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Synthesis and Characterization of Picket Porphyrin Receptors That Bind Phosphatidylglycerol, an Anionic Phospholipid Found in Bacterial Membranes

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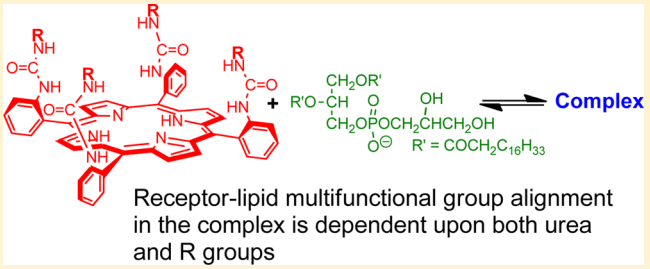
Supporting Information

ABSTRACT: The lipid binding ability of four urea-picket porphyrins designed to bind to both the phosphate anion portion as well as the glycerol hydroxyl groups of phosphatidylglycerol (PG) has been investigated. Isothermal titration calorimetry (ITC) and ^{1}H NMR were used to determine the receptor's stoichiometry of binding, association constants, and both the enthalpy and entropy of binding with the PG anion. Spectral evidence shows that the phosphate anion portion of PG is hydrogen bonded to the urea groups of the receptors. This binding interaction orients the PG anion in the receptor pocket such that its glycerol hydroxyl groups can align with a third urea picket, and results are furnished that suggest this multifunctional interaction does occur. The structure of the entire picket was found to influence the enthalpy and entropy of lipid binding. The synthesis of tetrabutlyammonium phosphatidylglycerol (TBAPG), and a detailed spectral characterization of its headgroup, is also presented.



INTRODUCTION

A continuous 30-year decrease in the number of newly approved antimicrobial agents for use in the United States and the concomitant emergence of many strains of multidrug-resistant bacteria (sometimes referred to as superbugs) has become a cause of concern to the medical community.[1] In light of these disturbing trends, there has been a growing interest in the use of antimicrobial peptides or their mimics as potential antibiotics.[2−8] Antimicrobial peptides (AMPs, members of a larger group of host defense peptides) are part of the innate immune system of all multicellular organisms and exhibit a broad spectrum of activity against Gram-positive and Gram-negative bacteria. The cationic peptides bind to the bacteria's anionic phospholipids located in their inner membrane, and complex formation is followed by insertion into the membrane, leading to eventual disruption of the membrane and to cell death[9,10] (while some AMPs have been shown to have intracellular targets, the recognition of membrane phospholipids is a required first step for entry into the prokaryote cell).

These peptides have recently garnered interest as antimicrobial therapeutics because bacteria have shown only limited resistance to these peptides even though they have been interacting with microbes since the beginnings of multicellular organism development. The method of interaction with the bacteria lowers the chances of resistance by target modification, as this would require the complete alteration of the bacterial membrane. However, native cationic peptides do not just kill bacteria; they have been shown to modulate several aspects of the immune response and, in high enough concentrations, exhibit host toxicity.[8,11−13] Partly due to this, the few selected cationic antimicrobial peptides presently licensed are for topical use only. Consequently, an important property for any AMP or synthetic mimic intended for *systemic bactericidal use* is that it must be highly selective in the disruption of prokaryotic membranes and not eukaryotic membranes.

For a synthetic antibiotic to exclusively interact with prokaryote membranes requires it to contain a recognition unit for unique bacterial membrane components. Our interest has been in the development of receptors that will bind to phosphatidylglycerol (PG), the major anionic phospholipid found in bacterial membranes (the outer leaflets of eukaryotic cell membranes, such as erythrocytes, are almost exclusively composed of zwitterionic phospholipids[14]). To impart selectivity in such receptors requires them to be multifunctional, containing numerous binding units positioned to interact with both the PG's phosphorus anion and glycerol dihydroxyl functionality. The linkage of these receptors to membrane disruptors could furnish AMP-like synthetic antibiotics that exhibit minimal host toxicity and thereby increase their potential for systemic use.

We recently published an article detailing a structural study of a family of charged bis-ammonium receptors where several of them utilized the same binding motif when forming complexes with inorganic phosphate anion or PG (via its phosphate anion portion).[15] In this comparative study, entropy was shown to be the driving force for the complexation of anions in both cases. However, the receptors' binding unit did not contain

