Dinucleotide Hydrolysis Promoted by Dinuclear Zn Complexes − The Effect of the Distance between Zn Ions in the Complexes on the Hydrolysis Rate

Shun-ichi Kawahara[a] and Tadafumi Uchimaru*[a]

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The rate of hydrolysis of an RNA dimer, uridylyl(3′→5′)uridine (UpU), promoted by dinuclear Zn complexes resulting from a series of ligands with differently spaced Zn ion binding sites, was examined. Although the ligands in the present study have in common two di-2-pyridylmethylamino moieties as the Zn binding sites, the activities of these ligands toward the hydrolysis were quite different. Our results suggest that the distance between metal ion coordination sites is an essential factor for the intrinsic activities of the dinuclear complexes.

Introduction

Biomimetic chemistry[1] involves the imitation of functional biomolecules, usually enzymes, by simple artificial molecules. Many studies have been carried out in this field, not only because investigations on the actions of simple artificial molecules are helpful for understanding the reaction mechanism of biomolecules, but also because biomimetic compounds have wide potential scope for medicinal and pharmaceutical applications. In particular, the design of an artificial molecule that promotes the hydrolysis of phosphodiester linkages in RNA under neutral conditions has attracted much attention, since such a molecule will provide a basic structure of anti-cancer and anti-viral drugs.

Many natural ribonucleases and ribozymes that catalyze the hydrolysis of phosphodiester bonds in RNA have metal ions in their active sites.[2] Recently, the double metal-ion mechanism has been widely accepted: the catalytic efficiency of natural nucleases is considered attributable to cooperative action of the two (or more) metal cations in the active sites.[2] Vallee et al. reported that cocatalytic motifs in the enzymes contain two or more ions, and that the distances between the ions range between 3 and 5 Å, based on the X-ray structure analyses.[2c] Our previous theoretical study supported the double metal-ion mechanism.[3] Inspired by this mechanism, several research groups have developed various dinuclear metal-ion complexes possessing high catalytic efficiency toward the hydrolysis of phosphodiester linkages.[4] The efficiency of the catalytic activity of the dinuclear complex can often be related to the distance between the two metal ions in the complex. However, very few systematic studies have been reported from this point of view. Only one exception was reported by Chapman et al.; namely, that the distance between the metals determines the selectivity of the catalysts.[4d]

We examined the rate of hydrolysis of an RNA dimer, uridylyl(3′→5′)uridine (UpU), promoted by dinuclear Zn complexes resulting from a series of ligands. Because of the lack of basicity of the uracil ring, the reaction of UpU is the simplest model of nucleotide hydrolysis. Di-2-pyridylmethylamine has a high affinity for the Zn ions: log of the binding constant (log k_{bind}) is 7.57 in 0.10 M KNO_3.[5] Our ligands have two differently distanced di-2-pyridylmethylamino groups at the benzylic positions as Zn coordination sites. Herein, we show that the efficiency of the hydrolytic activity of the Zn complexes is highly sensitive to the distance between the two Zn ions in the complexes.

Results and Discussion

Figure 1 shows the structures of the ligands used in this study. Ligands **1**−**5** have two di-2-pyridylmethylamino groups as Zn coordination sites, while ligand **6** has only one coordination site. In all the ligands, di-2-pyridylmethylamino groups are attached to the benzylic carbon atoms.

Semiempirical AM1[6] calculations suggested that the shortest distance between the two nitrogen atoms would be 4.4, 5.2, 7.7, 7.2, and 11.3 Å in **1a**, **2a**, **3a**, **4a**, and **5a**, respectively. The conformational analysis using Monte Carlo (MC) simulations was studied for bis(hydrogen dimethylammonium)dications (**1**−**5b**) as the models to confirm the distribution of the distance between the two intramolecular cations. Figure 2 shows the relationship between the distance of the two protons (horizontal axis, Å) and the energy (vertical axis, kcal/mol). The results of the conformers, which had energies within 20 kcal/mol for their most stable conformers, are shown. The order of the proton−proton distance was **1b** < **2b** < **4b** ≤ **3b** << **5b**. However, a widely spread distribution was observed in the case of **1b**, and the energy of the conformer of **1**, which has the minimum distance, was very high.

