Chemoselectivity in Metal Cation Mediated Hydrolysis of a Phosphonoformate Diester

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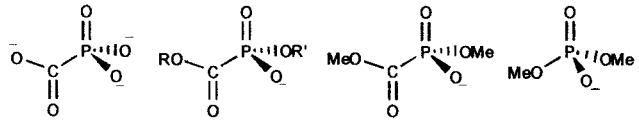
The phosphonoformate trianion, "foscarnet" (PFA, **1**), is an antiviral agent active against herpes simplex virus (HSV) and AIDS-related human cytomegalovirus.[1] However, PFA is trianionic at physiological pH,[2] resulting in poor membrane permeability and low bioavailability. Therefore, PFA mono-, di-, and triesters are of current interest as PFA prodrugs.[3−5]



1 (PFA) **2** **3** (DMPF) **4** (DMP)

Monoanionic PFA diesters (**2**) display anti-viral activity in PFA prodrug studies,[3,6] and have exhibited catalytically selective *C*-ester cleavage reactions with aminocyclodextrins.[4b] The parent PFA diester, dimethyl phosphonoformate (**3**, DMPF), bears some resemblance to dimethyl phosphate (**4**, DMP). Although DMP is notoriously resistant to hydrolysis,[7] enormous rate enhancements are obtainable in metal cation mediated hydrolyses. For example, Ce(IV),[8] Co(III)-cyclen,[9] and Cp_2MoCl_2[10] provide 10^8-10^{11} hydrolytic rate accelerations. Other highly charged metal cations such as Zr(IV), Hf(IV), and Th(IV), while not very reactive toward DMP, greatly speed the hydrolyses of more reactive phosphodiesters.[8c,11−13] Might we also expect accelerations in the metal cation mediated hydrolysis of DMPF?

Simple phosphonoformate diesters are relatively stable toward neutral or mildly acidic hydrolysis; e.g., hydrolysis of **2** (R = R′ = Et) at pH 7.0 proceeds with $k = 5.8 \times 10^{-8}$ s^{-1},[4b] although basic hydrolysis occurs rapidly at C−O.[4d] Here, we describe the first metal-mediated hydrolyses of phosphonoformates, as well as rate accelerations in the acidic hydrolyses of DMPF mediated by Zr(IV), Hf(IV), Th(IV), and Ce(IV) cations. Remarkably, we observe unprecedented chemoselectivity in these reactions.

Reactions were initiated by combining 10 mM DMPF[14,15] with 25 mM $ZrCl_4$, $HfCl_4$, $Ce(NH_4)_2(NO_3)_6$, or $Th(NO_3)_4{\cdot}4H_2O$ at 25 °C in D_2O (0.5 M $NaClO_4$) that contained pyrazine as a ^{1}H NMR integration standard. Solution pD values were established by the *natural* hydrolysis of the metal cations: Zr(IV), 1.7; Hf(IV) 2.2; Ce(IV), 1.9; Th(IV) 3.1. The reactions were monitored by ^{1}H and ^{31}P NMR, with products identified by NMR spiking experiments using authentic materials.[15]

With Zr(IV) and Hf(IV), DMPF hydrolyses proceeded mainly with P−OMe cleavage to *C*-monoester **5** (Scheme 1, branch A). Rather less C−OMe cleavage to *P*-monoester **6** occurred (branch *B*). The **5**:**6** product distributions were 79:21 from Zr(IV) and 90:10 from Hf(IV), after 15, 30, or 60 min of reaction.[16] The reaction kinetics were readily followed by ^{1}H NMR, monitoring the growth of singlets for MeOD at δ 3.33 and **5** at δ 3.79 (characteristically, no CH_3O−P coupling was observed for *C*-ester **5**). Rate constants and product distributions appear in Table 1.

Quite different behavior is observed in reactions of DMPF with Th(IV) or Ce(IV). Th(IV) affords only C−OMe scission to *P*-monoester **6** (which is stable to Th(IV) for 5 days) (Scheme 1, branch *B*). In the ^{1}H NMR spectrum, MeOD and **6** (δ 3.74, d, J_{P-H} =11.7 Hz) grow in; P−OMe cleavage to **5** is not competitive. Ce(IV) cleavage of DMPF begins similarly with C−OMe scission; *P*-monoester **6** is formed transiently, along with MeOD.[17] About 10% of *C*-monoester **5** is also produced by competitive P−OMe cleavage. Monoester **6**, however, is unstable to Ce(IV), which subsequently converts it to methyl phosphate **7** (^{31}P δ 3.07, q, J_{P-H} = 10.8 Hz) by oxidative decarboxylation and C−P cleavage. Rate constants and product distributions appear in Table 1.

