

# SUPPORTING INFORMATION

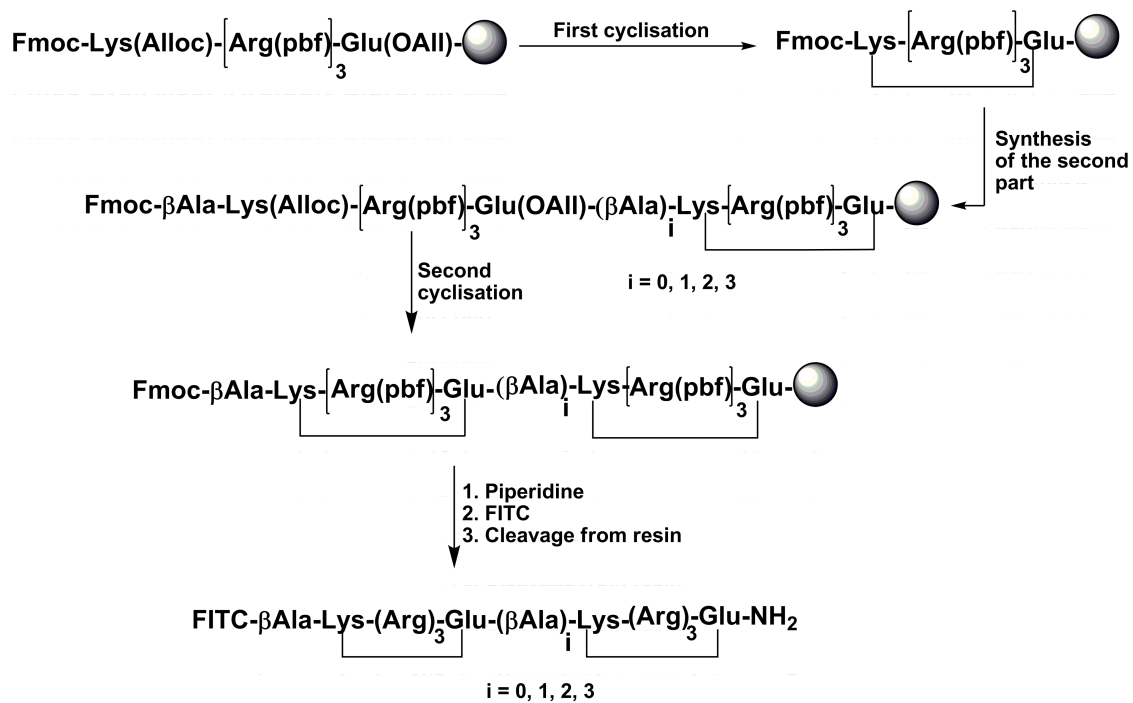
## **Macrocyclic Cell Penetrating Peptides: A Study of Structure-Penetration Properties.**

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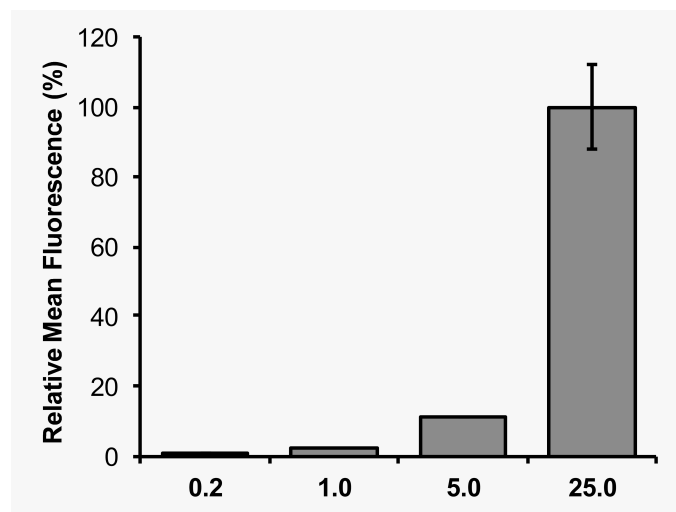
<b>A. Synthesis of Bicyclic Peptides</b>	<b>S2</b>
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## A. Synthesis of Bicyclic Peptides

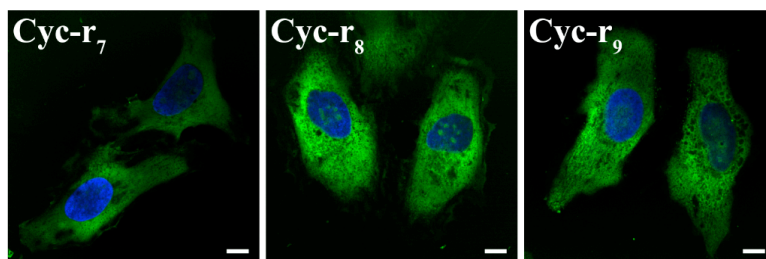


**Figure S1:** Synthetic scheme for preparing bicyclic polyarginine analogues.

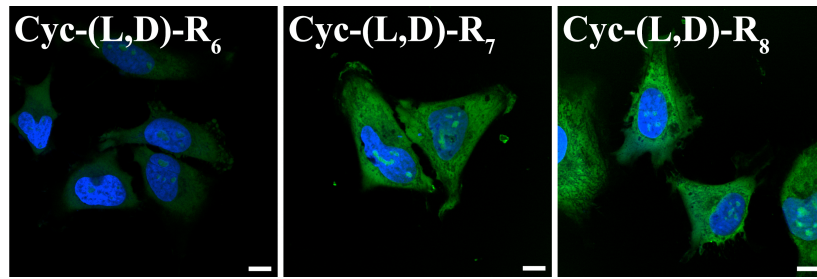
## B. Supplementary Flow Cytometry and Confocal Microscopy Figures



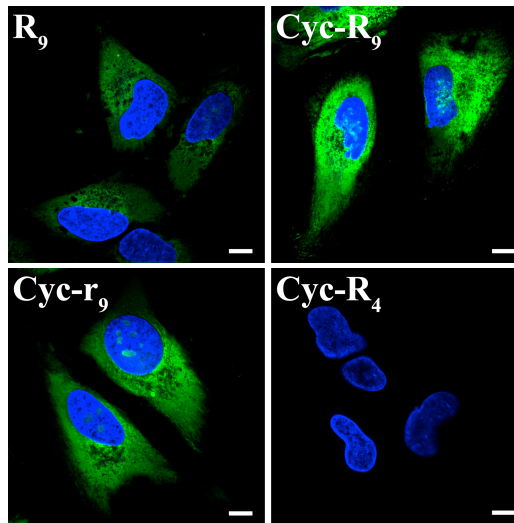
**Figure S2:** Concentration-dependent response for the uptake of Cyc-R<sub>9</sub> macrocycle (concentrations indicated in μM).



**Figure S3:** Confocal microscopy images of HeLa cells treated with macrocyclic homochiral (D)-peptides (in green). Nuclei are marked in blue. Scale bar, 10 μm.



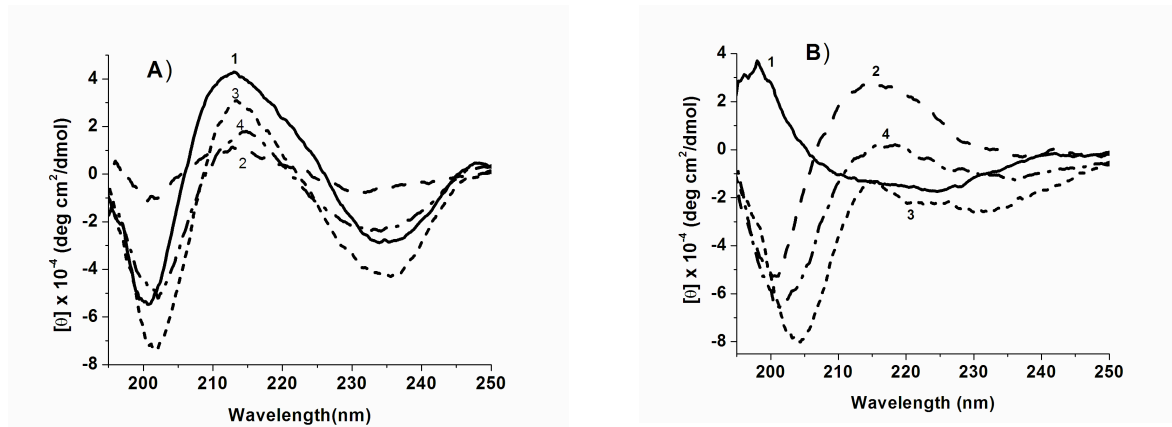
**Figure S4:** Confocal microscopy images of HeLa cells treated with (L,D)-peptides (in green). Nuclei are marked in blue. Scale bar, 10  $\mu$ m.



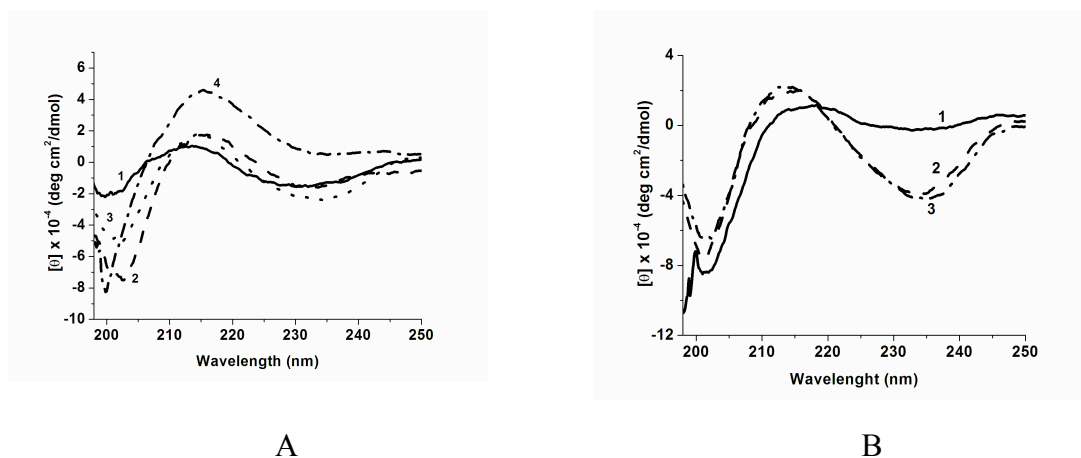
**Figure S5:** Live confocal microscopy images of HeLa cells incubated with FITC-labeled linear and cyclic peptides. Nuclei are marked in blue. Scale bar, 10  $\mu$ m.



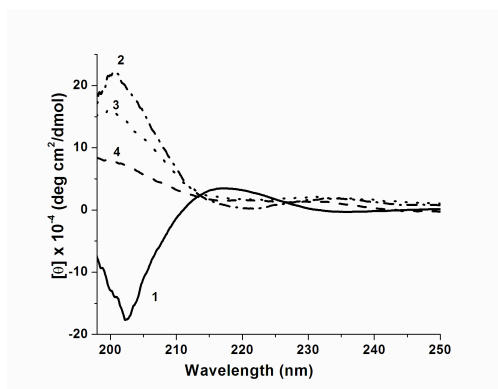
## C. Circular Dichroism Results



**Figure S6:** Circular dichroism spectra of **A)** linear and monocyclic peptides (1) R<sub>6</sub> ; (2) Cyc-R<sub>6</sub> ; (3) R<sub>7</sub> ; (4) Cyc-R<sub>7</sub> and **B)** bicyclic peptides where (1) Bicyc-0-R<sub>6</sub> ; (2) Bicyc-1-R<sub>6</sub> ; (3) Bicyc-2-R<sub>6</sub> ; (4) Bicyc-3-R<sub>6</sub>.



