JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

Upper Rim Bifunctional *cone*-Calix[4]arenes Based on a Ligated Metal Ion and a Guanidinium Unit as DNAase and RNAase Mimics

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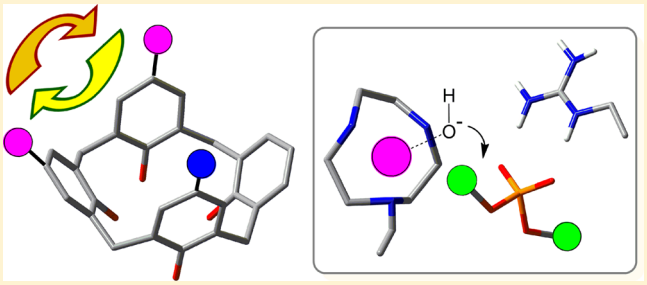
Supporting Information

ABSTRACT: The catalytic activity of an artificial phosphodiesterase that combines a ligated metal ion (Cu^{II}, Zn^{II}) with a guanidinium unit connected by a 1,2-vicinal calix[4]arene spacer was investigated in the transesterification of RNA models HPNP and four diribonucleoside 3′,5′-monophosphates. Comparison with previous data related to the 1,3-distal regioisomeric metal complexes confirms the superiority of the Cu^{II} complexes over the Zn^{II} analogs and shows that in the reactions of HPNP, GpU, and UpU, the catalytic efficiency depends very little on whether the substitution pattern is 1,2-vicinal or 1,3-distal. On the other hand, CpA turned out to be a good substrate for the Cu^{II} complex of the 1,2-vicinal catalyst and a bad substrate for the corresponding 1,3-distal regioisomer, whereas the opposite holds for GpA. Extension of the investigation to the cleavage of the DNA model BNPP showed that both Zn^{II} and Cu^{II} complexes exhibit good catalytic efficiency, with a superiority of the 1,2-vicinal catalyst in both cases. The data reported in this work show that rate accelerations over background for the best catalyst−substrate combinations at 0.5 mM catalyst concentration are 3.6×10^5-fold for HPNP, 1.1×10^6-fold for BNPP, and range from 1.3×10^6- to 1.3×10^7-fold for diribonucleoside monophosphates.



INTRODUCTION

The biological relevance of the phosphodiester bond in RNA and DNA has attracted the attention of many researchers that have designed and developed supramolecular catalysts with phosphodiesterase activity.[1−3] Some artificial phosphodiesterases feature one or more guanidinium units that act as the recognition site for the anionic phosphate.[1b,3]

In a recent paper,[4] we reported on the ribonuclease activity of the Zn^{II} and Cu^{II} complexes of compound **1b**, in which the upper rim of a *cone*-calix[4]arene was used as a spacer for connecting a guanidinium unit with a 1,4,7-triazacyclononane (TACN) ligand in 1,3-distal position. In these heterobifunctional catalysts, the metal complexed hydroxide ion acts as a general base, whereas both metal ion and guanidinium units act as electrophilic activators (Figure 1). The catalytic performance of the Cu^{II} complex turned out to be far superior to that of the Zn^{II} complex. The former was found to catalyze very effectively the transesterification of 2-hydroxypropyl *p*-nitrophenyl phosphate (HPNP, eq 1), as well as of a number of diribonucleoside monophosphates (*N*p*N*′, eq 2). The phosphodiesterase activity of the Cu^{II} complex of **1b** was also far superior to those of analogous catalysts in which the catalytic units are connected by xylylene spacers.[5,6] This superiority has been ascribed to a higher degree of adaptability of the more flexible calix[4]arene scaffold[7] to the altered substrate in the transition state.

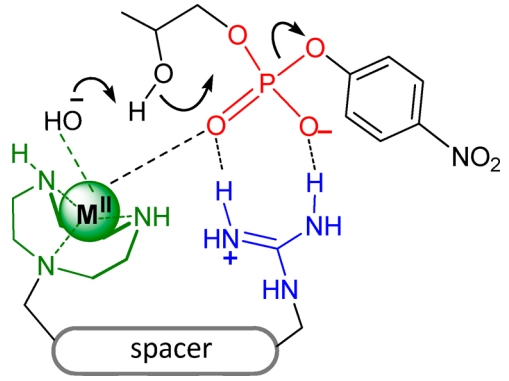
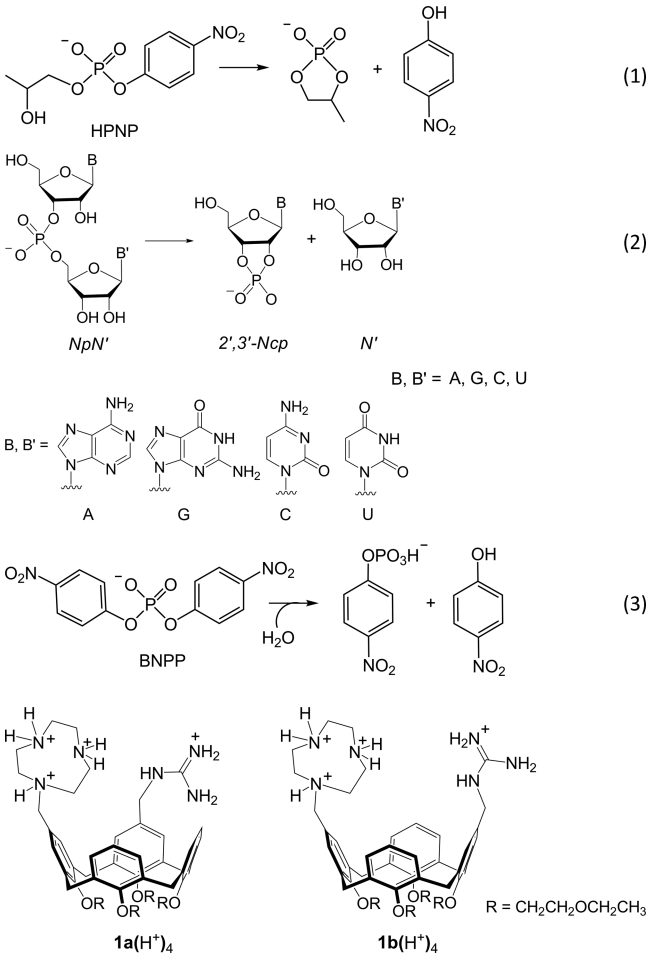


Figure 1. Proposed mechanism of HPNP cleavage catalyzed by the combined action of a guanidinium unit and a ligated metal ion (M^{II} = Cu^{II}, Zn^{II}).

