ChemPubSoc Europe

DOI: 10.1002/cmdc.201600400

CHEMMEDCHEM Communications

Designing Simple Lipidated Lysines: Bifurcation Imparts Selective Antibacterial Activity

Chandradhish Ghosh, Mohini Mohan Konai, Paramita Sarkar, Sandip Samaddar, and Jayanta Haldar*[a]

In the global effort to thwart antimicrobial resistance, lipopeptides are an important class of antimicrobial agents, especially against Gram-negative infections. In an attempt to circumvent their synthetic complexities, we designed simple membrane-active agents involving only one amino acid and two lipid tails. Herein we show that the use of two short lipid tails instead of a single long one significantly increases selective antibacterial activity. This study yielded several selective antibacterial compounds, and investigations into the properties of this compound class were conducted with the most active compound. Fluorescence spectroscopic studies revealed the capacity of the representative compound to cause depolarization and permeabilization of bacterial cell membranes. This membrane-active nature of the compound imparts superior activity against persister cells, biofilms, and planktonic cells. Topical application of the compound decreased bacterial burden in mice inflicted with burn-infections caused by *Acinetobacter baumannii*. We anticipate that the design principles described herein will direct the development of several antimicrobial agents of clinical importance.

The current predicament of antimicrobial resistance has not only put world health at risk, but is also expected to severely affect the global economy.[1] Natural antimicrobial peptides are an inspiration for the design of next-generation drugs.[2] The advent of synthetic membrane-active agents has bolstered the antimicrobial pipeline with a new class of drugs.[3] Although much remains to be achieved in terms of clinical success, their importance as future antibiotics cannot be overstated. Lipidated peptidomimetics have gained popularity as a successful method for producing broad-spectrum antibacterial agents.[4] We have designed several membrane-active agents in the past.[5] Lipidation of antibiotics in current use has also been reported.[6] Although there is no doubt about the efficacy of such designs, there is an argument for making a simpler and more cost-effective lipopeptide. More importantly, lipidation of antibiotics leads to compounds that generally lose their selective activity against bacteria relative to that of the parent antibiotics.[6] Herein we describe the development of simple lipidated lysine-based membrane-active molecules and one possible approach toward achieving selective antibacterial activity. Furthermore, the mechanism of antibacterial action, the ability to stall resistance development in bacteria, along with anti-biofilm properties of a representative compound were studied. Finally, the activity of the compound was also validated in a murine model of *Acinetobacter baumannii* burn infection.

We envisioned that the structure of a simple lysine-based membrane-active compound would be an alkyl chain conjugated to it. To have any significant activity, the alkyl chain must be sufficiently long, which will facilitate interactions with the bacterial membrane. In the first example, we chose to couple dodecylamine to lysine using simple HBTU coupling chemistry to obtain $C_{12}K$ (**1**). Gradual increase in alkyl chain length yielded compounds with tetradecyl ($C_{14}K$, **2**), hexadecyl ($C_{16}K$, **3**), octadecyl ($C_{18}K$, **4**) and eicosyl ($C_{20}K$, **5**) chains. The activity of the compounds (in their CF_3COO^- salt forms) were evaluated against *Staphylococcus aureus*, *Enterococcus faecium*, *Escherichia coli*, and *A. baumannii* (Table 1). The first three compounds were found to possess good antibacterial activity. However, in moving to $C_{18}K$ and $C_{20}K$, the activity of the compounds was lost. Evaluation of their toxicity against erythrocytes, as indicated by their HC_{50} values (concentration at which 50% of erythrocytes are lysed), revealed that all the active compounds possess substantial toxicity.

Table 1. Antibacterial and hemolytic activity of the compounds.