Next, the affinity of the ligands toward Zn ions was studied through competitive titration with 2-(5-bromo-2-

[a] National Institute of Advanced Industrial Science and Technology,
Tsukuba, Ibaraki 305-8565, Japan
Fax: (internat.) + 81-298/61-4487
E-mail: t-uchimaru@aist.go.jp

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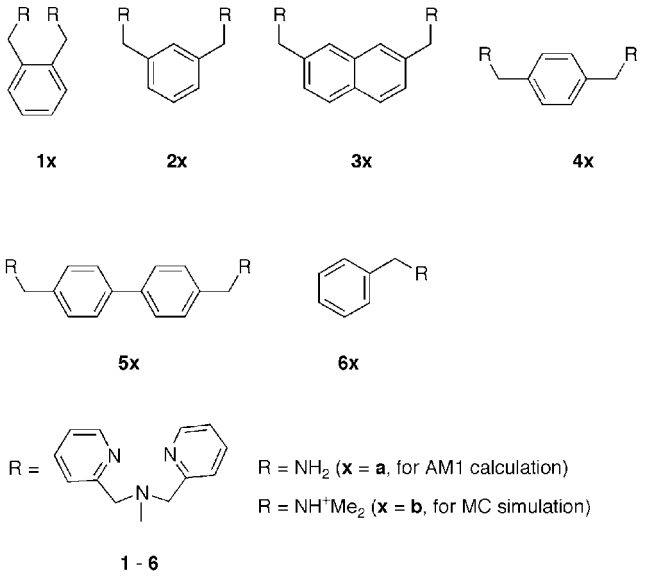

Figure 1. Structures of the ligands

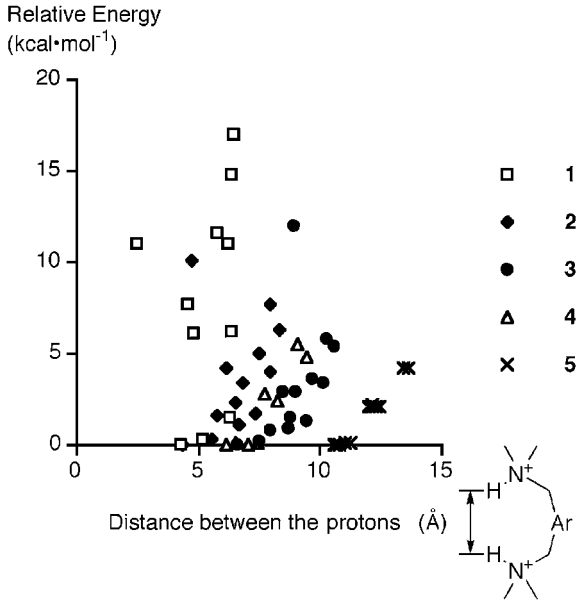

Figure 2. Relationship between the distance of the two protons (horizontal axis, Å) and the energy (vertical axis, kcal/mol)

pyridylazo)-5-[*N*-2-propyl-*N*-(3-sulfopropyl)amino]phenol (5Br-PAPS, see Supporting Information, Section 2). The titration results suggest that at least 95% of the ligands **2**−**5** in solution form a dinuclear complex under the experimental conditions in this report (typically, [Ligand] = 0.1 mM, [Zn] = 0.05 mM, see Figure 3 and Exp. Sect.). Hereafter, we refer to the dinuclear and mononuclear complexes as **L**-Zn_2 and **L**-Zn_1, respectively. We also carried out titration experiments by using Cu^{II} ion solution (see Supporting Information, Section 3). The Cu^{II} ion titration curves indicate that ligands **2**−**5** form the dinuclear Cu^{II} ion complexes and only **1** does not form a dinuclear complex. The binding affinity of the Zn ion with di-2-pyridylmethylamine is much less than that of the Cu^{II} ion (log k_{bind} = 9.31 in 0.10 M KNO_3 [5]). Additionally, from the result of the kinetics described below, it can be suggested that ligand **1** cannot form the dinuclear complex **1**-Zn_2 otherwise the amount of the dinuclear Zn ion complex of ligand **1** under the experimental conditions in this report would be considerably smaller than that of the dinuclear complex resulting from the other ligands.

