

Supporting information for:

## Semisynthetic polymyxins with potent antibacterial activity and reduced kidney cell toxicity

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## General Procedures

All reagents employed were of American Chemical Society (ACS) grade or finer and were used without further purification unless otherwise stated. Commercially sourced Polymyxin B was obtained as a mixture of isomers (Combi-Blocks, San Diego, USA), with polymyxin B<sub>1</sub>, B<sub>2</sub>, and B<sub>3</sub> accounting for >90% of the isomers.

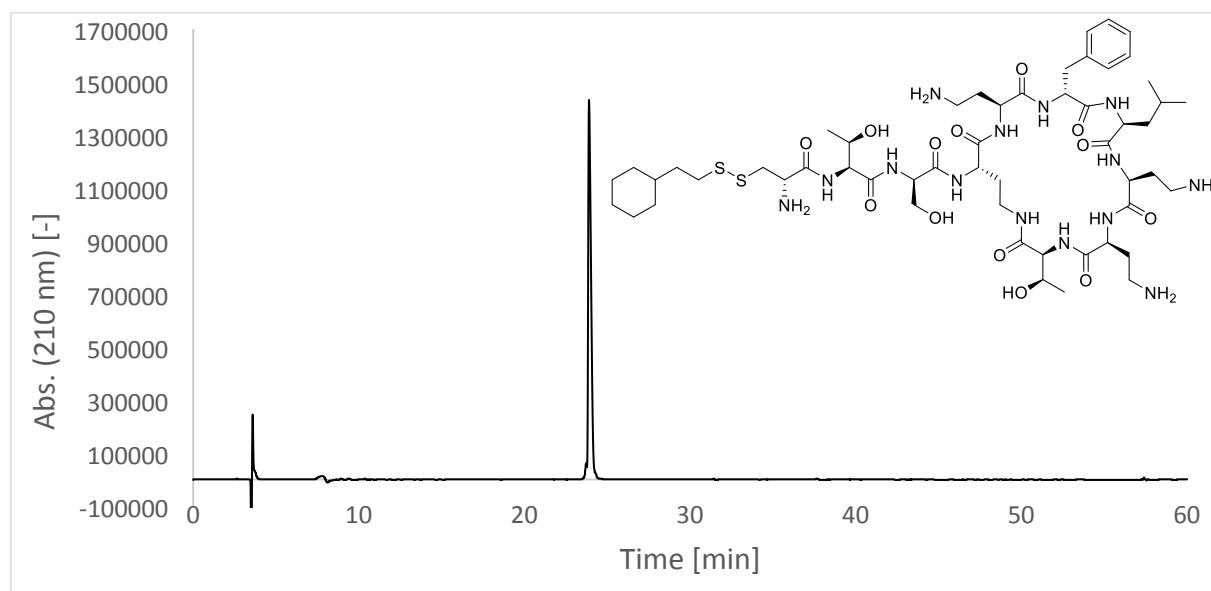
For compound characterization, HRMS analysis was performed on a Shimadzu Nexera X2 UHPLC system with a Waters Acquity HSS C18 column (2.1 × 100 mm, 1.8 μm) at 30 °C and equipped with a diode array detector. The following solvent system, at a flow rate of 0.5 mL/min, was used: solvent A, 0.1 % formic acid in water; solvent B, 0.1 % formic acid in acetonitrile. 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 of the peptides was confirmed to be ≥ 95% by analytical RP-HPLC using a Shimadzu Prominence-i LC-2030 system with a Dr. Maisch ReproSil Gold 120 C18 column (4.6 × 250 mm, 5 μm) at 30 °C and equipped with a UV detector monitoring at 214 nm. The following solvent system, at a flow rate of 1 mL/min, was used: solvent A, 0.1 % TFA in water/acetonitrile, 95/5; solvent B, 0.1 % TFA in water/acetonitrile, 5/95. Gradient elution was as follows: 100:0 (A/B) for 3 min, 100:0 to 0:100 (A/B) over 47 min, 0:100 (A/B) for 4 min, then reversion back to 100:0 (A/B) over 1 min, 100:0 (A/B) for 5 min.

## HPLC traces and HRMS data for final compounds

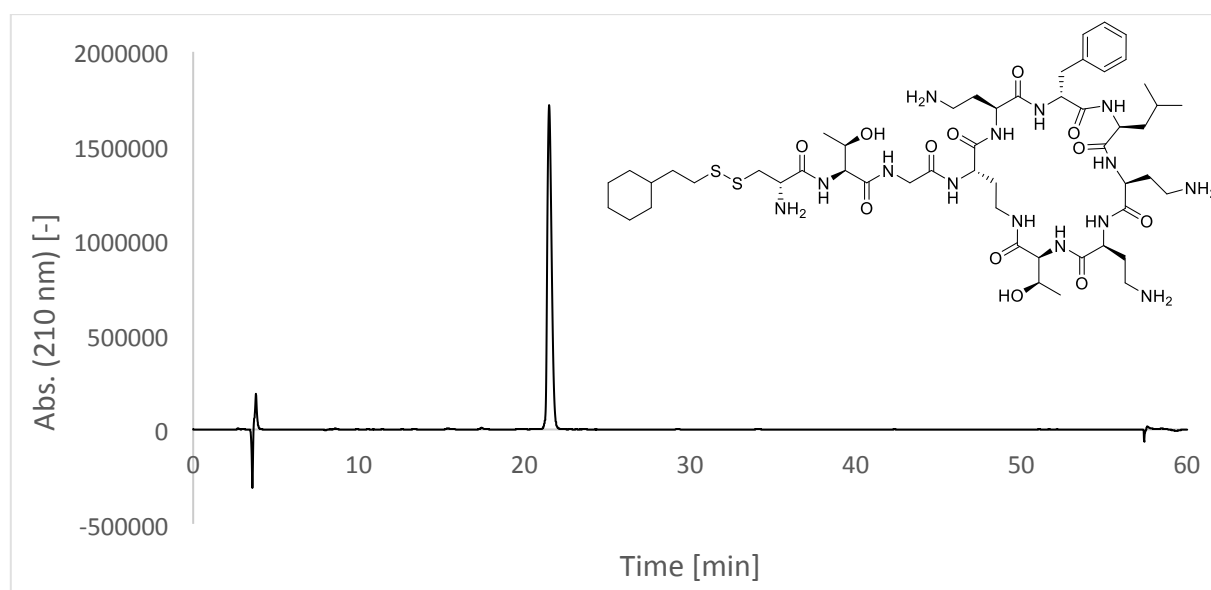
### Compound 7a

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>53</sub> H <sub>90</sub> N <sub>14</sub> O <sub>13</sub> S <sub>2</sub>	1194.6253	1195.6333	598.3207	1195.6334



