Preorganized Bis-Zinc Phosphodiester Cleavage Catalysts Possessing Natural Ligands: A Lesson Pertinent to Bimetallic Artificial Enzymes

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Abstract: Two preorganized bis-zinc receptors (**2** and **3**) were synthesized wherein the metals were ligated with ligands present in natural phosphodiesterases: imidazoles and carboxylates. The intrametallic distance is near 4.5 Å, that found in natural nucleases and other successful artificial nucleases. With only two imidazoles (**2**), the zinc binding affinities were not high enough to achieve cooperativity. Yet, with a third ligand, a carboxylate (**3**), cooperativity was found in the cleavage of HPNPP. The preorganization of **3** was achieved using a "steric gearing" strategy. The enhancement was 80-fold for cooperation between the two metals relative to a mono-metallic analogue (**5**). However, there was no observable enhancement in the hydrolysis of RNA using **3** relative to **5**. Therefore, we conclude that placing two zinc atoms that are ligated with natural ligands at the appropriate distance for catalysis is not sufficient to enhance the cleavage of RNA, but is successful for activated RNA substrate mimics.

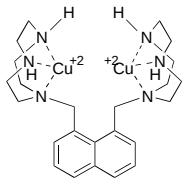
Keywords: enzyme models · hydrolysis · phosphoester · receptors

Introduction

A large number of bimetallic catalysts have been studied for the enhancement of phosphate ester cleavage and/or hydrolysis.[1] Many of these catalysts possess linkers between the ligands for the individual metals that have degrees of conformational freedom.[2] When rigid linkers are used, one can confidently predict the distances between the metals during the catalytic event. Correlations between metal distances and catalytic activity have been made. To date, the evidence suggests that the best catalysts are those where the distance between the metal centers is nearly the same as that found in bimetallic enzymes.[3]

A very good example is from the Chin laboratory (**1**).[4] This catalyst has a Cu–Cu distance near 4.5 Å. Although **1** is among the best synthetic catalysts in terms of rate enhancements for the cleavage/transesterification of RNA, the catalyst does not employ the same ligands and metals as in natural enzymes. In fact, a very large fraction of all artificial enzymes for phosphoester hydrolysis contain ligands other than those used by nature, such as amines, pyridines, and alkoxides.[1, 2] This is often an important synthetic advantage, and the use of these ligands in the development of a practical synthetic nuclease is certainly appropriate.

Alternatively, we are developing a series of catalysts modeled after and possessing similar intrametallic distances as **1**, but that use the same ligands and metals as nucleases: carboxylates/imidazoles and zinc respectively. In this manner, we seek to test if two zinc atoms ligated with natural ligands and possessing intrametallic distances similar to the enzymes and **1** would also show good catalysis. In this manner, we are mimicking a known artificial enzyme, not a particular natural enzyme. Here, we report that simply placing the Zn^{II} ions ligated with natural ligands in a geometry mimicking **1** does not give an efficient catalyst for RNA hydrolysis. Therefore, the appropriate metal–metal distance may be necessary, but is not sufficient, for good catalysis.



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Results and Discussion

Design criteria: As mimics of catalyst **1** but using Zn^{II} and natural ligands, compounds **2** and **3** were designed. Structure **2** uses fused rings in a tricyclic scaffold to place four imidazole

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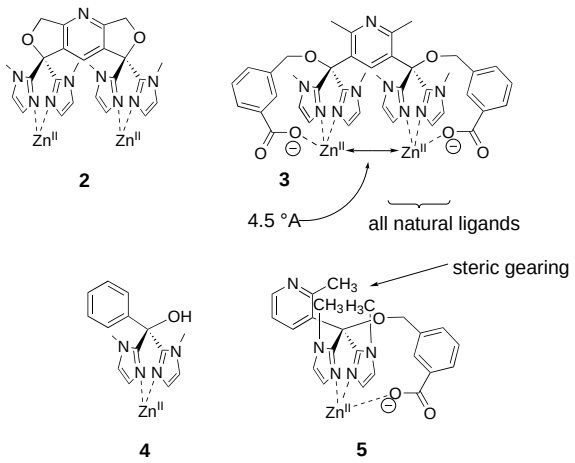
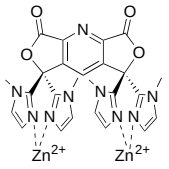


ligands in such a manner that the two zinc atoms are located approximately 4.5 Å apart. The fusing of the rings in the scaffold leads to a highly preorganized binding cleft, which positions the two metal centers at the required distances.

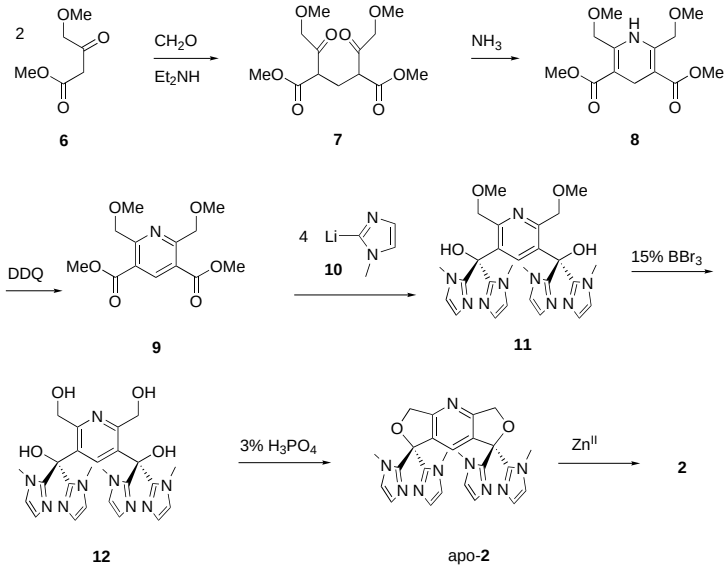
Compound **3** also places two zinc atoms at the distances required for our study, but is more flexible. This compound has two imidazoles and one carboxylate ligand for each metal center, and therefore has higher affinity constants for the zinc metals (as described below). In order to introduce a third ligand to each zinc, as in **3** compared to **2**, the scaffold in **2** could not be used. To incorporate another factor for preorganizing the zinc metals, thereby obtaining the intrametallic distance required for our analysis, we turned to a method we have been exploring for the creation of receptors and cavities, referred to as "steric gearing".[5] Here, alternating steric bulk is used to bias conformational preferences to those that possess the desired geometries. In the case of **3**, the lowest energy conformation found by molecular mechanics is with the methyl groups on the imidazoles placed above and below the methyl groups on the central pyridine spacer (see the analogous methyl groups explicity drawn in structure **5**). This "meshing" of the methyl groups was predicted to impart the desired conformation to the overall structure, leading to the required Zn–Zn distance. Further, the methyl groups on the pyridine spacer should impart a conformational bias to the carboxylate ligand via the linker, orienting the carboxylate toward the zinc center.

Synthesis: The syntheses of **2** and **3** are shown in Schemes 1 and 2, respectively. The synthesis of **2** commences from methyl methoxyacetoacetate (**6**) and formaldehyde. Cyclization of **7** to **8** occurs upon treatment with ammonia, followed by aromatization using DDQ to give **9**. Addition of four equivalents of lithium methylimidazole (**10**) results in **11**. The methyl groups are removed with BBr_3 giving **12**, which can be cyclized in the presence of phosphoric acid to give apo-**2**. Stirring with $ZnCl_2$ in water yields **2**.

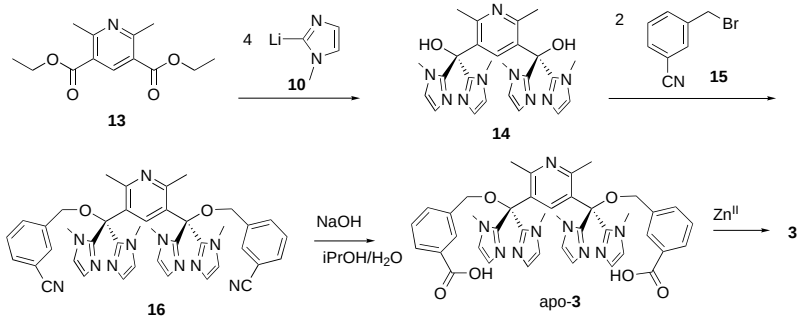
Scheme 2 shows the synthetic route used to obtain **3**. The synthesis of **3** starts with the combination of **13**[6] with four equivalents of lithium methylimidazole (**10**) to give **14**. Alkylation of **14** with **15** results in **16**, followed by hydrolysis of the cyano groups yields apo-**3**, which is simply allowed to stir with $ZnCl_2$ in water to give **3**. A very similar route was used in the synthesis of the mono-zinc receptor **5** (Scheme 3).



As described below, crystals suitable for crystallography could not be obtained for **2** and **3**, and therefore we sought another derivative. Specifically, as described below, compound **20** was

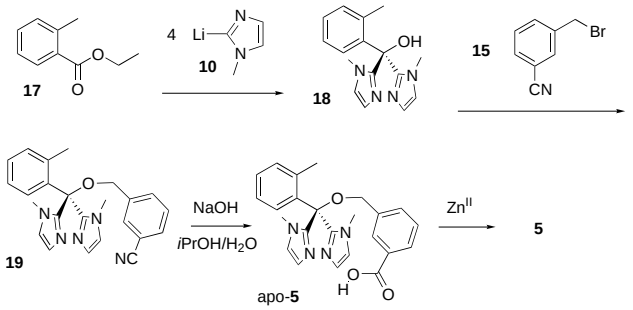


Scheme 1. Synthetic route to **2**.

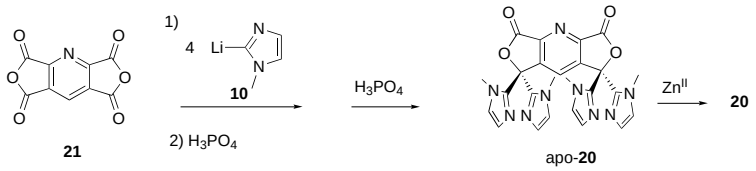


Scheme 2. Synthetic route to **3**.

0947-6539/03/0903-0742 $ 20.00+.50/0 *Chem. Eur. J.* **2003**, *9*, No. 3



Scheme 3. Synthetic route to **5**.



Scheme 4. Synthetic route to **20**.

crystalline. The synthesis of compound **20** is shown in Scheme 4. Addition of four equivalents of **10** to **21** gave apo-**20** in 30% yield, along with the *trans*-isomer in 65% yield. Again, simply stirring with $ZnCl_2$ gives **20**.

Structural analyses: We could not obtain X-ray quality crystals for either **2** or **3**, and hence we synthesized **20**, which was found to be crystalline. The crystal structure of **20** is shown in Figure 1A. It confirms the predicted distance between the zinc atoms in the kinds of structures reported herein, with a 3.98 Å separation, where a fluoride ligand bridges the two zinc atoms. However, we find that two equivalents of apo-**20** dimerize through the bridging of two bound zinc atoms, as shown in Figure 1B . This occurs although two full equivalents of $ZnCl_2$ per apo-**20** were added to the solution. This dimerization is a likely a structural motif for **2** also, and could in part explain the low activity of **2** in the hydrolysis of phosphoesters (see below).

In the design criteria, the possible steric gearing of the methyl groups in **3** (shown in **5**) was discussed as a means to preorganize the receptor and hold the metals in proximity. The proposed "meshing" of the methyl groups was confirmed with an NOE study on **3**, which showed a 15% enhancement of either set of methyl groups when the corresponding neighboring methyls were irradiated. This is best explained by the methyl groups being placed in the geometry for **3**, shown above, where the groups are near van der Waals distances.

Catalytic activity: We first analyzed compound **2** for catalysis of the cleavage of 2-hydroxypropyl-*p*-nitrophenylphosphate (HPNPP). Relative to control structure **4**, whose catalytic activity we have previously reported,[7] there was no rate enhancement in the cleavage of HPNPP or RNA, but instead even lower activity. Titrations of $ZnCl_2$ into solutions of apo-**2**, followed by UV/Vis spectroscopy, indicated that a second zinc is not bound in **2** to any significant extent at low millimolar concentrations of receptor and zinc. The binding constant of a zinc to **4** is $9 \times 10^4\,M^{-1}$.[6] Due to the electrostatic repulsion between the two adjacent zinc atoms in **2**, and the fact that only two imidazoles ligate each zinc, the second zinc binding constant to **2** must be significantly lower. The placement of only one zinc in apo-**2** then likely leads to a dimer in solution, as found for **20** in the solid state. The dimer possesses coordinately saturated zinc centers, and would be inactive as a catalyst for RNA hydrolysis.

As mentioned above, to improve upon the design of **2**, compound **3** was developed. Now three ligands, two imidazoles, and one carboxylate, ligate each zinc. This increases the affinity for zinc, and impedes dimerization because the coordination number of each metal is greater. An affinity constant of $7.2 \times 10^6\,M^{-1}$ for zinc with **5** was determined using a previously reported competition assay,[8] and we measured a Zn-bound water pK_a of 8.3 for that complex. Given the near 100-fold enhancement in zinc binding to apo-**5** relative to apo-**4**, we felt that the study of **3** was warranted.

The catalytic activities of **3** and **5** were examined using HPNPP and RNA. Compound **5** behaved similar to the

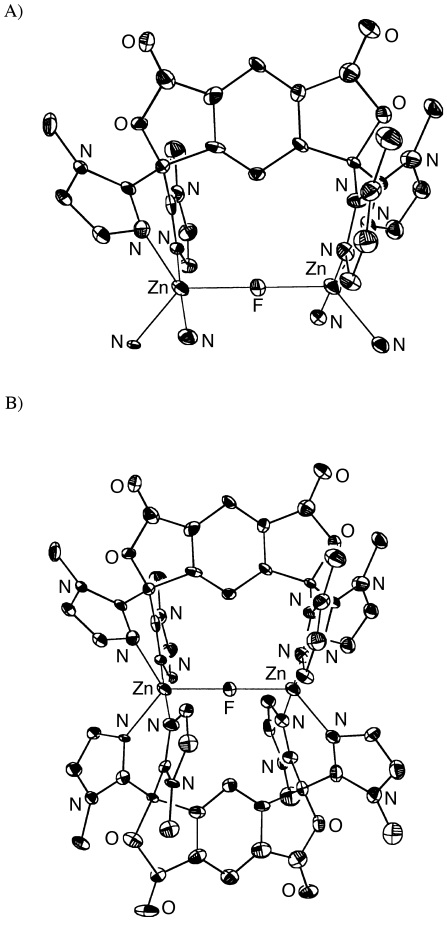


Figure 1. View of the Zn complex **20** showing a partial atom labeling scheme. Displacement ellipsoids are scaled to 30% probability level. A) Ortep diagram showing one unit of **20**. B) Ortep diagram showing the dimer found in the crystal structure of **20**.

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previously reported activity of **4** in the catalysis of HPNPP cleavage. We found a pH dependence on the rate constant for cleavage of HPNPP indicating general base catalysis (Figure 2A). A plateau was found above pH 8.7, close to the pK_a of the zinc bound water (see above).

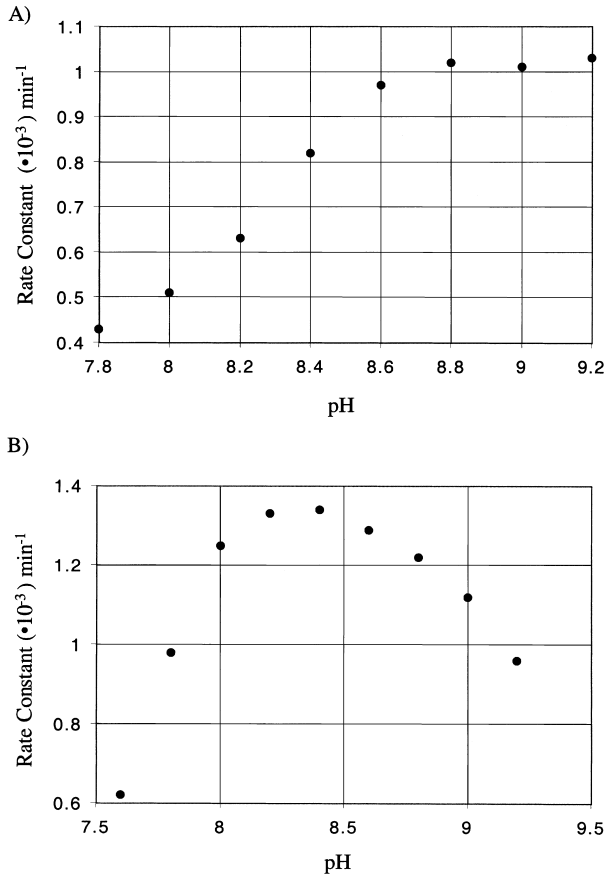

Figure 2. Kinetic runs (37 °C) showing k_{obs} versus pH profiles for **3** and **5** in methanol/water 1:2 solutions (MeOH added for solubility purposes, 50 mM tris buffer). All runs are with 0.4 mM HPNPP. A) Compound **3** at 3.8×10^{-4} M. B) Compound **5** at 2.6×10^{-3} M.

To create rates of cleavage of HPNPP with **3** and **5** that are similar, a more dilute solution of **3** was required because it is significantly more active. With a dilute solution of **3** (see caption to Figure 2B), we found similar rates constants, but now a bell-shaped pH versus rate constant profile was found. Such bell-shaped curves are common in the cleavage of phosphoesters, reflecting a combined general-acid/general-base catalyzed mechanism.[9] The peak of the bell-shaped curve is once again similar to the pK_a value of water bound to the zinc atom in **5**. Our proposed catalytic step is shown in Figure 3. The phosphate is activated by either one or two metals, and a zinc-bound hydroxide acts to deliver the intramolecular alcohol nucleophile.

Using the relative concentrations of compounds employed in the studies illustrated in Figure 2, and a factor of 2 due to the presence of two zinc atoms in **3**, we find an 80-fold advantage imparted to the catalysis of cleavage of HPNPP using **3** relative to **5**. This enhancement is significant, and

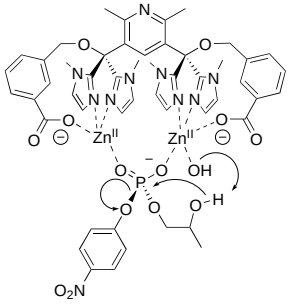

Figure 3. Hypothetical mechanism for the transesterification of HPNPP.

further confirms the preorganization and promixity of the zinc ions in **3**.

However, we found there is essentially *no* difference in the rate of cleavage of RNA when **3**, **4**, or **5** are used. At pH 8.4 (10 mM HEPES), the cleavage of UpU to give Up and U shows essentially identical time course plots in initial rate kinetic measurements. The lack of activity of **3** must be due to an improper orientation in the binding of RNA to the catalyst to promote cleavage/transesterification. Therefore, what ever geometry is achieved upon binding is insufficient to activate the phosphate of RNA for subsequent nucleophilic attack.

Conclusion

In summary, although compound **3** possesses similar intrametallic distances as bimetallic enzymes and **1**, a statement we can make based upon its rigid and preorganized structure, it is a significantly worse catalyst than **1** for RNA cleavage. This is despite the fact that we found it does act as a good bimetallic general acid/general base catalyst with an activated substrate (HPNPP). Therefore, other features of enzymes besides just the metal distance, such as proper orientation of the bound substrate and other activating groups, must also play major roles in the catalysis. Further, as noted by Chin,[4] compound **1** is likely a good synthetic catalyst not only due to the intrametallic distance, but also due to the higher electrophilicity of Cu^{II} compared with Zn^{II}, and the unnatural synthetic ligands which enhance metal binding relative to natural ligands. Our studies support his previous conclusions.

Experimental Section

General considerations: ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ or CD_3OD used as purchased. NMR spectra were recorded on a Bruker AC250 and a Varian Unity Inova (500 MHz, NOESY) spectrometer. Low resolution and high resolution mass spectra were measured with Finnigan TSQ70 and VG Analytical ZAB2-E instruments. Preparative flash chromatography was performed on Scientific Absorbents Incorporated Silica Gel 40 mm. Reversed-phase liquid chromatography was performed on RP18 modified silica gel 55-105 mm using a Pharmacia LKB-FRAC-100 LC system. Solvents and reagents were purchased from Aldrich, Sigma and Mallinckrodt and used without purification. Tetrahydrofuran (THF) was distilled from sodium/benzophenone.

pK_a Determination: Potentiometric titrations of the ligands apo-**3** and apo-**5** were performed in the presence and absence of Zn^{II}. The chloride salts (obtained by lyophilizing HCl acidic solutions of the ligands) were dissolved in volumetric standard sodium chloride solution (0.0973 M,

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Aldrich) and 30% methanol. The base (0.0975 M NaOH standard from Aldrich) was delivered using a programmable Harvard Apparatus syringe infusion pump 22. The pH readings were taken on an Orion Model 720A pH meter with an Orion Ross model 8103 combination pH electrode. The volume of titrant added, as well as the pH, was recorded during the experiment with the Graphit program on a PC.

Kinetic measurements on HPNPP: The studies were conducted in 50 mM Tris buffer, 30% methanol at 37 °C in a Beckman DU 640 spectrophotometer fitted with a temperature controller. Readings were taken at 401 nm every 30 min for 72 h. The buffer was prepared with distilled/deionized water and the pH adjusted with HCl before adding the methanol. The samples were prepared in 10 mm path length cuvettes which could be sealed with teflon stoppers. A typical sample would involve 900 mL buffer, 100 mL ligand + Zn^{II} and 500 mL methanol, equilibrated for 30 min at 37 °C followed by addition of 10 mL substrate stock solutions.

pH Studies: The pH studies were run at 2.6×10^{-3} M catalyst for **5** and 3.8×10^{-4} M for **3** in the range of pH 7.6–9.2 and 7.8–9.2, respectively.

Synthesis

Dimethyl 1,4-dihydro-2,6-dimethoxymethyl-3,5-pyridinedicarboxylate (8): Methyl methoxyacetoacetate (**6**; 56.45 g, 0.39 mol) was added to a 40% aqueous formaldehyde solution (15.25 g). The reaction mixture was cooled to 0 °C, then diethylamine (3 mL) was added dropwise. The reaction mixture was kept at 0 °C for 4 h, and then allowed to slowly warm to room temperature, followed by stirring for another 2 d. During this time, two layers formed, which were separated. The aqueous layer was extracted with three 100 mL portions of diethyl ether. The ether layers were combined with the previous organic layer, dried over sodium sulfate, and the solvents removed by rotary evaporation. The residue (**7**) was dissolved in absolute ethanol (100 mL), and cooled on an ice bath. The solution was saturated with ammonia gas, and the mixture was allowed to stir overnight. The ethanol was evaporated and the residue purified by silica gel chromatography (EtOAc:CH_2Cl_2 15:85), and then by recrystallization from ethanol (47% yield). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.22$ (s, 2H, C-CH_2-C), 3.32 (s, 6H, CH_2OCH_3), 3.59 (s, 6H, CO_2CH_3), 4.47 (s, 4H, CH_2OMe), 7.79 (s, 1H, NH); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): $\delta = 24.2$, 50.8, 58.8, 69.4, 96.8, 146.2, 167.5; HRMS-CI: m/z: calcd for $C_{13}H_{19}NO_6$: 284.1134; found: 284.1134 $[M+H]^+$.

Dimethyl 2,6-dimethoxymethyl-3,5-pyridinedicarboxylate (9):

Method A: A mixture of H_2O (21.6 g), concentrated nitric acid (sp. gr. 1.42, 5.8 g) and concentrated sulfuric acid (6.25 g) were added to **8** (18.05 g, 0.063 mol). The mixture was heated until all solids had dissolved, then heating was continued an additional 3 min; the reaction mixture turned yellowish orange. The mixture was then cooled on ice, while ice (10 g) was added; then the mixture was made alkaline (pH 9) by adding ammonium hydroxide. It was then extracted with CH_2Cl_2 and the combined extracts were dried over sodium sulfate. The residue after concentration was purified by silica gel chromatography (CH_2Cl_2: EtOAc 7:3) to yield the product (9.99 g, 56%).

Method B: A round bottom flask was charged with **8** (420 mg, 1.47 mmol) and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ; 401 mg, 1.77 mmol) in THF (20 mL). The mixture was stirred under nitrogen for 5 h, at which time TLC showed no starting material. The reaction mixture was then passed through the neutral alumina to remove excess DDQ and hyroquinone, then the alumina was washed with a large quantity of methylene chloride. The filtrate was concentrated and the residue purified by silica gel chromatography (EtOAc:CH_2Cl_2 3:7), yielding the product (304 mg, 73%). M.p. 45–47 °C; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.35$ (s, 3H, CH_2OCH_3), 3.36 (s, 3H, CH_2OCH_3), 3.84 (s, 3H, $COCH_3$), 3.85 (s, 3H, $COCH_3$), 4.83 (s, 2H, CH_2OMe), 4.84 (s, 2H, CH_2OMe), 8.50 (s, 1H, Ar-H); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): $\delta = 52.1$, 58.6, 73.6, 124.2, 139.9, 159.9, 165.3; HRMS-CI: m/z: calcd for $C_{13}H_{17}NO_6$: 284.1134; found: 284.1134 $[M+H]^+$.

2,6-Dimethoxymethyl-3,5-bis[di(1-methyl-2-imidazolyl)hydroxymethyl] pyridine (11): A solution of *n*BuLi (15.2 mL, 24.2 mmol of a 1.6 M solution in hexane) was added dropwise to a solution of 1-methylimidazole (1.92 g, 22 mmol) in THF (30 mL) at −78 °C for an hour, and then **9** (0.632 g, 2.21 mmol) in THF (200 mL) was added, which resulted in a dark red solution. The reaction mixture was allowed to warm to room temperature overnight. The reaction mixture was cooled to −78 °C and methanol (100 mL) was then added slowly. The solvent was removed in vacuo, yielding a dark red residue which was first purified by silica gel chromatography (100% EtOAc → EtOAc:MeOH(sat.NH_3) 80:20), then on an ion-exchange column (50 mM NH_4Oac, pH 6.8), yielding the title coompound (396 mg, 37%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.24$ (s, 6H, CH_2OCH_3), 3.33 (s, 12H, NCH_3), 4.64 (s, 4H, CH_2OCH_3), 5.69 (s, 2H, OH), 6.75 (s, 4H, C*H*=CH), 6.82 (s, 4H, C*H*=CH), 7.24 (s, 1H, Ar); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): $\delta = 34.4$, 58.4, 74.9, 75.9, 123.4, 126.1, 135.6, 135.9, 146.8, 154.8; HRMS-CI: m/z: calcd for $C_{27}H_{34}N_9O_4$: 548.2734; found: 548.2731 $[M+H]^+$.

2,6-Hydroxymethyl-3,5-bis[bis(1-methyl-2-imidazolyl)hydroxymethyl]pyridine (12): A round bottom flask was charged with **11** (86 mg, 0.16 mmol) in dry CH_2Cl_2 (5 mL) The reaction mixture was cooled to −78 °C and BBr_3 (1 M in CH_2Cl_2; 0.47 mL, 0.47 mmol) was then added dropwise. After the mixture was stirred for an hour, additional BBr_3 (0.47 mL) was added. The mixture was allowed to warm up to room temperature overnight. The mixture was then quenched with MeOH and then pH was adjusted to 7 using Na_2CO_3 (aq.). The mixture was concentrated and the residue was purified by silica gel chromatography (EtOAc:MeOH 80:20) to give **12** (12.9 mg, 15.8%). ^{1}H NMR (300 MHz, CD_3OD): $\delta = 3.38$ (s, 12H, NCH_3), 4.56 (s, 4H, C-CH_2-O), 6.32 (s, 1H, Ar-H), 6.84 (s, 4H, C*H*=CH), 7.07 (s, 4H, C*H*=CH); ^{13}C{^{1}H} NMR (75 MHz, CD_3OD): $\delta = 35.2$, 64.5, 76.5, 125.6, 126.7, 135.47, 136.1, 148.0, 158.2; HRMS-CI: m/z: calcd for $C_{25}H_{29}N_9O_4$: 520.2421; found: 520.2401 $[M+H]^+$.

Bis[2,2-bis(1-methyl-2-imidazolyl) furol][3.4-b:3′,4′-e] pyridine (apo-2): A flask containing 85% H_3PO_4 (3 mL) was heated at 150 °C for 30 min. To this flask was added **12** (35 mg, 0.067 mmol). The mixture was heated an additional 1.5 h, resulting in a greenish brown solution. After cooling the mixture to room temperature, water (3 mL) were added and the reaction mixture was basified to pH 8 by adding Na_2CO_3. The aqueous solution was extracted with five 10 mL portions of CH_2Cl_2. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by two silica gel chromatography (first CH_2Cl_2:MeOH(sat.NH_3) 95:5; then EtOAc:MeOH 85:15) to give the title compound (1.12 mg, 34%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.53$ (s, 12H, NCH_3), 5.10 (s, 4H, CCH_2O), 6.86 (s, 4H, C*H*=CH), 6.86 (s, 4H, C*H*=CH), 7.57 (s, 1H, Ar-H); ^{13}C{^{1}H} NMR (75 MHz, $CDCl_3$): $\delta = 34.4$, 71.0, 85.2, 123.7, 127.1, 130.6, 133.5, 145.0, 161.0; HRMS-CI: m/z: calcd for $C_{25}H_{25}N_9O_2$: 484.2187 $[M+H]^+$; found: 484.2199.

2,6-Dimethyl-3,5-bis-[di(1-methyl-2-imidazolyl)hydroxymethyl]pyridine (14): A solution of 1-methylimidazole (5.6 mL, 70.25 mM) in dry THF (70 mL) was cooled to −78 °C. A solution of *n*BuLi (42 mL, 1.6 M in hexanes, 67.2 mM) was added dropwise under argon. The reaction mixture was stirred for 1 h at −78 °C and a solution of 3,5-ethylcarboxy-2,6-dimethylpyridine (**13**; 1.2 g, 4.78 mM) was then added. The reaction mixture was allowed to warm up to room temperature over night and was cooled again to −78 °C followed by very slow addition of MeOH (60 mL). The solvent was evaporated and the yellow residue was purified by silica gel chromatography ($R_f = 0.35$; CH_2Cl_2:MeOH (sat. NH_3) 95:5). Yield 70%. ^{1}H NMR (250 MHZ, MeOD): $\delta = 2.27$ (s, 6H, CH_3), 3.33 (s, 12H, Im-CH_3), 6.57 (s, 1H, Ar-H), 6.83/6.84 and 7.06/7.07 (each d, 4H Im-CH=); ^{13}C NMR (62.9 MHz, MeOD): $\delta = 23.31$ (CH_3), 35.09 (Im-CH_3), 76.14 (C-OH), 125.28, 126.56 (Im-CH), 148.31 (Im-C), 134.64, 135.46, 158.38 (Ar-C); MS (CI): calcd for $C_{25}H_{29}N_9O_2$: 487; found: 487 $[M]^+$, 489 $[M+2]^+$.

2,6-Dimethyl-3,5-bis-[di(1-methyl-2-imidazolyl)(3-cyanobenzyloxy)methyl] pyridine (16): The reaction conditions were similar to **18**, but the residue after lyophilization was purified by silica gel chromatography (first wash with 100% CH_2Cl_2, then eluted with CH_2Cl_2:MeOH (sat. NH_3) 90:10, R_f = 0.52). Yield 65%. ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 2.52$ (s, 6H, CH_3Ar), 3.08 (s, 12H, CH_3-Im), 4.67 (s, 4H, Ar-CH_2), 6.74 and 6.91 (each s, 4H, Im-CH=), 7.36, 7.52, 7.76 (4H, AR-CH); MS (CI): calcd for $C_{41}H_{39}N_{11}O_2$: 717.833); found: 717, 719 $[M+2]^+$, 587 $[M - OBnzCN]^+$; HR-MS: calcd for: 718.3366; found: 718.3367 $[M+H]^+$.

Di(1-methyl-2-imidazolyl)(3-cyanobenzyloxy)methyl-2-methylbenzene (19): Alcohol **18** (220 mg) and KOH (360 mg) were ground together and dry DMSO (3 mL) was added. The light yellow mixture was stirred for 10 min at room temperature. Then, the supernatant was taken and α-bromotolunitrile (**15**; 211 mg) was added. The now dark yellow solution was stirred for 5 h at RT. H_2O (100 mL) and 1 N HCl (2 mL) were added and the mixture was lyophilized three times, adding water (50 mL) each time. The residue was purified by reversed phase liquid chromatography using a gradient mixer, column 1.0×35 cm, flow rate 3 mL min^{-1}). Yield 85%.

^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 2.14$ (s, 3H, CH_3Ar), 3.27 (s, 6H, CH_3-Im), 4.70 (s, 2H, Ar-CH_2), 6.88 and 7.03 (each br, 2H, Im-CH=), 7.11, 7.23, 7.37, 7.52, 7.71, (m, 8H, Ar-CH); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 21.03$, 34.57, 67.14, 82.12, 112.11,118.84, 123.29, 125.03, 126.23, 127.88, 128.82, 128.90, 130.84, 131.13, 132.06, 132.95, 135.91, 139.54, 140.18, 145.52; MS(CI): calcd for $C_{24}H_{23}N_{50}$: 397; found: 397, 398 $[M+H]^+$, 265 $[M-$ Obnz $-$ CN$]^+$.

CN-Hydrolysis—General Procedure

Di(1-methyl-2-imidazolyl)(3-carboxybenzyloxy)methyl]-2-methyl benzene and 2,6-dimethyl-3,5-bis-[di(1-methyl-2-imidazolyl)(3-carboxylbenzyloxy)-methyl] pyridine) (apo-3 and apo-5): The cyano compound (**16** or **19**), 300 mol % of NaOH and isopropanol/H_2O (5:1) were stirred in a sealed tube at 130 °C for 12 h. The mixture was neutralized with HCl in an ice bath. The organic phase was evaporated and the residue was purified via reversed phase liquid chromatography using a gradient of 90 % CH_3CN (100 mL each in a gradient mixer, column 1.0×35 cm, flow rate 3 mL min^{-1}. Yields: 93 and 91 %, respectively. **Apo-5**: ^{1}H NMR (250 MHz, $CDCl_3$/MeOD, 1:1): $\delta = 2.03$ (s, 3H, CH_3Ar), 3.37 (s, 6H, CH_3-Im), 7.94 and 7.98 (each s, 2H, Im-CH=), 7.14 – 7.45, 7.85 – 7.91 (m, 8H, Ar-CH); MS(CI): calcd for $C_{24}H_{24}NO_3$: 416; found: 416, 417 $[M+H]^+$, 418 $[M+2]^+$; HR-MS: calcd for: 417.192666; found: 417.191467 $[M+H]^+$. **Apo-3**: ^{1}H NMR (250 MHz, $CDCl_3$/MeOD): $\delta = 2.35$ (s, 6H, CH_3Ar), 3.30 (s, 12H, CH_3-Im), 4.50 (s, 4H, Ar-CH_2), 6.91 and 7.00 (each s, 4H, Im-CH=), 7.27(br, 4H), 7.48 (s, 1H), 7.85 (br, 4H, all Ar-CH); MS(CI): calcd for $C_{41}H_{41}N_9O_6$: 755; found: 755, 756 $[M+H]^+$; HR MS: calcd for: 756.3258, found: 756.3268 $[M+H]^+$.

2-Methylphenyl bis(1-methyl-2-imidazolyl) methanol (18): A solution of 1-methylimidazole (5.6 mL, 70.25 mM) in dry THF (70 mL) was cooled to -78 °C. A solution of *n*BuLi (42 mL, 1.6 M in hexanes, 67.2 mM) was added dropwise under argon. The reaction mixture was stirred for 1 h at -78 °C, at which time methyl-2-methyl benzoate (**17**; 3.2 mL, 20 mM) was added very slowly. The reaction mixture was allowed to warm to room temperature over night. It was then cooled again to -78 °C and MeOH (60 mL) were added slowly. The solvent was evaporated and the yellow residue was purified by silica gel chromatography ($R_f = 0.43$; CH_2Cl_2:MeOH (sat. NH_3) 90:10). Yield 67 – 72 %. ^{1}H NMR (250 MHz, $CDCl_3$): $\delta = 2.06$ (s, 3H, CH_3), 3.38 (s, 6H, Im-CH_3), 6.09 (s, 1H, Ar-H), 6.41 (d, 1H, Ar-H), 7.05 – 7.20 (m, 2H, Ar-H), 7.87 and 7.97 (d, 2H each, Im-HC=); ^{13}C NMR (62.9 MHz, $CDCl_3$): $\delta = 19.99$ (CH_3), 34.79 (Im-CH_3), 76.12 (q-C-OH), 125.43, 127.70, 128.67, 132.81 (Ar-CH), 138.86, 148.66 (Ar-C), 123.15, 125.93 (Im-HC=); MS (CI): calcd for $C_{16}H_{18}N_4O$: 282; found: 282, 283 $[M+H]^+$.

1,2,3,5,6,7-Hexahydro-3,3,5,5-tetra(1-methyl-2-imidazolyl)-2,6-dioxa-1,7-*s*-indacenedione (apo-20): A solution of *n*BuLi (3.4 mL, 5.1 mmol of a 1.5 M solution in hexane) was added dropwise to a solution of 1-methylimidazole (0.38 g, 4.6 mmol) in THF (20 mL) at -78 °C for an hour then pyromellitide anhydride (**20**; 0.2 g, 0.92 mmol) in THF (50 mL) was added dropwise to the reaction mixture. The mixture was allowed to warm to room temperature overnight. The mixture was cooled to -78 °C and methanol (50 mL) was added slowly. The solvent was removed in vacuo, yielding a dark red residue which was dissolved in 85 % H_3PO_4 (50 mL) and heated at 120 °C for an hour. After cooling the mixture to room temperature, water (30 mL) was added and the mixture was basified to pH 8 by adding concentrated NH_4OH. The aqueous solution was extracted with three 50 mL portions of CH_2Cl_2. The organic layer was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (CH_2Cl_2:MeOH(sat.NH_3) 97:3) to give **20** (197 mg, 42 %). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 3.45$ (s, 12H, NCH_3), 6.85 (s, 4H, NCH=C), 6.89 (s, 4H, NC*H*=C), 8.00 (s, 1H, Ar-H), 8.43 (s, 1H, Ar-H); ^{13}C{^{1}H} NMR (75 MHz, CD_3OD): $\delta = 34.4$, 83.7, 123.3, 124.6, 125.7, 127.4, 127.6, 141.4, 153.4, 167.0; HRMS-CI: *m*/*z*: calcd for $C_{26}H_{22}N_8O_4$: 511.1842; found: 511.1833 $[M+H]^+$.

X-ray Experimental for $[(C_{26}H_{22}N_8O_4)_2Zn_2F] \times 3BF_4^- \times 3CH_3OH \times 2H_2O$ (20): Crystals grew as colorless prisms from CH_3OH. The crystals decomposed rapidly when removed from the mother liquor and had to be stabilized by immersion in mineral oil. The data crystal had approximate dimensions; $0.23 \times 0.23 \times 0.57$ mm^3. The data were collected at 173 K on a Nicolet P3 diffractometer, equipped with a Nicolet LT-2 low-temperature device and using a graphite monochromator with $Mo_{K\alpha}$ radiation ($\lambda =$ 0.71073 Å). Details of crystal data, data collection and structure refinement are listed in Table 1. Three reflections (3,0, – 5;5,0, – 3;3,2,3) were remeasured every 97 reflections to monitor instrument and crystal stability. A smoothed curve of the intensities of these check reflections was used to scale the data. The scaling factor ranged from 0.940 to 1.00. The data were corrected for Lp effects but not absorption. Data reduction and decay correction were performed using the SHELXTL-Plus software package.[10] The structure was solved by direct methods[11] and refined on F^2 by full-matrix least-squares[12] with anisotropic thermal parameters for the non-H atoms of the Zn complex. Because only three BF_4^- ions were observed in the asymmetric unit and the ligands were neutral, the bridging atom between the Zn^{2+} ions had to have a -1 charge. Models were refined with the bridging ion as O, F and Cl. The best refinement as judged by the reasonableness of the isotropic temperature parameter was with F as bridge. The U_{iso} for the Cl refinement was 0.175, for O 0.002 and for F it was 0.033. The U_{iso} for F was close to the values observed for the other atoms of the complex. The atoms of the Zn dimer were well resolved and were refined anisotropically. Of the three BF_4^- ions, one was well behaved, one was disordered by rotation about an oxygen-boron bond and the third was badly disordered. This third BF_4^- ion and several solvate molecules could not be refined. The contribution to the observed structure factor amplitudes due to the disordered entities was removed by using the utility, SQUEEZE, in PLATON98.[12] The function, $\Sigma w(|F_o|^2 - |F_c|^2)^2$, was minimized, where $w = 1/[(\sigma(F_o))^2 + (0.02P)^2]$ and $P = (|F_o|^2 + 2|F_c|^2)/3$. $R_w(F^2)$ refined to 0.173, with $R(F)$ equal to 0.0980 and a goodness of fit, $S = 1.04$. Definitions used for calculating $R(F)$, $R_w(F^2)$ and the goodness of fit, *S*, are given below.[13] The data were checked for secondary extinction effects but no correction was necessary. Neutral atom scattering factors and values used to calculate the linear absorption coefficient are from the International Tables for X-ray Crystallography.[14] All figures were generated using SHELXTL/PC.[11] Tables of positional and thermal parameters, bond lengths and angles, torsion angles, figures and lists of observed and calculated structure factors are available from the CCDC.[15]

Acknowledgement

We gratefully acknowledge support for this work from the National Science Foundation.

Table 1. Crystal data and structure refinement for **20**.

empirical formula	$C_{55}H_{60}B_3F_{13}N_{16}O_{13}Zn_2$
F_w	1563.36
T [K]	173(2)
λ [Å]	0.71073
crystal system	monoclinic
space group	$P21/n$
a [Å]	18.367(3)
b [Å]	19.038(4)
c [Å]	19.453(4)
α [°]	90
β [°]	99.10(1)
γ [°]	90
V [Å^3]	6717(2)
Z	4
ρ_{calcd} [Mg m^{-3}]	1.546
μ [mm^{-1}]	0.823
$F(000)$	3192
crystal size [mm^3]	$0.57 \times 0.23 \times 0.23$
τ range for data collection [°]	2.25 to 22.51
index ranges	$0 \le h \le 19$
	$0 \le k \le 20$
	$-20 \le l \le 20$
reflections collected	8663
independent reflections	8449 [R(int) = 0.1003]
completeness to $\tau = 22.51°$	96.0 %
absorption correction	none
refinement method	full-matrix least-squares on F^2
data/restraints/parameters	8449/673/829
GOF on F^2	1.050
final R indices [$I > 2\sigma(I)$]	$R1 = 0.0980$, $wR2 = 0.1511$
R indices (all data)	$R1 = 0.2292$, $wR2 = 0.1726$
largest diff. peak and hole [e Å^{-3}]	0.813 and -0.532

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[15] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre: CCDC-197492 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336033; or deposit@ccdc.cam.uk).

Received: August 5, 2002 [F4317]