Supporting information for:

Vancomyxins: Vancomycin-Polymyxin Nonapeptide Conjugates That Retain Anti-Gram-Positive Activity with Enhanced Potency against Gram-Negative Strains

Emma van Groesen,[1,‡] Cornelis J. Slingerland,[1,‡] Paolo Innocenti,[1] Milos Mihajlovic,[2] Rosalinde Masereeuw,[2] Nathaniel I. Martin[1,*]

[1]Biological Chemistry Group, Institute of Biology Leiden, Leiden University, Sylviusweg 72, 2333 BE Leiden, The Netherlands.

[2]Division of Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, Universiteitsweg 99, 3584 CG Utrecht, The Netherlands

[‡]These authors contributed equally to this work.

*All correspondence should be directed to n.i.martin@biology.leidenuniv.nl

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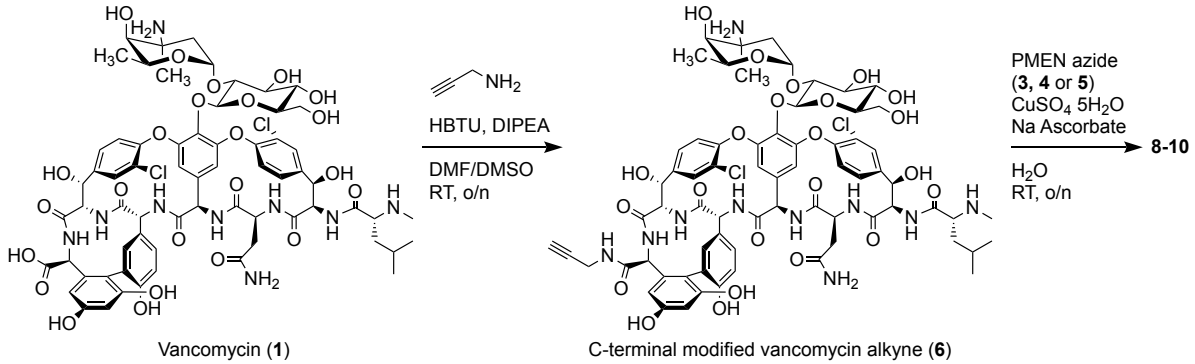
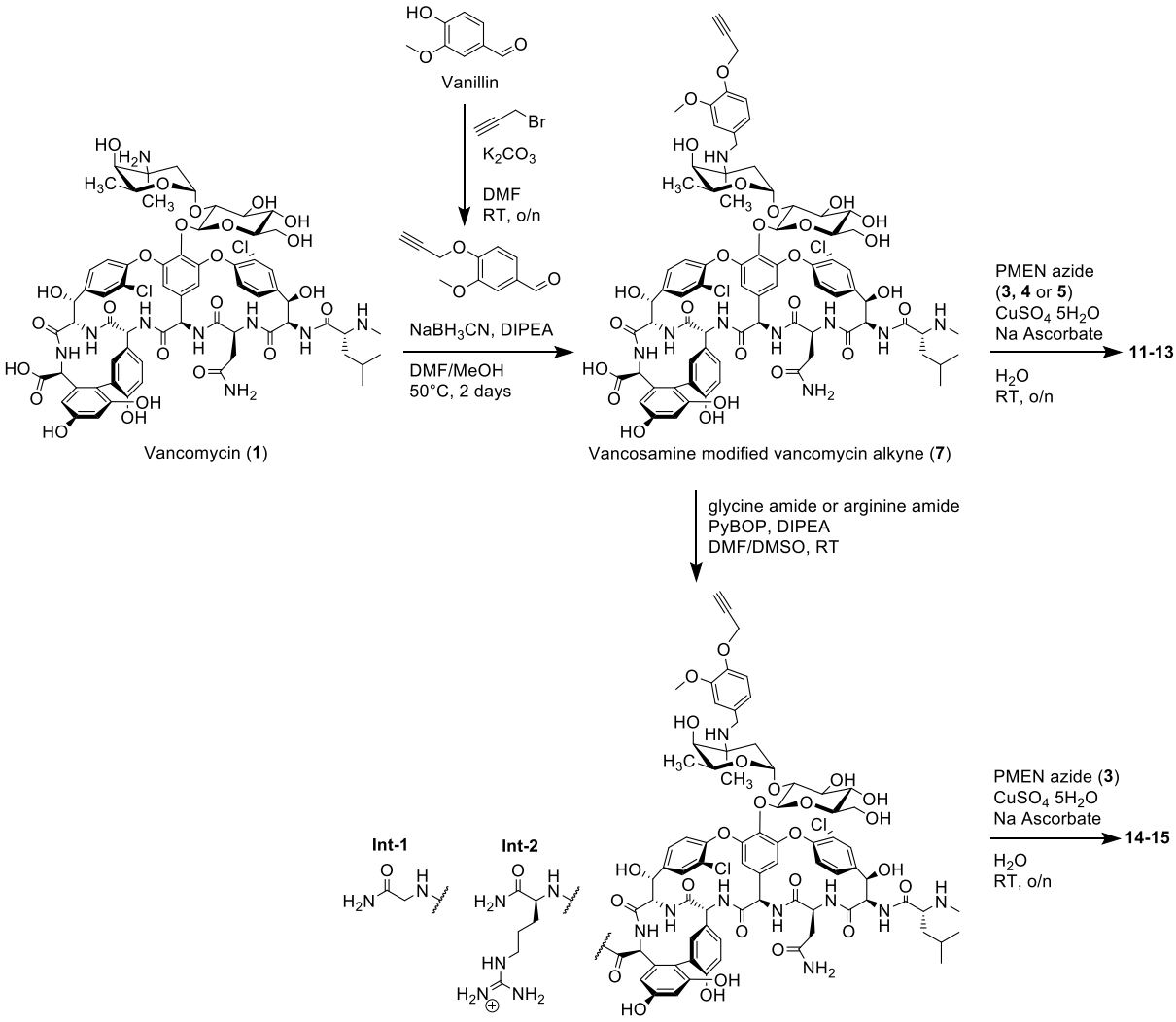
Synthetic schemes S1 and S2



