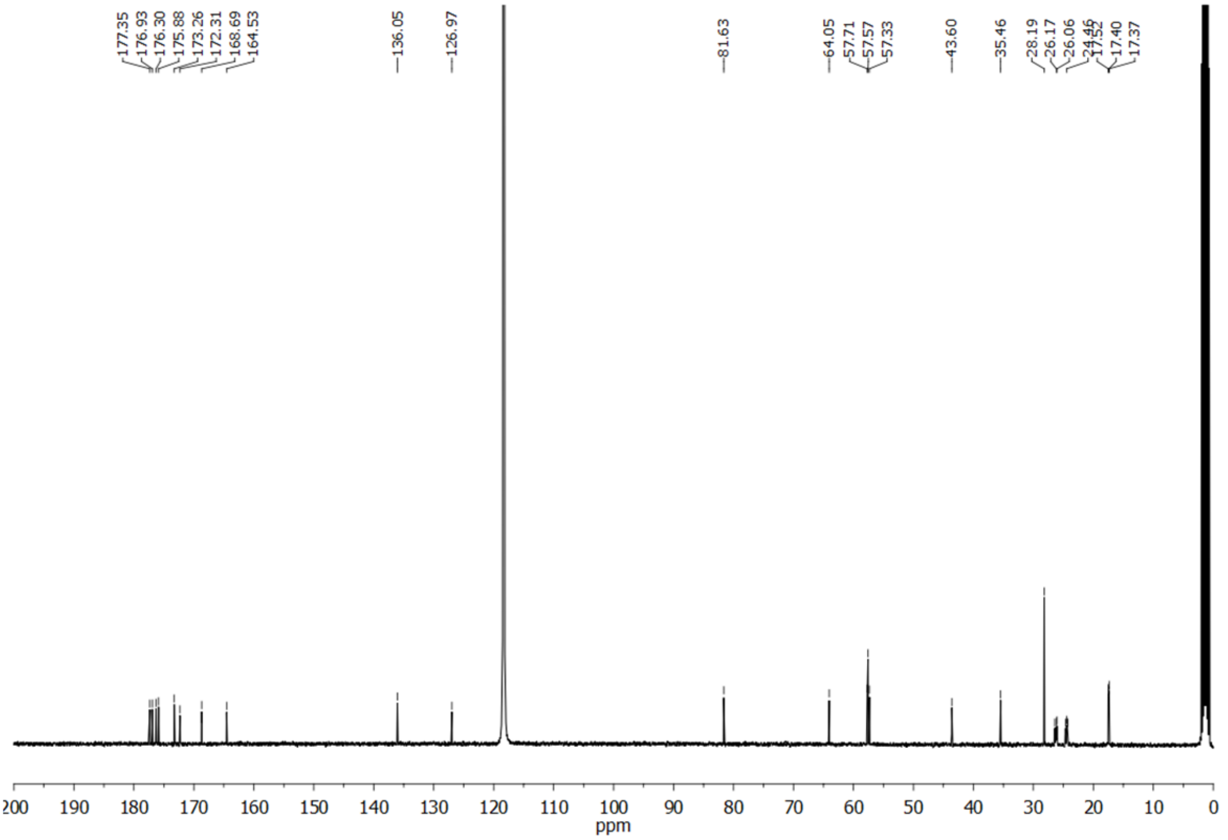


Figure S31. ^{13}C NMR (101 MHz) spectra of **3a** in CD_3CN.

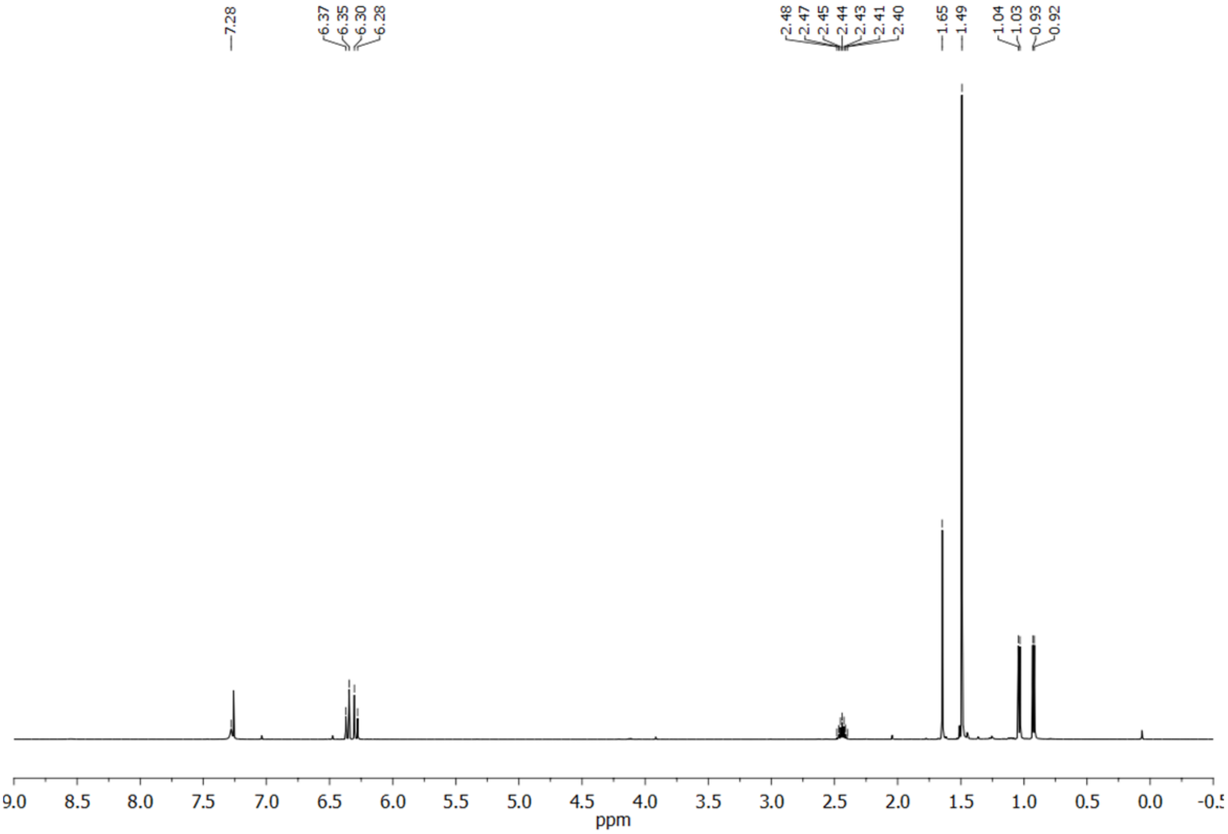


Figure S32. ^{1}H NMR (500 MHz) spectra of **15** in CDCl$_3$.

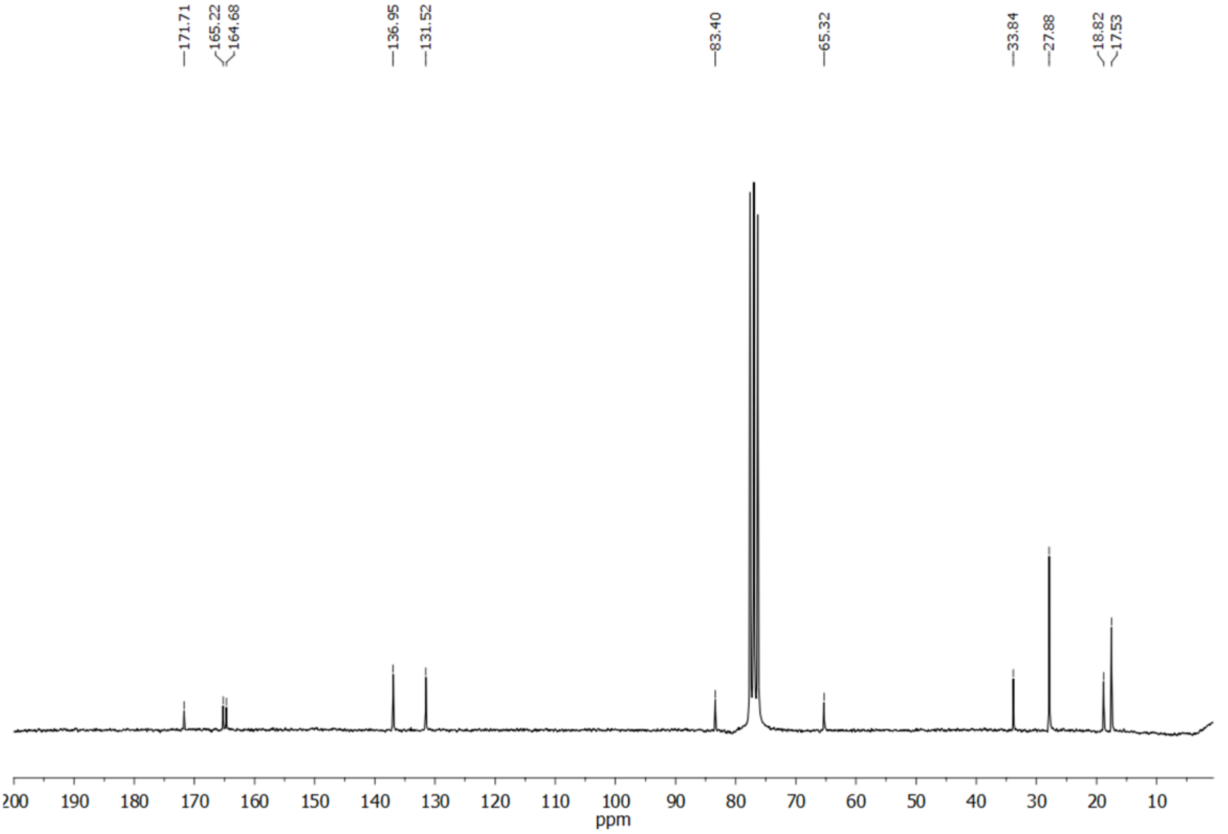


Figure S33. ^{13}C NMR (101 MHz) spectra of **15** in CDCl$_3$.

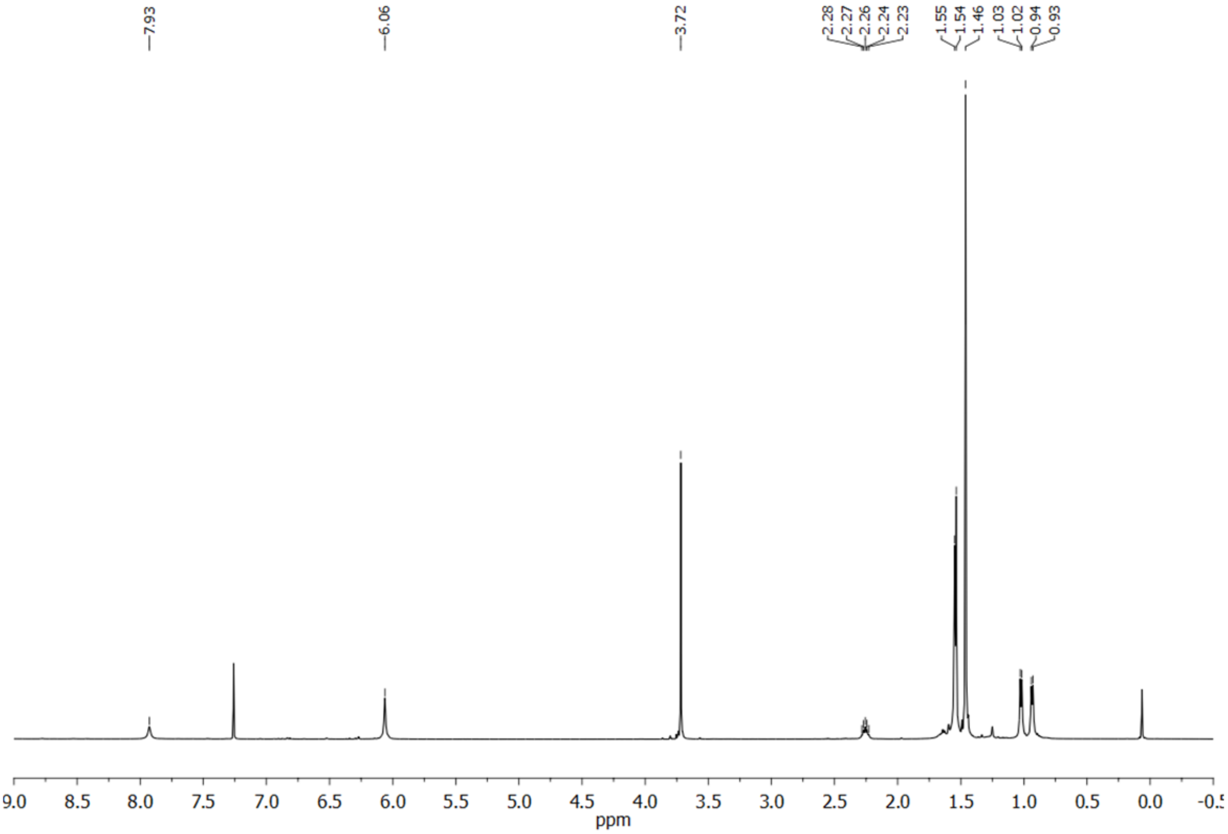


Figure S34. ^{1}H NMR (500 MHz) spectra of **3c** in CD_3CN.

