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An unusually reactive phosphodiester

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Abstract

4-Nitro-1,8-naphthyl phosphate (**4**, NNP) is 2–3 orders of magnitude more reactive to basic and metal cation-mediated hydrolysis than its acyclic analogue (**7**); origins of the rate enhancements are discussed.

Keywords: phosphoric acid diesters; kinetics; catalysis; metal cations.

Phosphodiesters are famously resistant to hydrolysis: for example, dimethyl phosphate (DMP) hydrolyzes with Me–O scission and $k=1.6\times10^{-13}s^{-1}$ ($t_{\frac{1}{2}}\sim137\,000$ years) at pH 7, 25°C.[1] P–O cleavage of DMP is even slower, with $k\leq10^{-15}s^{-1}$. Accordingly, bis(*p*-nitrophenyl) phosphate **1** (BNPP), with its more reactive *p*-nitrophenylate leaving groups, has become the most commonly employed substrate to gauge the potency of phosphodiester-lysing reagents and enzyme models.[2] We have used BNPP to study phosphodiester hydrolyses mediated by Ce^{4+},[3] Th^{4+},[4] and Zr^{4+}.[5] If $k=1.1\times10^{-11}s^{-1}$ for the pH 7, 25°C (presumably P–O)[2m] hydrolysis of BNPP,[2d] then the 2 *p*-nitrophenyl for methyl substitutions that 'convert' DMP into BNPP are worth ~2 orders of magnitude in actual hydrolytic reactivity at neutrality, or ≥4 orders of magnitude comparing only P–O cleavages.

Recently, we reported that the reactivity toward a variety of nucleophiles of the commonly employed phosphotriester substrate, diphenyl *p*-nitrophenyl phosphate (**2**, PNPDPP),[6] was enhanced 40–300 times by 'mutation' to the cyclic analogue, *p*-nitrophenyl 1,8-naphthyl phosphate (**3**, PNPNP).[7] A similar diphenyl→naphthyl fusion (with removal of a nitro group) transforms BNPP into 4-nitro-1,8-naphthyl phosphate (**4**, NNP). Will this parallel structural change elicit similar activation in the intrinsically unreactive phosphodiester series? And, if it does, what are the origins of the enhanced reactivity?

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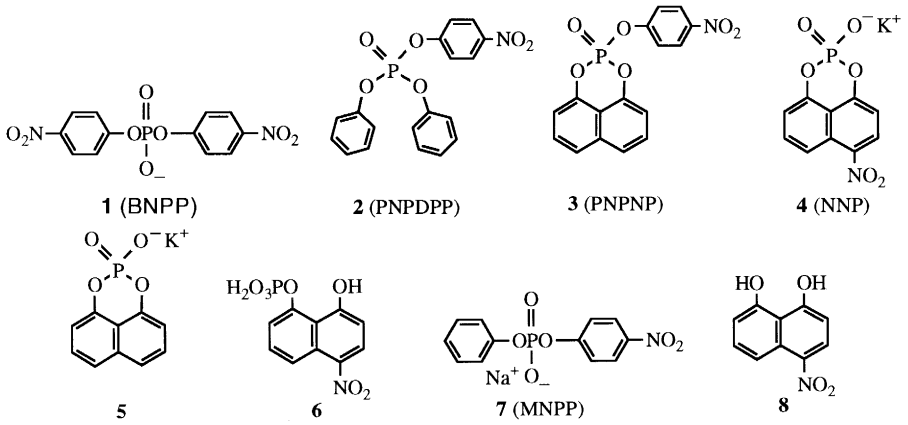

NNP was prepared from 1,8-naphthalene diol[8] by phosphorylation to **5** ($POCl_3$, Et_3N, Et_2O; then H_2O, $KHCO_3$, MeCN, 65% yield), followed by mononitration to **4** (1.3 equiv. of $NO_2{}^+BF_4{}^-$ in MeCN, 4°C, 90 min). The potassium salt of NNP was purified by sevenfold recrystallization from ethanol (7.5% yield), and characterized by IR, NMR, and elemental analysis (C, H, N, P).[9a] We prepared the partial cleavage product of NNP, **6**, by controlled hydrolysis of **4** (aq. KOH, pH 13, 37°C, 12 h), followed by neutralization, and acidification to precipitate **6** in 40% yield. Finally, **7** (MNPP), which is both the mononitro analogue of BNPP and the acyclic analogue of NNP, was prepared by reaction of (1:1) *p*-nitrophenyl phosphorodichloridate and phenol (Et_3N, Et_2O, 25°C, 24 h; then aqueous $NaHCO_3$, MeCN), followed by crystallization from warm ethanol (72% yield). Both **6** and **7** were characterized by NMR and elemental analysis.

The basic hydrolysis of NNP was first examined at pH 13 and 45°C, following the appearance of **6** at 480 nm.[10] k_{hydrol} for NNP was 7.5×10^{-4} s^{-1}, which is 160 times larger than k_{hydrol} (BNPP) [4.7×10^{-6} s^{-1}],[11] and 1700 times larger than k_{hydrol} (MNPP) [4.4×10^{-7} s^{-1}).[11] The basic hydrolysis of NNP at pH 13 is thus 1700 times faster than that of its acyclic relative, **7** (MNPP).

