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Dimerization and Anion Binding of a Fluorescent Phospholipid Analogue

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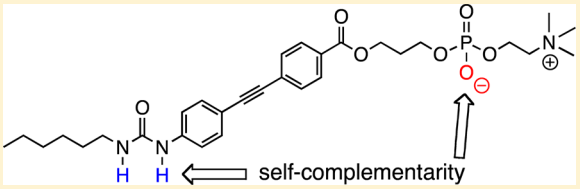
Supporting Information

ABSTRACT: A diarylacetylene fluorophore featuring spatially separated urea and phosphocholine (PC) groups forms a macrocyclic "head-to-tail" dimer stabilized by $NH_{urea}\cdots OP_{PC}$ hydrogen bonds. At concentrations above ~2×10^{-5} M in CH_2Cl_2, the emission intensity of the dimer is quenched by HCO_3^- and $H_2PO_4^-$ but not by Cl^- and NO_3^-. Under more dilute conditions, all four anions are bound unselectively with association constants on the order of 10^5 M^{-1}.

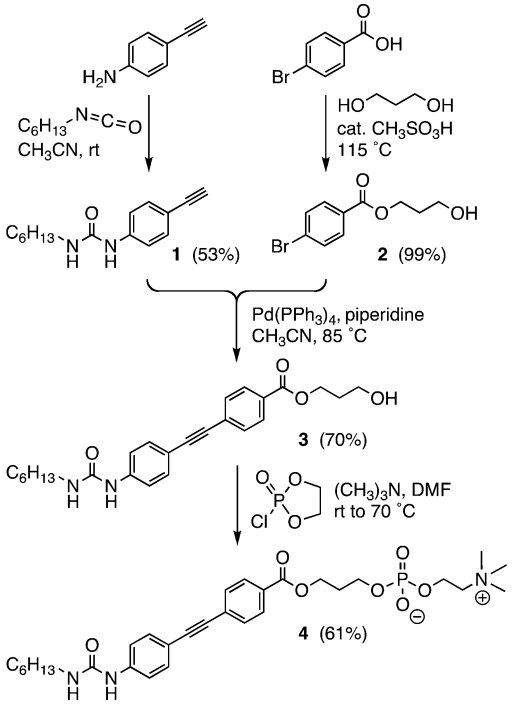


Poorly regulated movement of anions across cell membranes lies at the root of a number of diseases.[1,2] A small but operationally diverse set of synthetic transporters have been developed to facilitate diffusion of species like chloride and bicarbonate through lipid bilayers. Low molecular weight "shuttles" shield the ions of interest within a nonpolar envelope, yielding complexes that can traverse the hydrophobic bilayer core.[3−5] Larger, relatively immobile systems allow anions to pass among electrophilic sites in membrane-spanning single molecules[6,7] or through pores in noncovalent assemblies.[8,9] Regardless of the mechanism of transport, all such systems must recognize their targets near the aqueous interface, a milieu rich in electrically charged phosphate and ammonium groups. Here we evaluate the effect(s) of such groups on anion recognition in organic solution using a urea-based[10−12] fluorescent receptor that bears a biologically relevant phosphocholine (PC) unit.

Synthesis of the lipid analogue is shown in Scheme 1. 4-Ethynylaniline reacts slowly with hexyl isocyanate at room temperature to afford **1**. This alkyne was expected to impart organic solubility to subsequent products while setting the anion-binding site in conjugation with the final diarylacetylene reporter.[13,14] To ensure that any inductive effects of the PC would not alter the acidity of the urea, a simple 1,3-propanediol spacer, inspired by the glycerol found in naturally occurring lipids, was incorporated into **2**. Control compound **3** precipitates from a Sonogashira coupling mixture of **1** and **2** in sufficient purity for use in spectroscopic measurements. Electron-donor (:NHR) and electron-acceptor (C═O) groups are present at the *para*-positions; so-called "push-pull" chromophores are of interest for their nonlinear optical properties and environmental sensitivity.[15,16] The PC headgroup was attached to **3** by adapting previously published procedures.[17−19] Yields of **4** were less than 5% when acetonitrile was used as the reaction solvent instead of *N*,*N*-dimethylformamide.

Nonionic **3** emits violet light with a large Stokes shift in dichloromethane (λ_{ex} = 324 nm, λ_{em} = 414 nm, Φ_F = 0.43).

