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Hydrogen bonding association of a ruthenium(II) bipyridine barbituric acid guest to complementary 2,6-diamino-pyridine amide hosts: guidelines for designing high binding hydrogen bonding cavities in both high-and low-polarity solvents†

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ABSTRACT: The binding between a ruthenium polypyridine guest **RuG2**, (where **Ru** = 4,4′-di-tert-butyl-bpy)$_2$Ru (bpy = 2,2′-bipyridine) and **G2** = 5-[4-(4′-methyl)-2,2′-bipyridyl]methyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione, and a series of host acyl derivatives of 3,5-bis[(6-aminopyrid-2-yl) amino]carbonylpyridine (R/H = *n*-Pr/H, phenyl/H, CF_3/H, *t*-Bu/H, —$(CH_2)_3$-CO_2^-H) and 3,5-bis[(6-amino-4-isopropoxypyrid-2-yl)amino]carbonylpyridine diacetyl derivative (R/X = CH_3/*i*-OPr) was studied by fluorescence and NMR titrations. The **RuG2** (which exists in the enolate form in the presence of the hosts) forms a number of H-bonds involving the amide groups of the hosts and the carbonyl groups of the **G2** for all the hosts studied. Specific 1:1 association between **RuG2** and all the complementary hosts was observed with binding constants, K_a (l mol^{-1}), for R/H in CH_2Cl_2 of 3×10^5 (*t*-Bu/H), 5×10^6 (Ph/H), 3×10^7 (*n*-Pr/H), 9×10^7 (CF_3/H) and $>10^8$ [—$(CH_2)_3CO_2H$) and for R/X of 4×10^8 (Me/*i*-OPr). Similar, but weaker, binding was also observed in solvents of higher donor number such as d_6-acetone, d_3-acetonitrile and d_6-DMSO with R/X = Me/*i*-OPr host showing the highest binding constant in CH_2Cl_2, d_6-acetone and d_6-DMSO. Differences in the binding constants of the ruthenium guest **RuG2** to these hosts are analyzed in terms of the steric, electronic and solvational changes in the structure of the host amide substituents and the polarity of the solvents used.

KEYWORDS: Ruthenium (II) bipyridine barbituric acid guest; 2,6-diaminopyridine amide host; hydrogen bonding association; high-binding hydrogen bonding cavities

INTRODUCTION

Self-assembly and H-bonding between donors and acceptors are important determinants for charge-transfer pathways in biological molecules. Many enzymatic redox processes occur within protein interfacial environments where H-bonding, hydrophobic, and electrostatic interactions are collectively responsible for binding and molecular recognition[1,2] (for an interfacial electron transfer protein–protein–structure, see Ref. 1a). Several types of donor-acceptor molecules where charge-transfer processes occur across H-bonded interfaces have been described.[3–10a] Most relevant to this study is the work of Hamilton and others describing various types of H-bonded donor–acceptor complementary molecules.[10,11]

Recently, a new class of barbituric acids capable of undergoing keto–enol equilibria has been identified and shown to enhance this complementary binding further.[12] Additional increases in binding constants ranging from 10^3 to 10^4 l mol^{-1} were observed when these barbituric acids (which exist in the enolate form in the presence of the hosts) bind to 2,6-diaminopyridine amides. The host binding facilitates the enolization of the barbituric acid derivatives, which is also facilitated in the presence of electron-withdrawing groups (including ruthenium polypyridine complexes) attached to the C-5 position of the barbituric acid guest ring.[12]

In this paper we describe the binding of a series of 2,6-diaminopyridine hosts with different amide substituents that alter their steric, electronic and solvational properties and, therefore, their interactions with the barbituric acid–

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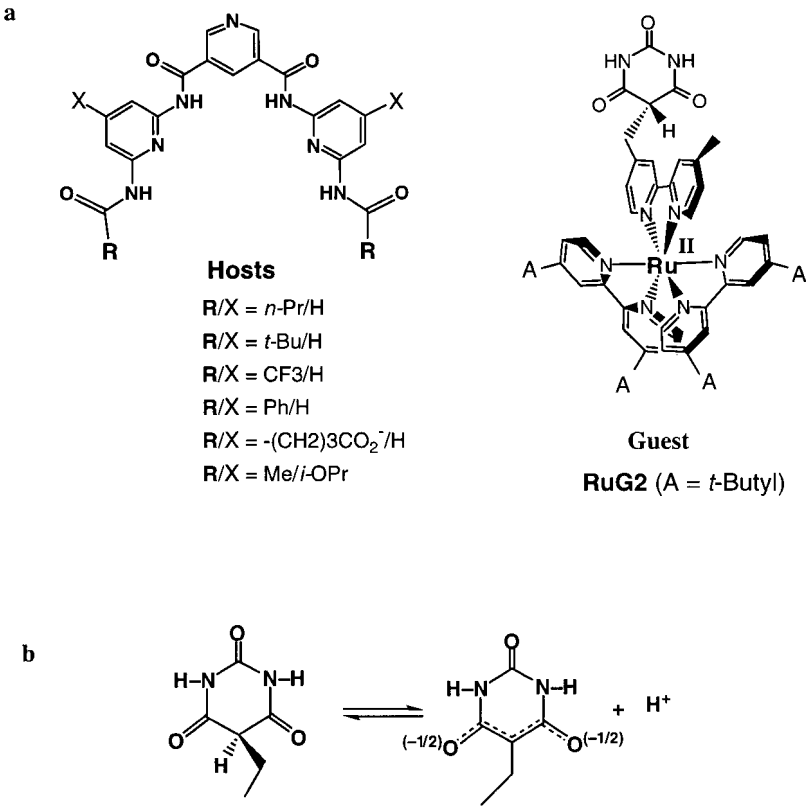


