Grauwels, G., Valkenier, H., Davis, A. P., Jabin, I., & Bartik, K. (2019). Repositioning Chloride Transmembrane Transporters: Transport of Organic Ion Pairs. *Angewandte Chemie*, 131(21), 6995-6999. <https://doi.org/10.1002/ange.201900818>

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Repositioning Chloride Transmembrane Transporters: Transport of Organic Ion Pairs

Glenn Grauwels, Hennie Valkenier,* Anthony P. Davis, Ivan Jabin,* and Kristin Bartik*

Abstract: Given the biological importance of organic cations, the facilitated transport of organic ion pairs could find many applications. We report here that calix[6]arene tris(thio)ureas, which possess a cavity that can accommodate primary ammonium ions, can not only act as carriers for $\text{Cl}^-/\text{NO}_3^-$ antiport but can also perform the cotransport of PrNH_3Cl . Transport was monitored by fluorescence spectroscopy and the presence of the different species inside the vesicles were characterized by ^1H and ^{35}Cl NMR experiments involving shift reagents. The cotransport of PrNH_3Cl was also observed by receptors deprived of a cavity, but the presence of the cavity conveys an advantage, as the cotransport by calix[6]arenes was observed to be more efficient than the $\text{Cl}^-/\text{NO}_3^-$ antiport, which is not the case with receptors without a cavity. The role played by the cavity was furthermore highlighted by the disappearance of this advantage when using a bulky ammonium ion which cannot be complexed within the cavity.

Transmembrane transport of charged species is crucial for most biological processes. These species cannot diffuse spontaneously through cell membranes and their transport is generally achieved *in-vivo* by specialized membrane proteins. Perturbed transport due to defects at the level of some of these proteins has been linked to different diseases, such as cystic fibrosis, which is caused by a deficiency in Cl^- transport.^[1] While many synthetic molecules have been shown to transport inorganic anions^[2–5] or metal-anion pairs,^[6–9] the transport of primary ammonium-anion pairs across lipid bilayers by synthetic compounds has, to the best of our knowledge, not been demonstrated.^[10] The transmembrane transport of primary ammonium neurotransmitters, such as dopamine and norepinephrine, indeed warrants attention as deficiencies in their transport is reported to lead to various psychiatric diseases, ranging from bipolar disorders to autism.^[11–12] The exact mechanism by which these neurotransmitters are transported *in-vivo* is not yet fully elucidated but seems to involve the transport of Cl^- as well as of Na^+ and K^+ .^[13–15]

Calix[6]arene-based receptors are known to efficiently host ammonium ions in their cavity and, if appropriately functionalized, can simultaneously bind anions.^[16–18] These calix[6]arenes are thus promising candidates for the transport of primary ammonium chloride contact ion pairs. As many of the effective Cl^- carriers reported to date bear multiple urea or thiourea groups,^[19–24] we decided to evaluate the potential of calix[6]arene tris(thio)ureas **1–4** (Figure 1a) for the transmembrane transport of RNH_3Cl ion pairs.

Figure 1. a) Structure of calixarenes **1–4**. b) Molecular model of complex **4** with PrNH_3Cl , with Cl^- bound by the thioureas and PrNH_3^+ in the calixarene cavity.^[25]

Previously reported calixarenes **1–3** (Figure 1a) are known to bind propylammonium ion-pairs in organic solvents.^[26] A Cl^- ion can be bound by the (thio)urea groups via H-bonds and an ammonium cation can then occupy the calixarene cavity, as illustrated for PrNH_3Cl in Figure 1b. Calixarene **4**, the thiourea analogue of **2**, was synthesized for this study (see SI for synthesis and characterization) and also shown to recognize PrNH_3Cl as confirmed by the presence of ^1H NMR signals at $\delta = -1.19$ ppm and -1.91 ppm characteristic of PrNH_3^+ inside the calixarene cavity (see SI, Figure S15).

