Supporting Information

Inhibition of β-amyloid aggregation in Alzheimer's disease: The key role of (pro)electrophilic warheads

Filippo Basagni [a], Marina Naldi [a], Tiziana Ginex [b], F. Javier Luque[b], Francesca Fagiani [c], Cristina Lanni [c], Matteo Iurlo [d], Massimo Marcaccio [d], Anna Minarini [a], Manuela Bartolini [a], Michela Rosini [a,*]

a) Department of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy

b) Department of Nutrition, Food Science, and Gastronomy, Institute of Biomedicine (IBUB) and Institute of Theoretical and Computational Chemistry (IQTCUB), University of Barcelona, Avinguda Prat de la Riba 171, 08921-Santa Coloma de Gramenet, Spain

c) Department of Drug Sciences (Pharmacology Section), University of Pavia, V.le Taramelli 14, 27100 Pavia, Italy

d) Department of Chemistry "Giacomo Ciamician", Alma Mater Studiorum - University of Bologna, Via Selmi 2, 40126 Bologna, Italy

Table of contents:

Corresponding author: Michela Rosini - Department of Pharmacy and Biotechnology, Alma Mater Studiorum - University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy; Email: michela.rosini@unibo.it, Phone: +39 051 2099722

Experimental section

No unexpected or unusually high safety hazards were encountered in all experiments of this study.

Chemistry. Chemical reagents were purchased from Merck, TCI and Fluorochem. Nuclear magnetic resonance spectra (NMR) were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C on Varian VXR 400 spectrometer in $CDCl_3$, DMSO-d_6 or CD_3OD as solvents. Chemical shifts (δ) are given in ppm from tetramethylsilane (TMS) with the solvent resonance as internal standard ($CDCl_3$: δ 7.26, DMSO-d_6: δ 2.50, CD_3OD: δ 3.31 for ^{1}H NMR and $CDCl_3$: δ 77.16, DMSO-d_6: δ 39.52, CD_3OD: δ 49.00 for ^{13}C NMR). For ^{1}H NMR, data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, dd = double of doublets, t = triplet, q = quartet, m = multiplet, p = pentet, dt = doublet of triplets, td = triplet of doublets, tt = triplet of triplets, qd = quartet of doublets, br s = broad singlet), coupling constants (Hz) and integration. Chromatographic separations were performed on silica gel columns by flash or gravity column (Kieselgel 40, 0.040-0.063 mm; Merck) chromatography. Reactions were followed by thin-layer chromatography (TLC) on Merck (0.25 mm) glass-packed pre-coated silica gel plates (60 F254) that were visualized in an iodine chamber, or with a UV lamp, $KMnO_4$, or bromocresol green. All the names were attributed by Chem BioDraw Ultra 20.0. Final compounds mass spectra were recorded on a Xevo G2-XS QTof apparatus with electrospray ionization (ESI) in positive mode. All final compounds were pure >95% as determined by HPLC. HPLC analyses were performed under reversed-phase conditions on a Phenomenex Jupiter C18 (150x4.6 mm I.D.) column, using as the mobile phase a binary mixture of H_2O/acetonitrile (50/50, v/v) with UV detection at λ = 302 nm and a flow rate of 1 mL/min. Analyses were performed on a liquid chromatograph model PU-1585 UV equipped with a 20 µL loop valve (Jasco Europe, Italy). Compounds **1** was prepared as reported in reference [1].

*General procedure for the synthesis of compounds **4** and **5**.* Malonic acid (1.1 eq) was added to a solution of aniline (0.03-0.05 mL), pyridine (1.5 eq) and selected benzaldehyde (1 eq) in 2-4 mL of toluene and the mixture stirred at reflux for 4h. Once cooled to room temperature, HCl 3N was added until pH=1. The obtained precipitate was filtered, washed with HCl 3N and dried.

(E)-3-(3,5-dimethoxyphenyl)acrylic acid (**4**). Compound **4** was synthesized from 3,5-dimethoxybenzaldehyde (500 mg, 3.01 mmol) and obtained as white powder (520 mg, 83%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 16 Hz, 1H), 6.68-6.67 (m, 2H), 6.51-6.50 (m, 1H), 6.41 (d, *J* = 16 Hz, 1H), 3.81 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.72, 161.03 (2C), 147.06, 135.85, 117.66, 106.19 (2C), 103.01, 55.44 (2C).

(E)-3-(2,5-dimethoxyphenyl)acrylic acid (**5**). Compound **5** was synthesized from 2,5-dimethoxybenzaldehyde (400 mg, 2.41 mmol) and obtained as yellowish powder (490 g, 98%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.27 (br s, 1H), 7.77 (d, *J* = 16 Hz, 1H), 7.22-7.21 (m, 1H), 7.00-6.94 (m , 2H), 6.52 (d, *J* =16 Hz, 1H), 3.77 (s, 3H), 3.71 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.78, 153.12, 152.01, 138.37, 122.94, 119.50, 117.46, 112.94, 112.50, 56.01, 55.48.

*General procedure for the synthesis of compounds **6** and **7**.* To a solution of corresponding acid **4** and **5** (1 eq) in 1.5-3.5 mL of dichloromethane at 0°C under nitrogen atmosphere, HOBt (1.3 eq) and EDC (1.3 eq) were added. Reaction mixture was left stirring at this temperature for 30 min. After confirming the complete conversion of starting material in activated complex, selected thiol (4 eq) was added and the reaction mixture left stirring overnight at room temperature. After evaporation of the solvent, the crude was purified through flash column chromatography using petroleum ether: ethyl acetate as mobile phase.

S-propyl (E)-3-(3,5-dimethoxyphenyl)prop-2-enethioate (**6**). Compound **6** was synthesized from **4** (520 mg, 2.50 mmol) and 1-propanethiol (0.91 mL, 9.99 mmol). Compound was eluted with petroleum ether: ethyl acetate (9:1), which afforded **6** as colourless oil (430 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 16 Hz, 1H), 6.67-6.64 (m, 3H), 6.49-6.47 (m, 1H), 3.80 (s, 6H), 2.98 (t, *J* = 7.2 Hz, 2H), 1.68-1.61 (m, 2H), 0.99 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 189.90, 161.00, 140.17, 136.03, 125.58, 106.15, 102.79, 55.44, 30.86, 22.99, 13.38.

S-propyl (E)-3-(2,5-dimethoxyphenyl)prop-2-enethioate (**7**). Compound **7** was synthesized from **5** (300 mg, 1.44 mmol) and 1-propanethiol (0.52 mL, 5.76 mmol). Compound was eluted with petroleum ether: ethyl acetate (9.2:0.8), which afforded **7** as yellowish oil (250 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (d, *J* = 15.8 Hz, 1H), 7.04-7.03 (m, 1H), 6.92-6.89 (m, 1H), 6.85-6.82 (m, 1H), 6.77 (d, *J* = 15.8 Hz, 1H), 3.83 (s, 3H), 3.78 (s, 3H), 2.98 (t, *J* = 7.2 Hz, 2H), 1.69-1.64 (m, 2H), 1.01 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 190.42, 153.58, 153,32, 135.66, 126.01, 123.75, 117.50, 113.40, 112.54, 56.16, 55.88, 30.88, 23.14, 13.49.

*General procedure for the synthesis of compounds **2** and **3**.* A solution of dimethoxy analogues **6** and **7** (1 eq) in 0.2-0.6 mL of anhydrous dichloromethane was cooled at 0°C. Boron tribromide 1M in dichloromethane (2.3 eq) was added to the solution and the reaction was left stirring at the same temperature until completion. 6-10 mL of cold water were added to terminate the reaction and the mixture extracted with ethyl acetate (3 x 8 mL). Organic phases, once

reunited, were dried with anhydrous sodium sulphate, concentrated in vacuo and the crude obtained purified by column chromatography using dichloromethane: methanol as mobile phase.
S-propyl (E)-3-(3,5-dihydroxyphenyl)prop-2-enethioate (**2**). Compound **2** was synthesized from **6** (430 mg, 1.61 mmol). Compound was eluted with dichloromethane: methanol (9.5:0.5), which afforded **2** as yellow powder (200 mg, 52%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.41 (d, *J* = 15.8 Hz, 1H), 6.67 (d, *J* = 15.8 Hz, 1H), 6.51 (s, 2H), 6.33-6.32 (m, 1H), 2.95 (t, *J* = 7.2 Hz, 2H), 1.66-1.59 (m, 2H), 0.99 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 191.65, 160.12 (2C), 141.97, 137.21, 125.76, 107.76 (2C), 106.16, 31.56, 24.18, 13.58. HRMS (ESI+) calcd for $C_{12}H_{14}O_3S$ $[M+H]^+$ 239.0736, found 239.0744.
S-propyl (E)-3-(2,5-dihydroxyphenyl)prop-2-enethioate (**3**). Compound **3** was synthesized from **7** (220 mg, 0.83 mmol). Compound was eluted with dichloromethane: methanol (9.6:0.4), which afforded **3** as yellow powder (66 mg, 34%). ^{1}H NMR (400 MHz, CD_3OD) δ 7.82 (d, *J* = 16 Hz, 1H), 6.89-6.88 (m, 1H), 6.79 (d, *J* = 16 Hz, 1H), 6.69-6.68 (m, 2H), 2.94 (t, *J* = 7.4 Hz, 2H), 1.65-1.60 (m, 2H), 0.99 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 190.87, 150.60, 149.93, 136.23, 124.07, 121.14, 119.10, 116.54, 113.38, 30.07, 22.84, 12.17. HRMS (ESI+) calcd for $C_{12}H_{14}O_3S$ $[M+H]^+$ 239.0736, found 239.0742.

Cyclic voltammetry study. Electrochemical experiments were carried out in an airtight single-compartment glass cell described elsewhere[2] by using glassy carbon (GC) as working electrode, a platinum spiral as counter electrode and a silver spiral surface modified by silver chloride as a reference electrode. For the measurements in aqueous solutions a 0.2M phosphate buffer was used as electrolyte solution. All the $E_{1/2}$ potentials have been directly obtained from CV curves as averages of the cathodic and anodic peak potentials for one-electron reversible peaks and by digital simulation for those processes either closely spaced in multielectron voltammetric peaks or for the irreversible electron transfers. The $E_{1/2}$ values are referred to an Ag/AgCl (KCl, 3M). The GC working electrode was a 3 mm diameter disk that was mechanically polished by an alumina slurry in water. Voltammograms were recorded with a Biologic potentiostat model SP-300. Digital simulations of the cyclic voltammetric curves were carried out either by Antigona or DigiSim 3.0.

Sample preparation for $A\beta_{42}$ self-aggregation. 1,1,1,3,3,3-Hexafluoro-2-propanol (HFIP)-pretreated $A\beta_{42}$ samples (Bachem AG, Switzerland) were resolubilized with a CH_3CN/0.3 mM Na_2CO_3/250 mM NaOH (48.4:48.4:3.2) mixture to have a stable stock solution ($[A\beta_{42}]$ = 500 μM).[3] Tested inhibitors were dissolved in MeOH and diluted in the assay buffer. Experiments were performed by incubating the peptide diluted in 10 mM phosphate buffer (pH 8.0) containing 10 mM NaCl at 30°C (Thermomixer Comfort, Eppendorf, Italy) for 24 h (final Aβ concentration = 50 μM) with and without inhibitor.