Figure 1. (a) Structure of the host and the dication of **RuG2** guest molecules; (b) keto and enolate forms of the barbituric acid derivatives

ruthenium bipyridine guests, **RuG2**, where **Ru** = 4,4′-di-*t*-butyl-bpy)$_2$Ru (bpy = 2,2′-bipyridine) and **G2** = 5-[4-(4′-methyl)-2,2′-bipyridyl]methyl-2,4,6-(1*H*,3*H*,5*H*)pyrimidinetrione) (Fig. 1). Binding constants for these host–guest pairs are reported in solvents of varying polarities. The large and unusual variation in guest host binding in the two solvents CH_2Cl_2 and d_6-DMSO will be discussed in terms of the steric and electronic effects of the host structures and the solvation of the amide substituents of the host cavities. The results are discussed in terms of guidelines for optimizing donor–acceptor H-bonding interactions.

RESULTS

Synthesis and characterization of guest and host molecules

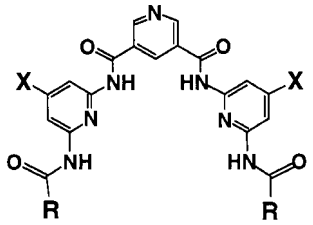
The guest molecule used in these studies is (4,4′-di-tert-butyl-bpy)$_2$Ru**G2 (RuG2)**, where **G2** = 5-[4-(4′-methyl)-2,2′-bipyridyl]methyl-2,4,6-(1*H*,3*H*,5*H*)pyrimidinetrione (Fig. 1). One of the important features of **RuG2** in its binding with the respective hosts is its ability to engage in keto–enol equilibria, strongly favoring the enolate form [Fig. 1(b)] as it binds to the hosts in solvents such as CH_2Cl_2, *d*-$CHCl_3$, d_3-CH_3CN and d_6-DMSO.[12]

The host molecules are derivatives of 2,6-diaminopyridine amides which are assembled from the reaction of the appropriate 2,6-diaminopyridine derivative with 3,5-pyridinedicarboxylic acid chloride. Two different amino hosts, 3,5-bis[(6-aminopyrid-2-yl)amino]carbonylpyridine and 3,5-bis[(6-amino-4-isopropoxypyrid-2-yl)-amino]carbonylpyridine, were used. They were acylated with the appropriate carboxylic acid derivative to give the corresponding host molecules which were used for the binding studies. The host molecules were characterized by NMR, UV and mass spectrometric analysis (see Experimental).

Binding studies of RuG2 to different hosts

The binding of **RuG2** to the different hosts (Table 1) was carried out in a variety of solvents using fluorescence and NMR methods.[12] An increase in the fluorescence intensity of the **RuG2** upon binding of the different hosts was used to calculate the 1:1 binding constants. Fluorescence methods were used mainly in CH_2Cl_2, where the large binding constants observed required the use of low concentrations of **RuG2** and host molecules.[13] A typical example of a large binding constant is shown for the host CF_3/H where the fluorescence intensity of **RuG2** increases upon the addition of host CF_3/H (as R/X) in CH_2Cl_2 (Fig. 2). Competition methods were also used

Table 1. Hydrogen bonding association constants for the different hosts with **RuG2** in different solvents equation



	K_a			
R/X	CH_2Cl_2	d_6-Acetone	d_3-CH_3CN	d_6-DMSO
t-Bu/H	$(3 \pm 1) \times 10^{5}$[a, b]	$(3.5 \pm 0.4) \times 10^{2}$[c]	—	$<0.1 \times 10^{2}$[c]
Ph/H	$(5 \pm 2) \times 10^{6}$[a]	—	—	—
n-Pr/H	$(3 \pm 1) \times 10^{7}$[a, b]	$(6.4 \pm 0.7) \times 10^{3}$[c]	$(8.0 \pm 1) \times 10^{3}$[c]	$(0.32 \pm 0.03) \times 10^{2}$[c]
Me/*i*-OPr	$(4 \pm 2) \times 10^{8}$[a, b]	$>1 \times 10^{4}$[c]	—	$(1.7 \pm 0.2) \times 10^{2}$[c]
—$(CH_2)_3CO_2^-$/H	$>1 \times 10^{8}$[a]	—[d]	$(3 \pm 1) \times 10^{5}$[a]	$(0.40 \pm 0.05) \times 10^{2}$[c]
CF_3/H	$(9 \pm 3) \times 10^{7}$[a, b]	$(2.5 \pm 0.3) \times 10^{3}$[c]	—[d]	$<0.1 \times 10^{2}$[c]

[a] Determined by direct fluorescence methods.
[b] Determined by direct and competition fluorescence methods.
[c] Determined by NMR titration.
[d] Insoluble at concentrations required to carry out NMR titration.

