



Cite this: *Org. Biomol. Chem.*, 2023, **21**, 7753

Formulation and evaluation of anion transporters in nanostructured lipid carriers†

Daniel Alonso-Carrillo, ^a Israel Carreira-Barral, ^a Marcin Mielczarek,^a Andrea Sancho-Medina,^a Enara Herran,^b Claudia Vairo,^b Angel Del Pozo,^b Iris Luzuriaga, ^b Nerea Lazcanoiturburu,^b Oihane Ibarrola,^b Sara Ponce, ^b María Villar-Vidal,^b María García-Valverde ^a and Roberto Quesada ^{*a}

Six novel click-tambjamins (**1–6**) bearing an alkyl chain of varying length linked to the imine moiety have been formulated in nanostructured lipid carriers (NLCs) to evaluate their transmembrane anion transport activity both when free (*i.e.*, not encapsulated) and nanoformulated. Nanostructured lipid carriers (NLCs) are an example of drug delivery systems (DDSs) that stand out because of their versatility. In this work we show that NLCs can be used to efficiently formulate highly lipophilic anionophores and experiments conducted in model liposomes reveal that these formulations are adequate to deliver anionophores without compromising their transport activity. This result paves the way to facilitate the study of highly lipophilic anionophores and their potential use as future drugs.

Received 26th July 2023,
Accepted 29th August 2023

DOI: 10.1039/d3ob01182h

rs.c.li/obc

Introduction

Facilitated ion transport across phospholipid membranes attracted the attention of the supramolecular chemistry community very early. Designing and studying artificial ionophores was one of the core concepts developed by pioneers in the field.^{1,2} More recently, the development of anion-selective ionophores, anionophores, has attracted renewed interest.^{3–5} In particular, potential applications of these compounds as antimicrobial, antiparasitic or anticancer agents have been reported.^{6–10} Applications as transmembrane protein surrogates as potential candidates for the treatment of diseases caused by defective transmembrane anion transport, like cystic fibrosis, have also been explored.^{11,12}

Designed to work embedded in the phospholipid bilayer, anionophores are necessarily highly lipophilic, as this characteristic is a key parameter determining their activity and potency.¹³ Consequently, anionophores are usually poorly soluble in water, making the study of their activity in model liposomes and *in vitro* difficult, thus representing a burden for future clinical applications. Usually, a small quantity of a stock solution of the compound of interest in DMSO or other organic solvent is used to deliver the anionophore to a suspen-

sion of vesicles or cell culture.¹⁴ For highly insoluble anionophores, strategies such as the pre-incorporation of anionophores into phospholipid solutions used to prepare vesicles have been employed to facilitate their study. On the other hand, this approach severely limits the applicability of the compounds.¹⁵ Davis and Sheppard have studied the use of liposomes as delivery vehicles for anionophores.¹⁶ More recently, Gale *et al.* reported the use of cyclodextrins as adjuvants to deliver lipophilic adamantyl-appended anionophores to vesicle suspensions in water.¹⁷

With the aim of exploring a versatile drug delivery system (DDS) for anionophores, we turned our attention to nanostructured lipid carriers (NLCs).¹⁸ NLCs present a lipid core composed of a mixture of liquid and solid lipids stabilised by surfactants. These nanoformulations present good biocompatibility, biodegradable properties, high drug loading, controlled drug release, long-term stability and scaling-up feasibility;¹⁹ in addition, they possess a well-established safety profile and toxicological data.^{20–22} The chemical nature of NLCs allows the incorporation of both hydrophilic and lipophilic drugs, and these carriers can be tailored according to the drug, the route to be followed by the drug upon its administration and the disease to be tackled.²³ NLCs have been employed in various applications, including wound healing enhancement,²⁴ central-nervous-system-related therapies,²⁵ treatments against multidrug-resistant bacteria,²⁶ and cancer chemotherapy.^{27,28}

The versatility of NLCs prompted us to investigate their applicability for the nanoformulation of a series of anionophores and to explore the impact of encapsulation in their anion transport properties in model vesicles.

^aDepartamento de Química, Universidad de Burgos, Burgos 09001, Spain.

