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Supporting Information

DOI: 10.1002/cbic.200900044

Supporting Information

for

Phenolic Oxime Oligomers Inhibit Alzheimer's Amyloid Fibril Formation and Disaggregate Fibrils in vitro

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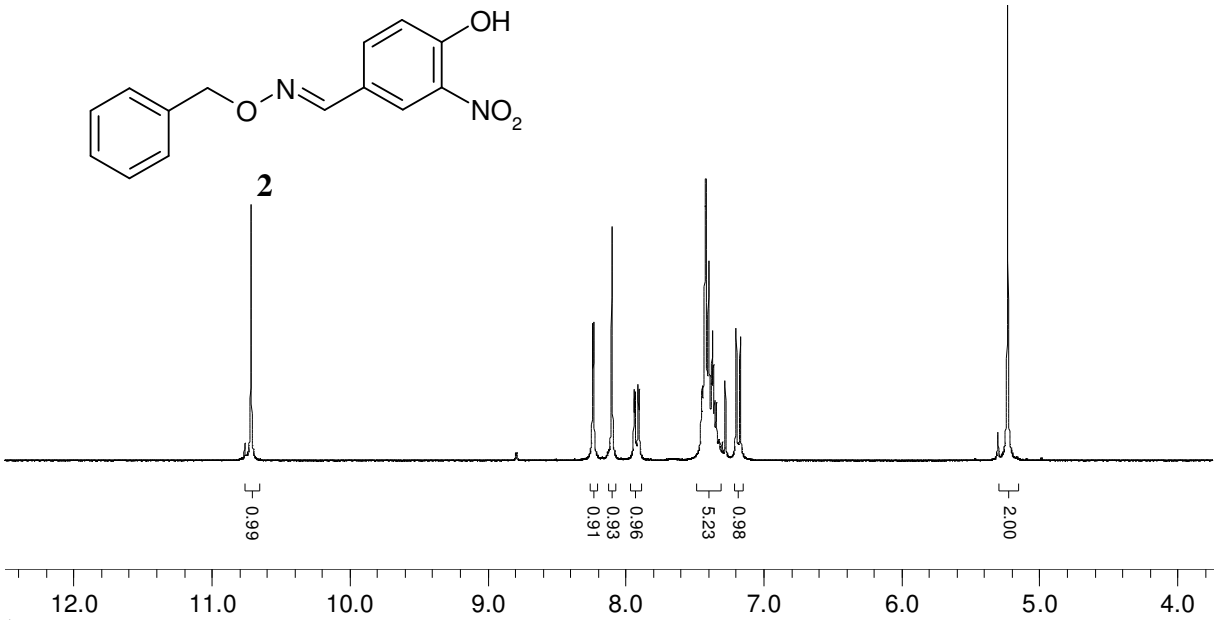
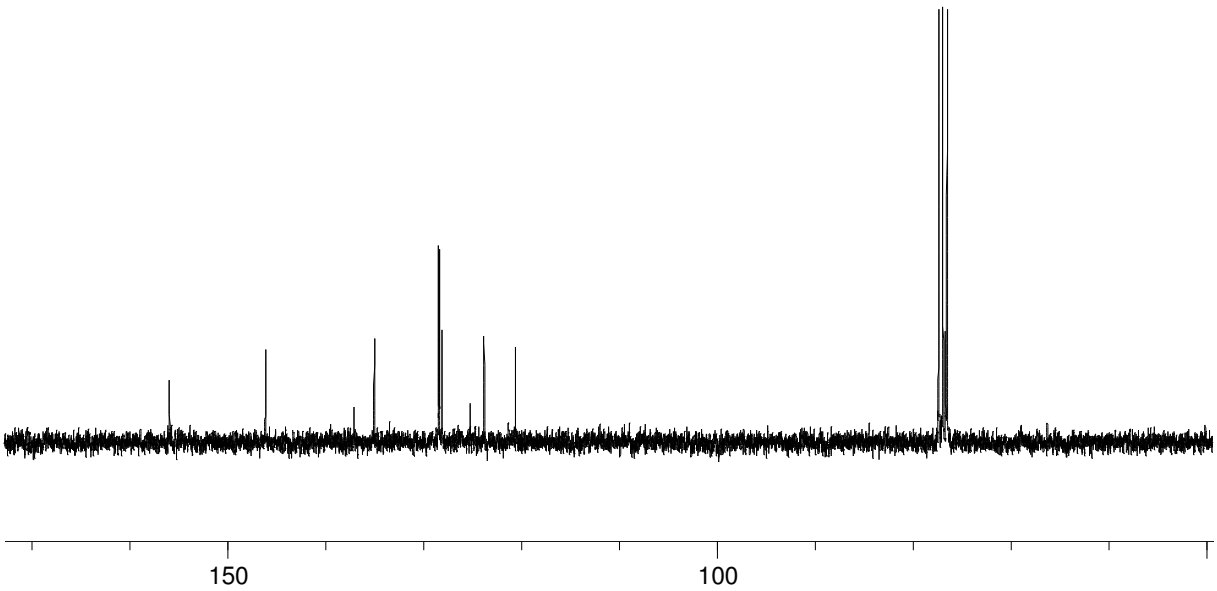
- Spectral data for new compounds (not featured in ref. [24])
- Procedure for HTS and AFM
- Figures S1 and S2

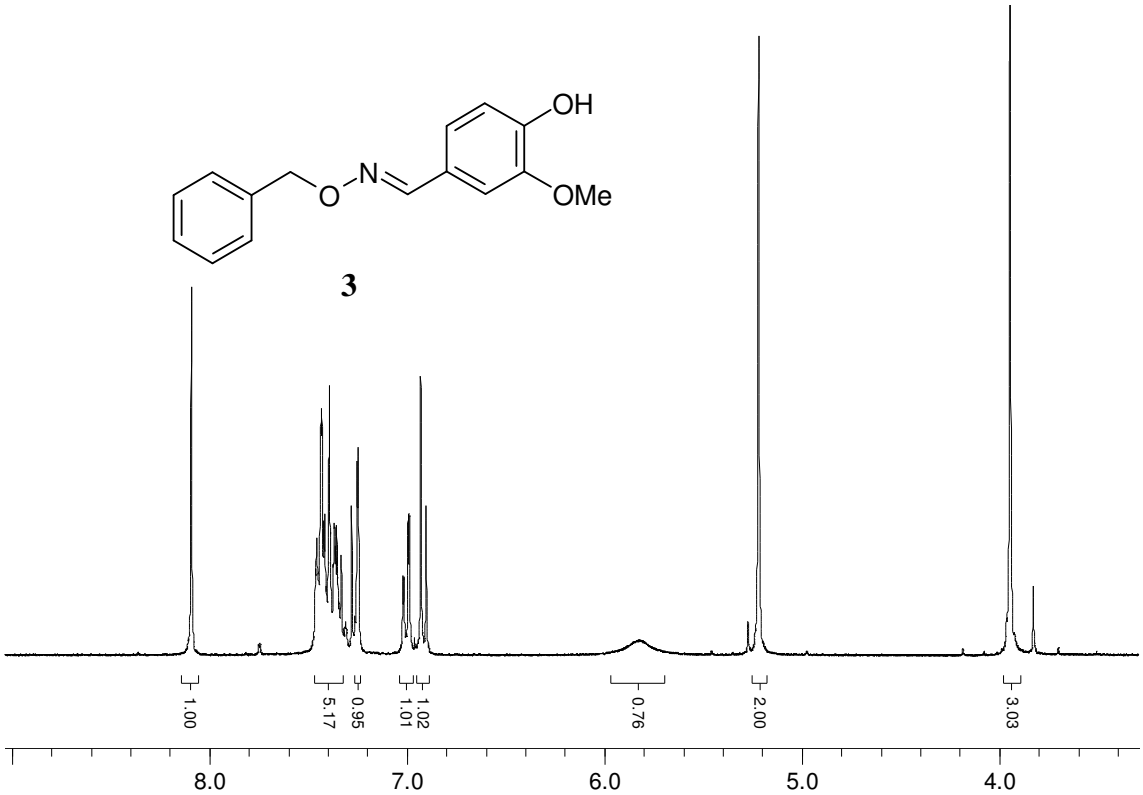


^{1}H NMR (300 MHz, $CDCl_3$) : δ ppm 10.71 (s, 1H), 8.23 (d, 1H, J = 2.0 Hz), 8.10 (s, 1H), 7.92 (dd, 1H, J = 2.0, 8.8 Hz), 7.45-7.35 (m, 5H), 7.18 (d, 1H, J = 8.8 Hz), 5.23 (s, 2H).