Scheme S1. *Synthesis of vancomyxins **8-10**.*



Scheme S2. *Synthesis of vancomyxins **11-15**.*

General procedures

All reagents were commercially available, American Chemical Society (ACS) grade or finer and used without further purification unless stated otherwise. For characterization of new compounds high resolution mass spectrometry (HRMS) was performed on a Shimadzu Nexera X2 UHPLC system with a Waters Acquity HSS C_{18} column (2.1 × 100 mm, 1.8 µm) at 30 °C and equipped with a diode array detector. At a flow rate of 0.5 mL/min, a solvent system with solvent A, 0.1% formic acid in water, and solvent B, 0.1% formic acid in acetonitrile, was used. Gradient elution was as follows: 95:5 (A/B) for 1 min, 95:5 to 15:85 (A/B) over 6 min, 15:85 to 0:100 (A/B) over 1 min, 0:100 (A/B) for 3 min, then reversion back to 95:5 (A/B) for 3 min. This system was connected to a Shimadzu 9030 QTOF mass spectrometer (ESI ionization) calibrated internally with Agilent's API-TOF reference mass solution kit (5.0 mM purine, 100.0 mM ammonium trifluoroacetate and 2.5 mM hexakis(1*H*,1*H*,3*H*-tetrafluoropropoxy)phosphazine) diluted to achieve a mass count of 10000. Purity and confirmation of the synthesis of small molecule building blocks, although previously reported in the literature, was assessed with nuclear magnetic resonance (NMR). Spectra were obtained from a Bruker DPX-300, super conducting magnet with a field strength of 7.0 Tesla, equipped with 5 mm BBO, Broadband Observe probe head, high resolution with Z- Gradient, and a 5 mm 19F / 1H dual high-resolution probe.

Compounds were purified using preparative high performance liquid chromatography (HPLC) using a BESTA-Technik system with a Dr. Maisch Reprosil Gold 120 C_{18} column (25 × 250 mm, 10 µm) and equipped with a ECOM Flash UV detector monitoring at 214 nm. All compounds were ran at a flow rate of 12 mL/min. For the vancomyxins and PMEN a solvent system with solvent A, 0.1% TFA in water/acetonitrile 95:5, and solvent B, 0.1% TFA in water/acetonitrile 5:95, was used. For the vancomyxins (**8-15**) the gradient elution was as follows: 95:5 (A/B) for 5 min, 95:5 to 40:60 (A/B) over 50 min, 40:60 to 0:100 (A/B) for 1 min, 0:100 (A/B) for 2 min, then reversion back to 95:5 (A/B) over 1 min, 95:5 (A/B) for 2 min. For PMEN the gradient elution was as follows: 100:0 (A:B) for 5 min, 100:0 to 70:30 (A:B) over 50 min, 70:30 to 0:100 (A:B) for 1 min, 0:100 (A:B) for 2 min, then reversion back to 100:0 (A:B) over 1 min, 100:0 (A:B) for 2 min.

The vancomycin building blocks (**7**, **Int-1, Int-2**) had an alternative solvent system of solvent A, 50 mM ammonium acetate, and solvent B, water/acetonitrile 5/95. Gradient elution was as follows: 95:5 (A/B)

for 2 min, 95:5 to 80:20 (A/B) for 5 min, 80:20 to 40:60 (A/B) over 40 min, 40:60 to 0:100 (A/B) for 1 min, 0:100 (A/B) for 2 min, then reversion back to 95:5 (A/B) over 1 min, 95:5 (A/B) for 2 min.

Purity of the vancomyxins was assessed by integration and confirmed to be >95% unless stated otherwise, using analytical reverse phase HPLC (RP-HPLC) using a Shimadzu Prominence-i LC-2030 system with a Dr. Maisch ReproSil Gold 120 C_{18} column (4.6 × 250 mm, 5 µm) at 30 °C and equipped with a UV detector monitoring at 214 nm. At a flow rate of 1 mL/min, a solvent system with solvent A, 0.1% TFA in water/acetonitrile 95:5, and solvent B, 0.1% TFA in water/acetonitrile 5:95, was used. Gradient elution was as follows: 95:5 (A/B) for 2 min, 95:5 to 0:100 (A/B) over 55 min, 0:100 (A/B) for 2 min, then reversion back to 95:5 (A/B) over 1 min, 95:5 (A/B) for 2 min.

Bacterial strains used for MIC assays

The following strains were obtained from BEI Resources, NIAID, NIH: *Staphylococcus aureus*, Strain HIP12864, NR-46074. *Staphylococcus aureus*, Strain LIM 2, NR-45881. *Staphylococcus aureus*, Strain HIP11983, NR-46411. *Staphylococcus aureus*, Strain HIP13419, NR-46413.

Synthesis of PMEN

PMEN was obtained by enzymatic digestion of colistin by ficin, by modification of a previously reported method.[1] Colistin sulphate (4.8 mmol, 1 eq) was dissolved in demiwater (180 mL). To the solution were added dithiothreitol (1.3 mmol, 0.3 eq) and ficin (~0.06 mmol, 0.01 eq). Enzymatic cleavage was run at 37 ^{o}C under nitrogen atmosphere overnight. Additional dithiothreitol (0.3 mmol 0.06 eq) and ficin (~0.01 mmol, 0.002 eq) were added, followed by incubation overnight. Once complete, the solution was heated to reflux for 20 min, cooled down and filtered. The filtrate was adjusted to pH 2 with 5 M HCl. Sample was extracted by *n*-butanol (5 x 50 mL). The pH of the aqueous layer was neutralized with 6 M NaOH. The resulting sample was lyophilized after addition of *t*-BuOH. Pure PMEN was obtained by reverse phase HPLC purification.

Synthesis of PMEN-Boc$_4$

PMEN-Boc_4 was prepared as previously described.[2] PMEN (semi-pure after extraction, 2.0 g, 2.2 mmol) was dissolved in water (13 mL). Triethylamine (13 mL) was added to it and the mixture was stirred for 5 min. 2-(Boc-oxyimino)-2-phenylacetonitrile (Boc-ON) was dissolved in dioxane (13 mL)

and added to the PMEN. Reaction was run at RT for 25 min. Reaction was quenched by the addition of methanolic NH_3 (7 M, 8 mL). The resulting mixture was concentrated on the rotavap. The residue was dissolved in MeOH (200 mL) and filtered. The filtrate was collected, concentrated and subjected to flash column chromatography (5% MeOH/DCM – 10% MeOH/DCM/0.5% Et_3N). Relevant fractions were combined and solvent was evaporated. Yield: 1.9 g (1.4 mmol, 65% (~ 90% pure)).

