ACADEMIA ROMÂNĂ
Revue Roumaine de Chimie
http://web.icf.ro/rrch/

Rev. Roum. Chim.,
2018, *63*(7-8), 665-672

Dedicated to Professor Bogdan C. Simionescu on the occasion of his 70th anniversary

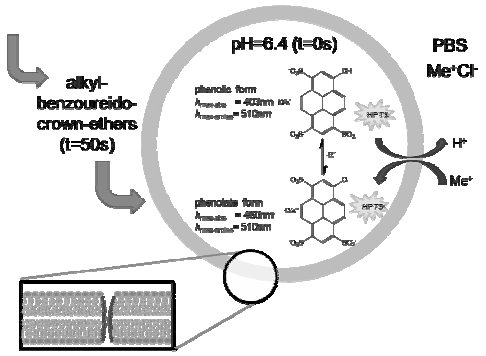
CATION TRANSPORT ACTIVITY OF THE ALKYL-UREIDO-BENZO-15--CROWN-5-ETHERS THROUGH DOUBLE LAYER LIPID MEMBRANES

Radu ZONDA,[a] Adina COROABA,[a,*] Sorin IBANESCU,[a,*] Tudor VASILIU,[a] Andrei NEAMTU,[a,b] Mihaela SILION,[a] Teodora RUSU[a] and Mariana PINTEALA[a]

[a] Centre of Advanced Research in Bionanoconjugates and Biopolymers, "Petru Poni" Institute of Macromolecular Chemistry, 41A Grigore Ghica Vodă Alley, Iaşi 700487, Roumania
[b] TRANSCEND Research Center, Regional Institute of Oncology, 2-4 General Henry Mathias Berthlot Street, Iaşi 700483, Roumania

Received July 31, 2017

Due to its many implications in biological processes, the ionic transport through double layer lipid membrane has generated a lot of attention from the supramolecular chemistry community. In this study, we report the synthesis of modified amino-benzo-crown-ethers with linear or optically active alkyl chains, in order to integrate them, by forming supramolecular structures, into the double layer lipid membranes. The cationic transport capability of the synthesized compounds through double layer lipid membranes was determined on the basis of fluorescence spectra. Furthermore, an *in-silico* study of alkyl-benzoureido-crown-ethers has been performed in order to better understand the mechanism of transport across a bilayer membrane.



INTRODUCTION

In living cells, the selective transport of water and ions through double layer lipid membranes is essential for maintaining the balance of electrolytes required for physiological processes.[1] This function is performed by specialized molecules like aquaporin for water[2] or gramicidin[3] and KcsA[4] for cations. But there are limitations of using natural molecules for transport of ions and water due to their cost, instability and difficulty in integration in different membrane systems.[5] Natural occurring ionic channels represented the starting point for artificial ionic channels integrated in simpler systems. These can be either modified complex natural molecules,[6] single molecule ionic channels[7] or monomers which form supramolecular structures and cross the double layer lipid membrane.[8] To form ionic channels within lipid membranes a synthetic molecule requires three types of constituents: (i) one should interact with desired ions and has to facilitate their transport, (ii) one has to assure the formation of supramolecular structures (by hydrogen bonding group, π- π stacking units, etc.) and (iii) the third should be responsible for the integration of the molecule within the lipid layer (usually a hydrophobic chain). Ionic channels containing crown ethers attracted a lot of interest because of their specific interaction with alkaline metal ions

* Corresponding authors: adina.coroaba@icmpp.ro; ibanescu.sorin@icmpp.ro

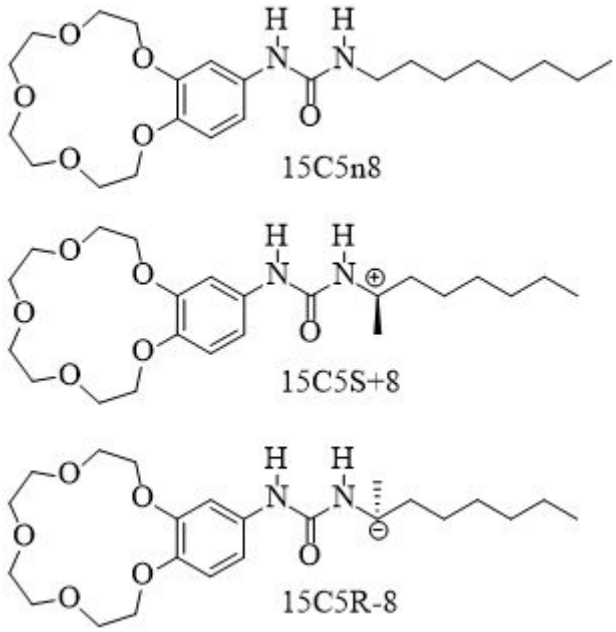


Fig. 1 – Structure of the three studied alkyl-benzoureido-crown-ethers.

