

# A Sub-Micromolar MraY<sub>AA</sub> Inhibitor with an Aminoribosyl Uridine Structure and a (S,S)-Tartaric Diamide: Synthesis, Biological Evaluation and Molecular Modeling

Martin Oliver<sup>1</sup>, Laurent Le Corre<sup>1</sup>, Mélanie Poinso<sup>1</sup>, Michaël Bosco<sup>1</sup>, Hongwei Wan<sup>1</sup>, Ana Amoroso<sup>2</sup>, Bernard Joris<sup>2</sup>, Ahmed Bouhss<sup>3</sup>, Sandrine Calvet-Vitale<sup>1,\*</sup> and Christine Gravier-Pelletier<sup>1,\*</sup>

<sup>1</sup> Université de Paris, Faculté des Sciences, UMR CNRS 8601, LCBPT, F-75006 Paris, France; [martin.cg.oliver@gmail.com](mailto:martin.cg.oliver@gmail.com) (M. O.); [laurent.le-corre@u-paris.fr](mailto:laurent.le-corre@u-paris.fr) (L. L. C.); [melanie.poinsot@u-paris.fr](mailto:melanie.poinsot@u-paris.fr) (M. P.); [michael.bosco@u-paris.fr](mailto:michael.bosco@u-paris.fr) (M.B.); [hongwei.wan@etu.parisdescartes.fr](mailto:hongwei.wan@etu.parisdescartes.fr) (H. W.)

<sup>2</sup> Unité de Physiologie et Génétique Bactériennes, Centre d'Ingénierie des Protéines, Département des Sciences de la Vie, Université de Liège, Sart Tilman, B4000 - Liège 1-Belgique; [amamoroso@ulg.ac.be](mailto:amamoroso@ulg.ac.be) (A. A.); [bjoris@uliege.be](mailto:bjoris@uliege.be) (B. J.)

<sup>3</sup> Université Paris-Saclay, INSERM U1204, Univ Evry, Structure-Activité des Biomolécules Normales et Pathologiques (SABNP), Evry-Courcouronnes, France; [ahmed.bouhss@univ-evry.fr](mailto:ahmed.bouhss@univ-evry.fr) (A. B.)

\* Correspondence: [sandrine.calvet-vitale@u-paris.fr](mailto:sandrine.calvet-vitale@u-paris.fr); Tel.: +33-176-534-246, [christine.gravier-pelletier@u-paris.fr](mailto:christine.gravier-pelletier@u-paris.fr); Tel.: +33-176-534-228

## ELECTRONIC SUPPLEMENTARY MATERIAL

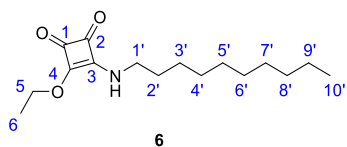
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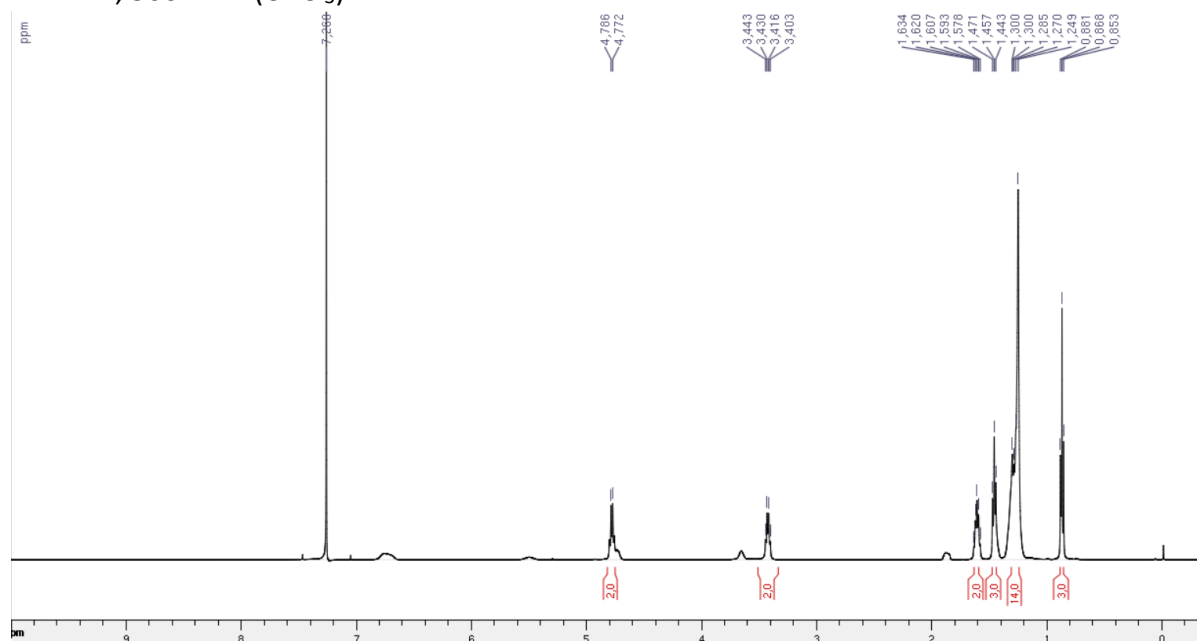
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# $^1\text{H}$ and $^{13}\text{C}$ Spectra

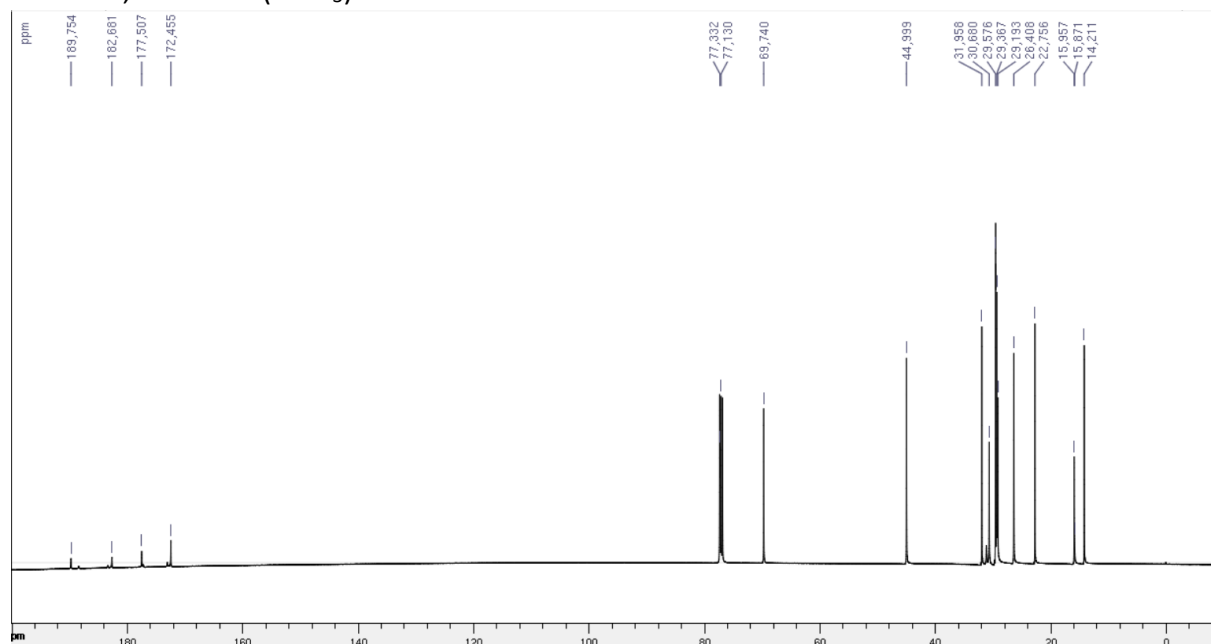
## 3-(Decylamino)-4-ethoxycyclobut-3-ene-1,2-dione **6**



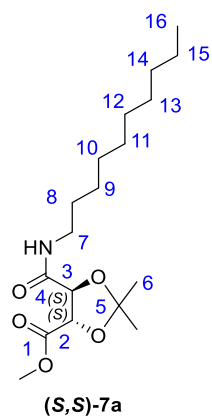
### $^1\text{H}$ NMR, 500 MHz (CDCl<sub>3</sub>)