Compound	MIC [μM][a]				HC_{50}
	SA[b]	EF[c]	EC[d]	AB[e]	
C_{12}-K (**1**)	23	46	23	23	120
C_{14}-K (**2**)	11	44	11	11	105
C_{16}-K (**3**)	5	21	5	10.5	73
C_{18}-K (**4**)	>50	>50	>50	>50	114
C_{20}-K (**5**)	>50	>50	>50	>50	185
C_6-K-C_6 (**6**)	23	46	23	>50	390
C_6-K-C_8 (**7**)	22	22	22	22	172
C_8-K-C_8 (**8**)	5	10.5	5	10.5	110
C_8-K-C_{10} (**9**)	5	10	5	10	108
C_{10}-K-C_{10} (**10**)	6	6	6	6	88
colistin	25	N.D.	0.4	0.4	N.D.
vancomycin	0.5	0.5	>100	>100	N.D.
CTAB	8.5	8.5	8.5	8.5	82

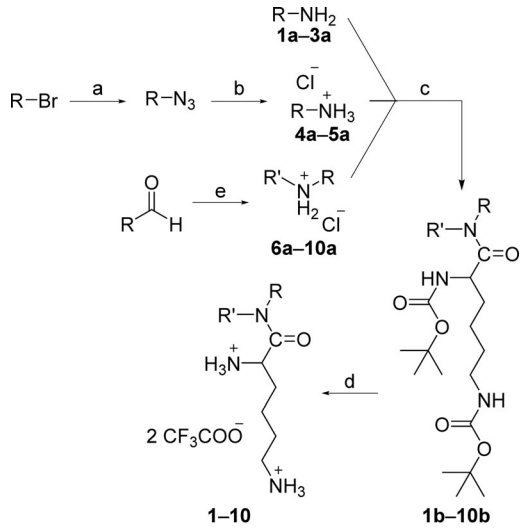
[a] Values are the average of at least two experiments, each done in triplicate (error <5%); N.D.: not determined. [b] *S. aureus* (MTCC 737). [c] *E. faecium* (ATCC 19634). [d] *E. coli* (MTCC 443). [e] *A. baumannii* (MTCC 737).

[a] C. Ghosh, M. M. Konai, P. Sarkar, Dr. S. Samaddar, Prof. Dr. J. Haldar
Chemical Biology and Medicinal Chemistry Laboratory, New Chemistry Unit, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Jakkur, Bengaluru 560064, Karnataka (India)
Fax: (+91) 80-2208-2627
E-mail: jayanta@jncasr.ac.in

Supporting information for this article can be found under http://dx.doi.org/10.1002/cmdc.201600400: synthesis and characterization of compounds and description of experimental protocols.

Although it is well accepted that optimum amphipathicity is necessary for potent antimicrobial activity, increase in alkyl chain length also leads to a compromise in selectivity.[7] We hypothesized that by decreasing the chain length but not compromising much on overall amphiphilicity, further selectivity could be achieved. One simple approach to do so was to bifurcate the alkyl chain, for example, by splitting the hexadecyl long chain to two octyl chains. With this in mind, we designed a set of new molecules containing two short alkyl chains instead of one long chain.

We symmetrically and asymmetrically varied the length of the two chains from hexyl to decyl. We deliberately avoided using chains with an odd number of methylene units to obtain asymmetry in the designs. The synthetic strategy is outlined in Scheme 1. In the first step of synthesis, alkanals were first reacted with alkylamines in dry methanol and then reduced by sodium borohydride to obtain dialkyl amines. These dialkyl amines were then coupled to Boc-Lys(Boc)-OH in a chloroform/*N*,*N*-dimethylformamide mixture using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) chemistry. In compounds **1**–**5**, single long-chain amines were directly coupled to Boc-Lys(Boc)-OH. After purifying the compounds by column chromatography, the Boc groups were subsequently deprotected with 50% trifluoroacetic acid in dichloromethane to yield the final compounds. They were purified by HPLC to >95% purity and were subsequently characterized by ^{1}H NMR, ^{13}C NMR, IR and HRMS.



