Supplemental figures and tables

Figure S1A: Far-UV CD spectrum of KZ144, Art-085 and Art-175. Both Artilysins and endolysin KZ144 do not show significant differences in secondary structure.

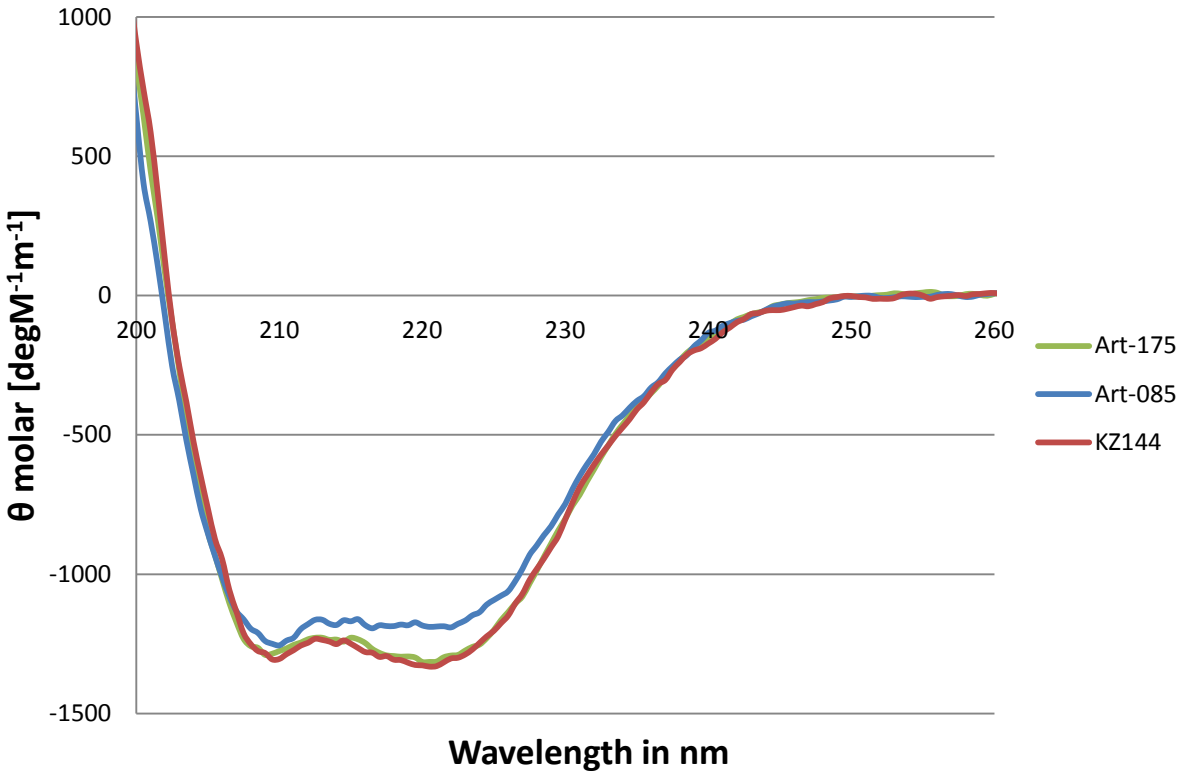

Figure S1B: SDS-PAGE with Art-085 and Art-175 under reducing and non-reducing conditions. Lane 1: Art-085 without β-mercapto-ethanol. Lane 2: Art-085 with β-mercapto-ethanol. Lane 3: Art-175 without β-mercapto-ethanol. Lane 4: Art-175 with β-mercapto-ethanol. Left marker: PageRuler (Thermo Scientific). Right marker: Low Molecular Weight Marker (GE Healthcare)

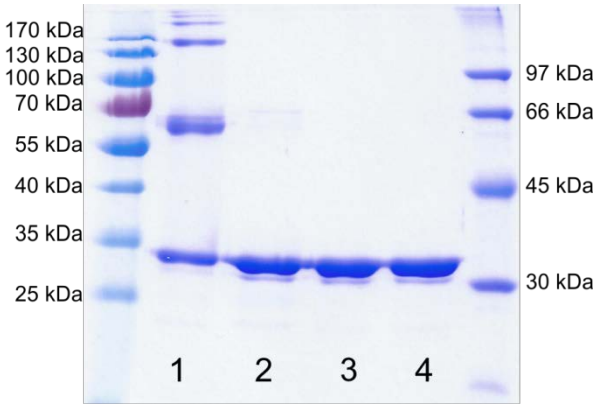

Figure S1C: Normalized CD ellipticity at 220 nm in function of temperature. Changes in secondary structure (α-helices) of Art-085 (squares) and Art-175 (diamonds) are recorded during heating from 25°C to 65°C. T_m of Art-085 (44.2°C) is lower than T_m of Art-175 (50.3°C), and Art-175 unfolds more cooperatively.

