Long-lived and highly conducting ion channels formed by lipophilic ethylenediamine palladium(II) complexes†

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The synthesis of a lipophilic ethylenediamine palladium(II) complex and its activity in planar bilayer membranes are reported. In acetonitrile solution containing one equivalent of 4,4-bipyridine the complex forms the expected metallosupramolecular tetrameric square. The same mixture provokes transmembrane conductance of three types: an initial period of erratic behavior, occasional short channel-like openings, and frequent very long-lived and highly conducting channel openings. The apparent pore radii of both short and long openings vary continuously over the range of 0.1–1.5 nm, and long-lived pores will form in the absence of 4,4′-bipyridine. The long-lived channels are presumed to involve an extended aggregate of the palladium complex and lipids surrounding a toroidal pore in the membrane.

Introduction

One of the longest standing design strategies for synthetic ion channels is the use of macrocycles to provide both a structural framework and a size selective portal for the channel.[1] The earliest examples were based on cyclodextrins,[2,3] but the most successful examples are Gokel's *hydraphile* channels[4,5] which utilize diaza-18-crown-6 units in these dual roles. In an elegant series of papers, Gokel and co-workers have explored structure–activity relationships,[6–8] probed details of the orientation and location of the macrocycles in the bilayer,[9] and have demonstrated these compounds are active in intact biological membranes in which the ion channel activity correlates with antibiotic activity.[10,11] Crown ethers have appeared in numerous other channel designs,[12–15] but none of these have yet to reach the standard of the hydraphiles either in terms of mechanistic understanding or of efficacy.

The main competing design strategy for ion channels exploits aggregation of smaller components to produce active membrane-spanning structures.[16] The best examples of these are the *p*-phenylene beta-barrels reported by Matile *et al.*,[17] and the macrocyclic DL-peptide nanopores reported by Ghadiri *et al.*[18,19] Both these systems use multi-point hydrogen bonding to assemble a tubular structure and enforce a large trans-membrane channel. The structures of the active species are inferred from an indirect but substantial body of experimental evidence.[17,20] Functional ion channels of even less defined structures can also result from the aggregation of a wide range of single- and double-headed amphiphiles.[21–24]

A drawback of many reported channels, especially of those based on pre-formed synthetic macrocycles, is the synthetic effort required to produce the active compounds. Even good macrocyclization reactions produce moderate amounts of compound and differential protection schemes required to produce functional ion channel compounds further lengthen the synthesis and diminish the yield.[25] Our own recent efforts have been directed solely to the goal of simplifying the required syntheses without compromising ion channel function, and a key to our recent progress was the decision to avoid macrocyclization reactions.[21,26]

The drive towards simple syntheses inevitably draws towards self-assembly processes, and our attention eventually settled on the system reported by Fujita and co-workers of the assembly of a supramolecular square macrocycle (**1**) from ethylenediamine palladium(II) nitrate (**2**) and 4,4′-bipyridine (**3**) at room temperature in aqueous solution.[27,28] We envisaged modifying one or both components to make them suitably lipophilic to partition to the bilayer membrane. Self-assembly in the membrane would then produce a macrocyclic portal to what we hoped would be a novel type of ion channel. Each palladium center is 2+, so the complex bears an overall 8+ charge. The internal dimensions from the crystal structure show a square cavity of 0.75 nm void space.[29] If these characteristics could be translated to a membrane, we would expect a very high conductance anion selective channel. Fig. 1 illustrates the conceptual design.

As appealing as this design might be, we immediately recognized that the differential partitioning of the components to the bilayer made it highly unlikely that we could control the relative palladium to bipyridine stoichiometry at the required 1 : 1 ratio. We therefore explored whether or not this was a critical issue: does it matter if the relative stoichiometry varies somewhat? Our efforts to explore this question using a computational model of speciation will be reported separately.[30] The key finding is that at the palladium concentration expected for a typical membrane transport experiment, the required square macrocycle is the dominant species formed between pH 4 and 7 at a bipy : Pd ratio between 0.4 to 4 : 1. We concluded that this window was large enough to work within, so we turned our attention to the chemical details of the design.

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† Dedicated to Professor George Gokel on the occasion of his 60th birthday.

