Water-Soluble Receptors for Cyclic-AMP and Their Use for Evaluating Phosphate–Guanidinium Interactions

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Received October 5, 1993®

Abstract: A water-soluble receptor for adenosine derivatives was synthesized for the study of molecular recognition in aqueous solution. The modular receptor makes use of hydrophobic interactions, Watson–Crick and Hoogsteen hydrogen-bonding, and a phosphate–guanidinium electrostatic interaction to bind cyclic adenosine monophosphates. Measured binding affinities of 2′,3′-cAMP are −3.65 and −3.26 kcal/mol at 51 and 501 mM ionic strength, respectively (H_2O/D_2O solution at 10 °C, pH 6.0). The phosphate–guanidinium interaction in this system is estimated to contribute on average 0.6 kcal/mol (51 mM ionic strength) and 0.3 kcal/mol (501 mM ionic strength) to binding. The maximum value of a phosphate–guanidinium electrostatic interaction is estimated to be 2.4 kcal/mol in water.

Introduction

Weak intermolecular interactions drive molecular recognition phenomena, and bioorganic chemistry continues to explore these forces with synthetic receptors. Adenine has been a particularly well-studied target for hydrogen-bonding and aromatic stacking in less competitive organic solvents.[1-7] We have recently developed a system for the recognition of adenine in aqueous media[8] and report here on the use of a similar system to evaluate the contribution of electrostatic interactions to the binding of cyclic adenosine phosphates.

The adenine-binding module of receptors **1** and **2** (Figure 1) combines the 3,6-diaminocarbazole spacer, previously described,[9-11] with the water-soluble version of the Kemp's triacid imide.[8] The concave surface of this module chelates the purine nucleus of adenine through simultaneous Watson–Crick and Hoogsteen hydrogen bonding and aryl stacking.[11] Introducing a phenyl group on N-9 of the diaminocarbazole offers increased hydrophobic contacts, decreased conformational flexibility, and increased ester stability in hydroxylic media.

The receptors were assembled as outlined in Schemes 1–3. The water-soluble derivative of Kemp's triacid imide (**5c**) was prepared as described previously (Scheme 1).[8] Monodeprotection of bicyclic guanidinium **6**[12] proceeded quantitatively under mild conditions to give alcohol **7** (Scheme 2). The scaffolding for receptors **1** and **2** was assembled by Ullmann-type coupling[13] of carbazole and methyl 4-iodobenzoate (**8**). Nitration of **9** with nitric acid proceeded easily in warm acetic acid to give dinitro **10a**. Diamine **10b**, generated by hydrogenation in THF, was air-sensitive and was immediately acylated with imide acyl chloride **5c** to generate the benzyl-protected **11a**. Deprotection of this with gaseous HBr in HCOOH gave cleft **2**. Hydrolysis of **11a**, followed by conversion to the acyl chloride (**11b**) gave protected **12a** after reaction with alcohol **7**. This product contained some **11b** as an inseparable impurity that could be removed only after deprotection of **12a** to the free alcohol (**12b**). Debenzylation with gaseous HBr yielded pure phosphate-binding cleft **1** after ion-exchange chromatography.

Results and Discussion

The binding free energies were determined by ^{1}H NMR titration (Table 1). The downfield shift (from 10.4 to 12.5 ppm) of the exchangeable imide proton was monitored during constant-host titration of the receptors (0.3–0.5 mM) with the adenosine derivatives (30 or 150 mM for 9-ethyladenine) at ionic strength (I) of 51 and 501 mM (NaCl) under conditions previously described.[8] The interaction between the cacodylate buffer and **1** was considered negligible since no proton shift of the dihydroxybicycloguanidinium was observed on the NMR upon varying cacodylate concentration. Furthermore, Haake *et al.* have shown that free guanidinium ($CH_6N_3^+$) and free phosphate ($H_2PO_4^-$) have an association constant of only 1.37 M^{-1} in water.[14] Reduced pH and temperature serve to sharpen the signal due to the imide protons by reducing the rate of chemical exchange with water. The water signal was suppressed using a $1\bar{3}3\bar{1}$ binomial pulse.[15,16] Association constants were obtained by nonlinear least-squares regression analysis and converted to free energies, ΔG^{283}. The binding constants were reproducible to within 10%. The statistical uncertainty in the curve-fitting procedure varied from ±2 to 17% at the 95% confidence level. When propagating error through the calculations though, the uncertainty was taken to be no less than 10%. For the charged guests with negligible dimerization constants (*i.e.* cAMPs), a simple 1:1 binding model was used.[17] For adenosine and 9-ethyladenine, guest dimerization was incorporated into the 1:1 binding model. Incorporation of this

* Abstract published in *Advance ACS Abstracts*, March 15, 1994.

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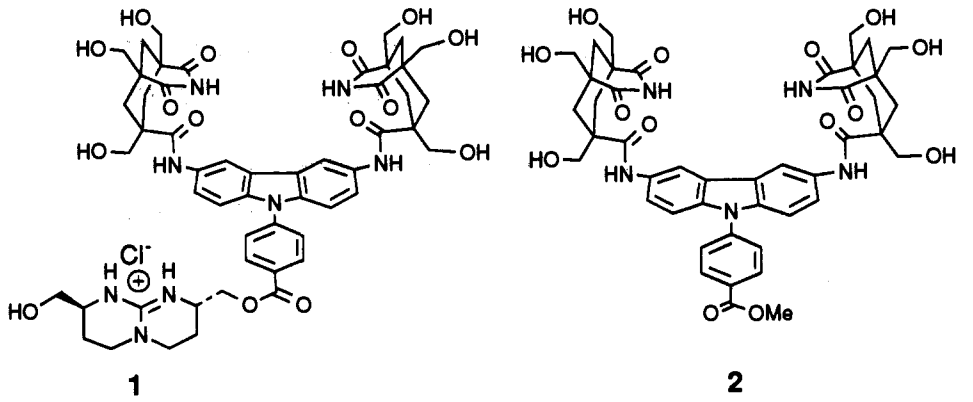
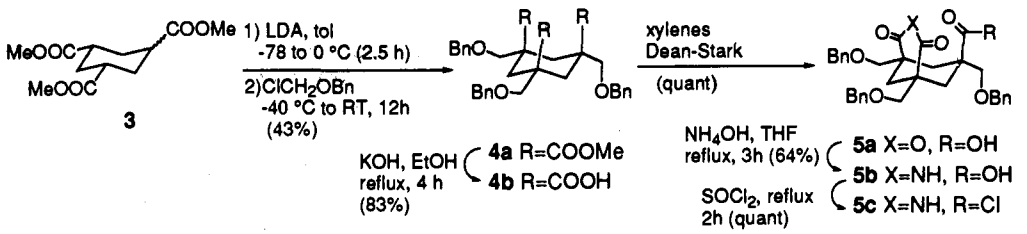


