N$^{\alpha}$-Methylation of Arginine: Implications for Cell-Penetrating Peptides

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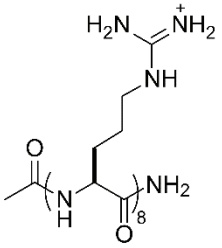
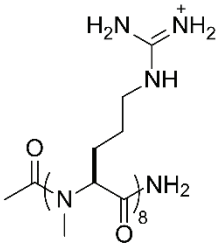
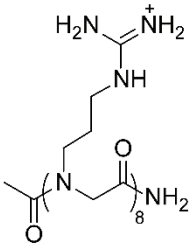
TABLE S1 Values of cLog*P* and TPSA for **R_8**, **Me-R_8**, and **$Narg_8$** as calculated with molinspiration.com.



R_8



Me-R_8



$Narg_8$

	cLog*P*	TPSA (Å^2)
R_8	−6.58	784.9
Me-R_8	−6.42	714.6
$Narg_8$	−6.63	714.6

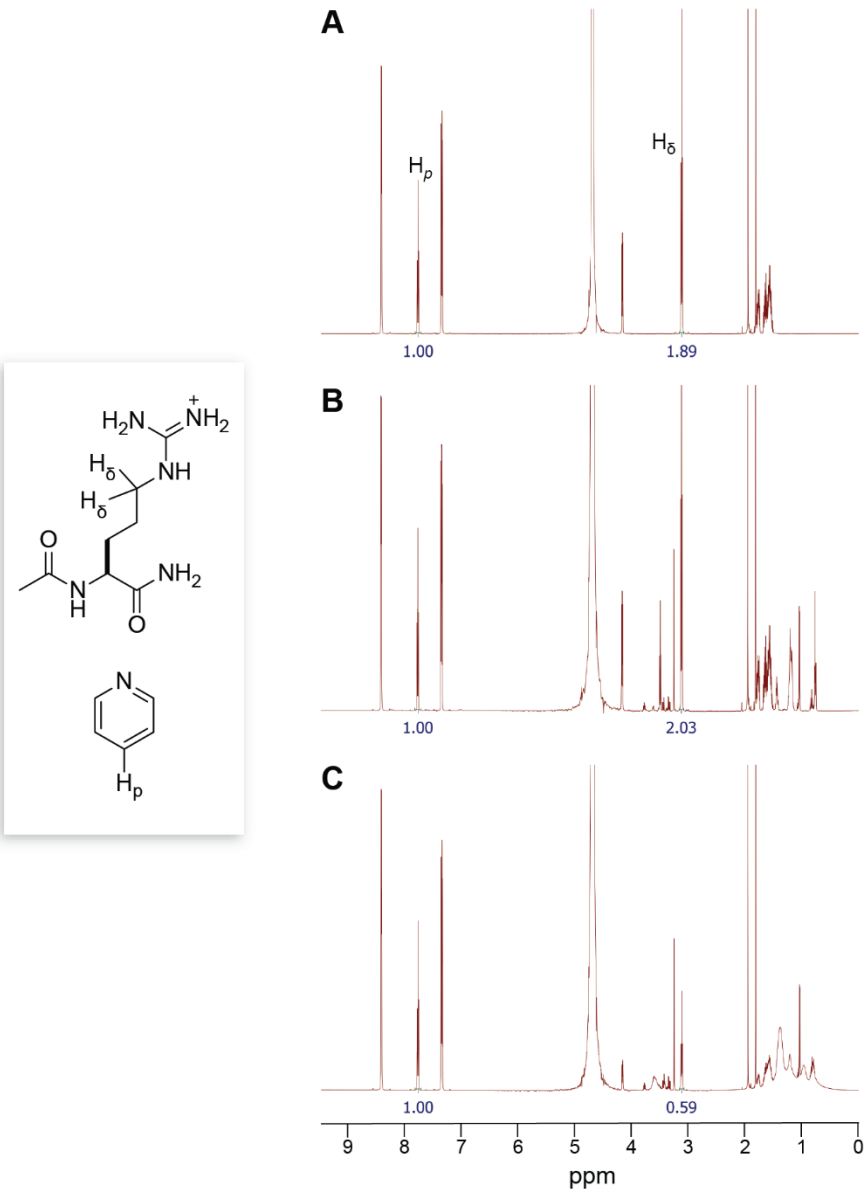


FIGURE S1 Representative ^{1}H NMR spectra of the aqueous layer from the octanol–water partitioning of Ac-Arg-NH_2·HCl (**1·HCl**) into octanol in the absence or presence of sodium dodecanoate showing 29% (= 0.59/2.03) remaining in the aqueous layer post-octanol + lipid wash. A. Pre-wash. B. Post-wash with octanol. C. Post-wash with octanol containing sodium dodecanoate.

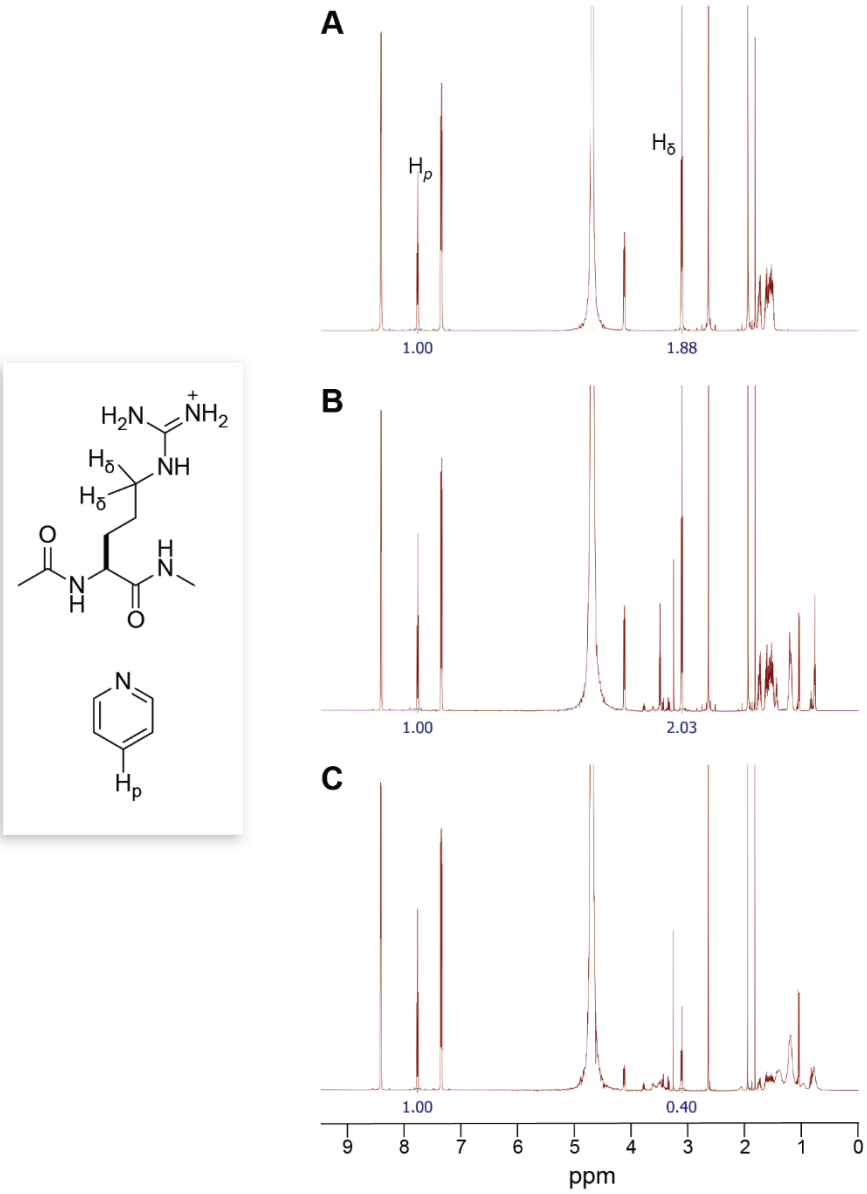


FIGURE S2 Representative ^{1}H NMR spectra of the aqueous layer from the octanol–water partitioning of Ac-Arg-NHMe·HCl (**2·HCl**) into octanol in the absence or presence of sodium dodecanoate showing 20% (= 0.40/2.03) remaining in the aqueous layer post-octanol + lipid wash. A. Pre-wash. B. Post-wash with octanol. C. Post-wash with octanol containing sodium dodecanoate.

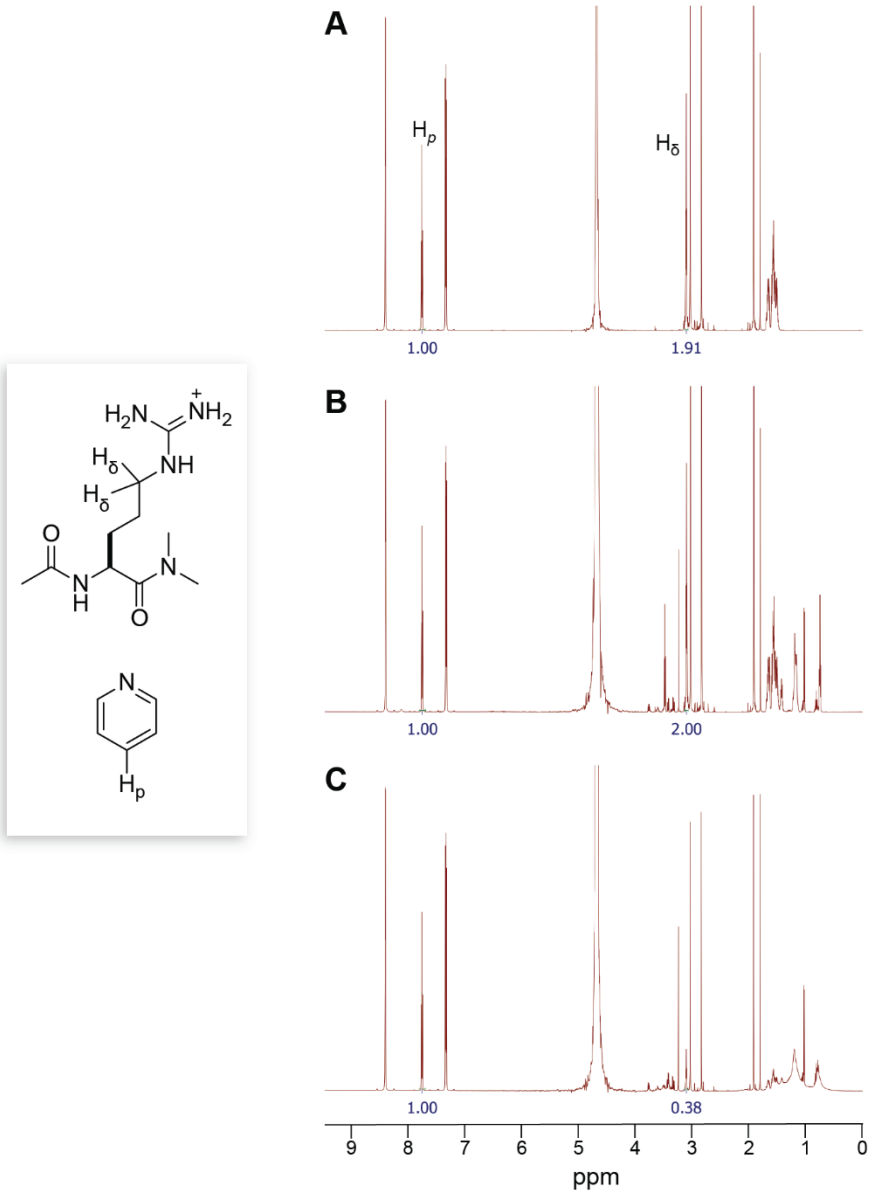


FIGURE S3 Representative ^{1}H NMR spectra of the aqueous layer from the octanol–water partitioning of Ac-Arg-NMe$_2$·HCl (**3·HCl**) into octanol in the absence or presence of sodium dodecanoate showing 19% (= 0.38/2.00) remaining in the aqueous layer post-octanol + lipid wash. The α hydrogen of **3·HCl** is buried beneath the water signal. A. Pre-wash. B. Post-wash with octanol. C. Post-wash with octanol containing sodium dodecanoate.

