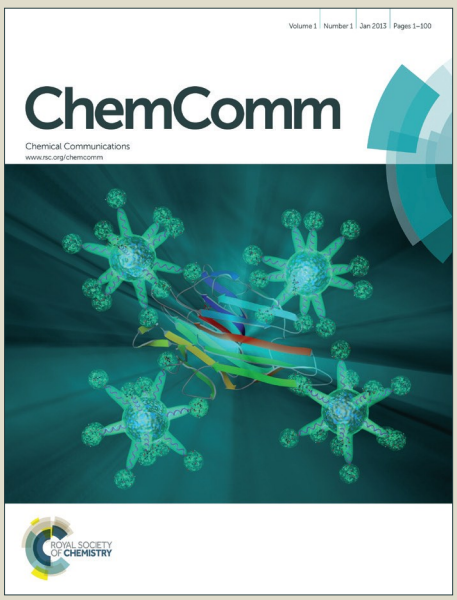
ChemComm

Accepted Manuscript



This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/chemcomm

Please do not adjust margins

Journal Name

COMMUNICATION

Chloride Transport Activities of *trans*- and *cis*- Amide-Linked Bisureas

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

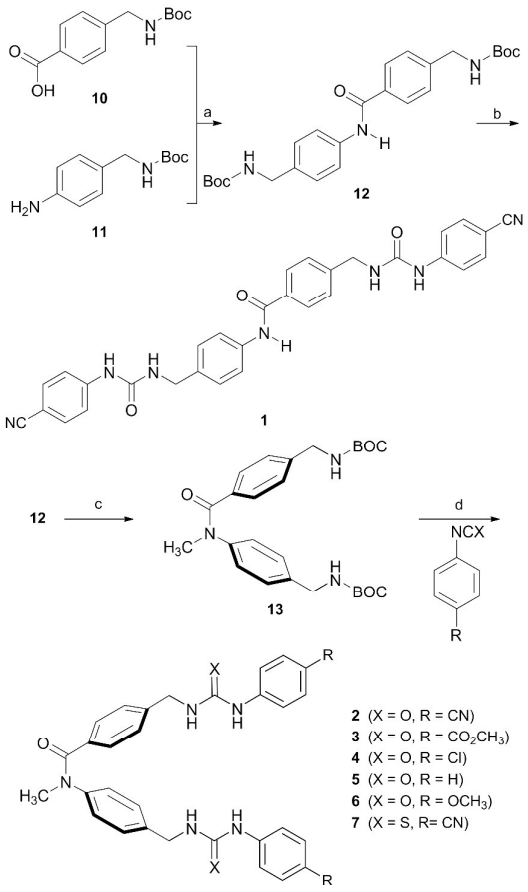
Eun Bit Park[a] and Kyu-Sung Jeong*[a]

Of bisurea compounds linked through *trans* and *cis* benzanilide spacers, the *cis* amide derivatives were found to be effective in the chloride transport, with which a stimuli-responsive mobile carrier was devised.

Nature uses amide bonds to connect amino acids and synthesize peptides and proteins. Most of the amide bonds adopt *trans* conformations predominantly but the population of *cis* conformations tends to increase in the tertiary amides prepared from proline and *N*-substituted amino acids such as sarcosine.[1] The relative stabilities of *trans* and *cis* conformers are reversed in some unnatural amides such as *N*-methyl acetanilide and benzanilide, showing strong preference of the *cis* conformation in the solid state and in solutions.[2] This feature has been nicely utilised for the construction of amide-based foldamers and molecular switches.[3]

Transmembrane anion transport is essential to control the cellular ion concentration, osmotic pressure, volume and pH in living organisms, and it is also associated with signal production, cell-to-cell communication, neuronal proliferation and differentiation, etc.[4] It has been well known that anion transport in the biological systems is facilitated by proteins functioning as anion channels, transporters, and exchangers. A variety of synthetic molecules that can facilitate anion transport across lipid and cell membranes has been described for the last decade.[5,6] These synthetic transporters may have applications in biomedical researches and potential therapeutics for diseases related to the defective anion transport.[7]

