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Synthesis of an azacrown template for phosphatidylinositol-4,5-bis(phosphate) recognition

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Abstract—An azacrown system has been developed for selective membrane binding of phosphatidylinositol-4,5-bis(phosphate) recognition. Neutral and cationic forms of the metacyclophane macrocycles have been synthesized by divergent routes in acceptable yields. Such diversity will be useful in identifying anion receptors that operate best at membrane interfaces.

Generation of molecular receptors for biologically relevant anions generally falls into two broad areas: charged receptors[1] that provide complementary electrostatic or metal–ligand interactions and neutral receptors[2] that utilize hydrogen bonding and/or ion-dipole attraction to bind their guests. Recognition of anions in biological systems is inherently more difficult than cation recognition due to the ubiquitous nature of negatively charged species at physiologic pH and the structural complexity of anions such as polyphosphates relative to simple, spherical cations such as sodium, potassium, and calcium. The entropic cost for binding the former class is more expensive due to additional degrees of freedom that must be restricted in these systems. Additionally, anions are much larger and generally have a more diffuse charge compared to cations. This demands a higher degree of design effort necessary to make receptors complementary to a specific anion target. Lehn's pioneering work on azacrowns showed the advantage of such preorganization in that these systems not only bind di- and triphosphates, but also catalyze the hydrolysis of these species.[3] Nature offers us the most selective receptors of biological anions as these macromolecules are able to overcome free energy lost upon dehydrating an anion with perfectly complementary binding sites.

We are interested in developing membrane-anchored receptors for phosphatidylinositol and its derivatives (these include the lipoarabinomannan (LAM) of *Mycobacterium tuberculosis* and the lipophosphoglycan (LPG) of *Leishmania* protozoa) as part of a two-pronged approach toward developing receptors for these glycoconjugates[4] to be used in drug targeting[5] toward their respective organisms. We are more broadly concerned with use of boronates in receptors for cell-surface carbohydrates,[6] inositols,[7] and α-hydroxycarboxylic acids.[8] Additionally, we are interested in studying both neutral and cationic receptors for the important second messenger phosphatidylinositol-4,5-bis(phosphate) using an azacrown core structure described here.

Our complete receptor design is shown in Figure 1. The azacrown is oriented symmetrically to charge balance the 4,5-bis(phosphate) while placing the aromatic ring beneath the hydrophobic face (composed of three axial hydrogens) of the inositol ring.[9] This symmetry element in the receptor reduces the entropic penalty of binding as interaction with either face of the benzene ring will situate its substituents identically. Anslyn has shown that the hexa-anion inositol 1,4,5-tris(phosphate) binds robustly to a hexaguanidium receptor on an aromatic core.[10] One binding element that can differentiate PIP_2 from other phosphorylated inositols, specifically those with phosphates at C-3, is a boronic acid. Reversible covalent binding to *cis*-vicinal diols has made boronic acids an important class of artificial carbohydrate receptors.[11] The boron may also interact with a phosphate oxygen at C1 in a manner similar to that observed with the N-acetyl group of sialic acid upon boronate binding of its glycerol tail.[12] As early as 1980, it was established that boronates had an

