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Alkylation of Inorganic Oxo Compounds and Insights on Preventing DNA Damage

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Abstract: Metabolism of food- and tobacco-borne procarcinogens results in the exposure of DNA to toxic alkylating agents. These assaults can bring about DNA alkylation damage, mutations, and cancer. Dietary inorganic compounds such as selenium and vanadium are known to prevent cancer, possibly by reacting directly with alkylating agents, thereby preventing DNA damage. To understand potential interactions between oxo species and alkylating toxins, we reacted a series of alkylating agents with varied classes of oxo compounds (i.e., vanadates, selenate, phosphate, sulfate, acetate, nitrate, and nitrite). A new organic-soluble selenate, $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$, was synthesized and characterized for these studies. Vanadates were found to convert ethylating agents into ethanol, whereas other anions formed esters upon alkylation. General trends show that oxo anions of the greatest charge density were the most reactive. These studies suggest that the design of new compounds for cancer prevention should incorporate reactive oxo groups with high anionic charge density.

Introduction

Humans are subjected to a daily onslaught of numerous chemical and physical agents that damage DNA. Alkylation damage is especially prevalent since alkylating chemicals or precursors are pervasive in cooked food[1] and tobacco smoke.[2] The toxic nature of nitrosamines and polycyclic aromatic hydrocarbons (PAHs) derives from their metabolism into deleterious alkylating agents.[3,4] For example, the enzymatic oxidation and subsequent decomposition of diethylnitrosamine yield the potent alkylating toxin ethyldiazonium ($^+N_2C_2H_5$).[5] Once formed, such alkylating agents react with nucleophilic sites on DNA.[3] Upon replication, these alkylated bases mispair, thereby bringing about DNA mutations and the subsequent carcinogenesis.[6]

In the ongoing search to prevent cancer, researchers have found promising results concerning the chemoprotective effects of dietary selenium and vanadium. Selenium, an essential trace nutrient, has more potent cancer-preventing properties than any other normal component of the human diet.[7] Many studies in the United States have shown an inverse relationship between the levels of dietary selenium uptake and cancer mortality.[8−10] The Selenium and Vitamin E Chemoprevention Trial (SELECT)[11] is an ongoing clinical trial involving over 30 000 men, investigating the efficacy of selenium and vitamin E, either alone or in combination, to prevent prostate cancer.

Although it does not have the same high profile as selenium, vanadium also exhibits significant potential to be a chemopreventive agent.[12] Rats provided with $VOSO_4$-supplemented feed prior to injection with *N*-methyl-*N*-nitrosourea (MNU) exhibited fewer tumors and reduced cancer incidence and mortality.[13] Other forms of vanadium (e.g., $NaVO_3$ and Na_3VO_4) have been used as dietary supplements and have shown effectiveness against chemically induced cancers from 7,12-dimethylbenzo-[*a*]anthracene (DMBA) as well as diethylnitrosamine.[14−16]

Despite these intriguing reports, the mechanism of cancer prevention by selenium and vanadium remains unknown. Although no significant data exist, various mechanisms have been proposed to explain the chemopreventive effects of selenium and vanadium, including alteration of peroxidase activities,[14,17] induction of detoxifying enzymes,[18,19] enhanced antioxidant levels,[7] and induction of apoptosis.[7,12,18,20] Many studies suggest that the protective benefits of selenium and

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vanadium against alkylating carcinogens are due to minimized DNA damage. Experiments in which rats were injected with the alkylating carcinogen DMBA revealed fewer DMBA−DNA adducts when the rats were given diets supplemented with Na_2SeO_3.[21,22] Sodium selenite-supplemented diets fed to rats also minimized DNA strand breaks, which are indicators of alkylated DNA.[23] Addition of selenium, copper, and zinc salts to the growth medium of Chinese hamster ovary cells enhanced cell survival upon exposure to alkylating agents.[24−26] Rats provided with NH_4VO_3-supplemented diets and then exposed to diethylnitrosamine were found to have fewer DNA strand breaks[27] and chromosomal aberrations.[28]

We have set out to understand the molecular basis of cancer prevention by selenium and vanadium. The aqueous chemistries of selenium[29] and vanadium[30,31] are dominated by oxo anions (e.g., $(SeO_4)^{2-}$, $(H_2VO_4)^-$, and $(V_4O_{12})^{4-}$), which may be nucleophilic. Perhaps these oxo anions act as nucleophiles toward the electrophilic alkylating agents, thereby consuming the toxin and preventing DNA damage. To investigate this "carcinogen interception" mechanism, we assayed inorganic salts for the ability to inhibit alkylation of bacterial DNA and found that only salts forming oxo anions in aqueous solution were able to protect DNA.[32,33] Vanadates, in particular, demonstrated the highest protective properties.

With the knowledge that oxo anions, and not simple salts, conferred protection to DNA, we investigated a limited collection of model reactions between inorganic oxo compounds and alkylating agents. We carried out model studies in organic solvents. Use of an organic solvent such as acetonitrile minimizes hydrolysis of alkylating agents. Additionally, performing these model reactions in acetonitrile simplifies the solution speciation of metal oxo compounds. For example, dissolution of Na_3VO_4 in water results in at least four different vanadate species.[30,31] In acetonitrile, however, we demonstrated the ability to work with one discrete vanadate anion.[34] Initial findings from these model reactions demonstrated that vanadates react and can convert alkylating agents to alcohols.[32]

To understand potential interactions between oxo species and alkylating carcinogens, we carried out the detailed study presented here. The results described below show that trends in alkylation susceptibility exist. We find that vanadates detoxify alkylating agents into alcohols, whereas the other oxo anions investigated form esters of the initial anion. The basic inorganic reactions and principles discussed below are presented in an effort to both understand the known beneficial properties of inorganics and aid in the design of new compounds for the future prevention of cancer.

Experimental Section

General Procedures. The solvent CD_3CN was dried, distilled according to standard procedures,[35] and stored under argon. The vanadates $[(C_4H_9)_4N]_3(V_3O_9)\cdot 0.5H_2O$,[34] $[(C_4H_9)_4N]_3(HV_4O_{12})\cdot CH_2Cl_2$,[34] $[(C_4H_9)_4N]_3(V_5O_{14})$,[36] $[(C_4H_9)_4N]_4(V_{10}O_{26})$,[37] and $[(C_4H_9)_4N]_3(H_3V_{10}O_{28})$[38] were synthesized according to literature procedures.

