Synthesis, Characterization, and Biological Activity of a New Potent Class of Anti-HIV Agents, the Peroxoniobium-Substituted Heteropolytungstates

Gyu-Shik Kim, Deborah A. Judd, Craig L. Hill,* and Raymond F. Schinazi

Department of Chemistry, Emory University, Atlanta, Georgia 30322, and Veterans Affairs Medical Center, and Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Decatur, Georgia 30033

Received August 9, 1993•

The mono- and trisubstituted peroxyniobium polyoxotungstates of formulas $[(CH_3)_3NH]_7[Si(NbO_2)_3W_9O_{37}]$, $Cs_7[Si(NbO_2)_3W_9O_{37}]$, α-$K_5[Si(NbO_2)W_{11}O_{39}]$ and α-$[(CH_3)_3NH]_5[Si(NbO_2)W_{11}O_{39}]$, have been prepared, purified, and characterized spectroscopically by ^{29}Si NMR, ^{183}W NMR, and IR. The presence of peroxo groups was verified by the yellow color of the product and quantified by iodometric titration. The potency of both the complexes and the precursor complexes was evaluated in human peripheral blood mononuclear cells (PBMC) acutely infected with human immunodeficiency virus type 1 (HIV-1). Hexaniobate ($K_7H[Nb_6O_{19}]$) was the least effective with a median effective concentration (EC_{50}) of >100 μM, while $Cs_7[Si(NbO_2)_3W_9O_{37}]$ was one of the most effective compounds with an EC_{50} of 1.0 μM. None of the compounds were toxic to uninfected PBMC with the exception of α-$K_8[SiW_{11}O_{39}]$, which had a median inhibitory concentration (IC_{50}) of 79 μM. The potency and selectivity of the complexes against HIV-1 reverse transcriptase was also evaluated and shown to be quite high (IC_{50} values from 0.03 to 0.06 μM). The trimethylammonium salts of the complexes were tested for their ability to inhibit the interaction between gp120 and CD4 using a cell-free system. The complex $[(CH_3)_3NH]_7[Si(NbO_2)_3W_9O_{37}]$ inhibited this interaction by 70% at 25 μM.

Introduction

Research in the field of anti-HIV agents has focused on nucleoside compounds. Unfortunately, the nucleoside chemotherapeutic agents approved for clinical use, 3′-azido-3′deoxythymidine (AZT), 2′,3′-dideoxycytidine (ddC), and 2′,3′-dideoxyinosine (ddI), all exhibit a range of toxic effects.[1] Moreover, many studies have indicated patients treated with these drugs for extended lengths of time develop a resistance to the drugs.[2] The development of new non-nucleoside drugs that do not suffer from the limitations exhibited by certain nucleosides is a vital goal of research. Such efforts have led to the discovery of a host of non-nucleoside reverse transcriptase (RT), Tat, and protease inhibitors.[1a] To date none of these compounds has shown significant promise in the clinic. A transition metal oxygen anion cluster, or polyoxometalate for convenience, HPA-23 (molecular formula = $(NH_4)_{17}H[NaSb_9W_{21}O_{86}]$) was found to be active against HIV-1 in cell culture.[3] HPA-23 proved to be too toxic and ineffective at the doses administered in clinical studies.[4] A beneficial consequence of the HPA-23 episode was that it provided impetus to investigate polyoxometalates as a new class of antiviral agents with multiple antiviral mechanisms. Subsequently, other polyoxometalates have proved to be far more active and less toxic in vitro and in vivo.[5] Polyoxometalates are attractive molecules since, in addition to inhibition of HIV-1 in vitro, certain compounds have been shown to affect herpesviruses.[6] Our group was the first to demonstrate that these compounds also inhibit the binding of HIV-1 to the receptor for this virus, CD4.[5c]

Polyoxometalates are large metal oxide clusters of the d^0 early transition metals V, Nb, Ta, Mo, and W.[7] A major limitation with the development of polyoxometalates as antiviral agents to date has been the incompatibility of many of these compounds with neutral aqueous media. Stability studies on the polyoxotungstates in buffered aqueous solution have demonstrated that at physiological pH many of these compounds fragment partially and, in a few cases, totally to lower nuclearity species (complexes with fewer transition metal ions per complex).[8] Acidic media favor the formation of polyoxotungstates such as the Keggin complexes of tetrahedral point group symmetry (formula = $[X^{n+}M_{12}O_{40}]^{(8-n)-}$, where X^{n+} is a p or d block heteroatom and M = Mo^{VI} or W^{VI}). $PW_{12}O_{40}^{3-}$, for example, is stable only below pH ~1.5.[7a] In contrast, basic media favor the formation of polyoxoniobates such as hexaniobate of octahedral point group symmetry (formula = $[Nb_6O_{19}]^{8-}$), which is prepared in molten KOH at pH >15. Dabbabi and Boyer reported the synthesis of a series of mixed addenda hexaniobotungstates of formula $[Nb_xW_{6-x}O_{19}]^{n-}$.[9] They found that lower pH conditions favored the formation of compounds enriched in tungsten relative to niobium, and higher pH conditions favored formation of compounds enriched in niobium relative to tungsten. The pH range of stability was also found to parallel the composition of the compound, with higher niobium content resulting in a higher pH range of stability. For example, the pH ranges of stability of $[Nb_2W_4O_{19}]^{4-}$ and $[Nb_4W_2O_{19}]^{6-}$ are 4.5–7.5 and >8.5, respectively.[9] The stability at pH values greater than 7 of the niobium-containing polyoxometalate with the formula $((CH_3)_3NH)_8[Si_2W_{18}Nb_6O_{77}]$, prepared and thoroughly characterized by Finke and Droege, further established the advantages of niobium substitution on the hydrolytic stability as a function of pH.[10]

The poor stability of niobium in aqueous solutions below pH 10 has limited the use of this element in synthetic procedures. Synthetic routes to niobium-containing polyoxometalates utilize niobium(V) solubilized by the addition of aqueous hydrogen peroxide forming soluble peroxoniobates.[9,10] All of the preparations involve reduction of the niobium peroxo species to the corresponding oxo species prior to isolation of the product. Recently, Finke and Droege have reported the synthesis of the triperox-

• Abstract published in *Advance ACS Abstracts*, February 1, 1994.