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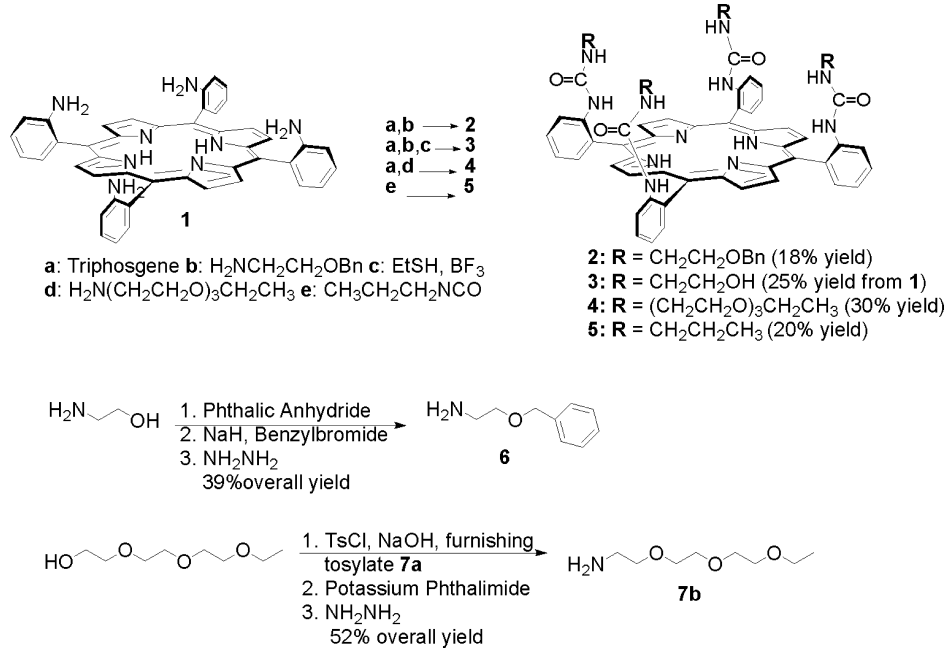
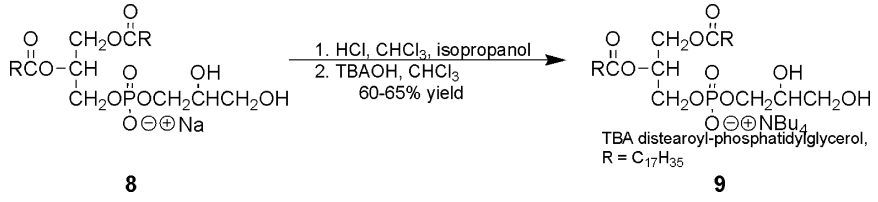
Scheme 1. Synthesis of Picket Porphyrin Receptors 2–5 and Their Precursors



Scheme 2. Synthesis of TBAPG



functionality to bind the glycerol headgroup but rather only the lipid's phosphate anion.

Herein we report on the synthesis and characterization of four neutral picket porphyrins designed to align their urea pickets with the phosphate anion and glycerol hydroxyl binding domains of the lipid (as a beginning for future iterative studies that will aid in the preparation of selective PG receptors). Experimental results are presented which (1) show that the multifunctional receptors do bind to the lipid's phosphate anion portion, (2) suggests that receptor functional groups also bind to the glycerol hydroxyl groups of PG, and (3) illuminate some of the initial structural and functional group requirements for receptor–lipid complex formation. The stoichiometry of binding was determined from ^{1}H NMR titrations, the K_a's were determined from both ^{1}H NMR titrations and isothermal titration calorimetry (ITC), and the thermodynamic driving force for complexation in polar organic solution was determined from ITC. The structure of the PG–receptor complex was inferred from a comparison of NMR solution studies of PG and the PG-receptor complex in DMF-d_7.

RESULTS

Receptor Syntheses. The syntheses of urea-picket porphyrins **2**–**5** are detailed in Scheme 1. CPK model analysis and minimization via molecular modeling with a modified MM2 force field illustrated that the porphyrin scaffold, when suitably functionalized, was the correct size and shape to furnish a binding pocket for the PG headgroup whereby the receptor and anion multifunctional groups would align (see S-Figure 26, Supporting Information).[16] The different pickets were designed to provide the receptors with varied sizes of binding pockets and, presumably, an accompanying difference in lipid accessibility and complex stability. As the targeted functional groups in the PG headgroup contain both negatively charged and neutral oxygens, it was decided to use receptor binding units capable of forming hydrogen bonds with both types of functional groups in the PG headgroup, thus the use of urea groups. The portion of the picket above the urea groups was expected to provide additional nonbonding stabilizing forces with the lipid, utilizing potential hydrophilic interactions in the case of the tetrahydroxy **3**, or hydrophobic interactions in the case of porphyrins **2** and **5**, or dipole–dipole and hydrophobic interactions in the case of the polyether **4**. While ultimately a functional receptor for antibiotic use must bind to a membrane-bound PG, it was believed that the use of solution NMR spectroscopy would most directly illustrate binding motifs and thereby allows for the rational synthesis of a PG receptor's binding units.

The porphyrin tetra-α atropisomer **1** was prepared as reported previously.[17] Two synthetic routes were utilized to construct the urea pickets on porphyrin **1**: (1) transform **1** into a tetraisocyanate with triphosgene followed by addition of the appropriate amine,[18] or (2) add isocyanate directly to **1**.[17] The yields for tetraurea picket porphyrins **2**–**5** were not optimized, as the determination of their usefulness as receptors for PG was the goal. The synthesis of compound **6** is referenced and **7** described in the Experimental Section.

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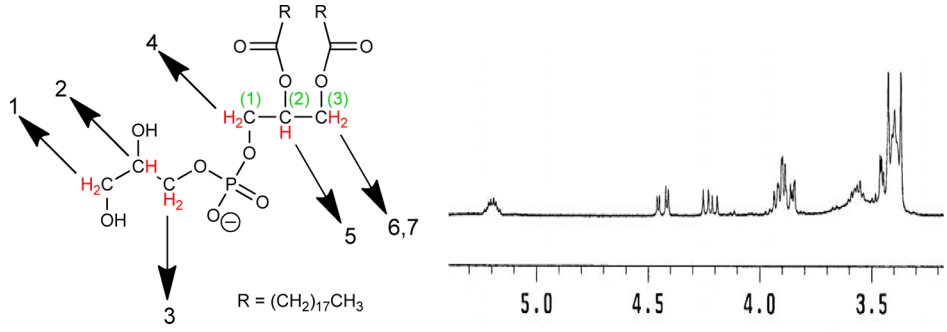

Figure 1. Left: PG structure and numbering of headgroup hydrogens and carbons (green = carbon). Right: ^{1}H NMR of headgroup region of TBAPG in DMF-d_7 (see text for details of proton assignments).