In recent years, we have synthesized small synthetic molecules that can facilitate anion transport across lipid membranes *via* antiport or symport.[8] It was also demonstrated that the transmembrane transport of chloride ions could be controlled by light stimulus using diazobenzene-based synthetic transporters.[9] As part of our continuing efforts to develop stimuli-responsive synthetic anion transporters,[10] we herein have prepared a series of bisurea compounds **1–7**



Scheme 1 Reagents and conditions: (a) EDCI, HOBt, Et_3N, CH_2Cl_2, r.t., 5 h, 72%; (b) TFA, then *p*-cyanophenyl isocyanate, anhydrous Et_3N, CH_2Cl_2, r.t., 6 h, 56% (for two steps); (c) KOH, MeI, acetone, reflux, 1 h, 97%; (d) TFA, then each isocyanate, anhydrous Et_3N, CH_2Cl_2, r.t., 4–7 h, 49–76% (for two steps).

[a] *Department of Chemistry, Yonsei University, Seoul, 120-749, Korea. E-mail: ksjeong@yonsei.ac.kr; Fax: +82-2-364-7050; Tel: +82-2-2123-2643*

† Electronic Supplementary Information (ESI) available: Synthetic procedures and characterization, experimental details for titrations and transports. See DOI: 10.1039/x0xx00000x

ChemComm Accepted Manuscript

Please do not adjust margins

containing *trans* or *cis* amides, and their transport abilities of chloride ions across a lipid membrane have been revealed. Furthermore, it is also demonstrated that the transport activity can be modulated by chemical and enzymatic reactions, leading to the conversion of *cis*-to-*trans* amide.

The syntheses of bisurea compounds **1**–**7** are outlined in Scheme 1. Coupling of **10** and **11** in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI) and 1-hydroxybenzotriazole (HOBt) gave compound **12**.[11] After removal of the *t*-butyloxycarbonyl (Boc) protecting groups, the resulting amine was directly coupled with *p*-cyanophenyl isocyanate to afford bisurea **1**. On the other hand, *N*-methylation of **12** yielded compound **13**, which was converted to bisurea compounds **2**–**7** *via* sequential deprotection and coupling reactions.

We first compared the chloride transport abilities of two urea compounds **1** and **2** which contain *trans* and *cis* amides, respectively. Both compounds have identical *p*-cyanophenyl substituents which are known to greatly enhance the anion transport activities of urea receptors across lipid membranes.[8a,9] The chloride transport abilities of **1** and **2** were estimated using unilamellar 1-palmitoyl-2-oleoyl-2-oleoylphosphatidyl-choline (POPC) vesicles which contain a phosphate buffer (10 mM, pH = 7.2), lucigenin (1 mM) and $NaNO_3$ (200 mM). These vesicles were suspended in a phosphate buffer (10 mM, pH = 7.2) containing $NaNO_3$ (200 mM) and NaCl (30 mM). Chloride influx into vesicles upon addition of each compound in a DMSO solution (2 mol% to lipid) was monitored by fluorescence quenching of lucigenin. As shown in Fig. 1a, compound **1** with a *trans* amide is nearly inactive while compound **2** with a *cis* amide is highly efficient to facilitate chloride transport across a POPC membrane. Hill plot afforded the EC_{50} value of 0.24 mol% (**2** to lipid), which is the concentration needed to achieve 50% completion of chloride transport at 300 sec. Dramatic difference in the chloride transport activities of **1** and **2** is attributed in part to their binding affinities with the chloride ion. In compound **2** with a *cis* amide, two urea functional groups can be involved in the binding of the chloride ion in a chelate manner, thus simultaneously forming four hydrogen bonds. The association constant between **2** and tetrabutylammonium chloride was determined to be 3950 M^{-1} in 10% (v/v) DMSO-d_6/$CDCl_3$ saturated with water (< 0.1%) at 24 ± 1 °C (Fig. S1, ESI†). However, such a chelate binding mode is not possible in compound **1** with a *trans* amide. Instead, compound **1** showed complex binding isotherms and therefore monourea compound **8** was prepared as a reference compound which showed the association constant of 170 M^{-1} under the identical conditions (Fig. S7, ESI†). Another possible reason for different transport activities is because compound **2** has a folded, C-shaped conformation with an internal binding cavity wherein the chloride ion binds. This geometric feature may reduce the contact area with the lipid surface, which may facilitate the shuttling movement of **2** and its chloride complex inside the lipid membrane.[9,12]