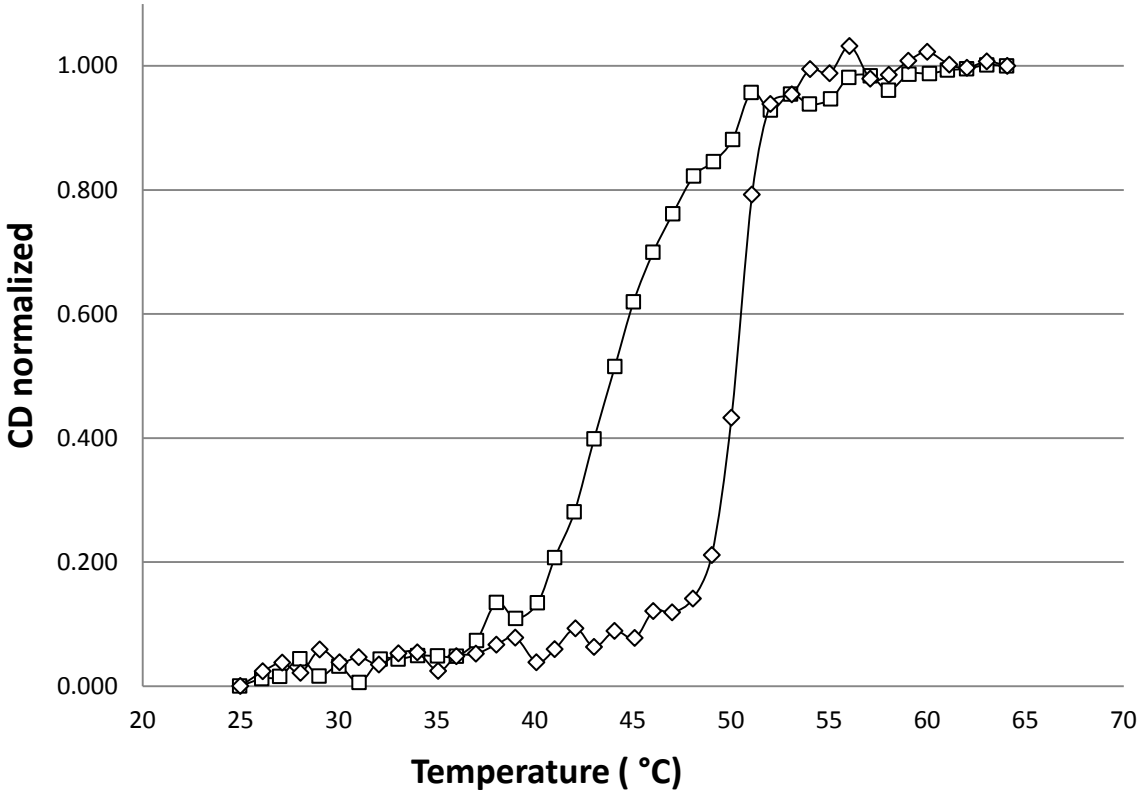

Figure S1D: Thermal aggregation curves of Art-085 and Art-175. Thermally induced aggregation of Art-085 (squares) and Art-175 (diamonds) is recorded by measurement of scattered length by absorption at 360 nm. Aggregation of Art-085 occurs at a lower temperature than for Art-175.

