Photoswitchable inhibitors of human β-glucocerebrosidase

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Electronic Supplementary Information (ESI)

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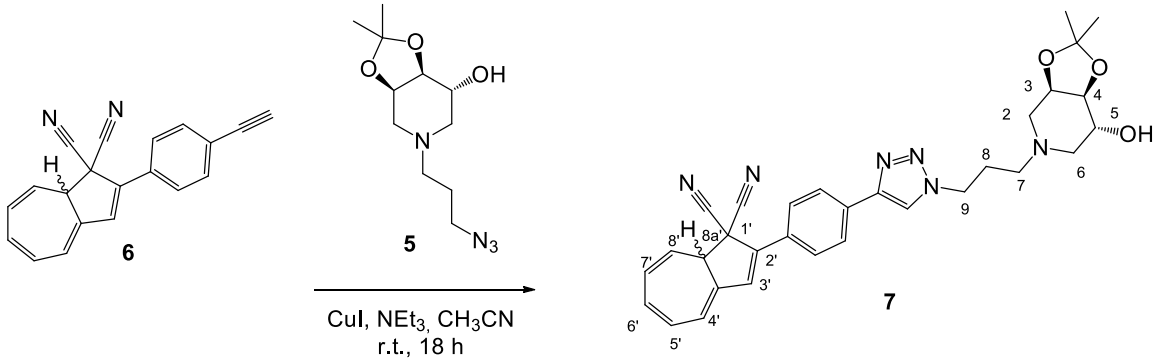
General methods: Commercial reagents were used as received. All reactions were carried out under magnetic stirring and monitored by TLC on 0.25 mm silica gel plates (Merck F254). Column chromatographies were carried out on Silica Gel 60 (32–63 μm) or on silica gel (230–400 mesh, Merck). Yields refer to spectroscopically and analytically pure compounds unless otherwise stated. NMR spectra were recorded on a Varian Gemini 200 MHz, a Varian Mercury 400 MHz or on a Varian INOVA 400 MHz instrument at 25 °C. Chemical shifts are reported relative to $CDCl_3$ (^{13}C: δ = 77.0 ppm), or to CD_3OD (^{13}C: δ = 49.0 ppm). Integrals are in accordance with assignments, coupling constants are given in Hz. For detailed peak assignments 2D spectra were measured (COSY, HSQC). IR spectra were recorded with a IRAffinity-1S SHIMADZU or IRAffinity-1 SHIMADZU system spectrophotometers. Optical rotation measurements were performed on a JASCO DIP-370 polarimeter. ESI-MS spectra were recorded with a Thermo Scientific™ LCQ fleet ion trap mass spectrometer. High Resolution Mass spectrometry (HRMS) were recorded with an ESP-MALDI-FT-ICR spectrometer equipped with a 7 T magnet (calibration of the instrument was done with NaTFA cluster ions) using Electrospray Ionization (ESI). Spectroscopic measurements were performed in a 1-cm path length quartz cuvette. UV-vis absorption spectra were recorded on a Varian Cary 50 UV-Vis spectrophotometer equipped with a Peltier heat exchange unit by scanning wavelengths from 800 to 200 nm at a rate of 600 nm/min. Photoswitching experiments were performed using ThorLabs M365L2 LED lamp for irradiation at 365 nm and ThorLabs M340L4 LED lamp for irradiation at 340 nm. Reaction and purification of light sensitive materials were performed shading the flask or the column with aluminium foil.

Compound **5** has been synthesized according to literary procedure.[1] Compound **6** has been synthesized according to literary procedure.[2] Compound **9** has been synthesized according to literary procedure.[3] Compound **10** has been synthesized according to literary procedure.[4] Compound **15** has been synthesized according to literary procedure.[5]

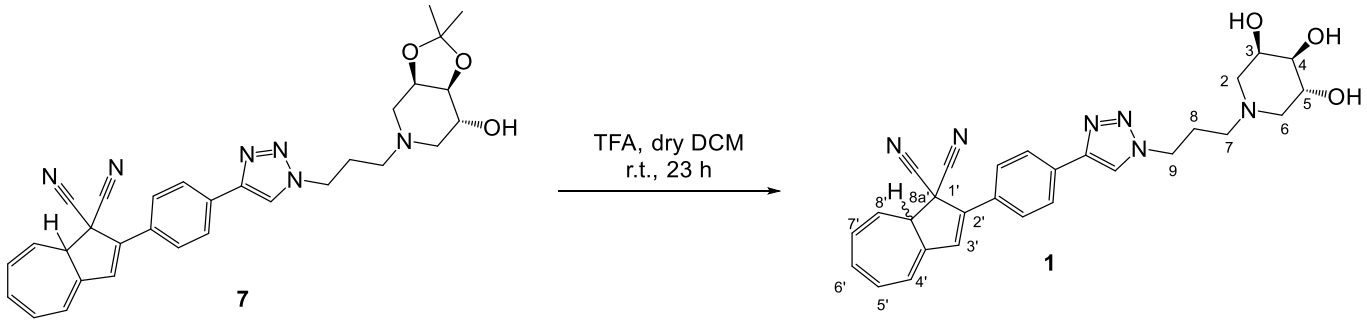
[1] C. Matassini, S. Mirabella, X. Ferhati, C. Faggi, I. Robina, A. Goti, E. M. Clavijo, A. J. Moreno-Vargas, F. Cardona, *Eur. J. Org. Chem*. **2014**, 5419.
[2] H. Lissau, S. L. Broman, M. Jevric, A. Ø. Madsen, M. B. Nielsen, *Aust. J. Chem.* **2014**, *67*, 531.
[3] K. Rustler, S. Pockes, B. König, *ChemMedChem* **2019**, *14*, 636.
[4] A. Vlasceanu, M. Koerstz, A. B. Skov, K. V. Mikkelsen, M. B. Nielsen, *Angew. Chem*. **2018**, *130*, 6177.
[5] S. H. Hwang, T. L. Choi, *Org. Lett.* **2020**, *22*, 2935.

Synthesis of compound 7



To a nitrogen-degassed solution of alkyne **6**[2] (55 mg, 0.20 mmol) in CH_3CN (8 ml), the azide **5**[1] (50 mg, 0.20 mmol), CuI (37 mg, 0.20 mmol), and degassed NEt_3 (54 µl, 0.39 mmol) were added. The mixture was stirred at room temperature for 18 hours until the disappearance of starting materials was assessed by a TLC control (AcOEt:MeOH 10:1). The mixture was concentrated under vacuum and subsequently the crude was treated with 'Quadrasil MP®' resin keeping the mixture under stirring at room temperature in the minimum amount of MeOH for 1 hour (1 g of resin for each mmol of copper). The crude was purified by FCC in the dark (SiO_2, DCM:MeOH 10:1) to give 72 mg of **7** (0.13 mmol, 69%) as a bright orange powder and indistinguishable mixture of diastereoisomers. **7**: R_f 0.21 (DCM:MeOH 10:1); δ_H (400 MHz, $CDCl_3$): 7.98 (s, 1H, triazole), 7.92 (d, J= 8.4 Hz, 2H, Ar), 7.76 (d, *J* = 8.4 Hz, 2H, Ar), 6.91 (s, 1H, H-3'), 6.60-6.52 (m, 1H), 6.49-6.43 (m, 1H), 6.36-6.26 (m, 2H), 5.81 (dd, *J* = 3.7, 10.2 Hz, 1H, H-8'), 4.48 (t, *J* = 6.6 Hz, 2H, CH_2-9), 4.29 (q, *J* = 4.9 Hz, 1H, H-3), 4.03 (t, *J* = 4.8 Hz, 1H, H-4), 4.00-3.94 (m, 1H, H-5), 3.81-3.75 (m, 1H, H-8a'), 2.73-2.62 (m, 2H, CH_2-7), 2.57-2.48 (m, 1H, Ha-2), 2.47-2.27 (m, 3H, Hb-2, CH_2-6), 2.16-2.03 (m, 2H, CH_2-8), 1.51 (s, 3H, Me), 1.37 (s, 3H, Me). δ_C (100 MHz, $CDCl_3$): 146.6, 139.6, 138.7, 132.4, 132.2, 131.0 (2C), 129.9, 127.8, 126.8 (2C), 126.3 (2C), 121.2 (triazole), 121.2, 119.5 (C-8'), 115.2 (C≡N), 112.8 (C≡N), 109.4 ($O\underline{C}(CH_3)_2$), 77.7 (C-4), 72.5 (C-3), 68.8 (C-5), 55.5 (C-7), 55.1 (C-2), 53.1 (C-6), 51.1 (C-1'), 47.9 (C-8a'), 45.1 (C-9), 28.5 (Me), 27.3 (C-8), 26.5 (Me). HRMS (ESP^+) calcd for $C_{31}H_{32}N_6O_3$ [$(M+H)^+$]: *m/z* = 537.26087; found 537.26286. UV-Vis (CH_2Cl_2): $\lambda_{max\text{-}\mathbf{DHA}}$/nm, ($\varepsilon$/$M^{-1}$ cm^{-1}) = 362 (2.50 x 10^4). UV-Vis (CH_2Cl_2): $\lambda_{max\text{-}\mathbf{VHF}}$/nm, ($\varepsilon$/$M^{-1}$ cm^{-1}) = 476 (2.40 x 10^4).

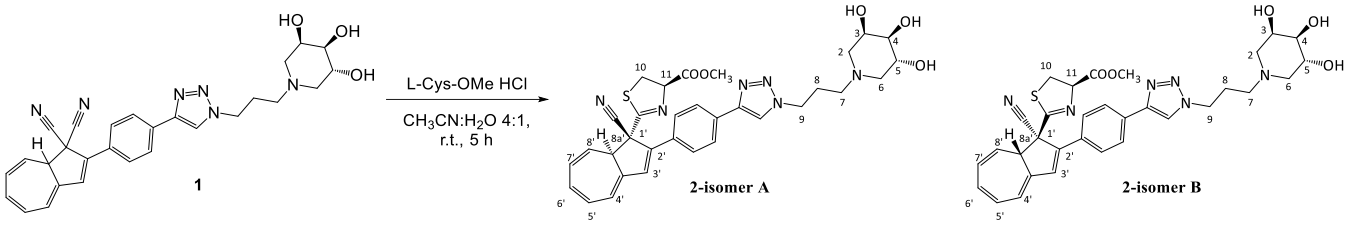
Synthesis of DHA 1



A solution of **7** (48 mg, 0.09 mmol) in DCM (1.5 mL) was left stirring with CF_3COOH (10 drops) at room temperature for 23 h until the disappearance of starting material was assessed by a TLC control (AcOEt:MeOH 5:1). Then, the crude mixture was concentrated and the crude residue was purified by FCC in the dark (SiO_2, DCM:MeOH 10:1) to give 32 mg of **1** (0.06 mmol, 71%) as an orange powder and

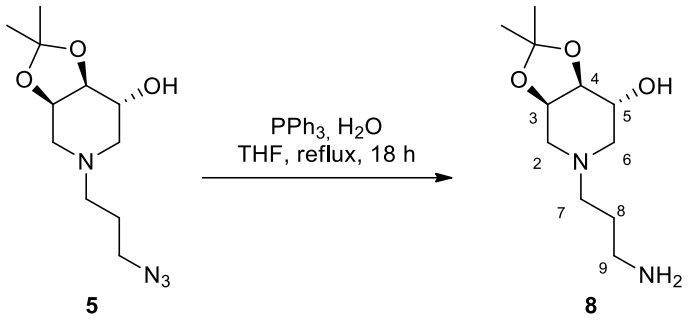
indistinguishable mixture of diastereoisomers. **1**: *Rf* 0.36 (DCM:MeOH 10:1); δ_H (400 MHz, CD_3OD): 8.52 (s, 1H, triazole), 7.97 (d, *J* = 7.4 Hz, 2H, Ar), 7.89 (d, *J* = 7.3 Hz, 2H, Ar), 7.23 (s, 1H, H-3'), 6.65-6.56 (m, 1H), 6.53-6.43 (m, 2H), 6.37-6.29 (m, 1H), 5.80 (dd, *J* = 10.2, 3.7 Hz, 1H, H-8'), 4.55 (t, *J* = 6.6 Hz, 2H, CH_2-9), 3.92 (br s, 1H, H-3), 3.86-3.78 (m, 2H, H-8a', H-5), 3.42 (br s, 1H, H-4), 2.88-2.67 (m, 2H, Ha-2, Ha-6), 2-46-2.27 (m, 3H, CH_2-7, Hb-2), 2.22-2.00 (m, 3H, CH_2-8, Hb-6). δ_c (100 MHz, CD_3OD): 147.6, 140.6, 140.3, 134.3 (C-3'), 133.1, 132.1, 131.9, 131.6, 128.8, 128.0 (2C), 127.0 (2C), 123.6 (triazole), 122.7, 120.6 (C-8'), 116.5 (C≡N), 114.2 (C≡N), 79.3 (C-4), 69.6 (C-5), 69.2 (C-3), 57.6 (2C, C-2, C-6), 55.0 (C-7), 52.4 (C-8a'), 49.3 (C-9), 46.5 (C-1'), 28.2 (C-8). HRMS (ESP+, *m/z*): calcd for $C_{28}H_{28}N_6O_3$ [M+H$^+$]: 497.22957, found 497.22950. UV-Vis (7% DMSO in water): $\lambda_{max\text{-}\mathbf{DHA}}$/nm, ($\varepsilon$/$M^{-1}$ cm^{-1}) = 370 (2.24 x 10^4). UV-Vis (7% DMSO in water): $\lambda_{max\text{-}\mathbf{VHF}}$/nm, ($\varepsilon$/$M^{-1}$ cm^{-1}) = 502 (2.31 x 10^4).

Synthesis of DHA 2



L-Cys-OMe·HCl (6 mg, 0.04 mmol) was added to a solution of compound **1** (17 mg, 0.03 mmol) in CH_3CN: H_2O 4:1 (5.5 mL). The mixture was stirred at room temperature for 5 hours until the disappearance of starting material **1** was assessed by a TLC control (DCM:MeOH 8:1). Then, the crude mixture was concentrated to dryness and the crude residue was purified by FCC in the dark (SiO_2, DCM:MeOH 8:1) to give 8 mg of compound **2** (0.01 mmol, 38%) as a 1:1 mixture of two inseparable diastereoisomers in the form of yellow powder. **2**: *Rf* 0.42 (DCM:MeOH 8:1). δ_H (400 MHz, CD_3OD): 8.49 (s, 1H, triazole, isomer A or B), 8.48 (s, 1H, triazole, isomer B or A), 7.89-7.80 (m, 3H, Ar), 7.76-7.71 (m, 1H, Ar), 7.18 (s, 1H, H-3', isomer A or B), 7.17 (s, 1H, H-3', isomer B or A), 6.63-6.56 (m, 1H), 6.48-6.39 (m, 2H), 6.30-6.23 (m, 1H), 5.85 (t, 1H, *J* = 3.6 Hz, 1H, H-8', isomer A or B), 5.83 (t, 1H, *J* = 3.6 Hz, 1H, H-8', isomer B or A), 5.35 (dd, *J* = 5.6, 9.6 Hz, 1H, H-11, isomer A or B), 5.29 (t, *J* = 8.9 Hz, 1H, H-11, isomer B or A), 4.55 (t, *J* = 6.8 Hz, 2H, CH_2-9), 3.92 (quint, *J* = 2.7 Hz, 1H, H-3), 3.84 (s, 3H, Me, isomer A or B), 3.76 (s, 3H, Me, isomer A or B), 3.83-3.78 (m, 1H, H-5), 3.74-3.62 (m, 2H, H-10), 3.45-3.38 (m, 2H, H-8a', H-4), 2.88-2.69 (m, 2H, Ha-2, Ha-6), 2.48-2.28 (m, 3H, CH_2-7, Hb-2), 2.23-2.05 (m, 3H, CH_2-8, Hb-6). δ_C (^{13}C 100 MHz and gHSQC ^{1}H/^{13}C 100/400 MHz, CD_3OD): 176.5 (C=O, isomer A or B), 175.8 (C=O, isomer B or A), 171.9 (C-S, isomer A or B), 171.8 (C-S, isomer B or A), 147.9, 147.8, 145.4 (triazole, isomer A or B), 145.3 (triazole, isomer B or A), 141.9 (isomer A or B), 141.8 (isomer B or A), 134.0 (isomer A or B), 133.8 (isomer B or A), 133.3 (C-3', isomer A or B), 133.2 (C-3', isomer B or A), 132.5 (isomer A or B), 132.2 (isomer A or B) 132.1 (isomer A or B), 131.3 (isomer A or B), 131.2 (isomer A or B), 128.2 (Ar, isomer A or B), 128.1 (Ar, isomer A or B), 126.9 (Ar, isomer A or B), 126.8 (Ar, isomer A or B), 123.3 (triazole), 121.8 (isomer A or B), 121.6 (isomer A or B), 121.2 (C-8', isomer A or B), 121.1 (C-8', isomer B or A), 118.0 (C≡N, isomer A or B), 117.9 (C≡N, isomer B or A), 79.1 (C-11, isomer A or B), 78.5 (C-11, isomer A or B), 75.4 (C-4), 69.7 (C-3), 69.3 (C-5), 60.4 (C-1', isomer A or B), 60.3 (C-1', isomer B or A), 57.6 (C-2), 55.1 55.0 (2C, C-6, C-7), 53.3 (Me, isomer A or B), 53.2 (Me, isomer A or B), 52.7 (C-8a', isomer A or B), 52.5 (C-8a', isomer B or A), 48.4 (C-9), 37.6 (C-10, isomer A or B), 37.3 (C-10, isomer B or A), 28.3 (C-8) (Some carbons are missing due to overlap). HRMS (ESP+, *m/z*): calcd for $C_{32}H_{34}N_6O_5S$ [M+H$^+$]: 615.23842, found: 615.23911. UV-Vis (2% DMSO in water): $\lambda_{max\text{-}\mathbf{DHA}}$/nm, ($\varepsilon$/$M^{-1}$ cm^{-1}) = 371 (1.91 x 10^4). UV-Vis (2% DMSO in water): $\lambda_{max\text{-}\mathbf{VHF}}$/nm, ($\varepsilon$/$M^{-1}$ cm^{-1}) = 385 (8.71 x 10^4).

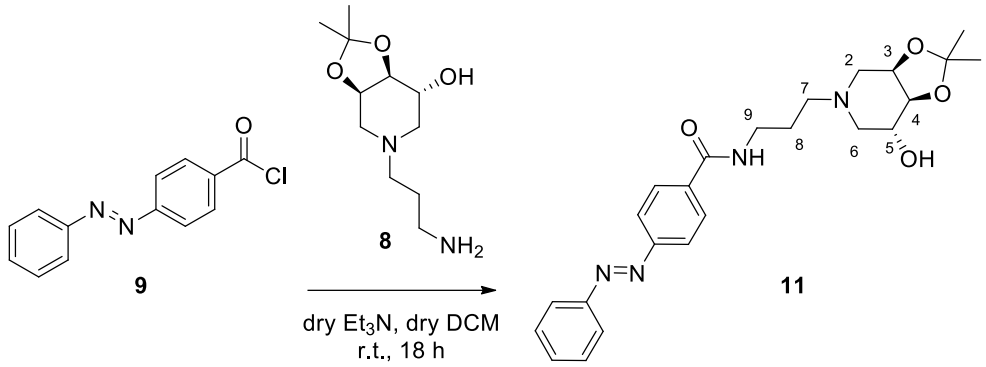
Synthesis of compound 8



5 8

PPh_3 (129 mg, 0.49 mmol) and water (15 µl) were added to a solution of **5** (105 mg, 0.41 mmol) in THF (7 ml), and the mixture was stirred at reflux for 18 hours. The disappearance of the starting material **5** was assessed via TLC (CH_2Cl_2:MeOH:(NH_4OH (6%) 10:1:0.1) and the reaction was concentrated under vacuum. The crude residue was purified by flash column chromatography on silica gel (gradient eluent CH_2Cl_2:MeOH:NH_4OH (6%) from 10:1:0.1 to 1:1:0.1) to give 85 mg of **8** (0.37 mol, 90%) as a waxy compound. **8**: *Rf* 0.05 (CH_2Cl_2:MeOH:NH_4OH (6%) 5:1:0.1). $[\alpha]_D^{25}$ = − 22.4 (c = 1, $CHCl_3$). δ_H (400 MHz, CD_3OD): 4.31 (dd, *J* = 4.2, 9.1 Hz, 1H, H-3), 3.92-3.80 (m, 2H, H-4, H-5), 3.05-2.89 (m, 3H, Ha-2, CH_2-9), 2.76-2.69 (m, 1H, Ha-6), 2.66-2.47 (m, 3H, Hb-2, CH_2-7), 2.20 (dd, *J* = 8.3, 11.5 Hz, 1H, Hb-6), 1.89-1.70 (m, 2H, CH_2-8), 1.48 (s, 1H, Me), 1.35 (s, 1H, Me). δ_C (50 MHz, CD_3OD): 110.3 (O<u>C</u>$(CH_3)_2$), 79.9 (C-4), 74.4 (C-3), 70.4 (C-5), 57.8 (C-6), 57.2 (C-7), 54.8 (C-2), 41.3 (C-9), 28.6 (Me), 26.6 (Me), 25.9 (C-8). HRMS (ESP+, *m/z*): calcd for $C_{11}H_{22}N_2O_3$ [M+H$^+$]: 231.17032, found: 231.17017. IR ($CDCl_3$): *v* = 3670, 3358, 3007, 2988, 2957, 2939, 2831, 2486, 1625, 1582, 1468, 1456, 1381, 1242, 1144, 1061 cm^{-1}.

Synthesis of compound 11

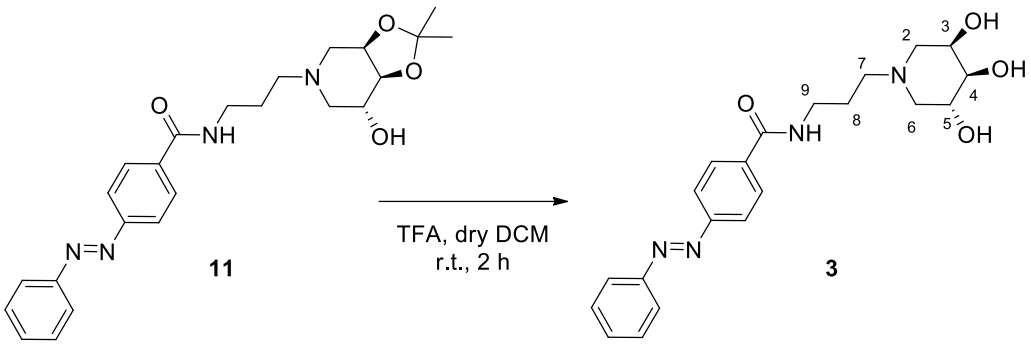


9 11

To a solution of amine **8** (35 mg, 0.15 mmol) in dry DCM (1.5 ml), dry NEt_3 (42 µl, 0.30 mmol) and diazo acyl chloride **9**[3] (45 mg, 0.18 mmol) were added. The reaction mixture was stirred at room temperature for 18 hours until the disappearance of **8** was assessed by a TLC control (DCM:MeOH:NH_4OH (6%) 5:1:0.1). The mixture was concentrated under vacuum and then the crude was purified by FCC in the dark (SiO_2, DCM:MeOH 20:1) to give 43 mg of **11** (0.10 mmol, 64%) as an orange waxy compound. **11**: *Rf* 0.17 (DCM:MeOH 20:1). δ_H (400 MHz, $CDCl_3$): 7.97-7.86 (m, 6H, Ar), 7.55-7.44 (m, 3H, Ar), 7.29 (t, *J* = 4.8 Hz, 1H, N<u>H</u>), 4.27 (q, *J* = 5.6 Hz, 1H, H-3), 4.02 (t, *J* = 4.6 Hz, 1H, H-4), 3.99-3.93 (m, 1H, H-5), 3.54 (q, *J* = 6.2 Hz, 2H, CH_2-9), 3.05 (br s, 1H, OH), 2.70 (dd, *J* = 5.5, 12.1 Hz, 1H, Ha-2), 2.62 (dd, *J* = 2.8, 11.8 Hz, 1H, Ha-6), 2.57-2.42 (m, 4H, Hb-2, Hb-6, CH_2-7), 1.79 (quint, *J* = 6.3 Hz, 2H, CH_2-8), 1.44 (s, 3H, Me), 1.33 (s, 3H, Me). δ_C (100 MHz, $CDCl_3$): 167.2 (NH<u>C</u>O), 154.2 (Ar), 152.6 (Ar), 136.5 (Ar), 131.6 (Ar), 129.2 (2C, Ar), 128.2 (2C, Ar), 123.2 (2C, Ar), 122.9 (2C, Ar), 109.3 (O<u>C</u>$(CH_3)_2$), 77.3 (C-4), 72.3 (C-3), 68.1 (C-5), 56.1 (C-7), 55.9 (C-6), 55.4

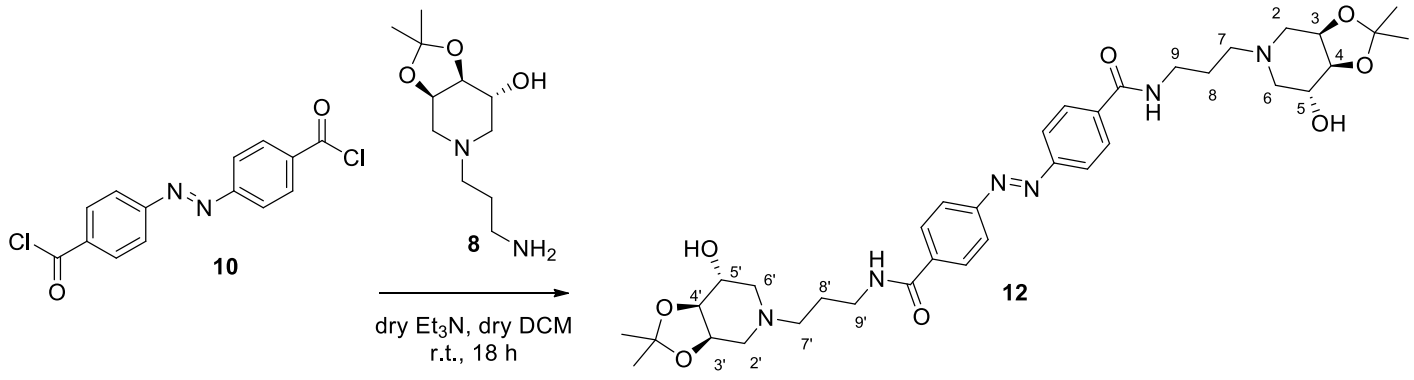
(C-2), 39.2 (C-9), 28.4 (Me) 26.4 (Me), 26.0 (C-8). HRMS (ESP+, *m/z*): calcd for $C_{24}H_{30}N_4O_4$ [M+H$^+$]: 439.23398, found: 439.23433.

Synthesis of *trans*-3



A solution of **11** (41 mg, 0.09 mmol) in dry DCM (3 mL) was left stirring with TFA (77 µl, 0.09) at room temperature for 2 h until the disappearance of starting material was assessed by a TLC control (DCM:MeOH:NH_4OH (6%) 5:1:0.1). Then, the crude mixture was concentrated and the crude residue was purified by FCC in the dark (SiO_2, DCM:MeOH:NH_4OH (6%) 8:1:0.1) to give 27 mg of **3** (0.07 mmol, 73%, >95% *trans* isomer) as an orange waxy compound. **3**: *Rf* 0.4 (DCM:MeOH:NH_4OH (6%) 8:1:0.1). δ_H (400 MHz, CD_3OD): 8.05-7.97 (m, 4H, Ar), 7.96-7.92 (m, 2H, Ar), 7.60-7.53 (m, 3H, Ar), 3.97-3.92 (m, 1H, H-3), 3.84 (td, *J* = 3.9, 7.7 Hz, 1H, H-5), 3.55-3.42 (m, 3H, H-4, CH_2-9), 2.93-2.76 (m, 2H, Ha-2, Ha-6), 2.57 (t, *J* = 7.0 Hz, 2H, CH-7), 2.44-2.33 (m, 1H, Hb-2), 2.28-2.11 (m, 1H, Hb-6), 1.87 (quint, *J* = 6.9 Hz, 2H, CH_2-8). δ_C (50 MHz, CD_3OD): 169.3 (NHCO), 155.6 (Ar), 154.0 (Ar), 137.7 (Ar), 132.8 (Ar), 130.3 (2C, Ar), 129.5 (2C, Ar), 124.0 (2C, Ar), 123.7 (2C, Ar), 75.0 (C-4), 69.6 (C-5), 68.9 (C-3), 57.9, 57.5 (2C, C-2, C-6), 56.7 (C-7), 39.6 (C-9), 26.9 (C-8). HRMS (ESP+, *m/z*): calcd for $C_{21}H_{26}N_4O_4$ [M+H$^+$]: 399.20268, found: 399.20298. UV-Vis (4% DMSO in water): λ_{max}/nm, (ε/M^{-1} cm^{-1}) = 325 (1.50 x 10^4), 428 (shoulder). IR (neat) ν = 3323, 2925, 2809, 1627, 1542, 1443, 1298, 1070, 1009, 862, 840 cm^{-1}.

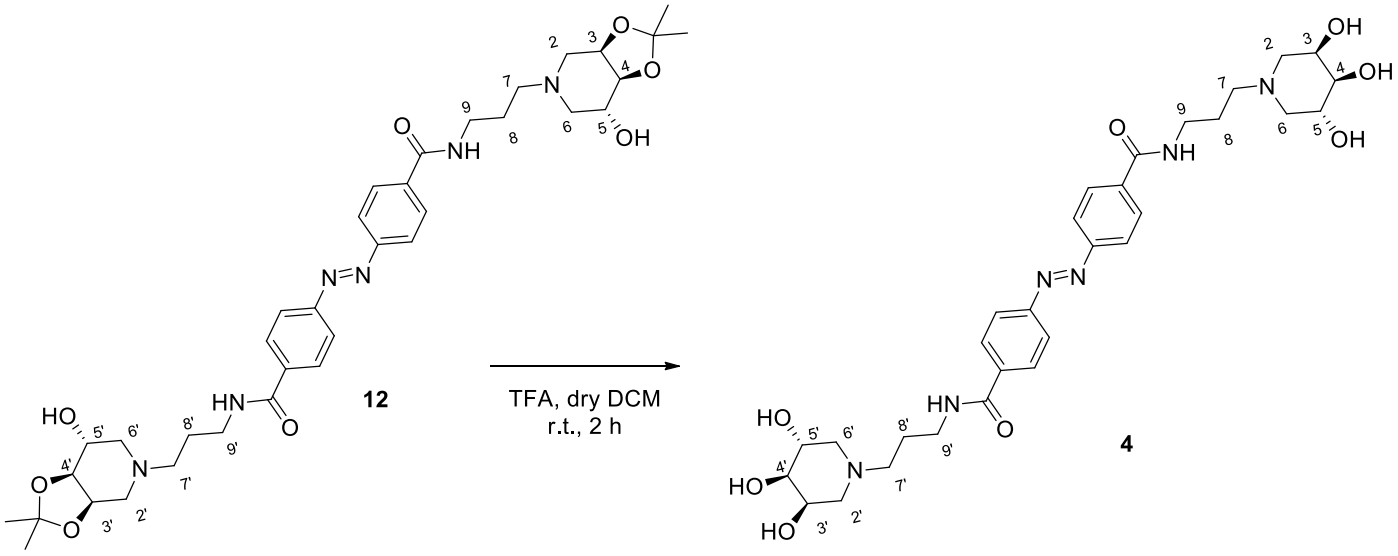
Synthesis of compound 12



To a solution of amine **8** (60 mg, 0.26 mmol) in dry DCM (2 ml), dry NEt_3 (55 µl, 0.39 mmol) and diazo acyl chloride **10**[4] (40 mg, 0.13 mmol) were added. The reaction mixture was stirred at room temperature for 18 hours until the disappearance of **8** was assessed by a TLC control (DCM:MeOH:NH_4OH (6%) 5:1:0.1). The mixture was concentrated under vacuum and then the crude was purified by FCC in the dark (SiO_2, AcOEt:MeOH 5:1) to give 46 mg of **12** (0.07 mmol, 51%) as an orange waxy compound. **12**: *Rf* 0.17

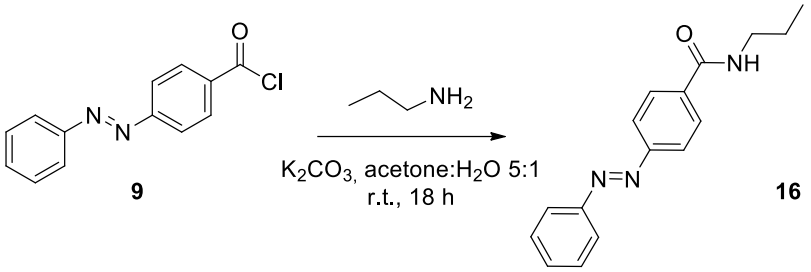
(AcOEt:MeOH 5:1). δ_H (400 MHz, $CDCl_3$): 7.87 (d, *J* = 7.9 Hz, 4H, Ar), 7.79 (d, *J* = 7.7 Hz, 4H, Ar), 7.53-7.46 (m, 2H, NH), 4.27 (q, *J* = 5.6 Hz, 2H, H-3, H-3'), 4.06 (t, *J* = 3.9 Hz, 2H, H-4, H-4'), 4.04-3.99 (m, 2H, H-5, H-5'), 3.61-3.53 (m, 4H, CH_2-9, CH_2-9'), 2.75 (dd, *J* = 5.6, 11.8 Hz, 2H, Ha-2, Ha-2'), 2.62-2.49 (m, 8H, CH_2-6, CH_2-7, CH_2-6', CH_2-7'), 2.45 (dd, *J* = 7.0, 11.8 Hz, 2H, Hb-2, Hb-2'), 1.82 (quint, *J* = 6.0 Hz, 4H, CH_2-8, CH_2-8'), 1.45 (s, 6H, 2xMe), 1.34 (s, 6H, 2xMe). δ_C (100 MHz, $CDCl_3$): 167.3 (2C, O<u>C</u>NH), 153.8 (2C, Ar), 136.7 (2C, Ar), 128.2 (4C, Ar), 123.1 (4C, Ar), 109.2 (2C, O<u>C</u>$(CH_3)_2$), 77.1 (2C, C-4, C-4'), 72.2 (2C, C-3, C-3'), 67.9 (2C, C-5, C-5'), 56.5, 56.1 (4C, C-6, C-7, C-6', C-7'), 55.7 (2C, C-2, C-2'), 39.7 (2C, C-9, C-9'), 28.4 (2C, Me), 26.5 (2C, Me), 25.7 (2C, C-8, C-8'). HRMS (ESP+, *m/z*): calcd for $C_{36}H_{50}N_6O_8$ [M+H$^+$]: 695.37629, found: 695.37786.