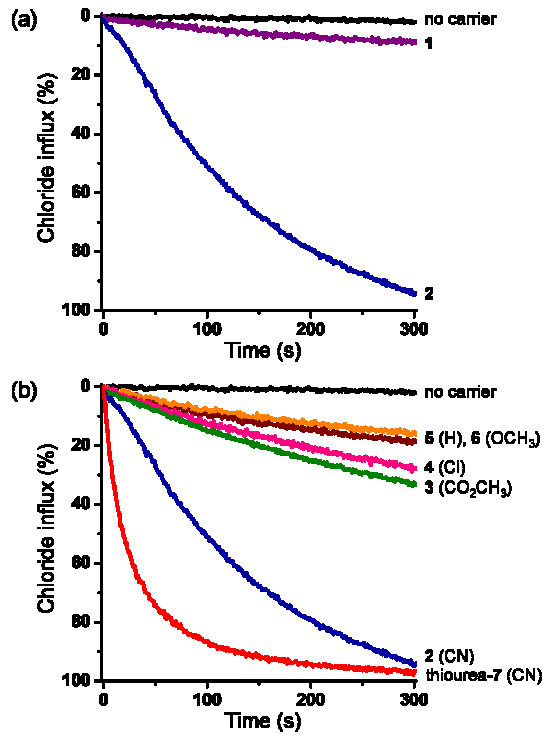
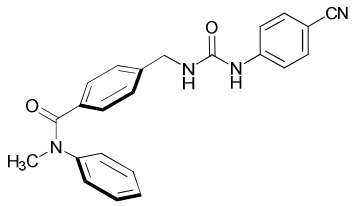


Fig. 1 (a) Chloride influxes facilitated by **1** and **2** (2 mol% to lipid) (b) Chloride influxes facilitated by **2**–**7** (2 mol% to lipid). Vesicles which contain a phosphate buffer (10 mM, pH = 7.2), lucigenin (1 mM) and $NaNO_3$ (200 mM) were suspended in a phosphate buffer (10 mM, pH = 7.2) containing $NaNO_3$ (200 mM) and NaCl (30 mM).



8

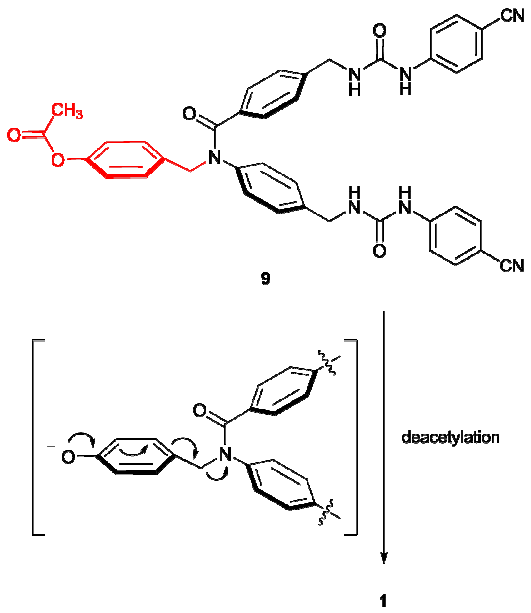
Next, we investigated substituent effects on the chloride transport activities using bisurea compounds **2**–**6** with different substituents on the terminal phenyl rings (Fig. 1b). The transport activities increase in the order of **6** (OCH_3) ~ **5** (H) < **4** (Cl) < **3** (CO_2CH_3) << **2** (CN). This trend nicely match with the increasing order of association constants between **2**–**6** and tetrabutylammonium chloride; that is **6** (250 M^{-1}) < **5** (370 M^{-1}) < **4** (860 M^{-1}) < **3** (1230 M^{-1}) < **2** (3950 M^{-1}) in 10% (v/v) DMSO-d_6/$CDCl_3$ saturated with water (< 0.1%) at 24 ± 1 °C (Fig. S1–S5, ESI†).[13] In addition, we also prepared thiourea compound **7** with the cyano substituent, which bound the chloride ion more strongly (9500 M^{-1}) than the corresponding urea compound **2** (Fig. S6, ESI†). Compound **7** was found to be most efficient in the chloride transport and the EC_{50} value was 0.08 molar ratio of **7** to POPC. These results suggest that the transport ability of synthetic transporters of the same molecular framework can be enhanced by increasing the