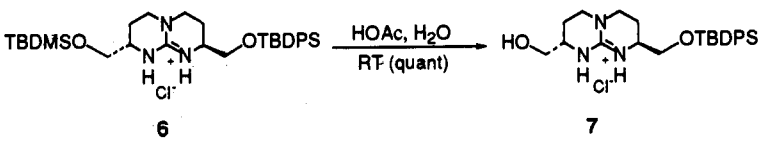
1 2

Figure 1.

Scheme 1



Scheme 2



additional equilibrium into the 1:1 binding isotherm gives the following nonlinear multivariable expression:

$$G_t = H_t\left(\frac{\delta - \delta_H}{\delta_{HG} - \delta_H}\right)^3 + \left(\frac{2K_d}{K_a^2} - G_t - 2H_t - \frac{1}{K_a}\right)\left(\frac{\delta - \delta_H}{\delta_{HG} - \delta_H}\right)^2 + \left(2G_t + H_t + \frac{1}{K_a}\right)\left(\frac{\delta - \delta_H}{\delta_{HG} - \delta_H}\right) \quad (1)$$

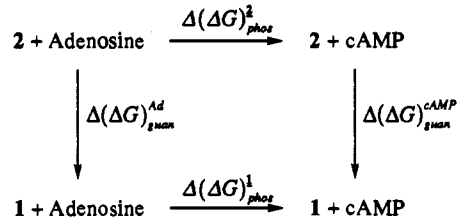
where G_t and H_t are the total concentration of guest and host at each titration point; K_d and K_a are the dimerization and 1:1 association constants, respectively; and δ, δ_H, and δ_{HG} are respectively the observed, free host, and complex chemical shifts.

The dimerization constant of 9-ethyladenine was determined at both 51 mM (11 M^{-1}) and 501 mM (16 M^{-1}) ionic strength. The respective values were incorporated into the binding constant determinations with 9-ethyladenine at both ionic strengths. The dimerization constant of adenosine was determined only at 51 mM. Moderate to high binding constants show only small changes with small variations in dimerization (confirmed with 9-ethyladenine data), and significantly lower concentrations of adenosine were used in titrations. The 5.2-M^{-1} value was used in the determination of binding constants at both 51 and 501 mM.

Receptor **2** shows nearly identical affinity to all four adenine derivatives with only slightly lower (0.03–0.08 kcal/mol) affinity at higher ionic strength. This reflects the sum of contributions from hydrogen-bonding and hydrophobic stacking with the purine nucleus, as well as the relative insensitivity of these forces to ionic strength. The similar affinity for 9-ethyladenine and adenosine shown by receptors **1** and **2** indicates that there is little or no interaction between the guanidinium and the hydroxyl groups of adenosine. With 2′,3′- and 3′,5′-cAMP, the phosphate–guanidinium interaction leads to significant (I = 51 mM) or moderate (I = 501 mM) increase in binding affinity (2′,3′- and 3′,5′-cAMP with **1**, Table 1).

Molecular modeling studies indicated that the lowest energy conformation of the complex of **1** and 2′,3′-cAMP features a hydrogen bond between the hydroxyl proton (exocyclic to the guanidinium) and phosphate and a coplanar arrangement of the two delocalized charges at the phosphate and guanidinium. That is, hydrogen-bonding can occur between the guanidinium protons and phosphate oxygens in addition to an electrostatic interaction (Figure 2). Conversely, the corresponding complex of **1** and 3′,5′-cAMP (Figure 3) suggests a perpendicular geometry between the two planes of delocalized charge, enabling electrostatic interactions but precluding a hydrogen-bonding interaction of a salt-bridge sort.

The individual contribution of this electrostatic interaction can be approximated through the use of the following thermodynamic cycle:



(2)

where each $\Delta(\Delta G)$ is the free energy difference associated with an alteration in the host–guest composition. The free energy change associated with the guanidinium–phosphate interaction alone can be derived from this cycle according to eq 3.

$$\Delta(\Delta G)_{phos\text{-}guan} = \Delta(\Delta G)^1_{phos} - \Delta(\Delta G)^2_{phos} = \Delta(\Delta G)^{cAMP}_{guan} - \Delta(\Delta G)^{Ad}_{guan} \quad (3)$$