Synthesis of *trans*-4



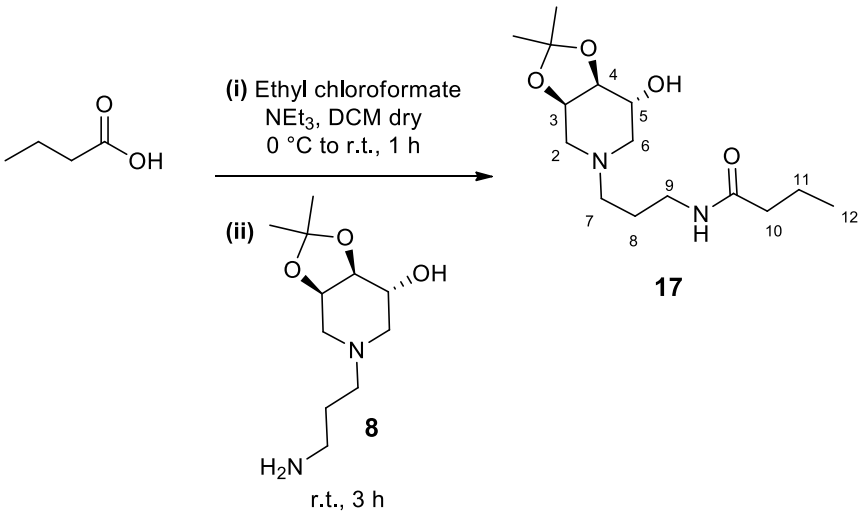
A solution of **12** (45 mg, 0.07 mmol) in dry DCM (2 mL) was left stirring with TFA (110 µl, 1.43 mmol) at room temperature for 2 h until the disappearance of starting material was assessed by a TLC control (DCM:MeOH:NH_4OH (6%) 5:1:0.1). Then, the crude mixture was concentrated and the crude residue was purified by FCC in the dark (SiO_2, DCM:MeOH:NH_4OH (6%) 3:1:0.1) to give 35 mg of fully *trans* isomer **4** (0.06 mmol, 88%) as an orange waxy compound. **4**: *Rf* 0.13 (DCM:MeOH:NH_4OH (6%) 3:1:0.1). δ_H (400 MHz, CD_3OD) 8.07-8.00 (m, 8H, Ar), 3.92 (quint, J = 2.9 Hz, 2H, H-3, H-3'), 3.81 (td, J = 4.0, 7.8 Hz, 2H, H-5, H-5'), 3.55-3.37 (m, 6H, H-4, H-4', CH_2-9, CH_2-9'), 2.92-2.71 (m, 4H, Ha-2, Ha-6, Ha-2', Ha-6'), 2.50 (t, J = 7.0 Hz, 4H, CH_2-7, CH_2-7'), 2.31 (d, J = 10.1 Hz, 2H, Hb-2, Hb-2'), 2.11 (br s, 2H, Hb-6, Hb-6'), 1.91-1.79 (m, 4H, CH_2-8, CH_2-8'). δ_C (50 MHz, CD_3OD) 169.1 (2C, O<u>C</u>NH), 155.5 (2C, Ar), 138.2 (2C, Ar), 129.5 (4C, Ar), 124.0 (4C, Ar), 75.4 (2C, C-4, C-4'), 69.7 (2C, C-5, C-5'), 69.3 (2C, C-3, C-3'), 58.2 57.8 (4C, C-2, C-6, C-2', C-6'), 56.9 (2C, C-7, C-7'), 39.8 (2C, C-9, C-9') 27.0 (2C, C-8, C-8'). HRMS (ESP+, *m/z*): calcd for $C_{30}H_{42}N_6O_8$ [M+H$^+$]: 615.31369, found: 615.31400. UV-Vis (4% DMSO in water): $\lambda_{max\text{-}AZB\text{-}trans}$/nm, ($\varepsilon$/$M^{-1}$ cm^{-1}) = 328 (2.65 x 10^4). UV-Vis (4% DMSO in water): $\lambda_{max\text{-}PSS}$/nm, (ε/M^{-1} cm^{-1}) = 328 (2.65 x 10^4), 430 (shoulder). IR (neat) ν = 3316, 2925, 2811, 1539, 1296, 1203, 1068, 1011, 862, 838 cm^{-1}.

Synthesis of compound 16



A solution of diazo acyl chloride (66 mg, 0.27 mmol) in acetone (2 ml) was added to a solution of propylamine (16 mg, 0.26 mmol) and K_2CO_3 (16 mg, 0.11 mmol) in H_2O (0.5 ml). The reaction mixture was stirred at room temperature for 18 hours until the disappearance of **9** was assessed by a TLC control (DCM:MeOH:NH_4OH (6%) 10:1:0.1). The mixture was concentrated under vacuum and then the crude was purified by FCC in the dark (SiO_2, EtP:AcOEt 3:1) to give 32 mg of **16** (0.12 mmol, 46%) as an orange solid. **16**: *Rf* 0.29 (EtP:AcOEt 3:1). M.p. = 155-157 °C. δ_H (400 MHz, $CDCl_3$) 7.98-7.86 (m, 6H, Ar), 7.55-7.45 (m, 3H, Ar), 6.55-6.47 (m, 1H, NH), 3.43 (q, J = 6.7 Hz, 2H, CH_2-NH), 1.66 (sext, J = 7.3 Hz, 2H, CH_2-CH_2-CH_3), 0.99 (t, J = 7.4 Hz, 3H, CH_2-CH_3). δ_C (100 MHz, $CDCl_3$) 167.0 (NHCO), 154.2 (Ar), 152.6 (Ar), 136.7 (Ar), 131.6 (Ar), 129.2 (2C, Ar), 128.0 (2C, Ar), 123.2 (2C, Ar), 123.0 (2C, Ar), 110.1 (interference), 42.0 (CH_2-NH), 23.0 (CH_2-CH_2-CH_3), 11.6 (CH_2-CH_3). HRMS (ESP+, *m/z*): calcd for $C_{16}H_{17}N_3O$ [M+H$^+$]: 268.14444, found: 268.14429. IR (neat) ν = 3312, 2961, 2872, 1631, 1539, 1483, 1325, 1152, 1011, 920, 860, 777 cm^{-1}.

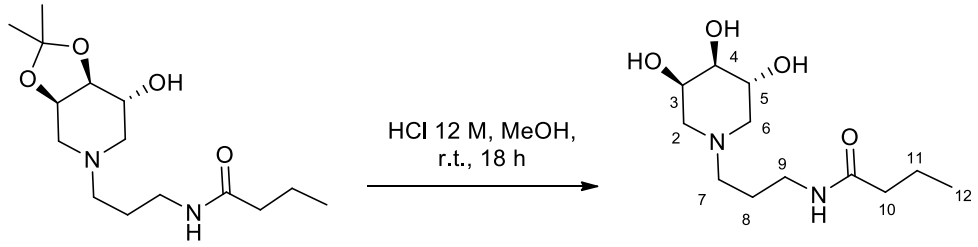
Synthesis of compound 17



Ethyl-chloroformate (16 µl, 0.17 mmol) was added to a solution of butyric acid (11 mg, 0.12 mmol) and dry Et_3N (28 µl, 0.20 mmol) in dry DCM (1 ml) at 0 °C. The mixture was cooled to room temperature and maintained under stirring for an hour. Then, the amine **8** (23 mg, 0.10 mmol) was added to the reaction mixture. The reaction was stirred for 3 hours until the disappearance of **8** was assessed by a TLC control (DCM:MeOH 10:1). The mixture was concentrated under vacuum and then the crude was purified by FCC (SiO_2, DCM:MeOH 10:1) to give 7 mg of **17** (0.02 mmol, 24%) as a pale yellow waxy compound. **17**: *Rf* 0.2 (DCM:MeOH 10:1). α_D^{21} = + 4.00 (c = 0.60, $CHCl_3$). δ_H (400 MHz, $CDCl_3$) 6.41 (br s, 1H, NH), 4.30 (q, J = 5.2 Hz, 1H, H-3), 4.01 (t, J = 5.0 Hz, 1H, H-4), 3.97-3.91 (m, 1H, H-5), 3.44-3.38 (m, 1H, Ha-9), 3.32-3.21 (m, 1H, Hb-9), 2.73-2.64 (m, 2H, Ha-2, Ha-6), 2.60 (dd, J = 5.0, 12.3 Hz, 1H, Hb-2), 2.49 (t, J = 6.4 Hz, 2H, H-7), 2.37 (dd, J = 6.6, 11.5 Hz, 1H, Hb-6), 2.13 (t, J = 7.3 Hz, 2H, H-10), 1.72-1.60 (m, 4H, H-8, H-11), 1.51 (s, 3H, Me), 1.36 (s,

3H, Me), 0.93 (t, J = 7.4 Hz, 3H, H-12). δ_C (50 MHz, $CDCl_3$) 173.2 (HN<u>C</u>O), 109.5 ($OC(CH_3)_2$), 77.2 (C-4), 72.6 (C-3), 68.8 (C-5), 56.4 (C-7), 56.3 (C-6), 55.0 (C-2), 38.8 (C-10), 38.5 (C-9), 28.5 (Me), 26.5 (Me), 26.3 (C-8), 19.4 (C-11), 14.0 (C-12). HRMS (ESP+, *m/z*): calcd for $C_{15}H_{28}N_2O_4$ [M+H$^+$]: 301.21218, found: 301.21231. IR ($CDCl_3$): ν = 3690, 3599, 3449, 2995, 2961, 2932, 1655, 1520, 1462, 1379, 1242, 1061 cm^{-1}.

Synthesis of compound 14



17 **14**

A solution of **17** (15 mg, 0.05 mmol) in MeOH (2 mL) was left stirring with 12 M HCl (15 µL) at room temperature for 18 h. The crude mixture was concentrated under vacuum and then the crude was purified by FCC (SiO_2, DCM:MeOH:NH_4OH (6%) 3:1:0.1) to give 6 mg of **14** (0.02 mmol, 46%) as a waxy compound. **14**: *Rf* 0.29 (DCM:MeOH:NH_4OH (6%) 3:1:0.1). α_D^{21} = − 8.90 (c = 0.55, MeOH). δ_H (400 MHz, CD_3OD) 4.17 (br s, 1H, H-3), 4.08-4.02 (m, 1H, H-4), 3.34-3.05 (m, 9H, H-2, H-5, H-6, H-7, H-9), 2.21 (t, J = 7.4 Hz, 2H, H-10), 1.94 (td, J = 7.5, 14.5 Hz, 2H, H-8), 1.65 (td, J = 7.4, 14.8 Hz, 2H, H-11), 0.96 (t, J = 7.4 Hz, 3H, H-12). δ_C (^{13}C 100 MHz and gHSQC $^1H/^{13}C$ 100/400 MHz, CD_3OD). 177.2 (HN<u>C</u>O), 67.4 (C-4), 64.1 (C-3), 55.4 (C-7), 52.6 (2C, C-2, C-6), 38.9 (C-10), 36.8 (C-9), 25.6 (C-8), 20.3 (C-11), 14.0 (C-12) (One carbon (C-5) is missing due to overlap). HRMS (ESP+, *m/z*): calcd for $C_{12}H_{24}N_2O_4$ [M+H$^+$]: 261.18088, found: 261.18171. Partial protonation of the piperidine cannot be excluded since H-2, H-6 and H-7 protons are slightly deshielded.

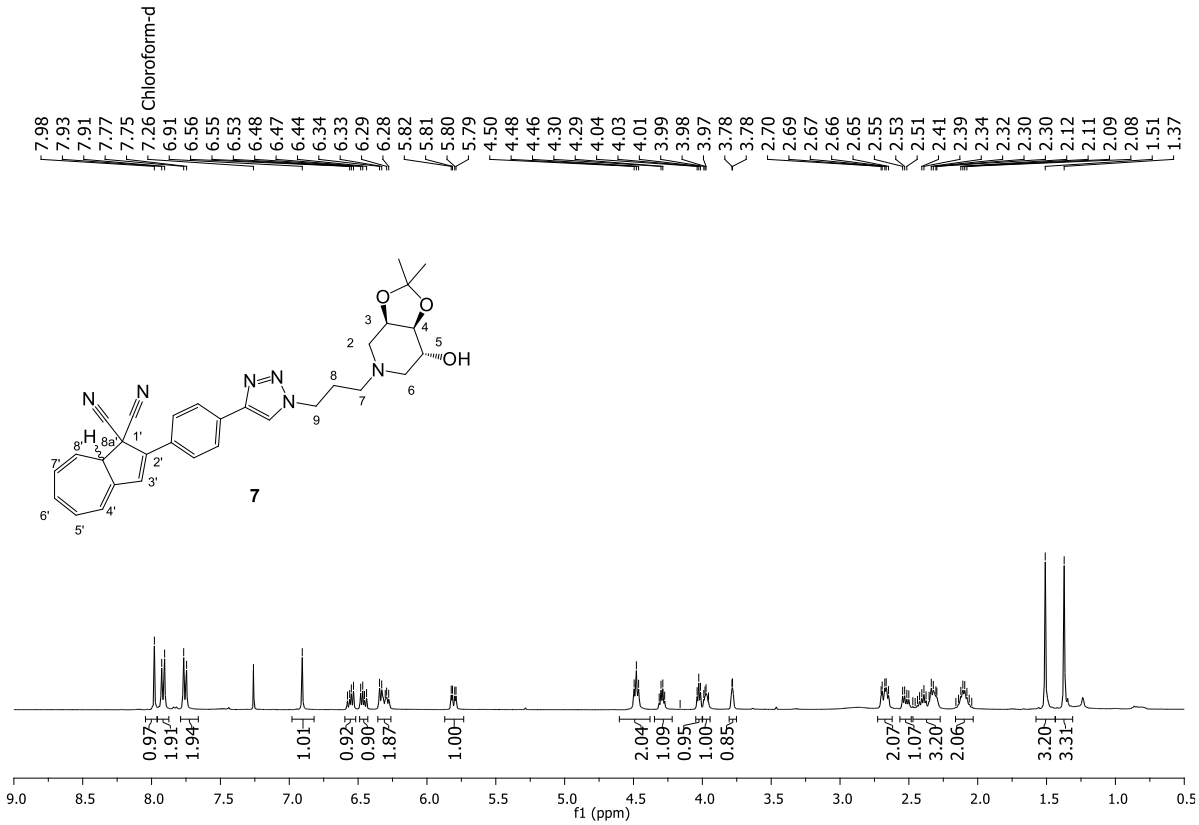


Figure S1: ^{1}H NMR spectrum of **7** (400 MHz, $CDCl_3$).

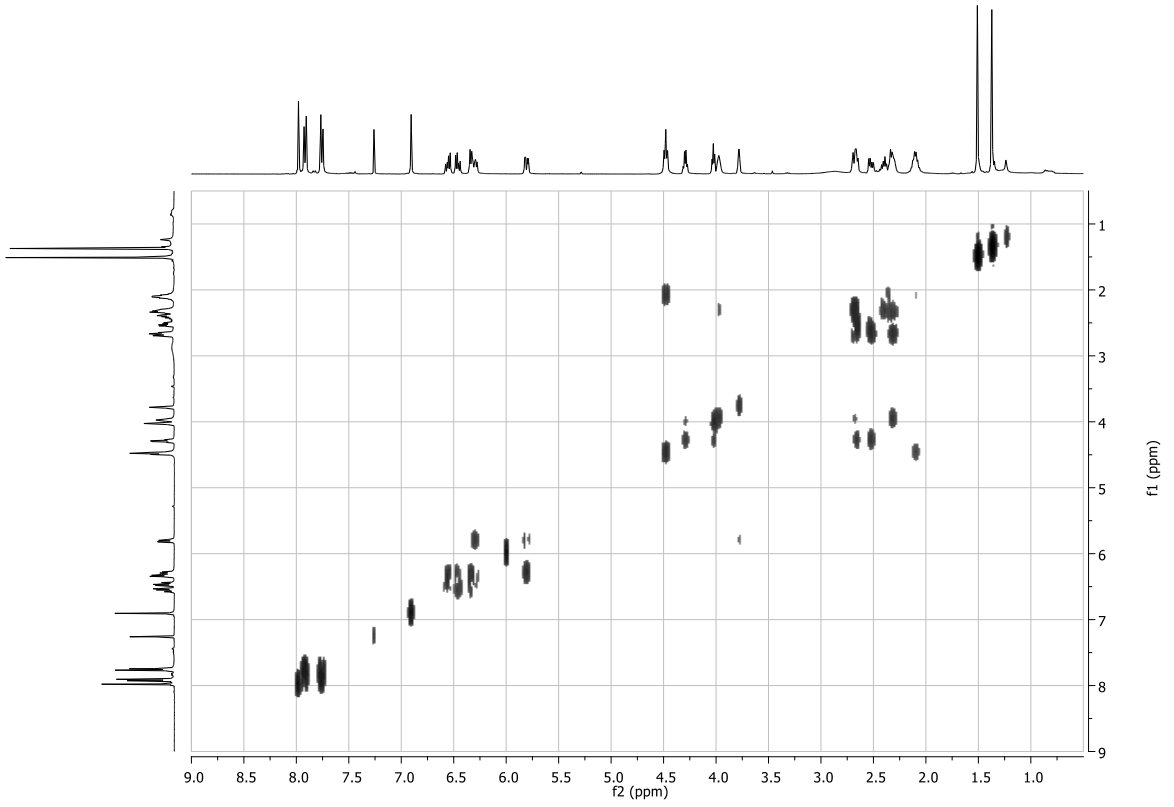


Figure S2: gCOSY spectrum of **7** (400 MHz, $CDCl_3$).

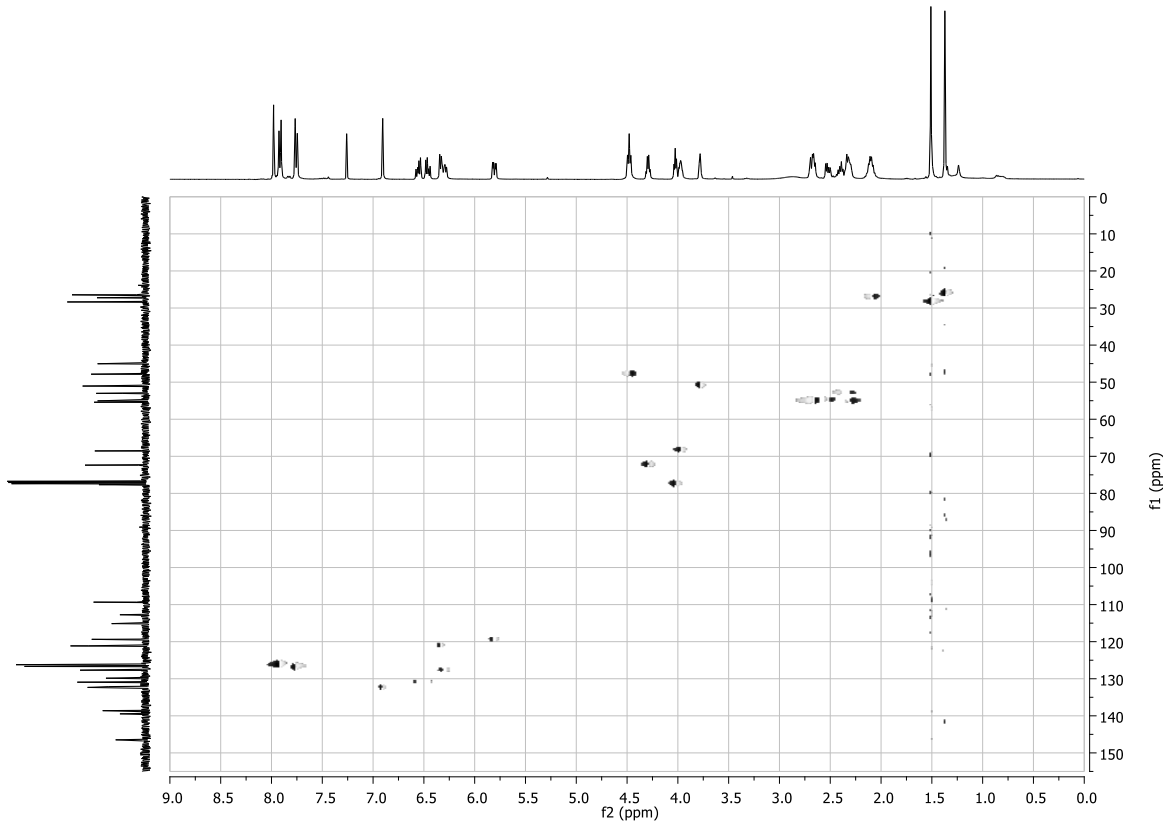


Figure S3: $^{1}H/^{13}C$ gHSQC spectrum of **7** (400/100 MHz, $CDCl_3$).

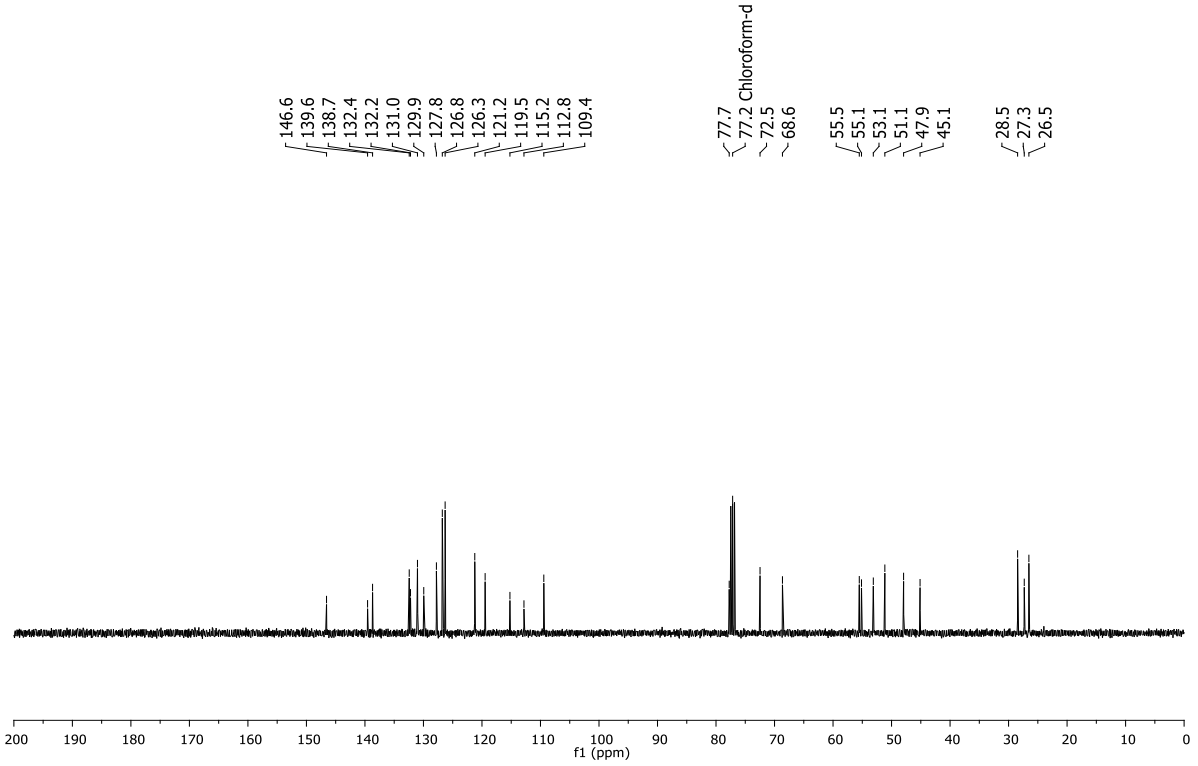


Figure S4: ^{13}C NMR spectrum of **7** (100 MHz, $CDCl_3$).

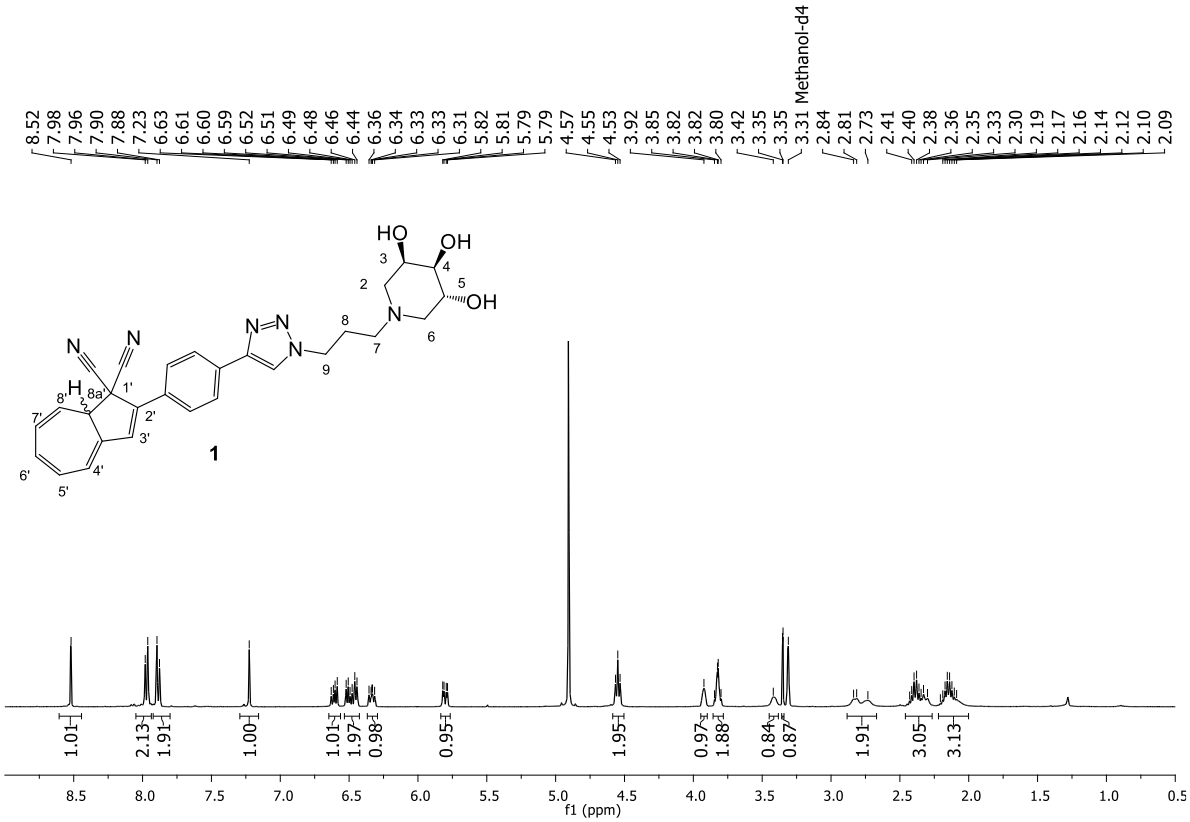


Figure S5: ^{1}H NMR spectrum of **1** (400 MHz, CD_3OD).

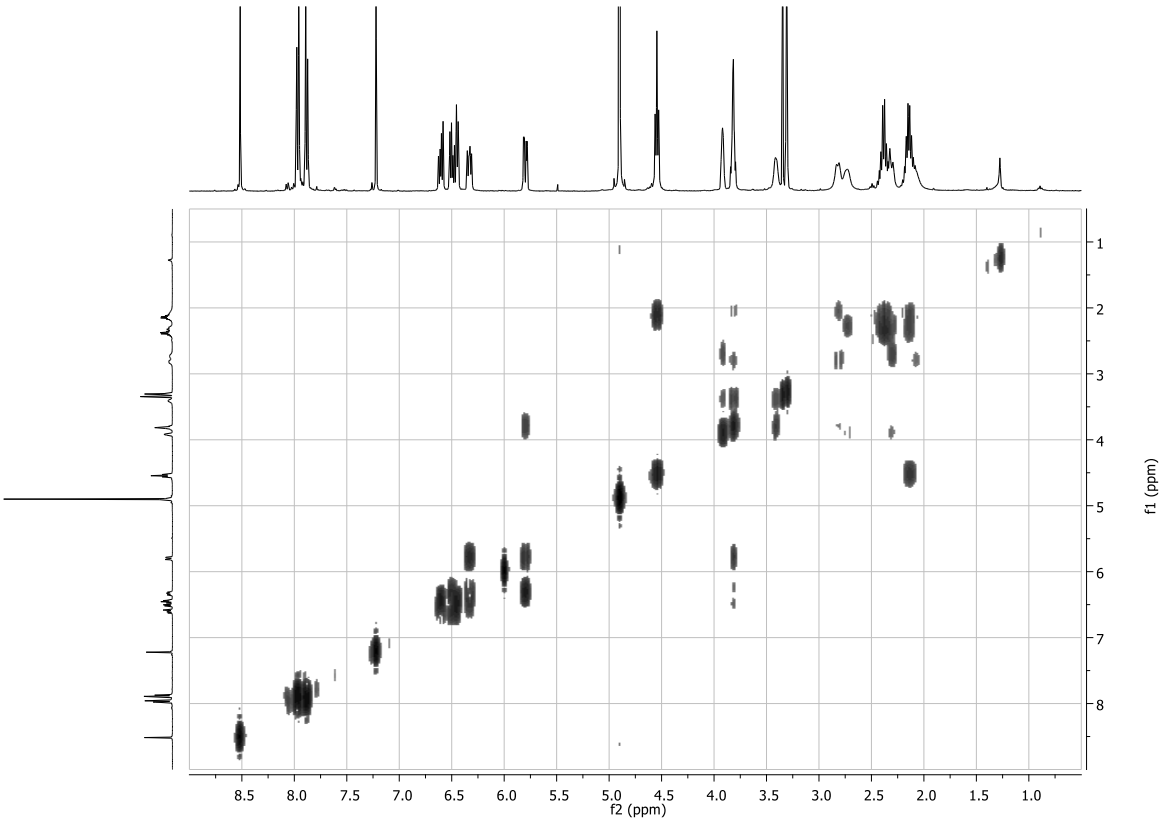


Figure S6: gCOSY spectrum of **1** (400 MHz, CD_3OD).

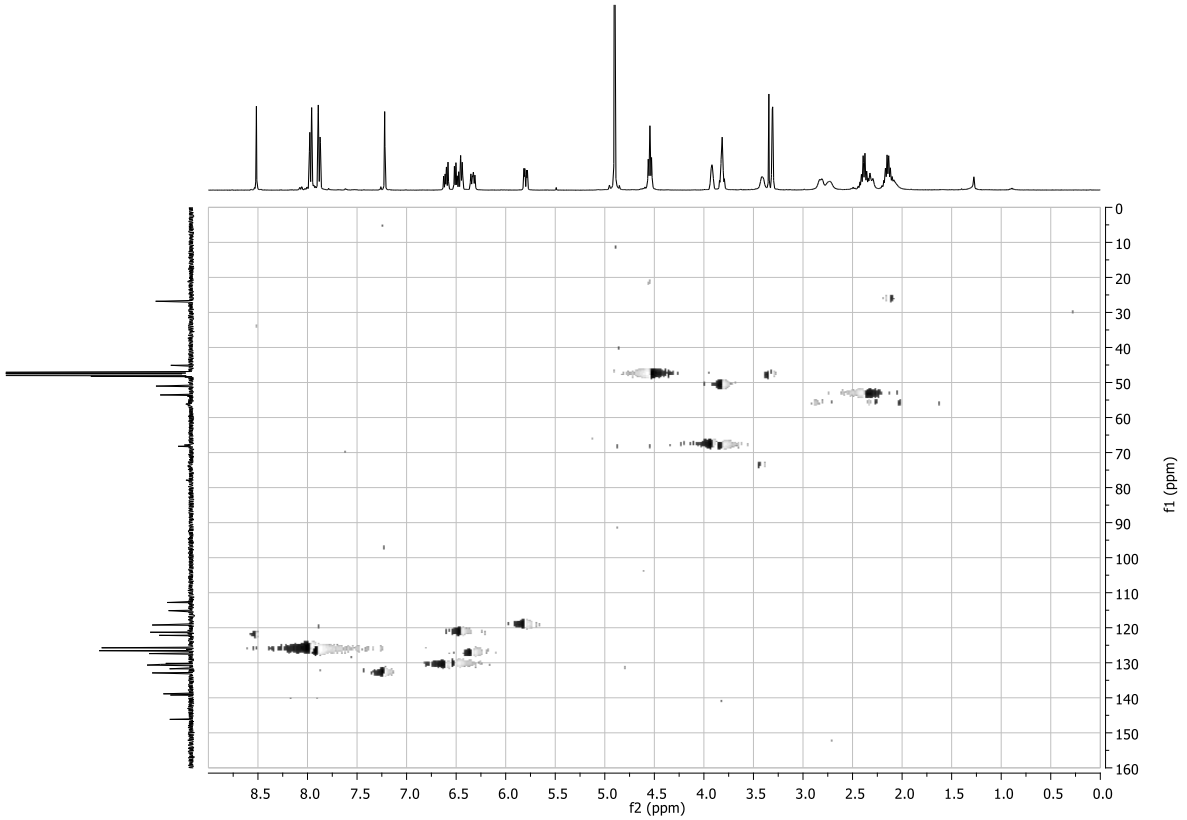


Figure S7: $^{1}H/^{13}C$ gHSQC spectrum of **1** (400/100 MHz, CD_3OD).

