

## *Supplementary Materials*

# **Development of 6'-N-acylated isepamicin analogs with improved antibacterial activity against isepamicin-resistant pathogens**

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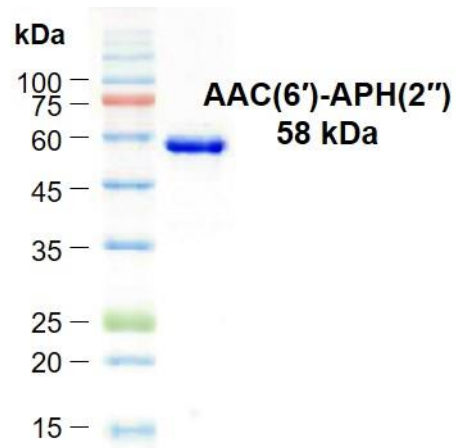
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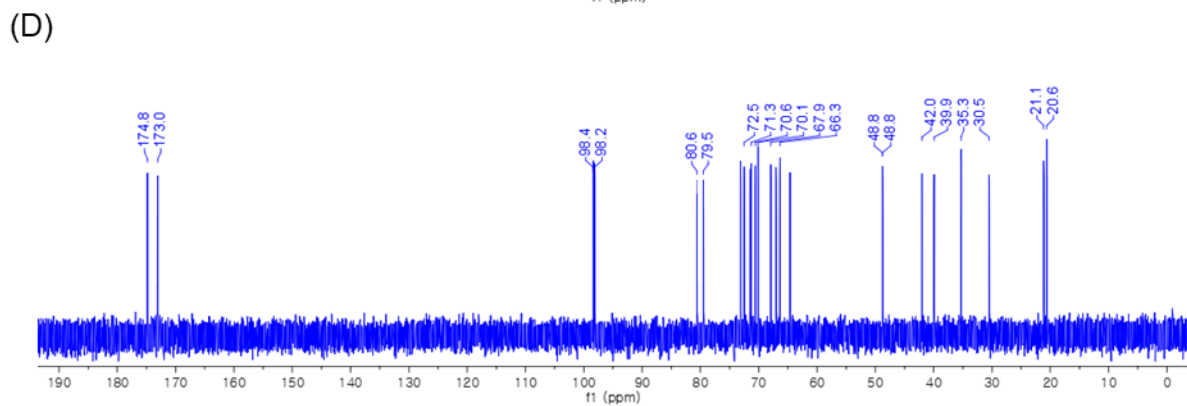
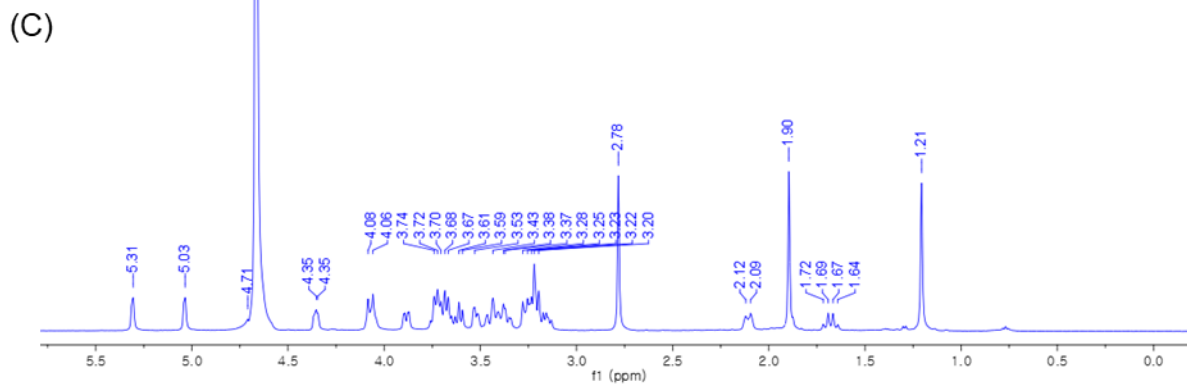