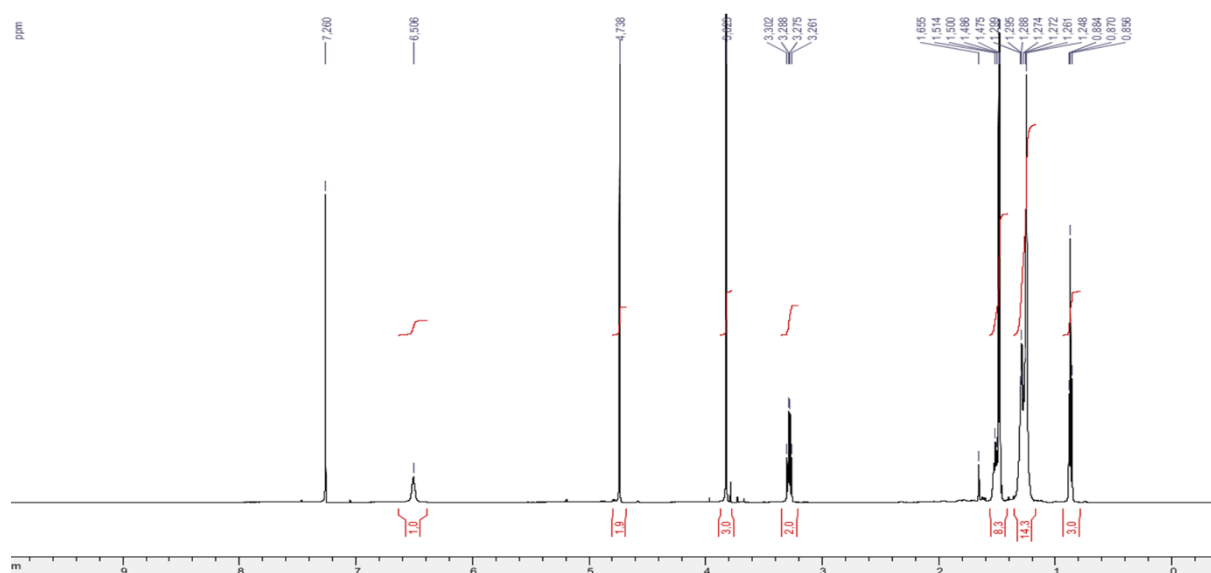
### $^{13}\text{C}$ NMR, 125 MHz (CDCl<sub>3</sub>)



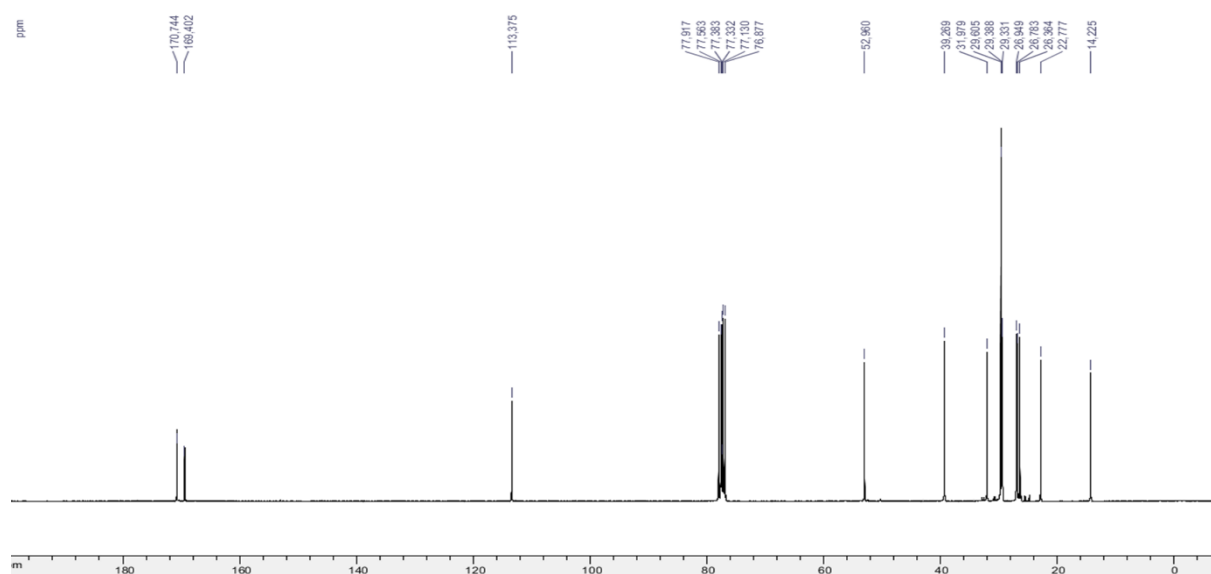
**Methyl (2*S*,3*S*)-2,3-*O*-isopropylidene-4-(decylamino)-2,3-dihydroxy-4-oxobutanoate (*S,S*)-7a**



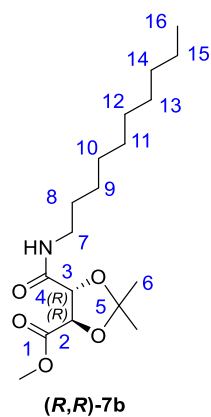
$^1\text{H}$  NMR, 500 MHz ( $\text{CDCl}_3$ )



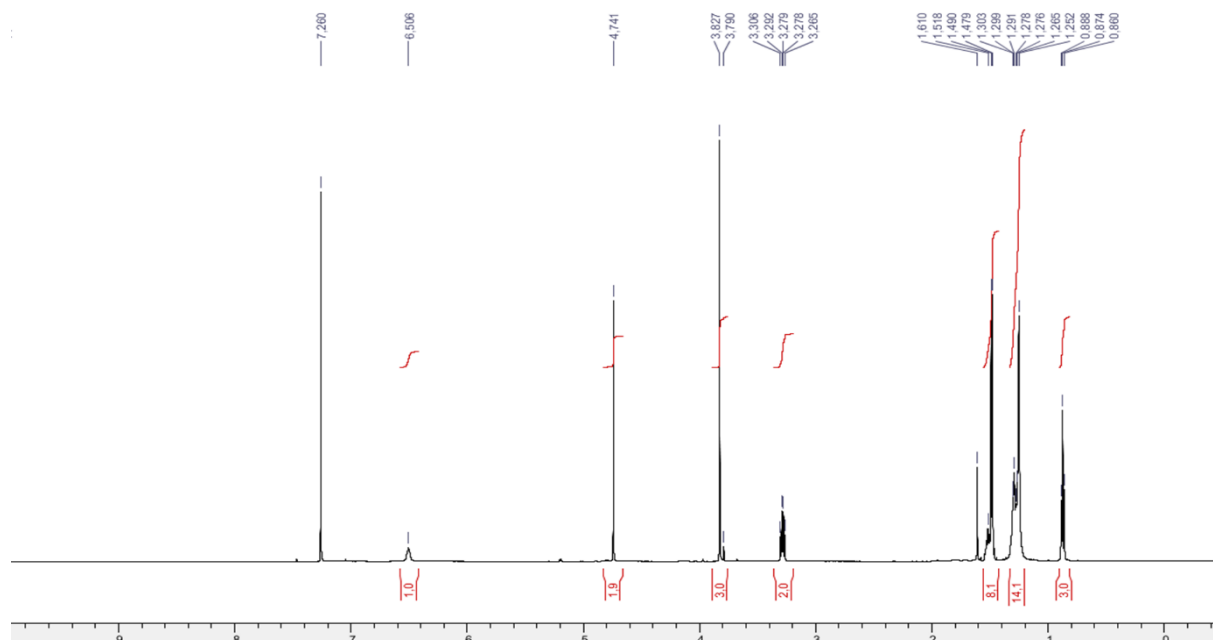
$^{13}\text{C}$  NMR, 125 MHz ( $\text{CDCl}_3$ )



