

Supporting Information

Fluorimetric detection of insulin misfolding by probes derived from functionalized fluorene frameworks

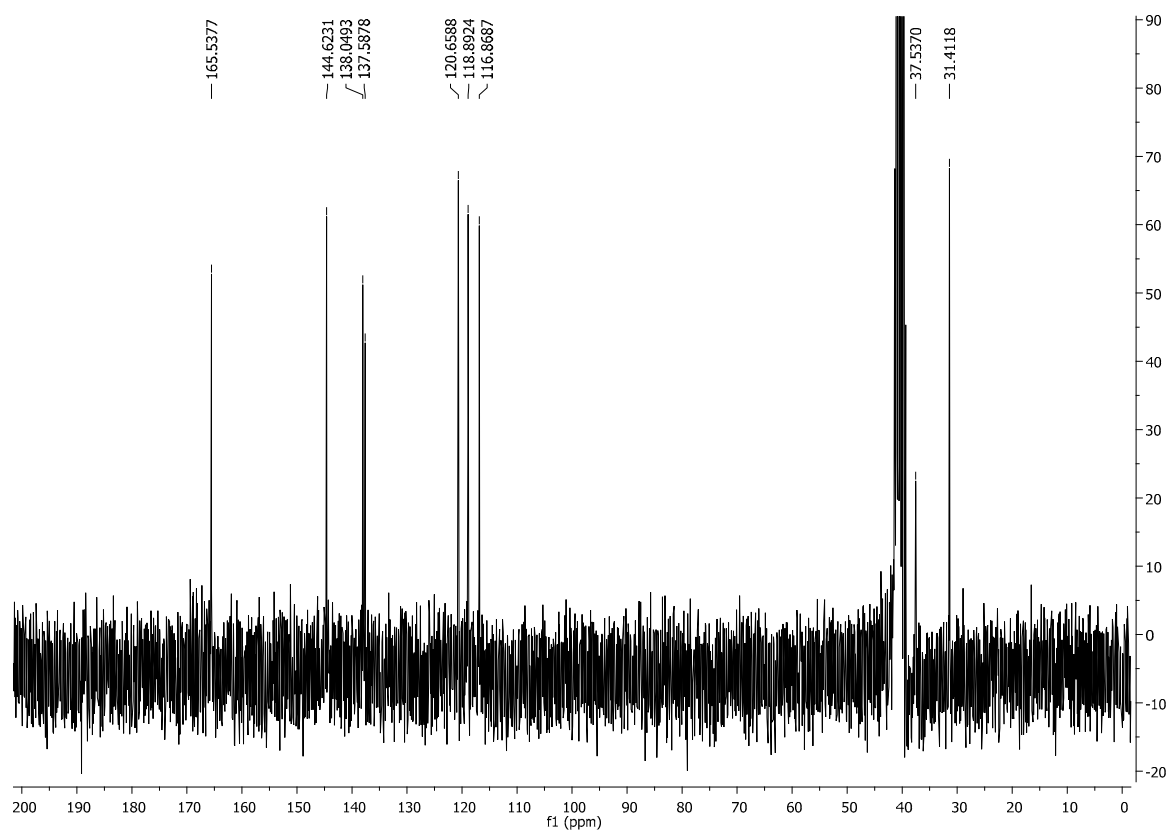
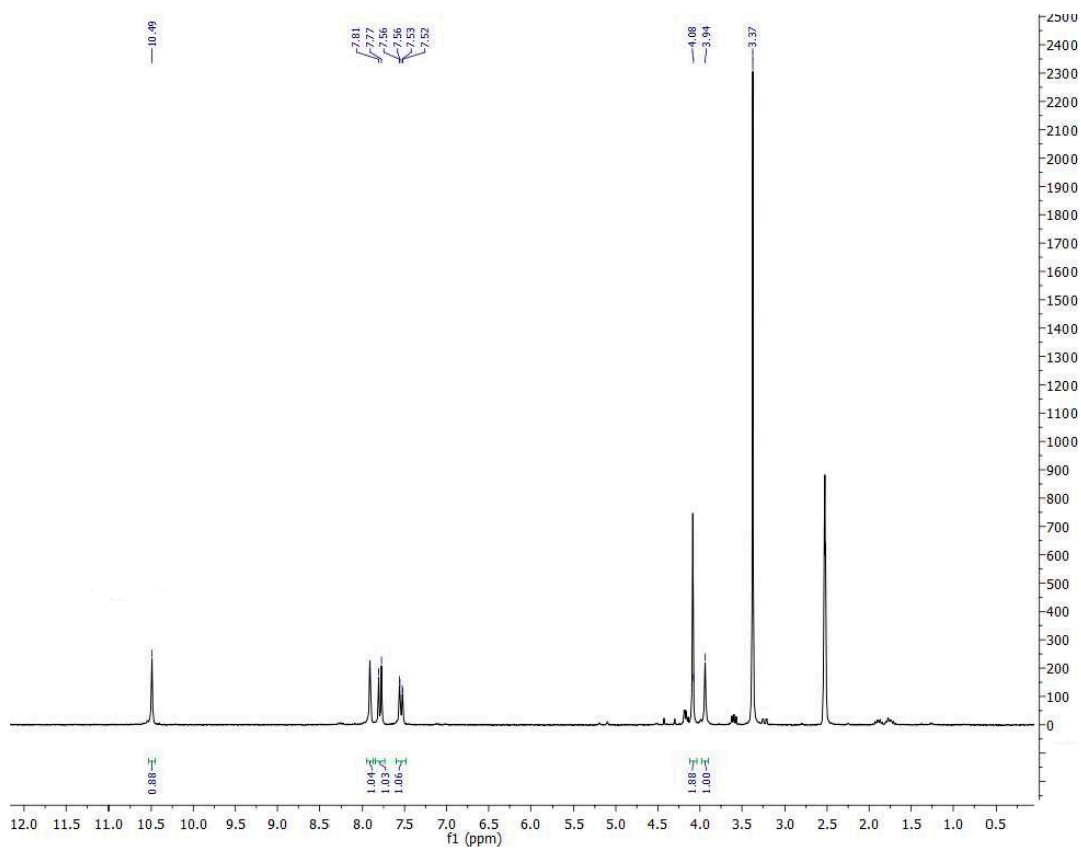
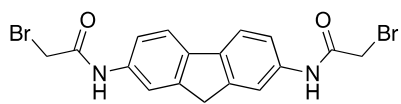
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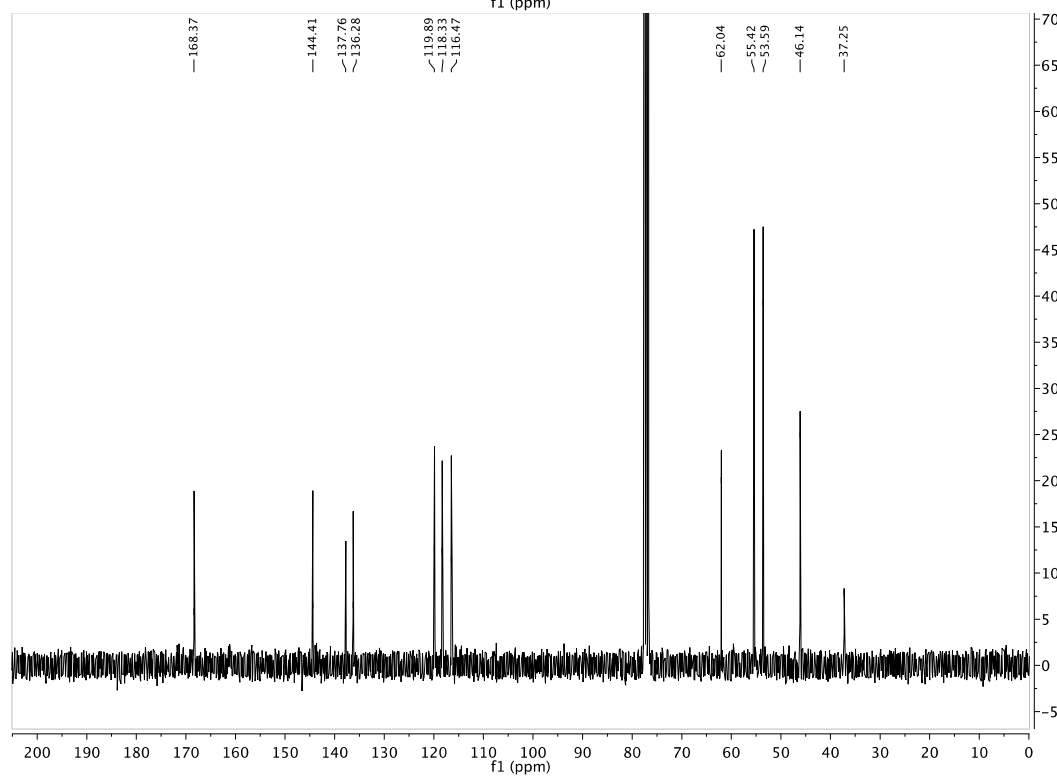