Scheme 1



Addition of 30 equiv of a tetraalkylammonium salt of HCO_3^-, $H_2PO_4^-$, Cl^-, or NO_3^- reduces the integrated fluorescence intensity F by 20% (for nitrate) to 70% (for dihydrogenphosphate), while causing λ_{em} to move by 3−5 nm to longer wavelengths. An isoemissive point appears at 505 nm during titration with HCO_3^- (Figure 1) and at 470 nm for NO_3^-, indicative of two-state behavior. Normalized outputs (F/F_0) were plotted vs $[anion]_{total}$ and fit to a hyperbolic function[20] to

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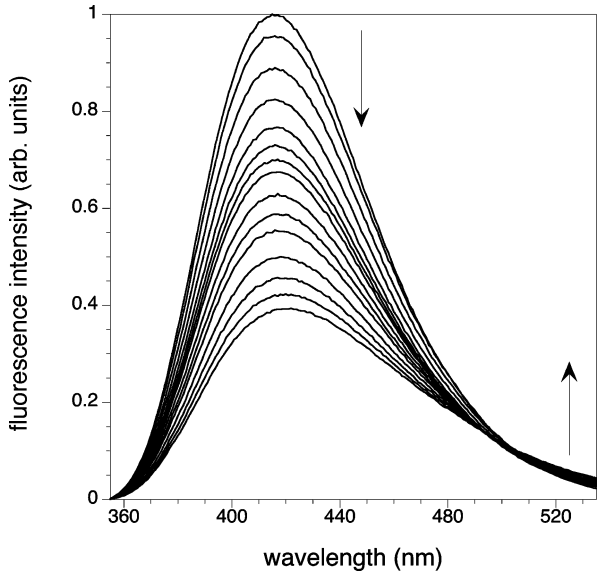

Figure 1. Normalized emission spectra of **3** (1.6×10^{-6} M in CH_2Cl_2) during titration with Et_4NHCO_3 (0 → 30 equiv); λ_{ex} = 320 nm.

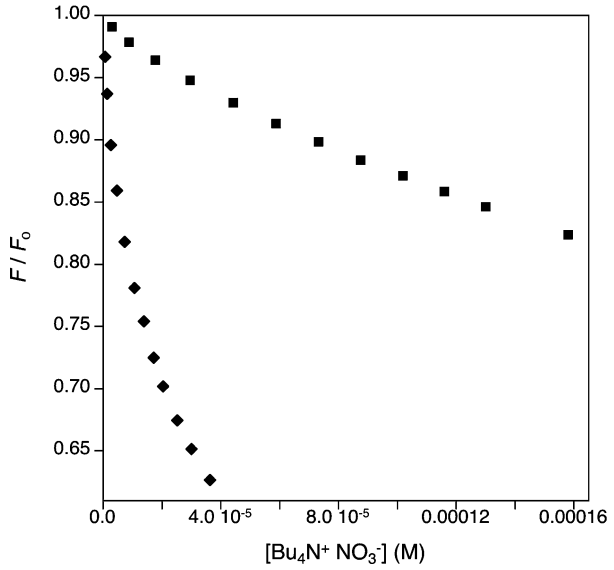

Figure 2. Fluorescence response of **3** (squares) and **4** (diamonds) during titration with Bu_4N^+ NO_3^- in CH_2Cl_2. The concentration of each sensor was 5.0×10^{-6} M.

derive the 1:1 association constants shown in Table 1. The K_{assoc} values for **3** increase with anion basicity.[21] Chloride

Table 1. Association Constants (M^{-1}) in CH_2Cl_2

	HCO_3^{-a}	$H_2PO_4^{-b}$	Cl^-	NO_3^{-b}
3	5.2×10^4	1.2×10^4	$9.6 \times 10^{3,a}$ 7.4×10^{3b}	4.7×10^3
4	$>10^{5c}$	3.7×10^5	2.9×10^{5a}	1.0×10^5

[a]Tetraethylammonium salt. [b]Tetrabutylammonium salt. [c]Titration curve shape is indicative of strong binding; fitting to the equation in ref 20 produced unacceptably large errors.