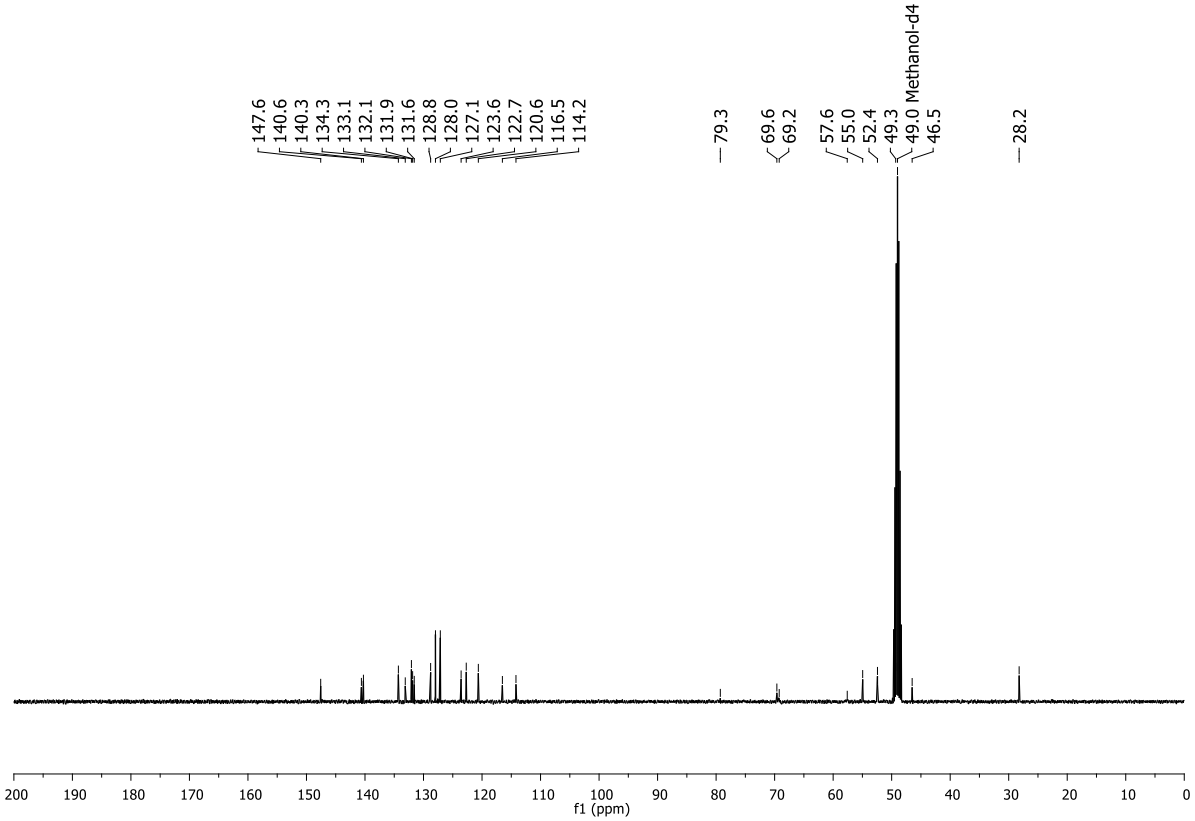


Figure S8: ^{13}C NMR spectrum of **1** (100 MHz, CD_3OD).

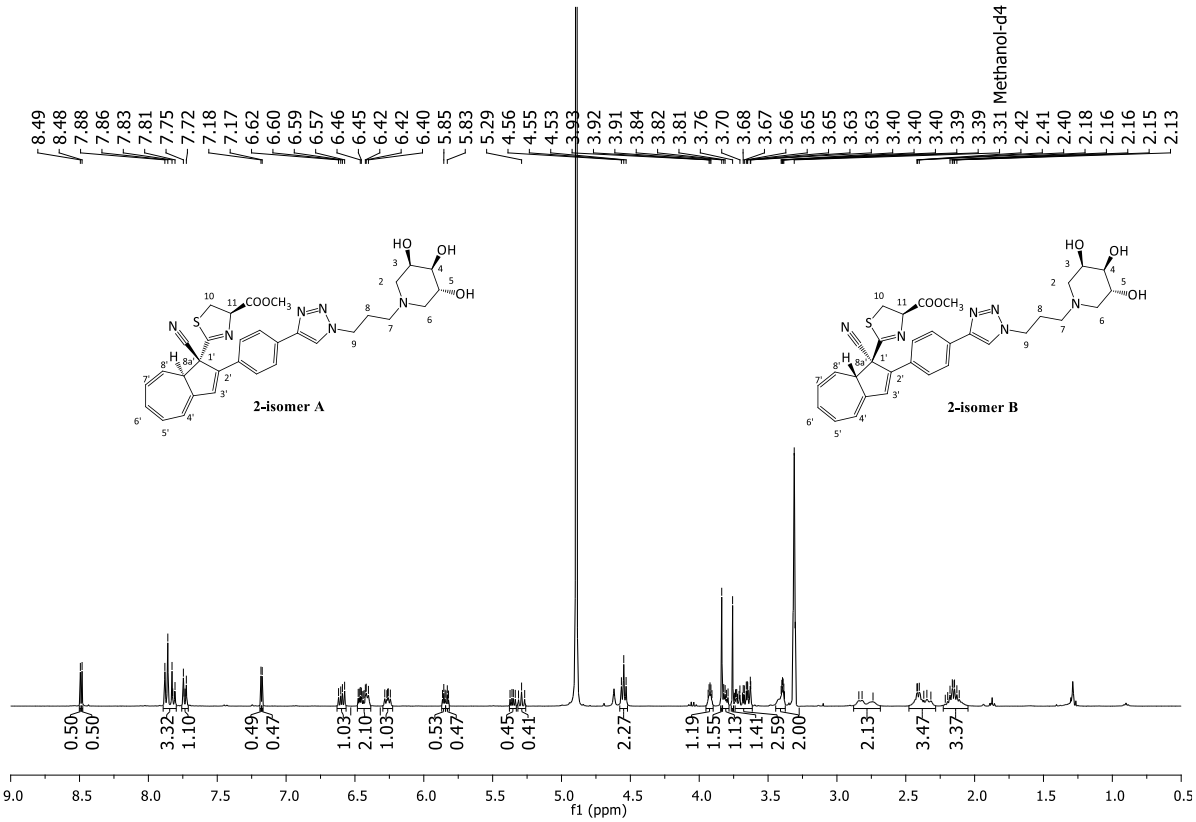


Figure S9: ^{1}H NMR spectrum of **2** (400 MHz, CD_3OD).

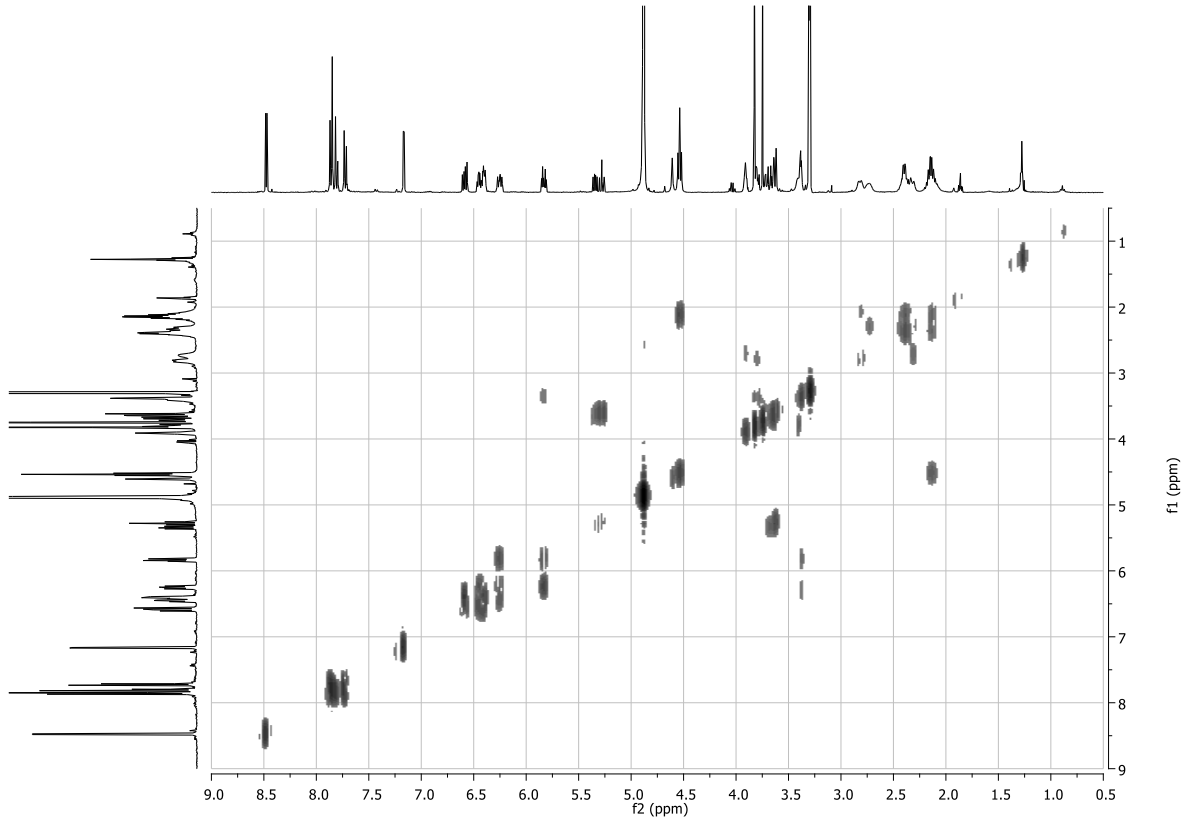


Figure S10: gCOSY spectrum of **2** (400 MHz, CD_3OD).

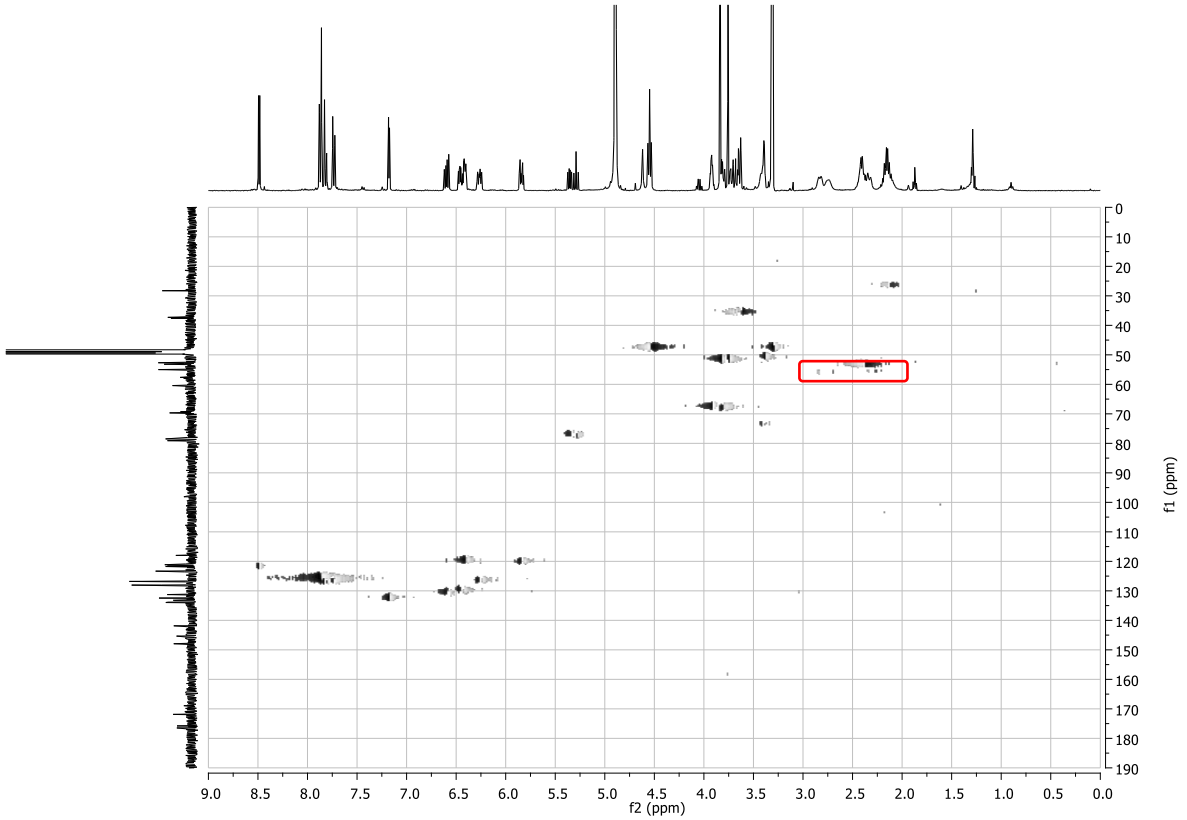


Figure S11: $^{1}H/^{13}C$ gHSQC spectrum of **2** (400/100 MHz, CD_3OD). Red frame highlights C-2.

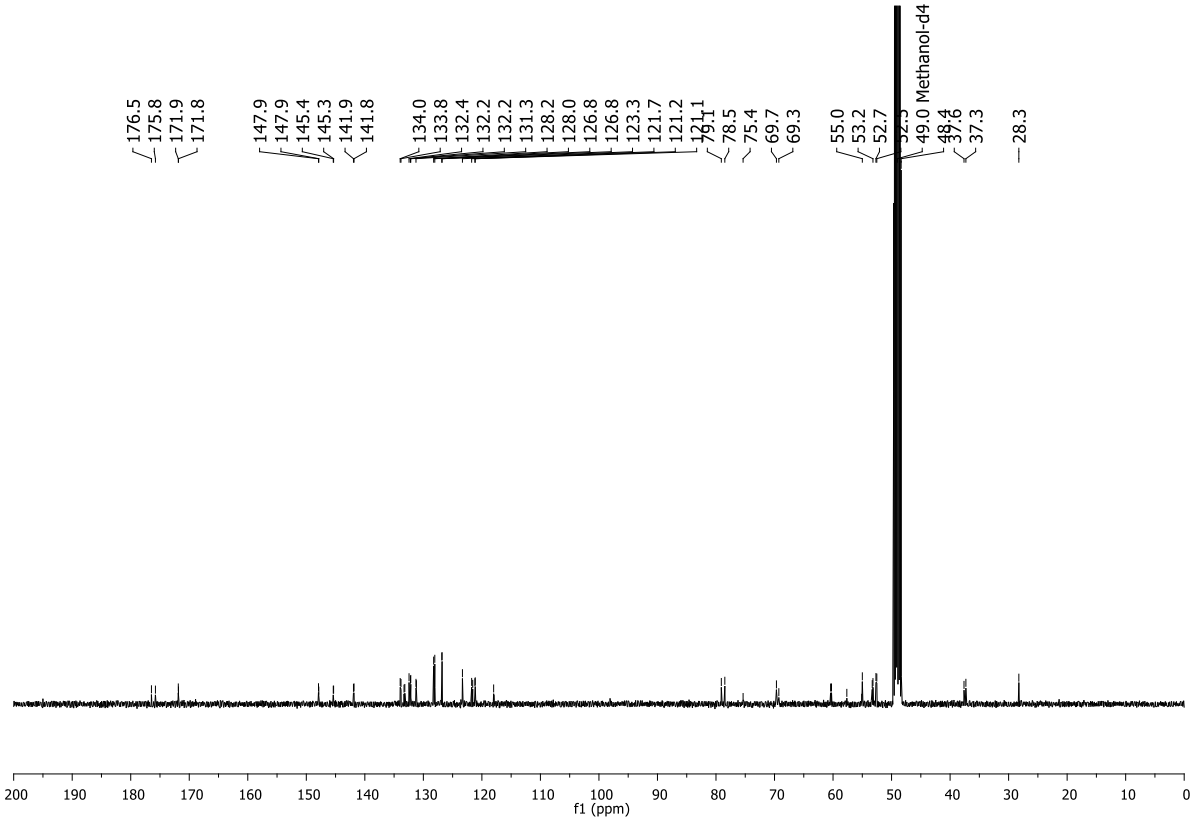


Figure S12: ^{13}C NMR spectrum of **2** (100 MHz, CD_3OD).

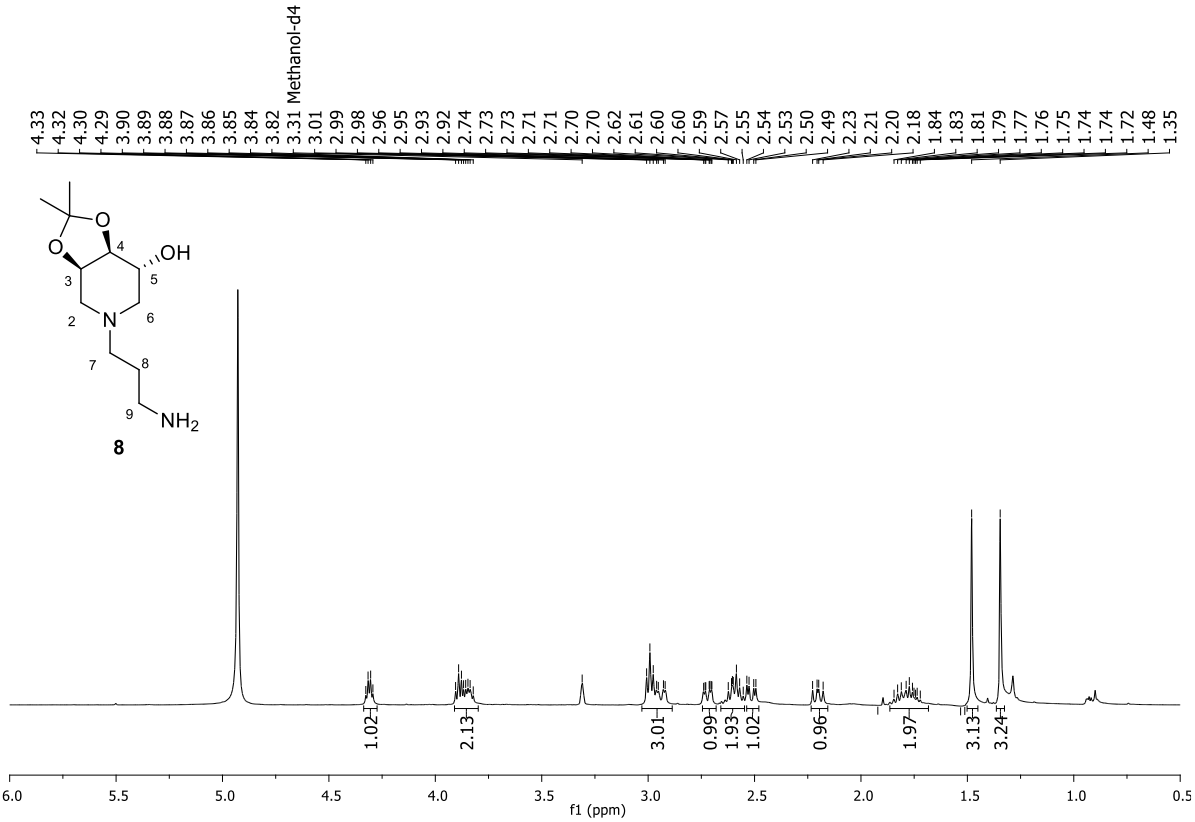


Figure S13: ^{1}H NMR spectrum of **8** (400 MHz, CD_3OD).

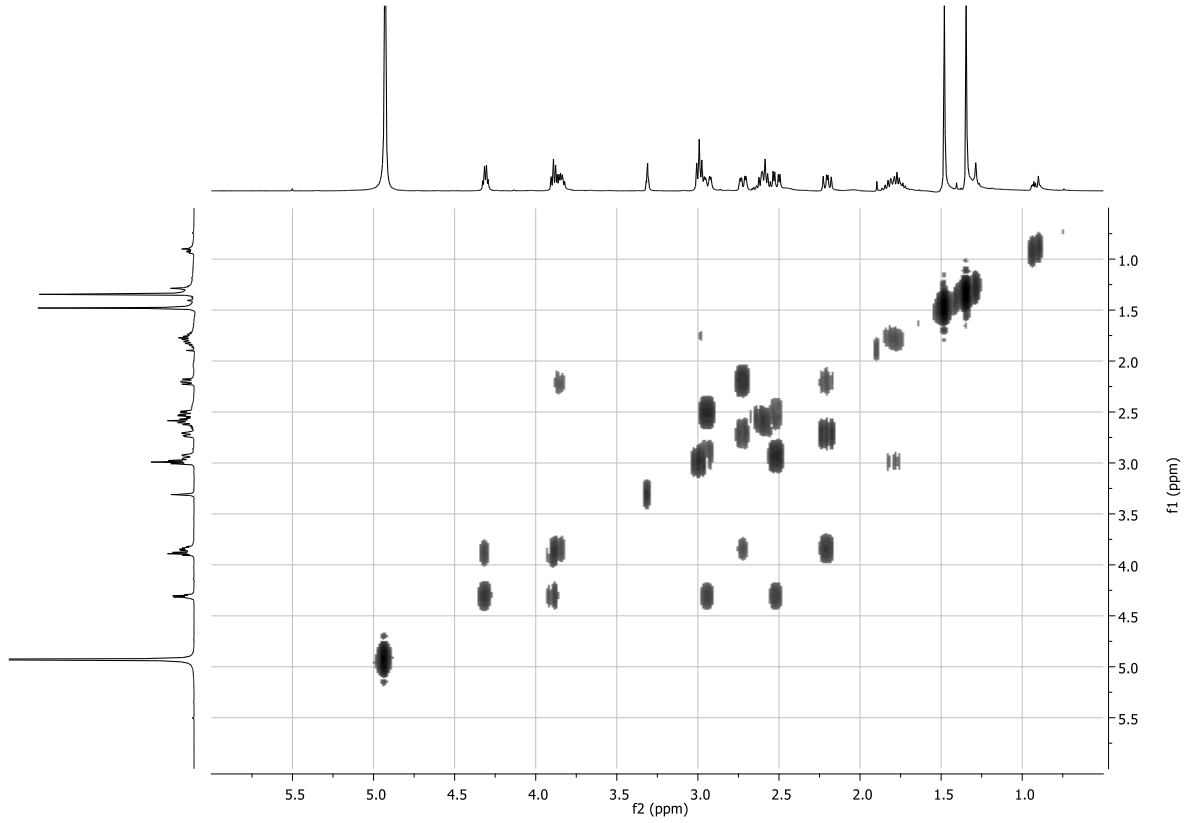


Figure S14: gCOSY spectrum of **8** (400 MHz, CD_3OD).

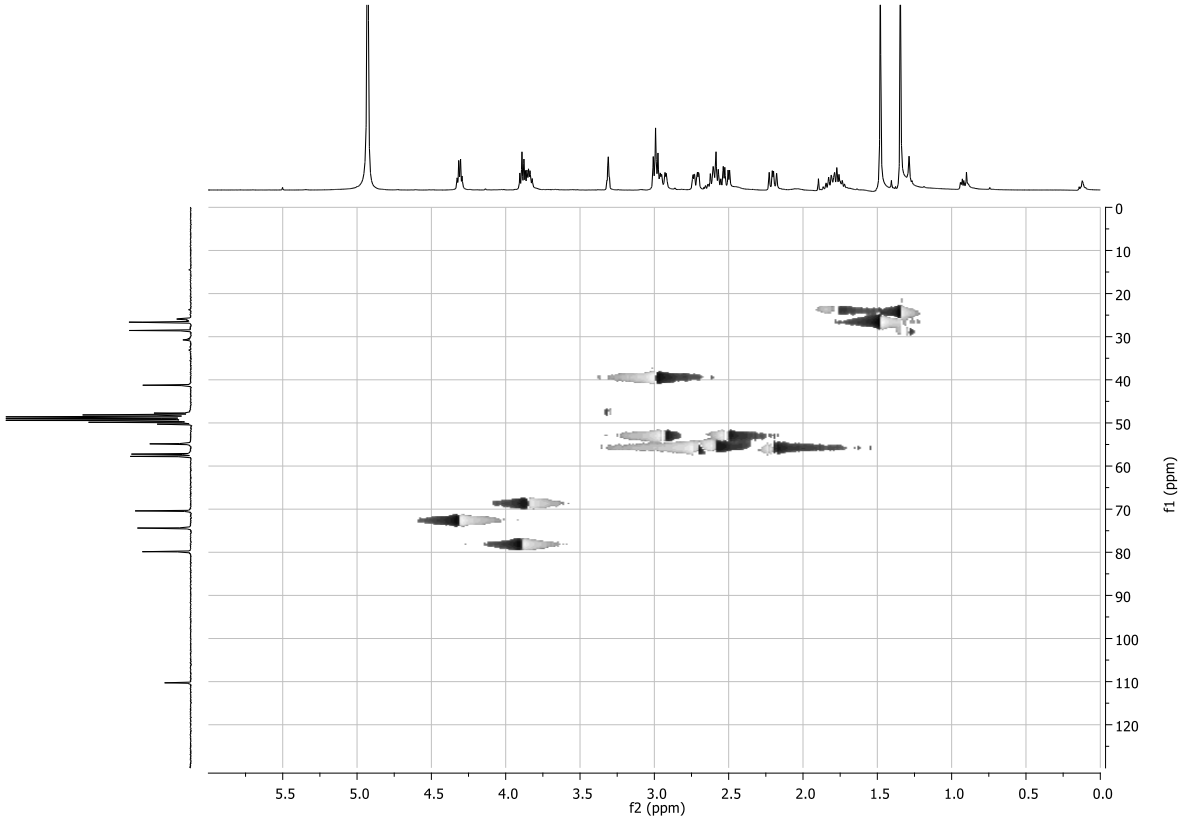


Figure S15: $^{1}H/^{13}C$ gHSQC spectrum of **8** (400/100 MHz, CD_3OD).

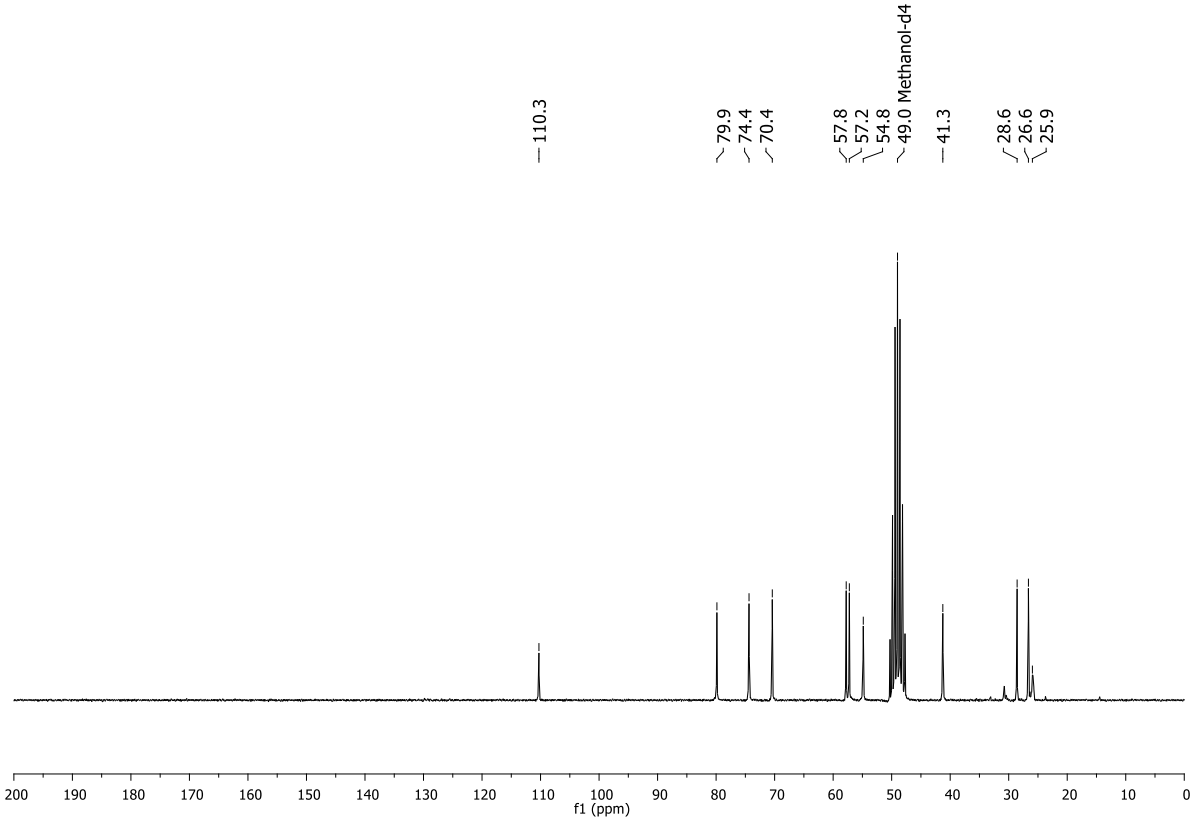


Figure S16: ^{13}C NMR spectrum of **8** (50 MHz, CD_3OD).

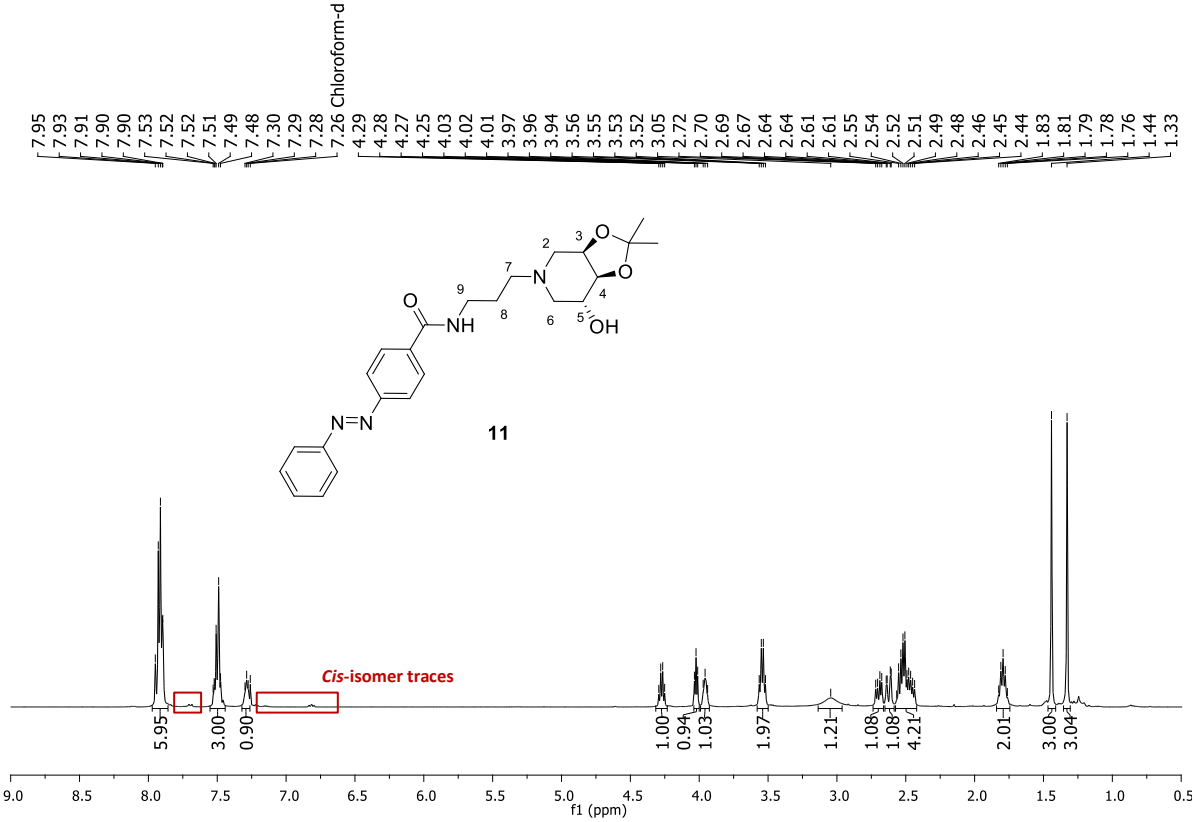


Figure S17: ^{1}H NMR spectrum of **11** (400 MHz, $CDCl_3$).

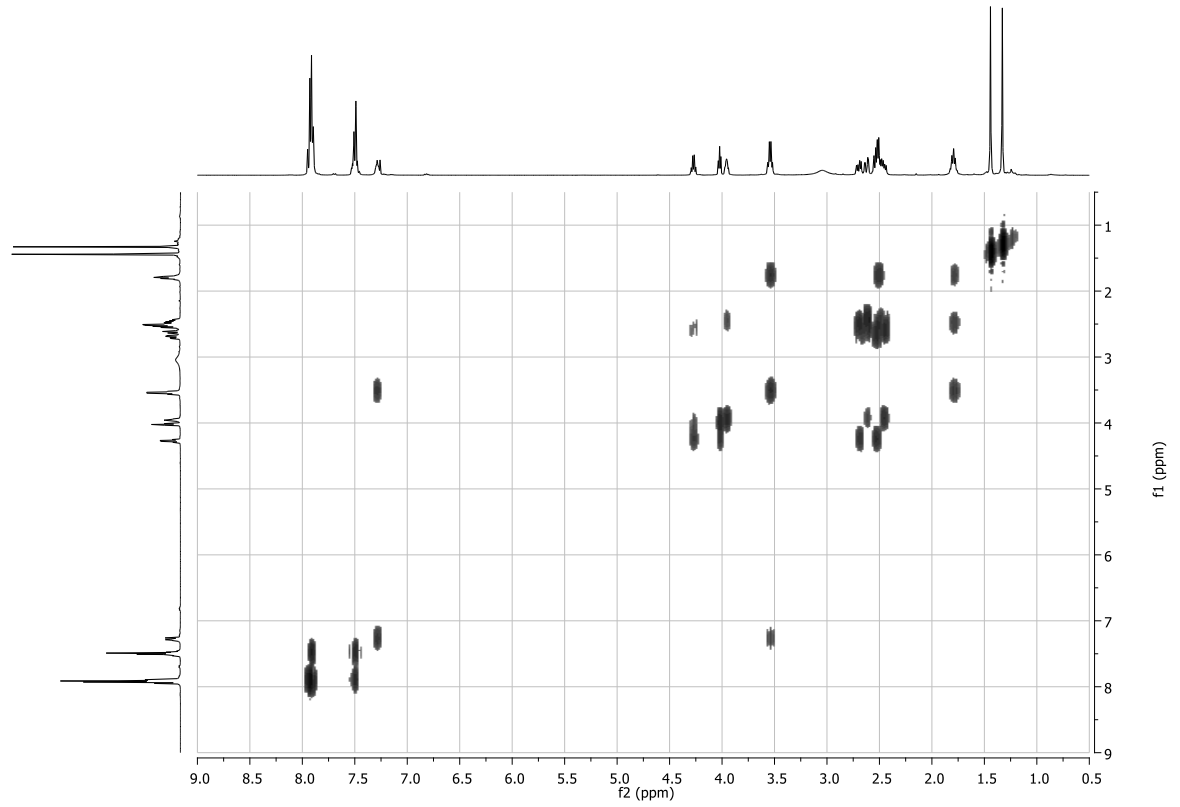


Figure S18: gCOSY spectrum of **11** (400 MHz, $CDCl_3$).