0022-2623/94/1837-0816$04.50/0

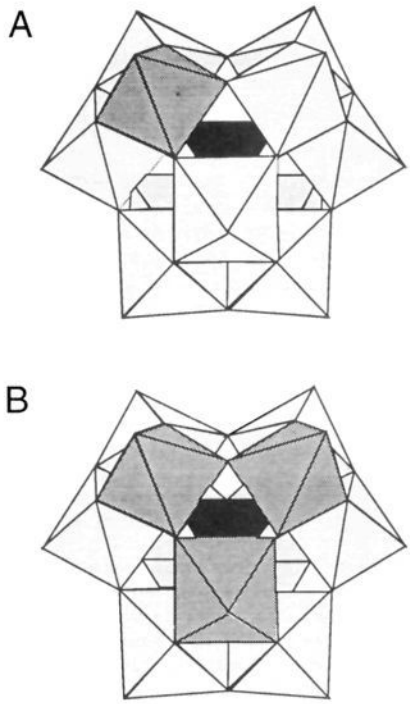

Figure 1. Polyhedral drawing of the niobopolyoxotungstate antiviral agents in this study. The darker shaded polyhedra represent niobium atoms. A: the monosubstituted complex, α-[Si(NbO$_2$)W$_{11}$O$_{39}$]$^{5-}$, of C_s point group symmetry. B: the trisubstituted complex, [Si(NbO$_2$)$_3$W$_9$O$_{37}$]$^{7-}$, of C_{3v} point group symmetry viewed from off the C_3 axis.

yniobium-containing polyoxometalate ((*n*-C$_4$H$_9$)$_4$N)$_7$[Si(NbO$_2$)$_3$W$_9$O$_{37}$] by omitting the reduction step before isolation of the product.[11] We report here the synthesis of two novel water-soluble salts of this polyoxometalate, [(CH$_3$)$_3$NH]$_7$[Si(NbO$_2$)$_3$W$_9$O$_{37}$], TMA(SiNb$_3$), and Cs$_7$[Si(NbO$_2$)$_3$W$_9$O$_{37}$], Cs(SiNb$_3$), and demonstrate this protocol may be generalized to the preparation of other peroxyniobium polyoxometalates from lacunary polyoxometalates: α-K$_5$[Si(NbO$_2$)W$_{11}$O$_{39}$], K(SiNb), and α-[(CH$_3$)$_3$NH]$_5$[Si(NbO$_2$)W$_{11}$O$_{39}$], TMA(SiNb) (Figure 1). We further report that all these complexes have attractive chemotherapeutic profiles against HIV in cell culture, and one of them, TMA(SiNb$_3$), inhibits the interaction of HIV-1 gp120 with CD4 by 70% at 25 μM.

Results and Discussion

Synthesis of Title Compounds. The attempted synthesis of [Si(NbO$_2$)W$_{11}$O$_{39}$]$^{5-}$ via the procedure successfully used by Finke and Droege to prepare the triniobium-substituted complex [Si(NbO$_2$)$_3$W$_9$O$_{37}$]$^{7-}$ failed. The Finke–Droege synthesis involved the addition of a stoichiometric amount of the lacunary complex A-β-Na$_9$H[SiW$_9$O$_{34}$] to an acidified aqueous solution of hydrogen peroxide and hexaniobate. Large quantities of α-K$_4$[SiW$_{12}$O$_{40}$] are formed as unequivocally indicated by a ^{29}Si NMR resonance at −86.3 ppm and a ^{183}W NMR resonance at −103.9 ppm.[7a] By changing the order of addition of the acid and lacunary complex; however, the amount of side product was greatly decreased. All the K$_4$[SiW$_{12}$O$_{40}$] impurity in K(SiNb) could be separated by differential precipitation or salting out using KCl. Precipitation using trimethylamine hydrochloride is far less satisfactory, consistently resulting in an impure precipitate that must be recrystallized. Recrystallization of TMA-SiNb was achieved from a DMF/water mixture.

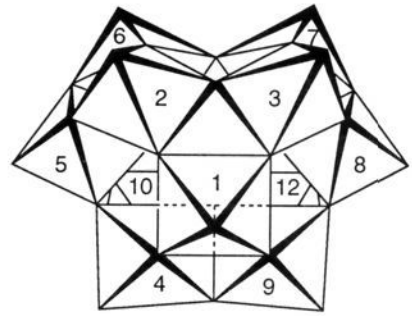

Figure 2. IUPAC numbering scheme of the α-Keggin anion. The substituted niobium atom occupies site 1 for α-[Si(NbO$_2$)W$_{11}$O$_{39}$]$^{5-}$.