E-mail: rquesada@ubu.es

^bBiokeralty Research Institute AIE, Hermanos Lumière 5, 01510 Miñano, Spain

† Electronic supplementary information (ESI) available: Experimental details, compound characterisation data, NLCs preparation and characterisation, anion transport data. See DOI: <https://doi.org/10.1039/d3ob01182h>



Results and discussion

Synthesis

Our group has recently described click-tambjamins as potent anionophores displaying biological activities.²⁹ Here, we selected a family of click-tambjamins (**1–6**) which differ in the length of the alkyl chain linked to the imine moiety (Fig. 1). We anticipated that these compounds would cover a range of lipophilicities and transmembrane anion transport potencies, which would be useful to assess and compare the encapsulation efficiency and performance of the compounds and their nanoformulated counterparts in transmembrane anion transport assays carried out in model liposomes (POPC). The use of these lipid-based formulations allows to keep or slightly increase (up to one order of magnitude) the potency (EC_{50}) of the studied anionophores. This is paramount when it comes to drug delivery, as encapsulation of these or similar systems, if developed as drugs, would permit their delivery without significantly affecting their anion transport ability.

Click-tambjamins **1–6** were synthesised by reaction of the appropriate amine (hexyl-, octyl-, decyl-, dodecyl-, tetradecyl- or hexadecylamine) with 5-(1-(4-(*tert*-butyl)phenyl)-1*H*-1,2,3-triazol-4-yl)-3-methoxy-1*H*-pyrrole-2-carbaldehyde in boiling chloroform, employing acetic acid as catalyst, in a similar procedure to that reported previously by our research group.²⁹ Solutions of the raw products in dichloromethane were treated with a 1 M HCl aqueous solution; this afforded the desired compounds as their hydrochloric salts (Fig. 1), which were fully characterised by nuclear magnetic resonance spectroscopy (1H , ^{13}C and DEPT-135 experiments) and high-resolution mass spectrometry (see ESI† for details).

1H NMR titration experiments

Interaction of the protonated click-tambjamins with chloride was investigated by means of 1H NMR spectroscopy, using serial dilution of a concentrated solution of the corresponding hydrochloric salt in a 9:1 $CDCl_3/DMSO-d_6$ mixture. This method allows the calculation of association constants without disturbance from potentially interfering counterions. The signals of the protons of the pyrrole and imine's N–H fragments and of the triazole's C–H group undergo a slight shielding upon dilution (Fig. 2). This is in agreement with the interaction of the chloride anion with the three hydrogen-bond donors of the molecules, as observed both in the solid state and in solution for similar structures.²⁹ The obtained profiles were fitted satisfactorily to the 1:1 (LH:Cl) binding model,

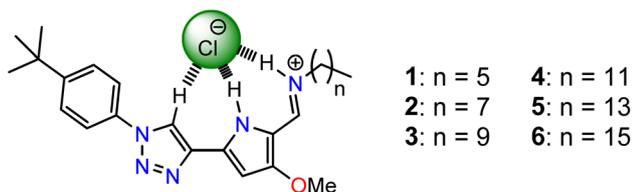


Fig. 1 Structures of the studied compounds.

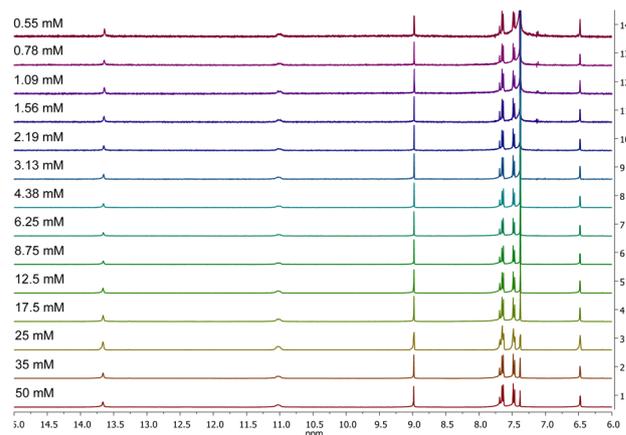


Fig. 2 Stack plot of partial 1H NMR spectra (400 MHz, 298 K) of compound **3** in a 9:1 $CDCl_3:DMSO-d_6$ mixture, obtained by serial dilution of a 50 mM solution of the compound.

where LH is the protonated receptor and Cl the chloride anion, with Bindfit software.^{30,31} The calculated K_a values (Table 1) are similar for all compounds.

