Supporting Information

An efficient chemical synthesis of carboxylate-isostere analogs of daptomycin

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I. Analytical HPLC method

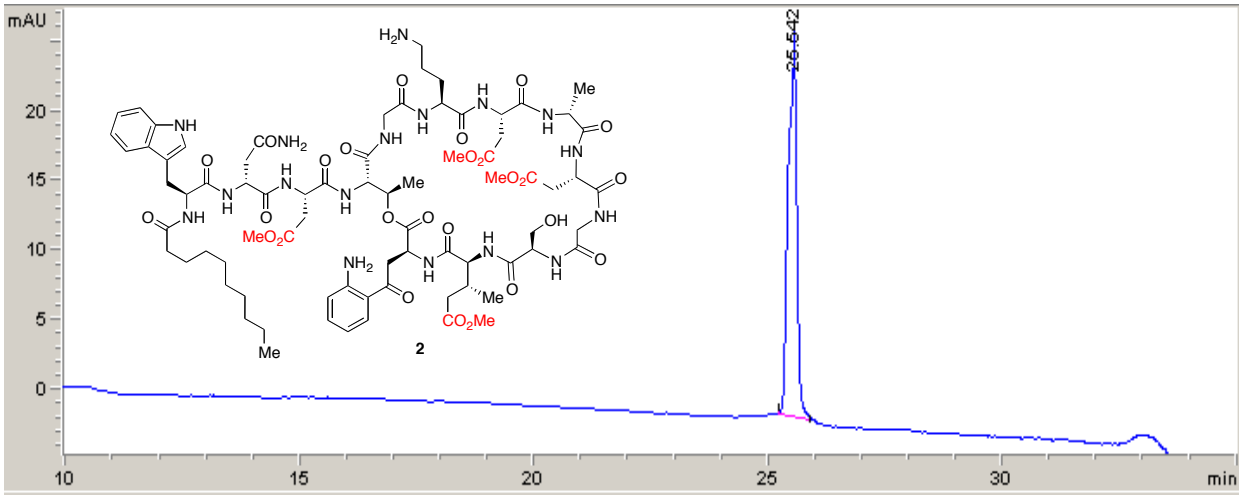
Method 3: Column = Phenomenex Luna 5 µm C_{18} (2), 250 × 10 mm; Temperature = 25 °C Solvent A = H_2O (0.1% formic acid); Solvent B = MeCN (0.1% formic acid); Flow Rate = 3.0 mL/min; Gradient: held at 5% B for 3 min, ramped to 20% B over 2 min, ramped to 60% B over 30 min, ramped to 95% B over 2 min, held at 95% B for 2 min, ramped to 5% B over 3 min and held for 5 min.

II. General procedure for the synthesis of linear daptomycin derivatives

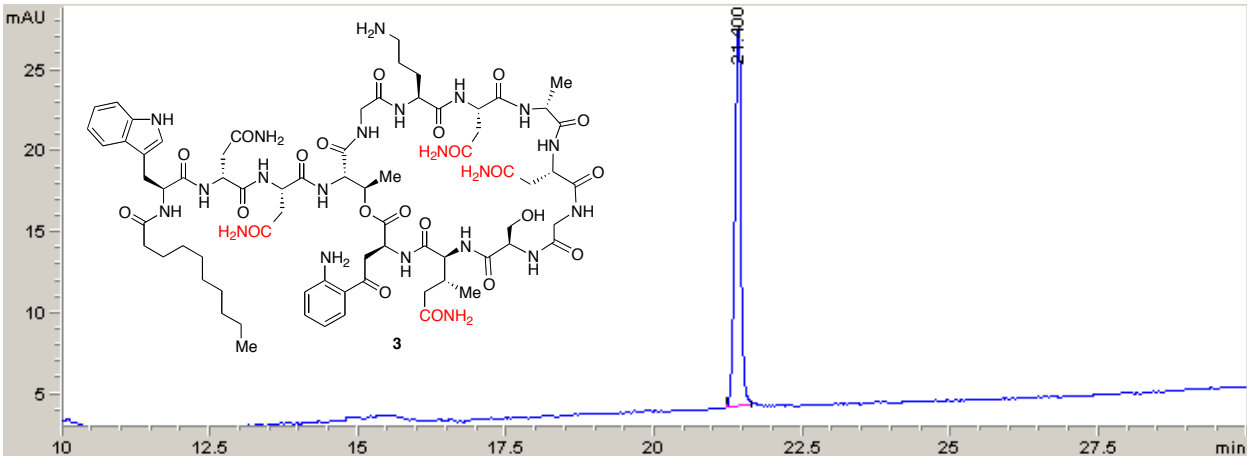
Procedure A: To a solution of daptomycin-tetra-methyl ester derivative (2.5 mg, 1.5 µmol) in MeOH (200 µL) and MeCN (200 µL) at 0 ºC was added 43 mM NaOMe in MeOH (20 µL). The reaction mixture was stirred for 30 min. At this time, the reaction mixture was acidified using 1 M HCl(aq) to adjust the pH to 3.0. The reaction mixture was analyzed by LC-MS/MS to characterize the linear product of interest.