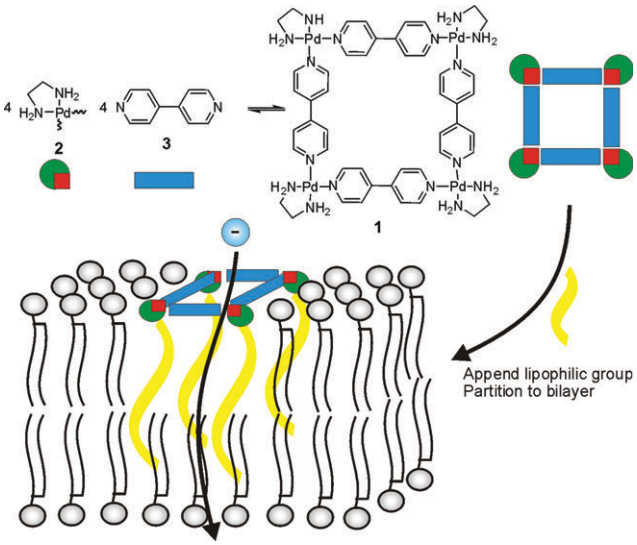
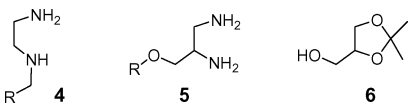


Fig. 1 Design of a channel based on a self-assembled metallosupramolecular square.

As noted, we require a suitable lipophilic derivative of either the ethylenediamine Pd(II) and/or of the 4,4′-bipyridine. We rejected *N*-substituted ethylenediamines such as **4** since we saw the steric requirements of the alkyl group position directly adjacent to the coordination center as having the potential to destabilize the square complex. Substitution on the bipyridine is certainly possible, but would not deal directly with the partitioning of the dicationic palladium to the membrane. Consequently we settled on a derivative in which one of the carbons of the ethylenediamine was functionalized with a long alkyl tail to achieve the required partioning (**5**). We chose to work with alkoxy substituted derivatives as these compounds are potentially available *via* a simple synthesis from solketal (**6**) (Scheme 1).

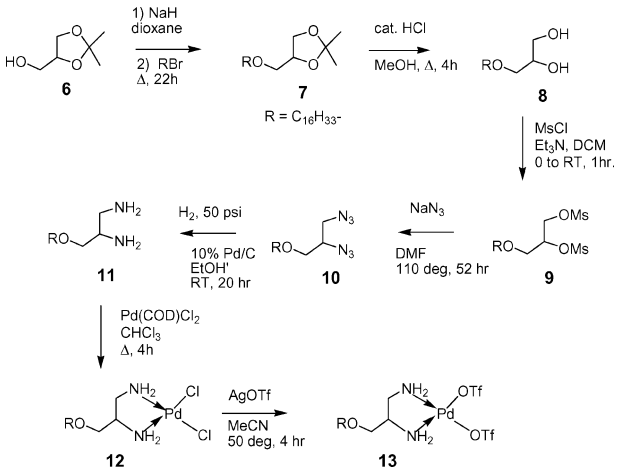


This paper outlines our exploration of the design of Fig. 1 through the synthesis of a suitable palladium complex of diamine **5** and the evaluation of the activity in a bilayer membrane. As we show below, we certainly generate highly conductive and long-lived pores. However, the experimental evidence does not support a channel of the type envisaged in Fig. 1, and points instead to another type of channel mechanism.

Results and discussion

Synthesis and characterization of a lipophilic ethylenediamine palladium(II) complex

The synthesis of a hexadecyl derivative is given in Scheme 1. The sodium salt of solketal (**6**) was alkylated in THF, or preferentially in dioxane, to give the ether **7**. Deprotection with catalytic acid in methanol produced the diol **8** that was then converted *via* the bis-mesylate **9** to the bisazide **10** which was reduced with hydrogen over Pd/C to the required diamine



Scheme 1 Synthesis of a lipophilic ethylenediamine and its palladium complex.

11. The azide displacement and the reduction both required relatively forcing conditions to achieve full conversion, but the method produced **11** from **8** in 92% yield without intermediate purification. The presumed dichloro palladium complex of **11** was prepared from $Pd(COD)Cl_2$, but the limited solubility of **12** in all solvents precluded any detailed analysis. Metathesis of the product with silver triflate produced the soluble palladium complex **13** which was characterized by NMR; the signals from the ligand showed the expected shifts and multiplicities for metal coordination.

The palladium complex is capable of forming the expected self-assembled square complex in the presence of 4,4′-bipyridine; a 15 mM solution of **13** in acetonitrile-d_3 with one equivalent of added 4,4′-bipyridine (**3**) showed the expected downfield coordination shifts of the bipyridine protons to give a pair of signals at 7.9 and 8.9 ppm. Fujita *et al.* report the same signals in complex **1** at 8.64 and 7.64 ppm with virtually identical peak shapes and couplings.[28] We attribute the additional 0.25 ppm downfield shift to a solvent difference between water and acetonitrile, and to a difference in anions (triflate *versus* nitrate). The spectroscopic data for the **13** : **3** mixture is entirely consistent with the formation of the expected square complex.