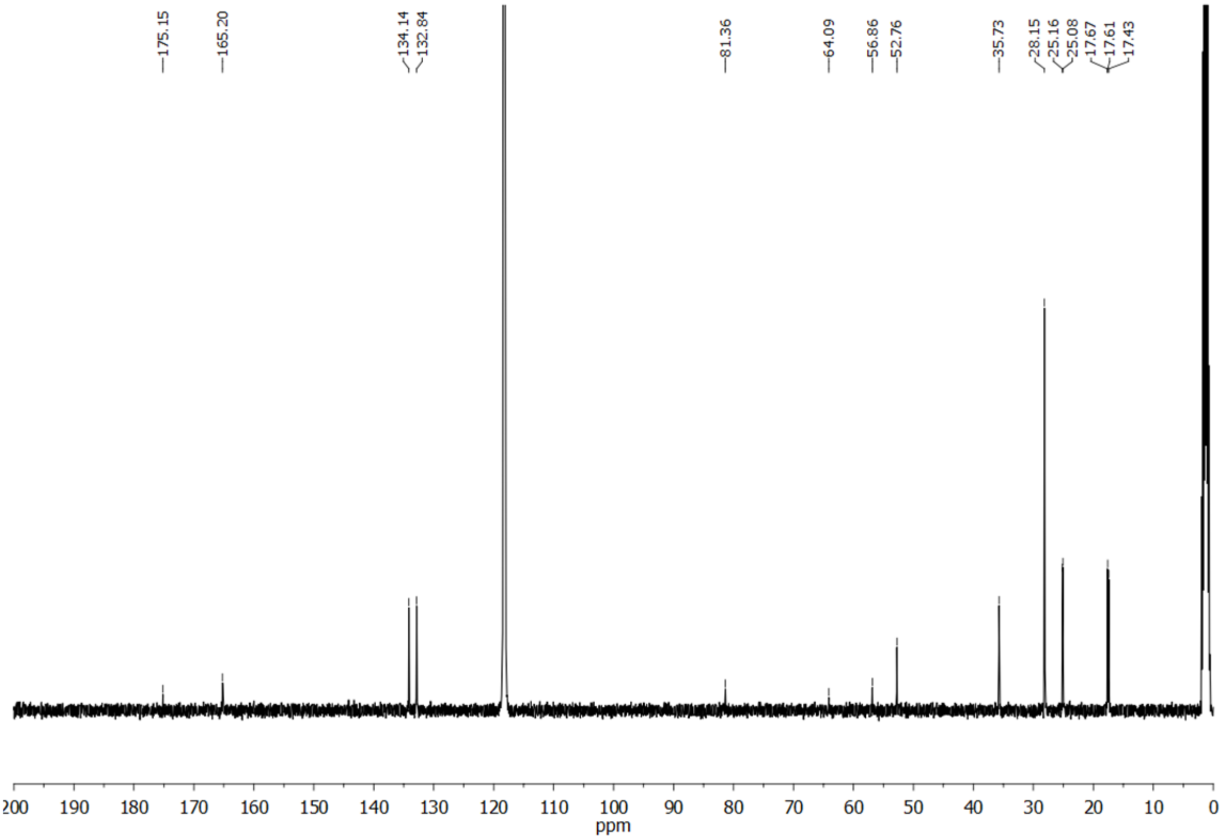


Figure S35. ^{13}C NMR (101 MHz) spectra of **3c** in CD_3CN.

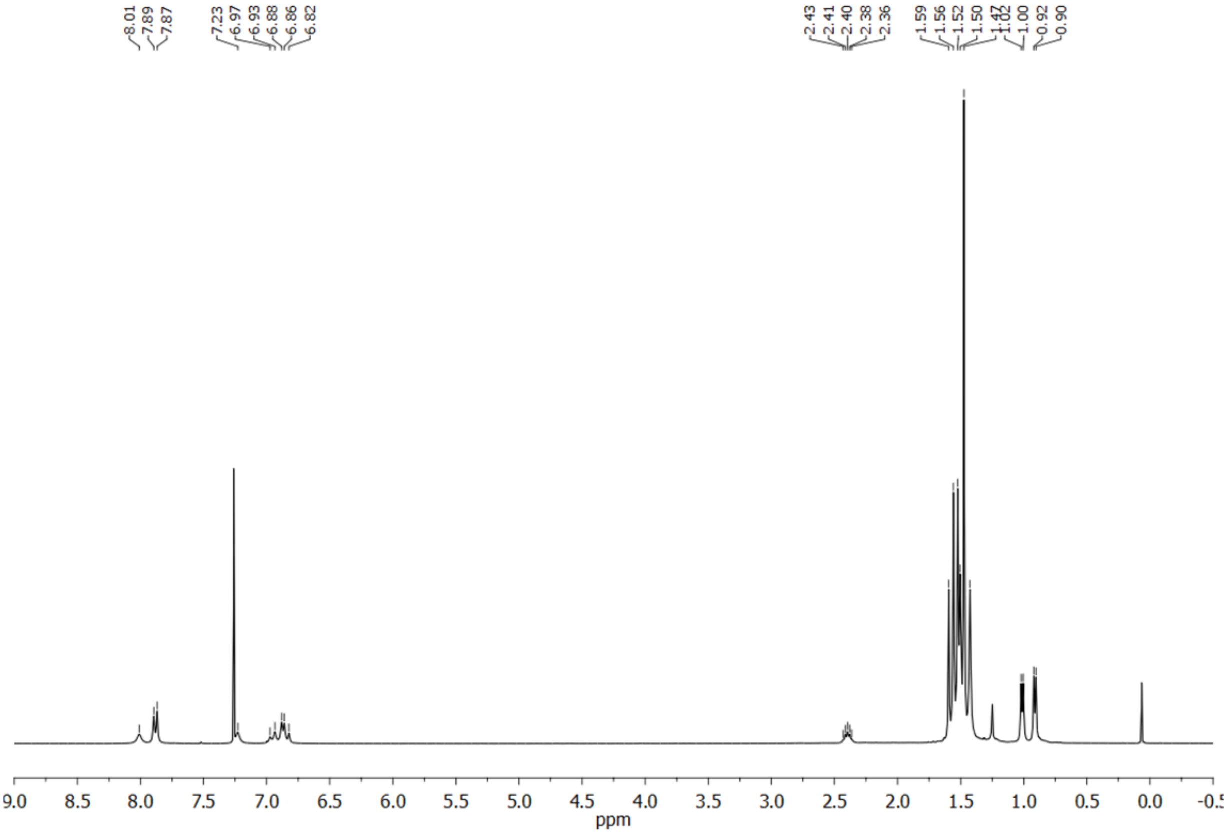


Figure S36. ^{1}H NMR (500 MHz) spectra of **4a** in $CDCl_3$.

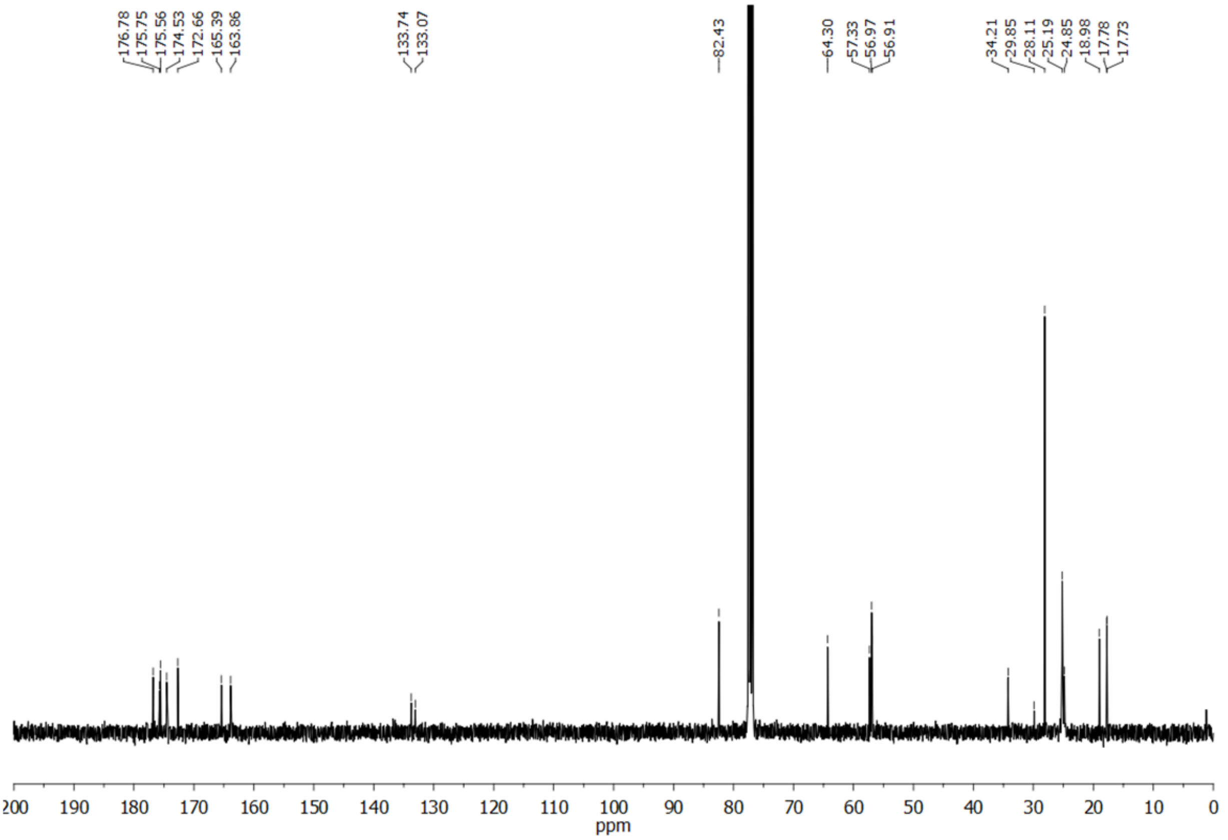


Figure S37. ^{13}C NMR (101 MHz) spectra of **4a** in $CDCl_3$.

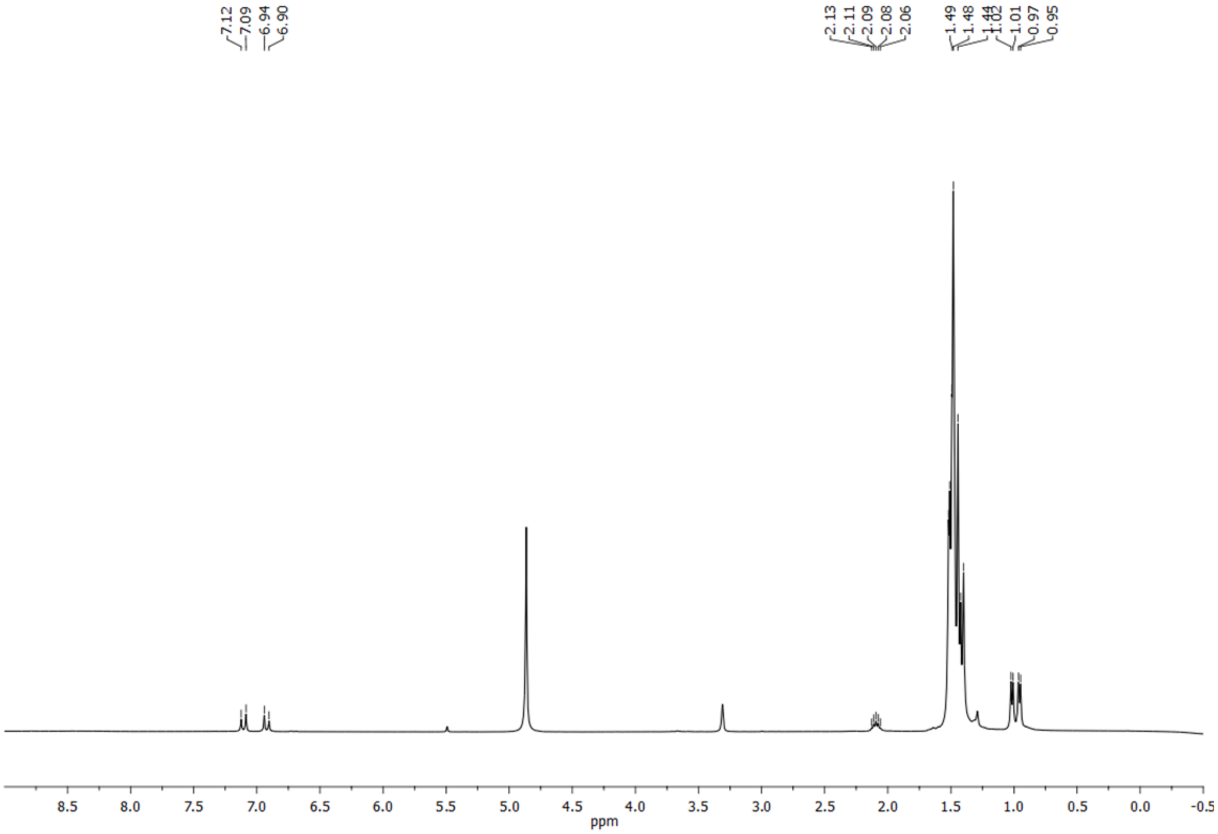


Figure S38. ^{1}H NMR (400 MHz) spectra of **4b** in MeOD.