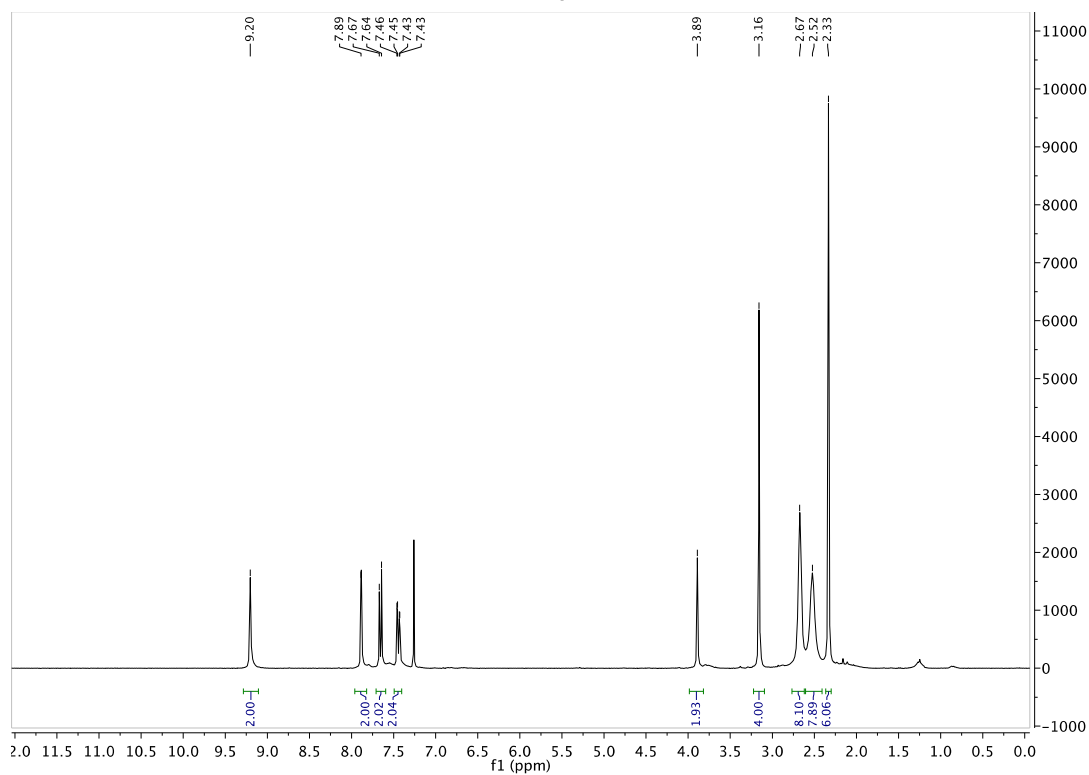
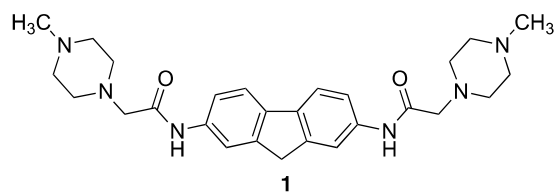
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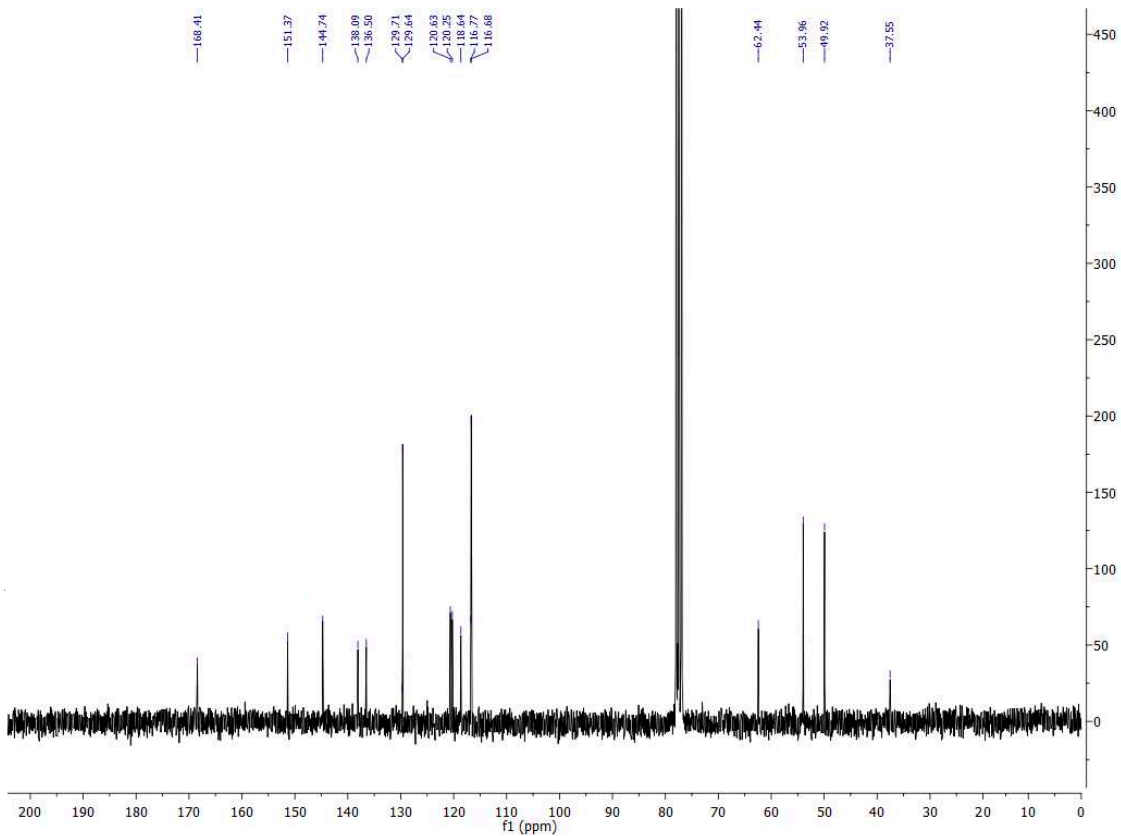
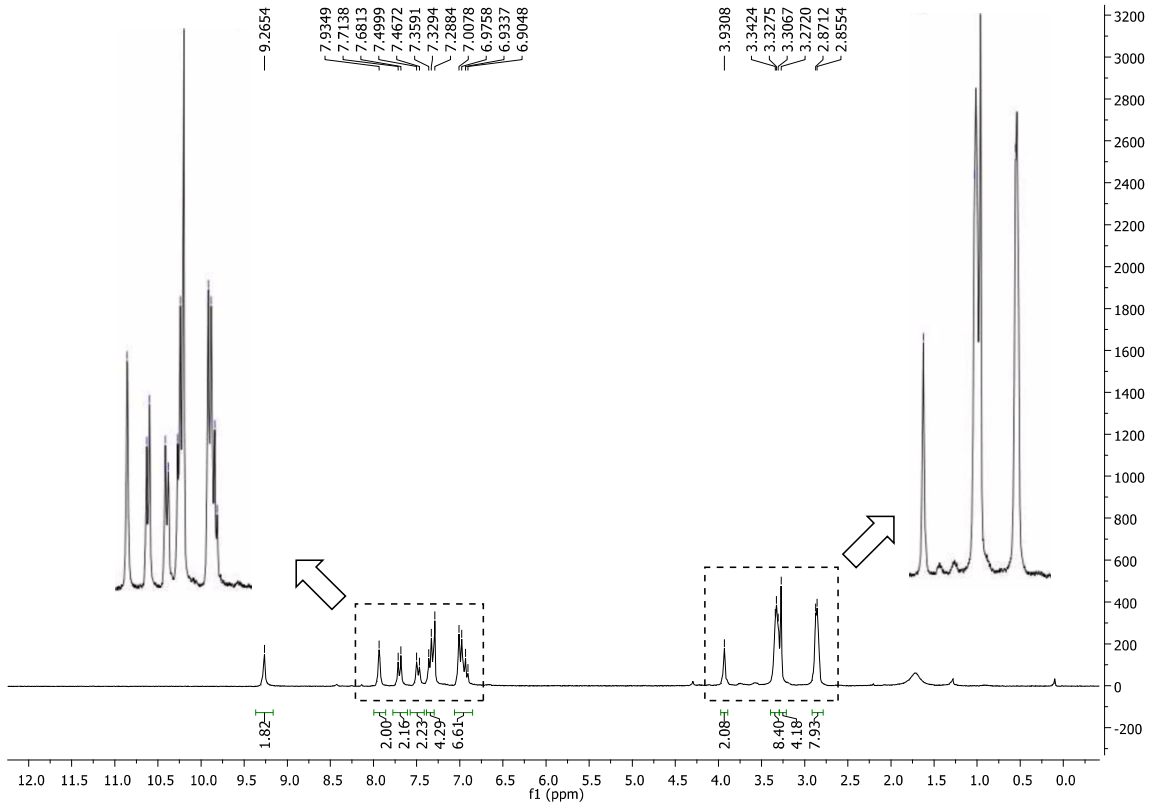
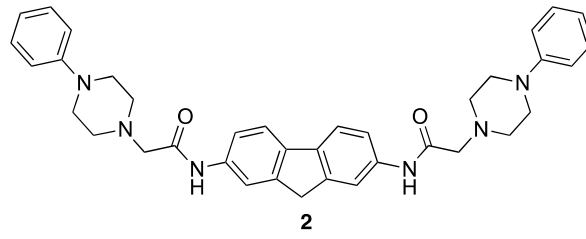
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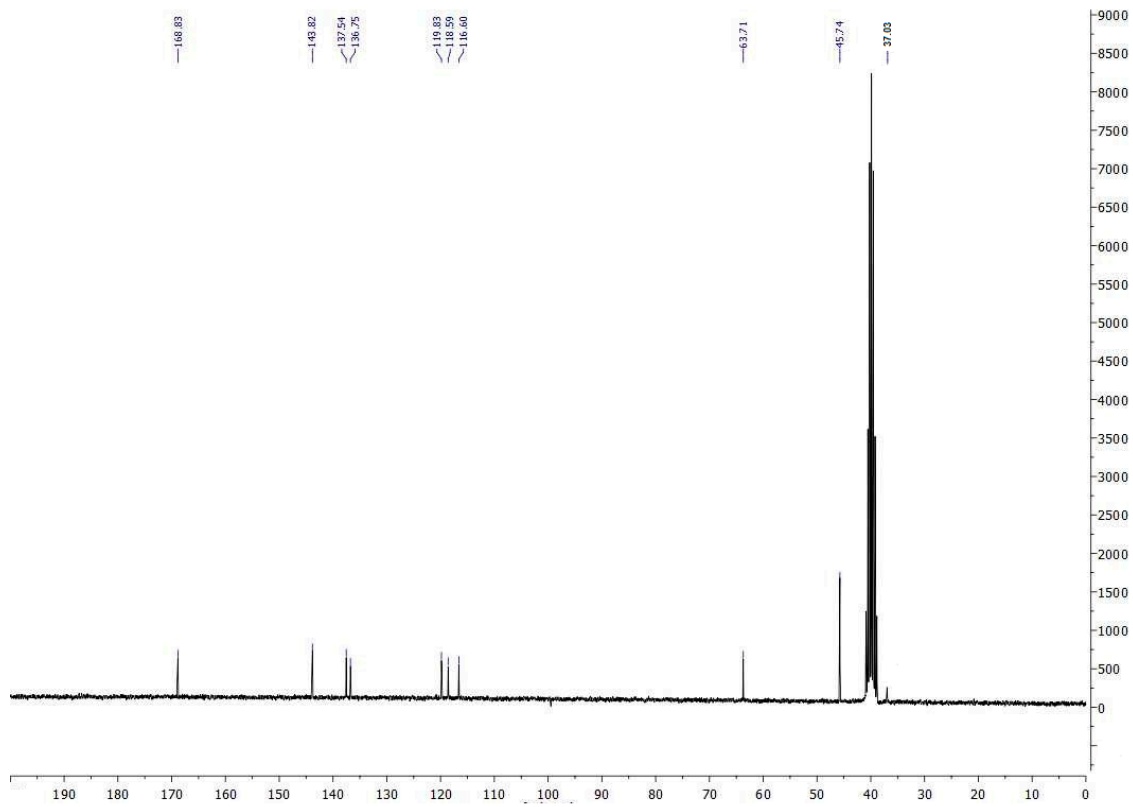