1a: R=$C_{12}H_{25}$, **1b** and **1**: R=H, R'=$C_{12}H_{25}$
2a: R=$C_{14}H_{29}$, **2b** and **2**: R=H, R'=$C_{14}H_{29}$
3a: R'=$C_{16}H_{33}$, **3b** and **3**: R=H, R'=$C_{16}H_{33}$
4a: R=$C_{18}H_{37}$, **4b** and **4**: R=H, R'=$C_{18}H_{37}$
5a: R=$C_{20}H_{41}$, **5b** and **5**: R=H, R'=$C_{20}H_{41}$
6a, **6b** and **6**: R=C_6H_{13}, R'=C_6H_{13}
7a, **7b** and **7**: R=C_6H_{13}, R'=C_8H_{17}
8a, **8b** and **8**: R=C_8H_{17}, R'=C_8H_{17}
9a, **9b** and **9**: R=$C_{10}H_{21}$, R'=C_8H_{17}
10a, **10b** and **10**: R=$C_{10}H_{21}$, R'=$C_{10}H_{21}$

Scheme 1. *Reagents and conditions*: a) NaN_3, MeOH, RT, 24 h; b) 1. Ph_3P/MeOH, 80 °C, 24 h, 2. HCl; c) Boc-Lys(Boc)-OH, HBTU, DIPEA, DMF/$CHCl_3$ (2:1), RT, 24 h; d) CF_3COOH (50%), CH_2Cl_2, RT, 6 h; e) 1. R'NH_2/MeOH, RT, 8 h, 2. $NaBH_4$, 0 °C→RT, 12 h, 3. HCl.

The activities of bifurcated compounds **6**–**10** were then tested against *S. aureus*, *E. faecium*, *E. coli*, *A. baumannii*, and human erythrocytes. Colistin and vancomycin were used as reference drugs for assays against Gram-negative and Gram-positive bacteria, respectively (Table 1). The classical surfactant cetrimonium bromide (CTAB) was also tested against the bacteria. Compound **6**, which is the bifurcated analogue of **1**, retained activity against *S. aureus* (MIC: 23 μM), *E. faecium* (MIC: 46 μM), and *E. coli* (MIC: 23 μM), but lost activity against *A. baumannii* (MIC > 50 μM). However, with an HC_{50} value of 390 μM, it was significantly less toxic toward erythrocytes than **1** (HC_{50}: 120 μM). Compound **7**, the asymmetric bifurcated analogue of **2**, consists of an octyl chain and a hexyl chain. With the exception of *E. faecium*, the parent compound **2** was active at 11 μM. The bifurcated compound **7** displayed two-fold lower activity (MIC: 22 μM) against *S. aureus*, *E. coli*, and *A. baumannii*, but it was more active than **2** against *E. faecium*. The HC_{50} value of compound **7** was 172 μM, while that of compound **2** was 105 μM. Like its single-chain counterpart **3**, compound **8** displayed good activity against all bacteria (MIC range: 5–10.5 μM), but was more active against *E. faecium* and relatively less toxic. Compound **4** was found to be inactive against all the bacteria tested up to 50 μM. In comparison, **9** turned out to be a very active compound in this series. It displayed MIC values of 5 μM against both *S. aureus* and *E. coli*, while against *E. faecium* and *A. baumannii* the MIC value was 10.5 μM. Compound **10** was as active as **9** (MIC: 6 μM) against *S. aureus*, *E. faecium*, and *E. coli*, but slightly more active against *A. baumannii*. Although the single-chain analogue **5** was less toxic (HC_{50}: 185 μM), it was inactive against all bacteria. The classical surfactant CTAB was active at 8.5 μM against all the bacteria tested. Because selective antibacterial activity is the most desired condition for membrane-active agents, it could be envisioned from this study that bifurcation of long chains into any membrane-active antibiotics containing such a feature should substantially increase the selectivity of the resulting compound.

To determine the potential of such compounds as antibacterial agents, we chose to carry out further studies with compound **9**. The minimum bactericidal concentration (MBC) of **9** was determined to be 2×MIC. The compound was also observed to be nontoxic toward human embryonic kidney (HEK) cells at its MBC, which emphasizes the selectivity of the compound at bactericidal concentrations (Figure 1). The kinetics of the bactericidal action of compound **9** were then studied. Within minutes of treatment at concentrations only three-fold its MIC value, **9** was able to lyse cells of both Gram-negative (Figure 2A) and Gram-positive bacteria (Supporting Information Figure S1). The capacity of the compound to lyse bacterial cells could be attributed to its membrane-active nature.

