Supporting Information

A Resorcinarene for Inhibition of Aβ Fibrillation

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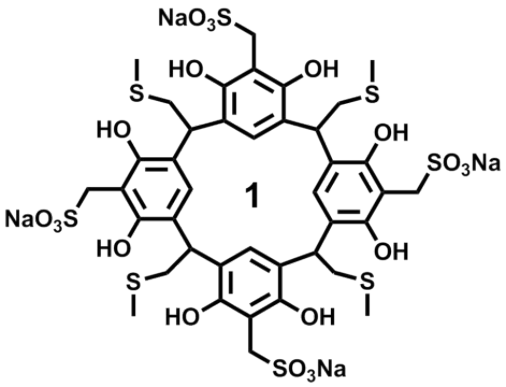
General methods. All solvents were purchased from commercial suppliers and used without further purification. Amyloid β-protein 40 (purity 95.77%) and 42 (purity 95.19%) were purchased from ChinaPeptides. All other reagents and starting materials were purchased from Aldrich or VWR and used without further purification unless otherwise noted. Solutions were prepared from nanopure water purified from Milli-Q plus system (Millipore Co.), with a resistivity over 18 MΩ cm^{-1}. Compound **1** were prepared according to our previously published literature procedures.[1] Deuterated solvents were purchased from Cambridge Isotope Laboratories Inc. and used as received. ThT fluorescence was measured by a fluorescence spectrophotometer (LS-55, Perkin Elmer) at 25 °C with a slit width of 5 nm for both excitation and emission. Far-UV CD spectroscopy was measured on JASCO J-810. Atomic force microscopy was used by tapping mode with cantilever having a resonance frequency of ~170 kHz and typical force constant of 7.5 N/m.



Scheme S1. The structure of resorcinarene

Table S1. The comparison of different inhibitor towards blocking Amyloid β-protein aggregation

	Molecule	Aβ 40[a]	Lag Time	$t_{1/2}$[b]	Aβ 42[a]	Lag Time	$t_{1/2}$[b]	Cytotoxicity[c]
Supramolecule	**1**	0.1	~240	~480	0.1	~360	~1200	Yes
	Cucurbit[7]uril[2]	10	~120	~150	100	< 60	~100	Yes
	CLR01[3]	-	-	-	1	-	-	-
Peptide	LK7[4]	-	-	-	0.5	< 60	360	Yes
	NV[5]	-	-	-	1	-	-	Yes
Small molecule	DC-AB1[6]	-	-	-	200	-	-	-
	Catechol derivative[7]	-	-	-	1	-	-	-
	Tanshinones[8]	-	-	-	1	-	-	-
Metal Chelator	Aromatic co-ligands[9]	0.1	-	-	-	-	-	-
	HL3 and HL4[10]	6	-	-	-	-	-	-

[a] The smallest reported effective molar ratio of inhibitor to Amyloid β-protein.

[b] The time (min) required to reach half the maximum fluorescence intensity at the reported ratio of inhibitor to Amyloid β-protein.

[c] Whether the inhibitor can reduce Amyloid β-protein cytotoxicity.

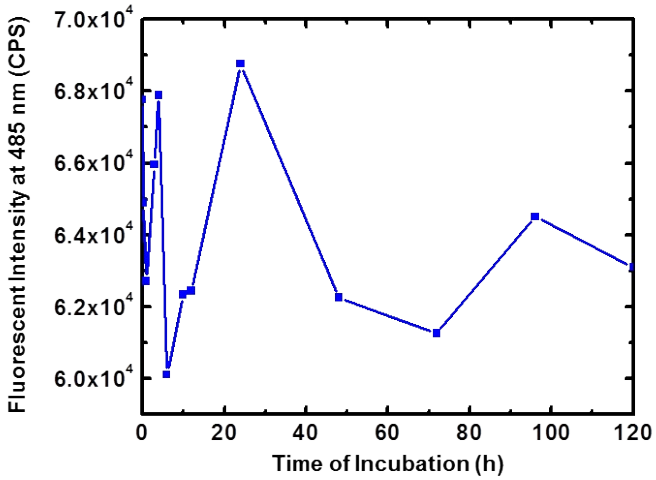


Figure S1. Fluorescence spectra of 0.15 mM **1** alone as a function of incubation time at 37°C in 25 mM PBS, pH 7.4. The emission was monitored at 480 nm with the excitation at 440 nm.