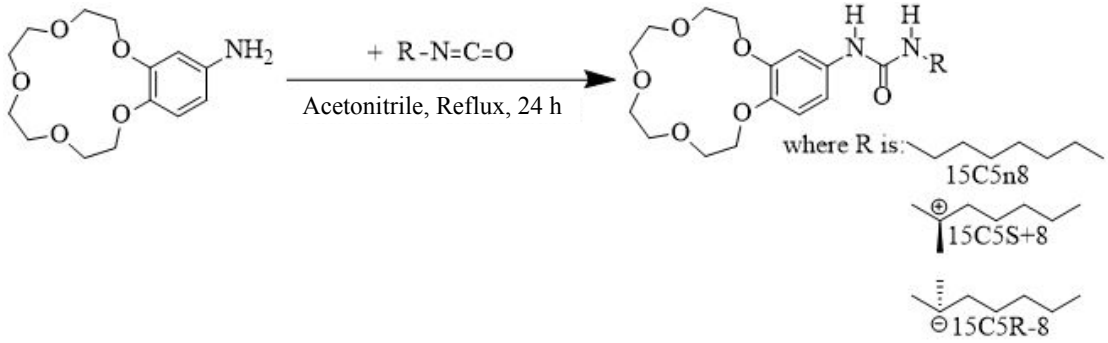
depending on the ionic radius.[9,10] In this context, alkyl-benzoureido-crown ethers are compounds that stand out for ionic transport applications, presenting a crown ether able to complex cations, a urea group, capable to form intermolecular hydrogen bonds, facilitating their self-assembling,[8] and a hydrophobic alkyl radical for integration within the lipid layer. The alkyl-benzoureido-crown ethers have been previously analyzed with respect to the selectivity of the transported cations depending on the ionic radius *versus* the diameter of the crown ether's ring.[11,12] However, the influence of the alkyl radical structure on their integration in the lipid membrane and their influence on the cation transport capacity has gained little attention. In this work, a series of alkyl-benzoureido-crown-ethers was prepared (Figure 1), by reacting amino-benzo-crown-ethers with different alkyl-isocyanates presenting either linear or optically active alkyl groups, to study their cationic transport capability through double layer lipid membranes.

RESULTS AND DISCUSSION

To facilitate the self-assembling and the integration of the crown ethers into the lipid membranes the designed compounds were synthesized by modifying the amino-benzo-15-crown-5-ether with three types of alkyl-isocyanates according to the Scheme 1. In this way, the amine group from the crown ether reacts with the isocyanate to form a urea, a group well known for its ability to generate hydrogen bonds. The added alkyl radical confers to the crown ether the hydrophobic character necessary for the integration in the lipid membrane. The reactions were carried out at reflux, in acetonitrile, with the reaction product readily precipitating out of the reaction solution.

The cationic transport capability of the alkyl-benzoureido-15-crown-5-ethers through double layer lipid membranes was tested by using 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS - a pH-sensitive dye) loaded large unilamellar vesicles (LUVs). The internal pH change inside the LUVs was monitored by the change in the fluorescence emission of HPTS. Figure 2a depicts a schematic representation of the HPTS experiment with the inserted graph presenting an ideal case of the measured fluorescence during the experiment (Figure 2b). At the beginning of the experiment HPTS loaded LUVs are suspended in phosphate-buffered saline (PBS) solution with a pH of 6.4. At this moment the pH values inside and outside of the vesicles is 6.4 and the recorded fluorescence ratio I_0/I_1 (where I_0 is the intensity of the emission of the HPTS protonated form and I_1 is the intensity of the emission of the HPTS deprotonated form) is at its minimum due to HPTS being fully deprotonated, as evidenced in the first region on the graph (Figure 2b). After 50 s of steering for homogenization the alkyl-benzoureido-crown-ether to be studied is added and a week spontaneous transport is recorded as seen in the second region on the graph (Figure 2b). This type of transport is of little interest for our work due to the difficulty of quantification. Our interest is focused mainly on

the cation transport under a pH-active gradient. To achieve this goal, after another 50 s the pH outside the vesicles was increased with approximately one unit in order to facilitate the cation migration. This leads to a gradual increase of the pH inside of the vesicle that can be seen by the increase of I_1 and the decrease of I_0 as seen in the third region of the spectrum (Figure 2b). This sharp increase in the intensity ratio is used to calculate the kinetic initial rates (k) of the cation transport, while the maximum plateau value allows the calculation of the fractional activity (Y). Also, in order to record the maximum of intensity of the deprotonated form of HPTS, as seen in the fourth region of the graph, after 600 seconds, a solution of a detergent is added for the lysis of the vesicles. The fluorescence data were used only to calculate k and Y with all important parameters being included in the subsequent figures (Figures 3, 4). Figure 2c and 2d represents typically recorded fluorescence spectra, at different concentration, for the linear alkyl-benzoureido-crown-ether (15C5n8) for Na^+ and K^+ cations, respectively.