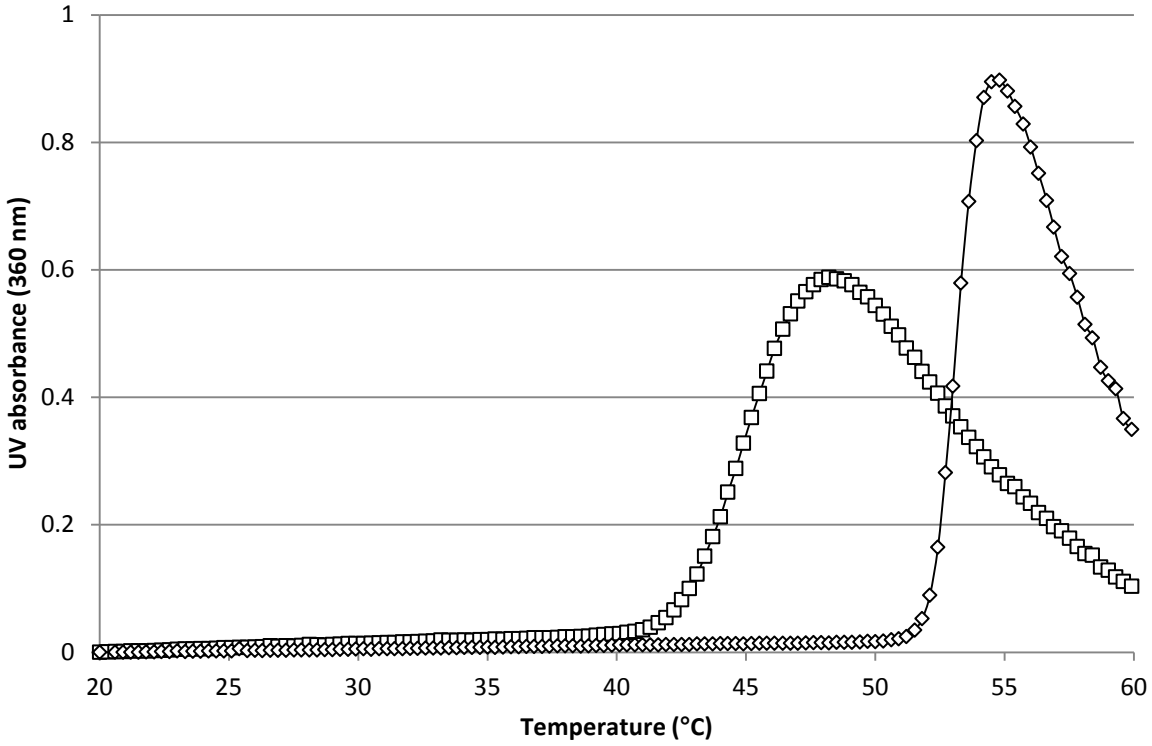

Figure S2: Cytotoxicity analysis of Art-175. Report delivered by Zwisler GmbH.

Commentary to Report N° 1308.1214

The following remarks are not part of the study report. They may only be considered as additional information for the sponsor without responsability. This commentary is valid without a signature.

Notice

In compliance with EN ISO 10993-5:2009 one specimen of the test item was tested in-vitro for cytotoxicity. Therefor L-929 cells were cultured as monolayer (6th split of DSMZ culture: DSM ACC2, mouse connective tissue fibroblasts clone of strain L). The culture medium (RPMI 1640, lot 1376274) was supplemented with 10% v/v fetal bovine serum (FBS, lot 1159166) and 100 U/ml penicillin and 100 µg/ml streptomycin (lot 1252676). All compounds were purchased from Life Technologies (Invitrogene) GmbH, Darmstadt. The sample was tested diluted 1:5, 1:10, 1:20 and 1:40 in cell culture medium, and was incubated with the cells for two days. Thereafter the degree of cell destruction was evaluated using microscopy and subsequent viability staining (MTT). The suitability of the test system was confirmed by positive and negative controls.

Sample-No.	Test Item	Quantitative Evaluation % viability (ISO)	Qualitative Evaluation reactivity grade (USP)
1308.1214	Proteinlösung Art-175, 5.17 mg/ml in 1xPBS+0.01%Tween 20	93+/- 1.4 %	0 (no reactivity)

Microscopy/Qualitative Evaluation: Not more than 10% of the cells were round and showed discrete intracytoplasmic granules (corresponding to no reactivity), no cell lysis and empty areas were observed.
Quantitative Evaluation: A sample with a cell viability above 90% is considered to be NON CYTOTOXIC.
Qualitative: The extract resulted in cell reactivities that characterize the sample to comply with USP<87>.

SUMMARY: The sample is considered to be NON CYTOTOXIC.

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Figure S3: Bactericidal effect of SMAP-29, KZ144, Art-085 and Art-175 against *Staphylococcus* DSM 346. A cell suspension was treated with equimolar amounts of SMAP-29, KZ144, Art-085 and Art-175 (3 µM) in the absence and presence of 0.125 and 0.5 mM EDTA. Bacterial reduction after 1h is expressed in $\log_{10}$ relative to the untreated control. Mean values (±SD) are shown. Detection limit was 4.88 log.

