SUPPLEMENTARY INFORMATION

Functional gallic acid-based dendrimers as synthetic nanotools to remodel amyloid-beta 42 into non-cytotoxic forms

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1 SYNTHESIS AND CHARACTERIZATION STUDIES

Synthesis of Ga-terminated dendrimers

Synthesis of Benzyl 3,4,5-tris(benzyloxy)benzoate: K_2CO_3 (12.04 g, 87.15 mmol), benzyl bromide (14.91 g, 87.15 mmol) and NaI (2.61 g, 17.43 mmol) were added to a stirred solution of gallic acid (3.0 g, 17.43 mmol) in MeOH (180 mL). The reaction was refluxed for 16 h, cooled down to rt and then, H_2O (30 mL) was added. After evaporation, the mixture was diluted with H_2O (50 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were dried and concentrated to give a crude product that was recrystallized from EtOH to give benzyl 3,4,5-tris(benzyloxy)benzoate as a white solid (8.19 g, 88%). ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ: 7.63-7.17 (m, 22H), 5.33 (s, 2H), 5.17 (s, 4H), 5.06 (s, 2H). ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ: 165.3, 152.3, 141.7, 137.5, 136.9, 136.4, 128.7, 128.6, 128.4, 128.2, 128.1, 127.9, 127.8, 124.8, 108.4, 74.3, 70.4, 66.2. HRMS (APCI-FIA-TOF, positive mode, *m/z*): 531.2166. Calculated for $[M+H]^+$, $C_{35}H_{31}O_5$: 531.2171. IR (neat, ATR) ν_{max}: 3091, 3033, 1713, 1590, 1498, 1109 cm^{-1}.

Synthesis of 3,4,5-Tris(benzyloxy)benzoic acid: A stirred solution of benzyl 3,4,5-tris(benzyloxy)benzoate (5.69 g, 10.7 mmol) in a 3M KOH solution in MeOH (285 mL) was refluxed overnight. The mixture was cooled down to rt and acidified (pH= 3-4) by addition of 3M HCl. The resulting precipitate was filtered, dissolved in EtOAc (80 mL) and washed with H_2O (40 mL). The aqueous phase was extracted with CH_2Cl_2 (3 x 30 mL). The combined organic phase was dried and concentrated. The crude product was purified by automated MPLC (gradient from hexane to EtOAc, silica, 40 min) to afford 3,4,5-tris(benzyloxy)benzoic acid as a white solid (4.20 g, 89%). ^{1}H NMR (300 MHz, DMSO-*d*$_6$) δ: 7.53-7.22 (m, 17H), 5.18 (s, 4H), 5.03 (s, 2H). ^{13}C NMR (75 MHz, DMSO-*d*$_6$) δ: 166.9, 152.0, 140.9, 136.9, 128.4, 128.2, 128.1, 127.9, 127.6, 126.2, 108.2, 74.2, 70.2. HRMS (APCI-FIA-TOF, positive mode, *m/z*): 441,1694. Calcd for $[M+H]^+$, $C_{28}H_{25}O_5$: 441.1702. IR (neat, ATR) ν_{max}: 3029, 1686, 1595, 1128 cm^{-1}.

Synthesis of 2[G0]-GaOBn: 3,4,5-Tris(benzyloxy)benzoic acid (334 mg, 0.76 mmol), HOBt (103 mg, 0.76 mmol) and EDC (145 mg, 0.76 mmol) were added to a solution of 2,2'-(ethylenedioxy)bis(ethylamine) (45 mg, 0.30 mmol) in DMSO (6 mL). After 20 h of stirring at rt, the reaction mixture was distributed between EtOAc (30 mL) and brine (30 mL). The aqueous phase was extracted with EtOAc (3 x 30 mL). The combined organic phase was washed

with H_2O (30 mL), dried and evaporated under vacuum. The crude product was purified by automated MPLC (gradient from hexane to 100% EtOAc and then to 100% CH_2Cl_2, neutral alumina, 25 min) to afford 2[G0]-GaOBn (255 mg, 85%) as a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.47-7.18 (m, 30H), 7.11 (s, 4H), 6.62-6.53 (m, 2H), 5.08 (s, 8H), 5.07 (s, 4H), 3.69-3.50 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.2, 152.8, 141.4, 137.5, 136.8, 129.9, 128.6, 128.2, 128.1, 128.0, 127.6, 107.1, 75.1, 71.4, 70.2, 69.8, 39.8. IR (neat, ATR) ν_{max}: 3310, 3064, 3033, 1634, 1581, 1112 cm^{-1}.

Synthesis of 2[G0]-GaOH: Pd/C (16 mg, 10%) was added to a solution of 2[G0]-GaOBn (160 mg, 0.16 mmol) in a mixture of DMF-MeOH 3:1 (4 mL). The resulting mixture was stirred under H_2 (1 atm) for 20 h. The catalyst was removed by filtration through Celite. The filtrate was concentrated and then purified by short column chromatography (MeOH-CH_2Cl_2-H_2O 3:3:0.2, silica) to give 2[G0]-GaOH as a green solid (71 mg, 98%). ^{1}H NMR (300 MHz, CD_3OD) δ: 6.85 (s, 4H), 3.67-3.57 (m, 8H), 3.50 (t, *J*=5.5 Hz, 4H). IR (KBr) ν_{max}: 3319, 1636, 1592, 1338, 1033 cm^{-1}.

Synthesis of 2[G1]-GaOBn: 3,4,5-Tris(benzyloxy)benzoic acid (466 mg, 1.06 mmol), Et_3N (0.224 mL, 1.06 mmol), HOBt (143 mg, 1.06 mmol) and EDC (210 mg, 1.06 mmol) were added to a solution of 2[G1]-NH_2·HCl (205 mg, 0.14 mmol) in DMSO (8 mL). After 24 h of stirring at rt, the reaction mixture was distributed between EtOAc (50 mL) and brine (50 mL). The aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic phase was washed with 0.1M HCl (30 mL) and H_2O (50 mL), dried and evaporated under vacuum. The crude product was purified by automated MPLC (gradient from EtOAc to 25% MeOH, neutral alumina, 25 min) to afford 2[G1]-GaOBn (428 mg, 75%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.40-7.10 (m, 102H), 6.97 (s, 4H), 5.03-4.94 (m, 36H), 4.07 (t, *J*=3.5 Hz, 4H), 3.96 (t, *J*=3.7 Hz, 8H), 3.75-3.38 (m, 72H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.2, 167.1, 152.5, 152.1, 140.9, 137.4, 136.7, 129.8, 128.4, 128.1, 127.8, 127.7, 127.4, 106.9, 75.0, 72.2, 71.1, 70.5, 70.3, 70.1, 70.0, 69.8, 69.6, 69.5, 68.6, 40.7, 39.7. IR (neat, ATR) ν_{max}: 3314, 3065, 3032, 1634, 1580, 1100 cm^{-1}.

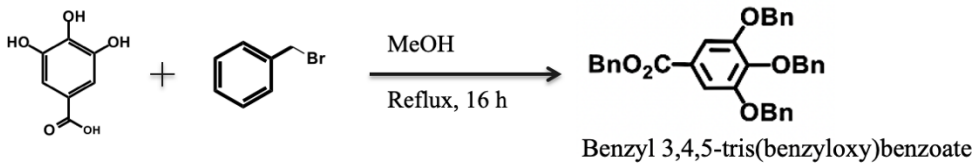
Synthesis of 2[G1]-GaOH: Pd/C (60 mg, 20%) was added to a solution of 2[G1]-GaOBn (300 mg, 0.075 mmol) in a mixture of EtOAc-MeOH 1:1 (8 mL). The resulting mixture was stirred under H_2 (1 atm) for 14 h. The catalyst was removed by filtration through Celite. The filtrate was concentrated and then purified by short column chromatography (MeOH-CH_2Cl_2-H_2O

3:3:0.2, silica) to give 2[G1]-GaOH as a green solid (133 mg, 82%). ^{1}H NMR (300 MHz, CD_3OD) δ: 7.10 (s, 4H), 6.84 (s, 12H), 4.15-4.03 (m, 12H), 3.82-3.43 (m, 72H). IR (KBr) ν_{max}: 3296, 1625, 1593, 1330, 1116, 1097 cm^{-1}.

Synthesis of 3[G1]-GaOBn: 3,4,5-Tris(benzyloxy)benzoic acid (268 mg, 0.61 mmol), Et_3N (0.128 mL, 0.61 mmol), HOBt (82 mg, 0.61 mmol) and EDC (120 mg, 0.61 mmol) were added to a solution of 3[G1]-NH_2·HCl (134 mg, 0.05 mmol) in DMSO (4.9 mL). After 36 h of stirring at rt, the reaction mixture was distributed between EtOAc (50 mL) and brine (50 mL). The aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic phase was washed with 0.1M HCl (30 mL) and H_2O (50 mL), dried and evaporated under vacuum. The crude product was purified by automated MPLC (gradient from EtOAc to 25% MeOH, neutral alumina, 25 min) to afford 3[G1]-GaOBn (413 mg, 84%) as a pale yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.37-7.08 (m, 153H), 6.89 (s, 6H), 5.91 (s, 3H), 4.93 (s, 54H), 4.04-3.81 (m, 24H), 3.67-3.33 (m, 120H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 167.2, 160.4, 152.6, 152.1, 140.9, 137.5, 136.7, 129.9, 129.8, 128.4, 128.1, 127.9, 127.5, 107.0, 106.8, 94.3, 75.1, 72.2, 71.2, 71.1, 70.5, 70.3, 70.1, 69.8, 69.5, 68.6, 67.3, 40.0. IR (neat, ATR) ν_{max}: 3319, 3069, 3034, 1635, 1581, 1108 cm^{-1}.

Synthesis of 3[G1]-GaOH: Pd/C (10 mg, 20%) was added to a solution of 3[G1]-GaOBn (49 mg, 0.008 mmol) in a mixture of DMF-MeOH 4:1 (3 mL). The resulting mixture was stirred under H_2 (1 atm) for 24 h. The catalyst was removed by filtration through Celite. The filtrate was concentrated and then purified by short column chromatography (MeOH-CH_2Cl_2-H_2O 3:3:0.2, silica) to give 3[G1]-GaOH as a green solid (26 mg, 90%). ^{1}H NMR (300 MHz, DMSO-d6) δ: 7.17 (s, 6H), 6.81 (s, 18H), 6.07 (s, 3H) 4.18-3.94 (m, 24H), 3.80-3.20 (m, 120H). IR (KBr) ν_{max}: 3334, 1629, 1597, 1336, 1109, 1097 cm^{-1}.

Characterization of Ga-terminated dendrimers



Benzyl 3,4,5-tris(benzyloxy)benzoate

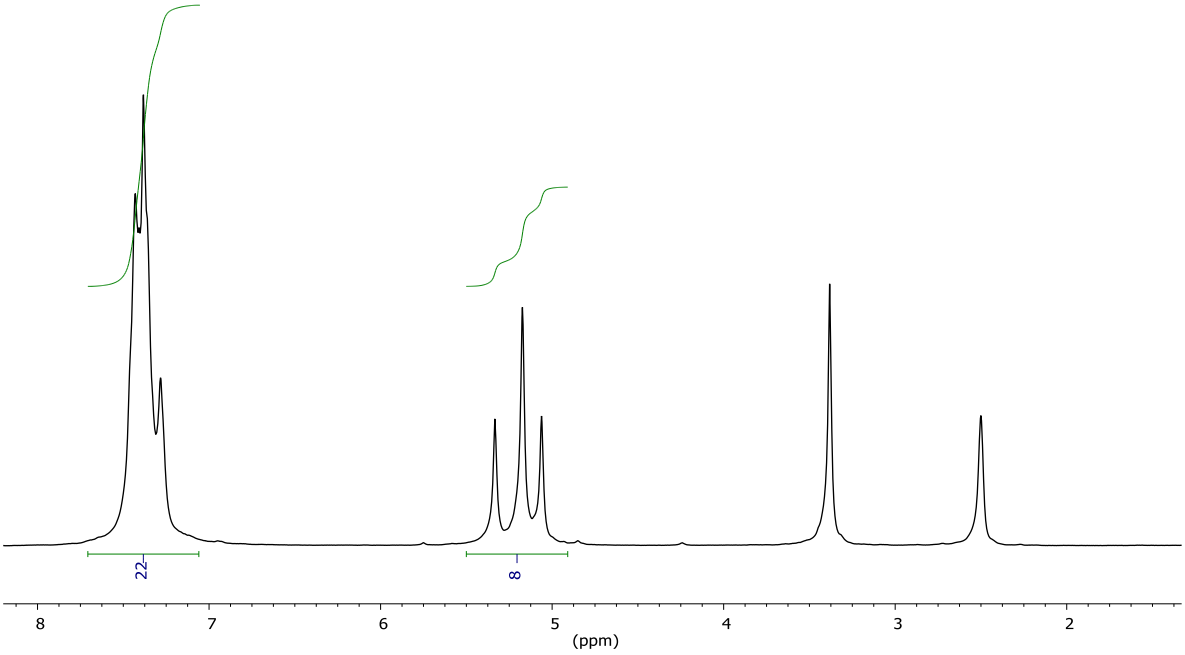


Figure S1. ^{1}H NMR spectrum (DMSO-d_6) of benzyl 3,4,5-tris(benzyloxy)benzoate**.**

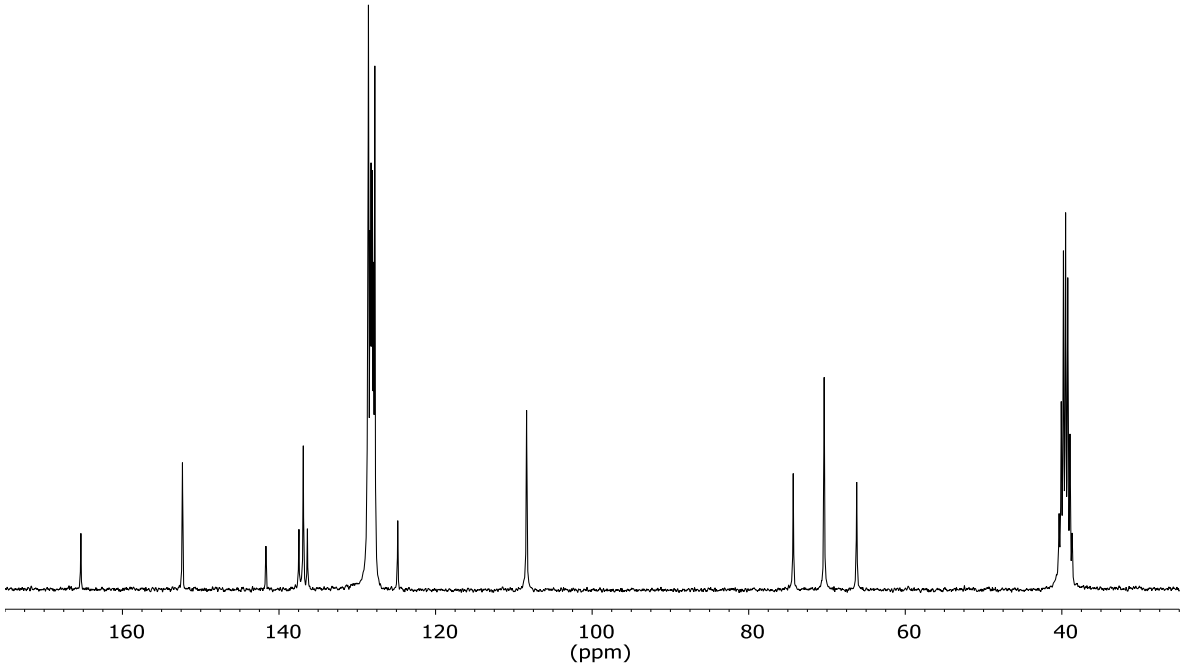


Figure S2. ^{13}C NMR spectrum (DMSO-d_6) of benzyl 3,4,5-tris(benzyloxy)benzoate.

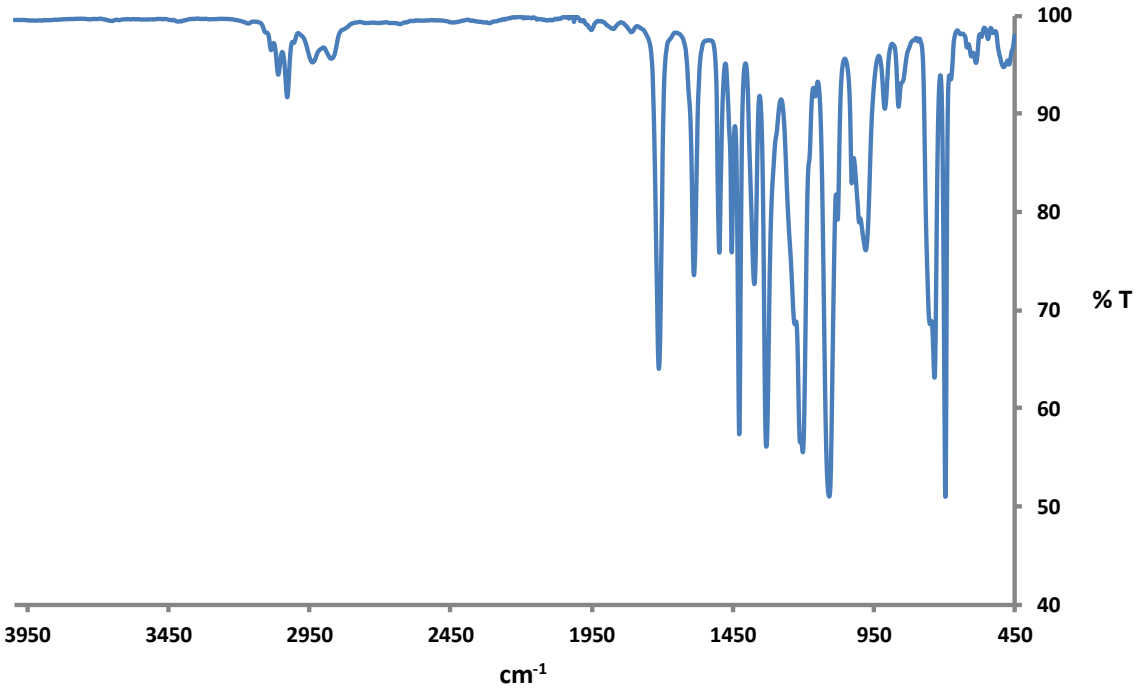


Figure S3. IR spectrum of benzyl 3,4,5-tris(benzyloxy)benzoate.