The spectroscopic (^{29}Si NMR, ^{183}W NMR, IR) and analytical data confirmed the high-yield preparation and purification of a monosubstituted derivative of the α-Keggin structure (C_s point group symmetry) of formula α-[Si(NbO$_2$)W$_{11}$O$_{39}$]$^{5-}$ (Figure 1A). The ^{29}Si NMR exhibited one resonance at −86.1 ppm compared to −85.8 ppm for [SiW$_{11}$O$_{39}$]$^{8-}$ and −86.3 ppm for [SiW$_{12}$O$_{40}$]$^{4-}$.[12] The complex [SiW$_{11}$O$_{39}$]$^{8-}$ of C_s point group symmetry exhibited six ^{183}W NMR resonances between −100.9 to −176.2 ppm in a ratio of 2:2:1:2:2:2,[7a] while the complex [SiW$_{12}$O$_{40}$]$^{4-}$ exhibited one resonance at −103.8 ppm.[7a] The product KSiNb exhibited six resonances between −92.4 and −128.7 ppm in a ratio of 2:2:2:1:2:2. The peaks in the spectrum were assigned by comparison to the assignments made by Domaille for [SiVW$_{11}$O$_{40}$]$^{5-}$.[13] The quadrupolar properties of ^{93}Nb (I = 9/2) and ^{51}V (I = 7/2) result in substantial broadening of the ^{183}W lines of the atoms adjacent to the site of substitution. This information may be used to assign the peaks in the spectra. Figure 2 shows the IUPAC numbering scheme of the monosubstituted α-Keggin structure.[13] Since W11 is a unique atom, this may be assigned on the basis of integration as the resonance at −118.1 ppm (line width = $\Delta\nu_{1/2}$ = 1.1 Hz). Domaille observed that in all spectra of the monovanadium-substituted Keggin complexes, the tungsten resonance due to W4 which shares two oxygen atoms with V(V) in the V containing triad is consistently the most deshielded. Therefore the resonance furthest downfield at −92.4 ppm ($\Delta\nu_{1/2}$ = 1.9 Hz) was assigned to W4 and W9. Another observation made by Domaille was that the tungsten atom sharing a corner with the vanadium atom in the adjacent triad, W2, was found to be shielded relative to the unsubstituted Keggin. In the case of [SiVW$_{11}$O$_{40}$]$^{5-}$, this resonance was the furthest upfield. On this basis, the resonance at −128.7 ppm ($\Delta\nu_{1/2}$ = 6.1 Hz) was assigned to W2 and W3. Domaille also observed that W6 was the second most deshielded resonance and futhermore, in all cases, was only slightly shifted from that of the parent compound. Consequently, the resonance at −106.2 ($\Delta\nu_{1/2}$ = 3.3 Hz) was attributed to W6 and W7 (note: δ for [SiW$_{12}$O$_{40}^{4-}$] is −103.9 ppm). The resonances at −108.6 ($\Delta\nu_{1/2}$ = 3.3 Hz) and −127.1 ppm ($\Delta\nu_{1/2}$ = 1.9 Hz) could not be unambiguously assigned. On the basis of line widths, however, the broader resonance at −108.6 ppm may be tentatively assigned to W5 and W8 and the resonance at −127.1 ppm to W10 and W12.

The IR of α-[Si(NbO$_2$)W$_{11}$O$_{39}$]$^{5-}$ exhibited features similar to that of the parent Keggin complex, α-[SiW$_{12}$O$_{40}$]$^{4-}$. All of the bands were shifted to lower frequencies by approximately 7–8 cm^{-1}, which was indicative of slightly lower overall stability and weaker bonds in α-[Si-

Table 1. Anti-HIV-1 Activity and Toxicity of the Mono- and Triperoxyniobium Polyoxotungstates and Their Precursor Complexes in Human PBMC

formula	$EC_{50} \pm SD^a$ (μM)	IC_{50},[b] (μM) (dThd/PBMC)
$K_7H[Nb_6O_{19}]$	>100	>100 (11)
α-$K_8[SiW_{11}O_{39}]$	1.2 ± 1.0	79.5
α-$K_5[Si(NbO_2)W_{11}O_{39}]$	1.6 ± 1.1	>100 (35)
α-$((CH_3)_3NH)_5[Si(NbO_2)W_{11}O_{39}]$	1.8 ± 1.4	>100 (36)
α-$Cs_7[Si(NbO_2)_3W_9O_{37}]$	1.0 ± 0.9	>100 (31)
α-$((CH_3)_3NH)_7[Si(NbO_2)_3W_9O_{37}]$	2.4 ± 0.6	>100 (8)
control; HPA-23	0.39[c]	35[c]

[a] EC_{50} = median effective (antiviral) concentration against HIV-1 in PBMC. [b] IC_{50} = median inhibitory (toxicity) concentration determined using radioactive thymidine uptake in PBMC. Number in parentheses indicates the percent inhibition at 100 μM. [c] See ref 5d.

Table 2. Inhibition of Reverse Transcriptase (RT) and DNA Polymerase α Activity by the Mono- and Triperoxyniobium Polyoxotungstates

formula	IC_{50}[a] (μM)	IC_{50}[b] (μM)
α-$K_5[Si(NbO_2)W_{11}O_{39}]$	0.04 (0.91)	6.9 (0.99)
α-$TMA_5[Si(NbO_2)W_{11}O_{39}]$[c]	0.06 (0.99)	5.0 (0.99)
α-$Cs_7[Si(NbO_2)_3W_9O_{37}]$	0.03 (0.97)	9.6 (0.98)
α-$TMA_7[Si(NbO_2)_3W_9O_{37}]$	0.23 (0.98)	6.3 (0.97)
control; PFA	0.06 (0.92)	>50

[a] IC_{50} = median inhibitory concentration to inhibit RT activity by 50%. Number in parentheses indicates the correlation coefficient derived by the Chou method.[23] [b] IC_{90} = median inhibitory concentration to inhibit DNA polymerase α activity by 50%. Number in parentheses indicates the correlation coefficient. [c] TMA = trimethylamine.