We examined the rate of hydrolysis of UpU at pH 7.0 and 60 °C in the presence of the ligands. The ligands **1**−**6** showed no ability to hydrolyze UpU without Zn ions. We kept the concentration of Zn ions at 0.1 mM and measured the pseudo-first order rate constant k_{obs} under different ligand concentrations (from 0 to 0.4 mM). The values of k_{obs} are plotted against ligand concentration in Figure 3-I.[7]

The value of k_{obs} in the presence of a 0.1 mM amount of the Zn ion without any ligand was $4.0 \times 10^{-4}\ h^{-1}$. For ligands **2**, **3**, and **4**, the value of k_{obs} increased with increase of the ligand concentration, and the largest values of k_{obs} for these ligands were observed under the conditions of a concentration ratio of [Zn]/[ligand] of 2:1. Hereafter, we refer to this condition as condition A. Further increase of the concentration of these ligands resulted in a decrease of k_{obs}. These results suggest that ligands **2**, **3**, and **4** should form a considerable amount of dinuclear Zn complexes under condition A and that, compared to the Zn ion itself and the mononuclear complexes, the resulting dinuclear complexes should be more active toward the hydrolysis. The enhancement of the rate of hydrolysis for ligand **2** under condition A was the most striking: the k_{obs} found for the condition A was $1.6 \times 10^{-2}\ h^{-1}$, which was around 40 times and 4 times as large as k_{obs} measured in the absence of the ligand and that observed for the condition of [Zn]/[**2**] = 1:1 (condition B), respectively. For ligand **5**, k_{obs} gradually increased with an increase of the ligand concentration, but the enhancement of the hydrolysis rate was much less significant than for ligands **2**, **3**, and **4**. The values of k_{obs} for ligands **1** and **6** were insensitive to the ligand concentration: these ligands did not substantially affect the rate of hydrolysis.

Figure 3-II shows the difference in k_{obs} from ligand to ligand under conditions A and B. The difference in k_{obs} under condition A was much more striking than those observed under condition B. Although ligands **1**−**5** commonly have two di-2-pyridylmethylamine moieties, ligand **2** showed substantially higher activity than the other ligands.

Next, the values of k_{obs} were measured under different concentrations of the Zn ion (from 0.1 to 1.0 mM) with a constant concentration (0.1 mM) of the ligands. The values of k_{obs} are plotted against the concentration of the Zn ion ([Zn]) in Figure 3-III. Figure 3-III clearly shows again that ligand **2** enhances the rate of hydrolysis under the condition of [Zn]/[**2**] = 2:1 (condition A). We also measured k_{obs} without the ligand under the same conditions. In the absence of the ligand, although the value of k_{obs} tends to increase slightly upon increasing [Zn], the value of k_{obs} is found to be relatively insensitive to [Zn] (see Figure 3-III). For ligand **2**, however, k_{obs} increased from 1.4×10^{-3} to $1.6 \times 10^{-2}\ h^{-1}$ on going from the condition of [Zn]/[ligand] = 1:1