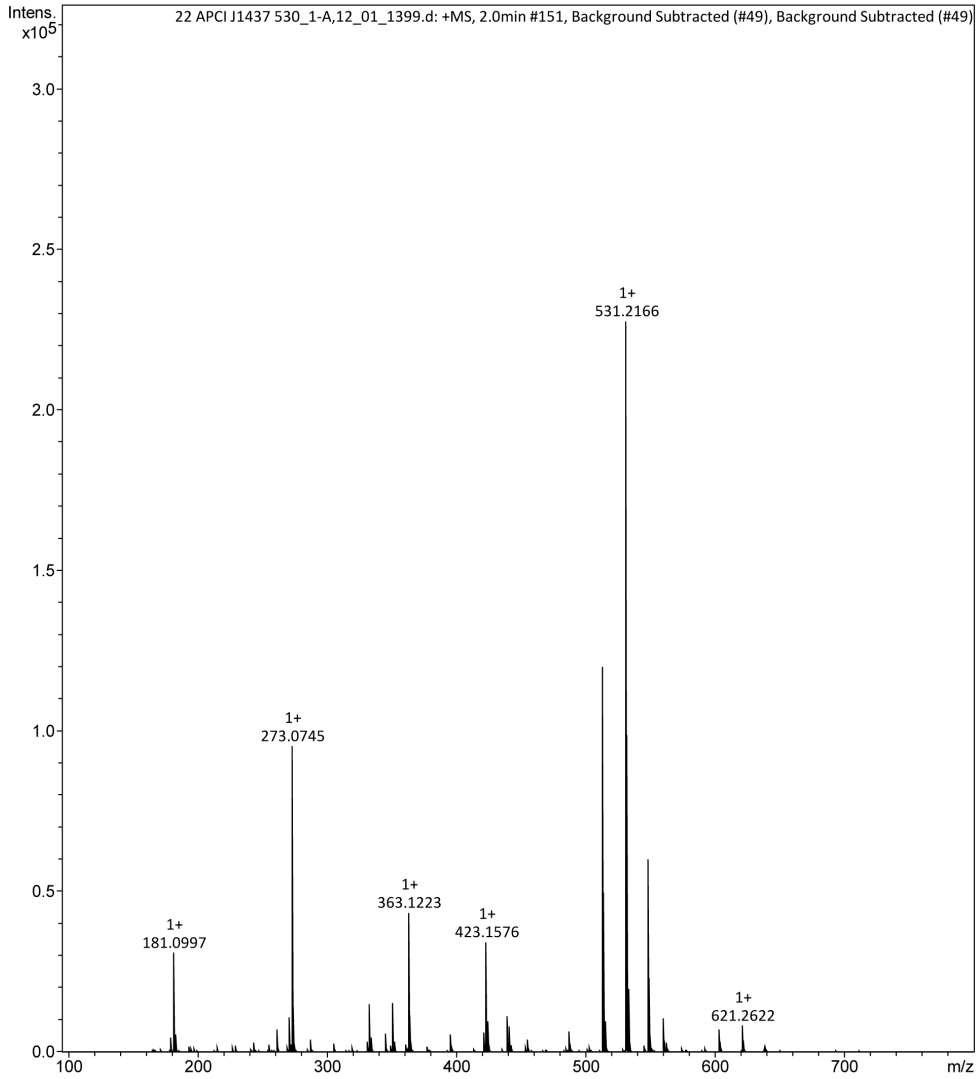
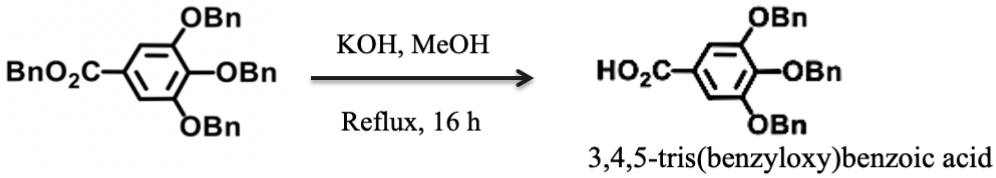


Figure S4. HR-MS of benzyl 3,4,5-tris(benzyloxy)benzoate.



3,4,5-tris(benzyloxy)benzoic acid

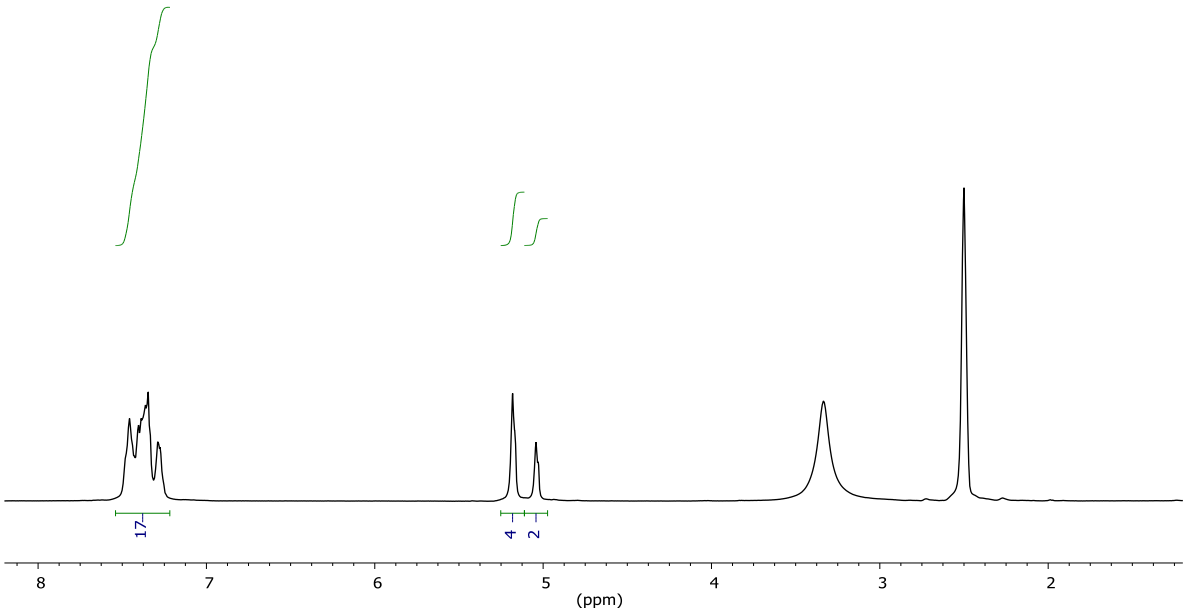


Figure S5. ^{1}H NMR spectrum (DMSO-d_6) of 3,4,5-tris(benzyloxy)benzoic acid.

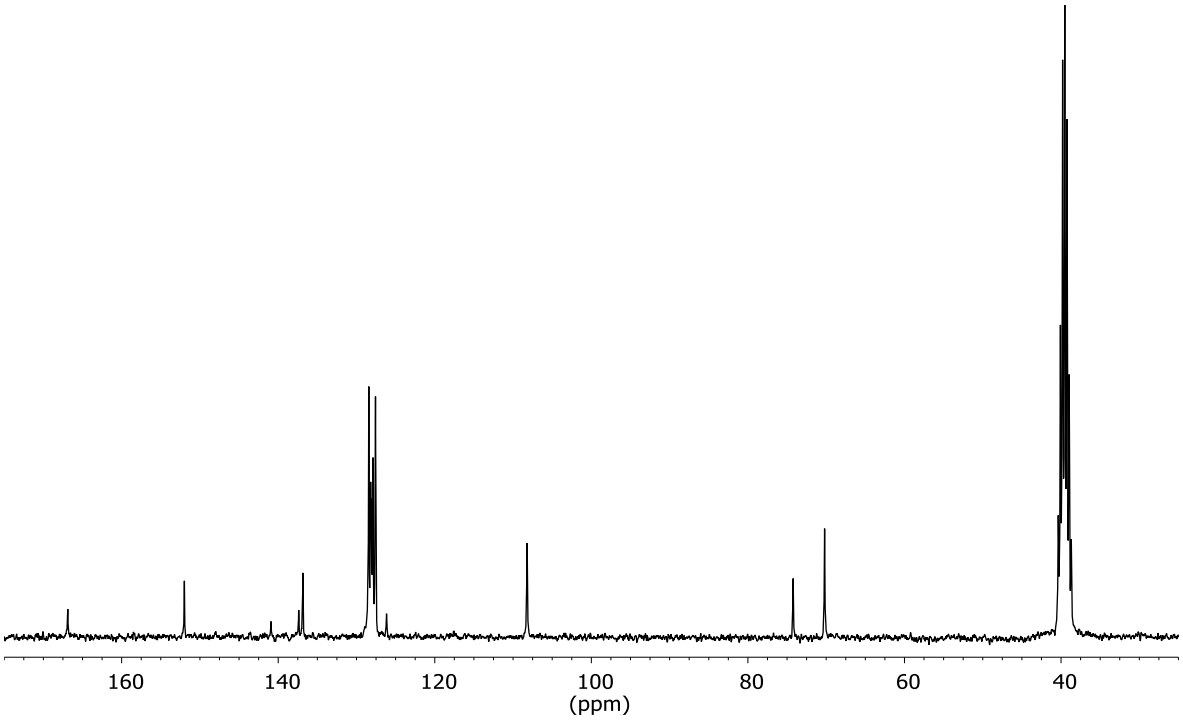


Figure S6. ^{13}C NMR spectrum (DMSO-d_6) of 3,4,5-tris(benzyloxy)benzoic acid.

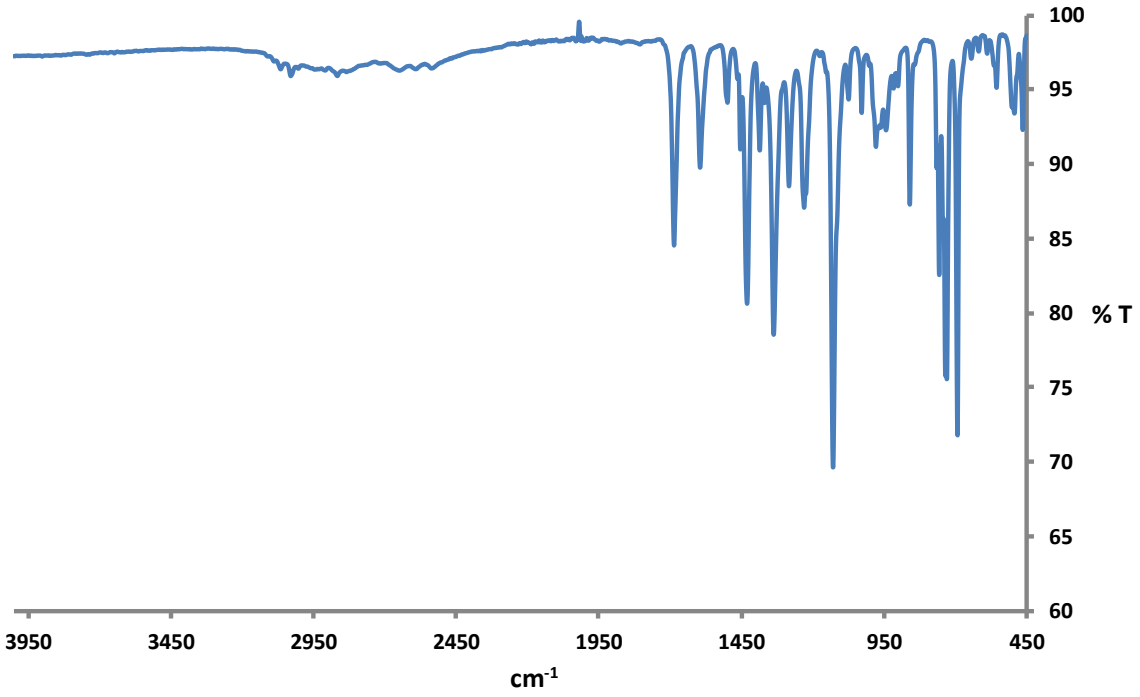


Figure S7. IR spectrum of 3,4,5-tris(benzyloxy)benzoic acid.

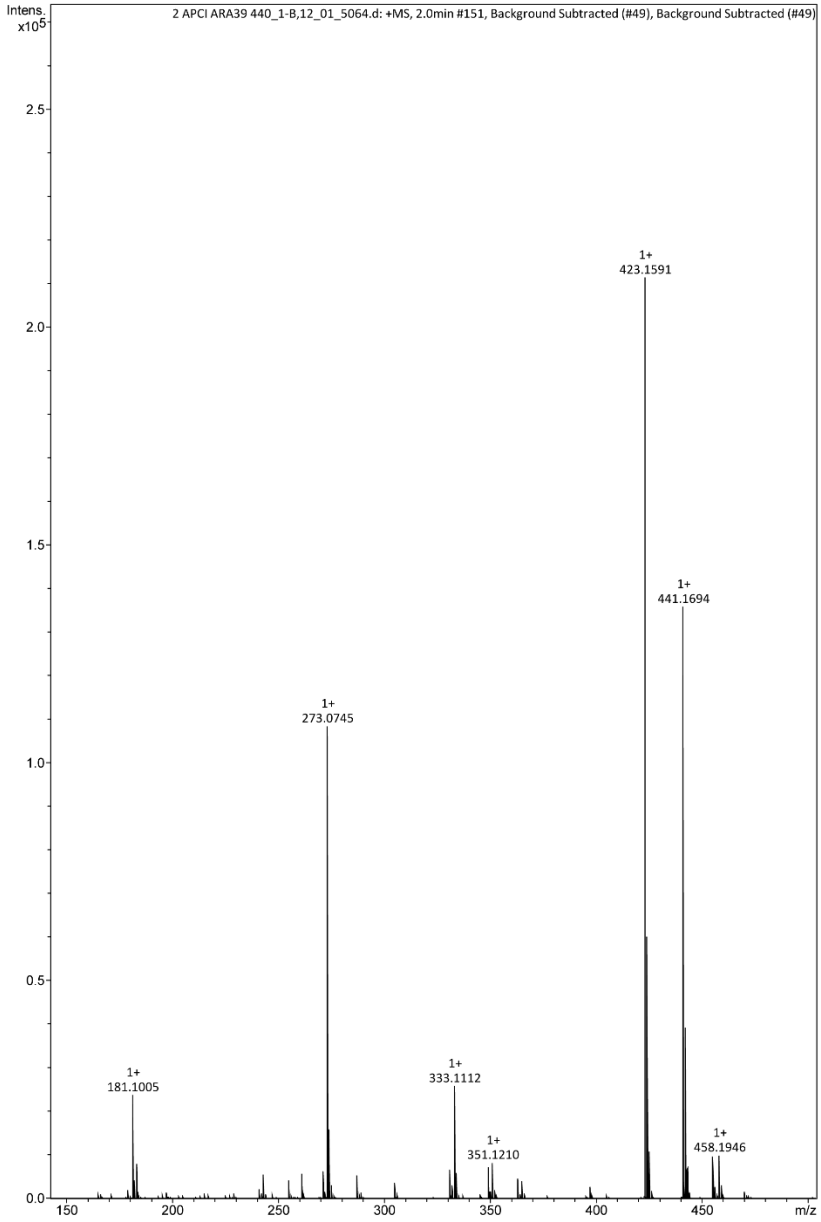


Figure S8. HR-MS of 3,4,5-tris(benzyloxy)benzoic acid.

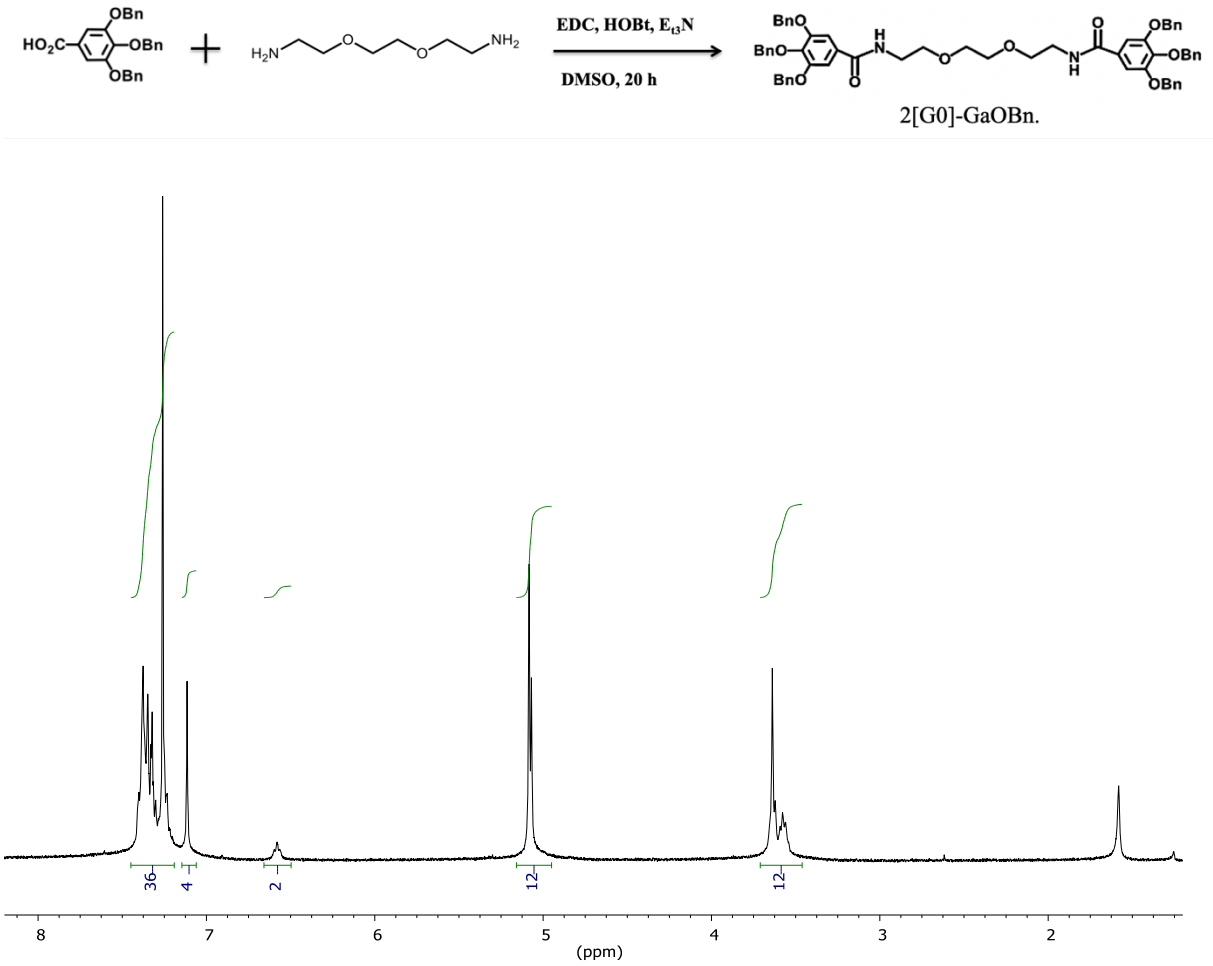


Figure S9. ^{1}H NMR spectrum ($CDCl_3$) of 2[G0]-GaOBn.

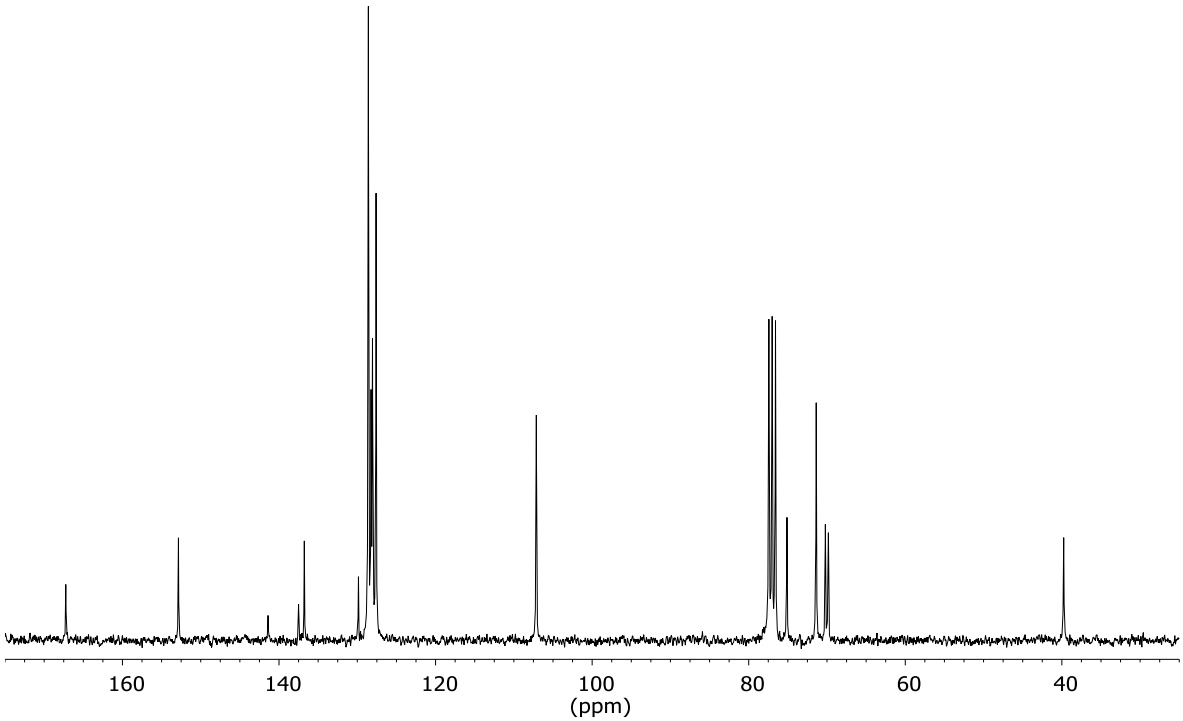


Figure S10. ^{13}C NMR spectrum ($CDCl_3$) of 2[G0]-GaOBn.

