Articles

Potent Anti-HIV (Type 1 and Type 2) Activity of Polyoxometalates: Structure−Activity Relationship and Mechanism of Action

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A series of polyoxometalates have been synthesized and evaluated for their inhibitory effects on HIV-1(III_B) and HIV-1(ROD) replication in MT-4 cells. All compounds showed activity against HIV-1 and HIV-2, but the antiviral potency of the heteropolytungstates varied considerably depending on their chemical structure. The antiviral activity of single, double, and triple Keggin-type of compounds against HIV-1(III_B) replication was comparable (IC_{50}: 0.4−0.5 μg/mL), whereas HIV-2(ROD) appeared to become less sensitive with the increasing number of Keggin structures per compound. The same trend was observed for single and double Dawson structures. Some of these compounds were examined for their inhibitory effect on the replication of HIV-1(RF) and SIV(MAC_{251}) in MT-4 cells. Their anti-HIV-1(RF) and anti-SIV(MAC_{251}) potencies were comparable to those for the HIV-1(III_B) or HIV-2(ROD) strain, respectively. The polyoxometalates represent a class of polyanionic compounds, which block the binding of the envelope glycoprotein gp120 of HIV to $CD4^+$ cells. The compounds interfered with the binding of anti-CD4 mAb to the OKT4A/Leu3a epitope of the CD4 receptor, compound **24** being the most active in this regard, and inhibited the binding of anti-gp120 mAb to infected MT-4 cells. None of the polyoxometalates inhibited the binding of a specific CXCR4 mAb to SUP-T1 cells, suggesting that they do not interact with CXCR4, the main co-receptor for T-tropic HIV strains, and thus act as virus binding, and not as fusion, inhibitors.

Introduction

From the diversity of drugs proposed as AIDS therapeutics, 14 compounds have now been formally approved by the U.S. Food and Drug Administration for the treatment of HIV infections: the nucleoside reverse transcriptase inhibitors zidovudine (AZT), didanosine (ddI), zalcitabine (ddC), stavudine (d4T), lamivudine (3TC), and abacavir (ABC); the non-nucleoside reverse transcriptase inhibitors nevirapine, delavirdine, and efavirenz; and the protease inhibitors saquinavir, ritonavir, indinavir, nelfinavir, and amprenavir. Derived from the inorganic chemistry field, the polyoxometalates were studied for more than a decade. However, with the exception of some initial attempts in France,[1] none of these inorganic agents have proceeded to AIDS clinical trials. The polyoxometalates are polyanionic oligomeric aggregates of transition metal ions held together by oxygen bridges, and rationalization of their structure−biological activity relationships was undertaken several times with only a limited success.[2] The research on inorganic polyanions as antiviral agents predates the investigation of their use in the field of anti-HIV therapy. These inorganic complexes were first described as antiviral agents against animal retroviruses,[3] based originally on the idea of biologically selective inorganic-phosphate precipitating compounds. This postulate was followed by studies of the polyoxometalates as inhibitors of several animal viruses and retroviruses. Since this initial work of French workers in the Pasteur Institute,[4] several groups[5−9] took on the development of this class of compounds in antiviral research. The hindrance in the development of the polyoxometalates in AIDS stems from the requirement in this disease for chronic administration of a therapeutic drug. We found that most polyoxometalates in our study were water soluble. Two representative compounds were studied elsewhere for their bioavailability in rats[10] and were found to be mostly excreted in urine; however, their breakdown into constituent metal-containing components contraindicated their therapeutic use. The compounds studied and their components measured by metal assay ratios were strongly nephrotoxic in rats. Similar results were reported in other studies.[11,12] This breakdown and/or accumulation under biological conditions, particularly in the kidneys and in the liver, accounts for the lack of interest in the further development of these potent anti-HIV agents. Here we report on the synthesis of a novel

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Table 1. Activity of Heteropolytungstate Compounds against the Replication of HIV-1 and HIV-2 in MT-4 Cells

compd	IC_{50}[a] (μg/mL) HIV-1(III_B)	HIV-2(ROD)	CC_{50}[b] (μg/mL)
	Keggin Structures		
1	4.3	1.8	>250
2	1.1	1.3	>200
3	17	4	>100
4	0.5	3.7	>250
	Dawson Structures		
5	6	6	>250
6	0.4	0.9	30
7	10		>100
8	7	14	>100
9	0.6	2.5	73
10	0.6	1.1	127
11	0.5	0.8	82
12	0.9	1.6	106
13	0.6	0.8	132
14	0.5	0.8	156
15	1.0	9.2	157
	"Double" Keggin Structures		
16	1.2	15.2	>250
17	0.4	3.4	131
18	0.8	2.5	144
19	7.5	>89	>89
20	0.4	3.3	124
21	2.3	16.3	112
22	0.8	29.2	168
23	3.9	94	>250
	"Double" Dawson Structures		
24	1.2	13.9	>250
25	4.7	12.7	>200
	"Triple" Keggin Structures		
26	0.5	121	112
27	0.9	71.8	125
	"Large" Ring Structures		
28	12.5	36.6	>250

[a] IC_{50}: 50% inhibitory concentration, or concentration required to inhibit HIV-induced cytopathicity in MT-4 cells by 50%. [b] CC_{50}: 50% cytotoxic concentration, or concentration required to reduce the viability of mock-infected MT-4 cells by 50%.

series of polyoxometalates. Their anti-HIV activity, and mechanism of action, was evaluated in detail.