to obtain these high binding constants (Table 1) (barbital was used as a competitive guest for R/X = *t*-Bu/H, and R/X = *t*-Bu/H host was used as a competitive host for R/X = *n*-Pr/H, CF_3/H and Me/*i*-OPr hosts). Binding studies were also carried out in more polar solvents such as d_6-acetone, d_3-acetonitrile and d_6-DMSO (Table 1). Binding constants below 10^4 $lmol^{-1}$ were determined by NMR titrations. The low binding constant ($K_a = 1.7 \times 10^2$ $lmol^{-1}$) was obtained by NMR titration of **RuG2** with the Me/*i*-OPr host (as R/X) in d_6-DMSO, where the changes in the chemical shifts of the amide protons were followed upon addition of **RuG2** ([Me/*i*-OPr] = 7.7 mM)

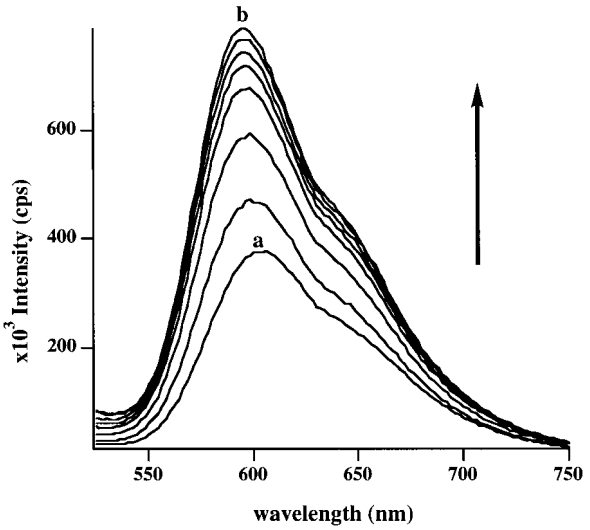


Figure 2. Fluorescence titration for the binding of **RuG2** to the host, R/X = CF_3/H, under anaerobic conditions: (a) [**RuG2**] = 2.4×10^{-7} M, no host present and (b) [**RuG2**] = 2.4×10^{-7} M with total host [CF_3/H] = 4.4×10^{-7} M

(Fig. 3). In some cases the low solubility of the hosts in solvents such as acetone and acetonitrile limited their binding studies to fewer solvents.

A number of control experiments were carried out to complement the binding studies. These resulted in the following observations: (a) the fluorescence intensity for host **RuG2** did not change upon addition of small amounts of the 2,6-lutidine base or trifluoroacetic acid in CH_2Cl_2; (b) addition of 2,6-lutidine (1 molar equivalent) to a 1:1 complex of **RuG2**–hosts [including R/X = —$(CH_2)_3CO_2^-$/H] showed no change in fluorescence intensity; (c) addition of trifluoroacetic acid to a solution of **RuG2**–host complexes caused the fluorescence intensity to decrease to that observed for the **RuG2** alone.

DISCUSSION

The binding of different host molecules to RuG2 guest

The different hosts selected for this study introduce varying steric, electronic and solvational effects on their binding cavity to **RuG2**. The binding was studied by both NMR and fluorescence methods. Changes in the NMR chemical shifts of the host were observed throughout the series upon titration of the guest into the different hosts. A detailed NMR analysis of the chemical shifts was given earlier for R = *t*-Bu/H.[12] The fluorescence titrations carried out under similar conditions, but at lower concentrations (where the stoichiometry was established to be 1:1) were assumed to have similar H-bonding patterns to those deduced from the NMR titrations above. The three hosts (where R/X = *t*-Bu/H, Ph/H, *n*-Pr/H) differ in

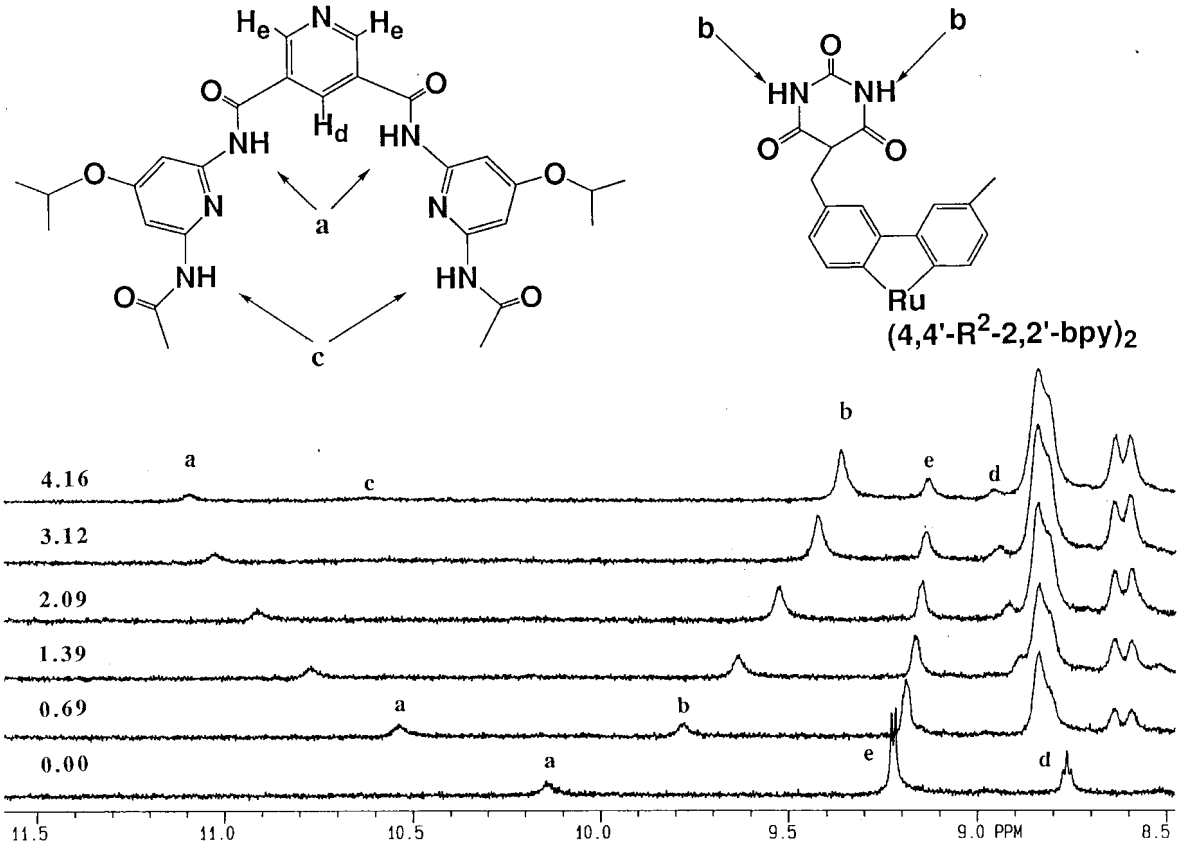


Figure 3. NMR titration of the amide protons of the Me/*i*-OPr host (4 mM) with 5–50 μl of **RuG2** stock solution (13 mM in 1.0 ml of solvent) (guest:host ratio = 0–4.16). The broad outer amide protons of the host were not observed

their respective side-chains, and the interaction between these side-chains of the host and the bipyridine rings of the guest alters the binding significantly (Table 1). The binding constants (K_a) for these hosts to **RuG2** in CH_2Cl_2 differ by more than two orders of magnitude with *t*-Bu/H <Ph/H <*n*-Pr/H. For Me/*i*-OPr (R/X) host, the introduction of an isopropoxy group *para* to the pyridine nitrogen of 2,6-diaminopyridine increases the basicity of the pyridine nitrogens and thus increases their association with the two amide groups of the barbituric acid. The Me/*i*-OPr host was found to exhibit the highest binding in both CH_2Cl_2 and d_6-DMSO (Table 1). Thus, in CH_2Cl_2 a discrimination factor of *ca* 10^3 is observed by manipulation of steric and electronic effects in these closely related host cavities with the groups R/X = *t*-Bu/H <Ph/H <*n*-Pr/H <Me/*i*-OPr.

The two hosts —$(CH_2)_3CO_2^-$/H and CF_3/H exhibit additional properties which become more apparent in solvents of high donor number.[14] The charge neutralization of the Ru(II) by the two carboxylate groups in —$(CH_2)_3CO_2^-$/H results in higher binding in CH_2Cl_2 (Table 1). However in d_6-DMSO the two carboxylate groups have virtually no effect on the binding constant [binding constant for R = *n*-Pr is equal to that for R/H = —$(CH_2)_3CO_2^-$/H]. This implies that the highly solvated carboxylate groups in d_6-DMSO are not contributing to charge neutralization from the host cavities and are not disrupting the binding of the guest. A minimized structure showing the binding of the barbituric acid ring to the host and the orientation of the carboxylate groups relative to the ruthenium bipyridine guest is shown in Fig. 4.

Unexpected effects are observed for the host with

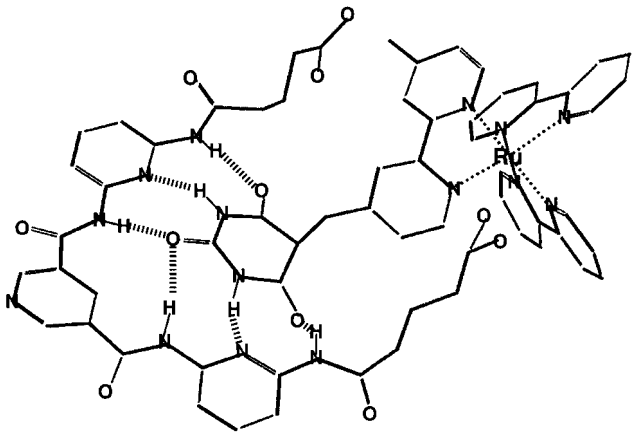


Figure 4. Minimized structure of —$(CH_2)_3CO_2^-$/H host–**RuG2** complex showing the cooperative electrostatic and H-bonding interactions

R = CF_3. Although the CF_3 electron-withdrawing group should increase the acidity of the outer amide groups of the host (those furthest from the central pyridine groups), making them more available for H-bonding, this is only observed in CH_2Cl_2. The finding that the CF_3 group reduces rather than enhances the host binding in both acetone and d_6-DMSO indicates that more than electronic effects are needed to explain its binding to **RuG2** in the more polar solvents. Additional solvation of the CF_3 must also increase solvent competition with the guest barbital ring, resulting in lower binding. Thus the solvation of (any or all parts of) the CF_3CONH group contributes to a decrease in the binding of this host to **RuG2**, such that this host experiences the largest drop in binding as the solvent is changed from CH_2Cl_2 to d_6-DMSO.

These binding studies show that some of the factors that enhance the H-bonding in a low polarity solvents (such as CH_2Cl_2) can decrease the H-bonding propensity in the more highly polar solvent (d_6-DMSO). The solvation of the groups next to the H-bonding cavity can have profound effects on the molecular recognition in high-polarity solvents. In order to design effective H-bonding interactions in donor–acceptor complexes which maintain their binding capacity in polar solvents, the steric and electronic properties of the guest and the host should be optimized[15] and, in addition to this, for the molecules chosen for this study, polar groups in the vicinity of the designed H-bonding cavity should be avoided.

EXPERIMENTAL

Solvents and starting material. Chloroform was distilled from $CaCl_2$, CH_2Cl_2 and triethylamine from CaH_2, and THF from Na–benzophenone and methanol from CaO. All deuterated solvents (Aldrich) were dried over 4 Å molecular sieves and then were used without further purification. 4,4′-Di-*t*-Bu-2,2′-bipyridine[16] and *cis*-(4,4′-R-bpy)$_2$RuCl$_2$·2H$_2$O (where R = *t*-Bu and bpy = bipyridine) and 4′-methyl-2,2′-bipyridine-4-carboxaldehyde[17] were prepared as described in the literature.[18] *cis*-(4,4′-R-bpy)$_2$Ru (**G2**)[PF_6]$_2$ (**RuG2**) (where R = *t*-Bu) was prepared as described earlier.[12]

Instrumentation. HPLC analyses (Waters instrument) were carried out by using 25–30% CH_3CN–H_2O with 0.1% TFA adjusted to pH 3.5 with NaOH and the effluent was monitored at 254 nm. NMR chemical shifts (Varian instrument, 200 MHz) are reported in ppm downfield from tetramethylsilane with coupling constants in hertz. Fast atom bombardment mass spectrometry (FAB-MS) was carried out at the Washington University Mass Spectrometry Resource Facilities and laser desorption mass spectrometry (LD-MS) at the Biomedical Research Core Facilities, University of Michigan; *m*/*z* values are reported for the protonated molecular ions, unless indicated otherwise. UV–visible spectra were acquired on a Hewlett-Packard model 8452A diode-array spectrophotometer. Fluorescence spectra were obtained using a FluoroMax spectrofluorimeter (SPEX).

Binding studies. The binding studies between **RuG2** and the hosts were investigated by ^{1}H NMR and fluorescence spectroscopy. The binding constants were obtained from non-linear least-squares curve-fitting of the data to the binding isotherms. The protocol used for each technique is detailed below.

All fluorescence binding studies were performed using CH_2Cl_2 (freshly distilled from CaH_2). To exclude oxygen and moisture, the samples were kept under argon using rubber septa and argon-filled balloons. A typical binding experiment involved the titration of **RuG2** (3.0 ml, *Ca* 0.2–5 μM) where aliquots of 2–5 μl of the host stock solution (*Ca* 2×10^{-4} M) were added. The increase in emission intensity at 610 nm (for excitation at 434 nm) was monitored as a function of host concentration. Addition of host solution was repeated until no further increase in emission intensity was observed. Different increases in fluorescence intensity were observed for the different hosts in their titration with **RuG2**. The percentage change in fluorescence intensity for **RuG2** upon binding the host molecules [ΔI_f (%)] for R/H = *t*-Bu/H, *n*-Pr/H, CF_3/H, phenyl/H, —$(CH_2)_3CO_2^-$/H was 53 ± 3, 82 ± 5, 107 ± 10, 67 ± 3, 145 ± 35%, respectively, and for R/X = Me/*i*-OPr it was 61 ± 3%. For the different host molecules several methods were used to determine the binding. For *t*-Bu/H, *n*-Pr/H, CF_3/H, phenyl/H and Me/*i*-OPr hosts, fluorescence titration with **RuG2** was carried out in CH_2Cl_2. Competition methods were used to carry out additional fluorescence titrations of **RuG2** with *t*-Bu/H, CF_3/H, *n*-Pr/H and Me/*i*-OPr hosts, where excess barbital was used (i.e. 500–600 times in the case of *t*-Bu/H). In a similar manner, excess *t*-Bu/H host (20–300 times) was used in the fluorescence titrations of **RuG2** with CF_3/H, *n*-Pr/H and Me/*i*-OPr hosts. The overall binding constants between **RuG2** and the hosts (R/X = CF_3/H, *n*-Pr/H and Me/*i*-OPr were calculated from the binding constants for *t*-Bu/H and barbital,[12] for *t*-Bu/H with **RuG2**[12] and the observed binding constants (determined under conditions of competition).

^{1}H NMR binding studies were carried out in d_1-chloroform, d_3-acetonitrile, d_6-acetone, or d_6-DMSO. In a typical experiment, the ^{1}H NMR spectrum of the host solution (4 mM in 0.5–0.7 ml of solvent) was recorded and then small aliquots (5–50 μl) of **RuG2** stock solution (13 mM in 1.0 ml of solvent) were added to the NMR tube via a gas-tight syringe. The chemical shifts of the amide protons of the host were monitored as a function of **RuG2** concentration. Addition of **RuG2** guest was continued until no further shifts of the amide protons were observed.

Molecular mechanics calculations. All calculations were carried out using the Molecular Mechanics program

(MM2),[22] part of the CAChe Work System (v. 3.8), from CAChe Scientific.

The initial structure of the host or guest molecule was constructed assuming that the molecule is planar in the conjugated regions. Local minima were avoided by choosing the lowest energy configuration from a sequential minimization by varying all the dihedral angles of the inter-ring bonds in 12° steps. All the N—H and O=C bonds responsible for the hydrogen bonding in the host molecule were oriented towards the center so that the hydrogen bonding network would be intact. The selected structure was further optimized to within 0.0003 kcal mol^{-1} (1 kcal = 4.184 kJ).

The $Ru^{II}(bpy)_2$ group was attached to the bipyridine side of the host molecules and the coordination environment of this ruthenium was taken to be that of the crystal structure.[23]

Once the individual host or guest molecules had been optimized, the oxygen atom of the pivotal O=C bond in the barbiturate ring was brought to a 2 Å distance from the *para*-H of the central pyridine of the host molecule with C=O H angle of 180°. For the starting configuration, the 2,4-Cs of the barbiturate ring of the guest molecules were placed in the same plane of the pyridine ring of the host and the structure was then minimized to a limit of 0.0003 kcal mol^{-1}.

Preparation of 2,6-diamino-4-isopropoxypyridine. Diethyl-4-isopropoxypyridine-2,6-dicarboxylate ester.[19–21]. Isopropyl iodide (20.0 ml, 34.0 g, 0.20 mol) was added to a suspension of the sodium salt of diethyl-4-hydroxypyridine-2,6-dicarboxylate ester (22.8 g, 87.3 mmol) in dry DMF (230 ml). The mixture was heated while stirring at 135 °C in an oil-bath for 5 h. The milky solution became transparent yellow, then brownish owing to the formation of iodine. It was cooled and the DMF was removed under vacuum. The oily residue obtained was dissolved in ethyl acetate (300 ml) and the solution was washed twice with 100 ml of 10% Na_2CO_3, then with 50 ml 20% sodium hydrogen sulfite (to bleach the iodine), to give a pale yellow solution that was dried over magnesium sulfate. Evaporation under reduced pressure gave a dark oily material, which was further dried under vaccum overnight to yield 11.5 g (46.8%) of a yellow oil. Analysis. Calculated for $C_{14}H_{19}NO_5$: C, 59.77; H, 6.81; N, 4.98. Found: C, 59.60; H, 6.99; N, 4.91%. NMR, δ (ppm) (200 MHz, $CDCl_3$): ^{1}H, 1.30–1.50 (m, 12H, CH_3), 4.43 (q, J = 7.08 Hz, 4H, OCH_3), 4.73 (m, J = 6.0 Hz, 1H, OCH), 7.69 (s, 2H, mHpyr); ^{13}C, 14.15, 21.58, 62.29, 71.25, 114.91, 150.12, 164.80, 165.95.

4-Isopropoxypyridine-2,6-dicarboxylic acid dihydrazide. The diester (11.5 g, 40.9 mmol) was dissolved in 100 ml of dry ethanol. Hydrazine (98%, 7.0 ml, 7.2 g, 1.4×10^2 mmol) was added to the solution and the mixture was refluxed for 5 h. After cooling, diethyl ether was added to precipitate a creamy product that was filtered off, washed with diethyl ether then dried *in vacuo* at 40 °C. The solid obtained was recrystallized from ethanol to yield 5.66 g (54.6%) of a white solid, m.p. 195 °C. Analysis. Calculated for $C_{10}H_{15}N_3O_3$: C, 47.42; H, 5.97; N, 27.65. Found: C, 47.42; H, 5.84; N, 27.54%. NMR, δ (ppm) (200 MHz, CD_3CN): ^{1}H, 1.35 (d, J = 6.06 Hz, 6H, CH_3), 4.20 (s, br, 4H, NH_2), 4.87 (m, J = 6.06 Hz, 1H, OCH), 7.64 (s, 2H, mHpyr), 9.30 (s br, 2H, NH).

4-Isopropoxy-2,6-dicarbethoxyamidopyridine. The dihydrazide (5.20 g, 20.5 mmol) was dissolved in 120 ml of 10% HCl at 10 °C and sodium nitrite solution (4.60 g, 66.4 mmol, in 20 ml of water) was added dropwise. The reaction mixture was kept at 10 °C for 20 min. A creamy white, gummy precipitate resulted with little foaming. The solid obtained was collected by filtration and dried by pressing it between pieces of filter-paper. The solid was dissolved in 200 ml of ethanol and the solution was heated under reflux conditions for 2 h, 50 ml of solvent were evaporated, then 50 ml of dry ethanol were added, and refluxing was continued for a total reflux time of 4 h. The solution was then concentrated to about 60 ml and water (300 ml) was added. The resulting creamy white precipitate was collected by filtration and dried under vacuum. It was recrystallized from aqueous ethanol to yield 5.88 g (92%) of a white solid, m.p. 112–114 °C. Analysis. Calculated for $C_{14}H_{21}N_3O_5$: C, 54.01; H, 6.80; N, 13.50. Found: C, 54.01; H, 6.66; N, 13.93%. NMR, δ (ppm) (200 MHz, $CDCl_3$): ^{1}H, 1.20–1.40 (m, 12H, CH_3), 4.24 (q, J = 7.08 Hz, 4H, CH_2), 4.68 (m, J = 5.8 Hz, 1H, OCH), 7.21 (s, 2H, mHpyr), 8.21 (s, 2H, NH).

2,6-Diamino-4-isopropoxypyridine. The dicarbamate (5.85 g, 18.8 mmol) was added to 10% sodium hydroxide solution (100 ml) and the mixture was refluxed for 3 h. The reaction mixture was cooled and the diamine precipitated as white crystals. It was filtered by suction and the mother liquor was extracted with ethyl acetate (4×200 ml), dried over sodium carbonate and evaporated under vacuum. The greenish solid obtained was added to the above filtered solid and the mixture was recrystallized from water to give pale yellow needle-like crystals, yield 1.25 g (40%), m.p. 154 °C. Analysis. Calculated for $C_8H_{13}ON_3$: C, 57.46; H, 7.84; N, 25.13. Found: C, 57.35; H, 7.71; N, 25.00%. NMR, δ (ppm) (200 MHz, $CDCl_3$): ^{1}H, 1.30 (d, J = 6.0 Hz, 6H, CH_3), 4.15 (s br, 4H, NH_2), 4.49 (m, J = 6.0 Hz, 1H, OCH), 5.46 (s, 2H, mHpyr).

*Preparation of 3,5-bis[(6-amino-4-isopropoxypyrid-2-yl)amino] carbonylpyridine [**1**].* A slurry of 3,5-pyridinedicarboxylic acid (1.00 g, 6.0 mmol) in chloroform (7 ml), thionyl chloride (16 ml, excess) and a drop of DMF was refluxed for 5 h under an inert atmosphere. A clear orange–yellow solution was obtained. The mixture was evaporated under vacuum to yield a light-orange

solid that was washed with benzene to remove excess thionyl chloride. The crude product was dissolved in CH_2Cl_2 (17 ml) and added to a vigorously stirred solution of 2,6-diamino-4-isopropoxypyridine (3.50 g, 21.0 mmol) and triethylamine (4.0 ml) in CH_2Cl_2 (70 ml) at 0 °C. The reaction mixture was allowed to warm to room temperature and then stirred for 24 h. The solvent was removed under reduced pressure. The light-green solid which formed was washed with water to remove excess of 2,6-diamino-4-isopropoxypyridine and triethylamine hydrochloride. The crude product obtained was purified by recrystallization from ethanol–water to yield 2.50 g (90%) of a light greenish yellow powder. FAB-MS: $[M+H]^+$ 466.2. NMR, δ (ppm) (200 MHz, d_6-DMSO): ^{1}H, 1.30 (d, CH_3, 12H), 4.03 (m, CH, 2H), 5.27 (s, NH_2, 4H), 5.90 (s, pyr-3H, 2H), 7.32 (s, pyr-5H, 2H), 8.92 (s, pyr-4H, 1H), 9.28 (s, pyr-2, 6H, 2H), 9.59 (s, NH, 2H).

*Preparation of 3,5-bis[(6-aminopyrid-2-yl)amino]carbonylpyridine[10b][**2**].* 3,5-Bis[(6-aminopyrid-2-yl)amino]-carbonylpyridine was prepared as described.[10b]

Preparation of the hosts. R/X = CF_3/H and R/X = Me/i-OPr. The corresponding diamino host **[1]** or **[2]** was dissolved in anhydrous pyridine and the corresponding acid anhydride RCOOCOR (R = CF_3 or CH_3) (3 equiv., excess) was added dropwise by a syringe while stirring, and the solution was left under nitrogen for 16 h. The volatiles were removed under vacuum and the residue was dissolved in a minimum amount of CH_2Cl_2. The crude acylated host was precipitated by the addition of heptane and filtered using a fritted glass funnel. This product was purified by chromatography (silica gel, 10% ethanol, 90% CH_2Cl_2), yield 65–80%. LD-MS: CF_3/H; $[M+H]^+$ 543.6. NMR, δ (ppm) (200 MHz, d_6-DMSO): 1H, 7.62 (d, J = 6.96 Hz, 2H, 5′py), 8.04 (m, J = 7.34 Hz, 4H, 3′–4′py), 8.85 (s, 1H, 4py), 9.28 (s, 2H, 2–6py), 11.06 (s, 2H, NH), 11.78 (s, 2H, NH′). Absorption spectra in CH_2Cl_2 [λ abs, nm (ε, 1 l mol^{-1} cm^{-1})]: 236 (52900), 300 (57600). FAB-MS: Me/*i*-OPr; $[M+H]^+$ 550.1. NMR, δ (ppm) (200 MHz, d_6-DMSO): ^{1}H, 1.35 (d, J = 5.84 Hz, 12H, $CHCH_3$), 2.13 (s, 6H, CH_3), 4.69 (septet, J = 5.84 Hz, 2H, CH), 7.47 (d, J = 3.02 Hz, 4H, 3′–5′py), 8.76 (s, 1H, 4py), 9.23 (s, 2H, 2–6py), 10.12 (s, 2H, NH), 10.72 (s, 2H, NH′). Absorption spectra in CH_2Cl_2 [λ abs, nm (ε, l mol^{-1} cm^{-1})]: 232 (42300), 292 (20300), 384 (800).