ChemComm Accepted Manuscript

This journal is © The Royal Society of Chemistry 20xx

binding affinity with a target ion. In order to reveal the transport mechanism, we investigated the chloride transport of compound **2** using POPC vesicles containing Na_2SO_4 (67 mM), instead of $NaNO_3$ (200 mM). The transport activity of **2** was practically ineffective under these conditions (Fig. S10, ESI†), indicating that the chloride transport occurs *via* Cl^-/NO_3^- antiport mechanism. Furthermore, we carried out transport experiments with vesicles prepared from a 7:3 POPC/cholesterol mixture. It has been known that cholesterol may reduce the membrane fluidity due to its rigid structure, thus slowing down the chloride transport.[14] Both compounds **2** and **7** exhibited noticeably decreased activities of chloride transport in the presence of cholesterol (Fig. S11, ESI†), supporting that the compounds function as mobile carriers. Furthermore, the Hill coefficients of **2** and **7** were determined to be 1.09 and 1.06, respectively, indicative of monomeric carriers (Table S1, ESI†).[15]

Utilizing different transport activities between *trans* and *cis* amide-linked bisureas, we have designed and prepared compound **9** as a stimulus-responsive anion transporter. To adopt a *cis* amide conformation, the *N*-methyl moiety is replaced by *p*-acetyloxybenzyl group which can be degradable *via* deacetylation to produce compound **1** with a *trans* amide (Scheme 2). We first examined the chloride transport ability of compound **9** using POPC vesicles under the conditions described earlier. As anticipated, compound **9** itself is very efficient to transport chloride ions *via* Cl^-/NO_3^- antiport (Fig. 2). To show stimuli-responsive chloride transport, we examined two different approaches. One was the addition of a good nucleophile, hydrazine for deacetylation. The transport ability of **9** was significantly dropped (Fig. 2) when treated for



Scheme 2 Plausible mechanism for the *cis*-to-*trans* isomerization of **9** upon deacetylation by chemical and enzymatic reactions.

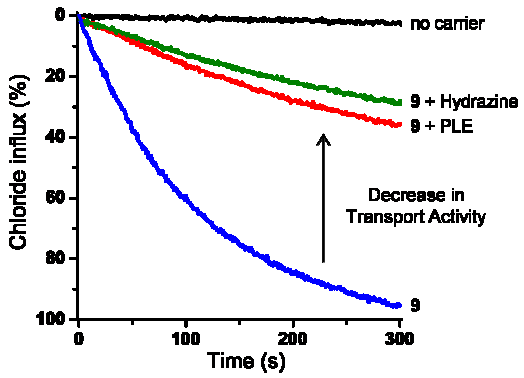


Fig. 2 Decrease in transport activity of **9** when treated with hydrazine in a 3:1 (v/v) DMSO:a phosphate buffer (pH = 7.2) at 21 °C for 10 min or PLE in a phosphate buffer (pH = 7.2) at 21 °C for 30 min.

10 min with hydrazine hydrate (10 equiv, 3:1 (v/v) DMSO:a phosphate buffer (pH = 7.2), 21 °C). Under these conditions, compound **9** was gradually converted to compound **1** with the *trans* conformation as demonstrated by ^{1}H NMR spectroscopy. (Fig. S12, ESI†) Another approach examined here was more bio-relevant degradation *via* the enzymatic hydrolysis of the ester residue. Upon incubation of **9** with porcine liver esterase (PLE) for 30 min in a phosphate buffer (pH = 7.2, 21 °C), the transport activity of chloride ions was significantly suppressed as a result of the conversion of **9** to **1** under the given conditions (Fig. 2).