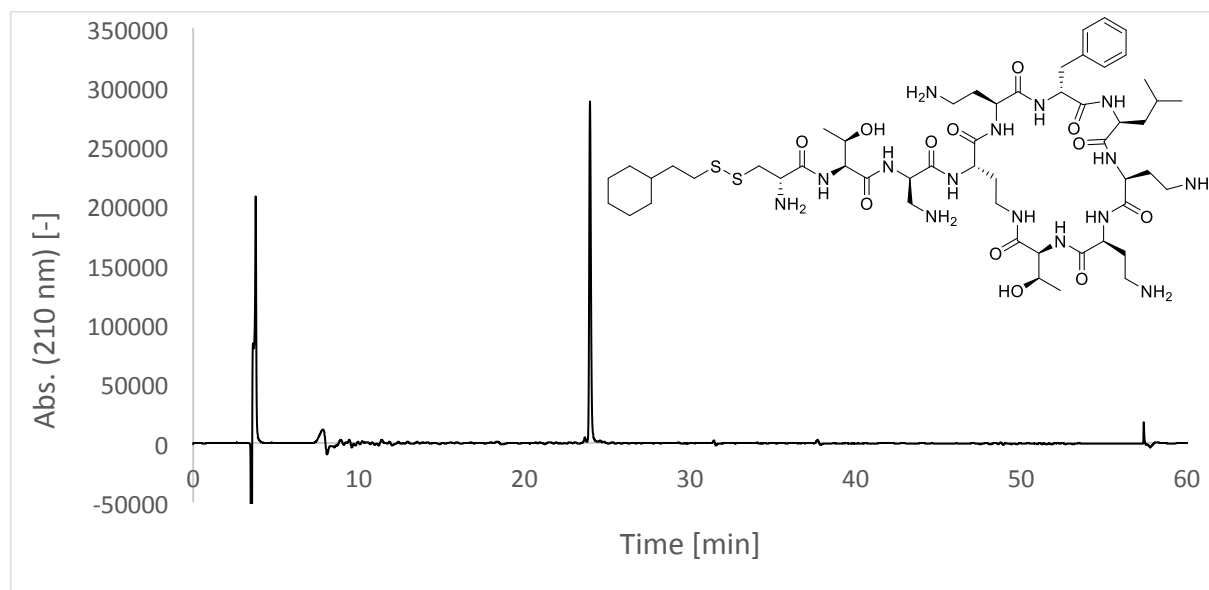
### Compound 7b

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>52</sub> H <sub>88</sub> N <sub>14</sub> O <sub>12</sub> S <sub>2</sub>	1194.6148	1165.6228	583.3154	1165.6224



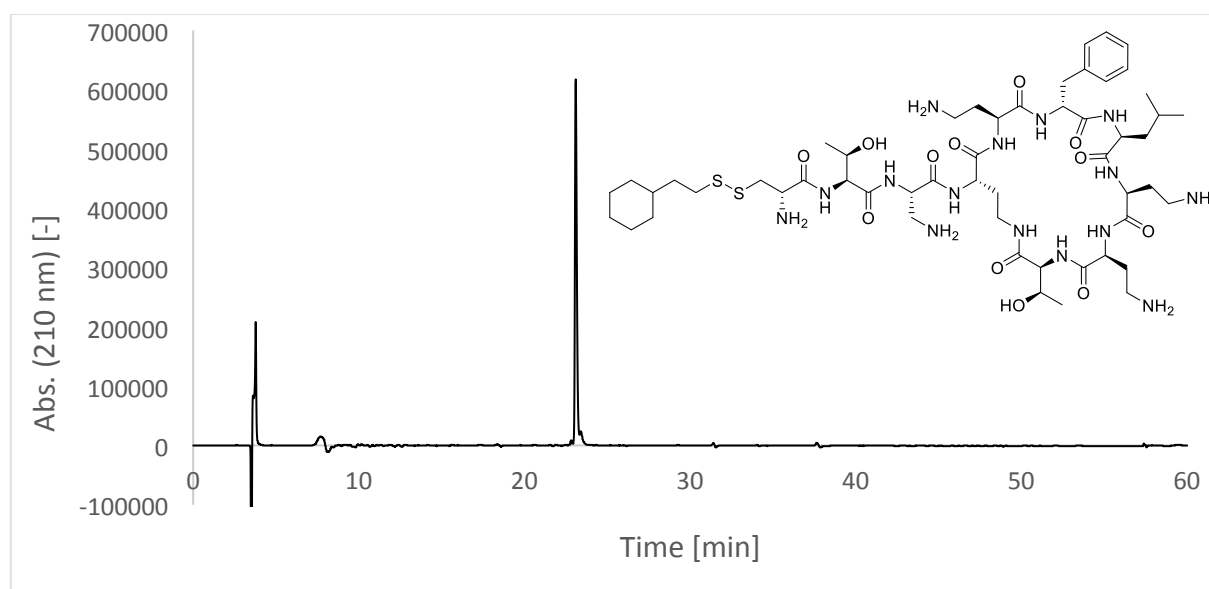
### Compound 7c

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>53</sub> H <sub>91</sub> N <sub>15</sub> O <sub>12</sub> S <sub>2</sub>	1193.6413	1194.6493	597.8287	1194.6489