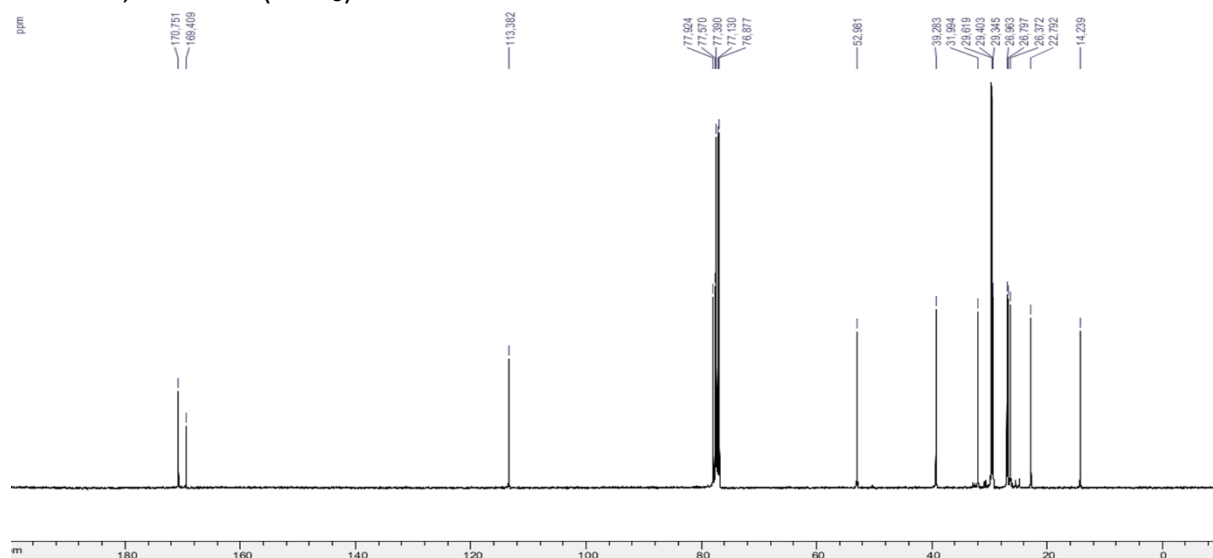
**Methyl (2R,3R)-2,3-O-isopropylidene-4-(decylamino)-2,3-dihydroxy-4-oxobutanoate (R,R)-7b**



$^1\text{H}$  NMR, 500 MHz ( $\text{CDCl}_3$ )



$^{13}\text{C}$  NMR, 125 MHz ( $\text{CDCl}_3$ )



Chemical structure of compound **8**, a complex glycoside derivative. The structure is labeled with blue numbers 1 through 10\* and 1' through 10'.

[illegible]

The chemical structure of (S,S)-9a is shown. It features a long branched alkyl chain (16\*, 15\*, 14\*, 13\*, 12\*, 11\*, 10\*, 9\*, 8\*, 7\*) attached to a carbamate group (NH, 4\*, 3\*, 5\*, 6\*). The carbamate is linked to a pyridine ring (1, 2, 3, 4, 5, 6) via a nitrogen atom (1'). The pyridine ring is also substituted with a TBSO group (2', 3', 4', 5', 6') and a TBSO group (1', 2', 3', 4', 5', 6'). The structure is labeled (S,S)-9a.

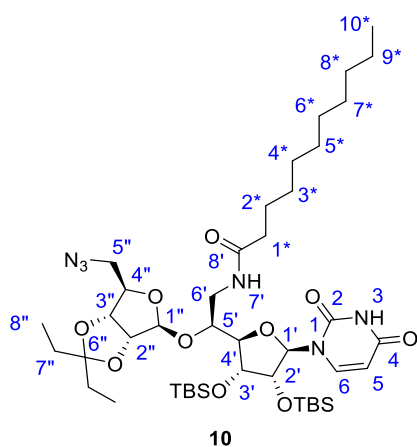
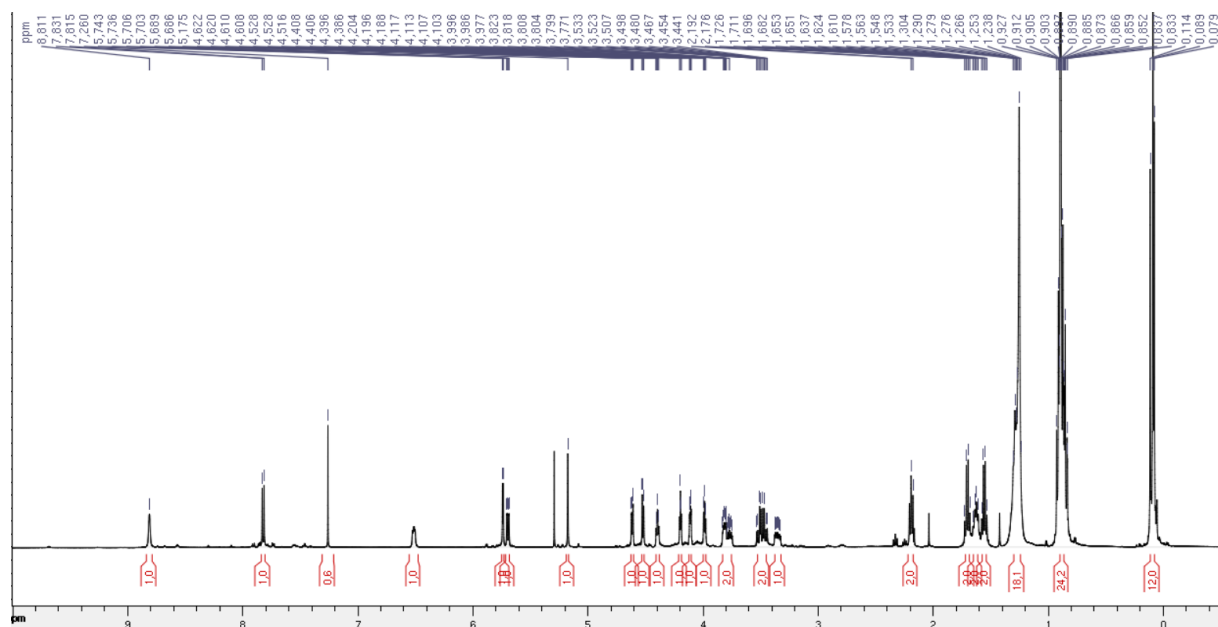
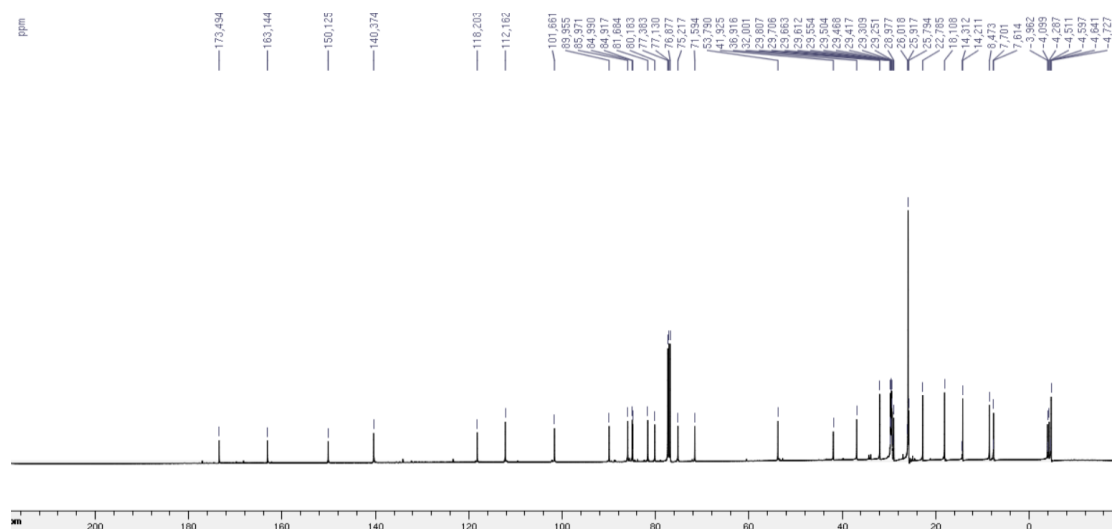
<sup>1</sup>H NMR spectrum of compound 10 in CDCl<sub>3</sub>. The spectrum shows peaks from 0 to 10 ppm. Key features include a triplet at 0.87 ppm (3H), a multiplet at 1.27 ppm (3H), a multiplet at 1.48 ppm (2H), a multiplet at 1.70 ppm (2H), a multiplet at 3.50 ppm (2H), a multiplet at 3.70 ppm (2H), a multiplet at 4.10 ppm (2H), a multiplet at 4.30 ppm (2H), a multiplet at 5.70 ppm (2H), and a multiplet at 7.80 ppm (2H). Integration values are shown below the peaks.

