Synthesis, Characterization, and Anti-Human Immunodeficiency Virus Activity of Water-Soluble Salts of Polyoxotungstate Anions with Covalently Attached Organic Groups

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The cesium and tetramethylammonium (TMA) salts of polyoxotungstate anions with covalently attached organosilyl groups of formula $[(RSi)_2O]SiW_{11}O_{39}^{4-}$, where R = $CH_2CH_2COCH_3$, $(CH_2)_3CN$, and $CH{=}CH_2$ (1-R, cesium salt, unless otherwise noted) have been prepared, purified, and spectroscopically characterized. The water solubility (25 °C) of these 10 new compounds ranges from 0.14 mM to 2.16 mM. All appear to be stable in aqueous media over a period of several hours as assessed by ^1H NMR. The activities (EC_{50}) of the new compounds against human immunodeficiency virus in primary human lymphocytes range from 3.3 μM to 39.0 μM. Their toxicities (IC_{50}) are all greater than 100 μM. The inhibition constants of the new compounds against purified virion-derived HIV-1 reverse transcriptase are in the 1–10 μM range.

Introduction

In the wake of the growing evidence that some of the most promising anti-HIV-1 agents, and in particular, 3′-azido-3′-deoxythymidine (AZT),[1,2] exhibit problems associated with toxicity, resistance, and drug delivery, efforts continue to mount to develop new nonnucleoside antiviral agents.[3] Our continuing efforts address the development of a large class of soluble inorganic compounds as antiviral agents. These compounds, known as polyoxometalates, are composed of early transition metal ions, usually in their d^0 electronic configurations, and oxide ions. The metal and oxide ions are typically arranged in MO_6 octahedra which are linked by edge sharing or corner sharing bridged O atoms.[4] The first member of this class of compounds to receive substantial attention as an antiviral and anti-HIV-1 agent, HPA-23 (molecular formula = $(NH_4^+)_{17}(H^+)$-$[NaSb_9W_{21}O_{86}^{18-}]$),[5,6] proved to be toxic in general and toxic to bone marrow cells in particular. At the limited levels of administration dictated by its toxicity, HPA-23 proved to be ineffective in clinical trials.[7] Subsequently, other structural classes of tungsten-based polyoxometalates (polyoxotungstates) proved to be as or more active against HIV-1 in cell culture, far more active in vitro, less toxic, and perhaps most significantly, orders of magnitude less toxic in some cases to human bone marrow cells (human granulocyte-macrophage precursor cells) than HPA-23.[8,9]

This paper addresses a class of complexes whose antiviral activities have yet to be evaluated—polyoxometalates containing covalently attached organic groups. Although several classes of polyoxometalates containing organic or organometallic groups have been reported,[10-14] all but perhaps two of these classes are very unstable to hydrolysis and as such would have effectively no chance of remaining intact in a biological milieu for the lengths of times requisite for pharmacological efficacy. The two exceptions are the organometallic derivatives RM (M = Si, Ge, Sn, or Pb, and R = an alkyl or aryl group) of some polyoxotungstates examined by the groups of Pope, and Knoth,[12a-c] and the cyclopentadienyl titanium derivatives of certain polyoxotungstates examined by the groups of Knoth and Keana.[12,13] Our general interest in polyoxometalates covalently derivatized with organic groups comes from the simple fact that such groups should permit a host of pharmacologically pertinent parameters of the polyoxometalates to be varied including, in principal, oral bioavailability and blood–brain barrier permeability as well as activity and toxicity.

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We report here the synthesis and properties of new water soluble cesium salts of Knoth-type covalently de-

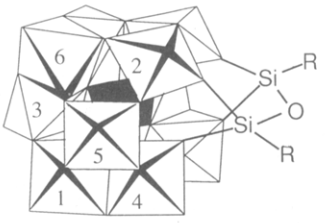
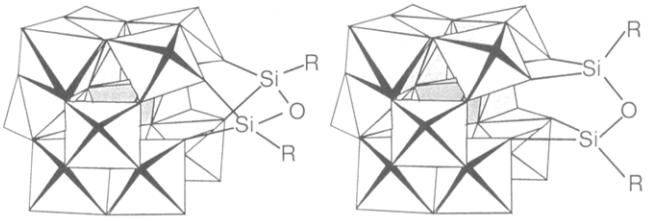


Figure 1. Structure of the α-$SiW_{11}O_{39}$ lacunary subunit showing the labeled tungstens which correspond to the indicated signals in Table II.