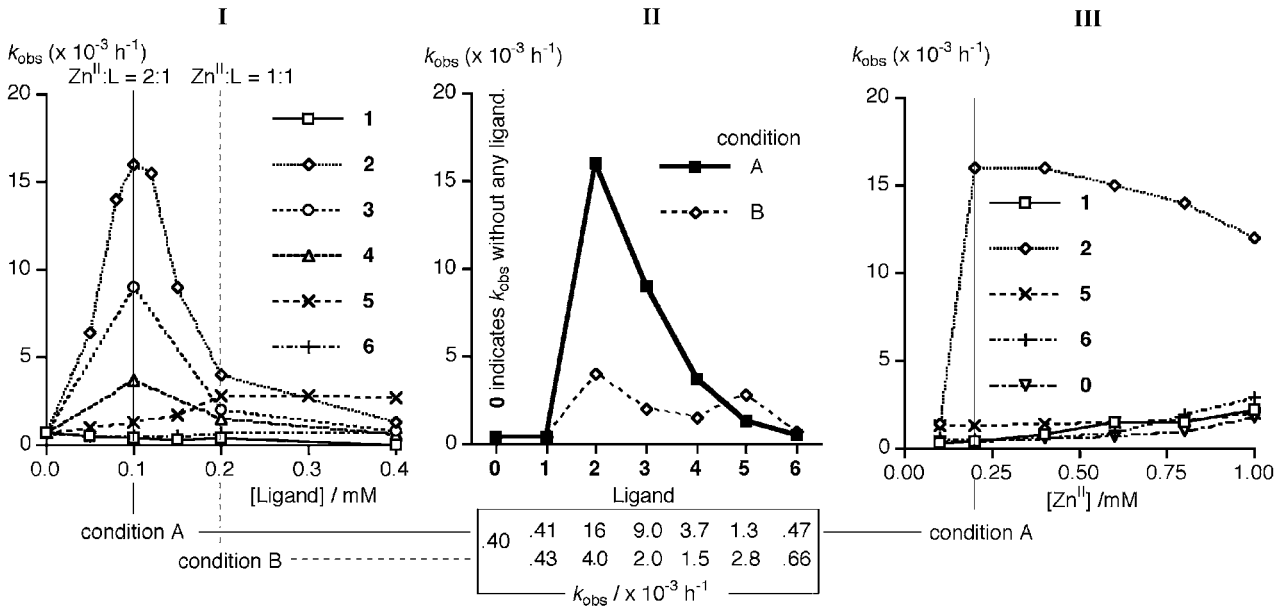

Figure 3. I) k_{obs} with various concentrations of **L** at a constant Zn ion concentration (0.2 mM); II) Relationship between k_{obs} and the backbone structure of the complexes under the conditions A and B; III) k_{obs} at a constant concentration of **L** (0.1 mM) with various Zn ion concentrations

to that of [Zn]/[ligand] = 2:1 (condition A). For ligand **2**, the value of k_{obs} decreased gradually in the presence of an excess amount of Zn ions. This finding can be interpreted as follows: Zn ions without the ligands, which have a much lower catalytic activity than that of **2**-Zn_2, interact with the substrate, and this interaction will inhibit electrostatically the approach of the dinuclear complex to the substrate. As expected from the results given in Figure 3-I, the k_{obs} values for ligands **5** and **6** were insensitive to [Zn] and these ligands showed no substantial enhancement of the rate of hydrolysis.

The binding constants of the ligands toward Zn ions and the structure of the ligands should both be related to the difference in k_{obs} values under condition A (Figure 3-II). As described above, at least 95% of the ligands form dinuclear Zn complexes under this condition. The k_{obs} values increased almost linearly with increase of [Zn] and [ligand] for both ligands **2** and **4**. This trend was observed regardless of the concentration of the dinuclear Zn complexes. We measured the rate of the hydrolysis under different concentrations of the Zn ion and ligands with the concentration ratio of [Zn]/[ligand] being kept at 2:1. As shown in Figure 4,[8] the k_{obs} value for ligand **2** was always greater than that for ligand **4**.

These results suggest that the differences in k_{obs} in Figure 3-II are attributable mainly to the difference in the structure of the ligands, and more specifically, the difference in the distance between the two metal coordination sites. The k_{obs} values under condition A in Figure 3-II are likely to be almost proportional to the intrinsic activities of the dinuclear complexes toward the hydrolysis. Ligands **2**, **3**, and **4** enhanced the hydrolysis rate under condition A and, thus, the two Zn ions in the dinuclear complexes of these

