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CONVERGENT FUNCTIONAL GROUPS XI. SELECTIVE BINDING OF GUANOSINE DERIVATIVES.

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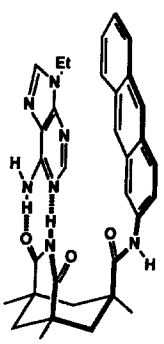
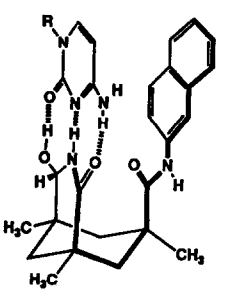
Abstract: New synthetic receptors for guanosine derivatives are introduced and evaluated using solubility titration methods. Base-pairing and some aromatic stacking effects are observed in $CDCl_3$.

Introduction Derivatives of Kemp's triacid[1] have been useful for selective recognition of nucleotide derivatives. In particular, imides provide base-pairing toward adenines, and the U-shaped relationship with other axial substituents presents possibilities for other intermolecular contacts.[2] Both hydrogen bonding and aromatic stacking interactions can be brought to bear on adenine derivatives (Eq. 1). Modifying the hydrogen bonding surfaces for complementarity to cytosines can also be accomplished,[3] while thymines can be complexed within the seamless curvature of macrocyclic structures.[4] Guanine has been a problem. The hydrogen-bonding complement is an acylated amidine, a function that is elusive in either context, although it has been successfully replaced in a macrocycle by a naphthyridine derivative.[5] Here we introduce two systems capable of selective binding to guanosine derivatives, and apply solubility titration[6] to determine the association constants.

Eq. 1

Complexation of adenine and cytosine derivatives

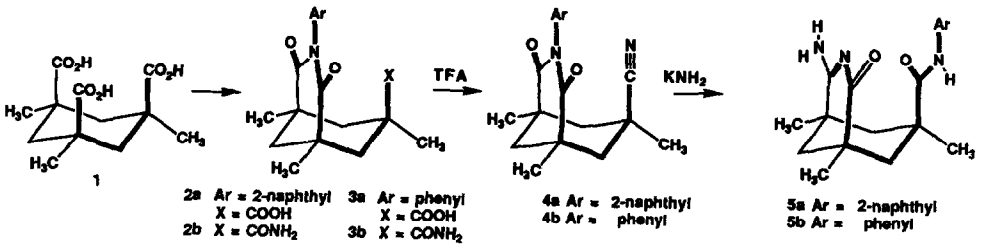




Synthesis. The synthetic receptors were prepared from Kemp's triacid **1** by conversion to its anhydride acid chloride,[1] then condensation with aromatic amines to give the imides **2** and **3** (Eq.

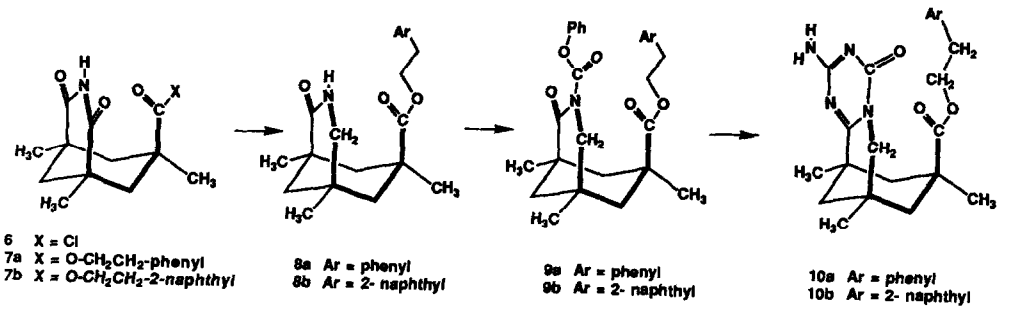
2). Sequential treatment with $SOCl_2$ and NH_3, then dehydration with trifluoroacetic anhydride (TFFA), gave the nitriles **4**. Exposure to KNH_2 in THF/NH_3 rearranged these materials to the acylated amidines **5**.

Eq. 2



A second set of receptors was prepared from the imide **6** by esterification to **7** and reduction to the lactam esters[7] **8** (Eq. 3). N-acylation with phenyl chloroformate followed by condensation with guanidine hydrochloride gave the azacytosines.[8] Both the phenyl ethyl **10a** and the 2-naphthyl ethyl ester **10b** were prepared by these means; all receptors were obtained in racemic form.

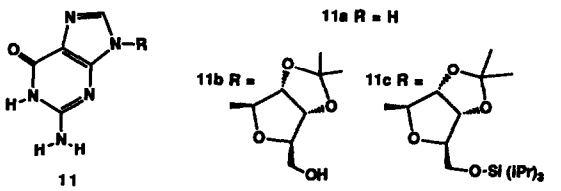
Eq. 3



Complexation. Before describing the complexation experiments, we note that self-association of guanosine derivatives encountered by Shaw[9] and others[10] cause solubility problems which render titration data difficult to interpret, particularly when diastereomeric complexes are formed. Accordingly, we have used the solubility titration method,[6] in which the synthetic receptor is used to solubilize the guanosine derivatives **11**.