binding strengths are effectively the same whether Et_4N^+ or Bu_4N^+ countercations are used, and agree well with the reported equilibrium constant for 1-(4-nitrophenyl)-3-octylurea in wet chloroform, ~8×10^3 M^{-1}.[7] For zwitterion **4**, at low concentrations appropriate for quantitative fluorescence measurements (i.e., ≤ 5×10^{-6} M in CH_2Cl_2), the absorption and emission maxima (329 nm, 415 nm) and quantum yield (0.36) are similar to those of its precursor. However, the emission intensity of **4** is strongly suppressed by all four species in Table 1. Figure 2 illustrates the enhancement in K_{assoc} for nitrate, which is typically a poor substrate for synthetic H-bond donors.[22−24] Titrations with bicarbonate yielded binding isotherms with a pronounced "L"-shape, consistent with tight association, that could not be reliably fit.[25] The sensor is significantly less radiative in a competitive solvent mixture (λ_{ex} = 339 nm, λ_{em} = 462 nm, Φ_F = 0.08 for a 4.1×10^{-6} M solution in DMSO containing 0.5% water by volume). Under these conditions, the presence of 50 equiv of a potent quencher, $H_2PO_4^-$, reduces F by just 10%. K_{assoc} is 6.7×10^3 M^{-1}, more than 1 order of magnitude below that observed in dichloromethane.

Electrically charged anion receptors with self-complementary groups are known to form cyclic dimers[26] or linear oligomers,[27] depending on the length and flexibility of the intervening spacer. Electrospray ionization MS of **4** in acetonitrile−water identified a species at m/z = 1175.64, matching the calculated mass of **4**·**4** + H^+, with about one-fifth of the abundance of the monomer at m/z = 588.29. Poor solubility in the NMR concentration regime prevented study of the self-association of **4** in the dichloromethane solvent used for fluorescence, so CD_3CN was used instead. The spectrum of a 2.0×10^{-3} M solution of **4** consists of one set of peaks. A very broad singlet at ~6.15 ppm and a sharper one at 8.68 ppm are assigned to the urea N*H*. At the same concentration, the corresponding protons in **3** appear at 5.29 and 7.36 ppm, respectively. Introducing aliquots of water into acetonitrile samples of **4** induces upfield shifts in both N*H* signals, consistent with a process that replaces relatively robust H-bonds (within the putative dimer) with weaker ones (to water). At 1.5% water by volume, for example, the sharper resonance is ~0.7 ppm removed from its starting point (see the Supporting Information). Density functional theory (DFT) analyses,[28−32] performed in a dielectric continuum corresponding to CH_2Cl_2, further support the conclusion that assembly of **4**·**4** involves hydrogen-bonding at the urea. Its predicted structure is a "head-to-tail" macrocycle (ΔE_{dimer} = −12.8 kcal/mol) with multiple $NH_{urea}\cdots OP_{PC}$ interactions (Figure 3).

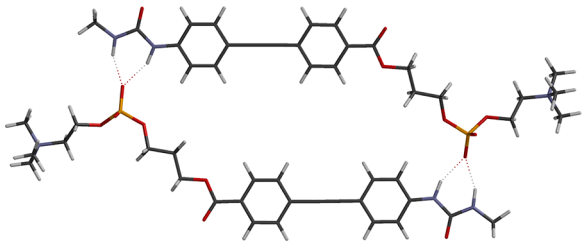

Figure 3. Calculated structure of **4**·**4** in a dichloromethane solvent field. Hexyl groups attached to urea were truncated to methyl prior to DFT optimization.

Electronic absorption spectra of **4** were acquired under several sets of conditions to determine if the absorbance values for the two bands near 280 and 330 nm would vary with the extent of dimerization (Figure 4). In dichloromethane, the ratio A_{281}/A_{332} remains constant at 0.68 as a sample is diluted from 5.3×10^{-5} M to 1.7×10^{-5} M, at which point it begins to fall.

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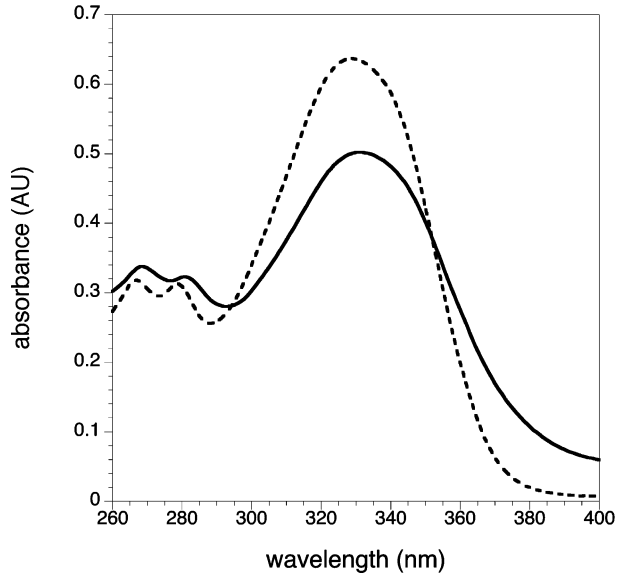

Figure 4. UV/vis traces of **4** in CH_2Cl_2 (—) and CH_3CN (---). [**4**] = 2.6×10^{-5} M.