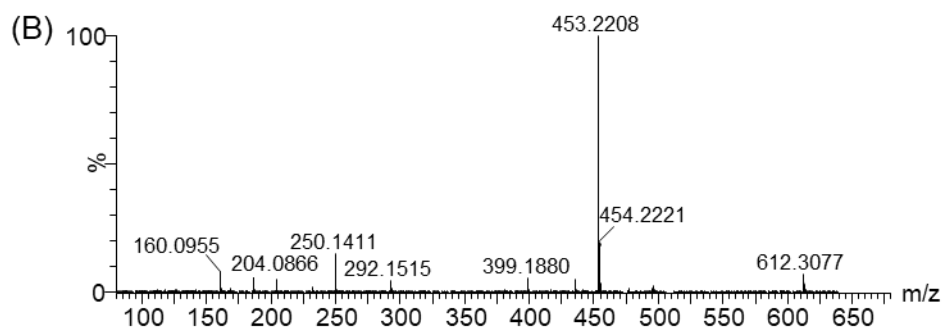
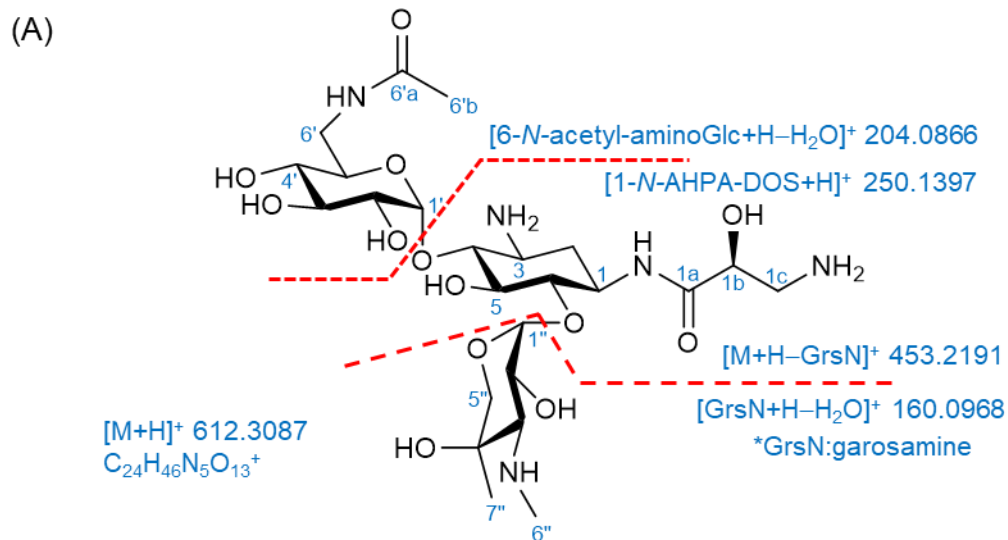
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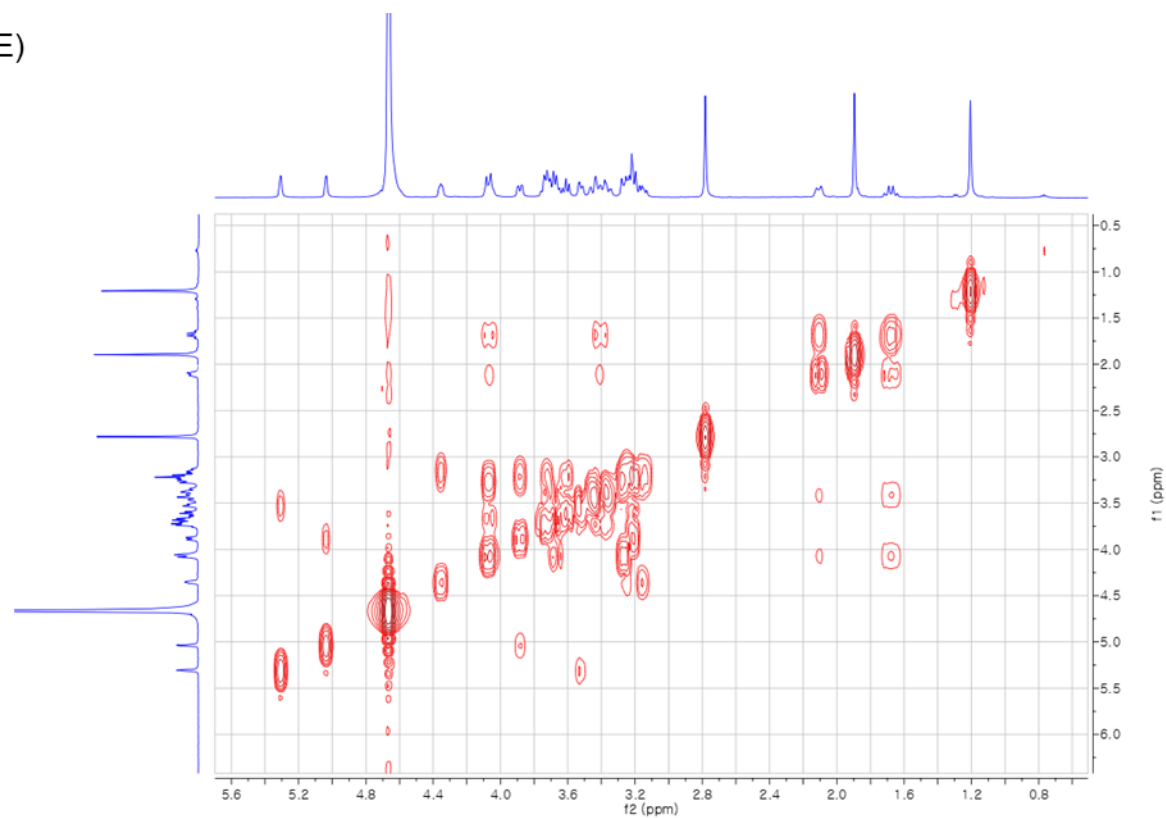
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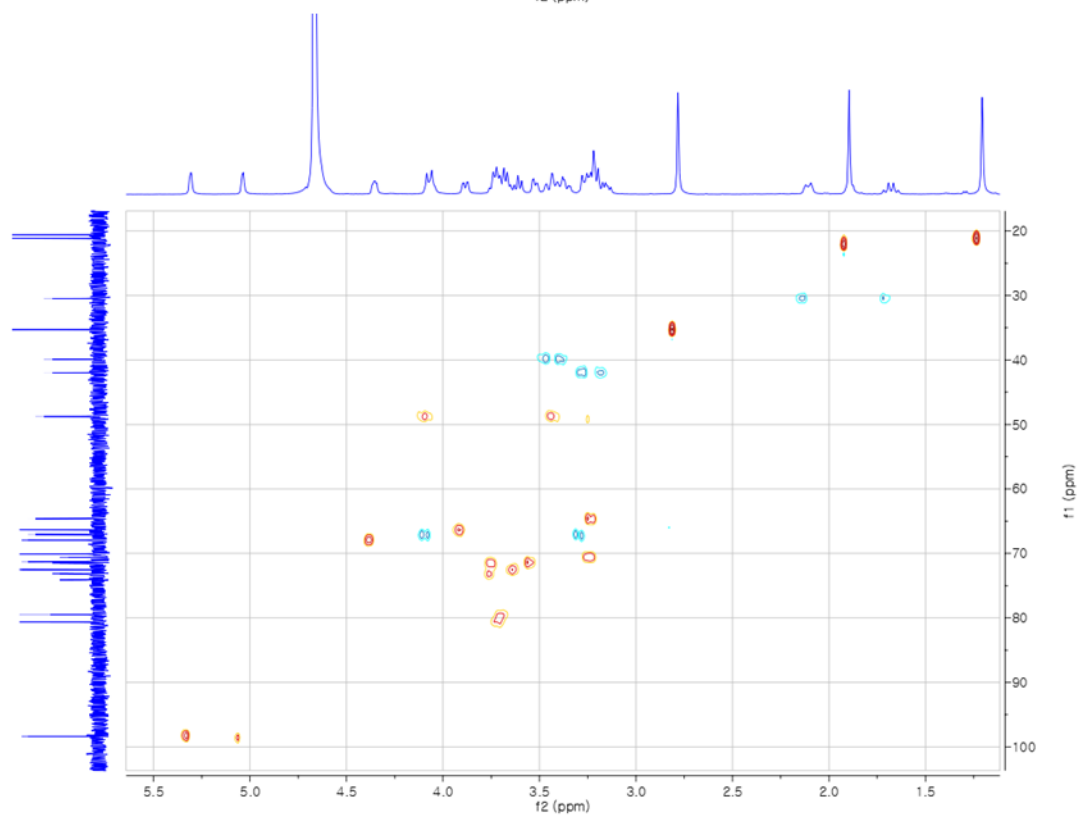
**Figure S1.** Coomassie blue-stained SDS-PAGE gel of purified C-terminal hexahistidine tagged AAC(6')-APH(2'').



