



Cite this: *Org. Biomol. Chem.*, 2021, **19**, 6797

Received 24th June 2021,

Accepted 16th July 2021

DOI: 10.1039/d1ob01227d

rsc.li/obc

New antimicrobial self-assembling short lipopeptides†

César Vicente-García ^a and Ignacio Colomer ^{*a,b}

Lipopeptides are an exceptional example of amphiphilic molecules that self-assemble into functional structures with applications in the areas of nanotechnology, catalysis or medicinal chemistry. Herein, we report a library of 21 short lipopeptides, together with their supramolecular characterization and antimicrobial activity against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) strains. This study shows that simple lipoamino acids self-assemble into micellar or vesicular structures, while incorporating dipeptides capable of establishing hydrogen bonds results in the adoption of advanced fibrillar structures. The self-assembly effect has proven to be key to achieve antimicrobial activity.

Peptide nanotechnology has emerged as a powerful tool that allows for the rational design of functional structures whose supramolecular properties can be engineered and controlled by manipulating the peptide sequence.¹ Amphiphilic peptides represent a heterogeneous category of these biomolecules that can be constituted of all amino acids, with alternating hydrophilic and lipophilic monomers, or that can incorporate appendant hydrophilic or hydrophobic chains. These amphiphiles self-assemble into miscellaneous supramolecular structures (*i.e.* micelles, vesicles, nanotubes, fibres, nanobelts) exhibiting versatile properties and applications.² Lipopeptides are a particular example of self-assembling amphiphilic peptides, formed with a polar hydrophilic head and a long hydrophobic chain that can be attached either to the N-terminus position (*i.e.* fatty acyl moiety, Fig. 1a) or at the C-terminus site (*i.e.* fatty amine or alcohol, Fig. 1b). Their self-organization is highly determined by the sequence of amino acids, something that prompted rational design to achieve advanced performances, controlling their morphology and activity, a field that has rapidly grown in recent years.³

Lipopeptides have been widely used as surfactants and low-molecular weight gelators.⁴ Alternatively, extensive investigations have shed light on how to control the supra-molecular properties by tuning the structural motifs of the amphiphilic monomers, using α - or β -amino acids,⁵ homo-chiral *vs.* heterochiral polymers⁶ or the nature and length of the hydrophobic component.⁷ Moreover, the influence of external stimuli on both the self-assembly and the properties displayed has been investigated, including concentration,⁸ solvent,⁹ saline¹⁰ or the pH effect.¹¹ Their ability to respond to these stimuli and adapt over time has been used to design synthetic advanced dynamic supramolecular systems,¹² nanostructured materials,¹³ and self-responsive delivery systems in the area of medical biotechnology¹⁴ or systems chemistry.¹⁵

Although great advances have been made in peptide engineering, identifying small (lipo)peptides with defined supramolecular behaviour,¹⁶ catalytic properties¹⁷ or biological activity¹⁸ is still a challenge. In this study, we aim to build a library of small lipopeptides to systematically explore the chemical space, covering different amino acids, complementary amino acid sequences, the number and length of the hydrophobic aliphatic chain, as well as the influence of its

^aIMDEA Nanociencia, Faraday 9, Campus UAM, 28049 Madrid, Spain

^bInstituto de Química Orgánica General (IQOG-CSIC), Juan de la Cierva 3, 28006 Madrid, Spain. E-mail: colomer@iqog.csic.es

†Electronic supplementary information (ESI) available: Experimental procedures, chemical and supramolecular characterization and antimicrobial assays. See DOI: 10.1039/d1ob01227d

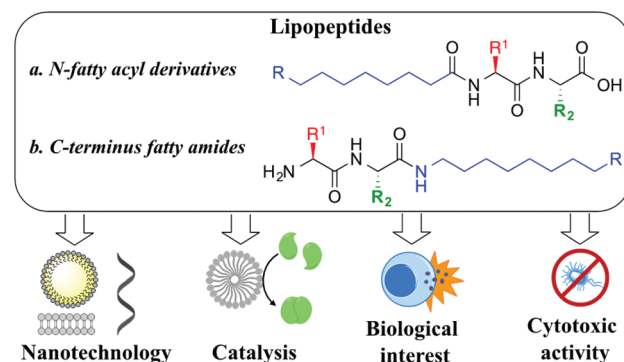


Fig. 1 Importance and uses of lipopeptides.



position (N- vs. C-terminus). Moreover, their biological activity will be investigated, in particular as antimicrobial agents.

