Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

A Minimalistic Hydrolase based on Co-Assembled Cyclic Dipeptides

Alexander J. Kleinsmann[b] and Boris J. Nachtsheim*[a]

[a] *Institut für Organische und Analytische Chemie, Universität Bremen, Leobener Straße 7, 28359 Bremen, Germany*

[b] *Institut für Organische Chemie, Universität Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, Germany*

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1.1 Materials

Unless otherwise stated, all chemicals were used as received from a commercial supplier. The water used for the preparation for the hydrogels and buffers was of Millipore Milli-Q grade. Buffer solutions were stored under exclusion from light and used up within five days. MES buffer (0.25 M) was used for pH 6.25 and 6.50, HEPES buffer (0.25 M) was used from pH 6.75 to 8.50.

For scanning electron microscopy (SEM), the DKP-samples were dissolved in water of Milli-Q grade in 4 ml screw-cap vials and the warm solution was applied to pre-cooled aluminum sheets. The samples were allowed to mature at 4°C for 20 minutes, lyophilized and coated with a thin layer of platinum by using a Balzers SCD 050 sputter coater. A Hitachi SU8030 scanning electron microscope was used to record the images of the xerogels with an accelerating voltage of 1 kV.

Transmission electron microscopy (TEM) images were recorded with a Hitachi SU8030 scanning electron microscope in STEM mode at an accelerating voltage of 30 kV. The hydrogel samples were prepared in a 4 ml screw-cap vial, lyophilized and distributed onto a TEM grid (200 mesh copper grid) that was coated with carbon film.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker Advance 400 MHz instrument in DMSO-d_6 or D_2O. The ^{1}H chemical shifts are reported as δ (parts per million) relative to the quintet signal of DMSO at 2.50 ppm or to the singlet signal of 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt in D_2O at 0.00 ppm. The ^{13}C chemical shifts are reported as δ (parts per million) relative to the DMSO septet at 39.43 ppm or to the singlet signal of 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt in D_2O at 0.00 ppm. The following abbreviations have been used to describe splitting patterns: br=broad, s=singlet, d=doublet, t=triplet, q=quartet, qi=quintet, m=multiplet. Coupling constants *J* are given in Hz.

^{1}H HR/MAS NMR NOE spectra of the hydrogels in D_2O were recorded on a Bruker ARX 400 MHz instrument with a 4 mm triple resonance HR/MAS probehead. The samples were measured at room temperature at spinning frequencies of 2.5 or 4.0 kHz.

IR spectra were recorded with a Jasco FT/IR-4100 spectrometer. UV/Vis spectra were recorded with a Perkin-Elmer Lambda 2 UV/Vis spectrometer. Mass spectra were recorded on a Finnigan MAT95 spectrometer. High-resolution mass spectra were recorded by using ESI method with a Bruker Daltonics Apex II FT-ICR mass analyzer. Optical rotations were measured with sodium light on a Jasco P-1020 polarimeter. Elemental analysis was carried out on an Elementar Vario MICRO Cube analyzer. Melting points were determined with a Büchi B-540 melting point analyzer.

1.2 UV/Vis experiments

Kinetic experiments

For experiments comprising DKPs with cysteine, the buffer was degassed before DKP addition by sparging argon through. DKPs were dissolved in the corresponding buffer (0.25 M) by heating in a 4 ml screw cap vial, the solution was allowed to reach room temperature and the pH of the mixture was checked and adjusted if necessary. The mixture was heated again, dipped in a water bath at 50°C for a few seconds to avoid burst of the vial and immediately cooled in an ice bath for 20 minutes. The hydrogel was subsequently allowed to reach room temperature for additional 20 minutes. The corresponding buffer (950 µl) was carefully added on top of the hydrogel followed by substrate solution (50 µl in DMF) and gently mixed by agitation. For higher substrate concentrations (more than 60 mM in the buffer-DMF solution), applied for the substrate concentration dependent initial rate measurements, the substrate was directly dissolved in buffer/DMF (950:50 µl) mixtures and placed on top of hydrogel. The reaction mixture was gently agitated every 10-15 seconds during the measurements and samples were diluted with the corresponding buffer. The product formation was measured at 406 nm and extinction coefficients for the calculation of the product concentration were determined experimentally (Table S1). All experiments were repeated at least three times and the average value was used for further calculations. In all experiments background hydrolysis of ANBS was measured in the corresponding buffers at the same pH with identical substrate concentration and subtracted from the measured values.

Table S 1: Experimentally determined pH dependent extinction coefficients of ANBS in 0.25 M HEPES buffer (measured at λ = 406 nm).

pH value	Molar Extinction Coefficient ε_λ [m^2/mol]
6.25	335.55
6.50	358.11
6.75	378.10
7.00	400.46
7.25	410.00
7.38	413.72
7.50	416.78
7.75	420.06
8.00	424.13
8.25	426.79
8.50	427.49

1.3 Synthesis

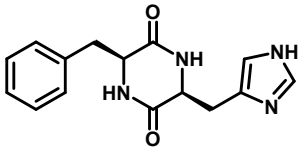
1.3.1 General procedures for the Synthesis of DKPs 1-4:

Boc-His(Boc)-OH was synthesized following the procedure by Castro and co-workers.[1]

DKP **1** and the *S*-4-methoxybenzyl (PMB) protected precursors of DKP **2**-**4** were prepared according to our previously reported procedure.[2]

The PMB-protected DKPs (2.50 mmol) were dissolved in 25 ml of a TFA/H_2O/phenol (90:5:5) mixture and refluxed for one hour. The reaction mixture concentrated to a fourth part and cooled to 4°C and an excess of diethylether was added. The precipitate collected by filtration, washed three times with diethylether and dried under reduced pressure. The crude product was dissolved in a dithiothreitol (DTT) solution (100 ml, 10 mM in THF/H_2O (8:2)) and 1.5 ml of saturated sodium bicarbonate solution were added. The solution was stirred for 30 minutes and subsequently THF was removed under reduced pressure. The resulting suspension was cooled in an ice bath and the precipitate was collected by filtration and washed with water.

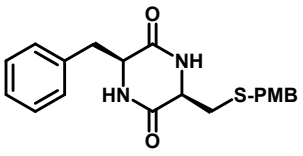
1.3.2 Cyclo[L-His-L-Phe] (DKP 1)



DKP **1** was synthesized according to the general procedure using Boc-Phe-OH (23.70 g, 89.33 mmol, 2.0 eq.) and Boc-His(Boc)-OH (47.62 g, 134.00 mmol, 3.0 eq.). The crude product solution in THF-water (8:1) was concentrated under reduced pressure until all volatile components were removed. The aqueous suspension was cooled in an ice bath under strong stirring and an excess of diethylether was added. The solid was collected by filtration and washed with water and diethylether. The crude product was recrystallized from water, subsequently recrystallized from methanol and dried under reduced pressure. DKP **1** was received as a white solid (6.48 g, 22.79 mmol, 51%).

^{1}H NMR (400 MHz, D_2O) δ 8.52 (s, 1H), 7.46-7.33 (m, 3H), 7.19 (d, *J*=6.9 Hz, 2H), 6.95 (s, 1H), 4.54-4.44 (m, 1H), 4.26-4.12 (m, 1H), 3.17 (dd, *J*=14.0, 3.2 Hz, 1H), 2.98 (dd, *J*=14.0, 4.4 Hz, 1H), 2.53 (dd, *J*=15.3, 4.4 Hz, 1H), 1.89 (dd, *J*=15.3, 7.6 Hz, 1H). **^{13}C NMR** (101 MHz, D_2O) δ 172.0, 170.7, 137.7, 136.7, 133.5 (2x), 131.9 (2x), 130.7, 130.1, 120.7, 58.6, 56.2, 41.2, 31.2. **MS (FAB)** calculated for $C_{15}H_{17}N_4O_2$ $[M+H]^+$: m/z 285.1, found: 285.2.[2]

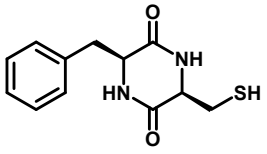
1.3.3 Cyclo[L-Cys(PMB)-L-Phe]



Cyclo[L-Cys(PMB)-L-Phe] was synthesized according to the general procedure using Boc-Phe-OH (17.46 g, 65.83 mmol, 2.0 eq.) and Boc-Cys(PMB)-OH (33.72 g, 98.75 mmol, 3.0 eq.). The crude product solution in THF-water (8:1) was concentrated under reduced pressure at 50°C, until the THF was removed completely, and stirred strongly at 4 °C. The product was collected by filtration, washed with water and dried under reduced pressure. Cyclo[L-Cys(PMB)-L-Phe] was received as a white solid (5.82 g, 15.72 mmol, 48%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.28-8.17 (m, 1H), 8.07-7.95 (m, 1H), 7.28-7.14 (m, 7H), 6.85 (d, *J*=8.6 Hz, 2H), 4.23-4.14 (m, 1H), 3.87-3.80 (m, 1H), 3.72 (s, 3H), 3.51 (s, 2H), 3.14 (dd, *J*=13.5, 4.7 Hz, 1H), 2.94 (dd, *J*=13.5, 4.9 Hz, 1H), 2.31 (dd, *J*=13.7, 3.9 Hz, 1H), 1.50 (dd, *J*=13.7, 7.4 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 166.2, 165.9, 158.1, 136.3, 130.2 (2x), 130.1, 130.0 (2x), 128.1 (2x), 126.7, 113.7, 55.4, 55.0, 53.6, 39.5, 35.2, 34.7. **MS (FAB)** calculated for $C_{20}H_{23}N_2O_3S$ $[M+H]^+$: m/z 371.1, found 371.2.[2]

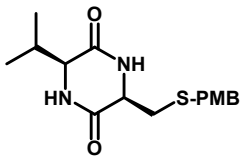
1.3.4 Cyclo[L-Cys-L-Phe] (DKP 2)



Cyclo[L-Cys(PMB)-L-Phe] (1.00 g, 2.70 mmol) was deprotected according to the general procedure. DKP **2** was received as a white solid (0.58 g, 2.30 mmol, 85%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (s, 1H), 8.04 (s, 1H), 7.30-7.14 (m, 5H), 4.24-4.17 (m, 1H), 3.93-3.86 (m, 1H), 3.15 (dd, *J*=13.6, 4.3 Hz, 1H), 2.92 (dd, *J*=13.6, 4.9 Hz, 1H), 2.36-2.29 (m, 1H), 2.02-1.92 (m, 1H), 1.74 (t, *J*=8.5 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 166.5, 165.5, 136.3, 130.3 (2x), 128.1 (2x), 126.6, 55.9, 55.2, 38.2, 27.4. **MS (FAB)** calculated for $C_{12}H_{14}N_2O_2SNa$ $[M+H]^+$: m/z 251.1, found: 251.1.[2]

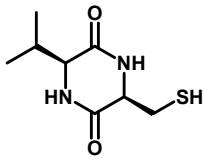
1.3.5 Cyclo[L-Cys(PMB)-L-Val]



Cyclo[L-Cys(PMB)-L-Val] was synthesized according to the general procedure using Boc-Cys(PMB)-OH (16.55 g, 48.48 mmol, 2.0 eq.) and Boc-Val-OH (15.80 g, 72.72 mmol, 3.0 eq.). The crude product solution in THF-water (8:1) was concentrated under reduced pressure at 50°C, until the THF was removed completely and stirred strongly at 4 °C. The product was collected by filtration, washed with water and dried under reduced pressure. Cyclo[L-Cys(PMB)-L-Val] was received as a white solid (3.36 g, 10.43 mmol, 43%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.18-8.11 (m, 1H), 8.11-8.06 (m, 1H), 7.27-7.20 (m, 2H), 6.90-6.81 (m, 2H), 4.19-4.13 (m, 1H), 3.76-3.65 (m, 6H), 2.84 (dd, *J*=13.8, 4.9 Hz, 1H), 2.75 (dd, *J*=13.8, 4.1 Hz, 1H), 2.26-2.15 (m, 1H), 0.98 (d, *J*=7.1 Hz, 3H), 0.89 (d, *J*=6.8 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 166.8, 166.5, 158.2, 130.3, 130.0 (2x), 113.8 (2x), 59.4, 55.0, 54.2, 35.5, 34.4, 31.2, 18.6, 17.3. **HRMS (ESI)** calculated for $C_{16}H_{22}N_2O_3SNa$ $[M+Na]^+$: m/z 345.12433, found: 345.12463. **FT-IR (cm^{-1})**: 3188.7, 3090.4, 3055.7, 2969.8, 1655.6, 1608.8, 1582.8, 1510.5, 1444.9, 1303.2, 1241.5, 1175.9, 1107.9, 1031.7, 831.7, 787.8, 676.9. α_{22}^{D} = -20.8 (*c*=0.67, DMSO). Mp: 219 -222 °C (decomp.).

