

Biotin[6]uril Esters: Chloride-Selective Transmembrane Anion Carriers Employing C—H···Anion Interactions

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Supporting Information

ABSTRACT: Biotin[6]uril hexaesters represent a new class of anionophores which operate solely through C—H···anion interactions. The use of soft H-bond donors favors the transport of less hydrophilic anions (e.g., Cl[−], NO₃[−]) over hard, strongly hydrated anions (e.g., HCO₃[−] and SO₄^{2−}). Especially relevant is the selectivity between chloride and bicarbonate, the major inorganic anions in biological systems.

Transmembrane anion transport by synthetic agents presents new opportunities for biology and medicine.¹ By analogy with cation transporters (cationophores),² anionophores could be valuable as research tools and might find therapeutic applications. For example, there is evidence that some anionophores possess anti-cancer activity.^{1d,3} In addition there is hope that synthetic transporters might be used to replace the activity of endogenous anion channels which are missing or defective.^{4,1b} Such deficiencies underlie a number of conditions including the widespread genetic disease cystic fibrosis.

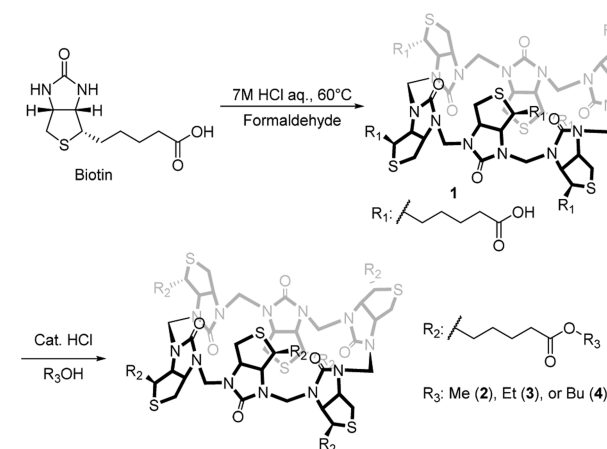
Recent research has yielded various structures which can transport anions through channel,⁵ relay,⁶ or mobile carrier mechanisms.^{3,7} High activities have been achieved in some cases,^{7e,f} but the control of anion selectivity is still underexplored.⁸ From a biological perspective the most relevant issue is the distinction between chloride and bicarbonate, the dominant inorganic anions in living systems.⁹ Chloride/bicarbonate selectivity may not be required for all applications,¹⁰ but for others it may be critical. Selective anionophores would be valuable as research tools, with potential to elicit new and specific biological effects.

Whatever their mechanism of action, anionophores must recognize their substrates through non-covalent interactions. The interaction most commonly applied is hydrogen bonding between anions and conventional donors (OH, NH). However, this may not be ideal for achieving Cl[−]/HCO₃[−] selectivity. Although bicarbonate is more strongly hydrated, it also binds well to O/NH in receptors. Thus, in studies of anion carriers employing NH···anion H-bonding, we and others have commonly observed transport of both substrates.^{7b,c,f,11} A promising alternative is the CH···anion hydrogen bond.¹² In contrast to OH and NH, CH is recognized as a soft H-bond donor.¹³ It might therefore favor binding to softer, more polarizable anions (e.g., Cl[−]) over hard anions such as HCO₃[−].

We now report the first anionophores which rely exclusively on CH···X[−] interactions, without any contribution from conventional H-bonds or electrostatic interactions.¹⁴ As predicted, we find that this system is effective for chloride transport but shows minimal activity for bicarbonate, demonstrating the potential of CH···anion interactions for moderating anionophore selectivity.