Our previous studies of the use of the upper rim of a *cone*-calix[4]arene platform in the construction of bifunctional catalysts capable of esterase and nuclease activity[1b,7b] have shown that catalytic efficiency may depend, even to a large extent, on whether the substitution pattern is 1,2-vicinal or 1,3-

Received: March 25, 2016
Published: May 2, 2016

DOI: 10.1021/acs.joc.6b00644
J. Org. Chem. 2016, 81, 4728−4735

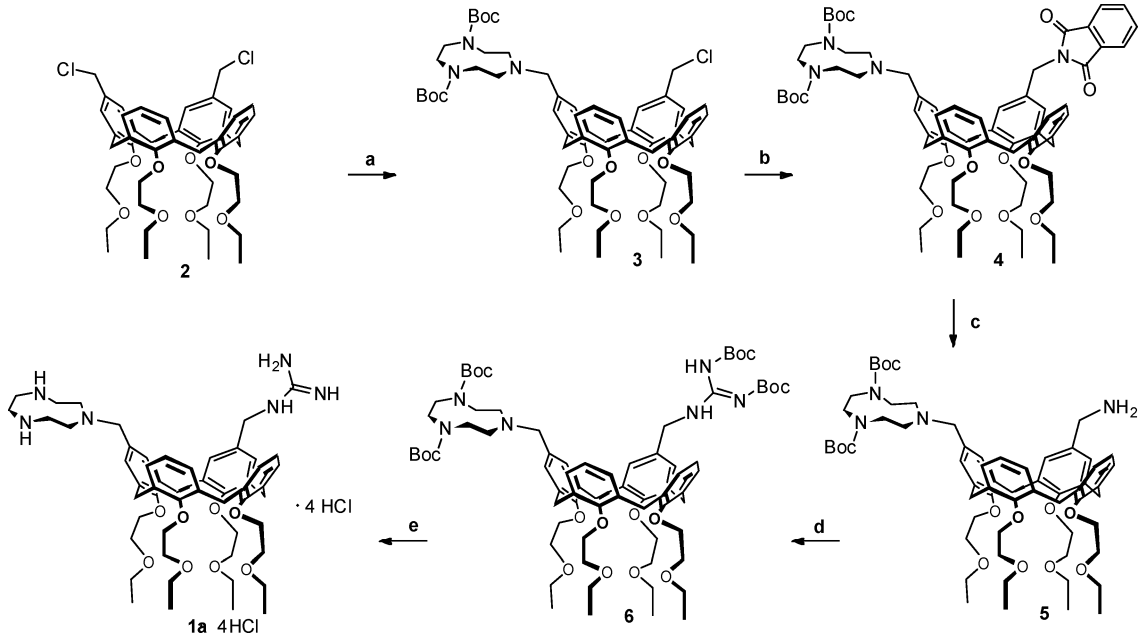


distal. Therefore, it seemed worthwhile to synthesize the 1,2-vicinal regioisomer **1a** and to compare the phosphodiesterase activity of its metal (Zn^{II}, Cu^{II}) complexes with that of the corresponding complexes of **1b**. The results of such an investigation are reported in this Article, together with an extension of the kinetic study to the hydrolytic cleavage of the DNA model compound bis(*p*-nitrophenyl) phosphate (BNPP, eq 3).[8,9]

RESULTS AND DISCUSSION

Synthesis of the Ligand. Compound **1a**·4HCl was synthesized starting from 5,11-bis(chloromethyl)-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene **2**[10] as illustrated in Scheme 1. The critical step of the synthesis is the desymmetrization of the two chloromethyl groups at the upper rim of **2** (Scheme 1, step *a*). An attempt to follow the reaction sequence used for the preparation of **1b**,[4] that is, treatment of the bis(chloromethyl) derivative with 1 mol equiv of potassium phthalimide to give **7** (see Scheme S1), followed by treatment with *N*,*N*′-bis(Boc)-1,4,7-triazacyclononane (bis(Boc)-TACN), was unsuccessful. Although the 1,2-monochloro-monophthalimidomethylcalix[4]arene **7** could be obtained in 65% yield, the following alkylation with bis(Boc)-TACN did not give product **4** even using strong bases, high temperatures, iodide as catalyst, and long reaction times. The introduction of bis(Boc)-TACN on the monophthalimidomethylcalixarene **7** suffers from severe steric problems that are, apparently, not present in its regioisomeric 1,3-derivative.[4] On the other hand, good results were obtained for the reaction of **2** with 1 mol equiv of bis(Boc)-TACN (Scheme 1). The monosubstituted compound **3** was even obtained in larger amounts (56% isolated yield) than the expected statistical ratio. From the reaction mixture, it was also possible to recover the unreacted starting material (27% isolated), while no trace of the disubstituted compound could be found. This also confirms that the low reactivity of the vicinal $-CH_2Cl$ group of both **7** and **3** with the highly hindered nucleophile most likely arises

Scheme 1. Synthesis of 1a[a]



[a](a) *N*,*N*′-Bis(Boc)-1,4,7-triazacyclononane, K_2CO_3; dry CH_3CN; (b) potassium phthalimide; dry DMF; (c) $N_2H_4{\cdot}H_2O$; dry MeOH, 60 °C; (d) *N*,*N*′-bis(Boc)-*N*″-triflylguanidine, NEt_3; dry DCM; (e) (1) TFA, Et_3SiH (TES); DCM; (2) HCl 1 M; EtOH.

DOI: 10.1021/acs.joc.6b00644
J. Org. Chem. 2016, 81, 4728−4735

from a steric effect exerted by the phthalimido group or the bis(Boc)-TACN unit, respectively.

Compound **5** was obtained according to the classical conditions of Gabriel synthesis, that is, reaction with potassium phthalimide followed by treatment with hydrazine, to give **5** in 83% overall yield. Guanidination of the $-CH_2NH_2$ group of **5** with *N*,*N*′-bis(Boc)-*N*″-triflylguanidine[11] gave compound **6** in 58% yield. Finally, removal of the Boc groups afforded compound **1a**·4HCl in 86% yield.

^1H and ^{13}C NMR spectra of compounds **3**−**6** are characterized by the presence of different patterns for each set of signals, compatible with the presence of comparable amounts of different rotamers in slow exchange on the NMR time-scale, as previously found with analogous compounds.[4,12] This phenomenon was ascribed to the steric bulk of the bis(Boc)-protected triazacyclononane units. After removal of protecting groups, the NMR spectra of **1a**$(H^+)_4$ become fully consistent with the presence of a single species.