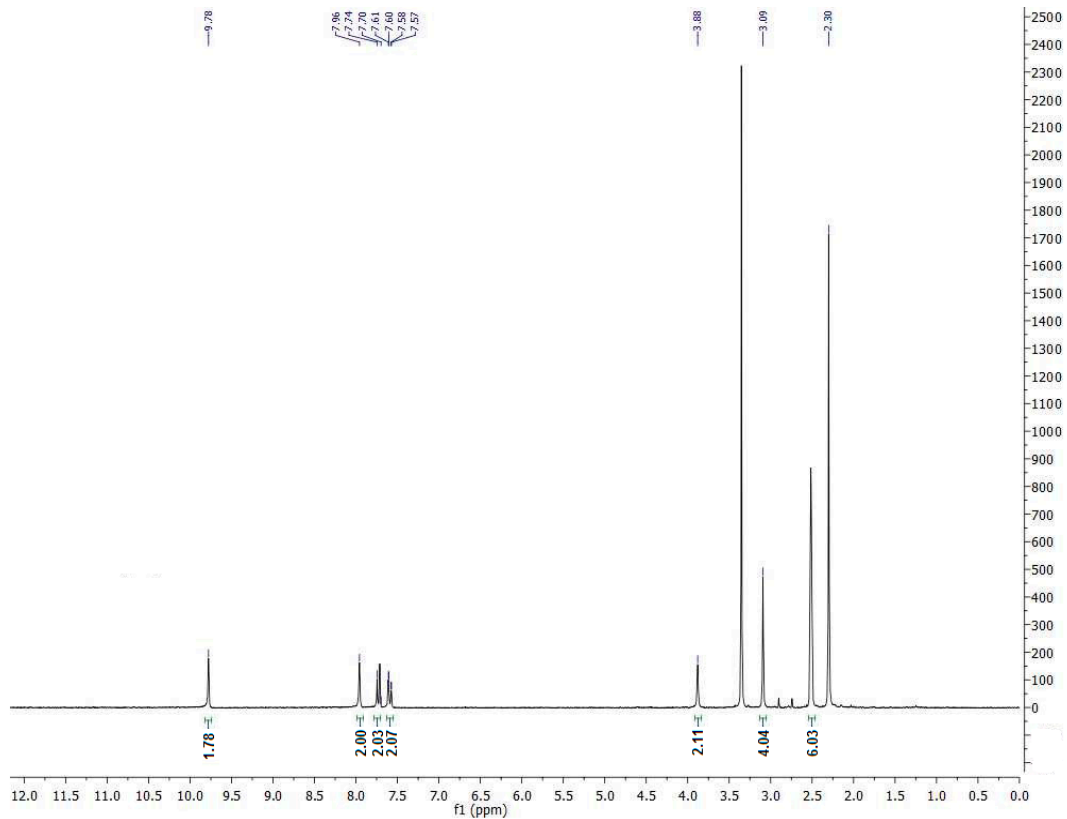
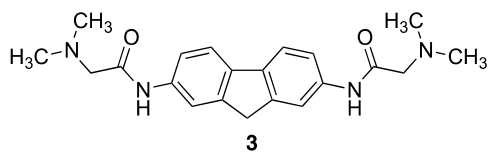
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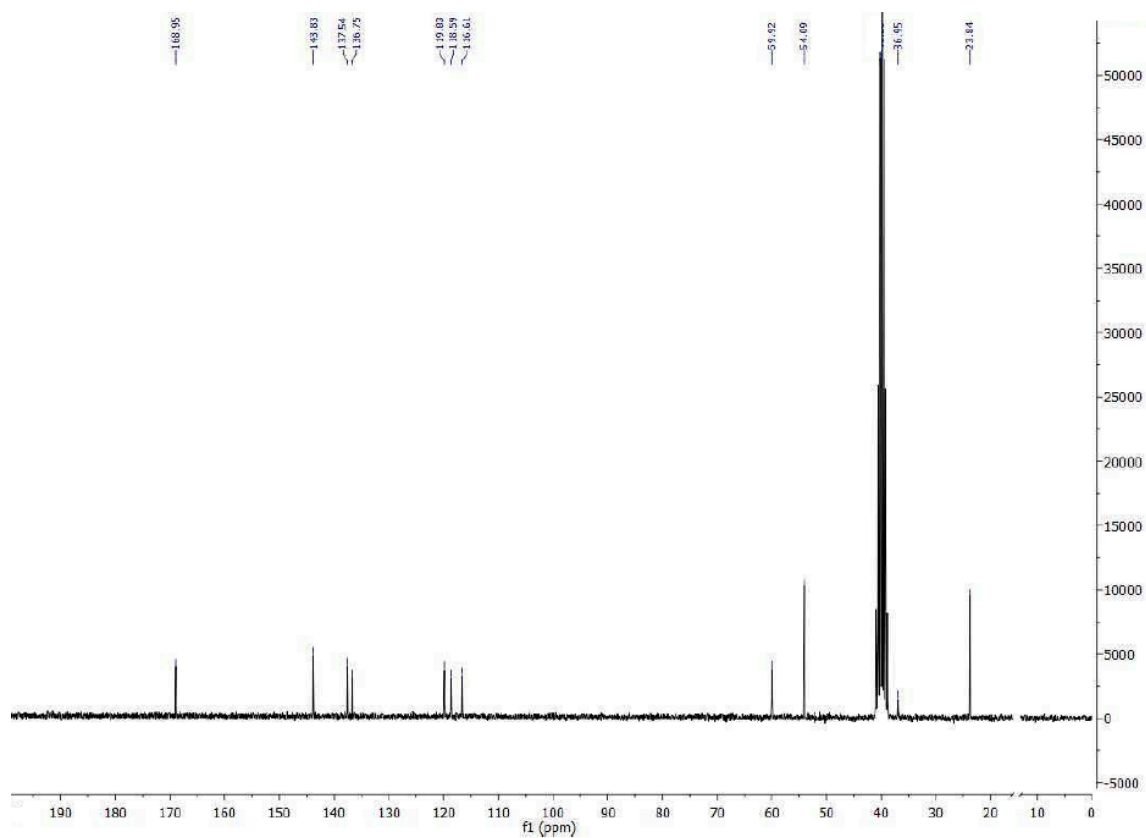
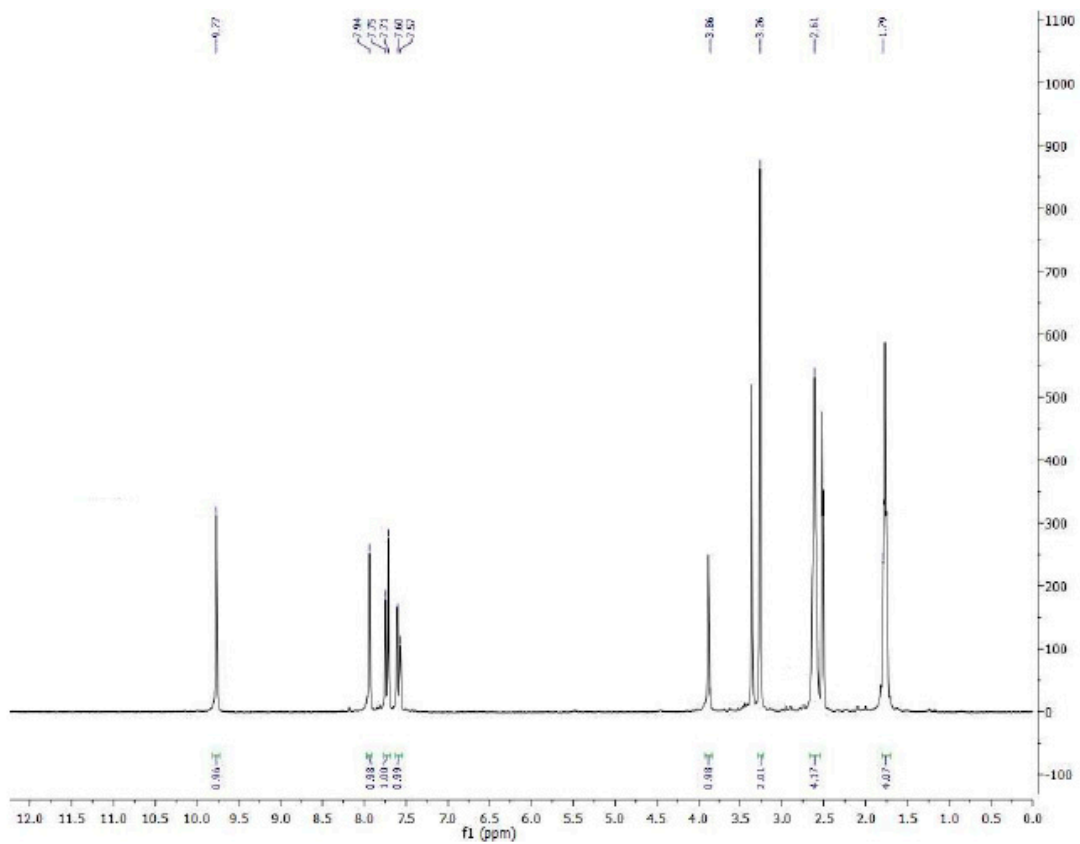
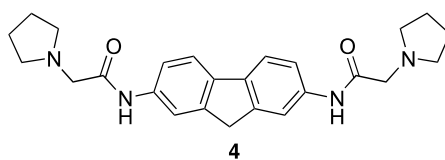
S1. Copies of NMR spectra