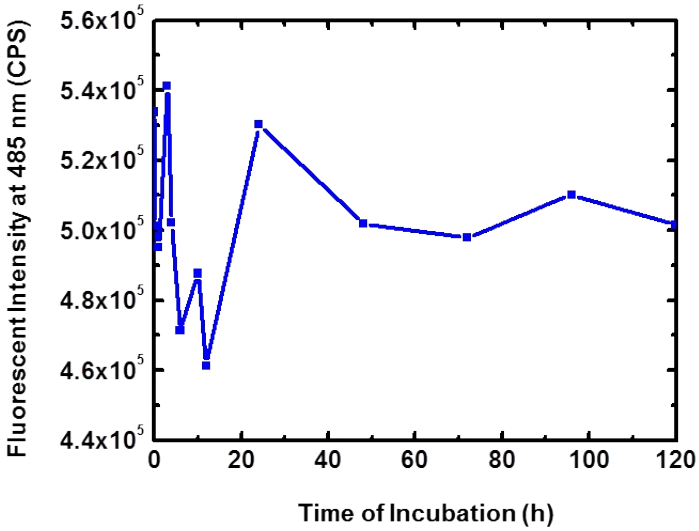


Figure S2 Fluorescence spectra of 0.15 mM **1** with 20 μM Thioflavin T (ThT) as a function of incubation time at 37°C in 25 mM pH = 7.4 PBS. The emission was monitored at 480 nm with the excitation at 440 nm.

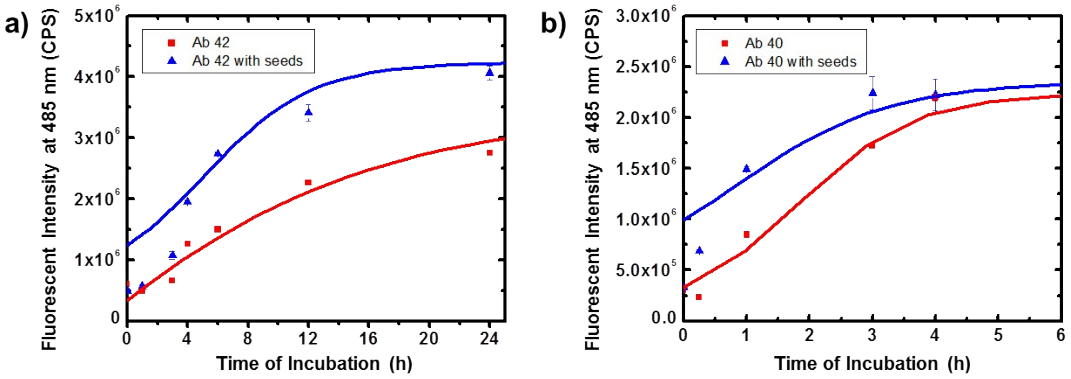


Figure S3. Normalized ThT fluorescence intensity of incubated a) Aβ 42 and b) Aβ 40 at 485 nm against incubation time: no seeds added (red), preformed seeds added (blue). Preformed seeds were collected after different hours incubation (2 h for Aβ 42 and 0.17 h for Aβ 40) of freshly made 10 μM Aβ 42 and Aβ 40 solution. After gel filtration by running through Phenogel™ 5 μm 10E4A column, the collected solution was lyophilized. Then the resulting products were added to Aβ 42 and Aβ 40 to prepare 0.3 μM seeds solution.