Scheme 1 – Synthesis of the studied alkyl-benzoureido-15-crown-5-ethers.

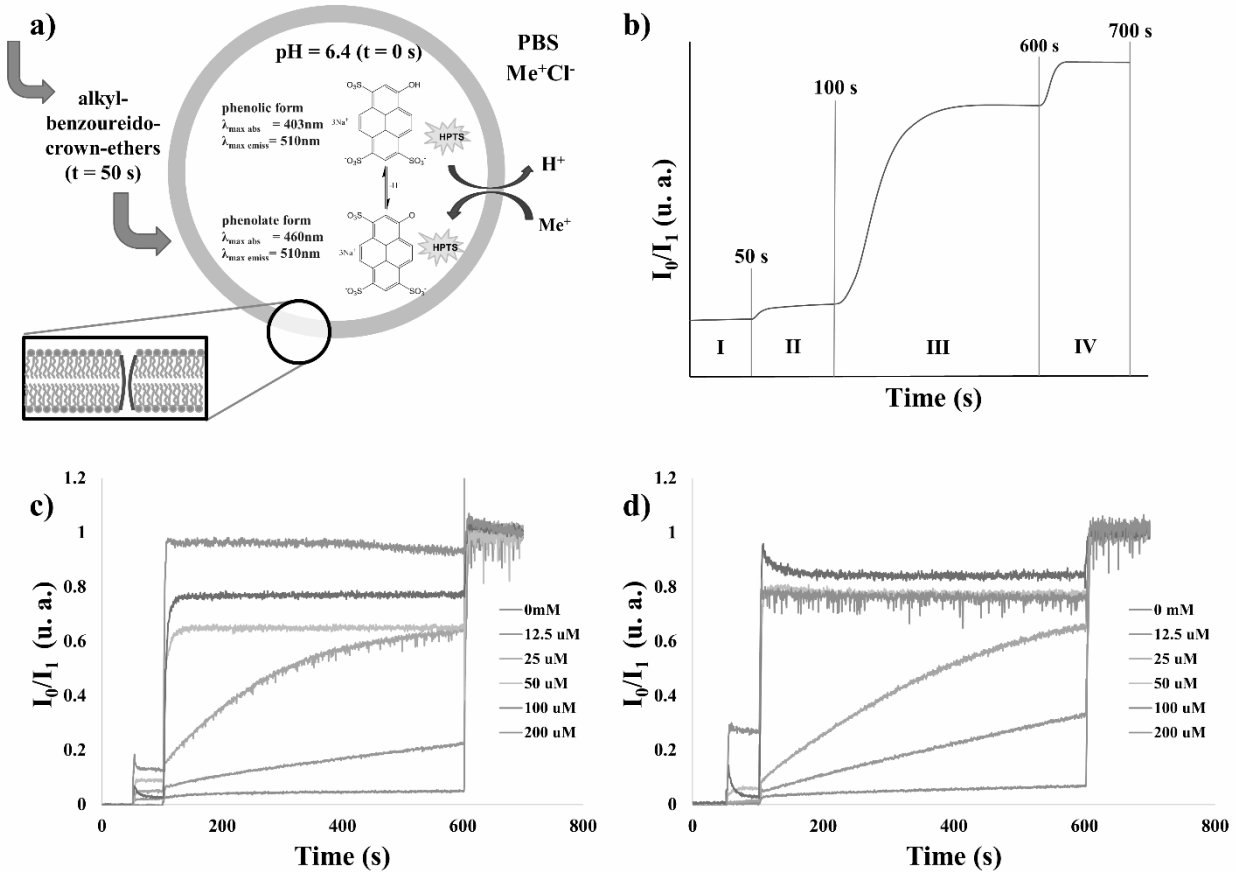


Fig. 2 – a) Principle of the HPTS experiment; b) Ideal case of recorded fluorescence ratio I_0/I_1; c) Na^+ transport activity for 15C5n8; d) K^+ transport activity for 15C5n8.