The design of the new anionophores is based on biotin[6]uril **1** (Scheme 1), a receptor for halide anions in water recently

Scheme 1. Synthetic Pathway to Biotin[6]Urils Hexaesters 2–4^a



^aEsterification of biotin[6]uril **1** to biotin[6]uril hexamethyl ester **2**, hexaethyl ester **3**, and hexabutyl ester **4** is catalyzed by HCl.

described by the Copenhagen group.¹⁵ Macrocycle **1** is prepared in a single step from biotin and formaldehyde in aqueous hydrochloric acid. The hexameric product consists of six biotin monomers in alternating orientation, connected through methylene bridges. Each biotin unit has two hydrogens on the convex face, pointing toward the center of the macrocycle. This creates a cavity bounded by 12 CH groups, positioned to bind spherical anions by CH···X[−] interactions (Figure 1). In aqueous solution **1** binds halides with affinities (*K*_a) ranging from ~2000 M^{−1} for I[−] to ~60 M^{−1} for Cl[−]. The mode of binding has been confirmed by an X-ray crystal structure of the 1-iodide complex.^{15a}

Received: March 4, 2015

Published: April 8, 2015

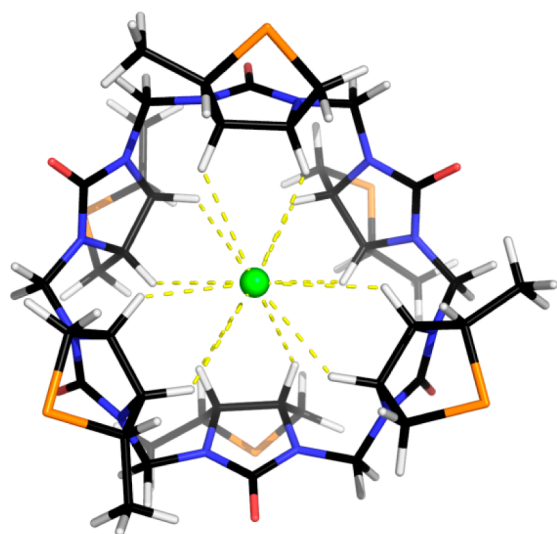


Figure 1. Macrocyclic core of biotin[6]urils 1–4, with side chains replaced by Me groups, modeled binding Cl^- . $\text{CH}\cdots\text{Cl}^-$ distances range from 2.9 to 3.2 Å. For details of the calculation, see SI.

To create hydrophobic analogues for transport studies, receptor **1** was treated with methanol, ethanol or butanol, with catalytic HCl, to yield hexaesters **2–4**. The binding of the hexaesters to Cl^- , NO_3^- , HCO_3^- , and SO_4^{2-} in an organic medium (CD_3CN) was first studied using ^1H NMR spectroscopy. As shown in Table 1, the affinities for chloride were

Table 1. Cl^- , NO_3^- , and HCO_3^- Binding Affinities (^1H NMR and ITC) in Acetonitrile

biotin[6]uril ester	$\log(K_a)$		
	Cl^-	NO_3^-	HCO_3^-
methyl ester ^a (2)	4.3 ^b , 4.5 ^c	2.3 ^c	2.1 ^c
ethyl ester ^a (3)	4.6 ^b	2.4 ^c	— ^d
butyl ester ^a (4)	4.5 ^b	— ^d	— ^d

^aJob's method and ITC indicated 1:1 binding stoichiometries for both $\text{Et}_4\text{N}^+\text{Cl}^-$ and $\text{Bu}_4\text{N}^+\text{NO}_3^-$. All data obtained had less than 11% error. ^b K_a obtained from ITC in CH_3CN at 25 °C. ^c K_a obtained by ^1H NMR titration in CD_3CN at 25 °C. ^dNot measured.

higher than those for nitrate and bicarbonate by roughly 2 orders of magnitude. No interaction with SO_4^{2-} could be detected. The selectivity for chloride vs nitrate contrasts with the results for **1** in water, where the two anions were bound with similar K_a .¹⁵ This solvent effect is not too surprising, as chloride is more hydrophilic than nitrate.¹⁶ More notable, however, are the almost identical K_a values for NO_3^- and HCO_3^- . The latter is by far the more basic, and therefore the better acceptor for conventional H-bonds. The similar affinities observed here, for similarly shaped anions, confirms the difference between conventional H-bonds and $\text{CH}\cdots\text{anion}$ binding.¹³ The result supported our expectation that **2–4** would not transport bicarbonate. If affinities were low in a non-competitive medium, the prospects for extracting hydrophilic HCO_3^- from water seemed very poor indeed.