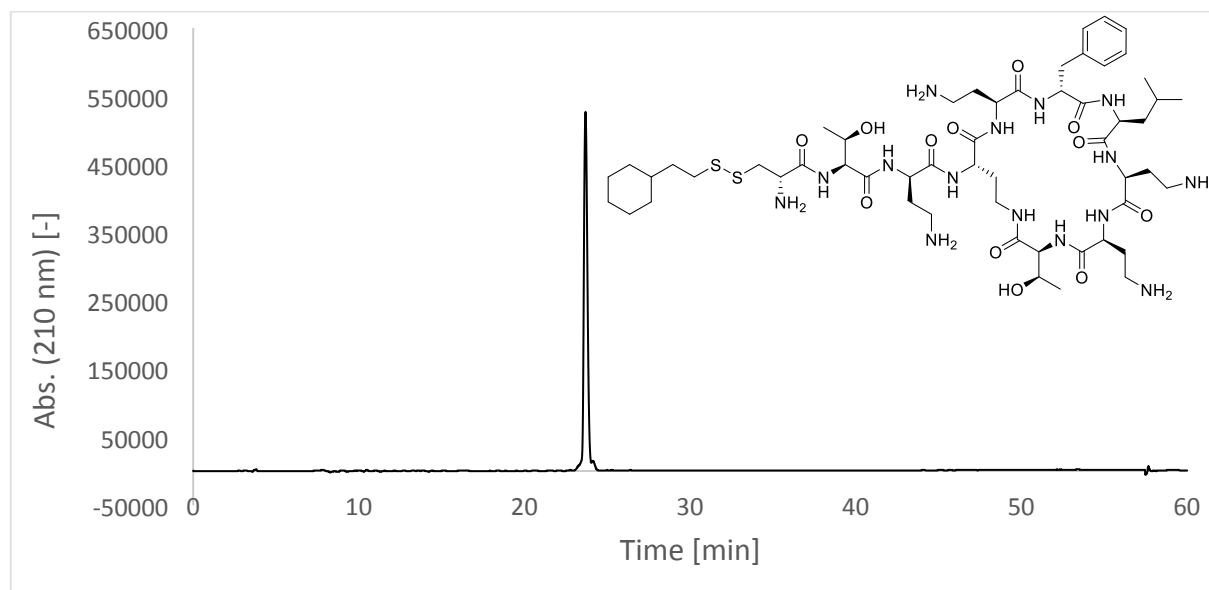
### Compound 7d

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>53</sub> H <sub>91</sub> N <sub>15</sub> O <sub>12</sub> S <sub>2</sub>	1193.6413	1194.6493	597.8287	1194.6492



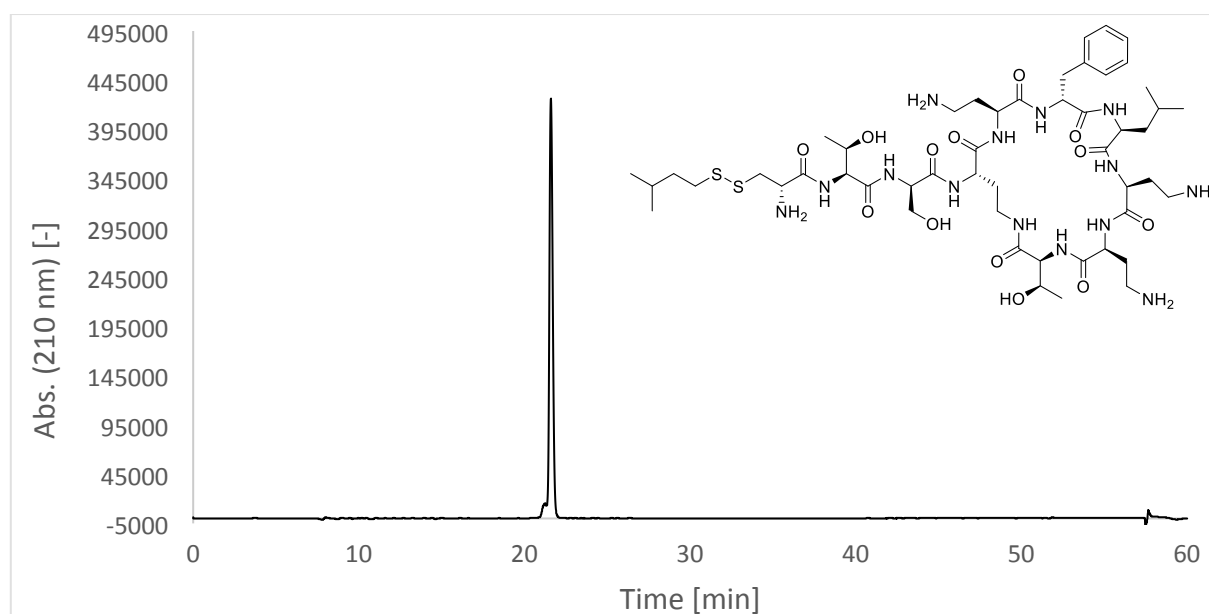
### Compound 7e

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>54</sub> H <sub>93</sub> N <sub>15</sub> O <sub>12</sub> S <sub>2</sub>	1207.6570	1208.6650	604.8365	604.8357



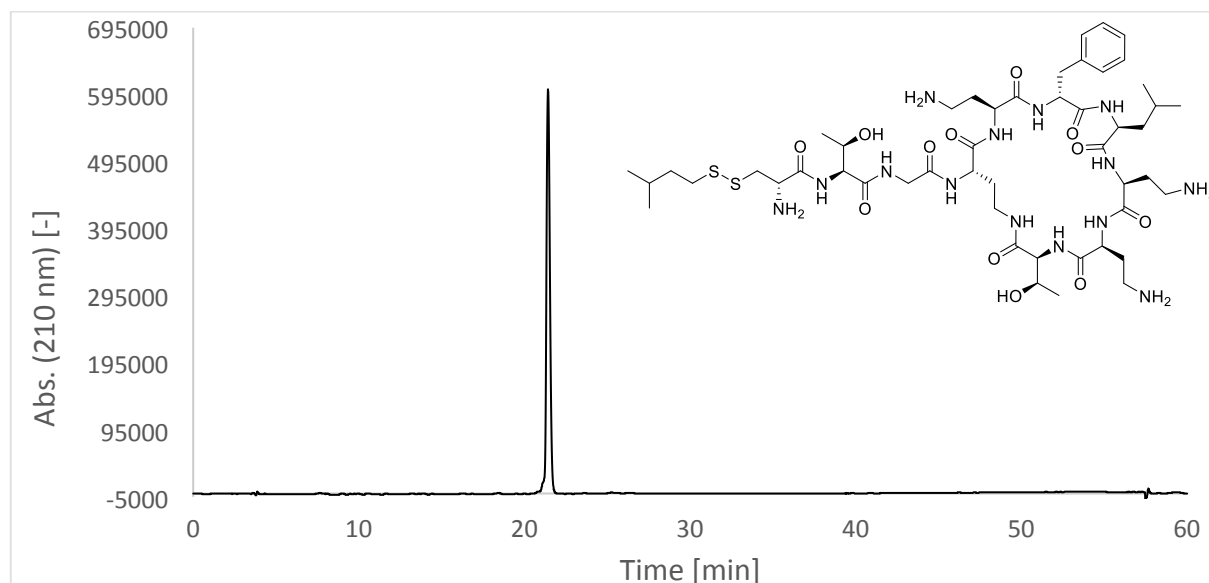
### Compound 8a

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>50</sub> H <sub>86</sub> N <sub>14</sub> O <sub>13</sub> S <sub>2</sub>	1154.5940	1155.6020	578.3050	578.3046