The ability of receptors **1–4** to transport chloride was verified to select the most promising compounds for experiments on the transport of organic ion pairs. Prior to the transport experiments, the binding of Cl^- by receptors **1–4** was quantified by ^1H NMR spectroscopy upon titrating with Bu_4NCl in $\text{DMSO}-d_6/\text{H}_2\text{O}$ (200:1) and monitoring the downfield shift of the (thio)urea NH proton signals. Fitting these shifts to a 1:1 binding model gave relatively low binding constants for all four systems ($< 50 \text{ M}^{-1}$; see SI, Table S1) with slightly higher constants for calixarenes **2** and **4** compared to **1** and **3**. Transmembrane transport of Cl^- was examined using the previously reported lucigenin assay.^[27] Calixarenes **1–4** were incorporated in the lipid bilayer of POPC/cholesterol (7:3) large unilamellar vesicles (LUVs) suspended in 225 mM NaNO_3 and with 0.8 mM lucigenin in the interior aqueous solution. Cl^- influx was assessed by monitoring the quenching of the fluorescence of lucigenin after addition of NaCl (25 mM). The results reported in Figure 2a show that calixarenes **2** and **4**, which bear CF_3 groups, are effective Cl^- transporters while **3** is scarcely active and no activity is observed for **1**. The higher rates can be due to the higher affinities of **2** and **4** for Cl^- and to the higher lipophilicity of these transporters, attributed to the presence of the fluorinated groups, making them more mobile in the bilayer than their non-fluorinated homologs.^[19] Faster transport is also observed for the calixarenes bearing thiourea groups compared to their urea analogues, as is commonly observed.^[20] Based on these results, calixarenes **2** and **4** were selected for further studies.

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Supporting information, including a description of used materials and equipment, experimental procedures, and data processing methods for this article is given via a link at the end of the document.

Figure 2. Normalized fluorescence traces for Cl^- transport at 25 °C in 200 nm POPC/cholesterol (7:3) LUVs (carrier:lipid = 1:1000) containing 0.8 mM lucigenin by: a) calixarenes 1–4, in 225 mM NaNO_3 upon addition of NaCl ; b) calixarene 4, in 112.5 mM Na_2SO_4 upon addition of NaCl or PrNH_3Cl ; c) calixarene 4, in water upon addition of NaCl or PrNH_3Cl .

To test if calix[6]arenes **2** and **4** act as mobile carriers or as channels, transport experiments were performed with DPPC LUVs below and above the lipid transition temperature (41 °C). No Cl^- transport was observed at 25 °C (gel phase) while transport was retrieved at 45 °C (fluid phase), which suggests that they indeed act as mobile carriers (see SI, Figures S17 and S18). To further support this, $\text{Cl}^-/\text{NO}_3^-$ exchange experiments in POPC/cholesterol (7:3) were performed with calixarene **4** at different calixarene:lipid ratios and a near linear trend was observed between the initial rates and the carrier concentration (Figure S19). As calixarenes (< 2 nm) are too small to span the lipid bilayer (ca. 4 nm), multiple calixarenes would be required to

form a channel, which is in contrast with the observed dependence on the concentration of **4**. All these experiments suggest that both calix[6]arenes **2** and **4** act as mobile carriers,^[28] in contrast to many other reported calixarene-based transmembrane transporters.^[29–31]

Ion transport in vesicles requires a mechanism for charge neutralization, to avoid the build-up of electrical potential across the membranes. For the lucigenin assay as performed here, electroneutrality can be maintained by countertransport of the relatively lipophilic nitrate anions. Indeed, when the NaNO_3 used to prepare the vesicles was replaced by Na_2SO_4 , chloride transport by **2** and **4** was no longer observed (Figures S20 and 2b respectively). This implies that (i) SO_4^{2-} is too hydrophilic for countertransport, and (ii) Na^+ cannot be cotransported by the receptors. With this result in hand, we were able to test for cotransport of other cations by adding them to the external solution and establishing whether they promoted chloride transport. We were pleased to find that when PrNH_3Cl was added to vesicles containing **2** or **4** in SO_4^{2-} , rapid chloride transport was detected (Figures S20 and 2b). Furthermore, when using NaCl as initial Cl^- source, transport was triggered by addition of $(\text{PrNH}_3)_2\text{SO}_4$ (Figure 2b, green curve). This suggests that the ditopic recognition feature of these calix[6]arenes enables the transport of organic ion-pairs.