Characterisation of NLC formulations

Nanostructured lipid carriers (NLCs) were prepared by the hot-melt homogenisation method, followed by freeze-drying. Briefly (for a detailed description of the procedure see ESI†), the oily phase containing the anion carrier was weighed and melted 5 °C above its melting point. In a parallel manner, the aqueous phase containing the surfactants was prepared and tempered. Both phases were mixed and sonicated for 30 seconds. The resulting emulsion was kept at 5 ± 3 °C for 2–3 hours to allow lipid solidification. Subsequently, a cryoprotecting agent was dissolved and added to the formulation and, finally, vials were filled and samples lyophilised. In order to characterise the obtained lipid nanoparticles, their size and zeta potential were determined (Table 2). Particles loaded with anionophores show a zeta potential close to 0 mV. Transmission electron microscopy (TEM) images of the formulations show nearly oval small particles that present a rough surface (ESI, Fig. S38†).

The concentrations of anionophore in the NLCs, as well as encapsulation efficacies, were determined experimentally by UV/vis spectroscopy. The relationship between absorbance and concentration was established for each pure compound, and a calibration plot obtained. It should be noted that lipid nanoparticles did not absorb in the region where anionophores show their maximum absorption band (300–400 nm). An aliquot of each formulation was taken and its absorption spectrum recorded; this procedure was performed in triplicate to check the homogeneity of the samples. Data for both determining the equations of the calibration plots and the concentrations of the compounds in the samples were taken at $\lambda = 342$ nm; the mean spectra of the formulations and the calculations performed to determine the concentration of the anio-



Table 1 Association constants K_a (M^{-1}) calculated for the chloride adducts derived from compounds 1–6 (9 : 1 $CDCl_3$: $DMSO-d_6$, 298 K), transport activities (Cl^-/NO_3^- and Cl^-/HCO_3^- exchanges) expressed as EC_{50} (nM) for those compounds, both free and formulated, and calculated lipophilicities

Compound	K_a	EC_{50} (Cl^-/NO_3^-)		EC_{50} (Cl^-/HCO_3^-)		Log P^g
		Free	Formulated	Free	Formulated	
1	1235 ± 162	25 ± 2	87 ± 4	201 ± 26	640 ± 72	5.57
2	1229 ± 103	29 ± 3	74 ± 4	155 ± 16	706 ± 78	6.56
3	1258 ± 101	71 ± 5	85 ± 7	8420 ± 536	1041 ± 23	7.18
4	1290 ± 88	2795 ± 68	183 ± 12	— ^a	— ^b	7.99
5	1480 ± 180	— ^a	— ^c	— ^d	— ^d	8.81
6	1344 ± 127	— ^e	— ^f	— ^d	— ^d	9.60

^a These values could not be determined reliably, since 50% chloride efflux was not reached, even at the highest concentration tried (15 μM). Therefore, in this case $EC_{50} > 15 \mu M$. ^b This value could not be determined reliably, since 50% chloride efflux was not reached, even at the highest concentration tried (10 μM). Therefore, in this case $EC_{50} > 10 \mu M$. ^c This value could not be determined reliably. However, at the highest concentration tried (5 μM), chloride efflux is close to 50%, so $EC_{50} \approx 5 \mu M$. ^d These values could not be determined reliably, since 50% chloride efflux was not reached. ^e This value could not be determined reliably. When [6] = 5 μM , chloride efflux is 31%. Higher concentrations were not tried given the low solubility of the compound in DMSO. ^f This value could not be determined reliably, since 50% chloride efflux was not reached, even at the highest concentration tried (10 μM). ^g Average log P was determined for the deprotonated form of the compound through Virtual Computational Chemistry Laboratory (VCCLAB).³²

Table 2 Size (nm) and zeta potential (mV) of lipid nanoparticles in the six formulations and of lipid nanoparticles alone, and encapsulation efficacies (%)

Compound	Size	Zeta potential	Encapsulation efficacy
1	155.5 ± 6.4	2.15 ± 0.18	85
2	174.9 ± 9.8	-5.93 ± 0.73	91
3	170.8 ± 13.8	-4.93 ± 0.63	92
4	176.6 ± 7.5	2.61 ± 0.27	91
5	162.8 ± 1.9	4.14 ± 0.22	96
6	158.6 ± 5.2	9.77 ± 0.26	94
Empty	214.0 ± 2.7	-15.53 ± 0.42	—

nophores in the formulations can be found in ESI†. As shown in Table 2, the encapsulation efficacies are very high. These results confirm that the method selected to prepare NLCs allows encapsulation of compounds displaying very different lipophilicities (the average log P varies from 5.57 for the hexyl-derivative to 9.60 for the compound bearing a hexadecyl-chain;³² see Table 1) with excellent encapsulation efficacy.

