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Air-water interfacial behavior of amphiphilic peptide analogs of synthetic chloride ion transporters

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Abstract

A family of heptapeptide-based chloride transporters (called synthetic anion transporters, SATs) were designed to insert into phospholipid membrane bilayers and form pores. Many of these compounds have proved to be chloride selective transporters. The transporters were designed to incorporate hydrophilic heptapeptides that could serve as headgroups and hydrocarbon tails that could serve as hydrophobic membrane anchors. Insertion of the SAT molecules into a bilayer requires approach to and insertion at the aqueous-membrane surface. The studies reported here were conducted to model and understand this process by studying SAT behavior at the air-water interface. A Langmuir trough was used to obtain surface pressure-area isotherm data. These data for amphiphilic SATs were augmented by Brewster angle microscopy (BAM), molecular modeling, and calculations of the hydrophobicity parameter $\log P$. The analyses showed that the heptapeptide (hydrophilic) module of the SAT molecule rested on the water surface while the dialkyl (hydrophobic) tails oriented themselves in the air, perpendicular to the water surface. Brewster angle microscopy visually confirmed a high order of molecular organization. Results from these studies are consistent with the previously proposed mechanism of SAT membrane insertion and pore formation.

Keywords

Chloride transporter; Langmuir trough; Brewster angle microscopy; Molecular monolayer; Amphiphile

1. Introduction

Transport of cations and anions through cellular phospholipid bilayer membranes is critical to maintain the vitality of most organisms [1]. The proteins that transport Na^+ , K^+ , Ca^{2+} , and Cl^- , are complex and quite different from each other. During recent years, we [2,3] and others [4-7] have developed synthetic models for ion channels that accomplish many of the functions of their natural counterparts, although they are typically less selective and/or efficient. We

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termed the family of Na⁺-selective transporters that we developed “hydraphiles” [8]. These are typically *tris*(crown) compounds designed to span a bilayer and conduct a cation through the bilayer by forming a uni-molecular pore [9]. Although the hydraphiles were designed and synthesized before the first cation channel structure [10] was known, the similarity in structural features is striking.

The remarkable complexity [11] of the CIC family of Cl⁻ transporting proteins [12,13] has presented an equally daunting challenge. Our approach [14,15] to this problem was to prepare an amphiphilic peptide of the general form (C₁₈H₃₇)₂N-COCH₂OCH₂CO-(Gly)₃-Pro-(Gly)₃-OR¹ that emulated a phospholipid monomer and incorporated four structural modules. A dialkylamine residue, typically *bis*(octadecyl)amine, mimicked the twin fatty acid chains typically present in a phospholipid [16]. The lipid’s glyceryl unit (mid-polar regime) was approximated first by diglycolic acid [17]. This diacid possesses three oxygens in locations similar to those of glycerol and is readily converted from its anhydride form to the dialkylamide monoacid R₂NCOCH₂OCH₂COOH in a single step. The third module is the peptide [18,19]. Our first studies used ~-(Gly)₃-Pro-(Gly)₃~ because the CIC family proteins have a conserved G-X-X-P unit within their presumed ion conduction pathway [20]. Finally, the peptide’s C-terminal carboxyl was protected either as an ester or amide. The latter residue often served as an additional membrane anchor [21].

The first compound in this family, (C₁₈H₃₇)₂N-COCH₂OCH₂CO-(Gly)₃-Pro-(Gly)₃-OCH₂Ph, mediated the release of Cl⁻ or carboxyfluorescein from phospholipid vesicles. Its proposed mechanism of membrane insertion is depicted in Fig. 1. Biophysical studies revealed that these synthetic anion transporters (SATs) were at least 10-fold selective for Cl⁻ over K⁺ and that the pores they formed were typically dimers [22]. Despite extensive structural modifications in conjunction with transport studies, many questions remain. Two points are of critical interest to us. First, what part, module, or fragment of the SAT is hydrophilic and which portion(s) is hydrophobic? It seems obvious that when the dialkyl chains are octadecyl this module is hydrophobic. When the alkyl groups are hexyl and the C-terminal anchor is heptyl or decyl, the clarity fades. Second, when the SAT inserts into the bilayer, what portion, fragment, or module remains at or near the bilayer-aqueous interface? We therefore decided to assess the surface interactions of SATs and several close relatives by measurement of surface pressure-area isotherms in a Langmuir trough. This effort was augmented by observing the monolayers using a Brewster angle microscope (BAM).

