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Ion Transport Across Membranes Facilitated by a Dynamic Combinatorial Library[‡]

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A dynamic combinatorial library (DCL) consisting of dialdehydes and diamines has been used to facilitate the transport of calcium ions across a supported liquid membrane (SLM). In a dual selection process, the calcium ions first select matching macrocycles **3** or **5** from the DCL. Then only one of the macrocycle–calcium complexes (**5**·Ca^{2+}) efficiently transports the calcium ions due to its better balance between lipo- and hydrophilicity. The special setup of a DCL combined with an SLM directly finds suitable carriers for ion transport starting from diamine and dialdehyde building blocks **1**, **2** and **4**. The synthesis of the new, more lipophilic 4-pentoxypyridine-2,6-dicarbaldehyde (**4**) is also described, and the first transport experiments with liposomes are discussed.

Introduction

Compartmentalization by membranes is most important for the existence of life. In the cell, numerous compounds must be transported from one location to another across membranes.[1] Nature has developed several tools to achieve this, from channels that allow diffusion all the way to specific carriers. These different approaches have also been investigated in supramolecular chemistry.[2] In particular, molecular recognition can also be exploited for the development of a selective transporting system. In a classic experiment, a host is chosen that may bind the specific guest to be transported across a membrane. As a model for the biological membrane either bulk organic layers[3] or polymer-supported thin liquid membranes[4] have been used. The guest must be recognized, taken from the (aqueous) source phase and be bound efficiently. The resulting host–guest complex must be soluble in the membrane, and finally the guest must be released into the aqueous receiving phase. Therefore, a fine balance between thermodynamic binding parameters and kinetic uptake and release rates must be found. This usually requires extensive optimization work.

One way to simplify the selection and optimization processes is the use of dynamic combinatorial chemistry (DCC).[5] At the beginning of DCC, the search for an optimal host for a given guest was the central focus, and many dynamic combinatorial libraries (DCLs) have been investigated in the search for a good host for a given guest. All DCC experiments have in common the interaction of the guest with a host to stabilize a particular host, and thus the dynamic equilibrium of several potential host molecules changes the composition of the DCL, and the most stable system is formed. A good match between a host and a guest can be used for several purposes, for example, for analysis (sensoring), purification (extraction) or transport. These applications have now become the focus of DCC. Besides sensoring[6] and extraction,[7] the first transport experiments across bulk liquid membranes have been described.[8,9] Herein we present the first example of transport across a supported liquid membrane (SLM) by a dynamic combinatorial library and experiments showing the selection of the most efficient transporter by its own guest. Moreover, we present the first preliminary transport experiment in which liposomes are used as cell membrane mimics.

Results and Discussion

In previous work[8] we showed that a DCL formed by a reversible reaction between diamine **1** and dialdehyde **2** can be used to effect calcium transport across a bulk membrane. When the building blocks react in the presence of calcium ions in the water source phase, macrocycle **3** is formed, along with linear oligo- and polymers, which transports calcium ions from the water source phase to the water receiver phase.[8] The good water solubility of complex **3**·Ca^{2+}[10] is supposed to be the weakness of its carrier activity. In fact, most of the complex was found in the water source phase.[8]

[‡] Dynamic Combinatorial Chemistry, Part 4. Part 3: Ref.[8]

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In this work we first repeated the same experiment using a supported liquid membrane[11] (SLM) (Figure 1; building blocks **1** and **2**). In an SLM experiment, the water source phase is separated from the water receiver phase by a polymeric membrane (polypropylene) soaked in an organic solvent [*o*-nitrophenyl octyl ether (NPOE)]. The solvent polarity of NPOE was determined by using a solvatochromic dye[12] and was found to be $E_T^N = 0.33$, slightly different from the value $E_T^N = 0.31$ of dichloromethane found in the literature[12] and less polar than that of dichloromethane (distilled but not dry) used in our laboratory ($E_T^N = 0.34$).[13]

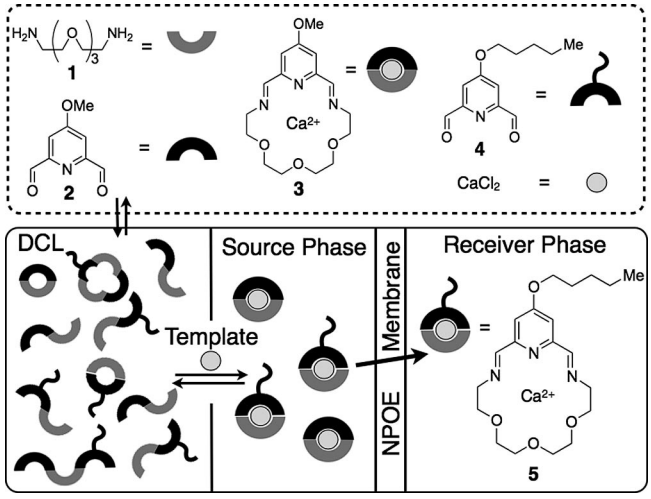


Figure 1. Building blocks (**1**, **2** and **4**), detected products (**3** and **5**) used and their cartoon representations. Setup of the transport experiment across a supported liquid membrane (SLM) facilitated by a dynamic combinatorial library (DCL).

