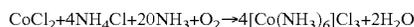


alphavirus (e.g., Sindbis virus), adenovirus, and/or cytomegalovirus (CMV). In preferred embodiments, the viral infection is by HIV or Ebola virus.

#### Preparation of Co(III) Hexammine

**[0033]** While Cohex is available commercially, its synthesis is fairly straight forward, using air to oxidize Co(II) to Co(III):



**[0034]** 9.6 g of  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (0.06 mol) and 6.4 g of  $\text{NH}_4\text{Cl}$  (0.12 mol) were added to 40 ml of water in a 250 ml Erlenmeyer flask with a side arm and shaken until most of the salts are dissolved. Then 1 g of fresh activated decolorizing charcoal and 20 ml concentrated ammonia were added. Next the flask was connected to the aspirator or vacuum line and air drawn through the mixture until the red solution becomes yellowish brown (usually 2-3 hours). The air inlet tube if preferably of fairly large bore (~10 mm) to prevent clogging with the precipitated  $\text{Co}(\text{NH}_3)_6^{3+}$  salt.

**[0035]** The crystals and charcoal were filtered on a Buchner funnel and then a solution of 6 ml of concentrated HCl in 75 ml of water was added. The mixture was heated on a hot plate to effect complete solution and filtered while hot. The hexamminecobalt (III) chloride was crystallized by cooling to 0° C. and by slowly adding 15 ml of concentrated HCl. The crystals were filtered, washed with 60% and then with 95% ethanol, and dried at 80-100° C.

#### Cohex Activity Against HIV

**[0036]** There are two known strains of HIV: HIV-1 and HIV-2, of which HIV-1 is the more virulent virus and is the major cause of HIV infections. The first clinically useful drugs developed for HIV-1 were the nucleoside reverse transcriptase (RT) inhibitors. AZT, or 3-azido-3-deoxythymidine, is a synthetic pyrimidine analog of thymidine was actually initially developed as an anticancer drug before it became known as a popular anti-HIV compound. The active form of AZT is its phosphorylated triphosphate (TP) form, which is a competitive inhibitor of RT because AZT-TP binds to the HIV-1 RT better than to the natural substrate deoxythymidine triphosphate (dTTP).

**[0037]** Cohex was tested in a standard PBMC cell-based microtiter anti-HIV assay against one CXCR4-tropic HIV-1 isolate and one CCR5-tropic HIV-1 isolate. For this study peripheral blood mononuclear cells (PBMCs) were pretreated with the compound for two hours prior to infection.

**[0038]** Cohex was stored at 4° C. as a powder and solubilized for tests. The solubilized stock was stored at -20° C. until the day of the assay. Stocks were thawed at room temperature on each day of assay setup and were used to generate working drug dilutions used in the assays. Working dilutions were made fresh for each experiment and were not stored for re-use in subsequent experiments performed on different days. Cohex was evaluated using a 3 mM (3,000 μM) highest concentration with 8 additional serial half-log dilutions in the PBMC assays.

#### PBMC Assay

**[0039]** Freshly prepared PBMCs were centrifuged and suspended in RPMI 1640 with 15% FBS, L-glutamine, penicillin, streptomycin, non-essential amino acids (MEM/NEAA; Hyclone; catalog #SH30238.01), and 20 U/ml recombinant

human IL-2. PBMCs were maintained in this medium at a concentration of  $1-2 \times 10^6$  cells/ml, with twice-weekly medium changes until they were used in the assay protocol. Monocyte-derived-macrophages were depleted from the culture as the result of adherence to the tissue culture flask.

**[0040]** For the standard PBMC assay, the cells were plated in the interior wells of a 96 well round bottom microplate at 50 μL/well ( $5 \times 10^4$  cells/well) in a standard format developed by the Infectious Disease Research department of Southern Research Institute. Each plate contains virus control wells (cells plus virus) and experimental wells (drug plus cells plus virus). Test drug dilutions were prepared at a 2x concentration in microtiter tubes and 100 μL of each concentration was placed in appropriate wells using the standard format. 50 μL of a predetermined dilution of virus stock was placed in each test well (final MOI ~0.1). Separate plates were prepared identically without virus for drug cytotoxicity studies using an MTS assay system (described below; cytotoxicity plates also include compound control wells containing drug plus media without cells to control for colored compounds that affect the MTS assay). The PBMC cultures were maintained for seven days following infection at 37° C., 5%  $\text{CO}_2$ . After this period, cell-free supernatant samples were collected for analysis of reverse transcriptase activity and compound cytotoxicity was measured by addition of MTS to the separate cytotoxicity plates for determination of cell viability. Wells were also examined microscopically and any abnormalities were noted.

#### Reverse Transcriptase Activity Assay

**[0041]** A microtiter plate-based reverse transcriptase (RT) reaction was utilized (detailed in Buckheit et al., AIDS Research and Human Retroviruses 7:295-302, 1991). Tritiated thymidine triphosphate (3H-TTP, 80 Ci/mmol, NEN) was received in 1:1  $\text{dH}_2\text{O}$ :Ethanol at 1 mCi/ml. Poly rA:oligo dT template:primer (Pharmacia) was prepared as a stock solution by combining 150 poly rA (20 mg/ml) with 0.5 ml oligo dT (20 units/ml) and 5.35 ml sterile  $\text{dH}_2\text{O}$  followed by aliquoting (1.0 ml) and storage at -20° C. The RT reaction buffer was prepared fresh on a daily basis and consisted of 125 μl 1.0 M EGTA, 125 μl  $\text{dH}_2\text{O}$ , 125 μl 20% Triton X100, 50 μl 1.0 M Tris (pH 7.4), 50 μl 1.0 M DTT, and 40 μl 1.0 M  $\text{MgCl}_2$ . The final reaction mixture was prepared by combining 1 part 3H-TTP, 4 parts  $\text{dH}_2\text{O}$ , 2.5 parts poly rA:oligo dT stock and 2.5 parts reaction buffer. Ten microliters of this reaction mixture was placed in a round bottom microtiter plate and 15 μl of virus-containing supernatant was added and mixed. The plate was incubated at 37° C. for 60 minutes. Following incubation, the reaction volume was spotted onto DE81 filter-mats (Wallac), washed 5 times for 5 minutes each in a 5% sodium phosphate buffer or 2xSSC (Life Technologies), 2 times for 1 minute each in distilled water, 2 times for 1 minute each in 70% ethanol, and then dried. Incorporated radioactivity (counts per minute, CPM) was quantified using standard liquid scintillation techniques.

#### MTS Staining for PBMC Viability to Measure Cytotoxicity

**[0042]** At assay termination, the uninfected assay plates were stained with the soluble tetrazolium-based dye MTS (CellTiter 96 Reagent, Promega) to determine cell viability and quantify compound toxicity. MTS is metabolized by the mitochondria enzymes of metabolically active cells to yield a soluble formazan product, allowing the rapid quantitative