Alkylation Reactions. To make direct comparisons with nitrosamine metabolism, ethyl diazonium would be the ideal alkylating agent, but the potentially explosive nature of this compound makes its use impractical. Instead, we selected the direct-acting $(CH_3CH_2O)_2SO_2$, which is a classic alkylating agent used in mutagenicity and carcinogenicity studies[6] and may be present in human diets.[39] We also examined the following alkylating agents with the expected order of relative reactivity: $CH_3CH_2OSO_2CF_3 > (CH_3CH_2O)_2SO_2 > CH_3CH_2I > CH_3CH_2Br$, where ethyl trifluoromethanesulfonate is the most reactive and ethyl bromide is the least.[40]

Reactions were carried out in 1 mL volumetric flasks that were oven-dried, flame-dried, and flushed with argon prior to use. Typical reaction conditions included a 1:1 ratio of inorganic compound:alkylating agent at a concentration of 50 or 200 mM each, depending on availability and solubility of the compound. For example, $(CH_3CH_2O)_2SO_2$ (6.5 μL, 0.050 mmol) was added to a stirred solution of $[(C_4H_9)_4N]_3(V_3O_9)\cdot 0.5H_2O$ (0.051 g, 0.050 mmol) in ~0.5 mL of CD_3CN. The reaction solution was kept under a stream of argon as the total volume was brought to 1 mL with CD_3CN. After complete mixing, the solution was transferred to an argon-flushed NMR tube. Reactions with *N*-ethyl-*N*-nitrosourea (ENU, $ONN(CONH_2)(CH_2CH_3)$) used a stock solution of ENU in CD_3CN and not the neat compound.

NMR Spectroscopy. NMR spectra were obtained at 25 ± 1 °C using a Varian INOVA 300 spectrometer at 300 MHz for ^{1}H, 121 MHz without ^{1}H decoupling for ^{31}P, 78.9 MHz with ^{1}H decoupling for ^{51}V, and 57.3 MHz with and without ^{1}H decoupling for ^{77}Se. For all non-proton NMR spectroscopies, chemical shift values were referenced to the following external standards: 85% H_3PO_4 ($\delta = 0$ ppm) for ^{31}P, neat $VOCl_3$ ($\delta = 0$ ppm) for ^{51}V, and neat $(CH_3)_2Se$ ($\delta = 0$ ppm) for ^{77}Se. In practice, we used a saturated aqueous solution of H_2SeO_3 ($\delta = 1300$ ppm) for ^{77}Se NMR experiments.[41] Typical ^{51}V NMR spectral parameters included 256 transients, 0.65 s acquisition time, 70 kHz spectral width, 13 μs pulse width, and 35 Hz line broadening. Typical ^{77}Se NMR spectral parameters included 17 000 transients, 0.16 s acquisition time, 17 kHz spectral width, 3.3 μs pulse width, and 10 Hz line broadening.

Synthesis of $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$. Silver selenate, Ag_2SeO_4 (1.81 g, 5.05 mmol), was added to a stirred solution of $[(C_6H_5)_4P]Br$ (4.22 g, 10.1 mmol) in dichloromethane (32 mL). The suspension was stirred in darkness for 4 h, after which time a green-yellow precipitate was removed by vacuum filtration through a pad of Celite. Solvent was removed by rotary evaporation to yield a white solid (4.00 g). Suitable crystals were obtained when the crude product was dissolved in acetonitrile, followed by slow vapor diffusion of acetone. Colorless plates were analyzed by single-crystal X-ray

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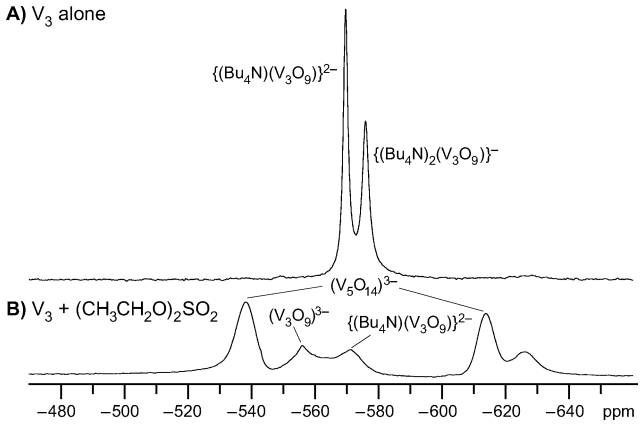

Figure 1. ^{51}V NMR spectra of 50 mM $[(C_4H_9)_4N]_3(V_3O_9)$, "V_3", in CD_3CN, (A) alone and (B) with 1 equiv of $(CH_3CH_2O)_2SO_2$ after 2.5 h.

diffraction methods and found to be $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$. The bridged dinuclear anion was disordered. Anal. Calcd for $C_{73}H_{63}O_{12}P_3Se_3$: C, 59.97; H, 4.34; N, 0. Found: C, 60.27; H, 4.35; N, not found. ^{1}H NMR (CD_3CN): δ 5.54 (s, 2H), 7.6−8.1 (m, 60H).

X-ray Crystal Structure. Single-crystal X-ray diffraction data were acquired through Purdue University in-house facilities. The experimental procedures for the X-ray diffraction studies can be found in the Supporting Information. Crystal data for $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$: colorless plate, space group $P\bar{1}$ (No. 2), $a = 11.2388(6)$ Å, $b = 14.2154(10)$ Å, $c = 22.1303(11)$ Å, $V = 3199.8(3)$ Å^3, $Z = 2$, $T = 150$ K, $R(F_o) = 0.068$, GOF = 1.057.

Results and Discussion

1. Reactions between $[(C_4H_9)_4N]_3(V_3O_9)\cdot 0.5H_2O$ and $(CH_3CH_2O)_2SO_2$. Our earlier biochemical studies demonstrated that vanadates were particularly effective at protecting plasmid DNA from an alkylation assault. These initial studies with DNA were performed in aqueous solutions at neutral pH, meaning that multiple vanadate species were present (e.g., $H_2VO_4^-$, $H_2V_2O_7^{2-}$, $V_4O_{12}^{4-}$, and $V_5O_{15}^{5-}$).[30,31] Any one or all of these species may have reacted with the alkylating agent to prevent DNA damage. Thus, we now focus on model reactions in organic solvents to understand the chemistry behind preventing DNA damage.

^{1}H NMR Spectroscopy. The 1:1 reaction of $[(C_4H_9)_4N]_3(V_3O_9)$ with $(CH_3CH_2O)_2SO_2$ (50 mM each) was approximately 50% complete after 15 min. The NMR spectrum of this reaction (Figure S1, Supporting Information) contained four new product resonances: two triplets at 1.11 and 1.18 ppm and two quartets at 3.53 and 3.84 ppm. The triplet at 1.18 ppm and quartet at 3.84 ppm were attributed to the $[(CH_3CH_2O)SO_3]^-$ anion on the basis of comparison with an authentic sample (sodium ethyl sulfate, $Na[(CH_3CH_2O)SO_3]$). The remaining set of peaks (1.11 ppm, t; 3.53 ppm, q) was assigned to ethanol. This assignment was made by comparison with a genuine sample, addition of ethanol to the reaction solution, and analysis by gas chromatography−mass spectrometry (GC−MS) of the reaction solution. Peaks in the final ^{1}H NMR spectrum remained sharp, an indication that the product was diamagnetic and all vanadium remained in the 5+ oxidation state. No ethanol was observed in a control solution of $(CH_3CH_2O)_2SO_2$ (50 mM) in CD_3CN.