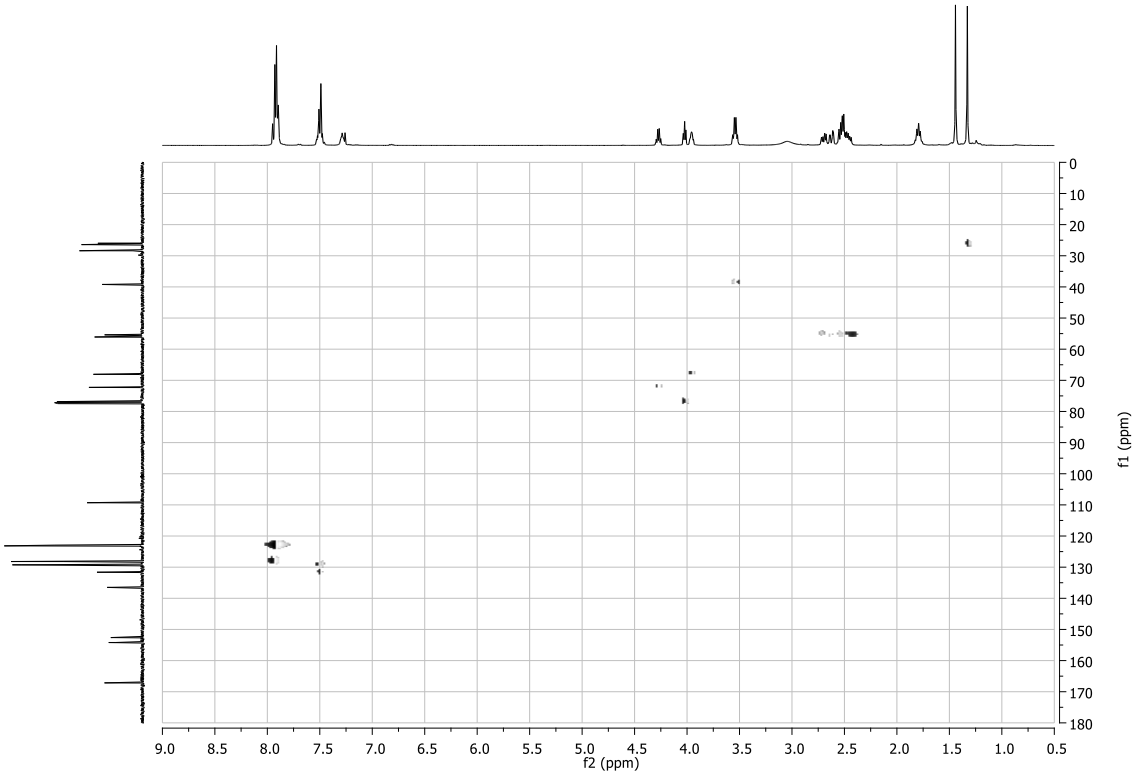


Figure S19: $^{1}H/^{13}C$ gHSQC spectrum of **11** (400/100 MHz, $CDCl_3$).

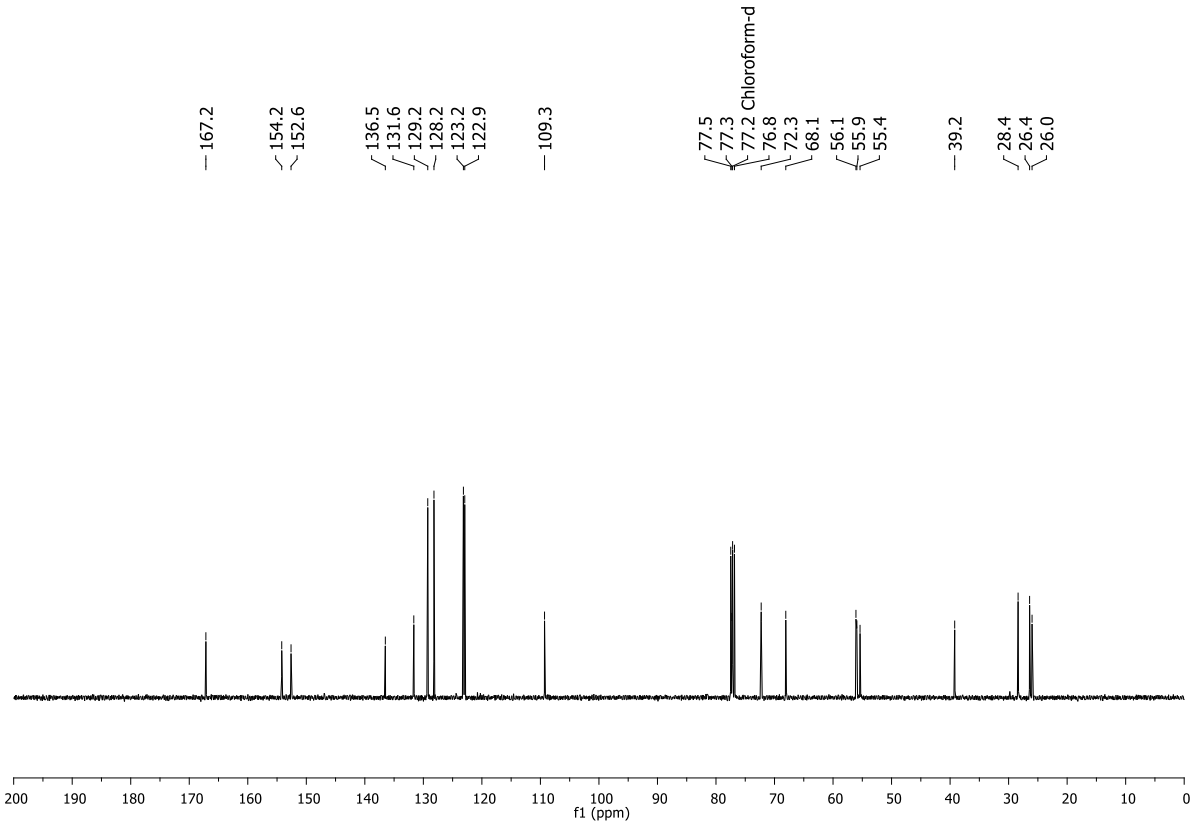


Figure S20: ^{13}C NMR spectrum of **11** (100 MHz, $CDCl_3$).

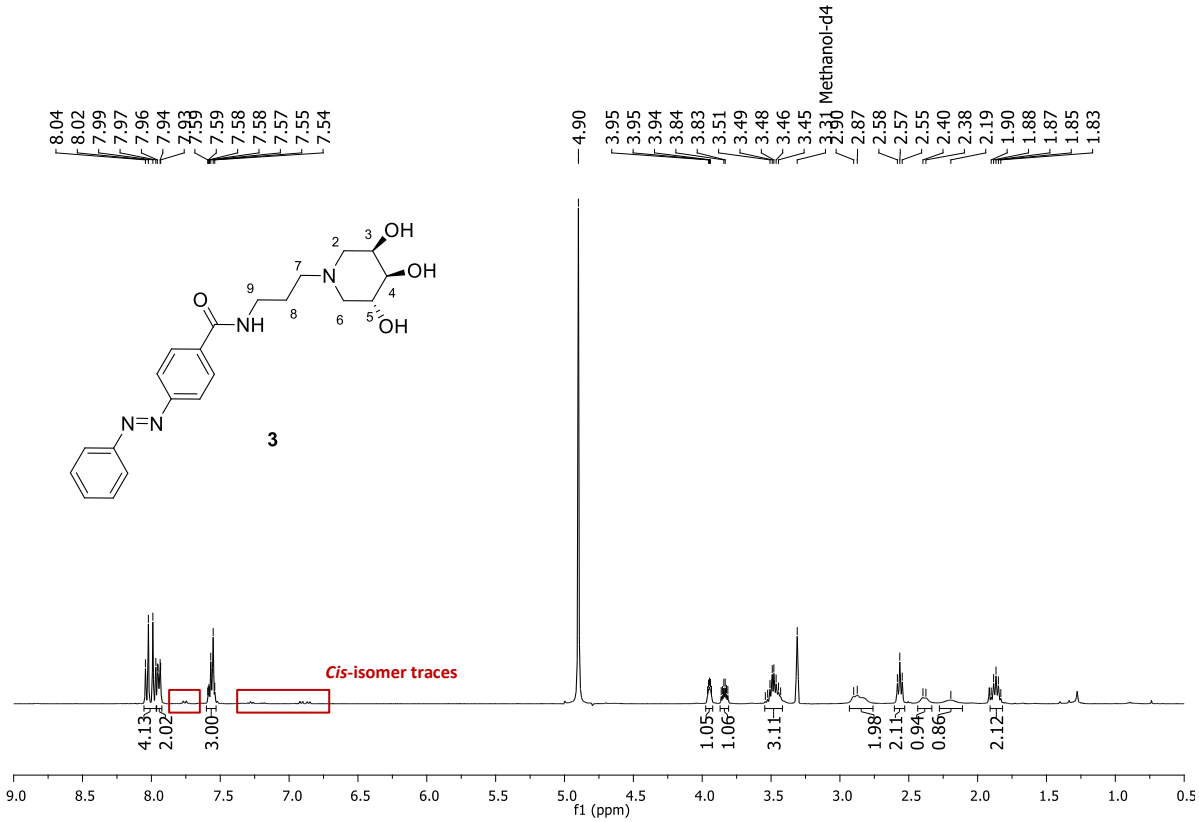


Figure S21: ^{1}H NMR spectrum of **3** (400 MHz, CD_3OD).

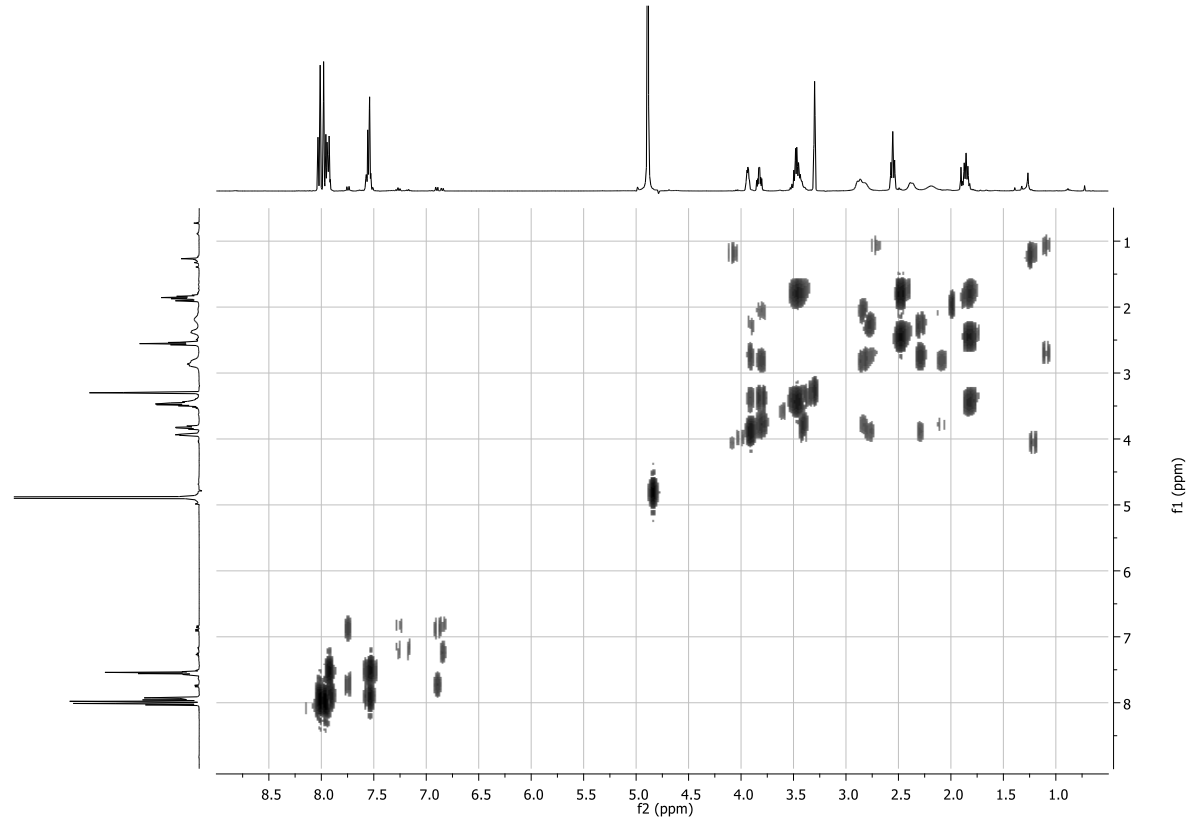


Figure S22: gCOSY spectrum of **3** (400 MHz, CD_3OD).

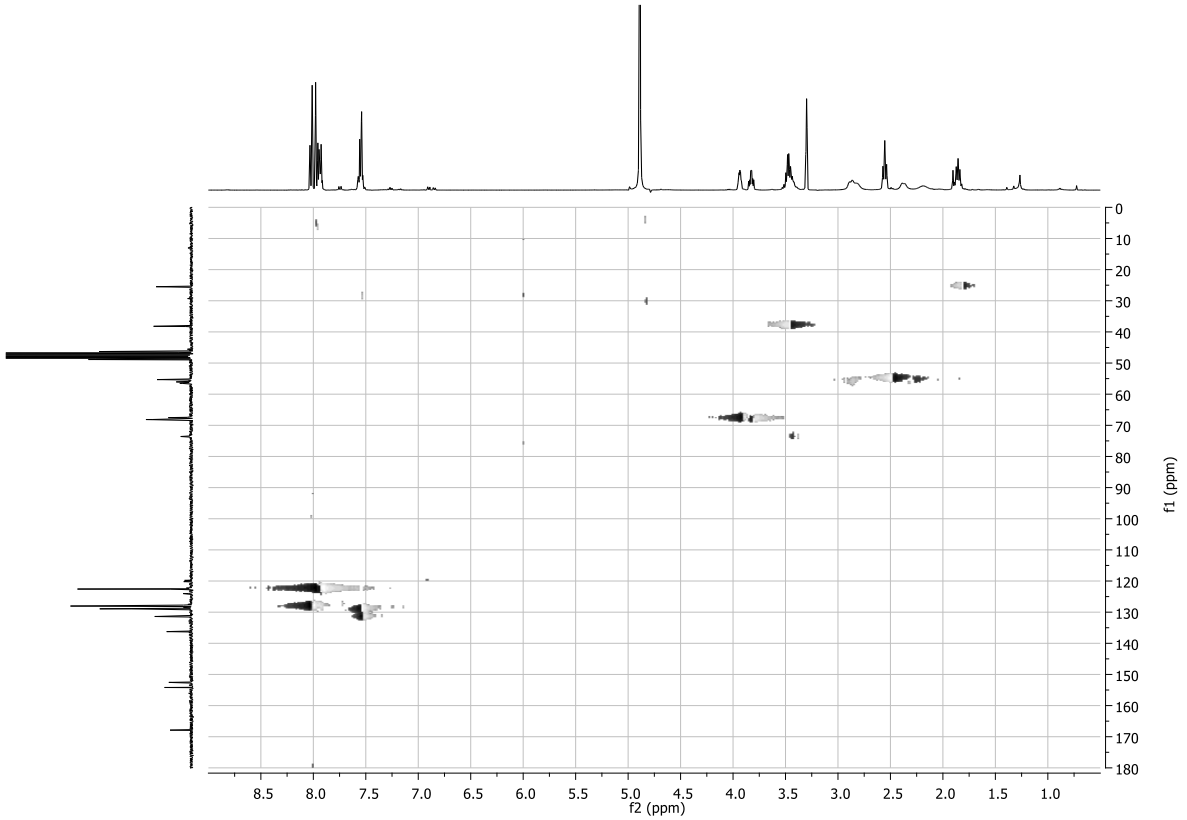


Figure S23: $^{1}H/^{13}C$ gHSQC spectrum of **3** (400/100 MHz, CD_3OD).

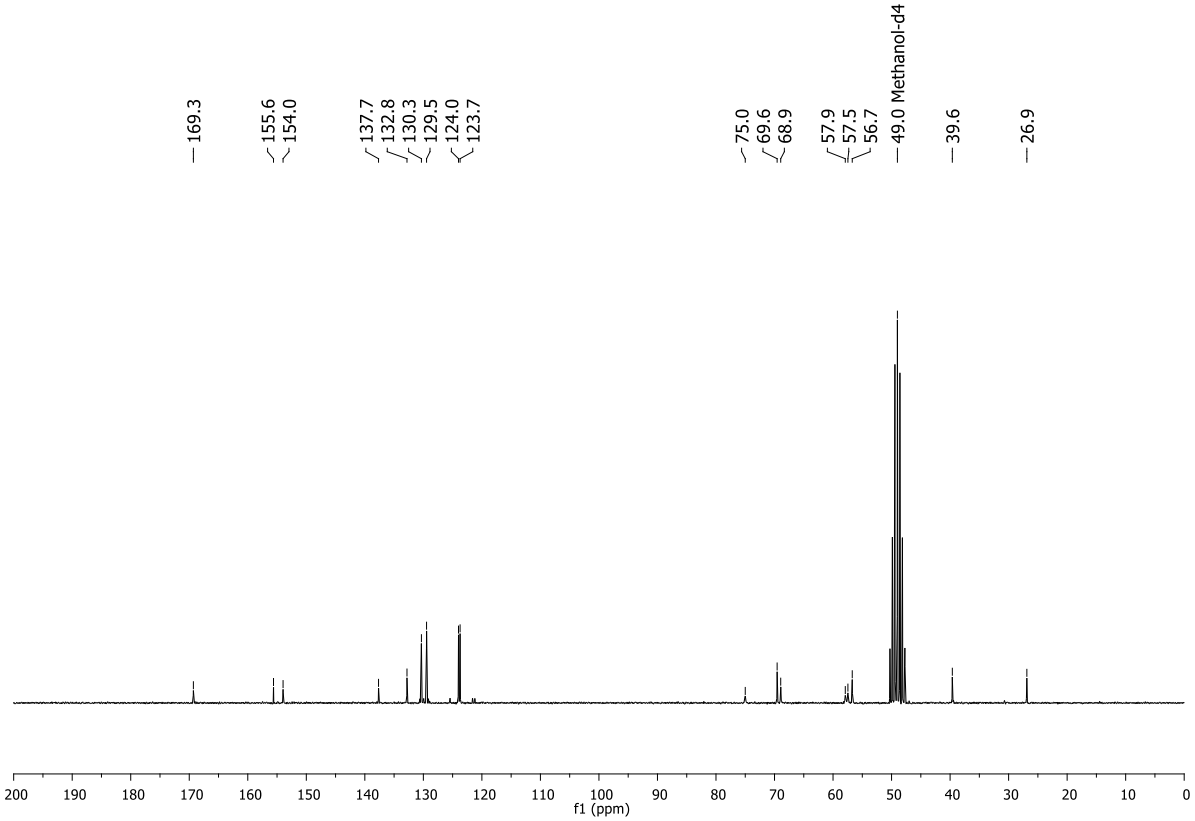


Figure S24: ^{13}C NMR spectrum of **3** (50 MHz, CD_3OD).

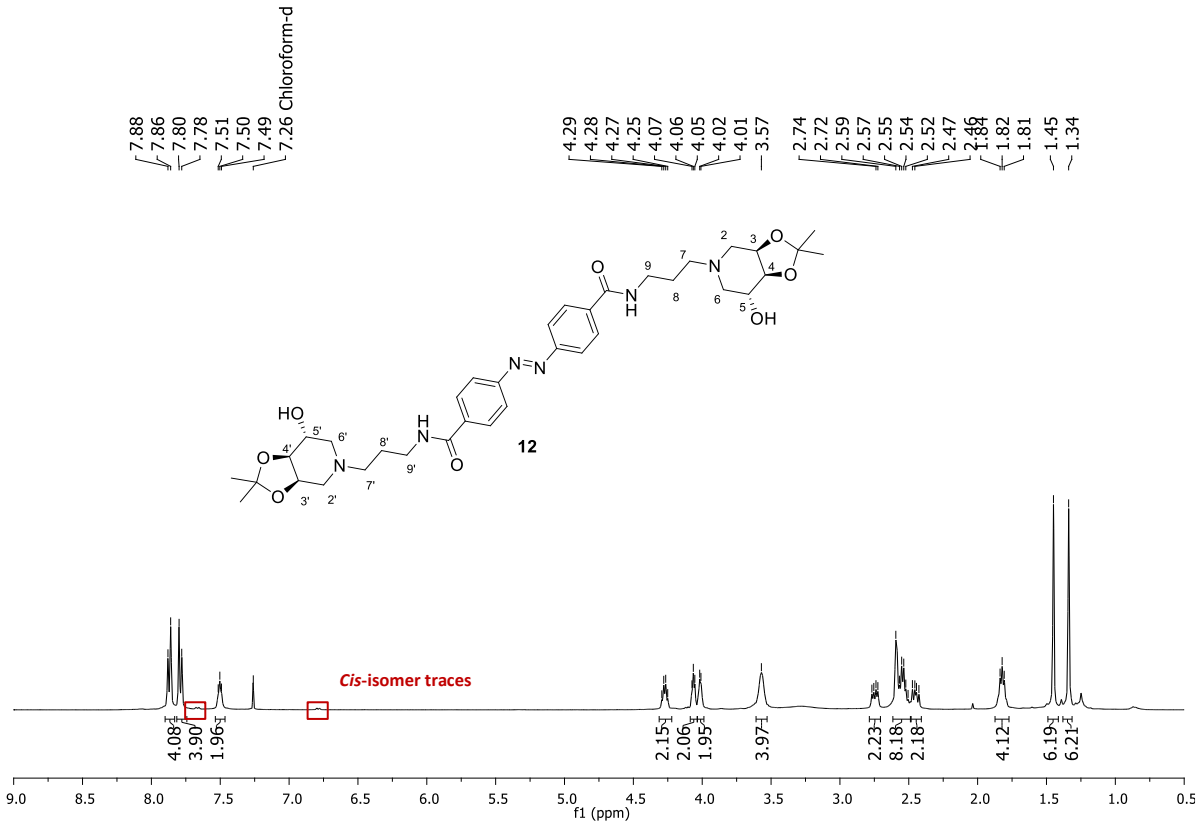


Figure S25: ^{1}H NMR spectrum of **12** (400 MHz, $CDCl_3$).

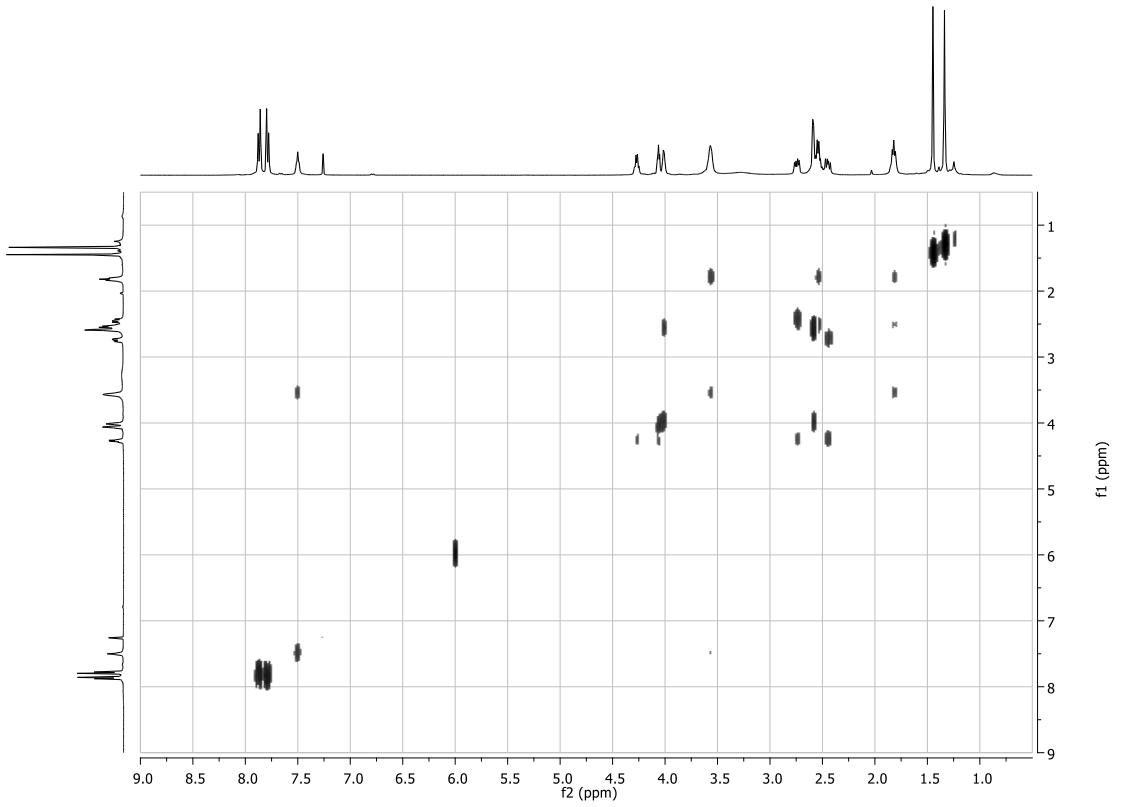


Figure S26: gCOSY spectrum of **12** (400 MHz, $CDCl_3$).

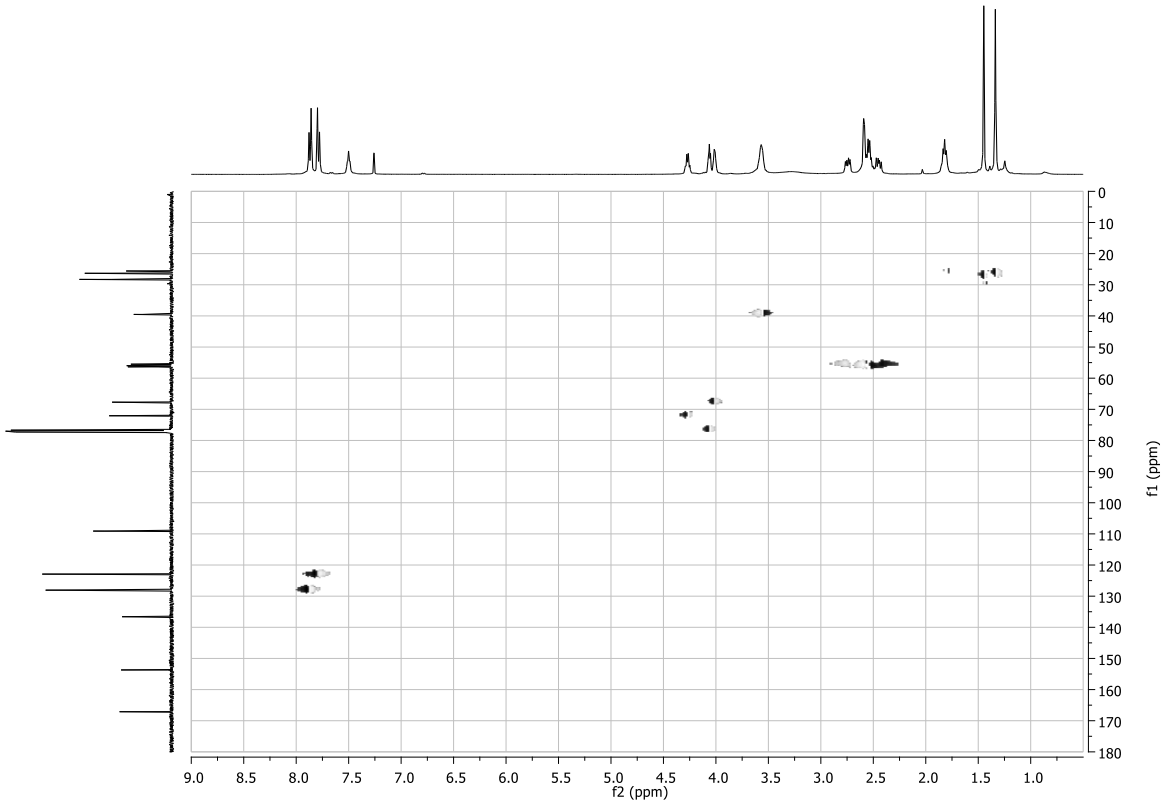


Figure S27: $^{1}H/^{13}C$ gHSQC spectrum of **12** (400/100 MHz, $CDCl_3$).

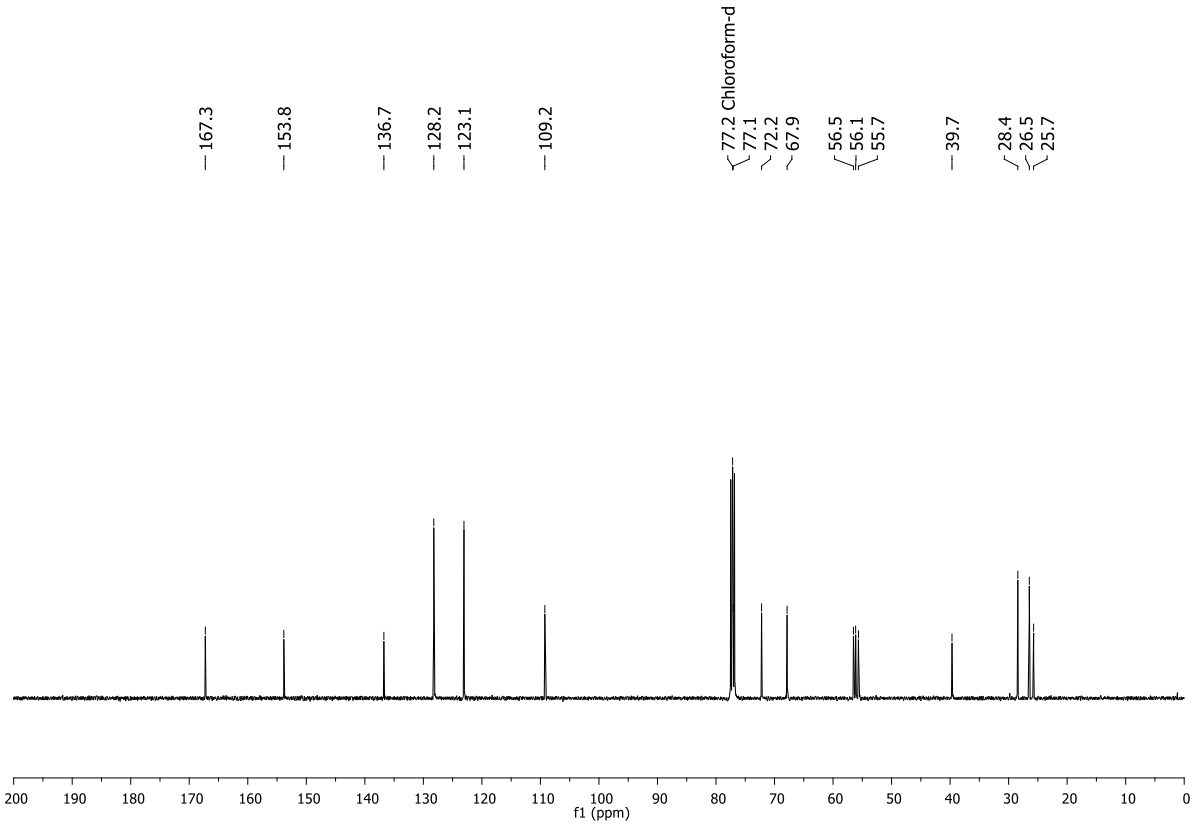


Figure S28: ^{13}C NMR spectrum of **12** (100 MHz, $CDCl_3$).

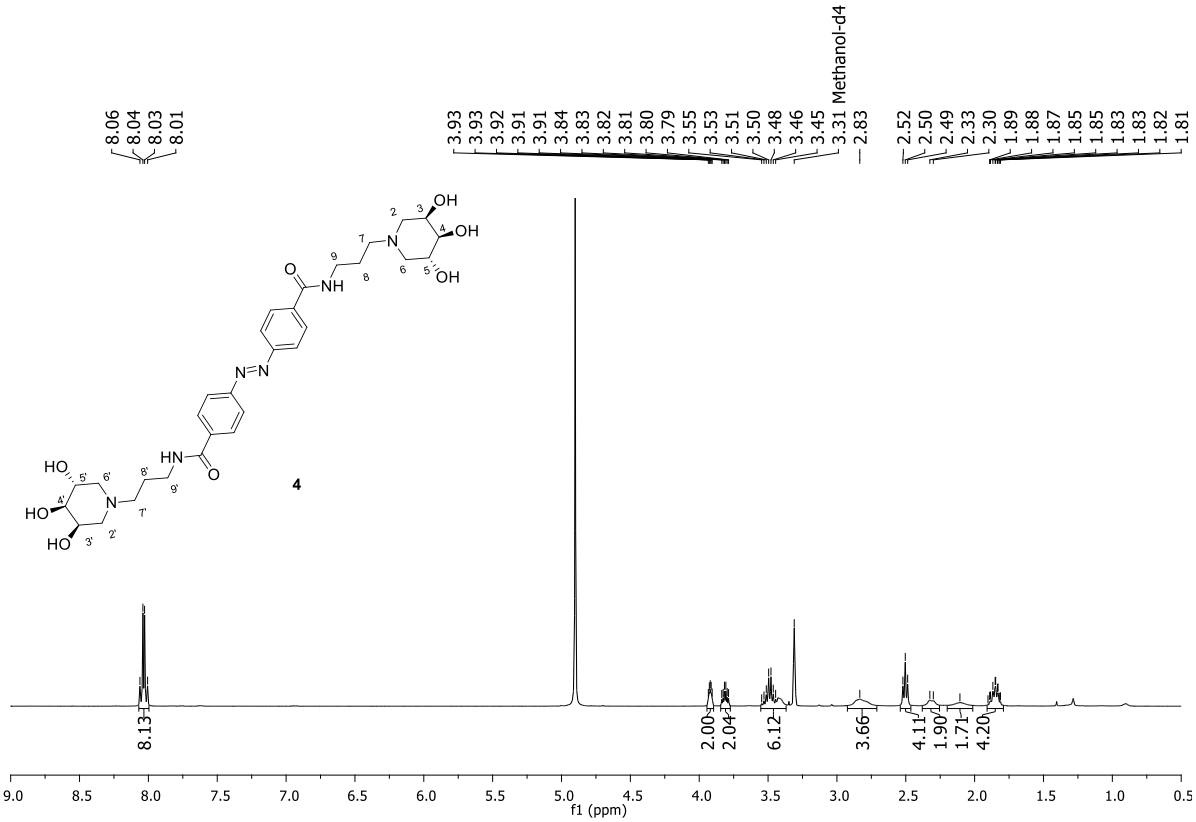


Figure S29: ^{1}H NMR spectrum of **4** (400 MHz, CD_3OD), 100% *trans.*

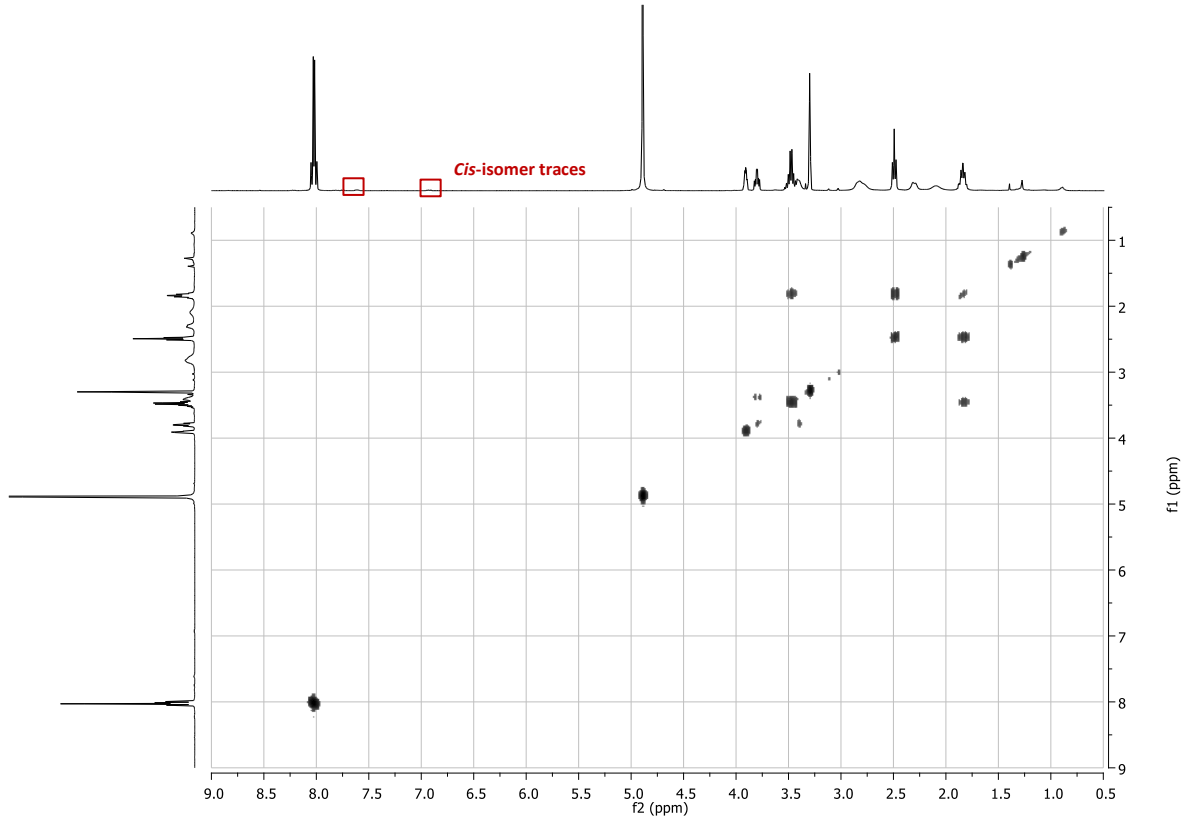


Figure S30: gCOSY spectrum of **4** (400 MHz, CD_3OD).