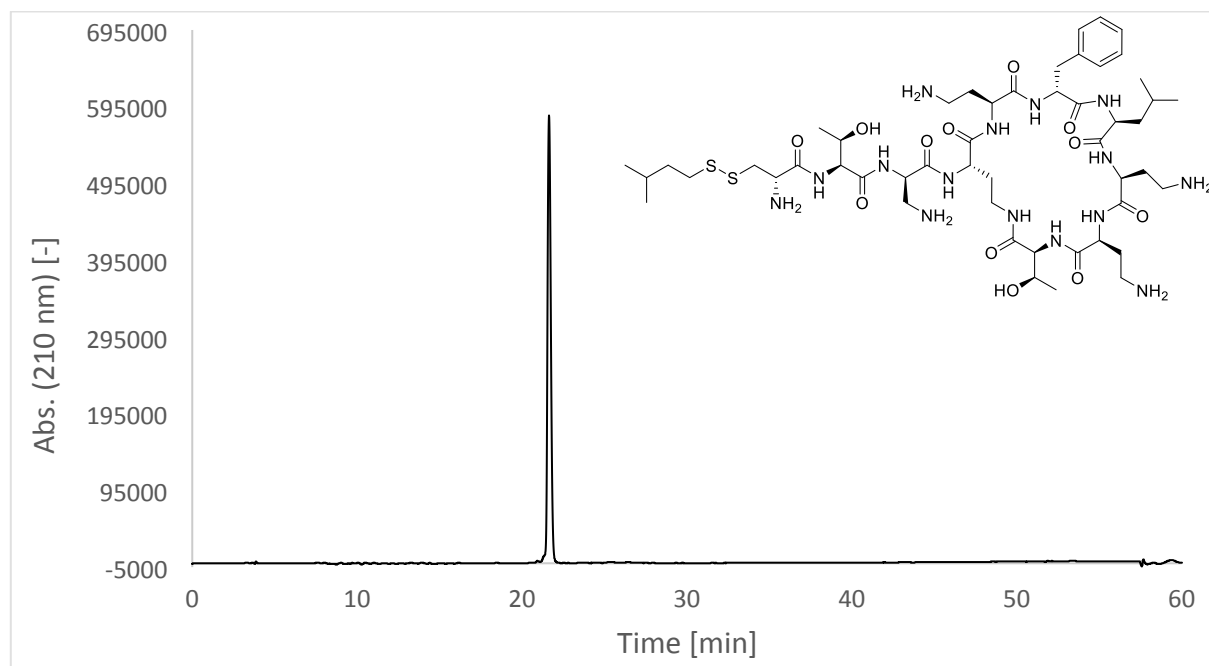
### Compound 8b

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>49</sub> H <sub>84</sub> N <sub>14</sub> O <sub>12</sub> S <sub>2</sub>	1124.5835	1125.5915	563.2998	563.2990



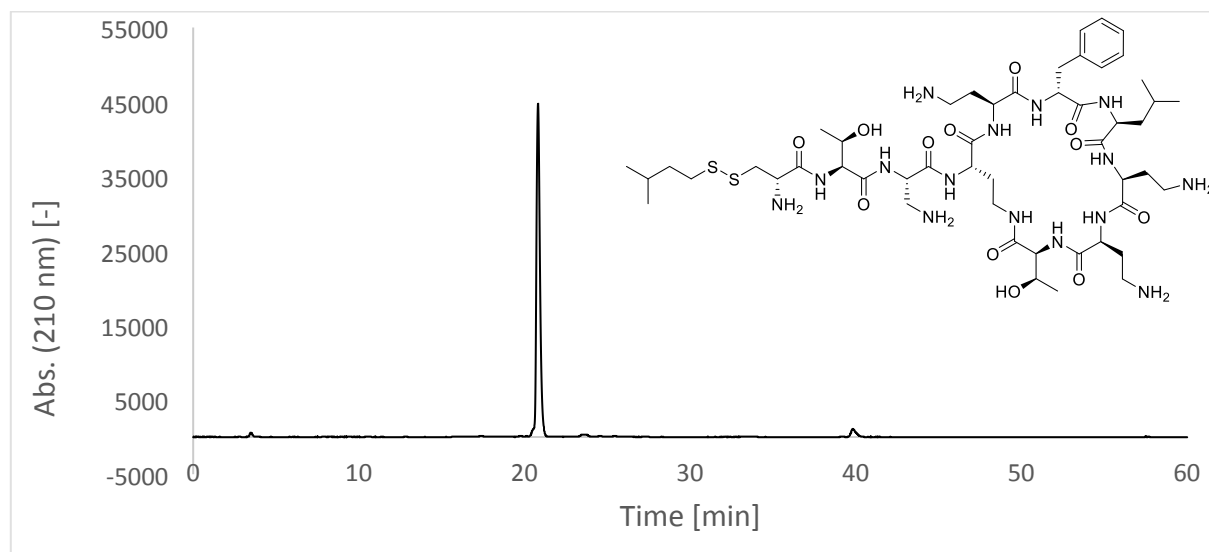
### Compound 8c

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>50</sub> H <sub>87</sub> N <sub>15</sub> O <sub>12</sub> S <sub>2</sub>	1153.6100	1154.6180	577.8130	577.8128



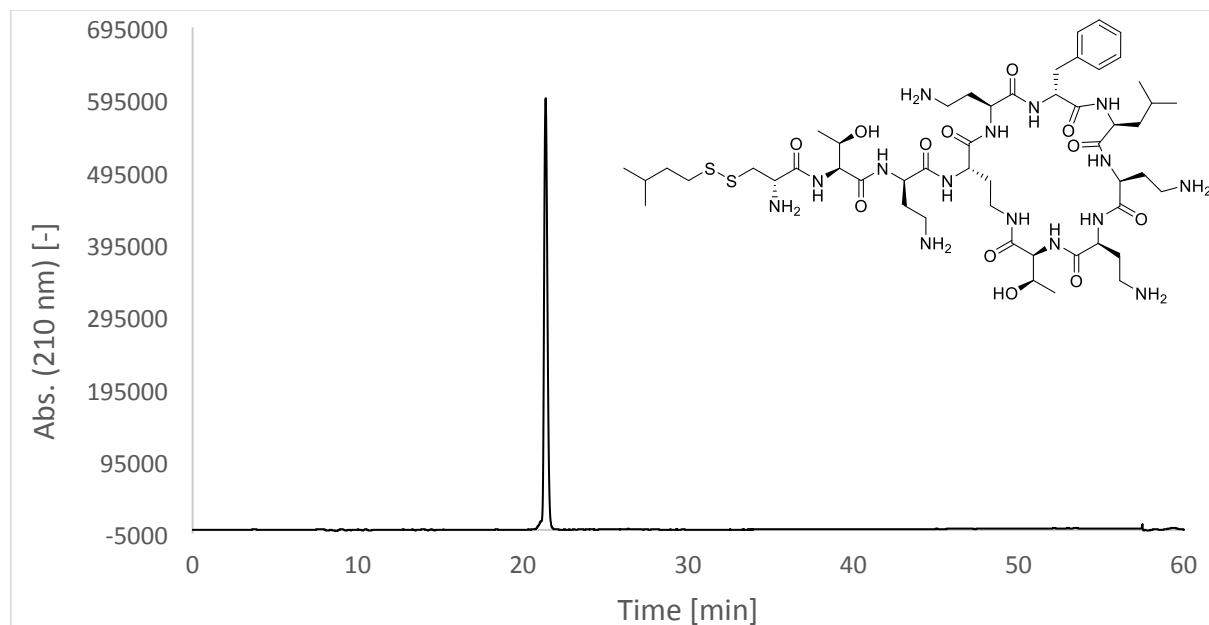
### Compound 8d

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>50</sub> H <sub>87</sub> N <sub>15</sub> O <sub>12</sub> S <sub>2</sub>	1153.6100	1154.6180	577.8130	577.8122