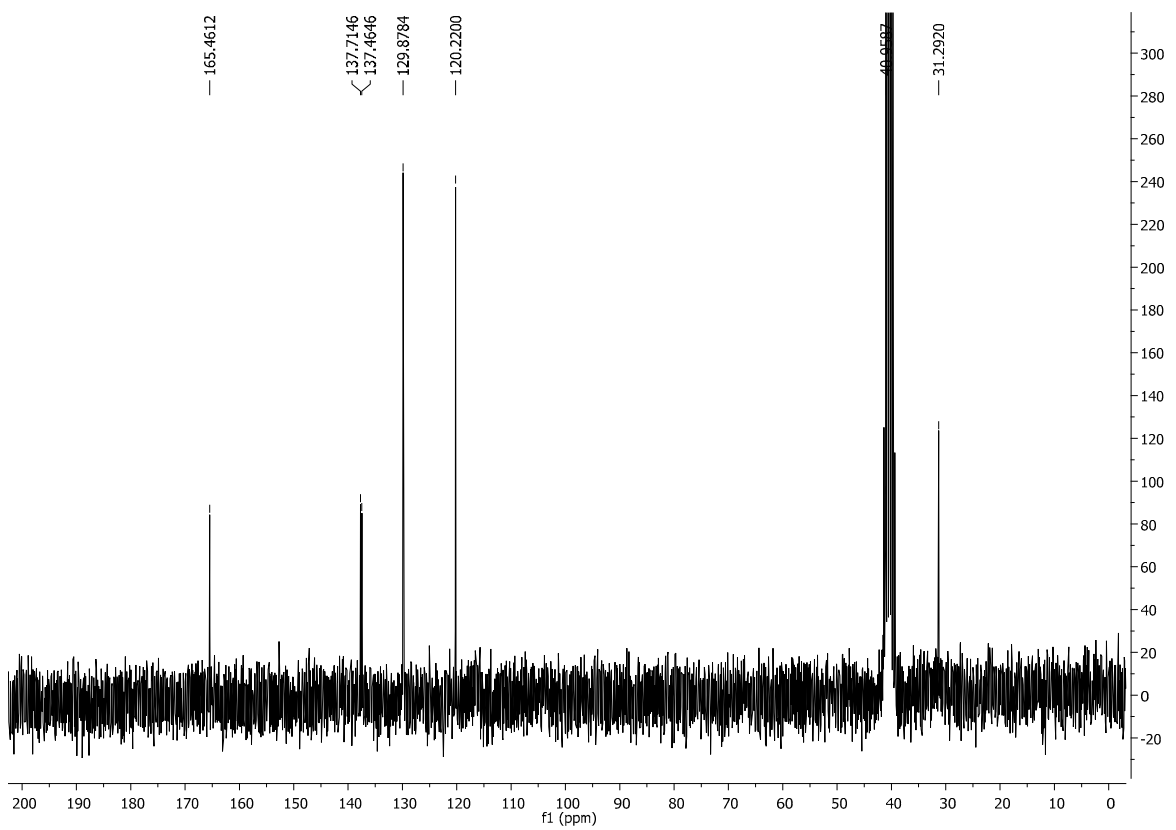
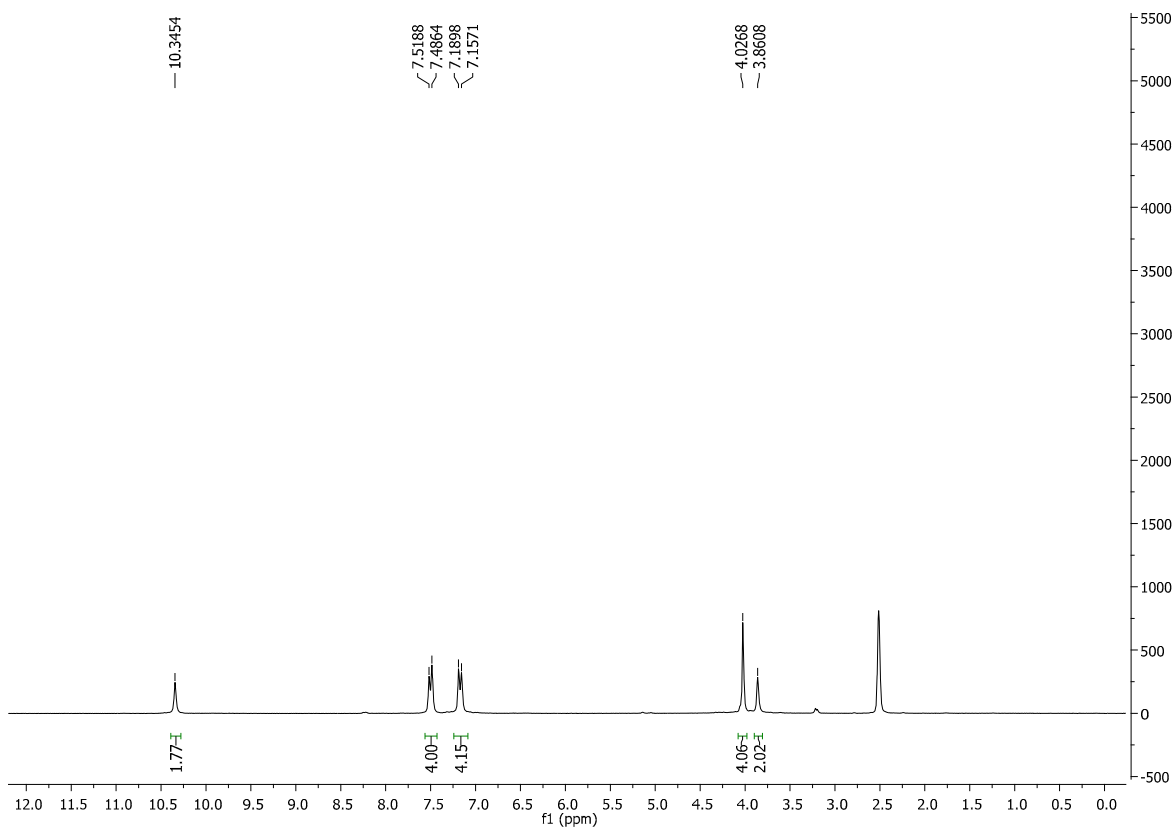
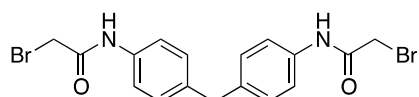