$(NbO_2)W_{11}O_{39}]^{5-}$ relative to α-$[SiW_{12}O_{40}]^{4-}$.[14] Most bands in the metal–oxygen regime in the IR were broadened as they contain contributions from both Nb–O and W–O modes. The band at 978 cm^{-1} was shifted to 969 cm^{-1} and has a small shoulder, reflecting a contribution of the terminal Nb–O bond. A single band observed at 913 cm^{-1} was attributed to the stretching fundamental of the internal SiO_4 unit, indicating that the symmetry around this structural unit was maintained to a considerable degree upon substitution of a terminal W oxo for a terminal Nb peroxo group. A band at 884 cm^{-1} present in α-$[Si(NbO_2)W_{11}O_{39}]^{5-}$ that is absent in the spectrum of $[SiW_{12}O_{40}]^{4-}$ was attributed to the O–O stretch of the peroxo group on the niobium.

Biological Results

Activity and Toxicity in Cell Culture. The four new polyoxometalates were evaluated in human peripheral blood mononuclear cells for their anti-HIV-1 activity and toxicity. Table 1 lists the median effective concentration (EC_{50}) and median inhibitory concentration (IC_{50}) for all four compounds as well as for the parent polyoxometalates. Although the niobium-substituted polyoxometalates exhibited similar antiviral activity when compared to the precursor polyoxometalate α-$K_8[SiW_{11}O_{39}]$, they were all markedly less toxic. Compound $Cs_7[Si(NbO_2)_3W_9O_{37}]$, $Cs(SiNb_3)$, was one of the most potent, with an EC_{50} of 1.0 μM, and showed no toxicity to the uninfected human lymphocytes when tested up to 100 μM. However, on the basis of data on replicate assay, this difference was not significantly greater than for the other Nb analogues.

Activity against HIV-1 RT. The complexes are quite potent against recombinant HIV-1 reverse transcriptase as indicated by the data presented in Table 2. IC_{50} values are significantly lower than for the values obtained with DNA polymerase α. However, they are lower than the cell culture EC_{50} values against HIV-1, suggesting that inhibition of HIV-1 RT may not be the primary mechanism of action.

Inhibition of gp120-CD4 Interaction. The ability of the trimethylammonium salts of the mono- and triperoxyniobium-substituted polyoxometalates to inhibit the interaction between HIV-1 gp120 and CD4 was evaluated using an enzyme immunosorbent assay. The triperoxyniobium-substituted analog was the most effective, inhibiting this interaction by 70% at a concentration of 25 μM, while the monoperoxyniobium analog, α-$[Si(NbO_2)W_{11}O_{39}]^{5-}$, was substantially less effective, inhibiting the interaction by only 38% at a concentration of 25 μM.

Experimental Section

Chemistry. Materials and Methods. All chemicals and organic solvents were commercially available, reagent grade, and used without further purification. The concentration of hydrogen peroxide was quantified by titration against potassium permanganate.[15] Aqueous reactions were carried out in deionized water. The pH was measured using colorPHast indicator strips unless otherwise indicated. The polyoxometalates $K_7H[Nb_6O_{19}]$,[16] α-$K_8[SiW_{11}O_{39}]$,[17] and A-β-$Na_9H[SiW_9O_{34}]$[18] were prepared by literature procedures. The purity and identification of the compounds were accessed by IR, ^{29}Si or ^{31}P NMR, ^{183}W NMR, and elemental analyses. IR spectra were obtained as KBr pellets (1–4 wt % in KBr) using a Nicolet 510M FTIR spectrometer. Elemental analyses were conducted by Atlantic Microlab Inc. (Norcross, Ga) for C, H, N. All other elements were analyzed by E + R Microanalytical Lab Inc. (Corona, NY) or Galbraith Laboratories (Knoxville, TN). The ^{29}Si, ^{31}P, and ^{183}W NMR spectra were run on a IBM WP-200SY FT spectrometer at 39.76, 81.02, and 8.34 MHz, respectively. The probe temperature was 295 K in all NMR experiments. In reporting NMR data, chemical shifts upfield from the references are reported as negative values. The number of nuclei causing the resonance and line widths are reported. A chromium(III) relaxation agent was used to reduce the relaxation delay in the ^{29}Si NMR experiments. These spectra were recorded on approximately 200 mM solutions of the polyoxometalate containing 5.6 mM Cr^{III}(diethylenetriaminepentaacetic acid, disodium salt) in Wilmad 513-7PP 10-mm-i.d. NMR tubes. The spectra were referenced to 3-(trimethylsilyl)-propionic acid, sodium salt. The pulse width was 7 μs, the relaxation delay was 2 s, and the acquisition time was 1.02 s. For ^{31}P NMR, the samples were approximately 20 mM in polyoxometalate and referenced to 85% H_3PO_4/D_2O. The spectra were run in Wilmad 513-7PP 10-mm-i.d. NMR tubes. The pulse width was 14.8 μs, the relaxation delay was 1 s, and the acquisition time was 0.5 s. The acquisition of ^{183}W NMR spectra required a custom-made probe, designed and made by Cryomagnetic Systems, which required the use of Wilmad 515-7PP 15-mm-i.d. NMR tubes. The concentration of polyoxometalate was approximately 200 mM, and the spectra were referenced to 2 M Na_2WO_4 in D_2O. The pulse width was 79.0 μs, the relaxation delay was 1 s, and the acquisition time was 4.096 s.