Results and Discussion

Antiviral Activity. The heteropolytungstate derivatives were evaluated for their inhibitory effects on HIV-1(III_B)- and HIV-2(ROD)-induced cytopathicity in MT-4 cells (Tables 1 and 2). All compounds showed activity against these retroviruses; but depending on their structure, the anti-HIV potency of the heteropolytungstates varied from one strain to another. Single Keggin structures proved to be equally active or up to 7-fold less active against HIV-2(ROD) compared to HIV-1(III_B). The anti-HIV activity of double Keggin structures was 3- to 40-fold lower for HIV-2(ROD) than for HIV-1(III_B). For the triple Keggin structures, the anti-HIV activity was 80- to 240-fold lower against HIV-2(ROD) than HIV-1(III_B). The activity of the three different Keggin-type of compounds against HIV-1(III_B) was the same, with 50% inhibitory concentration (IC_{50})-values of 0.5, 0.4, and 0.5 μg/mL for the single, double, and triple Keggin structures, respectively. In contrast, HIV-2(ROD) appeared to become less sensitive with an increasing number of Keggin structures per compound.

Dawson structures were evaluated for their anti-HIV activity as well. All single Dawson structures had a comparable activity against HIV-2(ROD) and against HIV-1(III_B), except for compound **15** ($K_8[P_2W_{15}Ti_3(H_2O)_2O_{60}]$) against which HIV-2(ROD) was 9-fold less sensitive. Again the double Dawson structures showed a comparable antiviral activity against HIV-1(III_B) in comparison to the single structures (IC_{50}: 0.4 and 1.2 μg/mL for the single and double Dawson structures, respectively), while HIV-2(ROD) was 3- to 12-fold less sensitive to these compounds.

Table 2. Heteropolytungstate Compounds

compd		ref
	Keggin Structures	
1	$K_5H[ZrW_{11}ZrO_{40}]$	
2	$K_7[Ti_2PW_{10}O_{40}]$	39
3	$K_6H[Zr_2W_{10}PO_{40}H]$	
4	$K_6[A\text{-}\alpha\text{-}SiW_9Fe_3(OH_2)_3O_{37}]$	
	Dawson Structures	
5	$\alpha_2\text{-}K_7[Fe(OH_2)P_2W_{17}O_{61}]$	31
6	$\alpha_2\text{-}K_8[Fe(OH)P_2W_{17}O_{61}]$	31
7	$\alpha_2\text{-}K_7H[TiP_2W_{17}O_{62}]$	
8	$\alpha_2\text{-}K_7[Zr(OH)P_2W_{17}O_{61}]$	
9	$\alpha_2\text{-}Na_7[C_5H_5\ TiP_2W_{17}O_{61}]$	40
10	$\alpha_2\text{-}K_7[C_5H_5TiP_2W_{17}O_{61}]$	40
11	$\alpha_2\text{-}(NH_4)_7[C_5H_5TiP_2W_{17}O_{61}]$	40
12	$\alpha_2\text{-}[NMe_3\ H]_7[C_5H_5TiP_2W_{17}O_{61}]$	40
13	$\alpha_2\text{-}[NMe_3H]_7\ [CH_3C_5H_4TiP_2W_{17}O_{61}]$	40
14	$K_8HP_2W_{15}V_3O_{62}$	41
15	$K_8[P_2W_{15}\ Ti_3(H_2O)_2O_{60}]$	
	"Double" Keggin Structures	
16	$Na_{12}[ZnFe_2O)W(ZnW_9O_{34})_2]$	42
17	$Li_{10}[Co_4(H_2O)_2(PW_9O_{34})_2]$	43
18	$K_{10}[Co_4(H_2O)_2(PW_9O_{34})_2]$	43
19	$Cs_{10}[Co_4(H_2O)_2(PW_9O_{34})_2]$	43
20	$[NH_4]_{10}[Co_4(H_2O)_2(PW_9O_{34})_2]$	43
21	$K_{10}[Zn_4(H_2O)_2(PW_9O_{34})_2]$	43
22	$[NH_4]_{10}[Zn_4(H_2O)_2(PW_9O_{34})_2]$	43
23	$Na_{20}[P_6W_{18}O_{79}]$	44
	"Double" Dawson Structures	
24	$Na_{16}[Mn_4(H_2O)_2(P_2W_{15}O_{56})_2]$	
25	$Na_{16}[Zn_4(H_2O)_2(P_2W_{15}O_{56})_2]$	32
	"Triple" Keggin Structures	
26	$K_{16}[Co_9(OH)_3(H_2O)_6(HPO_4)_2(PW_9O_{34})_3]$	45
27	$[NH_4]_{16}[Co_9(OH)_3(H_2O)_6(HPO_4)_2(PW_9O_{34})_3]$	45
	"Large" Ring Structure	
28	$K_{28}Li_5H_7P_8W_{48}O_{184}$	46

Only one compound with a "large" ring structure, compound **28** ($K_{28}Li_5H_7P_8W_{48}O_{184}$), was examined for its potential anti-HIV activity. This compound had an IC_{50} of 12.5 and 36.6 μg/mL, for HIV-1(III_B) and HIV-2(ROD), respectively.

The Dawson-type compound **14** ($K_8HP_2W_{15}V_3O_{62}$) was tested for its antiviral activity against HIV-1(RF) (data not shown). The compound was equally active against HIV-1(RF) as compared to its activity against HIV-1(III_B) (IC_{50}: 1.1 and 0.5 μg/mL, respectively). Three compounds (**9**, **11**, and **27**) were examined for their inhibitory effect on the replication of SIV(MAC_{251}) in MT-4 cells (data not shown). Their anti-SIV effects were comparable to those obtained for HIV-2(ROD).