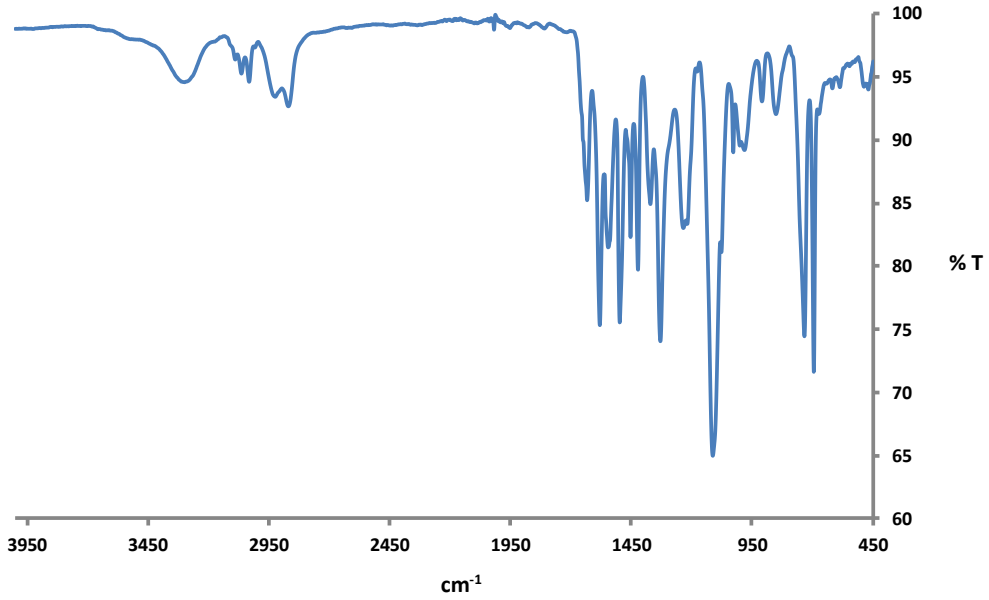


Figure S11. IR spectrum of 2[G0]-GaOBn.

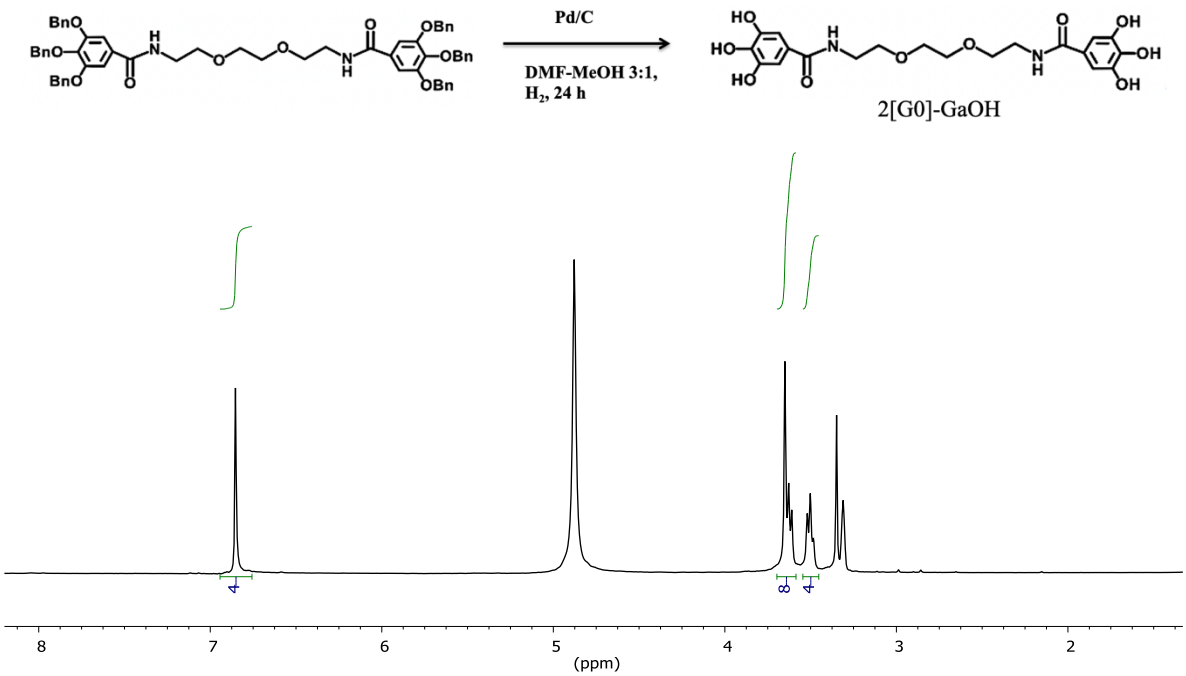


Figure S12. ^{1}H NMR spectrum (CD_3OD) of 2[G0]-GaOH.

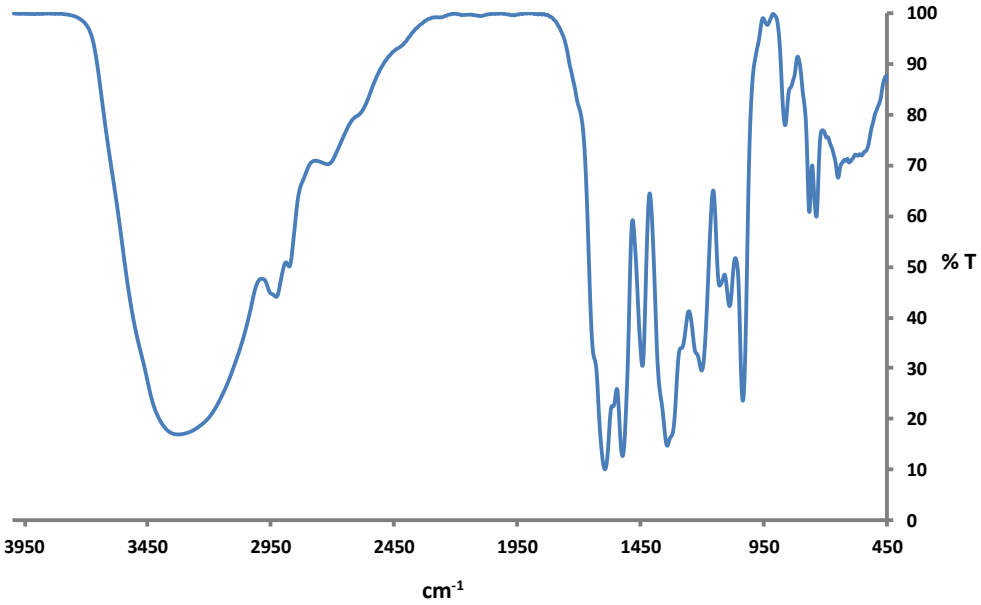


Figure S13. IR spectrum of 2[G0]-GaOH.

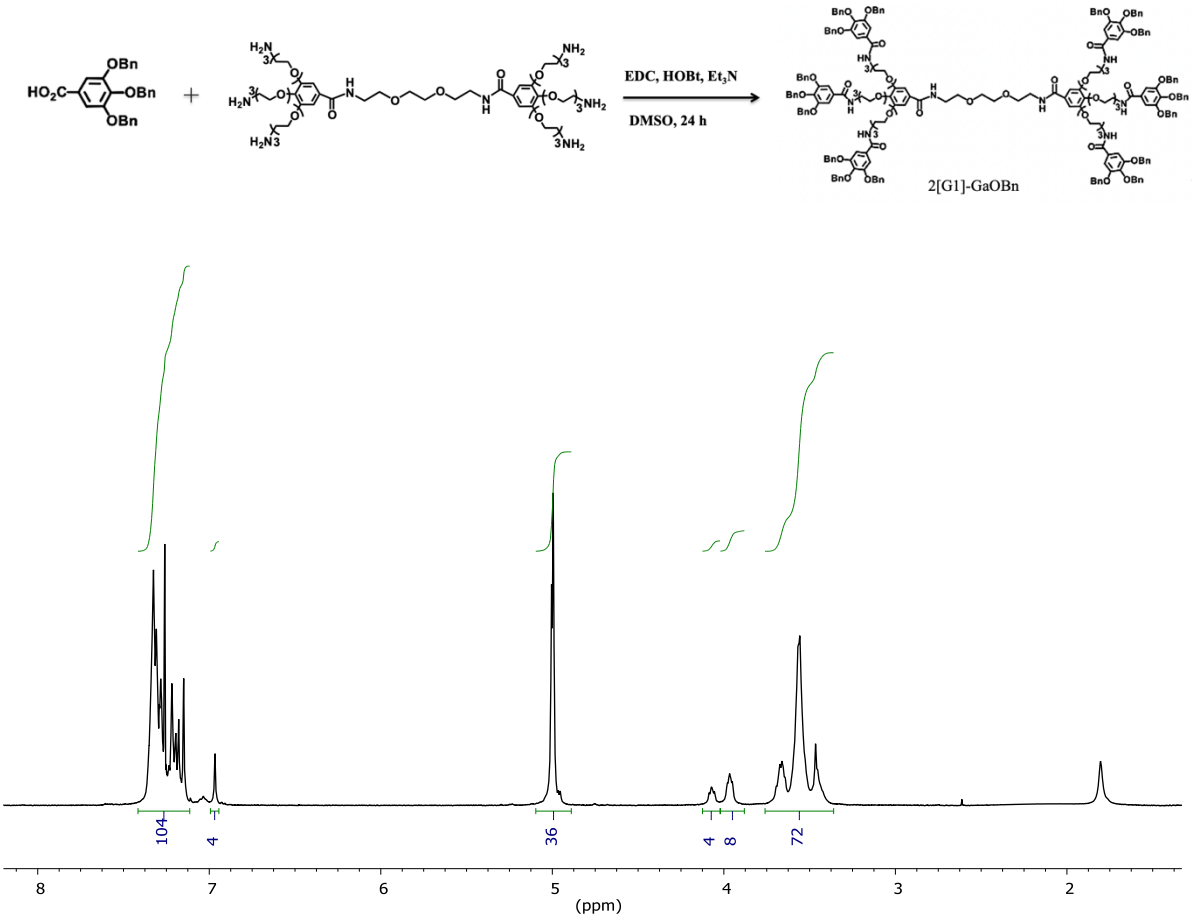


Figure S14. ^{1}H NMR spectrum ($CDCl_3$) of 2[G1]-GaOBn.

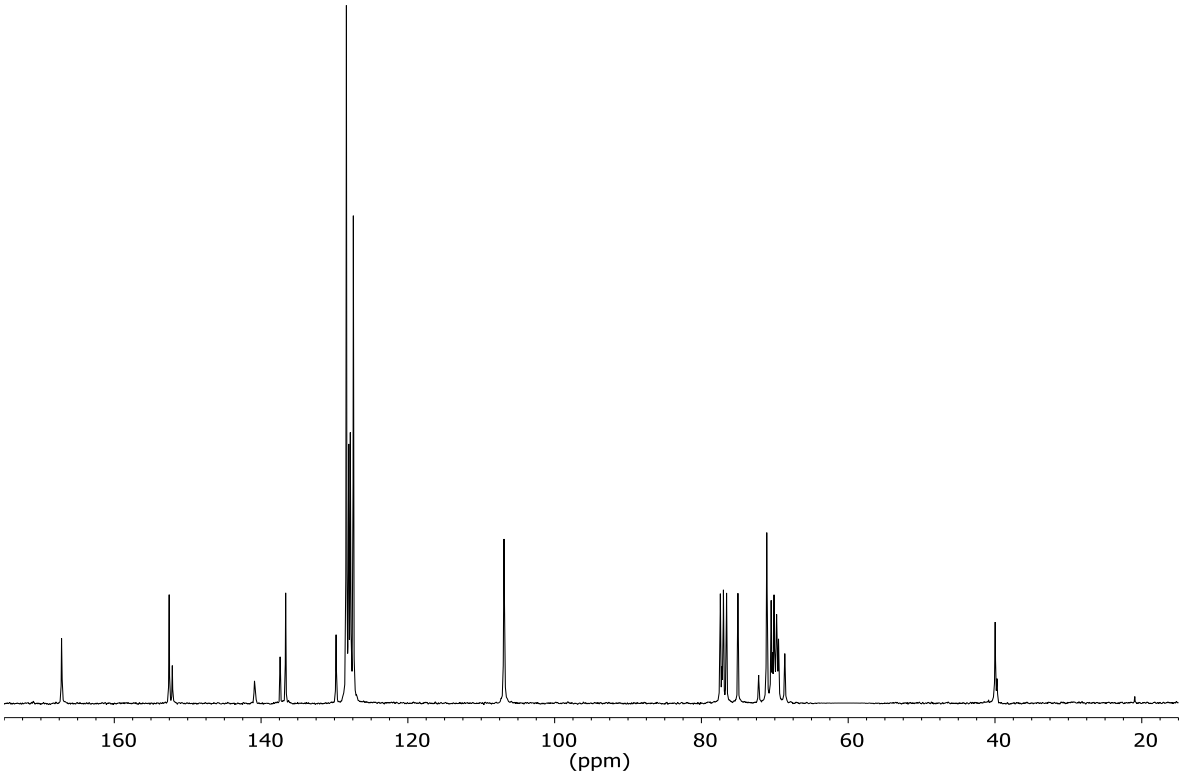


Figure S15. ^{13}C NMR spectrum ($CDCl_3$) of 2[G1]-GaOBn.

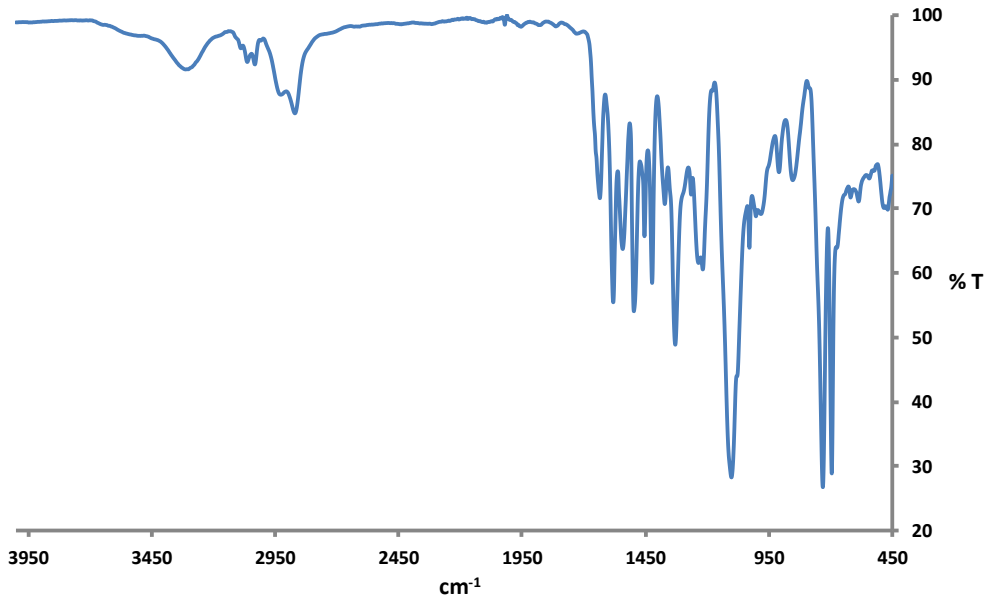


Figure S16. IR spectrum of 2[G1]-GaOBn.

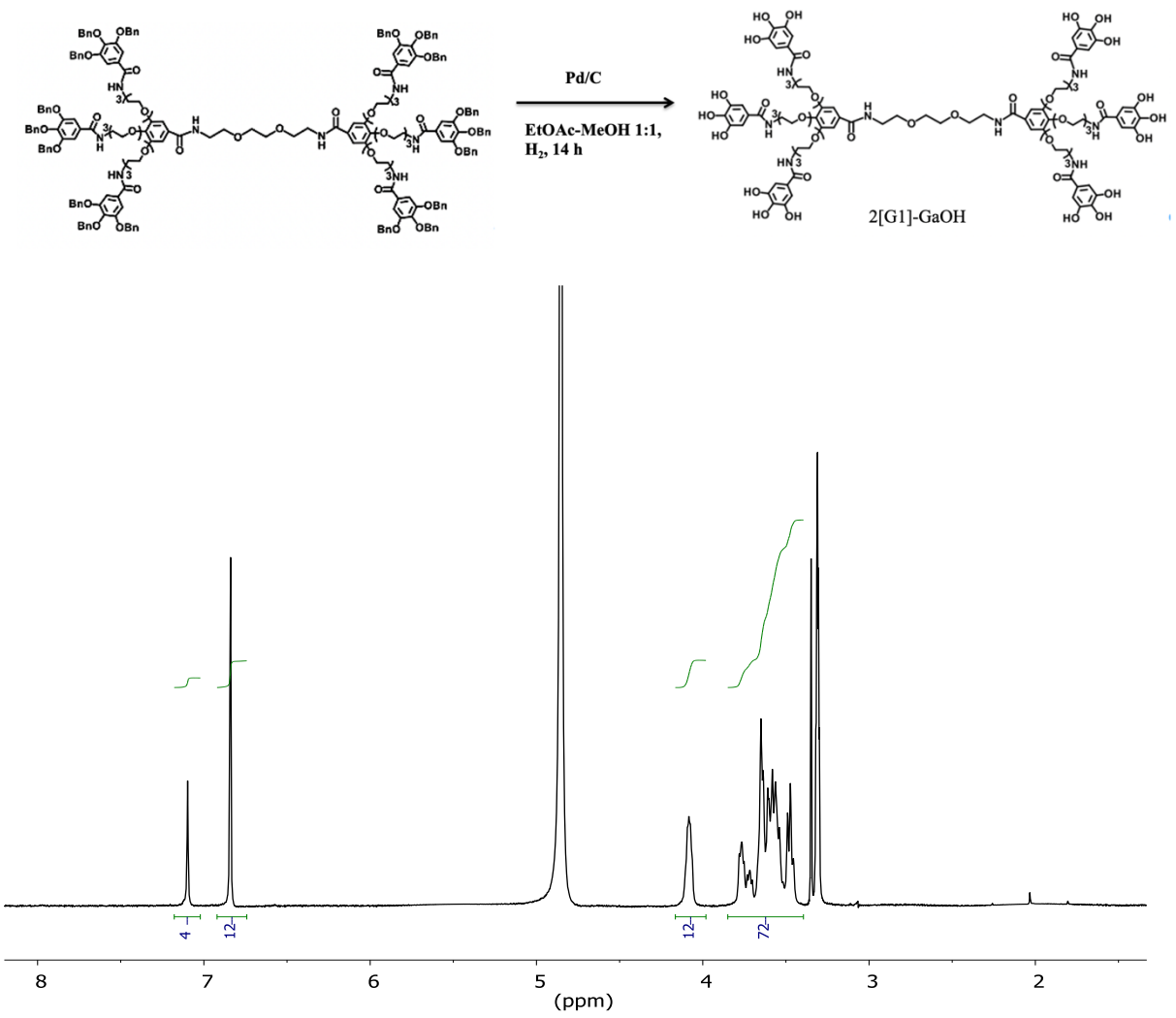


Figure S17. ^{1}H NMR spectrum (CD_3OD) of 2[G1]-GaOH.

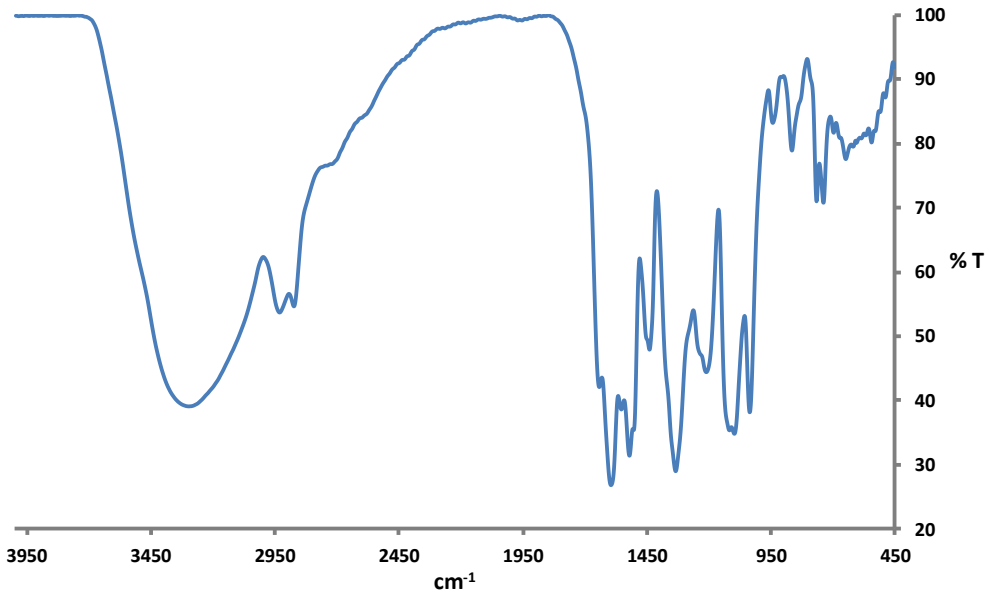


Figure S18. IR spectrum of 2[G1]-GaOH.