Inhibition of $A\beta_{42}$ self-aggregation by ThT. Inhibition studies were performed by incubating $A\beta_{42}$ samples in the assay conditions reported above, with and without tested inhibitors. Inhibitors were first screened at 50 μM in a 1:1 ratio with $A\beta_{42}$. To quantify amyloid fibril formation, the ThT fluorescence method was used.[4] After incubation, samples were diluted to a final volume of 2.0 mL with 50 mM glycine-NaOH buffer (pH = 8.5) containing 1.5 μM ThT. A 300 s-time scan of fluorescence intensity was carried out (λ_{exc} = 446 nm; λ_{em} = 490 nm), and values at plateau were averaged after subtracting the background fluorescence of 1.5 μM ThT solution. Blanks containing inhibitor and ThT were also prepared and evaluated to account for quenching and fluorescence properties. The fluorescence intensities were compared and the % inhibition was calculated. For selected compounds the IC_{50} value was also determined. To this aim four increasing concentrations were tested. IC_{50} value was obtained from the % inhibition vs log[inhibitor] plot.

Analysis of intact protein (Top-down analysis). ***Sample preparation.*** Studies were performed by incubating $A\beta_{42}$ samples under the assay conditions used for the ThT assay, with and without tested compounds (**1**-**3**) at 50 μM (tested compound/$A\beta_{42}$ = 1/1). Samples were incubated at 30°C for 24 h. At selected time points (0, 1, 3, 6 and 24h) 5 μL-aliquots of mixture were collected, diluted to 25 μL with reserpine 20 μg/mL (IS) in H_2O/MeOH (50/50; v/v) and subjected to LC-MS analysis. Experiments were performed in duplicate.
LC-MS analysis. LC-MS analyses were performed by an Agilent 1200 Series (Walbronn, Germany) coupled with an ESI-Q-ToF mass spectrometer equipped with a Z-spray ion source (Micromass, Manchester, UK). Flow injection analyses were performed by employing H_2O/AcCN/FA (70/30/0.1; v/v/v) as mobile phase at the flow rate of 0.1 mL/min. The injection volume was 5 μL. The capillary voltage was set at 3000 V, the cone voltage was 35 V, the capillary temperature was 120°C, while the desolvation temperature was 300°C. Mass spectra were recorded in total ion current (TIC), within 500 and 2000 *m/z*, in positive polarity. $A\beta_{42}$ baseline subtracted spectrum (m/z 650−1700) was deconvoluted onto a true mass scale using the maximum entropy (MaxEnt1)-based software supplied with MassLynx

4.1 software. The output parameters were mass range: 4000−5000 Da and resolution: 2 Da/channel. The uniform Gaussian model was used, with 0.65 Da width at half height.

Assessment of the inhibitory capacity. The ratio between intensity of the $[M+5H]^{5+}$ charge state of $A\beta_{42}$ monomer (at m/z 903.8) and the intensity of the internal standard (m/z at 609.4), namely $I_{A\beta42}/I_{IS}$, was used to assess the residual amount of $A\beta_{42}$ monomer. The $I_{A\beta42}/I_{IS}$ ratio at t0 was considered as 100% of the monomer content. $I_{A\beta42}/I_{IS}$ values at 24h were also calculated to assess the residual amount of monomer (R) as difference from the $I_{A\beta42}/I_{IS}$ value at 24h and t0. Percent inhibition calculated as follows:

% inhibition = $100-(100xR_{inh}/R_0)$

where R_{inh} and R_0 are the residual amount of amyloid monomer in the presence and in the absence of inhibitor.

Bottom-up analysis of $A\beta_{42}$ upon incubation with compound 3. ***Sample preparation.*** $A\beta_{42}$ 50 μM alone and co-incubated with **3** 50 μM were incubated for 6 h and digested overnight at 37°C upon dilution 1:2 with a solution of trypsin in ammonium bicarbonate 20 mM, pH 8; the final $A\beta_{42}$/trypsin ratio was 100/1 (w/w).

LC-MSMS analysis. $A\beta_{42}$ tryptic digest (10 μL) was analyzed by an Agilent 1100 Series HPLC system (Walbronn, Germany) coupled with a Q-ToF mass spectrometer (Micromass, Manchester, UK) equipped with a Z-spray ion source. Analyses were performed on a C18 column (Aeris peptide XB-C18; 150 × 2.1 mm; 3.5 μm; Phenomenex). Mobile phases A [water/acetonitrile/FA (99/1/0.1, v/v/v)] and B [water/acetonitrile/FA (1/99/0.1, v/v/v)] were used to develop a gradient. The solvent gradient was set as follows: A–B from (98:8, v/v) to (40:60, v/v) in 20 min; (40:60, v/v) for 2 min. The column was equilibrated with the mobile phase composition of the starting conditions for 10 min before the next injection. The ESI-Q-ToF source temperature was set at 100°C, the capillary voltage at 3.0 kV, and the cone voltage at 35 V. Peptide ions within a m/z 400–1700 survey scan mass range were analyzed for subsequent fragmentation. 2+, 3+, and 4+ charged ions exceeding a threshold abundance (TIC value: 10 counts/s) were selected for MS/MS analyses. From a single survey scan, 4 ions were selected for subsequent fragmentation. Scan returns to mass survey mode when the ion intensity falls below 5 counts/s or after 8 s. Scan time was 1 s for the parent ion and 1 s for the MS/MS ions. The collision energy was selected using charge state recognition.

Computational Methods. QM computations were performed to discern the feasibility of the nucleophilic addition of the methylamine to the *para*-quinone ring as well as to the vinyl position. Moreover, calculations were also performed to characterize the energetics of the addition to the *ortho*-quinone derivative.

All computations were performed at the M06-2X/6-31G(d) level.[5] Full geometry optimizations were performed for the different model systems, and in all cases the vibrational frequencies were analyzed to confirm the nature of the stationary points (no imaginary frequency for minimum energy structures, and 1 imaginary frequency for the transition states; atomic coordinates for the optimized structures of the stationary points are provided in the Supporting Information). The free energy profiles were obtained by adding thermal and entropy corrections in the gas phase. Hydration effects were determined by using the IEFPCM/MST method at the B3LYP/6-31G(d) level.[6]

All calculations were performed with a locally modified version of Gaussian 16.[7]

Sample preparation for cellular experiments. Compounds **1**-**3** were solubilized in dimethyl sulfoxide (DMSO) (at stock concentrations) and frozen (–20°C) in aliquots that were diluted immediately prior to use. For each experimental setting, one stock aliquot was thawed out and diluted to minimize compound damage due to repeated freeze and thaw cycles. The final concentration of DMSO in culture medium was < 0.1%.

SH-SY5Y Cell Cultures. Human neuroblastoma SH-SY5Y cells, purchased from the European Collection of Cell Cultures (ECACC No. 94030304), were cultured in a medium with equal amounts of Eagle's minimum essential medium and Nutrient Mixture Ham's F-12, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, 0.1 mg/mL streptomycin, 100 IU·mL penicillin and non-essential amino acids at 37°C in 5% CO_2-containing, and 95% air atmosphere. All culture media, supplements and FBS were purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). The experiments were carried out on passages 7–15. Cells were treated as reported in the figure legend.

Cell Viability. The mitochondrial dehydrogenase activity that reduces 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) (Sigma Aldrich, Merck KGaA, Darmstadt, Germany) was used to determine cell viability, using a quantitative colorimetric assay.[8] At day 0, SH-SY5Y cells were plated in 96-well plates at a density of 20×10^3 viable cells per well. After treatment, according to the experimental setting, cells were exposed to an MTT solution (1 mg/mL) in complete medium. After 4 hours of incubation with MTT, cells were lysed with DMSO and cell viability was

quantified by reading absorbance at 570 nm wavelength, using Synergy HT multi-detection microplate reader (Bio-Tek, Winooski, VT, USA).

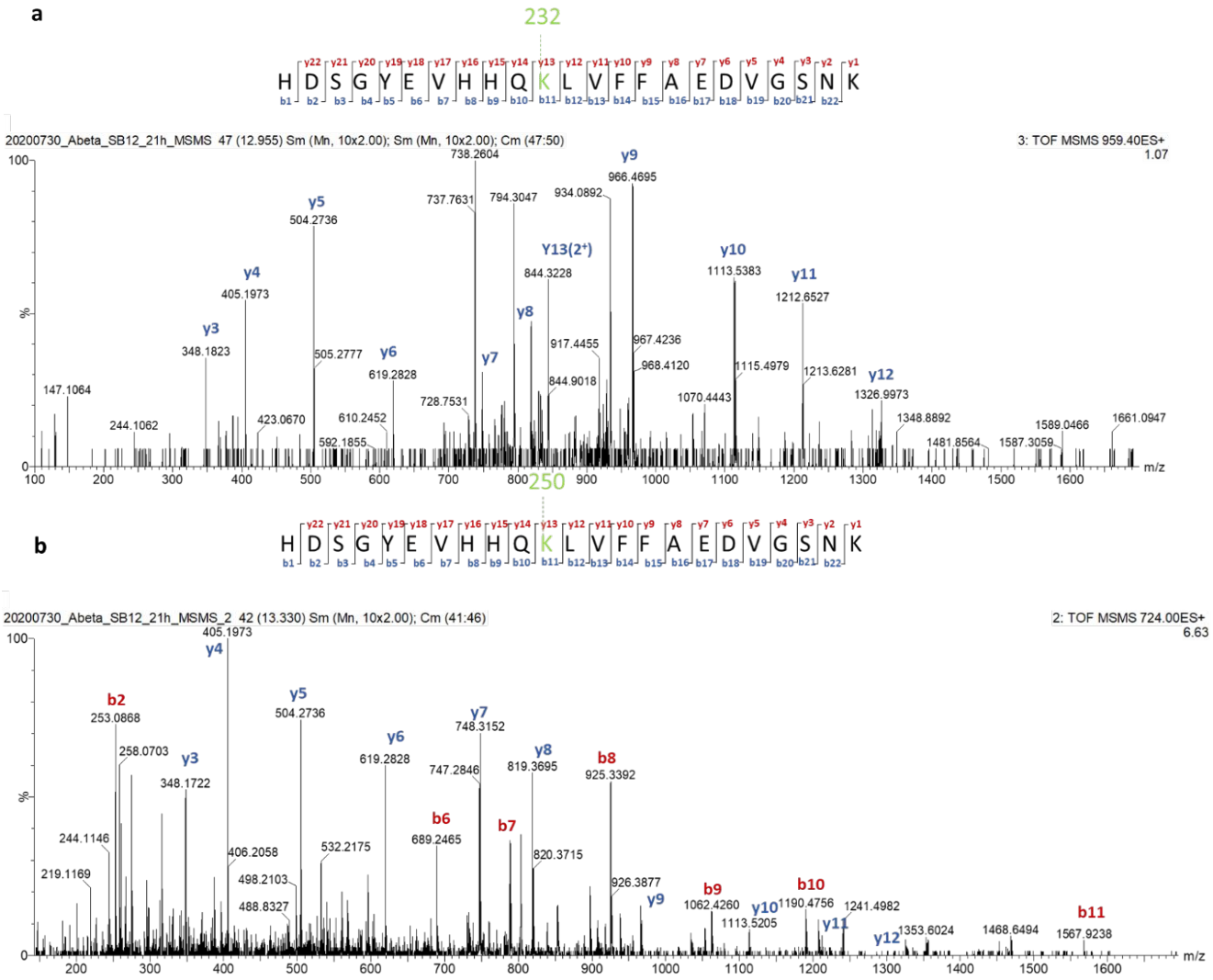


Figure S1. Bottom-up analysis of $A\beta_{42}$. MS/MS spectra of peptide 6-28 carrying a modification characterized by a mass increment of 232 (a) and 250 Da (b) at the level of $A\beta_{42}$ Lys16. In the mass spectra the identified fragments are labelled with the letter y or b, depending on the type of fragment, and with a number indicating the position of the fragmentation on the peptide sequence.