To elucidate the transport mechanism for PrNH_3Cl , a series of additional experiments were undertaken. Transport was still observed when the PrNH_3Cl experiment was performed in the absence of any salt buffer (Figure 2c, red curve), ruling out the possibility that the complex 4-PrNH_3^+ functions as a $\text{Cl}^-/\text{SO}_4^{2-}$ antiporter. The possibility that 4-PrNH_3^+ functions as a Cl^-/OH^- antiporter was also investigated. This mechanism has been described by Vargas Jentzsch *et al.*^[32] with a calix[4]arene- NMe_4^+ complex. As this mechanism would lead to a significant pH variation inside the vesicle, the PrNH_3Cl transport experiments were repeated with the commonly used pH-sensitive dye 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS)^[33] encapsulated in the vesicles (see SI, Figure S28). The pH changes observed upon addition of 25 mM PrNH_3Cl were insignificant compared to what would be expected with net transport of 25 mM OH^- out of the vesicles, making this mechanism at best a small side process. Global HCl cotransport can also be excluded on the basis of these results. All these experiments plead in favor of a net cotransport of PrNH_3Cl into the vesicles.

Transport experiments with vesicles containing anionic lipids (see SI for details) showed that the presence of anionic lipids had the same impact on the rates of $\text{Cl}^-/\text{NO}_3^-$ antiport and PrNH_3Cl cotransport. This provides more information on the complexation process occurring during transport and suggests that the complexation of chloride occurs prior to the complexation of PrNH_3^+ , making it the rate-determining step for transport. This behavior is in agreement with previous research on the binding process of organic ion-pairs with calix[6]arenes in organic solvents.^[26,39]

To confirm that PrNH_3Cl does indeed enter the vesicles, both ^1H and ^{35}Cl NMR experiments were undertaken with the aim of characterizing the interior of the vesicles, before and after transport. Paramagnetic species were used to distinguish between extra- and intravesicular species. Co^{2+} , a known shift reagent for Cl^- in the context of transmembrane transport,^[34,35] was used for the ^{35}Cl NMR experiments and thulium(III)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonate) (TmDOTP^{5-})^[36] for the ^1H NMR experiments. Figure 3a shows the ^1H NMR spectra with and without calixarene **4** preincorporated in the lipid bilayer of the vesicles, recorded immediately after addition of 100 mM PrNH_3Cl . Three ^1H signals are observed for the alkyl chain of PrNH_3^+ ($\delta = 2.95, 1.75$, and 0.95 ppm) and broad signals for the lipids because of the large size of the vesicles (400 nm POPC/cholesterol (7:3) vesicles). After 1 h at 25 °C, Na_5TmDOTP (2 mM) or CoSO_4 (20 mM) were added to the samples (Figure 3b and SI, Figure S31). Addition of

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the paramagnetic species led to a downfield shift ($\delta = 3.30, 1.90$, and 1.10 ppm) as well as a broadening of the three signals belonging to extravesicular PrNH_3^+ . Small signals of which the chemical shifts are characteristic of PrNH_3^+ before the addition of the paramagnetic species were however visible in the systems with carrier **4**, attesting of the presence of intravesicular PrNH_3^+ (Figure 3b, blue line). The two higher field signals overlap with the carbon satellites of the shifted extravesicular PrNH_3^+ signals but can be clearly seen when subtracting the spectra without carrier from the one with carrier **4** (Figure 3c). Similarly, in the ^{35}Cl NMR spectra, the extravesicular Cl^- signal undergoes a downfield shift upon addition of the shift reagent and a small signal corresponding to intravesicular Cl^- is observed in the system with calixarene **4** (see SI, Figure S31). All these NMR experiments provide direct proof that both PrNH_3^+ and Cl^- are carried into the interior of the vesicles.

Figure 3. ^1H NMR spectra of 10 mM POPC/cholesterol vesicles extruded through 400 nm membrane pores with preincorporated calixarene **4** (carrier:lipid = 1:100, blue lines) or no carrier (orange lines) in 50 mM Na_2SO_4 in D_2O and after addition of 100 mM PrNH_3Cl . Lipid signals ($\diamond$) are visible, and acetone (*) was used as reference. Spectrum c is a subtraction of spectra in b with and without carrier.

U-tube experiments were performed as ultimate proof that propylammonium chloride can be carried across a bulk liquid membrane composed of CHCl_3 by calix[6]arene **4** (see SI for details). The presence of chloride in the receiving phase after 72 h was confirmed by fluorescence spectroscopy (Figure S33), and that of PrNH_3^+ by ^1H NMR spectroscopy (Figure S35). This proves that both components of the ion pair are indeed transported via a carrier mechanism.