^{51}V NMR Spectroscopy. Figure 1A shows the ^{51}V NMR spectrum for a 50 mM solution of $[(C_4H_9)_4N]_3(V_3O_9)$ in CD_3CN. Our earlier work[34] revealed that the two resonances observed in the spectrum are a result of ion pairing by the vanadate anion: $\{[(C_4H_9)_4N](V_3O_9)\}^{2-}$ at −570 ppm and $\{[(C_4H_9)_4N]_2(V_3O_9)\}^-$ at −576 ppm. Addition of 1 equiv of $(CH_3CH_2O)_2SO_2$ to the colorless vanadate solution caused the solution to become light yellow within 15 min. This color change coincided with the appearance of new resonances in the ^{51}V NMR spectrum. Figure 1B shows a spectrum 2.5 h, or ∼10 half-lives, after the reaction began. The peaks at −538 and −613 ppm match the known vanadate $(V_5O_{14})^{3-}$.[36] The peaks at −556 and −570 ppm can be attributed to the bare $(V_3O_9)^{3-}$ anion and singly ion-paired $\{[(C_4H_9)_4N](V_3O_9)\}^{2-}$, respectively.[34] Although the peak at −626 ppm corresponds to the fully ion-paired $\{[(C_4H_9)_4N]_3(V_3O_9)\}$, this species exists only at high concentrations (>400 mM)[34] and would not be expected to exist under current reaction conditions. Thus, the vanadate species at −626 ppm is likely an unidentified vanadate with a chemical shift coincidental to that of the fully ion-paired $\{[(C_4H_9)_4N]_3(V_3O_9)\}$.

Ethanol Formation. Reactions between $(V_3O_9)^{3-}$ and $(CH_3CH_2O)_2SO_2$ yielded ethanol and $(V_5O_{14})^{3-}$. Ethanol generation may be best explained by considering the nature of the observed vanadate products. According to ^{51}V NMR data, the starting vanadate $(V_3O_9)^{3-}$ was converted to $(V_5O_{14})^{3-}$ upon alkylation. The trinuclear vanadate $(V_3O_9)^{3-}$ can be viewed as a trimer of the metavanadate ion, $(VO_3^-)_3$. The corresponding pentanuclear species $(V_5O_{15})^{5-}$ (i.e., $(VO_3^-)_5$) would be the expected vanadate upon rearrangement. However, the observed pentanuclear species is $(V_5O_{14})^{3-}$, which is one oxygen deficient of the $(V_5O_{15})^{5-}$ analogue. Thus, we propose that an oxygen of the trinuclear vanadate is ethylated and removed as ethoxide, initiating the vanadate rearrangement and ethanol formation. The source of the proton for ethanol formation may be adventitious water in the distilled solvent or water in the crystal lattice.

2. Reactions between $[(C_4H_9)_4N]_3(V_3O_9)\cdot 0.5H_2O$ and $(CH_3CH_2O)_2SO_2$ at Varied Ratios. ^{1}H NMR Spectroscopy. Various equivalents (0.5, 1.5, 2, 3, and 4) of $(CH_3CH_2O)_2SO_2$ were combined with 50 mM $[(C_4H_9)_4N]_3(V_3O_9)$. Just as in the 1:1 reaction (e.g., 50 mM $(CH_3CH_2O)_2SO_2$ and 50 mM $(V_3O_9)^{3-}$), the ethyl-containing products of these reactions were ethanol and $[(CH_3CH_2O)SO_3]^-$. The 1 equiv reaction was complete by ∼12 h, compared to 90 min for 0.5 equiv, 48 h for 1.5 equiv, and 13 d for 2 equiv. The 3 equiv reaction was one-half complete at 13 d, and the 4 equiv reaction was one-third complete at the same time.

In the 3 and 4 equiv reactions, two new multiplets appeared after ∼13 d at ∼2.1 ppm (possible doublet) and ∼3.4 ppm (possible quartet). Overall, peak broadening was also observed, indicating that a paramagnetic product was forming, most likely reduction of vanadium(V) to vanadium(IV), supported by the purple color observed in both final solutions.

^{51}V NMR Spectroscopy. Unlike the 0.5 or 1 equiv reactions, addition of 1.5 equiv of $(CH_3CH_2O)_2SO_2$ to 50 mM $[(C_4H_9)_4N]_3(V_3O_9)$ resulted in complete conversion of trinuclear vanadate to pentanuclear $(V_5O_{14})^{3-}$ (−538 and −613 ppm). By 6 h, three sharp signals grew at −590, −597, and −605 ppm, and the solution became honey yellow, corresponding to the vanadate $(V_{12}O_{32})^{4-}$.[42] These peaks continued to grow until ∼13 d, at which point there was a ∼2:1 ratio of $(V_{12}O_{32})^{4-}$ to $(V_5O_{14})^{3-}$ peaks (Figure 2A). For the 2:1 reaction, $(V_3O_9)^{3-}$ was converted

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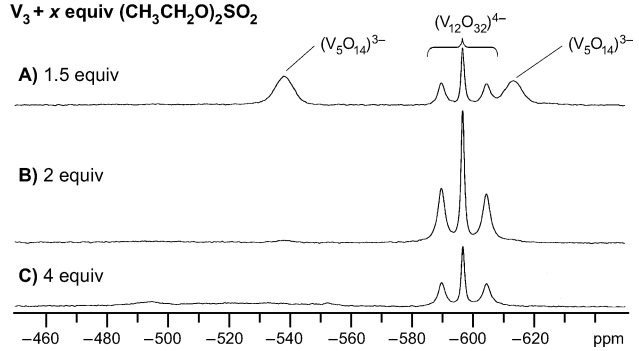

Figure 2. ^{51}V NMR spectra of $[(C_4H_9)_4N]_3(V_3O_9)$, "V_3", + $(CH_3CH_2O)_2SO_2$ in CD_3CN after ∼13 d with varied equivalents of alkylating agent: (A) 1:1.5, (B) 1:2, and (C) 1:4.

completely to $(V_5O_{14})^{3-}$. However, the pentanuclear vanadate was, in turn, converted completely to $(V_{12}O_{32})^{4-}$ (Figure 2B). For the 3:1 reaction at 13 d, the predominant product was $(V_{12}O_{32})^{4-}$, but two small, broad resonances were growing in at −495 and −520 ppm. Similarly, the 4:1 reaction displayed small, broad peaks growing between −490 and −560 ppm (Figure 2C). We could not attribute these signals to a specific compound.

When greater than stoichiometric amounts of $(CH_3CH_2O)_2SO_2$ were reacted with $(V_3O_9)^{3-}$, the final vanadium-containing product was not $(V_5O_{14})^{3-}$ but the higher order vanadate $(V_{12}O_{32})^{4-}$. This final product can also be viewed as oxygen-deficient. For example, the observed $(V_{12}O_{32})^{4-}$ product contains fewer oxygens than the dodecanuclear analogue of metavanadate, $(VO_3^-)_{12}$ or $(V_{12}O_{36})^{12-}$.