We began our study by preparing lipoamino acids (Fig. 2a) derived from amino acids with variable side chains, bearing no substitution (glycine, **1a**), and aliphatic (alanine, **1b** and valine, **1c**), aromatic (phenylalanine, **1d**), hydrogen bond donor (serine, **1e** and **1g**), pH responsive (histidine, **1f** and **1h**) and redox active (cysteine, **1i-k**) functional groups, following the Schotten-Baumann procedure, using free amino acids and decanoyl or oleoyl chlorides under basic conditions.¹⁹ Selective monofunctionalization of cysteine, **1i-j**, was achieved using benzotriazole to activate decanoic acid, leading to amide **1i** using a basic medium and a short reaction time (2 h), while using neutral pH and a longer time (12 h) afforded thioester **1j**

1j.²⁰ Aiming to increase the structural complexity that would allow for additional non-covalent interactions, we prepared a set of lipodipeptides. We decided to focus on Ser and His for their potential H-bonding capabilities, acid-base properties and π - π stacking. Using lipoamino acids derived from Ser or His (**1e-h**), the complementary amino acid (Ser or His) was coupled at the C-terminus position, leading to four new lipodipeptides (Fig. 2a, **2a-d**). The examples described, so far, display a fatty acyl moiety at the N-terminus position. Thus, we thought that evaluating complementary C-terminus derivatives will enrich the outcome of this library. A set of six new lipopeptides **3a-f** (Fig. 2b) could be accessed using fatty amines. For the Ser-His sequence, semi-protected common precursor **4** (ESI⁺) was reacted with decylamine (**3a**), oleylamine (**3b**) or bis-



Fig. 2 Library of lipopeptides synthesized, composed of: (a) N-terminus fatty acyl derivatives and (b) C-terminus fatty amides.



oleate amine derived from serinol (**3c**). For His-Ser derivatives, an alternative route was needed: first incorporating the fatty amine into the C-terminus amino acid (Ser) providing serine amides **5a–c** (Fig. 2b), followed by coupling of the N-terminus amino acid (His) to afford three new lipodipeptides, **3d–f**.

With these lipopeptides in hand, we first studied their self-assembling behavior by performing systematic characterization of their supramolecular properties. For every species, we have determined the minimal concentration needed for them to aggregate, which is the Critical Aggregation Concentration (CAC). We have also investigated the particle size of the supramolecular assemblies, as well as their stability in solution. Finally, we have conducted the study of the morphology of these supramolecular aggregates using electron microscopy.

The supramolecular characterization was started by determining the CAC, using an established spectrofluorimetric method (see the ESI†). A representative example for **1f** is presented in Fig. 3A. The CAC for lipoamino acids is in the range of 0.20–25.0 mM (Table 1), in agreement with the previous examples reported (see the ESI†). The lowest values of the CAC were observed for cysteine derivatives (**1i–k**). For lipodipeptides, the CAC data were more disperse (0.01–68.4 mM), with N-terminus acyl derivatives (**2a–d**) presenting much lower CAC than C-terminus fatty amides (**3a–f**). In general, lipoamino acids with non-polar side chains (**1a–d**) exhibit higher CAC values than examples bearing polar side chains capable of establishing additional non-covalent interactions. This reflects the well-known influence of the hydrogen bonding capacity and HLB to enhance the formation of supramolecular structures.²¹ Additionally, lipopeptides bearing longer aliphatic chains display lower CAC than their shorter counterparts, a trend extensively reported for similar lipopeptides.²²

Using Dynamic Light Scattering (DLS), we then moved to study the particle size, both by number and intensity to determine the probable polydispersity and to gain information

Table 1 Supramolecular characterization of lipopeptides

Compound	CAC (mM)	Size ^a (nm)		ZP ^a (mV)
		Number	Intensity	
1a	25.0	18	24, 142	—
1b	4.50	3	4, 161	−65.9
1c	12.0	4	5, 196	−60.0
1d	12.5	68	74, 241	−68.5
1e	8.90	28	30, 164	−85.3
1f	9.90	32	35, 120	−62.3
1g	6.50	31	13, 68	−75.3
1h	7.60	37	39, 215	−60.1
1i	0.20	10	15, 95	−67.3
1j	3.00	68	15, 210	—
1k	0.70	28	105	−74.2
2a	0.01	10	22, 255	+42.2
2b	0.06	37	40, 220	+69.9
2c	5.96	10	21, 181	+54.0
2d	0.01	190	8, 54, 278	+55.9
3a	68.4	68	70, 137	+61.3
3b	45.7	6	32, 236	+66.7
3c	—	32	7, 190	+91.2
3d	8.88	28	30, 130	+21.6
3e	7.17	5	24, 142	+43.3
3f	—	68	4, 161	+78.3

