Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.


# Electronic Supplementary Information

## Multivalent DNA recognition by self-assembled clusters: deciphering structural effects by fragments screening and evaluation as siRNA vectors

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1. Characterizations of scaffolds **A7**

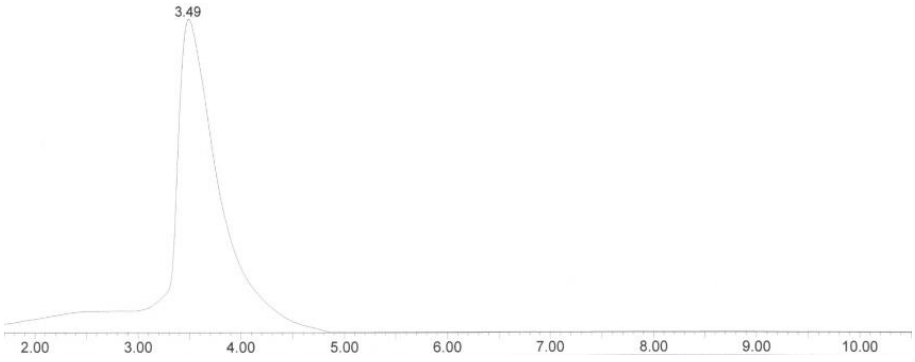


**Figure S1.** HPLC chromatogram of **A7**

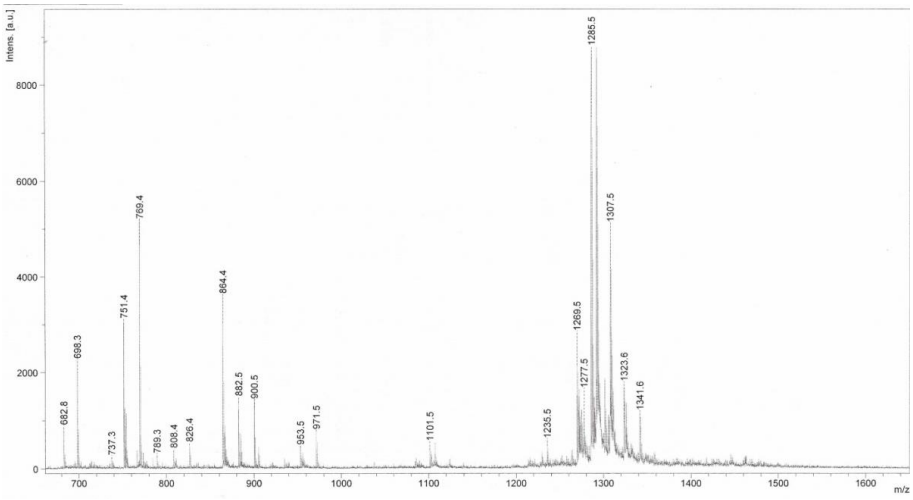


**Figure S2.** MALDI-ToF (HCCA) of **A7**. Calcd for $[M+K]^+$ 1285.58, found 1285.52.

2. Characterizations of hydrazide building blocks
    a. **GlyHyd**

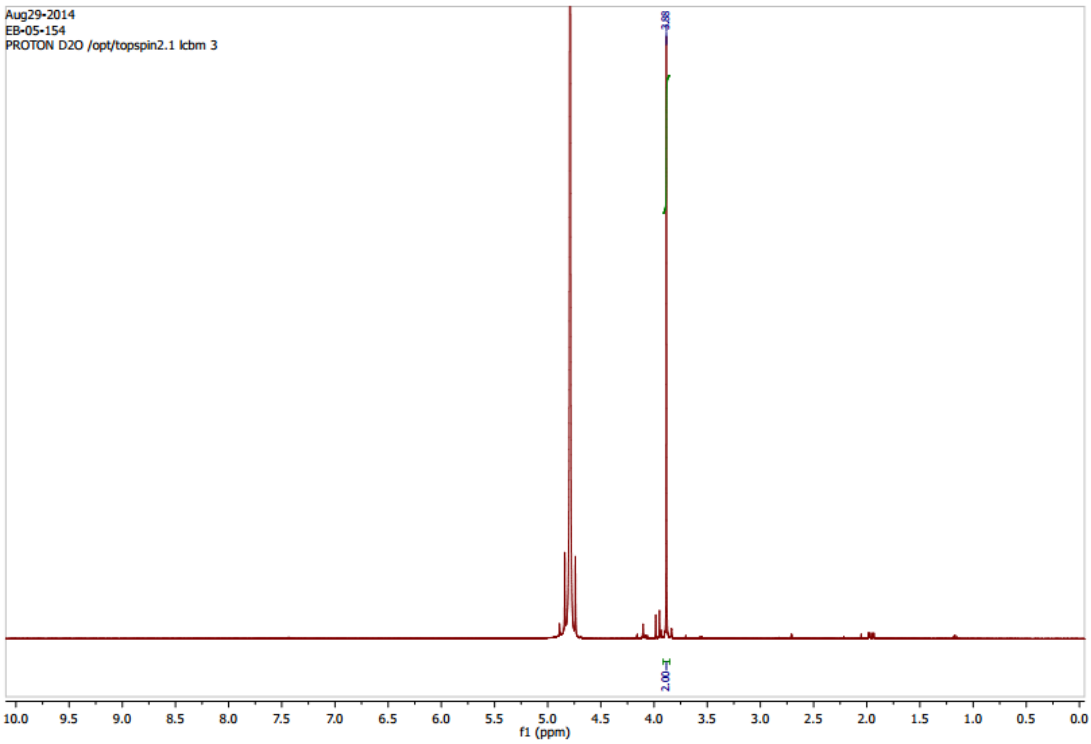


**Figure S3.** $^{1}$H NMR of **GlyHyd**

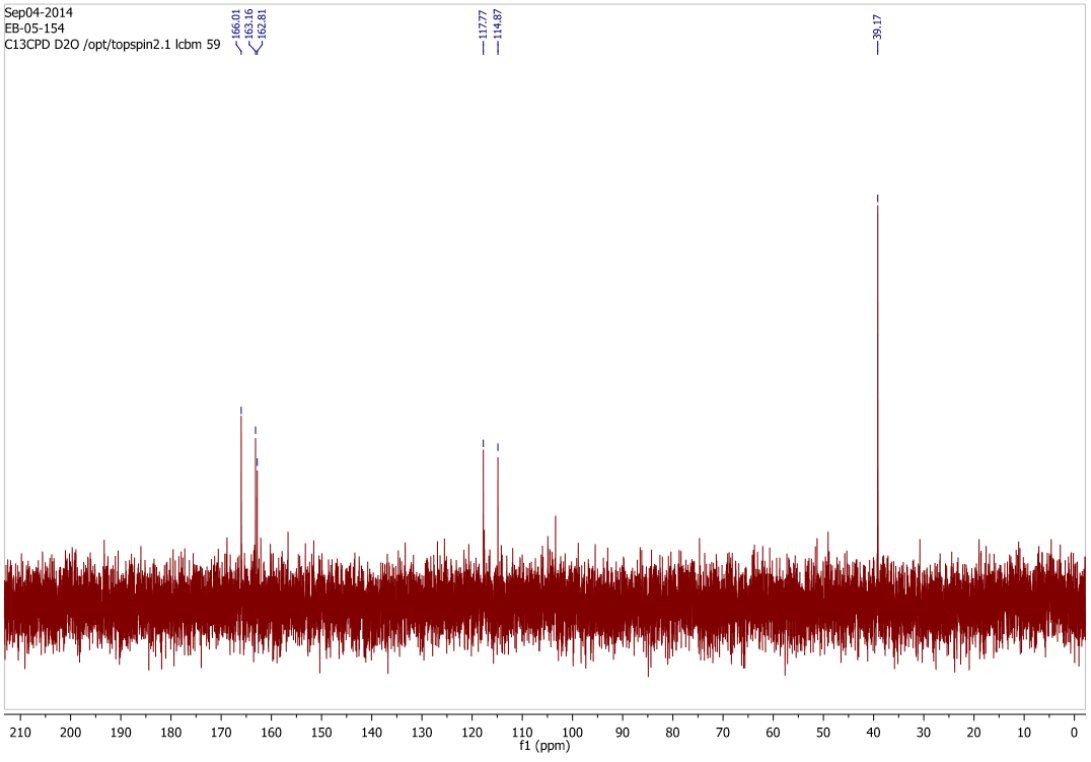


**Figure S4.** $^{13}$C NMR of **GlyHyd**

b. **AlaHyd**

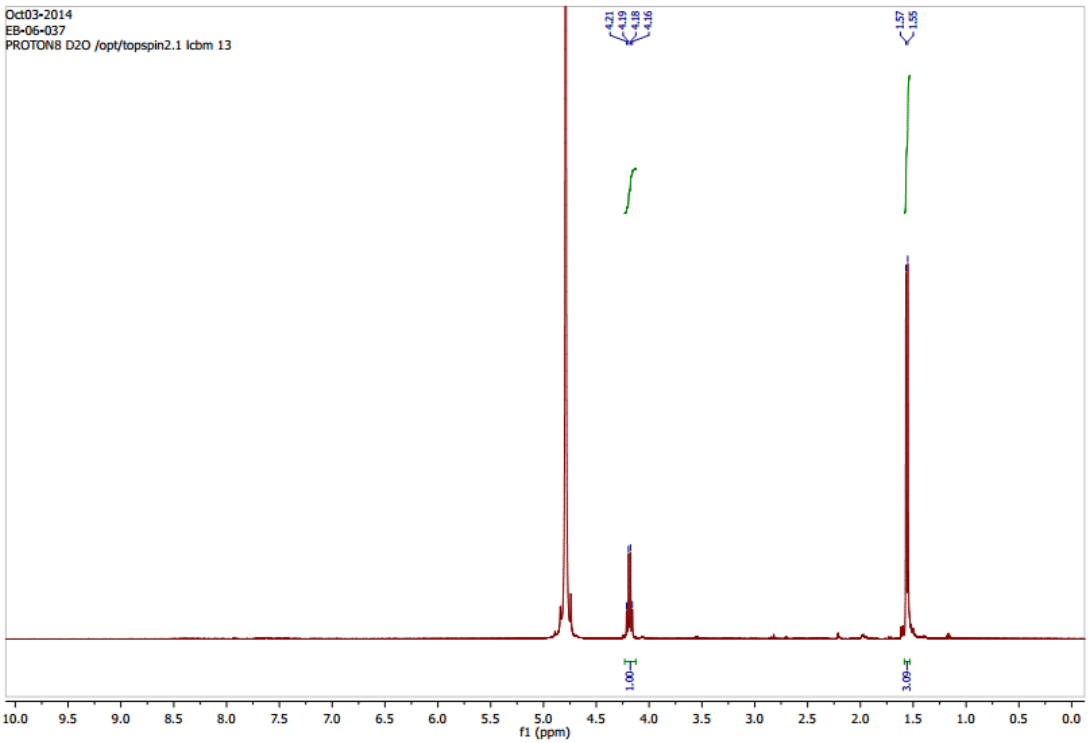


**Figure S5.** $^{1}$H NMR of **AlaHyd**

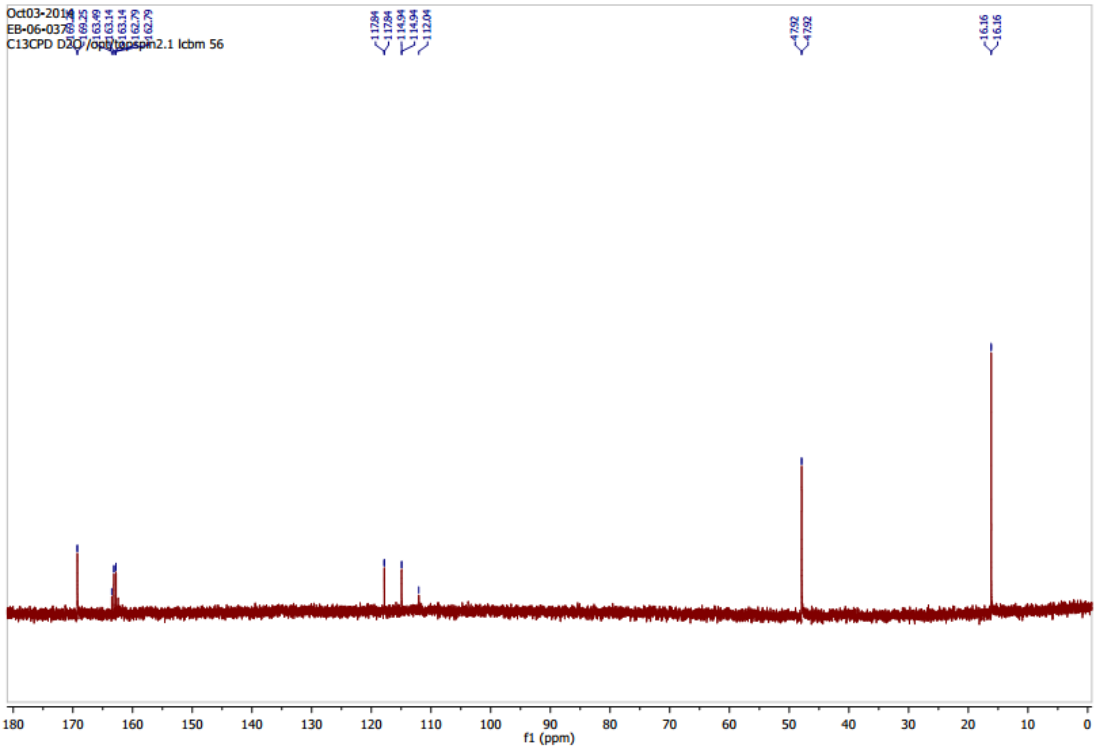


**Figure S6.** $^{13}$C NMR of **AlaHyd**

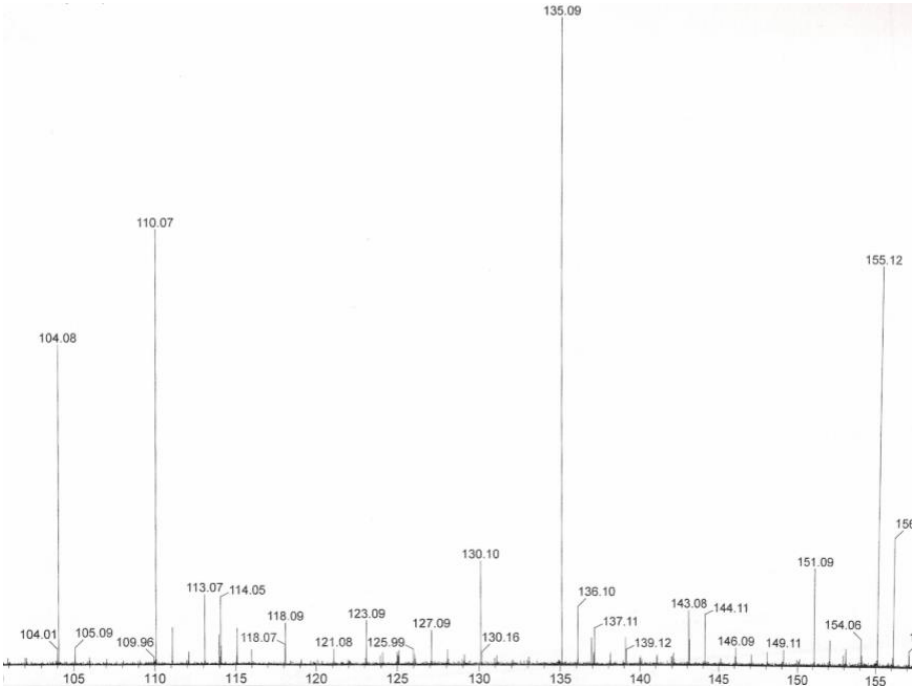