The ligand **11** introduces a chiral center and lowers the symmetry relative to the parent ethylenediamine Pd(II) fragment and inevitably leads to isomeric products in the final square complex. The four alkyl substituents in the square complex can lead to four unique possible orientations of the substituent groups above and below the plane of the square derived from **1** (all "up", 3 "up" plus 1 "down" *etc.*). Arranging a racemic mixture on this set of isomers then leads to 64 permutations of which 41 are unique. The ion channel design envisages that all four alkyl chains would be on the same face of the macrocyclic square. This arrangement occurs in 6 of the 41 possible structures (15%) based on racemic **11** which would increase to 10 of 41 if **11** were a single enantiomer. There would be little advantage of a chiral synthesis in this case since a mixture would result.

The NMR spectra of a **13** : **3** mixture indicate a high degree of apparent symmetry due to rapid equilibration of the various

isomers. Thus we expect that a similar equilibration of precursors leading to the active channel would be able to produce the required structure with all alkyl groups on the same face of the square macrocycle, no matter what the initial isomeric mixture in homogeneous solution.

Membrane activity

The membrane activity of **13** and mixtures with **3** was assessed using the voltage clamp technique under a wide range of conditions and electrolytes using a bilayer membrane from di-phytanoyl phosphatidyl choline (diPhyPC).[31] There was no lack of observable "activity" as the majority of experiments produced some type of conductance change in the bilayer. As in previous survey studies,[23] we started with a well-characterized bilayer of high capacitance and a very low incidence of small transient signals. The most effective way to introduce the active compound to the bilayer was to wet a small brush with a few microliters of an acetonitrile solution of **13** or **13** : **3** mixtures and to brush the material onto the bilayer. Breakage inevitably occurred, but as the bilayer re-formed with further brushing, the activity of **13** or **13** : **3** mixtures could be detected. The capacitance of these re-formed bilayers was uniformly as good as the initial state.

There are three main types of activity observed in this system. Virtually every experiment started with a phase of "erratic" openings as illustrated in Fig. 2A. This behavior is characterized by very short duration (<1 μs) "spikes" of current passage that vary widely in magnitude (<5 pA to >100 pA). There is no apparent order to this behavior with erratic periods extending from a few seconds to many minutes. Quiescent periods between periods of more vigorous activity were also observed. Although this activity is the simplest to observe for **13** : **3** mixtures we have not been able to develop any analytical tools to allow us to progress beyond this rather unsatisfactory qualitative description.

The initial irregular period of erratic activity eventually settled to one of two types of more regular activity. In a small proportion of cases (7 of 30), we were able to observe "short" openings of durations of the order of seconds as illustrated in Fig. 2B. In a larger proportion of cases (22 of 30) we observed "long" openings as discussed below.

In addition to being relatively rare, the period of the short openings (Fig. 2B) was also restricted to a few minutes or less. We were unable to find conditions under which the short openings could be reliably produced or prolonged, and as a consequence our ability to apply the usual analysis tools to these openings is very limited. Usually a series of observations is examined to derive current as a function applied potential and therefore a specific conductance for the channel.[31] This is not possible for the short openings as these observations are too rare and when they do appear, they occur over too limited a period. However, we can characterize the openings as individual observations. A complicating feature is the very significant variation in the current carried during the "open" state of these short openings (Fig. 2B). A histogram analysis of this type of opening produces a very broad distribution with the result that the derived mean current is very uncertain. The applied potential is known for each observation, thus a conductance value (with uncertainty) can be determined. On each occasion that short openings were observed, possibly with a unique combination of electrolyte and applied potential, the observation was converted to a conductance value. Thus over a number of experiments and openings we were able to generate a list of seventeen such observations.

The central question is: could some or all of these "short opening" observations be due to the proposed channel of Fig. 1? As a static structure we anticipate a box with internal face-to-face dimensions of 0.75 nm. The experimental conductance of a channel (g) can be used to calculate the so-called Hille radius[32] derived for a hemisphere-capped cylinder of length l and radius r containing a sample of electrolyte with bulk resistivity ρ according to eqn (1):

$$\frac{1}{g} = \left(l + \frac{\pi r}{2}\right)\left(\frac{\rho}{\pi r^2}\right) \quad (1)$$

Eqn (1) thus allows an estimate of the apparent radius of any pore that produces a short opening. For example, the dominant open level indicated on Fig. 2B has a mean current of 3.7 ± 1.4 pA which corresponds to a conductance of 37 ± 14 pS. We assume a length of 3.4 nm[16] and use the resistivity of 1.0 M KCl equal to 0.089 Ω m[43] to derive the Hille radius of 0.21 ± 0.04 nm. This is clearly well short of the expectation for a

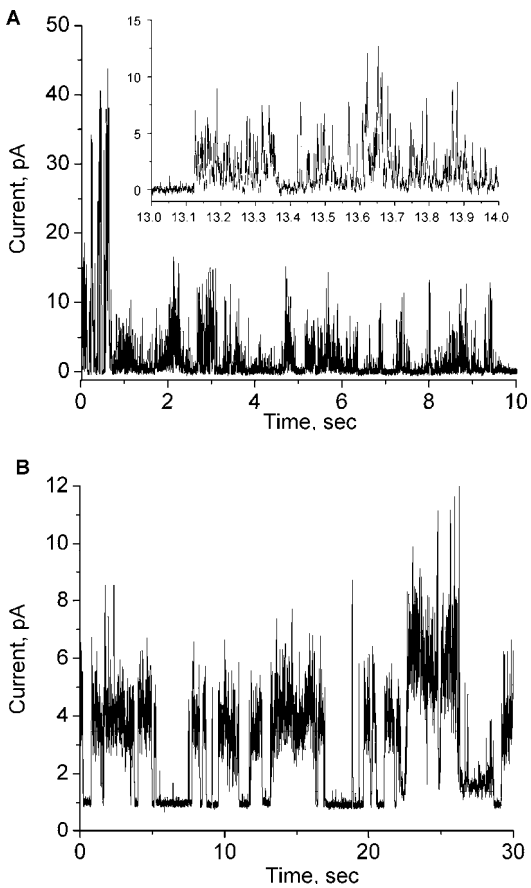


Fig. 2 Current–time records showing typical examples of "erratic" (A) and "short" (B) openings for a **13** : **3** mixture (diPhyPC, 1 M KCl, **13** : **3** = 1 : 1 mole ratio, +80 (A) and +100 (B) mV applied potential).

channel of the type of Fig. 1. The Hille radii calculated this way for the set of short opening observations range from 0.14 to 0.34 nm plus a single example at 0.63 nm (see Experimental). There is no defined value that accounts for a significant fraction of the observations within the corresponding uncertainties; rather the distribution of values appears to be a continuum. We therefore conclude that whatever circumstance produces this rare behavior, it is unlikely that it is due to a "square" channel as implied by Fig. 1.

As noted briefly above, a significant proportion of experiments proceed from the erratic behavior of Fig. 2A to a behavior we describe as "long openings".[33] This evolution occurs over a period of 30–90 min during which there is essentially no discernable activity. Indeed, it took some time for us to recognize the evolution of the system in the absence of defined activity. With patience, a long opening develops that appears as in the current–time data given in Fig. 3. These long openings are characterized by a relatively high and steady current that is a function of applied potential. These can be readily analyzed to produce a linear current–voltage relationship and a specific conductance for the opening. The conductance of these openings can be very significant with values ranging up to 5 nS.

The appearance we associate with a long opening is very similar to the current–time data expected for a carrier,[32] but a number of observations indicate that we have a channel not a carrier. Firstly, there are occasional minor step changes captured within a record, such as the one circled in Fig. 3. Given the slow and irregular startup period leading to long openings, it is rare to observe the significant step change in current that corresponds to the initial opening of a channel. We have seen such step changes, but have not successfully captured one in a digital record; fortunately, we have successfully captured an

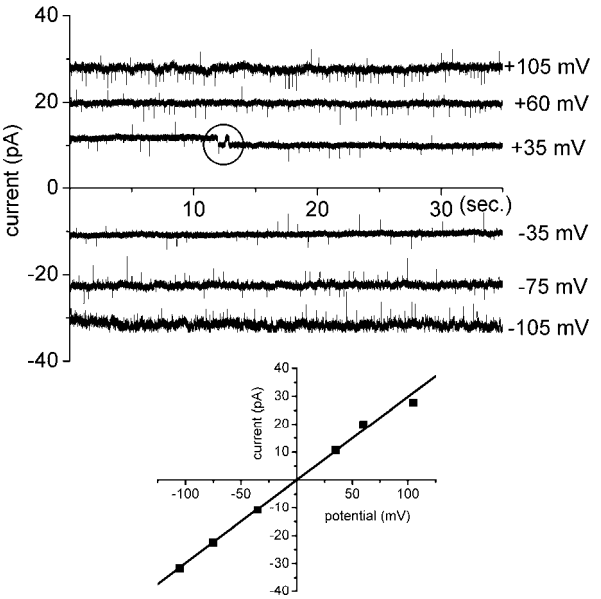


Fig. 3 Top: current–time recordings typical of "long" openings at various applied potentials (**13** : **3** = 1 : 1 mole ratio, diPhyPC, 0.1 M CsCl). Bottom: corresponding current–voltage diagram. Specific conductance 0.29 nS.

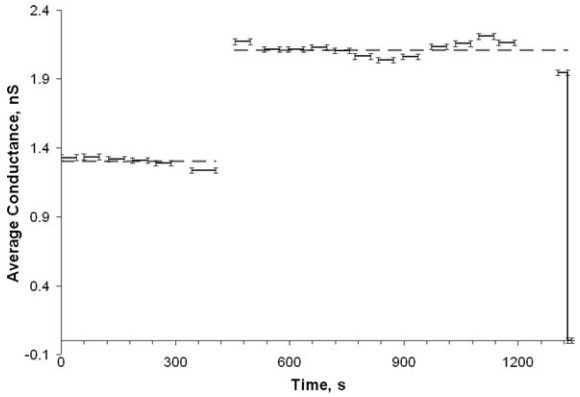


Fig. 4 Conductance as a function of elapsed time preceding a recorded closing of a long opening channel. Bars show duration and mean observed conductance of recorded periods.

example of a closing as shown in Fig. 4. The experimental data in Fig. 4 were recorded in a series of 40 s files over a 20 min period. The applied potential varied in this period as current–voltage relationships were being explored, thus it was not obvious until after the fact that there were two openings involved. The first 6 min segment had a mean conductance of 1.3 pS while the rest of the data were recorded for an opening with a mean conductance of 2.1 pS. We were fortunate to record a direct closing from the 2.1 pS level to the baseline, implying that a single species was involved in the closing. Since conductances of the two levels are not integer multiples, this implies the first opening was "enlarged" to form the second. Whatever the mechanistic interpretation, this record makes it clear that a carrier is not involved.

Long openings occur in a significant proportion of experiments, but once established there is usually only one conductance state observed. This is the converse of the situation with short openings since there is plenty of opportunity to characterize each opening. But as in the case of the short openings the number of independent observations is small. Here too we are reduced to computing the Hille radii in order to discern if some or all of the long openings could be due to the channel proposed in Fig. 1. The 9 available observations produce values that range from 0.13 to 14 nm diameter. Again the values appear to be uniformly distributed over the interval without a significant clustering about a defined value. And again we are forced to conclude that the proposal of Fig. 1 cannot account for the experimental observations.

Further evidence that the long openings are not due to the proposed square channel is provided by the observation in two cases of stable highly conductive openings formed by **13** in the absence of any added **3**. The Hille radii in these cases were at the low end of the range (0.19 nm) but these channels were otherwise indistinguishable from the behavior of other long openings found when **13** : **3** mixtures were used. Clearly, if bipyridine is not required for the formation of the channel, the channel cannot be formulated as a metallosupramolecular square complex. The palladium complex appears to be essential for activity since the diamine **11** failed to produce the chaotic initial activity even at relatively high concentrations.

The selectivity of the long opening channels can be assessed through the use of a membrane system in which an ionic gradient is imposed across the membrane. The applied potential to maintain a zero net current, the reversal potential, can be related to the permeability of the constituent ions through the Goldman–Hodgins–Katz equation.[31] We expected that the positive charges on the palladium centers would impart significant anion/cation selectivity. However we found only modest selectivity between Cs^+, K^+, Cl^- and Br^-. Since the channel conductance is high, the interaction of an ion with the walls, even if charged, would play a small role in determining the permeability. Thus the selectivity results further buttress the idea that we have large pores formed in the system.

Conclusions

Clearly the proposal of Fig. 1 is not supported by the observations, but it is not as clear what the active structures might be. We envisage the partition of **13** to the bilayer as if it were a classic amphiphile: the hydrocarbon would be in the non-polar region and the palladium head group would reside near the phosphocholine head groups. The bipyridine would partition as a non-polar electrolyte, thus would be distributed throughout the bilayer volume. This immediately alters the relative stoichiometry in the head group region of the bilayer since the volume accessible to the palladium center is much smaller than the volume accessible to the bipyridine. The solution speciation model shows the required cyclic 4 : 4 complex becomes a minor species at a mole ratio below 1 : 0.4 palladium : bipyridine.[30] Thus the failure to observe channels based on the square complex might simply be an effect of differential dilution and/or differential partition. It is possible that a more lipophilic or more surfactant-like bipyridine derivative could be designed to circumvent this issue.