A B

Figure 2. Proposed structures for the bridging $(SiR)_2O^{4+}$ unit. (A) Symmetry equivalent SiR groups, perpendicular to the mirror plane. (B) Symmetry inequivalent SiR groups, parallel to the mirror plane.

rivatized polyoxotungstates of formula $[(RSi)_2O]$-$SiW_{11}O_{39}^{4-}$, where R = one of three organic groups that

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Table I. Infrared Spectral Data

	Si–O–Si asymm stretch	Si–C stretch 1	Si–C stretch 2	C–H stretch	C–H bend 1	C–H bend 2
$Cs_4[(CH_3CO_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$	1040	1230	700	2980	1420	1440
$Cs_4[(NCCH_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$	1040	1250	710	2940	1420	1455
$Cs_4[(CH_2{=}CHSi)_2O]SiW_{11}O_{39}$	1050	1270	700	2960[a]	1405	a
$[(CH_3)_4N^+]_4[(CH_3CO_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$	1050	1225	720	2970	1420	1450
$[(CH_3)_4N^+]_4[(ClCH_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$	1050	1265	710	2970	1415	1450
$[(CH_3)_4N^+]_4[(CH_2{=}CHSi)_2O]SiW_{11}O_{39}$	1040	1290	700	2970	1420	1460
$[(CH_3)_4N^+]_4[(CH_3CH_2Si)_2O]SiW_{11}O_{39}$	1035	1240	720	2960	1410	1450
$[(CH_3)_4N^+]_4[(CH_3Si)_2O]SiW_{11}O_{39}$	1040	1260	700	2960	1410	1450

[a] Absent or very weak. All values are reported in wavenumbers (cm^{-1}).

Table II. ^{183}W NMR Chemical Shifts and Line Widths[a]

complex	signal rel intensities	1 2	2 2	3 1	4 2	5 2	6 2
$[(CH_3)_4N^+]_4[(CH_3CO_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$		−103.7	−105.6	−110.2	−125.3	−169.9	−249.0
		1.9	1.5	1.5	1.5	1.5	1.9
$[(CH_3)_4N^+]_4[(ClCH_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$		−104.4	−106.7	−111.0	−126.2	−171.9	−250.3
		2.3	2.3	2.7	1.9	1.9	2.3
$[(CH_3)_4N^+]_4[(CH_2{=}CHSi)_2O]SiW_{11}O_{39}$		−104.4	−106.7	−111.1	−125.5	−170.2	−247.1
		1.9	1.9	1.9	1.9	1.9	2.3
$[(CH_3)_4N^+]_4[(CH_3CH_2Si)_2O]SiW_{11}O_{39}$		−104.1	−107.6	−110.9	−126.9	−172.2	−252.3
		1.6	1.5	1.1	1.5	1.1	1.1
$[(CH_3)_4N^+]_4[(CH_3Si)_2O]SiW_{11}O_{39}$		−104.4	−108.0	−110.9	−125.8	−174.2	−255.2
		1.1	1.2	1.1	1.1	0.8	1.1

[a] All $\Delta\nu_{1/2}$ in hertz. All shifts in ppm relative to 2.0 M $Na_2WO_{4(aq)}$ external. The signal corresponds to the tungstens in Figure 1. All line widths are shown below their respective signals.

impart varying degrees of hydrophobic character or other potential points of attachment to other organic and/or biological molecules. The spectroscopic properties, hydrolytic stability, anti-HIV-1 activity and toxicity in cell culture, and activity against the HIV-1 reverse transcriptase enzyme (RT) of these compounds are given.