**Figure S7.** HR-ESI-MS of **AlaHyd**. Calcd for $[M+H]^+$ 104.0824, found 104.0822

c. **AspHyd**

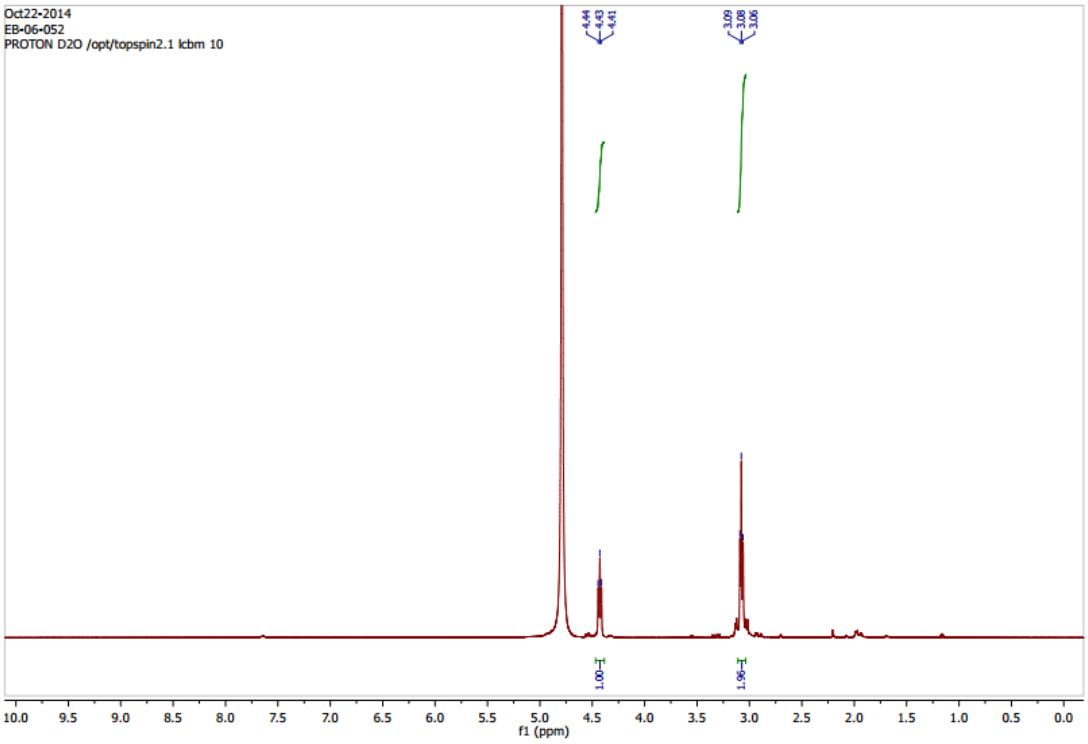


**Figure S8.** $^1$H NMR of **AspHyd**

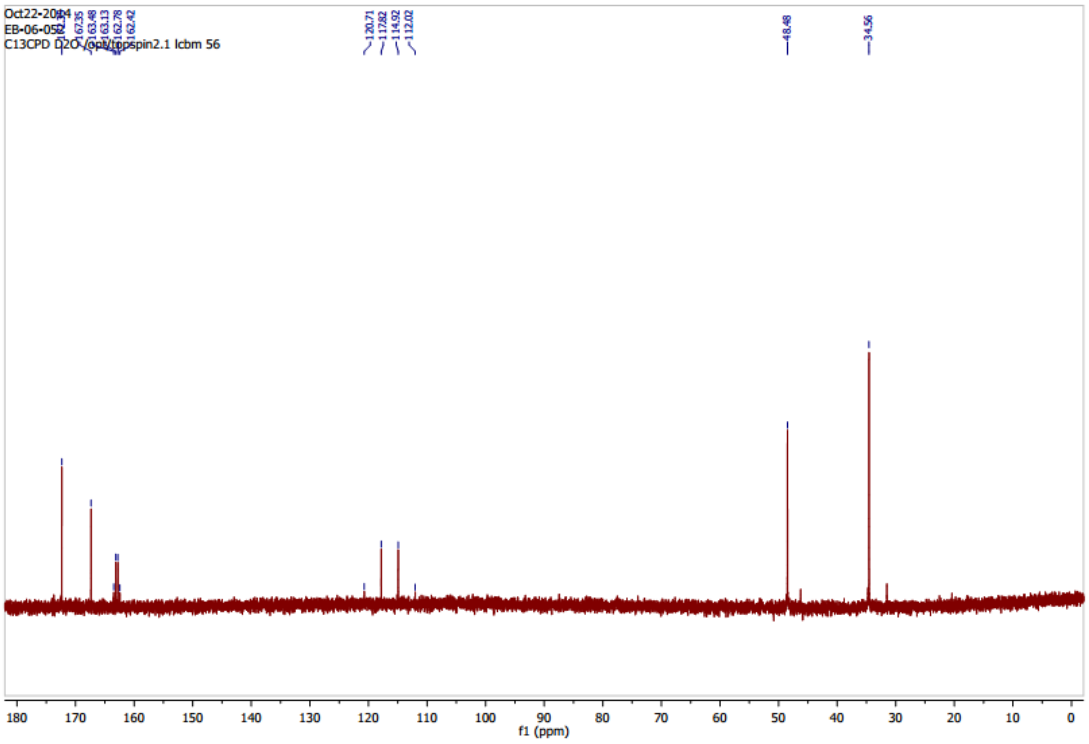


**Figure S9.** $^{13}$C NMR of **AspHyd**

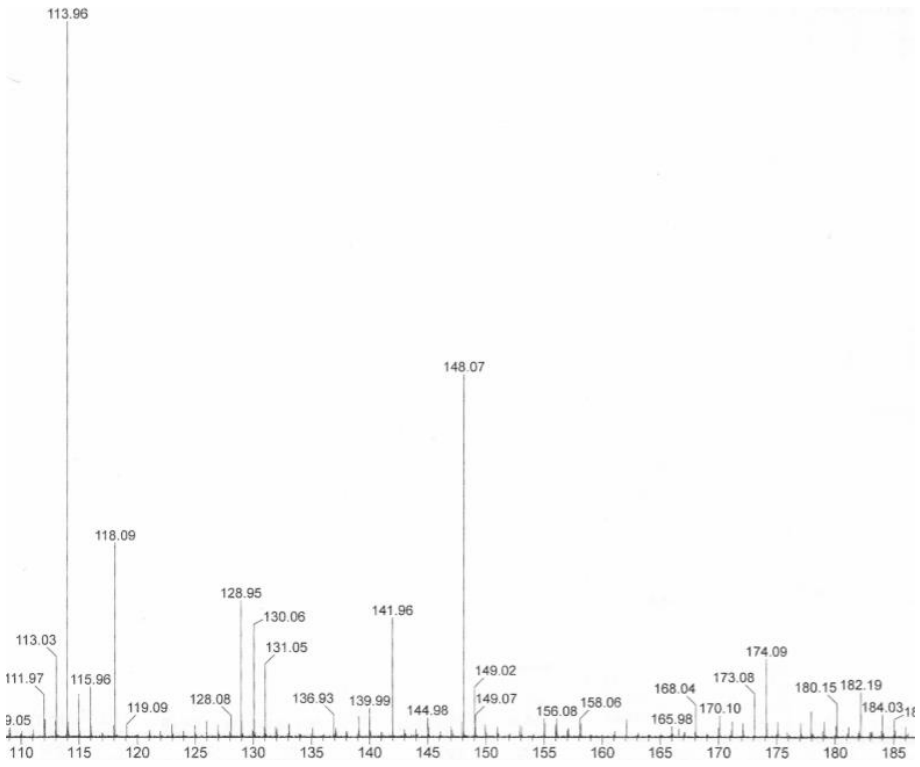


**Figure S10.** HR-ESI-MS of **AspHyd**. Calcd for $[M+H]^+$ 148.0722, found 148.0720

d. **HisHyd**

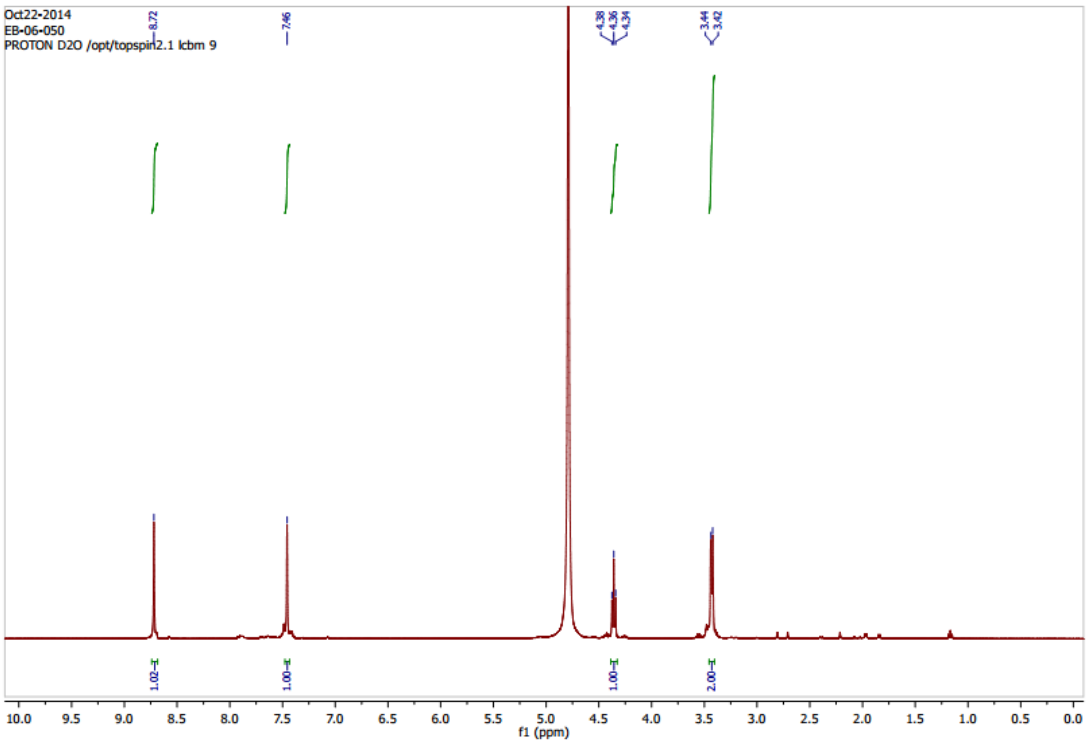


**Figure S11.** $^{1}$H NMR of **HisHyd**

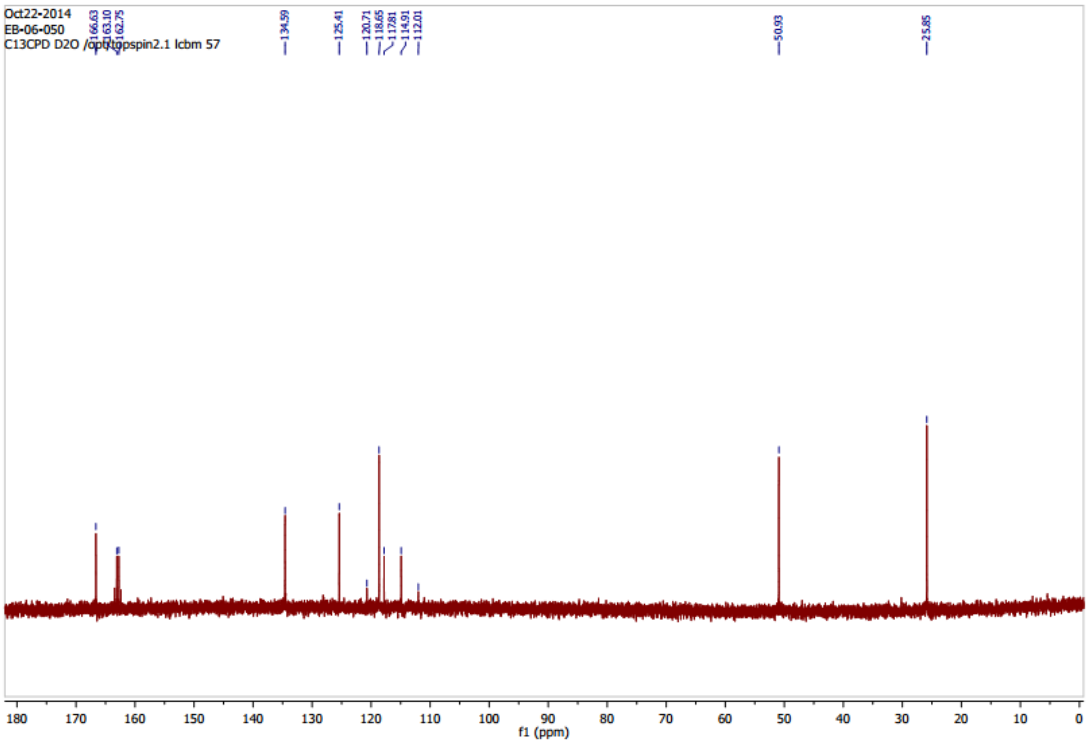


**Figure S12.** $^{13}$C NMR of **HisHyd**

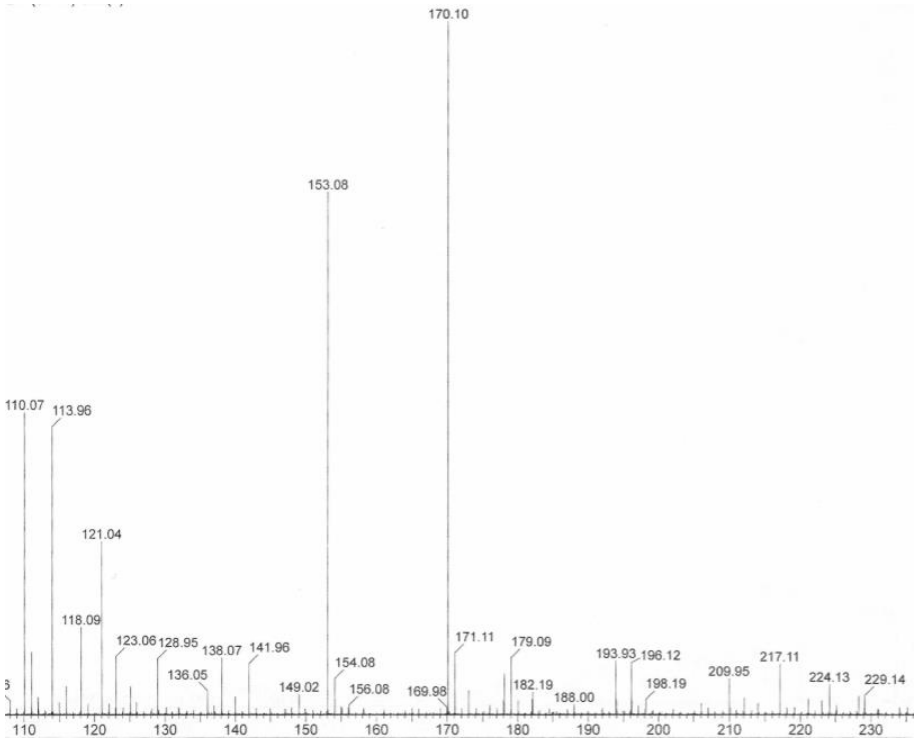


**Figure S13.** HR-ESI-MS of **HisHyd**. Calcd for $[M+H]^+$ 170.1042, found 170.1041

e. **LysHyd**

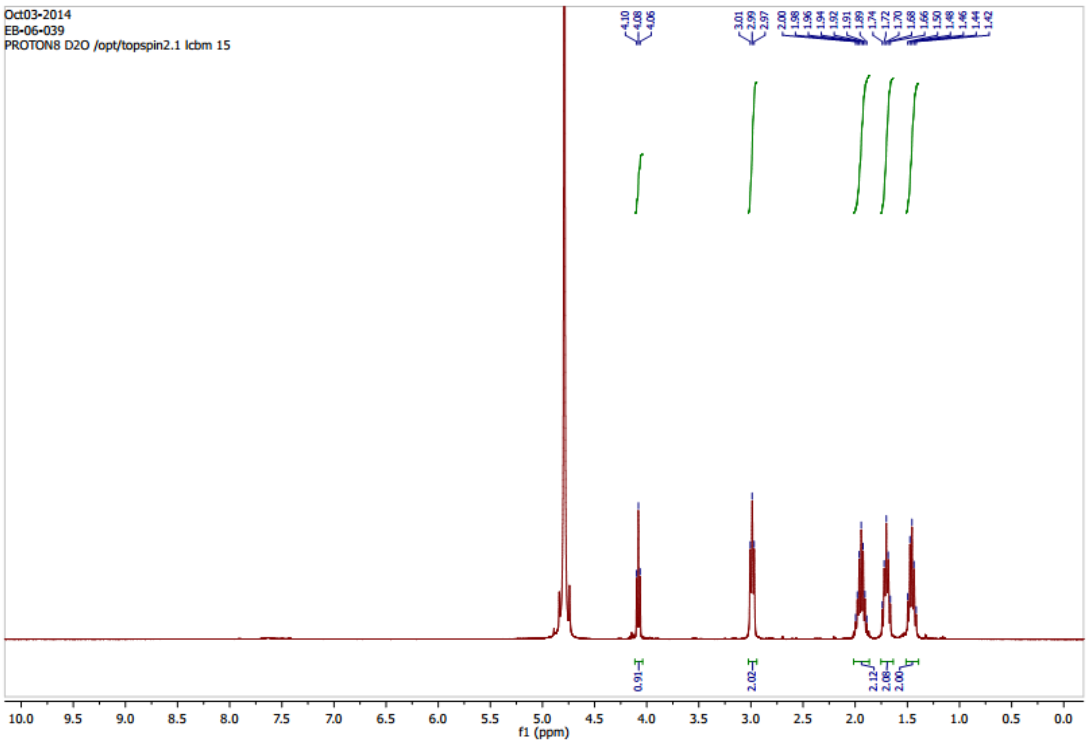


**Figure S14.** $^1$H NMR of **LysHyd**

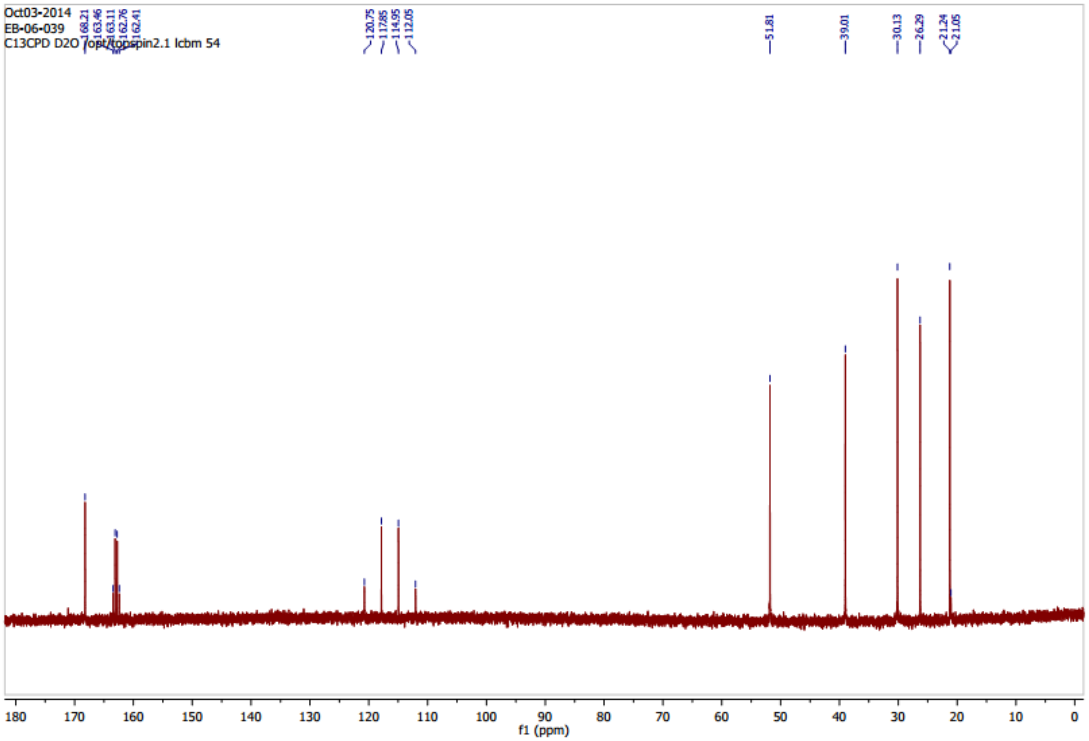


**Figure S15.** $^{13}$C NMR of **LysHyd**

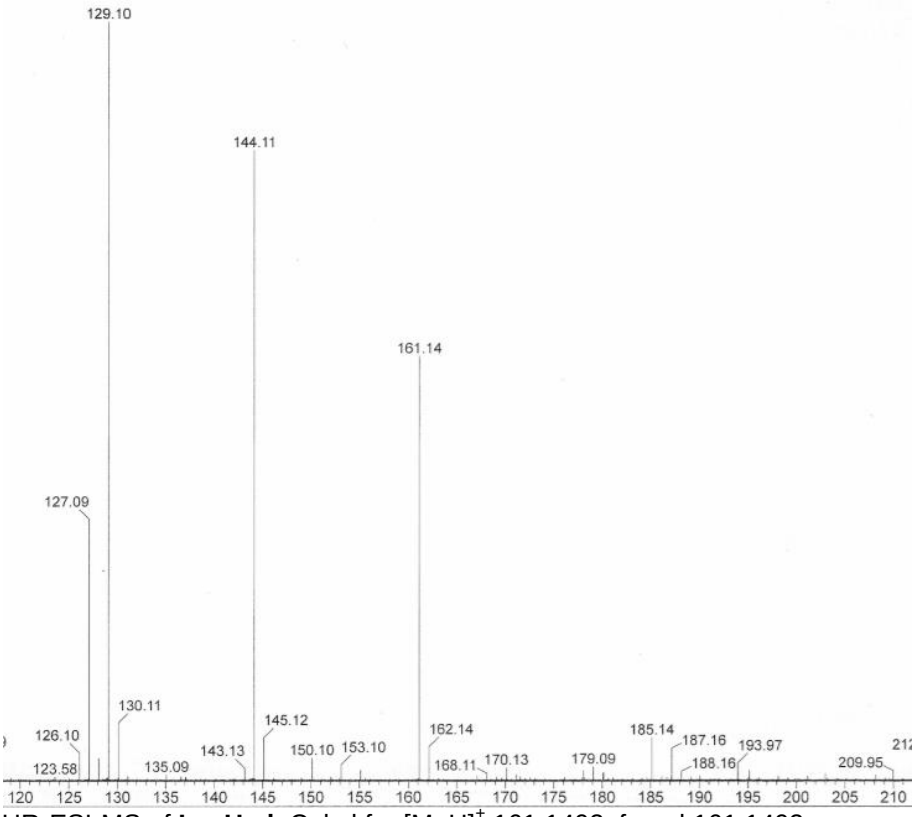


**Figure S16.** HR-ESI-MS of **LysHyd**. Calcd for $[M+H]^+$ 161.1402, found 161.1402

f. ***L*-ArgHyd**