The palladium complex is capable of forming supramolecular structures having internal pore diameters of greater than 10 nm. These are very large structures and by simple geometry must involve thousands of palladium centers. The inherent affinity between dicationic palladium complexes must be small, so some type of bridging interaction is likely. Bridged hydroxo complexes, such as bis-μ-hydroxo-bis(ethylendiamine palladium(II)) are dominant species in basic solutions of ethylenediamine palladium(II) aquo complexes,[34] so it is possible that species involving bridging hydroxide could form even though the bulk pH is nominally too acidic. Alternatively, the phospholipid phosphoryl groups, present in high local concentration in the region where the palladium is expected to reside, could form a monomeric or bridged species involving coordinated phosporyl oxygen (*i.e.* Pd–$^-$O–P). We have not found literature reporting any structural examples of this coordination type. On the contrary, the structures of ethylenediamine palladium complexes of nucleosides show *N*-coordinated bases, with the phosphate groups as outer-sphere ligands to coordinated water (*i.e.* Pd–OH_2 - $^-$O - P).[35] Another structural possibility is suggested by the complexes of one-dimensional mixed valence chlorobridged ethylenediamine platinum and palladium complexes $[M(en)_2][M(en)_2Cl_2]X_2$ with sulfonate amphiphiles.[36] This system forms supramolecular aggregates having rod- or tape-like morphologies. These aggregates are presumed to involve a core of the one-dimensional metal complex with an electrostatically bound coating of amphiphilic counterions.[36] Based on these structural precedents, it is likely that some type of complex of phospholipid and the lipophilic palladium complex can form within the membrane headgroup region. The proposed complex would be stabilized by electrostatic and hydrogen bonding interactions, and could involved more extended structures through bridging water and phosphoryl groups.

As for the nature of the channels formed, the available observations appear to require a continuum of radii. The channels must be able to expand above a threshold size depending on the available palladium concentration. This is reminiscent of the large channels formed by ceramides in which hydrogen-bonded stacks of ceramide monomers line a toroidal pore.[37,38] These channels show a lanthanum ion induced stepwise diassembly process in which the loss of individual stacks of molecules contract the shrinking pores by a fixed amount.[37] We have not observed any spontaneous disassembly, apart from the one closing event captured above, and in that case it appeared to occur in a single step. On the other hand, the pore sizes in our system, although "large", are smaller than in the ceramide system, and the postulated bridging interactions in our case are much less defined than in the hydrogen bonded stacks that support the ceramide channels, so it is possible that our channels are near a minimum size which cannot suffer incremental loss without complete diassembly.

Our working hypothesis is that a membrane-bound lipophilic ethylenediamine palladium aquo complex recruits adjacent phospholipids to form an aggregated network. As a surfactant, the single-chain palladium amphiphile is expected to have a shape parameter[39] that would be more stable in a positive curvature or micellar structure rather than in a lamellar bilayer structure. As a consequence, a sufficiently large patch of aggregate would be more stable as the lining to a toroidal pore in which the required positive curvature would be normal to the bilayer plane. The radius of the pore would be a function of the precise membrane concentration of the palladium complex. Melittin and other membrane-active peptides also form short-lived toroidal pores in which the peptide stabilizes the lipid to allow the required membrane curvature.[40] Such pores catalyze lipid flip-flop,[41] hence one implication of the working hypothesis is that a similar flippase activity would occur in this system.

Whatever the structure, the activity of the pores formed from this lipophilic palladium species is remarkable. The pores form reliably, albeit somewhat sluggishly, and once formed are stable for prolonged periods. As such they rival other large opening pores such as α-hemolysin[42] or the octiphenyl-β-barrels.[17] At the same time, these channels can be established through the investment of a tiny fraction of the preparative effort involved in other systems. Our design proposal of channel self-assembly according to Fig. 1 was certainly too naïve, and in retrospect failed to take into account the energetics and concentrations inherent in a bilayer membrane. Now that we have been alerted to these design deficiencies, we will be able to exploit related channels to explore the structural and functional features of this new type of synthetic channel.

Experimental

1,2-Dihydroxy-3-hexadecyloxypropane (8)

NaH (60% in oil; 0.734 g, 18.4 mmol, 1 eq) under N_2 was rinsed with two 3–5 ml aliquots of hexanes. To the NaH was added dry 1,4-dioxane (40 ml) followed by the dropwise addition of solketal **6** (2.588 g, 19.58 mmol, 1.06 eq). The rate of addition was regulated to keep the effervescing under control. Once the solketal addition was complete the cloudy, slightly yellow solution was stirred at rt for a further 10 min upon which the solution became clear. 1-Bromohexadecane (6.473 g, 21.2 mmol, 1.16 eq) was added and the mixture was heated to reflux for 22 h during which a white precipitate formed. The reaction was quenched with 5 ml of ice cold water. The reaction mixture was extracted three times with diethyl ether. The organic extracts were combined and dried with anhydrous $MgSO_4$. The crude material, a light yellow oil, was purified by column chromatography on silica gel (5 cm diameter, 600 ml silica), eluted with hexanes at first then increasing polarity to 4 : 1 hexanes : ethyl acetate. The product ketal **7** was recovered from the second band eluted from the column in 53% yield. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, J = 6.6 Hz), 1.2–1.4 (m, 26H), 1.57(m, 2H), 2.30 (broad s), 3.44–3.56 (m, 4H), 3.62–3.75 (m, 2H), 3.86 (m, 1H) ppm. ^{13}C NMR ($CDCl_3$): δ 14.1, 22.7, 26.0, 29.3–29.7, 31.9, 64.3, 70.4, 71.8, 72.5 ppm.