**(*R,R*)-9b**

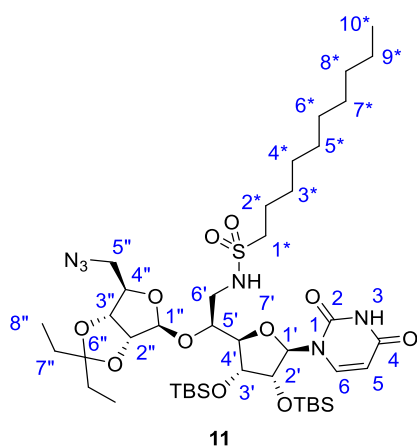
[illegible]

13C NMR spectrum of compound 1. The x-axis represents chemical shift in ppm, ranging from 0 to 180. The spectrum shows several sharp peaks. Key peaks are labeled with their chemical shift values: 170.039, 169.546, 162.754, 149.980, 140.299, 118.141, 112.473, 111.263, 110.633, 109.741, 106.038, 105.141, 104.867, 101.836, 79.473, 77.709, 77.318, 77.185, 77.130, 76.877, 75.377, 71.666, 53.483, 41.861, 39.445, 38.811, 39.332, 39.607, 39.404, 39.370, 39.334, 39.368, 35.959, 35.277, 35.184, 35.920, 35.902, 35.859, 35.838, 35.816, 35.791, 35.770, 35.754, 35.727, 35.704, 35.261, 34.434, 22.785, 22.763, 18.109, 15.069, 14.225, 14.188, 8.431, 7.600, -3.539, -4.304, -4.706, -4.752, -4.773.

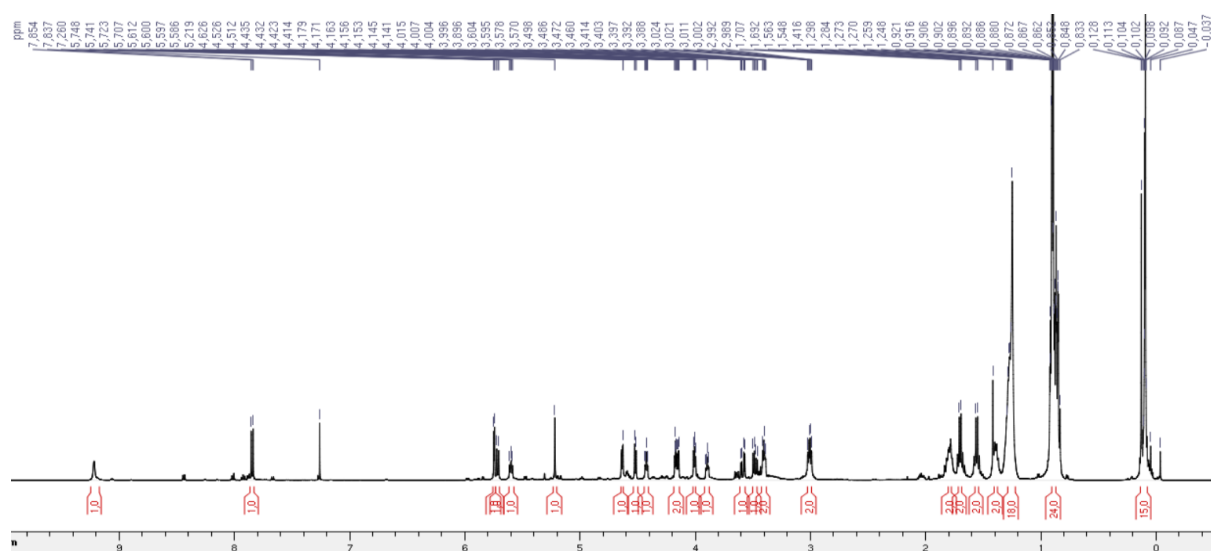
## Amide 10

<sup>1</sup>H NMR, 500 MHz (CDCl<sub>3</sub>) $^{13}\text{C}$  NMR, 125 MHz ( $\text{CDCl}_3$ )