The polyoxometalates represent a class of polyanionic compounds that have been credited with antiviral activity in vitro and in vivo against a number of enveloped RNA and DNA viruses.[7,13−25] We studied the potency of the heteropolytungstates against HIV-1, HIV-2, and SIV in MT-4 cells. Their antiviral activity varied considerably from one structural group to another. A marked decrease in anti-HIV-2 activity was observed

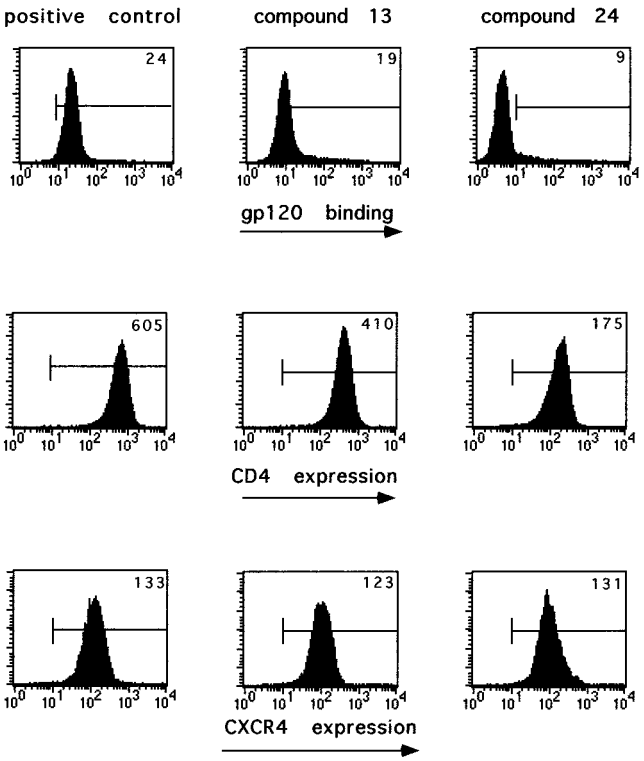

Figure 1. Effects of the single Dawson structure compound **13** and the double Dawson structure compound **24** (both at 20 μg/mL) on the binding of gp120 to the cells (upper panels), binding of anti-CD4 mAb Leu3a to the CD4 receptor (middle panels), and binding of anti-CXCR4 mAb (12G5) to CXCR4 (lower panels), all done in SUP-T1 cells. The mean fluorescence intensities (MFI) are added in each histogram. The MFI of the isotype control mAbs was between 5−6.

when the number of Dawson or Keggin structures per compound increased from one to two or three, while this increasing number had no effect on the anti-HIV-1 activity of the compounds. The inhibitory effects on the replication of HIV-1(RF) were comparable to the activity observed against HIV-(III_B). For SIV(MAC_{251}), an activity similar to that for HIV-2(ROD).

Mechanism of Action. The polyoxometalates represent a class of polyanionic compounds that block the binding of HIV particles to the $CD4^+$ cells.[7,13,14,18,23−25] The single Dawson structure compound **13** and the double Dawson structure compound **24** concentration-dependently blocked the binding of recombinant gp120 to SUP-T1 cells (Table 3 and Figure 1) in a concentration-dependent manner. These compounds interfered with the binding of the OKT4A/Leu-3a mAb to the CD4 receptor of SUP-T1 cells with compound **24** being the most active (Table 3 and Figure 1). They also inhibited the binding of anti-gp120 mAb to HIV-1-infected MT-4 cells (data not shown). The fact that some heteropolytungstates show a different inhibitory activity against HIV-1 and HIV-2 suggests that there may exist marked structural and conformational differences in the target molecules (i.e. V3 loop of the gp120) of HIV-1 and HIV-2 with which heteropolytungstates interact, as previously shown for sulfated polymers.[26,27] The single Dawson structure compound **13** and the double Dawson structure compound **24** did not inhibit the binding of a specific CXCR4 mAb to SUP-T1 cells at 20 μg/mL, as measured by flow cytometry (Figure 1), suggesting that they do not interact with CXCR4, the main co-receptor for T-tropic HIV strains.[28]

Table 3. Effects of Polyoxometalates, Dextran Sulfate MW 5000 (DS), and Aurintricarboxylic Acid (ATA) on the Binding of OKT4A/Leu3a mAb and gp120 to SUP-T1 Cells, as Detected by FACS Analysis

compd	concn (μg/mL)	IICD4[a]	IIgp120 binding[b]
13	100	61	78
	20	33	28
	4	10	0
	0.8	0	0
24	100	83	95
	20	73	83
	4	51	67
	0.8	21	11
DS	100	6	72
	20	6	33
	4	1	33
	0.8	1	0
ATA	100	96	ND[c]
	20	90	ND
	4	48	ND
	0.8	2	ND

[a] The inhibitory index for CD4 expression (IICD4). [b] The inhibitory index for gp120 binding (IIgp120). [c] Not determined.

Experimental Section

NMR spectra were measured on a Bruker AC250 spectrometer. Metal analyses were carried out using a Jobin Yvon ICP OES instrument, and the calibration solutions were obtained from Aldrich or Merck (ICP or AA standards). The compounds were usually only air-dried on a frit so that their moisture content would remain relatively constant between synthesis and use in the biological testing. Measurement of the moisture content of the compounds with a TG analyzer (Mettler TA3000) proved difficult as appreciable weight loss can occur during setting up of the experiments under the flow of dry nitrogen, even at temperatures below room temperature. Infrared spectra were measured in KBr discs on a Perkin-Elmer 377 spectrometer. Cation exchange experiments were carried out using Amberlite IR-120 resin. We used 0.05 M KOH in the pH titrations of protonated compounds, obtained by cation exchange, in determining the number of base-titratable protons in the structures.