**Figure S7 :** CD spectra in water of **A)** (1) Cyc-R<sub>4</sub> ; (2) R<sub>9</sub> ; (3) Cyc-R<sub>8</sub> ; (4) Cyc-(L-D)-R<sub>8</sub> and **B)** (1) Cyc-(L-D)-R<sub>7</sub> ; (2) Cyc-R<sub>3</sub>-R<sub>4</sub> and (3) Cyc-R<sub>4</sub>-R<sub>3</sub>



**Figure S8 :** CD spectra in water of (1) Cyc-R<sub>9</sub> ; (2) Cyc-r<sub>9</sub> ; (3) Cyc-r<sub>8</sub> ; (4) Cyc-r<sub>7</sub>

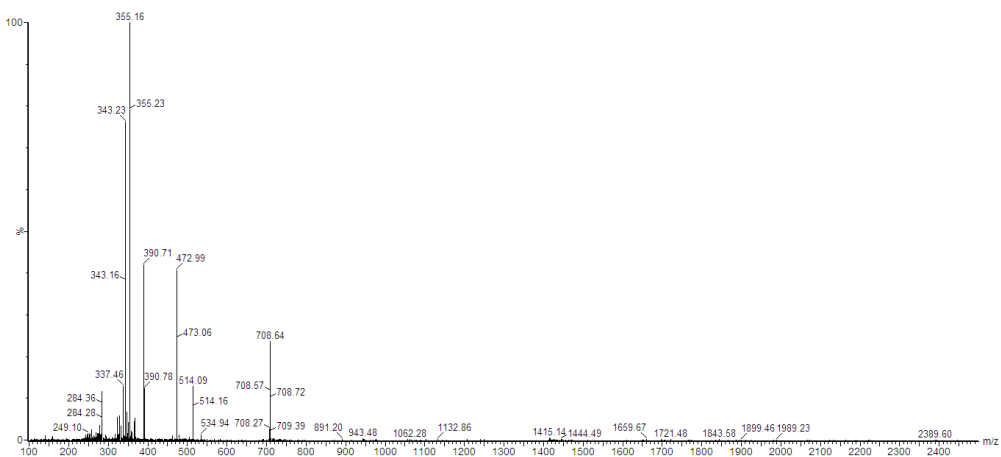
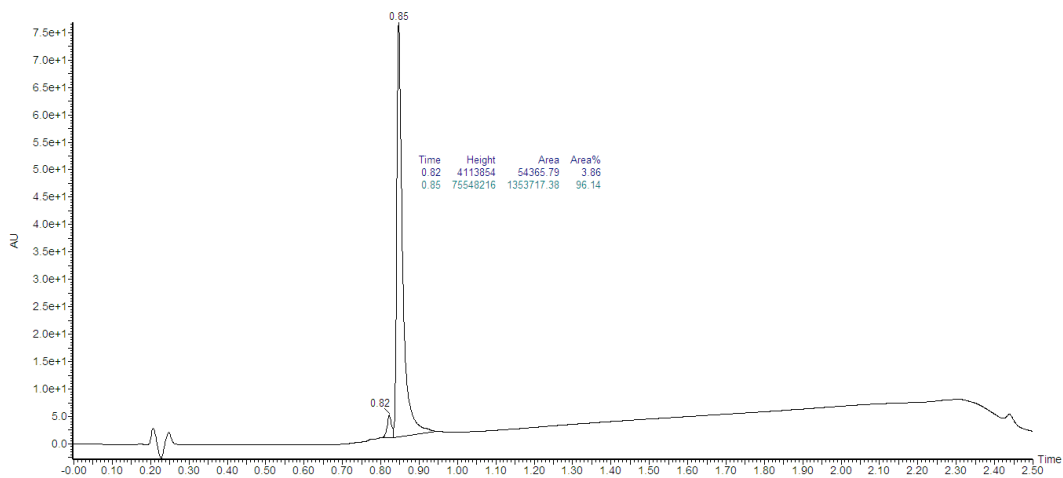
### D. Table of Characterization

**Table S1:** molecular weight, purity and UPLC retention time of new compounds

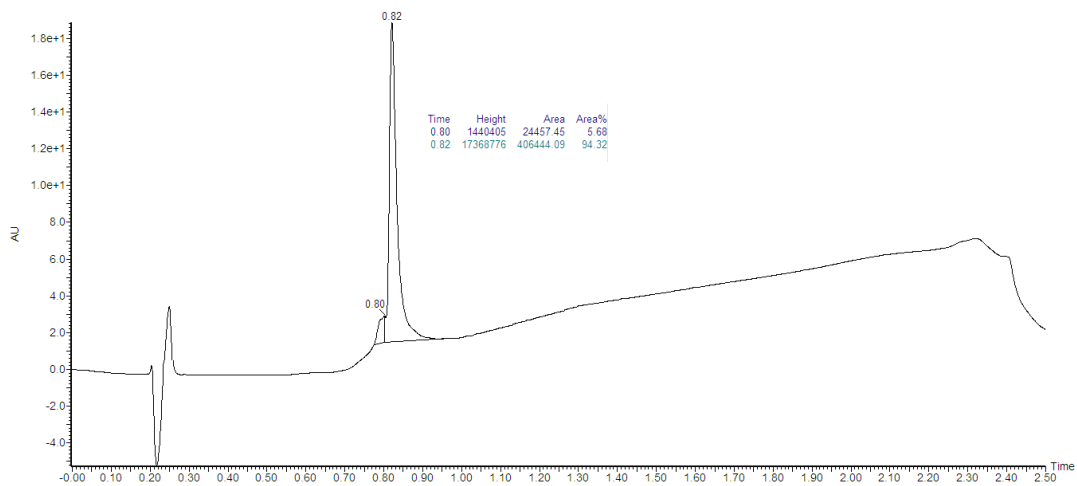
Compound	Calculated mass	Observed mass	Purity (UPLC-UV)	Retention time (UPLC)
Cyc-R <sub>6</sub>	(M+2)/2 = 827.95	(M+2)/2 = 828.20	97%	0.85
Cyc-R <sub>7</sub>	(M+2)/2 = 906.04	(M+2)/2 = 906.31	92%	0.82
Cyc-R <sub>8</sub>	(M+3)/3 = 656.42	(M+3)/3 = 656.82	96%	0.81
Cyc-R <sub>9</sub>	(M+3)/3 = 708.48	(M+3)/3 = 709.09	98%	0.84
Cyc-(L-D)-R <sub>6</sub>	(M+2)/2 = 827.95	(M+2)/2 = 828.42	93%	0.83
Cyc-(L-D)-R <sub>7</sub>	(M+2)/2 = 906.04	(M+2)/2 = 906.29	94%	0.81
Cyc-(L-D)-R <sub>8</sub>	(M+3)/3 = 656.42	(M+3)/3 = 656.89	92%	0.82
Cyc-r <sub>7</sub>	(M+3)/3 = 604.35	(M+3)/3 = 604.99	99%	0.83
Cyc-r <sub>8</sub>	(M+3)/3 = 656.42	(M+3)/3 = 657.04	99%	0.83
Cyc-r <sub>9</sub>	(M+3)/3 = 708.43	(M+3)/3 = 709.09	100%	0.84
Bicyc-0-R <sub>6</sub>	(M+3)/3 = 632.06	(M+3)/3 = 632.59	98%	0.85
Bicyc-1-R <sub>6</sub>	(M+3)/3 = 655.75	(M+3)/3 = 656.29	94%	0.85
Bicyc-2-R <sub>6</sub>	(M+3)/3 = 679.44	(M+3)/3 = 679.92	97%	0.86
Bicyc-3-R <sub>6</sub>	(M+3)/3 = 703.14	(M+3)/3 = 703.62	95%	0.86
Cyc-R <sub>3</sub> -R <sub>4</sub>	(M+2)/2 = 906.04	(M+2)/2 = 906.38	94%	0.82
Cyc-R <sub>4</sub> -R <sub>3</sub>	(M+2)/2 = 906.04	(M+2)/2 = 906.45	94%	0.83
Cyc-R <sub>5</sub> -R <sub>2</sub>	(M+2)/2 = 906.04	(M+2)/2 = 905.98	94%	0.82
Cyc-R <sub>6</sub> -R <sub>1</sub>	(M+2)/2 = 906.04	(M+2)/2 = 906.07	95%	0.83

## D. UPLC and MS results

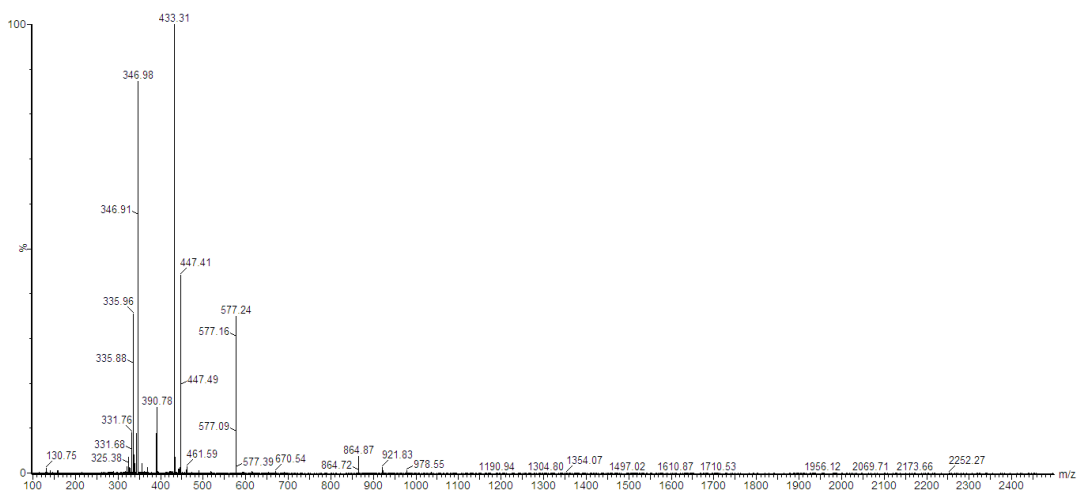
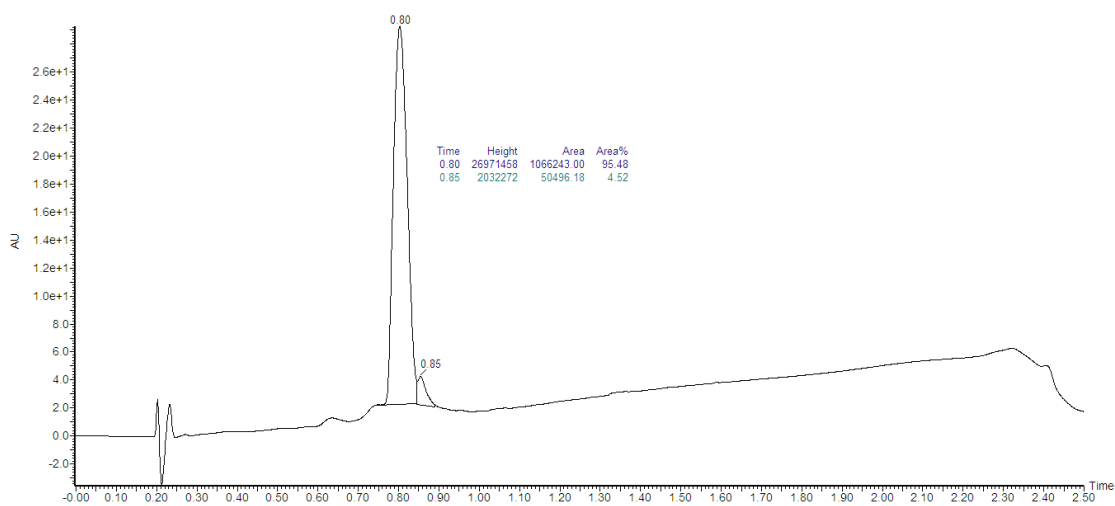
- R<sub>6</sub>