A systematically varied family of compounds related to **1** was prepared, and their behavior at the air-water interface was examined. The hydrocarbon chains were held constant in compounds **1-5** to investigate the hydrophilicity of the heptapeptide pore-forming moiety. Surface pressure-area isotherms, BAM, molecular modeling, and the hydrophobicity parameter log *P* were utilized to investigate the surface behavior of synthetic anion transporters.

2. Results and discussion

2.1. Compounds studied

Five compounds were used in the present study. They are shown as **1-5** in Fig. 2. Compounds **1-5** possess twin octadecyl groups at the N-terminal end of the molecule. The peptide chain was shortened from ~-(Gly)₃-Pro-(Gly)₃~ (**1**) to ~-(Gly)₃~ in **2** and to a single glycine in **3**. Compounds **4** and **5** possess no amino acids and may be represented as (C₁₈H₃₇)₂N-COCH₂OCH₂COX in which X = OH (**4**) or OCH₂Ph (**5**).

2.2. Surface pressure-area isotherm data

When an amphiphile is spread on water at low surface concentration, the initial molecular organization is random. As the barrier is drawn across the trough, the surface area available to the amphiphiles is reduced and the molecules are pushed closer together. The surface pressure (π) will increase, provided the molecules remain on the surface and do not form bulk aggregates. As the pressure increases and the amphiphiles are compressed into a smaller area, the molecular organization increases as molecules make contact and begin to interact. An increase in pressure (in mN m^{-1} on the ordinate) is observed as the mean molecular area (abscissa) decreases. An inflection point signals the transition from one phase (type) of organization to another. The first such inflection is typically from the liquid-expanded phase to the liquid-condensed phase. As surface area is further reduced, a point is reached at which structural collapse occurs and molecules are forced out of the two-dimensional plane.

In the studies described here, compounds **1-5** were individually spread on MilliQ® (>18 M) purified water in a commercial Langmuir trough (NIMA Technology) [23]. Fig. 3 shows data for **1** spread on pure water (solid gray line). Initially, the surface pressure is near zero. As the area per molecule is reduced to $\sim 165 \text{ \AA}^2$, the surface pressure begins to rise as **1** enters the liquid-expanded phase. A change in curve shape (inflection point) at $74 \text{ \AA}^2$ suggests that **1** is no longer randomly distributed on the aqueous surface and that an ordered phase is beginning to form. In the region of the isotherm between $74 \text{ \AA}^2$ and $52 \text{ \AA}^2$, **1** is in the liquid-expanded + liquid-condensed coexistence region. After $52 \text{ \AA}^2$, the liquid condensed phase is compressed further until the monolayer becomes solid-like. The lateral pressure increases until an area of $40 \text{ \AA}^2$ is reached, whereupon the monolayer collapses.

Compounds **1-5** contain the $(\text{C}_{18}\text{H}_{37})_2\text{N-COCH}_2\text{OCH}_2\text{COX}$ residue. Compounds **1-4** are benzyl esters and **5** is the free acid ($\text{X} = \text{OH}$). Although compound **5** is unique in this series because it is an acid, it is otherwise identical to **4**, its benzyl ester. Compounds **1-3** contain the $(\text{C}_{18}\text{H}_{37})_2\text{N-COCH}_2\text{OCH}_2\text{COX}$ subunit as well, but they are extended on the C-terminal side by $(\text{Gly})_3\text{-Pro-(Gly)}_3$ (**1**), triglycine (**2**), or glycine (**3**). Compounds **1-4** all possess C-terminal benzyl esters. In **1-5**, the hydrophobic dioctadecylamine tails are connected to the hydrophilic “headgroup” by a diglycolic acid residue. The isotherm data recorded for **1-5** (Fig. 3) show that as the polar head-group size increases, the corresponding isotherm displays an increase in the molecular area at which the surface pressure begins to rise. This initial rise is attributed to the monolayer phase with the least order. For compounds **1-4**, the area at which the surface pressure first rises represents the onset of the liquid-expanded phase. For compound **5**, the initial rise appears to represent direct onset of the liquid-condensed phase. There is also an increase in the pressure at the plateau region of the isotherms for **1-4** (liquid-condensed + liquid-expanded region) between $\sim 75 \text{ \AA}^2$ and $\sim 48 \text{ \AA}^2$ that seems to be proportional to the head-group size. A larger head-group size appears to postpone emergence of the liquid-condensed phase until higher surface pressures are reached.