*Synthesis of PMEN azides **3-5***

*PMEN-C_2-N_3 (**3**)*

PMEN-Boc_4 (0.45 g, 0.34 mmol) was dissolved in DCM and DMF (8:2 v:v, 10 mL). In a separate flask, 2-azidoacetic acid (68 mg, 0.68 mmol) and BOP (0.30 g, 0.68 mmol) were dissolved in DCM (8 mL). The mixture of 2-azidoacetic acid and BOP was then added to the PMEN-Boc_4, followed by addition of DIPEA (0.24 mL, 1.4 mmol). The reaction was left to stir overnight at RT under N_2 atmosphere. After completion, the solvent was evaporated and the residue treated with TFA/TIPS/H_2O (95:2.5:2.5, 8 mL) for 1.5 hours. The reaction mixture was added to ice-cold MTBE/PE (2/1, 120 mL). The resulting precipitate was washed with MTBE/PE (2/1). Crude peptide was lyophilized from t-BuOH/H_2O and HPLC purified. Yield: 130 mg, 0.13 mmol, 39%.

*PMEN-C_5-N_3 (**4**)*

Compound was prepared as PMEN-C_2-N_3 (**3**), starting from PMEN-Boc_4 and 5-azidopentanoic. Yield: 85 mg, 0.08 mmol, 36%.

*PMEN-$(PEG)_3$-N_3 (**5**)*

Compound was prepared as PMEN-C_2-N_3 (**3**), starting from PMEN-Boc_4 and 3-(2-(2-(2-azidoethoxy)-ethoxy)ethoxy)propanoic acid. Yield: 120 mg, 0.11 mmol, 43%.

*Synthesis of vancomycin alkynes **6-7***

Vancomycin alkyne building block **6** was synthesized as previously described[3] and used without any further purification. Vancomycin alkyne building block **7** was synthesized according to procedures described previously with minor alterations.[3,4] In short, 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde was synthesized starting from vanillin as described in the literature.[5] Subsequently, amine-derivatized

vancomycin **7** was prepared by dissolving vancomycin HCl (1.3 mmol, 1 eq) in 1/1 DMF/MeOH (40 mL). 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde (2.6 mmol, 2 eq) and DIPEA (6.5 mmol, 5 eq) were added and the reaction was stirred at 70 °C for 2 hours. Next the mixture was cooled to 50 °C and $NaBH_3CN$ (13 mmol, 10 eq) was added. After 5 hours another 10 eq of $NaBH_3CN$ were added and after 16 hours again 1 eq of 3-methoxy-4-(prop-2-yn-1-yloxy)benzaldehyde and 10 eq of $NaBH_3CN$ were added. The reaction was stirred for another 24 hours before a few mL of water were added. Solvent was evaporated and the mixture was dissolved in a minimum amount of DMF. Product was precipitated in cold Et_2O twice (2 x 600 mL). The precipitate was redissolved in HPLC buffer and purified using preparative HPLC. Fractions were analyzed using analytical HPLC and pure fractions were pooled and lyophilized.

*Synthesis of C-terminally modified vancomycin alkynes **Int-1** and **Int-2***

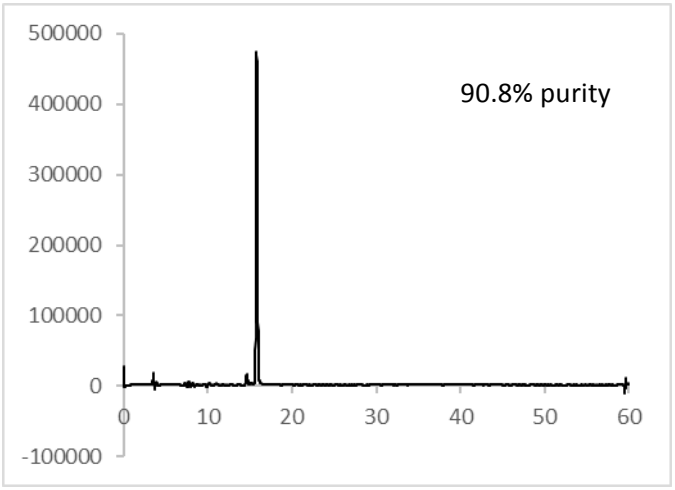
Int-1 and **Int-2** were synthesized according to a previous procedure.[6] In short, **7** (62 µmol, 1 eq) was dissolved in 1/1 DMF/DMSO (5 mL). Glycine amide HCl or arginine amide HCl (124 µmol, 2 eq) was added and the mixture was cooled to 0 °C. DIPEA (310 µmol, 5 eq) and PyBOP (93 µmol, 1.5 eq) were added. The mixture was allowed to warm to RT and stirred for 16 hours. Additional equivalents of glycine/arginine amide HCl, DIPEA, and PyBOP were added at 0 °C and the reaction was further stirred at RT until LCMS showed complete disappearance of starting material **7**. DMF was evaporated and 600 mL acetonitrile was added to precipitate the product. The mixture was passed over a filter and washed with 600 mL acetonitrile and 600 mL Et_2O. **Int-1** was used in the next reaction without intermediate purification. **Int-2** was dissolved in HPLC buffer and purified using preparative HPLC. Fractions were analyzed using analytical HPLC and pure fractions were pooled and lyophilized.