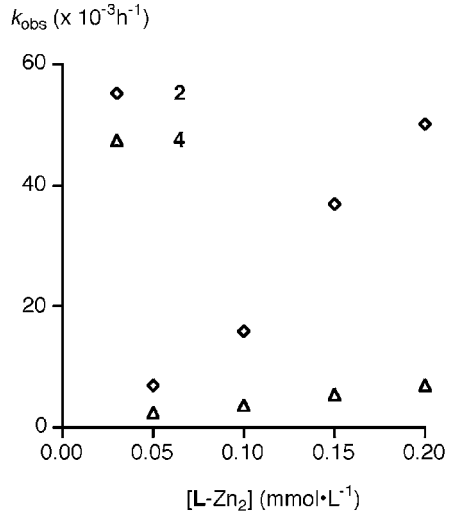

Figure 4. The k_{obs} values of **2**-Zn_2 and **4**-Zn_2 at various concentrations

ligands will act in a cooperative manner to promote the hydrolysis. According to the k_{obs} values in Figure 3-II, the intrinsic activity of the **2**-Zn_2 would be around 1.8 times and 4.3 times as large as those of **3**-Zn_2 and **4**-Zn_2, respectively. Meanwhile, ligands **1** and **5** did not enhance the hydrolysis under condition A. Formation of the dinuclear complex **1**-Zn_2 might be difficult (see above). Indeed, ligand **1** did not show enhancement of the rate of hydrolysis even under condition A. On the other hand, the two Zn ions in **5**-Zn_2 cannot act in a cooperative manner, probably because the distance between the two Zn ions is too large.

Previously, Chapman et al. examined systematically the activities of dinuclear Zn complexes toward the hydrolysis

of phosphodiesters.[4d] Their study covered a series of dinuclear complexes, with distances between the two metal ions in the binding sites similar to those for ligands **1**−**5**. They found that the dinuclear complex with a relatively large distance between the coordination sites, in which the distance between two bound metal ions would be comparable to that for ligand **5**, has the highest activity for the hydrolysis of phosphodiester, while the dinuclear complex with a short distance between the coordination sites has a higher activity for the hydrolysis of phosphomonoesters. They point out that a phosphodiester is sterically more hindered than a phosphomonoester, and that the phosphomonoester dianion is more basic than the phosphodiester monoanion. They then concluded that the phosphomonoester hydrolysis needs the two metal ions to be close, while the phosphodiester hydrolysis needs a larger separation between the two metal ions. In contrast, in the present study, ligand **2** was found to have the highest activity toward the phosphodiester hydrolysis. Obviously, a shorter distance between the two metals was preferred for the hydrolysis of phosphodiester, considering the fact that ligand **1** cannot bind two metal ions. The findings of Chapman et al. and ourselves may not seem to be in agreement with each other at first sight. However, a cyclic amine/imine structure, whose behavior of metal-ion binding will be quite different from that of the di-2-pyridylmethylamino group, was employed for the metal binding sites in their study. Consequently, their results and ours cannot be compared directly.[9] These findings show that dinuclear complexes, which have a different metal-ion binding site structure, react with the substrates in a different manner. We would like to point out that a ligand whose structure corresponds to **2** has been successfully applied as a metal coordination site for the sequence-selective hydrolysis of RNA:[4i,10] Matsuda et al. introduced the Zn ion binding site at the 5′-end of a DNA oligomer that recognizes the targeted sequence of RNA. They found that the ligand structure of **2** hydrolyzes the phosphodiester bonds in RNA at the targeted position in highly efficient manner.[4i] Xu et al. reported the X-ray structure of the phosphodiesterase (PDE4) active site.[11] They pointed out that the distance between the two ions in the active site is around 3.9 Å, and suggested that the two metal ions constitute a dinuclear motif similar to that found in other enzymes that catalyze the hydrolysis or formation of a phosphate bond. Meanwhile, our MC simulations suggested that the distance between the two ions in the dinuclear complexes resulting from ligands **1**−**5** would increase in the order **1** < **2** < **4** ≤ **3** < < **5**. For all ligands, the two metal ions would be more than 4 Å apart. However, the titration experiments showed that ligand **1** cannot form the dinuclear complex. Considering these findings, the metal complex resulting from ligand **2** (**2**-Zn_2) is likely to bear the closest resemblance to the dinuclear motif observed in enzymes: the distance between the two metal ions in **2**-Zn_2 will probably be similar to that in the dinuclear motif in PDE4. Thus, it is reasonable that **2**-Zn_2 was found to be the most efficient catalyst for the hydrolysis of UpU among the metal complexes studied in the present work.