Tetrabutylammonium Phosphatidylglycerol (TBAPG) Synthesis. The distearoyl-phosphatidylglycerol lipid **8** was purchased as its monosodium salt, which required a ternary solvent system of water, methanol, and chloroform to remain soluble. To gather useful binding data with receptors that were soluble in an organic solvent required the lipid salt to be soluble in preferably one organic solvent. Because of this the tetrabutylammonium salt of the lipid (TBAPG, **9**) was prepared (Scheme 2). At first this proved difficult due to the ease of hydrolysis of the fatty acid esters during the counterion exchange (and it was problematic to purify hydrolysis mixtures with chromatography). Mixtures of compounds and byproducts were easily spotted with the use of ^{13}C NMR (change in the number and positions of the carbonyl carbon resonances) and ^{31}P NMR (more than one resonance). Fortunately, it was possible to separate the lipid's sodium salt from its neutral acid, and the acid from the TBA salt by a judicious choice of solvent systems (see Experimental Section). Additionally, by running the reactions for a short period of time at approximately 20 °C, the hydrolysis of the lipid's ester groups was avoided.

Spectral Characterization of TBAPG. Unfortunately, it has not been possible to produce X-ray quality crystals of the lipid−receptor complex. Consequently, any description of the complex structure would only come from spectroscopic studies in solution. Critical to the determination of a binding motif when utilizing NMR is the observation of which protons shift upon titration of the receptor with the anion. The shifts and their direction (downfield or upfield) provide a good description of the (average) change in the molecular environment experienced during the binding event. To understand the significance of proton shifts observed upon binding of PG and receptors required a detailed proton assignment for receptor and anion structures alike. Because the anion was a lipid, it contained headgroup protons whose resonance shifts upon binding were potential descriptors of the complex structure. While the assignment of protons in the receptors was accomplished during their syntheses, the assignment of protons within the PG headgroup was not known. Therefore, several NMR studies of the TBAPG salt were undertaken.

The NaPG **8** was obtained from the vendor as a diastereomeric pair, and TBAPG **9** was prepared also as a mixture of the two diasteroisomers, complicating its spectrum somewhat (Figure 1; proton assignments are in the text). The hydrogens attached to the ester carbons 2 and 3 (proton no. 5, 6, 7 in Figure 1) resonate as a multiplet between 5.16 and 5.23 ppm (proton no. 5), and a multiplet between 4.19 and 4.25 ppm and a pair of doublets between 4.41 and 4.46 ppm (protons 6 and 7, respectively). All three resonances integrate to one proton each. The HMSQC and expanded HMQC spectra (S-Figures 27 and 28, Supporting Information) corroborate this assignment as proton no. 5 attached to one of two headgroup methine carbons resonating at 71 ppm, and protons no. 6 and no. 7 attached to the same methylene carbon resonating at 63 ppm. The other methine hydrogen (proton no. 2) resonates as a multiplet between 3.55 and 3.6 ppm, and the terminal methylene hydrogens (protons no. 1) resonate approximately between 3.45 and 3.49 ppm, partially buried underneath the multiplet belonging to the eight protons adjacent to the nitrogen on the TBA counterion. The four proton resonances (protons no. 3 and no. 4) belonging to the two carbons attached to the phosphorus ester resonate as a multiplet between 3.84 and 3.94 ppm. Thus, all proton resonances are typical for their associated functionality.

An expanded section of the COSY spectrum of the TBAPG salt (S-Figure 29, Supporting Information) shows methine proton no. 5 to be coupled to protons no. 4 as expected. Interestingly, proton no. 5 is apparently coupled to only proton no. 6 and not proton no. 7, although both reside on C(3) (Figure 1). Accordingly, the dihedral angle between proton no. 7 and proton no. 5 must be near 90°, which is consistent with a structure where the two hydrocarbon chains are parallel to each other and the headgroup is nearly orthogonal to the axis of the hydrocarbon chains. It is known that this is the conformation mammalian lipids adopt when in a biological membrane,[19] and apparently the PG can adopt in DMF-d_7 as well (presumably there must be rigidity between the C(2)−C(3) bond to allow for this as the major conformation in solution). As proton no. 7 is only geminal coupled to proton no. 6, the pair of doublets assigned to proton no. 7 arises from the existence of the two diastereoisomers. The COSY spectrum shows that proton no. 6 is coupled to both vicinal proton no. 5 and geminal proton no. 7, and considering the existence of the two stereoisomers, the multiplet is deceptively simple (estimated coupling constants show that the muliplet between 4.19 and 4.25 ppm is not a simple doublet of doublets). Additionally, the COSY spectrum shows the expected coupling within the spin system containing protons no. 1, no. 2, and no. 3. All of the above data is consistent with the proposed assignment of headgroup protons in TBAPG shown in Figure 1.

Receptor−PG Coordination Studies in Solution: The Determination of Binding Stoichiometry and Thermodynamics. The stoichiometry of binding for receptors **2**−**5** and TBAPG in DMF-d_7 were determined from Job plots using ^{1}H NMR[20,21] (Table 1). Of the four porphyrin receptors, only **2** and **4** exhibited well behaved 1:1 binding ratios with TBAPG. Porphyrins **3** and **5** exhibited complex binding stoichiometries,

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Table 1. Results from ^{1}H NMR Titrations

receptor	binding ratio[a]	K (M^{-1}) [error]
2	1:1	2400 [±300]
3	mixture	
4	1:1	6500 [±600]
5	mixture	

[a]Binding stoichiometry determined from Job plot.

and metalation of the porphyrin ring by Zn(II) did not alter these results.