^a Data collected at pH 11 for **1a–k** and at pH 6 for **2a–d** and **3a–f**.

about the different populations typically observed in this type of sample. A representative example for **1f** is shown in Fig. 3B, initially screening the effect of pH, where basic pH provided monodisperse nanoparticles with an average diameter of 32 nm (see the ESI† for a detailed study); however, below pH 5, the supramolecular species starts disassembling and precipitating. Small particles in the range of 3–68 nm were generally detected for the remaining lipopeptides (Table 1, size by number), together with some larger aggregates in the range of 105–278 nm, in different ratios depending on the example (Table 1, size by intensity). For every sample, we observed exceptionally good raw correlation data (see the ESI†), with a general trend: small structures identified as micelles (3–37 nm, size by number) coexist, in some cases, with larger populations (68–278 nm, size by intensity). DLS is a powerful and non-invasive technique that works particularly well for uniform, symmetric and spherical nanoparticles and allows for studying the particle size both by number and intensity. While size by intensity constitutes a general overview of the size from all particle populations, size by number refers to smaller and more abundant particle populations and should be taken into account with good correlation functions, as is the case here.

The stability of these nanoparticles was studied by measuring the magnitude of the zeta-potential (ZP). A representative study for **1f** is shown in Fig. 3C, with very stable particles above neutral pH, displaying a large absolute value of ZP (>50 mV), while they are much less stable (<30 mV) below neutral pH. Under optimal conditions of pH, a large absolute magnitude of ZP above 60 mV is obtained in most cases (Table 1). For lipoamino acids, **1a–k**, a large negative ZP would

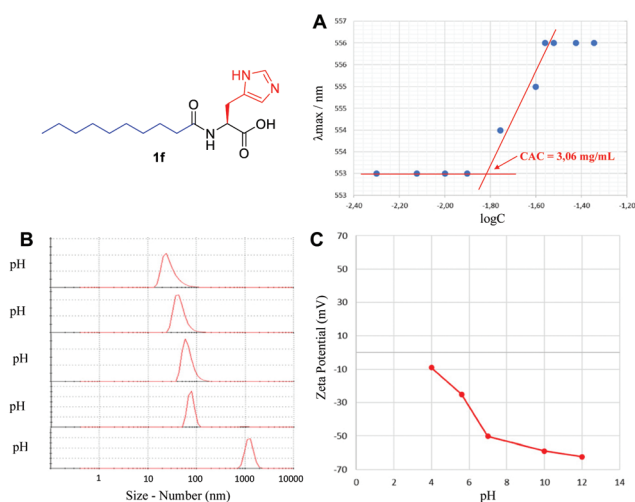


Fig. 3 Supramolecular characterization of **1f**, including (A) critical aggregation concentration, (B) particle size using DLS and (C) self-assembly stability via zeta-potential analysis.



agree with a deprotonated carboxylate, as a result of the basic pH used. In sharp contrast, for lipopeptides, **2a–d** and **3a–f**, measured at neutral pH, a positive value of ZP is observed, pointing towards a net positive charge, suggesting that this pH is below the isoelectric point. Overall, the ZP values for this library of lipopeptides demonstrate a general and exceptionally good stability ($ZP > |40|$ mV).

In an effort to improve this study, our attention was driven to study the infrared spectra of these lipopeptides, which exhibited the following interesting characteristics: (1) red-shifting of O–H and N–H stretching (from $3500\text{--}3000\text{ cm}^{-1}$ to $3300\text{--}2500\text{ cm}^{-1}$) and (2) band-reshaping, from smooth bell-shaped bands to speckled discrete peaks, typical of medium-to-strong hydrogen bond networks.²³ Such spectral characteristics provide insight into the overall ability of this pool of lipopeptides to self-assemble into supramolecular structures, promoted not only by the hydrophobic effect of long aliphatic chains, but also by establishing a hydrogen bonding network through the backbone sequence of amino acids.

The morphology of the self-assembling lipopeptides was studied using High Resolution Transmission Electron Microscopy (HR-TEM) and Field Emission Scanning Electron Microscopy (FE-SEM). While HR-TEM shows spherical and worm-like micelles between 20 and 100 nm for lipoamino acids (Fig. 4A–C and Fig. S12 in the ESI†), multilamellar vesicles of variable sizes, micelle clusters and fibers are observed for lipopeptides (Fig. 4D, E and Fig. S13, S14 in the ESI†). FE-SEM revealed the formation of fibers of different lengths for simple lipoamino acids, **1a–j**, in most cases (Fig. 4F and G), although amorphous aggregates have also been observed (see the ESI†). Interestingly, diacylated cysteine, **1k**, shows a more complex pattern (Fig. 4H), while beautiful cross-linked fibers and flower-like structures are observed for lipopeptides (Fig. 4I and J). Moreover, when fibers are detected, it is possible to visualize that they are formed by small spherical particles with an average diameter that ranges from 20 to 50 nm. These small spherical nanoparticles match the micellar struc-

tures observed in the HR-TEM images, which might imply a hierarchical organization.