With Zr(IV) or Hf(IV), *P*-monoester **6** is decarboxylatively cleaved to methyl *D*-phosphonate **8** (^{31}P δ 9.03, t, J_{P-D} = 97 Hz).[18] Rate constants for **8** → **9** (measured by MeOD release) were 2.4×10^{-4} or 2.1×10^{-4} s^{-1} with Zr(IV) or Hf(IV), respectively; P−OMe cleavages of **8** by these metal cations occurred at about half the P−OMe cleavage rate of DMPF.

C-monoester **5** hydrolyzed very slowly with Zr(IV) or Hf(IV), affording only PFA (**1**) (^{31}P δ 4.2) after ∼3 days. With Ce(IV), **5** was converted to phosphate ion (^{31}P δ 2.1) by net hydrolysis and oxidative decarboxylation. Finally, although Th(IV)-mediated hydrolysis of DMPF does not yield **5**, this monoester does slowly release MeOD upon reaction with Th(IV) in D_2O. ^{31}P NMR spectroscopy in the presence of tartrate reveals the formation of PFA (**1**) from this reaction.

Kinetics data are summarized in Table 1, and products are displayed in Scheme 1. As a basis for comparison, the acid-

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(14) NaDMPF was prepared by the NaI demethylation of commercial trimethyl phosphonoformate (refluxing acetone, 2 h): mp 179−181 °C. Anal. (C, H). ^{1}H NMR (δ, D_2O) 3.71 (s, 3H), 3.58 (d, J_{P-H} = 11.1 Hz, 3H). ^{31}P NMR (δ, D_2O) −2.6 (s, ^{1}H-decoupled).

(15) For ^{31}P NMR studies, M(IV) was first "removed" by chelation with EDTA to prevent line broadening. Reaction aliquots were then examined at various time intervals. The ^{31}P NMR chemical shifts varied slightly in the presence of the different metal cations.

(16) (a) ^{31}P NMR chemical shifts (δ, D_2O, ^{1}H-decoupled): **5**, −5.2; **6**, 6.9; **3**, −2.5. (b) A labeling experiment conducted in 47% $^{18}O-H_2O$ with DMPF (P−$O^{13}CH_3$)) and Hf(IV), monitored by ^{31}P and ^{13}C NMR,[8b] gave **5** with stoichiometric ^{18}O-incorporation and $^{13}CH_3{}^{16}OH$ (only); i.e., P−O−Me cleavage by Hf(IV) and (presumably) Zr(IV) occurred only by P−O scission.

(17) Precipitation occurred after ∼1000 s; the rate constant is therefore estimated from the initial rate.

(18) About 10% of PFA (**1**) also forms from **6**.

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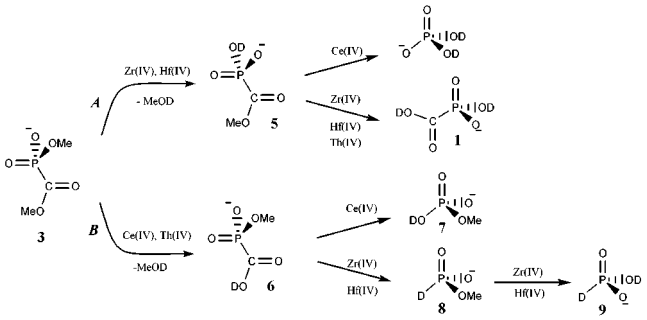
Scheme 1[a]



[a] Species are depicted in anticipated ionization states in D_2O at experimental pD's.

Table 1. Kinetic Data for Phosphonoformate Ester Cleavages[a]

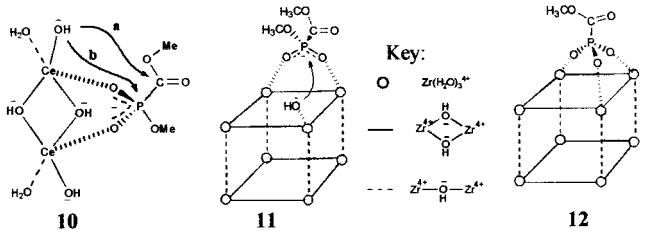
	$10^4\ k_{obs}$ (s^{-1})[b]			product distributions for cleavage of **3** (%)[c]	
M(IV)	DMPF (**3**)	**5**	$k_{M(IV)}/k_D{}^{+}$ [d]	**5**	**6**
Zr	4.4	0.022	3300	79	21
Hf	4.0	0.026	3100	90	10
Th	1.3	0.42	980		>95
Ce	5.2[e]	0.93[e]	3900	10	90

[a] Conditions: [M(IV)] = 25 mM, [substrate] = 10 mM, 0.5 M $NaClO_4$ in D_2O, 25 °C, pD 1.7 (Zr), 2.2 (Hf), 3.1 (Th), 1.9 (Ce). [b] Monitored by ^1H NMR integration of released MeOD relative to an internal pyrazine standard. Errors of duplicate runs, ± 8% (maximum). [c] From ^{31}P NMR spectra of aliquots quenched at 15, 30, or 60 min with EDTA; errors ±2%. [d] $k_D{}^{+} = 1.3 \times 10^{-7}\ s^{-1}$ for the acid-catalyzed cleavage of **3** at pD 1.7−2.2; see text. [e] See ref 17.