The surface compression data shown in Fig. 3 are expressed numerically in Table 1. By doing so, we can more readily compare the particular inflections that appear on the graph. Each such inflection is the transition between one phase of surface molecular organization and another. For compounds **1-4**, the first inflection represents the area at which the monolayer enters the liquid-expanded phase. The second inflection represents entry into the liquid-expanded + liquid-condensed coexistence region. The third represents exit from this region. The fourth transition represents either an apparent transition between two different liquid-condensed phases or a transition to a solid phase observed only for compound **3**. For compound **5**, the first inflection represents direct entry into the liquid-condensed phase. Within each row of Table 1, a larger molecular area represents a less organized phase on the aqueous surface.

The first transition observed for **1-5** varies according to head-group size. As the size (area) of X in **1-5** increases [$\text{OH} < \text{OCH}_2\text{Ph} < \text{Gly-OCH}_2\text{Ph} < (\text{Gly})_3\text{-OCH}_2\text{Ph} < (\text{Gly})_3\text{-Pro-(Gly)}_3\text{-OCH}_2\text{Ph}$], the molecular area at the first transition also increases. Compounds **1-4** also show second and third inflections, which are the same within experimental error. Compounds **1-5** have the same collapse area of $\sim 36 \pm 4 \text{ \AA}^2$, which corresponds to the known molecular cross-section of two hydrocarbon chains [24]. It thus appears that **1-5** are closely packed with the hydrophobic chains oriented perpendicular to the water surface immediately prior to collapse.

2.3. Behavior of compound 5

Fig. 3 shows that the surface pressure-area isotherm of **5** differs from those of **1-4**. Compound **5** does not exhibit a liquid-expanded phase. The first inflection point observed for **5** appears at $54 \text{ \AA}^2$ and collapse occurs at $36 \text{ \AA}^2$. At this stage, the twin alkyl chains are presumably vertical with respect to the aqueous plane. Thus, only the most polar fragment of the structure is expected to remain in contact with water. The first level of organization, i.e., $54 \text{ \AA}^2$, suggests a surface area corresponding to a square about $7\text{-}7.5 \text{ \AA}$ on a side or a circle of about $8\text{-}8.5 \text{ \AA}$ in diameter.

We constructed space-filling (CPK) molecular models and also used a simple molecular modeling program to assess the molecular size of **5**. An energy minimized model of $(\text{CH}_3)_2\text{N-COCH}_2\text{OCH}_2\text{COOH}$ was created in the ChemDraw/Chem 3D program; it is shown in Fig. 4. We used a dimethyl, rather than a dioctadecyl, derivative to simplify the calculations. The compound's molecular dimensions were obtained by using X-seed [25] and the molecular model shown in Fig. 4 was rendered using Pov-Ray [26]. The physical and computational models were measured in all dimensions; the maximum distance values were $8.7 \text{ \AA}$ and $4.2 \text{ \AA}$. Assuming that these dimensions define a rectangle, the area is $36.5 \text{ \AA}^2$. This corresponds well with the area at the collapse pressure of **5**. We infer that this represents the minimum size of all diglycoylamide derivatives.

The initial inflection point for this molecule, $54 \text{ \AA}^2$, is about 1.5 times larger than is the area at collapse. Further, it corresponds to an inflection point, usually the penultimate one, observed for all five of the molecules studied here. This transition at $\sim 54 \text{ \AA}^2$ marks the point at which compounds **1-5** enter the liquid-condensed phase. Compound **5** can bind to water at the interface through hydrogen bonding interactions. Fig. 5 shows only some of the possible ways in which compound **5** can interact with individual water molecules. The hydrocarbon tails of **5** are omitted for clarity. Both of the hydrogens in water can H-bond to oxygen atoms in the diglycolylamide moiety. Additionally, any water oxygen may interact with the electron-poor amide nitrogen through sigma-donation of the lone pair electrons on oxygen. These interactions could cause an overall polarization of the diglycolic acid headgroups, with resulting intermolecular repulsion between amphiphilic monomers. The transition at $54 \text{ \AA}^2$ seen in the isotherm may arise from this direct interaction between the diglycolic acid headgroup of **5** with the water subphase.

2.4. Pressure-area isotherm behavior of 1-3

Compounds **1-3** can all be described by the general formula $(\text{C}_{18}\text{H}_{37})_2\text{N-COCH}_2\text{OCH}_2\text{CO-(Aaa)}_n\text{-OCH}_2\text{Ph}$, where $(\text{Aaa})_n$ is glycine (**3**), triglycine (**2**), or $(\text{Gly})_3\text{-Pro-(Gly)}_3$ (**1**). All terminate in a benzyl ester and are linked to twin octadecyl chains by diglycolic acid. In all three cases, collapse is observed at an area of $36 \pm 4 \text{ \AA}^2$. Indeed, the collapse of **4**, which lacks any glycine, and **5**, which lacks both glycine and the benzyl ester, are within this range. As noted above, the polar diglycoyl residue occupies approximately this area with vertical, closely packed hydrophobic tails.