Because it is widely known that bacteria have difficulty in developing resistance against membrane-active agents, the ability of the compound to induce resistance development was studied (Figure 2B). Serial passage of bacterial culture (both *S. aureus* and *E. coli*) at sub-MIC concentrations of compound **9** yielded no resistant mutants. In comparison, norfloxacin (comparator drug for *S. aureus*) and colistin (comparator

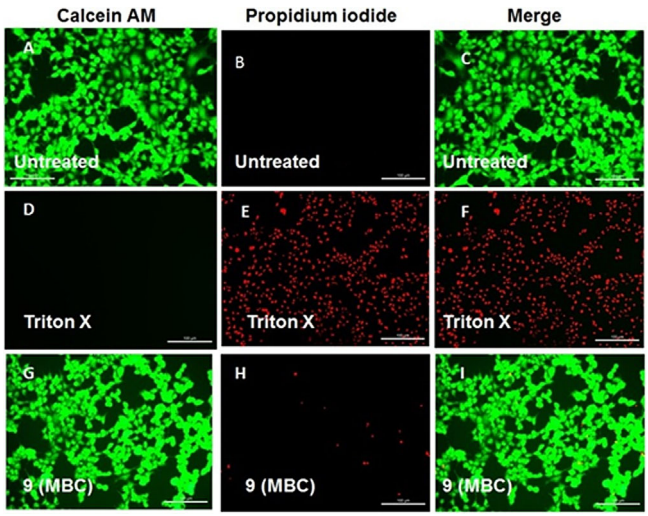


Figure 1. Toxicity studies: fluorescence microscopy images of HEK cells after treatment with or without Triton X and compound **9** for 24 h. Staining was done with calcein AM and propidium iodide (PI). (A–C) Untreated cells (negative control); (D–F) cells treated with 0.1% Triton X (positive control); (G–I) cells treated with compound **9** at MBC (2×MIC)

drug for *E. coli*), yielded resistant mutants within six passages, and in fourteen passages a 400-fold increase in MIC was observed for norfloxacin and an 800-fold increase was observed for colistin.

The membrane-disruptive properties of the compound was demonstrated by performing fluorescence studies with *E. coli* cells. Experiments with the membrane-potential-sensitive dye DiSC3(5) showed that **9** at 3×MIC is able to dissipate the potential of the *E. coli* membrane (Figure 2C). Studies with *N*-phenylnaphthylamine (NPN) showed that compromise of the *E. coli* outer membrane was achieved within minutes of treatment with **9** (Figure 2D). Treatment with **9** also allowed the entry of propidium iodide (PI) into *E. coli* cells, as evident by the increase in dye fluorescence intensity upon compound treatment (Figure 2E).

Next, to gain insight into the potential areas of application of compound **9**, we chose to study its antibacterial efficacy at various pH values (pH 5.5–8.5) and salinity (1–3%). Compound **9** was found to retain its activity against both *S. aureus* and *E. coli* under different physiological conditions (Supporting Information Table S1). At various percentages of salinity, **9** main-