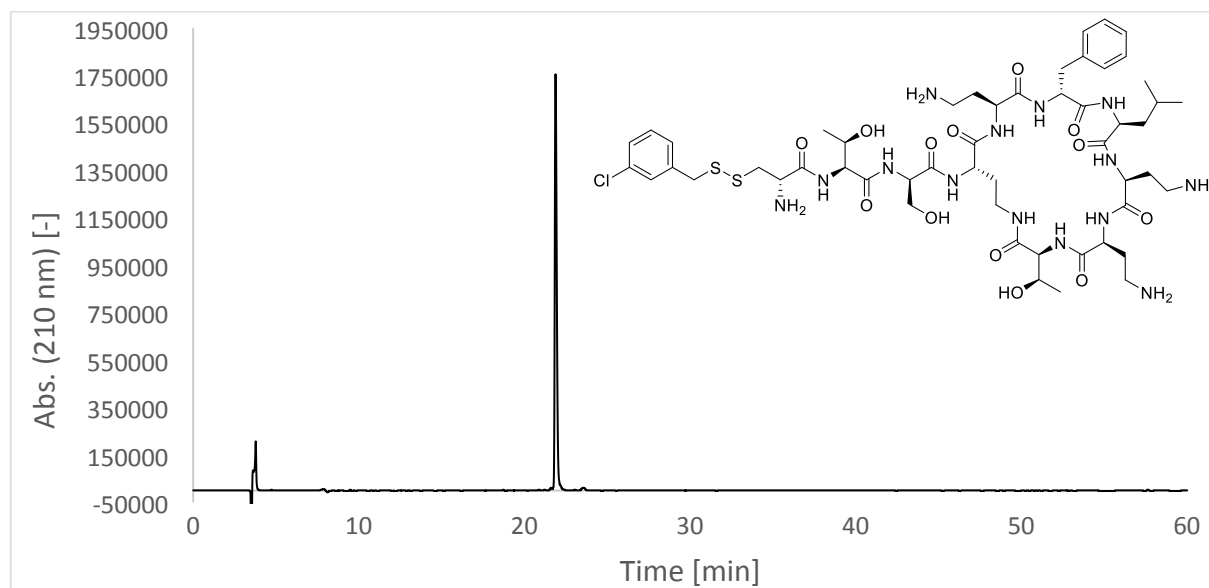
### Compound 8e

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>51</sub> H <sub>89</sub> N <sub>15</sub> O <sub>12</sub> S <sub>2</sub>	1167.6257	1168.6337	584.8209	584.8198



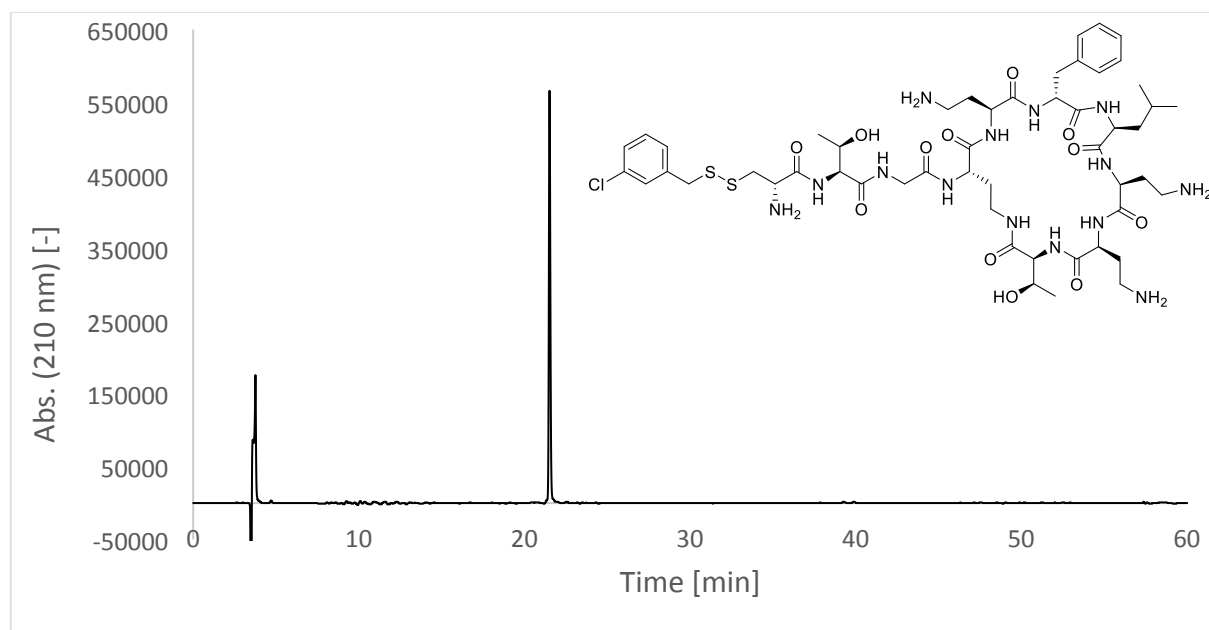
### Compound 9a

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>52</sub> H <sub>81</sub> ClN <sub>14</sub> O <sub>13</sub> S <sub>2</sub>	1208.5237	1209.5317	605.2699	1209.5311