The number of niobium peroxide groups in these compounds was quantified by an iodometric titration. Sodium thiosulfate was used as the titrant and was standardized against potassium iodate using starch as the indicator. The compounds were titrated using the procedure given by Day and Underwood for hydrogen peroxide.[15]

Synthesis of α-$K_5[Si(NbO_2)W_{11}O_{39}]$ [K(SiNb)]. $K_7H[Nb_6O_{19}]$ (1g, 0.88 mmol) was dissolved in 75 mL of deionized water. Hydrogen peroxide (2 mL of 30–35% aqueous solution or 11.6 M, 0.0232 mol) was added to the hexaniobate solution. Hydrochloric acid (3 M) was added to adjust the pH of the solution to approximately 7.[19] α-$K_8[SiW_{11}O_{39}]$ (15.78 g, 5.3 mmol) was added to the hexaniobate solution followed by 25 mL of H_2O resulting in a suspension. To this was added approximately 12 mL of 3 M HCl resulting in a final pH of around 1.[20] The solution was then stirred for 30 min while the pH was monitored. After

30 min a clear yellow solution of pH approximately 0–1 was produced. Potassium chloride (14 g, 0.188 mol) was added resulting in the formation of 6.2 g (2.06×10^{-3} mol, 38.9% yield based on α-$[SiW_{11}O_{39}^{8-}]$) of a yellow amorphous precipitate: IR (1200–400 cm^{-1}) 1009, 969, 913, 884 (sh), 779, 671 (sh), 597, 545, 526; ^{29}Si NMR (in D_2O) −86.22; ^{183}W NMR (in D_2O) −92.3 (2W), −106.2 (2W), −108.6 (2W), −118.0 (1W), −127.0 (2W), −128.6 (2W). In some cases a small quantity of α-$[SiW_{12}O_{40}]^{4-}$ (−103.6) was observed. The filtrate was saved and additional KCl (10 g, 0.134 mol) was added. The solution was refrigerated overnight resulting in the crystallization of 6.2 g (2.08×10^{-3} mol, 39.17% yield based on α-$[SiW_{11}O_{39}]^{8-}$) of a bright yellow crystalline product. In no case was α-$[SiW_{12}O_{40}]^{4-}$ detected in the second precipitate. IR (1200–400 cm^{-1}) 1011, 969, 914, 884 (sh), 779, 670 (sh), 597, 545, 525; ^{29}Si NMR (in D_2O) −86.06; ^{183}W NMR (in D_2O) −92.4 (2W), −106.2 (2W), −108.6 (2W), −118.1 (1W), −127.1 (2W), −128.7 (2W). Anal. Calcd (found): K 6.53 (6.53), Nb 3.10 (2.93), Si 0.94 (0.99), W 67.53 (67.65), O (by difference) 21.9 (21.9). The total yield was 12.4 g (4.14×10^{-3} mol), corresponding to a 78% yield based on α-$[SiW_{11}O_{39}]^{8-}$.

Synthesis of α-$[(CH_3)_3NH]_5[Si(NbO_2)W_{11}O_{39}]\cdot CH_3CN$ [TMA(SiNb)]. The above procedure was followed with the exception of using trimethylamine hydrochloride as the precipitating reagent. Addition of trimethylamine hydrochloride (13.7 g, 0.143 mol) resulted in the precipitation of 26.9 g (8.68×10^{-3} mol) of crude product, TMA(SiNb). The precipitate was collected and washed with ethanol followed by ether. Recrystallization was accomplished using water/DMF as a mixed solvent and by placing the solution in the freezer overnight. A whitish yellow amorphous film formed on the bottom of the crystallizing dish. The yellow solution was decanted from the solid, evaporated to half its volume, and returned to the freezer. A microcrystalline solid (3.78 g, 1.22×10^{-3} mol) formed and was collected: IR (3200–2500; 1200–400 cm^{-1}) 3137, 3045, 2965, 2929, 2851 (sh), 2972, 2523, 2471, 1099, 1050, 1009, 966, 913, 884 (sh), 783, 670 (sh), 597, 544 (sh), 527, 480 (sh); ^{183}W NMR (Li^+ salt in D_2O) −95.0 (2W), −108.8 (2W), −111.0 (2W), −120.3 (1W), −129.7 (2W), −132.0 (2W). The filtrate was evaporated to 75% of its original volume. The mother liquor was decanted away and left to dry at room temperature for 1 week. A yellow microcrystalline solid resulted (9.18 g, 2.96×10^{-3} mol): IR (3200–2500; 1200–400 cm^{-1}) 3442, 3140, 3036, 2966, 2929, 2752, 2519, 2468, 1106, 1049, 1008, 970, 915, 883 (sh), 782, 668 (sh), 598, 543 (sh), 527, 510 (sh); ^{183}W NMR (Li^+ salt in D_2O) −94.9 (2W), −108.7 (2W), −111.0 (2W), −120.4 (1W), −129.6 (2W), −131.9 (2W). Anal. Calcd (found): C 6.50 (6.81), H 1.70 (1.77), N 2.68 (2.57), Nb 2.96 (2.98), Si 0.89 (0.80), W 64.39 (64.98), O (by difference) 20.88 (20.09). The total yield was 12.96 g, 4.18×10^{-3} mol, and 40% yield based on $[SiW_{11}O_{39}]^{8-}$.