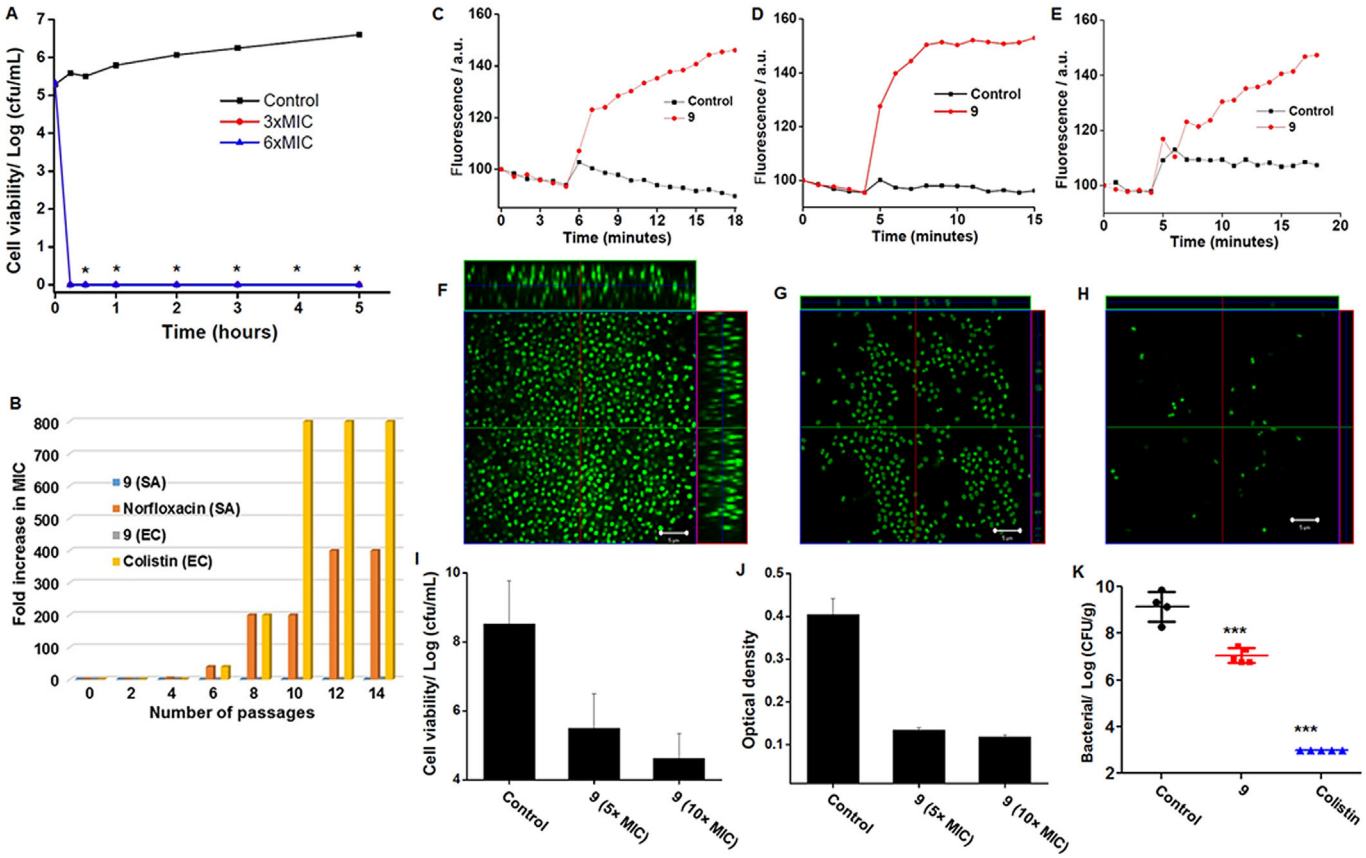


Figure 2. A) Bactericidal time–kill kinetics of compound **9** (concentrations at 3× and 6×MIC) against *E. coli* (*: < 50 CFU mL^{-1}). B) Ability to withstand resistance development (SA: *S. aureus*, EC: *E. coli*). The ability of compound **9** to act on *E. coli* membrane: C) membrane depolarization and D) outer and E) inner membrane permeabilization. SYTO-9 staining of *A. baumannii* biofilms: F) untreated control, and treatment with compound **9** at G) 5×MIC and H) 10×MIC. I) Decrease in bacterial viability within the biofilm. J) Decrease in biofilm mass as determined by crystal violet staining. K) In vivo activity against *A. baumannii* burn infection: the concentration of **9** was 40 mg kg^{-1} and that of colistin was 5 mg kg^{-1}. Statistical analysis was performed using Student's t-test. Differences are considered statistically significant from the untreated group with a value of $p < 0.05$ with 95% confidence intervals (***$p < 0.001$).

tained its MIC value of 5 μM against both *S. aureus* and *E. coli*. At pH 5.5, **9** was found to be active at 10 μM against *S. aureus* and at 5 μM against *E. coli*. No variation in MIC was observed against either bacteria at any other pH value. We also tested the activity of the compound in the presence of 4% bovine serum albumin (BSA). Compound **9** was active at 10 μM against both *S. aureus* and *E. coli* in the presence of BSA. The stability of the compound at such broad ranges of conditions suggests that this class of compounds is suited for use in treating bacterial infections at various regions in the body.