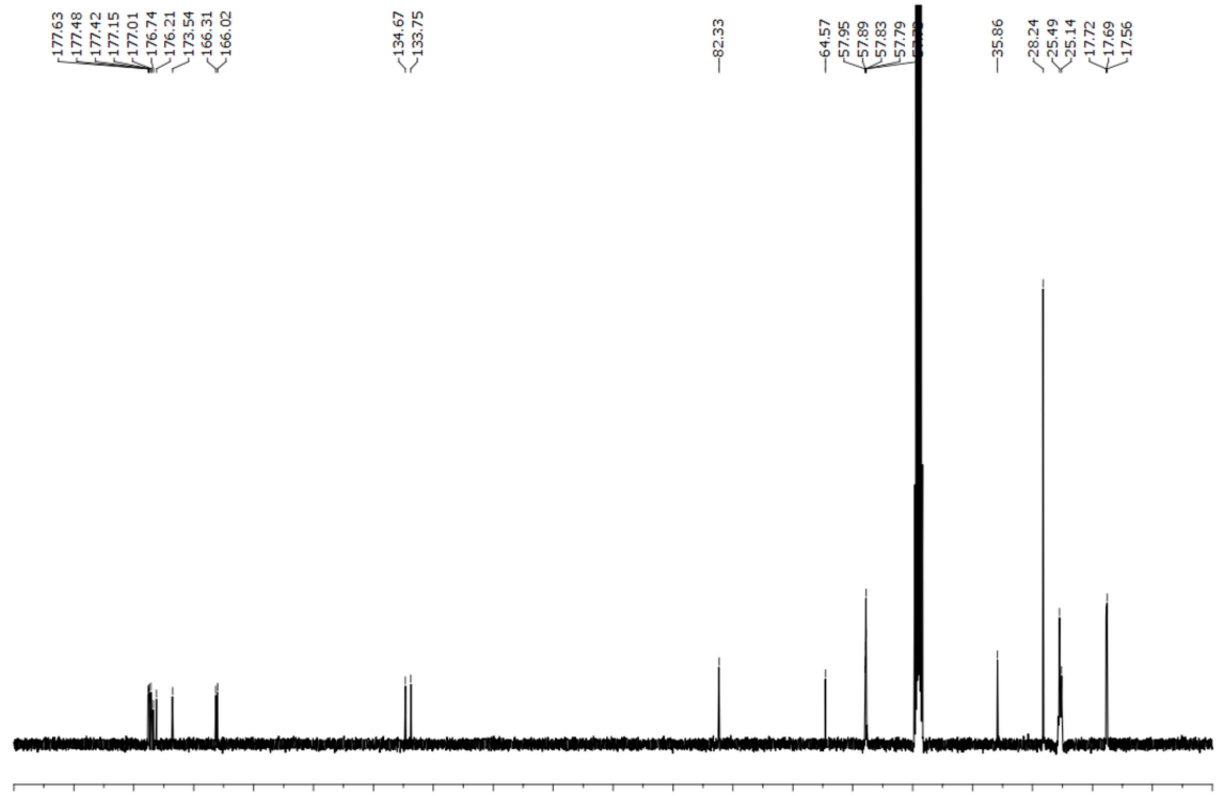


Figure S39. ^{13}C NMR (101 MHz) spectra of **4b** in MeOD.

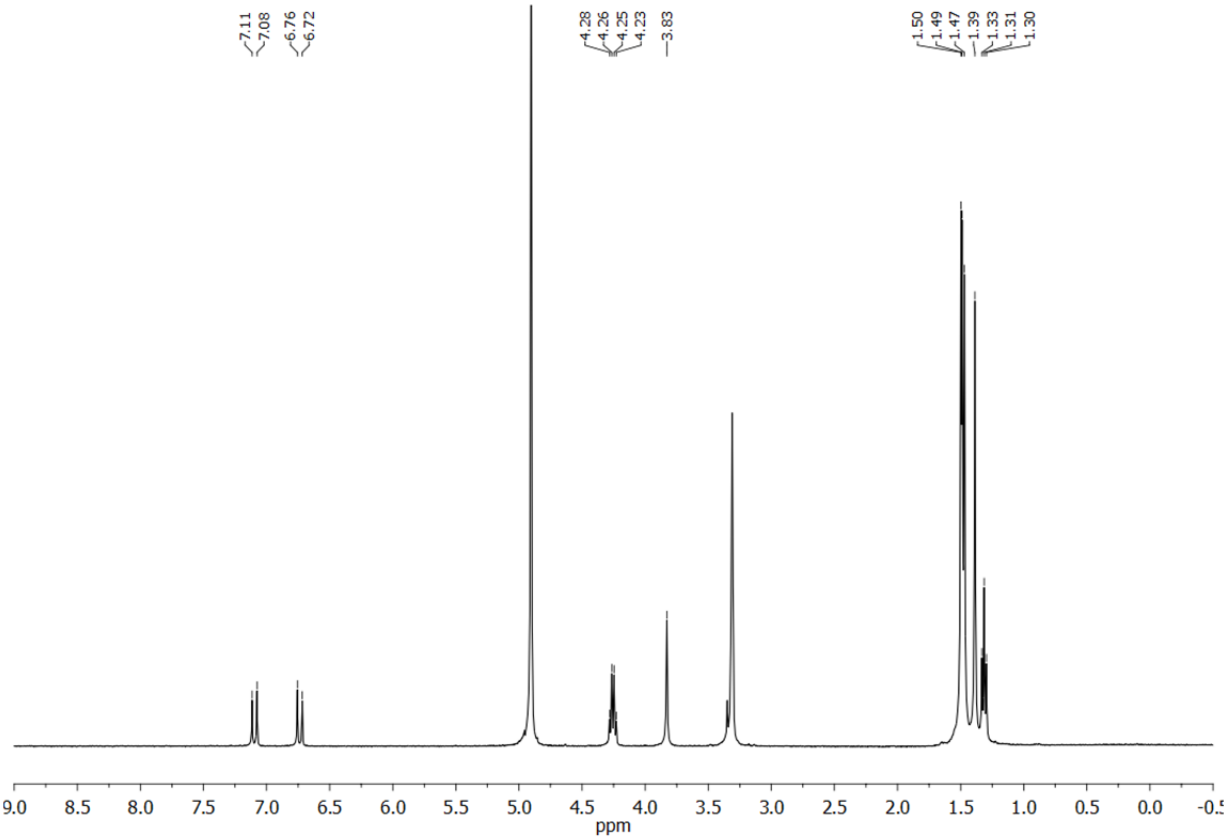


Figure S40. ^{1}H NMR (400 MHz) spectra of **16** in MeOD.

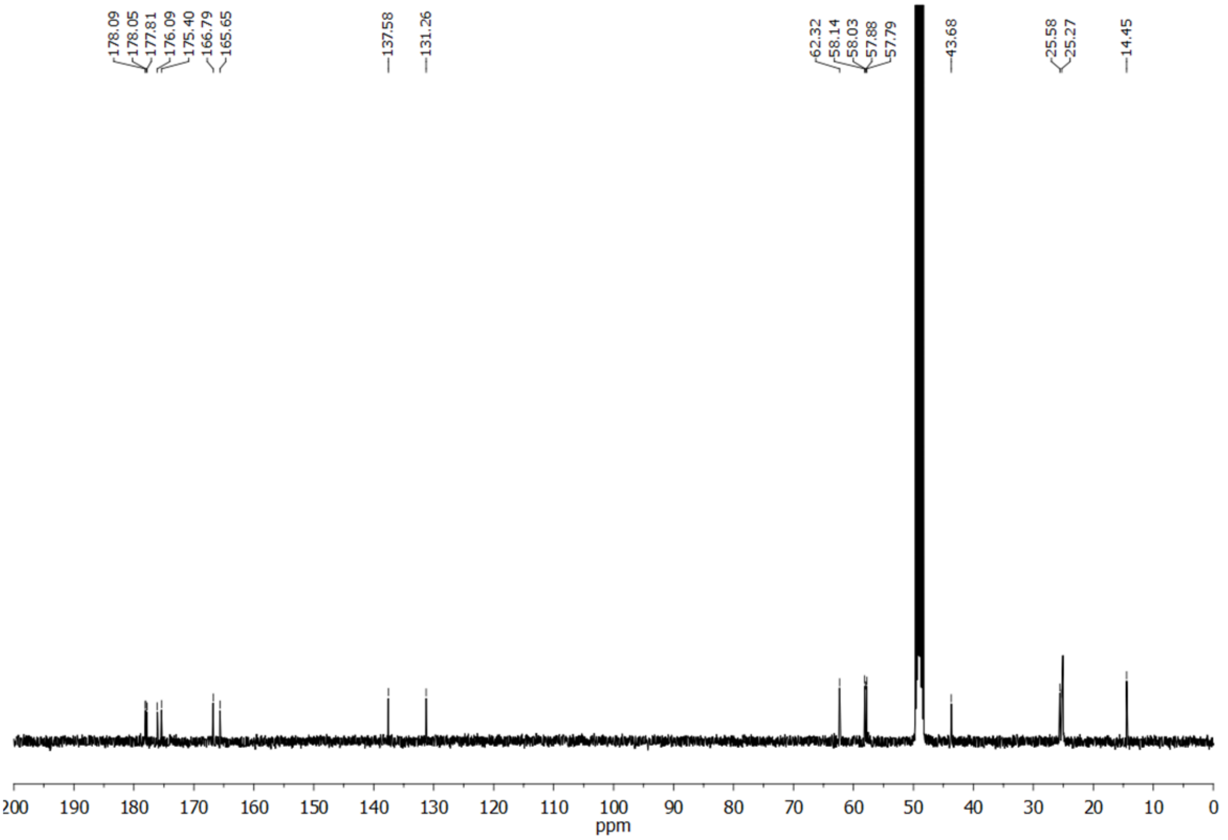


Figure S41. ^{13}C NMR (101 MHz) spectra of **16** in MeOD.