HRMS analysis and yields

Sample ID	Chemical formula	Calculated M + H	Calculated (M+2H)/2	Measured	Yield
3	$C_{42}H_{77}N_{17}O_{12}$	1012.6016	506.8047	506.8043	39%
4	$C_{45}H_{83}N_{17}O_{12}$	1054.6485	527.8282	527.8278	36%
5	$C_{49}H_{91}N_{17}O_{15}$	1158.6959	579.8519	579.8515	43%
6	$C_{69}H_{78}Cl_2N_{10}O_{23}$	1485.4696	743.2387	743.2379	65%
7	$C_{77}H_{85}Cl_2N_9O_{26}$	1622.5061	811.7570	811.7562	52%
8	$C_{111}H_{155}Cl_2N_{27}O_{35}$	2497.0634	1249.0356	1249.0345	54%
9	$C_{114}H_{161}Cl_2N_{27}O_{35}$	2539.1103	1270.0591	1270.0581	74%
10	$C_{118}H_{169}Cl_2N_{27}O_{38}$	2643.1577	1322.0828	1322.0821	55%
11	$C_{119}H_{162}Cl_2N_{26}O_{38}$	2634.0998	1317.5538	1317.5525	53%
12	$C_{122}H_{168}Cl_2N_{26}O_{38}$	2676.1468	1338.5773	1338.5762	38%
13	$C_{126}H_{176}Cl_2N_{26}O_{41}$	2780.1941	1390.6010	1390.6000	18%
Int-1	$C_{79}H_{89}Cl_2N_{11}O_{26}$	1678.5435	839.7757	839.7769	78%
Int-2	$C_{83}H_{98}Cl_2N_{14}O_{26}$	1777.6232	889.3155	889.3165	29%
14	$C_{121}H_{166}Cl_2N_{28}O_{38}$	2690.1373	1345.5726	1345.5742	44%
15	$C_{125}H_{175}Cl_2N_{31}O_{38}$	2789.2169	1395.1124	1395.1142	51%

Analytical HPLC analysis (purity values based on integration)

Compound 3



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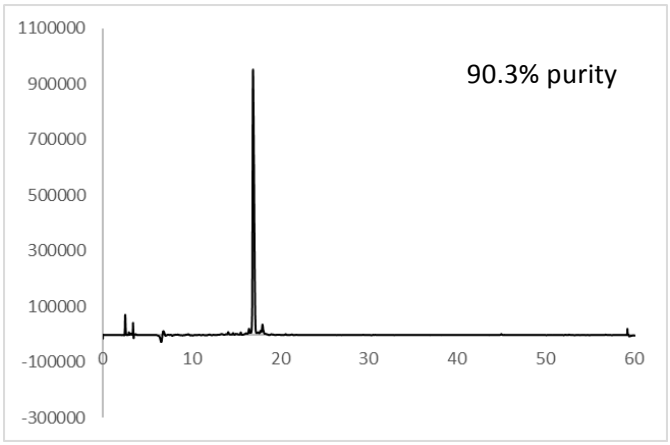

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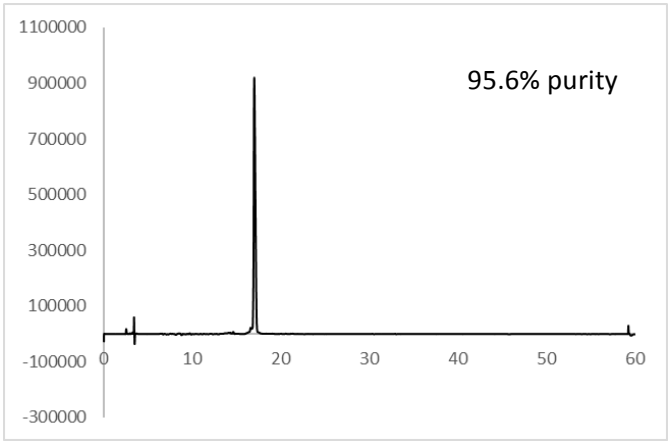

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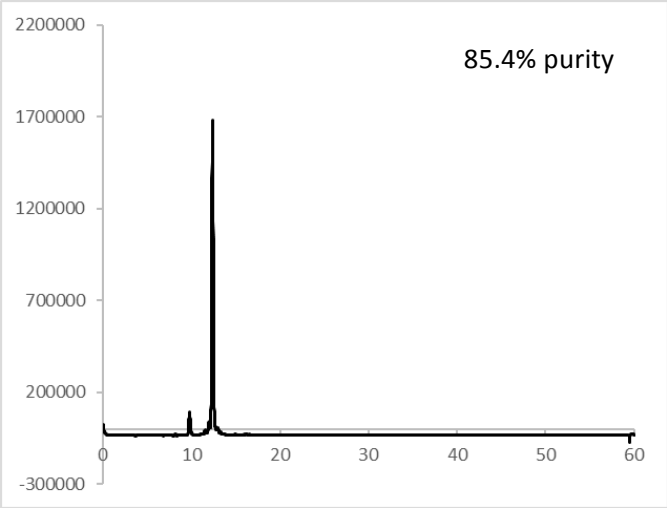

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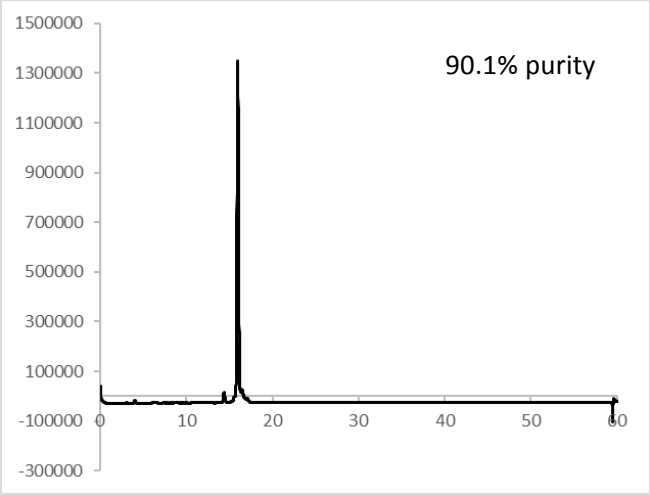
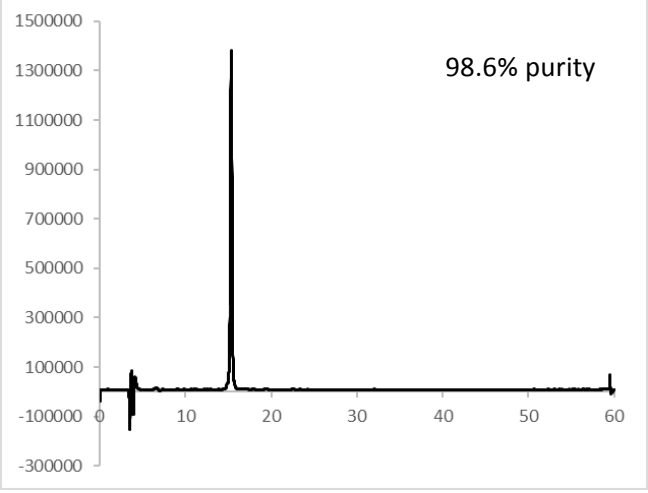