Scheme 3

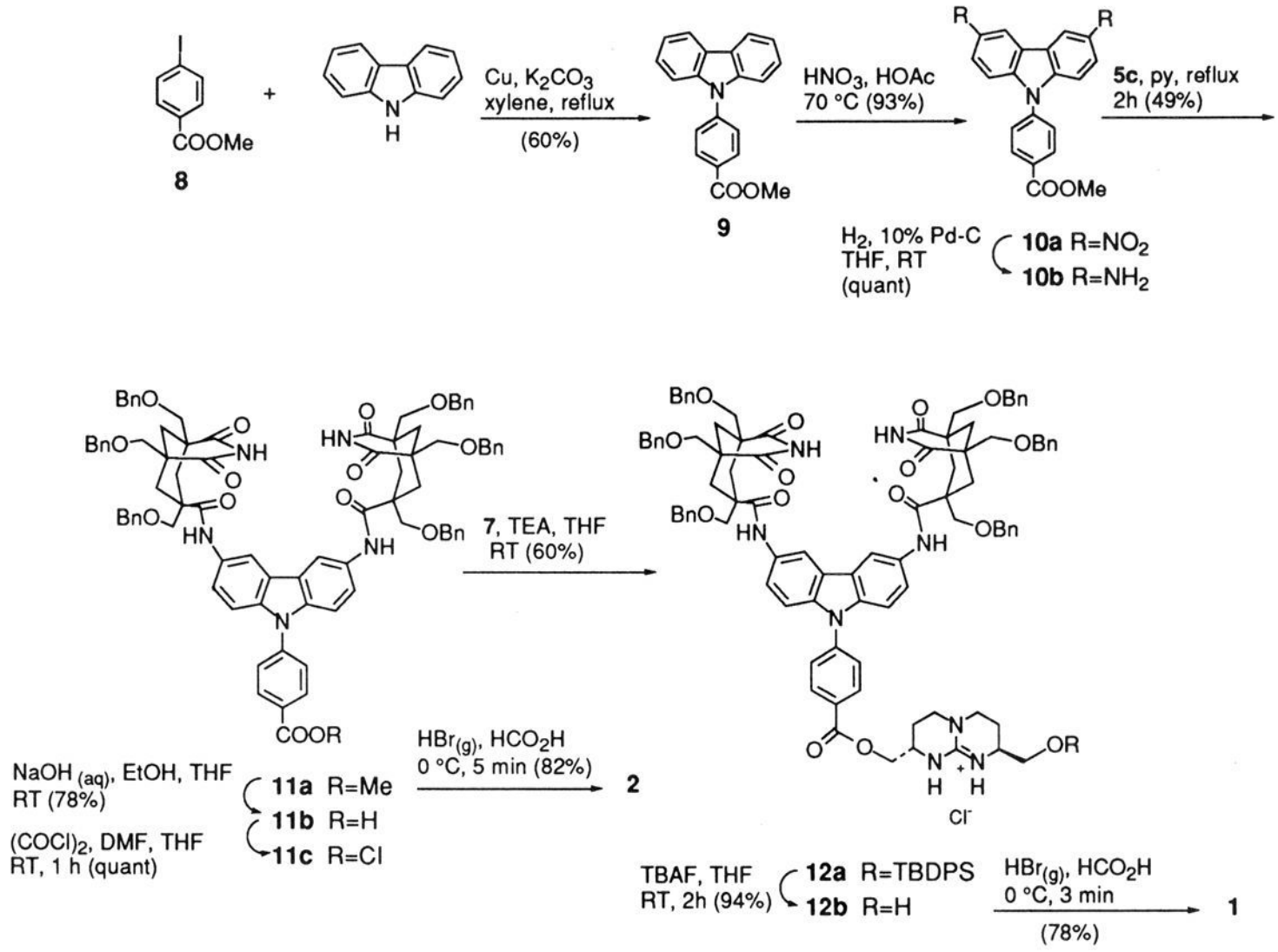


Table 1. Energy of Binding between Receptors and Adenosine Derivatives

		K_a (M^{-1}) ± % uncertainty		ΔG^{283} (kcal/mol)	
receptor	guest	I = 51 mM	I = 501 mM	I = 51 mM	I = 501 mM
1	9-ethyladenine	200 ± 6%[a]	180 ± 10%[b]	−2.98 ± 0.06[a]	−2.92 ± 0.06[b]
	adenosine	150 ± 5%[c]	130 ± 10%[c]	−2.82 ± 0.06[c]	−2.74 ± 0.06[c]
	2′,3′-cAMP	660 ± 17%	330 ± 9%	−3.65 ± 0.10	−3.26 ± 0.06
	3′,5′-cAMP	600 ± 6%	320 ± 3%	−3.60 ± 0.06	−3.24 ± 0.06
2	9-ethyladenine	190 ± 4%[a]	180 ± 7%[b]	−2.95 ± 0.06[a]	−2.92 ± 0.06[b]
	adenosine	150 ± 13%[c]	140 ± 14%[c]	−2.82 ± 0.07[c]	−2.78 ± 0.08[c]
	2′,3′-cAMP	190 ± 3%	180 ± 2%	−2.95 ± 0.06	−2.92 ± 0.06
	3′,5′-cAMP	250 ± 6%	220 ± 9%	−3.11 ± 0.06	−3.03 ± 0.06

[a] A 9-ethyladenine dimerization constant of 11 M^{-1} was incorporated into the calculation. [b] A 9-ethyladenine dimerization constant of 16 M^{-1} was incorporated into the calculation. [c] An adenosine dimerization constant of 5.2 M^{-1} was incorporated into the calculation.

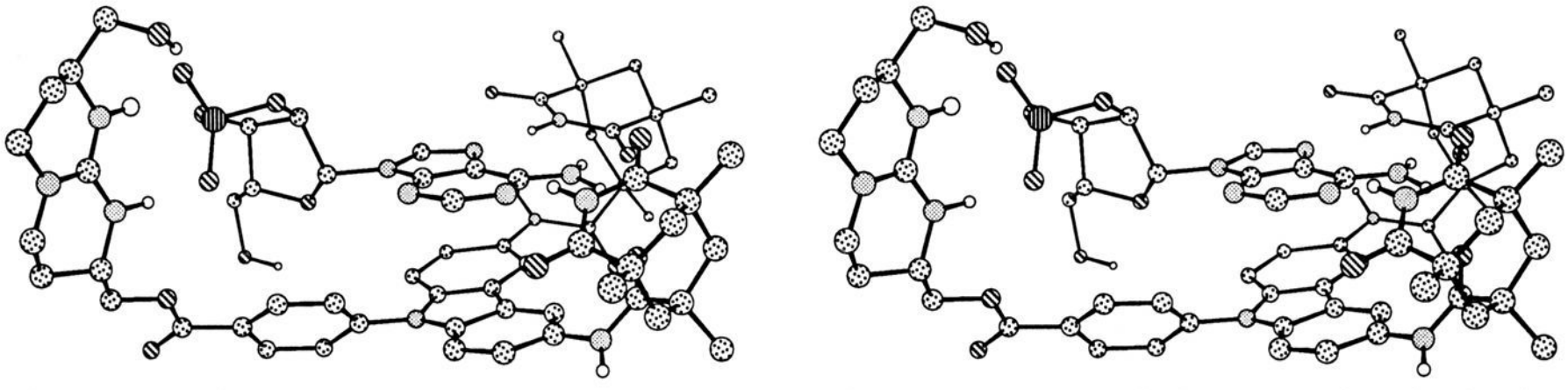


Figure 2. Predicted lowest energy conformation of the complex between **1** and 2′,3′-cAMP in stereoview. Hydrogens attached to carbon have been omitted for clarity.