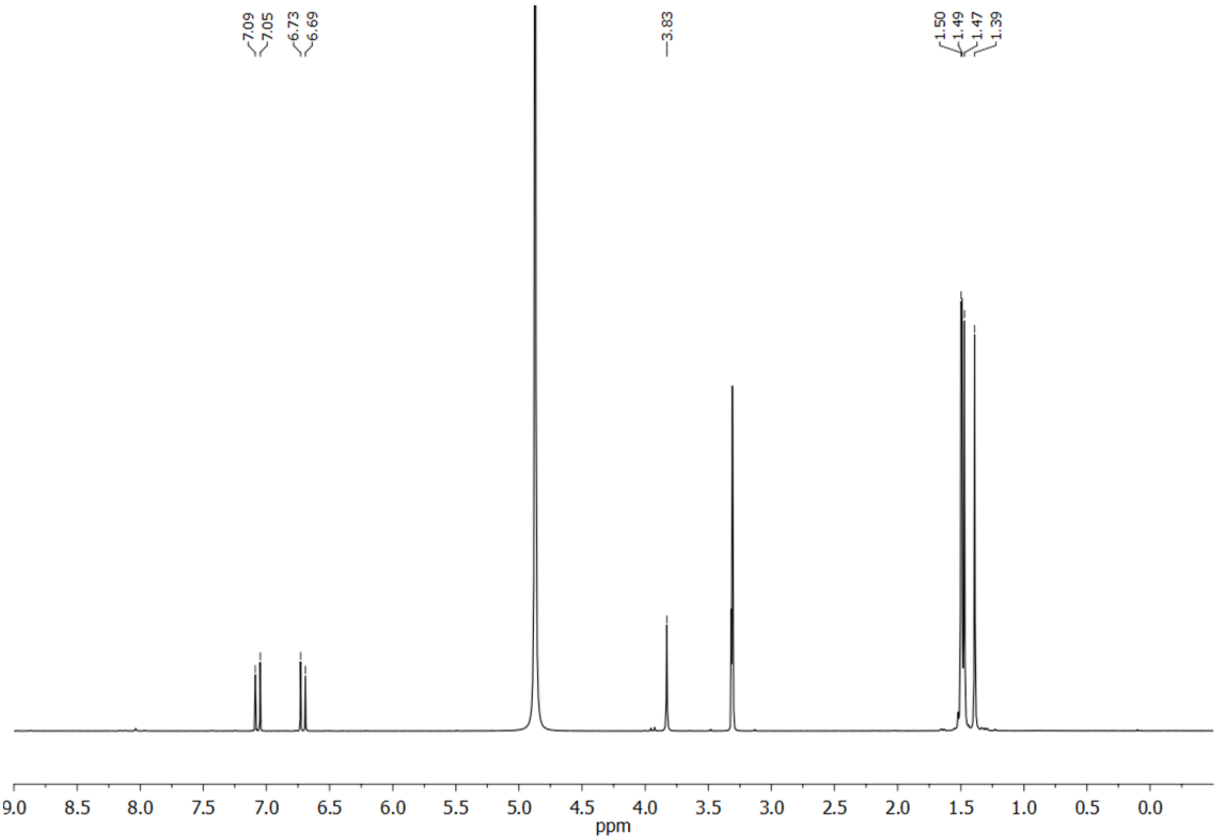


Figure S42. ^{1}H NMR (400 MHz) spectra of **10** in MeOD.

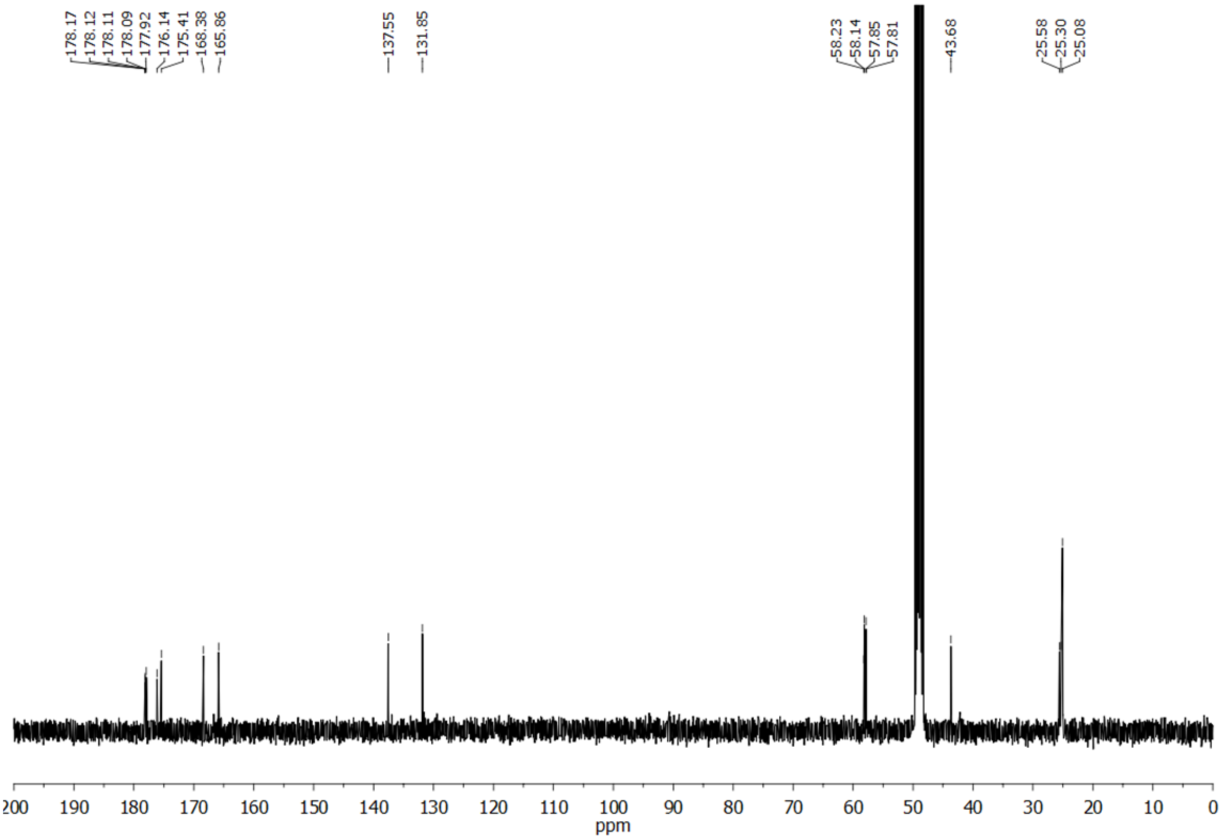


Figure S43. ^{13}C NMR (101 MHz) spectra of **10** in MeOD.

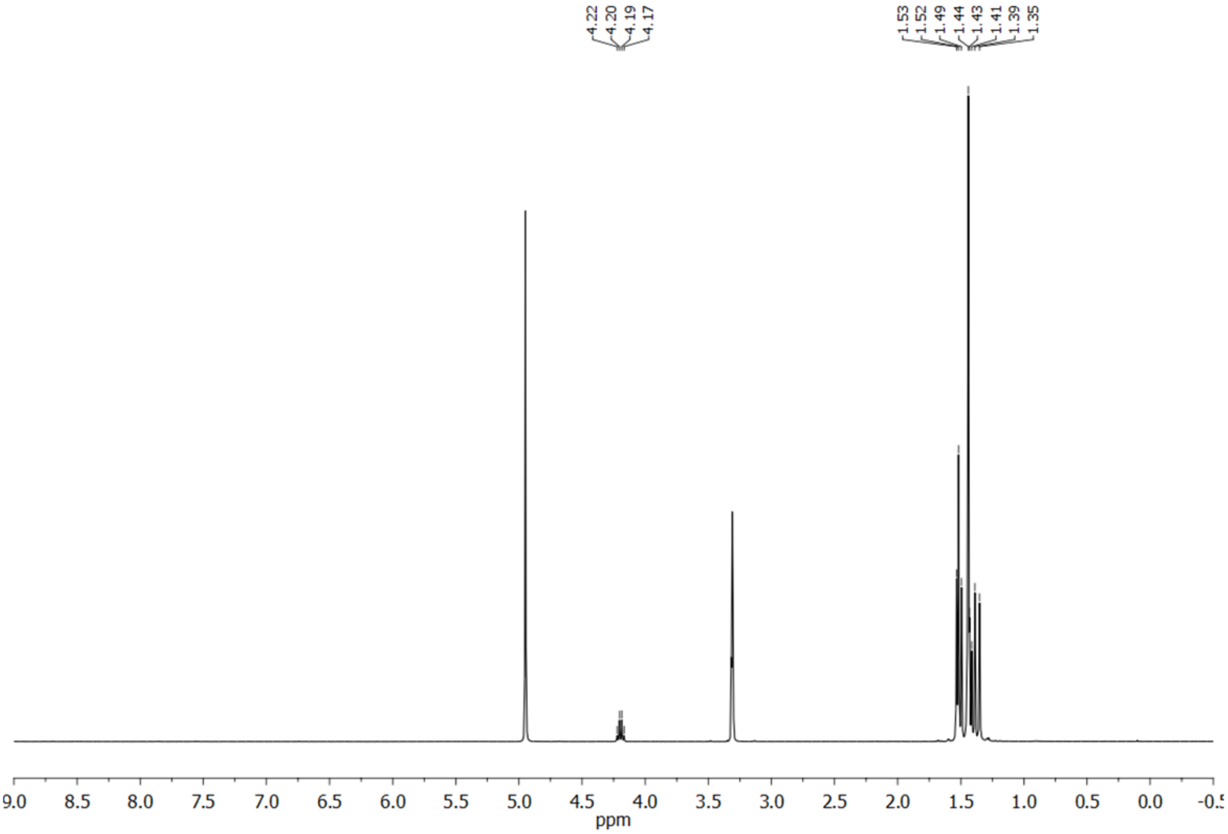


Figure S44. ^{1}H NMR (400 MHz) spectra of **17** in MeOD.

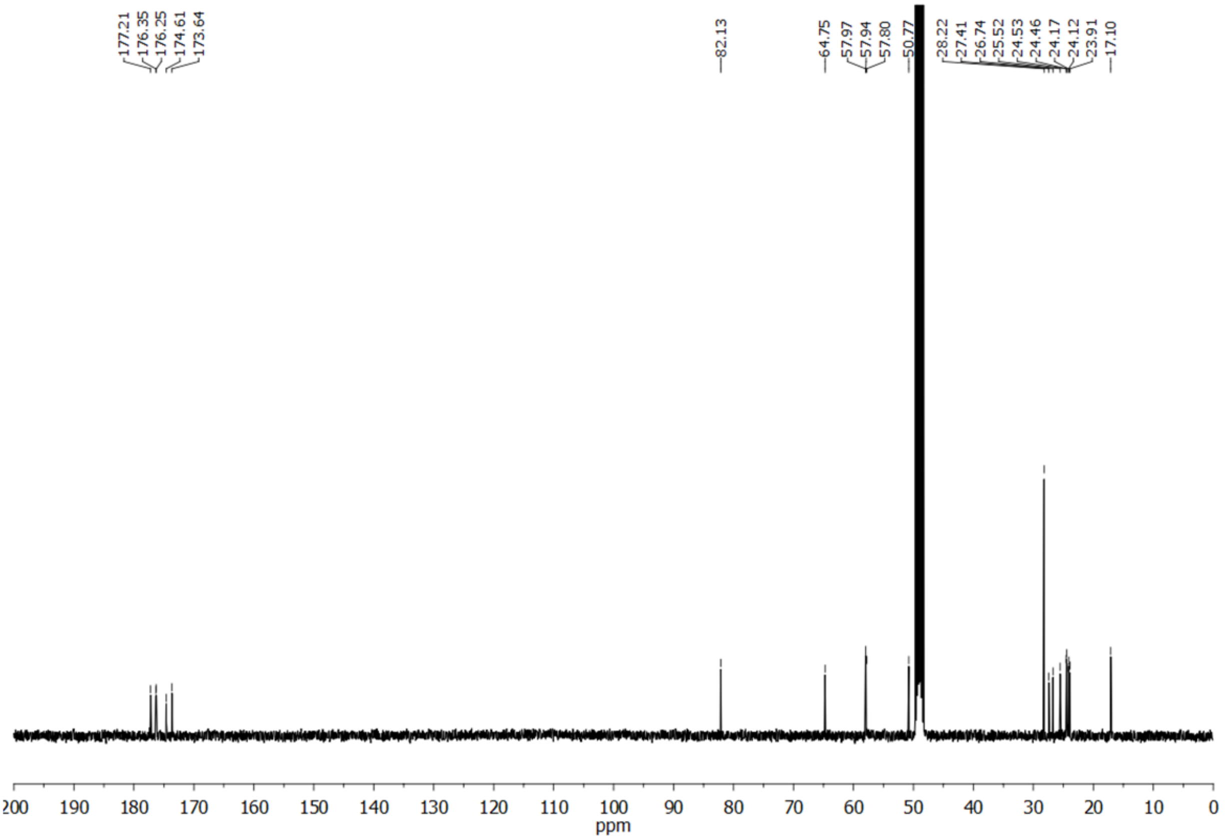


Figure S45. ^{13}C NMR (101 MHz) spectra of **17** in MeOD.