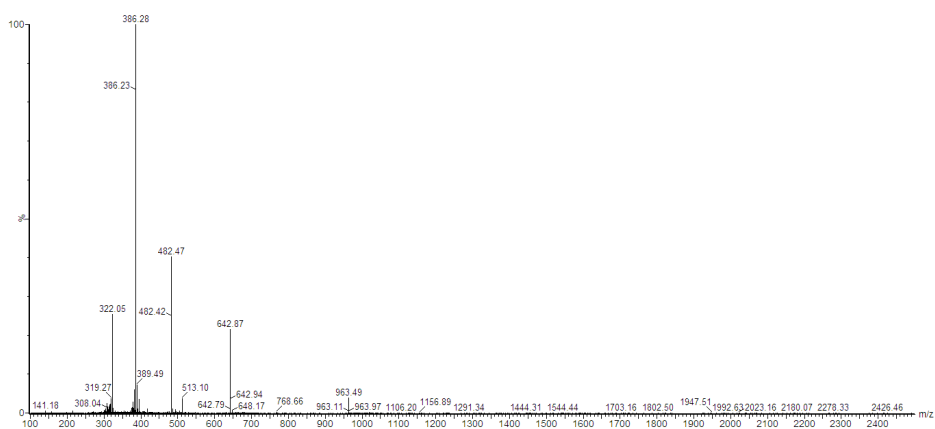
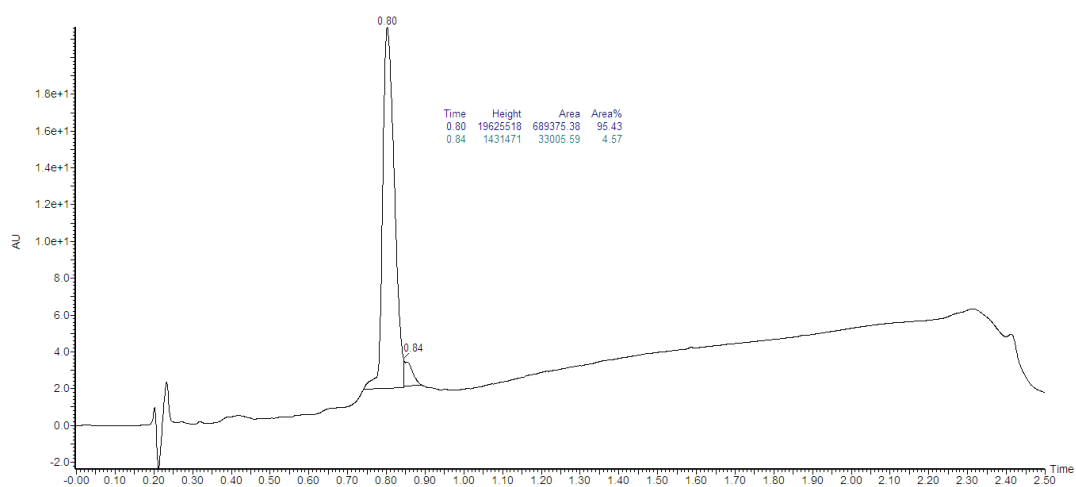
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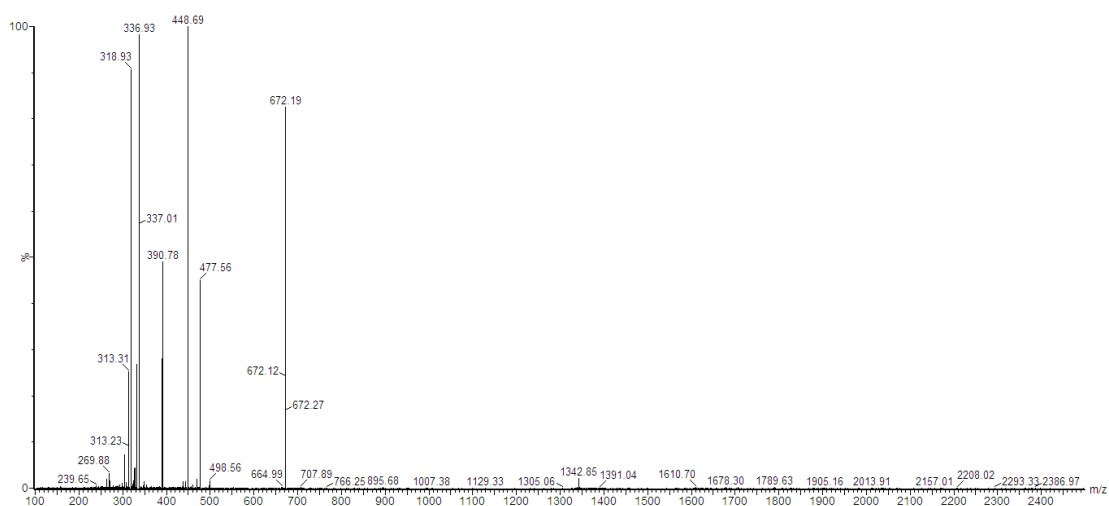
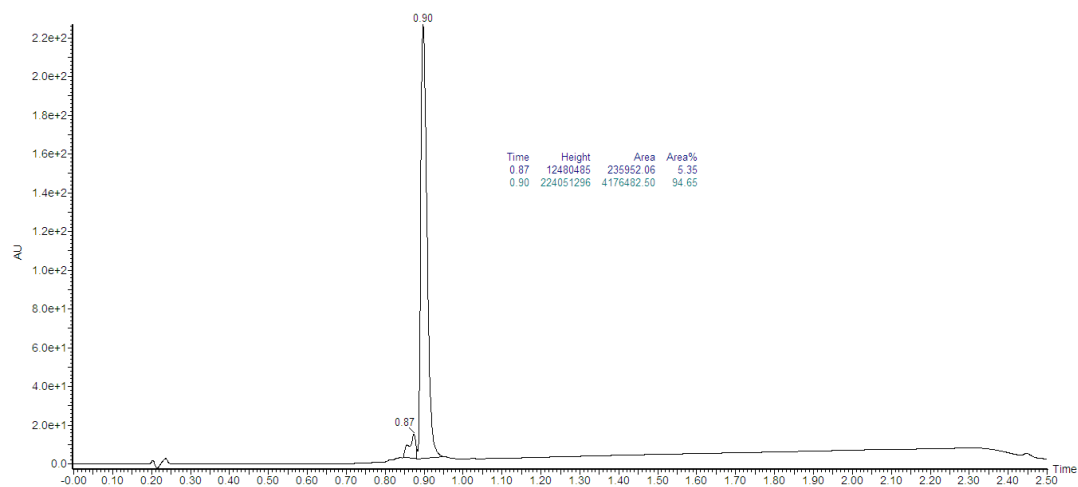
• R<sub>8</sub>



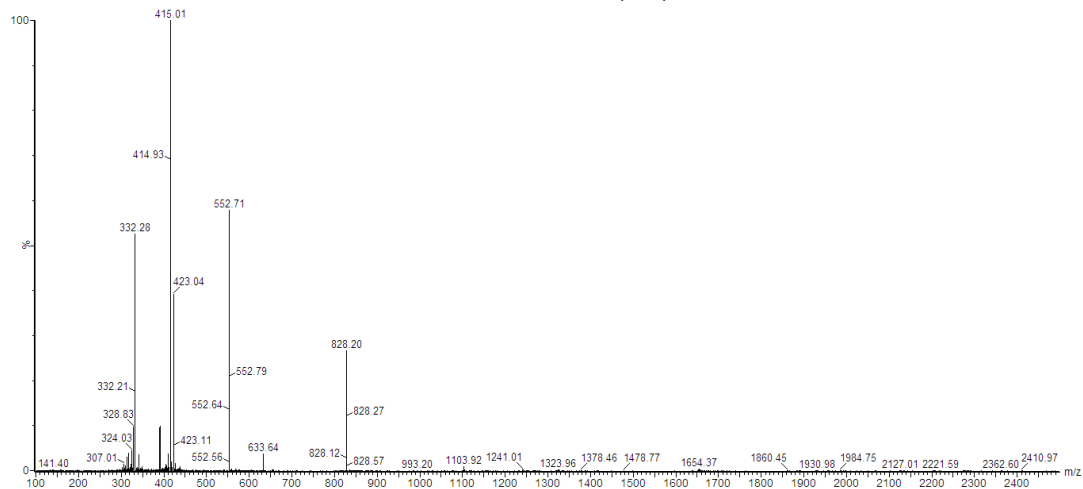
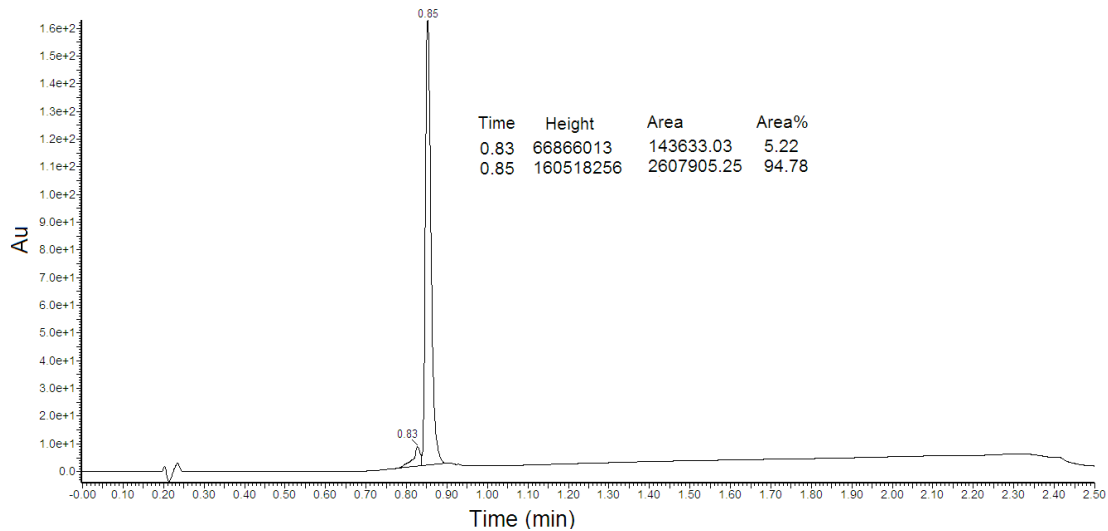
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- **Cyc-R<sub>4</sub>**