Results and Discussion

Synthesis and Basic Properties of the Title Complexes. The reactions of organosilyl trichlorides, $RSiCl_3$, where R = an aliphatic or aromatic hydrocarbon group, with the defect or "lacunary" complex, $SiW_{11}O_{39}^{8-}$, to yield product complexes containing two organosilyl groups and one polyoxotungstate unit are reported in a communication by Knoth[12] and have been reproduced in our laboratory. We report here that similar processes involving the reaction of one, two, or more equivalents of four organosilyl trichlorides with terminal functional groups, $RSiCl_3$, where R = $CH_2CH_2COCH_3$, $(CH_2)_3CN$, and $CH{=}CH_2$, with $SiW_{11}O_{39}^{8-}$ in unbuffered water leads in good yield to product complexes that have incorporated two organosilyl groups per undecatungstosilicate unit. The complexes were precipitated from aqueous solution as their Cs^+ salts by addition of CsCl. Knoth proposed that his bis(organosilyl) polyoxotungstate complexes contained a $(RSi)_2O^{4+}$ unit based principally on a strong stretching mode in the infrared at 1040 cm^{-1} attributed to an Si–O–Si stretch.[12] A more detailed analysis of the IR data provides further structural evidence for these complexes. Table I summarizes the relevant IR data. The Si–O–Si asymmetric stretch is present and intense in all the complexes. The Si–C stretch can be seen as a weak band at ~1250 cm^{-1} and as a shoulder at ~710 cm^{-1}.[15] The C–H stretch is seen as a weak band at ~2980 cm^{-1}, and the C–H bend is seen at ~1420 and 1450 cm^{-1}.[15] It should be noted that the influence of the Si–C bond on the C–H vibrations has been observed before, to lower the intensity of the C–H absorptions from one-third to one-fourth of their expected intensities.[15] In addition to these, there are other bands indicative of the functional groups present. The cesium and the TMA salts of the ester derivative both show a strong carbonyl stretch at 1735 cm^{-1}, and the nitrile derivative shows a nitrile stretch at 2250 cm^{-1}. The olefinic bands of the vinyl group, however, are obscured by polyoxometalate absorptions from 900 to 1000 cm^{-1} and at 1640 cm^{-1}. Elemental analyses of the purified product complexes are consistent with the presence of such a μ-oxo disilyl linking unit and a molecular formula of Cs_4-

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Table III. ^{29}Si NMR Chemical Shifts and Line Widths[a]

complex	Si(1)	$\Delta\nu_{1/2}$	Si(2)	$\Delta\nu_{1/2}$
$Cs_4[(CH_3CO_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$	-52.4	6.1	-86.3	4.9
$Cs_4[(NCCH_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$	-52.1	6.9	-86.3	4.9
$Cs_4[(CH_2{=}CHSi)_2O]SiW_{11}O_{39}$	-53.1	7.3	-86.1	7.8
$[(CH_3)_4N^+]_4[(CH_3CO_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$	-52.4	7.9	-84.3	7.9
$[(CH_3)_4N^+]_4[(ClCH_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$	-52.0	7.9	-84.3	6.9
$[(CH_3)_4N^+]_4[(CH_2{=}CHSi)_2O]SiW_{11}O_{39}$	-65.4	7.9	-84.2	7.9
$[(CH_3)_4N^+]_4[(CH_3CH_2Si)_2O]SiW_{11}O_{39}$	-51.3	5.9	-84.5	5.9
$[(CH_3)_4N^+]_4[(CH_3Si)_2O]SiW_{11}O_{39}$	-49.9	6.9	-84.3	6.9

[a] All shifts in ppm relative to 3-(trimethylsilyl)propionic acid, sodium salt, in D_2O. All $\Delta\nu_{1/2}$ in hertz. Si(1) is the signal for the silicon of the alkylsilyl group, and Si(2) is the signal for the polyoxometalate silicon.

$[(RSi)_2O]SiW_{11}O_{39}$, where R = $CH_2CH_2COCH_3$, $(CH_2)_3CN$, and $CH{=}CH_2$, henceforth referred to as 1-R for convenience (see Experimental Section).

