

## Supplementary information

### **Purification and biological activity of natural variants synthesized by tridecaptin M gene cluster and *in vitro* drug-kinetics of this antibiotic class**

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## Supplementary Tables

**Table S1** Mass spectrometric data of different variants of tridecaptin M

Peptide variant	Molecular formula [M+H] <sup>+</sup>	Observed molecular weight [M+H] <sup>+</sup>	Calculated monoisotopic mass [M+H] <sup>+</sup>	Error (ppm)
M <sub>1</sub> (or M)	C <sub>68</sub> H <sub>114</sub> N <sub>17</sub> O <sub>20</sub>	1488.8246	1488.8426	18.0
M <sub>2</sub>	C <sub>66</sub> H <sub>110</sub> N <sub>17</sub> O <sub>20</sub>	1460.8090	1460.8113	2.0
M <sub>3</sub>	ND	1482.6736	ND	ND
M <sub>4</sub>	ND	1460.6866	ND	ND
M <sub>5</sub>	C <sub>65</sub> H <sub>111</sub> N <sub>16</sub> O <sub>20</sub>	1435.8076	1435.8160	8.0
M <sub>6</sub>	C <sub>67</sub> H <sub>112</sub> N <sub>17</sub> O <sub>20</sub>	1474.7987	1474.8269	28.0
M <sub>7</sub>	C <sub>67</sub> H <sub>112</sub> N <sub>17</sub> O <sub>20</sub>	1474.8167	1474.8269	10.0
M <sub>8</sub>	C <sub>66</sub> H <sub>113</sub> N <sub>16</sub> O <sub>20</sub>	1449.7800	1449.8317	51.0
M <sub>9</sub>	ND	1607.7673	ND	ND
M <sub>10</sub>	ND	1563.8605	ND	ND
M <sub>11</sub>	ND	1569.8368	ND	ND

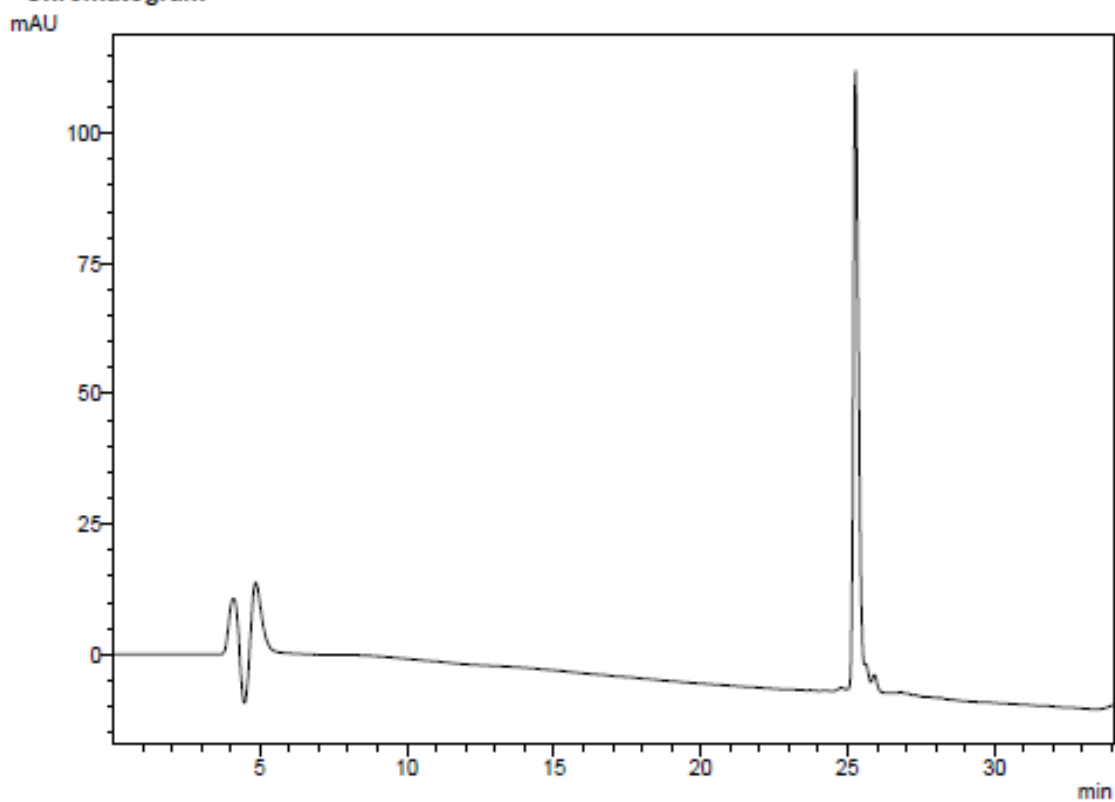
**Table S2** Synergistic activity of M<sub>11</sub> with efflux pump inhibitor, CCCP

Strains	Intrinsic Activity; MIC (μg/ml)		MIC (μg/ml) of Tri-M <sub>11</sub> in the presence of CCCP		FICI	
	Tri-M <sub>11</sub>	CCCP	1/4 <sup>th</sup> MIC	1/8 <sup>th</sup> MIC	1/4 <sup>th</sup> MIC	1/8 <sup>th</sup> MIC
<i>Acinetobacter baumannii</i> ATCC 19606	4	64	1	2	0.5	0.625
<i>Klebsiella pneumoniae</i> GMCH 13	4	32	2	2	0.75	0.625
<i>Klebsiella pneumoniae</i> GMCH 15	2	64	2	2	-	-

## Supplementary Figures

Sample Name : M152-P3 actual 32ug/ml (XBridge C18 1 ml/min)  
Sample ID : 10-30%ACN/Water 0.075% TFA  
Vial# : 157  
Injection Volume : 50 uL  
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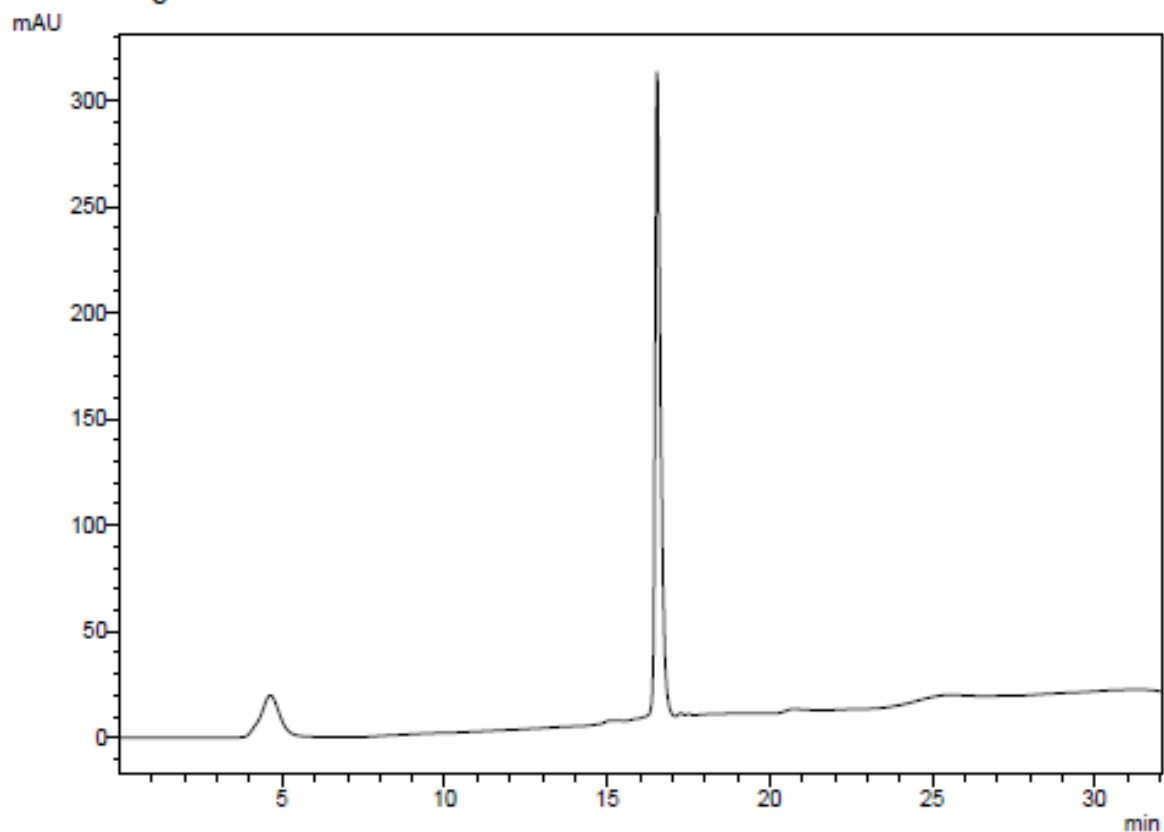
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**Figure S1** HPLC chromatogram of purified M<sub>1</sub> (The originally described major product of tridecaptin M). The peaks at 5 min in this chromatogram or other HPLC profiles belong to solvent.