Synthesis. $K_5H[ZrW_{11}ZrO_{40}]\cdot 30H_2O$ (1). $Na_2WO_4\cdot 2H_2O$ (120 mmol, 39.6 g) was dissolved in 120 mL of H_2O, to 55−60 °C, and 90 mmol of HCl was added followed by 2.92 g (10 mmol) of solid $(\eta^5\text{-}C_5H_5)_2ZrCl_2$, added over 30 min, and then a further 70 mmol of HCl. The reaction solution (pH < 1) was taken to reflux for 1.5 h, 14 g of KCl added, and the clear solution stored at 4 °C for several days. Next, 6.3 g of white product was collected and then redissolved in 5 mL of H_2O at 40 °C. On standing, the solid separated as a soft white mass from which the solution was decanted, and after addition of ethanol, the white powder (5 g) was collected and air-dried. Found: %W 56.87; Zr 5.00; K 5.24. For $K_5H[ZrW_{11}ZrO_{40}]\cdot 30H_2O$: %W 56.46; Zr 5.09; K 5.46. IR (cm^{-1}) 956, 890, 750 (br), 645 (br).

$K_6[Zr_2W_{10}PO_{40}H]\cdot 23H_2O$ (3) was prepared by reaction of 32.9 g (100 mmol) of $Na_2WO_4\cdot 2H_2O$ with 1.56 g (10 mmol) of $Na_2HPO_4\cdot 2H_2O$ in 100 mL of H_2O at room temperature, acidification of the solution with 9.41 g (80 mmol) of concentrated HCl (31−32% HCl) (pH = 8.6), and then treatment with $(\eta^5\text{-}C_5H_5)_2ZrCl_2$ (5.84 g, 20 mmol, 80 mL of THF) dropwise at 65−70 °C, at a rate so that the solution remained clear until ca. 80−90% of the $(\eta^5\text{-}C_5H_5)_2ZrCl_2$ had been added. After being refluxed for 1 h, the solution (pH 8.1) was filtered through Celite and decolorized with activated carbon. The solution (150−200 mL) was treated with 30 g of KCl, and the white precipitate obtained (23.5 g) was purified by reprecipitation from 100 mL of H_2O at 80 °C by addition of 25 g of KCl (recovered 22−23 g). After this method was repeated using 150 mL of H_2O at 90 °C and 25 g of KCl, the product was

collected, washed with 30−40 mL of cold water (recovered 20.2 g), recrystallized from 50 mL of H_2O warmed to 85−90 °C, and let stand to cool to room temperature overnight. The white (powder) product was collected, washed with methanol and ether, and air-dried on the frit. Yield = 12.7 g. ^{31}P NMR (D_2O, δ) sharp resonances at −9.61 (major) and −10.09 suggesting the presence of two isomers. The PO_4^{3-} stretching vibrations are at 1091, 1059, and 1031 cm^{-1}. The W=O stretch was a doublet with maxima at 957 cm^{-1} and 938 cm^{-1}. Two medium intense bands were found at 878 cm^{-1} and 720 cm^{-1}, with the strongest band of the spectrum centred around 802 cm^{-1}. Found: %W 55.02; Zr 5.57; P 1.05; K 6.87; H_2O 9.3. Calcd for $K_6[Zr_2W_{10}PO_{40}H]\cdot 23H_2O$: %W 55.01; Zr 5.46; P 0.93; K 7.02; H_2O 12.4. Titration of the fully protonated form of the compound indicated the presence of two sets of three acidic protons (titration curve inflection points at pH < 4 and pH 6.5−7.0).

$K_6H[A\text{-}\alpha\text{-}SiW_9Fe_3(OH_2)_3O_{37}]\cdot 14H_2O$ (4) was formed on reaction of $Na_{10}[\alpha\text{-}SiW_9O_{34}]\cdot nH_2O$[29] with an iron(III) species even when the Fe:W ratio was as low as 2:9. Thus, 7.1 g of the lacunary tungstate was placed in a beaker with 40 mL of water at room temperature and then treated with 0.9 g of solid, aerobically oxidized, anhydrous ferrous acetate. The reactants dissolved very rapidly to give a pale green-yellow solution. After 3−5 min, the trace of turbidity that may remain was removed by filtration. The solution, after standing at room temperature overnight, was treated with 10 g of KCl to yield a pale brown precipitate. This was collected and air-dried. Yield = 4.3 g. To purify, it was dissolved in 45 mL of water at room temperature, filtered, and reprecipitated with 10 g of KCl to give 3.7 g of product. On crystallization from 12 mL of water (65−70 °C), dark, red-brown crystals (2.2 g) were obtained on cooling to room temperature. Found: %W 55.75; Fe 5.48; K 7.45. Calcd for $K_6H[A\text{-}\alpha\text{-}SiW_9Fe_3(OH_2)_3O_{37}]\cdot 14H_2O$: %W 55.45; Fe 5.61; K 7.86. A further 0.9 g of product, greener in color and containing less K, was obtained at 4 °C. Found: W 56.45; Fe 5.50; K 7.17. Calcd for $K_5H_2[A\text{-}\alpha\text{-}SiW_9Fe_3(OH_2)_3O_{37}]\cdot 13H_2O$: W 56.51; Fe 5.72; K 6.68. The water molecules on the Fe atoms were labile and exchangeable with NH_3. The compound has also been prepared by Liu et al.[30] from ferric nitrate.