The two water phases were gently stirred, and the conductivity in the receiver phase was recorded with a conductivity meter. Calcium chloride was chosen for its low solubility in organic solvents compared with other salts (e.g., calcium nitrates or picrates are, as a result of the counterions, more lipophilic and thus more soluble in organic media).[14] Owing to the lipophobicity of calcium chloride, the control experiment with a solution of calcium chloride in the source phase showed that hardly any calcium ions pass across the membrane (Figure 2d; conductivity after 48 h: 0.25 $\mu S\,cm^{-1}$).

In contrast, when the two building blocks **1** and **2** were dissolved, equilibrated for 1 h and then used as the source phase, the imino macrocycle **3** that formed was able to transport calcium (conductivity after 48 h: 1.02 $\mu S\,cm^{-1}$; Figure 2b). In two separate control experiments, the building blocks were dissolved separately in the absence of the other building block in calcium solution (source phase). Although the two building blocks interact with the supported liquid membrane carrying the calcium to the receiver phase, the sum of their single transport activities is less than that of the macrocycle **3** (conductivity after 48 h: 0.86 $\mu S\,cm^{-1}$; Figure 2c).

To improve the transport activity of the macrocycle, a lipophilic dialdehyde was synthesized. Dialdehyde **4** was obtained in three steps from commercially available chelidamic acid (**6**; Scheme 1). The latter was treated with 1-pentanol in the presence of a catalytic amount of sulfuric acid to give the 4-pentoxy-substituted diester **7**. After reduction with sodium borohydride, alcohol **8** was obtained. This alcohol is a bench-stable product and can be stored at room temperature without any decomposition. A fast oxidation with selenium dioxide gave the desired dialdehyde **4** ready to be used after a simple purification.

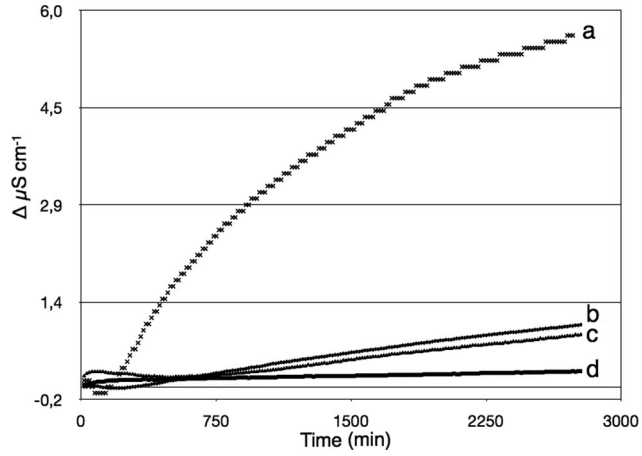
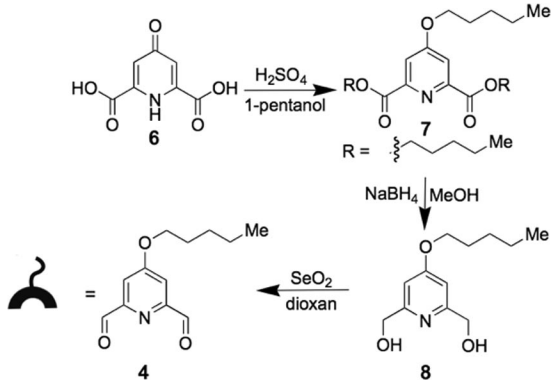


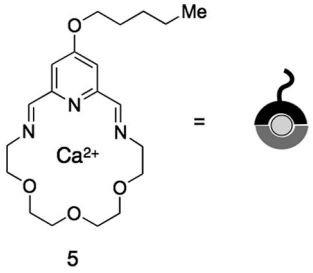
Figure 2. Transport of calcium ions through an SLM by using dialdehyde **2** or **4** and diamine **1**. The conductivity in the receiver phase is plotted against time. In all experiments, the water source phase consisted of: 10 mM solution of $CaCl_2$ with: (a) 0.1 mmol of **1** and 0.1 mmol of **4**, (b) 0.1 mmol of **1** and 0.1 mmol of **2**, (c) the calculated sum of the conductivities of **1** (0.1 mmol) and **2** (0.1 mmol) from two separate experiments in which they were dissolved separately in the absence of the other building block, (d) no addition of building blocks.