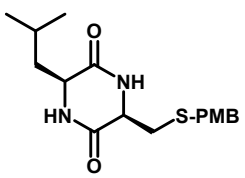
1.3.6 Cyclo[L-Cys-L-Val] (DKP 3)



Cyclo[L-Cys(PMB)-L-Val] (1.00 g, 3.10 mmol) was deprotected according to the general procedure. DKP **3** was received as a white solid (0.43 g, 2.13 mmol, 69%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (s, 1H), 8.03 (s, 1H), 4.20-4.15 (m, 1H), 3.78-3.74 (m, 1H), 2.96-2.86 (m, 1H), 2.80-2.72 (m, 1H), 2.28-2.19 (m, 1H), 2.18-2.11 (m, 1H), 0.97 (d, *J*=7.2 Hz, 3H), 0.87 (d, *J*=6.8 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 167.3, 166.4, 59.2, 55.3, 30.7, 26.7, 18.5, 17.2. **HRMS (ESI)** calculated for $C_8H_{14}N_2O_2SNa$ $[M+Na]^+$: m/z 225.06682, found: 225.06702. **FT-IR (cm^{-1}):** 3182.9, 3049.9, 2956.8, 2872.5, 2564.4, 1656.6, 1446.8, 1340.3, 1241.5, 1196.6, 1175.9, 1160.9, 1122.9, 1106.9, 973.4, 837.0, 800.3, 758.9, 737.2, 651.8. α_{22}^{D} = -89.9 (*c*=1.0, DMSO). **Mp:** 251-253°C (decomp.).

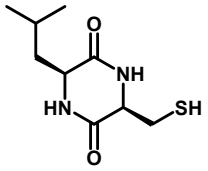
1.3.7 Cyclo[L-Cys(PMB)-L-Leu]



Cyclo[L-Cys(PMB)-L-Leu] was synthesized according to the general procedure using Boc-Leu-OH*H_2O (12.47 g, 50.00 mmol, 2 eq.) and Boc-Cys(PMB)-OH (25.61 g, 75.00 mmol, 3 eq.). The crude product solution in THF-water (8:1) was concentrated under reduced pressure at 50°C until the product did start to precipitate. Subsequently, an excess of n-hexane was added and the suspension was strongly stirred at 4°C until it became homogeneous. The product was collected by filtration and dried under reduced pressure. Cyclo[L-Cys(PMB)-L-Leu] was received as a white solid (2.27 g, 6.76 mmol, 27%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.39-8.30 (m, 1H), 8.14-8.06 (m, 1H), 7.27-7.18 (m, 2H), 6.91-6.82 (m, 2H), 4.17-4.09 (m, 1H), 3.83-3.76 (m, 1H), 3.75-3.68 (m, 5H), 2.86 (dd, *J*=13.9, 4.6 Hz, 1H), 2.71 (dd, *J*=14.0, 4.1 Hz, 1H), 1.94-1.83 (m, 1H), 1.72-1.58 (m, 2H), 0.90-0.84 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 167.9, 166.1, 158.2, 130.1, 130.0 (2x), 113.8 (2x), 55.0, 54.6, 52.6, 44.1, 35.5, 34.6, 23.4, 23.1, 21.8. **HRMS (ESI)** calculated for $C_{17}H_{24}N_2O_3SNa$ $[M+Na]^+$: m/z 359.13998, found: 359.14022. **FT-IR (cm^{-1}):** 3183.4, 3046.0, 2956.3, 2895.1, 1662.8, 1610.3, 1511.0, 1458.9, 1328.7, 1300.3, 1243.9, 1175.4, 1097.3, 1033.2, 827.3, 762.7, 690.4. α_{22}^{D} = -26.9 (*c*=1.0, DMSO). **Mp:** 177-180°C (decomp.).[3]

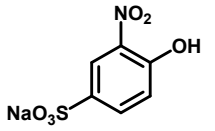
1.3.8 Cyclo[L-Cys-L-Leu] (DKP 4)



Cyclo[L-Cys(PMB)-l-Leu] (0.90 g, 2.67 mmol) was deprotected according to the general procedure. DKP 4 was received as a white solid (0.41 g, 1.89 mmol, 71%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.25 (s, 1H), 8.02 (s, 1H), 4.20-4.11 (m, 1H), 3.86-3.76 (m, 1H), 2.95-2.86 (m, 1H), 2.78-2.69 (m, 1H), 2.24-2.16 (m, 1H), 1.94-1.81 (m, 1H), 1.72-1.63 (m, 1H), 1.61-1.52 (m, 1H), 0.91-0.82 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 168.4, 166.0, 55.5, 52.4, 43.0, 26.9, 23.4, 23.0, 21.9. **HRMS (ESI)** calculated for $C_9H_{16}N_2O_2SNa$ $[M+Na]^+$: m/z 239.08247, found: 239.08257. **FT-IR (cm^{-1}):** 3480.4, 3430.7, 3185.4, 3045.5, 2956.3, 2873.4, 1662.3, 1459.9, 1386.1, 1364.9, 1324.9, 1117.6, 1094.9, 971.5, 846.6, 810.9, 681.7. α_{22}^{D} = -66.4 (*c*=1.0, DMSO). **Mp:** 221-224°C (decomp.).[3]

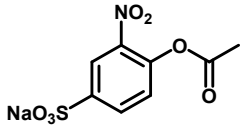
1.3.9 Sodium 4-hydroxy-3-nitrobenzenesulfonate



5.95 g of 2-Nitrophenol (42.77 mmol, 1.0 eq.) were dissolved in 50 ml dry carbon disulfide under argon atmosphere, the flask was sealed with septum and an injection needle was applied to provide hydrogen chloride removal during the reaction. The solution was cooled in an ice bath for 10 minutes and 2.84 ml of chlorosulfonic acid (42.77 mmol, 1.0 eq) were added dropwise. The reaction mixture was stirred for another 10 minutes at 4°C, allowed to reach room temperature and stirred for another 20 minutes. The resulting precipitate was collected by filtration and washed three times with hexane. The received 4-hydroxy-3-nitrobenzenesulfonic acid was evaporated to dryness, suspended in 10 ml of water and cooled in an ice bath. The suspension was treated carefully with saturated sodium bicarbonate solution under stirring until pH = 4-5 was reached. The crude product was collected by filtration and washed with acetone (x3) which was collected separately from the aqueous filtrate and disposed. The aqueous filtrate was concentrated and the former process was repeated. The resulting sodium 4-hydroxy-3-nitrobenzenesulfonate was dried under vacuum, dissolved in boiling acetic acid and filtered while hot. The product was recrystallized from acetic acid/benzene, washed with benzene and dried under vacuum to yield 8.35 g (34.63 mmol, 80%) of a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.15 (s, 1H), 8.02 (d, *J*=2.1 Hz, 1H), 7.72 (dd, *J*=8.6, 2.1 Hz, 1H), 7.09 (d, *J*=8.6 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO-d6) δ 152.3, 139.8, 135.3, 132.5, 122.2, 118.6. **Anal.** calcd. for $C_6H_4NNaO_6S$: C 29.88, H 1.67, N 5.81, S 13.29 %; found: C 29.48, H 1.59, N 5.86, S 13.18. **HRMS (ESI)** calculated for $[M]^-$: m/z 217.97648, found: 217.97636.[4]

1.3.10 Sodium 4-acetoxy-3-nitrobenzenesulfonate (ANBS)



3.00 g of sodium 4-hydroxy-3-nitrobenzenesulfonate (12.44 mmol) were suspended in 75 ml of acetic anhydride and refluxed for 15 hours. The

reaction mixture was cooled in an ice bath and the precipitate was collected by filtration. The crude product was first recrystallized from methanol and washed with ethanol, then from acetic acid/benzene and washed with benzene to yield 2.97 g (10.49 mmol, 84%) of sodium 4-acetoxy-3-nitrobenzenesulfonate (ANBS) as a white solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.22 (d, *J*=2.0 Hz, 1H), 7.98 (dd, *J*=8.4, 2.1 Hz, 1H), 7.44 (d, *J*=8.3 Hz, 1H), 2.34 (s, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 168.47, 147.19, 143.08, 140.61, 132.27, 125.26, 122.46, 20.55. **Anal.** calcd. for $C_8H_6NNaO_7S$: C 33.93, H 2.14, N 4.95, S 11.32; found: C 33.89, H 2.03, N 5.10, S 11.39. **HRMS (ESI)** calculated for $[M]^-$: m/z 259.98705, found: 259.98723.[5]

2 Nanofiber Diameter Determination with SEM Images

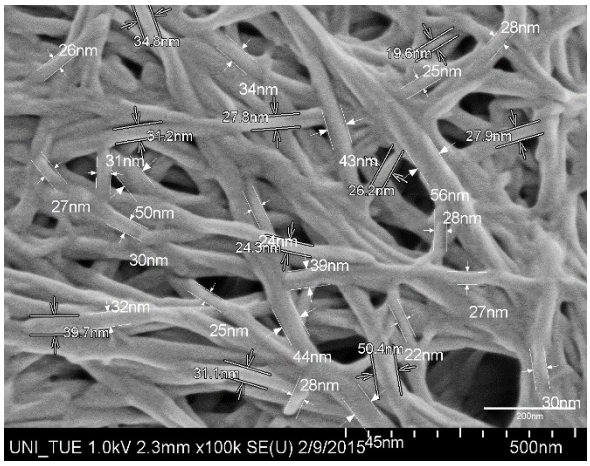


Figure S 1: SEM image of xerogel **1**; nanofiber diameter measurement represented.

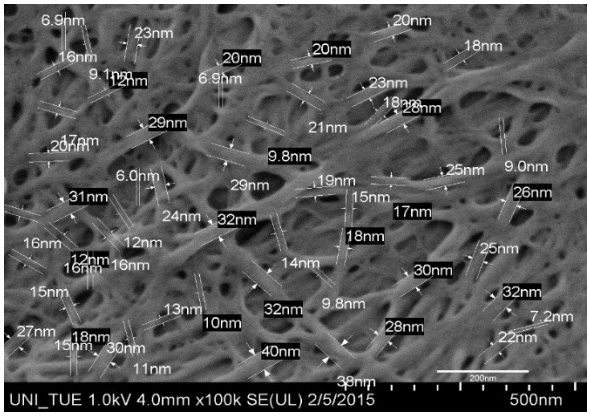


Figure S 2: SEM image of xerogel **2**; nanofiber diameter measurement represented.

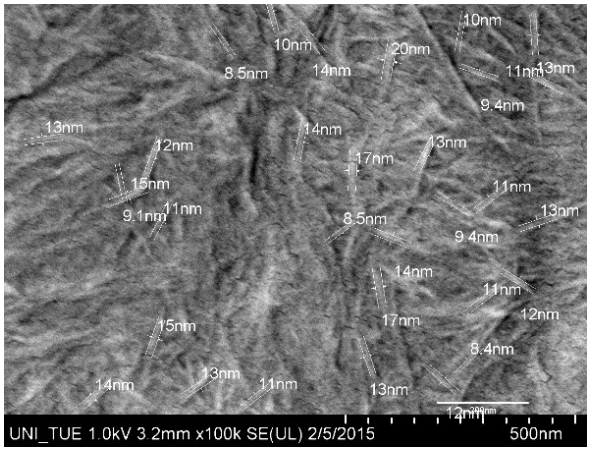


Figure S 3: SEM image of xerogel **[1+2]**; nanofiber diameter measurement represented.

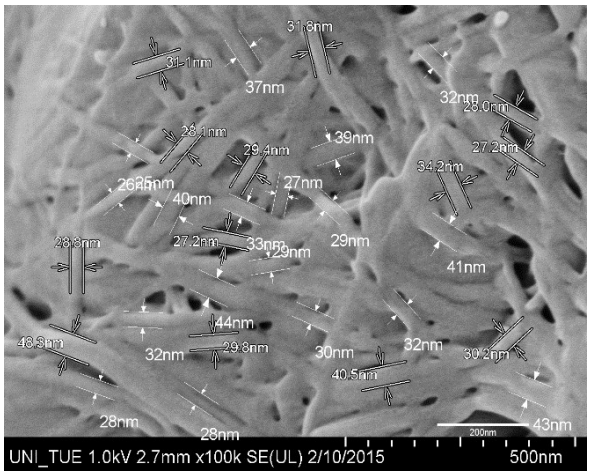


Figure S 4: SEM image of xerogel **[1+3]**; nanofiber diameter measurement represented.