Given the promising antibacterial efficacy displayed by compound **9** and its capacity to infiltrate bacterial membranes, the potential of **9** to treat metabolically inactive cells and biofilms was tested. Persister cells, which are known to down-regulate their metabolism to evade antibiotic stress, are often associated with chronic infection. Targeting their cell membrane is an efficient way of lysing such cells.[8] Owing to the inability of conventional antibiotics to treat such cells, a compound that can lyse persister cells would be medically very important. Persister cells of *S. aureus* and *E. coli* (the number of bacteria were 5 log CFU in each case) succumbed to lysis (limit of detection: 50 CFU mL^{-1}) when treated with **9** at 5×MIC, whereas in the control no change in number of cells were observed in either case. Upon performing the same spectroscopic experiments with *E. coli* persister cells, it was shown that **9** is able to carry out its lytic function by depolarization and disruption of the membranes of persister cells (Figure S2).

The ability of compound **9** to disrupt preformed biofilms of *S. aureus* and *E. coli* was then determined. Treatment of 24-hour-old preformed biofilms of *S. aureus* and 72-hour-old preformed biofilms of *E. coli* at 10×MIC were completely disrupted relative to biofilms left untreated (Figure S3). Because many antibiotics are intrinsically ineffective against Gram-negative pathogens, treatment of Gram-negative biofilms is extremely difficult. *A. baumannii* is a notorious example of an aggressive biofilm-forming bacteria. The capacity of **9** to obliterate preformed biofilms of a clinical isolate of *A. baumannii* (R674) was studied in detail (Figure 2F–J). The MIC value of **9** and colistin against this stain was 10 and 0.7 μM, respectively. Staining with SYTO-9 revealed that the thickness of the biofilms in the untreated case was 9.6 μm (Figure 2F). Compound **9** was effective in disrupting biofilms at 5×MIC, leaving behind a monolayer of cells (thickness: 2.4 μm), whereas at 10×MIC, almost no bacterial cells were observed (Figure 2G–H). Furthermore, treatment with compound **9** (5×MIC) also brought down the bacterial burden embedded within the biofilm by 3 log units, while a ~4 log decrease was observed upon treatment with **9** at 10×MIC (Figure 2I). Apart from that, decrease in biofilm mass was also observed upon treatment with compound **9**, as interpreted from the optical density (OD) values of the crystal violet (CV)-stained biofilms left after or without treatment with compound (Figure 2J).

Finally, to validate the antimicrobial potency of compound **9**, we tested its efficacy in a murine model of infection. Prior to that, acute dermal toxicity of the compound was done. The skin on the back of mice were shaved (~2 cm^2), and the shaved regions were treated with **9** at 200 mg kg^{-1} (single dose). The mice were observed for irritation, tremors, convulsions, salivation, or diarrhea for 14 days. Although the mice showed no sign of irritation or unnatural behavior, visual observation of the skin showed development of corrosion on the first and second days. However, the mice appeared to heal, and normal generation of fur was observed henceforth (within a week), proving that **9** possesses little dermal toxicity. To demonstrate its antibacterial efficacy, burn wounds of mice inflicted with *A. baumannii* were continuously treated for six days at a concentration of 40 mg kg^{-1} (no toxicity was observed at this concentration). Subsequently, the wounded portions were severed, homogenized and plated. Figure 2K shows that treatment with compound **9** decreased the bacterial burden by 2 log units relative to the untreated case in six days (*p* value: 0.0003). Colistin, which was used as a positive control, completely cleared the bacterial burden even at 5 mg kg^{-1}. Although the efficacy of the compounds in this study falls short of colistin, it should be kept in mind that colistin is prone to triggering resistance development in bacteria, unlike **9** (Figure 2B). The rapid development of resistance against colistin could be attributed to its lipopolysaccharide-mediated mechanism of action.[9] Moreover, colistin is used only against Gram-negative bacteria, but **9** is active against both Gram-positive and Gram-negative bacteria.