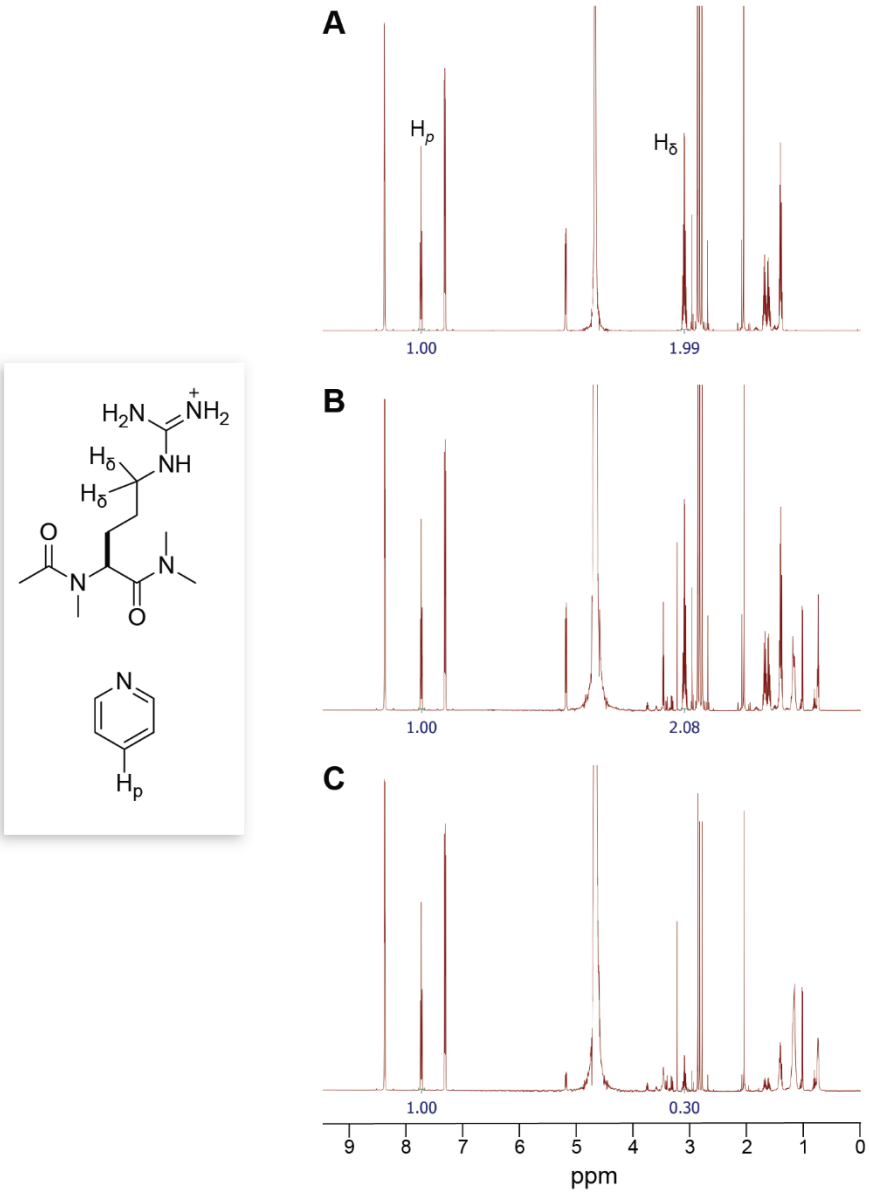


FIGURE S4 Representative ^{1}H NMR spectra of the aqueous layer from the octanol–water partitioning of Ac-(*N*-Me)Arg-NMe$_2$·HCl (**4·HCl**) into octanol in the absence or presence of sodium dodecanoate showing 14% (= 0.30/2.08) remaining in the aqueous layer post-octanol + lipid wash. A. Pre-wash. B. Post-wash with octanol. C. Post-wash with octanol containing sodium dodecanoate.

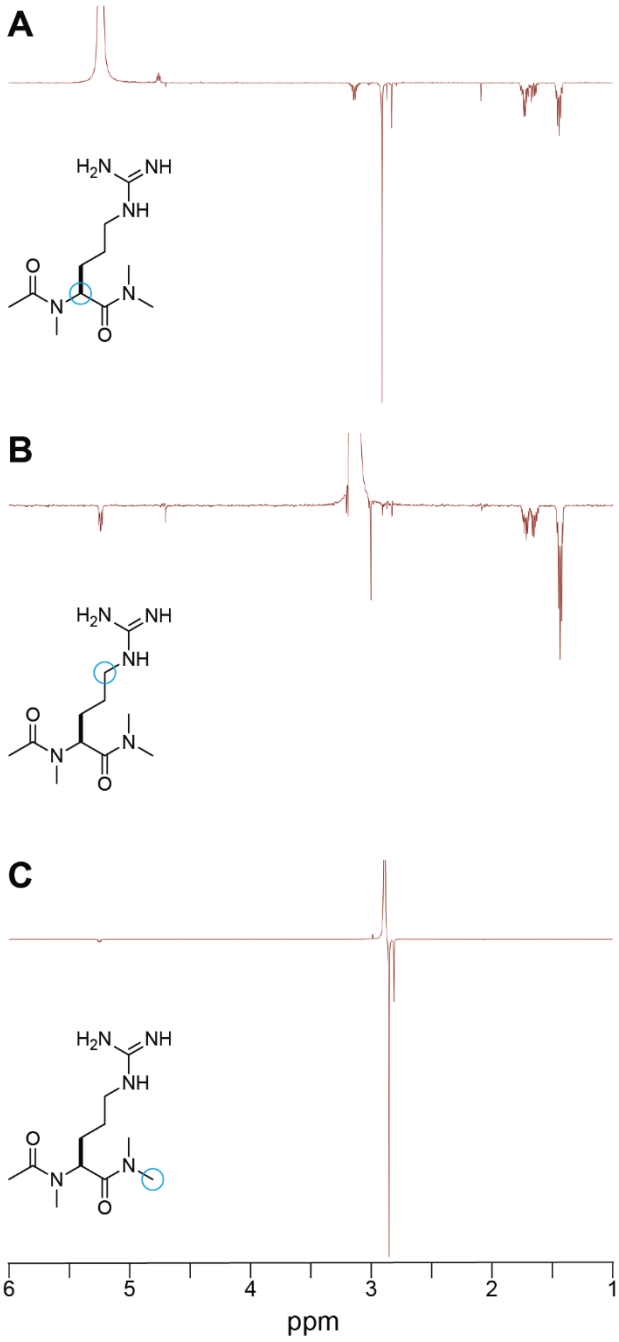


Figure S5, A–C Selective 1D NOESY experiments of compound **4** in D_2O. Blue circles highlight the protons excited, phased positive in the spectra. Through-space correlations to the excited proton are shown phased negative. (A) α-proton. (B) δ-protons. (C) One of the C-terminal methyl groups.

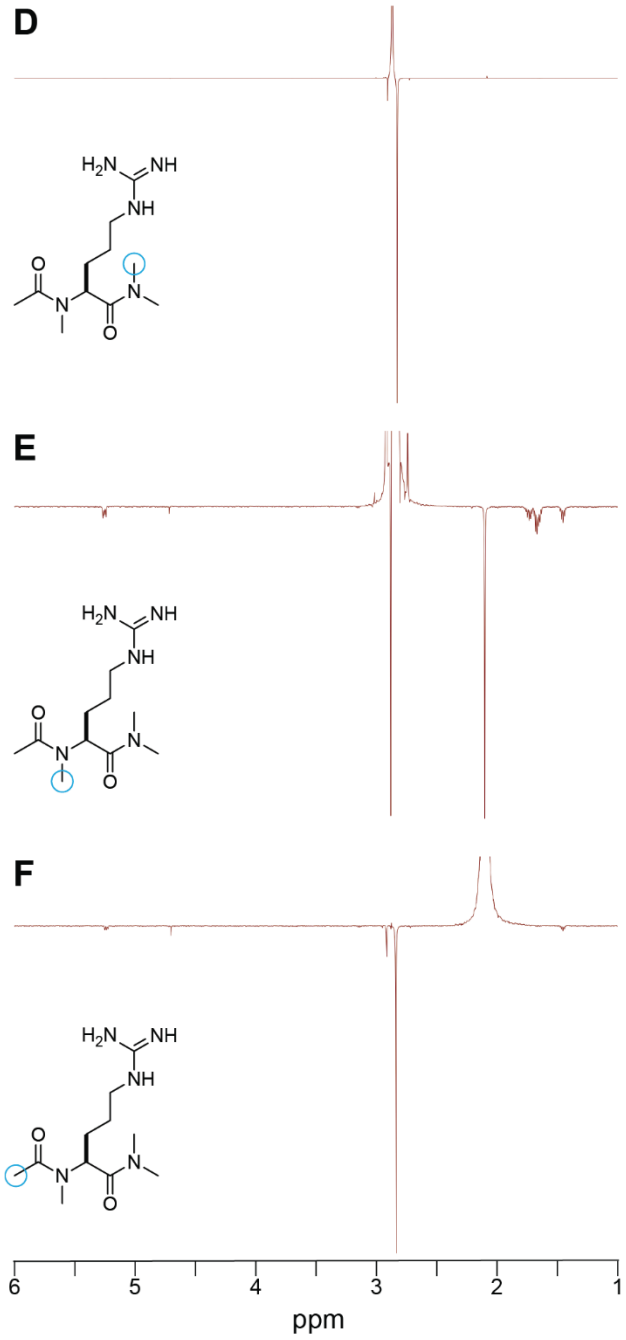


Figure S5, D–F Selective 1D NOESY experiments of compound **4** in D_2O. Blue circles highlight the protons excited, phased positive in the spectra. Through-space correlations to the excited proton are shown phased negative. (D) One of the C-terminal methyl groups. (E) N-Terminal methyl group. (F) Acetyl methyl group.