SDS Polyacrylamide Gel Electrophoresis After incubating 48 h, SDS-PAGE of Aβ - resorcinarene complex, including pure Aβ 40 and 42, Aβ 40 and 42 with 10 μM resorcinarene, and pure Aβ 40 and 42 mixed with 10 μM resorcinarene was performed. The component of 18 % SDS-PAGE gel contains: 1) 5 mL 18 % resolving gel which is made up of 0.6 ml H_2O, 3 ml 30% acrylamide mix, 1.3 ml 1.5 M Tris-HCl (PH 8.8), 50 μl 10% SDS, 50 μl 10% ammonium persulfate, 4 μl TEMED, and 2) 1 ml 5% stacking gel which consist of 0.68 ml H2O, 0.17 ml 30% acrylamide mix, 0.13 ml 1.0 M Tris-HCl (PH 6.8), 10 μl 10% SDS, 10 μl 10% ammonium persulfate, 1 μl TEMED. Each protein sample was prepared by dissolving 100 ng of pure protein in Laemmli sample buffer (Laemmli 1970) and boiling for 5 minutes before running on an 18% SDS-PAGE gel. After electrophoresis, the gel was fixed in fixing solution (30% ethanol, 10% acetic acid) for 45 minutes and then processed for silver staining using Pierce® Silver Stain for Mass Spectrometry kit (Thermo Fisher Scientific, Waltham, MA). Picture of gel was taken by using Epson Perfection V370 Photo scanner.

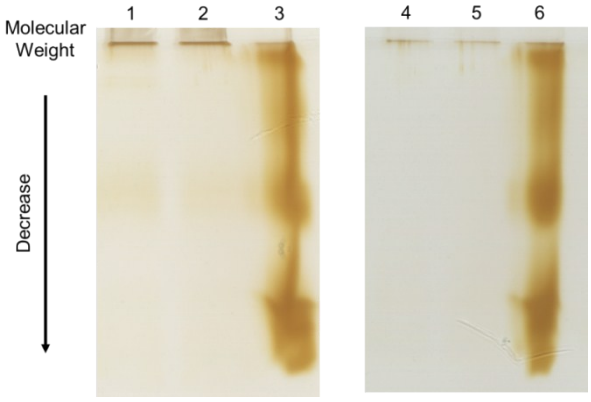


Figure S4. SDS-PAGE of Aβ and Aβ - resorcinarene complex. Each number represents 1) Aβ 42 at incubation time 48 h, 2) solution of 1) mixed with 10 μM resorcinarene, 3) Aβ 42 - 10 μM resorcinarene complex at incubation time 0, 4) Aβ 40 at incubation time 48 h, 5) solution of 4) mixed with 10 μM resorcinarene, 6) Aβ 40 - 10 μM resorcinarene complex at incubation time 0.

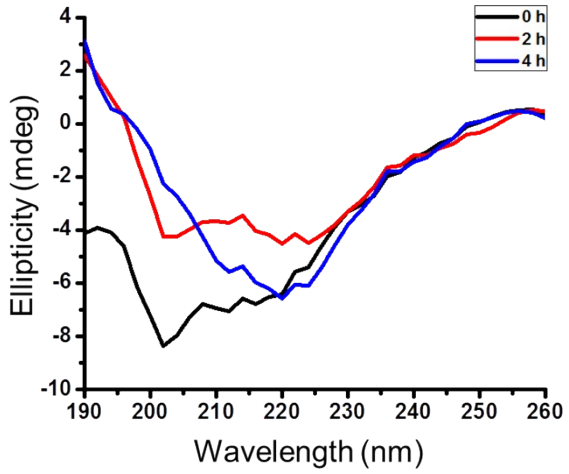


Figure S5. Far-UV circular dichroism spectra of 15 μM Aβ 40 alone in 25 mM pH = 7.4 PBS at 0, 2, 4 h.

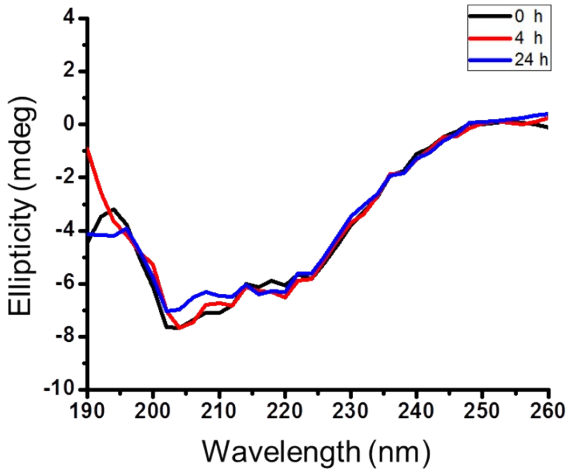


Figure S6. Far-UV circular dichroism spectra of 15 μM Aβ 40 incubated with arenes at the ratio of Aβ 40 to arenes 1:1, in 25 mM pH = 7.4 PBS at 0, 4, 24 h.