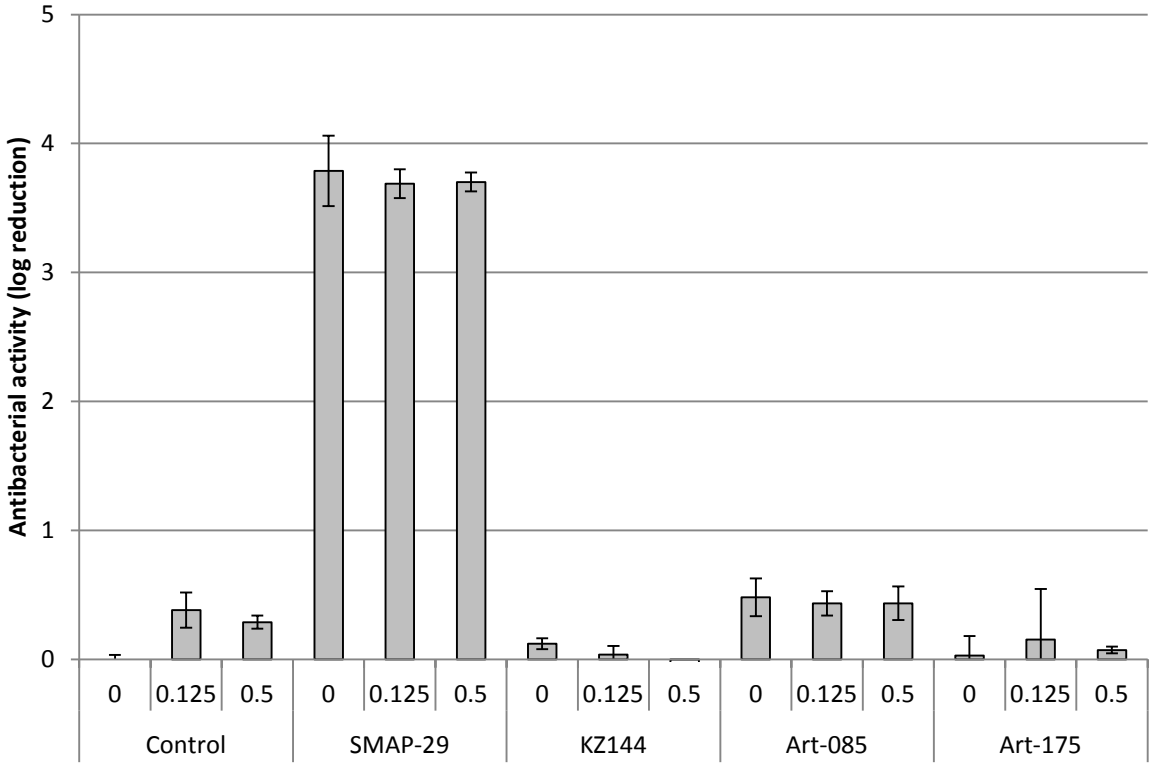

Table S1: MIC values of Art-085 and Art-175 against *P. aeruginosa isolates.* A panel of 79 *P. aeruginosa* isolates from diverse sources was used to analyze the MICs for Art-085 and Art-175 in the presence of 0.5 mM EDTA. The clinical (C) or environmental (E) origin is indicated. n.d. = not determined. MDR = multidrug-resistance or the number of resistances according to Table S2.

	MIC (µg/ml)						
Strain	**Art-085**	**Art-175**	**Clinical/ Environmental**	**MDR***	**Source**		
Br776	5	4	C	16	Pirnay et al., 2002a	patient isolate	throat
LiCC7	4	4			Lisando GmbH	unknown	
2575	9	4	C		Prof. Gessner, Medical Microbiology University Regensburg, Germany	patient isolate	
2747	4	4	C		Prof. Gessner, Medical Microbiology University Regensburg, Germany	patient isolate	
2572	8	4	C		Prof. Gessner, Medical Microbiology University Regensburg, Germany	patient isolate	
03.07-1.61	8	4	C		Prof. Gessner, Medical Microbiology University Regensburg, Germany	patient isolate	
03.10-181	6	4	C		Prof. Gessner, Medical Microbiology University Regensburg, Germany	patient isolate	
03.09-014	4	4	C		Prof. Gessner, Medical Microbiology University Regensburg, Germany	patient isolate	
03.09.177	6	4	C		Prof. Gessner, Medical Microbiology University Regensburg, Germany	patient isolate	
03.08-704	12	4	C		Prof. Gessner, Medical Microbiology University Regensburg, Germany	patient isolate	
Br257	4	4	E	8	Pirnay et al., 2002a	plant	rhizosphere
CFA	12.5	4			Military Hospital Nederoverheembeek, Brussels, Belgium	unknown	
GHb15	7	4	C		Prof. J. Verhaegen, UZ Gasthuisberg, Leuven, Belgium	patient isolate	
Krylov	7	4	C		Prof. V. Krylov, Mechnikov Research Institute for Vaccines and Sera, Russia	patient isolate	stool sample
Bo546	12.5	4	C	16	Pirnay et al., 2002a	patient isolate	burn wound
Bo548	8	4	C	12	Pirnay et al., 2002a	patient isolate	burn wound
Br908	6	4	C	9	Pirnay et al., 2002a	patient isolate	throat
Bu004	8	4	C	9	Pirnay et al., 2002a	patient isolate	throat
Bu007	16	4	C		Pirnay et al., 2002a	patient isolate	burn wound
C	4	4	C	10	Pirnay et al., 2002a	patient isolate	cystic fibrosis