Scheme 1. Synthesis of dialdehyde **4** and its cartoon representation.

The two dialdehydes **2** and **4** differ only in the pentyl chain at the 4-position of the pyridine. Thus, the formation of the imino macrocycle **5** (Figure 3) in the presence of calcium ions as the template and its complexing ability should be only marginally different from the imino macrocycle **3**.[8,10] As proof of the formation of the imino macrocycle **5**, the DCL composed of diamine **1** and dialdehyde **4** was screened by ^1H NMR spectroscopy in the presence and absence of calcium chloride (by using CD_3OD and H_2O/D_2O as solvent). When calcium was present, only one peak in the imine region (δ = 8.64 ppm) and one in the pyridine

region (δ = 7.55 ppm) could be detected in the NMR spectrum (see the Supporting Information).[15] Subsequently, the above-described SLM experiment was repeated by using dialdehyde **4** instead of **2**. Figure 2a clearly shows that the calcium transport activity of macrocycle **5** is approximately six times higher than that of macrocycle **3** (conductivity after 48 h: 5.70 $\mu S\,cm^{-1}$). The shape of this curve (Figure 2a) is not linear like those of the previous transport experiments (Figure 2b–d); however, the nonlinearity of transport curves has been observed before and has been discussed and explained as decomplexation rate-limited transport.[16,17]



5

Figure 3. Macrocycle **5** and its cartoon representation.

In addition to the conductivity measurements, the presence of calcium ions in the receiver phase was also verified by using a titration method (Aquamerck® Calcium Test). Although close to the detection limit (2 $mg\,L^{-1}$), this test revealed the presence of calcium ions in the receiver phase.

A control experiment with dialdehyde **4** alone dissolved in the calcium source solution was not possible due to its low solubility in water. However, when the dialdehyde **4** was dissolved in NPOE it did not show any carrier activity.

Experiments with magnesium and barium chloride were also carried out. A 10 mM solution of magnesium chloride was not able to dissolve the building blocks, and it was impossible to use the milky solution as the source phase. With a 10 mM solution of barium chloride, the solution became clear and an increase in conductivity in the receiver phase could be measured. In this case, the conductivity after 48 h was 2.33 $\mu S\,cm^{-1}$. This is less than when calcium is the ion in the source phase, which indicates a small preference of the imino macrocycle **5** to transport calcium over barium.[18,19]

In another experiment, a dynamic library composed of dialdehydes **2** and **4** (4 mM each) and 2 equiv. of diamine **1** was generated in a 12.5 mM solution of calcium chloride. The DCL formed was used as the source phase in a DCC-SLM experiment. The receiver phase, monitored by a conductivity meter, showed a flux comparable to the above experiment with only macrocycle **5** carrying calcium. The source phase was screened by ^1H NMR spectroscopy at intervals by analysing 550 µL samples of the source phase to which 50 µL of D_2O was added. The water residue peak in the NMR spectra was removed during acquisition by using arbitrary waveforms and a pulsed field gradient.[21] In the resulting spectra, two easily distinguishable peaks partially overlap in the imine region and two well-separated peaks for the pyridine hydrogen atoms could be easily assigned to the macrocycles **3** (δ = 7.35 ppm) and **5** (δ = 7.32 ppm) (Figure 4b). The ratio **3/5** could be calculated at different times from the integration of the respective peaks (Figure 4c). In Figure 4a, the ratio of **3/5** is plotted versus time and shows that macrocycle **5** is leaving the source phase. In a control experiment in which the solution containing the two macrocycles **3** and **5** was not used as the source phase, the ratio between the two macrocycles remained constant over time [Figure 4a (O)]. The shape of the + curve in the source phase is similar to the shape of the conductivity curve in the receiver phase (as an example see Figure 2a). Thus, two different detection methods, NMR in the source phase for macrocycles and conductivity in the receiver phase for the calcium complexes, prove the same fact: macrocycle **5** carrying calcium leaves the source phase for the receiver phase. Starting from a DCL of three building blocks and one template ion, two macrocycles are selected and amplified from the mixture of macrocycles, oligomers and polymers due to their calcium complex stabilities, and one shows a better transport activity than the other.

