Electronic Supplementary Material (ESI) for ChemComm.

Cooperative 5- and 10-membered ring interactions in the 10-helix folding of oxetin homo-oligomers

Sherif S. Ragab,[†,‡] Ahmad F. Kassir,[†] Régis Guillot,[‖] Thomas Boddaert[†] and David J. Aitken[†,*]

[†] CP3A Organic Synthesis Group, ICMMO, CNRS UMR 8182, Université Paris Sud, Université Paris Saclay, 15 rue Georges Clemenceau, 91405 Orsay Cedex, France. Fax: (+33)-1-69-15-62-78; phone: (+33)-1-69-15-32-38; e-mail: david.aitken@u-psud.fr.

[‡] Photochemistry Department, Chemical Industries Research Division, National Research Centre, P.O. Box 12622, Dokki, Giza, Egypt.

[‖] Services Communs, ICMMO, CNRS UMR 8182, Université Paris Sud, Université Paris Saclay, 15 rue Georges Clemenceau, 91405 Orsay Cedex, France.

Supporting Information

1. General Information

All chemical reagents and solvents were of commercial grade and were used without further purification, with the exception of MeCN which was distilled on P_2O_5, CH_2Cl_2 which was dried over activated alumina, and THF which was distilled from sodium/benzophenone. The starting compound Boc-[(*2R,3S*)-oxetin]-OH was prepared according to the literature procedure.[S1]

Flash chromatography was performed on columns of silica gel (35-70 µm). Analytical thin-layer chromatography (TLC) was performed on commercial 0.25 mm silica gel plates which were visualized by UV fluorescence at 254 nm then revealed using a phosphomolybdic acid solution (10% in EtOH) or a ninhydrin solution (0.3% in n-BuOH). Retention factors (R_f) are given for such TLC analyses. Melting points were obtained in open capillary tubes and are uncorrected. Optical rotations were measured using a 10 cm quartz cell; values for $[\alpha]_D^T$ were obtained with the D-line of sodium at the indicated temperature *T*, using solutions of concentration (c) in units of $g \cdot dL^{-1}$. Fourier-transform infrared spectra were recorded for neat samples using an ATR diamond accessory; maximum absorbances (ν) are given in cm^{-1}. Nuclear magnetic resonance (NMR) data were acquired on a spectrometer operating at 600/400/360/300/250 MHz for ^1H, at 100/90/75/63 MHz for ^{13}C. Chemical shifts (δ) are reported in ppm with respect to tetramethylsilane (δ = 0 ppm). Splitting patterns for ^1H NMR and ^{13}C NMR signals are designated as: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), or m (multiplet). Coupling constants (*J*) are reported in Hz. High-resolution mass spectrometry (HRMS) data were recorded using a spectrometer equipped with a positive mode an electrospray ionization source and a tandem Q-TOF analyzer.

Solution-state Fourier-transform infrared spectra (manuscript Fig. 3) were recorded for 10 mM solutions in $CHCl_3$ at 20 °C using NaCl solution cells of 1 cm path length.

For circular dichroism spectra (manuscript Fig. 4), samples were dissolved in an appropriate amount of HPLC-grade methanol to give stock solutions of known concentration (20 mM). Solutions for analysis were prepared by volumetric dilution to achieve sample solution of 0.2 mM in methanol. Data were collected at 20 °C in sample cells of 1 mm path length. Baseline spectra of pure solvent and were subtracted from the raw data. Observed ellipticity, θ_{obs} (mdeg), was converted into mean residue molar ellipticity, [θ] ($deg \cdot cm^2 \cdot dmol^{-1}$) using the equation $[\theta] = \frac{\theta_{obs}}{10 \times c \times n \times l}$, where c = concentration ($mol \cdot L^{-1}$), n = number of residues, l = cell path length (cm).

[S1] Kassir, A. F.; Ragab, S. S.; Nguyen, T. A. M.; Charnay-Pouget, F.; Guillot, R.; Scherrmann, M.-C.; Boddaert, T.; Aitken, D. J. *J. Org. Chem.* **2016**, *81*, 9983.

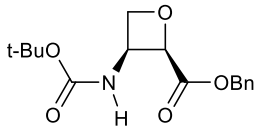
2. Synthetic procedures and product characterization

General procedure A for hydrogenolysis of benzyl esters

To a solution of Boc-[(*2R,3S*)-oxetin]$_n$-OBn (1.0 equiv.) in CH_2Cl_2 (13 mL/mmol) was added carefully the stated quantity of 10% Pd-C. The mixture was stirred under a H_2 atmosphere (rubber balloon) until the reaction was complete (TLC monitoring). The mixture was filtered through a Celite pad and washed with CH_2Cl_2. The filtrate was concentrated under reduced pressure to give the corresponding Boc-[(*2R,3S*)-oxetin]$_n$-OH, which was engaged in the next peptide coupling step without purification.

General procedure B for Boc group removal followed by peptide coupling

To a solution of Boc-[(2*R*,3*S*)-oxetin]$_n$-OBn (1.0 equiv.) in CH_2Cl_2 (10 mL/mmol) at 0 °C was added TFA (30 equiv.). After 3 h at room temperature, volatiles were evaporated under reduced pressure to leave the appropriate TFA salt. This material was dissolved in CH_2Cl_2 (2 mL) and added directly to a solution of Boc-[(*2R,3S*)-oxetin]$_m$-OH (1 equiv.) in DMF (2 mL) under an argon atmosphere. *N,N*-Diisopropylethylamine (DIPEA) (3.5 equiv.) and pentafluorophenyl diphenylphosphinate (FDPP) (1.2 equiv.) were added and the mixture was stirred for at 30 min at 0 °C then the solution was stirred at room temperature for 36 h. The solution was concentrated under reduced pressure then DMF was evaporated under reduced pressure. The residue was purified by flash column chromatography to give the corresponding peptide, Boc-[(2*R*,3*S*)-oxetin]$_{m+n}$-OBn.

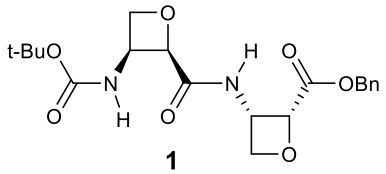


I

Boc-[(*2R,3S*)-oxetin]-OBn I:

To a solution of Boc-[(*2R,3S*)-oxetin]-OH (1.30 g, 6.0 mmol) and benzyl alcohol (1.8 mL, 18.0 mmol) in CH_2Cl_2 (100 mL) at 0 °C, were added 4-dimethylaminopyridine (DMAP) (73 mg, 0.6 mmol) and *N,N'*-dicyclohexylcarbodiimide (DCC) (1.48 g, 7.2 mmol). The reaction mixture was stirred for 1 h at 0 °C then for 24 h at room temperature. After filtration, the solvent was removed under reduced pressure and EtOAc (30 ml) was added. The organic layer was washed successively with brine (20 ml), 1 M HCl (20 ml), brine (20 ml), 5% $NaHCO_3$ (20 ml), and brine (20 ml). The organic solution was then dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The crude product was then purified by flash column chromatography (EtOAc/PE; 3/7) to give Boc-[(*2R,3S*)-oxetin]-OBn **I** (1.70 g, 92%) as a white solid.

R_f (EtOAc/PE; 1/4): 0.26; **Mp** 111-112 °C; **$[\alpha]_D^{25}$** –38 (*c* 0.50, $CHCl_3$); **IR** υ 3355, 2932, 1742, 1680, 1524, 1334; **^{1}H NMR (300 MHz, $CDCl_3$)** δ 7.36-7.28 (m, 5H), 5.35 (d, *J* = 9 Hz, 1H), 5.30-5.10 (m, 2H), 5.26 (A of AB quartet, *J* = 12.2 Hz, 1H), 5.17 (B of AB quartet, *J* = 12.2 Hz, 1H), 4.88 (t, *J* = 7.0 Hz, 1H), 4.65 (t, *J*= 6.7 Hz, 1H), 1.36 (s, 9H); **^{13}C NMR (75 MHz, $CDCl_3$)** δ 170.05 (C), 154.41 (C), 135.21 (C), 128.78 (2CH), 128.67 (2CH), 128.55 (CH), 84.29 (CH), 80.43 (C), 78.52 (CH_2), 67.17 (CH_2), 46.85 (CH), 28.29 ($3CH_3$); **HRMS** m/z: $[M + Na]^+$ Calcd. for $C_{16}H_{21}NNaO_5$ 330.1317; Found 330.1318.

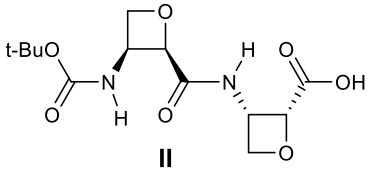


1

Boc-[(*2R,3S*)-oxetin]$_2$-OBn 1:

To a solution of Boc-[(*2R,3S*)-oxetin]-OBn **I** (1.23 g, 4.0 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added TFA (9.2 mL, 13.7 g, 120 mmol). After 2 h at room temperature, volatiles were evaporated under reduced pressure to leave the TFA salt. This material was added directly to a solution of Boc-[(*2R,3S*)-oxetin]-OH (868 mg, 4.0 mmol) in CH_2Cl_2 (25 mL) under an argon atmosphere. 1-Hydroxybenzotriazole hydrate (HOBt·H_2O) (648 mg, 4.8 mmol) and trimethylamine (1.95 mL, 1.42 g, 14.0 mmol) were added and the mixture was stirred for 5 min until all reagents had dissolved, then 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (EDCI·HCl) (1.15 g, 6.0 mmol) was added. The solution was stirred at room temperature for 36 h. The solvent was removed under reduced pressure and the residue was taken up in EtOAc (30 mL). The organic solution was washed successively with 1 M aqueous $KHSO_4$ solution (2 × 10 mL), saturated $NaHCO_3$ solution (2 × 10 mL) and brine (10 mL). The organic solution was then dried over Na_2SO_4 and concentrated under reduced pressure. The crude product was purified by flash column chromatography (EtOAc/PE; 9/1), to give the corresponding dipeptide Boc-[(*2R,3S*)-oxetin]$_2$-OBn **1** (1.61 g, 99%) as a white powder.