Simple molecular dynamics and energy minimization calculations conducted on $(\text{CH}_3)_2\text{N}-\text{COCH}_2\text{OCH}_2\text{CO}-\text{Gly}-\text{OCH}_2\text{Ph}$ (similar to **3**) and $(\text{CH}_3)_2\text{N}-\text{COCH}_2\text{OCH}_2\text{CO}-(\text{Gly})_3-\text{OCH}_2\text{Ph}$ (similar to **2**) suggested that both molecules can form compact structures. Fig. 6 shows space-filling representations of dimethyl (rather than dioctadecyl) derivatives of **2** and **3**. The monoglycyl analog of compound **3** is at the left and the triglycine analog of **2** is to the right. The structures are positioned so that the benzene rings are at the lower right in both cases.

In **3**, it is apparent that the N-terminal nitrogen is proximate to the benzene ring. To the extent that amide resonance renders the nitrogen positive, it may interact with the pi-electron rich benzene ring. A different interaction is apparent in the computed structure of **2**. In this case, the second amide NH hydrogen is pointed toward the benzene ring. The N-H...arene distance is ~ 3.6 Å. It is clear that the “height” of the two models is similar. The larger (like **2**, right) is almost circular and the measured distance across in any direction is 7-8 Å. A radius of 4 Å gives a circle with an area of ~ 50 Å², consistent with the molecular area at the third transition seen in the isotherm of **2**. The orientation of the two methyl groups, representative of the hydrophobic octadecyl chains, are turned toward the viewer in the structure shown for **2**.

2.5. Brewster angle microscopy

Fig. 3 shows that compounds **1-4** undergo several transitions before collapse. A main feature common to each isotherm is the plateau region between ~ 75 Å² and ~ 48 Å². To gain microscopic information about the surface behavior of these molecules, Brewster angle microscopy was performed at the air-water interface for compound **4**. Compound **4** shows well-defined, star-shaped domains at 48-50 Å² which is in the liquid-condensed + liquid-expanded coexistence region of the surface pressure-area isotherm (Fig. 7b-g). The bright, star-shaped domains are the ordered liquid-condensed regions. The observation of star-shaped domains indicates a well-ordered phase with significant lateral interactions between headgroups. The dark region between these bright domains is the less ordered liquid-expanded phase of the monolayer.

Beginning at 47 Å² (Fig. 7g), the bright domains coalesce to form a uniform liquid-condensed state (nearly featureless, not illustrated). At this point, no liquid expanded regions are seen. The different aggregation states illustrated correspond well with the pressure-area isotherm illustrated in panel (a) of Fig. 7. The value shown there for the phase transition to the condensed state is 46 Å². Upon further compression to 30 Å², disordered bright spots appear (Fig. 7h). Such disordered bright spots indicate three-dimensional aggregation and are characteristic of monolayer collapse.

2.6. Hydrophobic-hydrophilic balance

The N-terminal hydrocarbon anchor residues of **1-5** are obviously hydrophobic. In order to better understand the hydrophobic-hydrophilic balance, we used commercial programs to calculate log *P* values for various components of these amphiphiles. Log *P* is a hydrophobicity index that characterizes a compound's preference for the aqueous phase versus *n*-octanol. The latter is used as an approximation of phospholipid bilayer membrane polarity. Although the results obtained from several software programs were compared, the data discussed here were acquired by using the Internet version of A log *P* [27].

Log *P* values have both a sign (positive or negative) and a magnitude. A positive value means that the molecule is hydrophobic, while a negative number reflects hydrophilicity. Dioctadecylamine has a value of 10.51 (see Table 2), meaning that the molecule is quite hydrophobic. The hydrophilic diglycolic acid portion has a negative value of -1.66 or -1.18, depending on the substitution of the amidinitrogen. The most hydrophilic peptide fragment in Table 2 is GGGPGGG-OCH₂Ph; it has a log *P* value of -1.13. Compound **1** combines the

hydrophobic alkylamine, the hydrophilic diglycolyl fragment, and the hydrophilic peptide sequence resulting in an amphiphilic peptide.