Altogether, moving from simple amino acids to dipeptides incorporating functional groups promoting non-covalent interactions allows for switching the supramolecular structure from simple micelles to more complex vesicles or fibers (Fig. 5). Moreover, the size and the presence of a double bond in the aliphatic chain play a key role in the development of a more complex pattern of self-assembly (compare **2b** and **2d** vs. **2a** and **2c**). However, the sequence of amino acids can have a profound effect on the morphology (from vesicles to fibers), which cannot be anticipated, by simply reversing Ser and His residues (compare **2b** vs. **2d**).

One of the biggest challenges concerning public health is the emergence of multidrug resistant pathogens, in particular the growth of antibiotic resistant bacteria strains.²⁴ Understanding the resistance mechanisms²⁵ is key to tackling this problem from different perspectives, including health education,²⁶ optimization²⁷ and repurposing²⁸ of current drugs, development of new antibiotics²⁹ or incorporating advanced genomic techniques.³⁰ Lipopeptides have emerged as a good strategy to develop new antibacterial agents;³¹ most of the examples reported, so far, incorporated cationic amino acids, such as lysine or arginine.³² Mechanistic studies point towards bacterial membrane disruption;³³ however, alteration of the membrane electric potential is also invoked.³⁴



Fig. 5 Schematic self-assembly of lipopeptides.

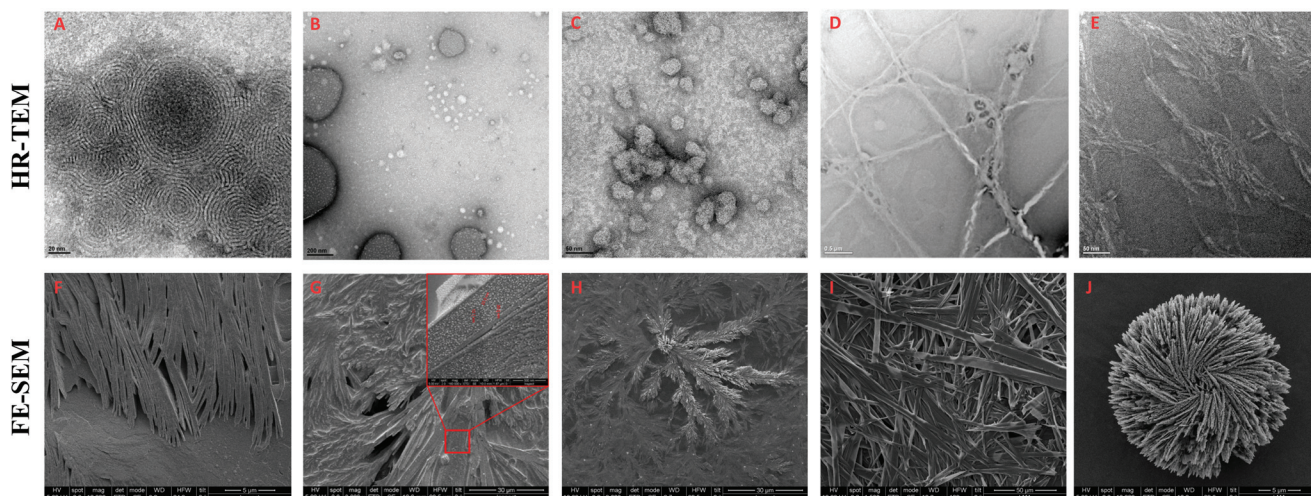


Fig. 4 Representative HR-TEM and FE-SEM images of: **1a** (A and F), **1f** (B and G), **1k** (C and H), **2d** (D and I) and **3f** (E and J).