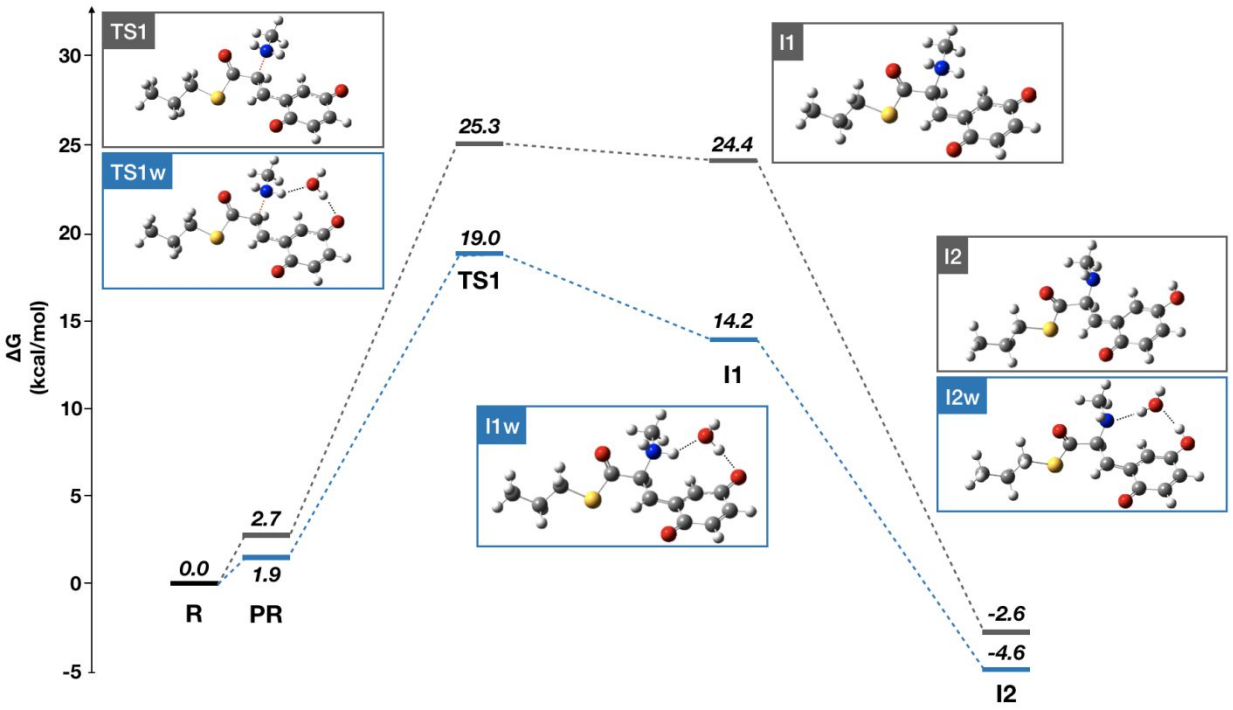


Figure S2. Free energy profiles in gas phase determined from density functional theory calculations for the attack to the vinyl position of **3** (see also route b, scheme 2 in the main text) and performed using a reduced model where the side chain of Lys16 was simulated with methylamine and assisted (or not) by a water molecule.