Sample Name : M152-P1 pure (XBridgeC18, 1.0 ml/min)  
Sample ID : 10-35%ACN/20%ACN0.075%TFA  
Vial# : 151  
Injection Volume : 30 uL  
Data Filename : Sample\_322.lcd  
Method Filename : manoj.lcm  
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**Figure S2** HPLC chromatogram of purified M<sub>2</sub>

Sample Name : M152-F3(1) pure (XBridgeC18, 1.0 ml/min)  
Sample ID : 10-35%ACN/20%ACN0.075%TFA  
Vial# : 151  
Injection Volume : 30 uL  
Data Filename : Sample\_323.lcd  
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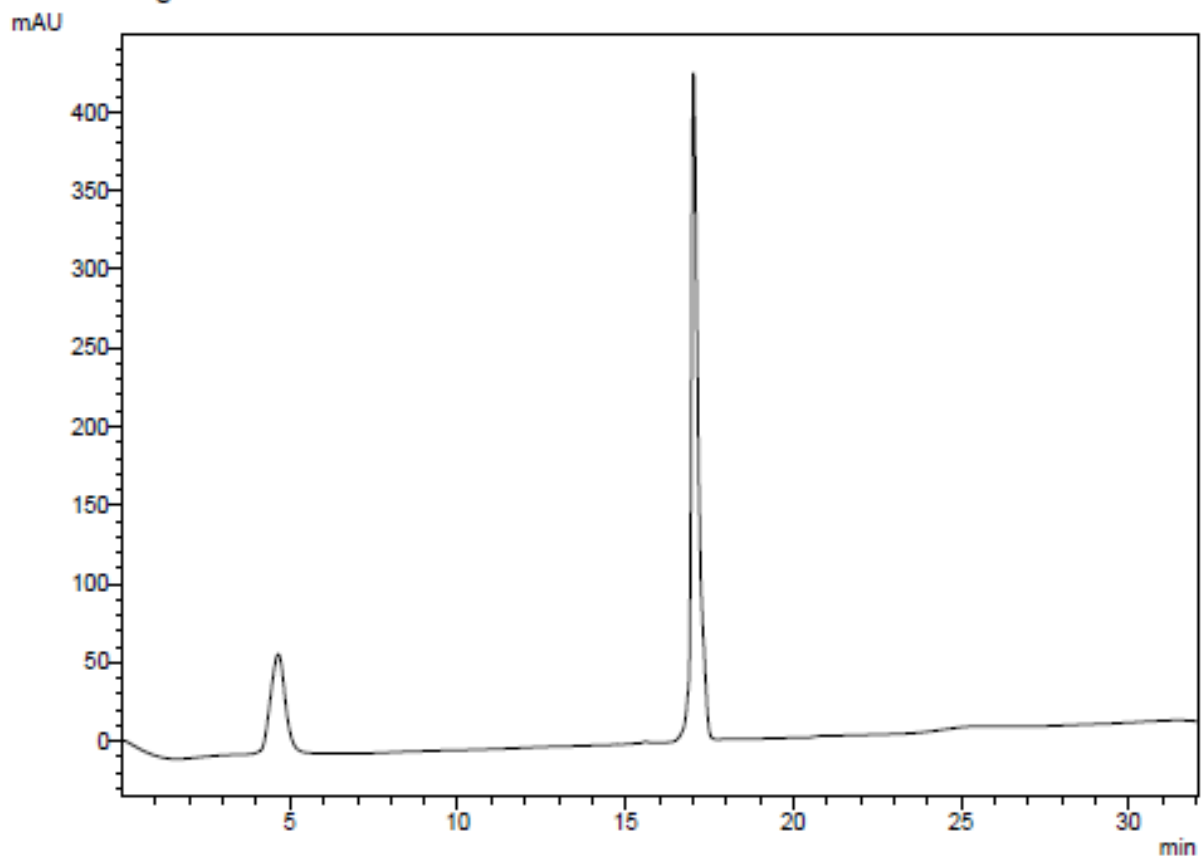
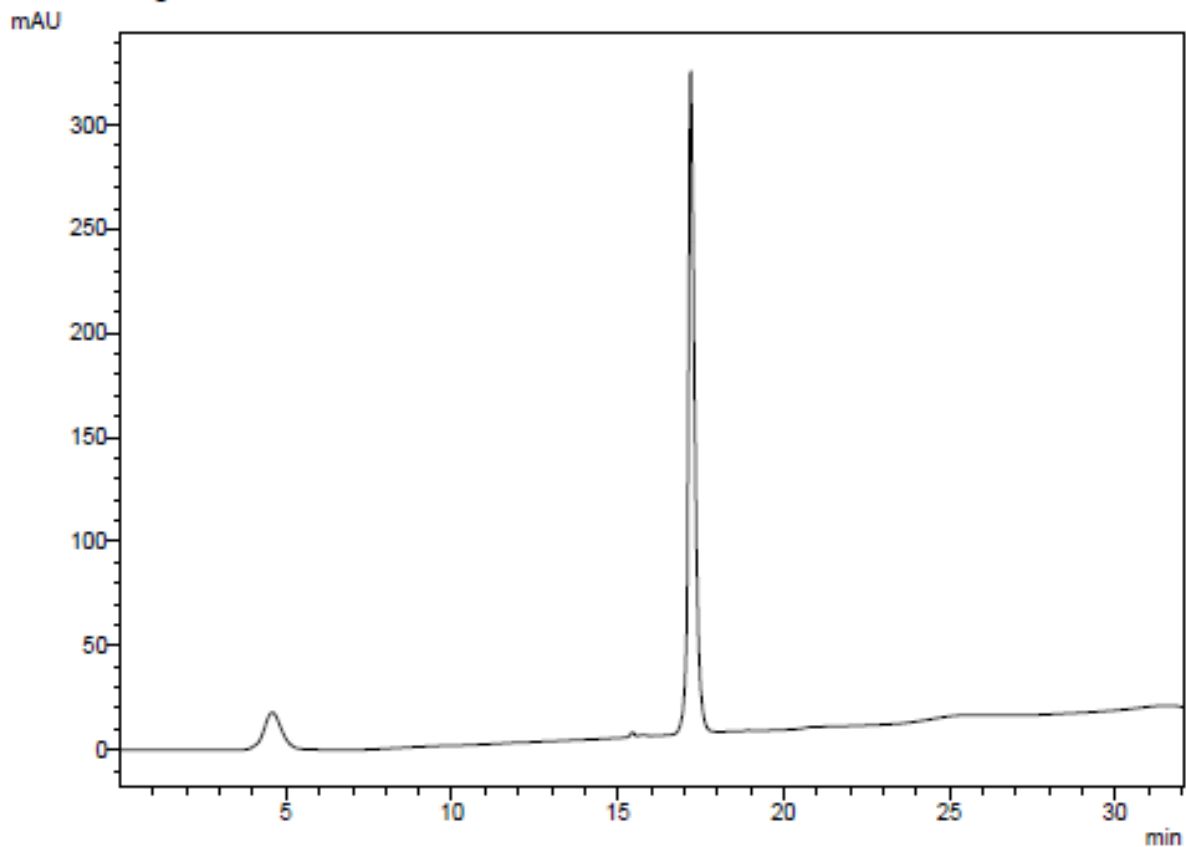


Figure S3 HPLC chromatogram of purified M<sub>3</sub>

Sample Name : M152-F3(2) pure (XBridgeC18, 1.0 ml/min)  
Sample ID : 10-35%ACN/20%ACN0.075%TFA  
Vial# : 151  
Injection Volume : 30 uL  
Data Filename : Sample\_324.lcd  
Method Filename : manoj.lcm  
Batch Filename :  
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**Figure S4** HPLC chromatogram of purified M<sub>4</sub>

Sample Name : M152-F4 pure (XBridgeC18, 1.0 ml/min)  
Sample ID : 10-35%ACN/20%ACN0.075%TFA  
Vail# : 151  
Injection Volume : 30 uL  
Data Filename : Sample\_325.lcd  
Method Filename : manoj.lcm  
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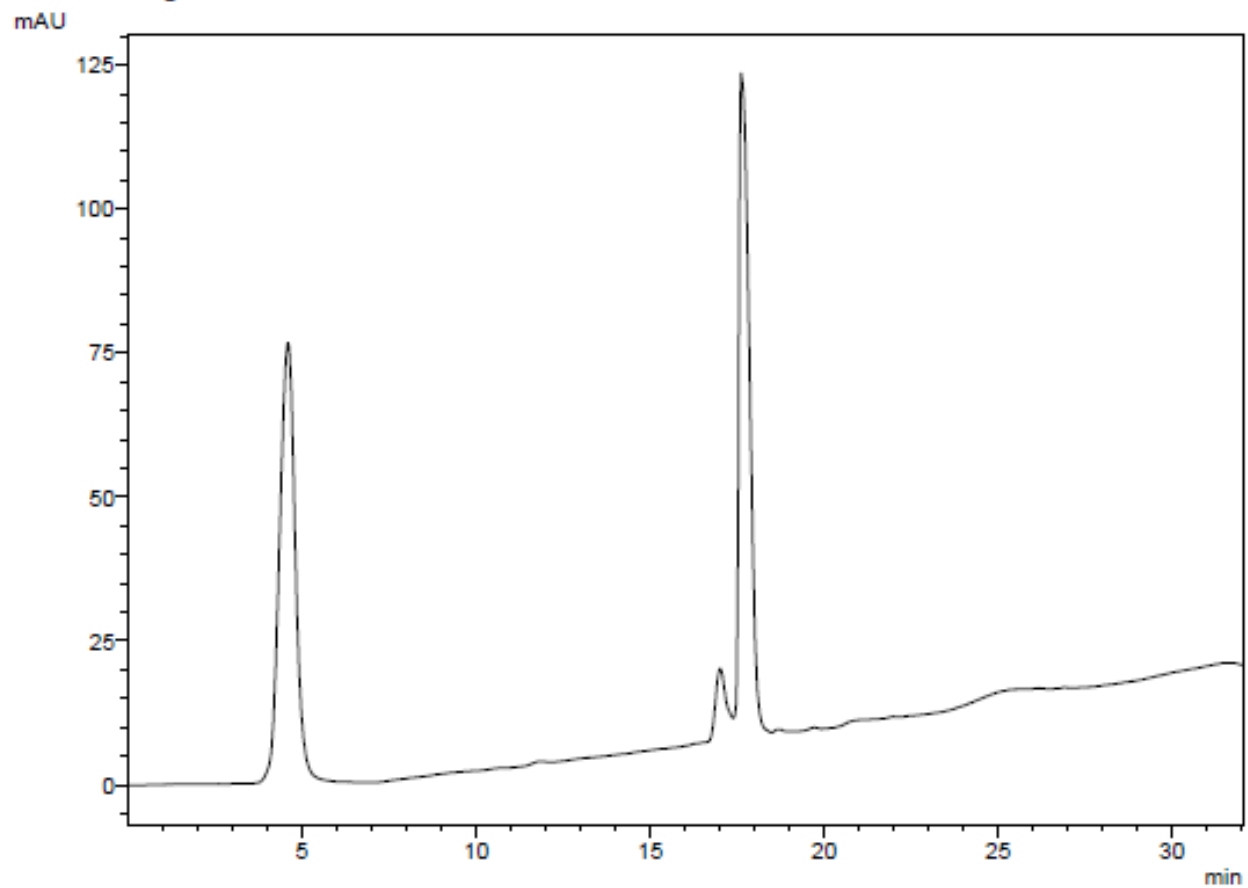
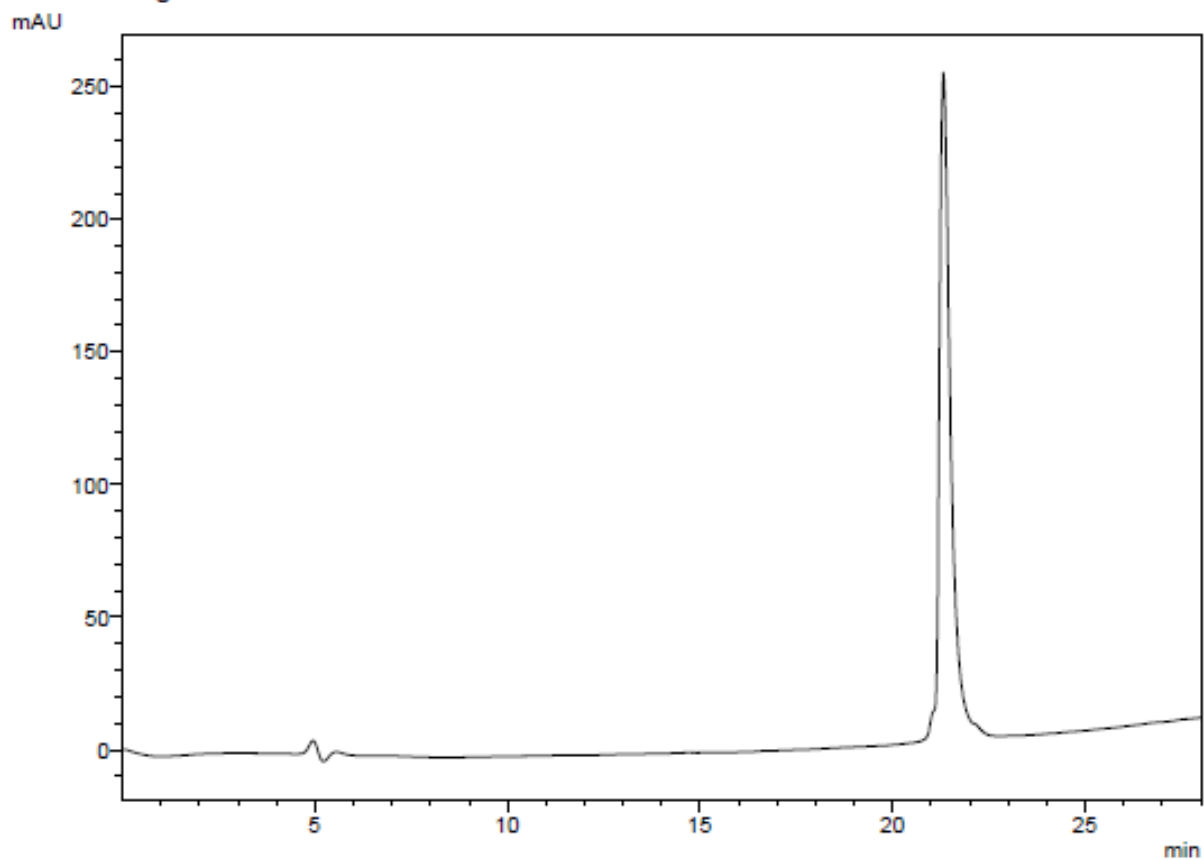