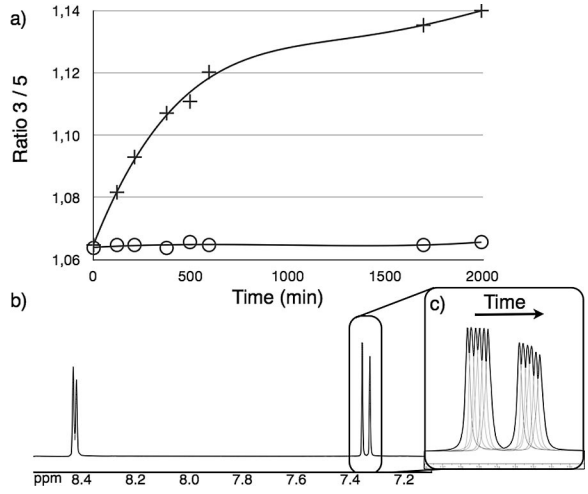


Figure 4. (a) Ratio of **3/5** vs. time (+) calculated from the integration of the pyridine peaks at δ = 7.35 and 7.32 ppm. (O): Control experiment (see text). (b) ^1H NMR spectrum of the water source phase. (c) Expanded section of the peaks at δ = 7.35 and 7.32 ppm over time.

After these results, we carried out some preliminary experiments to explore the possibility of screening for a carrier mediated by a DCL with a biologically important membrane. In this regard, liposomes are widely used as cell membrane mimics. Our approach was to use unilamellar phospholipid vesicles (1-palmitoyl-2-oleoyl-sn-*glycero*-3-phosphocholine, POPC) loaded with sodium chloride and dispersed in a sodium nitrate solution. The incorporation and movement of macrocycle **5** through the phospholipid membrane could promote counterion transport driven by the concentration gradient in a symport mechanism. Chloride release from the interior of the vesicles to the external medium can be easily monitored by using a chloride-selective electrode.[22]

Briefly, POPC vesicles loaded with NaCl were suspended in an $NaNO_3$ solution for a final lipid concentration of 1 mM. To this dispersion, 5 mol-% (calculated as building blocks/POPC) of the dynamic combinatorial library composed of diamine **1** and dialdehyde **4** previously equilibrated in a solution of $Ca(NO_3)_2$ was added, and the chloride ion efflux was monitored by a chloride ion selective electrode (ISE) over time. After 10 min, the vesicles were lysed by the addition of detergent and the final reading of the electrode was used to calibrate 100% release of chloride. For comparison purposes, control experiments with similar amounts of $Ca(NO_3)_2$ solution without any of the library components or just one of them, either **1** or **4**, were carried out. The results obtained are shown in Figure 5.

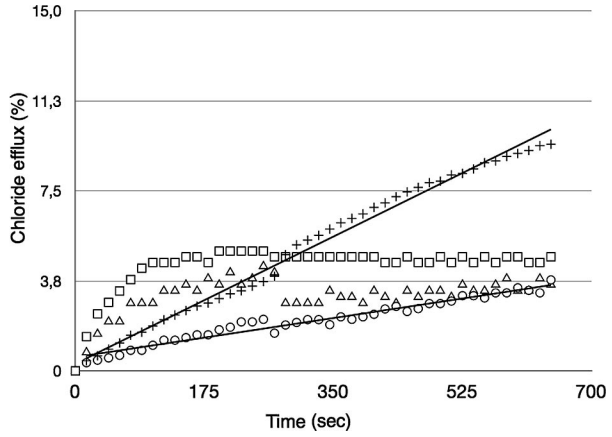


Figure 5. Chloride efflux from 100 nm POPC liposomes promoted by the addition of 40 μL of a 488 mM $Ca(NO_3)_2$ solution (O), 5 mol-% (per POPC) of dialdehyde **4** in 10 μL of DMSO (Δ), 5 mol-% (per POPC) of diamine **1** in 40 μL of a 488 mM $Ca(NO_3)_2$ solution (□) and 5 mol-% (per POPC) of the dynamic combinatorial library composed of diamine **1** and dialdehyde **4** in 40 μL of a 488 mM $Ca(NO_3)_2$ solution (+). The liposomes (1 mM POPC) contained NaCl (488 mM) and were immersed in $NaNO_3$ (488 mM) in a 5 mM phosphate buffer (pH = 7.2). 100% efflux was always achieved by destroying the liposomes upon addition of detergent. The straight lines through the data points are only drawn to guide the reader's eye.