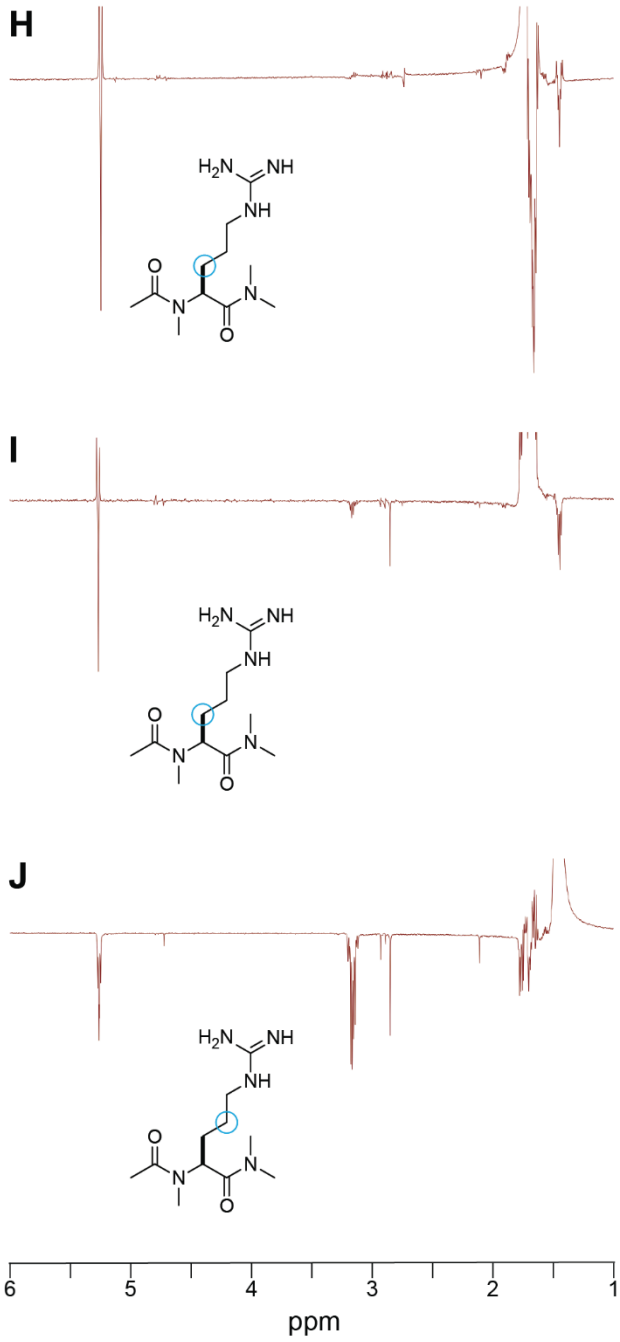


Figure S5, H–J Selective 1D NOESY experiments of compound **4** in D_2O. Blue circles highlight the protons excited, phased positive in the spectra. Through-space correlations to the excited proton are shown phased negative. (H) One of the β-protons. (I) One of the β-protons. (J) γ-Protons.

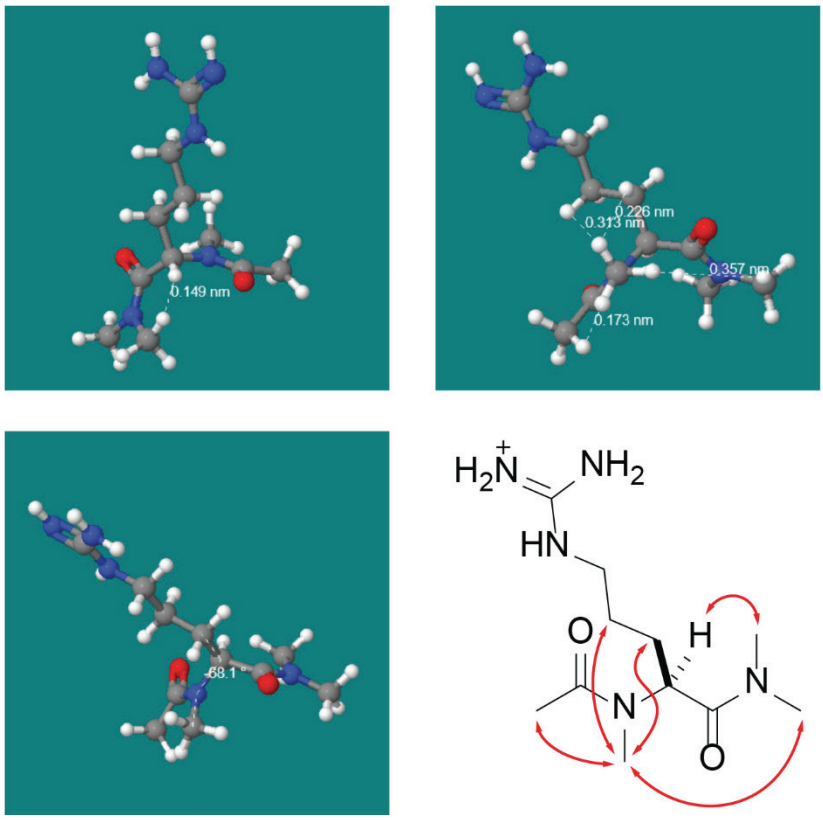


Figure S6 A model of compound **4** constructed with the CheMagic Virtual Molecular Model Kit according to the NOESY correlations found. (Note: This model is not optimized computationally.) Several angles of the model are shown with the distance between protons labeled (top) and the Me-*N*-C^{α}-CH_2^{β} dihedral angle labeled (bottom left). NOESY correlations can be observed up to ~4–5 Å. Significant NOESY correlations are noted with red arrows on a 2D representation of compound **4** (bottom right).

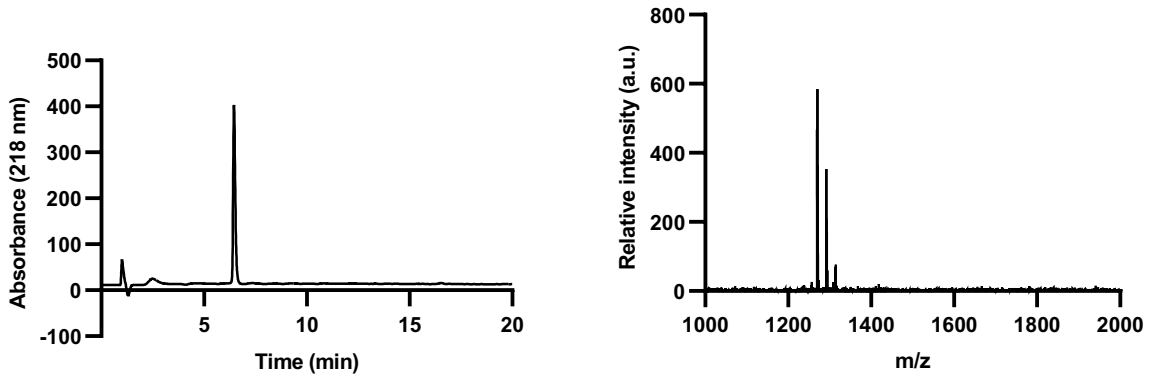


FIGURE S7 Analytical HPLC trace (left) and MALDI–TOF spectrum (right) of purified peptide **R$_5$**. RT, 6.45 min, (5–55% v/v B over 20 min); MALDI–TOF *m*/*z* expected [M + H]$^+$, 1269.67; found, 1270.2277.

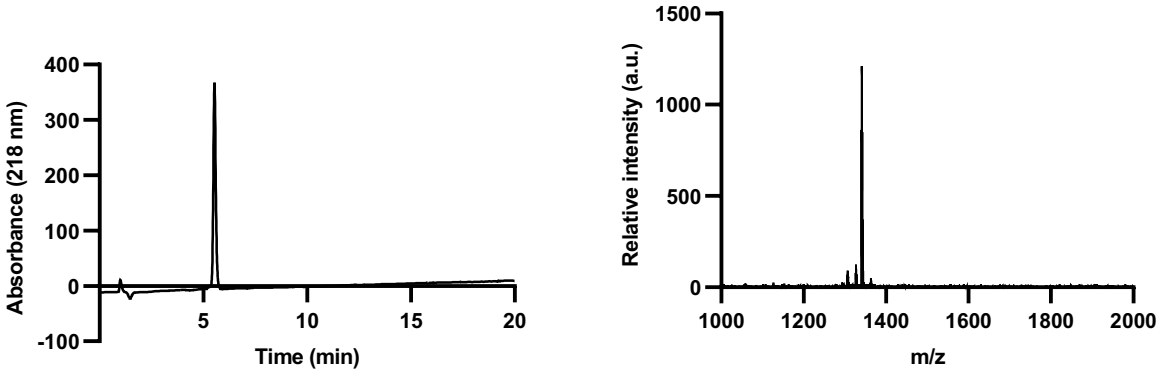


FIGURE S8 Analytical HPLC trace (left) and MALDI–TOF spectrum (right) of purified peptide **Me-R$_5$**. RT, 5.53 min, (5–55% v/v B over 20 min); MALDI–TOF *m*/*z* expected [M + H]$^+$, 1340.58; found, 1341.4020.

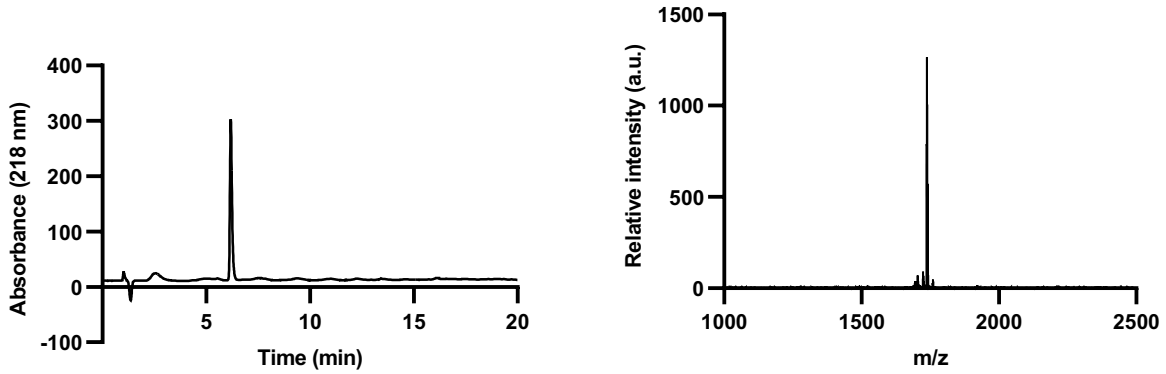


FIGURE S9 Analytical HPLC trace (left) and MALDI–TOF spectrum (right) of purified peptide **R**$_8$. RT, 6.18 min, (5–55% v/v B over 20 min); MALDI–TOF *m*/*z* expected, 1738.01; found, 1738.2543.

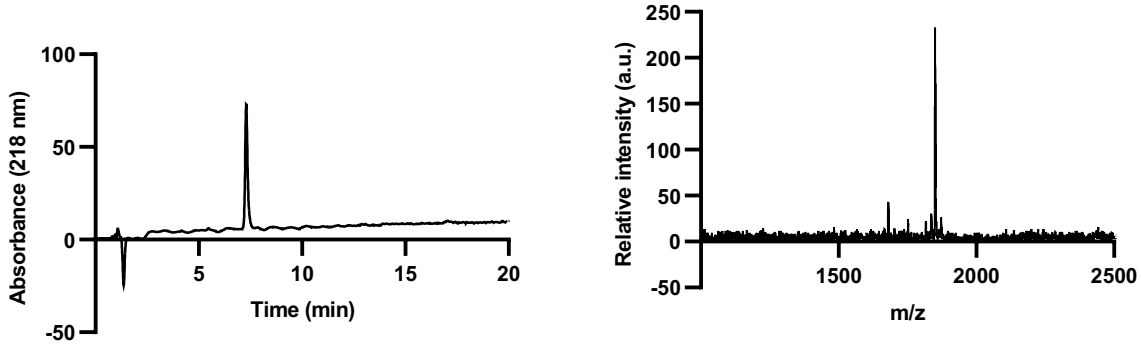


FIGURE S10 Analytical HPLC trace (left) and MALDI–TOF spectrum (right) of purified peptide **Me-R**$_8$. RT, 7.29 min, (5–55% v/v B over 20 min); MALDI–TOF *m*/*z* expected $[M + H]^+$, 1850.22; found, 1850.4494.