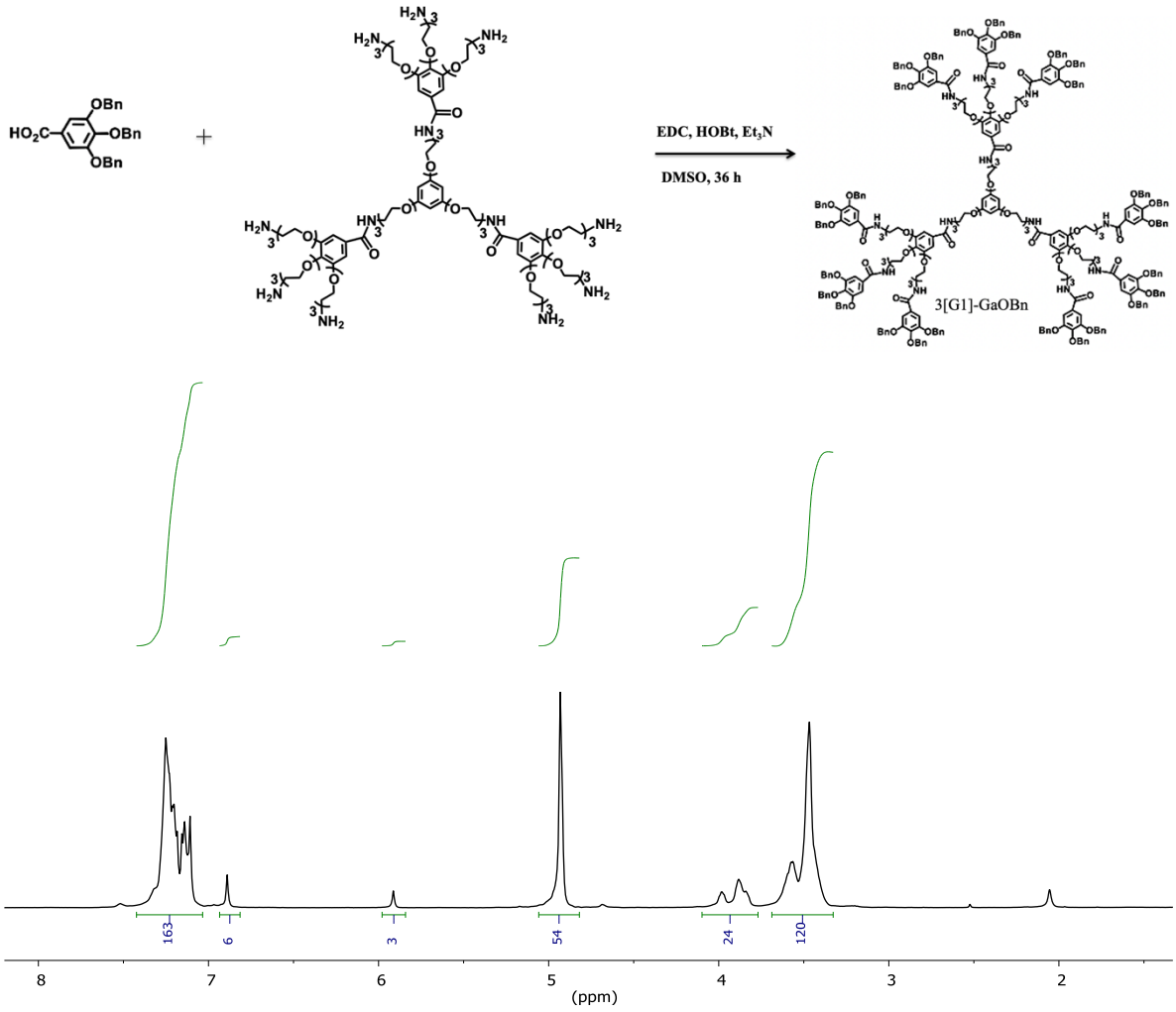


Figure S19. ^{1}H NMR spectrum ($CDCl_3$) of 3[G1]-GaOBn.

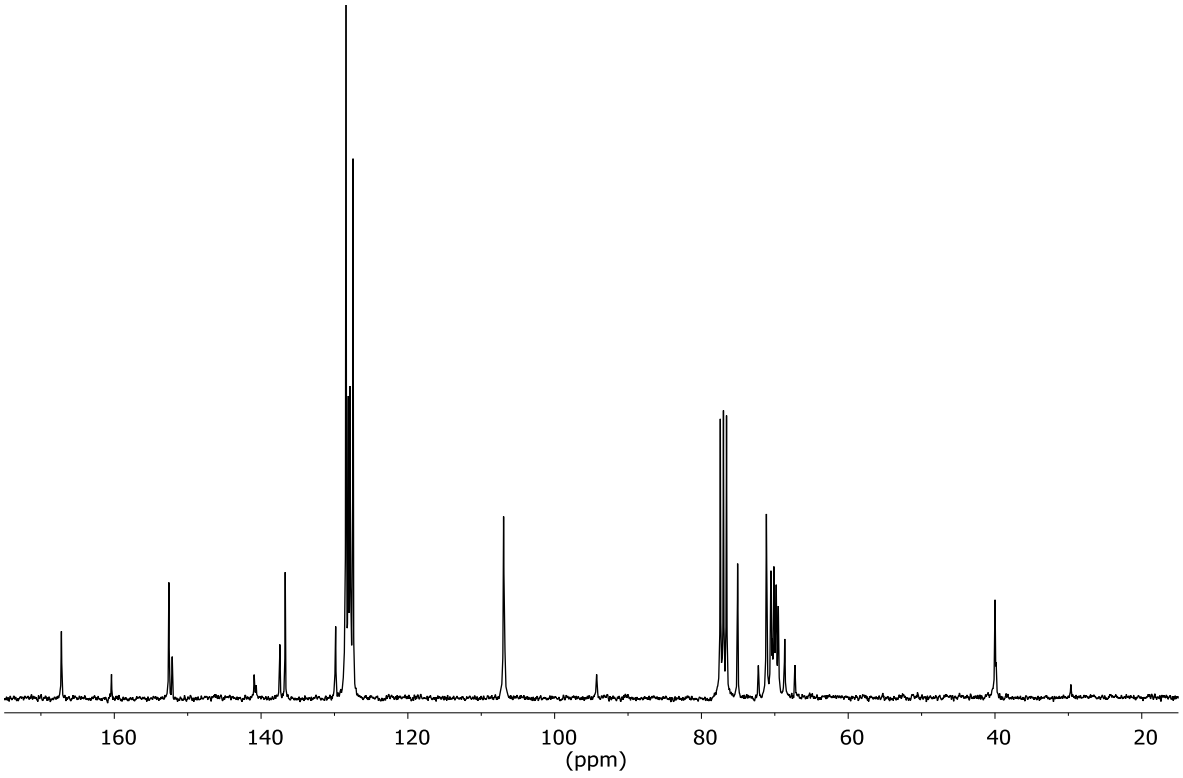


Figure S20. ^{13}C NMR spectrum ($CDCl_3$) of 3[G1]-GaOBn.

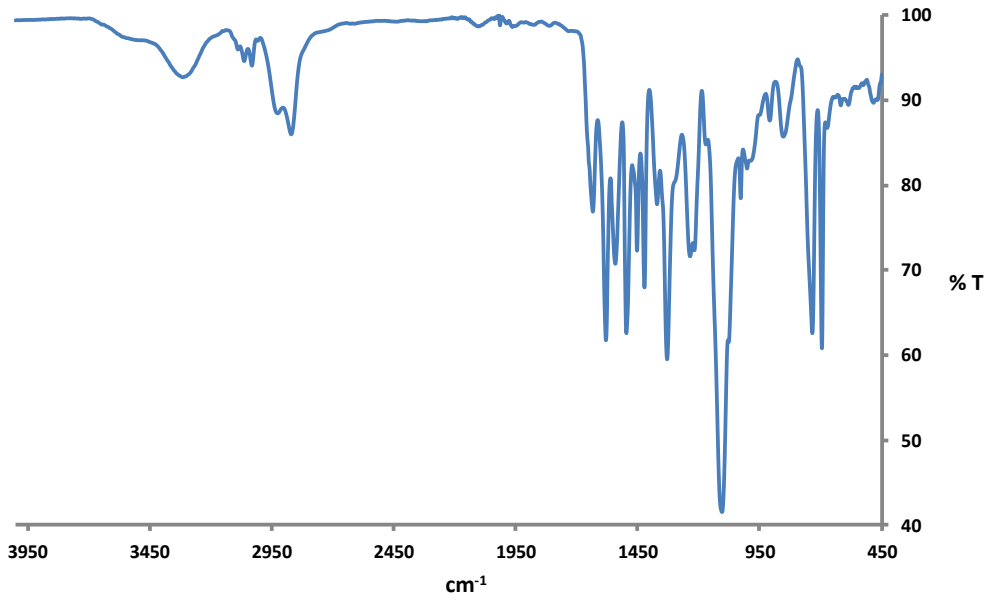


Figure S21. IR spectrum of 3[G1]-GaOBn.

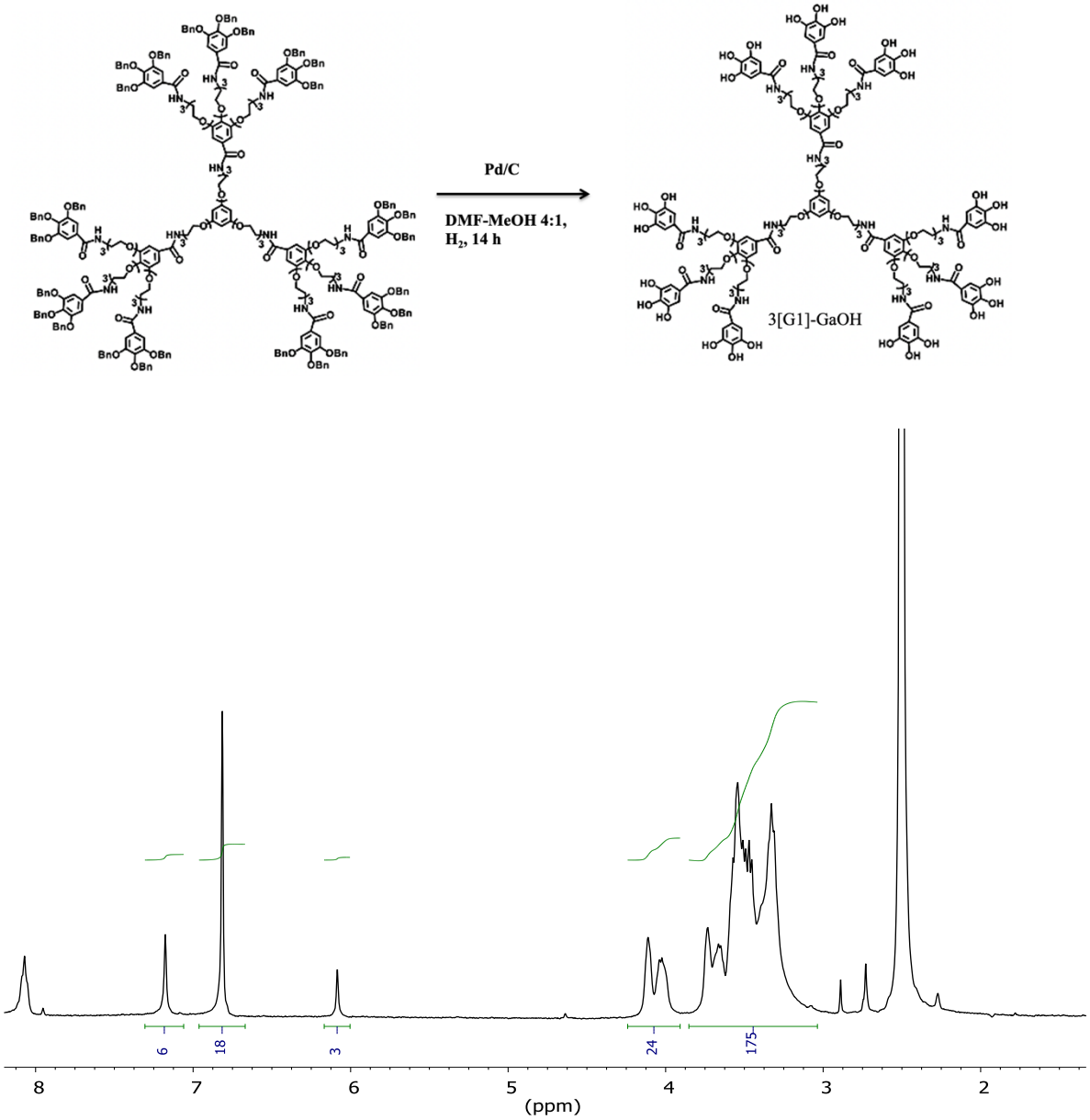


Figure S22. ^{1}H NMR spectrum (DMSO-d_6) of 3[G1]-GaOH.

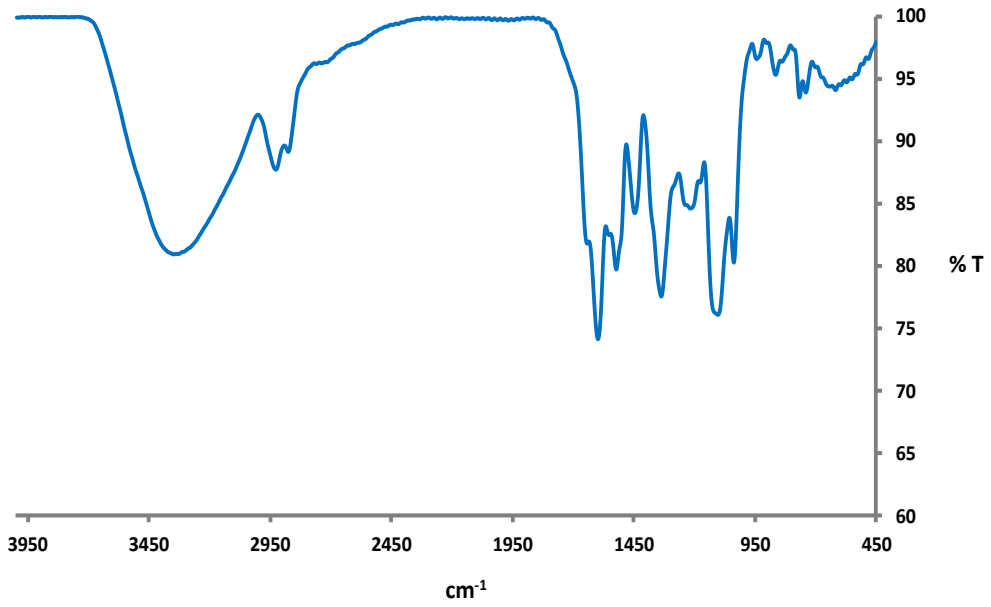


Figure S23. IR spectrum of 3[G1]-GaOH.

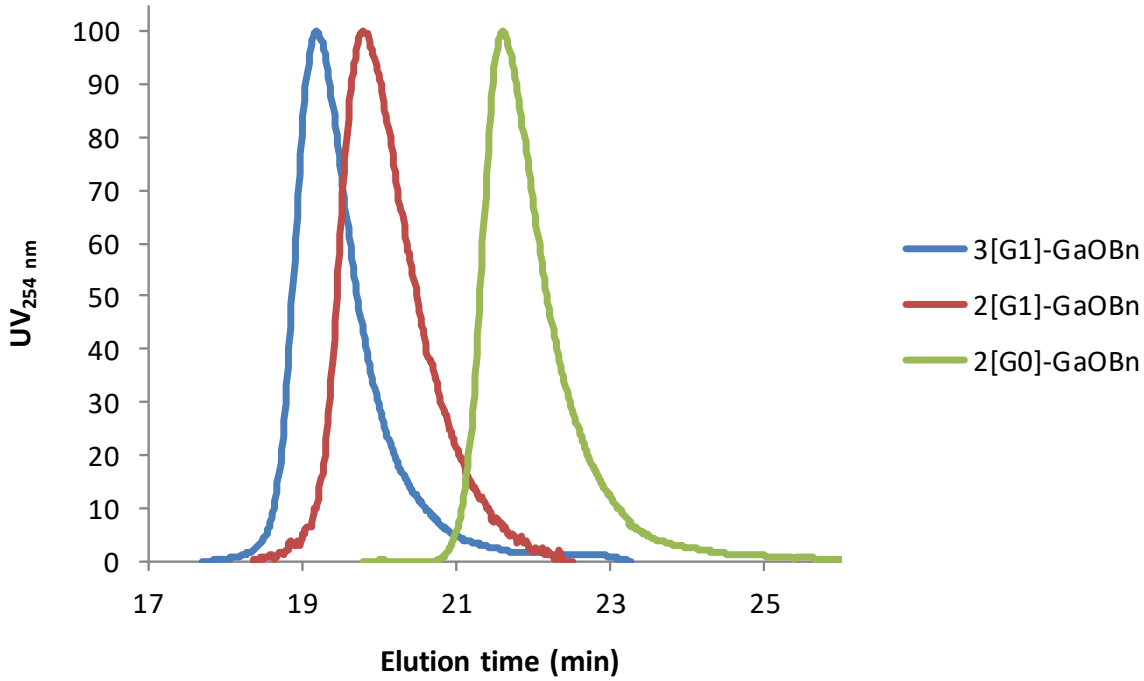


Figure S24. GPC elugrams of x[Gn]-GaOBn (THF).

Interference of the dendrimers in the Aβ supramolecular assembly

Molecular dynamics simulations of the dendrimer-Aβ interactions

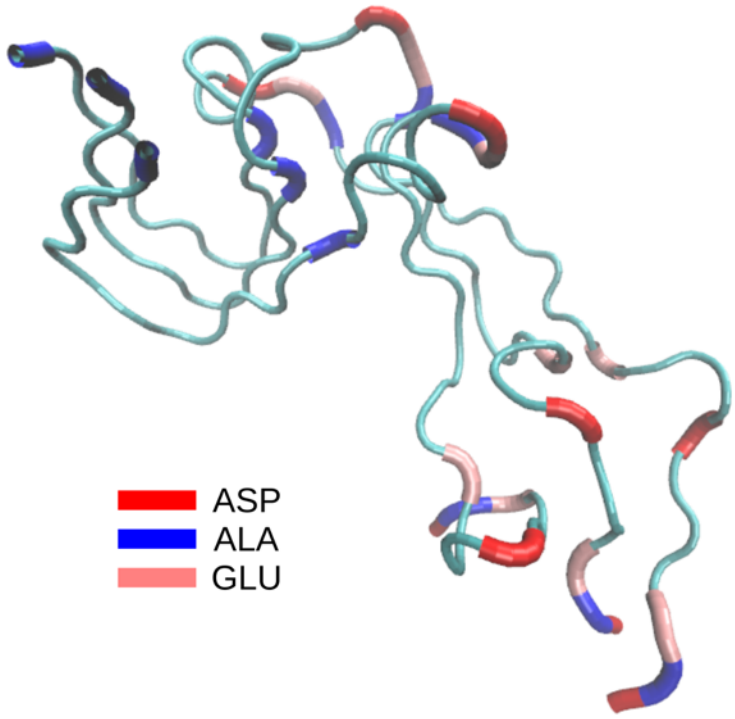


Figure S25. Optimized geometry from MD simulations of 2NAO model (full-length Aβf).

Table S1. MD simulation analysis of the interaction between bull-length $A\beta_f$ (2NAO pdb file) and dendrimers under a $A\beta_f$:dendrimers ratio of 1:1 showing the average number of H-bonds between the peptide chains of $A\beta_f$ and the corresponding dendrimer, as well as the solvent-accessible surface area (SASA) of the region where dendrimers are interacting.