Vancomyxin 8



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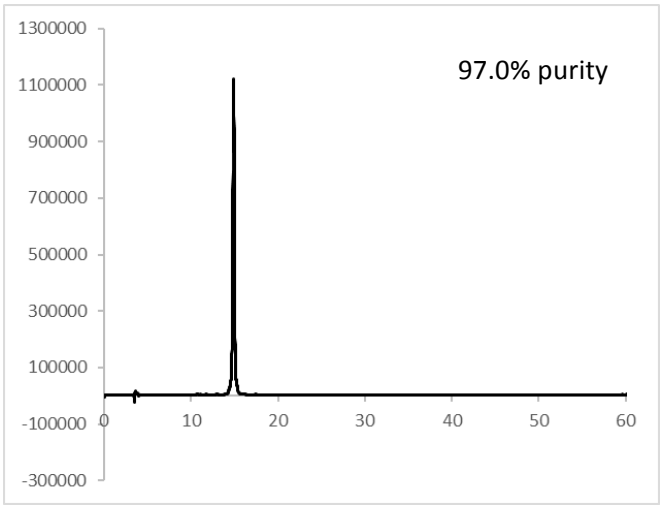

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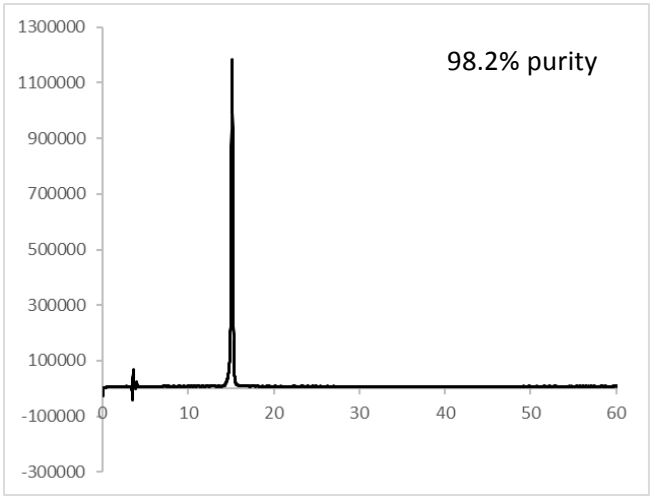

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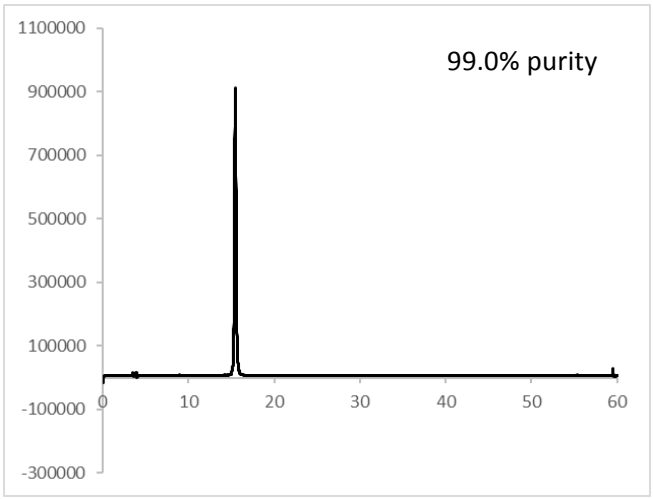

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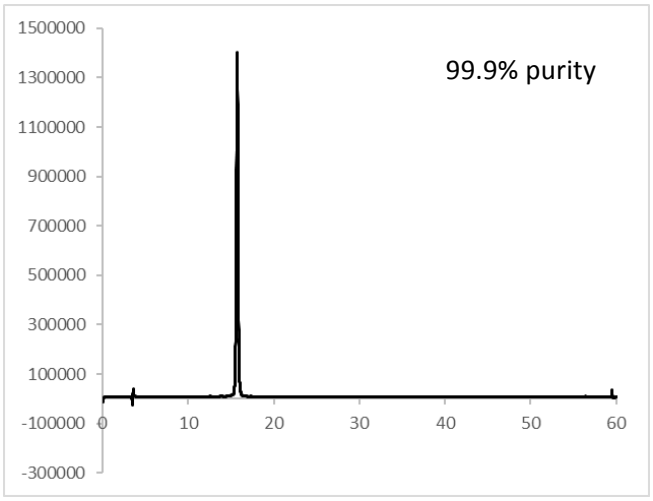

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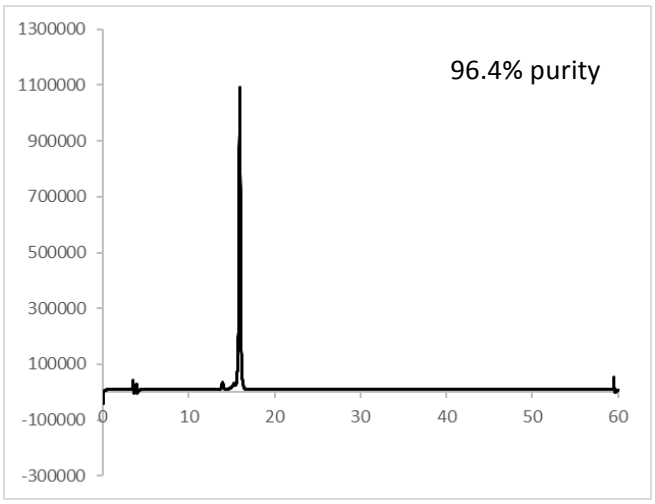