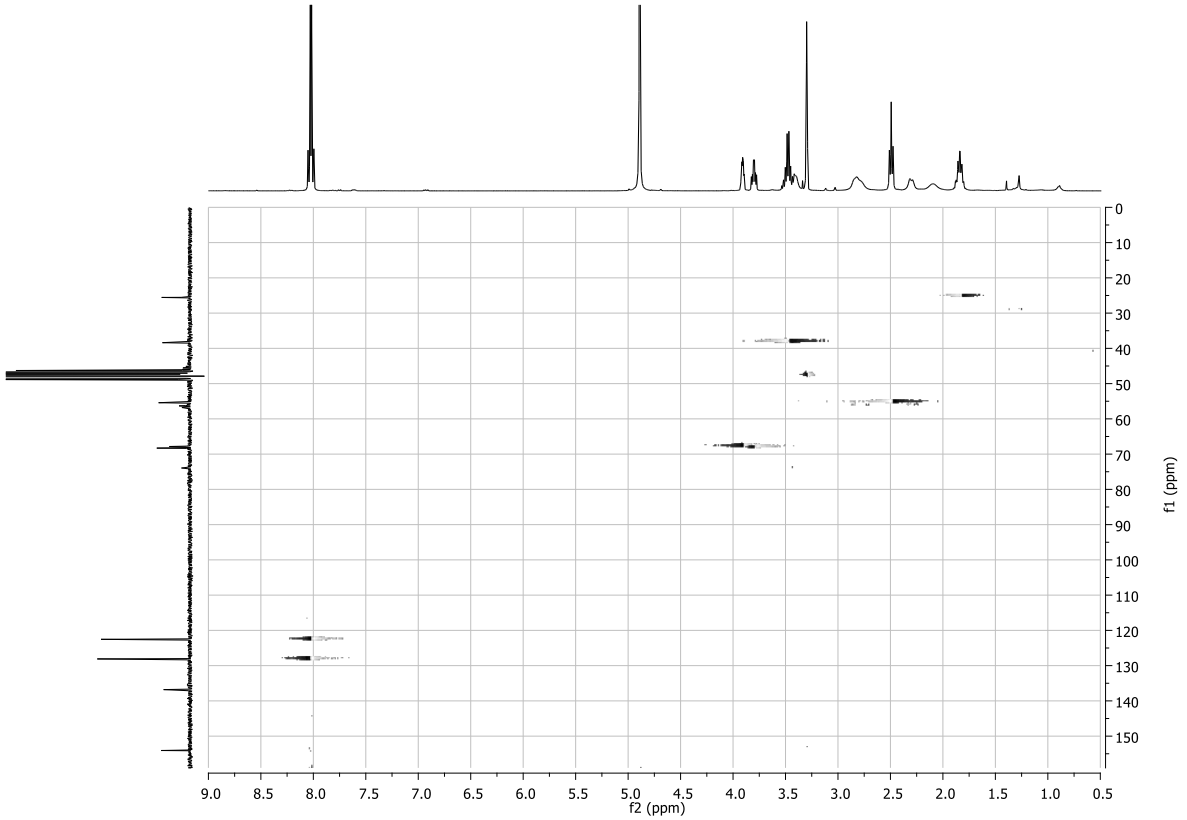


Figure S31: $^{1}H/^{13}C$ gHSQC spectrum of **4** (400/100 MHz, CD_3OD).

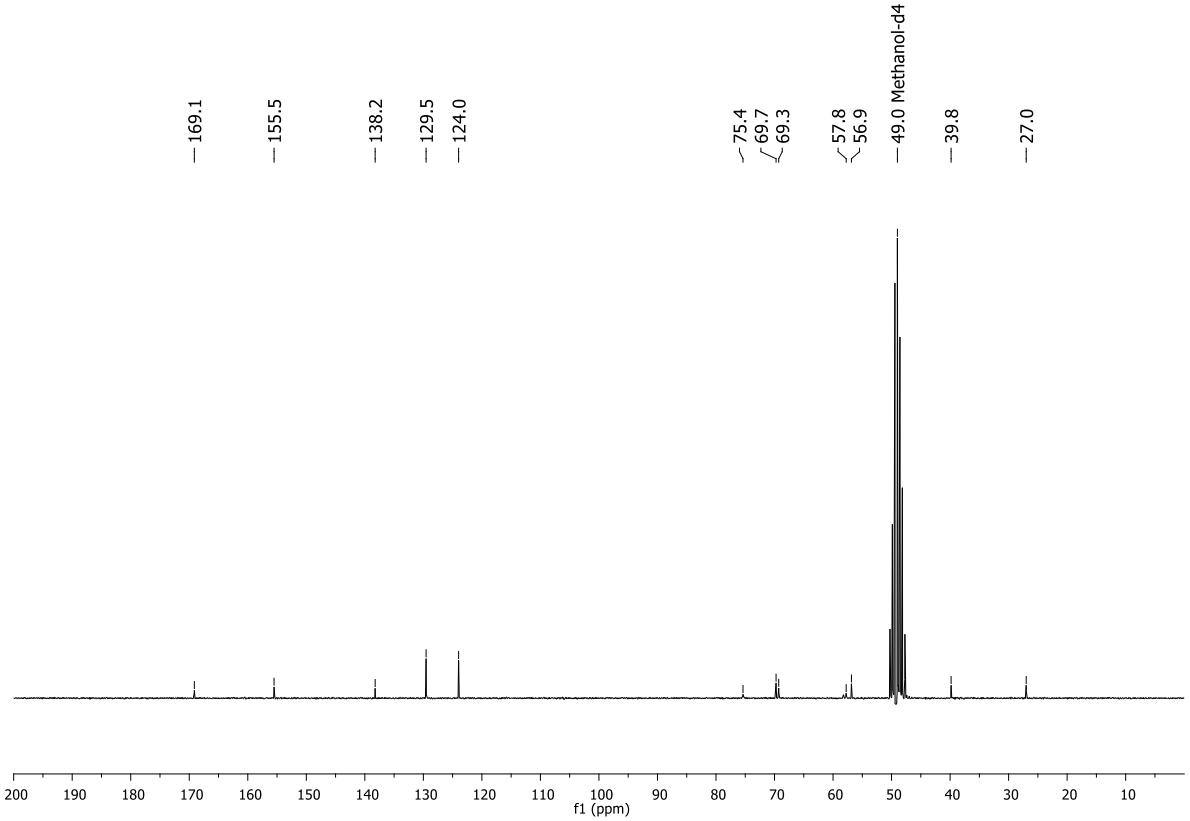


Figure S32: ^{13}C NMR spectrum of **4** (50 MHz, CD_3OD).

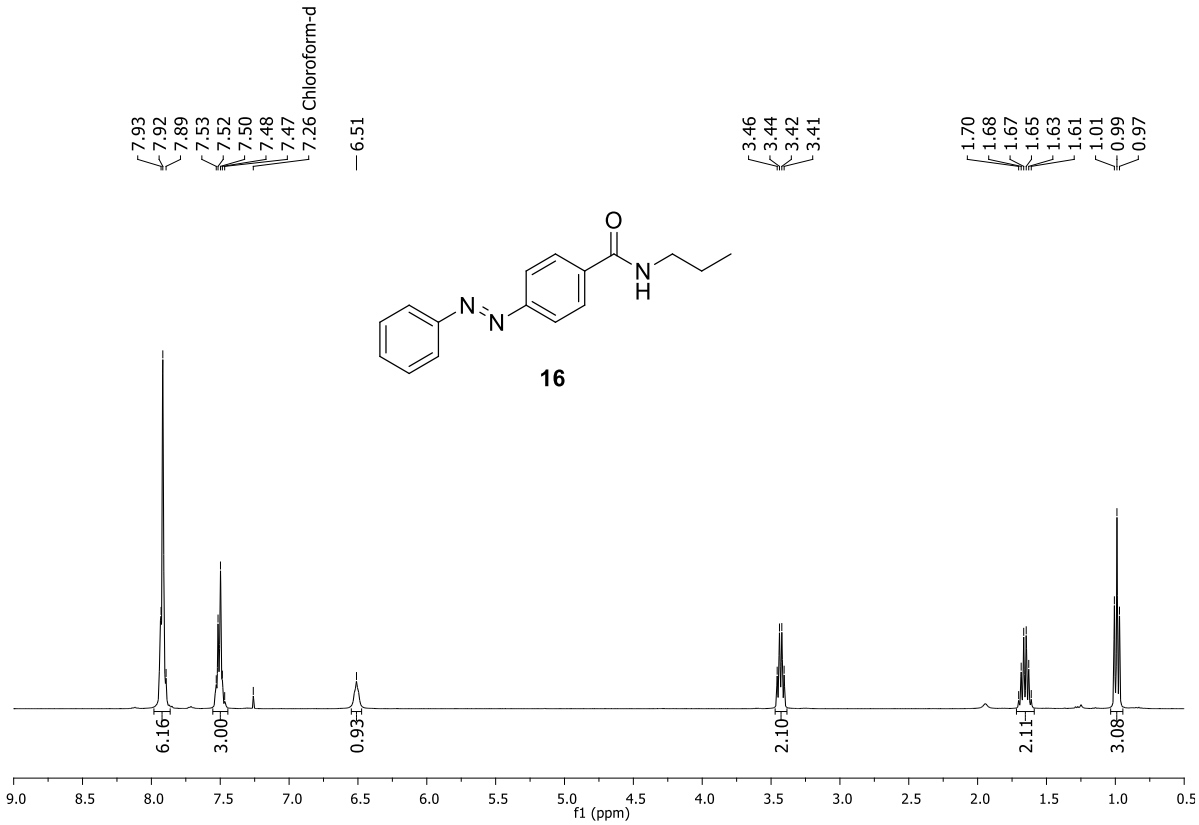


Figure S33: ^{1}H NMR spectrum of **16** (400 MHz, $CDCl_3$).

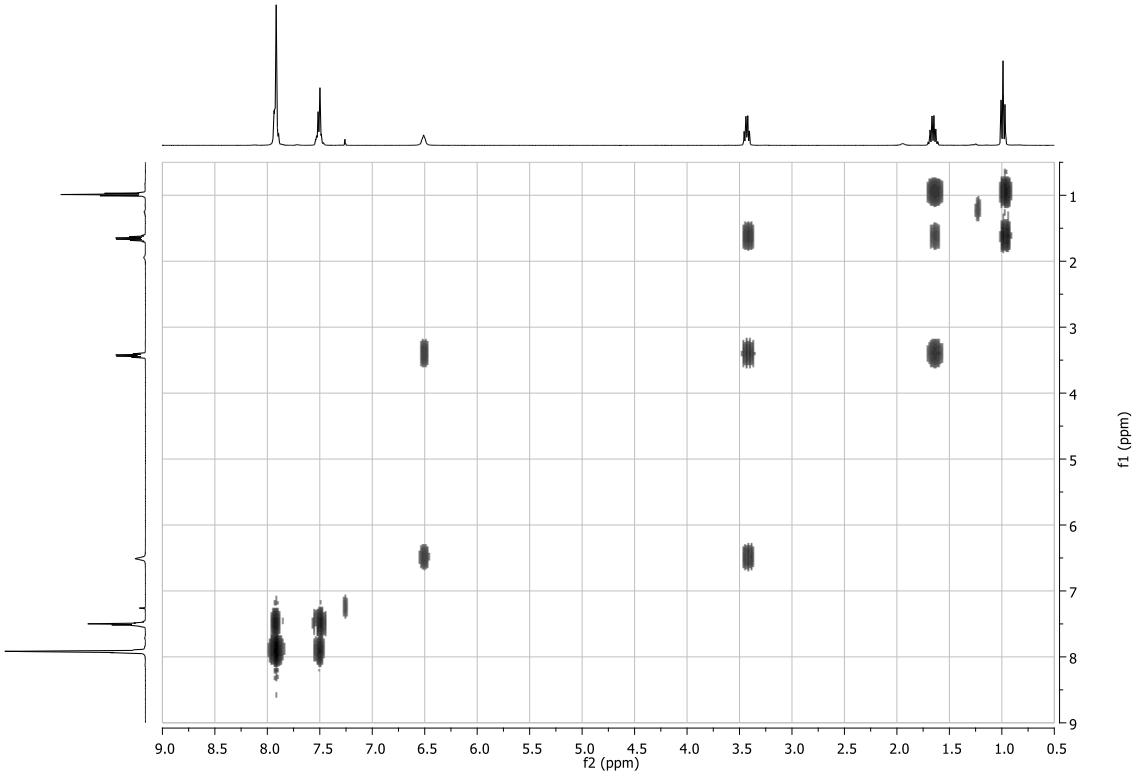


Figure S34: gCOSY spectrum of **16** (400 MHz, $CDCl_3$).

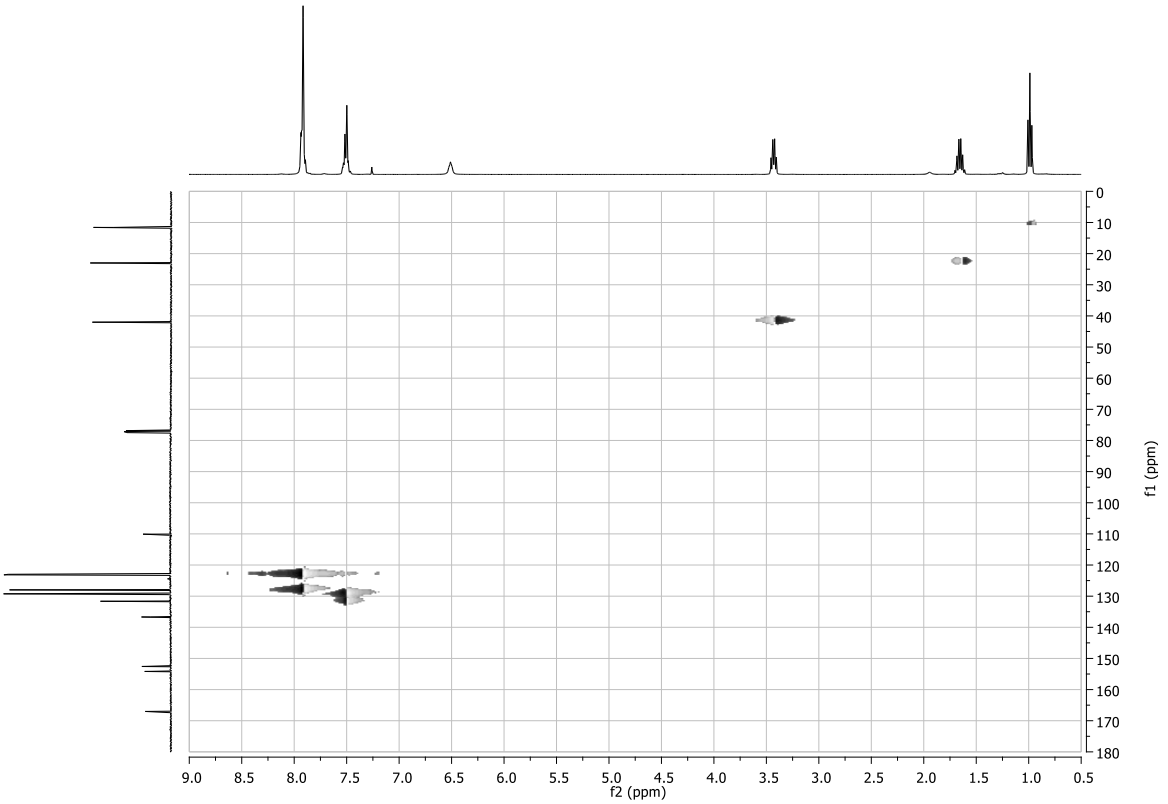


Figure S35: $^{1}H/^{13}C$ gHSQC spectrum of **16** (400/100 MHz, $CDCl_3$).

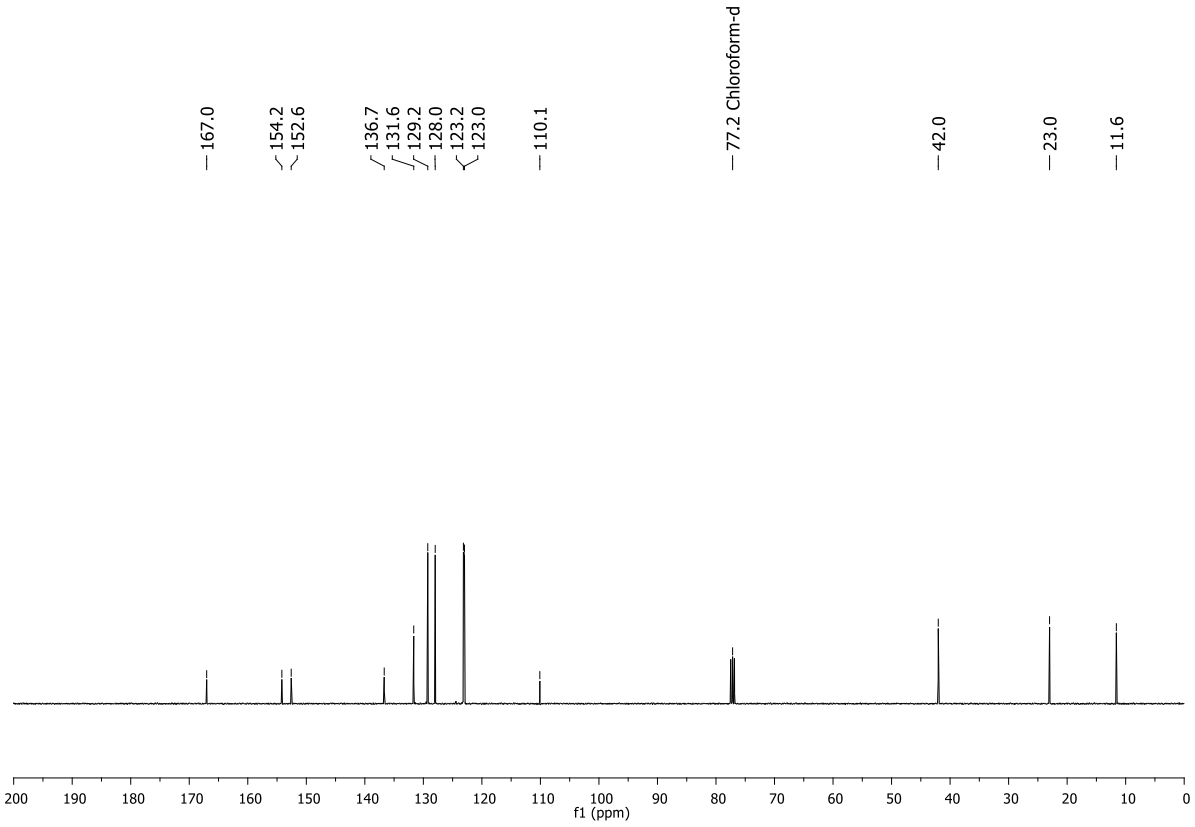


Figure S36: ^{13}C NMR spectrum of **16** (100 MHz, $CDCl_3$).

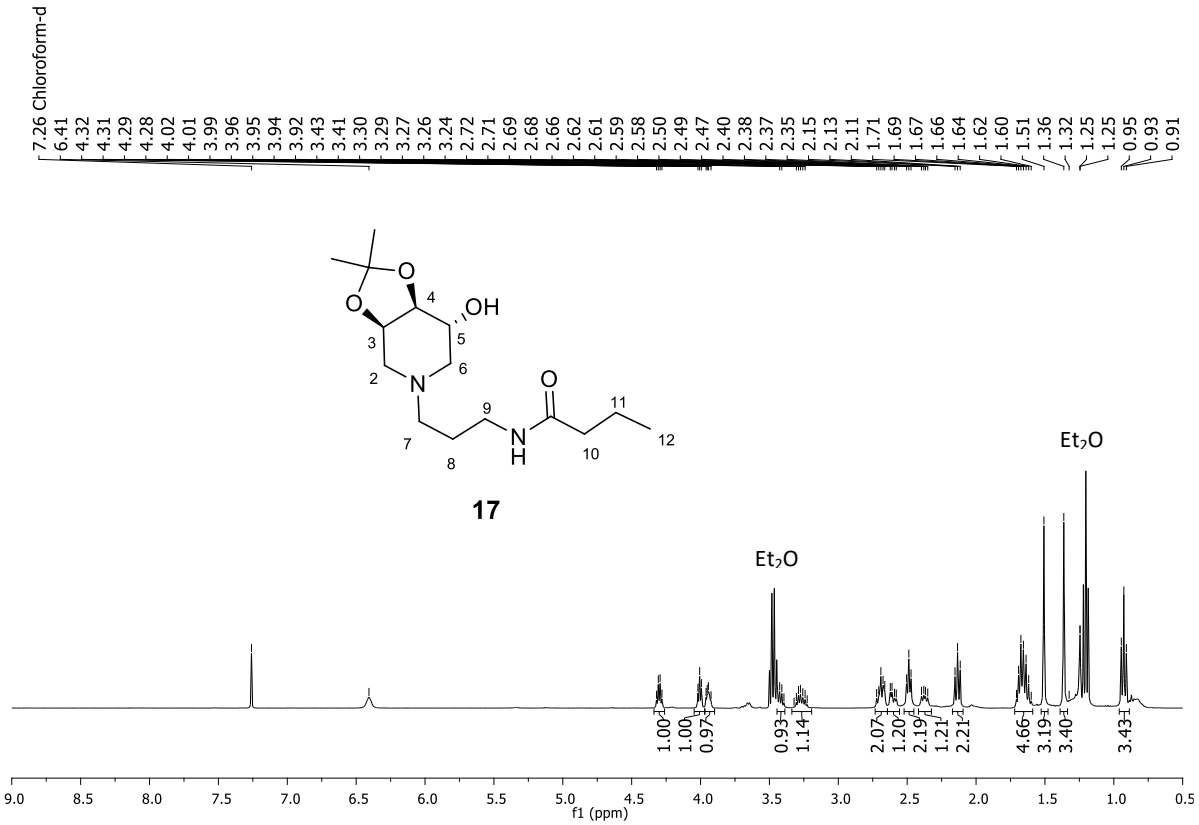


Figure S37: ^{1}H NMR spectrum of **17** (400 MHz, $CDCl_3$).

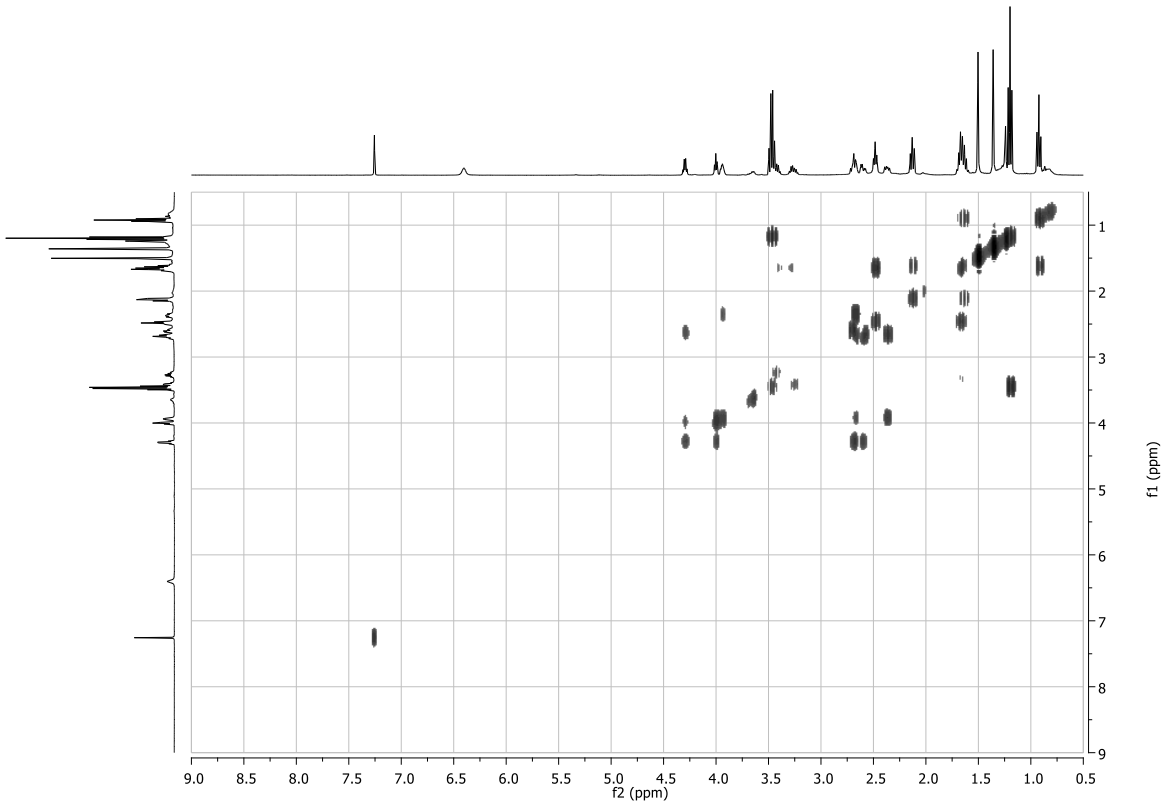


Figure S38: gCOSY spectrum of **17** (400 MHz, $CDCl_3$).

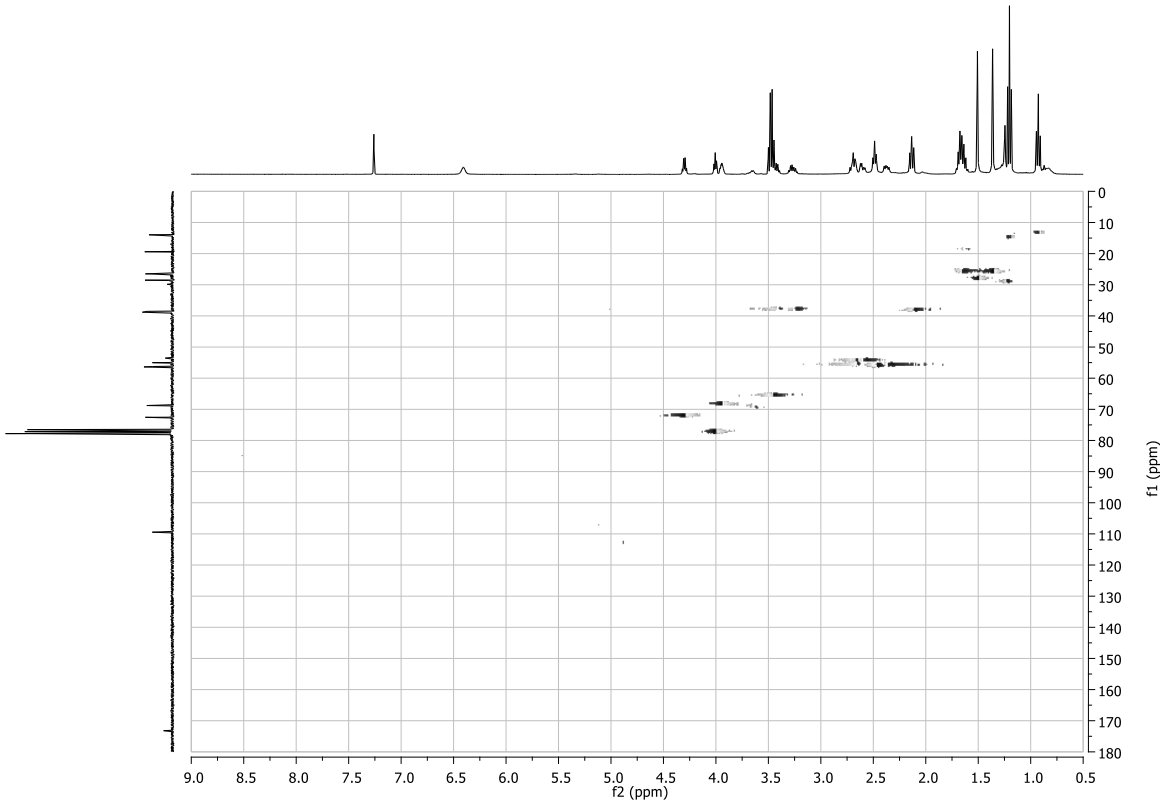


Figure S39: $^{1}H/^{13}C$ gHSQC spectrum of **17** (400/100 MHz, $CDCl_3$).

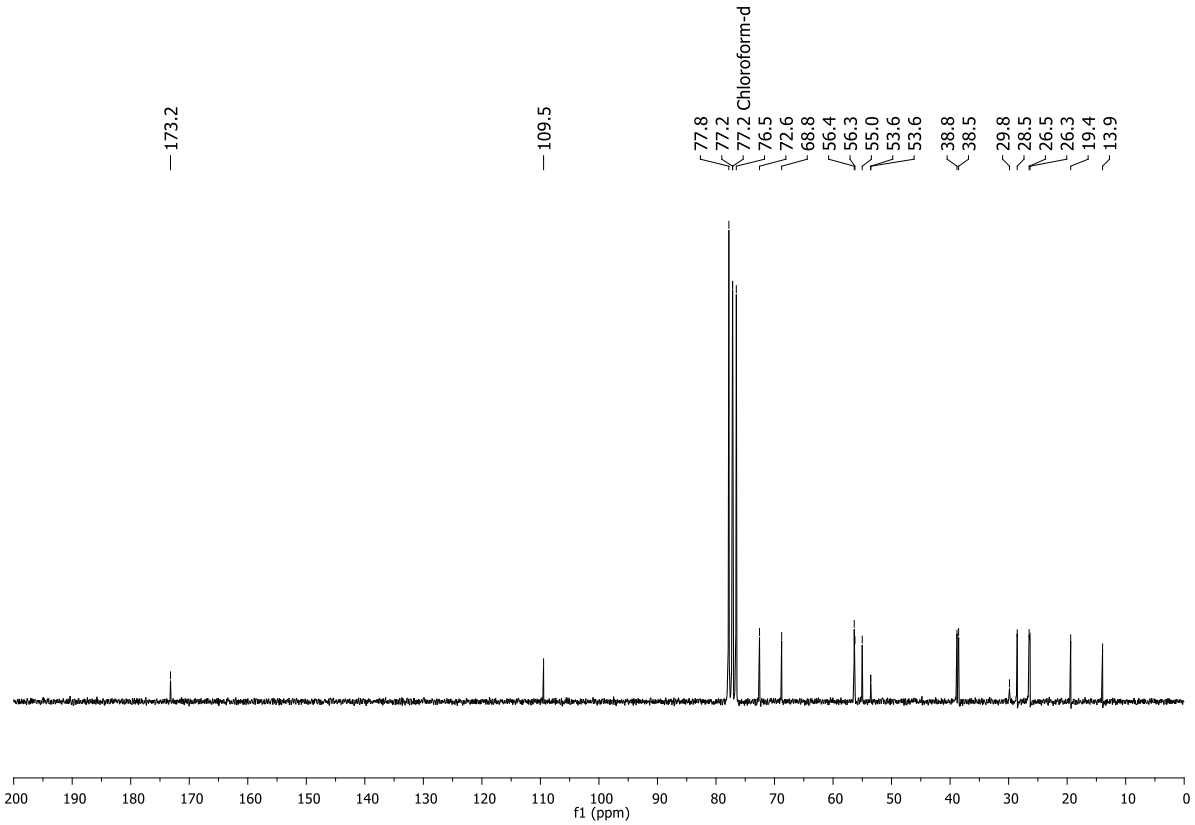
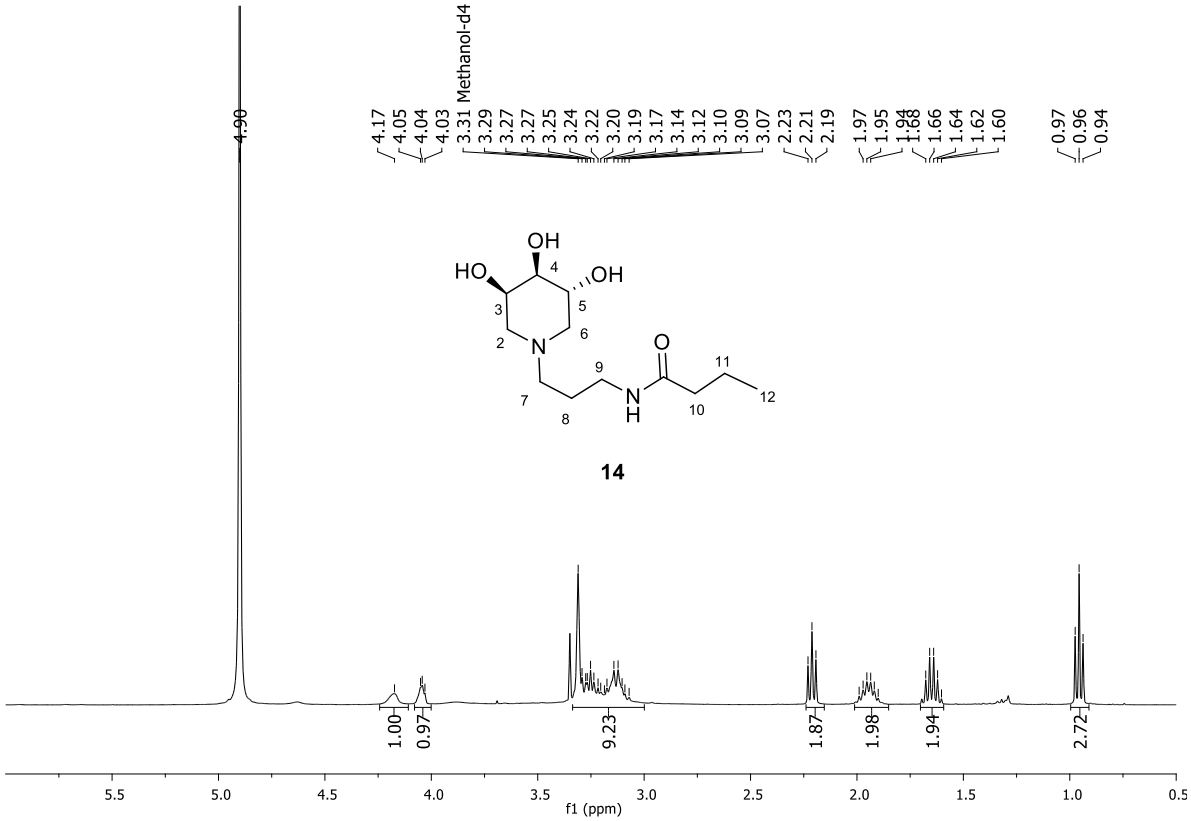


Figure S40: ^{13}C NMR spectrum of **17** (50 MHz, $CDCl_3$).