2.7. Inferences concerning SAT organization

The hydrophobicity study immediately preceding confirms that the peptide functions as the headgroup in these amphiphilic molecules. The systematic size variations presented in Table 1 and Fig. 3 confirm that the peptide is always present at the air-water interface. We further infer that the air-water interface reflects the aqueous-bilayer interface and that the peptide module of the SAT forms the headgroup for these amphiphiles. The surface pressure isotherm data show a size trend (at the first transition) that accords nicely with the peptide or amino acid present in amphiphiles **1-3**. As the surface pressure increases, a minimum area of about 36 Å² is reached. Molecular modeling shows that this conformation is highly condensed and it is unlikely to reflect the entry portal of a Cl²⁻ conducting pore.

3. Conclusions

Surface pressure-area isotherm measurements of synthetic anion transporter (SAT) **1** and its truncated analogs (**2-5**) agree with molecular size values predicted by molecular modeling. As the head-group size varies from 0 to 7 amino acids in length, the isotherms show an increase in both molecular area at the first transition and a pressure increase in the liquid-expanded + liquid-condensed coexistence ("plateau") region. Molecular areas elucidated from the isotherms reveal that the peptide modules of the SAT are present on the aqueous surface. The combination of log *P* calculations, air-water interface studies using the Langmuir trough, and Brewster angle microscopy all demonstrate that these SAT molecules are amphiphiles. Compelling visual evidence for the changes in molecular organization were obtained by BAM and these correlate well with the surface pressure-area isotherms. Thus, the peptide sequence Gly-Gly-Gly-Pro-Gly-Gly-Gly of **1** comprises the hydrophilic headgroup while the dialkylamine moiety serves as the hydrophobic tail in this amphiphilic, pore-forming monomer.

4. Experimental

4.1. General

¹H NMR spectra were recorded at 300 MHz in CDCl₃ solvents and are reported in ppm (δ) downfield from internal (CH₃)₃Si. ¹³C NMR spectra were recorded at 75 MHz in CDCl₃. Melting points were determined using a Thomas Hoover apparatus in open capillaries and are uncorrected. Solvents were distilled prior to use, and all reagents were the best (non-LC) grade commercially available.

(C₁₈H₃₇)₂N-COCH₂OCH₂CO-(Gly)₃-Pro-(Gly)₃-OCH₂Ph, **1** was prepared as previously reported [28].

(C₁₈H₃₇)₂N-COCH₂OCH₂CO-(Gly)₃-OCH₂Ph, **2** was prepared as previously reported [28].

(C₁₈H₃₇)₂N-COCH₂OCH₂CO-Gly-OCH₂Ph, **3**. 18₂N-[DGA]-OH, **5**, (0.087 g, 0.14 mmol), glycine benzyl ester-TsOH (0.070 g, 0.21 mmol), and triethylamine (0.021 g, 0.21 mmol) were dissolved in 6 mL 1:1 THF-DMF solution and cooled to 0 °C. 1-(3-Dimethylaminopropyl)-3-ethyl carbodiimide-HCl (0.040 g, 0.21 mmol) was added. The mixture was stirred for 30 min. Ice bath was removed and the reaction was stirred at RT overnight. The solvent was removed *in vacuo*. Preparative TLC (ethyl acetate/hexane/DMF 4:4:1) and crystallization from methanol gave a white powder in 47% yield, mp 38-40 °C. ¹H NMR CDCl₃: 0.88 (6H, t, -CH₃), 1.25 (60H, *pseudo-s*, CH₃(CH₂)₁₅CH₂CH₂N-), 1.53 (6H, bs, CH₃(CH₂)₁₅CH₂CH₂N-+H₂O), 3.08 (2H, t, CH₃(CH₂)₁₆CH₂N-), 3.29 (2H, t, CH₃(CH₂)₁₆CH₂N-), 4.12 (2H, s, Gly-NCH₂), 4.14 (2H, s, -CH₂OCH₂-), 4.26 (2H, s, -CH₂OCH₂-), 5.19 (2H, s, -OCH₂Ph), 7.35 (5H, m, HAr),

8.18 (1H, t, -CONH-). ^{13}C NMR: 14.1, 22.7, 27.0, 27.6, 28.9, 29.4, 29.7, 31.9, 40.8, 46.3, 46.9, 67.0, 69.5, 71.6, 128.3, 128.4, 128.6, 168.2, 169.5, 170.2. High-resolution mass spectral analysis for $\text{C}_{49}\text{H}_{88}\text{N}_2\text{O}_5$. Theoretical: $[\text{MX}]^+$ 784.66931. Found: 784.66931.