Potentiometric Titrations. The potentiometric determination of the acidity and binding constants of the ligand with the metal cations is a prerequisite for the study of the catalytic properties. The solvent mixture used for the potentiometric and kinetic measurements is 80:20 v/v DMSO:H_2O (hereafter referred to as 80% DMSO). This solvent mixture is known to be suitable for the study of hydrolytic reactions[1b,13] and for the determination of acidity constants.[14,15] The two-point hydrogen-bonding interaction of the phosphate to the guanidinium moiety[1b] is stronger than that in pure water;[13,16] therefore, a catalyst that operates with a mechanism involving a guanidinium unit as activator or binding site can be favored in this solvent mixture. The different autoprotolysis of water in 80% DMSO (pK_w = 18.4)[17] has to be taken into account in the treatment of potentiometric data (see Experimental Section). The strongly suppressed autoprotolysis of water results in a different pH value for neutrality that rises to 9.2.

Titration curves of the ligand in the absence and presence of Zn^{II} or Cu^{II} are shown in Figure 2, and the results of the elaboration of the curves are reported in Table 1. Titration of the tetrahydrochloride of **1a** showed, as expected, the presence of four titratable protons. The most acidic protons are those on the TACN nitrogen atoms, whereas the least acidic proton belongs to the guanidinium unit. Addition of 1 mol equiv of metal salt results in a deep modification of the titration curve, and the number of titratable protons rises to five. The apparent acidity of the three most acidic protons increases further as a result of the binding of the metal cations to the TACN unit (Table 1, footnotes d and e). The pK_a values of 9.8 and 8.9 determined in the presence of Zn^{II} and Cu^{II}, respectively, were assigned to the deprotonation of the metal coordinated water molecule, whereas the least acidic proton was again assigned to the guanidinium unit.

Not surprisingly, all of the pK_a values and the log *K* values related to the binding to the metal cations coincide within experimental errors with the corresponding values of the tetrahydrochloride of **1b**,[4] indicating the absence of significant differences in acid−base properties and complex stabilities between regioisomers.

Cleavage of RNA Models HPNP and *Np*N′. The first reaction investigated to test the performance of the bifunctional catalyst **1a**H^+−M was the transesterification of HPNP (eq 1). The pH of the solutions was adjusted to a value equal to the pK_a value of the water molecule coordinated to the metal ion. At the given pH, the guanidine unit of the catalyst is fully

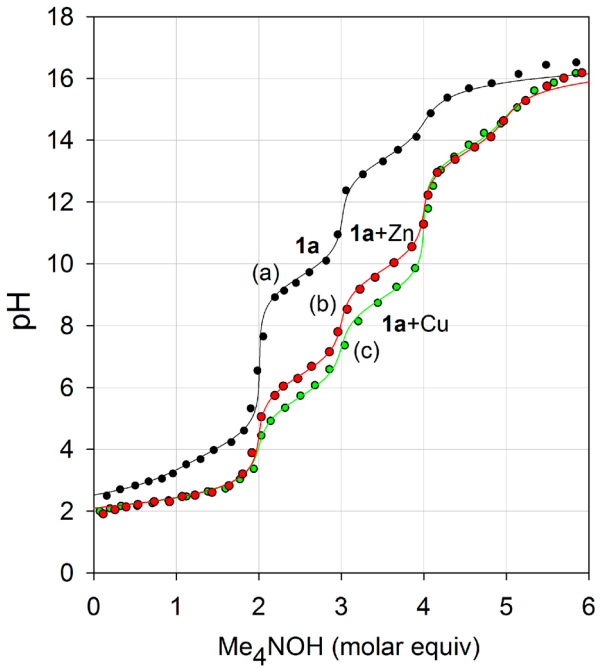


Figure 2. Potentiometric titration curves of 1.0 mM **1a**$(H^+)_4$ with Me_4NOH in 80% DMSO in the absence of any metal cation (a), and in the presence of 1 molar equiv of Zn^{II} (b) or Cu^{II} (c). Data points are experimental, and the lines are calculated.

Table 1. Acidity Constants (pK_a) of 1a·4HCl, in the Absence and Presence of 1 molar equiv of Metal Cations (80% DMSO, 25 °C)[a]

entry	additive	pK_{a1}	pK_{a2}	pK_{a3}	pK_{a4}	pK_{a5}
1	none	2.2[b]	4.0	9.6	13.4[c]	
2	Zn^{II}[d]	<2	<2	6.4	9.8	13.6
3	Cu^{II}[e]	<2	<2	5.7	8.9	13.7

[a]Data from potentiometric titration (Figure 2) of 1.0 mM **1a**$(H^+)_4$ with Me_4NOH in the presence of 10 mM Me_4NClO_4. Error limit = ±0.1 *pK* units, unless otherwise stated. [b]Error limit = ±0.3 *pK* units. [c]Under the same conditions, the pK_a of guanidinium chloride is 13.7 (see ref 6). [d]From the potentiometric titration: log K_{Zn} = 6.9 ± 0.5, for the binding of Zn^{II} to **1a**H^+. [e]From the potentiometric titration: log K_{Cu} = 8.8 ± 0.4, for the binding of Cu^{II} to **1a**H^+.

protonated, the metal ion is fully bound to the TACN unit, and 50% of the water molecules coordinated to the metal ion are deprotonated. Pseudo-first-order rate constants were calculated as $k_{obs} = v_0/[HPNP]$, where v_0 is the spectrophotometrically determined initial rate of *p*-nitrophenol release. These values are listed in Table 2 together with the corresponding values previously determined for the 1,3-distal regioisomer **1b**.[4]

The first and most important observation is that the catalytic performances of the metal complexes of the two regioisomers **1a** and **1b** are remarkably similar.[18] Comparison of entry 1 with entries 2 and 3, and entry 4 with entries 5 and 6, shows that the monofunctional controls TACN−Zn and TACN−Cu catalyze the cleavage of HPNP less effectively than the corresponding complexes of the bifunctional catalysts. These findings, together with the previous report[6] that the rate of cleavage of HPNP is unaffected by 5 mM guanidinium chloride in the pH range 9.0−9.8, indicate the existence of cooperation between catalytic units in the bifunctional catalysts. The degree of cooperation is modest for the Zn^{II} complexes, as previously found for the Zn^{II}

DOI: 10.1021/acs.joc.6b00644
J. Org. Chem. 2016, 81, 4728−4735

Table 2. Transesterification of the RNA Model HPNP in the Presence of Metal Complexes 1aH$^+$−M, 1bH$^+$−M, and TACN−M (M = Zn^{II}, Cu^{II})[a]

entry	pH	catalyst	$10^6 \times k_{obs}$ (s^{-1})[b]	k_{rel}[c]	k_{obs}/k_{bg}[d]
1[e]	9.9	TACN−Zn	20	1.0	400
2	9.8	**1a**H$^+$−Zn	73	3.6	1800
3[e]	9.9	**1b**H$^+$−Zn	110	5.5	2200
4[e]	8.8	TACN−Cu	1.4	1.0	350
5	8.9	**1a**H$^+$−Cu	1800	1300	360 000
6[e]	8.8	**1b**H$^+$−Cu	1300	930	320 000