This gives energies for phosphate–guanidinium interactions alone in aqueous solution at pH 6.0 and 10 °C (Table 2) which show that the 2′,3′-cAMP complex compared to the 3′,5′-cAMP complex has a 0.2 kcal/mol stronger interaction at either ionic strength. The molecular modeling studies indicated that there could be *up* to three additional hydrogen bonds in the complex of **1** with 2′,3′-cAMP. This suggests that the strength of a charged hydrogen bond exposed in water is lower than previously obtained in protein mutagenesis studies[18] (0.5–1.5 kcal/mol for neutral–neutral hydrogen bonds and 3–5 kcal/mol for neutral–charged hydrogen bonds). It should be noted that these values are indeed generalized values for interactions in free solution, in contrast to protein mutagenesis studies, where there are complications from

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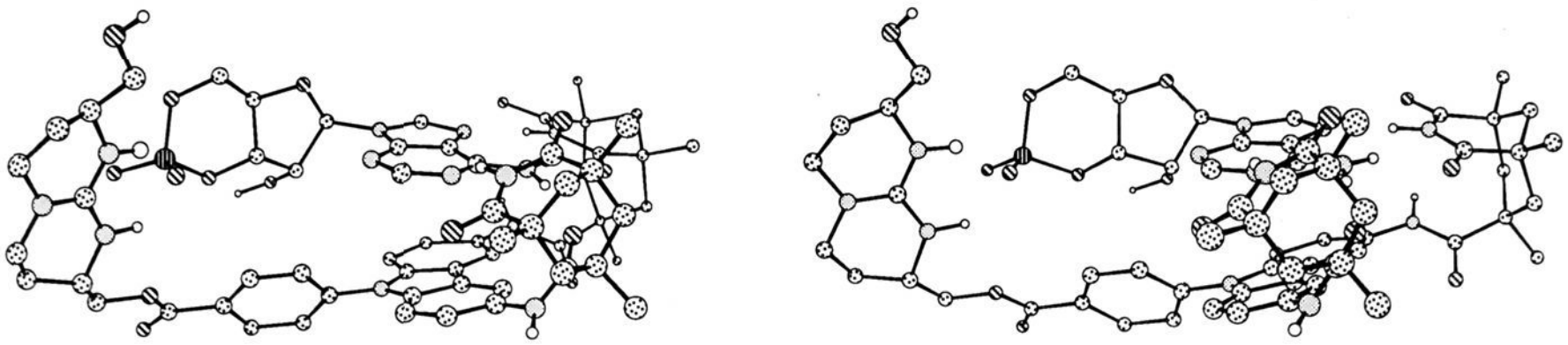

Figure 3. Predicted lowest energy conformation of the complex between **1** and 3′,5′-cAMP in stereoview. Hydrogens attached to carbon have been omitted for clarity.

Table 2. Free Energy Change for the Phosphate–Guanidinium Interaction at Different Ionic Strengths, Accurate tu ±0.1 kcal/mol

guest	$\Delta(\Delta G)_{phos-guan}$ at I = 51 mM	$\Delta(\Delta G)_{phos-guan}$ at I = 501 mM
2′,3′-cAMP	–0.7 kcal/mol	–0.4 kcal/mol
3′,5′-cAMP	–0.5 kcal/mol	–0.2 kcal/mol

altered backbone geometry, context-specific effects, and the nonaqueous environment of a protein interior.

By considering the complex between **1** and 3′,5′-cAMP, an estimate of the energy of a phosphate–guanidinium electrostatic interaction alone can be derived. According to the analyses of Williams,[19-23] based on the pioneering work of Jencks and Page,[24,25] the phosphate–guanidinium interaction can be considered as a combination of energy factors arising from intermolecular translational and rotational entropy (T + R), restriction of rotors (r), hydrophobicity (h), polar interactions (p), conformational strain (conf), and van der Waals interactions (vdW). Thus,

$$\Delta(\Delta G)_{phos-guan} = \Delta\Delta G_{T+R} + \Delta\Delta G_r + \Delta\Delta G_h + \Delta(\sum \Delta G_p) + \Delta\Delta G_{conf} + \Delta\Delta G_{vdW} \quad (4)$$

By virtue of the similarity of the two substrates involved in the comparison, adenosine and cyclic adenosine monophosphate, the terms associated with bimolecular association, hydrophobicity, conformational strain, and van der Waals interactions can all be estimated to have a negligible contribution to the interaction (*i.e.* $\Delta\Delta G_{T+R}, \Delta\Delta G_h, \Delta\Delta G_{conf}, \Delta\Delta G_{vdW} \approx 0$). The factorization, then, is represented by eq 5.

$$\Delta(\Delta G)_{phos-guan} = \Delta\Delta G_r + \Delta(\sum \Delta G_p) \quad (5)$$

In the complex between **1** and 3′,5′-cAMP, there are two bond rotors (the guanidinium exocyclic C–C and C–O bonds) that are constrained relative to the complex with adenosine. The cost of restricting a single rotor through noncovalent interactions has been estimated as 0.9 ± 0.2 kcal/mol.[26] So, the phosphate–guanidinium interaction can be estimated to contribute up to a *maximum* of 2.4 ± 0.2 kcal/mol at 51 mM ionic strength (or 2.1 ± 0.2 kcal/mol at 501 mM ionic strength). If either of these rotors is restricted in the uncomplexed state or not confined in the complexed state, then this value will be accordingly lower.

In conclusion, a highly organized synthetic receptor binds cyclic adenosine monophosphates through a combination of hydrophobic, electrostatic, and hydrogen-bonding forces. Assuming these effects act independently, the phosphate–guanidinium interaction in this system is calculated to contribute, on average, 0.6 kcal/mol (I = 51 mM) and 0.3 kcal/mol (I = 501 mM) to binding. The enthalpy of such an electrostatic interaction in water alone is estimated to be no more than 2.4 kcal/mol.

These receptors have been elaborated to recognize di- and trinucleotides, and we will report on these developments in due course.

Experimental Section

Synthesis. General. Air-/water-sensitive reactions were performed in flame-dried glassware under argon. Tetrahydrofuran was distilled from Na/benzophenone ketyl. Toluene was distilled from calcium hydride. Unless otherwise stated, all other commercially available reagents were used without further purification.