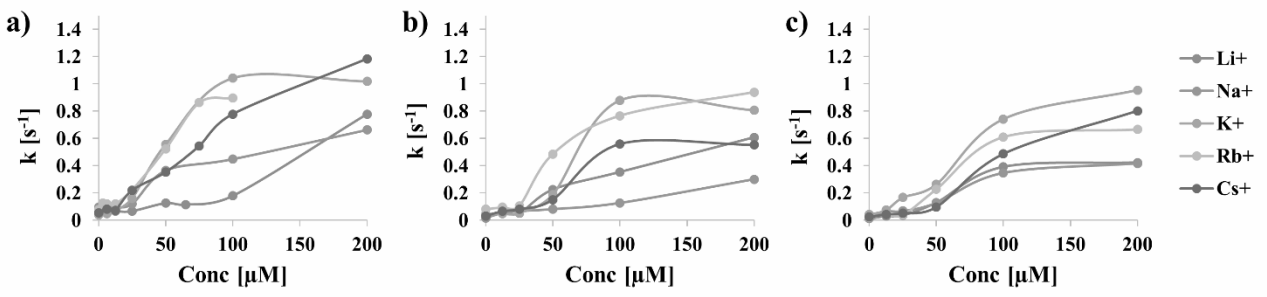


Fig. 3 – Pseudo-first order initial constant rate as a function of final concentration of alkyl-benzoureido-15-crown-5-ether in the bilayer membrane for: (a) 15C5n8, (b) 15C5S+8, (c) 15C5R-8.

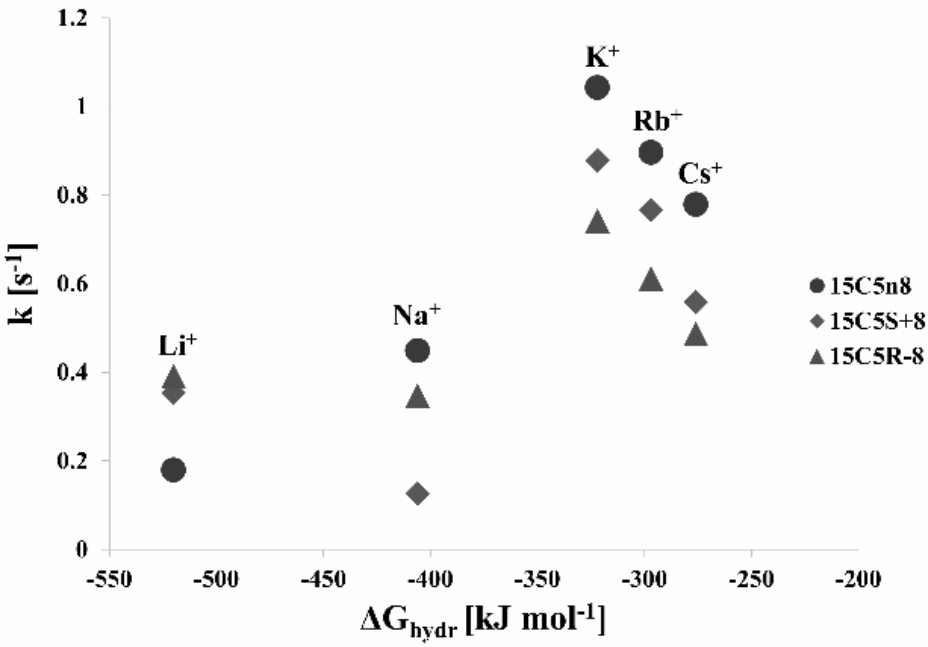


Fig. 4 – Initial constant rates of various cations as a function of hydration free enthalpy at 100 µM for 15C5n8, 15C5S+8, and 15C5R-8 compounds.

The capacity of ionic transport for all three synthesized compounds (15C5n8, 15C5S+8, 15C5R-8) was tested at different concentrations (from 0 to 200 µM). Generally, an increase of the alkyl-benzoureido-crown-ethers concentration induced an intensification of the transport capacity (Figure 2c and 2d). Figure 3 shows the pseudo-first order kinetic initial rates (k) of the cation transport through bilayer membrane as a function of concentration for all studied compounds and cations. It can easily be observed that for compound with the linear alkyl chain, 15C5n8, (Figure 3a), the smallest cations (Li^+ and Na^+) present a much lower activity than the bigger ones (K^+, Rb^+ and Cs^+). Moreover, a saturation behavior is observed at 100 µM for K^+, Rb^+ and Cs^+ while for the rest the plateau is not reached in the experimental concentration domain. Under the same conditions, the compound 15C5n8 at 100 µM presents significantly higher values of initial transport rate *k*, 1.042 s^{-1} for K^+ and 0.448 s^{-1} for Na^+, with kinetic selectivity of $SK^+/Na^+ \approx 2$. This observation is consistent with literature data[13] where 15-crown-5-ether was shown to slowly transport the smaller Li^+ and Na^+ cations while in an unexpected manner, higher transport rates were noticed for the K^+, Rb^+ and Cs^+ cations (having a bigger diameter than 15- crown-5 macrocycle). The same behavior is observed, also, for the two compounds with optically active alkyl chains, 15C5S+8 (Figure 3b) and 15C5R-8 (Figure 3c), where, again, for the larger cations the transport rate k reached a plateau at 100 µM. Comparing the three studied molecules it can be noticed a small decrease in activity when the alkyl chain is altered from linear to optically active one. Also, two sets of activity were observed, with a gap of conductance states between the smaller (Li^+ and Na^+) cations and the bigger (K^+, Rb^+ and Cs^+) cations.

