Electronic Supplementary Material (ESI) for Sensors & Diagnostics.

Supplementary Information

Turn-on Fluorescent Sensors for Cu-rich Amyloid β Peptide Aggregates

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I. Experimental Procedures

General Methods. All reagents were purchased from commercial sources and used as received unless stated otherwise. Solvents were purified prior to use by passing through a column of activated alumina using an MBRAUN SPS. All solutions and buffers were prepared using metal-free Millipore water that was treated with Chelex overnight and filtered through a 0.22 μm nylon filter. ^{1}H (500 MHz) NMR spectra were recorded on a Bruker 500 spectrometer (500 MHz). Chemical shifts are reported in ppm downfield from tetramethylsilane. UV-visible spectra were recorded on a Varian Cary 50 Bio spectrophotometer and are reported as λ_{max}, nm (ε, M^{-1} cm^{-1}). ESI-MS experiments were performed by the Mass Spectrometry Lab at UIUC using a Waters Q-TOF Ultima ESI mass spectrometer with an electron spray ionization source. **HYR-27**, **HYR-27-TACN** and **YL-1-6** were dissolved in DMSO to prepare 5.0 mM stock solutions.

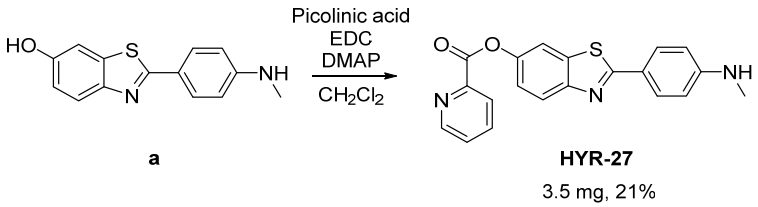
Amyloid β Peptide Experiments. Aβ_{42} powder was prepared by dissolving commercial Aβ_{42} peptide (GLbiochem) in ammonium hydroxide solution (1%, v/v). The solution was then aliquoted out and lyophilized overnight. The resulting aliquoted powder was stored at –80 °C. Aβ_{42} monomers were generated by dissolving Aβ_{42} powder in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, 1 mM) and incubating for 1 h at room temperature. The solution was then evaporated overnight and dried by vacuum centrifuge to result in monomeric films. Aβ$_{42}$ fibrils were generated by dissolving the monomeric Aβ_{42} films in DMSO, diluting into the appropriate buffer, and incubating for 24 h at 37 °C with continuous agitation (final DMSO concentration was < 2%). For preparation of Aβ_{42} oligomers, the peptides were suspended in PBS buffer and incubated overnight at 4 °C.

Fluorescence Spectra Measurements. All fluorescence measurements were performed by using a SpectraMax M2e plate reader (Molecular Devices). The fluorescence spectra of **HYR-27** (100 μL PBS, 5.0 μM) and **HYR-27-Cu** (100 μL PBS, 5.0 μM HYR-27, 5.0 μM $CuCl_2$) were recorded

with excitation at 340 nm and emission wavelengths from 400 to 600 nm. For the fluorescence turn-on effects of **HYR-27** towards $A\beta_{42}$ species, to PBS solutions (100 μL) of **HYR-27**, various $A\beta_{42}$ species and $CuCl_2$ were added to the solution, final concentrations of 5.0 μM, 5.0 μM, and 0-20 μM, and their fluorescence spectra and λ_{max} of emission were determined as described above.

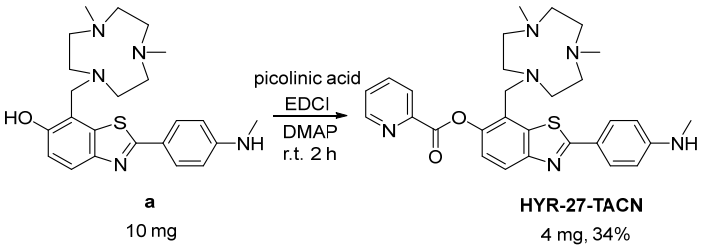
Histological Staining of 5xFAD Mice Brain Sections. To evaluate the Aβ binding specificity of the compound *ex vivo*, brain sections from 11-mon 5xFAD mice were washed with PBS (3 × 5 min) and blocked with bovine serum albumin (2% BSA in PBS, pH 7.4, 10 min). Then the sections were incubated with 5 μM **HYR-27** or 5 μM **HYR-27** with 50 μM $CuCl_2$, followed by extensive washings with buffer to remove any unbound Cu ions, and sequentially stained with the HJ3.4 antibody (Professor David Holtzman, Washington University School of Medicine) 1 μg/ml solution for 1 h respectively. For the curcumin derivatives, the brain sections were only stained with 50 μM compounds or 50 μM with 50 μM $CuCl_2$, followed by extensive washings with buffer to remove any unbound Cu ions. The brain sections were treated with 2% BSA-PBS again for 4 min to remove any compounds or antibodies that were non-specifically binding to the tissue. Finally, the sections were washed with PBS (3 × 2 min), DI water (2 min) and mounted with non-fluorescent mounting media (Vectashield). The primary antibodies were labeled with dye CF594 via Mix-n-Stain™ CF™ 594 Antibody Labeling Kit (Sigma Aldrich). The stained brain sections were observed by using EVOS FL Auto 2. Colocalization analysis and determination of the Pearson's correlation coefficient was performed with the imaging software Fiji (ImageJ 1.52p).

II. Synthesis of Cu-based Activatable Sensors



Scheme S1 Synthesis of **HYR-27**.

HYR-27. **S1a** (12 mg, 0.05 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated with 2-picolinic acid (27 mg, 0.20 mmol), DMAP (1 mg, 0.01 mmol), and EDC (15.8 mg, 0.08 mmol). The reaction was stirred at room temperature for 1 hr. After the completion of the reaction monitored by TLC, the resulting solution was concentrated, and the residue was purified via silica gel column chromatography) to afford HYR-27 (3.5 mg, 21%) as a yellow solid. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.87 (d, *J* = 4.6 Hz, 1H), 8.31 (d, *J* = 7.8 Hz, 1H), 8.00 (d, *J* = 8.8 Hz, 1H), 7.91 (d, *J* = 8.6 Hz, 2H), 7.76 (s, 1H), 7.58 (m, 1H), 7.33 (d, *J* = 10.5 Hz, 1H), 6.65 (d, *J* = 8.5 Hz, 2H), 5.30 (s, 1H), 2.91 (s, 3H). HR-ESI-MS: Calcd for $[M+H]^+$, 362.0963; Found, 362.0952.



Scheme S2 Synthesis of **HYR-27-TACN**.

HYR-27-TACN. **S2a** (10 mg, 0.023 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated with 2-picolinic acid (12 mg, 0.1 mmol), DMAP (1 mg, 0.01 mmol, 0.1 equiv), and EDC (7.9 mg, 0.04

mmol, 1.5 equiv). The reaction was stirred at room temperature for 1 hr. After the completion of the reaction monitored by TLC, the resulting solution was concentrated, and the residue was purified via silica gel column chromatography) to afford HYR-27-TACN (4 mg, 34%) as a yellow solid. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.90 (d, *J* = 7.7 Hz, 1H), 8.34 (d, *J* = 7.7 Hz, 1H), 8.05-7.98 (m, 2H), 7.97-7.91 (m, 2H), 7.68-7.65 (m, 1H), 7.34 (d, *J* = 8.7 Hz, 1H), 6.71 (d, *J* = 8.7 Hz, 2H), 3.97 (s, 2H), 3.04-2.85 (m, 12H), 2.46 (s, 6H), 2.20 (s, 3H). HR-ESI-MS: Calcd for $[M+H]^+$, 531.2542; Found, 531.2532.