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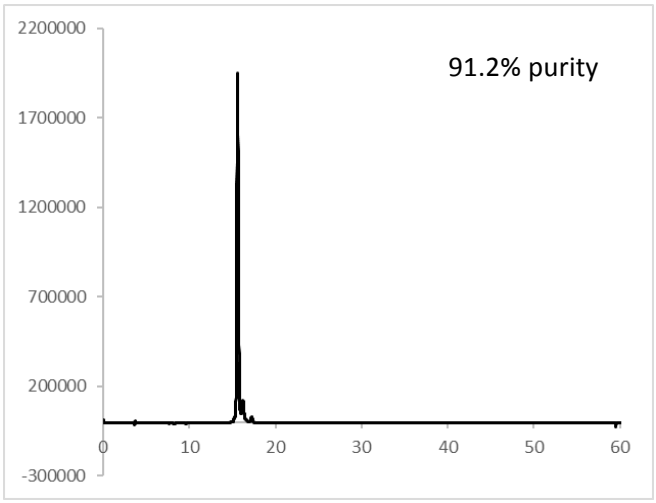

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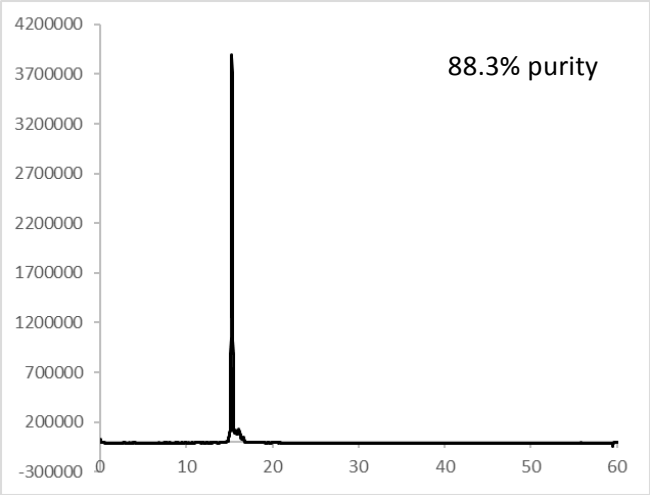

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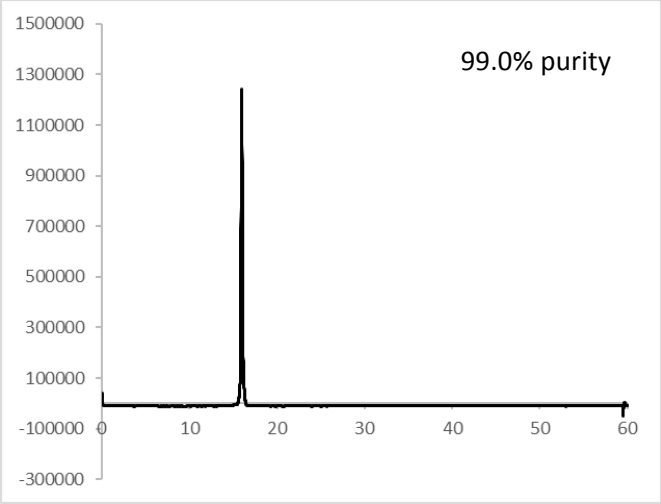

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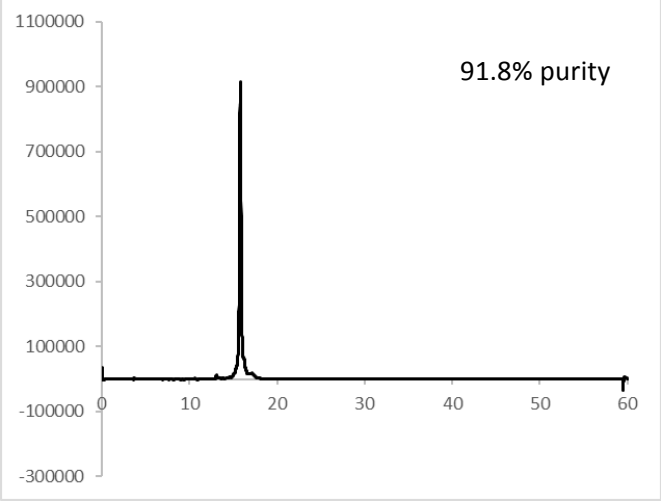

Tables S1, S2, S3 Expanded activity assessment and Table S4 LPS antagonization assay

Table S1. Expanded activity assessment of PMEN azide building blocks against Gram-positive and Gram-negative bacteria.

Bacterial strain		**MIC in µg/mL** (MIC in µM shown in brackets)					
Strain	**ID**	**Vancomycin**	**PMEN**	**Colistin**	**3**	**4**	**5**
Gram-negative bacteria							
E. coli	ATCC25922	>128 (>86)	>128 (>138)	0.5 (0.428)	64 (63)	8 (8)	128 (111)
	ATCC35218	128 (86)	>128 (>138)	0.125 (0.107)	64 (63)	8 (8)	32 (28)
K. pneumonia	ATCC13883	>128 (>86)	>128 (>138)	0.5 (0.428)	32 (32)	4 (4)	32 (28)
	ATCC27736	>128 (>86)	>128 (>138)	0.25 (0.214)	32 (32)	8 (8)	64 (55)
A. baumannii	BAA-747	>128 (>86)	>128 (>138)	0.125 (0.107)	>128 (>126)	>128 (>121)	>128 (>111)
P. aeruginosa	ATCC10145	>128 (>86)	>128 (>138)	1 (0.855)	8 (8)	4 (4)	16 (14)
Gram-positive bacteria							
S. aureus	MSSA ATCC29213	0.125 (0.084)	>128 (>138)	>128 (>109)	>16 (>16)	>16 (>15)	>16 (>14)
	MRSA USA300	0.25 (0.168)	>128 (>138)	>128 (>109)	>16 (>16)	>16 (>15)	>16 (>14)

MIC = Minimum inhibitory concentration, ND = not determined, PMEN = Polymyxin E nonapeptide, MSSA = Methicillin-sensitive *S. aureus*, MRSA = Methicillin-resistant *S. aureus.*

Table S2. Expanded activity assessment of PMEN, colistin, and the vancomyxin-alkyne building blocks against Gram-positive and Gram-negative bacteria.