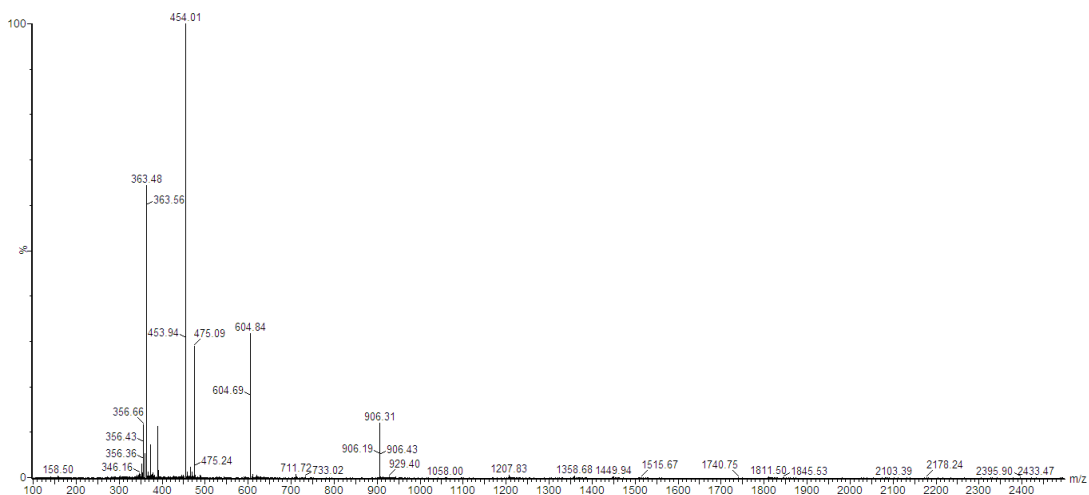
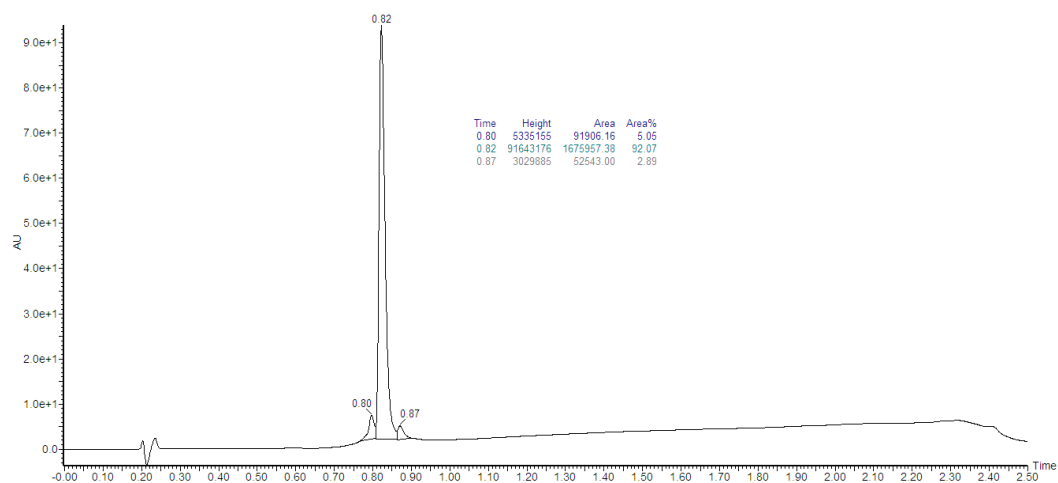


• Cyc-R<sub>6</sub>

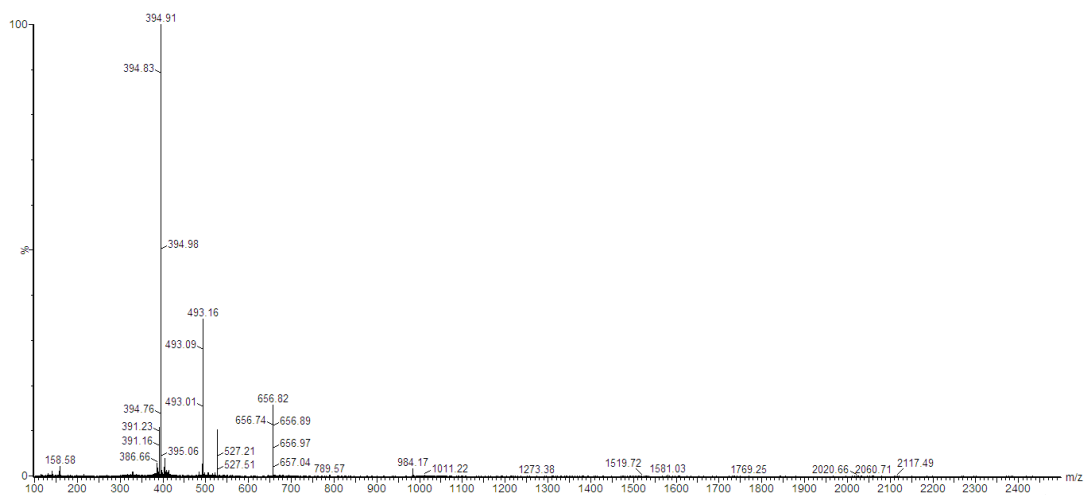
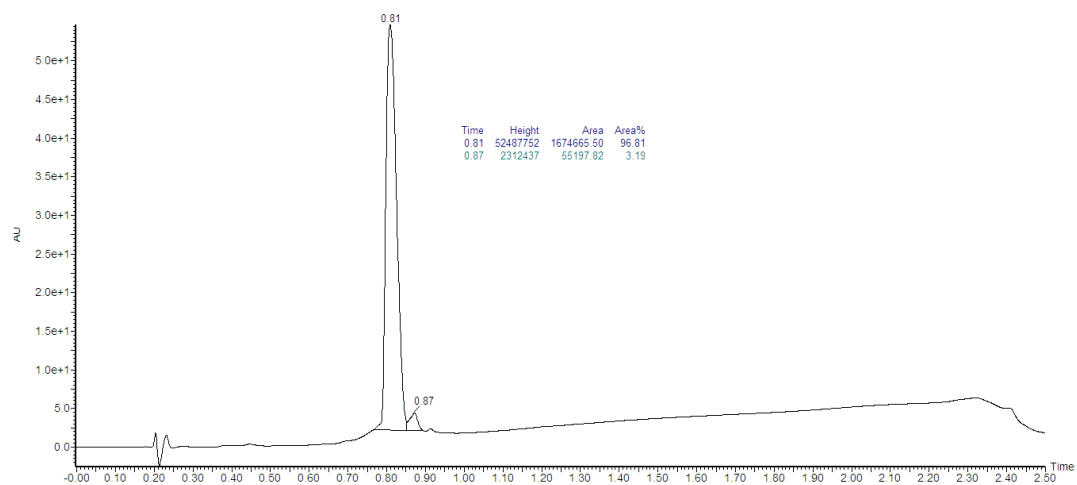