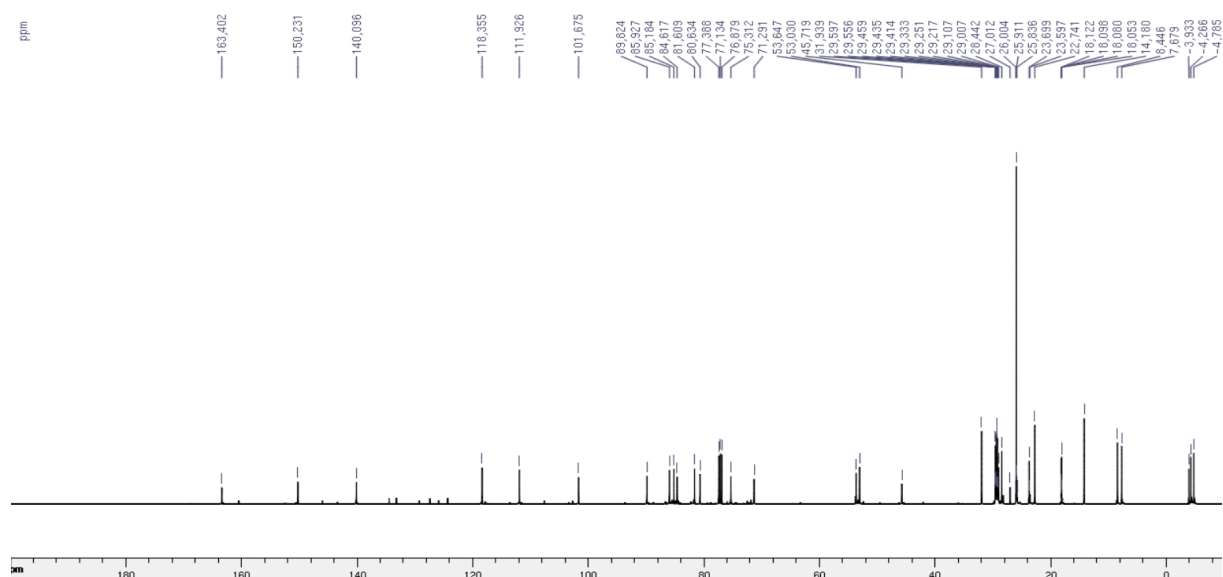
## Sulfonamide 11



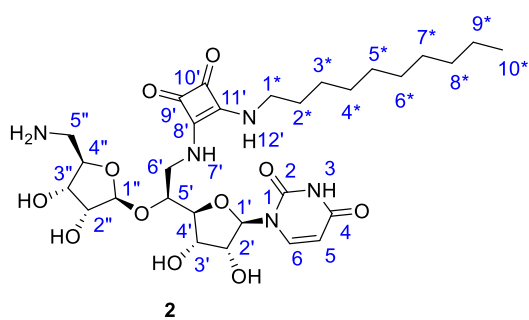
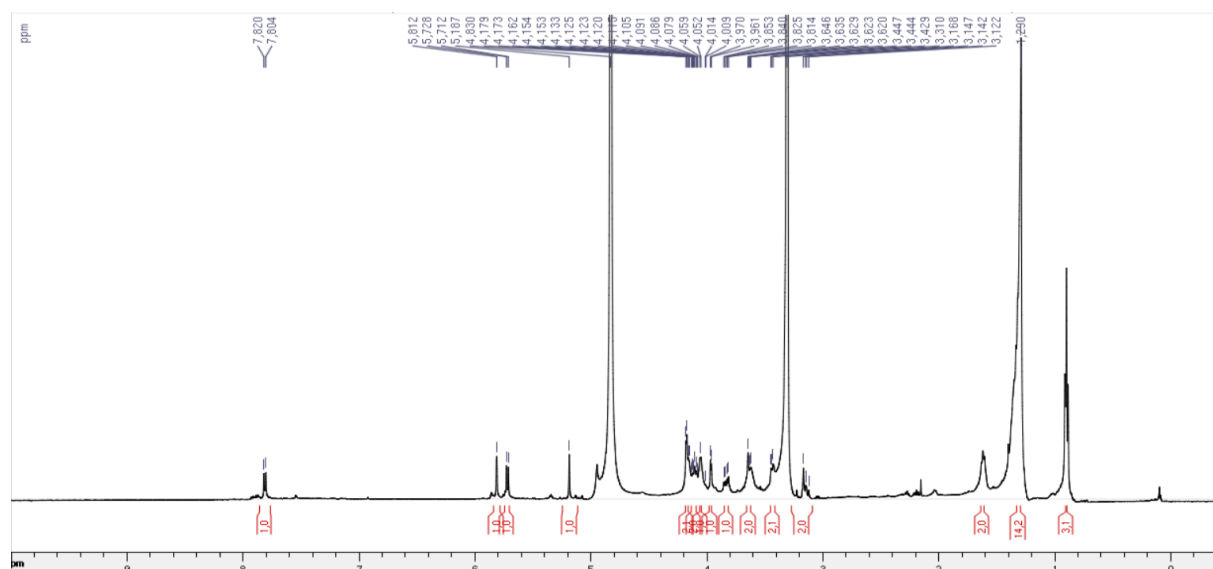
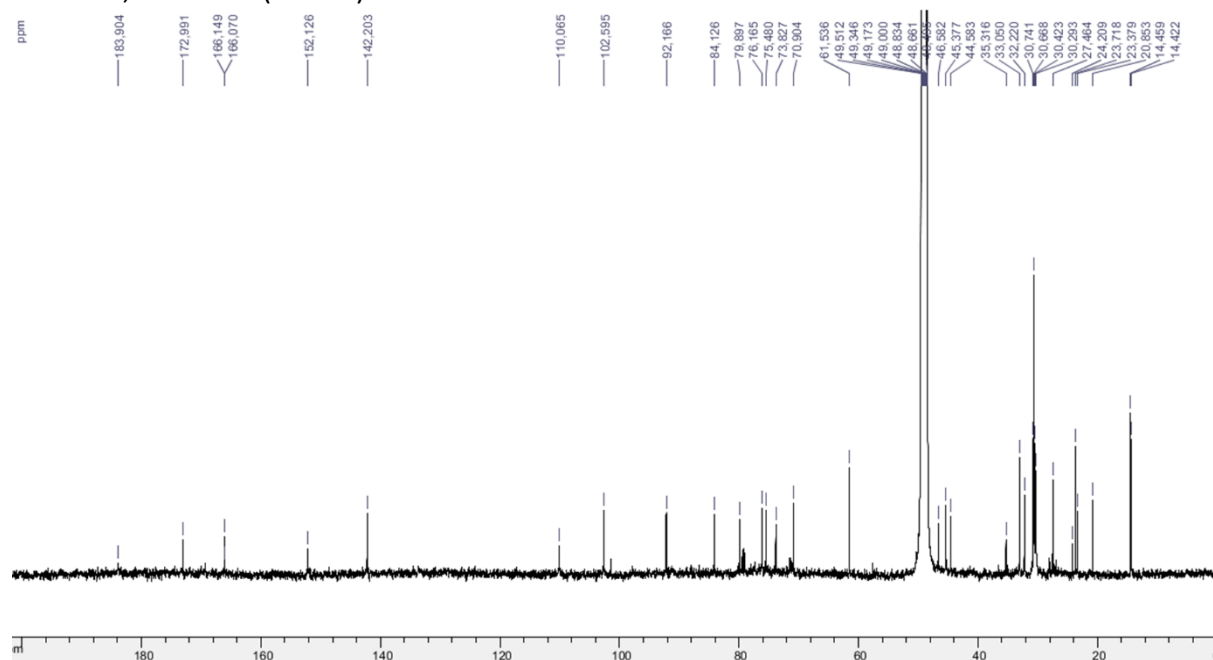
$^1\text{H}$  NMR, 500 MHz ( $\text{CDCl}_3$ )



$^{13}\text{C}$  NMR, 125 MHz ( $\text{CDCl}_3$ )



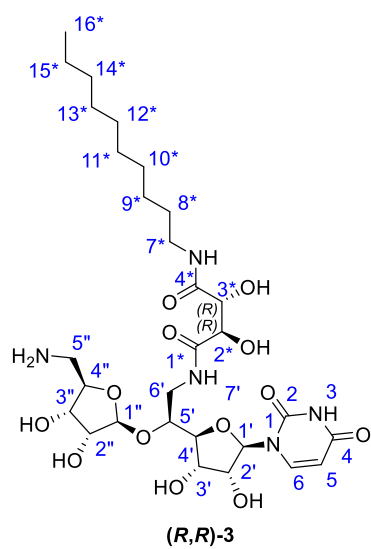
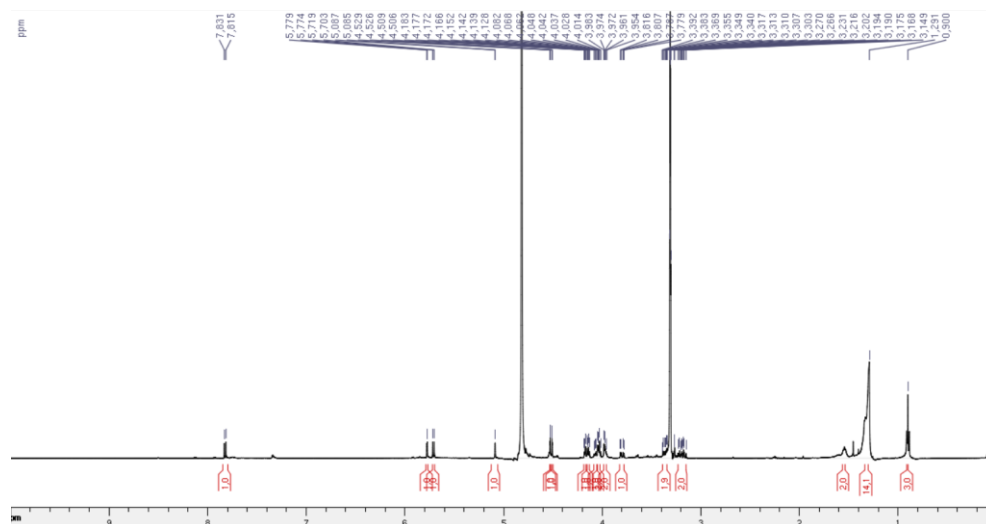
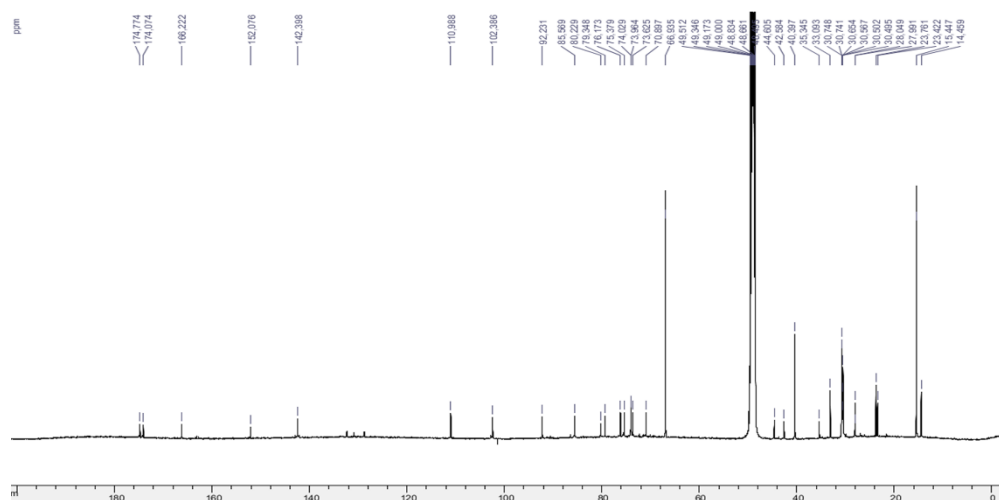
## Squaramide 2

<sup>1</sup>H NMR, 500 MHz (MeOD)<sup>13</sup>C NMR, 125 MHz (MeOD)