Figure S5 HPLC chromatogram of purified M<sub>5</sub>

Sample Name : P5-2 M152-P3 (Phenomenex C4 1.0ml/min)  
Sample ID : 3-35%ACN/Water 0.075%TFA  
Vail# : 153  
Injection Volume : 100 uL  
Data Filename : Sample\_195.lcd  
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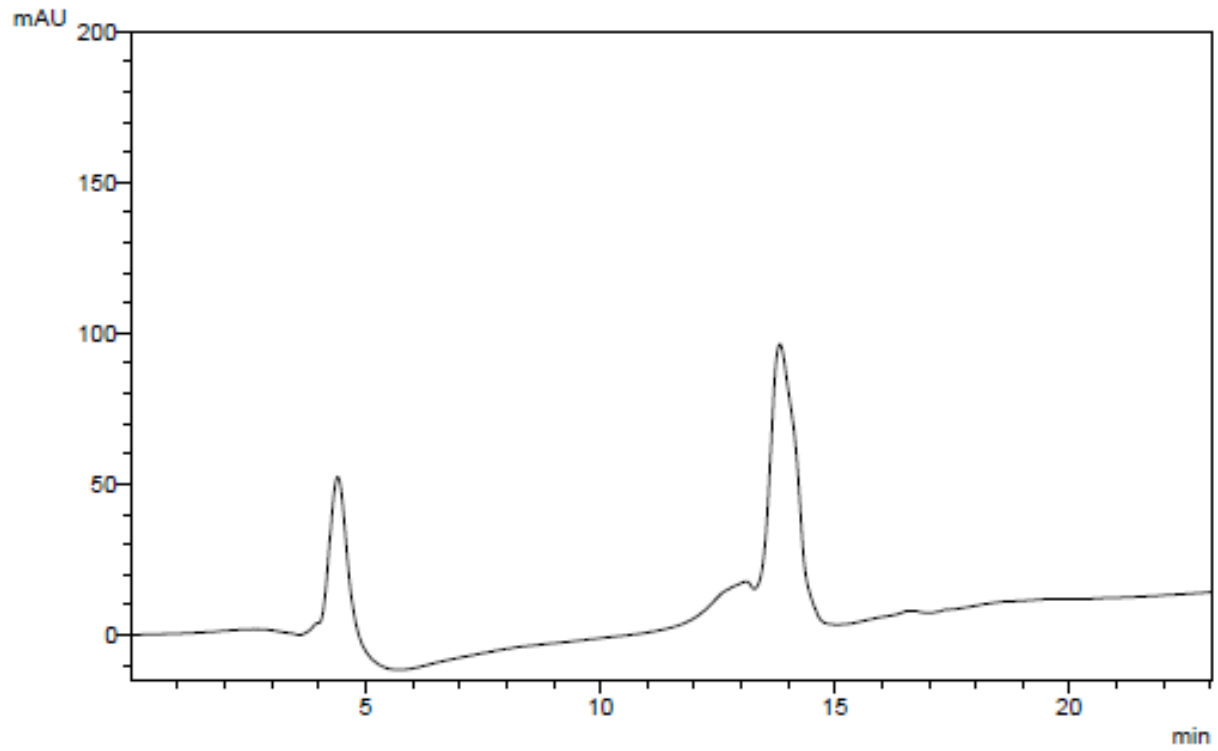


**Figure S6** HPLC chromatogram of purified M<sub>6</sub>



Sample Name : M152-P8 pure (Xbridge C18 1.0ml/min)  
Sample ID : 10-28%ACN/water 0.075%TFA  
Vail# : 151  
Injection Volume : 30 uL  
Data Filename : Sample\_233.Jcd  
Method Filename : manoj.lcm  
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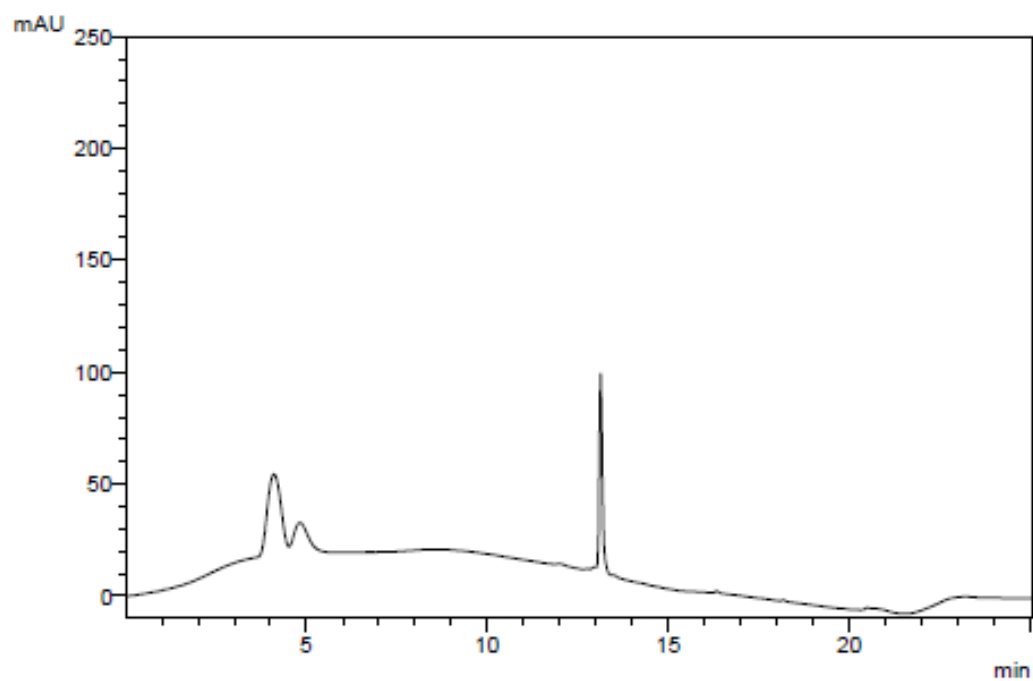
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**Figure S7** HPLC chromatogram of purified M<sub>7</sub>

Sample Name : P7  
Sample ID : Unknown conc  
Vail# : 152  
Injection Volume : 30 uL  
Data Filename : Sample\_116.lcd  
Method Filename : manoj.lcm  
Batch Filename : Manoj Q.lcb  
Date Acquired : 3/15/2019 9:24:43 PM  
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**Figure S8** HPLC chromatogram of purified M<sub>8</sub>