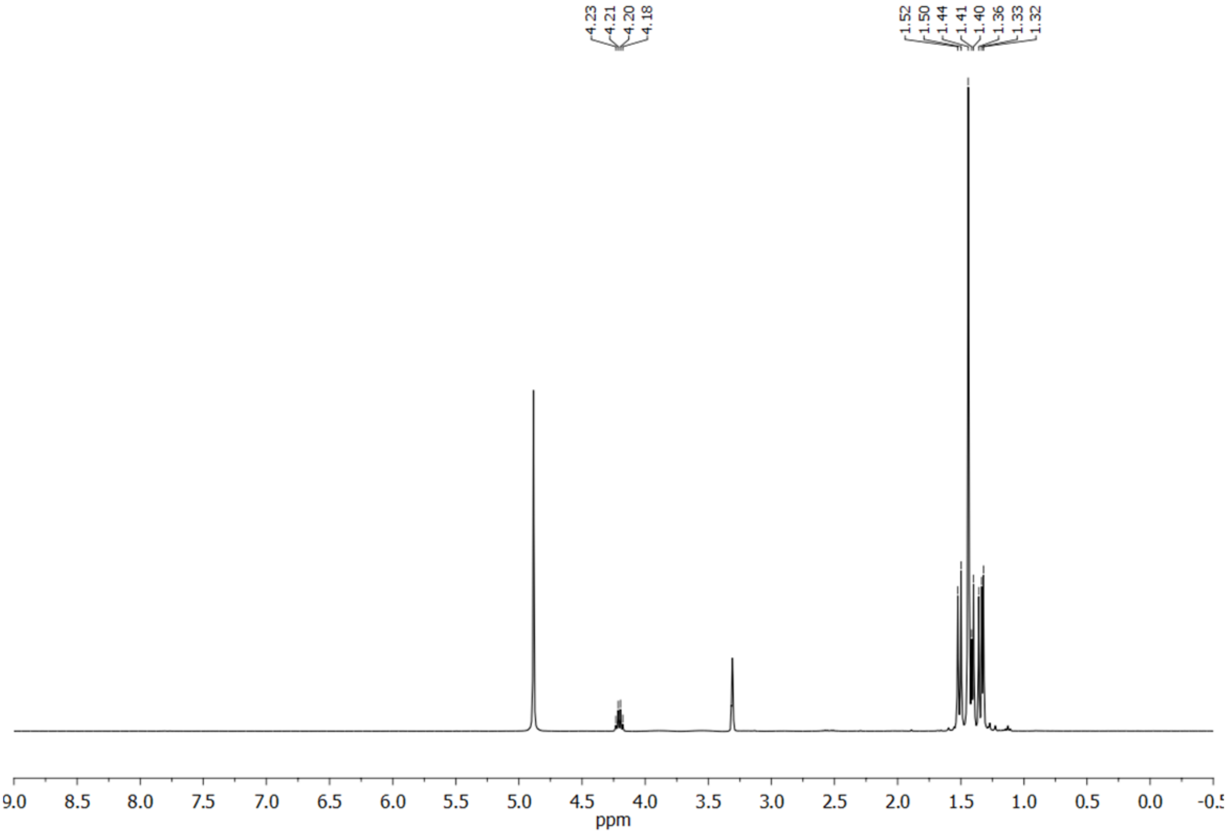


Figure S46. ^{1}H NMR (400 MHz) spectra of **11** in MeOD.

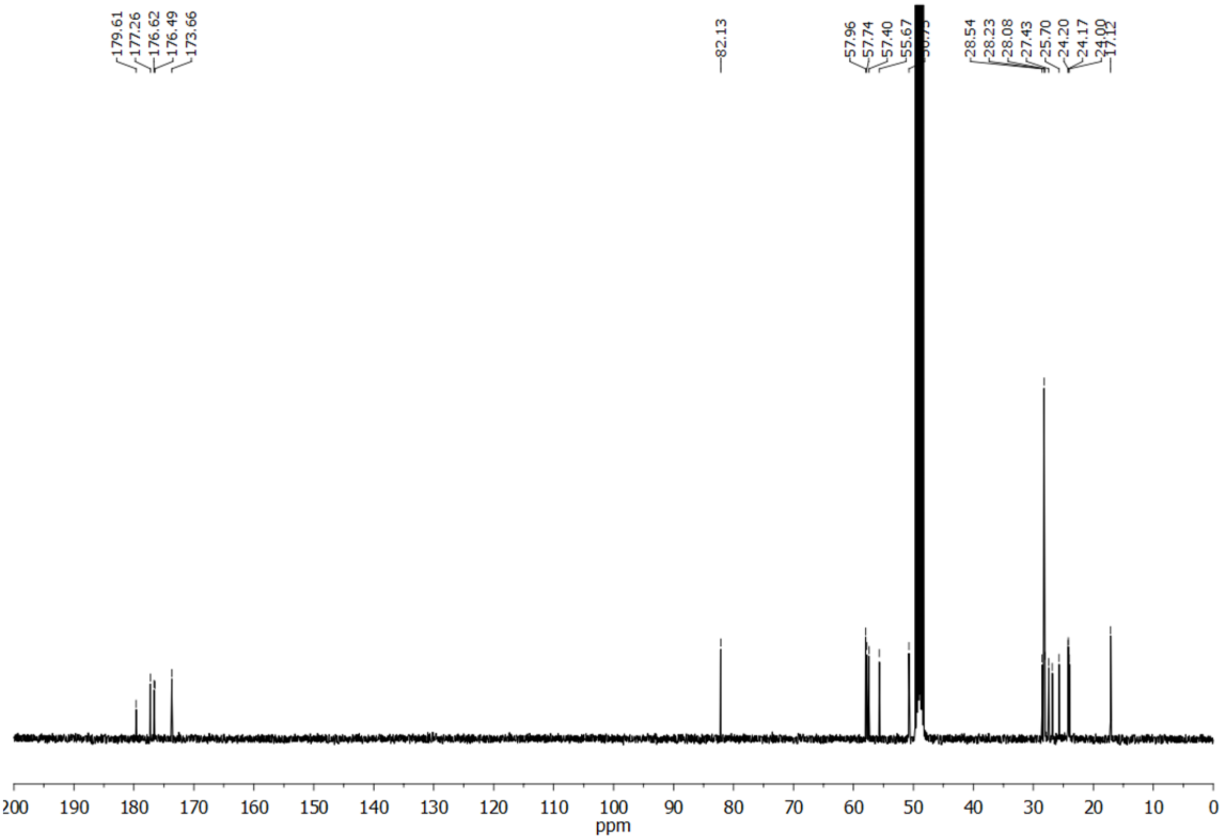


Figure S47. ^{13}C NMR (101 MHz) spectra of **11** in MeOD.

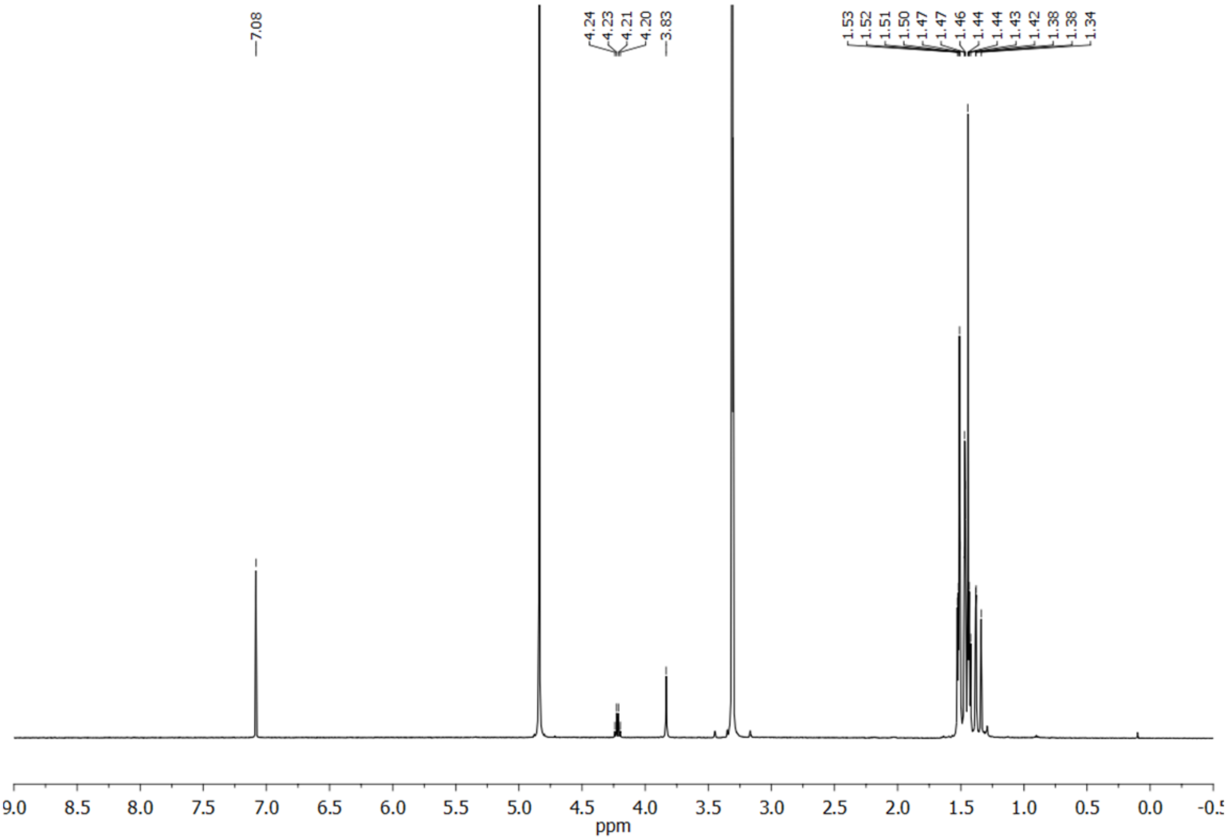


Figure S48. ^{1}H NMR (500 MHz) spectra of **12** in MeOD.

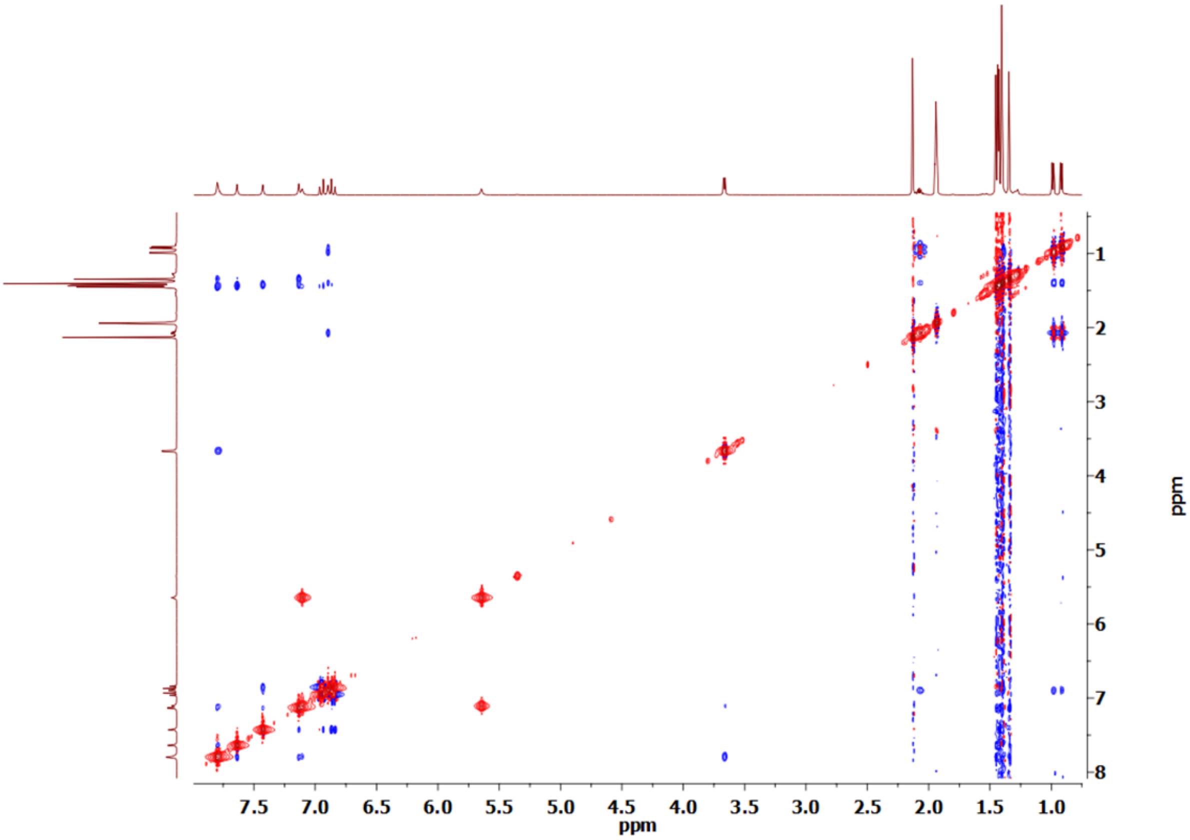


Figure S49. 2D NOESY NMR spectrum of **2a** (500 MHz in CD_3CN).