Synthesis of $[(CH_3)_3NH]_7[Si(NbO_2)_3W_9O_{37}]$ [TMA(SiNb$_3$)]. $K_7H[Nb_6O_{19}]$ (6.5 g, 5.7×10^{-3} mol) was dissolved in 400 mL of deionized water. Hydrogen peroxide (40 mL of 30–35% aqueous solution or 11.6 M, 0.464 mol) was added to the hexaniobate solution. The reaction mixture was acidified with HCl (20 mL, 3 M). To the acidified hexaniobate/peroxide solution (pH 1), A-β-$Na_9H[SiW_9O_{34}]$ (25.1 g, 9.98×10^{-3} mol) was added as a solid. The solution was diluted to a volume of 700 mL with H_2O, resulting in a clear, dark, orange-yellow solution. Addition of trimethylamine hydrochloride (70 g, 0.73 mol), followed by evaporation of the solvent at room temperature to approximately 350 mL, resulted in a yellow precipitate (12.38 g, 4.30×10^{-3} mol, 43% yield based on A-β-$Na_9H[SiW_9O_{34}]$): IR (3200–2500; 1200–400 cm^{-1}) 3118, 3029, 2956, 2851, 2738, 1056, 986, 958, 906, 870, 858 (sh), 794, 666, 594, 577 (sh), 535, 487, 474; ^{29}Si NMR (in D_2O) −84.29; ^{183}W NMR (Li^+ salt in D_2O) −115.4 (6W), −139.3 (3W). Anal. Calcd (found): C 8.22 (7.58), H 2.30 (2.64), N 3.19 (2.89), Nb 9.08 (8.79), Si 0.91 (1.00), W 53.89 (53.78), O (by difference) 22.41 (23.32).

Synthesis of $Cs_{6.7}H_{0.3}[Si(NbO_2)_3W_9O_{37}]\cdot 2H_2O$ [Cs(SiNb$_3$)]. The procedure for TMA(SiNb$_3$) above was followed on one-tenth the scale using 10 g (0.059 mol) of cesium chloride as the precipitating reagent. A yellow amorphous solid resulted (3.15 g, 9.28×10^{-4} mol, 93% yield based on A-β-$Na_9H[SiW_9O_{34}]$): IR (1200–400 cm^{-1}) 990, 956, 899, 866, 786, 668, 597, 577 (sh), 535, 518 (sh), 477; ^{183}W NMR (Li^+ salt in D_2O) −113.8 (6W), −140.0 (3W). Anal. Calcd (found): Cs 23.62 (23.43), Nb 7.62 (7.46), Si 0.77 (0.66), W 45.23 (45.76), O (by difference) 23.18 (22.24).

Virology/Biochemistry. Cell Culture Assays. The compounds were evaluated in human mitogen-stimulated peripheral blood mononuclear cells (PBMC) infected with the LAV-1 strain of HIV-1 as previously described.[21] Sample solutions were prepared immediately prior to testing at concentrations of 20 or 40 mM in H_2O. The diluted solutions of compounds were added to the infected cells 1 h after infection with HIV-1. The viruses were harvested 6 days later for quantitation as previously described.[21] The compounds were evaluated for their effect on uninfected mitogen-stimulated human PBMC using a radiolabeled thymidine uptake method as described previously.[22] The EC_{50} and IC_{50} were calculated using the median effect method.[23]

Activity against HIV-1 RT. The activity of the complexes against recombinant HIV-1 reverse transcriptase was evaluated using a poly $r(A)_n$·oligo$(T)_{12-18}$ template primer, as described elsewhere.[24] For these studies we used a p66/53/55 HIV-1 RT obtained from BioTechnology General Inc., Rehovot, Israel. The specific activity of the enzyme was 2000 units/mg protein. One unit is defined as the amount of enzyme which catalyzes the incorporation of 1 nmol of TMP into DNA in 10 min at 37 °C. DNA polymerase assays were performed as described previously.[24a]

gp120-CD4 Enzyme Immunosorbent Assay. The DuPont NENQUEST: HIV gp120/CD4 Receptor EIA (NED-006) designed to evaluate materials for activity in blocking the gp120/CD4 interaction was used.[25] The samples were assayed in triplicate as described by the manufacturer. The assay entailed using CD4 coated onto microtiter wells. The compounds tested were co-incubated with a fixed quantity of gp120. Unbound gp120 was washed, and the amount of gp120 bound to CD4 was colorimetrically detected by incubation with a monoclonal antibody against gp120 conjugated to horseradish peroxidase (HRP). The unbound HRP was washed, and color development was accomplished by addition of *o*-phenylenediamine (OPD) and hydrogen peroxide. The product of the enzymatic reaction was monitored at 492 nm. Since a fixed quantity of gp120 was added to each well, any inhibition of gp120 binding to CD4 will result in a decreased amount of gp120 captured and therefore, result in a decrease in the optical density after incubation of OPD with HRP. The optical density was measured at 492 nm on a Titertek Multiskan MC plate reader. The percent inhibition was then calculated.

Acknowledgment. We would like to thank the National Institutes of Health (grant no. AI-32903) for support and the Department of Veterans Affairs.

References

(1) (a) Schmazi, R. F.; Mead, J. R.; Feorino, P. M. Insights into HIV chemotherapy. *AIDS Res. Hum. Retroviruses* **1992**, *8* (6), 963–990. (b) Saag, M. S. Adverse Effects. *Hospital Practice* **1992**, *27* (Supp 2), 26–36.