Sample Name : M152-P10 pure (XBridgeC18, 1.0 ml/min)  
Sample ID : 10-35%ACN/20%ACN0.075%TFA  
Vail# : 151  
Injection Volume : 30 uL  
Data Filename : Sample\_326.lcd  
Method Filename : manoj.lcm  
Batch Filename :  
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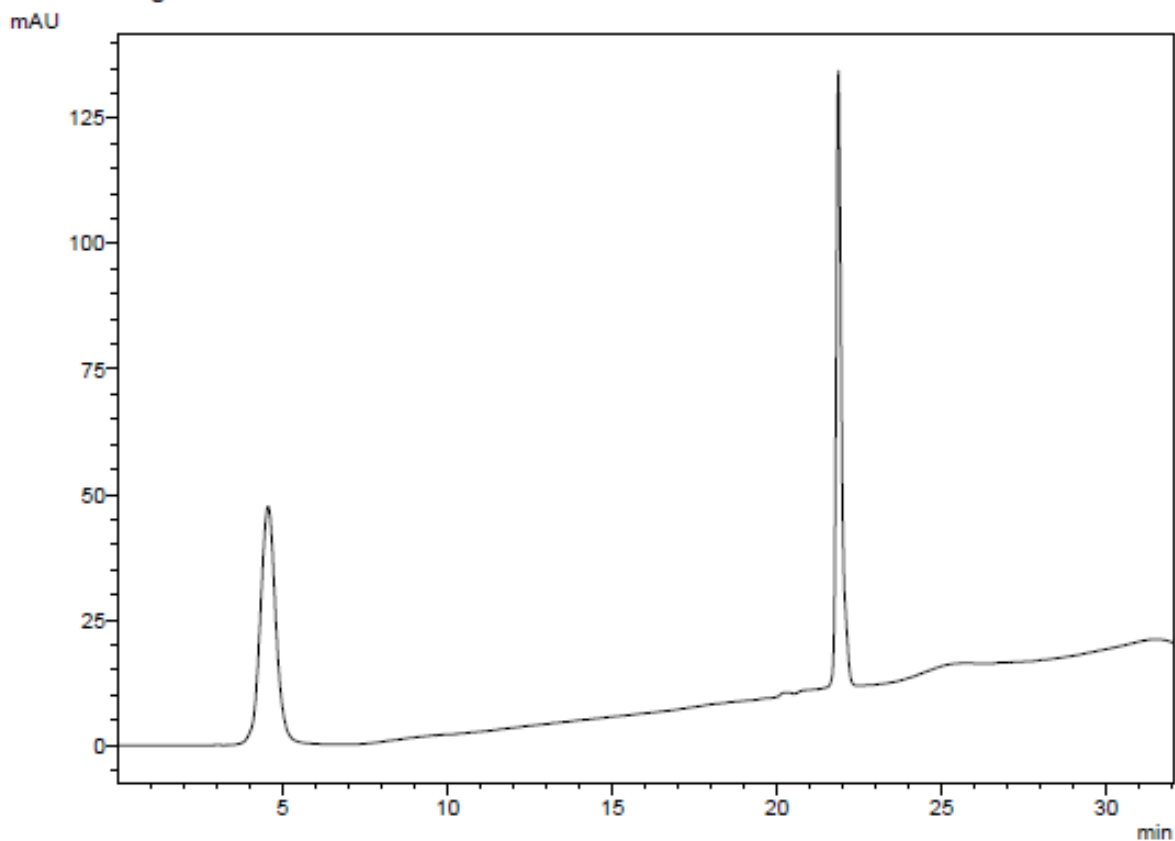
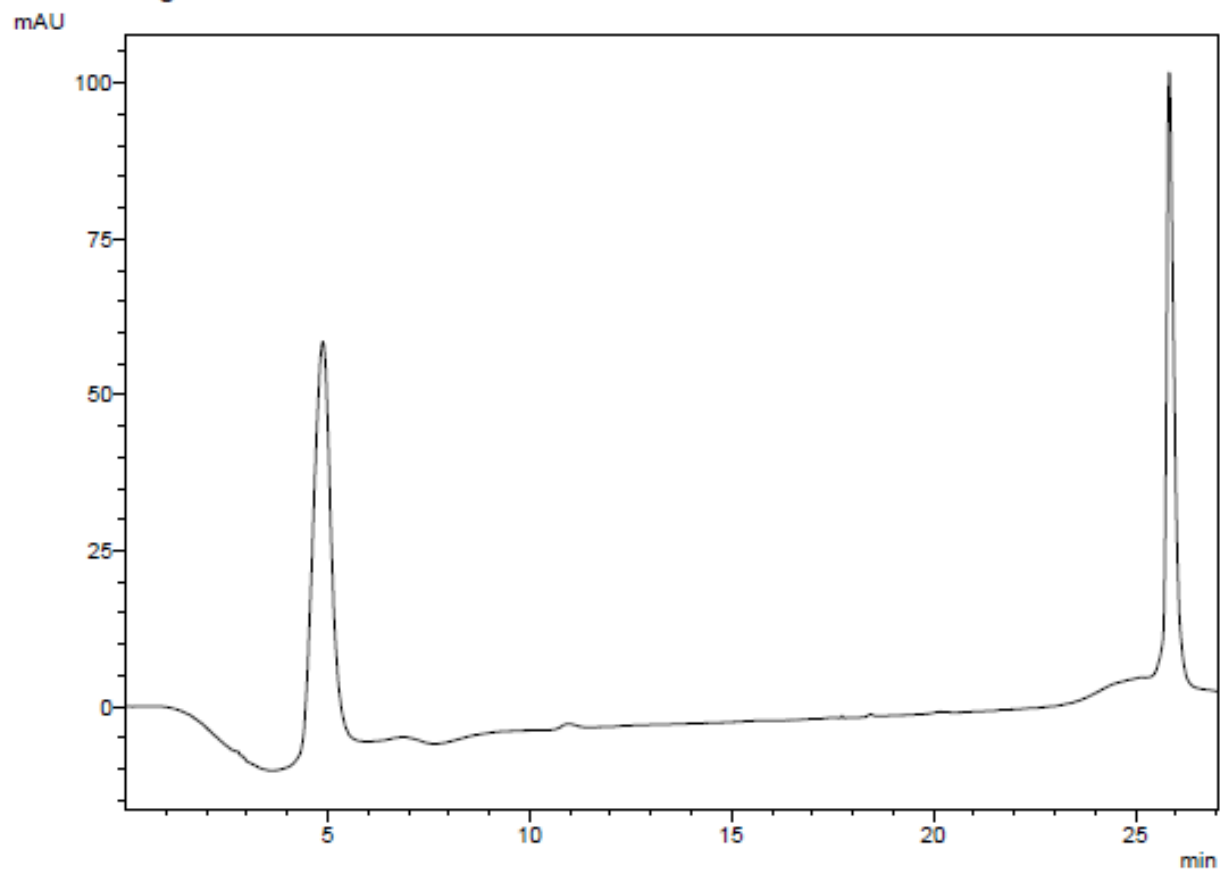


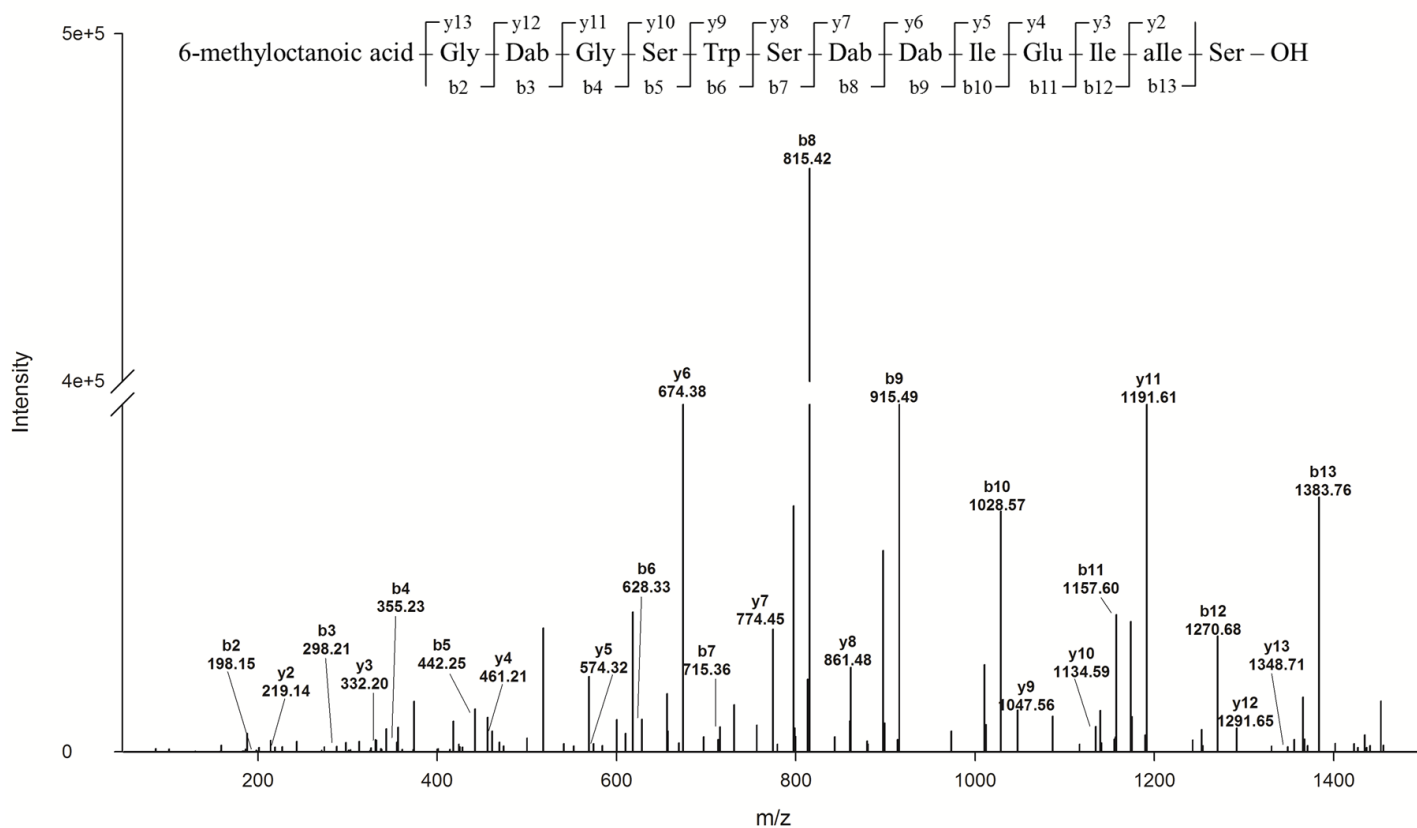
Figure S9 HPLC chromatogram of purified M<sub>10</sub>