Solubility titrations were particularly well suited to study the association of our molecules with guanosine for several reasons. As previously mentioned, higher order aggregates of guanosines (G) are unlikely under these conditions and the formation of G_2-C trimers is minimal below 1:1 stoichiometry[9]. Also $(G\text{-}C)_2$ tetramers are not present to any extent at low

concentration (10^{-3} M). Aggregation of guanosine can also be ignored, since g_0 contains all the higher order guanosine species and is a constant throughout the titration. To reach saturation in a typical NMR titration there must be a large excess of, in this case, the guanosine component. However, the analytical signal (i.e. the chemical shift of a relevant proton) is a compilation of all the guanosine species in solution, which in the case of molecules which self-associate and aggregate is now the time average of more than the two relevant species, the free and bound guanosine. It is often impossible to deconvolute the signal and assign the partial contributions to the overall observable. In the solubility method the increase in amount of guanosine present after addition of a cytosine analog is due solely to interactions with the host molecule making the analytical signal, the HPLC peak, directly interpretable.



11

The experiments are performed by preparing a stock solution of the soluble component (cytosine analogs **5** or **10**), and introducing increasing volumes of this solution into various vials. These solutions are saturated with the insoluble guanine, diluted to constant volume followed by shaking for 12 hrs. After filtration, HPLC analysis of the solutions gives the total amount of each component. The synthetic receptors generally solubilize from 0.05-0.5 equivalents of the guanine **11b**, and at these concentrations, the formation of higher order aggregates is minimal and can be ignored in the analysis. The calibration curves were obtained through a Beer's Law plot using a U.V. detector.

A plot of the concentration of the insoluble component *vs* the soluble component yields a straight line for which the slope is a function of the association constant K_a, and the intercept, g_o, is the limiting solubility of the guanine derivative in cases of 1:1 binding. This limiting solubility was also established for **11b** by shaking with pure $CDCl_3$. The appropriate equations are given below wherein H = host, G = guest.

$$K_a = [HG]/[H][G]$$

$$G_t = g_0 + [HG] \qquad H_t = [H] + [HG]$$

$$G_t = g_0 + g_0K[H] \qquad H_t = [H] + g_0K[H]$$

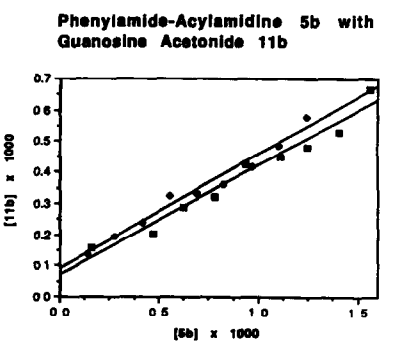
$$[H] = H_t/(1 + g_0K)$$

$$G_t = g_0 + (g_0\,K/(1 + g_0K))$$

$$\text{slope} = g_0K_a/(1 + g_0K_a) = \alpha \quad \text{y-int} = g_0$$

$$K_a = \alpha/(1 - \alpha)g_0$$

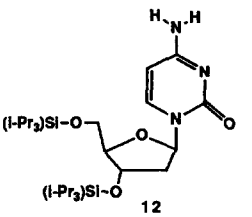
Fig. 1



Typical titration data is given (Figure 1) using guanosine **11b** as the solid component. The association constants are given in Table 1, and reasonable agreement was obtained on g_o, the limiting solubility of guanosine, using the various receptors.

Table 1. Association Constants Measured by Solubility Titration in $CDCl_3$

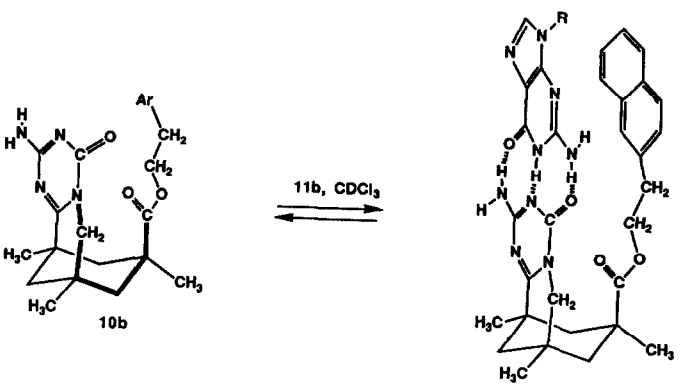
Host	Ar =	K_a M^{-1}
5b	Phenyl	8000
5a	2-Naphthyl	5500
10a	Phenyl	8000
10b	2-Naphthyl	13000
12		7900