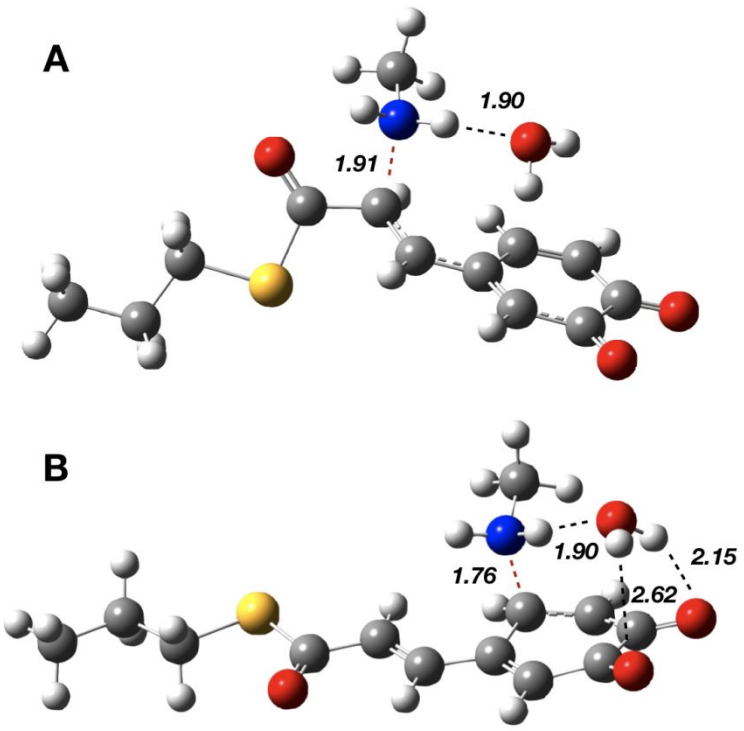
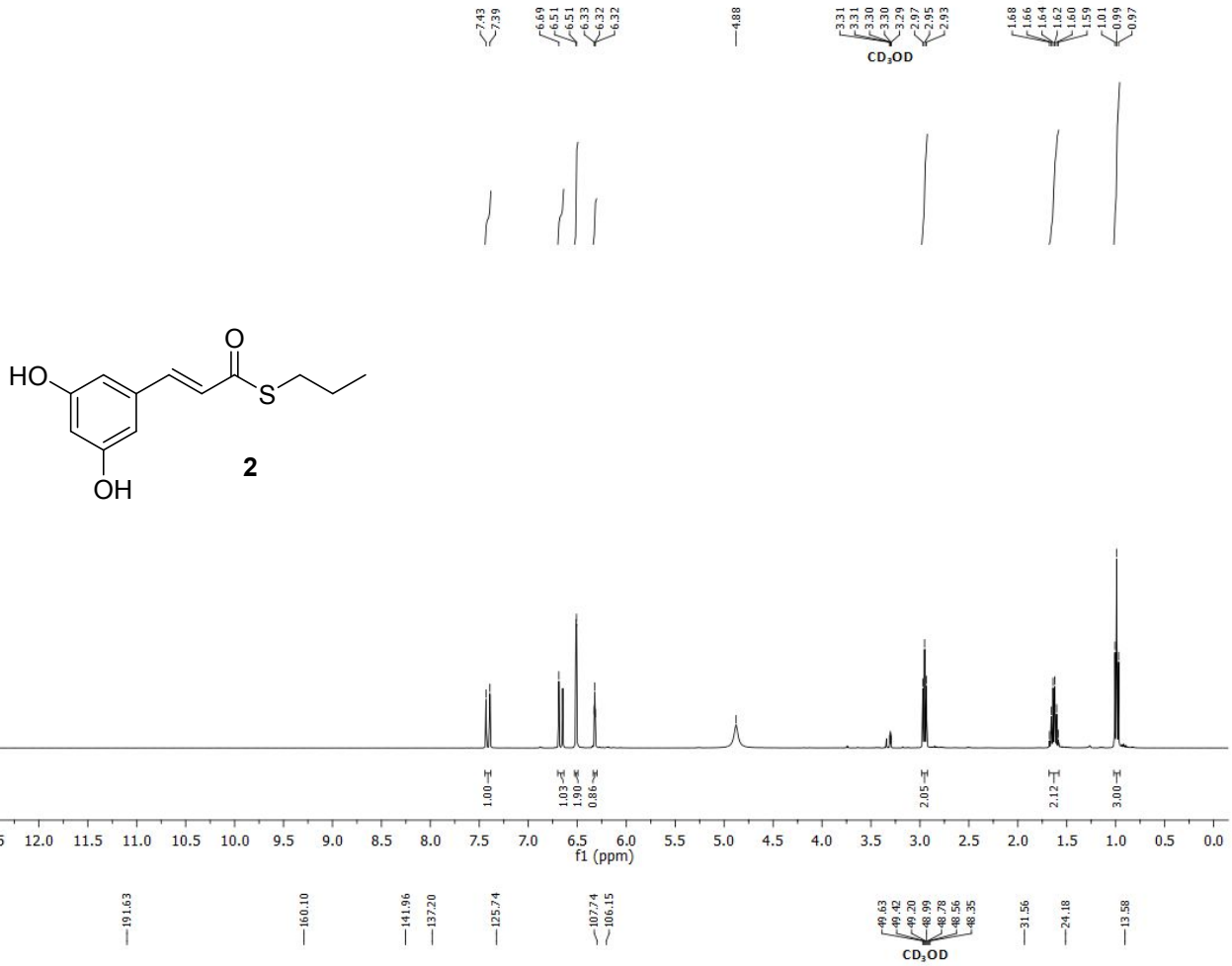
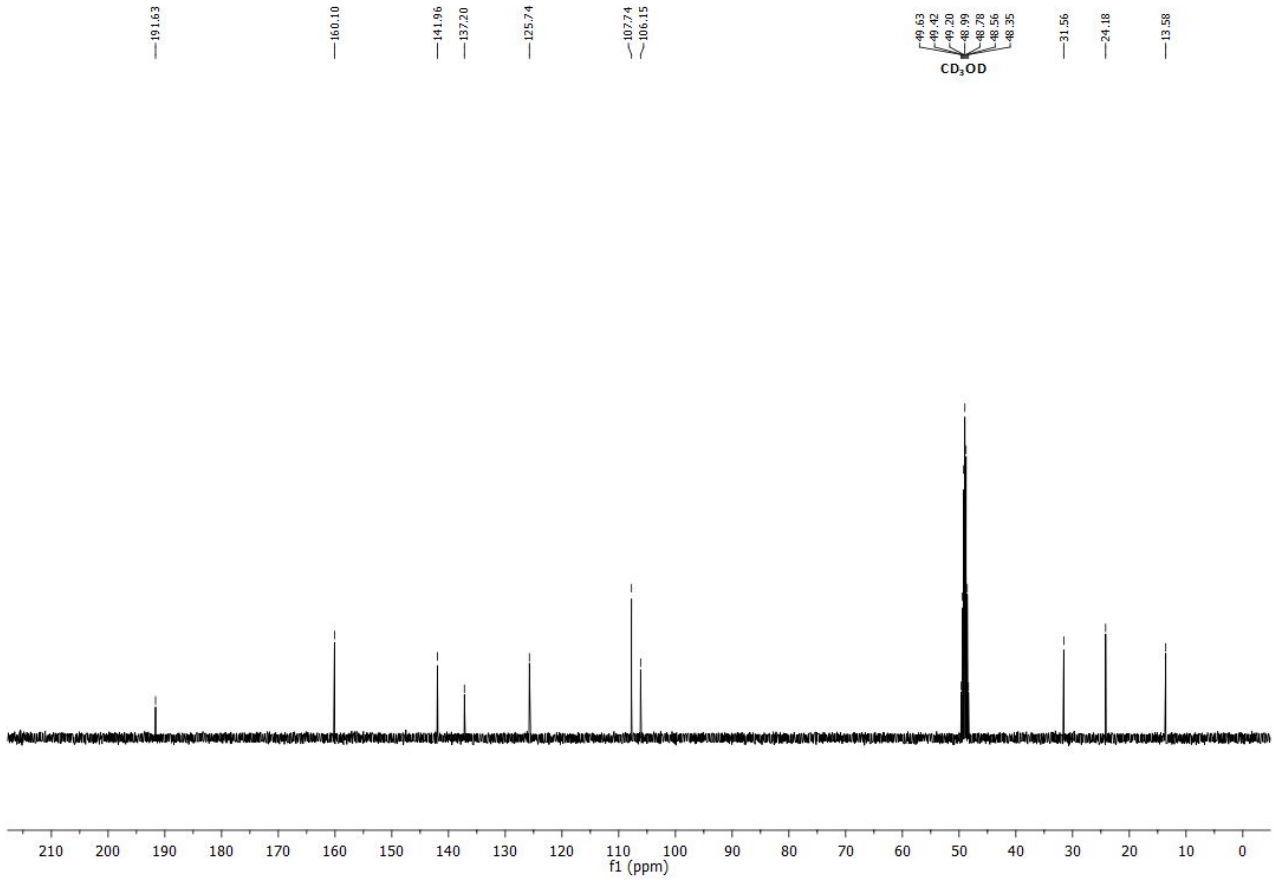
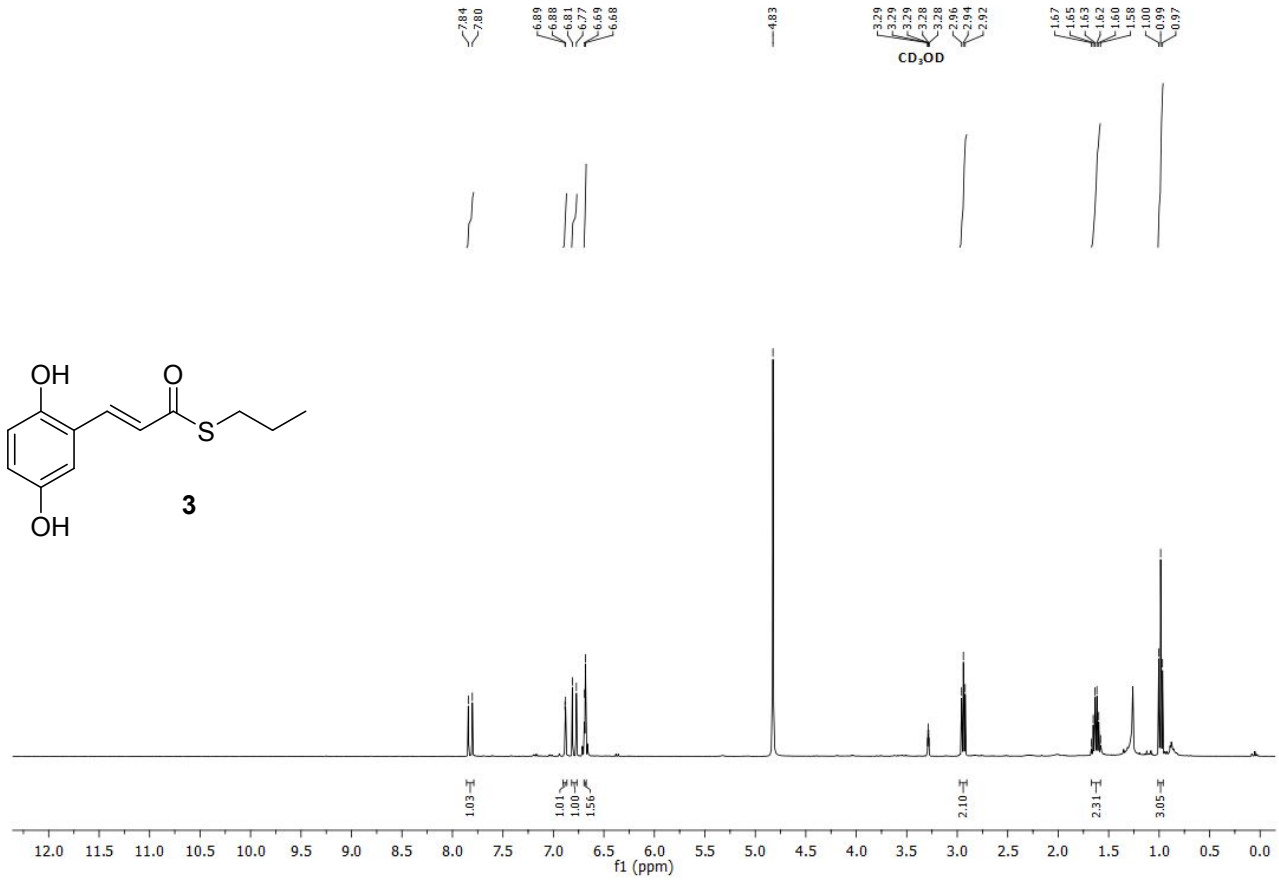


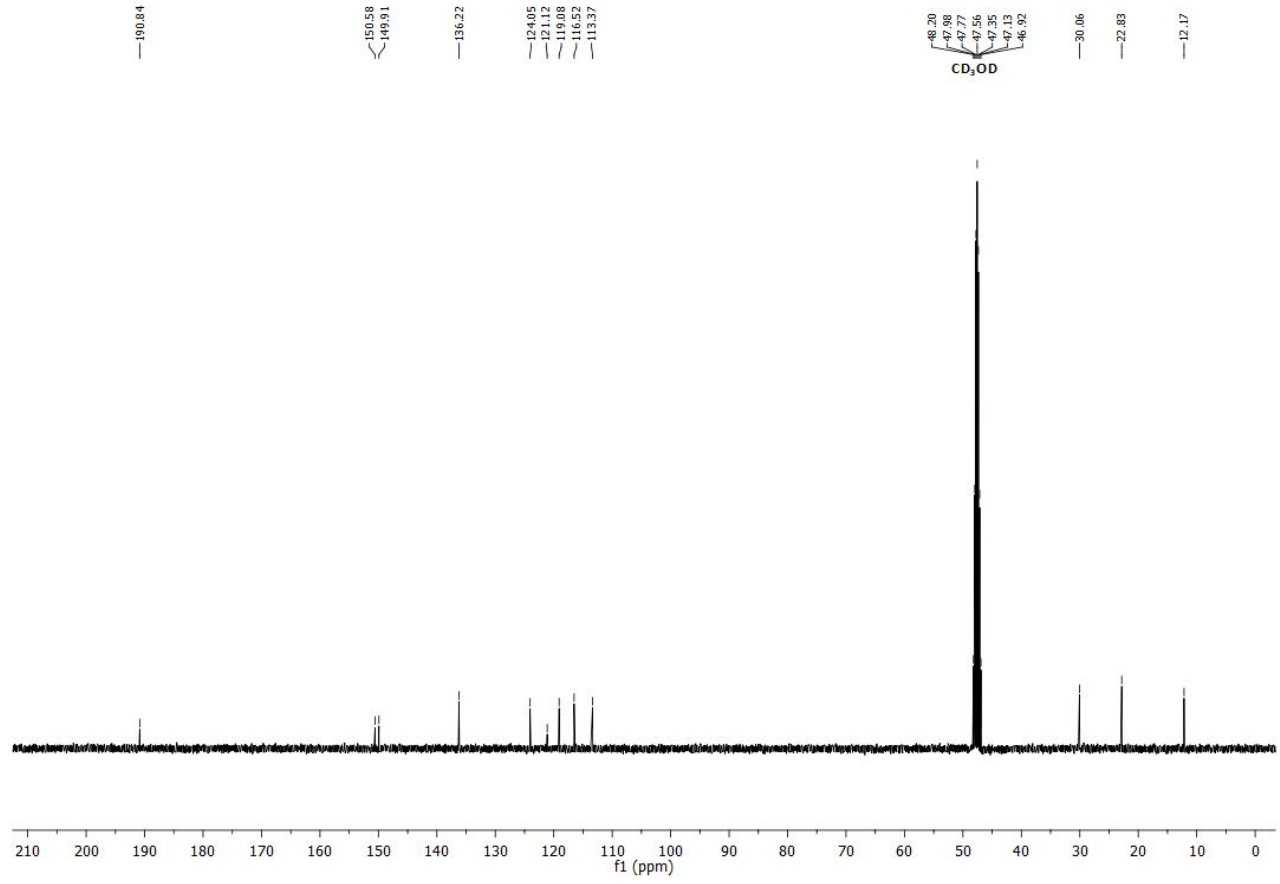
Figure S3. Transition (TS) structures from quantum mechanics (QM) calculations relative to the *water-assisted* addition of the methylamine to the vinyl (**A**) and to the quinone (**B**) moiety of the ortho-quinone derivative **1**.