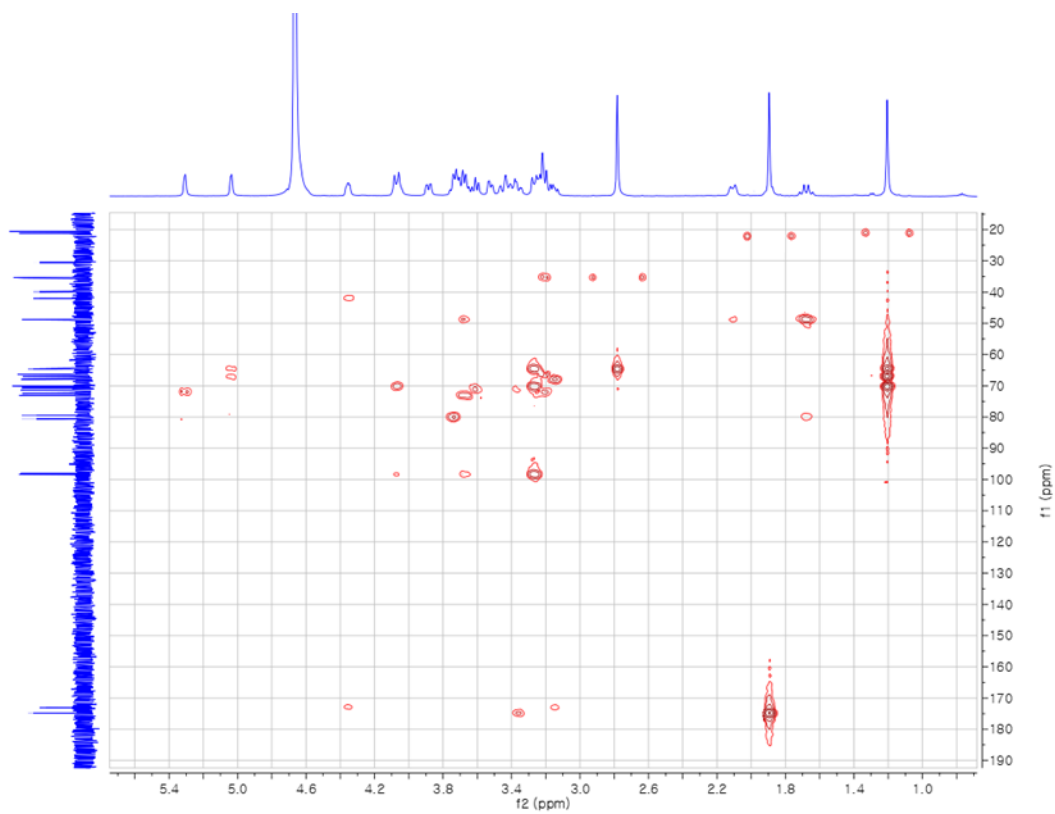
(E)