(2) (a) Richman, D. D. Zidovudine resistance of human immunodeficiency virus. *Rev. Infect. Dis.* **1990**, *12*, S507-S512. (b) Larder, B. A.; Darby, G.; Richman, D. D. HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* **1989**, *243*, 1731–1734. (c) Larder, B. A.; Chesebro, B.; Richman, D. D. Susceptibilities of zidovudine-susceptible and -resistant human immunodeficiency virus isolates to antiviral agents determined by using a quantitative plaque reduction assay. *Antimicrob. Agents Chemother.* **1990**, *34*, 436–441. (d) St. Clair, M. H.; Martin, J. L.; Tudor-Williams, G.; Bach, M. C.; Vavro, C. L.; King, D. M.; Kellam, P.; Kemp, S. D.; Larder, B. A. Resistance to ddI and sensitivity to AZT induced by a mutation in HIV-1 reverse transcriptase. *Science* **1991**, *253*, 1557–1559. (e) Gao, Q.; Gu, Z.; Parniak, M. A.; Li, X.; Wainberg, M. A. *In vitro* selection of variants of human immunodeficiency virus type 1 resistant to 3′-azido-3′-deoxythymidine and 2′,3′-dideoxyinosine. *J. Virol.* **1992**, *66*, 12–19.

(3) (a) Dormont, D.; Yeramian, P.; Lambert, P.; Spire, B.; Daveloose, D.; Barre-Sinoussi, F. C.; Chermann, J. C. Cellular and molecular mechanisms of anti-retroviral effects of HPA-23. *Cancer Detect. Prev.* **1988**, *12*, 181–94. (b) Rosenbaum, W.; Dormont, D.; Spire, B.; Vilmer, E.; Gentilini, M.; Griscelli, C.; Montagnier, L.; Barre-Sinoussi, F.; Chermann, J.-C. Antimoniotungstate (HPA 23) treatment of three patients with AIDS and one with prodrome. *Lancet* **1985**, 450–451.

(4) (a) Burgard, M.; Sansonetti, P.; Vittecoq, D.; Descamps, P.; Guetard, D.; Herson, S.; Rozenbaum, W.; Rouzioux, C. Lack of HPA-23 antiviral activity in HIV-infected patients without AIDS. *AIDS* 1989, *3*, 665–668. (b) Moskovitz, B. L. Clinical trial of tolerance of HPA-23 in patients with acquired immune deficiency syndrome. *Antimicrob. Agents Chemother.* 1988, *32*, 1300–1303.

(5) (a) Hill, C. L.; Kim, G.-S.; Prosser-McCartha, C. M.; Judd, D. A. Polyoxometalates in Catalytic Selective Homogeneous Oxidation and Anti-HIV Chemotherapy. In *Polyoxometalates: From Platonic Solids to Anti-retroviral Activity*; Pope, M. T., Muller, A., Eds.; Kluwer Academic Publishers: Dordecht, The Netherlands. 1994, in press. (b) Ni, L.; Boudinot, F. D.; Boudinot, S. G.; Henson, G. W.; Bossard, G. E.; Martellucci, S. A.; Ash, P. W.; Fricker, S. P.; Darkes, M. C.; Theobald, B. R. C.; Hill, C. L.; Schinazi, R. F. Pharmacokinetics of antiviral polyoxometalates in rats. *Antimicrob. Agents Chemother.* 1994, in press. (c) Hill, C. L.; Hartnup, M.; Faraj, M.; Weeks, M.; Prosser-McCartha, C. M.; Brown, R. B., Jr.; Kadkhodayan, M.; Sommadossi, J.-P.; Schinazi, R. F. Polyoxometalates as Inorganic anti-HIV-1 compounds. Structure activity relationships. In Advances in Chemotherapy of AIDS; Diasio, R. B., Sommadossi, J.-P., Eds. Pergamon Press, Inc.: New York, 1990; pp 33–41. (d) Yamamoto, N.; Schols, D.; De Clercq, E.; Debyser, Z.; Pauwels, R.; Balzarini, J.; Nakashima, H.; Baba, M.; Hosoya, M.; Snoeck, R.; Neyts, J.; Andrei, G.; Murrer, B. A.; Theobald, B.; Bossard, G.; Henson, G.; Abrams, M.; Picker, D. Mechanism of anti-human immunodeficiency virus action of polyoxometalates, a class of broad-spectrum antiviral agents. *Mol. Pharmacol.* 1992 ,*42*, 1109–1117.

(6) Souyri-Caporale, M.; Tovey, G.; Ono, K.; Jasmin, C.; Chermann, J.-C. Modulation by the polyoxotungstate HPA-23 of Epstein-Barr virus early antigen expression in Raji cells treated with iododeoxyuridine. *J. Gen. Virol.* 1984, *65*, 831–835. (b) Ablashi, D. V.; Twardzik, D. R.; Easton, J. M.; Armstrong, G. R.; Luetzeler, J.; Jasmin, C.; Chermann, J.-C. Effects of 5-tungsto-2-antimoniate in oncogenic DNA and RNA virus-cell systems. *Eurp. J. Genet.* 1977, *13*, 713–720.

(7) General reviews of polyoxometalates: (a) Pope, M. T. *Heterpoly and Isopoly Oxometalates*; Springer-Verlag: Berlin, 1983. (b) Pope, M. T.; Muller, A. Polyoxometalate chemistry: An old field with new dimensions in several disciplines. *Angew. Chem., Int. Ed. Engl.* 1991, *30*, 34–48. (c) Day, V. W.; Klemperer, W. G. Metal oxide chemistry in solution: The early transition metal polyoxoanions. *Science* 1985, *228*, 533.

(8) Hill, C. L.; Weeks, M. S.; Schinazi, R. F. Anti-HIV-1 activity, toxicity and stability studies of representative structural families of polyoxometalates. *J. Med. Chem.* 1990, *33*, 2767–2772.