14

Figure S41: ^{1}H NMR spectrum of **14** (400 MHz, CD_3OD).

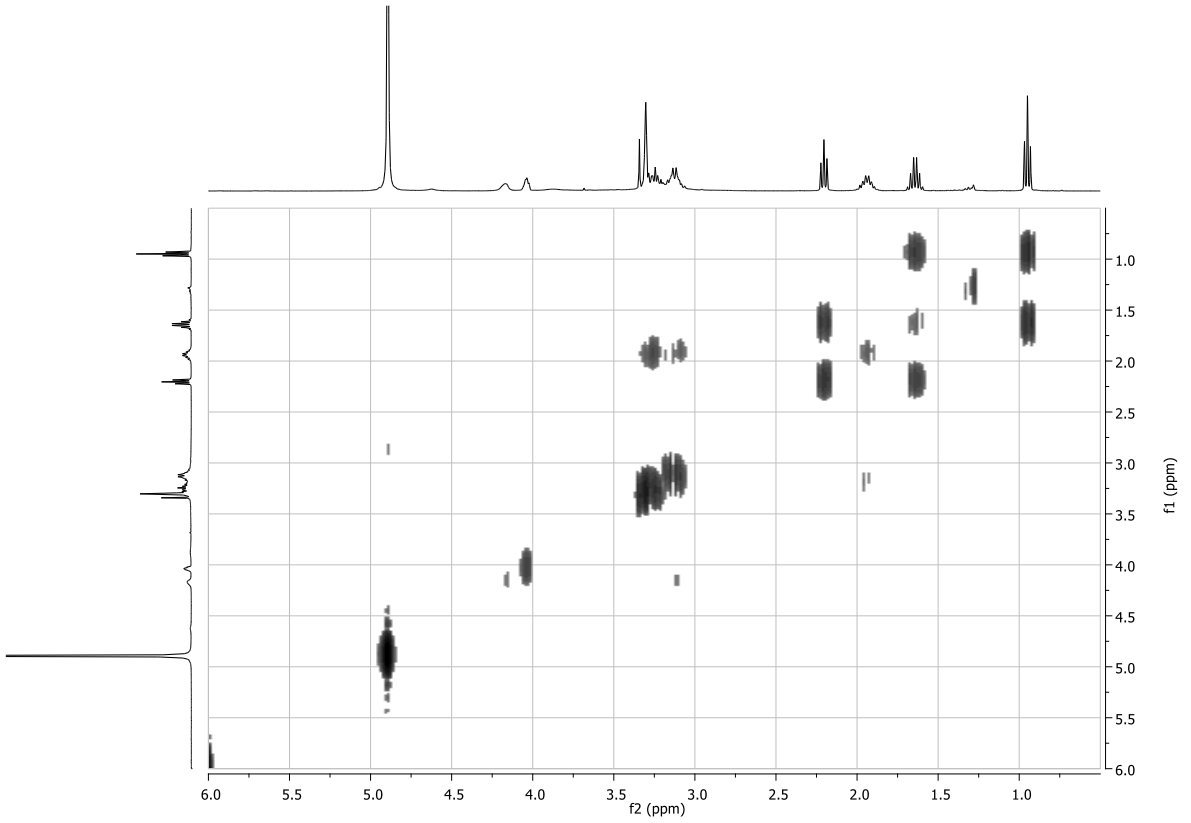


Figure S42: gCOSY spectrum of **14** (400 MHz, CD_3OD).

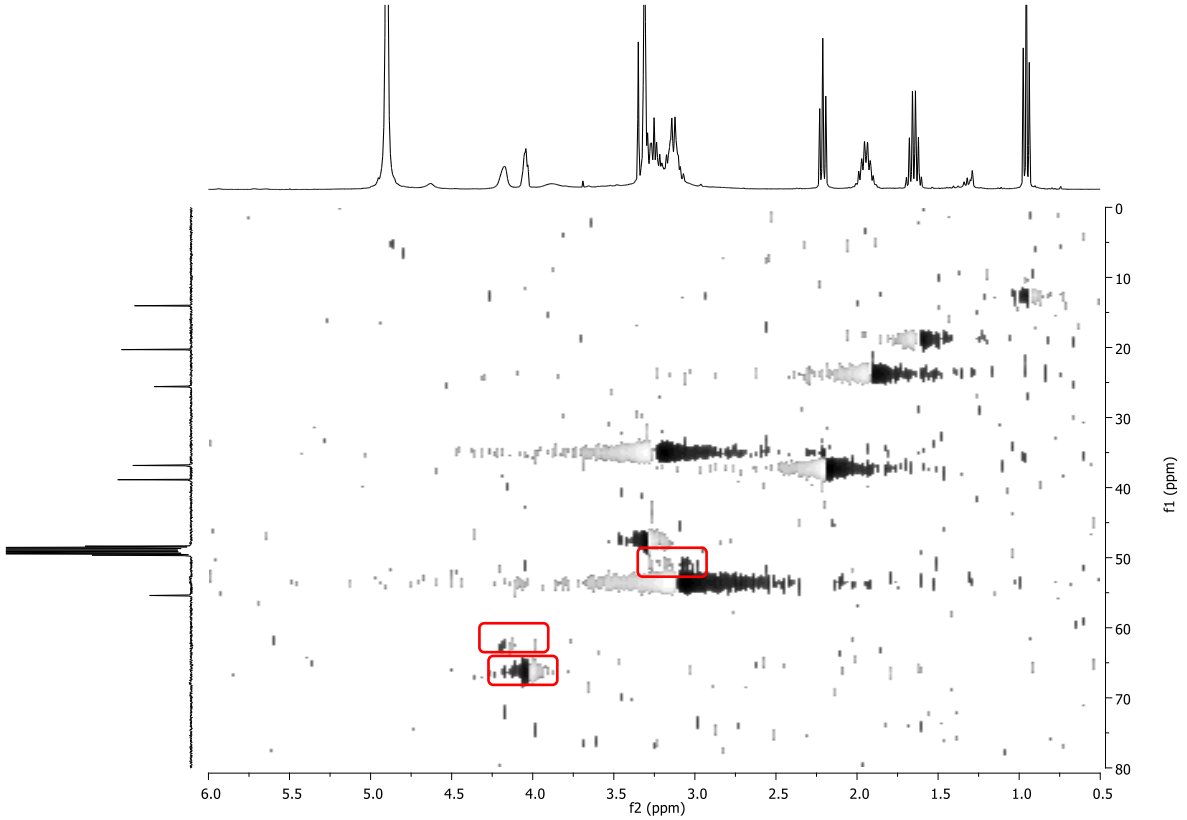


Figure S43: $^{1}H/^{13}C$ gHSQC spectrum of **14** (400/100 MHz, CD_3OD). Red frames highlight C-2, C-3, C-4, C-6.

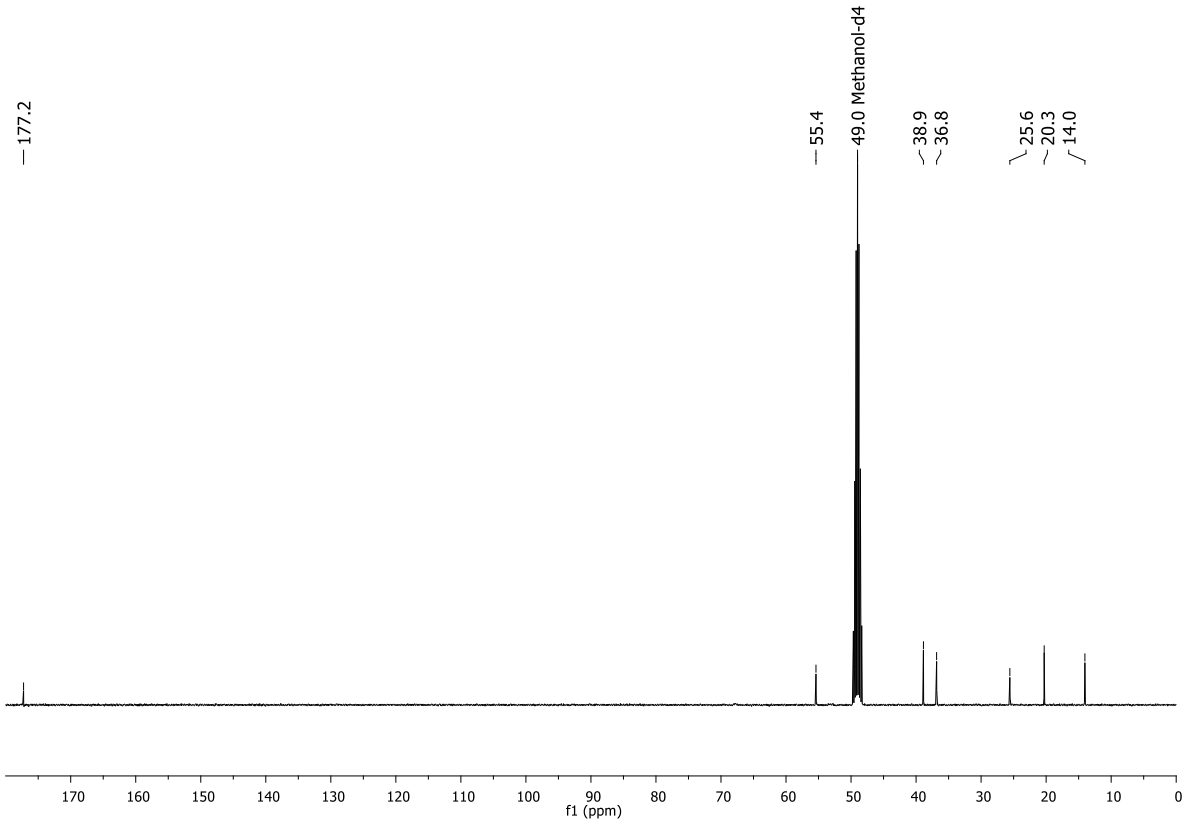


Figure S44: ^{13}C spectrum of **14** (100 MHz, CD_3OD).

Photostationary State (PSS) evaluation of 3 by ^{1}H-NMR with ThorLabs M340L4 LED lamp

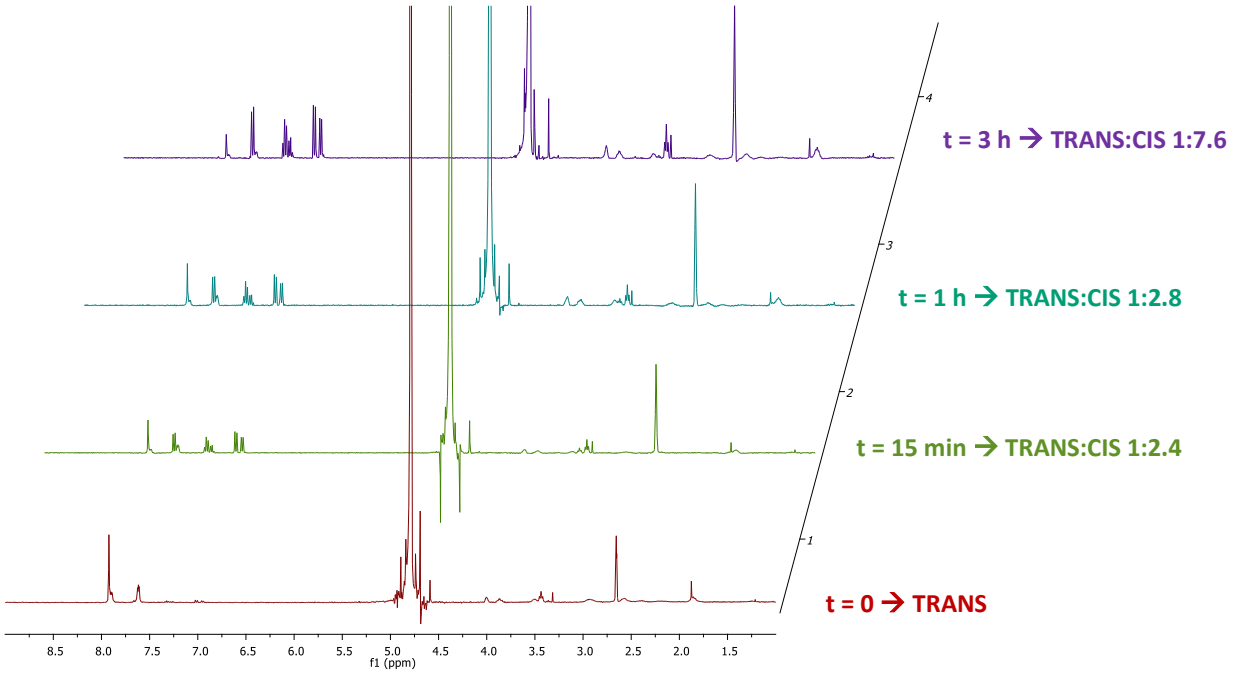


Figure S45: ^{1}H NMR spectra of *trans*-**3** (D_2O with 4% DMSO) at t = 0 (*trans* > 95%, bottom) and after 15 min, 1 h and 3 h of irradiation at 340 nm (increasing *cis/trans* ratio up to PSS).

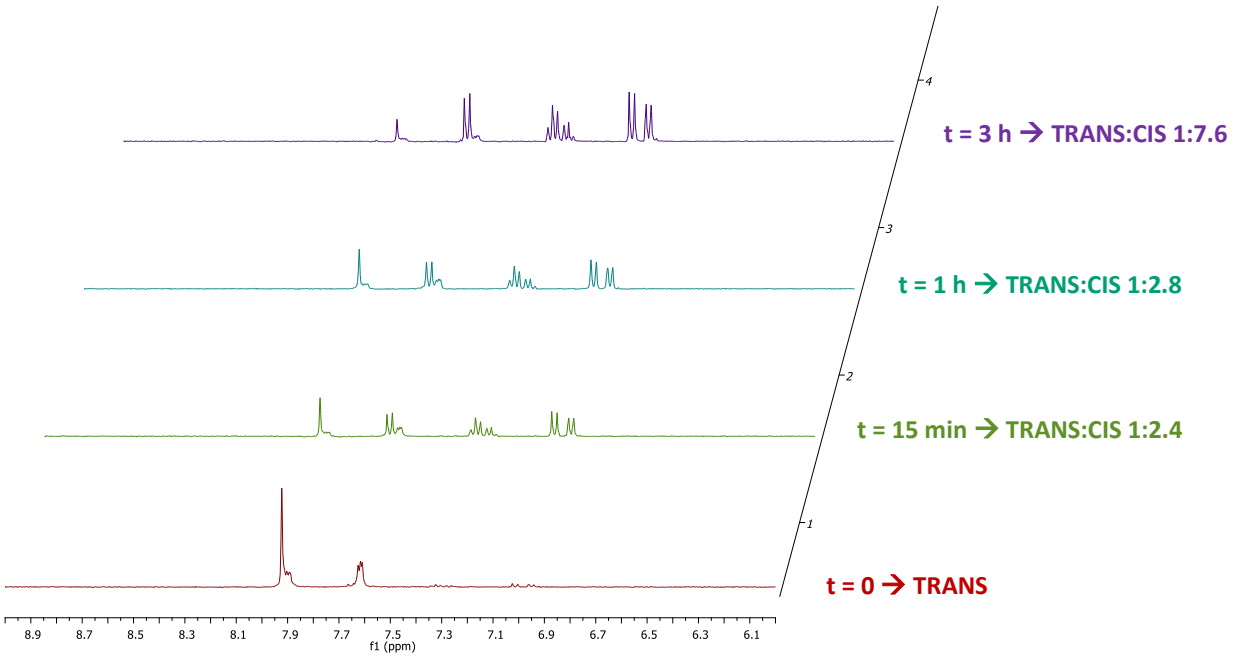


Figure S46: Selected region of ^{1}H NMR spectra of *trans*-**3** (D_2O with 4% DMSO) at t = 0 (100% trans, bottom) and after 15 min, 1 h and 3 h of irradiation at 340 nm (increasing *cis/trans* ratio up to PSS).

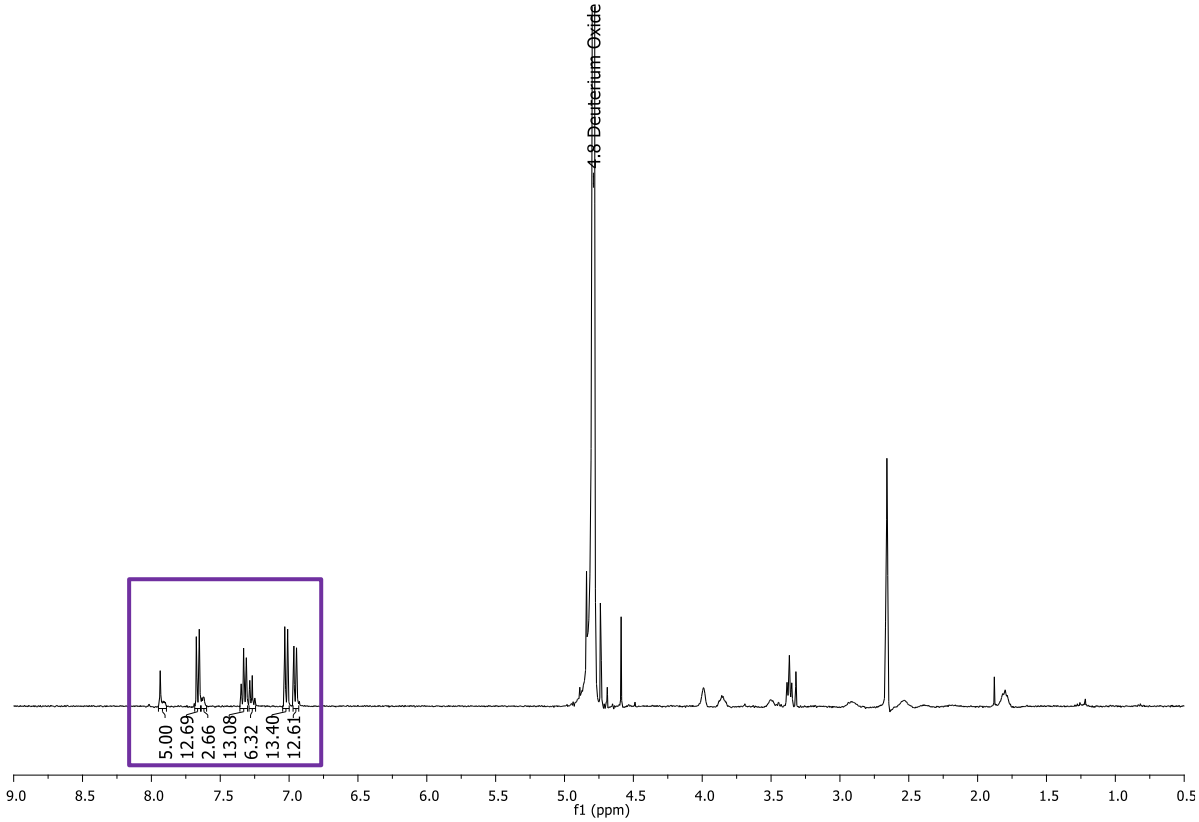


Figure S47: ^{1}H NMR spectrum of *trans*-**3** (D_2O with 4% DMSO) after 3 h of irradiation.

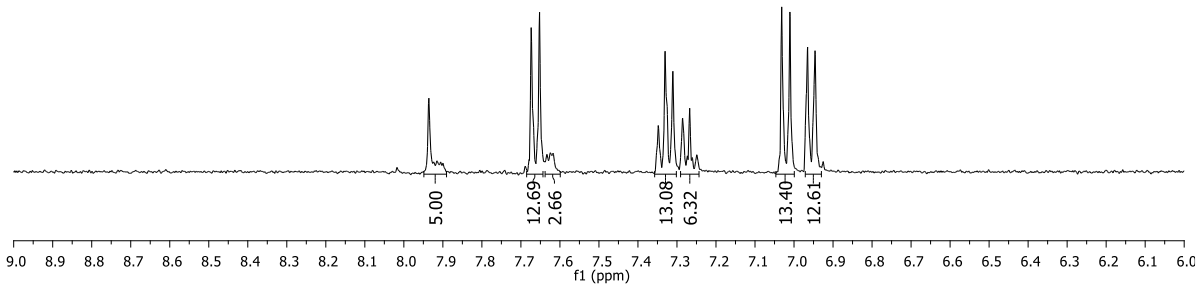


Figure S48: Selected region of ^{1}H NMR spectrum of *trans*-**3** (D_2O with 4% DMSO) after 3 h of irradiation.

Photostationary State (PSS) evaluation of 4 by ^{1}H-NMR with ThorLabs M340L4 LED lamp

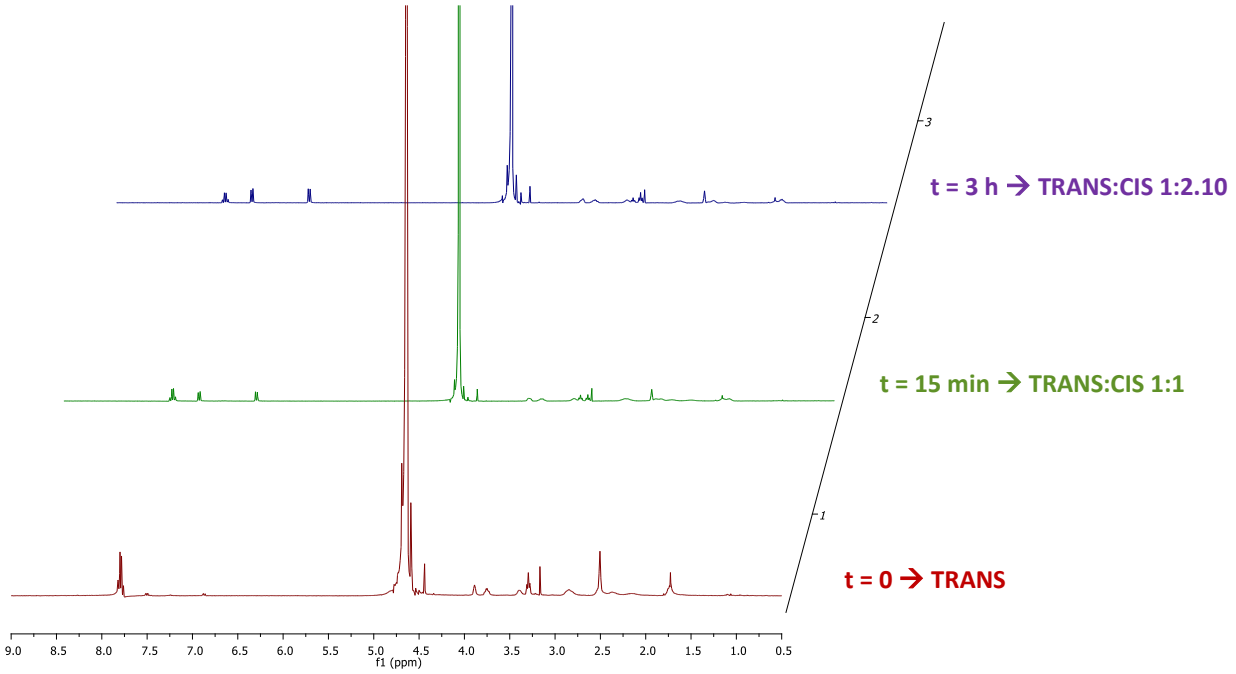


Figure S49: ^{1}H NMR spectra of *trans*-**4** (D_2O with 4% DMSO) at t = 0 (*trans* > 93%, the sample was slightly exposed to light, bottom) and after 15 min and 3 h of irradiation at 340 nm (increasing *cis/trans* ratio up to PSS).

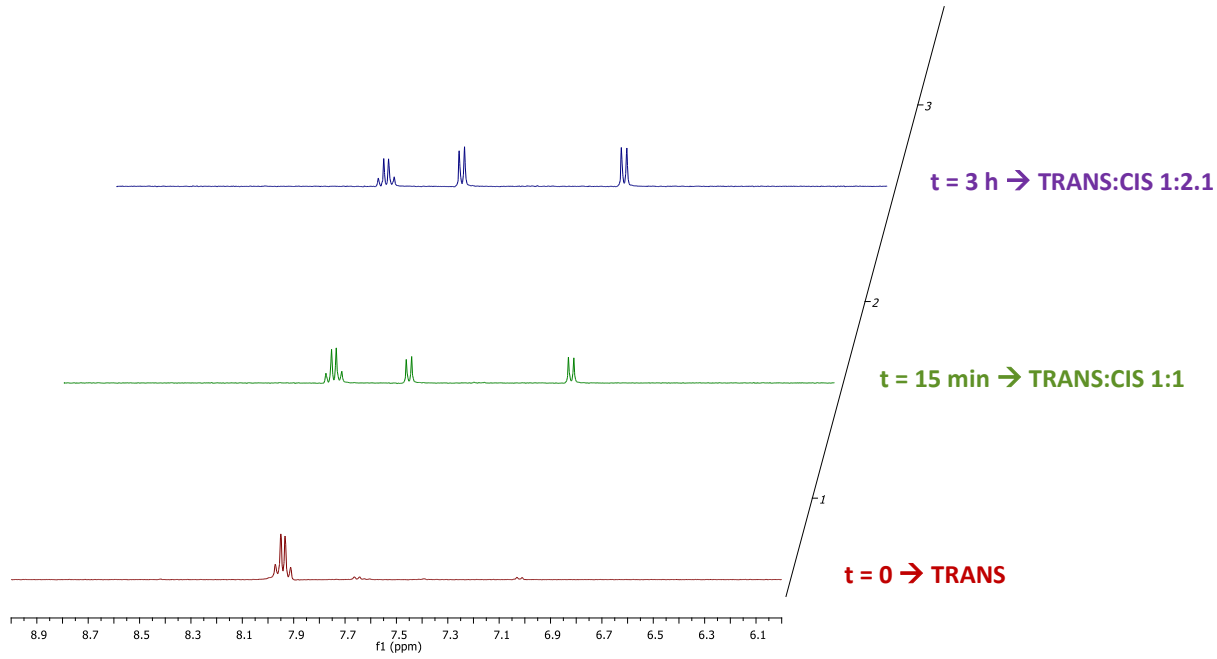


Figure S50: Selected region of ^{1}H NMR spectra of *trans*-**4** (D_2O with 4% DMSO) at t = 0 (*trans* > 93%, the sample was slightly exposed to light, bottom) and after 15 min and 3 h of irradiation at 340 nm (increasing *cis/trans* ratio up to PSS).

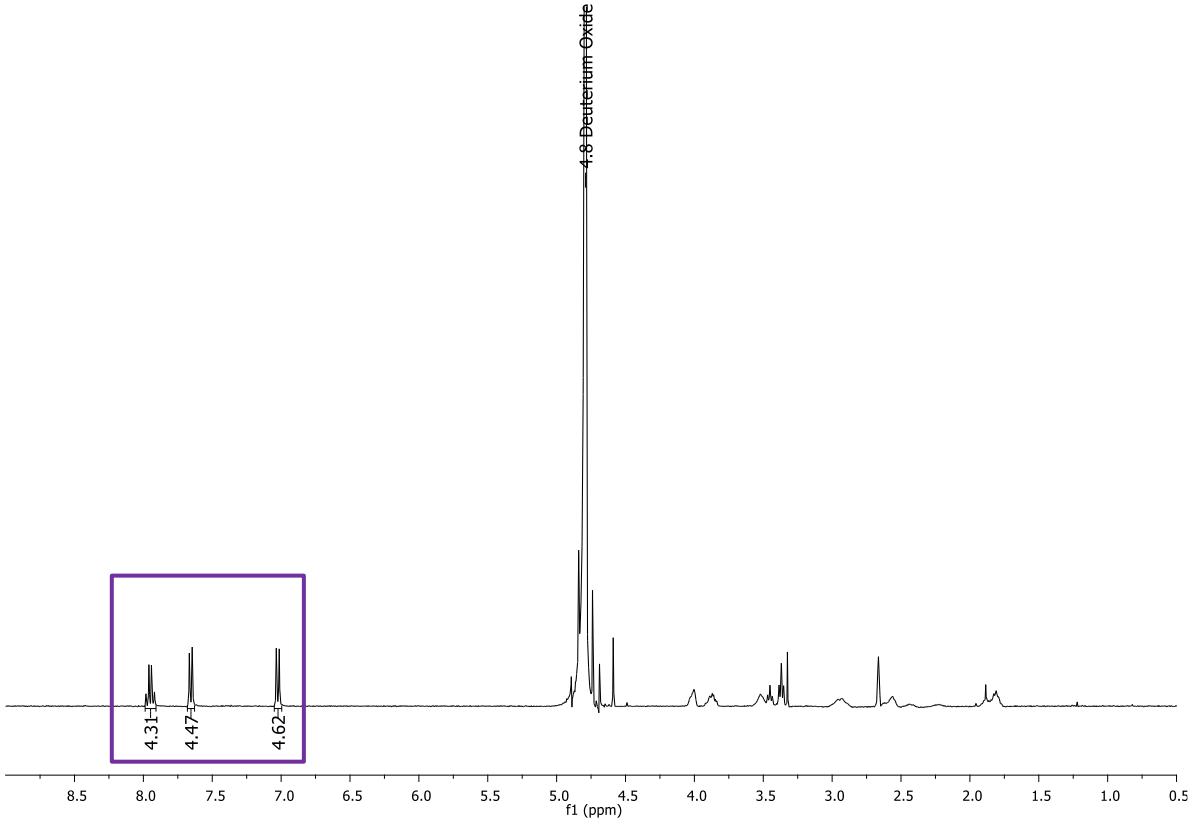


Figure S51: ^{1}H NMR spectrum of *trans*-**4** (D_2O with 4% DMSO) after 3 h of irradiation.

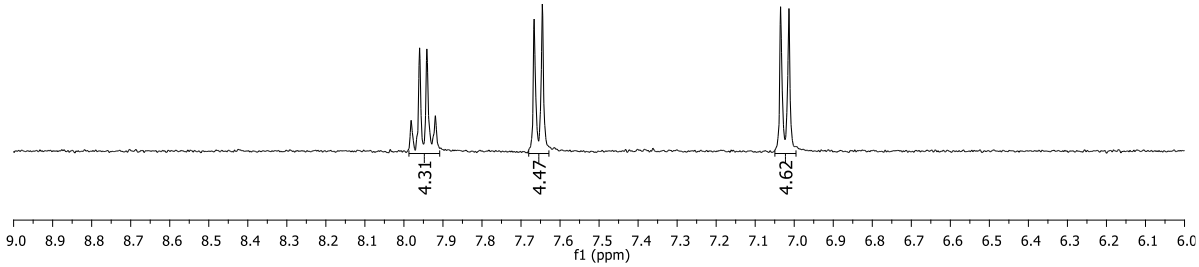


Figure S52: Selected region of ^{1}H NMR spectra of *trans*-**4** (D_2O with 4% DMSO) after 3 h of irradiation.

UV-vis absorption spectroscopy and switching studies – compound 1

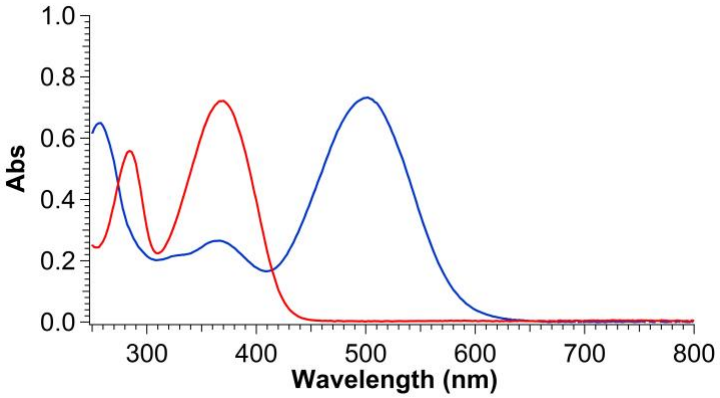


Figure S53: UV-Vis Absorption spectra of DHA **1** (red, solid line) and VHF **1** (blue line) in water (7% dmso) at 25 °C. The red dashed line shows the UV-Vis absorption spectrum after one light-heat cycle.