R_f (EtOAc/PE; 6/4): 0.31; **Mp** 148-149 °C. **$[\alpha]_D^{26}$** –68 (*c* 0.50, $CHCl_3$); **IR** υ 3369, 3321, 2980, 2932, 2890, 1742, 1709, 1694, 1661, 1524, 1367; **^{1}H NMR (360 MHz, $CDCl_3$)** δ 7.54 (br.d, *J* = 8.7 Hz, 1H), 7.42-7.30 (m, 5H), 5.52-5.41 (m, 2H), 5.37 (A of AB quartet, *J* = 12.1 Hz, 1H), 5.36 (d, *J* = 7.8 Hz, 1H), 5.20 (B of AB quartet, *J* = 12.1 Hz, 1H), 5.04-4.97 (m, 2H), 4.95 (t, *J* = 7.2 Hz, 1H), 4.83-4.76 (m, 1H), 4.64 (t, *J* = 6.7 Hz, 1H), 4.17 (t, *J* = 6.3 Hz, 1H), 1.39 (s, 9H); **^{13}C NMR (90 MHz, $CDCl_3$)** δ 169.8 (C), 169.0 (C), 155.1 (C), 135.0 (C), 128.9 (3CH), 128.6 (2CH), 83.1 (CH), 82.7 (CH), 80.3 (C), 78.9 (CH_2), 77.0 (CH_2), 67.5 (CH_2), 46.2 (CH), 44.9 (CH), 28.3 ($3CH_3$); **HRMS** m/z: $[M + Na]^+$ Calcd. for $C_{20}H_{26}N_2NaO_7$ 429.1637; Found 429.1617.

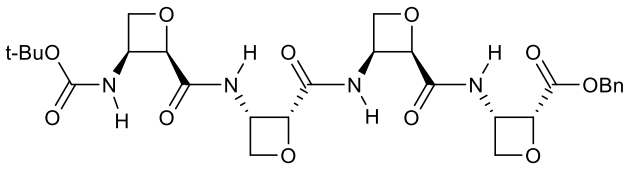


II

Boc-[(*2R,3S*)-Oxetin]$_2$-OH II:

General procedure **A** was applied using Boc-[(*2R,3S*)-oxetin]$_2$-OBn **1** (450 mg, 1.1 mmol) and Pd-C (500 mg) in CH_2Cl_2 (5 mL), to afford Boc-[(*2R,3S*)-oxetin]$_2$-OH **II** as a white solid (347 mg, 99%).

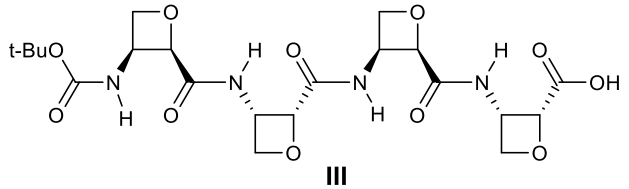
R_f (MeOH/CH_2Cl_2; 1/19): 0.16; **Mp** 179-180 °C; **$[\alpha]_D^{23}$** –69 (*c* 0.50, $CHCl_3$); **IR** υ 3355, 3293, 2975, 1785, 1680, 1657, 1502, 1362, 1249; **^{1}H NMR (250 MHz, $CDCl_3$)** δ 5.50-5.37 (m, 1H), 5.32 (d, *J* = 8.2 Hz, 1H), 5.15-5.01 (m, 2H), 4.95-4.82 (m, 2H), 4.75 (t, *J* = 6.5 Hz, 1H), 4.56 (t, *J* = 6.3 Hz, 1H), 1.40 (s, 9H), NH and COOH are not observed; **^{13}C NMR (63 MHz, $CDCl_3$)** δ 173.6 (C), 171.4 (C), 157.0 (C), 85.8 (CH), 84.4 (CH), 80.8 (C), 78.4 (CH_2), 76.8 (CH_2), 47.8 (CH), 46.2 (CH), 28.6 ($3CH_3$); **HRMS** m/z: $[M + Na]^+$ Calcd. for $C_{13}H_{20}N_2NaO_7$ 339.1168; Found 339.1149.



2

Boc-[(*2R,3S*)-oxetin]$_4$-OBn 2:

General procedure **B** was applied using Boc-[(*2R,3S*)-oxetin]$_2$-OBn **1** (450 mg, 1.1 mmol) and TFA (2.53 mL, 3.76 g, 33.0 mmol) in CH_2Cl_2 (3 mL), then DIPEA (0.67 mL, 497 mg, 3.85 mmol), FDPP (507 mg, 1.32 mmol) and Boc-[(*2R,3S*)-oxetin]$_2$-OH **II** (347 mg, 1.1 mmol) in DMF (2 mL), followed by flash column chromatography (EtOAc) to give Boc-[(*2R,3S*)-oxetin]$_4$-OBn **2** as a white solid (438 mg, 66%). ***R*$_f$** (EtOAc/PE; 9/1): 0.27; **Mp** 88-90 °C; **[α]$_D^{26}$** –123 (*c* 0.50, $CHCl_3$); **IR** υ 3684, 3669, 3653, 3312, 2976, 2895, 1713, 1667, 1515, 1367, 1250; **^{1}H NMR (360 MHz, $CDCl_3$)** δ 8.54 (d, *J* = 10.4 Hz, 1H), 7.96 (d, *J* = 10.1 Hz, 1H), 7.48 (d, *J* = 10.4 Hz, 1H), 7.46-7.30 (m, 5H), 6.62 (d, *J* = 10.3 Hz, 1H), 5.79-5.53 (m, 3H), 5.39 (A of AB quartet, *J* = 12.2 Hz, 1H), 5.33 (A of AB quartet, *J* = 12.2 Hz, 1H), 5.31 (d, *J* = 7.9 Hz, 1H), 5.26-5.16 (m, 1H), 5.13 (d, *J* = 8.6 Hz, 1H), 5.12 (d, *J* = 8.5 Hz, 1H), 5.03 (d, *J* = 8.5 Hz, 1H), 4.97 (t, *J* = 7.4 Hz, 1H), 4.89 (dd, *J* = 8.4, 6.5 Hz, 1H), 4.84 (dd, *J* = 8.1, 6.3 Hz, 1H), 4.77 (dd, *J* = 8.3, 7.2 Hz, 1H), 4.73-4.67 (m, 1H), 4.67 (t, *J* = 7.1 Hz, 1H), 4.52 (t, *J* = 6.8 Hz, 1H), 4.17 (t, *J* = 7.4 Hz, 1H), 1.42 (s, 9H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 170.1 (C), 170.0 (C), 169.34 (C), 169.28 (C), 155.5 (C), 135.2 (C), 129.03 (2CH), 128.98 (CH), 128.4 (2CH), 85.4 (CH), 84.4 (CH), 83.8 (CH), 83.0 (CH), 79.6 (C), 78.2 (CH_2), 76.6 (CH_2), 76.5 (CH_2), 76.2 (CH_2), 67.7 (CH_2), 46.8 (CH), 44.7 (CH), 43.9 (CH), 43.8 (CH), 28.5 ($3CH_3$); **HRMS** m/z: $[M + Na]^+$ Calcd. for $C_{28}H_{36}N_4NaO_{11}$ 627.2272; Found 627.2278.

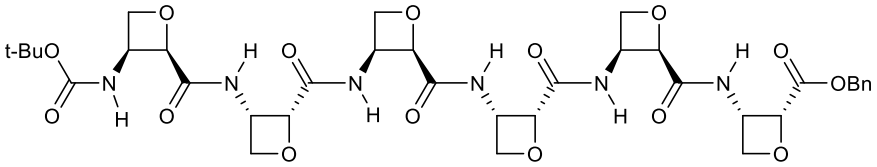


III

Boc-[(*2R,3S*)-oxetin]$_4$-OH III:

General procedure **A** was applied using Boc-[(*2R,3S*)-oxetin]$_4$-OBn **2** (163 mg, 0.27 mmol) and Pd-C (200 mg) in CH_2Cl_2 (5 mL), to give Boc-[(*2R,3S*)-oxetin]$_4$-OH **III** as a white solid (130 mg, 97%).

***R*$_f$** ($MeOH/CH_2Cl_2$; 1/9): 0.10; **Mp** 110–112 °C; **[α]$_D^{23}$** –133 (*c* 0.50, $CHCl_3$); **IR** υ 3311, 2967, 2931, 2895, 2454, 1714, 1658, 1524, 1432, 1360, 1247, 1165; **^{1}H NMR (360 MHz, $CDCl_3$)** δ 5.68-5.50 (m, 3H), 5.35 (d, *J* = 8.2 Hz, 1H), 5.21-5.02 (m, 4H), 4.98-4.76 (m, 7H), 4.63 (t, *J* = 6.9 Hz, 1H), 1.41 (s, 9H), NH and COOH are not observed; **^{13}C NMR (90 MHz, $CDCl_3$)** δ 173.4 (C), 171.9 (C), 171.6 (C), 171.5 (C), 157.1 (C), 86.4 (CH), 85.2 (CH), 84.8 (CH), 84.4 (CH),80.7 (C), 78.7 (CH_2), 77.2 (CH_2), 76.8 (CH_2), 76.3 (CH_2), 47.9 (CH), 45.9 (CH), 45.3 (2CH), 28.6 ($3CH_3$); **HRMS** m/z: $[M + Na]^+$ Calcd. for $C_{21}H_{30}N_4NaO_{11}$ 537.1809; Found 537.1784.



3

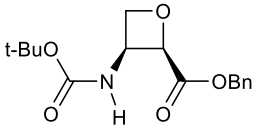
Boc-[(*2R,3S*)-oxetin]$_6$-OBn 3:

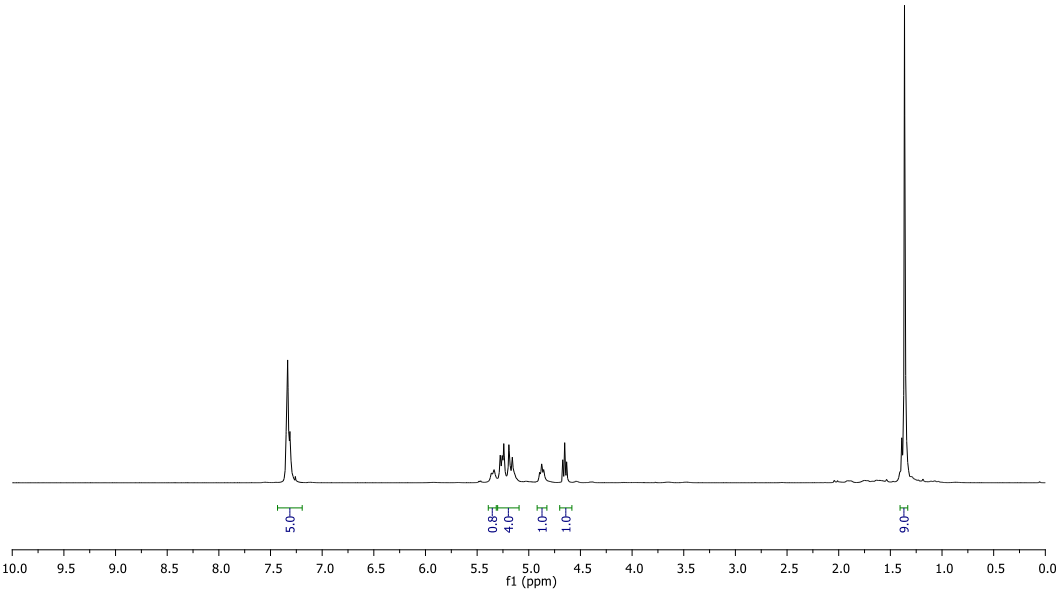
General procedure **B** was applied using Boc-[(*2R,3S*)-oxetin]$_2$-OBn **1** (166 mg, 0.41 mmol) and TFA (0.941, 1.4 g, 12.3 mmol) in CH_2Cl_2 (2 mL), then DIPEA (250 µL, 185 mg, 1.44 mmol), FDPP (189 mg, 0.49 mmol) and Boc-[(*2R,3S*)-oxetin]$_4$-OH **III** (210 mg, 0.41 mmol) in DMF (3 mL), followed by flash column chromatography (MeOH/EtOAc; 1/19) to give Boc-[(*2R,3S*)-oxetin]$_6$-OBn **3** as a white solid (290 mg, 88%).