YL-1. A solution of 2,4-pentanedione (100 mg, 1 mmol), $BF_3 \cdot OEt_2$ (213mg, 1.5 mmol) in Toluene (10 ml) were added to the reaction flask. The resulting solution was stirred at 65 °C for 2 h. After 2h reaction, 4-hydroxybenzylaldehyde (244 mg, 2 mmol), tributyl borate (460mg, 2 mmol) were added and then butylamine (14.6 mg, 0.2 mmol) was added subsequently. The mixture was stirred at 65 °C overnight. The solvent was removed, and the resulting residue was purified by silica gel column chromatography to afford YL-1 (17%, 60 mg) as a yellow solid. ^{1}H NMR (500 MHz, CD_3OD): δ (ppm): 7.84 (d, *J* = 15.2 Hz, 2H), 7.53 (d, *J* = 8.0 Hz, 4H), 6.82 (d, *J* = 8.3 Hz, 4H), 6.70 (d, *J* = 15.2 Hz, 2H), 6.22 (s, 1H). HR-ESI-MS: Calcd for $[M-H]^-$, 355.0958; Found, 355.0961.

YL-2. **YL-1** (18 mg, 0.2 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated with 2-picolinic acid (27 mg, 0.22 mmol), DMAP (0.7 mg, 0.01 mmol), and EDC (15.8 mg, 0.08 mmol). The reaction was stirred at room temperature for 1 hr. After the completion of the reaction monitored by TLC, the resulting solution was concentrated, and the residue was purified via silica gel column chromatography) to afford YL-2 (12 mg, 52%) as a yellow solid. ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.83 (s, 1H), 8.30 (d, *J* = 7.8 Hz, 1H), 8.14 – 8.03 (m, 3H), 7.99 (d, *J* = 8.7 Hz, 2H), 7.80 (d,

J = 8.8 Hz, 2H), 7.73 (s, 1H), 7.49 (d, *J* = 8.6 Hz, 2H), 7.15 (d, *J* = 18.4 Hz, 1H), 6.98 (m, 3H), 6.51 (d, *J* = 2.7 Hz, 1H). HR-ESI-MS: Calcd for $[M+H]^+$, 462.1324; Found, 462.1314.

YL-3. A solution of 2,4-pentanedione (300 mg, 3 mmol), $BF_3 \cdot OEt_2$ (639 mg, 4.5 mmol) in Toluene (30 ml) were added to the reaction flask. The resulting solution was stirred at 65 °C for 2 h. After 2h reaction, 4-(dimethylamino)benzaldehyde (447 mg, 3 mmol), tributyl borate (690 mg, 3 mmol) were added and then butylamine (21 mg, 0.3 mmol) was added subsequently. The mixture was stirred at 65 °C overnight. The solvent was removed, and the resulting residue was purified by silica gel column chromatography to afford half curcumin derivatives as a black solid. Then the half curcumin derivatives were dissolved in toluene, and vanillin (152 mg, 1 mmol), tributyl borate (230 mg, 1 mmol) were added and then butylamine (7.3 mg, 0.1 mmol) was added sequentially. The mixture was stirred at 65 °C overnight. The solvent was removed, and the resulting residue was purified by silica gel column chromatography to afford YL-3 (102 mg, 25%) as a dark red solid. ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.49 (s, 1H), 7.97 (d, *J* = 15.3 Hz, 1H), 7.87 (d, *J* = 15.6 Hz, 1H), 7.70 (d, *J* = 9.0 Hz, 2H), 7.46 (s, 1H), 7.34 (d, *J* = 8.2 Hz, 1H), 7.01-6.67 (m, 5H), 6.27 (s, 1H), 3.96 (s, 3H), 3.14 (s, 6H). HR-ESI-MS: Calcd for $[M-H]^-$, 412.1537; Found, 412.1521.

YL-4. **YL-3** (82 mg, 0.2 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated with 2-picolinic acid (27 mg, 0.22 mmol), DMAP (0.7 mg, 0.01 mmol), and EDC (15.8 mg, 0.08 mmol). The reaction was stirred at room temperature for 1 hr. After the completion of the reaction monitored by TLC, the resulting solution was concentrated, and the residue was purified via silica gel column chromatography) to afford YL-4 (42 mg, 41%) as a yellow solid. ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.83 (d, *J* = 4.6 Hz, 1H), 8.28 (d, *J* = 7.7 Hz, 1H), 8.12-8.02 (m, 2H), 7.93 (d, *J* = 15.7 Hz, 1H), 7.77-7.70 (m, 2H), 7.67 (d, *J* = 1.9 Hz, 1H), 7.51 (dd, *J* = 8.1, 2.0 Hz, 1H), 7.37 (d, *J* = 8.2

Hz, 1H), 7.12 (d, *J* = 15.8 Hz, 1H), 6.87-6.78 (m, 3H), 6.37 (s, 1H), 3.95 (s, 3H), 3.16 (s, 6H). HR-ESI-MS: Calcd for $[M+H]^+$, 519.1903; Found, 519.1894.

YL-5. A solution of 2,4-pentanedione (300 mg, 3 mmol), $BF_3 \cdot OEt_2$ (639mg, 4.5 mmol) in Toluene (30 ml) were added to the reaction flask. The resulting solution was stirred at 65 °C for 2 h. After 2h reaction, 4-(dimethylamino)benzaldehyde (447 mg, 1 mmol), tributyl borate (230 mg, 1 mmol) were added and then butylamine (21 mg, 0.1 mmol) was added subsequently. The mixture was stirred at 65 °C overnight. The solvent was removed, and the resulting residue was purified by silica gel column chromatography to afford half curcumin derivatives as a black solid. Then the half curcumin derivatives were dissolved in toluene, and 4-hydroxybenzylaldehyde (50 mg, 0.5 mmol), tributyl borate (115 mg, 0.5 mmol) were added and then butylamine (4 mg, 0.01 mmol) was added sequentially. The mixture was stirred at 65 °C overnight. The solvent was removed, and the resulting residue was purified by silica gel column chromatography to afford YL-5 (46 mg, 24%) as a dark red solid. ^{1}H NMR (500 MHz, Acetone-d_6) δ 7.98 (d, *J* = 15.4 Hz, 1H), 7.88 (d, *J* = 15.6 Hz, 1H), 7.74-7.68 (m, 4H), 6.98-6.94 (m, 2H), 6.89-6.81 (m, 3H), 6.78 (d, *J* = 15.3 Hz, 1H), 6.29 (d, *J* = 0.9 Hz, 1H), 3.14 (d, *J* = 0.9 Hz, 6H). HR-ESI-MS: Calcd for $[M-H]^-$, 382.1431; Found, 382.1417.

YL-6. **YL-5** (38 mg, 0.1 mmol) in anhydrous CH_2Cl_2 (10 mL) was treated with 2-picolinic acid (27 mg, 0.11 mmol), DMAP (0.7 mg, 0.005 mmol), and EDC (15.8 mg, 0.04 mmol). The reaction was stirred at room temperature for 1 hr. After the completion of the reaction monitored by TLC, the resulting solution was concentrated, and the residue was purified via silica gel column chromatography) to afford YL-6 (23 mg, 47%) as a dark red solid. ^{1}H NMR (500 MHz, Acetone-d_6) δ 8.83 (s, 1H), 8.29 (d, *J* = 7.8 Hz, 1H), 8.12-8.08 (m, 1H), 8.06 (d, *J* = 15.2 Hz, 1H), 7.96 (s,

3H), 7.79-7.69 (m, 3H), 7.47 (d, J = 8.6 Hz, 2H), 7.09 (d, J = 15.8 Hz, 1H), 6.88-6.78 (m, 3H), 6.39 (s, 1H), 3.16 (s, 6H). HR-ESI-MS: Calcd for $[M+H]^+$, 489.1797; Found, 489.1786.