In conclusion, we have shown that the chloride transport activities of amide-linked bisurea compounds strongly depend on the central amide conformation as well as the binding affinities with the chloride ion. The *trans* amide with an unfolded, extended structure shows negligible transport ability while the *N*-methylated *cis* amide with a folded, curved shape functions as a very potent mobile carrier across POPC membranes. Anion transports in living organisms are controlled by biological stimuli such as concentration gradient changes, ligand binding, and chemical reactions.[16] In this context, it is also demonstrated that the transport activity can be modulated by the *cis*-to-*trans* conversion by the chemical and enzymatic degradation.

This study was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (NRF-2013R1A2A2A05005796). E.B.P. acknowledges the fellowship of the BK 21-plus program from the Ministry of Education and Human Resources Development.

Notes and references

1 (*a*) Y. Takeuchi and G. R. Marshall, *J. Am. Chem. Soc.*, 1998, **120**, 5363–5372; (*b*) G. Fischer, *Chem. Soc. Rev.*, 2000, **29**, 119–127; (*c*) C. Dugave and L. Demange, *Chem. Rev.*, 2003, **103**, 2475–2532; (*d*) M. Kuemin, Y. A. Nagel, S. Schweizer, F. W. Monnard, C. Ochsenfeld and H. Wennemers, *Angew. Chem., Int. Ed.*, 2010, **49**, 6324–6327.

ChemComm Accepted Manuscript

2 (*a*) A. Itai, Y. Toriumi, N. Tomioka, H. Kagechika, I. Azumaya and K. Shudo, *Tetrahedron Lett.*, 1989, **30**, 6177–6180; (*b*) I. Azumaya, H. Kagechika, K. Yamaguchi and K. Shudo, *Tetrahedron*, 1995, **51**, 5277–5290.

3 (*a*) R. Yamasaki, A. Tanatani, I. Azumaya, S. Saito, K. Yamaguchi and H. Kagechika, *Org. Lett.*, 2003, **5**, 1265–1267; (*b*) A. Tanatani, A. Yokoyama, I. Azumaya, Y. Takakura, C. Mitsui, M. Shiro, M. Uchiyama, A. Maranaka, N. Kobayashi and T. Yokozawa, *J. Am. Chem. Soc.*, 2005, **127**, 8553–8561; (*c*) I. Okamoto, M. Nabeta, Y. Hayakawa, N. Morita, T. Takeya, H. Masu, I. Azumaya and O. Tamura, *J. Am. Chem. Soc.*, 2005, **129**, 1892–1893; (*d*) I. Okamoto, R. Yamasaki, M. Sawamura, T. Kato, N. Nagayama, T. Takeya, O. Tamura, H. Masu, I. Azumaya, K. Yamaguchi, H. Kagechika and A. Tanatani, *Org. Lett.*, 2007, **9**, 5545-5547; (*e*) H. Kakura, I. Azumaya, H. Masu, M. Matsumura, K. Yamaguchi, H. Kagechika and A. Tanatani, *Tetrahedron*, 2010, **66**, 8254–8260; (*f*) N. Fujimoto, M. Matsumura, I. Azumaya, S. Nishiyama, H. Masu, H. Kagechika and A. Tanatani, *Chem. Commun.*, 2012, **48**, 4809–4811.

4 (*a*) T. J. Jentsch, V. Stein, F. Weinreich and A. A. Zdebik, *Physio. Rev.*, 2002, **82**, 503–568; (*b*) L. Puljak, G. Kilic, *Biochim. Biophys. Acta*, 2006, **1762**, 404–413; (*c*) A. P. Davis, D. N. Sheppard and B. D. Smith, *Chem. Soc. Rev.*, 2007, **36**, 348.