3. Reactions between $[(C_4H_9)_4N]_3(V_3O_9)\cdot 0.5H_2O$ and Other Alkylating Agents. The ethyl-containing product for reactions of $(V_3O_9)^{3-}$ with CH_3CH_2Br, CH_3CH_2I, and $CH_3CH_2OSO_2CF_3$ was ethanol. The primary difference among these reactions was time. Half of CH_3CH_2Br was consumed at ∼2.5 h. For CH_3CH_2I the $t_{1/2}$ was ∼30 min, and $CH_3CH_2OSO_2CF_3$ was completely consumed in the time required to prepare the sample (∼10 min). The 1:1 reaction between $(V_3O_9)^{3-}$ and CH_3CH_2Br, CH_3CH_2I, or $CH_3CH_2OSO_2CF_3$ also produced the same results as $(CH_3CH_2O)_2SO_2$ when examined by ^{51}V NMR: partial conversion of trinuclear vanadate to $(V_5O_{14})^{3-}$.

The ethylating agents discussed to this point act through an S_N2 mechanism. To investigate if the nature of nucleophilic substitution affects the interaction between inorganic oxo anions and alkylating agents, we selected an S_N1 alkylating agent, ENU, to react with the trinuclear vanadate $[(C_4H_9)_4]_3(V_3O_9)$. Unlike the S_N2 alkylating agents, ENU is not the actual alkylating species. Instead, it must first decompose into the alkylating species ethyl diazonium, $C_2H_5N_2^+$.[43,44] This decomposition can occur by enzymatic metabolism or by a hydroxide-catalyzed process at physiological pH.

In a 1:1 reaction between $[(C_4H_9)_4]_3(V_3O_9)$ and ENU (50 mM each), ENU was completely consumed in <15 min, the time required to prepare the sample. The resulting ethyl-containing product was ethanol, as determined by ^{1}H NMR spectroscopy (Figure S2). The ^{51}V NMR spectrum (Figure S3) for this reaction was similar but not identical to the spectra for reactions between $(V_3O_9)^{3-}$ and $(CH_3CH_2O)_2SO_2$. The major vanadate product was $(V_5O_{14})^{3-}$ (−540 and −615 ppm), with a small amount of the singly ion-paired $\{[(C_4H_9)_4N](V_3O_9)\}^{2-}$ (−571 ppm) observed. Two new and unidentified resonances were detected at −545 and −568 ppm.

4. Reactions of Larger Vanadates with $(CH_3CH_2O)_2SO_2$. To test the generality of reactions between vanadates and alkylating agents, we reacted a series of vanadates with $(CH_3CH_2O)_2SO_2$. The final vanadium-containing product of each reaction varied, depending upon the nature of the starting vanadate, but ethanol was observed in each case.

Reactivity of $[(C_4H_9)_4N]_3(HV_4O_{12})\cdot CH_2Cl_2$. Addition of 1 equiv of $(CH_3CH_2O)_2SO_2$ to a 50 mM solution of $[(C_4H_9)_4N]_3(HV_4O_{12})$ in CD_3CN resulted in the formation of ethanol and $[(CH_3CH_2O)SO_3]^-$. This reaction progressed slower than the trinuclear vanadate alkylation, with consumption of half of the alkylating agent at ∼5.5 h. The ^{51}V NMR spectrum of a 50 mM solution of $[(C_4H_9)_4N]_3(HV_4O_{12})$ alone in CD_3CN revealed a mixture of vanadates (Figure S4A). Peaks were visible at −538, −555, −570, −614, and −626 ppm. The presence of $(V_5O_{14})^{3-}$ accounted for the resonances at −538 and −614 ppm.[36] The most intense and sharpest peak was at −570 ppm. We attributed this peak to the singly ion-paired species $\{[(C_4H_9)_4N](V_3O_9)\}^{2-}$.[34] The signal for the bare $(V_3O_9)^{3-}$ anion was at −555 ppm. The −626 ppm peak is not yet identified. A 1:1 reaction between $(HV_4O_{12})^{3-}$ and $(CH_3CH_2O)_2SO_2$, observed at 45 min, already had $(V_5O_{14})^{3-}$ (−538 and −614 ppm) as the main product. Other signals were visible at −556, −570, and −626 ppm, approximately equal in intensitiy to each other but much smaller than the $(V_5O_{14})^{3-}$ peaks. These resonances were gone after 5 h, and new signals emerged at −590, −597, and −605 ppm, attributed to $(V_{12}O_{32})^{4-}$.[42] The signals from $(V_{12}O_{32})^{4-}$ continued to grow while the $(V_5O_{14})^{3-}$ peaks decreased. After 16 d, the $(V_{12}O_{32})^{4-}$ peaks were approximately equal in height to the $(V_5O_{14})^{3-}$ peaks (Figure S4B).

Reactivity of $[(C_4H_9)_4N]_3(V_5O_{14})$. When forming ethanol, half of the diethyl sulfate was consumed by $(V_5O_{14})^{3-}$ within ∼7 d. A 50 mM solution of $[(C_4H_9)_4N]_3(V_5O_{14})$ produced a ^{51}V NMR spectrum with two peaks at −538 and −614 ppm, in agreement with literature values (Figure S5A).[36] Fifty minutes after addition of 1 equiv of $(CH_3CH_2O)_2SO_2$ to a 50 mM solution of $[(C_4H_9)_4N]_3(V_5O_{14})$, the −538 and −614 ppm peaks of $(V_5O_{14})^{3-}$ were present, as was the first sign of $(V_{12}O_{32})^{4-}$. By 19 h, the $(V_{12}O_{32})^{4-}$ peaks were roughly equal in height to the $(V_5O_{14})^{3-}$ peaks. The $(V_5O_{14})^{3-}$ peaks decreased dramatically by 16 d, leaving $(V_{12}O_{32})^{4-}$ as the predominant product (Figure S5B).

Comparison of $(V_3O_9)^{3-}$, $(HV_4O_{12})^{3-}$, and $(V_5O_{14})^{3-}$ Reactivities. Alkylation reactions with $(HV_4O_{12})^{3-}$ and $(V_5O_{14})^{3-}$ resembled the reactions of $(V_3O_9)^{3-}$ with excess alkylating agent: tetranuclear $(HV_4O_{12})^{3-}$ converted to $(V_5O_{14})^{3-}$, and then into $(V_{12}O_{32})^{4-}$. The starting pentanuclear $(V_5O_{14})^{3-}$ converted directly to $(V_{12}O_{32})^{4-}$. Results from alkylation reactions of $(HV_4O_{12})^{3-}$ and $(V_5O_{14})^{3-}$ provided insight on the $(V_3O_9)^{3-}$ reactions with excess alkylating agent. Once $(V_3O_9)^{3-}$ was consumed, the excess alkylating agent continued to react with the larger oligomers that formed.