Procedure B: To a solution of daptomycin derivative (1.5 µmol) in H_2O (200 µL) and MeCN (200 µL) at 0 ºC was added 1 M NaOH(aq) (~20 µL) to adjust the pH to 9 – 9.5. The reaction mixture was stirred for 30 min. At this time, the reaction mixture was acidified using 1 M HCl(aq) to adjust the pH to 3.0. The reaction mixture was analyzed by LC-MS/MS to characterize the linear product of interest.

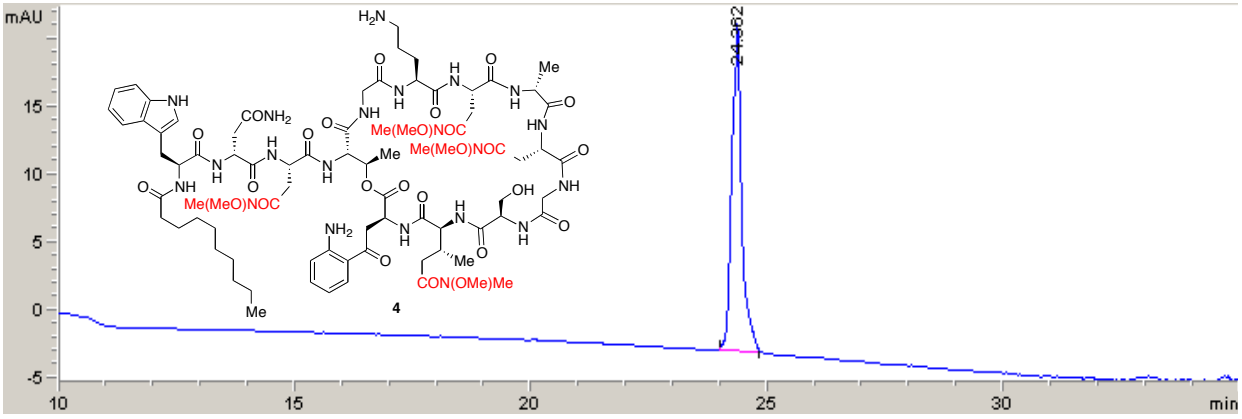
III. HPLC trace of tetra-methyl ester analog of daptomycin



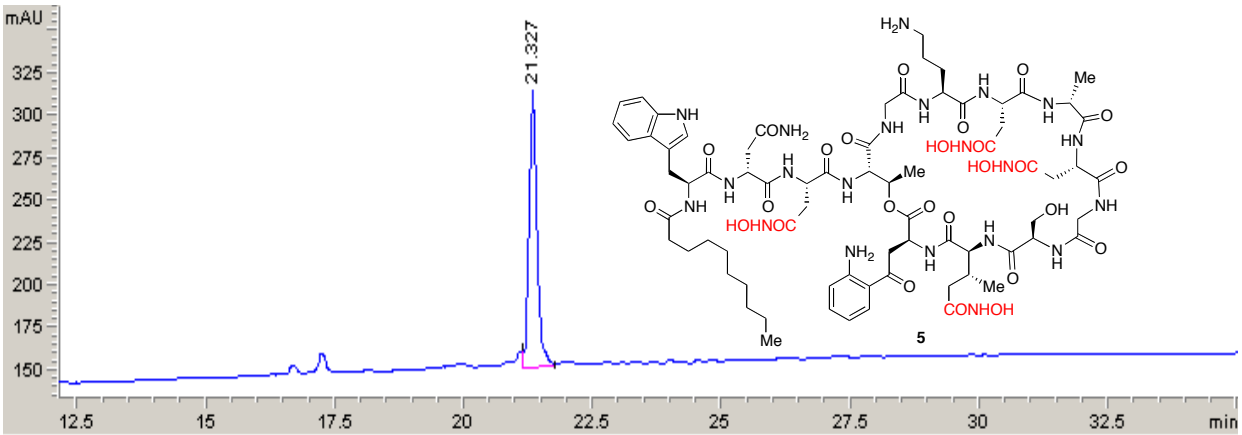
IV. HPLC trace of tetra-amide analog of daptomycin