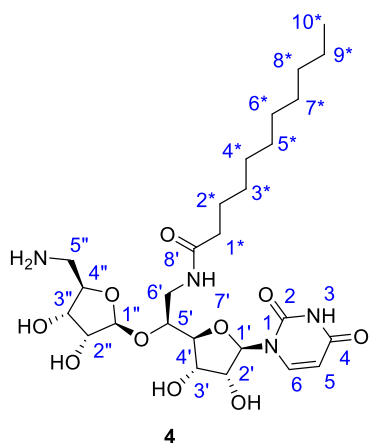
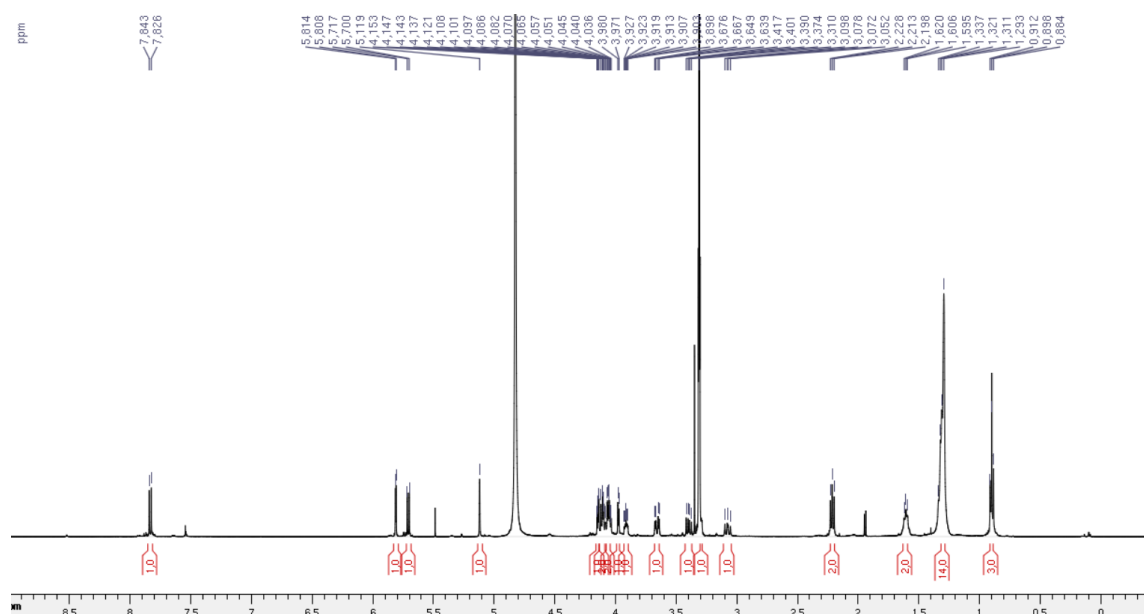
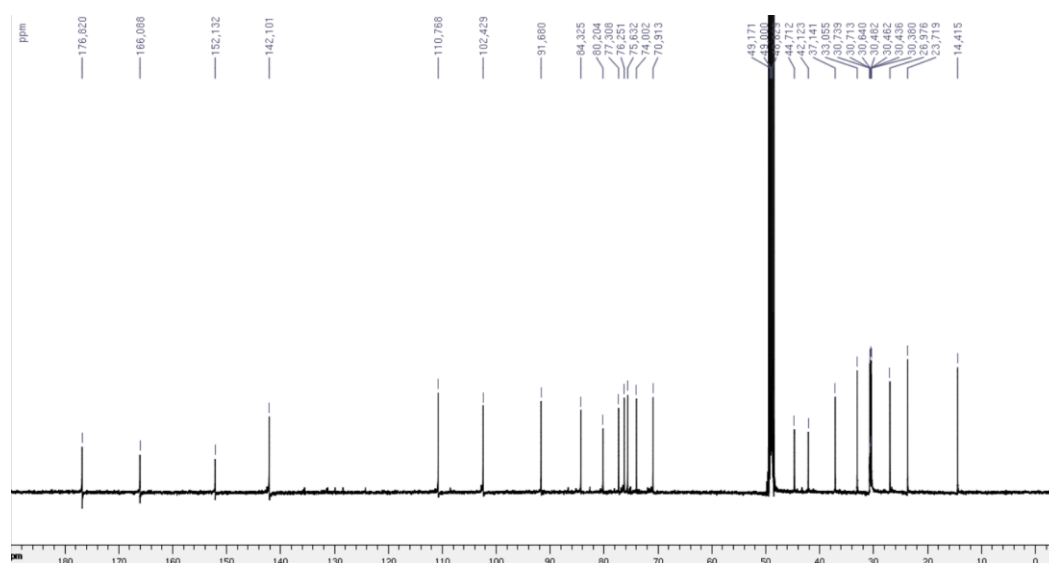
The chemical structure of **(R,R)-3a** is shown. It features a long, branched alkyl chain (16\* to 1\*) attached to a central amide group (NH). This amide group is linked to a central carbon atom (C3\*) which is part of a five-membered ring. This ring is further substituted with a hydroxyl group (OH), an amide group (NH), and a carbonyl group (C=O). The central carbon atom is also linked to another five-membered ring, which is substituted with a hydroxyl group (OH), an amide group (NH), and a carbonyl group (C=O). The structure is labeled **(R,R)-3a**.

<sup>13</sup>C NMR spectrum of compound 10a in CDCl<sub>3</sub>. The spectrum shows peaks from 15 to 175 ppm. Key peaks are labeled with their chemical shifts: 174.759, 174.095, 168.153, 152.090, 142.102, 111.378, 102.386, 91.921, 85.481, 79.767, 76.011, 75.516, 74.895, 74.690, 73.712, 70.094, 66.927, 49.905, 49.300, 49.166, 49.000, 48.884, 48.689, 44.893, 42.801, 42.625, 33.666, 30.733, 30.668, 30.488, 23.154, 15.440, and 14.451. The solvent peak for CDCl<sub>3</sub> is visible at 77.0 ppm.

**(R,R)-Diamide 3b**

<sup>1</sup>H NMR, 500 MHz (MeOD)<sup>13</sup>C NMR, 125 MHz (MeOD)

### Amide 4

<sup>1</sup>H NMR, 500 MHz (MeOD)<sup>13</sup>C NMR, 125 MHz (MeOD)

[illegible]

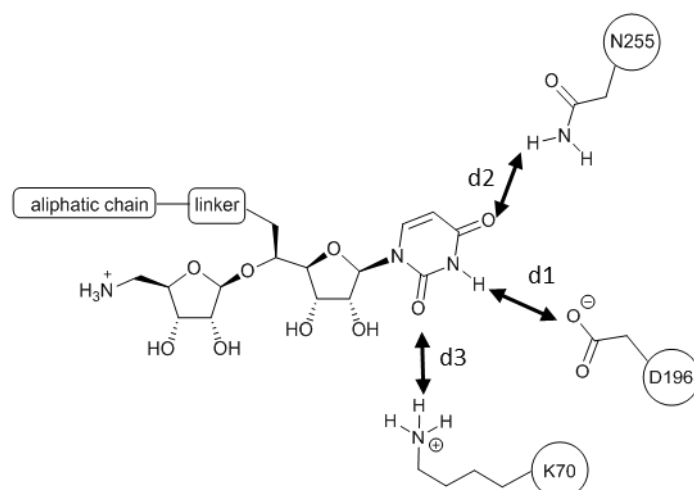
13C NMR spectrum of compound 10. The x-axis represents chemical shift in ppm, ranging from 0 to 180. The spectrum shows several sharp peaks, with the most intense at approximately 50 ppm. Labeled peaks are listed on the right side of the spectrum.