Transmembrane anion transport studies

Transmembrane anion transport was explored for both the free and encapsulated compounds by potentiometric (chloride-selective electrode) and emission spectroscopy techniques. In all cases 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocoline (POPC) liposomes were employed. For those assays involving the use of the chloride-selective electrode (ISE), liposomes were loaded with a sodium-chloride-buffered aqueous solution and suspended in a chloride-free isotonic solution (for the detailed procedure see ESI†). Chloride efflux induced by anionophores was monitored over time and chloride concentrations normalised by referring them to the total chloride concentration in liposomes, which was determined after lysing them with a detergent. In order to calculate EC_{50} values, *i.e.*, the concentration of anionophore required to promote 50%

chloride efflux in the timescale of these experiments (300 seconds), the chloride efflux at 300 seconds for different concentrations of the anion transporters was plotted against those concentrations and the resulting data fitted to Hill's equation. In order to perform the experiments, anionophores were added as DMSO solutions, while formulations were suspended in the extravesicular solution and used as such (for detailed procedure see ESI†). Since the concentration of compound in each formulation is known (see ESI†), it is possible to calculate the concentration of the anion transporters in such suspensions. Importantly, lipid nanoparticles containing no compound were also tested as blanks, eliciting minimal chloride efflux (ESI, Fig. S52†). The correlation between lipophilicity and transport activity found here is in line with previous studies conducted on structurally related molecules.¹³ The most active compounds are 1–3 derivatives, whereas an increase in the lipophilicity in 4–6 bearing dodecyl-, tetradecyl- and hexadecyl alkyl chains, respectively, resulted in a marked drop in their anionophoric potency. The same trend is observed for the NLC-formulated derivatives (Table 1, Fig. 3 and ESI†). A small increase in the EC_{50} values of the most active derivatives is observed. On the other hand, NLC-formulated carriers display improved activity compared to free derivatives in the case of the more lipophilic carriers 4 and 5 (Table 1, Fig. 3 and ESI†). Compound 6 displays very limited transport activity, possibly due to its reduced mobility within the membrane.³³ Internalisation of highly lipophilic compounds in lipid membranes is not straightforward and it appears that for moderately to highly lipophilic compounds, their formulation in NLCs eases, up to a point, their internalisation in the lipid bilayer, slightly increasing their transmembrane activity. It should also be noted that in a typical chloride-selective electrode experiment, the amount of lipids contributed by POPC vesicles and nanoparticles is similar. The fact that transport efficiency is essentially conserved/improved for the formulated carriers supports a fast and efficient deliv-



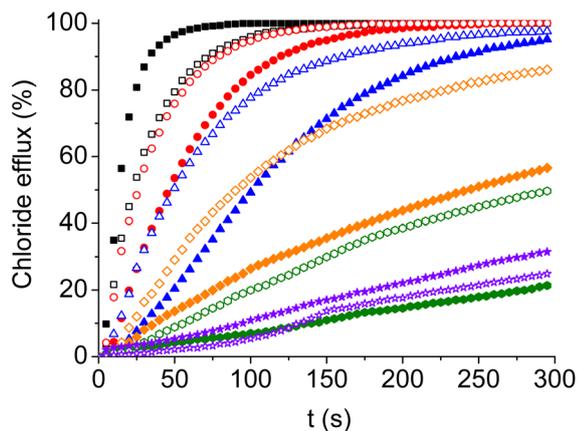


Fig. 3 Chloride efflux promoted by free (filled symbol) and encapsulated (hollow symbol) compounds ($5 \mu\text{M}$ in all cases) (1, black; 2, red; 3, blue; 4, orange; 5, green; 6, purple) in unilamellar POPC vesicles. Vesicles were loaded with a 489 mM NaCl solution buffered at $\text{pH } 7.2$ with 5 mM NaH_2PO_4 and dispersed in a 489 mM NaNO_3 solution buffered at $\text{pH } 7.2$ with 5 mM NaH_2PO_4 . Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