Photo-isomerization studies on compound **1**

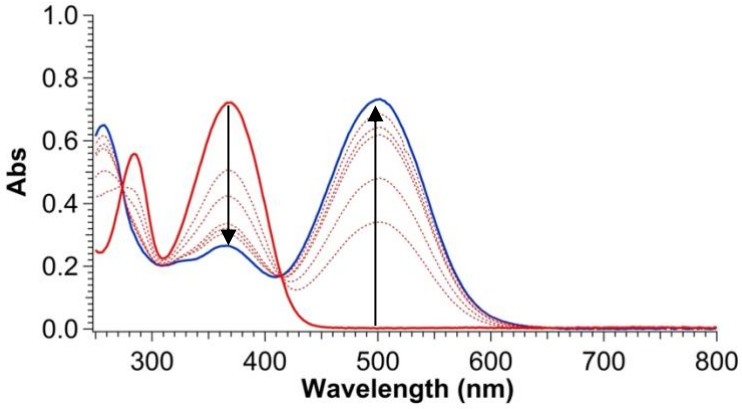


Figure S54: Spectral changes upon irradiation of **1** (DHA-to-VHF ring-opening, from red to blue) at 365 nm in water (7% dmso) for 0-15 sec.

Thermal studies on compound **1**

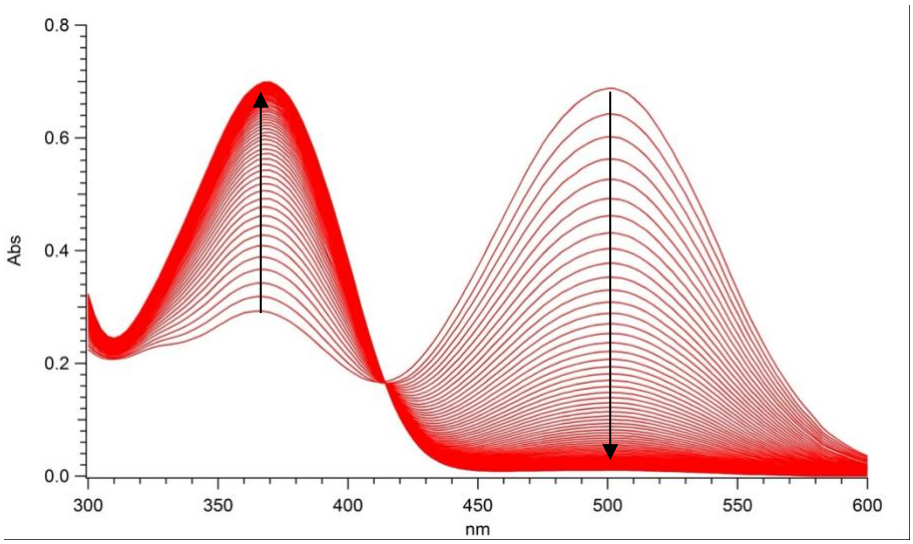


Figure S55: Spectral evolution during thermal back- reaction of **1** in water (7% dmso) at 25 °C.

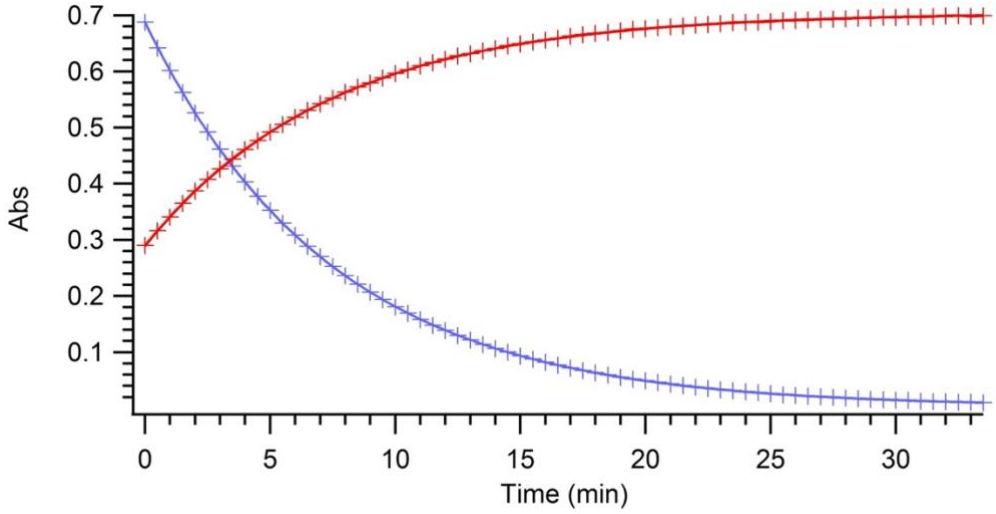


Figure S56: Exponential decays of absorbance at 370 nm (red) and 502 nm (blue) of $\mathbf{1}_{VHF}$ to $\mathbf{1}_{DHA}$, in water (7% dmso) at 25 °C ($t_{1/2}$ = 6 min).

UV-vis absorption spectroscopy and switching studies – compound 2

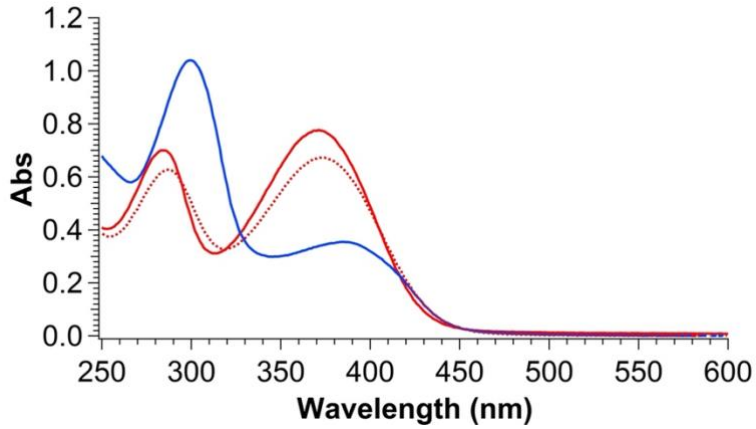


Figure S57: UV-Vis Absorption spectra of DHA **2** (red, solid line) and VHF **2** (blue line). The red dashed line shows the UV-Vis absorption spectrum after one light-heat cycle, indicating loss of photoactivity of **2** in water (2% dmso) at 37 °C.

Photo-isomerization studies on compound **2**

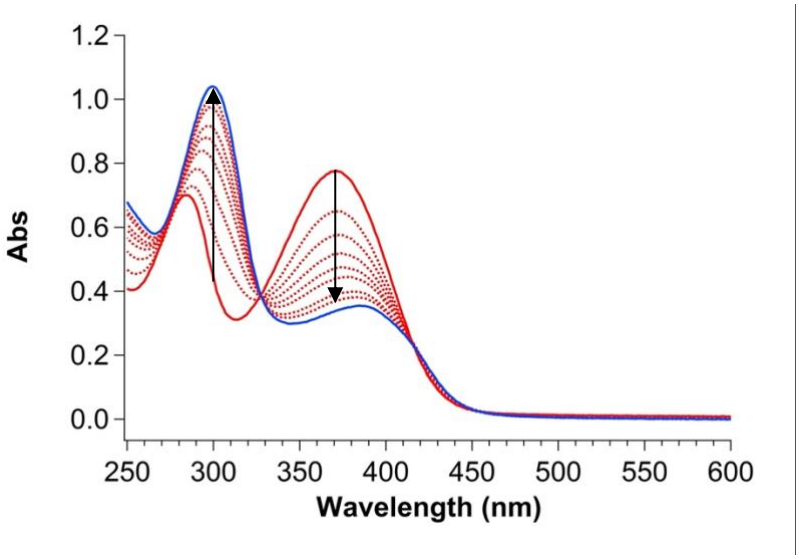


Figure S58: Spectral changes upon irradiation of **2** (DHA-to-VHF ring-opening, from red to blue) at 365 nm in water (2% dmso) for 0-3 min.

Thermal studies on compound **2**

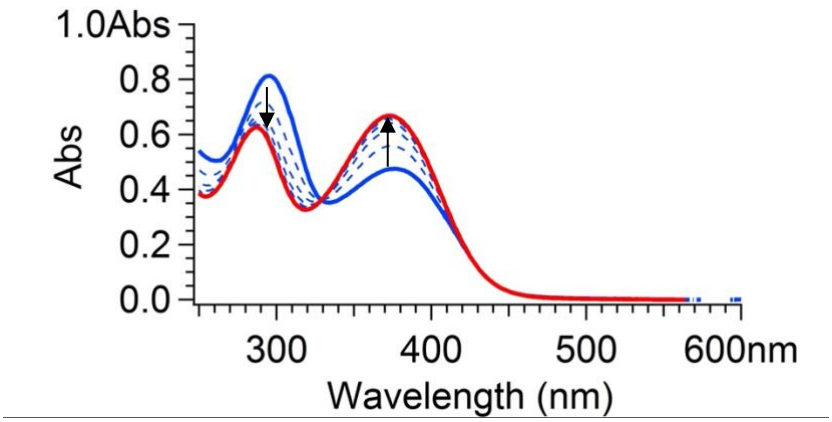


Figure S59: Spectral evolution during thermal back- reaction of **2** in water (2% dmso) at 37 °C.

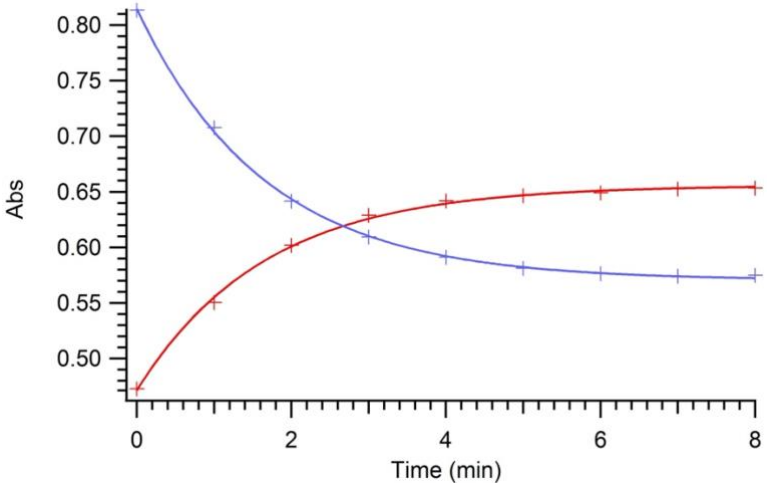


Figure S60: Exponential decays of absorbance at 295 nm (red) and 380 nm (blue) of $\mathbf{2}_{\mathbf{VHF}}$ to $\mathbf{2}_{\mathbf{DHA}}$, in water (2% dmso) at 37 °C ($t_{1/2}$ = 60 sec).

UV-vis absorption spectroscopy and switching studies – compound 3

Photo-isomerization studies on compound **3**

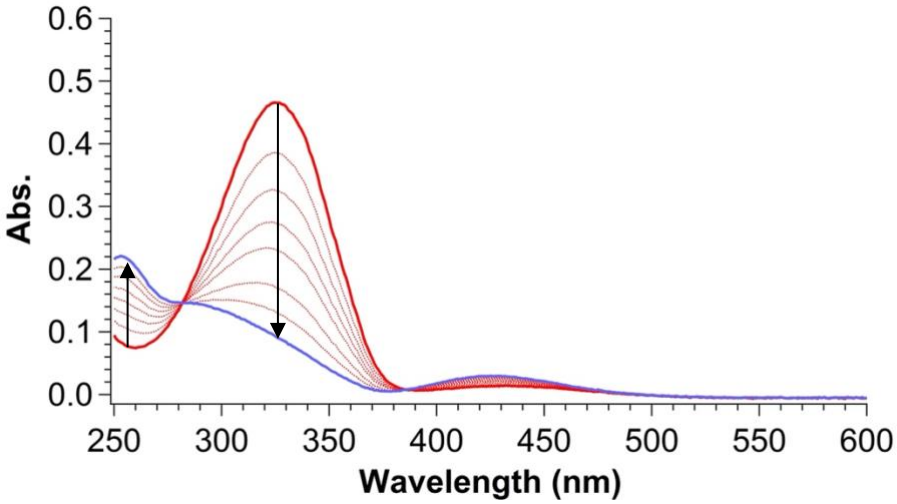


Figure S61: Spectral changes upon irradiation of AZB **3** (from red, > 95% *trans*, to blue, PSS) at 340 nm in water (4% dmso) for 0-60 sec.

Thermal studies on compound **3**

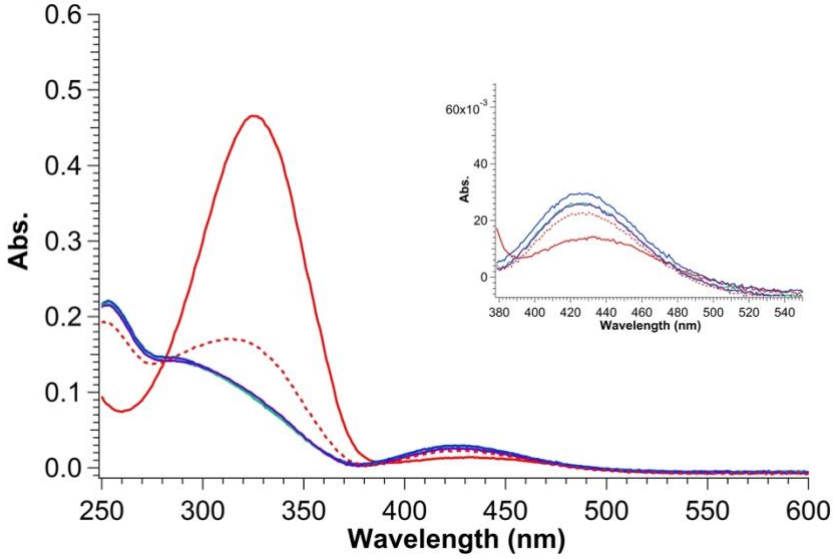


Figure S62: UV-Vis Absorption spectra of AZB **3** in water (4% dmso) before irradiation (red, solid line, >95% *trans*) and after irradiation at 340 nm for 1 min (blue line, PSS). Spectral evolution during thermal back-reaction at 37 °C after 1 h (green line) and 2 h (violet line) shows minimal changes. UV-Vis absorption spectrum after 20 h (red dotted line) at 37 °C indicates a $t_{1/2}$ > 20 h. The inset reports a zoomed characteristic region around 430 nm.

UV-vis absorption spectroscopy and switching studies – compound 4

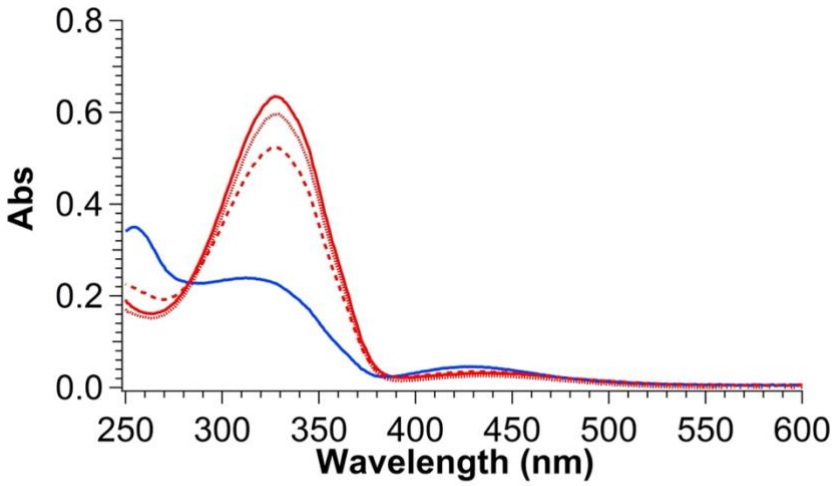


Figure S63: UV-Vis Absorption spectra of AZB **4** in water (4% dmso) before irradiation (red, solid line, 100% *trans*) and after irradiation at 340 nm for 6 min (blue line, PSS). UV-Vis absorption spectra after 5 d (red dashed line) and 12 d (red dotted line) of thermal relaxation in the dark at 37 °C are also shown.

Photo-isomerization studies on compound **4**

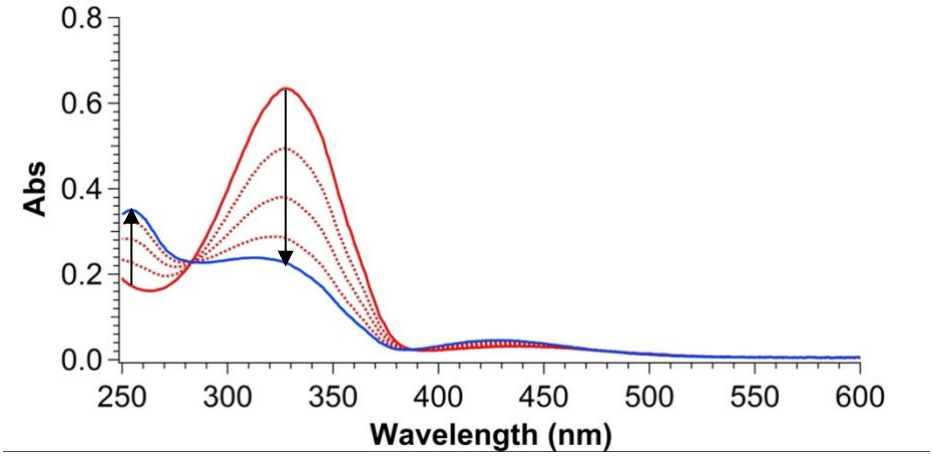


Figure S64: Spectral changes upon irradiation of AZB **4** (from red, 100% *trans*, to blue, PSS) at 340 nm in water (4% dmso) for 0-6 min.

Thermal studies on compound **4**

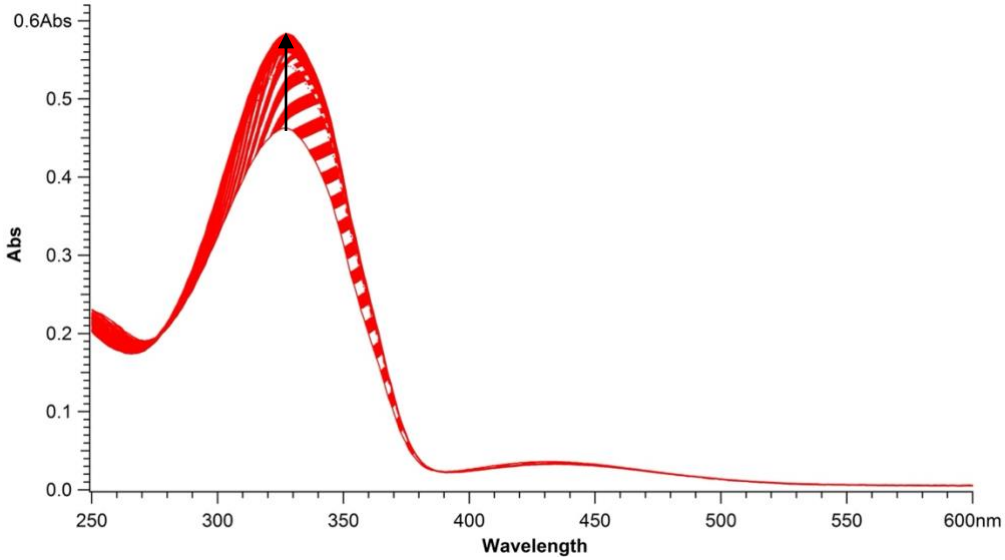


Figure S65: Spectral evolution during thermal back- reaction of **4** in water (4% dmso) at 37 °C recorded for 5 d ($t_{1/2}$ = 41 h).

Biochemical characterization

Inhibitory activity towards human GCase from leukocyte homogenates.

Compounds **1**, **2, *trans*- -3**, ***trans*-4**, **14**, **15** and **16** were screened towards GCase in leukocytes isolated from healthy donors (controls). Isolated leukocytes were disrupted by sonication, and a micro-BCA protein assay kit (Sigma–Aldrich) was used to determine the total protein amount for the enzymatic assay, according to the manufacturer instructions. Enzyme activity was measured in a flat-bottomed 96-well plate. Compound solution (3 µL), 4.29 µg/µL leukocytes homogenate (7 µL), and substrate 4-methylumbelliferyl-β-D-glucoside (3.33 mM, 20 µL, Sigma–Aldrich) in citrate/phosphate buffer (0.1:0.2, M/M, pH 5.8) containing sodium taurocholate (0.3%) and Triton X-100 (0.15%) were incubated for 1 h at 37 °C. The reaction was stopped by addition of sodium carbonate (200 µL; 0.5M, pH 10.7) containing Triton X-100 (0.0025 %), and the fluorescence of 4-methylumbelliferone released by GCase activity was measured in SpectraMax M2 microplate reader (λex=365 nm, λem=435 nm; Molecular Devices). For each compound a blank composed by a water solution containing 0.2% of bovine serum albumin (BSA), inhibitor and substrate (called "inhibitor blank") was tested and compared with the experiment blank, composed by BSA and substrate. No “inhibitor blank” differs from the experiment blank, demonstrating that the inhibitors do not interfere with the fluorescence of the hydrolyzed substrate. Percentage of GCase inhibition is given with respect to the control (without compound). Data are mean ± RSD (n=3).

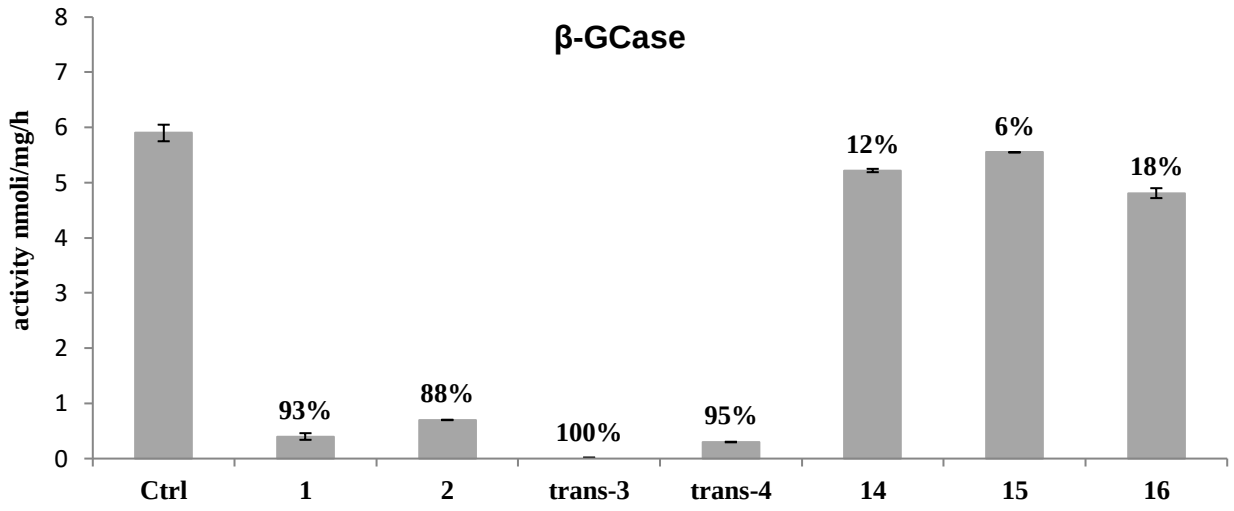


Figure S66: Activity of GCase in the presence of compounds **1**, **2**, *trans*-**3**, *trans*-**4**, **14**, **15** and **16** at 1 mM. The corresponding calculated percentage of inhibition is indicated above each bar.

For percentage of inhibition higher than 80% (at 1mM), the IC_{50} values of inhibitors against GCase were determined by measuring the initial hydrolysis rate with 4-methylumbelliferyl-β-D-glucoside (3.33 mM). Data obtained were fitted to the following equation using the Origin Microcal program.

$$\frac{Vi}{Vo} = \frac{Max - Min}{1 + \left(\frac{x}{IC_{50}}\right)^{slope}} + Min$$

where V_i/V_o, represent the ratio between the activity measured in the presence of the inhibitor (V_i) and the activity of the control without the inhibitor (V_0), "x" the inhibitor concentration, Max and Min, the maximal and minimal enzymatic activity observed, respectively.

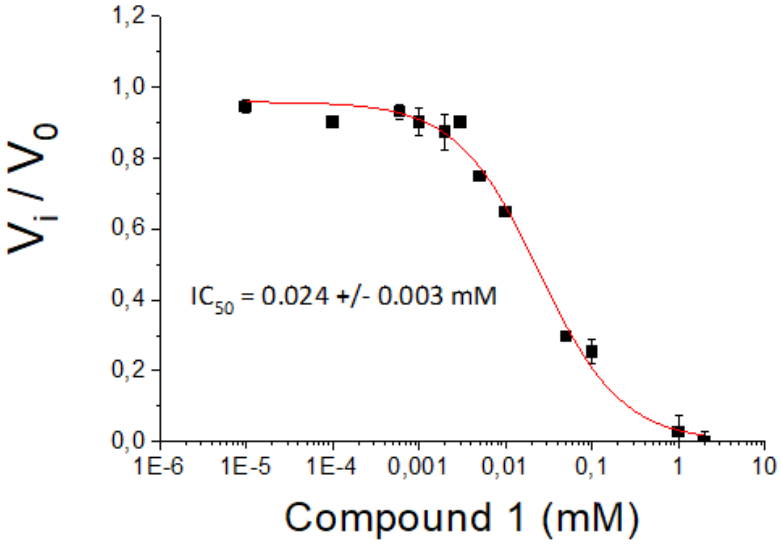


Figure S67: IC_{50} graph of compound **1** with 50% of DMSO**.**

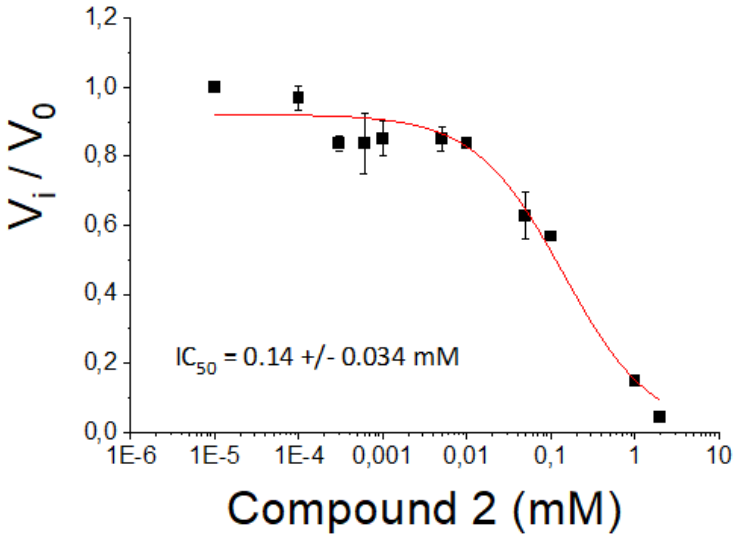


Figure S68: IC_{50} graph of compound **2.**

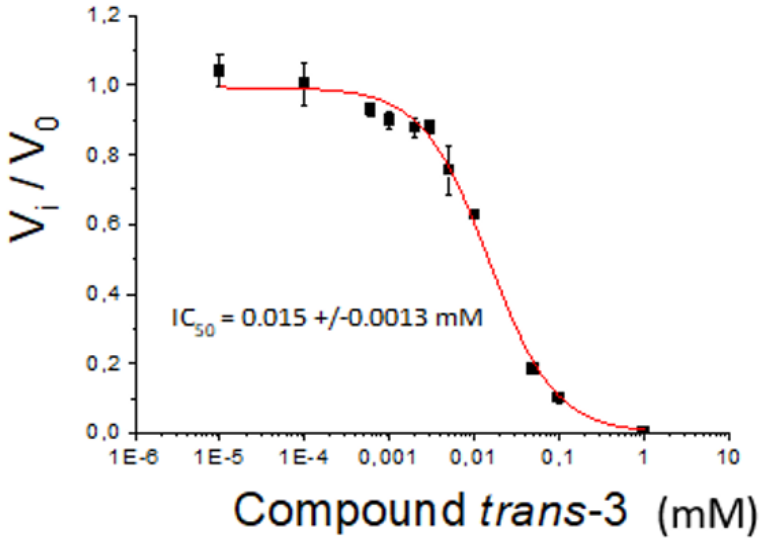


Figure S69: IC_{50} graph of compound *trans*-**3**.

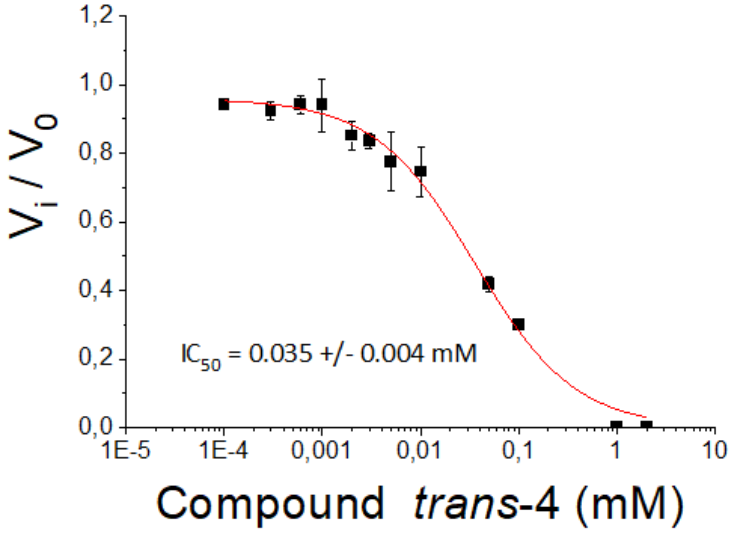


Figure S70: IC_{50} graph of compound *trans*-**4**.

Biochemical characterization towards human GCase for compounds **PSS-3** and **PSS-4** was carried using ThorLabs M340L4 LED lamp for irradiation at 340 nm. In a Micro Cell cuvette, a compound solution (10 mM, total volume 120 µL) was irradiated for 3 hours with the ThorLabs M340L4 LED at 340 nm.

The IC_{50} values of inhibitors **PSS-3** (88% *cis*) and **PSS-4** (68% *cis*) were determined by measuring the initial hydrolysis rate with 4-methylumbelliferyl-β-D-glucoside (see above). The percentage of inhibition for **PSS-3** (100%) and **PSS-4** (100%) at 1mM was extrapolated from the following IC_{50} graphs and reported in Table 2 of the main text.

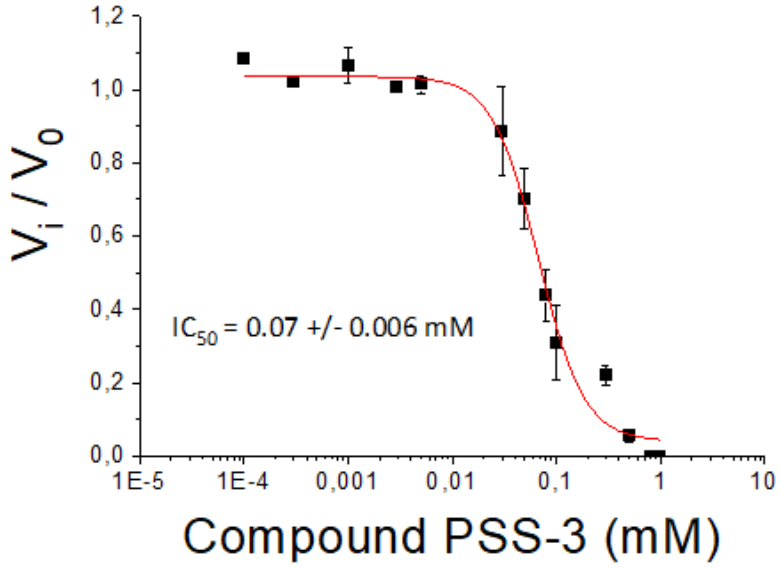


Figure S71: IC_{50} graph of compound **PSS-3.**

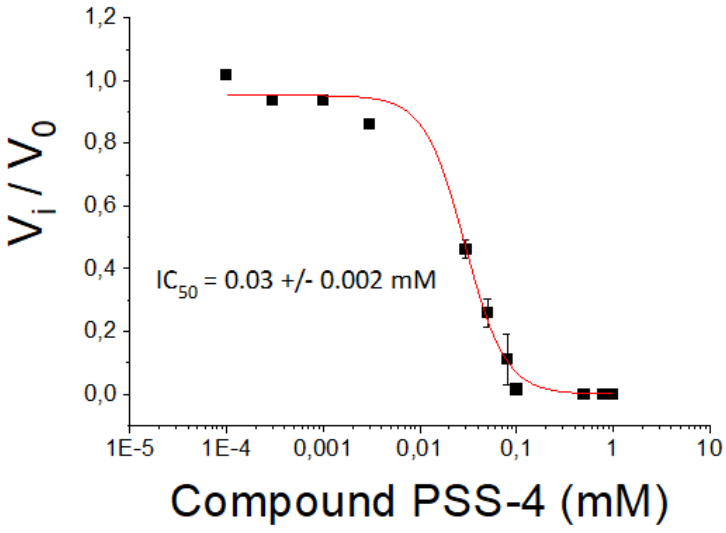


Figure 71: IC_{50} graph of compound **PSS-4.**

Kinetic Analysis for compound trans-3

The action mechanism of compound *trans*-**3** was determined studying the dependence of the main kinetic parameters (K_m and V_{max}) from the inhibitor concentration. Kinetic data were analysed using the *Lineweaver-Burk* plot (double reciprocal plot). We found that experimental points described straight lines intersecting one each other in a point of *y* axis, suggesting that compound *trans*-**3** behave as a non-competitive inhibitor.

