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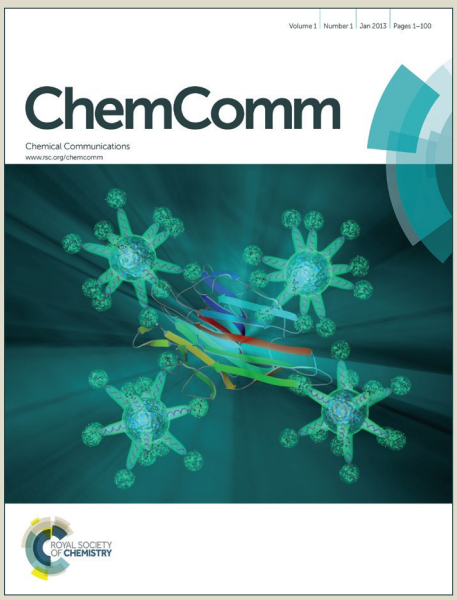

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Oligo(aryl-triazole)s CH···Cl^- Interactions Guide Chloride Efficient and Selective Transmembrane Transport†

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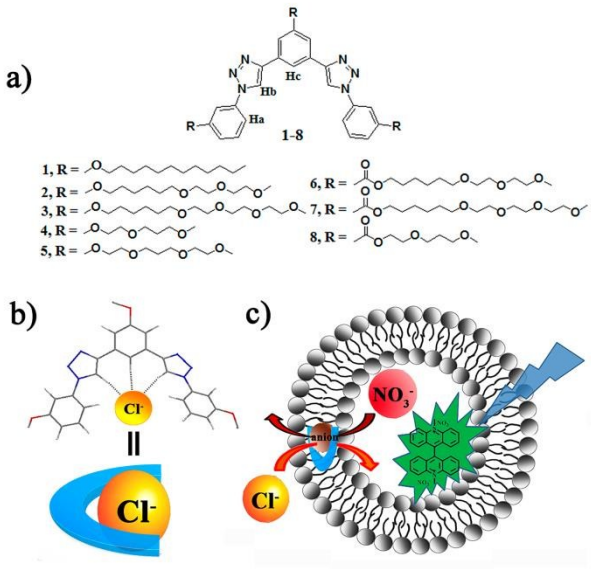
A series of oligo(aryl-triazole)s (compounds 1-8) have been synthesized and served as transmembrane anion transporters by only CH•••Cl^- interactions. The work confirms the roles for the activity of anion transport. By changing lipophilicity and anion affinity of the compounds, efficient anion transport with remarkable Cl^- vs HCO_3^- selectivity was achieved.

As known, ion transport across cell membranes is essential for the regulation of cellular metabolism, signal transduction, osmolyte homeostasis and so on.[1] Misregulation of ion transport, especially for anions, leads to a number of diseases known as "channelopathies",[2] such as cystic fibrosis (CF), Bartter's syndrome, Dent's disease and other diseases.[3] Therefore, synthetic molecules and assemblies that can replicate the activity of faulty anion channels either via a carrier, relay or channel mechanism present new opportunities for application as replacement therapy for relative diseases and tools for the study of transport processes in cells.[4]

As for nature transporters, ion translocation often involves high activity and very precise selectivity for specific ions. For example, Cl^- and HCO_3^- are well known anions in living systems and dominantly distribute over the cell membrane at millimolar concentrations. Different protein channels are in charge of transporting of them, ClC channel family for Cl^- selective transport,[5] AE1-3 protein for Cl^-/HCO_3^- exchange,[6] and CFTR for regulated Cl^- or HCO_3^- transport.[7] Up to now, many sophisticated molecules based on prodigiosin,[8] calixpyrrole,[9,4b] and steroidal[10] architectures have been designed for anion transport with high activities, and especially, some of them have been verified biological activity in anticancer and antibacterial therapy.[4b,8b-c,11] However, the effort for the control of anion selectivity is still limited.[12] Selectivity between Cl^-/HCO_3^- different anions, albeit not required for all applications, is of special importance to provide important insights into the biological function and effects of nature channels.