(9) Dabbabi, M.; Boyer, M. Syntheses et proprietes d'hexa niob(V)-tungstates(VI). (Syntheses and properties of hexaniobium(V) tungstates(VI).) *J. Inorg. Nucl. Chem.* 1976, *38*, 1011–1014.

(10) Droege, M. W.; Finke, R. G. Trisubstituted heteropolytungstates as soluble metal oxide analogues. 1. The preparation, characterization, and reactions of organic solvent soluble forms of $Si_2W_{18}Nb_6O_{77}^{8-}$, $SiW_9Nb_3O_{40}^{7-}$, and the $SiW_9Nb_3O_{40}^{7-}$ supported organometallic complex $[(C_5Me_5)Rh{\cdot}SiW_9Nb_3O_{40}]^{5-}$. *J. Am. Chem. Soc.* 1984, *106*, 7274–7277.

(11) Droege, M. W.; Finke, R. G. A novel triperoxyniobium-containing polyoxoanion, $SiW_9(NbO_2)_3O_{37}^{7-}$: Synthesis, characterization, catalytic allylic epoxidations with H_2O_2 and preliminary kinetic studies. *J. Mol. Catal.* 1991, *69*, 323.

(12) Weeks, M. S.; Hill, C. L.; Schinazi, R. F. Synthesis, characterization and anti-human immunodeficiency virus activity of water soluble salts of polyoxotungstate anions with covalently attached organic groups. *J. Med. Chem.* 1992, *35*, 1216–1221.

(13) Domaille, P. 1- and 2-Dimensional tungsten-183 and vanadium-51 NMR characterization of isopolymetalates and heteropolymetalates. *J. Am. Chem. Soc.*, 1984, *106*, 7677–7687.

(14) Rocchiccioli-Deltcheff, C.; Thouvenot, R. Metal complexes of heteropolyanions α-$XM_{11}O_{39}^{n-}$ with X = Si^{IV} or P^{V} and M = Mo^{VI} or W^{VI}: Study of structural modifications of ligand by infrared and raman spectrometry. *J. Chem. Res.* 1977, Part S, *2*, 46–47.

(15) Day, R. A.; Underwood, A. L. In *Quantitative Analysis*;5th ed.; Prentice Hall: Englewood Cliffs, 1986.

(16) Filowitz, M.; Ho, R. K. C.; Klemperer, W. G.; Shum, W. ^{17}O nuclear magnetic resonance spectroscopy of polyoxometalates. 1. Sensitivity and resolution. *Inorg. Chem.* 1979, *18*, 93.

(17) Hervé, G.; Tézé, A. Formation et isomerisation des undeca et dodeca tungstosilicates et germanates isomeres. (Formation and isomerization of undeca- and dodecatungstosilicate and -germanate isomers.) *J. Inorg. Nucl. Chem.* 1977, *39*, 999–1002.

(18) Finke, R. G.; Rapko, B.; Saxton, R. J.; Domaille, P. J. Trisubstituted heteropolytungstates as soluble metal oxide analogues. 3. Synthesis, characterization, ^{31}P, ^{29}Si, ^{51}V, and 1- and 2-D ^{183}W NMR, deprotonation, and H^+ mobility studies of organic solvent soluble forms of $H_xSiW_9V_3O_{40}^{(x-7)}$ and $H_xP_2W_{15}V_3O_{62}^{(x-9)}$. *J. Am. Chem. Soc.* 1986, *108*, 2957.

(19) If the hexaniobate solution became cloudy, the solution was discarded and a new solution was made.

(20) If the solution is filtered before the addition of hydrochloric acid, significant quantities of $SiW_{12}O_{40}^{4-}$ are formed upon acidification.

(21) Schinazi, R. F.; Sommadossi, J.-P.; Saalmann, V.; Cannon, D. L.; Xie, M. W.; Hart, G. C.; Smith, G. A.; Hahn, E. F. Activity of 3′-azido-3′-deoxythymidine nucleotide dimers in primary lymphocytes infected with human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* 1990, *34*, 1061–1067.

(22) Bardos, T. J., Schinazi, R. F.; Ling, K.-H. J.; Heider, A. R. Structure-activity relationships and mode of action of 5-mercapto-substituted oligo- and polynucleotides as antitemplates inhibiting replication of human immunodeficiency virus type 1. *Antimicrob. Agents Chemother.* 1992, *36*, 108–114.

(23) Chou, J.; Chou, T.-C. Dose-effect analysis with microcomputers: Quantitation of ED_{50}, LD_{50}, synergism, antagonism, low-dose risk, receptor binding, and enzyme kinetics. *A computer software for Apple II Series and IBM-PC and Instruction Manual Elsevier-Biosoft*; Elsevier Science Publishers: Cambridge, U.K., 1985.

(24) (a) Eriksson, B. F. H.; Schinazi, R. F. Combination of 3′-azido-3′-deoxythymidine (Zidovudine) and phosphonoformate (Forscarnet) against human immunodeficiency virus type 1 and cytomegalovirus in vitro. *Antimicrob. Agents Chemother.* 1989, *33*, 863–669. (b) Schinazi, R. F.; Eriksson, B. F. H.; Hughes, S. H. Comparison of inhibitory activity of various antiretroviral agents against particle derived and recombinant HIV-1 reverse transcriptase. *Antimicrob. Agents Chemother.* 1989, *33* 115–117.

(25) E. I. Du Pont de Nemours and Co. A information and instruction pamphlet for the NENQUEST Drug Discovery System: HIV gp120/CD4 Receptor EIA (catalog number NED-006).