The addition of dialdehyde **4** or diamine **1** (Figure 5; **4**: Δ, **1**: □) induced a fast initial chloride efflux that quickly reached a plateau. This result could be due to a small initial detergent effect exerted by these compounds. Nevertheless, the plateau shows that after the initial phase, the remaining liposomes are stable in the presence of starting materials for the DCL. Addition of both compounds **1** and **4**, that is, the library and carrier, resulted in a small yet constant chloride efflux from the liposomes (Figure 5; +). This efflux is more than twice as fast as in the blank experiment in which only the same aliquot of $Ca(NO_3)_2$ solution was added (Figure 5; O). Although the overall chloride efflux observed in these experiments is quite limited it must be stressed that macrocycle **5** has no affinity at all for anions, and therefore an efficient chloride transport was not expected. On the other hand, it seems clear that the addition of the carrier induces a significantly faster chloride efflux compared with those of the control experiments. This result shows promise for the development of dynamic combinatorial libraries for ion-pair transporters and the use of this approach in biomimetic environments.

Conclusions

The concept of using a DCL to find a matching carrier for a particle to be transported across a membrane (here: calcium ions) has been proven. From a DCL consisting of building blocks of varying lipo- and hydrophilicity, calcium ions select the appropriate host (**5**) for transport across a supported liquid membrane. In contrast to most DCC experiments, in this case the amplification process is dual: first, calcium ions amplify the formation of matching macrocycles **3** and **5** from the dynamic mixture of oligomers, polymers and hosts, and then the system selects complex **5**·Ca^{2+} over complex **3**·Ca^{2+} for the transport process due to the better balance of lipo- and hydrophilicity of **5**. Complex **5**·Ca^{2+} bears a long lipophilic chain but is still water-soluble and is able to pass through the NPOE membrane, thus transporting calcium ions. In future experiments, pyridinedicarbaldehydes such as **2** or **4** shall be mixed with diamines of varying length to find suitable carriers for different cations in this kind of double selection experiment. The DCC-SLM experiment described herein could also be used to analyse other complex DCL mixtures. The fittest species will cross the membrane and can then easily be detected in the receiver phase, separated from all other components of the library.

Preliminary experiments show that this methodology can also be used to screen the ability of carriers to cross phospholipid membranes. This is the first experiment on the way to the direct use of a DCL in a biomimetic system. The methodology of selecting a carrier from a dynamic combinatorial library therefore is very versatile: we started by screening its carrier ability with a bulk membrane[8] that has a thickness of centimetres, next we moved to a supported liquid membrane possessing a thickness of millimetres, and with the last experiment we used liposomes, which possess a thickness of nanometres.

Experimental Section

Remarks: *Trace*SELECT® water was obtained commercially from Fluka and was used for the source phase solution as well as for the receiver phase in the transport experiment. *o*-Nitrophenyl octyl ether (NPOE) was obtained from Aldrich and purified as described below before use in transport experiments. Polypropylene Accurel® PP was obtained from AkzoNobel. Aquamerck® Calcium Test was obtained from Merck; for its use, see ref.[8] 3,6,9-Trioxa-1,11-undecanediamine (**1**) and 4-methoxypyridine-2,6-dicarbaldehyde (**2**) were synthesized according to the literature.[23] Chelidamic acid (**6**) was synthesized by standard procedures or obtained from Aldrich. For a complete characterization of the imino macrocycle **3**, see ref.[10] An LF 340 conductimeter from WTW was used with automatic data storage (15 min) and non-linear compensation of the temperature set to 20 °C. During the transport experiments, the

temperature varied from 19 to 21 °C. An LR 325/01 probe (WTW) was used with a cell constant of 0.100 cm^{-1}. The molar conductance of calcium chloride is 271.56×10^{-4} $m^2 S mol^{-1}$ and 279.82×10^{-4} $m^2 S mol^{-1}$ for barium chloride.[20] The glassware (quartz) used for the transport experiment had a half-cell volume of 25 mL, a section of 8.553×10^{-4} m^2 and is described in the Supporting Information. NMR spectra were recorded with a Bruker DRX 500 or AV 600 instrument. Assignments are supported by COSY, HSQC and HMBC experiments. All chemical shifts are referenced to TMS or to the residual proton or carbon signal of the solvent (CD_3OD: ^1H: δ = 3.35 ppm, ^{13}C: δ = 49.0 ppm). Mass spectra were recorded with a Finnigan MAT 8200 or MAT 8230 spectrometer. ESI mass spectra were recorded with an Applied Biosystems Mariner Spectrometry Workstation. IR spectra were recorded with a Perkin–Elmer Paragon 1000 spectrometer equipped with an ATR unit. Elemental analyses were carried out with a EuroEA 3000 Elemental Analyzer from Euro Vector.