This trend explains how reactions of $(V_3O_9)^{3-}$ with increasing equivalents of $(CH_3CH_2O)_2SO_2$ did not follow an expected trend of more alkylating agent providing faster reactions. When the

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equivalents of $(CH_3CH_2O)_2SO_2$ relative to $(V_3O_9)^{3-}$ (held at 50 mM) were varied, we found the following trend in the time required to consume half of the alkylating agent: 0.5 equiv of $(CH_3CH_2O)_2SO_2$ > 1 equiv > 1.5 equiv > 2 equiv > 3 equiv > 4 equiv. As shown by our ^{51}V NMR data, the starting $(V_3O_9)^{3-}$ anion remains intact for the reactions with 0.5 and 1 equiv of $(CH_3CH_2O)_2SO_2$. When more $(CH_3CH_2O)_2SO_2$ was added (e.g., 1.5 or 2 equiv), the starting $(V_3O_9)^{3-}$ converted to $(V_5O_{14})^{3-}$ prior to the majority of alkylation. Even greater amounts of $(CH_3CH_2O)_2SO_2$ (e.g., 3 or 4 equiv) induced equilibration to $(V_{12}O_{32})^{4-}$. Thus, the higher levels of alkylating agent were left to react with compounds of lower charge density and, hence, less reactivity. For example, a starting 25 mM $(CH_3CH_2O)_2SO_2$ plus 50 mM $(V_3O_9)^{3-}$ solution appeared to proceed rapidly, with all alkylating agent reacting with the $(V_3O_9)^{3-}$ anion of high charge density. By contrast, starting with 200 mM $(CH_3CH_2O)_2SO_2$ and 50 mM $(V_3O_9)^{3-}$ seemed to proceed slowly, since once $(V_3O_9)^{3-}$ was converted to the larger, relatively unreactive vanadates (e.g., $(V_{12}O_{32})^{4-}$), the consumption of alkylating agent was slow.

Reactivity of $[(C_4H_9)_4N]_3(H_3V_{10}O_{28})$. No reaction was observed by ^1H NMR for addition of 1 equiv of $(CH_3CH_2O)_2SO_2$ to $(H_3V_{10}O_{28})^{3-}$ (50 mM each) until ~24 h. The ethyl product initially observed was $[(CH_3CH_2O)SO_3]^-$ without concomitant ethanol formation. Both the water and $(H_3V_{10}O_{28})^{3-}$ proton peaks (6.61 ppm, 1H; 9.31 ppm, 2H) broadened almost to the point of disappearing. Formation of ethanol became evident at ~17 d. The ^{51}V NMR spectrum for $(H_3V_{10}O_{28})^{3-}$ alone had four peaks: two equivalent, small, broad resonances at −392 and −429 ppm and two equivalent, large, sharp peaks at −506 and −525 ppm (Figure S6A).[45] Addition of 1 equiv of $(CH_3CH_2O)_2SO_2$ to $(H_3V_{10}O_{28})^{3-}$ produced no changes until ~16 d. At that time an orange precipitate formed in the NMR tube. The two small, broad peaks at −392 and −429 ppm merged to form a single broad peak from −380 to −440 ppm (Figure S6B), suggesting mutual site exchange behavior and loss of nonequivalency of the protonated sites on the vanadate.[45]

Reactions involving $(H_3V_{10}O_{28})^{3-}$ and $(CH_3CH_2O)_2SO_2$ initially showed only formation of $[(CH_3CH_2O)SO_3]^-$, without a corresponding ethyl group for ethanol or any other reaction product being observed. The protons of $(H_3V_{10}O_{28})^{3-}$ became too broad to observe when the $[(CH_3CH_2O)SO_3]^-$ peaks were formed. These changes suggest that a substrate was ethylated, but the ethyl peaks may be overlapped with other resonances. The ^1H NMR observations were accompanied by slight changes to the ^{51}V NMR spectrum. The −429 ppm peak remained stationary, while the neighboring −396 ppm signal broadened and moved to overlap the −429 ppm peak, suggesting a change in the protonation sites on the decavanadate.[45] Perhaps oxo sites once occupied by protons became ethylated, thereby preserving overall charge on the anion.

Reactivity of $[(C_4H_9)_4N]_4(V_{10}O_{26})$. The mixed-valence $(V_{10}O_{26})^{4-}$ anion is comprised of eight vanadium(V) and two vanadium(IV) atoms. Addition of 1 equiv of $(CH_3CH_2O)_2SO_2$ to 50 mM $[(C_4H_9)_4N]_4(V_{10}O_{26})$ yielded ethanol and $[(CH_3CH_2O)SO_3]^-$ within 1 h. Half of the $(CH_3CH_2O)_2SO_2$ had been consumed by ~17 d. A black-purple solution of $(V_{10}O_{26})^{4-}$ in CD_3CN produced a ^{51}V NMR spectrum containing three sharp resonances at −590, −597, and −604 ppm (Figure S7A), which correspond to $(V_{12}O_{32})^{4-}$.[42] After 4 weeks, small peaks at −538 and −614 ppm appeared, which corresponded to $(V_5O_{14})^{3-}$. The 1:1 reaction between $(CH_3CH_2O)_2SO_2$ and $(V_{10}O_{26})^{4-}$ produced new peaks at −493 and −554 ppm (Figure S7B), which were observable at 16 d. These peaks could not be assigned but may result from an alkylated vanadate.

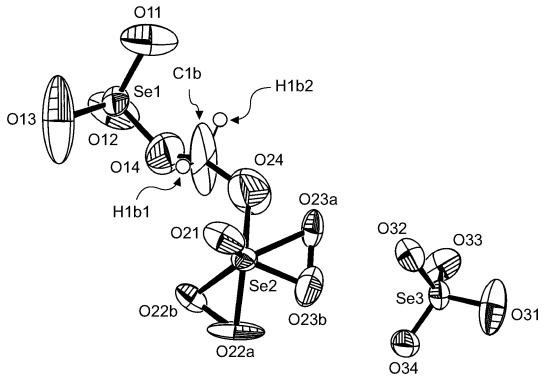

Figure 3. ORTEP representation of the $[(O_3SeOCH_2OSeO_3)(HSeO_4)]^{3-}$ anions, with thermal ellipsoids drawn at the 50% probability level. A portion of the dinuclear anion was found to be disordered and is shown with both locations of electron density (O22a/O22b and O23a/O23b).

Unlike the other vanadates studied here, $(V_{10}O_{26})^{4-}$ is a mixed-valence polyanion comprised of two vanadium(IV) and eight vanadium(V) ions. The two paramagnetic vanadium(IV) ions broadened the ^1H NMR spectrum of $[(C_4H_9)_4N]_4(V_{10}O_{26})$. The ^{51}V NMR spectrum, however, exhibited three distinct and relatively sharp peaks at −590, −597, and −606 ppm in a 1:1:1 ratio of intensities, corresponding to $(V_{12}O_{32})^{4-}$.[42] It is possible that the paramagnetic vanadium(IV) ions may prevent the remaining vanadium(V) atoms in $(V_{10}O_{26})^{4-}$ from being observed by ^{51}V NMR spectroscopy. This mixed-valence vanadate appears to be unstable in acetonitrile and equilibrates, at least partially, to $(V_{12}O_{32})^{4-}$.