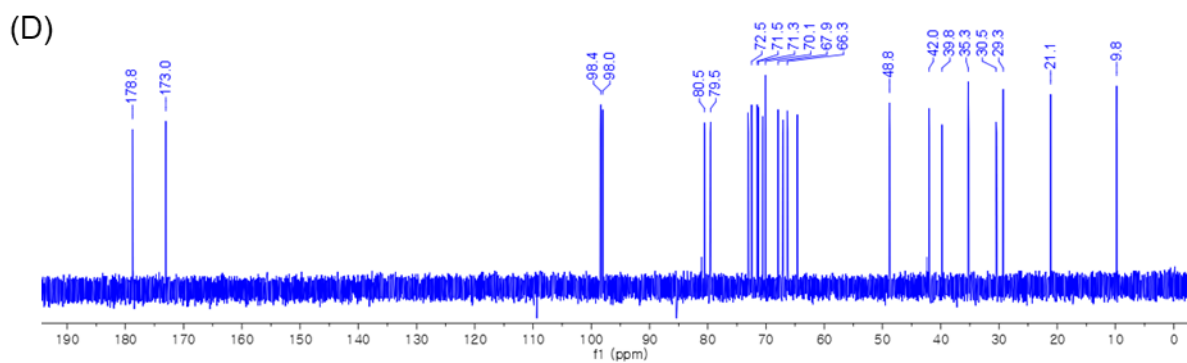
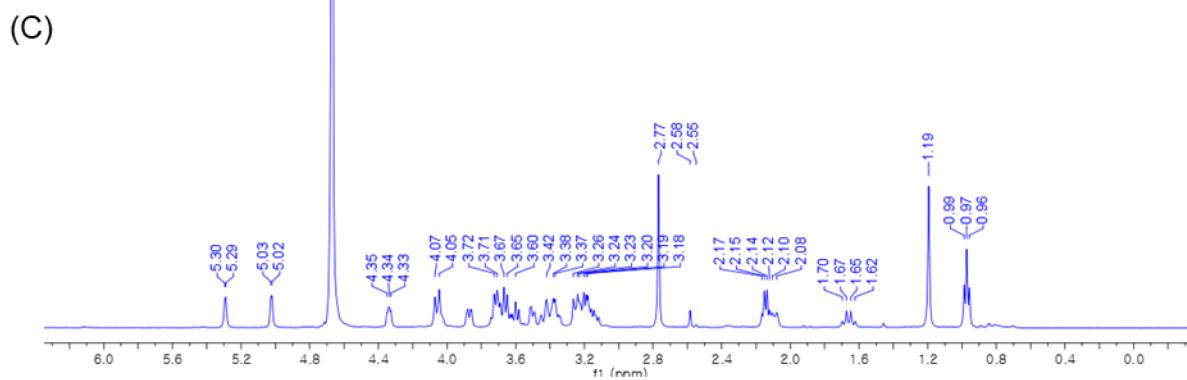
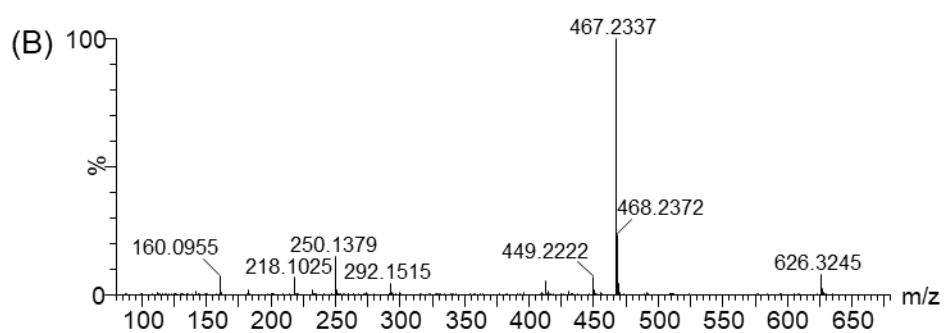
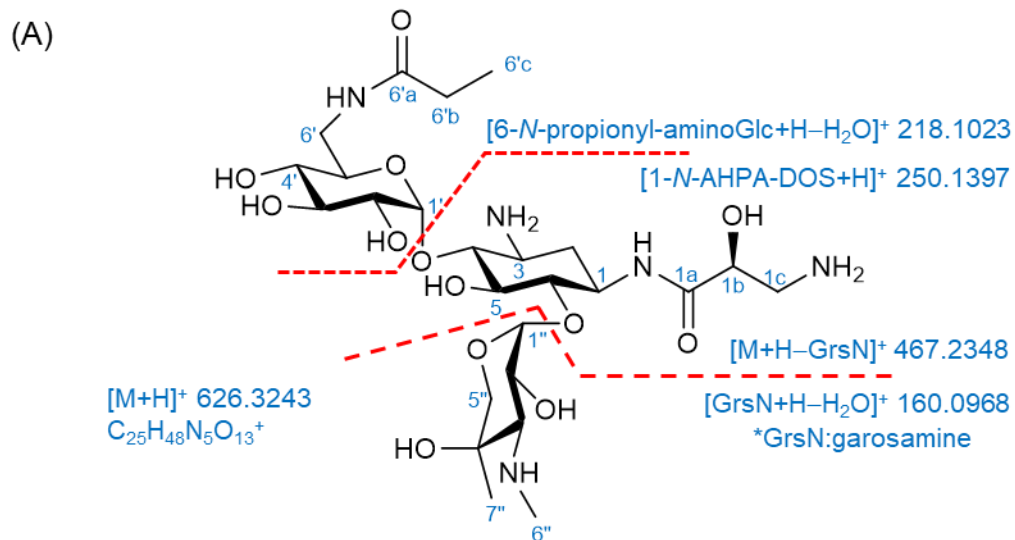
(F)



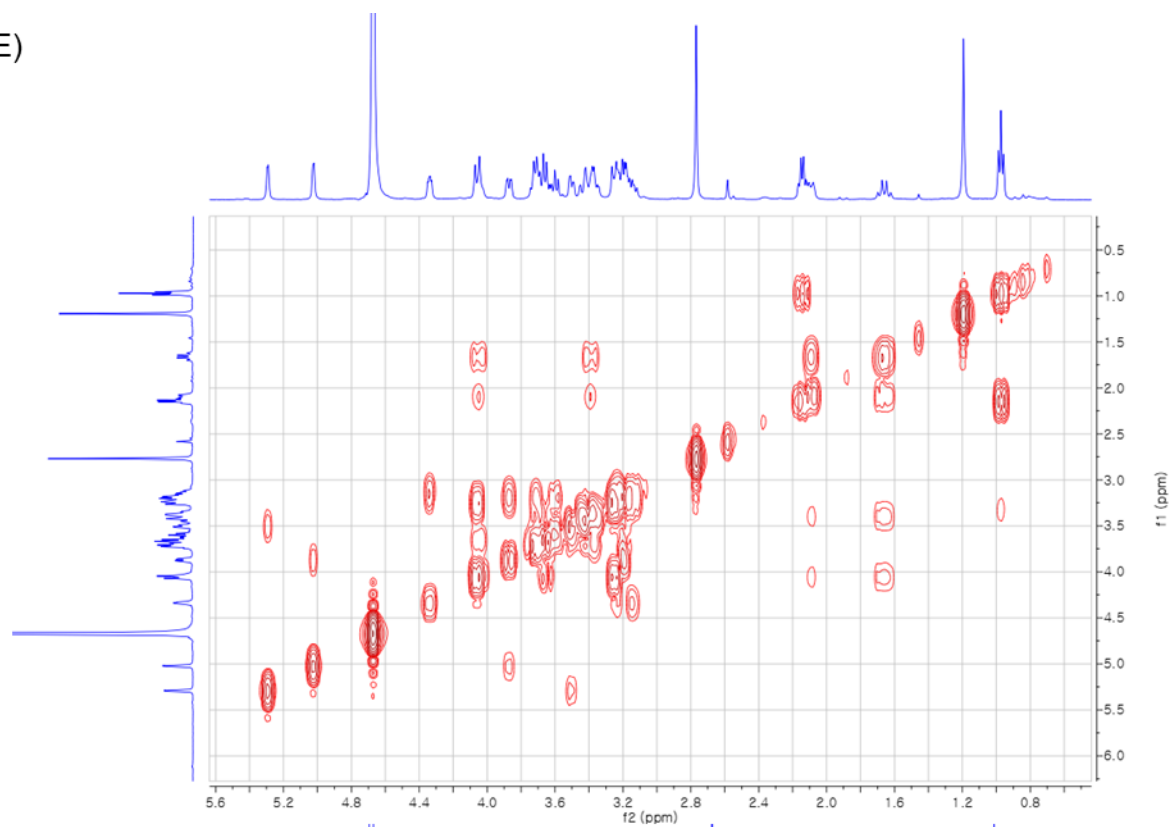
(G)