Transport Experiments: All glassware was washed with bidistilled water and rinsed with *Trace*SELECT® water before use. The experiments were repeated at least twice and also with different concentrations in the source phase (10 or 12.5 mM calcium chloride) to prove the reproducibility of the experiments.

Membrane: Commercial NPOE was purified by short-column chromatography [1:5 NPOE/silica gel (w/w), dichloromethane]. The solvent was removed under reduced pressure, and the oily residue was left under vacuum in an ultrasonic bath for 12 h before use. A square 4.5 × 4.5 cm of micropore polypropylene polymer Accurel® PP was cut and soaked in 300 mg of NPOE. The membrane was left under vacuum in a desiccator for 12 h before use.

Source Phase: Dialdehyde **2** or **4** (100 μmol) and 3,6,9-trioxa-1,11-undecanediamine (**1**; 19.1 mg, 100 μmol) were dissolved in a 10 mM calcium chloride solution (25 mL, *Trace*SELECT® water). The solution was vigorously stirred until complete dissolution of the building blocks. When the solution became clear (from 30 min to 2 h depending on the dialdehyde) it was used as source phase in the experiment.

Receiver Phase: *Trace*SELECT® water (25 mL), blank conductivity between 1 and 1.5 μS.

DCL Transport Experiment: The membrane was prepared as described above.

Source Phase: 4-Methoxypyridine-2,6-dicarbaldehyde (**2**; 16.5 mg, 100 μmol), 4-pentoxy-2,6-dicarbaldehyde (**4**; 22.1 mg, 100 μmol) and 3,6,9-trioxa-1,11-undecanediamine (**1**; 38.2 mg, 200 μmol) were dissolved in 12.5 mM calcium chloride solution (25 mL, *Trace*SELECT® water). The solution was vigorously stirred until complete dissolution of the building blocks. When the solution became clear it was used as the source phase in the experiment. An aliquot (550 μL) from the source phase was taken and mixed with D_2O (50 μL). The resulting solution was placed in an NMR tube, and ^1H NMR spectra were recorded at different times as the control experiment. At different times, aliquots (550 μL) were taken from both phases (source and receiver), the aliquot from the source phase was mixed with D_2O (50 μL), placed in an NMR tube, and the ^1H NMR spectra were recorded.

Receiver Phase: The receiver phase was set up as described above.

Preparation of Phospholipid Vesicles: The solvent of a solution of 1-palmitoyl-2-oleoyl-sn-*glycero*-3-phosphocholine (POPC, Genzyme) in chloroform (20 mg/mL) was evaporated to leave a lipid film. This was dried under vacuum for 12 h. The lipid film was rehydrated with a solution of sodium chloride (488 mM of NaCl, 5 mM of phosphate buffer, pH = 7.2) and shaken by vortex. The suspension was then subjected to nine freeze-thaw cycles and 29 extrusions through a 100 nm polycarbonate Nucleopore membrane by using a LiposoFast Basic extruder (Avestin) to obtain unilamellar vesicles with a mean diameter of 100 nm. Finally, the suspension was dialysed against an $NaNO_3$ solution (488 mM $NaNO_3$ and 5 mM phosphate buffer, pH = 7.2) to remove unencapsulated NaCl.

Ion-Selective Electrode Transport Assays: Unilamellar vesicles (POPC, 100 nm mean diameter) prepared as described above were suspended in a solution of $NaNO_3$ (488 mM, phosphate buffer, 5 mM, pH = 7.2) for a final POPC concentration of 1 mM. The dynamic combinatorial library composed of dialdehyde **4** and diamine **1** (6.25 mM each) was equilibrated in a solution of $Ca(NO_3)_2$ (488 mM, phosphate buffer, 5 mM, pH = 7.2) for 12 h before use. This solution (40 μL) was added to the vesicle suspension (5 mL, resulting in 5 mol-% of library to POPC), and the release of chloride ions was monitored by using an Accumet chloride-selective electrode. After 10 min, the vesicles were lysed by addition of detergent (Triton-X) to release all chloride ions (by definition giving 100% conductivity). The blank experiments with the $Ca(NO_3)_2$ solution and diamine **1** were carried out in the same way. Owing to the low solubility of dialdehyde **4**, it was dissolved (25 mM) in DMSO and this solution (10 μL, corresponding to 5 mol-% relative to POPC) was added to the vesicle suspension (5 mL). The experiment was repeated three times, and the average of the chloride efflux was plotted over time.