Table 1

Effective concentration (EC_{50}) and Hill coefficient (n)

	15C5n8		**15C5S+8**		**15C5R-8**	
	EC_{50} *(μM)*	*n*	EC_{50} *(μM)*	*n*	EC_{50} *(μM)*	*n*
Li^+	39.43	2.32	59.48	3.75	41.23	2.46
Na^+	24.81	1.27	68.78	3.38	48.60	2.99
K^+	14.81	2.98	22.63	1.27	20.88	1.53
Rb^+	9.98	1.22	43.65	1.08	23.49	1.84
Cs^+	22.26	1.98	37.54	2.70	50.25	4.46

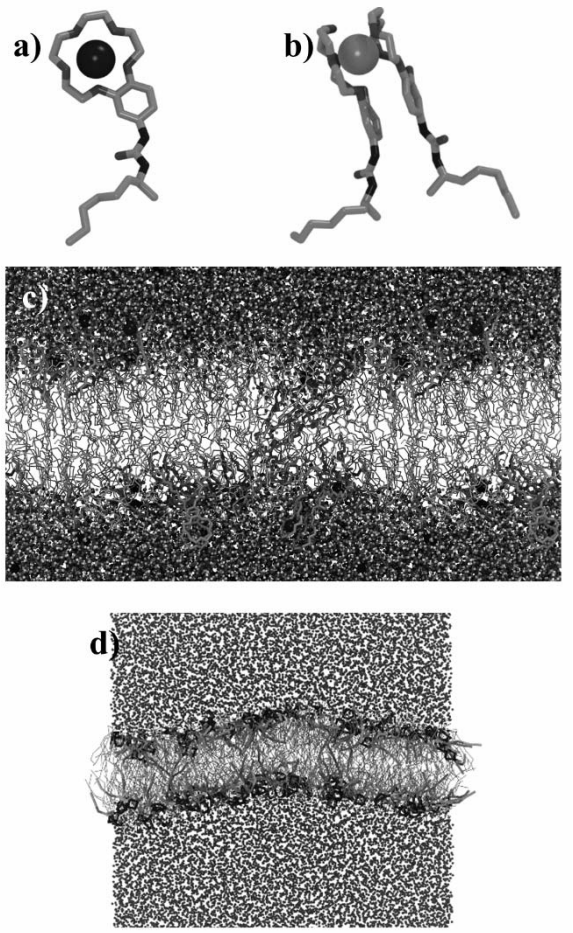


Fig. 5 –Atomistic simulations of compound 15C5S+8 with a) Na^+ and b) K^+ cations. c) Transversal section of double-layer lipidic membrane in atomistic simulation in presence of compound 15C5S+8 with Na^+. d) Transversal section of double-layer lipidic membrane in Course-Grain simulation, in presence of compound 15C5n8. The studied molecules have the crown ether colored blue, the alkyl tail colored yellow, the lipids are represented as thin teal lines and the water by red beads.

At 100 μM the transport activity of all the three synthesized compounds increases in the order $K^+ > Rb^+ > Cs^+ > Na^+ \geq Li^+$, corresponding to the Eisenman sequence IV[14] consistent with the energetic penalty for ions dehydration (Figure 4). This indicates that the binding to the crown ether is playing a more important role as compared to that of the dehydration.

A Hill analysis has been carried out to better understand the cations conductance behaviors. After determination of fractional activity (Y) at 600 seconds for each concentration, EC_{50} (the effective concentration needed to reach 50% activity), and Hill coefficient (n) were calculated, as mentioned in the Materials and Methods section. The results are summarized in Table 1. If the EC_{50} is smaller, it means that a smaller quantity is needed to form the supramolecular structures necessary to transport the cations.[15] In our cases, for all three compounds, the EC_{50} for K^+ is smaller than for Na^+, following the same trend as the initial

transport rates (k). Despite significant differences in transport activity, the Hill coefficients for all cations are higher than 1. The Hill coefficient (n) is a measure of cooperativity in a binding process. A Hill coefficient of 1 indicates independent binding, a value higher than 1 shows positive cooperativity binding, while a value lower than 1 shows negative cooperation.[15]