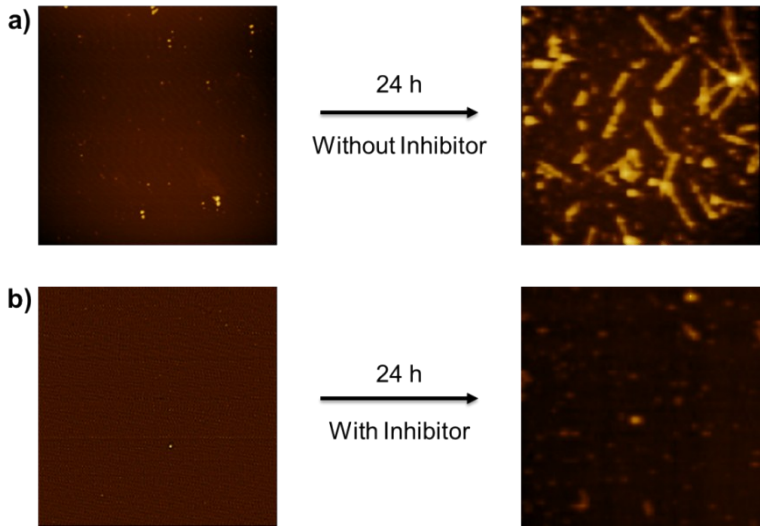


Figure S7. AFM images (size: 2.5 × 2.5 μm) of Aβ 40 incubated at 37°C in 25 mM PBS, pH 7.4 with the ration of Aβ 40 to the arenes at a) 1:0, and b) 1:1, respectively.

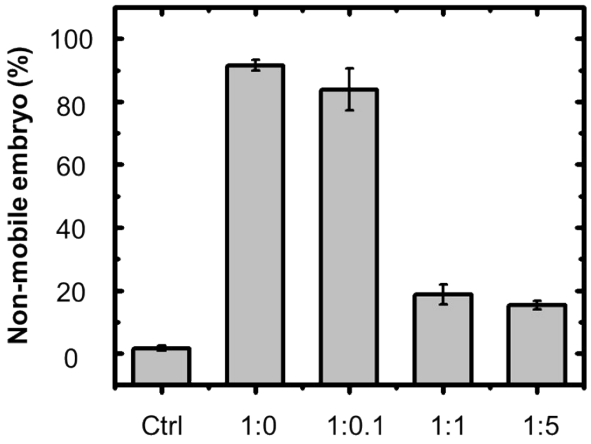


Figure S8. Percentage of non-mobile embryos at different molar ratios of Aβ 42 to resorcinarene.