### Compound 9b

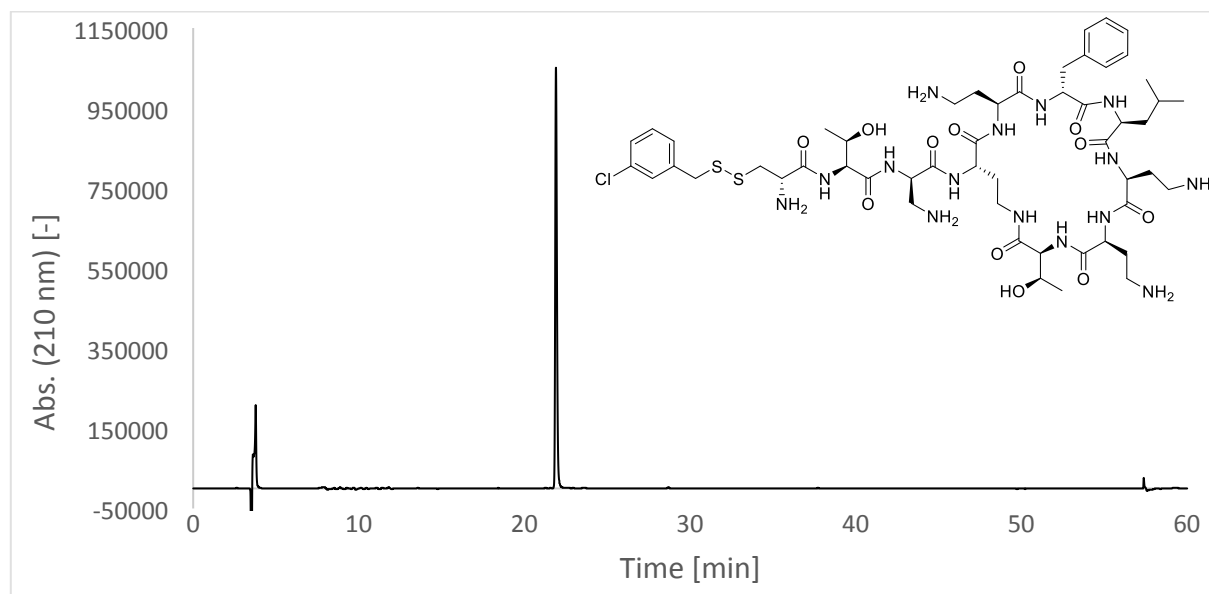
Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>51</sub> H <sub>79</sub> ClN <sub>14</sub> O <sub>12</sub> S <sub>2</sub>	1178.5132	1179.5212	590.2646	1179.5210





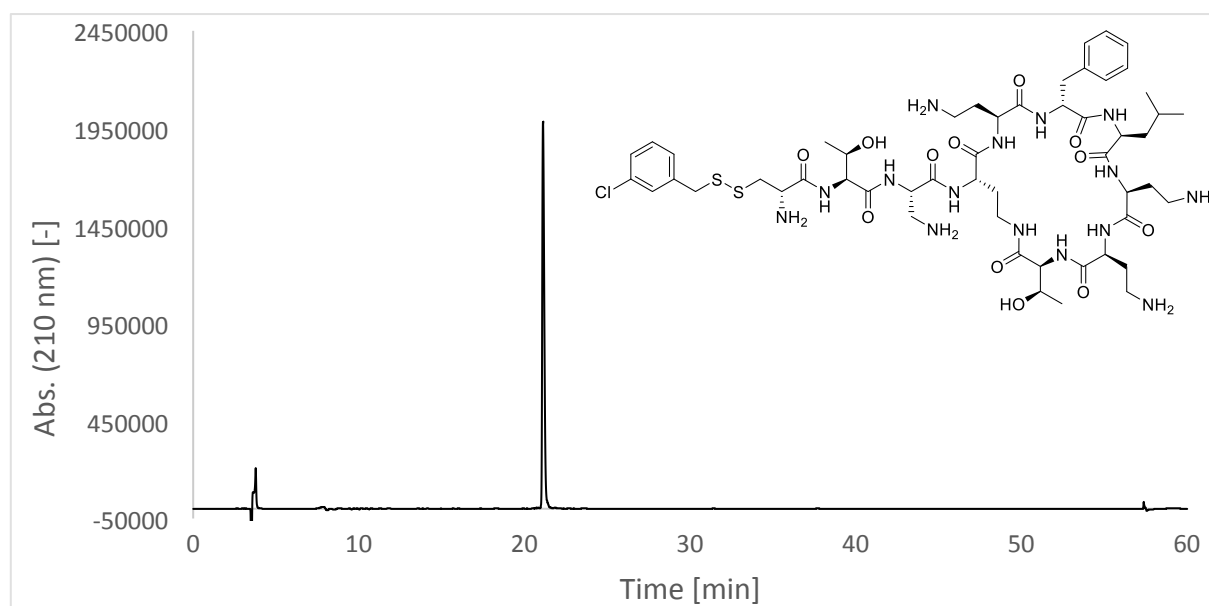
### Compound 9c

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>52</sub> H <sub>82</sub> ClN <sub>15</sub> O <sub>12</sub> S <sub>2</sub>	1207.5397	1208.5477	604.7779	1208.5476



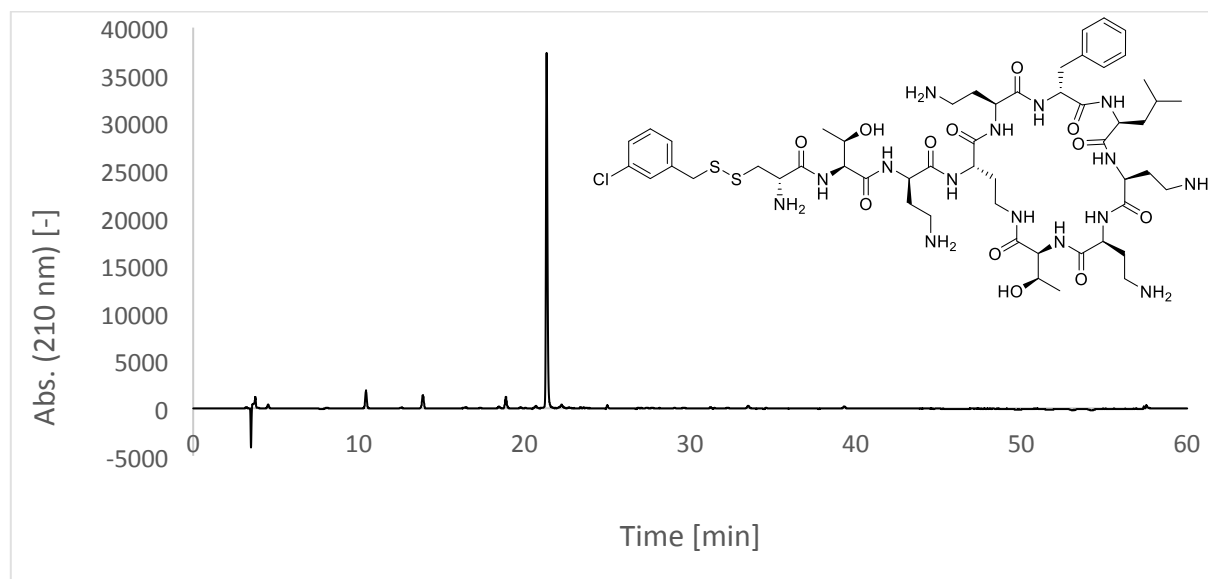
### Compound 9d

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>52</sub> H <sub>82</sub> ClN <sub>15</sub> O <sub>12</sub> S <sub>2</sub>	1207.5397	1208.5477	604.7779	1208.5477