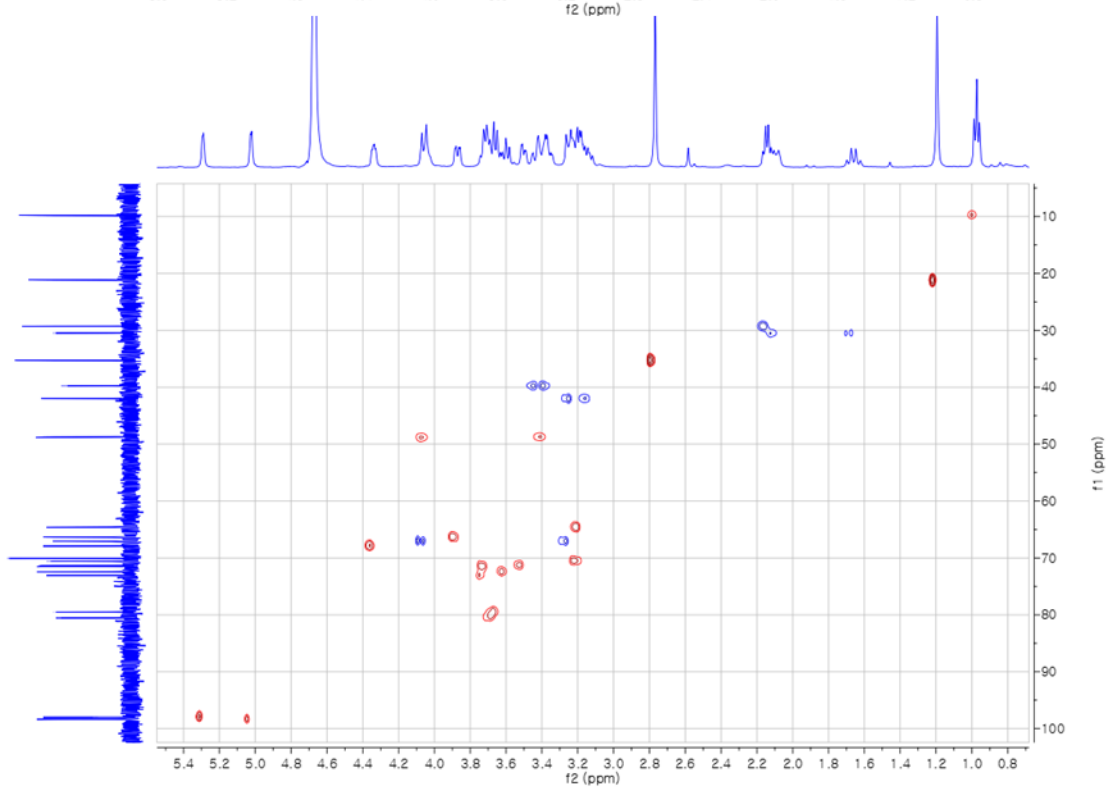
**Figure S2.** The structural determination of 6'-*N*-acetyl isepamicin. (A) MS/MS fragmentation pattern (B) MS/MS spectra (C) <sup>1</sup>H NMR data (D) <sup>13</sup>C NMR data (E) <sup>1</sup>H-<sup>1</sup>H gCOSY NMR data (F) <sup>1</sup>H-<sup>13</sup>C gHSQC NMR data (G) <sup>1</sup>H-<sup>13</sup>C gHMBC NMR data