Disassembly 1:1	**Net H-Bond (number #)**	**SASA (nm^2)**
2NAO	--	145 ± 4
2NAO - Ga	23 ± 3	141 ± 4
2NAO - 2G0-GaOH	29 ± 3	147 ± 5
2NAO - 2G1-GaOH	45 ± 4	153 ± 4
2NAO - 3G1-GaOH	64 ± 4	113 ± 3

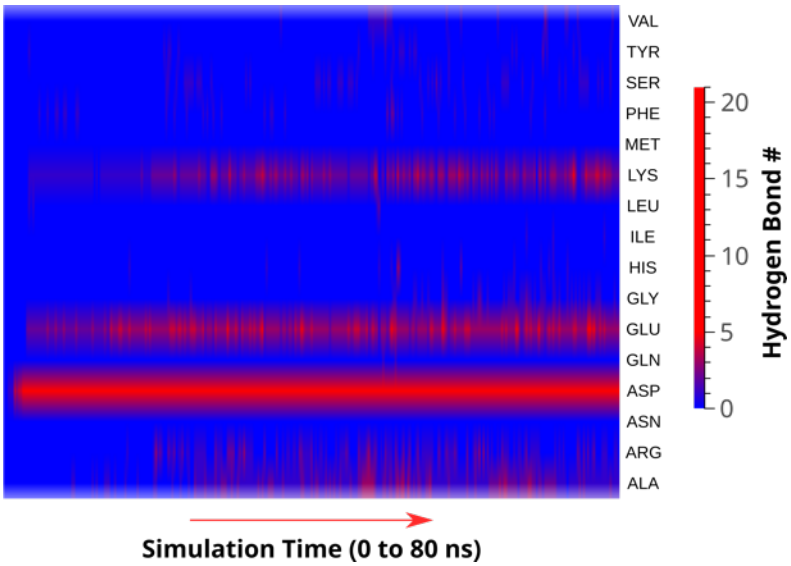


Figure S26. Dynamic interaction energy between full-length $A\beta_f$ (2NAO pdb file) and Ga (1:1 ratio). Preferential binding residues were calculated by the nonbonded interaction energy and the number of H-bonds between each $A\beta_f$ and Ga.

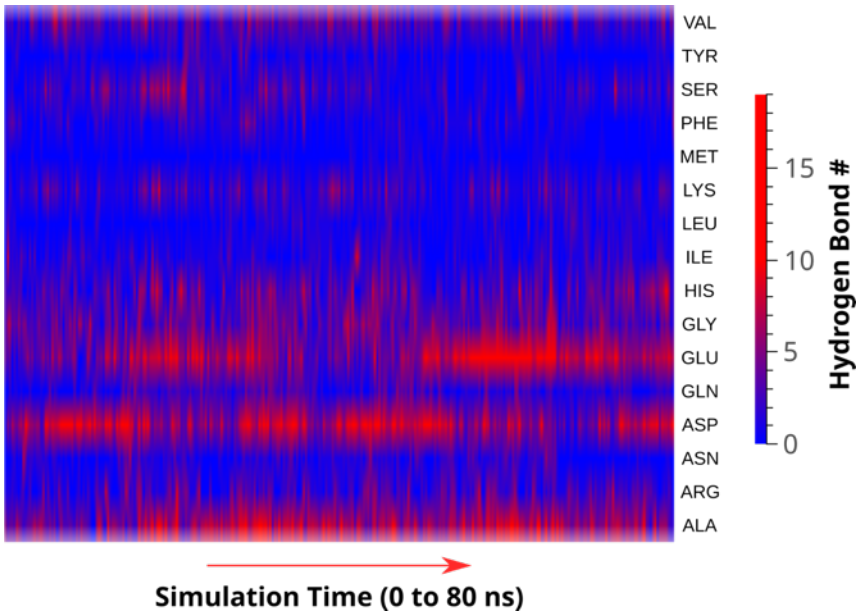


Figure S27. Dynamic interaction energy between full-length $A\beta_f$ (2NAO pdb file) and 2G0-GaOH (1:1 ratio). Preferential binding residues were calculated by the nonbonded interaction energy and the number of H-bonds between each full-length $A\beta_f$ and 2G0-GaOH.

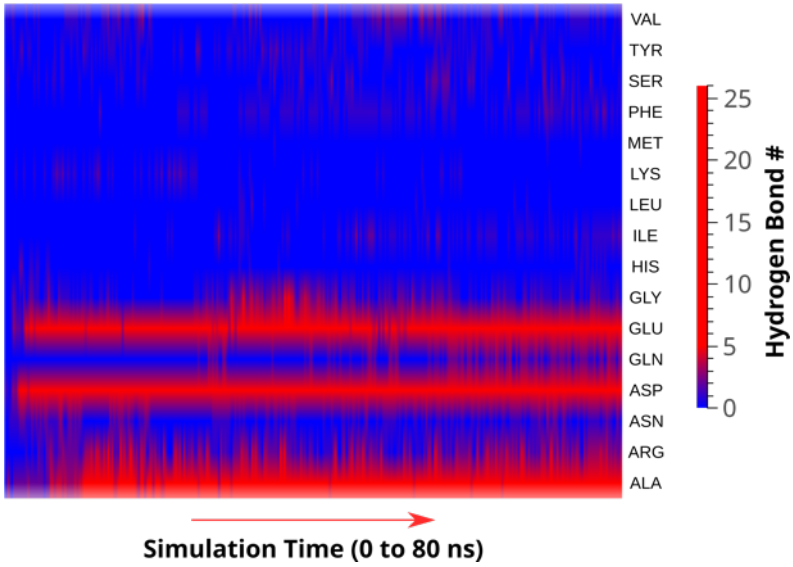


Figure S28. Dynamic interaction energy between full-length $A\beta_f$ (2NAO pdb file) and 2G1-GaOH (1:1 ratio). Preferential binding residues were calculated by the nonbonded interaction energy and the number of H-bonds between each full-length $A\beta_f$ and 2G1-GaOH.

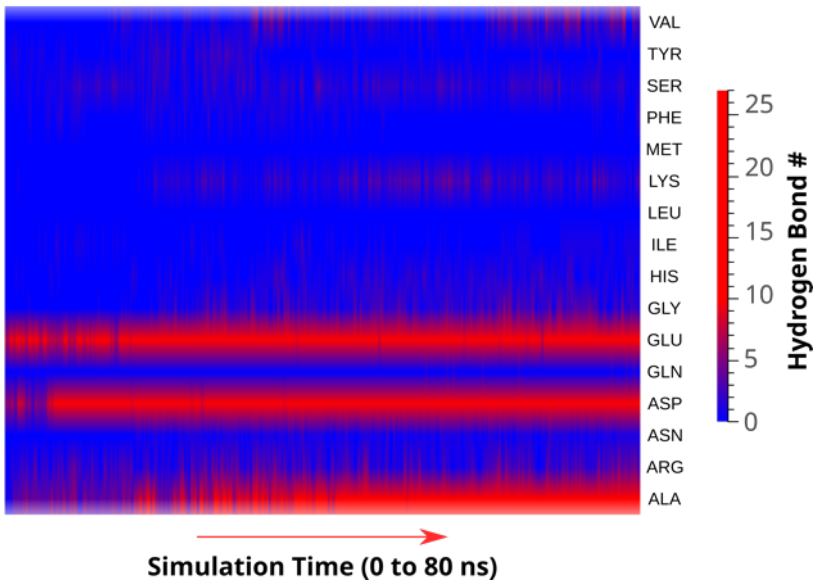


Figure S29. Dynamic interaction energy between full-length $A\beta_f$ (2NAO pdb file) and 3G1-GaOH (1:1 ratio). Preferential binding residues were calculated by the nonbonded interaction energy and the number of H-bonds between each full-length $A\beta_f$ and 3G1-GaOH.

Thioflavin T (ThT) assay

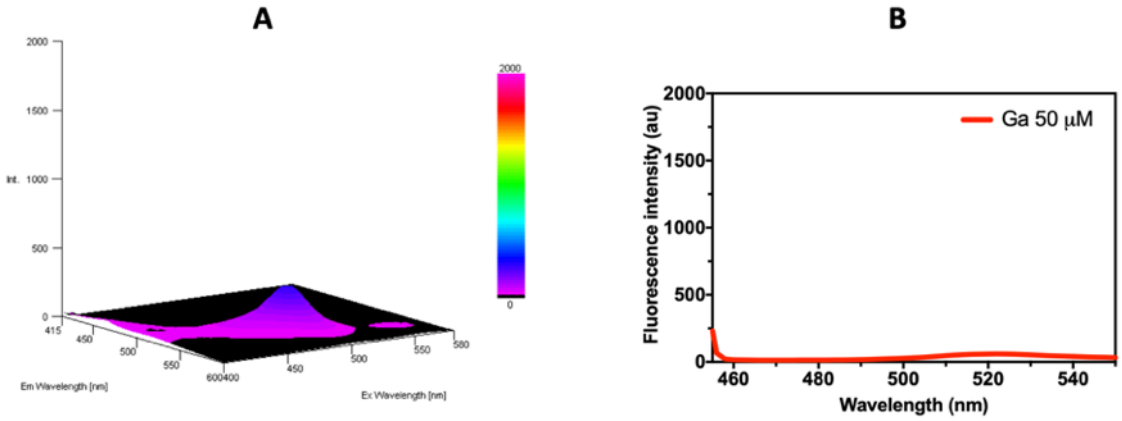


Figure S30. (A) 3D fluorescence spectra of Gallic acid (Ga, 50µM) using the following acquisition parameters: λ_{ex} = 400-580 nm; λ_{em} = 415-600 nm; (B) Fluorescence spectrum of Ga (50µM) acquired using a λ_{ex} = 435 nm and a λ_{em} = 445-600 nm; excitation bandwidth = 5nm; emission bandwidth = 10nm; response = 2s.

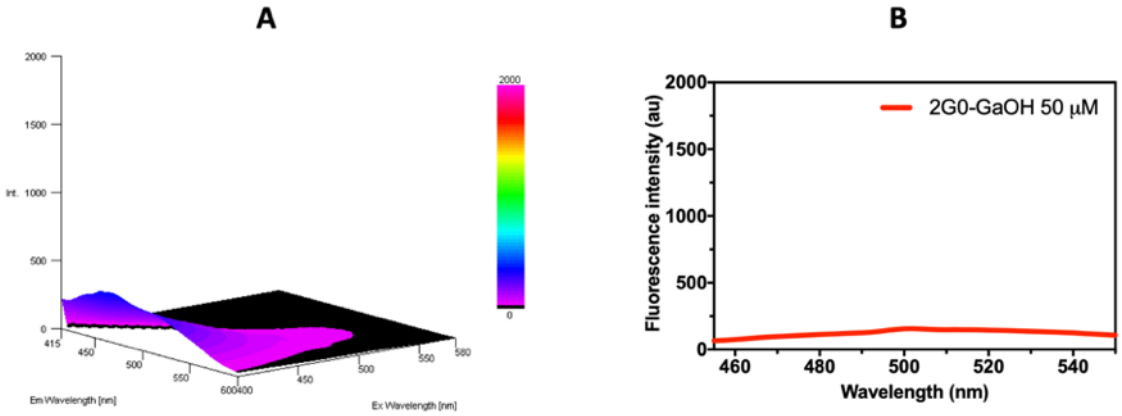


Figure S31. (A) 3D fluorescence spectra of 2G0-GaOH (50µM) using the following acquisition parameters: λ_{ex} = 400-580 nm; λ_{em} = 415-600 nm; (B) Fluorescence spectrum of 2G0-GaOH (50µM) acquired using a λ_{ex} = 435 nm and a λ_{em} = 445-600 nm; excitation bandwidth = 5nm; emission bandwidth = 10nm; response = 2s.

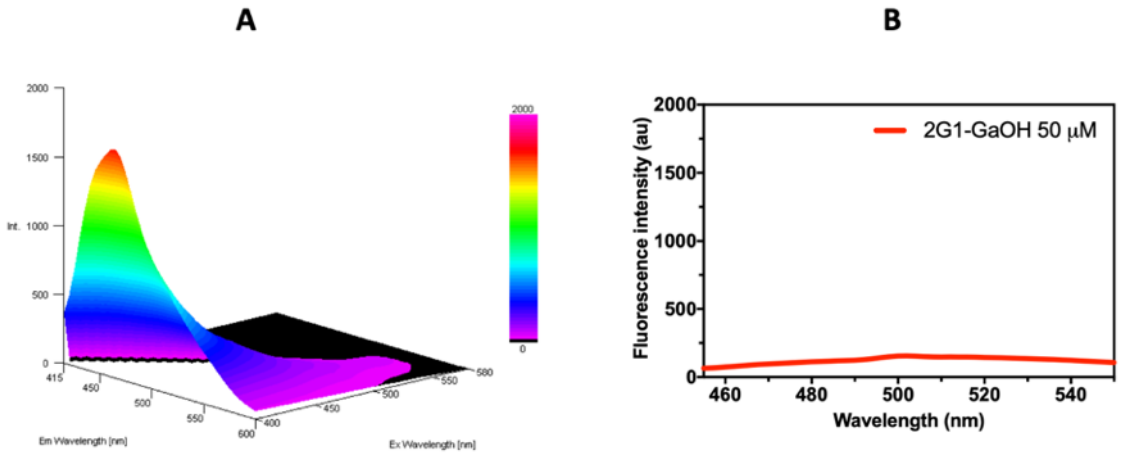


Figure S32. (A) 3D fluorescence spectra of 2G1-GaOH (50µM) using the following acquisition parameters: λ_{ex} = 400-580 nm; λ_{em} = 415-600 nm; (B) Fluorescence spectrum of 2G1-GaOH (50µM) acquired using a λ_{ex} = 435 nm and a λ_{em} = 445-600 nm; excitation bandwidth = 5nm; emission bandwidth = 10nm; response = 2s.

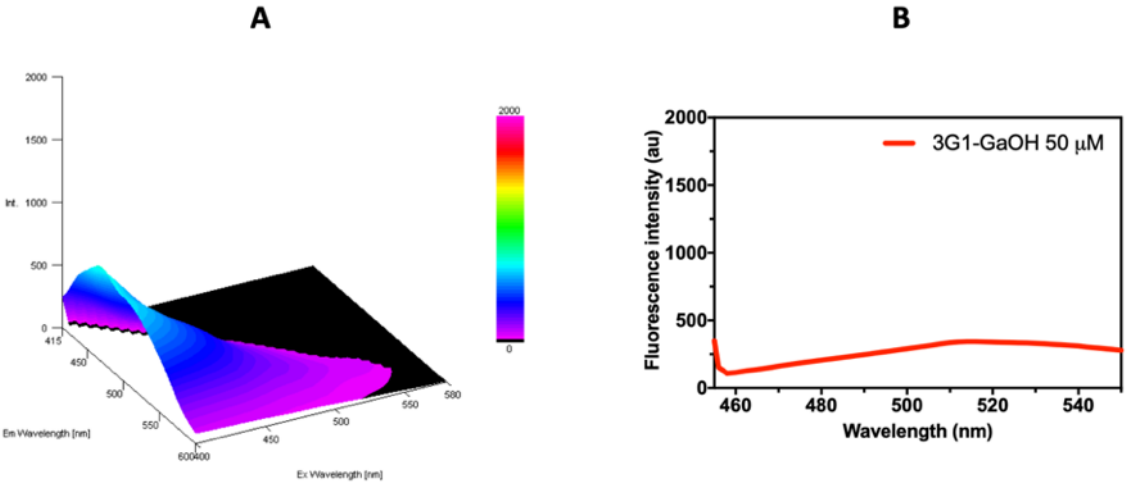


Figure S33. (A) 3D fluorescence spectra of 3G1-GaOH (50µM) using the following acquisition parameters: λ_{ex} = 400-580 nm; λ_{em} = 415-600 nm; (B) Fluorescence spectrum of 3G1-GaOH (50µM) acquired using a λ_{ex} = 435 nm and a λ_{em} = 445-600 nm; excitation bandwidth = 5nm; emission bandwidth = 10nm; response = 2s.

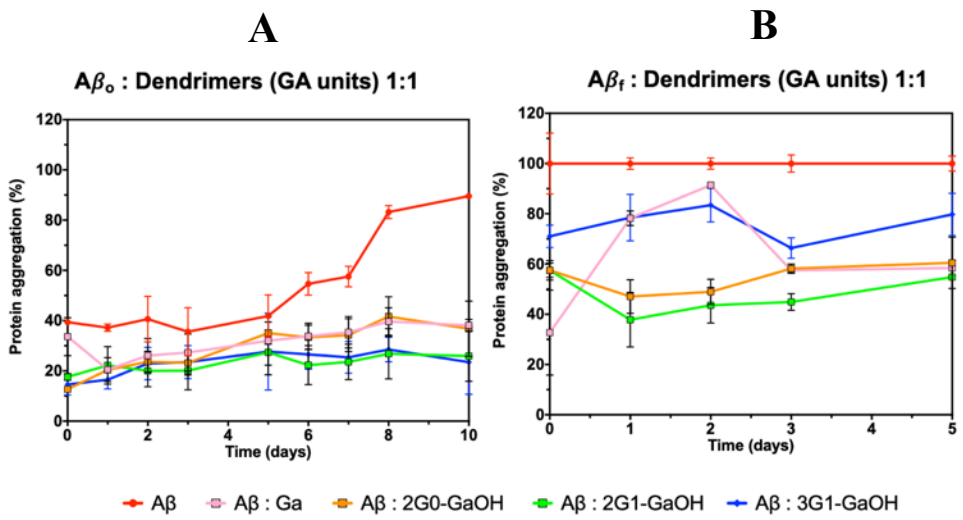


Figure S34. (A) Multivalent inhibition of the $A\beta_o$ aggregation kinetics evaluated using the ThT assay. Ga, 2G0-GaOH, 2G1-GaOH and 3G1-GaOH inhibited the Aβ fibril elongation at an Aβ:Ga molar ratio of 1:1. Dendrimers were mixed with $A\beta_o$ in the lag-phase of the Aβ fibril formation and fluorescence was measured over 10 days. All experiments were performed at room temperature. (B) Multivalent disassembling of $A\beta_f$ fibrils evaluated using the ThT assay. Ga, 2G0-GaOH, 2G1-GaOH and 3G1-GaOH promoted the disassembly of Aβ fibrils at an Aβ:Ga molar ratio of 1:1. Compounds were mixed with $A\beta_f$ at the plateau phase and fluorescence was measured over 5 days. All experiments were performed at room temperature.

Circular Dichroism (CD)

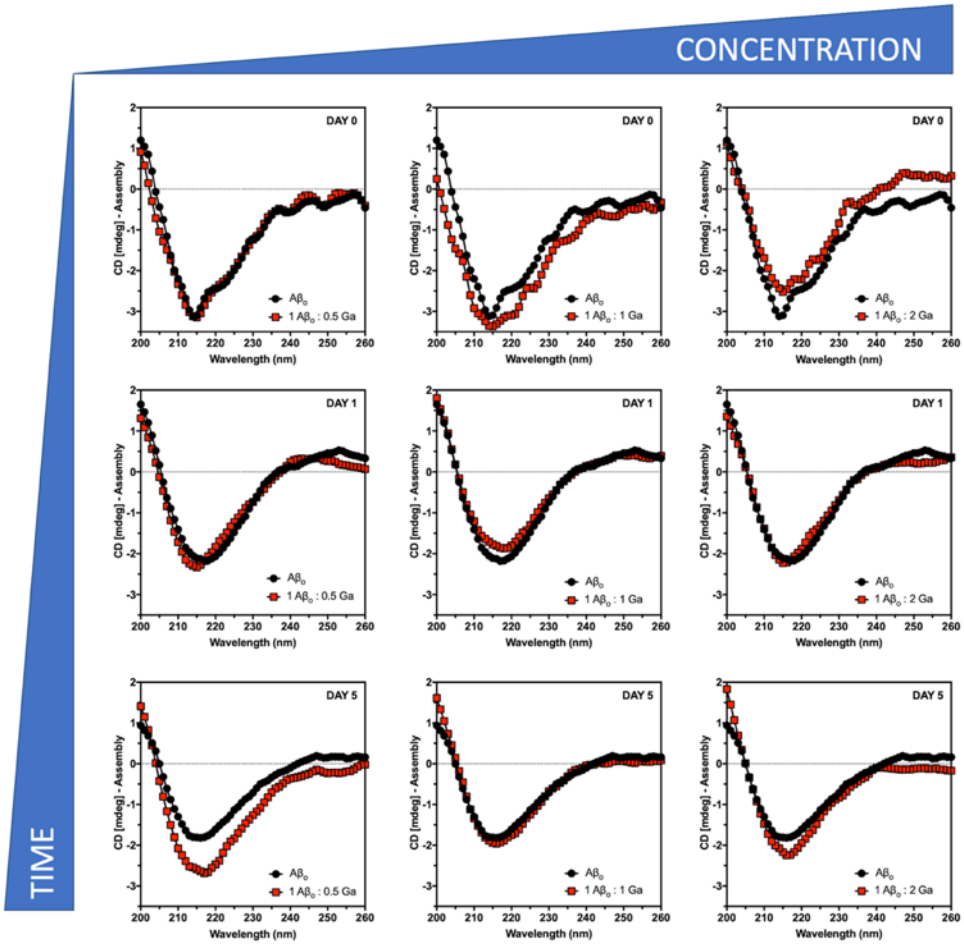


Figure S35. CD spectra of $A\beta_o$ recorded for 5 days in the presence of different molar ratios of Ga. In all the cases, incubation was made at room temperature, under constant agitation.