^{1}H NMR spectra were obtained on Bruker AC-250, Varian XL-300, Varian UN-300, and Varian VXR-500 spectrometers. All chemical shift values are reported in parts per million downfield from TMS for organic solvents, or DSS for H_2O/D_2O. Spectra taken in $CDCl_3$ were referenced to either TMS (0.00, for compounds with overlapping aromatic protons) or residual $CHCl_3$ (7.26). Spectra taken in DMSO-d_6 were referenced to residual solvent (2.50). Fourier transform infrared spectra were taken on a Perkin-Elmer infrared spectrometer. Flash chromatography was performed using Silica Gel 60 (EM Science, 230–400 mesh).

Trimethyl Cyclohexane-1,3,5-tricarboxylate (3) was prepared according to Askew *et al.*[27]

Tribenzyloxy Triester 4a. Trimethyl ester (5.003 g, 19.5 mmol) in toluene (100 mL) was added dropwise over a 30-min period to a –78 °C solution of LDA (1.5 M in cyclohexane, 45 mL, 67.5 mmol) in toluene (100 mL). Once the addition was complete, the solution was stirred at –78 °C for an additional 30 min. It was then brought to 0 °C in an ice bath and stirred for 30 min, during which time the solution became noticeably more viscous. The solution was cooled to –40 °C for 15 min. Benzylchloromethyl ether (8.5 mL, 62 mmol) was then added to the solution, which gradually cleared. The solution was stirred for another 45 min at –40 °C, 45 min at –20 °C, 45 min at 0 °C, and then for an additional 3 h at 25 °C. It was then quenched with saturated NH_4Cl (200 mL). The resulting solution was extracted with ether (300 mL). The ether layer was washed with HCl (2 × 150 mL, 1.2 M), NaOH (2 × 150 mL, 1 M), and then with brine (100 mL). The solution was dried (Na_2SO_4) and then evaporated *in vacuo* to give 12.266 g of brown, viscous oil, which was recrystallized from MeOH (100 mL) to yield 4.143 g of colorless crystals. A second crop of 1.022 g was obtained from the mother liquor. The overall yield was 5.165 g (43%): mp 110–112 °C; IR (KBr) 3050, 2950, 1740, 1730 cm^{-1}; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.4–7.1 (m, 15 H), 4.45 (s, 6 H), 3.70 (s, 6 H), 3.35 (s, 9 H), 2.70 (d, 3 H, J = 13 Hz), 1.19 (d, 3 H, J = 13 Hz); HRMS (EI) calcd for $C_{36}H_{42}O_9$, 618.2829; found, 618.2840.

Tribenzyloxy Triacid 4b. A solution of triester (1.635 g, 2.65 mmol) and KOH (4.568 g, 81 mmol) in EtOH (50 mL) was refluxed for 4 h. Ethanol was then removed *in vacuo*, and the resulting slurry was poured into water (100 mL). The aqueous layer was washed with ether (2 × 50 mL), acidified (concentrated HCl), and then extracted with ether (2 × 100 mL). Drying (Na_2SO_4) and evaporation of the organic layer gave

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1.253 g (83%) of the triacid as a pale yellow solid, which was used without further purification: ^{1}H NMR (250 MHz, DMSO-d_6) δ 12.15 (br s, 3 H), 7.4–7.1 (m, 15 H), 4.40 (s, 6 H), 3.42 (s, 6 H), 2.40 (d, 3 H, J = 13 Hz), 1.40 (d, 3 H, J = 13 Hz).

Anhydride Acid 5a. A solution of triacid (471 mg, 0.818 mmol) in xylenes (50 mL) was heated with azeotropic removal of water (80 mL of water was removed over a 1.5-h period). Removal of the remaining xylenes gave 455 mg (quant) of the anhydride acid as a pale yellow foam, which was used without further purification: ^{1}H NMR (250 MHz, DMSO-d_6) δ 11.20 (br s, 1 H), 7.4–7.1 (m, 15 H), 4.6–4.40 (m, 6 H), 3.82–3.75 (m, 2 H), 3.55–3.20 (m, 4 H), 2.6–2.4 (m, 3 H), 1.8–1.40 (m, 3 H).

Imide Acid 5b. A solution of 6.357 g (11.4 mmol) of anhydride **5a** and NH_4OH (5 mL) in THF (100 mL) was heated to reflux for 2 h. Solvent was removed *in vacuo*, and the resulting solid was dissolved in CH_2Cl_2 (150 mL). The organic layer was washed with 1 N HCl (100 mL) and then dried over Na_2SO_4. Removal of solvent provided 4.095 g (64%) of imide **5b** as an off-white solid: mp 138–140 °C; IR (KBr) 3166, 2860, 1713 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.48 (s, 1 H), 10.58 (s, 1 H), 7.34–7.24 (m, 15 H), 4.45 (d, 6 H, J = 19 Hz), 3.72 (d, 2 H, J = 8.4 Hz), 3.34–3.30 (m, 4 H), 2.33 (d, 1 H, J = 13 Hz), 2.20 (d, 2 H, J = 13 Hz), 1.51 (t, 3 H, J = 13 Hz); HRMS (EI) calcd for $C_{33}H_{35}NO_7$, 557.2414; found, 557.2416.

Imide Acid Chloride 5c. A solution of the imide acid **5b** (300 mg, 0.54 mmol) in thionyl chloride (15 mL) was heated to reflux for 2 h. The thionyl chloride was removed under reduced pressure to yield 312 mg of the imide acid chloride **5c** (quant), which was used without further purification.

(2*S*,8*S*)-2-(Hydroxymethyl)-8-[[(*tert*-butyldiphenylsilyl)oxy]methyl]-3,4,6,7,8,9-hexahydro-2*H*-pyrimido[1,2-*a*]pyrimidine Hydrochloride (7). Acetic acid (15 mL) was added to a solution of bicyclic guanidinium **6**[13] (252 mg, 0.428 mmol) in THF (5 mL) with stirring at room temperature for 24 h. The reaction mixture was concentrated *in vacuo*. Hexanes and diethyl ether were added, and the white precipitate was triturated. The solids were recrystallized from CH_2Cl_2 and hexanes to give 200 mg (quant) of clean **7**: mp 112–117 °C; $[\alpha]^{20}_D$ +44° (c = 0.0044, $CHCl_3$); IR (KBr) 3278, 2931, 1624, 1112, 703 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.29 (s, 1 H), 7.7–7.6 (m, 5 H), 7.45–7.35 (m, 6 H), 4.12 (br s, 1 H), 3.80 (m, 1 H), 3.75 (m, 1 H), 3.57 (m, 4 H), 3.25 (m, 4 H), 2.07 (m, 1 H), 1.90 (m, 3 H), 1.66 (s, 9 H); HRMS (EI) calcd for $C_{25}H_{36}N_3O_2Si$ (M – Cl), 438.2577; found, 438.2580.