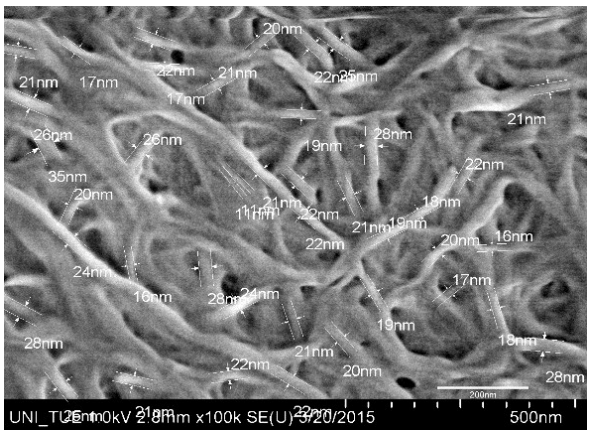


Figure S 5: SEM image of xerogel **[1+4]**; nanofiber diameter measurement represented.

3 Enzyme Kinetics Model

Table S 2: Initial rates of ANBS hydrolysis depending on the substrate concentration catalyzed by co-assembled hydrogels in 0,25 M HEPES buffer. Hydrogel **[1+2]** (1 : 1,5 (*n*/*n*); pH = 7.50; c (hydrogel) = 92 mM); c (total volume) = 18.4 mM. Hydrogel **[1+3]** (1 : 1 (*n*/*n*); pH = 7.25; c (hydrogel) = 92 mM); c (total volume) = 18.4 mM. Hydrogel **[1+4]** (1 : 1 (*n*/*n*); pH = 7.38; c (hydrogel) = 80 mM); c (total volume) = 16.0 mM.

c(ANBS) [mM]	Hydrogel **[1+2]** v_0 [mM/min]	Hydrogel **[1+3]** v_0 [mM/min]	Hydrogel **[1+4]** v_0 [mM/min]
10	0,436	-	0,83
20	0,559	1,10	1,04
40	0,665	1,33	1,20
60	0,712	1,48	1,25
90	0,727	1,53	1,30
100	0,739	1,58	1,31
120	-	1,60	-

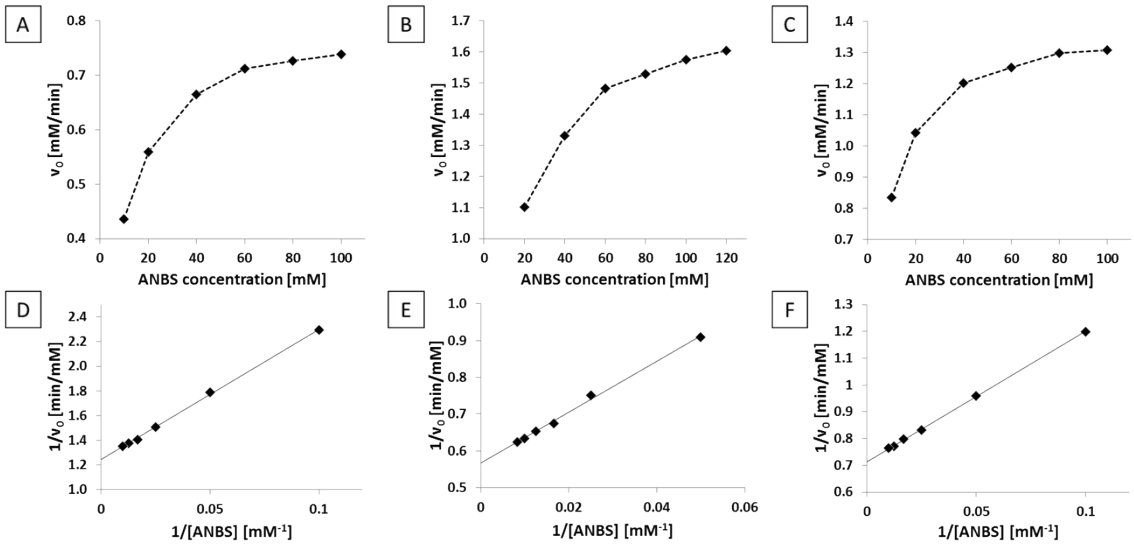


Figure S 6: Initial rates of ANBS hydrolysis plotted against the substrate concentration (top) and the corresponding Lineweaver-Burk plots (bottom). (A+D) Hydrogel **[1+2]** (1 : 1,5 (*n*/*n*); pH = 7.50; c (hydrogel) = 92 mM); c (total volume) = 18.4 mM. (B+E) Hydrogel **[1+3]** (1 : 1 (*n*/*n*); pH = 7.25; c (hydrogel) = 92 mM); c (total volume) = 18.4 mM. (C+F) Hydrogel **[1+4]** (1 : 1 (*n*/*n*); pH = 7.38; c (hydrogel) = 80 mM); c (total volume) = 16.0 mM.

4 Computational Studies

4.1 General Details

Structure calculations were performed by employing Orca 4.1 software.[6] The structures were m with Hartree-Fock/minimal basis set composite HF-3C.[7] Harmonic vibrational frequency calculations were performed at the same level of theory to characterize the nature of the stationary points along the reaction coordinates. For all optimized structures, no imaginary frequencies were found. The density-fitting RI-J approach for the Coulomb integrals was applied for the geometry optimization and frequencies calculations.[8]

4.2 Coordinates

4.2.1 [1+2]

C	1.64660503369833	0.71829933000705	-1.90593427631273
N	1.51085892125377	1.12244835856581	-0.51646986848090
C	0.49120335538741	0.65671641644035	0.29850071672167
C	-0.76141256304809	0.08532458316751	-0.43849829500061
N	-0.80426038401843	0.42266615110435	-1.84278883062057
C	0.26156562499085	0.81207849065733	-2.60898212354175
O	0.14506600487860	1.19681601682318	-3.75635481500535
O	0.52826847469930	0.71759068264233	1.50684583579751
C	2.24686600003256	-0.72674248574964	-2.01018698364000
C	2.22998722331780	-1.31462212691193	-3.41390569566369
C	3.38520267562308	-1.34044922642227	-4.18179804028309
C	3.37792773783534	-1.91167888874418	-5.44785824146546
C	2.20828512800229	-2.45806994752320	-5.95636128191051
C	1.04723897661953	-2.43256199642000	-5.19245912368440
C	1.06053683727092	-1.86422875081248	-3.92846295495896
C	-0.90084367074323	-1.45358017602799	-0.16946421449641
C	-2.15025340913867	-1.98746354175916	-0.83009240704359
C	-2.32107306535719	-2.82956289610631	-1.88261674016280
N	-3.67974727908135	-2.96195132200242	-2.16439094859990
C	-4.31049892969649	-2.21923348684530	-1.30261535880759
N	-3.41994905392255	-1.59781548466811	-0.45201522524692
H	2.30666563370193	1.41471520736752	-2.42259604715242
H	2.32392078098049	1.48850030553561	-0.04274696864257
H	-1.61218089536888	0.56368510113811	0.05093224475531
H	-1.70280454156177	0.33434037917251	-2.32678265432067
H	1.68768745165817	-1.37097449298647	-1.34485920496635
H	3.26568704304435	-0.68515745623729	-1.64249078773981
H	4.29677595417095	-0.91991274192387	-3.78656452927043
H	4.28349486926205	-1.92831780616441	-6.03614554454839
H	2.19879150475456	-2.89968833977376	-6.94193679400725
H	0.13015606795288	-2.85278690229709	-5.58074394302694

H	0.15567587453081	-1.85133268149010	-3.33990205242821
H	-0.04920309311397	-1.98946170471788	-0.56997971860710
H	-0.92178771529744	-1.61098363789290	0.90731965992073
H	-1.56520619796956	-3.33723892163097	-2.45766405124006
H	-5.38238510919346	-2.07805474508916	-1.22711445569619
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C	-4.52172734487733	-0.10163601505665	-5.29509073497136
N	-5.03302230717474	1.02446937882951	-6.05292413424848
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O	-4.62422693869664	2.62285144277786	-7.62900308922521
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H	-6.01764663810652	1.05890546976589	-6.27178483747476
C	-4.24497281422948	-1.33202499635258	-6.22937654142929
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H	-3.81192126324700	-0.97027108082032	-7.15762793563683
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H	-3.68784819668220	-2.77706531063072	-4.33442453198125
H	-5.25640032714105	-0.39498945197652	-4.54549520913612
H	-2.33744036566487	1.43817099133037	-8.57772831480508
H	-2.01707827804209	0.01814493201129	-7.58134657538323
H	-3.64540674106952	-0.96425357554104	0.30382580222999
C	-0.43200309346510	1.46890849224963	-7.58071898193529
C	0.01852431427203	2.61845883884382	-8.21996142343962
C	0.46071114814154	0.70375681513235	-6.84703578095652
C	1.34888774748983	2.99579470371671	-8.13088266276857
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C	2.24108373953952	2.22655212446780	-7.39218532556238
H	-0.67681817775375	3.21652078736218	-8.78871115948479
H	0.11894334044251	-0.18588447740971	-6.34045669340166
H	1.69086991896143	3.88789850237495	-8.63389327583496
H	2.47351646451629	0.47755937523624	-6.16727520694033
H	3.27689241370978	2.52089544857156	-7.31872570123643

4.2.2 [1+3]

C	1.51808168679845	0.50609125942352	-1.39576731152621
N	1.27898821320987	0.50893154661653	0.03598748747933
C	0.19557256100104	-0.10049023445895	0.64581783549675
C	-0.97191708971099	-0.48272904122959	-0.31322188831350
N	-0.95096725106844	0.28240603202795	-1.53743299296353
C	0.18436897403405	0.69057988751241	-2.18184952632196
O	0.17533348219148	1.20087327818711	-3.28685578619174
O	0.13572219785788	-0.30615637476712	1.83702144929831
C	2.28751359728117	-0.77769244827668	-1.85345512562246
C	2.46357849733890	-0.87164628487239	-3.36280981075565
C	3.12191780839248	0.13258666061324	-4.06417796153411
C	3.30018945181920	0.03019810804905	-5.43466777349874
C	2.82485067220024	-1.08291721991230	-6.11907507509162
C	2.16653621713943	-2.08631935776667	-5.42435597828764
C	1.98514382471633	-1.97631475650606	-4.05160401046036
C	-0.97850735798576	-2.03278190092548	-0.57124764800826
C	-2.03142593142656	-2.37313001962193	-1.59797148892286
C	-1.94435995220407	-2.50138891338098	-2.94896764312529
N	-3.21307394666411	-2.71587375836415	-3.47991228473689
C	-4.04344105011451	-2.71620625061655	-2.47753600252334
N	-3.36984223259846	-2.51057458594599	-1.29223795973998
H	2.12812868164652	1.37247393543175	-1.65142260534031
H	2.04461420597247	0.76228202227112	0.64291286650292
H	-1.89129854816945	-0.23945003054444	0.22123069335938
H	-1.84430911271107	0.38789088770125	-2.03249995505374
H	1.76623846923743	-1.65423455211575	-1.49650343065263
H	3.25974101054383	-0.76197740434301	-1.36778328698693
H	3.49572588351675	0.99744386133513	-3.53897180875097
H	3.81029732984543	0.81708978090183	-5.97086910475793
H	2.96284530809469	-1.16234429367950	-7.18720200802656
H	1.78931354964160	-2.95193446026367	-5.94896440501196
H	1.47347872939257	-2.75921161938749	-3.51424132596844
H	-0.01418019428908	-2.35032258806873	-0.94738319272127
H	-1.15599898660833	-2.54465070565767	0.37122469977012
H	-1.06397780138249	-2.44400510478601	-3.56711475163641
H	-5.11763440950286	-2.85498547345242	-2.52528579465346
C	-2.20966306362052	1.66073507701354	-6.14092698111233
N	-2.29171053775885	1.36218548323930	-4.72294794497013
C	-3.41005876295533	1.00467747475856	-4.03589600250354
C	-4.78871150279694	1.25857881560107	-4.71094832653188