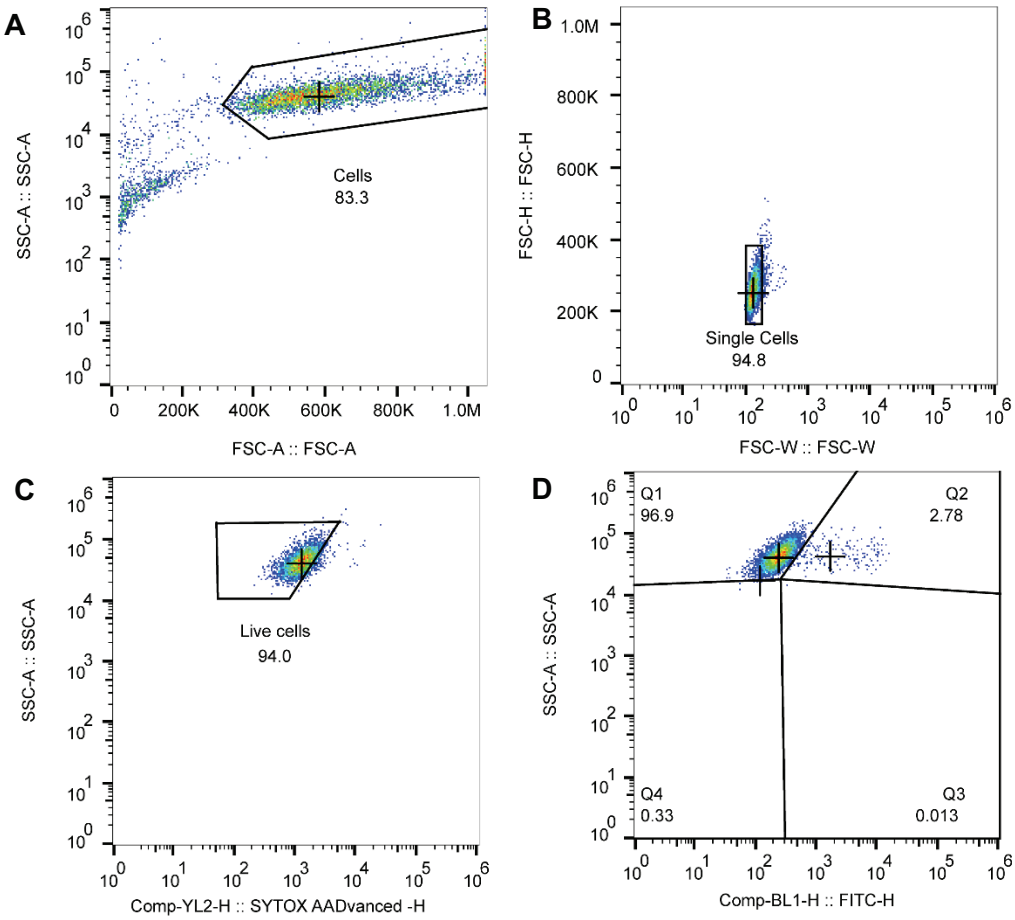


FIGURE S11 Gates used for flow cytometry data shown in Figure 2. An untreated cell sample was used to obtain gates for cells (A) and single cells (B). A control sample treated with only SYTOX AADvanced™ was gated for live cells (C). A fluorescein-treated control sample was used to obtain quadrants for analyses. Geometric mean values of green fluorescence from living single cells were recorded in each experiment.

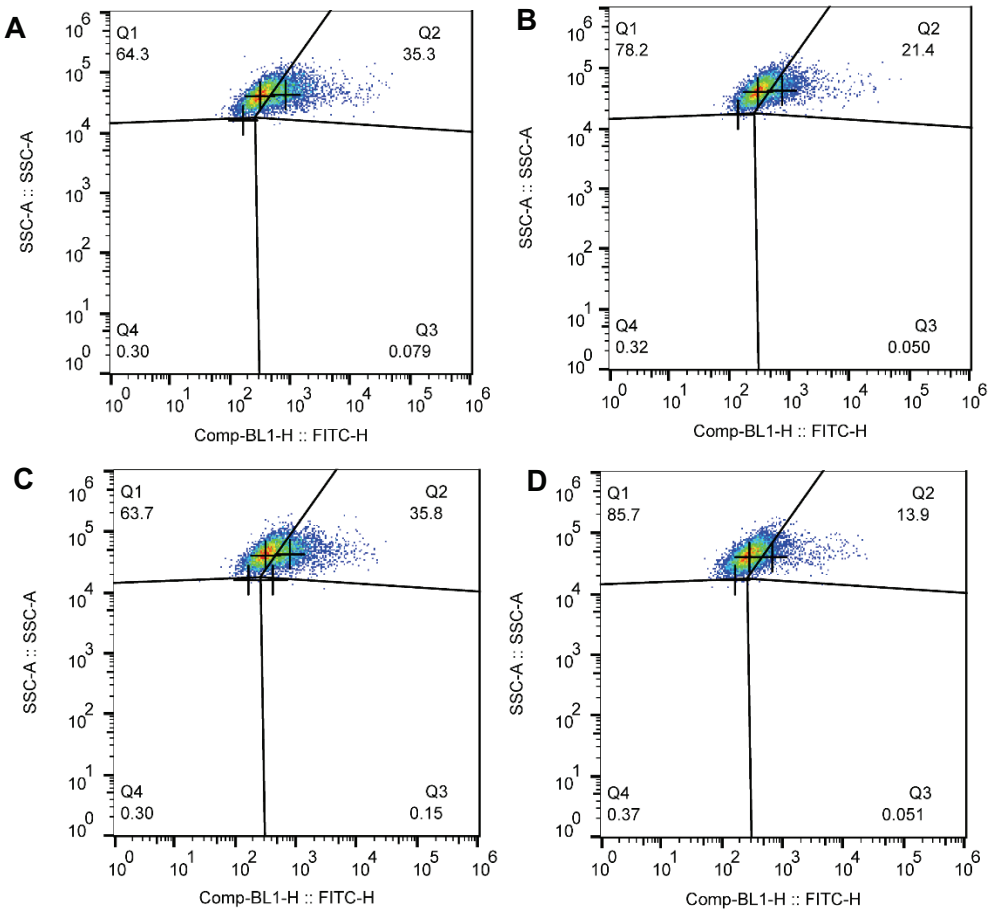


FIGURE S12 Representative flow cytometry data for peptides **Me-R$_5$** (A), **R$_5$** (B), **Me-R$_8$** (C), **R$_8$** (D). Note the increase in population in Q2 for *N*-methylated peptides (A, C) compared to their canonical congeners (B, D). Three biological replicates and two technical replicates are reported in Figure 2.

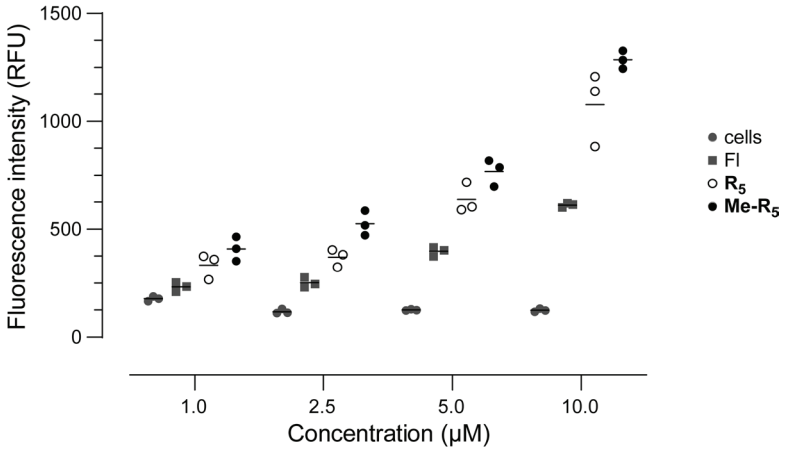


FIGURE S13 Graph showing the uptake of **Me-R$_5$** and **R$_5$** at increasing concentrations by live HeLa cells using flow cytometry. Cells were treated with the indicated concentration of carboxyfluorescein (Fl) or a fluorescein-labeled penta-arginine for 1 h at 37 °C. Geometric mean values of the green fluorescence from living, single cells were recorded by flow cytometry.

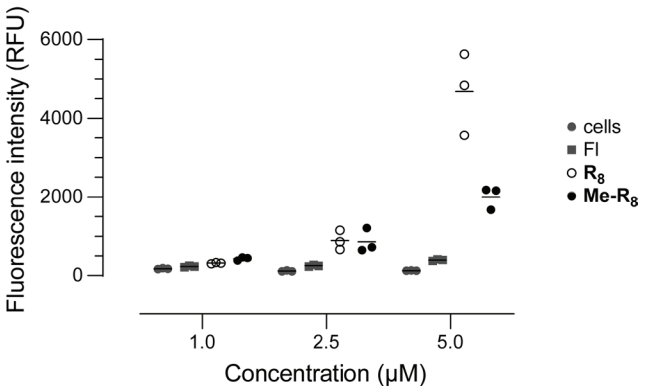


FIGURE S14 Graph showing the uptake of **Me-R$_8$** and **R$_8$** at increasing concentrations by live HeLa cells using flow cytometry. Cells were treated with the indicated concentration of carboxyfluorescein (Fl) or a fluorescein-labeled octaarginine for 1 h at 37 °C.