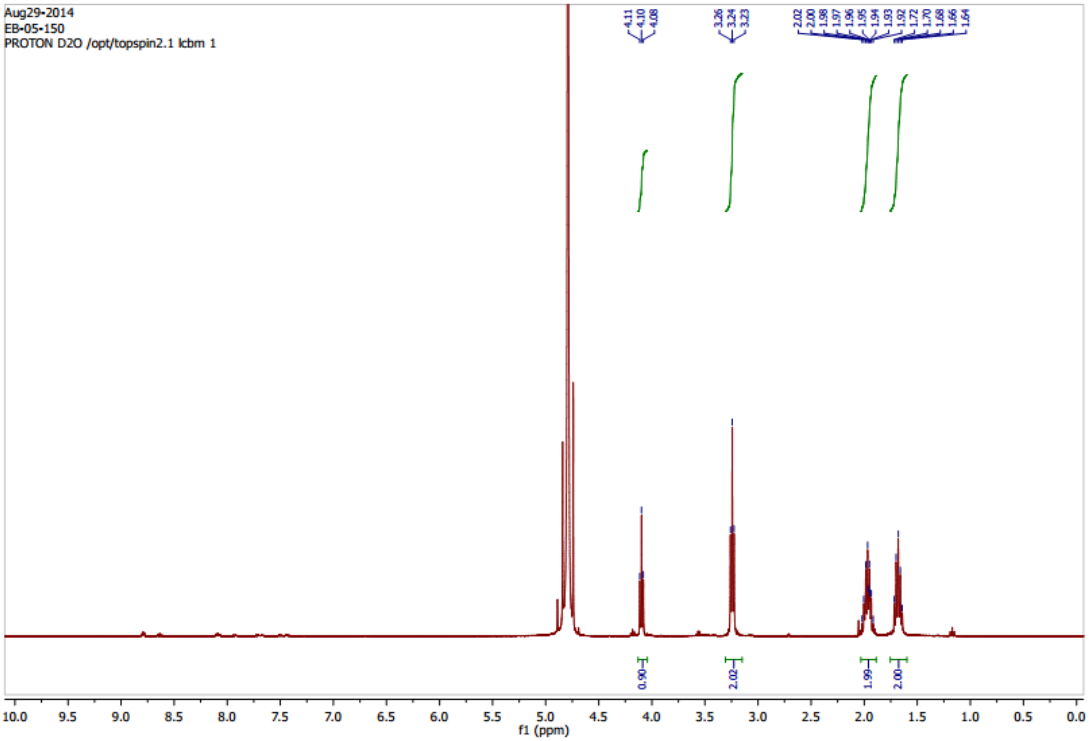


**Figure S17.** $^{1}$H NMR of ***L*-ArgHyd**

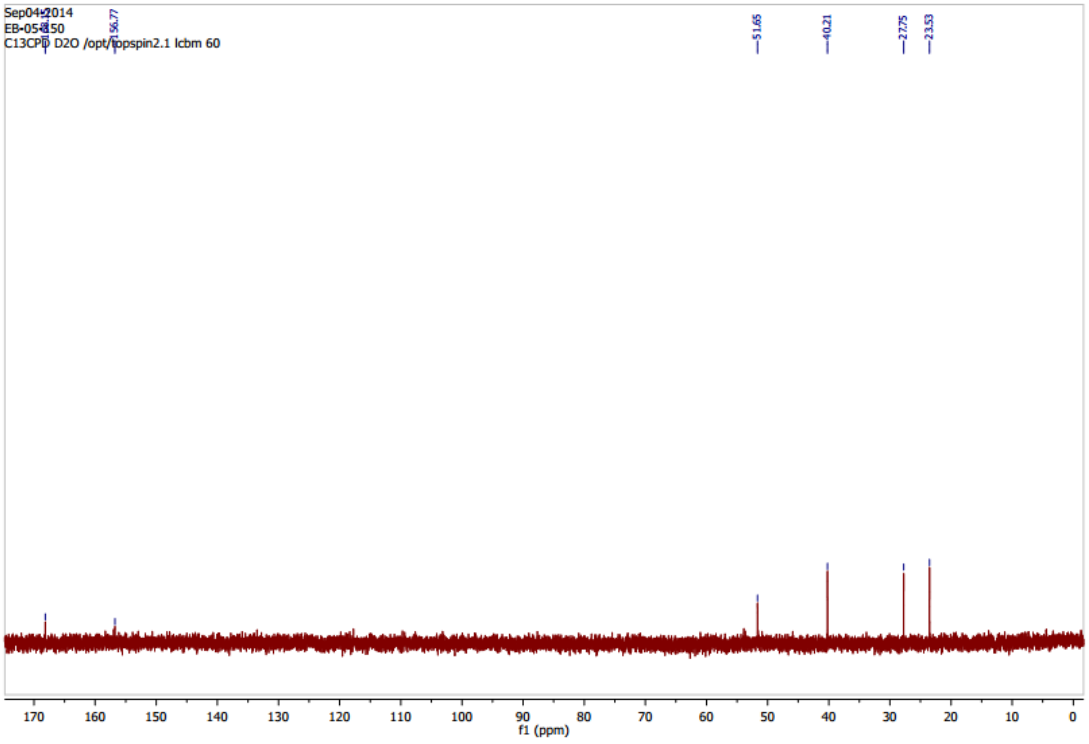


**Figure S18.** $^{13}$C NMR of ***L*-ArgHyd**

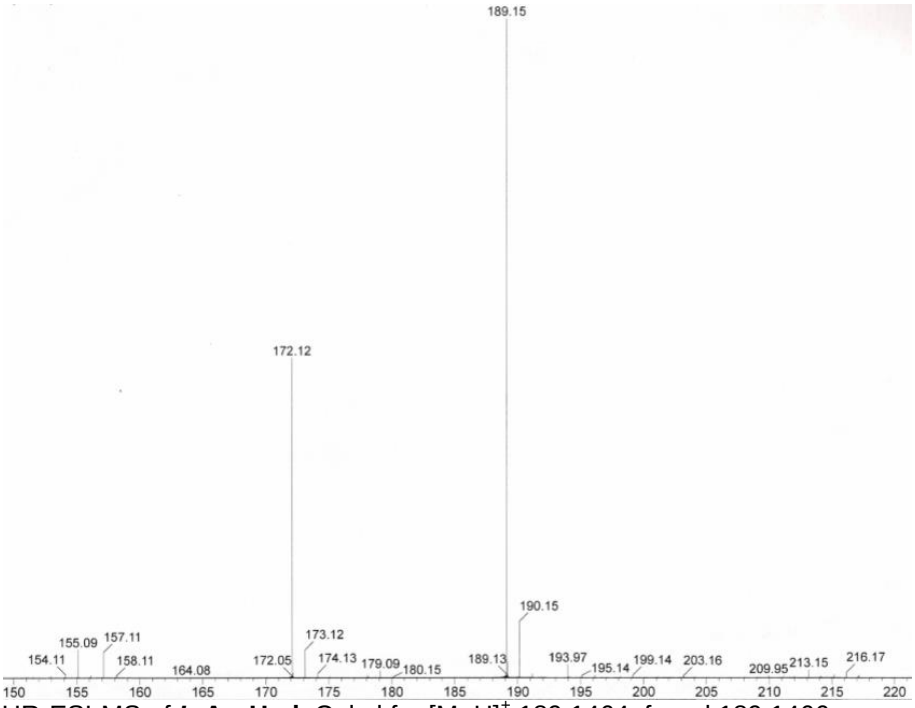


**Figure S19.** HR-ESI-MS of ***L*-ArgHyd**. Calcd for $[M+H]^+$ 189.1464, found 189.1466

g. ***D*-ArgHyd**

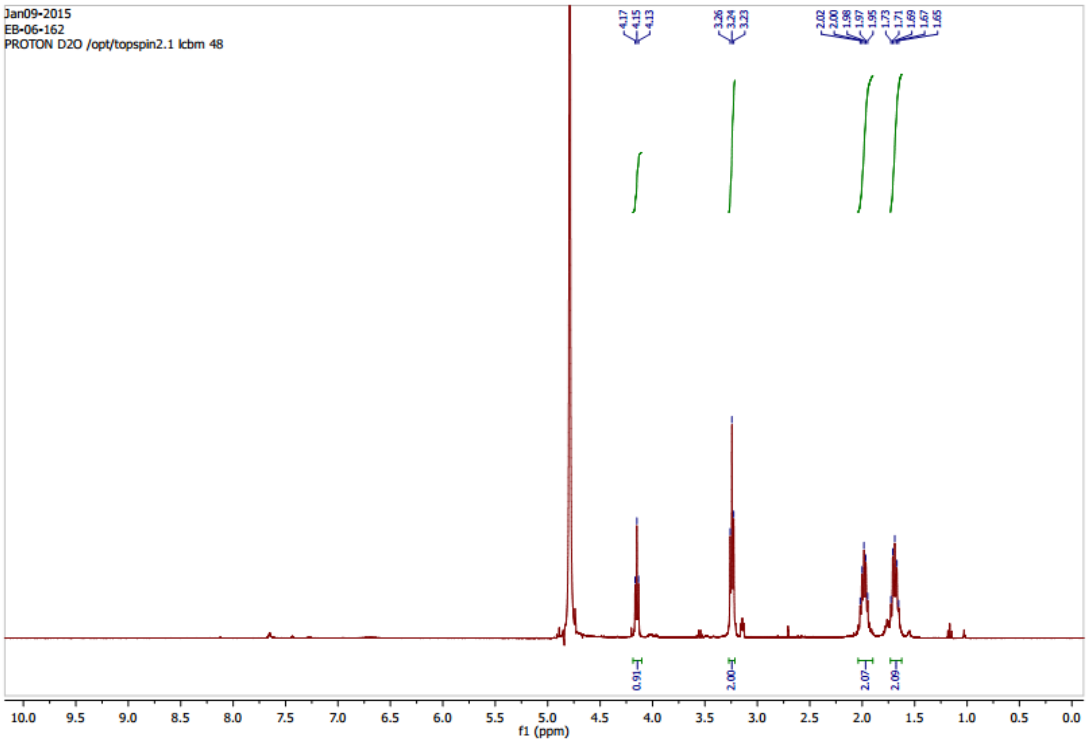


**Figure S20.** $^1$H NMR of ***D*-ArgHyd**

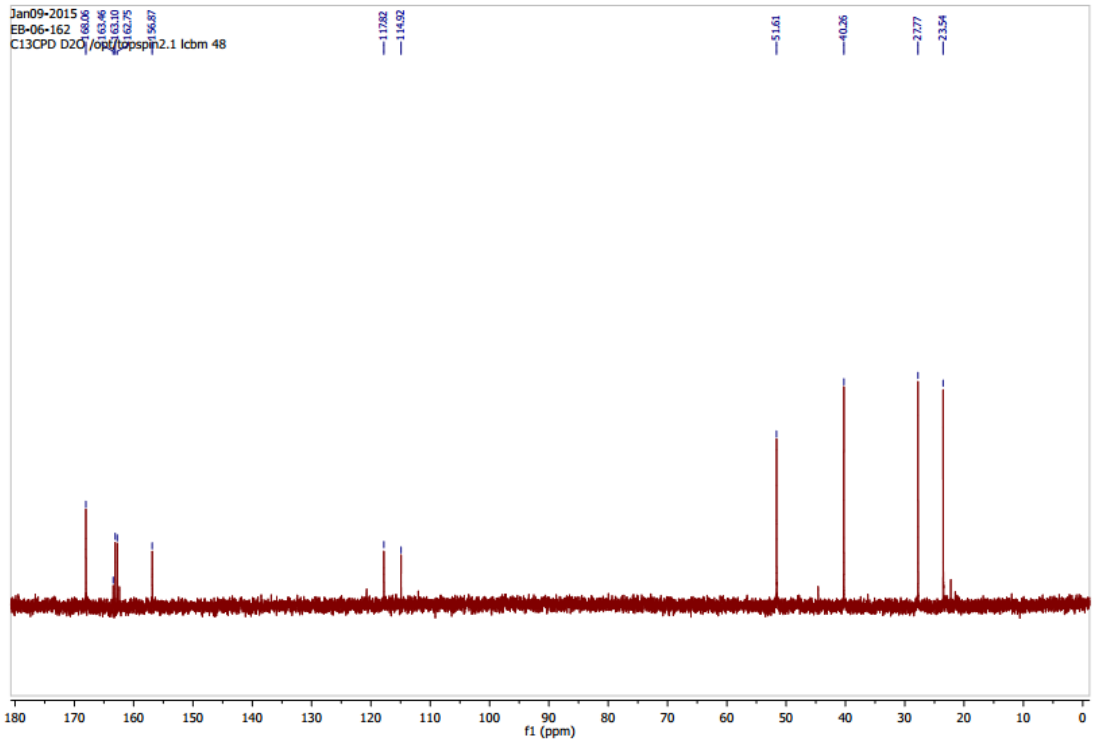


**Figure S21.** $^{13}$C NMR of ***D*-ArgHyd**

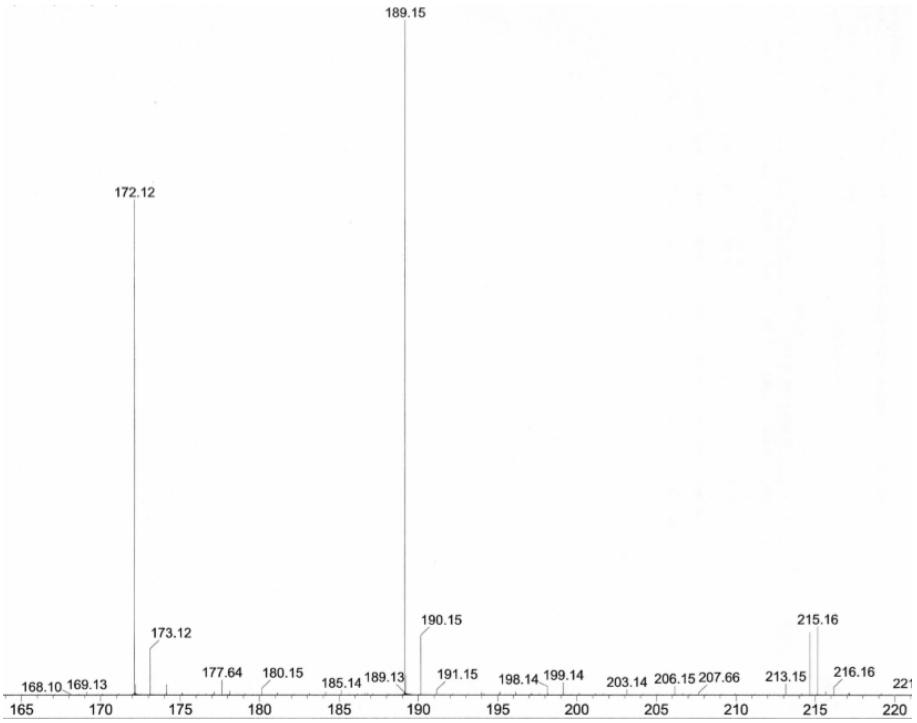


**Figure S22.** HR-ESI-MS of ***D*-ArgHyd**. Calcd for $[M+H]^+$ 189.1464, found 189.1464

h. **$Lys_3Hyd$**

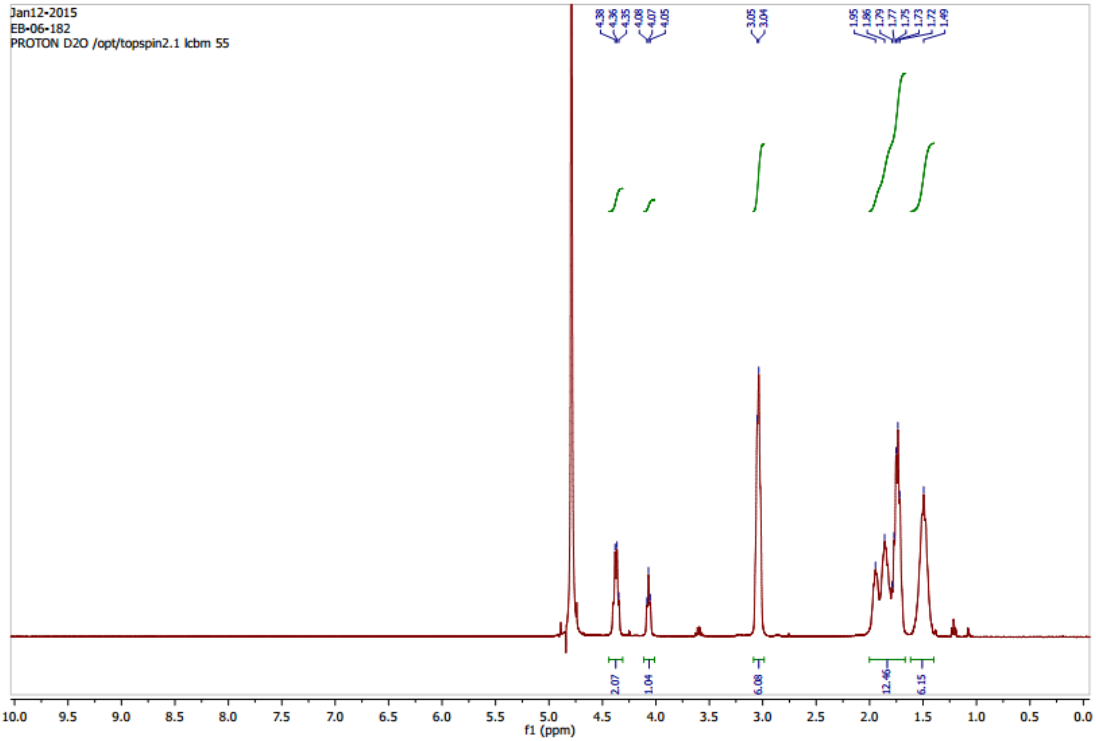


**Figure S23.** $^{1}$H NMR of **$Lys_3Hyd$**

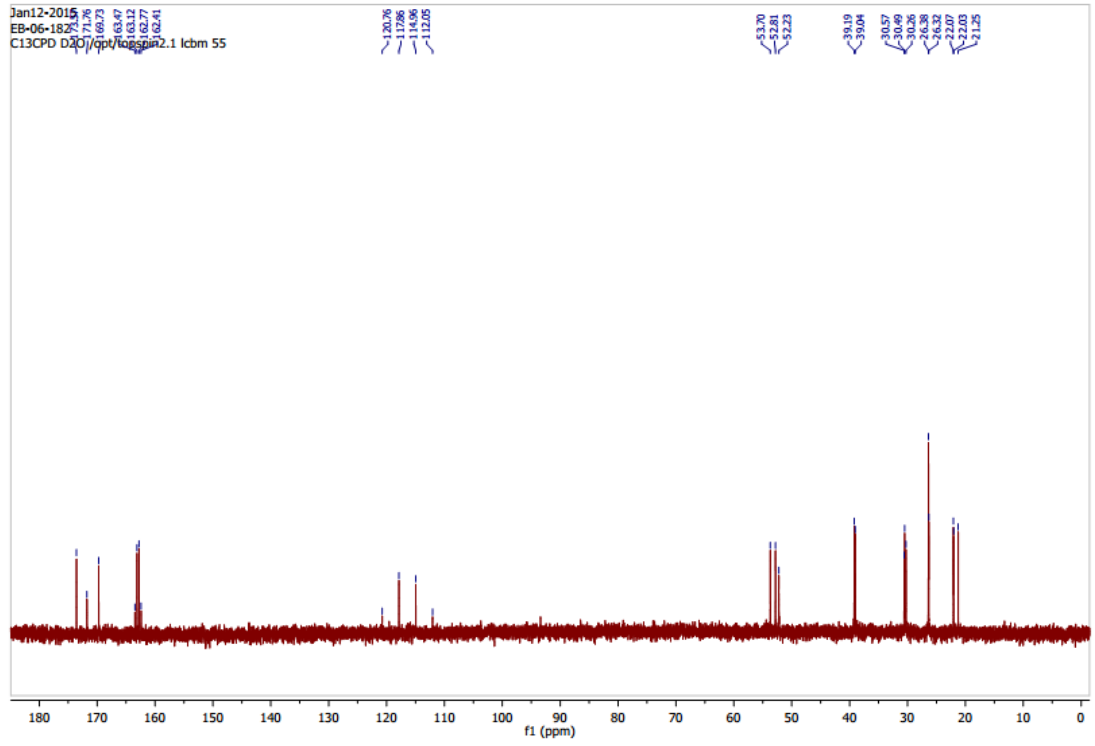


**Figure S24.** $^{13}$C NMR of **$Lys_3Hyd$**

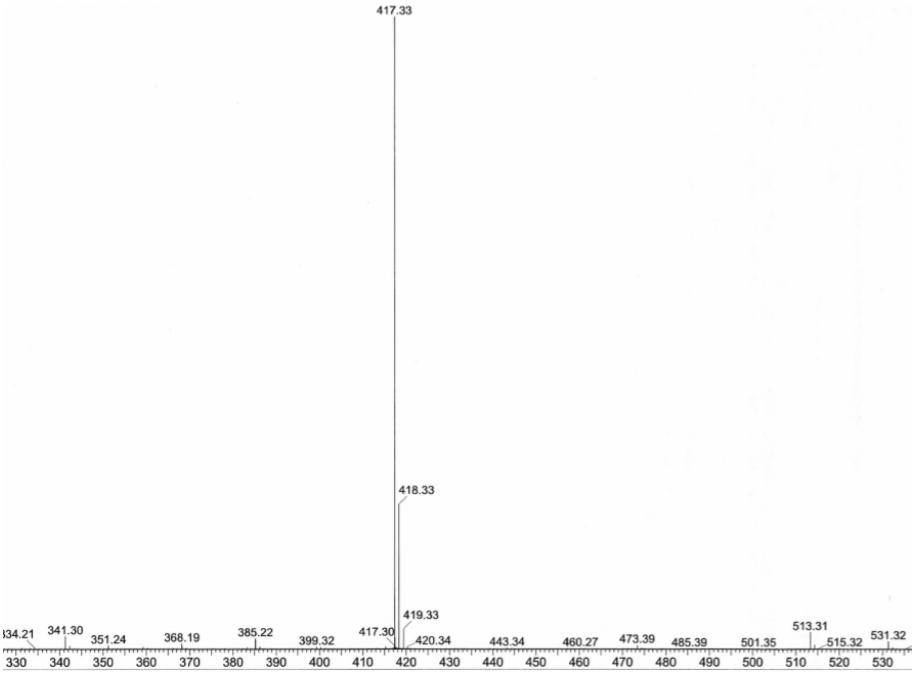


**Figure S25.** HR-ESI-MS of **$Lys_3Hyd$**. Calcd for $[M+H]^+$ 417.3302, found 417.3296

i. **$G_1$-LysHyd**