$\alpha_2\text{-}K_7H[TiP_2W_{17}O_{62}]\cdot 17H_2O$ (7) was formed when a solution of $TiCl_4$ (7.1 g, 15% in 15% HCl) was combined with a solution (50 mL) of NaOAc (20 g, pH 6.8 with HOAc) and added to 25 g of $\alpha\text{-}K_{10}P_2W_{17}O_{61}\cdot 22H_2O$ in 100 mL of H_2O at 70 °C. The clear solution became turbid after several minutes, and after 15−20 min at 70−75 °C it was cooled to room temperature (pH 5.7) and filtered through fine paper. On addition of 25 g of KCl, a dense, white solid (22.8 g) precipitated. This was redissolved in H_2O (80 mL, 70−80 °C), filtered, and reprecipitated with 25 g of KCl. This was repeated, and the recovered material (21.8 g) was recrystallized from 20 mL of H_2O cooled from 70 °C to room temperature overnight, collected, and air-dried. ^{31}P NMR (D_2O, δ) −9.42, −13.01. Found: %Ti 1.03; K 5.96; H_2O 6.60. Calcd for $\alpha_2\text{-}K_7H[TiP_2W_{17}O_{62}]\cdot 17H_2O$: %Ti 1.00; K 5.69; H_2O 6.37. On base titration of the protonated compound, 7.9 acidic protons/molecule were found. IR (cm^{-1}) 1085, 1017(w), 962(sh), 948, 914, 780.

$\alpha_2\text{-}K_7[Zr(OH)P_2W_{17}O_{61}]\cdot 18H_2O$ (8). $Zr(NO_3)_4\cdot 5H_2O$ (2.15 g, 5 mmol) was dissolved in a hot (85−90 °C) solution of NaOAc (20 g, 50 mL, pH ca. 5.6) and added to a hot aqueous (100 mL) solution of $\alpha_2\text{-}K_{10}P_2W_{17}O_{61}\cdot 22H_2O$ (24.7 g, 5 mmol). After the clear solution was heated for 40 min and filtered, it was cooled to room temperature and KCl (50 g) was added. The white precipitate (ca. 25 g) was twice taken up in 75−80 mL H_2O at 85 °C and precipitated with 25 g of KCl. After the precipitate was redissolved (22 g) in 50 mL of H_2O at 85 °C and stood at room temperature, the product separated, not as a dense precipitate, as was obtained in the presence of KCl, but as a white material occupying most of the volume of the mother liquor (pH 6.1). The product (18.4 g) was collected and air-dried. ^{31}P NMR (D_2O, δ) −8.69, −13.08. Found: %Zr 1.74; K 5.62; H_2O 6.72. Calcd for $\alpha_2\text{-}K_7[Zr(OH)P_2W_{17}O_{61}]\cdot 18H_2O$: %Zr 1.87, K 5.60, H_2O 6.64. Base titration of the proton exchanged species indicated that 6 protons/molecule were highly acidic, a further proton was neutralized at pH ca. 6.6, and another required a higher pH for its removal.

The corresponding Fe(III) compound **6** was obtained following the Zonnevijlle et al.[31] synthesis of $K_8[Fe(OH_2)P_2W_{17}O_{61}]$ (**5**), but carrying the reaction out in the presence of an equivalent of NaOAc.

$K_8[P_2W_{15}Ti_3(OH_2)_2O_{60}]\cdot 22H_2O$ (15) was obtained when $TiCl_4$ (1.2 mL, 11 mmol) was added dropwise to a suspension of $\alpha\text{-}Na_{12}P_2W_{15}O_{56}\cdot 18H_2O$ (14.4 g, 3.3 mmol) in 80 mL of H_2O at room temperature. The solution became clear after $^1/_3$ to $^1/_2$ of the $TiCl_4$ had been added, but a slight turbidity developed toward the end of the addition. After the solution was stirred at 70 °C for 2 h, 16 g of KCl was added to the clear, very pale yellow solution. The white precipitate (7 g) was collected at room temperature, redissolved in 30−35 mL of H_2O at 70 °C, and reprecipitated with 10 g of KCl. The product (6.7 g) was dissolved in 40 mL of H_2O at 80 °C, filtered through fine paper, and left overnight to crystallize (2 g, air-dried). Found: %W 59.5; Ti 3.04; K 6.62. Calcd for $K_8[P_2W_{15}Ti_3(OH_2)_2O_{60}]\cdot 22H_2O$: %W 59.07; Ti 3.08; K 6.70. ^{31}P NMR (D_2O, δ) −6.77, −13.44 and small peaks near δ − 5 and δ − 13, possibly due to an isomer. IR (cm^{-1}) 1087(s), 1065(w), 1015(w), 948(s), 915, 890, 830(s), 770(s), ca. 680(v br). While the formulation given above has water molecules on titanium atoms, structures in which titanium atoms bear hydroxyl groups are also possible.

$Na_{16}[Mn_4(H_2O)_2(P_2W_{15}O_{56})_2]\cdot 70H_2O$ (24) was synthesized by the method of Finke et al.[32] from 4.63 g of $\alpha\text{-}Na_{12}P_2W_{15}O_{56}\cdot 18H_2O$ and 0.46 g of $MnCl_2\cdot 6H_2O$ in 45 mL of H_2O containing 2.9 g of NaCl warmed to 30−40 °C for 10 min. The orange crystals (3.7 g) that formed on cooling were dissolved in 4 mL of H_2O at 35 °C, filtered through a G4 frit, and cooled to 4 °C to give 2.8 g (air-dried) of red-brown crystals. Found: %W 59.7; Mn 2.31; P 0.90; Na 3.58. Calcd for $Na_{16}[Mn_4(H_2O)_2(P_2W_{15}O_{56})_2]\cdot 70H_2O$: %W 59.20; Mn 2.36; P 1.33; Na 3.95. ^{31}P NMR (D_2O, δ) −10.5 ($W_{1/2}$ = 108 Hz). IR (cm^{-1}) 1085(s), 1041(m), 1007-(w), 945(sh), 931(s), 910(sh), 874, 829,770(s), 730(s).