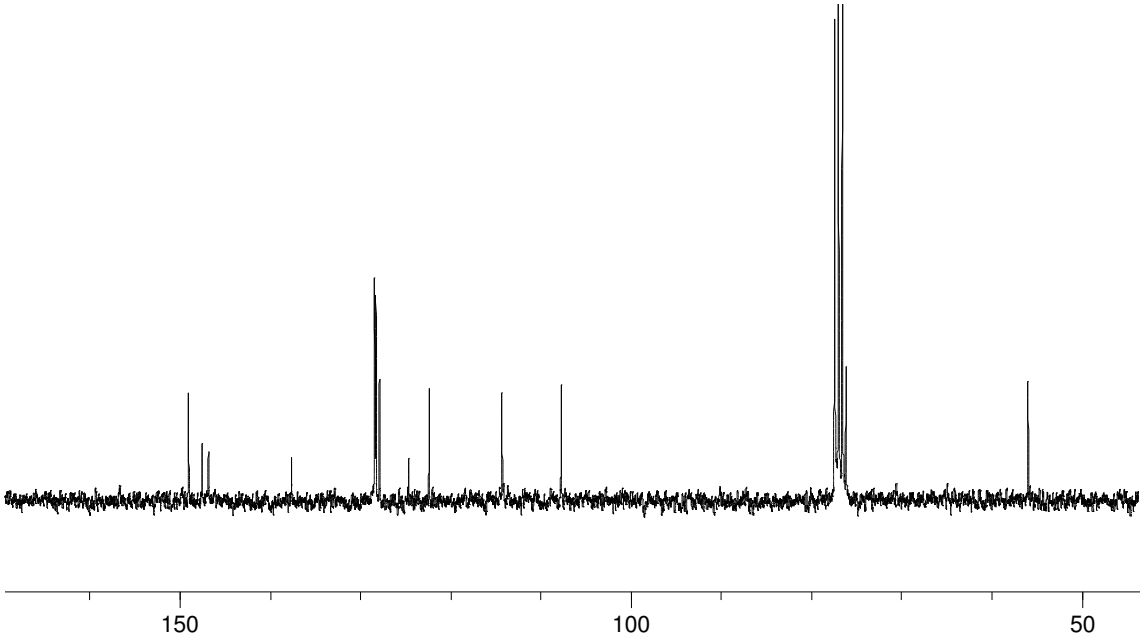


^{13}C NMR (75 MHz, $CDCl_3$) : δ ppm 155.9, 146.2, 137.2, 135.1, 128.6, 128.5, 128.4, 128.1, 125.3, 123.8, 120.6, 76.7.

HRMS analysis (ESI, positive mode): calcd. for $C_{14}H_{13}N_2O_4$: 273.0870, found: 273.0877.

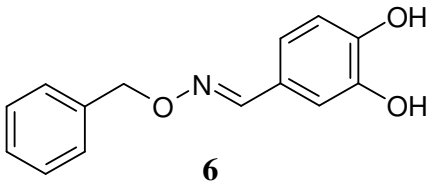


^{1}H NMR (300 MHz, $CDCl_3$) : δ ppm 8.09 (s, 1H), 7.46-7.33 (m, 5H), 7.25 (d, 1H, J = 1.7 Hz), 7.00 (dd, 1H, J = 1.7, 8.1 Hz), 6.91 (d, 1H, J = 8.1 Hz), 5.81 (bs, 1H), 5.22 (s, 2H), 3.95 (s, 3H).



^{13}C NMR (75 MHz, $CDCl_3$) : δ ppm 149.0, 147.6, 146.9, 137.6, 128.4, 128.3, 127.9, 124.6, 122.4, 114.3, 107.7, 76.2, 56.0.

HRMS analysis (ESI, positive mode): calcd. for $C_{15}H_{16}NO_3$: 258.1125, found: 258.1129.