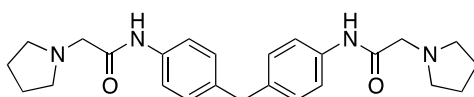




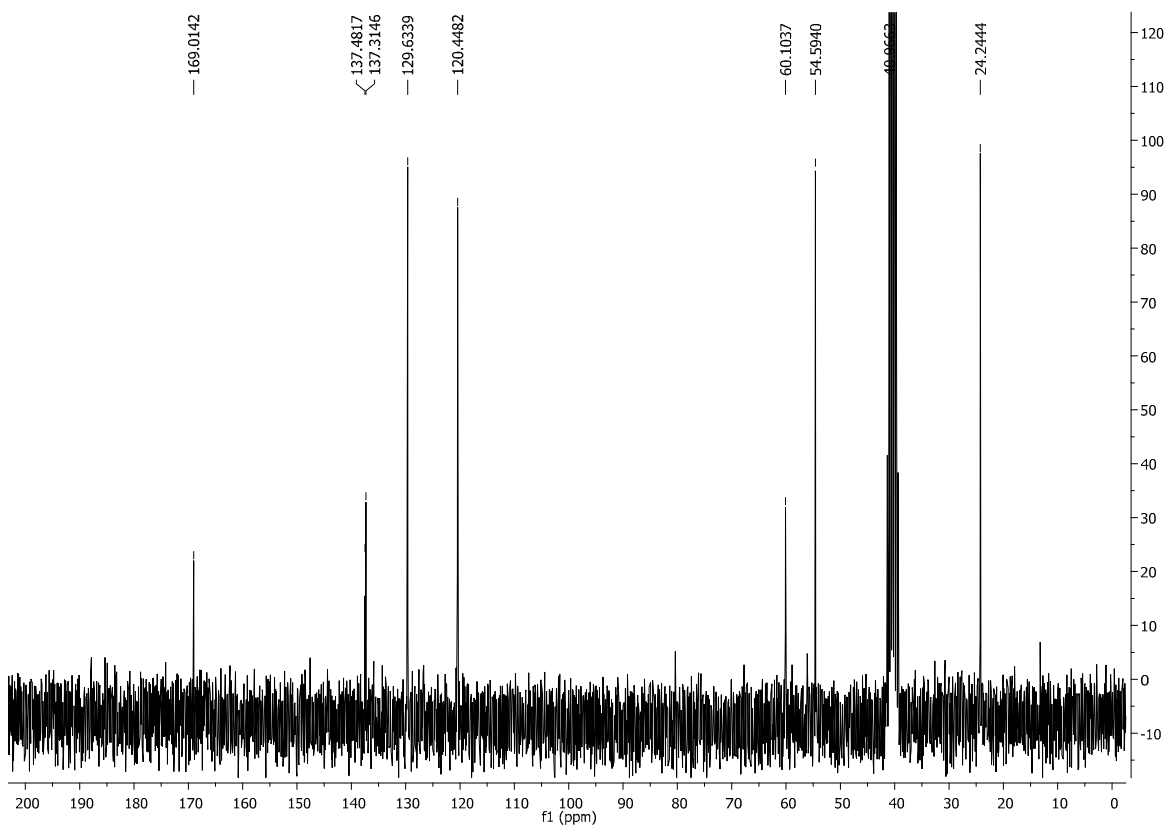
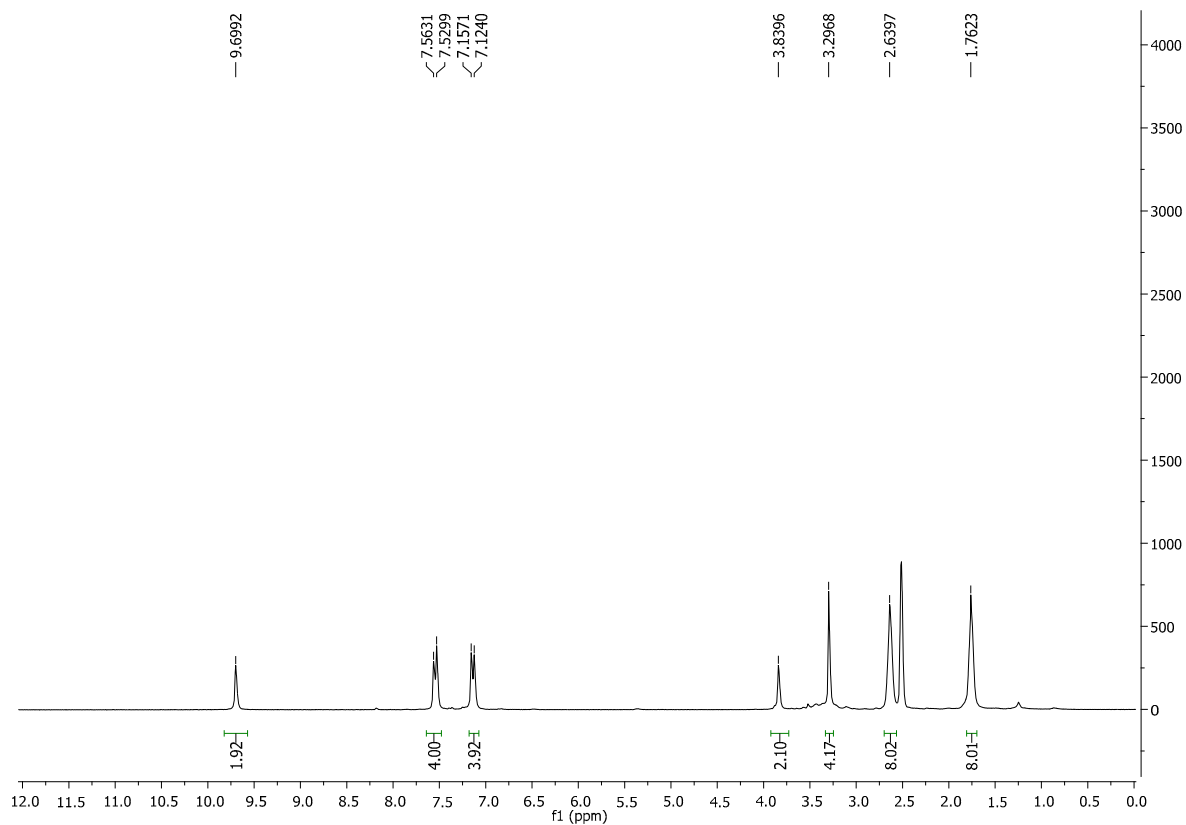








GN8



S2. UV-Vis and fluorescence experiments

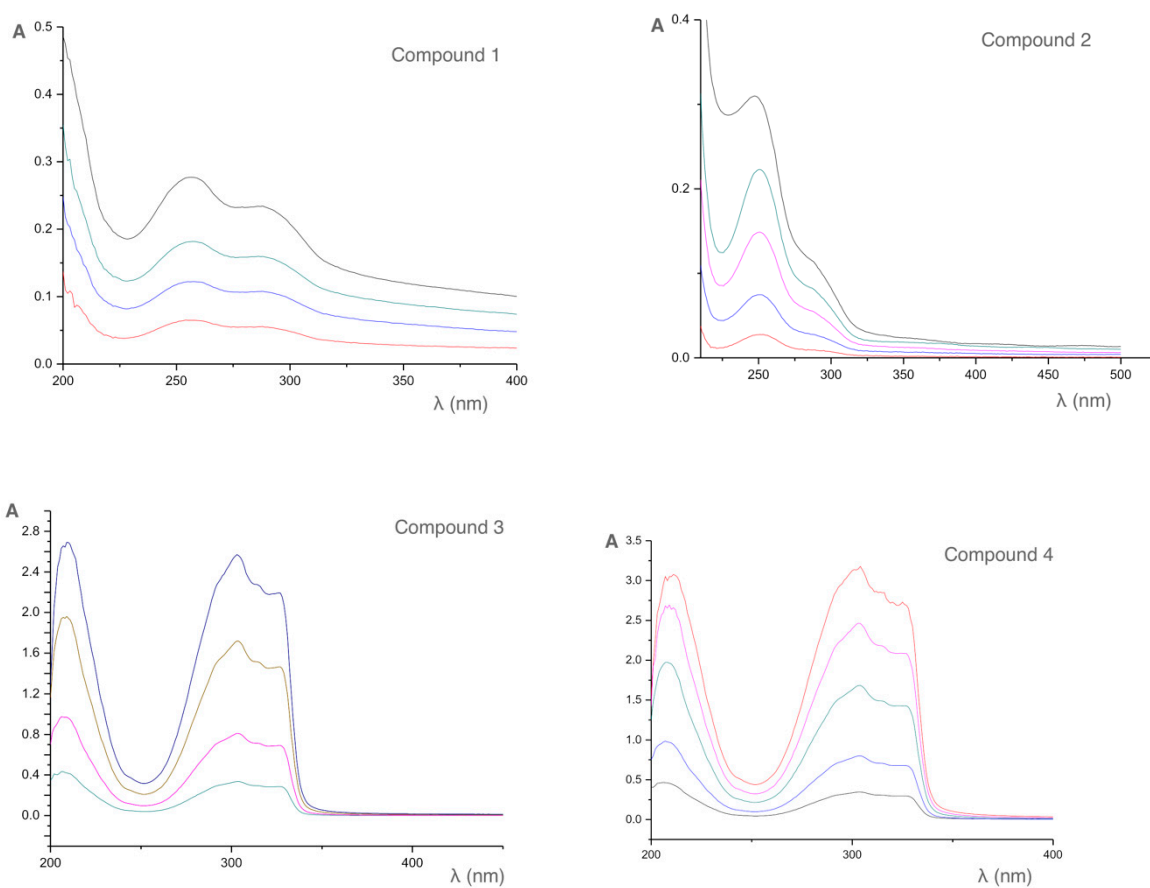


Figure S1: UV-Vis absorption spectra of fluorene sensors in ethanolic solution. Range of concentrations 1.0×10^{-5} M - 1.0×10^{-4} M.

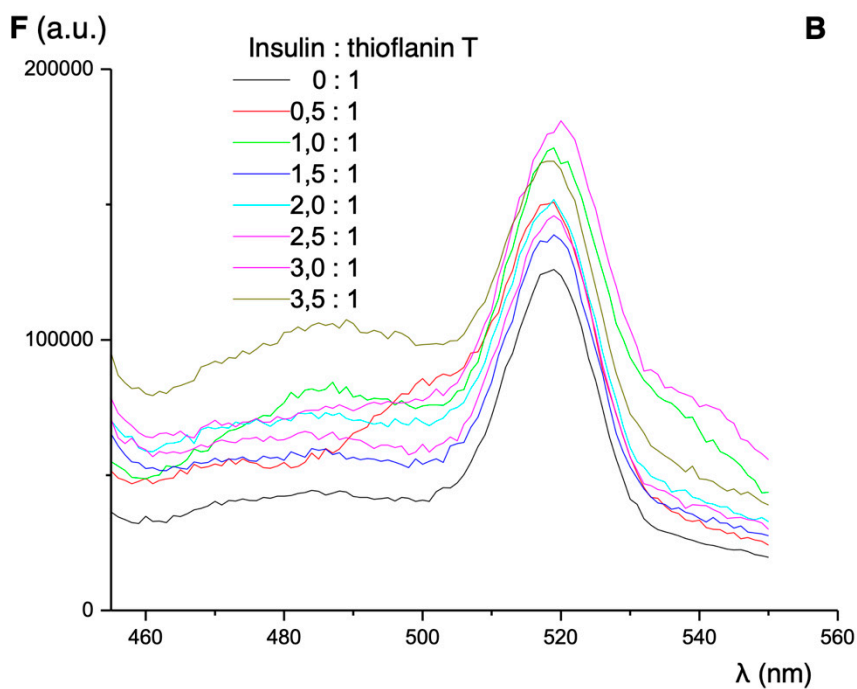
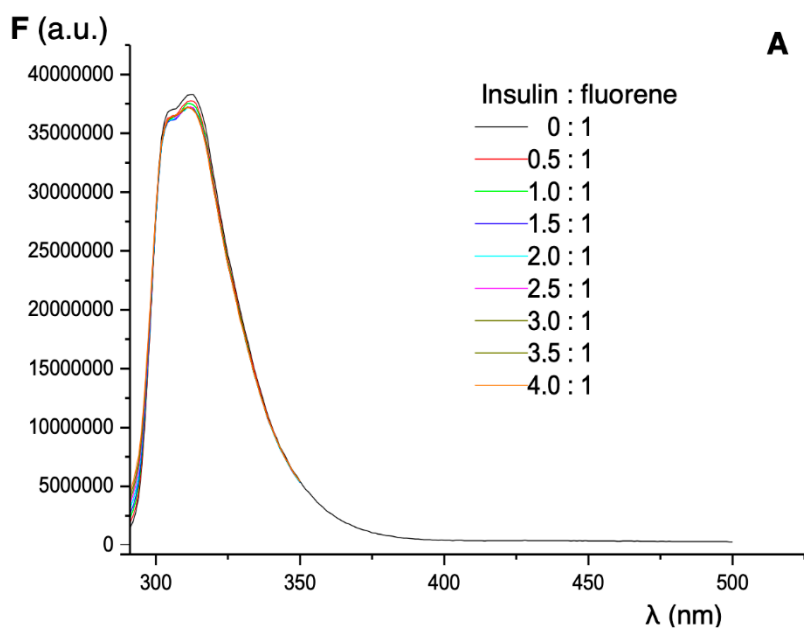