^{1}H NMR and ^{13}C NMR spectra of compounds **2** and **3**






7.84
7.80
6.89
6.88
6.81
6.77
6.69
6.68
4.83
3.29
3.29
3.29
3.28
3.28
2.96
2.94
2.92
CD3OD
1.67
1.65
1.63
1.62
1.60
1.58
1.00
0.99
0.97
OH
O
S
OH
3
1.03
1.01
1.00
1.56
2.10
2.31
3.05
f1 (ppm)


190.84
150.58
149.91
136.22
124.05
121.12
119.08
116.52
113.37
48.20
47.98
47.77
47.56
47.35
47.13
46.92
CD3OD
30.06
22.83
12.17
f1 (ppm)

Optimized geometries of the chemical species shown in **Figure 4**

A) Reactants (R)

p-Quinone

1	6	0	-4.684107	1.003908	-0.239094
2	6	0	-5.103002	-0.260884	-0.149892
3	6	0	-4.140645	-1.370763	0.085160
4	6	0	-2.704422	-1.022346	0.209142
5	6	0	-2.257461	0.241559	0.093938
6	6	0	-3.246316	1.357392	-0.106912
7	8	0	-2.882593	2.514640	-0.152749
8	8	0	-4.517341	-2.523095	0.172857
9	6	0	-0.850214	0.639025	0.185380
10	6	0	0.184903	-0.177605	-0.040313
11	6	0	1.571572	0.340353	0.101469
12	8	0	1.842329	1.474017	0.421638
13	16	0	2.817860	-0.884901	-0.248474
14	6	0	4.290039	0.144688	0.046042
15	6	0	5.558713	-0.671520	-0.175006
16	6	0	6.804255	0.178253	0.067763
17	1	0	-5.355317	1.840665	-0.404098
18	1	0	-6.146214	-0.547164	-0.238046
19	1	0	-2.039454	-1.857982	0.408623
20	1	0	-0.650021	1.680716	0.423983
21	1	0	0.060067	-1.214382	-0.343142
22	1	0	4.230698	0.527649	1.068053
23	1	0	4.239681	1.001619	-0.630568
24	1	0	5.568138	-1.064873	-1.197307
25	1	0	5.559549	-1.536602	0.496965
26	1	0	7.714138	-0.405425	-0.089487
27	1	0	6.822394	0.562021	1.091924
28	1	0	6.831532	1.035318	-0.611348

Energy: -1088.004018 au
Free energy: -1087.834121 au

Methylamine

1	7	0	-0.749150	-0.000000	-0.124550
2	6	0	0.702992	-0.000000	0.017776
3	1	0	1.079768	-0.000010	1.051895
4	1	0	1.113671	0.878796	-0.485958
5	1	0	1.113673	-0.878784	-0.485978
6	1	0	-1.140508	0.811408	0.342615
7	1	0	-1.140505	-0.811409	0.342616

Energy: -95.804301 au
Free energy: -95.762357 au

Water

1	8	0	0.000000	0.117813	0.000000
2	1	0	0.760185	-0.471279	0.000000
3	1	0	-0.760185	-0.471228	0.000000

Energy: -76.383939 au
Free energy: -76.380529 au

B) Attack without water

Pre-reactant state (PR)

1	6	0	4.168281	-1.588123	0.213187
2	6	0	4.655063	-0.488526	-0.365992
3	6	0	3.750706	0.523792	-0.972876
4	6	0	2.290774	0.272269	-0.891817
5	6	0	1.777987	-0.805225	-0.273069
6	6	0	2.707239	-1.832025	0.309820
7	8	0	2.275159	-2.836880	0.839731
8	8	0	4.187917	1.499508	-1.553135
9	6	0	0.345417	-1.080599	-0.134818
10	6	0	-0.619103	-0.157088	-0.221271
11	7	0	3.566281	1.828020	1.467172
12	6	0	2.619600	2.849041	1.023266
13	6	0	-2.040092	-0.563171	-0.075580
14	8	0	-2.408958	-1.698369	0.115097
15	16	0	-3.179262	0.805150	-0.208327
16	6	0	-4.729862	-0.120959	0.019471
17	6	0	-5.925539	0.822226	-0.052440
18	6	0	-7.235219	0.060266	0.138968
19	1	0	4.797604	-2.354990	0.653248
20	1	0	5.717935	-0.281483	-0.443886
21	1	0	1.664164	1.016114	-1.377188
22	1	0	0.064255	-2.107731	0.084902
23	1	0	-0.408832	0.897699	-0.381916
24	1	0	2.638680	3.784030	1.599384
25	1	0	1.605782	2.440548	1.076502
26	1	0	2.829686	3.086918	-0.023739
27	1	0	-4.676560	-0.630137	0.985178
28	1	0	-4.777212	-0.889995	-0.755811
29	1	0	-5.929661	1.335335	-1.020246
30	1	0	-5.828190	1.596908	0.715943
31	1	0	-8.093121	0.734621	0.087310
32	1	0	-7.257715	-0.440508	1.111217
33	1	0	-7.359588	-0.703849	-0.633829
34	1	0	3.382018	1.571005	2.431999
35	1	0	4.510513	2.201631	1.439316

Energy: -1183.819991 au

Free energy: -1183.591096 au

Transition state (TS0)

1	6	0	4.360657	-1.485164	0.096259
2	6	0	4.813461	-0.276863	-0.256841
3	6	0	3.866930	0.802076	-0.598898
4	6	0	2.389552	0.512731	-0.439663
5	6	0	1.945630	-0.797483	-0.179500
6	6	0	2.899265	-1.836316	0.159590
7	8	0	2.599709	-2.982949	0.468000
8	8	0	4.236003	1.908677	-0.945237
9	6	0	0.544889	-1.081417	-0.093520
10	6	0	-0.467707	-0.185941	-0.214284
11	7	0	2.045141	1.562620	1.076453
12	6	0	1.953148	3.011462	0.851081
13	6	0	-1.857686	-0.624289	-0.072315
14	8	0	-2.224563	-1.752660	0.170209
15	16	0	-3.044289	0.721374	-0.274981

16	6	0	-4.570000	-0.235440	-0.014189
17	6	0	-5.792377	0.667695	-0.132786
18	6	0	-7.082179	-0.121753	0.081221
19	1	0	5.031082	-2.300406	0.353288
20	1	0	5.868171	-0.028823	-0.322396
21	1	0	1.784533	1.148592	-1.088977
22	1	0	0.276698	-2.112743	0.130591
23	1	0	-0.305438	0.864974	-0.449001
24	1	0	1.731348	3.551246	1.774859
25	1	0	1.155780	3.195814	0.128846
26	1	0	2.896269	3.355061	0.425743
27	1	0	-4.511049	-0.702662	0.972140
28	1	0	-4.592490	-1.038837	-0.754855
29	1	0	-5.803485	1.140581	-1.120941
30	1	0	-5.723108	1.476562	0.603004
31	1	0	-7.959218	0.524444	-0.003320
32	1	0	-7.097619	-0.583749	1.072753
33	1	0	-7.178711	-0.920265	-0.660210
34	1	0	1.157689	1.154018	1.374529
35	1	0	2.751493	1.315539	1.767759

Energy: -1183,802958 au
Free energy. -1183.568774 au

Intermediate (I1)

1	6	0	4.387502	-1.469826	-0.311853
2	6	0	4.840806	-0.215426	-0.449519
3	6	0	3.934743	0.901504	-0.175263
4	6	0	2.431272	0.633005	-0.297618
5	6	0	1.998118	-0.764850	-0.030594
6	6	0	2.960009	-1.829195	0.008502
7	8	0	2.713944	-3.011470	0.235212
8	8	0	4.316641	1.997908	0.197257
9	6	0	0.627485	-1.046286	0.071504
10	6	0	-0.421366	-0.180441	-0.150354
11	7	0	1.766697	1.578687	0.714091
12	6	0	1.450301	2.940276	0.207300
13	6	0	-1.789312	-0.612244	0.069753
14	8	0	-2.144452	-1.673519	0.537400
15	16	0	-3.010676	0.644997	-0.407964
16	6	0	-4.515732	-0.273245	0.040835
17	6	0	-5.757327	0.564956	-0.241003
18	6	0	-7.030291	-0.192652	0.130832
19	1	0	5.053519	-2.324872	-0.391909
20	1	0	5.885422	0.030234	-0.610008
21	1	0	2.098151	1.016800	-1.278017
22	1	0	0.373429	-2.057142	0.388814
23	1	0	-0.290747	0.787254	-0.633959
24	1	0	0.983920	3.516784	1.005759
25	1	0	0.757068	2.841144	-0.628088
26	1	0	2.381794	3.402078	-0.111828
27	1	0	-4.444967	-0.541490	1.098012
28	1	0	-4.524497	-1.204571	-0.530973
29	1	0	-5.781666	0.837638	-1.301846
30	1	0	-5.702330	1.502371	0.323701
31	1	0	-7.920980	0.407160	-0.071814
32	1	0	-7.033464	-0.453380	1.193330
33	1	0	-7.112301	-1.122070	-0.440302

34	1	0	0.897842	1.099656	1.008024
35	1	0	2.397428	1.654827	1.515802

Energy: -1183.810064 au
Free energy: -1183.572434 au

Intermediate (I2)

1	6	0	4.241573	-1.598558	-0.222647
2	6	0	4.853606	-0.403920	-0.248549
3	6	0	4.057705	0.811514	0.000386
4	6	0	2.802784	-1.742285	-0.032189
5	8	0	2.304484	-3.003798	0.057260
6	6	0	1.963722	-0.673286	0.019321
7	6	0	2.539365	0.716108	-0.214527
8	8	0	4.575304	1.846465	0.368956
9	6	0	0.535399	-0.920597	0.101705
10	6	0	-0.468885	-0.030165	0.014400
11	7	0	1.833755	1.780152	0.474793
12	6	0	1.823735	3.043852	-0.258679
13	6	0	-1.859156	-0.530997	0.065412
14	8	0	-2.182715	-1.691079	0.191790
15	16	0	-3.077154	0.775891	-0.085044
16	6	0	-4.576733	-0.249626	0.016710
17	6	0	-5.823708	0.620956	-0.090377
18	6	0	-7.093153	-0.224036	-0.007157
19	1	0	2.420984	0.896780	-1.299723
20	1	0	4.826860	-2.511499	-0.325694
21	1	0	5.930208	-0.298335	-0.321532
22	1	0	0.248323	-1.963978	0.211317
23	1	0	1.345251	3.805668	0.360402
24	1	0	1.215654	2.924509	-1.162247
25	1	0	2.820805	3.399377	-0.545719
26	1	0	-4.548325	-0.794245	0.964073
27	1	0	-4.534120	-0.989518	-0.786852
28	1	0	-5.804240	1.173609	-1.036098
29	1	0	-5.818833	1.367596	0.711272
30	1	0	-7.140289	-0.766037	0.941934
31	1	0	-7.125291	-0.961419	-0.814631
32	1	0	-7.987370	0.399072	-0.084094
33	1	0	2.275514	1.924619	1.378462
34	1	0	3.021277	-3.644265	0.108900
35	1	0	-0.302336	1.036274	-0.069517

Energy: -1183.828158 au
Free energy: -1183.593517 au

C) Attack assisted by a water molecule

Pre-reactant state (PRw)