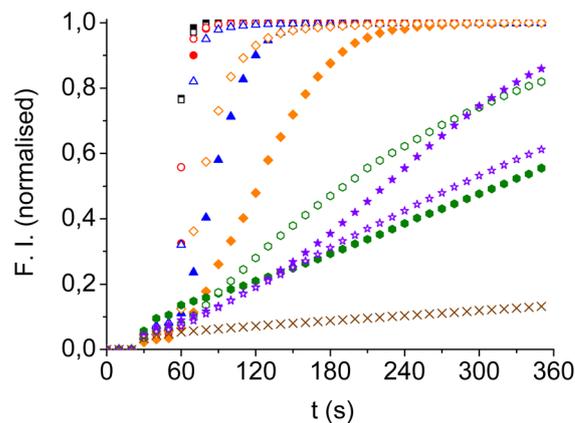


Fig. 4 Emission changes induced by free (filled symbol) and encapsulated (hollow symbol) compounds ($0.5 \mu\text{M}$ in all cases) (1, black; 2, red; 3, blue; 4, orange; 5, green; 6, purple; blank, brown) in 7:3 POPC: cholesterol vesicles. Vesicles (loaded with a 126.2 mM NaNO_3 solution buffered at $\text{pH } 7.2$ with 10 mM NaH_2PO_4 and containing 1 mM HPTS; I.S. 150 mM) were suspended in a NaNO_3 solution (126.2 mM NaNO_3 buffered at $\text{pH } 7.2$ with 10 mM NaH_2PO_4 ; I.S. 150 mM). At $t = 30 \text{ s}$ an aliquot of NaOH solution ($20 \mu\text{L}$, 0.5 M) was added, and at $t = 60 \text{ s}$ the anion carrier, either free or formulated, was added. The blank is DMSO ($6.25 \mu\text{L}$) or lipid nanoparticles containing no compound ($38 \mu\text{L}$). Each trace represents the average of at least three trials, performed with at least three batches of vesicles.

ery of the formulated anionophores to the vesicles. Overall, the results show that NLC formulations represent an effective delivery system for the explored anionophores in these assays. The results obtained in the $\text{Cl}^-/\text{HCO}_3^-$ exchange assays follow the same trends described above (Table 1 and ESI†). Bicarbonate is more hydrophilic than nitrate, and therefore it is more difficult to extract into the membrane. This explains why the calculated EC_{50} values are higher (usually about one order of magnitude) for the assays involving the $\text{Cl}^-/\text{HCO}_3^-$ exchange compared to the $\text{Cl}^-/\text{NO}_3^-$ exchange assays and are in agreement with an antiport mechanism accounting for the transmembrane activity of these anionophores.

Emission spectroscopy experiments were also performed both with the free and formulated compounds, employing two fluorescent probes, carboxyfluorescein (CF) and pyranine (HPTS). In the case of the former, liposomes were loaded with a sodium-chloride-buffered aqueous solution containing CF and suspended in an isotonic sodium sulfate solution. The anion carrier, either free or encapsulated, was added, and emission changes were recorded for 300 seconds. At the end of the experiments a surfactant was added, leading to the release of all the entrapped fluorophore and, consequently, to its dilution, which provoked a dramatic increase in emission that was used to normalise the data (see ESI† for the complete procedure). The minimal emission changes observed upon addition of solutions of the free anion carriers and of suspensions of their formulations (0.5% mol carrier to lipid in all cases) confirm that neither the free click-tambjamins nor the encapsulated ones create large non-selective pores in the membrane, and therefore they do not act as detergents (see ESI†).

HPTS-based experiments were conducted in order to ascertain whether the free anionophores and their formulations were able to discharge pH gradients across lipid membranes.³⁴

For this purpose, 7:3 POPC: cholesterol liposomes were filled with a sodium-nitrate-buffered aqueous solution containing HPTS and suspended in an isotonic sodium nitrate solution. A sodium hydroxide aqueous solution was added to create a pH gradient, followed by the addition of anionophores, both free and encapsulated. Emission changes were recorded for five minutes and, at the end of the assay, a detergent was added; this value was employed to normalise the data (for the detailed procedure see ESI†). The results are consistent with the trends discussed in the ISE-based assays (Fig. 4). In general, NLC formulations of the compounds show activities comparable to or an improvement on those of free compounds, especially for the highest concentrations. Moreover, encapsulation did not seem to slow the transport process.