To assess the influence of the calixarene cavity on the transport process and to determine if PrNH_3Cl cotransport is specific to our calix[6]arene-based receptors, transport experiments were also performed with known tripodal Cl^- carriers^[19,37,38] that are deprived of a cavity for the complexation of ammonium ions (compounds **5-7**).

Figure 4. Structures of carriers **5-7**.

As for calixarenes **2** and **4**, Cl^- transport was observed for **5-7** when using PrNH_3Cl and Na_2SO_4 in the lucigenin assay (see Table 1 and SI, Figures S23-25). This suggests that PrNH_3^+ transport might not necessarily require a calixarene cavity and that its transport across the lipid bilayer is driven by the flux of Cl^- ; the PrNH_3^+ could be complexed at the level of the phenyl moieties or might even not interact with the carrier at all. When comparing the half-lives and initial rates of PrNH_3Cl cotransport and $\text{Cl}^-/\text{NO}_3^-$ antiport (Table 1), a significant increase in transport efficiency of PrNH_3Cl cotransport over $\text{Cl}^-/\text{NO}_3^-$ antiport is however observed with calixarenes **2** and **4**, which is not the case for transporters **5-7**. The enhancement factor, defined as the ratio between half-lives of $\text{Cl}^-/\text{NO}_3^-$ antiport and PrNH_3Cl cotransport, is higher than 2 in the case of calixarenes **2** and **4** while it is around 1 for receptors **5-7**. A similar trend is observed when comparing the initial rates (see SI, Table S2). These results clearly show that the presence of a complexing cavity has a positive impact on the cotransport of PrNH_3Cl .

Table 1. Transport data for **2** and **4-7**

Receptor (carrier:lipid ratio)	Half-life, $t_{1/2}$ [s] ^[a]		
	NaCl in NaNO_3	PrNH_3Cl in Na_2SO_4	Enhancement factor ^[b]
2 (1:1000)	162	55	2.9
4 (1:1000)	47	20	2.4
5 (1:250)	159	139	1.1
6 (1:25k)	42	44	1.0
7 (1:25k)	108	86	1.3

^[a] Half-lives are calculated from fitting the inverse of normalized fluorescence curves (carrier:lipid = 1:1000) to single exponential decay equations. Errors are generally within 10% (see SI, Table S1, for details). ^[b] The enhancement factor is defined as a ratio between half-lives of $\text{Cl}^-/\text{NO}_3^-$ antiport and PrNH_3Cl cotransport.

To further support the benefit of the cavity of calix[6]arenes for PrNH_3Cl transport, we tested the transport of $t\text{BuNH}_3\text{Cl}$ by **4** and **7** using the lucigenin assay in a SO_4^{2-} solution (see SI Figures S22 and S25). $t\text{BuNH}_3^+$ is more sterically hindered than PrNH_3^+ and cannot be complexed by the calixarene cavity (see SI, Figure S16).^[39] While for receptor **7**, which is devoid of a cavity, the half-life of 98 s is similar to that observed for $\text{Cl}^-/\text{NO}_3^-$ antiport, a longer half-life of 71 s was observed in the case of calixarene **4**. These results confirm the advantage of the calix[6]arene cavity for the transport of PrNH_3Cl .

In conclusion, we report here that anion receptors able to transport Cl^- across lipid bilayers can also function as cotransporters of organic ion-pairs, such as PrNH_3Cl and $t\text{BuNH}_3\text{Cl}$. This cotransport was observed for receptors without a cavity, but the presence of a cavity in calix[6]arenes able to complex the PrNH_3^+ cation, has a positive impact on the rate of transport. The use of a thulium complex as shift reagent in ^1H NMR spectroscopy allowed to demonstrate that the PrNH_3^+ cation is indeed carried into the vesicles. We note that this method could also find applications in transmembrane transport studies of other organic cations. Furthermore, to the best of our knowledge, this is the first demonstration of calix[6]arenes to function as anion carriers. The ability of ditopic calix[6]arene-based transporters to efficiently cotransport PrNH_3Cl paves the way towards tailored cavity-based transporters for more biologically relevant ammonium compounds, like catecholamines and lysine.