All the results obtained from the analysis of rate transport (*k*), the effective concentration needed to reach 50% activity (EC_{50}) and Hill coefficient (*n*) suggests a complex transport behavior with a combination of dynamic channels and carrier-type mechanism. In order to better understand the ion transport mechanism, an atomistic molecular dynamic simulation was run for 500 ns. All studied crown ethers form complexes with Na^+ and K^+, as exemplified in Figures 5a for Na^+ and 5b for K^+. A main difference between the two cases is the number of crown ether coordinated by the different cations: Na^+ forms a more stable complex with one, and K^+ with two crown ether molecules. However, structures with different cation crown ether ratio were also observed for short periods of time. Moreover, in the case of 15C5S+8 a transient channel like structure could be evidenced as presented in Figure 5c. This was, also, observed for 15C5n8 and 15C5R-8 compounds but only for a brief time period, most likely due to the high mobility of the alkyl chain which is responsible for a dynamic channel formation. As well, a Course-Grain (CG) model was constructed allowing us to extend the time of the simulation to 10 μs. Nevertheless, this did not reveal any channel like structures (Figure 5d) in the period of the simulation, either due to the temporal instability of the formed channels or the CG model selected. Over the course of the CG simulation, several crown ether molecules were seen performing a "flip-flop" action inside the membrane, suggesting a carrier-type mechanism (figure 5d). These observations suggest a more complex transport behavior with a combination of dynamic channels and carriers, being in some part involved in the cation transport, which is in a good agreement with the experimental results.

EXPERIMENTAL

Materials and methods

Amino-benzo-15-crown-5-ether was acquired from TCI, n-octyl-isocyanate from Acros Organics, (S)-(+)-2-octyl-isocyanate and (R)-(-)-2-octyl-isocyanate from Alfa Aesar and the other compounds were acquired from Sigma-Aldrich. L-α-phosphatidylcholine (egg, chicken – EYPC) was purchased from Avanti Polar Lipids. All chemicals were used as received unless specified otherwise.

Electrospray ionization mass spectrometry (ESI-MS) was performed on an Agilent 6520 Series Accurate-Mass Q-TOF LC/MS. The NMR spectra were recorded on an ARX 300 MHz Bruker. For the fluorescence experiments, a Perkin-Elmer LS 55 Fluorescence Spectrofluorimeter was used.

Synthesis of alkyl-ureido-benzo-15-crown-5-ethers

n-Octyl-benzoureido-15-crown-5-ether (15C5n8) was prepared by refluxing for 24 h 50 mg (0.1766 mmol) of amino-benzo-15-crown-5-ether with 1.1 equivalents of n-octyl-isocyanate (0.195 mmol, 30 mg, 36.8 μL) in 2 mL acetonitrile, under nitrogen atmosphere. The resulting mixture was cooled for 24 h at -20 °C and then filtered under the vacuum to collect the precipitate, **yield:** 80%.
ESI-MS: m/z (%): $[M+Na]^+$ = 461.61. ***^{1}H-NMR:*** ($(CD_3)_2S$=O, ppm) δ = 0.86 (t, 3H, C**H$_3$**), 1.26 (m, 10H, C**H$_2$**), 1.40 (m, 2H, **CH$_2$**), 3.05 (m, 2H, **CH$_2$**), 3.60 (m, 8H, -O-C**H$_2$**-C**H$_2$** -O- crown ether), 3.74 (m, 4H, -O-C**H$_2$**-C**H$_2$**-O- crown ether), 3.97 (m, 4H, -O-C**H$_2$**-C**H$_2$**-O- crown ether), 6.00 (t, 1H, N**H**-Alkyl), 6.79 (m, 2H, **H**-Ar), 7.13 (d, 1H, **H**-Ar), 8.18 (s, 1H, N**H** -Ar).

(S)-(+)-2-Octyl-benzoureido-15-crown-5-ether (15C5S+8) and **(R)-(-)-2-Octyl-benzoureido-15-crown-5-ether** (15C5R-8) were prepared similarly to 15C5N8, but using (S)-(+)-2-Octyl-isocyanate and (R)-(-)-2-Octyl-isocyanate respectively, instead of n-octyl-isocyanate, in the same ratios as for the **15C5n8** synthesis.
15C5S+8,* yield:** 82%. ***ESI-MS: m/z (%): $[M+Na]^+$ = 461.16. ***^{1}H-NMR:*** ($(CD_3)_2S$=O, ppm) δ = 0.86 (t, 3H, C**H$_3$**), 1.06 (d, 3H, =N-CH(C**H$_3$**)-alkyl), 1.26 (s, 8H, C**H$_2$**), 1.35 (m, 1H, =N-C**H**(CH_3)- alkyl), 3.61 (m, 8H, -O-C**H$_2$**-C**H$_2$** -O- crown ether), 3.75 (m, 4H, -O-C**H$_2$**-C**H$_2$**-O- crown ether), 3.97 (m, 4H, -O-C**H$_2$**-C**H$_2$**-O- crown ether), 5.85 (d, 1H, N**H**-Alkyl), 6.79 (dd, 2H, **H**-Ar), 7.15 (s, 1H, **H**-Ar), 8.08 (s, 1H, N**H** -Ar).
15C5R-8,* yield:** 81%. ***ESI-MS: m/z (%): $[M+Na]^+$ = 461.17. ***^{1}H-NMR:*** ($(CD_3)_2S$=O, ppm) δ = 0.86 (t, 3H, C**H$_3$**), 1.06 (d, 3H, =N-CH(C**H$_3$**)-alkyl), 1.26 (s, 8H, C**H$_2$**), 1.36 (m, 1H, =N-C**H**(CH_3)- alkyl), 3.60 (m, 8H, -O-C**H$_2$**-C**H$_2$** -O- crown ether), 3.75 (m, 4H, -O-C**H$_2$**-C**H$_2$**-O- crown ether), 3.97 (m, 4H, -O-C**H$_2$**-C**H$_2$**-O- crown ether), 5.85 (d, 1H, N**H**-Alkyl), 6.79 (dd, 2H, **H**-Ar), 7.15 (s, 1H, **H**-Ar), 8.09 (s, 1H, N**H** -Ar).