It is noteworthy that the two porphryin receptors that exhibited a 1:1 binding stoichiometry were also the most stable atropisomers, exhibiting no isomerization during variable temperature ^{1}H NMR experiments up to 100 °C. These experimental results indicate that the urea pickets of receptors **2** and **4** were conformationally stable and able to provide a well-defined binding pocket to the lipid. On the other hand, receptors **3** and **5** in comparison were relatively unstable atropisomers, whose short and more conformationally flexible pickets began to isomerize at 40−50 °C, resulting in the formation of several atropisomers.

Association constants (Table 1) were determined for receptors **2** and **4** by following titrations with the TBAPG salt in DMF-d_7 using ^{1}H NMR.[22] The association constants of the two receptors ranged roughly between 2000 and 7000 (M^{-1}) in DMF-d_7, with the binding constant of receptor **4** approximately three times greater than that of receptor **2**. Isothermal titration calorimetry (ITC) was also used to determine the association constants and thermodynamic properties of receptor−PG anion complex formation for receptors **2** and **4** as shown in Table 2. The use of ITC

Table 2. Results from Isothermal Titration Calorimetry

receptor	K (M^{-1}) [error]	ΔH, cal/mol [error]	ΔS, cal/mol/°C
2	2100 [±100]	−2830 [±70]	6
4	3700 [±450]	−1210 [±40]	12

required a modification of the solvent to 95% DMF/5% $CHCl_3$, and the association constants, which varied from 2000 to 4000 (M^{-1}), were very similar to those determined by NMR titrations for the two receptors. The ITC data showed that the binding process for receptors **2** and **4** titrated with TBAPG was enthalpy driven (1−3 kcal/mol^{-1}), as the strongest stabilizing forces were due to the formation of neutral hydrogen bonds, even in such a competitive solvent. Interestingly, the binding event was also entropy driven in the organic solvent mixture, with an increase between 6 and 12 cal/mol^{-1}/°C.

Receptor−PG Coordination Studies in Solution: ^{1}H NMR Spectral Data That Provides Clues to Receptor−Lipid Complex Structure. A large downfield shift in the urea protons of receptors **2** and **4** (nearly 0.5 ppm; see S-Figure 30, Supporting Information) was observed upon titration with the lipid. The magnitude of urea proton shifts matches those seen with our previously reported urea-picket porphyrin receptor[17,20] upon its binding of inorganic phosphate anion, and the X-ray crystal structure of the porphyrin−dihydrogenphosphate anion complex showed that the phosphate anion was hydrogen bonded to two urea pickets.[20] Thus, the ^{1}H NMR titration data for receptors **2** and **4** strongly suggest that the phosphate anion portion of the lipid binds to the urea protons in both receptors. In the present case, titration of receptors **2**−**5** with inorganic phosphate anion exhibited only complex binding stoichiometries; consequently, a direct comparison of association constants of lipid and inorganic phosphate anion was not possible. Since the lipid's proton resonance change upon complex formation is also descriptive of the receptor−lipid complex structure, an inverse addition was undertaken to observe the change in proton resonances of PG when it was titrated with porphyrin **4** (Figure 2). While the entire set of PG

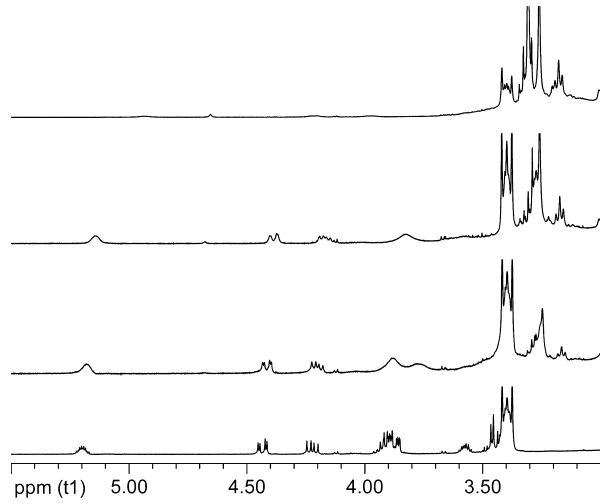

Figure 2. Stacked plot of the inverse titration of TBAPG with receptor **4**, bottom to top: 0 equiv, 0.2 equiv, 0.4 equiv, and 2 equiv of **4**. The lipid headgroup protons 1−3 (3.4−3.6 and 3.8−3.9 ppm) quickly move upfield upon titration with porphyrin **4**.

headgroup protons 1−7 moved upfield, protons 1−3 were observed to move farther and more quickly upfield. Unfortunately, the glycerol hydroxyl protons disappeared into the baseline upon titration.

DISCUSSION

The receptor's four urea pickets provide a complementary binding pocket for PG, as porphyrins **2** and **4** strongly hydrogen bond to the lipid in DMF. While ^{1}H NMR titration experiments demonstrated that the lipid did bind to receptors **3** and **5** (downfield movement of urea protons upon titration), Job plot analysis showed only complex binding stoichiometries in solution. Receptors **2** and **4** pickets furnished the more conformationally stable binding pockets of the four receptors as demonstrated by variable temperature ^{1}H NMR experiments. This larger, more sterically constrained picket framework was apparently necessary for well behaved 1:1 receptor−anion binding stoichiometry.

The most plausible explanation for these observed differences is that multifunctional groups in receptors **2** and **4** were aligned with those in PG in the receptor−anion complex, i.e., the receptor's urea groups were bound to the lipid's phosphate anion portion, while a third urea picket was interacting with the lipid's glycerol hydroxyl(s). A complementary fit of lipid to receptor would prevent the complex's multiply aligned functional groups from interacting with a second receptor or lipid. Receptors **3** and **5**, with their more flexible urea pickets, offered the lipid a less preorganized binding pocket, and the receptors were apparently unable to orient their urea pickets to match all of the lipid's functional groups. *Without the proper alignment of all binding groups, any nonbound functional group,*

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whether part of the lipid or receptor, could interact with a third partner and as a result exhibit a complex binding stoichiometry (see S-Figures 20−23, Supporting Information, for Job plots). Indeed, receptors **3** and **5** never exhibited a well-behaved 1:1 (or 1:2 or 2:1, for that matter) stoichiometry of binding. All four receptors contained the same phenylurea binding unit, and only the top part of the pickets was different. This contrast in receptor−PG anion binding stoichiometry makes little sense unless the multifunctional groups in **2**'s and **4**'s lipid−receptor complexes were fully engaged with each other while this was not the case in **3**'s and **5**'s lipid−receptor complexes.

An alignment in the receptor−lipid complex whereby the glycerol hydroxyl would hydrogen bond to a third urea picket while the phosphate anion portion concomitantly hydrogen bonds to two urea pickets would necessitate the positioning of the lipid headgroup directly above and across the porphyrin ring. In this orientation one would expect PG headgroup protons 1−3 to shift upfield due to the positioning of these protons within the porphyrin's large, shielding ring current. The inverse titration experiment (Figure 2) shows the dramatic upfield shift of PG protons 1−3 during the formation of the receptor−lipid complex (as well as smaller upfield shifts of protons 4−7) which convincingly demonstrates that the lipid headgroup's glycerol protons are located directly above the receptor's porphyrin ring. Thus, ^{1}H NMR spectroscopy confirms that receptor **4** orients the lipid headgroup in such a way that at least one of its hydroxyl groups would be positioned to hydrogen bond with one of the receptor's other two urea pickets while the phosphate anion portion is bound to two urea pickets.

Interestingly, the ITC data show that the thermodynamic driving force for complex formation was both enthalpic and entropic. The negative enthalpies are a result of the stabilization afforded by hydrogen bonding in the receptor−lipid complex even in the competitive solvent DMF. Receptor **2** exhibited larger negative enthalpy but smaller positive entropy upon binding with TBAPG than did receptor **4** (Table 2). The observed divergence in the thermodynamic forces driving complex formation can be attributed to the substantially longer pickets contained in receptor **4**, which would presumably require the release of larger numbers of solvent molecules upon formation of the receptor−anion complex. This indicates that the entire picket is involved in binding to the lipid, i.e., enthalpic stabilization that is furnished via the receptor's urea groups and an increase in entropy that is dependent upon the picket structure above the urea groups.

CONCLUSION

When taken all together, the above results point to a receptor−lipid complex of **2** or **4** and PG where the lipid headgroup lies directly above the porphyrin ring's interior in such a way that one of its hydroxyl groups would be positioned to hydrogen bond with one of the receptor's other two urea pickets while the phosphate anion portion is bound to two urea pickets. The sensitivity of receptor−lipid stoichiometry of binding to the rigidity in the receptor's pickets suggests that a complementary, preorganized binding pocket, which allows for all the lipid and receptor functional groups to fully align, is necessary for well behaved, 1:1 complex formation. Thus, it is very likely that at least one hydroxyl functional group in the lipid's headgroup is hydrogen bonded to a third urea picket on the receptor.

The complementary binding pocket afforded by the urea picket porphyrins suggests that other kinds of conformationally stable pickets with similarly placed binding units would also provide a complementary binding pocket for the lipid. As such, we are currently synthesizing structurally similar picket porphyrins but with binding pockets that contain ammonium groups to prepare water-soluble receptors.

EXPERIMENTAL SECTION

Materials and Procedures. All chemicals were dried and/or purified according to literature procedures before use (all solvents were dried unless otherwise noted),[23] and reactions were performed under a nitrogen atmosphere. Receptors **2**−**5** were dried in a vacuum desiccator over P_2O_5, and TBAPG was dried in a vacuum desiccator over drierite. All melting points (Mel-Temp) are uncorrected. Purity affirmation and compound identity were accomplished by HRMS (TOF ES) . Compound **6** (2-*O*-benzylethanolamine) was synthesized from 2-aminoethanol by the method of Lehn and co-workers.[24]

^{1}H NMR Titrations. ^{1}H NMR titrations to determine association constants were performed as described in ref 17. In this case, the movements of the proton resonances were averaged from a minimum of three titration experiments, and each experiment's value was averaged using several proton resonances (urea protons, *β*-protons, and *meso*-phenyl protons). Nonlinear regression analysis using EQNMR[22] furnished the receptor−lipid association constants.

Job Plots. Both receptor and lipid were dried in a vacuum desiccator over P_2O_5. The lipid and receptor were weighed on a microbalance and each placed into a 2 mL volumetric flask; the amounts used allowed for identical molar concentrations. Aliquots of receptor solution and lipid solution (DMF-d_7) were placed into separate NMR tubes, such that one tube contained 1 equiv of receptor and 0 equiv of lipid; the next tube contained 0.9 equiv of receptor and 0.1 equiv of lipid, and the next tube contained 0.8 equiv of receptor and 0.2 equiv of lipid and so forth down to 0.1 equiv of receptor and 0.9 equiv of lipid. In this way, the sum of the molar equivalents of both anion and receptor was always the same. Once the solutions had been added to the tube, they were diluted with DMF-d_7 so that the volume in each tube was 0.45 mL. A spectrum was then obtained for each tube, and the change in chemical shift of receptor proton resonances relative to the shift recorded from the tube with no added lipid was determined. The mole fraction of receptor was plotted vs the change in chemical shift multiplied by the mole fraction of receptor in the tube to afford the Job plots. Job experiments were performed at slightly different concentrations to remove any artifacts caused by different concentrations when using ^{1}H NMR.

Isothermal Titration Calorimetry. ITC experiments were performed with a MicroCal iTC200 to determine the enthalpy of association. All data analysis was performed with Origin software supplied by MicroCal, using a model for the stoichiometry of binding that was determined from the Job plots. Control titrations were performed by the addition of lipid solution to a solution of 95% DMF/5% $CHCl_3$, and these control titrations were subtracted from the titrations of lipid to receptor to remove the effects of heats of dilution. All ITC experiments were run at 30 °C. The results are averaged from a minimum of three ITC experiments, and all experiments were performed at slightly different concentrations to detect problems associated with possible impurities such as from crystalline solvent.

***α,α,α,α*-5,10,15,20-Tetrakis(2-(*N*-[(2-phenylmethoxy)ethylureido])phenyl)porphyrin (2).** Dry *α,α,α,α*-tetra(*o*-aminophenyl)porphyrin (**1**) (0.253 g, 0.375 mmol) was dissolved in dry CH_2Cl_2 (178 mL) and dry triethylamine (0.42 mL, 3.01 mmol) in a glovebox. Triphosgene (0.167 g, 0.563 mmol) in dry CH_2Cl_2 (6 mL) was added to the reaction mixture, and it was stirred at ambient temperature for 1.5 h. Dry 2-*O*-benzylethanolamine (0.284 g, 1.88 mmol) in dry CH_2Cl_2 (4 mL) was then added via syringe in one portion, and the reaction mixture was stirred at ambient temperature for 48 h. The solution was washed with water (2 × 75 mL), and the organic layer was dried over sodium sulfate and then concentrated under reduced pressure to yield a crude purple solid. The crude reaction material was purified by silica gel chromatography with 50% CH_2Cl_2 in ethyl acetate to remove the impurities and then 100% ethyl acetate to elute product.

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The resulting solid was recrystallized from CH_2Cl_2/hexane to afford a pure purple solid (0.095 g, 0.0687 mmol, 18%): mp 150−152 °C; UV/vis $CHCl_3$ λ_{max} (ln ε): 422.5 (9.64), 517 (9.27), 552 (8.17), 592 (8.24), 653.5 (7.55); ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.70 (s, 8H), 8.44 (d, 4H, J = 8.8 Hz), 7.72 (t, 4H, J = 7.9 Hz), 7.59 (d, 4H, J = 6.6 Hz), 7.36 (s, broad, 4H), 7.29 (t, 4H, J = 7.1 Hz), 7.14−7.03 (m, 20H), 6.20 (t, broad, 4H, J = 5.4 Hz), 4.18 (s, 8H), 3.16 (t, 8H, J = 5.4 Hz), 2.91(m, 8H,), −2.71 (s, 2H); ^{13}C NMR (100 MHz, DMSO) δ 155, 140, 138, 135, 131, 129, 128, 127.32, 127, 121.1, 120.9, 116, 72, 69; HRMS (ESI) calcd for $C_{84}H_{79}N_{12}O_8$ $(M + H)^+$ 1383.6144, found 1383.6151; HRMS (ESI) cald for $C_{84}H_{78}N_{12}O_8Na$ $(M + Na)^+$ 1405.5963, found 1405.5918.

α,α,α,α,-5,10,15,20-Tetrakis-(2-(*N*-(2-hydroxyethyl)ureido)-phenyl)porphyrin (3). To a reaction mixture containing α,α,α,α-5,10,15,20-tetrakis-(2-(*N*-[(2-phenylmethoxy)ethylureido])phenyl)-porphyrin (**2**) (0.165 g, 0.119 mmol) dissolved in ethanethiol (22.14 mL, 309 mmol) was added BF_3·etherate (0.83 mL, 6.749 mmol).[25] The reaction was kept under nitrogen and allowed to stir at room temperature for 4 h. At this time, the reaction was quenched with a saturated solution of $NaHCO_3$, and the mixture extracted with ethyl acetate (3 × 50 mL). The organic layers were combined, and a precipitate formed that was collected by filtration. This resultant crude solid was dissolved in a minimum amount of DMF and added to a radial chromatography plate and air-dried overnight. The product was purified using silica gel radial chromatography (CH_2Cl_2/methanol/water; 84/15/1), affording a pure purple solid (0.031 g, 0.030 mmol, 25%): mp 218 °C, decomp; UV/vis DMF λ_{max} (ln ε): 422.5 (14.31), 515 (11.91), 550 (10.66), 590 (10.76), 647 (10.13); ^{1}H NMR (400 MHz, DMSO-d_6) δ −2.66 (s, broad, 2H), 2.80−2.81 (m, 8H), 3.09−3.10 (m, 8H), 4.35 (s, broad, 4H), 6.16 (t, broad, 4H, J = 5.4 Hz), 7.29 (t, 4H, J = 7.4 Hz), 7.38 (s, broad, 4H), 7.61 (d, 4H, J = 7.6 Hz), 7.72 (t, 4H, J = 7.9 Hz), 8.48 (d, 4H, J = 8 Hz), 8.73 (s, 8H); ^{13}C NMR (100 MHz, DMSO) δ 42, 60, 116, 120.6, 120.7, 129, 130, 135, 140, 155; HRMS (ESI) cald for $C_{56}H_{55}N_{12}O_8$ $(M + H)^+$ 1023.4266, found 1023.4265.

2-(2-(2-Ethoxyethoxy)ethoxy)ethyl Tosylate (7a).[26] Triethylene glycol monoethyl ether (10 g, 56.11 mmol) was dissolved in THF (20 mL) and the solution brought to 0 °C in an ice bath, whereupon a solution of sodium hydroxide (4.6 g, 115 mmol) dissolved in water 18.4 mL) was added to the stirred, cold mixture that was kept under nitrogen. A solution of toluensulfonyl chloride (13.799 g, 72.38 mmol) dissolved in THF (20 mL) was added dropwise over 15 min, and the reaction mixture was allowed to warm to ambient temperature and stirred for 2 h. Diethyl ether (150 mL) was added to the reaction mixture, and the two layers were separated. The organic layer was washed with 1 M NaOH (3 × 12.5 mL) without shaking and washed with water (2 × 12.5 mL). The organic layer was dried with sodium sulfate and concentrated under reduced pressure to afford a yellow liquid (15.61 g, 46.97 mmol, 84%), which was used in the next reaction without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (t, 3H, J = 7 Hz), 2.42 (s, 3H), 3.49 (quartet, 2H, J = 6.9 Hz), 3.61−3.53 (m, 8H), 3.66 (t, 2H, J = 4.8 Hz), 4.14 (t, 2H, J = 4.8 Hz), 7.32 (d, 2H, J = 8.4 Hz), 7.78 (d, 2H, J = 8.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15, 22, 67, 68.9, 69.5, 70, 70.7, 70.9, 71, 128, 130, 133, 145.00.

2-(2-(2-Ethoxyethoxy)ethoxy)ethylamine (7b).[26] 2-(2-(2-Ethoxyethoxy)ethoxy)ethyl tosylate (**10a**) (3 g, 9.02 mmol) was dissolved in DMF (6.2 mL) under a nitrogen atmosphere, and to this solution was added potassium phthalimide (2.22 g, 11.99 mmol). The reaction mixture was heated to 110 °C and stirred for 3 h. After cooling to rt, diethyl ether (39 mL) was added to the solution and the resultant precipitate was filtered. The filtrate was washed with 1 M NaOH (2 × 13 mL) and water (1 × 13 mL). The organic layer was dried with sodium sulfate and concentrated under reduced pressure. The crude phthalimide product was dissolved in 6.4 mL of a hydrazine monohydrate/ethanol (1/1 v/v) mixture and heated to 110 °C overnight under a nitrogen atmosphere. The reaction mixture was cooled to rt and extracted with toluene (4 × 21 mL). The combined organic layers were concentrated under reduced pressure to afford **7b** as a yellow oil (0.99 g, 5.56 mmol, 62%), which was used in the next reaction without further purification: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.19 (t, 3H, J = 7.1 Hz), 1.59 (s, broad, 2H), 2.84 (t, 2H, J = 5 Hz), 3.66−3.47 (m, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15, 42, 67, 70, 70.5, 70.8, 70.9, 74.

α,α,α,α,-5,10,15,20-Tetrakis-(2-(*N*-(3,6,9-trioxaundecyl)-ureido)phenyl)porphyrin (4). Dry α,α,α,α-tetra-(*o*-aminophenyl)-porphyrin (**1**) (0.192 g, 0.285 mmol) was dissolved in dry CH_2Cl_2 (137.2 mL) in a glove box. To the solution was added dry triethylamine (0.32 mL, 2.27 mmol) followed by the addition of triposhgene (0.127 g, 0.427 mmol). The mixture was stirred for 1 h at rt. Dry 2-(2-(2-ethoxyethoxy)ethoxy)ethylamine (**7b**) (0.262 g, 1.478 mmol), dissolved in dry CH_2Cl_2 (5 mL), was then added to the reaction mixture via syringe in one portion, and the reaction was stirred for 24 h. The reaction mixture was then concentrated under reduced pressure, and the resulting crude material was purified using silica gel chromatography (4% ethanol in CH_2Cl_2). The resultant product was recrystallized with CH_2Cl_2/hexanes to yield a pure purple solid (0.125 g, 0.084 mmol, 30%): mp 173−175 °C; UV/vis $CHCl_3$ λ_{max} (ln ε): 422 (12.14), 516.5 (9.62), 545 (8.72), 590 (8.54), 648 (7.78); ^{1}H NMR (400 MHz, DMSO-d_6) δ −2.68 (s, broad, 2H), 0.98 (t, 12H, J = 7 Hz), 2.86−2.94 (m, 8H), 3.13−3.40 (m, 48H), 6.21 (t, broad, 4H, J = 5.0 Hz), 7.29 (t, 4H, J = 7.4 Hz), 7.36 (s, broad, 4H), 7.60 (d, 4H, J = 7.6 Hz), 7.72 (t, 4H, J = 7.9 Hz), 8.45 (d, 4H, J = 8.4 Hz), 8.73 (s, 8H); ^{13}C NMR (100 MHz, DMSO) δ 15, 65, 69, 69.2, 69.4, 69.5, 69.6, 116, 120.8, 120.9, 129, 130, 135, 140, 155; HRMS (ESI) calcd for $C_{80}H_{103}N_{12}O_{16}$ $(M + H)^+$ 1487.7615, found 1487.7577.

α,α,α,α,-5,10,15,20-Tetrakis-(2-(*N*-(propylureido))phenyl)-porphyrin (5). To a solution of dry α,α,α,α-tetra-(*o*-aminophenyl)-porphyrin (**1**) (0.413 g, 0.612 mmol) dissolved in dry CH_2Cl_2 (12.2 mL) was added propyl isocyanate (0.46 mL, 4.90 mmol) via syringe in one portion, and the reaction mixture was stirred overnight in a glovebox. The solution was removed from the box and then washed with water (3 × 25 mL) and with brine (1 × 50 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure. The crude solid was purified using silica gel chromatography (20% CH_2Cl_2 in ethyl acetate), and the resultant solid was recrystallized from CH_2Cl_2/hexanes to yield a pure purple solid (0.126 g, 0.124 mmol, 20%): mp 232 °C, decomp; UV/vis $CHCl_3$ λ_{max} (ln ε): 422 (12.12), 516 (9.51), 554 (8.40), 590 (8.45), 646 (7.86);^{1}H NMR (400 MHz, DMSO-d_6) δ −2.685 (s, broad, 2H), 0.50 (t, 12H, J = 7.4 Hz), 1.01 (quartet, 8H, J = 7.3 Hz), 2.64 (quartet, 8H, J = 6.4 Hz), 5.89 (t, broad, 4H), 6.95 (s, broad, 4H, J = 5.4 Hz), 7.35 (t, 4H, J = 11.3 Hz), 7.77−7.72 (m, 8H), 8.35 (d, 4H, J = 8.8 Hz), 8.74 (s, 8H); ^{13}C NMR (100 MHz, DMSO) δ 11, 23, 41, 116, 121.1, 121.5, 129, 131, 135, 140, 155; HRMS (ESI) cald for $C_{60}H_{63}N_{12}O_4$ $(M + H)^+$ 1015.5095, found 1015.5139; HRMS (ESI) cald for$C_{60}H_{62}N_{12}O_4Na$ $(M + Na)^+$ 1037.4915, found 1037.4960.

1,2-Distearoyl-sn-glycero-3-phospho-sn-1-glycerol, Tetrabutylammonium Salt (9). 0.52 g (0.65 mmol) amount of 1,2-distearoyl-sn-glycero-3-phospho-sn-1-glycerol, sodium (**8**) (Genzyme Pharmaceuticals, Liestal, Switzerland) was placed in a ternary mixture of 2 mL of 1.5 M HCl, 12 mL of 2-propanol, and 11 mL of chloroform. The mixture was stirred at room temperature until complete solvation occurred (usually around 5 min), at which time the solvents were removed under reduced pressure (using a rotovap attached to a Welch pump) at room temperature, which furnished a white slurry. The neutral phospholipid was precipitated using cold acetone and collected by filtration as a white, waxy solid (0.47 g, 0.6 mmol, 93% yield). This material was characterized by ^{31}P NMR, ^{13}C NMR, and ^{1}H NMR. The ^{31}P NMR spectrum showed a single, well-resolved peak at 0.8 ppm (in ternary mixture of 3:3:0.5 CD_3OD:$CDCl_3$:D_2O) while the peak from that of the sodium salt was at 1.6 ppm in the same solvent mixture. The melting point of the neutral acid was 134−136 °C, while that of the sodium salt was over 200 °C. The material (0.47 g, 0.6 mmol) was resuspended in 30 mL of chloroform, and 0.125 g (0.48 mmol) of tetrabutylammonium hydroxide was added to the mixture. The mixture was stirred at room temperature for 5 min to dissolve the base and stirred an additional 5 min before removal of the solvent under high vacuum at

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room temperature. The resultant semisolid was resuspended in 50:50 acetone:hexanes, and the solution was filtered to remove neutral phospholipid. The solvents were removed from the filtered solution under high vacuum, and the resultant material was taken up into warm hexanes and transferred to the freezer, where it was left overnight. The next day, a precipitate had set up and was collected by filtration, furnishing 0.36 g (0.35 mmol, 60% yield) of a white, semicrystalline solid: mp 53−55 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, 6H, J = 6.8 Hz); 1.00 (t, 12H, J = 7.2 Hz); 1.24 (broad singlet, 62H); 1.44 (quartet, 8H, J = 7.2 Hz); 1.65 (broad singlet, 10H); 2.28 (quartet, 4H, J = 6.4 Hz); 3.29 (t, 8H, J = 8.0 Hz); 3.61−3.76 (m, 4H); 3.89−4.05 (m, 3H); 4.15−4.20 (m, 1H); 4.40−4.42 (m, 1H); 5.21 (broad singlet,1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.9, 14.3, 20.0, 22.9, 24.2, 25.1, 29.4, 29.6, 29.7, 29.9, 32.1, 34.4, 34.5, 59.0, 63.1, 63.5, 65.2, 66.3, 70.8, 71.8, 173.3, 173.7; ^{31}P NMR (162 MHz, $CDCl_3$) δ 3.06; FTIR (KBr) ν 1472, 1734, 3421 cm^{-1}; MS (ESI) m/z 242.2 $(M)^+$, 777.6 $(M)^-$; HRMS (ESI) calcd for $C_{16}H_{36}N$ $(M)^+$ 242.2848, found $(M)^+$ 242.2821; HRMS (ESI) calcd for $C_{42}H_{82}O_{10}P$ $(M)^-$ 777.5646, found $(M)^-$ 777.5641.

■ ASSOCIATED CONTENT

Supporting Information

HRMS, ^{1}H NMR and ^{13}C NMR spectra of **2**−**5**, ^{1}H NMR and ^{13}C NMR spectra of **7a**, **7b**, **9**, ^{1}H NMR of **9** + CD_3OD, Job plots of TBAPG and of receptors **2**−**5**, ITC binding isotherms of receptors **2** and **4** with TBAPG, HyperChem model demonstrating that the PG headgroup and simplified urea-picket porphryin have proper dimensions for complex formation, HSQC, HMQC, and COSY of TBAPG, stacked plot of **4** titrated with TBAPG, and stacked plot of TBAPG titrated with **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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