Conclusion

We examined the activities of the ligands **1**−**6** toward the hydrolysis of UpU in the presence of Zn ions. In agreement with the double metal-ion mechanism, ligands **2**, **3**, and **4** showed the highest activity under dinuclear complex forming conditions. Interestingly, although ligands **1**−**5** have two di-2-pyridylmethylamino moieties as Zn ion binding sites in common, the activities of these ligands toward the hydrolysis are quite different. Our results suggest that the difference in the k_{obs} value for ligands **2**−**5** under the dinuclear complex forming condition (condition A) should be almost proportional to the intrinsic activity of the dinuclear complexes toward the hydrolysis, which implies that the distance between the metal ion coordination sites will be an essential factor for the intrinsic activities of the dinuclear complexes.

Experimental Section

General: Silica gel TLC and column chromatography were performed using Merck Kieselgel 60F-254 and Wakogel C-200, respectively. All NMR spectra were recorded with a Varian Gemini-300BB spectrometer. ^1H spectra were referenced to the TMS peak, and ^{13}C spectra were referenced to the $CDCl_3$ peak. IR spectra were recorded with a Jasco FT/IR-5300 instrument. Mass spectra were recorded with a Hitachi N80E instrument. HPLC purity analysis of the ligands was carried out using Luna-Si column (Phenomenex, 4.6 × 150 mm, 3 μm) using $CHCl_3$/MeOH (1:1, v/v) as an eluent. Figure 5-I in the Supporting Information shows the HPLC profiles of the free ligands. Over 99% purity was guaranteed for all ligands in the analysis.

Preparation of the Ligands: Ligand **1** was prepared by *N*-alkylation of *o*-xylylenediamine dihydrochloride,[12] in a modification of the procedure reported by Sato et al.[13] A solution of 2-(chloromethyl)-pyridinium hydrochloride (3.30 g, 20.12 mmol) and *o*-xylylenediamine dihydrochloride (1.05 g, 5.02 mmol) in water (12 mL) was added to aqueous NaOH solution (5.0 M, 12 mL). Then, hexadecyltrimethylammonium chloride (0.16 g, 0.50 mmol) and $CHCl_3$ (10 mL) were added successively to the solution. The resulting mixture was heated to reflux in $CHCl_3$ for 12 h with vigorous stirring. The precipitate was separated by filtration and the filtrate was extracted three times with $CHCl_3$ (20 mL portions). The organic layer was collected and dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on silica gel by elution with $CHCl_3$/MeOH (the content of MeOH was increased from 0 to 2%). The yield of the product was 44% (1.1 g).

***N*,*N*,*N*′,*N*′-Tetra-(2-pyridylmethyl)-*o*-xylylenediamine (1a):** IR(KBr): $\tilde{\nu}$ = 1591, 1570, 1473, 1433 (C=C, C=N), 1047 cm^{-1} (C−N). − ^1H NMR ($CDCl_3$): δ = 3.67 (s, 4 H, PhCH_2 × 2), 3.70 (s, 8 H, PyCH_2 × 4), 7.12−7.16 (m, 4 H, Ph), 7.18−7.65 (m, 12 H, Py^{3-5} × 4), 8.50−8.52 (m, 4 H, Py^6 × 4). − ^{13}C NMR ($CDCl_3$): δ = 55.87 (PhCH_2), 60.28 (PyCH_2), 121.97 (Py^3), 122.87 (Py^5), 126.96 ($Ph^{3\ or\ 4}$), 129.49 ($Ph^{4\ or\ 3}$), 136.52 (Py^4), 137.79 (Ph^1), 148.97 (Py^6), 159.77 (Py^2). − HRMS for $C_{32}H_{33}N_6$ $[M]^+$: calcd. 501.2767; found 501.2754.

Ligands **2**−**6** were prepared in yields of 80−95% by *N*-alkylation of 2,2′-dipyridylmethylamine with the corresponding arylmethyl-(di)bromides. A typical procedure is given for preparation of ligand

2: *m*-Xylylenedibromide (1.32 g, 5.00 mmol), di-2-pyridylmethylamine (2.00 g, 9.63 mmol), and *N*,*N*-diisopropylethylamine (1.30 g, 10.06 mmol) were dissolved in $CHCl_3$ (50 mL). After 4 h of stirring at room temperature, the resulting solution was washed once with water (25 mL) and dried over Na_2SO_4. The product was purified by silica gel chromatography with $CHCl_3$/MeOH (the content of MeOH was increased from 0 to 2%) as the eluent. The yield of the product was 95% (2.37 g).

