Electronic Supplementary Information

Peptoid Transporters: Effects of Cationic, Amphipathic Structures on their Cellular Uptake

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Materials and methods

Synthesis and purification: Peptoids were synthesized using an ABI 433A peptide synthesizer (Applied Biosystems, Inc.) on Rink amide MBHA resin (EMD Biosciences, Gibbstown, NJ) using the submonomer protocol. Briefly, the amine on the nascent chain is bromoacetylated followed by S_N2 displacement of bromide by a primary amine to form a side chain(1, 2). These two steps were iterated with appropriate amines until the desired peptoid sequence was obtained. Resin-bound peptoids were then exposed to a mixture of trifluoroacetic acid (TFA): triisopropylsilane: water (95:2.5:2.5, volume ratio) for 10 minutes to cleave peptoids from the resin. Crude peptoids were purified by reverse-phase high performance liquid chromatography (RP-HPLC) (Waters Corporation) using a C18 column and a linear acetonitrile/water gradient. A final purity > 95% as measured by analytical RP-HPLC (Waters Corporation) was achieved, and the identity of each peptoid was confirmed using electrospray ionization mass spectrometry (ESI/MS). Unless indicated otherwise, all reagents were purchased from Sigma Aldrich (St. Louis, MO). Among the submonomers used, *N*spe was derived from (*S*)-*N*-(1-phenylethyl)amine; *N*pm from benzylamine (1-phenylethylamine); *N*ssb from (*S*)-(*sec*-butyl)amine; *N*Leu from isobutylamine; *N*Lys from *N*-*tert*-butoxycarbonyl-1,4-butanediamine (CNH Technologies, MA). Guanidinylation of *N*Lys was carried out according to the reported procedure(3). 5(6)-Carboxyfluorescein was used to label the N-terminus of all the constructs on solid-phase (4) .

RP-HPLC traces and ESI data of construct **1**-**11** are summarized in Figure S1 and S2.

Cell cultures: MCF-7 cells were kindly provided by Professor Branimir Sikic's lab at Stanford University, and were cultured in McCoy's 5A media (GIBCO) with 10% FBS (Hyclone, US

sources) and antibiotics(Sigma). Cells were cultured in cell incubators (Thermo Scientific) at 37°C with 5% CO_2.

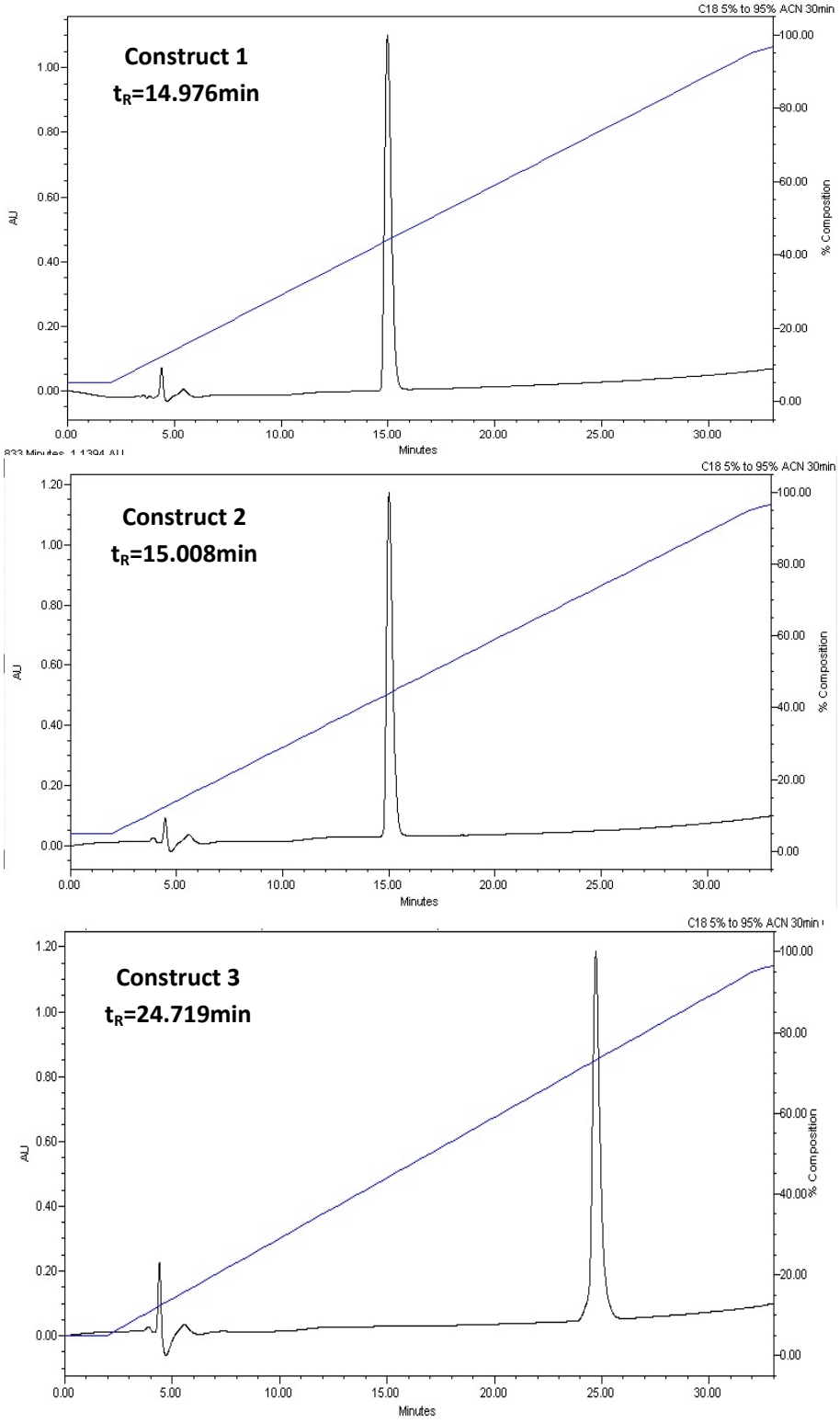
The MTS assays: Aliquots of 100µl media containing 1×10^4 cells were distributed into each well of a 96-well plate (BD Falcon). The following day, when cell density reaches about ~40% confluency, the cell media were removed and replaced with serial dilutions of peptoid stocks in culturing media. For peptoid dilutions, peptoid stocks were initially diluted in media at 100µM and then diluted by half in series using a multichannel pipette, and maintained in 100µl media for each concentration with triple repeats. After peptoid solutions were transferred onto cells in 96-well plates, cells were incubated at 37°C for certain time periods. Then 20µl of the CellTiter 96® Aqueous Non-Radioactive cell proliferation assay (Promega) reagent which contains a tetrazolium compound, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS(a)], was added to each well and cells were further incubated for 2h to metabolize. The absorbance of formazan products were measured at 490nm in microplate reader (Molecular Devices). Percentage of cell viability = $(A - A_{\text{testblank}})/(A_{\text{control}} - A_{\text{blank}})\times100$, where A is the absorbance of the test well and A_{control} the average absorbance of wells with cells not treated with peptoids. $A_{\text{testblank}}$ (media, MTS, and diluted peptoids) and A_{blank} (media and MTS) were background absorbances measured in the absence of cells (2, 5).