Sample Name : M152-P11 pure (XBridgeC18, 1.0 ml/min)  
Sample ID : 10-35%ACN/20%ACN0.075%TFA  
Vial# : 151  
Injection Volume : 30 uL  
Data Filename : Sample\_388.lcd  
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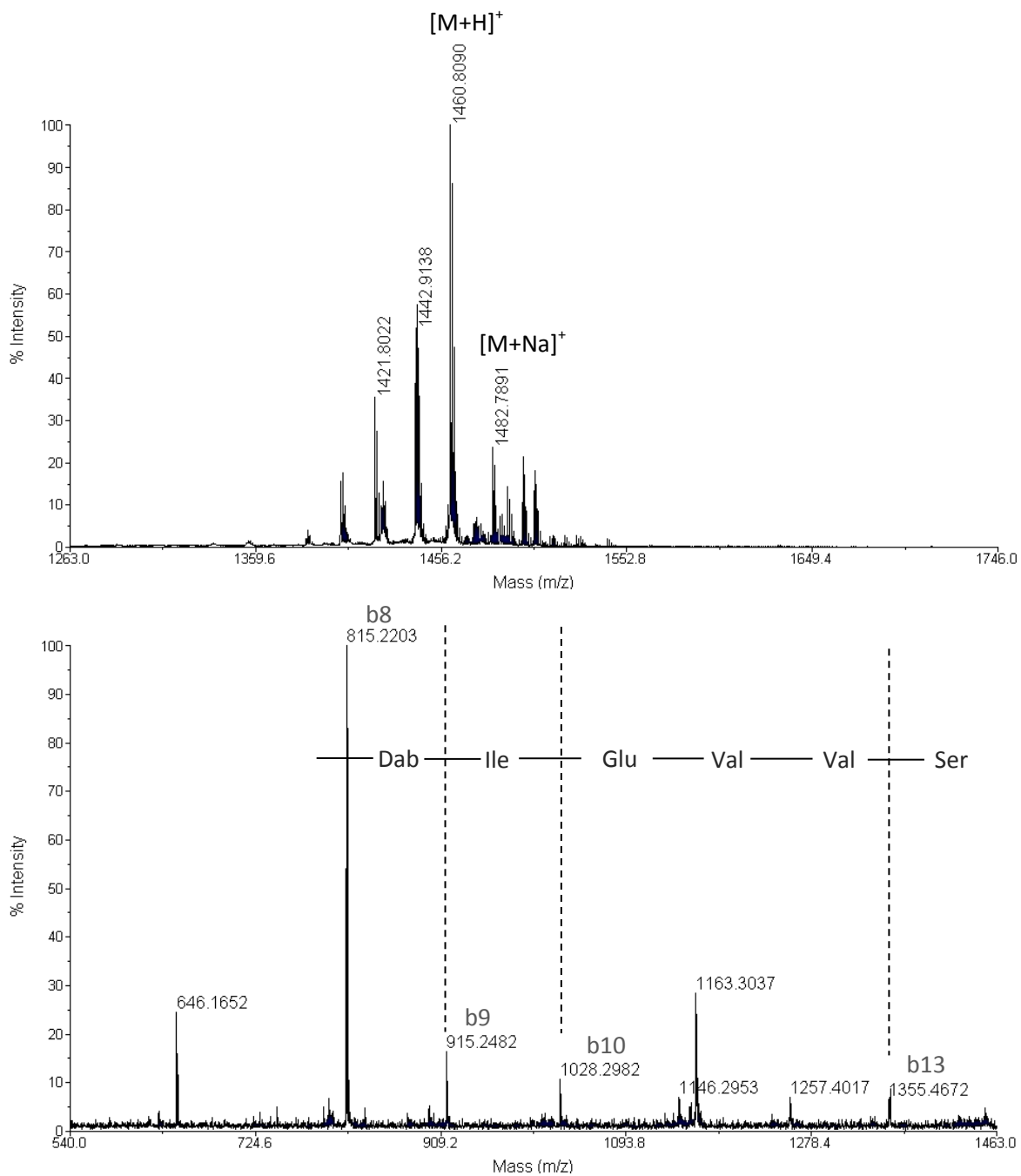
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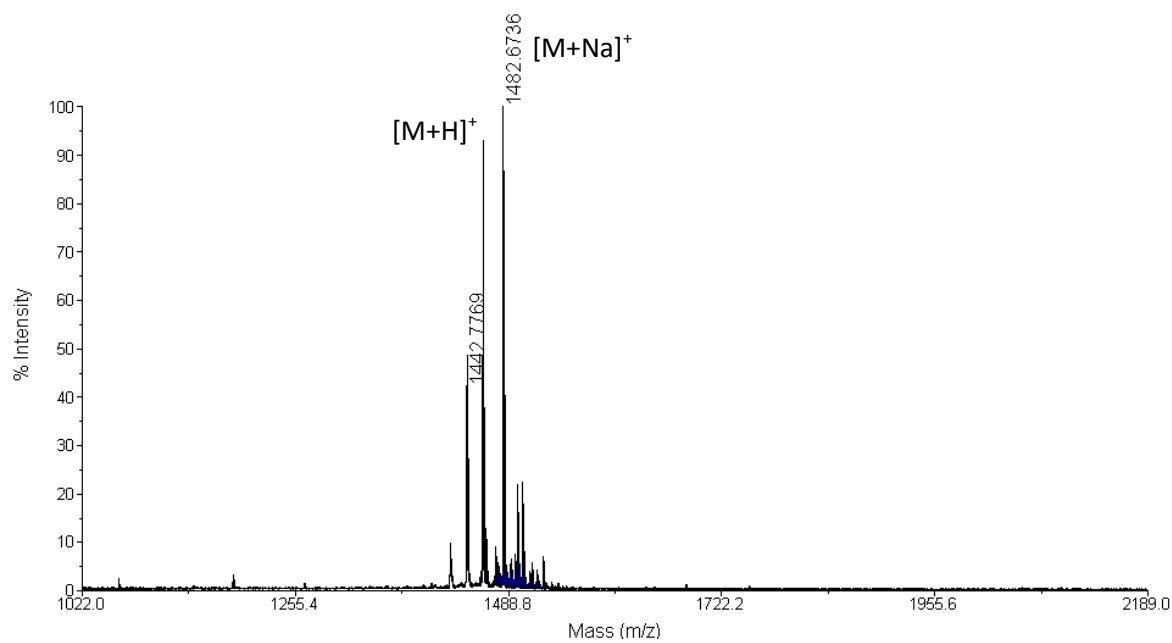
**Figure S10** HPLC chromatogram of purified M<sub>11</sub>



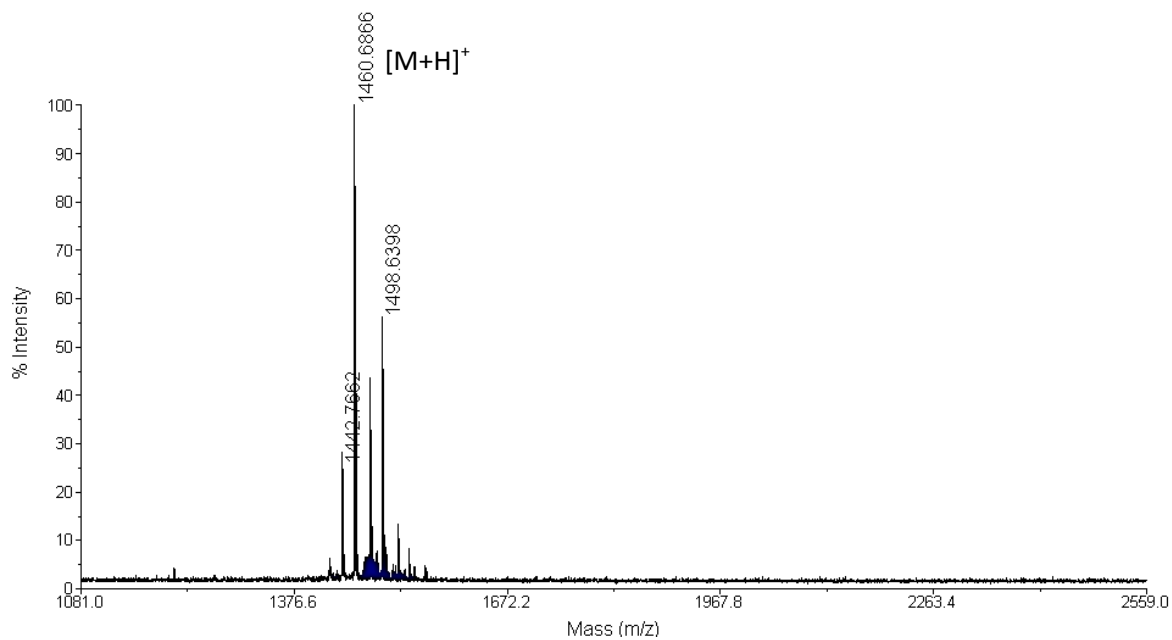
**Figure S11** Tandem MS spectroscopic data of tridecaptin M (or M<sub>1</sub>). This data is already published by our group in original research paper on tridecaptin M<sup>1</sup>. The MS/MS data of rest of the variants was analyzed relative to this spectrum.