Scheme 1 a) Molecular structures of compounds **1-8**; b) the schematic representation of complexation of compounds;[14] and c) the anion transport vesicle assay monitored using the lucigenin dye, in which aryl-triazoles promote transport of Cl^- into the vesicle and NO_3^- out of the vesicle.

Up to date, most commonly applied anion transporters are based on H-bonding mechanism, in which conventional OH, NH are always introduced as donors. These kinds of donors can both bind to Cl^- and HCO_3^- and result in co-transport of two anions.[13] Therefore, more specific donors are needed for achieving selectivity between Cl^-/HCO_3^- anions. Recently, Davis et al. reported a new class of cyclic biotinuril ester based anionophores which exhibited chloride-selective transport employing specific C-H···Cl^- interactions.[12a] Such kind of soft H-bond favors the transport of softer, more polarizable anions (e.g., Cl^-, NO_3^-) over harder anions such as HCO_3^-. However, due to the high lipophilic character (calculated logp, clogp≈11.35), the biotinuril esters exhibited moderate activities only by pre-incorporating to a lipid bilayer. Herein,

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we focused on conceptually similar oligo(aryl-triazoles) based compounds, which have been proven to bind Cl^- via specific CH···Cl^- interactions.[14,15] Oligo(aryl-triazoles) can be synthesized in high yields and easy to functionalise, making them attractive binding motifs for anionic guests. By the efficient "click" coupling of alkyl azides with alkynes, a series of aryl-triazoles (compounds **1-8**, as illustrated in scheme 1) were reported in this paper and applied as anion acceptors to selectively transport Cl^- anions through liposomal membranes by exclusively CH···Cl^- interactions. Different substitutions were applied to optimize the transport activity to achieve both of high activity and selectivity, mimicking the functionalities as nature ones, without pre-incorporation into a lipid bilayer.

As for anion transporters, especially for anion carriers, the trend in transporter efficiency was influenced by 1) the partition of transporters from the aqueous phase to the lipid bilayer, 2) mobility within the membrane and 3) "solubilising" the anion in the lipid membrane (the affinity for binding and release of anion).[16] It suggested that lipophilicity and anion affinity have great effects on the activity of transporters. Therefore, different substitutions were introduced in compounds **1-8** to adjust lipophilicity by changing substituted alkyloxy side chains (**1-5**, with clogp from 16.85 to 2.44), and anion binding affinity by introducing electron-withdrawing carbonyl ester substitution (**6-8**).[9a,17] All compounds were prepared by a single click reaction between corresponding azide and alkyne derivatives (ESI†, section 3.1-3.2). The binding of the compounds to anions, including Cl^- and NO_3^-, in an organic medium ($CDCl_3$) was explored using ^1H NMR spectrometer. Job's Plot method was performed for the binding stoichiometry at 1:1 ratio (ESI†, Fig. S1-3) as reported for similar analogs.[14] A summary of the results is presented in Table 1. Despite the different clogp values, compounds **1-5** showed similar and low affinities to Cl^- or NO_3^-, and compounds **6-8** with similar clogp values as those **2-4** exhibited

Table 1 Binding affinities for anions in chloroform, as determined from 1:1 fit mode from ^1H NMR titration data in $CDCl_3$.