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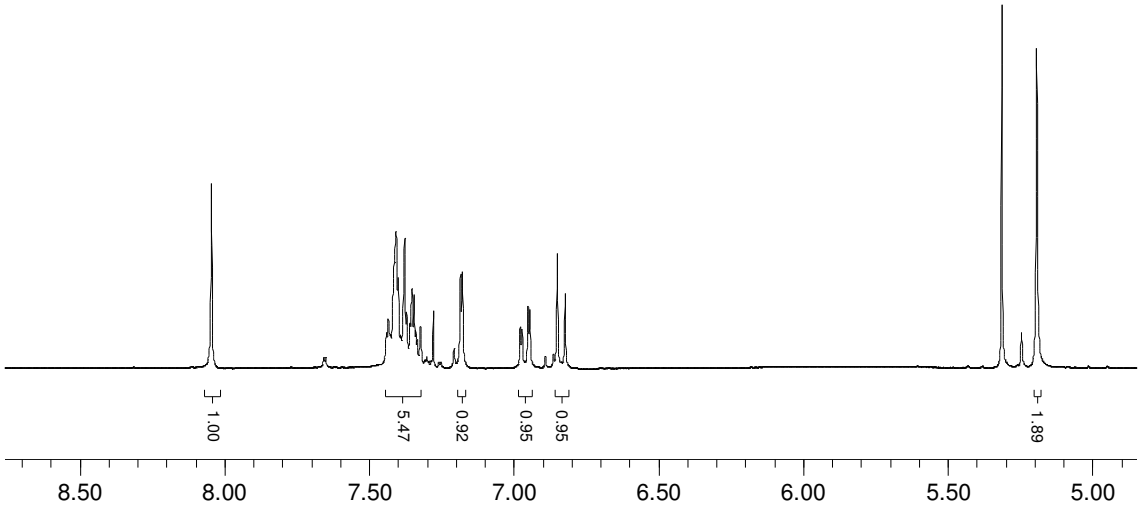
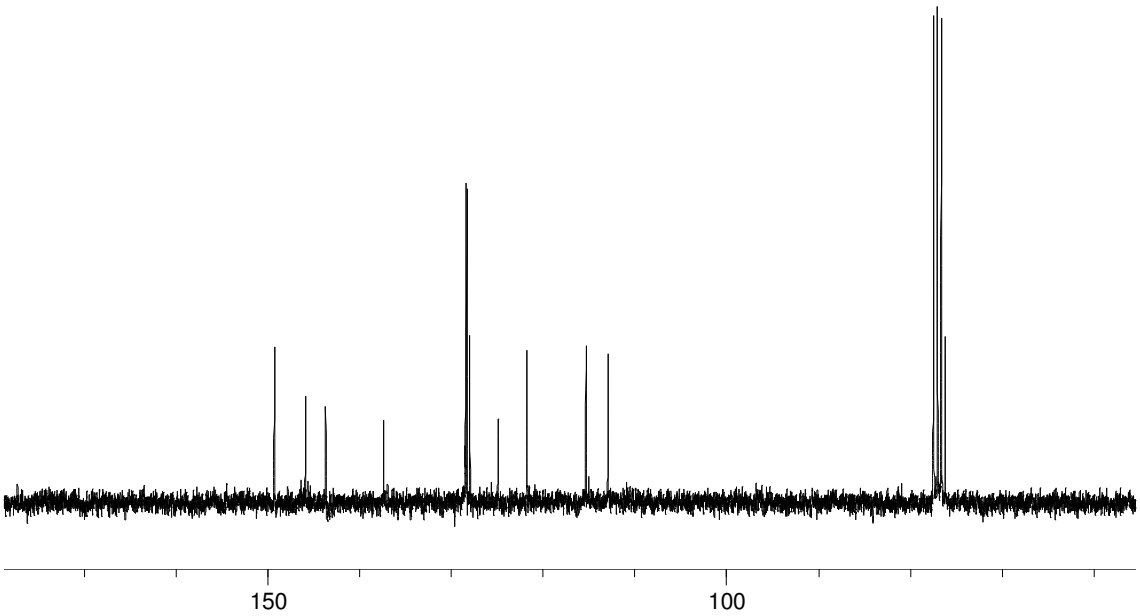
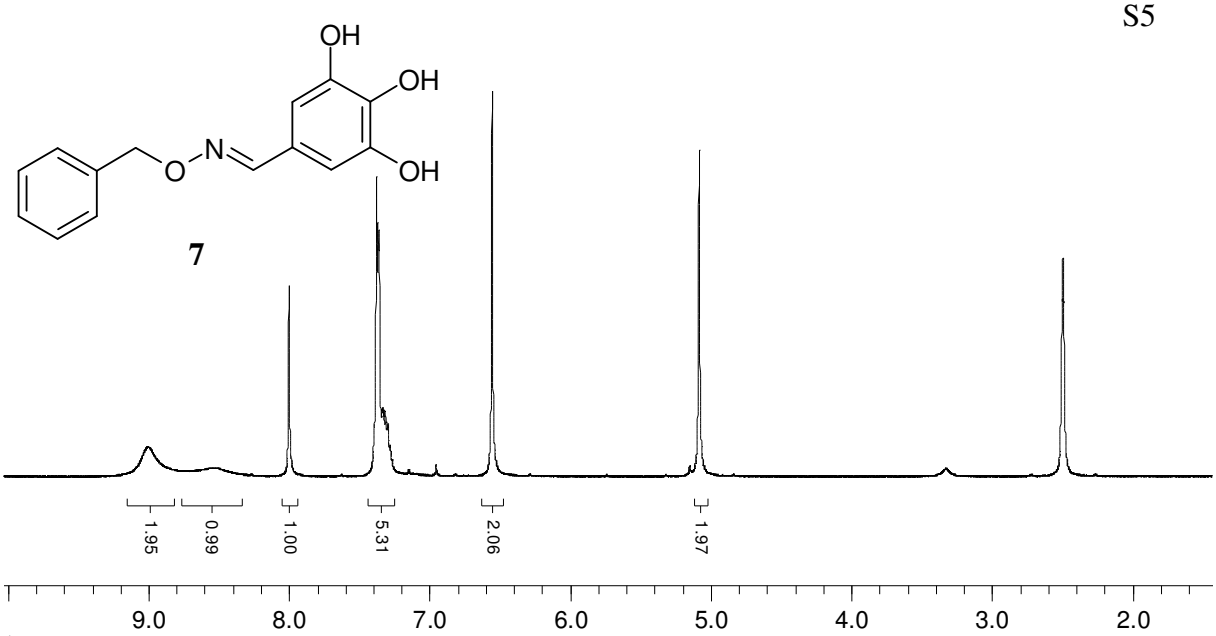


^{1}H NMR (300 MHz, $CDCl_3$) : δ ppm 8.04 (s, 1H), 7.44-7.32 (m, 5H), 7.18 (d, 1H, J = 1.9 Hz), 6.96 (dd, 1H, J = 1.9, 8.2 Hz), 6.83 (d, 1H, J = 8.2 Hz), 5.19 (s, 2H).



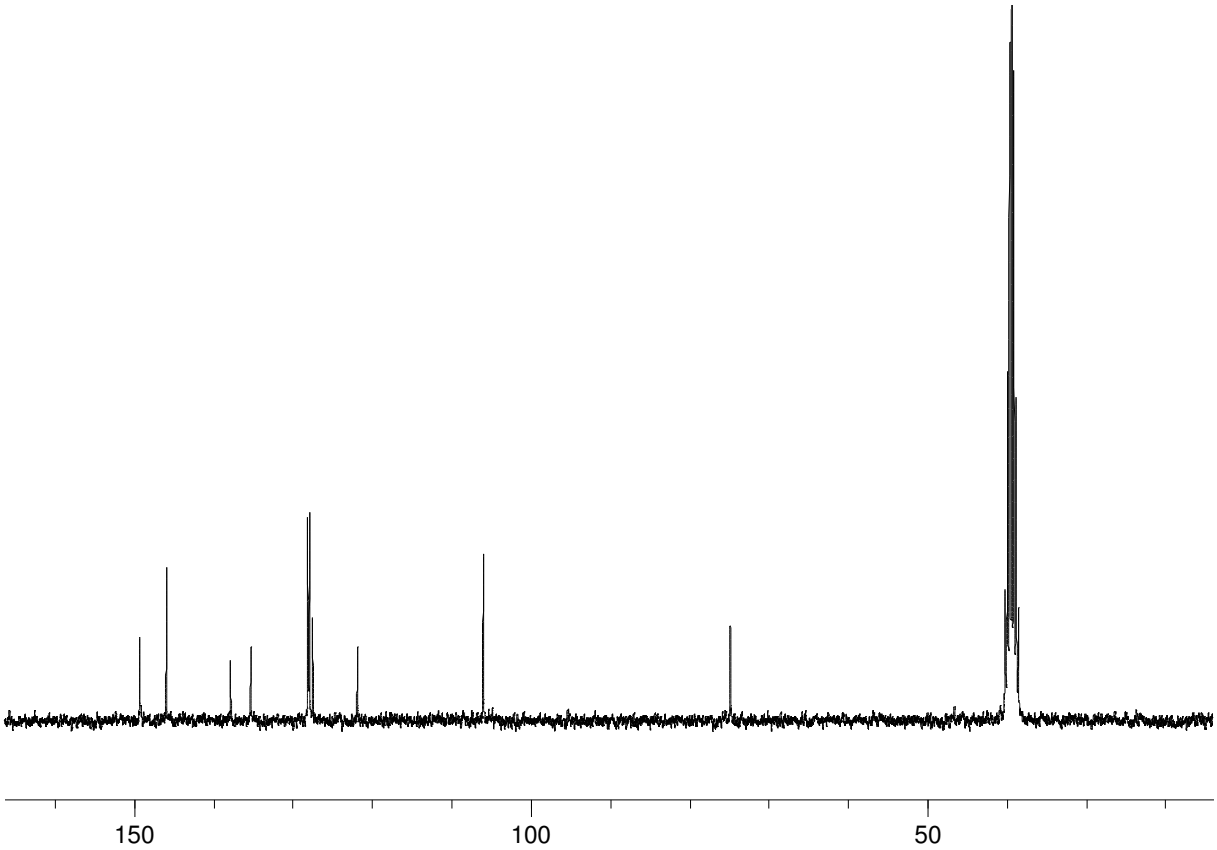
^{13}C NMR (75 MHz, $CDCl_3$) : δ ppm 149.3, 145.9, 143.7, 137.4, 128.5, 128.3, 128.0, 125.0, 121.8, 115.3, 113.0, 76.2.