V. HPLC trace of tetra-Weinreb amide analog of daptomycin



VI. HPLC trace of tetra-hydroxamic acid analog of daptomycin



VII. LC-MS/MS analysis of linear daptomycin

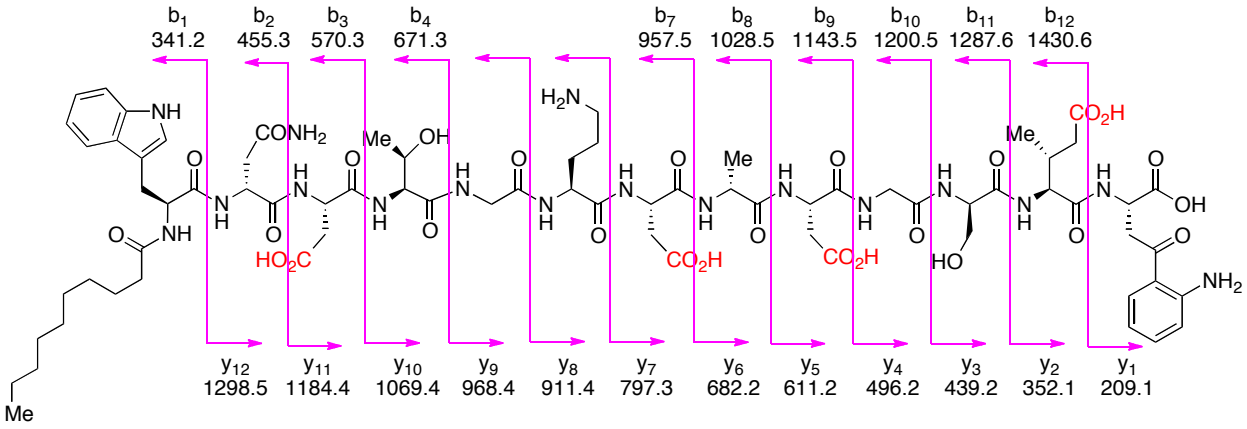


Figure SI-1. UPLC-MS/MS fragmentation analysis of linear daptomycin

VIII. LC-MS/MS analysis of linear penta-methyl ester analog of daptomycin

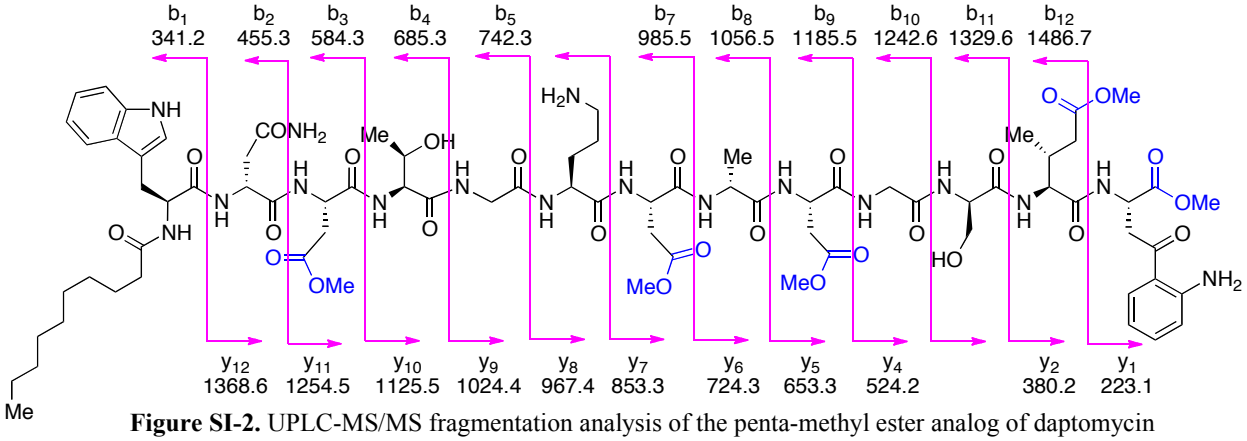


Figure SI-2. UPLC-MS/MS fragmentation analysis of the penta-methyl ester analog of daptomycin