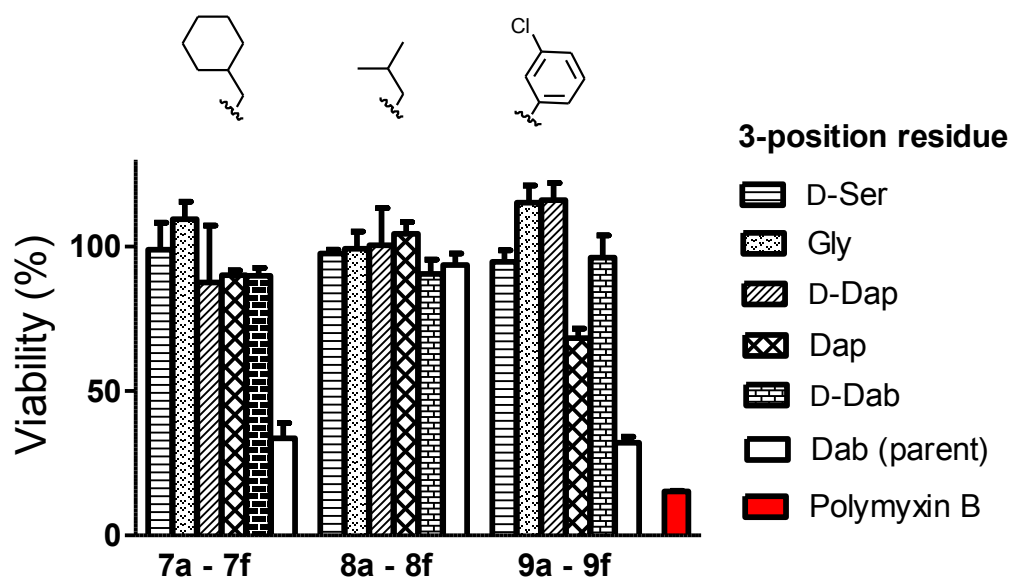
### Compound 9e

Composition	Exact mass	M + H	(M+2H)/2	Found value
C <sub>53</sub> H <sub>84</sub> ClN <sub>15</sub> O <sub>12</sub> S <sub>2</sub>	1221.5554	1222.5634	611.7857	1222.5620

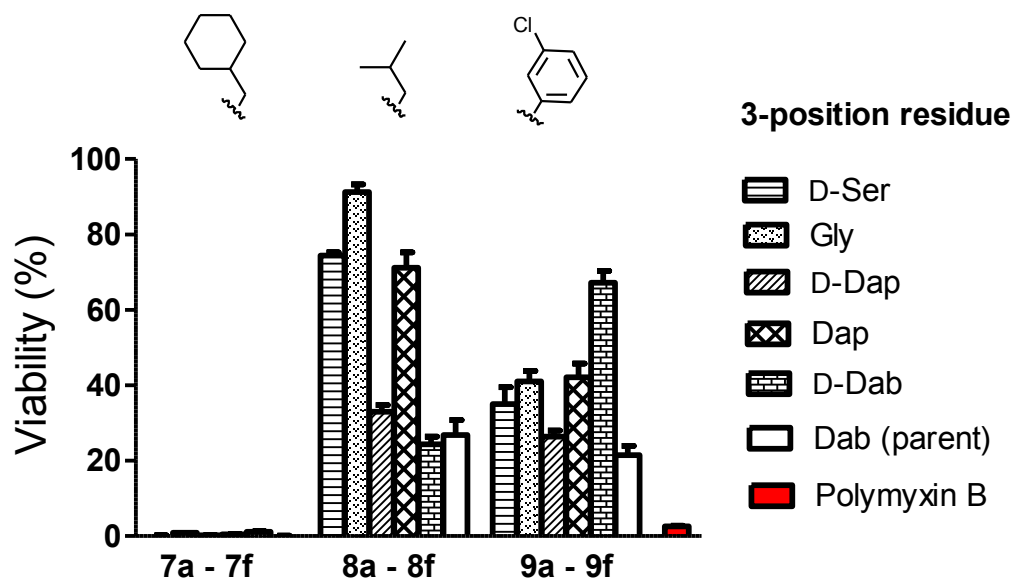


Analytical data for compounds **7f**, **8f** and **9f** have been previously reported.<sup>1</sup>

## Extended toxicity data on all analogues

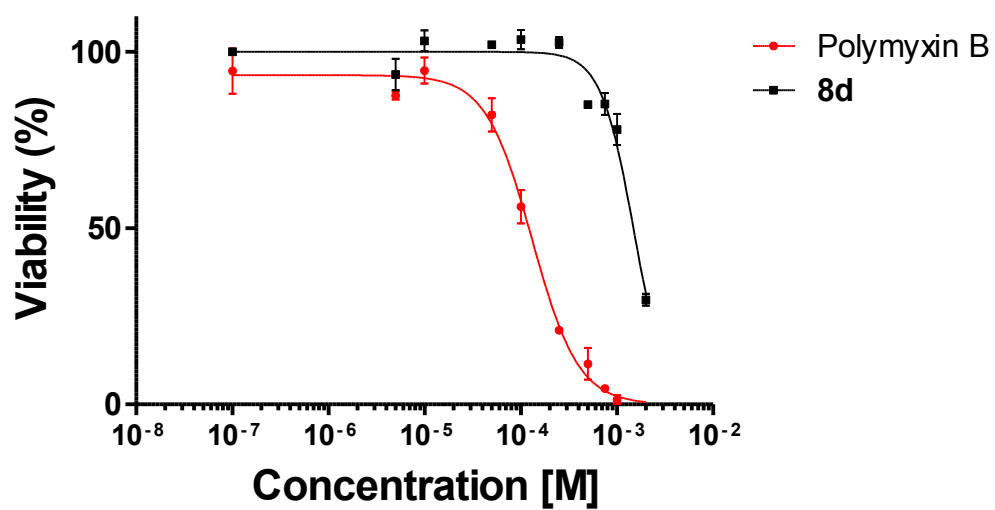


**Figure S1.** Viability of PTECs upon incubation with polymyxin analogues. Cells were exposed to the compound at 100  $\mu\text{M}$  for 24 hours, followed by washing, incubation with resazurin (PrestoBlue<sup>TM</sup>) and fluorescence read-out. Data are normalized to positive control (medium treated cells). Represented data are from triplicates.