12

Structure. Derivatized cytosine **12** which is unable to simultaneously hydrogen bond and aryl stack exhibits a K_a of 7900 M^{-1}. A value of ΔH = -5.6 kcal/mol has been reported for a similarly protected G-C pair in $CHCl_3$.[11] Hydrogen bonding is the major contributor in low dielectric strength aprotic solvents like chloroform while stacking is thought to dominate in water[12]. Compounds **5b** and **10a** in which the both interactions are possible associate to the same extent as **12** which suggests that the phenyl ring is not suitably disposed to interact with the purine surface. Naphthyl ester **10b** shows the expected increase in binding upon favorable interactions with the aromatic surface. This may be due to the extra degrees of freedom allowed in the ethylene spacer (Eq. 4). In the case of amide **5a** there are unfavorable steric interactions between the aromatic surface and the acetonide of the ribose on the guanosine which can be avoided in the more extended ester **10b**. Nonetheless, these models give association constants of the right order of magnitude for a system containing three hydrogen bonds[13] and enhancement of binding is observed in cases bearing a well-placed aromatic surface.

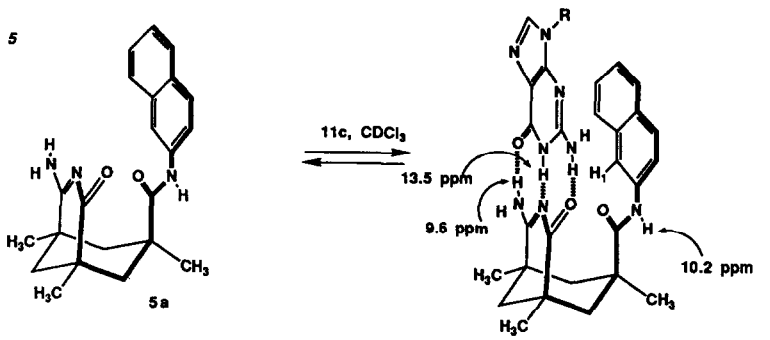
Eq. 4



10b

While NMR did not allow quantification of the association constant it did give some structural information regarding the complexes. Because synthetic receptors are racemic mixtures and the guanosines are optically active, they form diastereomeric complexes upon mixing. In the NMR spectrum this results in a duplication of signals for those resonances which are in sufficiently different environments. The downfield region of the spectrum of a 1:1 mixture of **5a** and **11c** taken at - 25 °C contains five resonances, three of which are paired peaks from diastereomeric complexes with the remaining two appearing as broadened signals. The resonances at 10.2 and 8.8 ppm were assigned as the amide NH and the H-1 proton of the naphthalene ring. The resonances at 13.5, 9.6, and 8.4 ppm were assigned as the imino NH of **11c**, the amino NH of **5a**, and the amino NH of **11c**. These assignments are in agreement with values reported by Shaw[9] for **12** complexed with **11c**. A structure which is consistent with these findings is shown in Eq. 5. A naphthalene configuration in which H-1 is adjacent to the amide carbonyl is suggested by its chemical shift.

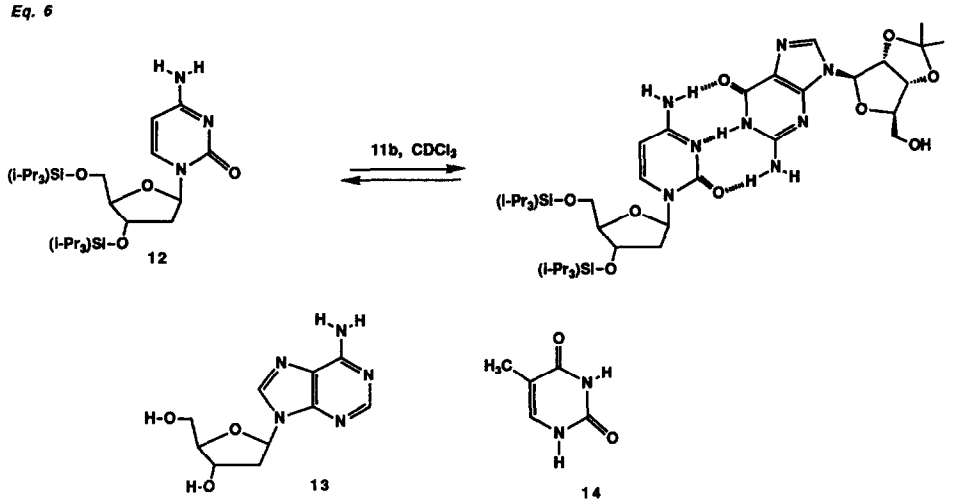
Eq. 5



It can be concluded that the simple acyl amidines express their affinity for guanosine derivatives by base pairing. The azacytosines, which sport the extended ethylene spacer could permit stacking interactions and affinities twice that of the simple base pair derivatives.