4-Iodobenzoic Acid Methyl Ester (8). Thionyl chloride (10 mL) was added dropwise (1.5 h) to a cooled (0 °C) heterogeneous solution of 4-iodobenzoic acid (10.0 g, 40.0 mmol) in MeOH (100 mL). Upon complete addition, the solution was warmed to room temperature to yield white solids **8** (8.67 g). A second crop was obtained from the remaining reaction mixture to give another 947 mg of **8**. The overall yield of **8** was 9.62 g (92%): mp 115.4–115.7 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, 2 H, J = 8.7 Hz), 7.74 (d, 2 H, J = 8.7 Hz), 3.91 (s, 3 H); HRMS (EI) calcd for $C_8H_7O_2I$, 261.9491; found, 261.9493.

***N*-(4-(Methoxycarbonyl)phenyl)carbazole (9).** A paste consisting of carbazole (6.14 g, 36.7 mmol), **8** (9.92 g, 36.7 mmol), K_2CO_3 (5.07 g), copper powder (190 mg), and xylene (10 mL) was stirred with a mechanical stirrer at 200 °C for 1 h. Benzene (100 mL) was added, and the whole was refluxed for 2 h. Upon cooling the reaction mixture to room temperature, solids were removed and the brown filtrate was concentrated. Recrystallization from MeOH yielded 6.67 g (60%) of clean aromatic **9**: mp 120.7–121.5 °C; IR (KBr) 3050, 2946, 1721, 1602, 1513, 1452, 1432, 1290, 1280, 1225, 1168, 1115, 1102 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (d, 2 H, J = 8.5 Hz), 8.15 (dd, 2 H, J = 7.5, 0.5 Hz), 7.69 (d, 2 H, J = 8.0 Hz), 7.48 (dd, 2 H, J = 8.2, 0.7 Hz), 7.43 (dt, 2 H, J = 7.6, 1.0 Hz), 7.32 (dt, 2 H, J = 7.2, 0.8 Hz), 4.00 (s, 3 H); HRMS (EI) calcd for $C_{20}H_{15}NO_2$, 301.1103; found, 301.1100.

***N*-(4-(Methoxycarbonyl)phenyl)-3,6-dinitrocarbazole (10a).** To a stirred mixture of **9** (2.94 g, 9.75 mmol) and 48 mL of acetic acid was added nitric acid (70.4%, 29 mL) over 12 min. The mixture was stirred at 70 °C for 4.5 h and cooled to room temperature. Addition of water yielded precipitates which were filtered, washed with water, and dried *in vacuo* to yield 3.55 g (93%) of crude **10a**, which was used without further purification: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.66 (d, 2 H, J = 2.4 Hz), 8.42 (dd, 2 H, J = 9.2, 2.3 Hz), 8.30 (d, 2 H, J = 8.7 Hz), 7.91 (d, 2 H, J = 8.4 Hz), 7.62 (d, 2 H, J = 9.3 Hz), 3.94 (s, 3 H).

***N*-(4-(Methoxycarbonyl)phenyl)-3,6-diaminocarbazole (10b).** A heterogeneous solution of **10a** (486 mg, 1.24 mmol) and 10% Pd–C (55.1 mg) in THF (50 mL) was stirred under a H_2 atmosphere at room temperature for 24 h. The reaction mixture was filtered through Celite and then concentrated *in vacuo* to give 510.3 mg (quant) of crude **10b** as a brown film, which was used without further purification: ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.14 (d, 2 H, J = 9.0 Hz), 7.69 (d, 2 H, J = 8.5 Hz), 7.25 (d, 2 H, J = 8.5 Hz), 7.13 (d, 2 H, J = 2.0 Hz), 6.71 (dd, 2 H, J = 8.4, 2.0 Hz), 4.93 (br s, 4 H), 3.31 (s, 3 H).

***N*-(4-(Methoxycarbonyl)phenyl)-3,6-bis[((*cis*,*cis*-2,4-dioxo-1,5,7-tris((benzyloxy)methyl)-3-azabicyclo[3.3.1]non-7-yl)carbonyl)amino]carbazole (11a).** A solution of diaminocarbazole **10b** (1.23 g, 3.73 mmol) and acid chloride **5c** (5.37 g, 9.33 mmol) in pyridine (180 mL) was stirred at room temperature under argon for 2 h. The whole was concentrated *in vacuo*, and the dark residue was taken up in CH_2Cl_2. The organic phase was washed with 1.2 N HCl (300 mL), then dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The resulting residue was flash-chromatographed through a silica gel column (20% EtOAc/CH_2Cl_2) to give 2.55 g (49%) of **11a**: mp 125–145 °C (dec); IR (KBr) 3384, 2861, 1702, 1604, 1466, 1281, 1195, 1101, 738, 698 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.63 (s, 2 H), 9.42 (s, 2 H), 8.31 (s, 2 H), 8.23 (d, 2 H, J = 8.5 Hz), 7.79 (d, 2 H, J = 8.5 Hz), 7.49 (d, 2 H, J = 9.5 Hz), 7.42 (d, 2 H, J = 8.8 Hz), 7.28–7.00 (m, 30 H), 4.51 (s, 8 H), 4.46 (s, 4 H), 3.41 (d, 4 H, J = 9.1 Hz), 2.58 (d, 4 H, J = 13.5 Hz), 2.34 (d, 2 H, J = 11.9 Hz), 1.61 (d, 4 H, J = 14.3 Hz), 1.60 (d, 2 H, J = 9.9 Hz); HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{86}H_{84}N_5O_{14}$ (M + H), 1410, 6015; found 1410.6007.