Computational Details

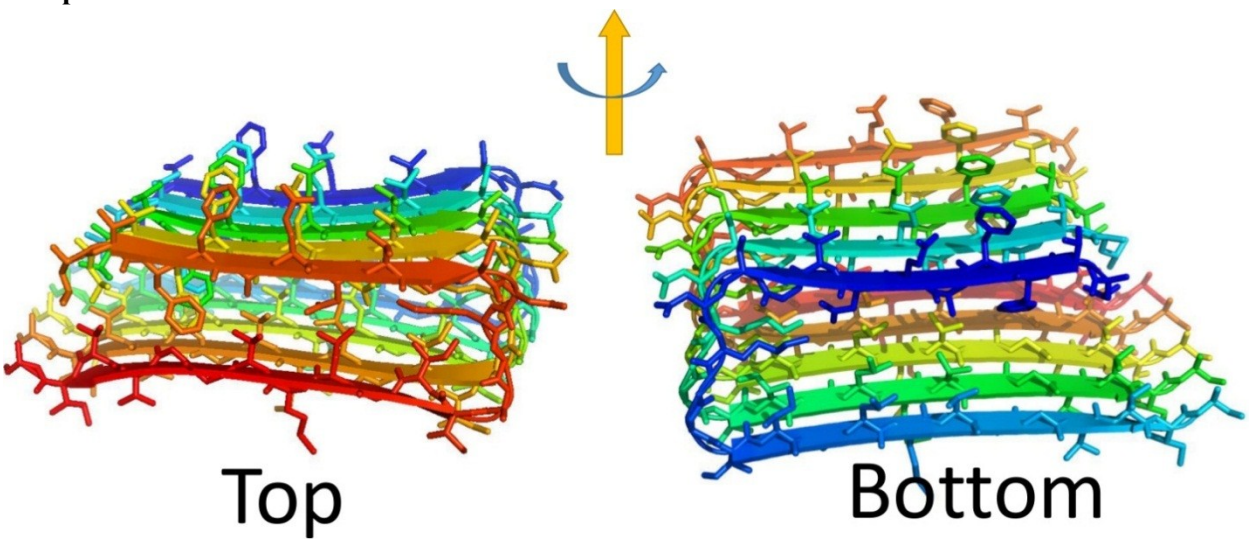


Figure S8. The top and the bottom of the Aβ42 fibril (PDB Code: 2BEG).

Details of the PELE simulations

Detailed molecular structure of the amyloid filament (PDB Code: 2BEG) was retrieved from Protein Data Bank (PDB). The amyloid fibril structure consists of five chains of Aβ protein, spanning from Leu17 to Ala42. Using the amyloid fibril and a resorcinarene, 40 PELE simulations were repeated to produce > 24,000 ligand bound conformations.

Once the PELE simulations were finished, we analyzed the conformations of the Aβ42 fibril bound to resorcinarene. First, we analyzed the center-of-mass (CM) position of the bound resorcinarene on top or the bottom of the filament. We considered 1 Å^3 bins near the ends of the fibril. For each bin, the total number of occurrences that the CM of resorcinarene were bound was counted. The most populated spatial bins on the top and the bottom of the fibril were chosen and the corresponding conformations were used as the initial configurations of the molecular dynamics (MD) simulations.

Analysis of solvent accessible surface area (SASA) and trans-rotational entropy

We used VMD software for the computations of solvent accessible areas of the resorcinarene bound complex ($^{SASA_{cmpl}}$), the amyloid fibril ($^{SASA_{prot}}$) and the ligand ($^{SASA_{lig}}$). The buried solvent accessible surface area is defined:

$\Delta SASA = SASA_{cmpl} - (SASA_{prot} + SASA_{lig})$.

From the MD trajectories of the complex, we separated the amyloid fibril and the ligand to compute corresponding SASA values. The probe radius of the SASA value was 1.6 Å.

The translational and the rotational entropies are defined[11]:

$$S_{trans} = \frac{k_B}{2}\left[5 + 3\log\left(\frac{2\pi m}{\beta h^2}\right) - 2log(\rho)\right]$$

$$S_{rotation} = \frac{k_B}{2}\left[3 + \log\left(\pi I_A I_B I_C\right) + 3\log\left(\frac{8\pi^2}{\beta h^2}\right) - 2log(\sigma)\right]$$

, where m is the mass of molecule, T is temperature, $\beta = k_B T$ is the Boltzmann constant, h is Planck constant, ρ is the number density (in units of mole), σ is the symmetry factor of the molecule, and $I_{A,B,C}$ are three rotational moments of inertia. The rotational moments of inertia were averaged over the last 20 ns of MD simulations, using VMD software. The entropic contribution of free energy (-TS) is computed at 300 K.

Table S2. Optimized coordinates and atomic partial charges of resorcinarene that are used for MD simulations