The "natural" base-pairing (Eq. 6) is reasonably well imitated by the new receptor for guanosines, but their *selectivity* can also be assessed with the methodology here. Accordingly, a set of experiments was carried out to determine the extent to which **5b** would solubilize nucleoside bases to which it is not complementary. The insoluble components used were 2-deoxyadenosine **13** and thymine **14**. The technique gave limiting solubilities of 13×10^{-5} M for **13** and a K_a with **5b** of 600 M^{-1} while the corresponding numbers for **14** were 1.0×10^{-5} M and K_a = 850 M^{-1}. In either case, it appears that two hydrogen bonds are formed with some additional contact likely from aryl stacking. Thus the selectivity of the new receptors for guanosine is about one order of magnitude over the other bases under these conditions, a factor that probably reflects the contribution of the third hydrogen bond.

Eq. 6



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Experimental

Naphthylimide-acid 2a

The anhydride-acid chloride of Kemp's triacid,[1] (0.9 g, 3.5 mmol), β-naphthylamine (500 mg, 3.5 mmol), and pyridine (290 µl, 3.6 mmol) were dissolved in CH_2Cl_2 and stirred at RT for 2 hr. The mixture was diluted with pyridine (10 mL) and heated under reflux overnight. The pyridine was removed under vacuum and the residue was treated with 10 % HCl (5 mL). The precipitate was filtered yielding imide-acid (1.1 g) in 85 % yield. This material decomposed above 250°C. ^{1}H-NMR ($CDCl_3$): δ 7.75-7.84 (mult, 4 H-Ar), 7.39-7.43 (mult, 3 H- Ar), 2.73 (d, J = 13.8 Hz, 2 H_{eq}), 2.17 (d, J = 13.8 Hz, 1 H_{eq}), 1.49 (d, J = 13.2 Hz, 1 H_{ax}), 1.35 (s, 6 H), 1.20 (d, J = 12.6 Hz, 2 H_{ax}), 1.18 (s, 3 H). IR (2 % KBr): 3445, 3146, 1730, 1714, and 1678 cm^{-1}.

Phenylimide-acid 3a was prepared by a similar procedure in 82% yield on a 7.5 mmol scale: mp 251-252° C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.29-7.39 (mult, m, p-Ar), 7.05-7.08 (mult, o-Ar), 2.82 (d, J = 13.5 Hz, 2 H_{eq}), 2.14 (d, 13.5 Hz, 1 H_{eq}), 1.49 (d, J = 13.5 Hz, 1 H_{ax}), 1.34 (s, 6 H), 1.33 (s, 3 H), 1.28 (d, J = 14.4 Hz, 2 H_{ax}). IR (2 % KBr): 3196, 2980, 1730, 1709, and 1678 cm^{-1}. Anal Calcd for $C_{18}H_{21}NO_4$: C, 68.40; H, 6.90. Found C, 68.55; H, 6.71.

Naphthylimide-amide 2b

Imide-acid **2a** (0.9 g, 2.5 mmol) was refluxed overnight in $SOCl_2$ (10 mL). Excess $SOCl_2$ was removed under vacuum and the residue was taken up in dry THF (10 mL). Ammonia was bubbled through the solution for 30 min then the inlet was discontinued while the reaction was allowed to stir 1 hr at RT. The reaction solution was concentrated to dryness and the residue was extracted with hot $CHCl_3$ (2 X 50). The combined organic extracts were concentrated to give the imide-amide (1 g) in quantitative yield which was used without further purification. ^{1}H-NMR ($CDCl_3$): δ 7.83-7.86 (mult, 4 H-Ar), 7.44-7.49 (mult, 3 H- Ar), 5.28 (br s, NH_2), 2.56 (d, J = 14.1 Hz, 2 H_{eq}), 2.18 (d, J = 13.2 Hz, 1 H_{eq}), 1.49 (d, J = 13.2 Hz, 1 H_{ax}), 1.35 (s, 6 H), 1.28 (d, J = 12.6 Hz, 2 H_{ax}), 1.20 (s, 3 H). IR (2 % KBr): 3480, 1728, 1680, and 1192 cm^{-1}.

Phenylimide-amide 3b was prepared similarly (71% yield). mp 273-275 °C
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.34-7.43 (mult, m, p-Ar), 7.20 (br s, o-Ar), 5.55 (br s, NH), 5.23 (br s, NH), 2.68 (d, J = 13.9 Hz, 2 H_{eq}), 2.17 (d, 13.2 Hz, 1 H_{eq}), 1.50 (d, J = 13.2 Hz, 1 H_{ax}), 1.36 (s, 6 H), 1.35 (d, J = 14.9 Hz, 2 H_{ax}), 1.31 (s, 3 H). IR (2 % KBr): 3404, 3200, 1732, and 1684 cm^{-1}. Anal Calcd for $C_{18}H_{22}N_2O_3$: C, 68.53; H, 7.01. Found C, 68.77; H, 7.05.