Anti-HIV Activity Assays. The human immunodeficiency virus strains used were HIV-1(III_B and RF)[33] and HIV-2 (ROD).[34] The simian immunodeficiency virus strain used was SIV(MAC_{251}).[35] Anti-retroviral activity and cytotoxicity measurements were carried out in parallel. They were based on the viability of MT-4 cells that had been infected with HIV and then exposed to various concentrations of the test compounds. After the MT-4 cells were allowed to proliferate for 5 days, the number of viable cells was quantified by a tetrazolium-based colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) procedure in 96-well microtrays.[36] In all of these assays, viral input (viral multiplicity of infection, moi) was 0.01, or 100 times the 50% cell culture infective dose ($CCID_{50}$). The 50% antiviral inhibitory concentration (IC_{50}) was defined as the compound concentration required to protect 50% of the virus-infected cells against viral cytopathicity. The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration required to reduce the viability of mock-infected cells by 50%. The > symbol is used to indicate the highest concentration at which the compounds were tested and still found noncytotoxic. Average IC_{50} and CC_{50} values for several separate experiments are presented as defined above. As a rule, the individual values did not deviate by more than 2-fold up or down from the IC_{50} and CC_{50} values indicated in Table 1.

Gp120 Binding Assay. The inhibitory effect of the test compounds on recombinant gp120 (HIV-1, III_B) (Intracel, London, U.K.) binding was measured by indirect immunofluorescence-laser flow cytometric method which had been specifically designed for this purpose.[37] Briefly, SUP-T1 cells were exposed to gp120 (10 μg/mL), in the presence or absence of the different concentrations of test compounds. The compounds were added 10−20 s before the virus was added. The cells were processed for gp120 binding using an anti-gp120 mAb (9284, DuPont de Nemours, Brussels, Belgium) and analyzed for cell-bound gp120 by flow cytometry.

CD4 Immunofluorescence Assay. CD4 expression was

determined by a FACScan (Becton Dickinson) analysis, as described previously.[38] Briefly, MT-4 cells were incubated for 10−20 s at room temperature in PBS in the absence of serum with or without test compound. The cells were then stained with optimal concentrations of the mAbs OKT4A-FITC (Ortho Diagnostics) or Leu3a-FITC (Becton Dickinson) and Simultest immune monitoring kit control (FITC-labeled IgG_1-Phycoerythrin (PE)-labeled IgG_{2a}) (Becton Dickinson) for 20 min at 4 °C, washed once in PBS, and fixed in 0.5 mL of 0.5% paraformaldehyde in PBS.

Analysis of CXCR4 Expression. SUP-T1 cells were incubated with the compounds (at different concentrations) or PBS for different time periods (15 min) and at different temperatures (at room temperature), and the cells were washed once with PBS. The 12G5 mAb, which reacts specifically with the human CXCR4 (R&D Systems Europe Ltd., Abingdon, U.K.), was then added (at 10 μg/mL) for 30 min at room temperature. The cells were washed twice in PBS and then incubated with FITC-conjugated goat anti-mouse Ab (Caltag Labs, San Francisco, CA) for 30 min at room temperature and washed twice in PBS. Cells were analyzed by a FACScan flow cytometer. The percentage of positive cells and the mean fluorescence intensity (MFI) values are indicated in each histogram. The region for positivity was defined using a control isotype mAb (Becton Dickinson, San Jose, CA).

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References

(1) Dormont, D.; Yeramian, P.; Lambert, P.; Spire, B.; Daveloose, D.; Barré-Sinoussi, F. C.; Chermann, J. C. Cellular and Molecular Mechanisms of Antiretroviral Effects of HPA. *Cancer Detect. Prev*. **1988**, *12*, 181−194.

(2) 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 compounds. Structure-activity relationships. In *Advances in Chemotherapy of AIDS*; Diasio, R. B., Sommadossi, J.-P., Eds.; Pergamon Press, Inc.: New York, 1990; pp 33−42.

(3) Jasmin, J.-C.; Raybaud, N.; Chermann, J.-C.; Haapala, D.; Sinoussi, F.; Boy-Laustau, C.; Kona, P.; Raynaud, M. In vitro effects of silicotungstate on some RNA viruses. *Biomedicine* **1973**, *18*, 319−327.

(4) Jasmin, C.; Chermann, J.-C.; Hervé, G.; Teze, A.; Souchay, P.; Boy-Loustau, C.; Raybaud, N.; Sinoussi, F.; Raynaud, M. In vivo inhibition of murine leukaemia and sarcoma viruses by heteropolyanion 5-tungsto-2- antimoniate. *J. Natl. Cancer Inst*. **1974**, *53*, 469−474.

(5) Inouye, Y.; Take, Y.; Tokutake, Y.; Yoshida, T.; Yamamoto, A.; Yamase, T.; Nakamura, S. Inhibition of replication of human immunodeficiency virus by a heteropolytungstate (PM-19). *Chem. Pharm. Bull*. **1990**, *38*, 285−287.