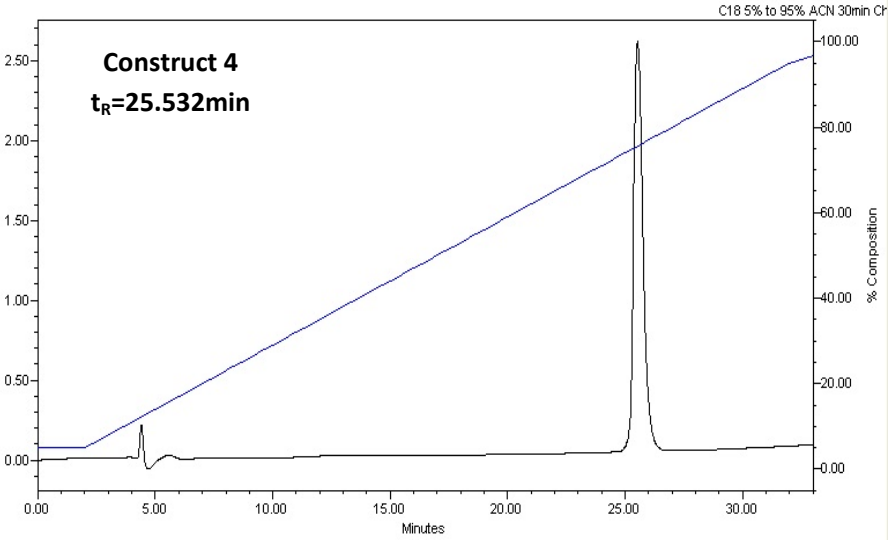
The Guava flow cytometry cellular uptake assay: All the fluorescently labeled constructs were dissolved in water as stock solutions, and their concentrations were further matched by their absorbances of fluorescein at 490nm. Cells were plated as described in MTS assays. Constructs were diluted in McCoy's 5A media with 10% FBS at desired concentrations. MCF-7 cells were incubated with constructs for certain time period. Cells incubated with 5(6)-Carboxyfluorescein dissolved in DMSO was measured as the control. After the supernatant was removed, cells were washed with PBS for three times, each wash incubated at 37°C at 5min. Cells were then trypsinized and suspended in fresh media. Cellular fluorescent signals were quantified in Guava Easycyte Plus® flow cytometry system (Millipore). Each sample was repeated three times, and the data presented are the mean fluorescent signal for 1500 cells.