1	6	0	4.278104	-0.710645	-0.491324
2	6	0	4.750750	0.515400	-0.249267
3	6	0	3.856501	1.703418	-0.253781
4	6	0	2.412190	1.468154	-0.486682
5	6	0	1.919555	0.236148	-0.700354
6	6	0	2.847714	-0.940346	-0.818509
7	8	0	2.465200	-2.008958	-1.262315
8	8	0	4.290586	2.822419	-0.053394
9	6	0	0.501670	-0.090110	-0.825759
10	6	0	-0.501346	0.572281	-0.238313

11	7	0	2.246351	-1.226845	1.701941
12	6	0	2.133594	-0.042308	2.535726
13	6	0	-1.872533	0.019338	-0.348773
14	8	0	-2.108693	-1.117044	-0.713232
15	16	0	-3.153419	1.152962	0.114961
16	6	0	-4.591403	0.064347	-0.140598
17	6	0	-5.875168	0.791517	0.244304
18	6	0	-7.093379	-0.105152	0.033592
19	1	0	4.908723	-1.594071	-0.510257
20	1	0	5.797712	0.713070	-0.041903
21	1	0	1.777287	2.349460	-0.453860
22	1	0	0.267970	-0.989950	-1.385992
23	1	0	-0.351552	1.475634	0.347638
24	1	0	1.885431	-0.238869	3.587591
25	1	0	1.355549	0.609077	2.126355
26	1	0	3.075814	0.518193	2.509715
27	1	0	-4.439693	-0.833754	0.463425
28	1	0	-4.600779	-0.239455	-1.190358
29	1	0	-5.974485	1.703359	-0.354523
30	1	0	-5.818145	1.105025	1.292257
31	1	0	-8.013947	0.413946	0.309672
32	1	0	-7.021848	-1.011650	0.641486
33	1	0	-7.178794	-0.409595	-1.013522
34	1	0	1.352428	-1.701700	1.564634
35	1	0	2.890551	-1.898114	2.107542
36	8	0	0.118095	-2.865592	0.402624
37	1	0	0.776296	-2.882824	-0.306159
38	1	0	-0.677277	-2.486116	0.002246

Energy: -1260.229686 au
Free energy: -1259.974897 au

Transition state (TS0w)

1	6	0	4.273167	-1.386954	-0.281871
2	6	0	4.780983	-0.156524	-0.413218
3	6	0	3.879884	1.000265	-0.620894
4	6	0	2.402738	0.718901	-0.615121
5	6	0	1.915787	-0.578586	-0.716516
6	6	0	2.799152	-1.663887	-0.319963
7	8	0	2.406175	-2.775960	0.026614
8	8	0	4.292601	2.137006	-0.740071
9	6	0	0.501891	-0.828334	-0.671145
10	6	0	-0.505900	0.068477	-0.754218
11	7	0	2.098976	1.277056	1.303572
12	6	0	1.677029	2.670548	1.424928
13	6	0	-1.840293	-0.366280	-0.337568
14	8	0	-2.044784	-1.343273	0.366042
15	16	0	-3.175534	0.685782	-0.881854
16	6	0	-4.552120	-0.153380	-0.035279
17	6	0	-5.852322	0.611375	-0.257261
18	6	0	-7.021458	-0.079847	0.441134
19	1	0	4.897976	-2.257422	-0.104023
20	1	0	5.844462	0.057302	-0.374830
21	1	0	1.816547	1.560933	-0.976622
22	1	0	0.224750	-1.858046	-0.451385
23	1	0	-0.371763	1.104309	-1.053064
24	1	0	1.571149	2.990942	2.466000
25	1	0	0.714983	2.789022	0.922671

26	1	0	2.415560	3.302413	0.924644
27	1	0	-4.301195	-0.220735	1.026412
28	1	0	-4.617132	-1.173065	-0.422796
29	1	0	-6.053140	0.689779	-1.331360
30	1	0	-5.743564	1.634446	0.118973
31	1	0	-7.953455	0.468053	0.283726
32	1	0	-6.848006	-0.147886	1.519095
33	1	0	-7.159087	-1.095922	0.060077
34	1	0	1.399660	0.584029	1.606861
35	1	0	2.953410	1.097065	1.826451
36	8	0	0.572066	-1.189218	2.145199
37	1	0	1.077976	-1.911472	1.744381
38	1	0	-0.313131	-1.288714	1.759117

Energy: -1260.211592 au
Free energy: -1259.954709 au

Intermediate (I1w)

1	6	0	4.460097	-1.304425	-0.379670
2	6	0	4.833133	-0.024015	-0.521346
3	6	0	3.845654	1.041145	-0.326301
4	6	0	2.360515	0.678404	-0.445100
5	6	0	2.017472	-0.762882	-0.290267
6	6	0	3.042286	-1.758172	-0.156304
7	8	0	2.854336	-2.951274	0.074865
8	8	0	4.147496	2.178710	-0.008043
9	6	0	0.663865	-1.109963	-0.245158
10	6	0	-0.387381	-0.305581	-0.643532
11	7	0	1.657170	1.504508	0.642858
12	6	0	1.020438	2.761055	0.175332
13	6	0	-1.708379	-0.564744	-0.154616
14	8	0	-1.953934	-1.205869	0.871166
15	16	0	-3.040293	0.190575	-1.092034
16	6	0	-4.426004	-0.208127	0.019589
17	6	0	-5.722239	0.387420	-0.518446
18	6	0	-6.900898	0.057413	0.394984
19	1	0	5.188367	-2.110978	-0.399943
20	1	0	5.866849	0.286969	-0.632798
21	1	0	2.010118	1.113488	-1.394898
22	1	0	0.427317	-2.083560	0.186125
23	1	0	-0.241456	0.524950	-1.328704
24	1	0	0.588912	3.273645	1.035080
25	1	0	0.231573	2.506060	-0.530767
26	1	0	1.786297	3.377964	-0.292949
27	1	0	-4.188753	0.181590	1.013032
28	1	0	-4.491314	-1.295528	0.102251
29	1	0	-5.911728	0.001037	-1.525959
30	1	0	-5.614928	1.473705	-0.612995
31	1	0	-7.830377	0.483698	0.009955
32	1	0	-6.738887	0.454809	1.401381
33	1	0	-7.036566	-1.024599	0.481597
34	1	0	0.963789	0.898397	1.158799
35	1	0	2.378944	1.769588	1.318586
36	8	0	0.089498	0.026725	2.418182
37	1	0	0.653957	-0.713709	2.671201
38	1	0	-0.688134	-0.409803	2.009206

Energy: -1260.223321 au
Free energy: -1259.962719 au

Intermediate (I2w)

1	6	0	4.249954	-1.410953	-0.279122
2	6	0	4.754089	-0.177732	-0.431374
3	6	0	3.845119	0.962317	-0.672928
4	6	0	2.363147	0.761028	-0.356105
5	6	0	1.893013	-0.673690	-0.374743
6	6	0	2.809966	-1.671980	-0.295159
7	8	0	2.538954	-2.991153	-0.234356
8	8	0	4.259660	2.047915	-1.030227
9	6	0	0.474016	-0.925558	-0.264464
10	6	0	-0.508917	-0.035429	-0.492451
11	7	0	2.060532	1.314613	0.978300
12	6	0	2.255786	2.755969	1.113535
13	6	0	-1.889217	-0.410700	-0.136510
14	8	0	-2.165346	-1.356679	0.578383
15	16	0	-3.137610	0.657570	-0.818375
16	6	0	-4.603675	-0.130285	-0.079706
17	6	0	-5.863861	0.636609	-0.465264
18	6	0	-7.105372	-0.009634	0.145807
19	1	0	4.886299	-2.281534	-0.152195
20	1	0	5.820252	0.019927	-0.452809
21	1	0	1.810215	1.365305	-1.085222
22	1	0	0.141994	-1.890945	0.119521
23	1	0	-0.328935	0.961234	-0.882665
24	1	0	1.967348	3.045186	2.126156
25	1	0	1.590450	3.265260	0.410105
26	1	0	3.277846	3.100191	0.916113
27	1	0	-4.460907	-0.151189	1.003730
28	1	0	-4.642301	-1.165629	-0.427501
29	1	0	-5.956555	0.665458	-1.556432
30	1	0	-5.778062	1.675052	-0.127443
31	1	0	-8.009174	0.538608	-0.129719
32	1	0	-7.040397	-0.026061	1.237601
33	1	0	-7.218845	-1.041746	-0.198365
34	1	0	2.605718	0.806964	1.674155
35	8	0	0.305782	-0.554027	2.593027
36	1	0	-0.546023	-0.863741	2.260773
37	1	0	0.515549	0.212116	2.038478
38	1	0	1.586872	-3.146068	-0.252107

Energy: -1260.231856 au

Free energy: -1259.973534 au

Optimized geometries for the chemical species in **Figure S2**.

A) Attack without wáter

Pre-reactant state (PR)

1	6	0	-3.943357	-2.284669	0.191102
2	6	0	-4.768881	-1.252141	0.002720
3	6	0	-4.236587	0.123734	-0.195132
4	6	0	-2.766487	0.304667	-0.203560
5	6	0	-1.918975	-0.726656	-0.022728
6	6	0	-2.467156	-2.105433	0.210418
7	8	0	-1.731372	-3.050748	0.414235
8	8	0	-4.987454	1.070751	-0.338458
9	6	0	-0.456789	-0.602790	-0.009110
10	6	0	0.194155	0.513699	-0.358725
11	7	0	-1.497401	3.422997	-0.224187
12	6	0	-0.709205	3.460536	1.010078
13	6	0	1.664728	0.683053	-0.274784
14	8	0	2.174892	1.782500	-0.248856
15	16	0	2.645184	-0.810726	-0.229599
16	6	0	4.278758	-0.031755	-0.027925
17	6	0	5.363915	-1.101475	0.028230
18	6	0	6.745412	-0.471093	0.191249
19	1	0	-4.290501	-3.301246	0.346395
20	1	0	-5.849652	-1.352451	-0.009189
21	1	0	-2.414164	1.325543	-0.355675
22	1	0	0.081420	-1.489973	0.317096
23	1	0	-0.333780	1.413184	-0.675788
24	1	0	-0.714300	4.428365	1.528431
25	1	0	0.326153	3.197243	0.777719
26	1	0	-1.093556	2.703148	1.700382
27	1	0	4.259680	0.567874	0.885677
28	1	0	4.431350	0.651168	-0.867347
29	1	0	5.332990	-1.702536	-0.887024
30	1	0	5.163724	-1.783956	0.861305
31	1	0	7.524113	-1.236266	0.230971
32	1	0	6.801657	0.115398	1.112958
33	1	0	6.971374	0.197174	-0.644812
34	1	0	-1.112716	4.074379	-0.901370
35	1	0	-2.450623	3.725492	-0.043190

Energy: -1183.821196 au
Free energy: -1183.592145 au

Transition state (TS1)

1	6	0	4.084353	-1.967612	0.262942
2	6	0	4.777931	-1.027440	-0.382799
3	6	0	4.153851	0.269411	-0.795699
4	6	0	2.753528	0.460715	-0.482784
5	6	0	1.980141	-0.494747	0.140119
6	6	0	2.653731	-1.768517	0.604137
7	8	0	2.048990	-2.607368	1.243384
8	8	0	4.843388	1.111972	-1.360394
9	6	0	0.604335	-0.390726	0.432044
10	6	0	-0.108672	0.747980	0.017030
11	7	0	0.358079	2.214107	0.974854
12	6	0	0.252424	3.489886	0.254832
13	6	0	-1.612044	0.758431	0.211865

14	8	0	-2.212432	1.668856	0.745006
15	16	0	-2.403488	-0.672365	-0.446153
16	6	0	-4.131364	-0.193105	-0.109523
17	6	0	-5.075918	-1.275266	-0.622451
18	6	0	-6.532034	-0.904851	-0.349033
19	1	0	4.515082	-2.910346	0.585805
20	1	0	5.827575	-1.142931	-0.637084
21	1	0	2.343147	1.413905	-0.808546
22	1	0	0.149251	-1.126483	1.082587
23	1	0	0.177862	1.180773	-0.950867
24	1	0	0.633079	4.322668	0.850028
25	1	0	-0.798563	3.653112	0.017744
26	1	0	0.830231	3.419318	-0.669438
27	1	0	-4.320570	0.767583	-0.595647
28	1	0	-4.238596	-0.046701	0.968240
29	1	0	-4.836237	-2.229209	-0.140447
30	1	0	-4.921609	-1.416789	-1.697639
31	1	0	-7.208730	-1.679693	-0.716150
32	1	0	-6.796356	0.035034	-0.842376
33	1	0	-6.709923	-0.782384	0.723295
34	1	0	-0.253727	2.194549	1.788848
35	1	0	1.316198	1.994039	1.248070

Energy: -1183.790889 au
Free energy: -1183.556182 au

Intermediate (I1)

1	6	0	4.160965	-1.842568	0.334339
2	6	0	4.835850	-0.800375	-0.160153
3	6	0	4.159740	0.443766	-0.659729
4	6	0	2.735103	0.498800	-0.529426
5	6	0	1.967298	-0.565384	-0.015949
6	6	0	2.686072	-1.813144	0.458137
7	8	0	2.080442	-2.753505	0.937385
8	8	0	4.858168	1.335443	-1.144799
9	6	0	0.604362	-0.556621	0.177633
10	6	0	-0.112210	0.708623	-0.071798
11	7	0	0.488700	1.807488	0.827622
12	6	0	0.279338	3.200249	0.366971
13	6	0	-1.594252	0.659521	0.273974
14	8	0	-2.062074	1.351395	1.155958
15	16	0	-2.525532	-0.475945	-0.689918
16	6	0	-4.171994	-0.132207	0.018692
17	6	0	-5.219557	-0.996557	-0.675400
18	6	0	-6.609118	-0.735659	-0.098062
19	1	0	4.641831	-2.752707	0.679838
20	1	0	5.918874	-0.802420	-0.250077
21	1	0	2.268526	1.349873	-1.025344
22	1	0	0.100791	-1.397549	0.633782
23	1	0	0.018450	1.102843	-1.093107
24	1	0	0.734311	3.888440	1.078718
25	1	0	-0.791960	3.382230	0.297197
26	1	0	0.756003	3.312761	-0.607157
27	1	0	-4.382595	0.932861	-0.107234
28	1	0	-4.126424	-0.340823	1.090295
29	1	0	-4.958215	-2.053614	-0.557977
30	1	0	-5.215306	-0.786069	-1.750292
31	1	0	-7.360130	-1.353736	-0.594731

32	1	0	-6.896557	0.311992	-0.225871
33	1	0	-6.638069	-0.964171	0.971153
34	1	0	0.072466	1.671765	1.752381
35	1	0	1.503755	1.583697	0.850036

Energy: -1183.794995 au
Free energy: -1183.557624 au

Intermediate (I2)

1	6	0	3.958448	-2.079732	0.215329
2	6	0	4.760258	-1.077378	-0.176249
3	6	0	4.238177	0.261447	-0.426687
4	6	0	2.928760	0.553235	-0.290637
5	6	0	1.988115	-0.480728	0.109296
6	6	0	2.510856	-1.865965	0.409873
7	8	0	1.785941	-2.770211	0.788331
8	8	0	5.196704	1.148591	-0.795420
9	6	0	0.652311	-0.324031	0.198030
10	6	0	-0.113796	0.950460	-0.082528
11	7	0	0.456381	2.102903	0.588999
12	6	0	-0.195808	3.356516	0.222246
13	6	0	-1.584733	0.723095	0.322391
14	8	0	-2.021721	1.100185	1.381690
15	16	0	-2.560701	-0.164797	-0.870828
16	6	0	-4.108205	-0.286894	0.085044
17	6	0	-5.180071	-0.995518	-0.735654
18	6	0	-6.485473	-1.105393	0.049469
19	1	0	4.328935	-3.079108	0.414124
20	1	0	5.827937	-1.217811	-0.316659
21	1	0	2.551024	1.558995	-0.441895
22	1	0	0.083036	-1.208482	0.477111
23	1	0	-0.091203	1.144216	-1.166904
24	1	0	0.267163	4.173266	0.779372
25	1	0	-1.276831	3.372554	0.416335
26	1	0	-0.032451	3.543353	-0.844004
27	1	0	-4.413410	0.726887	0.356064
28	1	0	-3.890460	-0.826422	1.010290
29	1	0	-4.827058	-1.993874	-1.015539
30	1	0	-5.349459	-0.446460	-1.668288
31	1	0	-7.254325	-1.613331	-0.537173
32	1	0	-6.865133	-0.115290	0.318125
33	1	0	-6.339121	-1.670402	0.974567
34	1	0	0.366221	1.952005	1.591289
35	1	0	4.791248	2.013125	-0.932920

Energy: -1183.834147 au
Free energy: -1183.600620 au

B) Attack assisted by a water molecule

Pre-reactant state (PRw)

1	6	0	3.391720	-2.907068	-0.095120
2	6	0	4.331218	-1.972164	0.058286
3	6	0	3.964960	-0.535423	0.153150
4	6	0	2.535313	-0.163458	0.124999
5	6	0	1.570199	-1.096678	-0.012265
6	6	0	1.952052	-2.544796	-0.169636
7	8	0	1.111420	-3.400658	-0.356330
8	8	0	4.848883	0.305141	0.243905

9	6	0	0.130779	-0.821220	-0.047636
10	6	0	-0.408227	0.350742	0.310602
11	7	0	1.226540	3.088371	0.546549
12	6	0	0.768632	3.296271	-0.825454
13	6	0	-1.859969	0.648368	0.213056
14	8	0	-2.271123	1.785885	0.149354
15	16	0	-2.968989	-0.755378	0.215996
16	6	0	-4.527209	0.156915	-0.017931
17	6	0	-5.705800	-0.810455	-0.013360
18	6	0	-7.025486	-0.066085	-0.205271
19	1	0	3.613941	-3.965621	-0.183455
20	1	0	5.392067	-2.195577	0.107509
21	1	0	2.308293	0.899188	0.224268
22	1	0	-0.489524	-1.648092	-0.385543
23	1	0	0.197186	1.198087	0.647250
24	1	0	0.911409	4.316139	-1.209121
25	1	0	-0.294362	3.047804	-0.895286
26	1	0	1.314163	2.613418	-1.486100
27	1	0	-4.463060	0.705375	-0.961243
28	1	0	-4.608219	0.892837	0.785931
29	1	0	-5.722044	-1.362408	0.932682
30	1	0	-5.575767	-1.551705	-0.809346
31	1	0	-7.870938	-0.757960	-0.201344
32	1	0	-7.035638	0.473355	-1.156890
33	1	0	-7.182777	0.663454	0.594496
34	1	0	0.723835	3.705955	1.175957
35	1	0	2.218781	3.311338	0.598706
36	8	0	4.071224	3.016822	-0.150375
37	1	0	4.451100	2.160368	0.101021
38	1	0	4.237268	3.094290	-1.094220

Energy: -1260.224535 au
Free energy: -1259.973903 au

Transition state (TS1w)

1	6	0	3.687016	-2.517113	0.218509
2	6	0	4.486692	-1.580861	-0.298412
3	6	0	3.961115	-0.227281	-0.640541
4	6	0	2.537939	-0.017983	-0.573829
5	6	0	1.681706	-0.926159	-0.004291
6	6	0	2.242404	-2.251141	0.455609
7	8	0	1.545470	-3.080255	1.005493
8	8	0	4.755425	0.680256	-0.912898
9	6	0	0.312427	-0.686606	0.258744
10	6	0	-0.286161	0.493625	-0.166765
11	7	0	0.404189	2.091145	0.770899
12	6	0	0.273253	3.306392	-0.033720
13	6	0	-1.763221	0.682648	0.095514
14	8	0	-2.254892	1.675035	0.585386
15	16	0	-2.734569	-0.703731	-0.430291
16	6	0	-4.382723	-0.036971	-0.026732
17	6	0	-5.459289	-1.044916	-0.415675
18	6	0	-6.852534	-0.512566	-0.087298
19	1	0	4.037187	-3.500342	0.516363
20	1	0	5.550788	-1.732491	-0.452490
21	1	0	2.195808	0.965558	-0.877010
22	1	0	-0.207865	-1.365859	0.923582
23	1	0	0.041883	0.934500	-1.112080

24	1	0	0.695120	4.179293	0.471813
25	1	0	-0.783192	3.473119	-0.244896
26	1	0	0.824985	3.167129	-0.966204
27	1	0	-4.507581	0.908912	-0.560412
28	1	0	-4.407663	0.181837	1.043846
29	1	0	-5.285895	-1.988586	0.112794
30	1	0	-5.385856	-1.264480	-1.486334
31	1	0	-7.623853	-1.234338	-0.365542
32	1	0	-7.052382	0.418823	-0.625101
33	1	0	-6.951394	-0.309228	0.982965
34	1	0	-0.152338	2.140750	1.619302
35	1	0	1.391660	1.925191	0.982986
36	8	0	3.238599	2.730005	0.354350
37	1	0	3.852553	2.173138	-0.160870
38	1	0	3.783260	3.422009	0.740329

Energy: -1260.202359 au
Free energy: -1259.946712 au

Intermediate (I1w)