Syntheses

Dipentyl 4-Pentoxypyridine-2,6-dicarboxylate (7): Chelidamic acid (**6**; 500 mg, 3.62 mmol) was dissolved in 1-pentanol (30 mL), and concentrated sulfuric acid (0.2 mL) was added. The solution was attached to a Dean–Stark trap and heated at reflux while stirring for 4 h. Then the solvent was removed under reduced pressure, and the residue was dissolved in chloroform and washed with water (2 × 20 mL). The organic phase was dried with $MgSO_4$. Chloroform was removed under reduced pressure, and the oily residue was purified by column chromatography (silica gel, chloroform) to give 869 mg (2.21 mmol, 61%) of the final product **7** as a colourless oil. R_f ($CHCl_3$) = 0.12. IR: $\tilde{\nu}$ = 2953 (alkyl), 1716 (ester), 1591, 1442 (arom.) cm^{-1}. ^1H NMR (500 MHz, 298 K, $CDCl_3$, TMS): δ = 7.74 (s, 2 H, Py), 4.39 (t, J = 7 Hz, 4 H, $COOCH_2$), 4.12 (t, J = 6.5 Hz, 4 H, OCH_2), 1.87–1.80 (m, 6 H, OCH_2CH_2 and $COOCH_2CH_2$), 1.48–1.36 (m, 12 H, $CH_2CH_2CH_3$), 0.96–0.91 (m, 9 H, CH_3) ppm. ^{13}C NMR (125 MHz, 298 K, $CDCl_3$, TMS): δ = 166.98 (C-4, Py), 164.89 (C-2,6, Py), 150.21 (*C*OO), 114.22 (C-3,5, Py), 68.99 (OCH_2), 66.39 ($COOCH_2$), 28.45, 28.24, 28.04, 27.99, 22.36, 22.34 ($CH_2CH_2CH_2$), 13.96 (CH_3) ppm. MS (CI, isobutane): calcd. for $C_{22}H_{35}NO_5$ 393.25; found 394.0 $[M + H]^+$. $C_{22}H_{35}NO_5$ (393.25): C 67.15, H 8.96, N 3.56; found C 66.98, H 9.24, N 3.51.

4-Pentoxypyridine-2,6-bis(methanol) (8): Dipentyl 4-pentoxypyridine-2,6-dicarboxylate (**7**; 800 mg, 2.05 mmol) was dissolved in dry methanol (15 mL). $NaBH_4$ (1.5 g, 41 mmol) was slowly added to the solution at 0 °C. When the hydride had completely dissolved, the reaction mixture was heated at reflux for 4 h. Then at room temperature, a saturated aqueous solution of Na_2CO_3 (5 mL) was added, and the solution was heated at reflux for an additional 30 min. The solvent was removed under reduced pressure, and the residue was dissolved in $CHCl_3$ and washed with water (2 × 15 mL). The organic phase was dried with $MgSO_4$, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel and eluting with $CHCl_3$/MeOH (96:4, v/v) as eluent. Alcohol **8** was obtained pure as a white solid. Yield: 424 mg (1.88 mmol, 92%). R_f ($CHCl_3$/

MeOH, 96:4) = 0.20. M.p. 82–83 °C. IR: $\tilde{\nu}$ = 3341 (OH), 2973 (alkyl), 1597, 1496 (arom.) cm^{-1}. ^1H NMR (500 MHz, 298 K, $CDCl_3$,TMS): δ = 6.63 (s, 2 H, Py), 4.62 (s, 4 H, CH_2OH), 3.95 (t, J = 6.5 Hz, 2 H, OCH_2), 3.65 (br., 2 H, OH), 1.72 (quint, J = 7.2 Hz, 2 H, OCHCH_2CH$_2$), 1.39–1.28 (m, 4 H, CH_2CH_2CH$_3$), 0.86 (t, J = 7.1 Hz, 3 H, CH$_3$) ppm. ^{13}C NMR (125 MHz, 298 K, $CDCl_3$, TMS): δ = 165.77 (C-4, Py), 159.09 (C-2,6, Py), 104.67 (C-3,5, Py), 67.31 (OCH_2), 63.26 (CH_2OH), 27.52 (OCH$_2$$CH_2$), 27.00 (OCH$_2CH_2$$CH_2$), 21.34 ($CH_2CH_3$), 12.95 (CH$_3$) ppm. MS (ESI): calcd. for $C_{12}H_{19}NO_3$ 225.14; found 226.14 [M + H$^+$], 248.12 [M + Na$^+$]. $C_{12}H_{19}NO_3$ (225.14): C 63.98, H 8.50, N 6.22; found C 64.18, H 8.86, N 6.04.