IX. LC-MS/MS analysis of linear tetra-amide analog of daptomycin

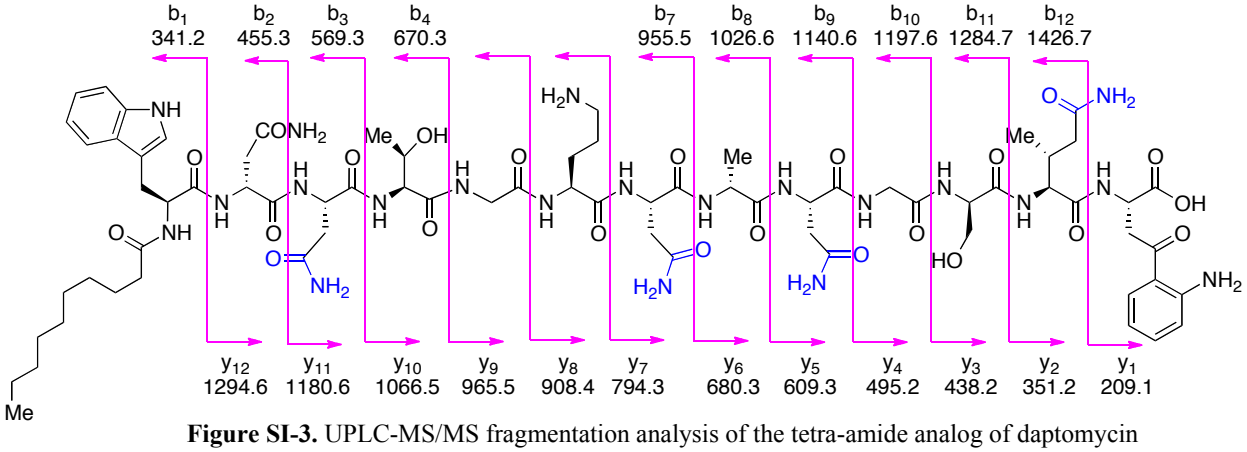


Figure SI-3. UPLC-MS/MS fragmentation analysis of the tetra-amide analog of daptomycin

X. LC-MS/MS analysis of a linear tri-amide analog of daptomycin

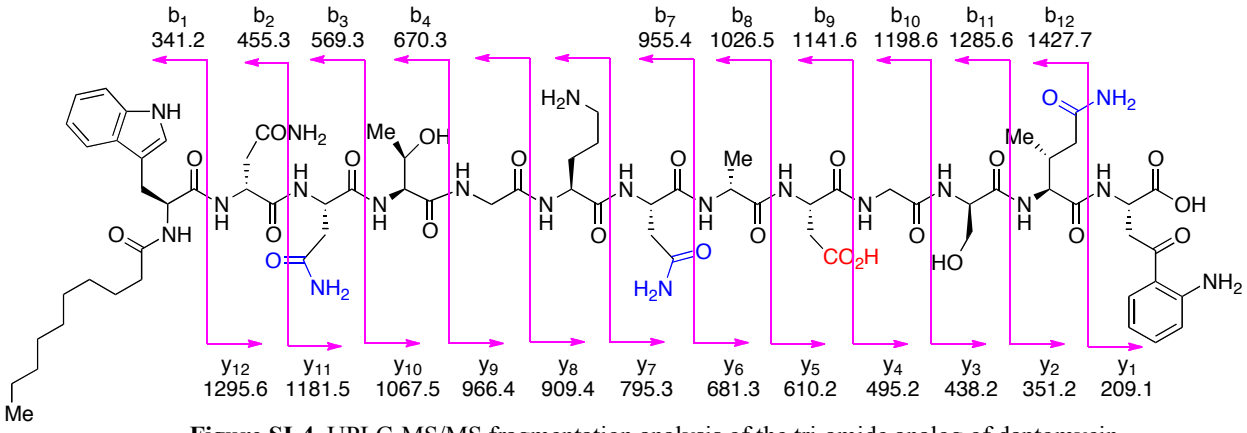


Figure SI-4. UPLC-MS/MS fragmentation analysis of the tri-amide analog of daptomycin

XI. LC-MS/MS analysis of linear tetra-Weinreb amide analog of daptomycin

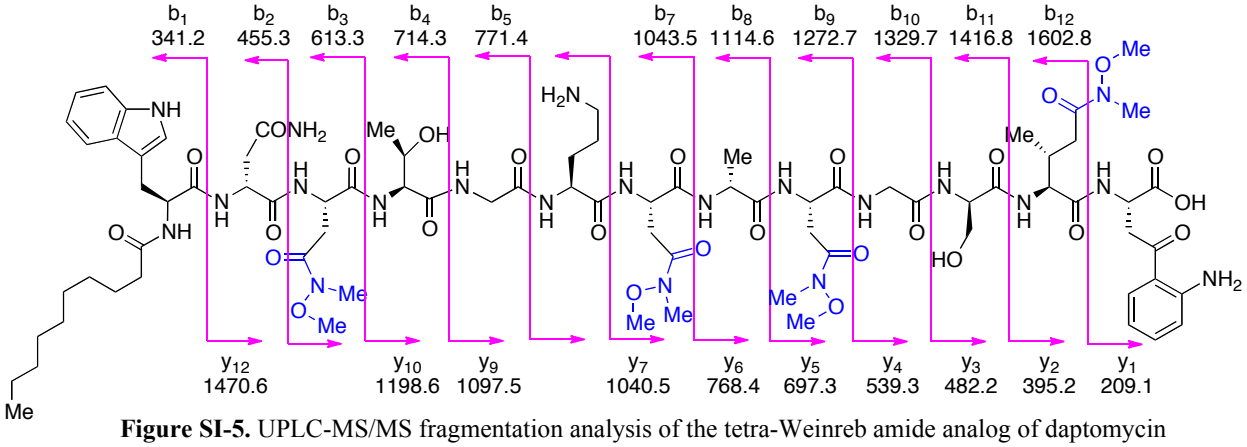


Figure SI-5. UPLC-MS/MS fragmentation analysis of the tetra-Weinreb amide analog of daptomycin