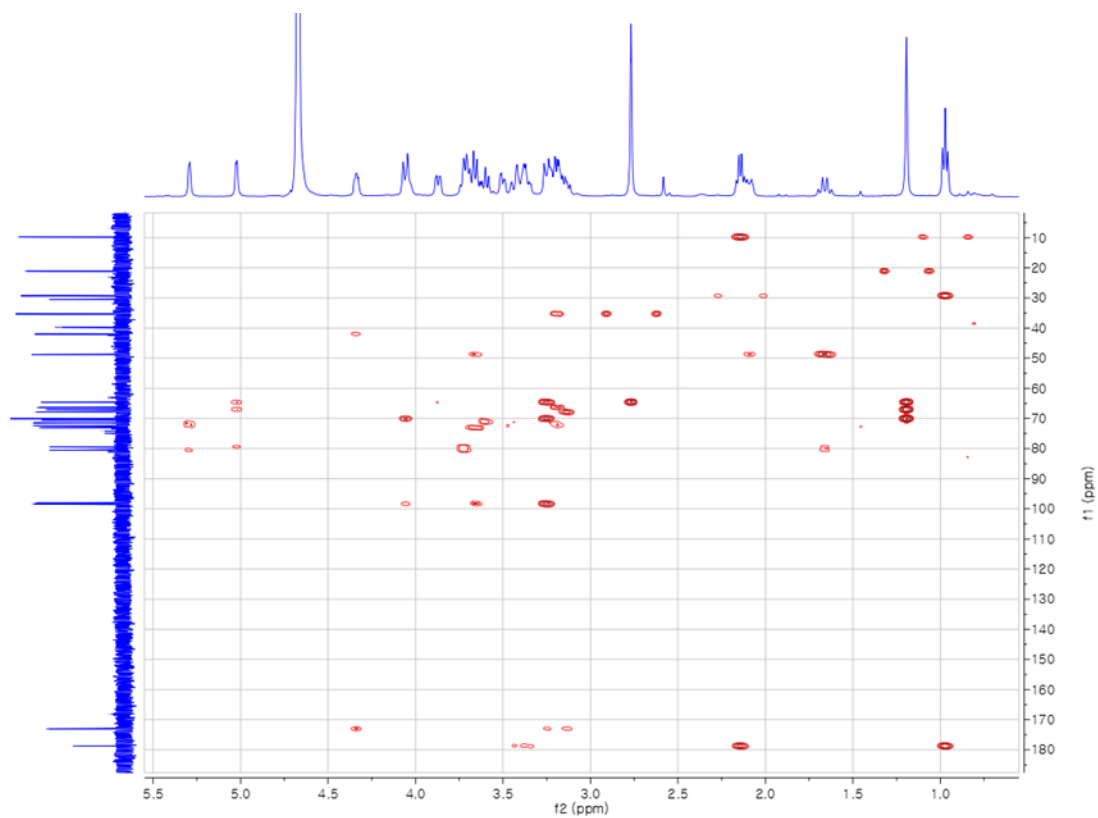
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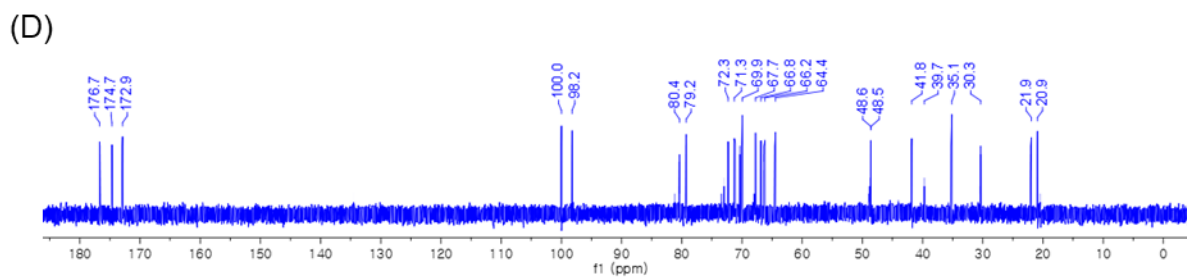
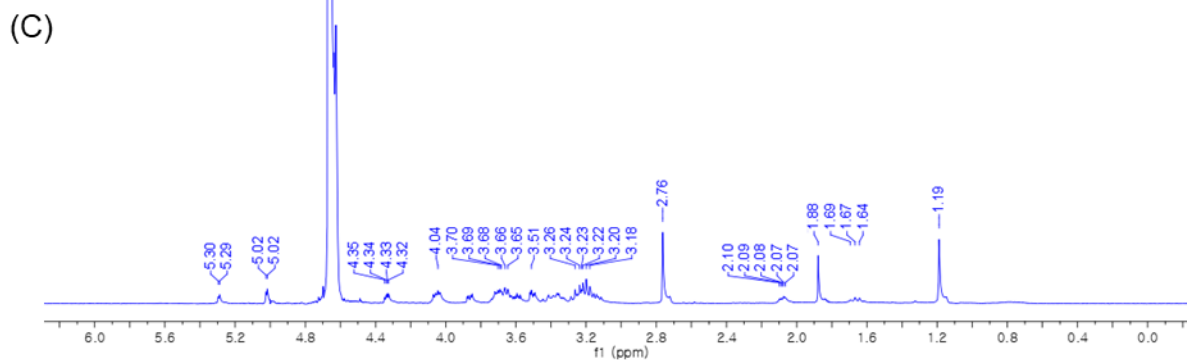
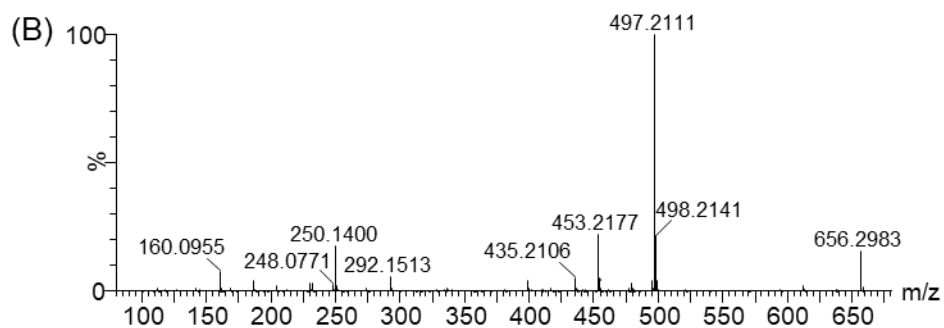
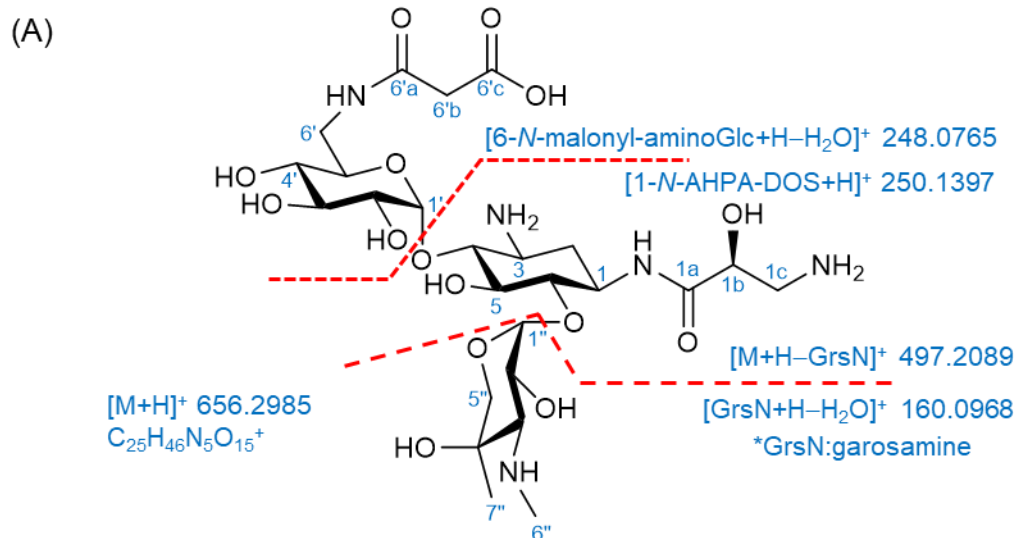