III. UV-vis and Emission Spectra

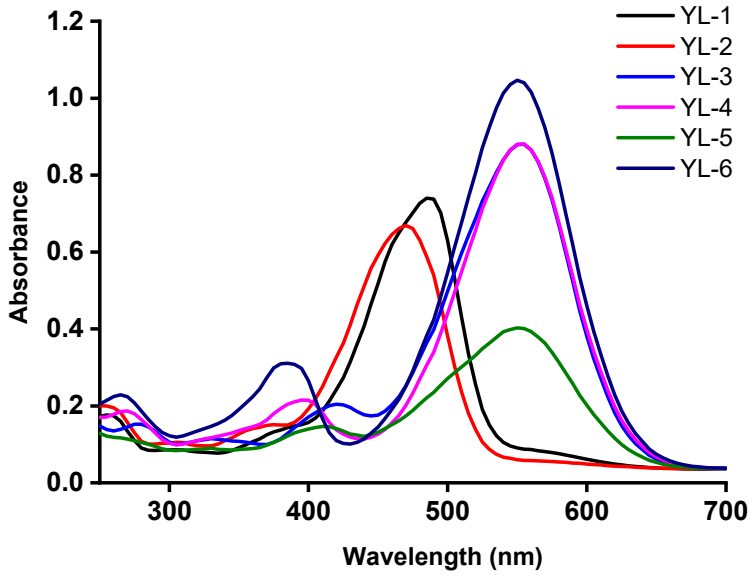
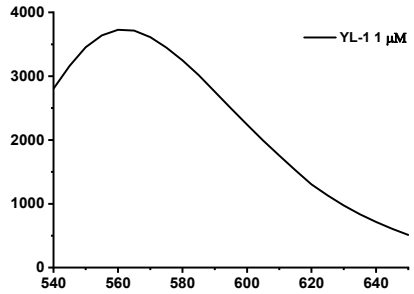
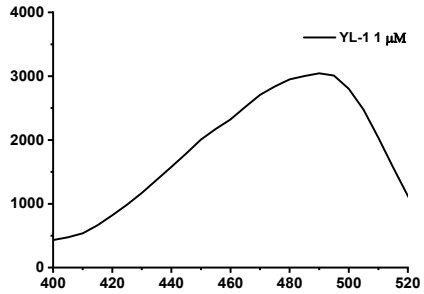
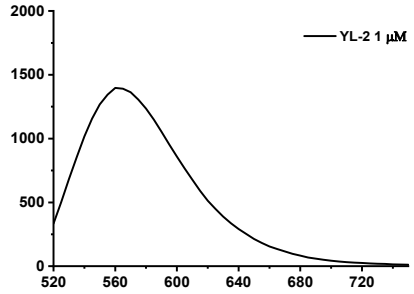
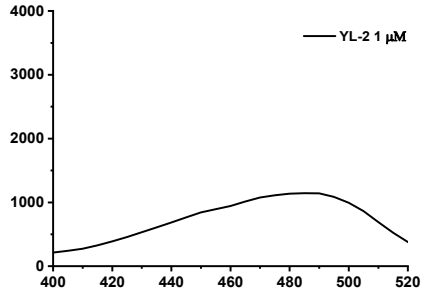
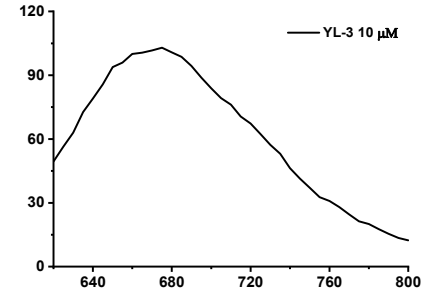
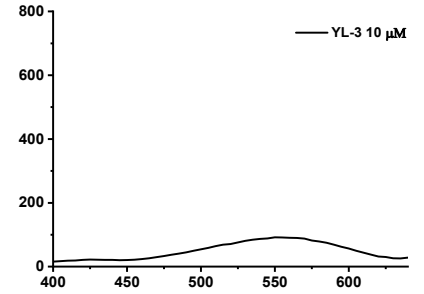


Fig. S1 UV-vis spectra of investigated sensors (10 μM in MeOH with 0.2% DMSO).

Comp.	Fluorescence	Em fixed/Ex sweep
YL-1		
YL-2		
YL-3		

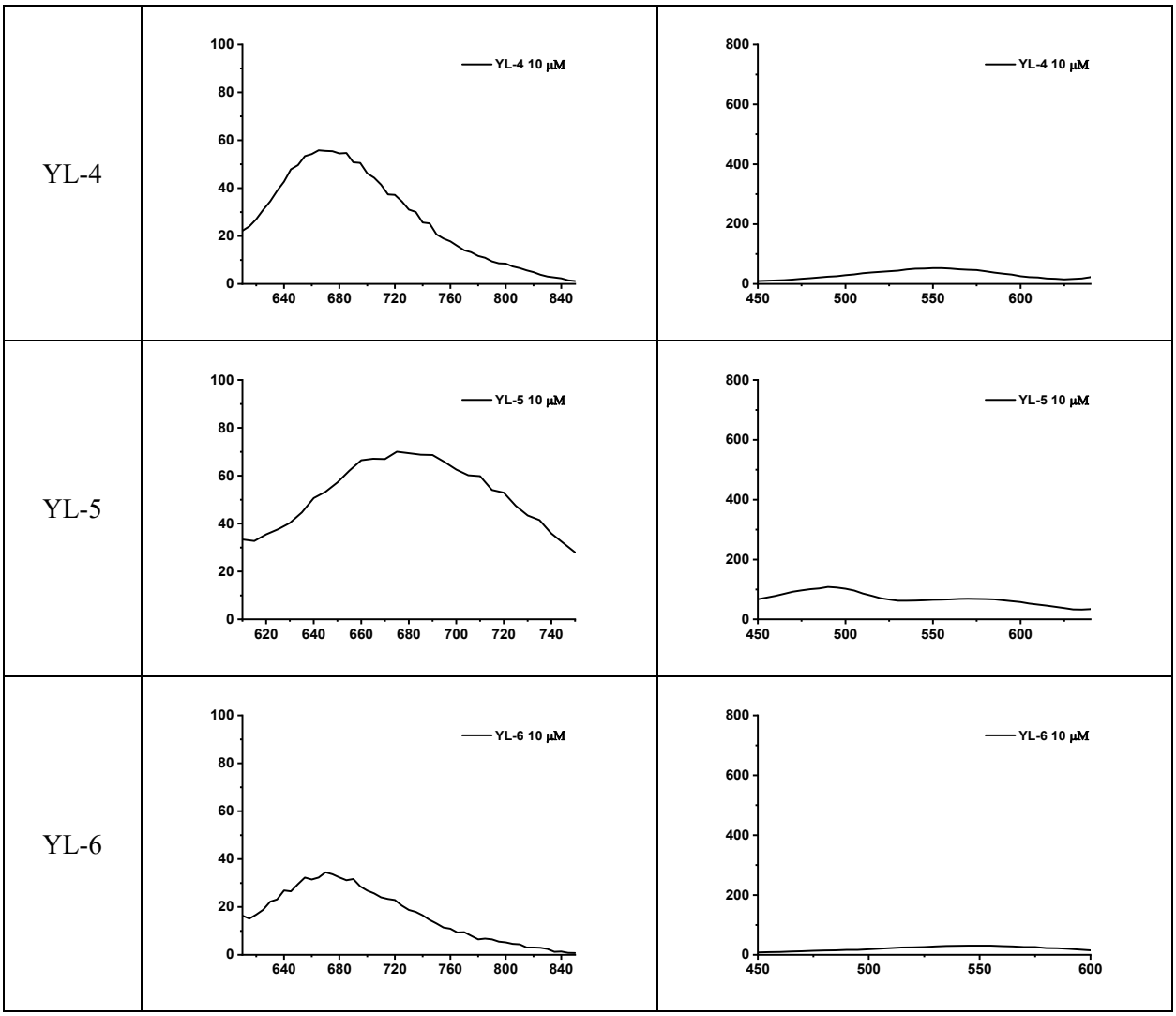


Fig. S2 Fluorescence spectra of investigated sensors (10 μM in MeOH with 0.2% DMSO).