Large unilamellar vesicles (LUVs) preparation

The LUVs were prepared according with previously published protocol.[13] Briefly, 1 ml solution of 20 mg EYPC in $CHCl_3$/MeOH 1:1 was dried to form a thin film. The film was hydrated with 400 μL 10 mM PBS solution (pH = 6.4) containing HPTS (8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt) (10 μM) and NaCl (100 mM). The completed hydrated solution was subjected to 7 freeze-thaw cycles, then to extrusion to form HPTS loaded LUVs, using an Avanti Mini Extruder (25 times, 100 nm polycarbonate membranes). The HPTS from external buffer was removed using a Dextran cross-linked G-50 column, washed with 10 mM PBS (pH=6.4) with 100 mM NaCl solution.

Ion transport experiment

1.85 mL solution of 10 mM PBS/100 mM NaCl and 100 μL solution of the LUVs were added in a cuvette. After 50 s, 20 μL of the alkyl-benzoureido-crown-ether solved in

DMSO, with concentration ranging between 0 and 20 mM, depending on the final desired concentration, was added. After another 50 s the pH outside the vesicles was increased with approximately one unit by the addition of 20 µL solution of NaOH 0.5 M. At the moment t = 600 s, 40 µL Triton X100 5%, was added in order to lyse the vesicles and determine the maximum fluorescence level. During all experiments, the temperature of the vesicle solution was kept at 20 °C. The emission at 510 nm was recorded simultaneously with the excitation at 460 (I_0) and 403 nm (I_1) throughout the entire timeline of the experiment.

The **pH values inside the vesicles** during the assay were calculated for each point from the emission intensities according to the equation $pH = 1.1684 * log(I_0/I_1) + 6.9807$[16]. Normalized values of the ratio I_0/I_1 were calculated using the following formula: $(I_0/I_1)_{norm} = ((I_0/I_1) - (I_0/I_1)_{initial})/((I_0/I_1)_{final} - (I_0/I_1)_{initial})$. In a similar way, the normalized pH was calculated by the equation: $pH_{norm} = (pH - pH_{initial})/(pH_{final} - pH_{initial})$. The **rate of the transport (k)** in the first moment of the pH change was calculated as a pseudo-first order rate from the slope of the linear sector after the moment t = 100 s. The **fractional activity Y** was calculated for each curve using the normalized values of I_0/I_1, between 0 and 1, where 0 was considered the I_0/I_1 ratio of the blank just before lysis of the vesicles and 1 the highest I_0/I_1 ratio obtained, i.e. the plateau value[13]. **EC_{50}** and **Hill coefficient (n)** were calculated by expressing $log[Y/(1-Y)] = n * log(C) - n * log(EC_{50})$ where ***n*** is the slope of the curve and $n*log(\boldsymbol{EC_{50}})$ is the $log(C) = 0$ intercept value.

The protocol described is used for analyzing the Na^+ transport. For the study of the other alkali cations the PBS outside the vesicles was prepared using a chloride of the desired alkali instead of NaCl.

Molecular dynamics simulations

Molecular dynamics simulations were performed using the Gromacs software, in order to study the self-assembly properties of the studied compounds in a lipid bilayer. Two methods were chosen: atomistic simulations and Course-Grain (CG). For the atomistic simulations, two systems were created where 150 phosphatidylcholine and 40 alkyl-benzoureido-crown-ether molecules were randomly dispersed in a simulation box. Afterwards, the box was filled with 8940 water molecules and 30 Na^+, K^+ and Cl^- ions. The simulations were done under the same conditions (temperature 310K, pressure 1 bar) and using the same forcefield (CHARMM-FF) for 10 µs. In order to obtain a higher simulation time, a CG simulation using the Martini force field[17] was performed. To this end, 520 phosphatidylcholine (POPC) and 130 alkyl-benzoureido-crown-ether (15C5n8) molecules were randomly dispersed in a simulation box. Afterwards, the box was filled with 18000 water molecules. The POPC molecule was built from 12 beads according to the literature.[18] The 15C5n8 molecule was built from 10 beads and beads were chosen according to Marrink *et al.*[17] The simulation was done under atmospheric pressure (1 bar) and at a temperature of 310 K.