(C₁₈H₃₇)₂N-COCH₂OCH₂COOCH₂Ph, 4. 18₂N-[DGA]-OH, **5**, (0.20 g, 0.31 mmol) and KOH (0.02 g, 0.3 mmol) were dissolved in hot methanol (30 mL) and stirred for 20 min at RT. Methanol was removed *in vacuo*. Toluene (20 mL), tetrabutylammonium bromide (0.02 g, 0.06 mmol), and benzyl bromide (75 μL , 0.62 mmol) were added to the white residue. The reaction mixture was heated to reflux under N₂ for 6 h. After cooling to RT, the mixture was washed with H₂O (25 mL) and saturated NaCl (25 mL), then dried over MgSO₄, filtered, and concentrated. Crystallization in methanol afforded a white powder (100.5 mg, 45%), mp 42–44 °C. ^1H NMR CDCl₃: 0.88 (6H, t, -CH₃), 1.25 (60H, *pseudo-s*, CH₃(CH₂)₁₅CH₂CH₂N-), 1.51 (4H, bs, CH₃(CH₂)₁₅CH₂CH₂N-), 3.17 (2H, t, CH₃(CH₂)₁₆CH₂N-), 3.28 (2H, t, CH₃(CH₂)₁₆CH₂N-), 4.30 (4H, s, -CH₂OCH₂-), 5.19 (2H, s, -OCH₂Ph), 7.35 (5H, m, HAr). ^{13}C NMR: 14.1, 22.7, 26.8, 27.0, 27.5, 28.9, 29.3, 29.5, 29.6, 29.7, 31.9, 45.8, 47.0, 66.6, 68.0, 69.4, 128.3, 128.4, 128.6, 135.4, 167.8, 170.0. High-resolution mass spectral analysis for $\text{C}_{47}\text{H}_{85}\text{NO}_4$. Theoretical: $[\text{MX}]^+$ 727.64783. Found: 727.64783.

(C₁₈H₃₇)₂N-COCH₂OCH₂COOH, 5 was prepared as previously reported [28].

4.2. Surface pressure-area isotherm studies of monolayers at the air-water interface

HPLC grade chloroform from Aldrich (St. Louis, MO) was used to prepare amphiphile solutions with a concentration of $\sim 1 \text{ mg mL}^{-1}$ as determined by weight. Surface pressure-area isotherm experiments were carried out on a Langmuir trough (Nima, UK). Surface pressure was measured with a Wilhelmy plate made out of filter paper. Sub-phase temperature was maintained at 23.0 ± 0.1 °C by an Isotemp 3016 circulating thermostat. The subphase contained ultrapure water with a resistivity of 18.2 M (Millipore). Monolayers were formed by spreading 50 μL of a CHCl₃ solution of compounds **1–5** ($\sim 1.0 \text{ mg mL}^{-1}$) onto the subphase and allowing 10 min for the solvent to evaporate. Trough barriers were compressed at a constant speed of $\leq 0.3 \text{ nm}^2 \text{ molecule}^{-1} \text{ min}^{-1}$. Data are plotted as surface pressure (mN m^{-1}) versus molecular area ($\text{\AA}^2$). Isotherm data were collected in triplicate on each of 4 separate days, resulting in a total of 12 individual trials for each compound to obtain accurate isotherm information.

4.3. Brewster angle microscopy

BAM images were collected using a MicroBAM2 (Nanofilm Technology, Göttingen, Germany) fitted over the Langmuir trough. The light source is a 659 nm laser diode with 30 mW maximum optical power. The images were captured by a CCD camera and stored on a PC. Field of view for raw image is approximately $3.6 \text{ mm} \times 4.0 \text{ mm}$. Images were resized to $1.8 \text{ mm} \times 2.0 \text{ mm}$ in Fig. 7. Barrier compression speed was $5 \text{ \AA}^2 \text{ molecule}^{-1} \text{ min}^{-1}$.