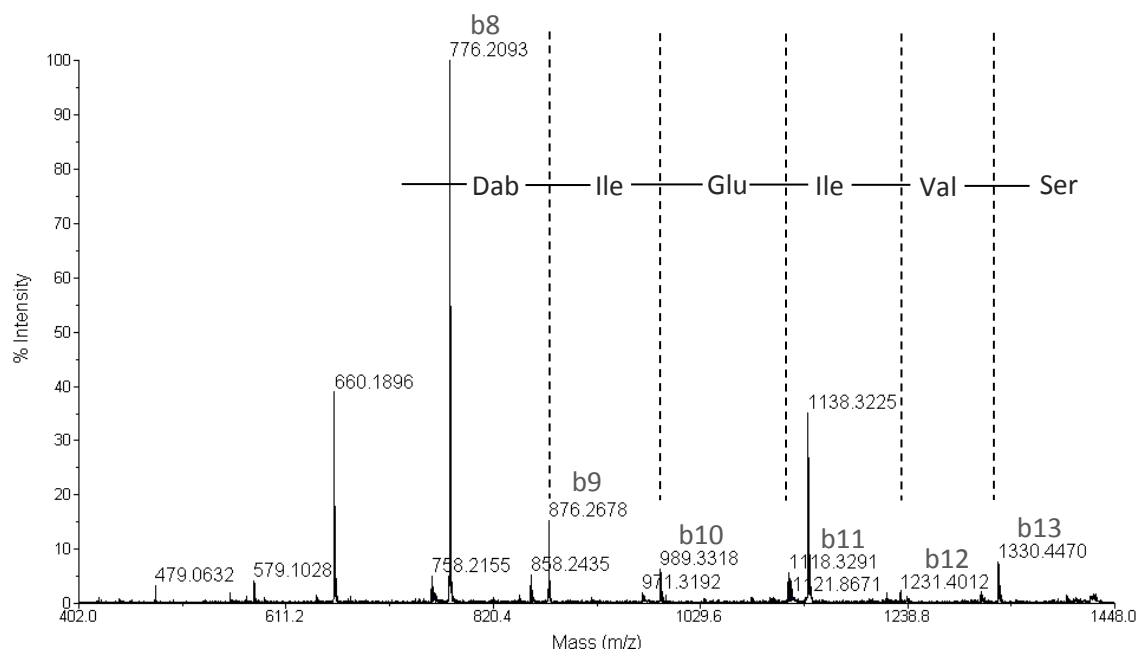
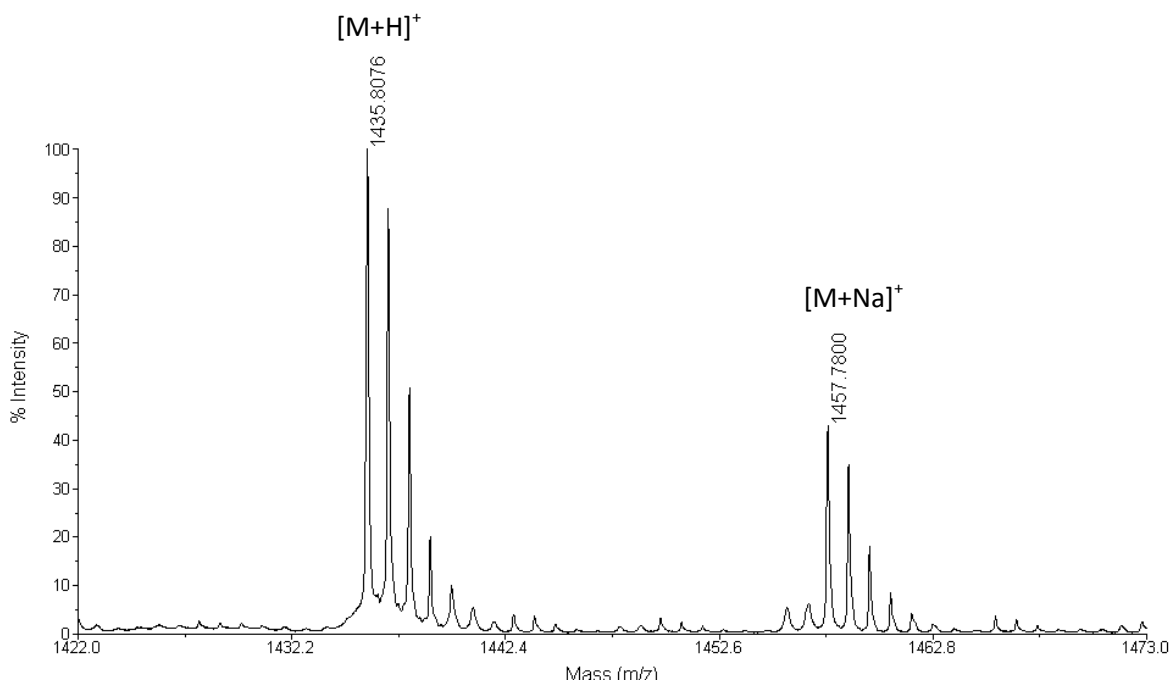
**Figure S12** Mass spectroscopic data of M<sub>2</sub>. Top, MS data; bottom, MS/MS spectrum