Without further purification, **7** was added to MeOH (50 ml) and 6 drops concentrated HCl, and 15 drops H_2O. The mixture was heated to reflux for 4 h and the solvent was removed to give a white, slightly waxy solid recovered in quantitative yield. The product was characterized and used without further purification. ^{1}H NMR ($CDCl_3$): δ 0.87 (t, 3H, J = 6.6 Hz), 1.2–1.4 (m, 26H), 1.57(m, 2H), 2.30 (broad s), 3.41–3.58 (m, 4H), 3.60–3.76 (m, 2H), 3.81–3.90 (m, 1H) ppm. ^{13}C NMR ($CDCl_3$): δ 14.1, 22.7, 26.0, 29.3–29.7, 31.9, 64.3, 70.4, 71.8, 72.5 ppm. FT-IR (KBr): 3369 (m, br), 2954 (sh, m), 2919 (m), 2850 (m), 1471 (s), 1328 (m), 1123 (s), 1060 (s) cm^{-1}. MS (EI) m/z: 316 [2%], 285 [22%], 225 [50%]. Anal. Calc. for $C_{19}H_{40}O_3$: C, 72.10; H, 12.74. Found: C, 72.71; H, 13.16.

1,2-Diamino-3-hexadecyloxypropane (11)

Compound **8** (2.726 g, 8.1 mmol, 1 eq), was dissolved in DCM (35 ml) and NEt_3 (9.6 ml, 68.9 mmol, 8 eq). The solution was cooled to 0 °C in an ice bath and a solution of DCM (5 ml) and methanesulfonyl chloride (2.963 g, 25.8 mmol, 3 eq) was added dropwise at a rate that maintained a constant temperature of 0 °C. Once the addition was complete the solution was stirred at 0 °C for 30 min then allowed to warm to rt and stirred at rt for 30 min. The reaction was quenched with a small amount of water, and extracted with three 20 ml portions of saturated $NaHCO_3$ followed by three 20 ml portions of 1 M HCl(aq). The organic phase was dried with anhydrous $MgSO_4$, and then solvent was removed by rotary evaporation. To this material was added DMF (40 ml) and NaN_3 (2.102 g, 32.3 mmol, 5.5 eq). This solution was heated with stirring to 110 °C for 52 h. The mixture was concentrated on the rotary evaporator to about 5–10 ml and extracted with diethyl ether and water. The organic phase was dried with anhydrous $MgSO_4$ and the solvent removed by rotary evaporation. **(CAUTION: organic azides are known to be explosive and are typically handled only in solution.[43] However, a small amount ($\ll$ 50 mg) of 10 was handled as the pure material on several occasions without incident. Larger amounts were handled as solutions only.)** A diethyl ether solution of **10** (5–10 ml) was diluted in 100% ethanol (25 ml) and added to a low pressure hydrogenation bottle containing 10% Pd on carbon (0.170 g) wetted with 100% EtOH (15 ml). The bottle was purged three times with H_2 and sealed under 50 psi of H_2 and shaken for 20 h. The solution was then filtered through a pad of Celite and the solvent was removed from the filtrate by rotary evaporation. The recovered material was then dried under vacuum. The yield of **11** from **8** was 92%. The product could be further purified by careful recrystalization from warm DCM. ^{1}H NMR ($CDCl_3$): δ 0.88 (t, 3H, J = 6.6 Hz), 1.25 (m, 26H), 1.55 (m, 2H), 2.02 (broad s), 2.66 (m, 1H), 2.84 (m, 1H), 3.00 (m, 1H), 3.25–3.53 (m, 4H) ppm. ^{13}C NMR ($CDCl_3$): δ 14.1, 22.7, 26.1, 29.3–29.7, 31.9, 44.4, 45.6, 53.2, 71.7, 74.5 ppm. FT-IR (KBr): 3361 (m, br) , 3301 (m, br), 2918 (m), 2850 (m), 1645 (m), 1468 (m), 1123 (s) cm^{-1}. MS (FAB) m/z: 597.6 [18%], 343.3 [16%], 316.3 [100%].