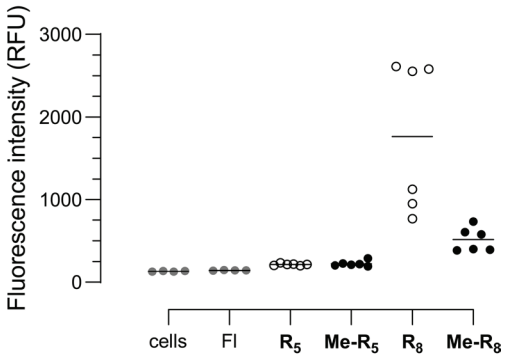
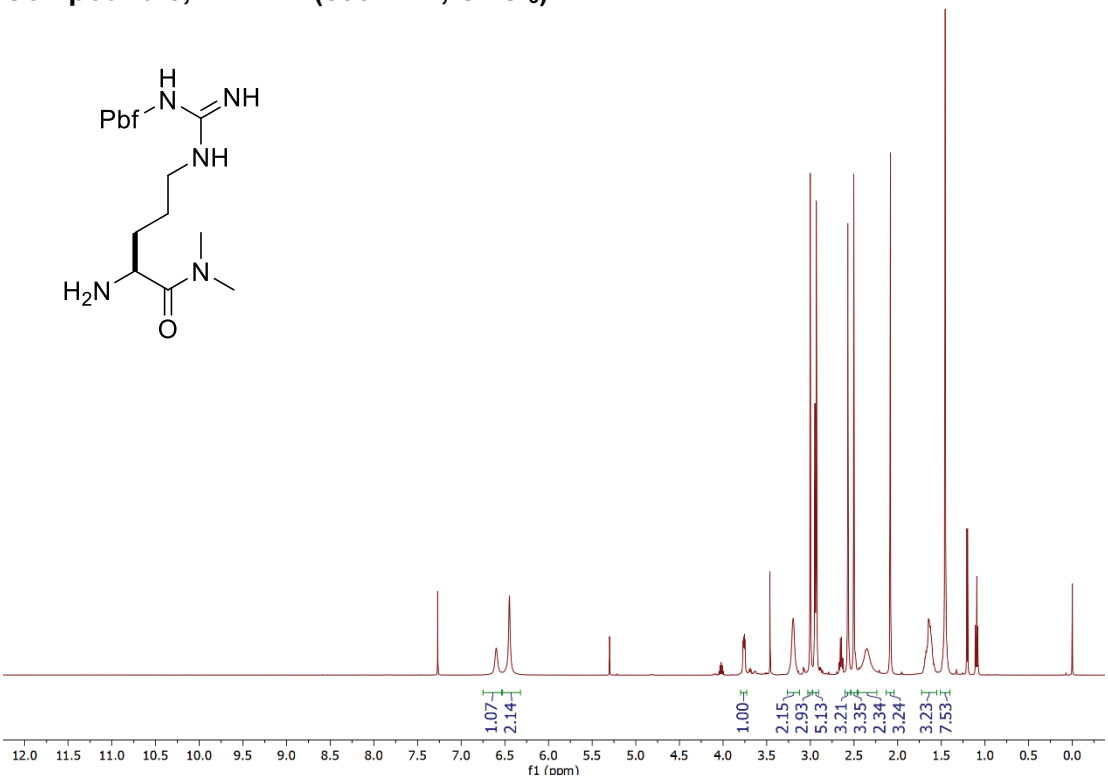


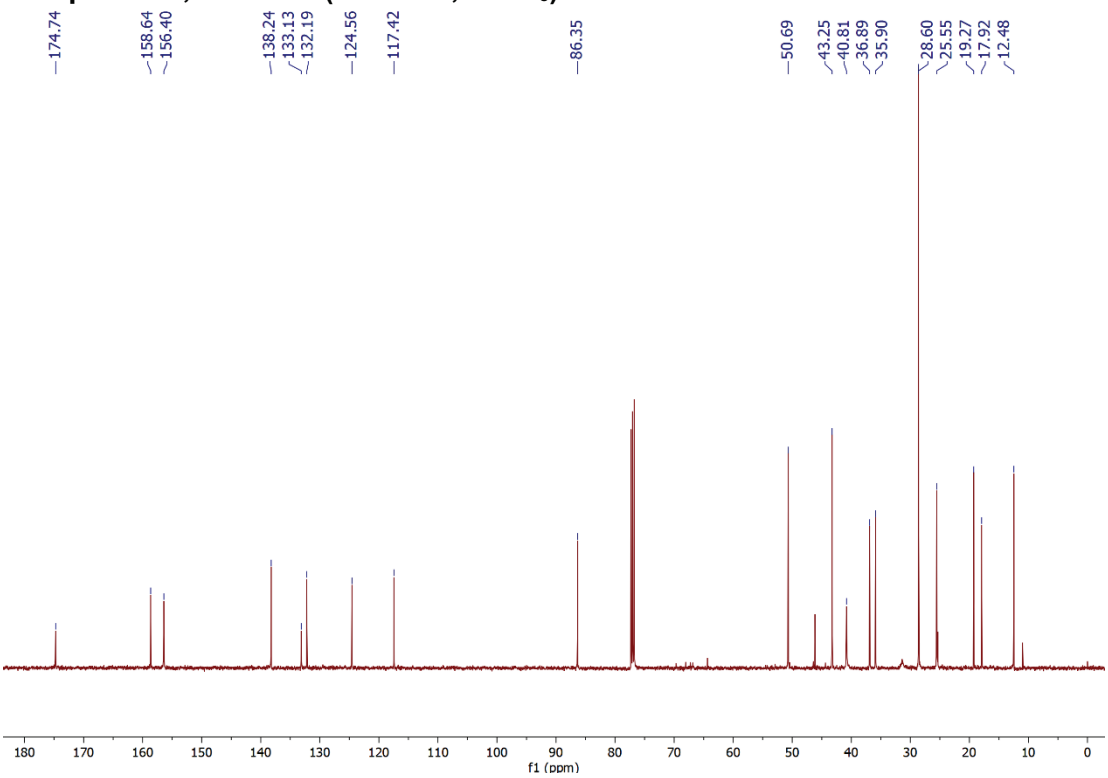
FIGURE S15 Graph showing the uptake of CPPs at 4 °C by live HeLa cells using flow cytometry. Cells were treated with 5 μM of carboxyfluorescein (Fl) or a fluorescein-labeled oligoarginine for 1 h at 4 °C.

NMR Spectra

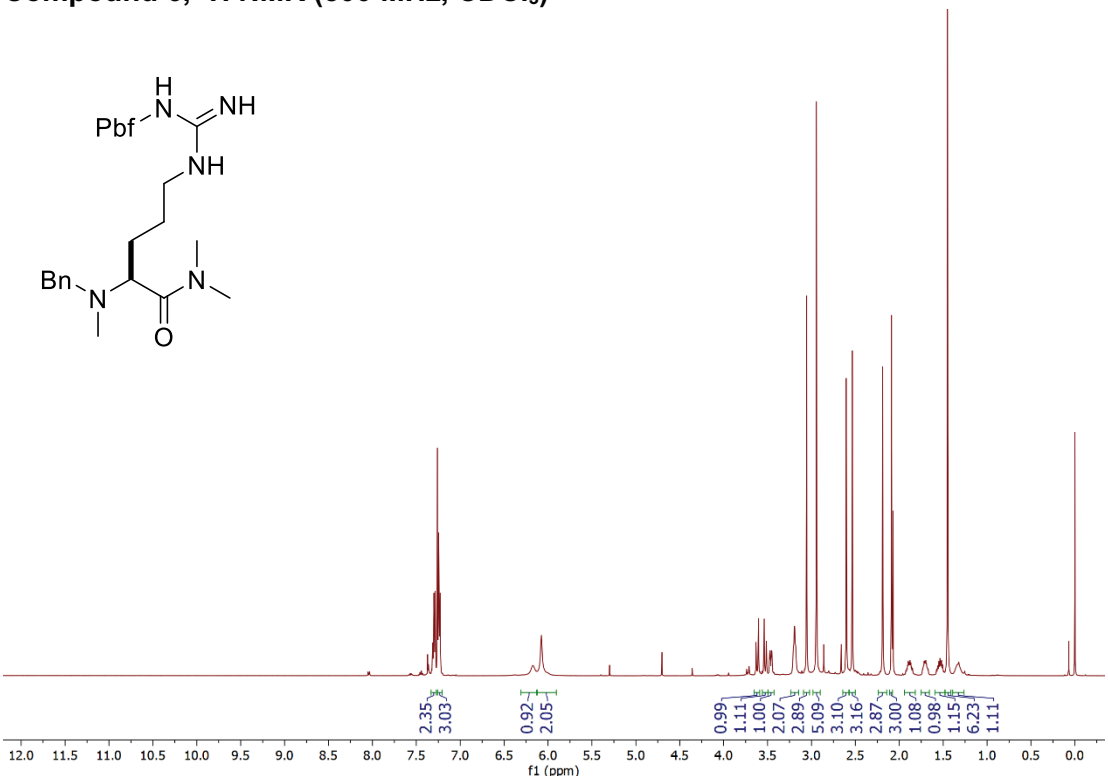
Compound 5, ^{1}H NMR (500 MHz, $CDCl_3$)



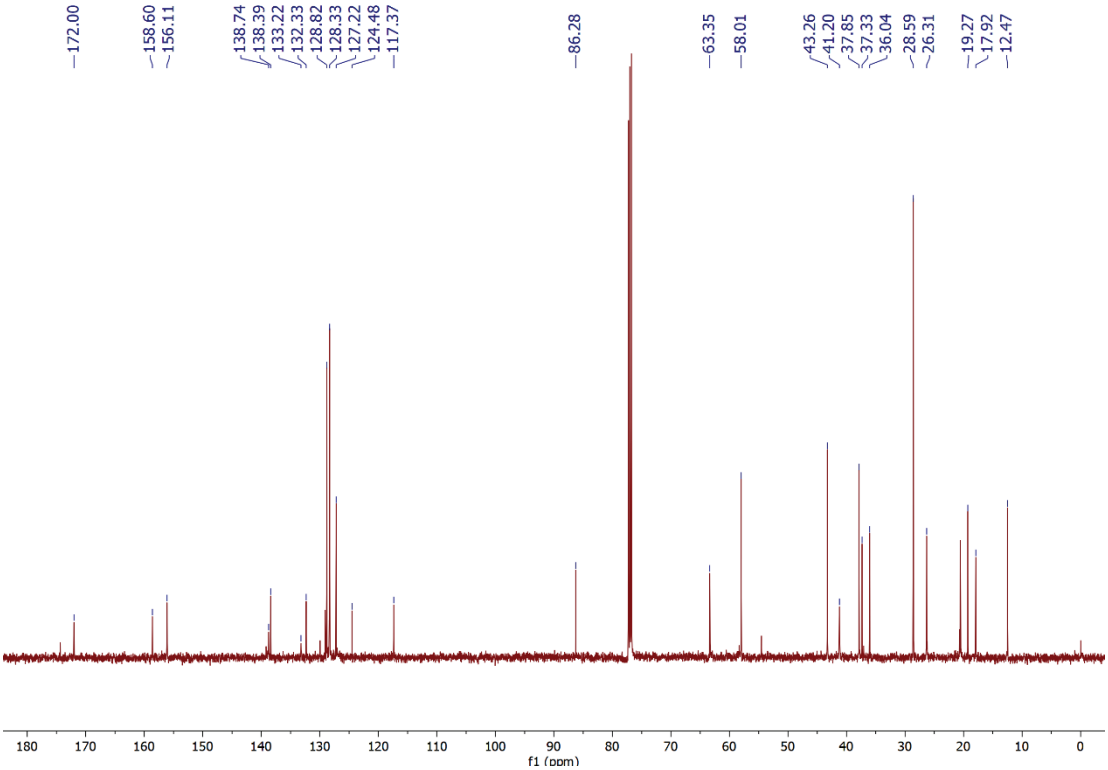
Compound 5, ^{13}C NMR (500 MHz, $CDCl_3$)