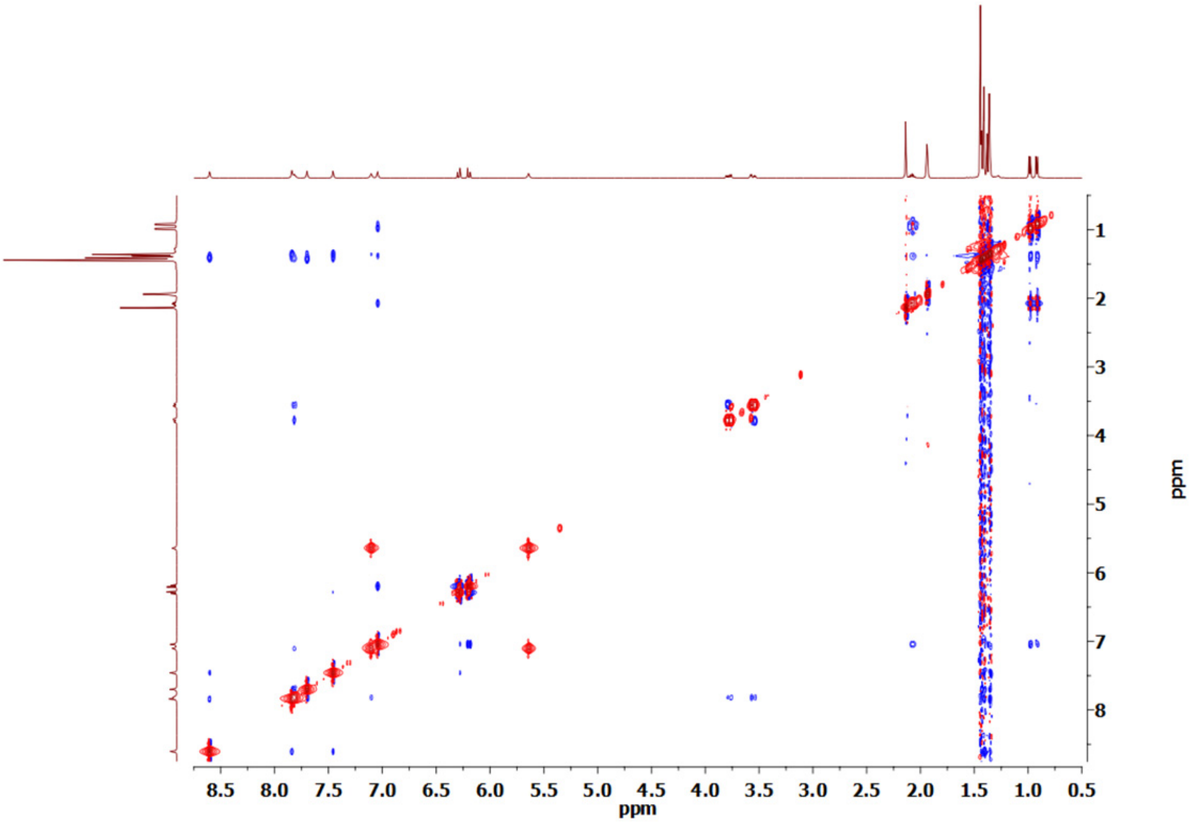


Figure S50. 2D NOESY NMR spectrum of **3a** (500 MHz in CD_3CN).

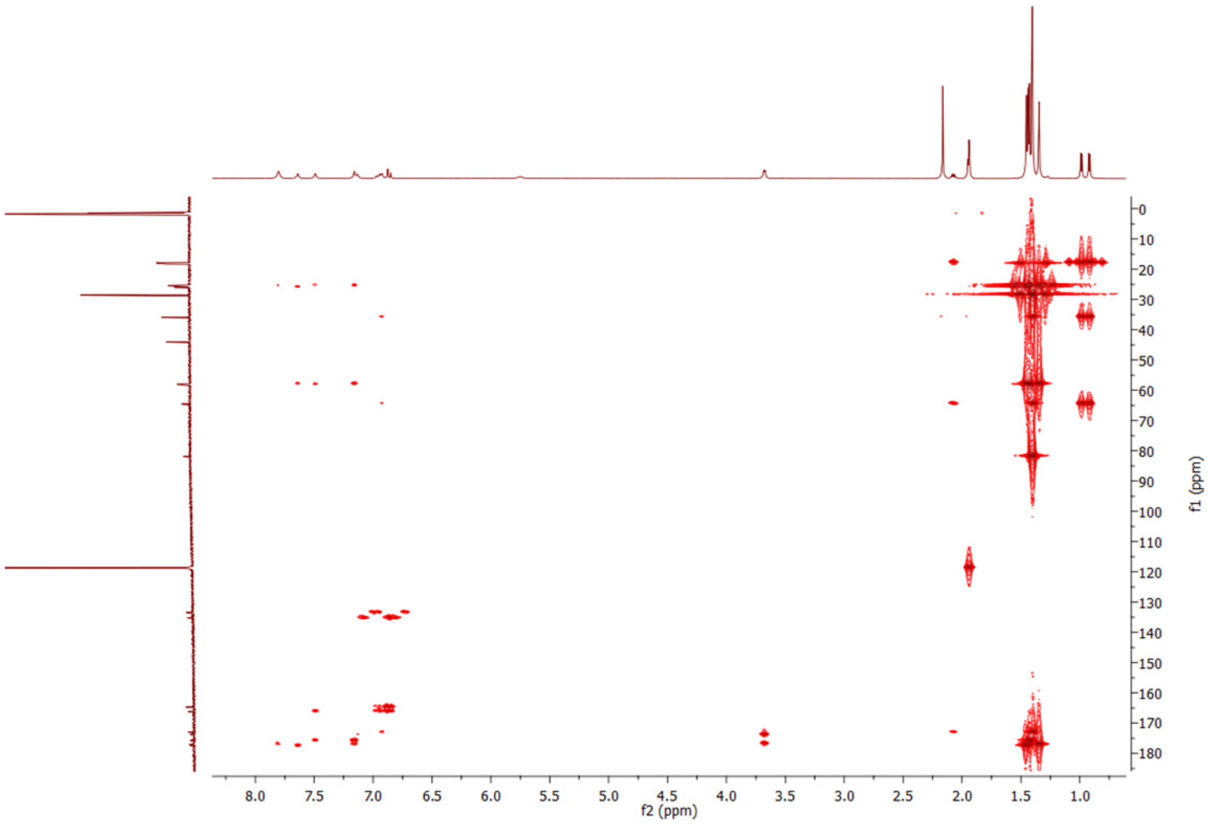


Figure S51. HMBC spectrum of **2a** (600 MHz in CD_3CN).

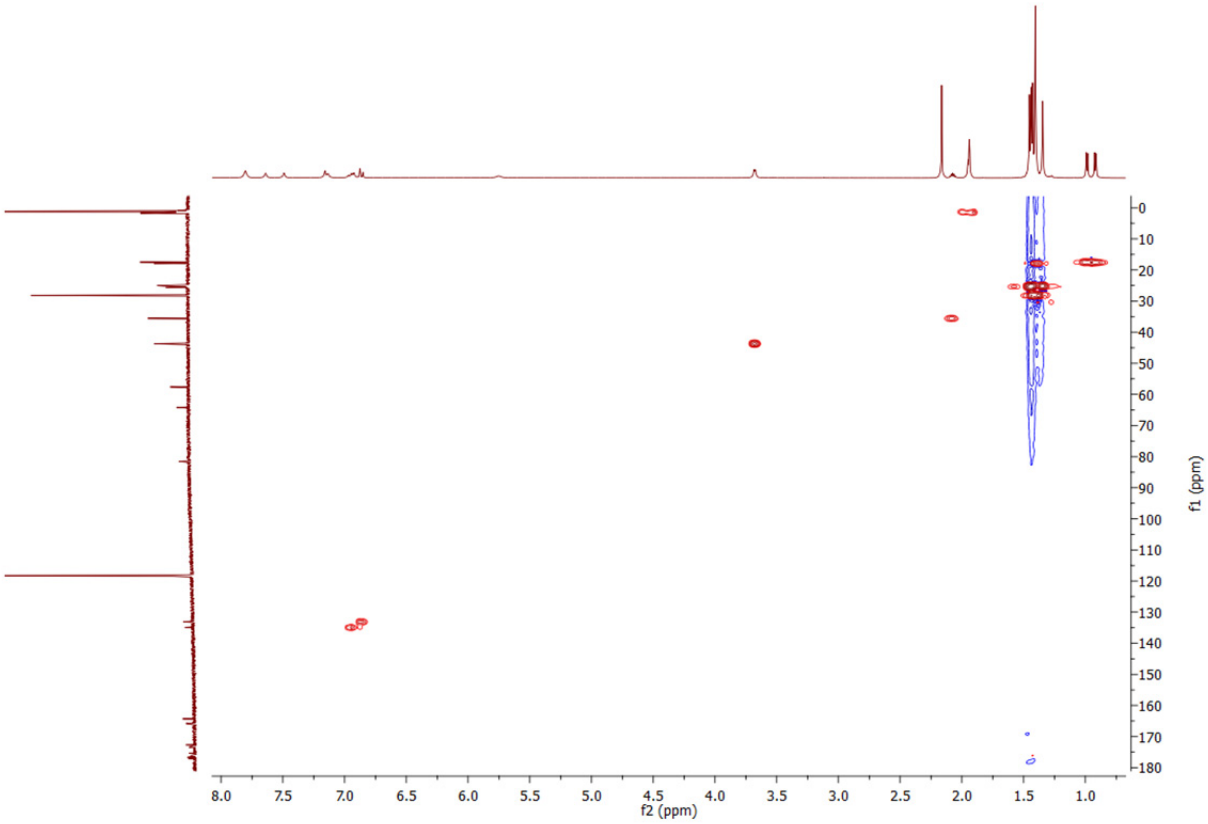


Figure S52. HMQC spectrum of **2a** (600 MHz in CD_3CN).

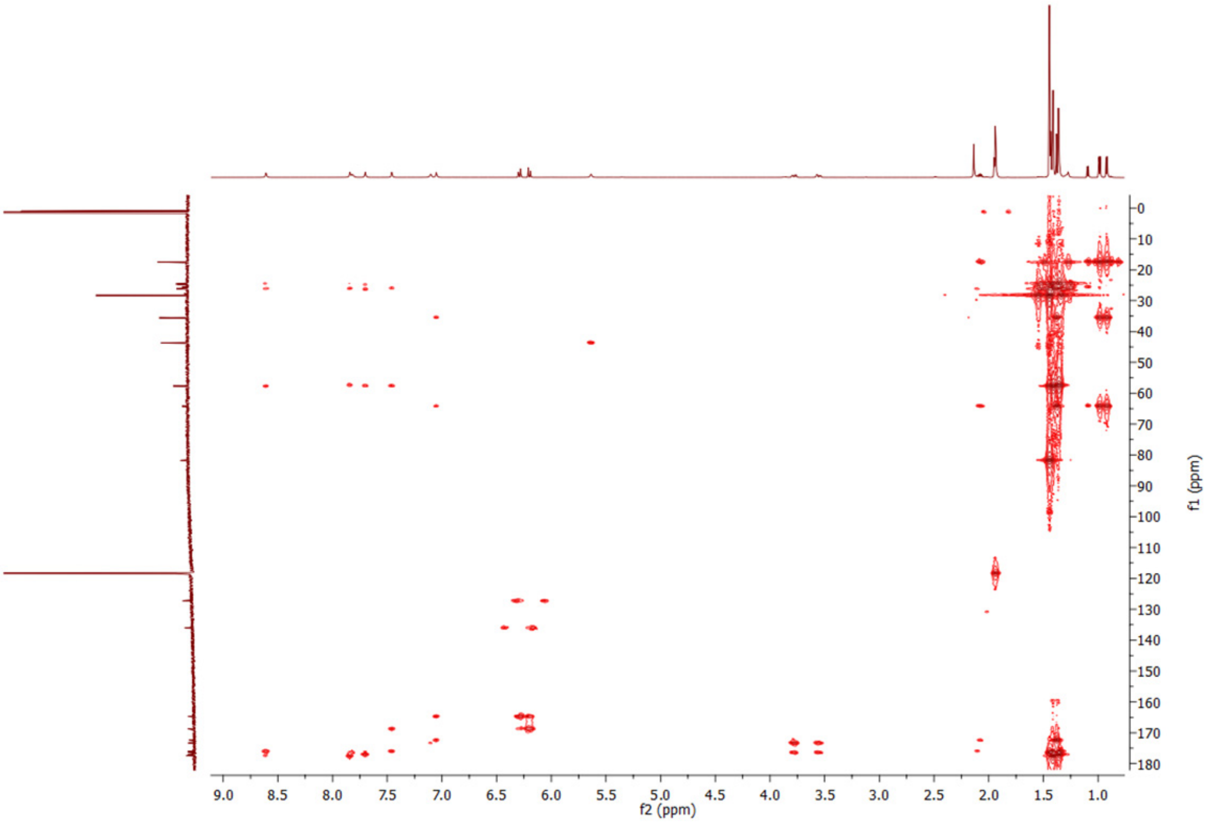


Figure S53. HMBC spectrum of **3a** (600 MHz in CD_3CN).