***N*-(4-(Methoxycarbonyl)phenyl)-3,6-bis[((*cis*,*cis*-2,4-dioxo-1,5,7-tris(hydroxymethyl)-3-azabicyclo[3.3.1]non-7-yl)carbonyl)amino]carbazole (2).** HBr(g) was bubbled for 5 min through a solution of the benzylated cleft **11a** (8.9 mg, 0.00631 mmol) in formic acid (1 mL) at 0 °C, followed by argon for 5 min. The whole was concentrated *in vacuo* to give a brown solid, which upon washing with a minimum amount of MeOH (2 mL) yielded 4.5 mg (82%) of white solid **2**: mp 260–270 °C (dec); IR (KBr) 3421, 3209, 1700, 1466, 1400, 1290, 1200, 1057 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.41 (s, 2 H), 9.26 (s, 2 H), 8.38 (d, 2 H, J = 2.1 Hz), 8.24 (d, 2 H, J = 8.4 Hz), 7.80 (d, 2 H, J = 8.4 Hz), 7.52 (dd, 2 H, J = 2.2, 8.8 Hz), 7.43 (d, 2 H, J = 9.2 Hz), 5.12 (br 2 H), 4.77 (br, 4 H), 3.92 (s, 3 H), 3.79 (d, 4 H, J = 10.6 Hz), 2.42 (d, 4 H, J = 14.1 Hz), 2.21 (d, 2 H, J = 11.9 Hz), 1.51 (d, 4 H, J = 14.3 Hz), 1.35 (d, 2 H, J = 13.1 Hz); HRMS (FAB in glycerol) calcd for $C_{44}H_{48}N_5O_4$ (M + H), 870.3198; found, 870.3201.

***N*-(4-Carboxyphenyl)-3,6-bis[((*cis*,*cis*-2,4-dioxo-1,5,7-tris((benzyloxy)methyl)-3-azabicyclo[3.3.1]non-7-yl)carbonyl)amino]carbazole (11b).** To a solution containing **11a** (361 mg, 0.256 mmol) in ethanol (10 mL) and THF (10 mL) was added 1 N NaOH (1.55 mL, 6 equiv). The solution was stirred under argon at room temperature for 20 h. The basic solution was acidified to approximately pH 2 with 5% HCl to precipitate the product. The whole was concentrated *in vacuo*, and the solids were taken up in CH_2Cl_2 (50 mL) and brine (50 mL). The organic layer was separated, dried over $MgSO_4$, filtered, and concentrated to give crude acid **11b** (304 mg), which was flash-chromatographed through a silica gel column (5–7% MeOH/CH_2Cl_2) to give 277 mg (78%) of clean **11b**: mp 141.5–143.0 °C; IR (KBr) 3134, 1700, 1603, 1100 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.19 (s, 2 H), 8.03 (s, 2 H), 7.94 (s, 2 H), 7.75 (d, 2 H, J = 8.2 Hz), 7.40–7.23 (m, 34 H), 7.18 (d, 2 H, J = 8.4 Hz), 7.08 (d, 2 H, J = 8.8 Hz), 4.62–4.52 (m, 12 H), 3.85 (d, 4 H, J = 9.1 Hz), 3.57 (d, 4 H, J = 9.1 Hz), 3.48 (s, 4 H), 2.60 (d, 4 H, J = 14.2 Hz), 2.50 (d, 2 H, J = 13.3 Hz), 1.79 (d, 2 H, J = 13.0 Hz), 1.67 (d, 4 H, J = 14.2 Hz); HRMS (FAB in 3-nitrobenzyl alcohol) calcd for $C_{85}H_{82}N_5O_{14}$ (M + H), 1396.5858; found, 1396.5857.

***N*-(4-(Chlorocarbonyl)phenyl)-3,6-bis[((*cis*,*cis*-2,4-dioxo-1,5,7-tris((benzyloxy)methyl)-3-azabicyclo[3.3.1]non-7-yl)carbonyl)amino]carbazole (11c).** To a solution of **11b** (410 mg, 0.293 mmol) and DMF (6 μL) in THF (9 mL) was added dropwise oxalyl chloride (400 μL). The yellow solution was stirred for 1 h and concentrated *in vacuo* to give 414 mg (quant) of yellow solid **11c**, which was used without further purification.

Hexakis(benzyloxymethyl)carbazolediimide *t*BDPS Bicyclic Guanidinium Ester Hydrochloride (12a). Monohydroxy guanidinium **7** (154 mg, 0.322 mmol), acid chloride **11c** (414 mg, 0.293 mmol), TEA (400 μL), and THF (17 mL) were stirred at room temperature under Ar for 20 h. The reaction mixture was concentrated and then taken up in $CHCl_3$ (70 mL) and 1.2 N HCl (50 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated *in vacuo* to give crude material which was flash-chromatographed once through a silica gel column (10% MeOH/$CHCl_3$) to give a mixture of **11b** and **12a** (1:12, 310 mg), which was used without further purification: ^{1}H NMR (300 MHz, DMSO-d_6, 13a only) δ 10.60 (s, 2 H), 9.42 (s, 2 H), 8.29 (m, 4 H), 7.84 (d, 2 H, J = 8.4 Hz), 7.62 (m, 3 H), 7.53–7.26 (m, 43 H), 4.53–4.48 (m, 12 H), 3.98 (dd, 1 H, J = 6.9, 1.5 Hz), 3.86 (m, 1 H), 3.79 (d, 4 H, J = 9.0

Hz), 3.68–3.55 (m, 2 H), 3.46 (d, 4 H, J = 10 Hz), 3.41 (s, 4 H), 2.59 (d, 4 H, J = 13 Hz), 2.36 (d, 2 H, J = 13 Hz), 1.99 (m, 4 H), 1.62 (m, 6 H), 1.02 (s, 9 H).