C17	7	4	E	9	Pirnay et al., 2002a	hospital environment	
Is580	5	4	C	15	Pirnay et al., 2002a	patient isolate	burn wound
LMG2107	12.5	4	E	9	Pirnay et al., 2002a	shallow well	
LMG5031	5	4	E	9	Pirnay et al., 2002a	Chinese evergreen	
LMG14083	9	4	E		Pirnay et al., 2002a	unknown	
SG17M	5	4	E		Pirnay et al., 2002a	river water	
So099	12.5	4	C		Pirnay et al., 2002a	patient isolate	burn wound
US449	4	4	C	9	Pirnay et al., 2002a	patient isolate	sputum
C13	4	4	C		Pirnay et al., 2002a	patient isolate	cystic fibrosis
Lo053	9	4	C		Pirnay et al., 2002b	patient isolate	burn wound
Alexis3	6	4	C		Lisando GmbH	dog	otitis
ATCC 27853	7.5	4	C		Prof. Gessner, Medical Microbiology University Regensburg, Germany	unknown	
MH57	9	4	C	multiresistant	Prof. Schobert, Technical University Braunschweig, Germany	patient isolate	urinary tract infection
GH06_25135	<5	4	C		Prof. Schobert, Technical University Braunschweig, Germany	patient isolate	chronic wound infection
BT73	8	4	C		Prof. Schobert, Technical University Braunschweig, Germany	patient isolate	chronic lung infection/cystic fibrosis
11161	4	4			Prof. Tateda, University Toho, Tokyo, Japan	unknown	
11159	6	4			Prof. Tateda, University Toho, Tokyo, Japan	unknown	
11170	4	4			Prof. Tateda, University Toho, Tokyo, Japan	unknown	
Colja 6	8	4	C		Lisando GmbH	dog	otitis
va32824_2	n.d.	4	C		Lisando GmbH	patient isolate	inguinal region
va4672	n.d.	4	C		Lisando GmbH	dog	otitis
va49882	n.d.	4	C		Lisando GmbH	dog	otitis
PAO1	5	5	C	9	Pirnay et al., 2002a (laboratory reference strain)	patient isolate	stool sample
Br667	>20	6	C	19	Pirnay et al., 2002a	patient isolate	burn wound
2573	6	6	C		Prof. Gessner, Medical Microbiology University Regensburg, Germany	patient isolate	
Be136	12.5	6	C	9	Pirnay et al., 2002a	patient isolate	sputum
Bo559	15	6	C		Pirnay et al., 2002a	patient isolate	burn wound
Br229	>15	6	E	18	Pirnay et al., 2002a	hospital	