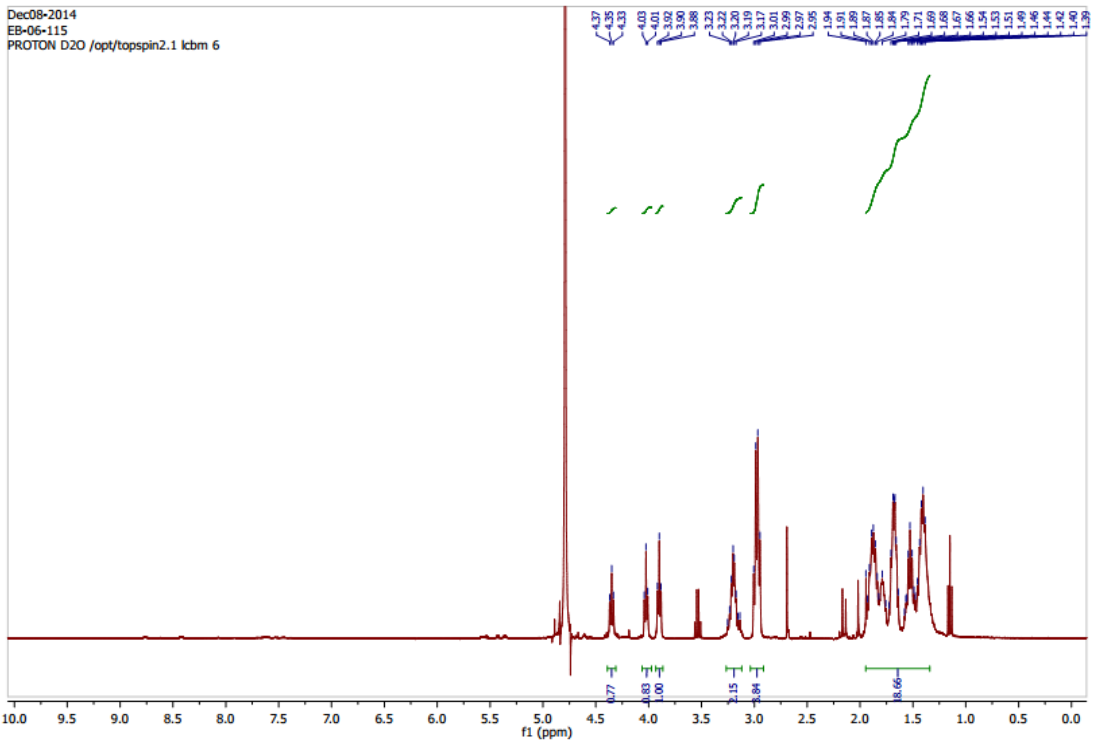


**Figure S26.** $^1H$ NMR of **$G_1$-LysHyd**

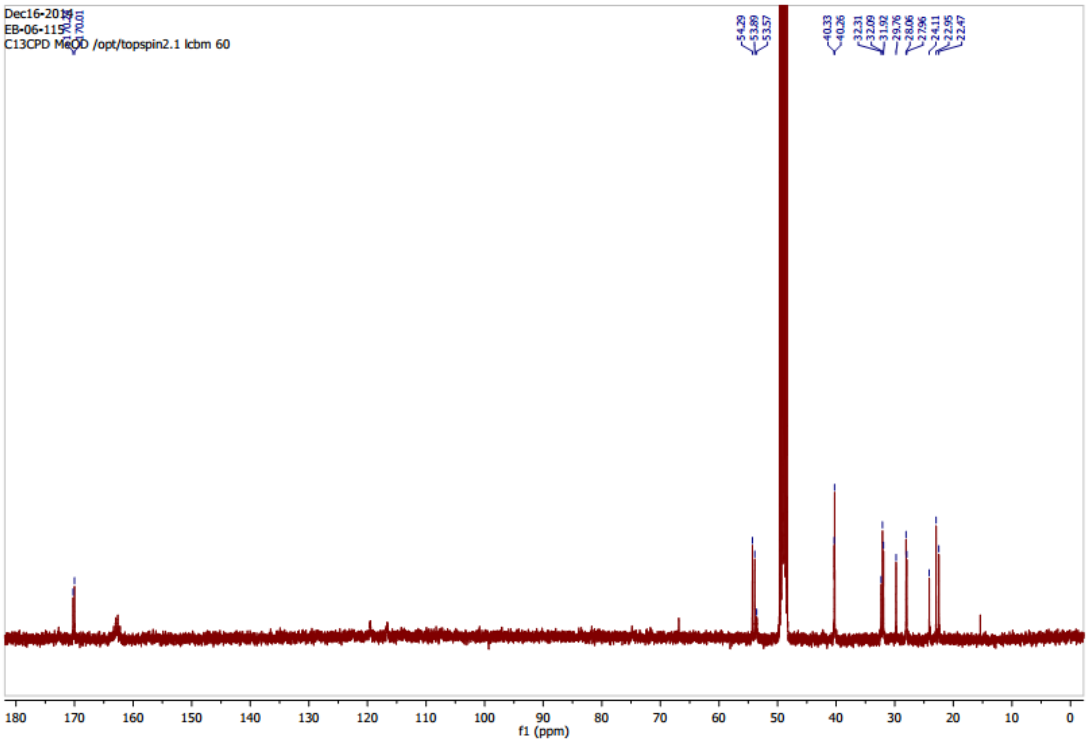


**Figure S27.** $^{13}$C NMR of **$G_1$-LysHyd**

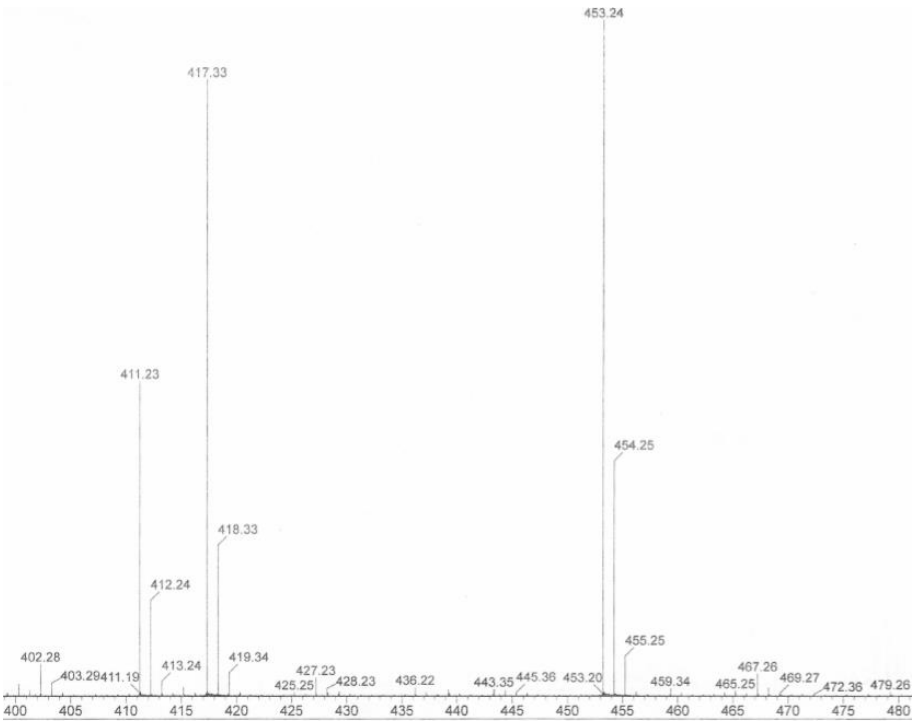


**Figure S28.** HR-ESI-MS of **$G_1$-LysHyd**. Calcd for $[M+H]^+$ 417.3302, found 417.3299

j. **$Arg_3Hyd$**

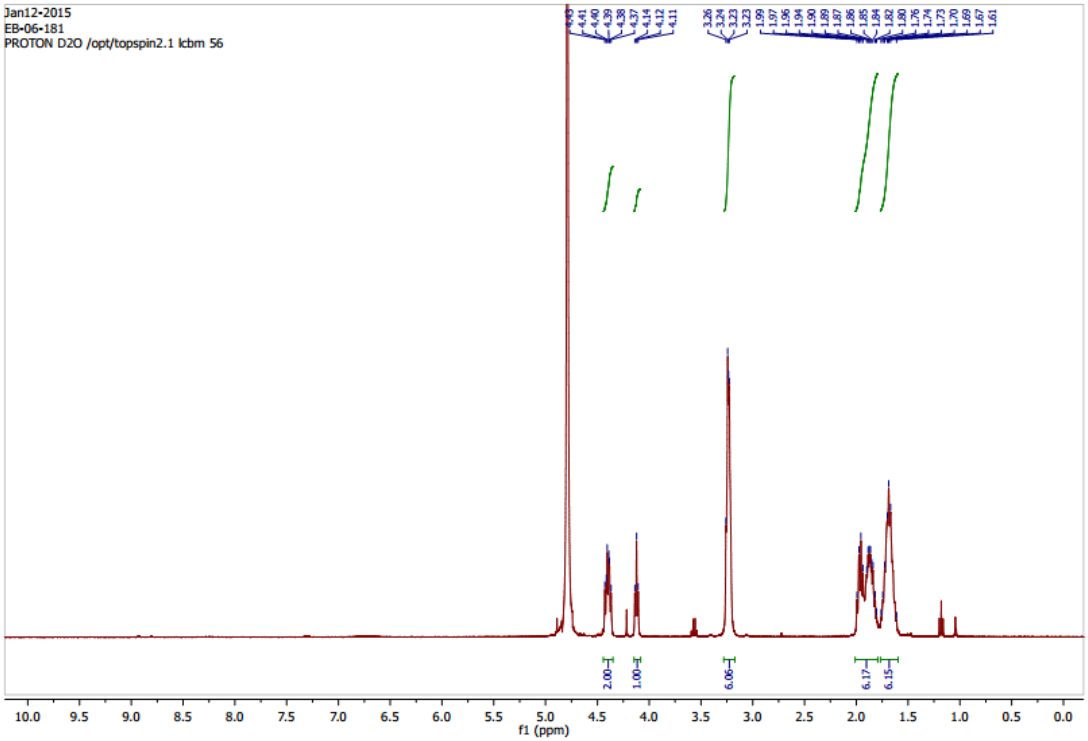


**Figure S29.** $^{1}$H NMR of **$Arg_3Hyd$**

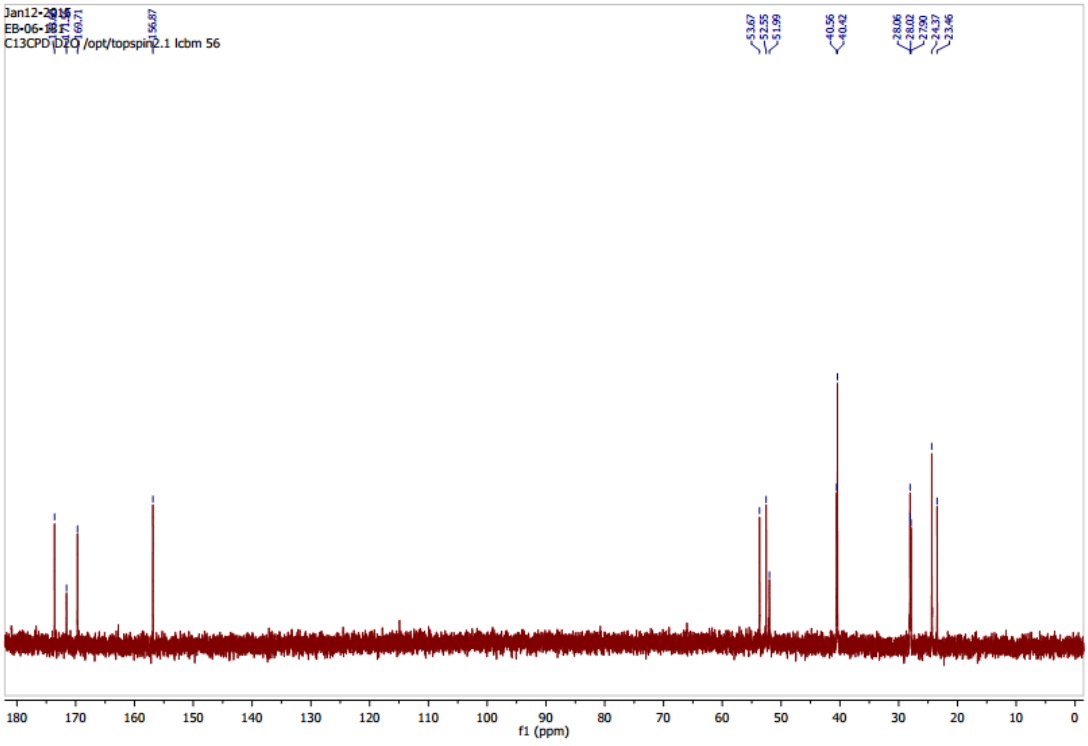


**Figure S30.** $^{13}$C NMR of **$Arg_3Hyd$**

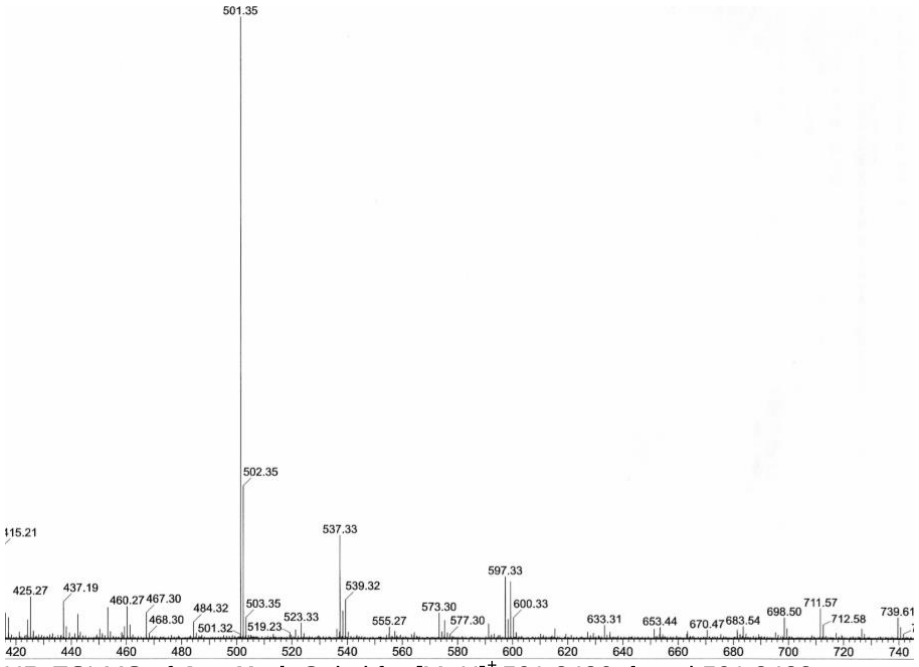


**Figure S31.** HR-ESI-MS of **$Arg_3$Hyd**. Calcd for $[M+H]^+$ 501.3486, found 501.3482

3. Characterizations of clusters **A.Hyd**
    a. **A8.Gly**

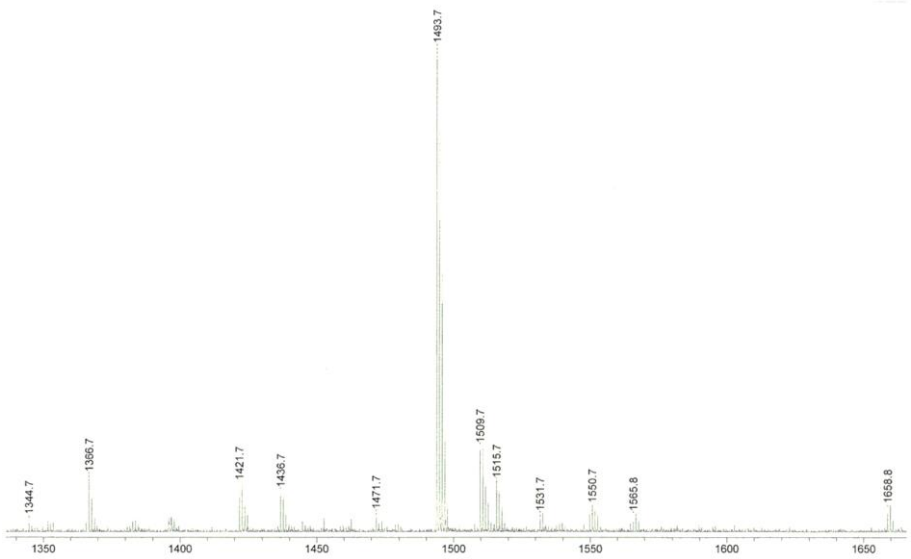


**Figure S32.** MALDI-ToF (HCCA) of **A8.Gly**. Calcd for $[M+Na]^+$ 1493.76, found 1493.72

    b. **A8.Gir**

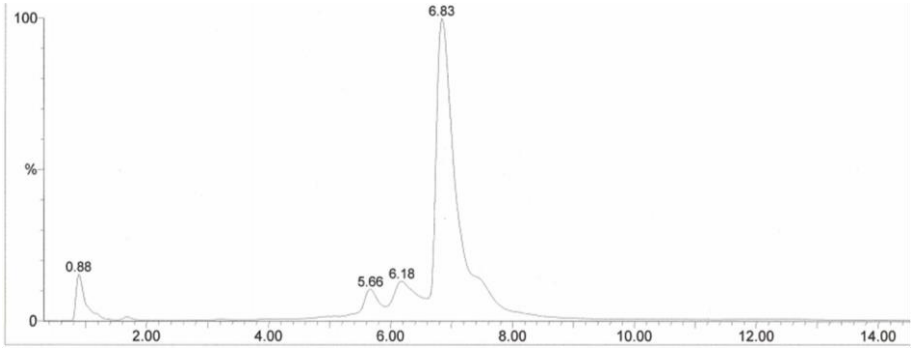


**Figure S33.** HPLC chromatogram of **A8.Gir**

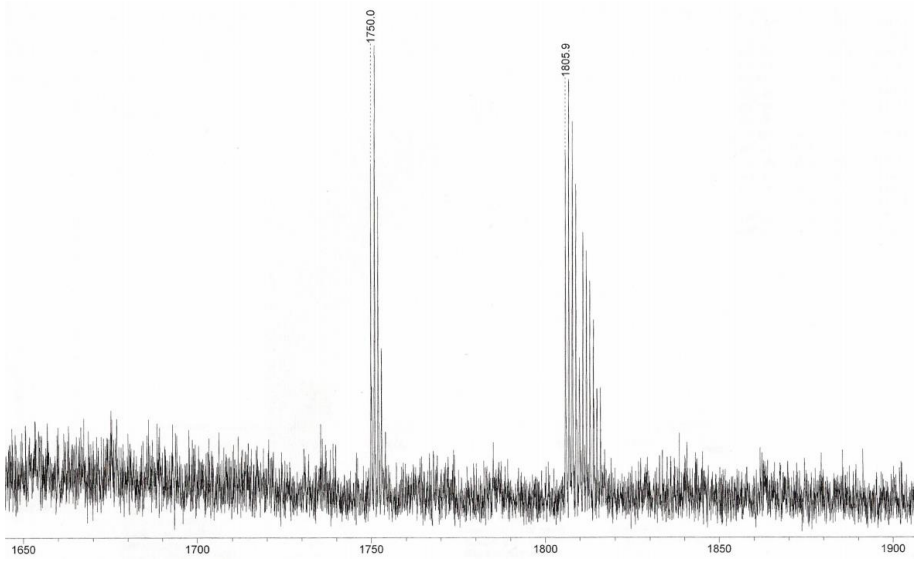