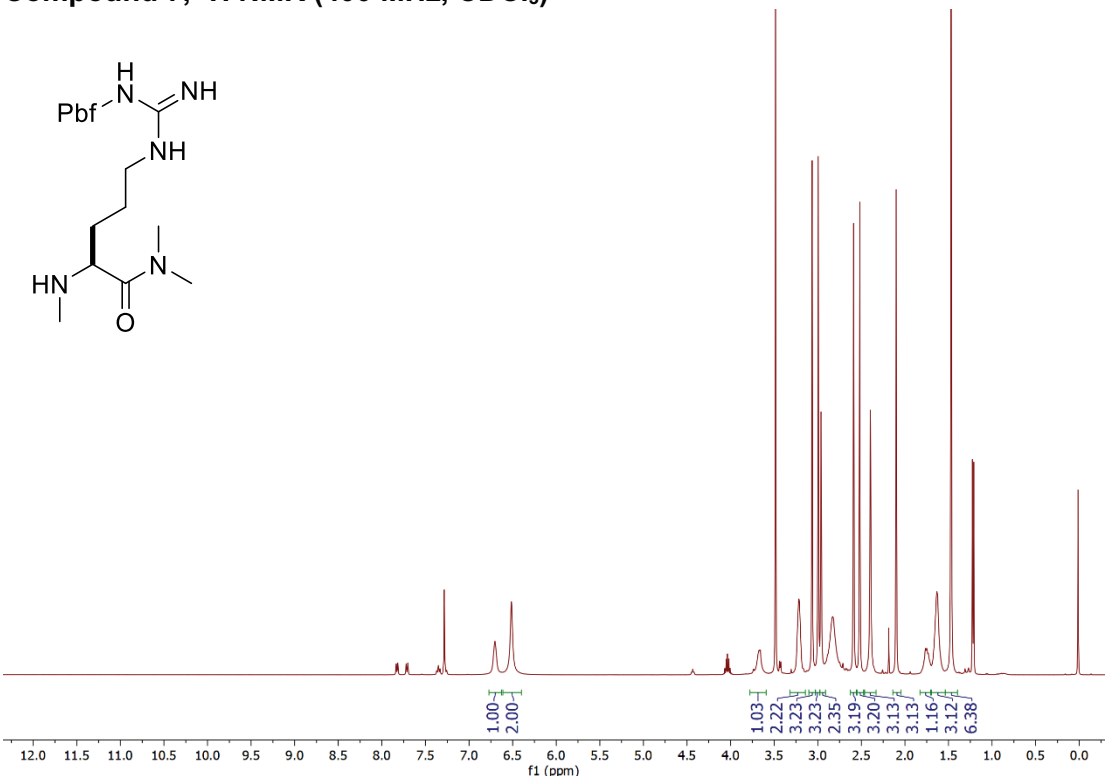
Compound 6, ^{1}H NMR (500 MHz, $CDCl_3$)



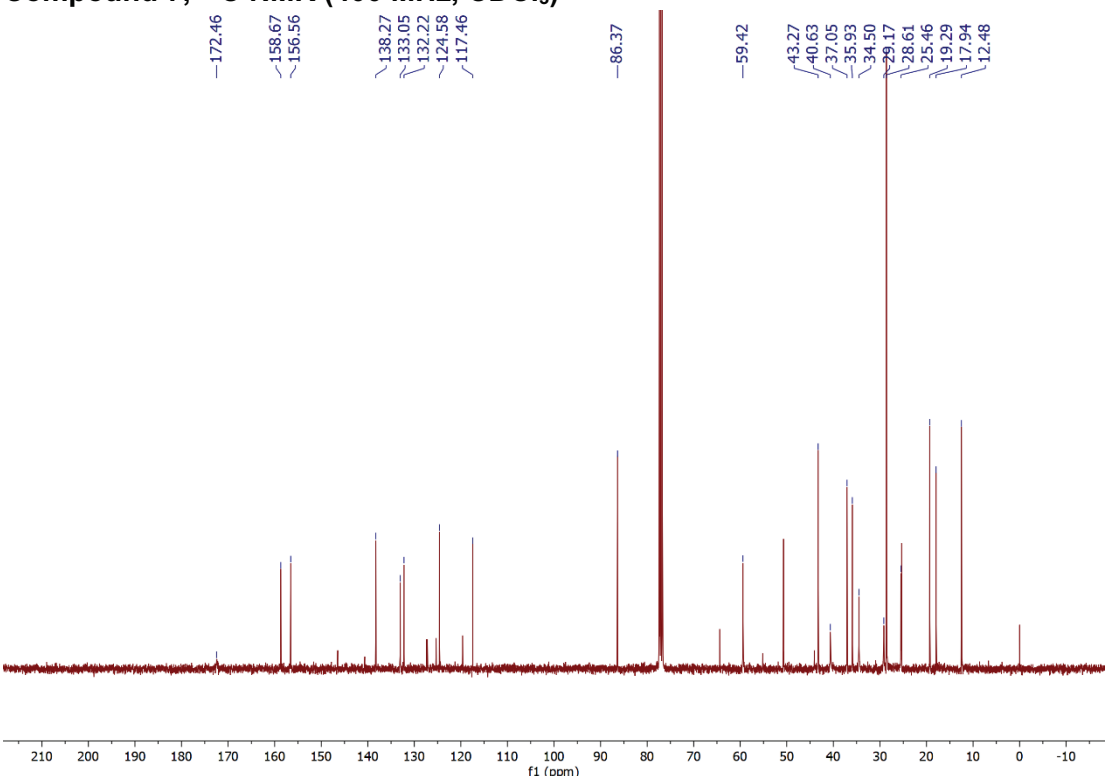
Compound 6, ^{13}C NMR (500 MHz, $CDCl_3$)



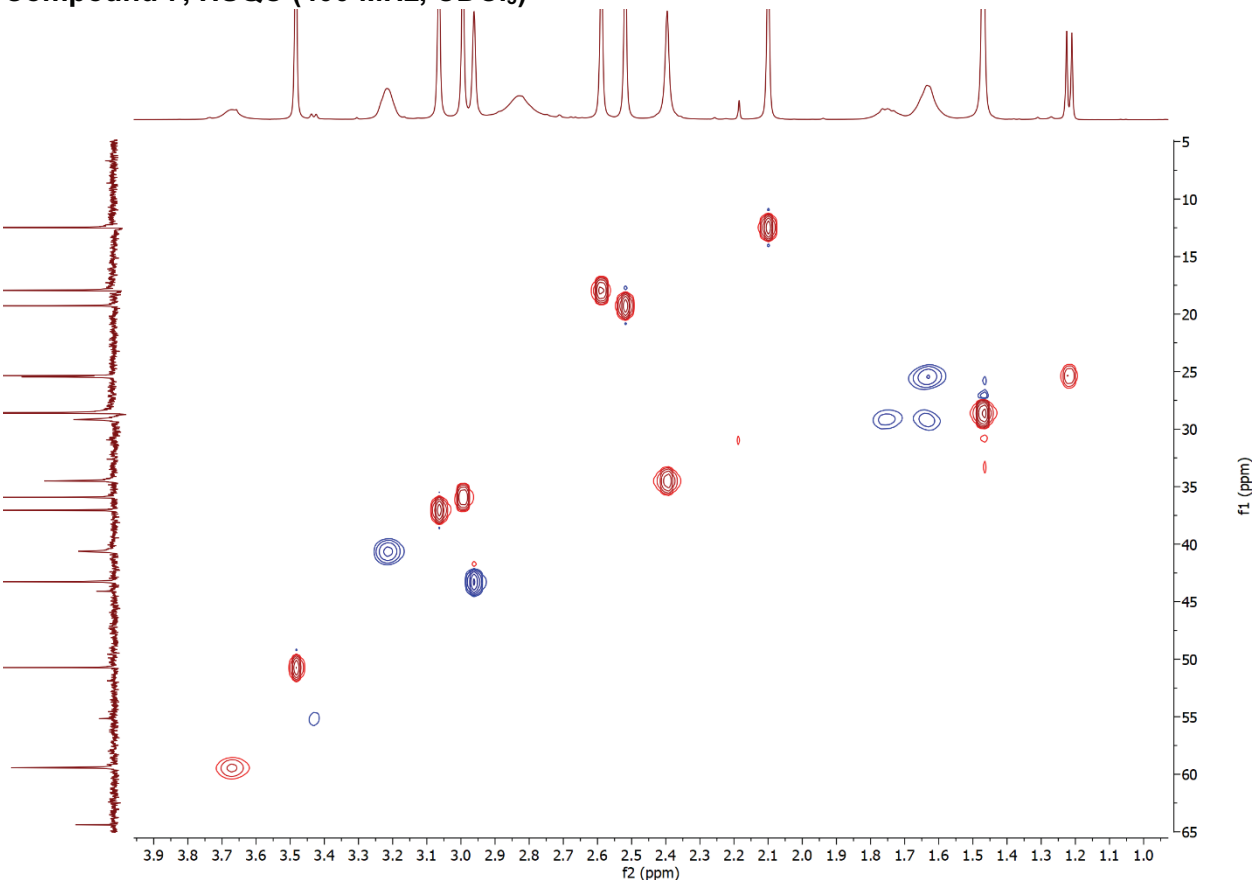
Compound 7, ^{1}H NMR (400 MHz, $CDCl_3$)



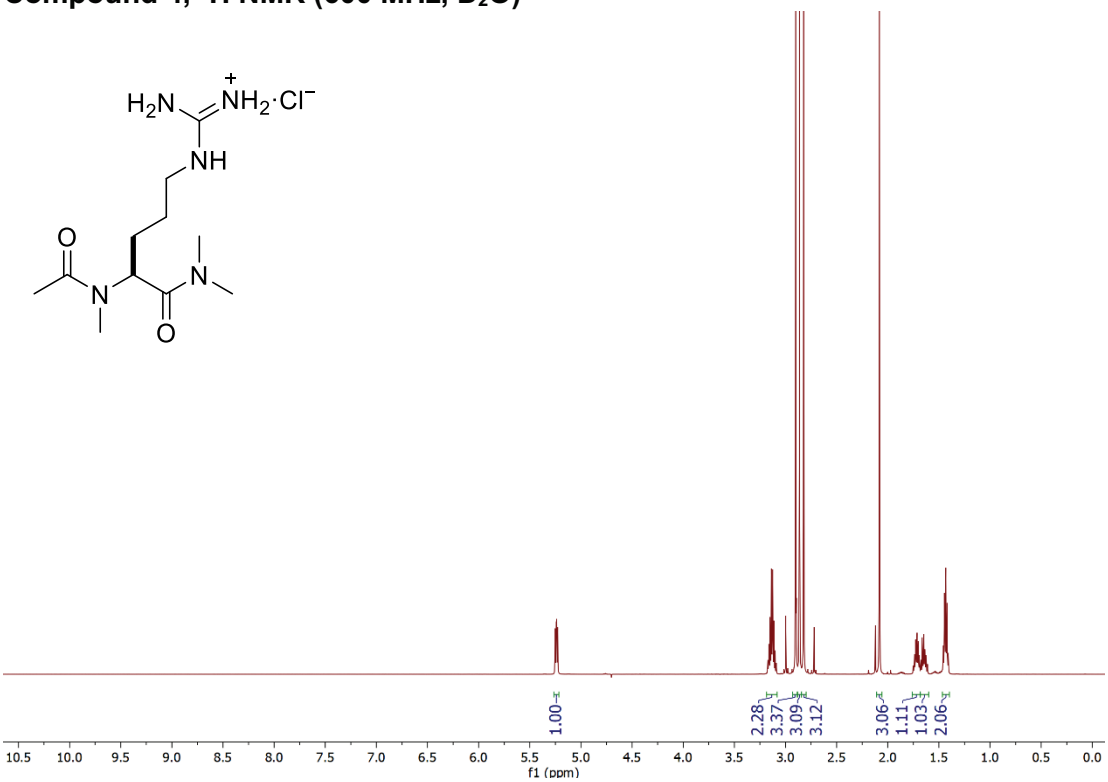
Compound 7, ^{13}C NMR (400 MHz, $CDCl_3$)