XII. LC-MS/MS analysis of linear tetra-hydroxamic acid analog of daptomycin

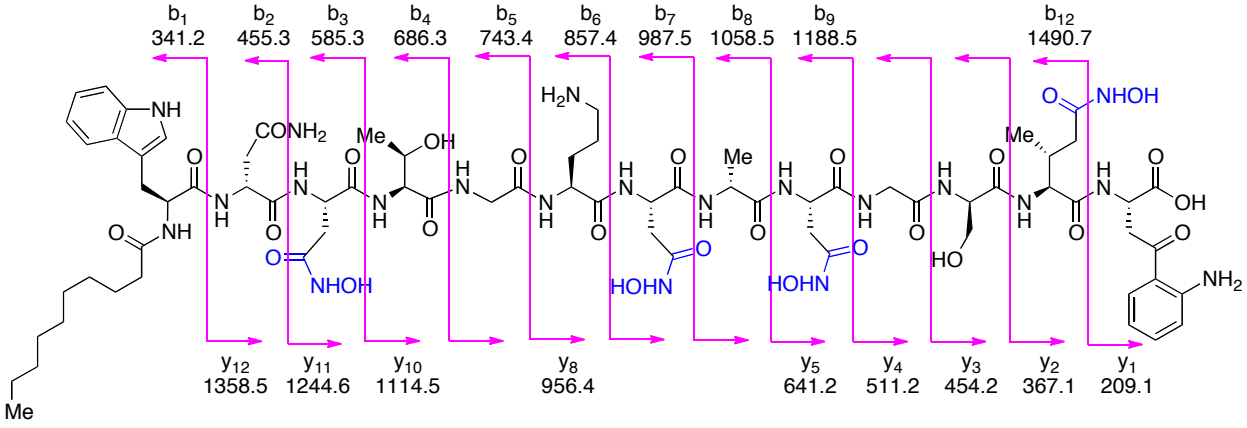


Figure SI-6. UPLC-MS/MS fragmentation analysis of the tetra-hydroxamic acid analog of daptomycin

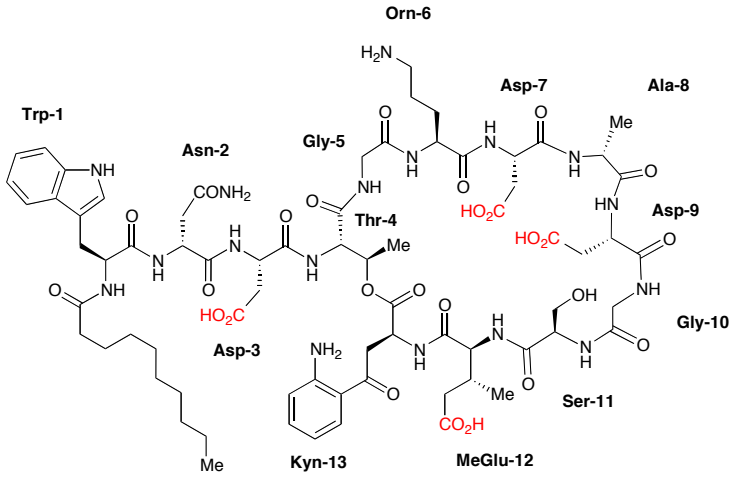
XIII. NMR spectra of tetra-modified daptomycin analogs

All NMR experiments were performed on a 600 MHz Bruker Avance III NMR Spectrometer equipped with a 1.7 mm Cryo-TCI probe. The samples were inserted into the magnet using a SampleJet accessory.

To prepare the samples, approximately 1 mg of material was dissolved in 50 μL of DMSO-d_6 and bath sonicated for one minute to ensure proper dissolution before it was pipetted into the NMR tube. The tube was sealed with a plastic ball and was stored at room temperature prior to the experiments. All NMR experiments were performed at 298 K. All NMR data was processed using Mnova (MestReLab Research, Santiago de Compostela, Spain).