***N*,*N*,*N*′,*N*′-Tetra-(2-pyridylmethyl)-*m*-xylylenediamine (2):** IR (neat): $\tilde{\nu}$ = 1589, 1570, 1473, 1433 (C=C, C=N), 1045 cm^{-1} (C−N). − ^{1}H NMR ($CDCl_3$): δ = 3.69 (s, 4 H, PhCH_2 × 2), 3.81 (s, 8 H, PyCH_2 × 4), 7.10−7.15 (m, 4 H, Ph), 7.27−7.65 (m, 12 H, Py$^{3-5}$ × 4), 8.50−8.52 (m, 4 H, Py^6 × 4). − ^{13}C NMR ($CDCl_3$): δ = 58.46 (Ph*C*H$_2$), 59.97 (Py*C*H$_2$), 121.91 (Py^3), 122.73 (Py^5), 127.58 (Ph^4), 128.29 (Ph$^{2\ or\ 5}$), 129.21 (Ph$^{2\ or\ 5}$), 136.40 (Py^4), 139.13 (Ph^1), 149.01 (Py^6), 159.93 (Py^2). − HRMS for $C_{32}H_{32}N_6$ $[M]^+$: calcd. 500.2688; found 500.2653.

2,7-Di(aminomethyl)-*N*,*N*,*N*′,*N*′-tetra-(2-pyridylmethyl)naphthalene (3): IR (KBr): $\tilde{\nu}$ = 1589, 1568, 1473, 1431 (C=C, C=N), 1047 cm^{-1} (C−N). − ^{1}H NMR ($CDCl_3$): δ = 3.84 (s, 4 H, PhCH_2 × 2), 3.86 (s, 8 H, PyCH_2 × 4), 7.11−7.16 (m, 4 H, Naph), 7.52−7.79 (m, 12 H, Py$^{3-5}$ × 4), 7.76 (br. s, 2 H, Naph^8), 8.51−8.53 (m, 4 H, Py^6 × 4). − ^{13}C NMR ($CDCl_3$): δ = 58.73 (Ph*C*H$_2$), 60.00 (Py*C*H$_2$), 122.03 (Py^3), 122.92 (Py^5), 126.94 (Naph^3), 127.54 (Naph$^{1\ or\ 4}$), 127.80 (Naph$^{4\ or\ 1}$), 129.21 (Ph$^{2\ or\ 5}$), 136.52 (Py^4), 136.66 (Naph$^{9\ or\ 10}$), 136.85 (Naph$^{10\ or\ 9}$), 149.10 (Py^6), 159.87 (Py^2). − HRMS for $C_{36}H_{35}N_6$ $[M + H]^+$: calcd. 551.2923; found 551.2881.

***N*,*N*,*N*′,*N*′-Tetra-(2-pyridylmethyl)-*p*-xylylenediamine (4):** IR (KBr): $\tilde{\nu}$ = 1591, 1568, 1512, 1473, 1433 (C=C, C=N), 1045 cm^{-1} (C−N). − ^{1}H NMR ($CDCl_3$): δ = 3.66 (s, 4 H, PhCH_2 × 2), 3.80 (s, 8 H, PyCH_2 × 4), 7.10−7.14 (m, 4 H, Ph), 7.27−7.65 (m, 12 H, Py$^{3-5}$ × 4), 8.50−8.51 (m, 4 H, Py^6 × 4). − ^{13}C NMR ($CDCl_3$): δ = 58.26 (Ph*C*H$_2$), 60.04 (Py*C*H$_2$), 121.99 (Py^3), 122.83 (Py^5), 128.89 (Ph^2), 136.52 (Py^4), 149.01 (Py^6), 159.97 (Py^2). − HRMS for $C_{32}H_{32}N_6$ $[M]^+$: calcd. 500.2688; found 500.2688. − $C_{32}H_{32}N_6$ (500.65): C 76.77, H 6.44, N 16.79; found C 76.58, H 6.44, N 16.73.