Chemical Shift (ppm)
186.098
152.073
142.304
131.168
129.055
110.387
102.524
92.168
84.785
79.952
78.621
76.213
75.521
72.861
70.996
53.266
49.000
45.536
44.477
33.021
31.623
30.467
30.405
30.279
29.442
24.696
23.696
14.406

**Table S1.** Antibacterial activity of compounds **2-5**, **13** and reference compounds.

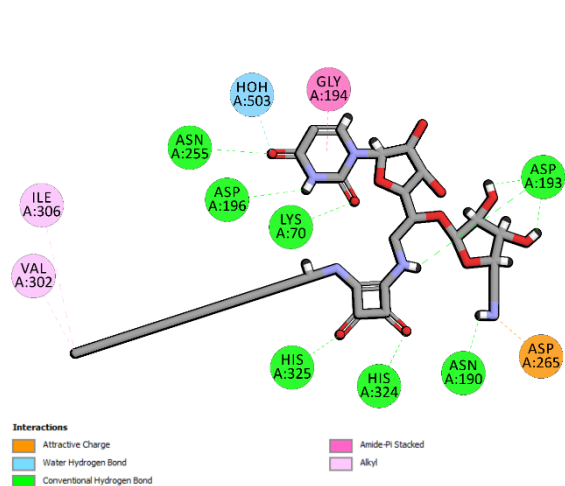
Compound	Linker	CMI (μg/mL)					
		Gram -			Gram +		
		<i>Escherichia coli</i> ATCC 8730	<i>Citrobacter freundii</i> ATCC 8090	<i>Pseudomonas aeruginosa</i> ATCC 27853	<i>Staphylococcus aureus</i> ATCC 25923	<i>Staphylococcus aureus</i> MRSA ATCC 43300	<i>Enterococcus faecium</i> ATCC 19434
Piperacillin		4	4	8	4	>128	4
Vancomycin		-	-	-	1	1	0.5
<b>13</b>	Urea	>128	>128	>128	>128	>128	>128
<b>2</b>	Squaramide	>128	>128	>128	>128	>128	>128
( <i>S,S</i> )- <b>3a</b>	( <i>S,S</i> )-Diamide	>128	>128	>128	128	>128	>128
( <i>R,R</i> )- <b>3b</b>	( <i>R,R</i> )-Diamide	>128	>128	>128	128	>128	>128
<b>4</b>	Amide	>128	>128	>128	128	128	128
<b>5</b>	Sulfonamide	>128	>128	>128	>128	>128	>128

**Table S2:** Distance measurement (Å) between hits and interacting residues of MrpY<sub>AA</sub> binding site from docking experiments in model 5CKR and 6OYH.

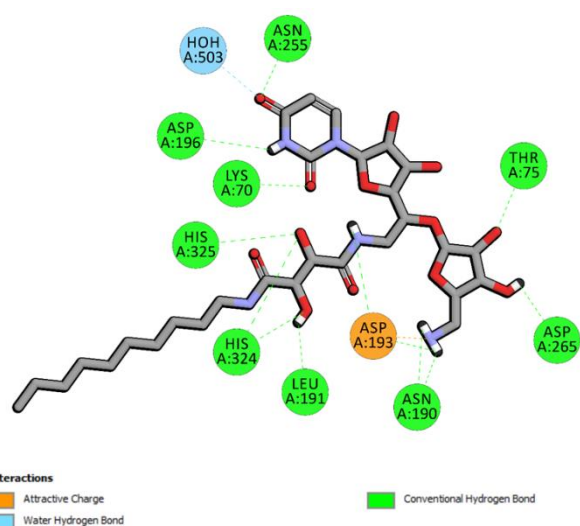


Compound	IC <sub>50</sub>	d1		d2		d3	
		5CKR	6OYH	5CKR	6OYH	5CKR	6OYH
<b>13</b>	1.93 ± 0.13	1.90	2.00	1.94	2.21	1.69	- <sup>a</sup>
<b>2</b>	17.97 ± 0.04	1.86	1.92	2.44	1.84	1.69	2.43
( <i>S,S</i> )- <b>3a</b>	0.37 ± 0.01	1.85	1.85	2.34	2.29	1.79	1.65
( <i>R,R</i> )- <b>3b</b>	1.38 ± 0.05	1.87	1.95	2.43	2.40	1.66	1.73
<b>4</b>	6.49 ± 0.21	2.04	1.94	1.84	2.48	-	3.00
<b>5</b>	2.91 ± 0.13	1.87	1.94	2.46	2.12	2.70	- <sup>a</sup>

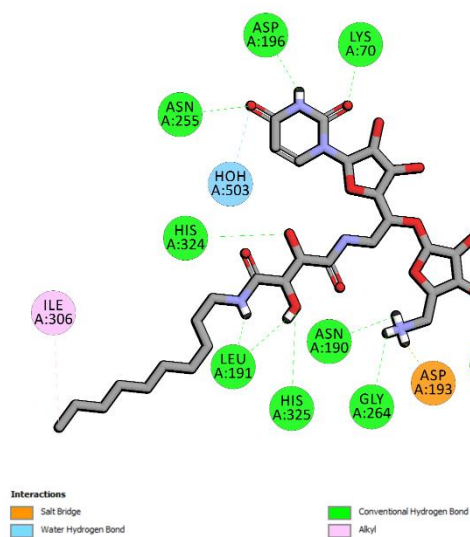
<sup>a</sup> not observed.



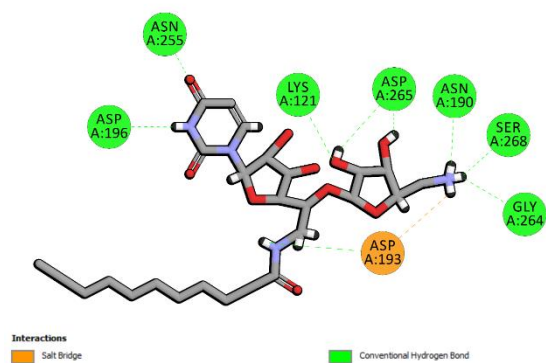
2



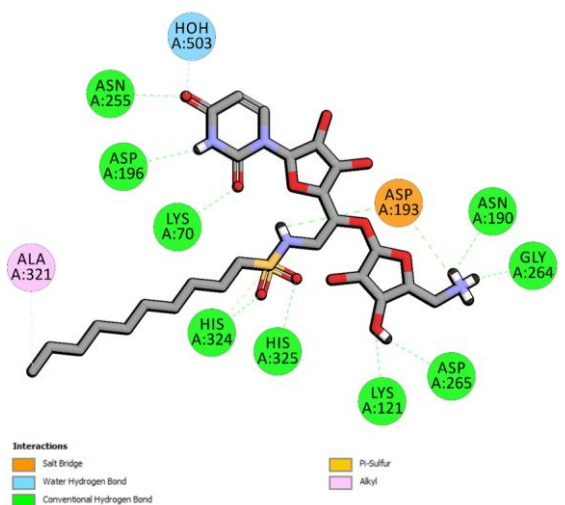
3a



3b

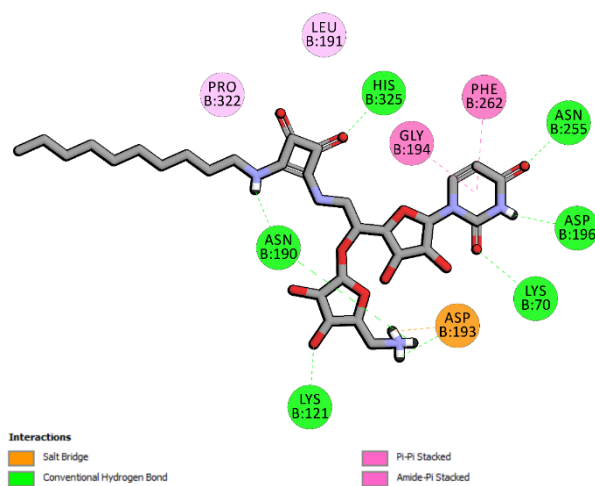


4

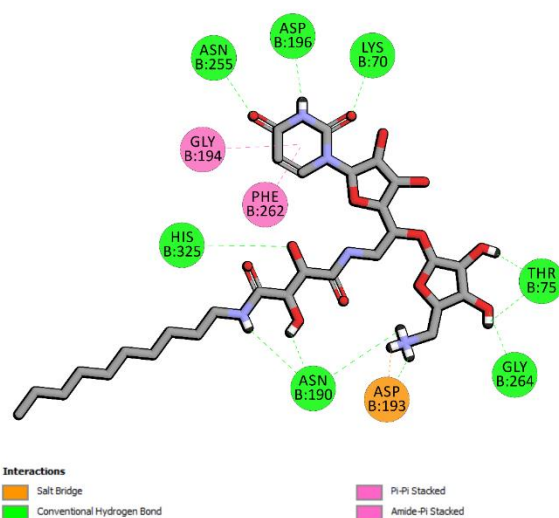


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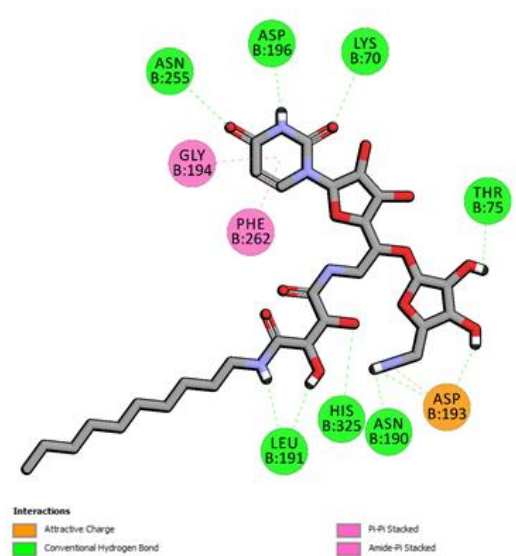
Figure S1. 2D-diagram of ligands interactions in MraY<sub>AA</sub> from docking experiments (PDB: 5CKR)