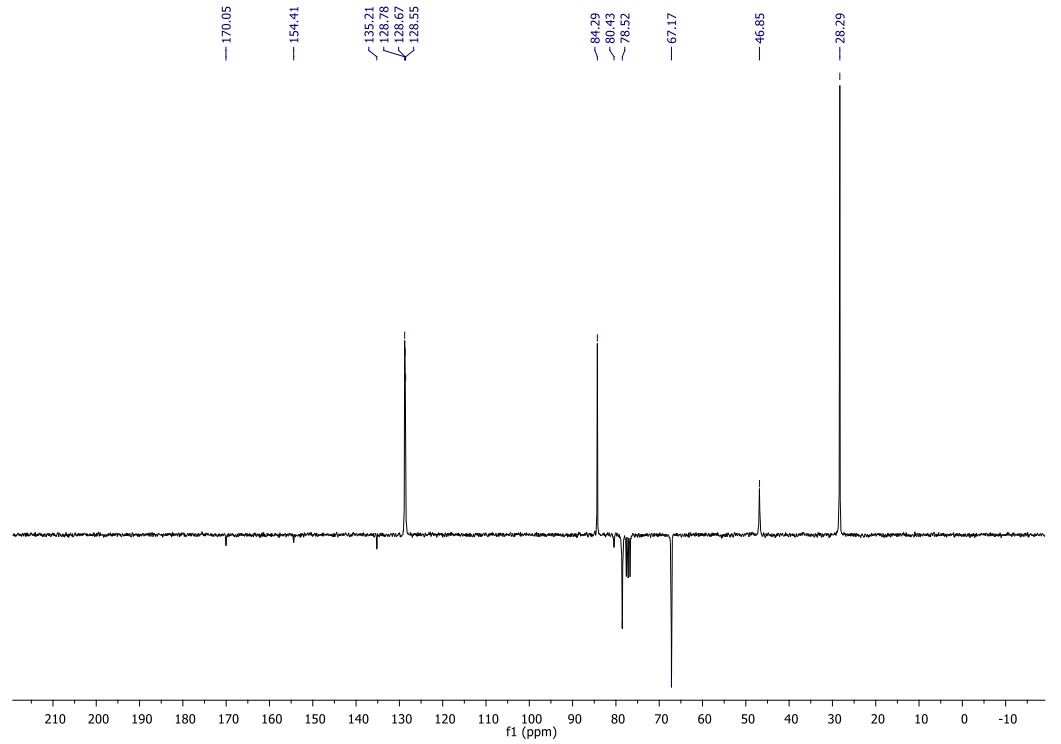
R$_f$ (MeOH/CH_2Cl_2; 1/9): 0.28; **Mp** 221-223 °C $[\alpha]_D^{24}$ -264 (*c* 0.50, $CHCl_3$) **IR** υ 3661, 3291, 2980, 2967, 1714, 1658, 1509, 1360, 1252, 1237, 1160; **^{1}H NMR (600 MHz, $CDCl_3$)** δ 8.81 (d, *J* = 10.3 Hz, 1H), 8.78 (d, *J* = 10.4 Hz, 1H), 8.61 (d, *J* = 9.4 Hz, 1H), 7.95 (d, *J* = 10.2 Hz, 1H), 7.49 (d, *J* = 10.1, 1H), 7.46-7.41 (m, 2H), 7.41-7.32 (m, 3H), 6.65 (d, *J* = 8.7 Hz, 1H), 5.78-5.68 (m, 3H), 5.67-5.58 (m, 2H), 5.39 (A of AB quartet, *J* = 12.2 Hz, 1H), 5.34 (B of AB quartet, *J* = 12.2 Hz, 1H), 5.31 (d, *J* = 8.0 Hz, 1H), 5.26-5.17 (m, 1H), 5.12 (d, *J* = 8.1 Hz, 2H), 5.06-5.00 (m, 3H), 4.97 (t, *J* = 7.4 Hz, 1H), 4.89 (t, *J* = 7.5 Hz, 1H), 4.87-4.80 (m, 4H), 4.80-4.73 (m, 3H), 4.67 (t, *J* = 6.9 Hz, 1H), 4.59 (t, *J* = 6.6 Hz, 1H), 4.22 (t, *J* = 7.2 Hz, 1H), 1.44 (s, 9H); **^{13}C NMR (100 MHz, $CDCl_3$)** δ 170.5 (C), 170.3 (C), 170.1 (C), 170.0 (C), 169.5 (C), 169.4 (C), 155.5 (C), 135.1 (C), 129.0 (3CH), 128.3 (2CH), 85.6 (CH), 84.7 (CH), 84.5 (CH), 84.1 (CH), 83.7 (CH), 83.0 (CH), 79.5 (C), 78.1 (CH_2), 76.24 (CH_2), 76.20 (CH_2), 75.9 (CH_2), 75.82 (CH_2), 75.73 (CH_2), 67.7 (CH_2), 46.7 (CH), 44.7 (CH), 44.1 (CH), 44.0 (CH), 43.9 (CH), 43.7 (CH), 28.4 ($3CH_3$); **HRMS** m/z: $[M + Na]^+$ Calcd. for $C_{36}H_{46}N_6NaO_{15}$ 825.2913; Found 825.2907.