5 For reviews, see: (*a*) P. R. Brotherhood and A. P. Davis, *Chem. Soc. Rev.*, 2010, **39**, 3633–3647; (*b*) J. T. Davis, O. Okunola and R. Quesada, *Chem. Soc. Rev.*, 2010, **39**, 3843–3862; (*c*) P. A. Gale, *Acc. Chem. Res.*, 2011, **44**, 216–226; (*d*) S. Matile, A. V. Jentzsch, J. Montenegro and A. Fin, *Chem. Soc. Rev.*, 2011, **40**, 2453–2474; (*e*) F. De Riccardids, I. Izzo, D. Montesarchio and P. Tecilla, *Acc. Chem. Res.*, 2013, **46**, 2781–2790; (*f*) A. V. Jentzch, A. Henning, J. Mareda and S. Matile, *Acc. Chem. Res.*, 2013, **46**, 2791–2800; (*g*) P. A. Gale, R. Pérez-Tomás and R. Quesada, *Acc. Chem. Res.*, 2013, **46**, 2801–2813; (*h*) G. W. Gokel and S. Negin, *Acc. Chem. Res.*, 2013, **46**, 2824–2833; (*i*) H. Valkenier and A. P. Davis, *Acc. Chem. Res.*, 2013, **46**, 2898–2909; (*j*) D. S. Kim and J. Sessler, *Chem. Soc. Res.*, 2015, **44**, 532–546.

6 For some selected recent papers, see: (*a*) S. J. Moore, C. J. E. Haynes, J. Gonazález, J. L. Sutton, S. J. Brroks, M. E. Light, J. Herniman, G. J. Langley, V. Soto-Cerrato, R. Pérez-Tomás, I. Marques, P. J. Costa, V. Félix and P. A. Gale, *Chem. Sci.*, 2013, **4**, 103–117; (*b*) I. Alfonso and R. Quesada, *Chem. Sci.*, 2013, **4**, 3009–3019; (*c*) C.-R. Elie, A. Hébert, M. Charbonneau, A. Haiun and A. R. Schmitzer, *Org. Biomol. Chem.*, 2013, **11**, 923–928; (*d*) C. Chhun, J. Richard-Daniel, J. Kempf and A. R. Schmitzer, *Org. Biomol. Chem.*, 2013, **11**, 6023–6028; (*e*) A. V. Jentzsch and S. Matile, *J. Am. Chem. Soc.*, 2013, **135**, 5302–5303; (*f*) P. B. Cranwell, J. R. Hiscock, C. J. E. Haynes, M. E. Light, N. J. Wells and P. A. Gale, *Chem. Commun.*, 2013, **49**, 874–876; (*g*) N. Busschaert, S. J. Bradberry, M. Wenzel, C. J. E. Haynes, J. R. Hiscock, I. L. Kirby, L. E. Karagiannidis, S. J. Moore, N. J. Wells, J. Herniman, G. J. Langley, P. N. Horton, M. E. Light, I. Marques, P. J. Costa, V. Félix, J. G. Frey and P. A. Gale, *Chem. Sci.*, 2013, **4**, 3036–3045; (*h*) C. Chhun, J. Richard-Daniel, J. Kempf and A. R. Schmitzer, *Org. Biomol. Chem.*, 2013, **11**, 6023–6028; (*i*) C. J. E. Haynes, N. Busschaert, I. L. Kirby, J. Herniman, M. E. Light, N. J. Wells, I. Marques, V. Félix and P. A. Gale, *Org. Biomol. Chem.*, 2014, **12**, 62–72; (*j*) E. Hernando, V. Soto-Cerrato, S. Cortés-Arroyo, R. Pérez-Tomás and R. Quesada, *Org. Biomol. Chem.*, 2014, **12**, 1771–1778; (*k*) J. Shang, W. Si, W. Zhao, Y. Che, J.-L. Hou and H. Jiang, *Org. Lett.*, 2014, **16**, 4008–4011; (*l*) J. A. Cooper, S. T. G. Street and A. P. Davis, *Angew. Chem., Int. Ed.*, 2014, **53**, 5609–5613; (*m*) H. Valkenier, L. W. Judd, H. Li, S. Hussain, D. N. Sheppard and A. P. Davis, *J. Am. Chem. Soc.*, 2014, **136**, 12507–12512; (*n*) H. Valkenier, N. L. Mora, A. Kros and A. P. Davis, *Angew. Chem., Int. Ed.*, 2015, **54**, 2137–2141.

7 (*a*) N. Busschaert and P. A. Gale, *Angew. Chem., Int. Ed.*, 2013, **52**, 1374–1382; (*b*) S.-K. Ko, S. K. Kim, A. Share, V. M. Lynch, J. Park, W. Namkung, W. V. Rossom, N. Busschaert, P. A. Gale, J. L. Sessler and I. Shin, *Nat. Chem.*, 2014, **6**, 885–892.