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References

- [1]. Hille, B. Ionic channels of excitable membranes. 3rd ed.. Sinauer Associates; Sunderland, MA: 2001. p. 814
- [2]. Gokel GW, Mukhopadhyay A. Synthetic models of cation-conducting channels. *Chem. Soc. Rev* 2001;30:274–286.
- [3]. Gokel GW, Schlesinger PH, Djedovic NK, Ferdani R, Harder EC, Hu J, Leevy WM, Pajewska J, Pajewski R, Weber ME. Functional, synthetic organic chemical models of cellular ion channels. *Bioorg. Med. Chem* 2004;12:1291–1304. [PubMed: 15018901]

- [4]. Fyles TM. Synthetic ion channels in bilayer membranes. *Chem. Soc. Rev* 2007;36:335–347. [PubMed: 17264934]
- [5]. Biron E, Otis F, Meillon JC, Robitaille M, Lamothe J, Van Hove P, Cormier ME, Voyer N. Design, synthesis, and characterization of peptide nanostructures having ion channel activity. *Bioorg. Med. Chem* 2004;12:1279–1290. [PubMed: 15018900]
- [6]. Sakai N, Mareda J, Matile S. Rigid-rod molecules in biomembrane models: from hydrogen-bonded chains to synthetic multifunctional pores. *Acc. Chem. Res* 2005;38:79–87. [PubMed: 15709727]
- [7]. Yoshii M, Yamamura M, Satake A, Kobuke Y. Supramolecular ion channels from a transmembrane bischolic acid derivative showing two discrete conductances. *Org. Biomol. Chem* 2004;2:2619–2623. [PubMed: 15351826]
- [8]. Gokel GW. Hydraphiles: design, synthesis, and analysis of a family of synthetic, cation-conducting channels. *Chem. Commun* 2000:1–9.
- [9]. Abel E, Maguire GEM, Murillo O, Suzuki I, Gokel GW. Hydraphile channels: structural and fluorescent probes of position and function in a phospholipid bilayer. *J. Am. Chem. Soc* 1999;121:9043–9052.
- [10]. Doyle DA, Cabral JM, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R. The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science* 1998;280:69–77. [PubMed: 9525859]
- [11]. Dutzler R, Campbell EB, Cadene M, Chait BT, MacKinnon R. X-ray structure of a ClC chloride channel at 3.0 Å reveals the molecular basis of anion selectivity. *Nature* 2002;415:287–294. [PubMed: 11796999]
- [12]. Jentsch TJ, Neagoe I, Scheel O. ClC chloride channels and transporters. *Curr. Opin. Neurobiol* 2005;15:319–325. [PubMed: 15913981]
- [13]. Dutzler R. The ClC family of chloride channels and transporters. *Curr. Opin. Struct. Biol* 2006;16:439–446. [PubMed: 16814540]
- [14]. Schlesinger PH, Ferdani R, Liu J, Pajewska J, Pajewski R, Saito M, Shabany H, Gokel GW. Scmtr: a chloride-selective, membrane-anchored peptide channel that exhibits voltage gating. *J. Am. Chem. Soc* 2002;124:1848–1849. [PubMed: 11866586]
- [15]. Schlesinger PH, Ferdani R, Pajewski R, Pajewska J, Gokel GW. A hydrocarbon anchored peptide that forms a chloride-selective channel in liposomes. *Chem. Commun* 2002:840–841.
- [16]. Schlesinger PH, Djedovic NK, Ferdani R, Pajewska J, Pajewski R, Gokel GW. Anchor chain length alters the apparent mechanism of chloride channel function in scmtr derivatives. *Chem. Commun* 2003:308–309.
- [17]. Pajewski, R.; Pajewska, J.; Li, R.; Fowler, EA.; Gokel, GW. *New J. Chem.* The effect of midpolar regime mimics on anion transport mediated by amphiphilic heptapeptides. in press
- [18]. Pajewski R, Ferdani R, Pajewska J, Li R, Gokel GW. Cation dependence of chloride ion complexation by open-chained receptor molecules in chloroform solution. *J. Am. Chem. Soc* 2005;126:18281–18295. [PubMed: 16366583]
- [19]. Ferdani R, Pajewski R, Pajewska J, Schlesinger PH, Gokel GW. Glycine position permutations and peptide length alterations change the aggregation state and efficacy of ion-conducting, pore-forming amphiphiles. *Chem. Commun* 2006:439–441.
- [20]. Maduke M, Miller C, Mindell JA. A decade of ClC chloride channels: structure, mechanism and many unsettled questions. *Annu. Rev. Biomol. Struct* 2000;29:411–438.
- [21]. Djedovic N, Ferdani R, Harder E, Pajewska J, Pajewski R, Weber ME, Schlesinger PH, Gokel GW. The C- and N-terminal residues of synthetic heptapeptide ion channels influence transport efficacy through phospholipid bilayers. *New J. Chem* 2005;29:291–305.
- [22]. Pajewski R, Ferdani R, Pajewska J, Djedovic N, Schlesinger PH, Gokel GW. Evidence for dimer formation by an amphiphilic heptapeptide that mediates chloride and carboxyfluorescein release from liposomes. *Org. Biomol. Chem* 2005;3:619–625. [PubMed: 15703797]
- [23]. URL: www.nima.co.uk/.
- [24]. Alexander AE. The role of hydrogen bonds in condensed monolayers. *Proc. Roy. Soc. Lond. Ser. A: Math. Phys. Sci* 1942;179(979):470–485.
- [25]. URL: www.X-Seed.net.