HRMS analysis (ESI, positive mode): calcd. for $C_{14}H_{14}NO_3$: 244.0968, found: 244.0971.



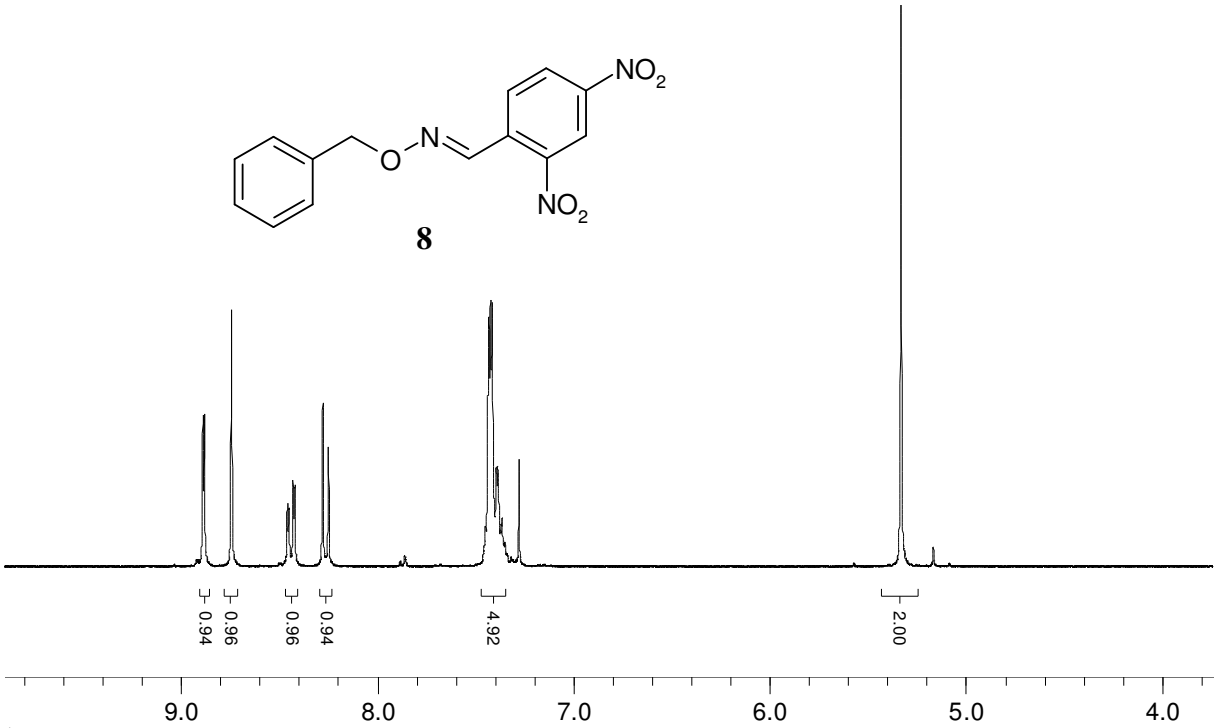
7

^{1}H NMR (300 MHz, $CDCl_3$) : δ ppm 9. 01 (bs, 1H), 8.53 (bs, 1H), 8.00 (s, 1H), 7.38-7.30 (m, 5H), 6.56 (s, 2H).



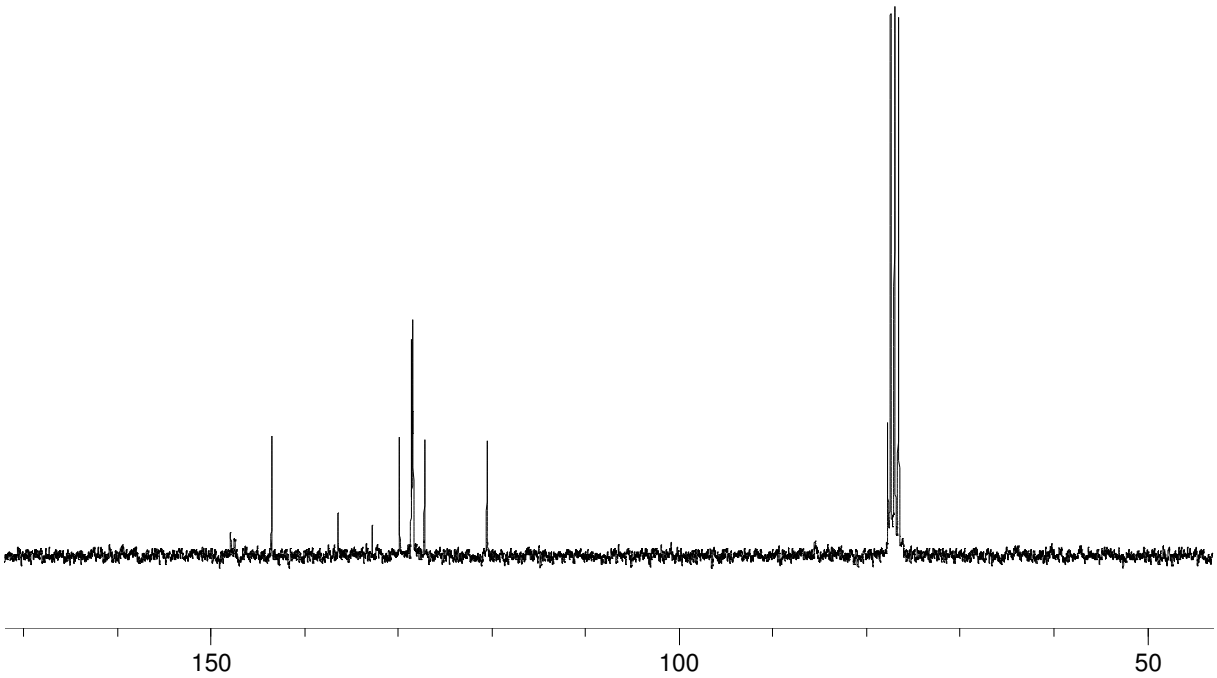
^{13}C NMR (75 MHz, $CDCl_3$) : δ ppm 149.4, 146.9, 137.9, 135.4, 128.1, 127.9, 127.6, 121.9, 106.0, 74.9.

HRMS analysis (ESI, positive mode): calcd. for $C_{14}H_{14}NO_4$: 260.0917, found: 260.0924.