The ^{183}W NMR spectra of all the TMA salts establish that all of the complexes retain the α lacunary unit, α-$SiW_{11}O_{39}^{8-}$, derived from removal of one $W{=}O^{4+}$ unit from the parent "Keggin" structure, α-$SiW_{12}O_{40}^{4-}$, of T_d point group symmetry. All show six tungsten resonances in a 2:2:1:2:2:2 ratio indicative of the α-$SiW_{11}O_{39}^{8-}$ lacunary subunit (Table II). The signals have been assigned to their respective tungsten atoms based on previous NMR studies of lacunary Keggin structures (Figure 1).[16] Low solubility of the cesium salts (1-R = 0.142 mM in D_2O at 25 °C) precluded obtaining satisfactory ^{183}W NMR spectra. The low receptivity of the ^{183}W nucleus (1.04×10^{-5} relative to ^1H at constant field) requires tungsten concentrations of at least 100 mM for satisfactory spectra. Both ^1H and ^{183}W NMR show that the $SiW_{11}O_{39}^{8-}$ isomers of C_1 point group symmetry derived from removal of a $W{=}O^{4+}$ unit from the β isomer of the parent complex, β-$SiW_{12}O_{40}^{4-}$, are not present in any of the complexes 1-R. The β isomers have never before been shown to be formed from the α-$SiW_{11}O_{39}$ starting material under these conditions. Indeed the β isomers spontaneously rearrange in aqueous solution to the thermodynamically more stable α isomer.[17]

Although no X-ray crystal structures are available on any of the complexes reported by Knoth and Pope, a preliminary 2-fold disordered structure of a bis(phenylphosphonyl)lacunary complex, $[(C_6H_5PO)_2PW_{11}O_{39}]^{3-}$, derived from condensation of phenylphosphonyl dichloride with the lacunary complex α-$PW_{11}O_{39}^{7-}$, shows that coordination of the two electrophilic phenylphosphonyl groups is to the four oxygens defining the hole left by removal of the $W{=}O^{4+}$ unit.[18] It is very likely that these oxygens are also the sites of attachment of the Si atoms of the $(RSi)_2O^{4+}$ unit in the complexes 1-R.[19] Even so, this leaves two possible binding modes of the $(SiR)_2O^{4+}$ unit to the polyoxometalate surface. The structure proposed by Knoth has equivalent SiR groups bound perpendicular to the mirror plane bisecting the α-$SiW_{11}O_{39}^{8-}$ subunit, but the possibility exists for these groups to be bound parallel to, or in, the plane of the mirror, creating inequivalent SiR groups (Figure 2). With this in mind we set out to study the SiR groups more closely by ^{29}Si and high field ^1H NMR.

The acquisition of ^{29}Si NMR is made quite difficult by three factors. First, ^{29}Si requires relatively long relaxation delays, with T_1's varying from 5 to 150 s dictating long total acquisition times. Second, the silicon signal in organosilicon compounds is complicated by two and three bond coupling; non-decoupled spectra are of little value. Third, ^{29}Si has a negative gyromagnetic ratio which causes the nuclear Overhauser effect to be negative; this can give rise to weak, negative, or even null signals.[20] Decoupling the spectra of these derivatized silicotungstates resulted in a negative signal for the organosilicon ^{29}Si signal and a positive signal for the polyoxoanion ^{29}Si signal. We solved these problems by using a paramagnetic relaxation reagent, either $Cr^{III}(acac)_3$ (acac = acetylacetonate) in DMSO or Cr^{III}DTPA (DTPA = diethylenetriaminepentaacetic acid) in D_2O.[20] Addition of these agents reduced the relaxation delay to 2 s, improving the total acquisition time, and eliminated one relaxation mechanism that gave rise to the negative signal [the (^{29}Si, ^1H) dipole–dipole contribution

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(18) Crystallographic data: $C_{44}H_{83}N_2O_{41}P_3W_{11}$ orthorhombic, space group: P_{bcn}, λ = 0.71073 Å, a = 19.680 (11) Å, b = 16.446 (13) Å, c = 23.398 (13) Å, V = 7573 (8) Å^3, Z = 4, R = 8.96%, R_w = 9.15%, GOF = 2.42. Absorption correction by empirical psi scan method. The bis(phenylphosphenyl) lacunary anionic unit is 2-fold disordered. Kim, G. S.; Hill, C. L.; Hagen, K. S., unpublished work.

(19) Although considerable efforts thus far have provided single crystals of 1-R or related organic soluble derivatives of 1-R, all the crystals have proved to be sufficiently disordered (cubic space groups) to not be of any value in assigning the exact mode of attachment of the organosilyl groups to the polyoxometalate.