In summary, this study describes the development of simple membrane-active agents using two long chains and one amino acid (L-lysine). An important conclusion of the study is that the use of two short chains instead of a single long chain can effectively increase the selective antibacterial activity of the resulting compounds. Through the study, one potent membrane antimicrobial agent was discovered which acts on planktonic cells, persister cells, and bacterial biofilms. Active in murine models of infection, the compound retains activity under various physiological conditions and does not select resistant mutants rapidly. Although many more studies need to be done, initial data reveal the potential of this compound class for the treatment of topical Gram-negative infections.

Acknowledgements

We thank Prof. C. N. R. Rao, F.R.S. (JNCASR) for his constant support and encouragement.

Keywords: antibiotics · antimicrobial resistance · biofilms · drug design · peptidomimetics

[1] a) *Antimicrobial Resistance: Global Report on Surveillance*, World Health Organization, **2014**: www.who.int/drugresistance/documents/surveillancereport/en/; b) S. Gandra, D. M. Barter, R. Laxminarayan, *Clin. Microbiol. Infect.* **2014**, *20*, 973–980.

[2] a) C. Ghosh, J. Haldar, *ChemMedChem* **2015**, *10*, 1606–1624; b) S. Rotem, A. Mor, *Biochim. Biophys. Acta Biomembranes* **2009**, *1788*, 1582–1592; c) M. Zasloff, *Nature* **2002**, *415*, 389–395; d) R. E. W. Hancock, H. G. Sahl, *Nat. Biotechnol.* **2006**, *24*, 1551–1557; e) B. Findlay, G. G. Zhanel, F. Schweizer, *Antimicrob. Agents Chemother.* **2010**, *54*, 4049–4058.

[3] a) B. Mensa, G. L. Howell, R. Scott, W. F. DeGrado, *Antimicrob. Agents Chemother.* **2014**, *58*, 5136–5145; b) J. Isaksson, B. O. Brandsdal, M. Engqvist, G. E. Flaten, J. S. M. Svendsen, W. Stensen, *J. Med. Chem.* **2011**, *54*, 5786–

5795; c) H. D. Thaker, A. Som, F. Ayaz, D. H. Lui, W. X. Pan, R. W. Scott, J. Anguita, G. N. Tew, *J. Am. Chem. Soc.* **2012**, *134*, 11088 – 11091; d) B. P. Mowery, S. E. Lee, D. A. Kissounko, R. F. Epand, R. M. Epand, B. Weisblum, S. S. Stahl, S. H. Gellman, *J. Am. Chem. Soc.* **2007**, *129*, 15474 – 15476; e) H. Mohammad, S. Thangamani, M. N. Seleem, *Curr. Pharm. Des.* **2015**, *21*, 2073 – 2088.

[4] a) I. S. Radzishevsky, S. Rotem, D. Bourdetsky, S. Navon-Venezia, Y. Carmeli, A. Mor, *Nat. Biotechnol.* **2007**, *25*, 657 – 659; b) Y. G. Hu, M. N. Amin, S. Padhee, R. S. E. Wang, Q. Qiao, G. Bai, Y. Q. Li, A. Mathew, C. H. Cao, J. F. Cai, *ACS Med. Chem. Lett.* **2012**, *3*, 683 – 686; c) N. P. Chongsiriwatana, T. M. Miller, M. Wetzler, S. Vakulenko, A. J. Karlsson, S. P. Palecek, S. Mobashery, A. E. Barron, *Antimicrob. Agents Chemother.* **2011**, *55*, 417 – 420; d) S. S. Shankar, S. N. Benke, N. Nagendra, P. L. Srivastava, H. V. Thulasiram, H. N. Gopi, *J. Med. Chem.* **2013**, *56*, 8468 – 8474.