**Figure S34.** MALDI-ToF (HCCA) of **A8.Gir**. Calcd for $[M+4Cl+Na]^+$ 1805.98, found 1805.86

c. **A8.Ala**

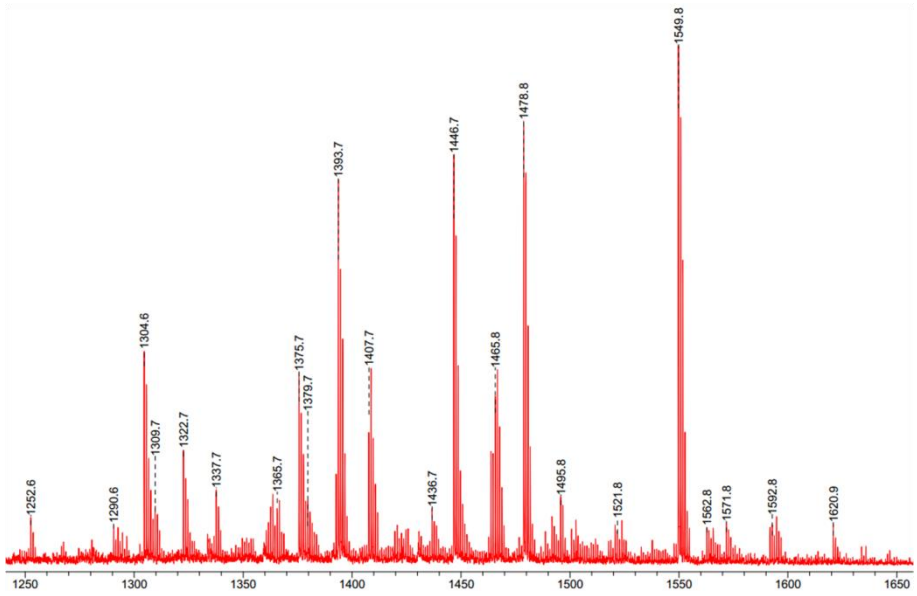


**Figure S35**. MALDI-ToF (HCCA) of **A8.Ala**. Calcd for $[M+Na]^+$ 1549.82, found 1549.81

d. **A8.Asp**

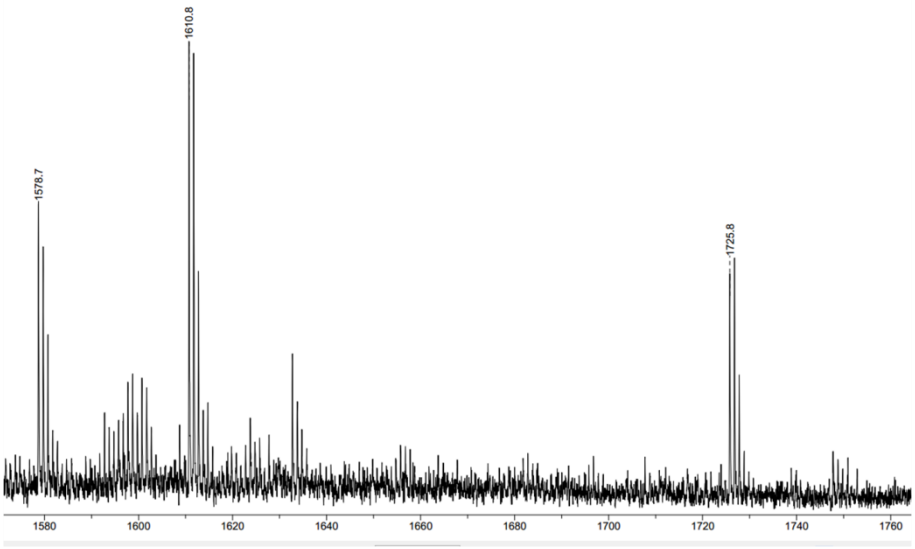


**Figure S36.** MALDI-ToF (HCCA) of **A8.Asp**. Calcd for $[M+Na]^+$ 1726.78, found 1725.76

e. **A8.His**

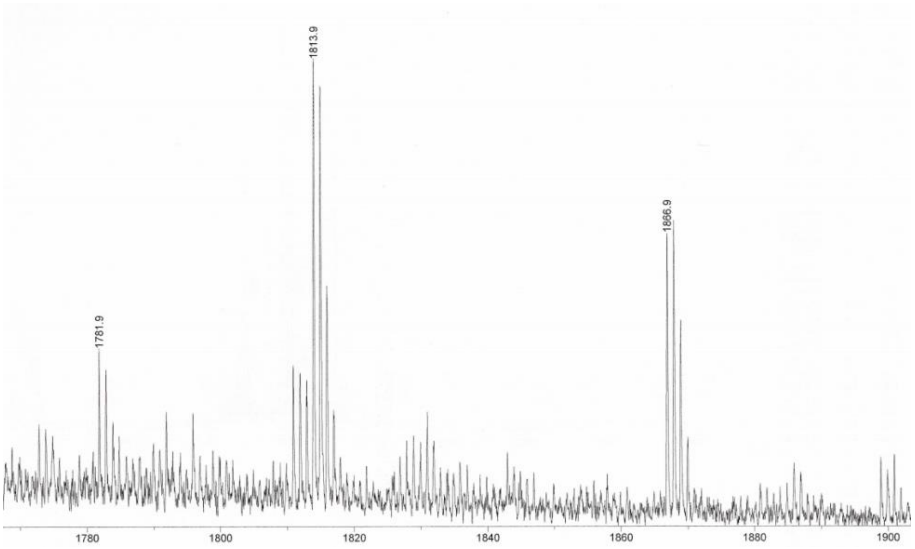


**Figure 37.** MALDI-ToF (HCCA) of **A8.His**. Calcd for $[M+Na]^+$ 1814.91, found 1813.92

f. **A8.Lys**

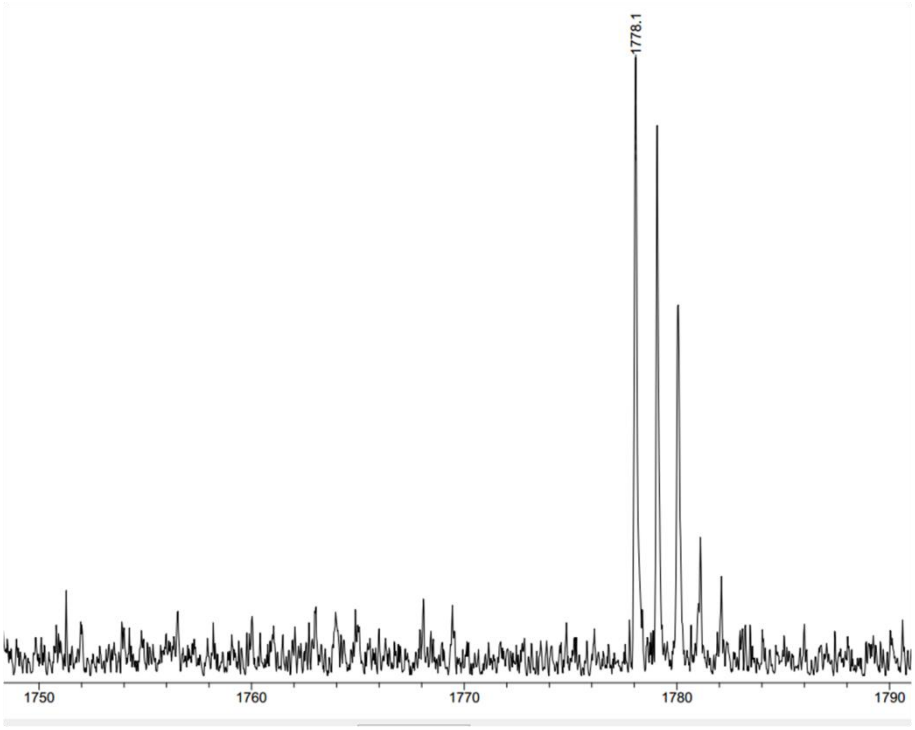


**Figure S38.** MALDI-ToF (HCCA) of **A8.Lys**. Calcd for $[M+Na]^+$ 1778.05, found 1777.89

g. **A8.D-Arg**

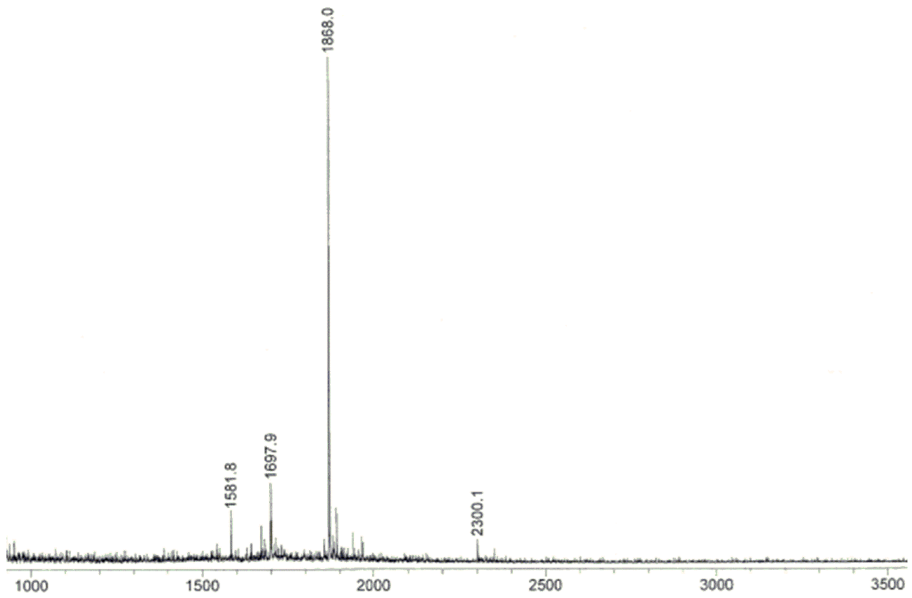


**Figure S39.** MALDI-ToF (HCCA) of **A8.*D*-Arg**. Calcd for $[M+Na]^+$ 1868.07, found 1868.05

h. **A8.Lys$_3$**

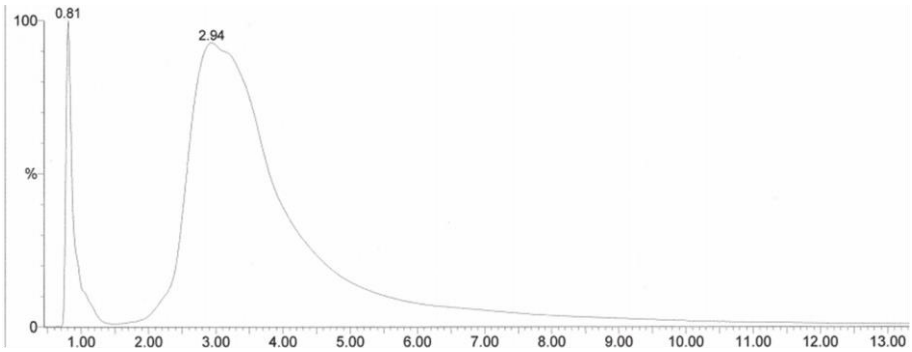


**Figure S40.** HPLC chromatogram of **A8.Lys$_3$**

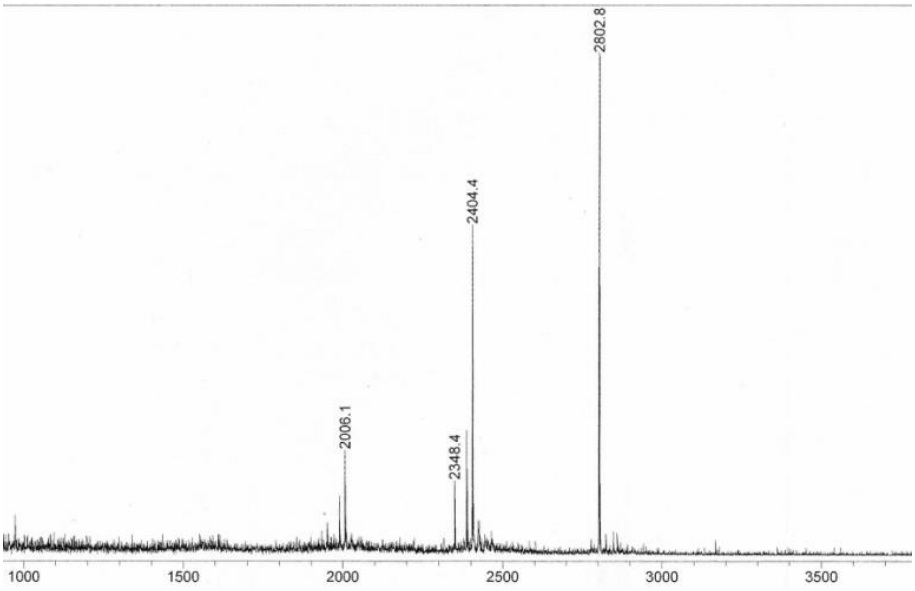