Metal cation mediated hydrolyses of NNP were studied with Eu^{3+}, Th^{4+} and Zr^{4+}. Rate constants were determined for the NNP→**6** cleavage and for the (slower) **6**→4-nitro-1,8-naphthalene diol (**8**) cleavage. NNP hydrolysis was monitored by following the decreases in NNP absorptions at 259 and 383 nm; while hydrolysis of **6** was tracked by observing the appearance of absorptions at 476 nm (Eu^{3+}), 480 nm (Zr^{4+}), or 475 nm (Th^{4+}) due to formation of the metal complexes of **8**,[9b] the hydrolysis product of **6**. These complexes were separately prepared for spectroscopic characterization. Rate constants were reproducible to ±10%, and all reactions were followed for more than eight half-lives. For reactions with k>0.05 s^{-1}, stopped-flow methods were employed. Rate constants and reaction conditions appear in Table 1; note the use of the micellar polyoxyethylene Brij 35 surfactant with the Eu^{3+} and Th^{4+} cations.

NNP is 1700 times more reactive toward OH^- at pH 13 than acyclic analogue MNPP, whereas the metal cation mediated hydrolyses of NNP at pH ≤7 are ~50–100 times more rapid (Table 1). Clearly, the 'fusion' of BNPP to NNP substantially enhances phosphodiester reactivity, just as the parallel structural change does in the phosphotriester series.[7] What are the origins of these accelerations? We believe that several factors are at work.

Westheimer cited the ring strain in cyclic phosphodiesters, particularly the five-membered ethylene phosphate (endocyclic O–P–O angle, ~98°), as a major factor in its very rapid base-catalyzed hydrolysis, which is ~10^7 times faster in 1N NaOH than the hydrolysis of acyclic DMP (O–P–O angle, 104.8°).[12,13] In contrast, the six-membered cyclic phosphate, trimethylene phosphate (O–P–O angle, ~104.6°) is essentially strain-free and only 7.5 times more reactive than DMP.[12a] An X-ray crystal structure of **5**[14]

Table 1
Metal cation mediated hydrolyses of phosphodiesters[a]

Cation	pH	$10^3 k_{hydrol}$ (s^{-1}) for substrate				k_4/k_6	k_4/k_7
		4	**6**	**7**	**1**		
Eu^{3+}	7.0	12.	3.0	0.14	0.16	4.0	86
Zr^{4+}	3.5[b]	170	11.	3.3	8.1[c]	15.	52
Th^{4+}	6.0	320	12.	3.0	28.[d]	27.	107

[a]Conditions: [Brij 35] = 2 mM; [cation] = 1 mM; [HEPES] = 10 mM; [KCl] = 10 mM; [substrate] = 0.05 mM; 37 °C. [b]No Brij, buffer, or KCl. [c]From ref. 5. [d]From ref. 4.

gives the endocyclic O–P–O angle as 100.4°, significantly strained relative to either DMP or trimethylene phosphate. If the O–P–O angle of NNP (**4**) is similar to that of **5**, then ground state strain will contribute to NNP's high reactivity vs **7** in basic hydrolysis. Additionally, as we noted for phosphotriester **3** (unstrained, with O–P–O=105.8°),[7] the P center is ideally suited for nucleophilic attack because the aromatic rings are 'tied back' into the naphthyl unit.[7] This feature, carried over to NNP, should contribute to its kinetic advantage over acyclic **7**.

Similar factors underlie the enhanced reactivity of NNP toward metal cations. In these reactions, the cations bind the substrate's $P–O^-$, lessen the negative charge, and simultaneously provide a metal-bound OH nucleophile to attack the P atom.[2–5] The structure based enhanced reactivity of NNP operates in both the metal-mediated and basic hydrolyses. Additionally, we expect a small contribution of micellar binding in the Eu^{3+} and Th^{4+} cleavages, where Brij micelles are used.[4]

Recently, the correlation of enhanced phosphorolytic reactivity with ground state 'angle strain'[12] has been questioned. Karplus presented computational evidence that much of the observed rate acceleration of the five-membered cyclic phosphodiester stemmed from preferential solvation of its hydrolysis transition state.[15] A similar proposal was also offered in the phosphotriester series.[16] Regardless of the ultimate weightings assigned to the ring strain or preferential solvent explanations, the enhanced reactivity of NNP vs MNPP (or BNPP) remains. NNP is an attractive, sensitive phosphodiester that should prove useful in the quantitative evaluation of new phosphorolytic reagents.

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9. (a) IR; 1516, 1328 cm^{-1} (NO_2). NMR (400 MHz, D_2O, δ): 7.09 (d, J=8.0 Hz, H_7), 7.18 (d, J=8.0 Hz, H_2); 7.70 (t, J=8.0 Hz, H_6); 8.28 (d, J=8.0 Hz, H_5); 8.36 (d, J=8.0 Hz, H_3). The 2 low-field protons, H_3 and H_5, rule out the 2-nitro-1,8-naphthyl phosphate isomer, for which only 1 low-field proton would be expected. (b) Product **8** was prepared by exhaustive hydrolysis of **4**. NMR (dipotassium salt, 400 MHz, D_2O, δ): 6.40 (d, J=8.0 Hz, H_7); 6.41 (d, J=8.0 Hz, H_2); 6.81 (d, J=8.0 Hz, H_5); 7.23 (t, J=8.0 Hz, H_6); 7.68 (d, J=8.0 Hz, H_3).
10. At pH 13, the hydrolysis of **4** to **6** is faster than the subsequent hydrolysis of **6** to 4-nitro-1,8-naphthalene diol; hydrolysis of **4** at pH 13 for 36 h affords **6** after acidification.
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