						environment	
Br906	12.5	6	C	9	Pirnay et al., 2002a	patient isolate	nose
Is573	>15	6	C	19	Pirnay et al., 2002a	patient isolate	burn wound
Li004	>15	6	C		Pirnay et al., 2002a	patient isolate	cystic fibrosis
PhDW6	7.5	6	C		Pirnay et al., 2002a	patient isolate	wound
Pr335	>15	6	E		Pirnay et al., 2002a	hospital environment	
TuD199	>15	6	C		Pirnay et al., 2002a	patient isolate	sputum
PAK	9	6	E		Prof. L. Debarbieux, Institut Pasteur, Paris, France	unknown	
Bobby 6	8	6	C		Lisando GmbH	dog	otitis
MH39	15	6	C	multiresistant	Prof. Schobert, Technical University Braunschweig, Germany	patient isolate	urinary tract infection
GH06_41910	<5	6	C		Prof. Schobert, Technical University Braunschweig, Germany	patient isolate	chronic wound infection
va32872_1	n.d.	6	C		Lisando GmbH	patient isolate	nose
3-8 1.68	4	8	C		Prof. Gessner, Medical Microbiology University Regensburg, Germany	patient isolate	
K	9	8			Military Hospital Nederoverheembeek, Brussels, Belgium	unknown	
US448	15	8	C	9	Pirnay et al., 2002a	patient isolate	urine
Br680	10	8	C	9	Pirnay et al., 2002a	patient isolate	burn wound
PA6	10	8	C	13	Pirnay et al., 2002a	patient isolate	urine
PAO23	>15	8	E		Pirnay et al., 2002a	river water	
PT31M	8	8	E		Pirnay et al., 2002a	drinking water	
11177	12	8			Prof. Tateda, University Toho, Tokyo, Japan	unknown	
B10-03.07.159	4	10	C		Prof. Gessner, Medical Microbiology University Regensburg, Germany	patient isolate	
Aa249	>15	10	C		Pirnay et al., 2002a	patient isolate	burn wound
Be128	15	10	C	9	Pirnay et al., 2002a	patient isolate	sputum
Li009	13	10	C	8	Pirnay et al., 2002a	patient isolate	cystic fibrosis
Aa245	>15	12	C		Pirnay et al., 2002b	patient isolate	burn wound
Bobby 5	6	12	C		Lisando GmbH	dog	otitis
B11-09.07.229	20	12	C		Prof. Gessner, Medical Microbiology University Regensburg, Germany	patient isolate	cystic fibrosis
ur08107	n.d.	20	C		Prof. Gessner, Medical Microbiology University Regensburg	patient isolate	urinary tract infection

Tu843	15	22			Military Hospital Nederoverheembeek, Brussels, Belgium	unknown	
11178	12	22			Prof. Tateda, University Toho, Tokyo, Japan	unknown	
MH27	>20	24	C	multiresistant	Prof. Schobert, Technical University Braunschweig, Germany	patient isolate	urinary tract infection
1631	20	28			Prof. Tateda, University Toho, Tokyo, Japan	unknown	

Table S2: Comparison of MIC-values for Art-175 and commonly used antibiotics against a panel of clinical MDR *P. aeruginosa* isolates. The MIC of Art-175 (in µg/ml) was determined for *P. aeruginosa* PAO1 and 24 clinical and environmental isolates in Mueller-Hinton medium with 0.5 mM EDTA. Art-175 was dialyzed against 20 mM HEPES 0.5 M NaCl pH 7.4 and tested at a concentration between 4 and 15 µg/ml. Plates were incubated for 18h at 37°C. In addition, the susceptibility (in µg/µl; S = sensitive (green), I = intermediate (yellow) and R = resistant (red)) of these strains for 12 therapeutically used antibiotics was indicated, using the CLSI breakpoint system (CLSI M100-S24): PIP = piperacillin, TZP = piperacillin + tazobactam, CTX = cefotaxime; CAZ = ceftazidime, FEP = cefepime, MEM = meropenem, AMK = amikacin, GEN = gentamicin, TOB = tobramycin, CIP = ciprofloxacin, NOR = norfloxacin, OFX = ofloxacin. The different strains are ranked according to the number of resistances. Averages of (at least) three repeats are presented here.