The antimicrobial activity of these new lipopeptides was evaluated *in vitro* against Gram-negative (*E. coli* CECT 516) and Gram-positive (*S. aureus* CECT 240) strains. The Minimal Inhibitory Concentration (MIC) was determined following the broth microdilution method (see the ESI†). While all lipoamino acids bearing a single fatty acyl chain, **1a–j**, are not active at a realistic concentration (up to 1000 $\mu\text{g mL}^{-1}$), diacylated cysteine **1k** shows a notable activity and selectivity against *S. aureus* (Table 2). For dipeptide derivatives, we first screened non-lipidated Ser-His and His-Ser dipeptides, which proved inactive against both strains, a control experiment that demonstrates the importance of the amphiphilic nature, that allows self-assembling, key to achieving antimicrobial activity (ESI†). Considering N-terminus fatty acyl derivatives (**2a–d**), both *N*-decanoyl Ser-His (**2a**) and His-Ser (**2c**) showed modest and similar inhibitory activities against both strains (250–500 $\mu\text{g mL}^{-1}$). However, *N*-oleyl derivatives (**2b** and **2d**) were inactive. Finally, C-terminus fatty amides lead to interesting results, where all examples bearing a single hydrophobic chain (**3a–b** and **3d–e**) are active against the two bacterial strains. From this, oleyl derivatives (**3b** and **3e**) are exceptionally active (40 $\mu\text{g mL}^{-1}$). Unfortunately, double hydrophobic chain derivatives, **3c** and **3f**, proved to be inactive.

From this, we can draw the following conclusions:

(1) Diacylated cysteine **1k** is an outstanding example, as the only active lipoamino acid, and the only example in the library with such an impressive selectivity towards *S. aureus*. As far as we are aware, there is no precedent for such a simple and short lipoamino acid with this level of activity and selectivity. Shedding light on the mechanism of action of this diacylated cysteine is part of our current efforts.

(2) For dipeptides, the most active ones (**2a**, **2c**, **3b** and **3e**) correlate with the smallest values of particle size (up to 10 nm size by number). However, we are aware that the Hydrophilic-Lipophilic Balance (HLB) might also play an important role.²¹

(3) C-terminus fatty amides **3**, bearing the N-terminal ammonium group, are significantly more active than their ana-

logous *N*-fatty acyl derivatives **2**. Remarkably, fatty amides **3** show MIC values comparable to, or even lower than, highly cationic and structurally more complex lipopeptides previously reported.³² Previous antimicrobial lipopeptides containing both serine and histidine residues are embedded within a polycationic chain of several arginine or lysine residues.³⁵ Alternatively, histidine gemini-lipopeptides with a highly complex pattern of substitution have been reported.³⁶

(4) No straightforward correlation has been found between the CAC of these lipopeptides and their activity, suggesting that the antimicrobial mechanism might not be related to the detergent effect, but rather to the membrane and electrochemical disruption quenching the cell viability. This is in agreement with the higher activity displayed by polycationic peptides and their increased ability to disrupt the bacterial membrane.^{32,33} Moreover, elucidating whether these lipopeptides are bacteriostatic or bactericidal agents will need further investigation.

Conclusions

In conclusion, we have synthesized a library of amphiphilic short lipopeptides that combine *N*-fatty acyl amino acids and all possible combinations of N- and C-lipidated dipeptides from Ser and His. Their supramolecular behaviour has been systematically studied, and their Critical Aggregation Concentration (CAC), particle size, stability (zeta-potential) and morphology have been reported. In addition, their antimicrobial activity has been evaluated, showing promising results with a respectable selectivity towards Gram-positive strains for some examples. This study shows the importance of new structurally simple and short lipopeptides as a tool to develop novel, potent and selective antimicrobial agents, where the self-assembling nature is indispensable for their bioactivity.

Author contributions

C.V.G. performed the experiments. C.V.G. and I.C. contributed to designing and analysing the experiments, and writing, discussing and editing the manuscript. I.C. conceived and guided the research.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This project received funding from the La Caixa Foundation under the Junior Leader Program (No. LCF/BQ/PI19/11690020, ID 100010434), CAM-Atracción de Talento (2019-T1/IND-12683) and Spanish MICINN (PID2019-106327GA-I00). I. C. holds a

Table 2 Antimicrobial activity results using lipopeptides

Compound	MIC ^a ($\mu\text{g mL}^{-1}$)		Compound	MIC ^a ($\mu\text{g mL}^{-1}$)	
	<i>E. coli</i> CECT 516	<i>S. aureus</i> CECT 240		<i>E. coli</i> CECT 516	<i>S. aureus</i> CECT 240
1a	>1000	>1000	2a	250	250
1b	>1000	>1000	2b	>1000	>1000
1c	>1000	1000	2c	500	500
1d	>1000	1000	2d	>1000	>1000
1e	>1000	>1000	3a	500	125
1f	>1000	>1000	3b	40	40
1g	>1000	>1000	3c	>1000	>1000
1h	>1000	>1000	3d	250	250
1i	>1000	>1000	3e	40	40
1j	>1000	>1000	3f	>1000	>1000
1k	>1000	40	Gentamicin	4.0 ^{37a}	1.56 ^{37b}

^a Minimum concentration that inhibits proliferation after overnight incubation.