Bacterial strain		**MIC in μg/mL** (MIC in μM shown in brackets)						
Strain	**ID**	**Vancomycin**	**PMEN**	**Colistin**	**6**	**7**	**Int-1**	**Int-2**
Gram-negative bacteria								
E. coli	ATCC25922	>128 (>86)	>128 (>138)	0.5 (0.428)	64 (43)	>128 (>79)	64 (38)	16 (9)
	ATCC35218	128 (86)	>128 (>138)	0.125 (0.107)	32 (22)	>128 (>79)	32 (19)	8 (4)
K. pneumonia	ATCC13883	>128 (>86)	>128 (>138)	0.5 (0.428)	>128 (>86)	>128 (>79)	>128 (>76)	128 (72)
	ATCC27736	>128 (>86)	>128 (>138)	0.25 (0.214)	>128 (>86)	>128 (>79)	>128 (>76)	128 (72)
A. baumannii	ATCC17978	>128 (>86)	>128 (>138)	0.25 (0.214)	128 (86)	>128 (>79)	128 (76)	128 (72)
	BAA-747	>128 (>86)	>128 (>138)	0.125 (0.107)	128 (86)	>128 (>79)	128 (76)	128 (72)
P. aeruginosa	ATCC10145	>128 (>86)	>128 (>138)	1 (0.855)	>128 (>86)	>128 (>79)	>128 (>76)	>128 (>72)
	ATCC27853	>128 (>86)	>128 (>138)	0.5 (0.428)	>128 (>86)	>128 (>79)	>128 (>76)	>128 (>72)
Gram-positive bacteria								
B. subtilis	168	0.25 (0.168)	>128 (>138)	8 (7)	0.125 (0.084)	0.5 (0.308)	0.125 (0.074)	0.016 (0.009)
S. simulans	22	0.125 (0.084)	>128 (>138)	4 (3)	0.25 (0.168)	1 (0.616)	0.25 (0.149)	0.125 (0.07)
S. aureus	MSSA ATCC29213	0.125 (0.084)	>128 (>138)	>128 (>109)	0.5 (0.336)	2 (1)	0.5 (0.298)	0.5 (0.281)
	MRSA USA300	0.25 (0.168)	>128 (>138)	>128 (>109)	0.5 (0.336)	2 (1)	0.5 (0.298)	0.5 (0.281)
	VISA LIM-2	4 (3)	>128 (>138)	>128 (>109)	2 (1)	16 (10)	4 (2)	2 (1)
	VISA NRS402	8 (5)	>128 (>138)	>128 (>109)	4 (3)	16 (10)	4 (2)	4 (2)
	VRSA 2 (vanA)	128 (86)	>128 (>138)	>128 (>109)	>128 (>86)	>128 (>79)	>128 (>76)	>128 (>72)
	VRSA 3b (vanA)	>128 (>86)	>128 (>138)	>128 (>109)	>128 (>86)	>128 (>79)	>128 (>76)	>128 (>72)
E. faecalis	VRE E1246 (vanA)	>128 (>86)	>128 (>138)	>128 (>109)	>128 (>86)	>128 (>79)	>128 (>76)	>128 (>72)
	VRE E74064 (vanB)	32 (22)	>128 (>138)	>128 (>109)	16 (11)	32 (20)	64 (38)	32 (18)
E. faecium	VSE E980	0.5 (0.337)	>128 (>138)	>128 (>109)	1 (0.673)	1 (0.616)	0.5 (0.298)	0.25 (0.141)
	VRE E155 (vanA)	>128 (>86)	>128 (>138)	>128 (>109)	>128 (>86)	>128 (>79)	>128 (>76)	64 (36)
	VRE E7314 (vanB)	128 (86)	>128 (>138)	>128 (>109)	128 (86)	64 (39)	32 (19)	4 (2)

MIC = Minimum inhibitory concentration, ND = not determined, PMEN = Polymyxin E nonapeptide, MSSA = Methicillin-sensitive *S. aureus*, MRSA = Methicillin-resistant *S. aureus*, VISA = Vancomycin-intermediate *S. aureus*, VRSA = Vancomycin-resistant *S. aureus*, VSE = Vancomycin-sensitive *Enterococci*, VRE = Vancomycin-resistant *Enterococci*

Table S3. Expanded assessment of **8**, **9**, **11**, **12**, **14**, and **15** against Gram-negative and Gram-positive bacteria.