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to T_1 (^{29}Si)].[20] This allowed us to simultaneously decouple the spectrum while retaining the phase of the organosilicon signal.[20] The final proton-decoupled ^{29}Si NMR data are summarized in Table III. All the spectra showed a single resonance for the organosilicon ^{29}Si atoms and a single resonance for the polyoxometalate ^{29}Si atoms. This technique renders the signals nonintegrable, however, due to the presence of paramagnetic species in solution. This combined with the 500 MHz ^{1}H NMR spectra of the tetramethylammonium salts of 1-CH_2CH_3 and 1-CH_3 which showed clean multiplets for the protons indicate that the predominant structure is most probably the symmetrical structure with equivalent SiR groups bound perpendicular to the mirror plane. ^{17}O NMR spectra of the 1-R complexes were not measured because the oxygen atoms exposed to the solvent are labile, and the label would wash out under the aqueous conditions in which the complexes are prepared and manipulated.

The solubilities and stabilities of these new compounds in water were assessed at the outset as certain threshold levels of both solubility and stability in water are required for thorough characterization of the complexes and for rigorous interpretation of a range of necessary biological and pharmacological studies based on the compounds. Polyoxometalates, unlike nucleosides and most other classes of antiviral agents, are susceptible, in principle, to facile fragmentation into complexes of lower nuclearity, which renders the interpretation of results from some biological evaluations of these compounds difficult at best. The only pharmacokinetics study of any polyoxotungstate to date, the demonstration by Bountiff that the ^{185}W from HPA-23 radiolabelled with ^{185}W accumulates in the liver, kidneys, spleen, and brain,[21] suffers from a related and real ambiguity—there is no hard evidence as to what the tungsten-containing species being detected here actually are. In our previous paper, we illustrated the complexity that can arise in some studies of polyoxometalates in aqueous media as a result of hydrolytic instability and lability with respect to formation of several discrete polyoxometalate species.[8b]

The solubilities in water (25 °C) of the three new Cs^+ salts of 1-R, range from 0.142 mM for $Cs_4[(CH_3CO_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$] to 2.16 mM for $Cs_4[(NCCH_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$. Examination of these three compounds by ^{1}H NMR in D_2O showed them all to be completely stable to decomposition in both aprotic and aqueous media over a period of several hours. The ester derivative appears to be fairly stable to decomposition over a period of days with approximately 20% decomposition being observed over a period of 7 days. The nitrile derivative showed 50% decomposition over 24 h, increasing to 70% after 48 h. The vinyl derivative was stable to decomposition even after 8 days.

Anti-HIV-1 Activity and Toxicity of 1-R in Cell Culture. Table IV lists the median effective concentration (EC_{50}), and the median inhibitory concentration (IC_{50}) for human peripherial blood mononuclear cells (PBMC) and for purified cell-free virion-derived HIV-1 reverse transcriptase and DNA polymerase for all four of the title compounds compared to phosphonoformate (PFA), an antiviral agent used as a positive control. Compound $Cs_4[(CH_3CO_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$ (HS-72) was the most potent in the series with an EC_{50} of 3.3 μM and no toxicity to the uninfected host human lymphocytes when tested up to 100 μM.

Table IV. Anti-HIV-1 Activity and Toxicity of Organosilyl Functionalized Polytungstosilicate Complexes (1-R) in Human PBM Cells and Activity against HIV-1 Reverse Transcriptase and DNA Polymerase

1-R	HIV-1 in PBMC: EC_{50}[a]	toxicity in PBMC: IC_{50}[b]	HIV-1 RT: IC_{50}[c]	DNA pol.: IC_{50}[c]
1 1-$CH_2CH_2COOCH_3$	3.3	>100	13.2	17.8
2 1-$CH_2CH_2CH_2CN$	19.8	>100	0.91	39.9
3 1-$CH{=}CH_2$	35.3	>100	0.57	14.9
4 TMA 1-$CH_2CH_2COOCH_3$ phosphonoformate	39.0	>100	1.1	65.2
	21.0	>100	0.15	160

[a] EC_{50} = median effective (antiviral) concentration in μM. [b] IC_{50} = median inhibitory (toxicity) concentration in μM. [c] IC_{50} = median inhibitory concentration for cell free HIV-1 reverse transcriptase or DNA polymerase in μM.