N	-4.68985733653564	1.94097076440993	-5.97966268530409
C	-3.54540819310990	2.21925744159463	-6.70646139458826
O	-3.59212237125628	2.85088479885196	-7.73927283818731
O	-3.38748963257435	0.56350250777811	-2.89614654289976
C	-1.75716129195863	0.43163579164860	-7.00472426844734
H	-1.46378832205219	2.44508515960845	-6.27395991878365
H	-1.39825011064139	1.27677520652569	-4.23155095880085
H	-5.54924674681106	2.27067877154249	-6.39226965393414
C	-5.61406015455317	-0.06745652934129	-4.78017612992917
S	-5.00467965499002	-1.28806368042201	-6.00954315036964
H	-6.63759155704826	0.18835919368132	-5.04470640037539
H	-5.62837798165340	-0.50285930471746	-3.78372180945850
H	-4.13893671721996	-1.95221500927912	-5.15562849576705
H	-5.31730473931509	1.90379399344188	-4.00053021083875
H	-2.61549571232459	-0.21144689884182	-7.15556881370443
H	-3.77444580112171	-2.45337030892007	-0.36598119726549
C	-0.66084018988513	-0.37315608624677	-6.26833952900768
H	-1.05121612449416	-0.82198503436724	-5.36395073974449
H	-0.29577827457560	-1.16377479973191	-6.91205495358927
H	0.17522184638222	0.26380882102616	-6.00276994693171
C	-1.24183542374906	0.92306255212584	-8.37794586066276
H	-0.34561462208194	1.51742130314967	-8.24676997712270
H	-1.00207149497481	0.07494689564952	-9.00808065027702
H	-1.98976401375987	1.53181571909356	-8.87482771361471

4.2.3 [1+4]

C	1.47850710030332	0.50201924909328	-1.53622521535497
N	1.23924653047458	0.65579066667923	-0.11240904452897
C	0.15967770544121	0.10711218153402	0.55955360965050
C	-1.02108332259017	-0.34230496337237	-0.35205414025225
N	-0.99144440301670	0.30087227867214	-1.64316204087760
C	0.14511921571087	0.60794422240282	-2.33894821788326
O	0.13336853299350	0.97499088220466	-3.49954096534541
O	0.11166693932376	0.00616980838943	1.76471945858018
C	2.23704754053863	-0.82876518937435	-1.85497911983508
C	2.54564022295821	-0.99478241688103	-3.33524783497589
C	3.45362600631817	-0.14825228068369	-3.96094622685852
C	3.76093341969903	-0.31650791910291	-5.30172228341751
C	3.16118902928677	-1.33703481363769	-6.03061137864201
C	2.24885353839546	-2.17931083132692	-5.41237908197909
C	1.94251374985078	-2.00514246250405	-4.06930509986620

C	-1.06466734629375	-1.90962907964498	-0.46283964638807
C	-2.14042628097165	-2.32148944703151	-1.43999551554543
C	-2.09547251539826	-2.49878214743011	-2.78795138177836
N	-3.37306421982840	-2.77582498236602	-3.26563014563870
C	-4.16860064094444	-2.76453322975583	-2.23548515114698
N	-3.46245914436103	-2.49069801945172	-1.08305270612425
H	2.09808951565713	1.33013871600503	-1.88148627606805
H	2.01159256378052	0.95598637735665	0.46399236838662
H	-1.93215356347306	-0.02951212640313	0.16019050901942
H	-1.88660474838412	0.37065178440942	-2.14185856026298
H	1.65342265823816	-1.66944841218875	-1.50832602818283
H	3.16190646061345	-0.81714384238701	-1.28555246244494
H	3.92606301308199	0.64010113683923	-3.39598519182061
H	4.46890008072462	0.34486042129266	-5.77959739709833
H	3.40272467306569	-1.47063701375341	-7.07452204644644
H	1.77516883204692	-2.97155425080132	-5.97341904258332
H	1.23759124076948	-2.66575335784509	-3.58943335389859
H	-0.11120332636699	-2.28209048494368	-0.81620709355945
H	-1.24130542358017	-2.32713214172316	0.52495677242369
H	-1.24136253118350	-2.43202233379424	-3.44082119631249
H	-5.23809074611081	-2.94183133971965	-2.23921668377559
C	-2.41709993303089	1.56237368431517	-6.29417871381452
N	-2.41134033056882	1.17913091383462	-4.89237008630293
C	-3.49506805506614	0.90631310537132	-4.11678093150394
C	-4.91214775288887	1.15063389054633	-4.70853692953523
N	-4.89750052109886	1.82220364465318	-5.98578910839818
C	-3.81249597328456	2.00038445654240	-6.82290836899372
O	-3.92827419190739	2.48949940442842	-7.92475500488377
O	-3.41596612601344	0.50830819195693	-2.96386152811655
C	-1.88565331982364	0.44261630305657	-7.23555452810267
H	-1.77343727545277	2.43555035355742	-6.41385543436104
H	-1.49223881147953	1.06163933990125	-4.45813967994234
H	-5.78805693843355	2.09670063026023	-6.37254057561486
C	-0.43734010885813	-0.00533598549686	-6.92496580247341
C	-5.70370792957513	-0.19786280993492	-4.73480462900809
S	-5.04078681453522	-1.42707397775902	-5.92929202091185
H	-6.73458369472504	0.02062002512167	-5.00385682719349
H	-5.70166085568093	-0.60971737219978	-3.72832677788577
H	-4.23741410031718	-2.09694863728581	-5.01987039248546
H	-5.41280336141007	1.78929804084652	-3.97361780393816
C	-0.03216469619443	-1.08806654889696	-7.95649316885740

H	-0.40436046975220	-0.44138285689019	-5.93312573837931
C	0.55726962930490	1.17949885275122	-6.96942325035702
H	-1.93409046825465	0.82727430634316	-8.24824399446056
H	-0.70592749363537	-1.93522825459316	-7.90493837729301
H	0.97580750187682	-1.43285916194549	-7.76776848076259
H	-0.07328601242384	-0.67914853935013	-8.95892100825461
H	0.48125412128622	1.70051181178572	-7.91625855379707
H	1.56990306278718	0.81301497534995	-6.85418930068344
H	0.36567355528972	1.87988937602027	-6.16608408020875
H	-2.54890035225954	-0.41157569939251	-7.17312522220129
H	-3.83613164064384	-2.41410010165347	-0.14526887044718

5 NMR Spectra

5.1 ^{1}H and ^{13}C NMR spectra of ANBS and DKPs 1-4

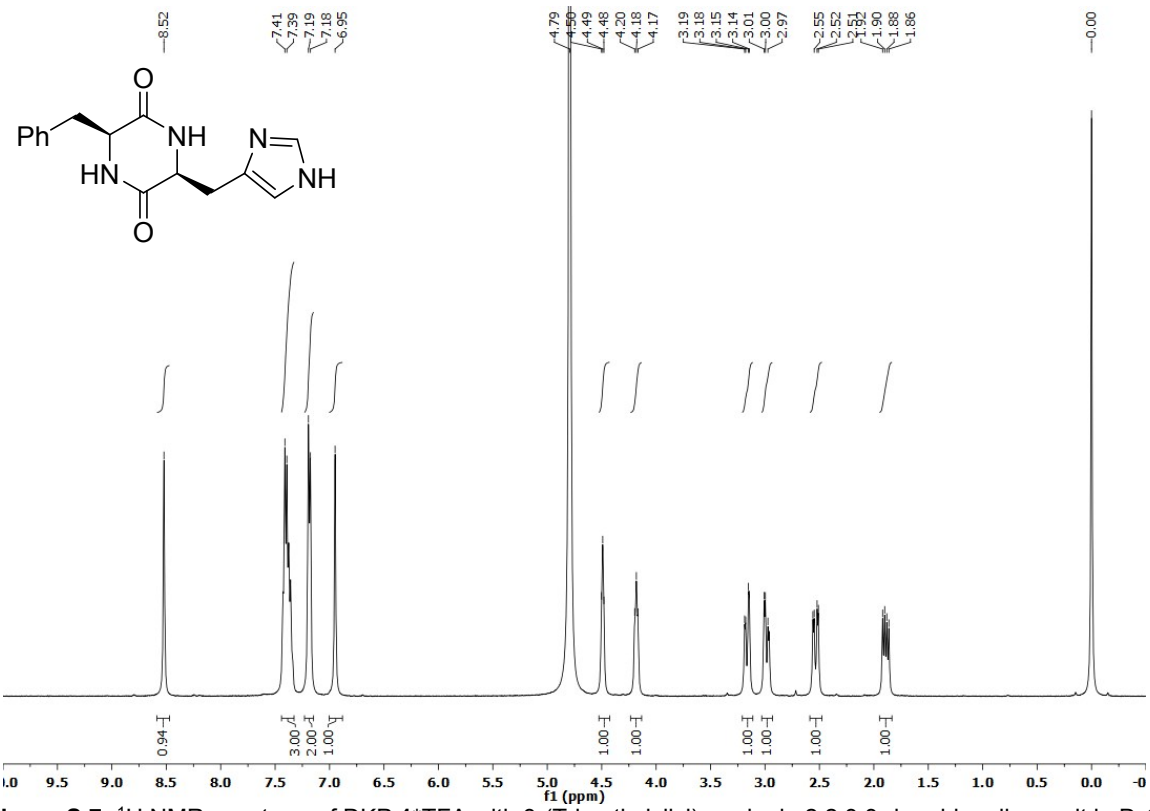

Figure S 7: ^{1}H NMR spectrum of DKP **1***TFA with 3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt in D_2O.

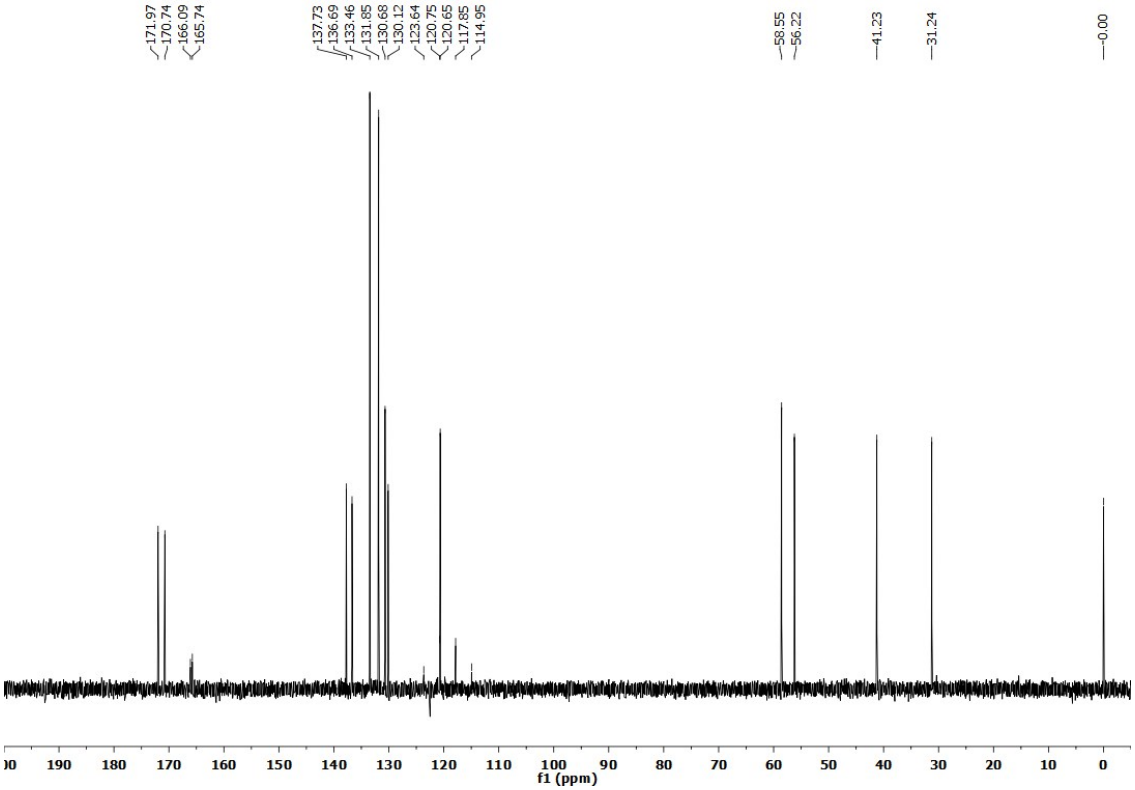

Figure S 8: ^{13}C NMR spectrum of DKP **1***TFA with 3-(Trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt in D_2O.