At a concentration within the range above (2.4×10^{-5} M) in 1-octanol, a solvent with a comparable dielectric constant[33] but with hydrogen-bonding potential, $A_{281}/A_{335} = 0.53$. The ratio is lowest for **4** in acetonitrile (0.49 at 2.2×10^{-5} M). Control compound **3**, which is incapable of phosphodiester-mediated dimerization, has a similar value ($A_{280}/A_{324} = 0.49$ in CH_2Cl_2). For comparison, an electronic excitation profile of **4** in its monomeric form was generated using time-dependent DFT. Calculated transitions occur at 274 and 374 nm with oscillator strengths of 0.2962 and 1.4819, respectively, for a ratio of 0.20. These results seem to define a trend in which samples with higher dimer content have higher absorbance quotients. However, an unexpectedly large value is observed when **4** is dissolved in DMSO-0.5% water ($A_{282}/A_{339} = 0.67$ at 4.1×10^{-5} M). Therefore, the absorbance ratio is perhaps better viewed as an approximate gauge of H-bond strength at the anion recognition site, and not strictly as an indicator for the self-association of **4**.

DFT treatments show that poorly basic anions are not likely to disrupt the hydrogen bonds of **4**·**4**. Starting from the structure of Figure 3, the four anions in turn were placed close to a peripheral $RN(CH_3)_3^+$ unit of an intact dimer, and the resulting complexes were optimized in a CH_2Cl_2 solvent field. Calculated ΔE values for ion-pairing (labeled "Closed" in Table 2) are −6 kcal/mol or greater across the series. When the macrocycle is opened on one side, there is less driving force for binding of Cl^- and NO_3^-. Here, a set of $NH_{urea}\cdots OP_{PC}$ H-bonds was broken, an anion was docked to the free urea, and the nearby choline group was rotated into proximity of the guest ("Opened," Table 2). The best H-bond acceptor, HCO_3^-, benefits from this mode of association, while the energy changes for $H_2PO_4^-$ are too similar to assign a clear preference.[28] Qualitative fluorescence determinations of anion sensitivity, acquired at relatively high sensor concentrations in CH_2Cl_2, are in general agreement with the computational results. Large excesses of Cl^- or NO_3^- do not diminish F for 2.6×10^{-5} M solutions of **4**, but rather cause negligible increases in intensity. Bicarbonate and dihydrogenphosphate ions quench such samples. In previous work with synthetic receptors that feature anion recognition sites isolated from (positive) charges, high affinities were ascribed to entropic gains that occur upon substrate binding.[34,35] Rigidity in the receptors is relieved and an ensemble of energetically low-lying configurations is generated. Separation of **4**·**4** into its component monomers would indeed free the PC groups from conformational restriction. Nevertheless, any positive changes in entropy that may accompany the dissociation event are apparently not large enough to offset the enthalpic cost, at least in a nonpolar solvent.

Table 2. Calculated Binding Energies (kcal/mol) for Anion Complexes in a CH_2Cl_2 Dielectric Continuum

	HCO_3^-	$H_2PO_4^-$	Cl^-	NO_3^-
closed (**4**·**4**·anion$_{choline}$)	−7.0	−6.2	−7.2	−7.3
opened (**4**·anion$_{chol+urea}$·**4**)	−8.2	−5.7	−5.0	−5.5
monomer (**4**·anion$_{urea}$)	−12.0	−10.2	−9.1	−8.8