Naphthylimide-nitrile 4a

To a suspension of amide **2b** (0.9 g, 2.5 mmol) and pyridine (490 μL, 6.0 mmol) in THF (10 mL) was added dropwise $(CF_3CO)_2O$ (425 μL, 3.0 mmol) at RT. After 2 hr the mixture became homogeneous at which time it was diluted with 10 % HCl (60 mL) and EtOAc (100 mL) The organic and aqueous layers were separated and the aqueous layer was extracted with EtOAc (3 X 40mL). The combined organic layers were dried over Na_2SO_4 and concentrated to give imide-nitrile in quantitative yield. mp 232-233 °C. ^{1}H-NMR ($CDCl_3$): δ 7.80-7.90 (mult, 4 H-Ar), 7.44-7.49 (mult, 3 H- Ar), 2.50 (d, J = 13.5 Hz, 2 H_{eq}), 2.30 (d, J = 13.2 Hz, 1 H_{eq}), 1.54 (s, 3 H), 1.51 (d, J = 13.2 Hz, 1 H_{ax}), 1.41 (d, J = 14.4 Hz, 2 H_{ax}), 1.39 (s, 3 H). IR (2 % KBr): 2231, 1732, and 1682 cm^{-1}. Anal. Calcd for $C_{22}H_{22}N_2O_2$: C, 76.28; H, 6.39. Found C, 75.95; H, 6.52.

Phenylimide-nitrile 4b was prepared similarly (80% yield). mp 206-208 °C.
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.34-7.46 (mult, o, m, p-Ar), 2.47 (d, J = 14.9 Hz, 2 H_{eq}), 2.26 (d, 13.2 Hz, 1 H_{eq}), 1.52 (s, 3 H), 1.49 (d, J = 13.2 Hz, 1 H_{ax}), 1.41 (d, J = 14.9 Hz, 2 H_{ax}), 1.38 (s, 6 H). IR (2 % KBr): 2971, 2227, 1731, and 1682 cm-1. Anal Calcd for $C_{18}H_{20}N_2O_2$: C, 72.95; H, 6.80. Found C, 72.47; H, 6.68.

Naphthylimide-amidine 5a

Potassium amide was prepared by dissolving K metal (225 mg, 5.8 mmol) in liquid ammonia (25 mL) and adding a small amount of $FeCl_3$. At this point the solution turns from blue to grey. The nitrile **5a** (250 mg, 0.72 mmol) was added as a slurry in THF (10 mL). The reaction was stirred at reflux for 3 hr before quenching with NH_4Cl. The ammonia was allowed to boil off and the

residue was taken up in aqueous acid, then washed with CH_2Cl_2 (2 X 60mL). The aqueous layer was then made basic (pH = 12) causing the product to precipitate. Filtration gave 217 mg of crude material which could be further purifed by recrystallization from $CHCl_3$. mp 266-268 °C. ^{1}H-NMR (300 MHz, CD_3OD): δ 8.06 (s, NH), 7.75 (d, J = 8.4 Hz, 1 Ar), 7.51 (dd, J = 2.1 and 8.7 Hz, 2 Ar), 7.40 (mult, 4 Ar), 2.76 (d, J = 14.4 Hz, 2 H_{eq}), 1.83 (d, J = 13.0 Hz, H_{eq}), 1.20-1.53 (mult, 3 H_{ax}), 1.30 (s, CH_3), 1.28 (s, CH_3), 1.19 (s, CH_3). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 8.15 (s, NH), 7.72 (mult, 3 Ar), 7.38-7.46 (mult, 4 Ar), 2.71 (d, J = 14 Hz, 1 H_{eq}), 2.53 (d, J = 14 Hz, 1 H_{eq}), 1.36-1.58 (mult, 3 H_{ax}),1.32 (s, CH_3), 1.27 (s, CH_3), 1.22 (s, CH_3). IR (2 % KBr): 3333, 3192, 1682, 1603, and 1540 cm^{-1}. HRMS Calcd for $C_{22}H_{25}N_3O_2$: 363.147. Found 363.1946.

Phenylamide-amidine 5b was prepared similarly (80% yield). mp 276-278 °C. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 7.47 (d, J = 6.9 Hz, o-Ar), 7.27 (dd, J = 7.0 Hz, m-Ar), 7.0 (t, J = 7 Hz, p-Ar), 2.74 (d, J = 15.0 Hz, 1 H_{eq}), 2.52 (d, J = 13.0 Hz, 1 H_{eq}), 1.96 (d, J = 13.0 Hz, 1 H_{eq}), 1.23-135 (mult, 3 H_{ax} and 3 CH_3). IR (1 % KBr): 3338, 3175, 2961, 1676, 1599, and 1536 cm^{-1}. HRMS Calcd for $C_{18}H_{23}N_3O_2$: 313.1789. Found 313.1789.