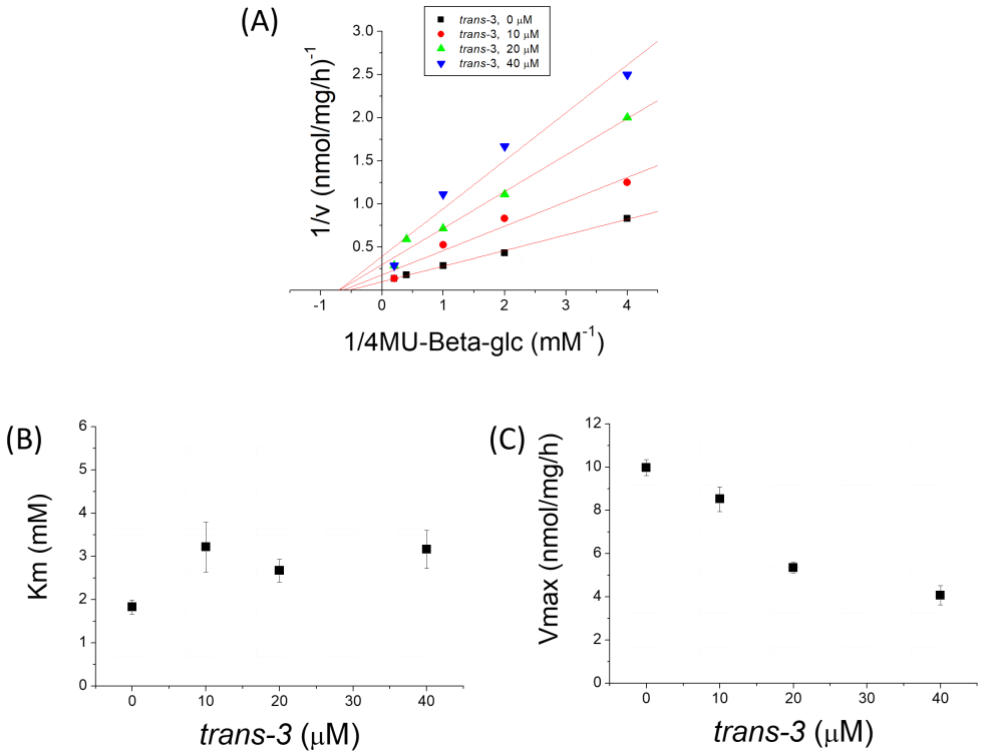


Figure S72: Kinetic analysis of compound *trans*-**3** (A) Double reciprocal plots. 4-Methylumbelliferyl-β-D-glucoside was employed as a substrate. The concentrations of compound *trans*-**3** are: ■, 0 µM; ♦, 10 µM; ▲, 20 µM; ▼, 40 µM. Data reported in the figures represent the mean values — S.E.M. (n = 3). (B, C) Behaviour of Km and Vmax at different concentrations of compound *trans*-**3**.

Ki value for compound *trans*-**3** was calculated, resulting 14.6 ± 0.3 µM.

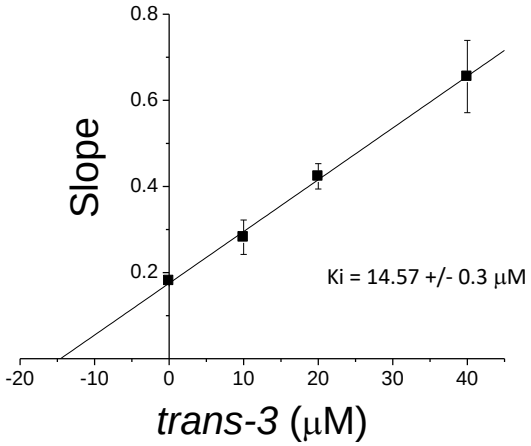


Figure S73: Plots for the determination of the Ki values of compound *trans*-**3**.

Thermal stabilization[6] of recombinant wild-type human GCase by compound *trans*-3

Recombinant wild-type human GCase enzyme (VPRIV® 1.0 x 10^{-9} mg/mL) aliquots (100 µL) with 0 (control), 1, 10, 50, 100 µM of compound *trans*-**3** were incubated at pH 7.0 for 20 minutes at 0 °C and then for 0 minutes or 20 minutes or 40 minutes or 60 minutes at 48 °C.

Subsequently, 100 µL of water were added to each aliquot. Then 10 µL of each aliquot was incubated at 37 °C for 1 h with 20 µL of substrate 4-methylumbelliferyl-β-D-glucoside (3.33 mM, Sigma–Aldrich), in citrate/phosphate buffer (0.1:0.2, M/M, pH 5.8) containing sodium taurocholate (0.3%) and Triton X-100 (0.15%). The reaction was stopped by addition of sodium carbonate (200 µL; 0.5M, pH 10.7) containing Triton X-100 (0.0025 %), and the fluorescence of 4-methylumbelliferone released by β-glucosidase activity was measured in SpectraMax M2 microplate reader (λex=365 nm, λem=435 nm; Molecular Devices). Data are mean ± RSD (n=3).

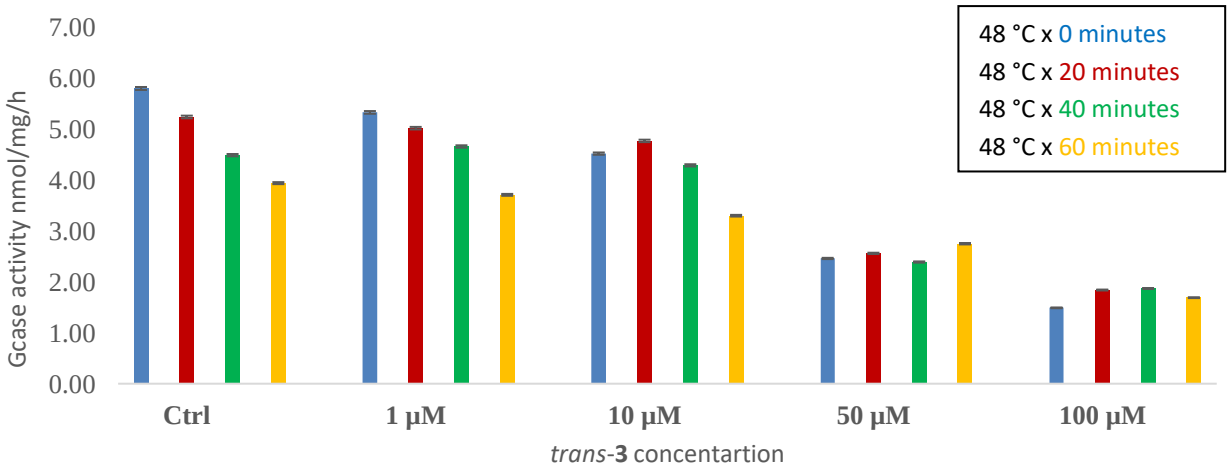


Figure S74: Activity of recombinant human GCase enzyme was determined after incubation for 0 minutes or 20 minutes or 40 minutes or 60 minutes at 48 °C with or without (Ctrl) different concentrations of *trans*-**3** by measuring the hydrolysis rate with 4-methylumbelliferyl-β-D-glucoside.

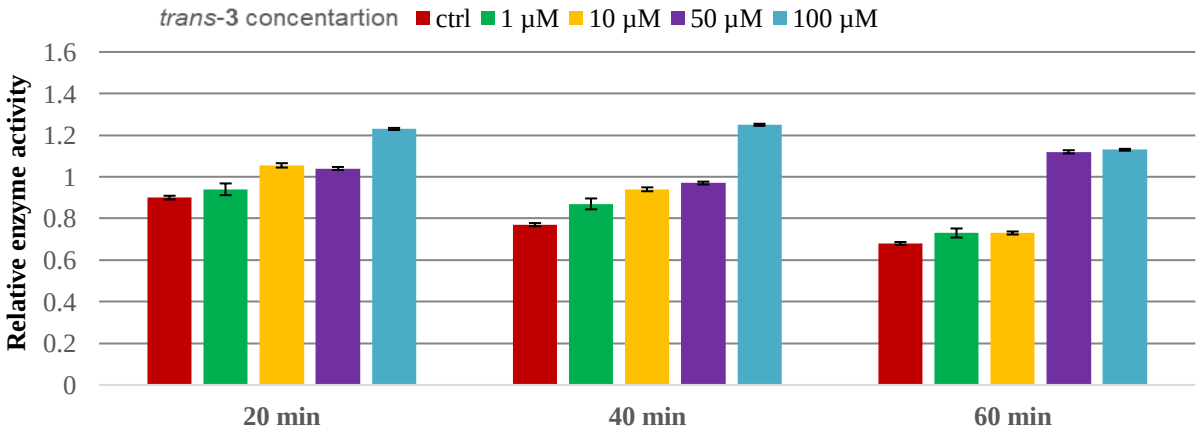


Figure S75: Stabilization of recombinant human GCase enzyme using heat inactivation. Relative enzymatic activity after thermal denaturation (48 °C) for 20 minutes, 40 minutes or 60 minutes at the indicated inhibitor concentrations respect to the corresponding assay at 37 °C. Data for control (ctrl) are obtained as above except that no inhibitor is present.

[6] L. Dìaz, J. Bujons, J. Casas, A. Llebaria, A. Delgado, *J. Med. Chem*. **2010**, *53*, 5248–5255.

Cytotoxicity Assays

MTT test was carried out using human fibroblasts wild type at different concentrations of compound (**1**, *trans*-**3** and *trans*-**4**). Fibroblasts were grown in the presence of Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (FBS), 1% glutamine, and 1% penicillin-streptomycin, at 37 °C in controlled atmosphere with 5% CO_2. For the experiments, cells were seeded at a density of 20000 cells per well in 24-well plates and grown for 24 h (or 48 h) before adding compound. Each compound was dissolved in DMSO (mother solution) and then diluted in the growth medium to reach the final concentrations of 5, 10, 25, 50 and 100 µM. To preserve sterility of solutions, the samples were filtered with 0.22 µm filters before adding to the dishes containing fibroblasts. Then, cells were incubated at 37 °C in 5% CO_2 for 24 h (or 48 h). After this time, the media were replaced with medium containing 0.5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); the cells were incubated for an additional 1 h at 37 °C in 5% CO_2. Finally, the number of viable cells was quantified by the estimation of their dehydrogenase activity, which reduces MTT to water-insoluble formazan. Growth medium was removed and substituted with 300 µL of DMSO to dissolve the formazan produced. The quantitation was carried out measuring the absorbance of samples at 570 nm with the iMark microplate absorbance reader (BIO RAD) in a 96-well format.

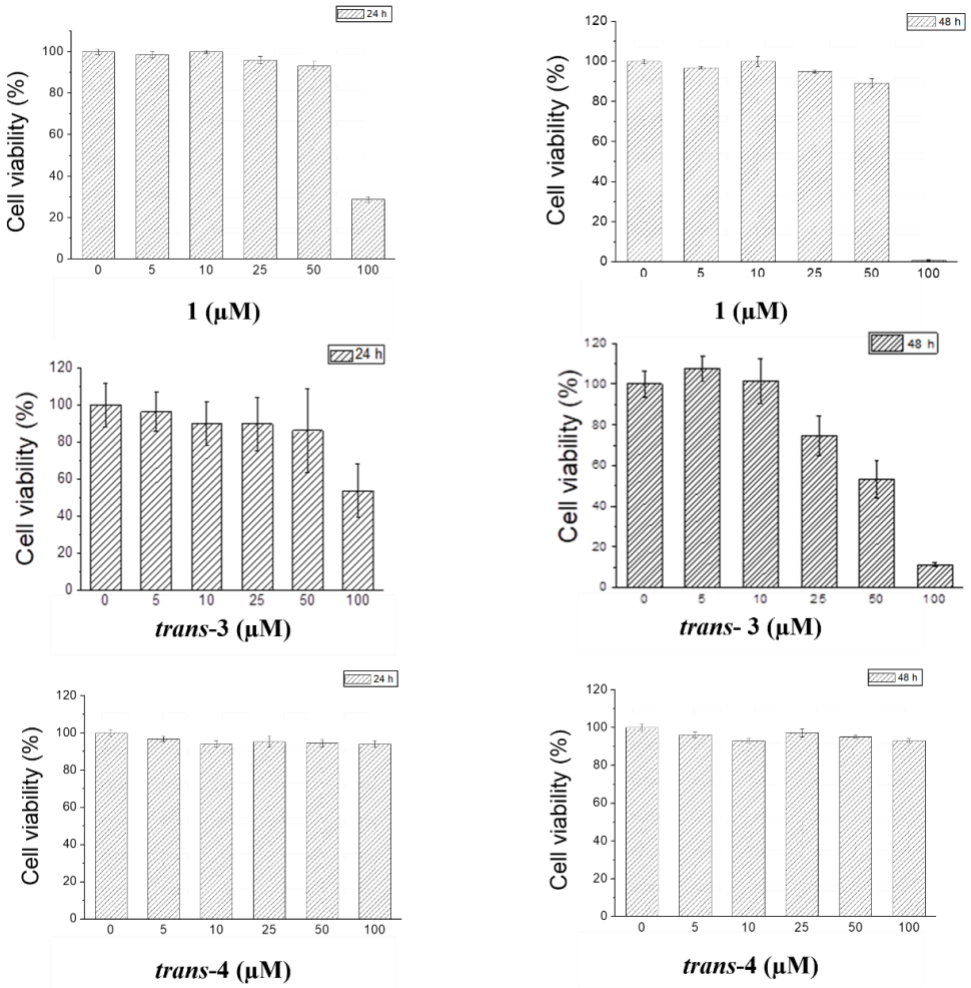


Figure S76: Viability assay results. WT Fibroblasts were incubated for 24 h (right) and 48 h (left) in the presence of **1** (top), *trans*-**3** (middle) and *trans*-**4** (bottom) at different concentrations. The viability of cells was evaluated using MMT assay. Obtained values were normalized with respect to control experiments.

GCase activity assay with constant illumination of the system (without inhibitors)

To probe the feasibility of performing the enzymatic essay under constant irradiation, the typical set-up of the experiment was changed. The flat-bottomed 96-well plate, usually employed for the incubation at 37 °C inside the spectrophotometer was replaced by a PCR plate, incubated at 37 °C in a PCR thermal cycler, which allowed to place the irradiation lamp on the top (Figure S77). Water (3 µL), 4.29 µg/µL leukocytes homogenate (7 µL), and substrate 4-methylumbelliferyl-β-D-glucoside (3.33 mM, 20 µL, Sigma–Aldrich) in citrate/phosphate buffer (0.1:0.2, M/M, pH 5.8) containing sodium taurocholate (0.3%) and Triton X-100 (0.15%) were incubated for 1 h at 37 °C (control, ctrl) under irradiation at 365 nm with a VILBER VL-6.LC TLC lamp. BSA albumin (10 µL) and substrate (20 µL) were also incubated in the same conditions (blank). The control as well the blank measurements were performed in triplicate. In addition, to validate the new set-up, the same experiment was performed in parallel without irradiation (Figure S77). The reaction was stopped by addition of sodium carbonate (200 µL; 0.5M, pH 10.7) containing Triton X-100 (0.0025 %), and the fluorescence of 4-methylumbelliferone released by GCase activity was measured in SpectraMax M2 microplate reader (λex=365 nm, λem=435 nm; Molecular Devices).

Figure S77: Novel set-up for the enzymatic activity assay under continuous irradiation at 365 nm (left) and without irradiation (right). During the experiment the left side was completely covered with foil to avoid visible light illumination.

Table S1: Results from the non-irradiated experiment (n =6)

SAMPLE	VALUES	RESULT	STD. DEV.	CV%	Activity (nmol/mg/h)
ctrl	2844.38	0.15	0.012	9.2	4.5
	2808.63	0.14			
	2464.40	0.13			
	2512.72	0.13			
	2803.50	0.14			
	2277.85	0.12			
blank	199.60				
	160.51				
	173.83				
	228.24				
	205.03				
	176.40				
Group blank	190.60				

Table S2: Results from the irradiated experiment (n=6)

SAMPLE	VALUES	RESULT	STD. DEV.	CV%	Activity (nmol/mg/h)
ctrl	2022.50	0.10	0.023	26.3	2.9
	2331.63	0.12			
	1737.33	0.09			
	1146.82	0.06			
	1369.76	0.07			
	1624.46	0.08			
blank	714.12				
	870.94				
	711.51				
	627.05				
	844.92				
	659.23				
Group blank	738.00				

Data reported in Table S1 for GCase activity value and the relative error (CV%) are consistent with those of the experiment performed with the traditional set-up. For the irradiated experiment, as reported in Table 2, a higher value of group blank was obtained (738.00 *vs* 190.60), probably due to the continuous absorption by the fluorescent 4-methylumbelliferyl-β-D-glucoside substrate. Moreover, a set of very different read-out values was obtained, leading to a high error (CV% = 26.3) which compromises the reliability of the datum. More importantly, a GCase activity value out of the reference data range (4.00-7.00 nmol/mg/h) was calculated in this case (2.9 nmol/mg/h), suggesting that is impossible to determine the enzyme activity in these conditions.

Inhibitory activity assay of pre-irradiated 1 (DHA/VHF-1)

In a Micro Cell cuvette, a compound **1** solution (10 mM H_20/DMSO 9:1, total volume 100 µL) was irradiated for 30 minutes and 60 minutes with the ThorLabs M365L3-C4 LED at 365 nm. Although it was not possible to check the DHA/VHF-**1** ratio of the irradiated solution via ^{1}H-NMR, the reversible color change (Figure S78) confirmed the conversion of **1** into unknown DHA/VHF-**1** mixtures.

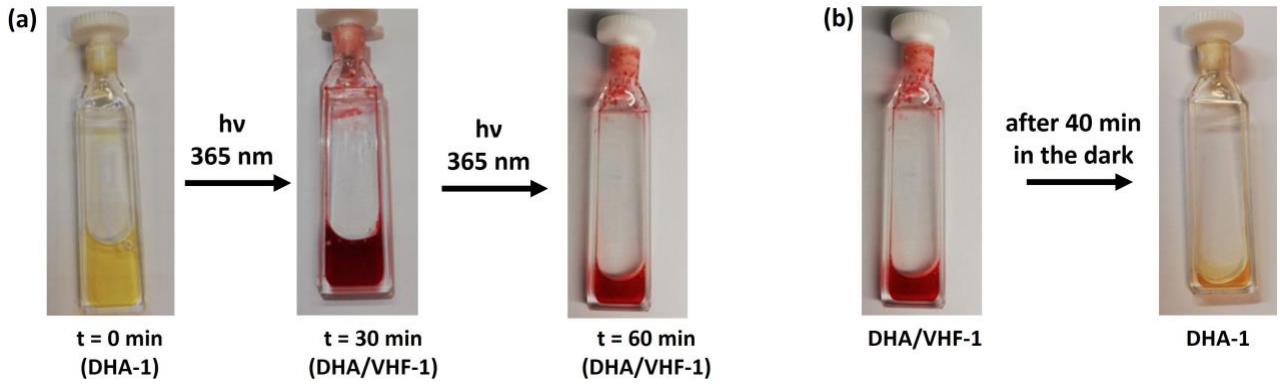


Figure S78: Visual change in color of a 10 mM solution of: (a) **1** before (t=0 min) and after irradiation (t=30 min and 60 min) with ThorLabs M365L3-C4 LED at 365 nm; (b) DHA/VHF-**1** kept in the dark for 40 min.

GCase enzyme activity in the presence of different concentrations of not irradiated **1** (DHA-**1**, 0 min) and irradiated **1** (DHA/VHF-**1**, 30 min and 60 min) was measured in parallel, in a flat-bottomed 96-well plate. Compound solution (10% DMSO in H_2O, 3 µL), 4.29 µg/µL leukocytes homogenate (7 µL), and substrate 4-methylumbelliferyl-β-D-glucoside (3.33 mM, 20 µL, Sigma–Aldrich) in citrate/phosphate buffer (0.1:0.2, M/M, pH 5.8) containing sodium taurocholate (0.3%) and Triton X-100 (0.15%) were incubated for 1 h at 37 °C. The reaction was stopped by addition of sodium carbonate (200 µL; 0.5M, pH 10.7) containing Triton X-100 (0.0025 %), and the fluorescence of 4-methylumbelliferone released by GCase activity was measured in SpectraMax M2 microplate reader (λex=365 nm, λem=435 nm; Molecular Devices). Data are mean ± RSD (n=3). The obtained results are shown in Figure S79.

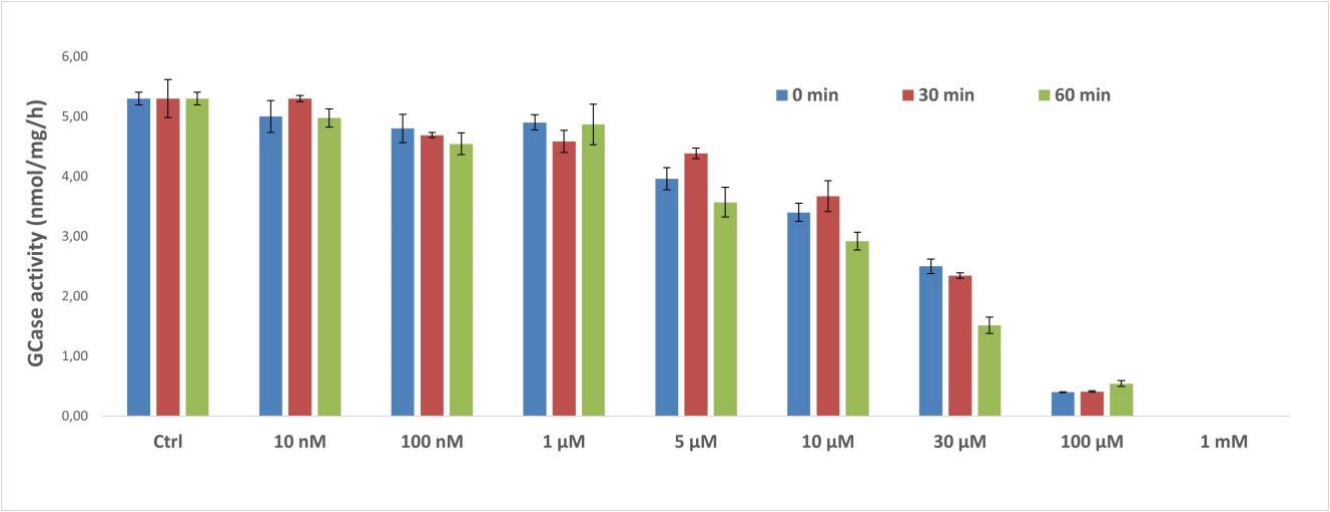


Figure S79: GCase activity without (control, Ctrl) and with different concentrations (from 10 nM to 1 mM) of **1** (t=0 min) and DHA/VHF-**1** (30 min and 60 min).

The IC_{50} values of **1** (t = 0 min) and DHA/VHF-**1** (t = 30 and 60 min) were determined by measuring the initial hydrolysis rate with 4-methylumbelliferyl-β-D-glucoside (see above).

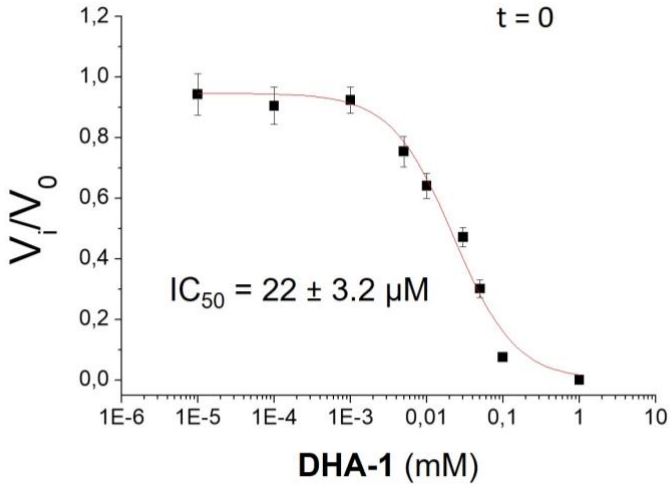


Figure S80: IC_{50} graph of compound **1** with 10% of DMSO**.**

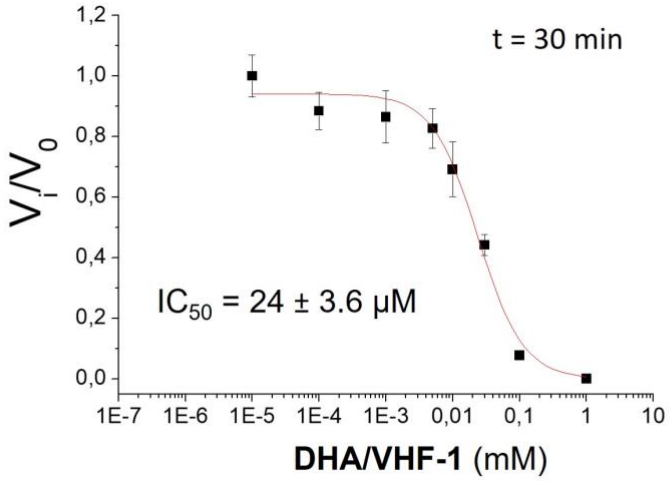


Figure S81: IC_{50} graph of compound DHA/VHF-**1** with 10% of DMSO (after 30 min irradiation).

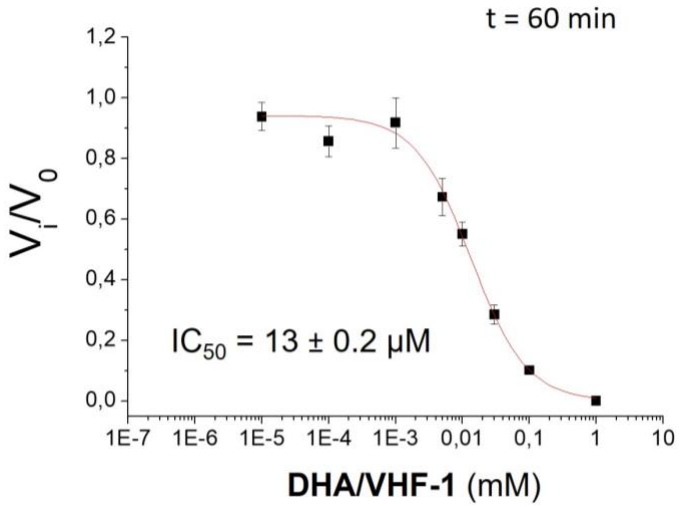


Figure S82: IC_{50} graph of compound DHA/VHF-**1** with 10% of DMSO (after 60 min irradiation).

Stability studies of DHA-Ph in dmso-d6 by ^{1}H-NMR spectroscopy

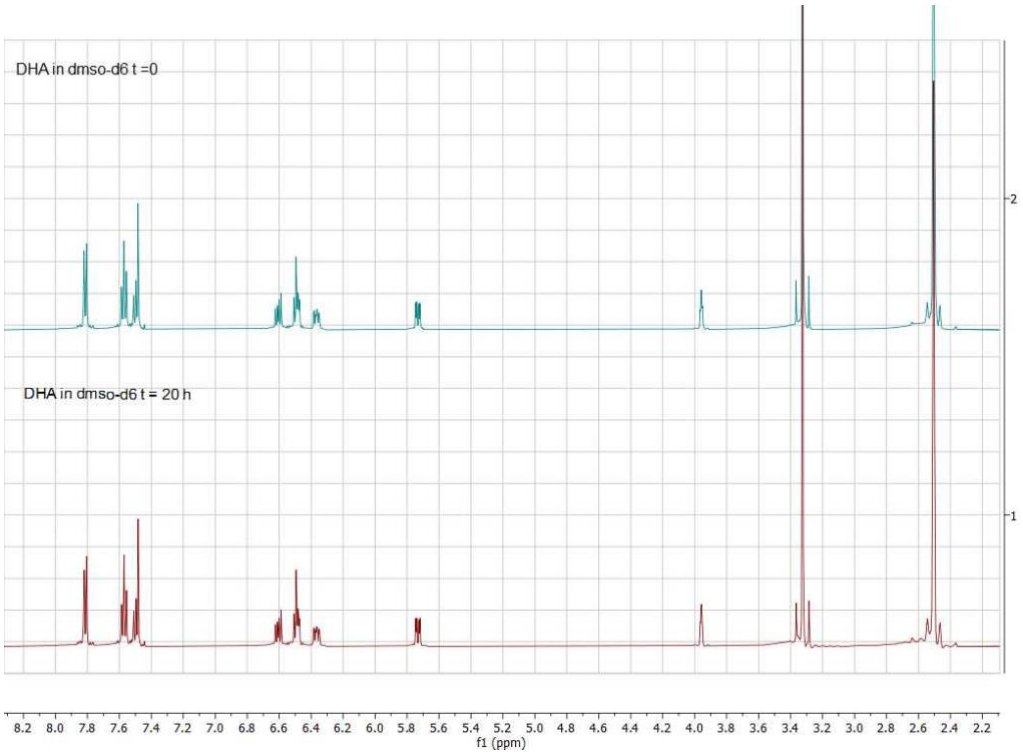


Figure S83: ^{1}H-NMR spectra at 500 MHz of **DHA-Ph** in dmso-d6 in the dark at t = 0 (top) and t = 20 h (bottom). No changes are observed.

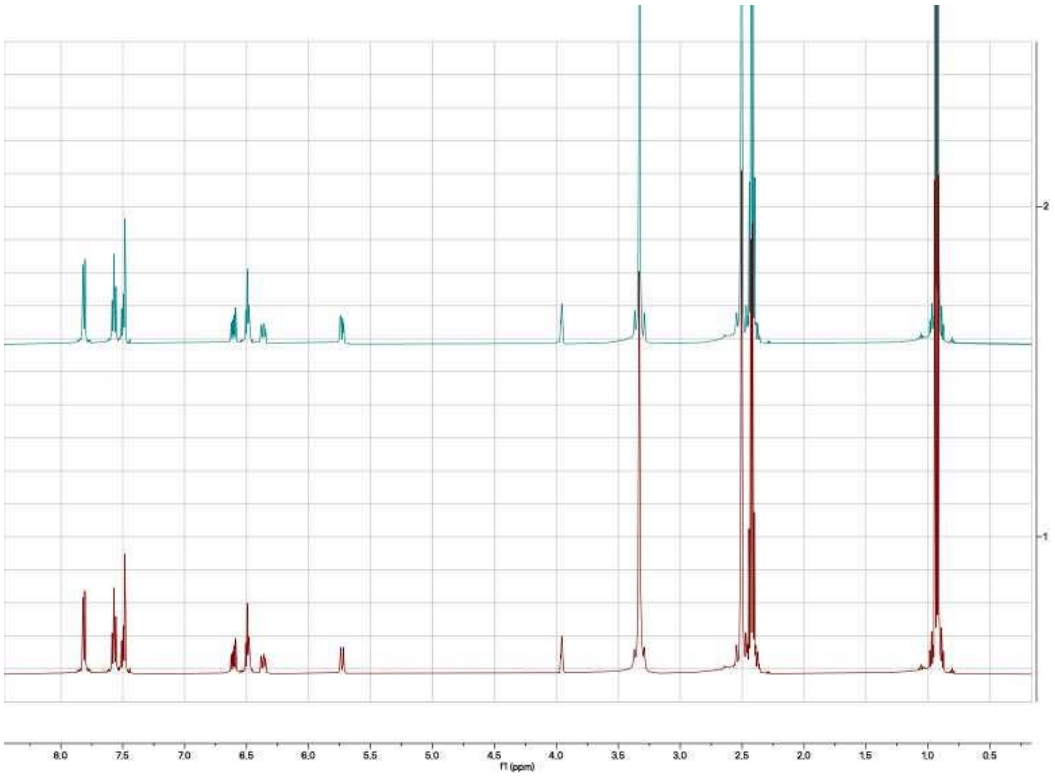


Figure S84: ^{1}H-NMR spectra at 500 MHz of **DHA-Ph** in dmso-d6 in the dark in the presence of NEt_3 (c.a. 1 eq.) at t = 0 (top) and t = 24 h (bottom). No changes are observed.

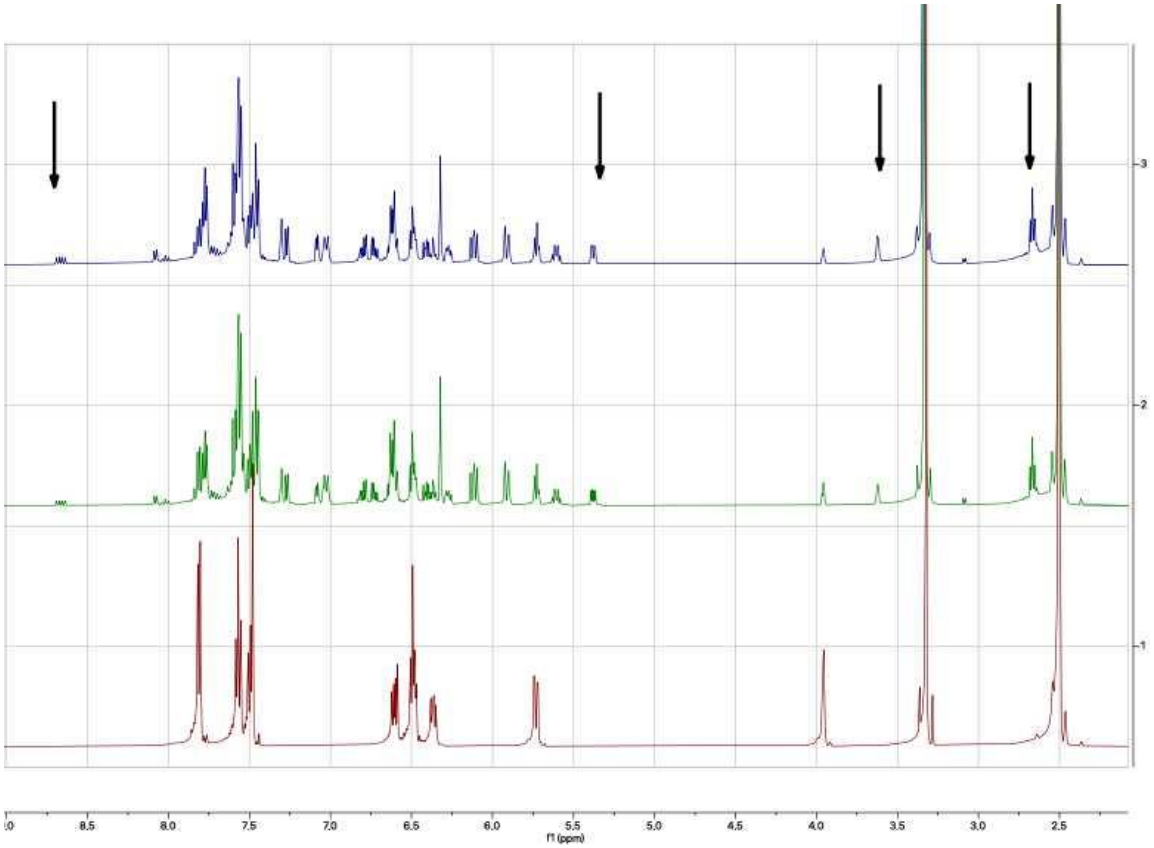


Figure S85: ^{1}H-NMR spectra at 500 MHz of **DHA-Ph** in dmso-d6 irradiated at 365 nm for 0 s (no irradiation, bottom), 45 min (middle) and 70 min (top). Relevant changes that point towards degradation and conspicuous formation of unknown side-products are observed and indicated by black arrows. Possible side-products could be azulene derivatives (depicted by the aromatic signals above 8 ppm) and products rising from sigmatropic rearrangement at the 7-membered ring (depicted by the formation of aliphatic peaks at 2.6 ppm and 3.6 ppm). Few cases of sigmatropic rearrangement have been reported, mainly rising from oxidative reaction conditions of DHA derivatives (see *Org. Biomol. Chem*., **2016**, *14*, 2403).