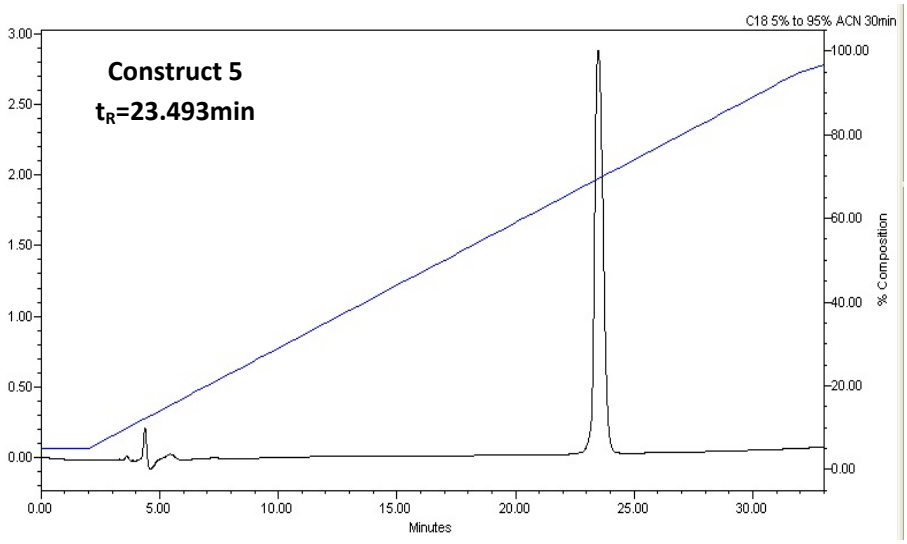
The temperature study: The uptake assays were performed as described above with the exception that for the 4°C experiment, the cells and all the reagents were pre-incubated at 4°C

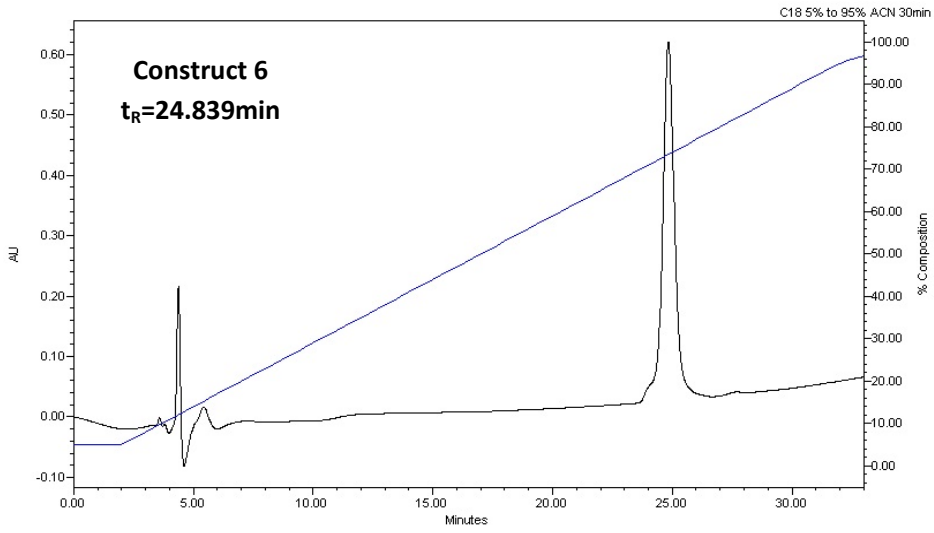
for 30min. Cells were incubated with constructs for 1h at either 37°C or 4°C before flow cytometry analysis(6).