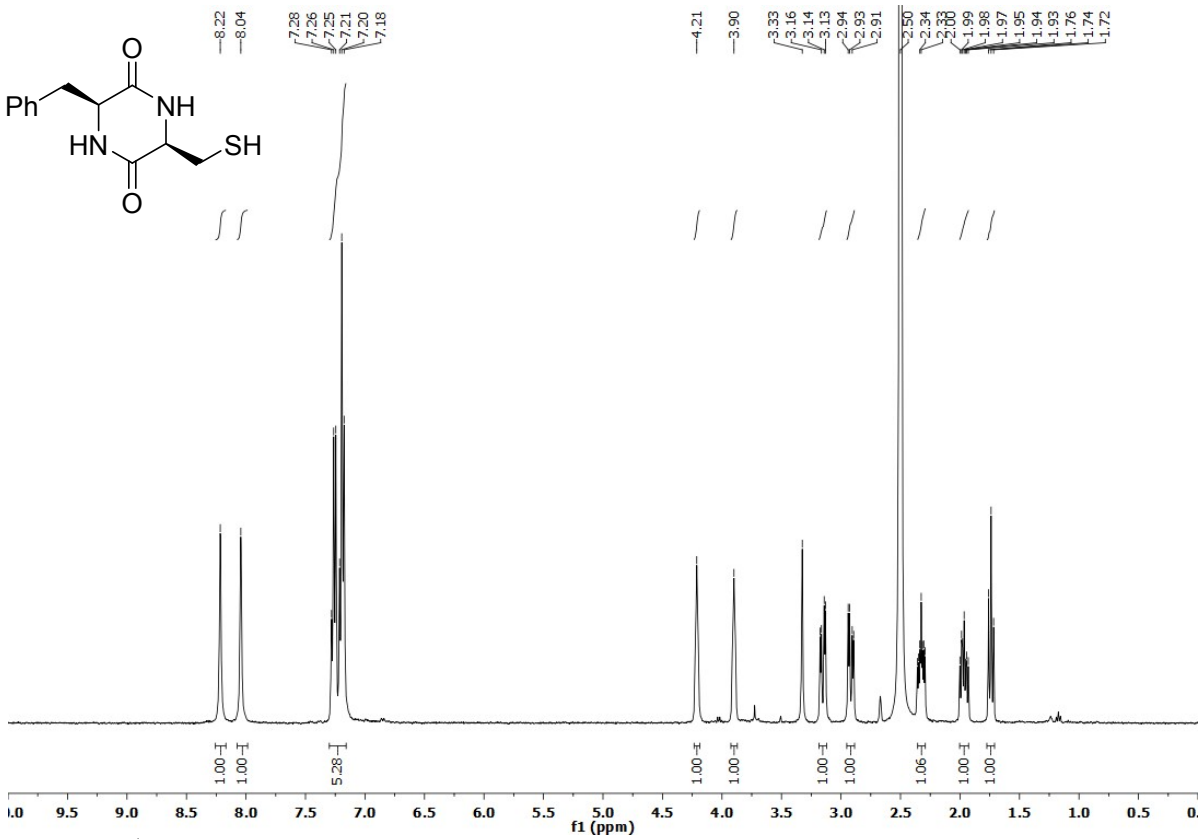

Figure S 9: ^{1}H NMR spectrum of DKP **2** DMSO-d_7.

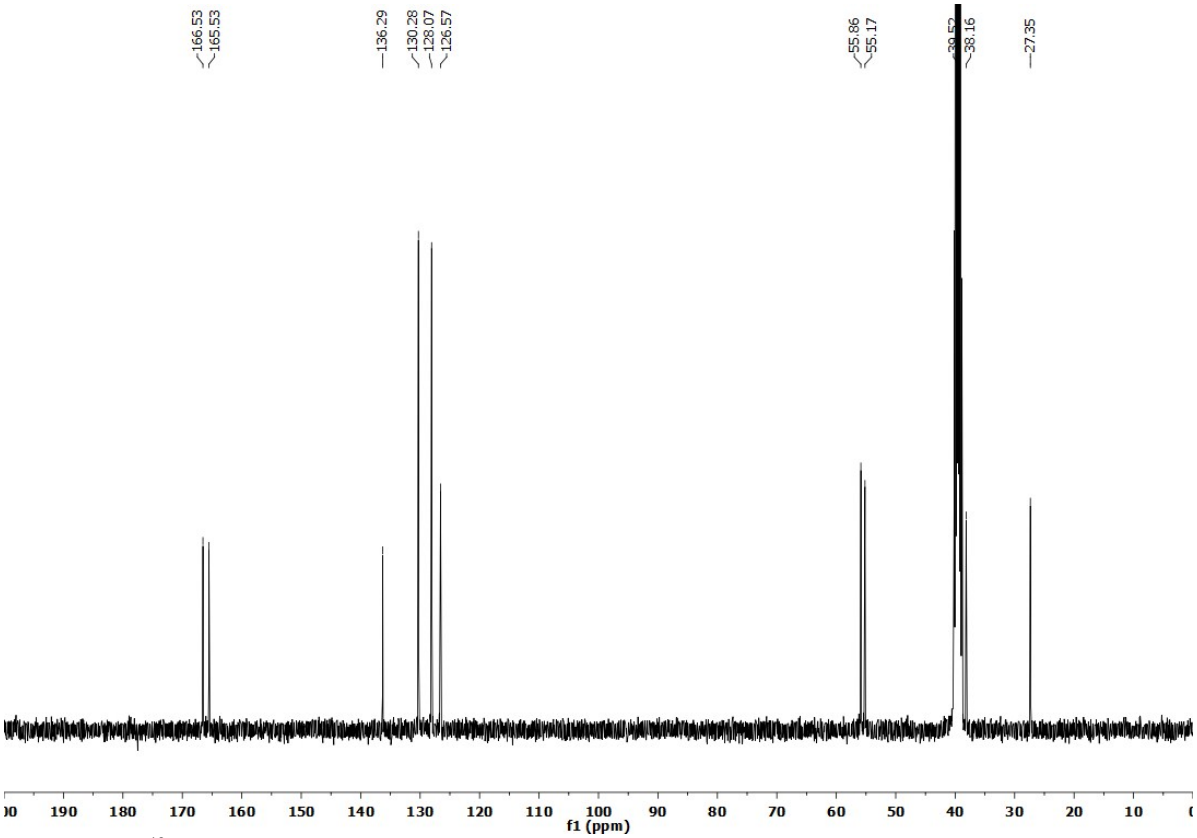

Figure S 10: ^{13}C NMR spectrum of DKP **2** DMSO-d_7.

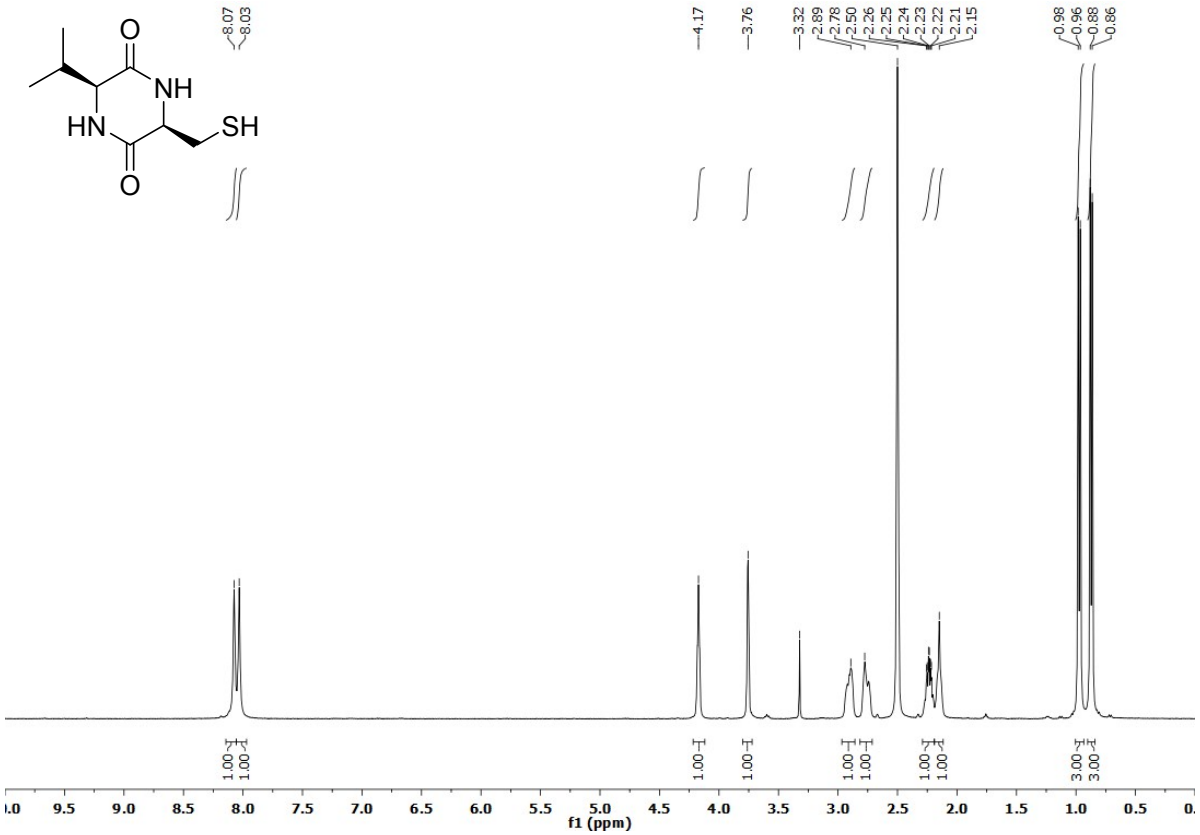

Figure S 11: ^{1}H NMR spectrum of DKP **3** DMSO-d_7.

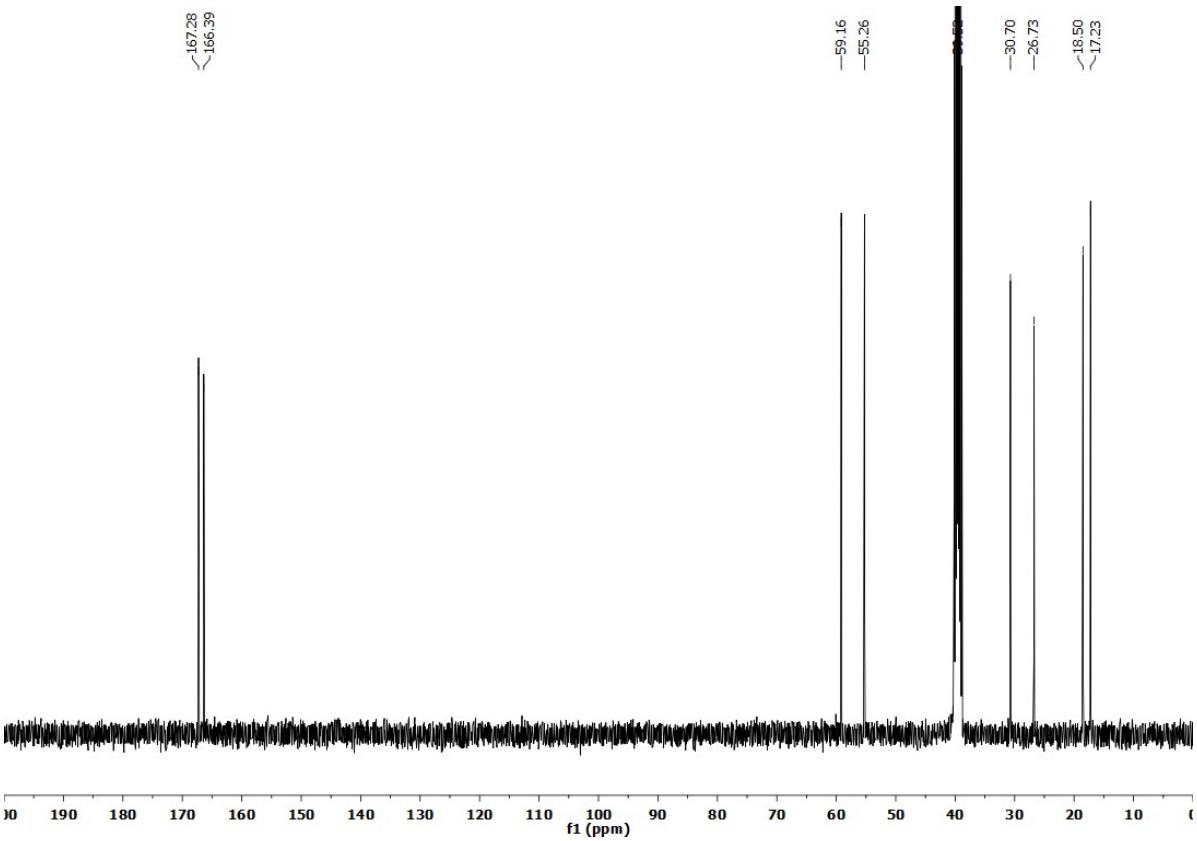

Figure S 12: ^{13}C NMR spectrum of DKP **3** DMSO-d_7.

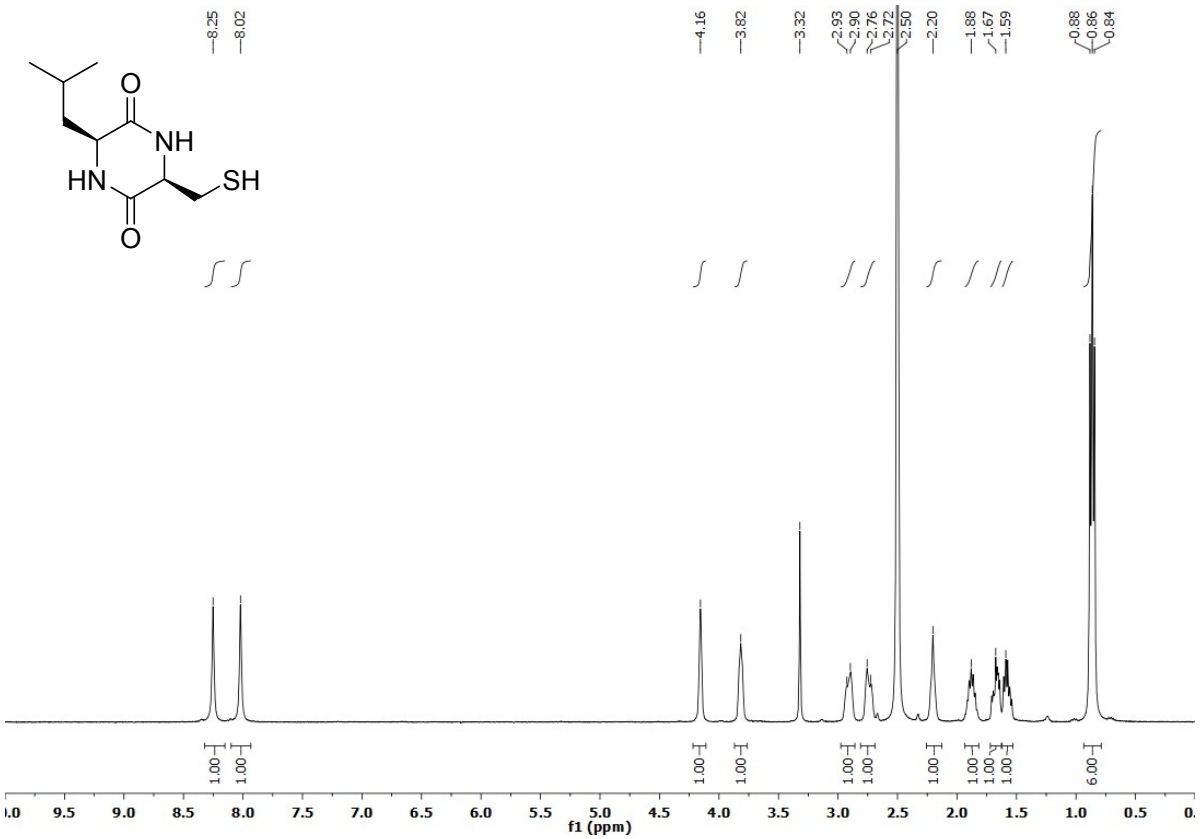

Figure S 13: ^{1}H NMR spectrum of DKP **4** DMSO-d_7.

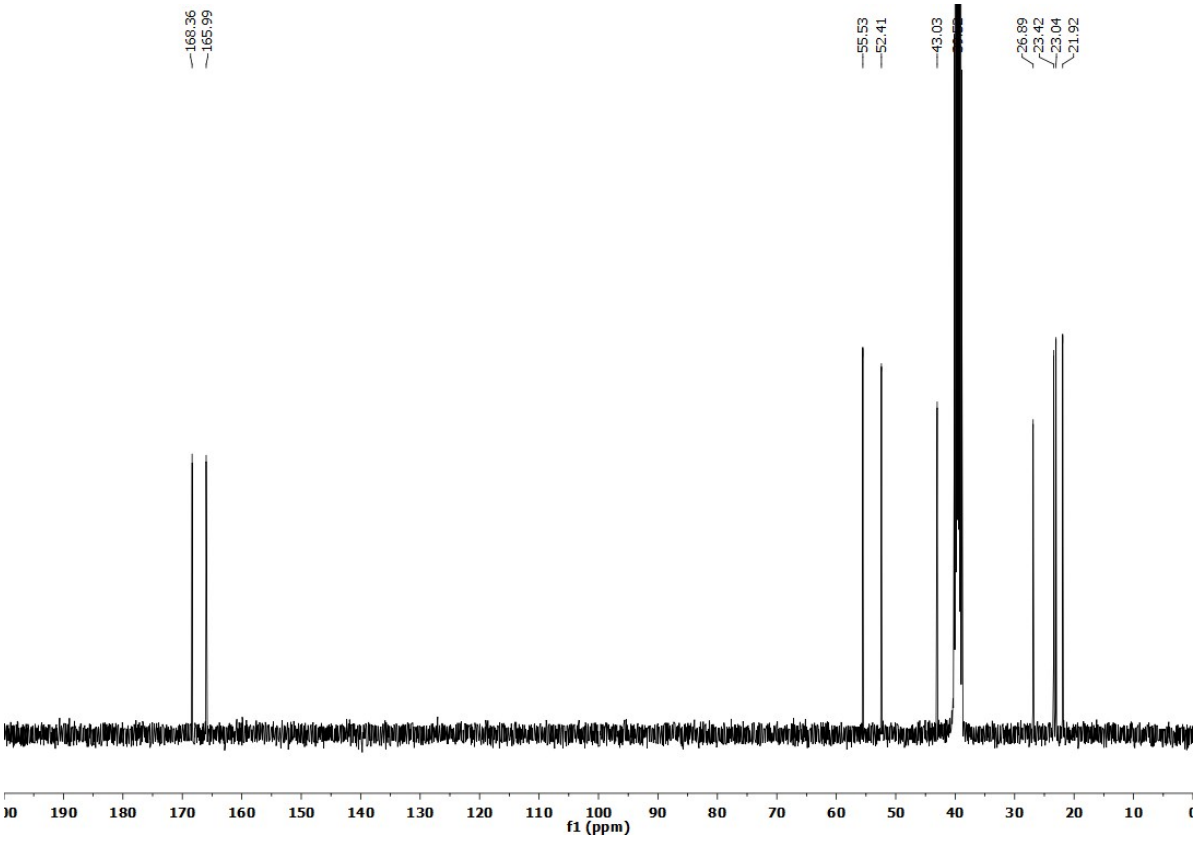

Figure S 14: ^{13}C NMR spectrum of DKP **4** DMSO-d_7.

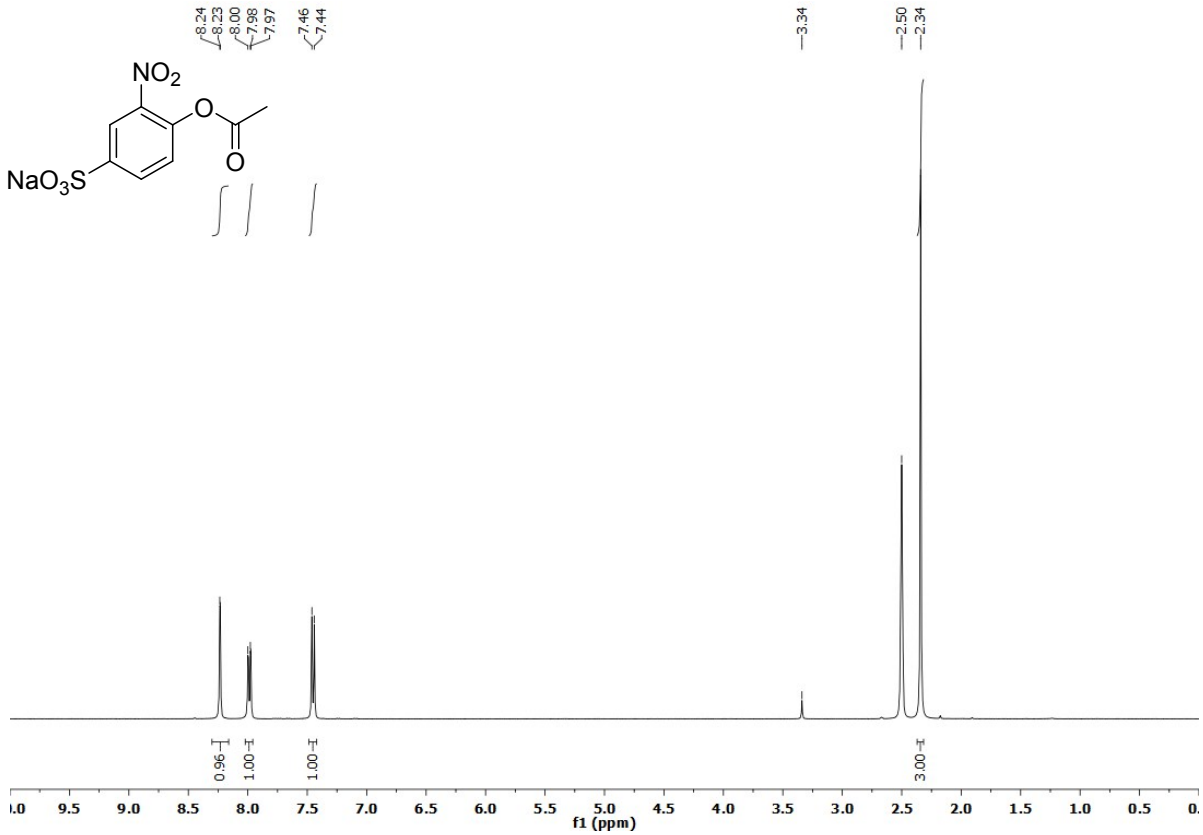

Figure S 15: ^{1}H NMR spectrum of **ANBS** DMSO-d_7.

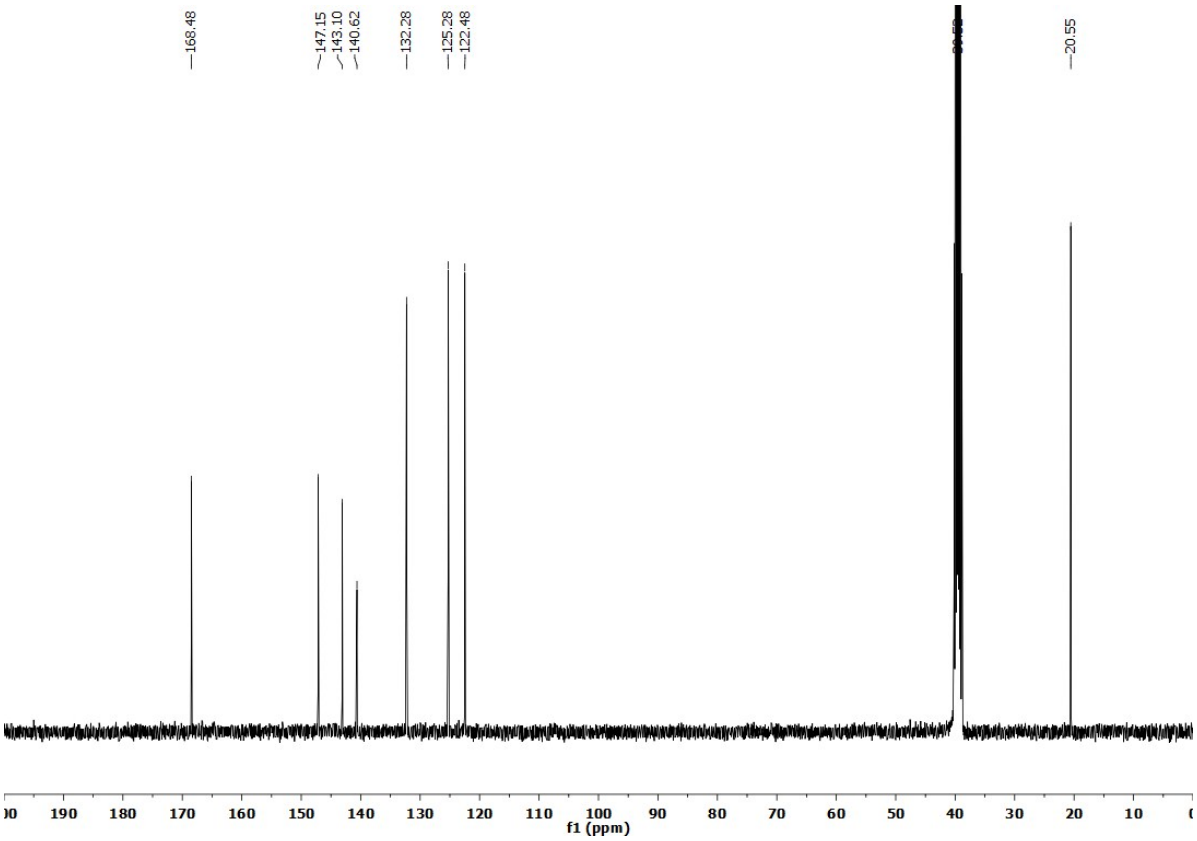

Figure S 16: ^{13}C NMR spectrum of **ANBS** DMSO-d_7.