Atom	X	Y	Z	AM1-BCC Partial Charge
C1	0.305	-3.151	0.263	-0.1463
C2	1.728	-3.096	0.784	0.04915
C3	2.52	-1.954	0.181	-0.1463
H1	2.23	-4.063	0.489	0.0822
C4	-0.047	-4.1	-0.724	0.156975
C5	-0.701	-2.333	0.787	-0.0675
C6	-1.368	-4.237	-1.203	-0.10905
C7	-2.345	-3.414	-0.604	0.156975
C8	-2.038	-2.475	0.401	-0.1463
C9	-3.152	-1.693	1.056	0.04915
C10	-3.237	-0.289	0.493	-0.1463
H2	-4.135	-2.21	0.798	0.0822
H3	-0.441	-1.576	1.541	0.12275
C11	-4.197	-0.004	-0.505	0.156975
C12	-4.373	1.294	-1.03	-0.10905
C13	-2.425	0.747	0.96	-0.0675
C14	-3.557	2.31	-0.485	0.156975
C15	-2.587	2.06	0.506	-0.1463
H4	-1.654	0.531	1.713	0.12275
C16	-1.774	3.207	1.062	0.04915
C17	-0.415	3.263	0.392	-0.1463
H5	-2.314	4.175	0.8	0.0822
C18	3.532	-2.219	-0.765	0.156975
C19	4.399	-1.221	-1.259	-0.10905
C20	4.173	0.092	-0.803	0.156975
C21	3.179	0.401	0.151	-0.1463
C22	2.352	-0.63	0.606	-0.0675
H6	1.561	-0.397	1.333	0.12275
C23	3.115	1.803	0.723	0.04915
C24	1.919	2.573	0.215	-0.1463
H7	4.046	2.351	0.364	0.0822
C25	2.084	3.482	-0.851	0.156975
C26	1.047	4.332	-1.294	-0.10905

C27	0.655	2.464	0.804	-0.0675
H8	0.503	1.74	1.618	0.12275
C28	-0.203	4.176	-0.665	0.156975
O1	0.981	-4.884	-1.172	-0.5311
H9	0.664	-5.721	-1.71	0.457125
O2	-3.629	-3.596	-1.068	-0.5311
H10	-4.296	-2.989	-0.571	0.457125
O3	-4.94	-1.077	-0.912	-0.5311
H11	-5.799	-0.818	-1.447	0.457125
O4	-3.778	3.568	-0.996	-0.5311
H12	-3.167	4.267	-0.553	0.457125
C29	-1.744	-5.171	-2.296	-0.24705
S1	-1.708	-6.823	-1.75	1.4213
H13	-1.087	-5.012	-3.194	0.085825
H14	-2.743	-4.893	-2.73	0.085825
O5	-2.554	-6.996	-0.523	-0.752533
O6	-2.171	-7.747	-2.83	-0.752533
O7	-0.257	-7.118	-1.396	-0.752533
O8	4.94	1.157	-1.214	-0.5311
H15	5.513	0.941	-2.02	0.457125
O9	3.663	-3.535	-1.154	-0.5311
H16	4.313	-3.644	-1.923	0.457125
O10	3.273	3.607	-1.531	-0.5311
H17	3.982	2.951	-1.178	0.457125
O11	-1.301	4.929	-1.006	-0.5311
H18	-1.177	5.45	-1.863	0.457125
C30	-5.33	1.605	-2.123	-0.24705
S2	-6.975	1.524	-1.563	1.4213
H19	-5.154	0.929	-3.003	0.085825
H20	-5.092	2.601	-2.588	0.085825
O12	-7.919	1.946	-2.643	-0.752533
O13	-7.165	2.383	-0.347	-0.752533
O14	-7.221	0.071	-1.184	-0.752533
C31	-3.047	-1.668	2.581	0.0202
S3	-3.187	-3.321	3.316	-0.37845
H21	-3.816	-0.997	3.013	0.067575
H22	-2.068	-1.265	2.929	0.067575
C32	-4.776	-3.943	2.741	-0.0235
H23	-4.774	-4.184	1.67	0.069033
H24	-5.001	-4.871	3.288	0.069033
H25	-5.599	-3.243	2.927	0.069033
C33	5.519	-1.552	-2.175	-0.24705