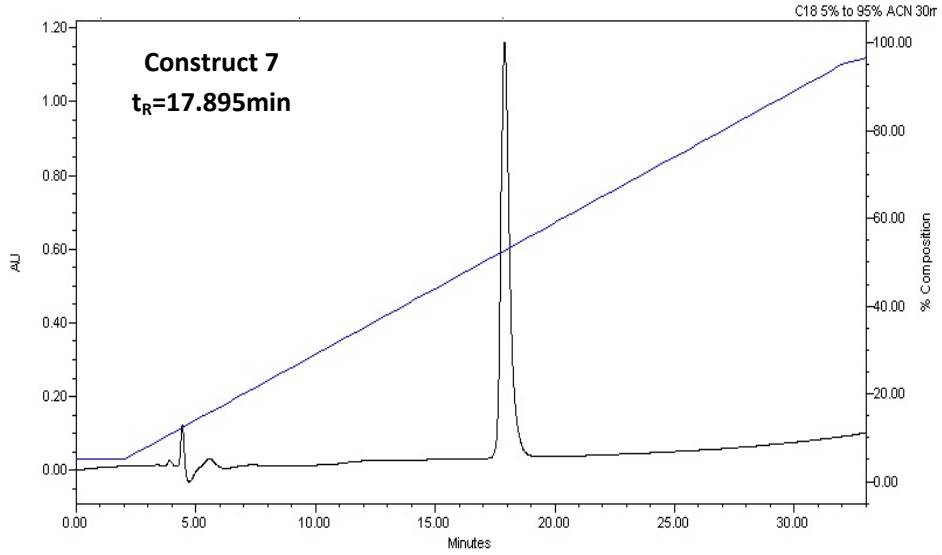
Figure S1

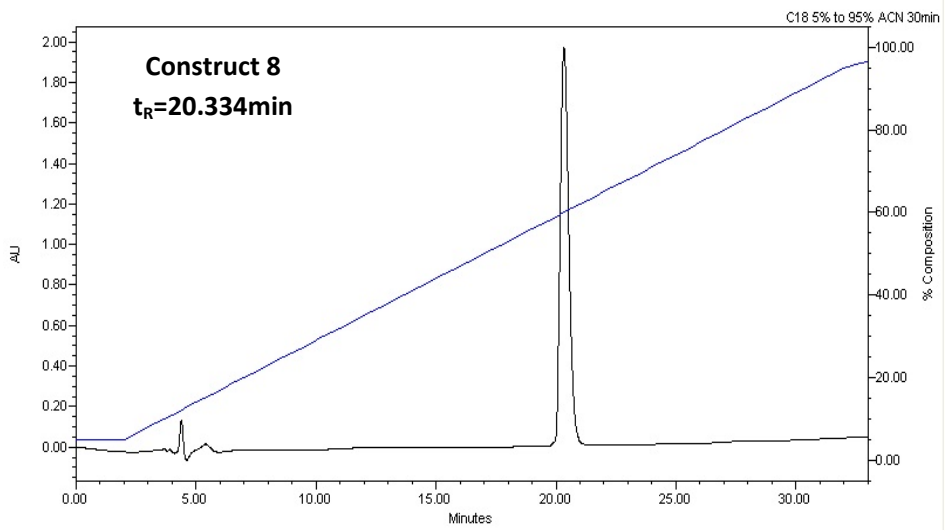


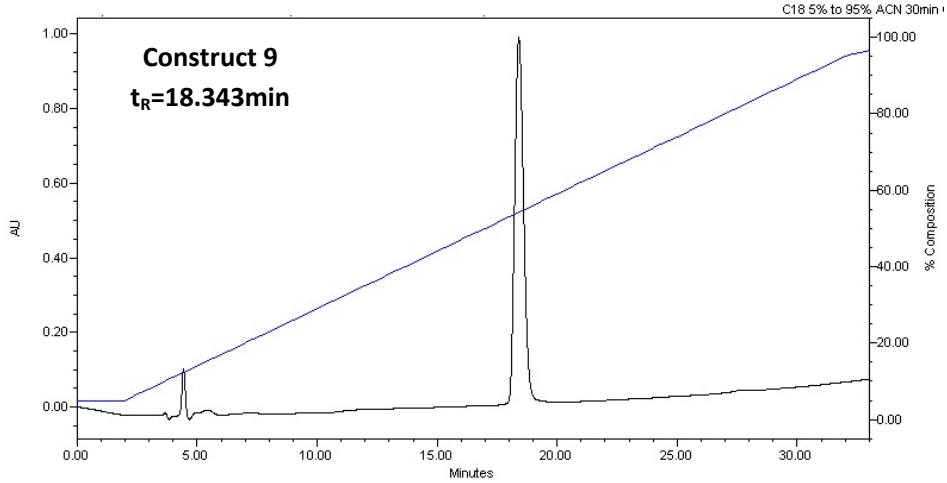