4,4′-Di(aminomethyl)-*N*,*N*,*N*′,*N*′-tetra-(2-pyridylmethyl)biphenyl (5): IR (neat): $\tilde{\nu}$ = 1591, 1570, 1496, 1473, 1433 (C=C, C=N), 1047 cm^{-1} (C−N). − ^{1}H NMR ($CDCl_3$): δ = 3.73 (s, 4 H, PhCH_2 × 2), 3.84 (s, 8 H, PyCH_2 × 4), 7.12−7.17 (m, 8 H, Ph), 7.27−7.70 (m, 12 H, Py$^{3-5}$ × 4), 8.52−8.54 (m, 4 H, Py^6 × 4). − ^{13}C NMR ($CDCl_3$): δ = 58.10 (Ph*C*H$_2$), 59.95 (Py*C*H$_2$), 122.00 (Py^3), 122.84 (Py^5), 126.98 (Ph$^{2\ or\ 3}$), 129.30 (Ph$^{3\ or\ 2}$), 136.51 (Py^4), 137.98 (Ph$^{1\ or\ 4}$), 139.83 (Ph$^{4\ or\ 1}$), 149.08 (Py^6), 159.86 (Py^2). − HRMS for $C_{38}H_{37}N_6$ $[M + H]^+$: calcd. 577.3080; found 577.2999.

Di-(2-pyridylmethyl)benzylamine (6): IR (neat): $\tilde{\nu}$ = 1589, 1570, 1494, 1473, 1433 (C=C, C=N), 1045 cm^{-1} (C−N). − ^{1}H NMR ($CDCl_3$): δ = 3.69 (s, 2 H, PhCH_2 × 2), 3.81 (s, 4 H, PyCH_2 × 4), 7.12−7.61 (m, 11 H, Ph, Py$^{3-5}$ × 4), 8.51−8.53 (m, 2 H, Py^6 × 2). − ^{13}C NMR ($CDCl_3$): δ = 55.46 (Ph*C*H$_2$), 59.94 (Py*C*H$_2$), 122.00 (Py^3), 122.82 (Py^5), 127.12 (Ph^4), 128.38 (Ph$^{2\ or\ 3}$), 128.91 (Ph$^{3\ or\ 2}$), 136.52 (Py^4), 139.06 (Ph^1), 149.08 (Py^6), 159.94 (Py^2). − HRMS for $C_{19}H_{19}N_3$ $[M]^+$: calcd. 289.1579; found 289.1583.

Computational Methods: All MO calculations were carried out using SPARTAN 5.11.[14] The most stable conformers of **1**−**5b** were studied in a systematic conformer search: The dihedral angle in freely rotatable bonds (two Ar−CH_2 and two CH_2−NH_2) was varied in 120° steps in the initial structures. The structures of **1**−**5b** were then optimized in an AM1 Hamiltonian. Monte Carlo simulations were carried out using Macro Model 6.0.[15] Each structure in the simulation was optimized using the Amber* force field.[16] The effect of solvation was included in the simulations by implicit treatment of solvent water with the GB/SA model.[17]

Kinetic Analysis of the UpU Hydrolysis: Reaction conditions of the UpU hydrolysis: $[UpU]_0$ = 0.02 mM in 100 mM HEPES buffer (pH = 7.0), reaction temperature = 60 °C. The reaction was monitored by reverse-phase HPLC (Phenomenex, Luna 3μ C8(2), 4.6 × 150 mm, 3 μm. Conditions: a linear gradient of 1−9% MeCN in 0.1 M aqueous $AcONH_4$ (pH = 7.0) in 8 min. at a flow of 1.0 mL·min^{-1} at 50 °C). The eluent was monitored at 258 nm by a SOMA S-310A UV/Vis detector. The pseudo-first order reaction rate constants (k_{obs}) were obtained as slopes of ln($[UpU]_0$/[UpU]) vs. time, where $[UpU]_0$ is the initial concentration of the substrate. The pseudo-first order reaction profile and its reproducibility are shown in the Supporting Information (Section 4).

Acknowledgments

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