Imide phenethyl ester 7a

A mixture of imide acid chloride **6** (1.00 g, 3.90 mmol), DMAP (952 mg, 7.8 mmol), and phenethyl alcohol (1.5 mL) in dry CH_2Cl_2 (20 mL) under a nitrogen atmosphere was heated to reflux for 66 hr. The reaction was poured into 1 N HCl (50 mL) and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2 X 60 mL). Combined extracts were washed with 1 N HCl (50 ml), dried ($MgSO_4$) and concentrated. Chromatography on silica gel with CH_2Cl_2 resulted in partial purification. Phenethyl alcohol was easily removed by recrystalization from *n*-hexane. This gave 1.07 g (80 %) of imide-ester **7a**. mp 160-161 °C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.12 (s, CH_3), 1.15 (d, J = 16.8 Hz, 2 H), 1.26 (s, 2 CH_3), 1.36 (d, J = 13.3 Hz, 1 H), 1.98 (d, J = 13.3 Hz, 1 H), 2.68 (d, J = 13.3 Hz, 2 H), 2.91 (t, J = 7.2 Hz, 2 H), 4.21(t, J = 7.3 Hz, 2 H), 7.19-7.32 (mult, 5 H), 7.47 (br s, NH). IR (thin film): 1734, 1701, and 1718 cm^{-1}. LRMS m/e =344(MH+) by chemical ionization with NH_3 matrix.

Imide β-Naphthylethyl ester 7b was prepared similarly in 89% yield: mp 187-188 °C (recrystallized from toluene) ^{1}H-NMR (300 MHz, CDCl3): δ 1.10 (s,CH_3), 1.14 (d, J = 14.3 Hz, 2 H), 1.26 (s, 2 CH_3), 1.36 (d, J = 13.4 Hz, 1 H), 1.98 (d, J = 13.4 Hz, 1 H), 2.70 (d, J = 13.0 Hz, 2 H), 3.08 (t, J = 7.1 Hz, 2H), 4.30 (t, J = 7.2 Hz,2H), 7.32-7.82 (mult, 8H). IR (thin film): 1729 and 1696 cm^{-1}. HRMS Calcd for $C_{24}H_{27}NO_4$: 393.1940. Found 393.1939.

Lactam phenethyl ester 8a

Imide phenethyl ester **7a** (604 mg, 1.76 mmol) and $LiBH_4$ (310 mg) at 0 °C were dissolved in dry THF (15 mL). After 4 h at 0 °C additional portions of $LiBH_4$ (110 mg) and THF (15 mL) were added. The reaction was kept in the refrigerator overnight. The reaction was quenched with satd NaCl

solution and extracted with CH_2Cl_2. Flash chromatography on silica gel column (CH_2Cl_2 : ether :: 9 : 1 to 4:1) gave 418 mg, (68 %). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.94 (d, J = 16.2 Hz, 1 H), 1.05 (s, CH_3), 1.12 (s, CH_3), 1.16 (s, CH_3), 1.29 (d, J = 13.5 Hz, 2 H), 1.76 (d, J = 13.4 Hz, 1 H), 2.62 (d, J = 13.7 Hz, 2 H), 2.96 (t, J = 7.0 Hz, 2 H), 4.15-4.35 (mult, 2 H), 4.53-4.70 (d, J = 13.0 Hz, 1 H) 4.70 (d, J = 7.0 Hz, 1 H), 5.39 (br s, NH), 7.18-7.33 (mult, 5 H). HRMS Calcd for $C_{20}H_{25}NO_3$ (M-H2O): 327.1834. Found 327.1832. This material, (405 mg, 1.17 mmol) in dry CH_2Cl_2 (5 mL) was treated with trifluoroacetic acid (500 μL) and triethyl silane (500 μL). After 3.5 h, the reaction was diluted with CH_2Cl_2 (30 mL) and poured into sat'd $NaHCO_3$ (50 ml). The aqueous and organic layers were separated, the aqueous layer was extracted with CH_2Cl_2 (3 X 50 mL). The combined organics were washed with sat'd $NaHCO_3$ (50 ml), dried ($MgSO_4$), and concentrated to give 379 mg (98 %) of **8a**. mp 145-146°C. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.94 (s, CH_3), 1.03 (d, J = 13.9 Hz, 2 H), 1.09 (s, CH_3), 1.16 (s, CH_3), 1.22 (d, J = 12.8 Hz,1 H), 1.70 (d, J = 12.9 Hz, 1 H), 2.46 (d, J = 14.0 Hz, 1 H), 2.61 (d, J = 13.9 Hz, 1 H, 2.93-2.98 (mult, 4 H), 4.10-4.31 (mult, 2 H), 5.1 (br s, NH), 7.18-7.31(mult, 5 H). IR (thin film): 1726 and 1665 cm^{-1}. HRMS Calcd for $C_{20}H_{27}NO_3$: 329.1991. Found 329.1990.