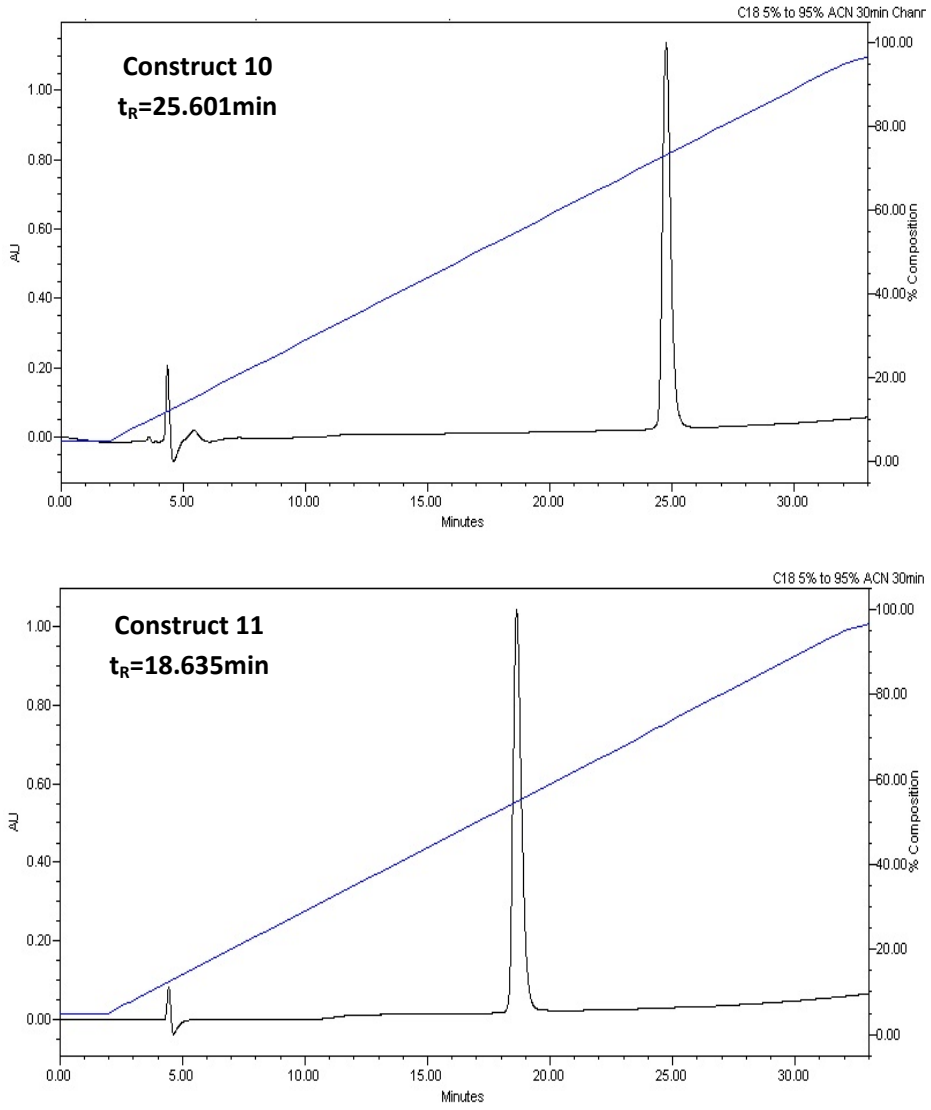
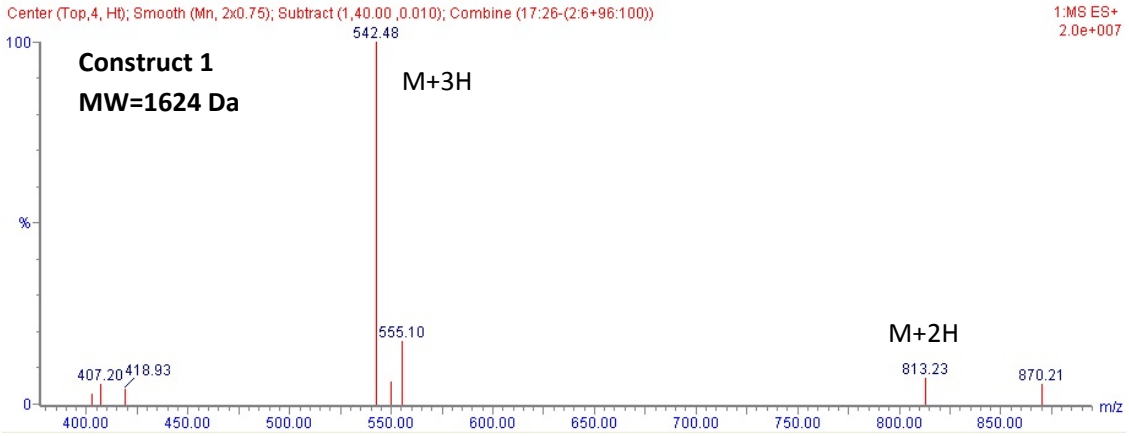
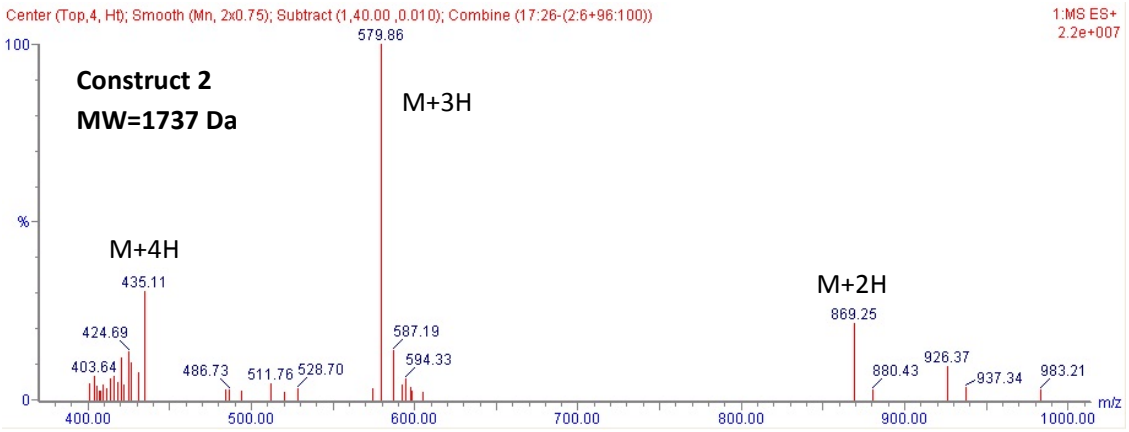
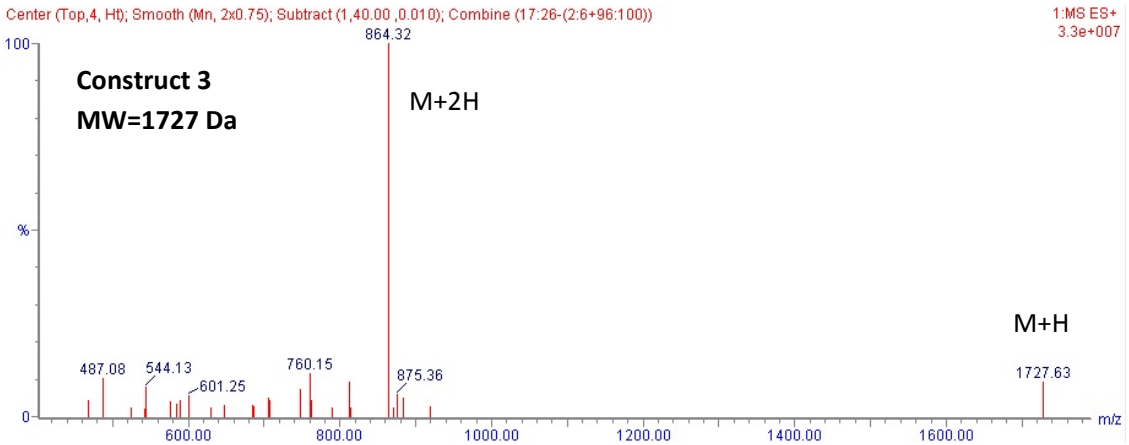