Affinities for chloride were also measured by isothermal titration calorimetry (ITC) (Table 1). The binding interactions were all shown to be enthalpically and entropically favorable. This is different from the trend observed for the biotin[6]uril

hexaacid (**1**) in water where the entropy change is unfavorable.¹⁵

Anion transport by esters **2–4** was studied in large unilamellar vesicles with a mean diameter of 200 nm, employing the previously reported “lucigenin assay” (Figure 2).¹⁷ The vesicles were prepared from 1-palmitoyl-2-oleoyl-*sn*-

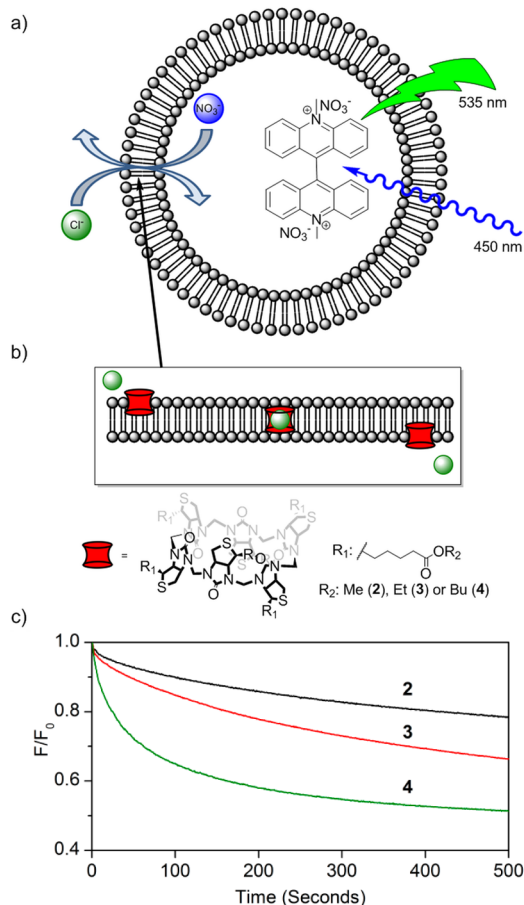


Figure 2. (a) Schematic representation of the vesicles used in this study. The transport activity is monitored using the lucigenin assay. Biotin[6]uril hexaesters promote transport of Cl^- into the vesicles and NO_3^- out of the vesicle. This process is observed as the quenching of lucigenin fluorescence caused by the increasing amount of chloride inside the vesicle. (b) Part of the vesicle membrane illustrating the carrier mechanism employed by the biotin[6]uril hexaesters. (c) Chloride/nitrate exchange by **2**, **3**, and **4** at a transporter-to-lipid ratio of 1:1000.

glycero-phosphocholine (POPC) and cholesterol in a 7:3 ratio, with the biotin[6]uril hexaesters pre-incorporated at receptor:lipid ratios from 1:250 to 1:25 000. The vesicles enclosed NaNO_3 (225 mM) with the halide-sensitive dye lucigenin (0.8 mM), and were suspended in NaNO_3 (225 mM). After addition of sodium chloride (24 mM), the decay in lucigenin fluorescence caused by chloride influx was monitored. Traces from experiments at receptor:lipid = 1:1000 are shown in Figure 2c. All three hexaesters showed activity, but with substantial differences depending on the length of side chain (**4** \gg **3** > **2**). As with some other systems,¹⁸ it seems that lipophilicity promotes anionophore effectiveness. This result might simply reflect different distributions between membrane and aqueous phases. However, leaching tests¹⁹ confirmed that all carriers were exclusively located in the membrane (see

Supporting Information (SI)). It thus seems that increased lipophilicity enhances the intrinsic rate of anion transport.²⁰ The most active transporter **4** promotes chloride influx with $t_{1/2} = 180$ s at transporter:lipid = 1:2500 (see SI). This rate is ~ 100 times lower than the highest reported,^{7c} but compares well with many published systems and is remarkable for a transporter which relies solely on CH \cdots anion interactions.