3. Copies of standard ^{1}H and ^{13}C NMR spectra of all compounds

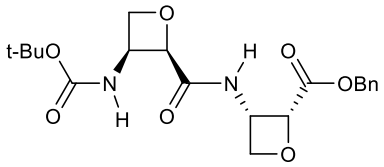
Boc-[(*2R,3S*)-oxetin]-OBn I

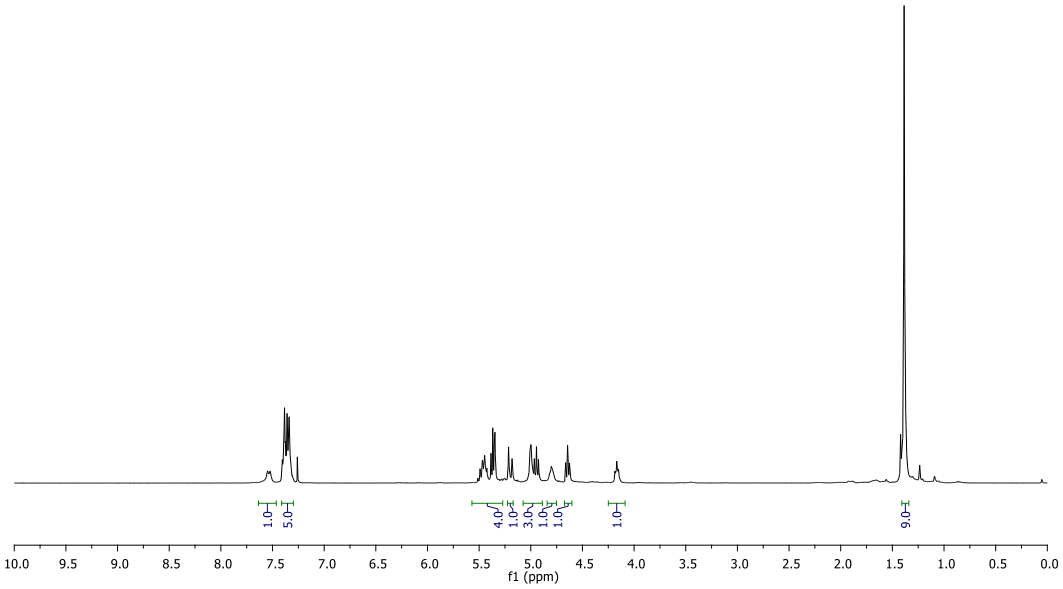


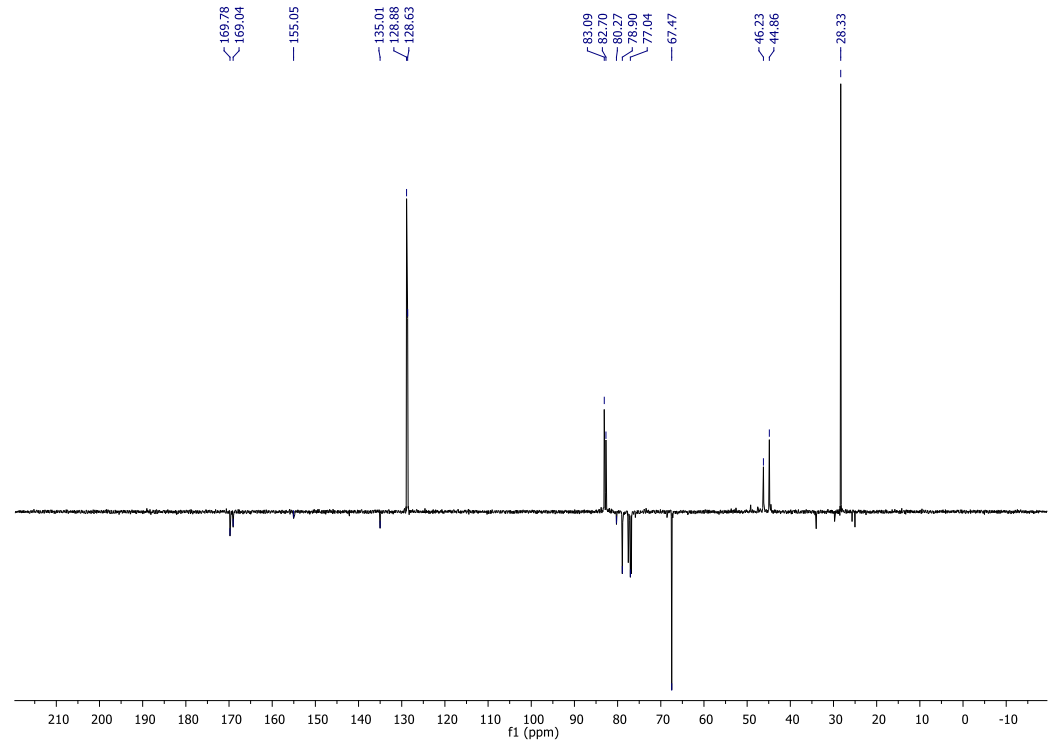




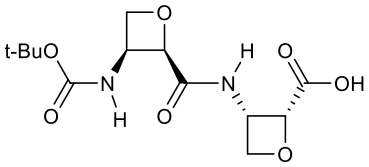
Boc-[(*2R,3S*)-oxetin]$_2$-OBn 1

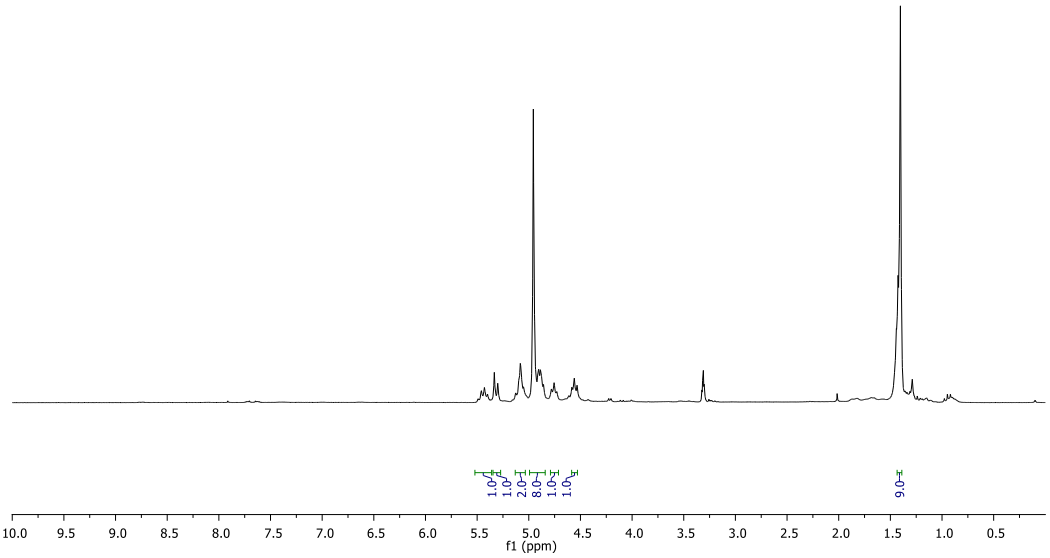


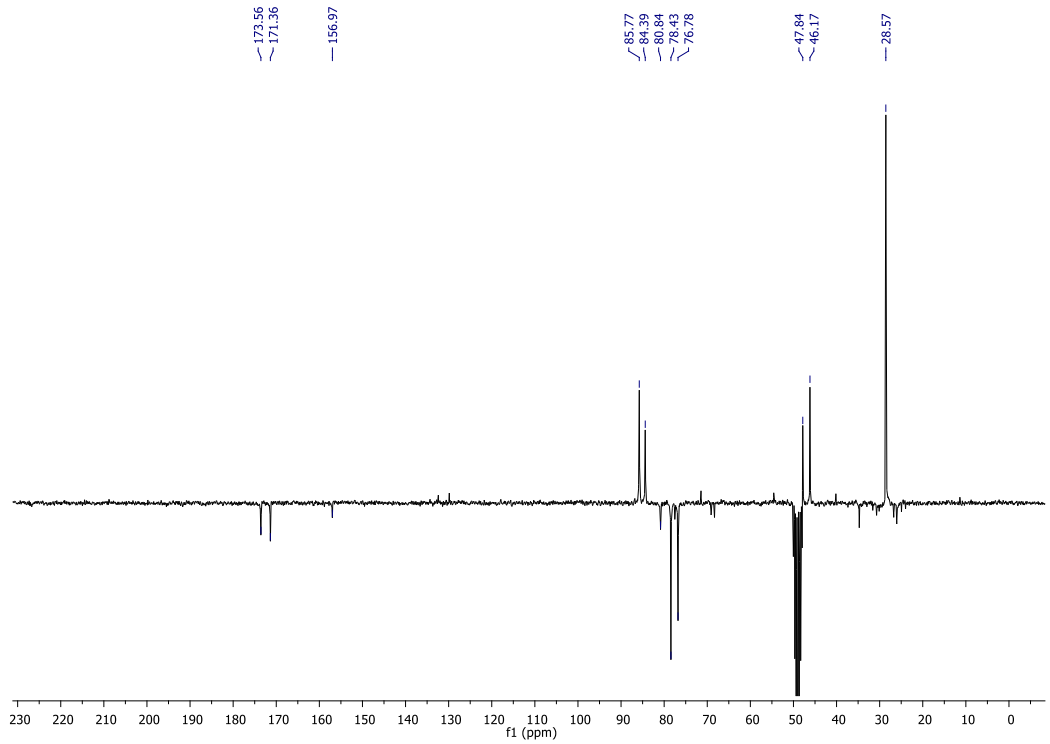




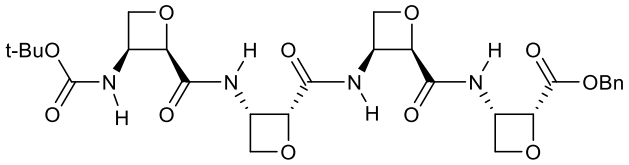
Boc-[(*2R,3S*)-oxetin]$_2$-OH II

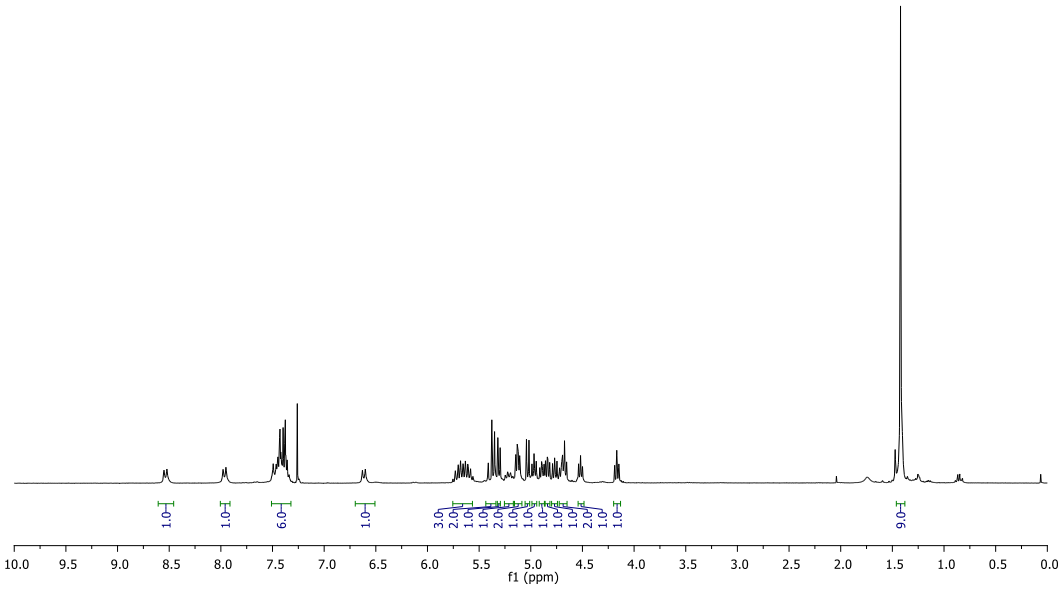


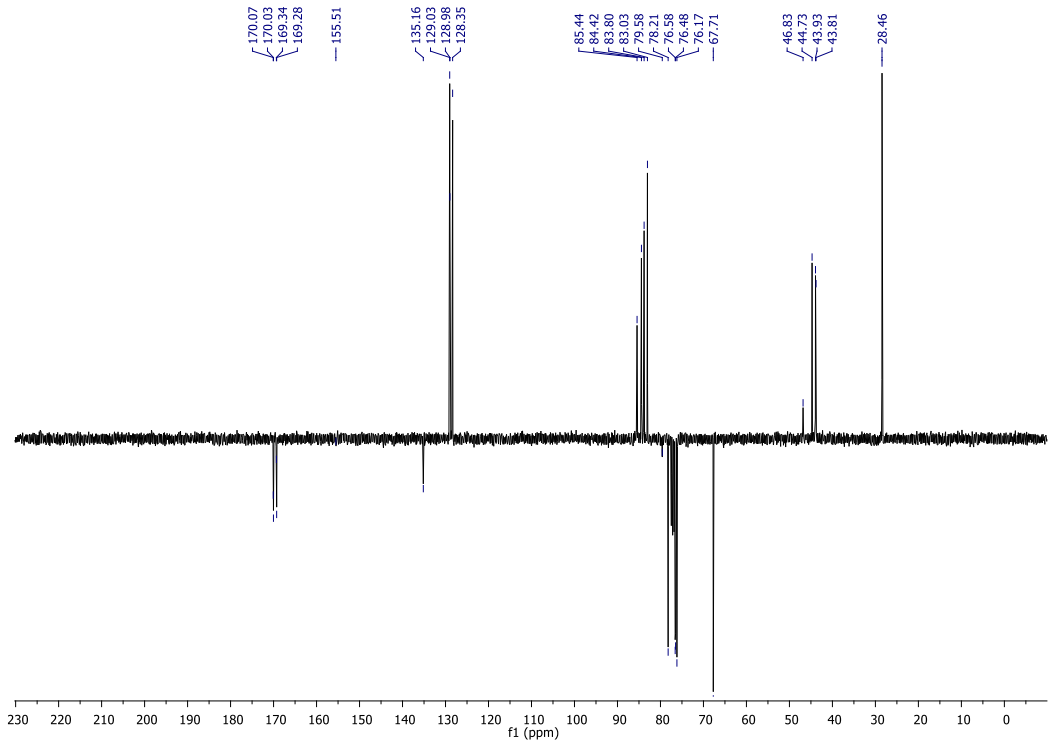