**Figure S13** Mass spectroscopic data of  $M_3$

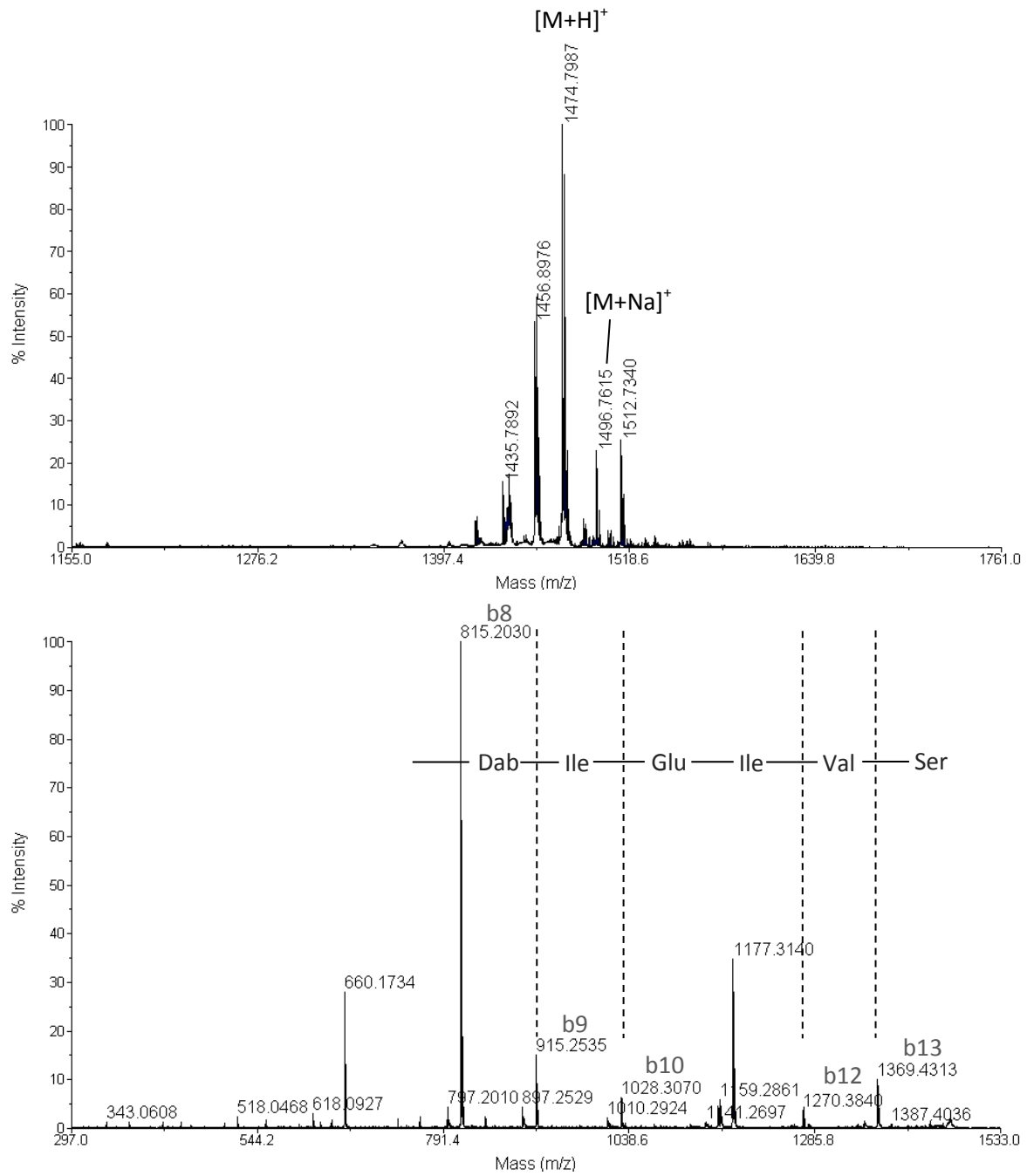


**Figure S14** Mass spectroscopic data of  $M_4$

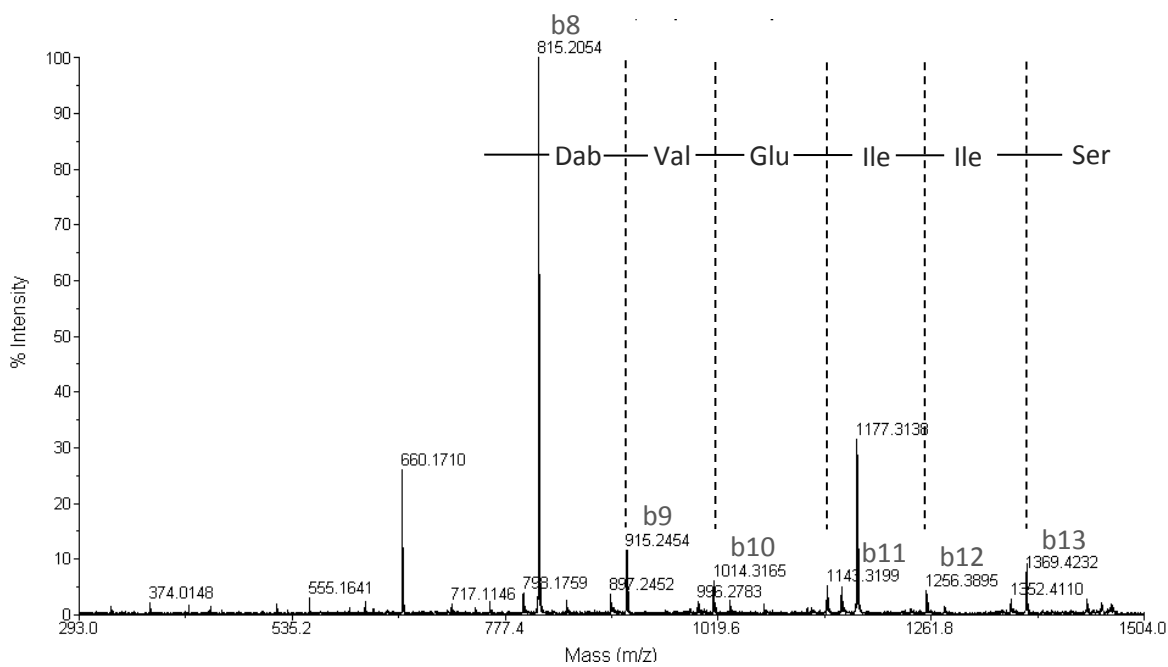
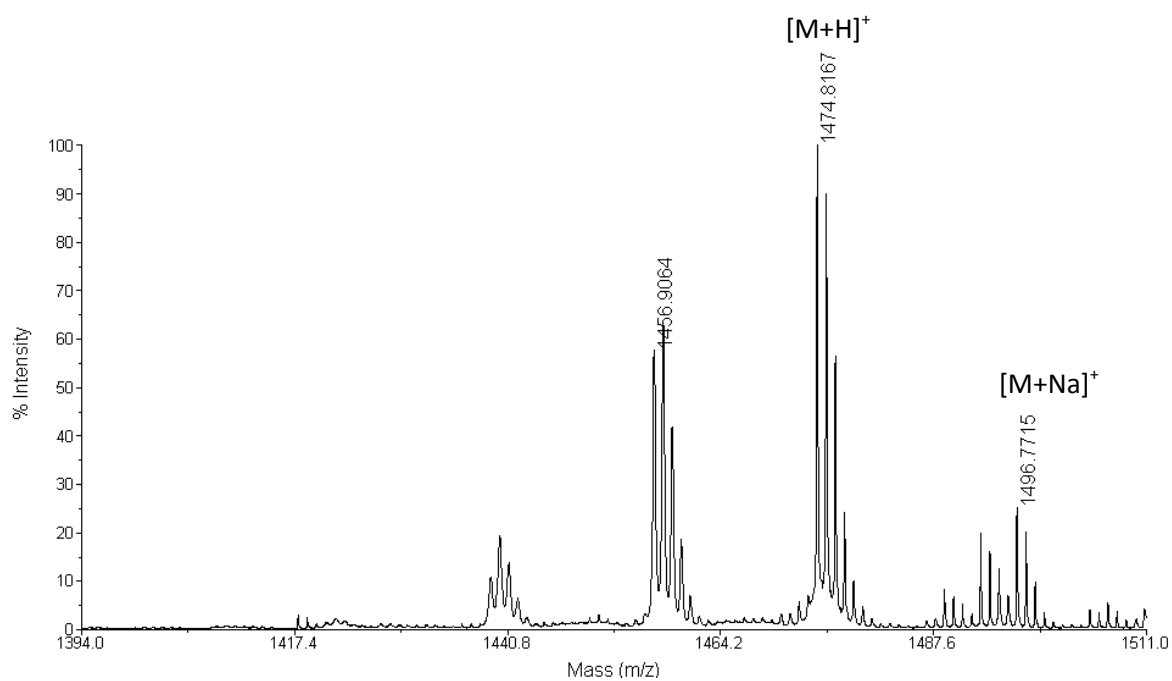


**Figure S15** Mass spectroscopic data of M<sub>5</sub>. Top, MS data; bottom, MS/MS spectrum

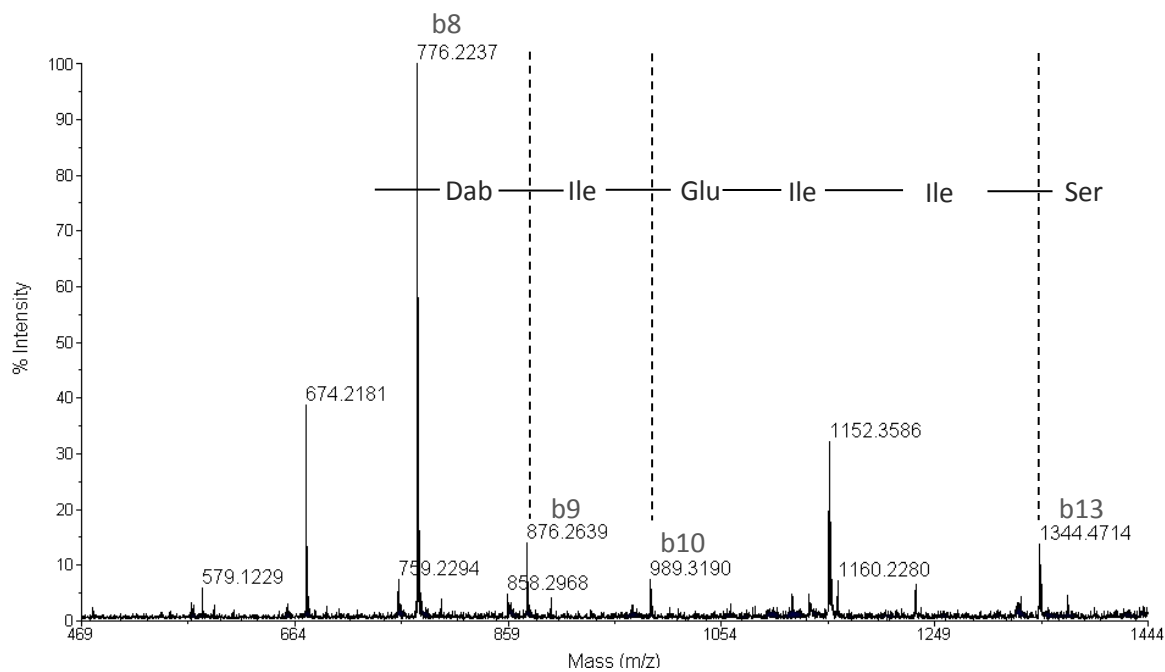
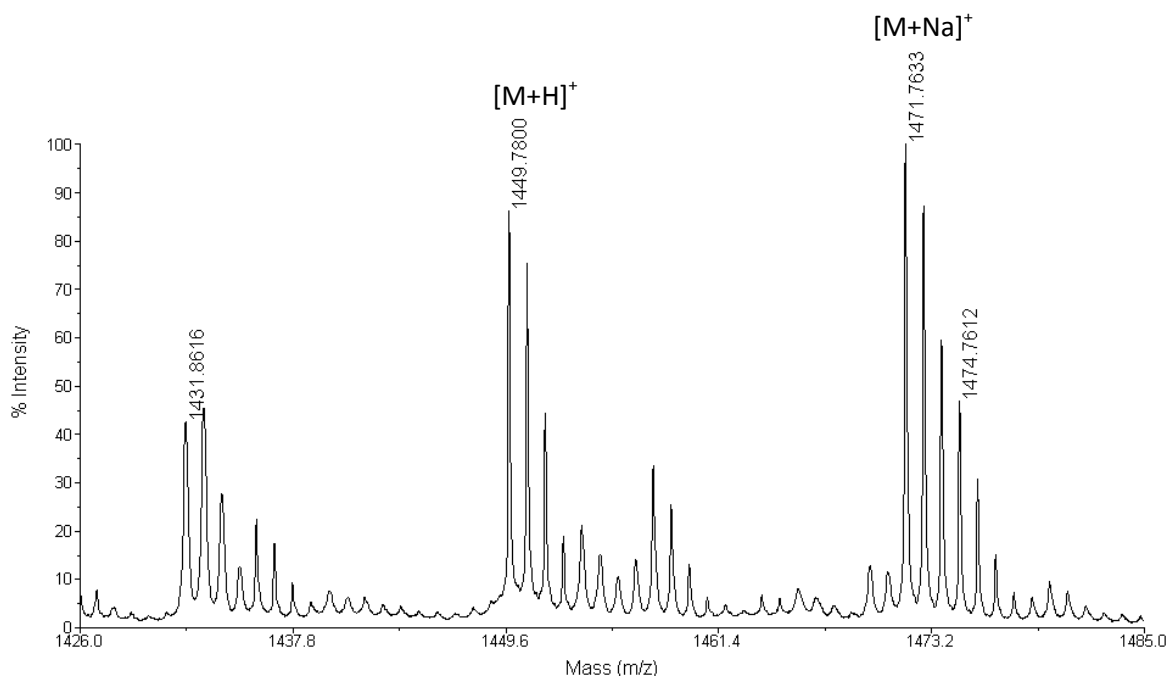




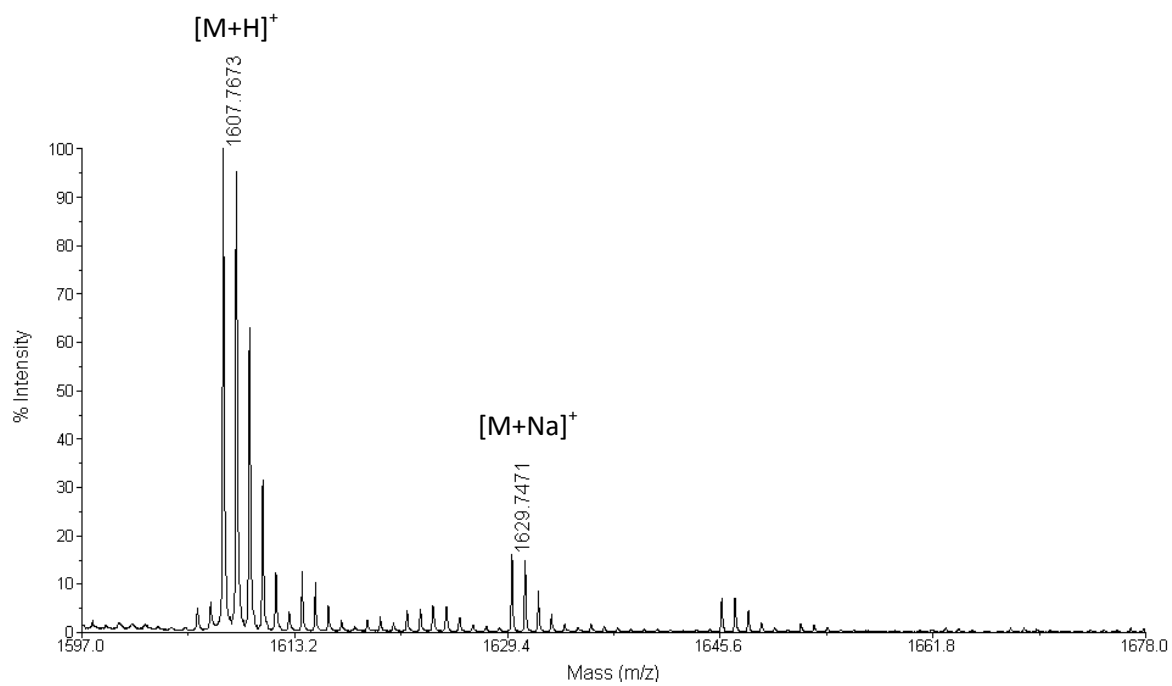
**Figure S16** Mass spectroscopic data of  $M_6$ . Top, MS data; bottom, MS/MS spectrum