Vanadate Reactivity. We observed the following trend in reactivity toward $(CH_3CH_2O)_2SO_2$: $(V_3O_9)^{3-} > (HV_4O_{12})^{4-} > (V_5O_{14})^{3-} \gg (V_{10}O_{26})^{4-}/(V_{12}O_{32})^{4-} \gg (H_3V_{10}O_{28})^{3-}$. This trend gives rise to the conclusion that the reactivity of such metal oxo compounds is likely dictated by charge density, with higher anionic density (e.g., $(V_3O_9)^{3-}$) providing greater nucleophilicity than more diffuse anions (e.g., $(H_3V_{10}O_{28})^{3-}$).

5. Reactivity of Selenates. To provide a comparison with our vanadate results, we sought to work with organic-soluble selenates. The lack of solubility for Na_2SeO_3 and Na_2SeO_4 in acetonitrile led us to prepare an anionic selenium oxo compound with suitable solubility. By reacting Ag_2SeO_4 and $[(C_6H_5)_4P]$-Br, we obtained crystals of a new selenate species, $[(C_6H_5)_4P]_3$-$(O_3SeOCH_2OSeO_3)(HSeO_4)$. Figure 3 shows an ORTEP diagram of this selenate. We have written the formula so that the monoselenate anion is protonated. This proton was not crystallographically assignable but was required for charge balance. Although it is possible that the proton may be on the bridged diselenate anion, the greater charge density of $(SeO_4)^{2-}$ relative to $(O_3SeOCH_2OSeO_3)^{2-}$ indicates a more reasonable assignment of the proton on the $(SeO_4)^{2-}$ anion.

^1H NMR Spectroscopy. A 25 mM solution of the selenate $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$ in CD_3CN produced a ^1H NMR spectrum (Figure S8) containing a singlet at 5.54 ppm with splitting ($^3J_{H-Se} = 11.7$ Hz), allowing assignment to the

(45) Day, V. W.; Klemperer, W. G.; Maltbie, D. J. *J. Am. Chem. Soc.* **1987**, *109*, 2991−3002.

bridging methylene group. All selenate reactions were performed at 25 mM concentrations due to solubility limitations. Addition of 1 equiv of $(CH_3CH_2O)_2SO_2$ to a 25 mM solution of $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$ resulted in loss of the methylene singlet and appearance of ethanol and $[(CH_3CH_2O)SO_3]^-$, along with an ethyl resonance (1.21 ppm, t; 4.03 ppm, q) (Figure S9B) attributable to monoethyl selenate $(CH_3CH_2O)Se(OH)O_2$ (vide infra). A number of small singlet peaks between 4.4 and 5.3 ppm also formed as the reaction progressed, none of which exhibited H−Se splitting. The reaction between the selenate and $(CH_3CH_2O)_2SO_2$ was slow, with only a hint of products visible in the spectrum after 24 h and ∼25% of the diethyl sulfate consumed after 18 days.

This selenate was significantly more reactive toward $CH_3CH_2OSO_2CF_3$, with the reaction being complete in ∼24 h. Similar to the $(CH_3CH_2O)_2SO_2$ reaction, addition of 1 equiv of $CH_3CH_2OSO_2CF_3$ resulted in loss of the methylene peak at 5.54 ppm and formation of a number of small singlet peaks between 4.4 and 5.3 ppm. Ethanol and diethyl ether were observed, along with two sets of ethyl peaks attributed to alkylated selenate products. The triplet at 1.24 ppm and quartet at 4.11 ppm were assigned to monoethyl selenate, $[(CH_3CH_2O)SeO_3]^-$, while the triplet at 1.38 ppm and quartet at 4.47 ppm were assigned to diethyl selenate, $(CH_3CH_2O)_2SeO_2$, in accord with literature assignments.[46]

The presence of CH_3CH_2OH and $(CH_3CH_2)_2O$ can be explained by the chemistries of the diethyl and monoethyl selenates. Ethanol may form as a result of excess alkylating agent reacting with residual water in the dried solvent, but the lack of ethanol in control solutions disfavors this possibility. The $(CH_3CH_2O)_2SeO_2$ may hydrolyze by breaking the Se−O bond and creating an ethoxide anion,[47] which could then be protonated by adventitious water in the dried solvent. Once formed, ethanol may be alkylated by $(CH_3CH_2O)_2SeO_2$ to generate $(CH_3CH_2)_2O$ and $[(CH_3CH_2O)SeO_3]^-$.[47] The $[(CH_3CH_2O)SeO_3]^-$ may in turn alkylate any ethoxide anions present to form $(CH_3CH_2)_2O$ and selenate $(SeO_4)^{2-}$.[47]

The 1:1 reaction between $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$ and ENU (25 mM each) yielded no products (Figure S10). As stated earlier, the hydroxide-catalyzed decomposition of ENU is required to form the alkylating species $C_2H_5N_2^+$. Control reactions revealed that ENU was stable in CD_3CN and D_2O for at least 2 days. However, ENU did react immediately with $(V_3O_9)^{3-}$. Perhaps the trinuclear vanadate is basic enough to deprotonate ENU, thereby initiating decomposition to $C_2H_5N_2^+$. In comparison, the bridged selenate species did not react, suggesting a lower basicity and lesser ability to deprotonate ENU and form the alkylating species. The related compound MNU has been shown to react with the oxo anion $(HPO_4)^{2-}$ in methanolic D_2O (pH maintained at 8.2), producing methanol (major product), methyl phosphate, dimethyl phosphate, and methyl carbonate.[43] In this reaction, $(HPO_4)^{2-}$ acted as a base to deprotonate the amine group of MNU, thereby initiating decomposition to the alkylating species methyl diazonium.[43,48]

^{77}Se NMR Spectroscopy. The proton-decoupled ^{77}Se NMR spectrum of $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$ in CD_3CN yielded two peaks at 1065 and 1046 ppm in a ∼2:1 ratio (Figure S11A). With the decoupler off, the peak at 1065 ppm was revealed to be a triplet and the peak at 1046 ppm remained a singlet (Figure S12). The triplet at 1065 ppm was assigned to the symmetrically bridged dinuclear anion $(O_3SeOCH_2OSeO_3)^{2-}$ and the singlet peak at 1046 ppm to $(HSeO_4)^-$.

Addition of 1 equiv of $(CH_3CH_2O)_2SO_2$ to a 25 mM solution of $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$ in CD_3CN produced a proton-decoupled ^{77}Se NMR spectrum (Figure S11B) with one peak, a singlet at 1041 ppm. This peak remained a singlet even when a proton-coupled spectrum was collected. This peak was attributed to a monoselenate anion of unknown protonation state, $(H_nSeO_4)^{(2-n)-}$.