Ion transport in vesicles can only take place if electro-neutrality is maintained, either by counter-transport of an ion of similar charge (antiport) or co-transport of a counterion (symport). As implied by Figure 2a, the esters **2–4** were expected to act as antiporters, exchanging chloride for intravesicular nitrate. To confirm this hypothesis, the lucigenin assay on **4** was performed with nitrate replaced by hydrophilic sulfate. As shown in Figure 3, the rate of fluorescence decay was

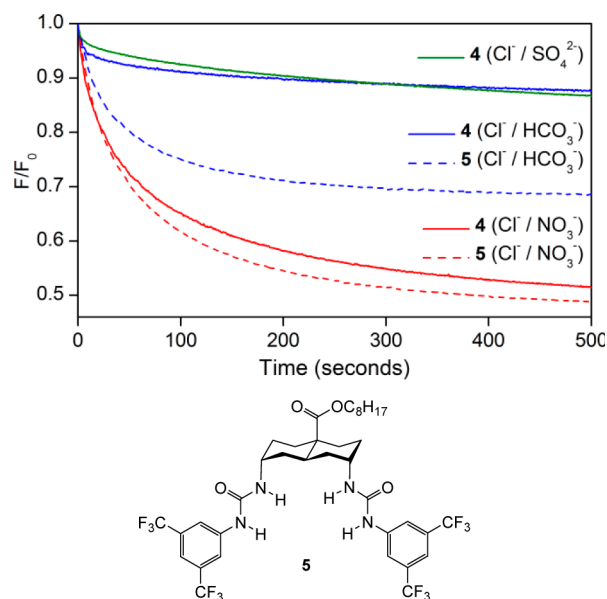


Figure 3. Exchange of chloride for different anions by biotin[6]uril ester (**4**, solid lines) and bis-urea **5** (dashed lines). Red = NO_3^- ; green = SO_4^{2-} ; blue = HCO_3^- (Na^+ salts, 225 mM). Transporter-to-lipid ratios were 1:1000 for **4** and 1:25 000 for **5**. Transport by compound **5** was assessed at a lower loading because it is ~ 25 times more active than **4**. At equilibrium (24 mM chloride both inside and outside of the vesicle), $F/F_0 \approx 0.5$ in NaNO_3 and Na_2SO_4 , but ≈ 0.7 in NaHCO_3 , due to the partial quenching of lucigenin by HCO_3^- .

negligible after an initial small drop. The result implies that the inward flow of charge cannot be balanced under these conditions, and quickly stops due to the developing electrical potential. In common with many other anion carriers, it thus seems that **4** transports both chloride and nitrate, but neither sulfate nor Na^+ .

We next tested for bicarbonate transport by repeating the lucigenin assay with HCO_3^- as the background anion, available for counter-transport. In similar experiments with anionophores employing conventional H-bonds, we have previously observed two types of result. In some cases fluorescence decay profiles are similar to those for $\text{Cl}^-/\text{NO}_3^-$ exchange, implying that HCO_3^- is freely transported. One such example is the bis-urea **5** (see Figure 3).^{7c} In other cases, results for bicarbonate antiport have been intermediate between those for nitrate and sulfate, suggesting that bicarbonate is transported but only slowly.^{7b,d} The result for biotin[6]uril **4** is shown in Figure 3 (blue solid line). The trace is almost indistinguishable from that

for sulfate counter-transport (green solid line), implying that the membrane is impermeable to HCO_3^- . As expected, it thus seems that **4** shows very high selectivity for chloride vs bicarbonate.

Finally, we performed experiments to confirm that transport was occurring via the “mobile carrier” mechanism (Figure 2b), and not by self-assembly into channels.²¹ The lucigenin assay ($\text{Cl}^-/\text{NO}_3^-$ exchange) was applied to **4** using vesicles prepared with different levels of cholesterol. The increase of cholesterol in a membrane decreases the fluidity, and thereby hampers the movement of carriers. In contrast, channels should be unaffected.²² As expected, the transport rate fell dramatically when the proportion of cholesterol was raised to 40% (see SI). Assays were also conducted in vesicles composed of dipalmitoylphosphatidylcholine (DPPC), which exists as a gel phase at room temperature and a liquid crystalline (fluid) phase above 41 °C.²³ Transport was only observed at 45 °C and not at 25 °C, supporting the carrier mechanism. Further support was obtained from the dependence of transport rates on anionophore loading. The data suggested that aggregation was counter-productive, the opposite of that expected for self-assembling channels.