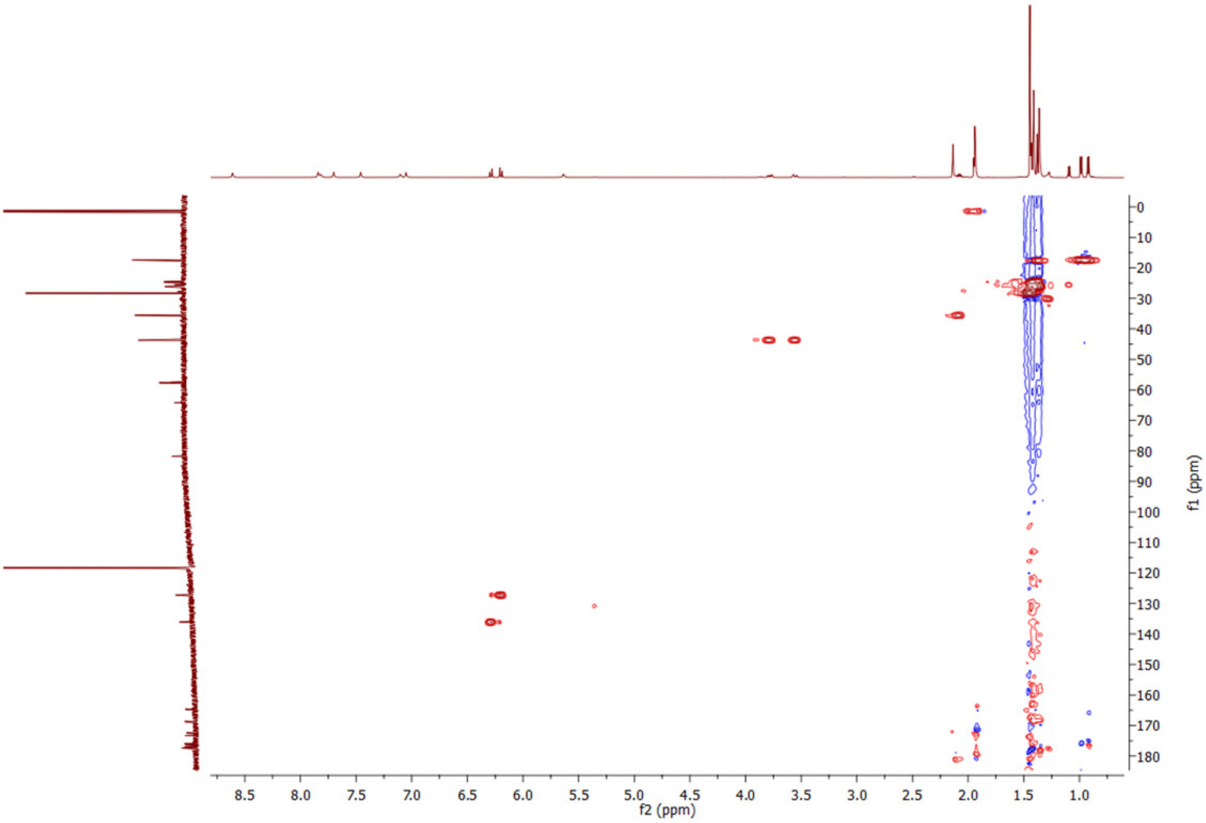


Figure S54. HMQC spectrum of **3a** (600 MHz in CD_3CN).

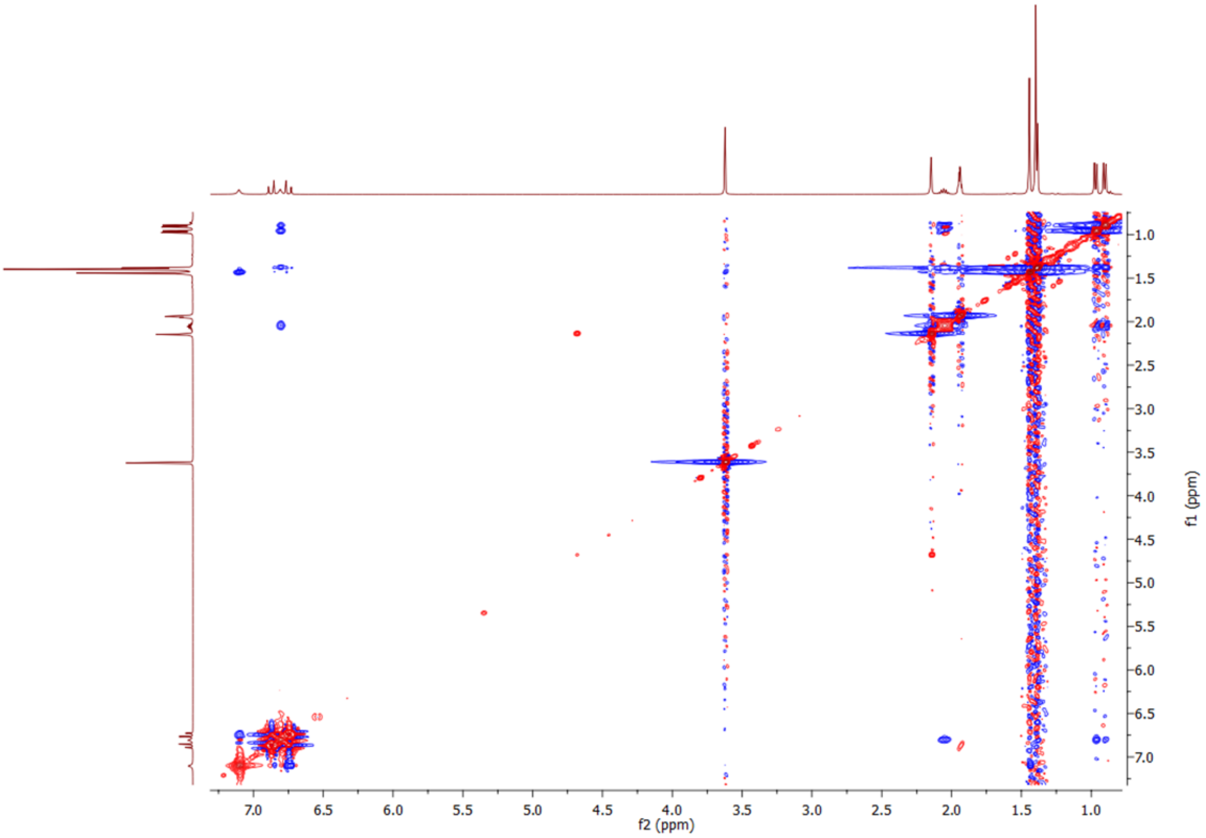


Figure S55. 2D NOESY NMR spectrum of **2c** (400 MHz in CD_3CN).

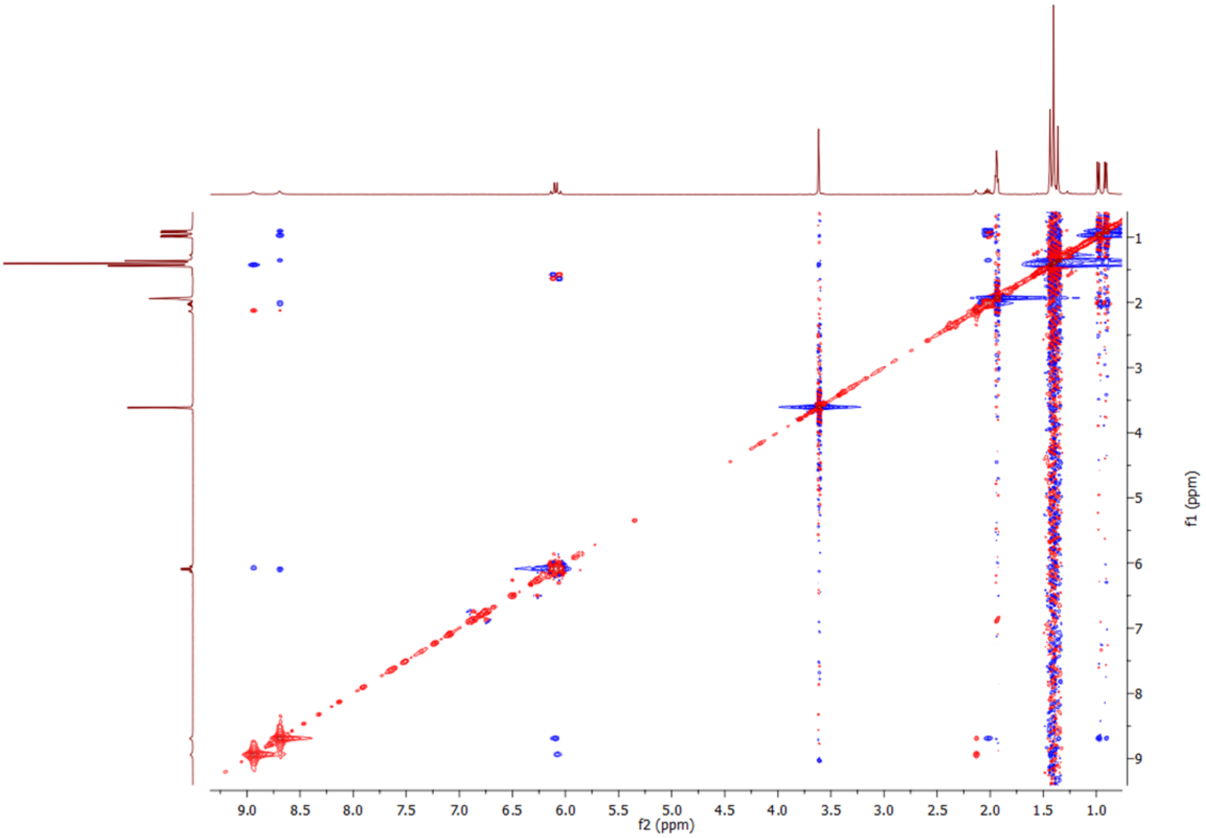


Figure S56. 2D NOESY NMR spectrum of **3c** (400 MHz in CD_3CN).