(G)

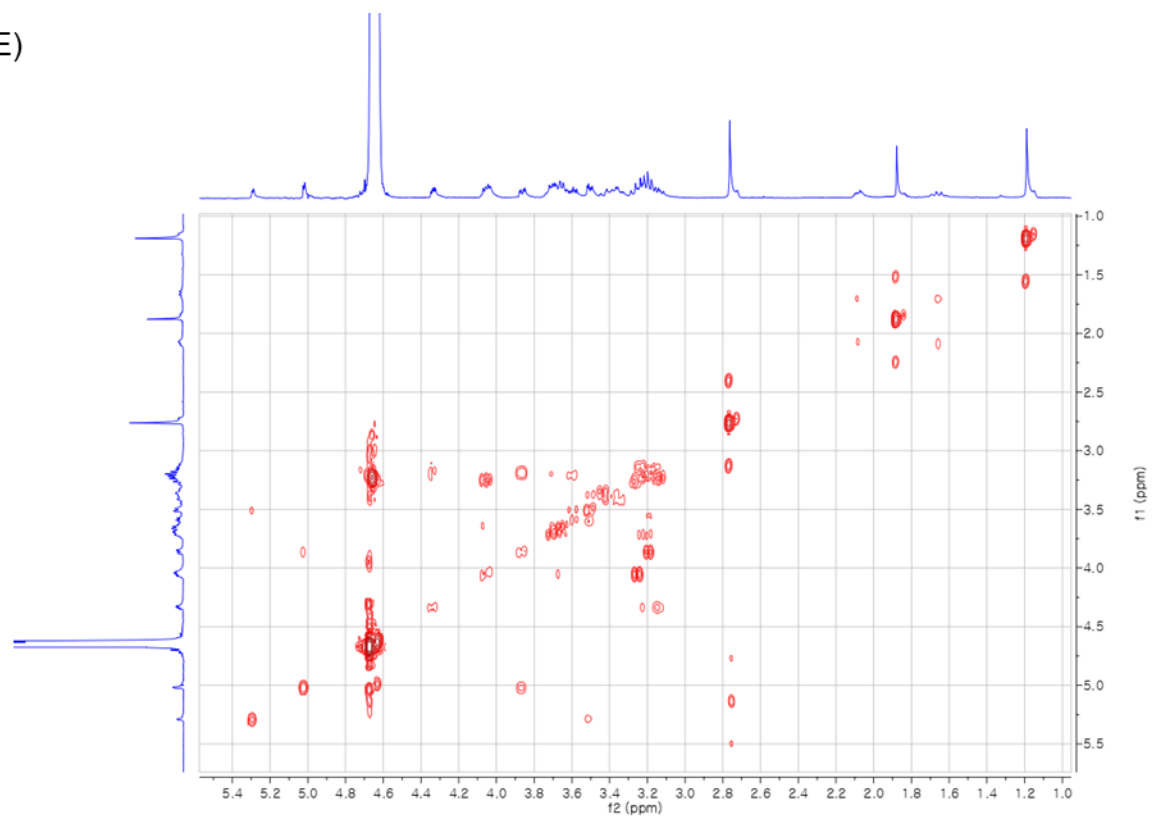


**Figure S3.** The structural determination of 6'-*N*-propionyl isepamicin. (A) MS/MS fragmentation pattern (B) MS/MS spectra (C) <sup>1</sup>H NMR data (D) <sup>13</sup>C NMR data (E) <sup>1</sup>H-<sup>1</sup>H gCOSY NMR data (F) <sup>1</sup>H-<sup>13</sup>C gHSQC NMR data (G) <sup>1</sup>H-<sup>13</sup>C gHMBC NMR data