Structures of the sensor-anion assemblies that are present in the dilute samples used for K_{assoc} determinations are unknown. Proton NMR experiments upon acetonitrile solutions may shed some light on the complexes, since the monomeric state of **4** is accessible in this solvent at millimolar concentrations. Continuous variations plots were generated for **4** in the presence of tetraethylammonium bicarbonate and chloride in acetonitrile-d_3. Maxima for both appear at a sensor mole fraction of 0.4, corresponding to a binding stoichiometry that is intermediate between 1:1 and 1:2 (**4**-to-anion). Ion-pairing and urea H-bonding are present. When 10 equiv of Et_4NCl is added to a 2.0×10^{-3} M CD_3CN solution of **4**, the choline methyl protons at 3.08 ppm experience a downfield shift of 0.07 ppm.[36,37] The N*H* resonances appear at 7.23 and 10.26 ppm under these conditions. Treating **3** with the same amount of chloride ion yields δ_{NH} of 7.19 and 10.18 ppm, suggesting that the coordination environments surrounding Cl^- are similar for both sensors. The urea signals are further downfield for **4** with 10 equiv of Et_4NHCO_3, at 8.56 and 10.97 ppm, in accord with the greater calculated binding energy for bicarbonate vs chloride ("Monomer," Table 2). An NMR-derived association constant for **4** + Cl^- is 2.4×10^3 M^{-1}, a value typical for uncharged urea-containing receptors in acetonitrile.[38,39] Thus, the presence of a PC headgroup does not guarantee unusually strong anion binding in polar solvents.

In summary, dimerization of a fluorescent lipid analogue that bears urea and phosphocholine groups can render it unresponsive toward weakly basic anions. Under conditions where a fraction of the analogue is monomeric (i.e., at micromolar concentrations in CH_2Cl_2, or when it is dissolved in CH_3CN or DMSO), strong anion binding is observed only in nonpolar dichloromethane solvent. Future work will focus on incorporating the sensor into liposomal membranes and evaluating its ability to promote anion transport. Presumably, the directionally organized nature of bilayer molecules will disfavor self-association of the sensor.

EXPERIMENTAL SECTION

1-(4-Ethynylphenyl)-3-hexylurea (1). 4-Ethynylaniline (2.78 g, 23.7 mmol) and hexyl isocyanate (3.08 g, 24.2 mmol) were combined in 60 mL of acetonitrile to afford a tea-colored solution. The mixture was stirred at room temperature under N_2 for 4 d, during which time a precipitate appeared. The solution volume was reduced to approximately 30 mL using a rotary evaporator, and the crude product was collected by filtration and washed with chilled CH_3CN.

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Urea **1** (3.10 g, 53%) was obtained as an off-white powder: mp 129−131 °C; ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H), 1.27 (s, 6H), 1.48 (m, 2H) 3.02 (s, 1H), 3.21 (q, 2H), 5.10 (t, 1H), 6.91 (s, 1H), 7.27 (d, 2H), 7.39 (d, 2H); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.5, 26.6, 30.0, 31.5, 40.5, 76.2, 83.5, 116.6, 119.5, 133.1, 139.4, 155.4; HRMS (ESI-QToF) calcd for $C_{15}H_{21}N_2O$ $(M + H)^+$ 245.1654, found 245.1644.

3-Hydroxypropyl 4-Bromobenzoate (2). A suspension of 4-bromobenzoic acid (0.83 g, 4.1 mmol) and methanesulfonic acid (≪1 drop) in 1,3-propanediol (20 mL, 280 mmol) was heated with stirring to 115 °C for 4 h. The solid disappeared. Upon cooling to room temperature, the contents were poured into 200 mL of water, and the resultant mixture was extracted with EtOAc. The organic phases were combined, washed several times with water and then with saturated aqueous $NaHCO_3$, and dried over anhydrous Na_2SO_4. Filtration and evaporation of the filtrate provided 1.06 g (99%) of **2** as a colorless oil/low-melting white solid: ^{1}H NMR ($CDCl_3$) δ 2.01 (m, 2H), 2.48 (br s, 1H), 3.78 (t, 2H), 4.48 (t, 2H), 7.57 (d, 2H), 7.89 (d, 2H); ^{13}C NMR ($CDCl_3$) δ 32.0, 59.3, 62.4, 128.4, 129.2, 131.3, 132.0, 166.4; HRMS (ESI-QToF) calcd for $C_{10}H_{12}BrO_3$ $(M + H)^+$ 258.9970, found 258.9977.