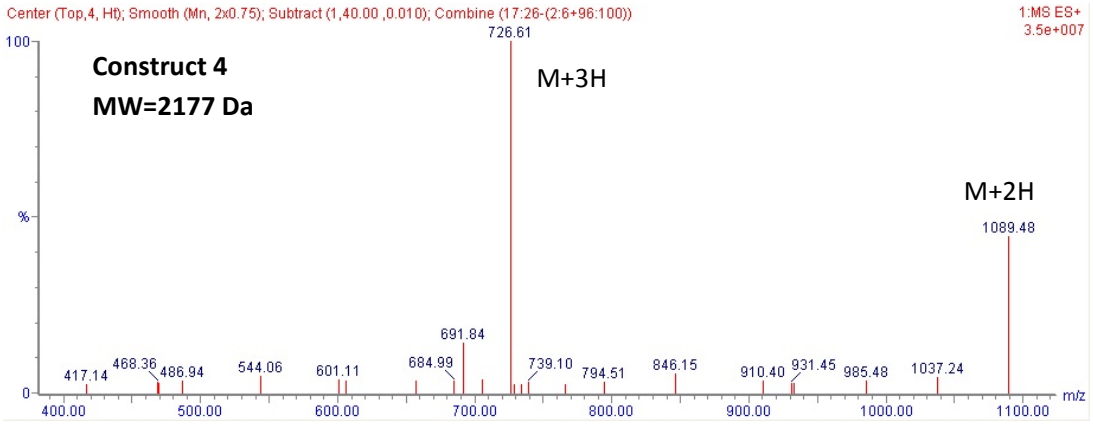
Figure S1: Analytical RP-HPLC traces of construct **1-11**, measured at 220nm. The retention time (t_R) of each construct was labeled in the chromatogram.

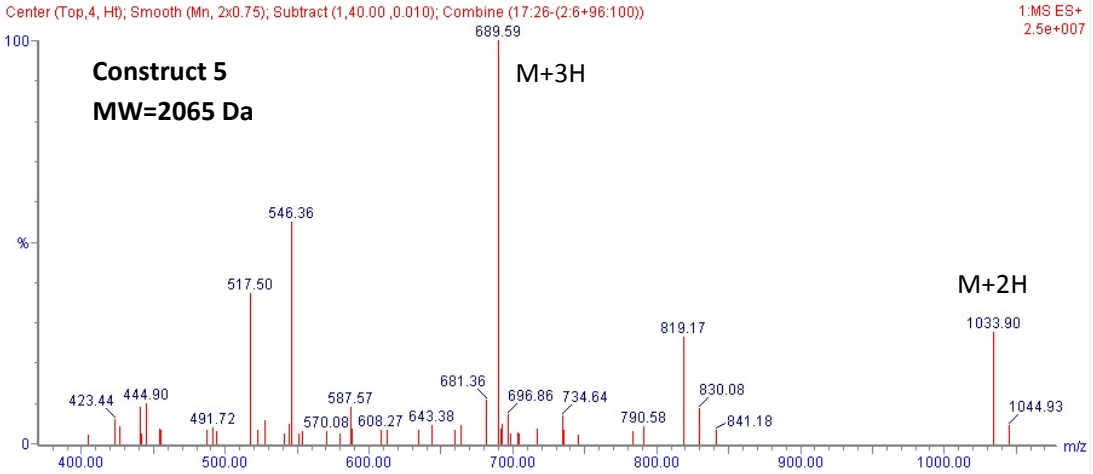
Figure S2:

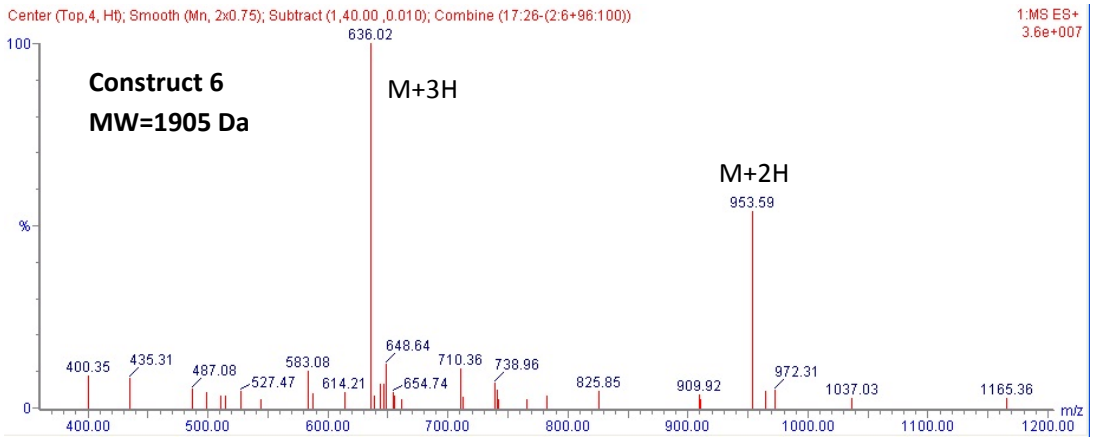


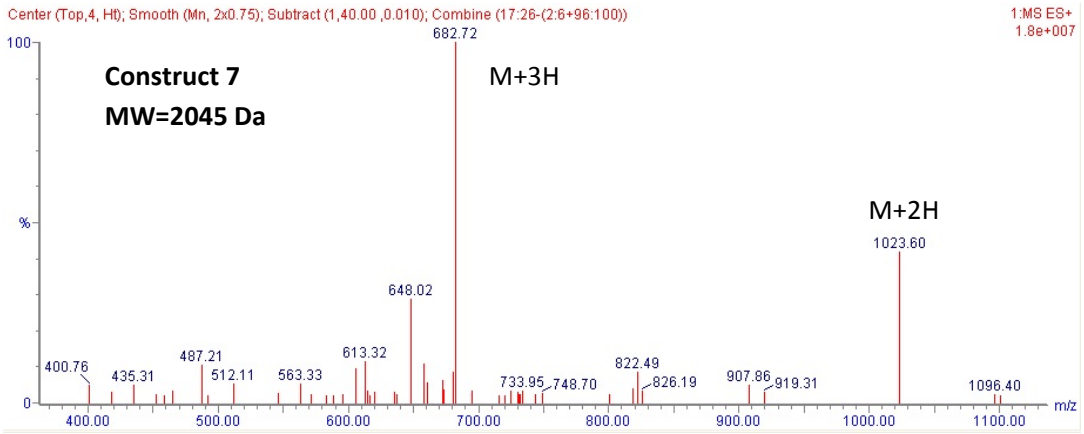


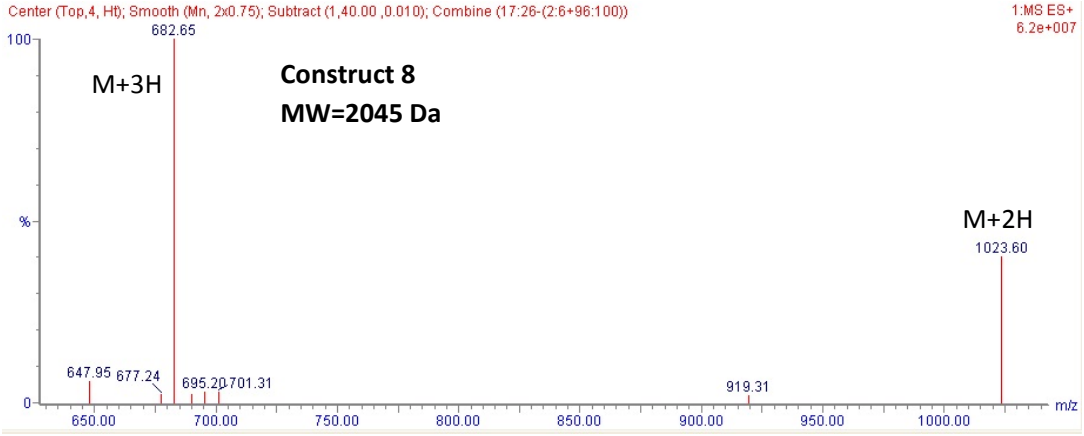


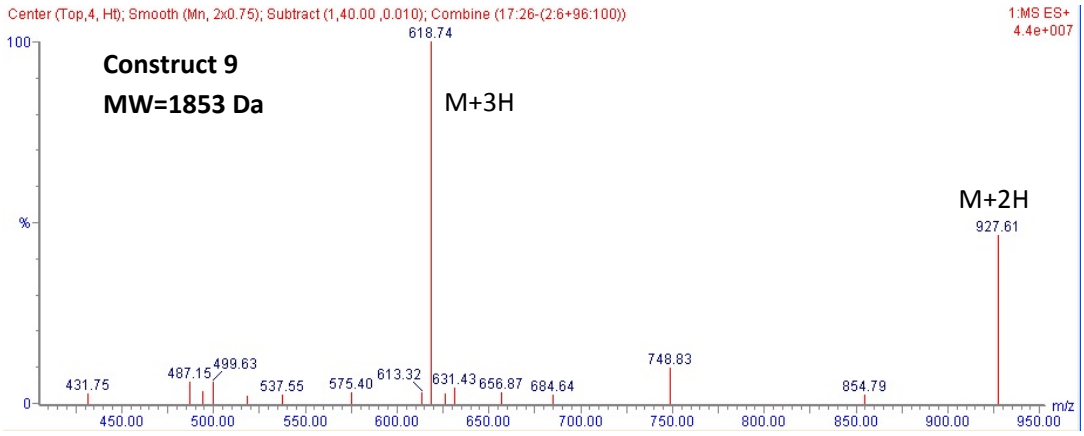












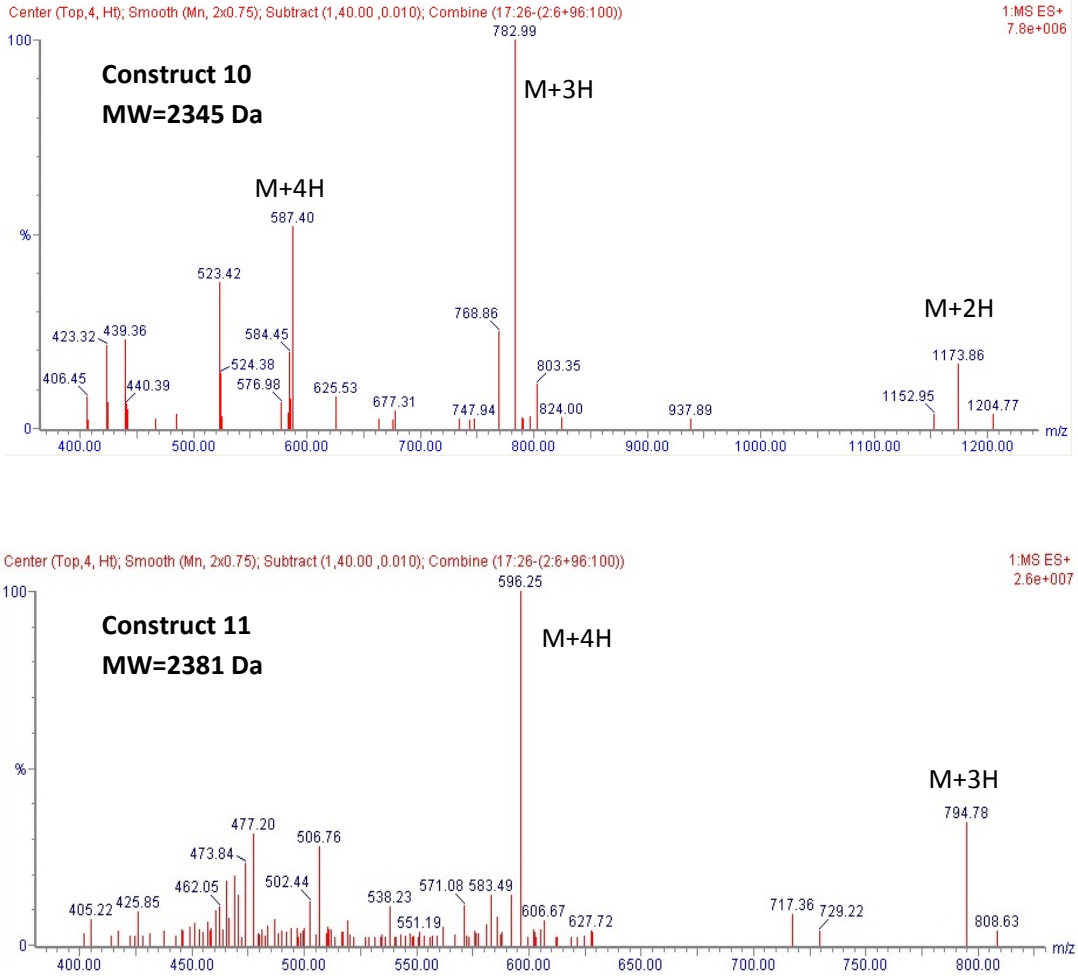


Figure S2: ESI data of construct **1-11**. Construct molecular weight (MW) and the corresponding peaks were indicated in the mass spectrum.

Reference:

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