Compounds	Ions	K [M^{-1}]	$\Delta\delta_{max}$[Hz][a]	clogp[b]
1	Cl^-	6.5 (±0.1)	656.4	16.80
	NO_3^-	9.0 (±0.3)	262.6	
2	Cl^-	6.6 (±0.3)	546.3	6.80
3	Cl^-	5.8 (±0.1)	592.2	6.33
	NO_3^-	6.1 (±0.1)	346.4	
	HCO_3^-	--[c, nd]	--[c, nd]	
	SO_4^{2-}	102 (±12)[c]	144.2[c]	
4	Cl^-	8.7 (±0.6)	624.7	2.97
5	Cl^-	7.1 (±0.4)	645.1	2.44
6	Cl^-	22 (±2)	344.1	6.83
7	Cl^-	20 (±2)	281.6	6.43
8	Cl^-	18 (±1)	350.0	2.95

[a], Difference in chemical shift for Hb proton of compounds (ESI†) for free vs. complex; [b], calculated logp, an estimate of lipophilicity where p is the partition coefficient between octanol and water. Values were obtained using ALOGPS 2.1; [c], titration was processed upon addition of ammonium salt in DMSO-d6/0.5% water; [nd], not determined.

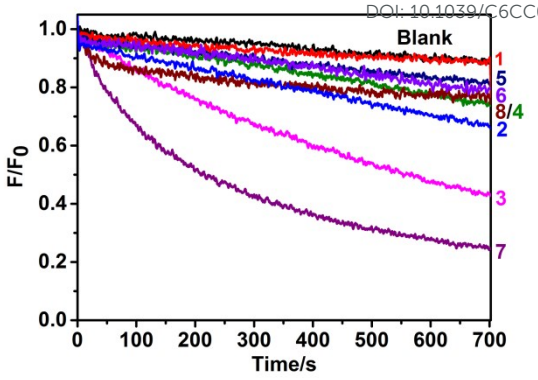

Fig. 1 a) Comparison of chloride transport by compounds **1-8** into vesicles containing $NaNO_3$ (225 mM) and Lucigenin (1 mM), the concentrations were at 0.25 mol% (molar ratio of transporter to lipid).

obviously stronger affinities to Cl^-, suggesting electron-withdrawing substitution increased affinities as expected. The variant in lipophilicity and anion affinity would reveal the relationships between molecular structures and transport activity thus provide valuable experience for designing effective ion transporters.

Chloride transport by compounds **1-8** was explored using unilamellar vesicles composed of 1-palmitoyl-2-oleoylphosphatidylcholine and cholesterol in a 9:1 ratio with a mean diameter of ~200 nm. $NaNO_3$ (225 mM in 5 mM PB) was used as the suspension solution for internal and external aqueous solution and a Cl^- sensitive dye lucigenin (1 mM) was enwrapped in the interior of vesicles (ESI†, section 5.1-5.2). Sodium chloride (133 mM) was added to the suspension to generate Cl^- concentration difference. Then compounds **1-8** were added as solutions in THF and the resulting chloride transport from vesicles monitored through the decay in lucigenin fluorescence (ESI†, Fig. S4-11). Selected traces from the experiments are shown in Fig. 1, all compounds showed activities but with substantial differences depending on the substitution of side chains. Table 2 summarized the data of EC_{50} and k_{ini} (ESI†, Fig. S12), two sets of data were reciprocally consistent except for **8.** Considering the complex kinetic behaviour of transporters, we selected $EC_{50,700}$ as the key data

Table 2 Quantification analysis of transport assays (Cl^-/NO_3^-) of compounds **1-8**.

Compounds	$EC_{50,700s}$[a]	k_{ini}[b]	n[c]
1	--[nd]	0.19	--[nd]
2	--[nd]	0.31	--[nd]
3	0.09±0.02	0.91	0.49
4	--[nd]	0.21	--[nd]
5	--[nd]	0.15	--[nd]
6	--[nd]	0.16	--[nd]
7	0.025±0.004	2.43	0.82
8	2.22±0.11	2.65	0.75

[a], $EC_{50,700s}$ defined as concentration (mol% carrier to lipid) needed to obtain 50% fluorescent quench after 700 s; [b], specific initial rate of chloride transport (s^{-1}) is defined as initial slope of F_0/F vs time t, devided by the transporter/lipid ratio and averaged over a range of experiments at different ratios (ESI†, Fig. S12); [c], Hill coefficient derived from the activity vs concentrations assays, [nd], not determined.