In conclusion, we have shown that receptors **2–4**, employing only CH \cdots X $^-$ interactions, can serve as transmembrane anion carriers with remarkable $\text{Cl}^-/\text{HCO}_3^-$ selectivity. We propose that this selectivity results from the “soft” nature of CH as a hydrogen bond donor, which should favor the polarizable, more hydrophobic anions (e.g., Cl^- , NO_3^-) over harder, more basic anions (e.g., HCO_3^-).¹³ The exploitation of CH \cdots anion interactions in anionophores has further advantages: donor CH groups are not hydrophilic, nor inclined to provoke aggregation. Thus, we believe this motif can make useful contributions to anionophore design, especially where chloride selectivity is a priority.

■ ASSOCIATED CONTENT

Supporting Information

Binding studies, Job plots, and experimental details for the measurement of the chloride transport assays. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge financial support from the Lundbeck Foundation for a Young Group Leader Fellowship (M.P.) and from the EPSRC (grant no. EP/F03623X/1). The support and sponsorship provided by COST Action CM1005, Augustinus Fonden and Oticon Fonden, is acknowledged. We thank Birgitte O. Milhøj for insightful discussions.

■ REFERENCES

- (1) (a) Ashcroft, F. M. *Ion Channels and Disease*; Academic Press: London, 2000. (b) Davis, A. P.; Sheppard, D. N.; Smith, B. D. *Chem. Soc. Rev.* **2007**, 36, 348. (c) Davis, J. T.; Okunola, O.; Quesada, R. *Chem. Soc. Rev.* **2010**, 39, 3843. (d) Busschaert, N.; Gale, P. A. *Angew. Chem., Int. Ed.* **2013**, 52, 1374. (e) Matile, S.; Fyles, T. *Acc. Chem. Res.* **2013**, 46, 2741–2742.