CONCLUSIONS

Three alkyl-benzoureido-15-crown-5-ethers with linear or optically active alkyl chains were prepared from the amino-benzo-crown-ethers and adequate alkyl isocyanates. The cation transport ability was tested by using HPTS loaded LUVs and quantified in respect with their kinetic transport rate (k) and EC_{50} values. For all studied compounds the transport activity at 100 µM is lower for smaller cations (Li^+, Na^+) and higher for the bigger ones (K^+, Rb^+, Cs^+), as evidenced by lower k and higher EC_{50} values. By replacing the alkyl chains, from linear to optically active, no significant difference in ion transport was observed, with only a small decrease in activity being noticed. At the selected concentration (100 µM) the 15C5n8, 15C5S+8, and 15C5R-8 compounds can be considered as selective for K^+ over Na^+ even if the K^+ cation has a bigger diameter than 15-crown-5 macrocycle. This suggests a complex cation transport mechanism and in order to elucidate it an *in-silico* study was performed. The molecular dynamic simulations suggest a more intricate transport behavior with a combination of dynamic channels and carriers being in some part involved in the cation transport, which is in a good agreement with the experimental results. Further investigations are needed to be able to fully understand how the cation transport occurs through the double-layer lipidic membrane.

Acknowledgements. The authors would like to thank Dr. Mihai Barboiu and Dr. Arnauld Gilles from the Institut Européen des Membranes, Montpellier, France for useful discussions and support during the development of this work. This publication is part of a project that has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No. 667387 WIDESPREAD 2-2014 SupraChem Lab. This work was also partially supported by a grant of the Roumanian National Authority for Scientific Research and Innovation, CNCS/CCCDI – UEFISCDI, project number PN-III-P3-3.6-H2020- 2016-0011, within PNCDI III program.

REFERENCES

1. P. K. Whelton and J. He, *Curr. Opin. Lipidol.,* **2014**, *25*, 75–79.
2. P. Agre, *Biosci. Rep.,* **2004**, *24*, 127–163.
3. D. A. Kelkar and A. Chattopadhyay, *Biochim. Biophys. Acta - Biomembr.,* **2007**, *1768*, 2011–2025.
4. L. G.Cuello, V Jogini, D. M. Cortes, A. Sompornpisut, M. D. Purdy, M. C. Wiener and E. Perozo, *FEBS Lett.,* **2010**, *584*, 1133–1138.
5. M. Barboiu, *Angew. Chemie - Int. Ed.,* **2012**, *51*, 11674–11676.
6. S. K. Bagal, A. D. Brown, P. J. Cox, K. Omoto, R. M. Owen, D. C. Pryde, B.Sidders, S. E. Skerratt, E. B. Stevens, R. I. Storer and N. A. Swain, *J. Med. Chem.,* **2013**, *56*, 593–624.

7. X. Hu, Z. Chen, G. Tang, J.-L. Hou and Z. *J.* Li, *Am. Chem. Soc.,* **2012**, *1*, 10–13.
8. A. Cazacu, C. Tong, A. Van Der Lee, T. M. Fyles and M. Barboiu, *J. Am. Chem. Soc.,* **2006**, *128*, 9541–9548.
9. N. Sakai and S. Matile, *Langmuir,* **2013**, *29*, 9031–9040.
10. J. W. Steed, *Coord. Chem. Rev.,* **2001**, *215*, 171–221.
11. A. Cazacu, Y.-M. Legrand, A. Pasc, G. Nasr, A. Van der Lee, E. Mahon and M. Barboiu, *Proc. Natl. Acad. Sci.,* **2009**, *106*, 8117–8122.
12. M. Barboiu, G. Vaughan and A. Van Der Lee, *Org. Lett.,* **2003**, *5*, 3073–3076.
13. A. Gilles and M. Barboiu, *J. Am. Chem. Soc.,* **2016**, *138*, 426–432.
14. G. Eisenman and R. Horn, *J. Membr. Biol.,* **1983**, *76*, 197–225.
15. C. Schalley, "Analytical methods in supramolecular chemistry", Wiley-VCH Verlag, **2012**, p. 711–739.
16. V. Sidorov, F. W. Kotch, J. L. Kuebler, Y. F. Lam and J. T. Davis, *J. Am. Chem. Soc.,* **2003**, *125*, 2840–2841.
17. S. J. Marrink, H. J. Risselada, S. Yefimov, D. P. Tieleman and A. H. De Vries, *J. Phys. Chem., B* **2007**, *111*, 7812–7824.
18. T. A. Wassenaar, H. I. Ingólfsson, R. A. Böckmann, D. P. Tieleman and S. J. Marrink, *J. Chem. Theory Comput.* **2015**, *11*, 2144–2155.