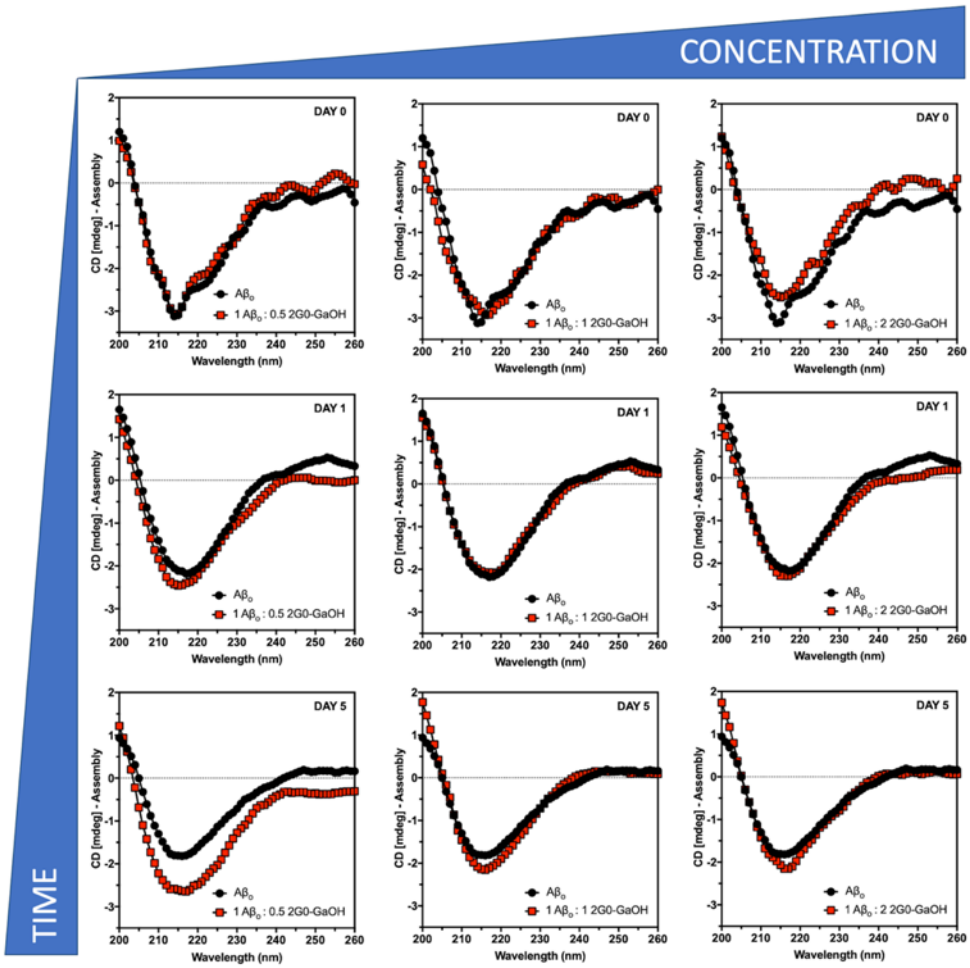


Figure S36. CD spectra of $A\beta_o$ recorded for 5 days in the presence of different molar ratios of 2G0-GaOH. In all the cases, incubation was made at room temperature, under constant agitation.

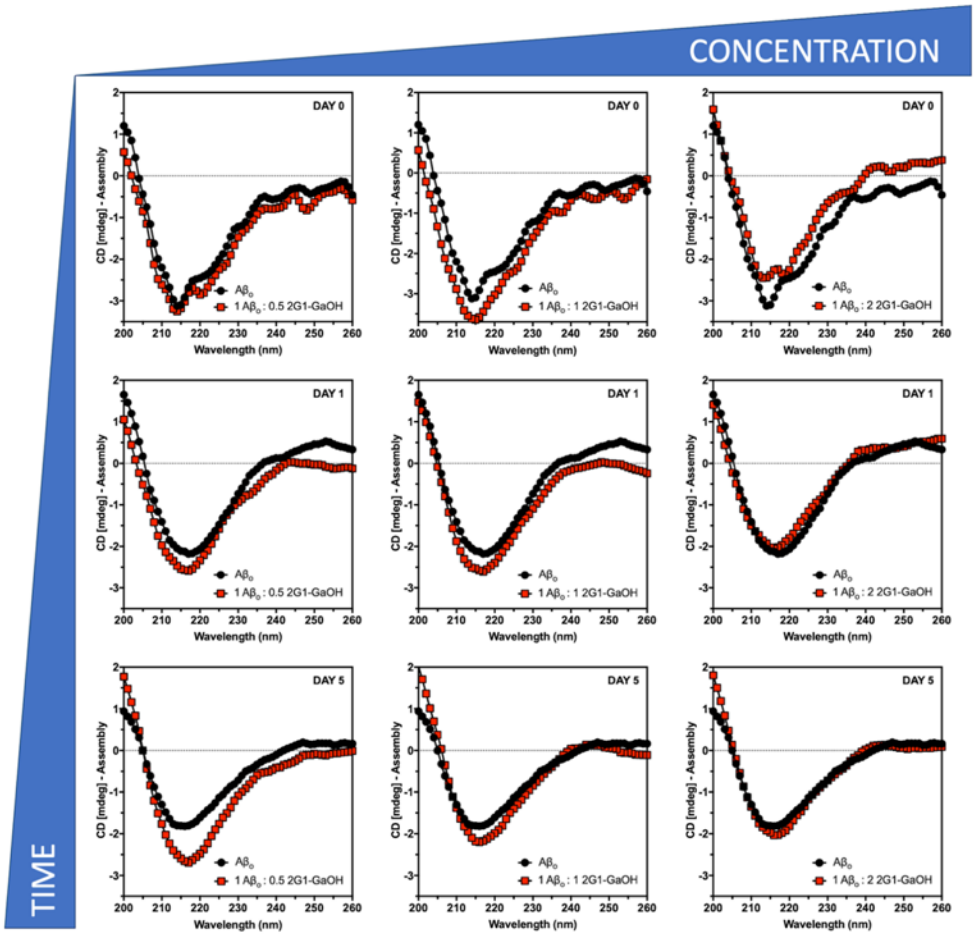


Figure S37. CD spectra of Aβ$_o$ recorded for 5 days in the presence of different molar ratios of 2G1-GaOH. In all the cases, incubation was made at room temperature, under constant agitation.

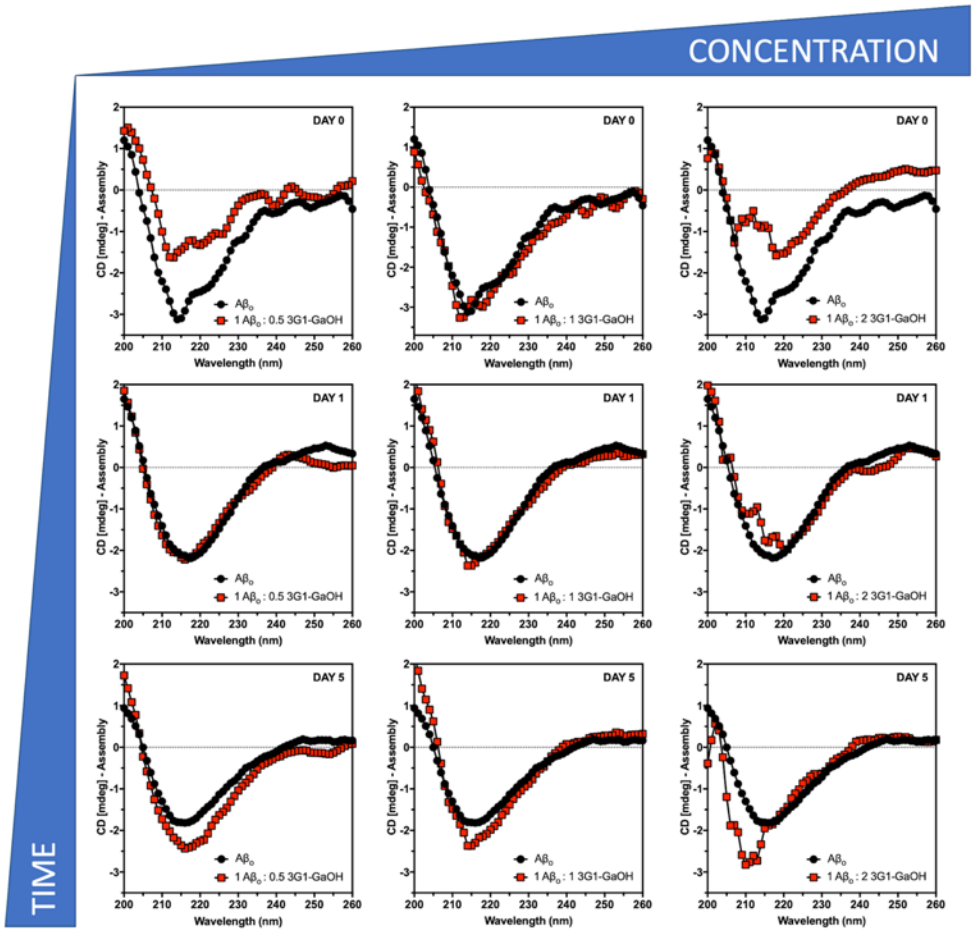


Figure S38. CD spectra of $A\beta_o$ recorded for 5 days in the presence of different molar ratios of 3G1-GaOH. In all the cases, incubation was made at room temperature, under constant agitation.

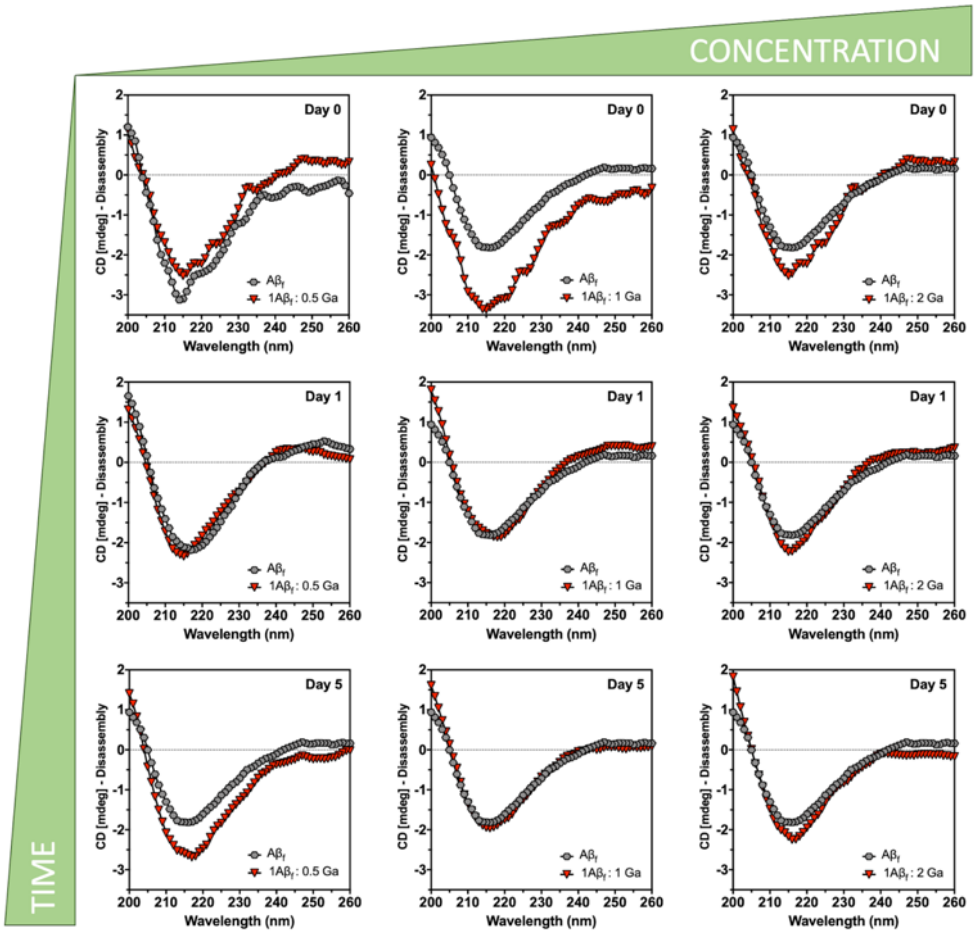


Figure S39. CD spectra of $A\beta_f$ recorded for 5 days in the presence of different molar ratios of Ga. In all the cases, incubation was made at room temperature, under constant agitation.

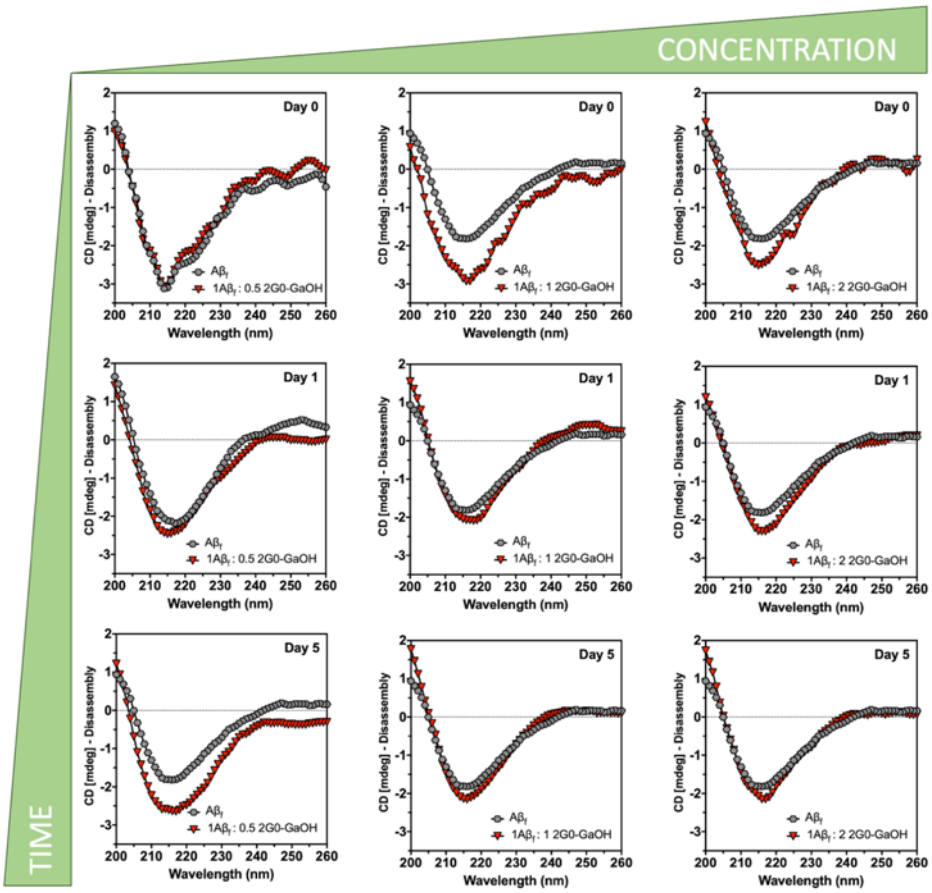


Figure S40. CD spectra of $A\beta_f$ recorded for 5 days in the presence of different molar ratios of 2G0-GaOH. In all the cases, incubation was made at room temperature, under constant agitation.

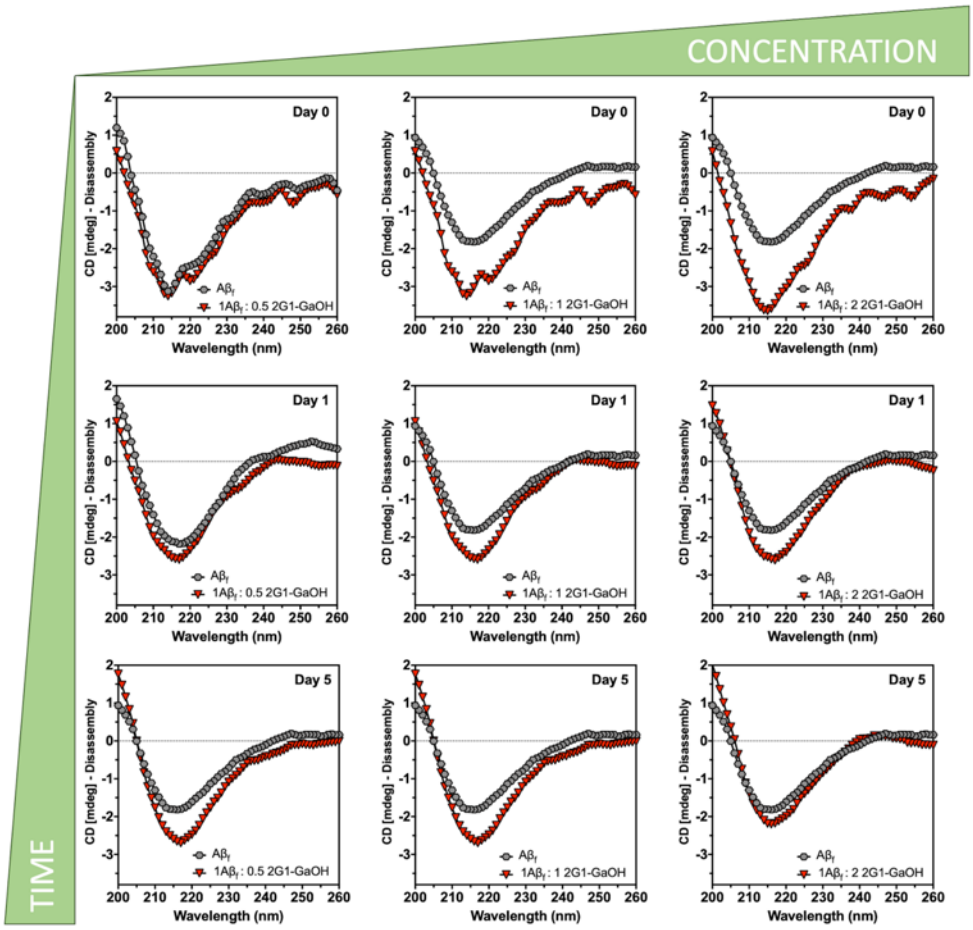


Figure S41. CD spectra of $A\beta_f$ recorded for 5 days in the presence of different molar ratios of 2G1-GaOH. In all the cases, incubation was made at room temperature, under constant agitation.