Figure S2: Effect of the additions of increasing volumes of native insulin on the fluorescence emission spectra of reference compounds. A. fluorene at $\lambda_{\text{ex}} = 266$ nm and B. Thioflavin T at $\lambda_{\text{ex}} = 440$ nm. Protein:sensor molar ratios are in the inset. The peak at 520 nm corresponds to Raman dispersion.

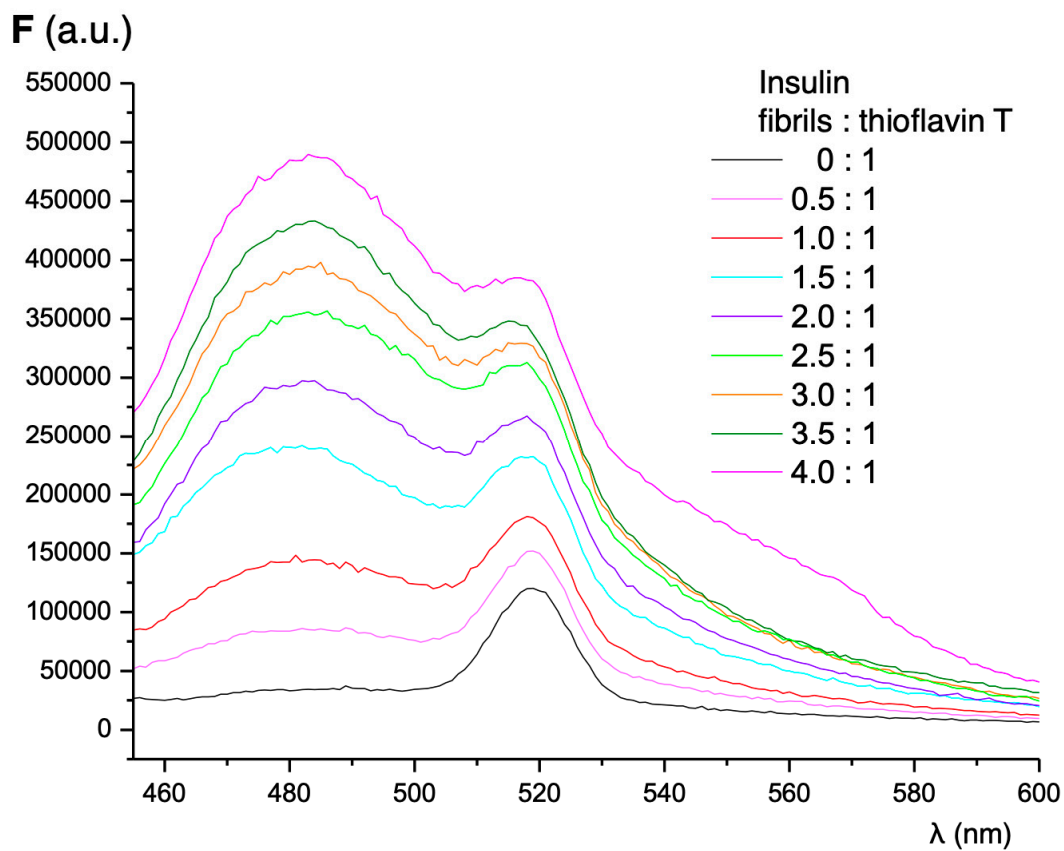


Figure S3: Effect of the additions of increasing volumes of insulin fibrils/aggregates on the fluorescence emission spectra of reference compound thioflavin T at $\lambda_{\text{ex}} = 440 \text{ nm}$ employing method A for protein aggregation. Protein:sensor molar ratios are in the inset. The peak at 520 nm corresponds to Raman dispersion.

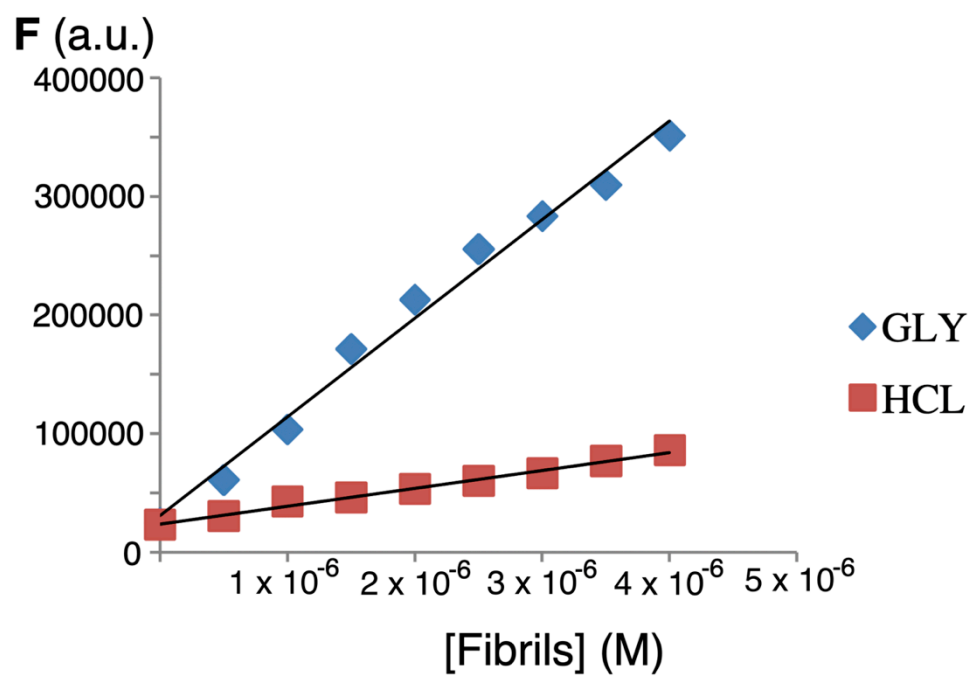


Figure S4: Comparison of the increase in the fluorescence intensity of the reference compound thioflavin T ($\lambda_{\text{ex}} = 440$; $\lambda_{\text{em}} = 490$ nm) employing method A (GLY) and method B (HCL) for inducing insulin β -amyloid protein aggregation.

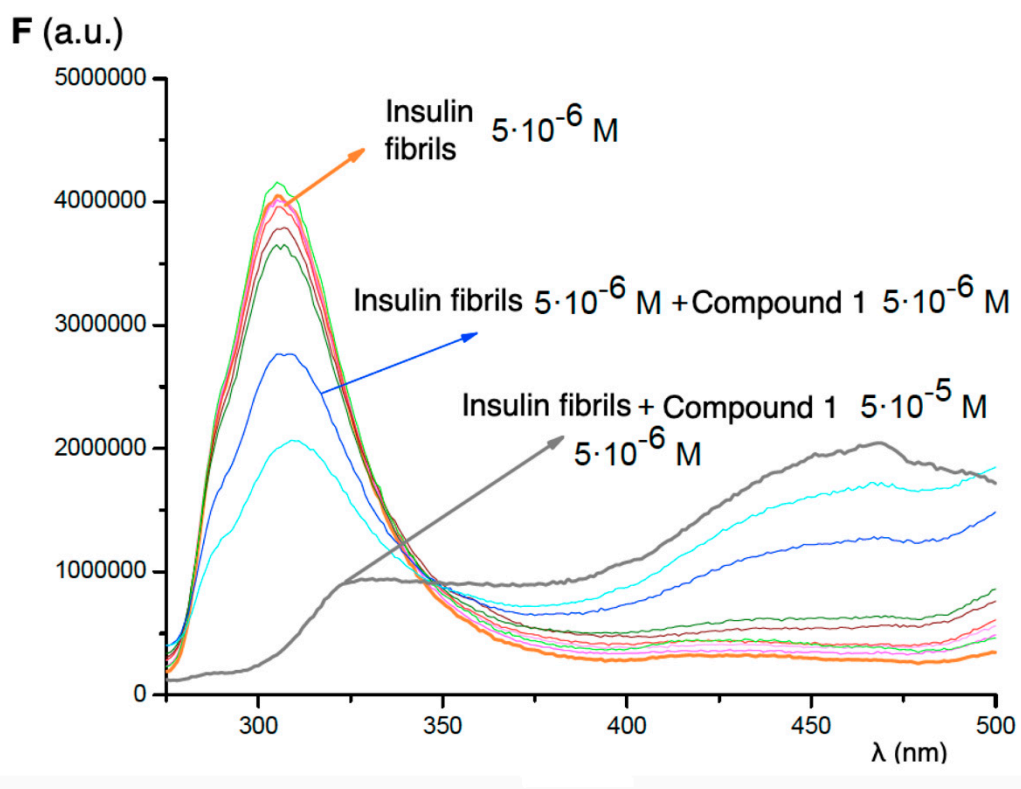


Figure S5: Fluorescence emission spectra of compound **1** (concentration varies from 0 to 5×10^{-5} M) at $\lambda_{\text{ex}} = 260$ nm and fixed concentration of amyloid fibrils of insulin (5×10^{-6} M).