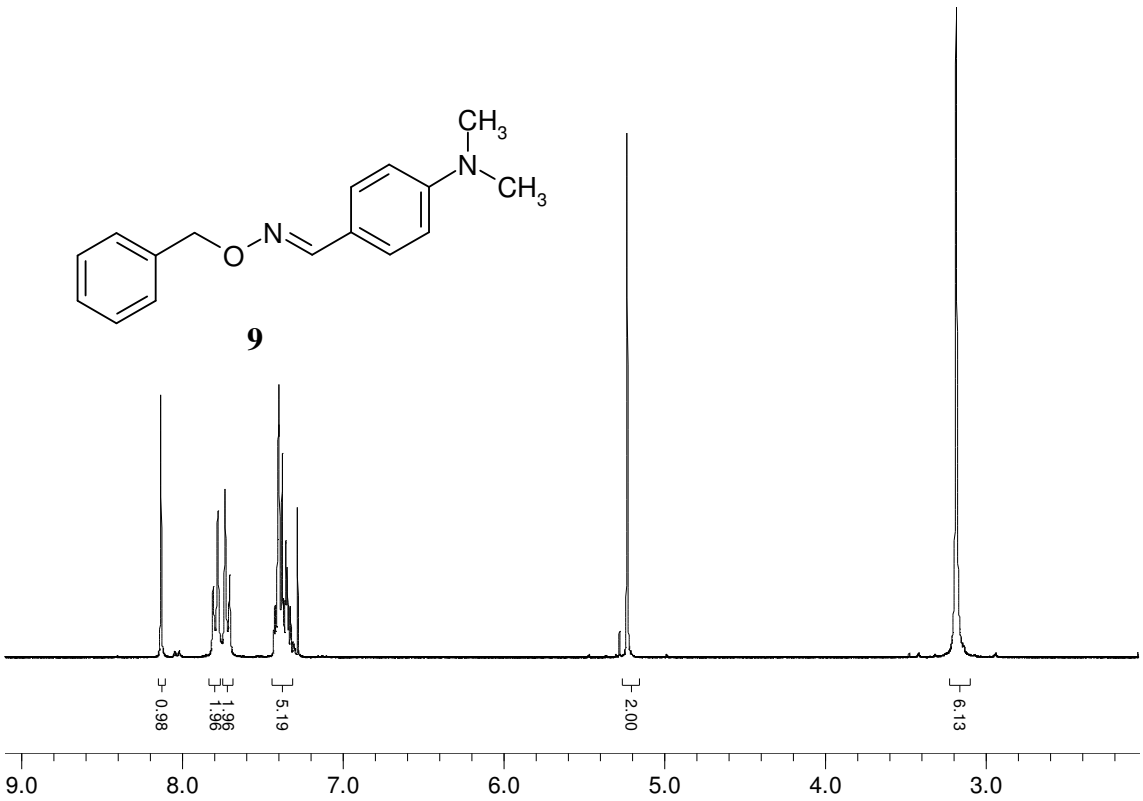
8

^{1}H NMR (300 MHz, $CDCl_3$) : δ ppm 8.89 (d, 1H, J = 2.3 Hz), 8.74 (s, 1H), 8.44 (dd, 1H, 2.3, 8.7 Hz), 8.25 (d, 1H, J = 8.7 Hz), 7.45-7.37 (m, 5H), 5.33 (s, 2H).



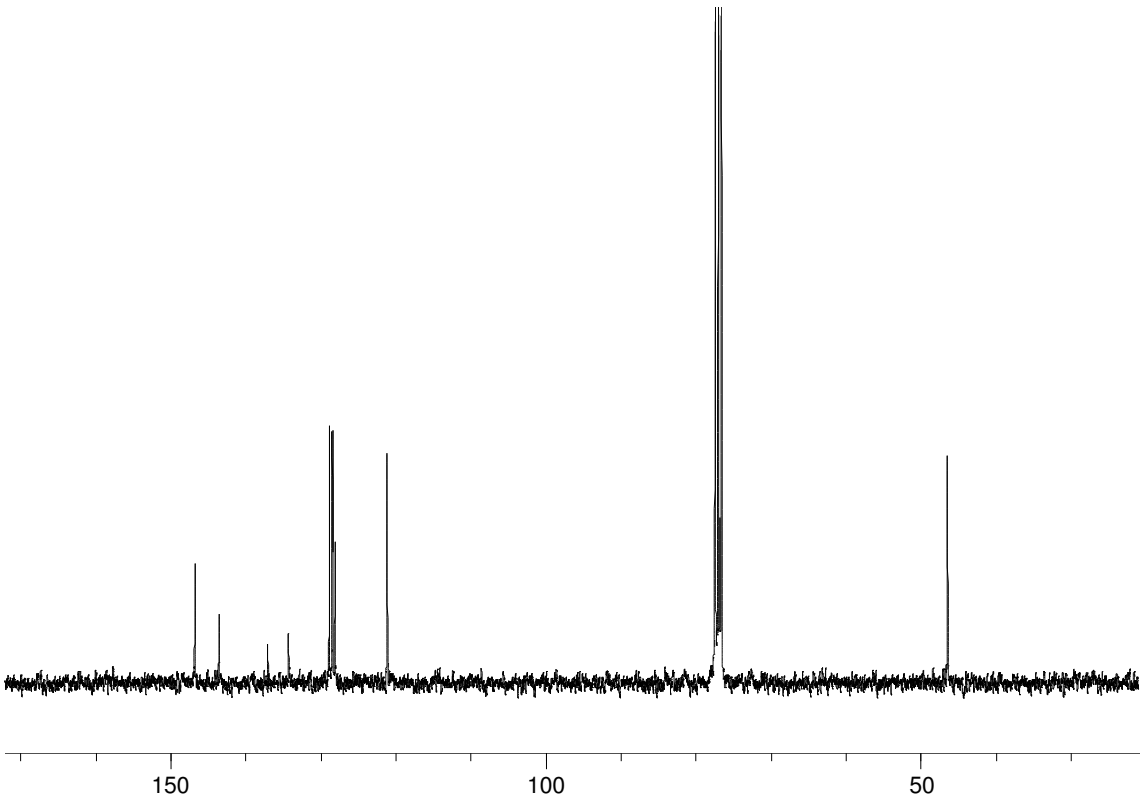
^{13}C NMR (75 MHz, $CDCl_3$) : δ ppm 147.9, 147.5, 147.4, 143.5, 136.5, 132.8, 129.9, 128.6, 128.5, 128.4, 127.2, 120.5, 77.7.

HRMS analysis (ESI, positive mode): calcd. for $C_{14}H_{11}N_3O_5Na$: 324.0591, found: 324.0596.



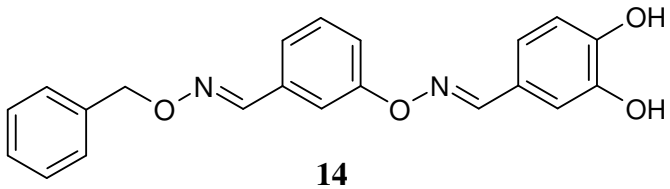
9

^{1}H NMR (300 MHz, $CDCl_3$) : δ ppm 8.13 (s, 1H), 7.79 (d, 2H, AA'BB' system), 7.72 (d, 2H, AA'BB' system), 7.42-7.32 (m, 5H), 5.23 (s, 3H), 3.18 (s, 6H).