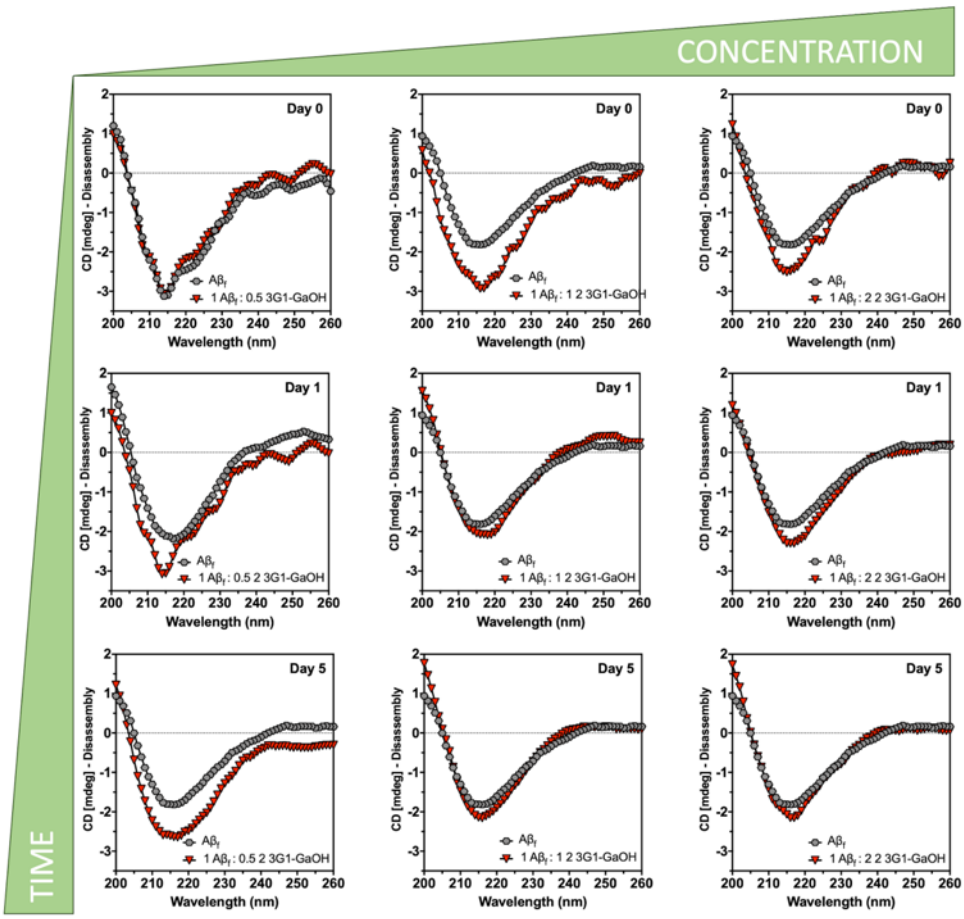


Figure S42. CD spectra of $A\beta_f$ recorded for 5 days in the presence of different molar ratios of 3G1-GaOH. In all the cases, incubation was made at room temperature, under constant agitation.

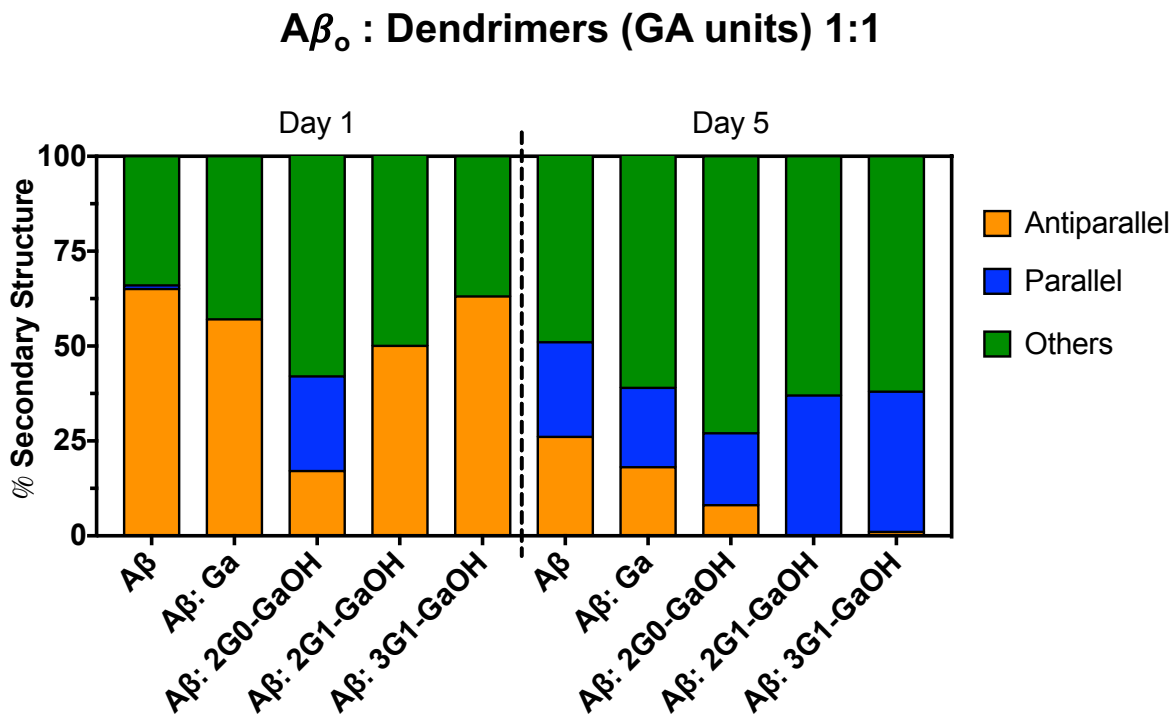


Figure S43. Loss of antiparallel β-sheets for the aggregation pathway ($A\beta_o$) followed by CD during 1 and 5 days. All experiments were done using an Aβ:Ga molar ratio of 1:1; [Aβ]=25μM; and under constant agitation at 37 °C. Error bars = SD; n = 3. CD data were fitted using BeStSel - RMSD: 1.0283; NRMSD: 0.04966.

Scanning transmission electron microscopy (STEM)

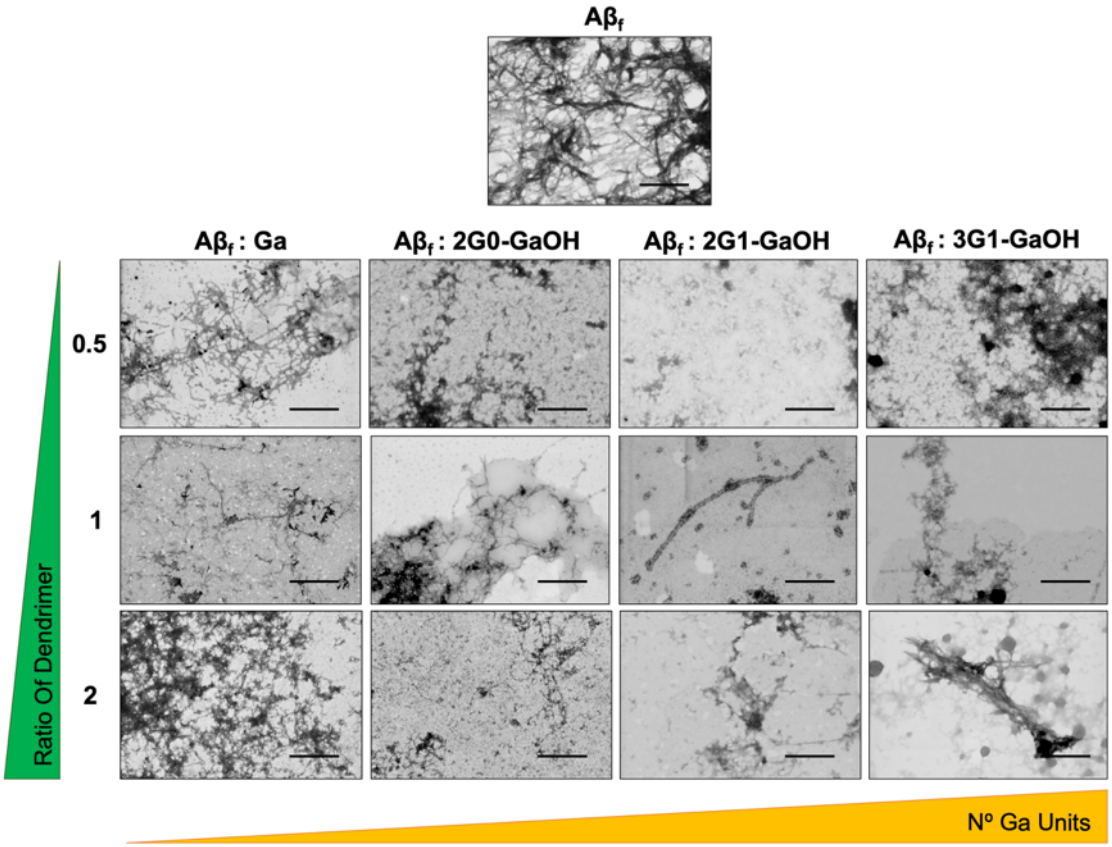


Figure S44. Representative STEM images of $A\beta_f$ samples. Each dendrimer was added into an $A\beta_f$ solution (under Aβ:dendrimer concentration ratios of 1:0.5, 1:1 and 1:2) and left to incubate for 24h under constant agitation. Both compounds directly altered $A\beta_f$ morphological presentation. Scale bars = 500 nm.

Atomic Force Microscopy (AFM)

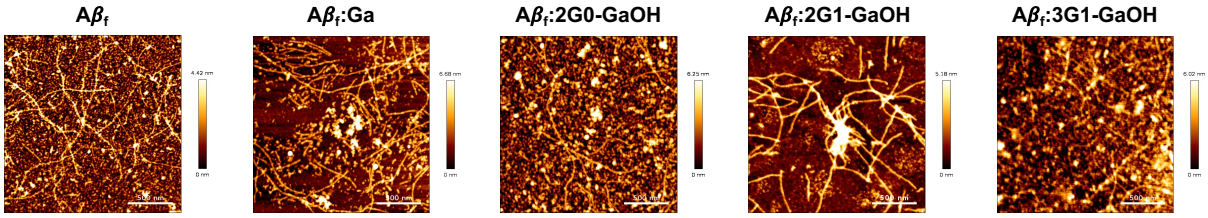


Figure S45. Representative AFM images of $A\beta_f$. Each dendrimer was added into an $A\beta_f$ solution (Aβ:Ga molar ratio of 1:1) and left to incubate for 24h under constant agitation. The dendrimers with higher number of Ga units (i.e., 2G1-GaOH and 3G1-GaOH) were more effective than Ga to alter the $A\beta_f$ presentation. Scale bars = 500 nm.

Western Blot (WB) analysis

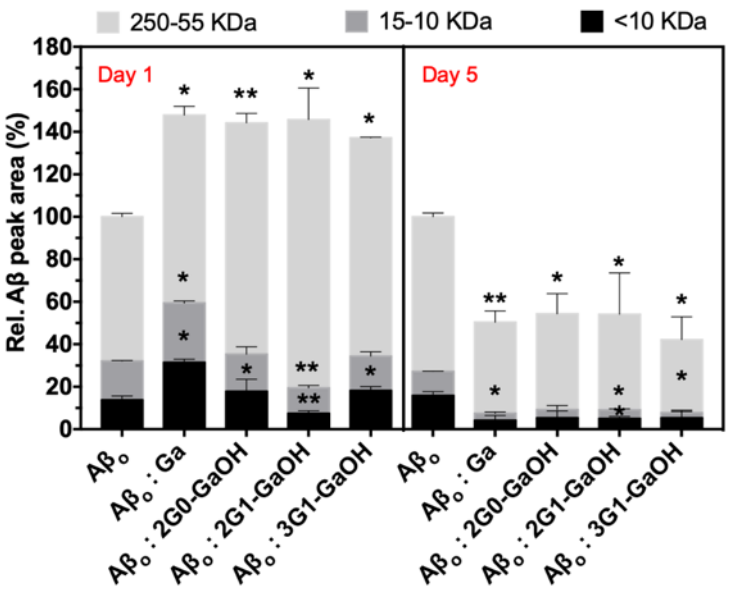


Figure S46. Relative densitometric bar graphs of $A\beta_o$ (25μM) assembly quantified by WB (using the antibody 6E10); experiments were executed at 37°C in PBS, during 1 and 5 days. Error bars = SD, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs control 25μM $A\beta_o$; n=3.

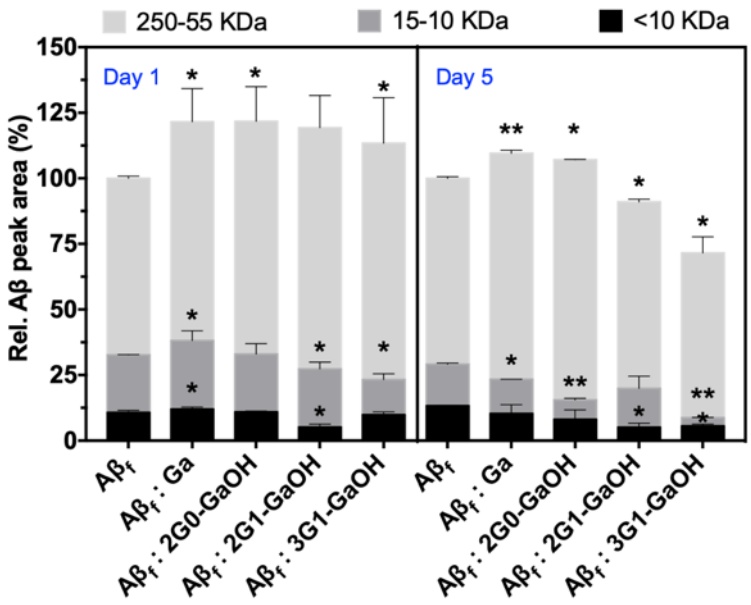


Figure S47. Relative densitometric bar graphs of $A\beta_f$ (25μM) disassembly quantified by WB (using the antibody 6E10); experiments were executed at 37°C in PBS, during 1 and 5 days. Error bars = SD, * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs control 25μM $A\beta_f$; n=3.

2 CELL STUDIES

Ability of the dendrimers to modulate the Aβ cytotoxicity

AlamarBlue® and Live/Dead assays

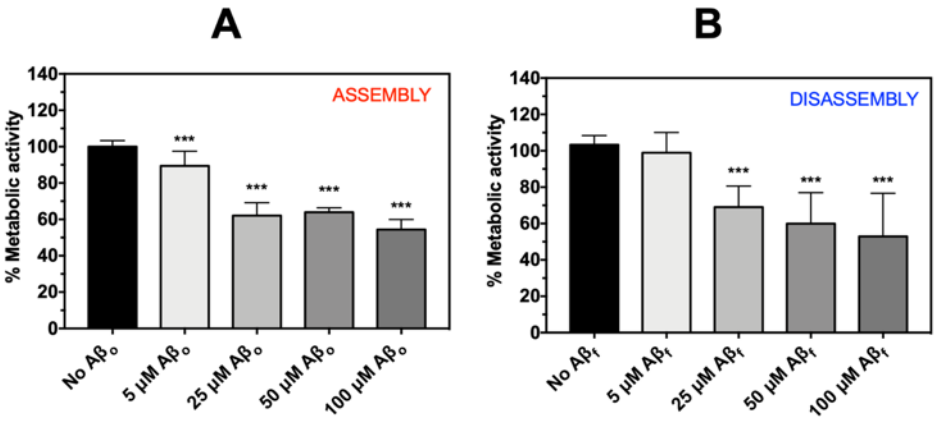


Figure S48. SH-SY5Y cell viability in the presence of (A) Aβ oligomers ($A\beta_o$, 25 μM) and (B) Aβ fibrils ($A\beta_f$, 25 μM) during 24h (using AlmarBlue® assay). Cells were incubated during 24h with different concentrations of Aβ. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (vs control); n=3.

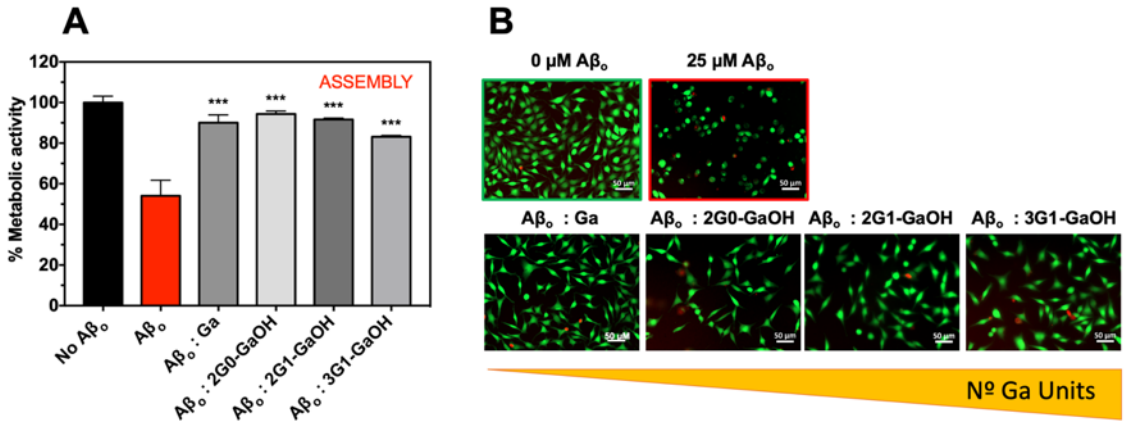


Figure S49. (A) Metabolic activity measured by AlamarBlue® assay of SH-SY5Y in the presence of a solution of $A\beta_o$ oligomeric species (25μM) during 24h, for a Aβ:dendrimers concentration ratio of 1:1. *** $p < 0.001$ (vs fibrillar Aβ); n=3; (B) Representative images of the Live/Dead assay. Scale bar = 50μm; n=3.

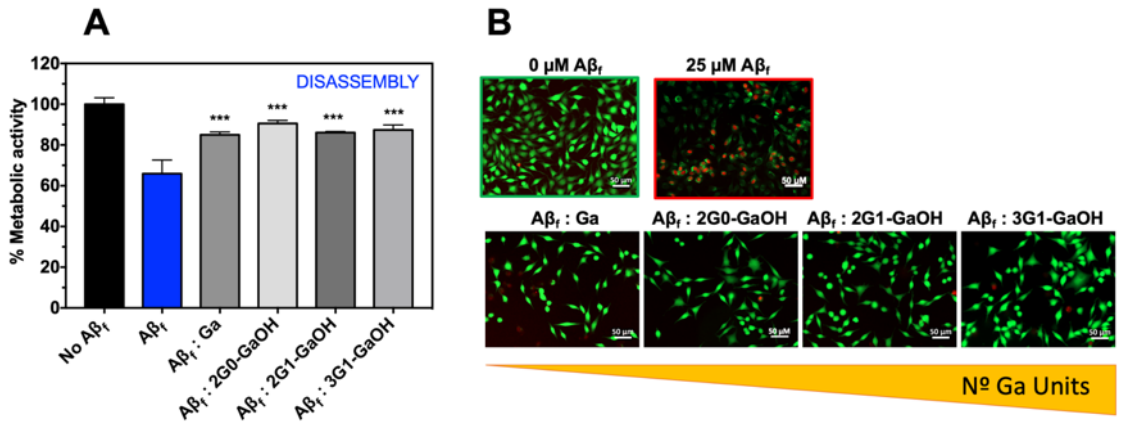


Figure S50. (A) Metabolic activity measured by AlamarBlue® assay of SH-SY5Y in the presence of a solution of $A\beta_f$ pre-formed fibrils (25μM) during 24h, for a Aβ:dendrimers concentration ratio of 1:1. *** $p < 0.001$ (vs No Aβ); n=3. (B) Representative images of the Live/Dead assay. Scale bar = 50μm; n=3.

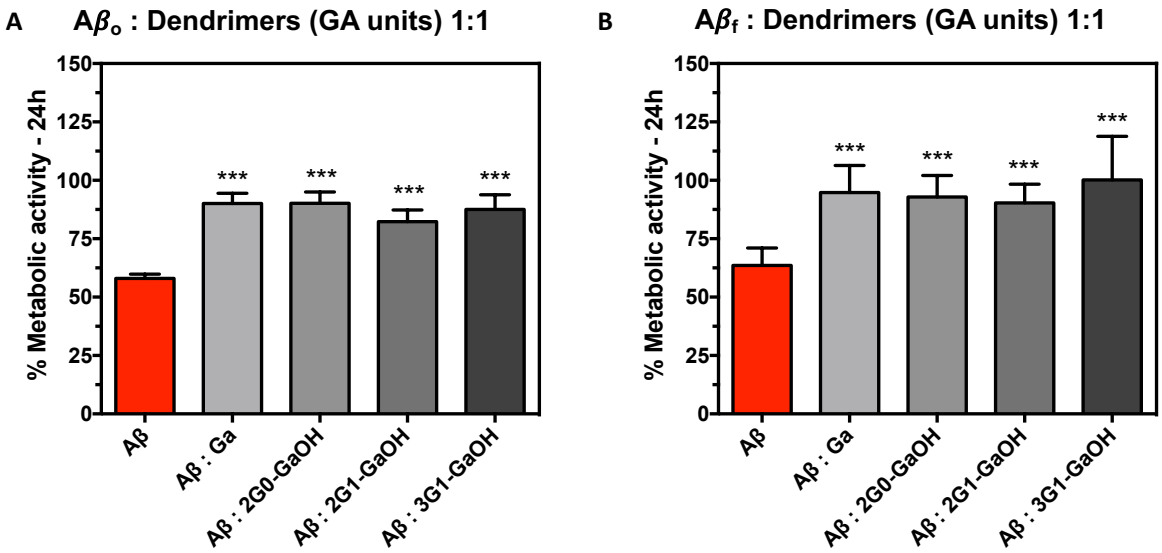


Figure S51. Metabolic activity measured by AlamarBlue® assay of SH-SY5Y in the presence of a solution of (A) $A\beta_o$ and (B) $A\beta_f$ pre-formed fibrils (25μM) during 24h. Experiments done at an Aβ:Ga molar ratio of 1:1. *** $p < 0.001$ (vs Aβ); n=3.