[5] a) M. M. Konai, C. Ghosh, V. Yarlagadda, S. Samaddar, J. Haldar, *J. Med. Chem.* **2014**, *57*, 9409 – 9423; b) M. M. Konai, J. Haldar, *ACS Infect. Dis.* **2015**, *1*, 469 – 478; c) M. M. Konai, U. Adhikary, S. Samaddar, C. Ghosh, J. Haldar, *Bioconjugate Chem.* **2015**, *26*, 2442 – 2453; d) J. Hoque, M. M. Konai, S. Gonuguntla, G. B. Manjunath, S. Samaddar, V. Yarlagadda, J. Haldar, *J. Med. Chem.* **2015**, *58*, 5486 – 5500.

[6] a) V. Yarlagadda, P. Akkapeddi, G. B. Manjunath, J. Haldar, *J. Med. Chem.* **2014**, *57*, 4558 – 4568; b) I. M. Herzog, K. D. Green, Y. Berkov-Zrihen, M. Feldman, R. R. Vidavski, A. Eldar-Boock, R. Satchi-Fainaro, A. Eldar, S. Garneau-Tsodikova, M. Fridman, *Angew. Chem. Int. Ed.* **2012**, *51*, 5652 – 5656; *Angew. Chem.* **2012**, *124*, 5750 – 5754; c) Y. Berkov-Zrihen, I. M. Herzog, M. Feldman, A. Sonn-Segev, Y. Roichman, M. Fridman, *Bioorg. Med. Chem.* **2013**, *21*, 3624 – 3631; d) N. Thamban Chandrika, K. D. Green, J. L. Houghton, S. Garneau-Tsodikova, *ACS Med. Chem. Lett.* **2015**, *6*, 1134 – 1139; e) M. P. Mingeot-Leclercq, J. L. Decout, *MedChemComm* **2016**, *7*, 586 – 611.

[7] a) C. Ghosh, G. B. Manjunath, P. Akkapeddi, V. Yarlagadda, J. Hoque, D. S. Uppu, M. M. Konai, J. Haldar, *J. Med. Chem.* **2014**, *57*, 1428 – 1436; b) D. S. S. M. Uppu, P. Akkapeddi, G. B. Manjunath, V. Yarlagadda, J. Hoque, J. Haldar, *Chem. Commun.* **2013**, *49*, 9389 – 9391.

[8] a) C. Ghosh, G. B. Manjunath, M. M. Konai, D. S. S. M. Uppu, J. Hoque, K. Paramanandham, B. R. Shome, J. Haldar, *PLoS One* **2015**, *10*, e014409; b) J. G. Hurdle, A. J. O'Neill, I. Chopra, R. E. Lee, *Nat. Rev. Microbiol.* **2011**, *9*, 62 – 75; c) C. Ghosh, G. B. Manjunath, M. M. Konai, D. S. S. M. Uppu, K. Paramanandham, B. R. Shome, R. Ravikumar, J. Haldar, *ACS Infect. Dis.* **2016**, *2*, 111 – 122.

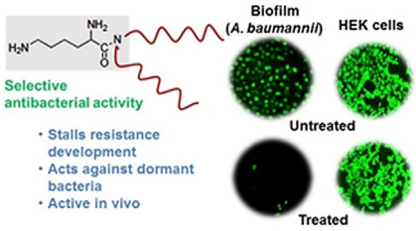
[9] J. H. Moffatt, M. Harper, P. Harrison, J. D. Hale, E. Vinogradov, T. Seemann, R. Henry, B. Crane, F. St Michael, A. D. Cox, B. Adler, R. L. Nation, J. Li, J. D. Boyce, *Antimicrob. Agents Chemother.* **2010**, *54*, 4971 – 4977.

Received: August 9, 2016
Published online on ■■ ■■, 0000

*C. Ghosh, M. M. Konai, P. Sarkar, S. Samaddar, J. Haldar**

■■ – ■■

Designing Simple Lipidated Lysines: Bifurcation Imparts Selective Antibacterial Activity



How simple but selective antibacterial membrane-active agents can be designed is presented herein. Bifurcation of long alkyl chains into two short chains leads to selective antibacterial activity. A representative bifurcated compound exhibits broad-spectrum bactericidal activity and disrupts biofilms. It also treats burn wounds in mice infected with *Acinetobacter baumannii*. This principle could be applied to further designs of membrane-active agents.