Lactam β-Naphthylethyl Ester 8b was prepared similarly in 85% overall yield: mp 186-187 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (s, CH_3), 1.02 (d, J = 13.9 Hz, 2 H), 1.08 (s, CH_3), 1.16 (s, CH_3), 1.20 (d, J = 13.0 Hz, 1 H), 1.68 (d, J = 13.0 Hz, 1 H), 2.46 (d, J = 14.0 Hz, 1 H), 2.62 (d, J = 13.9 Hz, 1 H), 2.82-2.95 (mult, 2 H), 3.12 (t, J = 7.0 Hz, 2 H), 4.22-4.36 (mult, 2 H), 5.08 (br s, NH), 7.35-7.82 (mult, 7 H). IR (thin film): 1725, 1665 cm^{-1}. HRMS Calcd for $C_{24}H_{29}NO_3$: 379.2147. Found 379.2148.

N-phenoxycarbonyllactam phenethyl ester 9a

A mixture of lactam **8a** (519 mg, 1.37 mmol), phenylchloroformate (800 μl) and diisopropylethyl amine (800 μL) in dry CH_2Cl_2 (25 mL) was heated to reflux for 15 h. The reaction was diluted to 100 mL with CH_2Cl_2, washed with 1 N HCl (50 mL), dried ($MgSO_4$) and concentrated. Chromatographic separation (Hex : $CHCl_3$: ether, 5 : 2 : 1) gave 608 mg (89 %) of **9a** (solidifies upon standing when pure) . ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.05 (s, CH_3), 1.09 (s, CH_3), 1.16 (d, J = 12.0 Hz, 2 H), 1.24 (s, CH_3), 1.35 (dd, J = 13.0 and 2.4 Hz, 1 H), 1.84 (d, J = 13.0 Hz, 1 H), 2.59 (d, J = 14.2 Hz, 1 H), 2.71 (d, J=14.2 Hz, 1 H), 2.84-2.90 (mult, 2 H), 3.40 (d, J=12.3 Hz, 1 H), 3.79 (dd, J = 12.4 and 2.4 Hz, 1 H), 4.21 (t, J = 7.1 Hz, 2 H), 7.08-7.37 (mult, 10 H). IR (thin film): 1786 and 1728 cm^{-1}. HRMS Calcd for $C_{21}H_{26}NO_4$ (M-PhO): 356.1862. Found 356.1862.

N-Phenyloxycarbonylactam β-Naphthylethyl Ester 9b was similarly prepared in 89% yield: mp 110.5-112 °C ^{1}H-NMR (300 MHz, $CDCl_3$): δ, 1.04 (s, CH_3), 1.08 (s, CH_3), 1.16 (d, J = 12.5 Hz, 2H), 1.25 (s, CH_3), 1.35 (dd,J = 13.1 and 2.6 Hz, 1H), 1.84 (d, J = 13.1Hz, 1H), 2.60 (d, J = 14.0 Hz, 1H), 2.72 (d, J = 14.1 Hz, 1H), 3.02-3.08 (mult, 2H), 3.40 (dd, J = 12.0 and 1.8 Hz, 1H), 3.80 (dd, J = 12.4 and 2.6 Hz, 1H), 4.26-4.33 (mult, 2H), 7.17-7.79 (mult, 12 H).

Azacytosine phenethyl ester 10a

To a suspension of guanidine hydrochloride (143 mg, 1.46 mmol) and sodium hydride(60 % dispersion, 60 mg, 1.46 mmol) in dry THF (25 mL) under nitrogen atmosphere were slowly added N-phenoxycarbonyl lactam phenethyl ester **9a** (655 mg, 1.46 mmol) and triethylamine (1.2 mL). The resulting yellowish solution was heated to reflux for 20 h. The reaction was concentrated and chromatographed directly. Elution with ether followed by 5% ethanol in $CHCl_3$ gave 291 mg (50%) of azacytosine **10a** as white solid. mp 184-185 °C

^{1}H-NMR ($CDCl_3$, 300 MHz): δ 1.05-1.36 (mult, 12 H), 1.70 (d, J = 13.0 Hz, 1 H), 2.47 (d, J = 14.2 Hz, 1 H), 2.60 (d, J = 13.8 Hz, 1 H), 2.87 (t, J = 7.1 Hz, 2 H), 3.20 (dd, J = 14.6 and 1.4 Hz, 1 H), 3.94-4.12 (mult, 3 H), 5.30 (br s, 1 H), 6.06 (br s, 1 H), 7.17-7.30 (mult, 5 H). IR (thin film): 3300 (br), 3200 (br), 1721, 1684, 1622, 1581, and 1458 cm^{-1}. HRMS Calcd for $C_{22}H_{28}N_4O_3$: 396.2161. Found 396.2163.