5.2 ^{1}H HR-MAS NOE spectra of co-assembled hydrogels

For ^{1}H HR-MAS NOESY experiments 75 µl of the warm DKP solution in D_2O were transferred to a spinner and cooled in an ice bath for 20 minutes. The hydrogel in the spinner was allowed to reach room temperature for 20 minutes and subsequently measured at room temperature.

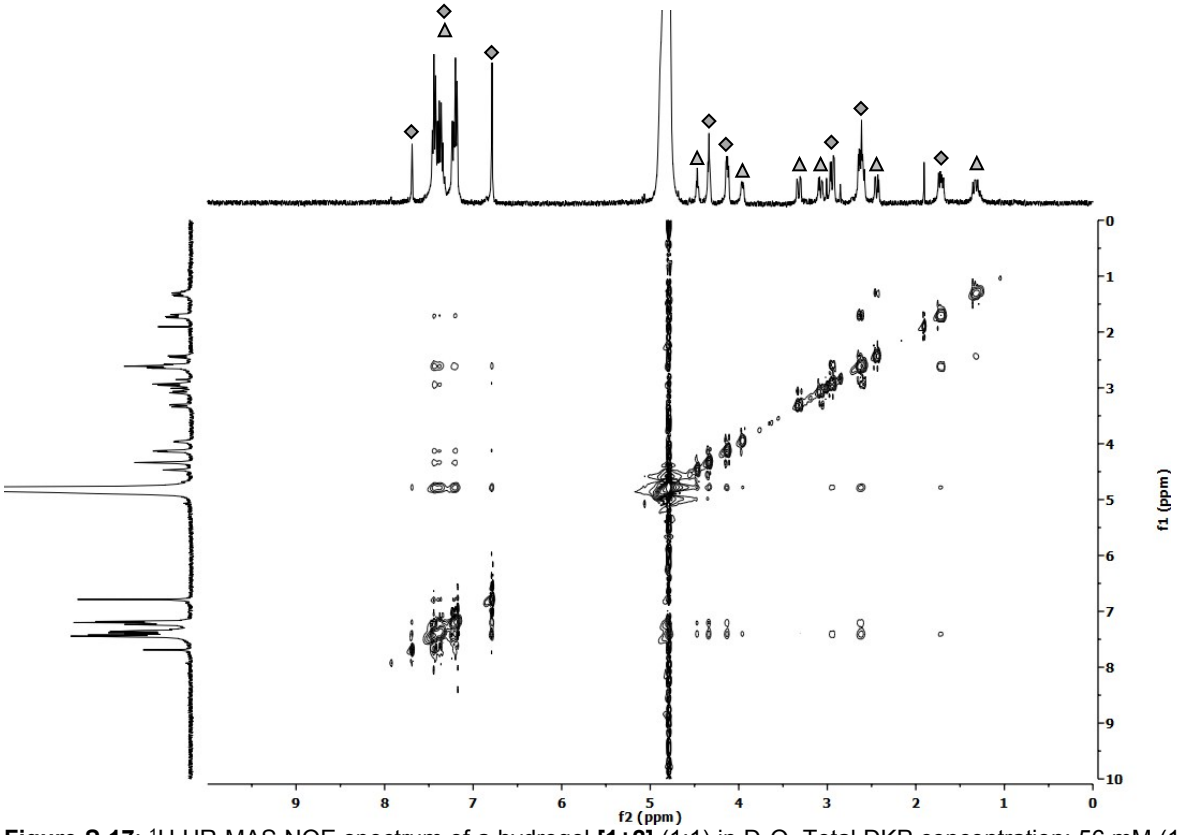

Figure S 17: ^{1}H HR-MAS NOE spectrum of a hydrogel **[1+2]** (1:1) in D_2O. Total DKP concentration: 56 mM (1.50 wt%); Spinning frequency: 2.5 kHz; Diamonds indicate DKP **1** protons; Triangles indicate DKP **2** protons.

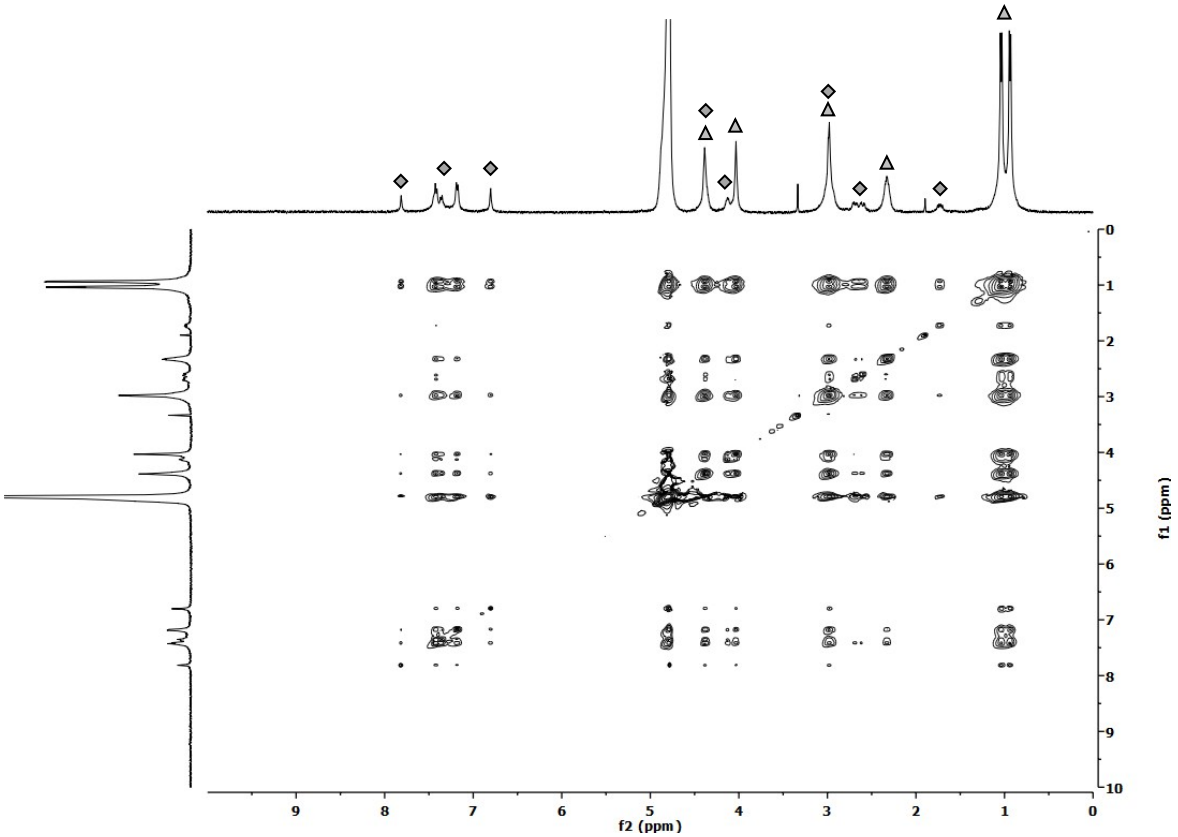

Figure S 18: ^{1}H HR-MAS NOE spectrum of hydrogel **[1+3]** (1:1) in D_2O. Total DKP concentration: 80 mM (1.94 wt%); Spinning frequency: 2.5 kHz; Diamonds indicate DKP **1** protons; Triangles indicate DKP **3** protons.

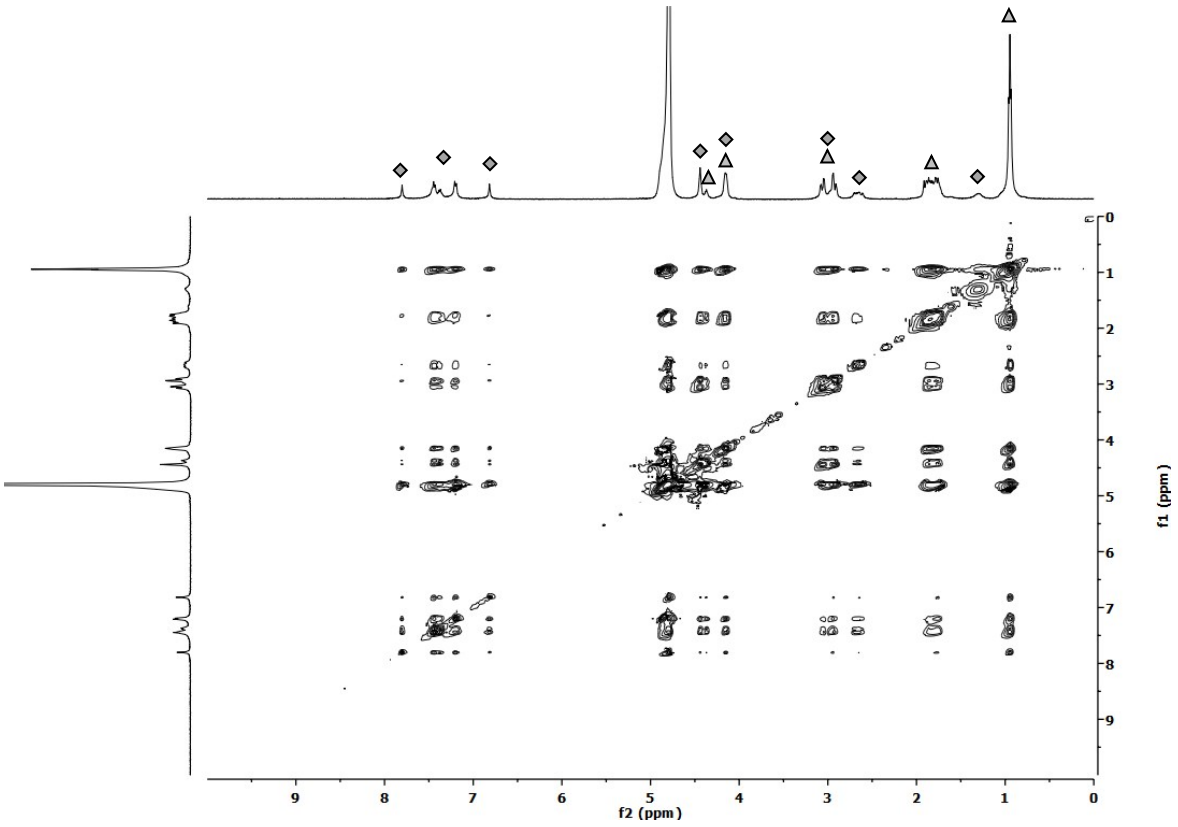

Figure S 19: ^{1}H HR-MAS NOE spectrum of hydrogel **[1+4]** (1:1) in D_2O. Total DKP concentration: 80 mM (2.00 wt%); Spinning frequency: 4.0 kHz; Diamonds indicate DKP **1** protons; Triangles indicate DKP **4** protons.

5.3 ^{1}H NOE spectra of blended DKP solutions

As reference for ^{1}H HR-MAS NOESY experiments, less concentrated DKP solutions in DMF-d_7/D_2O (1:1) were measured correspondingly.