Ramón y Cajal fellowship from Spanish MICINN (RyC2019-026674-I). We also thank Prof. Isabel Rodriguez (IMDEA-Nanociencia) for providing bacterial strains and Dr Jose Miguel González (ICB-CSIC) for helping with the microscopy analysis.

Notes and references

- (a) R. de la Rica and H. Matsui, *Chem. Soc. Rev.*, 2010, **39**, 3499–3509; (b) M. Zelzer and R. V. Ulijn, *Chem. Soc. Rev.*, 2010, **39**, 3351–3357; (c) A. Lampel, R. V. Ulijn and T. Tuttle, *Chem. Soc. Rev.*, 2018, **47**, 3737–3758.
- (a) D. W. P. M. Löwik and J. C. M. van Hest, *Chem. Soc. Rev.*, 2004, **33**, 234–245; (b) I. W. Hamley, *Soft Matter*, 2011, **7**, 4122–4138; (c) S. Fleming and R. V. Ulijn, *Chem. Soc. Rev.*, 2014, **43**, 8150–8177.
- (a) I. W. Hamley, *Chem. Commun.*, 2015, **51**, 8574–8583; (b) P. Hendricks, K. Sato, L. C. Palmer and S. I. Stupp, *Acc. Chem. Res.*, 2017, **50**, 2440–2448; (c) N. Battista, M. Bari and T. Bisogno, *Biomolecules*, 2019, **9**, 822.
- (a) M. C. Morán, A. Pinazo, L. Pérez, P. Clapés, M. Angelet, M. T. García, M. P. Vinardell and M. R. Infante, *Green Chem.*, 2004, **6**, 233–240; (b) R. Bordes and K. Holmberg, *Adv. Colloid Interface Sci.*, 2015, **222**, 79–91; (c) X. Du, J. Zhou, J. Shi and B. Xu, *Chem. Rev.*, 2015, **115**, 13165–13307.
- D. Sivaramakrishna and M. J. Swamy, *Chem. Phys. Lipids*, 2016, **201**, 1–10.
- (a) A. Ohta, M. Shirai, T. Asakawa and S. Miyagishi, *J. Oleo Sci.*, 2008, **57**, 659–667; (b) S. Lin, J. Qin, Y. Li, B. Li and Y. Yang, *Langmuir*, 2017, **33**, 8246–8252.
- Selected examples: (a) G. D. Smith and R. E. Barden, *Chem. Phys. Lipids*, 1975, **14**, 1–14; (b) A. Pinazo, L. Pérez, M. Lozano, M. Angelet, M. R. Infante, M. P. Vinardell and R. Pons, *J. Phys. Chem. B*, 2008, **112**, 8578–8585; (c) C. Zheng, S. Lin, Y. Chen, Y. Li, B. Li and Y. Yang, *Langmuir*, 2019, **35**, 11406–11413.
- C. W. Harrell, M. E. McCarroll, K. F. Morris, E. J. Billiot and I. M. Warner, *Langmuir*, 2003, **19**, 10684–10691.
- (a) C. Boettcher, B. Schade and J.-H. Fuhrhop, *Langmuir*, 2001, **17**, 873–877; (b) Y. Li, B. Li, Y. Fu, S. Lin and Y. Yang, *Langmuir*, 2013, **29**, 9721–9726.
- G. A. Rothbauer, E. A. Rutter, C. Reuter-Seng, S. Vera, E. J. Billiot, Y. Fang, F. H. Billiot and K. F. Morris, *J. Surfactants Deterg.*, 2018, **21**, 139–153.
- (a) T. Kar, S. Debnath, D. Das, A. Shome and P. K. Das, *Langmuir*, 2009, **25**, 8639–8648; (b) M. Hatip Koc, G. Cinar Ciftci, S. Baday, V. Castelletto, I. W. Hamley and M. O. Guler, *Langmuir*, 2017, **33**, 7947–7956.
- Selected recent examples: (a) T. J. Moyer, J. A. Finbloom, F. Chen, D. J. Toft, V. L. Cryns and S. I. Stupp, *J. Am. Chem. Soc.*, 2014, **136**, 14746–14752; (b) A. T. Preslar, F. Tantakitti, K. Park, S. Zhang, S. I. Stupp and T. J. Meade, *ACS Nano*, 2016, **10**, 7376–7384; (c) C. Gao, H. Li, Y. Li, S. Kewalramani, L. C. Palmer, V. P. Dravid, S. I. Stupp, M. Olvera De La Cruz and M. J. Bedzyk, *J. Phys. Chem. B*, 2017, **121**, 1623–1628.