		MIC (µg/ml)											
	Art-175	Antibiotics											
		PIP	TZP	CTX	CAZ	FEP	MEM	AMK	GEN	TOB	CIP	NOR	OFX
Br667	6	≥128 (R)	≥128 (R)	≥64 (R)	8 (S)	32 (R)	2 (S)	≥64 (R)	≥16 (R)	≥16 (R)	≥4 (R)	≥16 (R)	≥8 (R)
Is573	6	≥128 (R)	≥128 (R)	≥64 (R)	16 (I)	≥64 (R)	≥16 (R)	≥64 (R)	≥16 (R)	4 (S)	≥4 (R)	≥16 (R)	≥8 (R)
Br229	6	≥128 (R)	≥128 (R)	≥64 (R)	4 (S)	8 (S)	1 (S)	≥64 (R)	≥16 (R)	≥16 (R)	≥4 (R)	≥16 (R)	≥8 (R)
Br776	4	≥128 (R)	≥128 (R)	≥64 (R)	≥64 (R)	32 (R)	≥16 (R)	16 (S)	4 (S)	≤1 (S)	2 (I)	8 (I)	≥8 (R)
Bo546	4	≥128 (R)	64 (S)	≥64 (R)	≥64 (R)	≥64 (R)	≤0,25 (S)	≥64 (R)	≥16 (R)	≥16 (R)	≤0,25 (S)	≤0,5 (S)	1 (S)
Is580	4	≥128 (R)	64 (S)	≥64 (R)	32 (R)	4 (S)	1 (S)	≤2 (S)	≥16 (R)	≥16 (R)	2 (I)	4 (S)	≥8 (R)
Pa6	8	≥128 (R)	≥128 (R)	32 (I)	4 (S)	8 (S)	≤0,25 (S)	≤2 (S)	≥16 (R)	≥16 (R)	0.5 (S)	≤0,5 (S)	1 (S)
Bo548	8	≥128 (R)	64 (S)	≥64 (R)	32 (R)	8 (S)	2 (S)	8 (S)	4 (S)	≤1 (S)	≤0,25 (S)	1 (S)	2 (S)
C	4	≤4 (S)	≤4 (S)	8 (S)	4 (S)	8 (S)	≤0,25 (S)	4 (S)	≥16 (R)	8 (I)	1 (S)	2 (S)	4 (I)
Li012	8	≤4 (S)	≤4 (S)	4 (S)	2 (S)	≤1 (S)	≤0,25 (S)	≤2 (S)	≤1 (S)	≤1 (S)	2 (I)	4 (S)	≥8 (R)
Us449	4	8 (S)	8 (S)	32 (I)	4 (S)	2 (S)	1 (S)	4 (S)	2 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	2 (S)
Us448	8	≤4 (S)	≤4 (S)	32 (I)	4 (S)	2 (S)	≤0,25 (S)	4 (S)	2 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	1 (S)
Be128	10	8 (S)	8 (S)	32 (I)	4 (S)	2 (S)	4 (S)	≤2 (S)	2 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	1 (S)
Br908	4	8 (S)	8 (S)	32 (I)	4 (S)	2 (S)	1 (S)	≤2 (S)	≤1 (S)	≤1 (S)	0.5 (S)	≤0,5 (S)	2 (S)
Bu004	4	≤4 (S)	≤4 (S)	16 (I)	≤1 (S)	≤1 (S)	0.5 (S)	≤2 (S)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	2 (S)
C18	6	≤4 (S)	≤4 (S)	16 (I)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤2 (S)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	1 (S)
PAO1	6	≤4 (S)	≤4 (S)	16 (I)	2 (S)	2 (S)	≤0,25 (S)	≤2 (S)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	1 (S)
LMG 5031	5	8 (S)	8 (S)	32 (I)	2 (S)	2 (S)	≤0,25 (S)	≤2 (S)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	0.5 (S)
LMG 2107	4	≤4 (S)	≤4 (S)	16 (I)	2 (S)	≤1 (S)	≤0,25 (S)	≤2 (S)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	1 (S)
Be136	6	8 (S)	8 (S)	32 (I)	4 (S)	2 (S)	≤0,25 (S)	≤2 (S)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	1 (S)
Br906	6	8 (S)	8 (S)	32 (I)	4 (S)	2 (S)	≤0,25 (S)	≤2 (S)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	1 (S)
C17	4	≤4 (S)	≤4 (S)	8 (S)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤2 (S)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	1 (S)
Br680	8	≤4 (S)	≤4 (S)	4 (S)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤2 (S)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	0.5 (S)
Br257	4	≤4 (S)	≤4 (S)	8 (S)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤2 (S)	≤1 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	≤0,25 (S)
Li009	10	≤4 (S)	≤4 (S)	4 (S)	4 (S)	≤1 (S)	≤0,25 (S)	4 (S)	2 (S)	≤1 (S)	≤0,25 (S)	≤0,5 (S)	0.5 (S)

Table S3: MIC values of Art-085 and Art-175 against a broad panel of bacterial species. Gram-negative species belonging to the families Pseudomonadaceae, Enterobacteriaceae and Bacteroidaceae, and diverse Gram-positive species were tested for their susceptibility to up to at least 20 µg/ml Art-085 and Art-175 in the absence of EDTA. Growth conditions and source of the strains are indicated.