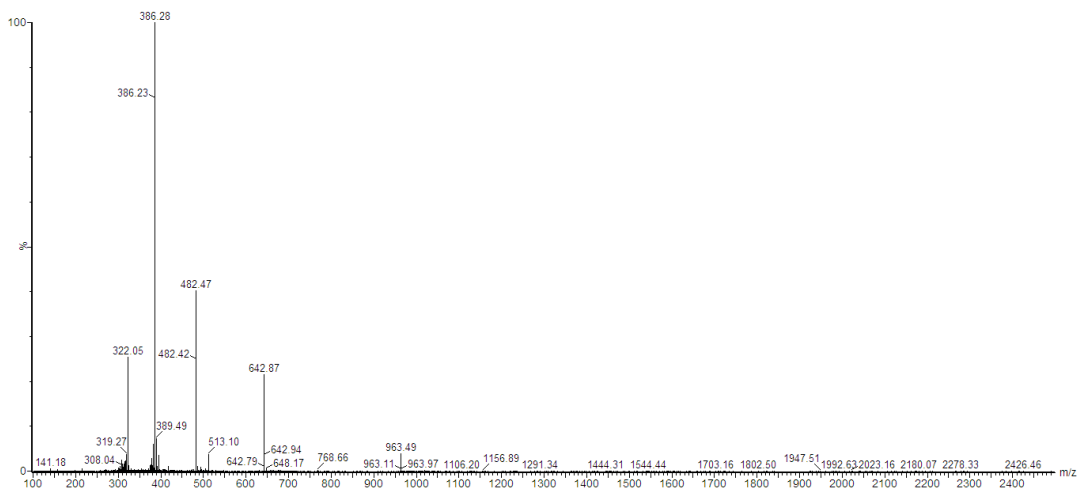
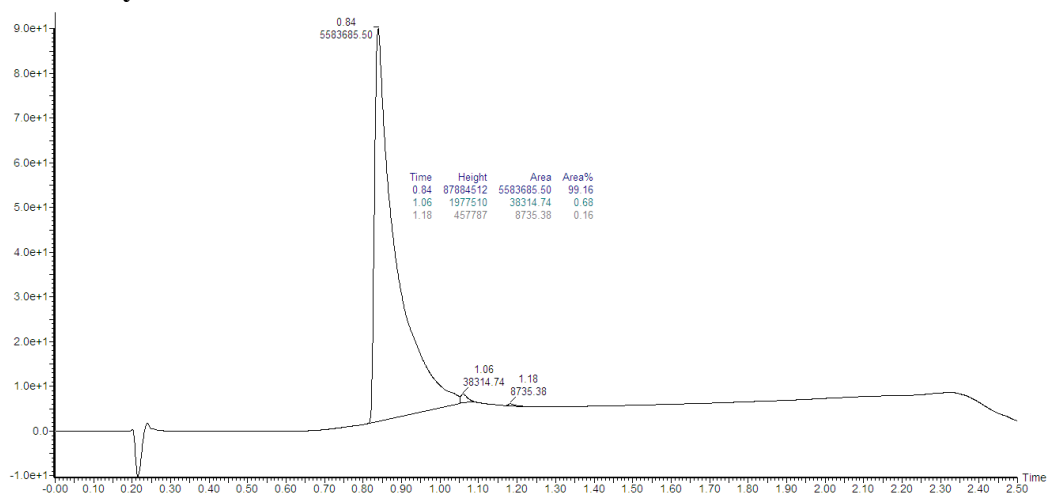
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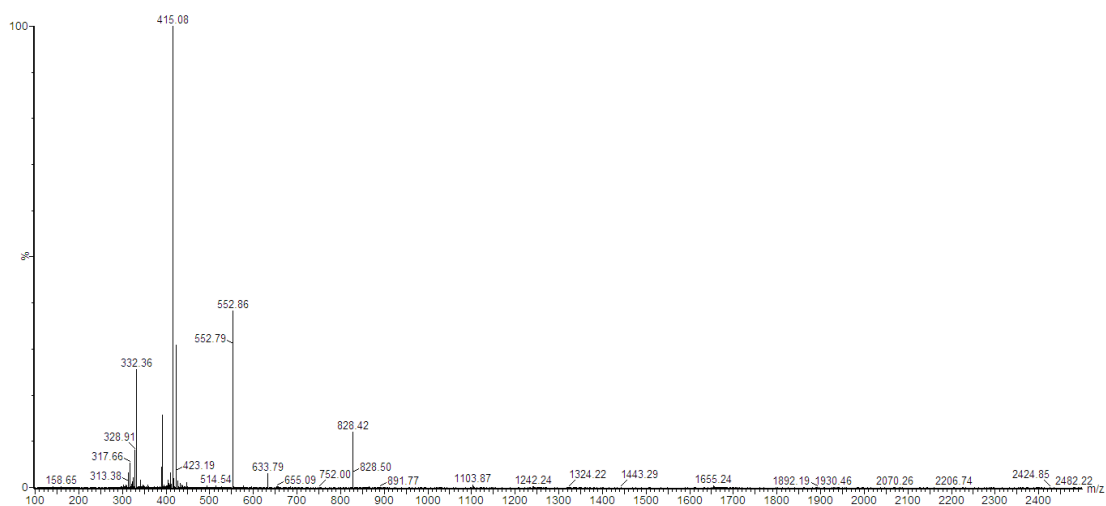
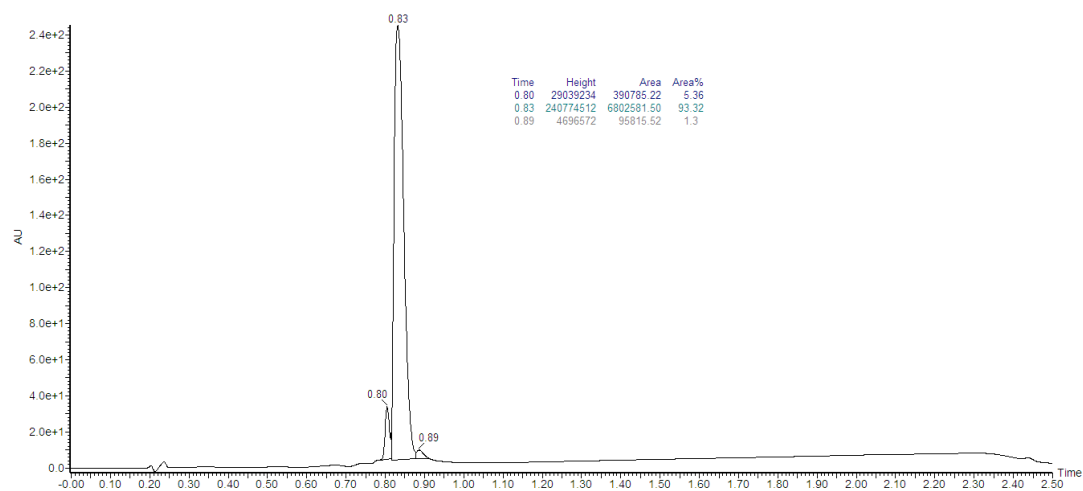
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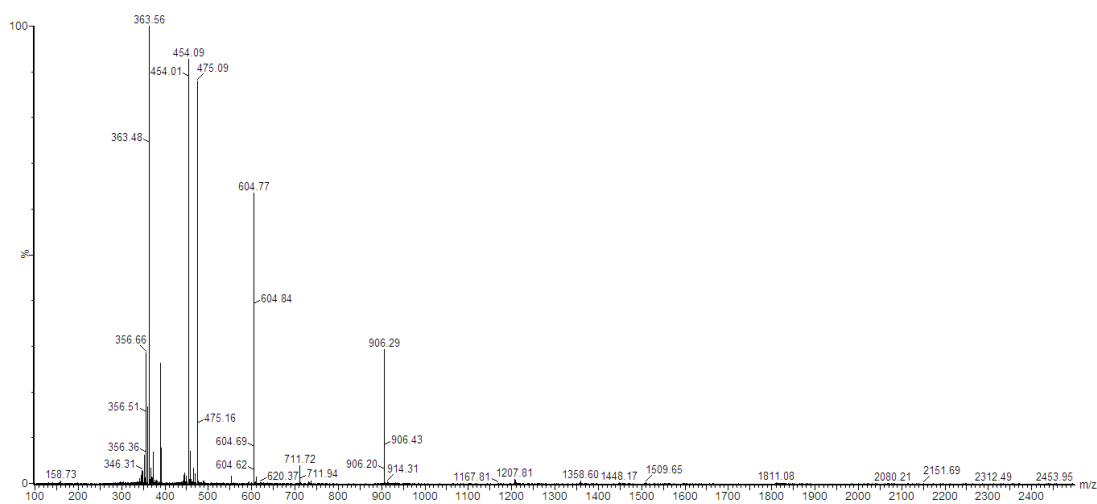
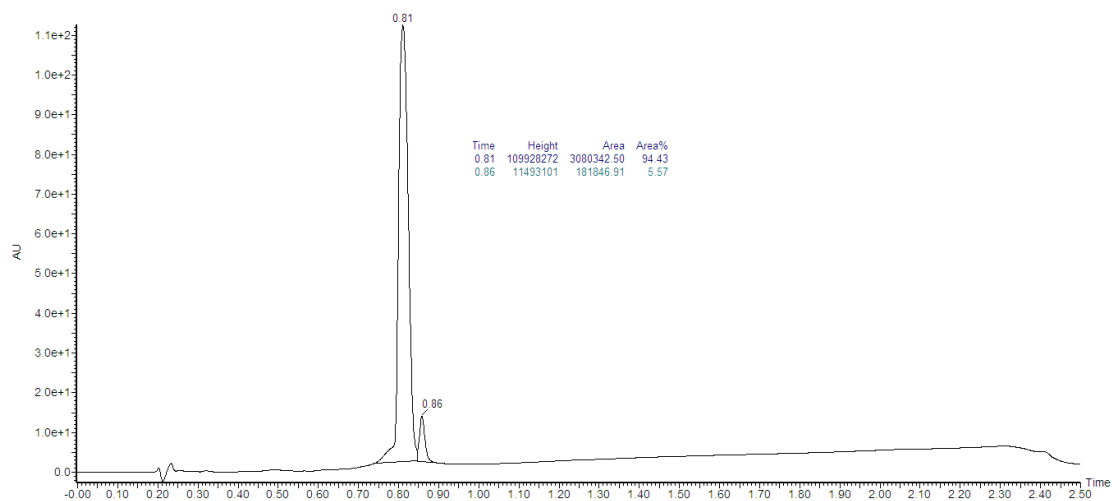
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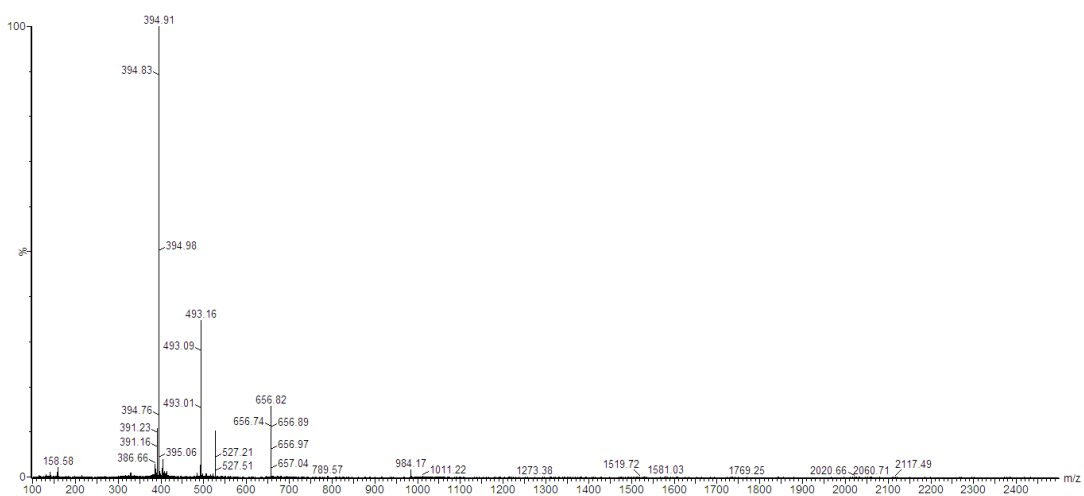