- [26]. URL: www.povray.org.
- [27](a). Tetko IV, Tanchuk VY. Application of associative neural networks for prediction of lipophilicity in alogps 2.1 program. J. Chem. Inf. Comput. Sci 2002;42:1136–1145. [PubMed: 12377001]URL: www.vcclab.org/lab/alogps/.
- [28]. Schlesinger PH, Ferdani R, Pajewska J, Pajewski R, Gokel GW. Replacing proline at the apex of heptapeptide-based chloride ion transporters alters their properties and their ionophoretic efficacy. New J. Chem 2003;27:60–67.

Fig. 1.
Proposed mechanism by which two $(C_{18}H_{37})_2N-COCH_2OCH_2CO-(Gly)_3-Pro-(Gly)_3-OCH_2Ph$ (SAT) molecules insert into the membrane bilayer to form a pore.

Fig. 2.
Structures of Compounds 1-5.

Fig. 3.
Surface pressure-area isotherms for **1-5** on pure water.

Fig. 4.
3D model of $(\text{CH}_3)_2\text{NCOCH}_2\text{OCH}_2\text{COOH}$.

Fig. 5.
Hydrogen bonding interactions between H_2O and $(\text{C}_n\text{H}_{2n+1})_2\text{N}-\text{COCH}_2\text{OCH}_2\text{CO}-\text{OH}$, 5.

Fig. 6. Space-filling representations of (CH₃)₂N-COCH₂OCH₂CO-Gly-OCH₂Ph (left) and (CH₃)₂N-COCH₂OCH₂CO-(Gly)₃-OCH₂Ph (right), dimethyl analogs of **3** and **2**, respectively.

Fig. 7.

(a) Pressure-area isotherm of **4**; arrows indicate regions of captured images. Brewster angle micrographs of **4** at (b) 51 Å², (c) 50 Å², (d) 49 Å², (e) and (f) 48 Å², (g) 47 Å², and (h) 30 Å². Image field of view is 2.0 mm × 2.0 mm.

Table 1
Summary of transition points in pressure-area isotherms obtained by using a Langmuir-Blodgett trough

Compound	X in (C ₁₈ H ₃₇) ₂ N-COCH ₂ OCH ₂ COX	L-B trough transitions (Å ²)				Collapse
		1st	2nd	3rd	4th	
1	-GGPGGG-OCH ₂ Ph	161 ± 2	74 ± 1	52 ± 1	n/a	40 ± 1
2	-GGG-OCH ₂ Ph	133 ± 11	76 ± 6	46 ± 3	n/a	36 ± 3
3	-Gly-OCH ₂ Ph	121 ± 1	72 ± 1	51 ± 2	41 ± 1	31 ± 2
4	-OCH ₂ Ph	115 ± 7	77 ± 5	46 ± 3	n/a	34 ± 2
5	-OH	54 ± 3	n/a	n/a	n/a	36 ± 1

Table 2Calculations of log *P* hydrophobicity parameters

Molecule	<i>A log P_s</i> ^a
(C ₁₈ H ₃₇) ₂ NH	10.51
H ₂ N-COCH ₂ OCH ₂ CO-NHCH ₃	-1.66
(CH ₃) ₂ N-COCH ₂ OCH ₂ CO-NHCH ₃	-1.18
(C ₁₈) ₂ N-COCH ₂ OCH ₂ CO-NHCH ₃	9.71
(C ₁₈) ₂ N-[DGA]-G-OCH ₂ Ph, ^b 3	10.28
(C ₁₈) ₂ N-[DGA]-GGG-OCH ₂ Ph, 2	9.53
(C ₁₈) ₂ N-[DGA]-GGGPGGG-OCH ₂ Ph, 1	8.03
H ₂ N-[DGA]-GGGPGGG-OCH ₂ Ph	-1.11
GGGPGGG-OCH ₂ Ph	-1.13
GGG-OCH ₂ Ph	-0.58
G-OCH ₂ Ph	0.71

^a See Ref. [27].^b [DGA] represents the diglycolic acid fragment -COCH₂OCH₂CO-.