**Figure S41.** MALDI-ToF (HCCA) of **A8.Lys$_3$**. Calcd for $[M+Na]^+$ 2803.82, found 2802.76

i. **A8.G$_1$-Lys**

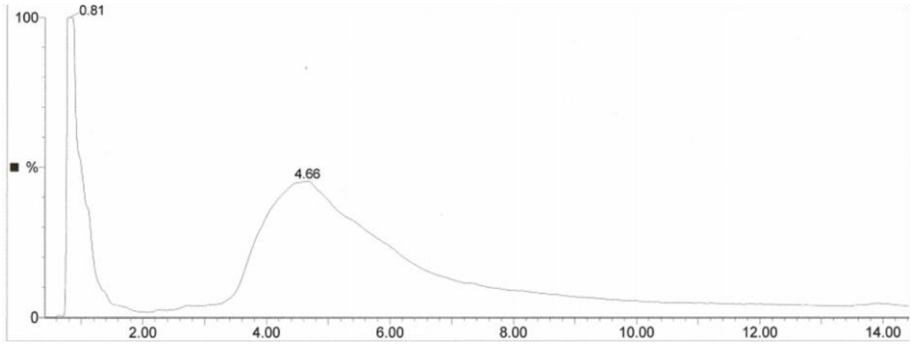


**Figure S42.** HPLC chromatogram of **A8.G$_1$-Lys**

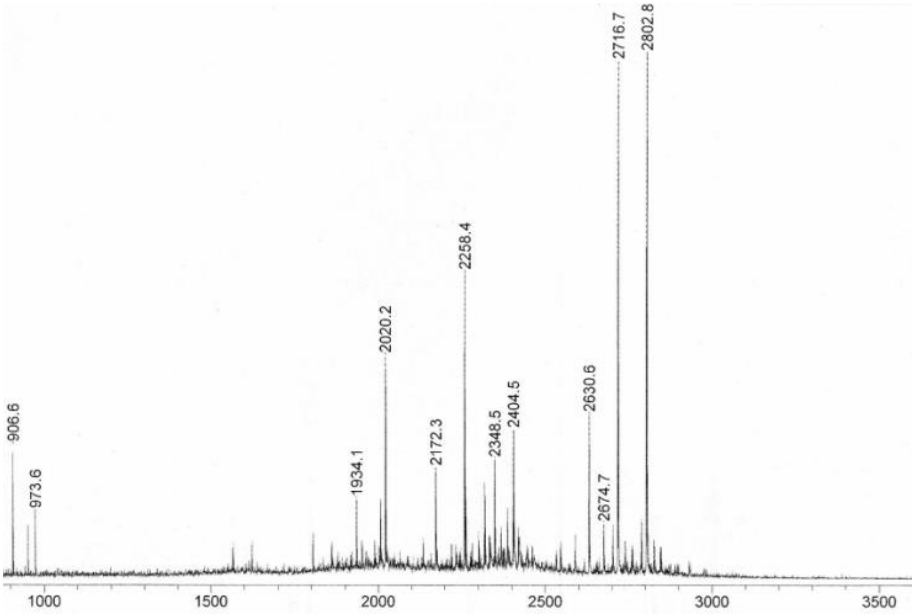


**Figure S43.** MALDI-ToF (HCCA) of **A8.G$_1$- Lys**. Calcd for $[M+Na]^+$ 2803.82, found 2802.79

j. **A8.Arg$_3$**

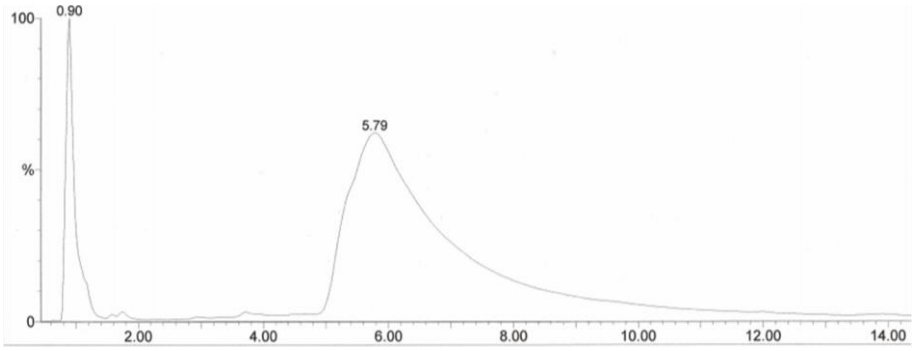


**Figure S44.** HPLC chromatogram of **A8.Arg$_3$**

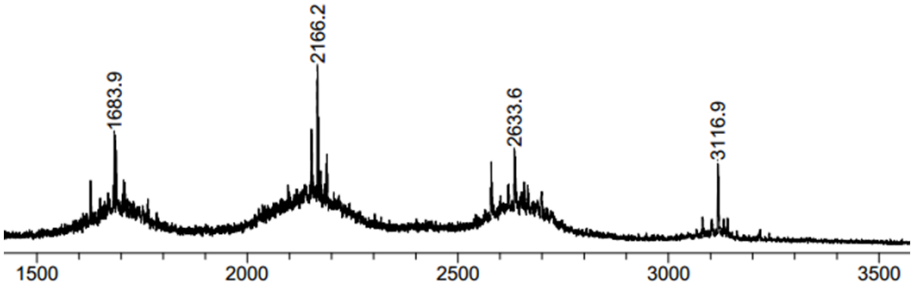


**Figure S45.** MALDI-ToF (HCCA) of **A8.Arg$_3$**. Calcd for $[M+H]^+$ 3116.82, found 3116.86

k. **A1.Ac**

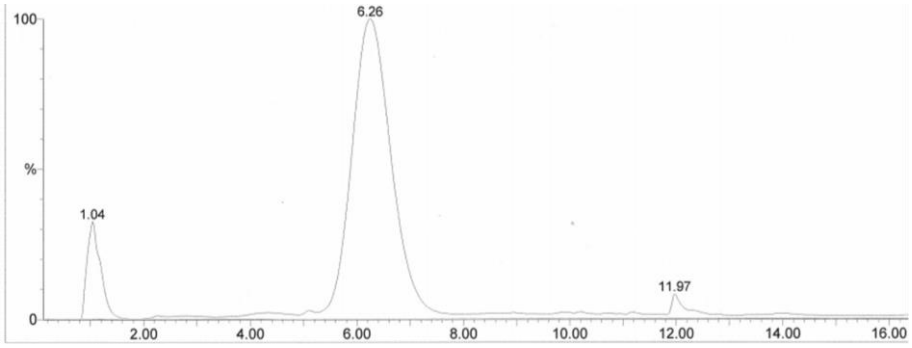


**Figure S46.** HPLC chromatogram of **A1.Ac**

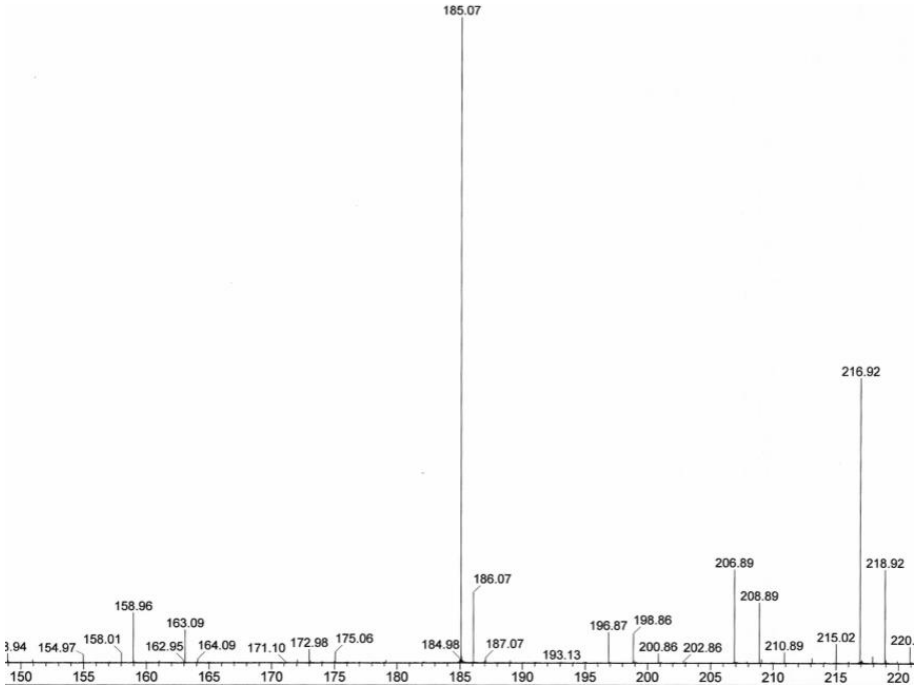


**Figure S47.** HR-ESI-MS spectra of **A1.Ac**. Calcd for $[M+Na]^+$ 185.0871, found 185.0870

l. **A2.Ac**

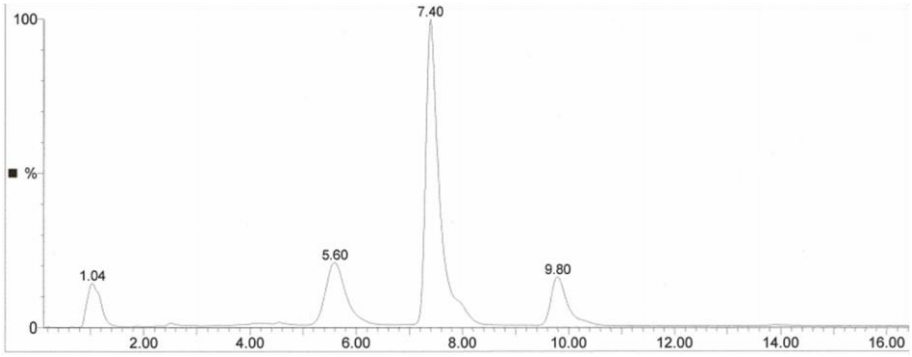


**Figure S48.** HPLC chromatogram of **A2.Ac**

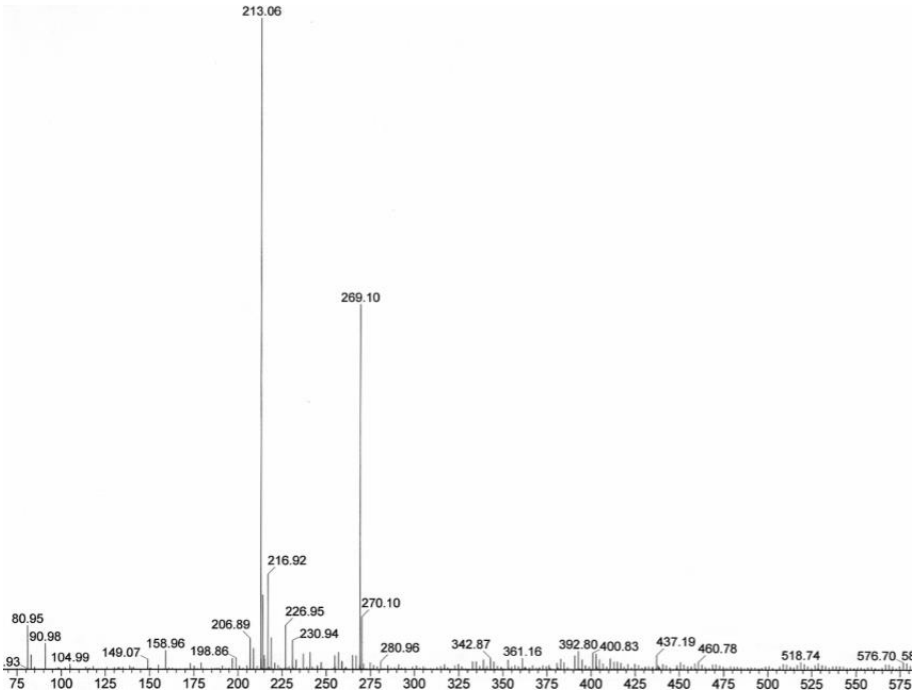