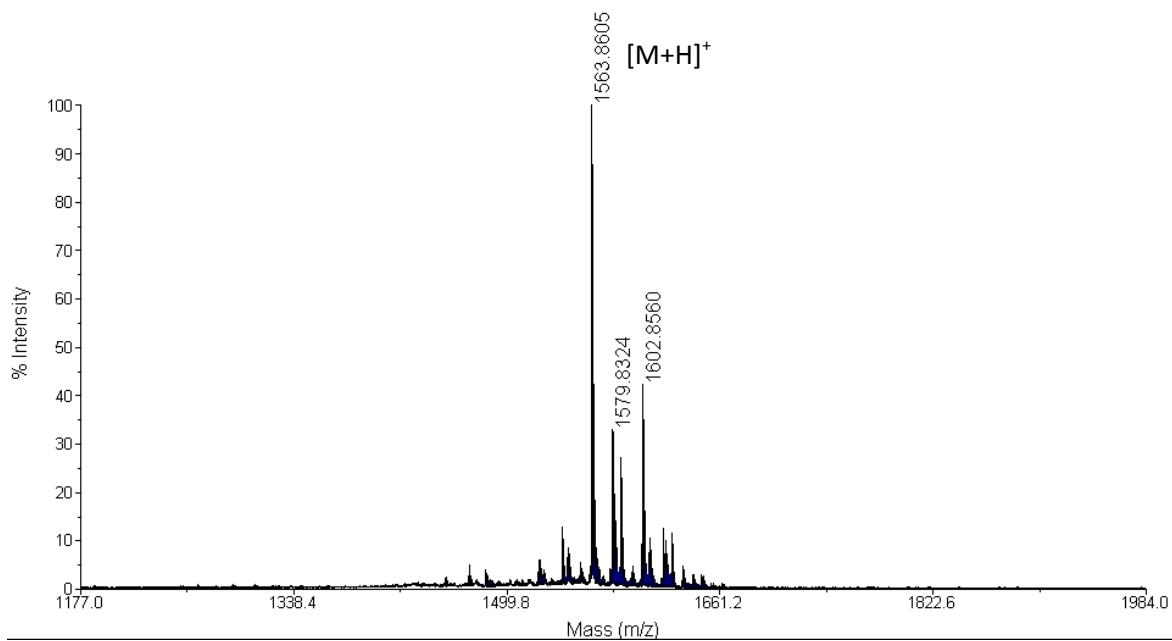
**Figure S17** Mass spectroscopic data of M<sub>7</sub>. Top, MS data; bottom, MS/MS spectrum



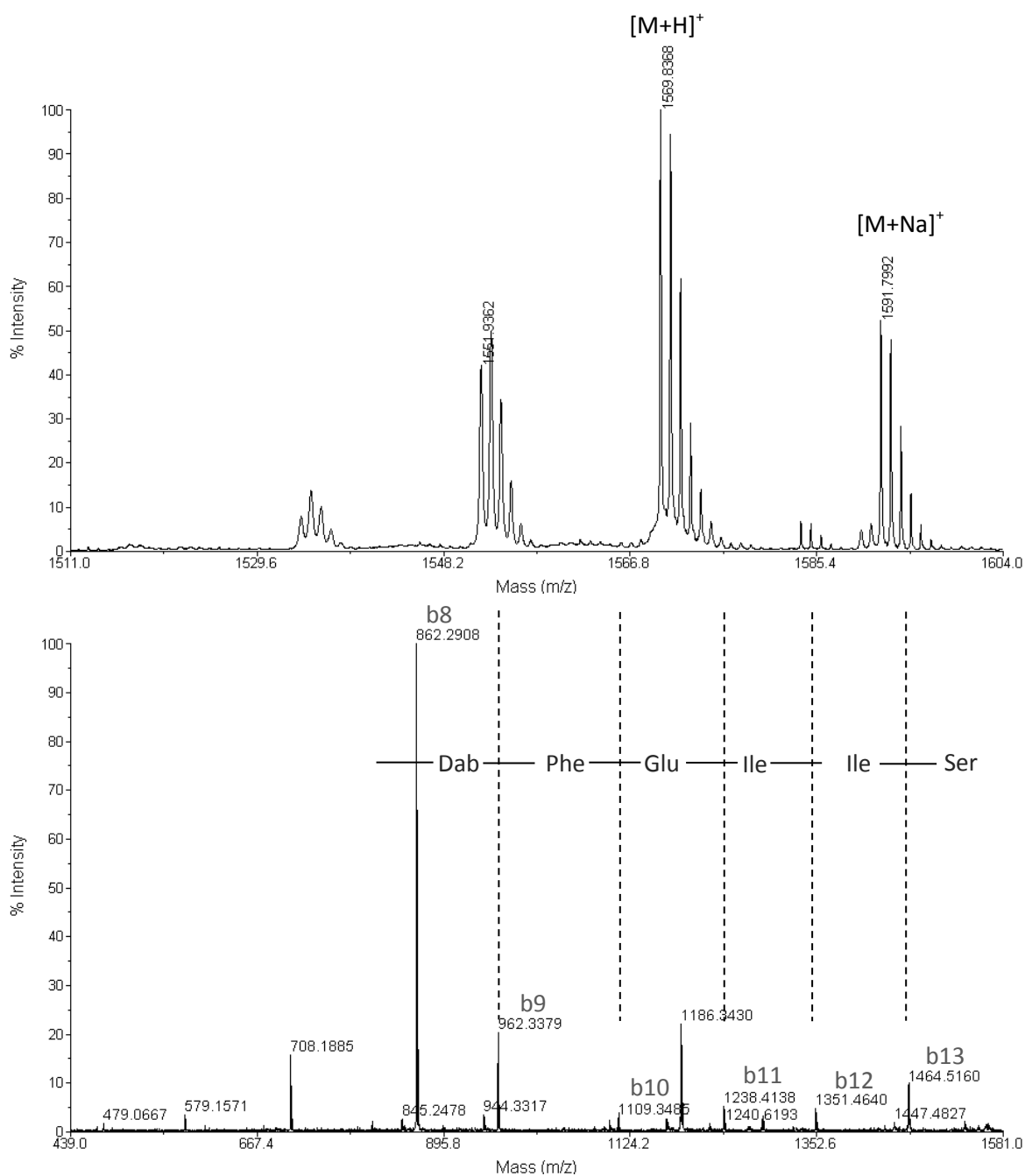
**Figure S18** Mass spectroscopic data of  $M_8$ . Top, MS data; bottom, MS/MS spectrum



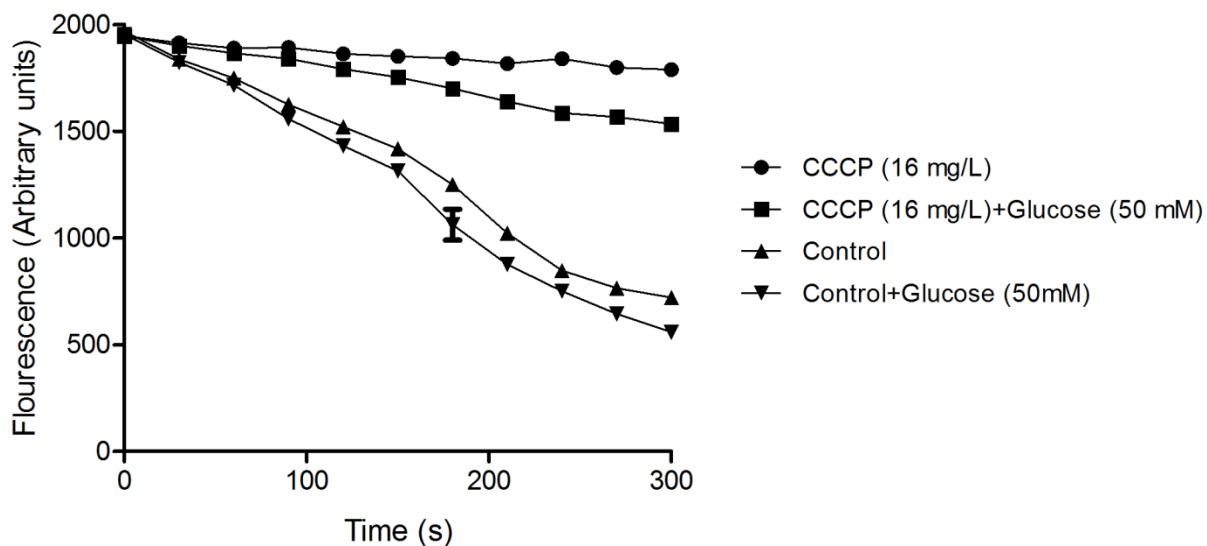
**Figure S19** Mass spectroscopic data of  $M_9$



**Figure S20** Mass spectroscopic data of  $M_{10}$



**Figure S21** Mass spectroscopic data of  $M_{11}$ . Top, MS data; bottom, MS/MS spectrum



**Figure S22** Nile red efflux inhibition assay in *A. baumannii* ATCC 19606 to determine the presence of efflux pumps. *A. baumannii* was incubated with Nile red dye in the presence or absence of efflux pump inhibitor, CCCP, and the fluorescence of the intracellular dye was measured. Treated cells showed inhibition of efflux which indicates the presence of efflux pumps in the bacteria. Glucose re-energizes the efflux pumps and thereby reducing the activity of CCCP.

## References

- 1 Jangra, M. *et al.* Tridecaptin M, a new variant discovered in mud bacterium, shows activity against colistin-and extremely drug-resistant Enterobacteriaceae. *Antimicrob. Agents Chemother.* **63**, e00338-00319 (2019).