R/X = t-Bu/H and R/X = phenyl/H. The diamino host **[2]** was dissolved in dry THF and 3 equiv. of triethylamine and cooled to 0 °C. The corresponding acid chloride RCOCl (R = *tert*-butyl or phenyl) (2.5 equiv.) was added dropwise with a syringe while stirring, then left under nitrogen for 12 h. The volatiles were removed under vacuum and the residue was dissolved in a minimum amount of THF and water was added to precipitate the desired host, which was filtered using a fritted glass funnel and washed with water, yield 70–90%. FAB-MS: *t*-B/H; $[M+H]^+$ 518.1. NMR, δ (ppm) (200 MHz, d_6-DMSO): ^{1}H, 1.25 [s, 18H, $(CH_3)_3$], 7.73 (m, 2H, 3′py), 7.87 (m, 4H, 4′5′py), 8.82 (s, 1H, 4py), 9.26 (s, 2H, 2-6py), 10.15 (s, 2H, NH), 10.84 (s, 2H, NH′). Absorption spectra in CH_2Cl_2 [λ abs, nm (ε, l mol^{-1} cm^{-1})]: 232 (44900) 302 (34400). LD-MS: phenyl/H; $[M+H]^+$ 567.2. NMR, δ (ppm) (200 MHz, d_6-DMSO): ^{1}H, 7.60 (m, 6H, 3′4′5′py), 7.98 (m, 10H, Ph), 8.87 (s, 1H, 4py), 9.29 (s, 2H, 2–6py), 10.50 (s, 2H, NH), 11.10.95 (s, 2H, NH′). Absorption spectra in CH_2Cl_2 [λ abs, nm (ε, l mol^{-1} cm^{-1})]: 232 (44700), 306 (36200).

R/X = —$(CH_2)_3CO_2^-$/H. The diamino host **[2]** (70 mg, 0.21 mmol) was dissolved in anhydrous pyridine (30 ml) with glutaric anhydride (2.5 equiv.), then stirred under nitrogen at 100 °C for 16 h. The volatiles were removed under vacuum and water was added to the oily residue. Using a fritted glass funnel, a creamy brown solid was collected and washed with water, ethanol and diethyl ether. The crude solid was dissolved in 20% sodium hydrogencarbonate solution (10 ml), then precipitated with 4M HCl–acetic acid solution (1:1) (pH 4). The light creamy solid was collected by filtration and dried under vacuum, yield 75 mg (64%). FAB-MS: —$(CH_2)_3CO_2^-$/H; $[M+H]^+$ 578.1. NMR, δ (ppm) (200 MHz, d_6-DMSO): ^{1}H, 1.84 (q, J = 7.26 Hz, 4H, —CH_2—), 2.29 (t, J = 7.34 Hz, 4H, —CH_2CONH), 2.48 (t, J = 7.34 Hz, 4H, —CH_2COOH), 7.85 (d, J = 3.30 Hz, 6H, 3′4′5′py), 8.80 (s, 1H, 4py), 9.26 (s, 2H, 2–6py), 10.18 (s, 2H, NH), 10.83 (s, 2H, NH′), 12.09 (s, 2H, COOH). Absorption spectra in CH_2Cl_2 [λ abs, nm (ε, l mol^{-1} cm^{-1})]: 214 (50100), 232 (39700), 300 (30600).

R/X = n-Pr. The diamino host **[2]** (70 mg, 0.21 mmol) was dissolved in anhydrous pyridine and 30 μl of butyric anhydride (2.5 equiv.) were added dropwise while stirring, then the solution was kept at 100 °C for 16 h under nitrogen. The volatiles were removed under vacuum and diethyl ether was added to the oily residue. Using a fritted glass funnel, a creamy white solid was collected and washed with diethyl ether and 10% sodium hydrogencarbonate. The crude solid was purified by chromatography (neutral alumina, 0–10% ethanol, CH_2Cl_2), yield 88 mg (90%). FAB-MS: *n*-Pr/H; $[M+H]^+$ 490.1. NMR, δ (ppm) (200 MHz, d_6-DMSO): ^{1}H, 0.93 (t, J = 7.32 Hz, 6H, CH_3), 1.62 (m, J = 7.32 Hz, 4H, —CH_2—), 2.40 (t, J = 7.32 Hz, 4H, —CH_2CONH), 7.84 (m, J = 5.22 J' = 4.84, J'' = 4.96 Hz, 6H, 3′4′5′py), 8.79 (s, 1H, 4py), 9.26 (s, 2H, 2–6py), 10.17 (s, 2H, NH), 10.86 (s, 2H, NH′). Absorption spectra in CH_2Cl_2 [λ abs, nm (ε, l mol^{-1} cm^{-1})]: 232 (21200), 302 (15200).

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