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

(7) 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.

(8) Judd, D. A.; Schinazi, R. F.; Hill, C. L. Relationship of the molecular size and charge density of polyoxometalates to their anti-gp120-CD4-binding activity. *Antiviral Chem. Chemother*. **1994**, *5*, 410−414.

(9) Moore, P. S.; Jones, C. J.; Mahmood, N.; Evans, I. G.; Goff, M.; Cooper, R.; Hay, A. J. Anti-(human immunodeficiency virus) activity of polyoxotungstates and their inhibition of human immunodeficiency virus reverse transcriptase. *J. Biochem*. **1995**, *307*, 129−134.

(10) Thomas, S. G. A Series of inorganic antiviral polyoxometalates: Determination and comparison of their pharmacological properties. Ph.D. Thesis, Monash University Pharmacology Department, Melbourne, 1997.

(11) Ni, L.; Boudinot, F. D.; Boudinot, S. G., H. 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. *Antimicrob. Agents Chemother*. **1994**, *38*, 504−510.

(12) Thomas, S. G.; Beveridge, A. A.; Weigold, H.; Holan, G. Tissue residue studies of an antiviral compound MB1532 in rats. *Clin. Exp. Pharmacol. Physiol*. **1993**, (Suppl. 1) 72.

(13) 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.

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

(15) Fukuma, M.; Seto, Y.; Yamase, T. *In vitro* antiviral activity of polyoxotungstate (PM-19) and other polyoxometalates against herpes simplex virus. *Antiviral Res*. **1991**, *16*, 327−339.

(16) Inouye, Y.; Tokutake, Y.; Yoshida, T.; Yamamoto, A.; Yamase, T.; Nakamura, S. Antiviral activity of polyoxomolybdoeuropate PM-104 against human immunodeficiency virus type 1. *Chem. Pharm. Bull*. **1991**, *39*, 1638−1640.

(17) Take, Y.; Tokutake, Y.; Inouye, Y.; Yoshida, T.; Yamamoto, A.; Yamase, T.; Nakamura, S. Inhibition of proliferation of human immunodeficiency virus type 1 by novel heteropolyoxotungstates *in vitro*. *Antiviral Res*. **1991**, *15*, 113−124.

(18) De Clercq, E. New perspectives for the chemotherapy and chemoprophylaxis of AIDS (acquired immune deficiency syndrome). *Verh. K. Acad. Geneesk. België* **1992**, *54*, 57−89.

(19) Inouye, Y.; Tokutake, Y.; Kunihara, J.; Yoshida, T.; Yamase, T.; Nakata, A.; Nakamura, S. Suppressive effect of polyoxometalates on the cytopathogenicity of human immunodeficiency virus type 1 (HIV-1) *in vitro* and their inhibitory activity against HIV-1 reverse transcriptase. *Chem. Pharm. Bull*. **1992**, *40*, 805−807.

(20) 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.

(21) Ikeda, S.; Neyts, J.; Yamamoto, N.; Murrer, B.; Theobald, B.; Bossard, G.; Henson, G.; Abrams, M.; Picker, D.; De Clercq, E. *In vitro* activity of a novel series of polyoxosilicotungstates against human myxo-, herpes-, and retroviruses. *Antiviral Chem. Chemother*. **1993**, *4*, 253−262.

(22) Ikeda, S.; Nishiya, S.; Yamamoto, A.; Yamase, T.; Nishimura, C.; De Clercq, E. Activity of the Keggin polyoxotungstate PM-19 against herpes simplex virus type 2 infection in immunosuppressed mice: role of peritoneal macrophage activation. *J. Med. Virol*. **1993**, *41*, 191−195.

(23) Inouye, Y.; Tokutake, Y.; Yoshida, Y.; Seto, T.; Hujita, Y. H.; Dan, K.; Yamamoto, A.; Nishiya, S.; Yamase, T.; Nakamura, S. *In vitro* antiviral activity of polymolybdates. Mechanisms of inhibitory effect of $PM_{10}4$ $(NH_4)_{12}H_2(Eu_4(MoO_4)(H_2O)_{16}$ $(Mo_7O_{24})_4 \cdot 3H_2O$ on human immunodeficiency virus type 1. *Antiviral Res*. **1993**, *20*, 317−331.

(24) Kim, G. S.; Judd, D. A.; Hill, C. L.; Schinazi, R. F. Synthesis, characterization and biological activity of a new potent class of anti-HIV agents, the peroxoniobium-substituted heteropolytungstates. *J. Med. Chem*. **1994**, *37*, 816−820.

(25) Shigeta, S.; Mori, S.; Watanabe, J.; Baba, M.; Khenkin, A. M.; Hill, C. L.; Schinazi, R. F. *In vitro* antimyxovirus and anti-human immunodeficiency virus activities of polyoxometalates. *Antiviral Chem. Chemother*. **1995**, *6*, 114−122.

(26) Schols, D.; De Clercq, E.; Witvrouw, M.; Nakashima, H.; Snoeck, R.; Pauwels, R.; Van Schepdael, A.; Claes, P. Sulphated cyclodextrins are potent anti-HIV agents acting synergistically with 2′,3′-dideoxynucleoside analogues. *Antiviral Chem. Chemother*. **1991**, *2*, 45−53.

(27) Witvrouw, M.; Schols, D.; Andrei, G.; Snoeck, R.; Hosoya, M.; Pauwels, R.; Balzarini, J.; De Clercq, E. Antiviral activity of low-MW dextran sulphate (derived from dextran MW 1000) compared to dextran sulphate samples of higher MW. *Antiviral Chem. Chemother*. **1991**, *2*, 171−179.