Acknowledgements

GG thanks the Fonds pour la Formation à la Recherche dans l'Industrie et dans l'Agriculture (FRIA-FRS, Belgium) for his PhD grant. HV is Chercheuse Qualifiée of the Fonds de la Recherche Scientifique-FNRS and thanks the European Research Council (grant 802727). GG, HV and KB also thank the ULB, "Fonds Van

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Buuren" and "Fonds Defay" for grants which enabled the purchase of the fluorescence spectrometer.

Keywords: Calix[6]arene • Ion transport • Membranes • Receptors • Supramolecular Chemistry

References

- [1] C. A. Hübner, T. J. Jentsch, *Hum. Mol. Genet.* **2002**, *11*, 2435.
- [2] A. P. Davis, D. N. Sheppard, B. D. Smith, *Chem. Soc. Rev.* **2007**, *36*, 348.
- [3] G. W. Gokel, M. M. Daschbach, *Coord. Chem. Rev.* **2008**, *252*, 886.
- [4] S. Matile, A. Vargas Jentzsch, J. Montenegro, A. Fin, *Chem. Soc. Rev.* **2011**, *40*, 2453.
- [5] P. A. Gale, J. T. Davis, R. Quesada, *Chem. Soc. Rev.* **2017**, *46*, 2497.
- [6] A. V. Koulou, J. M. Mahoney, B. D. Smith, *Org. Biomol. Chem.* **2003**, *1*, 27.
- [7] C. C. Tong, R. Quesada, L. Sessler, P. A. Gale, *Chem. Commun.* **2008**, 6321.
- [8] J. H. Lee, J. H. Lee, Y. R. Choi, P. Kang, M. G. Choi, K. S. Jeong, *J. Org. Chem.* **2014**, *79*, 6403.
- [9] D. S. Kim, J. L. Sessler, *Chem. Soc. Rev.* **2015**, *44*, 532.
- [10] We note that experiments on transport of alkylammonium anion pairs through bulk liquid have been reported in the past, such as: R. M. Izatt, B. L. Nielsen, J. J. Christensen, J. D. Lamb, *J. Memb. Sci.* **1981**, *9*, 263; M. Paugam, L. S. Valencia, B. Boggess, B. D. Smith, *J. Am. Chem. Soc.* **1994**, *116*, 11203–11204, L. Mutihac, R. Mutihac, H.-J. Buschmann, *J. Inclusion Phenom. Mol. Recognit. Chem.* **1995**, *23*, 167.
- [11] A. S. Kristensen, J. Andersen, T. N. Jørgensen, L. Sørensen, J. Eriksen, C. J. Loland, K. Strømgaard, *Pharmacol. Rev.* **2011**, *63*, 585.
- [12] F. Herborg, T. F. Andreassen, F. Berlin, C. J. Loland, U. Gether, *J. Biol. Chem.* **2018**, *293*, 7250.
- [13] E. Zomot, A. Bendahan, M. Quick, Y. Zhao, J. A. Javitch, B. I. Kanner, *Nature* **2007**, *449*, 726.
- [14] L. Borre, T. F. Andreassen, L. Shi, H. Weinstein, U. Gether, *J. Biol. Chem.* **2014**, *289*, 25764.
- [15] L. J. De Felice, *Pflügers Arch* **2016**, *468*, 503.
- [16] M. Ménand, I. Jabin, *Chem. - A Eur. J.* **2010**, *16*, 2159.
- [17] S. Le Gac, I. Jabin, O. Reinaud, in *Bioinspiration Biomimicry Chem. Reverse-Engineering Nat.*, **2012**, pp. 367–395.
- [18] G. De Leener, S. Moerkerke, R. Lavendomme, O. Reinaud, I. Jabin, in *Calixarenes and Beyond*, **2016**, pp. 113–140.
- [19] N. Busschaert, M. Wenzel, M. E. Light, P. Iglesias-Hernández, R. Pérez-Tomás, P. A. Gale, *J. Am. Chem. Soc.* **2011**, *133*, 14136.
- [20] H. Valkenier, L. W. Judd, H. Li, S. Hussain, D. N. Sheppard, A. P. Davis, *J. Am. Chem. Soc.* **2014**, *136*, 12507.
- [21] L. E. Karagiannidis, C. J. E. Haynes, K. J. Holder, I. L. Kirby, S. J. Moore, N. J. Wells, P. A. Gale, *Chem. Commun.* **2014**, *50*, 12050.