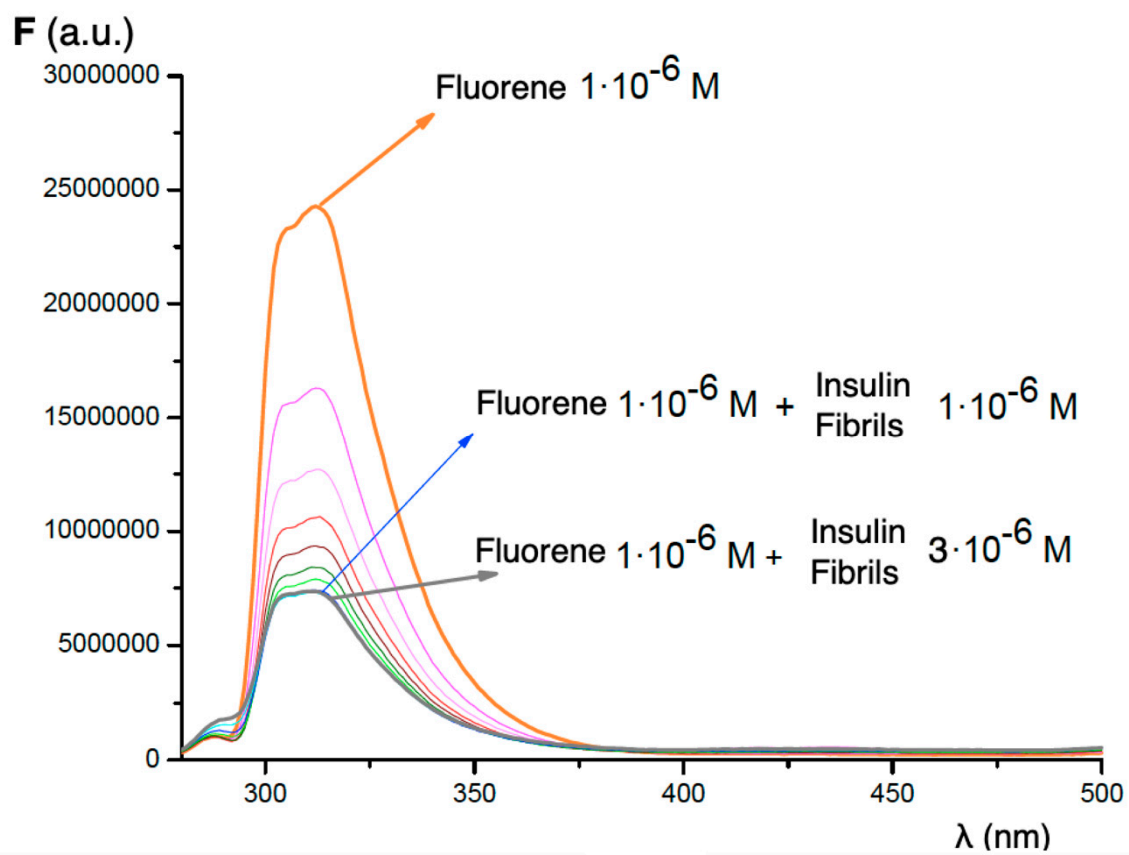


Figure S6: Fluorescence emission spectra of fluorene (5×10^{-6} M) in the presence of increasing concentrations of amyloid fibrils of insulin (concentration varies from 0 to 3×10^{-6} M) at $\lambda_{\text{ex}} = 261$ nm.

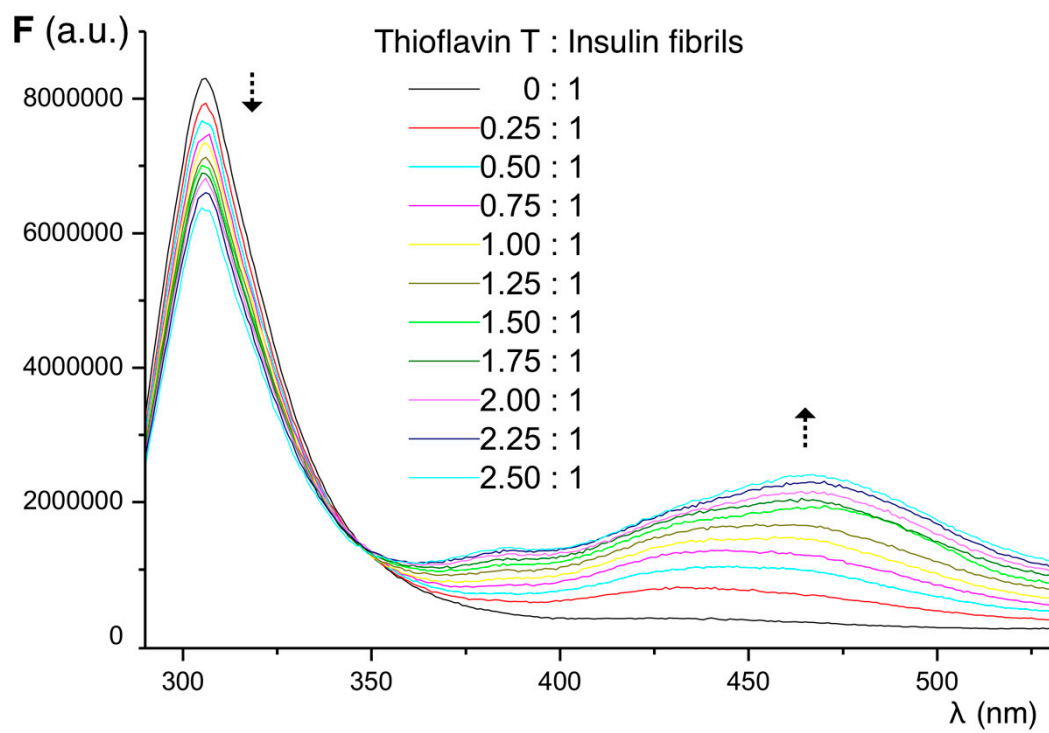


Figure S7: Fluorescence of amyloid insulin quenched by thioflavin T. Amyloid insulin fibrils obtained according to method A. Concentration of insulin fibrils 5×10^{-6} M. The concentration of thioflavin varies from 0 to 12.5×10^{-6} M; at $\lambda_{\text{ex}} = 275$ nm.

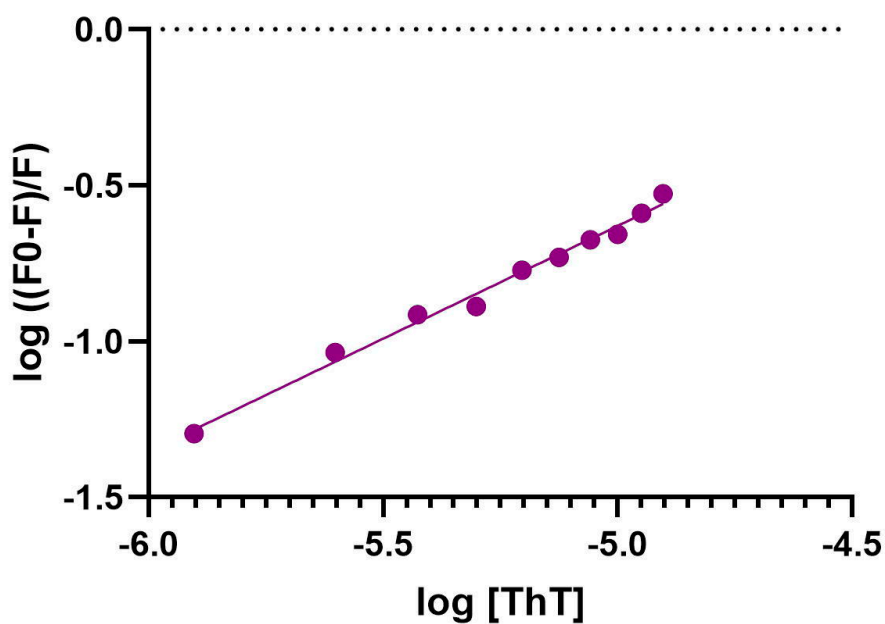


Figure S8. Calculation of the binding constant for the quenching by thioflavin T of the amyloid insulin fluorescent emission at 305 nm