3-Hydroxypropyl 4-((4-(3-Hexylureido)phenyl)ethynyl)-benzoate (3). A pressure tube was charged with **1** (0.71 g, 2.9 mmol), **2** (0.75 g, 2.9 mmol), piperidine (1.5 mL, 15 mmol), and 8 mL of CH_3CN. With stirring, a stream of N_2 was gently bubbled into the gold suspension for 5 min, and then tetrakis(triphenylphosphine)-palladium(0) (0.067 g, 0.058 mmol) was added. The tube was immediately sealed and lowered into an oil bath that had been preheated to 85 °C. After 15 h, the reaction mixture was allowed to cool to room temperature with slow stirring. The cream-colored precipitate was collected by filtration and washed with chilled CH_3CN to afford 0.86 g (70%) of **3**. Analytical samples were recrystallized from hot EtOAc: mp 188−190 °C; ^{1}H NMR (DMSO-d_6) δ 0.90 (t, 3H), 1.31 (s, 6H), 1.48 (m, 2H), 1.93 (m, 2H), 3.14 (q, 2H), 3.64 (q, 2H), 4.39 (t, 2H), 4.48 (t, 1H), 6.08 (t, 1H), 7.40, (d, 2H), 7.44 (d, 2H), 7.56 (d, 2H), 7.97 (d, 2H), 8.52 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 14.4, 22.6, 26.5, 30.1, 31.5, 32.0, 57.7, 62.7, 87.7, 93.7, 113.9, 117.8, 128.0, 129.8, 131.8, 132.8, 142.2, 155.3, 165.7; FTIR (ATR, solid) ν 3314 cm^{-1}; HRMS (ESI-QToF) calcd for $C_{25}H_{31}N_2O_4$ $(M + H)^+$ 423.2284, found 423.2301; UV/vis (CH_2Cl_2) λ_{max} (ε, M^{-1}cm^{-1}) 324 nm (31000), 280 (16000).

3-(4-((4-(3-Hexylureido)phenyl)ethynyl)benzoyloxy)propyl 2-(Trimethylammonio)ethyl Phosphate (4). Alcohol **3** (0.42 g, 1.0 mmol) was dissolved in 3 mL of stirring *N*,*N*-dimethylformamide in a pressure tube. A solution of 2-chloro-1,3,2-dioxaphospholane 2-oxide (0.71 g, 5.0 mmol) in 1 mL of DMF was added, followed immediately by neat liquid trimethylamine (1 mL, stored at −40 °C). The tube was sealed, and the contents were stirred at room temperature for 4 h. During this time, the gold mixture became opaque. The tube was then heated to 70 °C for an additional 6 h. Upon cooling, the reaction vessel was cautiously opened, and the liquid portion was transferred directly onto a silica gel flash chromatography column that had been saturated with CH_2Cl_2−CH_3OH−H_2O (30:60:10, respectively, by volume). Fractions containing the desired compound (R_f = 0.17) were combined and evaporated to a viscous yellow oil that solidified upon standing under vacuum. The yield of **4** was 0.36 g (61%). Analytical samples were purified by RP-HPLC on a C_{18} column using a gradient of 50:50 CH_3CN−H_2O → 80:20 CH_3CN−H_2O, then were lyophilized to afford a white fluffy solid: mp 134−148 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H), 1.28 (br s, 6H), 1.43 (m, 2H), 2.04 (m, 2H) 3.08 (m, 2H), 3.13 (s, 9H), 3.59 (m, 2H), 4.00 (m, 2H), 4.24 (br s, 2H), 4.37 (t, 2H), 6.35 (t, 1H), 7.44 (d, 2H), 7.47 (d, 2H), 7.65 (d, 2H), 7.98 (d, 2H), 8.82 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 14.4, 22.6, 26.5, 30.1, 31.5, 44.7, 52.7, 62.5, 63.2, 65.6, 87.7, 94.0, 113.6, 117.7, 128.3, 129.1, 130.1, 131.8, 132.8, 142.4, 155.4, 165.6; HRMS (ESI-QToF) calcd for $C_{30}H_{43}N_3O_7P$ $(M + H)^+$ 588.2839, found 588.2836; UV/vis (dry CH_2Cl_2, 2.6 × 10^{-5} M) λ_{max} (ε, M^{-1} cm^{-1}) 332 nm (19000), 281 (12000), 268 (13000), (DMSO, 5.0 × 10^{-5} M) λ_{max} (ε, M^{-1} cm^{-1}) 339 nm (12500), 281 (8800), 270 (9200); fluorescence (CH_2Cl_2, < 5 × 10^{-6} M) λ_{em} 415 nm, (CH_2Cl_2, > 2 × 10^{-5} M) λ_{em} 425 nm.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^{1}H/^{13}C NMR spectra for new compounds, representative fluorescence titration data, binding and Job plots from NMR, and atomic coordinates of DFT-calculated structures. 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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