- For a seminal outstanding contribution: J. D. Hartgerink, E. Beniash and S. I. Stupp, *Science*, 2001, **294**, 1684.
- K. Sato, M. P. Hendricks, L. C. Palmer and S. I. Stupp, *Chem. Soc. Rev.*, 2018, **47**, 7539–7551.
- (a) S. Bal, K. Das, S. Ahmed and D. Das, *Angew. Chem., Int. Ed.*, 2019, **58**, 244–247; (b) S. P. Afrose, S. Bal, A. Chatterjee, K. Das and D. Das, *Angew. Chem., Int. Ed.*, 2019, **58**, 15783–15787.
- Selected studies on short peptides' supramolecular behaviour: (a) L. Chen, K. Morris, A. Laybourn, D. Elias, M. R. Hicks, A. Rodger, L. Serpell and D. J. Adams, *Langmuir*, 2010, **26**, 5232–5242; (b) L. Chen, S. Revel, K. Morris, L. C. Serpell and D. J. Adams, *Langmuir*, 2010, **26**, 13466–13471; (c) P. W. J. M. Frederix, R. V. Ulijn, N. T. Hunt and T. Tuttle, *J. Phys. Chem. Lett.*, 2011, **2**, 2380–2384; (d) P. W. J. M. Frederix, G. G. Scott, Y. M. Abul-Haija, D. Kalafatovic, C. G. Pappas, N. Javid, N. T. Hunt, R. V. Ulijn and T. Tuttle, *Nat. Chem.*, 2015, **7**, 30–37; (e) J. K. Gupta, D. J. Adams and N. G. Berry, *Chem. Sci.*, 2016, **7**, 4713–4719; (f) A. Lampel, S. A. McPhee, H.-A. Park, G. G. Scott, S. Humagain, D. R. Hekstra, B. Yoo, P. W. J. M. Frederix, T.-D. Li, R. R. Abzalimov, S. G. Greenbaum, T. Tuttle, C. Hu, C. J. Bettinger and R. V. Ulijn, *Science*, 2017, **356**, 1064–1068; (g) V. Castelletto, J. Seitsonen, K. M. Tewari, A. Hasan, R. M. Edkins, J. Ruokolainen, L. M. Pandey, I. W. Hamley and K. H. A. Lau, *ACS Macro Lett.*, 2020, **9**, 494–499.
- For reviews on catalytic small peptides: (a) E. A. C. Davie, S. M. Mennen, Y. Xu and S. J. Miller, *Chem. Rev.*, 2007, **107**, 5759–5812; (b) A. J. Metrano and S. J. Miller, *Acc. Chem. Res.*, 2019, **52**, 199–215 For a review partially covering catalytic lipopeptides: (c) O. Zozulia, M. A. Dolan and I. V. Korendovych, *Chem. Soc. Rev.*, 2018, **47**, 3621–3639.
- For an example of bioactive small lipopeptides: (a) A. Makovitzki, D. Avrahami and Y. Shai, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 15997–16002 For reviews: (b) R. Jerala, *Expert Opin. Invest. Drugs*, 2007, **16**, 1159–1169; (c) J.-J. Koh, S. Lin, R. W. Beuerman and S. Liu, *Amino Acids*, 2017, **49**, 1653–1677.
- Comprehensive Organic Name Reaction and Reagents*, ed. Z. Wang, John Wiley and Sons, Weinheim, 2009, p. 2536.
- A. R. Katritzky, S. R. Tala, N. E. Abo-Dya, K. Gyanda, B. E.-D. M. El-Gendy, Z. K. Abdel-Samii and P. J. Steel, *J. Org. Chem.*, 2009, **74**, 7165–7167.
- For a perspective on HLB: (a) R. C. Pasquali, M. P. Taurozzi and C. Bregni, *Int. J. Pharm.*, 2008, **356**, 44–51 For the suggested relationship between HLB and antibacterial activity: (b) K.-M. Park, S. J. Lee, H. Yu, J.-Y. Park, H.-S. Jung, K. Kim, C. J. Lee and P.-S. Chang, *Food Sci. Biotechnol.*, 2018, **27**, 401–409; (c) A. Maneedaeng, S. Phoemboon, P. Chanthasena and N. Chudapongse, *Korean J. Chem. Eng.*, 2018, **35**, 2313–2320.
- P. U. Sinare and J. D. Mhatre, *Am. J. Chem.*, 2012, **2**, 186–190.