**Figure S49.** HR-ESI-MS spectra of **A2.Ac**. Calcd for $[M+Na]^+$ 213.0821, found 213.0819

m. **A3.Ac**

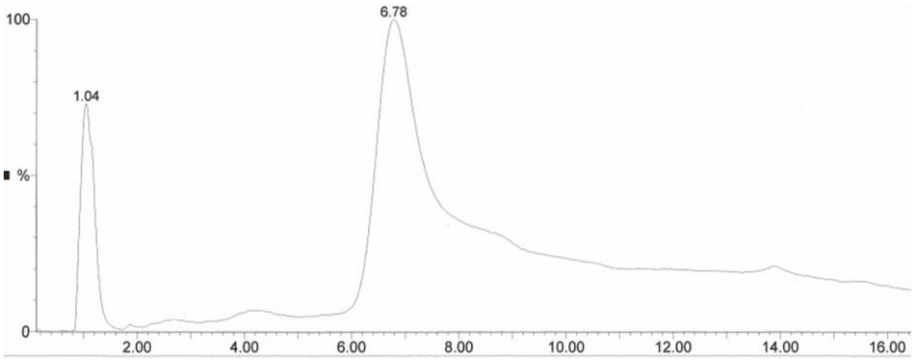


**Figure S50.** HPLC chromatogram of **A3.Ac**

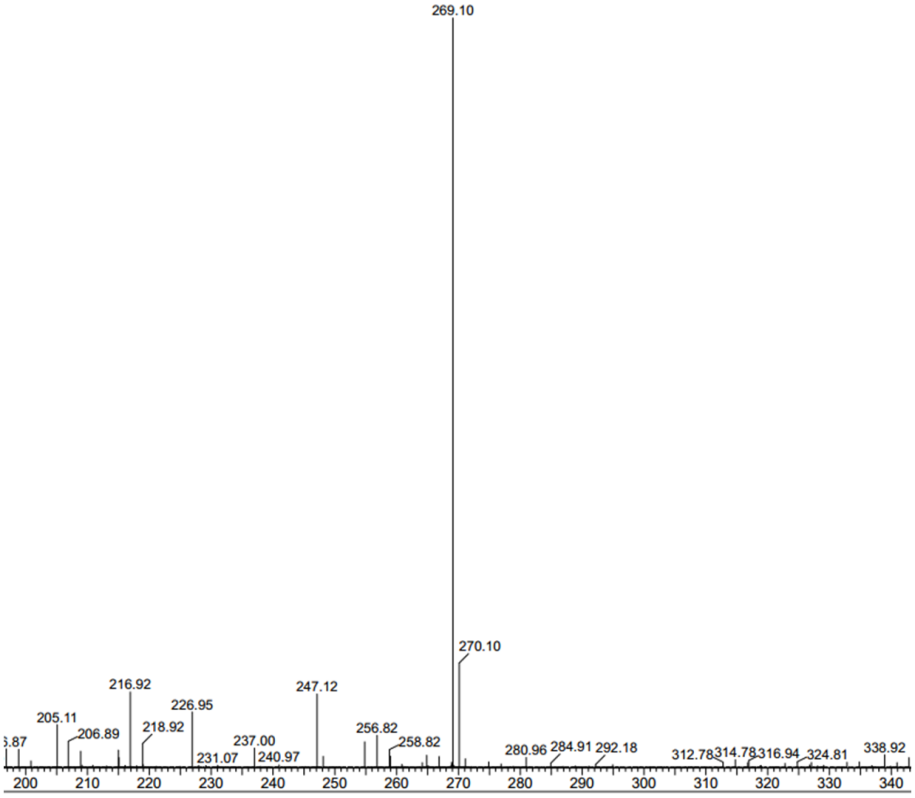


**Figure S51.** HR-ESI-MS spectra of **A3.Ac**. Calcd for $[M+Na]^+$ 269.1195, found 269.1194

n. **A4.Ac**

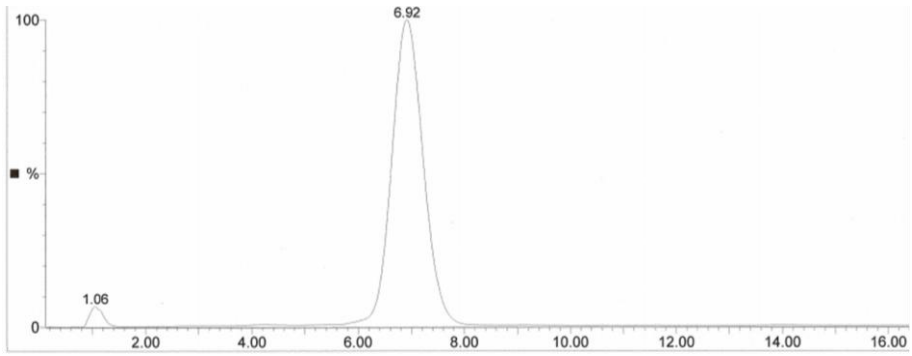


**Figure S52.** HPLC chromatogram of **A4.Ac**

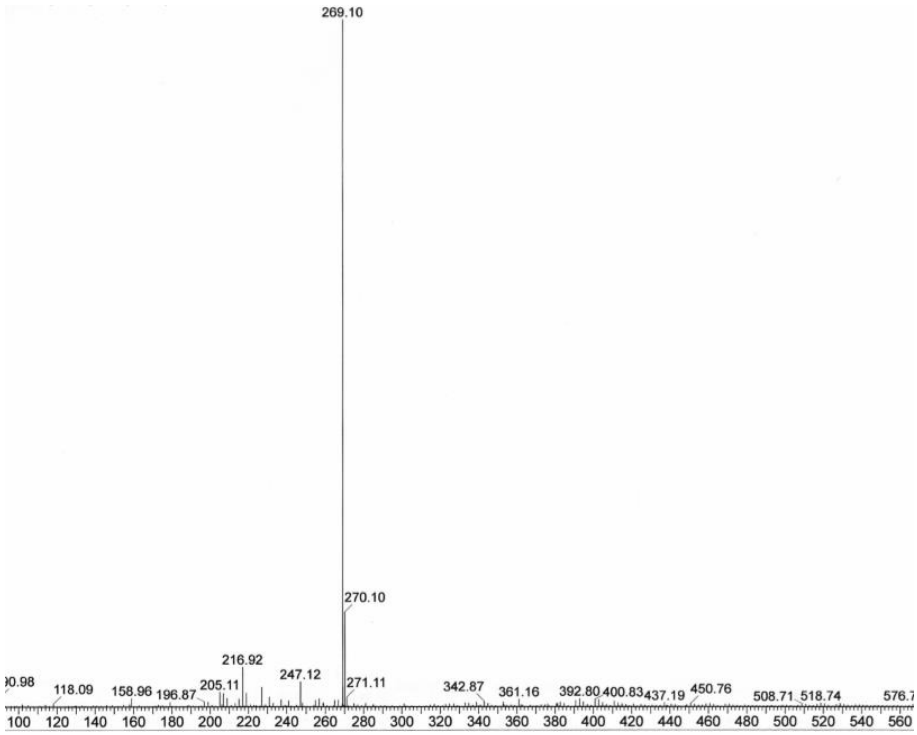


**Figure S53.** HR-ESI-MS spectra of **A4.Ac**. Calcd for $[M+Na]^+$ 269.1195, found 269.1197

o. **A5.Ac**

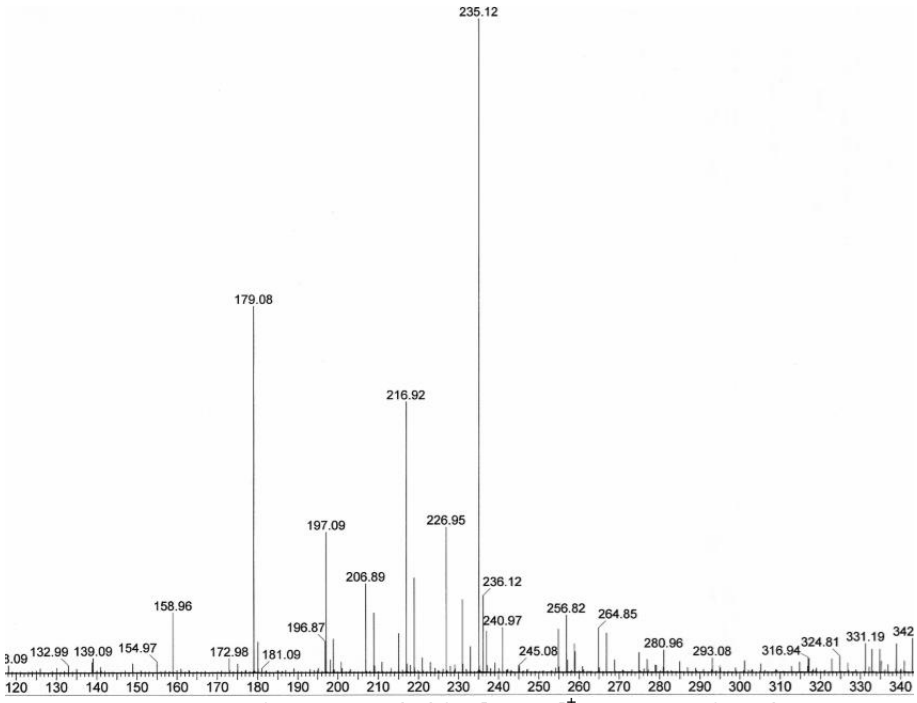


**Figure S54.** HR-ESI-MS spectra of **A5.Ac**. Calcd for $[M+Na]^+$ 235.1171, found 235.1169

p. **A6.Ac**

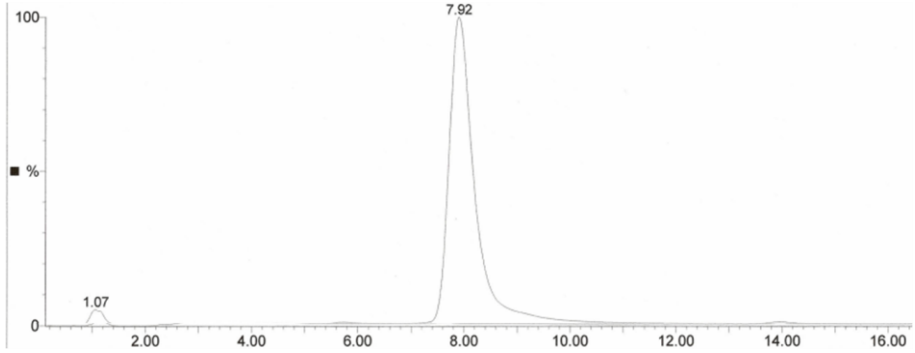


Figure S55. HPLC chromatogram of **A6.Ac**

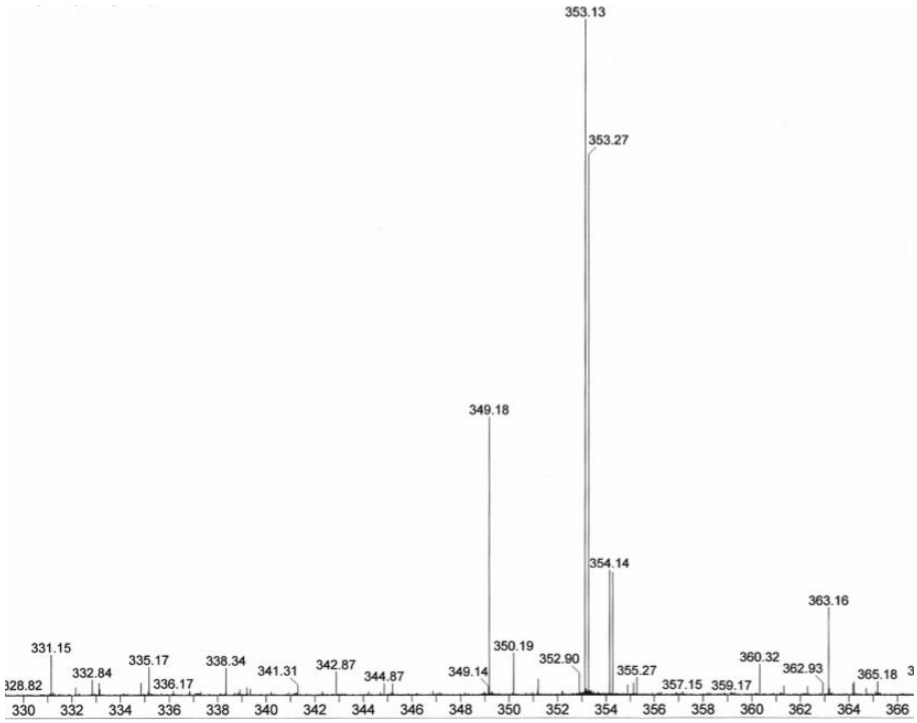


**Figure S56.** HR-ESI-MS spectra of **A6.Ac**. Calcd for $[M+Na]^+$ 353.1519, found 353.1516

q. **A7.Ac**

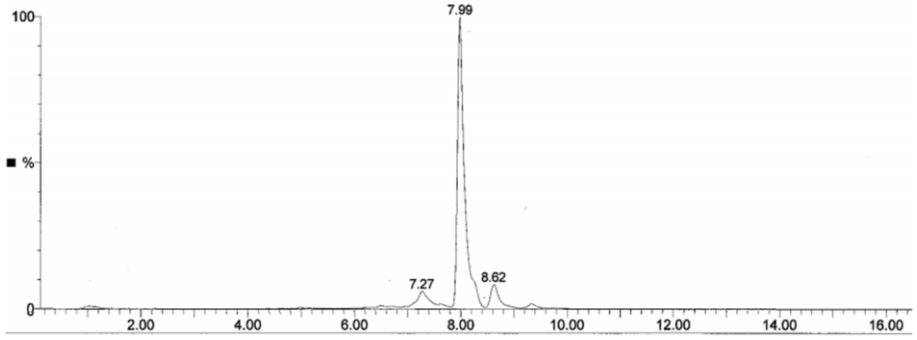


**Figure S57.** HPLC chromatogram of **A7.Ac**

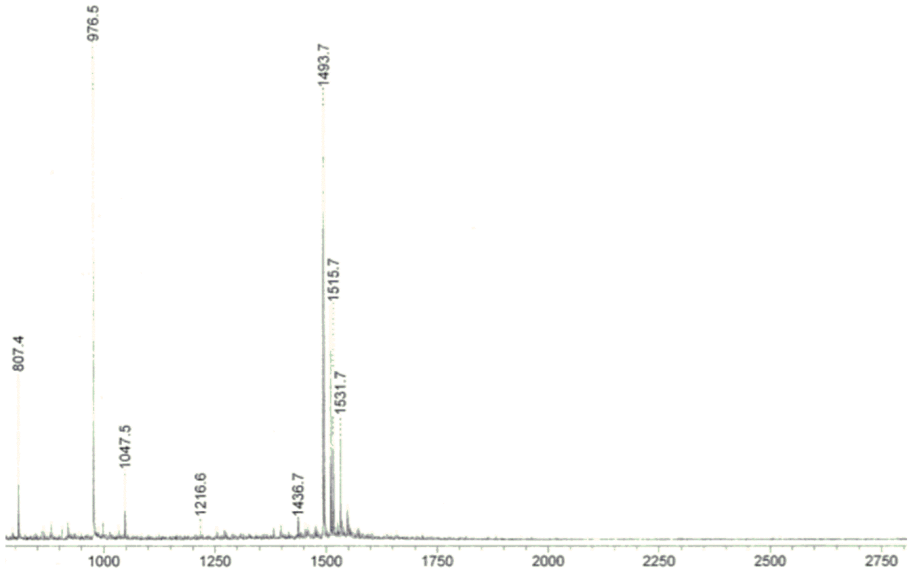