for the comparison of transport activity while taking k_{ini} as reference. It suggested lipophilicity (increased clogp) promoted Cl^- transporter (**3>4,5**) because of the enhanced distribution in lipids;[18] however, further increase in lipophilicity significantly decreased the transport activity (**1**,**2<3**) attributed to the solubility problems (precipitation appeared in the system),[19] despite similar anion affinities for **1-5**. As expected, electron-withdrawing substitutions induced enhanced transport activity, especially for compound **7** with very low EC_{50} value at ~0.025 mol%. It is evident that lipophilicity and anion affinity have profound impacts on the transport abilities of these compounds, as also illustrated by the chloride selective electrode assay (ESI†, Fig. S13-15).

The transmembrane transport in Fig. 1 is a passive process, with charge balance across the membrane by a Cl^-/NO_3^- antiport process or a Na^+/Cl^- symport process. To test which of these process is predominant, lucigenin assay with NO_3^- replaced by SO_4^{2-}, a significantly hydrophilic anion ($\Delta Gh(SO_4^{2-})$ = -1080 kJ mol^{-1}) that cannot pass through the lipid bilayer membrane, was explored. As implied in Fig. 2a, taking compound **7** as example, the fluorescence decay was negligible under this condition, indicating that the inward flow of charge couldn't be balanced and chloride transport stopped by the developing electrical potential. Same result was monitored using a chloride selective electrode (Fig. 2b). All these suggested the Cl^-/NO_3^- antiport, not Na^+/Cl^- symport, mechanism, in which compound **7** acted as antiporter to exchange chloride with intravesicular nitrate.

Since HCO_3^- is the another dominant anions existed in living systems as Cl^-, the selectivity between Cl^-/HCO_3^- was explored by using HCO_3^- as the background anion. Chloride transport was also monitored by using both a lucigenin fluorescence assay and a chloride selective electrode. As shown in Fig. 2a-b, the transport by **7** was almost negligible, implying that the

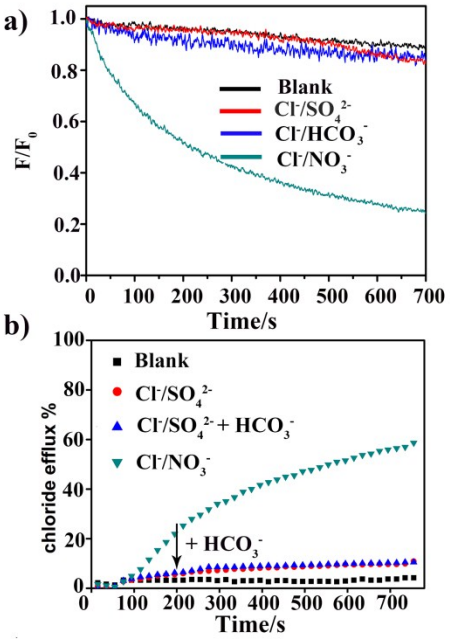

Fig. 2 Exchange of chloride for different anions by **7**: a) Lucigenin vesicle fluorescence assay at 0.25 mol%, b) Chloride efflux determined by Cl^- selective electrode assay at 0.50 mol%.