catalyzed decomposition of DMPF with DCl at pD 1.7, 1.9, or 2.2, in the absence of M(IV), afforded MeOD and methyl *D*-phosphonate **8** with $k_{obs} = 1.3(\pm 0.08) \times 10^{-7}\ s^{-1}$. From Table 1, we note accelerations of ~1000−4000 for M(IV) vs D^+ hydrolyses of DMPF at pD ~ 2.[19]

Most dramatically, Zr(IV) and Hf(IV) are chemoselective for the P−OMe cleavage of DMPF, while Ce(IV) and Th(IV) are chemoselective for C−OMe cleavage. This unprecedented motif continues in cleavages of monoesters **5**, **6**, and **8**. Thus, *P*-monoester **6** is not appreciably cleaved by Th(IV) or Ce(IV). On the other hand, *C*-monester **5** is cleaved 16−42 times more rapidly by Th(IV) or Ce(IV) than by Zr(IV) or Hf(IV).

How does this chemoselectivity operate? At pD 1.7−3.1, DMPF is probably present in aqueous solutions as the P−O^- monoanion.[2] The chemoselectivity of DMPF cleavage, however, must depend on which M-OH species are present under the specific reaction conditions and the particular mode of DMPF binding in each case. Using the equilibria given for the hydrated Ce(IV) species by Baes and Mesmer,[20] and the species distribution program of Martell and Motekaitis,[21] we calculate that Ce(IV) should be mainly dimeric, with lesser amounts of hexameric species, under our experimental conditions (25 mM, pD 1.9). This accords with Komiyama's assignment of a dimeric Ce(IV)-hydroxo species $[Ce^{IV}_2(OH)_4]^{4+}$ as the reactive entity in cAMP cleavage in acidic Ce(IV) solutions.[22] Th(IV) is similarly calculated[20,21] to be either monomeric or dimeric at pD 3; a dimeric species is implicated by X-ray scattering results in solution.[20,23] We thus represent[8b,22] Ce(IV) cleavage of DMPF by **10**, where intracomplex hydroxide attack at the carbonyl center (a) occurs via a five-membered transition state, whereas attack at phosphorus (b) requires a more strained 4-centered transition state. We therefore suggest that C−O cleavage is kinetically preferred to P−O scission in this case. By implication, other dimeric or monomeric metal complexes (cf., Th(IV)) should also be C−O selective. Indeed, we find that 10 mM DMPF is hydrolyzed at pD 6.8 by 25 mM Co(III)cyclen[9] ($k_{obs} = 9.5 \times 10^{-4}\ s^{-1}$) to yield only **6** by C−OMe cleavage.



For Zr(IV), the appropriate equilibria,[20,24a] coupled with the Martell algorithm,[21] predict Zr(IV) octamers as the dominant species at pD 1.7. These octamers are most readily represented as stacked hydroxyl bridged tetramers.[24a] Such tetramers also occur as the unit cell in the crystal structure of $ZrOCl_2 \cdot 8H_2O$.[25] Binding of DMPF to the Zr(IV) octamer can be represented as in **11**, where intracomplex OH^- cleavage can now readily occur at phosphorus via a six-membered transition state. The tripodal phosphonate product, **12**, reproduces the binding scheme of the lamellar zirconium phosphonates.[26] Cleavage of DMPF by Hf(IV) presumably follows an analogous P−O selective course.

This mechanism implies that alteration of the Zr(IV) octamers should impact the expressed P−O chemoselectivity. Indeed, addition of 1 or 2 equiv of tris(2-hydroxymethyl)aminomethane (Tris) to the Zr reaction mixture of Table 1 changes the **5**/**6** product distribution from ~80:20 to 40:60 or 15:85, respectively; the P−O chemoselectivity shifts to C−O selectivity. Tris is reported to form 1:1 complexes with Zr(IV) that are able to cleave bis(*p*-nitrophenyl)phosphate in acidic aqueous solution.[11c] With Hf(IV), similar additions of 1 or 2 equiv of Tris change the **5**/**6** distribution from 90:10 (Table 1) to 66:34 or 19:81, respectively.[27] Thus, the P−O selectivity of Zr(IV) and Hf(IV) toward DMPF appears to be a property of M(IV) tetramers or octamers, and can be altered toward C−O selectivity by destruction of these complexes.

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(19) Phosphonoformate diesters (e.g., DMPF) are ~10^5−10^7 times more reactive than phosphodiesters[4b,d] (e.g., DMP), probably because of ground-state destabilization due to unfavorable carbonyl/phosphoryl electronic interactions. Thus, catalytic enhancements of DMPF hydrolysis by metal cations are much compressed (~10^3) relative to those observed with DMP.

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