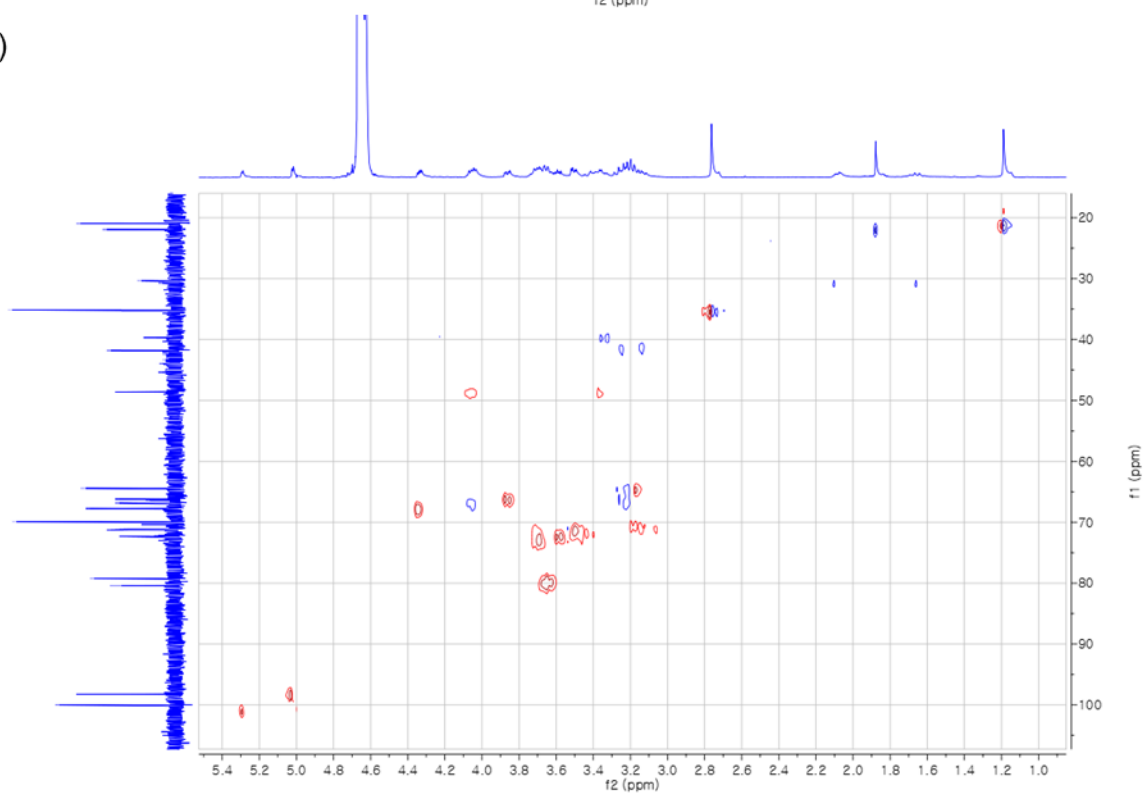




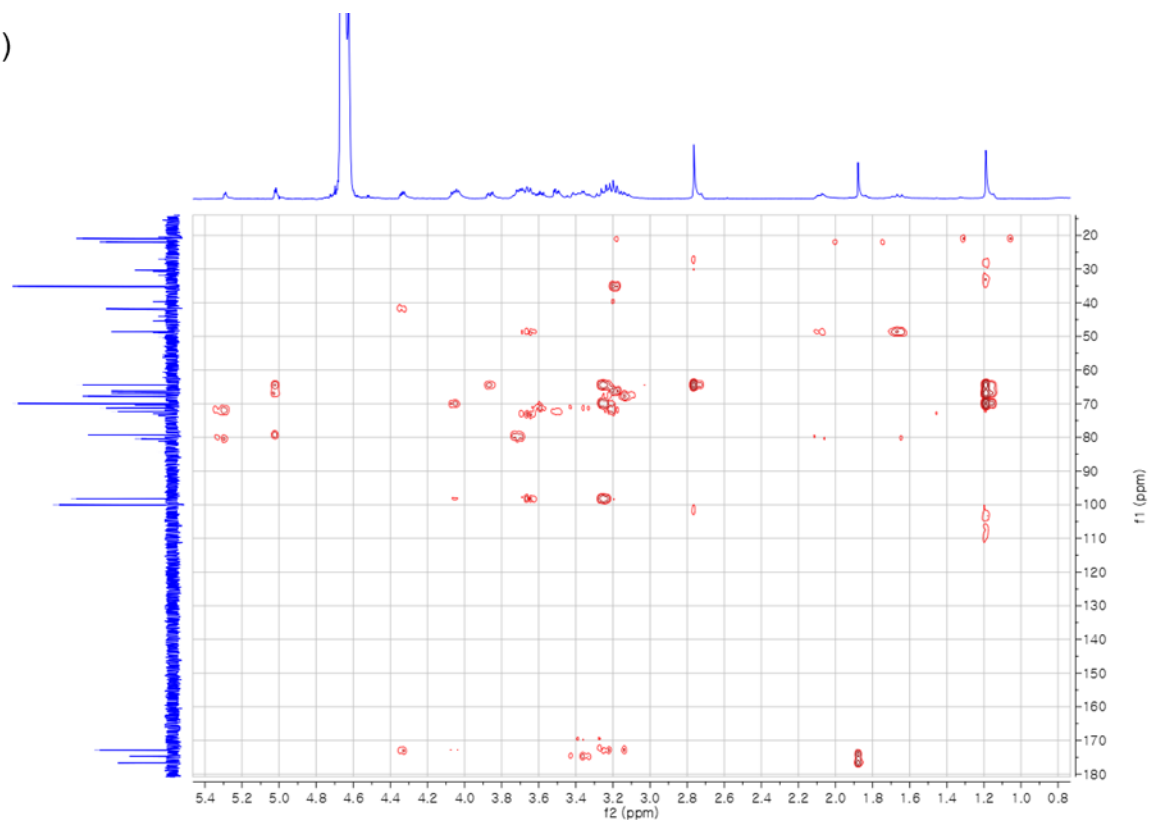
(E)



(F)



(G)



**Figure S4.** The structural determination of 6'-*N*-malonyl isepamicin. (A) MS/MS fragmentation pattern (B) MS/MS spectra (C) <sup>1</sup>H NMR data (D) <sup>13</sup>C NMR data (E) <sup>1</sup>H-<sup>1</sup>H gCOSY NMR data (F) <sup>1</sup>H-<sup>13</sup>C gHSQC NMR data (G) <sup>1</sup>H-<sup>13</sup>C gHMBC NMR data