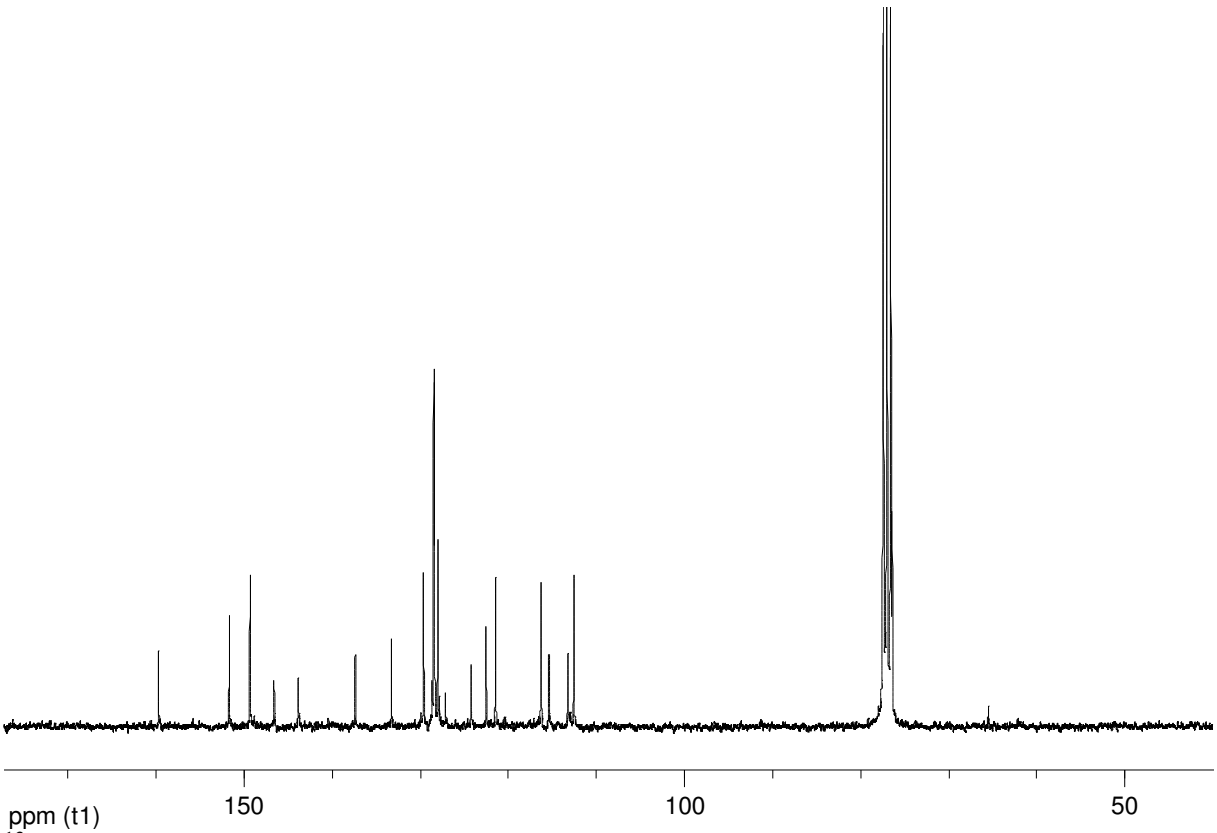
^{13}C NMR (75 MHz, $CDCl_3$) : δ ppm 146.8, 143.6, 137.0, 134.3, 128.9, 128.5, 128.4, 128.1, 121.2, 76.8, 46.5.

HRMS analysis (ESI, positive mode): calcd. for $C_{16}H_{19}N_2O$: 255.1492, found: 255.1497.



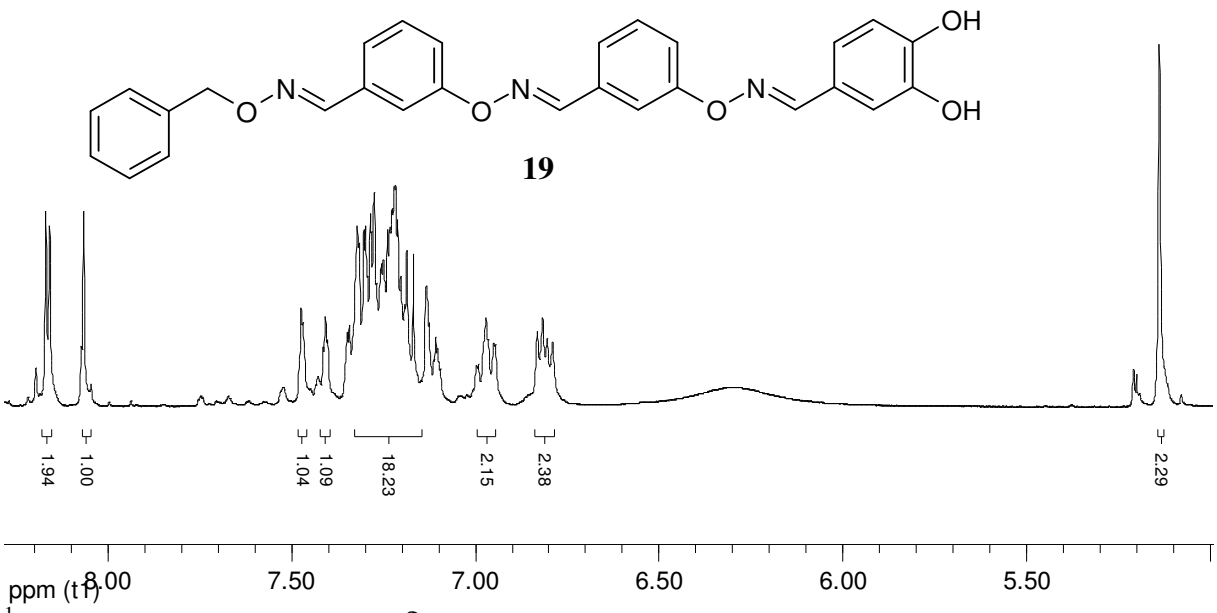
14

^{1}H NMR (300 MHz, $CDCl_3$) : δ ppm 8.18 (s, 1H), 8.07 (s, 1H), 7.42 (bs, 1H), 7.36-7.21 (m, 9H), 7.15-7.12 (2H), 6.99 (bd, 1H, J = 7.4 Hz), 6.81 (bd, 1H, J = 7.4 Hz), 5.15 (s, 2H).



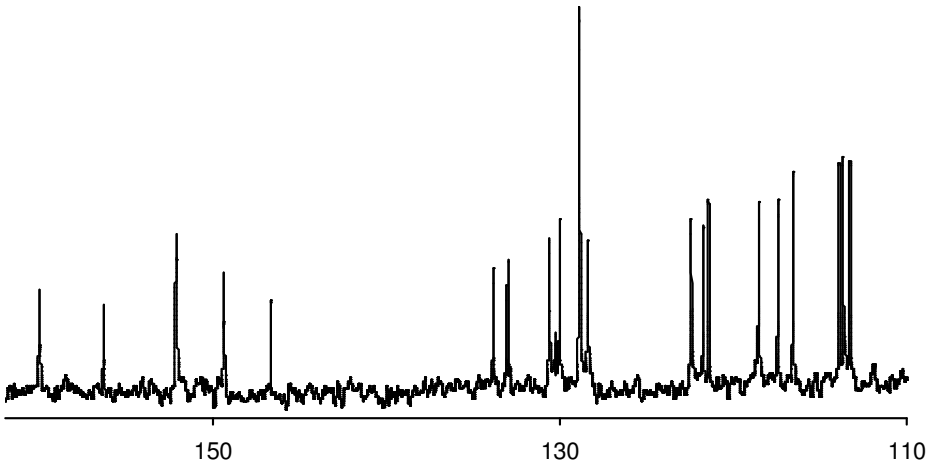
^{13}C NMR (75 MHz, $CDCl_3$) : δ ppm 159.7, 151.7, 149.3, 146.6, 143.8, 137.4, 133.3, 129.6, 128.5, 128.4, 128.0, 124.2, 122.5, 121.4, 116.3, 115.4, 113.2, 112.5, 76.6.

HRMS analysis (ESI, positive mode): calcd. for $C_{21}H_{19}N_2O_4$: 363.1339, found: 363.1345.