In order to examine one of the potential sites of inhibition of these compounds, their activity against HIV-1 RT was determined. The inhibitory activity of the compounds against the RT proved to be submicromolar in the nitrile and the vinyl derivatives, slightly above 10 μM for the cesium salt of the ester derivative, and 1.1 μM for the tetramethylammonium salt of the ester derivative. With the exception of compound 1-$CH_2CH_2CO_2CH_3$, these compounds were at least 26-fold more potent against HIV-1 RT than DNA polymerase. Interestingly, the IC_{50} for HIV-1 RT was significantly lower than the EC_{50} for HIV-1 inhibition in human PBM cells for compounds **2–4**.

Conclusions

The complexes 1-R are a few representatives of what is, in principal, a large family of antiviral polyoxometalates containing organic groups covalently attached to the surface oxygen atoms of the polyoxometalate units through thermodynamically strong Si–O linkages that exhibit substantial hydrolytic stability. The Cs^+ salts of these compounds exhibit high therapeutic indices in cell culture against HIV-1. Although inhibition of HIV-1 RT is a possible target for these compounds, other targets such as interference of binding of gp120 to the lymphocyte CD4 receptor are possible for this class of compounds.[8] Inasmuch as 1-R and related complexes should be accessible in quantity and the pendant organic functions should be amenable to extensive derivatization, the further assessment and development of these types of complexes as antiviral agents is warranted.

Experimental Section

Materials. Cesium chloride, salts of monomeric tungstate, WO_4^{2-}, $Cl_3SiCH_2CH_2CH_2CN$, $Cl_3SiCH_2CH_2CH_2Cl$, $Cl_3SiCH_2CH_3$, Cl_3SiCH_3, $Cl_3SiCH{=}CH_2$, and $Cl_3SiCH_2CH_2CO_2CH_3$ were commercial samples and used without further purification. The water was deionized, and the organic solvents were Burdick and Jackson glass-distilled grade. The complexes α-$H_4SiW_{12}O_{40}$[22] and α-$K_8SiW_{11}O_{39}$[17a] were made by literature procedures. The purity of the polyoxometalates was assessed by ^{1}H NMR and infrared spectroscopies. PFA, used as a positive control for the biological assays, was obtained from Astra Alab, Södertälje, Sweden.

Instrumentation and Methods. High- and medium-field ^{1}H NMR spectra were run on a GE QE-500 and a GE QE-300, respectively. ^{29}Si and ^{183}W were both run on an IBM WP200SY spectrometer. The ^{183}W NMR spectra required the use of a special probe for this application designed and made for us by Cryomagnetic Systems, Inc. The ^{183}W spectra were recorded at 8.34 MHz in Wilmad 515-7PP 15-mm id NMR tubes and referenced to external 2 M Na_2WO_4 in D_2O. The pulse width was 79 μs in all cases. Samples were ~100 mM in DMSO-d_6.

The ^{29}Si were recorded at 39.76 MHz in Wilmad 513-7PP 10-mm id NMR tubes and referenced to 3-(trimethylsilyl)-

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propionic acid, sodium salt. The pulse width was 7 μs, relaxation delay was 2 s, and repetition rate was 2.01 s. Sample concentrations were approximately 200 mM in polyoxometalate and 4.8 mM in $Cr^{III}(acac)_3$ for the DMSO soluble TMA salts, or saturated in polyoxometalate and 5.6 mM in Cr^{III}DTPA for the D_2O soluble cesium salts, as discussed above. The chromium(III) relaxation reagents were used to reduce the relaxation delay and maintain the phase of the organosilicon signal while decoupling the spectra. The probe temperature in all NMR measurements was 296 K. Infrared spectra were recorded on a Perkin-Elmer 1430 ratio recording spectrophotometer as KBr pellets (2–4 wt% in KBr).

Synthesis of 1-R Compounds. The infrared, ^{183}W NMR, and ^{29}Si NMR spectra for the following complexes are found in Tables I, II, and III, respectively.