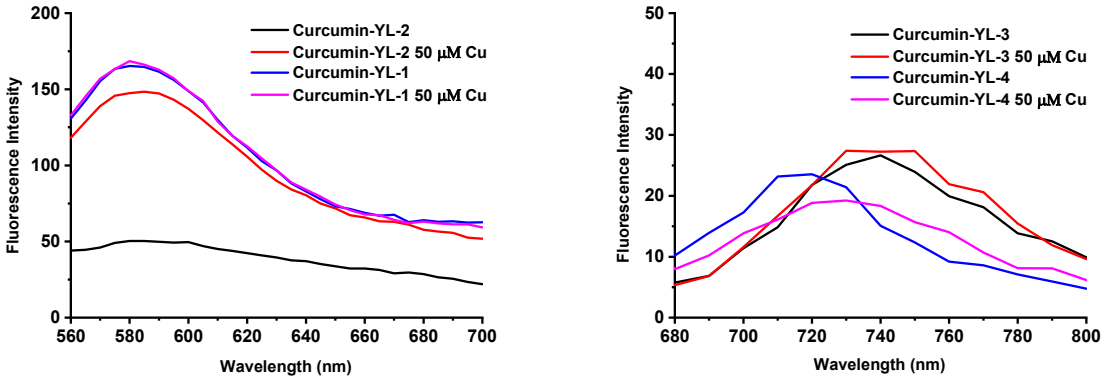


Fig. S3 Fluorescence "turn-on" spectra of investigated sensors (in 40% DMSO and PBS).

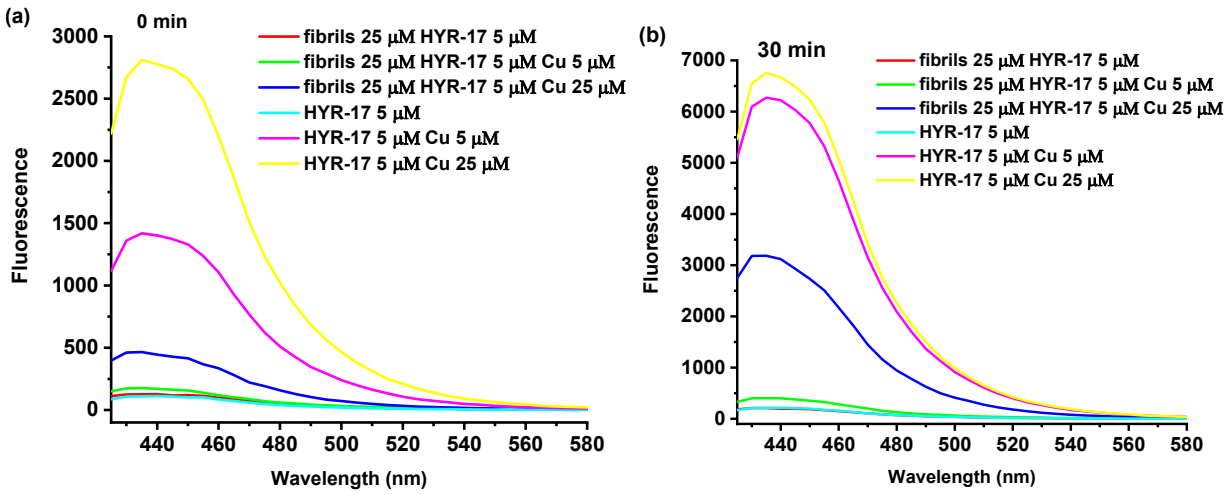
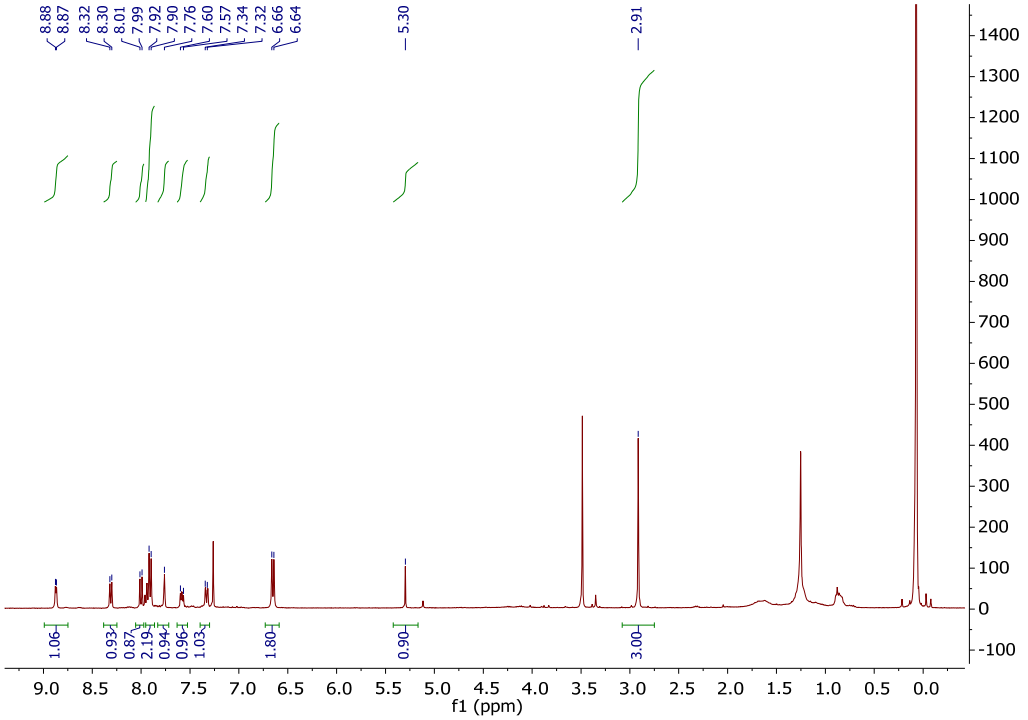