1	6	0	3.806883	-2.320508	0.336604
2	6	0	4.540337	-1.302763	-0.129877
3	6	0	3.924764	-0.034766	-0.628468
4	6	0	2.527263	-0.007535	-0.759371
5	6	0	1.697287	-0.957890	-0.128287
6	6	0	2.328901	-2.231849	0.394097
7	8	0	1.659940	-3.133581	0.863278
8	8	0	4.673718	0.948473	-0.853034
9	6	0	0.371096	-0.774127	0.152998
10	6	0	-0.254906	0.534422	-0.170691
11	7	0	0.415957	1.681507	0.587125
12	6	0	0.134313	3.029338	0.028155
13	6	0	-1.726018	0.577616	0.232931
14	8	0	-2.110231	1.267670	1.154150
15	16	0	-2.775254	-0.467577	-0.716282
16	6	0	-4.357278	-0.062673	0.098240
17	6	0	-5.491026	-0.835169	-0.568037
18	6	0	-6.828720	-0.518770	0.097441
19	1	0	4.240126	-3.245114	0.704932
20	1	0	5.626349	-1.336017	-0.152743
21	1	0	2.108083	0.888313	-1.210060
22	1	0	-0.193868	-1.532406	0.679435
23	1	0	-0.155296	0.810132	-1.230893
24	1	0	0.637822	3.772042	0.642554
25	1	0	-0.942742	3.187661	0.017049
26	1	0	0.555032	3.070043	-0.976059
27	1	0	-4.510325	1.017108	0.026646
28	1	0	-4.265265	-0.319086	1.156410
29	1	0	-5.287660	-1.909553	-0.506775
30	1	0	-5.532958	-0.580049	-1.632402
31	1	0	-7.641560	-1.071641	-0.378344
32	1	0	-7.059561	0.548058	0.026066
33	1	0	-6.812364	-0.788951	1.157156
34	1	0	0.061772	1.626929	1.546253
35	1	0	1.453612	1.568735	0.591161
36	8	0	3.020428	2.695475	0.368884
37	1	0	3.656074	2.185688	-0.200506
38	1	0	3.566898	3.022545	1.090133

Energy: -1260.215285 au
Free energy: -1259.954422 au

Intermediate (I2w)

1	6	0	-3.710191	-2.420200	-0.324722
2	6	0	-4.475283	-1.419515	0.144018
3	6	0	-3.894498	-0.161333	0.610248
4	6	0	-2.555645	0.009160	0.643665
5	6	0	-1.676419	-0.954934	0.020929
6	6	0	-2.241751	-2.280481	-0.419650
7	8	0	-1.538172	-3.174733	-0.856109
8	8	0	-4.778039	0.786457	0.977397
9	6	0	-0.395757	-0.678352	-0.288703
10	6	0	0.233246	0.671801	-0.011815
11	7	0	-0.431518	1.749705	-0.750013
12	6	0	0.071705	3.072349	-0.362009
13	6	0	1.724755	0.592953	-0.374673
14	8	0	2.145337	0.989719	-1.434954
15	16	0	2.748843	-0.174601	0.854228
16	6	0	4.326184	-0.158067	-0.060380
17	6	0	5.432924	-0.770767	0.790836
18	6	0	6.764019	-0.764403	0.042035
19	1	0	-4.126392	-3.365351	-0.655485
20	1	0	-5.556552	-1.505863	0.201039
21	1	0	-2.139035	0.922533	1.049033
22	1	0	0.188857	-1.444997	-0.791389
23	1	0	0.150975	0.903237	1.060136
24	1	0	-0.398587	3.829658	-0.992752
25	1	0	1.160181	3.171787	-0.451630
26	1	0	-0.225434	3.272454	0.671532
27	1	0	4.549126	0.878725	-0.324426
28	1	0	4.180993	-0.714102	-0.990017
29	1	0	5.161193	-1.796609	1.061785
30	1	0	5.527830	-0.210271	1.727205
31	1	0	7.558039	-1.202592	0.650876
32	1	0	7.062127	0.255480	-0.217935
33	1	0	6.693834	-1.339876	-0.885517
34	1	0	-0.226476	1.596100	-1.736518
35	8	0	-3.081158	2.835118	0.046914
36	1	0	-3.445235	3.404077	-0.638042
37	1	0	-4.318489	1.646396	0.894161
38	1	0	-2.361306	2.323133	-0.374281

Energy: -1260.244187 au
Free energy: -1259.984370 au

Optimized geometries for the chemical species in **Figure S3**.

Transition state for the water-mediated addition to the vinyl position of the *o*-quinone species

1	6	0	3.820304	-1.559883	0.551856
2	6	0	4.420854	-0.867393	-0.694824
3	6	0	3.514069	-0.009852	-1.495548
4	6	0	2.231889	0.153647	-1.142521
5	6	0	1.633973	-0.486303	0.054895
6	6	0	2.429789	-1.287181	0.844740
7	6	0	0.269253	-0.233149	0.371661
8	6	0	-0.515205	0.761047	-0.213460
9	7	0	-0.192516	2.500239	0.510222
10	6	0	-0.355986	3.581203	-0.462167
11	6	0	-2.018494	0.710064	0.006115
12	8	0	-2.704099	1.672050	0.273581
13	16	0	-2.684404	-0.909366	-0.237711
14	6	0	-4.447521	-0.504194	-0.005536
15	6	0	-5.295415	-1.758431	-0.191067
16	6	0	-6.778766	-1.449193	-0.000412
17	1	0	1.593642	0.772145	-1.766661
18	1	0	-0.127908	-0.736892	1.248623
19	1	0	-0.285497	1.093121	-1.227629
20	1	0	-0.083464	4.555674	-0.047430
21	1	0	-1.396627	3.601634	-0.787610
22	1	0	0.286697	3.381178	-1.324092
23	1	0	-4.716431	0.271965	-0.726898
24	1	0	-4.570165	-0.082566	0.995388
25	1	0	-4.978538	-2.524429	0.524974
26	1	0	-5.125084	-2.171236	-1.191306
27	1	0	-7.386294	-2.347104	-0.133504
28	1	0	-7.118821	-0.701761	-0.723112
29	1	0	-6.971136	-1.057557	1.002654
30	1	0	-0.847533	2.602945	1.281638
31	1	0	0.771834	2.427287	0.862496
32	8	0	2.576049	2.077411	1.357236
33	8	0	4.546165	-2.268087	1.227567
34	1	0	3.938101	0.453409	-2.381441
35	1	0	3.238703	2.155036	0.661508
36	1	0	2.015399	-1.775406	1.721737
37	8	0	5.583239	-1.019983	-0.994719
38	1	0	2.597889	1.137326	1.590784

Energy: -1260.183951 au
Free energy: -1259.928630 au

Transition state for the water-mediated addition to the *o*-quinone ring

1	6	0	3.768235	-1.360770	-0.021503
2	6	0	4.216066	-0.119962	-0.834623
3	6	0	3.177952	0.661993	-1.374350
4	6	0	1.854505	0.540503	-0.864242
5	6	0	1.396570	-0.798663	-0.347803
6	6	0	2.321206	-1.700386	0.023327
7	6	0	-0.022566	-1.066011	-0.137493
8	6	0	-1.031646	-0.202615	-0.333374
9	7	0	1.717277	1.468252	0.626965
10	6	0	1.872162	2.906818	0.374464
11	6	0	-2.422890	-0.627028	-0.032901
12	8	0	-2.720454	-1.717778	0.396595

13	16	0	-3.631365	0.641356	-0.360116
14	6	0	-5.124164	-0.277246	0.133516
15	6	0	-6.365187	0.586423	-0.062920
16	6	0	-7.626575	-0.169641	0.349056
17	1	0	1.088131	1.057355	-1.445478
18	1	0	-0.284223	-2.053903	0.239059
19	1	0	-0.888472	0.803649	-0.719623
20	1	0	1.983734	3.457559	1.309329
21	1	0	1.003619	3.276854	-0.174007
22	1	0	2.771238	3.032983	-0.231678
23	1	0	-5.003024	-0.576917	1.177621
24	1	0	-5.167874	-1.188123	-0.468832
25	1	0	-6.437414	0.890212	-1.112803
26	1	0	-6.270573	1.504661	0.526916
27	1	0	-8.516772	0.447474	0.208275
28	1	0	-7.581509	-0.461485	1.402270
29	1	0	-7.749256	-1.079325	-0.245737
30	1	0	0.854014	1.261329	1.133035
31	1	0	2.534202	1.134228	1.171049
32	8	0	4.561824	-2.013536	0.623737
33	1	0	3.440400	1.516103	-1.987347
34	1	0	2.051195	-2.663931	0.448966
35	8	0	5.433079	0.095919	-0.880827
36	8	0	4.315069	0.877544	1.810747
37	1	0	4.519739	0.044244	2.252593
38	1	0	4.935585	0.879891	1.060549

Energy: -1260.192348 au

Free energy: -1259.934872 au

Bibliography

(1) Simoni, E.; Serafini, M. M.; Caporaso, R.; Marchetti, C.; Racchi, M.; Minarini, A.; Bartolini, M.; Lanni, C.; Rosini, M. Targeting the Nrf2/Amyloid-Beta Liaison in Alzheimer's Disease: A Rational Approach. *ACS Chem Neurosci* **2017**, *8* (7), 1618-1627. DOI: 10.1021/acschemneuro.7b00100.
(2) Mobbili, G.; Crucianelli, E.; Barbon, A.; Marcaccio, M.; Pisani, M.; Dalzini, A.; Ussano, E.; Bortolus, M.; Stipa, P.; Astolfi, P. Liponitroxides: EPR study and their efficacy as antioxidants in lipid membranes. *RSC Adv.* **2015**, *5*, 98955-98966.
(3) Bartolini, M.; Naldi, M.; Fiori, J.; Valle, F.; Biscarini, F.; Nicolau, D. V.; Andrisano, V. Kinetic characterization of amyloid-beta 1-42 aggregation with a multimethodological approach. *Anal Biochem* **2011**, *414* (2), 215-225. DOI: 10.1016/j.ab.2011.03.020. Bartolini, M.; Bertucci, C.; Bolognesi, M. L.; Cavalli, A.; Melchiorre, C.; Andrisano, V. Insight into the kinetic of amyloid beta (1-42) peptide self-aggregation: elucidation of inhibitors' mechanism of action. *Chembiochem* **2007**, *8* (17), 2152-2161. DOI: 10.1002/cbic.200700427.
(4) Naiki, H.; Higuchi, K.; Hosokawa, M.; Takeda, T. Fluorometric determination of amyloid fibrils in vitro using the fluorescent dye, thioflavin T1. *Anal Biochem* **1989**, *177* (2), 244-249. DOI: 10.1016/0003-2697(89)90046-8.
(5) Zhao, Y.; Truhlar, D. G. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theor. Chem. Acc.* **2008**, *120*, 215-241.
(6) Curutchet, C.; Orozco, M.; Luque, F. J. Solvation in octanol: parametrization of the continuum MST model. *J. Comput. Chem.* **2001**, *22*, 1180-1193. Soteras, I.; Curutchet, C.; Bidon-Chanal, A.; Orozco, M.; Luque, F. J. Extension of the MST model to the IEF formalism: HF and B3LYP parametrizations. *J. Mol. Struct.* **2005**, *727*, 29-40.
(7) *Gaussian 16*; Gaussian Inc.: Wallington CT, 2016.
(8) Kumar, P.; Nagarajan, A.; Uchil, P. D. Analysis of Cell Viability by the MTT Assay. *Cold Spring Harb Protoc* **2018**, *2018* (6). DOI: 10.1101/pdb.prot095505.