**Figure S58.** MALDI-ToF spectra of **A7.Ac**. Calcd for $[M+H]^+$ 1493.73, found 1493.70

4. Gel electrophoresis analysis of **A6.Hyd**

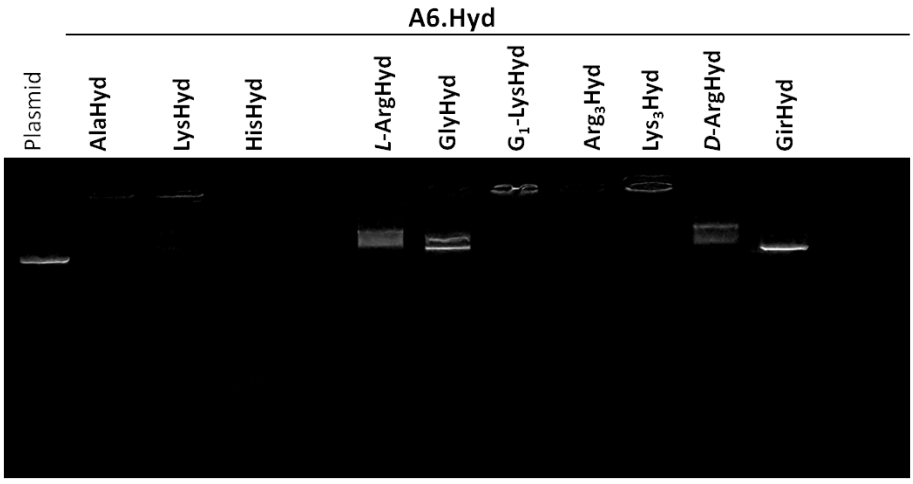


**Figure S59.** Gel electrophoresis analysis showing the complexation of plasmid DNA by clusters made of scaffold **A6** with different hydrazides. Experiments carried out at N/P=30

5. Gel electrophoresis analysis of cluster **A8.Gly**

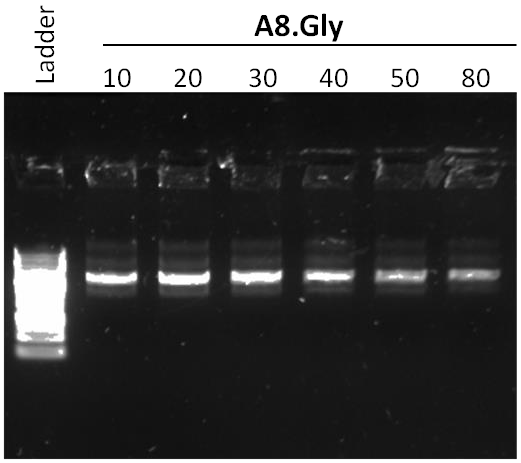


**Figure S60.** Gel electrophoresis analyses of mixture of plasmid DNA and cluster **A8.Gly** at different N/P

6. Fluorescence microscopy

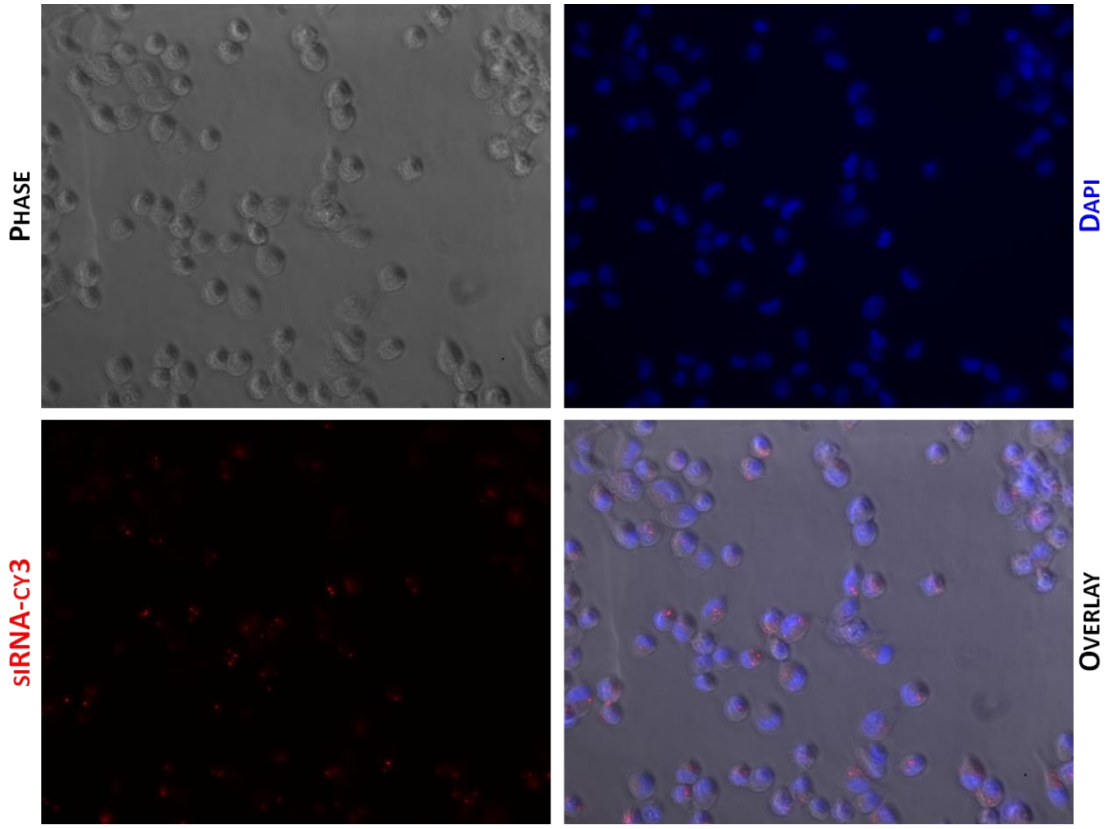


**Figure S61.** Fluorescence imaging (magnification 40x) of MDA-MB-231 breast cancer cells transfected by the complex formed between cluster **A8.Arg$_3$** and Cy3-labeled non-coding siRNA at N/P=9. The blue fluorescence (top right) indicates the nuclei (DAPI stained), and red fluorescence (bottom left) indicates the Cy3-labeled siRNA.