- 23 (a) S. Saha and G. R. Desiraju, *J. Am. Chem. Soc.*, 2018, **140**, 6361–6373; (b) *Vibrational dynamics of hydrogen bonds*, ed. E. T. Nibbering, J. Dreyer, O. Kühn, J. Bredenbeck, P. Hamm and T. Elsaesser, Springer, Berlin, Heidelberg, 2007, vol. 87.
- 24 (a) H. Nikaido, *Annu. Rev. Biochem.*, 2009, **78**, 119–146; (b) M. Frieri, K. Kumar and A. Boutin, *J. Infect. Public Health*, 2017, **10**, 369–378.
- 25 (a) C. Walsh, *Nature*, 2000, **406**, 775–781; (b) M. N. Alekshun and S. B. Levy, *Cell*, 2007, **128**, 1037–1050.
- 26 S. Hernando-Amado, T. M. Coque, F. Baquero and J. L. Martínez, *Nat. Microbiol.*, 2019, **4**, 1432–1442.
- 27 M. Tyers and G. D. Wright, *Nat. Rev. Microbiol.*, 2019, **17**, 141–155.
- 28 M. A. Farha and E. D. Brown, *Nat. Microbiol.*, 2019, **4**, 565–577.
- 29 (a) D. J. Payne, M. N. Gwynn, D. J. Holmes and D. L. Pompliano, *Nat. Rev. Drug Discovery*, 2007, **6**, 29–40; (b) K. Lewis, *Nat. Rev. Drug Discovery*, 2013, **12**, 371–387; (c) U. Theuretzbacher, K. Outterson, A. Engel and A. Karlén, *Nat. Rev. Microbiol.*, 2020, **18**, 275–285.
- 30 M. Boolchandani, A. W. D'Souza and G. Dantas, *Nat. Rev. Genet.*, 2019, **20**, 356–370.
- 31 (a) A. Pinazo, M. A. Manresa, A. M. Marques, M. Bustelo, M. J. Espuny and L. Pérez, *Adv. Colloid Interface Sci.*, 2016, **228**, 17–39; (b) F. Azmi, A. G. Elliott, Z. G. Khalil, W. M. Hussein, A. Kavanagh, J. X. Huang, M. Quezada, M. A. T. Blaskovich, R. J. Capon, M. A. Cooper, M. Skwarczynski and I. Toth, *Nanomedicine*, 2015, **10**, 3359–3371.
- 32 Selected examples: (a) Y. Fang, W. Zhong, Y. Wang, T. Xun, D. Lin, W. Liu, J. Wang, L. Lv, S. Liu and J. He, *Eur. J. Med. Chem.*, 2014, **83**, 36–44; (b) B. Mishra, T. Lushnikova and G. Wang, *RSC Adv.*, 2015, **5**, 59758–59769; (c) M. A. Padászyska, M. Maciejewska, D. Neubauer, K. Golacki, M. Szymukowicz, M. Bauer and W. Kamysz, *Pharmaceutics*, 2019, **11**, 506.
- 33 S. Nasompag, P. Dechsiri, N. Hongsing, P. Phonimdaeng, S. Daduang, S. Klaynongsruang, T. A. Camesano and R. Patramanon, *Biochim. Biophys. Acta, Biomembr.*, 2015, **1848**, 2351–2364.
- 34 Y. Shai, *Biopolym. – Pept. Sci. Sect.*, 2002, **66**, 236–248.
- 35 (a) S. R. Nagarajan, B. Devadas, M. E. Zupec, S. K. Freeman, D. L. Brown, H.-F. Lu, P. P. Mehta, N. S. Kishore, C. A. McWherter, D. P. Getman, J. I. Gordon and J. A. Sikorski, *J. Med. Chem.*, 1997, **40**, 1422–1438; (b) H. Yu, K. Park and P. Chang, *Food Chem.*, 2020, **319**, 126533.
- 36 M. Ahn, R. N. Murugan, B. Jacob, J. Hyun, C. Cheong, E. Hwang, H. Park, J. Seo, G. Srinivasrao, K. S. Lee, S. Y. Shin and J. K. Bang, *Eur. J. Med. Chem.*, 2013, **68**, 10–18.
- 37 (a) H.-K. Sun, A. Pang, D. C. Farr, T. Mosaiab, W. J. Britton, S. Anoopkumar-Dukie, I. D. Grice, M. J. Kiefel, N. P. West, G. D. Grant and T. A. Houston, *Aust. J. Chem.*, 2018, **71**, 716–719; (b) T. S. Graziano, M. C. Cuzzullin, G. C. Franco, H. O. Schwartz-Filho, E. D. de Andrade, F. C. Groppo and K. Cogo-Müller, *PLoS One*, 2015, **10**, e0128098.