- [22] C. Lang, X. Zhang, Q. Luo, Z. Dong, J. Xu, J. Liu, *Eur. J. Org. Chem.* **2015**, *2015*, 6458.
- [23] C. M. Dias, H. Valkenier, A. P. Davis, *Chem. Eur. J.* **2018**, *24*, 6262.
- [24] M. J. Spooner, H. Li, I. Marques, P. M. R. Costa, X. Wu, E. N. W. Howe, N. Busschaert, S. J. Moore, M. E. Light, D. N. Sheppard, V. Félix, P. A. Gale, *Chem. Sci.* **2019**, Advance Article.
- [25] Lowest energy conformation obtained from a conformational search (Monte Carlo Molecular Mechanics) in MacroModel 10.3 using the OPLS2005 force field.
- [26] M. Hamon, M. Ménand, S. Le Gac, M. Luhmer, V. Dalla, I. Jabin, *J. Org. Chem.* **2008**, *73*, 7067.
- [27] B. A. McNally, A. V. Koulou, B. D. Smith, J.-B. Joos, A. P. Davis, *Chem. Commun.* **2005**, *0*, 1087.
- [28] Calix[4]arenes acting as mobile carriers for anions have been reported, for example: S. Lichen, V. Bagnacani, L. Baldini, A. Casnati, F. Sansone, M. Giannetto, P. Pengo, P. Tecilla, *Supramol. Chem.* **2013**, *25*, 631; O. Y. Shatursky, L. A. Kasatkina, R. V. Rodik, S. O. Cherenok, A. A. Shkrabak, T. O. Veklich, T. A. Borisova, S. O. Kosterin, V. I. Kalchenko, *Org. Biomol. Chem.* **2014**, *12*, 9811.
- [29] V. Sidorov, F. W. Kotch, G. Abdrakhmanova, R. Mizani, J. C. Fetting, J. T. Davis, *J. Am. Chem. Soc.* **2002**, *124*, 2267.
- [30] J. L. Seganiash, P. V. Santacroce, K. J. Salimian, J. C. Fetting, P. Zavalij, J. T. Davis, *Angew. Chem. Int. Ed.* **2006**, *45*, 3334.
- [31] R. Zappacosta, A. Fontana, A. Credi, A. Arduini, A. Secchi, *Asian J. Org. Chem.* **2015**, *4*, 262.
- [32] A. Vargas Jentzsch, D. Emery, J. Mareda, P. Metrangolo, G. Resnati, S. Matile, *Angew. Chem. Int. Ed.* **2011**, *50*, 11675.
- [33] K. Kano, J. H. Fendler, *BBA - Biomembr.* **1978**, *509*, 289.
- [34] Y. Shachar-Hill, R. G. Shulman, *Biochemistry* **1992**, *31*, 6272.
- [35] A. V. Koulou, T. N. Lambert, R. Shukla, M. Jain, J. M. Boon, B. D. Smith, H. Li, D. N. Sheppard, J. B. Joos, J. P. Clare, A. P. Davis, *Angew. Chem. Int. Ed.* **2003**, *42*, 4931.
- [36] I. Lazar, D. C. Hrnčir, W. D. Kim, G. E. Kiefer, A. D. Sherry, *Inorg. Chem.* **1992**, *31*, 4422.
- [37] A. L. Jowett, E. Howe, X. Wu, N. Busschaert, P. A. Gale, *Chem. Eur. J.* **2018**, *24*, 10475.
- [38] H. Valkenier, C. M. Dias, K. L. Porter Goff, O. Jurček, R. Puttreddy, K. Rissanen, A. P. Davis, *Chem. Commun.* **2015**, *51*, 14235.
- [39] E. Brunetti, J.-F. Picron, K. Flidrova, G. Bruylants, K. Bartik, I. Jabin, *J. Org. Chem.* **2014**, *79*, 6179.

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**Repositioning chloride
transmembrane transporters:
transport of organic ion pairs**

Chloride carriers as cotransporters for organic ion pairs: Calix[6]arene tris(thio)ureas bearing a cavity that can accommodate primary ammonium ions can perform the cotransport of PrNH_3Cl across a lipid bilayer as well as act as carriers for $\text{Cl}^-/\text{NO}_3^-$ antiport. The advantage of the cavity is highlighted by comparing calixarenes to receptors deprived of a cavity, and also by testing bulkier alkylammonium ions which cannot be complexed in the cavity.