$Cs_4[(CH_3CO_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$. Twenty milliliters of a 0.0335 M aqueous solution of α-$K_8SiW_{11}O_{39}$ was heated to 50 °C with stirring. To this solution was added an acetonitrile solution containing 2 equiv (0.22 mL) of [2-(carbomethoxy)ethyl]trichlorosilane per equivalent of the undecatungstosilicate. The solution was left on low heat for approximately 2 more min, and then 5–10 equiv of cesium chloride was added, producing a white precipitate. The solution was cooled slightly, and the precipitate was collected on a medium fritted glass funnel and air-dried overnight to yield 1.57 g (70%) of a white solid: 300-MHz ^1H NMR (D_2O 296 K) δ 3.78 (s, 3 H, OCH_3), 2.75 (t, 2 H, CH_2CO_2), 1.19 (t, 2 H, $SiCH_2$). Anal. Calcd for $C_8H_{14}Cs_4O_{44}Si_3W_{11}$: C, 2.78; H, 0.41; Cs, 15.4; O, 20.4; Si, 2.44; W, 58.6. Found: C, 2.72; H, 0.42; Cs, 15.6; O (by diff), 22.3; Si, 2.44; W, 56.5.

$[(CH_3)_4N]_4[(CH_3CO_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$. The procedure was the same as that for the cesium salt of the 2-(carbomethoxy)ethyl derivative above except that approximately 3 equiv of tetramethylammonium chloride were used to salt out the complex: ^1H NMR (DMSO-d_6 296 K) δ 3.60 (s, 3 H, OCH_3), 3.14 (s, 48 H, $(CH_3)_4N^+$), 2.44 (t, 2 H, CH_2CO_2), 0.88 (s, 2 H, $SiCH_2$). Anal. Calcd for $C_{24}H_{62}N_4O_{44}Si_3W_{11}$: C, 8.56; H, 1.94; N, 1.74; O, 21.9; Si, 2.62; W, 62.9. Found: C, 8.91; H, 1.91; N, 1.71; O (by diff), 22.2; Si, 2.61; W, 62.7.

$Cs_4[(NCCH_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$. The procedure was the same as that for the cesium 2-(carbomethoxy)ethyl derivative above except that (3-cyanopropyl)trichlorosilane was used as the alkyltrichlorosilane: ^1H NMR (D_2O 296 K) δ 2.70 (t, 2 H, CH_2CN), 2.04 (m, 2 H, CH_2), 1.08 (t, 2 H, $SiCH_2$). Anal. Calcd for $C_8H_{12}N_2Cs_4O_{40}Si_3W_{11}$: C, 2.81; H, 0.35; N, 0.82; Cs, 15.6; O, 18.7; Si, 2.47; W, 59.2. Found: C, 2.78; H, 0.37; N, 0.76; Cs, 16.2; O (by diff), 18.6; Si, 2.35; W, 58.9.

$[(CH_3)_4N]_4[(ClCH_2CH_2CH_2Si)_2O]SiW_{11}O_{39}$. The procedure was the same as that for the TMA 2-(carbomethoxy)ethyl derivative above except that (3-chloropropyl)trichlorosilane was used for the alkyltrichlorosilane: ^1H NMR (DMSO-d_6 296 K) δ 3.70 (t, 2 H, CH_2Cl), 3.14 (s, 48 H, $(CH_3)_4N^+$), 1.93 (m, 2 H, CH_2), 0.70 (t, 2 H, $SiCH_2$). Anal. Calcd for $C_{22}H_{60}N_4Cl_2O_{40}Si_3W_{11}$: C, 8.26; H, 1.89; N, 1.75; Cl, 2.22; O, 20.0; Si, 2.63; W, 63.2. Found: C, 8.35; H, 1.92; N, 1.66; Cl, 2.31; O (by diff), 20.2; Si, 2.69; W, 62.9.

$Cs_4[(CH_2{=}CHSi)_2O]SiW_{11}O_{39}$. The procedure was the same as that for the cesium 2-(carbomethoxy)ethyl derivative above except that vinyltrichlorosilane was used for the alkyltrichlorosilane: ^1H NMR (D_2O, 296 K) δ 6.41–5.99 (m). Anal. Calcd for $C_4H_6Cs_4O_{40}Si_3W_{11}$: C, 1.44; H, 0.18; Cs, 16.0; O, 19.2; Si, 2.53; W, 60.7. Found: C, 1.31; H, 0.22; Cs, 16.9; O (by diff), 19.2; Si, 2.43; W, 59.9.