**Figure S2.** Viability of PTECs upon incubation with polymyxin analogues. Cells were exposed to the compound at 500  $\mu\text{M}$  for 24 hours, followed by washing, incubation with resazurin (PrestoBlue<sup>TM</sup>) and fluorescence read-out. Data are normalized to positive control (medium treated cells). Represented data are from triplicates.

### Extended toxicity data on 8d



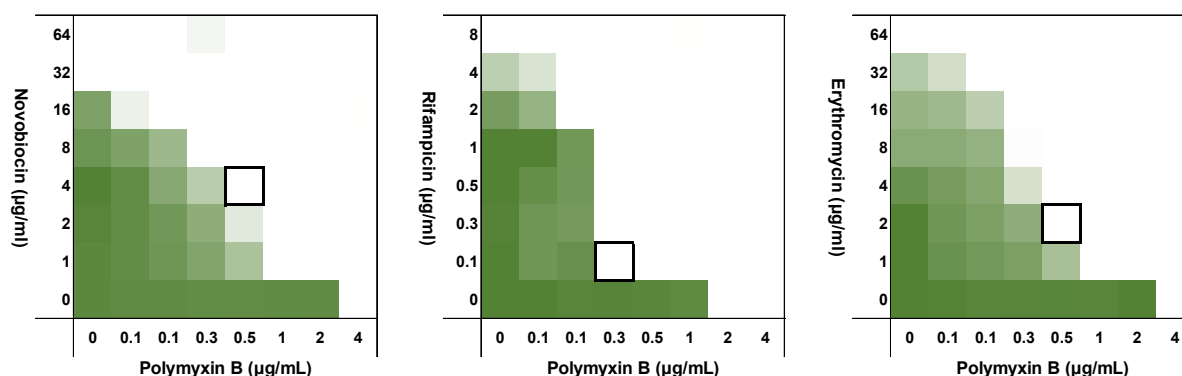
**Figure S3.** Viability of PTECs upon incubation with polymyxin B and analogue 8d. Cells were exposed to the compound for 24 hours, followed by washing, incubation with resazurin (PrestoBlue™) and fluorescence read-out. Data are normalized to positive control (medium treated cells). Represented data are from triplicates.

## Extended MIC data

**Table S1.** Extended MIC data on compound **8d**.

	Strain	Resistance	MIC values (µg/mL)	
			8d	Polymyxin B
<i>E. coli</i>	NCTC 13846	<i>mcr-1</i> , others (not specified)	4	4
	2158	OXA	4	2
	RC089	NDM-1	0.25	0.125
	2018-014	IMP-4	0.5	0.25
	1062	NDM-5	0.5	0.25
	M0072	Ciprofloxacin, doxycycline, trimethoprim	0.5	0.25
	JS136	OXA-48	0.5	0.25
	NCTC 12486	-	0.5	0.25
	<i>mcr-1</i>	<i>mcr-1</i>	4	4
	EQAS <i>mcr-1</i>	<i>mcr-1</i>	4	2
EQAS <i>mcr-2</i>	<i>mcr-2</i>	4	4	
<i>K. pneumoniae</i>	2048	OXA-1, SHV-11, CTX-M-15, TEM-1C	2	0.5
	JS-022	NDM-1	2	2
	1124	VIM-1	2	1
<i>P. aeruginosa</i>	RC0060	VIM-2, blaPAO	4	4
	ATCC 10145	-	2	2
	2251	VIM-2	2	4
	2018-011	NDM-1	2	1
	JS-080	IMP-13, IMP-37, blaPAO	2	1
	2173	VIM-2, blaPAO, OXA-50	2	2
<i>A. baumannii</i>	RUH-134	Multiple <sup>2</sup>	0.5	0.25
	9955	-	0.5	0.25
	NRZ-00687	NDM-2	1	0.25
	ATCC 17961	-	0.25	0.125
	BAA-747	-	0.5	0.25

## Synergy data on polymyxin B and 8d with Gram-positive selective antibiotics



**Figure S4.** Checkerboard assays results, exploring synergetic activity between polymyxin B and antibiotics typically used for Gram-positive bacteria only: novobiocin (left), rifampicin (middle) and erythromycin (right). Experiments are done on *mcr-2* positive *E. coli*. Color intensity corresponds to growth (as read by OD<sub>600</sub> measurements), with white areas indicating no growth. Each square represent data from a technical triplicate. The bounded box indicates the combination of antibiotic and synergist with the lowest FIC.

**Table S2.** MIC and MSC values for synergist-antibiotic concentrations as shown in Figure 4, Table 2 and Figure S4. MIC<sub>ant</sub>: MIC of the antibiotic. MIC<sub>syn</sub>: MIC of the synergist. MSC<sub>ant</sub>: synergistic concentration of the antibiotic corresponding to the indicated FIC value. MSC<sub>syn</sub>: synergistic concentration of the synergist corresponding to the indicated FIC value. FIC index: fractional inhibitory concentration index. MIC and MSC values are expressed in µg/mL.

Synergist	Antibiotic	MIC <sub>ant</sub>	MIC <sub>syn</sub>	MSC <sub>ant</sub>	MSC <sub>syn</sub>	FIC index
8d	Novobiocin	32	4	2	0.5	0.19
8d	Rifampicin	8	4	0.13	0.5	0.14
8d	Erythromycin	64	4	1	1	0.27
Polymyxin B	Novobiocin	32	4	4	0.5	0.25
Polymyxin B	Rifampicin	8	4	0.13	0.25	0.08
Polymyxin B	Erythromycin	64	4	2	0.5	0.16

### Bacterial strains used for MIC assays

The following strains were obtained from BEI Resources, NIAID, NIH:  
*E. Coli* M0072, strain MVA0072, NR-51488.

**Table S3.** Background of the strains used for MIC testing, as shown in Table 1.  
NDM: New Dehli Metallobetalactamase; OXA: oxacillinase; IMP: imipenemase.

Strain	Resistance	
<i>E. coli</i>	ATCC 25922	-
	1313	NDM-1
	NCTC 13846	<i>mcr-1</i> , others (not specified)
<i>K. pneumoniae</i>	ATCC 13883	-
	JS-123	NDM
	NCTC 13443	NDM-1
<i>A. baumannii</i>	ATCC 19606	-
	ATCC 17978	-
	MDR	Multi drug resistant (not specified)
	2018-006	NDM/OXA-023/OXA-051
<i>P. aeruginosa</i>	ATCC 27853	-
	NRZ 08418	NDM-1
	NRZ 03961	IMP-1

### References

- 1 C. J. Slingerland, C. M. J. Wesseling, P. Innocenti, K. G. C. Westphal, R. Masereeuw and N. I. Martin, *J. Med. Chem.*, 2022, **65**, 15878–15892.
- 2 S. J. Nigro and R. M. Hall, *J. Antimicrob. Chemother.*, 2012, **67**, 335–338.