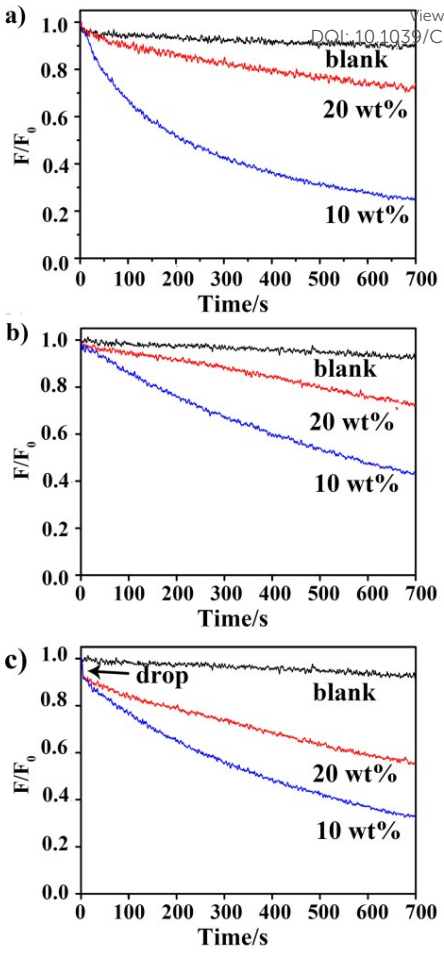

Figure 3 Effect of amount of cholesterol in EYPC/cholesterol membrane on transport by a) compound **7** at 0.125 mol% and b-c) compound **3** at different concentrations (0.125 mol% for b and 2.0 mol% for c).

membrane is impermeable to HCO_3^-. Combining the bad and almost no binding interactions for receptor- HCO_3^-, the high selectivity for Cl^- vs HCO_3^- should be resulted from the specific CH···Cl^- interactions and was quite different as those transporters by traditional NH H-bonding interactions.[13]

Finally, the transport mechanism of the compounds, by either a mobile carrier or a channel formation was explored. Addition of cholesterol, to increase bilayer viscosity, is one method to test mobile carrier activity, since the increase of cholesterol in a membrane decreases the fluidity and thereby hampers the movement of carriers; in contrast, channels should be unaffected.[13] Therefore, the lucigenin assay (Cl^-/NO_3^-exchange) was applied to **7** using vesicles prepared with different levels of cholesterol. As illustrated in Fig. 3a, the results clearly indicated a significant reduction in the transport activity of **7** when the vesicle bilayer composition included more cholesterol, suggesting a carrier mechanism was likely for chloride transport. Further evidence for a carrier mechanism was obtained through a classic U-tube experiment on compound **7** (ESI†, Fig. S16). The increased chloride concentration in the receiving phase indicated that compound **7** acted as mobile carriers for Cl^- transport.

Unlike compound **7**, it was noticed that most of other compounds with moderate transport ability exhibited different dynamic decay processes. Taking compound **3** as example (ESI†, Fig. S6), the fluorescence was gradually reduce after addition of transporter at low concentrations, however, the fluorescence appeared a rapid drop followed by gradually decrease when the concentration was up to 1.25 mol%. And the drop was enhanced with concentrations. All these guided us to suggest that different mechanism maybe appear for compound **3** at high concentrations. To confirm the hypothesis, both low (0.125 mol%) and high concentrations (2.0 mol%) of **3** were applied for variant cholesterol assay. As illustrated in Fig. 3b, the transport showed typical carrier mechanism at 0.125 mol% by the obviously decreased activity with higher ratio of cholesterol, coinciding with the U-tube experiment (ESI†, Fig. S16). However, at high concentration of 2.0 mol% as shown in Fig. 3c, the drop value kept constant despite the change of cholesterol content, which guided us to speculate the channel contribution by the stacking of compound **3** in membrane.[20] Inferential evidence for channel stacking in membrane was supported by the self-assembly of compound **3** in $CHCl_3$ in the presence of anions (ESI†, Fig. S17).

In conclusion, we have demonstrated that aryl-triazole based oligomers can mediate the transmembrane transport by anion recognition. The distinctive soft CH···Cl^- interactions exhibited excellent Cl^-/HCO_3^- selectivity and the adjustment of lipophilicity and affinity optimized an efficient activity at very low transporter/lipid molar ratio (EC_{50} of **7** at ~0.025 mol%). With the facile click-based synthesis, we believe the oligotriazoles provide new opportunities for developing more potent anion transporters with high activity and selectivity.