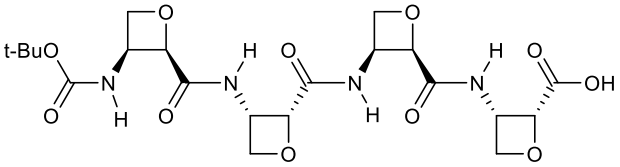
Boc-[(*2R,3S*)-oxetin]$_4$-OBn 2

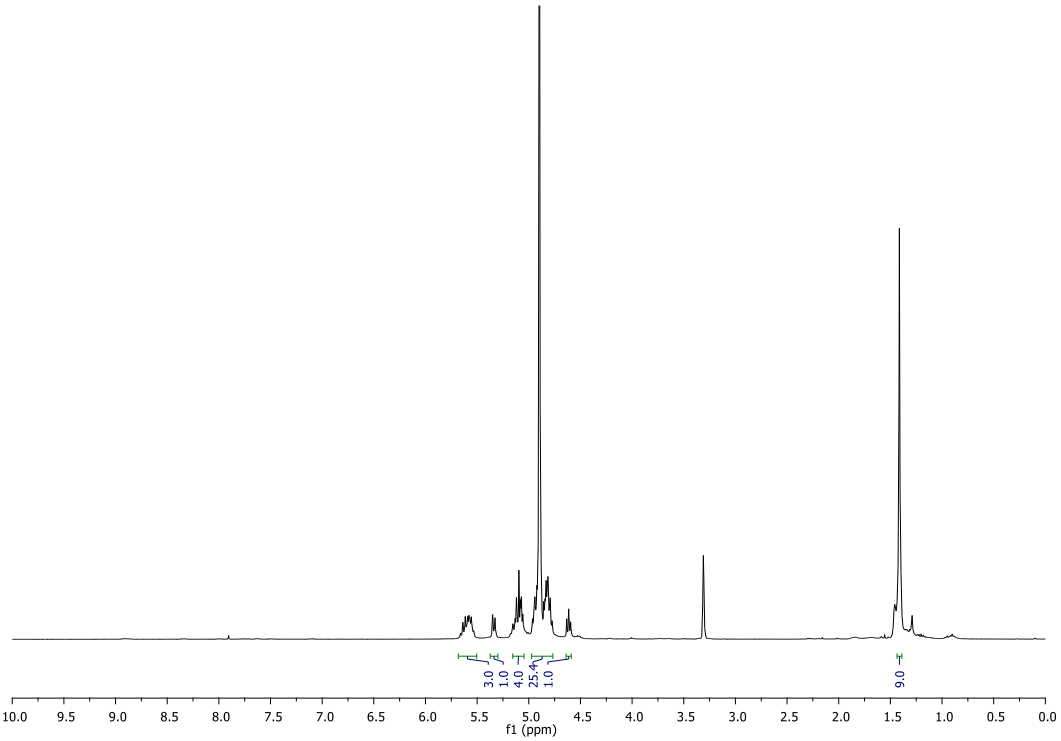


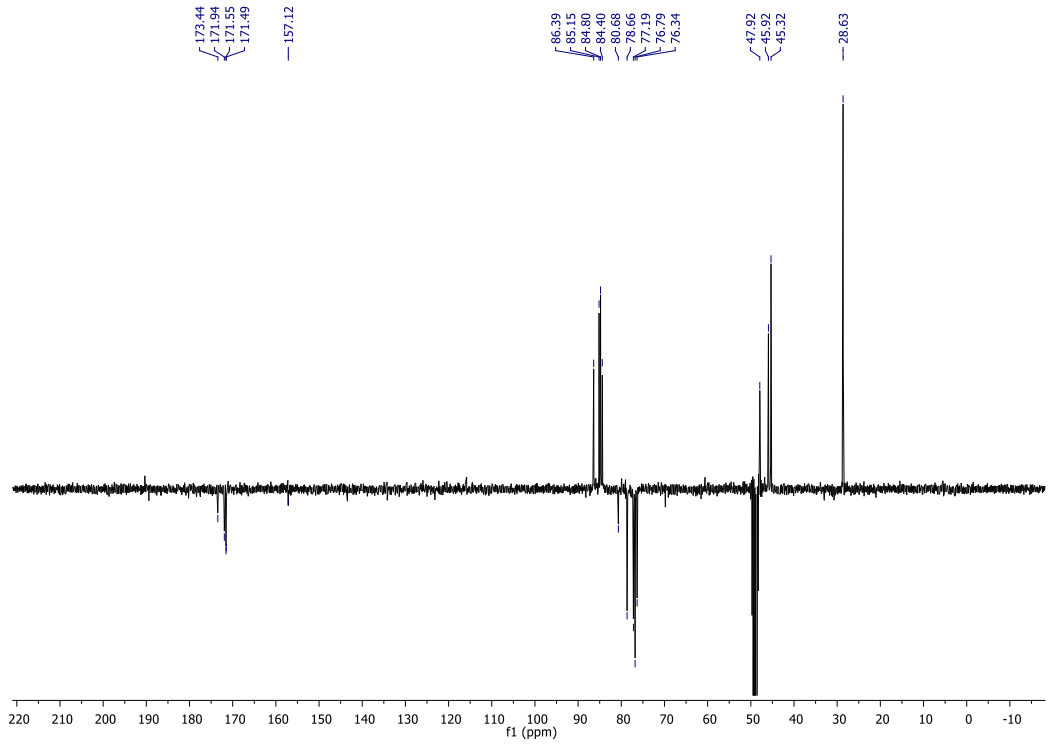




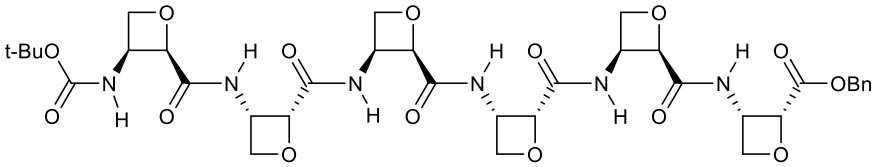
Boc-[(*2R,3S*)-oxetin]$_4$-OH III

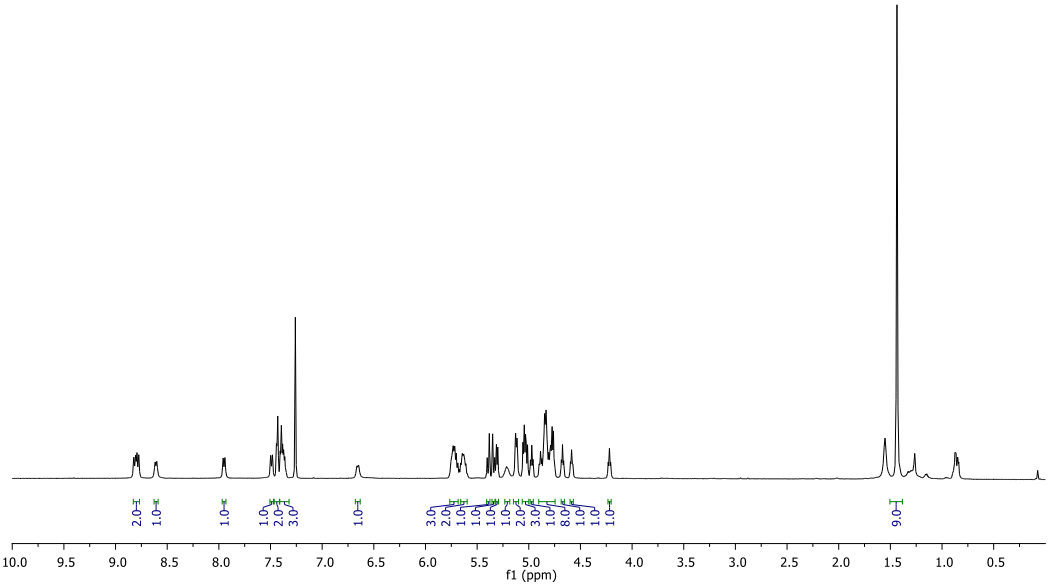


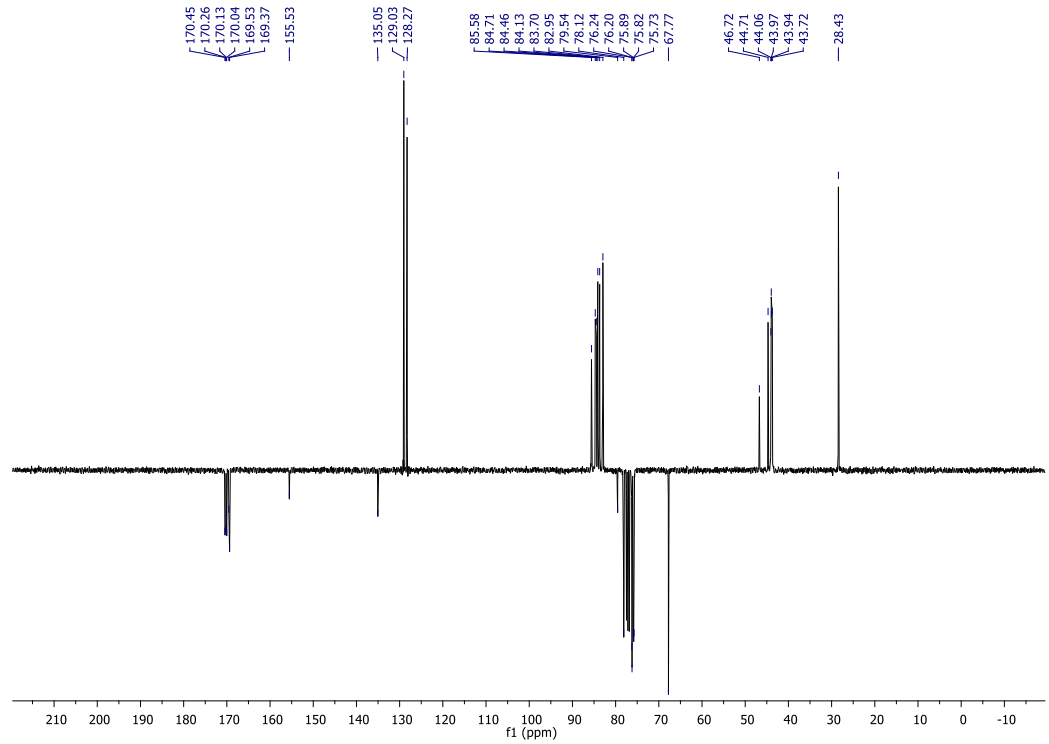




Boc-[(*2R,3S*)-oxetin]$_6$-OBn 3







4. Further NMR spectroscopic analysis of tetramer 2 and hexamer 3

a. NOESY/ROESY correlations:

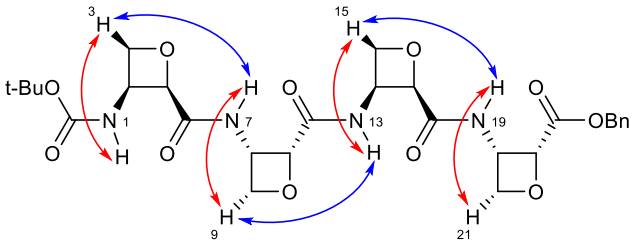
Tetramer 2:

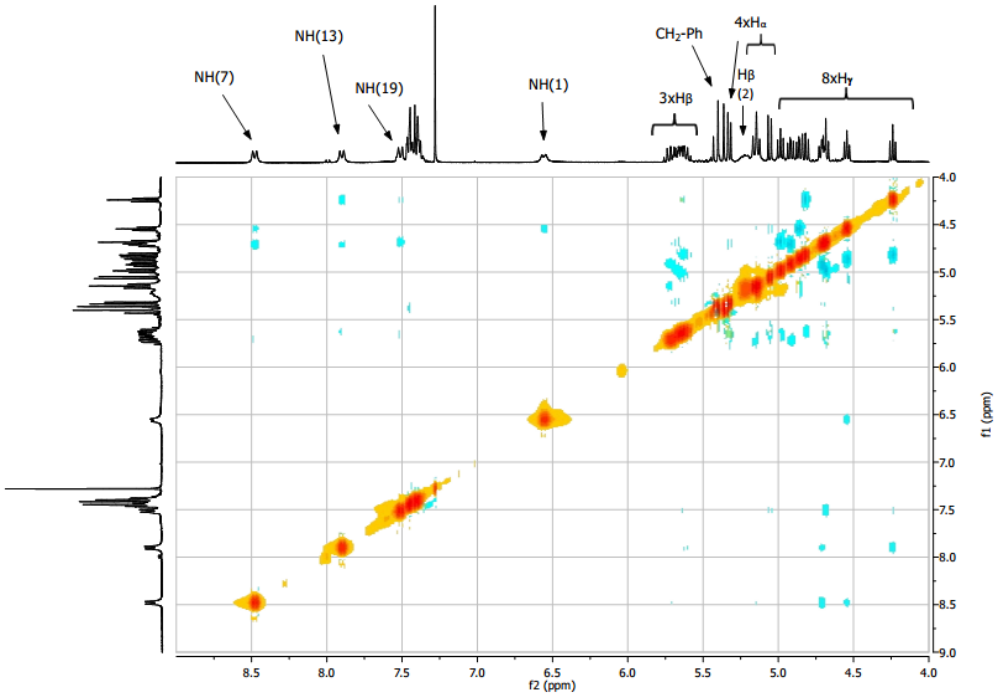
NOESY spectra were recorded at 312 K on a Bruker 400 MHz spectrometer. Samples were prepared in $CDCl_3$ at a concentration of 10 mM. The pulse sequence was noesygpph with gradient pulses in mixing time. The experiment was performed by collecting 9578 points in f2 and 256 points in f1.

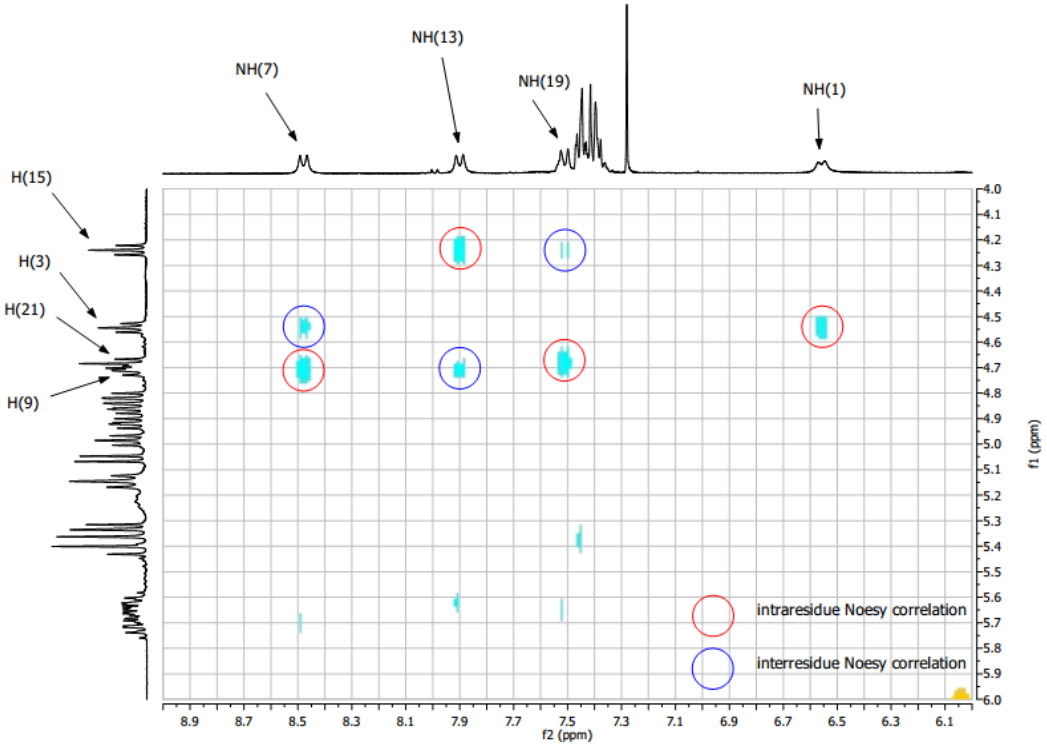
Hexamer 3:

ROESY spectra were recorded at 312 K on a Bruker 600 MHz spectrometer. Samples were prepared in $CDCl_3$ at a concentration of 10 mM. The pulse sequence was roesyph with 400 ms mixing time. The experiment was performed by collecting 12018 points in f2 and 512 points in f1.