The proton-decoupled ^{77}Se NMR spectrum of the selenate (25 mM) reacted with $CH_3CH_2OSO_2CF_3$ (1 equiv) in CD_3CN (Figure S11C) contained four peaks: 995, 1036 (major), 1049, and 1058 ppm. The proton-coupled spectrum was obtained, and only two peaks were observed: a singlet at 1036 ppm and a triplet at 1058 ppm. The peaks at 995 and 1049 ppm may not be observable in the proton-coupled spectrum, as the signals may be split into multiplets, thereby depleting the signal intensity, making it indistinguishable from baseline noise. The major product, the singlet at 1036 ppm, was ascribed to the monoselenate anion of unknown protonation state, $(H_nSeO_4)^{(2-n)-}$. The triplet at 1058 ppm was tentatively assigned to the monoethyl selenate, $[(CH_3CH_2O)SeO_3]^-$, due to this splitting pattern. The 1049 ppm peak observed in the proton-decoupled spectrum was assigned to the diethyl selenate, $(CH_3CH_2O)_2SeO_2$, on the basis of literature data.[49]

Selenate Structure and Reactivity. To study the alkylation reactivity of selenate under controlled conditions, we prepared and characterized the new compound $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$. This selenate contains two distinct anions: the bridged diselenate $(O_3SeOCH_2OSeO_3)^{2-}$ and the monoselenate $(HSeO_4)^-$. On the basis of the ^{1}H and ^{77}Se NMR spectra, the reactivity of this selenate appears to be due to the bridged diselenate anion. The bridging methylene disappeared from the ^{1}H NMR spectra after the addition of alkylating agent. The ^{77}Se NMR spectra showed disappearance of the bridged diselenate resonance upon alkylation and appearance of a singlet attributable to a monoselenate anion. These data suggest that the alkylating agents attack the bridged selenate, causing it to split into two monoselenate anions. The alkylated selenate product, $[(CH_3CH_2O)SeO_3]^-$, may be alkylated again to form diethyl selenate, $(CH_3CH_2O)_2SeO_2$.

Both ethanol and diethyl ether were generated from alkylation reactions of $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$. Ethanol is likely a product of a multistep process in which $(SeO_4)^{2-}$ is alkylated twice to yield $(CH_3CH_2O)_2SeO_2$. Subsequent hydrolysis of this alkylation product yielded ethanol and $[(CH_3CH_2O)SeO_3]^-$. Reaction of this ethanol with an alkylating agent then generated the ether.

This selenate and all the vanadates examined here formed ethanol upon alkylation. The slower reaction kinetics of selenate alkylation allowed us to observe an intermediate between the starting materials and ethanol. In the reactivity of $(V_3O_9)^{3-}$, for example, we could only infer alkylation of the vanadate and subsequent decomposition to yield ethanol and a rearranged

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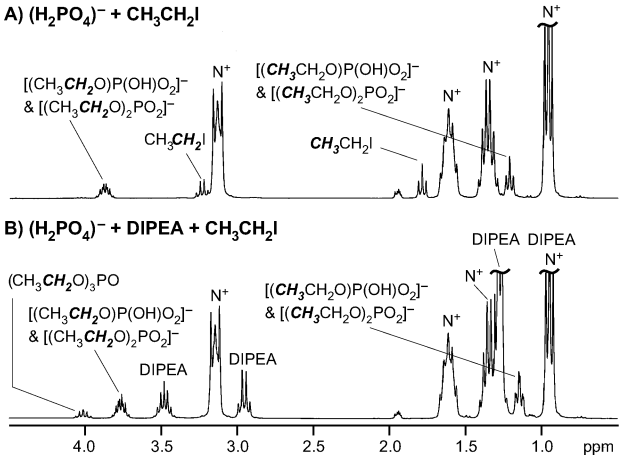

Figure 4. ^1H NMR spectra of 200 mM $[(C_4H_9)_4N](H_2PO_4)$ in CD_3CN, (A) with 1 equiv of CH_3CH_2I after 4 weeks and (B) with 1 equiv each of base (DIPEA) and CH_3CH_2I after 4 weeks.

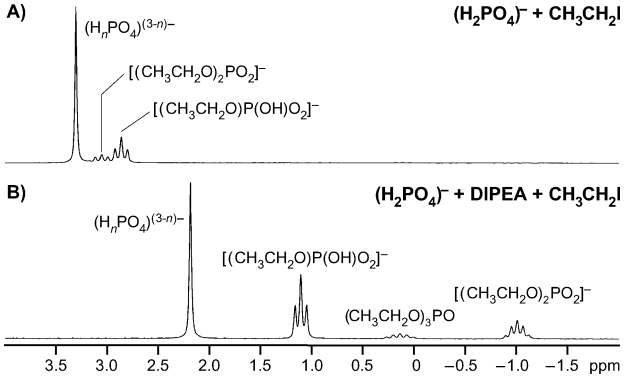

Figure 5. ^{31}P NMR spectra of 200 mM $[(C_4H_9)_4N](H_2PO_4)$ in CD_3CN, (A) with 1 equiv of CH_3CH_2I after 4 weeks and (B) with 1 equiv each of base (DIPEA) and CH_3CH_2I after 4 weeks.

vanadate. This diminished reactivity of selenate, relative to vanadate, may help to explain our prior biochemical work in which we observed that selenium salts inhibited DNA alkylation damage with less potency than vanadate.

6. Reactivity of $[(C_4H_9)_4N](H_2PO_4)$. ^1H NMR Spectroscopy. The spectrum of a 200 mM solution of $[(C_4H_9)_4N](H_2PO_4)$ in CD_3CN exhibited a broad singlet at ∼9.1 ppm which was assigned to the protons of $(H_2PO_4)^-$. For alkylation reactions, we favored CH_3CH_2I owing to peak overlaps when using $(CH_3CH_2O)_2SO_2$. Addition of 1 equiv of CH_3CH_2I resulted in the appearance of a triplet at 1.14 ppm and a quintet at 3.75 ppm (Figure 4A). Half of the ethyl iodide was consumed by ∼8 h. At that time, the triplet and quintet began to split, indicating that two distinct triplet−quintet pairs were present. A third, very small multiplet at 4.03 ppm appeared after ∼8 h.

N,*N*-Diisopropylethylamine (DIPEA) was used to deprotonate $(H_2PO_4)^-$ and compare the reactivity of $(H_2PO_4)^-$, $(HPO_4)^{2-}$, and $(PO_4)^{3-}$. Control solutions showed no reaction between DIPEA and CH_3CH_2I. In the ^1H NMR spectrum of a 1:1:1 reaction of $(H_2PO_4)^-$:DIPEA:CH_3CH_2I (Figure 4B), initial product peaks were observed at 1.15 (triplet) and 3.75 ppm (quintet). Half of the ethyl iodide was consumed by ∼4 h. Time revealed the splitting of the triplet and quintet, as well as a third product at 4.03 ppm (quintet). Addition of 2 or 5 equiv of DIPEA yielded similar results.