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Notes and references

1 (a) S. Y. Chiu and G. F. Wilson, *J. Physiol*., 1989, **408**, 199-222; (b) T. E. DeCoursey, K. G. Chandy, S. Gupta and M. D. Cahalan, *Nature*, 1984, **307**, 465-468; (c) B. Legutko, M. Staufenbiel and K. Krieglstein, *Int. J. Dev. Neurosci.*, 1998, **16**, 347-352.

2 F. M. Ashcroft, *Ion Channels and Disease*, Academic Press, San Diego, 2000.

3 (a) A. Fürstner, *Angew. Chem., Int. Ed*., 2003, **42**, 3582-3603; (b) C. A. Hübner and T. J. Jentsch, *Hum. Mol. Genet*., 2002, **11**, 2435-2445; (c) B. Dworakowska and K. Dołowy, *Acta Biochim. Pol.*, 2000, **47**,685–703.

4 (a) J. T. Davis, O. Okunola and R. Quesada, *Chem. Soc. Rev*., 2010, **39**, 3843-3862; (b) S.-K. Ko, S. K. Kim, A. Share, V. M. Lynch, J. Park, W. Namkung, W. Van Rossom, N. Busschaert, P. A. Gale, J. L. Sessler and I. Shin, *Nat. Chem*., 2014, **6**, 885-892; (c) G. W. Gokel and S. Negin, *Acc. Chem. Res*., 2013, **46**, 2824-2833; (d) P. R. Brotherhood and A. P. Davis, *Chem. Soc. Rev*., 2010, **39**, 3633-3647; (e) 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.

5 R. Dutzler, E. B. Campbell, M. Cadene, B. T. Chait and R. MacKinnon, *Nature*, 2002, **415**, 287-294.

6 S. L. Alper, R. B. Darman, M. N. Chernova and N. K. Dahl, *J. Nephrol*., 2002, **15**, 41-53.

7 P. M. Q. MallaReddy M Reddy, *JOP. J. Pancreas*, 2001, **2**, 212-218.

8 (a) J. T. Davis, *Top. Heterocycl. Chem*., 2010, **24**, 145-176; (b) R. I. S. Diaz, J. Regourd, P. V. Santacroce, J. T. Davis, D. L. Jakeman and A. Thompson, *Chem. Commun*., 2007, 2701-2703; (c) J. L. Sessler, L. R. Eller, W.-S. Cho, S. Nicolaou, A. Aguilar, J. T. Lee, V. M. Lynch and D. J. Magda, *Angew. Chem., Int. Ed*., 2005, **44**, 5989-5992.

9 (a) P. A. Gale, C. C. Tong, C. J. E. Haynes, O. Adeosun, D. E. Gross, E. Karnas, E. M. Sedenberg, R. Quesada and J. L. Sessler, *J. Am. Chem. Soc*., 2010, **132**, 3240-3241; (b) C. C. Tong, R. Quesada, J. L. Sessler and P. A. Gale, *Chem. Commun*., 2008, 6321-6323.

10 (a) H. Valkenier, L. W. Judd, H. Li, S. Hussain, D. N. Sheppard and A. P. Davis, *J. Am. Chem. Soc*., 2014, **136**, 12507-12512; (b) D. S. Kim and J. L. Sessler, *Chem. Soc. Rev*., 2015, **44**, 532-546; (c) H. Valkenier and A. P. Davis, *Acc. Chem. Res*., 2013, **46**, 2898-2909.

11 (a) P. A. Gale, R. Pérez-Tomás and R. Quesada, *Acc. Chem. Res*., 2013, **46**, 2801-2813; (b) V. Soto-Cerrato, P. Manuel-Manresa, E. Hernando, S. Calabuig-Fariñas, A. Martínez-Romero, V. Fernández-Dueñas, K. Sahlholm, T. Knöpfel, M. García-Valverde, A. M. Rodilla, E. Jantus-Lewintre, R. Farràs, F. Ciruela, R. Pérez-Tomás and R. Quesada, *J. Am. Chem. Soc*., 2015, **137**, 15892-15898.