		MIC (µg/ml)			
Species	Strain	Art-085	Art-175	Growth conditions	Source
Gram-negative species					
Pseudomonads					
Pseudomonas aeruginosa	PAO1	18	10	37°C (18h)	Pirnay et al., 2002a (Laboratory reference strain)
Pseudomonas aeruginosa	GHb15	12	10	37°C (18h)	Prof. J. Verhaegen, UZ Gasthuisberg, Leuven, Belgium
Pseudomonas aeruginosa	SG17M	7.5	7.5	37°C (18h)	Pirnay et al., 2002a
Pseudomonas aeruginosa	Alexis3	16	7.5	37°C (18h)	Lisando GmbH
Pseudomonas aeruginosa	FRDI	10	7.5	37°C (18h)	Prof. M. Schobert, Technical University Braunschweig, Germany
Pseudomonas aeruginosa	11160	7.5	5	37°C (18h)	Prof. K. Tateda, University of Toho, Tokyo, Japan
Pseudomonas putida	RD3SR1	18	16	37°C (18h)	Prof. R. De Mot, Centre of Microbial and plant genetics, Leuven, Belgium
Pseudomonas putida	RW1P1	20	18	37°C (18h)	Prof. R. De Mot, Centre of Microbial and plant genetics, Leuven, Belgium
Pseudomonas putida	RW1P2	20	18	37°C (18h)	Prof. R. De Mot, Centre of Microbial and plant genetics, Leuven, Belgium
Pseudomonas putida	RW5P2	20	18	37°C (18h)	Prof. R. De Mot, Centre of Microbial and plant genetics, Leuven, Belgium
Pseudomonas syringae	DSM 50281	5	5	30°C (18h)	German Collection of Microorganisms and Cell Cultures
Pseudomonas syringae	DSM 10604	7.5	7.5	30°C (18h)	German Collection of Microorganisms and Cell Cultures
Enterobacteriaceae					
Escherichia coli O26:H11	RKI 07-05313	>20	>20	37°C (18h)	Dr. A. Fruth, Robert Koch Institute, Wernigerode, Germany
Escherichia coli O103:H2	RKI 06-08410	>20	>20	37°C (18h)	Dr. A. Fruth, Robert Koch Institute, Wernigerode, Germany
Escherichia coli O111:H-	09-06443	>20	>20	37°C (18h)	Dr. A. Fruth, Robert Koch Institute, Wernigerode, Germany
Escherichia coli O145:H-	06-05741	>20	>20	37°C (18h)	Dr. A. Fruth, Robert Koch Institute, Wernigerode, Germany
Escherichia coli O157:H7	RKI 03-07953	>20	>20	37°C (18h)	Dr. A. Fruth, Robert Koch Institute, Wernigerode, Germany
Salmonella Typhimurium	DSM 17058	>20	>20	37°C (18h)	German Collection of Microorganisms and Cell Cultures

Salmonella Enteritidis	DSM 17420	14	>20	37°C (18h)	German Collection of Microorganisms and Cell Cultures
Proteus mirabilis	B12-11.20.0190	>20	>20	37°C (18h)	Prof. Gessner, Medical Microbiology University Regensburg, Germany
Klebsiella pneumoniae	ATCC 13883	7.5	10	37°C (18h)	ATCC
Bacteroidaceae					
Bacteroides fragilis	DSM 2151	>20	>20	37°C (20h), anaerobic	German Collection of Microorganisms and Cell Cultures
Bacteroides fragilis	DSM 9669	>20	>20	37°C (20h), anaerobic	German Collection of Microorganisms and Cell Cultures
Gram-positive species					
Staphylococcus aureus	DSMZ 346	>20	>20	37°C (18h)	German Collection of Microorganisms and Cell Cultures
Staphylococcus epidermidis	DSM 20041	>20	>20	37°C (18h)	German Collection of Microorganisms and Cell Cultures
Streptococcus pneumoniae	DSM 14378	>20	>20	37°C (20h), microaerophilic	German Collection of Microorganisms and Cell Cultures
Listeria monocytogenes	DSMZ 15675	>20	>20	37°C (18h)	German Collection of Microorganisms and Cell Cultures
Bacillus subtilis subsp. spizizenii	DSM 347	>20	>20	37°C (18h)	German Collection of Microorganisms and Cell Cultures
Enterococcus faecalis	DSM 6134	>20	>20	37°C (20h), microaerophilic	German Collection of Microorganisms and Cell Cultures
Clostridium perfringens	DSM 756	>20	>20	37°C (20h), anaerobic	German Collection of Microorganisms and Cell Cultures
Yeast					
Candida albicans	DSM 1386	>20	>20	25°C (40h)	German Collection of Microorganisms and Cell Cultures

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2. **Pirnay J-P, De Vos D, Mossialos D, Vanderkelen A, Cornelis P, Zizi M.** 2002b. Analysis of the *Pseudomonas aeruginosa oprD* gene from clinical and environmental isolates. Environ. Microbiol. **4:**872-882.