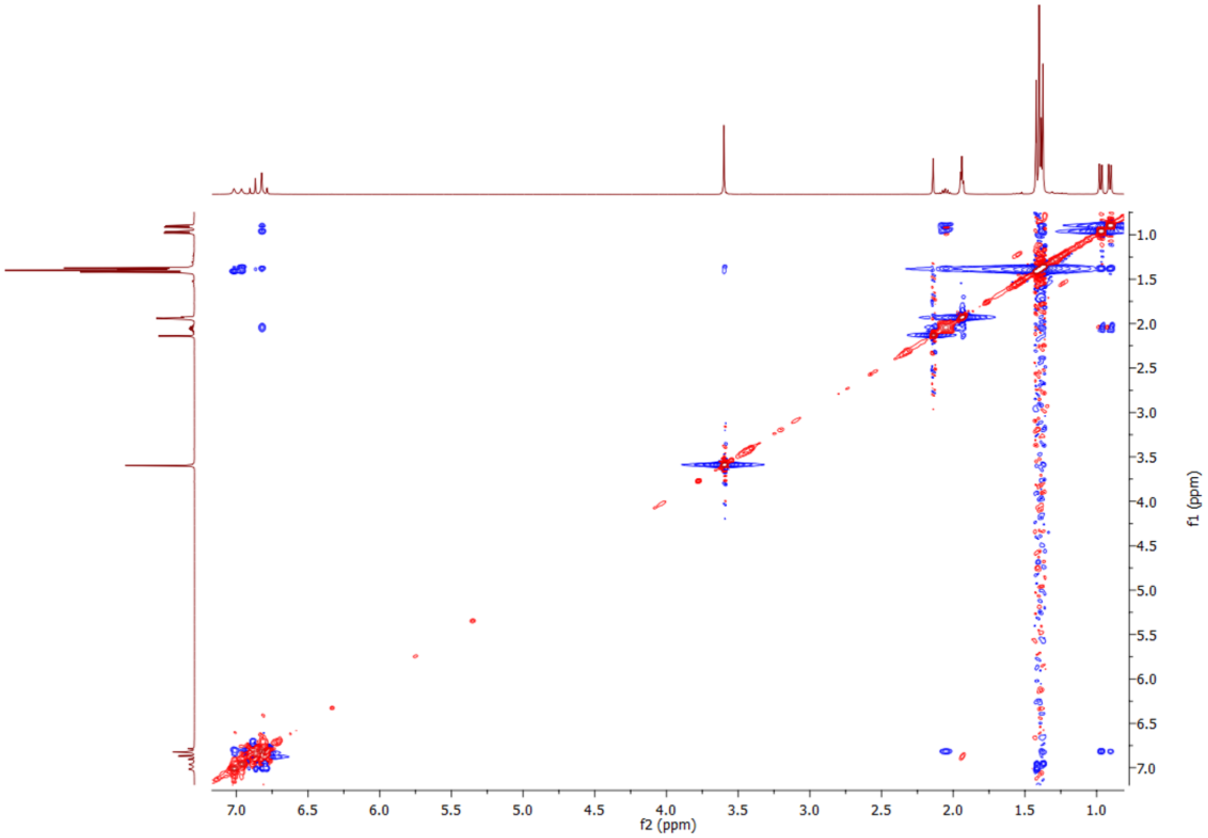


Figure S57. 2D NOESY NMR spectrum of **2d** (400 MHz in CD_3CN).

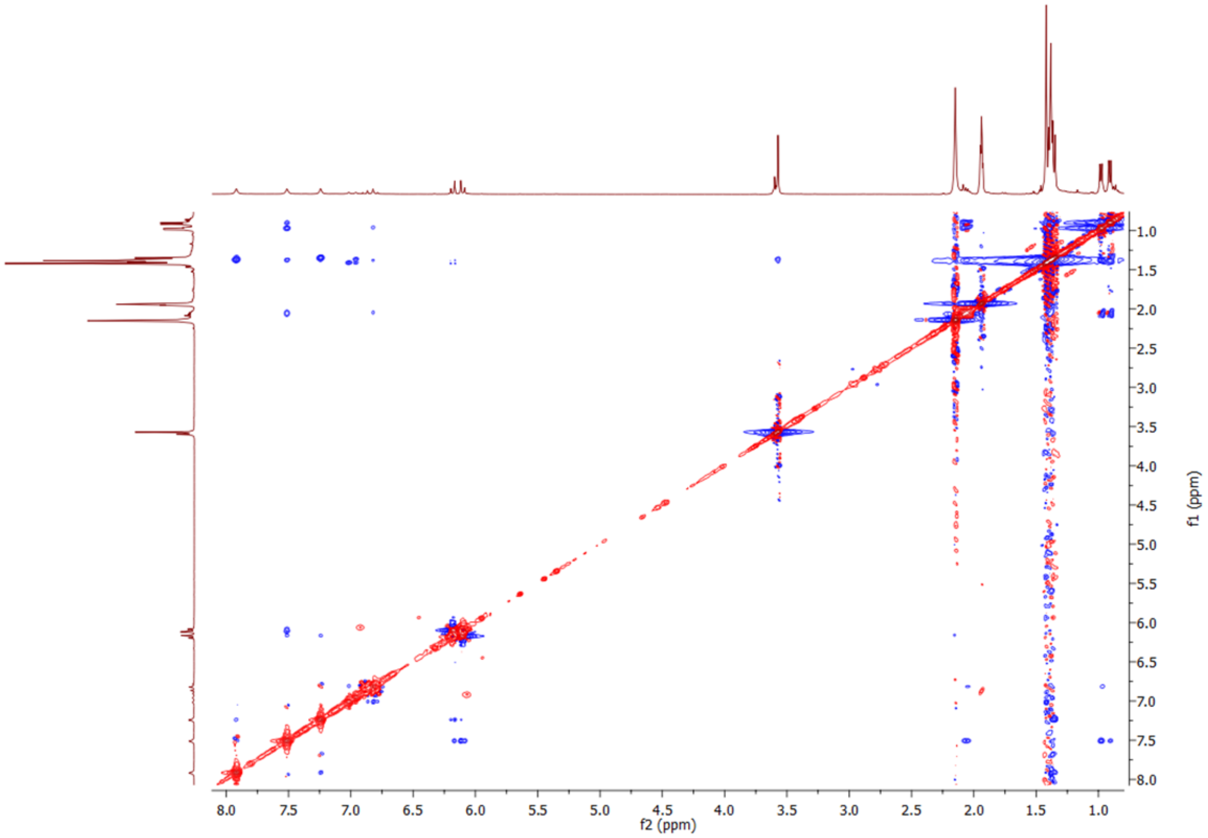


Figure S58. 2D NOESY NMR spectrum of **2d** after irradiation (400 MHz in CD_3CN).

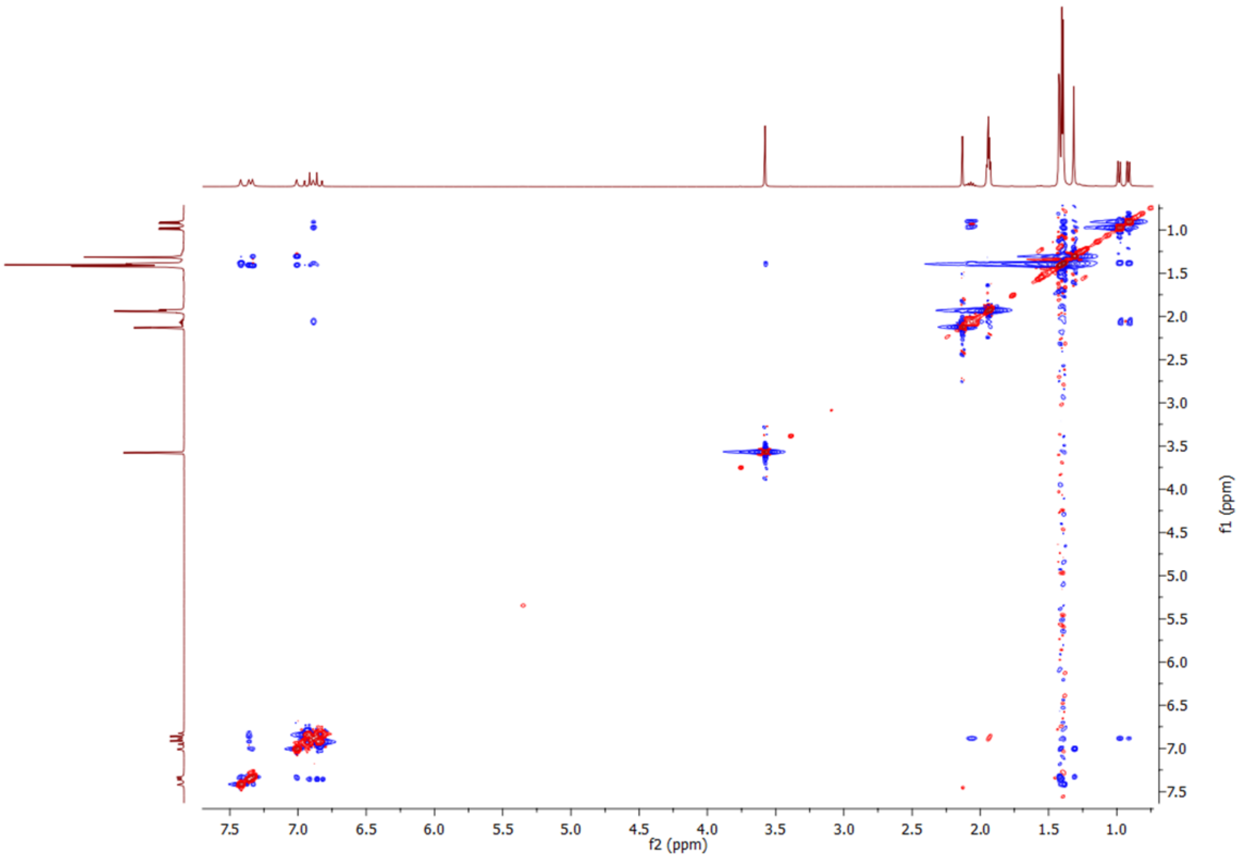


Figure S59. 2D NOESY NMR spectrum of **2e** (400 MHz in CD_3CN).

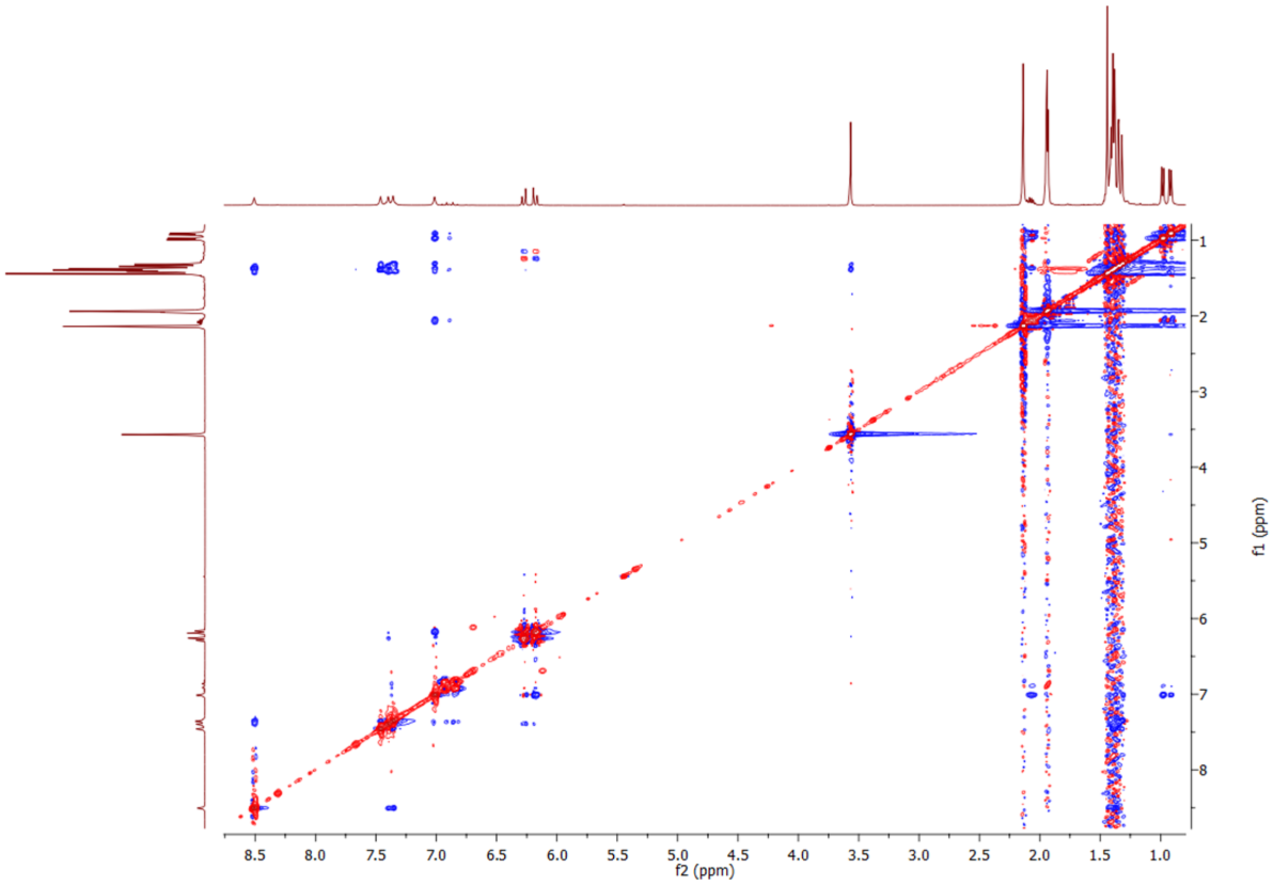


Figure S60. 2D NOESY NMR spectrum of **2e** after irradiation (400 MHz in CD_3CN).

Supporting References

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