1,2-Diamino-3-hexadecyloxypropane palladium(II) dichloride (12)

$Pd(COD)Cl_2$ (0.072 g, 25 mmol) was dissolved in $\sim$3 ml $CHCl_3$ and **11** (0.080 g, 25 mmol) was added. The mixture was heated to reflux for 3 h, cooled to rt and filtered to recover a beige solid. The solid was washed with small portions of acetone and ethanol. The isolated solid, presumed to be **12** (47% yield), was not soluble enough to be characterized by NMR spectroscopy. FT-IR (KBr): 3276 (m), 3193 (m), 2917 (s), 2850 (s), 1557 (m), 1468 (m), 1124 (m) cm^{-1}. MS (EI) m/z: 419.2 [100%]. Anal. Calc. for $C_{19}H_{42}Cl_2N_2OPd$: C, 46.39; H, 8.61; N, 5.70. Found: C, 46.62; H, 8.55; N, 6.00.

1,2-Diamino-3-hexadecyloxypropane palladium(II) bis-trifluoromethylsulfonate (13)

Dichloro complex **12** (0.0709 g, 14 mmol) was suspended in 5 ml ACN and AgOTf (0.7688 g, 30 mmol) dissolved in 2 ml ACN was added to the suspension. The mixture was warmed at 40–50 °C for at least 4 h protected from light. During this time a white precipitate formed and the solution became light yellow. The suspension was cooled to rt and filtered to recover a light yellow solution which was transferred to a 10 ml volumetric flask and diluted to the mark with ACN. The solution was used in experiments without further treatment. This procedure was assumed to be quantitative. The reaction carried out in d_3-ACN was used to characterize the product. ^{1}H NMR (d_3-ACN): δ 0.88 (t, 3H, J = 6.7Hz), 1.2–1.4 (m, 28H), 1.57 (m, 2H), 2.71 (br, 1H), 3.24 (broad s), 3.47 (m, 4H) ppm.

The 1 : 1 complex of **13** and **3** was formed by mixing equimolar amounts of the components at ambient temperature in ACN solution at a concentration of 15 mM. The reaction carried out in d_3-ACN was used to characterize the product. ^{1}H NMR (d_3-ACN): δ 0.88 (t, 3H, J = 6.7 Hz), 1.2–1.4 (m, 28H), 1.57 (m, 2H), 2.71 (br, 1H), 3.3 (broad s), 3.5 (m, 4H), 7.9 (d, 4H, J = 8 Hz) and 8.9 (d, 4H, J = 8 Hz) ppm.

Bilayer clamp experiment

The general experimental procedure used a previously described system and followed previous protocols.[21,44] Membranes were formed from diphtanoyl phosphatidyl choline (Avnti Polar Lipids; 5 mg in 200 μl of decane) by brushing and/or dipping as previously described. Active channels were formed by brushing a solution of **13** and **3** (10 mM in Pd) in ACN across a pre-formed bilayer membrane. Membrane activity and the analysis of short and long openings was described in the text using published solution resistivity data.[43]

The following short openings were observed for **13** : **3** mixtures (1 : 1 mole ratio; electrolyte, conductance in nS, Hille radius in nm): 1 M KNO_3, 0.123, 1.0; 1 M KCl, 0.009, 0.10; 1 M KCl, 0.010, 0.10; 1 M KNO_3, 0.148, 0.11; 1 M KNO_3, 0.150, 0.11; 1 M KNO_3, 0.197, 0.13; 1 M KNO_3, 0.212, 0.13; 1 M KCl, 0.016, 0.13; 1 M KCl, 0.016, 0.13; 0.1 M NaCl, 0.017, 1.3; 1 M KNO_3, 0.248, 1.4; 1 M KNO_3, 0.265, 1.5; 1 M KNO_3, 0.318, 0.16; 1 M KNO_3, 0.381, 0.18; 1 M KCl, 0.040, 0.21; 0.1 M NaCl, 0.046, 0.23; 1 M KNO_3, 0.638, 0.23; 1 M KNO_3, 0.750, 0.25; 1 M KNO_3, 0.813, 0.26; 0.1 M NaCl, 0.087, 0.32; 0.1 M NaCl, 0.349, 0.68.

The following long openings were observed for **13** : **3** mixtures (1 : 4 mole ratio; electrolyte, conductance in nS, Hille radius in nm): 0.5 M Cs_2SO_4, 0.12, 0.13; 0.1 M CsBr, 0.066, 0.25; 0.1 M CsCl, 0.29, 0.51; 0.1 M Cs_2SO_4, 0.8, 0.73; 0.1 M NaCl, 0.52, 0.81; 0.1 M Cs_2SO_4, 1.3, 0.96; 1 M KNO_3, 7.21, 1.25; 0.1 M Cs_2SO_4, 2.1, 1.30; 0.1 M CsCl, 1.7, 1.45.

The following long openings were observed for **13** alone (electrolyte, conductance in nS, Hille radius in nm): 1 M KNO_3, 0.251, 0.19; 1 M KNO_3, 0.26, 0.19.

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