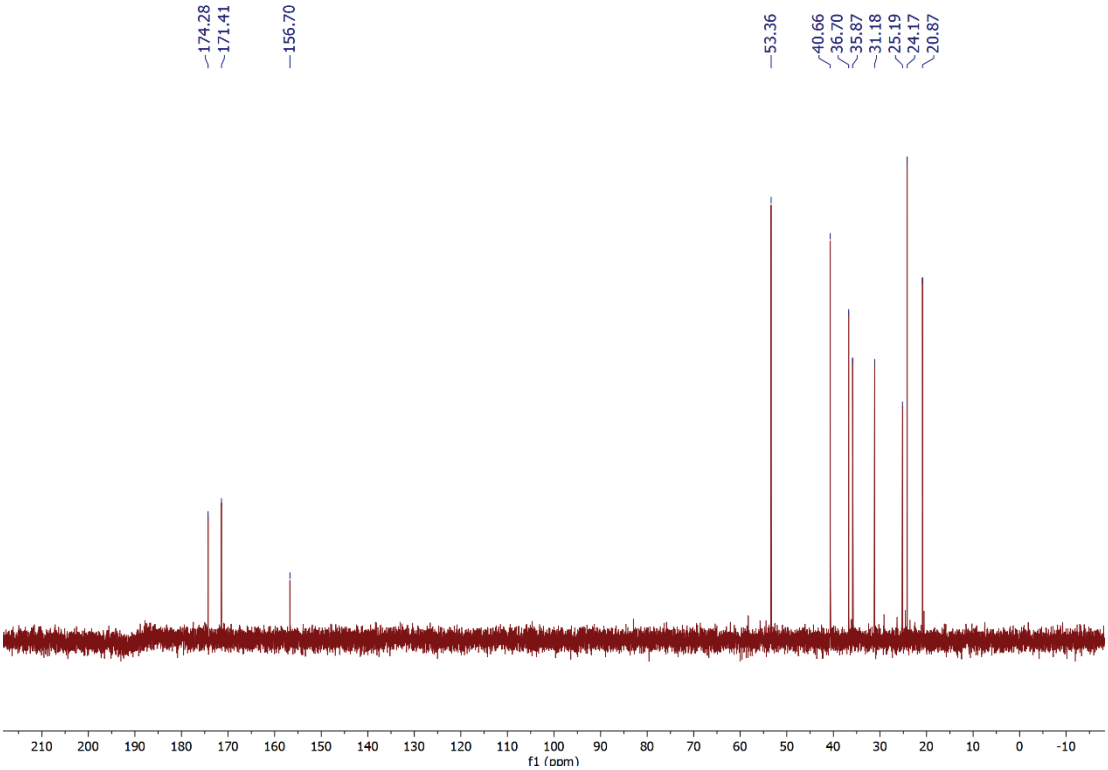
Compound 7, HSQC (400 MHz, $CDCl_3$)



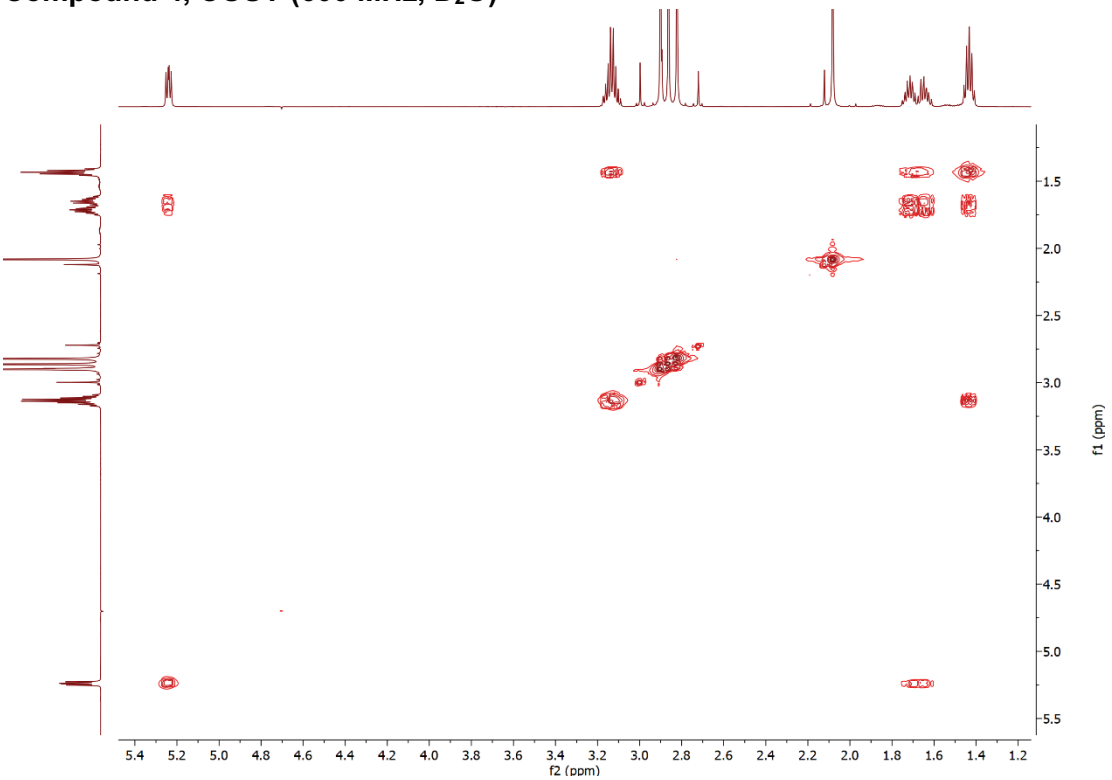
Compound 4, ^{1}H NMR (600 MHz, D_2O)



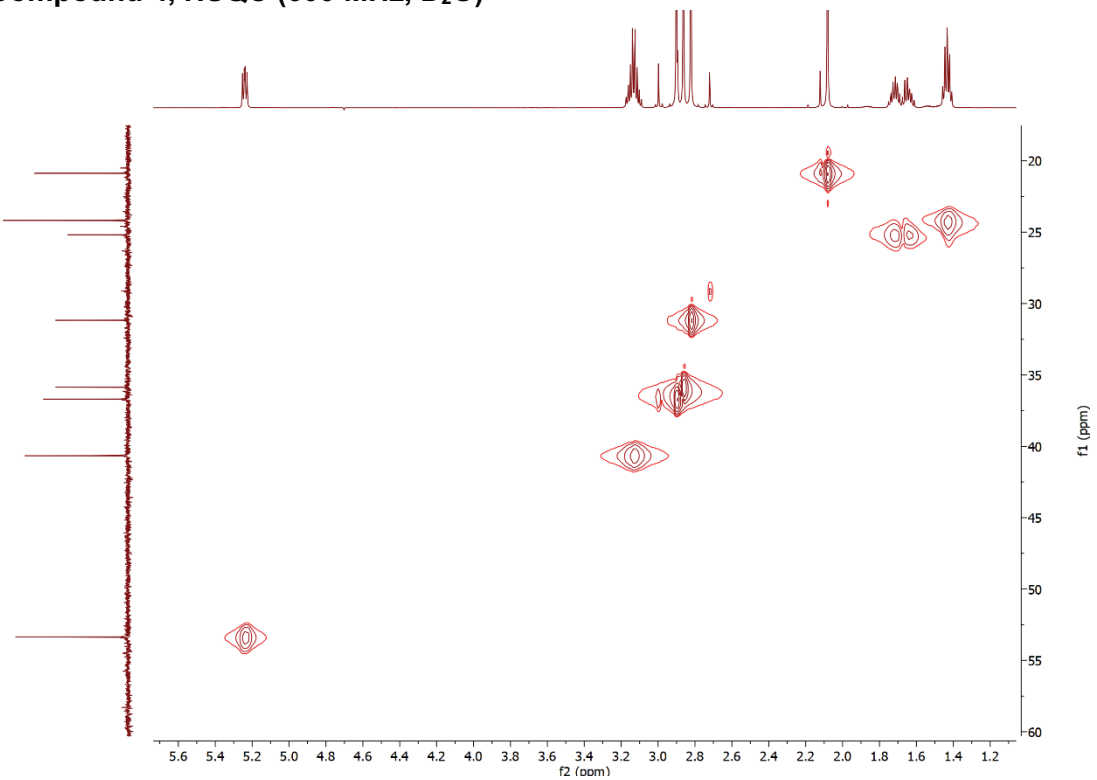
Compound 4, ^{13}C NMR (600 MHz, D_2O)



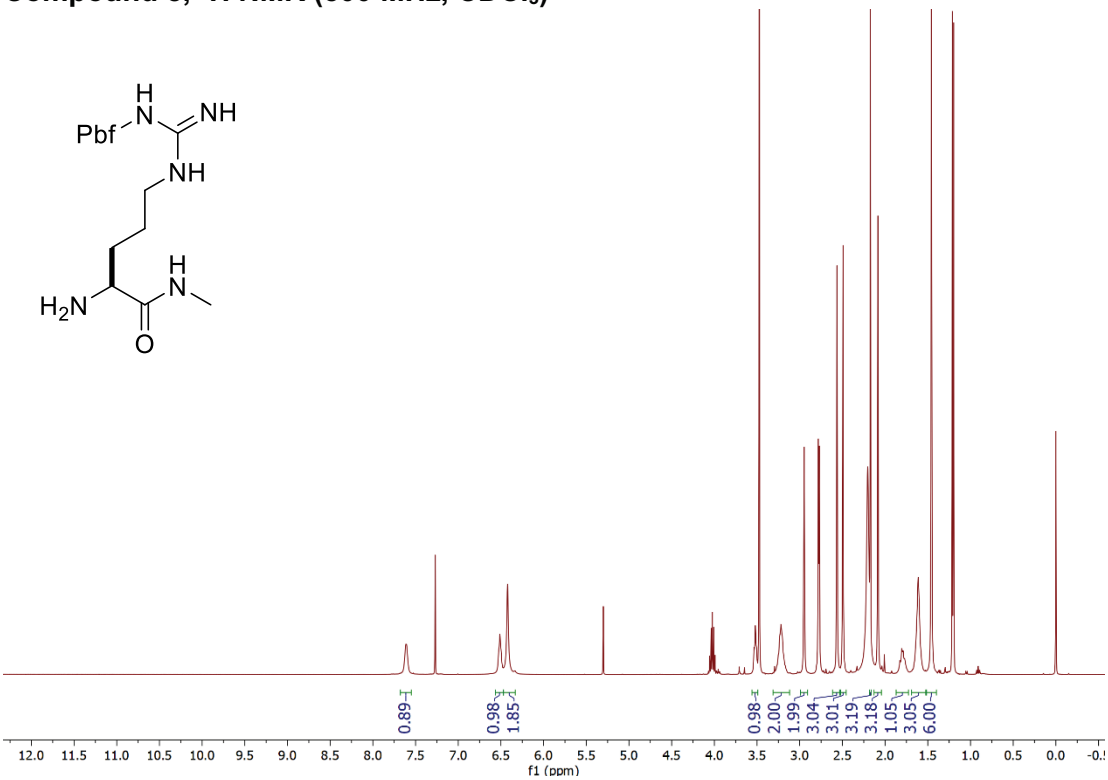
Compound 4, COSY (600 MHz, D_2O)