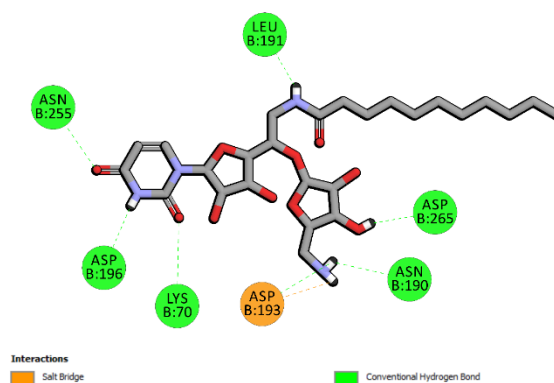
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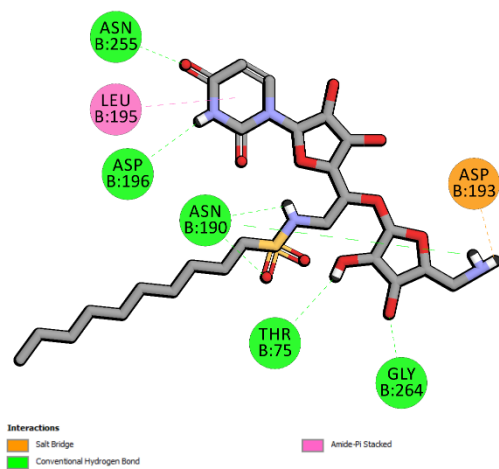
(*S,S*)-3a



(*R,R*)-3b

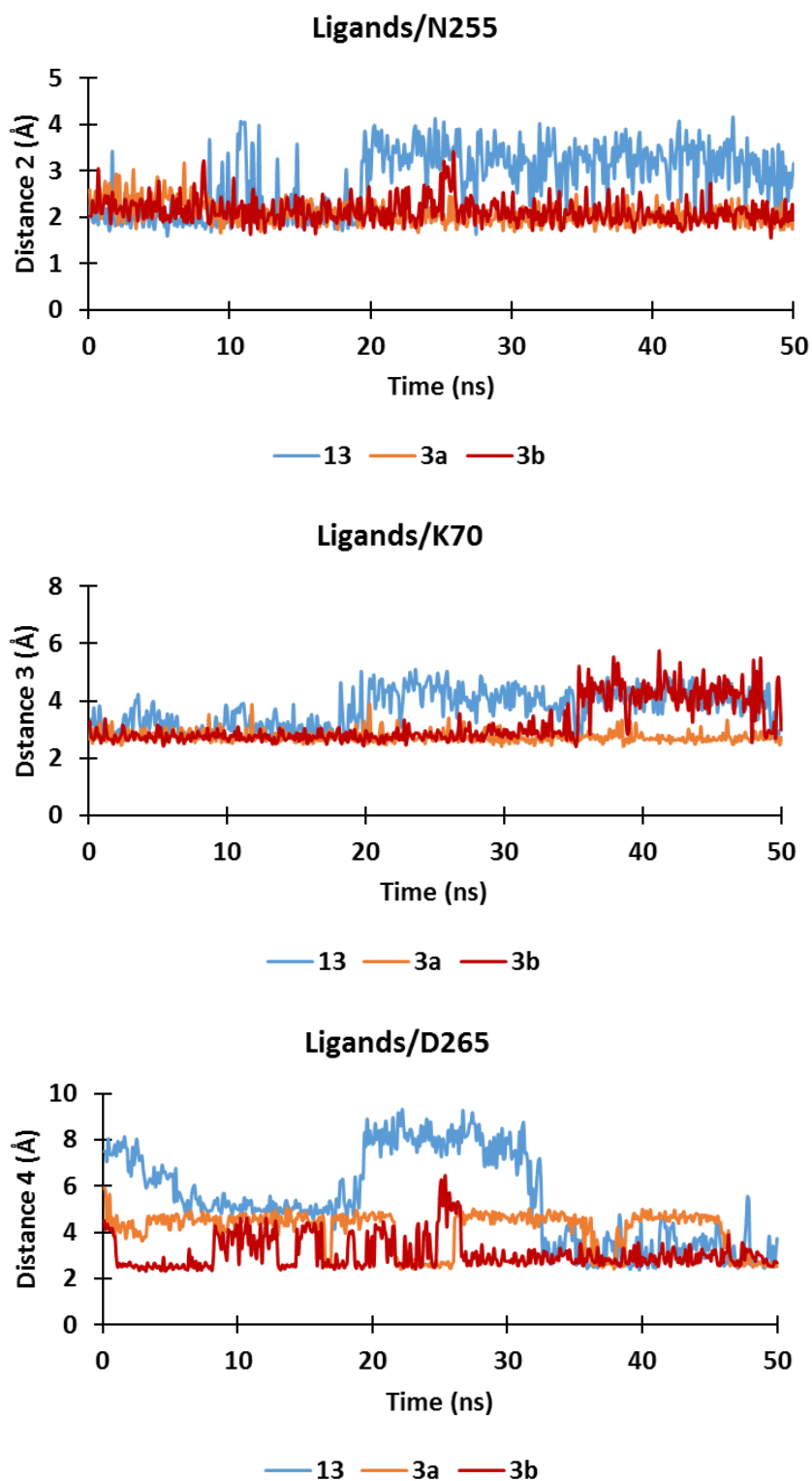


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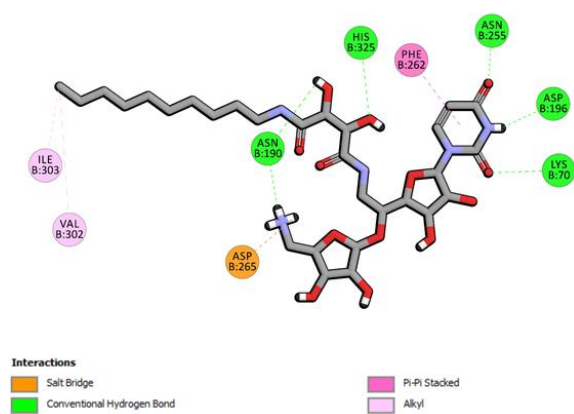


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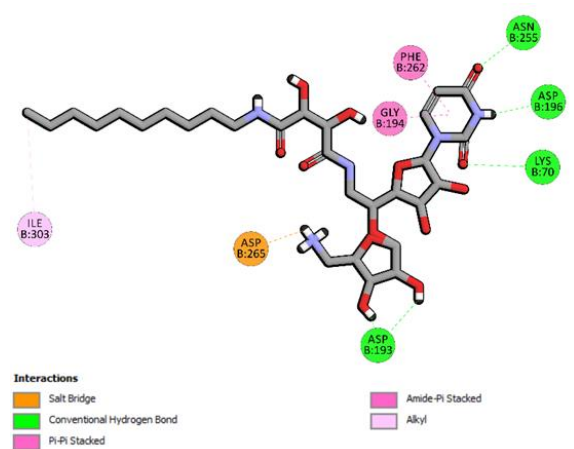
**Figure S2.** 2D-diagram of ligands interactions in MraY<sub>AA</sub> from docking experiments (PDB: 6OYH)



**Figure S3.** Time evolution of the distances between binding site residues of MraY<sub>AA</sub> and ligands atoms. A) Distance d2 between the NH<sub>2</sub> group of N255 and the NH group of the uridine moiety. B) Distance d3 between the centroid of the amino group of K70 and O40 atom of the uridine moiety. C) Distance d4 between the OD1 atom of D265 and the centroid of the amino group of ligands.



**(*S,S*)-3a**



**(*R,R*)-3b**

**Figure S4.** 2D-diagram of ligands interactions in MraY<sub>AA</sub> from 50 ns MD simulations (PDB: 6OYH)