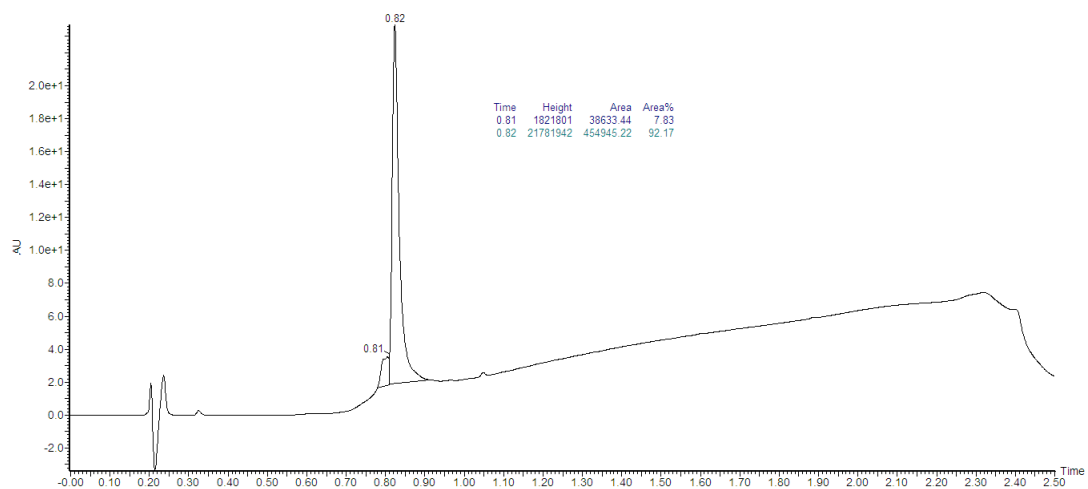
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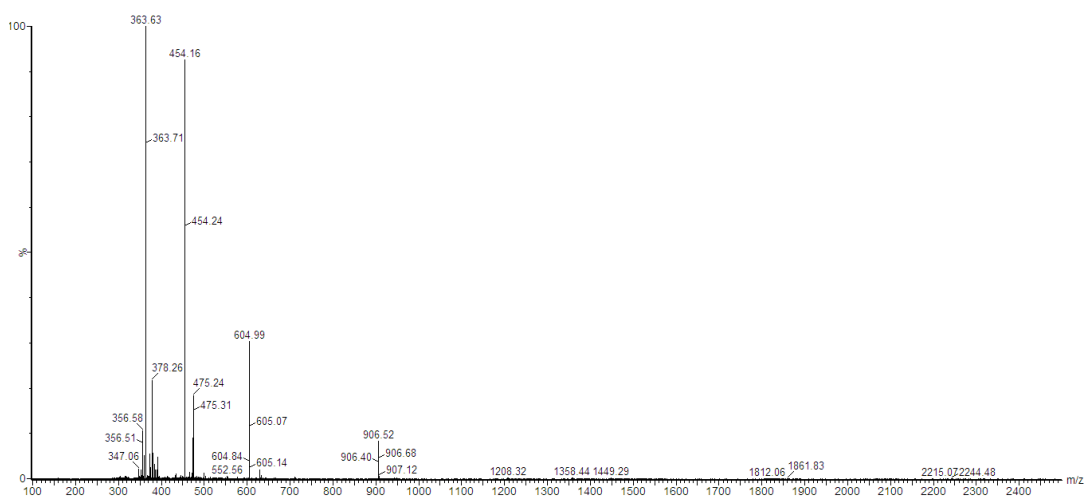
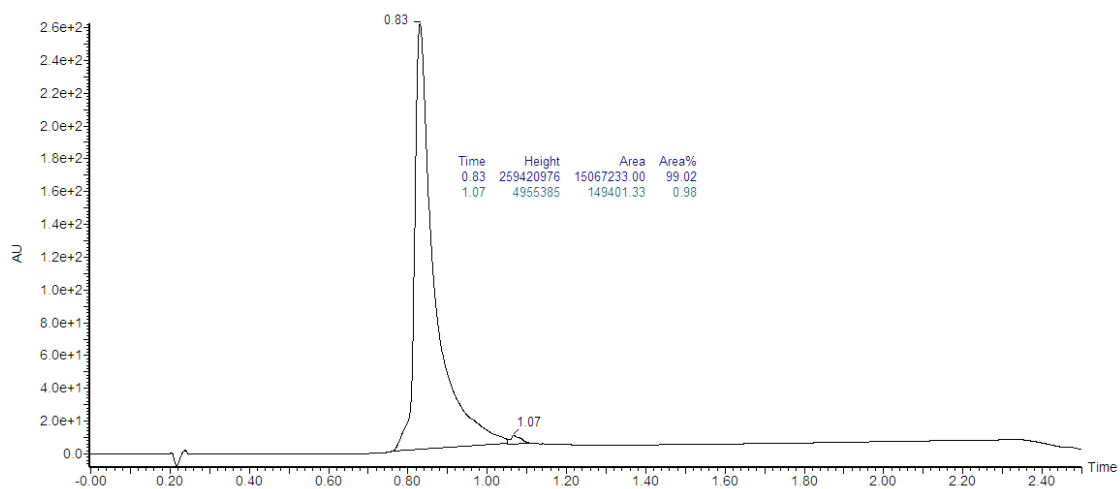
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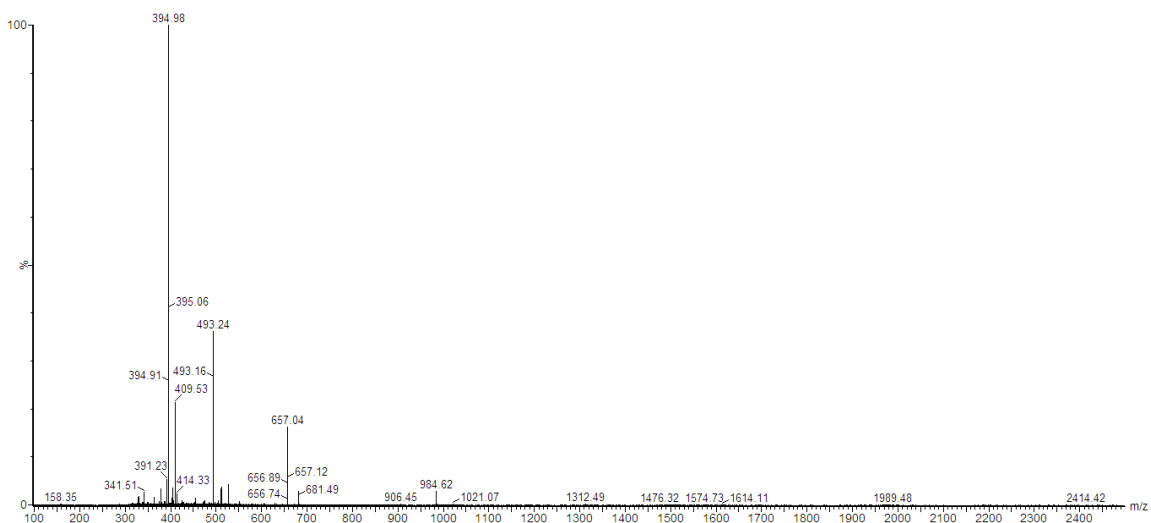
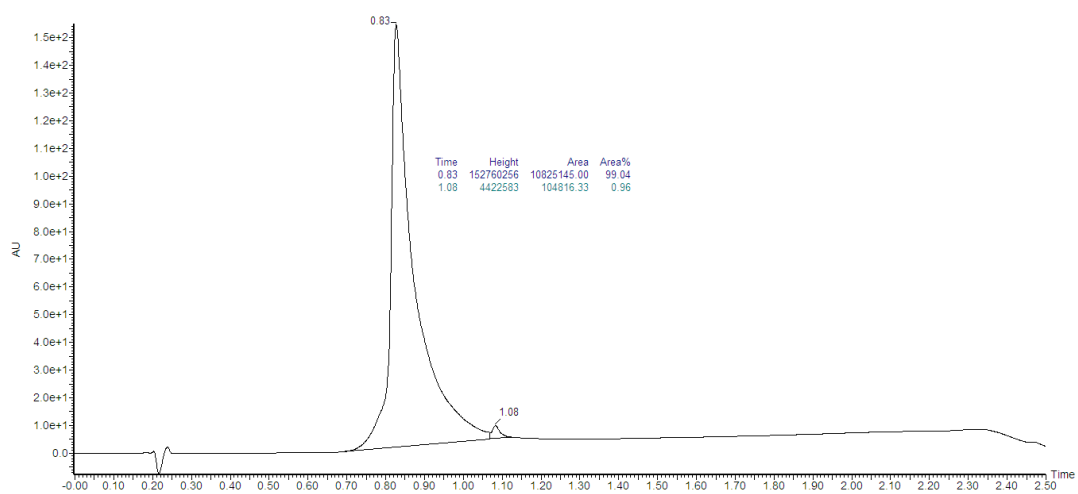
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- **Cyc-r<sub>7</sub>**