Conclusions

In this work, we have proved the usefulness of nanostructured lipid carriers (NLCs) as a suitable drug delivery system for the encapsulation and study of small-molecule anionophores. Effective encapsulation in NLCs of six click-tambjamins with different alkyl chain lengths on the imine moiety and therefore different lipophilicity was achieved. NLC nanoformulated anionophores efficiently facilitate anion transport in model liposomes, maintaining and even in some cases improving the potency of the compounds added as DMSO solutions. Use of NLCs should facilitate the study of highly lipophilic anionophores and ease their development as future drugs.



Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This research has been financially supported by Consejería de Educación de la Junta de Castilla y León and European Regional Development Fund (ERDF) (project BU067P20), Ministerio de Ciencia e Innovación (project PID2020-117610RB-I00), Gobierno Vasco (Elkartek program 2021 KK-2021/00052), and Alava Innova program 2021 (INNOAS-2021/00003) of the Provincial Council of Alava. D. A.-C. and I. C.-B. thank Consejería de Educación de la Junta de Castilla y León, ERDF and European Social Fund (ESF) for their pre-doctoral and post-doctoral contracts, respectively.

References

- G. W. Gokel, W. M. Leevy and M. E. Weber, *Chem. Rev.*, 2004, **104**, 2723–2750.
- T. M. Fyles, *Chem. Soc. Rev.*, 2007, **36**, 335–347.
- J. T. Davis, P. A. Gale and R. Quesada, *Chem. Soc. Rev.*, 2020, **49**, 6056–6086.
- G. Picci, S. Marchesan and C. Caltagirone, *Biomedicines*, 2022, **10**, 885.
- A. Mondal, M. Ahmad, D. Mondal and P. Talukdar, *Chem. Commun.*, 2023, **59**, 1917–1938.
- I. Carreira-Barral, C. Rumbo, M. Mielczarek, D. Alonso-Carrillo, E. Herran, M. Pastor, A. Del Pozo, M. García-Valverde and R. Quesada, *Chem. Commun.*, 2019, **55**, 10080–10083.
- A. M. Rodilla, L. Korrodi-Gregório, E. Hernando, P. Manuel-Manresa, R. Quesada, R. Pérez-Tomás and V. Soto-Cerrato, *Biochem. Pharmacol.*, 2017, **126**, 23–33.
- N. Busschaert, S.-H. Park, K.-H. Baek, Y. P. Choi, J. Park, E. N. W. Howe, J. R. Hiscock, L. E. Karagiannidis, I. Marques, V. Félix, W. Namkung, J. L. Sessler, P. A. Gale and I. Shin, *Nat. Chem.*, 2017, **9**, 667–675.
- S.-H. Park, S.-H. Park, E. N. W. Howe, J. Y. Hyun, L.-J. Chen, I. Huang, G. Vargas-Zuñiga, N. Busschaert, P. A. Gale, J. L. Sessler and I. Shin, *Chem*, 2019, **5**, 2079–2098.
- R. Herráez, R. Quesada, N. Dahdah, M. Viñas and T. Vinuesa, *Pharmaceutics*, 2021, **13**, 705.
- H. Li, H. Valkenier, A. G. Thorne, C. M. Dias, J. A. Cooper, M. Kieffer, N. Busschaert, P. A. Gale, D. N. Sheppard and A. P. Davis, *Chem. Sci.*, 2019, **10**, 9663–9672.
- A. Gianotti, V. Capurro, L. Delpiano, M. Mielczarek, M. García-Valverde, I. Carreira-Barral, A. Ludovico, M. Fiore, D. Baroni, O. Moran, R. Quesada and E. Caci, *Int. J. Mol. Sci.*, 2020, **21**, 1488.