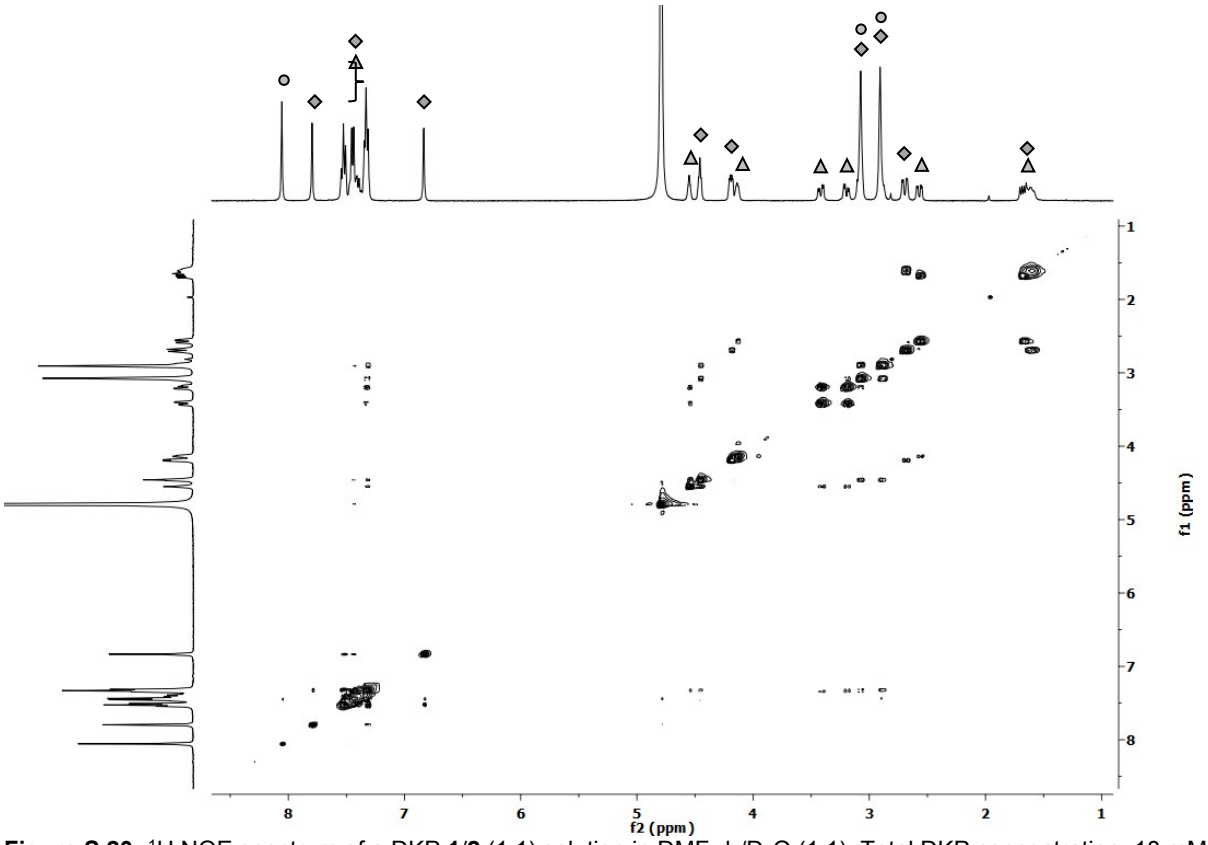

Figure S 20: ^{1}H NOE spectrum of a DKP **1**/**2** (1:1) solution in DMF-d_7/D_2O (1:1). Total DKP concentration: 18 mM; Diamonds indicate DKP **1** protons; Triangles indicate DKP **2** protons. Circles indicate DMF protons.

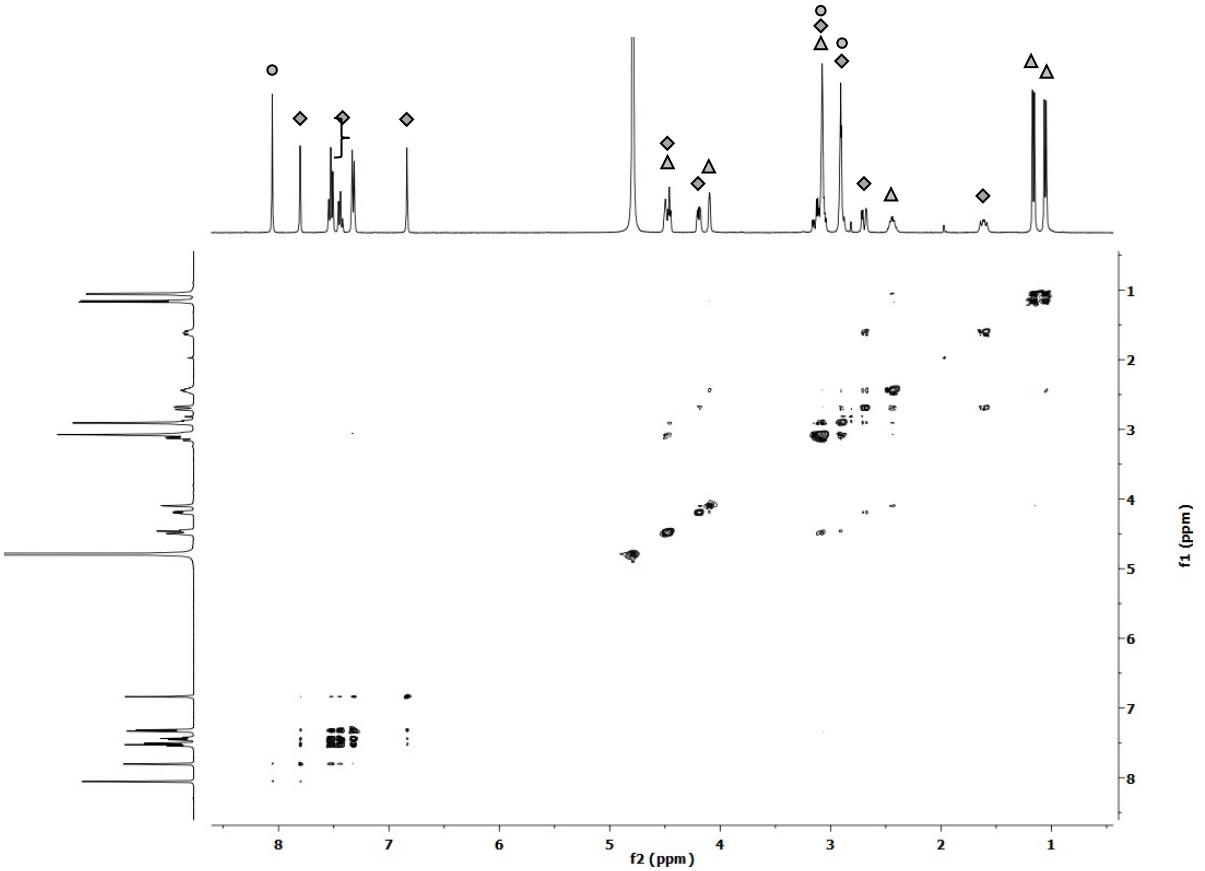

Figure S 21: ^{1}H NOE spectrum of a DKP **1**/**3** (1:1) solution in DMF-d_7/D_2O (1:1). Total DKP concentration: 18 mM; Diamonds indicate DKP **1** protons; Triangles indicate DKP **3** protons.

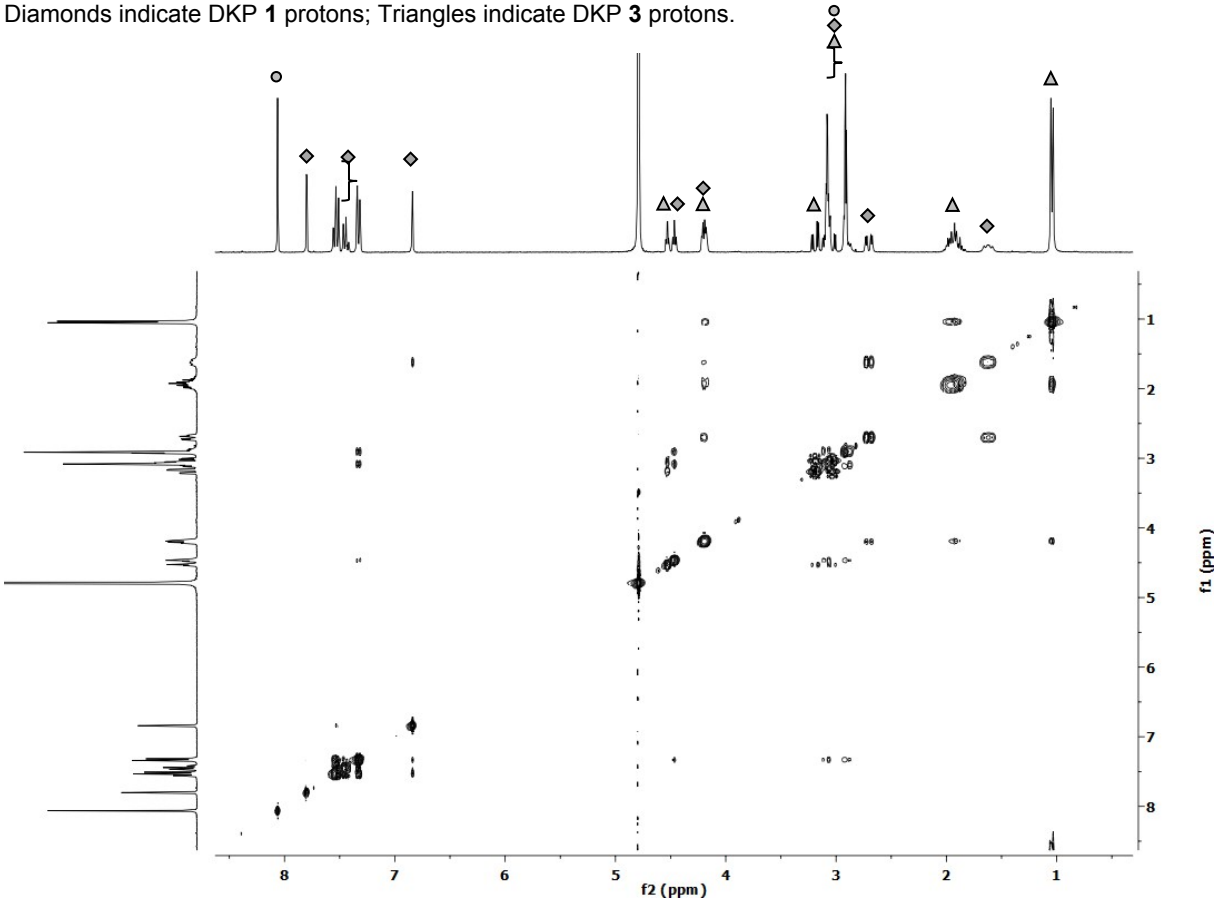

Figure S 22: ^{1}H NOE spectrum of a DKP **1**/**4** (1:1) solution in DMF-d_7/D_2O (1:1). Total DKP concentration: 18 mM; Diamonds indicate DKP **1** protons; Triangles indicate DKP **4** protons.

6 References

[1] D. Le Nguyen, R. Seyer, A. Heitz, B. Castro, *J. Chem. Soc., Perkin Trans. 1* **1985**, 1025.
[2] A. J. Kleinsmann, B. J. Nachtsheim, *Chem. Commun.* **2013**, *49*, 7818.
[3] T. Furukawa, T. Akutagawa, H. Funatani, T. Uchida, Y. Hotta, M. Niwa, Y. Takaya, *Bioorg. Med. Chem.* **2012**, *20*, 2002.
[4] A. Arcelli, C. Concilio, *J. Org. Chem.* **1996**, *61*, 1682.
[5] T. C. Bruice, J. Katzhendler, L. R. Fedor, *J. Am. Chem. Soc.* **1968**, *90*, 1333.
[6] a) F. Neese, *WIREs Comput. Mol. Sci.* **2012**, *2*, 73; b) F. Neese, *WIREs Comput. Mol. Sci.* **2018**, *8*, e1327.
[7] R. Sure, S. Grimme, *J. Comput. Chem.* **2013**, *34*, 1672.
[8] a) K. Eichkorn, O. Treutler, H. Öhm, M. Häser, R. Ahlrichs, *Chem. Phys. Lett.* **1995**, *240*, 283; b) K. Eichkorn, F. Weigend, O. Treutler, R. Ahlrichs, *Theor. Chem. Acc.* **1997**, *97*, 119.