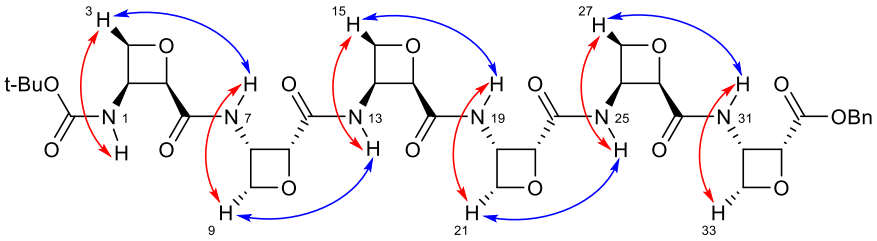
Boc-[(2*R*,3*S*)-oxetin]$_4$-OBn 2

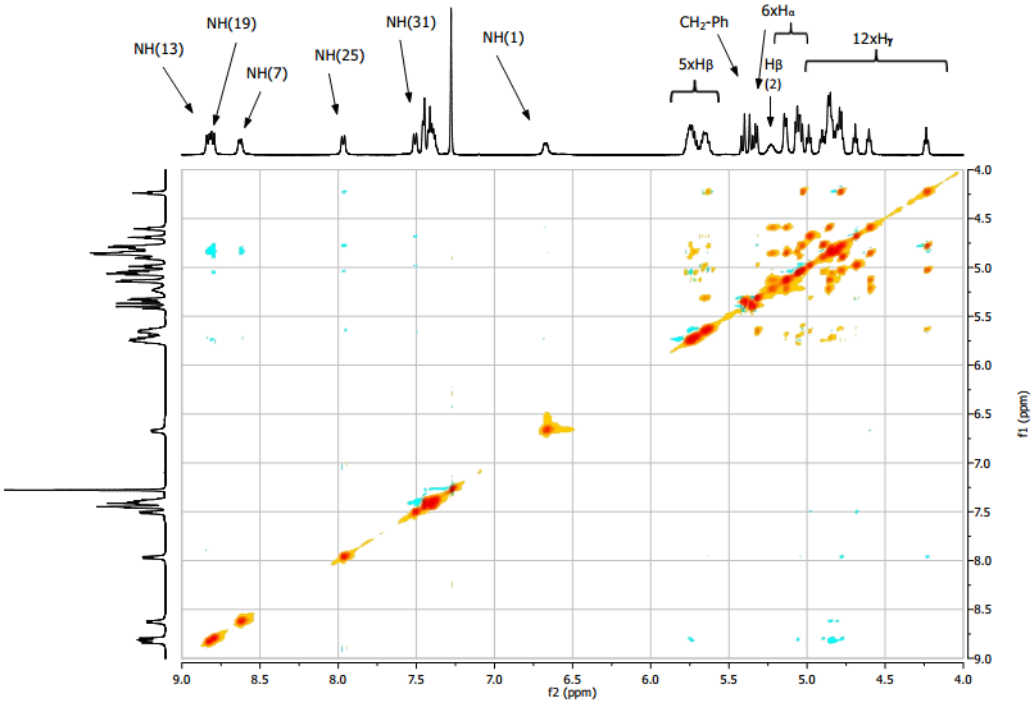


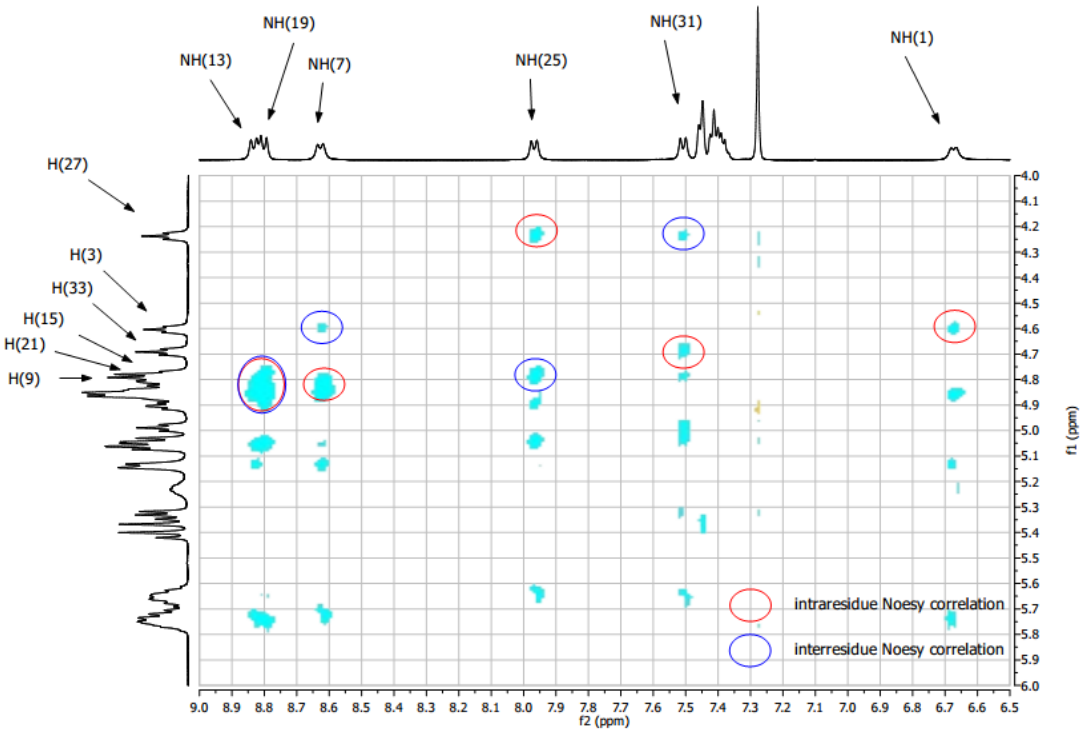




Boc-[(2*R*,3*S*)-oxetin]$_6$-OBn 3



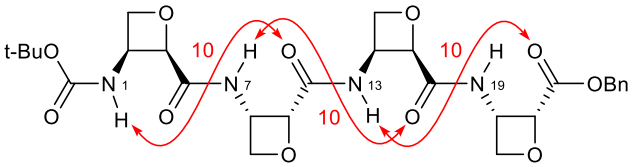


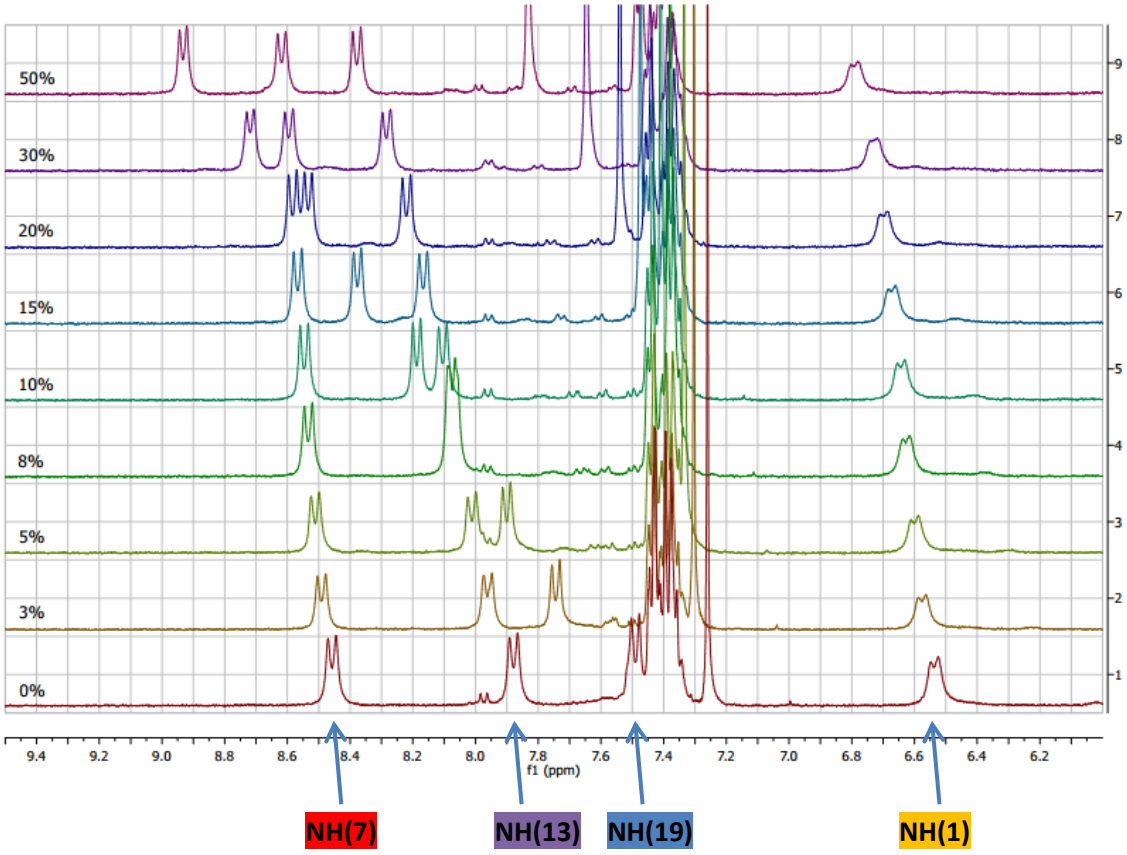


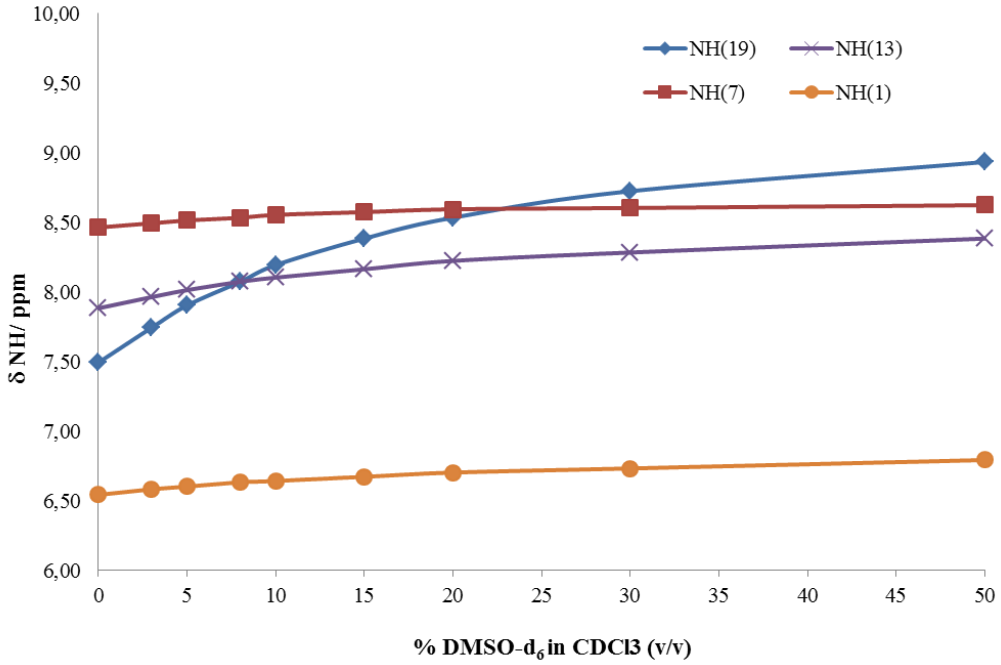
b. DMSO-d_6 titrations:

^{1}H Spectra were recorded at 312 K on spectrometers operating at 400 MHz and 600 MHz, for tetramer **2** and hexamer **3**, respectively. Samples were dissolved in $CDCl_3$ (500 µL) to give solutions of concentration 10 mM. Aliquots of DMSO-d_6 (15 µL (3%), 10 µL (5%), 15 µL (8%), 10 µL (10%), 25 µL (15%), 25 µL (20%), 50 µL (30%) and 100 µL (50%)) were added successively to the NMR tube followed, after each addition, by rapid agitation then re-recording of the ^{1}H spectra.