In order to obtain complete connectivities for the parent daptomycin, a standard ^{1}H NMR spectrum, a ^{1}H-^{1}H gCOSY spectrum, and a ^{1}H-^{1}H TOCSY spectrum were used to assign the individual spin systems. A two-dimensional ^{1}H-^{1}H NOESY experiment (150 msec mixing time) was used to confirm the sequential connectivities where possible. Finally, a ^{1}H-^{13}C gHSQC spectrum, a ^{1}H-^{13}C gHMBC spectrum, a ^{1}H-^{15}N fHSQC and a ^{1}H-^{15}N gHMBC were recorded. These enabled some resolution in cases where spectral overlap was an issue. Because of the apparent pH of the solution, the exchangeable amide protons were not observed in these experiments.

(1) Chemical shift assignment for the parent daptomycin (**1**):



Daptomycin (**1**)

Figure SI-7. Structure of daptomycin

Table SI-1. Chemical shift assignment for daptomycin (**1**) in DMSO-d_6.

Residue	H^{α} (C^{α})	H^{β}(C^{β})	Sidechain
Tail		Tail not assigned in these experiments	
Trp-1	*4.74 (48.14)*	3.39/3.60 (39.50)	γ2- 7.16 (123.51); δ4- 7.57 (118.13); δ5- 6.96 (117.93); δ6- 7.04 (120.57); δ7- 7.31 (111.01); H_N- 10.79
Asn-2	*4.58 (49.59)*	*2.37/2.48 (36.77)*	---
Asp-3	*4.58 (49.59)*	*2.43/2.80 (35.71)*	---
Thr-4	4.50 (55.18)	5.16 (69.99)	γ- 1.06 (15.45)
Gly-5	3.67/3.83 (42.02)	---	---
Orn-6	4.20 (52.68)	1.72 (27.81)	γ-1.62 (23.29); δ-2.81 (38.16)
Asp-7	*4.58 (49.59)*	*2.63 (36.26)*	---
Ala-8	4.19 (48.39)	1.23 (17.35)	---
Asp-9	*4.58 (49.59)*	*2.63 (36.26)*	---
Gly-10	3.60 (39.50)	---	---
Ser-11	4.38 (54.15)	3.63 (61.40)	---
Mglu-12	4.50 (55.18)	1.98 (37.68)	*γ- 2.33 (37.44),* βMe- 0.84 (14.09)
Kyn-13	*4.38 (55.12)*	2.90/3.03 (26.98)	γ3- 6.75 (116.63); γ4- 7.23 (134.13); γ5- 6.54 (114.22); γ6- 7.76 (131.05)

Assignments in italics are ambiguous due to spectral overlap.

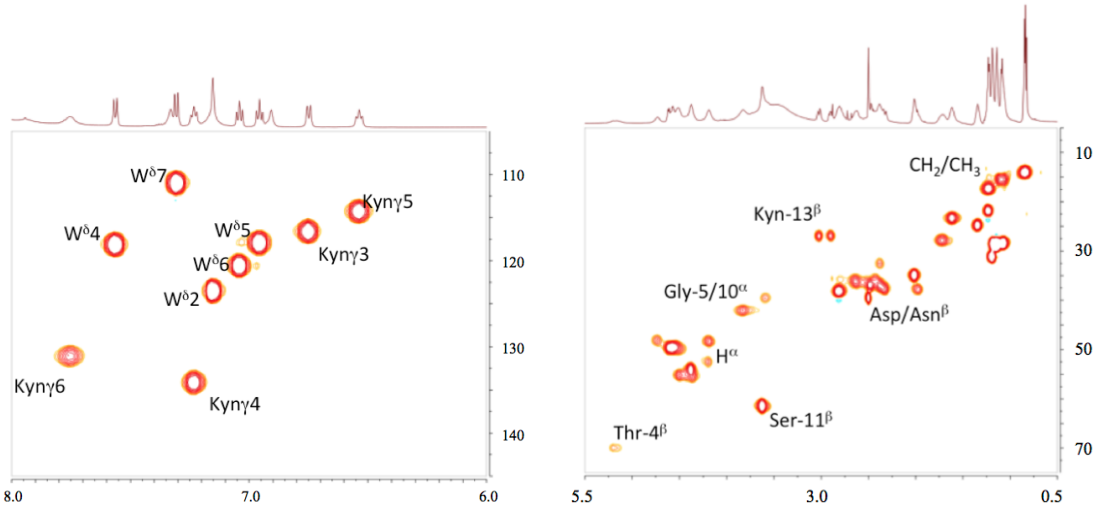


Figure SI-8. The ^{1}H-^{13}C gHSQC correlation data for daptomycin in DMSO-d_6.

(2) Comparative analysis of the ^{1}H-^{13}C gHSQC correlations of the tetra-modified analogs **2** – **5**.

For all of the ^{1}H-^{13}C gHSQC correlation experiments, the overall chemical shift patterns are preserved relative to daptomycin. The aromatic CH correlations are unchanged. In the aliphatic region of the spectra extra CH resonances are observed for the extra –O-Me and –N-Me groups. In addition, subtle shifts of the Asp H^{β} resonances are also observed, consistent with the modification of these residues. Those changes are highlighted on the spectra.

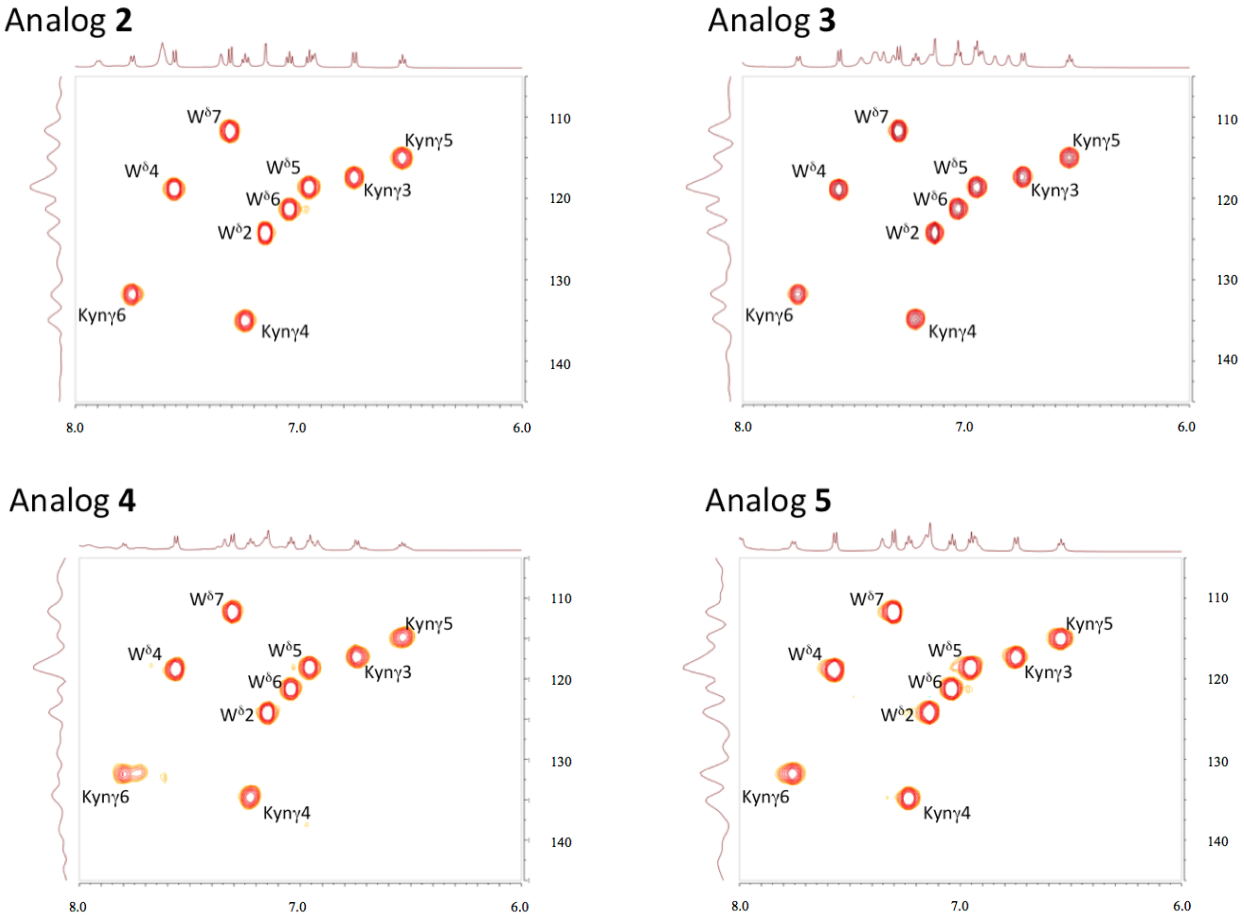


Figure SI-9. Aromatic region of the ^{1}H-^{13}C gHSQC correlation data for **2** – **5** in DMSO-d_6.

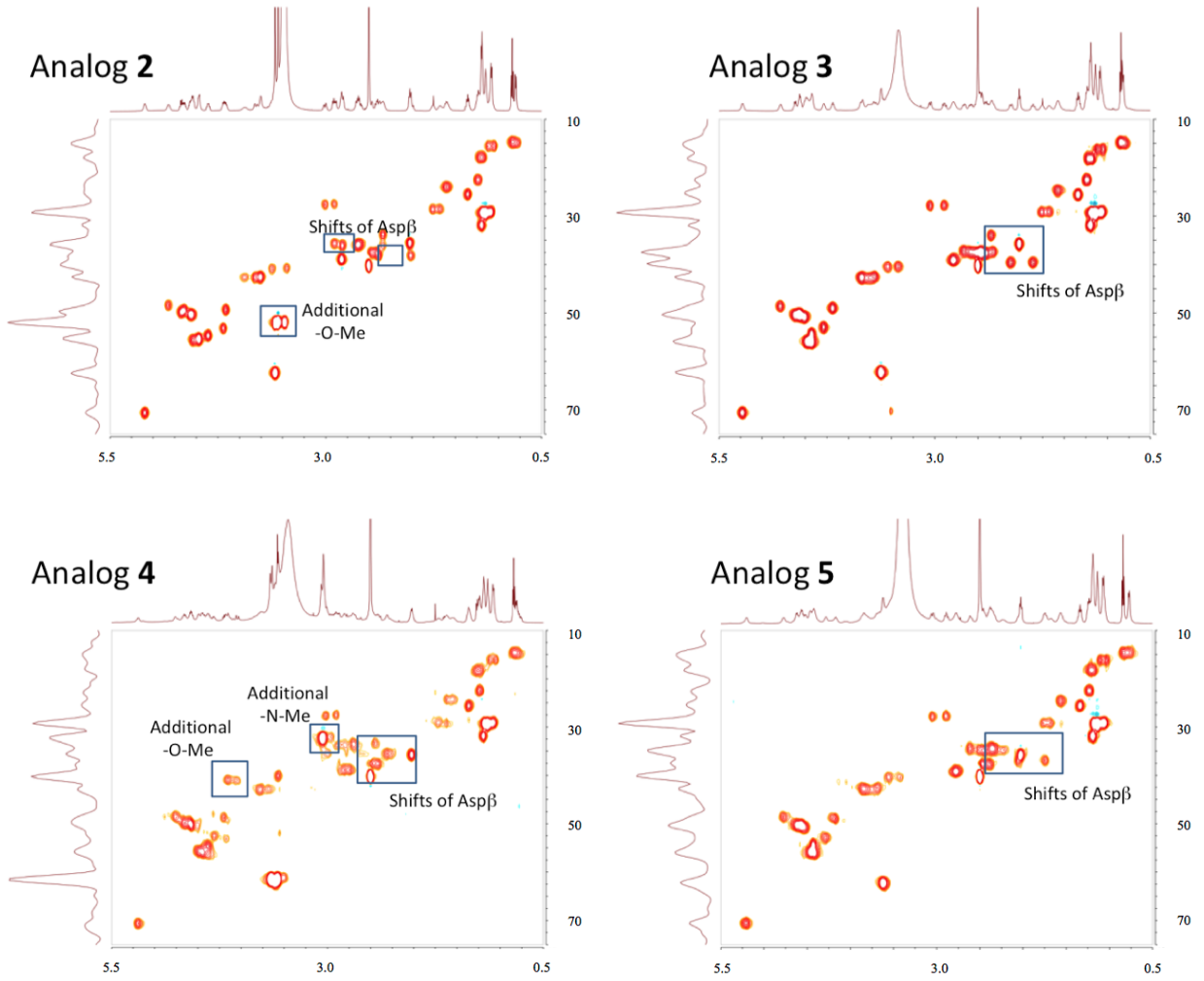


Figure SI-10. Aliphatic region of the ^{1}H-^{13}C gHSQC correlation data for **2** – **5** in DMSO-d_6.

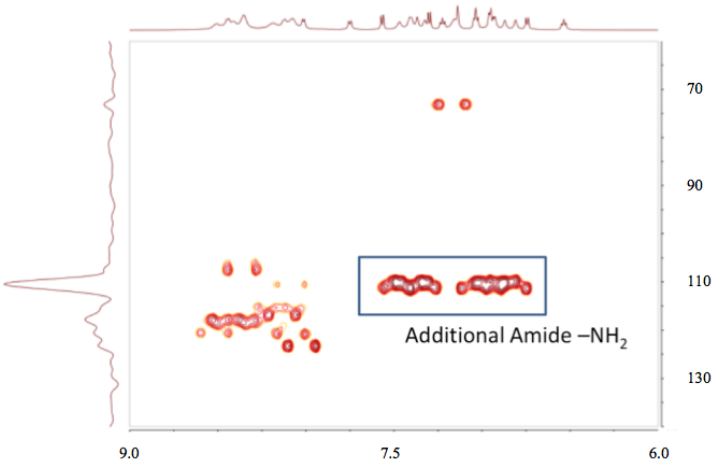


Figure SI-11. ^{1}H-^{15}N HSQC correlation data for daptomycin analog **3** in DMSO-d_6.