[a]0.10 M *N*,*N*′-diisopropyl ethanolamine buffer (80% DMSO, 25.0 °C); 0.20 mM HPNP; 0.50 mM catalyst. [b]k_{obs} calculated as v_0/[HPNP]; error limits on the order of ±10%. [c]k_{rel} calculated as k_{obs}(bifunctional catalyst)/k_{obs}(TACN−M). [d]The background rate constant (k_{bg}, s^{-1}) for the hydroxide-catalyzed cleavage is a function of pH, and it is given by the following expression: $k_{bg} = 10^{(pH-17.2)}$ (see ref 3c). [e]Data from ref 4.

complex of an analogous catalyst based on a *m*-xylylene spacer.[6] Better results are found for the Cu^{II} complexes, whose catalytic performances exceed that of the monofunctional control TACN−Cu by a factor of 10^3, and that of the bifunctional catalyst based on the *m*-xylylene spacer by a factor of about 30.[6]

In a second set of rate measurements, the transesterification of GpU, CpA, UpU, and GpA (eq 2) was carried out in the presence of the Cu^{II} complex of **1a** under the same conditions used for HPNP, with the sole difference that the temperature was raised from 25 to 50 °C because diribonucleoside monophosphates are more reluctant than HPNP to undergo transesterification. The reaction progress was monitored up to about 10% conversion by HPLC analysis of samples withdrawn at selected times. Pseudo-first-order rate constants were calculated as $k_{obs} = v_0/[NpN']$, where v_0 is the initial rate of formation of the nucleoside *N*′ (eq 2). These values are listed in Table 3, together with the acceleration factors relative to

Table 3. Cleavage of Diribonucleoside 3′,5′-Monophosphates *NpN*′ by 1aH$^+$−Cu (This Work)[a] and Comparison with the Activity of 1bH$^+$−Cu (Data from Ref 4)

	1aH$^+$−Cu (1,2-vicinal)		**1b**H$^+$−Cu (1,3-distal)	
	$10^6 \times k_{obs}$ (s^{-1})	k_{obs}/k_{bg}[b]	$10^6 \times k_{obs}$ (s^{-1})[c]	k_{obs}/k_{bg}[c]
GpU	93	3.0×10^6	85	3.4×10^6
CpA	48	2.7×10^6	1.2	8.6×10^4
UpU	15	5.8×10^5	26	1.3×10^6
GpA	1.0	6.7×10^4	160	1.3×10^7

[a]0.50 mM **1a**H$^+$−Cu; 0.050 mM *NpN*′; 0.10 M *N*,*N*′-diisopropyl ethanolamine buffer; 80% DMSO, pH 8.9, 50.0 °C. Error limits on the order of ±10%. [b]The k_{bg} values (s^{-1}) were calculated from data measured in the presence of 1.0 mM Me_4NOH in 80% DMSO, 50.0 °C, and extrapolated to pH 8.9. k_{bg} (GpU) = 3.1×10^{-11} s^{-1}; k_{bg} (CpA) = 1.8×10^{-11} s^{-1} (from ref 19); k_{bg} (UpU) = 2.6×10^{-11} s^{-1} (from ref 20); k_{bg} (GpA)= 1.5×10^{-11} s^{-1} (from ref 4). [c]k_{obs} and k_{bg} values at pH 8.8 from ref 4.

background k_{obs}/k_{bg}, where k_{bg} is the first-order rate constant for the spontaneous cleavage at the same pH. For comparison purposes, the corresponding data for the same reactions catalyzed by the Cu^{II} complex of the 1,3-distal regioisomeric catalyst **1b** are also listed in Table 3.

The 1,2-vicinal catalyst **1a**H$^+$−Cu effectively cleaves all of the investigated substrates, with catalytic rates spanning almost 2 orders of magnitude. The reactivity order GpU > CpA > UpU ≫ GpA considerably differs from that found in the presence of the 1,3-distal regioisomer, for which the reactivity order was GpA > GpU > UpU ≫ CpA. Whereas GpU and UpU are cleaved by both catalysts with comparable efficiency, GpA is a bad substrate for the 1,2-vicinal catalyst, but a good substrate for the 1,3-distal regioisomer, and exactly the opposite is true for CpA. The net result is that the 1,2-vicinal catalyst cleaves CpA 40 times more effectively than the 1,3-distal regioisomer, but the relative rate in the cleavage of GpA drops to 1/160. Thus, data in Table 3 show that catalytic rates may depend on the substrate−catalyst combination even to a very significant extent. As previously suggested,[4] lower rates of catalytic cleavage are possibly ascribable to unfavorable steric interactions between the catalyst and the altered substrate in the transition state. These interactions presumably involve nonreacting part-structures, and are either absent or less important for the most reactive substrate−catalyst combinations.

It has been argued[4] that in the cleavage of phosphodiesters, upon replacement of a good leaving group with a poor one, the transition state bears a closer resemblance to a pentavalent phosphorane dianion, independent of whether the reaction occurs via a two-step (A_N+D_N) or a concerted (A_ND_N) mechanism.[1c] Consequently, larger rate enhancements are predicted for reactions of substrates less activated than HPNP, as a result of a stronger role played by the metal ion and the neighboring guanidinium as electrophilic/electrostatic activators. The k_{obs}/k_{bg} values in Tables 3 and 2 provide a convenient basis for a comparison of rate accelerations in the cleavage of diribonucleoside monophosphates versus HPNP, respectively, under the reasonable assumption that k_{obs}/k_{bg} values of the reactions of HPNP (Table 2), measured at 25 °C, provide an acceptable estimate of the values that would be measured at 50 °C.

The data reported in Table 4 are the rate accelerations over background for the reactions of the catalyst−*NpN*′ pairs

Table 4. Comparison of Rate Accelerations over Background of Catalysts 1aH$^+$−Cu and 1bH$^+$−Cu in the Cleavage of *NpN*′ and HPNP Substrates[a]

entry	catalyst/substrate	$(k_{obs}/k_{bg})_{NpN'}/(k_{obs}/k_{bg})_{HPNP}$
1	**1b**H$^+$−Cu/GpA	41
2	**1b**H$^+$−Cu/GpU	11
3	**1a**H$^+$−Cu/GpU	8.3
4	**1a**H$^+$−Cu/CpA	7.5
5	**1b**H$^+$−Cu/UpU	4.1
6	**1a**H$^+$−Cu/UpU	1.6
7	**1b**H$^+$−Cu/CpA	0.27
8	**1a**H$^+$−Cu/GpA	0.19

[a]Data from Tables 2 and 3.

relative to the corresponding quantities for the cleavage of HPNP. The 1,3-distal catalyst **1b**H$^+$−Cu cleaves GpA (entry 1) with a rate acceleration over background that exceeds by a factor of 41 the rate acceleration measured for the cleavage of HPNP by the same catalyst. This is clearly in keeping with the idea that the cleavage of a less activated substrate benefits from a greater electrophilic activation. Such an advantage tends to become smaller for the less efficient catalyst−substrate combinations. Although too much emphasis cannot be placed on exact figures, for the reactions of CpA catalyzed by **1b**H$^+$−

DOI: 10.1021/acs.joc.6b00644
J. Org. Chem. 2016, 81, 4728−4735

Cu and GpA catalyzed by **1a**H$^+$−Cu (entries 7 and 8, respectively), rate accelerations are lower than those for the corresponding reactions of HPNP, showing that in these cases the advantage arising from a larger electrophilic activation is more than offset by adverse factors, which are presumably of steric origin.

Cleavage of the DNA Model BNPP. The phosphodiesterase activity of regioisomeric metal complexes **1a**H$^+$−M and **1b**H$^+$−M and of model complexes TACN−M (M = Zn^{II}, Cu^{II}) was also tested in the hydrolysis of the DNA model BNPP (eq 3). In consideration of the low reactivity of this substrate, lacking the benefit of an intramolecular nucleophile group, kinetic experiments were carried out at 50 °C. Reaction solutions were buffered at a pH coincident with the pK_a of the metal-coordinated water molecule in the given complex. Pseudo-first-order rate constants k_{obs}, calculated from initial rate values of *p*-nitrophenol release (spectrophotometric monitoring), are listed in Table 5 together with rate enhancement over background values (k_{obs}/k_{bg}).

Table 5. Cleavage of the DNA Model BNPP in the Presence of Metal Complexes 1aH$^+$−M, 1bH$^+$−M, and TACN−M (M = Zn^{II}, Cu^{II})[a]

entry	pH	catalyst[b]	$10^6 \times k_{obs}$ (s^{-1})[c]	k_{rel}[d]	(k_{obs}/k_{bg})[e]
1	9.9	TACN−Zn	0.74	1.0	430
2	9.8	**1a**H$^+$−Zn	180	240	130 000
3	9.9	**1b**H$^+$−Zn	84	110	49 000
4	8.8	TACN−Cu	0.14	1.0	1100
5	8.9	**1a**H$^+$−Cu	190	1400	1 100 000
6	8.8	**1b**H$^+$−Cu	20	140	140 000

[a]0.20 mM BNPP; 0.50 mM catalyst; 0.10 M *N*,*N*′-diisopropyl ethanolamine buffer (80% DMSO, 50.0 °C). [b]In the presence of 0.50 mM guanidinium chloride (pH range 8.8−9.9), no detectable liberation of *p*-nitrophenol was observed within 12 h. [c]k_{obs} calculated as v_0/[HPNP]; error limits on the order of ±10%. [d]k_{rel} calculated as k_{obs}(bifunctional catalyst)/k_{obs}(TACN−M). [e]k_{bg} data from extrapolation to the given pH value of the hydroxide catalyzed cleavage of BNPP measured in the presence of 1.0 mM Me_4NOH (k_{obs} = 5.4 × 10^{-4} s^{-1}). The following k_{bg} values (s^{-1}) were obtained (pH value in parentheses): 1.7 × 10^{-9} (9.9); 1.4 × 10^{-9} (9.8); 1.7 × 10^{-10} (8.9); 1.4 × 10^{-10} (8.8).

Kinetic data show a very good performance of the bifunctional metallocatalysts in the BNPP cleavage, with large rate enhancements over background (49 000 ≤ k_{obs}/k_{bg} ≤ 1 100 000), a marked superiority of the 1,2-vicinal arrangement of the catalytic dyad, and a widespread high level of cooperation of the two active moieties. The bifunctional catalysts are indeed from 110-fold (**1b**H$^+$−Zn) up to 1400-fold (**1a**H$^+$−Cu) more active than the corresponding monofunctional models TACN−M, the other monofunctional control guanidinium chloride being totally ineffective (footnote b to Table 5). The significant level of cooperation of the two active units in the cleavage of BNPP catalyzed by **1a**H$^+$−Zn and **1b**H$^+$−Zn is at variance with the modest degree of cooperation observed with the same Zn^{II} complexes in the transesterification of HPNP (Table 2). It is worth noting that the specific hydrolytic rate of BNPP cleavage with **1b**H$^+$−Zn is even 4-fold higher than with **1b**H$^+$−Cu. Despite the higher catalytic rate of **1b**H$^+$−Zn, the rate enhancement over background turns out to be higher with **1b**H$^+$−Cu, because this metal catalyst operates at a pH value lower by 1 unit.

On the grounds of the simple argument that the reaction center of BNPP is electronically more activated than that of HPNP,[21] one would expect larger rate accelerations over background for the cleavage of the latter substrate. Such prediction, however, is contradicted by facts: comparison of Table 5 with Table 2 shows that k_{obs}/k_{bg} values for the two substrates are either comparable in magnitude (entries 1, 4−6) or even much larger for the cleavage of BNPP catalyzed by the Zn^{II} complexes of **1a**H$^+$ and **1b**H$^+$ (entries 2 and 3), whose rate accelerations exceed those found for the cleavage of HPNP by 72- and 22-fold, respectively. Similar findings have been reported in the literature[8f,22] for metal ion-catalyzed cleavage of BNPP and HPNP. In a recent paper,[8f] Yatsimirsky et al. ascribe the generally more efficient catalysis of metal complexes in BNPP hydrolysis to the entropic advantage arising from the conversion of an otherwise intermolecular reaction into an intramolecular nucleophilic attack of a metal coordinated hydroxide ion. Here, we suggest that a reasonable contribution to the lower than expected rate accelerations exhibited by the less electronically activated HPNP may also arise from stringent steric and geometric requirements imposed by the participation of the neighboring nucleophile of the 2-hydroxypropyl side chain. It seems likely that these requirements are particularly stringent in the cleavage of HPNP catalyzed by the bifunctional Zn^{II} complexes (Figure 3).

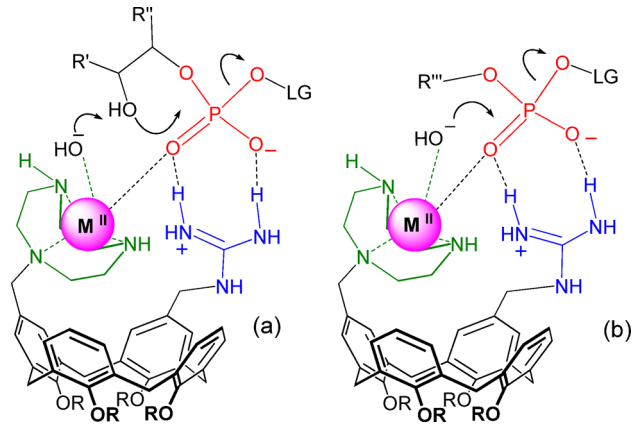


Figure 3. Suggested mechanism for the transesterification of RNA models (a) and for the hydrolysis of DNA models (b), catalyzed by the metal complex of calix[4]arene **1a**H$^+$.

Although alternative mechanisms were proposed for the cleavage of HPNP catalyzed by phosphodiesterases provided with metal centers,[8f,23] on the basis of the comparison of kinetic and potentiometric data previously reported for similar systems under the same experimental conditions,[4,6] we believe that in the present case the operating mechanism is a general base catalysis as indicated in Figure 3a.

CONCLUSIONS

In this work, we have reported that the Cu^{II} and Zn^{II} complexes of **1a**H$^+$ exhibit a catalytic efficiency very similar to that of its 1,3-distal regioisomer in the cleavage of the RNA model compound HPNP, with a marked superiority of the Cu^{II} complexes over the Zn^{II} analogues. A similar situation was found for the cleavage of GpU and UpU catalyzed by the Cu^{II} complexes, but CpA proved to be a good substrate for the 1,2-vicinal catalyst and a bad substrate for the 1,3-distal regioisomer, while the reverse situation was found for GpA.

DOI: 10.1021/acs.joc.6b00644
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Investigation of the catalytic performances of the bifunctional metallocatalysts has been extended to the DNA model compound BNPP. In this case, both Zn^{II} and Cu^{II} complexes exhibit good catalytic efficiency as the result of a high degree of synergism between catalytic functions. The best results were obtained for the Cu^{II} complexes of the 1,2-vicinal regioisomer, which cleaves BNPP with a catalytic acceleration over background as high as one million-fold, and a degree of synergism of 1400. The latter value compares well with the values reported for the most efficient synthetic phosphodiesterases[1b,3f,24] and is definitely the highest for enzyme mimics based on calixarene scaffolds.[7b] In general, however, a meaningful quantitative comparison with the catalytic efficiency of other guanidinium-based artificial phosphodiesterases is hampered by the fact that available data often consist of erratic examples scattered over diverse experimental conditions, as well as by the paucity of reliable k_{bg} values required for the calculation of k_{obs}/k_{bg} values.

In contrast to the cleavage of HPNP, for which the two regioisomeric Cu^{II} complexes show very nearly the same catalytic efficiency, BNPP is cleaved by **1a**H^+−Cu an order of magnitude more rapidly than by the 1,3-distal regioisomer.

To sum, if one chooses the best substrate−catalyst combination, rate accelerations over background for the cleavage of the model compounds reported in this work range from 3.6×10^5 to 1.3×10^7. The data reported in this work offer a varied phenomenological picture, in which catalytic efficiency and degree of synergism between catalytic units may depend, even to a large extent, on the structure of the phosphodiester undergoing hydrolytic cleavage, the identity of the metal ion, as well as on whether the substitution pattern is 1,2-vicinal or 1,3-distal.

EXPERIMENTAL SECTION

Instruments. NMR spectra were recorded on either a 300 or a 400 MHz spectrometer. Partially deuterated solvents were used as internal standards to calculate the chemical shifts (δ values in ppm). High-resolution mass spectra were obtained by an electrospray ionization (ESI) single-quadrupole spectrometer. Potentiometric titrations were performed by an automatic titrator equipped with a pH electrode.

Spectrophotometric measurements of *p*-nitrophenol liberation were carried out at 400 nm on a double beam spectrophotometer. HPLC analyses were performed on a liquid chromatograph equipped with a UV−vis detector operating at 254 nm.

Materials and General Procedures. All reactions, with the sole exception of the deprotection of **6**, were carried out under a nitrogen atmosphere. Flash chromatography was carried out on 230−240 mesh silica gel. Anhydrous CH_2Cl_2 (DCM) was obtained by distillation over $CaCl_2$. DMSO, purged 30 min with argon, and mQ water were used in the preparation of 80% DMSO used in kinetic and potentiometric experiments. HPNP[25] and 5,11-bis(chloromethyl)-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene **2**[10] were prepared according to literature procedures. All other solvents and reagents were commercial samples and used as such. Commercial samples of *NpN′* and their aqueous solutions were stored at −20 °C.

1. Warning! Care was taken when handling tetramethylammonium perchlorate because it is potentially explosive.[26] No accident occurred in the course of the present work.

*2. 5-Chloromethyl-11-[N,N′-bis(tert-butoxycarbonyl)-1,4,7-triazacyclonon-1-ylmethyl] -25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (**3**).* To a solution of **2** (0.084 g, 0.10 mmol) in dry CH_3CN (5 mL) were added 1,4-bis(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane (0.034 g, 0.10 mmol) and K_2CO_3 (0.014 g, 0.10 mmol). The reaction mixture was stirred at room temperature for 6 days. In the course of the reaction, additional amounts of 1,4-bis(*tert*-butoxycarbonyl)-1,4,7-triazacyclononane (2 × 0.010 g, 0.030 mmol) were added. The reaction was then quenched by evaporating the solvent under reduced pressure. The residue was dissolved with DCM (20 mL), and the organic phase was washed with distilled water (2 × 20 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The crude material was purified by flash chromatography (hexane/AcOEt/DCM 7:2:1−hexane/AcOEt 7:3) to recover unreacted **2** (0.022 g, 0.027 mmol; 27% yield) and give **3** as a colorless oil (0.064 g, 0.058 mmol; 56% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.80 (m, 1H), 6.71−6.65 (m, 3H), 6.62−6.55 (m, 2H), 6.51−6.4 (m, 3H), 6.38 (d, 1H, J = 5.1 Hz), 4.51 (d, 2H, J = 12.6 Hz), 4.48 (d, 2H, J = 13.2 Hz), 4.32−4.05 (m, 10H), 3.84−3.82 (m, 8H), 3.55−3.44 (m, 14H), 3.15−3.08 (m, 8H), 2.59−2.37 (m, 4H), 1.49 (s, 9H), 1.45 (s, 9H), 1.23−1.17 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 156.8−155.2, 135.7−133.9, 133.3−133.1, 128.7−127.8, 122.2, 79.4, 79.3, 73.4, 73.2, 72.9, 69.7, 66.4, 66.3, 60.2, 59.8, 53.6−52.9, 50.8−49.0, 46.6, 30.8, 28.6, 28.5, 15.3. HR ES-MS: m/z calcd for $C_{62}H_{89}O_{12}N_3Cl$ [(**3** + H)$^+$] 1102.61293, found 1102.61236.

*3. 5-(N-Phthalimidomethyl)-11-[N,N′-bis(tert-butoxycarbonyl)-1,4,7-triazacyclo-non-1-ylmethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (**4**).* To a solution of **3** (0.060 g, 0.055 mmol) in dry DMF (8 mL) was added potassium phthalimide (0.011 g, 0.060 mmol). The reaction mixture was stirred overnight at room temperature and quenched by evaporating the solvent under reduced pressure. The crude material was dissolved in AcOEt (20 mL), and the organic phase was washed with a saturated solution of NaCl (3 × 20 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. Compound **4** was obtained as a pale white oil (0.060 g, 0.049 mmol; 90% yield), pure enough to avoid further purifications. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.83−7.81 (m, 2H), 7.71−7.67 (m, 2H), 7.04, 6.93, 6.88, 6.78, 6.72, 6.63, 6.54, 6.45, 6.32 (9 m, 10H), 4.65−4.38 (m, 6H), 4.23−3.97 (m, 8H), 3.90−3.76 (m, 8H), 3.64−3.34 (m, 14H), 3.26−3.06 (m, 6H), 3.00−3.70 (m, 2H), 2.49−2.29 (m, 4H), 1.50 (s, 9H), 1.44 (s, 9H), 1.22−1.10 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 167.7, 156.9−155.7, 135.9, 135.5, 134.2, 133.8, 133.1, 129.9, 128.7−127.9, 123.1, 122.1, 79.2, 73.3, 72.9, 69.6, 66.3, 60.0, 53.4, 50.3, 50.4−49.1, 41.8, 30.8, 28.6, 15.2. HR ES-MS: m/z calcd for $C_{70}H_{93}N_4O_{14}$ [(**4** + H)$^+$] 1213.66828, found 1213.66847; m/z calcd for $C_{70}H_{92}N_4O_{14}Na$ [(**4** + Na)$^+$] 1235.65022, found 1235.65075.

*4. 5-(Aminomethyl)-11-[N,N′-bis(tert-butoxycarbonyl)-1,4,7-triazacyclonon-1-yl-methyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (**5**).* To a solution of **4** (0.034 g, 0.028 mmol) in dry MeOH (4 mL) was added $N_2H_4{\cdot}H_2O$ (35 μL, 0.72 mmol). The reaction mixture was heated to 65 °C and stirred for 3 h, and then was quenched by evaporating the solvent under reduced pressure. The residue was dissolved in DCM (20 mL), and the organic phase was washed with a solution of NaOH 1 M (20 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. Compound **5** was obtained as a colorless oil (0.028 g, 0.026 mmol; 92% yield). Because of the instability of the amino group,[27] the compound was not fully characterized and used as such in the following reaction. HR ES-MS: m/z calcd for $C_{62}H_{91}N_4O_{12}$ [(**5** + H)$^+$] 1083.66280, found 1083.66238.

*5. 5-N-[N′,N″-Bis(tert-butoxycarbonyl)guanidine]methyl-11-[4,7-bis(tert-butoxycarbonyl)-1,4,7-triazacyclonon-1-ylmethyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene (**6**).* To a solution of **6** (0.028 g, 0.026 mmol) in dry DCM (5 mL) were added *N,N′*-bis(*tert*-butoxycarbonyl)-*N″*-triflylguanidine (0.015 g, 0.039 mmol) and triethylamine (5.4 μL, 0.039 mmol). The reaction mixture was stirred overnight at room temperature, and then quenched by adding distilled water (20 mL) and vigorously stirred for an additional 30 min. The aqueous phase was separated and washed with DCM (2 × 20 mL), and then the combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The crude material was purified by preparative TLC plates (hexane/AcOEt/DCM 6:3:1) to give **6** as a yellow oil (0.020 g, 0.018 mmol; 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 11.55 (s, 1H), 8.38, 8.31, 8.25 (3 t, 1H, J = 4.8 Hz), 6.77−6.72 (m, 1H), 6.66−6.61 (m, 4H), 6.58−6.52 (m, 4H), 6.49−6.3 (m, 1H), 4.52−4.45 (m, 4H), 4.37, 4.29, 4.24 (3 t, 2H, J = 4 Hz), 4.22−4.04 (m, 8H), 3.91−3.84 (m,

DOI: 10.1021/acs.joc.6b00644
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8H), 3.72−3.40 (m, 14H), 3.32−3.08 (m, 6H), 2.98−2.78 (m, 2H), 2.53−2.25 (m, 4H), 1.54−1.46 (m, 36H), 1.24−1.20 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 163.6, 156.2, 155.9, 155.8, 155.7, 155.2, 153.1, 150.8, 135.7−134.1, 133.1, 130.4, 128.3, 128.2, 127.6, 127.4, 122.2, 82.9, 79.3, 73.5−72.8, 69.5, 66.3, 60.2, 53.4−52.9, 50.5−49.2, 45.0, 30.9, 28.7, 28.6, 28.3, 28.1, 15.3. HR ES-MS: m/z calcd for $C_{73}H_{109}N_6O_{16}$ [(**6** + H)$^+$] 1325.78946, found 1325.78946; m/z calcd for $C_{73}H_{108}N_6O_{16}Na$ [(**6** + Na)$^+$] 1347.77140, found 1347.77186.

*6. 5-N-Guanidinomethyl-11-N-[1,4,7-triazacyclononane-1-methyl]-25,26,27,28-tetrakis(2-ethoxyethoxy)calix[4]arene Tetrakis-hydrochloride (**1a**·4HCl).* In a mixture of DCM/TFA/TES 95:2.5:2.5 (10 mL) was dissolved **6** (0.020 g, 0.015 mmol). The reaction mixture was stirred overnight at room temperature and quenched by evaporating the solvent under reduced pressure. The residue was first treated with a solution of 1 M HCl in EtOH (3 mL), and vigorously stirred for 30 min three times, to exchange the TFA anion, then recrystallized from DCM/hexane to give **1**·4HCl as a waxy white solid (0.012 g, 0.013 mmol; 86% yield). ^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 6.92−6.87 (m, 4H), 6.77−6.72 (m, 2H), 6.64−6.57 (m, 4H), 4.62 (d, 2H, J = 12.8 Hz), 4.61 (d, 1H, J = 13.2 Hz), 4.60 (d, 1H, J = 12.8 Hz), 4.27−4.22 (m, 6H), 4.08−4.07 (m, 4H), 3.99−3.96 (m, 4H), 3.90−3.85 (m, 4H), 3.65−3.56 (m, 14H), 3.25−3.18 (m, 4H), 3.03−3.00 (m, 4H), 2.55 (m, 4H), 1.27−1.20 (m, 12H). ^{13}C NMR (100 MHz, CD_3OD) δ (ppm): 157.1, 156.8, 156.7, 155.8, 136.3, 136.2, 135.7, 135.6, 134.8, 134.6, 134.5, 134.2, 130.0, 129.9, 129.6, 128.3, 128.2, 128.0, 127.9, 127.8, 127.5, 127.4, 122.0, 73.8, 73.7, 73.0, 72.8, 69.9, 69.8, 69.7, 69.6, 66.0, 58.2, 47.4, 44.6, 43.2, 42.1, 30.4, 14.3. HR ES-MS: m/z calcd for $C_{53}H_{77}N_6O_8$ [(**1** + H)$^+$] 925.57974, found 925.57982.

*7. 5-(N-Phthalimidomethyl)-11-chloromethyl-25,26,27,28-tetrakis(2-ethoxyethoxy) calix[4]arene (**7**).* To a solution of **2** (0.255 g, 0.31 mmol) in dry toluene (8 mL) were added potassium phthalimide (0.058 g, 0.31 mmol) and dicyclohexyl-18-crown-6 (0.117 g, 0.31 mmol). The reaction mixture was stirred at 110 °C under nitrogen atmosphere. After 3 h, 25 mL of 1 M HCl and 10 mL of toluene were added, and the mixture was stirred for 20 min. The solvent was removed from the separated organic phase under reduced pressure, and the residue was submitted to flash chromatography (SiO_2:hexane/AcOEt (6/4)). Pure compound **7** was isolated as a white foam after evaporation of the solvent (0.185 g, 0.201 mmol; 65% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.86−7.84 (m, 2H), 7.71−7.77 (m, 2H), 6.85−6.78 (m, 4H), 6.71 (t, J = 7.2 Hz, 1H), 6.40−6.36 (m, 5H), 4.63 (s, 2H), 4.49 (d, J = 13.4 Hz, 2H), 4.45 (d, J = 13.4 Hz, 2H), 4.12 (m, 6H), 4.04−3.98 (m, 4H), 3.85−3.78 (m, 8H), 3.56−3.45 (m, 8H), 3.15−3.07 (m, 4H), 1.25−1.11 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm): 168.1, 157.1, 153.5, 155.8, 135.9, 135.6, 134.4, 134.2, 133.9, 132.2, 130.9, 129.7, 128.7, 123.2, 122.5, 73.3, 73.1, 72.9, 69.7, 69.6, 66.4, 66.3, 46.8, 41.3, 30.8, 30.7, 15.3. HR ES-MS: m/z calcd for $C_{54}H_{62}ClNO_{10}Na$, [(**7** + Na)$^+$] 942.39544, found 942.39552.

Titrations. Potentiometric titrations were carried out according to a previously reported procedure.[3c,4] A freshly prepared solution of 50−100 mM Me_4NOH in 80% DMSO was added in small increments under an argon atmosphere to 5 mL of a 1 mM solution (80% DMSO) of **1a**$(H^+)_4$, 10 mM Me_4NClO_4 as ionic strength buffer, in the absence or presence of 1 mol equiv of $ZnCl_2$ or $CuCl_2$ (80% DMSO, 25 °C). For details about the electrode calibration, see the previously reported procedure.[4,6] Elaboration of the titration plots was carried out with the software HYPERQUAD 2000.[28]

Kinetic Measurements. Metal complexes were formed in situ by addition of the calculated stoichiometric amount of a concentrated aqueous solution of the metal salt ($ZnCl_2$ or $CuCl_2$) to the reaction mixture. The solutions were incubated for 1 h before the start of the kinetic run by fast addition of a small volume of the substrate solution.

HPNP and BNPP. Observed rate constants were obtained by UV−vis monitoring of *p*-nitrophenol liberation at 400 nm (initial rate method). Experiments were carried out in the presence of 0.50 mM precatalyst **1a**/**1b**·4HCl, 10 mM Me_4NClO_4, 0.50 mM $CuCl_2$ or $ZnCl_2$, and 0.10 M *N*,*N*′-diisopropyl ethanolamine buffer (80% DMSO, 25.0 °C). The pH of the solution was adjusted with a 50−100 mM solution of $HClO_4$ in 80% DMSO.

Diribonucleoside Monophosphates. HPLC monitoring of nucleoside liberation (eq 3) was carried out on solutions with the following composition: 0.50 mM precatalyst **1a**·4HCl, 10 mM Me_4NClO_4, 0.50 mM $CuCl_2$, 0.10 mM *Np*N′, 0.10 M *N*,*N*′-diisopropyl ethanolamine buffer (80% DMSO, pH 8.9, 50.0 °C); the pH of the solution was adjusted with a 50−100 mM solution of $HClO_4$ in 80% DMSO. Aliquots of the reaction mixture (60−80 μL) were withdrawn at appropriate time intervals and quenched with the same volume of a 15 mM solution of $HClO_4$ in 80% DMSO. After addition of *p*-hydroxybenzoic acid (internal standard) in 80% DMSO, the solution was filtered and analyzed by HPLC (C-18 DB column, Supelcosil) using the following eluent: H_2O (0.1% trifluoroacetic acid)/MeCN, gradient from 100:0 to 85:15 in 28 min, flow 0.9 mL/min.

The pseudo-first-order rate constants for the spontaneous background reaction (k_{bg}) of the cleavage of BNPP were obtained by extrapolation to the required pH values of the pseudo-first-order constant measured in the presence of 1.0 mM Me_4NOH (pH 15.4), 10 mM Me_4NClO_4 (80% DMSO, 50.0 °C).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b00644.

NMR spectra for the products and Scheme S1 (PDF)

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Thanks are due to the Ministero dell'Istruzione e dell'Università e della Ricerca (PRIN 2010JMAZML MultiNanoIta), Ateneo 2015, and Consiglio Nazionale delle Ricerche (CNR) for financial support. Thanks are also due to CIM (Parma University) for the use of NMR and mass spectrometry facilities.

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DOI: 10.1021/acs.joc.6b00644
J. Org. Chem. 2016, 81, 4728−4735