Boc-[(2*R*,3*S*)-oxetin]$_4$-OBn 2

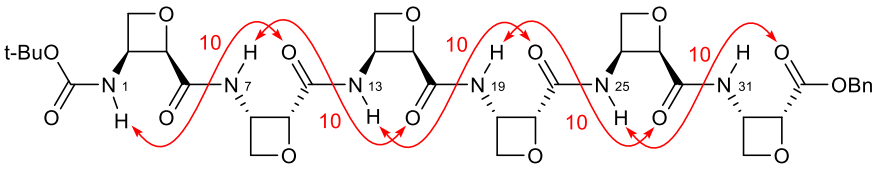


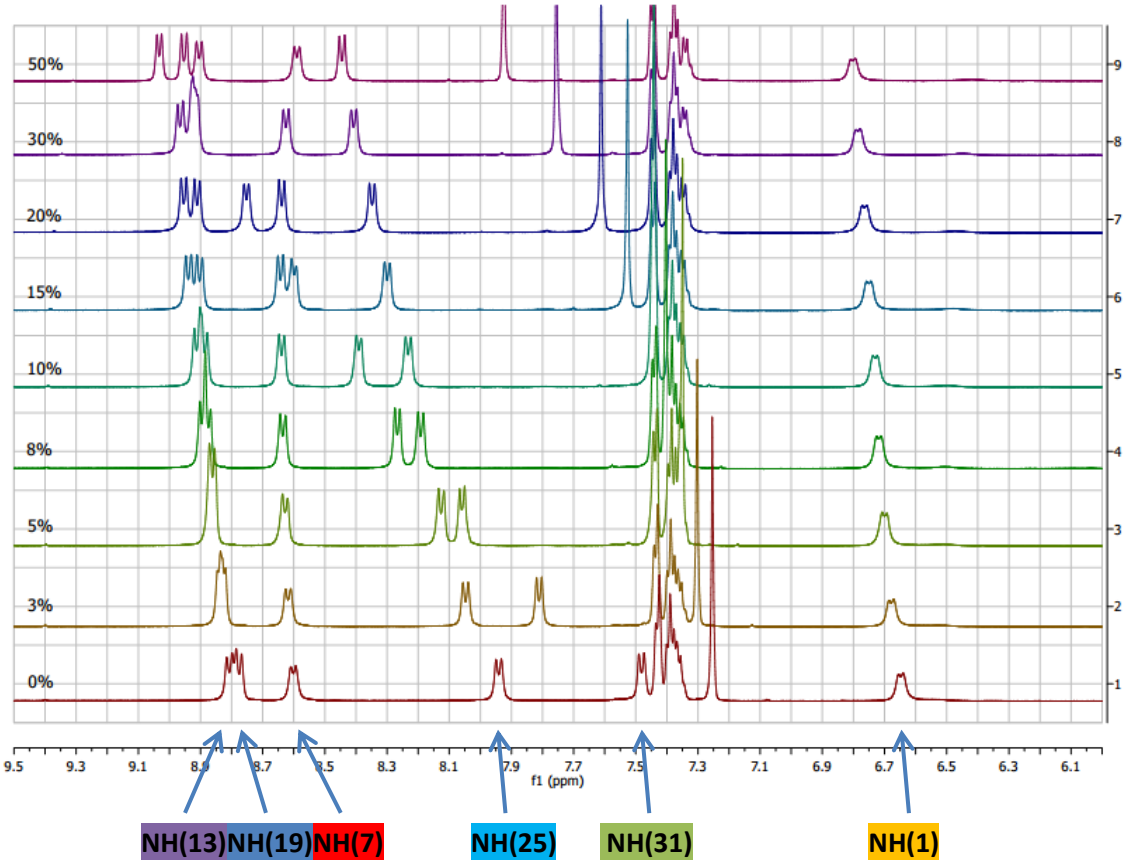


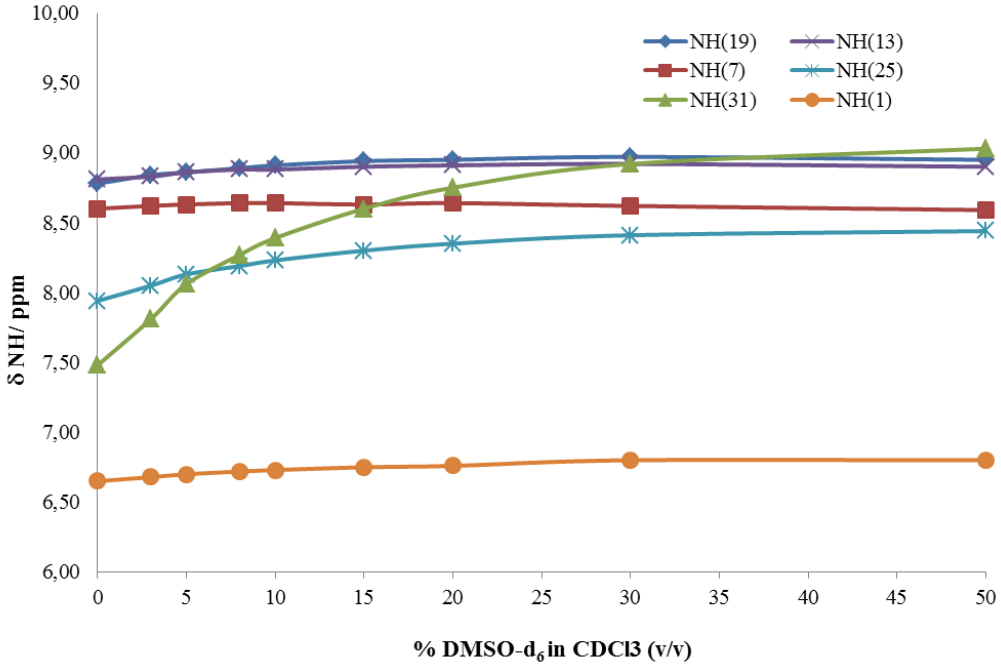


DMSO-d_6 (v/v)	NH(1)	NH(7)	NH(13)	NH(19)
0%	6.54	8.46	7.88	7.49
3%	6.58	8.49	7.96	7.74
5%	6.60	8.51	8.01	7.90
8%	6.63	8.53	8.07	8.07
10%	6.64	8.55	8.10	8.19
15%	6.67	8.57	8.16	8.38
20%	6.70	8.59	8.22	8.53
30%	6.73	8.60	8.28	8.72
50%	6.79	8.62	8.38	8.93
Δδ (10% DMSO-d_6)	**0.10**	**0.09**	**0.22**	**0.70**

Boc-[(2*R*,3*S*)-oxetin]$_6$-OBn 3







DMSO-d_6 (v/v)	NH(1)	NH(7)	NH(13)	NH(19)	NH(25)	NH(31)
0%	6.65	8.60	8.81	8.78	7.94	7.48
3%	6.68	8.62	8.83	8.84	8.05	7.81
5%	6.70	8.63	8.86	8.86	8.13	8.06
8%	6.72	8.64	8.88	8.89	8.19	8.27
10%	6.73	8.64	8.88	8.91	8.23	8.39
15%	6.75	8.63	8.90	8.94	8.30	8.60
20%	6.76	8.64	8.91	8.95	8.35	8.75
30%	6.80	8.62	8.92	8.97	8.41	8.92
50%	6.80	8.59	8.90	8.95	8.44	9.03
Δδ (10% DMSO-d_6)	**0.08**	**0.05**	**0.07**	**0.13**	**0.29**	**0.91**

5. X-ray crystal structure analysis of hexamer 3

X-ray diffraction data for compound **3** were collected by using a VENTURE PHOTON100 CMOS Bruker diffractometer with Micro-focus IμS source $Cu_{K\alpha}$ radiation. Crystals were mounted on a CryoLoop (Hampton Research) with Paratone-N (Hampton Research) as cryoprotectant and then flashfrozen in a nitrogen gas stream at 100 K. The temperature of the crystal was maintained at 100 K by means of a N-Helix Cryosystems cooling device to within an accuracy of ±1 K. The data were corrected for Lorentz polarization, and absorption effects. The structures were solved by direct methods using SHELXS-97[S2] and refined against F^2 by full-matrix least-squares techniques using SHELXL-2017[S3] with anisotropic displacement parameters for all non-hydrogen atoms. Hydrogen atoms were located on a difference Fourier map and introduced into the calculations as a riding model with isotropic thermal parameters. All calculations were performed by using the Crystal Structure crystallographic software package WINGX.[S4]

The absolute configuration was determined by refining the Flack parameter[S5] using a large number of Friedel's pairs.

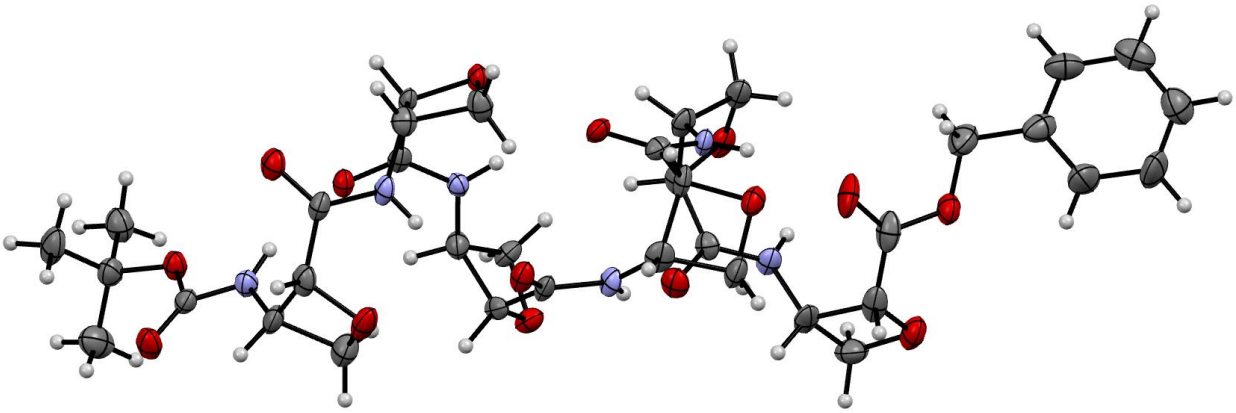
The crystal data collection and refinement parameters are given in the following Table.

CCDC 1588632 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/Community/Requestastructure.

[S2] Sheldrick, G. M. *SHELXS-97, Program for Crystal Structure Solution*, University of Göttingen, Göttingen, Germany, **1997**.
[S3] G. M. Sheldrick, *Acta Cryst*., **2008**, *A64*, 112.
[S4] Farrugia, L. J. *J. Appl. Cryst*., **1999**, *32*, 837.
[S5] Flack H. D. *Acta Cryst*. **1983**, *A39*, 876.

Crystallographic data and structure refinement details for hexamer **3**

Compound	**3**
CCDC number	1588632
Empirical Formula	$2(C_{36}H_{46}N_6O_{15})$, $3(CHCl_3)$, 1.5(O)
M_r	2003.67
Crystal size, mm^3	0.13 × 0.08 × 0.02
Crystal system	monoclinic
Space group	*P 2₁*
a, Å	13.7293(9)
b, Å	20.0802(14)
c, Å	16.8259(11)
α, °	90
β, °	101.186(3)
γ, °	90
Cell volume, $Å^3$	4550.6(5)
Z	2
T, K	100(1)
Radiation type; wavelength, Å	$Cu_{K\alpha}$; 1.54178
F_{000}	2084
μ, mm^{-1}	3.293
θ range, °	2.677 - 67.185
Reflections collected	51 276
Unique reflections	15 990
R_{int}	0.0673
GOF	1.031
Refl. obs. ($I > 2\sigma(I)$)	14428
Parameters	1168
wR_2 (all data)	0.2316
R value ($I > 2\sigma(I)$)	0.0864
Flack parameter	0.021(14)
Largest diff. peak and hole ($e^- \cdot Å^{-3}$)	0.786; –0.482



ORTEP diagram (30% probability thermal ellipsoids) of one of the two molecules of hexamer **3** present in the asymmetric unit ($CHCl_3$ and H_2O molecules have been removed for clarity).

Two non-identical molecules (A and B) were present in the asymmetric unit.

Torsion angle (°)	molecule	Residue 1	Residue 2	Residue 3	Residue 4	Residue 5	Residue 6
φ	A	*–131.4*	–90.0	–111.0	–100.9	–95.1	–118.0
	B	*–133.6*	–93.7	–114.8	–100.4	–101.1	–116.9
θ	A	–13.4	–11.2	–15.0	–6.4	–14.3	*9.5*
	B	–15.0	–8.3	–15.4	–10.8	–11.2	*9.0*
ψ	A	–97.8	–97.8	–103.4	–110.4	–99.1	*–135.6*
	B	–100.6	–100.2	–99.3	–101.8	–101.1	*–127.2*
ω	A	175.1	170.1	172.0	167.5	171.4	-
	B	177.8	171.3	173.8	169.0	172.5	-

10-mr H-bonding	molecule	1→2	2→3	3→4	4→5	5→6
N–H···O=C H-bond length (Å)	A	2.087	2.496	2.150	2.179	2.320
	B	2.093	2.640	2.116	2.297	2.267
N–H···O H-bond angle (°)	A	171.43	147.20	158.83	150.19	148.70
	B	166.68	147.32	164.56	156.40	151.62

5-mr H-bonding	molecule	1←2	2←3	3←4	4←5	5←6
N–H···O(oxtane) H-bond length (Å)	A	2.272	2.296	2.272	2.311	2.246
	B	2.275	2.301	2.293	2.297	2.242
N–H···O H-bond angle (°)	A	108.84	108.49	108.83	106.76	109.82
	B	108.95	108.13	108.57	107.75	109.19

6. Molecular modelling of hexamer 3

A hybrid Monte Carlo Molecular Mechanics (MCMM) conformational search was carried out on hexamer **3** in a chloroform medium using Macromodel 04 from Schrödinger software and the MMFF force field without restraints. 10000 conformers were generated from which 1033 low-energy conformers (up to 10 $kJ \cdot mol^{-1}$ of relative energy) were retained and sorted into different types of conformer families, differentiated by the H-bonded ring systems they displayed.

6 conformer families were observed, all of which feature 10-membered ring H-bonds. They were designated as 10/10/10/10/10, 10/10/10/10/X, 10/10/10/X/10, 10/10/X/10/10, 10/X/10/10/10 and 10/10/X/X/10, in which discreet successive 10-membered ring H-bonds are separated by the symbol '/' and the symbol 'X' is used when no H-bond is observed. The 10/10/10/10/10 conformer constitutes the complete 10-helix.

The relative abundance (expressed as %) of each conformer family is given in the Table below. The 52 lowest-energy conformers were all members of the 10/10/10/10/10 conformer family.

Hexamer **3**	number of conformers	relative abundance
conformers total (< 10 $kJ \cdot mol^{-1}$)	1033	100.00 %
10/10/10/10/10 (10-helix)	927	89.74 %
10/10/10/10/X	34	3.29 %
10/10/X/10/10	45	4.36 %
10/10/X/X/10	17	1.65 %
10/X/10/10/10	8	0.77 %
10/10/10/X/10	7	0.19 %