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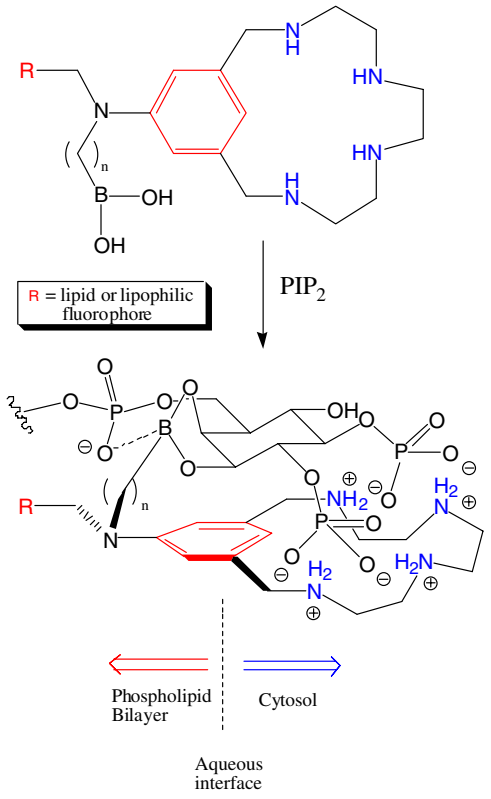
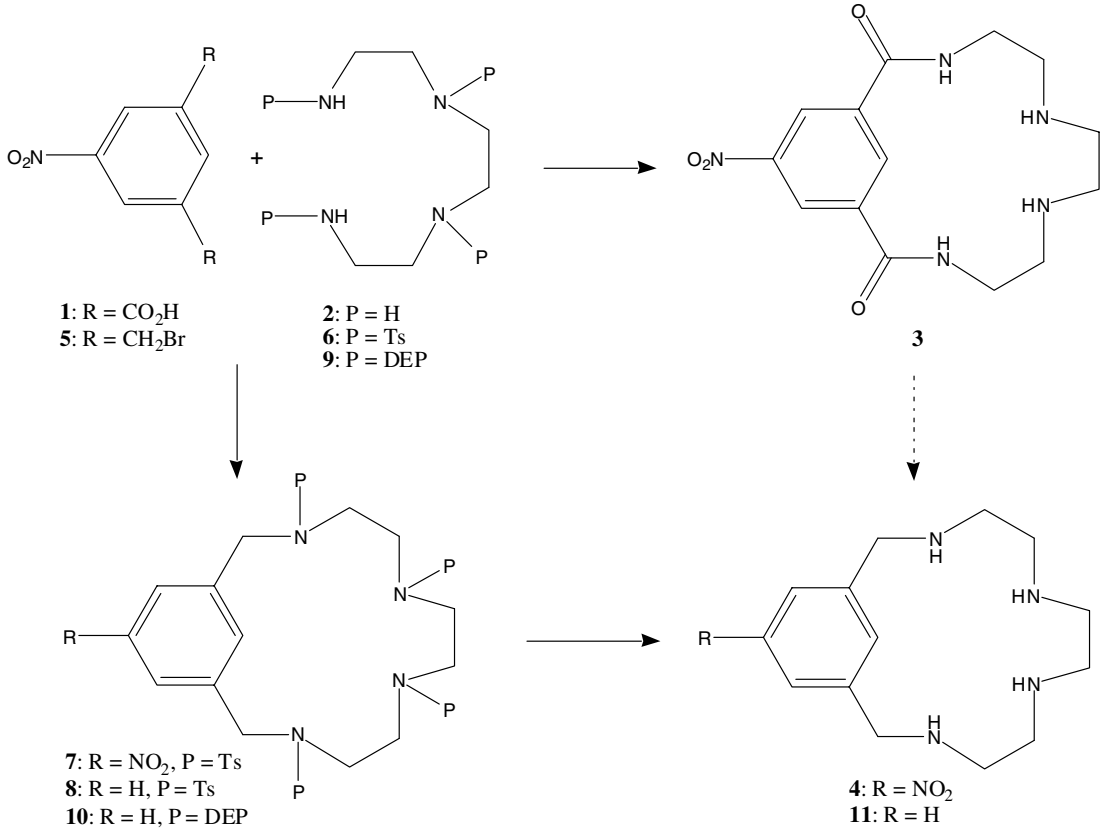


Figure 1. PIP_2 receptor with hydrophobic (red) and hydrophilic (blue) recognition components.

appreciable affinity for cell surfaces.[13] Liposomes loaded with boronates have improved affinity for erythrocytes relative to those without.[14] Wang has reported development of bis(boronates) whose affinity has been tuned to bind sialyl Lewis X on the cell surface.[6b] Coupling boronates with cationic groups can greatly enhance the affinity of a receptor for a desired target.[15] By positioning a six-membered aromatic template beneath the cyclohexyl ring of the target guest, it is readily identifiable where addition of a hydrophobic group ('R') is necessary in order to orient our receptor at the membrane interface to bind PIP_2. Synthesis of this core azacrown template in both neutral and cationic forms, as well as a mixture of these two types, is delineated in Scheme 1.

First, attempts were made to couple **1** with unprotected triethylenetetramine (**2**) by taking advantage of the *meta* disposition of the phenylene linker in the azacrown target structures. By forming an amide bond with a primary amine in **2**, cyclization through a second amide bond formation should be favored at the distal primary amine to provide **3** as the major product. Cyclization at an internal secondary amine to yield a 12-membered macrocycle was expected to be disfavored due to ring strain (compared to the 15-membered ring in **3**) and distortion of both amide conformations. Indeed, slow addition of **2** to a solution of **1** and EDC in $CHCl_3$ yielded **3** in modest yield (23%). This macrocycle contains a mixture of both neutral and cationic anion-bind-



Scheme 1. Synthesis of neutral, cationic, and 'mixed' azacrown templates.

ing functionality in the pairs of amides and amines. As the nitrogen atoms closest to the aromatic ring of the receptor will be nearer the membrane interface if the proposed binding model is correct, neutral groups may interact with the phosphates as effectively as charged species. This system seemed a useful synthetic precursor to the fully cationic azacrowns; however, attempts to selectively (to furnish **4**) or globally reduce this macrocycle to the target structures met with only limited success. Unfortunately, **3** decomposed slowly in air to brown biproducts that were not identified and this severely compromised its usefulness as a synthetic intermediate.

Next, an alkylative macrocyclization route based on literature precedent was attempted with dibromide **5**[16] and tetratosylate **6**[17] that produced a reasonable yield of the target compound **7** (67%) and the simplified structure **8**[17] (65%) from α,α′-dibromo-*m*-xylene. This first of our desired core structures contain a nitrogen functionality that harbors no hydrogens capable of hydrogen bonding anions such as phosphates, but the strong dipole of the sulfonamides may still bind PIP_2 at membrane interfaces. It will be interesting to compare binding of this neutral compound in relation to the free amine structures described below. Deprotection of this compound under a variety of conditions (e.g., HBr/HOAc,[18] sodium napthalide[19]) and temperatures produced only disappointing conversion to the cationic azacrown. Harsher conditions produced inextractable mixtures, while milder conditions provided limited deprotection. For this, an alternate protecting group strategy was explored using phosphonamide **9**.[20a]

Reaction of **9** with α,α′-dibromo-*m*-xylene furnished **10** and the deprotection now proceeded smoothly with HCl/dioxane to generate the second target core structure **11** (33% for two steps).[20] Thus, the framework is in place to generate families of neutral and cationic receptors for membrane-sequestered phosphoinositides. Whether any of these azacrowns alone have appreciable affinity for inositol phosphates remains to be determined. At present there are still no de novo synthetic receptors capable of selective recognition of PIP_2 at membrane interfaces, but the work described here is being applied to this end. Such a compound will be useful in dissecting the intricacies of complex cell-signaling pathways where PIP_2 occupies a crucial message branch point. It will provide complementary information to signal disruption by polycations, such as neomycin, whose affinity for PIP_2 and IP_3 is less discretionary.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.02.073.

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N,N′,N″,N‴-Tetratosyl-5′-nitro-2,6,9,13-tetraaza[14]metacyclophane (**7**): To a stirred solution of tetratosyl-triethylentetraamine **6** (0.679 g, 0.890 mmol) in 60 mL MeCN was added excess potassium carbonate. The mixture was heated to reflux at which time α,α′-dibromo-5-nitro-*m*-xylene (**5**) (0.275 g, 0.890 mmol) in 50 mL of heated MeCN was added dropwise over 1.5 h via addition funnel. The mixture was left to reflux for 18 h at which time potassium carbonate was filtered from the mixture. The filtrate was concentrated to give a brown residue. The product was isolated in 67% yield on a silica column in 99:1 DCM/ethyl acetate. Theoretical: 0.810 g, experimental: 0.543 g; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (s, 2H), 7.77 (d, $J = 8.1$ Hz, 4H), 7.65 (d, $J = 8.1$ Hz, 4H), 7.40 (d, $J = 7.5$ Hz, 4H), 7.31 (d, $J = 7.5$ Hz, 4H), 7.27 (s, 1H), 4.25 (s, 4H), 3.09–3.06 (m, 4H), 2.97–2.94 (m, 4H), 2.76 (s, 4H), 1.57 (s, 4H). ESI-MS $[M+H]^+$, calculated: 910.2, found: 910.3.
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2,5,8,11-Tetraaza[14]metacyclophane (**11**): To a stirred solution of commercial α,α′-dibromo-*m*-xylene (1.0 g, 3.8 mmol) in 60 mL MeCN was added excess potassium carbonate and this mixture was heated to reflux. Polyamine **9** (2.38 g, 3.8 mmol) was dissolved in hot MeCN (50 mL) and added to the mixture dropwise via addition funnel. The mixture was left to reflux for 8 h and was then cooled and diluted with deionized water (50 mL). The aqueous solution was extracted with DCM (4×20 mL). The organic extracts were combined, dried over sodium sulfate, and concentrated to give a gelatinous residue. The residue was dissolved in 20 mL dioxane saturated with HCl gas. This was allowed to stir overnight at room temperature. Diethyl ether was added and the azacrown precipitated as the hydrochloride salt to give **11** in 33% yield. Theoretical: 0.958 g, experimental: 0.316 g; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.69 (s, 1H), 7.37–07.11 (m, 3H), 3.92 (s, 4H), 2.71 (m, 8H), 2.65 (s, 4H).