- N. J. Knight, E. Hernando, C. J. E. Haynes, N. Busschaert, H. J. Clarke, K. Takimoto, M. García-Valverde, J. G. Frey, R. Quesada and P. A. Gale, *Chem. Sci.*, 2016, **7**, 1600–1608.
- L. A. Jowett and P. A. Gale, *Supramol. Chem.*, 2019, **31**, 297–312.
- L. Martínez-Crespo, J. L. Sun-Wang, P. Ferreira, C. F. M. Mirabella, G. Aragay and P. Ballester, *Chem. – Eur. J.*, 2019, **25**, 4775–4781.
- H. Li, H. Valkenier, L. W. Judd, P. R. Brotherhood, S. Hussain, J. A. Cooper, O. Jurček, H. A. Sparkes, D. N. Sheppard and A. P. Davis, *Nat. Chem.*, 2016, **8**, 24–32.
- D. A. McNaughton, T. Y. To, B. A. Hawkins, D. E. Hibbs and P. A. Gale, *Org. Biomol. Chem.*, 2021, **19**, 9624–9628.
- A. Khosa, S. Reddi and R. N. Saha, *Biomed. Pharmacother.*, 2018, **103**, 598–613.
- S. Weber, A. Zimmer and J. Pardeike, *Eur. J. Pharm. Biopharm.*, 2014, **86**, 7–22.
- A. Beloqui, M. A. Solinís, A. Delgado, C. Évora, A. del Pozo-Rodríguez and A. Rodríguez-Gascón, *Eur. J. Pharm. Biopharm.*, 2013, **84**, 309–314.
- S. B. Lim, A. Banerjee and H. Önyüksel, *J. Controlled Release*, 2012, **163**, 34–45.
- M. Gobbi, F. Re, M. Canovi, M. Beeg, M. Gregori, S. Sesana, S. Sonnino, D. Brogioli, C. Musicanti, P. Gasco, M. Salmona and M. E. Masserini, *Biomaterials*, 2010, **31**, 6519–6529.
- M. Pastor, J. Basas, C. Vairo, G. Gainza, M. Moreno-Sastre, X. Gomis, A. Fleischer, E. Palomino, D. Bachiller, F. B. Gutiérrez, J. J. Aguirre, A. Esquisabel, M. Igartua, E. Gainza, R. M. Hernandez, J. Gavaldà and J. L. Pedraz, *Nanomedicine*, 2019, **18**, 101–111.
- C. Vairo, M. Collantes, G. Quincoces, S. Villullas, I. Peñuelas, M. Pastor, A. G. Gil, E. Gainza, R. M. Hernandez, M. Igartua and G. Gainza, *Int. J. Pharm.*, 2019, **569**, 118484.
- O. Gartzandia, E. Herran, J. L. Pedraz, E. Carro, M. Igartua and R. M. Hernandez, *Colloids Surf.*, 2015, **134**, 304–313.
- M. Moreno-Sastre, M. Pastor, A. Esquisabel, E. Sans, M. Viñas, A. Fleischer, E. Palomino, D. Bachiller and J. L. Pedraz, *Int. J. Pharm.*, 2016, **498**, 263–273.
- S. Selvamuthukumar and R. Velmurugan, *Lipids Health Dis.*, 2012, **11**, 159.
- S. Guo, Y. Zhang, Z. Wu, L. Zhang, D. He, X. Li and Z. Wang, *Biomed. Pharmacother.*, 2019, **118**, 109225.
- I. Carreira-Barral, M. Mielczarek, D. Alonso-Carrillo, V. Capurro, V. Soto-Cerrato, R. Pérez-Tomás, E. Caci, M. García-Valverde and R. Quesada, *Chem. Commun.*, 2020, **56**, 3218–3221.
- P. Thordarson, *Chem. Soc. Rev.*, 2011, **40**, 1305–1323.
- <https://www.supramolecular.org> (last access: October 2020).
- Average log *P* values were determined for the deprotonated form of the compounds through Virtual Computational Chemistry Laboratory (VCCLAB: <https://www.vcclab.org>; last access: August 2021).
- H. Valkenier, C. J. E. Haynes, J. Herniman, P. A. Gale and A. P. Davis, *Chem. Sci.*, 2014, **5**, 1128–1134.
- A. M. Gilchrist, P. Wang, I. Carreira-Barral, D. Alonso-Carrillo, X. Wu, R. Quesada and P. A. Gale, *Supramol. Chem.*, 2021, **33**, 325–344.