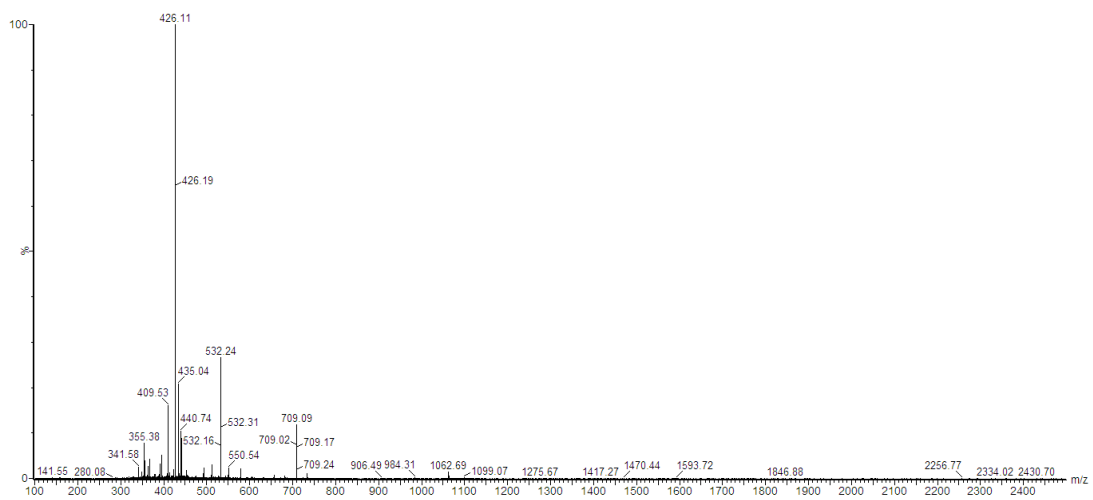
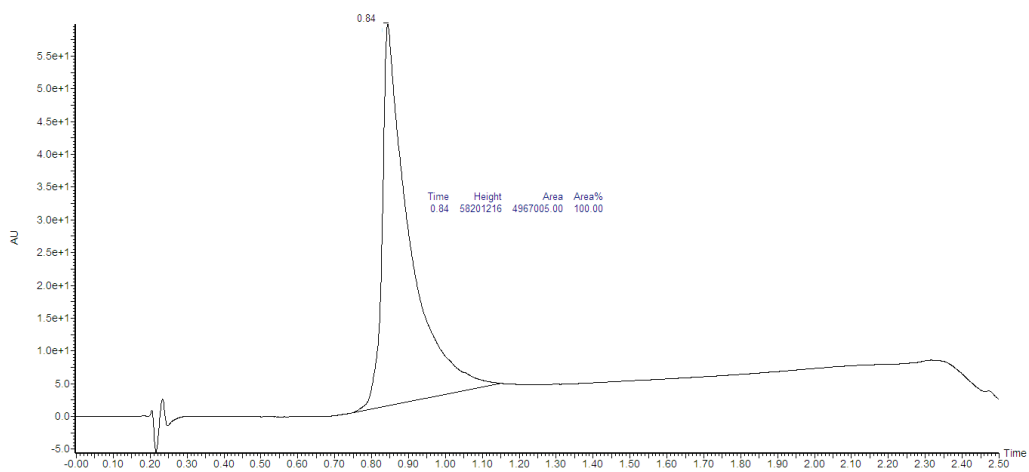


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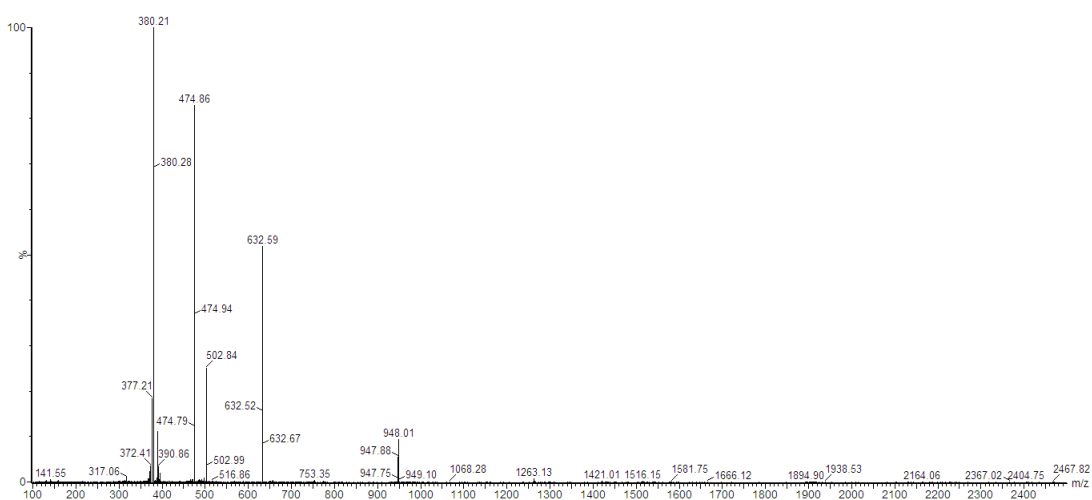
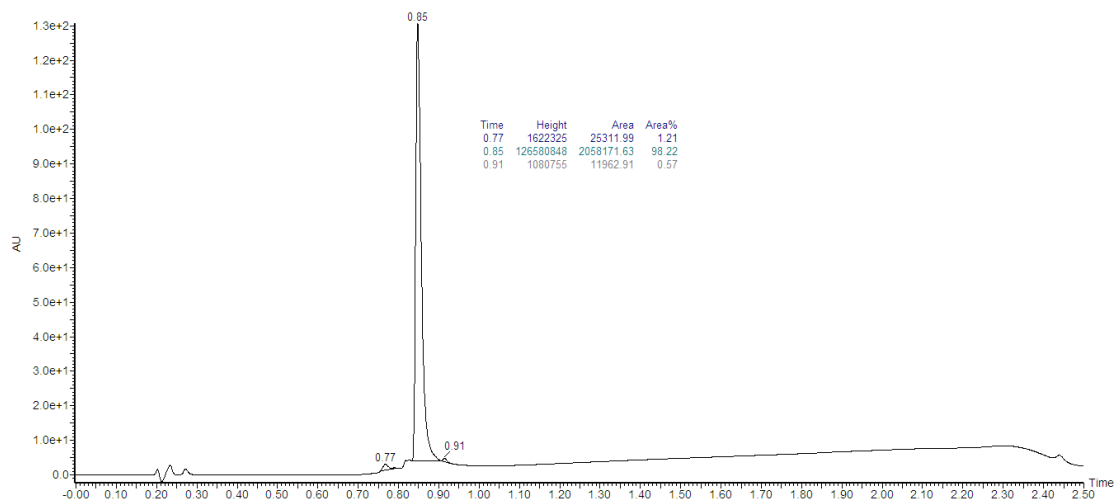