S4	6.954	-1.964	-1.262	1.4213
H26	5.231	-2.366	-2.897	0.085825
H27	5.704	-0.722	-2.91	0.085825
O15	6.66	-3.173	-0.41	-0.752533
O16	8.084	-2.268	-2.198	-0.752533
O17	7.321	-0.813	-0.366	-0.752533
C34	1.74	-3.031	2.315	0.0202
S5	3.465	-3.056	2.895	-0.37845
H28	1.161	-3.866	2.754	0.067575
H29	1.267	-2.102	2.699	0.067575
C35	3.578	-4.608	3.805	-0.0235
H30	3.405	-5.483	3.168	0.069033
H31	2.876	-4.657	4.646	0.069033
H32	4.594	-4.692	4.221	0.069033
C36	1.28	5.334	-2.364	-0.24705
S6	1.811	6.859	-1.695	1.4213
H33	0.378	5.452	-3.021	0.085825
H34	2.018	4.94	-3.114	0.085825
O18	0.764	7.38	-0.745	-0.752533
O19	2.029	7.834	-2.813	-0.752533
O20	3.093	6.664	-0.927	-0.752533
C37	-1.638	3.168	2.584	0.0202
S7	-3.239	3.324	3.424	-0.37845
H35	-0.948	3.962	2.932	0.067575
H36	-1.199	2.211	2.944	0.067575
C38	-3.897	4.9	2.855	-0.0235
H37	-4.801	5.127	3.44	0.069033
H38	-4.185	4.884	1.796	0.069033
H39	-3.195	5.731	2.999	0.069033
C39	3.189	1.744	2.25	0.0202
S8	3.502	3.372	2.989	-0.37845
H40	3.971	1.027	2.573	0.067575
H41	2.241	1.376	2.703	0.067575
C40	5.13	3.833	2.37	-0.0235
H42	5.446	4.751	2.889	0.069033
H43	5.889	3.066	2.563	0.069033
H44	5.13	4.042	1.293	0.069033

References

1. Sanabria, E.; Esteso, M. A.; Perez-Redondo, A.; Vargas, E.; Maldonado, M. *Molecules* **2015**, *20*, 9915.
2. Lee, H. H.; Choi, T. S.; Lee, S. J. C.; Lee, J. W.; Park, J.; Ko, Y. H.; Kim, W. J.; Kim, K.; Kim, H. I. *Angew. Chem. Int. Ed.* **2014**, *53*, 7461.
3. Zheng, X. Y.; Liu, D.; Klarner, F. G.; Schrader, T.; Bitan, G.; Bowers, M. T. *J. Phys. Chem. B* **2015**, *119*, 4831.
4. Xiong, N.; Dong, X. Y.; Zheng, J.; Liu, F. F.; Sun, Y. *ACS Appl. Mater. Interfaces* **2015**, *7*, 5650.
5. Biswas, A.; Kurkute, P.; Saleem, S.; Jana, B.; Mohapatra, S.; Mondal, P.; Adak, A.; Ghosh, S.; Saha, A.; Bhunia, D.; Biswas, S. C.; Ghosh, S. *ACS Chem. Neurosci.* **2015**, *6*, 1309.
6. Liu, D. X.; Xu, Y. C.; Feng, Y.; Liu, H.; Shen, X.; Chen, K. X.; Ma, J. P.; Jiang, H. L. *Biochemistry* **2006**, *45*, 10963.
7. Simoni, E.; Serafini, M. M.; Bartolini, M.; Caporaso, R.; Pinto, A.; Necchi, D.; Fiori, J.; Andrisano, V.; Minarini, A.; Lanni, C.; Rosini, M. *ChemMedChem* **2016**, *11*, 1309.
8. Wang, Q. M.; Yu, X.; Patal, K.; Hu, R. D.; Chuang, S.; Zhang, G.; Zheng, J. *ACS Chem. Neurosci.* **2013**, *4*, 1004.
9. Man, B. Y. W.; Chan, H. M.; Leung, C. H.; Chan, D. S. H.; Bai, L. P.; Jiang, Z. H.; Li, H. W.; Ma, D. L. *Chem. Sci.* **2011**, *2*, 917.
10. Scott, L. E.; Telpoukhovskaia, M.; Rodriguez-Rodriguez, C.; Merkel, M.; Bowen, M. L.; Page, B. D. G.; Green, D. E.; Storr, T.; Thomas, F.; Allen, D. D.; Lockman, P. R.; Patrick, B. O.; Adam, M. J.; Orvig, C. *Chem. Sci.* **2011**, *2*, 642.
11. Simon, D. A., and J. D. McQuarrie. "Physical Chemistry: A Molecular Approach." University Science Book (1997).