- (2) (a) Pressman, B. C. *Annu. Rev. Biochem.* **1976**, *45*, 501–530. (b) Fyles, T. M. In *Inclusion Aspects of Membrane Chemistry*; Osa, T., Atwood, J., Eds.; Kluwer: Dordrecht, 1991; Vol. 2, pp 59–110.
- (3) (a) Sessler, J. L.; Eller, L. R.; Cho, W. S.; Nicolaou, S.; Aguilar, A.; Lee, J. T.; Lynch, V. M.; Magda, D. J. *Angew. Chem., Int. Ed.* **2005**, *44*, 5989–5992. (b) Gale, P. A.; Perez-Tomas, R.; Quesada, R. *Acc. Chem. Res.* **2013**, *46*, 2801–2813. (c) Ko, S. K.; Kim, S. K.; Share, A.; Lynch, V. M.; Park, J.; Namkung, W.; Van Rossom, W.; Busschaert, N.; Gale, P. A.; Sessler, J. L.; Shin, I. *Nat. Chem.* **2014**, *6*, 885–892.
- (4) Wallace, D. P.; Tomich, J. M.; Eppler, J. W.; Iwamoto, T.; Grantham, J. J.; Sullivan, L. P. *Biochim. Biophys. Acta* **2000**, *1464*, 69–82.
- (5) (a) Gokel, G. W.; Negin, S. *Acc. Chem. Res.* **2013**, *46*, 2824–2833. (b) Vargas Jentzsch, A.; Hennig, A.; Mareda, J.; Matile, S. *Acc. Chem. Res.* **2013**, *46*, 2791–2800.
- (6) (a) McNally, B. A.; O’Neil, E. J.; Nguyen, A.; Smith, B. D. *J. Am. Chem. Soc.* **2008**, *130*, 17274. (b) Hennig, A.; Fischer, L.; Guichard, G.; Matile, S. *J. Am. Chem. Soc.* **2009**, *131*, 16889.
- (7) (a) Valkenier, H.; Davis, A. P. *Acc. Chem. Res.* **2013**, *46*, 2898. (b) Koulov, A. V.; Lambert, T. N.; Shukla, R.; Jain, M.; Boon, J. M.; Smith, B. D.; Li, H. Y.; Sheppard, D. N.; Joos, J. B.; Clare, J. P.; Davis, A. P. *Angew. Chem., Int. Ed.* **2003**, *42*, 4931–4933. (c) Hussain, S.; Brotherhood, P. R.; Judd, L. W.; Davis, A. P. *J. Am. Chem. Soc.* **2011**, *133*, 1614–1617. (d) Cooper, J. A.; Street, S. T. G.; Davis, A. P. *Angew. Chem., Int. Ed.* **2014**, *53*, 5609. (e) Valkenier, H.; Judd, L. W.; Li, H.; Hussain, S.; Sheppard, D. N.; Davis, A. P. *J. Am. Chem. Soc.* **2014**, *136*, 12507–12512. (f) Moore, S. J.; Haynes, C. J. E.; Gonzalez, J.; Sutton, J. L.; Brooks, S. J.; Light, M. E.; Herniman, J.; Langley, G. J.; Soto-Cerrato, V.; Perez-Tomas, R.; Marques, I.; Costa, P. J.; Felix, V.; Gale, P. A. *Chem. Sci.* **2013**, *4*, 103–117. (g) Busschaert, N.; Elmes, R. B. P.; Czech, D. D.; Wu, X.; Kirby, I. L.; Peck, E. M.; Hendzel, K. D.; Shaw, S. K.; Chan, B.; Smith, B. D.; Jolliffe, K. A.; Gale, P. A. *Chem. Sci.* **2014**, *5*, 3617. (h) Vargas Jentzsch, A.; Emery, D.; Mareda, J.; Nayak, S. K.; Metrangolo, P.; Resnati, G.; Sakai, N.; Matile, S. *Nat. Commun.* **2012**, *3*, 905.
- (8) For notable exceptions see: (a) Santacrose, P. V.; Okunola, O. A.; Zavalij, P. Y.; Davis, J. T. *Chem. Commun.* **2006**, 3246–3248. (b) Busschaert, N.; Karagiannidis, L. E.; Wenzel, M.; Haynes, C. J. E.; Wells, N. J.; Young, P. G.; Makuc, D.; Plavec, J.; Jolliffe, K. A.; Gale, P. A. *Chem. Sci.* **2014**, *5*, 1118–1127.
- (9) Fahlke, C. *Am. J. Physiol. Renal Physiol.* **2001**, *280*, F748–F757.
- (10) Selectivity is not always observed for natural systems. See: Quinton, P. M. *Am. J. Physiol. Cell Physiol.* **2010**, *299*, C1222–C1233.