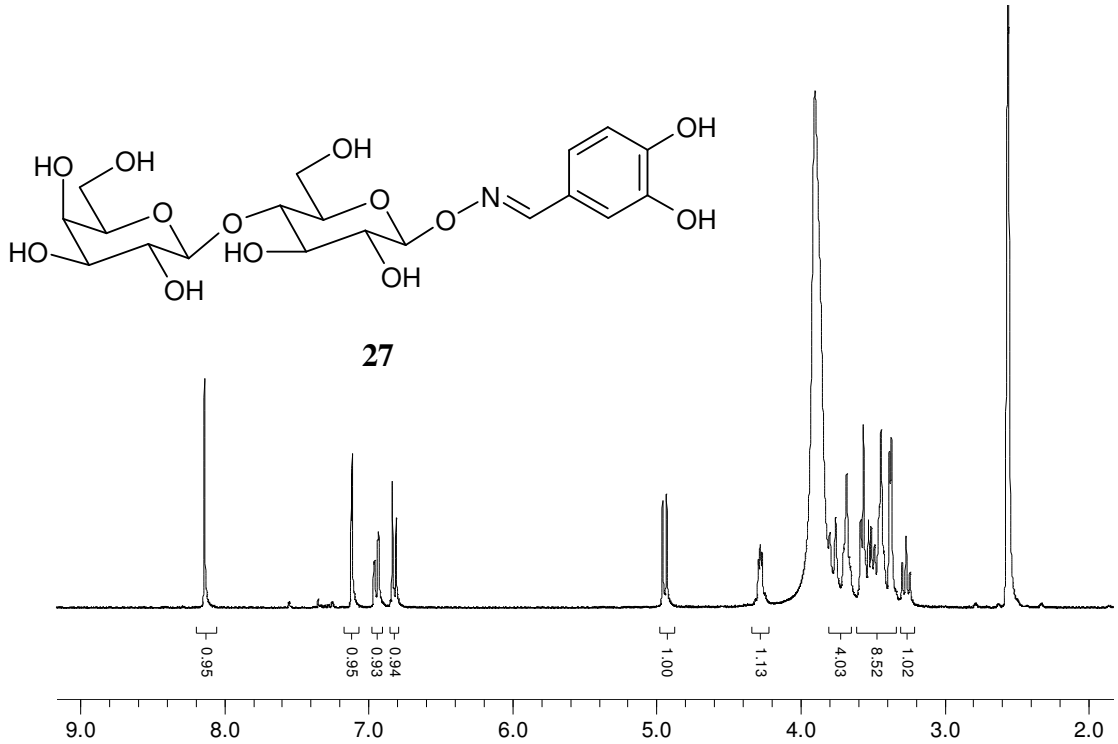
19

^{1}H NMR (300 MHz, $CDCl_3$): δ ppm 8.17 (s, 1H), 8.16 (s, 1H), 8.06 (s, 1H), 7.47 (bt, 1H), 7.41 (bt, 1H), 7.35-7.11 (m, 12H), 7.99-7.95 (m, 2H), 6.83-6.79 (m, 2H), 5.14 (s, 2H).



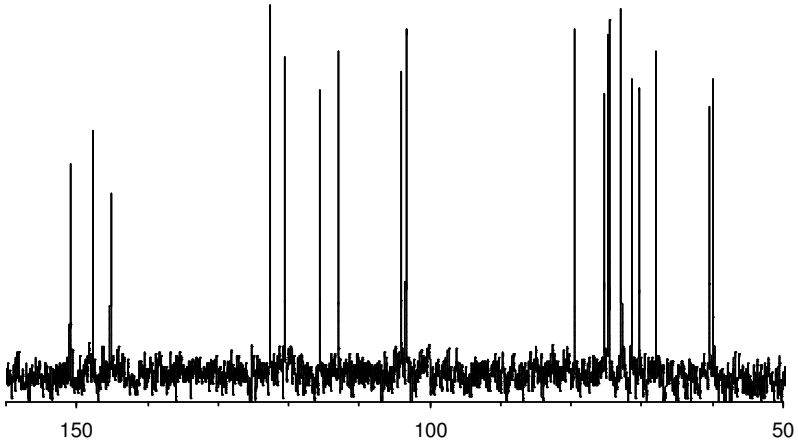
^{13}C NMR (75 MHz, $CDCl_3$): δ ppm 160.1, 160.0, 156.3, 152.2, 152.1, 149.4, 146.7, 133.8, 133.1, 132.9, 130.6, 130.2, 128.9, 128.8, 128.4, 122.4, 121.7, 121.4, 118.5, 117.4, 116.5, 113.9, 113.6, 113.3, 76.9.

HRMS analysis (ESI, positive mode): calcd. for $C_{28}H_{23}N_3O_5Na$: 504.1530, found: 504.1532.



27

^{1}H NMR (300 MHz, DMSO-$_{d6}$) : δ ppm 8.14 (s, 1H), 7.11 (d, 1H, J = 1.9 Hz), 6.94 (dd, 1H, J = 1.9, 8.0 Hz), 6.82 (d, 1H, J = 8.0 Hz), 4.95 (d, 1H, J = 8.3 Hz), 4.28 (bp, 1H, 3.9 Hz), 3.80-3.37 (m, 12H), 3.27 (t, 1H, 8.1 Hz).



^{13}C NMR (75 MHz, DMSO-$_{d6}$) : δ ppm 150.9, 147.6, 145.2, 122.6, 120.5, 115.6, 112.9, 104.1, 103.4, 79.5, 75.3, 74.8, 74.6, 72.9, 71.4, 70.4, 68.0, 60.4, 59.8.

HRMS analysis (ESI, positive mode): calcd. for $C_{19}H_{27}NO_{13}Na$: 500.1375, found: 500.1381.

Materials: All chemical reagents and solvents were purchased from Sigma Aldrich, Fluka, Acros or Carlo-Erba and were used without further purification. Synthetic $A\beta_{1-40}$ was prepared as previously described.[1]

Preparation of $A\beta_{1-40}$ peptide stock solution: For aggregation assays, a stock solution of $A\beta_{1-40}$ was prepared as follow: 2.7 mg was dissolved in 200 µL of 1,1,1,3,3,3-hexafluoro-2-propanol to disassemble preformed aggregates, thereafter it was lyophilized. One mL of pure water was added to the lyophilized peptide and the solution was centrifuged at 12000 g to remove eventual aggregates. The stock solution was divided into portions and stored at -20 °C until use. The stock concentration of $A\beta_{1-40}$ was 500 µM.

Preparation of oxime oligomer and inhibitor stock solutions: compounds were dissolved in DMSO. Stock solutions of 10 mM were first prepared, thereafter they were diluted with DMSO to the following concentrations 5 mM, 500 µM, 50 µM, 5 µM and 0.5 µM. Final concentration of DMSO in inhibition studies was 2%.

Aggregation measurement of $A\beta_{1-40}$: Aggregation of $A\beta_{1-40}$ was performed in 96-well black polypropylene microplates (Geriner). To each well an aliquot of the peptide stock solution was mixed into the aggregation buffer giving a final composition of $A\beta_{1-40}$ (50 µM) in sodium phosphate (50 mM) and NaCl (100 mM) pH 7.4. Thereafter 2 µL aliquots of the inhibitor compounds were added, giving the aggregation mixture a total volume 100 µL. Microplates were sealed with a plastic sheet and incubated in a Molecular Devices Spectra MAX Gemini XS microplate reader at 37 °C. Kinetic data were fitted with the stretched exponential function: $F(t)=F(\infty)-\Delta F\exp(-(kt)^n)$, where $F(t)$ is the fluorescence at time t, $F(\infty)$ is the fluorescence after complete fibril formation, ΔF is the difference in fluorescence between $t(0)$ and $t(\infty)$, k is the rate constant and values larger than 1 for the parameter n indicate a sigmoidal transition with an initial lag-phase.[2]

Aggregation measurement with preformed fibrils: The seeding experiment was identical to the aggregation measurement of $A\beta_{1-40,}$ with the addition of preformed fibrils (2.5 µM). The preformed fibrils of $A\beta_{1-40}$ were prepared from the aggregation measurement of $A\beta_{1-40}$ without inhibitor compounds (described above). Fibrils were sonicated for 10 seconds immediately before use. Kinetic data were fitted with an exponential function.

Fibril destabilization: The preformed fibrils of $A\beta_{1-40}$ were prepared as described above, by following the formation of fibrils with the ThT assay. The fibrils were mixed and sonicated (2 times 10 seconds) and left overnight before the destabilization study. The experimental conditions were identical as the aggregation measurement of $A\beta_{1-40}$.