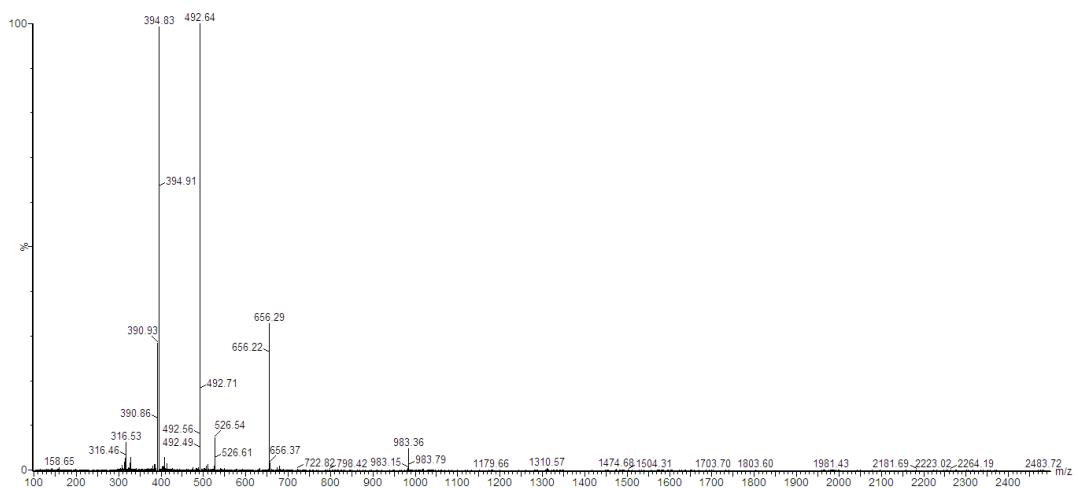
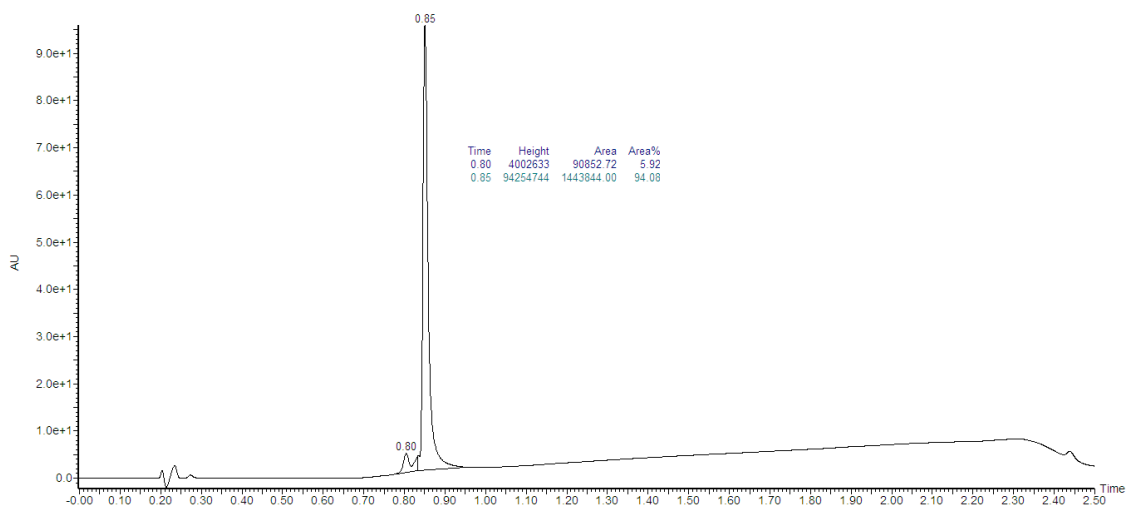
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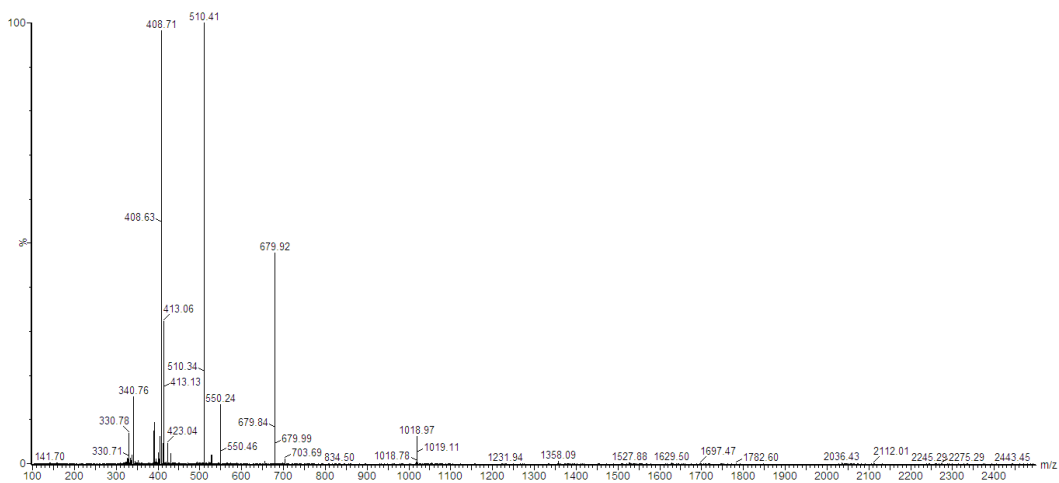
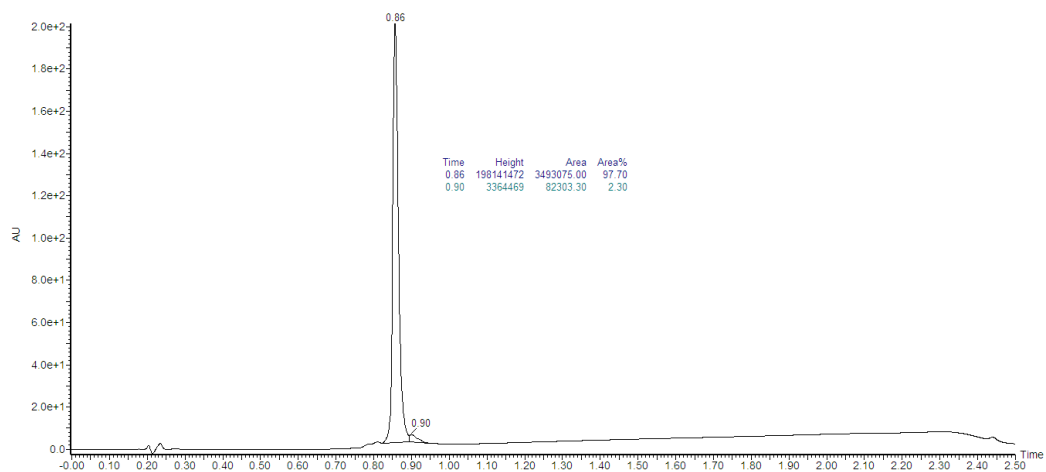
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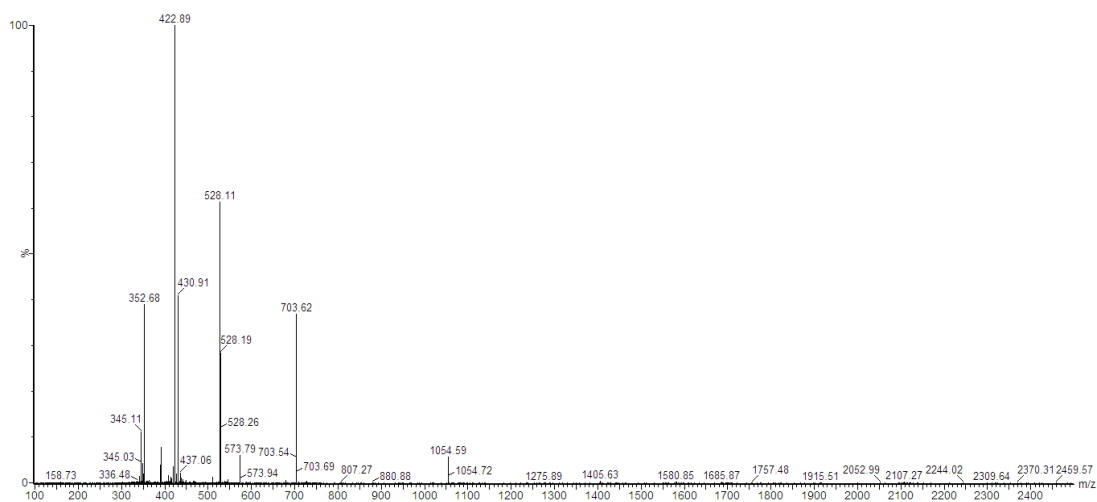
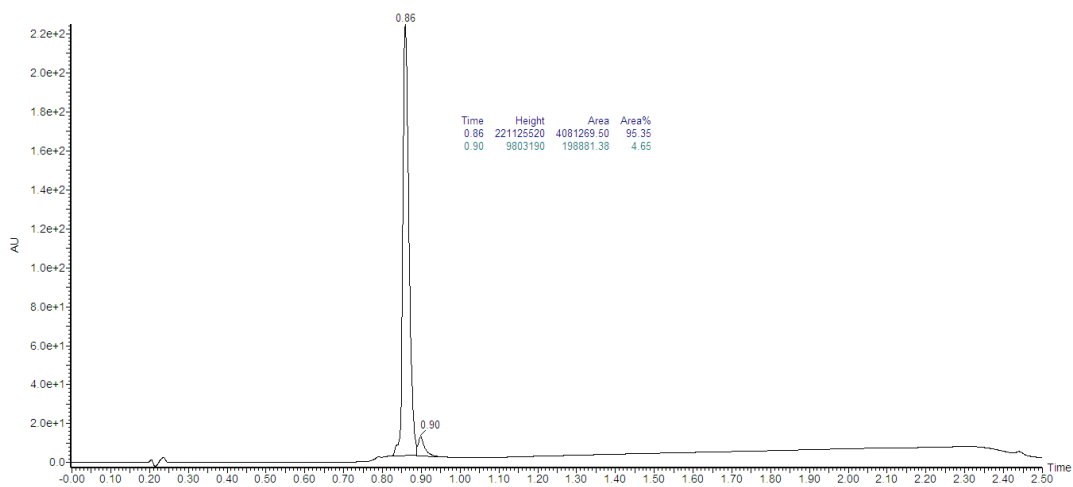
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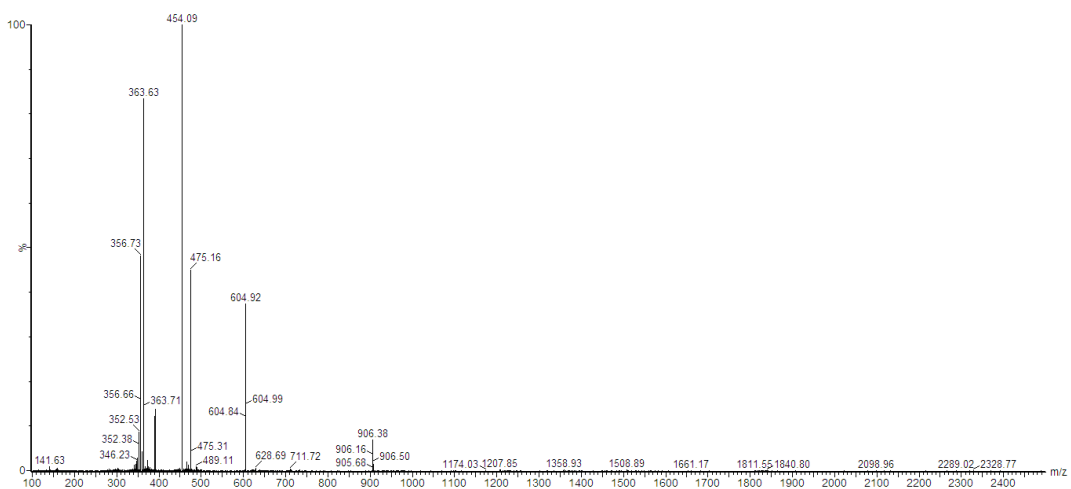
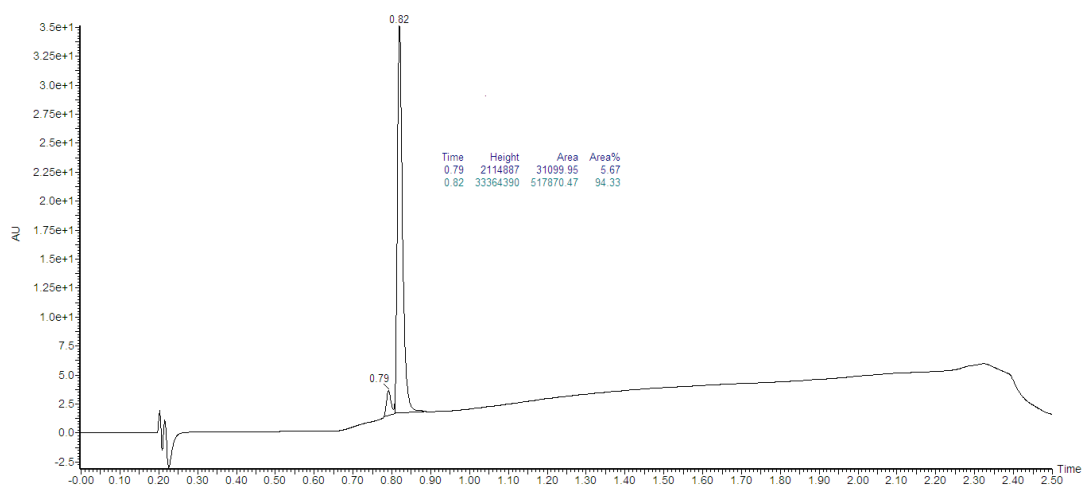
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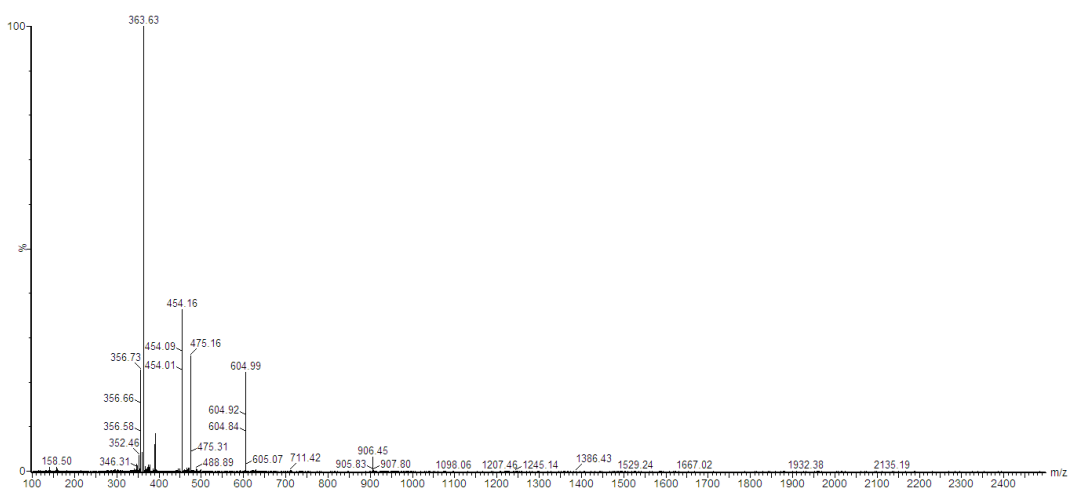
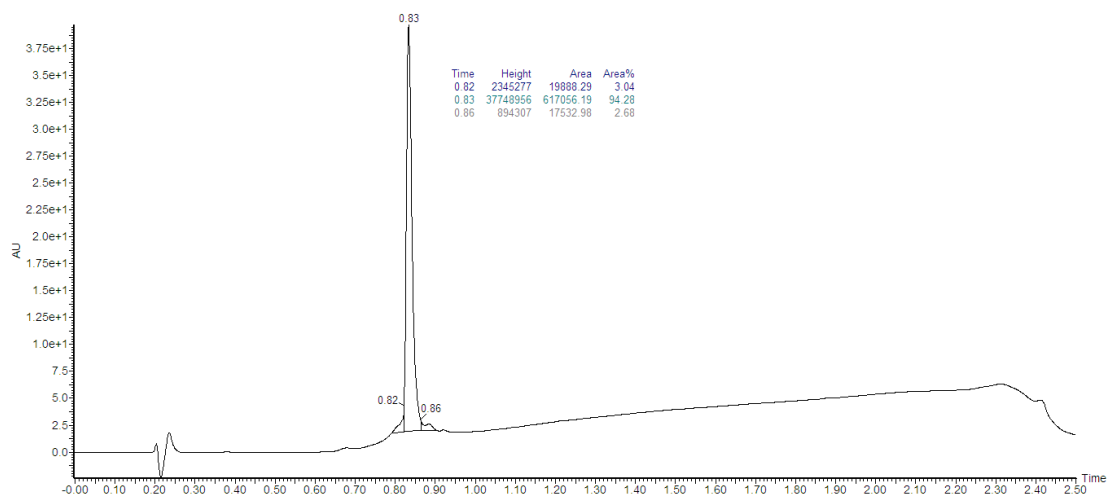
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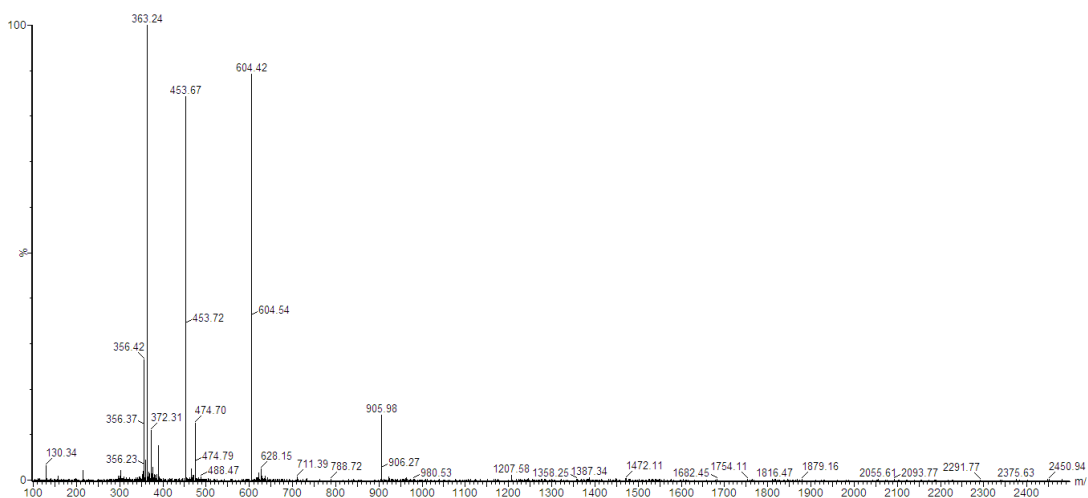
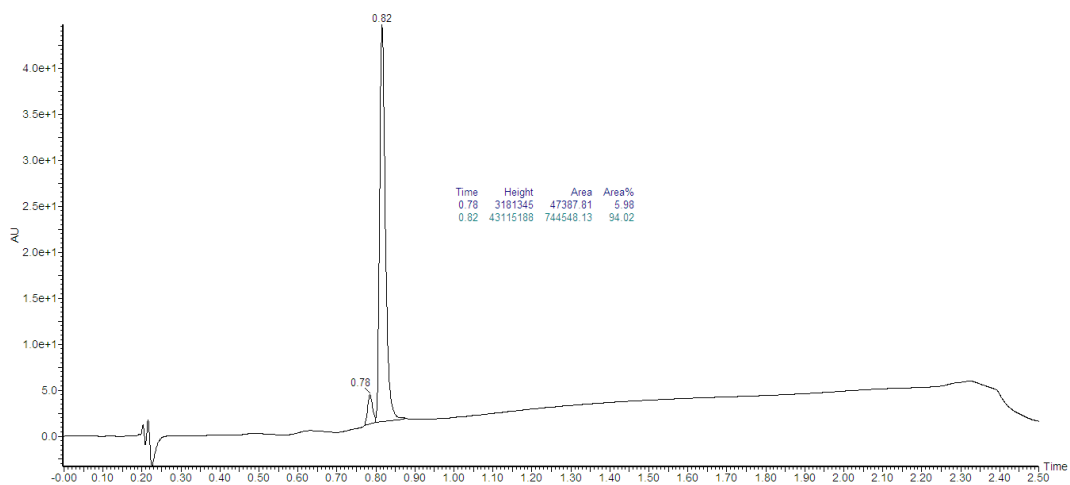
- Cyc-R<sub>3</sub>-R<sub>4</sub>



- Cyc-R<sub>4</sub>-R<sub>3</sub>



- Cyc-R<sub>5</sub>-R<sub>2</sub>





- **Cyc-R<sub>6</sub>-R<sub>1</sub>**