Quantification of the β-sheet content under cell culture determined by ThT assay

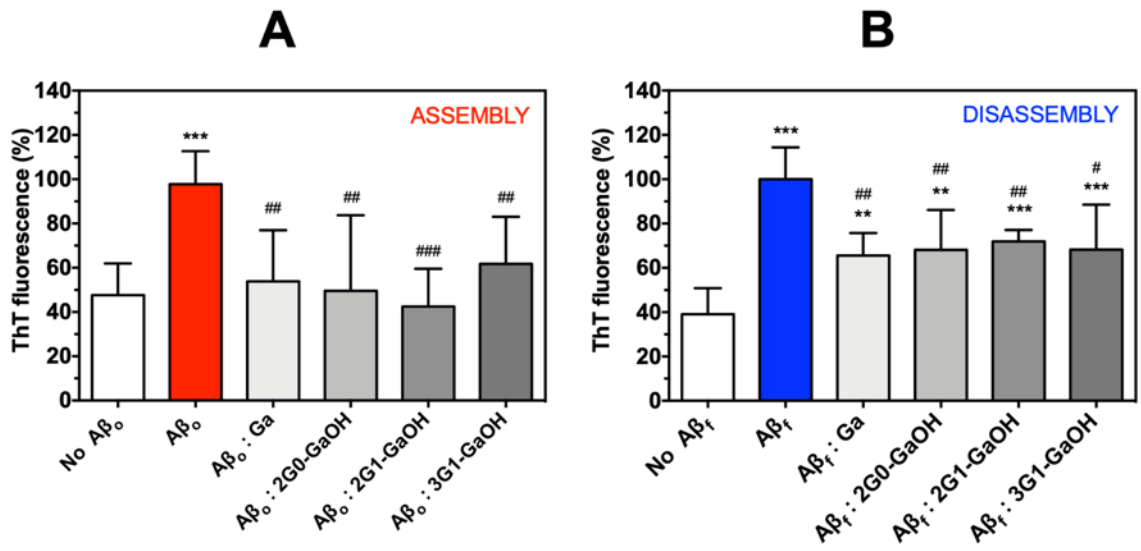


Figure S52. ThT fluorescence measurements in SH-SY5Y cell culture, after incubation with $A\beta_o$ (A) and $A\beta_f$ (B) species and dendrimers for a Aβ:dendrimers concentration ratio of 1:1, during 1 day of culture. Error bars = SD, $^{###}$ $p < 0.01$ (vs control 25μM Aβ); * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ vs No Aβ; n=3.

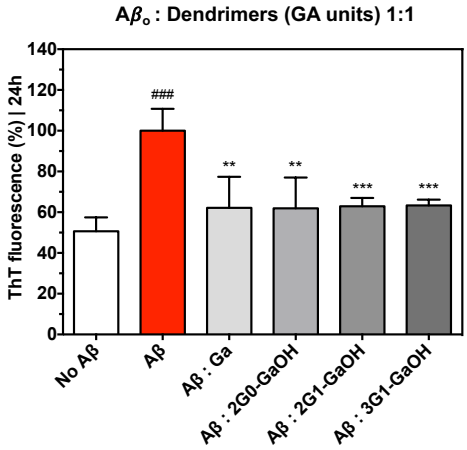


Figure S53. ThT fluorescence measurements in SH-SY5Y cell culture, after incubation with $A\beta_o$ species and dendrimers (Aβ:Ga molar ratio of 1:1), during 1 day of culture. Error bars = SD, $^{###}$ $p < 0.01$ (vs No Aβ); ** $p < 0.01$ and *** $p < 0.001$ vs control 25μM Aβ; n=3.

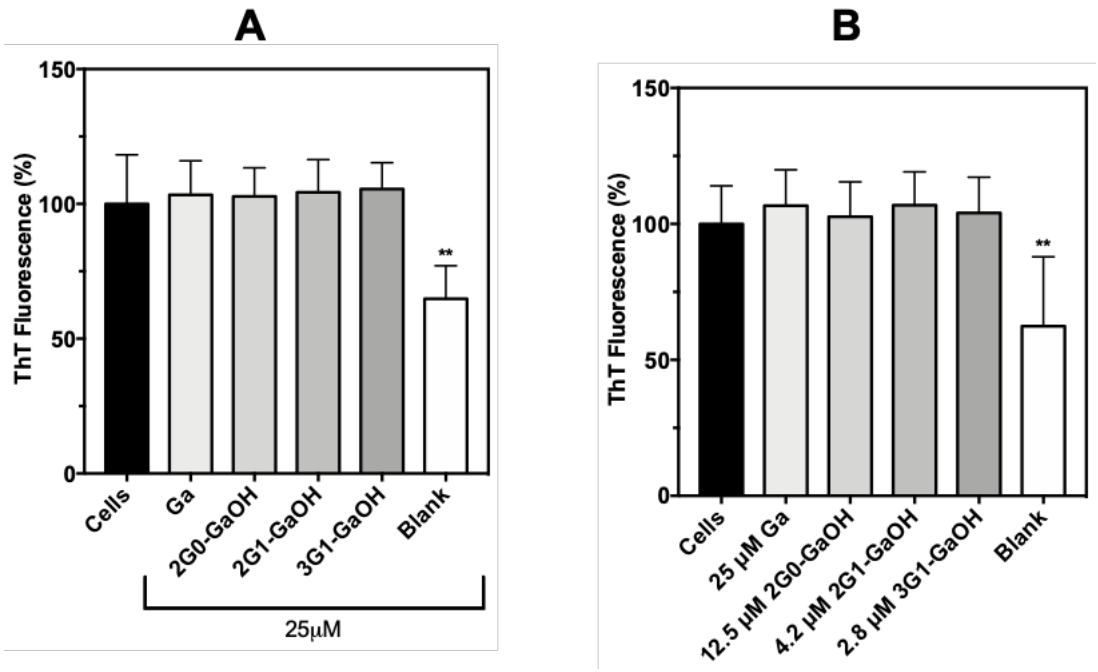


Figure S54. ThT fluorescence measurements in SH-SY5Y cell culture, after 1 day of incubation with A. solution of dendrimers at 25 μM concentration and B. solution of dendrimers at the concentrations used in the multivalency experiments (i.e., Aβ:Ga 1:1 molar ratio, per gallate). Error bars = SD, ** $p < 0.01$; n=3.

Protein expression

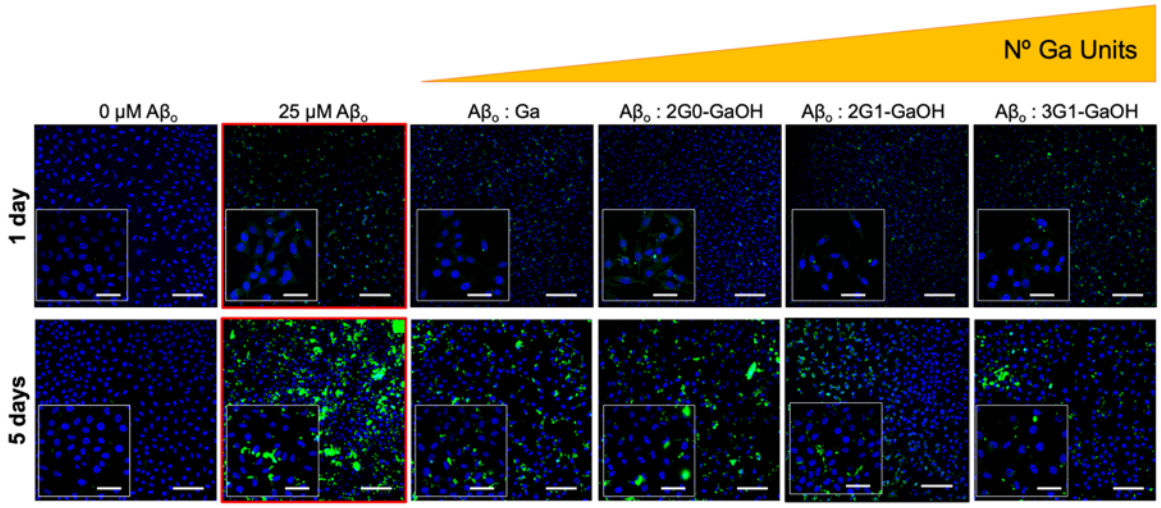


Figure S55. Immunofluorescence analysis of $A\beta_o$ species in the SH-SY5Y cell culture visualized by confocal microscopy (mAb 6E10, green) after incubation with dendrimers for a Aβ:dendrimer concentration ratio of 1:1, during 1 and 5 days (Aβ: green, cell nuclei: blue). Scale bar: 200μm (50μm for the insets).

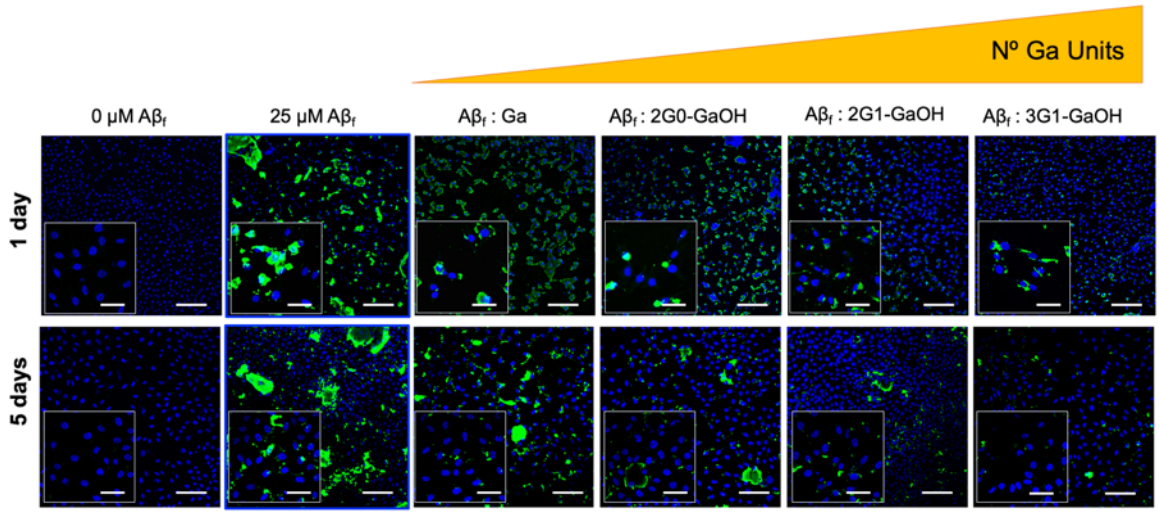


Figure S56. Immunofluorescence analysis of $A\beta_f$ species in the SH-SY5Y cell culture visualized by confocal microscopy (mAb 6E10, green) after incubation of dendrimers for a Aβ:dendrimers concentration ratio of 1:1, during 1 and 5 days (Aβ: green, cell nuclei: blue). Scale bar: 200 μm (50 μm for the insets).

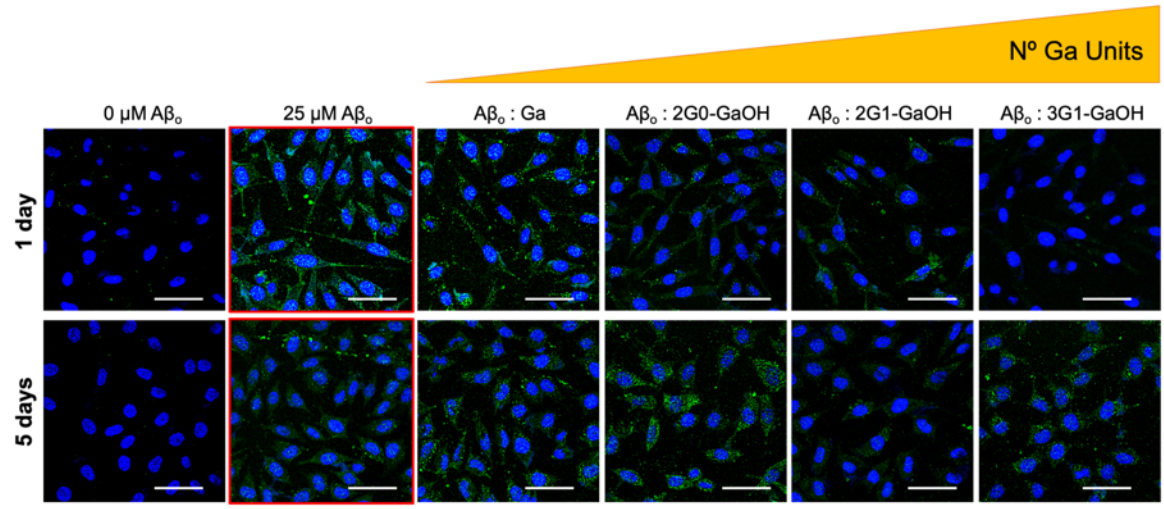


Figure S57. Immunofluorescence analysis of $A\beta_o$ species in the SH-SY5Y cell culture visualized by confocal microscopy (A11 antibody, green) after incubation of dendrimers for a Aβ:dendrimers concentration ratio of 1:1, during 1 and 5 days (Aβ: green, cell nuclei: blue). A11 antibody specifically bind to oligomeric Aβ species. Scale bar: 50μm.

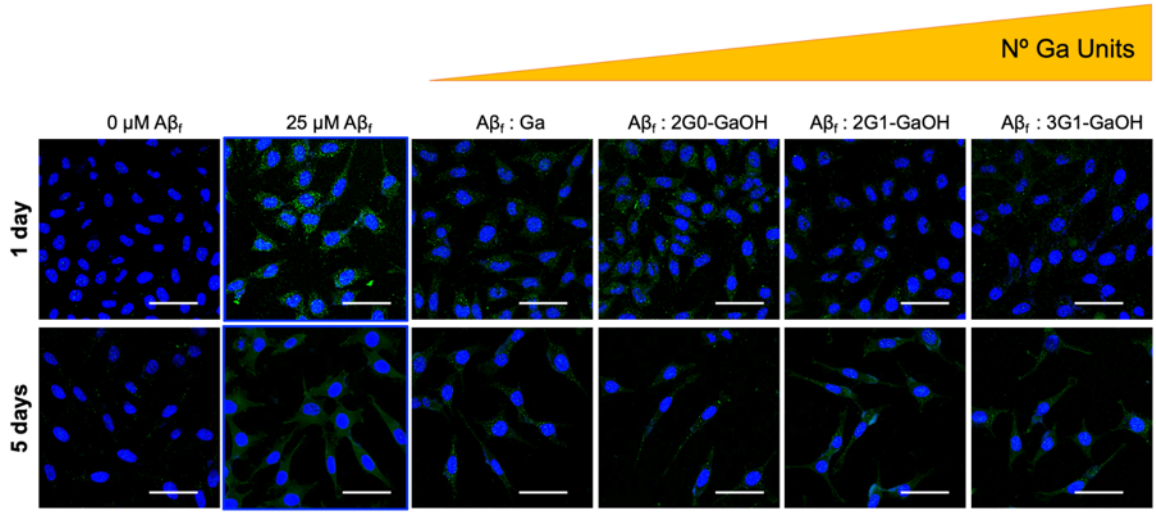


Figure S58. Immunofluorescence analysis of $A\beta_f$ species in the SH-SY5Y cell culture visualized by confocal microscopy (A11 antibody, green) after incubation of dendrimers to a 1:1 ratio, for 1 and 5 days (Aβ: green, cell nuclei: blue). A11 antibody specifically bind to oligomeric Aβ species. Scale bar: 50 μm.

Bio-AFM experiments

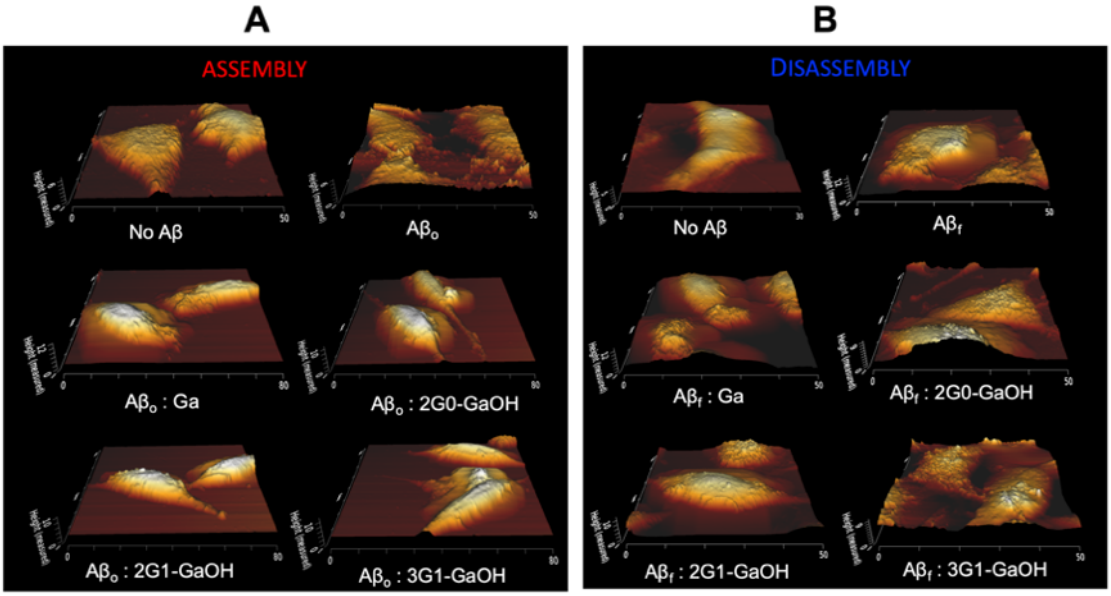


Figure S59. Representative AFM topographic images of SH-SY5Y cells cultured during 24h in the presence of (A) $A\beta_o$ (assembly) and (B) $A\beta_f$ (disassembly) and Ga, 2G0-GaOH, 2G1-GaOH and 3G1-GaOH using a Aβ:dendrimers ratio of 1:1.

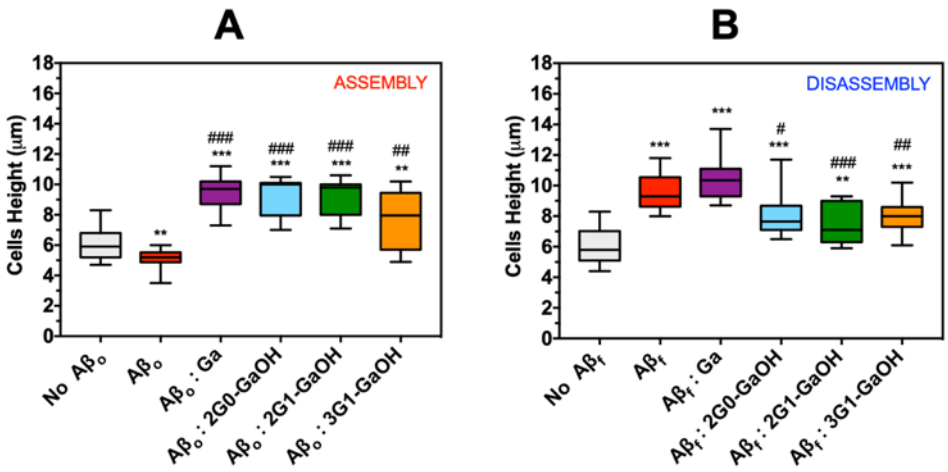


Figure S60. Statistical comparison of the cells height profile from the nanomechanical properties obtained by AFM topographic images of normal SH-SY5Y cells with 1:1 ratio of Aβ:dendrimers, under different conditions: (A) assembly pathway ($A\beta_o$) and (B) disassembly pathway ($A\beta_f$). All experiments were conducted after 24h of incubation with Aβ (25μM). Error bars = SD, $^{###}$ $p < 0.01$ (vs No Aβ); ** $p < 0.01$ and *** $p < 0.001$ vs control 25μM $A\beta_o$/$A\beta_f$; n=3.