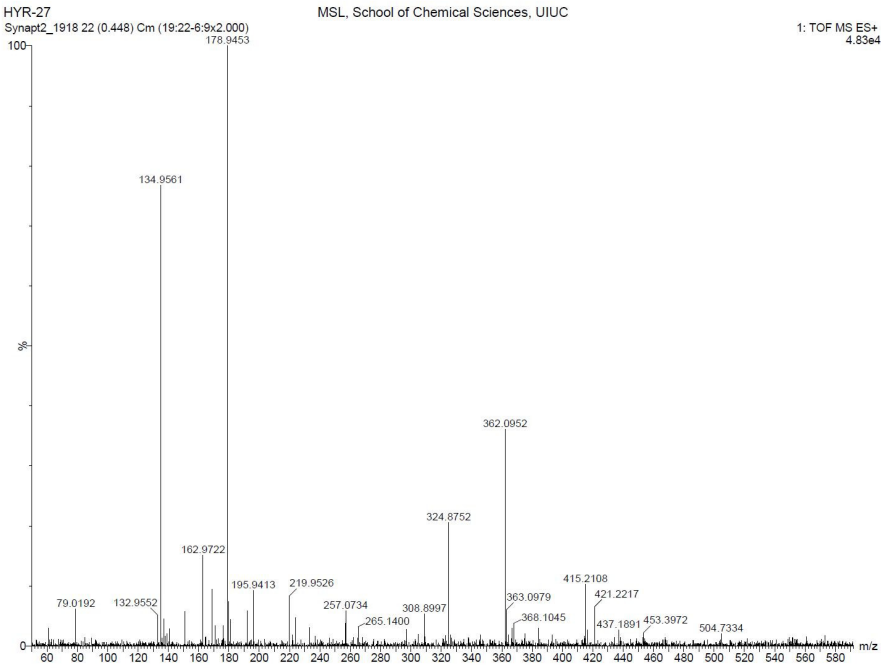
Fig. S4 Control Cu(II) fluorescence turn-on studies in the absence and presence of Aβ fibrils after a) 0 min and b) 30 min. [**HYR-27**] = 5 μM, [Aβ] = 0 or 25 μM, [Cu] = 0, 5, or 25 μM in PBS buffer (pH =7.4).

IV. Characterization of Cu-based Activatable Sensors

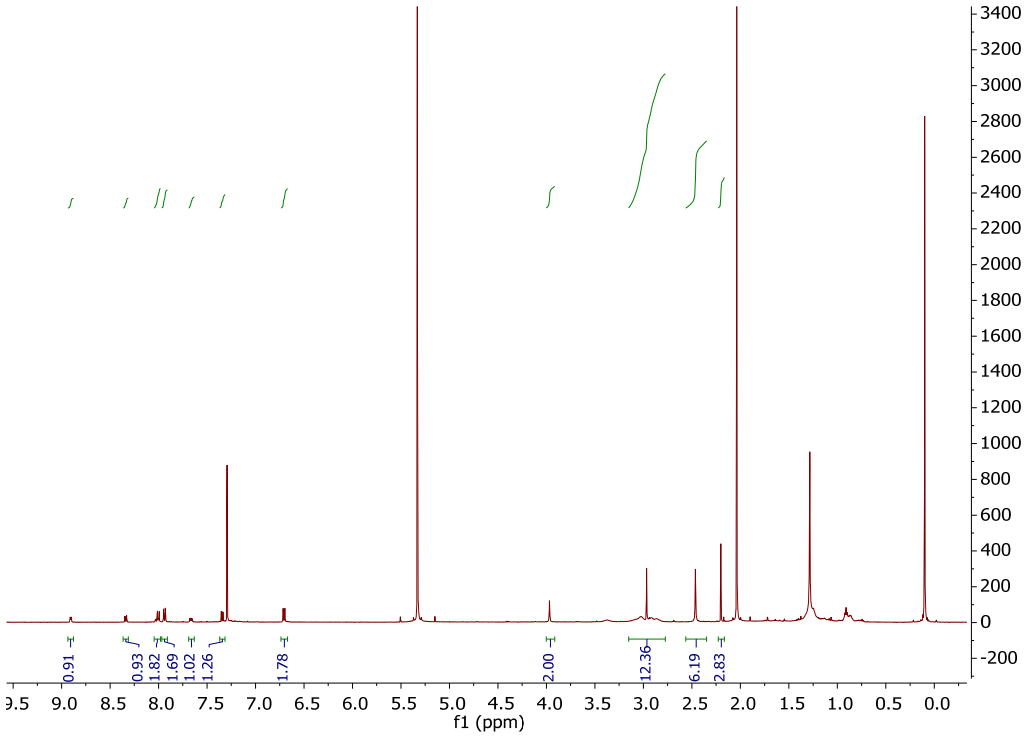
^{1}H NMR of HYR-27:



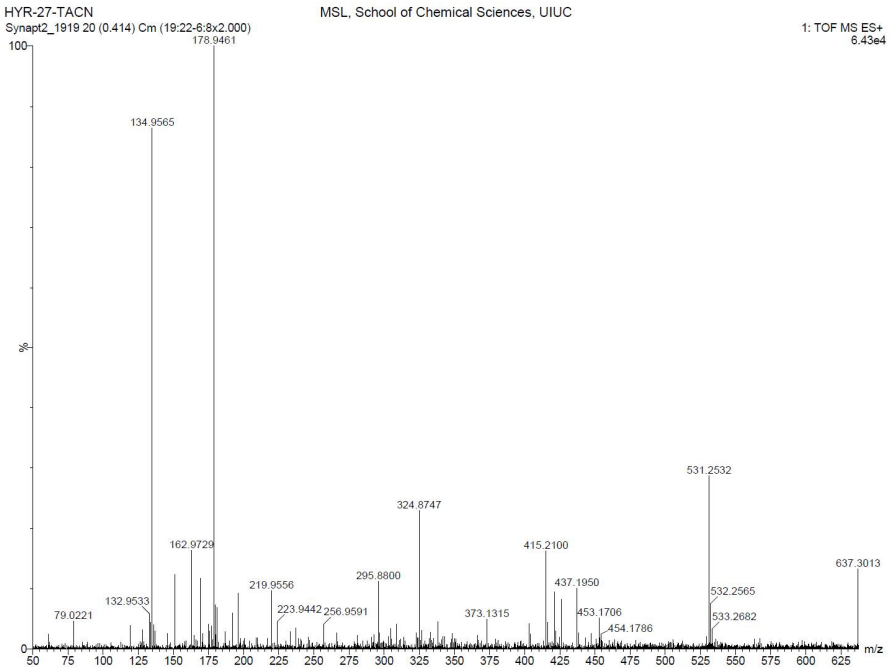
High-res ESI of HYR-27:



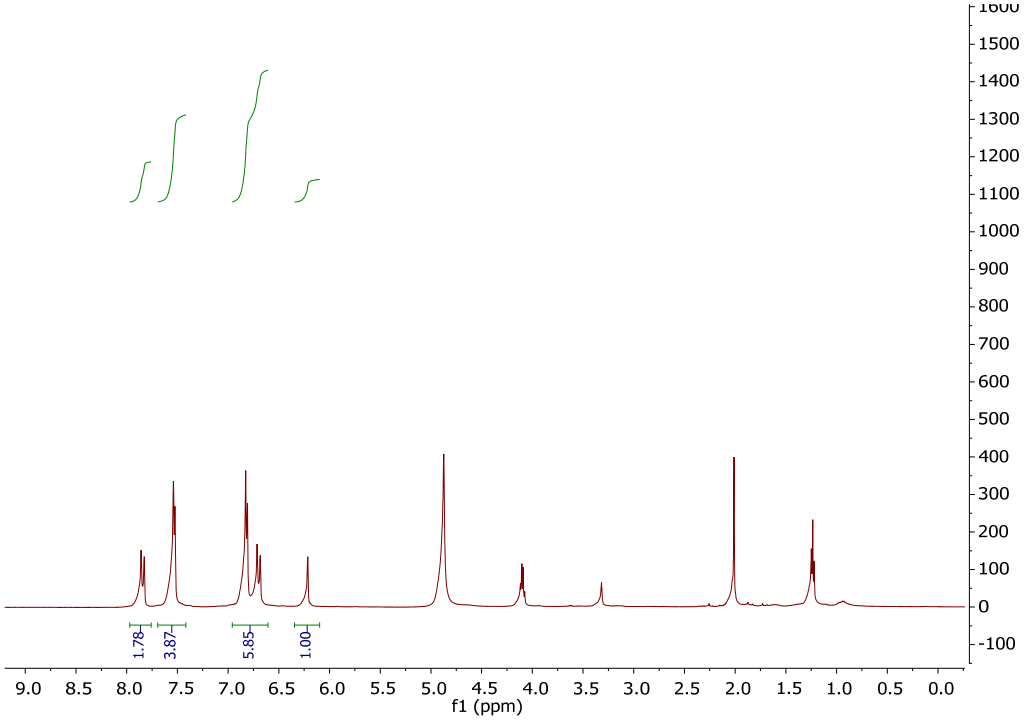
^{1}H NMR of HYR-27-TACN:



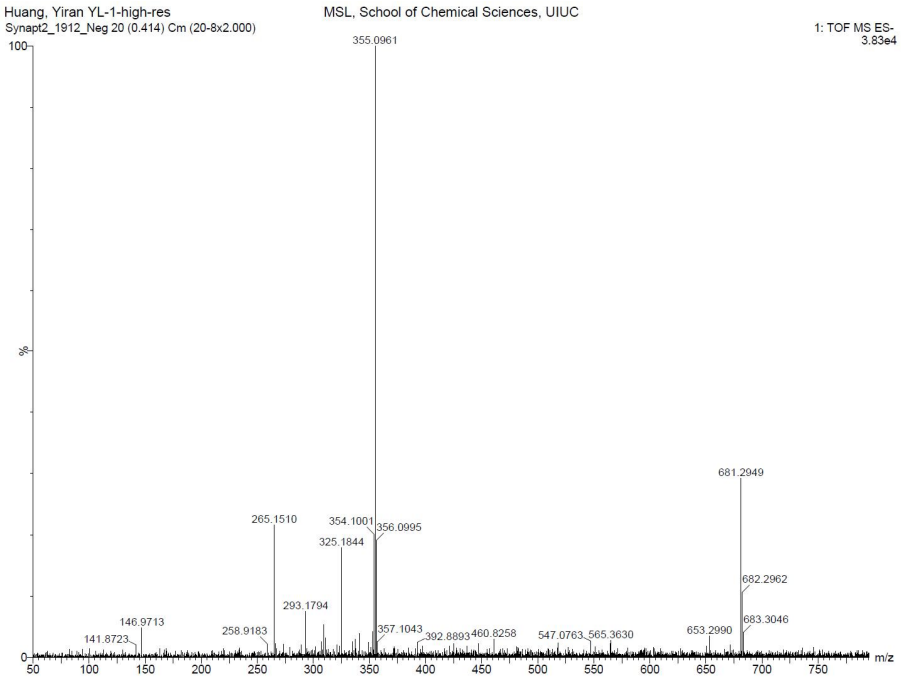
High-res ESI of HYR-27-TACN:



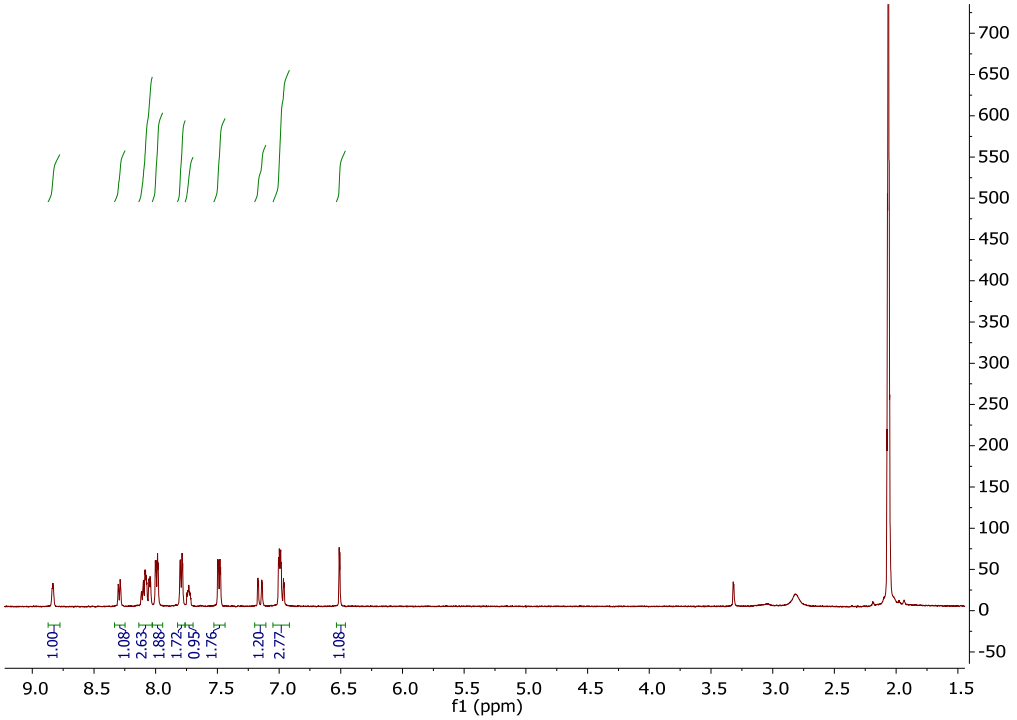
^{1}H NMR of YL-1:



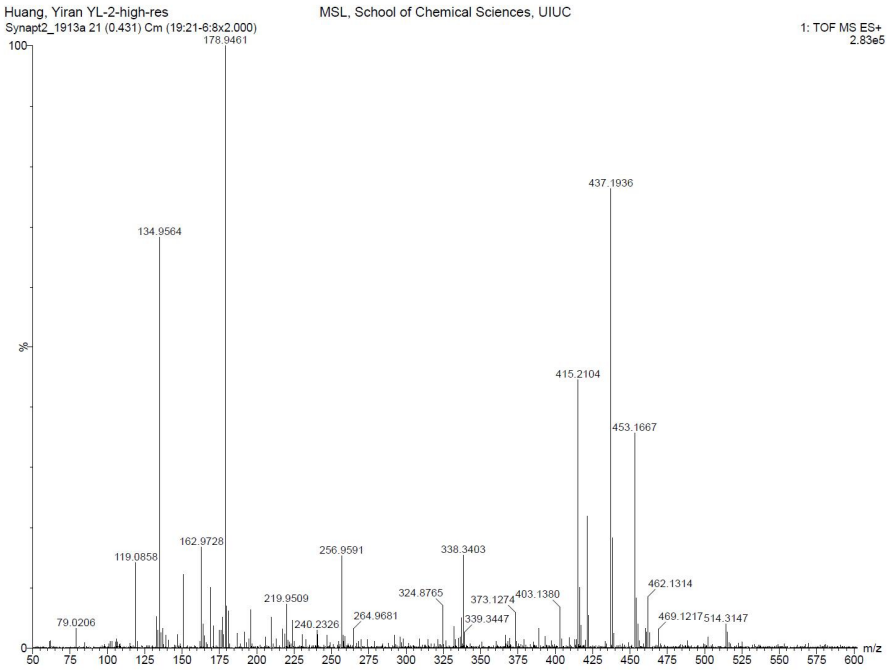
High-res ESI of YL-1:



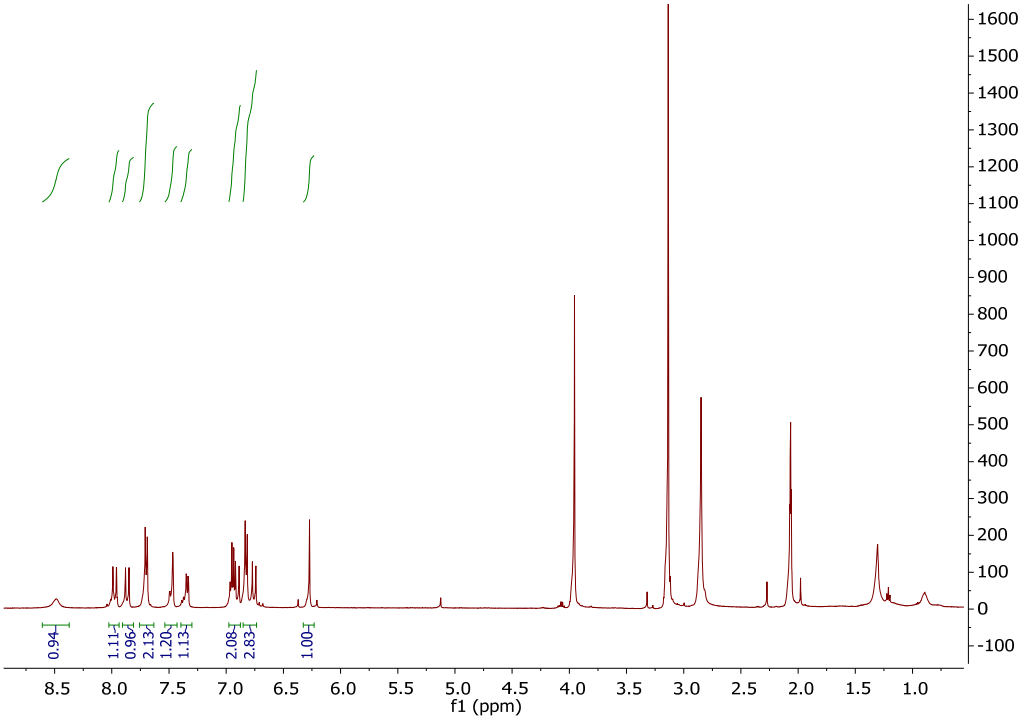
^{1}H NMR of YL-2:



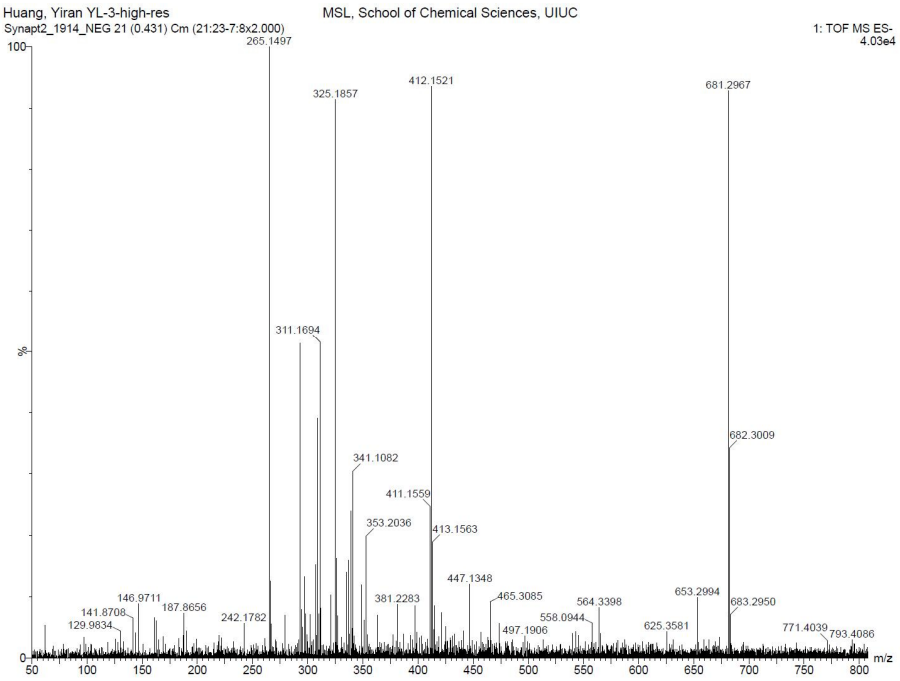
High-res ESI of YL-2:



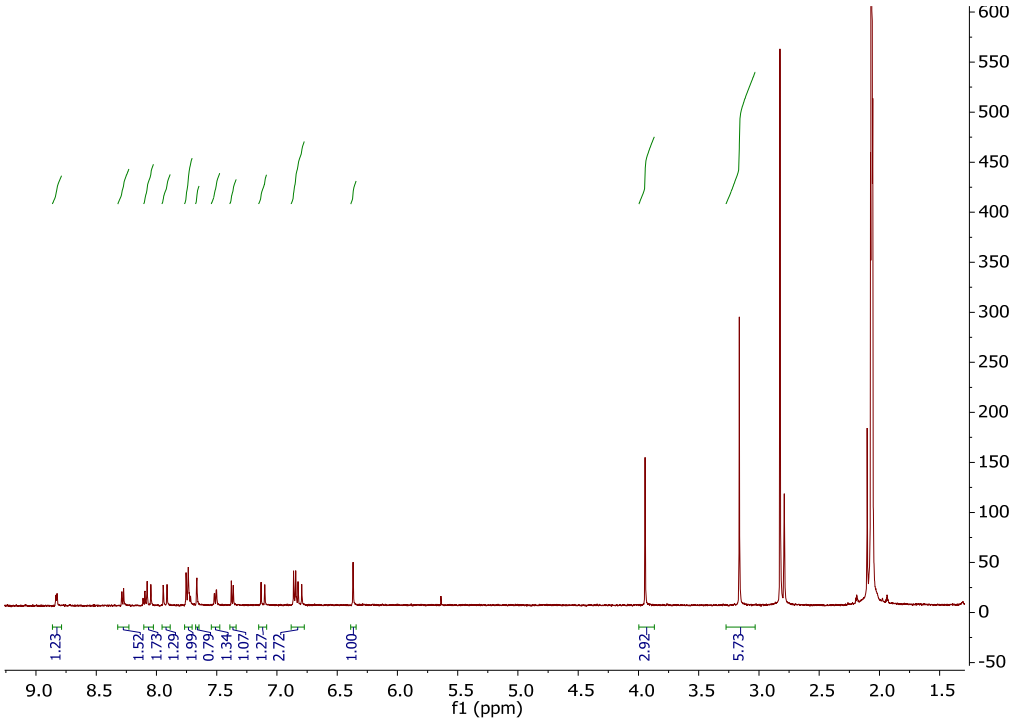
^{1}H NMR of YL-3:



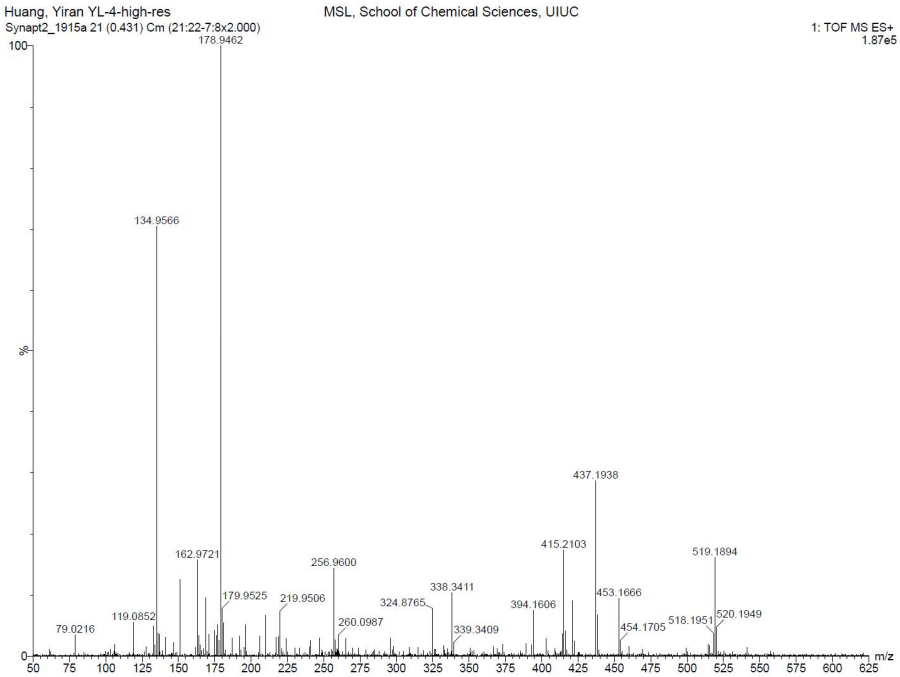
High-res ESI of YL-3:



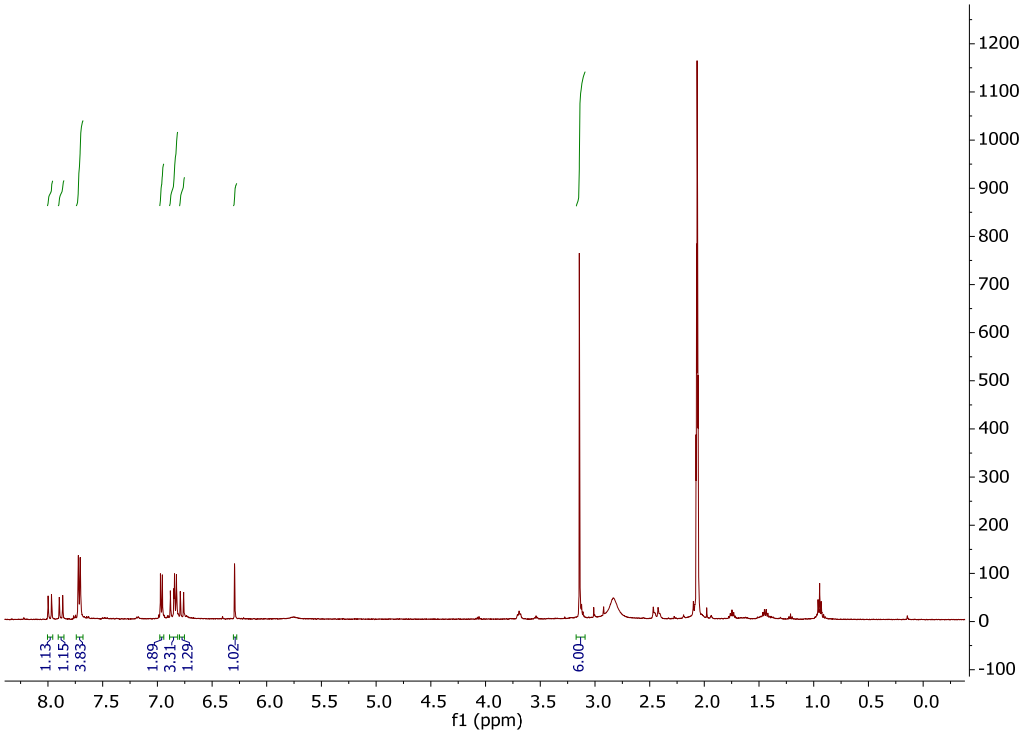
^{1}H NMR of YL-4:



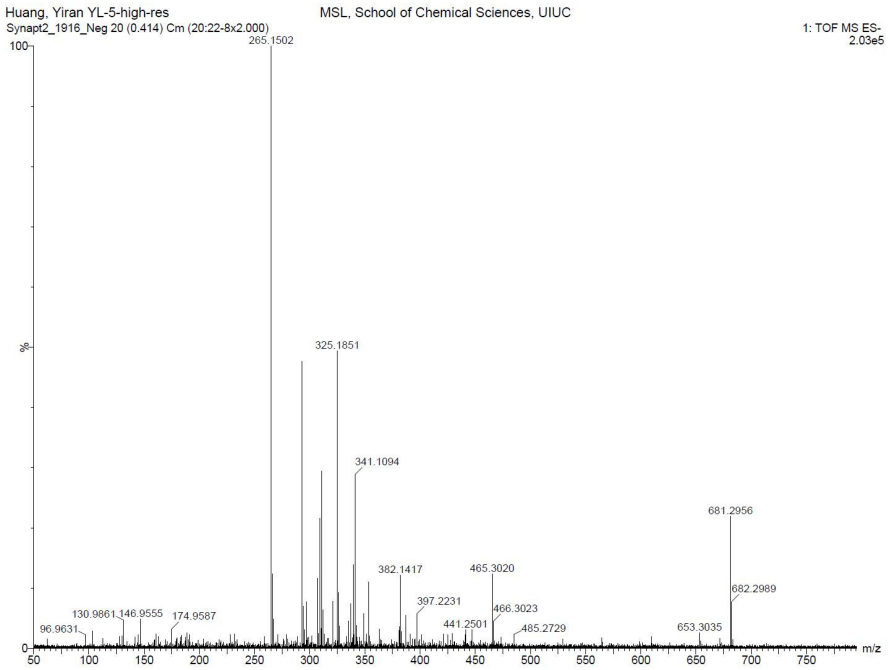
High-res ESI of YL-4:



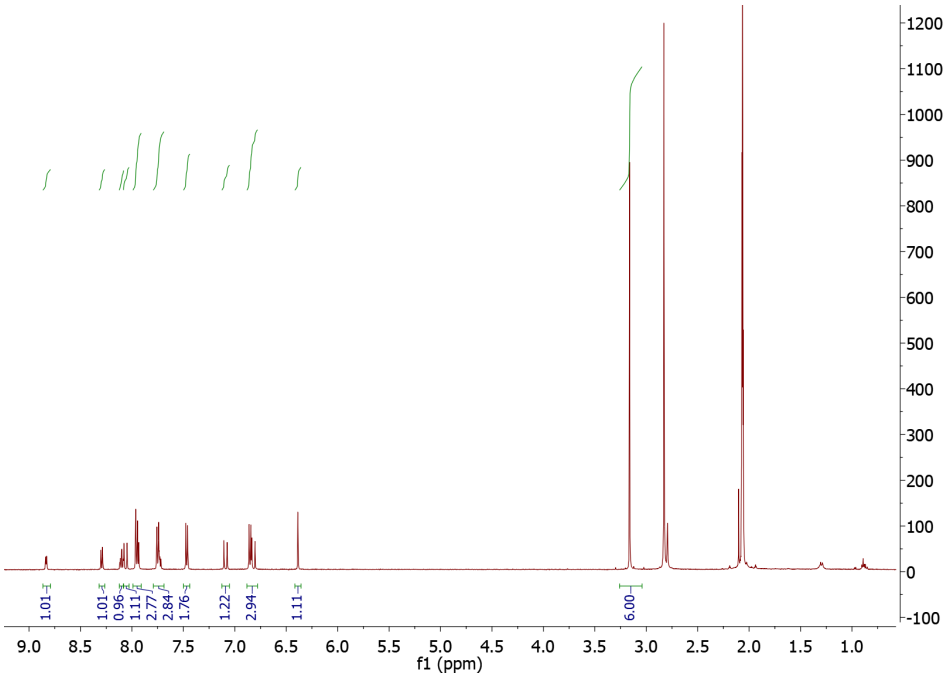
[1]H NMR of YL-5:



High-res ESI of YL-5:



^{1}H NMR of YL-6:



High-res ESI of YL-6: