Pergamon

Tetrahedron Letters 43 (2002) 4179–4182

TETRAHEDRON
LETTERS

Comparative nucleophilic reactivities in phosphodiester cleavage

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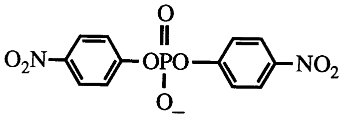
Received 20 March 2002; accepted 17 April 2002

Abstract—*o*-Iodosobenzoate (**2**) and 2,3-iodosonaphthoate (**4**) cleave phosphodiester substrate **3** in cationic micelles at pH 9 with rate accelerations of 1200 or 5800, respectively. © 2002 Published by Elsevier Science Ltd.

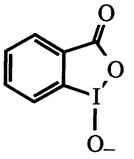
Phosphodiesters strongly resist hydrolysis: P–O cleavage of dimethyl phosphate at pH 7 and 25°C has $k<10^{-15}$ s^{-1},[1] and even the 'activated' phophodiester, bis(*p*–nitrophenyl) phosphate, **1** (BNPP) displays only $k\sim1.1\times10^{-11}$ s^{-1} for unassisted hydrolysis under these conditions.[2] It appears to be difficult for an anionic nucleophile to attack a phosphodiester's negatively charged O–P–O triad. Accordingly, most useful reagents for phosphodiester cleavage feature cationic metal centers designed to both bind P–O^- and to deliver a metal-bound nucleophile, frequently hydroxide.[3,4]

Although *o*-iodosobenzoate (**2**, IBA) is a remarkably effective catalyst for the hydrolysis of *neutral phosphotriesters* and related substrates in aqueous micellar media,[5] it was considered unreactive toward BNPP.[5a] However, our recent observation that IBA in cationic cetyltrimethylammonium (CTA) micelles cleaves BNPP with $k=1.7$–2.3×10^{-6} s^{-1} at pH 9, reopens the question of nucleophilic reactivity toward phosphodiesters.[6] Although IBA-mediated BNPP hydrolysis is slow, the observed rate constant represents an acceleration of $\sim2\times10^{5}$ over the unassisted hydrolysis at pH 7.[6]

How general is phosphodiester hydrolysis by those nucleophiles typically associated with phosphotriester cleavage?[5b] What is the kinetic range of these reactions? To conveniently probe these questions, we chose the highly reactive and sensitive phosphodiester substrate, 4-nitro-1,8-naphthyl phosphate (**3**, NNP).[7] We then selected several nucleophiles known to be potent reagents for the cleavage of neutral phosphotriester substrates in aqueous CTA micelles.[5b] These included OH^- in (CTA)Cl at pH 9, IBA (**2**)[5a,8] in (CTA)Cl, cetyltrimethylammonium iodosobenzoate (CTA)IBA (no chloride ions presents),[6] iodosonaphthoate (**4**, INA) in (CTA)Cl,[5a,9] monoperoxyphthalate (**5**, MPP)[10] in (CTA)Cl, cetyltrimethylammonium hydroperoxide (CTA)OOH (chloride present),[11] and the cupric complex of *N*-hexadecyl-*N*,*N*′,*N*′-trimethylethylene diamine (**6**, Cu-HTMED).[12,13]



1 (BNPP)



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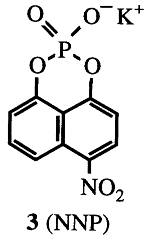

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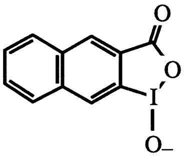
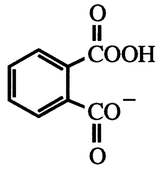
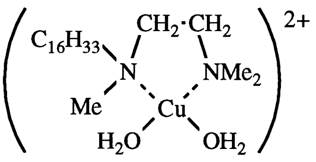


4 (INA)



5 (MPP)



6 (Cu-HTMED)

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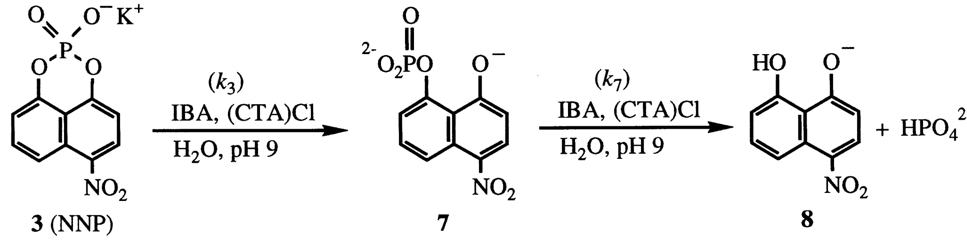
PII: S0040-4039(02)00777-3

The IBA-mediated hydrolysis of **3** is representative of our results. At pH 9.0 in 20 mM CHES buffer, 0.1 mM **3** undergoes a two-step cleavage mediated by 1.0 mM IBA in 10 mM (CTA)Cl; cf., Eq. (1). The phosphate monoester **7** forms initially, and subsequently hydrolyzes to 4-nitro-1,8-naphthalene diol (**8**)[7] and phosphate. The reactions can be followed by UV and ^{31}P NMR spectroscopy, permitting both observation of the products and extraction of the rate constants k_3 and k_7; cf., Eq. (1). Cleavage of **7** to **8** (k_7) is monitored in the UV at 470 nm (disappearance of **7**) and 520 nm (appearance of **8**).



3 (NNP) **7** **8**

(1)

The isosbestic point for this reaction falls at 485–497 nm (depending on the nucleophile) and, in separate runs, this wavelength is used to follow the appearance of **7** from the cleavage of **3** (k_3). We thus find $k_3 = 5.1\times10^{-5}$ s^{-1} and $k_7 = 5.3\times10^{-5}$ s^{-1} for IBA in (CTA)Cl micelles. Values of k_3 and k_7 for the other nucleophiles appear in Table 1.

The hydrolysis of **3** by OH$^-$ in micellar (CTA)Cl at pH 9 was examined by ^{31}P NMR with 5.0 mM **3** and 33 mM (CTA)Cl in 0.1 M 2-(cyclohexylamino)-ethanesulfonic acid (CHES) buffer; 5 mM methyl methylphosphonate was present as an internal standard.[14] There was *no* detectable hydrolysis to **7** over two weeks. Assuming that 5% of hydrolysis could have been detected, we can estimate $k_3 \leq 4.3\times10^{-8}$ s^{-1} as the *maximum* rate constant for the pH 9 micellar (CTA)Cl hydrolysis of **3**. As shown in Table 1, this value allows estimates of the *minimum* rate accelerations (k_3/k_{OH}) for phosphodiester cleavage brought about by the various nucleophiles, relative to micellar (CTA)Cl.

Table 1. Rate constants for the cleavages of substrates **3** and **7**[a]

Nucleophile	$10^4\ k_{obs}$ (s^{-1})[b]		k_3/k_7	k_3/k_{OH}[d]
	k_3 (**3**→**7**)	k_7 (**7**→**8**)[c]		
OH$^-$[e]	≤0.00043	0.56		1
IBA (**2**)	0.51	0.53	0.96	1200
INA (**4**)	2.5	0.57	4.4	5800
MPP (**5**)	[f]	[f]		
(CTA)IBA[g]	5.0	1.3	3.8	11600
(CTA)OOH[h]	0.11	0.51	0.22	260
Cu-HTMED (**6**)	12.8	8.6	1.5	29800

[a] Conditions: [substrate] = 0.10 mM; [nucleophile] = 1.0 mM; [(CTA)Cl] = 10 mM; [CHES] = 20 mM, pH 9.0, 25°C.

[b] Reactions were performed in duplicate with ±5% or better reproducibility in k_{obs}.

[c] Determined separately on substrate **7**.

[d] Based on $k_{OH} \leq 4.3\times10^{-8}$ s^{-1}; see text.

[e] NMR study; see text.

[f] No reaction was observed (UV, NMR) over two weeks.

[g] (CTA)IBA is present at 10 mM; no chloride is present.

[h] [(CTA)Cl] = 9.0 mM; [NaCl] = 1.0 mM.

Inspection of Table 1 reveals that IBA (**2**) and INA (**4**) are the most reactive nucleophiles toward phosphodiester **3** among those reagents typically employed to cleave phosphotriesters. In (CTA)Cl micellar solution at pH 9, IBA and INA accelerate the hydrolysis of **3** by factors of (at least) 1200 and 5800, respectively, relative to hydroxide ion. (CTA)OOH is considerably less reactive and monoperoxyphthalate is unreactive (although it is quite reactive toward phosphotriesters[5b,10]). Micellar (CTA)IBA, in the absence of chloride, presents the highest k_3 value among the anionic reagents of Table 1, but [IBA] is 10 times higher here than for the other iodoso reagents.

Cu-HTMED (**6**) is the most reactive reagent in Table 1. This is not surprising: metal cations which can both bind phosphodiesters at P–O$^-$ and deliver a metal-bound hydroxide ion to P=O are generally the reagents of choice for phosphodiester hydrolysis.[3,4,7,12] What is striking, however, is that INA is only 5 times less reactive toward **3** than Cu-HTMED. In fact, INA and IBA appear to be the most reactive non-metallic, anionic nucleophiles yet deployed against phosphodiesters.

The data of Table 1 also indicate that the hydrolysis of phosphate monoester **7** is *not* catalyzed by IBA, INA, MPP or (CTA)OOH. The rate constant for hydrolysis of **7** (~5–6×10^{-5} s^{-1}) is similar with each of these reagents, and similar to the value obtained with micellar (CTA)Cl alone at pH 9. In each case, we must be observing the pH 9 (CTA)Cl micelle-mediated hydroxide ion cleavage of **7**. Acceleration is observed, however, in the Cu-HTMED hydrolysis of **7**, where we see a rate enhancement of ~15.

Two other points should be briefly noted. IBA in CTA micelles at pH 9 cleaves **3** about 26 times more rapidly than it cleaves BNPP (**1**); the higher reactivity of **3** versus **1** observed in metal cation or hydroxide mediated hydrolyses,[7] persists with IBA. Secondly, as expected, IBA and INA are much less reactive toward phosphodiester **3** than (e.g.) toward phosphotriester **9**. For the latter in micellar (CTA)Cl at pH 8, $k_9 = 2.8$ (IBA) and 4.8 (INA) s^{-1},[5b] factors that are 55,000 and 19,000 times greater than k_3 for the comparable reactions of phosphodiester **3**.

Table 2. Rate constants for cleavages in various media[a]

Case	Reaction medium	$10^4\ k_{\mathrm{obs}}\ (\mathrm{s}^{-1})$			
		IBA (**2**)		INA (**4**)	
		3	**7**	**3**	**7**
1	(CTA)Cl (10 mM)	0.51	0.53	2.5	0.57
2	12-2-12 2Cl (5 mM)[b]	0.99	0.65	6.1	0.54
3	(CTA)Br (10 mM)	0.47	0.51	1.3	0.51
4	12-2-12 2Br (5 mM)	0.91	0.63	4.7	0.50
5	Brij-35 (10 mM)	0.18[c]	0.086[c]	0.12[c]	0.10[c]
6	$\mathrm{Me_4N^+Cl^-}$ (10 mM)	0.053[c]	[d]	0.047[c]	[d]

[a] Conditions as in Table 1.

[b] Contains 1% $\mathrm{Br^-}$ counterions.

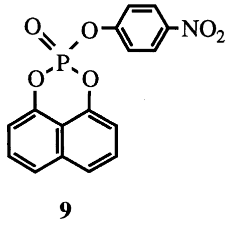
[c] Followed for ~4.5 half-lives.

[d] No reaction after 48 h.

IBA 'turns over' in the cleavage of excess substrate **3**. When [IBA]/[**3**] = 10:1, the complete hydrolysis of **3** to **8** and P_i requires 19 h.[15] This time (T) increases as the ratio (R) of IBA to **3** decreases: with $R=1$, $T=36$ h; $R=0.4$, $T=55$ h; and $R=0.2$, $T=92$ h. The strong dependence of hydrolysis time on R suggests that it is the subsequent hydrolysis of intermediate phosphorylated IBA,[16] rather than the initial cleavage of **3** by IBA, that governs the turnover rate of the IBA catalyst when the substrate is in excess. The key observation, however, is that IBA in micellar (CTA)Cl does turn over and is a true catalyst for the total hydrolysis of excess substrate **3**.

We also examined the influence of the reaction medium on the cleavage rates of **3** and **7** by IBA and INA; data appear in Table 2. The hydrolysis of phosphodiester **3** is most effectively mediated by INA (**4**) and is most rapid in aggregates of gemini surfactant[17] 12-2-12 2Cl (**10**);[18] cf., Table 2, cases 1 and 2. The enhanced reactivity in the cationic gemini medium might reflect activation of both the anionic substrate and the anionic nucleophiles by ion pairing to the dual cationic centers of a single surfactant molecule (within the micelle). Bromide counterions as in (CTA)Br or 12-2-12 2Br, slightly depress the hydrolysis of substrate **3** mediated by either IBA or INA; cf., cases 1 versus 3 and 2 versus 4.

Cationic micelles are critical for efficient cleavages of anionic **3** by anionic IBA or INA. With the neutral micellar alkylpolyether surfactant Brij-35, cleavage rates are significantly depressed (case 5). In the absence of micelles (case 6), IBA and INA are 1-2 orders of magnitude less reactive toward **3** than they are in cationic micelles of (CTA)Cl or 12-2-12 2Cl. The pH 9 hydroxide-mediated cleavage of phosphomonoester **7**, occurring with $k \sim 0.50$–0.65×10^{-4} s^{-1} in cationic micellar media (cases 1–4), is ~5–6 times slower in Brij-35 micelles (case 5), and is absent when micelles are not present (case 6).



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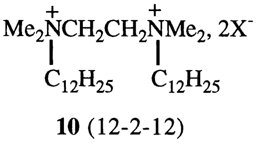

$$\mathrm{Me_2\underset{C_{12}H_{25}}{\overset{+}{N}}CH_2CH_2\underset{C_{12}H_{25}}{\overset{+}{N}}Me_2,\ 2X^-}$$

10 (12-2-12)

In conclusion, IBA and INA, known for their reactivity toward neutral phosphotriester substrates,[5] can also cleave anionic phosphodiesters (e.g. **3** or **1**[6]) in cationic micellar media. The most rapid hydrolysis, observed for substrate **3** with these reagents, involves excess INA in gemini surfactant micelles of 12-2-12 2Cl at pH 9, where $k_3 = 6.1\times10^{-4}$ s^{-1}. Under these conditions, the product phosphate monoester **7** is further hydrolyzed to naphthalene diol **8** by hydroxide ion with $k_7 = 0.54\times10^{-4}$ s^{-1}.

Acknowledgements

We thank Professor Larry Romsted for a helpful discussion and Professor Gene Hall for the X-ray fluorescence analysis of 12-2-12 2Cl. We are grateful to the US Army Research Office for financial support.

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14. Relative to external 85% phosphoric acid, the relevant ^{31}P NMR chemical shifts are **3** ($\delta = -14.5$), **7** ($\delta = -5.0$), and P_i ($\delta = 0.1$). In the case of nucleophile **5** (see Table 1, note *e*), [**5**] = 33 mM. The internal standard, methyl methylphosphonate, does not hydrolyze under the reaction conditions.
15. Conditions: [**3**] = 0.5 mM, [(CTA)Cl] = 10 mM, [CHES] = 20 mM, [IBA] = 5.0–0.1 mM, pH 9.0, 25°C.
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