5-Azacytosine 2-naphthyl ester 10b

In a flame dried 2-necked 50 mL flask equipped with a condenser were placed guanidine hydrochloride (73 mg, 0.76 mmol) and 60 % NaH (30.5 mg). Dry THF (13 mL) was added (vigorous hydrogen evolution!). After 5 min N-phenoxylactam **9b** (380 mg, 0.76 mmol) in dry THF (25mL) was added via double tipped needle. After 5 min diisopropylethylamine (663 μL) was added. The flask was heated for 17 h at 90 °C. The reaction was cooled, filtered and concentrated. The resulting crude product was loaded on a silica gel column and side products including lactam **4a** were removed by washing with ether. Elution with 5% ethanolic chloroform gave 256 mg (75%) of amorphous solid **10b**. mp 227-228 °C

^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.03-1.72 (mult, 13 H), 2.45 (d, J = 14.2 Hz, 1 H), 2.61 (d, J = 13.7 Hz, 1 H), 3.05 (t, J = 6.9 Hz, 2 H), 3.20 (d, J = 15.9 Hz, 1 H), 3.99 (d, J = 12.8 Hz, 1 H), 4.11-4.21(mult, 2H), 5.13 (br s, NH_2), 7.34-7.81 (mult, 7 H). IR (thin film): 1721, 1622, 1581, 1539, 1514, 1462 cm^{-1}. HRMS Calcd for $C_{26}H_{30}N_4O_3$: 446.2318. Found 446.2319.

3,5-bis(triisopropylsilyl)-2-deoxycytosine 12

Cytosine (100 mg, 0.39 mmol) and imidazole (235 mg, 3.46 mmol) were dissolved in dry DMF (1 mL). The silyl chloride (337 mL, 1.57 mmol) was added neat and dropwise with stirring at RT. After stirring overnight the reaction mixture was diluted with H_20 (15 mL) and extracted with $CHCl_3$ (5 X 30mL). The combined organic extracts were dried over Na_2SO_4 and concentrated. Chromatography on SiO_2 eluting with 5 % MeOH/$CHCl_3$ gave material which was further recrystallized from $CHCl_3$/Hex to 75 mg (36 % yield) of pure material. mp 193-194 °C. ^{1}H-NMR (250 MHz, $CDCl_3$): δ 9.48 (br s, NH), 8.52 (br s, NH), 7.93 (d, J = 7.5 Hz, C=CH), 6.35 (d, J = 7.5 Hz, C=CH), 6.20 (dd, J = 6.8 and 6.8 Hz, OCHN), 4.57 (ddd, Σ J = 22 Hz, HCOSi), 4.03 (d, J = 2.3 Hz, $CHCH_2$), 3.93 (dd, J = 11.5 and 2.7 Hz, CHHO), 3.87 (dd, J = 11.5 and 2.1 Hz, CHHO), 2.44 (ddd, S J = 21.6 Hz, CHH), 1.98 (ddd, J = 6.2, 6.2, and 12.4 Hz; CHH), 1.01-1.12 (mult, $2Si(CH(CH_3)_2)_3$. IR (1 % KBr): 3327, 3107, 2944, 2866, 1723, and 1652 cm^{-1}.

Solubility Titrations.

The methodology is exemplified by the titration of Phenylamide-Acylamidine (**5b**) with the 2,3-isopropylidene derivative of guanosine (**11b**). Standard HPLC conditions were 65:35 MeOH:H_2O containing 0.1% Et_3N with a reverse phase column. With a flow rate of 1 mL/min, the retention times for **5b** and **11b** were 4 min and 2.5 min, respectively. The peaks were observed using the UV detector set to 254 nm. An attenuation value of 1.0 aufs was used throughout the titration.

A 1.56×10^{-3} M solution of **5b** was prepared by dissolving 2.42 mg in 5 mL of $CDCl_3$. This solution was distributed into 10 vials containing 700 μL ranging in concentration from 1.56×10^{-3} M to 0.156×10^{-3} M (dilution was with $CDCl_3$). These solutions were then saturated with **11b** (~ 2 mg) and shaken overnight. The resulting slurry was filtered and analyzed by HPLC for concentration of **11b**. To accomplish this it was necessary to determine the ratio of the molarity of the solution to the area of the detected peak. Thus a solution of **11b** in MeOH:H_2O was prepared (3.49 mg/5 mL, 2.16×10^{-3} M). Using molar ratios **[11b]/[5b]** of 0.37 and 0.25 gave area ratioes of 0.526 and 0.386 respectively. This yields a value of 0.793 for ([**11b**]/[**5b**]) / (area **11b**/area **5b**). The molarity of each vial was then calculated. From the slope of a plot of [**11b**] vs [**5b**] the K_a can be determined using the following relationship: $K_a = \alpha/g_o(1-\alpha)$ where α = slope of the graph and g_o is the solubility of **11b**. The value of g_o for **11b** was independently determined to be 6.8×10^{-5} M, which is in reasonable agreement with values obtained graphically. Using the α value of 0.350 gives a K_a of 7900 M^{-1}. A second determination using slightly different initial concentrations gave $K_a = 8500\ M^{-1}$. The experimental data is plotted in the graph of Fig. 1 which is shown in the text.

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