(28) Feng, Y.; Broder, C. C.; Kennedy, P. E.; Berger, E. A. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G-protein-coupled receptor. *Science* **1996**, *272*, 872−877.

(29) Tézé, A.; Hervé, G. α, *β* and *γ*-Dodecatungstosilicic Acids: Isomers and Related Lacunary Compounds. In *Inorganic Syntheses*, Vol. 27; Ginsberg, A. P., Ed.; Wiley-Interscience Publication Co.: New York, 1990; pp 87−96.
(30) Liu, J.; Ortéga, F.; Sethuraman, P.; Katsoulis, D. E.; Costello, C. E.; Pope, M. T. Trimetallo Derivatives of Lacunary 9-Tungstosilicate Heteropolyanions. Part 1. Synthesis and Characterization. *J. Chem. Soc., Dalton Trans.* **1992**, 1901.
(31) Zonnevijlle, F.; Tourné, C. M.; Tourné, G. F. Preparation and Characterization of Iron(III)- and Rhodium(III)- Containing Heteropolytungstates. Identification of Novel Oxo-Bridged Iron-(III) Dimers. *Inorg. Chem*. **1982**, *21*, 2751−2757.
(32) Finke, R. G.; Droege, M. W.; Domaille, P. J. Trivacant Heteropolytungstate Derivatives. 3. Rational Syntheses, Characterization, Two-Dimensional ^{183}WNMR, and Properties of $P_2W_{18}M_4(H_2O)_2O_{68}^{10-}$ and $P_4W_{30}M_4(H_2O)_2O_{112}^{16-}$ (M = Co, Cu, Zn). *Inorg. Chem*. **1987**, *26*, 3886−3896.
(33) Popovic, M.; Sarngadharan, M. G.; Read, E.; Gallo, R. C. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **1984**, *224*, 497−500.
(34) Clavel, F.; Guyader, M.; Guetard, D.; Salle, M.; Montagnier, L.; Alizon, H. Molecular cloning and polymorphism of the human immunodeficiency virus type 2. *Nature* **1986**, *324*, 691−695.
(35) Franchini, G.; Gurgo, C.; Guo, H. G.; Gallo, R. C.; Collalti, E.; Fargnoli, K. A.; Hall, L. F.; Wong-Staal, F.; Reitz, M. J., Jr. Sequence of simian immunodeficiency virus and its relationship to the human immunodeficiency viruses. *Nature* **1987**, *328*, 539−543.
(36) Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, M. R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. *J. Virol. Methods* **1988**, *20*, 309−321.
(37) Schols, D.; Baba, M.; Pauwels, R.; De Clercq, E. Flow cytometric method to demonstrate whether anti-HIV-1 agents inhibit virion binding to $T4^+$ cells. *J. Acquired Immune Defic. Syndr*. **1989**, *2*, 10−15.
(38) Schols, D.; Baba, M.; Pauwels, R.; Desmyter, J.; De Clercq, E. Specific interaction of aurintricarboxylic acid with the human immunodeficiency virus/CD4 cell receptor. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 3322−3326.
(39) Domaille, P. J.; Knoth, W. H. $Ti_2W_{10}PO_{40}^{7-}$ and $[CpFe(CO)_2Sn]_2$-$W_{10}PO_{38}^{5-}$. Preparation, Properties, and Structure Determination by Tungsten-183 NMR. *Inorg. Chem*. **1983**, *22*, 818−822.
(40) Weigold, H.; Holan, G.; Marcuccio, S. M.; Birch, C. J.; Gust, I. A. Patent: Antiviral Agents. PCT/AU/00282, 28 June, 1991.
(41) 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 $H_2SiW_9V_3O_{40}^{x-7}$ and $H_2P_2W_{15}$-$V_3O_{62}^{x-9}$. *J. Am. Chem. Soc*. **1986**, *108*, 2947−2960.
(42) Tourné, C. M.; Tourné, G. F.; Zonnevijlle, F. Chiral Polytungstates $[WM_3(H_2O)_2(XW_9O_{34})_2]^{12-}$ (X = M = Zn or Co^{II}) and their M-Substituted Derivatives. Syntheses, Chemical, Structural and Spectroscopic Study of some d,l Sodium and Potassium Salts. *J. Chem. Soc., Dalton Trans*. **1991**, 143−155.
(43) Evans, H. T.; Tourné, C. M.; Tourné, G. F.; Weakley, T. J. R. X-Ray Crystallographic and Tungsten-183 Nuclear Magnetic Resonance Structural Studies of the $[M_4(H_2O)_2(XW_9O_{34})_2]^{10-}$ Heteropolyanions (M = CoII or Zn, X = P or As). *J. Chem. Soc., Dalton Trans*. **1986**, 2699−2705.
(44) Fuchs, J.; Palm, R. Strukturen und Schwingungsspektren neuer Wolframatophosphate. (Structures and IR-spectra of new Walframophosphate.) *Z. Naturforsch*. **1988**, *43b*, 1529−37.
(45) Weakley, T. J. R. The Identification and X-Ray Structure of the Diphosphatotris(nonatungstophosphato)nonacobaltate(II) Heteropolyanion. *J. Chem. Soc., Chem. Commun*. **1984**, 1406−1407.
(46) Contant, R. Potassium Octadecatungstodiphosphates(V) and Related Lacunary Compounds. In *Inorganic Syntheses*, Vol. 27; Ginsberg, A. P., Ed.; Wiley-Interscience Publication Co.: New York, 1990; pp 104−111.

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