4-Pentoxypyridine-2,6-dicarbaldehyde (4): 4-Pentoxypyridine-2,6-bis(methanol) (**8**; 424 mg, 1.88 mmol) was dissolved in dry dioxane (10 mL), and SeO_2 (207 mg, 1.88 mmol) was added. The solution was heated at reflux while stirring for 6 h. Then the heterogeneous solution was passed through a pad of Celite and eluted with a small portion of dioxane. The solution was recovered, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography [silica gel, cyclohexane/ethyl acetate (1:1, v/v)] to give a slightly yellow solid. Yield: 376 mg (1.70 mmol, 90%). R_f (cyclohexane/ethyl acetate, 1:1) = 0.25. M.p. 67–68 °C. IR: $\tilde{\nu}$ = 2931 (alkyl), 1704 (C=O), 1591, 1448 (arom.) cm^{-1}. ^1H NMR (500 MHz, 298 K, $CDCl_3$, TMS): δ = 10.11 (s, 2 H, CHO), 7.63 (s, 2 H, Py), 4.14 (t, J = 6.5 Hz, 2 H, OCH$_2$), 1.88–1.82 (m, 2 H, OCH$_2$CH_2), 1.47–1.39 (m, 4 H, CH_2CH_2CH$_3$), 0.94 (t, J = 7.0 Hz, 3 H, CH$_3$) ppm. ^{13}C NMR (125 MHz, 298 K, $CDCl_3$, TMS): δ = 192.46 (CHO), 167.14 (C-4, Py), 154.72 (C-2,6, Py), 111.50 (C-3,5, Py), 69.34 (OCH_2), 28.37 (OCH$_2$$CH_2$), 27.94 (OCH$_2CH_2$$CH_2$), 22.32 ($CH_2CH_3$), 13.95 (CH$_3$) ppm. MS (ESI): calcd. for $C_{12}H_{15}NO_3$ + Na$^+$ 244.09; found 244.09 [M + Na$^+$]. $C_{12}H_{15}NO_3$ (221.10): C 65.14, H 6.83, N 6.33; found C 64.99, H 7.04, N 6.10.

1^4-Pentoxy-6,9,12-trioxa-3,15-diaza-1(2,6)-pyridinahexadecacyclophan-2,15-diene (5): 4-Pentoxypyridine-2,6-dicarbaldehyde (**4**; 11.0 mg, 50 μmol), 3,6,9-trioxa-1,11-undecanediamine (**1**; 11.5 mg, 60 μmol) and $CaCl_2$ (5.5 mg, 50 μmol) were dissolved in CD_3OD (10 mL). After 12 h, the solution was analysed by NMR, ESI MS and HR-MS. ^1H NMR (500 MHz, 298 K, CD_3OD): δ = 8.64 (t, J = 1.3 Hz, 2 H, CH=N), 7.55 (s, 2 H, Py), 4.31 (t, J = 6.4 Hz, 2 H, 4-OCH_2CH$_2$), 4.05 (t, J = 4.6 Hz, 4 H, CH=NCH_2), 3.96–3.90 (m, 12 H, CH=NCH$_2$CH_2 and OCH_2CH_2O), 1.94–1.89 (m, 2 H, 4-OCH$_2$CH_2), 1.56–1.44 (m, 4 H, CH_2CH_2CH$_3$), 1.00 (t, J = 7.2 Hz, 3 H, CH$_3$) ppm. ^{13}C NMR (125 MHz, 298 K, CD_3OD): δ = 170.47 (C-2,6, Py), 164.70 (CH=N), 154.40 (C-4, Py), 115.46 (C-3,5, Py), 72.52, 71.39, 79.83, 70.03 (CH_2OCH_2 and CH=NCH_2), 58.25 (Py-OCH_2), 29.55 (Py-OCH$_2$$CH_2$), 29.13 (Py-OCH$_2CH_2$$CH_2$), 23.40 ($CH_2CH_3$), 14.32 (CH$_3$) ppm. MS (ESI): calcd. for $C_{20}H_{31}N_3O_4$ + Na$^+$ 400.21; found 400.22 [M + Na$^+$]. HR-MS (EI, 70 eV): calcd. for $C_{20}H_{31}N_3O_4$ 377.23145, found 377.23146; calcd. for $C_{19}{}^{13}CH_{31}N_3O_4$ 378.23480, found 378.23545.

Supporting Information (see footnote on the first page of this article): Glassware used for the experiments, ^1H and ^{13}C NMR spectra of **4**, **5**, **7** and **8**, ^1H NMR spectra of the DCL of **4** and **1** in the presence and absence of $CaCl_2$, ^1H NMR spectra (ratio/time) of the DCC-SLM source phase and COSY of **5**.

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