Fluorescence spectroscopy: Microplates, 96-well, were analyzed in a Molecular Devices Spectra MAX Gemini XS microplate spectrophotometer at 37°C. For HTS measurements, ThT binding were recorded using bandpass filters of 440 nm for excitation and 480 nm for emission with a cut-off filter of 475 nm. For inhibition, seed and destabilisation studies of $A\beta_{1-40}$ fibril formation the kinetics were monitored by the bound ThT fluorescence at 485 nm with excitation at 444 nm with a cut-off filter of 475 nm.

Atomic force microscopy: An aliquot, 3 µL, from each inhibition study was withdrawn at the end of the aggregation reaction and deposited onto freshly cleaved mica sheets for AFM inspection. The sample was incubated on mica for 2 minutes followed by four washes with 5 µL water to gently remove the material not adsorbed to the substrate. The mica was dried in air for one hour. In-air AFM images were acquired in non-contact mode in a vibration insulated environment, using a PicoPlus microscope (Molecular Imaging), equipped with a PicoScan-3000 controller. For imaging, we used single beam aluminum-coated cantilevers (type NSC36/ALBS, µmasch) with Rc < 10 nm, 100 µm length and nominal spring constant 0.06 N/m. The drive frequency was between 200 and 400 kHz.

Bibliography:

[1] G. T. Dolphin, M. Ouberai, P. Dumy, J. Garcia, *ChemMedChem* **2007**, *2*, 1613.
[2] S. S. S. Wang, Y.-T. Chen, P.-H. Chen, K.-N. Liu, *Biochemical Engineering Journal* **2006**, *29*, 129.

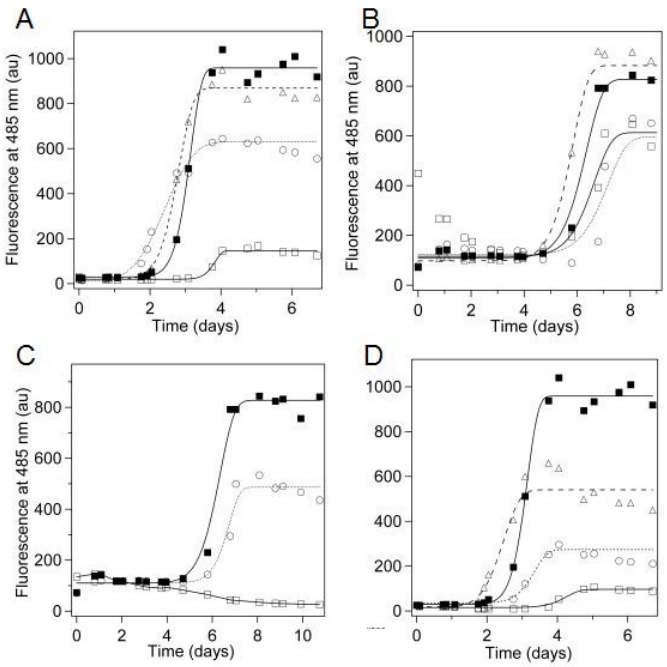


Figure S1. Representive inhibition studies of $A\beta_{1\text{-}40}$ (50 µM) fibril formation with oxime compounds. (A) **19**, (B) **20**, (C) **22**, (D) **27**. Concentrations of the oxime compounds are; 0 µM (solid squares, solid lines), 1 µM (triangles, dashed lines), 10 µM (circles, dotted lines) and 100 µM (squares, solid lines). Kinetics was monitored by ThT fluorescence at λ_{em} = 485 nm (λ_{ex} = 444 nm). Lines are extended-exponential functions fitted to the data. Data are representative of three independent experiments. Conditions: stagnant aq. solution of NaCl (100 mM), HPO_4^{2-} (50 mM) pH 7.4 at 37°C, 10 µM ThT. Assays were performed in in 96-well micro plates.

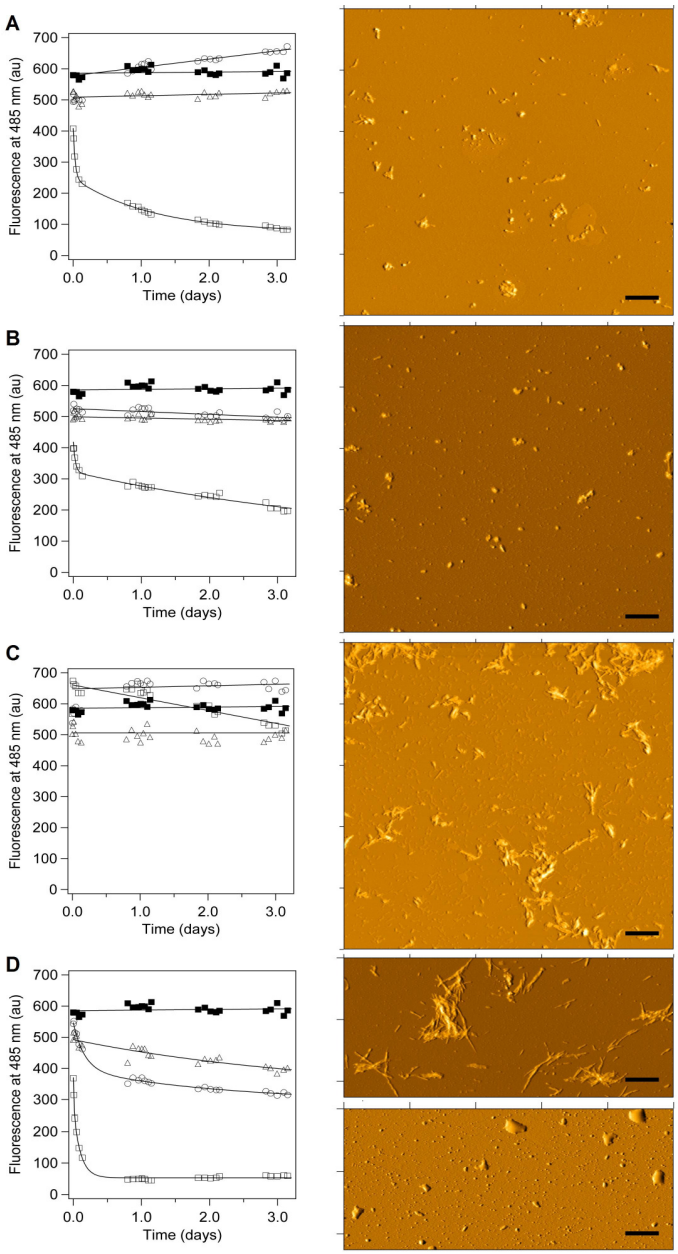


Figure S2. Disaggregation of $A\beta_{1\text{-}40}$ protofibrils (25 μM) followed in HPO_4^{2-} (50 mM), pH 7.4 and NaCl (100 mM) at 37℃ by the ThT assay (left) and AFM (Right) with **19** (A), **22** (B), **20** (C), NDGA (D, lower), and no added cpds (D, upper). Test concentration in the ThT assay: 0 μM (solid squares), 1 μM (triangles), 10 μM (circles) and 100 μM (squares). AFM studies are performed, after an incubation time of six days, on the mixtures with 100 μM compound concentrations (scan size 10x10 μm). **19** (A) and **22** (B) show good destabilizing properties at 100 μM with a decrease of the ThT fluorescence and no fibrils in the AFM images. (C) **20** has weak destabilizing properties and distorted fibrils are observed in the AFM image. (D) In the lower AFM image, the destabilization with 100 μM NDGA, very few fibrils are seen. The small drops are residual DMSO. In the Upper control image with no inhibitor compounds, many short fibrils are observed. Solid lines are non-linear fitting of the data with double exponential or linear functions. Other experimental details as in Figure 2. Scale bars indicate a length of 1 μm. Data are representative of two independent experiments.