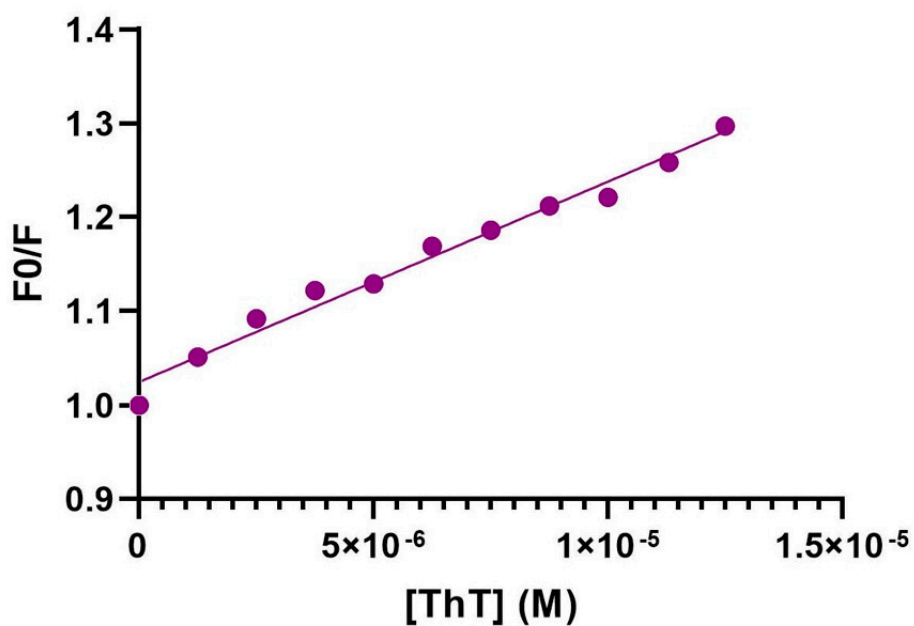


Figure S9. Calculation of the Stern-Volmer constant (K_{SV}) for the quenching by thioflavin T of the amyloid insulin fluorescent emission at 305 nm

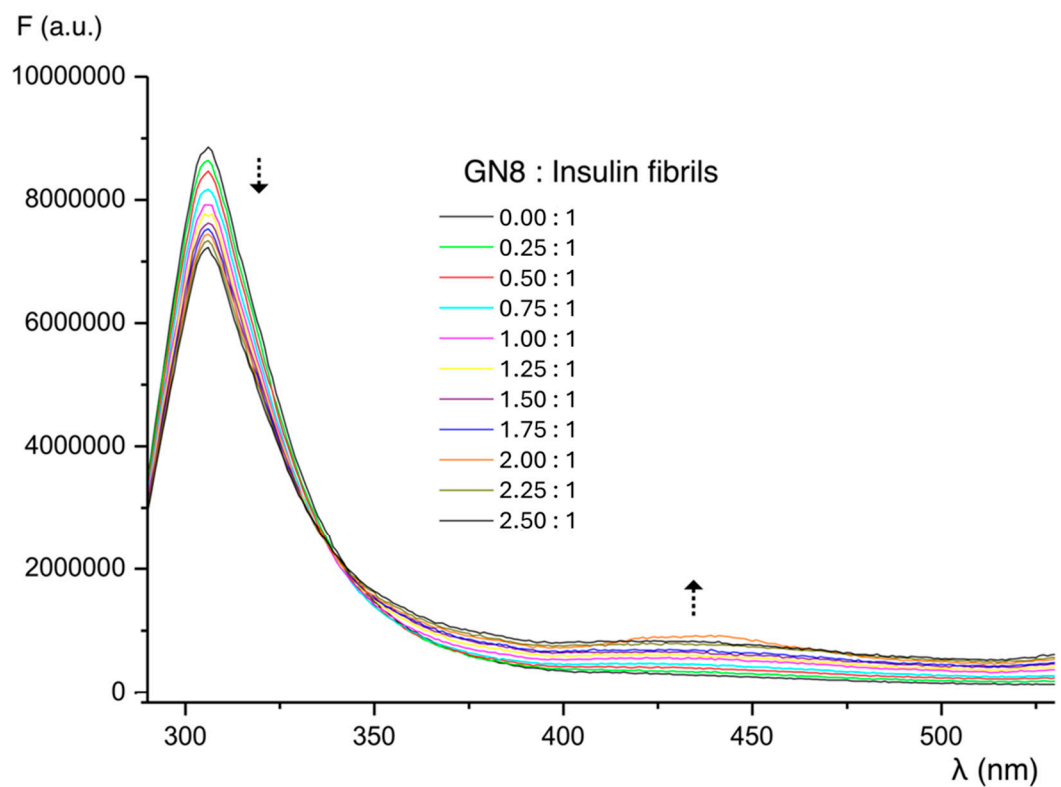


Figure S10: Fluorescence of amyloid insulin quenched by GN8. Amyloid insulin fibrils obtained according to method A. Concentration of insulin fibrils 5×10^{-6} M. Concentration of GN8 varies from 0 to 12.5×10^{-6} M; at $\lambda_{\text{ex}} = 275$ nm.

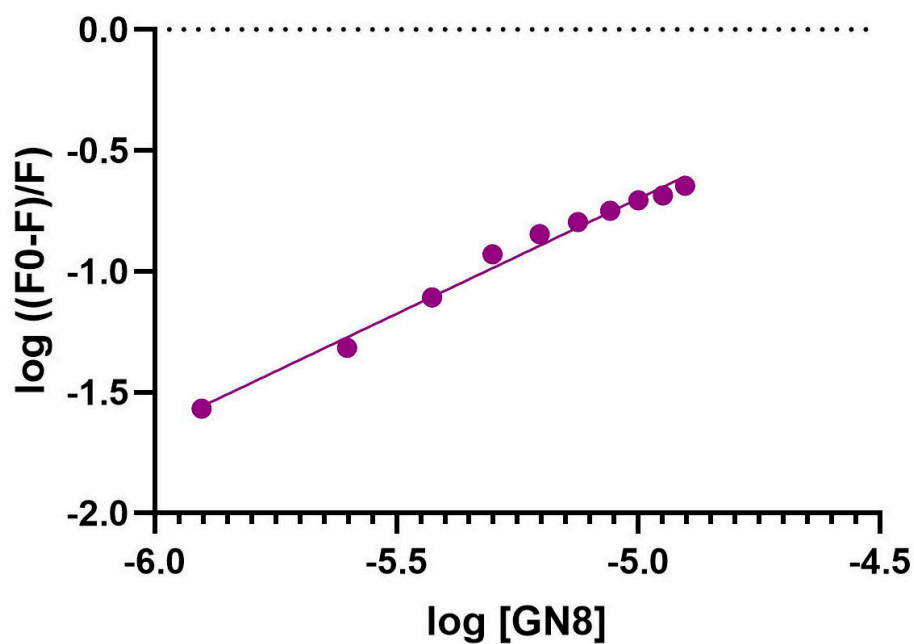


Figure S11. Calculation of the binding constant for the quenching by GN8 of the amyloid insulin fluorescent emission at 305 nm

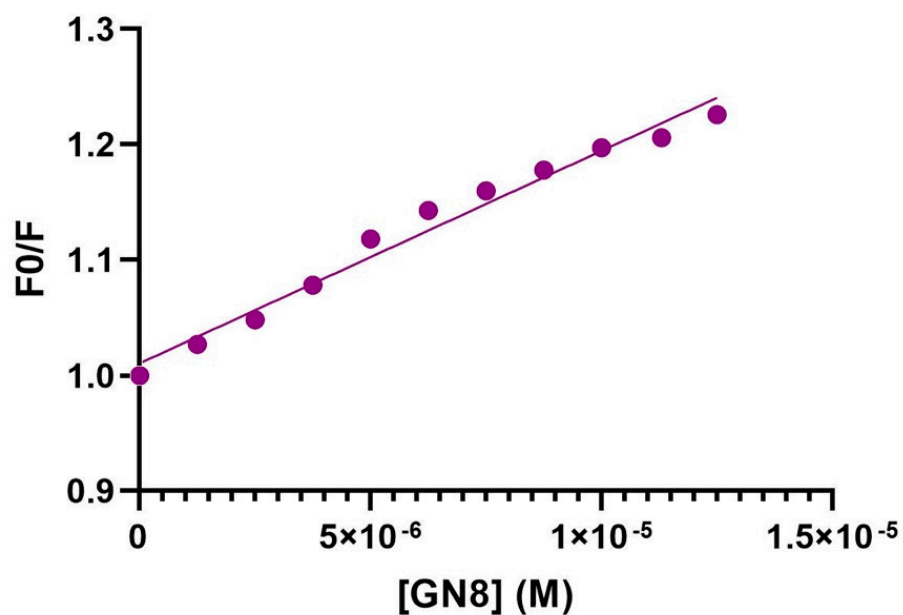


Figure S12. Calculation of the Stern-Volmer constant (K_{SV}) for the quenching by GN8 of the amyloid insulin fluorescent emission at 305 nm

S3. Tables

Table S1: Molar absorptivities at the maximum absorption wavelengths obtained in pure ethanol for the fluorene derivatives studied

Compound	λ_{max} (nm) and molar absorptivity (log ϵ)
1	256 (3.446), 289 (3.368)
2	250 (3.469), 290 (2.884)
3	304 (4.520), 327 (4.454)
4	304 (4.503), 326 (4.432)

Table S2: UV-Visible absorption maxima of the fluorene derivatives studied in different solvents. Fluorene derivatives concentration 2.0×10^{-5} M

Compound	UV-Visible absorption maxima (nm)			
	Cyclohexene	Ethanol	Acetonitrile	Water
1	260, 287	256, 289	257, 287	254, 287
2	252, 287 (sh)	250, 290	248, 290 (sh)	257, 289 (sh)
3	303, 315	304, 327	305, 326	296, 311
4	305, 329	304, 326	305, 326	296, 311

sh: shoulder

Table S3: Fluorescence emission maxima (excitation wavelength in parenthesis) of the fluorene derivatives studied in different solvents. Fluorene derivatives concentration 1.0×10^{-6} M

Compound	Cyclohexene	Ethanol	Acetonitrile	DMSO	Water
1	304, 312 (260)	305, 313 (290)	305, 315, 340 (sh) (257)	340 (260)	305, 313, 425 (sh) (254)
2	---	305, 315, 340 (254)	340 (sh), 435 (258)	340 (266)	425 (255)
3	336, 350, 429 (310)	336, 352, 425 (305)	336, 352, (302)	336 (308)	348 (296)
4	336, 353, 400, 429, 450 (305)	337, 352, 409, 429 (305)	340, 354 (302)	335, 354 (sh) (305)	353 (284)

sh: shoulder