- (11) (a) Davis, J. T.; Gale, P. A.; Okunola, O. A.; Prados, P.; Iglesias-Sánchez, J. C.; Torroba, T.; Quesada, R. *Nat. Chem.* **2009**, *1*, 138. (b) Harrel, W. A., Jr.; Bergmeyer, M. L.; Zavalij, P. Y.; Davis, J. T. *Chem. Commun.* **2010**, 46, 3950. (c) Gale, P. A.; Tong, C. C.; Haynes, C. J. E.; Adeosun, O.; Gross, D. E.; Karnas, E.; Sedenberg, E. M.; Quesada, R.; Sessler, J. L. *J. Am. Chem. Soc.* **2010**, *132*, 3240. (d) Andrews, N. J.; Haynes, C. J. E.; Light, M. E.; Moore, S. J.; Tong, C. C.; Davis, J. T.; Harrell, W. A., Jr.; Gale, P. A. *Chem. Sci.* **2011**, *2*, 256. (e) Busschaert, N.; Gale, P. A.; Haynes, C. J. E.; Light, M. E.; Moore, S. J.; Tong, C. C.; Davis, J. T.; Harrell, W. A., Jr. *Chem. Commun.* **2010**, 46, 6252.
- (12) Cai, J.; Sessler, J. L. *Chem. Soc. Rev.* **2014**, *43*, 6198.
- (13) (a) Desiraju, G. R.; Steiner, T. *The Weak Hydrogen Bond. In Structural Chemistry and Biology*; Oxford University Press: Oxford, 1999; pp 86–89. (b) Nishio, M. *Phys. Chem. Chem. Phys.* **2011**, *13*, 13873–13900.
- (14) It is fairly common for CH \cdots anion bonding to supplement stronger interactions in anionophores. For examples, see ref 12 and the following: (a) Yano, M.; Tong, C. C.; Light, M. E.; Schmidtchen, F. P.; Gale, P. A. *Org. Biomol. Chem.* **2010**, *8*, 4356. (b) Fisher, M. G.; Gale, P. A.; Hiscock, J. R.; Hursthouse, M. B.; Light, M. E.; Schmidtchen, F. P.; Tong, C. C. *Chem. Commun.* **2009**, 3017.
- (15) (a) Lisbjerg, M.; Jessen, B. M.; Rasmussen, B.; Nielsen, B. E.; Madsen, A. Ø.; Pittelkow, M. *Chem. Sci.* **2014**, *5*, 2647. (b) Lisbjerg, M.; Nielsen, B. E.; Milhøj, B. O.; Sauer, S. P. A.; Pittelkow, M. *Org. Biomol. Chem.* **2015**, *13*, 369.
- (16) Sisson, A. L.; Clare, J. P.; Taylor, L. H.; Charmant, J. P. H.; Davis, A. P. *Chem. Commun.* **2003**, 2246–2247.
- (17) McNally, B. A.; Koulov, A. V.; Smith, B. D.; Joos, J.; Davis, A. P. *Chem. Commun.* **2005**, 1087.
- (18) (a) Haynes, C. J. E.; Busschaert, N.; Kirby, I. L.; Herniman, J.; Light, M. E.; Wells, N. J.; Marques, I.; Félix, V.; Gale, P. A. *Org. Biomol. Chem.* **2014**, *12*, 62. (b) Saggiomo, V.; Otto, S.; Marques, I.; Félix, V.; Torroba, T.; Quesada, R. *Chem. Commun.* **2012**, *48*, 5274. (c) Busschaert, N.; Bradberry, S. J.; Wenzel, M.; Haynes, C. J. E.; Hiscock, J. R.; Kirby, I. L.; Karagiannidis, L. E.; Moore, S. J.; Wells, N. J.; Herniman, J.; Langley, G. J.; Horton, P. N.; Light, M. E.; Marques, I.; Costa, P. J.; Félix, V.; Frey, J. G.; Gale, P. A. *Chem. Sci.* **2013**, *4*, 3036.
- (19) Valkenier, H.; Haynes, C. J. E.; Herniman, J.; Gale, P. A.; Davis, A. P. *Chem. Sci.* **2014**, *5*, 1128–1134.
- (20) For another example of this phenomenon, see ref 7c.
- (21) The biotin[6]uril hexaesters are not large enough to form unimolecular channels. The height of the biotin[6]uril hexaacid measured from the crystal structures is 16.5–17.2 Å,^{15a} while the vesicle membrane thickness is ~3.4–4.0 nm.²⁴
- (22) (a) Moore, S. J.; Haynes, C. J. E.; González, J.; Sutton, J. L.; Brooks, S. J.; Light, M. E.; Herniman, J.; Langley, G. J.; Soto-Cerrato, V.; Pérez-Tomás, R.; Marques, I.; Costa, P. J.; Félix, V.; Gale, P. A. *Chem. Sci.* **2013**, *4*, 103. (b) Busschaert, N.; Karagiannidis, L. E.; Wenzel, M.; Haynes, C. J. E.; Wells, N. J.; Young, P. G.; Makuc, D.; Plavec, J.; Jolliffe, K. A.; Gale, P. A. *Chem. Sci.* **2014**, *5*, 1118.
- (23) Deng, G.; Dewa, T.; Regen, S. L. *J. Am. Chem. Soc.* **1996**, *118*, 8975.
- (24) (a) Gullingsrud, J.; Schulten, K. *Biophys. J.* **2004**, *86*, 3496. (b) Leekumjorn, S.; Sum, A. K. *J. Phys. Chem. B* **2007**, *111*, 6026.