To assist with product identification, spectra of known ethylated phosphates were collected. A 200 mM solution of $(CH_3CH_2O)_3PO$ exhibited resonances at 1.28 (triplet) and 4.03 ppm (quintet) (Figure S13A). The related diethyl phosphate, $(CH_3CH_2O)_2POOH$, showed peaks at 1.29 (triplet), 4.06 (quintet), and ∼9.2 ppm (singlet) (Figure S13B). These two compounds were virtually identical. DIPEA (1 equiv) was used to deprotonate $(CH_3CH_2O)_2POOH$, which resulted in the triplet and quintet peaks shifting to 1.15 and 3.77 ppm, respectively (Figure S13C). Diethyl phosphate was also subjected to alkylation. Addition of 1 equiv of CH_3CH_2I to a 200 mM solution of $(CH_3CH_2O)_2POOH$ in CD_3CN resulted in no reaction. However, if 1 equiv of DIPEA was added prior to the addition of CH_3CH_2I, $(CH_3CH_2O)_3P$ formed (Figure S13D).

^{31}P NMR Spectroscopy. The ^{31}P NMR spectrum for 200 mM $[(C_4H_9)_4N](H_2PO_4)$ in CD_3CN exhibited one singlet at 4.31 ppm. Addition of CH_3CH_2I (1 equiv) to this phosphate solution resulted in formation of two product peaks (Figure 5A). A triplet appeared at 2.58 ppm and eventually moved downfield to 3.1 ppm. A multiplet for the second product peak was at 3.3 ppm and moved downfield to 3.5 ppm.

Addition of 1 equiv of DIPEA to a 200 mM solution of $[(C_4H_9)_4N](H_2PO_4)$ prior to addition of 1 equiv of CH_3CH_2I resulted in formation of three products (Figure 5B): a triplet at 1.24 ppm, followed by a quintet at −0.74 ppm, and a multiplet at 0.20 ppm. When 2 equiv of DIPEA was used, the final spectrum was essentially the same as that resulting from reaction with 1 equiv of DIPEA. The final spectrum of the reaction with 5 equiv of DIPEA yielded the same spectral pattern, but the chemical shifts were farther upfield: triplet at 1.08 ppm, multiplet at 0.08 ppm, and quintet at −1.07 ppm.

The control spectrum of 200 mM $(CH_3CH_2O)_3PO$ (Figure S14A) showed a septet at 0.28 ppm, and 200 mM $(CH_3CH_2O)_2POOH$ (Figure S14B) displayed a quintet at 1.45 ppm. Deprotonation of $(CH_3CH_2O)_2POOH$ with 1 equiv of DIPEA produced a quintet at −0.89 ppm (Figure S14C). Alkylation reactions with $(CH_3CH_2O)_2POOH$ were also performed. A 1:1 reaction between $(CH_3CH_2O)_2POOH$ and CH_3CH_2I produced no products. Addition of 1 equiv of DIPEA to $(CH_3CH_2O)_2POOH$, followed by addition of 1 equiv of CH_3CH_2I, generated a septet signal at 0.22 ppm (Figure S14D) attributed to $(CH_3CH_2O)_3PO$.

These results were combined with further control studies (see Supporting Information) to show that, in general, the reaction products of these phosphate alkylations were $[(CH_3CH_2O)P(OH)O_2]^-$, $[(CH_3CH_2O)_2PO_2]^-$, and $(CH_3CH_2O)_3PO$. Figures 4 and 5 show the appropriate peak assignments. Phosphate alkylation reactions yielded esters that remained stable rather than rearranging to yield ethanol.

7. Reactivity of Sulfate, Acetate, Nitrate, and Nitrite. To provide comparisons with vanadates, selenate, and phosphate, we examined the alkylation reactions of the following anions: $(SO_4)^{2-}$, $(HSO_4)^-$, $(CH_3COO)^-$, $(NO_3)^-$, and $(NO_2)^-$ (data not shown). Each anion began with $[(C_4H_9)_4N]^+$ and was reacted stoichiometrically with $(CH_3CH_2O)_2SO_2$. In all cases, the anions were alkylated to produce stable products. Alkylation of both $(SO_4)^{2-}$ and $(HSO_4)^-$ yielded $(CH_3CH_2O)SO_3^-$, albeit at a faster rate for the dianion. Acetate was converted to $CH_3CH_2OOCCH_3$. The product of nitrate alkylation was $CH_3CH_2ONO_2$. Similarly, the reaction of $(NO_2)^-$ with $(CH_3CH_2O)_2SO_2$ provided CH_3CH_2ONO. Consumption of $(CH_3CH_2O)_2SO_2$ by $(NO_2)^-$ was significantly faster (∼40×) than that observed for $(NO_3)^-$.

Phosphate, sulfate, acetate, nitrate, and nitrite all reacted with $(CH_3CH_2O)_2SO_2$ to yield the corresponding esters. Somewhat like selenate, but unlike the vanadates, these ester reaction products appeared stable and did not rearrange or decompose to yield ethanol. The relative reactivity of these anions was found to be consistent with our hypothesis of higher negative charge density leading to greater reactivity. Fully deprotonated $(PO_4)^{3-}$ reacted faster than $(HPO_4)^{2-}$, which, in turn, was more rapid than $(H_2PO_4)^-$. Likewise, $(HSO_4)^-$ was easier to alkylate upon addition of base to form $(SO_4)^{2-}$. Similarly, $(NO_2)^-$ is of higher charge density and alkylated more rapidly than $(NO_3)^-$.

Conclusions

In an effort to gain mechanistic insights on the cancer-preventing properties of vanadium and selenium, the alkylation reactions of various oxo compounds were studied. Vanadates converted alkylating toxins into the relatively harmless product ethanol. In the cases of selenate, phosphate, sulfate, acetate, nitrate, and nitrite, the immediate ester products of alkylation were observed. Subsequent decomposition of alkylated selenates also generated ethanol. When comparing the ethyl iodide alkylations of phosphates to vanadates, we found that the smaller vanadates (e.g., $(V_3O_9)^{3-}$) were more reactive than those protonated phosphates likely to be found in vivo (e.g., $(H_2PO_4)^-$, $(RO_2PO_2)^-$ of DNA). Indeed, $(V_4O_{12})^{4-}$ is a common vanadate formed under aqueous conditions of neutral pH. It is tempting to propose that vanadates may prevent cancer by being more reactive, even in the presence of high cellular phosphate concentrations. However, the selenate examined here, $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$, was less reactive toward CH_3CH_2I than the phosphates. This selenate may not be a good model for cellular selenium. We have observed a general trend in which reactivity is greatest with anions of highest charge density. Our findings indicate that future efforts in cancer prevention should focus upon nucleophilic compounds with high negative charge densities and oxo groups for accepting alkylating toxins.

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Supporting Information Available: Complete crystal structure and ^1H and ^{77}Se NMR data for $[(C_6H_5)_4P]_3(O_3SeOCH_2OSeO_3)(HSeO_4)$ as well as NMR data for select alkylation reactions (PDF, CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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