Hexakis(benzyloxymethyl)carbazolediimide Hydroxy Bicyclic Guanidinium Ester Hydrochloride (12b). A solution of the above mixture (310 mg, 0.167 mmol), THF (800 μL), and TBAF (600 μL) was stirred at room temperature for 2 h, concentrated, and taken up in $CHCl_3$ (40 mL) and water (30 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to give a yellow residue. Diethyl ether (5 mL) was added to dissolve *t*BDPSF and precipitate the desired product. The solids were collected by centrifugation and then flash-chromatographed through a silica gel column (15–20% MeOH/$CHCl_3$) to yield 254 mg (56% yield from **11c**) of **12b**: IR (KBr) 3262, 2923, 2854, 1702, 1624, 1100, 739, 698 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 10.59 (s, 2 H), 9.42 (s, 2 H), 8.32–8.28 (m, 4 H), 7.84 (d, 2 H, J = 8.5 Hz), 7.62 (s, 1 H), 7.51 (d, 2 H, J = 9.5 Hz), 7.49 (s, 1 H), 7.41 (d, 2 H, J = 8.5 Hz), 7.35–7.20 (m, 30 H), 5.74 (m, 1 H), 4.54–4.44 (m, 12 H), 4.30 (dd, 1 H, J = 8.0, 9.7 Hz), 3.86 (m, 1 H), 3.78 (d, 4 H, J = 8.5 Hz), 3.54 (m, 1 H), 3.46 (s, 4 H), 3.15 (m, 1 H), 2.58 (d, 4 H, J = 14 Hz), 2.35 (d, 2 H, J = 13 Hz), 2.12 (m, 1 H), 1.92 (m, 2 H), 1.63–1.59 (m, 7 H); HRMS (FAB in glycerol) calcd for $C_{94}H_{97}O_{15}N_8$ (M – Cl), 1577.7073; found, 1577.7066.

Hexahydroxy-Kemp-imide *p*-Carbazole Phenyl Hydroxybicyclic Guanidinium Ester Hydrochloride (1). HBr(g) was bubbled for 3 min through the benzylated cleft **12b** (253 mg, 0.157 mmol) in formic acid (12 mL) at 0 °C, followed by Ar for 5 min. The solution was concentrated *in vacuo* to give orange solids which upon trituration in $CHCl_3$ and THF gave a pale orange solid, which was further purified by passing through an anion-exchange resin (Dowex 1 × 8-200, prepared by flushing saturated $NH_4Cl_{(aq)}$, eluted with water) to give 131 mg (78%) of receptor **1**: mp 240–280 °C (dec); $[\alpha]^{20}_D$ +66° (c = 0.0028, H_2O); IR (KBr) 3207, 1696, 1618, 1513, 1464, 1400, 1274, 1196, 1052 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 10.38 (s, 2 H), 9.26 (s, 2 H), 8.36 (s, 2 H), 8.30 (d, 2 H, J = 8.4 Hz), 7.84 (d, 2 H, J = 8.7 Hz), 5.17 (t, 1 H, J = 4.8 Hz), 5.11 (t, 1 H, J = 4.8 Hz), 4.77 (t, 4 H, J = 5.4 Hz), 4.48 (dd, 1 H, J = 3.7, 10 Hz), 4.31 (t, 1 H, J = 9.7 Hz), 3.89–3.75 (m, 5 H), 3.59–3.46 (m, 1 H), 3.07–3.03 (m, 2 H), 2.42 (d, 4 H, J = 15 Hz), 2.21 (d, 2 H, J = 13 Hz), 1.92 (m, 2 H), 1.65 (m, 1 H), 1.49 (d, 4 H, J = 13 Hz), 1.35 (d, 2 H, J = 13 Hz); HRMS (FAB in glycerol) calcd $C_{52}H_{61}O_{15}N_8$ (M – Cl), 1037.4256; found, 1037.4248.

Titrations. Titrations were performed at 283 K on a Varian VXR-500 instrument, in a 9:1 mixture of H_2O/D_2O with 1.0 mM DSS buffered with 10 mM cacodylate buffer to pH = 6.0. Ionic strength of the solution was adjusted with NaCl to 51 or 501 mM. All titrations were done at constant host concentration: aliquots of a solution of 0.35 mM (or 0.50 mM) host and 200 mM (or 30 mM) guest were added to a 0.35 mM (or 0.50 mM) host solution. The resulting curves obtained by following the chemical shift of the imide proton were fitted to the 1:1 binding isotherm[17] with (for 9-ethyladenine and adenosine) or without (for 2′,3′-cAMP and 3′,5′-cAMP) incorporating guest dimerization.

Nonlinear least-squares regression was used to curve-fit the experimental data with the Simplex algorithm as implemented in Systat 5.2.[28]

Guest Dimerization Determination. The change in chemical shifts of C^2H and C^8H purine protons was monitored at 500 MHz as the concentration of 9-ethyladenine (up to 250 mM) or adenosine (up to 30 mM) was increased in D_2O (or a 9:1 mixture of H_2O/D_2O for 9-ethyladenine at 501 mM ionic strength) with 1.0 mM DSS buffered with 10 mM cacodylate buffer to pD (or pH) = 6.0. Ionic strength of the solution was adjusted with NaCl to 51 or 501 mM. For 9-ethyladenine at 51 mM ionic strength, the dimerization constant (K_{dim}) at 283 K was extrapolated from K_{dim} measurements made at 3, 9, 15, 21, and 27 °C. For 9-ethyladenine at 501 mM ionic strength and adenosine at 51 mM ionic strength, the change in chemical shift was observed at 283 K. The resulting curves were fitted to a dimerization curve using the Simplex algorithm as implemented. The resulting dimerization constants from the two protons (C^2H and C^8H) were averaged.

Molecular Modeling. All molecular modeling was performed on a Silicon Graphics 4D30G+ with MacroModel 3.5X.[29] The conformations of the complexes between **1** and the cyclic adenosine monophosphates were derived by minimization (using the MULT routine and the TNCG algorithm[30]) of 300 structures sampled during a 60-ps stochastic dynamics simulation (300 K, 1.5-fs time step, all-atom AMBER* force field modified with 6,12-Lennard Jones hydrogen-bonding potential and McDonald–Still amide parameters,[31] and GB/SA continuum water solvation[32]) following a 5-ps equilibration period. For simplicity. the hydroxymethyl groups on the Kemp's imide moieties were approximated as methyl groups.

Acknowledgment. We acknowledge the contributions of Ghislain Deslongchamps, Kazuya Kobiro, Vincent Rotello, and Neil Branda for preliminary synthetic investigations. We thank Brian A. Murray for assistance in the determination of binding constants and Profs. J. Williamson and D. Williams for stimulating discussions. This work was supported by the National Institutes of Health. M.M.C. thanks the Natural Science and Engineering Research Council of Canada for a predoctoral fellowship.

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