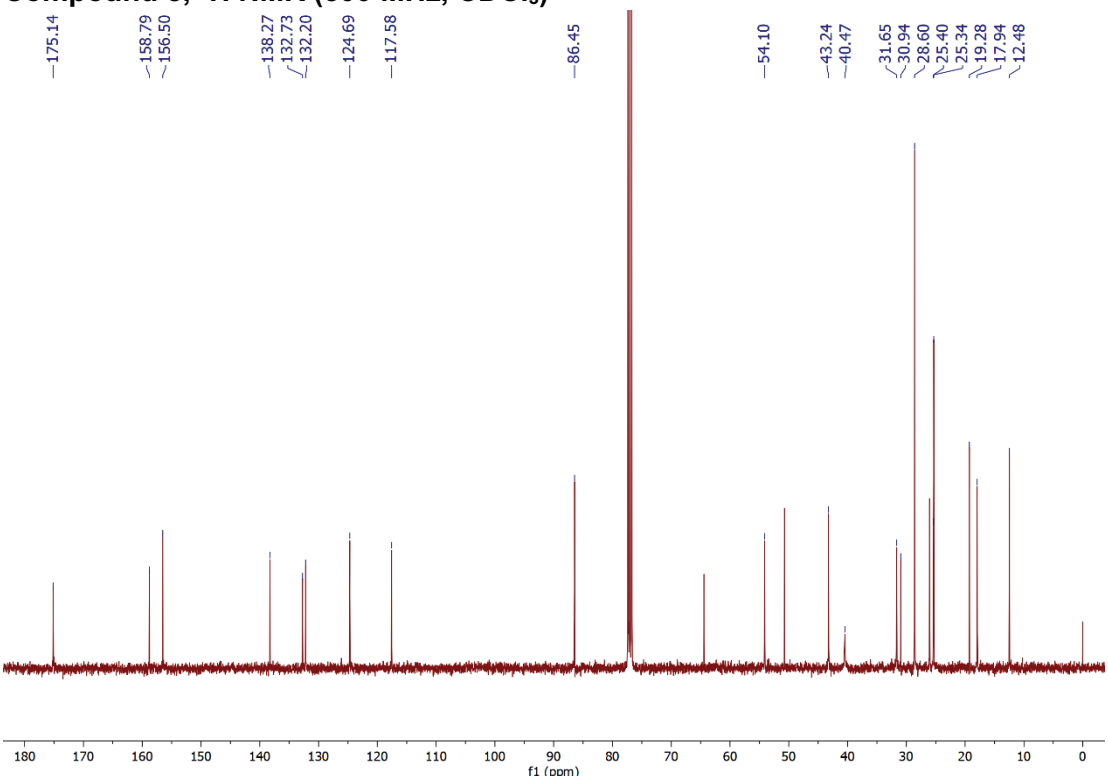
Compound 4, HSQC (600 MHz, D_2O)



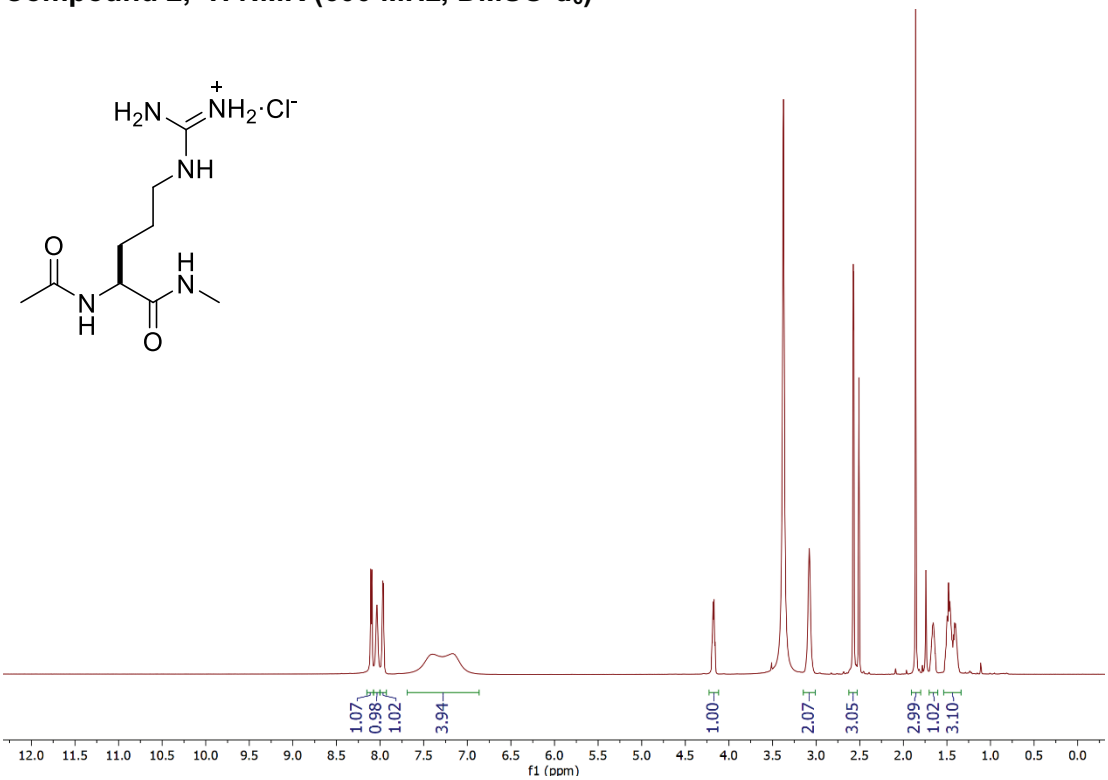
Compound 8, ^{1}H NMR (500 MHz, $CDCl_3$)



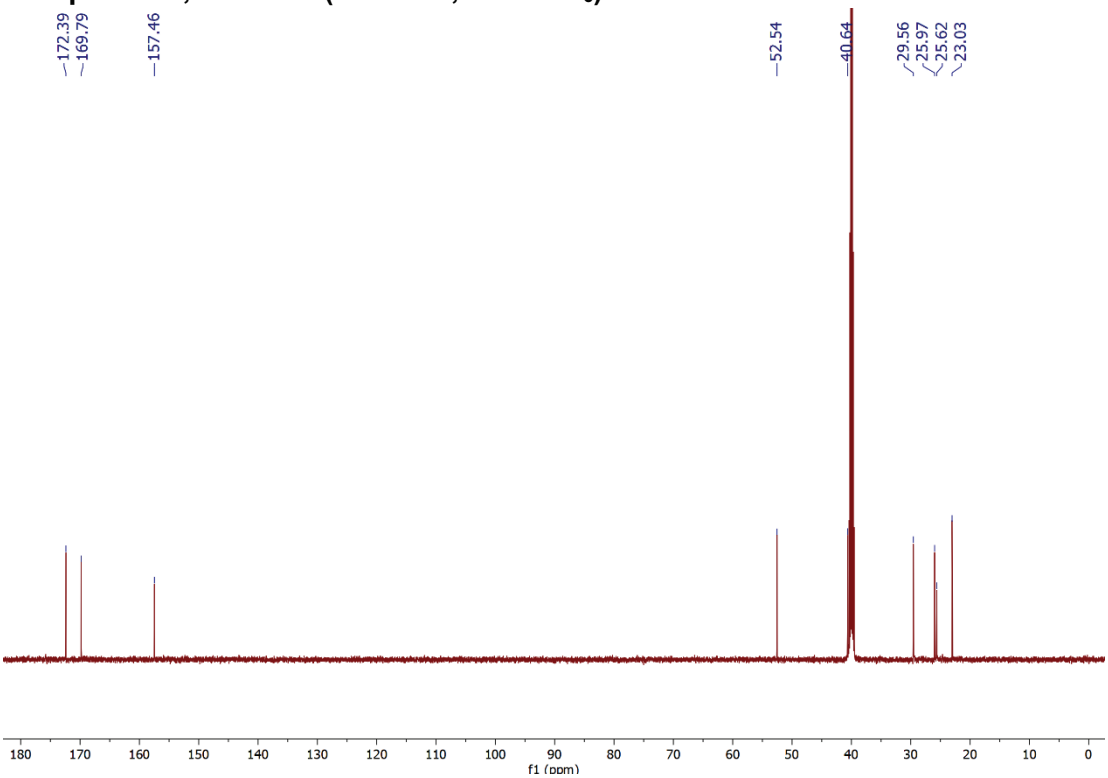
Compound 8, ^{1}H NMR (500 MHz, $CDCl_3$)



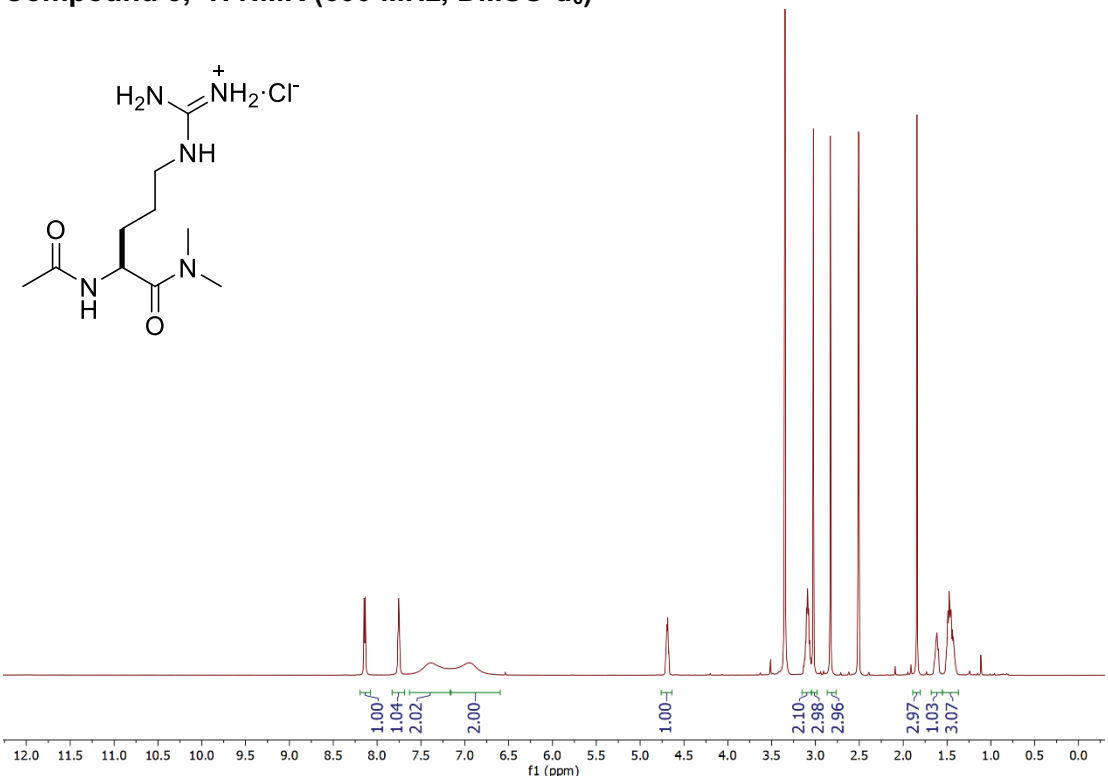
Compound 2, ^{1}H NMR (600 MHz, DMSO-d_6)



Compound 2, ^{13}C NMR (600 MHz, DMSO-d_6)



Compound 3, ^{1}H NMR (600 MHz, DMSO-d_6)



Compound 3, ^{1}H NMR (600 MHz, DMSO-d_6)