Bacterial strain					**MIC in µg/mL** (MIC in µM shown in brackets)					
Strain	**ID**	**Vancomycin**	**PMEN**	**Vancomycin + 8 µg/mL PMEN**	**8**	**9**	**11**	**12**	**14**	**15**
Gram-negative bacteria										
E. coli	ATCC25922	>128 (>86)	>128 (>138)	32 (22)	16 (6)	16 (6)	16 (6)	16 (6)	16 (6)	16 (6)
	ATCC35218	128 (86)	>128 (>138)	32 (22)	16 (6)	16 (6)	16 (6)	16 (6)	8 (3)	8 (3)
K. pneumonia	ATCC13883	>128 (>86)	>128 (>138)	128 (86)	32 (13)	32 (13)	8 (3)	8 (3)	16 (6)	32 (11)
	ATCC27736	>128 (>86)	>128 (>138)	128 (86)	32 (13)	16 (6)	8 (3)	16 (6)	16 (6)	16 (6)
A. baumannii	ATCC17978	>128 (>86)	>128 (>138)	128 (86)	128 (51)	64 (25)	32 (12)	32 (12)	128 (48)	128 (46)
	BAA-747	>128 (>86)	>128 (>138)	128 (86)	32 (13)	64 (25)	32 (12)	32 (12)	16 (6)	32 (11)
P. aeruginosa	ATCC10145	>128 (>86)	>128 (>138)	16 (11)	>128 (>51)	>128 (>50)	64 (24)	64 (24)	32 (12)	32 (11)
	ATCC27853	>128 (>86)	>128 (>138)	4 (3)	16 (6)	32 (13)	16 (6)	16 (6)	16 (6)	16 (6)
Gram-positive bacteria										
B. subtilis	168	0.25 (0.168)	>128 (>138)	ND	0.5 (0.2)	0.25 (0.098)	0.25 (0.095)	0.25 (0.093)	≤0.008 (≤0.003)	≤0.008 (≤0.003)
S. simulans	22	0.125 (0.084)	>128 (>138)	ND	≤0.008 (≤0.003)	0.031 (0.012)	0.031 (0.012)	0.016 (0.006)	≤0.008 (≤0.003)	≤0.008 (≤0.003)
S. aureus	MSSA ATCC29213	0.125 (0.084)	>128 (>138)	ND	0.25 (0.1)	0.25 (0.098)	0.25 (0.095)	0.25 (0.093)	0.25 (0.093)	1 (0.358)
	MRSA USA300	0.25 (0.168)	>128 (>138)	ND	0.5 (0.2)	0.25 (0.098)	0.25 (0.095)	0.25 (0.093)	0.25 (0.093)	1 (0.358)
	VISA LIM-2	4 (3)	>128 (>138)	4 (3)	2 (0.8)	2 (0.79)	2 (0.76)	4 (1)	2 (0.74)	8 (3)
	VISA NRS402	8 (5)	>128 (>138)	8 (5)	16 (6)	8 (3)	8 (3)	2 (0.75)	8 (3)	4 (1)
	VRSA 2 (vanA)	128 (86)	>128 (>138)	128 (86)	32 (13)	64 (25)	64 (24)	128 (48)	>128 (>48)	>128 (>46)
	VRSA 3b (vanA)	>128 (>86)	>128 (>138)	>128 (>86)	32 (13)	>128 (>50)	32 (12)	>128 (>48)	>128 (>48)	>128 (>46)
E. faecalis	VRE E1246 (vanA)	>128 (>86)	>128 (>138)	>128 (>86)	>128 (>51)	>128 (>50)	>128 (>49)	>128 (>48)	>128 (>48)	>128 (>46)
	VRE E7406 (vanB)	32 (22)	>128 (>138)	32 (22)	8 (3)	64 (25)	64 (24)	128 (48)	128 (48)	128 (46)
E. faecium	VSE E980	0.5 (0.337)	>128 (>138)	ND	0.25 (0.1)	0.25 (0.098)	0.25 (0.095)	0.25 (0.093)	0.125 (0.046)	0.125 (0.045)
	VRE E155 (vanA)	>128 (>86)	>128 (>138)	>128 (>86)	64 (26)	64 (25)	64 (24)	64 (24)	64 (24)	8 (3)
	VRE E7314 (vanB)	128 (86)	>128 (>138)	128 (86)	2 (0.8)	8 (3)	2 (0.76)	8 (3)	0.5 (0.19)	0.031 (0.01)

MIC = Minimum inhibitory concentration, ND = not determined, PMEN = Polymyxin E nonapeptide, MSSA = Methicillin-sensitive *S. aureus*, MRSA = Methicillin-resistant *S. aureus*, VISA = Vancomycin-intermediate *S. aureus*, VRSA = Vancomycin-resistant *S. aureus*, VSE = Vancomycin-sensitive *Enterococci*, VRE = Vancomycin-resistant *Enterococci*

Table S4. LPS antagonization assay of compound **11** and colistin against *E. coli* ATCC25922

	MIC in µg/mL (MIC in µM shown in brackets)	
	No LPS	1 mg/mL LPS
Colistin	0.5 (0.428)	>16 (>14)
Vancomyxin **11**	16 (6)	>128 (>86)

MIC = Minimum inhibitory concentration

Figure S1 Hemolysis assessment

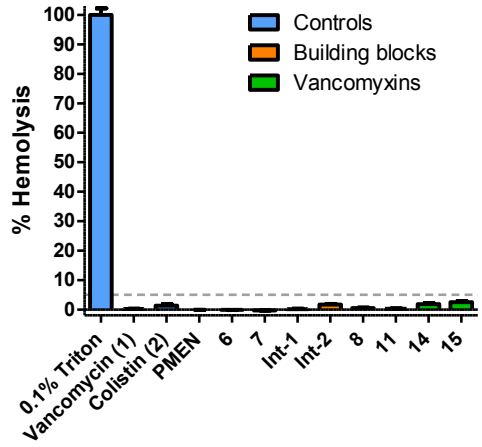

Figure S1. *Percent hemolysis at 512 µg/mL. All tested compounds including controls (blue), vancomycin building blocks (orange) and vancomyxins (green) are not hemolytic at 512 µg/mL as the percent hemolysis is below 5% (grey dotted line). Data are normalized based on 0.1% Triton-X100 as positive control for 100% hemolysis.*

Figure S2 Toxicity assessment on ciPTECs

Sample Id	**TC_{50} (mM)**
Vancomycin (**1**)	> 1
PMEN	> 1
8	0.37
11	0.23
14	0.18
15	0.11
Polymyxin B	0.07

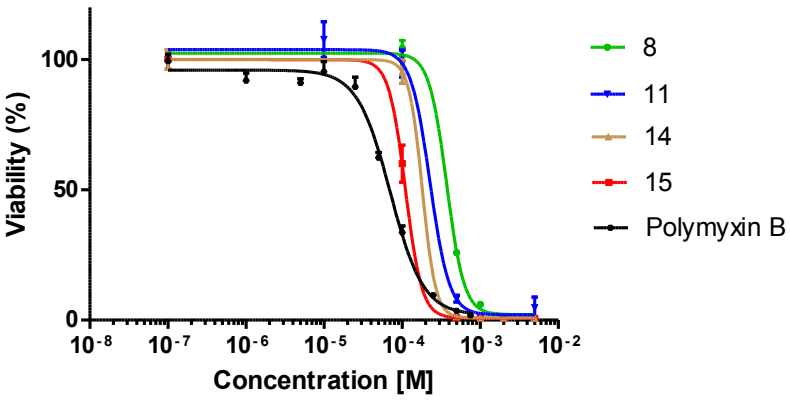

Figure S2. *Toxicity assessment on proximal tubular epithelial cells performed using PrestoBlue™ assay. Left: summarized TC_{50} values for vancomycin, PMEN and for vancosamine linked vancomyxins, either with or without modification of the C-terminus. Values for vancomyxins and polymyxin B are derived after non-linear regression analysis on cell viability data (right). Data presented as mean ± S.E.M, n = 3. TC_{50} values for compounds 8, 11, 14 and 15 are significantly higher ($p<0.0005$) compared to polymyxin B.*

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