12 (a) M. Lisbjerg, H. Valkenier, B. M. Jessen, H. Al-Kerdi, A. P. Davis and M. Pittelkow, *J. Am. Chem. Soc.*, 2015, **137**, 4948-4951; (b) P. V. Santacroce, O. A. Okunola, P. Y. Zavalij and J. T. Davis, *Chem. Commun.*, 2006, 3246-3248.

13 (a) A. V. Koulou , T. N. Lambert, R. Shukla, M. Jain, J. M. Boon, B. D. Smith, H. Y. Li, D. N. Sheppard, J. B. Joos, J. P. Clare, A. P. Davis, *Angew. Chem. Int. Ed.*, 2003, **42**, 4931-4933; (b) S. Hussain, P. R. Brotherhood, L. W. Judd, A. P. Davis, *J. Am. Chem. Soc.*, 2011, **133**, 1614-1617; (c) S. J. Moore, C. J. E. Haynes, J. Gonzalez, J. L. Sutton, S. J. Brooks, M. E. Light, J. Herniman, G. J. Langley, V. Soto-Cerrato, R. Perez-Tomas, I. Marques, P. J. Costa, V. Felix, P. A. Gale, *Chem. Sci.*, 2013, **4**, 103-117; d) N. J. Andrews, C. J. E. Haynes, M. E. Light, S. J. Moore, C. C. Tong, J. T. Davis, W. A. Harrell Jr, P. A. Gale, *Chem. Sci.*, 2011, **2**, 256-260.

14 (a) H. Juwarker, J. M. Lenhardt, D. M. Pham and S. L. Craig, *Angew. Chem., Int. Ed.*, 2008, **47**, 3740-3743; (b) R. M. Meudtner and S. Hecht, *Angew. Chem., Int. Ed.*, 2008, **47**, 4926-4930.

15 (a) Y. Li and A. H. Flood, *Angew. Chem. Int. Ed.*, 2008, **47**, 2649-2652; (b) Y. Hua, Y. Liu, C.-H. Chen and A. H. Flood, *J. Am. Chem. Soc.*, 2013, **135**, 14401-14412; (c) Y. Li and A. H. Flood, *J. Am. Chem. Soc.*, 2008, **130**, 12111-12122.

16 (a) A. P. Davis, D. N. Sheppard and B. D. Smith, *Chem. Soc. Rev.*, 2007, **36**, 348-357; (b) P. A. Gale, *Acc. Chem. Res*., 2011, **44**, 216-226.

17 J. Cai and J. L. Sessler, *Chem. Soc. Rev.*, 2014, **43**, 6198-6213.

18 (a) C. J. E. Haynes, N. Busschaert, I. L. Kirby, J. Herniman, M. E. Light, N. J. Wells, I. Marques, V. Felix and P. A. Gale, *Org. Biomol. Chem.*, 2014, **12**, 62-72; (b) V. Saggiomo, S. Otto, I. Marques, V. Felix, T. Torroba and R. Quesada, *Chem. Commun*., 2012, **48**, 5274-5276.

19 N. Busschaert, M. Wenzel, M. E. Light, P. Iglesias-Hernandez, R. Perez-Tomas and P. A. Gale, *J. Am. Chem. Soc*., 2011, **133**, 14136-14148.

20 (a) C. R. Yamnitz, S. Negin, I. A. Carasel, R. K. Winter and G. W. Gokel, *Chem. Commun.*, 2010, **46**, 2838-2840; (b) T. Liu, C. Bao, H. Wang, Y. Lin, H. Jia and L. Zhu, *Chem. Commun.*, 2013, **49**, 10311-10313.