8 (*a*) Y. R. Choi, M. K. Chae, D. Kim, M. S. Lah and K.-S. Jeong, *Chem. Commun.*, 2012, **48**, 10346–10348; (*b*) J. H. Lee, J. H. Lee, Y. R. Choi, P. Kang, M.-G. Choi and K.-S. Jeong, *J. Org. Chem.*, 2014, **79**, 6403–6409.

9 Y. R. Choi, G. C. Kim, H.-G. Jeon, J. Park, W. Namkung and K.-S. Jeong, *Chem. Commun.*, 2014, **50**, 15305–15308.

10 (*a*) P. V. Santacroce, J. T. Davis, M. E. Light, P. A. Gale, J. C. Iglesias-Sánchez, P. Prados and R. Quesada, *J. Am. Chem. Soc.*, 2007, **129**, 1886–1887; (*b*) O. A. Okunola, J. L. Seganish, K. J. Salimian, P. Y. Zavalij and J. T. Davis, *Tetrahedron*, 2007, **63**, 10743–10750; (*c*) P. V. Jog and M. S. Gin, *Org. Lett.*, 2008, **10**, 3693–3696; (*d*) N. Busschaert, R. B. P. Elmes, D. D. Czech, X. Wu, I. L. Kirby, E. M. Peck, K. D. Hendzel, S. K. Shaw, B. Chan, B. D. Smith, K. A. Jolliffe and P. A. Gale, *Chem. Sci.*, 2014, **5**, 3617–3626.

11 R. Hrdina, E. C. Müller, R. C. Wende, L. Wanka and P. R. Schreiner, *Chem. Commun.*, 2012, **48**, 2498–2500.

12 L. W. Judd and A. P. Davis, *Chem. Commun.*, 2010, **46**, 2227–2229.

13 (*a*) A. V. Koulov, T. N. Lambert, R. Shukla, M. Jain, J. M. Boon, B. D. Smith, H. Li, D. N. Sheppard, J. B. Joos, J. P. Clare and A. P. Davis, *Angew. Chem., Int. Ed.*, 2003, **42**, 4931–4933; (*b*) B. A. MacNally, A. V. Koulov, B. D. Smith, J.-B. Joos and A. P. Davis, *Chem. Commun.*, 2005, 1087–1089; (*c*) N. Busschaert, I. L. Kirby, S. Young, S. J. Coles, P. N. Horton, M. E. Light and P. A. Gale, *Angew. Chem., Int. Ed.*, 2012, **51**, 4426–4430; (*d*) L. Adriaenssens, *J. Am. Chem. Soc.*, 2013, **135**, 8324–8330.

14 (*a*) W. F. D. Bennett, J. L. MaCallum and D. P. Tieleman, *J. Am. Chem. Soc.*, 2009, **131**, 1972–1978; (*b*) N. Busschaert, M. Wenzel, M. E. Light, P. Iglesias-Hernández, R. Pérez-Tomás and P. A. Gale, *J. Am. Chem. Soc.*, 2011, **133**, 14136–14148.

15 (*a*) S. Matile and N. Sakai, in *Analytical Methods in Supramolecular Chemistry*, ed. C. A. Schalley, Wiley, Weinheim, 2007, pp. 391–418; (*b*) A. V. Jentzsch, D. Emery, J. Mareda, S. K. Nayak, P. Metrangolo, G. Resnati, N. Sakai and S. Matile, *Nat. Commun.*, 2012, **3**, 905–913.

16 (*a*) M. Chebib and G. A. R. Johnston, *J. Med. Chem.*, 2000, **43**, 1427–1447; (*b*) A. S. Verkman and L. J. V. Galietta, *Nat. Rev. Drug Discov.*, 2009, **8**, 153–171; (*c*) K. T. Kahle, T. Z. Deeb, M. Puskarjov, L. Silayeva, B. Liang, K. Kaila and S. J. Moss, *Trends Neurosci.*, 2013, **36**, 726–737.

ChemComm Accepted Manuscript

This journal is © The Royal Society of Chemistry 20xx