$[(CH_3)_4N]_4[(CH_2{=}CHSi)_2O]SiW_{11}O_{39}$. The procedure was the same as that for the TMA 2-(carbomethoxy)ethyl derivative above except that vinyltrichlorosilane was used for the alkyltrichlorosilane: ^1H NMR (DMSO-d_6 296 K) δ 6.18–5.89 (m), 3.14 (s, 48 H, $(CH_3)_4N^+$). Anal. Calcd for $C_{20}H_{54}N_4O_{40}Si_3W_{11}$: C, 7.76; H, 1.76; N, 1.81; O, 20.7; Si, 2.72; W, 65.3. Found: C, 7.70; H, 1.73; N, 1.75; O (by diff), 20.9; Si, 2.67; W, 65.3.

$[(CH_3)_4N]_4[(CH_3CH_2Si)_2O]SiW_{11}O_{39}$. The procedure was the same as that for the TMA 2-(carbomethoxy)ethyl derivative above except that ethyltrichlorosilane was used for the alkyltrichlorosilane: ^1H NMR (DMSO-d_6 296 K) δ 3.15 (s, 48 H, $(CH_3)_4N^+$), 1.06 (t, 3 H), 0.55 (q, 2 H). Anal. Calcd for $C_{20}H_{58}N_4O_{40}Si_3W_{11}$: C, 7.75; H, 1.89; N, 1.81; O, 20.6; Si, 2.72; W, 65.2. Found: C, 7.76; H, 1.89; N, 1.77; O (by diff), 21.3; Si, 2.88; W, 64.4.

$[(CH_3)_4N]_4[(CH_3Si)_2O]SiW_{11}O_{39}$. The procedure was the same as that for the TMA 2-(carbomethoxy)ethyl derivative above except that methyl trichlorosilane was used for the alkyltrichlorosilane: ^1H NMR (DMSO-d_6 296 K) δ 3.14 (s, 48 H, $(CH_3)_4N^+$), 0.09 (s, 3 H). Anal. Calcd for $C_{18}H_{54}N_4O_{40}Si_3W_{11}$: C, 7.03; H, 1.77; N, 1.82; O, 20.8; Si, 2.74; W, 65.8. Found: C, 7.05; H, 1.75; N, 1.74; O (by diff), 22.4; Si, 2.84; W, 64.2.

Solubility and Stability Studies. The solubility of the complexes were assessed by preparing saturated solutions of the complexes in the solvent and subtracting the weight of undissolved material (compacted by centrifugation) from the weight of the sample prior to dissolution. The stability studies were conducted by monitoring saturated solutions of the complexes in D_2O as a function of time by ^1H NMR. Measurements were made every 24 h for 8 days. Integration of the spectra provided the quantity of the impurity(ies) and facilitated calculation of the percent decomposition.

Cell Culture Assays. The compounds were evaluated in human mitogen-stimulated PBMC infected with HIV-1 (strain LAV-1), as described previously.[23] Stock solutions (2 mM) of the compounds were freshly prepared in water prior to testing. Compounds were added to the cultures 1 h after infection. Virus was harvested 6 days later and quantitated by a reverse transcriptase assay using poly(rA)$_n$·oligo(dT)$_{12-18}$ as the template primer, as described previously.[23] The compounds were evaluated for their potential effects on uninfected mitogen-stimulated human PBMC. Cells were maintained for 6 days in the presence or absence of compounds at different concentrations. Cell viability was assessed by the trypan blue dye-exclusion method using a hemacytometer.[23] The EC_{50} and IC_{50} were calculated using the median effect method.[24]

Enzyme Assays. Assays for determining the degree of inhibition of the compounds against recombinant p66 HIV-1 reverse transcriptase and *Escherichia coli* DNA polymerase were performed as described previously.[25] The protein concentration for the DNA polymerase and viral enzyme used in the assays were similar. Phosphonoformate (PFA) provided by Astra ALab, Södertälje, Sweden, was used as a positive control.

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